Fisheries and Oceans Canada | Pêches et Océans Canada

Ecosystems and Oceans Science | Sciences des écosystèmes et des océans

Canadian Science Advisory Secretariat (CSAS)

Research Document 2016/038

Pacific Region

Report on the Performance Evaluation of the Fluidigm BioMark Platform for High-Throughput Microbe Monitoring in Salmon

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May 2016

Canada

Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

Research documents are produced in the official language in which they are provided to the Secretariat.

Published by:

Fisheries and Oceans Canada
Canadian Science Advisory Secretariat
200 Kent Street
Ottawa ON K1A 0E6

http://www.dfo-mpo.gc.ca/csas-sccs/
csas-sccs@dfo-mpo.gc.ca

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ISSN 1919-5044

Correct citation for this publication:

Miller, K.M., Gardner, I.A., Vanderstichel, R., Burnley, T., Schulze, A.D., Li, S., Tabata, A., Kaukinen, K.H., Ming, T.J., Ginther, N.G. 2016. Report on the Performance Evaluation of the Fluidigm BioMark Platform for High-Throughput Microbe Monitoring in Salmon. DFO Can. Sci. Advis. Sec. Res. Doc. 2016/038. xi +282 p.

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ABSTRACT

A formal evaluation of the Fluidigm BioMark™ high throughput microfluidics platform for use in microbe surveillance research was undertaken. Forty-seven assays to 46 microbes suspected or known to cause disease in salmon worldwide were developed or derived from the literature, and appropriate controls obtained. The evaluation completed stage-1 of the OIE pathway, ascertaining the analytical sensitivity, analytical specificity, and repeatability and consistency between platforms within a laboratory for all 47 assays. Note, however, that our repeatability analyses took place over less than two months, so we may not have adequately factored in time. The microfluidics platform can assess duplicates of all 47 microbes at once across 96 samples, but to do so, due to the very small size of the reaction chambers (7nl), requires an enrichment step that includes a multiplex with all assay primers. Therefore, we developed a unique approach to additionally assess the role of the serial targeted enrichment on the specificity and relative quantitation of the assays.

The full undertaking of this study entailed 349,440 qPCR reactions over 37 dynamic arrays on the BioMark platform and 11-384 well plates on the ABI 7900. By all measures, the assays assessed on the platform performed to high standards, demonstrating a high degree of sensitivity (limit of detection under 40 copies per reaction chamber, with most assays less than10copies), specificity (most assays demonstrating >98% sensitivity and specificity to the targeted microbes), and repeatability (averaging 96% over all assays for samples run by two technicians and near perfect agreement for scoring alone). Consistency of data between the two platforms was also high, with a concordance correlation coefficient (CCC) of 0.95 after correction for the 8-10 Ct difference between platforms. If samples were only scored positive for duplicate detections and appropriate Ct cutpoints were applied, specificity and sensitivity between platforms rose to >0.99. Finally, there was no evidence that the STA enrichment step had any great impact the analytical sensitivity or specificity of the assays.

In the process of evaluation, while we found that the platform provided high caliber, reliable data, we did identify a few assays that performed relatively more poorly than others on one or more measures. Most were still within reasonable limits to use for research purpose to document variance in prevalence and load, but a few were targeted for removal or replacement. Issues with amplification curve quality appeared to have the largest effect on assay performance, and affected six of the 47 assays. Optimization of primer/probe concentrations could resolve this issue for most, however.

The BioMark platform proved to provide reliable, rapid and inexpensive quantitative data on microbe presence and load. We did not evaluate the platform for diagnostic use, which would require re-assessment under different criteria to ascertain whether diagnostic testing would be an appropriate use of this technology in salmon. We note, however, that the platform is already being applied for human viral and bacterial diagnostics and water quality testing.

Rapport sur l'évaluation du rendement de la plateforme BioMark de Fluidigm pour la surveillance à haute capacité des microbes chez le saumon

RÉSUMÉ

Une évaluation officielle de la plateforme microfluidique à haute capacité de BioMarkMC de Fluidigm aux fins d'utilisation dans le cadre de la recherche de suivi a été entreprise. On a procédé à quarante-sept essais, tirés de la littérature ou non, sur 46 microbes suspectés ou reconnus pour causer des maladies chez le saumon à travers le monde, et obtenus des contrôles appropriés. L'évaluation a terminé l'étape 1 du processus de l'Organisation mondiale de la santé animale (OIE), en vérifiant la sensitivité analytique, la spécificité analytique ainsi que la répétabilité et l'uniformité entre les plateformes d'un laboratoire pour les 47 essais. Toutefois, il convient de noter que les analyses de la répétabilité ont été réalisées en moins de deux mois, ce qui ne permet peut-être pas de bien tenir compte du facteur temps. La plateforme microfluidique peut évaluer les duplicats des 47 microbes en même temps au moyen de 96 échantillons mais, pour ce faire, compte tenu de la très petite taille des chambres de réaction (7 nl), il faut ajouter une étape d'enrichissement qui comprend une analyse multiplexe de toutes les amorces d'essai. En conséquence, nous avons élaboré une approche unique visant à évaluer davantage le rôle de l'enrichissement en série ciblé en ce qui a trait à la spécificité et à l'analyse quantitative relative des essais.

En tout, cette étude a nécessité 349 440 réactions en chaîne par polymérase quantitative (qPCR) pour 37 jeux ordonnés dynamiques avec la plateforme BioMark et 11 384 plaques à puits avec la plateforme ABI 7900. Selon toutes les mesures, les essais évalués sur la plateforme ont respecté les normes élevées et démontré un haut niveau de sensitivité (limite de détection pour 40 copies par chambre de réaction, avec moins de 10 copies pour la majorité des essais), de spécificité (la plupart des essais ont affiché une sensitivité et une spécificité supérieures à 98 % aux microbes ciblés) et de répétabilité (en moyenne 96 % de l'ensemble des essais pour les échantillons réalisés par deux techniciens, et une concordance presque parfaite pour le pointage). L'uniformité dans les données entre les deux plateformes était également élevée, avec un coefficient de corrélation de concordance (CCC) de 0,95 après correction pour une différence de 8-10 Ct entre les plateformes. Si les échantillons n'obtenaient un résultat positif qu'aux détections de duplicats et que des points de seuils Ct appropriés étaient appliqués, la spécificité et la sensitivité entre les plateformes s'élevaient à plus de 0,99 %. Enfin, il n'y avait aucune preuve que l'étape d'enrichissement par amplification de cibles spécifiques ait un impact quelconque sur la sensitivité ou la spécificité analytique des essais.

Au cours de l'évaluation, lorsque nous avons observé que la plateforme offrait des données fiables de plus haut niveau, nous avons décelé quelques essais qui ont obtenu de moins bons résultats à une ou plusieurs mesures. La plupart pouvaient toujours être utilisées dans des limites raisonnables à des fins de recherche pour documenter l'écart dans la prévalence et la charge, mais quelques-uns de ces essais ont été supprimés ou remplacés. Les problèmes liés à la qualité de la courbe d'amplification semblent avoir l'effet le plus important sur le rendement des essais, et ont touché 6 des 47 essais. L'optimisation des concentrations amorce/sonde pourrait toutefois résoudre ce problème la majorité du temps.

La plateforme BioMark s'est révélée en mesure de fournir des données quantitatives de manière fiable, rapide et peu coûteuse sur la présence et les charges des microbes. Nous n'avons pas évalué la plateforme à des fins d'analyse diagnostique, qui nécessiterait une nouvelle évaluation en fonction de différents critères pour vérifier si les diagnostics sont appropriés pour cette technologie en ce qui concerne le saumon. Nous avons toutefois remarqué que la plateforme est déjà appliquée aux diagnostics bactériologiques et viraux chez les humains ainsi qu'aux analyses de la qualité de l'eau.

1 INTRODUCTION

Over 90% of juvenile salmon migrating from freshwater into the ocean will die before returning to freshwater to spawn. Although mortality is believed to be highest during the first few months in the marine environment, the relative importance of infectious and non-infectious causes remains largely unknown. Current knowledge of infectious diseases in salmon is derived almost exclusively from observations of cultured fish (both enhancement and commercial). Consequently, we have a fair understanding of pathogens and clinical disease impacting salmon in freshwater hatcheries and sea-water net pens, but a much poorer understanding of pathogens affecting wild Pacific salmon in the ocean. Motivated by the need to fill this gap and the recognition that novel genomic approaches may provide a depth of resolution not previously available, the DFO Molecular Genetics Laboratory developed a 4-phase project with the Pacific Salmon Foundation and Genome British Columbia to describe the microbes (defined as viral, bacterial, fungal and protozoan micro-organisms) in British Columbia (BC) salmon, and identify those that may influence productivity and performance of BC's wild, hatchery and farmed salmon populations. The Project focuses principally on microbes that are recognized globally to cause clinical disease (or related to opportunistic infections of immune compromised fish) in salmonids, and utilizes genomic methods to identify and verify which microbes are presently detectable in wild and cultured (federal hatcheries and salmon farms) fishes in BC, Canada. Later phases of the project will develop challenge studies on top ranking microbes that have the greatest potential to negatively impact wild salmon to better understand under what conditions, if any, these microbes cause disease.

The stated strategic goal of the Project is "to discover the microbes and potential diseases that may undermine the productivity and performance of BC (wild) salmon and to determine what exchanges may happen between wild and cultured salmon in the evolution of these microbes". The four sequential Phases are set up as follows:

PHASE 1 (2012 - 2013) established a large-scale sampling program for wild, hatchery and aquaculture salmon. The initial sampling was conducted in 2012 and early 2013 but extends into other phases to include multi-year data. Collection archives are also available for hatchery and wild salmon from 2008-2011, which was supported by a previous Genome BC project.

PHASE 2 (2013 - 2016) is developing, testing, evaluating and ultimately applying a novel genomic technology to determine which microbes associated with disease in salmon worldwide are carried by wild and cultured salmon in BC. This phase also involves epidemiological studies supported by microbe distributional data, and next generation sequencing, histopathology, and functional genomics studies to identify the microbes that carry the highest probability of impact on wild fish.

PHASE 3 (2015 - 2017) will focus on the microbes identified in Phase 2, with an emphasis on microbes that have not been extensively researched in BC and that carry the highest potential for disease in wild salmon. Laboratory challenge studies will be carried out to assess under what conditions specific microbes are associated with disease in Pacific salmon. Additional studies assessing transmission dynamics of specific microbes will also take place. Genomic data on host response and adaptability to disease will be integrated into these studies. This phase is moving to funding from Genome Canada and will be largely carried out in academic laboratories.

PHASE 4 (2017 - 2018) will include reporting of research and presentations to management agencies on the potential utility of methods developed and the application of outcomes to future monitoring.

It is important to note that the first two *Phase*s of the research program focus on microbes rather than specifically on disease, which is the focus in the third *Phase* of our program. We take this approach because we rarely, if ever, have the opportunity to observe wild fish die, especially in the ocean; hence, it would be rare to sample wild fish in their final stages of disease. However, because many microbes can be carried by fish before (and after) clinical disease occurs, identification of some infectious disease agents is possible in a representative sample of wild fish. In taking this approach, we recognize that while many microbes can cause disease in wild salmonids, not all diseases are caused by microbes (note: this program is specifically focused on infectious disease) and not all microbes necessarily cause disease. It is also recognized that the diagnostic performance for simultaneous detection of large numbers of microbes, particularly in healthy fish populations, will be very difficult to quantify and may not reflect active infection (i.e. the assay is detecting components of the microbe and not necessarily reflecting live organisms), thus limiting the interpretation of results to trend comparisons. Hence, in *Phase* 2 of this program, we need to be careful to avoid assuming that all microbes detected in BC salmon are causing disease and mortality. However, we argue that by identifying microbes detectable in wild Pacific salmon populations, and by restricting our surveys largely to microbes known to be associated with disease or mortality in salmon, we can begin to define the types of diseases that *may* affect them and to define the temporal and spatial distribution patterns of selected microbes. Further, statistical analyses contrasting microbe prevalence and load among salmon populations, life-history stages, and/or years may also inform the ecological interaction of specific microbes, or suites of microbes, and salmon population dynamics.

Genomic science provides a foundation upon which this project will build a greater understanding of salmon microbes and disease processes. Over the course of the project, we will use three genomic approaches:

1. a nano-fluidic quantitative PCR platform (Fluidigm BioMark®; hereafter BioMark) for microbe monitoring,
2. next generation sequencing (NGS) for microbe sequence confirmations, epidemiological studies and novel microbe (mostly viral) discovery, and
3. gene expression profiling on microarrays and the BioMark platform to assess transcriptional signatures associated with microbe carrier states (Phase 2) and disease (Phase 3).

For microbe monitoring, we intended to use a combination of published and newly developed assays for up to 46 unique microbes that are suspected or known to associate with disease or decreased marketability (e.g. *Kudoa*) of salmon worldwide.

The objective of this Canadian Science Advisory Secretariat (CSAS) National Peer Review (NPR) Process is to provide managers advice pertaining to the reliability of information on microbe presence and load that would emanate from the BioMark platform. This advice was motivated, in part, by the recognized need for information on the health status of wild BC salmon, the high public and media interest in this research, and the wide ranging implications of such research. As such, it is imperative that the assays run on the platform be carefully evaluated for sensitivity (limit of detection), specificity (range of species and strains amplified), and repeatability so that the limitations of what can and cannot be concluded are well established and measures are implemented to minimize the potential for false positive or negative results. This corresponds to evaluation of the analytical performance of assays to stage 1 of the World Organisation for Animal Health (OIE) as described in its Manual of Diagnostic Tests for Aquatic Animals (OIE 2013).

Briefly, the OIE Manual describes a 4-stage pathway to assess a test's "fitness-for-purpose" in a designated target population/species (e.g. Pacific or Atlantic Salmon in BC): stage 1 - analytical

characteristics, stage 2 - diagnostic sensitivity and specificity, stage 3 - reproducibility among laboratories, and stage 4 - program implementation. Completion of stages 1, 2, and 3 is interpreted as being fit for the originally intended purpose, usually at a national level, and would be expected for tests used in a diagnostic laboratory setting. After successful implementation in disease control or surveillance programs, the assay might be considered for international recognition by OIE but this is usually in the context of trade related to OIE-listed diseases. To our knowledge, there are no tests for aquatic animals validated to stage 4.

The purposes listed in the OIE Manual are:

1. to demonstrate population 'freedom' from infection (zero prevalence);
2. to demonstrate freedom from infection or agent in individual animals or products for trade purposes;
3. to eradicate infection;
4. to confirm a diagnosis of clinical cases;
5. to estimate prevalence of infection to facilitate risk analysis; and
6. to determine immune status in individual animals or populations.

Research purposes are not mentioned in the Manual but as a minimum, most scientists would expect evaluation of the analytical characteristics of an assay (stage 1) as described herein.

2 OBJECTIVES

We were asked to provide an analysis that includes the following:

1. Determine whether the methodology, including design and statistical analyses, used to assess the assays appropriate to demonstrate their fitness for large scale research monitoring for microbes in wild Pacific and farmed Atlantic Salmon.
2. Determine the analytical sensitivity, specificity, and repeatability of each microbe assay.
3. Evaluate to what level the assay results are comparable across the Fluidigm BioMark and ABI 7900 platforms (within the Molecular Genetics Laboratory at the Pacific Biological Station).
4. Evaluate the effect of the specific target amplification (STA) of multiple independent target species on the analytical sensitivity specificity, and repeatability of the assays. Specifically:
   a. whether the STA introduces biases in the relative abundance of targets, and
   b. whether it generates spurious (false) targets.
5. Provide a synthesis of strengths and weaknesses for the intended application of this platform.

The design of the evaluation process was developed within our core research team (input from Miller, Gardner, Hammell) and collaborators (Clouthier, Johnson, Jones, Winton), with input from the National Aquatic Animal Health Program (NAAHP) (Veniot, Garver, Stephen, Gagné), and went through two rounds of formal peer review with Genome BC. The research funded by Genome BC pertaining to platform evaluation was carried out under six objectives:

1. Assay development (original objective B);
2. Assay sensitivity and optimization on the BioMark (original objective A.iii);
3. STA impact on assay specificity (ASP; original objective A.ii);

4. STA impact on relative quantification (original objective A.i) ;
5. Repeatability of assay results on the BioMark (original objective A.iv);
6. Consistency across platforms (original objective A.v).

3 FLUIDIGM BIOMARK PLATFORM

The Fluidigm BioMark platform uses nano-fluidics to increase the numbers of samples and assays run simultaneously, decrease the sample volumes by 1000-fold and increase the speed and sensitivity of the assays when compared with the ABI 7900 platform, resulting in a significant reduction in reagent use and cost (Blow 2007; Devonshire *et al.* 2011). The platform gene expression dynamic array simultaneously performs 96 assays on 96 samples, providing 9,216 data points per run (Figure 1a). Evaluation of the BioMark against the ABI 7900 in Devonshire *et al.* (2011) showed that the linear detection range and precision of replicate measurements was similar between instruments. Moreover, the BioMark was recently shown to be able to detect a 1.25 fold difference in DNA copy number (when 18 to 40 replicates are applied), better than most other platforms (Weaver *et al.* 2010). The assays run on this platform, and use of appropriate controls, can be the same as one would run on other quantitative PCR platforms. Hence, evaluation of platform performance is fairly straightforward.

The major distinguishing feature of the BioMark from other qPCR platforms is the very small sample volumes (0.007 μl compared to 6-10 μl for traditional platforms). These small volumes necessitate the use of a pre-amplification (STA) step to enrich for targeted sequences of interest. While this protocol has been extensively evaluated for gene transcriptional studies, it will need to be thoroughly evaluated for any effects it may have on the relative quantification and specificity of microbe assays.

We should note that the BioMark platform is being developed for human pathogen and disease diagnostics in a few large biotechnology companies in the USA; hence, we are on the cutting edge by applying this platform in salmon at an early stage of adoption for human health. The first publications on use of this platform for assessment of presence and load of bacteria and pathogens came out as we were performing these analyses (Ishii *et al.* 2013; Dhoubhadel *et al.* 2014; Michelet *et al.* 2014), and results are very positive. There is a strong potential that this system could, once appropriate levels of validation are met, be applied broadly to fish health diagnostics and regulatory diagnostics, bringing about considerable cost and time savings for the application of molecular assays - the cost to run four assays in duplicate on 90 fish samples on the ABI 7900 is similar to the cost of running 48 assays in duplicate on 90 fish samples on the BioMark, with a time commitment similar to running only a single assay. The BioMark is capable of 3-4 runs per day, based on normal working hours; hence up to 360 individuals for 47 duplicated microbe assays.

4 MICROBES OF INTEREST

Our team of experts identified 46 microbes of interest to this project, including 12 viruses, 12 bacterial pathogens, and 22 parasites (Table 1). For 30 of these microbes, TaqMan assays were available from the published primary literature, 2 were available through other labs but not yet published, and 15 required that assays be developed.

While most of the microbes on our list are known or hypothesized to be salmon pathogens, not all are known to be endemic to BC salmon. Of the viruses, salmon alphavirus (SAV), piscine myocarditis virus (PMCV), infectious salmon anemia virus (ISAV) and infectious pancreatic necrosis virus (IPNV) are associated with salmon diseases in European and/or East Coast

salmon, and the salmon herpesvirus (OMV) associated with disease in Japan and elsewhere, have not been detected in Pacific salmon. Viral encephalopathy and retinopathy virus (VER) is a betanodavirus that is associated with disease worldwide in many marine teleost species, including those in Pacific waters, and although laboratory challenge studies have shown that salmon can be susceptible to disease and mortality associated with VER (Korsnes *et al.* 2005), there have to our knowledge been no direct studies on this virus in salmon. Of the microbial pathogens, the two chlamydia species, an uncharacterized gill chlamydia species (Sch) and *Piscichlamydia salmonis*, have recently been identified in diseased salmon in Europe but not extensively studied, and not previously assessed for presence in BC salmon. Several of the parasites are Reportable or Immediately Notifiable to the CFIA, including *Ceratomyxa shasta*, *Myxobolus cerebralis* and *Gyrodactylus salaris,* the latter two of which are not known to occur in BC. Twelve other parasites included in our panel occur in BC and have been associated with disease in salmon either in Canada or, in the case of *Desmozoon lepeophtherii*, in Norway. Four other parasites have not been reported from Canada but are included on our panel because of their association with significant disease in salmon elsewhere. Two of these are important pathogens of salmon in waters of the United States adjacent to Canada: *Neoparamoeba perurans* is the causative agent of amoebic gill disease in salmon and *Nanophyetus salmincola*, associated with reduced survival of juvenile Pacific salmon.

Kudoa thyrsites is included not because it causes clinical disease in salmon, but because severe infections lead to severely diminished post-mortem flesh quality. While this parasite is known to be economically problematic to the BC Atlantic Salmon farming industry, the parasite is also detected in other salmon and marine fish species, although little is known about its distribution and severity in wild fish.

One virus on our list, Pacific salmon parvovirus, was only recently identified by full genome sequence in sockeye salmon by K. Miller's laboratory; this virus has not been amenable to culture. Testing by a qPCR assay to this virus has shown that it emanates from the freshwater environment, with the highest prevalence and load present in the early marine environment; prevalence and load appear to diminish in the fall across multiple years. The virus has also been detected in return-migrating adults. A single challenge study has been performed on sockeye fry, demonstrating infectivity but not mortality at this life-history stage. We chose to include this virus in our survey to discover more about its distribution and potential for association with disease in the early marine environment.

5 METHODS

5.1 ASSAYS

Principles of Assay Design

If assays developed in reputable molecular/fish health laboratories were available in the published literature, we used them. If there was a choice in assays, we tended to select those with good specificity (demonstrated *in situ* or *silico*) that had been validated for other platforms according to the OIE pathway (OIE Chapter 1.1.2 "Principles and Methods of Validation of Diagnostic Assays for Infectious Diseases" in the OIE Manual of Diagnostic Tests for Aquatic Animals). For the reportable viruses, we are using the NAAHP assays.

For microbes with no published assays, we developed TaqMan assays based on the following principles:

1. Minimize size of amplified product (70-150 bases);

2. Amplify all known sequence variants (conserved region for the microbe in question, divergent for other related microbes);
3. Do not amplify related species (alignments of strains and related species; primers and probes blasted against sequence database to ensure they are specific);
4. Universal set of cycling conditions (same dynamic temperature);
5. Minimize secondary structure that can impede the efficiency or efficacy of PCR.

Microbe assays were developed using all available sequence data from strain variants and closely-related species (>80% homology), with sequences attained using BLAST searches. PrimerQuest (online IDT assay design tool) and Primer Express 3.0.1 were used to develop the TaqMan assays, with specificity checked *in silico* by BLASTN. Ideally, each primer/probe set showed 100% identity exclusively to its specific target sequence(s). When possible, 2-4 primer sets were developed for each microbe and underwent initial efficiency testing; assays with good efficiency were further tested for specificity using a small number of related species (depending upon availability) and strain variants on the ABI 7900 before moving to the BioMark for further evaluation.

As most microbes that required assay development were parasites or bacteria with limited sequence data, we preferentially design assays to 16S rRNA for bacteria and 18S rDNA for parasites, as these are often the most characterized sequences within and between species and strains, and are widely used in phylogenetic analyses. As such, these sequences provide the greatest potential to design assays that ensure inclusivity (detection of all known strain variants of the targeted microbe) and exclusivity (no detection of untargeted microbe species). However, we note that rDNA is multi-copy; hence analytical sensitivity can be affected.

RNA assays to specific highly expressed genes were also pursued as they have the potential advantage to reflect microbes in an active state; in some cases, showing a high correlation with culture ability (see *Renibacterium salmoninarum* Msa assay in Suzuki and Sakai 2007). Targeting mRNA can increase analytical sensitivity for genes that are highly transcribed (RNA copy number >DNA copy number--e.g. Msa), but can distort the calculation of the actual number of microbes present in a sample.

Note: published assays were not modified to precisely match the universal Temperature of hybridization (Ta) (Table 1) on the BioMark, but were rather tested empirically for their performance under universal (annealing T 60 ^{o}C) conditions. Assays that did not perform well were removed or replaced.

5.2 POSITIVE SAMPLES

Positive control samples were acquired from fish health laboratories throughout the world (see Table 2) that included strain variants within microbes of interest and closely-related species. “Real sample” control material was provided in the form of nucleic acids from cell lines or tissue samples. RNA samples were either received as purified RNA on dry ice or RNA precipitated in 95% ethanol. These were recovered according to the shippers’ instructions and re-suspended in RNase-free sdH2O (Gibco BRL, Gaithersburg, MD). DNA samples were either received as tissue culture, purified DNA, precipitated DNA in 95% ethanol or dried (spores). The precipitated samples were recovered and re-suspended in nuclease-free sdH2O. For tissue culture and dried spore samples, the DNA extractions were performed using the DNeasy spin column kit according to manufacturer’s instructions (Qiagen, Maryland).

Where we could not obtain “real” positive samples through reputable sources, we used those from our own laboratory (MGL) which had been previously screened through traditional qPCR or

PCR and verified using Sanger sequencing. RNA and DNA extraction method for these samples is provided below.

The remainder of the samples for which positive control material could not be found were acquired as 1000 bp synthetic constructs (gBlocks) through IDT DNA (Coralville, IA, USA) which contained an APC probe sequence (Snow *et al.* 2009) labeled with NED™ dye (Applied Biosystems, Foster City, California) in order to track contamination. These constructs were designed *in silico* to surround the assay of interest and based on all available and relevant GenBank microbe sequences.

The acquired positive control materials (not including gBlocks) were kept in a separate, locked laboratory with restricted access. A negative pressure UV laminar flow hood was used whenever handling these and they were never brought into the main labs where field samples were stored and handled. The gBlocks were maintained and handled in another negative pressure laminar flow hood which is also housed in a separate room from all other positive control and "real" samples (see Supplemental Figure 1 to follow the segregation of labs).

5.3 TISSUE SAMPLE HANDLING AND NUCLEIC ACID EXTRACTION

All tissue samples collected for our program are either flash frozen in liquid nitrogen or on dry ice in the field and kept in -80 ^{o}C degree freezers until extraction or preserved in RNAlater, held at 4 ^{0}C overnight, and then frozen at -80 ^{o}C until extraction. Some fish are also collected whole and flash frozen in the field, with tissue dissections occurring in the laboratory. Note that samples did not go through more than 3 freeze-thaw cycles during the course of these analyses; most samples were only thawed 1-2 times.

We utilized Tri-reagent™ homogenates for all extractions so that DNA and RNA were extracted from the same pieces of tissue. Because smolt tissues were small, in general the entire tissue was extracted as a single homogenate. Depending upon the tissue size, there may be enough material for 1-3 extractions from each homogenate. We utilized these same extractions for functional genomic analyses on microarrays or via qPCR of host genes.

DNA was extracted using the DNeasy spin column kit (Qiagen, Maryland) and RNA was extracted using the Magmax™-96 for Microarrays RNA kit (Ambion Inc, Austin, TX, USA) with a Biomek NXP™ (Beckman-Coulter, Mississauga, ON, Canada) automated liquid-handling instrument, both based on manufacturer's instructions. The quantity of DNA and RNA was analyzed using the Qubit 2.0 fluorometer (Invitrogen, Carlsbad, CA) or spectrophotometer readings following manufacturer's instructions with the appropriate components (high sensitivity components for tissue culture and pure samples where quantities are limited and broad range for fish tissue positive samples).

All RNA was reverse transcribed into cDNA using the superscript VILO master mix kit (Invitrogen, Carlsbad, CA) following manufacturer's instructions. In order to provide enough cDNA for controls in samples with relatively lower loads, we added 2.5 µg of concentrated RNA and increased the incubation time to 2 hrs. CHSE (Chinook embryo cell control nucleic acids) RNA was added to boost the input quantity up to 1.0 µg when reverse transcribing RNA from viral cell cultures.

5.4 ARTIFICIAL CONTROL STANDARDS (APC)

Positive reference control constructs (APC clones) were developed for all microbes according to the methods outlined in Snow *et al.* 2009 (Supplemental Table 1). This approach, which utilizes artificial constructs of microbe nucleic acid which contain an additional "vector" probe synthesized within the amplified fragment, enables the simultaneous detection of vector

contamination in all assays, an important validation step to track contamination of high-load positive controls that is currently being applied in many fish health laboratories throughout the world. Serial dilutions of these controls spiked into negative salmon CHSE cell culture extracts were used to estimate the analytical sensitivity (under objective 2) for each assay on the BioMark following the methods of Devonshire *et al.* 2011 (see section on assay sensitivity and LOD on the BioMark below). These controls were added to the dynamic array last, and handled in a separate room from where DNA/RNA samples are stored and worked with to limit potential contamination between controls, which will contain high copy numbers of APC control nucleic acids, and samples. During monitoring, microbe controls within each level of serial dilution will be combined so that on the BioMark, clone controls for all microbes take up only 5 of the total 96 "sample" wells. This is done not only for efficiency, but also to emulate the situation in samples where multiple microbes may be present. However, in the actual BioMark analysis, each assay is amplified in a separate well (assays are not multiplexed) for samples and clones controls. Hence, with the 9,216 assays being performed simultaneously on the BioMark dynamic array, 480 (5 x 96) of the qPCR assays will comprise the serially-diluted microbe reference controls.

5.5 SPECIFIC TARGET AMPLIFICATION (STA)

While we are not multiplexing assays on the BioMark, the STA (run on a conventional PCR machine) does involve multiplexing low concentrations (1/20th of normal concentrations) of all primers to be run on a single dynamic array; these unincorporated primers are degraded in a purification step (ExoSAP-IT has no interference in downstream application, 100% recovery of PCR products) before individual assays are run.

The 5 μl STA reaction contains 1.3 μl of cDNA/DNA, 1X TaqMan PreAmp master mix (Applied Biosystems, Foster City, California) and 0.2 μM of each of the 48 primers (47 microbes and 1 housekeeping gene from Miller et al. 2011). For objectives aimed at assessing the impact of the STA on sensitivity/specificity/repeatability of microbe assays, multiple STAs were performed generally using more than one PCR machine (either a PTC-100 PCR instrument [Bio-Rad, Hercules, CA] or a Tetrad2 DNA engine instrument [Bio-Rad, Hercules, CA]). During the evaluation process, we found the Tetrad2 produced more consistent results (the PTC instruments were over 10 years old); hence the PTC instruments were only utilized in objectives 2 and the first two experimental sets for objective 4. The STA cycling program was performed according to manufacturer's instructions for TaqMan Gene Expression assays (Fluidigm Corporation, CA, USA) and included 14 cycles. Note the addition of the components was completed in separate work stations from the STA itself.

Upon completion of the STA, excess primers were removed by treating with Exo-SAP-IT™ (Affymetrix, Santa Clara, CA) according to manufacturer's instructions and then diluted 1/5 in DNA re-suspension buffer (Teknova, Hollister, CA). Attempts to amplify samples post treatment with Exo-Sap-It by adding probes with no primers have not yielded any detections in >900 samples analyzed.

5.6 96.96 DYNAMIC ARRAY PREPARATIONS

The 96.96 gene expression dynamic array (Fluidigm Corporation, CA, USA) was brought to the Fluidigm room where the IFC controller HX (Fluidigm Corporation, CA, USA) was used to prime the dynamic array with control line fluid and then loaded with samples and assay mixes in the appropriate inlets according to manufacturer's instructions. Specifically, a 5 µl sample mixture was prepared for each sample containing 1 × TaqMan Universal Master Mix (No UNG), 1 × GE Sample Loading Reagent (Fluidigm PN 85000746) and each of diluted STA'd sample mixtures. Five µl of Assay mix was prepared with 1 × each of the appropriate TaqMan qPCR assay

(microbe in FAM-MGB and APC in NED-MGB, 10 µM of primers and 3 µM of probes) and 1 × Assay Loading Reagent (Fluidigm PN 85000736).

Prior to running the dynamic array, both the sample loading tray and the dynamic array were brought to the APC clone hood whereby 2.7 µl of the serially diluted APC clone mixtures were pipetted into their appropriate wells in the sample loading plate. These were mixed and then 5 µl pipetted into the appropriate inlet wells from dilute to concentrated. Note all 47 of the APC clones were contained in a serially diluted pool, minimizing the likelihood of contamination of any single APC clone.

Once mixing and loading of the dynamic array was completed by the IFC HX controller, it was transferred to the BioMark HD instrument and processed using the GE 96x96 Standard TaqMan program for qPCR which includes a hot start followed by 40 cycles at 95°C for 15 sec and 60°C for 1 min (Fluidigm Corporation, CA, USA). The data were analyzed with Real-Time PCR Analysis Software (Fluidigm Corporation, CA, USA) and multiple dynamic array runs on the same sample groups were merged using GenEx5 from MultiD.

5.7 CONTROLS RUN IN EACH DYNAMIC ARRAY

1. A set of 5 or more serial dilutions containing all APC clones was run on every dynamic array both as a positive control for all assays and to estimate copy number.
2. While artificial constructs can be quantified for RNA and DNA assays, the DNA extracts are more consistent and most laboratories have moved to using them exclusively (Dr. M. Purcell, personal communication). Given that we have used both DNA and RNA assays, and the complexity in working with both DNA and RNA, we have chosen to use DNA extracts from APC clones for consistency.
3. A “plasmid” control probe labeled in NED was multiplexed into all assays to track contamination of the APC (same probe for all assays; as described in Snow *et al.* 2009). Note that the BioMark does not have a NED setting, so we used the VIC setting, which is the most similar wavelength, to score the NED probe detections. Hereafter we refer to these detections as VIC. The impact of this plasmid on assay efficiency and performance was assessed in the ASe study.
4. Negative processing controls were used to detect contamination within the DNA/RNA extraction phase (n=3).
5. No template controls for STA were used to detect contamination in STA-PCR reagents (n=2).
6. No template controls for PCR reaction were used to detect contamination in PCR reagents (n=2).
7. A pooled sample was constructed by merging samples from column A of each DNA/RNA extraction plate in a project, and used across the entire series of dynamic arrays. This sample should contain low levels of a large number of microbes and can be used to assess repeatability of low copy number samples between dynamic arrays and between platforms (n=2).
8. The RNA pooled sample was used as a no RT control (same pool, but no RT performed) (n=2).
9. An endogenous reference gene used to assess false negative results due to poor RNA quality or inefficient cDNA synthesis. We do not intend to normalize with this reference sample.

6 METHODOLOGICAL APPROACH AND DATA ANALYSIS ASSOCIATED WITH SPECIFIC OBJECTIVES

6.1 ASSAY SENSITIVITY AND OPTIMIZATION ON THE BIOMARK

The limit of detection (LOD) is a measure of the analytical sensitivity (ASe) of an assay. The LOD is the estimated amount of analyte (e.g. number of copies) in a specified host tissue or fluid that would produce a positive result at least a specified percent of the time. The greater the number of replicates for each dilution, the more precise the estimate will be of LOD. Recommendations of the working group on microbiological methods (AOAC, 2006) suggest that a 50% endpoint be estimated. The selected endpoint can be estimated using the Spearman-Karber non-parametric approach, or by logistic regression or probit analysis (AOAC, 2006). In all cases, point estimates and 95% confidence intervals are reported. We chose to use a more conservative estimate based on a 95% endpoint, interpreted as the concentration of the analyte in the sample matrix that would be detected with high statistical certainty (95% of the time). We also used data from the LOD experiment to estimate the efficiency, linearity, and linear range of each assay. Linearity is reflected by the correlation coefficient, R^2, which ideally is >0.985; although values >0.9 are acceptable. Efficiency is optimal when each cycle of amplification doubles the amount of product (efficiency 100%). 100% efficiency is obtained when the slope is -3.32. Efficiencies ranging from 90% to 110%, representing slopes between -3.1 to -3.6, are generally acceptable. Slopes outside this range may indicate non-specificity, mutations under primer/probe, assay not optimized due to inhibitors, primer-dimer, etc.

Minigenes™ that covered the full sequence amplified by each microbe assay were ordered from IDT. These were provided as purified double-stranded DNA in circularized plasmids, which contain restriction enzyme sites and RNA polymerase promoter sites on each side of the specific assay sequence. An APC probe sequence was inserted between assay specific probe and one of its primers as in Snow *et al.* (2009) in order to track potential contamination of high concentration controls. The minigenes were re-suspended according to manufacturer's instructions and transformed into One Shot® TOP10 Chemically Competent E. coli cells (Invitrogen, Carlsbad, CA). Plasmids were purified with QIAprep Miniprep (Qiagen) and linearized with 40 units of SpeI-HF (New England Biolabs 20,000 U/ml). Linearized plasmids (dsDNA) were quantitated through spectrophotometry with a Qubit 2.0 Fluorometer (Invitrogen), and copy numbers were calculated based on the concentration and size of the plasmid. Each plasmid was diluted to as close as possible $1x10^9$ copies/µl. 5 µl aliquots of each microbe plasmid were pooled into a 47 microbe mixture (containing plasmids for all microbe assays). The clone pool was serially diluted into 15 dilution mixtures, with the first three dilutions at 10x, followed by four at 5x and seven at 2x, with a final average copy number per microbe per µl range of 0.2 to $1.9x10^7$ (Supplemental Table 2; Figure 2). To maintain consistent salmon nucleic acids in each dilution, 2 µl of CHSE DNA (50ug/ml) was spiked-in. The full dilution series underwent six independent STAs over two independent PCR plates (the older PCR machines were used for this, which did increase the variance between plates compared to the newer Tetrad machines). Five STA and one Non-STA set were run in duplicate on each of four dynamic arrays, providing 40 replicates for each dilution overall STAs, 6-8 replicates within an STA, and 8 replicates of each Non-STA dilution (Figure 2). Given this design, we also had the ability to assess variability within assays over STA reactions within and between dynamic arrays, and associated with different PCR machines.

There was some concern from the CSAS review that our use of a single dilution series followed by intense replication (40x) by STA and dynamic array would not adequately address technical variation in pipetting through across dilution series. Hence, post-review, we conducted an additional analysis whereby three independent dilution series were made, each undergoing 6

STA replicates, and analyzed across 3 dynamic arrays (STAs were semi-randomized between dynamic arrays). In total, we ran an additional 18 replicates, which included variance by dilution series (3), STA (6), and dynamic array (3) to more fully evaluate assay-specific LOD and efficiencies. Note that after the CSAS review, we removed the assay for ye_ruc and switched assays for three of the microbes (VHSV, ISA7, and PMCV; Table 1); we ran the newer assays in this additional analysis.

As the actual starting concentrations of APC clones analyzed herein were not precisely $1x10^9$ copies per µl starting material, when calculating copy number, we adjusted the serial dilution concentrations to the actual measured concentrations for each APC clone, and these are the values we report – i.e. the LOD of an assay based on the copy number of the microbe plasmid present per µl prior to the STA reaction. We also provide the LOD per reaction chamber; to adjust for the actual copy number pipetted into each reaction chamber, we needed to consider both the volume of nucleic acid sample pipetted (starting copy number / µl x [2.25 µl nucleic acids/ 5 µl total assay components] x [6.075 nl pipetted per inlet] / 1000 nl/µl) and for the STA samples, the expected level of amplification of the sample (calculated according to manufacturer's instruction by the formula [1.25 µl nucleic acid /25 µl assay volume] x [14 cycles minus 1 to the power of 2] x [volume of nucleic acid sample pipetted (above)]). In essence, the final copy number that goes into each chamber on the BioMark after the STA is estimated to be 1.12 times that per µl of the starting solution. The final copy number into each chamber for a Non-STA sample is 0.00273 times that per µl of the starting solution. Hence, the STA is developed to produce approximately a 1000-fold amplification of the starting material.

For statistical LOD analyses, control samples (such as water and blanks) and assays (such as 'housekeeping' genes) were excluded, and analyses were stratified by their amplification status (STA vs. Non-STA). The observed LOD in terms of copy number and Ct value was determined as the last dilution in which 95% or more samples were positive. We had initially also included a calculated Ct, based on a logistic regression with the Pass/Fail status of the sample as the outcome and the concentration (log10) as the predictor, but we found that this approach had a tendency to provide tenuous estimates when any outlier samples were included, hence were removed from the final document. Given the relatively large numbers of dilutions and repeats, direct estimates were deemed sufficiently precise.

All statistical analyses were performed in Stata 13 (Stata 2013), and unless otherwise stated, statistical significance was always set as $p<0.05$.

Optimization of assays is more difficult on platforms on which multiple assays are run at once, as assays must use the same cycling conditions and share common buffers. In the case of the BioMark, while primer and probe concentrations could technically be varied among assays, it would be difficult to change other components unless done universally for all assays. We began an optimization testing process for a small number of assays later in our analysis after we became aware that a small number of assays had poor quality amplification curves on the BioMark, but not on the ABI7900, and were difficult and time consuming to score. Hence, we tested a variety of primer and probe concentrations to determine if the assay amplification curves could be improved (Supplemental Figure 2). We found that by shifting our primer/probe concentrations from 10µM /3µM to 9µM/2µM, we gained considerable improvement in our problematic assays, with little change in good assays. As we were over half way through our evaluation process, we decided not to change conditions within our studies. However, in future we will assess the impact of universally shifting all primer/probe combinations to this optimal concentration on LOD and repeatability before deciding whether to undertake this change universally.

6.2 STA IMPACT ON ASSAY SPECIFICITY

Analytical specificity (ASp) assesses the degree to which the assay detects all intended strains of the microbe in question (inclusivity/sensitivity) and does not detect (amplify) other microbes (exclusivity/specificity). ASp is closely considered in the design of all microbe assays, with care to ensure that primers and probes are unique to the intended microbe. Ideally, primers and probes comprising a microbe assay are developed in regions that contain no known sequence variation between strains, but that show variance between closely-related species sufficient to prevent their amplification. This is the case for most of the assays evaluated on the BioMark platform, however, there are some assays that rely on a modest number of mismatches to discriminate among closely-related species (e.g. *Vibrio* spp., *Myxobolus* spp.) or whereby there may be a small number of variable nucleotides amongst strains that, depending upon their location (under primers or probe), may or may not interfere with their amplification (e.g. IPNV).

We evaluated whether the STA affects the ASp of the TaqMan assays on the BioMark in three sets comprising

1. bacterial microbes,
2. viruses, and
3. parasites.

Within each set, we utilized the "real" and synthesized gBlocks control material (with spiked-in CHSE cDNA to provide a background of host nucleic acids) from strains showing sequence variation under the assay in question and for closely-related species (See Table 2 and Supplemental Tables 3-5 for control details). Within each of these sets, all assays were run across all control samples, providing a robust estimation of ASp that includes both closely- and distantly-related species (note that some of the tissue controls provided to us carried more than one microbe; this was most problematic for the parasites). Four to six replicates of each microbe set were run both with STA (including all microbe primers) and Non-STA on one or two 96.96 dynamic arrays (Figure 3).

For statistical analysis, control samples (such as water and blanks) and the housekeeping gene assay were excluded from all analyses related to analytical specificity, and analyses were stratified by their amplification status (STA vs. Non-STA). An expected Pass/Fail status was assigned to each observation, based on the intended targeted microbe, and samples were designated as 'false positive' if they were observed as 'Pass' when their expected status was 'Fail'. Control samples for which co-infections were confirmed via sequencing (see below) were re-scored as 'Pass' for final estimates of ASp.

6.3 VALIDATION OF ANALYTICAL SPECIFICITY USING NGS

The Fluidigm Access Array™ system was used to validate the Asp qPCR data and to verify if distantly-related species detections were the result of co-infections within tissue samples or cross-amplification. This system allows for the efficient generation of 2,304 targeted sequences on each 48.48 Access Array IFC by combining amplicon generation (primers only-no probes) with NGS library preparation (addition of barcode).

By utilizing two 48.48 Access Array IFCs, we sequenced all of the positive control samples used in this study, with the exception of those derived from gBlocks. The 4-primer amplicon tagging approach to generate Illumina compatible sequences was utilized whereby tagged target-specific (TS) primer pairs were combined with sample-specific primer pairs that contain Illumina adaptor sequences and barcodes. Note, that the NGS target specific primer pairs included the primers used in the study with the exception of ye_ruc and included the newer primers for

replacement assays to isav7, pmcv and vhsv. As in the other analyses, all samples were STA'd, because the GE IFC well size was only 7 nl.

The priming and loading steps were performed on an IFC Controller AX, the amplification step was performed on the BioMark system and finally the harvest step was performed using barrier tape in the IFC Controller AX. The post-harvest sample pool PCR products were evaluated for quality and equal volumes of the 48 sample pools within an Access Array IFC were subsequently pooled and purified utilizing AMPure XP beads (Beckman-Coulter, Brea, CA). The purified PCR product pools were then quantified using the Qubit dsDNA HS assay kit (Invitrogen, Carlsbad, CA) and qualified using the Bioanalyzer HS DNA chip (Agilent, Santa Clara, CA). The final libraries were normalized to 4nM and run on a MiSeq 600 cycle v3 chip (Illumina, San Diego, CA) according to manufacturer's instructions when using custom primers. A BWA BAM alignment utilizing a reference file corresponding to all of our APC amplicon sequences minus the APC probe region was performed within the MiSeq Reporter software. Finally, the data was analyzed utilizing both MiSeq Reporter (Illumina, San Diego, CA) and Partek Genomics software (Partek Inc., St. Louis, MO, USA). In the qPCR/NGS comparison, FAM data was analyzed in terms of less than or greater than the assay-specific Ct-cutpoint (> or < LOD), no VIC data cutoffs were applied and a minimum 30 read cutoff for sequences matching (or nearly matching) microbe reference sequences was applied to the NGS data.

6.4 STA IMPACT ON RELATIVE QUANTITATION

The threshold cycle (Ct) is the point in the amplification plot whereby the fluorescence exceeds background levels in the no template control and where exponential growth of the PCR product ensues, with lower Ct values indicating higher sample loads. We assessed the impact of the STA on relative quantification in the presence of controlled mixtures of microbe samples to ensure that there was no cross-amplification of spurious products (in which negatives may come up as positive) or inhibitory competition between microbes (in which low copy number microbes may come up negative or at higher Cts than expected). To assess for competition effects, we focused on the detection and quantification of low copy number microbes in the presence of multiple high copy number microbes, and determined the level of divergence in Ct from expectation. This is similar to the approach taken in Dhoubhadel *et al.* (2014). To test for cross-amplification of spurious products, we incorporated 276 to 372 samples per microbe that should be negative (i.e. measured but not present in the mixture in question; for details, see further below). Repeatability was measured on high- and medium-load samples (see definitions below), assessing the variance in Ct among replicates and mixtures (24-84 for each microbe).

To assist setting up mixtures and determining expected loads (by Ct values), we ran each control sample alone against all microbes on the BioMark to determine the Ct of the targeted microbe and assess whether there were any additional "background" microbes that may be present but not identified by the source lab in tissue samples. Samples with Cts < 18 were targeted as "high-load", with copy numbers generally exceeding 1,000 per µl of starting material. These samples could be serially diluted in mixtures to assess performance of the assay at high and low-load in a mixture. For simulated low-load samples, we diluted the sample to obtain an expected Ct of 24-26 (4-15 copies/µl). Controls with Cts between 18-23 contained only "moderate" loads (10s to 100s of copies) and could not be serially diluted (in 10-fold increments) to create low-load samples, hence these were generally run at a constant load, although some dilutions were attempted.

Concentrated samples (cDNA or DNA) were diluted in DNA suspension buffer to obtain a "high-load" sample, "medium-load" sample, and "low-load" sample. High-load DNA samples that came from pure cultures were diluted in DNA suspension buffer and spiked with CHSE cells (final concentration of 100 ng); whereas those that came from fish tissue samples were diluted

in DNA suspension buffer alone. The remainder of the dilutions was carried out in DNA suspension buffer except for the final low-load dilution for each sample which included a spike-in of 100ng of CHSE DNA. For mixtures 35 to 42 where there were only 2 low-load samples, 100 ng of CHSE DNA was also spiked in.

We developed a series of 8-microbe mixtures (Figure 4a) with most containing six microbes at high-load (light blue), one microbe at moderate load (dark blue), and one microbe at low-load (bright blue), with the low-load microbe alternating amongst the six high-load microbes. Additional mixtures included all low-load all high-load (with one moderate), and negative CHSE controls. Due to background microbe carrier states in some tissue samples (which we could not control), there was often additional microbes present, and hence our described complexity reflected the minimal complexity of each mixture. On each dynamic array, we assessed two blocks of eight microbes each (16 targeted microbes), with each "high-load" microbe being run as a "low-load" against each 8-microbe set (Figure 4b). In all, there were 42 sample mixture sets run on each dynamic array, with each set undergoing four independent STAs produced on two separate PCR instruments and run in triplicate over two dynamic arrays (Figure 5). In order to capture all of the microbes in a mixture set (consisting of the 8 targeted microbes), we repeated the process for four sets of 16 targeted microbes. Consequently, 14 of the microbes were assessed in two separate mixtures.

For maximum efficiency on the BioMark, we ran these analyses in blocks of 16 microbe assays plus 14 additional assays, which included positive and negative samples in the mixtures, one housekeeping gene assay and one negative control assay with no primers and/or probes (NRP). These 32 assays were run in triplicate on each 96.96 dynamic array over the 42 sample mixtures. Note, that while each sample mixture generally contained a maximum of 8 targeted positive microbes, the STA was performed with all intended 47 microbe assays plus the housekeeping assay.

For each microbe, more than half of the 42 samples were designed to be negative. As the samples were run in triplicate with four separate STAs across two 96.96 dynamic arrays, there were 276 or 372 samples (microbes 1-7 and 8, respectively) per microbe that was expected to be negative, 48 or 24 samples (microbes 1-7, and 8, respectively) of each microbe at a low-load, 84 or 180 samples (microbe1 and 2-7, respectively) at a high-load and 96 or 108 samples (microbes 1 and 8, respectively) at a moderate load.

In a final run, we created a mixture of all positive microbe samples serially diluted into 14 concentrations and ran both with and without STA to test the influence of the 47 microbes on the amplification efficiency of each individual microbe within the mixture. Thirteen serial dilutions of the artificial constructs were also run on the same dynamic array (0.2-1.9E5). All samples were repeated on a second dynamic array without the plasmid control APC probe to determine the potential effect of the extra probe (labeled in NED, scored in VIC) on ASe; the absence of the plasmid control APC probe was deemed to have a negligible influence on the results.

The efficiency of each assay was calculated using the ASe LOD serially-diluted artificial construct clone data in GenEx software from MultiD Analysis.

The statistical analyses were focused on assessing if there was a reduction in the ability of an assay to detect the presence of a low (or medium) concentration microbe diluted in a solution containing seven other microbes in high (or medium) concentrations. The microbe dilutions were carried from the same sample solutions that were used to create the high concentrations. As such, the dataset contained samples with observed Cts, and dilutions (i.e. 1/2, 1/4, 1/6, 1/8, 1/10, 1/50, 1/100, 1/200, 1/400, 1/1000, 1/2000, and 1/4000). Using the estimated corresponding assay efficiencies (expressed as a percent, and determined in the limit of detection study), expected Cts were calculated as such:

$$Ct_{expected} = [log_{10}(dilution)(-3.32)(efficiency)] + Ct_{high}$$

Where the *dilution* is based on the dilution factor (e.g. 1/10 = 0.1) of the high-concentration sample, and Ct_{high} is the median Ct value for the all high concentration Cts within an assay within a group. The $Ct_{expected}$ values were subsequently aggregated to produce one median $Ct_{expected}$ within assays, groups, and sample batches.

The *observed* Cts (one median value per group, assay, and sample) were compared to the *expected* Cts (one median value per group and assay) with a concordance correlation coefficient analysis (including a Bland-Altman plot), and a nonparametric Wilcoxon matched-pairs signed-ranks test. A simple linear regression with $Ct_{observed}$ as the outcome and $Ct_{expected}$ as the sole predictor (with no intercept) was used to describe the overall percent difference between the observed and expected Cts. Additional linear regressions were used to model the effect of assays and serial dilutions on the observed Ct values.

The Pass/Fail status of each sample was used to determine the probability of false negatives, based on the expected outcome (indicated as negative, low, medium or high microbe concentration); for this task, the expected outcome was dichotomized (Pass: low, medium, and high concentrations; Fail: negative). Varying Ct cutpoints for the observed values were used to define the Pass/Fail status; these were selected based on appropriate Ct values determined from the limit of detection analyses (mean, median, and assay-specific Ct-cutpoints). The 95% confidence intervals for prevalence, sensitivity, and specificity estimates were also calculated for each assay (Stata 13, 'diagt' command).

Logistic regression models were used to assess the relationship of the probability of detection (observed positive results) between the assays and across the various serial dilutions, for those samples that were expected to be positive. Model assumptions were evaluated with the Hosmer-Lemeshow goodness-of-fit test (Dohoo et al. 2009).

6.5 REPEATABILITY OF ASSAY RESULTS ON THE BIOMARK

Because of the extensive replication in all of our studies, repeatability can be calculated under each objective; however, we conducted an additional study in which we assessed repeatability of microbe results between technicians.

We began the analysis on endemic microbes for which positive samples from BC are readily available. For repeatability analysis, we developed a study design that maximized the number of microbes we could assess in the smallest number of samples with a minimum requirement of at least 20 positive samples/microbe of interest and that reflected a range of Ct values for each microbe. To identify appropriate samples, we used the data from some of the "proof of concept" case studies (published in Miller et al. 2014) and a previous IHNV challenge study (Miller et al. 2007), a portion of which we had verified microbe results with Sanger sequencing. These studies involved a variety of species and tissues (Figure 6). Two hundred and forty samples were identified that contained >20 positive detections for 19 microbe assays, but also included dozens of samples with no microbe detections.

The study was set-up by a biologist who was not involved in the Fluidigm analysis, and who prepared triplicate plates of the RNA-containing aqueous layers from previously characterized known-positive tissue samples, assigning each sample a random number so that all testing was conducted blinded. Annotation information on individual samples was added after samples had been scored. Part 1 of the repeatability study focused on two different RNA extractions for each of the 3 x 80 samples which were performed by two different technicians following the same standard operating procedure (SOP). The cDNA from these extractions was assessed across three 96.96 dynamic array runs per technician (Figure 7). Each dynamic array also contained

16 control samples, including 7 APC clone dilutions, pooled samples (RT positive/negative), and processing and qPCR negative samples. In Part 2, one RNA extraction was processed by two different technicians (Figure 8). Part 3 assessed the repeatability of scoring, with three different technicians scoring six 96.96 dynamic array runs (Figure 9).

Repeatability and reproducibility (between dynamic arrays) estimates of samples were determined with linear mixed models assessing the relationship between Ct values and samples. The statistical methods followed closely the recommendation from the International Organization for Standardization (ISO) Technical Report on designing, implementing and statistically analyzing inter-laboratory repeatability and reproducibility results (ISO/TR 22971:2005(E)). In brief, Parts 1 and 2 followed this statistical model for *i* technicians, *j* samples of varying concentrations, and *k* replicates:

$$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + \varepsilon_{ijk}$$

Where: $A_i \sim \mathrm{N}(0, \sigma_A^2), AB_{ij} \sim N(0, \sigma_{AB}^2), \epsilon_{ijk} \sim N(0, \sigma^2)$

Formulae for estimates: $\text{repeatability} = \hat{r} = 2\sqrt{2}\sqrt{\hat{\sigma}^2}$

$$\text{reproducibility} = \hat{R} = 2\sqrt{2}\sqrt{\hat{\sigma}_A^2 + \hat{\sigma}^2}$$

These estimates represent the repeatability and reproducibility limits, respectively, to assess the validity or relevance of results produced by the measurement method. These estimates can be interpreted as the difference between two results, obtained from the same product under repeatability (or reproducibility) conditions that will not be exceeded 95 times out of 100.

As part of that statistical model diagnostics, best linear unbiased predictions (BLUPs) were calculated for each random effect (A_i, and AB_{ij}), and were subsequently plotted against the predicted outcome to verify the assumption of homoscedasticity (equal finite variance). The residuals from the repeated measures were also evaluated by plotting the standardized residuals against the predicted outcome. Normality assumptions were verified by plotting the quantiles of the BLUPs, for both random effects, against quantiles of normal distribution. Similarly, the quantiles from the residuals (repeated measures) were plotted against quantiles of the normal distribution (Dohoo *et al.* 2009).

In Part 1, the repeatability estimated the Ct difference between two results within a technician (within the same chip and RNA plate) that will not be exceeded 95% of the time, and the reproducibility estimated the Ct difference between two results between two RNA extractions that will not be exceeded 95% of the time. Similarly, in Part 2, the repeatability estimated two results within a technician (within the same chip) and the reproducibility estimated two results between two chips (on the same RNA plate and RNA extraction). The interpretation of repeatability and reproducibility were different between Parts 1 and 2, despite having a similar statistical model, because the layout of the technician replicates within and between chips were different – please refer to Figures 7 and 8 for detailed schematics of the two experimental designs.

In addition to estimating the repeatability and reproducibility, the level of agreement between two technician readings was assessed with a concordance correlation coefficient (CCC). Values close to 1 indicated very good agreement while values approaching zero reflected very poor agreement (Dohoo *et al.* 2009). A CCC is similar to a Pearson correlation coefficient, with the exception that the CCC also accounts for both location-shift (how far data are from the equality line) and scale-shift (difference between the slope for the sample data and the equality line) (Lin 1989). Within the CCC analysis, a Bradley-Blackwood F test compares the mean and variance between the two series (Bradley and Blackwood 1989), where $p<0.05$ indicates that either the mean or the variance (or both) were unequal between the series. Bland-Altman plots

(Bland and Altman 1986) were created to compare average differences in values between technicians.

The agreement of observed Pass/Fail status for samples between technicians was assessed using a McNemar's test.

A more intuitive way of presenting these data from Parts 1 and 2 was to predict the probabilities of disagreement for each assay. A dichotomous variable was created so that each sample in the dataset was marked as either 1 (when technicians disagreed on the Pass/Fail sample result) or 0 (when both technicians agreed on the result). The predicted probabilities of disagreement (Pr(Disagreement)) for each assay were modeled with a mixed-effect logistic regression with the dichotomous disagreement variable as the outcome, the assays as a fixed effect, and the samples as a random effect.

Although not specifically designed to assess repeatability, data from the LOD study were also able to provide estimates for repeatability and reproducibility, describing the within- and between-chip variability for each assay.

The data analyses and statistical methods, as described for the repeatability study, were similarly applied to the LOD study to produce repeatability and reproducibility estimates. The linear mixed model for the LOD data was designed to estimate the repeatability of each assay within chips, and the reproducibility between chips, by modeling the Ct values (outcome) from the sample concentrations, for i chips, j concentrations, and k replicates. These analyses were stratified for both STA and Non-STA samples.

6.6 CONSISTENCY OF ASSAY RESULTS BETWEEN THE BIOMARK AND ABI 7900 PLATFORMS

Consistency can be assessed by calculating the agreement among test results when the same sample is tested on different platforms. We tested consistency between the Fluidigm BioMark and ABI 7900 in the Miller laboratory using a portion of the samples used for repeatability, with one of the extraction plates run in duplicate for each microbe on the ABI 7900. As well, we assessed the serially-diluted artificial constructs both to determine the linearity, efficiency, and ASe of each assay and to determine the Ct variance among platforms (as the ABI 7900 is a standard platform for microbe quantification around the world). Previous verifications between these two platforms have shown a Ct variance of 8-10 Ct units, with lower values for the BioMark platform (Devonshire *et al.* 2011). For fourteen of the microbe assays assessed for repeatability on the BioMark, >20 samples with positive detections on the BioMark were identified amongst the 80 available samples on a single plate, while seven microbes had positive detections in 9-19 samples surveyed. Single extractions for each were used to assess consistency of results on the ABI 7900.

q PCR was performed on ABI 7900HT (ABI) in 384-well optical plates. The qPCR reaction volume was 12 µl, which comprised 6 µl of 2X TaqMan Gene Expression Master Mix (ABI PN 4369016), 4.04 µl of water, 0.24 µl of mixed forward and reverse primers (1 µM final concentration of each), 0.36 µl of each probe (assay specific probe and APC probe, 0.3 µM final concentration of each), and 1 µl of cDNA template. Temperature cycles included one 2-min hold (50°C); a 10-min denaturation (95°C); and 40 cycles of denaturation (95°C for 15s), annealing and extension (60°C for 60s). Amplification conditions on the ABI 7900 were not optimized for this platform, but rather closely reflected those used on the BioMark platform. Samples run on the ABI did not undergo STA enrichment.

Standard curves were constructed using the same APC clone standards spiked in with CHSE DNA as on the BioMark. Serial dilutions were made to obtain concentrations of 4, 8, 16, 1.5×10^2,

$7.7x10^2$, $3.9x10^3$, $1.9x10^4$, $1.9x10^5$, $1.9x10^6$ and $1.9x10^7$ copies of the clone per reaction. Clone standards, unknown samples, positive and negative controls were all run in duplicate.

The Ct values between the BioMark and the ABI 7900 platforms were compared with CCC analyses. Since CCCs account for shifts in both location and scale, and the pre-amplification step was used for samples run on the BioMark platform, we anticipated the CCC on raw Ct values to be different across both platforms. Adjustments on Ct values for the BioMark samples consisted of a global location-shift (correcting all BioMark Ct values by one factor), based on the average difference between BioMark and ABI 7900 Cts. Similar adjustments on Ct values for the BioMark samples were also made for each assay, as opposed to one global factor for all assays. These adjusted Cts for the BioMark samples were compared to ABI7900 Cts using CCCs.

Nonparametric receiver operating characteristic (ROC) analyses were carried out to quantify the agreement for the dichotomous 'Pass/Fail' status for each sample between BioMark and ABI7900 platforms, at various Ct cutpoints. Typically, ROC curves are used to plot the sensitivity of a test versus the false positive rate (1-Sp) computed at a number of different cutpoints (Greiner *et al.* 2000); this is used to select the appropriate Ct cutpoint for distinguishing animals that are specific pathogen free from those that test positive for a pathogen. Since the pathogen status of the animals from which the samples were collected is not known, we assessed the agreement between the two platforms by artificially assigning a "pathogen load" status set at four Ct cutpoints (25, 30, 35, and 40) to classify ABI7900 samples as Pass/Fail. The nonparametric ROC analysis was used to determine the optimal BioMark Ct value for maximum agreement, as determined by the highest area under the ROC curve with the highest specificity value.

7 RESULTS

This section is set-up to follow the line of inquiry as outlined in the Terms of Reference for the National CSAS process. The first question related to the validity of the methods, which is not specifically presented, as this is a judgment call that naturally follows when assessing any research paper. Hence, we devote our presentation of results to the measurable entities of our research.

In total, 349,440 qPCR reactions were run over the course of our platform evaluation studies, generating 505,728 data points and resulting in 243,092 analyzed sample observations not including controls (Supplemental Table 6). To accomplish this large undertaking, 41 dynamic arrays were processed on the BioMark and 11 x 384 well plates were processed on the ABI 7900. Given the complexity of the studies, there was far more time spent setting up the samples for the studies than the technical time and physical running of the experiments. This whole process took approximately 6 months to accomplish. Unique to our study is the ability to assess diverse and extremely distant microbe species within a single run, such as the data presented within the sensitivity study. Our ability to do so is also unique to the BioMark, as it is one of the few high-throughput fluidics systems that allow the flexibility to dynamically load different suites of assays for different runs. Our study is the largest analytical evaluation for the BioMark to date (see Ishii *et al.* 2013; Dhoubhadel *et al.* 2014; Michelet *et al.* 2014 for other validation studies), and likely the largest "validation" studies performed for qPCR research.

7.1 ASSAY DEVELOPMENT

We developed 15 novel microbe TaqMan assays (2 to viruses, 2 to bacterial microbes, and 10 to parasites), and obtained 29 assays from the published literature and 3 unpublished assays from research colleagues (J. Winton for VEN and S. Clouthier for IPNV, N. Gagné for isav8)

(Table 1). For many species where assays had not yet been developed (especially parasites), there was only limited sequence available for the species of focus or for closely-related species, and when there was, it was largely restricted to 16S and 18S rDNA, classically used for barcoding studies. Development of species-specific assays can be difficult when limited to sequence information from rDNA. Therefore for a few assays, we knew at the development stage that they may not be 100% exclusive to the species infecting salmon (e.g. Loma assay). However, as our program will ultimately conduct HTS on many of the microbes, we will not only be able to validate the identifications made, but will provide, for some species, more in depth sequencing that may be useful in future, to develop more species-specific assays. However, we have to be cognizant of the fact that even if we have more in depth sequence data for our species of interest, until similar depth of sequencing is available for closely-related species, which may or may not infect salmon, it is difficult to know *in silico* that assays will, in fact, be specific.

For all but ISAV, we undertook a complete OIE stage 1-level evaluation for a single assay per microbe. For ISAV, we evaluated the performance of two assays, the segment 8 assay developed by the National Aquatic Animal Health Program in DFO and implemented for regulatory purposes by the Canadian Food Inspection Agency (Ms. N. Gagné, personal communication) and the Plarre segment 7 assay developed in Europe (Plarre *et al.* 2005). In total, the performance of 47 microbe assays (to 46 unique microbes) was evaluated on the Fluidigm BioMark™ nano-fluidics platform.

Details on the design of novel microbe assays are included below and in Table 1.

Pacific salmon parvovirus (final REP68-2 assay length -65 bp)

The Pacific salmon parvovirus was recently discovered through NGS in the Miller laboratory and is highly divergent from all known viruses (closest homology <48% to the parvovirus REP and CAP protein coding regions). A full length sequence of the virus was obtained using multiple individual salmon samples, and identified two distinct viral lineages with <3% divergence between them over the full length of the viral sequence. Based on these two genogroups, eight TaqMan assays each in the REP and CAP protein encoding regions were designed, all of which were identical to the both genogroups identified at the time. TaqMan assays were tested in DNA and RNA for a range of smolt and adult liver sample “unknowns” to determine the consistency of amplification between primers and DNA/RNA. Variance in amplification of samples among assays was observed, prompting us to do a more complete assessment of sequence variation using various combinations of the primers developed to amplify large sections of the virus. Sequenced products again clustered into the two previously defined genogroups, but re-examination of the assays based on additional sequence information revealed that five of the eight were an identical match to all known sequence variants. Of these, we chose the assay in the REP gene that had the highest efficiency and that amplified both in DNA (parvovirus is a DNA virus) and RNA (indicating that the segment amplified was in a protein coding region). *In situ* testing that included the two viral lineages verified the specificity of the REP68-2 assay.

Salmonid herpesvirus / Oncorhynchus masou herpes virus (final capsid-6 assay length 85 bp)

Five TaqMan assays were designed within the terminase gene of the salmonid herpesvirus, and three within the major capsid protein gene (sequences from Bernard and Mercier 1993). All passed the specificity check with BLASTN and were initially tested with real samples and APC clones. The OMV capsid-6 assay was selected because it gave the best efficiency. In 2001, Aso *et al.* also developed a sensitive PCR method for OMV detection based on the capsid

sequence. The OMV capsid-6 assay is in the region between their selected primer set F10 and R5. *In situ* testing verified that the capsid-6 assay was specific to OMV.

***Vibrio anguillarum* (*Listonella anguillarum*)** (final VirA-1 assay length 92 bp)

Attempts to design a specific assay from the extensive sequences available for 16s rDNA failed. Instead, we developed two TaqMan assays designed in the same region of the VirA gene (accession number LO8012) as the SYBR assay developed by Prol *et al.* (2009). Blast searches identified the primers and probe as unique to *V. anguillarum*, however, as there are no sequences available for VirA in other *Vibrio* species, specificity was further tested *in situ*. One assay yielded a highly specific product with good efficiency.

Vibrio salmonicida (final lux-2 assay length 138 bp)

Difficulties designing species-specific assays in the 16s rDNA region for *Vibrio* spp. again necessitated looking outside of this region for genes that might be more specific. As such, we attempted to design TaqMan assays in the gapA and lux genes (5-6 assays each). BLASTN searches revealed non-specificity of the gapA primer, but all 6 of the lux gene assays designed between O-EN26 and O-EN23 were specific. The lux gene is associated with bioluminescence (Nelson et al 2007). After initial testing with a small number of closely-related species on the ABI7900, we chose the assay which gave the best efficiency.

Cryptobia salmositica (final CYS-4 assay length 91 bp)

Five TaqMan assays were designed based on *Cryptobia salmositica* cysteine proteinase (CYS) mRNA (AY713477.2) and *Cryptobia salmositica* 49 kDa cysteine proteinase Cysp1 mRNA (AY090898.1) sequences reported in Jesudhasan *et al.* (2007). Two TaqMan assays were also designed to the SSU subunit. All assays passed the BLASTN specificity check and were tested with real samples and APC clones. The SSU assays were more sensitive, but detected the picked host reference gene with naïve salmon cells (CHSE culture). The CYS4 assay was selected based on performance (specificity and efficiency).

Dermocystidium salmonis (final 18S-1 assay length 60 bp)

Only one *D. salmonis* sequence exists in GenBank. It is a close relative of *I. hoferi* and *S. destruens* (DRIP clade – see Ragan et al 1996) A TaqMan assay was designed in the 18S region already chosen for *S. destruens* due to the relatively large amount of between species variability in that region. The assay was specific to *D. salmonis* within the clade alignment. BLASTN analysis showed that the probe was not entirely specific, the F primer matched to *Amphibiocystidium* sp. (a freshwater frog pathogen) and *Rhinosporidium seeberi* (a human pathogen), and the R primer matches *Amphibiocystidium*, all species that are unlikely to be picked up in fish.

Loma salmonae (final lsq-1 assay length 87 bp)

Limited sequence information for *L. salmonae* and closely-related species was problematic in the design of a specific assay. The *L. salmonae* TaqMan assay was developed in the same SSU region covered by the LS1-LS2 assay developed by Docker *et al.* (1997). *In silico* analysis suggests that this assay will be specific to *Loma* spp. but not species specific to *L. salmonae*. Attempts to develop a more specific assay *in silico* in the EF1 gene, the only other gene available from *Loma*, failed.

Ichthyophthirius multifiliis (final 18S-1 assay length 98 bp)

Two TaqMan assays for *I. multifiliis* were designed in the same 18S rDNA region as the SYBR assay described in Jousson *et al.* (2005), and *in silico* analyses through BLAST searches

suggested the primers and probe were all specific to *I. multifiliis*. We picked the assay with the best efficiency.

Sphaerothecum destruens (final 18S-2 assay length 59 bp)

Two TaqMan assays to *S. destruens* were developed in the same 18S rDNA region as the SYBR assay published by Andreou *et al.* (2011). *In silico* sequence analysis of Mesomycetozoan species similar to Arkush *et al.* (2003) showed the primers and probes to be specific to *S. destruens*. We picked the assay with the best efficiency.

Spironucleus salmonicida (final SSU-1 assay length 61bp)

There were only two *Spironucleus* species for which 18s rDNA sequences aligned with *S. salmonicida*, S. *torosa* and S. *barkhanus*, and strains of these, along with the 29 available *S. salmonicida* sequences were used to design the TaqMan assay. Two assays were designed, with the one with the best performance being a perfect match for 25 of the 29 *S. salmonicida* (one mismatch in the other strains) but also matched one *S. barkhanus* sequence whose identification has been questioned in the literature (see Keeling and Doolittle 1997).

Facilispora margolisi (final 18S-3 assay length 58 bp)

An alignment of SSU sequences from related microsporidia species outlined in Jones *et al.* (2012) was used to design three TaqMan assays specific to *F. margolisi*. All were specific based on BLASTN searches and in initial testing; we chose the assay with the best efficiency.

Nanophyetus salmincola (final ND1-1 assay length 67 bp)

The only sequence data available for *N. salmincola* are for the NADH dehydrogenase (ND1) gene, for which there were 91 sequences available. ND1 did not align easily to other closely-related trematode species (Deropegus, Plagioporus; Criscione and Blouin 2004). It was impossible to develop an assay that was a 100% match in primer sequence to all *N. salmincola* sequences. We developed two assays whereby the probe exactly matched all sequences, but there was some strain-specific variation under the primers. The assay chosen, based on efficiency and the closest match to all strains had a single mismatch in the F1 for 4/91 sequences and 1-3 mismatches in the R1 primer in 17/91 sequences, one of which was close to the 3' end of the primer and may diminish inclusivity of these strains. A single degenerate position was therefore synthesized for the R1 primer.

Myxobolus arcticus (final 18S-1 assay length 68 bp)

All available 18s rDNA sequences were aligned to develop species-specific assays for *M. arcticus* and *M. insidiosus*. The *M. arcticus* assay developed is a 100% match to all BC sequences and 5 of 6 Japanese sequences, but is not a sufficient match to the highly divergent Norwegian strains. It should not pick up other *Myxobolus* species.

Myxobolus insidiosus (final 18S-2 assay length 63bp)

Using all available *Myxobolus* species 18s rDNA sequences, it was difficult to design an assay that was specific to *M. insidiosus* that would not pick up *M. fryeri*, a very close relative. Specificity testing *in silico* revealed the assay did, in fact, pick up *M. fryeri* and *M. squamalis*, and perhaps weakly *M. cerebralis*.

Parvicapsula kabatai (final SSU-2 assay length 62 bp)

Parvicapsula kabatai was first described in Jones *et al.* (2006). SSU sequences for all *Parvicapsula* spp. were aligned to develop two TaqMan assays specific to *P. kabatai*. The high degree of divergence between species made it easy to design a specific assay. The assay with the best efficiency in initial testing was chosen.

7.2 ANALYTICAL SENSITIVITY

We estimated assay efficiency across multiple studies, including the LOD study (described in 6.1) in which we calculated efficiency for APC controls, the STA impacts on relative quantification study (described in 6.3) in which efficiency of the STA for real samples was calculated, and the consistency between platforms study (described in 6.5) in which efficiencies on the ABI 7900 were calculated (Tables 3 and 4). Optimal efficiencies are generally between 0.9-1.1. In our initial analysis (which did not include technical variance in dilution series), 9 of the 47 assays (19%) had values outside of this range for the APC controls on the BioMark, and 10 of 47 (21%) on the ABI 7900, with all but one divergent assay showing reduced rather than enhanced efficiency, with the average efficiency of divergent assays of 0.85. In the additional analysis that included replication of independent dilution series, the range of calculated efficiencies for all but one assay (de_sal) was within optimal, including three newly added assays (vhsv1, pmcv1, snow7 (for ISAV); Table 3). Efficiency of assays based on real samples was complicated by the fact that we did not have high copy number controls for all samples, hence could only calculate efficiency over 37 of the assays. In general, divergence in efficiency was higher for real samples, with 11 of 40 assays (30%) containing efficiencies outside of the optimal range (Table 3). While most of these showed only modest levels of divergence (eight with efficiencies $\geq$0.85), two were substantially lower (pa_ther and sav <0.71, isav8 0.74). None of the assays was divergent based on all measures. These data suggest that the efficiency of assays, in general, was fairly high, and not highly disparate between the two platforms, and that in general, efficiencies of the APC are a good approximation for real samples.

Correlation coefficients were high for the APC samples on both the BioMark and the ABI 7900 (based on the initial serial dilution), with no assays consistently performing below the optimal level of 0.98 (Table4). The new isav7 assay (snow7) was slightly below optimal, but the range intersected the optimal $R^2 \leq 0.98$ (data not shown). Given the reduced dynamic range of loads for the real samples, it was not surprising that the R^2 was somewhat lower for some of these assays, but all but one (pa_min) was above 0.9 (Table 3).

For the LOD analyses, there were a total of 64,512 observations across 47 assays (1140 observations per assay). Figure 2 provides a schematic representation of the breakdown over the serial dilutions, chip allocation, amplification status, and number of replicates over parts 1 and 2 of the study. Included in our analysis were 40 replicates of 15 serially diluted concentrations of artificial constructs for each assay under the first dilution series, and 6 replicates each on three additional dilution series, bringing the total replication (n) to 58 and enabling a precise estimation of LOD (Supplemental Figure 3 and 4 show the changes in Ct over the first series of serially diluted concentrations). Herein, we provide LOD estimates based on the starting material (concentration of sample before being processed by STA or loaded onto the dynamic array); the concentration present in the individual reaction chamber for STA samples can be calculated by multiplying the starting material by 1.12. The LOD of the starting material was calculated from the last dilution in which at least 95% of replicates were detected (i.e. directly reflecting the measured concentration).

The median LOD copy number for starting material across assays was 8 copies, with a mean of 13.4 copies and range of 1.1 to 39.2 (Table 3). This translated to a chamber LOD of 8.9 (median) to 15.0 (mean) copies.

In contrast, whereas the chamber LOD was similar or slightly (but not significantly) lower for Non-STA (range 1.2-28.5; data not shown), the median LOD of starting material was 1185. On average (across assays), the LOD of the Non-STA was 310 times that of the STA. However, for both STA and Non-STA reactions, the Ct associated with the LOD was generally between 25 and 29.

The linear dynamic range of all assays on the BioMark performed with the STA was minimally from 10^1 to 10^6, with most assays going to 10^7 (Supplemental Table 2).

Taken together, our results show that efficiencies and the linear dynamic range on the BioMark are similar to those on the ABI 7900. The within chamber detection limit on the BioMark was <10 copies per μl for 2/3rds of the assays, and between 10 and 39 copies per μl for the remaining 1/3rd of assays. These values were similar for STA and Non-STA samples, but the starting concentration for Non-STA samples must be at a concentration of approximately 300-1000 copies per μl to achieve 1-10 copies per chamber.

7.3 ANALYTICAL SPECIFICITY

The objectives of these analyses were to evaluate potential cross-reactions of assays with unintended microbes (false positives). Reasons for apparent false positives include: cross-reactions with closely-related species (CRS), co-infections in samples (defined as true co-occurrence, not an active infective state, which cannot be resolved via PCR), spurious amplifications (typically occur at high Ct's and often inconsistent across all replicates) and laboratory contamination. The origins of the samples (gBlock, tissue, or culture) were accounted for to explain potential co-infections and closely-related species and broader species groupings (e.g. protozoan within parasite or gamma proteobacteria within bacteria) were accounted for to explain potential cross-reactions.

Our analytical specificity testing was conducted over all assays (published and developed herein), with a total of 63,840 observations across the three major microbe groups (bacteria, viruses, and parasites), 82,944 including controls. *In silico*, we identified all closely-related species (defined as carrying homology of >90% over the assay gene region [not just the assay itself]) and developed gBlocks (1000 base sequences) or obtained real samples for each.

In our first specificity analysis, we tested all assays against all control samples (tissue, gBlock, cultures and spores) within each of the viral, bacterial, and parasite groupings (Figure 3). There were 12 assays representing the bacteria, 13 for the viruses, and 22 for the parasites, and each sample was replicated within its respective microbe group 6, 6, and 4 times (fewer replicates for parasites due to a larger number of assays under assessment), respectively, in STA and Non-STA. There was a very large sample size of negative controls and (corresponding qPCR reactions), for the parasites, 24 (576), bacteria, 54 (864) and viruses 492 (7872), resulting in only 2 spurious amplifications with Ct's >32.

In our second specificity analysis, we tested all microbe assays (with the exception of ye_ruk) against all samples (not including gBlocks) to rule out the cross reactivity between assays and more distantly related species. In this case, there were 46 assays replicated 2 times over 85 STA samples. The 4 negative controls run in this analysis represented 384 reactions, of which only 2 were detected at Ct's of 27 or greater.

The use of tissue samples for specificity testing, a necessity for most parasites, non-culturable viruses, and some bacteria, adds a level of complexity to the analysis of analytical specificity when multiple microbes are being assessed at once because tissue samples may contain co-infections involving more than one microbe. In order to more accurately calculate the assay specificities in our study, it was necessary to determine which results were due to co-infections and subsequently remove these as false detections in the ASp estimates; these data are presented in Tables 5-7.

7.3.1 NGS Validation of co-infections

In order to validate the results from the two specificity analyses, NGS was performed on the same set up as the second analysis, tissue and pure positive samples only versus all assays not including ye_ruk. It is important to note, however, that the replacement assays for isav7, pmcv and vhsv were used in the NGS library prep as opposed to the qPCR analysis, so that these assays were not evaluated in this comparison. Also, as no VIC Ct cutoffs were applied to any of the qPCR/NGS comparative data and a majority of the VIC+ Ct's > 27, if one were to eliminate any sample with VIC+ results from the analysis, the number of qPCR samples with NGS validation would have only increased. As in FAM detections, we expect some of the inconsistent high Ct VIC+ detections to be due to spurious amplifications, and more analysis will need to be done in order to determine where and when to apply a VIC assay-specific Ct-cutpoint.

Sequences were confirmed for virtually all controls for individual assays; exceptions were ISAV_HPR4 (NA) which did not yield isav8 sequences and IPNV_Canada1, 2, and 3 none of which yielded ipnv sequences. We speculate that these 4 libraries may have failed, as very few sequences, if any, were obtained from them at all.

Most tissue sample controls (86%) that amplified additional species were confirmed by NGS sequencing as co-infections (Figure 10) with a -0.86 correlation between the detected Ct and # of NGS reads. Many tissue samples contained multiple co-infections with up to 8 found in the 22Csal_CYS4_1072 sample. Interestingly, 21% of the qPCR+/ NGS validations corresponded to detections above the assay-specific Ct-cutpoint, with the highest being fl_psy at 9.0 Ct's above its cutpoint.

In a majority of the detections of the remaining non-NGS confirmed tissue sample controls (14%), the Ct was also above the assay-specific Ct-cutpoint. We consider some of these to be failed NGS reactions due to insufficient microbe load for NGS library prep, particularly those with no corresponding VIC+ detections (n=6) (Figure 10). Alternately, 2 assays, de_sal and ihnv, were detected below their assay-specific LOD in 1 and 3 samples, respectively. We consider the ihnv detections as false qPCR positives and likely the result of contamination, as they all had corresponding VIC+ detections (n=5) (Figure 10).

Thirty five percent of the pure positive controls that amplified additional species were confirmed by NGS sequencing as co-infections and only two of these samples, 11M_cerebralis and 11M_insidiosus, contained more than one co-infection. Interestingly, a majority of these detections were above their assay-specific Ct-cutpoint (Figure 10) which may be indicative of more successful NGS library preparation with lower load samples when they are more pure.

As in the tissue samples, a majority of the detections of the remaining non-NGS confirmed pure sample controls (65%) were also above their assay-specific Ct-cutpoint. Those with no corresponding VIC+ detections (n=5) (Figure 10) were deemed to be false negatives while those assays detected below their assay-specific Ct-cutpoint with corresponding VIC + detections (n=5) were deemed to be false positives and likely indicative of contamination. These assays included ae_hyd, de_sal, my_arc and ven; each with one detection.

From the NGS data it appears that not all "pure" samples were indeed pure. Four "pure" parasite spore samples with FAM only detections above their assay-specific Ct-cutpoints of distantly-related species (parasites or bacteria), were confirmed by NGS sequencing; both fl_psy and ic_mul were detected in the 11M_cerebralis control, ce_sha, ic_mul and rlo were detected in the 11M_insidiosus control, ic_mul was detected in the 11M_fryeri control and ic_mul was additionally found in the 11M_squamalis control. The ic_mul detections ranged between 72-16,511 reads per sample and varied from our ic_mul tissue control sample by 1

SNP in the 11M_fryeri sample, 2 different SNPs in the M_squamalis control sample and 3 SNPs in both the 11M_cerebralis and 11M_insidiosus control samples. *Ichthyophthirius multifiliis* (ic_mul) is a common freshwater ciliate parasite that is observed in co-infections with *Myxobolus* spp; however, in our repeatability study, there was no strong correlation between ic_mul and *Myxobolus* species detections, indicating that impure spores were the most likely explanation for the co-occurrence of these species.

Finally, despite the lack of sequence confirmation for the ipnv assay in the IPNV_Canada1 cell-culture control, a low level detection (Ct 27) of lo_sal was confirmed with 3,846 reads. As no other IPNV samples showed detection of lo_sal, and the fact that this assay had a low VIC+ Ct, we conclude that this must have been from contamination rather than cross-amplification.

7.3.2 Specificity Analysis

Given the enormity of this analysis, and complexity of samples for inclusivity and exclusivity required to assess up to 47 viral, bacterial and parasite assays, the data and analyses are presented over 7 tables (Tables 2, 5-7, and Supplemental Tables 3-5). Sourcing of control material is presented in Table 2, and *in silico* analysis for % homology in Supplemental Tables 3-5. A detailed presentation of specificity data for viruses, bacteria and parasites is found in Tables 5-7, respectively. This analysis provides information on the positive control samples to consider for each assay (i.e. species positives and closely-related species) and their origin (gBlock, tissue, culture), sensitivity calculations, specificity calculations, as well as a variance explanation for each assay and their respective confirmed co-infections.

By removing the data from suspected co-infections, we determined that 45 of the 47 assays displayed 100% sensitivity (amplified all sequence variants tested) while 11 of 12 bacterial assays, 13 of 13 viral assays, and 18 of 22 parasite assays displayed 100% specificity (did not detect CRS) to the microbe species they were designed to detect. Although two other parasite assays, de_sal and sp_des, did not detect any CRS, they did detect other microbes within the protozoan group. In a majority of cases where the overall specificity of an assay was calculated at <100%, the amplification(s) responsible were at Ct's >27 and/or above their respective assay-specific Ct-cutpoint (>LOD) and likely the result of either a spurious event or a low level co-infection that was not validated with NGS. There were a few problem samples including the 07_Loma_positive tissue sample which had three assays detected at Ct's < LOD and no NGS validation. Both ihnv and vhsv assays had corresponding VIC+ detections at Ct's <27, likely representing contamination of this sample.

The few assays that cross-amplified closely-related species or that did not detect all strains were largely predicted based on *in silico* analyses. Below, we outline only the results that showed some deviation from 100% species specificity or sensitivity for which cross amplification is the most likely cause of reduced specificity. We include in this presentation the three assays that were expected *in silico* to be genus- rather than species-specific (*Kudoa, Loma,* and *Ichthyophonus*).

7.3.3 Viruses

For specificity testing, we attempted to account for all known genogroup sequence variation by obtaining cell cultures or gBlocks, especially where assays were not a 100% match to the genogroup sequence. For some viruses, the assays were designed as a 100% match, making it unnecessary to incorporate multiple variants in testing. For recently discovered viruses (e.g. VEN, PRV, PSPV and PMCV), there is a possibility that sequence variants have not been fully characterized. The two assays that were not 100% inclusive (sensitive) were the isav7 assay

from Plarre *et al.* (2005) and the ipnv assay recently developed in DFO by Tamara Schroeder. Below, we highlight only the results from assays showing divergent patterns of amplification.

isav7 assay

The isav7 assay did not detect any replicates of the North American HPR4 sample in either specificity analysis, and only amplified the STA replicates of the North American HPR21 sample much more weakly than the isav8 assay (average Ct 25.4 vs. 5.2), which amplified all isolates with low Ct values (Cts between 5-11). This result was not unexpected as sequence variation over this assay region exists in 45% of sequences analyzed *in silico* (n=76). We re-ran a dilution series for isav7 over Non-STA and STA replicates for all genogroups and found that while the Non-STA replicates of the 2 NA genogroups still did not amplify, the concentrated STA replicates of the North American HPR4 sample finally amplified. The isav7 assay also amplified weak products above its respective LOD in 6/6 IHNV_M STA replicates, 5/6 VHSV type IIIb gBlock STA replicates and 1/6 VER gp6 Cp gblock and VHSV IVb_KG Non-STA replicates. These results likely represent spurious amplifications as no isav7 gBlocks exist in the lab, there were no VIC+ amplifications, this result occurred inconsistently in STA and Non-STA replicates, and none of the pure samples were sequence confirmed with the replacement assay for isav7.

isav8 assay

The isav8 assay detected all genogroups in the STA samples, but in our initial analysis, none in the Non-STA samples were detected. Because our initial analysis used undiluted cDNA, we re-ran both the isav7 and isav8 assays using serial dilutions and found that all but the highest concentration sample amplified product using the isav8 assay in the STA and Non-STA; we conclude that this assay may be more sensitive to template concentrations or PCR inhibitors than others, but it does detect all known genogroups.

ipnv assay

Two of the IPNV clones were not detected by the ipnv assay (group VI Hecht clone and group II Ab clone) in either the STA or Non-STA samples in our original analysis (based on a single high-concentration sample of each), indicating that this was not an effect of the STA. This result did not change when re-run using a dilution series of each control sample (starting with 105 ng/µl, estimated to be 10^7 copies). Both of these genogroup samples were from plasmid DNA clones encoding the target sequence provided by Dr. S. Clouthier. These two genogroups were the most divergent to the assay; group II had with 3 mismatches in each of the F- and R-primers and five mismatches to the probe whereas group VI sequence did not align in this region, hence, the results were anticipated. Other genogroups were either perfect matches (III), or contained 3 or 4 mismatches in the F-primer only (IV and I, respectively). The Ab and Hecht plasmids also did not amplify on the NAAHP Mx3005P platform (Dr. S. Clouthier, personal communication), consistent with our findings on the BioMark.

7.3.4 Bacteria

We note that while our characterization of strain variation was high for the viruses (i.e. viral strain sequence variation known to date was taken into account), for some bacterial species, this can be difficult as there can be thousands of sequences in Genbank for a single bacterial species, and some may be misidentified (i.e. there can exist an overlap in sequences purported to be of different species). On the other hand, depending on the gene, there may only be 1 or 2 sequences available for assay design and determination of inclusivity and exclusivity using *in silico* analysis. For assays developed in house, care was taken to design primers and probes to

match all known sequence variants within the species of interest; however, this may not have been the case for published assays. While our specificity testing suggested that all bacterial assays detected all tested positive controls and virtually none detected closely-related species or distantly-related species for which co-infection was not the likely source, a re-examination of *in silico* data suggested that our testing may not have been quite thorough enough for some assays. Moreover, we were unable to evaluate the inclusivity of three assays, rickettsial-like organism (rlo), *Vibrio salmonicida* (vi_sal) and gill chlamydia (sch), as there was only 1 or 2 sequences and positive control samples available. In the case of the *Aeromonas salmonicida* (ae_sal) assay, although there were a great number of GenBank sequences for the genus *Aeromonas*, there were only 2 species (*A. salmonicida* and *A. hydrophila*) represented at this particular gene (vapA) and our results showed that this assay did weakly amplify one of its CRS control samples (*A. sobria*) in STA samples only (Ct range of 25.8-28.2). Specificity results for each assay are detailed in Table 6. Below, we highlight only the assays showing divergent patterns of amplification, or suspected to be less than 100% inclusive or exclusive based on sequence data.

***Aeromonas hydrophila* (ae_hyd) assay**

This qPCR assay published by Lee et al. (2006) was designed to the adhesion gene (aha1) for the purpose of quantifying *A. hydrophila* in municipal wastewater. No specificity testing was performed in Lee et al. (2006) but in a 2007 publication the qPCR efficiency and assay sensitivity were determined (Shannon et al. 2007). Sixteen of the 18 (89%) strains analyzed *in silico* would not amplify with this assay as they were <90% homologous and contained multiple mismatches to primers and probe (see Supplemental Table 4a/b). Consequently, we designed a gBlock to this particular strain as a positive reference control, which was amplified (both STA and Non-STA) by this assay while none of the closely related strains (CRS) were. The inclusivity of this assay should be followed up in future to determine whether it is inclusive to all strains that infect salmonids.

***Yersinia ruckeri* (ye_ruc) assay**

Yersinia ruckeri (ye_ruc), the causative agent for enteric redmouth disease (ERM), has been detected in many fish species as well as in other aquatic and terrestrial organisms world-wide. The ye_ruc assay developed by Glenn *et al.* (2011) was designed to identify infectivity of 2 strains of *Y. ruckeri* (Hag and SC) in cultured Chinook salmon (*Oncorhynchus tshawytscha*) and steelhead trout (*Oncorhynchus mykiss*). In this publication, the assay demonstrated exclusivity from tested bacterial species (*A. salmonicida, A.* h*ydrophila, F. psychrophilum*) including enterobacteriales (*E. tarda, E. ictaluri and H. alvei)* and inclusivity with three strains of *Y. ruckeri* (Carson, Sc and Hag). This assay also appears to display 100% inclusivity of *Y. ruckeri* strains based on *in silico* analysis (33 sequences), and our single positive control sample (*Yersinia ruckeri* type 2) was amplified in STA and Non-STA samples. However, upon blasting the 119 bp amplicon, some published sequences for *Yersinia kristensenii*, *Rahnella spp.* and *Serratia spp.* were detected with >95% homology and based on their *in silico* analysis (Supplemental Table 4a/b) could potentially be amplified. All three of these species were deemed as fish pathogens in a study by Austin and Austin (2007) and were also detected in BC Chinook salmon in Evans and Neff (2009). We recognize that the specificity of this assay needs to be tested further based on our findings.

Flavobacterium psychrophilum (fl_psy) assay

Flavobacterium psychrophilum is a salmon pathogen that causes rainbow trout fry syndrome (RFTS) and bacterial cold water disease (BCWD). This qPCR assay was

published by Duesund *et al.* (2010) but there was no assay validation performed upon which we could compare results. The fl_psy assay demonstrated specificity as our positive control sample (purified nucleic acid from ATCC 49511D) STA and Non-STA amplified, while the closely related species *Flavobacterium columnare* and *Flavobacterium branchiophilum* (gBlocks) did not. However, based on *in silico* analysis (137 sequences), the inclusivity of this assay may not be 100% as approximately 26% of the sequences would likely not amplify with this assay due to primer/probe mismatches. We may carry out further testing to determine which, if any, strains would not be detected, although based on preliminary population-level analyses, it appears we are picking up dominant strains in BC.

***Piscichlamydia salmonis* (pch_sal) and gill chlamydia (sch) assays**

The pch_sal assay was published by Nylund *et al.* (2008), while the sch assay was published by Duesund *et al.* (2010); however no assay performance validation was performed in either publication upon which we could compare results. On the BioMark, the pch_sal assay detected the *P. salmonis* positive control sample in the STA and Non-STA, while the Sch assay detected the gill chlamydia (sch) positive control sample as well as the sch gBlock in the STA and Non-STA. The *P. salmonis* assay did not amplify products for the gill chlamydia (sch) positive control sample, sch gBlock, or the related species gBlock (Chlamydiales gp1), but weakly amplified the other closely-related Chlamydiales species gBlock in the STA only (Ct 25). Given that the gBlocks were diluted similarly, the higher Ct for the Chlamydiales gBlocks relative to the *P. salmonis* gBlock suggests that the sensitivity of detection is comparably lower for the *Chlamydiales* species. Importantly, the sch assay did not amplify the pch positive control sample or the related species gBlocks (Chlamydiales gp1 and Chlamydiales gp2), hence allowing the distinction between the only two known *Chlamydia spp*. infecting salmon (pch_sal and sch).

7.3.5 Parasites

As with the bacteria, most parasite controls that were not gBlocks were obtained as tissue samples, increasing the chances for detecting any co-infections present. In the NGS analysis, co-infections were confirmed in 83% of the parasite tissue control samples and 88% of those were of assays detected below their respective LOD (Table 7).

As the parasite data were complex, with many closely-related species assessed, we present the results of our *in silico* analysis to identify closely-related species (CRS) in Supplemental Table 5. Of the 22 parasite assays assessed, four were not specific to a single species (*L. salmonae, M. insidiosus, I. hoferi*, and *K. thyrsites*); these results were expected based on BLASTN *In silico* analyses. For all assays, specificity was highly consistent between STA and Non-STA reactions; hence results were due to the assay design rather than an artefact of the STA.

***Loma salmonae* (lo_sal) assay**

The *L. salmonae* assay was designed herein, and as expected from *in silico* analyses, the assay amplified all tested *Loma* species (*L. salmonae, L. lingcodae, L. embiotocia, L. richardi*) in the STA and Non-STA samples. The assay did not detect any other microsporidian species tested. *Loma* species parasites are not known to have a broad host range, most coevolving within a host-species (Brown *et al.* 2010). While *L. salmonae* is the only *Loma* species known to infect salmon, if others exist, this assay will not likely differentiate them. Hence, the assay is deemed to be a *Loma* spp. specific assay.

***Myxobolus insidiosis* (my_ins) assay**

Six closely-related *Myxobolus* species were tested as well as one distantly related species (*M. cerebralis*) for cross reactivity to the *M. insidiosis* assay designed herein. As expected from *in silico* analyses, the assay was also detected in the STA and Non-STA of the gBlock based on the closely-related *M. fryeri* sequence, which carries a 95% sequence homology to M. insidiosis. *M. fryeri* spores are morphologically similar to *M. insidiosus*, but are differentially found infecting the nerves of skeletal muscle rather than the myocytes, although both are commonly detected in the same fish (Ferguson *et al.* 2008). The my_ins assay also detected *M. squamalis* in the STA and Non-STA (100% homology to the assay) which is known to infect salmon skin (Ferguson *et al.* 2008). The assay did not detect *M. cerebralis*, *M. neurobias, M. kisutchi, M. neurotropis* or *M. arcticus*. Fortunately, the other *Myxobolus* species assays (to *M. cerebralis* and *M. arcticus*) were species-specific; hence these three salmon-infecting species can be differentiated with the three available assays.

***Kudoa thyrsites* (ku_thy) assay**

The *K. thyrsites* assay was designed herein, and as expected based on *in silico* analysis, the assay detected all closely-related *Kudoa* species equally in the STA and Non-STA. The species tested were all 89-95% homologous to the assay developed, with 0 to 3 mismatches under each of the primers and probe, and a maximum of 5 mismatches (for *K. miniauriculatus*), but did not detect other microsporidian species. The assay is therefore considered a *Kudoa* spp. assay and additional sequencing would be required to differentiate species. However, none of the co-amplifying species are known to infect salmon.

***Ichthyophonus hoferi* (ic_hof) assay**

The *I. hoferi* assay was developed by White *et al.* (2013), and while they conducted *in silico* analyses to ensure specificity, they did not test the ability of the assay to cross-amplify closely-related species in the laboratory. In our analysis, we tested seven closely-related species (18S sequence homology >90%, including mezomycetazoan and protozoan species), and only the second species within the *Ichthyophonus* genus, *I. irregularis*, a morphologically distinct species isolated from yellowtail flounder, was detected (STA and Non-STA); this species showed 100% homology to the assay and is not known to infect salmon. *Ichthyophonus* was originally classified as a protozoan (later a fungi) before it was classified as a fungal-like mezomycetazoan. The more distantly related salmon infecting protozoans, *Dermocystidium salmonis*, *Sphaerothecum destruens*, and *Cryptobia salmositica* were not detected by the *I. hoferi* assay and each of these assays were all species-specific; therefore we can differentiate all known protozoan and mezomycetazoan infecting salmon. *I. hoferi* is the most common species of *Ichthyophonus*, infecting a wide range of host species. We deem this assay an *Ichthyophonus* spp specific assay.

7.4 ASSAY REPEATABILITY ON THE BIOMARK

We analyzed microbe assays using real samples from BC salmon to assess repeatability of assays to prevalent, endemic microbes. For this analysis, we assess

1. repeatability starting from tissue homogenates all the way through RNA extraction, STA, BioMark amplification and scoring over two technicians and 3 dynamic arrays,
2. repeatability starting from cDNA, encompassing STA, BioMark amplification, and scoring over two technicians and 3 dynamic arrays, and

3. repeatability of scoring over three technicians and 6 dynamic arrays.

Note that we were only able to perform this analysis on RNA, as there was not enough sample available to repeatedly extract DNA. For microbe monitoring applications, we will generally combine DNA and cDNA to maximize the breadth of microbes that can be assessed at once, although studies that combine microbes with host genes will be based solely on RNA, as was done here.

In the repeatability analyses, Parts 1 and 2 each produced 24,960 observations, and Part 3 produced 74,880 observations (24,960 samples read by three technicians); please refer to Figures 7-9 for detailed experimental schematics. While similar in design, the major difference between Part 1 and Part 2 was that a different RNA extraction was the starting point in Part 1 and the same RNA extraction was the starting point in Part 2.

In Parts 1 and 2, the McNemar's chi square test had a value of 11.71 ($p<0.001$) and 7.76 ($p<0.001$) respectively, indicating that there are differences between technician results in both studies. In Part 1, when calculated for each assay and excluding controls, McNemar's chi square test was significant for only 7 of the 26 assays ($p<0.05$), two after multiple test (Bonferroni) correction ($\alpha<0.05$; Cabin and Mitchell, 2000) (Table 8). In Part 2, only 3 assays were significant, one after multiple test correction ($\alpha<0.05$).

In Parts 1 and 2, assays with high disagreement were flagged if the Pr(disagreement) was greater than or equal to the 75th percentile (Part 1, 0.051; Part 2, 0.049). For Part 1, the flagged assays include (in order from most to least extreme): c_b_cys, cr_sal, ic_hof, ic_mul, my_arc, pa_min, and pmcv (Figure 11a), while for Part 2, the flagged assays include: ae_sal, c_b_cys, cr_sal, ic_hof, ic_mul, pmcv, and ven (Figure 11b). This part of the study represented the greatest potential for variance between replicates. Many of the affected assays were limited to low load (high Ct) samples (Figure 11c), and most of the individual samples in disagreement were measured at high Cts for one technician and not detected in the alternate technician's analysis. This is exemplified by cr_sal, ic_mul and ic_hof, where the Cts were above 30 for one technician and undetectable for the alternate technician. Indeed, if LODs were to be applied, this variance would disappear. Of note, there were very low numbers of samples assessed for ic_hof, my_arc, na_sal, pa_min, pmcv, and ven. Moreover, for pmcv we have no verification that this virus is present in BC, and most detections were singletons at very high Cts. There were only a couple of positive samples that agreed among the two technicians, and we need to follow up with sequencing of these samples. For ae_sal the placement of the Ct threshold was the source of the disagreement between technicians.

Repeatability (within technicians) and reproducibility (between technicians/dynamic arrays) of the Ct averaged over all assays was 0.91 and 2.96, respectively, for Part 1 (Table 8) and 1.52 and 2.95 for Part 2 (Table 10), with medians slightly lower. In both studies, repeatability and reproducibility were reduced in the high- versus low-load samples, as expected (Tables 8 and 9). High-load samples (Ct<20) generally showed repeatability of 0.2-0.4 Ct and reproducibility of 1.4-1.5 Ct.

A close examination of the assays showing the lowest repeatability suggested that the quality of the amplification plot (curve) and technician variance in the placement of the threshold likely played a significant role in the disagreements between technicians in Parts 1 and 2. In general, curve quality had the strongest effect on repeat scoring of high Ct samples, while threshold placement affected both Ct variance and positive detections. Assays that were most highly affected by this in the repeatability study included pmcv, c_b_cys, ye_ruc, ae_sal, and ven; we expect that the vhsv assay would be similarly affected given its poor curve quality, although they were not included in the repeatability study. To circumvent this problem, for Part 3, the technicians developed assay-specific scoring SOPs (see Figures 12-14), and applied these to

blindly score the same six dynamic arrays produced in repeatability studies 1 and 2. Only three assays showed significant McNemar's *p*-values, and based on Kappa values of 0.86-0.97, they were in near perfect agreement (Table 10; Supplemental Figures 10 to 13).

Repeatability (within dynamic arrays) and reproducibility (between dynamic arrays) in the LOD study was assessed at high, low and overall concentrations (loads) in the STA and Non-STA samples (Table 11). Reproducibility increased by at least 1 Ct for the low versus high-load samples, while increases in repeatability were more modest; this pattern was similar for the STA and Non-STA treatments. Over all assays, there was a close to 3 Ct variance in reproducibility for low-load samples and a 1.5-2 Ct variance for high-load samples.

In conclusion, sources of variance arise from each step within the experimental process. The first repeatability study assessed the maximum level of potential variance between replicate samples, while the second phase assessed two technicians using a single RNA extraction and the third study assessed the effectiveness of scoring rules developed within the project. Even with the highest level of expected disagreement in Part 1, there was over 96% agreement, while the establishment of scoring rules enabled very good to perfect agreement when scoring 74,880 data points (3 technicians; 6 chips; 80 samples; 26 replicate assays).

7.5 CONSISTENCY OF RESULTS ACROSS THE FLUIDIGM BIOMARK AND ABI 7900 PLATFORMS (WITHIN THE MOLECULAR GENETICS LABORATORY AT THE PACIFIC BIOLOGICAL STATION)

We assessed the consistency of assay results across the BioMark and ABI 7900 platform (considered the "gold standard" in that it is the most highly applied qPCR instrument on the market for disease diagnostics). We ran 96 samples (including controls) from the same RNA extraction on both instruments across 22 microbe assays run in the repeatability study Part 1. Microbe assays run on the ABI 7900 were divided among two technicians (each responsible for 10-12 assays) and three of the repeatability plates, with each technician assessing consistency with the same extraction plate that they generated. We used the 384-well format on the ABI 7900 fast-HT to run duplicates of two assays simultaneously. In all, 6,930 PCR reactions (excluding controls) were compared across the BioMark and ABI 7900 platforms (Figure 15).

There exists a distinct and definitive Ct shift between the ABI7900 and BioMark. The unadjusted model, not taking into account this 8-10 Ct shift produced a concordance correlation coefficient (95% CI) of 0.376 (Supplemental Figure 14), while the assay specific adjustment produced a CCC of 0.948 (Supplemental Figure 15), indicating a high level of consistency across platforms (Table 12 for overall CCC). Only two assays showed significant differences (either differences in means, variance, or both) between platforms after assay-specific adjustments (and multiple test correction), ce_sha and pa_pse ($p<0.001$) (Table 13). Assays with considerably lower CCCs that were not significant included fl_psy (CCC 0.657) and pa_ther (CCC 0.765) (Table 3 and 13).

Sensitivity and specificity analysis was carried out on the assay-specific adjusted BioMark Ct values using five ABI7900 Ct value cutpoints (20, 25, 30, 25, and 40) for classifying tests as positive or negative. Over all assays, sensitivity ranged from 60.8 to 94.3 and specificity from 63.1 to 93% for ABI Ct cutpoint of 40 to 25, with values between 82-93 for cutpoints of 35-30 (Table 14). The area under the ROC curve (AUC) in this range was 0.90 to 0.96 (Supplemental Figures 16 to 19). The optimum cutpoint overall was around 30 Ct, but retained decent values up to 35 Ct. Table 14 shows the sensitivity and specificity analysis over each assay, and highlights that sensitivity and specificity is generally maximized by a cutpoint of 30 to 35. Actual cutpoints to be used, however, would depend upon the purpose of testing and how the tests are to be applied.

Looking at the data another way, over all assays and a continuous Ct range, there were 123 detections exclusive to the ABI 7900 and 78 to the BioMark. Sixty-six of the detections on the ABI 7900 contained a Ct>37, beyond the detection limit for most assays, while only 15 had a Ct<35, the typical detection limit used for many molecular assays. Moreover, 39 of the 123 ABI-exclusive detections were singletons, leaving 42 detections in duplicate samples. Alternately, 12 of the detections exclusive to the BioMark contained Ct>30 (beyond the detection limit for this platform), with only 22 with Ct<27, the average LOD for most assays (detecting 1-4 copies per chamber). Thirty-two of the 78 detections exclusive to the BioMark were singletons, leaving 23 detections in duplicate samples.

If all discordant results were considered with no cutpoints (maximum Ct scored as positive) specificity (calculated as the percentage of samples positive only on the BioMark) would be 0.981 and sensitivity (calculated as the percentage of samples positive only on the ABI) 0.965. However, given that we would normally score as positive only samples detected in duplicate samples (real scores), the consistency moderately improves based on real scores. If no Ct cutpoint were applied, overall specificity increased to 0.987 and sensitivity to 0.976. The real improvements in consistency come if cutpoints and duplicate positive scores are combined; based on an ABI 7900 Ct cutpoint of 35 and a BioMark cutpoint of 30, specificity enhances to 0.993 and sensitivity to 0.996.

Most assays with poor quality curves on the BioMark had normal curves on the ABI 7900; hence we expect that scoring may be somewhat more reliable on the ABI instrument for these assays (ye_ruc, pmcv, c_b_cys, ae_sal, my_arc, and ven), although empirically, we did not observe a higher variance between platforms for these assays. However, one assay, pa_pse, had very poor quality amplification curves on the ABI but not the BioMark, resulting in very poor consistency of results for this assay between platforms (Figure 16; Table 13).

7.6 IMPACT OF STA ON THE ANALYTICAL SENSITIVITY AND SPECIFICITY OF THE ASSAYS

The STA targeted enrichment is required to match the sensitivity of traditional platforms due to the small volumes upon which the microfluidics qPCR reactions are run on the BioMark platform, essentially 1/1,000th the volumes used on traditional single-assay instruments. The STA reaction includes a multiplex of primers for all assays run over 14 PCR cycles to facilitate a 1000-fold amplification of targeted product. After the STA, samples are cleaned up to remove all primers and enzymes, followed by individual (non-multiplexed) q-PCR performed on the BioMark instrument.

The fact that this targeted enrichment comprises a multiplex of primers opens the possibility for assay competition and the production of spurious products. As such, we conducted a series of analyses to determine if the STA introduced biases in the relative abundance of targets, and whether or not this had an influence on analytical sensitivity, specificity, and repeatability. STA and Non-STA samples were available for studies described in 6.1 (LOD), 6.2 (ASp), and 6.3 (ASe); below is a detailed description for the methods and results to determine the effect of STA on

a) sensitivity,

b) specificity, and

c) repeatability.

7.6.1 STA impact on Analytical Sensitivity

We were asked to evaluate whether the STA introduces biases in the relative abundance of targets. We assessed this in two ways. First, we assessed the efficiencies and LOD for STA and Non-STA samples from study 6.1 using the nonparametric Wilcoxon matched-pairs signed-ranks test and the paired t-test. There were no significant differences in efficiencies between the STA and Non-STA assay-specific groups (Table 4), but there were differences in the minimum number of copies necessary in the starting material to achieve 95% detection between STA and Non-STA samples ($p<0.001$). As presented in the section 7.2, the LOD of starting material for STA samples ranged between 1-39 copies per µl (median 8), whereas the LOD of starting material was considerably higher for the Non-STA, ranging between 72-7698 copies per µl (median 1185) (Table 3). There were, however, no difference in the minimum number of copies in the chamber to detect 95% of the time between STA and Non-STA samples ($p=0.453$). For both STA and Non-STA reactions, the Ct associated with the LOD was between 25 and 29.

Taken together, our results show that the detection limit of the Fluidigm BioMark platform is generally 1-10 copies per chamber over all assays, regardless of whether the assay is an STA or Non-STA, but the starting concentration for the Non-STA must be at a concentration of roughly 1000 copies per µl to achieve 1 copy per chamber. These data clearly demonstrate that the STA enriches targeted assays by approximately 1,000-fold.

The second way in which we tested the STA impact on analytical sensitivity was to assess the relative quantification of microbes at low-loads in mixtures that contained high-loads of other microbes, as described in 6.3. This particular objective was uniquely designed to test the performance of assays on the high throughput/microfluidic platform; as many platforms are now going to a similar multi-assay format with some level of pre-enrichment, our approach may prove useful as technologies continue to move towards high throughput in future. In total, 168 mixtures were evaluated over four STAs in triplicate over two dynamic arrays each, with the entire analysis encompassing 73,728 PCR reactions, 60,984 reactions excluding controls (Figure 5).

The CCC between expected and observed Ct values was 0.819 (n=539), with a statistical difference between the two Cts (Bradley-Blackwood F test, $p<0.001$), which was corroborated with a nonparametric Wilcoxon matched-pairs signed-ranks test ($p<0.001$). The linear regression with only the observed Ct as the outcome and the expected Ct as the predictor (no intercept) estimated that observed Cts were approximately 3% (2.7 to 3.5%) higher than expected Cts, which is less than one Ct difference.

A linear regression was used to estimate the predicted Cts for each assay used in the ASe study (Figure 17; Table 15). The observed median Cts were modeled as the outcome, with the expected Cts and assays as fixed effects. No random effects were required to model this relationship, and five assays were deemed potential outliers (sp_sal, ye_ruc, vhsv, ihnv, and fa_mar, in order of importance); they were left in the model to show their estimates. Of the outlier assays, observed Cts were higher than expected for three (vhsv, sp_sal, ihnv) and lower than expected for two (fa_mer, ye_ruc).

A logistic regression estimated the predicted probability of microbe detection for all samples in the ASe study with an expected positive outcome by microbe assay (Figure 18). Numerous assays predicted the outcome 100% (perfect detection). In all cases where predictions were less than 96%, the observed and/or expected Cts were above the assay-specific LOD.

Taken together, our results show that there is limited measurable impact of the STA on relative quantification, efficiency, and the LOD within the reaction chamber. The only highly significant

impact is on the LOD of the starting material, which must contain ~1,000 as opposed to <10 copies for reliable detection. These data clearly demonstrate that the STA enriches targeted assays by approximately 1,000-fold.

7.6.2 STA impact on Analytical Specificity (ASp)

We were asked to determine whether the STA generates spurious (false) targets (i.e. cross-amplifications between microbe assays). To assess the impact of the STA on analytical specificity, we compared the specificity results between STA and Non-STA control samples. In this analysis, because there is a 1000x difference in sensitivity between STA and Non-STA, we considered all detections by the Non-STA but for the STA, limited comparisons to samples with an STA Ct<18 (the average LOD for Non-STA detection). We then used a nonparametric Wilcoxon matched-pairs signed-ranks test to assess the false-positive frequencies, by assays, for all three combined microbe groups (bacteria, viruses, and parasites). There were no significant differences between STA and Non-STA frequencies of false positives across the assays.

In all, there were no indications that the STA was impacting analytical specificity of the assays in any consistent way; most assays run on the BioMark showed a high degree of specificity for the microbe of interest in both STA and Non-STA samples as long as microbe loads were high enough. The weak detection of CRS for my_ins, sp_sal that were exclusive to the STA were generally above the LOD for the assays, so would not readily be scored as positive.

7.7 FALSE POSITIVE DETECTIONS

Most of the analyses presented herein involved the removal of negative control samples. Over the ASe and ASp studies, there were 16,512 negative control samples assessed. Of these false detections, 19 (0.115%) occurred in FAM and 8 (0.048) in VIC (Table 16). These are very low false positive detection rates, consistent with the high quality of data emanating from the BioMark platform. Because each chamber is sealed, there is little potential for contamination during PCR.

7.8 OVERVIEW OF ASSAY QUALITY AND PERFORMANCE ON THE BIOMARK

Forty-seven microbe assays were assessed for efficiency, sensitivity, specificity, and repeatability on the BioMark, and for consistency in results between the BioMark and ABI 7900. The general results for each of these analyses are presented in Table 3. This presentation allowed us to assess the performance of each assay under each of these measures, and to rank the quality of each assay by the level of deviation from ideal expectations observed. Note that this ranking is subjective, and we applied “cutoffs” associated with each analytical measure that would identify at least some underperforming assays (i.e. greatest deviation from perfect or 100%). Optimal ranges for each measure are provided in Table 3 caption. Thirteen assays performed within ideal levels by all measures and were ranked as excellent, 15 showed only minor variance from ideal measures and were ranked very good and 13 showed reduced performance for no more than two measures and were ranked good. For the remaining assays, two were ranked adequate, one was ranked poor, and we decided to replace three for future applications. The three that were replaced (vhsv, isav7, pmcv) generally had curve quality issues making them more difficult to score, as well as other issues, and we had other assays available that had better amplification plots and good efficiencies (vhsv—Jonstrup et al. 2013, isav7—Snow et al. 2011, pmcv—Wiik-Nielsen et al. 2013). We will undertake additional analytical validations of these assays in future. We may apply both the NAAHP vhsv assay and the Jonstrup et al. 2013 assay, as this is a reportable. Moreover, the replacement of the isav7 was consistent with recommendations by the review panel that we use OIE-approved assays

(which snow7 is, although it is not the assay applied by the CFIA in the NAAHLS. Pa_pse was an extreme outlier for the CCC under consistency across platforms but was still ranked "good" as there was a curve issue on the ABI 7900 rather than the BioMark that created this variance. The single "poor" assay (ye_ruc) was very difficult to score and performed relatively poorly for multiple measures and was removed for future applications. A new assay may need to be developed for ye_ruc.

We should note that assays with poor quality amplification curves on the BioMark can still provide reliable data when loads were not approaching the LOD, and can be enhanced by altering the primer/probe concentrations.

7.9 SYNTHESIS OF THE STRENGTHS AND WEAKNESSES OF THE BIOMARK PLATFORM FOR THE INTENDED APPLICATION

STRENGTHS

1. **Depth: Ascertainment of results of dozens of assays at once can increase the depth of microbe** assessments, enabling a more thorough understanding of the role of co-infections on disease development.
2. **Cost**: Cheaper cost per assay if over 4 assays are required; it costs roughly the same amount to run 96 assays on the BioMark as to run 4 assays on a traditional qPCR platform.
3. **Time savings**: One technician can run over 27,000 PCR reactions in two days when starting from nucleic acids.
4. **Efficiency**: All assays are run in a 96-well format, enabling a smooth, efficient work-flow from robotic sample preparations.
5. **Flexibility**: The composition of microbe assays can be easily modified to meet the needs for different applications; refinements could include parsing out assays by environment, tissue, species, or life-history stage, among others. As well, there are different dynamic array formats that can accommodate fewer assays over more samples (24.192 array) or fewer assays over fewer samples (48.48 array).
6. **Expandability**: It is easy to incorporate additional assays to microbes or even to host biomarkers, although one might want to consider doing so in a separate STA; merging microbe monitoring with transcriptomics of host immune-related genes would enable assessments of the relative risks for disease imposed by various microbes assuming that the strength of such responses are associated with propensity for damage (under Matzinger's damage hypothesis for pathogenesis; Matzinger 2007). Studies to make these assessments contrasting host immune stimulation with histopathology are underway.
7. **Refinement of purpose**: While we developed and tested a broad range of salmon microbe assays to assess which microbes were detectable in BC salmon, one could quite easily focus development on strain- or genogroup typing within microbes as has been done for human *Streptococcus pneumoniae* bacteria (Dhoubhadel *et al.* 2014). One could also design a gill panel that included infectious agents and harmful algal bloom species.
8. **Analytical Sensitivity**: Both our analysis and those from literature show that the BioMark is, for most assays, more sensitive than other platforms, reliably detecting 3-10 copies per µl of starting material for most assays as opposed to 30-100 copies for most single-assay qPCR platforms. While this enhanced sensitivity may not be desirable for disease diagnostics (something that we did not assess herein), it could provide valuable information on the early transmission of microbes, microbe carrier states and ascertaining dilute microbes present in the water column or sediment.

9. **Interchangeability between platforms**: The BioMark uses the same assays developed for any qPCR platform, whether they are TaqMan, as applied herein, SYBR, EVA, and others.
10. **Limited tissue requirements**: Given the very small assay volumes (0.007 µl), hundreds of assays can be performed on the BioMark with as little as 1 µg of nucleic acids. This makes possible the use of non-destructive samples and sampling from very small specimens. We have utilized the platform with a single smolt gill filament smaller than a pin head as the starting material to run three dynamic arrays with microbes and host immune and stress biomarkers, the caveat being the smaller the tissue, the greater potential for false negatives for the microbes. Profiling microbes present in the stomachs of microparasites (e.g. sea lice) is also realizable. Other labs are performing single cell analyses (generally using the digital array capability of the BioMark).
11. **Upgradable to facilitate PCR-sequencing**: The BioMark can be upgraded to run the access array system that enables sequencing of all products produced on a single dynamic array, with barcoding of individual samples. This could facilitate rapid strain typing of multiple microbes simultaneously if assays were developed for such purposes or provide an extra level of validation of quantitative assay results.
12. **Simultaneous RNA quality assessments**: Housekeeping genes are often used as controls for RNA quality, and can easily be incorporated into the workflow on the BioMark.
13. **Detection of a second probe**: We utilized the Snow *et al.* (2009) positive control strategy that incorporates a second probe labeled in a different wavelength to facilitate detection of contamination of high copy number positive control material from artificial constructs. This is done seamlessly on the BioMark, which is capable of detecting multiple wavelengths at once.
14. **Controls**: The same controls as used on traditional qPCR platforms can be applied on the BioMark.

WEAKNESSES

1. **Poor amplification curves for some assays**: We identified a small percentage of assays that were difficult to score due to poor quality amplification curves. We assume that this is an issue with the software rather than the platform, but further testing and working with the software company will be required to verify this assumption. In some cases, assays had to be removed due to curve quality issues.
2. **Assesses presence and load, not disease**: As with any PCR-based assay, a molecular assay alone cannot discern whether an animal is diseased or if a microbe is infective, although with validation, it may be possible in future to predict disease states by ascertaining the activation of microbe and host biomarkers.
3. **Enhanced training requirements**: Because so many assays are being run at once, there is likely a greater need for training of technical staff to accurately score a broad range of microbe assays simultaneously. We ran our first analyses with no assay-specific scoring rules, and for a small percentage of assays, these required refinement to increase the repeatability of assay results between technical staff.
4. **STA introduces some enhanced variance in Ct**: We found that the individual STAs could introduce significant Ct variance, especially when different (and older) PCR instruments were used that may vary in ramp times. To limit these effects, we suggest that STAs are performed only on newer PCR instruments with closely-matched cycling conditions.

8 DISCUSSION

For validation of assays for important infectious diseases in aquatic and terrestrial animals, it is well accepted internationally that the OIE pathway (Figure 19) provides a suitable framework for such an evaluation (Purcell et al. 2011). Our evaluation was to level-1 of the pathway but completion of stages 2 and 3 would be necessary if the purpose was use in a diagnostic setting. We contend that the purpose of the assay, in its current application, is research rather than diagnosis. In addition, we followed standards for reporting PCR experiments (Bustin et al. 2009) and subjected the resultant data to rigorous statistical evaluation as outlined in prior sections of the report.

In our experiments, we have demonstrated that quantitative microbe TaqMan assays performed on the Fluidigm BioMark platform produce results that are congruent with those from other platforms, but at a much enhanced throughput, reduced cost, and considerable time savings. Case in point is the fact that we could undergo a highly intensive OIE stage-1 evaluation encompassing over 300,000 individual PCR reactions to assess sensitivity, specificity, repeatability and consistency of results for 47 microbe assays in less than 6 months' time at a cost that is a fraction of that required to assess assays independently (0.2 million versus estimated 2.4 million). Moreover, while our analysis showed that the STA was necessary to attain sufficient assay sensitivity, there was no evidence that the STA negatively impacted the specificity or relative quantification of the microbe assays.

Evaluation, or validation, of assay integrity and performance is an important step in ascertaining interpretation limits and provides a basis upon which running conditions and scoring rules can be developed to minimize false negative and positive results. We learned that while most assays could quite readily detect as little as a few microbe copies per µl starting material (at around Ct 26-28), for a small number of assays, enhanced variance in repeatability of low copy number samples, and the potential for the detection of false positives necessitated the implementation of scoring rules that would decrease the sensitivity to 20-30 copies per µl (around Ct 25). We also found that the software developed for the BioMark platform did not visually optimize the amplification curves, making it more difficult to score some of the assays than experienced on other platforms. Curve quality issues accounted for a large percentage of variance in repeatability between technicians. With the development of scoring SOPs for difficult assays, we were able to enhance repeatability considerably. We are now working with the software company to make changes in their algorithms to facilitate easier scoring. On the positive side, the automated amplification curve quality scores do limit false detections for most assays, with few requiring manual intervention.

While there were no assays for which profound effects of competition were observed, there are a few lower performance assays that might benefit from being in a more restricted STA set. While we did not have time to pursue this for the report, we will conduct some testing before the CSAS meeting to determine if having two independent STA sets improves the performance of these assays.

In the microbe monitoring studies to be performed in future, we will use a set of 5-serial dilutions of APC clones as positive controls to assess array-to-array variability in assay performance, and, where desired, to calculate microbe copy numbers. In future, these sets of serially-diluted artificial constructs can be made available to other laboratories to assess sensitivity between platforms and laboratories.

9 ACKNOWLEDGEMENTS

The analyses performed on 46 microbes potentially known or suspected to cause disease in salmon worldwide would not have been possible without the generosity of the international scientists who provided positive control material to us. We sincerely thank Dr. Terge Steinum from Norway (Candidatus *Branchiomonas cysticola*, *Piscichlamydia salmonis*), Dr. Hisae Kasai from Japan (Salmonid herpes virus), Dr. Jerri Bartholomew from the USA (*Ceratomyxa shasta*), Dr. Sharon Clouthier from Canada (Infectious pancreatic necrosis virus), Dr. Fred Kibenge from Canada (Infectious salmon anemia virus), Nellie Gagné from Canada (Infectious salmon anemia virus), Dr. Simon Jones from Canada (*Aeromonas salmonicida*, *Ceratomyxa Shasta*, *Kudoa thyrsites*, *Neoparamoeba perurans, Paranucleospora theridion*, *Parvicapsula* spp., *Piscirickettsia salmonis*, *Renibacterium salmoninarum*, *Spironucleus salmonicida*, *Tetracapsuloides bryosalmonae*, *Yersinia ruckeri*, *Vibrio anguillarum*), Dr. Duane Barker from Canada (*Tenacibaculum*), Dr. Stephen Atkinson from USA (*Myxobolus* spp.), Dr. Paul Savage from Ireland (Salmon alpha virus), and Dr. Kyle Garver from Canada (Infectious haematopoietic necrosis virus, Viral hemorrhagic septicemia virus), Dr. Cathryn Abbott from Canada (*Gyrodactylus salaris*), and Dr. Jim Winton (Viral erythrocytic necrosis virus). We would also like to thank individuals in the NAAHLS from DFO who provided initial feedback on the experimental design. Finally, a huge thank you to Drs. Brad Popovich, Marcia MacDonald, Sally Greenwood, Jenny Boon, and Tony Brooks from Genome BC, and Dr. Brian Riddell, CEO of the Pacific Salmon Foundation, and our overall project lead, Timothy Sucic, and Mike Meneer also from the Pacific Salmon Foundation, for their immense support, both financial and scientific, in bringing this project to fruition.

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11 TABLES

Table 1. TaqMan™ assays evaluated on the BioMark and their design origin. MGL refers to an in-house assay design. Throughout the document, assays are referred to by their organism abbreviations. Assays with an asterix (*) were added to replace poorly performing assays (isav7, vhsv, pmcv) after the initial platform evaluation; herein these were only evaluated for efficiency and LOD (see Table 3).

Microbe Full Name	Type	Organism Abbreviation	Assay Reference	Gene	Gene Function	Accession No.	Forward Primer Sequence (5'-3') Reverse Primer Sequence (5'-3') Probe Sequence (FAM-5'-3'-MGB)	Ta	Amplicon Size (bp)
Aeromonas hydrophila	Bacterium	ae_hyd	Lee *et al*. 2006	aha1	Major adhesin	AY165026	F: ACCGCTGCTCATTACTCTGATG R: CCAACCCAGACGGGAAGAA P: TGATGGTGAGCTGGTTG	68	60
Aeromonas salmonicida	Bacterium	ae_sal	modified from Keeling *et al*. 2013	vapA	Surface array protein	M64655	F: TAAAGCACTGTCTGTTACC R: GCTACTTCACCCTGATTGG P: ACATCAGCAGGCTTCAGAGTCACTG	60	144
Candidatus Branchiomonas cysticola	Bacterium	c_b_cys	Mitchell *et al*. 2013	16S	Ribosomal	JQ723599	F: AATACATCGGAACGTGTCTAGTG R: GCCATCAGCCGCTCATGTG P: CTCGGTCCCAGGCTTTCCTCTCCCA	65	122
Flavobacterium psychrophilum	Bacterium	fl_psy	Duesund *et al.* 2010	16S	Ribosomal		F: GATCCTTATTCTCACAGTACCGTCAA R: TGTAAACTGCTTTTGCACAGGAA P: AAACACTCGGTCGTGACC	66	72
Gill chlamydia	Bacterium	sch	Duesund *et al.* 2010	16S	Ribosomal	FJ897519	F: GGGTAGCCCGATATCTTCAAAGT R: CCCATGAGCCGCTCTCTCT P: TCCTTCGGGACCTTAC	67	66
Piscichlamydia salmonis	Bacterium	pch_sal	Nylund *et al*. 2008	16S	Ribosomal	EU326495	F: TCACCCCCAGGCTGCTT R: GAATTCCATTTCCCCCTCTTG P: CAAAACTGCTAGACTAGAGT	64	60
Piscirickettsia salmonis	Bacterium	pisck_sal	Corbeil *et al*. 2003	23S	Ribosomal	U36943	F: TCTGGGAAGTGTGGCGATAGA R: TCCCGACCTACTCTTGTTTCATC P: TGATAGCCCCGTACACGAAACGGCATA	67	77
Renibacterium salmoninarum	Bacterium	re_sal	Powell *et al*. 2005	P57		AF123890	F: CAACAGGGTGGTTATTCTGCTTTC R: CTATAAGAGCCACCAGCTGCAA P: CTCCAGCGCCGCAGGAGGAC	67	95
Rickettsia-like organism	Bacterium	rlo	Lloyd *et al.* 2011	16S	Ribosomal	EU555284	F: GGCTCAACCCAAGAACTGCTT R: GTGCAACAGCGTCAGTGACT P: CCCAGATAACCGCCTTCGCCTCCG	69	149
Vibrio anguillarum	Bacterium	vi_ang	MGL	VirA		L08012	F: CCGTCATGCTATCTAGAGATGTATTTGA R: CCATACGCAGCCAAAAATCA P: TCATTTCGACGAGCGTCTTGTTCAGC	64	93
Vibrio salmonicida	Bacterium	vi_sal	MGL	lux	Bioluminescence protein	AF452135	F: GTGTGATGACCGTTCCATATTT R: GCTATTGTCATCACTCTGTTTCTT P: TCGCTTCATGTTGTGTAATTAGGAGCGA	64	138

Microbe Full Name	Type	Organism Abbreviation	Assay Reference	Gene	Gene Function	Accession No.	Forward Primer Sequence (5'-3') Reverse Primer Sequence (5'-3') Probe Sequence (FAM-5'-3'-MGB)	Ta	Amplicon Size (bp)
Nanophyetus salmincola	Fluke	na_sal	MGL	ND1	NADH dehydrogenase	AY269674	F: GATCTGCATTTGGTTCTGTAACA R: CCAACGCCACAATGATAGCTATAC P: TGAGGCGTGTTTTATG	66	66
Ceratomyxa shasta	Parasite	ce_sha	Hallett and Bartholomew 2006	18S	Ribosomal	AF001579	F: CCAGCTTGAGATTAGCTCGGTAA R: CCCCGGAACCCGAAAG P: CGAGCCAAGTTGGTCTCTCCGTGAAAAC	67	71
Cryptobia salmositica	Parasite	cr_sal	MGL	cys	Cysteine protease		F: TCAGTGCCTTTCAGGACATC R: GAGGCATCCACTCCAATAGAC P: AGGAGGACATGGCAGCCTTTGTAT	65	91
Dermocystidium salmonis	Parasite	de_sal	MGL	18S	Ribosomal	U21337	F: CAGCCAATCCTTTCGCTTCT R: GACGGACGCACACCACAGT P: AAGCGGCGTGTGCC	66	60
Facilispora margolisi	Parasite	fa_mar	MGL	18S	Ribosomal	HM800849	F: AGGAAGGAGCACGCAAGAAC R: CGCGTGCAGCCCAGTAC P: TCAGTGATGCCCTCAGA	69	58
Gyrodactylus salaris	Parasite	gy_sal	Collins *et al.* 2010	ITS rDNA	Ribosomal		F: CGATCGTCACTCGGAATCG R: GGTGGCGCACCTATTCTACA P: TCTTATTAACCAGTTCTGC	66	63
Ichthyophonus hoferi	Parasite	ic_hof	White *et al.* 2013	18S	Ribosomal	AF467793	F: GTCTGTACTGGTACGGCAGTTTC R: TCCCGAACTCAGTAGACACTCAA P: TAAGAGCACCCACTGCCTTCGAGAAGA	68	72
Ichthyophthirius multifiliis	Parasite	ic_mul	MGL	18S	Ribosomal	IMU17354	F: AAATGGGCATACGTTTGCAAA R: AACCTGCCTGAAACACTCTAATTTTT P: ACTCGGCCTTCACTGGTTCGACTTGG	64	98
Kudoa thyrsites	Parasite	ku_thy	Funk *et al.* 2007	18S	Ribosomal	AF031412	F: TGGCGGCCAAATCTAGGTT R: GACCGCACACAAGAAGTTAATCC P: TATCGCGAGAGCCGC	67	82
Loma salmonae	Parasite	lo_sal	MGL	SSU	Ribosomal	HM626243	F: GGAGTCGCAGCGAAGATAGC R: CTTTTCCTCCCTTTACTCATATGCTT P: TGCCTGAAATCACGAGAGTGAGACTACCC	66	87
Myxobolus arcticus	Parasite	my_arc	MGL	18S	Ribosomal	HQ113227	F: TGGTAGATACTGAATATCCGGGTTT R: AACTGCGCGGTCAAAGTTG P:CGTTGATTGTGAGGTTGG	66	68
Myxobolus cerebralis	Parasite	my_cer	Kelley *et al.* 2004	SP	serine protease	AY27570-8	F: GCCATTGAATTTGACTTTGGATTA R: ACCATTCATGTAAGCCCGAACT P: TCGAAGCCTTGACCATCTTTTGGCC	63	73
Myxobolus insidiosus	Parasite	my_ins	MGL	18S	Ribosomal	EU346375	F: CCAATTTGGGAGCGTCAAA R: CGATCGGCAAAGTTATCTAGATTCA P: CTCTCAAGGCATTTAT	64	111
Neoparamoeba perurans	Parasite	ne_per	Fringuelli *et al.* 2012	18S	Ribosomal	EF216905	F: GTTCTTTCGGGAGCTGGGAG R: GAACTATCGCCGGCACAAAAG P: CAATGCCATTCTTTTCGGA	68	137
Nucleospora salmonis	Parasite	nu_sal	Foltz *et al.* 2009	ITS-1	Ribosomal	AF186009	F: GCCGCAGATCATTACTAAAAACCT R: CGATCGCCGCATCTAAACA P: CCCCGCGCATCCAGAAATACGC	66	78
Paranucleospora theridion (syn. Desmozoon lepeophtherii)	Parasite	pa_ther	Nylund *et al.* 2010	SSU	Ribosomal	FJ59481	F: CGGACAGGGAGCATGGTATAG R: GGTCCAGGTTGGGTCTTGAG P: TTGGCGAAGAATGAAA	68	59
Parvicapsula kabatai	Parasite	pa_kab	MGL	SSU	Ribosomal	DQ515821	F: CGACCATCTGCACGGTACTG R: ACACCACAACTCTGCCTTCCA P:CTTCGGGTAGGTCCGG	69	62
Parvicapsula minibicornis	Parasite	pa_min	Hallett and Bartholomew 2009	18S	Ribosomal	AF201375	F: AATAGTTGTTTGTCGTGCACTCTGT R: CCGATAGGCTATCCAGTACCTAGTAAG P: TGTCCACCTAGTAAGGC	68	72
Parvicapsula pseudobranchicola	Parasite	pa_pse	Jørgensen *et al.* 2011	18S	Ribosomal	AY308481	F: CAGCTCCAGTAGTGTATTTCA R: TTGAGCACTCTGCTTTATTCAA P: CGTATTGCTGTCTTTGACATGCAGT	62	187
Sphaerothecum destructuens	Parasite	sp_des	MGL	18S	Ribosomal	AY267346	F: GGGTATCCTTCCTCTCGAAATTG R: CCCAAACTCGACGCACACT P: CGTGTGCGCTTAAT	66	59
Spironucleus salmonicida	Parasite	sp_sal	MGL	18S	Ribosomal	AY677182	F: GCAGCCGCGGTAATTCC R: CGAACTTTTTAACTGCAGCAACA P: ACACGGAGAGTATTCT	65	61
Tetracapsuloides bryosalmonae	Parasite	te_bry	Bettge *et al.* 2009	18S	Ribosomal	AF190669	F: GCGAGATTTGTTGCATTTAAAAAG R: GCACATGCAGTGTCCAATCG P: CAAAATTGTGGAACCGTCCGACTACGA	62	73

Microbe Full Name	Type	Organism Abbreviation	Assay Reference	Gene	Gene Function	Accession No.	Forward Primer Sequence (5'-3') Reverse Primer Sequence (5'-3') Probe Sequence (FAM-5'-3'-MGB)	Ta	Amplicon Size (bp)
Atlantic salmon paramyxovirus	Virus	aspv	Nylund *et al.* 2008	P/C/V gene	P,C,V,W proteins	EF646380	F: CCCATATTAGCAAATGAGCTCTATCTT R: CGTTAAGGAACTCATCATTGAGCTT P: AGCCCTTTTGTTCTGC	65	97
Infectious hematopoietic necrosis virus	Virus	ihnv	Purcell *et al.* 2013	N gene	Nucleocapsid protein	NC_001652	F: AGAGCCAAGGCACTGTGCG R: TTCTTTGCGGCTTGGTTGA P: TGAGACTGAGCGGGACA	66	80
Infectious pancreatic necrosis virus	Virus	ipnv	S. Clouthier pers. comm.	segA VP2			F: GCAACTTACTTGAGATCCATTATGCT R: AGACCTCTAAGTTGTATGACGAGGTCTCT P: CGAGAATGGGCCAGCAAGCA	66	115
Infectious salmon anemia virus	Virus	isav7	Plarre *et al.* 2005	S7	Nonstructural protein		F: TGGGATCATGTGTTTCCTGCTA R: GAAAATCCATGTTCTCAGATGCAA P: CACATGACCCCTCGTC	67	81
Infectious salmon anemia virus	Virus	snow7*	Snow *et al.* 2006	S7	Segment 7		F: CAGGGTTGTATCCATGGTTGAAATG R: GTCCAGCCCTAAGCTCAACTC P: CTCTCTCATTGTGATCCC	66	155
Infectious salmon anemia virus	Virus	isav8	LeBlanc *et al.* 2010	S8	Segment 8 - matrix protein	EU118822	F: TGGGCAATGGTGTATGGTATGA R: GAAGTCGATGAACTGCAGCGA P: CAGGATGCAGATGTATGC	67	169
Pacific salmon parvovirus	Virus	pspv	MGL	rep			F: CCCTCAGGCTCCGATTTTTAT R: CGAAGACAACATGGAGGTGACA P: CAATTGGAGGCAACTGTA	65	65
Piscine myocarditis virus		pmcv	Løvoll *et al.*. 2010	RdRP	RNA dependent RNA polymerase	HQ401057	F: TTCCAAACAATTCGAGAAGCG R: ACCTGCCATTTTCCCCTCTT P: CCGGGTAAAGTATTTGCGTC	67	141
Piscine myocarditis virus	Virus	pmcv1*	Wiik-Nielsen *et al.* 2013	RdRP	RNA dependent RNA polymerase	HQ339954	F: AGGGAACAGGAGGAAGCAGAA R: CGTAATCCGACATCATTTTGTGA P: TGGTGGAGCGTTCAA	64	61
Piscine reovirus	Virus	prv	Wiik-Nielsen et al. 2012	L1	Core shell protein		F: TGCTAACACTCCAGGAGTCATTG R: TGAATCCGCTGCAGATGAGTA P: CGCCGGTAGCTCT	67	59
Salmon alphavirus 1, 2, and 3	Virus	sav	Andersen *et al.* 2007	nsP1	Non-structural gene	AY604235	F: CCGGCCCTGAACCAGTT R: GTAGCCAAGTGGGAGAAAGCT P: TCGAAGTGGTGGCCAG	68	107
Salmonid herpesvirus / *Oncorhynchus masou* herpes virus	Virus	omv	MGL	capsid6	Capsid6		F: GCCTGGACCACAATCTCAATG R: CGAGACAGTGTGGCAAGACAAC P: CCAACAGGATGGTCATTA	67	85
Viral encephalopathy and retinopathy virus	Virus	ver	Korsnes *et al.* 2005	RNA2	Capsid protein	AJ245641	F: TTCCAGCGATACGCTGTTGA R: CACCGCCCGTGTTTGC P: AAATTCAGCCAATGTGCCCC	67	70
Viral erythrocytic necrosis virus	Virus	ven	J. Winton, pers. comm.				F: CGTAGGGCCCCAATAGTTTCT R: GGAGGAAATGCAGACAAGATTTG P: TCTTGCCGTTATTTCCAGCACCCG	65	100
Viral hemorrhagic septicemia virus	Virus	vhsv	Garver *et al.* 2011	N gene	Nucleoprotein	Y18263	F: ATGAGGCAGGTGTCGGAGG R: TGTAGTAGGACTCTCCCAGCATCC P: TACGCCATCATGATGAGT	67	82
Viral hemorrhagic septicemia virus	Virus	vhsv1*	Jonstrup et al. 2013	N gene	Nucleoprotein	Z93412	F: AAACTCGCAGGATGTGTGCGTCC R: TCTGCGATCTCAGTCAGGATGAA P: TAGAGGGCCTTGGTGATCTTCTG	69	77
Si:dkey-78d16.1 protein [Danio rerio]	HouseKeeping gene	hkg	MGL	We used statistical ananlysis to examine multiple Salmon microarray database, found this gene that was stably expressed in various species, tissues and migration states.		CA056739	F: GTCAAGACTGGAGGCTCAGAG R: GATCAAGCCCCAGAAGTGTTTG P: AAGGTGATTCCCTCGCCGTCCGA	67	102

Table 2. Control samples used in the assay specificity study. Controls are broken down by their classification (virus, bacterium, parasite) and type, and for specificity testing, whether the organism is a "true" positive (T) for one of the microbe assays or a closely-related species (CRS); in some instances, the same sample may be both (when more than one assay for closely-related species are being tested). Control samples were classified at the species level and genus level () for specificity testing in Loma salmonae, Kudoa thyrsites and Ichthyophonus hoferi parasites. Real control samples were provided as pure bacterial cultures, tissue cultures, cell cultures, sera, or fish tissues. Where these were not found, we obtained DNA plasmids or synthesized gBlocks containing 1000 bases of sequence including the assay region.

Type of microbe	Classification of Microbe	Specificity Sample Run Name	Species	Asp Test or CRS	Control Source	Fish Tissue species
Bacteria	Proteobacteria_γ	Ahyd_aha1	*Aeromonas hydrophila*	T	gBlocks	
Bacteria	Proteobacteria_γ	Asob_FH	*Aeromonas sobria*	CRS	Pure bacterial culture	
Bacteria	Proteobacteria_γ	Asal_atypicalBC_FH	*Aeromonas salmonicida subsp. achromogenes, masoucida* or *smithia*	T	Pure bacterial culture	
Bacteria	Proteobacteria_γ	Asal_atypicalYukon_FH	*Aeromonas salmonicida subsp. achromogenes, masoucida* or *smithia*	T	Pure bacterial culture	
Bacteria	Proteobacteria_γ	Asal_FH	*Aeromonas salmonicida*	T	Pure bacterial culture	
Bacteria	Proteobacteria_γ	Asal_typicalBC_FH	*Aeromonas salmonicida subsp. salmonicida*	T	Pure bacterial culture	
Bacteria	Proteobacteria_γ	Bcy2_TMS	*Candidatus Branchiomonas cysticola*	T	Fish tissue	Atlantic salmon
Bacteria	gram pos_actinobacteria	BKD_FH	*Renibacterium salmoninarum*	T	Fish tissue	Chinook salmon
Bacteria	CFB group	Flavo_ATCC_49511D	*Flavobacterium psychrophilum*	T	ATCC purified nucleic acid	
Bacteria	CFB group	F._branchiophilum_16S_gblock_APC	*Flavobacterium branchiophilum*	CRS	gBlock	
Bacteria	CFB group	F._columnare_16S_gblock_APC	*Flavobacterium columnare*	CRS	gBlocks	
Bacteria	Chlamydiae/Verrucomicrobia	Pch1_TMS	*Piscichlamydia salmonis*	T	Fish tissue	Atlantic salmon
Bacteria	Chlamydiae/Verrucomicrobia	Sch_gblock	*gill chlamydia* (Sch)	T	gBlocks	
Bacteria	Chlamydiae/Verrucomicrobia	Sch_Onne2010_3223	*gill chlamydia* (Sch)	T	Fish tissue	Sockeye salmon
Bacteria	Chlamydiae/Verrucomicrobia	Chlamydiales_gp1_16S	*Chlamydiales sp.*	CRS	gBlocks	
Bacteria	Chlamydiae/Verrucomicrobia	Chlamydiales_gp2_16S	*Chlamydiales sp.*	CRS	gBlocks	
Bacteria	Proteobacteria_γ	Piscik_1_MR	*Piscirickettsia salmonis*	T	Tissue culture S/N	
Bacteria	Proteobacteria_γ	Pisck_2_SR	*Piscirickettsia salmonis*	T	Tissue culture S/N	
Bacteria	Proteobacteria_α	RLO_OntsSE838	*Rickettsia-like organism*	T	Fish tissue	Chinook salmon
Bacteria	Proteobacteria_γ	Vang1_SJ	*Vibrio anguillarum*	T	Pure bacterial culture	
Bacteria	Proteobacteria_γ	Vang2_SJ	*Vibrio anguillarum*	T	Pure bacterial culture	
Bacteria	Proteobacteria_γ	Vang3_SJ	*Vibrio anguillarum*	T	Pure bacterial culture	
Bacteria	Proteobacteria_γ	Vang4_SJ	*Vibrio anguillarum*	T	Pure bacterial culture	
Bacteria	Proteobacteria_γ	Vang5_SJ	*Vibrio anguillarum*	T	Pure bacterial culture	
Bacteria	Proteobacteria_γ	Vibrio_145	*Photobacterium sp.* SL13	CRS	Pure bacterial culture	
Bacteria	Proteobacteria_γ	Vibrio_148	*Moritella marina*	CRS	Pure bacterial culture	
Bacteria	Proteobacteria_γ	Vibrio_153	*Vibrio pectenicida* Ifremer A365	CRS	Pure bacterial culture	
Bacteria	Proteobacteria_γ	Vibrio_187	*Vibrio logei* NCIMB 2252	CRS	Pure bacterial culture	
Bacteria	Proteobacteria_γ	Vibrio_21	*Vibrio logei* ATCC 15382	CRS	Pure bacterial culture	
Bacteria	Proteobacteria_γ	Vibrio_279	*Vibrio logei* NCIMB 2252	CRS	Pure bacterial culture	
Bacteria	Proteobacteria_γ	Vibrio_296	*Vibrio splendidus* VC1	CRS	Pure bacterial culture	
Bacteria	Proteobacteria_γ	Vibrio_423	*Vibrio parahaemolyticus*	CRS	Pure bacterial culture	
Bacteria	Proteobacteria_γ	Vibrio_481	*Vibrio splendidus* LMG 4042	CRS	Pure bacterial culture	
Bacteria	Proteobacteria_γ	Vibrio_495	*Vibrio pectenicida* Ifremer A365	CRS	Pure bacterial culture	
Bacteria	Proteobacteria_γ	Vibrio_60	*Vibrio sp.* QY101	CRS	Pure bacterial culture	
Bacteria	Proteobacteria_γ	Vibrio_76	*Vibrio sp.* OC25	CRS	Pure bacterial culture	
Bacteria	Proteobacteria_γ	Vibrio_9	*Vibrio sp.* R-3884	CRS	Pure bacterial culture	
Bacteria	Proteobacteria_γ	Vibrio_94	*Vibrio sp.* NAP-4	CRS	Pure bacterial culture	
Bacteria	Proteobacteria_γ	Vsal_ATCC_43839	*Aliivibrio salmonicida*	T	ATCC purified nucleic acid	
Bacteria	Proteobacteria_γ	Yersinia ruckeri type2_FH	*Yersinia ruckeri* (Enteric redmouth disease)	T	Pure bacterial culture	

Type of microbe	Classification of Microbe	Specificity Sample Run Name	Species	Asp Test or CRS	Control Source	Fish Tissue species
Virus	ssRNA (-) Paramyxovirus	ASPV_2_gblock	Atlantic salmon paramyxovirus	T	gBlocks	
Virus	ssRNA (-) Rhabdovirus	IHNV_M_KG	Infectious haematopoietic necrosis virus	T	Cell culture	
Virus	ssRNA (-) Rhabdovirus	IHNV_MGL_L_HK_B29	Infectious haematopoietic necrosis virus	T	Fish tissue	Atlantic salmon
Virus	ssRNA (-) Rhabdovirus	IHNV_U_KG	Infectious haematopoietic necrosis virus	T	Cell culture	
Virus	ssRNA (-) Rhabdoviridae	VHSV type Ib_N_gblock	Viral hemorrhagic septicemia virus	T	gBlocks	
Virus	ssRNA (-) Rhabdoviridae	VHSV type IIIa_N_gblock	Viral hemorrhagic septicemia virus	T	gBlocks	
Virus	ssRNA (-) Rhabdoviridae	VHSV type IIIb_N_gblock	Viral hemorrhagic septicemia virus	T	gBlocks	
Virus	ssRNA (-) Rhabdoviridae	VHSV type IV_N_gblock	Viral hemorrhagic septicemia virus	T	gBlocks	
Virus	ssRNA (-) Rhabdoviridae	VHSV_Iva_KG	Viral hemorrhagic septicemia virus	T	Cell culture	
Virus	ssRNA (-) Rhabdoviridae	VHSV_Ivb_KG	Viral hemorrhagic septicemia virus	T	Cell culture	
Virus	ssRNA (-) Orthomyxovirus	ISAV_E_HPR14	Infectious Salmon Anemia virus	T	Cell culture	
Virus	ssRNA (-) Orthomyxovirus	ISAV_E_HPR2	Infectious Salmon Anemia virus	T	Cell culture	
Virus	ssRNA (-) Orthomyxovirus	ISAV_E_HPR3	Infectious Salmon Anemia virus	T	Cell culture	
Virus	ssRNA (-) Orthomyxovirus	ISAV_E_HPR7b	Infectious Salmon Anemia virus	T	Cell culture	
Virus	ssRNA (-) Orthomyxovirus	ISAV_E_new_13_aa_deleted	Infectious Salmon Anemia virus	T	Cell culture	
Virus	ssRNA (-) Orthomyxovirus	ISAV_EU_HPR5	Infectious Salmon Anemia virus	T	Cell culture	
Virus	ssRNA (-) Orthomyxovirus	ISAV_NA_HPR21	Infectious Salmon Anemia virus	T	Cell culture	
Virus	ssRNA (-) Orthomyxovirus	ISAV_NA_HPR4	Infectious Salmon Anemia virus	T	Cell culture	
Virus	ssRNA (+) Togavirus	SAV- 1_DG	Salmon alphavirus/ Salmon pancreas disease virus (PD)	T	Cell culture	
Virus	ssRNA (+) Togavirus	SAV-2_DG	Salmon alphavirus/ Sleeping disease virus (SD)	T	Cell culture	
Virus	ssRNA (+) Togavirus	SAV-3_DG	Salmon alphavirus/ Norwegian Salmon alphavirus (PD)	T	Cell culture	
Virus	ssRNA (+) Togavirus	SAV-4_DG	Salmon alphavirus/ Salmon pancreas disease virus (PD)	T	Sera	
Virus	ssRNA (+) Togavirus	SAV-5_DG	Salmon alphavirus/ Salmon pancreas disease virus (PD)	T	Sera	
Virus	ssRNA (+) Togavirus	SAV-6_DG	Salmon alphavirus/ Salmon pancreas disease virus (PD)	T	Sera	
Virus	ssRNA (+) Togavirus	SAV_1_2_3 nSP_gblock	Salmon alphavirus	T	gBlocks	
Virus	ssRNA (+) Nodavirus	VER gp1 Cp_gblock	Viral encephalopathy and retinopathy virus	T	gBlocks	
Virus	ssRNA (+) Nodavirus	VER gp2 Cp_gblock	Viral encephalopathy and retinopathy virus	T	gBlocks	
Virus	ssRNA (+) Nodavirus	VER gp3 Cp_gblock	Viral encephalopathy and retinopathy virus	T	gBlocks	
Virus	ssRNA (+) Nodavirus	VER gp4 Cp_gblock	Viral encephalopathy and retinopathy virus	T	gBlocks	
Virus	ssRNA (+) Nodavirus	VER gp5 Cp_gblock	Viral encephalopathy and retinopathy virus	T	gBlocks	
Virus	ssRNA (+) Nodavirus	VER gp6 Cp_gblock	Viral encephalopathy and retinopathy virus	T	gBlocks	
Virus	dsRNA Birnavirus	IPNV_groupI_Jasper_SC	Infectious pancreatic necrosis virus	T	Cell culture	
Virus	dsRNA Birnavirus	IPNV_groupI_VR299_SC	Infectious pancreatic necrosis virus	T	Cell culture	
Virus	dsRNA Birnavirus	IPNV_groupII_Ab_clone	Infectious pancreatic necrosis virus	T	DNA plasmid	
Virus	dsRNA Birnavirus	IPNV_groupIII_Sp_clone	Infectious pancreatic necrosis virus	T	DNA plasmid	
Virus	dsRNA Birnavirus	IPNV_groupIV_Canada1_SC	Infectious pancreatic necrosis virus	T	Cell culture	
Virus	dsRNA Birnavirus	IPNV_groupV_Canada2_SC	Infectious pancreatic necrosis virus	T	Cell culture	
Virus	dsRNA Birnavirus	IPNV_groupV_Canada3_SC	Infectious pancreatic necrosis virus	T	Cell culture	
Virus	dsRNA Birnavirus	IPNV_groupVI_Hecht_clone	Infectious pancreatic necrosis virus	T	DNA plasmid	
Virus	dsRNA Birnavirus	IPNV_groupVII_Japan_clone	Infectious pancreatic necrosis virus	T	DNA plasmid	
Virus	dsDNA Herpesvirus	OMV _HK	*Oncorhynchus Masou* Herpes Virus/ Salmonid herpesvirus	T	Cell culture	
Virus	dsRNA Totivirus	PMCV_RdRp_gblock	Piscine myocarditis virus (CMS)	T	gBlocks	
Virus	dsRNA Reovirus	PRV_L1_gblock	Piscine reovirus (HSMI)	T	gBlocks	
Virus	dsRNA Reovirus	PRV_MGL_Creat_ L_1055	Piscine reovirus (HSMI)	T	Fish tissue	Chinook salmon
Virus	dsRNA Reovirus	PRV_MGL_OnkiAKT2012_3112	Piscine reovirus (HSMI)	T	Fish tissue	Coho salmon
Virus	ssDNA (+) Parvovirus	PSPV_MGL_liver2	Pacific salmon parvovirus	T	Fish tissue	Sockeye salmon
Virus	ssDNA (+) Parvovirus	PSPV_MGL_liver3065	Pacific salmon parvovirus	T	Fish tissue	Sockeye salmon
Virus	dsDNA Iridovirus	VEN_JW	Viral erythrocytic necrosis virus	T	Fish tissue	Pacific herring

Type of microbe	Classification of Microbe	Specificity Sample Run Name	Species	Asp Test or CRS	Control Source	Fish Tissue species
Parasite	Amoeba	01Neo_FH	*Neoparamoeba perurans*	T	Fish tissue	Unknown
Parasite	Amoeba	01Neo_gblock_CHSE	*Neoparamoeba perurans*	T	gBlocks	
Parasite	Ciliate	02IMF_gblock_CHSE	*Ichthyophthirius multifiliis*	T	gBlocks	
Parasite	Ciliate	02IMF_OntsSE1756	*Ichthyophthirius multifiliis*	T	Fish tissue	Chinook salmon
Parasite	Ciliate	02IMF_OntsSE1756_old	*Ichthyophthirius multifiliis*	T	Fish tissue	Chinook salmon
Parasite	Ciliate	02IMF_Gchattoni_CHSE	*Glaucoma chattoni* X56533.1	CRS	gBlocks	
Parasite	Ciliate	02IMF_Lambornella_CHSE	*Lambornella* JQ723973.1	CRS	gBlocks	
Parasite	Ciliate	02IMF_Taustralis_CHSE	*Tetrahymena australis* M98015	CRS	gBlocks	
Parasite	Ciliate	02IMF_Tthermophila_CHSE	*Tetrahymena thermophila* X56165.1	CRS	gBlocks	
Parasite	Ciliate	02IMF_Ttropicalis_CHSE	*Tetrahymena tropicalis* M98023	CRS	gBlocks	
Parasite	Ecto-worm	03Gysa_Gsal_CHSE	*Gyrodactylus salaris*	T	gBlocks	
Parasite	Ecto-worm	03GysaAS_Gsal_CHSE	*Gyrodactylus salaris*	T	gBlocks	
Parasite	Flagellate	04SpiroSal_gblock_CHSE	*Spironucleus salmonicida* (Diplomonadida; Hexamitidae); aka *Hexamita salmonis;*	T	gBlocks	
Parasite	Flagellate	04SpiroSal_salmonicida	*Spironucleus salmonicida* (Diplomonadida; Hexamitidae); aka *Hexamita salmonis*;	T	Fish tissue	Atlantic salmon
Parasite	Flagellate	04SpiroSal_Spirobark_CHSE	*Spironucleus barkhanaus*	CRS	gBlocks	
Parasite	Flagellate	04SpiroSal_Storosa_CHSE	*Spironucleus torosa* EF050055.1	CRS	gBlocks	
Parasite	Fluke	05Nanosal_OntsSE1198	*Nanophyetus salmincola*	T	Fish tissue	Chinook salmon
Parasite	Fluke	05Nanosal_OntsSE1198_old	*Nanophyetus salmincola*	T	Fish tissue	Chinook salmon
Parasite	Microsporidian	06Faci3_OntsSE3665	*Facilispora margolisi*	T	Fish tissue	Chinook salmon
Parasite	Microsporidian	06Faci3_OntsSE3665_old	*Facilispora margolisi*	T	Fish tissue	Chinook salmon
Parasite	Microsporidian	07Loma_positive	*Loma salmonae*	T	Fish tissue	
Parasite	Microsporidian	07Loma_Lembiotocia_CHSE	*Loma embiotocia*	CRS (T)	gBlocks	
Parasite	Microsporidian	07Loma_Llingcod_CHSE	*Loma lingcodae*	CRS (T)	gBlocks	
Parasite	Microsporidian	07Loma_Lrichardi_CHSE	*Loma richardi*	CRS (T)	gBlocks	
Parasite	Microsporidian	08Nsp_gblock_CHSE	*Nucleospora salmonis*	T	gBlocks	
Parasite	Microsporidian	08NspAS_gblock_CHSE	*Nucleospora salmonis*	T	gBlocks	
Parasite	Microsporidian	09NUC_Onne2010-2090	*Paranucleospora theridion*	T	Fish tissue	Sockeye salmon
Parasite	Microsporidian	09NUC_Onne2010-2090_old	*Paranucleospora theridion*	T	Fish tissue	Sockeye salmon
Parasite	Myxozoan	10Cs_K19#3	*Ceratomyxa shasta*	T	gut tissue	Rainbow trout
Parasite	Myxozoan	10Cs_positive	*Ceratomyxa shasta*	T	Fish tissue	Rainbow trout
Parasite	Myxozoan	11M_fryeri	*Myxobolus fryeri*	CRS for 12, 14	Parasite spores	
Parasite	Myxozoan	11M_squamalis	*Myxobolus squamalis*	CRS for 12, 14	Parasite spores	
Parasite	Myxozoan	11M_arcticus	*Myxobolus arcticus*	T for12; CRS for 14	Fish tissue	Unknown
Parasite	Myxozoan	12MA_Mfry_CHSE	*Myxobolus fryeri* EU346370	CRS	gBlocks	
Parasite	Myxozoan	12MA_Minsid_CHSE	*Myxobolus insidiosus*	CRS	gBlocks	
Parasite	Myxozoan	12MA_Mkis_CHSE	*Myxobolus kisutchi* AB469988	CRS	gBlocks	
Parasite	Myxozoan	12MA_Mneurobius_CHSE	*Myxobolus neurobius* AB469987	CRS	gBlocks	
Parasite	Myxozoan	12MA_Mneurotropus_CHSE	*Myxobolus neurotropus* DQ84666.1	CRS	gBlocks	
Parasite	Myxozoan	12MA_Msquam_CHSE	*Myxobolus squamalis*	CRS	gBlocks	
Parasite	Myxozoan	13Myx-SP_gblock_CHSE	*Myxobolus cerebralis*	T	gBlocks	
Parasite	Myxozoan	11M_cerebralis	*Myxobolus cerebralis*	T for 13	Parasite spores	
Parasite	Myxozoan	14Mi2_Minisid_CHSE	*Myxobolus insidiosus*	T	gBlocks	
Parasite	Myxozoan	11M_insidiosus	*Myxobolus insidiosus*	T for 14; CRS for 12	Parasite spores	
Parasite	Myxozoan	14Mi2_Mfry_CHSE	*Myxobolus fryeri* EU346370	CRS	gBlocks	
Parasite	Myxozoan	14Mi2_Mkis_CHSE	*Myxobolus kisutchi* AB469988	CRS	gBlocks	
Parasite	Myxozoan	14Mi2_Mneurobius_CHSE	*Myxobolus neurobius* AB469987	CRS	gBlocks	
Parasite	Myxozoan	14Mi2_Mneurotropus_CHSE	*Myxobolus neurotropus* DQ84666.1	CRS	gBlocks	
Parasite	Myxozoan	14Mi2_Msquam_CHSE	*Myxobolus squamalis*	CRS	gBlocks	
Parasite	Myxozoan	15Pkab2_gblock_CHSE	*Parvicapsula kabatai*	T	gBlocks	
Parasite	Myxozoan	15Pkab2_positive	*Parvicapsula kabatai*	T	Fish tissue	Unknown
Parasite	Myxozoan	15Pkab2_Pspinach_CHSE	*Parvicapsula spinachiae*	CRS	gBlocks	
Parasite	Myxozoan	16Pm_dup_positive	*Parvicapsula minibicornis*	T	Fish tissue	Unknown
Parasite	Myxozoan	17Parvi2_OntsSE9682	*Parvicapsula pseudobranchicola*	T	Fish tissue	Chinook salmon
Parasite	Myxozoan	17Parvi2_OntsSE9682_old	*Parvicapsula pseudobranchicola*	T	Fish tissue	Chinook salmon
Parasite	Myxozoan	18PKDtaq_positive	*Tetracapsuloides bryosalmonae*	T	Fish tissue	Unknown
Parasite	Myxozoan	23Kud_18S_positive	*Kudoa thyrsites*	T	Fish tissue	Unknown
Parasite	Myxozoan	23Kud_18S_inicapsula_CHSE	*Kudoa inicapsula*	CRS (T)	gBlocks	
Parasite	Myxozoan	23Kud_18S_miniauriculata_CHSE	*Kudoa miniauriculata*	CRS (T)	gBlocks	
Parasite	Myxozoan	23Kud_18S_neurophila_CHSE	*Kudoa neurophila*	CRS (T)	gBlocks	
Parasite	Myxozoan	23Kud_18S_thalassomi_CHSE	*Kudoa thalassomi*	CRS (T)	gBlocks	
Parasite	Protozoan	19Dsal_gblock_CHSE	*Dermocystidium salmonis*	T	gBlocks	
Parasite	Protozoan	19DsalAS_gblock_CHSE	*Dermocystidium salmonis*	T	gBlocks	
Parasite	Protozoan	19Dsal_Aparasiticum_CHSE	*Amoebidium parasiticum* AF2740	CRS	gBlocks	
Parasite	Protozoan	19Dsal_Sphaer_CHSE	*Sphaerothecum destructens*	CRS	gBlocks	
Parasite	Protozoan	20White_Ich_OntsSE9870	*Ichthyophonus hoferi*	T	Fish tissue	Chinook salmon
Parasite	Protozoan	20White_Ich_OntsSE9870_old	*Ichthyophonus hoferi*	T	Fish tissue	Chinook salmon
Parasite	Protozoan	20White_Ich_Anurofeca_CHSE	*Anurofeca richardsi* AF070445.1	CRS	gBlocks	
Parasite	Protozoan	20White_Ich_Dgrandis_CHSE	*Diaphanoeca grandis* DQ059033.1	CRS	gBlocks	
Parasite	Protozoan	20White_Ich_Dpercae_CHSE	*Dermocystidium percae*	CRS	gBlocks	
Parasite	Protozoan	20White_Ich_Ich-irreg_CHSE	*Ichthyophonus irregularis* AF2323	CRS (T)	gBlocks	
Parasite	Protozoan	20White_Ich_Pseud-tap_CHSE	*Pseudoperkinsus tapetis* AF192386.1	CRS	gBlocks	
Parasite	Protozoan	21Sphaer2_M1069	*Sphaerothecum destructens*	T	Fish tissue	Chinook salmon
Parasite	Protozoan	21Sphaer2_M1069_old	*Sphaerothecum destructens*	T	Fish tissue	Chinook salmon
Parasite	Protozoan	21Sphaer2_gblock_CHSE	*Sphaerothecum destructens*	T	gBlocks	
Parasite	Protozoan	21Sphaer2_Anurofeca_CHSE	*Anurofeca richardsi* AF070445.1	CRS	gBlocks	
Parasite	Protozoan	21Sphaer2_Aparasiticum_CHSE	*Amoebidium parasiticum* AF2740	CRS	gBlocks	
Parasite	Protozoan	21Sphaer2_Demo-salmonis_CHSE	*Dermocystidium salmonis*	CRS	gBlocks	
Parasite	Protozoan	21Sphaer2_Dgrandis_CHSE	*Diaphanoeca grandis* DQ059033.1	CRS	gBlocks	
Parasite	Protozoan	21Sphaer2_Dpercae_CHSE	*Dermocystidium percae*	CRS	gBlocks	
Parasite	Protozoan	21Sphaer2_Ich-hof_CHSE	*Ichthyophonus hoferi*	CRS	gBlocks	
Parasite	Protozoan	21Sphaer2_Ich-irreg_CHSE	*Ichthyophonus irregularis* AF2323	CRS	gBlocks	
Parasite	Protozoan	21Sphaer2_Pseud-tap_CHSE	*Pseudoperkinsus tapetis* AF192386.1	CRS	gBlocks	
Parasite	Protozoan	22Csal_CYS4_1072	*Cryptobia salmositica*	T	Fish tissue	Chinook salmon
Parasite	Protozoan	22Csal_CYS4_1072_old	*Cryptobia salmositica*	T	Fish tissue	Chinook salmon
Parasite	Protozoan	22Csal_CYS4_gblock_CHSE	*Cryptobia salmositica*	T	gBlocks	

Table 3. Overview of main results of all studies to provide a synthesis of the performance of each assay on the BioMark. While efficiencies and R^2 are only presented herein, most of the remaining columns are redundant with the detailed tables for each study. Measures deviating from ideal ranges (efficiency 0.9-1.1, R^2 > 0.98, repeatability pass-fail and CCC > 0.90 for Part 1 and 2, >95 for Part 3, consistency > 0.85) are highlighted in light grey or if extreme in dark grey. Outlier values that are considered inaccurate are highlighted in grey. Assays were rated qualitatively by the number and strength of unwanted variance. The last column summarizes the key deficiencies in each assay, if any exist.

Microbe full name	Assay	Efficiency				R^2			Analytical Sensitivity (LOD)			Analytical Specificity		Repeatability					STA impact	Comparability between Platforms			Rating	Delinquencies
		APC Initial Dilution	APC Multiple Dilutions	APC Range	Real Sample	Initial Dilution	Multiple Dilutions	Real Sample	Estimated Copy Number Starting Material		CT of last dilution with >95% detection	STA / Closely-related species		Pass-Fail agreement all samples Part 1	CCC all samples Part 1	N positive real samples	Pass-Fail different STAs Part 2	High CT variation	Sensitivity	N	CT Variance Real Samples	CCC		
		BioMark							STA	non STA	STA	Inclusivity	Exclusivity	Repeatability Study						Comparability Study				
Aeromonas hydrophila	ae_hyd	0.97	1.03	1.01-1.06	0.98	0.99	1.00	0.95	2.0	1094	28.7	100[e]	99.7	0.995	0.981	0							Good[c]	slightly low R^2 for real sample, suspect <100% ASe from *in silico* analysis
Aeromonas salmonicida	ae_sal	1.19	1.09	1-1.21	1.06	0.99	0.98	1.00	31.4	587	26.5	100	98.9[g]	0.934	0.882	85	0.935	Yes		54	6.257	0.901	Good	poor curve quality slightly enhances high Ct false positives
Atlantic salmon paramyxovirus	aspv	0.97	0.93	0.85-0.98	0.96	1.00	0.99	1.00	30.1	600	26.4	100	100	1	0.994	0							Excellent[c]	
Candidatus Branchiomonas cysticola	c_b_cys	0.99	1.06	0.99-1.16	0.87	1.00	0.99	1.00	33.0	4069	26.9	100[f]	100	0.889	0.886	272	0.933			80	9.251	0.813	Good	poor curve quality enhances high Ct false positives
Ceratomyxa shasta	ce_sha	1.07	1.01	0.98-1.03	0.92	1.00	0.99	0.99	8.6	2146	28.2	100	100	0.939	0.866	185	0.992	Yes		84	8.141	0.867	Good	repeatability and comparability low as reduced efficiency at high Ct
Dermocystidium salmonis	de_sal	0.99	0.97	0.91-1.02	0.95	1.00	0.99	1.00	3.9	4896	27.5	100[f]	98.5	0.974	0.981	38	0.985			20	11.54	0.998	Excellent	
Facilispora margolisi	fa_mar	0.92	1.14	1.13-1.15	0.80	0.97	0.99	0.95	2.1	1049	29.1	100	99.4	0.99	0.945	0			Yes				Good[d]	efficiency variation
Flavobacterium psychrophilum	fl_psy	0.83	0.87	0.85-0.9	1.07	1.00	1.00	0.96	14.7	829	28.3	100[e]	100	0.934	0.92	210	0.950			80	10.172	0.657	Adequate	low efficiency, CCC comparability low (most samples high Ct), suspect <100% Ase from *in silico* analysis
Gill chlamydia (Sch)	sch	0.95	N/A	N/A	0.90	0.99	N/A	0.97	4.1	127	27.7	100[f]	100	0.957	0.927	6	0.971	Yes		8	8.004	0.865	Very Good	high Ct variance but good repeatability overall
Gyrodactylus salaris	gy_sal	0.98	1.03	1.02-1.07	0.99	1.00	1.00	1.00	2.0	1073	26.3	100	100	1	0.967	0							Excellent[c]	
Ichthyophonus hoferi (Ichthyophonus Spp)	ic_hof	1.10	1.07	1.02-1.1	N/A	1.00	0.99	N/A	1.1	754	25.4	100	93.9 (94.9)	0.901	0.966	46	0.925			39	12.46	0.948	Good	genus-specific, STA variation in repeatability, weak detection CRS
Ichthyophthirius multifiliis	ic_mul	1.05	0.97	0.9-1.02	N/A	0.99	0.99	N/A	3.9	488	25.2	100	96.3	0.938	0.899	188	0.902			166	11.203	0.984	Very Good	false positives at high Ct
Infectious hematopoietic necrosis virus	ihnv	0.88	0.93	0.87-0.98	0.90	1.00	1.00	1.00	31.2	1033	27.9	100	98.4	0.955	0.966	42	0.960		Yes	42	10.81	0.997	Very Good	modest STA impact
Infectious pancreatic necrosis virus	ipnv	0.92	0.97	0.92-1.06	1.07	1.00	0.99	0.93	30.3	2293	27.7	77.8	100	1	0.977	0							Good[c]	2 strains not detected
Infectious salmon anemia virus	isav7, snow7*	0.97	1.13*	0.99-1.25*	0.83	0.99	0.96	0.92	4.7/34.2*	2243	27.9/27.6*	87.5	98.4	0.955	0.856	0							Replace[c]	isav7 assay had poor R^2, does not detect NA strains, low CCC repeatability; replace with snow7 assay
Infectious salmon anemia virus	isav8	1.09	1.00*	0.89-1.13	0.74	0.99	0.98	0.96	31.1	1324	21.3	100	100	1	0.953	0							Very Good[c]	low efficiency on ABI
Kudoa thyrsites (Kudoa Spp)	ku_thy	0.93	0.96	0.88-1.04	0.89	1.00	0.99	1.00	33.5	2808	26.2	100	96.0 (100)	0.97	0.902	128	0.990			70	10.643	0.981	Very Good	genus-specific assay
Loma salmonae (Loma Spp)	lo_sal	1.03	0.95	0.89-1	0.92	1.00	0.99	0.98	4.0	1315	26.1	100	95.6 (98.2)	0.96	0.963	48	0.958			34	10.463	0.94	Very Good	genus-specific assay
Myxobolus arcticus	my_arc	0.85	0.89	0.86-0.95	0.86	0.99	1.00	0.98	7.7	4091	26.9	100	99.2	0.92	0.927	41	0.967			33	13.329	0.952	Good	low efficiency, weak detection CRS, some poor curve quality
Myxobolus cerebralis	my_cer	1.02	1.01	0.94-1.07	N/A	0.96	1.00	N/A	8.4	72	26.4	100	100	1	0.984	0							Very Good[c]	
Myxobolus insidiosus	my_ins	0.99	0.96	0.87-1.02	1.03	0.99	0.99	0.93	2.1	931	26.6	100	97.6	0.997	0.982	0							Good[c]	cross-detection of a few CRS
Nanophyetus salmincola	na_sal	1.04	0.97	0.92-1.01	N/A	0.99	0.99	N/A	2.3	603	25.8	100	100	0.948	0.968	2	0.944						Very Good[c]	
Neoparamoeba perurans	ne_per	1.07	1.06	0.91-1.24	0.92	0.99	0.99	1.00	39.2	1402	26.9	100	100	1	0.988	0							Excellent[c]	
Nucleospora salmonis	nu_sal	1.06	1.06	0.99-1.1	1.02	0.99	1.00	0.91	0.9	889	25.3	100	100	0.998	0.982	0							Good[c]	low ABI efficiency
Pacific salmon parvovirus	pspv	0.97	0.96	0.93-0.98	0.99	1.00	1.00	0.96	7.6	683	27.0	100[f]	99.2	0.962	0.98	23	0.981			28	11.528	0.909	Excellent	
Paranucleospora theridion	pa_ther	0.91	0.95	0.88-0.99	N/A	1.00	0.99	0.98	7.7	1544	28.6	100	100	0.96	0.909	88	0.977				10.94	0.765	Very Good	relatively low comparability CCC not significant
Parvicapsula kabatai	pa_kab	0.90	0.95	0.91-0.99	N/A	1.00	1.00	N/A	8.5	24622[a]	27.0	100	100	0.986	0.986	2	0.983						Very Good[d]	
Parvicapsula minibicornis	pa_min	0.87	0.95	0.9-0.99	0.85	1.00	1.00	0.88	8.6	3198	28.7	100	100	0.924	0.978	146	0.948			72	9.657	0.948	Very Good	slightly low repeatability due to STA variation
Parvicapsula pseudobranchicola	pa_pse	0.93	1.01	0.91-1.23	0.89	1.00	0.97	0.96	30.5	977	25.7	100	100	0.97	0.725	60	0.983	Yes		64	7.989	0.488	Adequate	poor ABI performance, poor CCC in repeatability Part 1 due to high Ct variance
Piscichlamydia salmonis	pch_sal	0.81	0.98	0.92-1.09	N/A	0.98	0.96	1.00	3.8	54115[a]	29.2	100	100	0.981	0.897	7	0.983	Yes					Good[c]	high Ct variance
Piscine myocarditis virus	pmcv	0.98	0.97*	0.92-0.99*	0.97	0.99	1.00	1.00	2.2/1.0*	1163	26.3/25.6*	100[f]	98.9	0.917	0.979	0							Replace[c]	poor curve quality enhances high Ct false positives; replace with pmcv1 assay
Piscine reovirus	prv	1.04	1.00	0.96-1.05	0.86	1.00	1.00	0.98	0.5	427	25.4	100[f]	100	0.97	0.976	8	0.979						Very Good[c]	slight reduction in efficiency for real sample
Piscirickettsia salmonis	pisck_sal	0.94	0.98	0.92-1.03	1.05	0.99	1.00	0.96	7.4	1976	25.6	100	100	0.997	0.956	0							Excellent[c]	
Renibacterium salmoninarum	re_sal	1.00	0.97	0.91-1.02	0.86	1.00	0.99	1.00	8.2	743	26.0	100	99.6	1	0.984	0							Excellent[c]	
Salmon alphavirus 1, 2, and 3	sav	0.88	0.98	0.94-1.07	N/A	1.00	0.99	1.00	31.0	1960	25.6	100	100	1	0.979	0							Good[f]	enhanced variance at high Ct in real sample
Salmonid herpesvirus	omv	0.98	0.98	0.93-1.04	1.00	1.00	0.99	0.99	8.4	33879[a]	27.4	100	100	0.995	0.987	0							Very Good[c]	
Sphaerothecum destructuens	sp_des	0.92	0.95	0.88-1.01	0.89	1.00	1.00	0.99	4.3	10348[a]	27.0	100	98.5	0.964	0.929	19	0.981			7	12.164	0.99	Excellent	
Spironucleus salmonicida	sp_sal	0.94	0.89	0.84-0.94	N/A	1.00	0.99	N/A	8.4	62479[a]	29.0	100	99.9	0.99	0.943	0			Yes				Good[c]	STA impact
Strawberry disease	rlo	0.96	1.02	0.95-1.09	1.05	1.00	0.99	0.97	31.8	468	26.5	100	100	0.964	0.986	70	0.967			28	12.417	0.979	Excellent	
Tetracapsuloides bryosalmonae	te_bry	1.00	1.08	1.05-1.12	0.97	0.99	1.00	0.99	2.4	1332	25.4	100	100	0.953	0.971	56	0.964			32	14.983	0.879	Excellent	
Cryptobia salmonistica	cr_sal	1.03	1.09	1-1.16	0.87	1.00	0.99	0.97	3.8	549	27.3	100	100	0.931	0.95	33	0.927			19	9.291	0.902	Very Good	
Vibrio anguillarum	vi_ang	1.07	1.03	0.92-1.11	1.01	0.99	0.99	0.99	2.2	3098	26.5	100	100[g]	1	0.985	0							Excellent[c]	
Vibrio salmonicida	vi_sal	1.07	1.02	0.9-1.09	0.92	1.00	0.99	0.99	29.8	7909	25.7	100[f]	100[g]	1	0.987	0							Excellent[c]	
Viral encephalopathy and retinopathy virus	ver	1.01	1.02	0.99-1.04	0.94	1.00	1.00	1.00	7.6	1738	26.4	100	99.4	0.997	0.983	2							Excellent[d]	
Viral erythrocytic necrosis virus	ven	1.08	0.98	0.91-1.02	0.98	0.99	0.99	1.00	8.6	1206	25.4	100[f]	97.9	0.941	0.948	44	0.941			26	10.903	0.988	Very Good	minor curve quality issues impact high Ct repeatability
Viral hemorrhagic septicemia virus	vhsv	0.84	0.97*	0.91-1.02*	0.78	1.00	0.99	0.96	17.6/31.4*	3546	31.8/25.5*	100	99.7	0.986	0.978	0			Yes				Replace[c]	high background (difficult to score), STA impact; replaced with vhsv1 assay
Yersinia ruckeri	ye_ruc	0.85	N/A	N/A	N/A	0.99	N/A	N/A	30.1*	7698	28.9	100	100	0.99	0.89	0			Yes				Poor[c]	poor curve quality, difficulty scoring, low efficiency, high LOD, STA impact

a. unreliable estimate as too few samples
b. poor ABI curve and difficult scoring
c. no repeatability/comparability study
d. no comparability study
e. in silico analysis suggests that not all strains may be detected
f. Limited sequence data on strain variation may impact ASp estimates
g. limited sequence data on closely-related species may impact Ase estimates

Table 4. Efficiency calculations for assays on the BioMark and ABI 7900. Note that the values shown are only for the initial dilution series, as multiple dilution series of the Non-STA were not analyzed on the BioMark, nor were they analyzed on the ABI 7900.Efficiency calculations for assays on the BioMark and ABI 7900.

	BioMark STA				BioMark Non-STA				ABI 7900			
Microbe	**Efficiency**	**Slope**	**Y-Intercept**	**R^2**	**Efficiency**	**Slope**	**Y-Intercept**	**R^2**	**Efficiency**	**Slope**	**Y-Intercept**	**R^2**
ae_hyd	0.97	-3.40	28.57	0.99	0.92	-3.54	30.54	1.00	0.95	-3.46	39.29	1.00
ae_sal	1.19	-2.93	26.67	0.99	1.53	-2.48	32.20	1.00	0.96	-3.42	38.15	1.00
aspv	0.97	-3.40	28.02	1.00	1.01	-3.29	29.63	1.00	0.94	-3.47	41.84	1.00
c_b_cys	0.99	-3.34	27.91	1.00	1.08	-3.15	30.07	1.00	0.93	-3.50	39.78	1.00
ce_sha	1.07	-3.17	29.82	1.00	1.02	-3.28	35.30	1.00	0.97	-3.39	38.78	1.00
cr_sal	1.03	-3.25	26.38	1.00	1.10	-3.09	28.94	1.00	1.00	-3.33	37.59	1.00
de_sal	0.99	-3.34	26.32	1.00	0.95	-3.44	28.28	1.00	0.96	-3.42	38.14	1.00
fa_mar	0.92	-3.52	31.61	0.97	0.95	-3.45	33.82	0.99	0.91	-3.55	41.01	1.00
fl_psy	0.83	-3.80	32.38	1.00	0.89	-3.62	32.11	1.00	0.97	-3.41	41.47	0.99
gy_sal	0.98	-3.36	28.09	1.00	0.94	-3.47	31.44	1.00	0.96	-3.43	39.99	1.00
ic_hof	1.10	-3.11	25.98	1.00	1.01	-3.29	29.85	1.00	0.98	-3.37	37.82	1.00
ic_mul	1.05	-3.21	25.64	0.99	0.99	-3.34	28.94	1.00	0.80	-3.90	42.46	0.97
ihnv	0.88	-3.66	31.18	1.00	1.01	-3.29	32.45	1.00	0.91	-3.56	42.88	1.00
ipnv	0.92	-3.54	29.35	1.00	0.98	-3.38	30.64	1.00	0.89	-3.61	41.13	1.00
isav7	0.97	-3.39	28.76	0.99	1.02	-3.27	30.57	1.00	1.05	-3.21	40.68	0.94
isav8	1.09	-3.12	25.51	0.99	1.25	-2.85	28.95	1.00	0.80	-3.90	41.82	1.00
ku_thy	0.93	-3.50	27.52	1.00	1.00	-3.33	28.44	1.00	0.95	-3.45	38.64	1.00
lo_sal	1.03	-3.26	26.10	1.00	0.96	-3.42	28.58	1.00	0.96	-3.43	39.14	1.00
my_arc	0.85	-3.73	30.15	0.99	0.97	-3.40	31.25	0.99	0.86	-3.70	41.99	1.00
my_cer	1.02	-3.28	25.83	0.96	0.98	-3.37	28.19	1.00	1.01	-3.31	38.43	1.00
my_ins	0.99	-3.34	26.88	0.99	1.01	-3.31	29.42	1.00	0.90	-3.57	40.07	1.00
na_sal	1.04	-3.22	25.60	0.99	0.95	-3.45	28.17	1.00	0.88	-3.66	40.59	1.00
ne_per	1.07	-3.16	26.61	0.99	0.94	-3.48	30.33	1.00	0.91	-3.56	40.17	0.99
nu_sal	1.06	-3.18	25.13	0.99	1.04	-3.24	26.91	1.00	0.82	-3.83	41.82	0.99
omv	0.98	-3.38	28.97	1.00	1.12	-3.06	31.69	1.00	0.87	-3.68	42.81	0.99
pa_kab	0.90	-3.60	29.49	1.00	0.96	-3.42	30.45	1.00	0.88	-3.64	41.21	0.99
pa_min	0.87	-3.68	31.70	1.00	1.01	-3.30	32.32	0.99	0.91	-3.55	42.63	1.00
pa_pse	0.93	-3.50	27.95	1.00	1.22	-2.89	28.81	1.00	0.87	-3.67	44.01	0.99
pa_ther	0.91	-3.55	31.22	1.00	0.95	-3.45	33.80	1.00	0.94	-3.48	42.75	0.99
pch_sal	0.81	-3.87	35.09	0.98	2.21	-1.97	37.19	0.99	0.88	-3.64	43.43	1.00
pisck_sal	0.94	-3.48	26.02	0.99	1.00	-3.33	27.16	1.00	0.94	-3.46	38.52	1.00
pmcv	0.98	-3.37	27.91	0.99	1.24	-2.85	30.72	1.00	0.90	-3.60	42.55	0.99
prv	1.04	-3.22	25.04	1.00	0.99	-3.34	26.89	1.00	1.04	-3.22	39.71	0.99
pspv	0.97	-3.39	27.95	1.00	0.96	-3.43	30.14	1.00	0.95	-3.44	40.54	1.00
re_sal	1.00	-3.31	26.68	1.00	0.96	-3.42	28.86	1.00	0.98	-3.37	38.34	1.00
rlo	0.96	-3.42	26.84	1.00	0.99	-3.35	28.48	1.00	0.92	-3.54	40.24	1.00
sav	0.88	-3.65	29.79	1.00	0.99	-3.35	29.68	1.00	0.90	-3.59	42.46	0.99
sch	0.95	-3.45	30.37	0.99	0.88	-3.66	33.51	1.00	0.92	-3.54	41.61	1.00
sp_des	0.92	-3.52	28.77	1.00	0.96	-3.41	30.16	1.00	0.95	-3.45	39.97	0.99
sp_sal	0.94	-3.47	29.11	1.00	1.03	-3.26	31.37	0.99	0.99	-3.35	42.00	0.97
te_bry	1.00	-3.32	25.78	0.99	0.97	-3.39	27.71	1.00	0.98	-3.38	38.56	1.00
ven	1.08	-3.15	25.65	0.99	0.97	-3.40	29.11	1.00	0.92	-3.54	39.69	1.00
ver	1.01	-3.30	28.32	1.00	1.06	-3.19	31.05	1.00	0.94	-3.48	41.38	1.00
vhsv	0.84	-3.76	31.31	1.00	1.14	-3.03	30.64	1.00	0.87	-3.67	44.72	0.99
vi_ang	1.07	-3.17	27.25	0.99	1.02	-3.28	30.96	1.00	0.95	-3.46	39.74	1.00
vi_sal	1.07	-3.18	26.20	1.00	1.00	-3.32	30.64	1.00	0.92	-3.54	39.68	1.00
ye_ruc	0.85	-3.73	31.95	0.99	1.58	-2.43	35.45	0.99	0.90	-3.58	40.73	1.00

Table 5. Results of Analytical Specificity testing for viruses. The sensitivity results are reported in terms of the STA and (Non-STA) replicates and ^ indicates when this difference was over all replicates within a sample and likely due to low load in the viral control samples (e.g. STA Ct >16). New sensitivity calculations ($) are displayed for both isav7 and isav8 where subsequent dilution series were run. All of the Non-STA isav8 sample replicates amplified once diluted, indicating sensitivity to inhibition in this assay. In the first specificity analysis, each viral assay was run against 48 samples STA and Non-STA, in replicates of 6 for a total of 576. In the second specificity analysis, each viral assay was run against 85 unique samples (STA only) each with 2 replicates, for a total of 170, which included parasites, viruses and bacteria for tissue and pure positive controls only (no gBlocks). The positive controls for each virus generally cover the full range of known sequence variation at the genogroup-level (see Supplemental Table 3). The samples responsible for specificity variation within an assay and those with assay-specific NGS confirmed co-infections, were classified in terms of their Ct as less than the assay-specific Ct-cutpoint (<LOD) or greater than the assay-specific Ct-cutpoint (>LOD). Two qPCR positive samples in the Specificity Variance >LOD row are indicated with a (*) as they both contained NGS reads below the applied cutoff of 30. Not including controls, 28,500 q-PCR sample reactions were conducted for viral analytical specificity testing.

	Viral hemorrhagic septicemia virus	Infectious haematopoietic necrosis virus	Salmon alphavirus 1, 2, and 3	Piscine Myocarditis Virus	Atlantic salmon paramyxovirus	Infectious salmon anemia virus	Infectious salmon anemia	Viral encephalopathy and retinopathy virus	Infectious pancreatic necrosis virus	Viral erythrocytic necrosis virus	Piscine reovirus	Pacific salmon parvovirus (MGL)	*Oncorhynchus masou* virus (MGL)
Assay	VHSV Rhabdovirus	IHNV Rhabdovirus	SAV Togaviridae	PMCV Totiviridae	ASPV Paramyxoviridae	ISAV7 Orthomyxoviridae	ISAV8 Orthomyxoviridae	VER Nodaviridae	IPNV Birnaviridae	VEN Iridoviridae	PRV Reoviridae	PSPV Parvoviridae	OMV Herpesviridae
Closely-related species	IHNV	VHSV	None	None	None	None	None	None	None	IPNV	None	None	None
In silico analysis						Sequence variation over this assay on 45% of available sequences (n=76)			Poor alignment to group II (3 mismatches on primers and 5 on probe) and VI (1-2 mismatches on primers and 3 on probe)				
Positive controls	6 genogroups in culture (IVa, IVb) and 4 gblocks (Ib, IIIa, IIIb, IV)	2 genogroups in culture (U, M) and 1 in BC tissue (L)	6 genogroups in culture and 1 gblock	1 gblock	1 gblock	8 cultured genogroups (NA-HPR4 and 21, EU-HPR 2, 3, 5, 7b, 14 and new)	8 cultured genogroups (NA-HPR4 and 21, EU-HPR 2, 3, 5, 7b, 14 and new)	6 genogroups in gblocks	5 genogroups in culture (I-Jasper and VR299, IV-Canada1, V-Canada2 and Canada3) and 4 plasmids (II-Ab, III-Sp, VI-Hecht, VII-Japan)	1 tissue sample from herring	2 tissue (BC) and 1 gblock sample	2 genogroups in tissue	1 cultured sample from Japan
Sensitivity-STA (Non-STA)	100.0% (97.2%)	100.0% (94.4%)	100.0% (97.6%)	100.0% (83.3%)	100.0% (100.0%)	100.0%$ (75.0%)^	100.0%$ (100.0%)	100.0% (94.4%)	77.8% (77.8%)	100.0% (100.0%)	100.0% (94.4%)	100.0% (91.7%)	100.0% (83.3%)
Specificity-Analysis 1	100.0%	100.0%	100.0%	98.9%	100.0%	76.0%	100.0%	100.0%	100.0%	98.2%	100.0%	99.1%	100.0%
Specificity-Analysis 2	98.8%	93.9%	100.0%	N/A	100.0%	98.1%	100.0%	98.8%	100.0%	96.4%	100.0%	98.8%	100.0%
Specificity-Overall	99.7%	98.4%	100.0%	N/A	100.0%	98.4%	100.0%	99.7%	100.0%	97.9%	100.0%	99.2%	100.0%
LOD's	31.8	27.9	25.6	26.3	26.4	27.9	21.2	26.4	27.7	25.4	25.4	27	27.4
Specificity Variance <LOD	07Loma_positive	07Loma_positive				07Loma_positive				11M_cerebralis		ISA-HPR7b cell culture	
Specificity Variance >LOD		04SpiroSal_salmonicida, 10Cs_positive*, 11M_arcticus, 15Pkab2_positive, 17Parvi2_OntsSE9682		SAV_1_2_3 nSP_gblock		10Cs_K19#3*, IHNV_M, VER gp6_Cp_gblock, VHSV type IIIb_N_gblock, VHSV_IVb_KG		IPNV_VR299		ISAV_E_HPR3, PRV_L1_gblock, SAV-4_DG			
Confirmed Co-infections <LOD												11M_arcticus, 16Pm_dup_positive	
Confirmed Co-infections >LOD										Parvo_liver3065			
CRS amplifies in VIC but not FAM, indicating specificity of probe important for species specificity													

Table 6. Results of analytical specificity testing for bacteria. The sensitivity results are reported in terms of the STA and (Non-STA) replicates and ^ indicates when this difference was over all replicates within a sample and likely due to low load in the bacterial control samples (e.g. STA Ct >16). In the first specificity analysis, each bacterial assay was run against a total of 480 bacterial control samples (STA and Non-STA of 40 samples in replicates of 6). In the second specificity analysis, each bacterial assay was run against a total of 170 control samples (STA only for 2 replicates of 85 samples) which included parasites, viruses and bacteria for tissue and pure positive controls only (no gBlocks). For the most part, bacterial assays were designed to be identical, or nearly identical to all known sequence variants, reducing the need for the testing of multiple variants within a species. Closely-related species (homology >90%) to most bacterial species assays were included in testing. The samples responsible for specificity variation within an assay and those with assay-specific NGS confirmed co-infections, were classified in terms of their Ct as less than the assay-specific Ct-cutpoint (<LOD) or greater than the assay-specific Ct-cutpoint (>LOD). Not including controls, 8,796 q-PCR sample reactions were conducted for bacterial analytical specificity testing. Genus and species names are not italicized to enhance readability.

Assay	Aeromonas hydrophila Ae_hyd	Aeromonas salmonicida Ae_sal	Piscirickettsia salmonis Pisck_sal	Vibrio anguillarum (MGL) Vi_ang	Vibrio salmonicida (MGL) Vi_sal	Yersinia ruckeri Ye_ruc	Strawberry disease RLO	Candidatus Branchiomonus cysticola c_b_cys	Flavobacterium psychrophilum Fl_psy	Piscichlamydia salmonis Pch_sal	Gill chlamydia (Sch) Sch	Renibacterium salmoninarum Re_sal
Group	Proteobacteria gamma	Proteobacteria gamma	Proteobacteria gamma	Proteobacteria gamma	Proteobacteria gamma	Proteobacteria gamma	Proteobacteria alpha	Proteobacteria beta	CFB group	Chlamydiae/ Verrucomicrobia group	Chlamydiae/ Verrucomicrobia group	gram pos Actinobacteria
Closely-related species	A. salmonicida (2), A. sobria pure culture	A. hydrophila gblock, A. sobria pure culture	None	Bacterial cultures containing 16 (14) Vibrio spp.[a] and 1 nucleic acid (DNA) sample to the genome of Alivibrio salmonicida-ATCC_43839	bacterial cultures to Vibrio anguillarum (5) and other Vibrio spp. (14)	None	None	None	F. columnare (gBlock), F. branchiophilum (gBlock)	Chlamydiales sp. gp1 (gBlock), Sch	P. salmonis, Chlamydiales sp. gp1 and gp2 (gBlocks)	None
In silico analysis	Mismatches (11-13) in all primers/probe of Ahyd aha1 assay to Ae_sal sequences; not inclusive of all A. hydrophila strains in GenBank--up to 7 mismatches in F primer	Limited; Only 2 vapA sequences for Ae_hyd and these would not align with the Ae_sal vapA assay primers/probe							6-7 probe mismatches to other Flavobacterium spp.	4-8 mismatches in F/R primers and 4-9 in probe for CRS		
Positive controls	1 gblock (aha1)	4 strain cultures (atypical-BC, atypical-Yukon, typical-BC, and other-BC)	2 tissue cultures	5 bacterial cultures	1 purified nucleic acid (ATCC_43839)	1 bacterial culture	1 tissue from BC	1 tissue from Norway	1 purified nucleic acid (ATCC_49511D)	1 tissue from Norway	1 gblock, 1 tissue from BC	1 tissue from BC
Sensitivity	100.0% (100.0%)	100.0% (100.0%)	100.0% (66.7%)	100.0% (100.0%)	100.0% (100.0%)	100.0% (100.0%)	100.0% (0.0%)	100.0% (0.0%)	100.0% (100.0%)	100.0% (50.0%)	100.0% (50.0%)^	100.0% (100.0%)
Specificity-Analysis 1	100.0%	98.6%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Specificity-Analysis 2	98.8%	98.8%	100.0%	100.0%	100.0%	N/A	100.0%	100.0%	100.0%	100.0%	100.0%	98.8%
Specificity-Overall	99.7%	98.9%	100.0%	100.0%	100.0%	N/A	100.0%	100.0%	100.0%	100.0%	100.0%	99.6%
LOD's	28.7	26.5	25.6	26.5	25.7	28.9	26.5	26.9	28.3	29.2	27.7	26
Specificity Variance <LOD												
Specificity Variance >LOD	11M_cerebralis	A.sob_FH										ISAV_NA_HPR4
Confirmed Co-infections <LOD		21Sphaer2_M1069						RLO_OntsSE838, Sch_Onne2010_3223, PRV_OnkiAKT2012_3112, 05Nanosal_OntsSE1198, 09NUC_Onne2010-2090, 17Parvi2_OntsSE9682, 20White_Ich_OntsSE9870, 21Sphaer2_M1069, 22Csal_CYS4_1072	Bcy2_TMS, PRV_OnkiAKT2012_3112, 10Cs_positive, 11M_cerebralis, 15Pkab2_positive, 18PKDtaq_positive, 22Csal_CYS4_1072, 21Sphaer2_M1069		01Neo_FH	
Confirmed Co-infections >LOD							11M_insidiosus	Pch1_TMS, 02IMF_OntsSE1756, 06Faci3_OntsSE3665	01Neo_FH			
CRS gblock amplifies in VIC but not FAM, indicating specificity of probe important for species specificity									Detection of F. branchicola and F. columnar, STA and non-STA			

[a]14 Vibrio-related cultures were included in the CRS for V. salmonicida and V. anguillarum [V. anguillarum, V. pectenicida (2), V. logei (3 varients), V. splendidus, V. parahaemolyticus, V. splendidus, V. sp QY101, OC25, R-3884, NAP-4, Moritella marina, and Photobacterium sp. SL13], as well as our target Vibrio species [V. anguillarum (5) and V. salmonicida].

Table 7. Results of analytical specificity testing for parasites. The sensitivity results are reported in terms of the STA and (Non-STA) replicates and ^ indicates when this difference was over all replicates within a sample and likely due to low load in the parasite control samples (e.g. STA Ct >16). In the first specificity analysis, each parasite assay was run against a total of 680 parasite control samples (STA and Non-STA of 85 samples in replicates of 4). In the second specificity analysis, each parasite assay was run against a total of 170 control samples (STA only for 2 replicates of 85 samples) which included parasites, viruses and bacteria for tissue and pure positive controls only (no gBlocks). For Loma salmonae, Kudoa thyrcites and Ichthyophonus hoferi, species level and (genus level) specificity calculations are displayed for the first and overall analyses. The samples responsible for specificity variation within an assay and those with assay-specific NGS confirmed co-infections, were classified in terms of their Ct as less than the assay-specific Ct-cutpoint (<LOD) or greater than the assay-specific Ct-cutpoint (>LOD). Assays that were classified as genus-specific in in silico analyses were still measured against specificity to the salmon microbe in question rather than genus-specificity. Not including controls, 17,664 q-PCR sample reactions were conducted for parasite analytical specificity testing. Genus and species names are not italicized to enhance readability.

	Neoparamoeba perurans	Ichthyophthirius multifiliis (MGL)	Gyrodactylus salaris	Spironucleus salmonicida (MGL)	Nanophyetus salminicola (MGL)	Facilispora margolisi (MGL)	Loma salmonae (MGL)	Nucleospora salmonis	Paranucleospora theridion	Kudoa thyrcites	Ceratomyxa shasta	Myxobolus arcticus (MGL)	Myxobolus cerebralis	Myxobolus insidiosus (MGL)	Parvicapsula kabatai (MGL)	Parvicapsula minibicornis	Parvicapsula pseudobranchicola	Tetracapsuloides bryosalmonae	Dermocystidium salmonis (MGL)	Ichthyophonus hoferi	Sphaerothecum destruens (MGL)	Cryptobia salmositica (MGL)
Assay Parasite Family	Ne_per Amoeba	Ic_mul Ciliate	Gy_sal Ecto-worm	Sp_sal Flagellate	Na_sal Fluke	Fa_mar Microsporidian	Lo_sal Microsporidian	Nu_sal Microsporidian	Pa_ther Microsporidian	Ku_thy Microsporidian	Ce_sha Myxozoan	My_arc Myxozoan	My_cer Myxozoan	My_ins Myxozoan	Pa_Kab Myxozoan	Pa_min Myxozoan	Pa_pse Myxozoan	Te_bry Myxozoan	De_sal Protozoan	Ic_hof Protozoan	Sp_des Protozoan	Cr_sal Protozoan
Closely-related species (CRS) identified >90% similarity in silico	None	5 ciliate species (Tetrahymena australis, thermophila, and tropicalis; Glaucoma chattoni, and Lambornella)	None	Spironucleus barkhanaus, S. torisa (80%)	None	None	3 Loma spp. (lingcodae, embiotocia, richardi)	None	None	4 Kudoa species (incapsula, miniauriculata, neurophila, thalassomi)	None	6 Myxobolus spp. (fryeri, kisutchi, neurobias, neurotropis, squamalis, insidiosis)	None	6 Myxobolus spp. (fryeri, kisutchi, neurobias, neurotropis, squamalis, arcticus	Parvicapsula spinachiae (85%)	None	None	None	Amoebidium parasiticum, Sphaerothecum destruens	Ichthyophonus irregularis, Dermocystidium percae, Amoebidium parasiticum, Anurofeca richardsi, Diaphanoeca grandis, Pseudoperkinsus tapetis, Sphaeroforma arctica Ichthyophonus spp.	Dermocystidium salmonis, Ichthyophonus irregularis	Trypanoplasma borreli
In silico analysis							Loma Spp.			Kudoa Spp.												
Positive controls	1 BC tissue, 1 gBlock	2 samples from 1 BC tissue, 1 gBlock	2 samples from 1 gBlock	1 BC tissue, 1 gBlock	2 samples from 1 BC tissue	2 samples from 1 BC tissue	1 BC tissue	2 samples from 1 gBlock	2 samples from 1 BC tissue	1 BC tissue	2 US tissues	1 BC tissue	1 spore sample and 1 gBlock	1 gBlock	1 BC tissue, 1 gBlock	1 BC tissue	2 samples from 1 BC tissue	1 BC tissue	2 samples from 1 gBlock	2 samples from 1 BC tissue	2 samples from one BC tissue, 1 gBlock	2 samples from 1 BC tissue, 1 gBlock
Sensitivity	100.0% (100.0%)	100.0% (100.0%)	100.0% (100.0%)	100.0% (100.0%)	100.0% (12.5%)^	100.0% (100.0%)	100.0% (75.0%)	100.0% (100.0%)	100.0% (100.0%)	100.0% (0.0%)^	100.0% (50.0%)^	100.0% (100.0%)	100.0% (87.5%)	100.0% (100.0%)	100.0% (100.0%)	100.0% (25.0%)	100.0% (12.5%)^	100.0% (100.0%)	100.0% (100.0%)	100.0% (100.0%)	100.0% (100.0%)	100.0% (83.3%)
Specificity-Analysis 1	100.0%	96.2%	100.0%	99.8%	100.0%	99.2%	94.8% (98.1%)	100.0%	100.0%	95.2% (100.0%)	99.7%	99.4%	100.0%	97.1%	100.0%	100.0%	100.0%	100.0%	98.2%	93.1% (94.2%)	98.5%	100.0%
Specificity-Analysis 2	100.0%	97.6%	100.0%	100.0%	100.0%	98.8%	100.0%	100.0%	100.0%	100.0%	100.0%	98.8%	100.0%	98.8%	100.0%	100.0%	100.0%	100.0%	100.0%	96.4%	98.8%	100.0%
Specificity-Overall	100.0%	96.3%	100.0%	99.9%	100.0%	99.4%	95.6% (98.2%)	100.0%	100.0%	96.0% (100.0%)	99.8%	99.2%	100.0%	97.6%	100.0%	100.0%	100.0%	100.0%	98.5%	93.9% (94.9%)	98.5%	100.0%
LOD's	26.9	25.2	26.3	29	25.8	29.1	26.1	25.3	28.6	26.2	28.2	26.9	26.4	26.6	27	28.7	25.7	25.4	27.5	25.4	27	27.3
Specificity Variance <LOD							All Loma Spp.			All Kudoa spp.				11M_fryeri, 14Mi2_Mfry, 14Mi2_Msquam					21Sphaer2_Demo-salmonis	20White_Ich_Ich-irreg, 21Sphaer2_Ich-hof, 21Sphaer2_Ich-irreg PRV_OnkiAKT2012_3112, 02IMF_OntsSE1756, 02IMF Gchattoni, 03Gysa_Gsal, 09NUC_Onne2010-2090, 10Cs_positive, 11M cerebralis, 20White_Ich_Pseud-tap, 22Csal_CYS4_1072	19Dsal_Sphaer	
Specificity Variance >LOD		ISAV_E_HPR2, ISAV_NA_HPR21, 03Gysa Gsal, 05Nanosal_OntsSE1198, 06Faci3, OntsSE3665, 07Loma_Llingcod, 13Myx-SP gblock, 14Mi2 Mkis, 14Mi2_Msquam, 20White_Ich_Anurofeca		14Mi2_Msquam		20White_Ich_Dgrandis, 23Kud_18S_positive	02IMF_OntsSE1756, 04SpiroSal_gblock, 15Pkab2_gblock				21Sphaer2_Demo-salmonis	17Parvi2_OntsSE9682, Vang1_SJ		11M_cerebralis					20White_Ich_OntsSE 9870		15Pkab2_gblock, IHNV_M	
Confirmed Co-infections >LOD		PRV_OnkiAKT2012_3112					Sch_Onne2010_3223, PRV_OnkiAKT2012_3112, 15Pkab2_positive, 18PKDtaq_positive		PRV_MGL_Creat_L_1055, 06Faci3_OntsSE3665, 20White_Ich_OntsSE9870		PRV_OnkiAKT2012_3112, 11M_fryeri, 21Sphaer2_M1069, 22Csal_CYS4_1072	02IMF_OntsSE1756, 22Csal_CYS4_1072			18PKDtaq_positive	RLO_OntsSE838, Sch_Onne2010_3223, PRV_OnkiAKT2012_3112, 10Cs_positive, 21Sphaer2_M1069, 22Csal_CYS4_1072	05Nanosal_OntsSE1198, 06Faci3_OntsSE3665	02IMF_OntsSE1756, 15Pkab2_positive, 21Sphaer2_M1069, 22Csal_CYS4_1072		17Parvi2_OntsSE9682	22Csal_CYS4_1072	
Confirmed Co-infections <LOD		Bcy2_TMS, Pch1_TMS, Sch_Onne2010_3223, 11M_cerebralis, 11M_fryeri, 11M_insidiosus, 11M_squamalis, 16Pm dup positive, 22Csal_CYS4_1072			Vibrio_495	09NUC_Onne2010-2090	IPNV_Canada1, PRV Creative liver 1055, 05Nanosal_OntsSE1198, 10Cs_K19#3, 17Parvi2_OntsSE9682		Bcy2_TMS, RLO_OntsSE838, PRV_OnkiAKT2012_3112, 17Parvi2_OntsSE9682	PRV_OnkiAKT2012_3112	11M_insidiosus, 17Parvi2_OntsSE9682	RLO_OntsSE838, 06Faci3_OntsSE3665			11M_arcticus					20White_Ich_OntsSE9870	06Faci3_OntsSE3665	
CRS amplifies in VIC but not FAM, indicating specificity of probe important for species specificity		Some Ichthyophtherius sp.		Spironucleus barkhanaus and S. torisa								M. neurobius, M. neurotropus and M. kisutchi		weakly to M. neurobiusand M. neurotropus						P. kabatai and P. spinach		

Table 8. Repeatability Part 1. Summary of agreement, repeatability, and reproducibility (between dynamic arrays) estimates for Part 1 of the repeatability analyses. Two results taken within a technician were on the same chip and RNA plate (repeatability), while two results between technicians were taken between two RNA extractions (reproducibility). Concordance correlation coefficients (CCC) were used to estimate the level of agreement between the two technicians. Significant p-values (<0.05) on the McNemar's test indicate differences in agreement (Pass/Fail) between the two technicians (no agreement), and on the Bradley-Blackwood F test indicate differences of Cts in either their mean or variance (or both). The repeatability and reproducibility estimates were further stratified by high (<20 Ct) and low (≥20 Ct) microbe concentrations

								Overall		High Load		Low Load	
Assay	McNemar's P-value	N	CCC	CCC 95%CI	N	Ave. Diff.	Bradley-Blackwood P-value	Repeat (r)	Reproduce (R)	Repeat (r)	Reproduce (R)	Repeat (r)	Reproduce (R)
ae_sal	0.210	480	0.90	(0.86-0.94)	97	-0.217	0.636	<0.01	4.31	<0.01	1.41	<0.01	3.96
c_b_cys	0.067	480	0.87	(0.84-0.90)	280	-0.789	<0.001	1.10	3.01	0.66	1.67	0.92	2.45
ce_sha	0.052	480	0.77	(0.72-0.83)	185	-1.312	<0.001	1.80	4.27	1.08	1.91	<0.01	2.45
cr_sal	0.256	480	0.76	(0.62-0.89)	33	-0.854	0.013	0.93	3.19	<0.01	0.94	1.83	3.16
de_sal	1.000	480	0.98	(0.97-0.99)	40	-0.719	<0.001	0.98	1.76	0.66	1.14	1.38	2.34
fl_psy	0.007	480	0.84	(0.80-0.88)	212	-0.765	<0.001	1.03	3.44	0.72	2.57	<0.01	1.35
ic_hof	0.011	480	0.95	(0.92-0.98)	52	-0.236	0.103	<0.01	2.25	0.00	1.39	<0.01	2.42
ic_mul	0.864	480	0.89	(0.86-0.91)	195	-1.908	<0.001	2.63	4.87	1.77	2.00	2.25	4.02
ihnv	0.143	480	0.97	(0.96-0.99)	42	-0.873	<0.001	1.21	1.92	1.34	1.70	<0.01	2.11
ku_thy	1.000	480	0.86	(0.82-0.90)	128	-1.805	<0.001	2.51	3.56	2.45	3.25	1.26	2.14
lo_sal	0.791	480	0.90	(0.86-0.95)	56	-0.179	0.626	<0.01	2.45	<0.01	1.48	<0.01	2.47
my_arc	0.066	480	0.89	(0.83-0.95)	41	0.823	0.001	0.92	3.42	<0.01	1.25	2.37	4.83
na_sal	0.815	480	0.00	(0.00-0.00)	2	-1.410	<0.001	<0.01	1.59	1.43	2.00	<0.01	1.48
pa_kab	0.688	480	0.82	(0.59-1.05)	4	0.137	0.247	<0.01	2.73			<0.01	2.73
pa_min	1.000	480	0.97	(0.97-0.98)	149	-0.386	<0.001	0.54	1.81	0.46	0.76	0.60	2.03
pa_pse	0.004	480	0.35	(0.17-0.53)	68	-1.487	<0.001	1.92	5.45	<0.01	1.15	<0.01	2.96
pa_ther	0.263	480	0.79	(0.71-0.86)	93	0.362	0.213	<0.01	2.85	<0.01	0.26	<0.01	2.85
pch_sal	0.625	480	0.94	(0.85-1.02)	9	1.070	0.311	1.31	3.57	1.49	1.55	<0.01	4.57
pmcv	0.302	480	0.00	(0.00-0.00)	0	0.000	<0.001	<0.01	2.63	<0.01	0.57	<0.01	2.84
prv	0.754	480	0.81	(0.61-1.01)	8	-0.051	0.167	<0.01	2.10	<0.01	2.18	3.36	3.53
pspv	0.424	480	0.85	(0.73-0.96)	22	-0.355	0.088	<0.01	2.11	0.63	0.71	0.90	1.94
rlo	0.001	480	0.96	(0.95-0.98)	72	-0.047	0.613	<0.01	1.47	<0.01	0.96	<0.01	1.52
sch	0.481	480	0.67	(0.42-0.91)	11	4.029	<0.001	5.36	5.83			5.36	5.86
sp_des	0.001	480	0.86	(0.73-0.98)	20	0.459	0.641	<0.01	2.43	0.01	0.07	<0.01	1.68
te_bry	0.041	480	0.97	(0.95-0.99)	58	-0.009	0.537	<0.01	1.39	<0.01	1.38	0.34	1.30
ven	0.024	480	0.95	(0.93-0.98)	50	-0.990	<0.001	1.34	2.56	1.70	2.02	0.75	2.76
Median			0.87					0.730	2.683	0.237	1.403	<0.001	2.464
Mean			0.79					0.907	2.961	0.600	1.430	0.821	2.760
# Assays			26					26	26	24	24	26	26

Table 9. Repeatability Part 2. Summary of agreement, repeatability, and reproducibility (between dynamic arrays) estimates for Part 2 of the repeatability analyses. Two results taken within a technician were on the same chip (repeatability), while two results between technicians were taken on the same RNA plate and RNA extraction (reproducibility). Concordance correlation coefficients (CCC) were used to estimate the level of agreement between the two technicians. Significant p-values (<0.05) on the McNemar's test indicate differences in agreement (Pass/Fail) between the two technicians (no agreement), and on the Bradley-Blackwood F test indicate differences of Cts in either their mean or variance (or both). The repeatability and reproducibility estimates were further stratified by high (<20 Ct) and low (≥20 Ct) microbe concentrations.

								Overall		**High Load**		**Low Load**	
Assay	McNemar's P-value	N	CCC	CCC 95%CI	N	Ave. Diff.	Bradley-Blackwood P-value	Repeat (r)	Reproduce (R)	Repeat (r)	Reproduce (R)	Repeat (r)	Reproduce (R)
ae_sal	0.029	478	0.88	(0.83-0.92)	90	-2.260	<0.001	3.36	5.13	2.21	2.83	2.97	4.53
c_b_cys	0.001	478	0.94	(0.92-0.95)	276	-0.430	<0.001	0.58	2.08	0.39	1.59	0.61	2.20
ce_sha	1.000	478	0.85	(0.82-0.88)	190	-1.661	<0.001	2.32	4.04	1.62	2.70	6.07	7.22
cr_sal	0.500	478	0.77	(0.66-0.88)	36	-1.631	<0.001	2.22	3.52	<0.01	1.82	2.48	3.67
de_sal	0.453	478	0.98	(0.97-0.99)	40	-0.559	0.002	0.74	1.56	0.60	1.10	0.79	1.98
fl_psy	0.541	477	0.98	(0.97-0.98)	210	-0.061	0.117	<0.01	1.35	<0.01	1.33	0.98	1.68
ic_hof	0.243	478	0.95	(0.93-0.98)	46	-0.150	0.346	<0.01	2.12	<0.01	1.59	<0.01	2.28
ic_mul	0.771	478	0.96	(0.95-0.97)	188	-1.551	<0.001	2.24	3.12	1.75	1.98	2.66	3.79
ihnv	0.359	478	0.98	(0.97-0.99)	42	-0.807	<0.001	1.11	1.86	1.18	1.43	<0.01	2.27
ku_thy	1.000	478	0.96	(0.95-0.98)	131	-0.720	<0.001	1.02	1.86	1.24	1.57	<0.01	2.06
lo_sal	1.000	478	0.82	(0.75-0.90)	52	-1.639	<0.001	2.35	3.68	<0.01	1.52	2.62	3.83
my_arc	0.210	478	0.97	(0.95-0.99)	45	0.057	0.705	<0.01	2.40	<0.01	1.75	<0.01	2.89
na_sal	0.248	478	-0.44	(0.00-0.00)	2	-0.048	<0.001	<0.01	1.46	<0.01	0.11	<0.01	1.56
pa_kab	1.000	478	0.01	(0.00-0.00)	2	0.552	<0.001	<0.01	2.59			<0.01	2.59
pa_min	1.000	477	0.98	(0.97-0.98)	154	-0.157	<0.001	0.14	1.69	0.75	1.13	0.47	1.80
pa_pse	0.008	478	0.32	(0.19-0.44)	62	-4.365	<0.001	6.33	7.88	3.18	3.35	4.20	5.80
pa_ther	0.227	478	0.71	(0.61-0.80)	90	-0.510	0.001	0.57	3.76	<0.01	0.07	0.75	3.76
pch_sal	0.727	478	0.89	(0.72-1.06)	7	1.357	0.220	1.81	2.76	0.73	2.76	1.10	2.50
pmcv	0.791	478	0.00	(0.00-0.00)	0	0.000	<0.001	7.67	7.95	<0.01	0.93	7.66	7.95
prv	0.754	478	0.89	(0.72-1.06)	8	0.248	0.825	<0.01	1.73	<0.01	1.86	<0.01	1.61
pspv	1.000	477	0.81	(0.69-0.93)	23	-1.184	<0.001	1.63	2.10	<0.01	0.13	1.58	2.07
rlo	1.000	478	0.94	(0.92-0.97)	77	0.197	0.015	0.06	2.01	<0.01	1.52	<0.01	1.86
sch	0.013	478	0.62	(0.20-1.04)	6	-2.827	0.071	4.57	5.44			4.57	5.44
sp_des	0.508	478	0.98	(0.95-1.00)	19	0.194	0.636	<0.01	1.33	0.39	0.49	<0.01	1.42
te_bry	0.629	478	0.97	(0.95-0.98)	56	0.366	<0.001	0.45	1.50	0.82	1.13	<0.01	1.97
ven	0.851	478	0.97	(0.95-0.99)	45	-0.305	0.282	0.34	1.79	0.44	0.91	<0.01	2.17
Median			0.91					0.661	2.106	0.393	1.516	0.681	2.273
Mean			0.76					1.520	2.950	0.637	1.483	1.520	3.111
# Assays			26					26	26	24	24	26	26

Table 10. Repeatability Part 3. Summary of agreement estimates for Part 3 of the repeatability analyses, where the three technician scores were analyzed with three pairwise comparisons (technician 1 vs. 2, 2 vs. 3, and 1 vs. 3). Concordance correlation coefficients (CCC) were used to estimate the level of agreement between two technicians. Significant p-values (<0.05) on the McNemar's test indicate differences in agreement (Pass/Fail) between the two technicians, and on the Bradley-Blackwood F test indicate differences of Cts in either their mean or variance (or both).

	Comparison between Technician 1 and 2							Comparison between Technician 2 and 3							Comparison between Technician 1 and 3								
Assay	McNemar's P-value	N	CCC	CCC 95%CI	N	Ave. Diff.	Bradley-Blackwood P-value	McNemar's P-value	N	CCC	CCC 95%CI	N	Ave. Diff.	Bradley-Blackwood P-value	McNemar's P-value	Cohen's Kappa	Kappa 95%CI	N	CCC	CCC 95%CI	N	Ave. Diff.	Bradley-Blackwood P-value
ae_sal	0.383	960	0.96	(0.94-0.97)	197	-0.990	<0.001	0.302	960	0.99	(0.98-0.99)	200	-0.633	<0.001	1.000	0.93	(0.90-0.96)	960	0.93	(0.91-0.95)	194	-1.502	<0.001
c_b_cys	0.070	960	0.96	(0.96-0.97)	599	-1.096	<0.001	0.375	960	0.99	(0.99-0.99)	602	0.504	<0.001	0.250	0.99	(0.99-1.00)	960	0.98	(0.98-0.99)	600	-0.597	<0.001
ce_sha	0.007	960	0.94	(0.93-0.95)	388	-1.643	<0.001	0.007	960	0.98	(0.98-0.98)	388	1.019	<0.001	1.000	0.98	(0.97-0.99)	960	0.98	(0.97-0.98)	397	-0.592	<0.001
cr_sal	1.000	960	0.94	(0.93-0.96)	96	-1.109	<0.001	1.000	960	0.96	(0.94-0.97)	97	-0.198	0.006	1.000	0.98	(0.96-1.00)	960	0.94	(0.92-0.96)	96	-1.275	<0.001
de_sal	1.000	960	0.99	(0.98-0.99)	88	-0.971	<0.001	1.000	960	0.99	(0.99-1.00)	88	0.487	<0.001	1.000	1.00	(1.00-1.00)	960	1.00	(1.00-1.00)	88	-0.484	<0.001
fl_psy	0.500	960	0.99	(0.99-0.99)	460	-0.592	<0.001	1.000	960	0.99	(0.99-0.99)	459	0.460	<0.001	0.500	1.00	(0.99-1.00)	960	1.00	(1.00-1.00)	460	-0.126	<0.001
ic_hof	0.057	960	0.97	(0.96-0.98)	141	-1.011	<0.001	0.039	960	0.98	(0.97-0.99)	144	0.443	<0.001	1.000	0.98	(0.96-1.00)	960	0.99	(0.99-0.99)	142	-0.592	<0.001
ic_mul	0.383	960	0.97	(0.97-0.98)	400	-1.598	<0.001	0.503	960	0.98	(0.98-0.98)	400	1.254	<0.001	1.000	0.99	(0.98-1.00)	960	1.00	(1.00-1.00)	410	-0.359	<0.001
ihnv	0.250	960	0.97	(0.96-0.98)	93	-1.716	<0.001	1.000	960	0.98	(0.98-0.99)	95	0.554	<0.001	0.625	0.98	(0.95-1.00)	960	0.98	(0.97-0.99)	92	-1.132	<0.001
ku_thy	0.250	960	0.97	(0.97-0.98)	266	-0.650	<0.001	0.125	960	0.99	(0.98-0.99)	266	0.639	<0.001	1.000	1.00	(0.99-1.00)	960	0.99	(0.99-0.99)	269	-0.016	0.912
lo_sal	0.125	960	0.97	(0.96-0.98)	124	-0.654	<0.001	0.180	960	0.99	(0.99-0.99)	123	0.152	0.013	1.000	0.98	(0.96-1.00)	960	0.99	(0.98-0.99)	128	-0.503	<0.001
my_arc	1.000	960	0.97	(0.97-0.98)	115	-1.233	<0.001	1.000	960	0.99	(0.99-1.00)	117	0.439	<0.001	0.500	0.99	(0.98-1.00)	960	0.99	(0.98-0.99)	118	-0.803	<0.001
na_sal	1.000	960	0.97	(0.96-0.99)	29	-0.802	<0.001	1.000	960	0.98	(0.96-0.99)	29	0.702	<0.001	1.000	1.00	(1.00-1.00)	960	0.99	(0.99-1.00)	29	-0.100	0.433
pa_kab	1.000	960	0.93	(0.86-1.00)	14	-1.160	0.002	1.000	960	0.95	(0.90-1.00)	14	0.688	0.073	1.000	1.00	(1.00-1.00)	960	0.99	(0.99-1.00)	14	-0.472	<0.001
pa_min	0.688	960	0.99	(0.99-0.99)	336	-0.607	<0.001	0.289	960	0.98	(0.98-0.99)	334	0.844	<0.001	0.500	1.00	(0.99-1.00)	960	1.00	(1.00-1.00)	336	0.234	<0.001
pa_pse	0.125	960	0.98	(0.97-0.98)	143	0.212	0.015	0.008	960	0.81	(0.77-0.85)	135	-2.382	<0.001	0.000	0.95	(0.92-0.98)	960	0.80	(0.75-0.85)	135	-2.112	<0.001
pa_ther	0.189	960	0.92	(0.90-0.93)	188	-1.378	<0.001	0.146	960	0.98	(0.97-0.98)	192	0.502	<0.001	1.000	0.97	(0.95-0.99)	960	0.96	(0.96-0.97)	197	-0.877	<0.001
pch_sal	1.000	960	0.98	(0.96-1.00)	24	-0.768	0.007	1.000	960	0.97	(0.94-0.99)	24	-1.224	<0.001	1.000	1.00	(1.00-1.00)	960	0.93	(0.89-0.98)	24	-1.992	<0.001
pmcv	0.250	960	0.69	(0.55-0.84)	46	-0.823	0.058	1.000	960	0.74	(0.63-0.84)	46	-1.720	<0.001	0.688	0.93	(0.88-0.99)	960	0.54	(0.39-0.69)	44	-2.487	<0.001
prv	1.000	960	1.00	(0.99-1.00)	24	-0.084	0.458	1.000	960	1.00	(1.00-1.00)	24	0.286	<0.001	1.000	1.00	(1.00-1.00)	960	1.00	(0.99-1.00)	24	0.201	0.052
pspv	1.000	960	0.92	(0.89-0.95)	55	-1.254	<0.001	0.250	960	0.97	(0.96-0.99)	59	0.244	0.010	0.125	0.96	(0.93-1.00)	960	0.91	(0.87-0.95)	58	-0.900	<0.001
rlo	1.000	960	1.00	(0.99-1.00)	160	-0.048	0.001	0.500	960	1.00	(1.00-1.00)	158	0.180	<0.001	0.500	0.99	(0.98-1.00)	960	1.00	(1.00-1.00)	158	0.121	<0.001
sch	0.289	960	0.98	(0.96-0.99)	29	-0.951	<0.001	0.549	960	0.90	(0.84-0.97)	28	-0.680	0.087	1.000	0.75	(0.63-0.87)	960	0.92	(0.87-0.98)	24	-1.079	0.002
sp_des	0.002	960	0.91	(0.88-0.95)	57	-1.875	<0.001	0.109	960	0.95	(0.92-0.97)	57	1.302	<0.001	0.016	0.94	(0.90-0.99)	960	0.99	(0.98-0.99)	65	-0.609	<0.001
te_bry	1.000	960	1.00	(0.99-1.00)	139	-0.300	<0.001	1.000	960	0.99	(0.99-1.00)	139	0.306	<0.001	1.000	1.00	(1.00-1.00)	960	0.99	(0.99-0.99)	139	0.006	0.004
ven	0.625	960	0.97	(0.95-0.98)	131	-0.762	<0.001	1.000	960	0.98	(0.97-0.99)	132	-0.301	0.006	1.000	0.98	(0.96-1.00)	960	0.97	(0.96-0.98)	130	-0.978	<0.001

Table 11. LOD Repeatability and Reproducibility (between dynamic arrays; r and R, respectively), categorized by microbe load (High: Ct<20; Low: Ct≥20) for amplified (STA) and non-amplified (Non-STA) samples found in the limit of detection study.

	STA						Non-STA					
	Overall		High Load		Low Load		Overall		High Load		Low Load	
Assay	r[a]	R[b]	r[c]	R[d]	r[c]	R[d]	r[a]	R[b]	r[e]	R[f]	r[e]	R[f]
ae_hyd	1.69	3.68	1.83	2.57	1.63	4.27	2.49	3.14	1.74	2.03	2.72	3.39
ae_sal	2.44	3.42	2.12	2.54	2.60	3.86	3.49	4.59	<0.01	0.18	3.41	4.47
aspv	0.30	1.72	0.52	0.70	<0.01	2.32	0.49	1.32	<0.01	0.51	0.58	1.56
c_b_cys	0.82	2.25	0.68	1.08	0.93	2.86	1.02	1.75	1.14	1.44	1.33	1.92
ce_sha	1.82	3.17	1.58	2.06	2.03	3.71	1.94	2.94	0.89	1.28	2.16	3.08
cr_sal	1.66	2.80	1.41	1.59	1.88	3.55	2.67	3.09	2.69	2.85	2.82	3.23
de_sal	0.71	2.28	0.89	1.18	0.48	2.98	0.52	2.37	0.48	0.71	0.23	3.05
fa_mar	3.24	6.23	2.32	3.99	3.33	6.78	3.56	5.36	3.05	3.31	3.54	5.61
fl_psy	0.69	2.25	0.58	0.89	0.77	2.79	0.34	1.00	0.71	0.95	0.16	1.02
gy_sal	1.05	2.14	0.87	1.30	1.22	2.62	2.28	2.89	1.44	1.73	2.57	3.24
ic_hof	1.06	2.20	0.77	1.07	1.27	2.76	1.84	2.53	1.48	1.86	1.89	2.72
ic_mul	1.78	3.98	1.43	1.64	2.04	5.36	1.72	2.61	2.34	2.51	1.08	2.37
ihnv	0.82	2.20	0.83	1.38	0.82	2.60	1.87	2.83	1.41	2.14	1.97	2.84
ipnv	1.07	2.74	0.97	1.39	1.10	3.09	1.53	2.51	0.84	1.10	1.86	2.95
isav7	0.31	2.18	0.49	1.25	0.30	2.68	0.79	1.58	0.86	1.38	1.04	1.68
isav8	2.33	3.32	2.20	2.56	2.39	3.83	4.08	4.51	2.23	2.46	4.40	4.76
ku_thy	0.17	2.34	0.06	1.86	<0.01	2.73	0.21	1.21	0.38	0.56	<0.01	1.51
lo_sal	1.60	2.86	1.28	1.36	1.89	3.76	1.81	2.73	2.07	2.29	1.51	2.98
my_arc	0.93	2.79	0.85	2.08	0.86	3.23	<0.01	3.57	1.67	1.92	1.84	2.48
my_cer	1.07	1.98	1.26	1.47	0.83	2.41	1.49	2.38	0.99	1.20	1.79	2.95
my_ins	0.95	2.18	0.70	1.28	1.17	2.79	1.50	2.81	1.58	2.08	1.62	2.32
na_sal	0.06	1.81	0.34	0.68	0.14	2.45	0.81	2.17	0.93	2.21	0.57	2.12
ne_per	0.37	2.20	0.54	0.90	<0.01	2.92	1.39	2.40	0.86	1.63	1.37	2.55
nu_sal	1.10	1.78	1.12	1.20	1.06	2.18	1.66	2.19	1.25	1.33	1.93	2.67
omv	1.76	2.70	1.34	1.62	2.01	3.20	2.97	3.59	1.57	1.99	3.32	3.90
pa_kab	0.53	1.85	0.43	0.85	0.61	2.31	0.75	2.01	0.79	1.24	0.74	2.27
pa_min	1.63	2.92	1.59	1.76	1.64	3.43	2.47	2.96	2.80	2.81	2.52	3.00
pa_pse	1.02	2.66	1.82	2.17	0.17	3.06	1.42	2.22	1.14	2.54	1.13	1.91
pa_ther	1.26	2.40	1.26	1.85	1.23	2.62	1.74	2.59	0.76	1.30	1.93	2.78
pch_sal	3.71	5.48	2.06	3.35	4.00	5.87	5.27	7.37	3.71	5.48	5.27	7.37
pisck_sal	1.42	3.18	1.48	2.55	1.32	3.75	1.68	3.42	1.73	1.93	1.48	4.20
pmcv	1.67	2.96	1.47	1.80	1.81	3.63	2.47	3.07	2.75	2.94	2.61	3.05
prv	0.74	1.45	0.67	0.77	0.83	1.87	<0.01	3.01	0.76	0.86	<0.01	3.79
pspv	1.05	2.48	0.88	1.60	1.12	2.96	0.69	2.49	1.76	2.79	<0.01	2.35
re_sal	0.72	1.89	0.81	0.93	0.65	2.54	1.06	1.83	0.93	1.07	1.08	2.29
rlo	0.40	1.57	0.39	0.70	0.37	2.15	<0.01	2.46	0.47	0.74	0.73	3.26
sav	0.53	2.48	0.51	0.99	0.64	3.27	0.23	1.77	0.39	0.79	0.74	2.09
sch	1.82	2.95	1.54	2.06	1.90	3.28	2.87	3.71	<0.01	1.78	3.14	3.97
sp_des	0.13	2.06	0.13	0.73	0.25	2.66	0.58	1.68	0.63	0.77	0.78	1.92
sp_sal	2.93	4.69	2.70	2.87	3.05	5.58	3.90	4.07	4.62	4.68	3.80	3.98
te_bry	0.08	2.18	0.20	0.98	<0.01	2.74	<0.01	1.87	0.62	1.68	<0.01	2.01
ven	0.61	2.17	0.46	0.95	0.82	2.91	1.95	2.57	1.12	1.56	2.30	2.98
ver	0.84	2.10	0.76	1.11	0.98	2.66	1.19	2.38	0.51	1.02	1.47	2.80
vhsv	1.74	6.25	1.18	1.48	3.61	6.65	1.64	2.20	2.18	2.35	1.44	2.04
vi_ang	1.21	2.35	1.14	1.42	1.28	2.87	2.36	3.03	1.75	2.53	2.59	3.24
vi_sal	0.85	2.20	0.54	0.94	1.07	2.92	0.51	2.11	1.41	1.69	<0.01	2.31
ye_ruc	3.89	7.32	2.42	3.30	4.59	8.76	3.99	6.20	3.89	7.32	3.99	6.20
Median	1.05	2.35	0.89	1.39	1.10	2.92	1.64	2.57	1.14	1.73	1.62	2.84
Mean	1.25	2.82	1.09	1.59	1.33	3.41	1.69	2.83	1.43	1.95	1.78	3.00

Table 12. Concordance correlation coefficients (CCC) for unadjusted, globally-adjusted, and pathogen grouping-adjusted models.

ABI7900 vs Fluidigm Model	Concordance Correlation Coefficient (95% CI)	n	*p*-value (Bradley Blackwood)
Unadjusted Model	0.376 (0.353, 0.398)	1027	0
Global Adjustment[1]	0.905 (0.894, 0.916)	1027	0.427
Assay Specific Adjustment[2]	0.948 (0.942, 0.954)	1027	0.004

[1] Fluidigm Ct values adjusted by +10.293 units

[2] Fluidigm Ct values adjusted by each assay

Table 13. Concordance correlation coefficients (CCC) generated from assay specific comparisons of the ABI 7900 vs. BioMark platform using assay-specific adjusted BioMark Ct values.

Assay	CCC	95% CI		n	p-value (Bradley Blackwood)
ae_sal	0.901	0.850	0.953	53	0.936
c_b_cys	0.813	0.738	0.887	80	0.565
ce_sha	0.867	0.821	0.913	84	<0.001
cr_sal	0.902	0.814	0.989	19	0.780
de_sal	0.998	0.996	0.999	28	0.508
fl_psy	0.657	0.534	0.780	80	0.161
ic_hof	0.960	0.933	0.986	36	0.450
ic_mul	0.985	0.981	0.990	166	0.006
ihnv	0.997	0.995	0.999	42	0.235
ku_thy	0.985	0.977	0.992	70	0.439
lo_sal	0.940	0.900	0.980	34	0.903
my_arc	0.952	0.922	0.982	30	0.022
pa_min	0.948	0.925	0.972	70	0.196
pa_pse	0.488	0.344	0.632	62	<0.001
pa_ther	0.766	0.660	0.872	56	0.070
pspv	0.909	0.838	0.980	25	0.907
rlo	0.979	0.964	0.995	28	0.625
sch	0.865	0.666	1.063	8	0.901
sp_des	0.994	0.985	1.004	7	0.529
te_bry	0.879	0.799	0.959	32	0.656
ven	0.988	0.979	0.997	25	0.166

Table 14. Optimum assay-specific Ct value cut-points with corresponding sensitivity, specificity, Area under the Curve-ROC and number of observations for three ABI 7900 cut-points for classifying positive/negative test results.

Assay Grouping	ABI7900 Cut-point	BioMark Cut-Point	Raw BioMark Ct Value	Sensitivity	Specificity	Correctly Classified	AUC-ROC	N
ae_sal	Ct25	24.25	18.1	100	88.89	98.74	0.984	159
	Ct30	31.18	25.04	100	92.86	98.74	0.995	159
	Ct35	35	28.86	96.77	100	97.48	0.996	159
	Ct40	56.14	49.99	99	89.83	95.6	0.948	159
c_b_cys	Ct25	23.87	14.62	99.29	52.63	93.75	0.900	160
	Ct30	31.13	21.88	99.02	87.93	95	0.972	160
	Ct35	34.23	24.8	98.9	91.3	95.63	0.976	160
	Ct40	59.25	50	85.71	88.89	87.5	0.929	160
ce_sha	Ct25	25.64	17.5	93.67	100	96.53	0.991	144
	Ct30	58.14	50	100	100	100	1.000	144
	Ct35	58.14	50	100	100	100	1.000	144
	Ct40	58.14	50	100	100	100	1.000	144
cr_sal	Ct25	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	160
	Ct30	30.66	21.4	100	100	100	1.000	160
	Ct35	35.59	26.3	100	100	100	1.000	160
	Ct40	59.29	51.15	91.34	57.58	84.37	0.750	160
de_sal	Ct25	26.07	14.53	100	100	100	1.000	160
	Ct30	30.22	18.68	100	100	100	1.000	160
	Ct35	36.35	24.81	98.53	100	98.75	0.994	160
	Ct40	61.54	50	0	100	20.63	0.921	160
fl_psy	Ct25	23.93	13.76	100	86.36	96.25	0.961	160
	Ct30	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	160
	Ct35	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	160
	Ct40	60.17	50	100	100	100	1.000	160
ic_hof	Ct25	29.67	16.85	100	100	100	1.000	160
	Ct30	32.58	19.76	100	100	100	1.000	160
	Ct35	37.93	25.11	97.06	100	97.5	0.995	160
	Ct40	41.46	28.64	98.35	87.18	95.63	0.945	160
ic_mul	Ct25	25.56	14.36	100	100	100	1.000	298
	Ct30	30.2	19	98.1	100	98.66	1.000	298
	Ct35	36.13	24.93	95.95	100	97.65	0.999	298
	Ct40	61.2	50	94.49	97.08	95.97	0.982	298
ihnv	Ct25	26.56	15.75	98.58	100	98.75	1.000	160
	Ct30	32.03	21.22	100	100	100	1.000	160
	Ct35	36.06	25.25	98.37	100	98.75	1.000	160
	Ct40	60.81	100	100	100	100	1.000	160
ku_thy	Ct25	25.48	14.84	100	100	100	1.000	144
	Ct30	30.58	19.94	100	100	100	1.000	144
	Ct35	35.53	24.89	97.56	100	98.61	1.000	144
	Ct40	40.47	29.83	100	100	100	1.000	144
lo_sal	Ct25	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	160
	Ct30	32.87	22.41	100	100	100	1.000	160
	Ct35	35.48	25.02	98.68	88.89	98.13	0.993	160
	Ct40	40.78	30.32	97.56	91.89	96.25	0.953	160
my_arc	Ct25	27.33	13.08	100	100	100	1.000	160
	Ct30	29.15	14.9	100	100	100	1.000	160
	Ct35	34.28	20.03	99.28	90.91	98.12	0.996	160
	Ct40	40.24	25.9	98.41	88.24	96.25	0.937	160
pa_min	Ct25	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	160
	Ct30	30.55	20.88	100	75	99.38	0.817	160
	Ct35	35.58	25.91	96.58	93.02	95.63	0.979	160
	Ct40	38.37	28.7	100	91.67	96.25	0.984	160
pa_pse	Ct25	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	160
	Ct30	29	21.5	99.26	66.67	94.37	0.908	160
	Ct35	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	160
	Ct40	57.5	50	100	79.49	90	0.897	160
pa_ther	Ct25	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	160
	Ct30	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	160
	Ct35	34.5	23.61	100	89.47	98.75	0.991	160
	Ct40	42.85	31.96	93.07	89.93	91.87	0.933	160
pspv	Ct25	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	160
	Ct30	30.97	19.46	99.37	100	99.38	0.994	160
	Ct35	35.61	24.1	99.33	80	98.13	0.887	160
	Ct40	40.16	28.65	99.24	85.71	96.88	0.944	160
rlo	Ct25	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	160
	Ct30	31.36	18.94	100	100	100	1.000	160
	Ct35	34.96	22.54	100	93.33	99.38	0.963	160
	Ct40	41.44	29.02	100	87.5	97.5	0.937	160
sch	Ct25	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	160
	Ct30	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	160
	Ct35	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	160
	Ct40	58	50	97.44	100	97.5	0.994	160
sp_des	Ct25	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	N/A[1]	160
	Ct30	34.08	21.98	100	100	100	1.000	160
	Ct35	39.28	27.18	99.35	71.43	98.12	0.848	160
	Ct40	41.68	29.58	99.34	77.78	98.13	0.883	160
te_bry	Ct25	28.57	13.59	100	100	100	1.000	160
	Ct30	30.8	15.82	100	100	100	1.000	160
	Ct35	35	20.02	99.28	96.24	98.75	0.999	160
	Ct40	64.983	50	98.44	96.88	98.13	0.983	160
ven	Ct25	25.72	14.83	100	100	100	1.000	160
	Ct30	30.6	19.71	100	100	100	1.000	160
	Ct35	37.17	26.28	100	100	100	1.000	160
	Ct40	38.97	28.08	99.24	86.21	96.21	0.927	160

[1]*Outcome does not vary at specific ABI7900 cut-points, cannot estimate.*

Table 15. Expected and observed Ct of the low and medium dilutions from the sensitivity study assessing impacts of the STA on relative quantitation. Estimated Cts were calculated based on the Ct of the high load samples, the dilution factor, and the assay-specific efficiency. For a few microbes, the observed and/or expected Ct was above the LOD, which impacted the probability of detection shown in Figure 18. Note this analysis was performed on the original efficiencies and LOD's calculated from the first dilution series only. The new LODs based on three dilution series are also shown; for the most part, the Cts did not change substantively, hence the general conclusions still hold.

Assay	Group	Load	N	High Ct	Med Ct	Dilution	Efficiency	Expected Ct	Median Observed Ct	Standard Deviation Ct	Original LOD	New LOD	Predicted Probability of Detection
ae_hyd	2	Low	4	15.80		1/1000	0.967	25.43	26.04	0.61	28.67	28.7	100
ae_sal	1	Low	4	12.61		1/200	1.193	21.72	21.53	1.10	25.61	26.5	100
ae_sal	3	Low	4	9.56		1/200	1.193	18.68	18.51	0.89	25.61	26.5	100
aspv	1	Low	4	13.73		1/1000	0.967	23.35	24.09	0.36	26.23	26.4	100
aspv	2	Low	4	13.82		1/1000	0.967	23.45	24.59	0.25	26.23	26.4	100
c_b_cys	3	Medium	8	21.17	22.77	1/2	0.993	22.16	22.70	0.43	25.71	26.9	99
c_b_cys	3	Low	4	21.17	22.77	1/4	0.993	24.76	24.74	0.86	25.71	26.9	99
ce_sha	1	Medium	8	16.05	19.05	1/4	1.069	18.19	18.87	0.47	28.5	28.2	100
ce_sha	1	Low	4	16.05	19.05	1/8	1.069	22.26	21.72	0.65	28.5	28.2	100
cr_sal	2	Low	4	16.14		1/200	1.029	24.00	24.64	0.61	24.34	27.3	100
de_sal	4	Low	4	15.45		1/200	0.992	23.02	23.12	0.18	25.49	27.5	100
fa_mar	4	Low	2		20.86	1/4	0.924	22.71	21.50	2.33	30.55	29.1	100
fl_psy	4	Low	4	13.76		1/1000	0.834	22.06	24.19	0.29	29.46	28.3	100
gy_sal	2	Low	4	12.43		1/1000	0.983	22.22	23.00	0.60	26.42	26.3	100
gy_sal	4	Low	4	12.99		1/1000	0.983	22.78	23.15	0.30	26.42	26.3	100
ic_hof	1	Low	4	17.55		1/100	1.098	24.84	24.56	0.82	24.17	25.4	100
ic_mul	1	Low	2		20.22	1/6	1.048	22.92	23.72	1.30	23.7	25.2	100
ihnv	2	Low	4	16.02		1/1000	0.876	24.74	28.49	1.22	27.64	27.9	96
ihnv	4	Low	4	15.56		1/1000	0.876	24.28	26.66	0.30	27.64	27.9	96
ipnv	1	Low	4	16.17		1/1000	0.916	25.30	26.95	0.28	27.63	27.7	98
ipnv	2	Low	4	16.60		1/1000	0.916	25.72	27.48	1.04	27.63	27.7	98
isav7	4	Medium	8	20.19	22.00	1/4	0.973	22.13	22.02	0.12	27	27.9	100
isav7	4	Low	4	20.19	22.00	1/10	0.973	25.23	25.20	0.92	27	27.9	100
isav8	2	Medium	8	21.96	23.94	1/4	1.094	24.15	24.01	0.24	26.12	21.3	99
isav8	2	Low	4	21.96	23.94	1/10	1.094	27.57	27.72	0.76	26.12	21.3	99
ku_thy	2	Low	4	15.49		1/400	0.932	23.54	25.29	0.88	26.23	26.2	99
lo_sal	2	Medium	8	16.52	19.58	1/8	1.025	19.59	19.62	0.29	25.42	26.1	100
lo_sal	2	Low	4	16.52	19.58	1/10	1.025	22.98	23.13	0.27	25.42	26.1	100
my_arc	4	Low	4	20.26		1/400	0.855	27.65	28.34	0.50	26.8	26.9	90
my_cer	4	Medium	8	21.46	22.51	1/2	1.019	22.47	22.48	0.46	26.18	26.4	100
my_cer	4	Low	4	21.46	22.51	1/4	1.019	24.55	24.16	0.71	26.18	26.4	100
my_ins	1	Low	4	15.70		1/400	0.991	24.26	25.05	0.87	26.43	26.6	100
my_ins	3	Low	4	15.68		1/400	0.991	24.25	25.33	0.47	26.43	26.6	100
na_sal	1	Low	2		25.05	1/2	1.045	26.10	26.20	0.98	24.3	25.8	100
ne_per	1	Low	4	12.38		1/1000	1.074	23.08	23.11	0.91	25.39	26.9	100
nu_sal	1	Low	4	15.51		1/400	1.062	24.68	24.69	0.46	26.07	25.3	99
nu_sal	3	Low	4	15.24		1/400	1.062	24.41	24.18	0.12	26.07	25.3	99
omv	1	Low	4	7.99		1/1000	0.977	17.71	17.79	1.13	26.59	27.4	100
omv	3	Low	4	8.87		1/1000	0.977	18.60	19.23	0.51	26.59	27.4	100
pa_kab	4	Low	2		23.57	1/4	0.896	25.36	24.53	0.67	25.58	27	100
pa_min	3	Low	2		26.64	1/4	0.871	28.38	28.29	0.30	29.62	28.7	96
pa_pse	2	Low	1		26.53	1/10	0.931	29.62	31.46		25.16	25.7	64
pa_ther	2	Low	2		20.67	1/10	0.913	23.70	24.84	0.54	28.16	28.6	100
pch_sal	3	Low	2		22.13	1/4	0.814	23.75	25.57	0.87	30.72	29.2	100
pisck_sal	3	Low	4	15.12		1/400	0.937	23.22	25.12	0.61	23.32	25.6	99
pmcv	2	Low	4	15.04		1/1000	0.982	24.82	25.85	1.16	26.29	26.3	100
pmcv	4	Low	4	13.38		1/1000	0.982	23.16	23.93	0.93	26.29	26.3	100
prv	2	Low	4	18.27		1/200	1.042	26.23	26.35	0.36	26.11	25.4	100
prv	3	Medium	8	18.07	19.54	1/4	1.042	20.16	19.51	0.08	26.11	25.4	100
prv	3	Low	4	18.07	19.54	1/50	1.042	25.42	26.47	0.63	26.11	25.4	100
pspv	1	Medium	8	16.62	20.59	1/10	0.972	19.85	20.50	0.20	26.42	27	98
pspv	1	Low	4	16.62	20.59	1/100	0.972	27.04	26.95	0.47	26.42	27	98
re_sal	1	Low	4	15.90		1/1000	1.003	25.89	26.30	0.68	25.91	26	95
re_sal	4	Low	4	16.54		1/1000	1.003	26.53	26.80	0.62	25.91	26	95
rlo	1	Low	4	17.24		1/400	0.959	25.52	25.87	0.38	25.23	26.5	100
sav	2	Low	4	15.25		1/400	0.878	22.84	25.38	1.15	26.28	25.6	100
sav	3	Low	4	16.00		1/400	0.878	23.59	25.18	0.83	26.28	25.6	100
sch	2	Low	4	15.37		1/400	0.950	23.58	24.87	0.80	27.9	27.7	100
sch	3	Low	4	15.33		1/400	0.950	23.54	23.94	0.84	27.9	27.7	100
sp_des	4	Low	4	16.11		1/400	0.924	24.09	24.85	0.33	26.5	27	100
sp_sal	3	Low	4	18.05		1/400	0.940	26.18	31.12	1.98	26.05	29	90
te_bry	3	Low	4	17.04		1/400	1.000	25.68	26.20	0.81	24.98	25.4	100
ven	4	Low	4	13.63		1/1000	1.077	24.36	24.56	0.42	24.85	25.4	100
ver	2	Low	4	13.29		1/1000	1.011	23.36	23.99	0.17	26.21	26.4	100
vhsv	1	Low	4	14.27		1/4000	0.845	24.37	27.47	2.91	26.86	31.8	94
vhsv	4	Low	4	14.25		1/4000	0.845	24.36	28.44	1.91	26.86	31.8	94
vi_ang	3	Low	4	13.16		1/400	1.066	22.37	22.13	0.48	26.41	26.5	100
vi_ang	4	Low	4	13.29		1/400	1.066	22.50	22.28	0.43	26.41	26.5	100
vi_sal	3	Low	4	9.72		1/400	1.065	18.92	19.30	0.36	25.84	25.7	100
vi_sal	4	Low	4	10.75		1/1000	1.065	21.36	21.46	0.27	25.84	25.7	100
ye_ruc	1	Low	1	14.61		1/2000	0.853	23.96	20.64		25.81	28.9	100

Table 16. False positive detections from negative control samples included inASe and ASp studies.

FAM		Total	Passes	%	Assays	Ct
FAM	qPCR	12864	2	0.016	isav7,pmcv	Ct>32
	STA	2016	9	0.446	ven,isav7,ven, sp_sal	Ct>26, 24.8, 28, 30.1
	STA_CHSE	1632	8	0.490	Pa_min, ku_thy	Ct>26, 26.9
	Total	16512	19	0.115		
VIC	qPCR	12864	0	0.000		
	STA	2016	4	0.198	ven, sp_sal	Ct>26.5, 31.6
	STA_CHSE	1632	4	0.245	pa_kab	Ct>28.2
	Total	16512	8	0.048		

12 FIGURES

1 (a) 1(b)

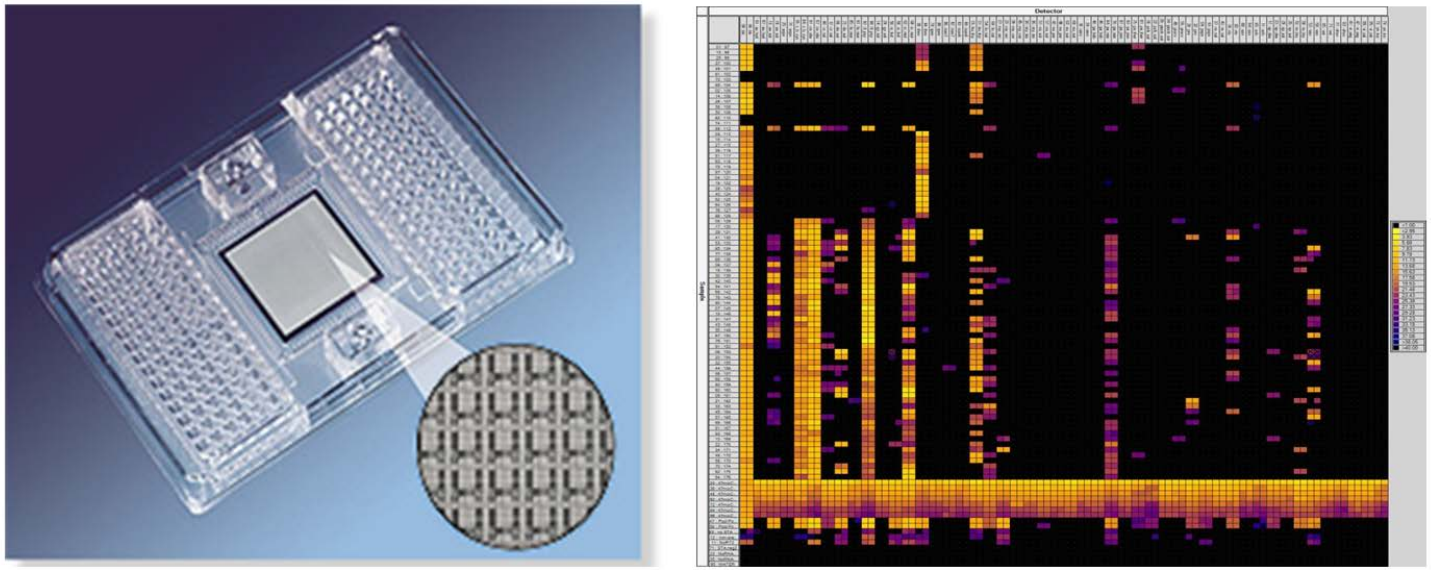


Figure 1. Fluidigm BioMark™ Platform. The dynamic array (a) is a microfluidic system that can run 96 samples across 96 assays. Heatmap (b) representing the output of the BioMark microbe screening technology applied to livers of adult sockeye salmon at the spawning grounds. This panel included quadruplicates of 23 TaqMan qPCR assays to 18 salmon microbes. Assays are shown on the horizontal axis and samples on the vertical axis. Brighter spots indicate higher microbe loads. The far left column was a reference gene control and the lower 7 rows were serial dilutions of microbe positive controls. Blanks indicate microbes for which controls were still in development.

(A3) Limit of Detection – Part A

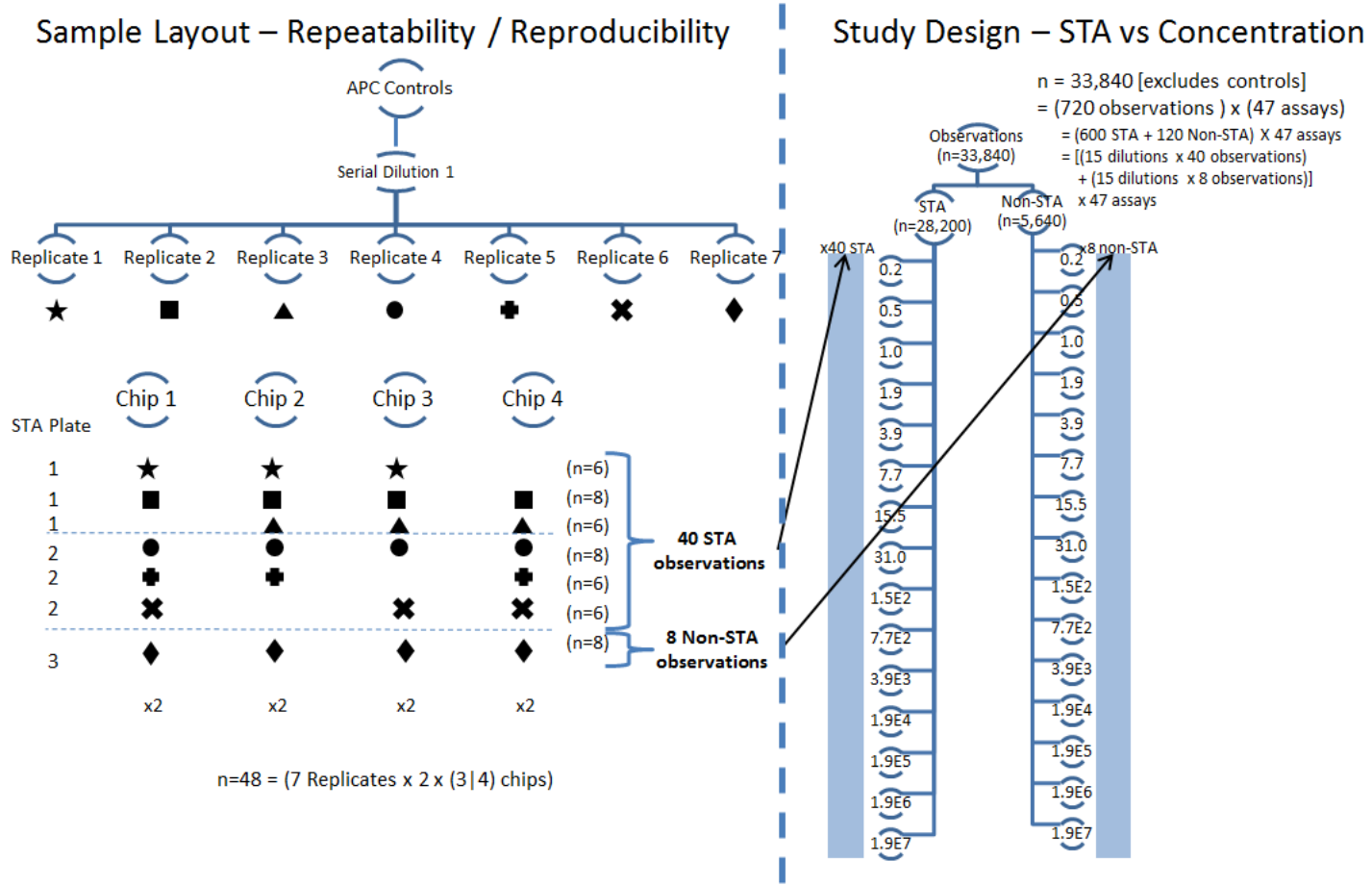


Figure 2. Schematic representation of limit of detection (LOD) study, parts A and B.

(A3) Limit of Detection – Part B

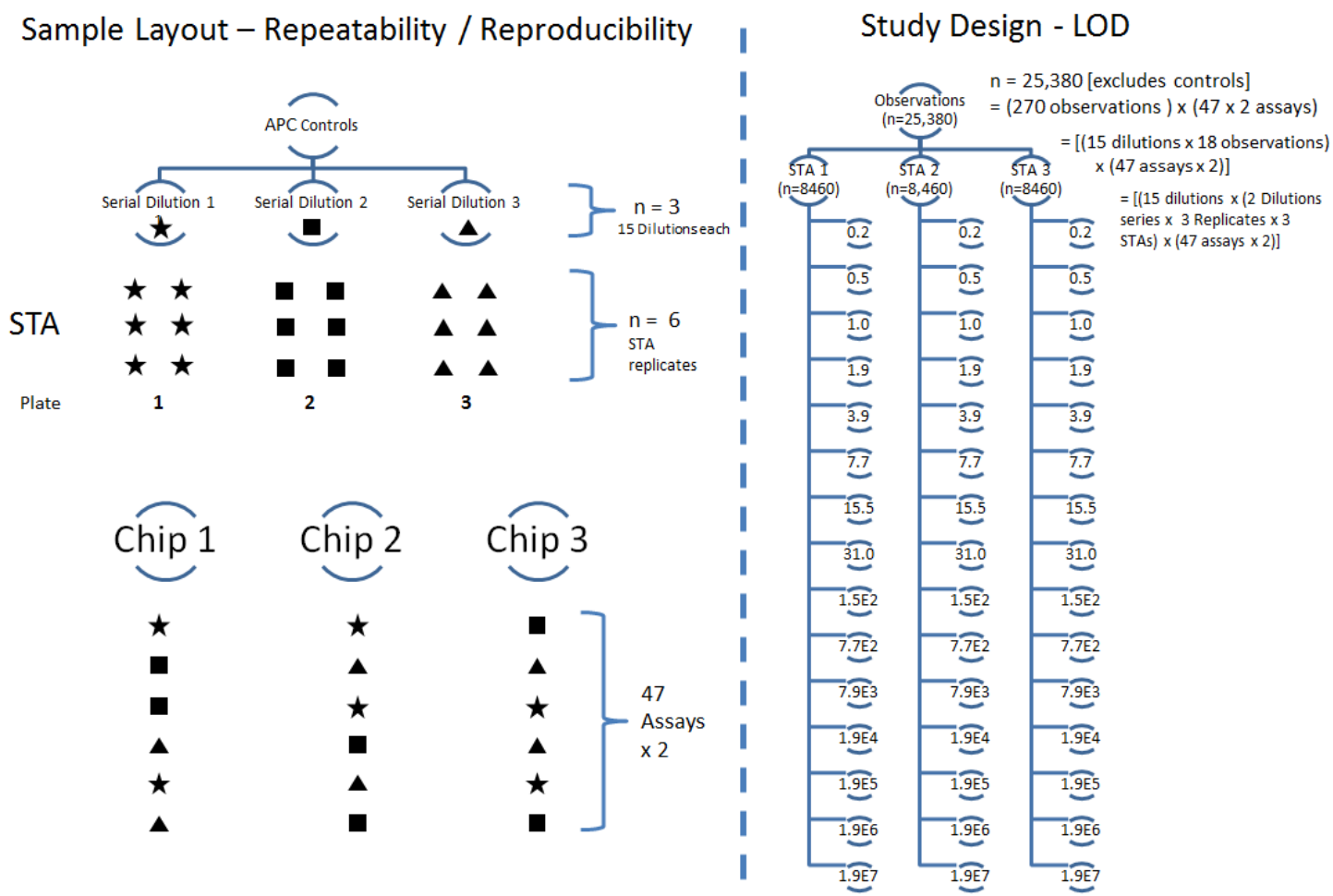
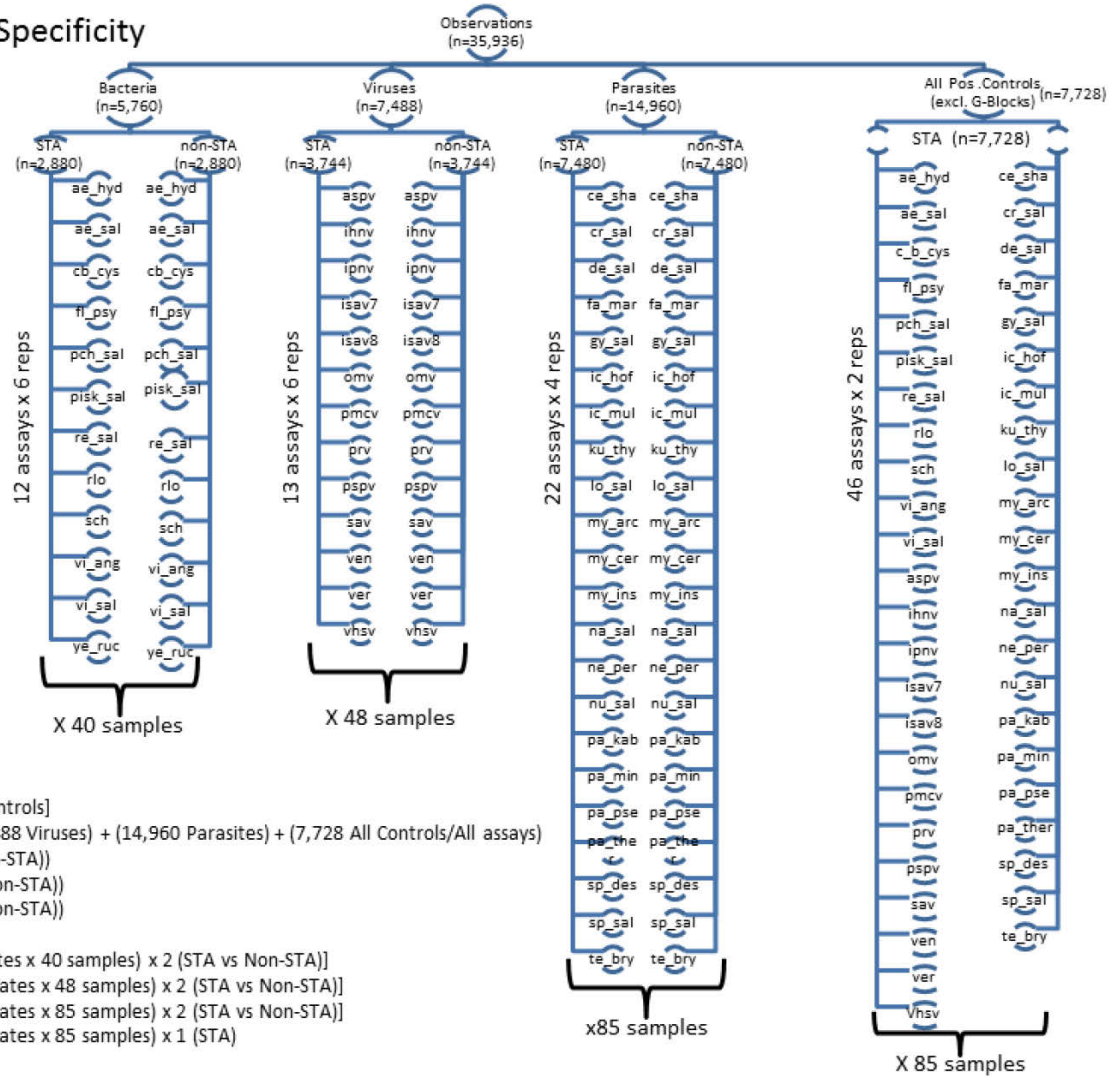


Figure 2. (Continued).

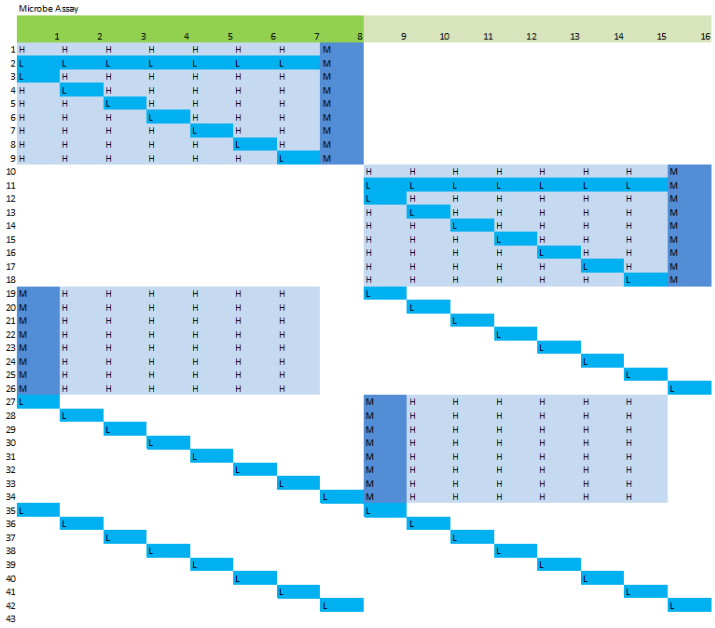
(A2) Analytical Specificity



n = 35,936 [excludes controls]
= (5,760 Bacteria) + (7,488 Viruses) + (14,960 Parasites) + (7,728 All Controls/All assays)
= (2,880 x 2 (STA vs Non-STA))
+ (3,744 x 2 (STA vs Non-STA))
+ (7,480 x 2 (STA vs Non-STA))
+ (7,728 x 1)
= [(12 assays x 6 replicates x 40 samples) x 2 (STA vs Non-STA)]
+ [(13 assays x 6 replicates x 48 samples) x 2 (STA vs Non-STA)]
+ [(22 assays x 4 replicates x 85 samples) x 2 (STA vs Non-STA)]
+ [(46 assays x 2 replicates x 85 samples) x 1 (STA)

Figure 3. Schematic representation of analytical specificity study.

A



B

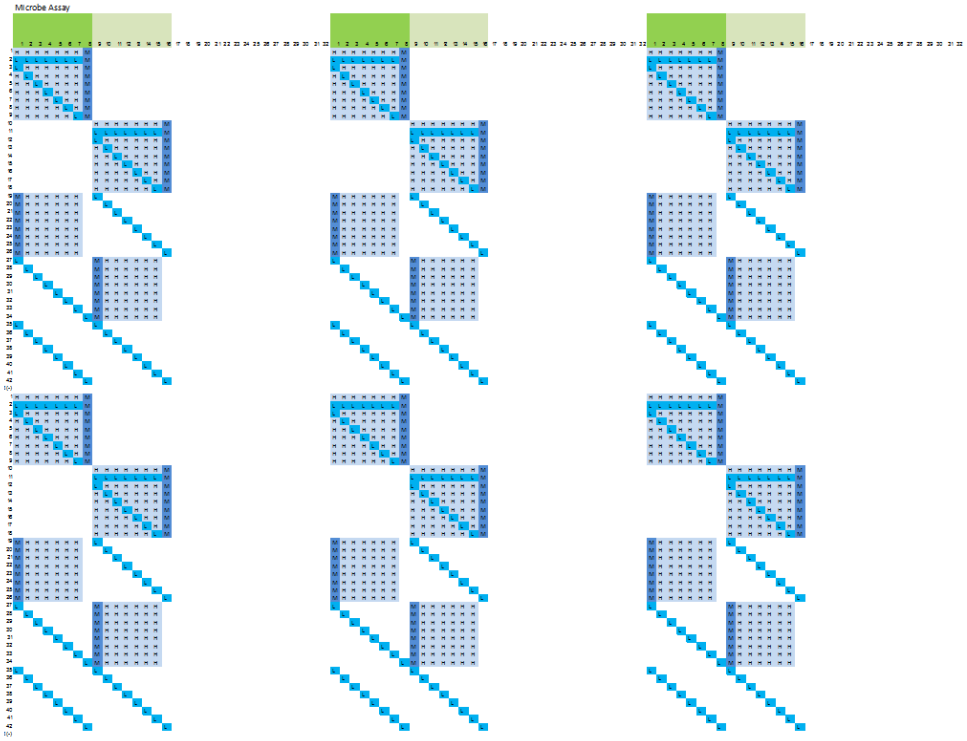


Figure 4. Assessment of STA impact on relative quantitation showing set-up of 42 sample mixture over 16 assays (A), repetition of mixtures over a single 96.96 dynamic array (B). Mixtures are denoted on the y-axis and microbe assays on the x-axis. Wells are colour-coded by load, with bright blue depicting low-load (Ct 24-26) samples, light blue high-load (Ct<18), and dark moderate load (Ct 19-23). The dark and light green along the top depicts the two 8-microbe groups repeated 6 times across each dynamic array. White space between mixtures was filled with controls.

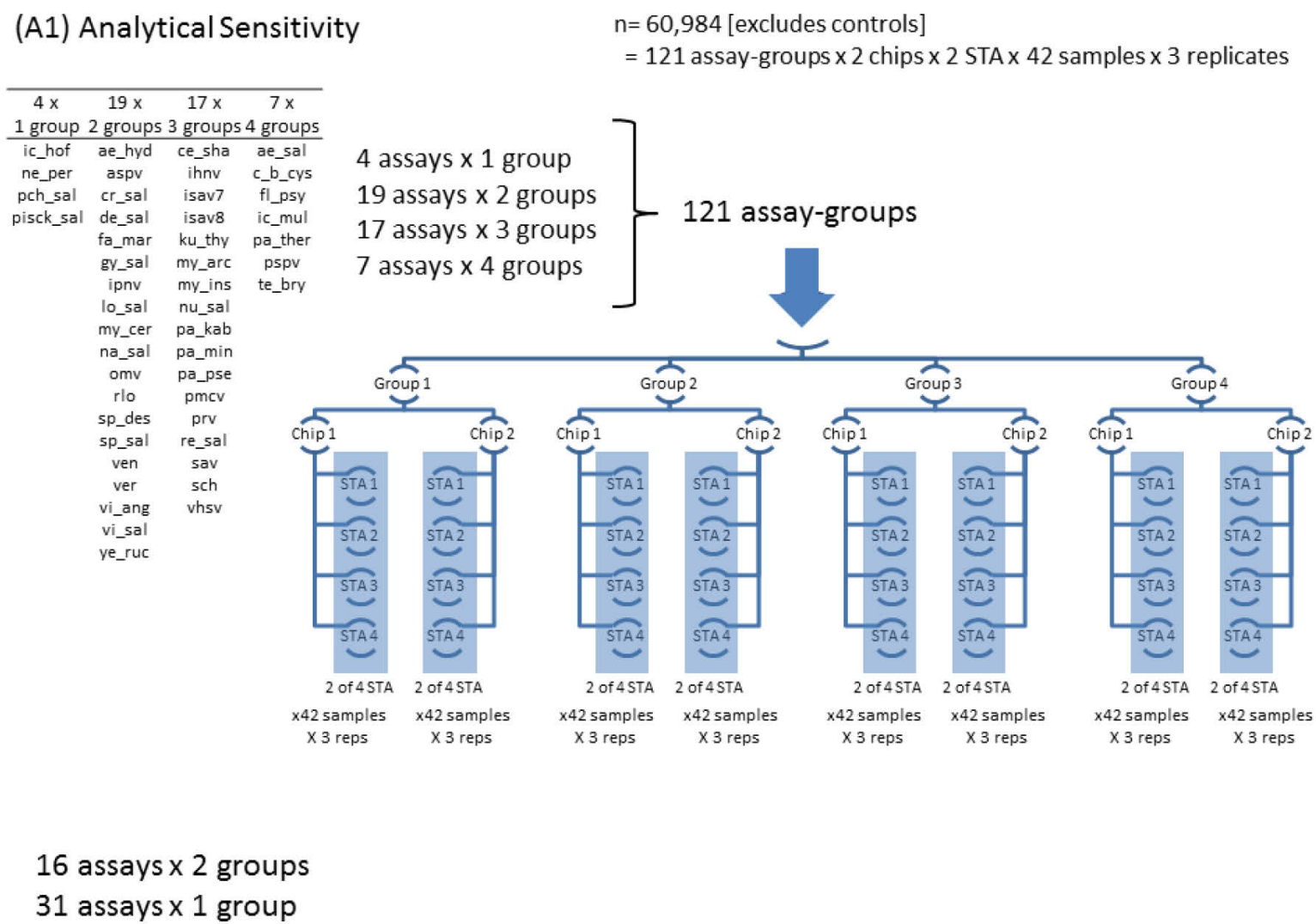


Figure 5. Schematic representation of analytical sensitivity study.

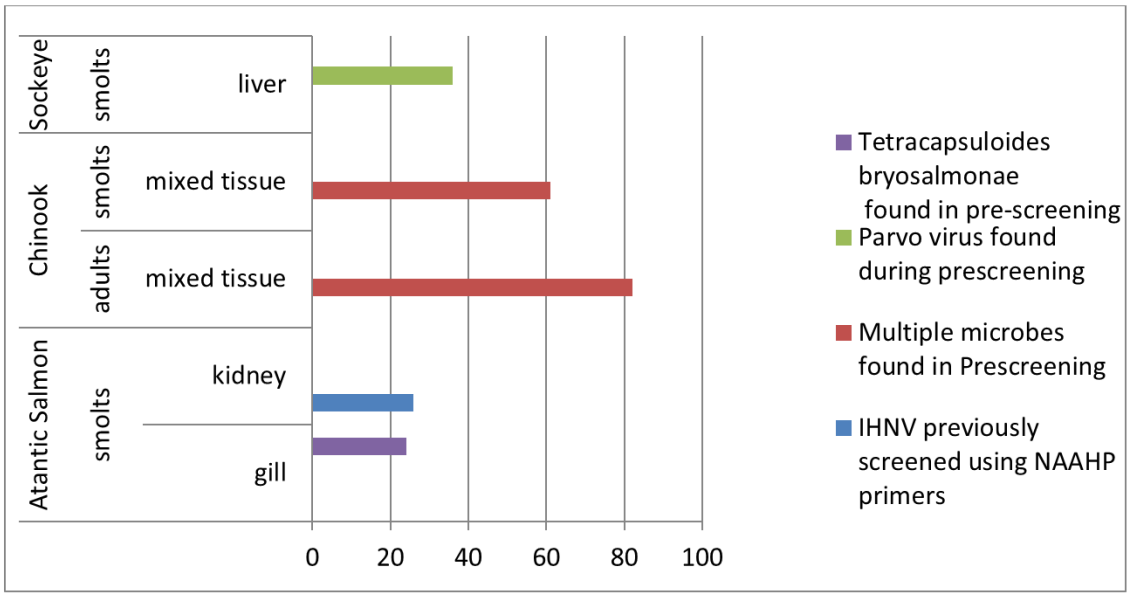
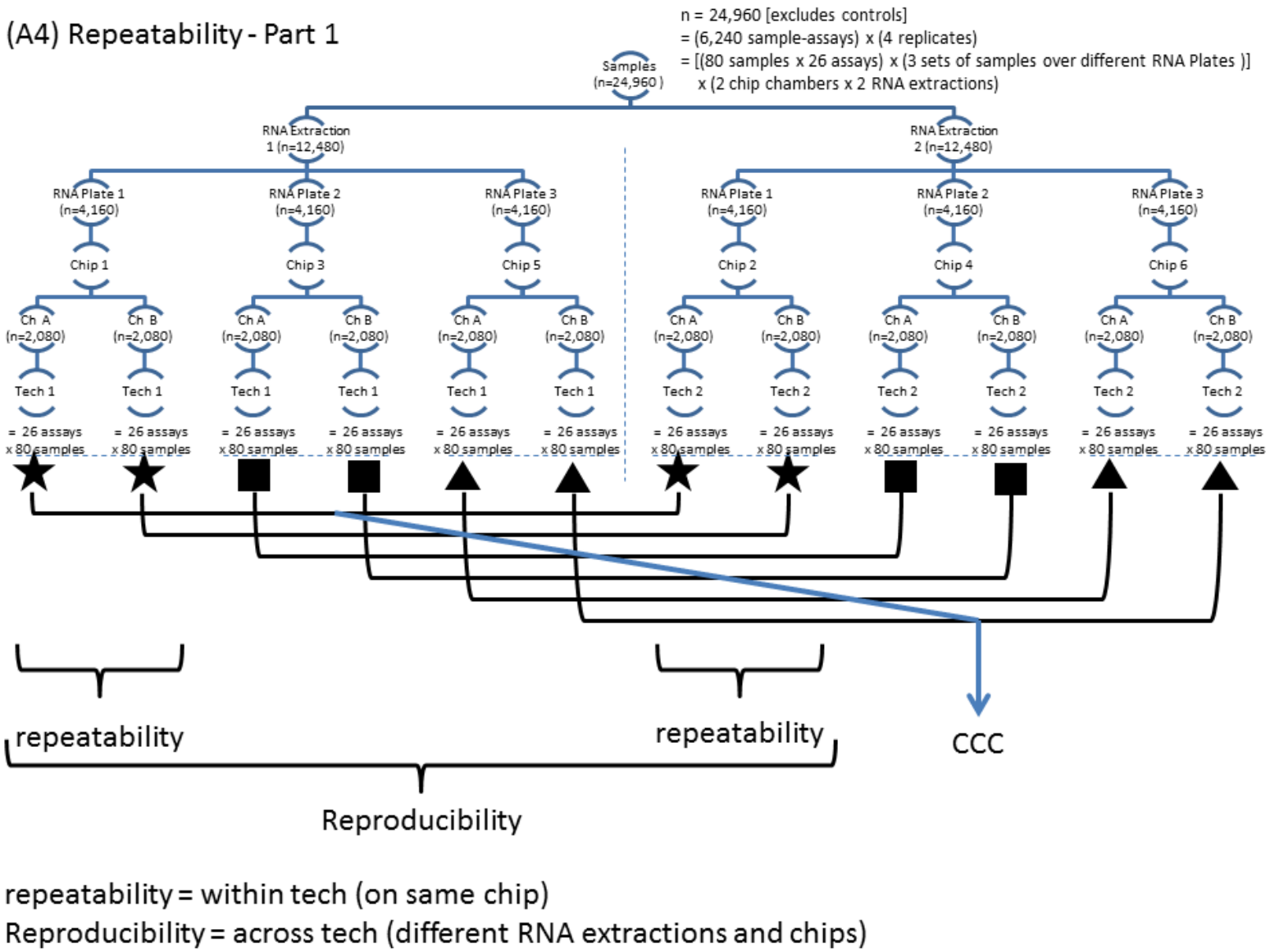


Figure 6. Pre-screened samples selected from mixed tissue (gill/liver/kidney/heart/brain) from Chinook smolts and adults provided a large number of microbe containing samples. Sockeye smolt liver and Atlantic Salmon kidney and gill samples were added to obtain the minimum 20 positives per microbe, samples positive for parvovirus, IHNV, and Tetracapsuloides bryosalmonae were added.



repeatability = within tech (on same chip)
Reproducibility = across tech (different RNA extractions and chips)

Figure 7. Schematic representation of repeatability study Part 1.

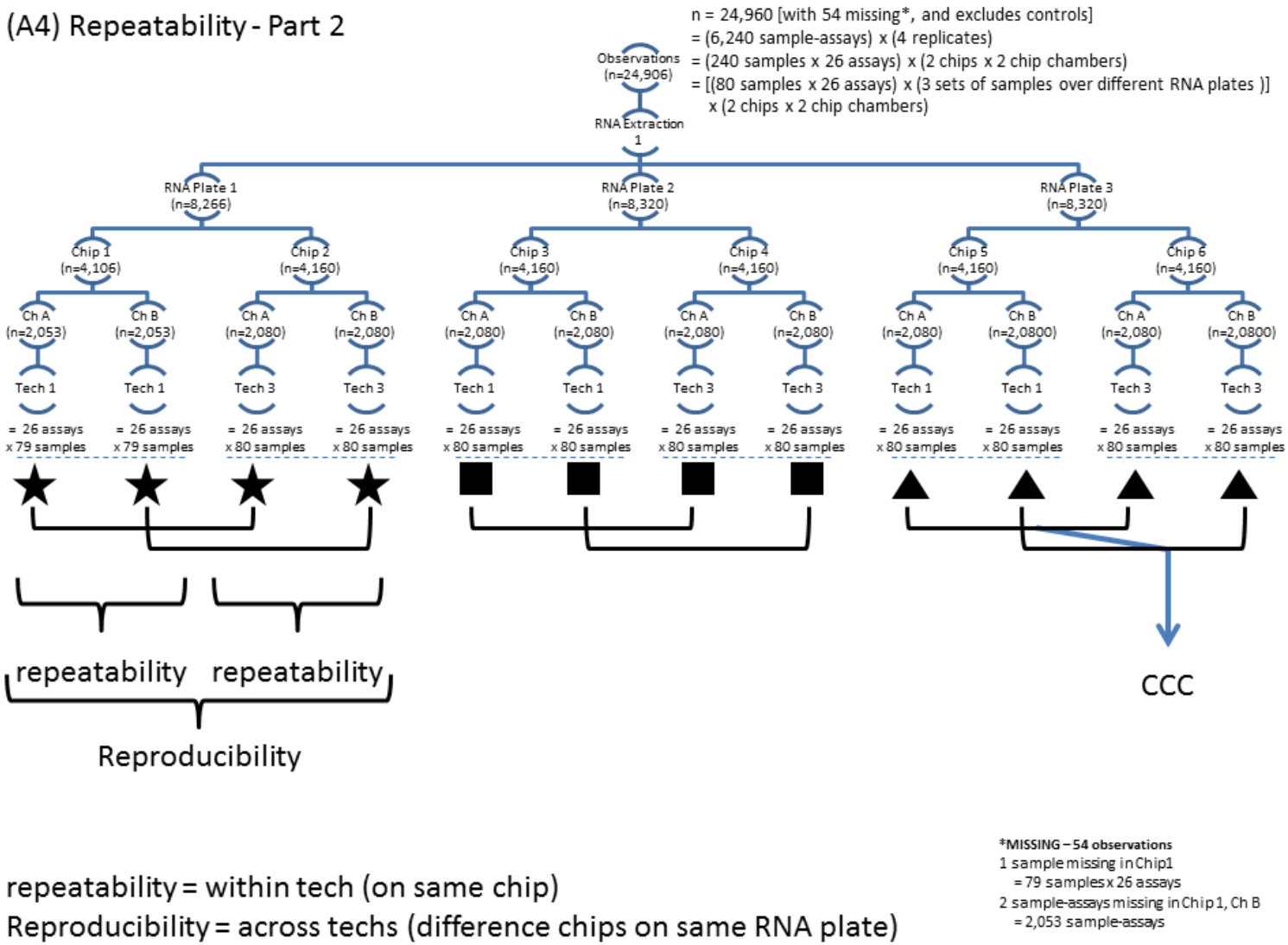
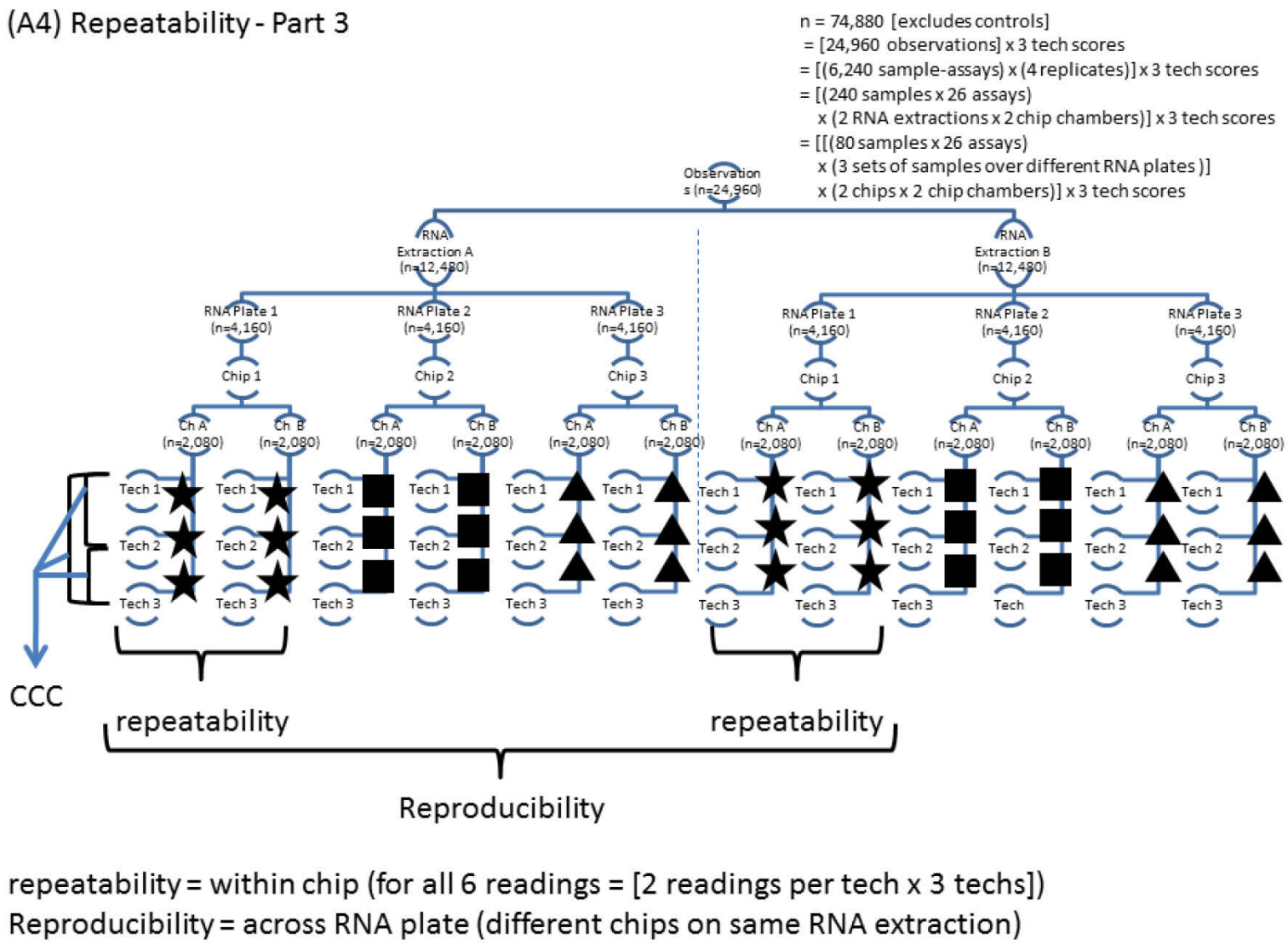


Figure 8. Schematic representation of repeatability study Part 2.



repeatability = within chip (for all 6 readings = [2 readings per tech x 3 techs])
Reproducibility = across RNA plate (different chips on same RNA extraction)

Figure 9. Schematic representation of repeatability study Part 3.

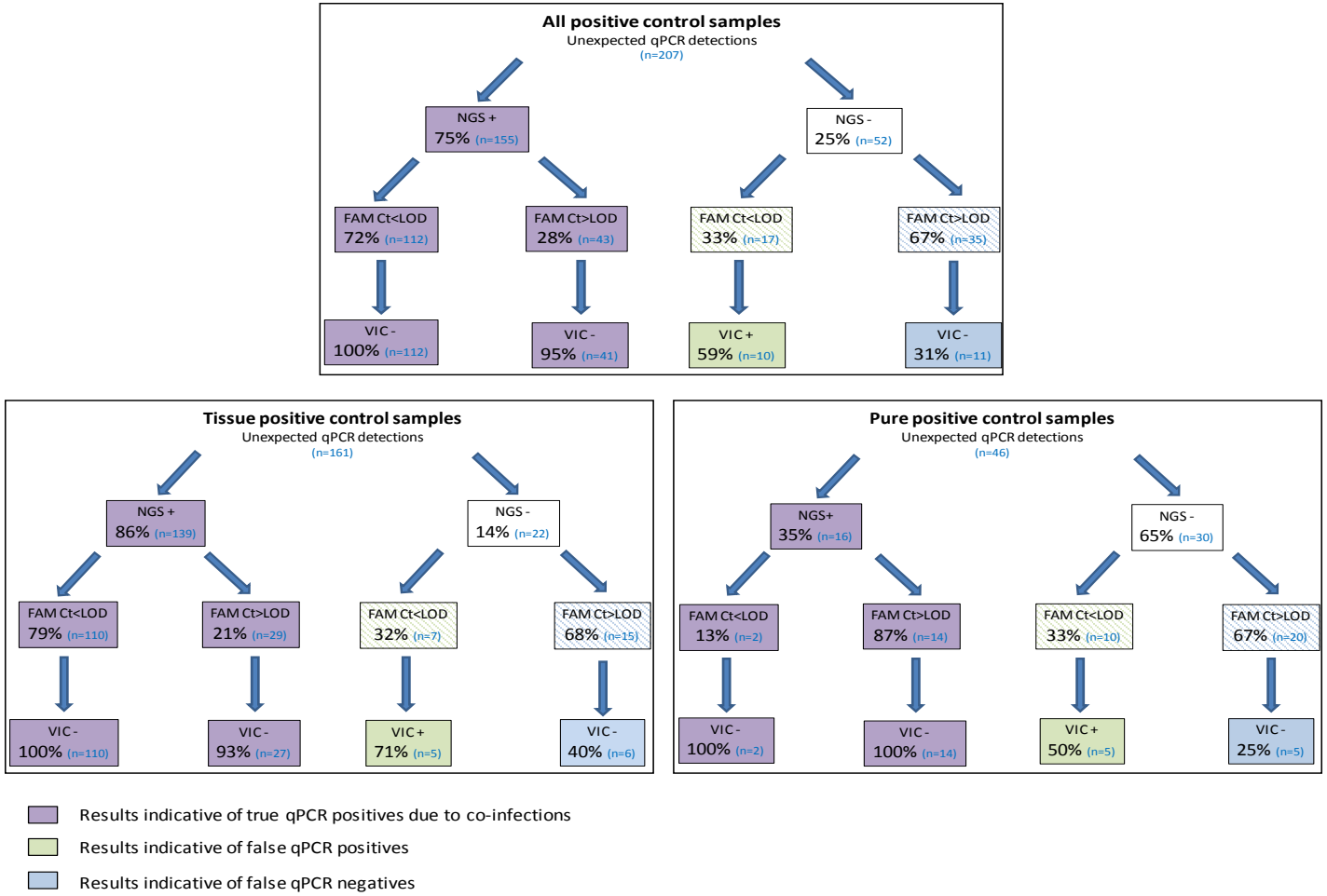


Figure 10. Schematic representation of the NGS validation results from the unexpected qPCR detections in the second analytical specificity analysis over all tissue and pure positive control samples (not including gBlocks). This particular analysis incorporates 85 samples analyzed over 43 assays in duplicate and does not include the following assays in the NGS library prep; ye_ruk assay was eliminated and the assays for pmcv, isav7 and vhsv were replaced. Note, that in this schematic the number (n) represents detections and not samples, but as the assays are run in duplicate, (n=2) typically refers to 1 sample. Validated (NGS +) and non-validated (NGS -) detections are further classified into those that are less than their assay-specific Ct-cutpoint (<LOD) or greater than their assay-specific Ct-cutpoint (>LOD) as well as by their corresponding VIC results. Finally, the results indicative of co-infections are colour-coded purple, those more likely indicative of false negatives or inconclusive are colour-coded blue, while those most likely indicative of contamination are colour-coded green.

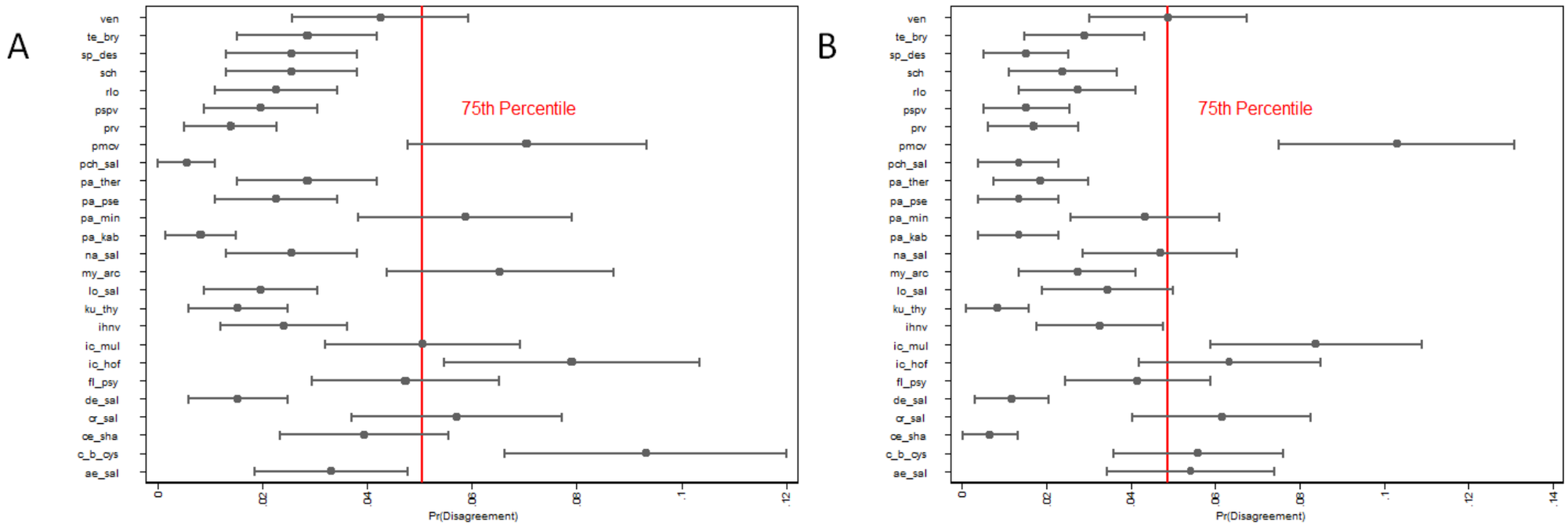


Figure 11. Probability of disagreement plots for each of the assays under Part 1 (A) and Part 2 (B) of the repeatability study based on only the real samples (no controls). Red line for indicates the 75th percentile of probability of disagreement (Pr(disagreement)=0.051 for (A) and 0.049 for (B)). Those assays to the right of the line are the worst 25%. %. Boxplots showing the Ct distribution for each microbe in the repeatability study (C).

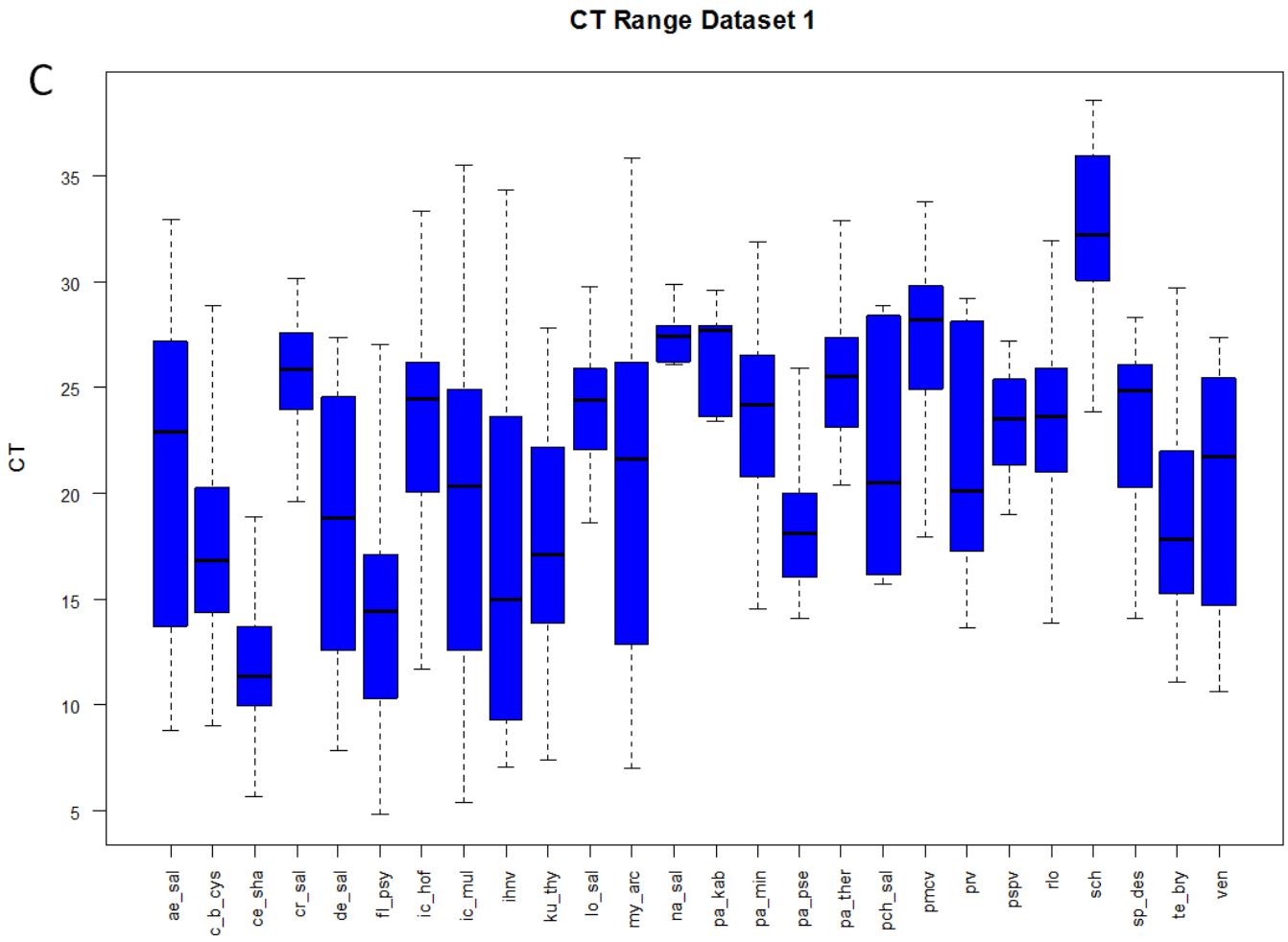


Figure 11. (Continued.)

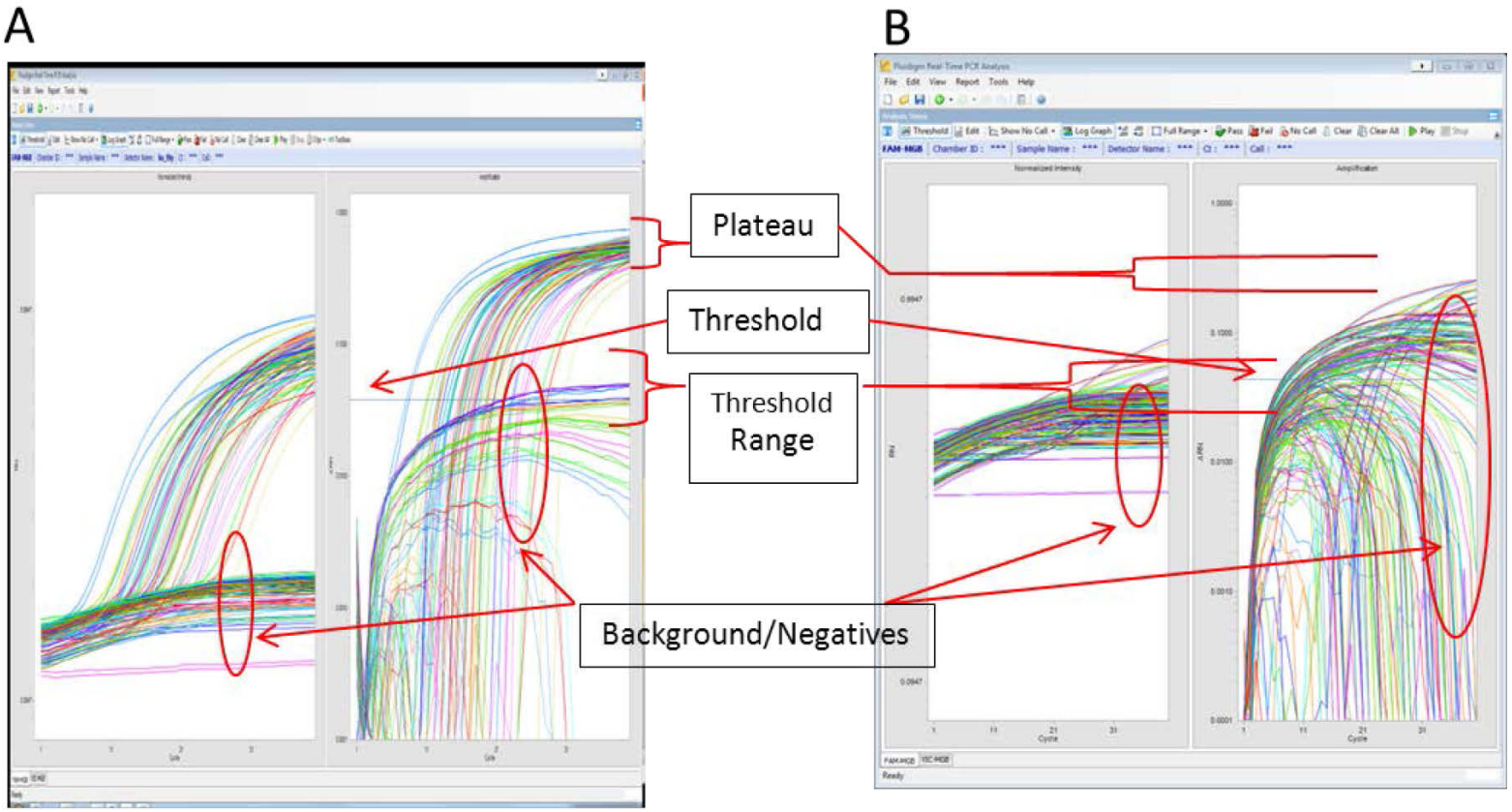


Figure 12. Amplification plots from the BioMark showing an overview of scoring and terminology. An overview of amplification plots produced by the BioMark Real Time PCR Analysis software. (A) is a "Good" assay, represented by ku_thy and (B) is a poor quality assay represented by pch_sal. Good assays have a long, vertical linear portion of the exponential phase, an even plateau region, and low background amplification. Less ideal assays show very little distance between background/negative samples and the plateau region, and the linear phase is short and not very vertical. The threshold is determined by the user for each assay. Given the short linear phase of assays with poor quality amplification curves, the placement of the threshold is critical to the dynamic range of the assay.

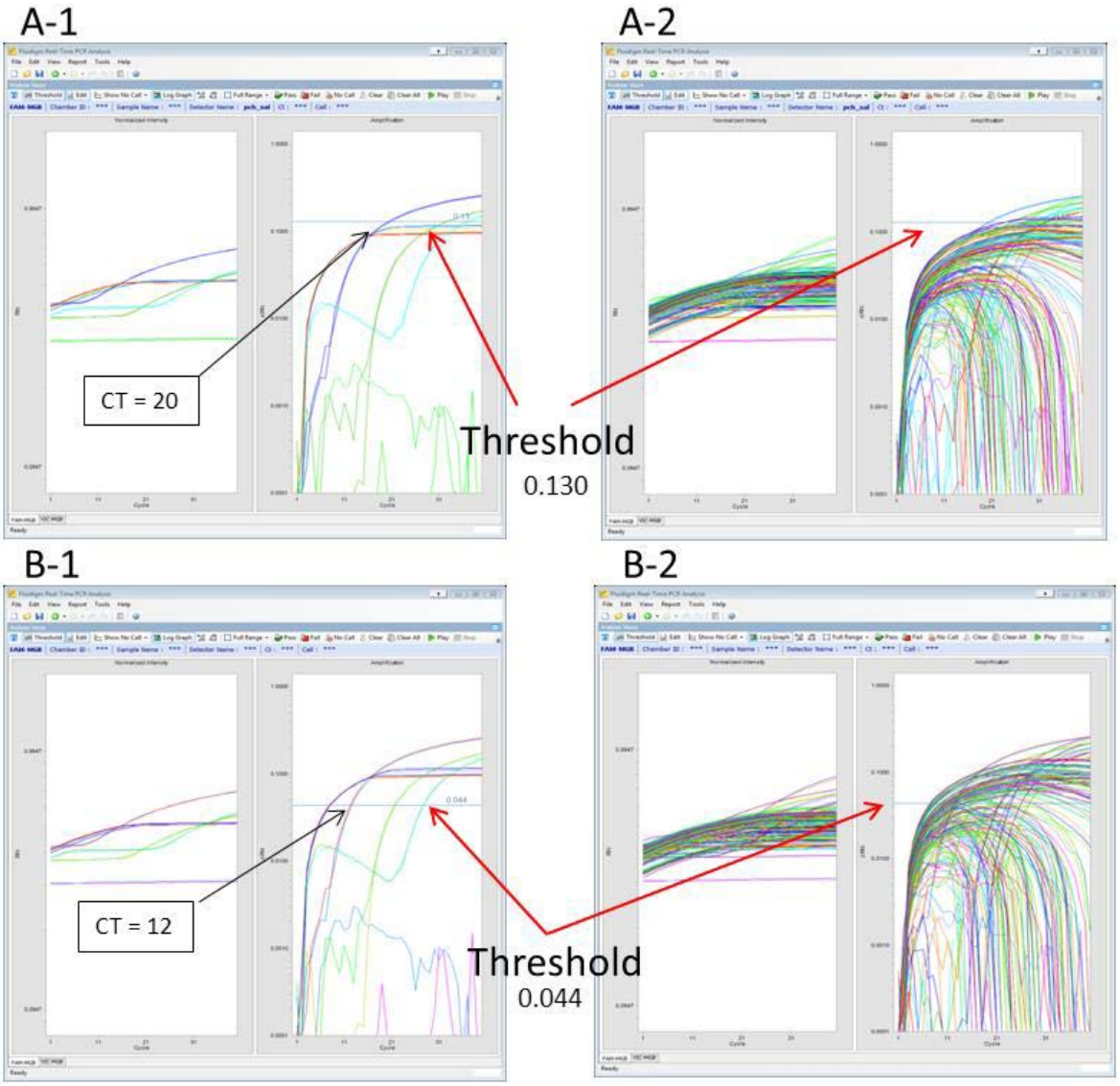


Figure 13. Amplification plots from the BioMark demonstrating the differences resulting from creating qPCR scoring SOPs. An example of changes in scoring SOPs developed to increase repeatability using pch_sal as an example. (A-1) (Selected control samples) and (A-2) (all samples from one dynamic array) as scored prior to creating a scoring protocol. Normally, the threshold is placed just above the background or negative amplification curves. This generally resulted in a higher threshold, which in turn can result in much higher CT values as the threshold may be placed above the linear portion of the curve. This effect is amplified when the exponential phase of an assay is not very linear, as it severely reduces the dynamic range. This method is also then more susceptible to run-to-run variation, as for some assays, the background levels can change with the quality of samples being analyzed. The new scoring SOP places the threshold in the linear portion of the curve, regardless of the background/negative plots as seen in B-1 (selected controls) and B-2 (all samples from the same dynamic array). This method provides a more consistent threshold placement, and therefore enhances the dynamic range and reduces run-to-run variation in Ct as it is no longer dependant on background/negative samples. The software will automatically assess the curve quality of each sample to determine if the sample is a pass or fail, which removes most background negative samples; the user can confirm or can adjust this determination.

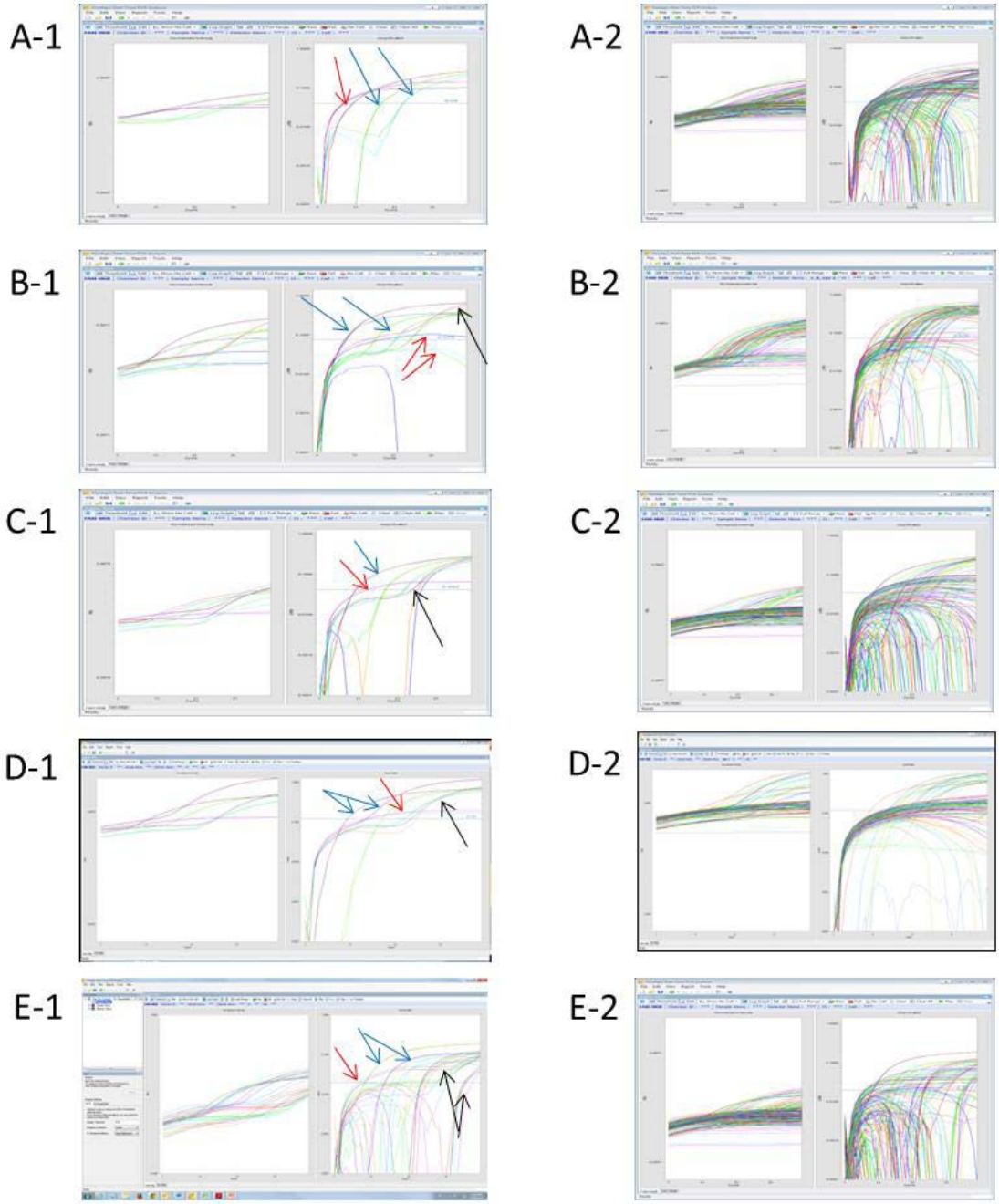


Figure 14. BioMark outputs for five assays with atypical amplification curves. A – ae_sal, B – c_b_cys, C – pmcv, D – ven and E - sch. Panel 1 shows selected samples and Panel 2 shows all samples for the selected assay from one chip run. Red arrows indicate negatives, blue arrows indicate positive samples and black arrows highlight samples in which the threshold placement may also affect the pass/fail determination of a sample, in addition to a change in CT value. These assays often have a very short, and not very vertical, linear phase. Small changes in threshold placement can lead to significant changes to CT values, and assay-specific scoring protocols have been deemed to be necessary to ensure accuracy and repeatability.

(A5) Comparability

n = 6,930 [excludes controls]
= 3,465 on each BioMark and ABI7900 system
= (21 assays) x (~80 samples) x (2 replicates) x (2 PCR systems)

Assay	Replicate		Total
	1	2	
ae_sal	79	80	159
c_b_cys	80	80	160
ce_sha	72	72	144
cr_sal	80	80	160
de_sal	80	80	160
fl_psy	80	80	160
ic_hof	80	80	160
ic_mul	149	149	298
ihnv	80	80	160
ku_thy	72	72	144
lo_sal	80	80	160
my_arc	80	80	160
pa_min	80	80	160
pa_pse	80	80	160
pa_ther	80	80	160
pspv	80	80	160
rlo	80	80	160
sch	80	80	160
sp_des	80	80	160
te_bry	80	80	160
ven	80	80	160
Total	1,732	1,733	3,465

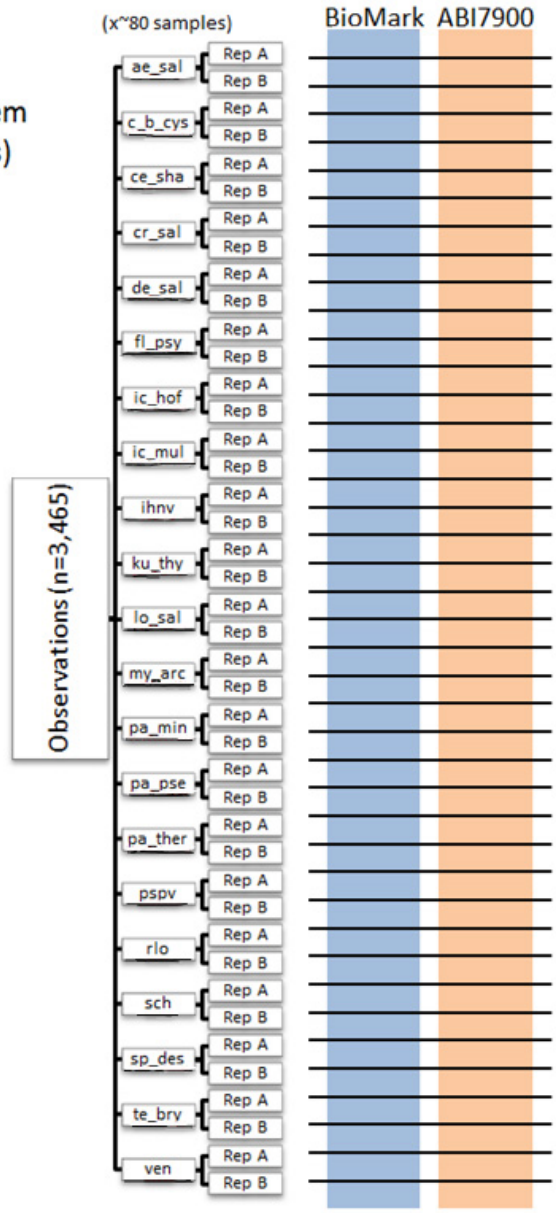


Figure 15. Schematic representation of consistency across platforms (i.e. comparability) study.

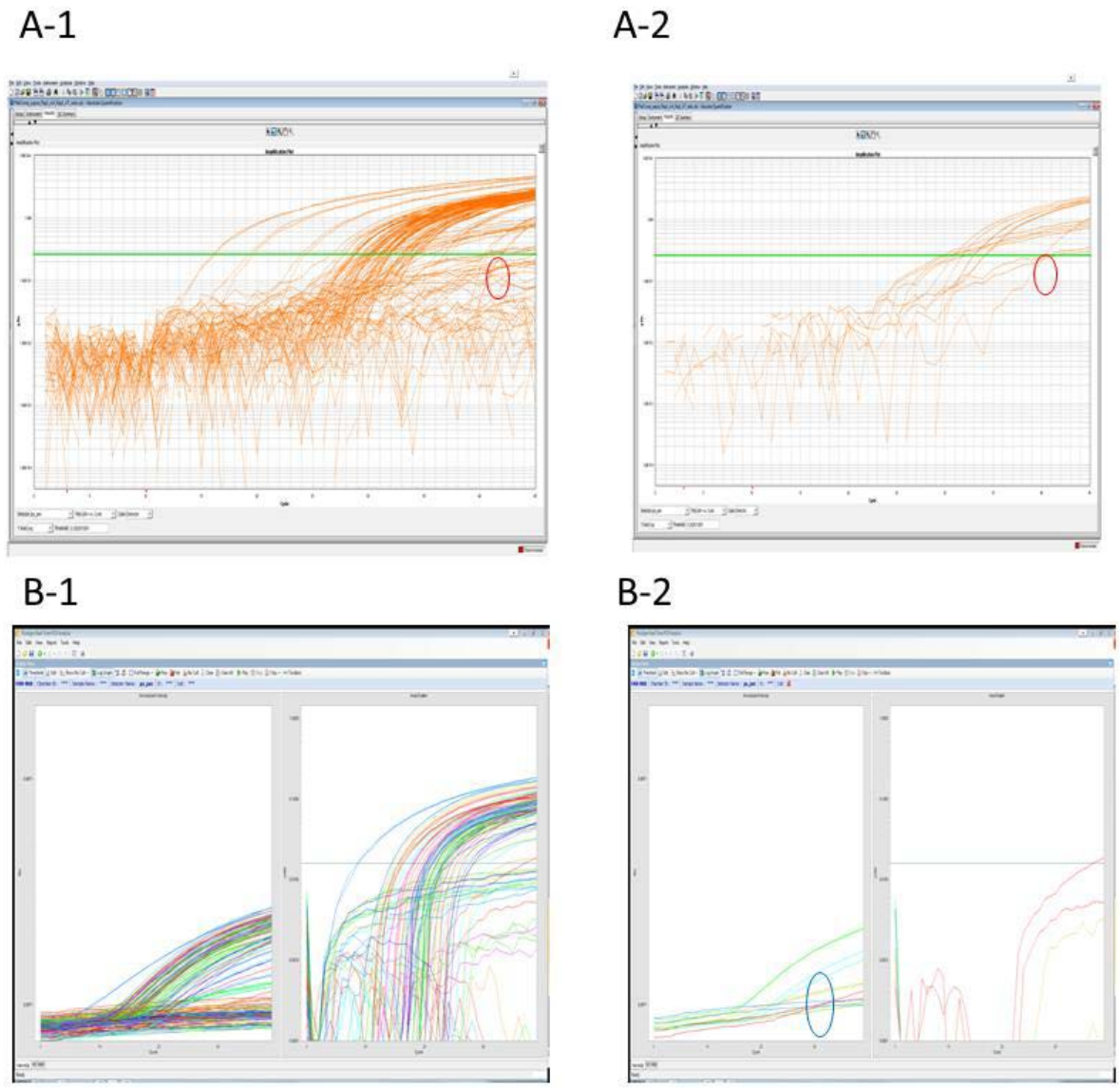


Figure 16. Amplification plots from the ABI 7900 (A) and BioMark (B) demonstrating some reasons for platform-to-platform variation of the pa_pse assay. A-1 (all samples) and A-2 (selected samples with ABI/BioMark discrepancies) from one ABI 7900 pa_pse run as scored by the SDS software (ABI), with user intervention for baseline and threshold adjustments. Red circles highlight samples with poor amplification plots on the ABI7900, but which were still scored by the ABI software as positive (and negative according to the BioMark output). Samples exhibiting these poor amplification plots should be flagged and regarded as unreliable. B-1 (all samples) and B-2 (selected samples with ABI/BioMark discrepancies) as scored by Real Time PCR Analysis software (Fluidigm), with user intervention for threshold adjustment. The blue circle highlights samples appear to show an increase in fluorescence (left side of B-2) consistent with a positive sample, however the amplification plot (right side of B-2) has these samples as negative. These samples showed positive amplification plots on the ABI 7900, and in this case the BioMark results should be flagged and regarded as unreliable. This demonstrated the importance of technician expertise to recognize abnormalities in the data outputs of each platform. Each of these issues will be brought to the attention of the respective software companies.

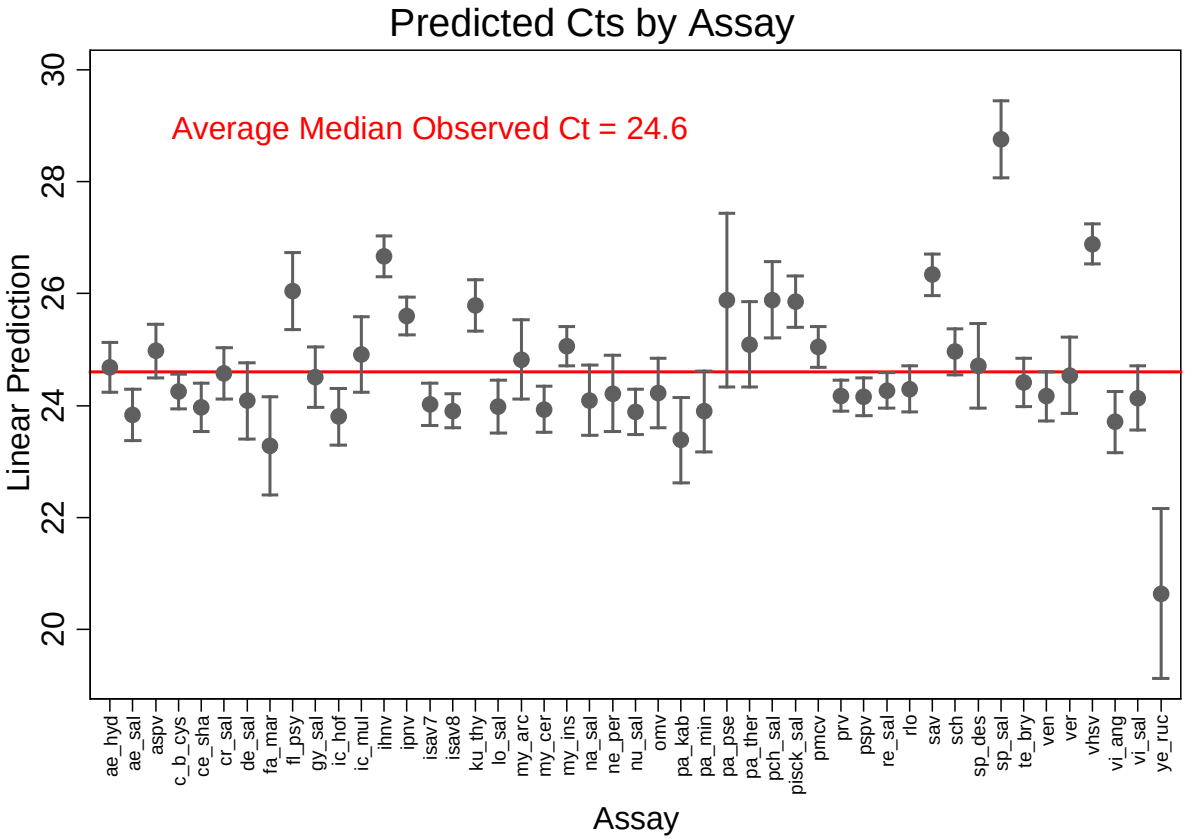


Figure 17. Predicted Cts for each assay used in the ASe study, based on modeling the observed median Cts (n=670) by linear regression with expected Cts and assays as fixed effects. No random effects were required to model this relationship. Four assays were deemed potential outliers (vhsv, sp_sal, ihnv, and fa_mar), but were left in the model to show their estimates.

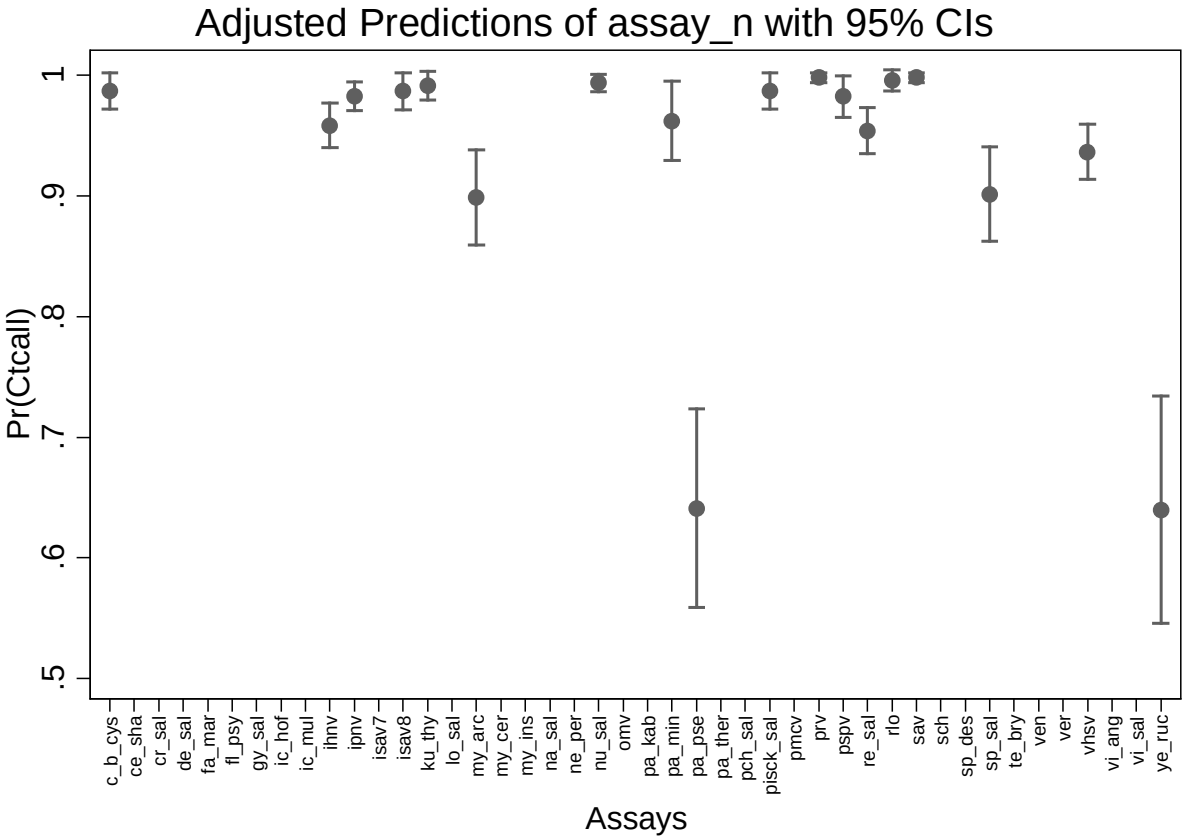


Figure 18. Predicted probability of microbe detection for all samples in the ASe study with an expected positive outcome (n=13,776), by microbe assay. A logistic regression was used with machine derived Pass/Fail as the outcome and the microbe assay as the categorical predictor. Missing estimates (e.g. ce_sha to ic_mul) indicate a perfect prediction (100% detection) for those assays.

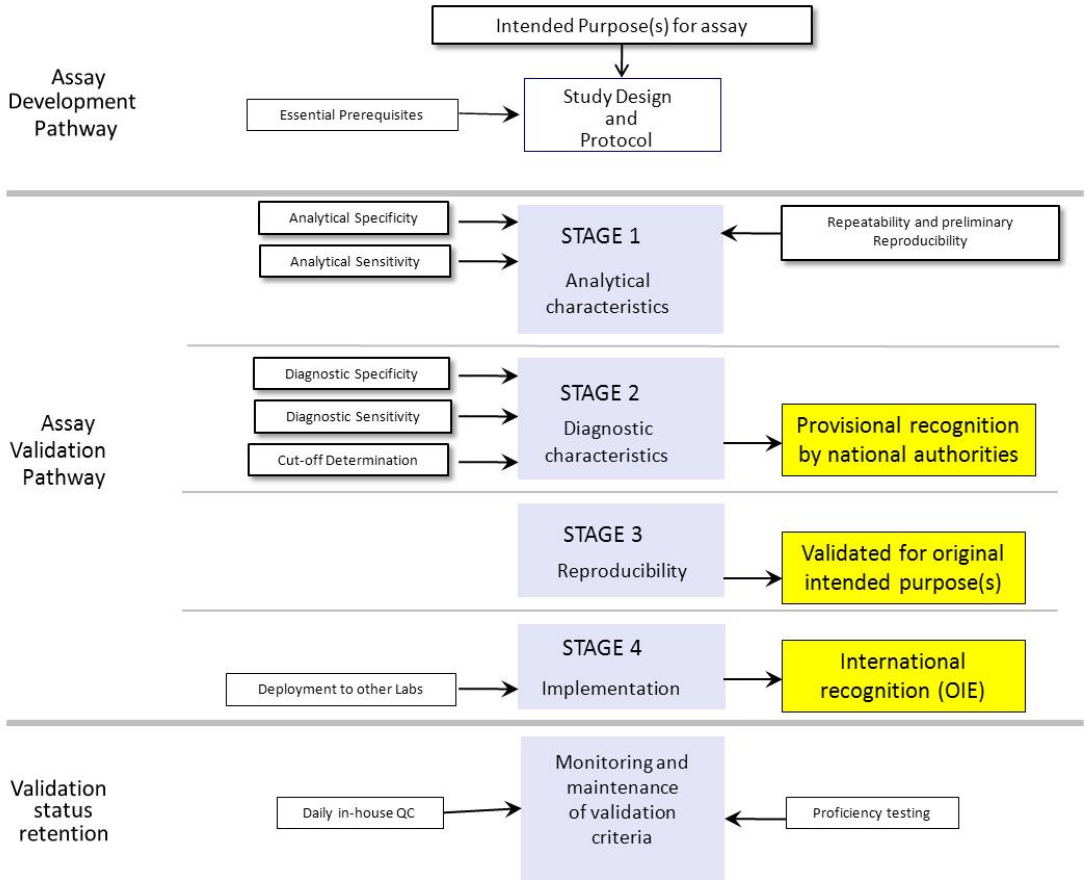


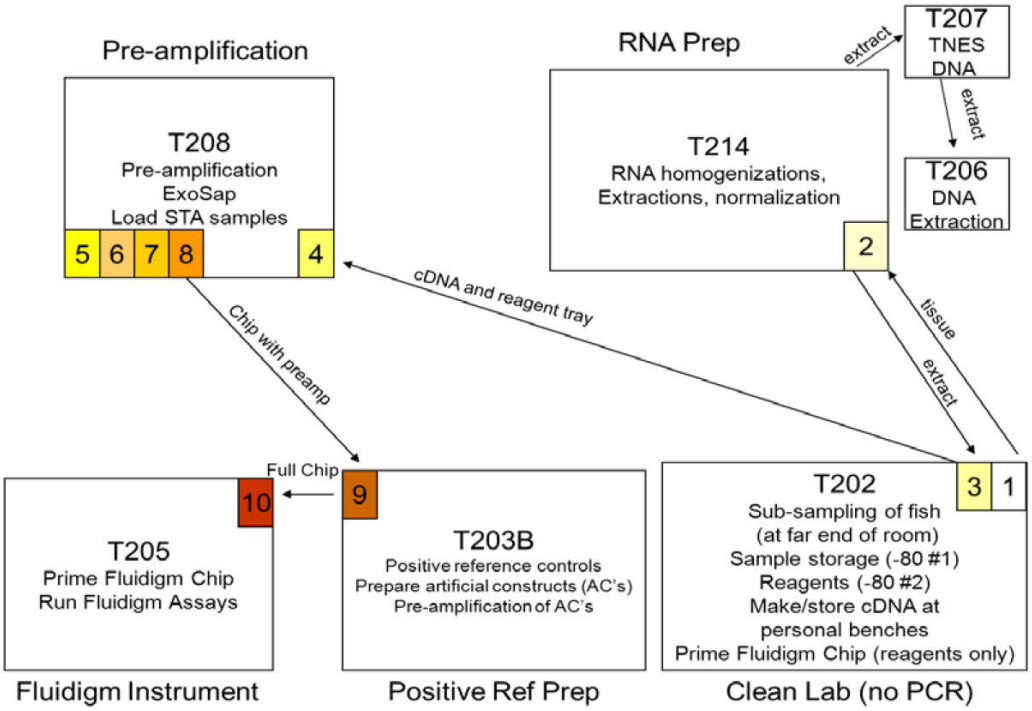
Figure 19. Stages of assay validation according to the OIE pathway, modified from Figure 1 in Chapter 1.1.2 (Principles and methods of validation of diagnostic assays for infectious diseases) of the Manual of Diagnostic Tests for Aquatic Animals (OIE 2013).

APPENDIX

CONTROL SAMPLES

Supplemental Table 1. APC clone sequences for microbe assays evaluated herein. The APC probe was inserted as small letters without bold. Newly added (replacement) assays are shown with an asterix (*).

Microbe full name	Type	Organism abbreviation	APC clone size	APC clone seq
Aeromonas hydrophila	Bacterium	ae_hyd	145	CCATGGTAATACGACTCACTATA#GGGCGATCCTGGT**ACCGCTGCTCATTACTCTGATG**T**tgatggtgagctggttg**Gaccgtctagcatccagt**TTCTTCCCGTCTGGGTTGG**TCCGGTTTC#TATAGTGTCACCTAAATACTAGT
Aeromonas salmonicida	Bacterium	ae_sal	218	CCATGGTAATACGACTCACTATA#GGGCGA**TAAAGCACTGTCTGTTACCC**TGCCAAGCGGTGGTG**cagtgactctgaagcctgctgatgt**TGCTGCTGACGTTGGTGCTaccgtctagcatccagtTCTATCACTGCTGGCCGTCAGGCTCGCTTCCTGTTTGAAGTTGAAA**CCAATCAGGGTGAAGTAGC**TTTC#TATAGTGTCACCTAAATACTAGT
Candidatus Branchiomonas cysticola	Bacterium	c_b_cys	205	CCATGGTAATACGACTCACTATA#GGGCGATGAGT**AATACATCGGAACGTGTCTAGTG**GTGGGGGATAACTTAGCGAaccgtctagcatccagtAAGTTAAGCTAATACCGCATAAGCT**TGGGAGAGGAAAGCCTGGGACCGAG**AGGCCAGGCGC**CACATGAGCGGCTGATGGC**TGATTTTC#TATAGTGTCACCTAAATACTAGT
Flavobacterium psychrophilum	Bacterium	fl_psy	161	CCATGGTAATACGACTCACTATA#GGGCGACTATGGAT**TGTAAACTGCTTTTGCACAGGAA**G**aaacactcggtcgtgacc**GAaccgtctagcatccagtGC**TTGACGGTACTGTGAGAATAAGGATC**GGCTAACTCTTC#TATAGTGTCACCTAAATACTAGT
Gill chlamydia	Bacterium	sch	153	CCATGGTAATACGACTCACTATA#GGGCGAAGCTGATAT**CCCATGAGCCGCTCTCTCT**CC**gtaaggtcccgaagga**TCCaccgtctagcatccagtCCT**ACTTTGAAGATATCGGGCTACCC**CGGATCTTC#TATAGTGTCACCTAAATACTAGT
Piscichlamydia salmonis	Bacterium	pch_sal	150	CCATGGTAATACGACTCACTATA#GGGCGAATCTTGGGGC**TCACCCCCAGGCTGCTT**C**caaaactgctagactagagt**Taccgtctagcatccagt**CAAGAGGGGGAAATGGAATTC**CACAAGTATTC#TATAGTGTCACCTAAATACTAGT
Piscirickettsia salmonis	Bacterium	pisck_sal	160	CCATGGTAATACGACTCACTATA#GGGCGAAACAC**TCTGGGAAGTGTGGCGATAG**AGGG**tgatagccccgtacacgaaacggcata**TTaccgtctagcatccagtA**GATGAAACAAGAGTAGGTCGGGA**CACGTTTC#TATAGTGTCACCTAAATACTAGT
Renibacterium salmoninarum	Bacterium	re_sal	180	CCATGGTAATACGACTCACTATA#GGGCGAACATTTTT**CAACAGGGTGGTTATTCTGCTTTC**TTTCACGAGTTAAaccgtctagcatccagtGGCCTGACGGGAC**ctccagcgccgcaggaggac**CAG**TTGCAGCTGGTGGCTCTTATAG**TTCTTTC#TATAGTGTCACCTAAATACTAGT
Rickettsia-like organism	Bacterium	rlo	228	CCATGGTAATACGACTCACTATA#GGGCGACTTG**GGCTCAACCCAAGAACTGCT**TTTGAAACTGCTAAGCTTGAGTACTGTAGAGGATAGCGGAATTCCTAGTGTAGAGGTGAAaccgtctagcatccagtATTCGTAGATATTAGGAGGAACAC**cggaggcgaaggcggttatctggg**C**AGTCACTGACGCTGTTGCAC**GAATTC#TATAGTGTCACCTAAATACTAGT
Vibrio anguillarum	Bacterium	vi_ang	176	CCATGGTAATACGACTCACTATA#GGGCGAACTGT**CCGTCATGCTATCTAGAGATGTATTTGA**TAGGTATGG**tcatttcgacgagcgtcttgttcagc**TACaccgtctagcatccagtAGGATTT**TGATTTTTGGCTGCGTATGG**CCTCTATTC#TATAGTGTCACCTAAATACTAGT
Vibrio salmonicida	Bacterium	vi_sal	218	CCATGGTAATACGACTCACTATA#GGGCGATAAGTGTGATGACCGTTCCATATTTAATGTCGCTTCATGTTGTGTAATTAGGAGCGATACTCTGTAAAGTTATACAGGTTTACCTAAATAATTACCCTGTTATAGTTTTCTAAACCGTCTAGCATCCAGTAATAAAGAAACAGAGTGATGACAATAGCTTTATTC#TATAGTGTCACCTAAATACTAGT
Yersinia ruckeri	Bacterium	ye_ruc	196	CCATGGTAATACGACTCACTATA#GGGCGAA**TGCCGCGTGTGTGAAGAA**GGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAGGAAGGGTTAAGTGTT**aatagcactgaacattgac**GTTAaccgtctagcatccagtCTCGCAGAAG**AAGCACCGGCTAACTCCGT**GCCATTC#TATAGTGTCACCTAAATACTAGT



Supplemental Figure 1. BioMark Microbe Assay laboratory flow chart. "Chip" refers to the Fluidigm dynamic array.

Laboratory Design

We utilize seven separate rooms to conduct microbe monitoring research (Figure 9) and evaluate test performance as described in prior sections.

1. The first is the clean room (T202), where our individual benches are located and reagents are stored. This is a long room, and at one bench at the far end of the room is where fish dissections occur; these are well separated from individual lab bench spaces. Individual technician lab bench spaces are used to prepare assay reagents (minus nucleic acids). cDNA is made in a dedicated PCR machine. Two of our -80 freezers are in this room, one dedicated to kits/reagents, the other to working tissue storage (no amplified products).
2. The RNA extraction room (T214) is where tissue homogenates are obtained (in a hood), plates are prepared for RNA/DNA extraction, robotic RNA extractions are performed, RNA/DNA is normalized, and cDNA trays are set-up in a dedicated UV hood with dedicated pipettes. DNA extractions are performed in our dedicated DNA extraction rooms (T206 and T207).
3. Our sample preparation/pre-amplification room (T208) is used to prepare samples for pre-amplification, conduct ExoSap reactions, and to load pre-amplified (STA) samples onto the Fluidigm dynamic array. This prep work is done in a UV hood that is UV sterilized between uses. Pre-amplification occurs in an adjoining room; no other PCR occurs in this room. A separate bench top UV hood is used handle pre-amplified samples.
4. Plasmids and other positive control samples are prepared in a room separate from all others (T203B). Pre-amplification of plasmids also occurs in this room separately from other samples. Serially diluted plasmid controls are added last to the Fluidigm dynamic array in this room.

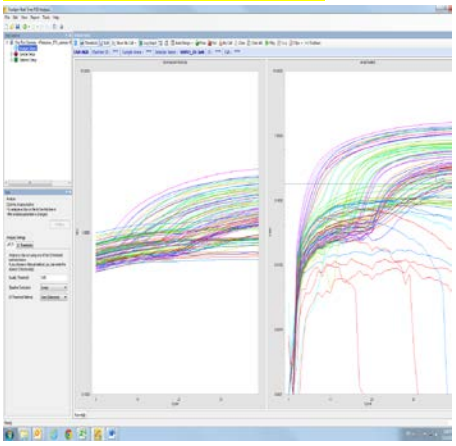
5. The BioMark is located in an equipment room (T205), along with the ABI 7900. Dynamic arrays are primed and run in the same room. Once primed, samples are enclosed in a plastic case and are not exposed to the environment. Hence, there is no potential for post-PCR contamination.

UV lamps were initiated prior to and the following the use of any laminar flow hoods. In addition to this, 10% bleach disinfection, RNase-Zap (Ambion Inc, Austin, TX, USA) and 95% EtOH was used for cleaning of lab benches and equipment prior to RNA work and 10% bleach and 95% EtOH was used prior to all DNA work. All equipment, labcoats, gloves and labware were allocated to specific work areas and did not travel between those.

ANALYTICAL SENSITIVITY

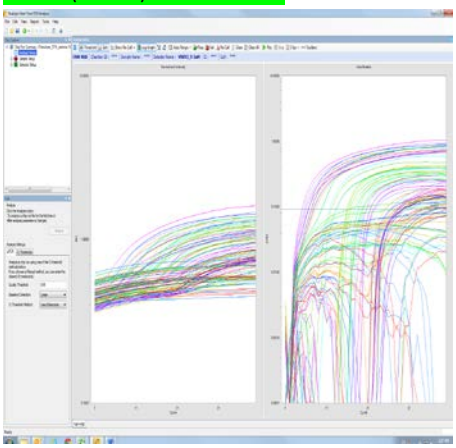
VHSV

10-3uM (VHSV2) current condition

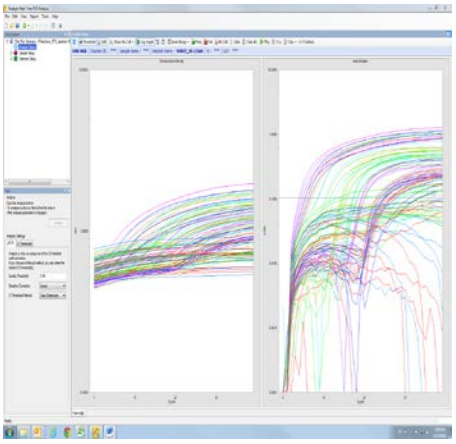


VHSV

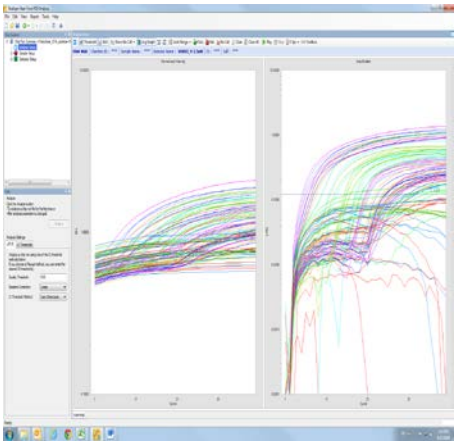
9-2uM (VHSV2) best condition



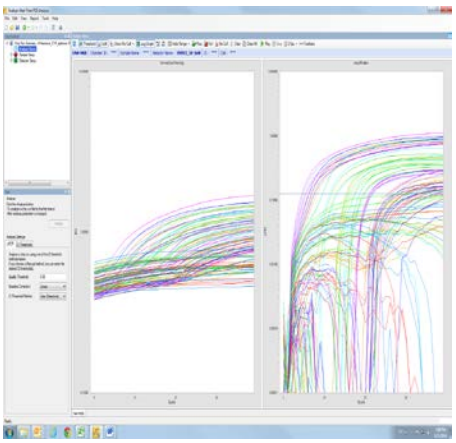
10-2.5uM(VHSV2)



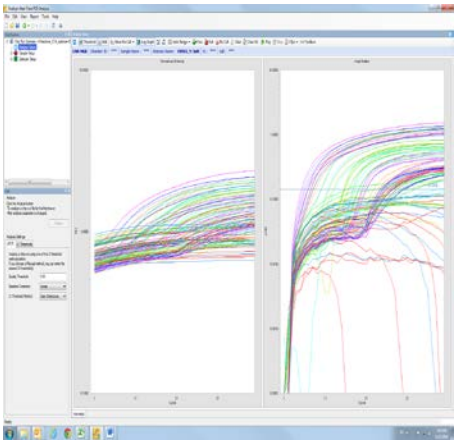
9-2.5uM (VHSV2)



10-2uM (VHSV2)



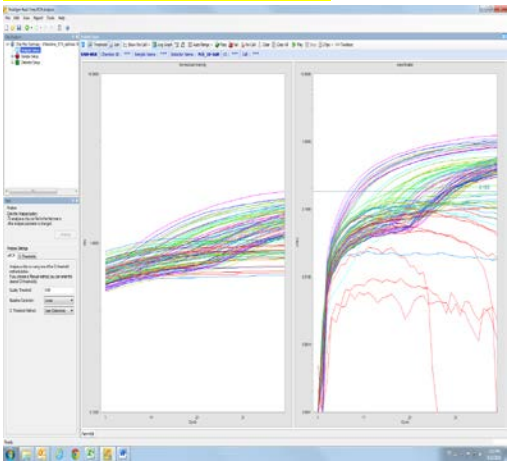
9-3uM(VHSV2)



Supplemental Figure 2. Five assays with high background and/or poor quality amplification curves were tested with different primer/probe concentrations in attempt to optimize performance and reduce background. Primer concentration was varied between 9 and 10µM and probe at 2, 2.5, and 3 µM. Currently we use 10µM/3µM primers/probe concentration, but found that 9µM/2µM primers/probe concentration was best for most assays, optimally separating true curves from background. By reducing background, this optimization enhanced the linear dynamic range by allowing the threshold to be placed lower in the linear portion of the curve. This worked especially well for problematic assays, as shown in the figures below. We also compared existing with alternate assays for vhsv and pmcv under both conditions.

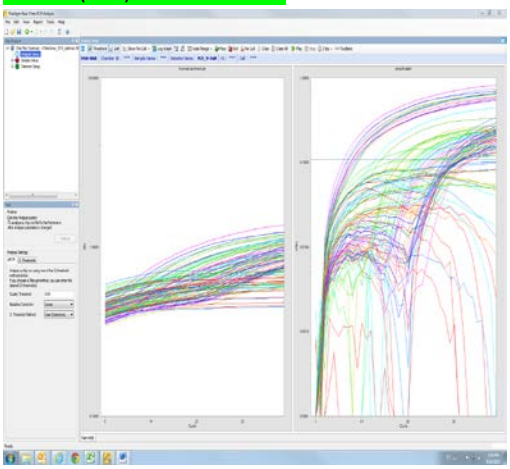
pch_sal

10-3uM (Pch) current condition

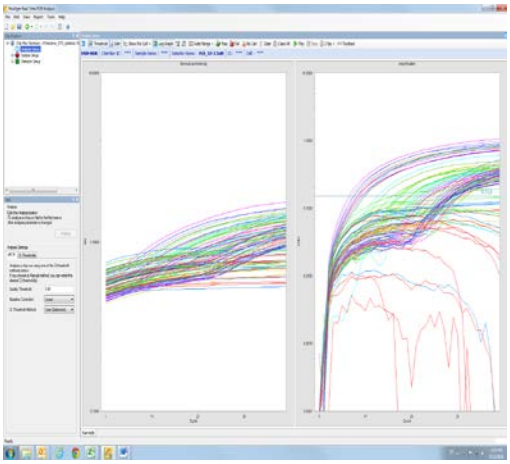


pch_sal

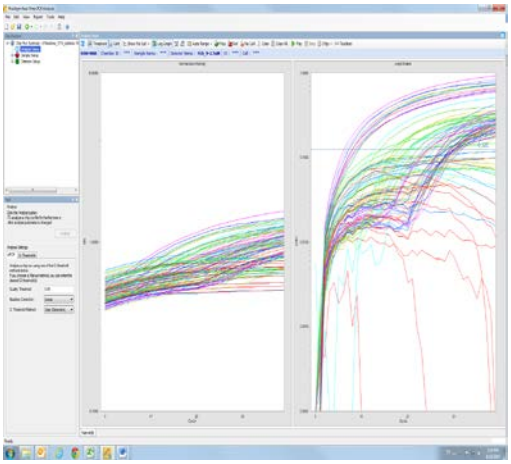
9-2uM (Pch) best condition



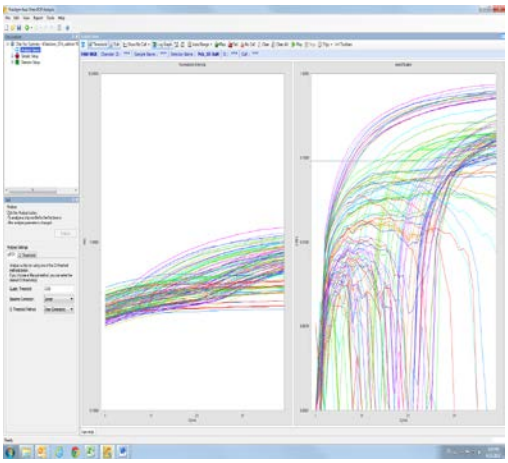
10-2.5uM (Pch)



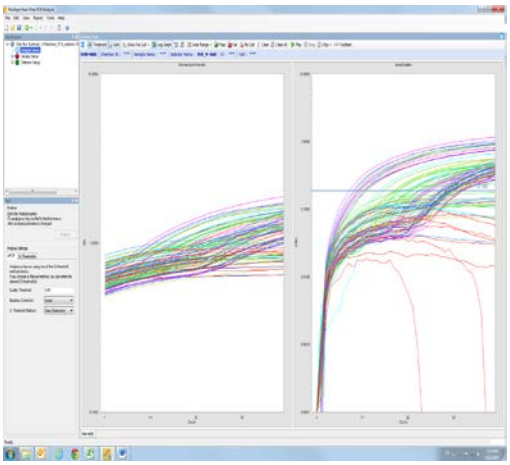
9-2.5uM (Pch)



10-2uM (Pch)



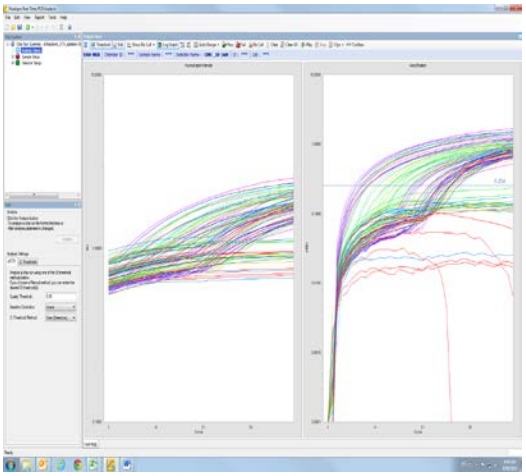
9-3uM (Pch)



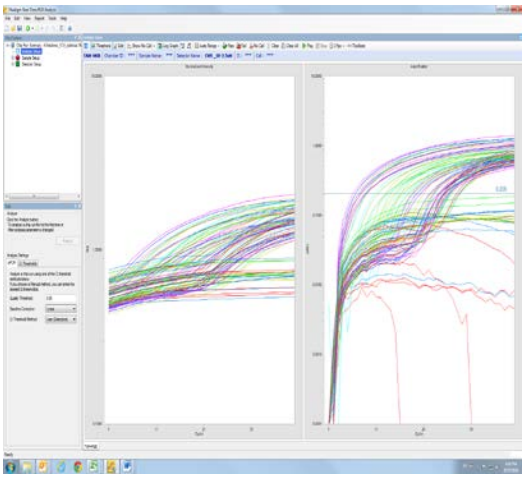
Supplemental Figure 2 (Continued)

PMCV

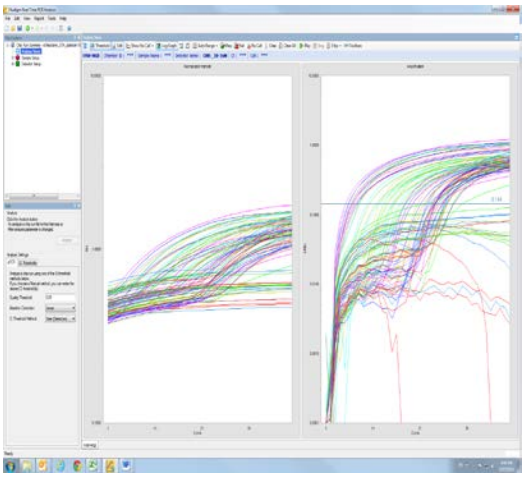
10-3uM (CMS) current condition



10-2.5uM(CMS)

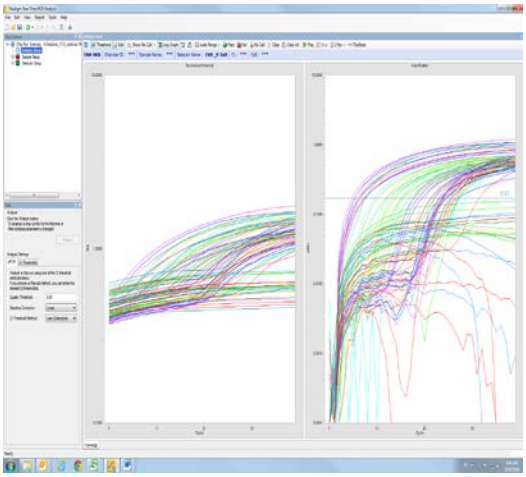


10-2uM (CMS)

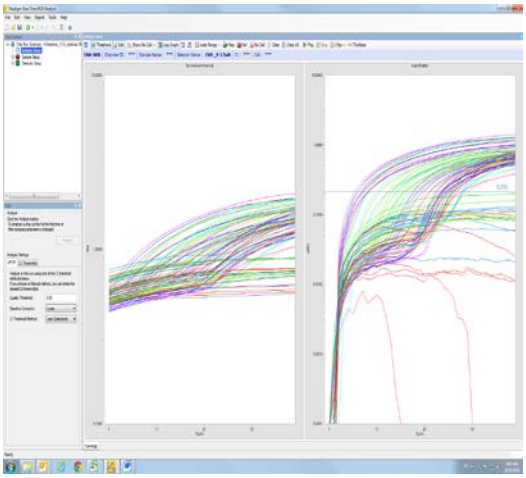


PMCV

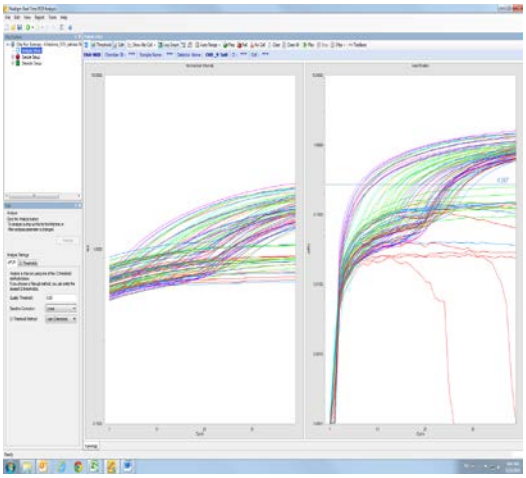
9-2uM (CMS) best condition



9-2.5uM (CMS)



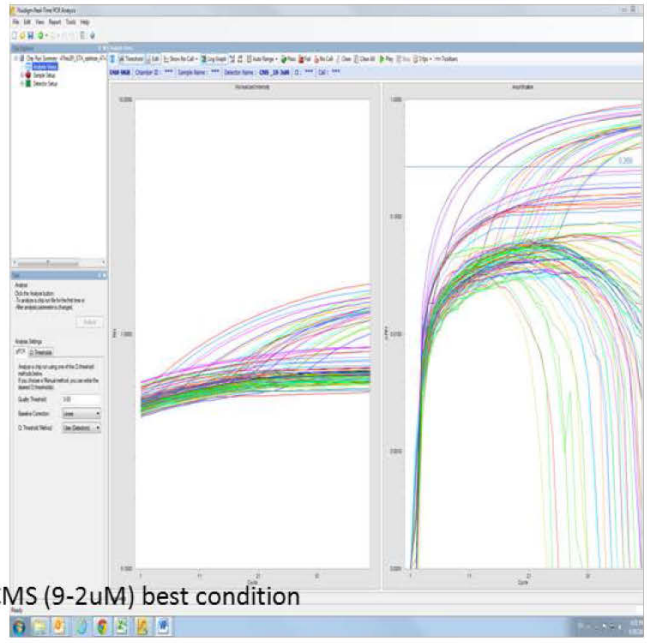
9-3uM (CMS)



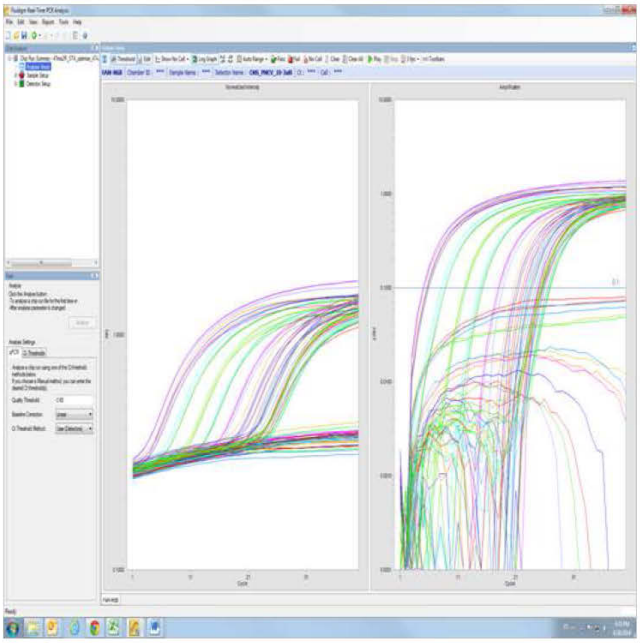
Supplemental Figure 2 (Continued)

PMCV (old)

CMS (10-3uM) current condition



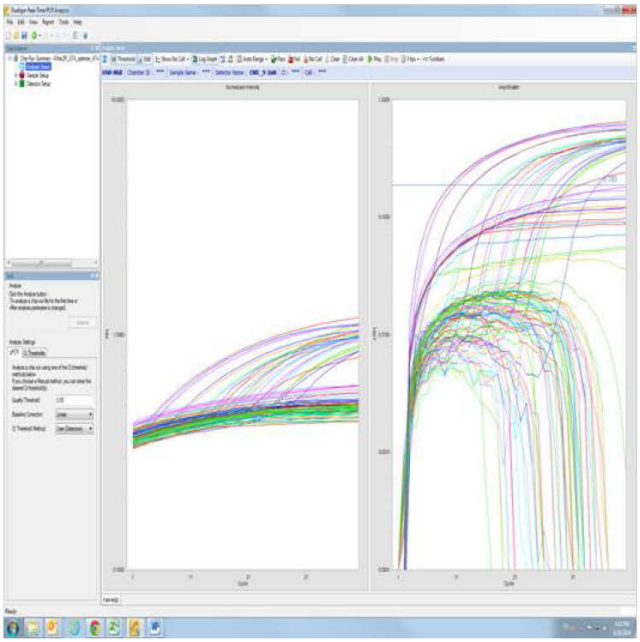
CMS (9-2uM) best condition



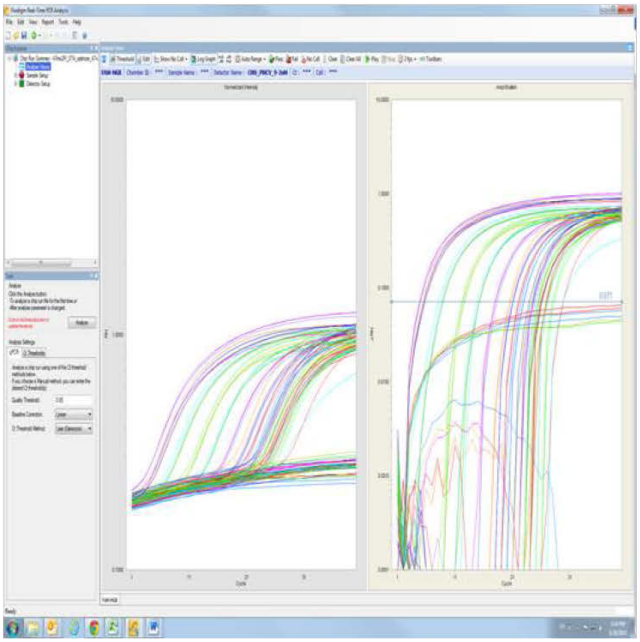
CMS (9-2uM) best condition

PMCV (new)

CMS_PMCV (10-3uM) current condition



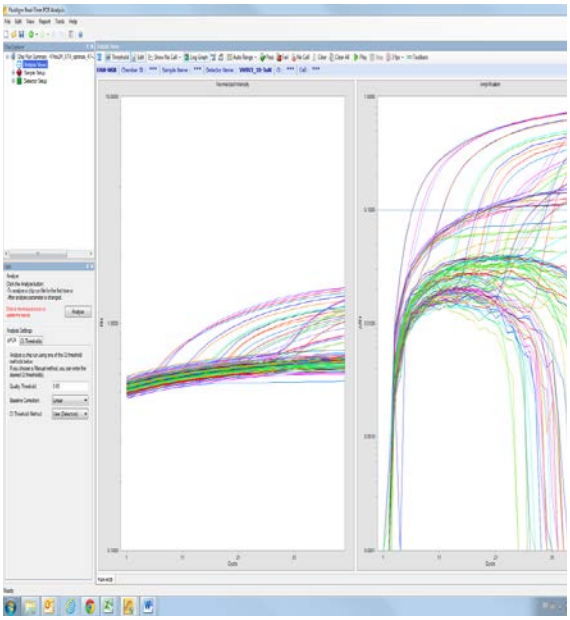
CMS_PMCV (9-2uM) best condition



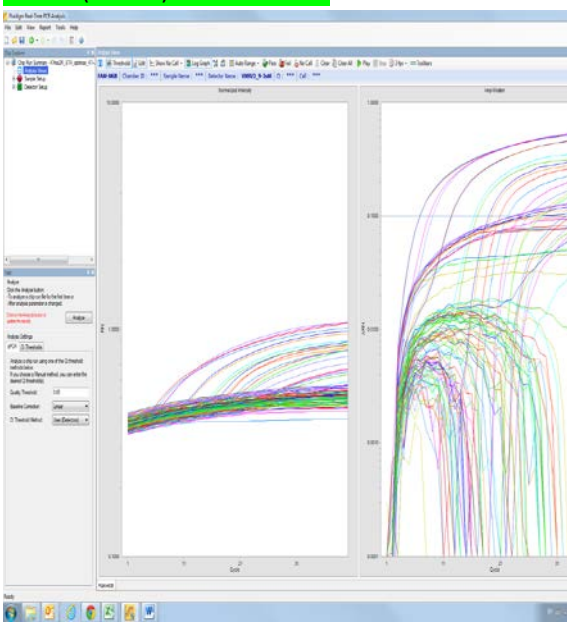
Supplemental Figure 2 (Continued)

VHSV (old)

VHSV2 (10-3uM) current condition

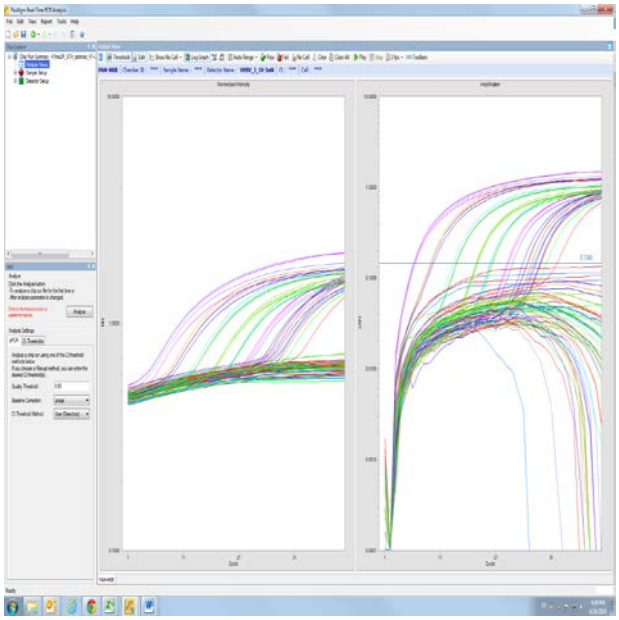


VHSV2 (9-2uM) best condition

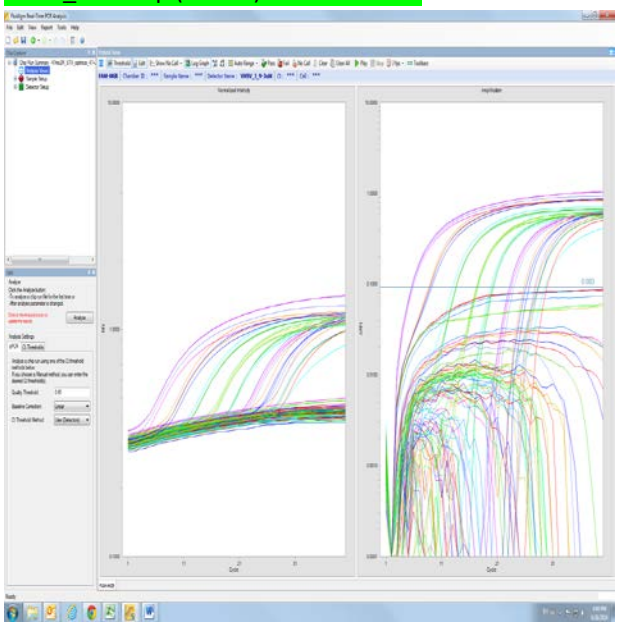


VHSV (new)

VHSV_Jonstrup (10-3um) current condition



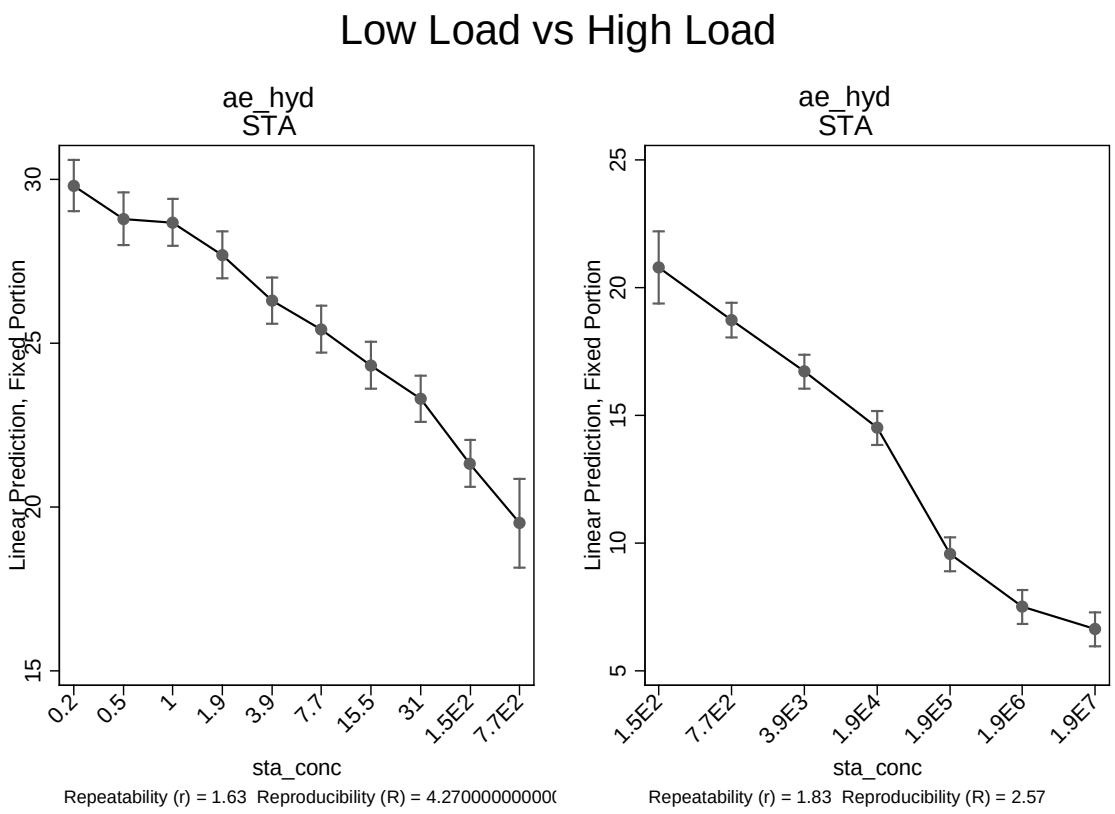
VHSV_Jonstrup (9-2um) best condition



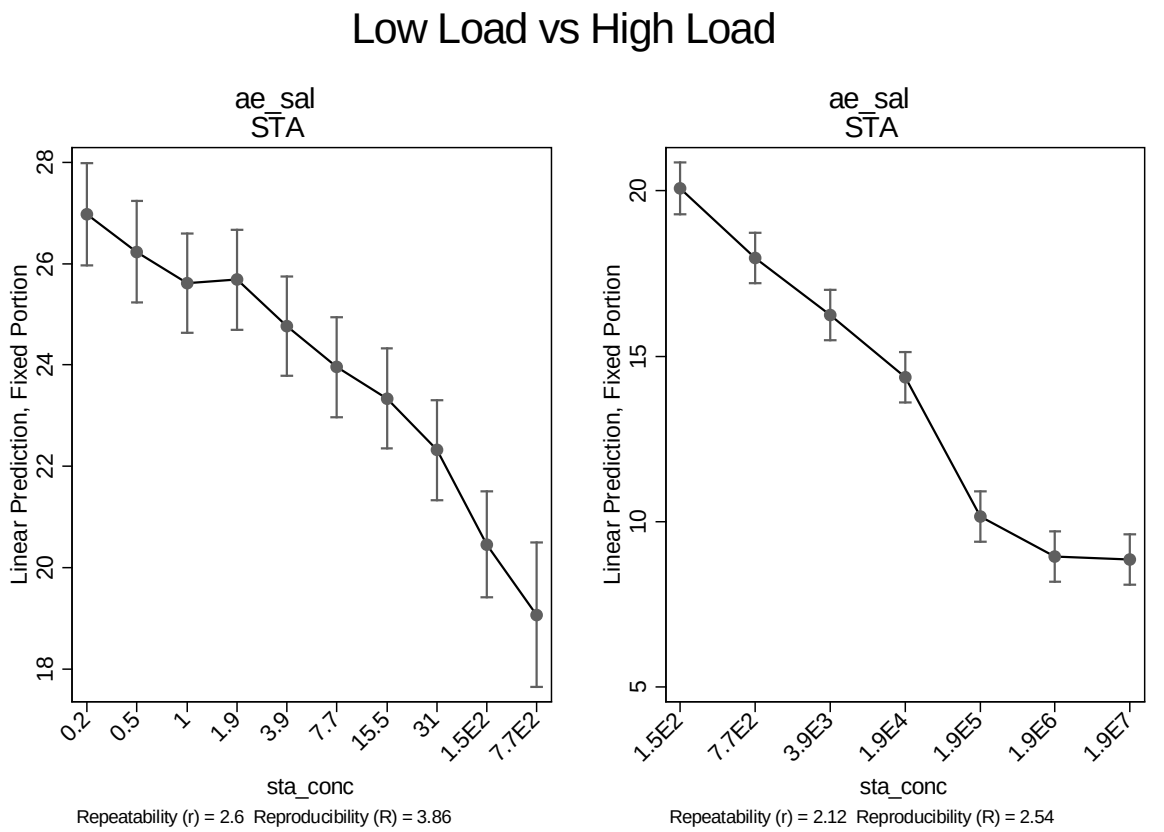
Supplemental Figure 2 (Continued)

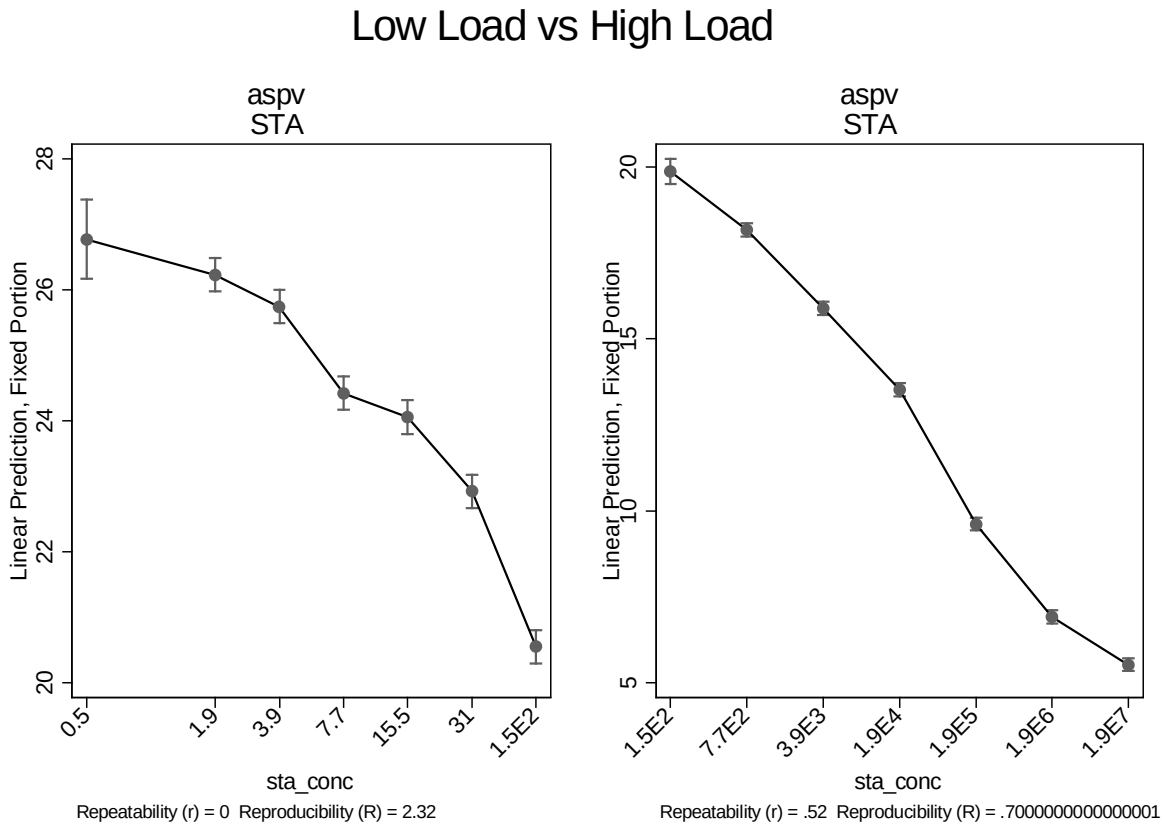
Supplemental Table 2. Average Ct values by assay for each serial dilution from the LOD study for STA and Non-STA samples run on the BioMark. Starting concentration is averaged over all assays, hence there may be minor assay-specific variances in concentrations among assays (generally within 95% of estimates). Values reflect the first dilution series only (averaged over 40 replicates per dilution).

	Mean Starting Concentration for STA Assays														Mean Starting Concentration for Non-STA Assays							
Assay	0.5	1	1.9	3.9	7.7	15.5	31	1.5×10^2	7.7×10^2	3.9×10^3	1.9×10^4	1.9×10^5	1.9×10^6	1.9×10^7	31	1.5×10^2	7.7×10^2	3.9×10^3	1.9×10^4	1.9×10^5	1.9×10^6	1.9×10^7
ae_hyd	28.80	28.67	27.72	26.30	25.43	24.33	23.30	21.32	18.83	16.71	14.51	9.58	7.51	6.64	30.44	30.52	29.30	26.88	24.46	21.04	17.50	13.60
ae_sal	26.40	25.61	25.68	24.77	23.95	23.34	22.32	20.25	18.10	16.24	14.37	10.16	8.95	8.85	33.65	33.82	31.55	29.45	28.05	25.52	22.52	20.76
aspv	26.77	N/A	26.23	25.74	24.42	24.06	22.92	20.54	18.16	15.89	13.53	9.62	6.92	5.53	N/A	29.36	28.44	26.24	24.14	20.78	17.36	14.03
re_sal	26.79	26.27	25.91	25.70	23.37	23.15	21.85	19.06	17.01	14.76	12.45	8.76	6.37	5.47	27.74	27.76	28.25	24.95	22.88	19.33	16.02	12.95
c_b_cys	26.88	26.83	27.59	25.72	24.97	23.94	23.06	20.64	18.20	15.97	13.65	9.71	7.38	6.49	31.64	30.65	28.72	26.81	24.95	21.81	18.25	14.95
pmcv	26.78	26.13	26.29	25.33	24.57	23.76	23.05	20.61	18.06	16.04	13.87	9.18	7.25	6.70	29.89	30.23	30.06	27.79	25.73	22.70	19.78	17.67
cr_sal	26.88	25.67	25.22	24.34	22.82	22.45	21.61	19.44	16.96	14.81	12.42	8.40	6.54	5.97	29.92	29.55	27.99	25.45	23.73	20.78	17.45	14.16
ce_sha	27.83	28.77	27.35	28.48	26.13	26.20	24.92	22.75	20.70	18.68	16.65	12.41	10.19	9.49	35.18	34.85	33.87	32.05	29.83	26.76	23.03	19.59
de_sal	27.82	26.40	26.15	25.49	24.27	22.37	21.60	19.02	16.69	14.08	12.07	7.99	5.98	5.27	29.35	27.75	27.42	24.60	22.11	18.96	15.45	12.08
ye_ruc	29.55	30.21	29.12	30.16	28.88	27.89	25.81	24.10	20.95	18.32	17.08	11.16	8.90	8.25	37.16	35.04	34.25	33.28	31.40	29.24	25.88	24.07
fa_mar	30.33	30.68	30.55	29.78	28.73	26.51	25.70	24.24	22.02	20.14	18.04	11.66	8.97	7.76	35.40	33.29	31.90	30.36	28.33	25.22	21.41	16.63
fl_psy	30.27	31.41	31.02	30.00	29.46	27.72	26.71	24.03	21.25	19.20	16.62	11.90	8.45	6.25	33.51	31.53	30.48	28.75	26.02	22.38	18.64	14.82
gy_sal	27.00	26.69	26.43	26.08	24.60	24.36	23.12	20.65	18.30	15.97	13.55	9.80	7.54	6.68	N/A	30.91	30.09	27.83	25.67	22.31	18.49	14.87
prv	25.09	26.11	24.14	23.54	22.49	21.52	20.40	17.91	15.50	13.38	11.10	7.49	5.44	4.82	30.80	28.71	25.73	23.46	21.24	17.91	14.41	11.09
ihnv	27.64	28.35	28.20	27.64	27.55	26.56	25.84	22.92	20.66	18.56	16.11	11.06	8.27	7.07	N/A	30.18	30.84	29.54	27.04	23.57	20.01	16.87
ic_mul	25.87	25.07	25.60	23.70	22.96	22.08	21.17	18.51	16.23	13.82	11.71	8.05	6.29	5.78	30.51	28.92	28.10	25.37	23.07	19.79	16.43	13.31
ipnv	27.22	27.91	27.63	27.10	26.58	25.36	23.95	21.12	19.53	16.79	14.41	10.01	7.41	6.14	N/A	29.73	29.41	27.16	25.01	21.49	18.00	14.63
isav7	27.86	28.43	27.45	27.00	25.87	24.28	23.53	21.67	19.17	17.06	14.67	10.07	7.56	6.13	N/A	30.52	29.24	27.35	24.97	21.88	18.54	14.83
isav8	25.46	26.12	24.35	23.92	22.96	21.94	20.84	18.79	16.37	14.53	12.25	7.99	6.72	6.31	29.81	29.77	27.91	26.26	24.00	21.23	18.13	15.63
ku_thy	26.63	26.63	26.98	26.23	24.51	23.69	22.23	19.92	17.46	15.11	11.88	8.66	6.23	5.36	32.61	29.07	27.37	25.15	22.46	19.52	16.03	12.70
lo_sal	25.64	25.42	24.51	24.53	23.14	22.43	21.30	18.93	16.54	14.40	12.09	8.33	6.14	5.39	30.91	28.21	27.97	24.67	22.53	19.24	15.75	12.65
my_arc	27.18	28.09	26.65	26.80	26.18	25.57	24.80	22.03	18.94	17.16	14.80	9.43	7.17	6.27	N/A	29.81	30.66	27.95	24.51	21.91	18.60	15.53
my_ins	27.45	26.43	26.30	24.99	23.90	23.24	22.18	19.39	16.90	14.86	12.42	8.57	6.67	6.12	N/A	29.41	28.50	26.08	23.20	20.73	17.11	13.76
my_cer	25.23	26.04	25.24	24.13	24.00	23.17	21.69	15.88	16.67	14.34	11.90	8.31	5.65	4.92	N/A	25.43	27.06	24.87	22.19	19.25	15.33	12.38
na_sal	26.08	25.79	24.30	23.65	23.00	21.70	21.36	18.67	16.12	13.74	11.59	8.05	6.16	5.65	28.79	29.56	26.97	24.69	22.32	18.84	15.00	12.04
ne_per	27.06	26.83	25.23	25.39	24.45	23.40	21.74	19.86	17.18	15.14	12.99	9.24	7.60	7.10	N/A	29.57	29.65	26.47	24.15	20.73	17.24	14.14
nu_sal	25.45	26.07	25.04	23.10	22.83	21.90	20.45	18.00	15.60	13.60	11.39	7.81	5.85	5.18	27.50	28.09	25.63	23.83	21.38	18.10	14.82	11.59
pa_ther	28.03	27.81	28.16	28.73	27.44	26.76	25.79	23.45	21.14	18.85	16.60	11.69	8.99	7.55	N/A	32.90	32.40	30.30	27.96	24.77	21.02	17.21
omv	27.33	27.12	26.59	26.50	26.04	24.59	23.92	21.85	19.40	17.10	14.66	10.38	8.03	6.93	33.96	31.16	30.58	28.66	26.23	23.74	20.30	17.10
pa_pse	26.81	30.54	27.76	27.09	25.16	23.58	22.56	20.43	17.91	15.86	13.37	8.58	6.18	5.52	N/A	28.09	28.06	25.88	23.61	20.99	17.74	15.45
pch_sal	29.78	29.18	30.49	30.72	29.89	29.11	28.99	27.09	24.85	22.23	19.85	13.04	10.49	9.50	37.00	35.82	36.05	35.47	34.17	31.70	29.96	27.66
pisck_sal	26.26	26.34	25.55	24.21	23.32	22.13	21.30	18.42	16.03	12.39	11.00	7.30	5.00	4.32	N/A	28.59	26.53	23.48	21.19	18.14	14.46	11.79
pa_kab	27.36	26.77	26.73	26.85	25.58	25.33	24.03	21.60	19.09	16.69	14.31	9.85	7.24	6.08	28.80	29.85	29.05	27.18	24.71	21.22	17.65	14.25
te_bry	26.91	25.19	24.98	24.72	22.72	22.50	20.63	18.67	15.87	13.41	11.44	7.76	5.69	5.12	29.90	28.52	26.71	24.15	21.88	18.49	14.81	11.86
pa_min	29.33	29.49	28.86	29.62	28.67	27.37	26.11	23.62	21.12	18.64	16.32	11.80	8.77	7.09	30.68	31.24	30.54	29.63	26.71	23.54	19.91	16.56
pspvv	26.87	27.57	26.42	26.29	24.97	23.71	22.81	20.73	18.30	16.10	13.54	9.37	6.95	5.76	31.05	30.27	29.13	26.56	24.04	20.98	17.47	13.85
rlo	26.52	26.32	26.49	25.23	23.59	23.08	21.78	19.27	16.69	14.54	12.19	8.37	5.76	4.95	N/A	29.97	27.30	25.27	22.67	19.25	15.86	12.78
sav	27.32	27.82	27.59	27.15	26.28	25.24	24.13	21.84	19.44	17.09	14.63	9.80	6.88	5.63	N/A	28.98	28.58	26.22	23.89	20.65	17.24	13.77
sch	27.76	27.76	28.13	27.90	27.46	25.75	24.95	23.18	20.81	18.64	16.03	10.97	8.96	8.12	N/A	32.31	32.55	29.35	27.01	23.97	20.19	15.90
sp_des	27.26	28.14	27.86	26.50	25.33	24.65	23.51	21.04	18.68	16.23	13.75	9.55	7.10	5.95	N/A	30.57	29.08	26.55	24.36	20.83	17.52	14.00
sp_sal	30.03	29.07	28.53	29.28	26.05	25.48	23.93	21.13	19.03	16.33	14.57	10.26	7.74	6.57	31.85	32.19	30.94	27.09	25.90	22.67	19.21	16.00
vi_ang	26.03	26.81	26.41	25.42	23.99	24.21	22.34	20.20	17.68	15.81	13.61	9.98	8.00	7.27	30.89	31.10	29.55	27.68	25.70	22.12	18.63	15.34
ven	26.01	25.58	24.85	24.54	22.92	22.17	21.23	18.83	16.25	14.17	11.84	8.45	6.71	6.08	N/A	29.69	28.21	25.25	23.35	20.00	16.43	13.03
ver	26.13	26.86	26.22	26.21	25.47	24.34	23.23	21.18	18.91	16.73	14.57	10.23	7.81	6.84	N/A	29.03	29.97	27.85	25.44	22.56	19.15	15.94
vhsv	30.31	28.81	30.35	30.85	31.96	26.86	25.90	22.77	20.44	17.95	15.60	10.89	7.95	6.39	N/A	29.79	29.15	27.76	25.65	22.77	19.29	16.03
vi_sal	25.98	26.92	25.84	25.06	23.92	22.67	21.34	19.48	16.93	14.72	12.46	8.78	6.91	6.27	N/A	N/A	30.11	26.89	24.54	21.70	18.13	15.22
ic_hof	26.56	25.42	25.36	24.17	23.79	22.37	21.45	19.33	16.88	14.79	12.59	8.97	7.05	6.59	N/A	29.89	28.80	26.37	24.18	21.06	17.53	14.26

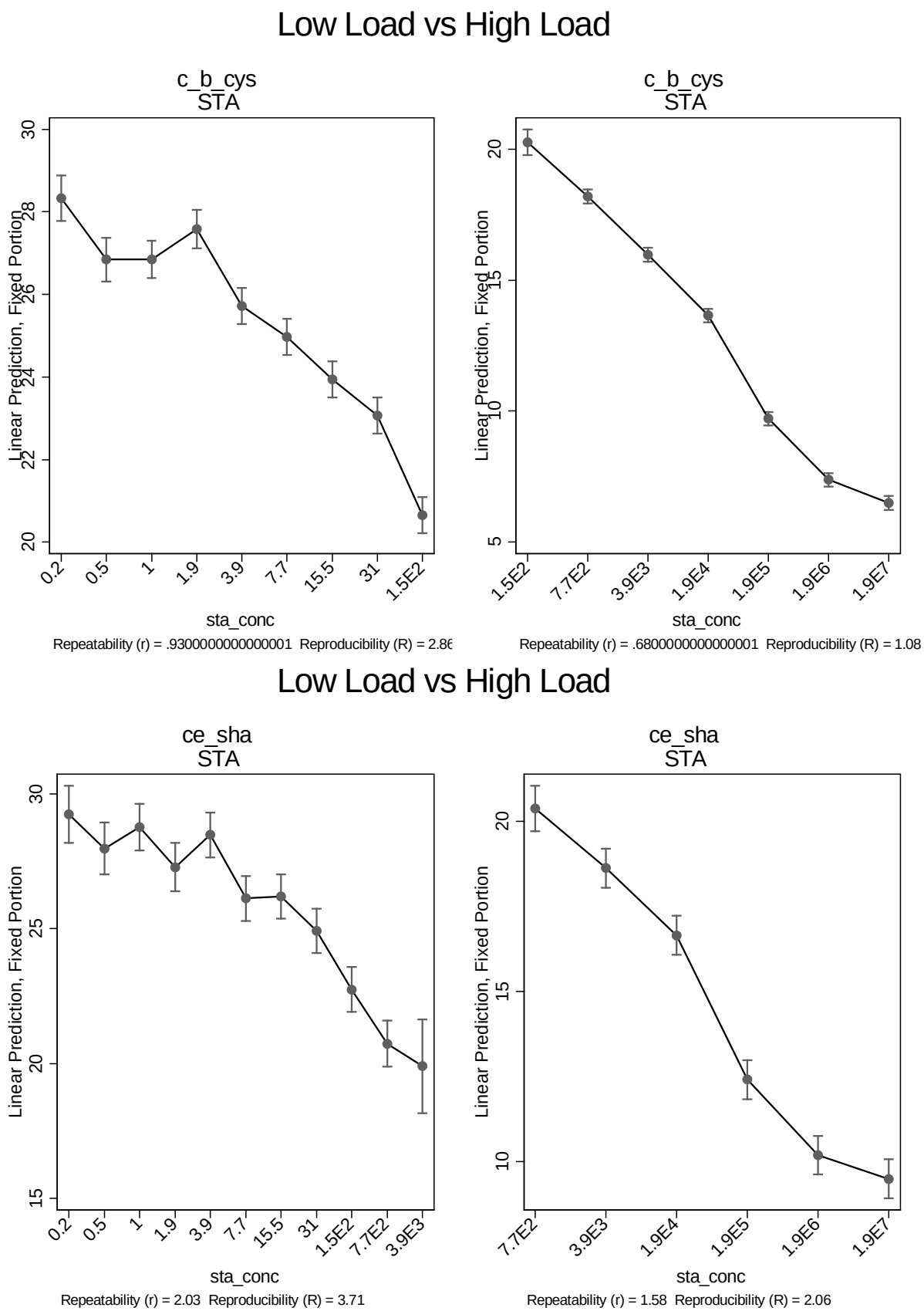


Supplemental Figure 3. Predicted Cts versus microbe concentration, from linear regression models, stratified by low (Ct>=20) and high (Ct<20) microbe load, for each assay (new page for each assay: ae_hyd, ae_sal, aspv, c_b_cys, ce_sha, cr_sal, de_sal, fa_mar, fl_psy, gy_sal, ic_hof, ic_mul, ihnv, ipnv, isav7, isav8, ku_thy, lo_sal, my_arc, my_cer, my_ins, na_sal, ne_per, nu_sal, omv, pa_kab, pa_min, pa_pse, pa_ther, pch_sal, pisck_sal, pmcv, prv, pspv, re_sal, rlo, sav, sch, sp_des, sp_sal, te_bry, ven, ver, vhsv, vi_ang, vi_sal, and ye_ruc).

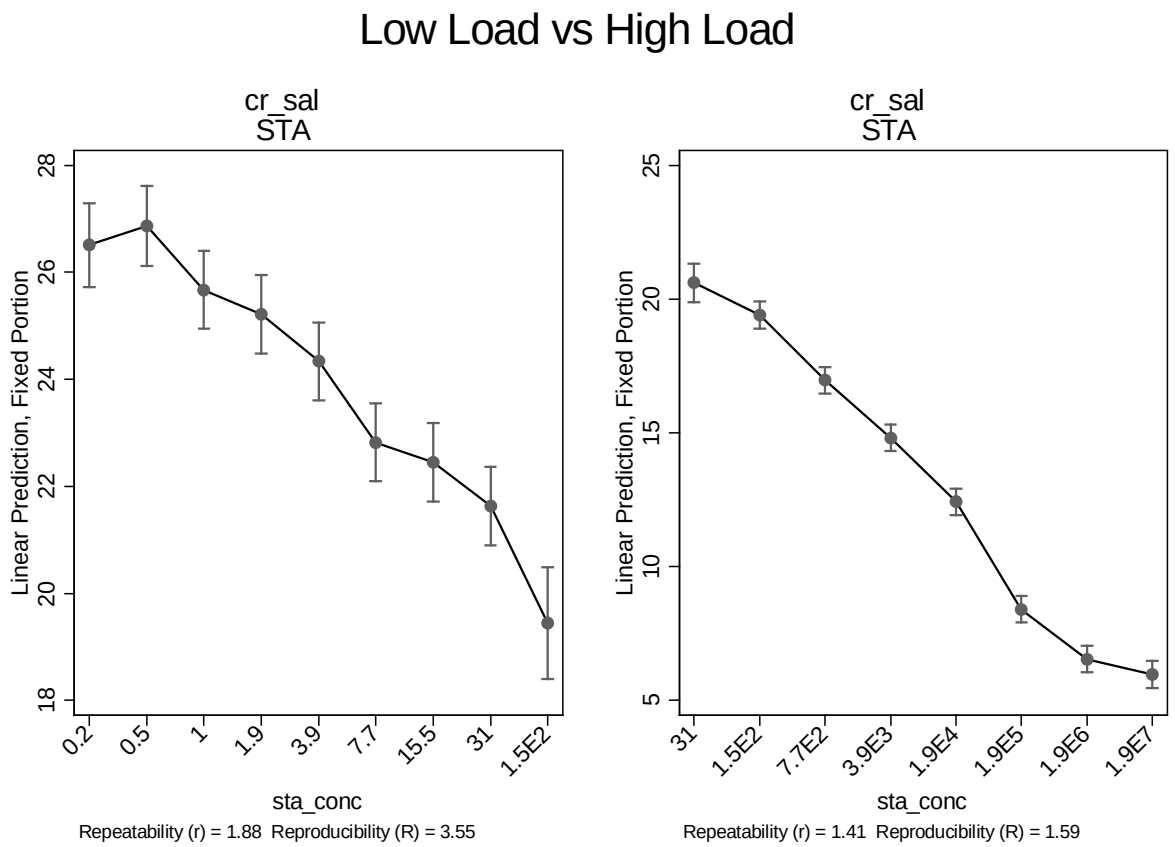


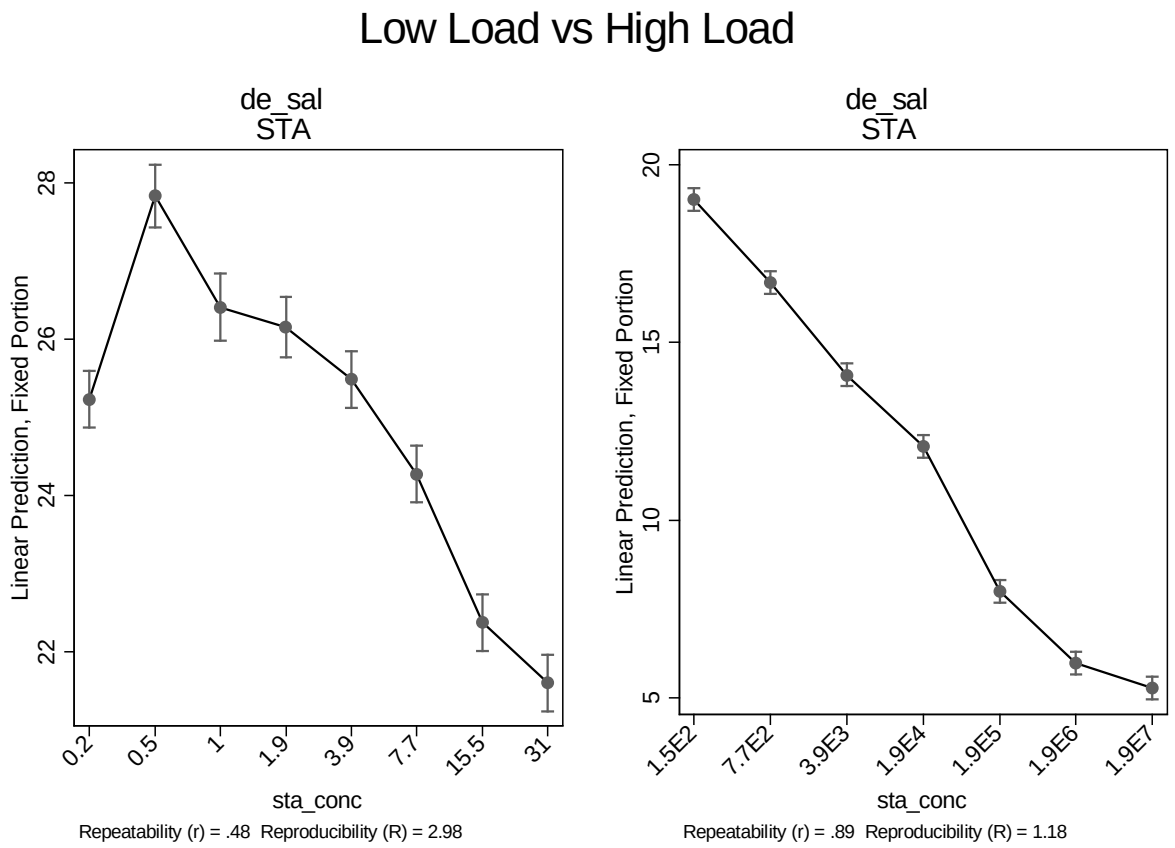


Supplemental Figure 3 (Continued)

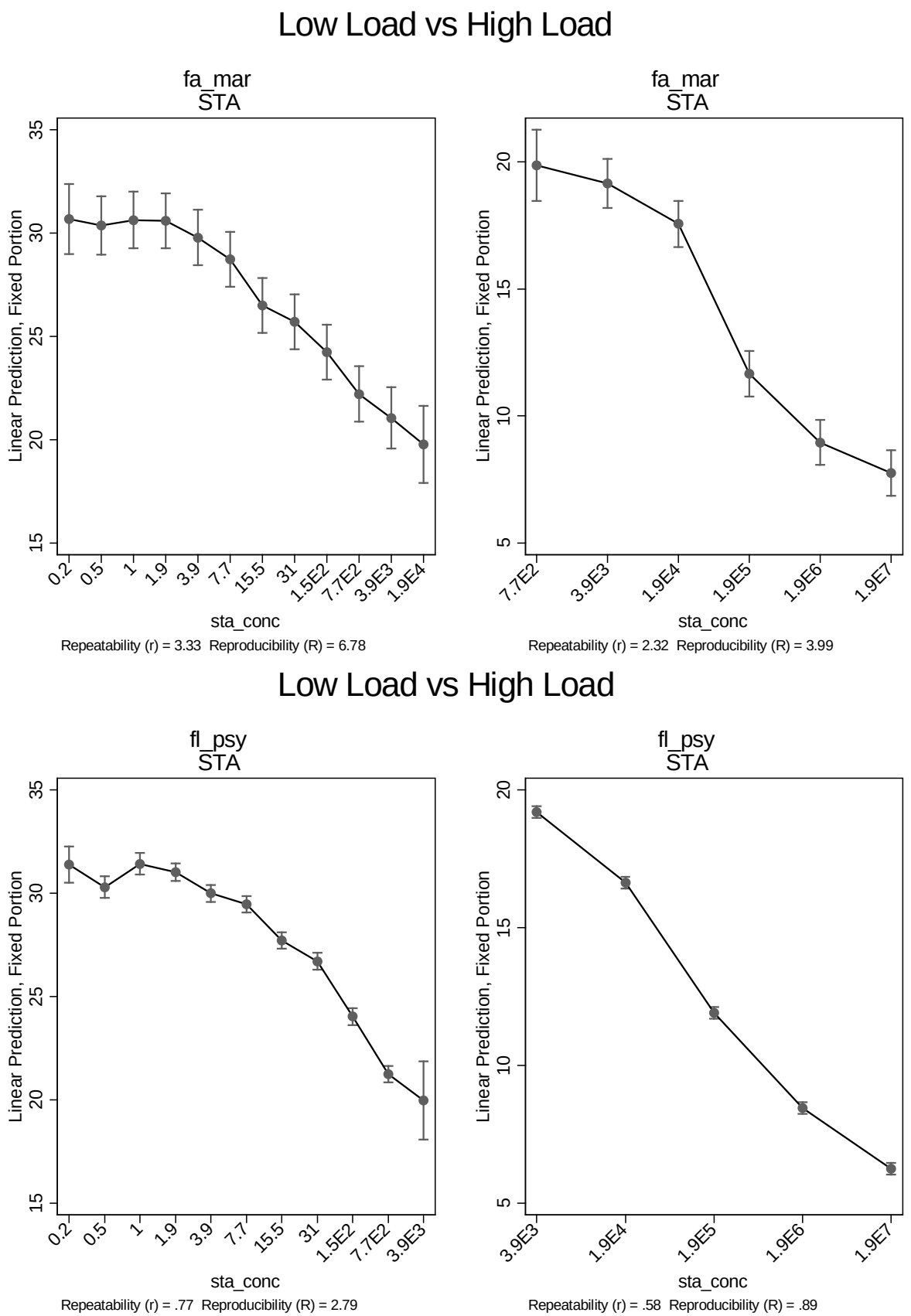


Supplemental Figure 3 (Continued)

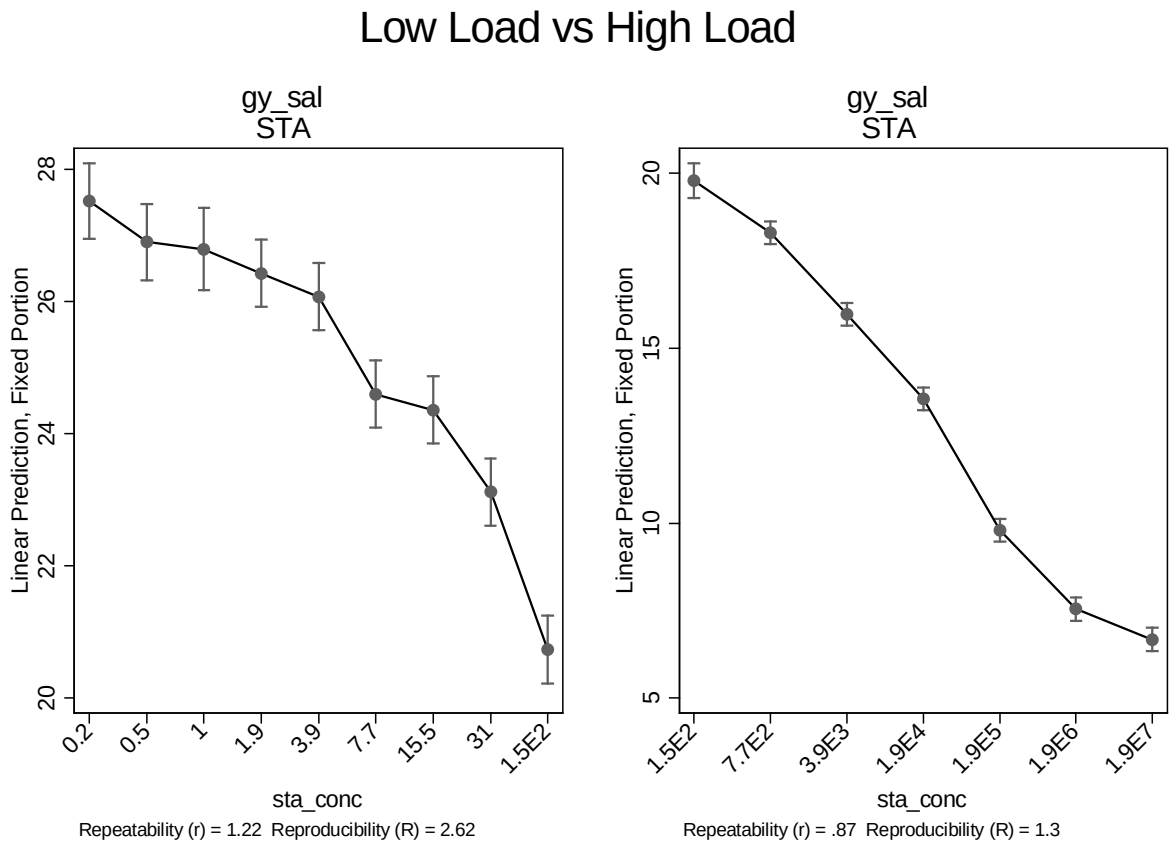


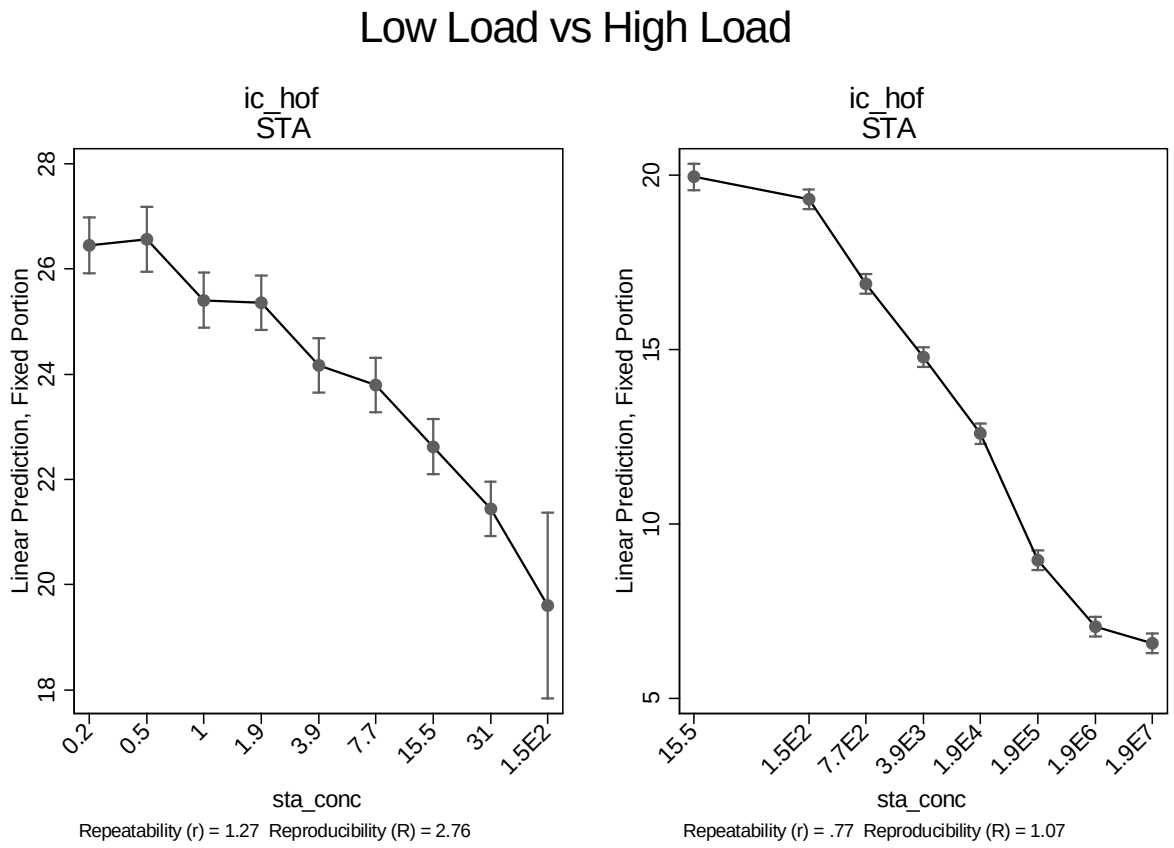


Supplemental Figure 3 (Continued)

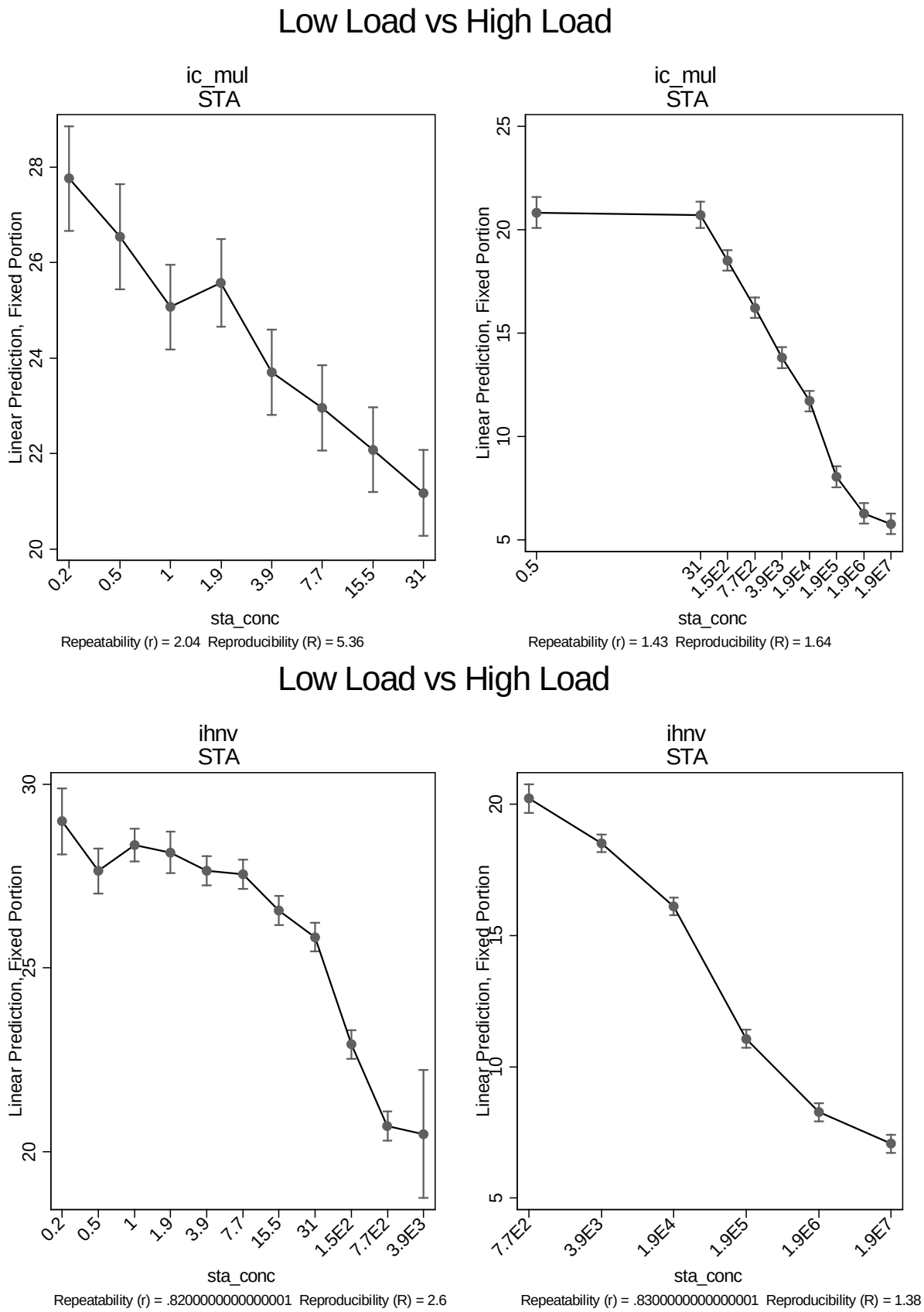


Supplemental Figure 3 (Continued)

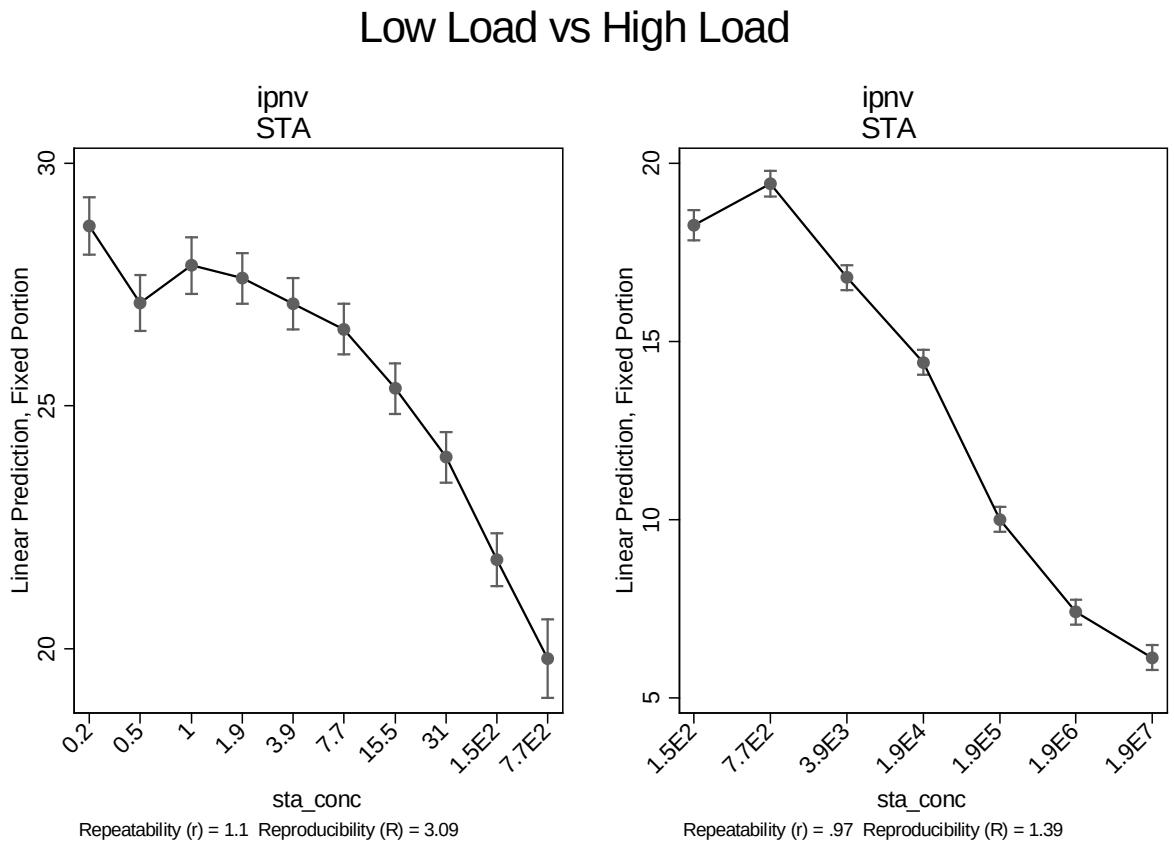


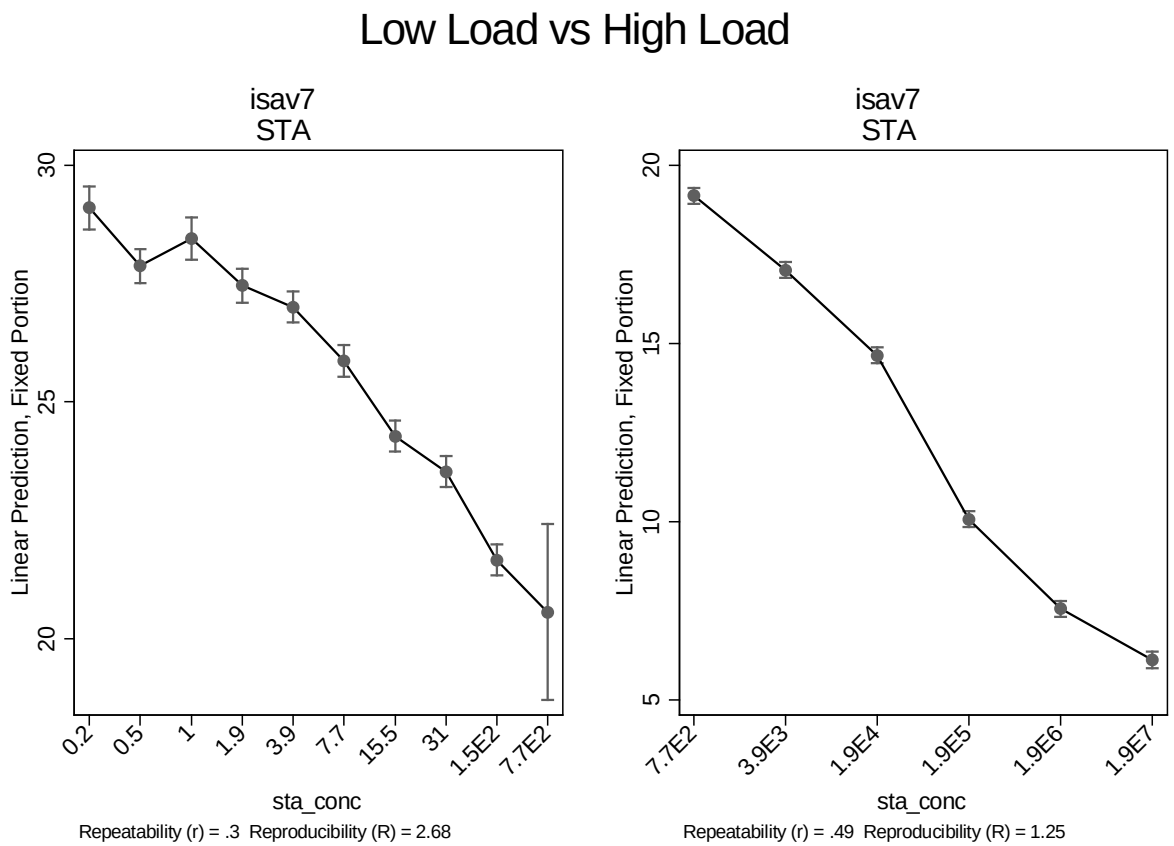


Supplemental Figure 3 (Continued)

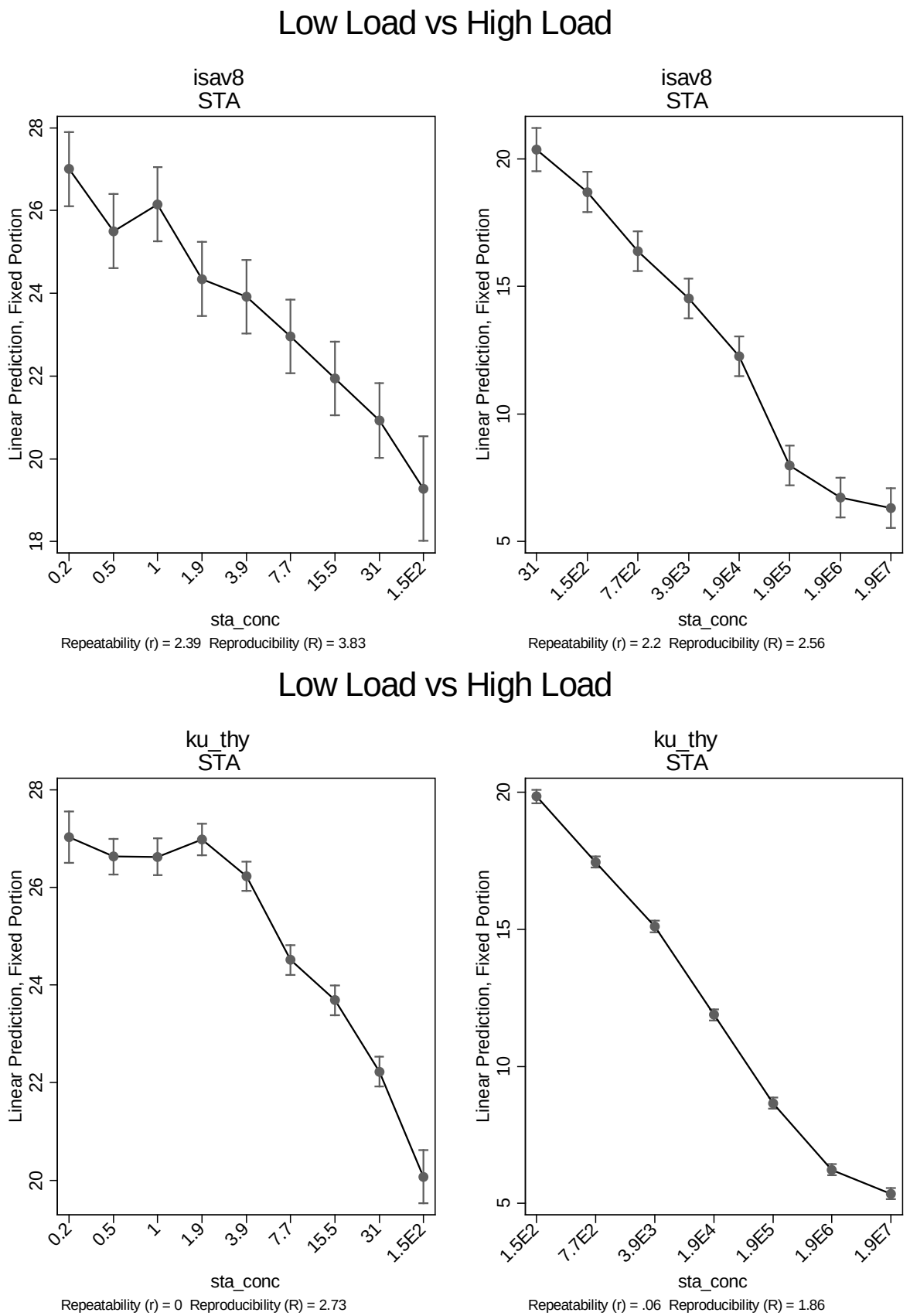


Supplemental Figure 3 (Continued)

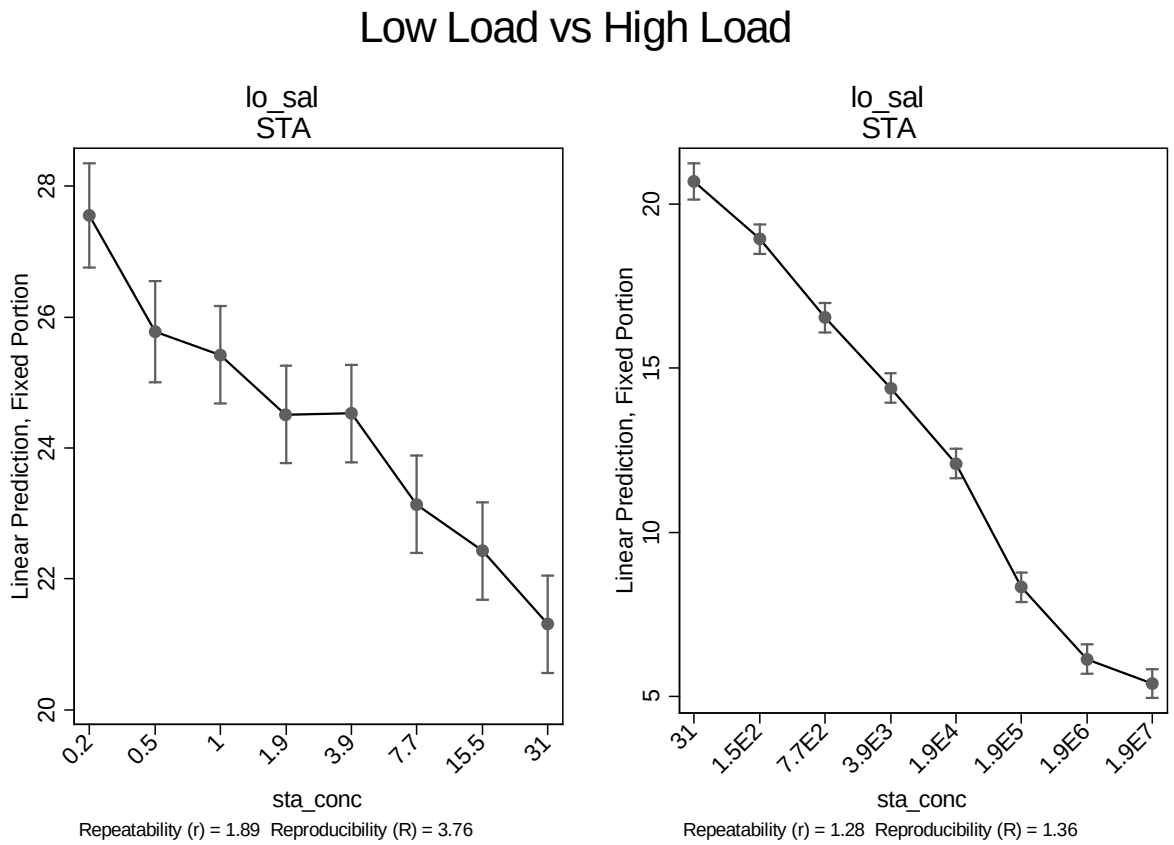


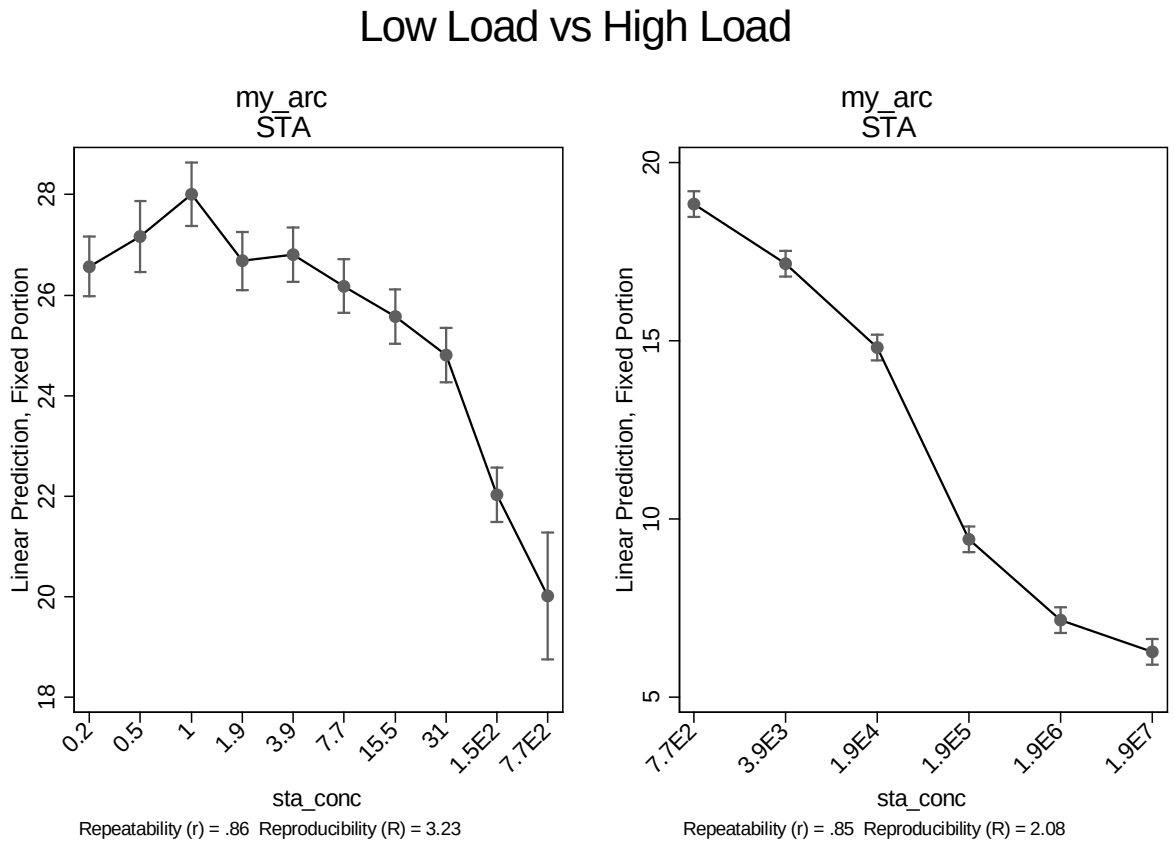


Supplemental Figure 3 (Continued)



Supplemental Figure 3 (Continued)





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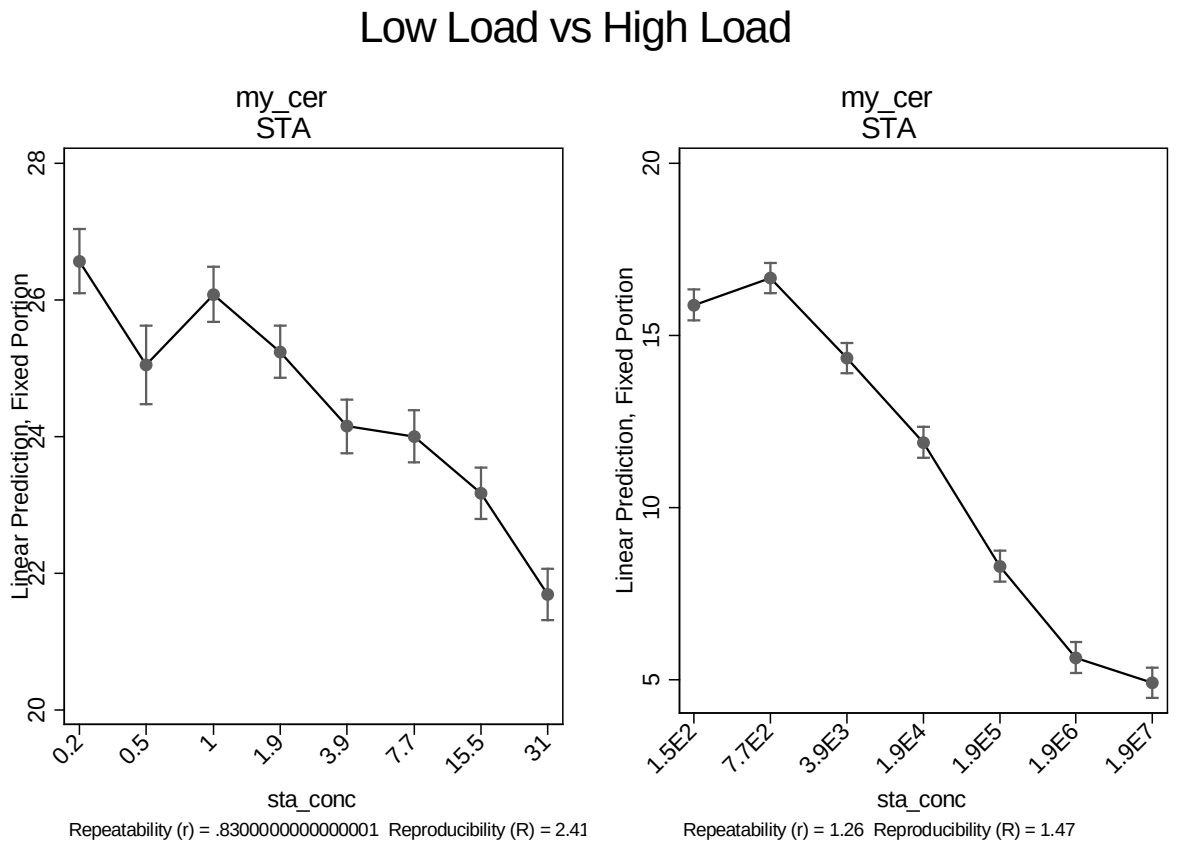

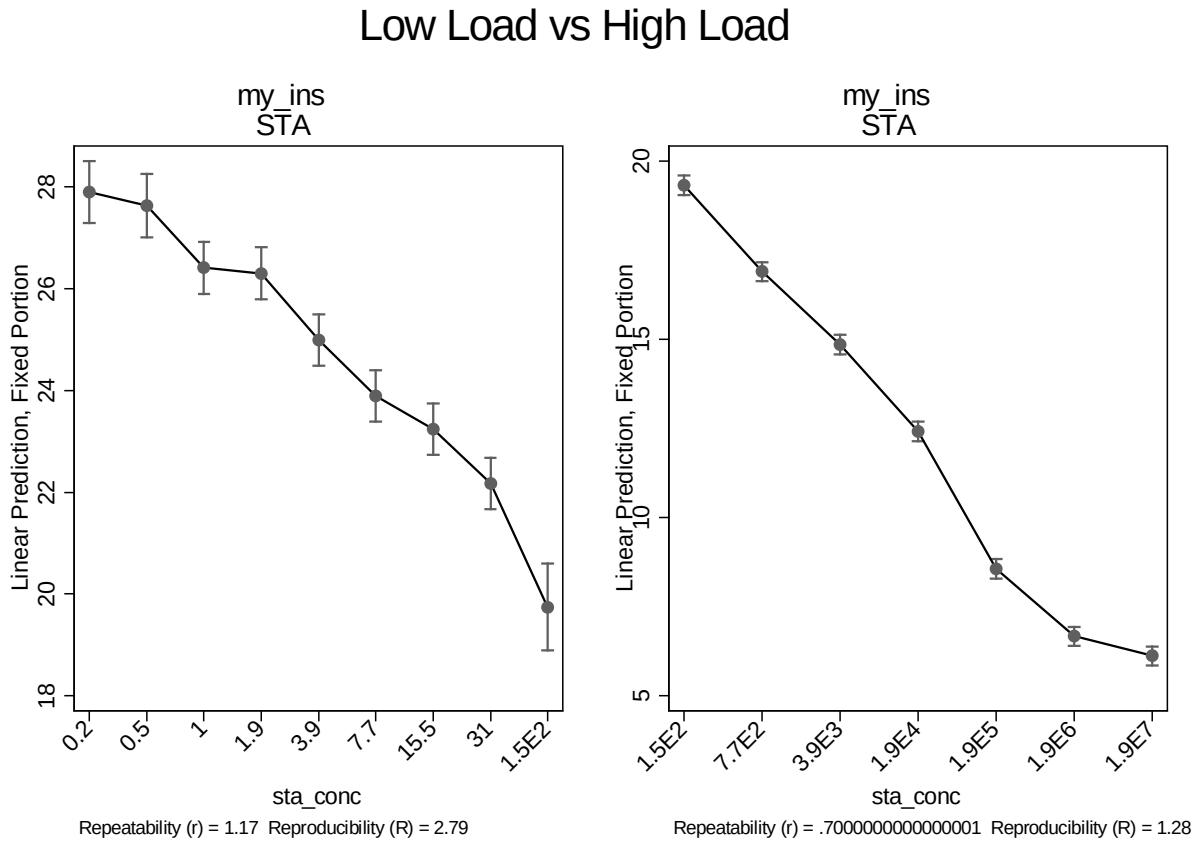

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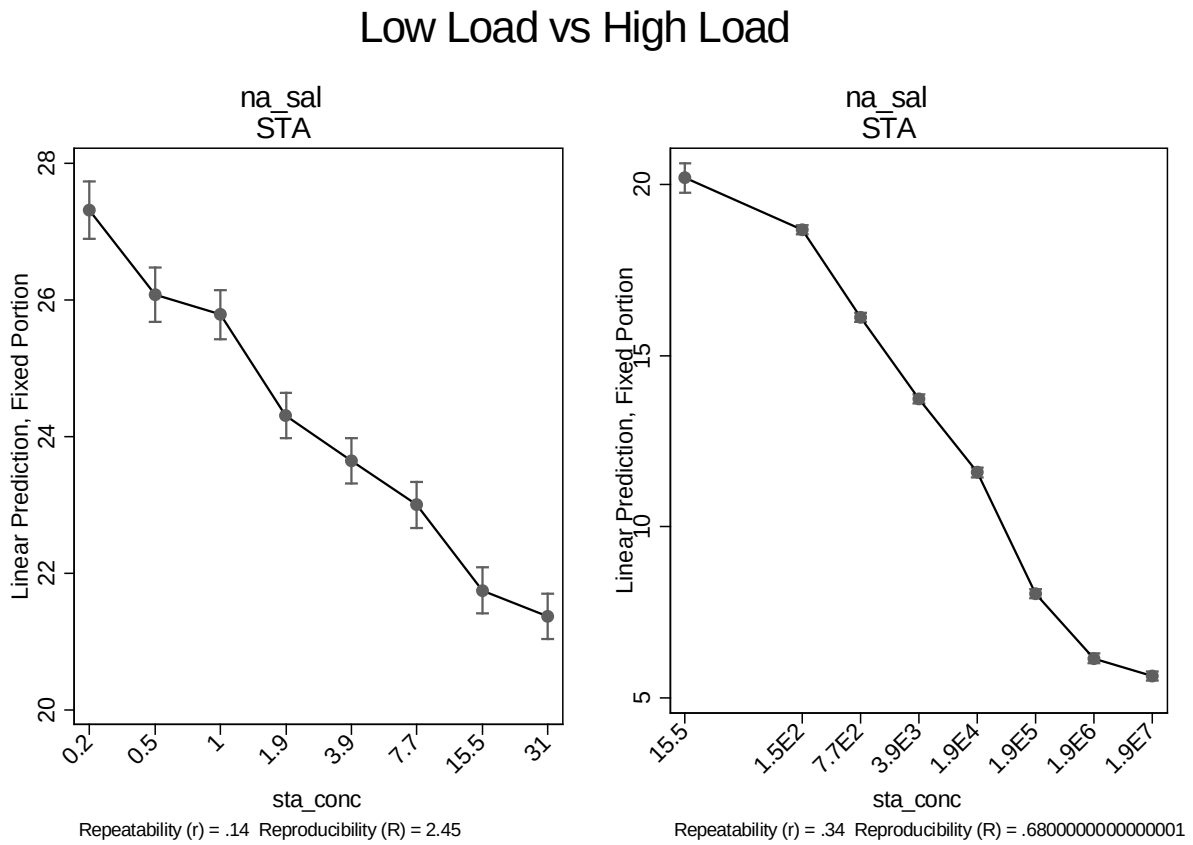

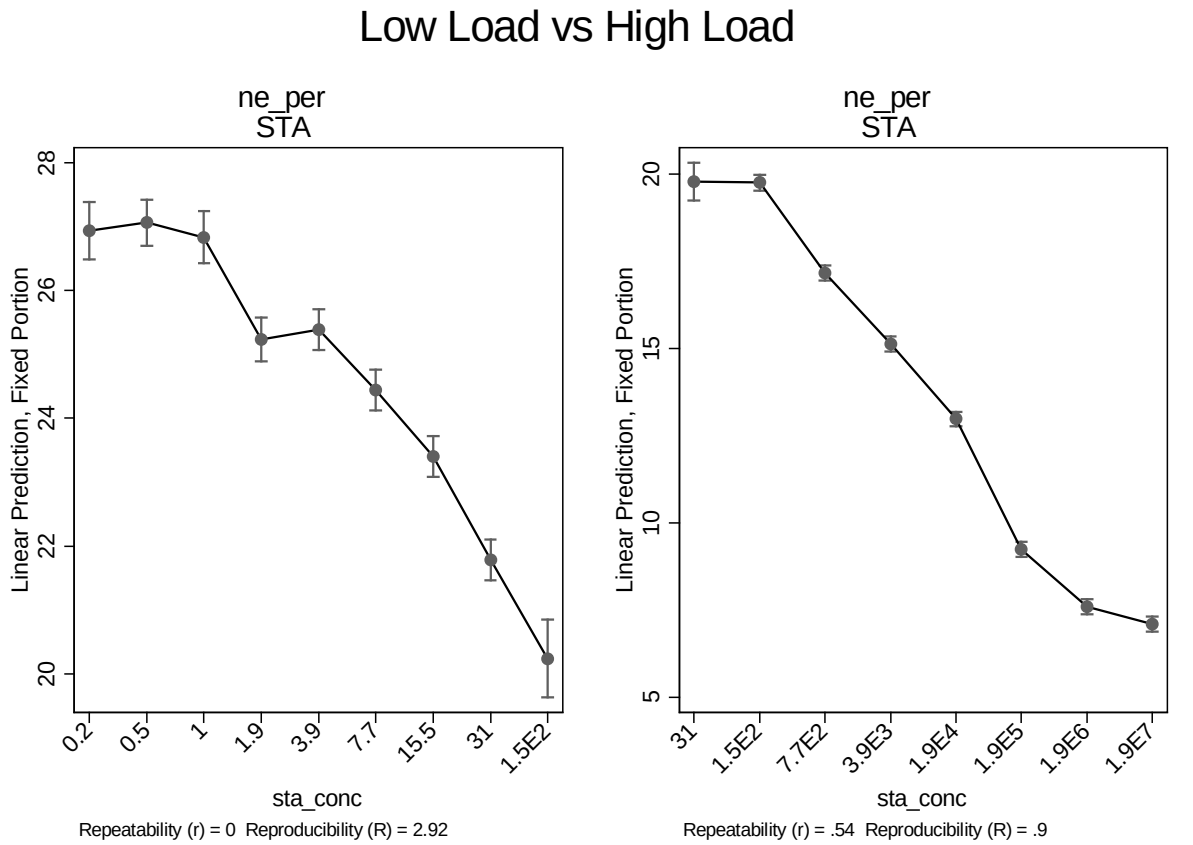

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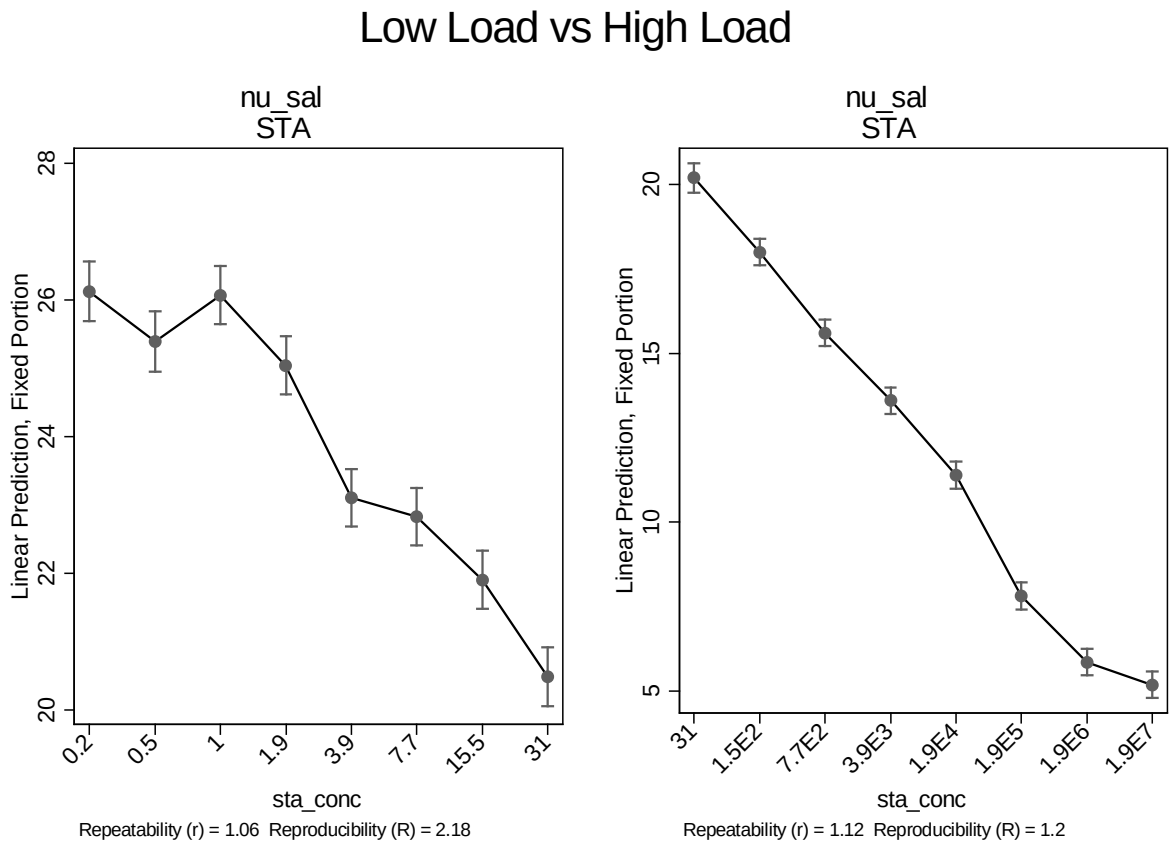

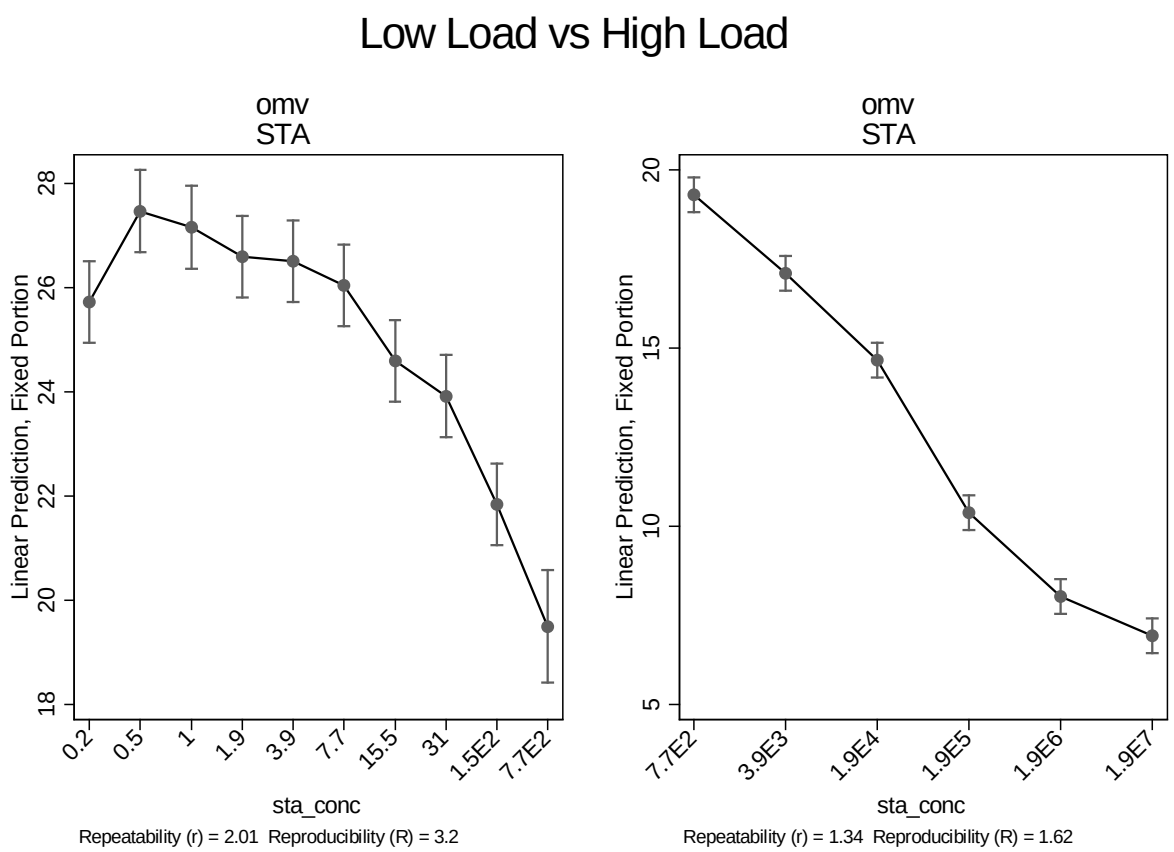

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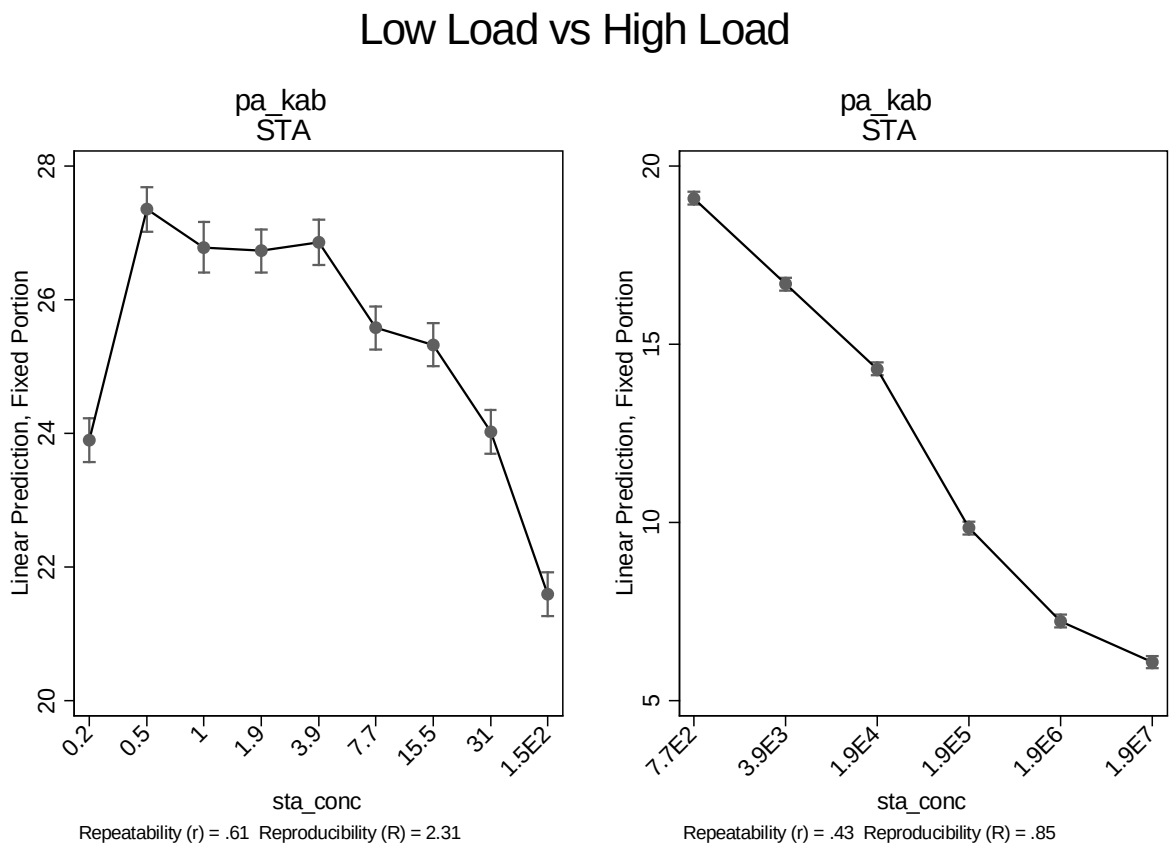

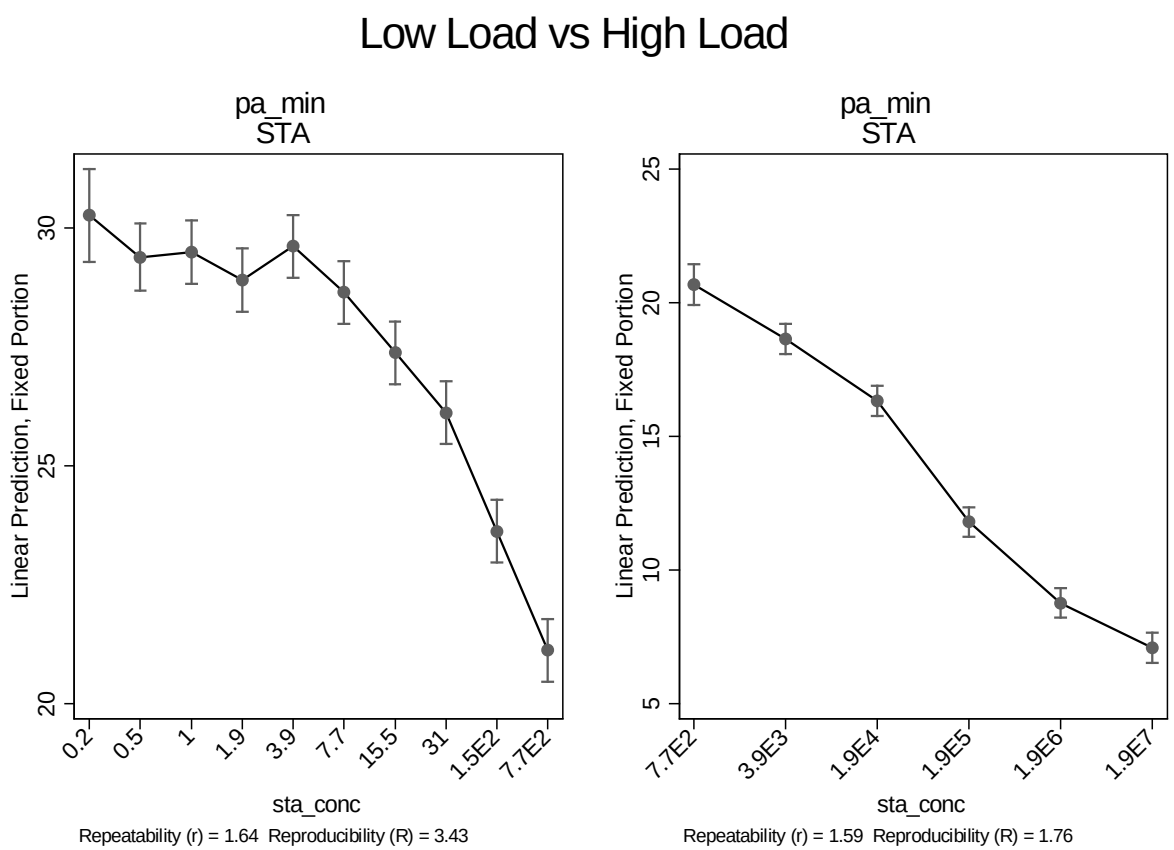

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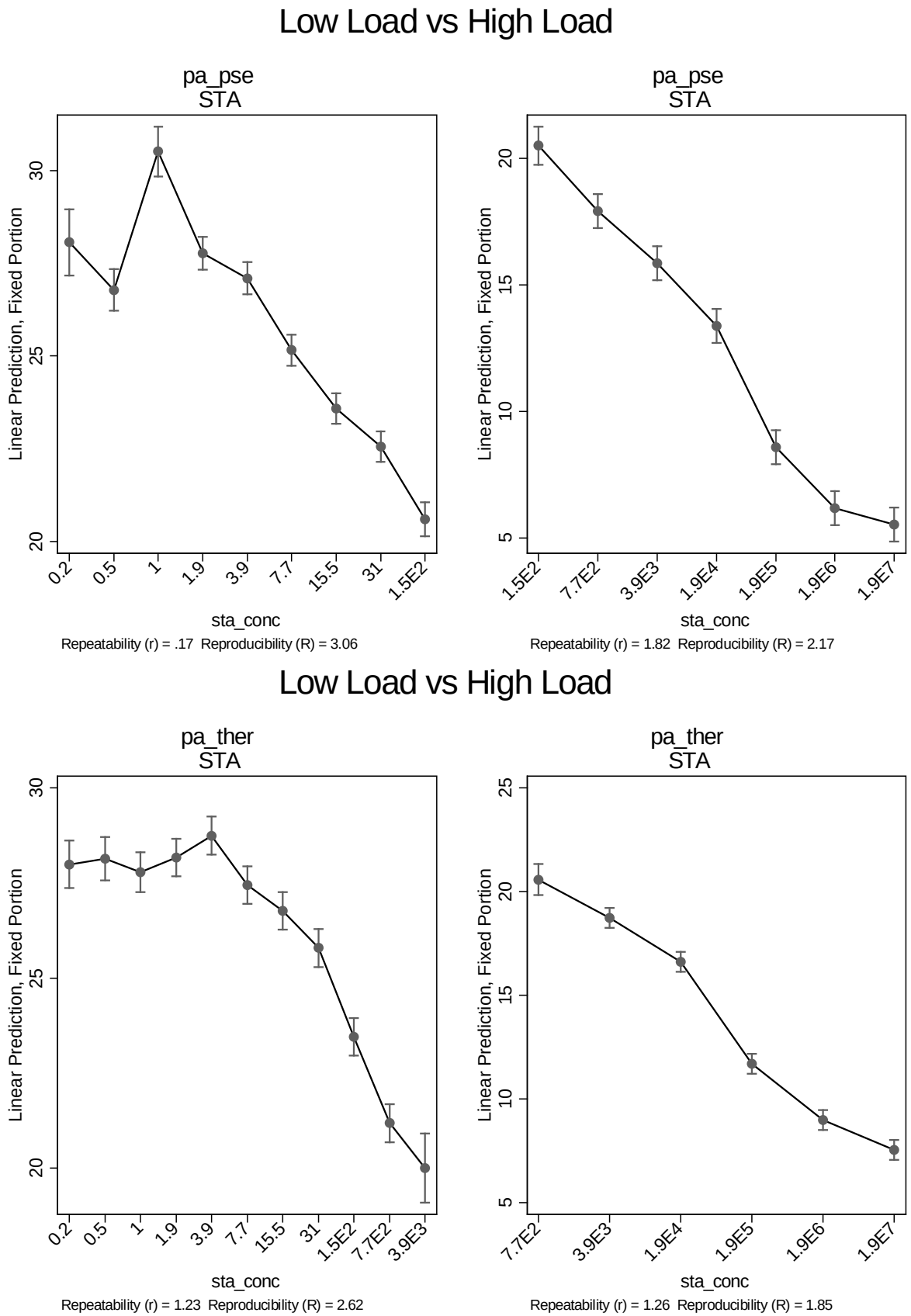

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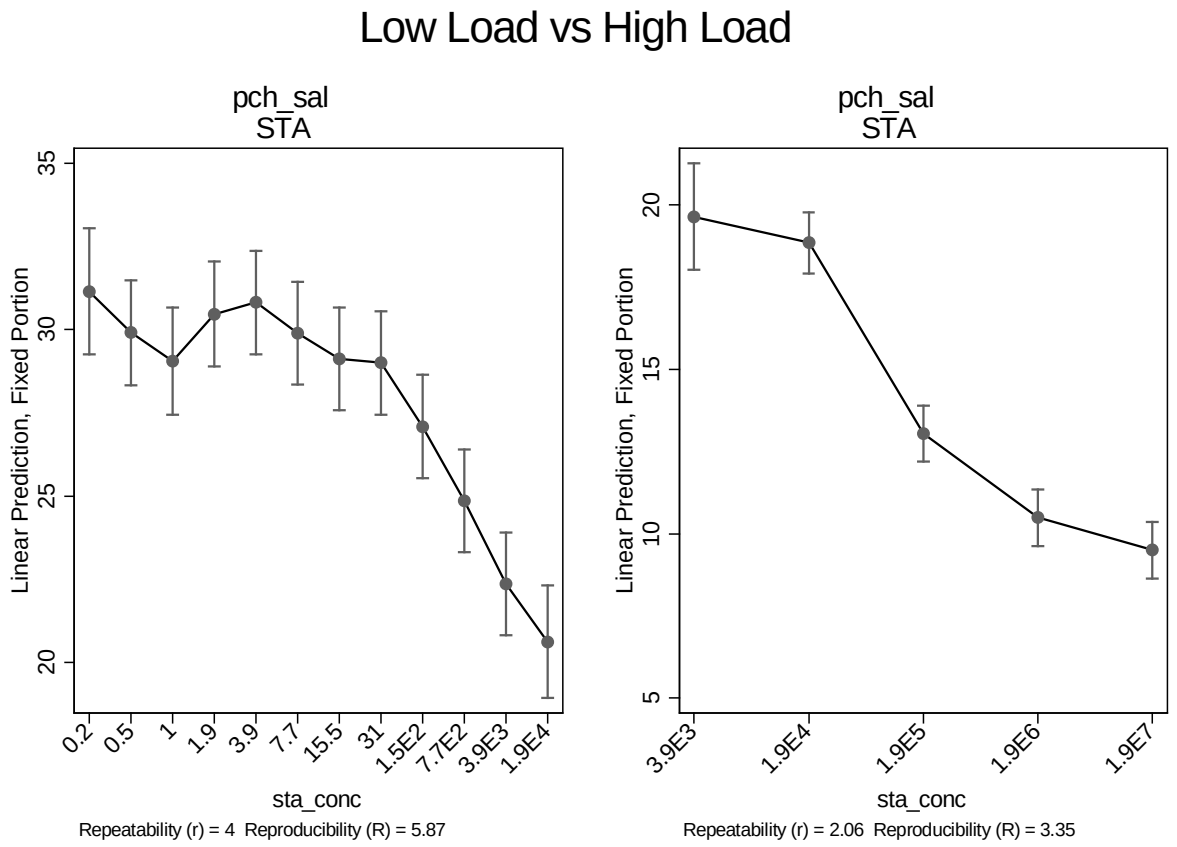

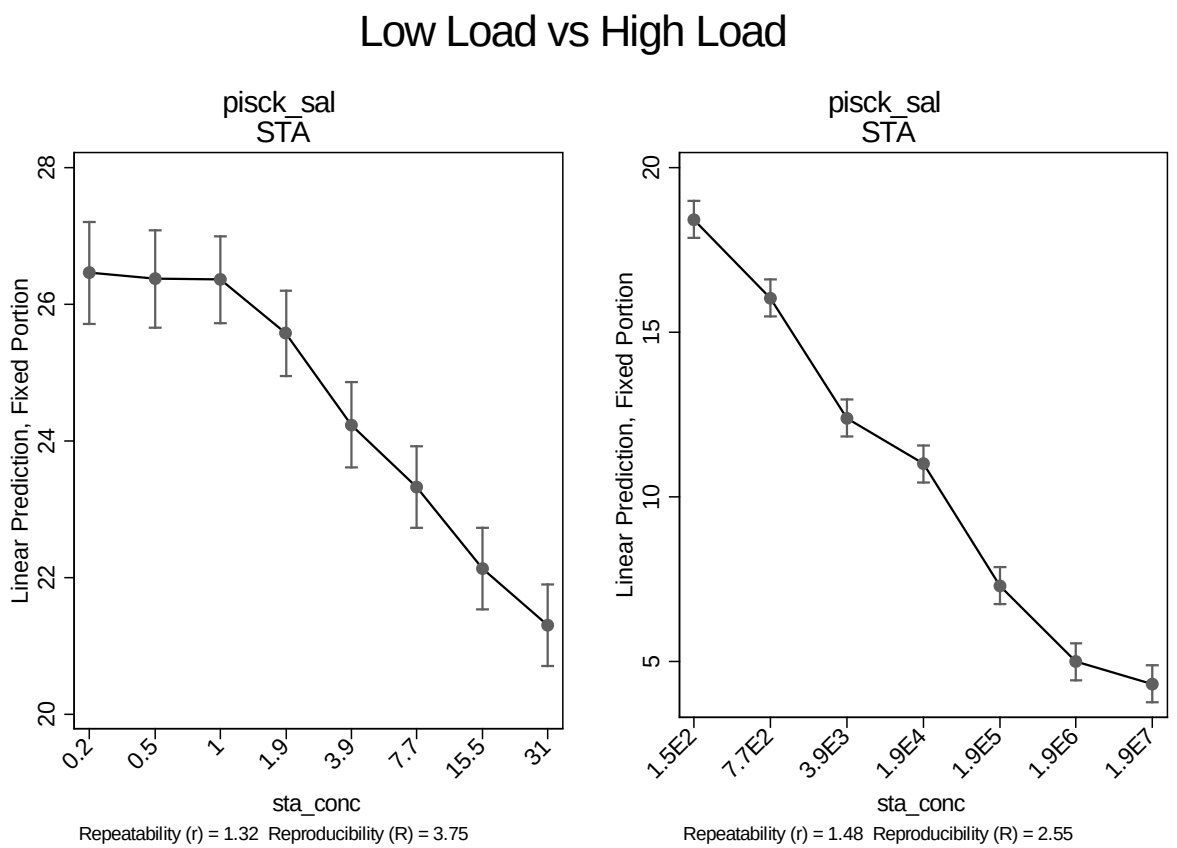

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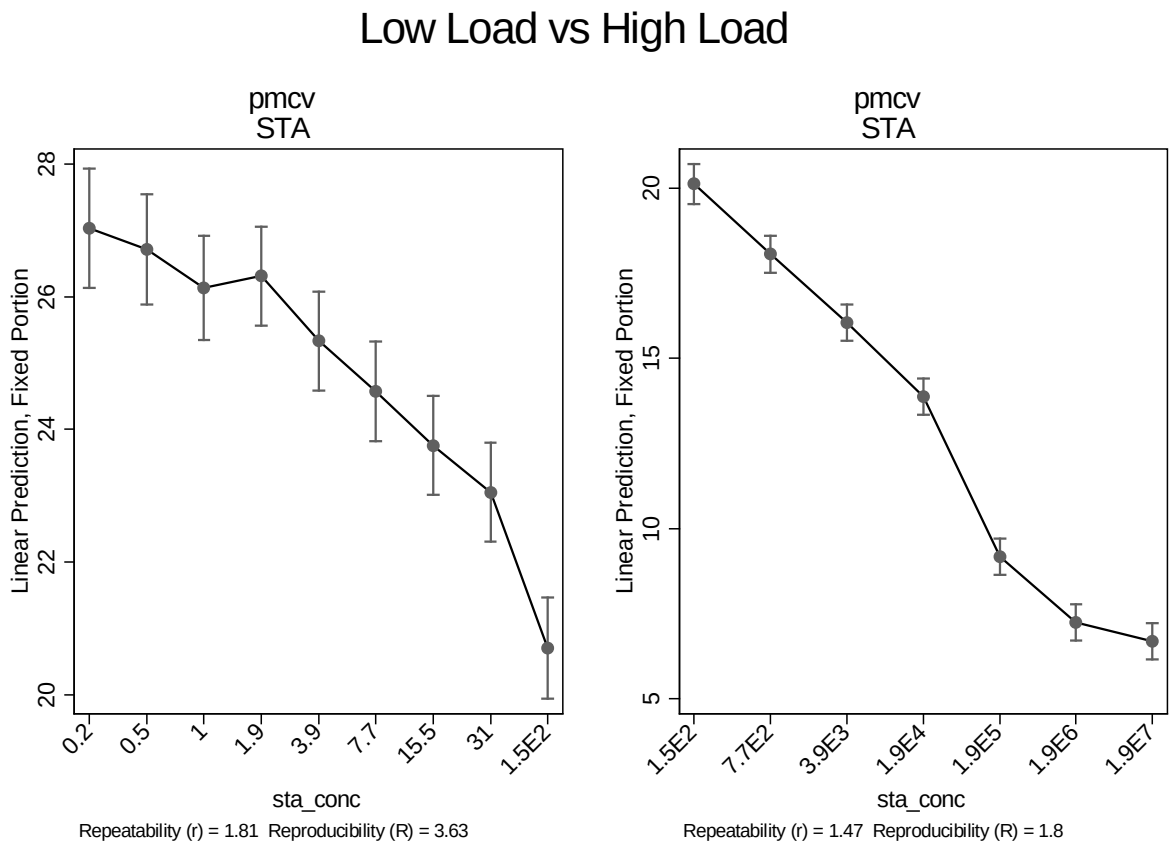

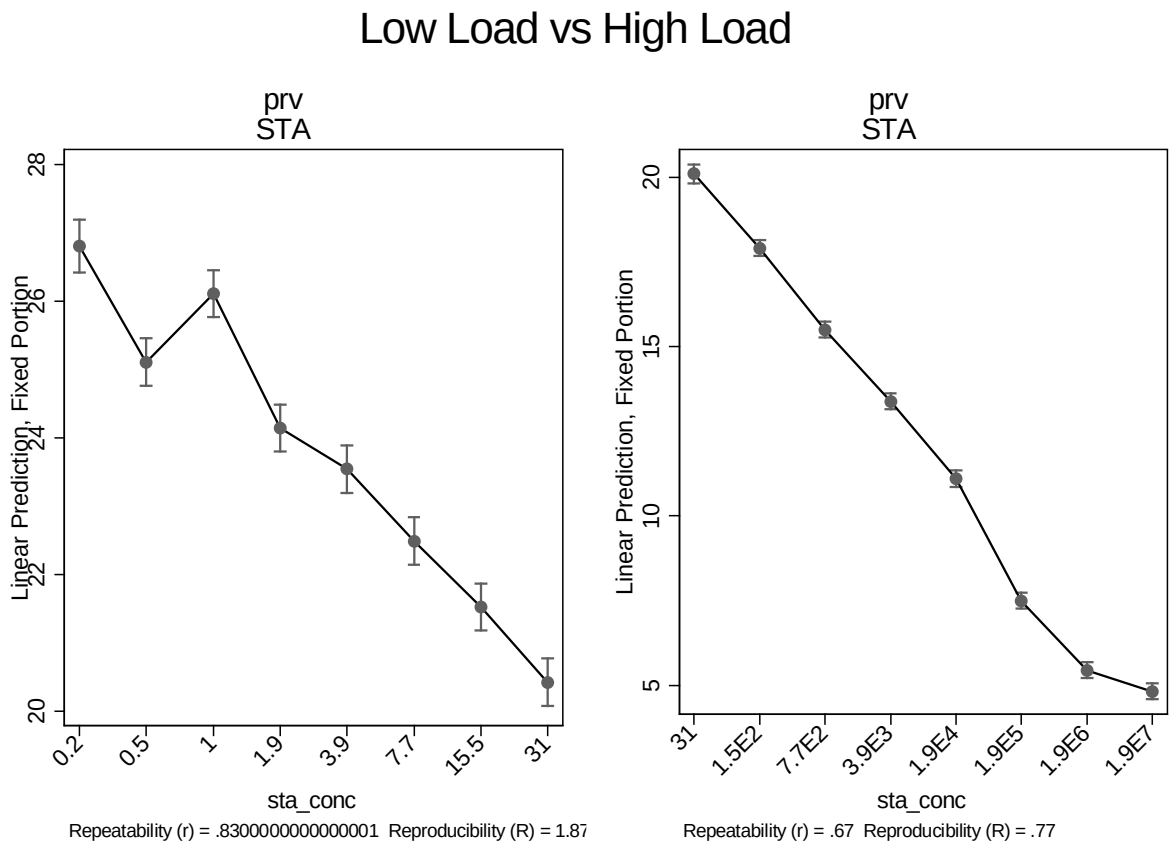

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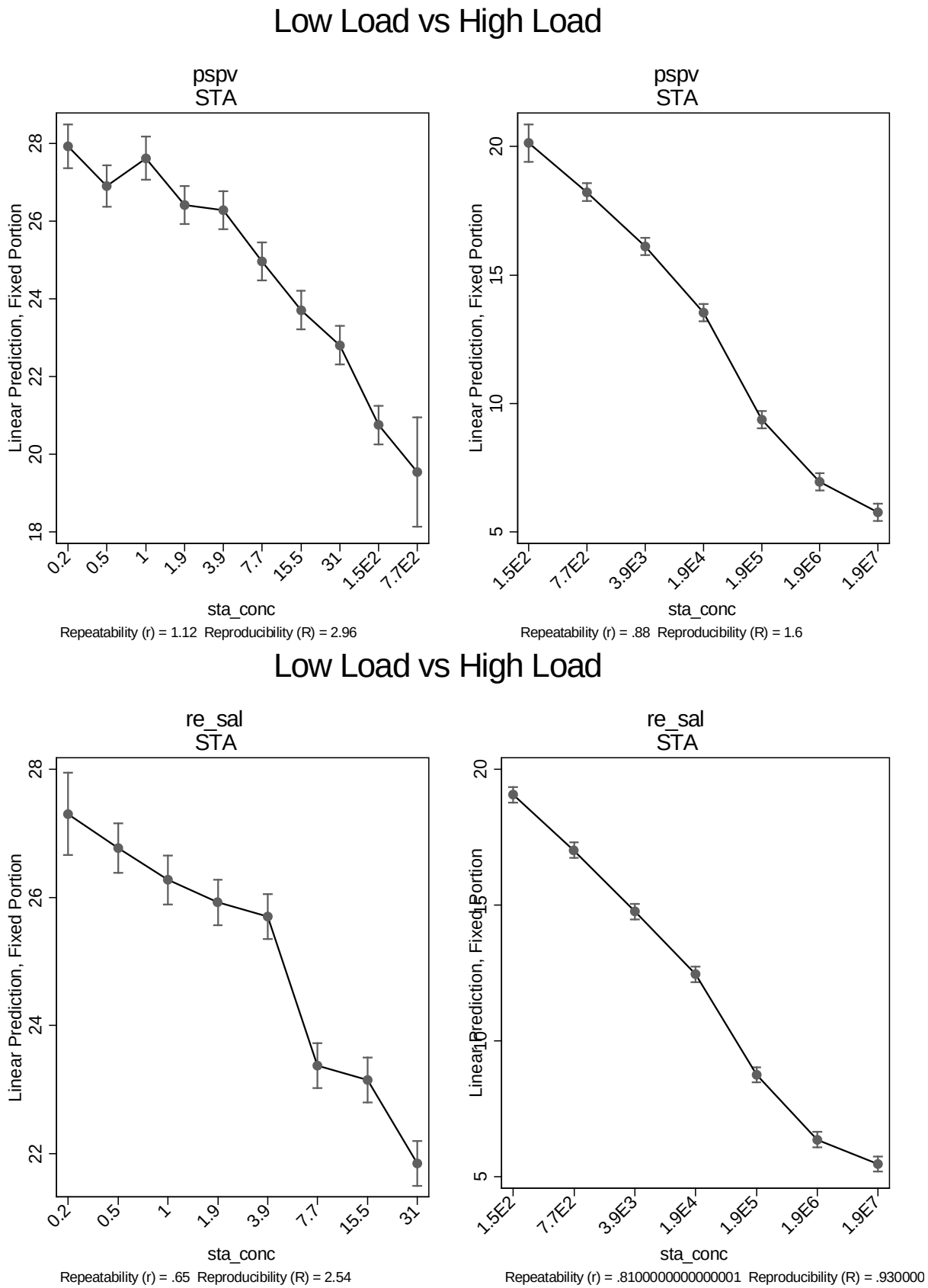

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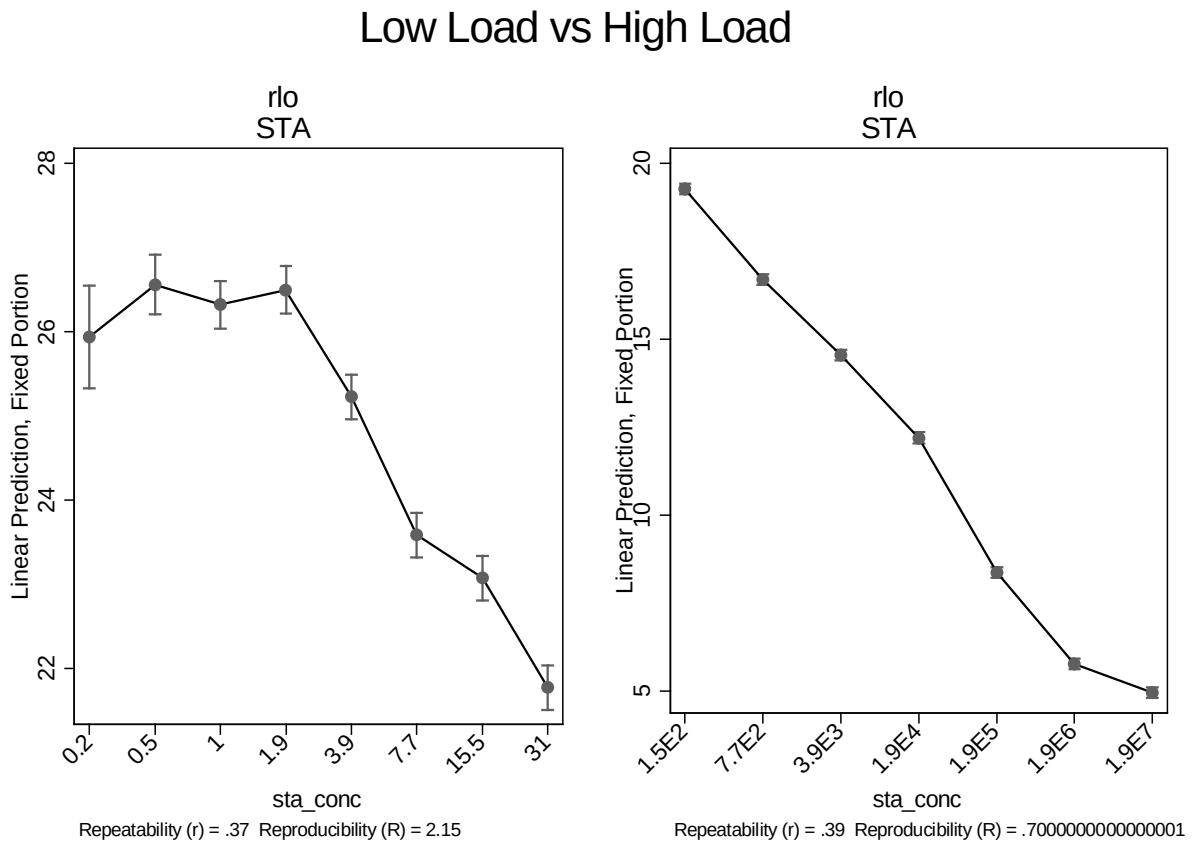

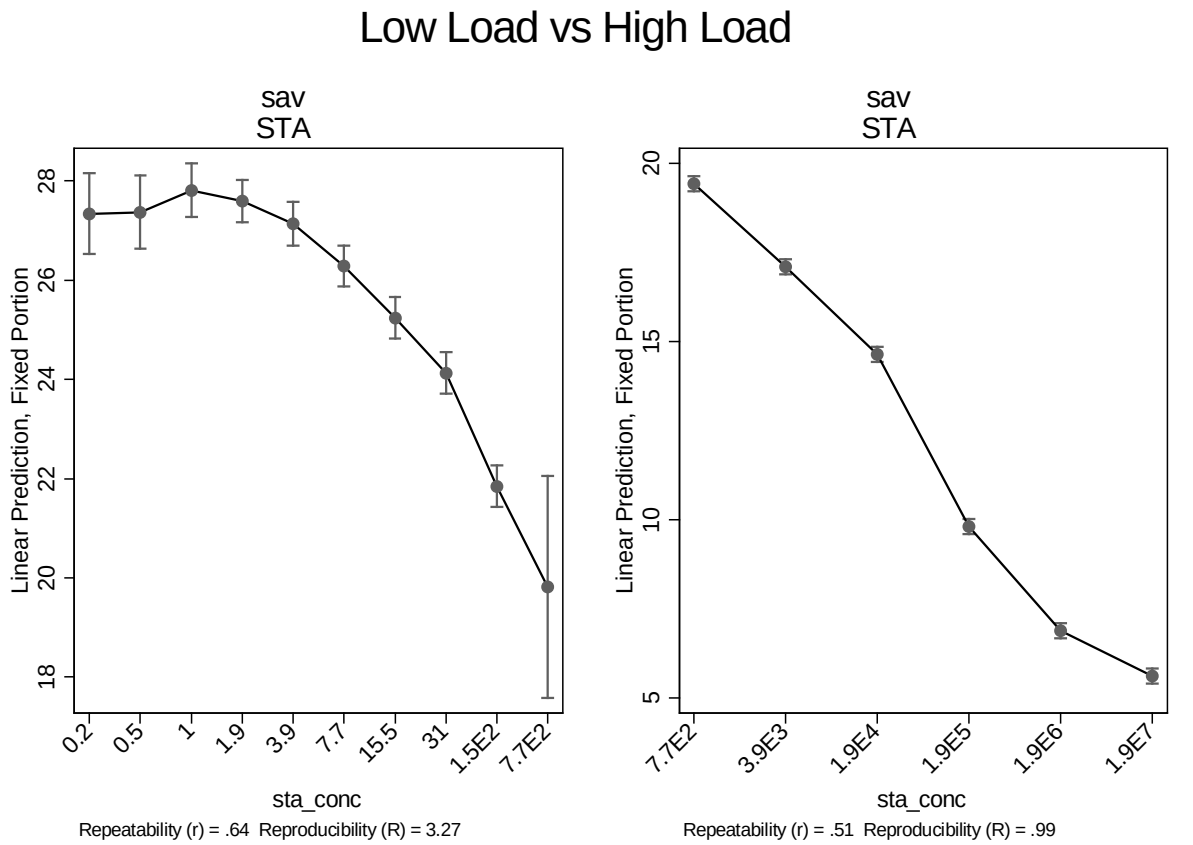

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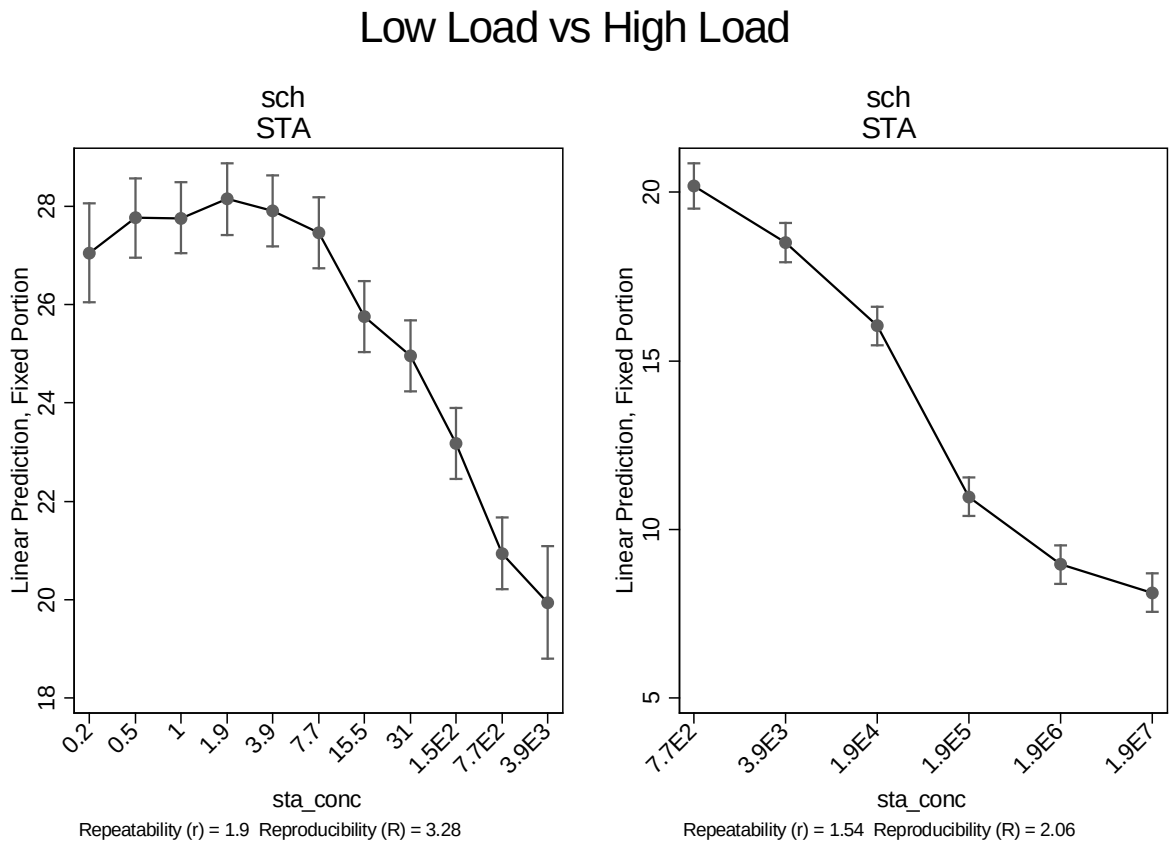

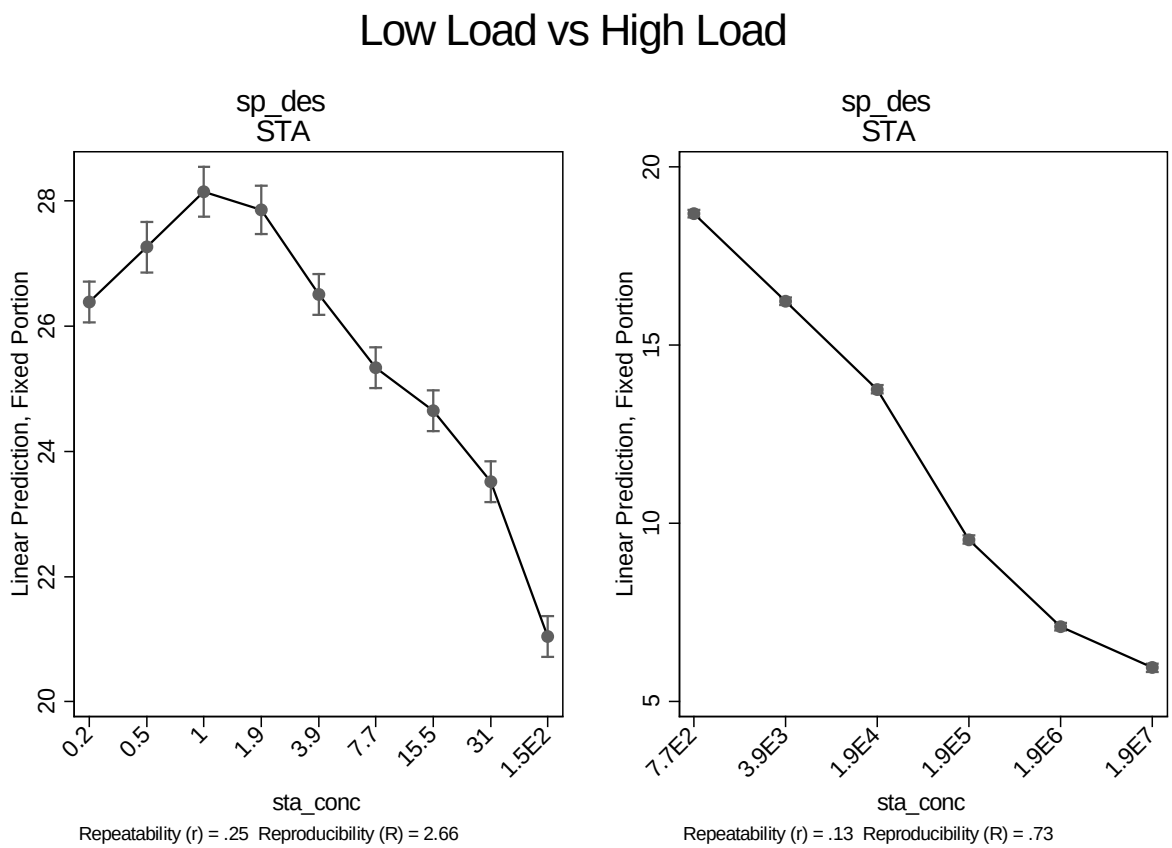

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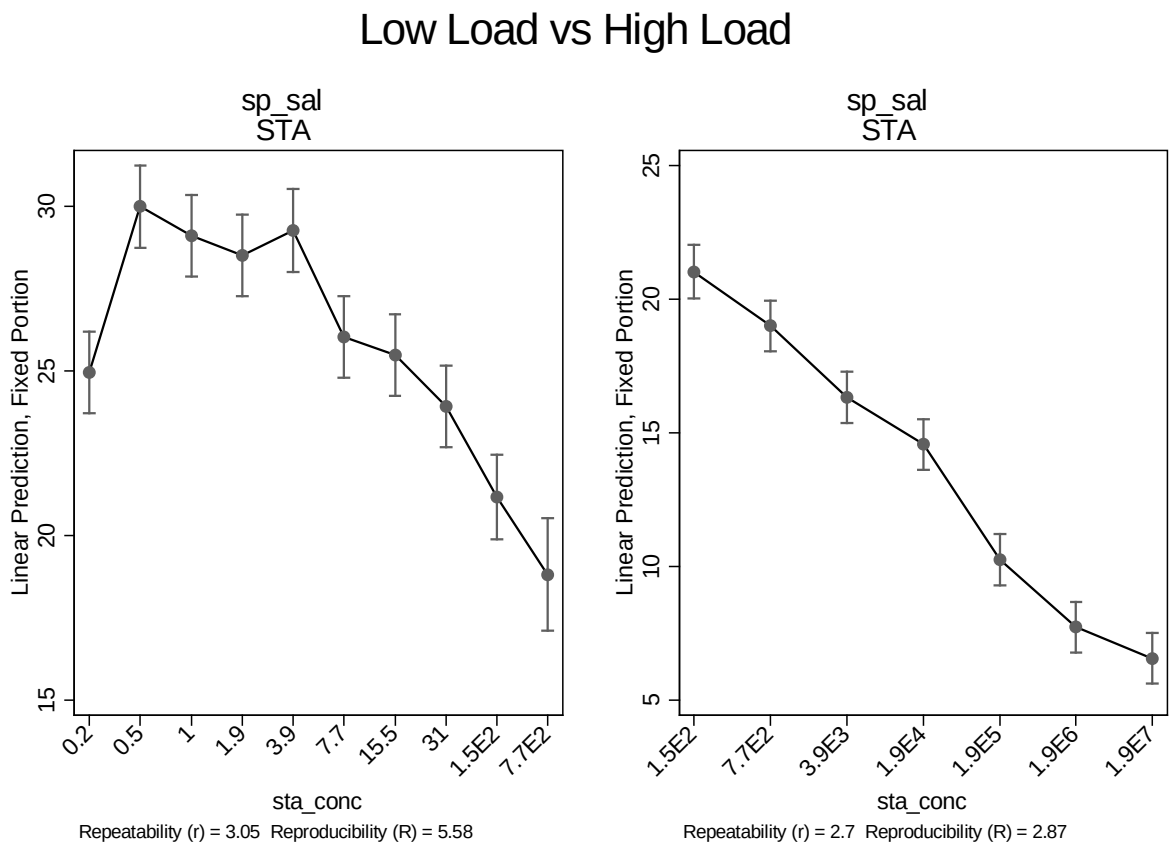

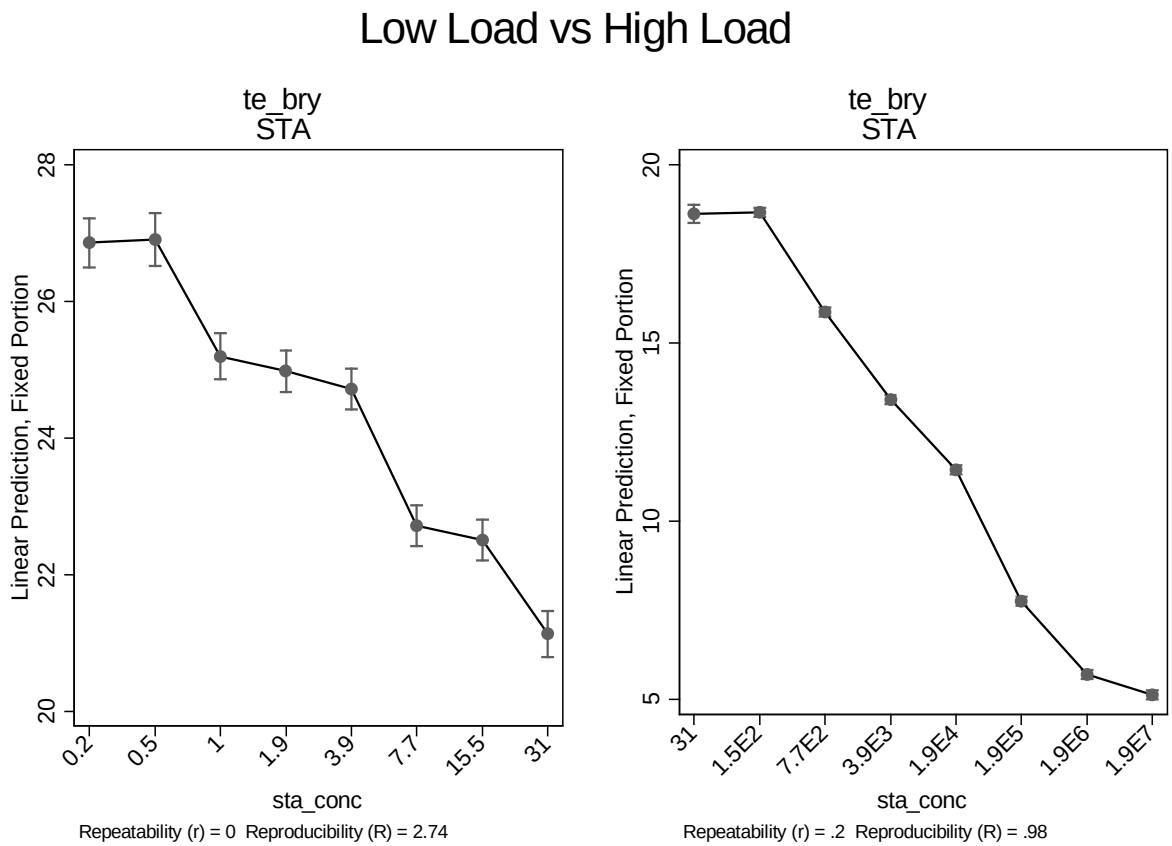

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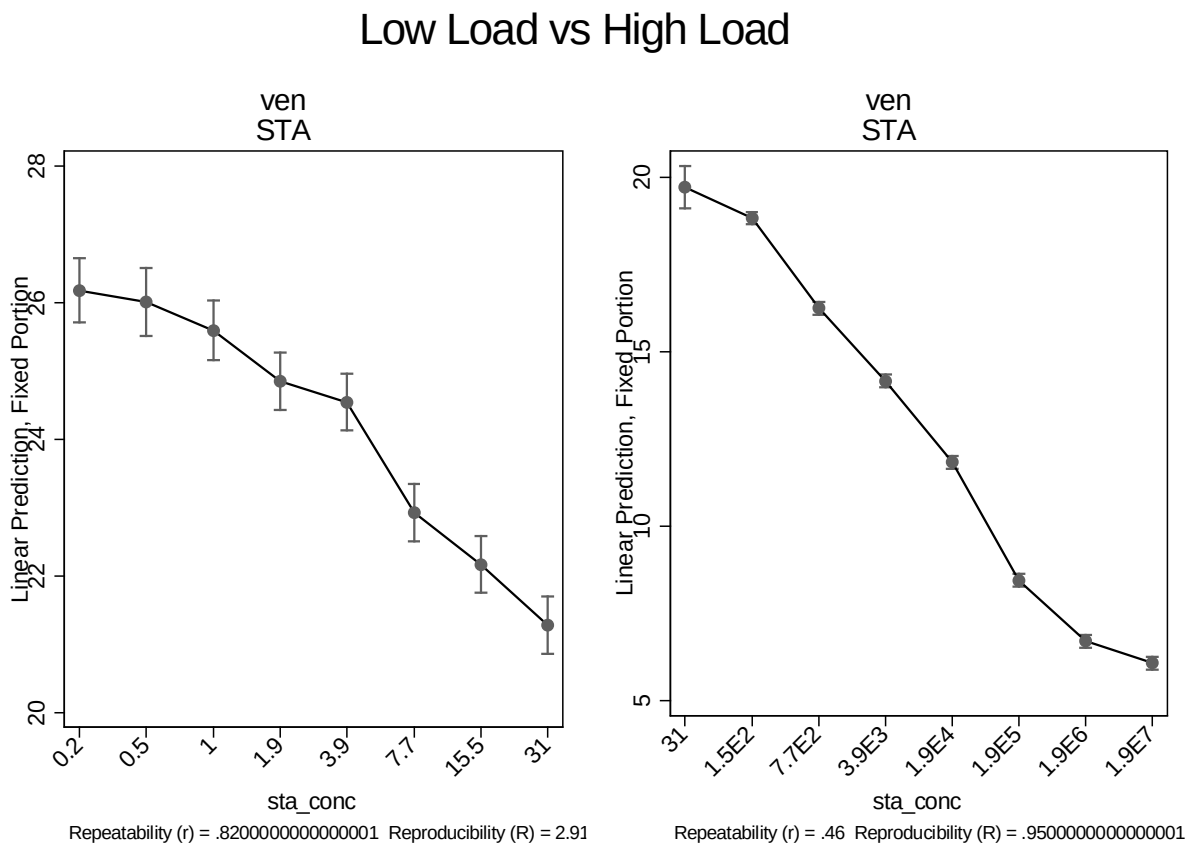

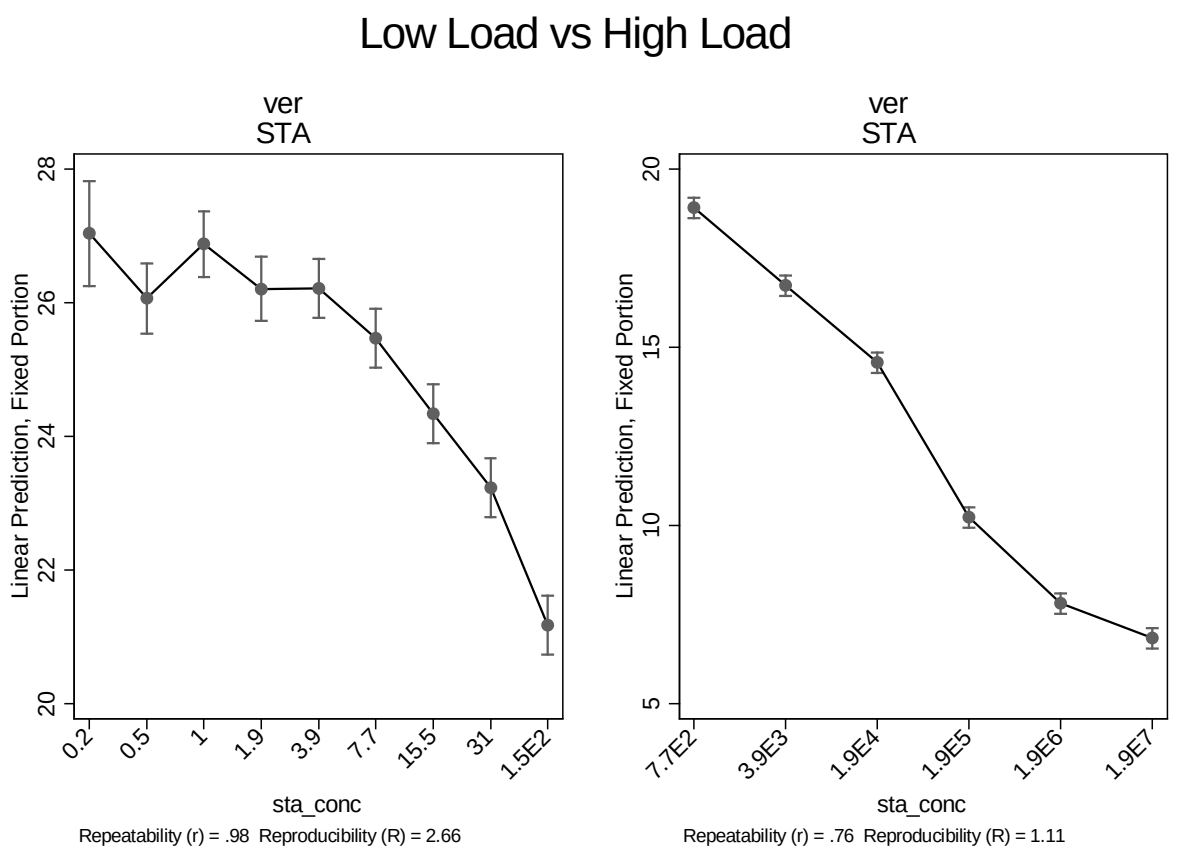

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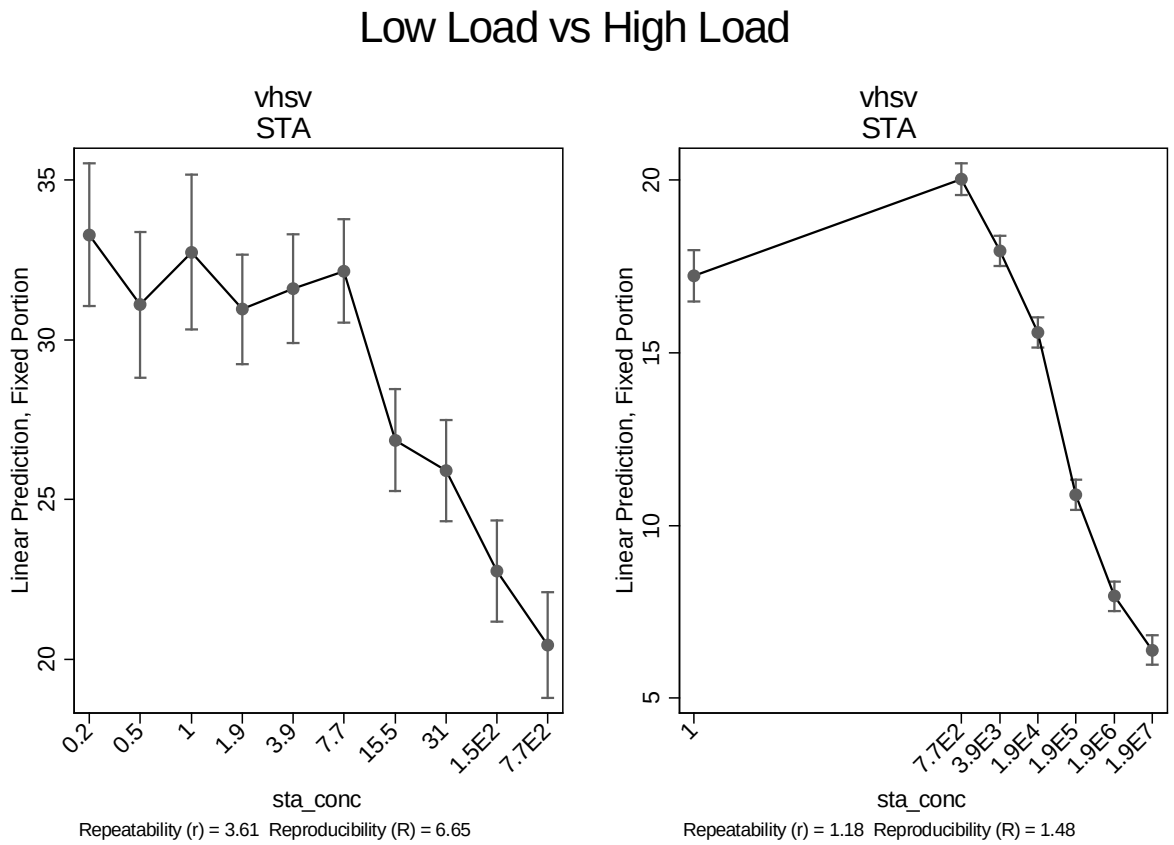

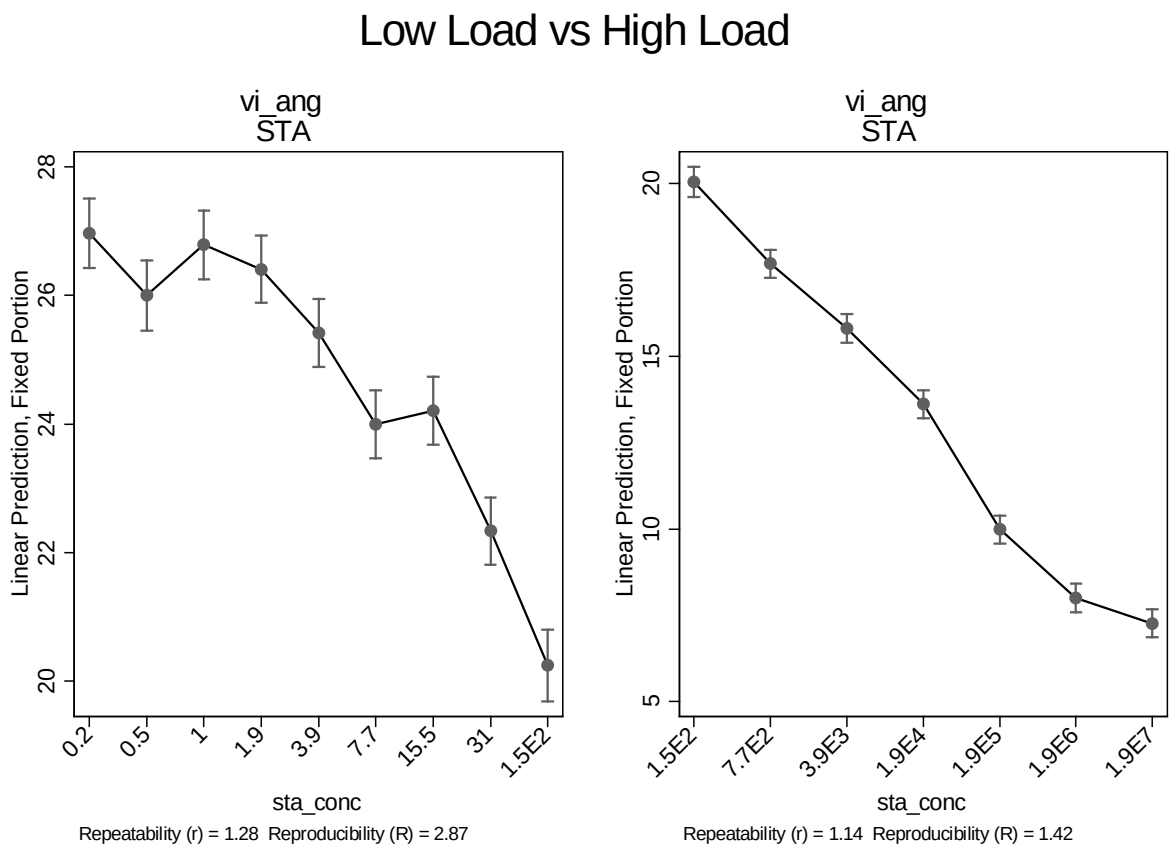

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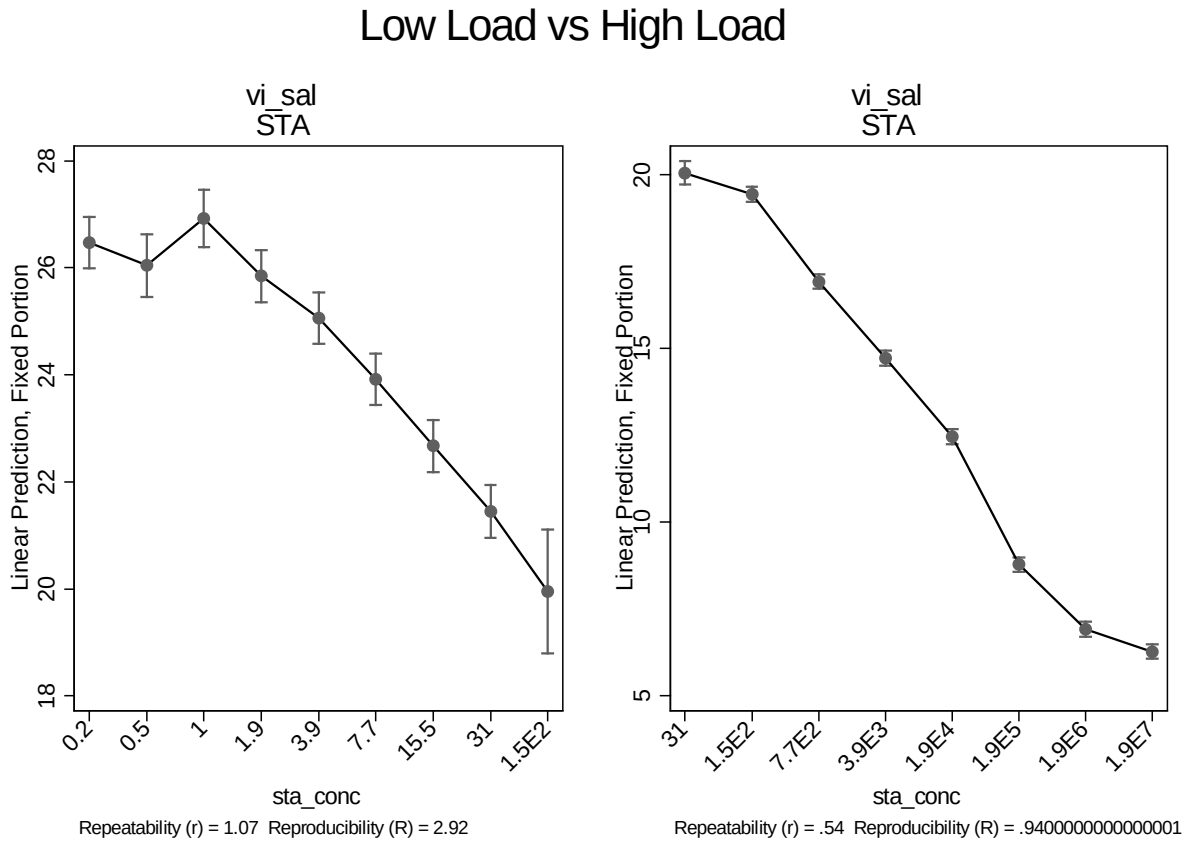

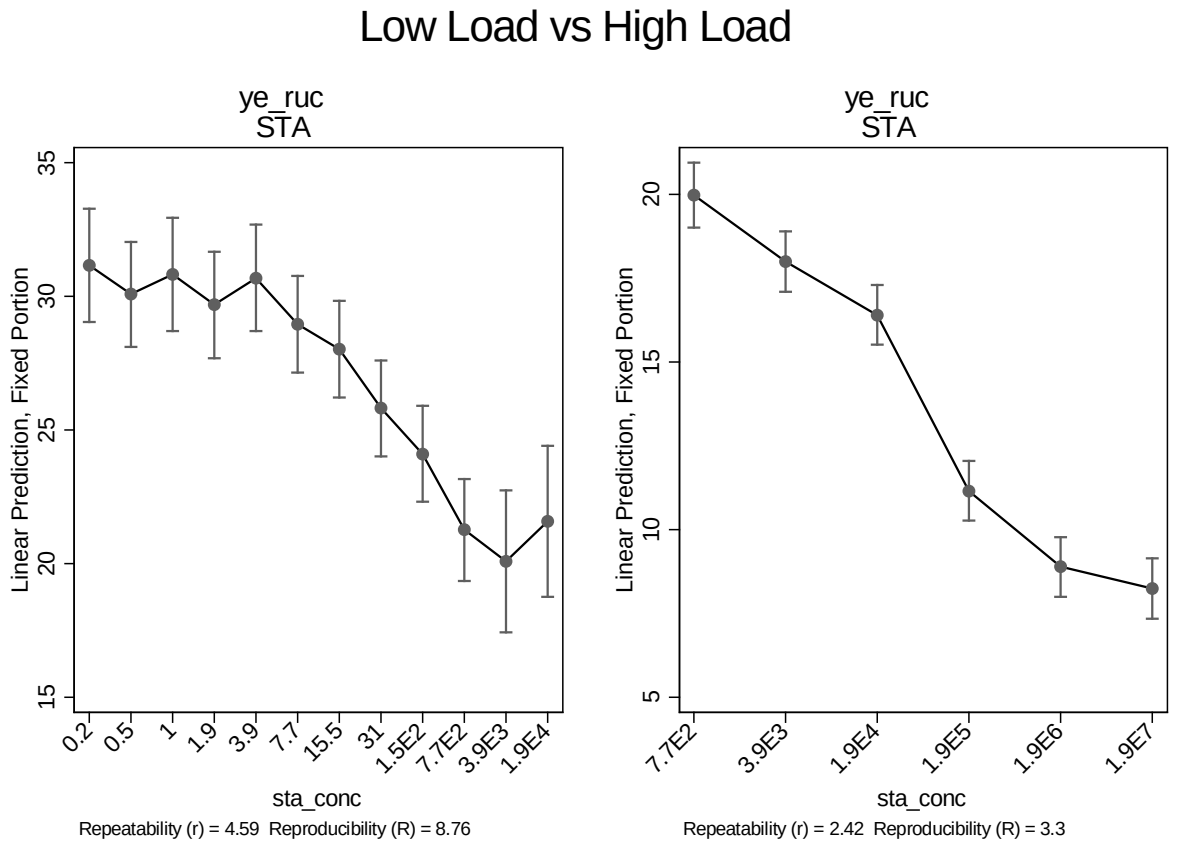

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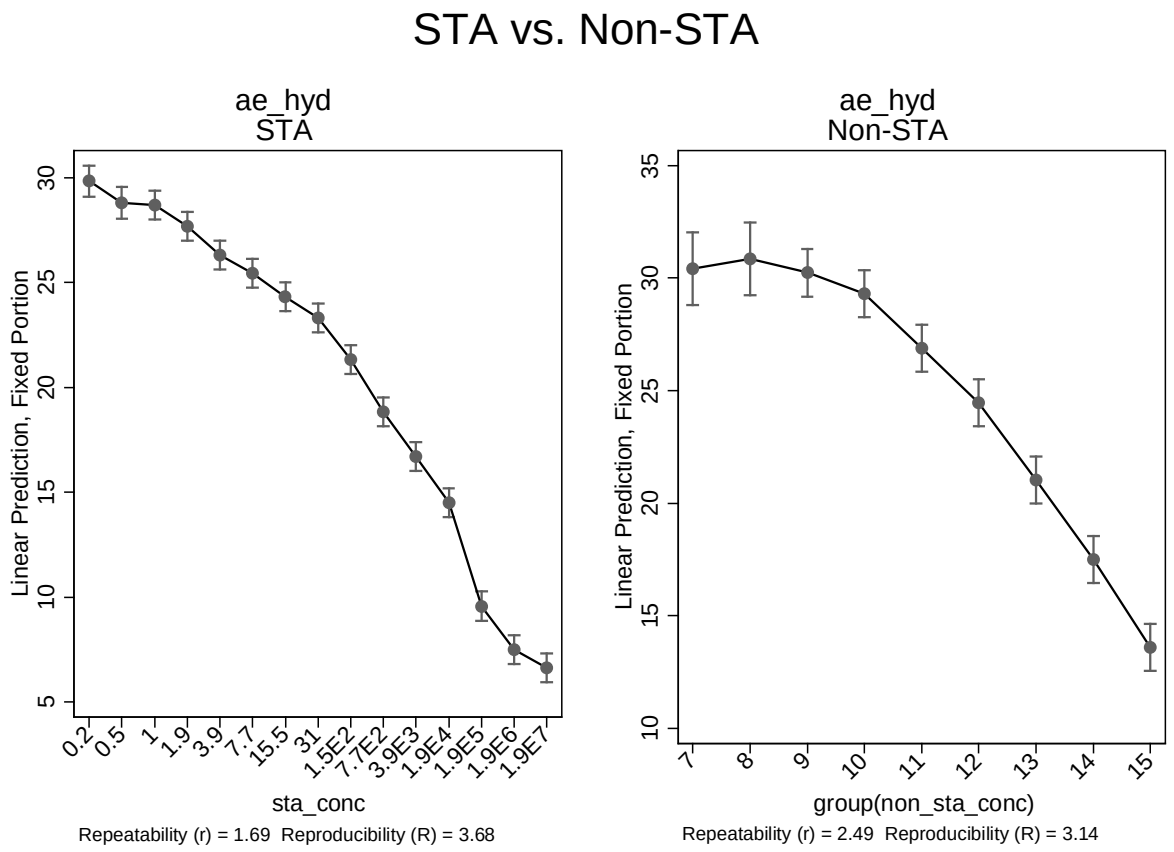
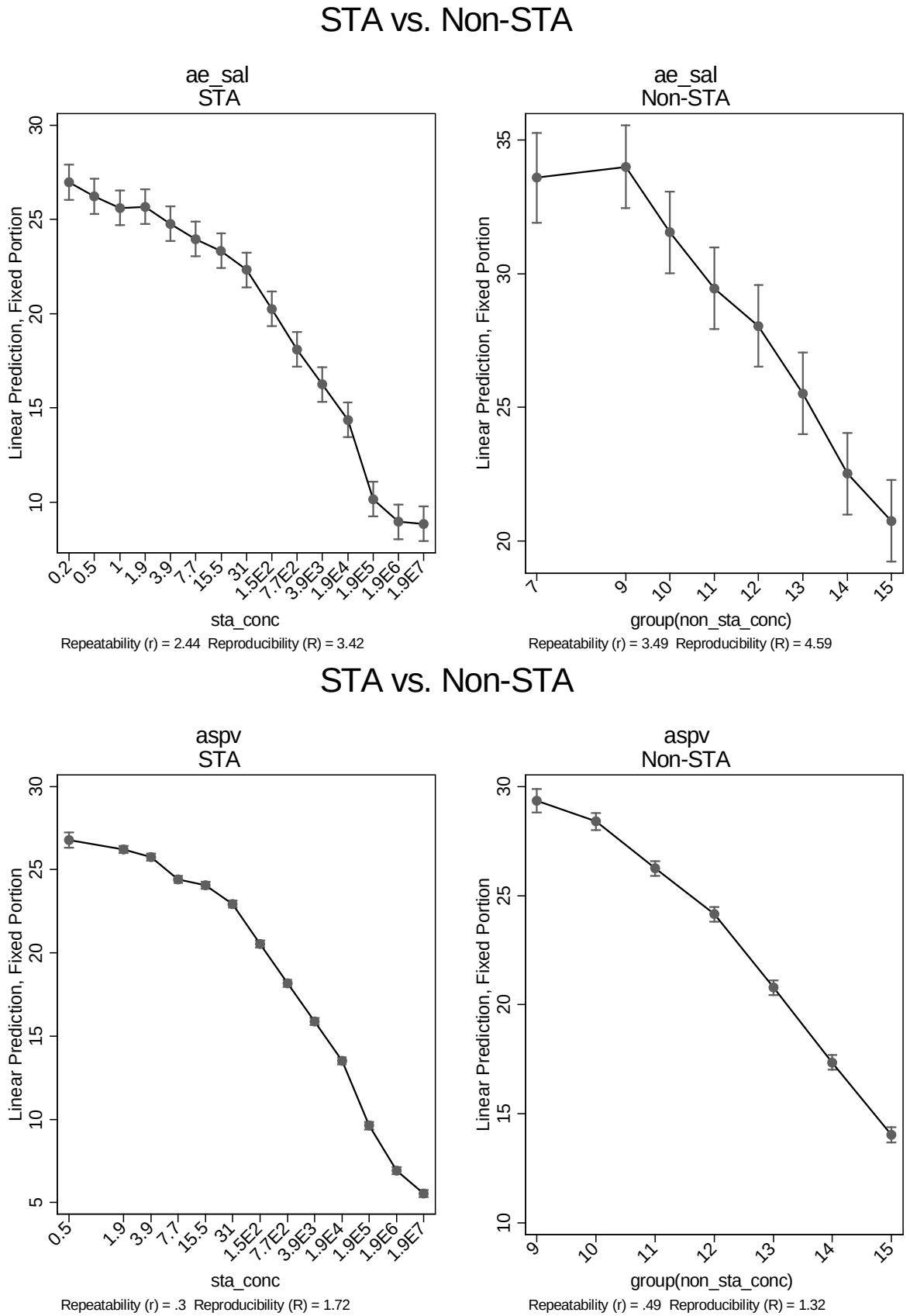


Supplemental Figure 4. Predicted Cts versus microbe concentration, from linear regression models, stratified by pre-amplification status (STA and Non-STA), for each assay (new page for each assay: ae_hyd, ae_sal, aspv, c_b_cys, ce_sha, cr_sal, de_sal, fa_mar, fl_psy, gy_sal, ic_hof, ic_mul, ihnv, ipnv, isav7, isav8, ku_thy, lo_sal, my_arc, my_cer, my_ins, na_sal, ne_per, nu_sal, omv, pa_kab, pa_min, pa_pse, pa_ther, pch_sal, pisck_sal, pmcv, prv, pspv, re_sal, rlo, sav, sch, sp_des, sp_sal, te_bry, ven, ver, vhsv, vi_ang, vi_sal, and ye_ruc).



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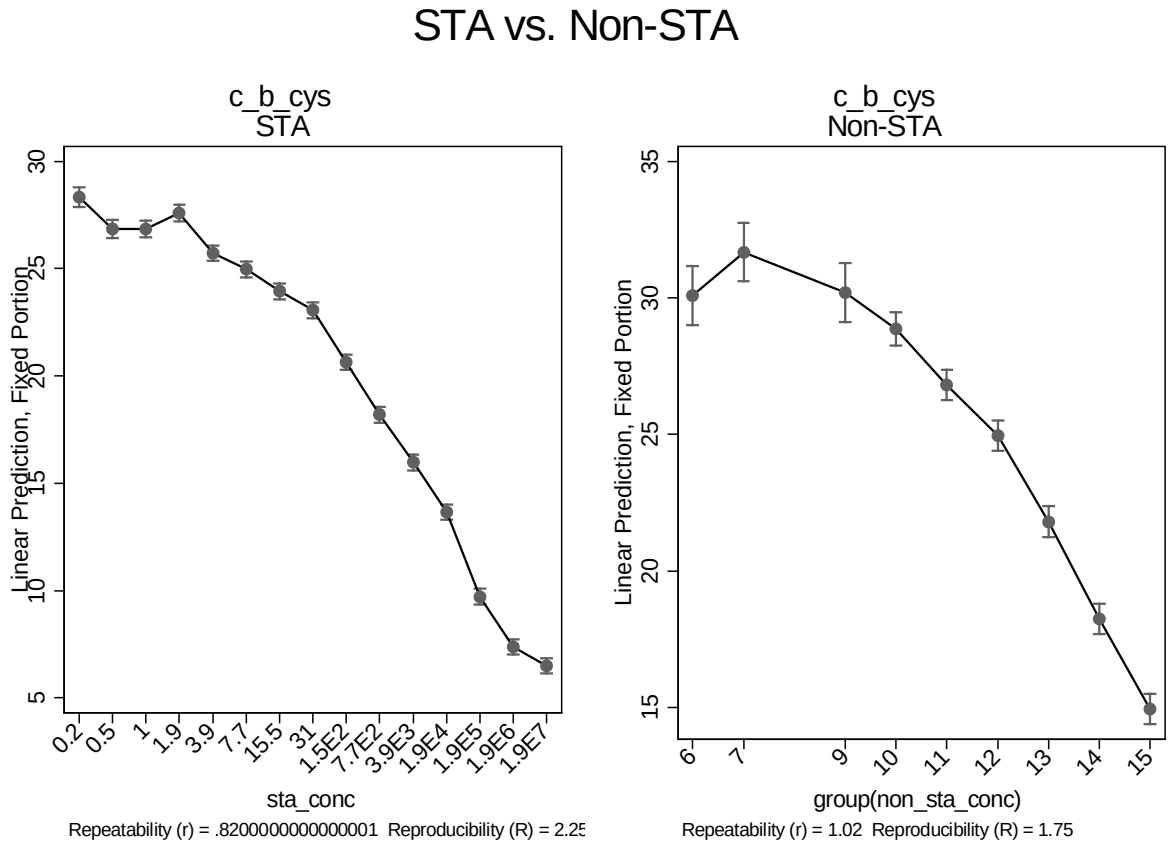

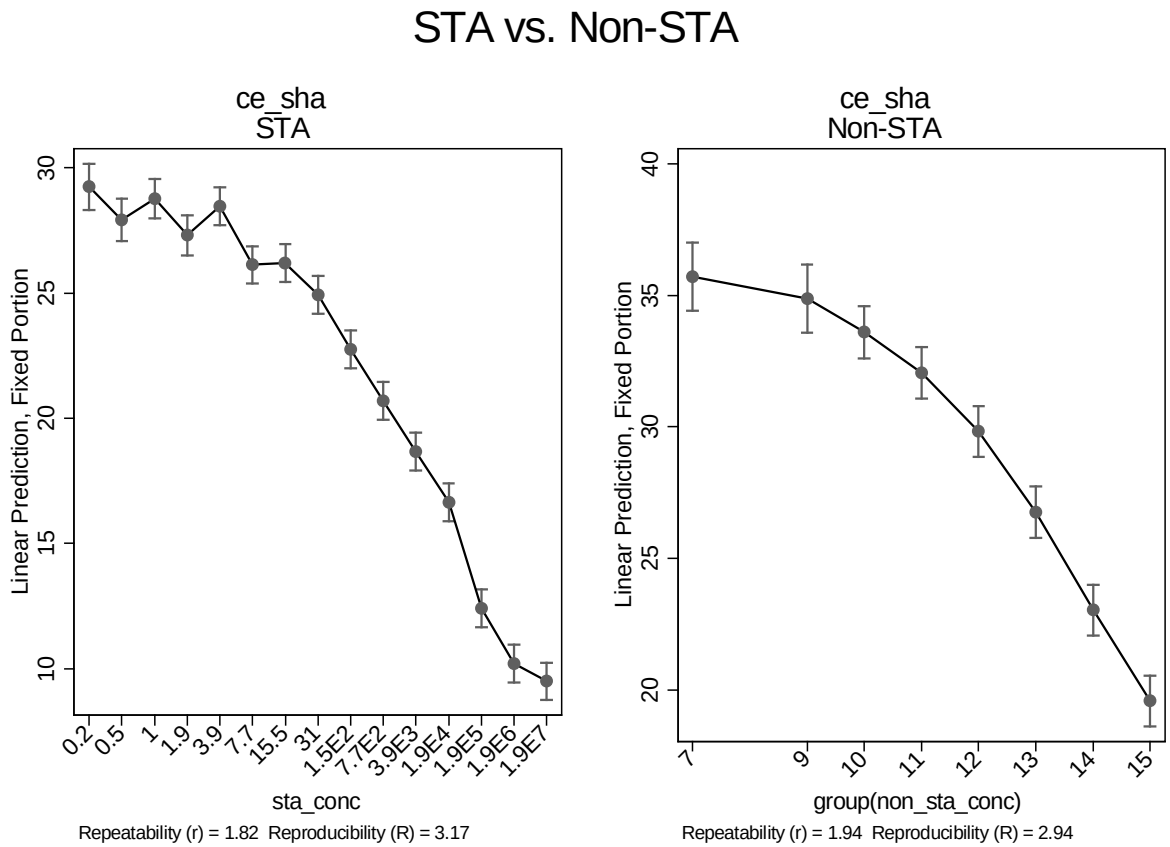

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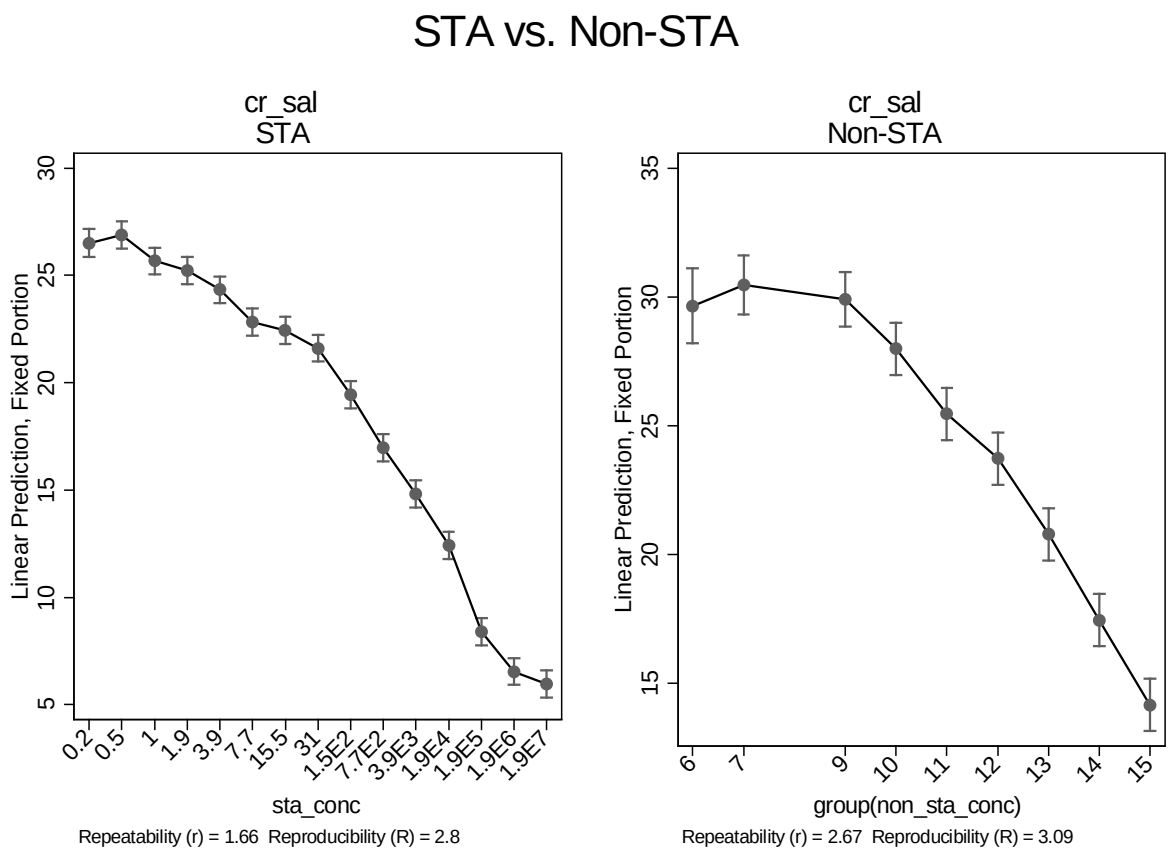

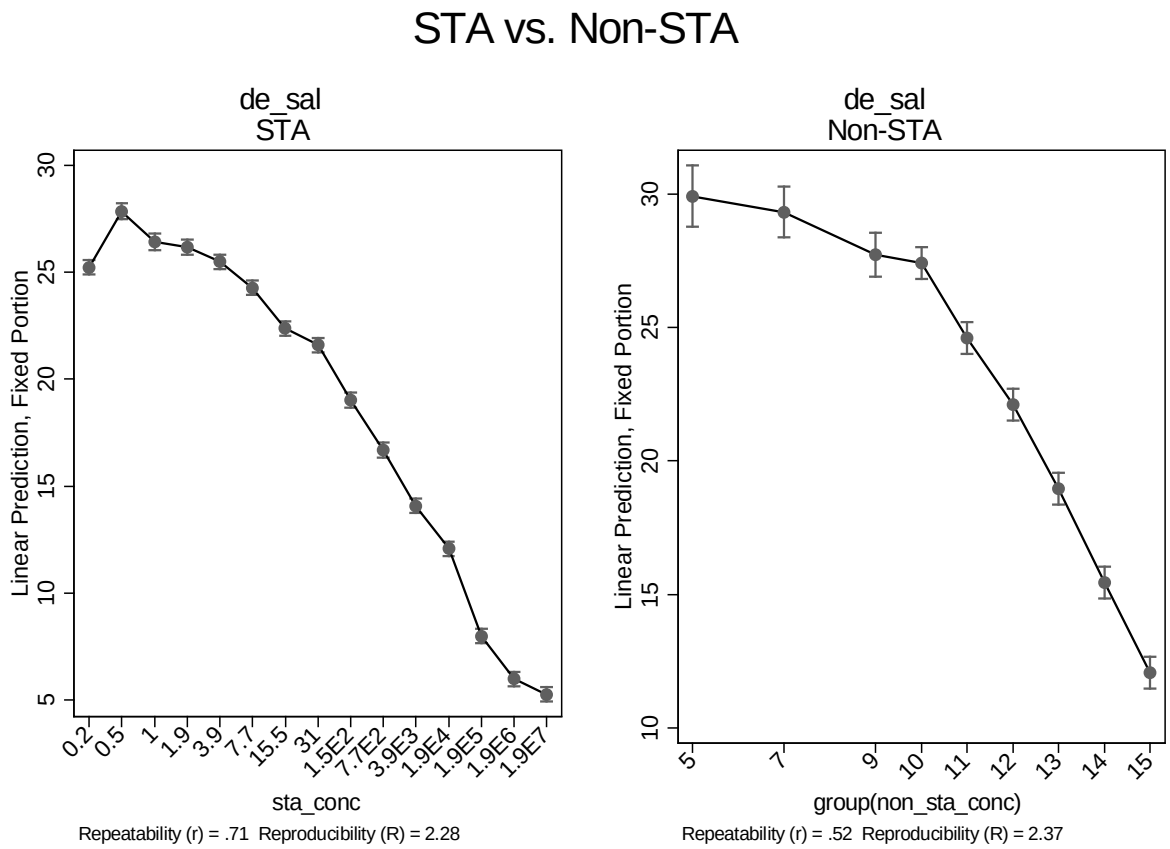

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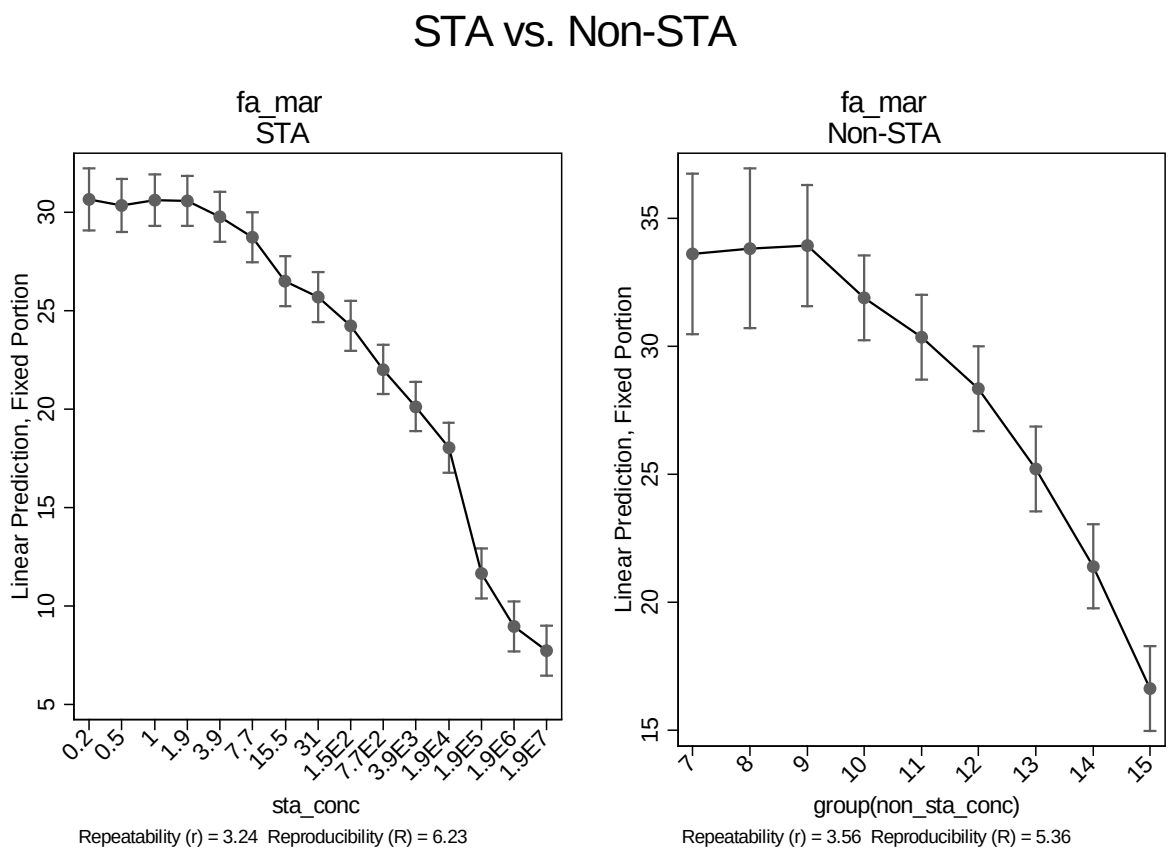

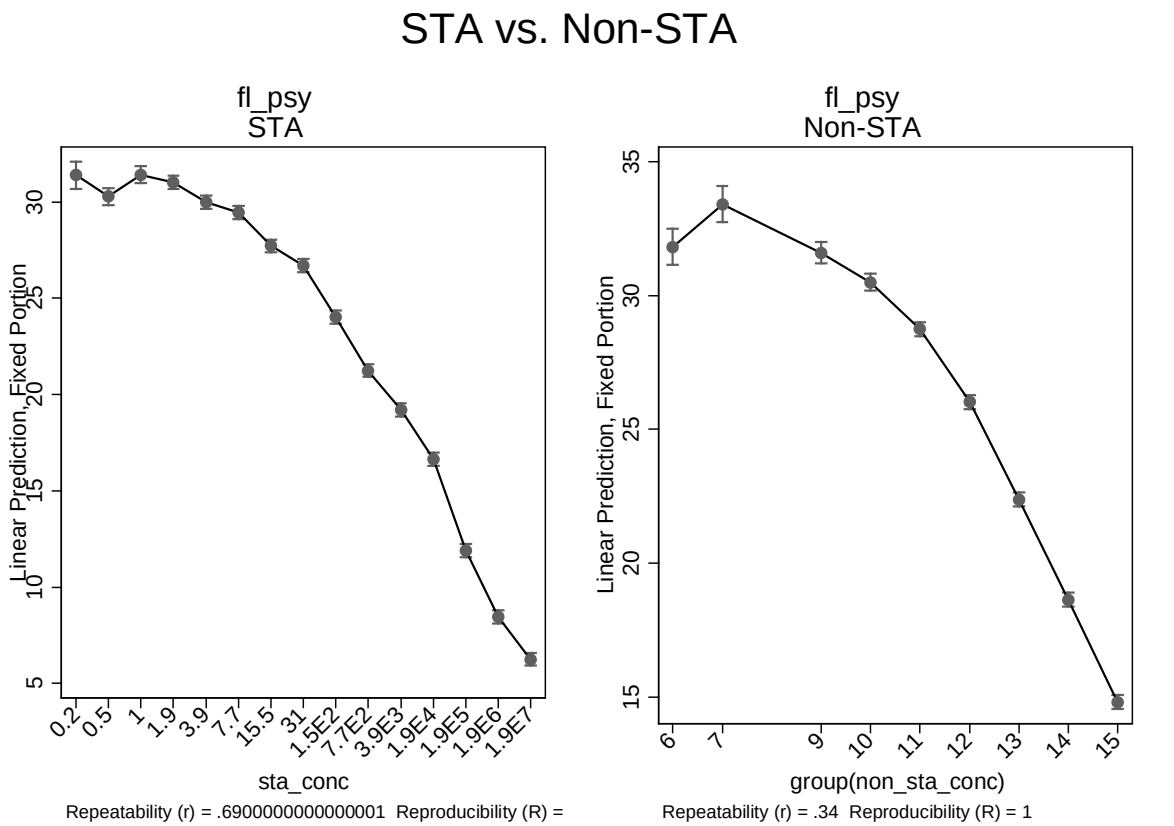

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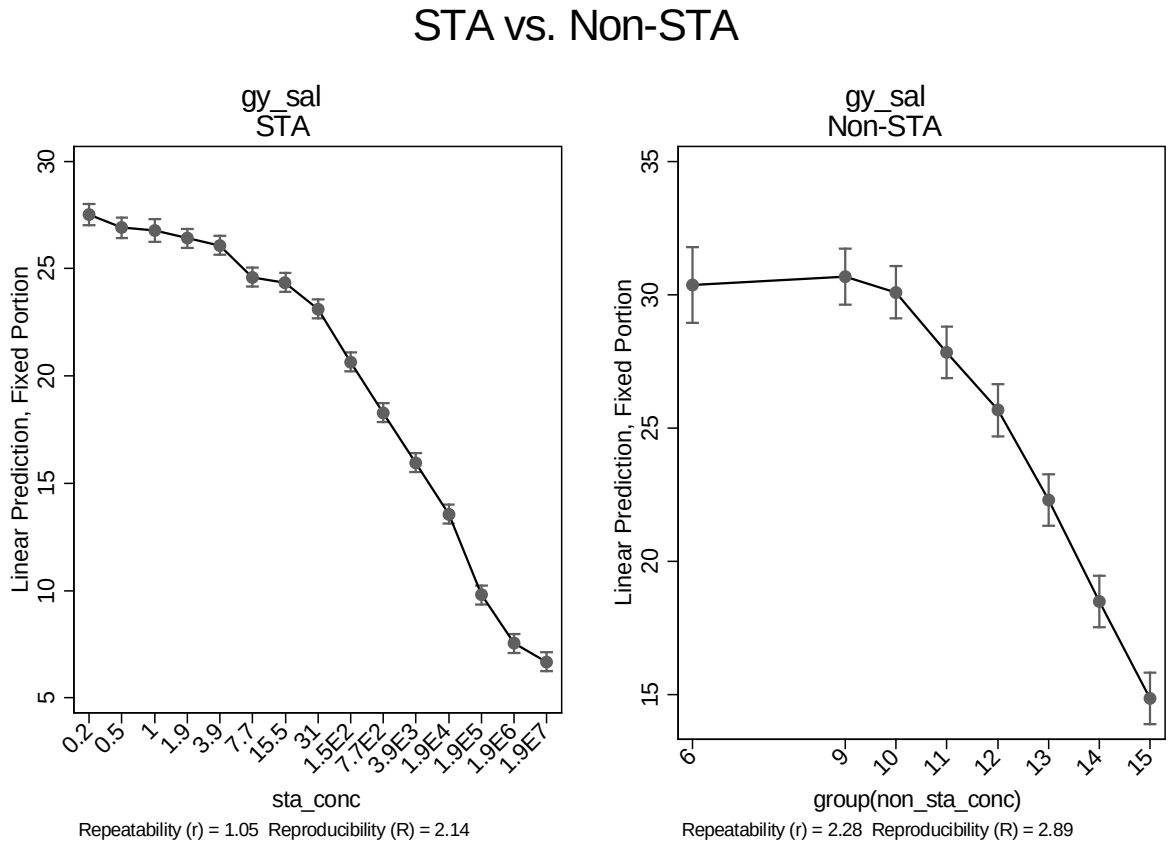

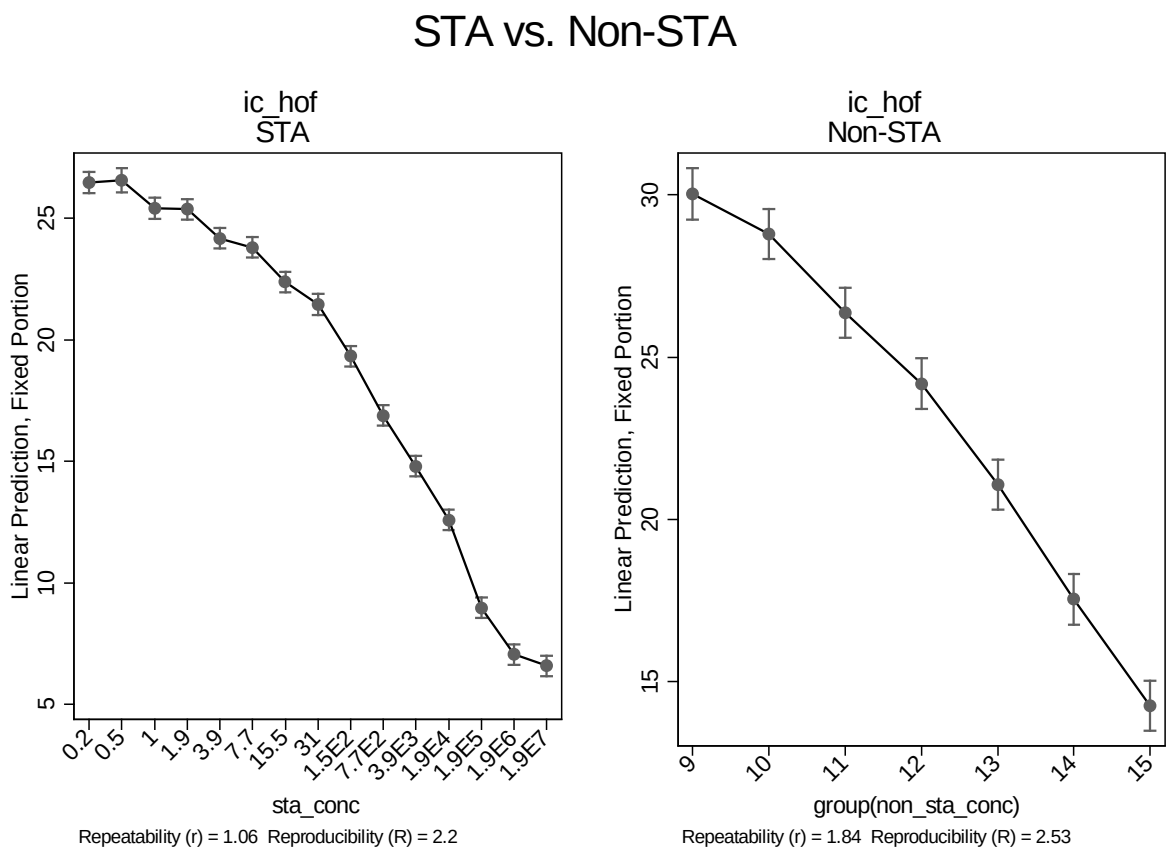

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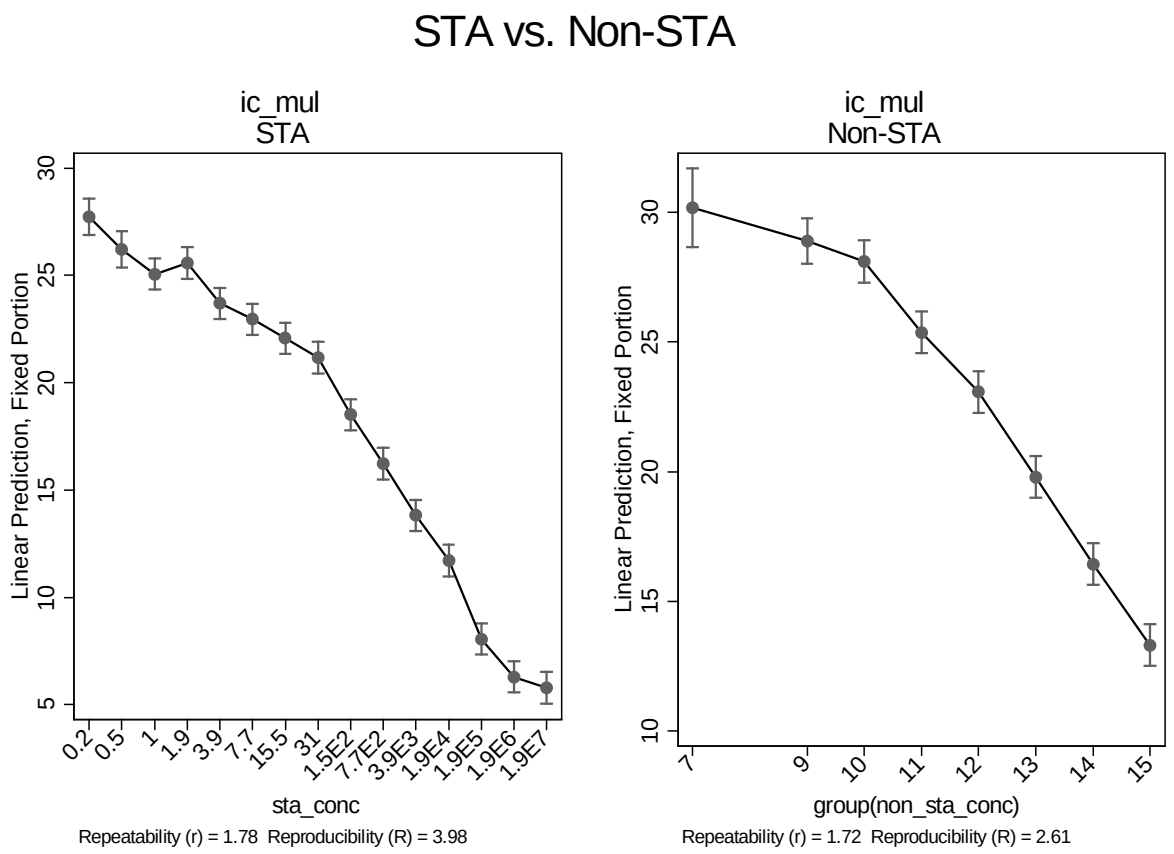

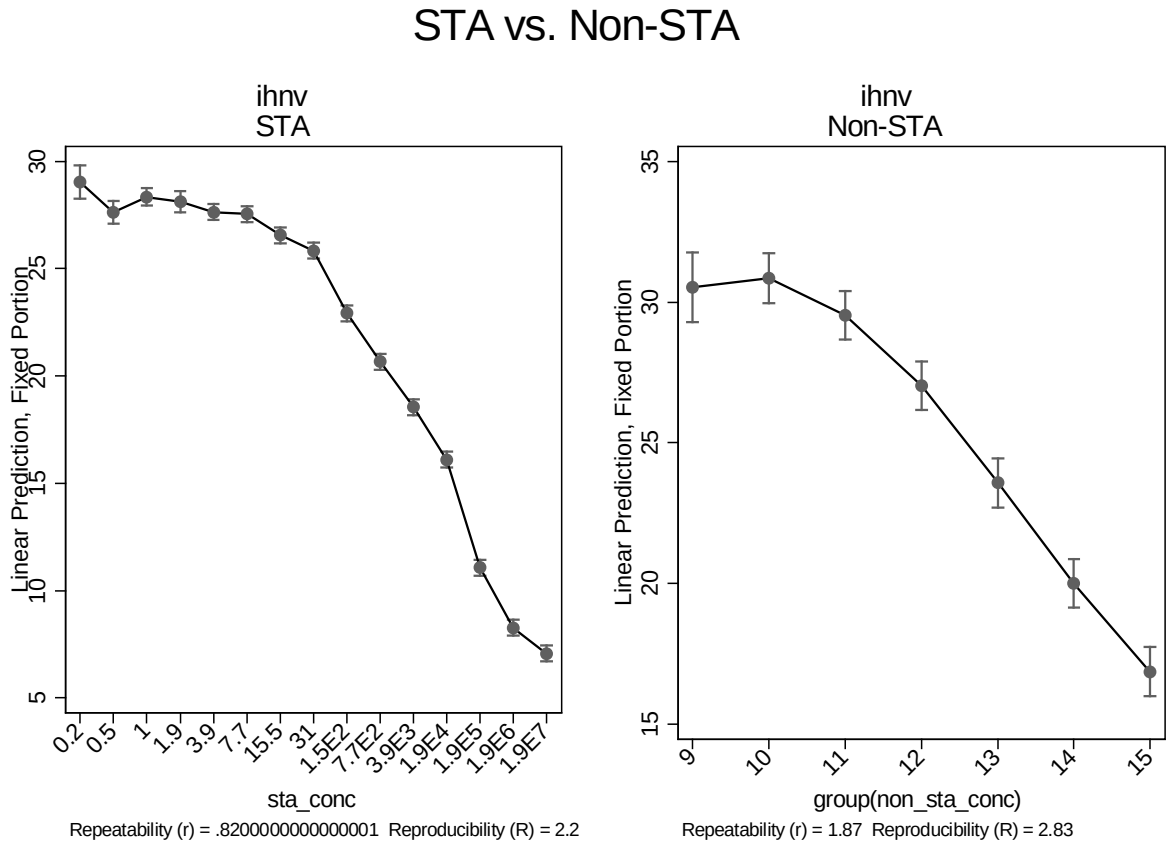

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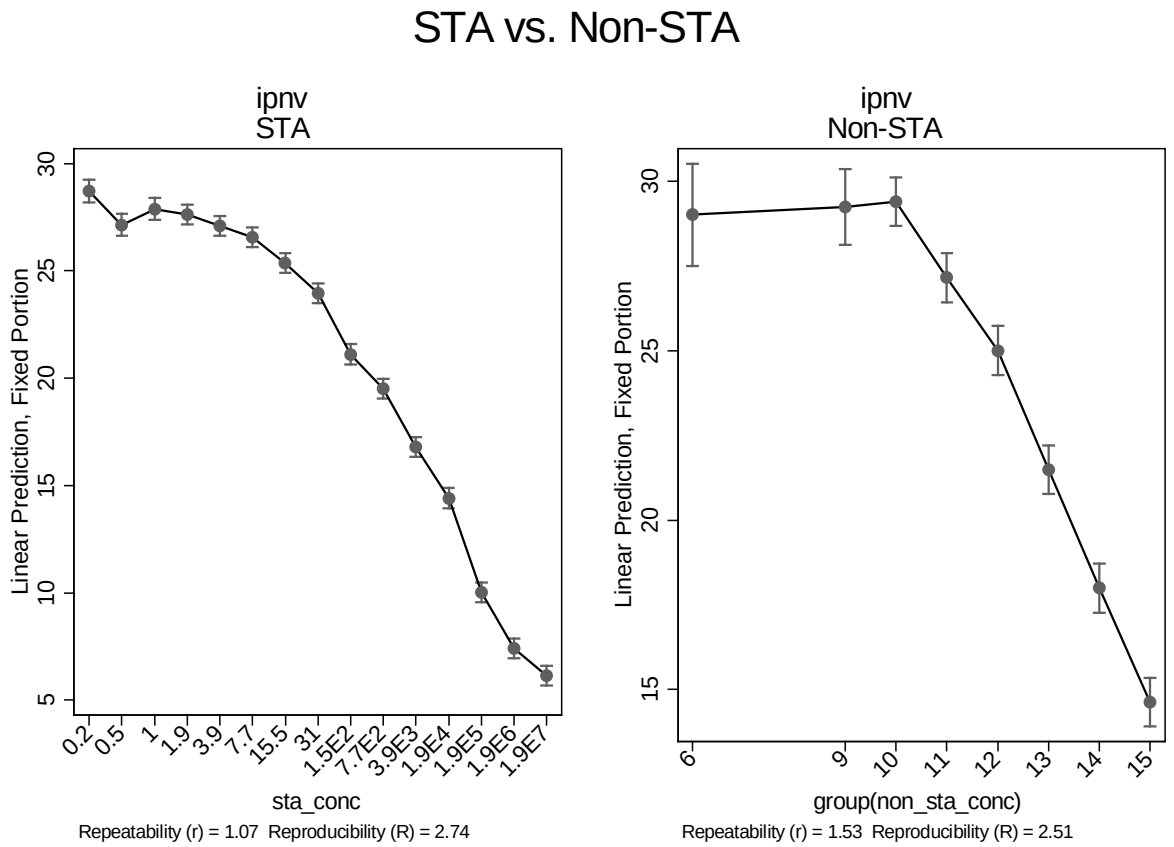

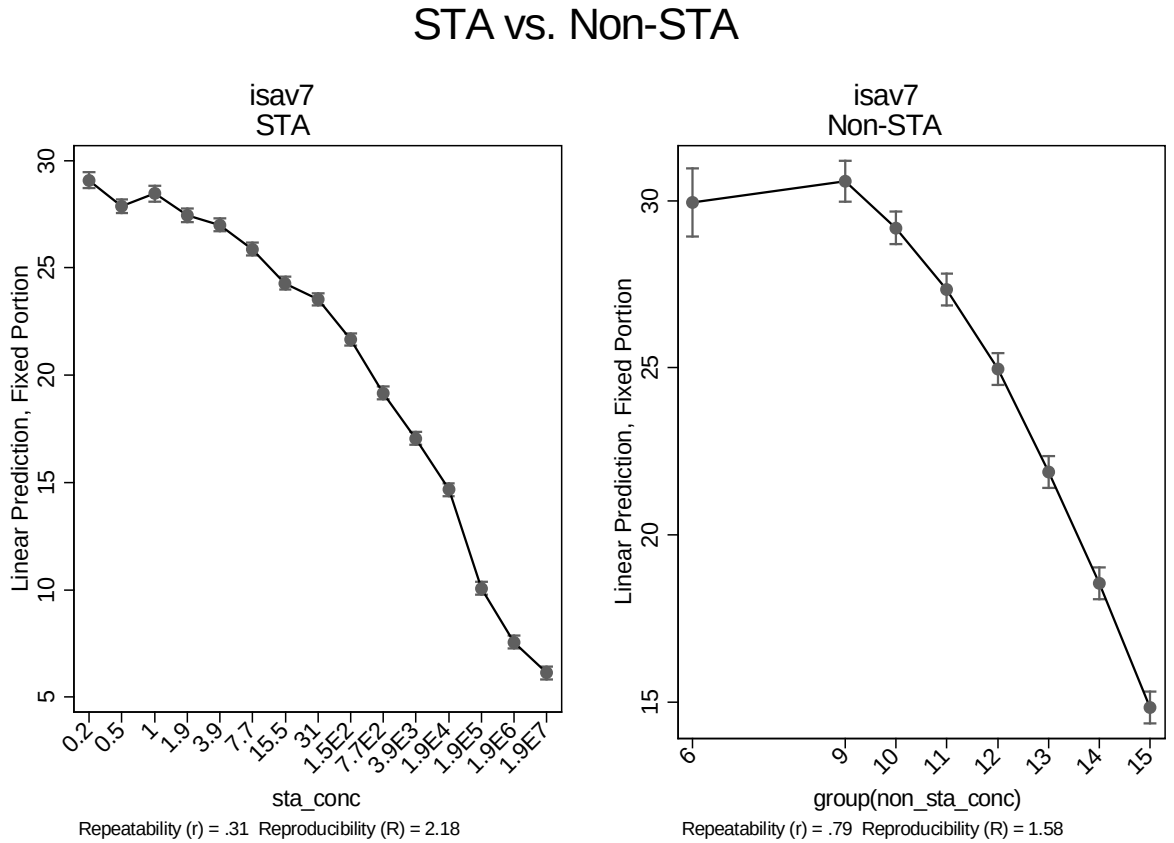

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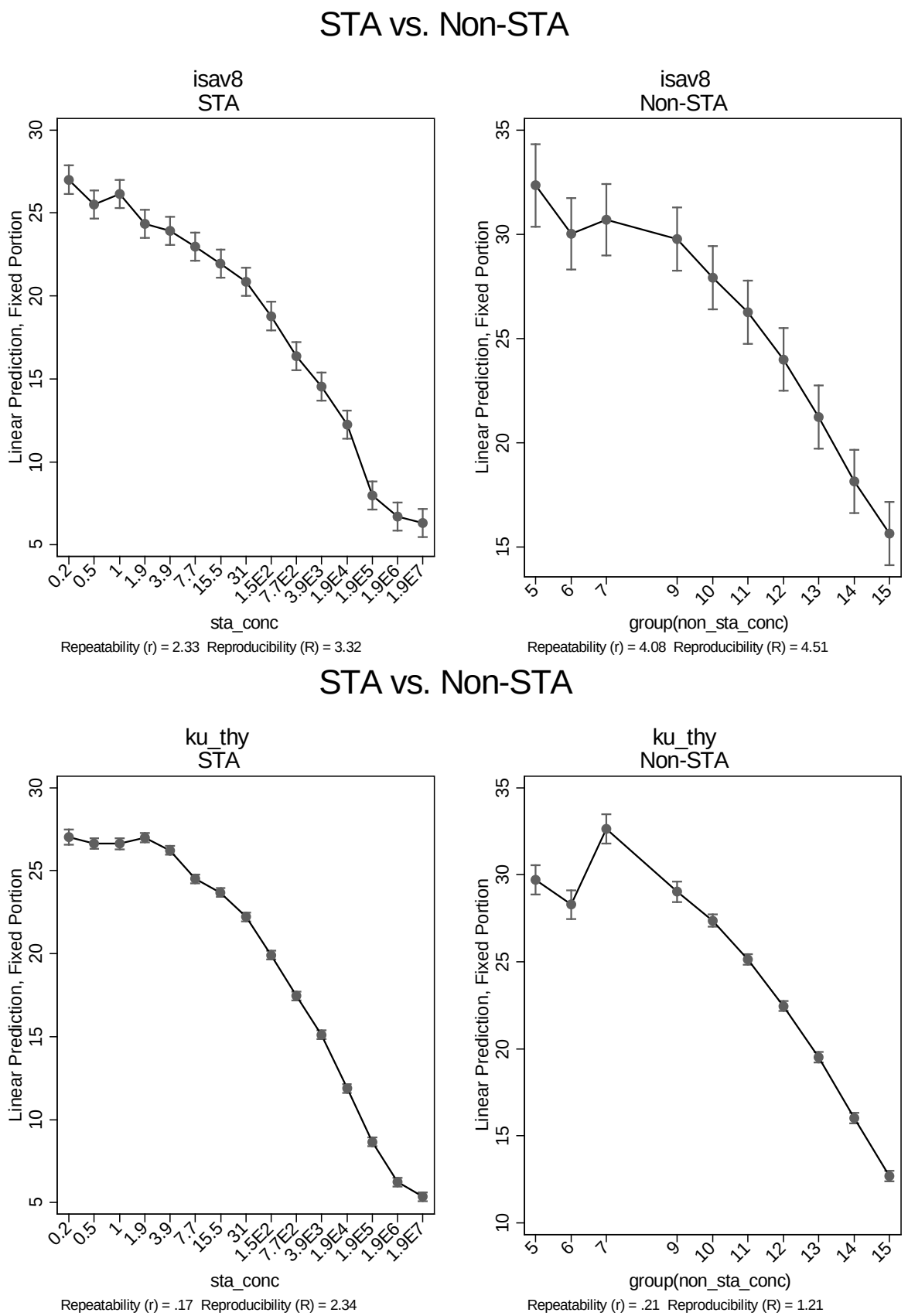

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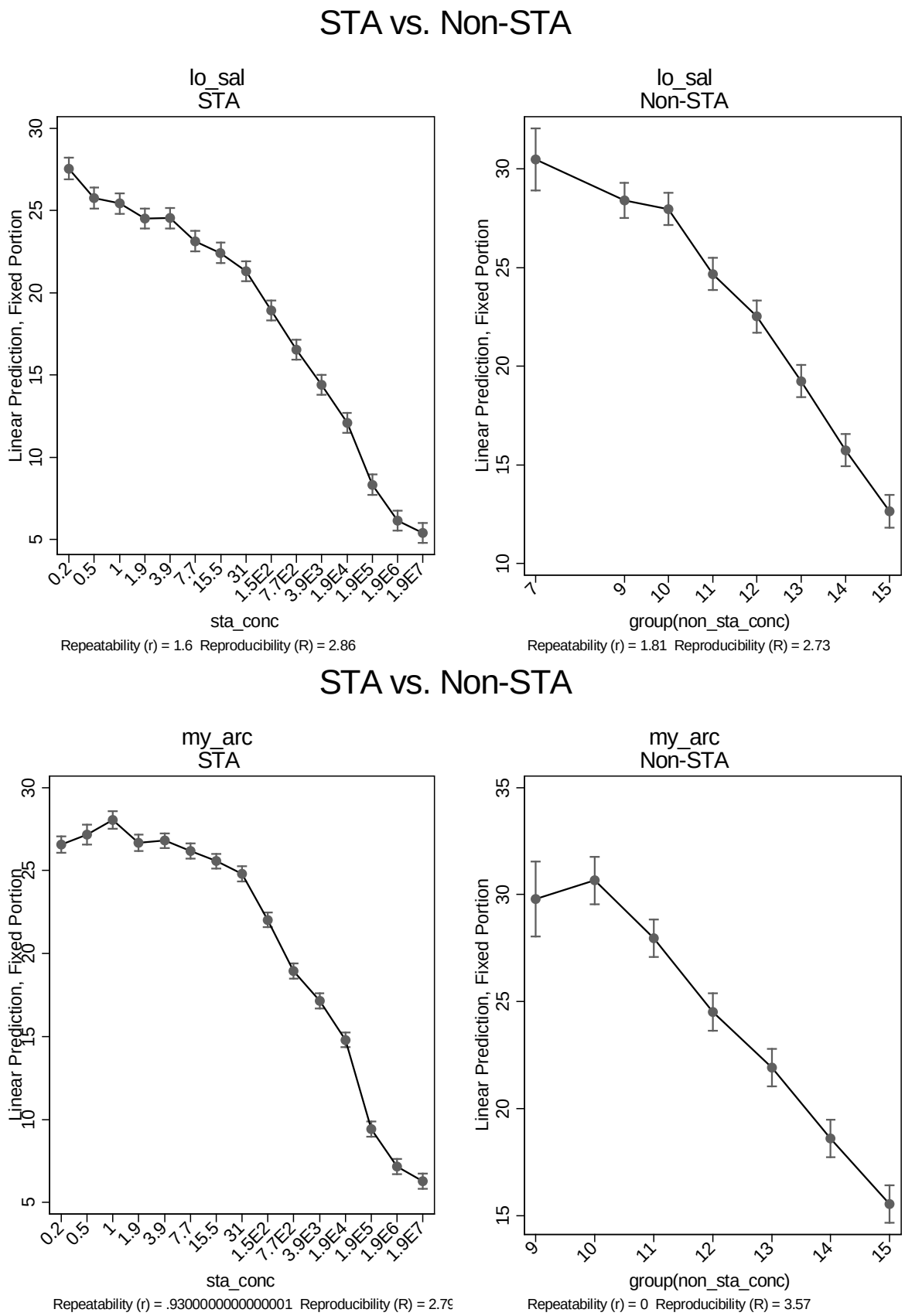

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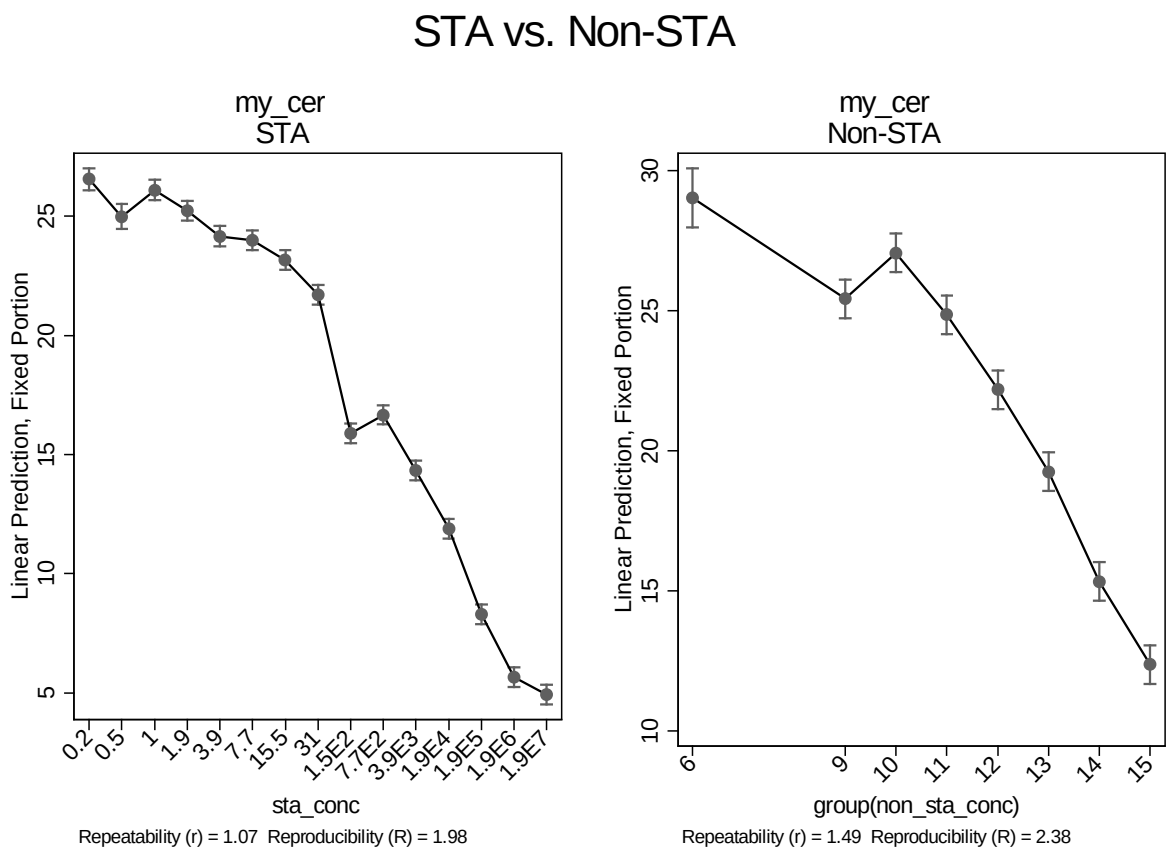

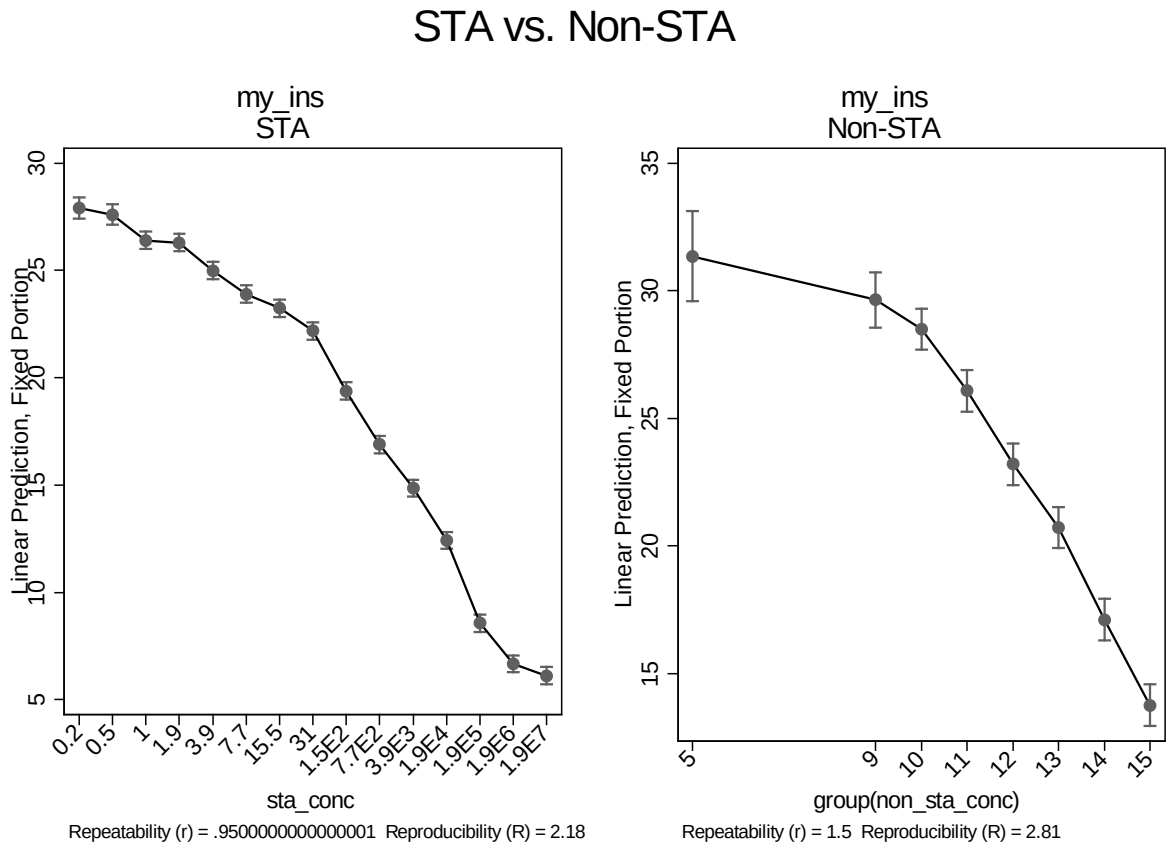

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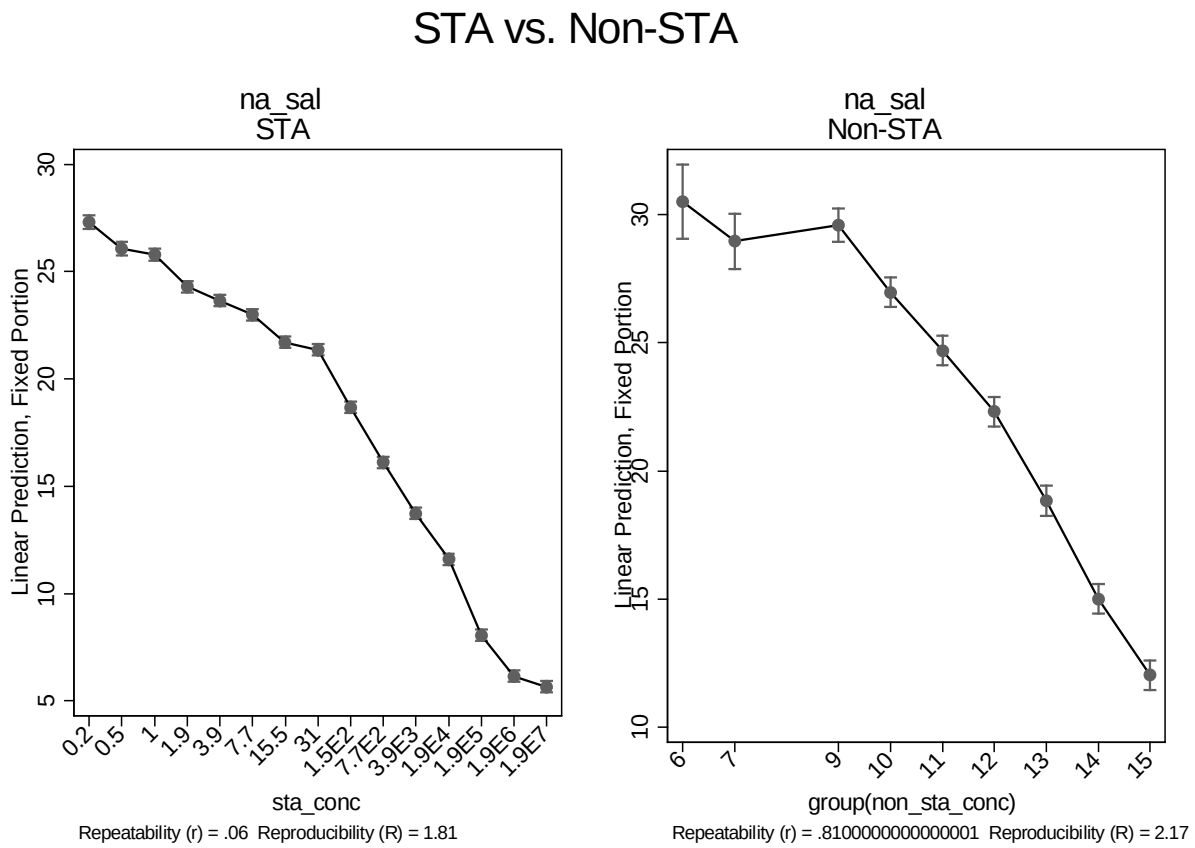

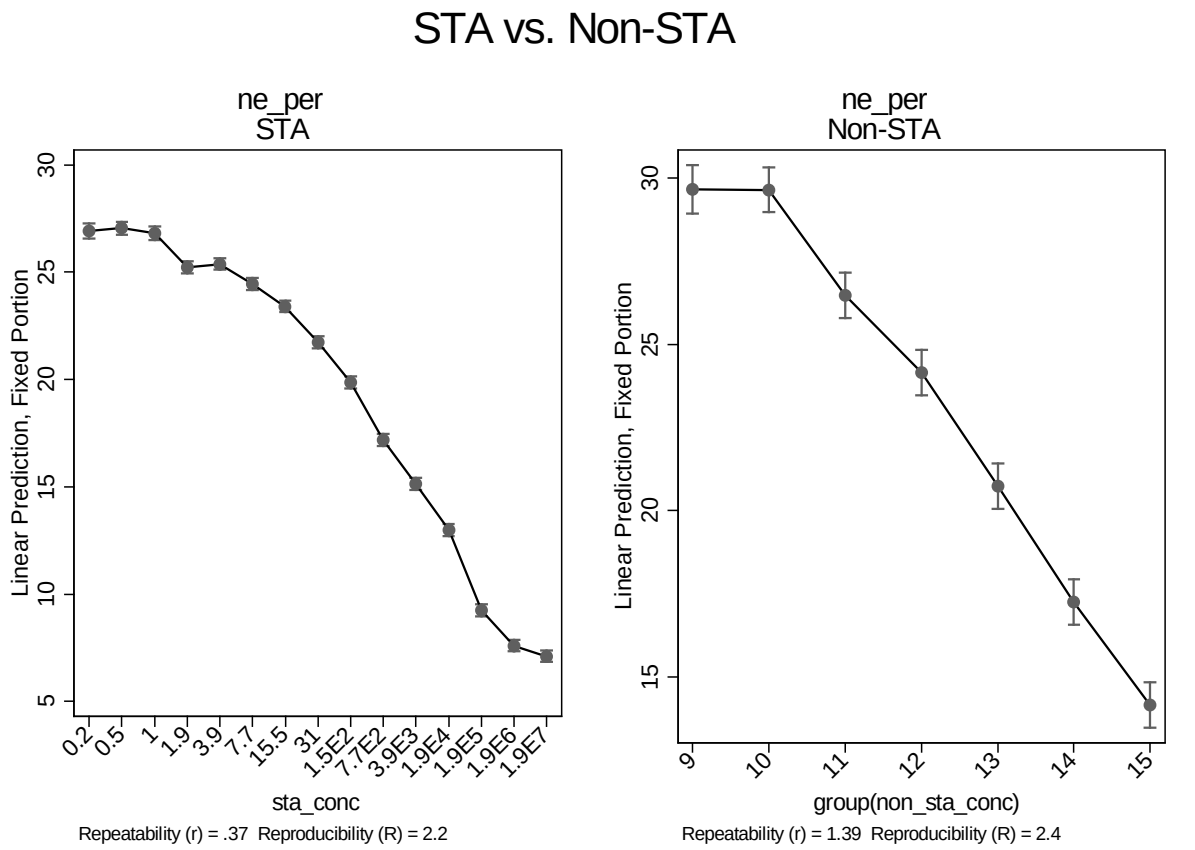

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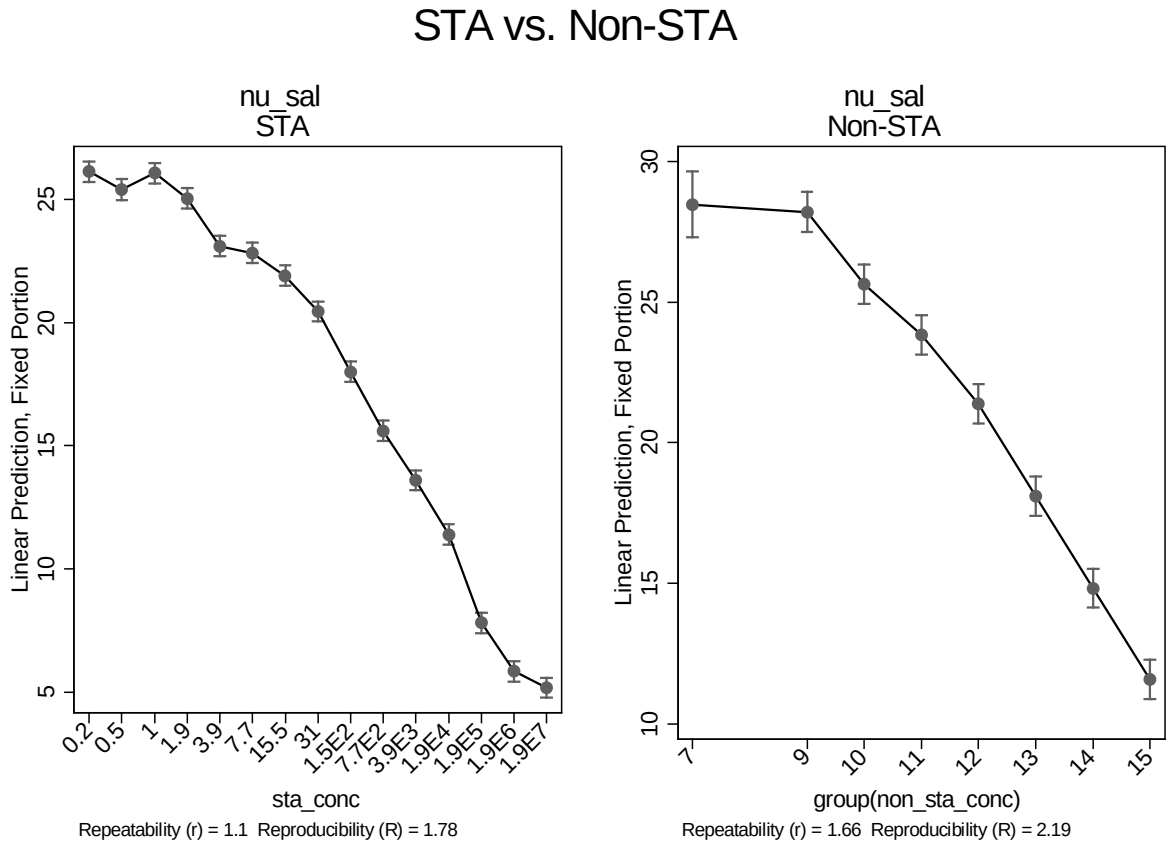

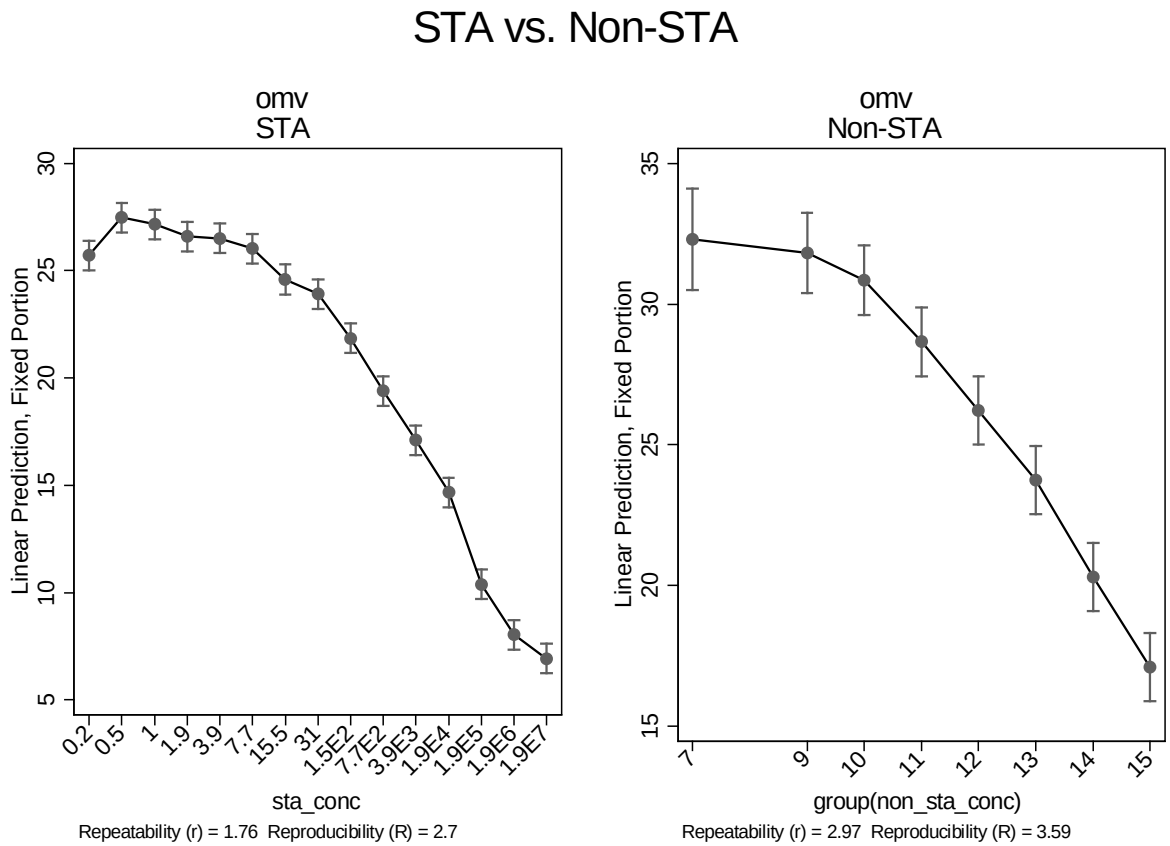

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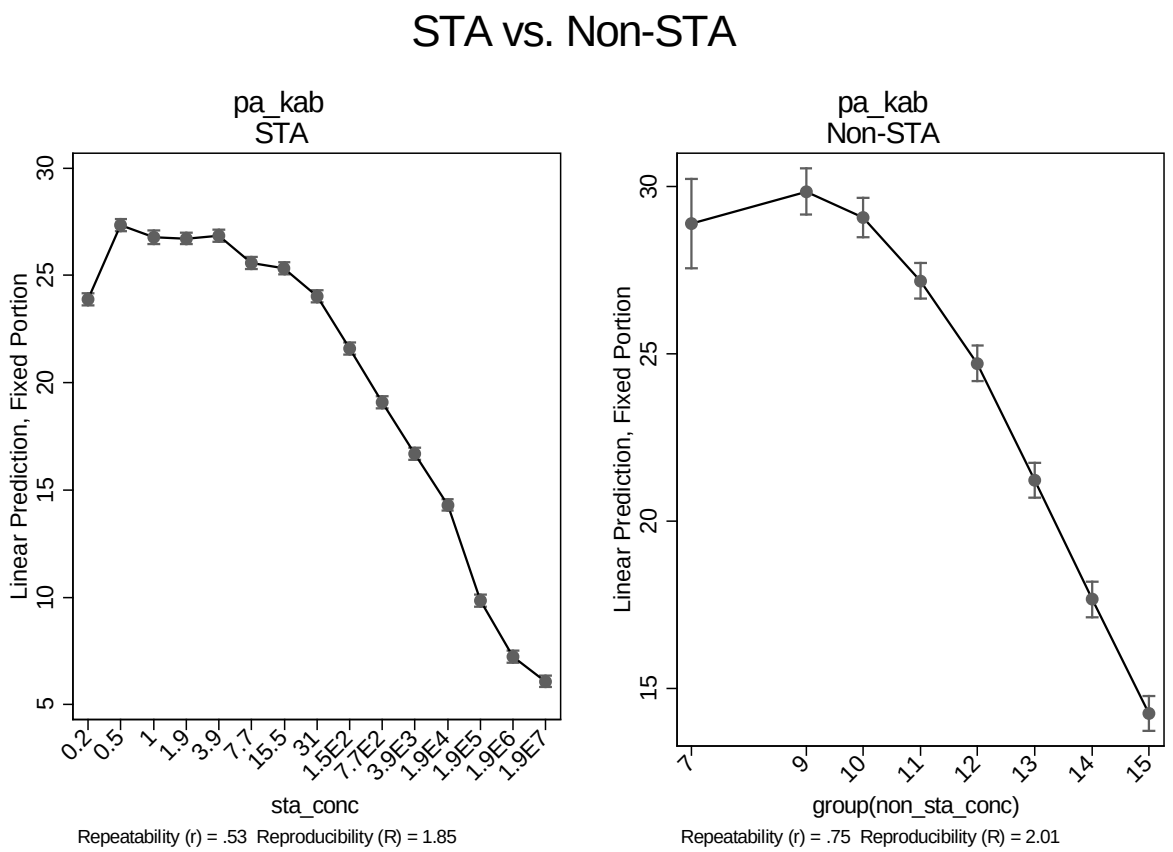

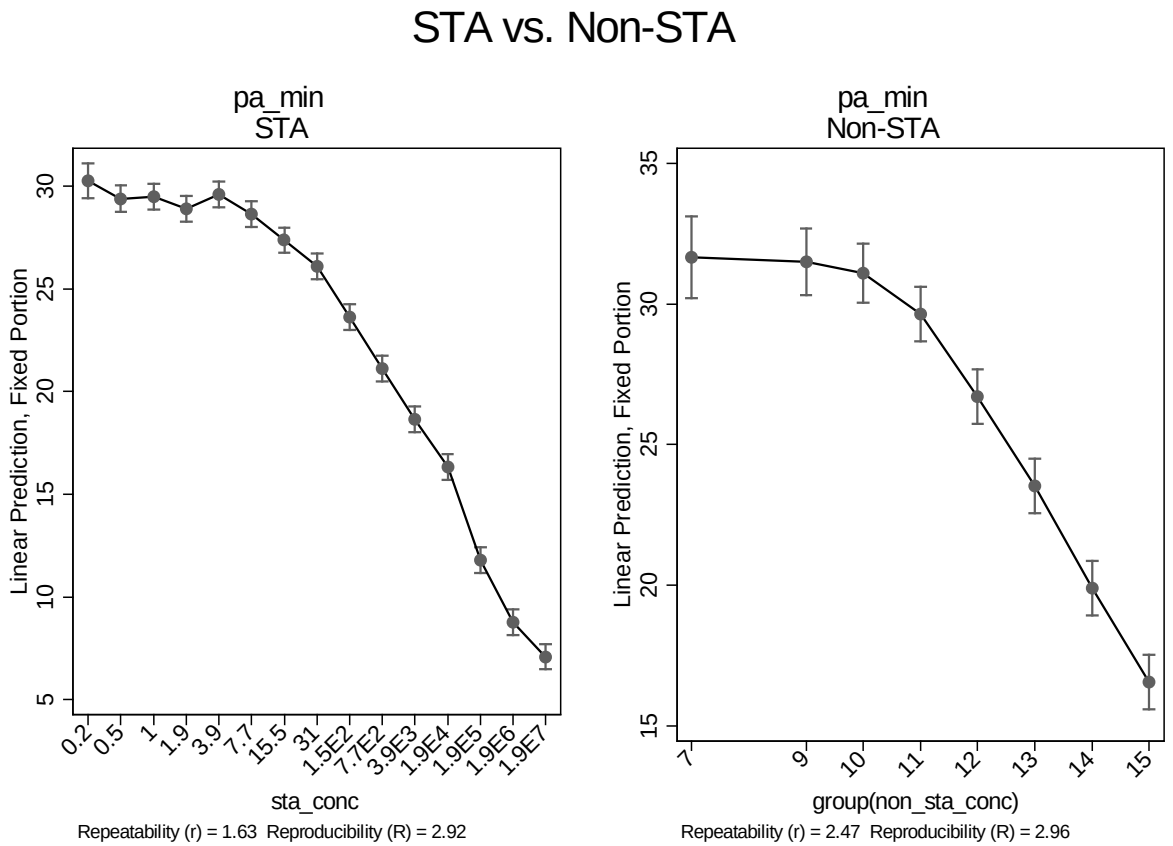

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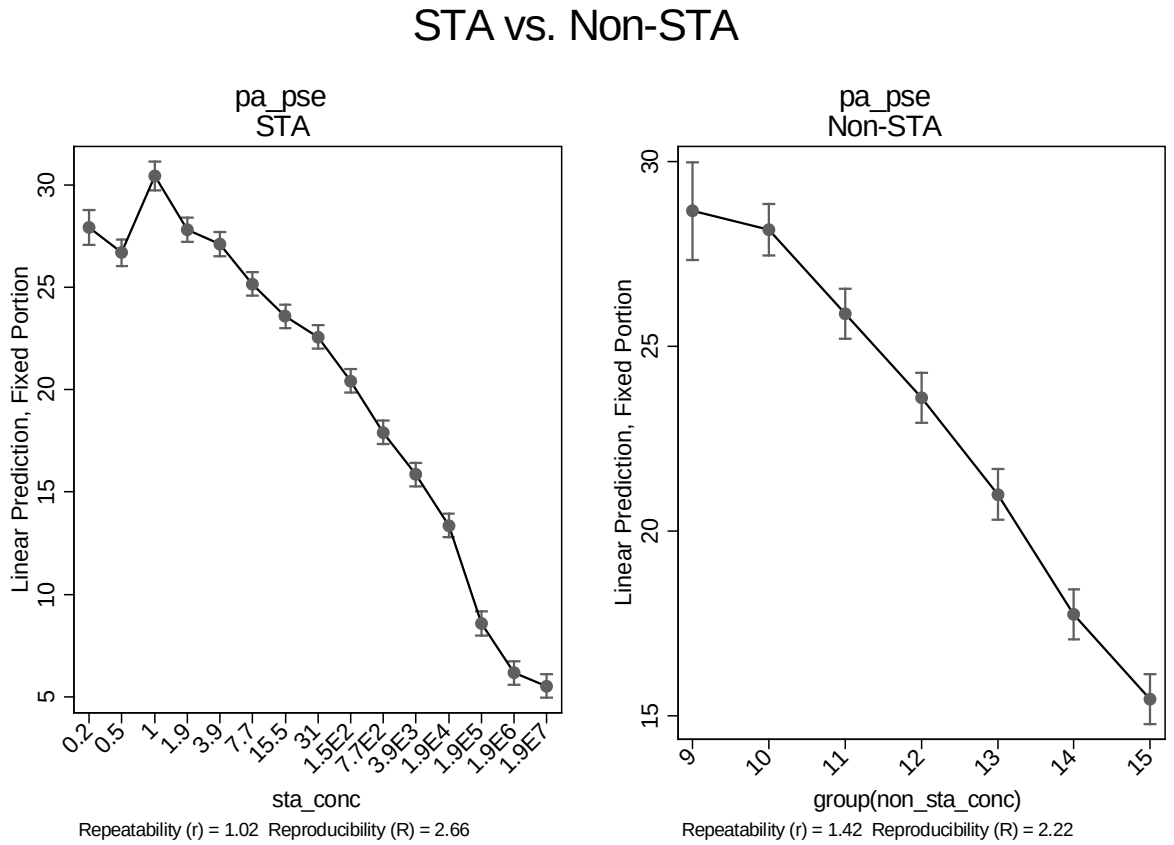

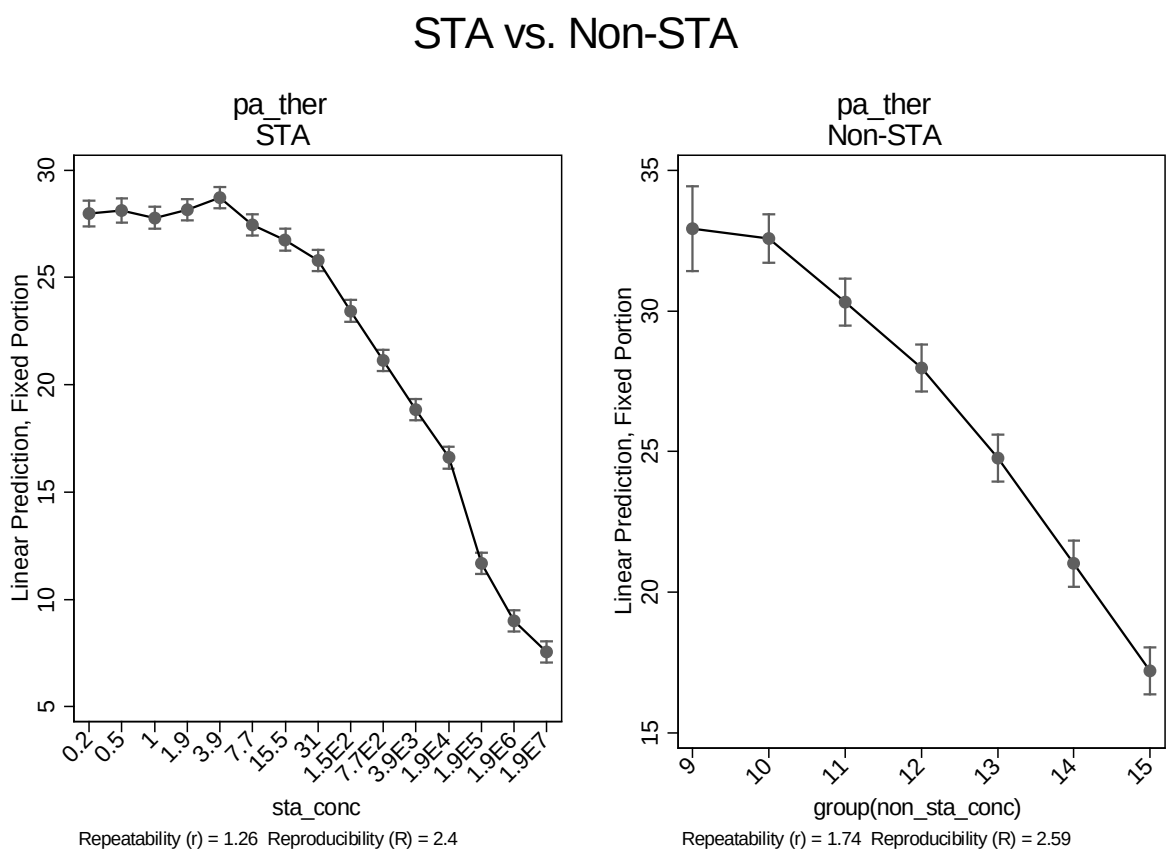

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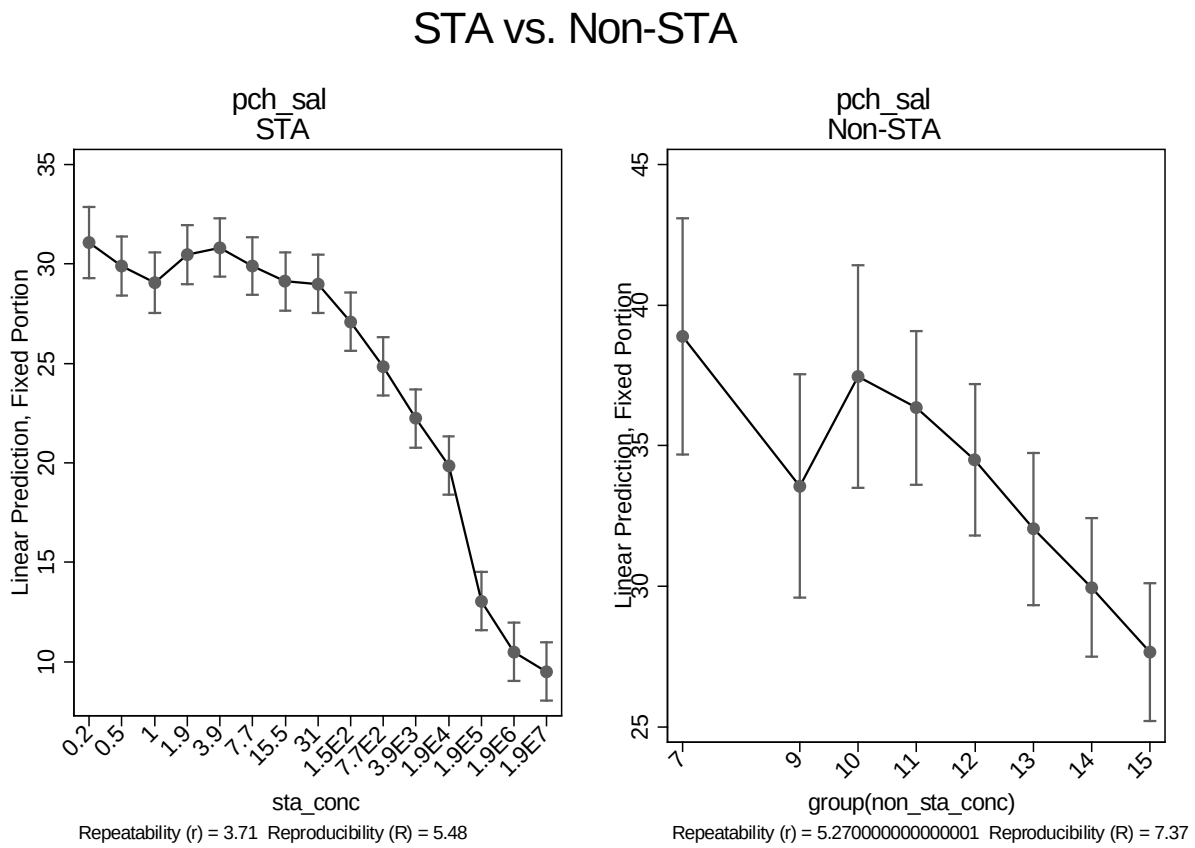

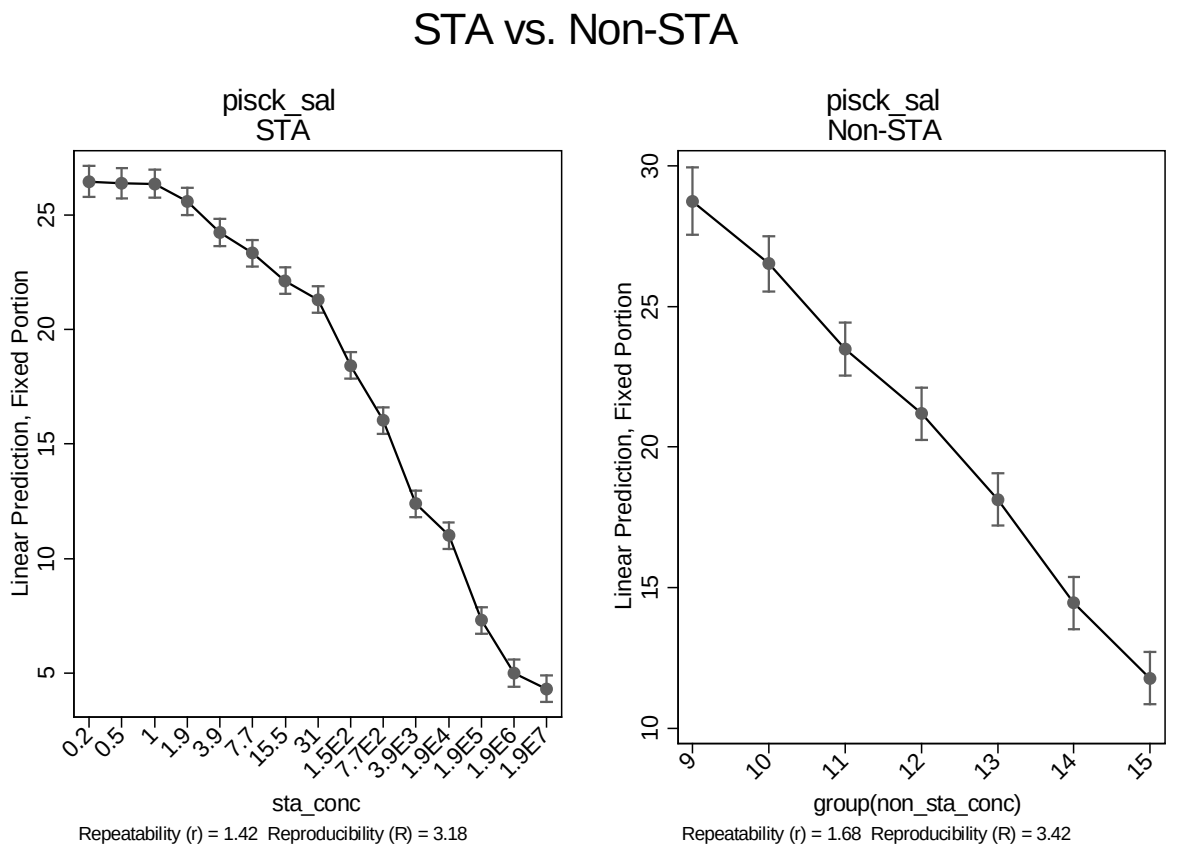

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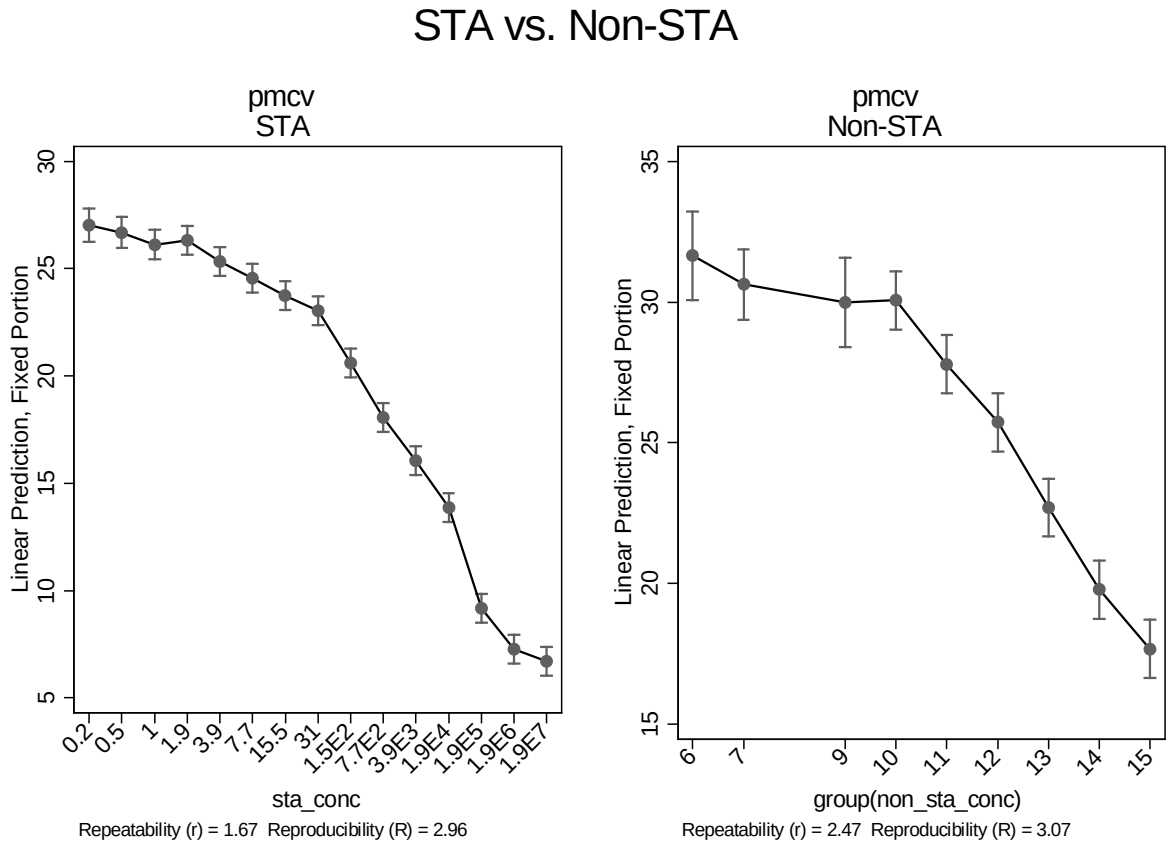

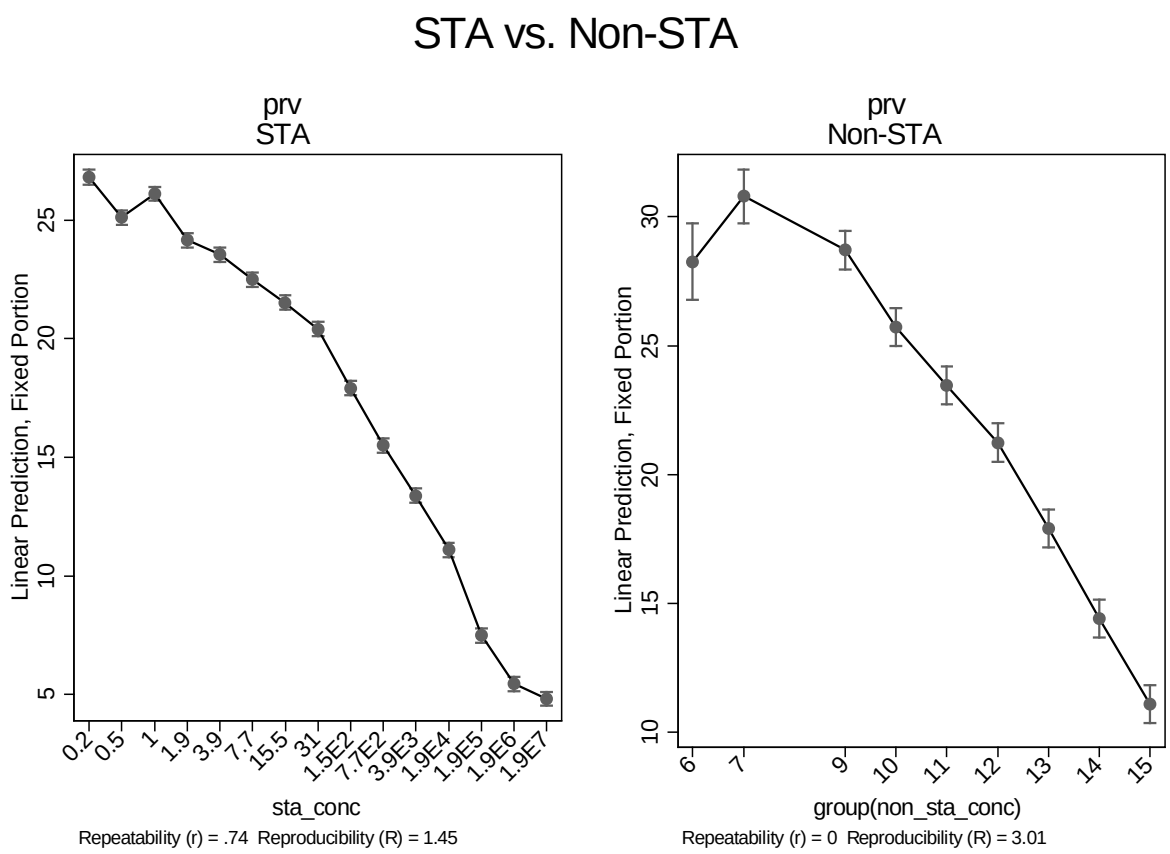

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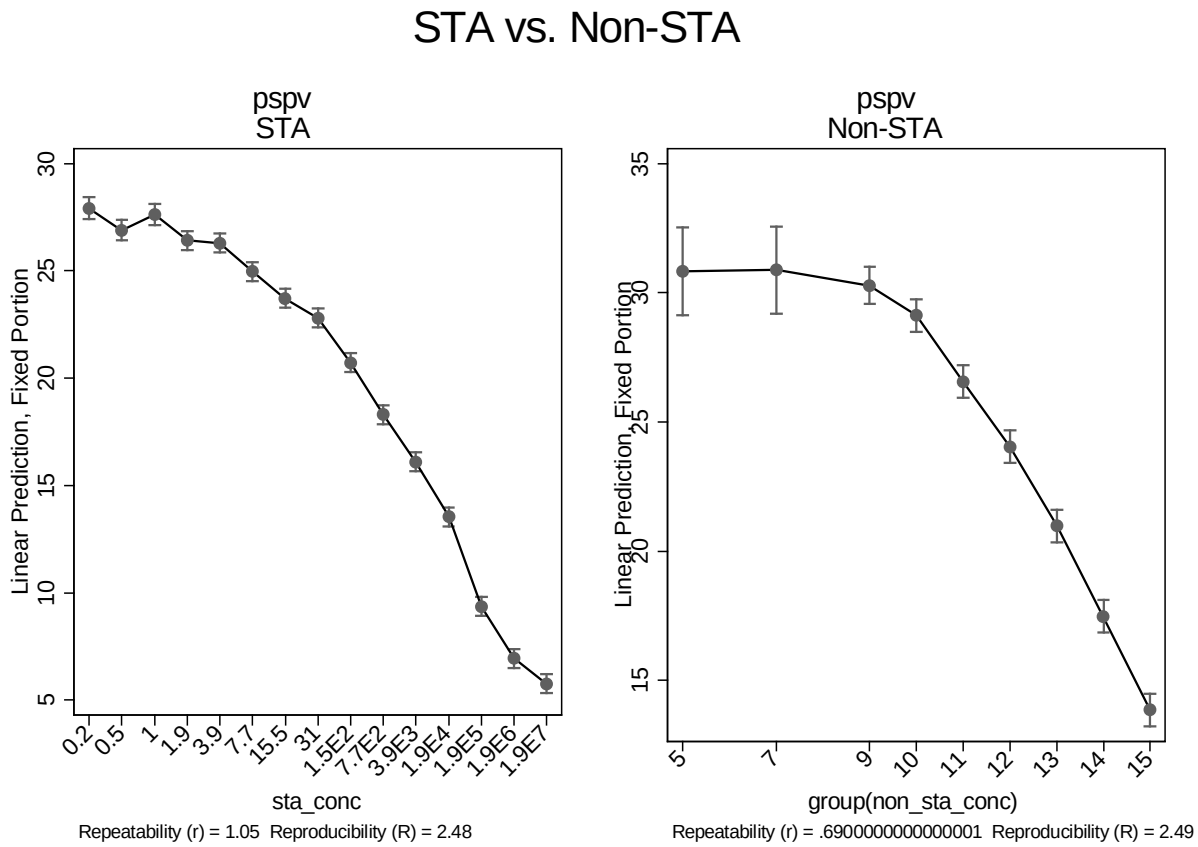

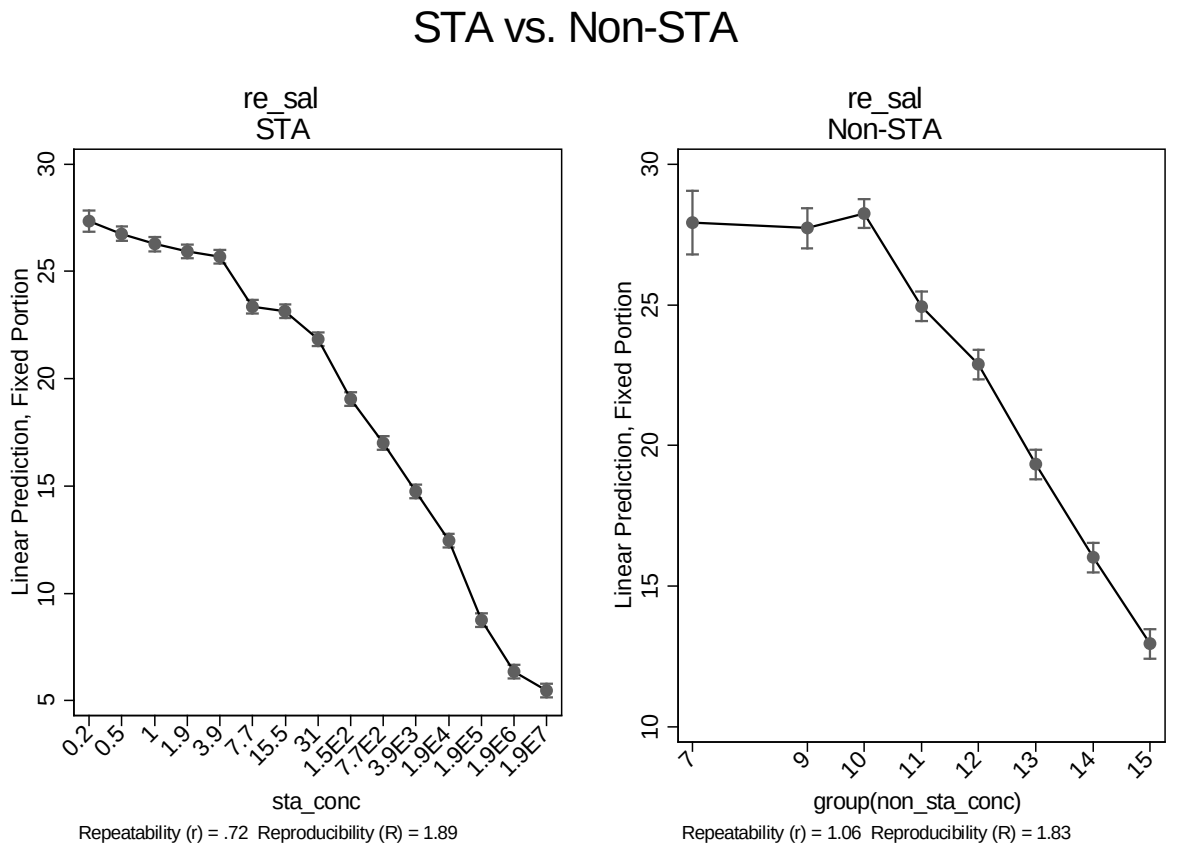

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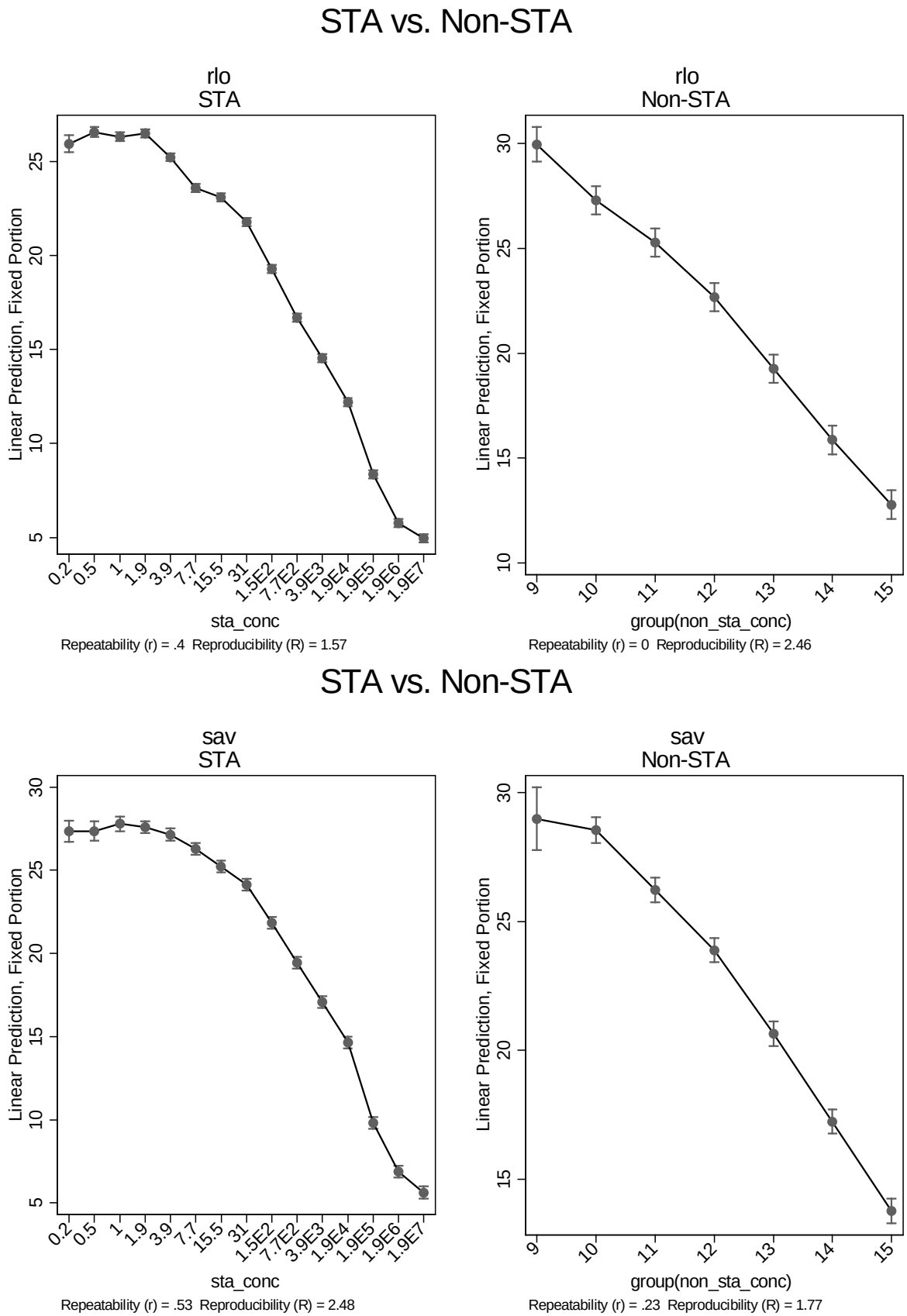

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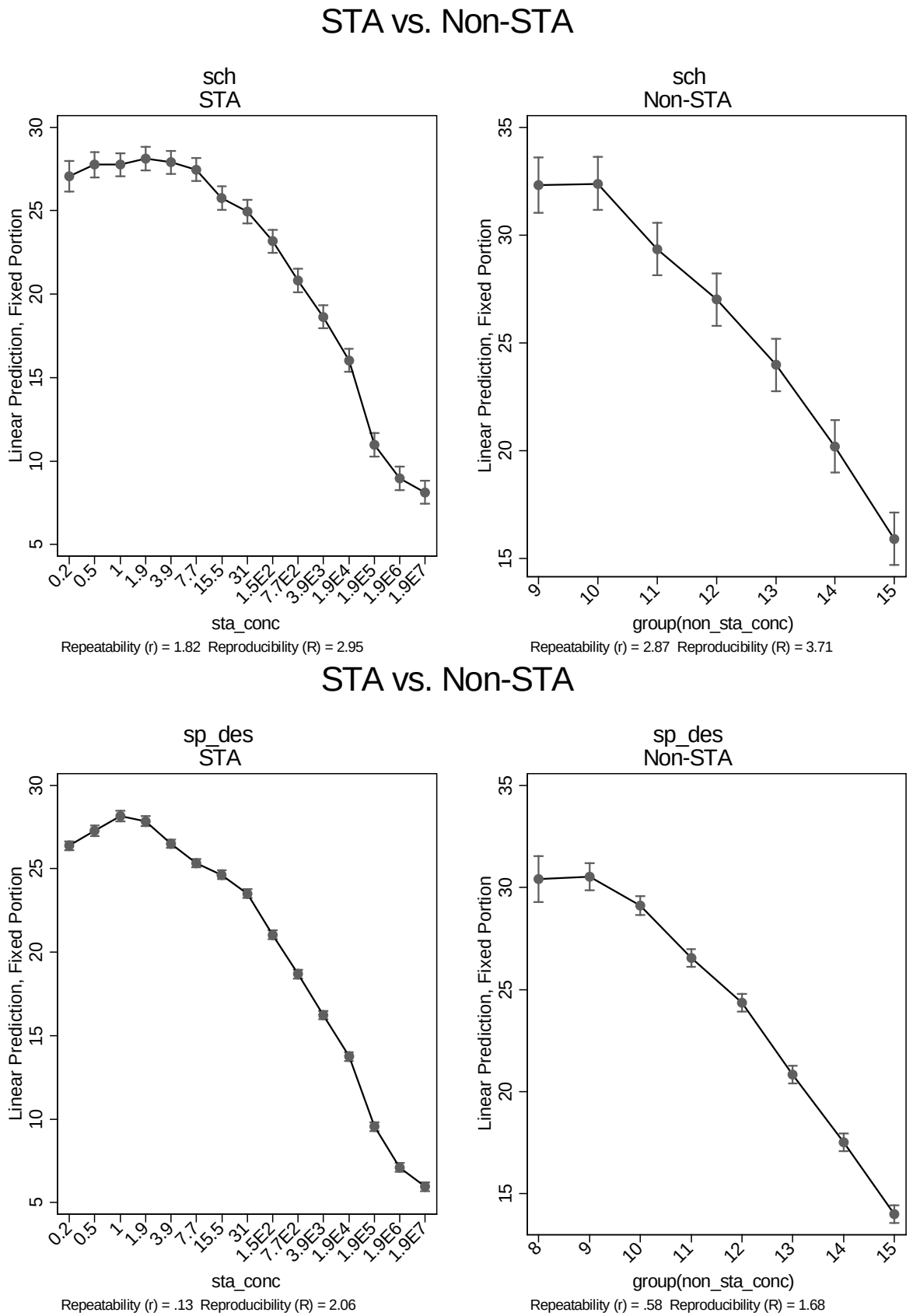

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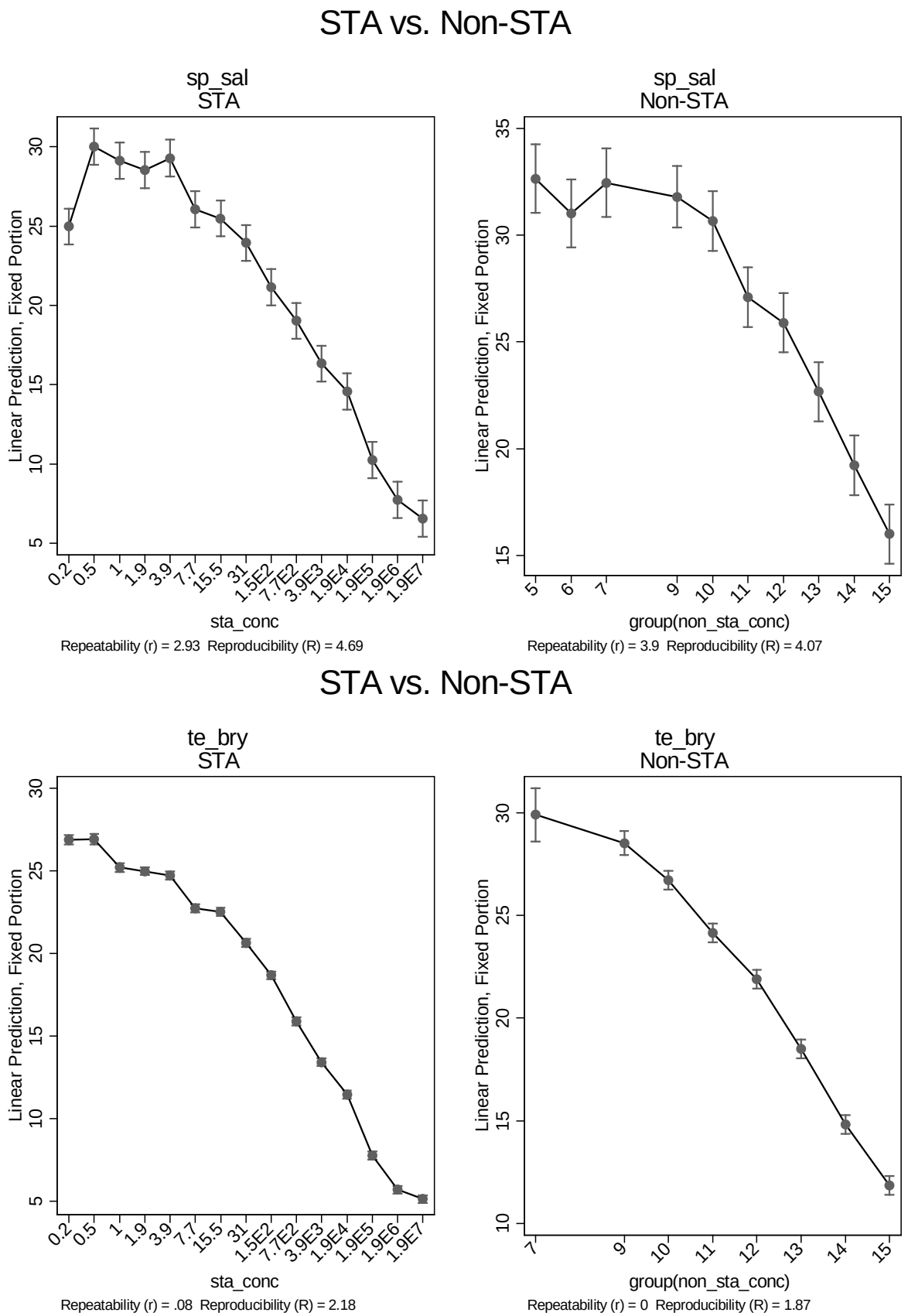

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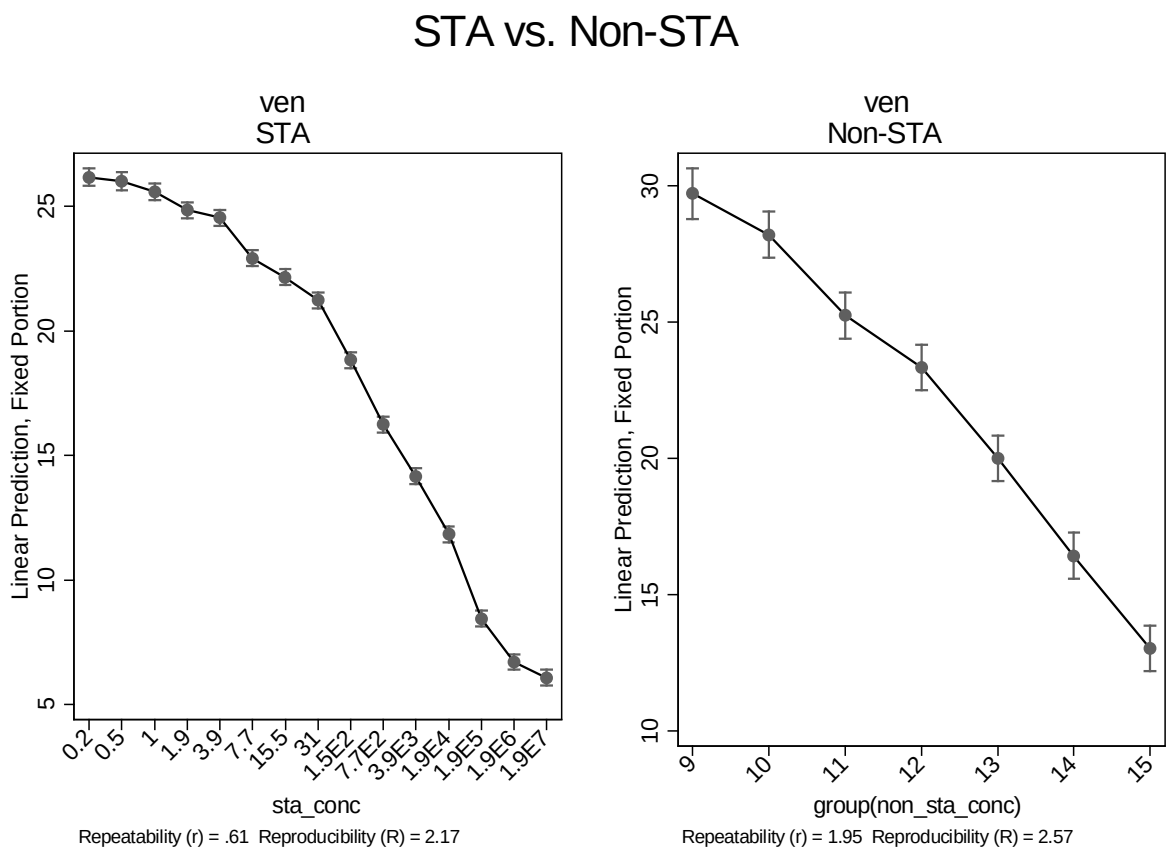

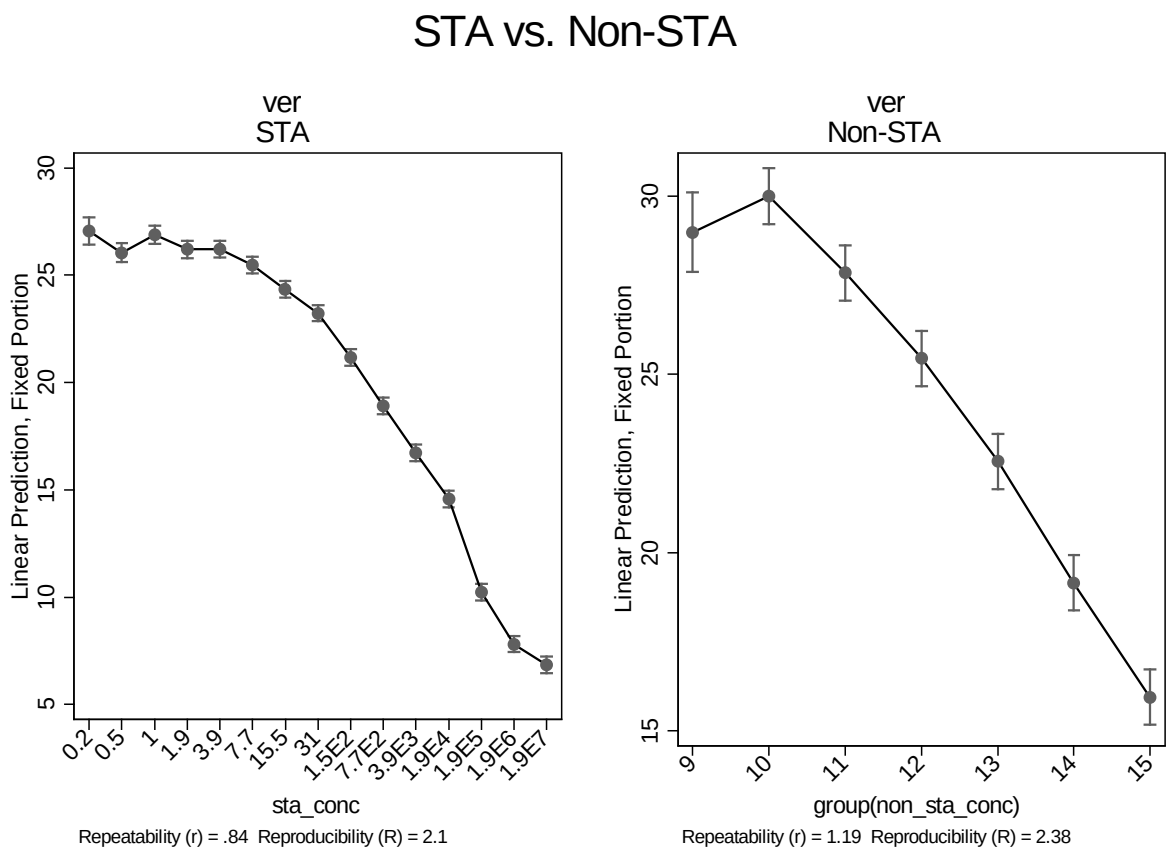

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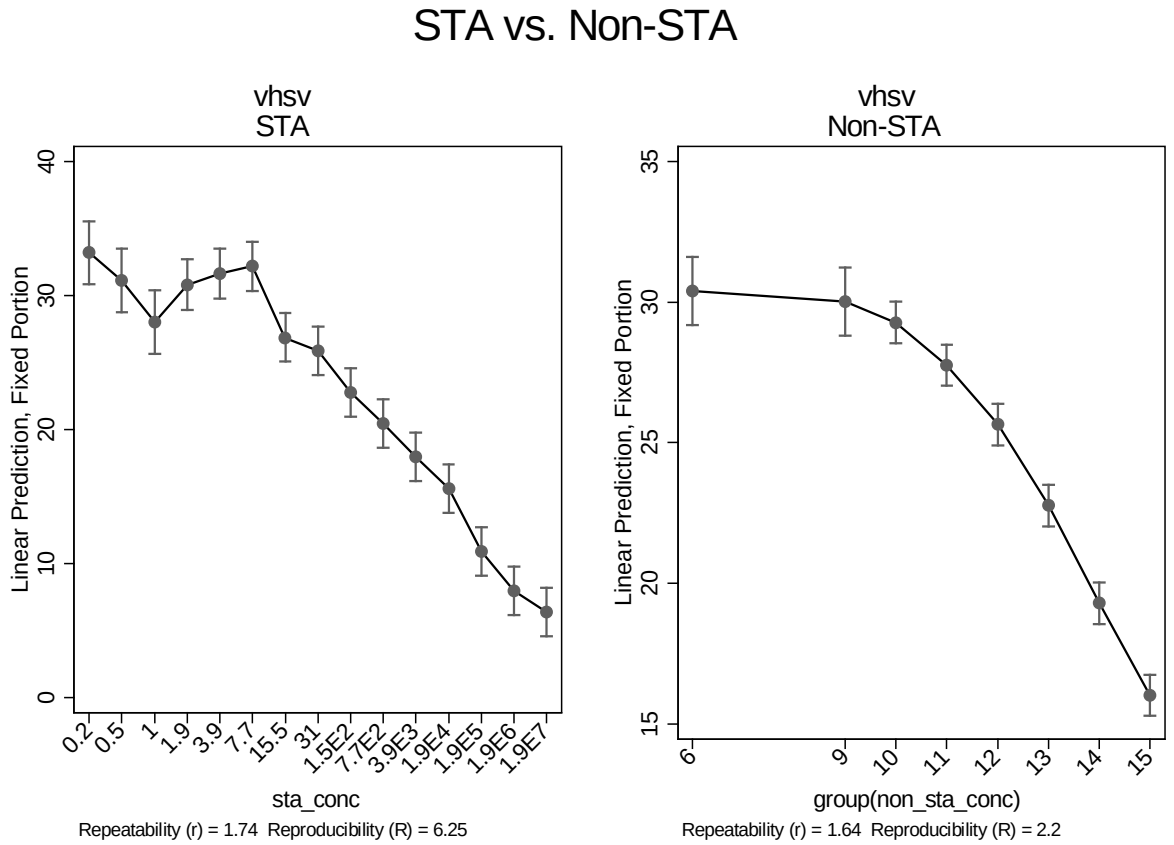

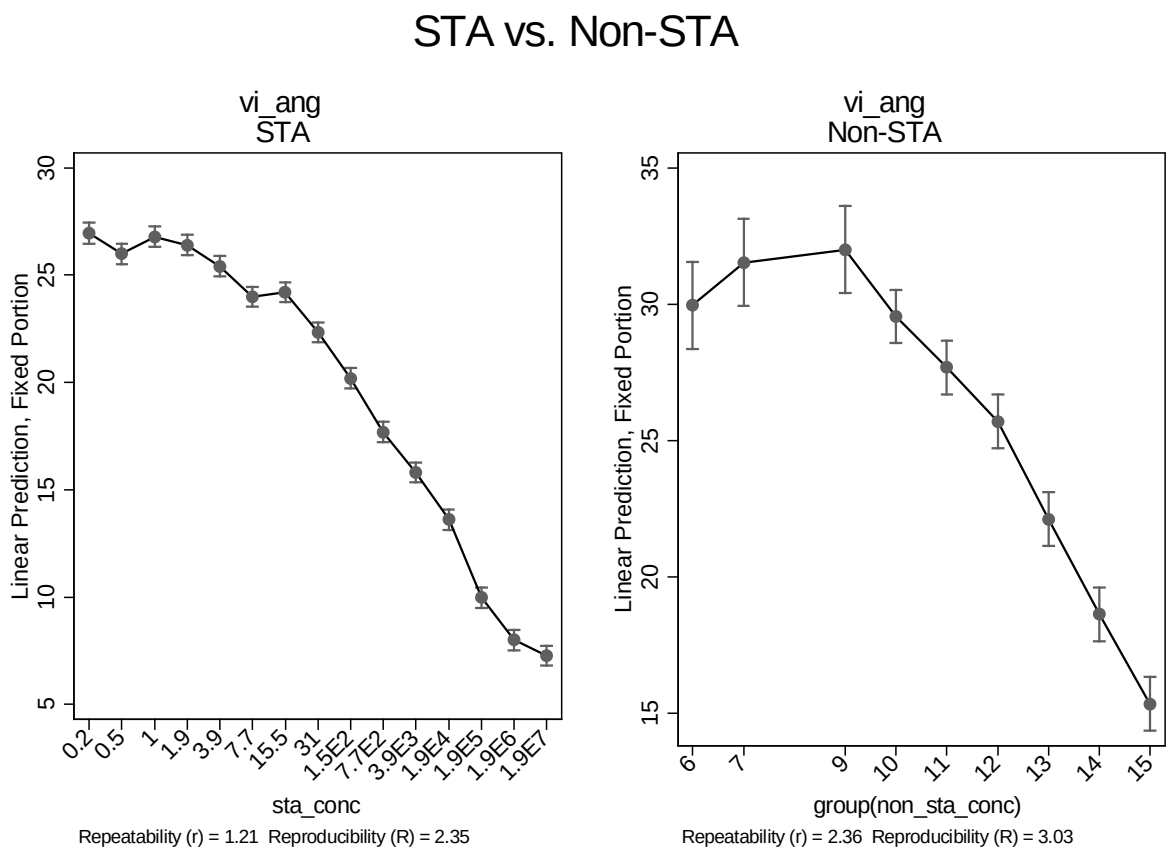

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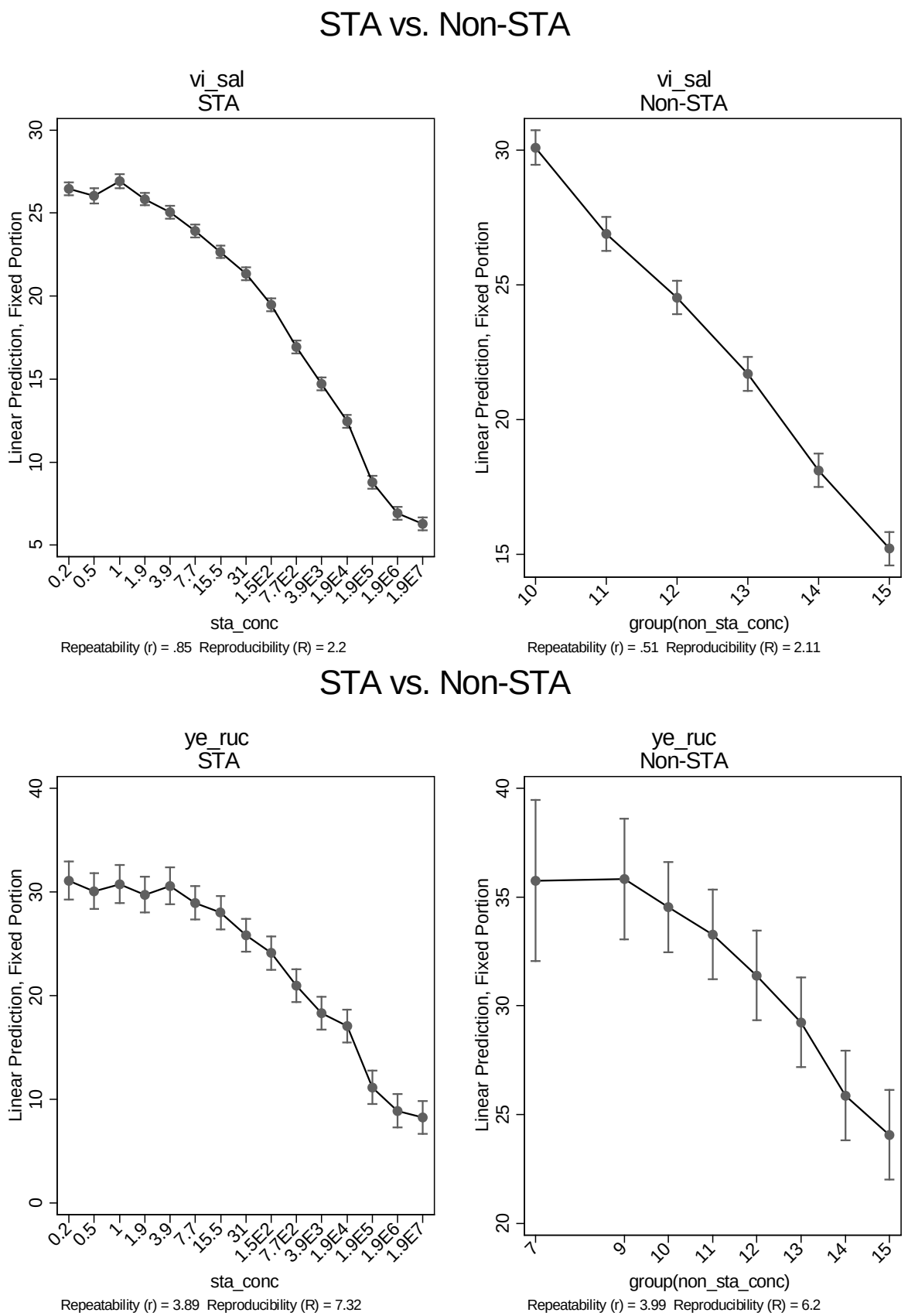

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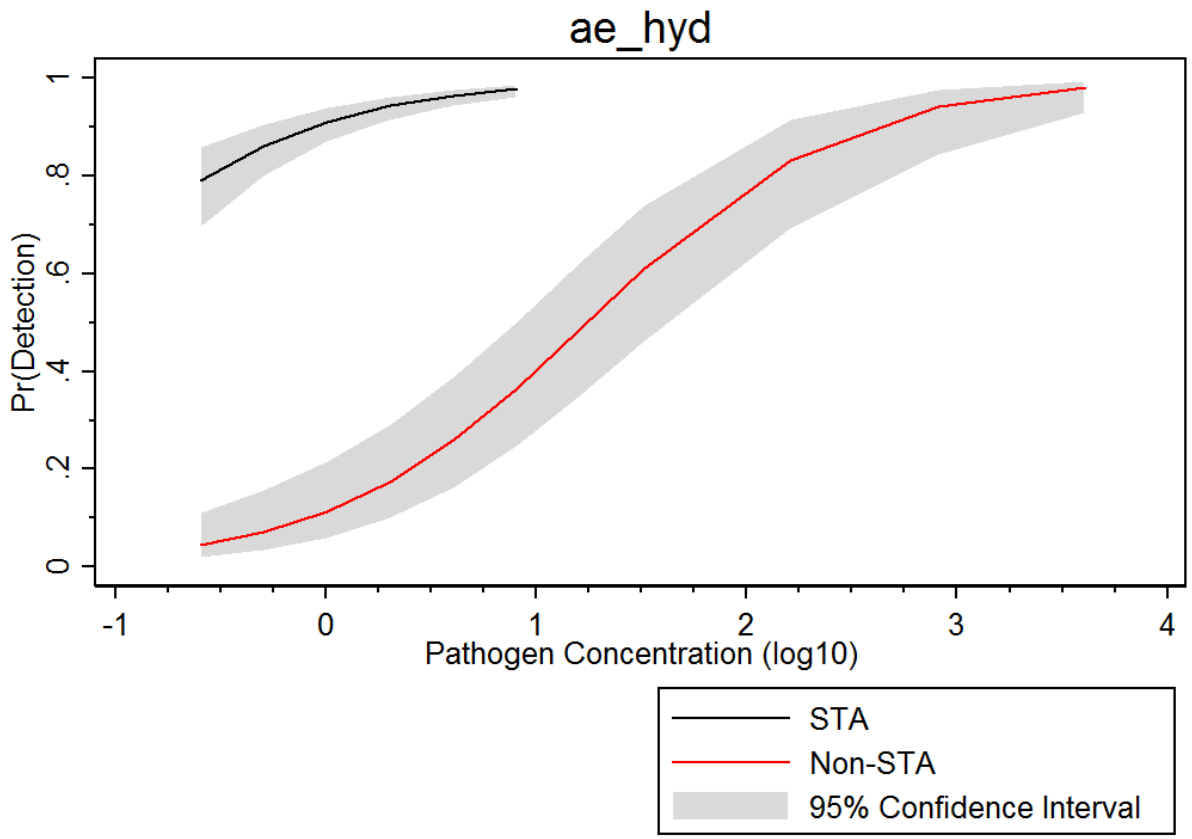
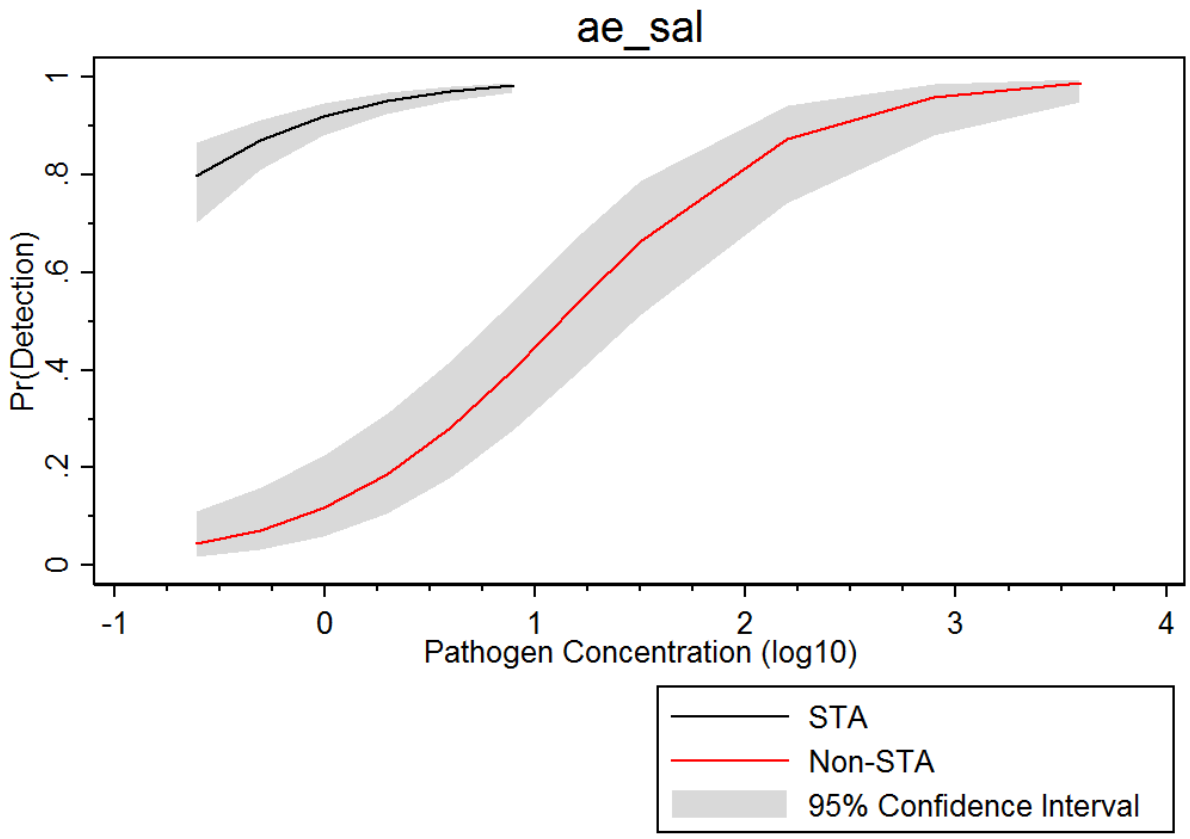
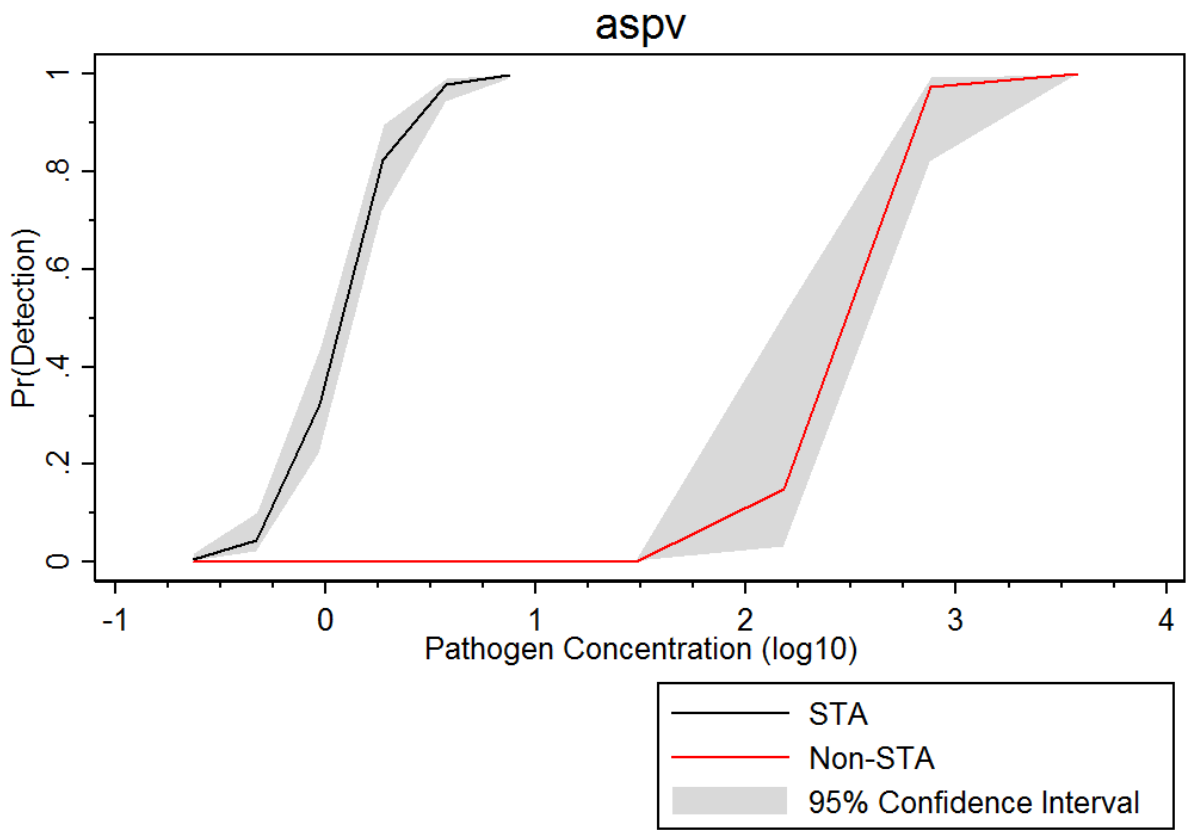


Supplemental Figure 5. Predicted probability of detection versus microbe concentration, from logistic regression models, stratified by the pre-amplification status (STA vs Non-STA), for each assay (new page for each assay: ae_hyd, ae_sal, aspv, c_b_cys, ce_sha, cr_sal, de_sal, fa_mar, fl_psy, gy_sal, ic_hof, ic_mul, ihnv, ipnv, isav7, isav8, ku_thy, lo_sal, my_arc, my_cer, my_ins, na_sal, ne_per, nu_sal, omv, pa_kab, pa_min, pa_pse, pa_ther, pch_sal, pisck_sal, pmcv, prv, pspv, re_sal, rlo, sav, sch, sp_des, sp_sal, te_bry, ven, ver, vhsv, vi_ang, vi_sal, and ye_ruc).





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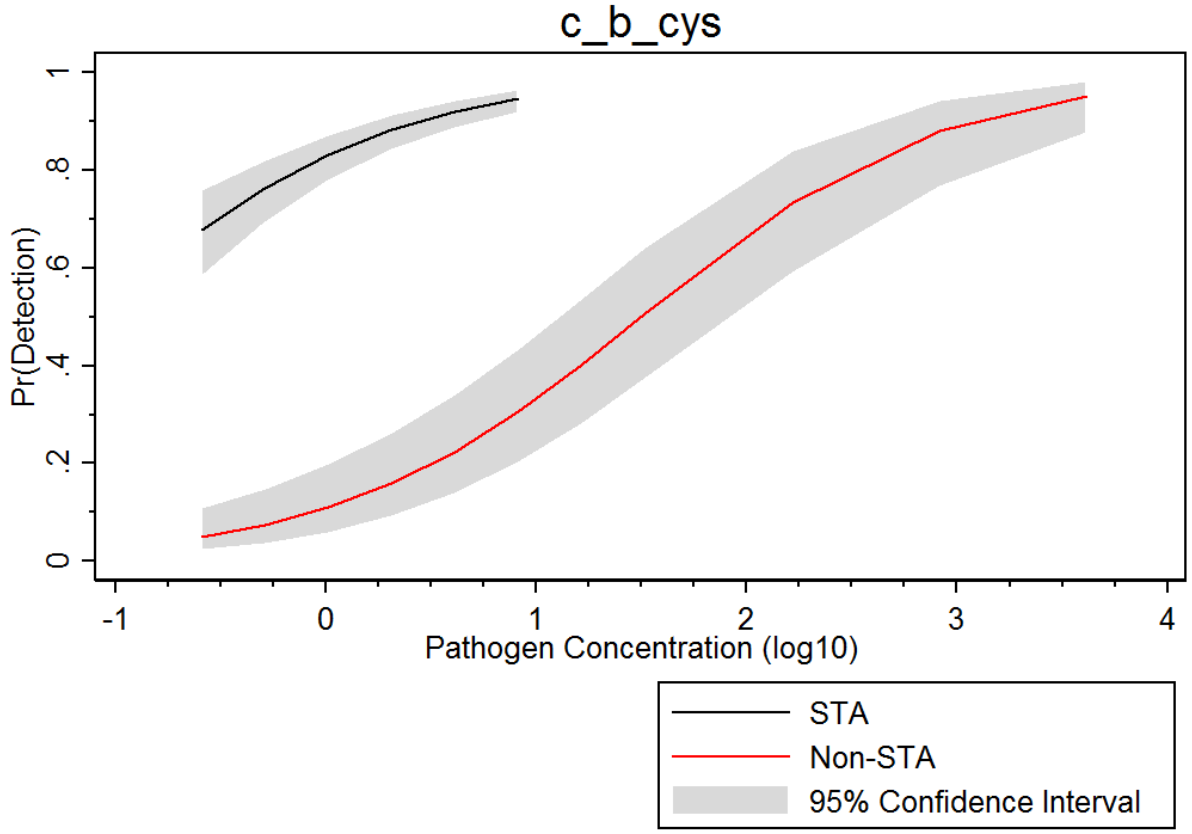

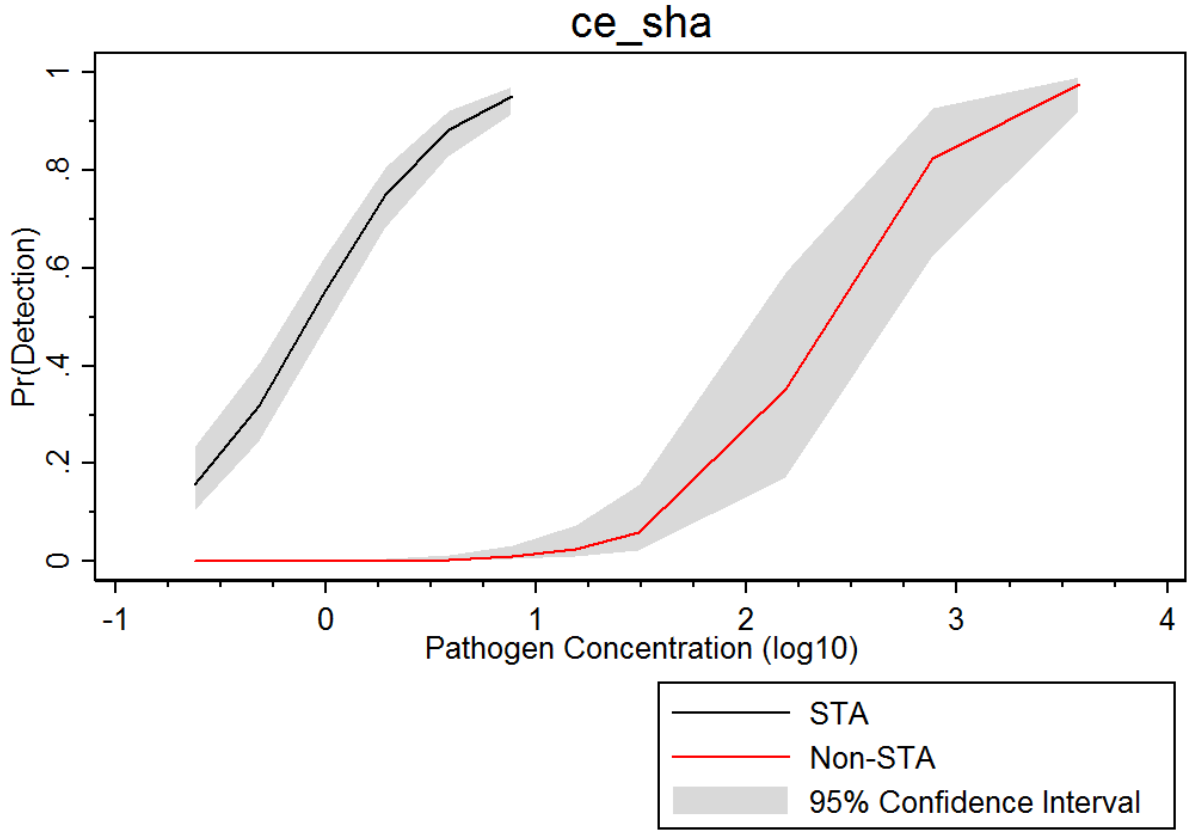

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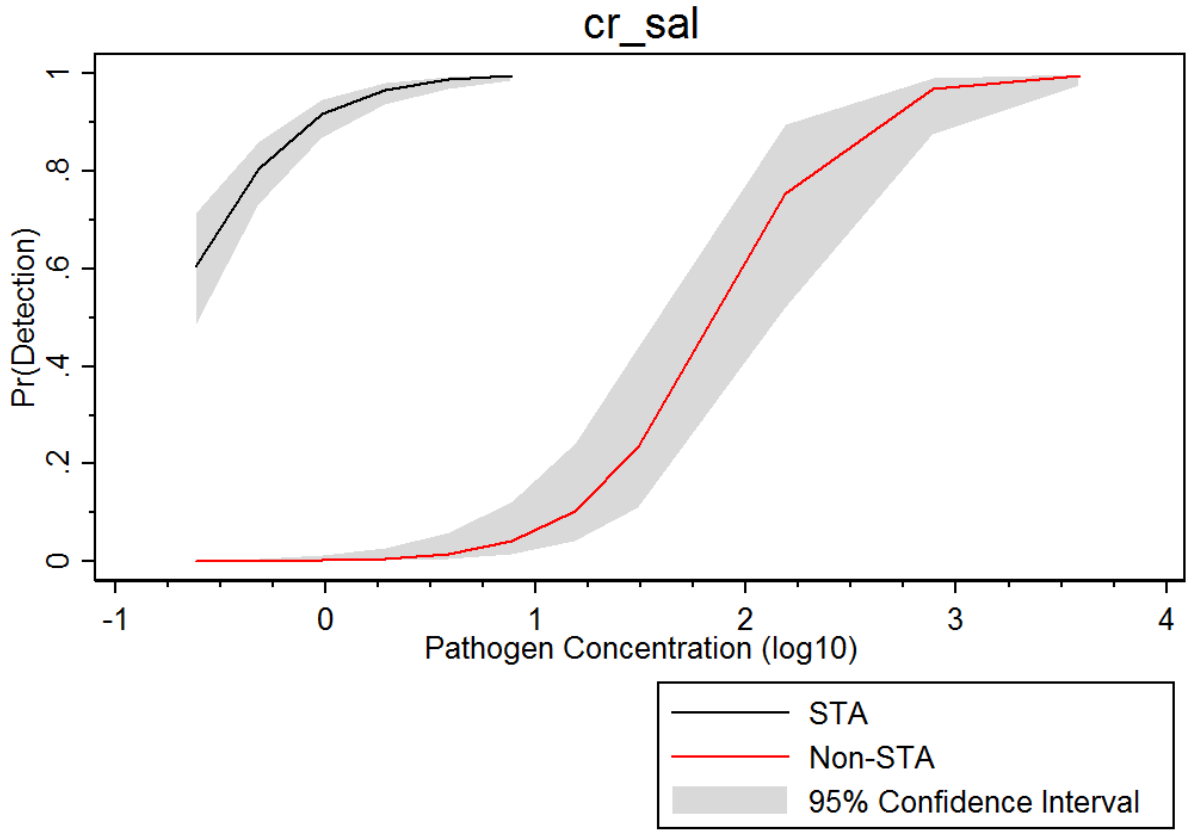

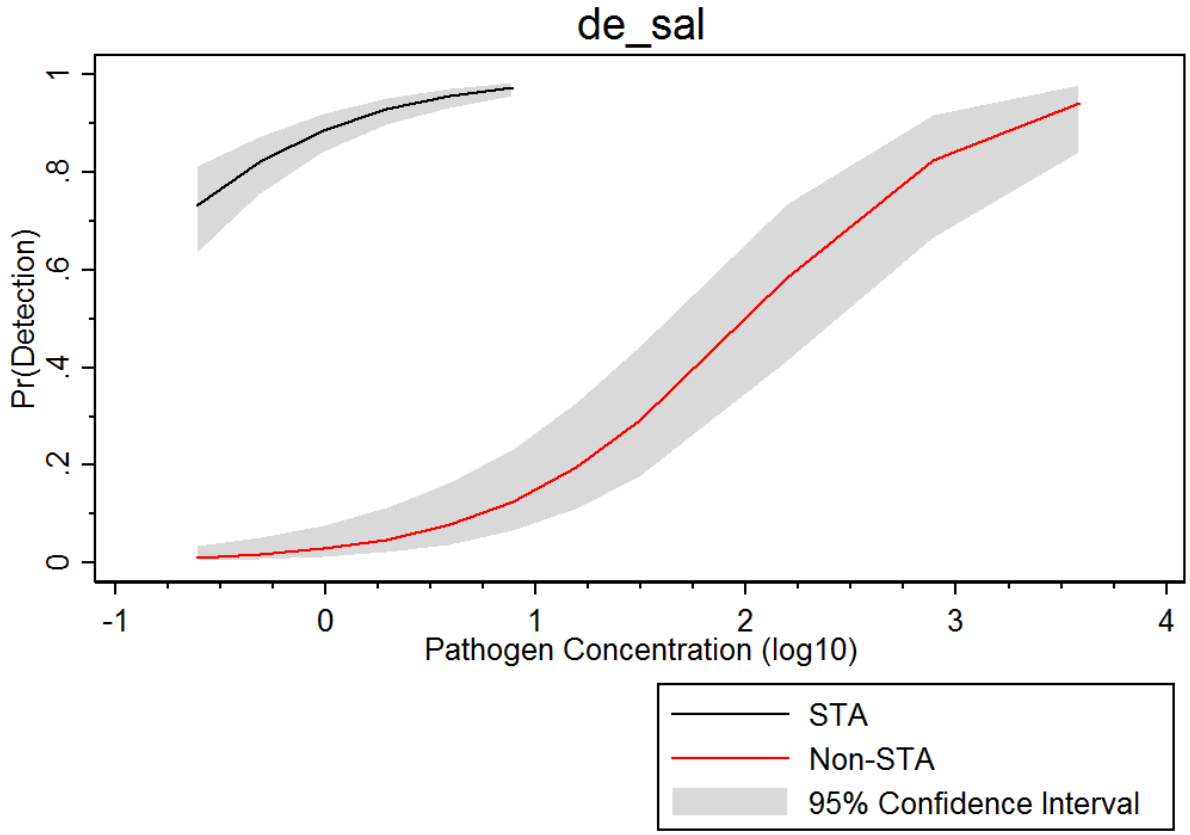

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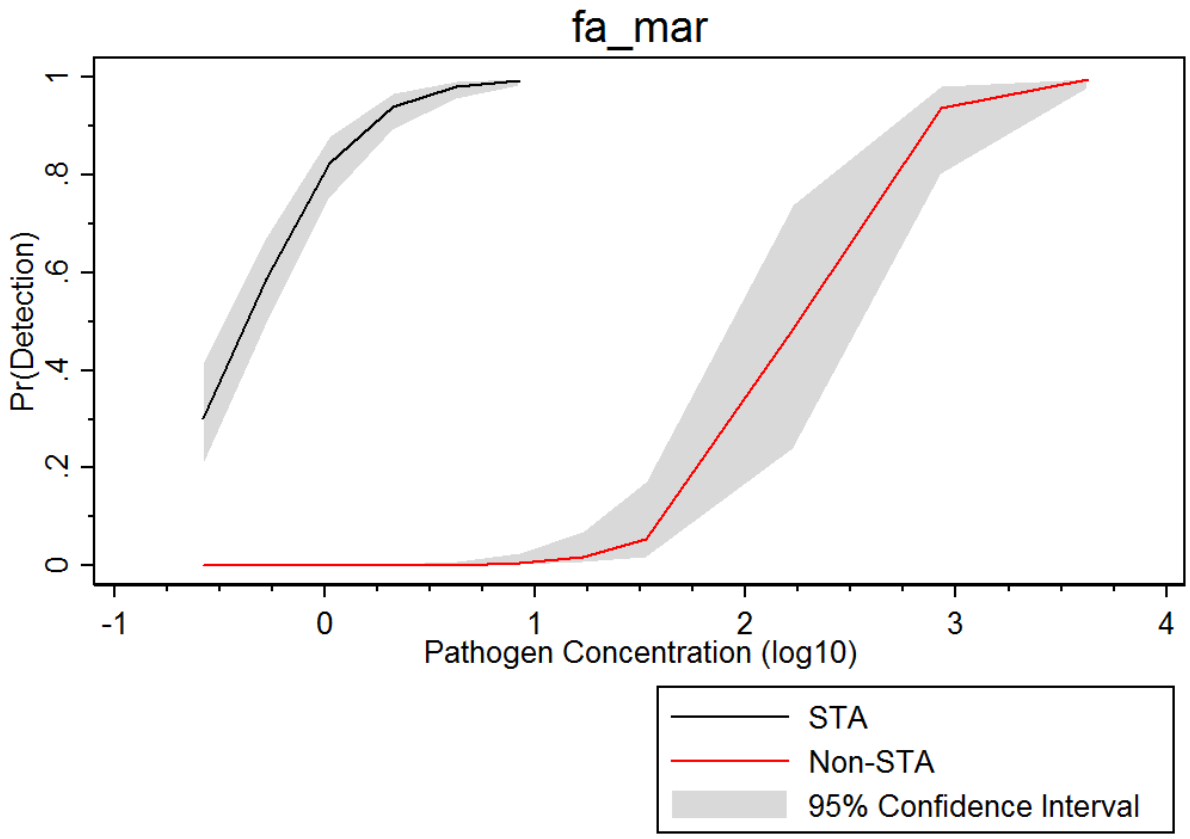

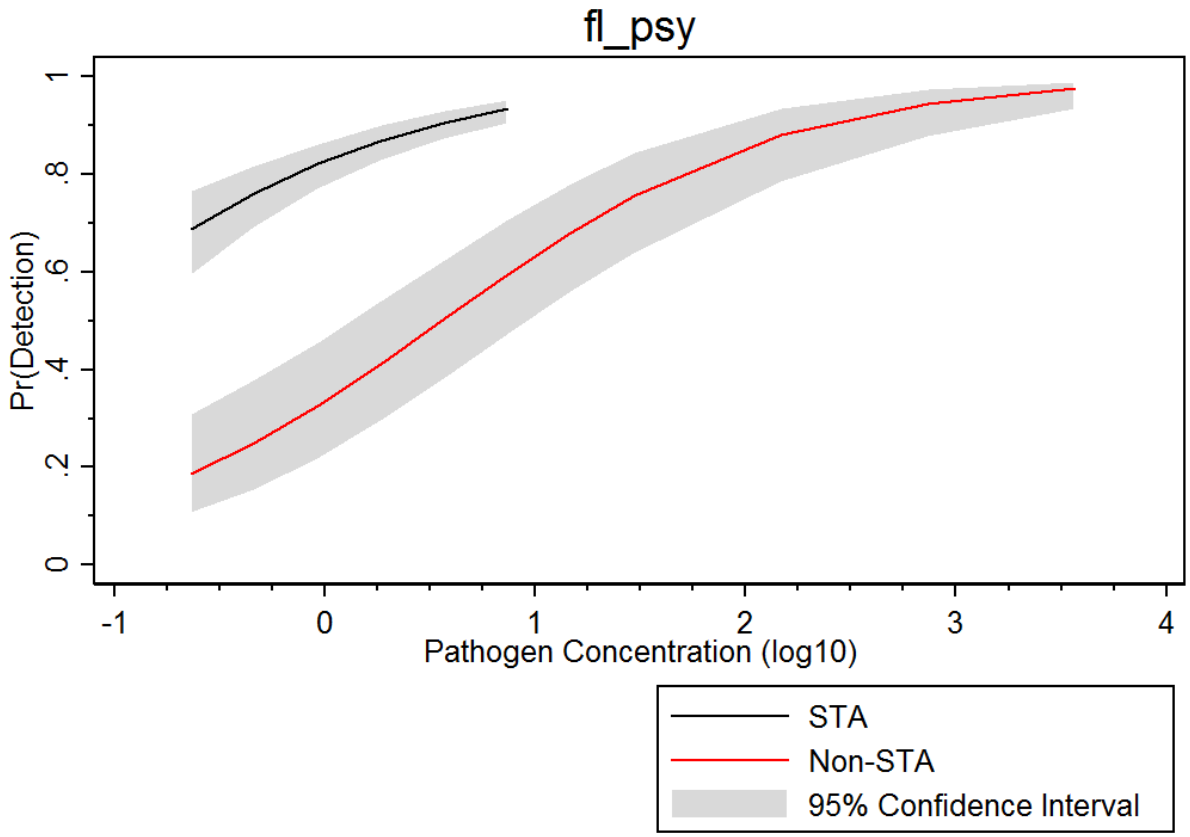

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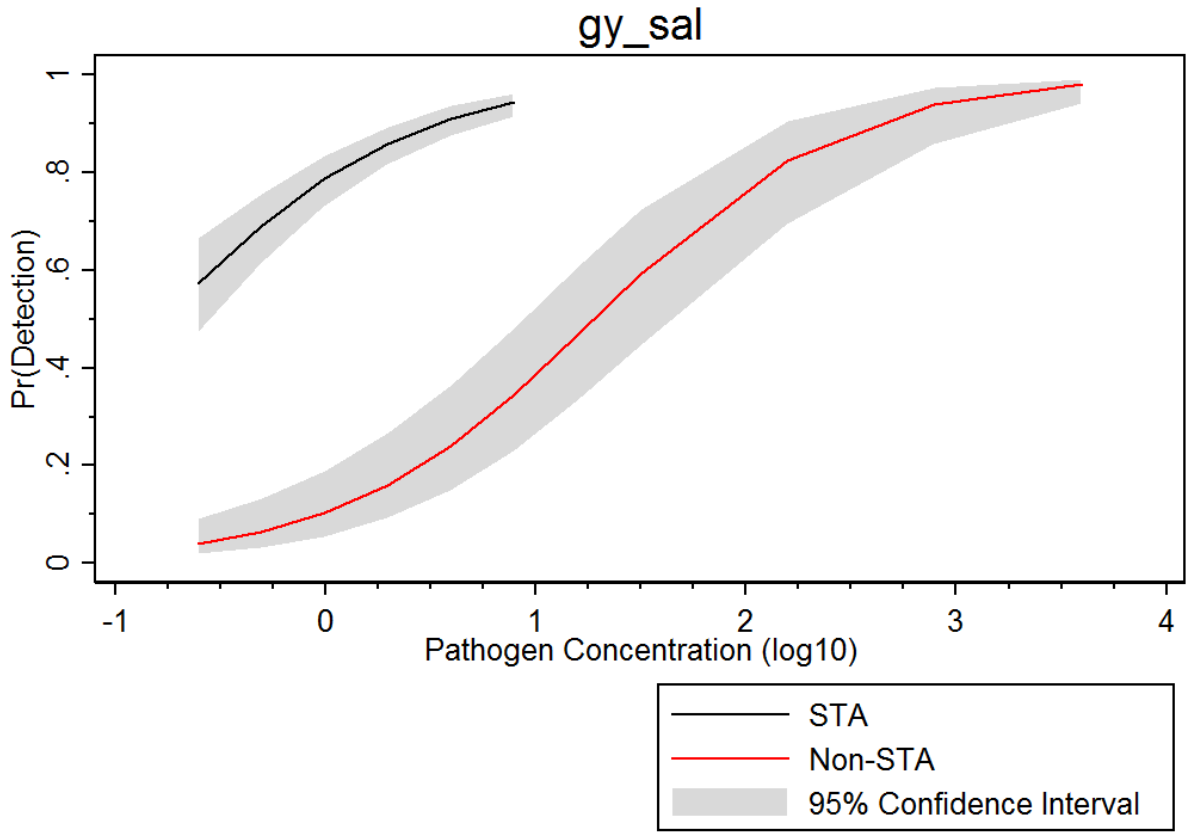

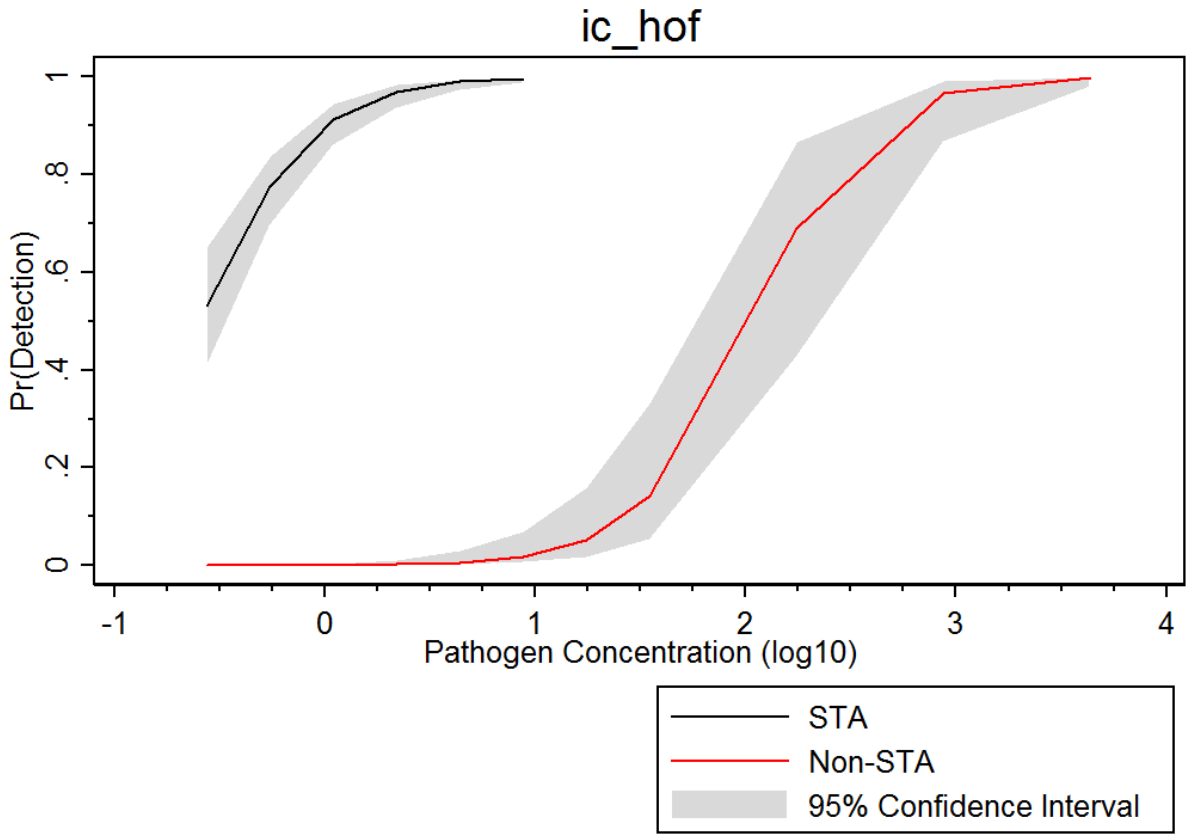

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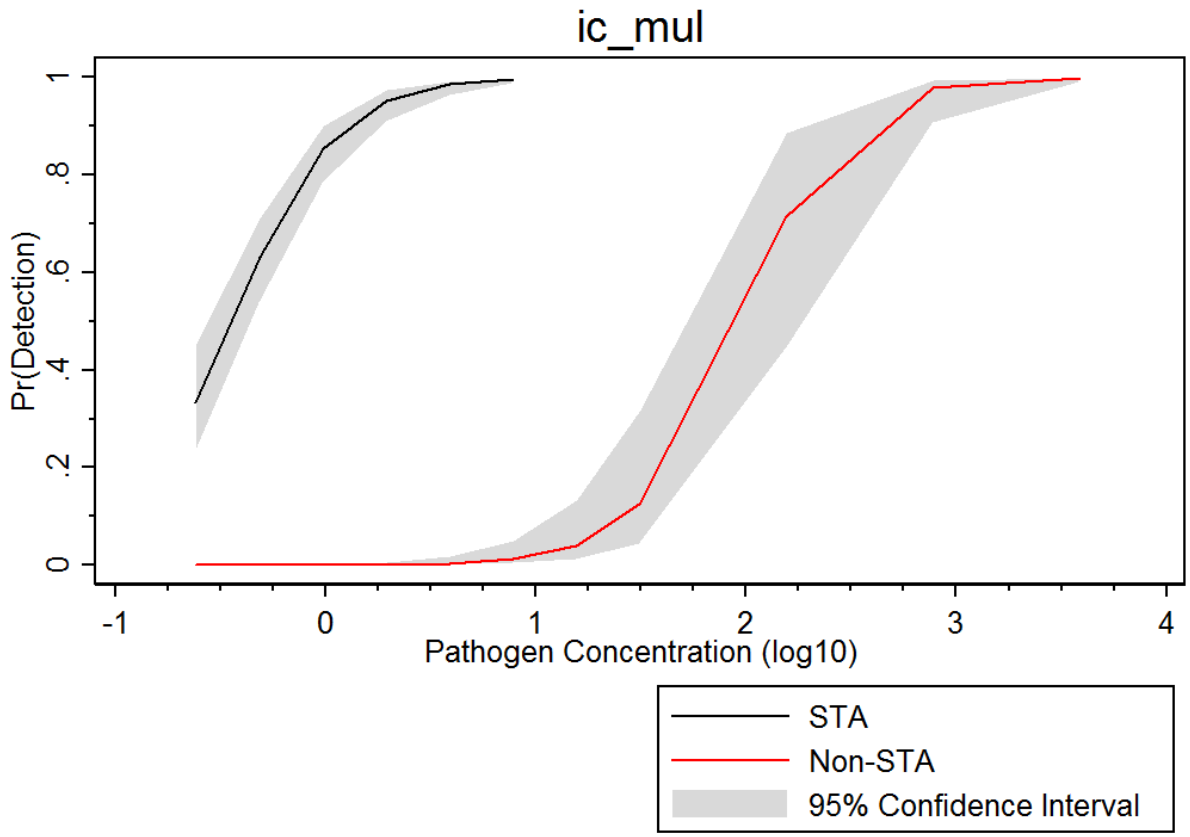

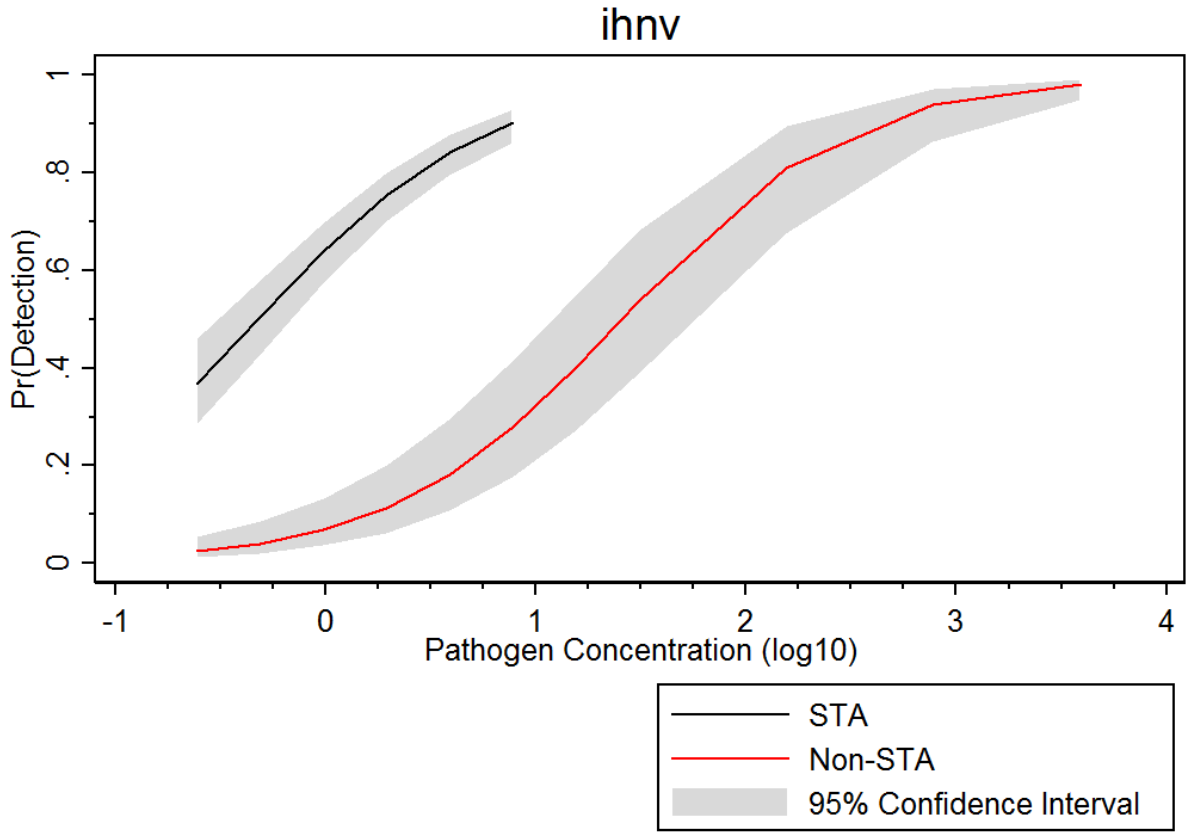

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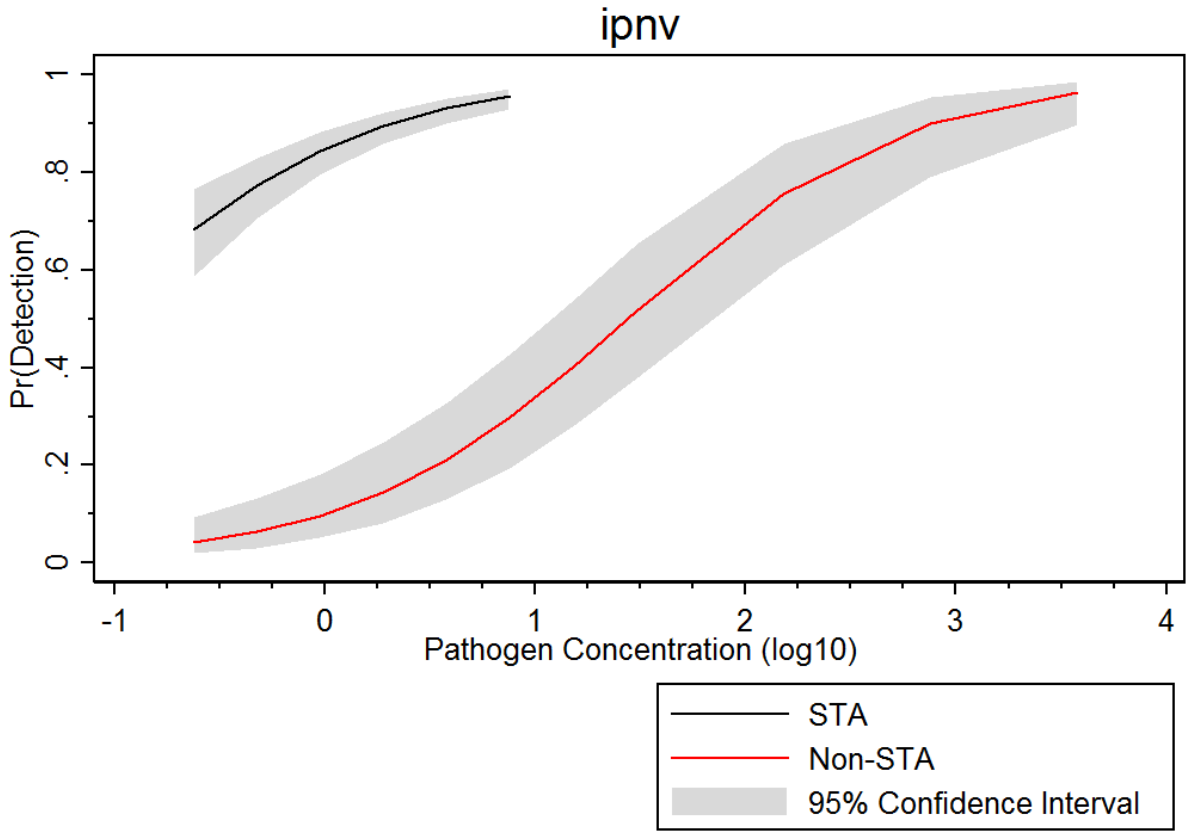

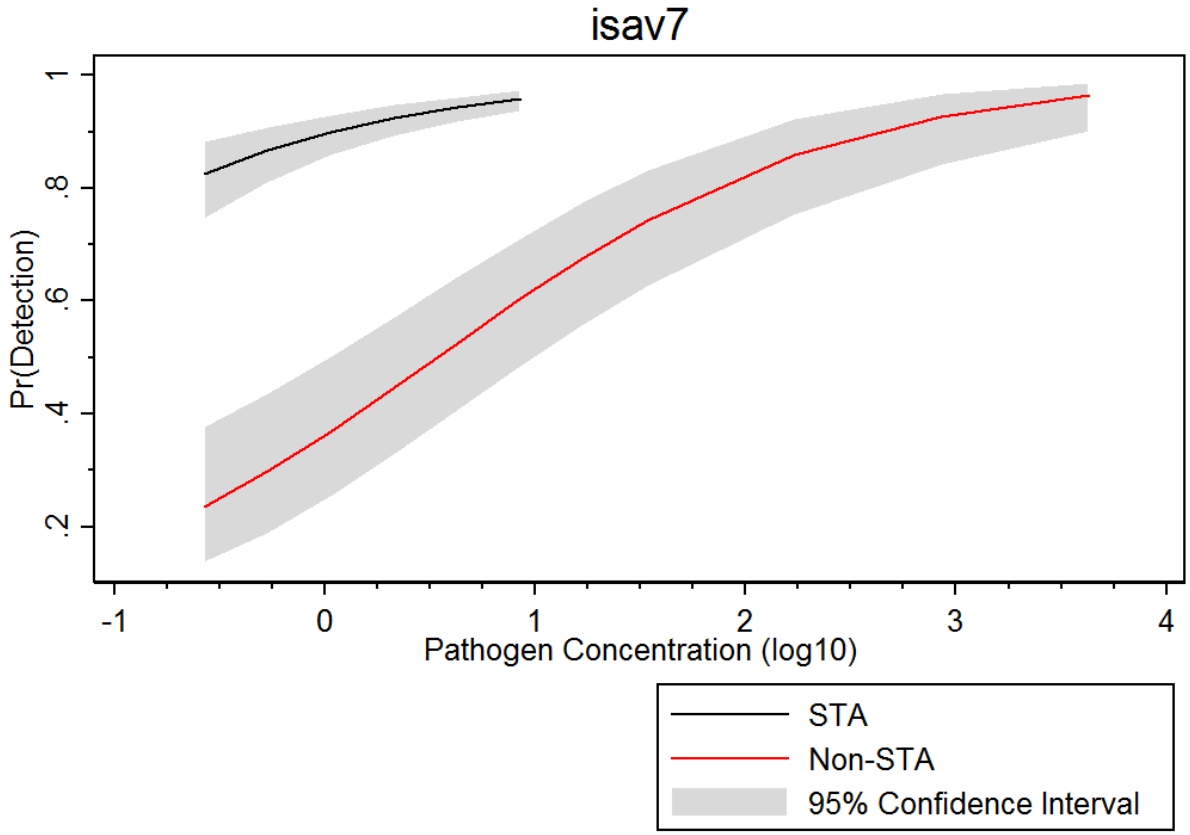

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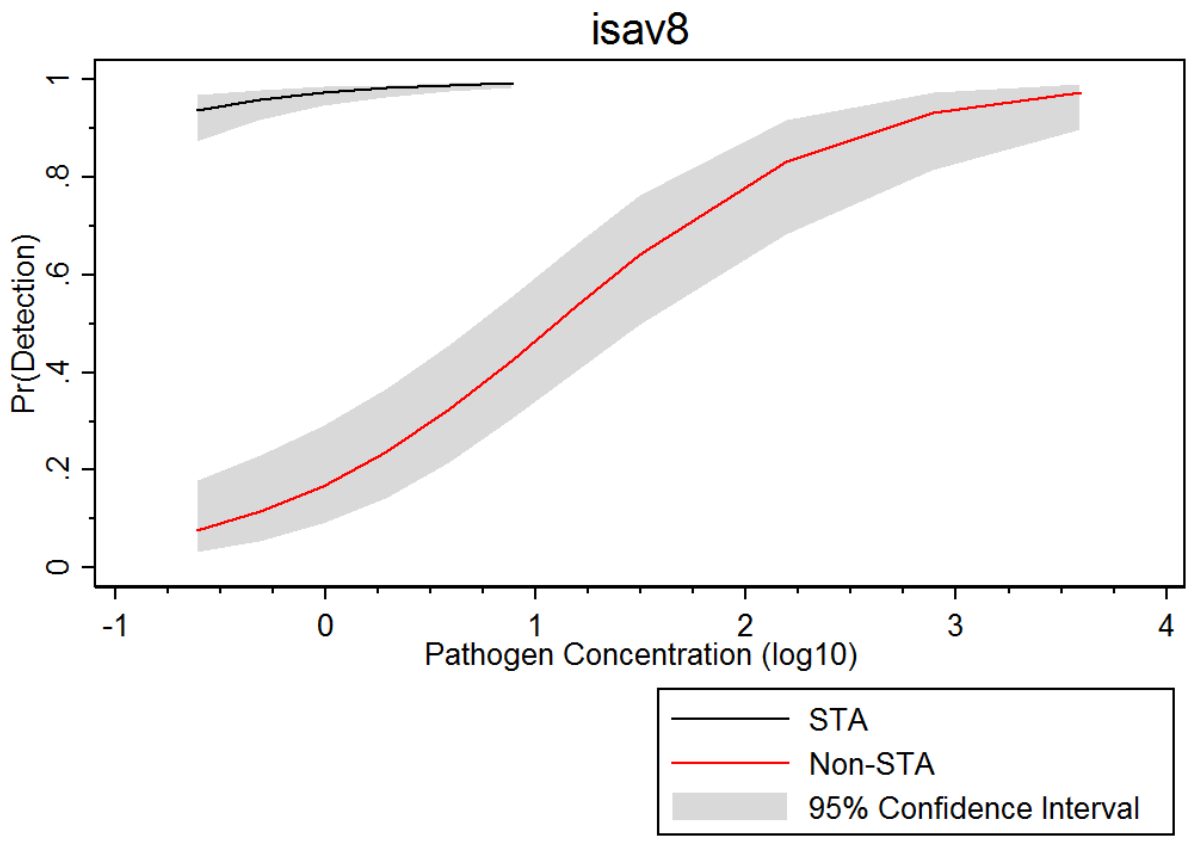

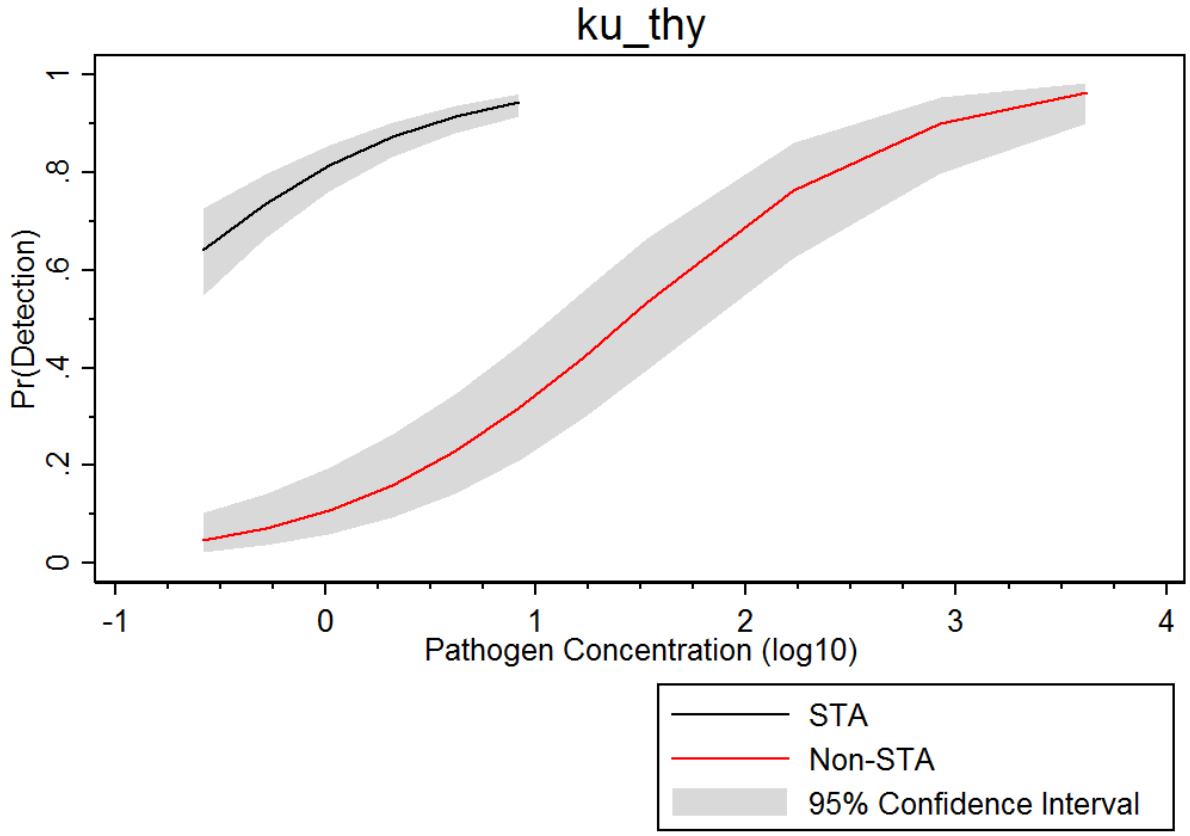

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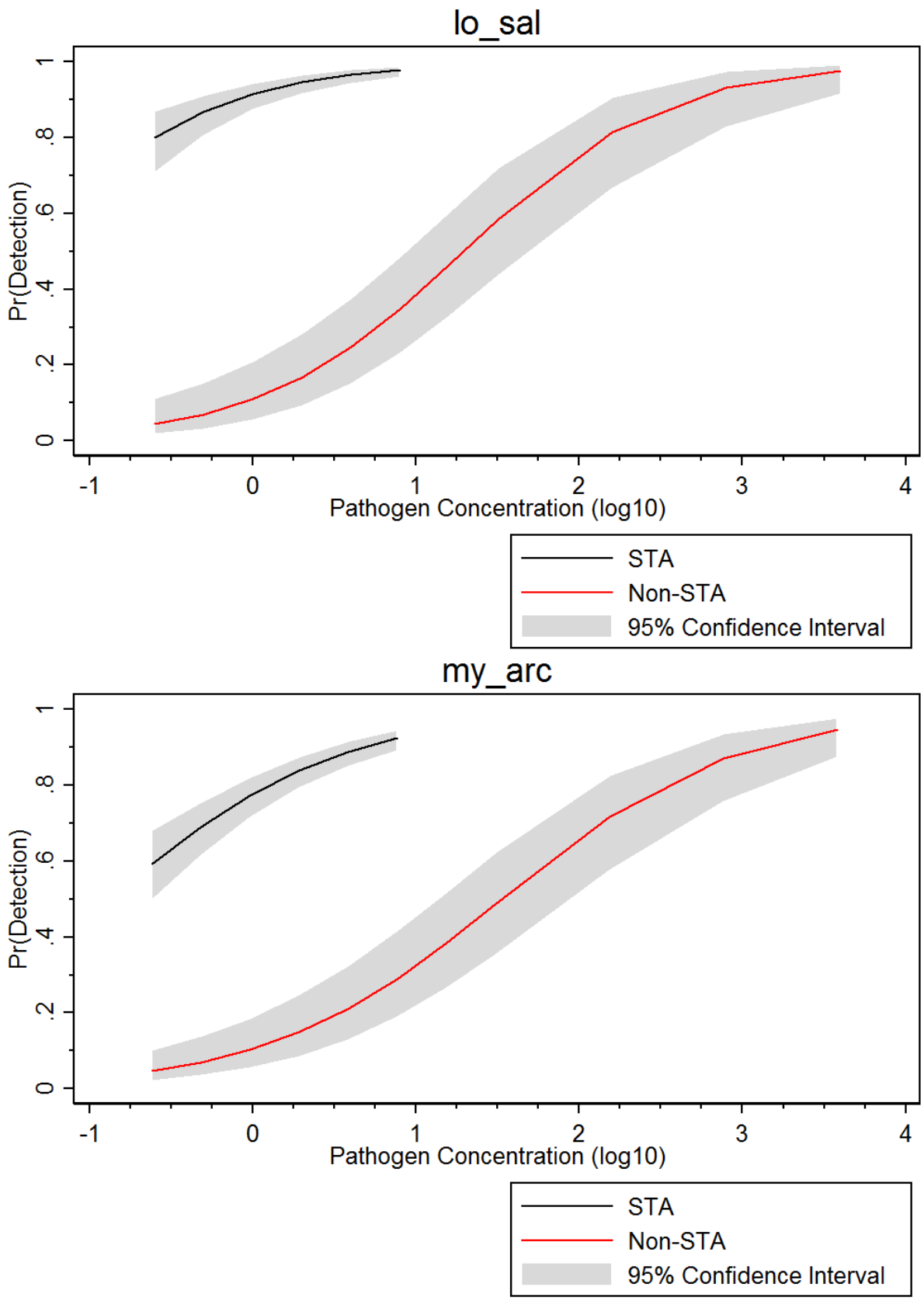

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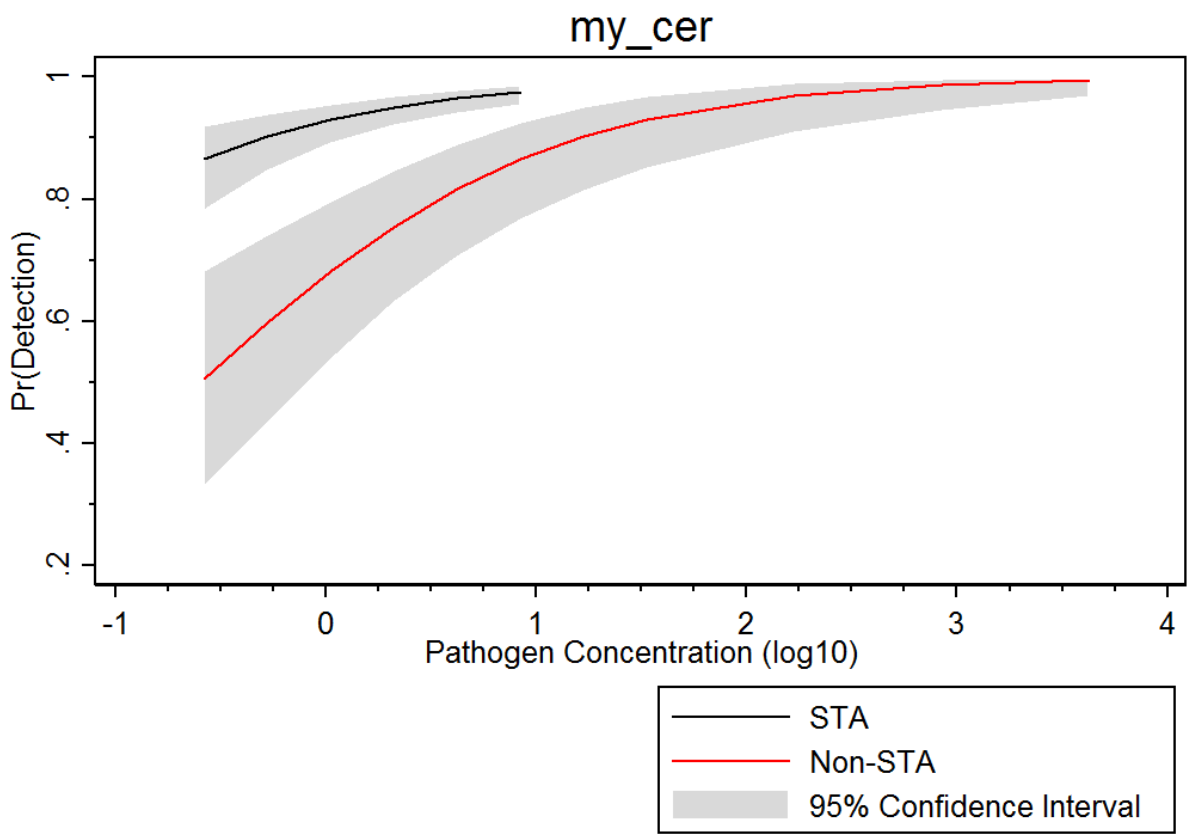

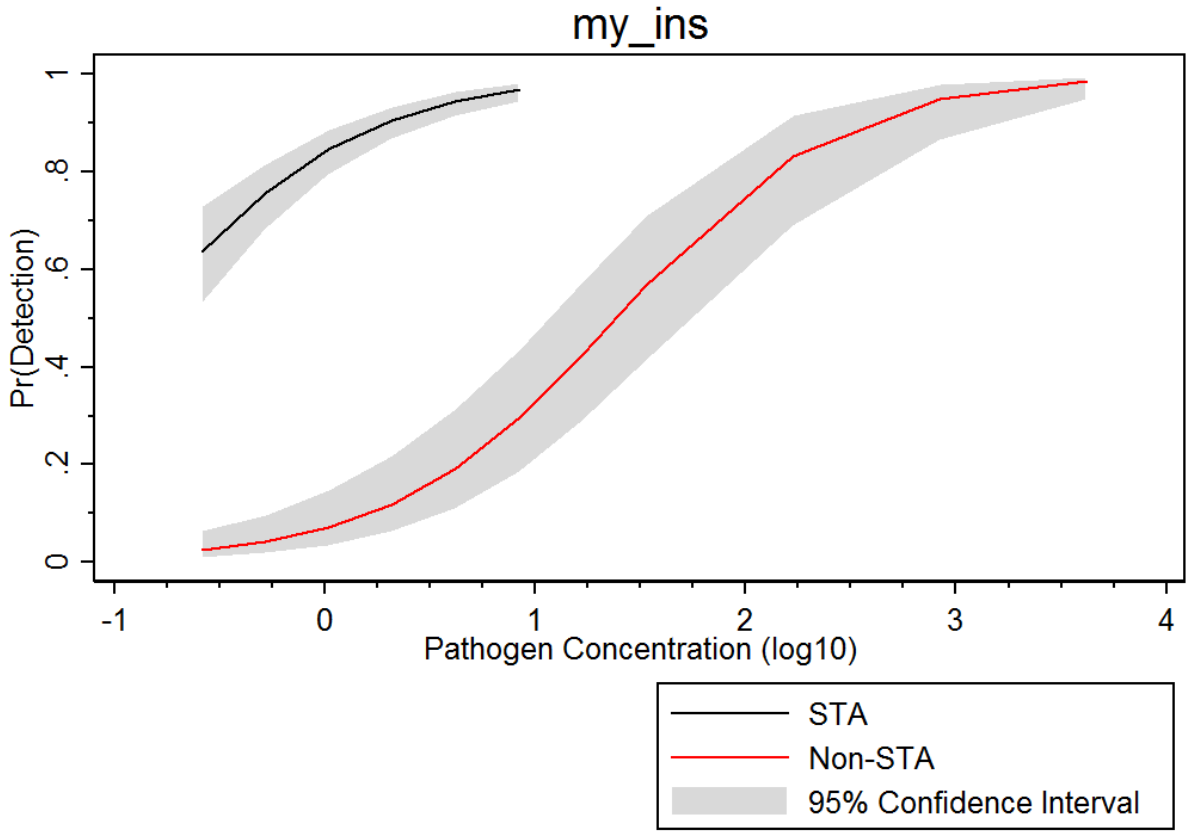

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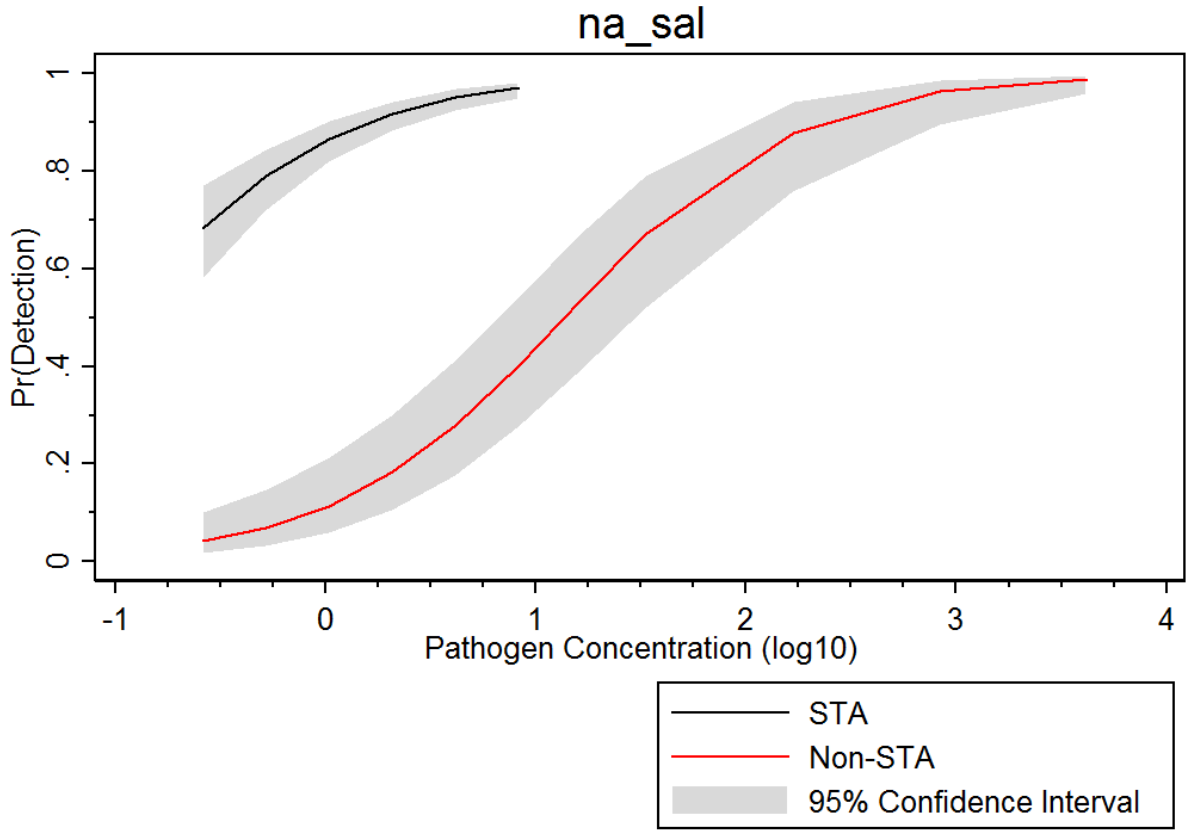

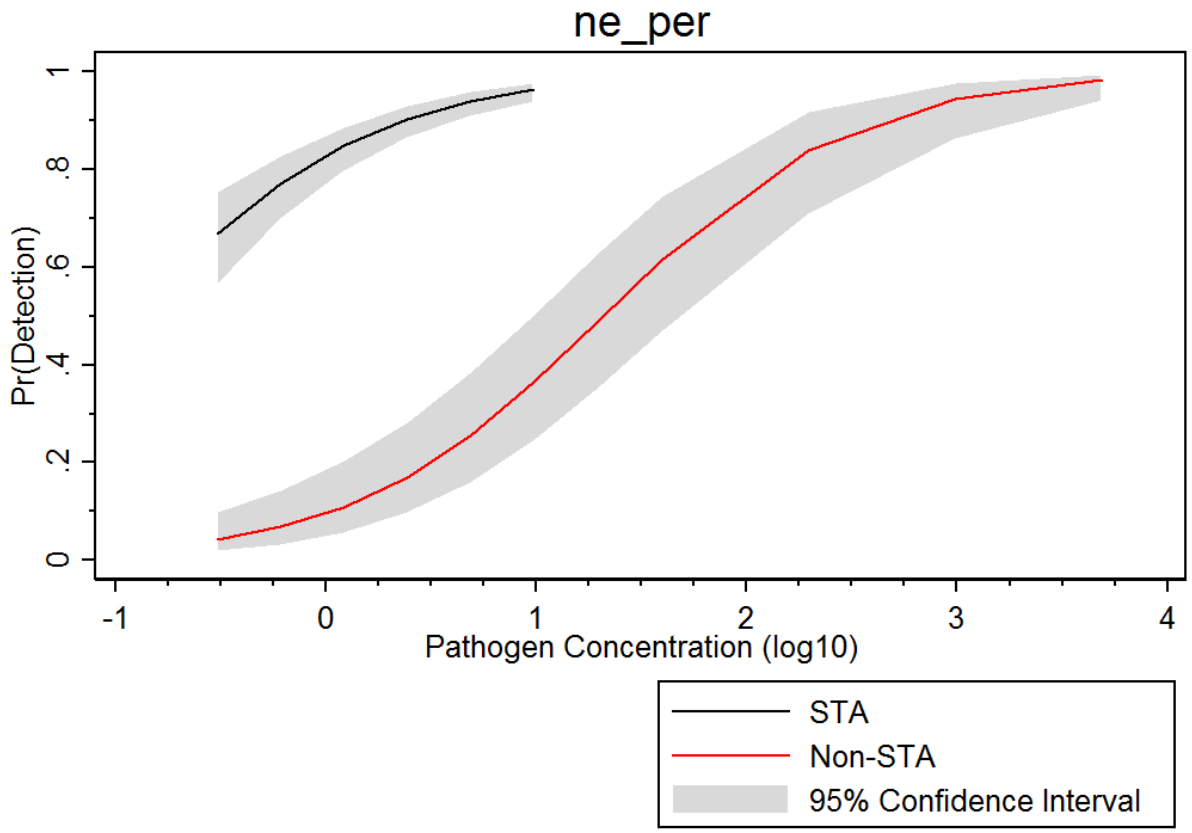

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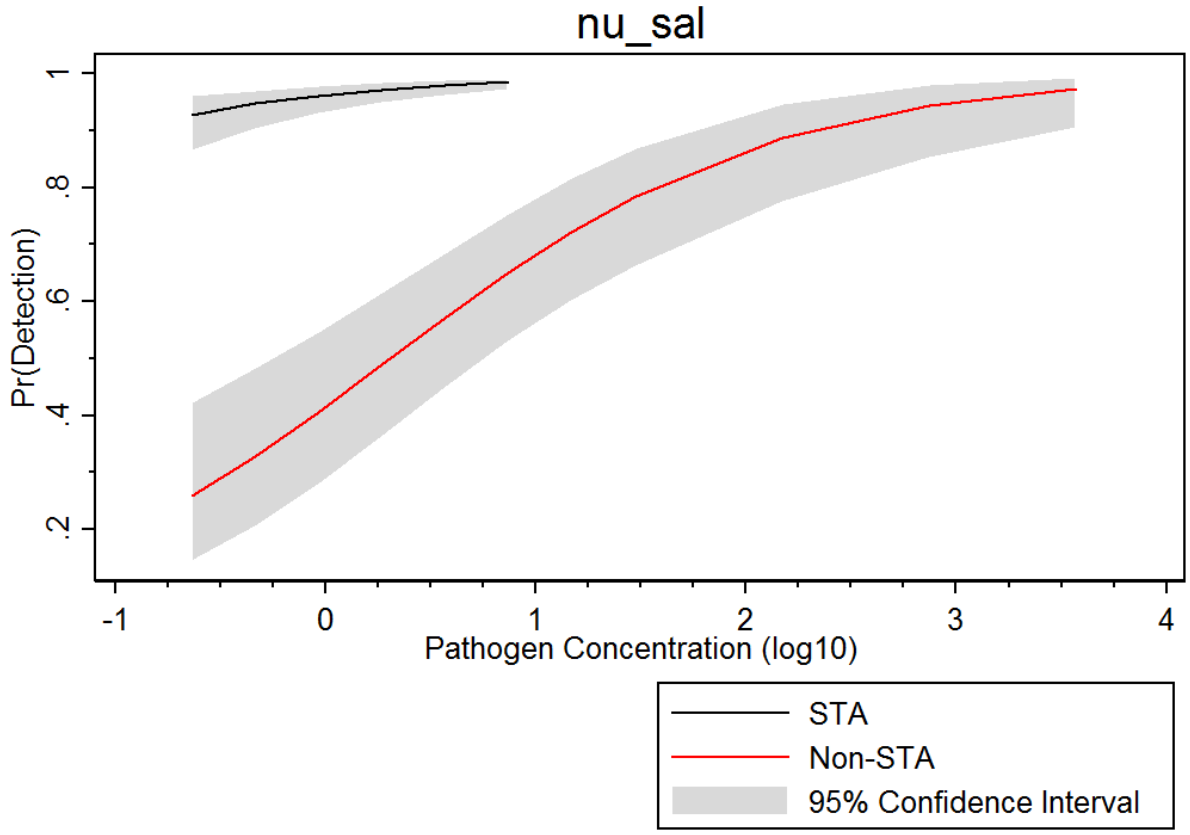

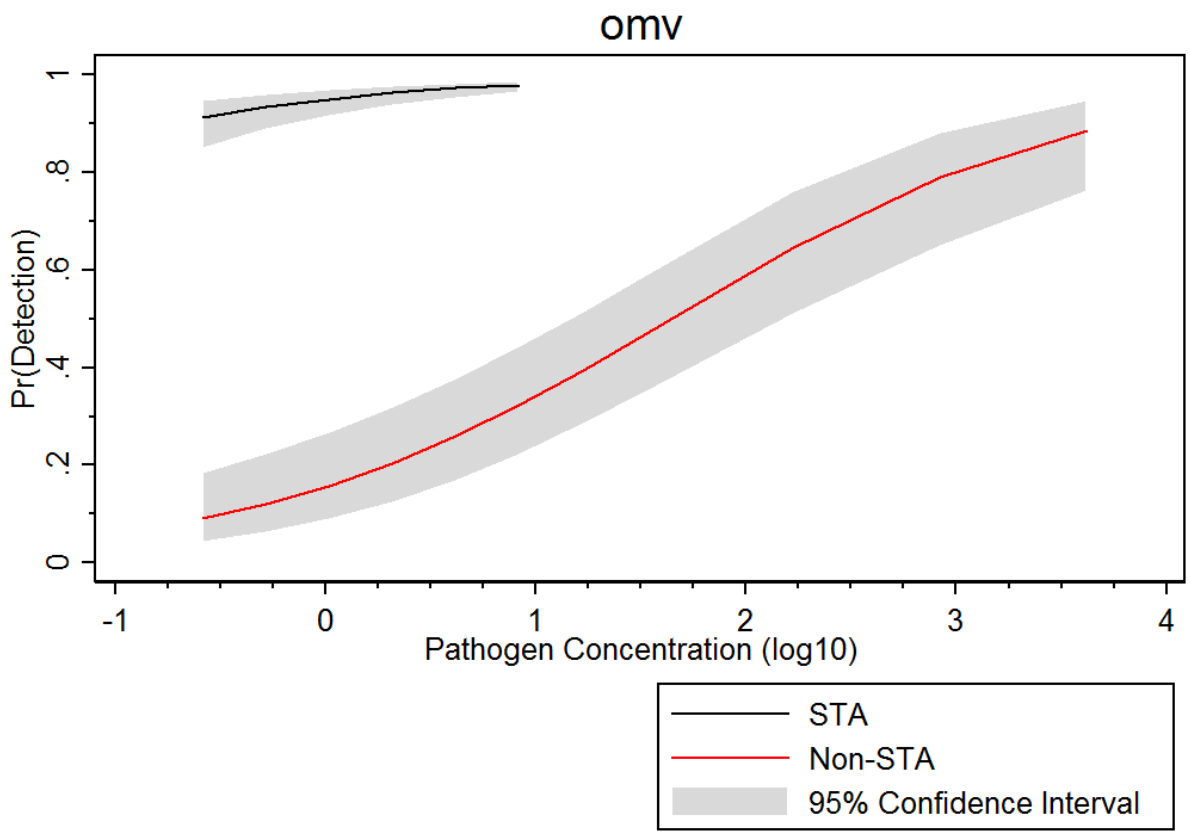

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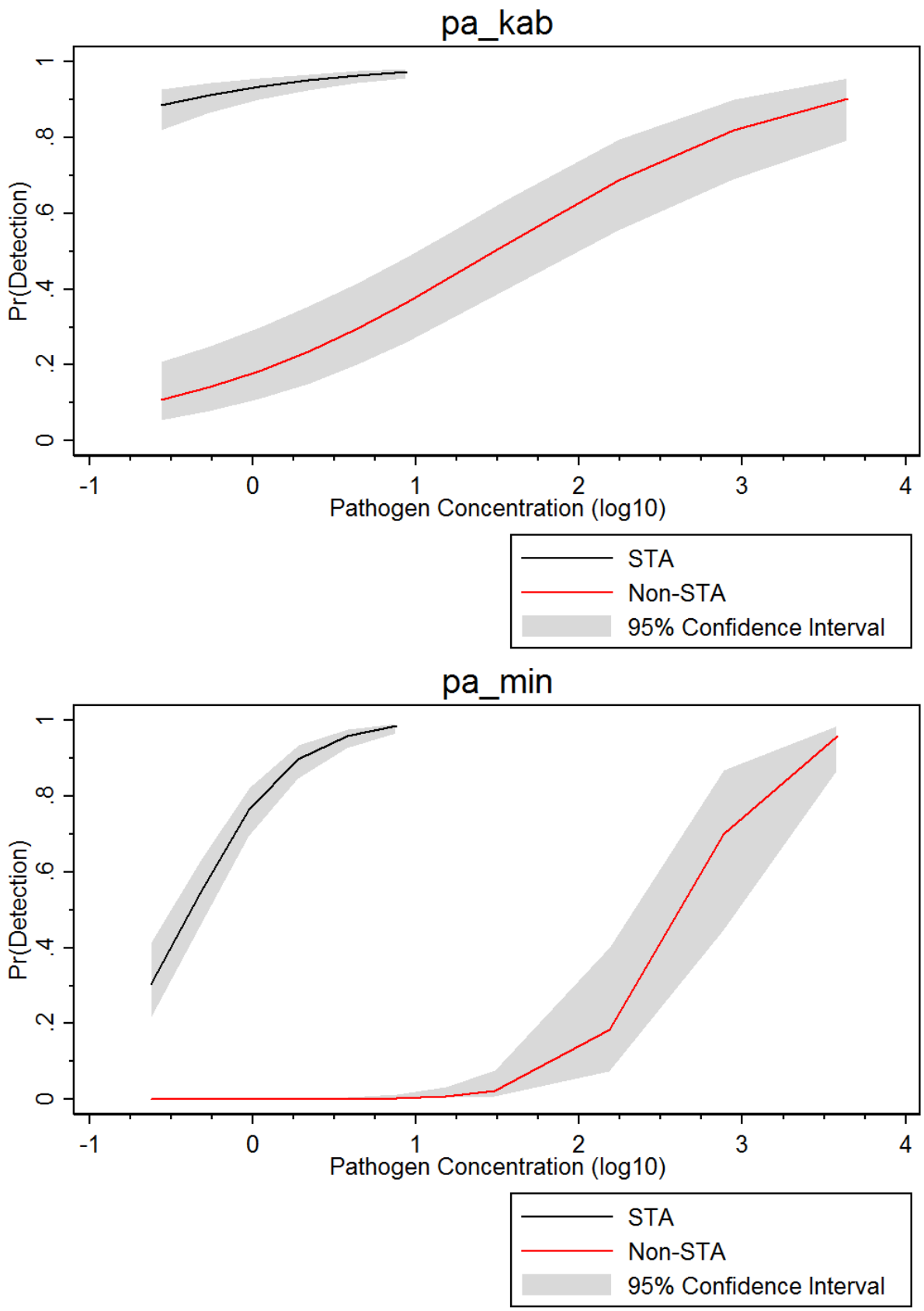

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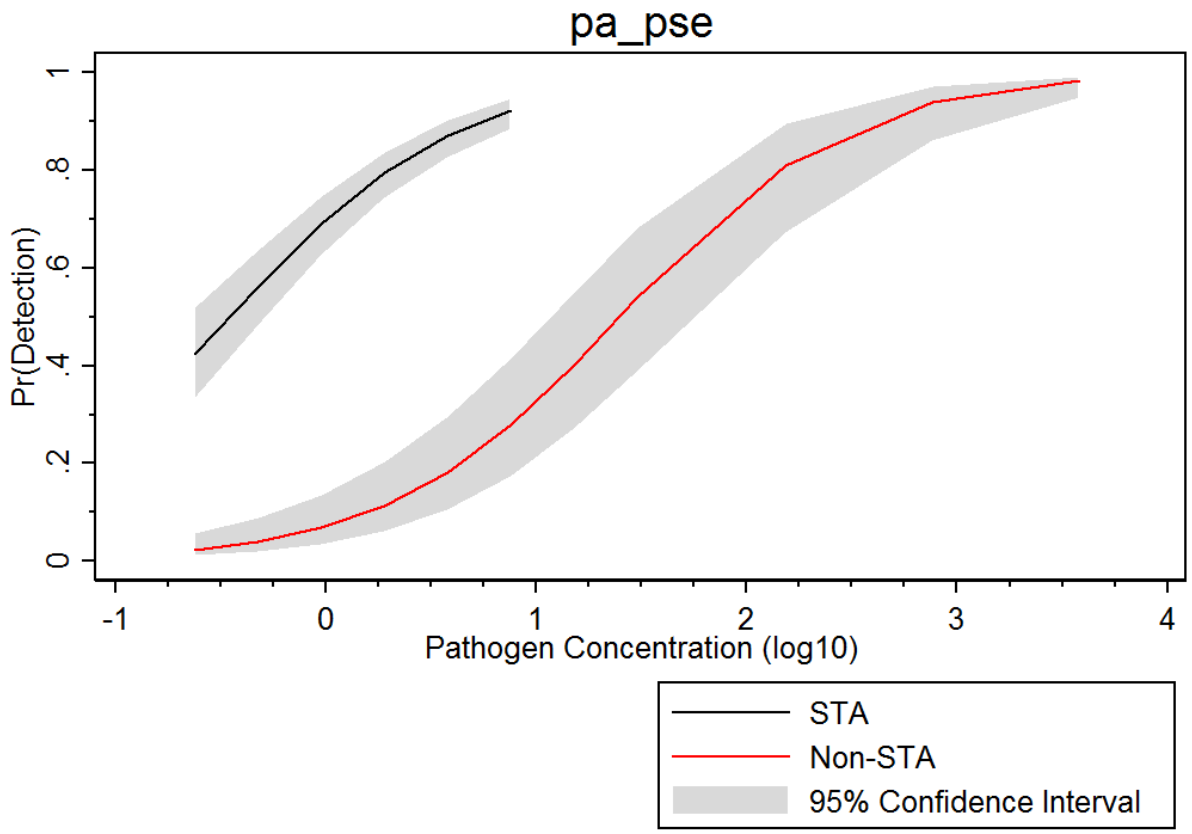

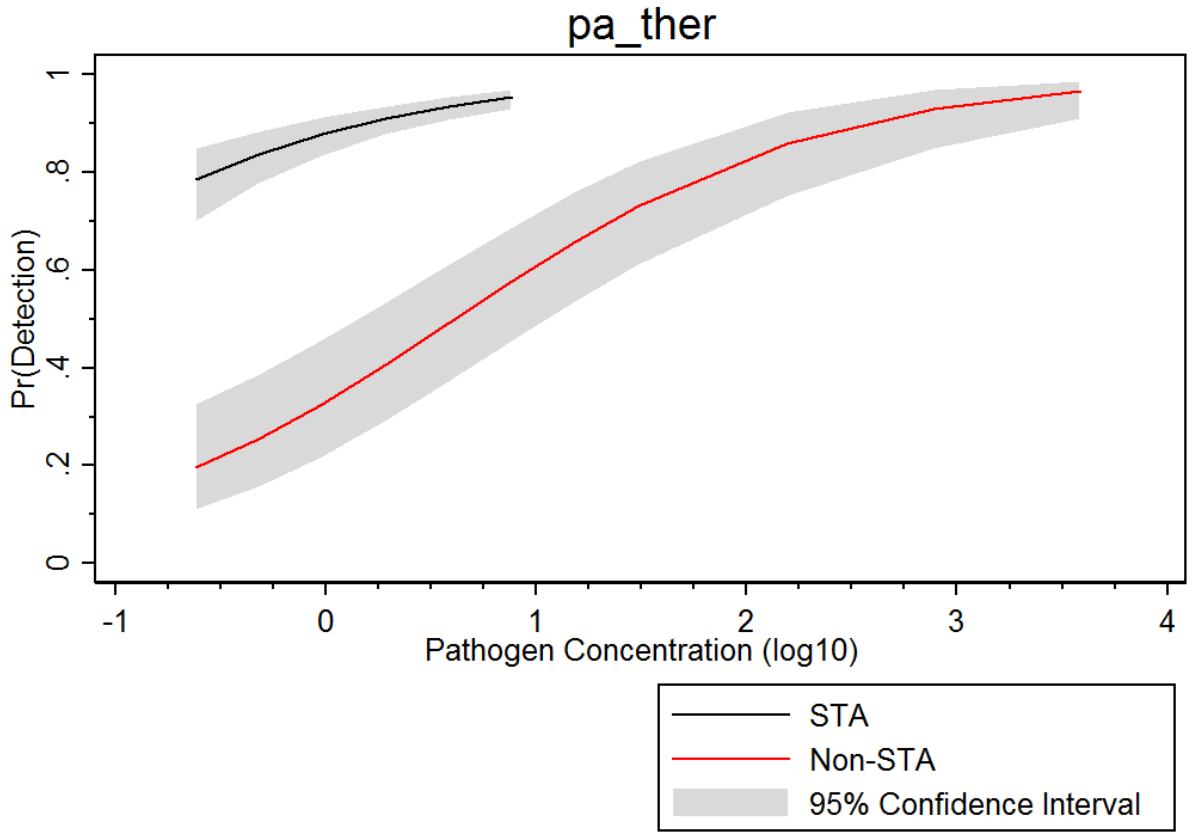

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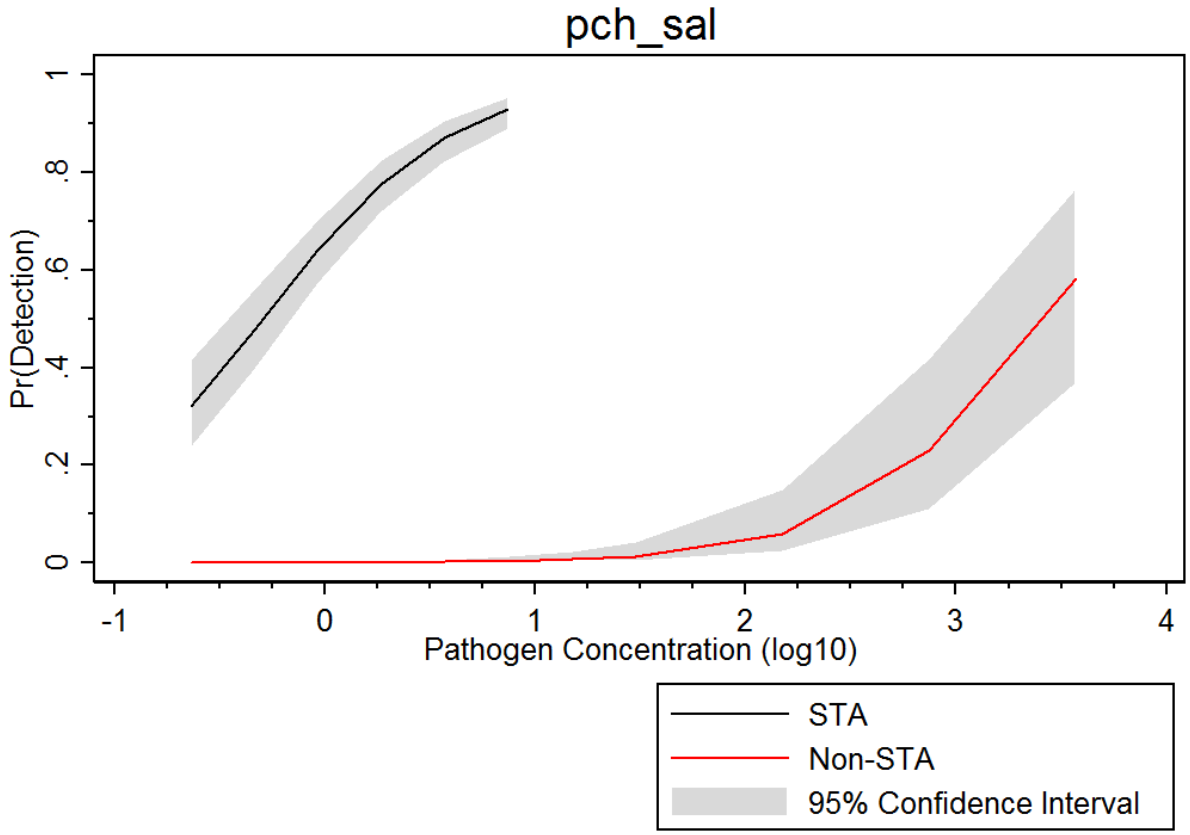

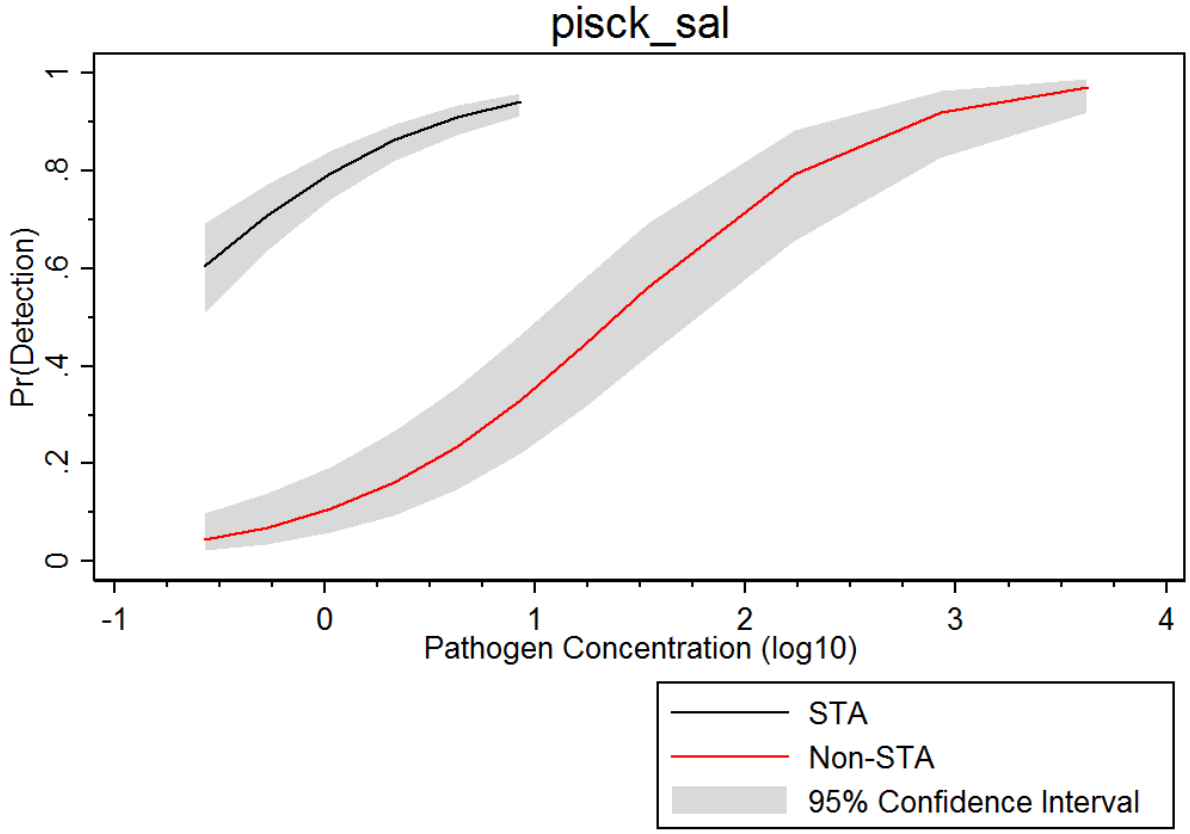

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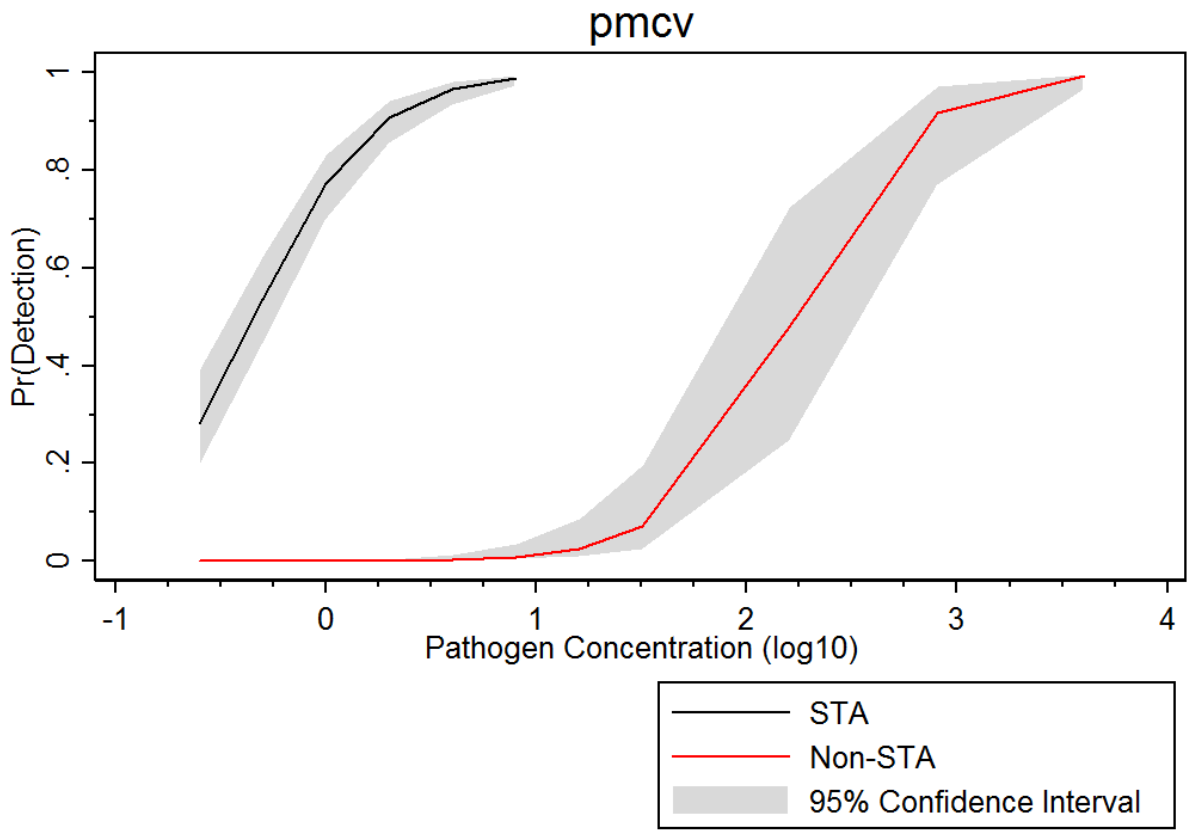

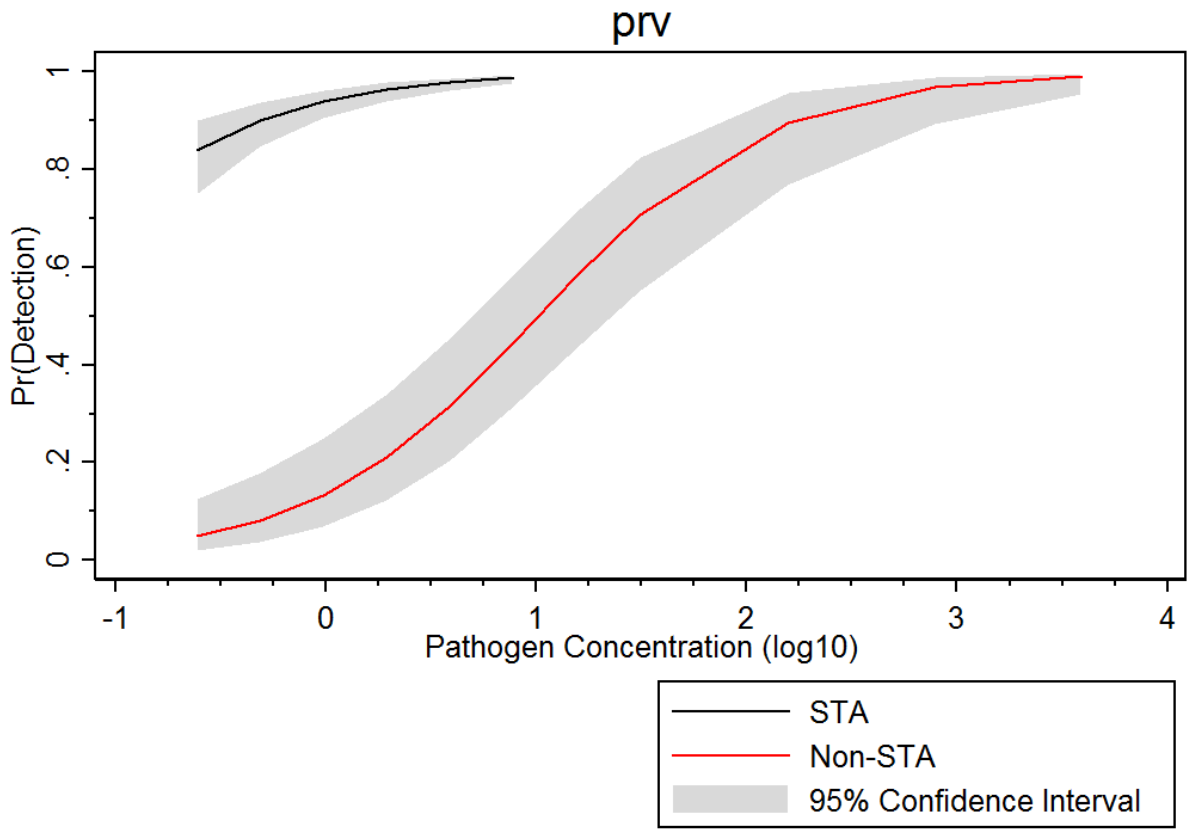

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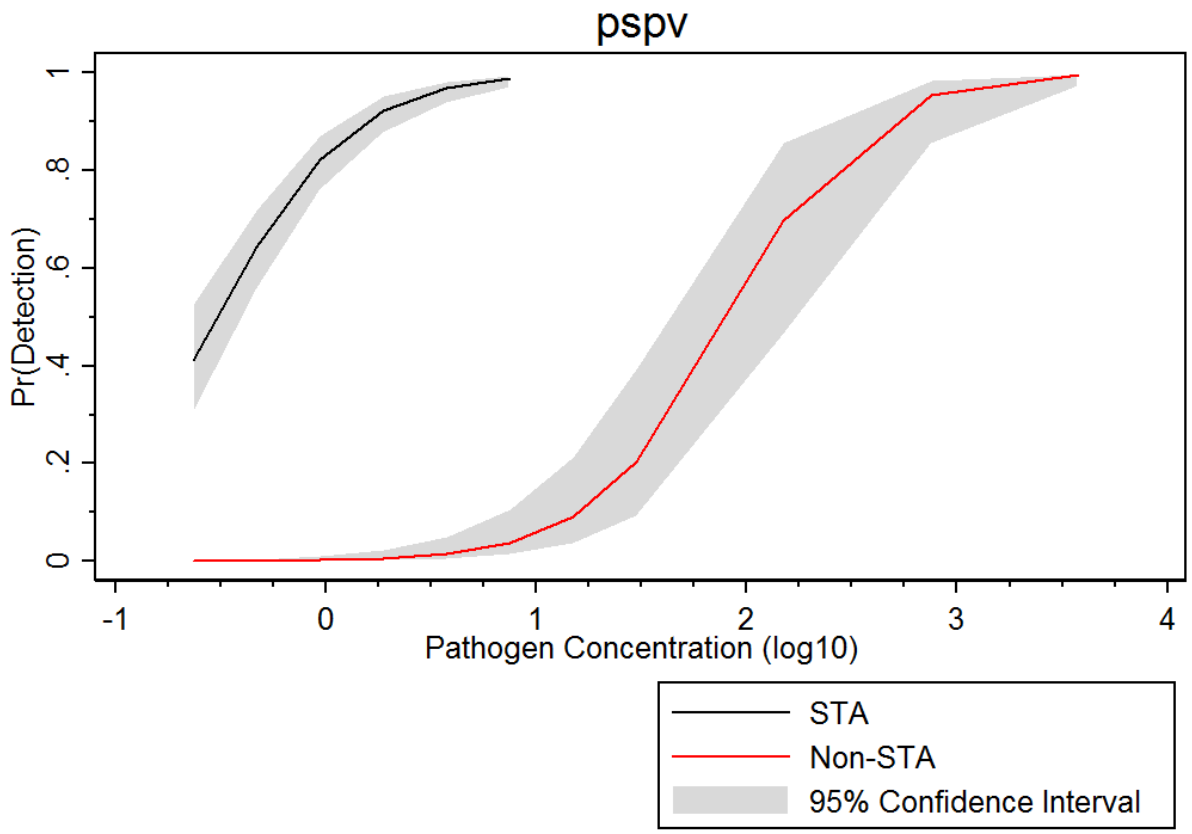

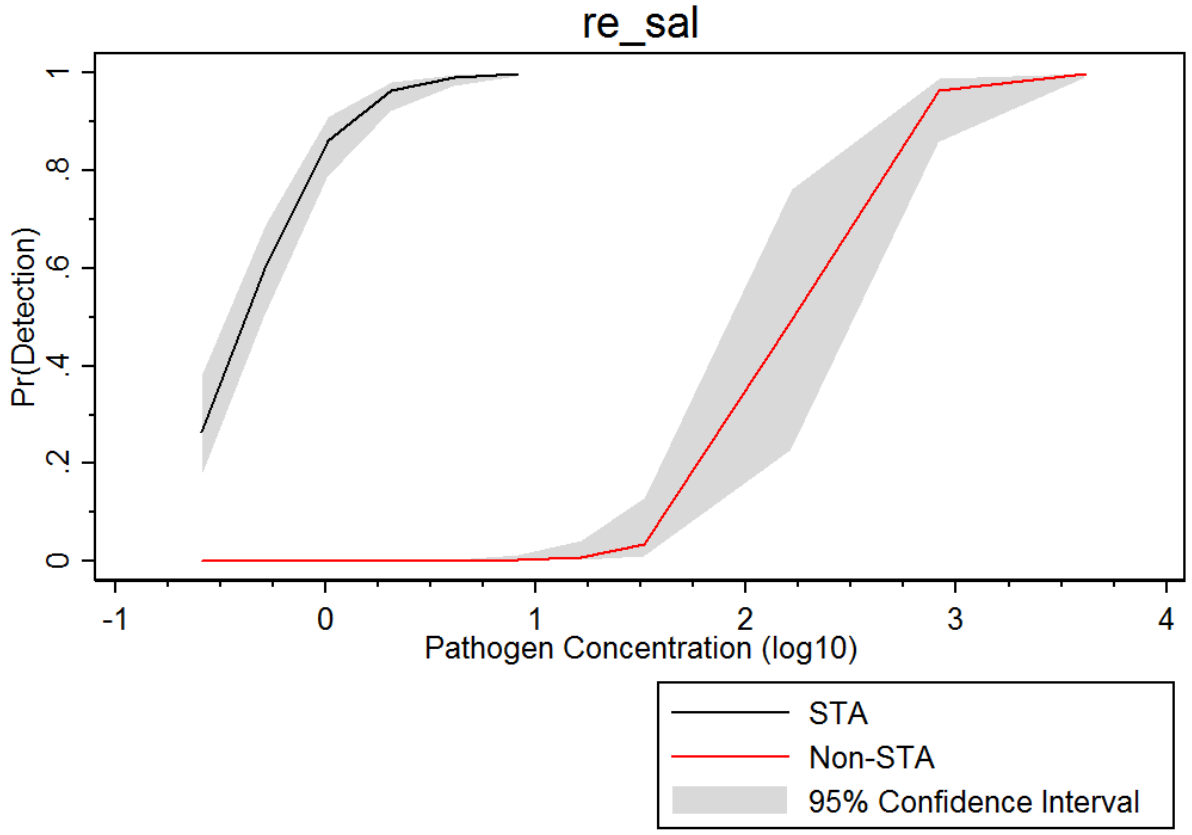

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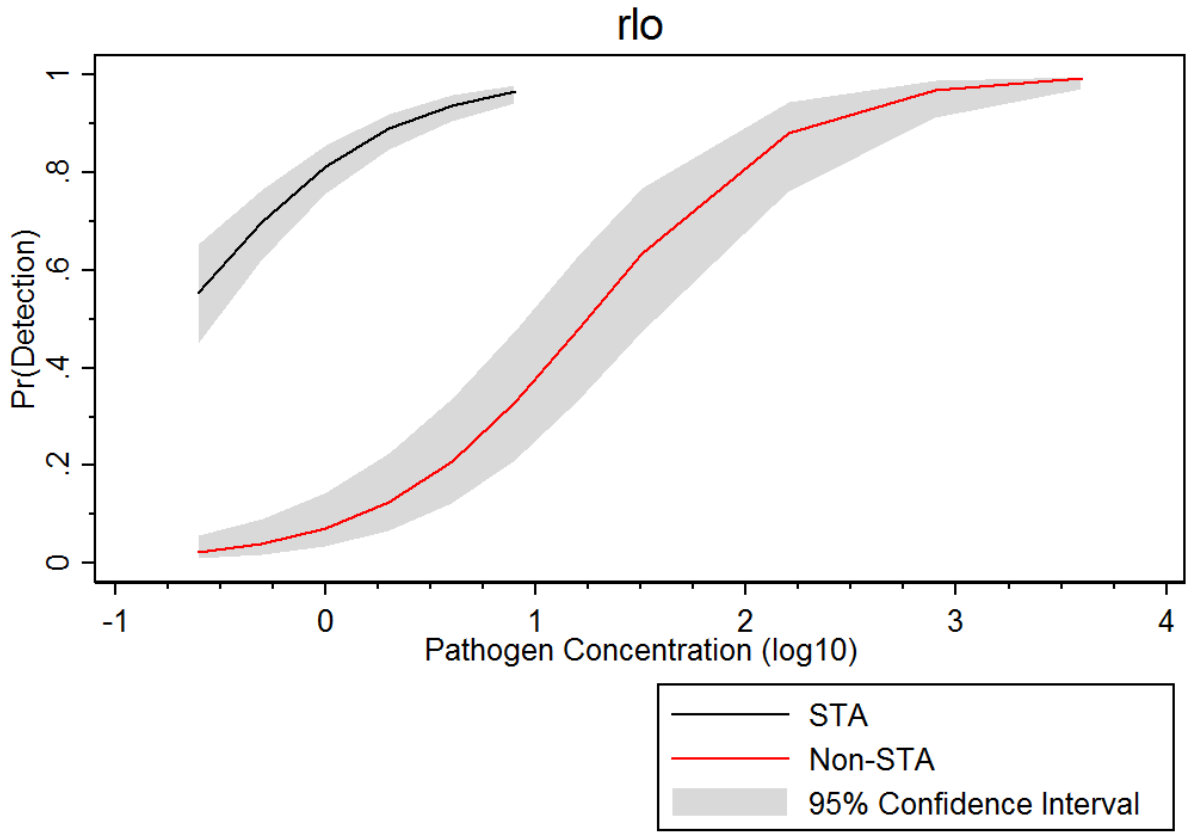

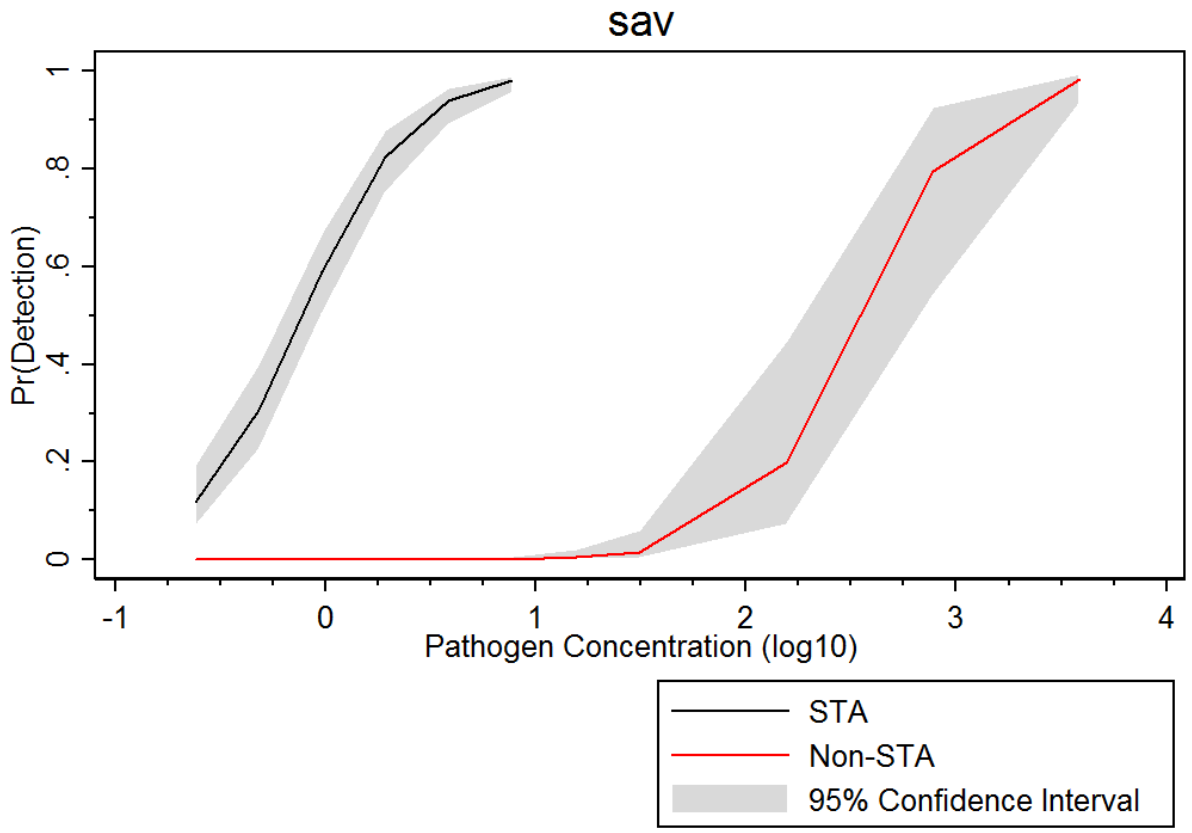

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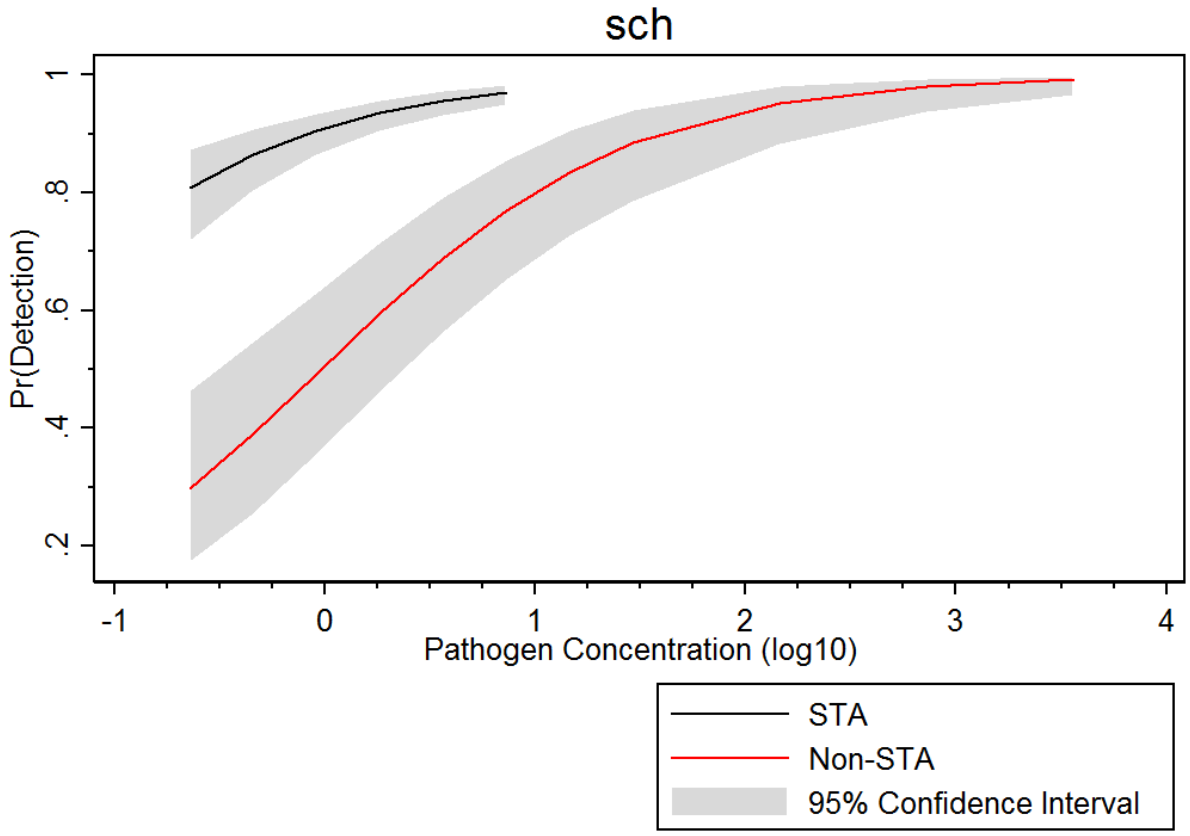
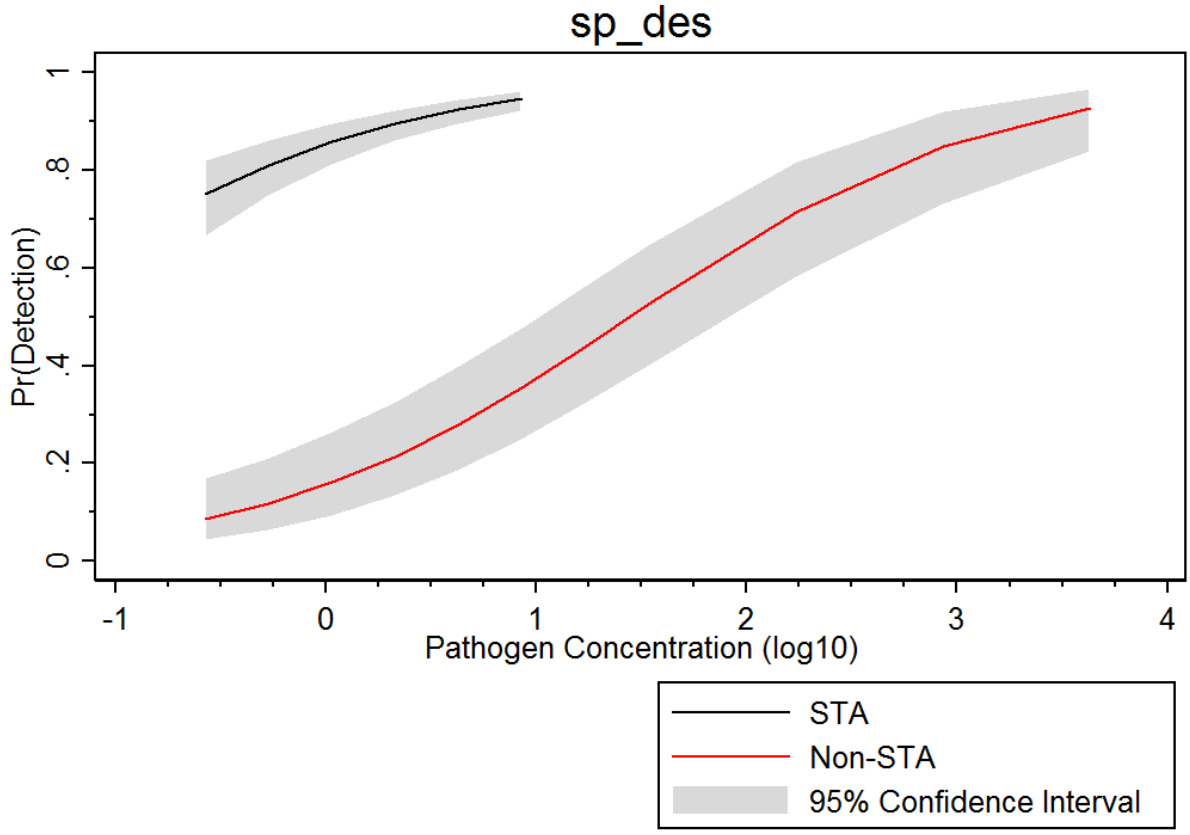
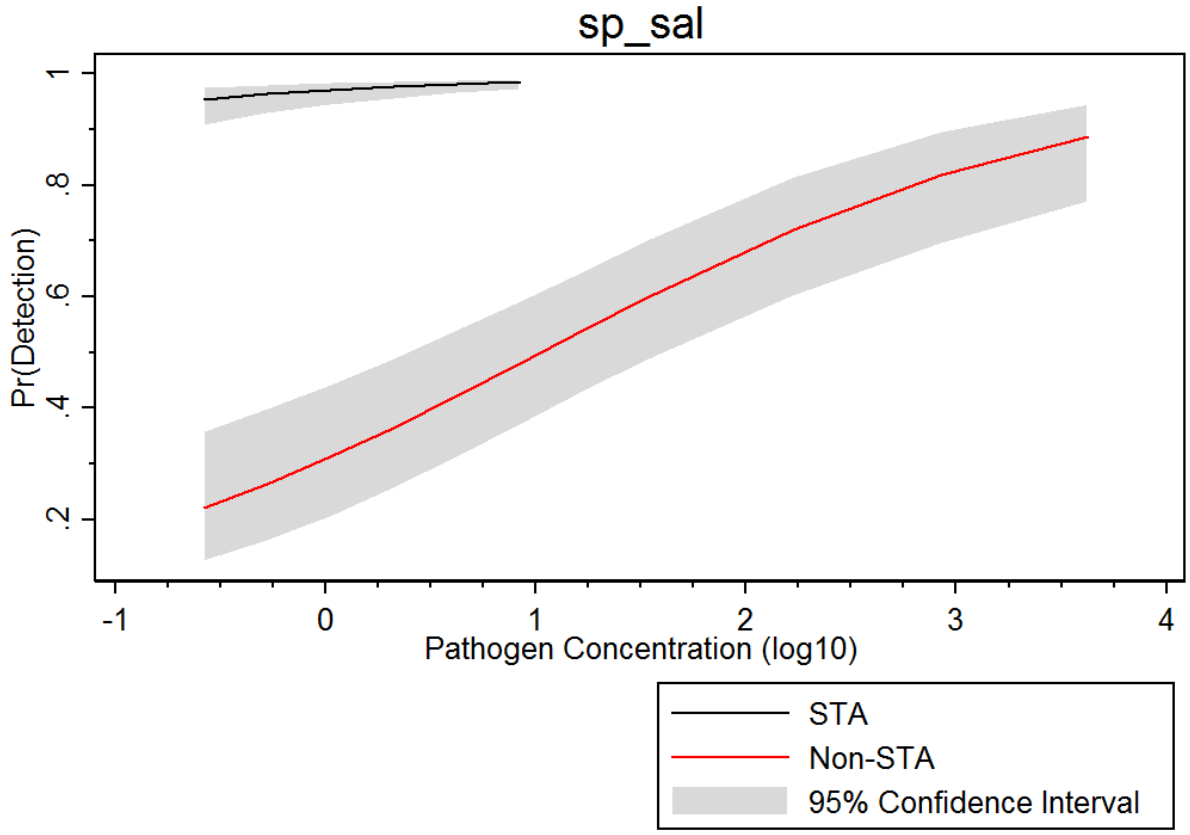
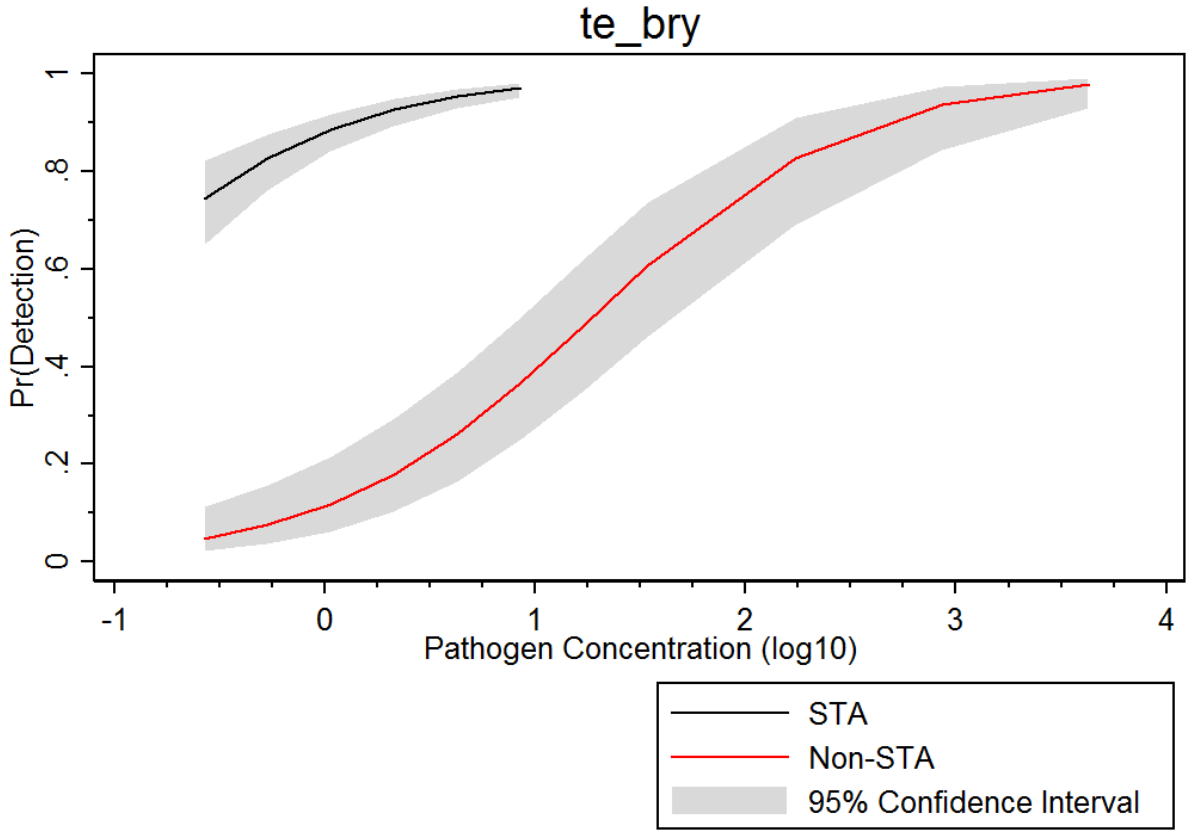


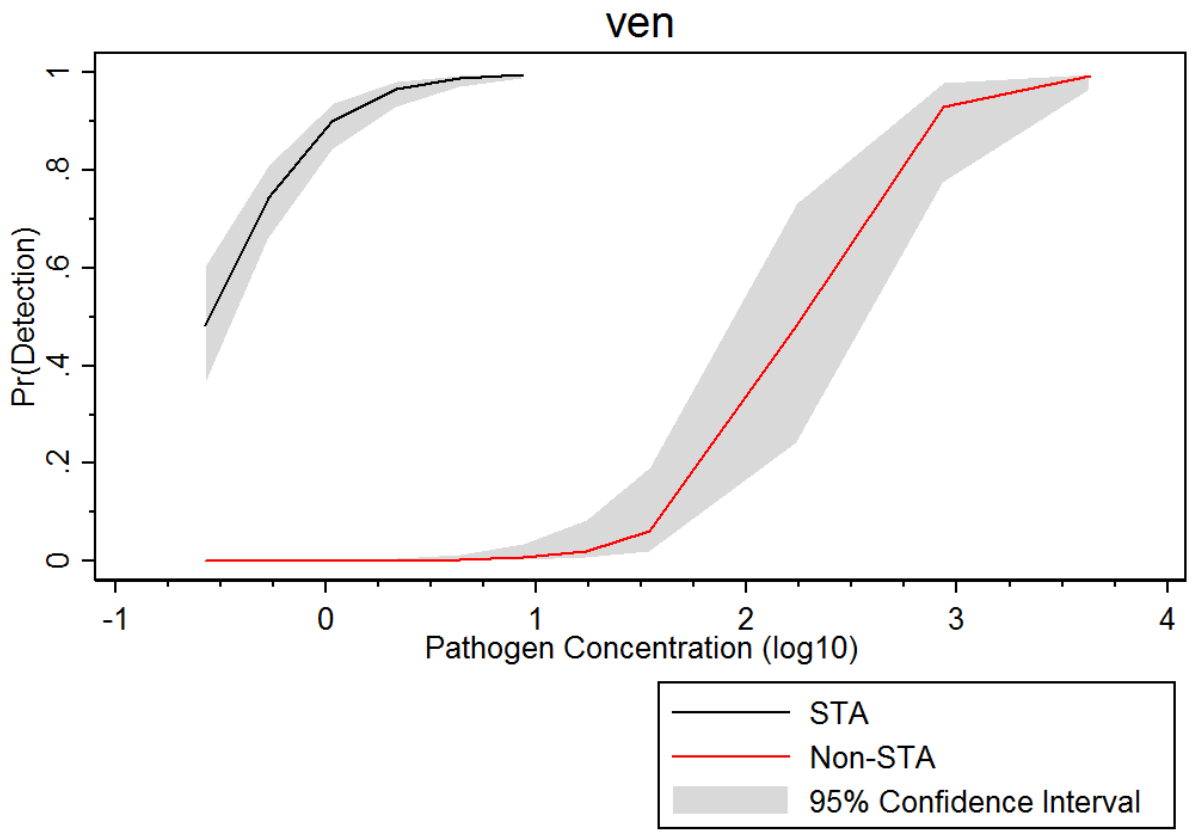


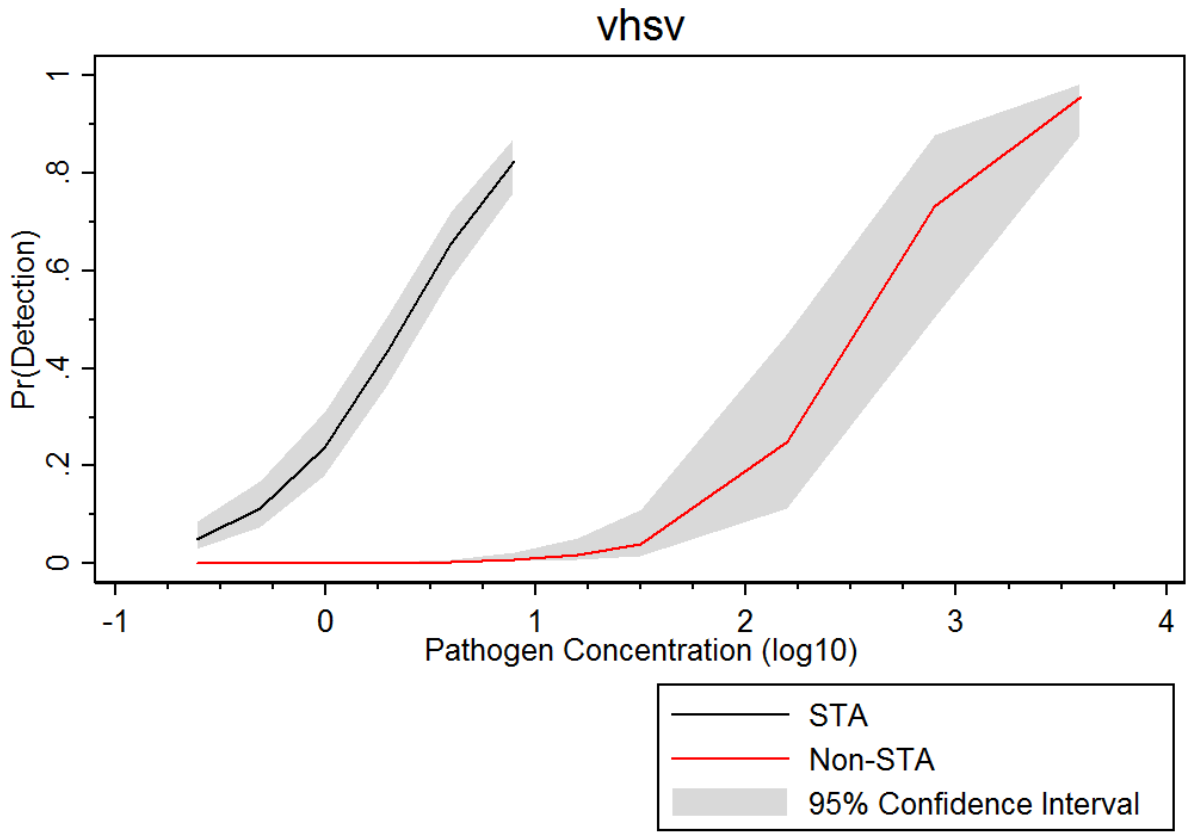
Supplemental Figure 5 (Continued)



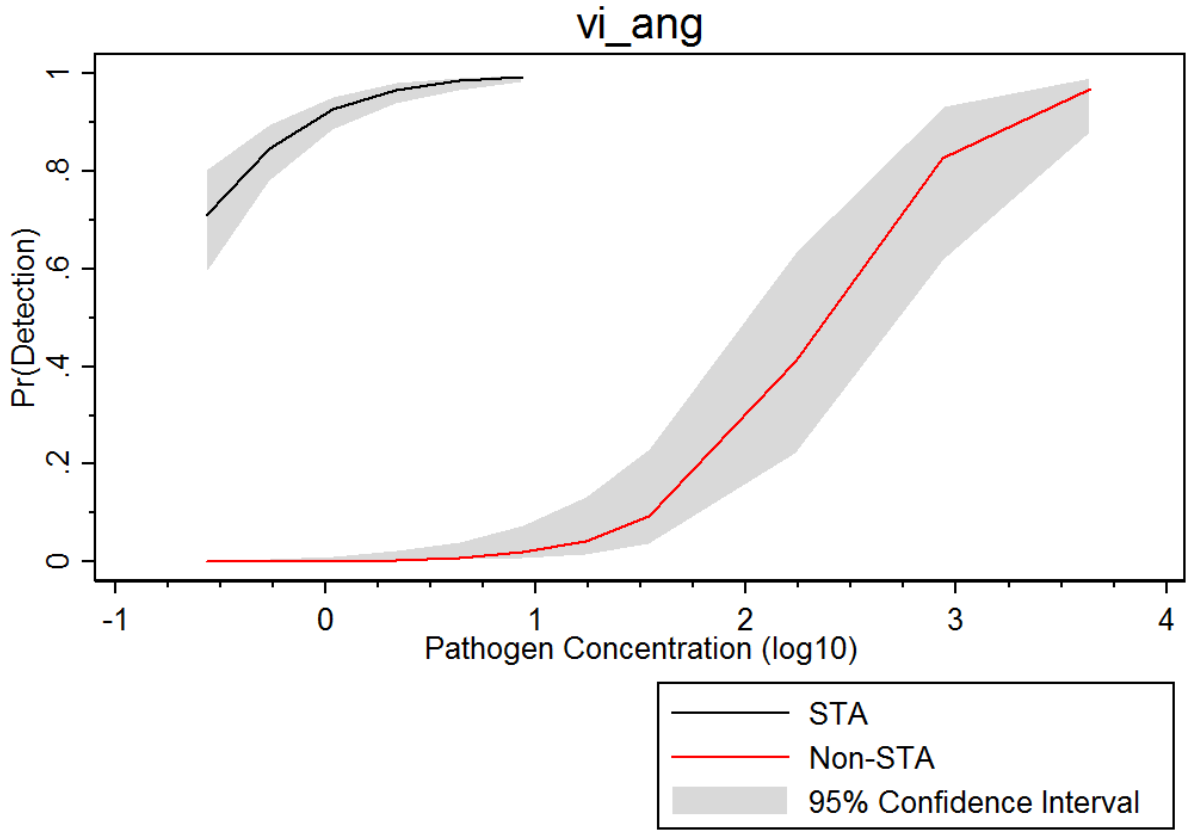


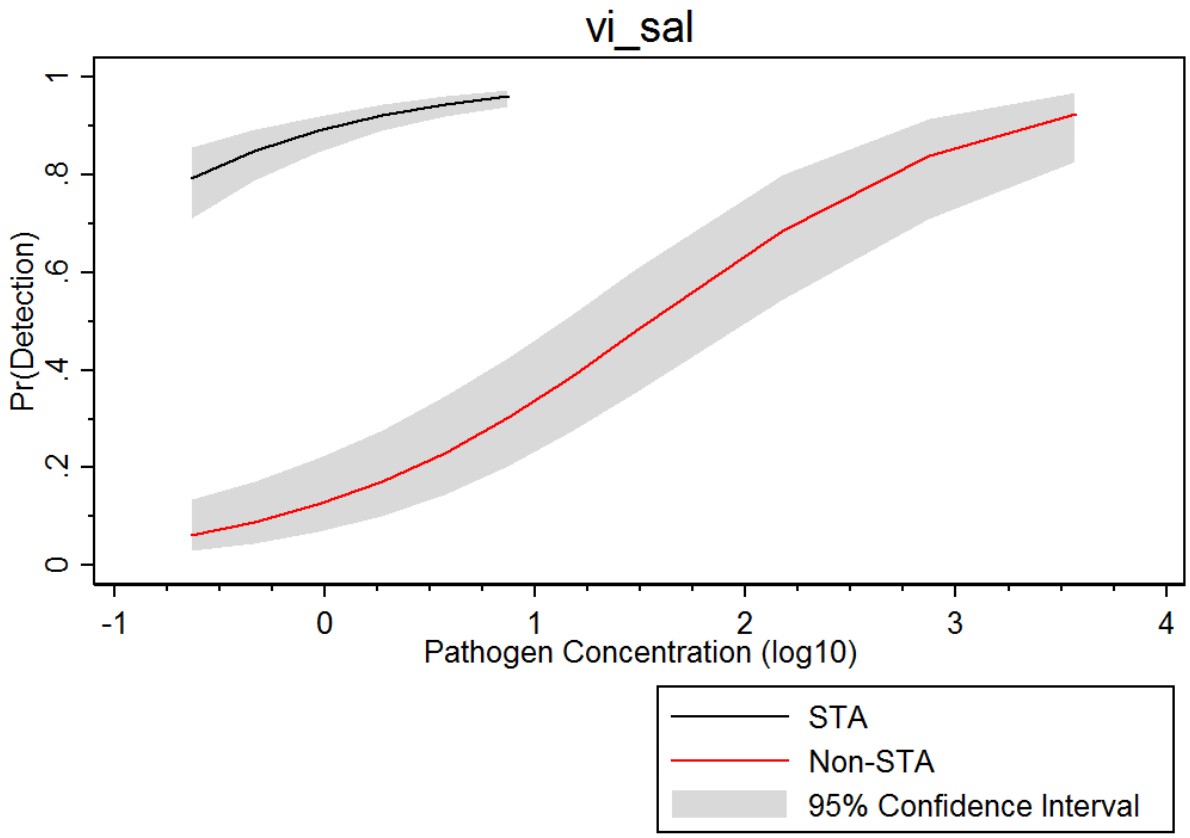
Supplemental Figure 5 (Continued)



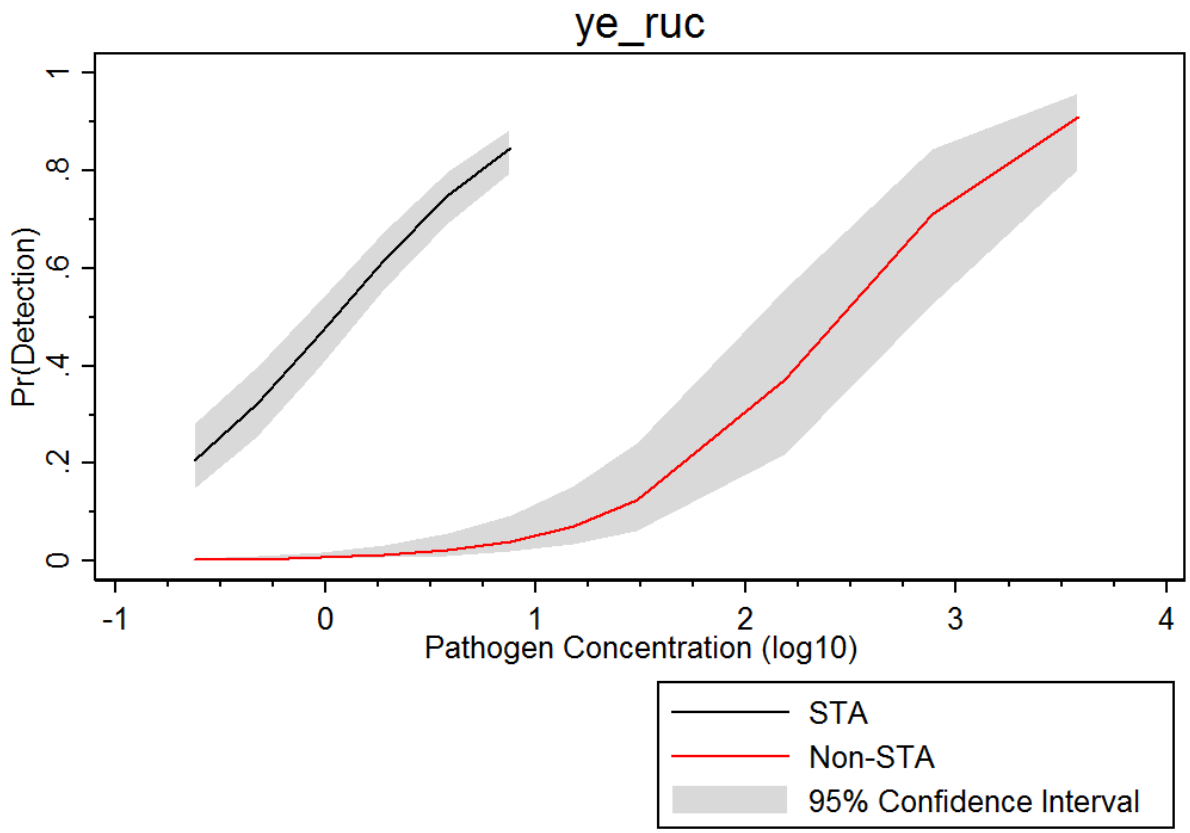


Supplemental Figure 5 (Continued)





Supplemental Figure 5 (Continued)



Supplemental Figure 5 (Continued)

Supplemental Table 3. In silico analysis of genogroup variation under the viral assays evaluated herein. N refers to the # of sequences that overlap the corresponding assay region. SNP mismatches are represented by numbers in the F (Forward primer), P (Taqman Probe) and R (Reverse primer).

Viral class	Microbe	Assay gene	Amplicon size (bp)	N	F	P	R	Amplification likely?	Accession #	Asp tested	Comments
Rhabdovirus [ssRNA(-)]											
	IHNV genogroup U	Nucleoprotein	80	4	0	0	0	yes	AY442508, AY442515, AY442516, AB231658	Yes	
	IHNV genogroup M	Nucleoprotein	80	4	0	0	0	yes	AY442511, AY442512, AY442514, AY442518	Yes	
	IHNV genogroup L	Nucleoprotein	80	3	0	0	0	yes	AY442509, AY442510, AY442517	Yes	strain 93-057 from IHNV study was used
	IHNV genogroup WRAC	Nucleoprotein	80	1	0	0	0	yes	L40833	No	low virulence
	IHNV genogroup 220-90	Nucleoprotein	80	2	0	0	0	yes	HM461966, GQ413939	No	virulent to Rainbow Trout
Rhabdovirus [ssRNA(-)]											
	VHSV genogroup Ib	Nucleocapsid	82	4	2	0	0	yes	FJ460590, FJ460591, AB672614, AF143862	Yes	no sequence data at N for groups 1a, c,d,e
	VHSV genogroup II	Nucleocapsid	N/A	3	N/A	N/A	N/A	?	no sequence over assay region; AY356743, AY356690, AY356691	No	
	VHSV genogroup III	Nucleocapsid	82	5	2	0 or 3	0 or 2	yes for most	NC00085, FN665788, FJ362510, EU481506, AF143863	Yes: a & b	two contigs based on sequence alignments
	VHSV genogroup IV	Nucleocapsid	82	7	0	0	0	yes	AB490792, JF792424, AB179621, GQ385941	Yes; a & b	IVa-North P. ocean; IVb-Great Lakes and E. Canada
Paramyxovirus [ssRNA (-)]		Nucleoprotein									
	ASPV		97	3	0	0	0	yes	EF646380, EU1561471, AY229975	Yes	gblock designed to sequence
Orthomyxovirus [ssRNA(-)]										Yes	we have multiple strains from N. America and Europe (seg6 sequence)
	ISAV s7 gp1	segment7	81	33	0-1	0-1	0-1	yes			contains European and NA strains (Norway, Scotland, Canada)
	ISAV s7 gp2	segment7	81	34	5	5	2-3	no			contains European and NA strains (Norway, Canada)
	ISAV s8 gp1	segment8	169	31	0	0	0	yes			contains European strains (Norway, Scotland)
	ISAV s8 gp2	segment8	169	11	0	0	0	yes			contains NA strains (Canada)
Nodavirus [ssRNA(+)]											
	VER Cp group 1	Coat protein	70	115	0-1	0-1	0-1	yes		Yes	<5% divergency within group
	VER Cp group 2	Coat protein	70	5	0	0	0	yes		Yes	<2% divergency within group; >10% divergency from group1
	VER Cp group 3	Coat protein	70	6	0	0	0	yes		Yes	<2% divergency within group; >15% divergency from group1
	VER Cp 4 group	Coat protein	70	1	0	0	0	yes	GQ402013	Yes	<2% divergency within group; >5% divergency from group1
	VER Cp 5 group	Coat protein	70	2	0	0	0	yes	AY547548, AY547549	Yes	<2% divergency within group; >15% divergency from group1
	VER Cp 6 group	Coat protein	70	2	0	0	0	yes	AF445800, AY547547	Yes	<2% divergency within group; >15% divergency from group1
Togavirus [ssRNA (+)]											
	SAV 1	non-structural protein (nSP1)	107	1	0	0	0	yes	AJ316244	Yes	
	SAV 2	non-structural protein (nSP1)	107	1	0	0	0	yes	AJ316246	Yes	
	SAV 3	non-structural protein (nSP1)	107	5	0	0	0	yes	JQ799139, AY604235, AY604236, AY604237, AY604238	Yes	
	SAV 4	non-structural protein (nSP1)	N/A	N/A	N/A	N/A	N/A	yes		Yes	Fringuelli et al. (2008) found assay identified all genogroups1-6
	SAV 5	non-structural protein (nSP1)	N/A	N/A	N/A	N/A	N/A	yes		Yes	Fringuelli et al. (2008) found assay identified all genogroups1-6
	SAV 6	non-structural protein (nSP1)	N/A	N/A	N/A	N/A	N/A	yes		Yes	Fringuelli et al. (2008) found assay identified all genogroups1-6
	SAV-1-2-3 nSP gblock	non-structural protein (nSP1)	107	7	0	0	0	yes		Yes	
Birnavirus [dsRNA]											
	IPNV genogroup I West Buxton	Viral capsid protein (VP2)	116	2	0	0	0	yes	AF342727	No	we have VR299 strain from genogroup 1
	IPNV genogroup I VR299	Viral capsid protein (VP2)	116	1	1	0	1	yes	AF343572	Yes	
	IPNV genogroup I Jasper	Viral capsid protein (VP2)	116	1	1	1	0	yes	AF342735	Yes	
	IPNV genogroup II Abild	Viral capsid protein (VP2)	116	1	3	5	3	no	AF342729	Yes	
	IPNV genogroup III Sparjarup	Viral capsid protein (VP2)	116	1	1	2	1	?	AF342728	Yes	
	IPNV genogroup IV Canada1	Viral capsid protein (VP2)	116	1	2	3	1	?	AF342732	Yes	
	IPNV genogroup V Canada2 3	Viral capsid protein (VP2)	116	2	2	1-2	1-2	?	AF342733, AF342734	Yes	
	IPNV genogroup VI Hecht	Viral capsid protein (VP2)	116	1	5	5	7	no	AF342730	Yes	
	IPNV genogroup VII	Viral capsid protein (VP2)	N/A	0	N/A	N/A	N/A	N/A		Yes	Japanese isolates
Totivirus [dsRNA]											
	PMCV isolated from Salmo salar	RNA dep RNA polymerase (RdRp)	141	3	0	0	0	yes	HQ339954, NC 015639, HQ401057	Yes	
	PMCV isolated from Argentina silus	RNA dep RNA polymerase (RdRp)	141	1	1	2	3	?	JN624781	No	strain86% similar to those isolated from *Salmo salar*
Reovirus [dsRNA]											
	PRV	core shell (L1)	59	4	0	0	0-1	yes	KC795565, KC776256, KC715679, GU994013	Yes	tested samples from Chinook and Coho tissue and gblock to published sequence
Parvovirus [ssDNA (+)]										Yes	
	PSPV lineage1	replication protein (rep68)	65	1	0	0	0	yes	unpublished		All lineages match 100% so only tested 2 samples
	PSPV lineage2	replication protein (rep68)	65	1	0	0	0	yes	unpublished		
	PSPV lineage3	replication protein (rep68)	65	1	0	0	0	yes	unpublished		
Herpesvirus [dsDNA]											
	SalHV2 (OM)	capsid protein (cap6)	85	1	0	0	0	yes	X81060	Yes	*Oncorhynchus masou* herpes virus
	SalHV1 (SHV/HPV)	capsid protein (cap6)	N/A	0	N/A	N/A	N/A	?	no sequence available over this gene	No	Herpes virus salmonis (Steelhead Herpes virus)
	SalHV3 (EEDV)	capsid protein (cap6)	N/A	0	N/A	N/A	N/A	?	no sequence available over this gene	No	Epizootic epitheliotropic disease virus
Iridovirus [dsDNA]											
	VEN		100	1	0	0	0	yes	1000bp sequence from Dr. Jim Winton	Yes	

Supplemental Table 4a. Table showing bacterial assay mismatches to corresponding species and closely-related species. N refers to the # of sequences that overlap the corresponding assay region. SNP mismatches are represented by numbers in the F(Forward primer), P (TaqMan primer, and R (Reverse Primer). A mismatch at the 3' end of a primer is deemed fatal.

Bacterial Class	Microbe	Assay Gene	Species or CRS	N	F	P	R	Amplification Likely?	Comments
Proteobacteria γ	*Aeromonas salmonicida*	VapA	*Aeromonas salmonicida*	16 (20)	0	0	0	Yes	20 ae_sal sequences match 100%, but 4 missing complete R primer overlap
Proteobacteria γ		VapA	*Aeromonas salmonicida subsp. salmonicida* (typical)	5	0	0	0	Yes	
Proteobacteria γ		VapA	*Aeromonas salmonicida subsp. achromogenes* (atypical)	1	0	0	0	Yes	
Proteobacteria γ		VapA	*Aeromonas salmonicida subsp. masoucida* (atypical)	1	0	0	0	Yes	
Proteobacteria γ		VapA	*Aeromonas salmonicida subsp. smithia* (atypical)	1	0	0	0	Yes	
Proteobacteria γ		VapA	*Aeromonas hydrophila*	0	N/A	N/A	N/A	N/A	no alignment of vapA gene sequences in both species
Proteobacteria γ	*Aeromonas hydrophila*	aha1	Aeromonas hydrophila AY165026 gblock	1	0	0	0	Yes	
Proteobacteria γ		aha1	Aeromonas hydrophila EF189590	1	1	1	0	?	
Proteobacteria γ		aha1	Aeromonas hydrophila EU518465	1	6	1	0	No?	
Proteobacteria γ		aha1	Ahyd contig1 a	4	8	1	0	No	
Proteobacteria γ		aha1	Ahyd contig1 b	4	9	0-1	0-2	No	
Proteobacteria γ		aha1	Ahyd contig2	2	7	3	2	No	
Proteobacteria γ		aha1	Ahyd genome condensed overall	2	10	1	0-2	No	
Proteobacteria γ		aha1	*Aeromonas salmonicida*	2	11-13	8-12	5-11	No	
Proteobacteria γ	*Piscirickettsia salmonis*	23S	*Piscirickettsia salmonis*	6	0-1	0-1	0-1	Yes	
Proteobacteria γ		23S	*Cycloclasticus sp.*	4	N/A	x	x	No	
Proteobacteria γ		23S	*Thiomicrospira crunogena*	2	3	8	9-fatal	No	
Proteobacteria γ		23S	*Thioalkalimicrobium cyclicum*	1	4	8	6-fatal	No	
Proteobacteria γ		23S	*Thiomicrospira arctica*	6	4	10	5	No	
Proteobacteria γ		23S	*Methylophaga lonarensis*	2	x	x	x	No	
Proteobacteria γ		23S	*Methylophaga thiooxidans*	5	N/A	N/A	N/A	N/A	no alignment of genes between sequences
Proteobacteria γ		23S	*Methylophaga sp.*	5	N/A	N/A	N/A	N/A	no alignment of genes between sequences
Proteobacteria γ		23S	*Methylophaga aminisulfidivorans*	2	N/A	N/A	N/A	N/A	no alignment of genes between sequences
Proteobacteria γ		23S	*Thioalkalimicrobium aerophilum*	1	4	8	5-fatal	No	
Proteobacteria γ	*Vibrio anguillarum/Listonella anguillarum*	virA	*Vibrio anguillarum*	7	0	0	0	Yes	All full genomes/ Vang1-5 sequenced over 16S and all matched each other 100%
Proteobacteria γ	*Vibrio salmonicida*	lux2	*Aliivibrio salmonicida AF452135*	1	0	0	0	Yes	Only 1 sequence available for in silico analysis
Proteobacteria γ		lux2	*Aliivibrio logei HQ450520*	1	3	6	4	No	
Proteobacteria γ		lux2	*Aliivibrio fischeri AB107370*	1	7	11	8	No	
Proteobacteria γ	*Yersinia ruckeri*	16S	*Yersinia ruckeri*	33 (36)	0	0	0	Yes	36 Ye_ruc sequences match 100%, but 3 missing complete R primer overlap
Proteobacteria γ		16S	*Yersinia kristensenii*	2	0	1	0	?	future-should be tested with gBlocks
Proteobacteria γ		16S	*Serratia proteamaculans*	1	0	1	0	?	future-should be tested with gBlocks
Proteobacteria γ		16S	*Serratia sp.*	8	0	0-1	0	Yes	future-should be tested with gBlocks
Proteobacteria γ		16S	*Rahnella aquatilis*	2	0	0	0	?	future-should be tested with gBlocks
Proteobacteria γ		16S	*Rahnella sp.*	3	0	0-1	0	Yes	future-should be tested with gBlocks
Proteobacteria α	*Rickettsia*-like organism	16S	Uncultured Rickettsiales ID25L EU555284	1	0	0	0	Yes	
Proteobacteria α		16S	*Rickettsiales sp.* It86 AF585482	1	2	1	3	No	
Proteobacteria α		16S	*Candidatus midichloria*	40 (42)	2 or 3	1 or 2	0-2	No	
Proteobacteria α		16S	Endosymbiont of *Acanthamoeba sp.* AF069962	1	3	1	4	No	
Proteobacteria α		16S	*Candidatus Nicolleia massiliensi DQ788562*	1	2	2	1	No	
Proteobacteria β	*Candidatus Branchiomonus cysticola*	16S	*Branchiomonus cysticola*	2	0	0	0	Yes	
Proteobacteria β		16S	uncultured beta proteobacterium_JN807444	1	0	0	0	Yes	
CFB group	*Flavobacterium psychrophilum*	16S	*F. psychrophilum* 16S group1 contig	101	0	0	0	Yes	Approximately 74% of sequences available match 100%
CFB group		16S	*F. psychrophilum* 16S group1 contig	32	0	0, 1 or 5	0-2	?	Most of these samples will not amplify
CFB group		16S	*F. psychrophilum* 16S group2 contig	4	0, 2 or 8	1,7 or 8	0, 2 or 3	No	Probably none of these would amplify
CFB group		16S	*F. columnare* 16S contig1	89 (139)	1	3-6	1	No	
CFB group		16S	*F. columnare* 16S contig2	15 (19)	1	7	1	No	
CFB group		16S	*F. columnare* 16S contig3	22 (32)	1	6-7	1	No	
CFB group		16S	*F. branchiophilum* 16S	5	1	6	1	No	
CFB group		16S	*F. branchiophilum* 16S gblock	N/A	1	6	1	No	
CFB group		16S	*F. columnare* 16S gblock	N/A	1	3	1	No	
Chlamydiae / Verrucomicrobia	Gill chlamydia	16S	Gill chlamydia	2	0-3	0	0	Yes	100% match to isolate from Salmo salar/mismatch in the isolate from a worm (Xenoturbella westbladi)
Chlamydiae / Verrucomicrobia		16S	Uncultured *Chlamydiales sp.*	1	3	0	0	?	
Chlamydiae / Verrucomicrobia		16S	*Piscichlamydiales salmonis*	10	11	9-10	5-6	No	
Chlamydiae / Verrucomicrobia		16S	*Chlamydiales sp.* gp1 contig	15	7-14	6-10	3-7	No	
Chlamydiae / Verrucomicrobia		16S	Chlamydiales gp1 gblock		10	6	3	No	APC in Sch assay
Chlamydiae / Verrucomicrobia		16S	*Chlamydiales sp.* gp2 contig	2	10	3	8	No	
Chlamydiae / Verrucomicrobia		16S	Chlamydiales gp2 gblock		10	3	6	No	APC in Pch assay
Chlamydiae / Verrucomicrobia		16S	*Clavochlamydia salmonicola*	1	9	5	6	No	fatal matches in both F/R primers
Chlamydiae / Verrucomicrobia	*Piscichlamydia salmonis*	16S	*Piscichlamydia salmonis*	6	0	0	0	Yes	all 100% match
Chlamydiae / Verrucomicrobia		16S	Uncultured *Chlamydiaceae sp.*	1	0	0	0	Yes	100% match
Chlamydiae / Verrucomicrobia		16S	gill chlamydia (Sch)	2	6	8	6	No	
Chlamydiae / Verrucomicrobia		16S	*Chlamydiales sp.* gp1 contig	15	4-7	4-9	6-8	No	
Chlamydiae / Verrucomicrobia		16S	*Chlamydiales sp.* gp1 gblock		5	5	5	No	APC in Sch assay
Chlamydiae / Verrucomicrobia		16S	*Chlamydiales sp.* gp2 contig	0	N/A	N/A	N/A	N/A	
Chlamydiae / Verrucomicrobia		16S	*Chlamydiales sp.* gp2 gblock	N/A	N/A	N/A	N/A	N/A	not relevant for this assay, APC in Pch assay
Chlamydiae / Verrucomicrobia		16S	Uncultured *Chlamydiales sp.*	0	N/A	N/A	N/A	N/A	
Chlamydiae / Verrucomicrobia		16S	*Clavochlamydia salmonicola*	0	N/A	N/A	N/A	N/A	
Gram positive actinobacteria	*Renibacterium salmoninarum*	msa2	*R. salmonarum* contig1	3	0	0	0	Yes	
		msa2	*R. salmonarum* contig2	3	0	0	0	Yes	

Supplemental Table 4b. In silico analysis of species phylogenetically similar to bacterial species for which assays were developed. Sequences from these species were used in alignments to design the MGL assays and for those with homology of >90%, they were tested in the Specificity study. Color coding of cells reflects the degree of homology with the targeted parasite, with red=targeted parasite, green= >95% homology, yellow=>90% homology, blue=85% homology, and teal=80% homology.

			Proteobacteria γ						Proteobacteria α	Proteobacteria β	CFB group	Chlamydiae/ Verrucomicrobia group		Gram positive actinobacteria
Assay	Organism	Assay (species)	Aeromonas salmonicida	Aeromonas hydrophila	Piscirickettsia salmonis	Vibrio anguillarum / Listonella anguillarum	Vibrio salmonicida	Yersinia ruckeri	Rickettsia-like organism	Candidatus Branchiomonus cysticola	Flavobacterium psychrophilum	Gill chlamydia	Piscichlamydia salmonis	Renibacterium salmonarum
		Amplicon size	144bp	54bp	77bp	93bp	138bp	119bp	149bp	122bp	72bp	66bp	60bp	137bp
ae_sal	*Aeromonas salmonicida*	Proteobacteria γ	12	2										
ae_sal	*Aeromonas salmonicida subsp. salmonicida*	Proteobacteria γ	4											
ae_sal	*Aeromonas salmonicida subsp. smithia*	Proteobacteria γ	1											
ae_sal	*Aeromonas salmonicida subsp. achromogenes*	Proteobacteria γ	1											
ae_sal	*Aeromonas salmonicida*	Proteobacteria γ	8											
ae_sal	*Aeromonas salmonicida subsp. masoucida*	Proteobacteria γ	1											
ae_hyd	*Aeromonas hydrophila* AY165026	Proteobacteria γ	0	1										
ae_hyd	*Aeromonas hydrophila* strains	Proteobacteria γ	0	13										
pisc_sal	*Piscirickettsia salmonis*	Proteobacteria γ			1									
pisc_sal	*Piscirickettsia salmonis*	Proteobacteria γ			5									
pisc_sal	*Methylophaga lonarensis*	Proteobacteria γ			2									
pisc_sal	*Cycloclasticus_sp.*	Proteobacteria γ			4									
pisc_sal	*Thiomicrospira crunogena*	Proteobacteria γ			2									
pisc_sal	*Thioalkalimicrobium cyclicum*	Proteobacteria γ			1									
pisc_sal	*Thiomicrospira arctica*	Proteobacteria γ			6									
pisc_sal	*Thioalkalimicrobium aerophilum*	Proteobacteria γ			1									
pisc_sal	*Methylophaga thiooxidans*	Proteobacteria γ			5									
pisc_sal	*Methylophaga sp.*	Proteobacteria γ			5									
pisc_sal	*Methylophaga aminisulfidivorans*	Proteobacteria γ			2									
vi_ang	*Vibrio anguillarum/Listonella anguillarum*	Proteobacteria γ				4								
vi_sal	*Alivibrio salmonicida*	Proteobacteria γ					2							
vi_sal	*Alivibrio logei*	Proteobacteria γ					1							
ye_ruc	*Yersinia ruckeri*	Proteobacteria γ						33						
ye_ruc	*Yersinia kristensenii*	Proteobacteria γ						2						
ye_ruc	*Serratia sp.*	Proteobacteria γ						8						
ye_ruc	*Rahnella sp.*	Proteobacteria γ						3						
ye_ruc	*Rahnella aquatilis*	Proteobacteria γ						2						
ye_ruc	*Serratia protemaculans*	Proteobacteria γ						1						
rlo	*Rickettsia-like organism* EU555284	Proteobacteria α							1					
rlo	*Rickettsiales sp.*	Proteobacteria α							7					
rlo	*Uncultured Rickettsiales sp.*	Proteobacteria α							1					
rlo	*Candidatus midichloria*	Proteobacteria α							37					
rlo	*Candidatus Nicolleia massiliensi*	Proteobacteria α							1					
c_b_cys	*Candidatus Branchiomonus cysticola*	Proteobacteria β								2				
c_b_cys	*Uncultured ß proteobacterium*	Proteobacteria β								1				
fl_psy	*Flavobacterium psychrophilum*	CFB group									100			
fl_psy	*Flavobacterium sp.*	CFB group									3			
fl_psy	*Uncultured flavobacterium sp.*	CFB group									10			
fl_psy	*Flavobacterium psychrophilum*	CFB group									8			
fl_psy	*Flavobacterium psychrophilum*	CFB group									26			
fl_psy	*Flavobacterium branchiophilum*	CFB group									5			
fl_psy	*Flavobacterium columnare*	CFB group									129			
fl_psy	*Flavobacterium columnare*	CFB group									1			
sch	Gill chlamydia symbiont EU326493	Chlamydiae/ Verrucomicrobia										1	1	
sch	Uncultured *Chlamydiales sp.* FJ817591	Chlamydiae/ Verrucomicrobia										1		
sch	Uncultured *Chlamydiales sp.* EU400613	Chlamydiae/ Verrucomicrobia										1		
sch	*Chlamydiales* symbiont EF177461 (*Xenoturbella westbladi*)	Chlamydiae/ Verrucomicrobia										1	1	
sch	*Clavochlamydia salmonicola*	Chlamydiae/ Verrucomicrobia										1	1	
pi_sal	*Piscichlamydia salmonis*	Chlamydiae/ Verrucomicrobia										9	6	
pi_sal	Uncultured *Chlamydiales sp.* EF153480	Chlamydiae/ Verrucomicrobia										1	1	
re_sal	*Renibacterium salmonarum*	Gram positive actinobacteria												18

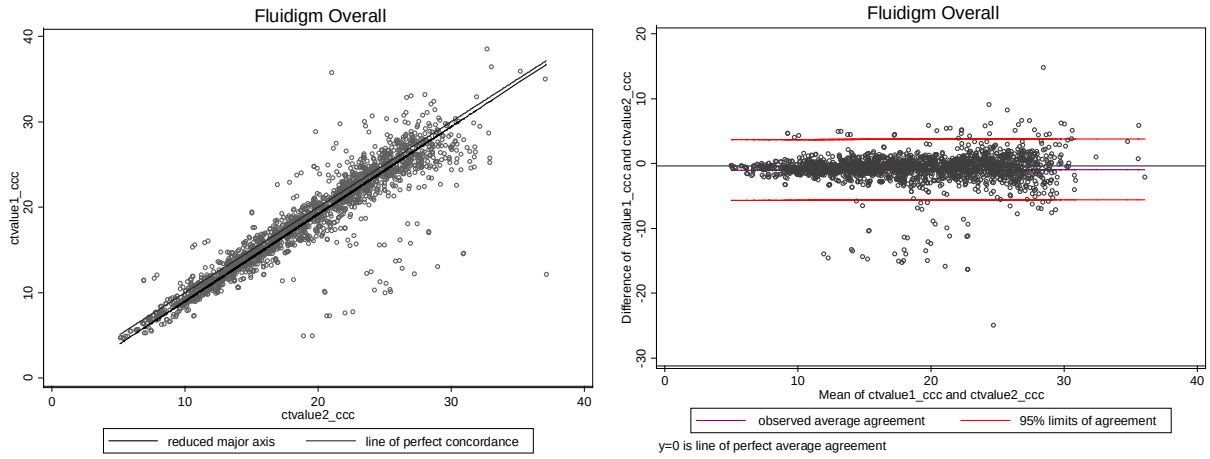
KEY:
% homology over assay region
100
>95-99
>90-95
85-90
>80-85
<80
no alignment

Supplemental Table 5. In silico analysis of species phylogenetically similar (closely-related) to parasite species for which assays were developed. Sequences from these species were used in alignments to design the MGL assays and for those with homology of >90%, they were tested in the Specificity study. Color coding of cells reflects the degree of homology with the targeted parasite, with red=targeted parasite, yellow= >95% homology, green=>90% homology, blue=85% homology, and teal=80% homology and grey indicates no alignment.

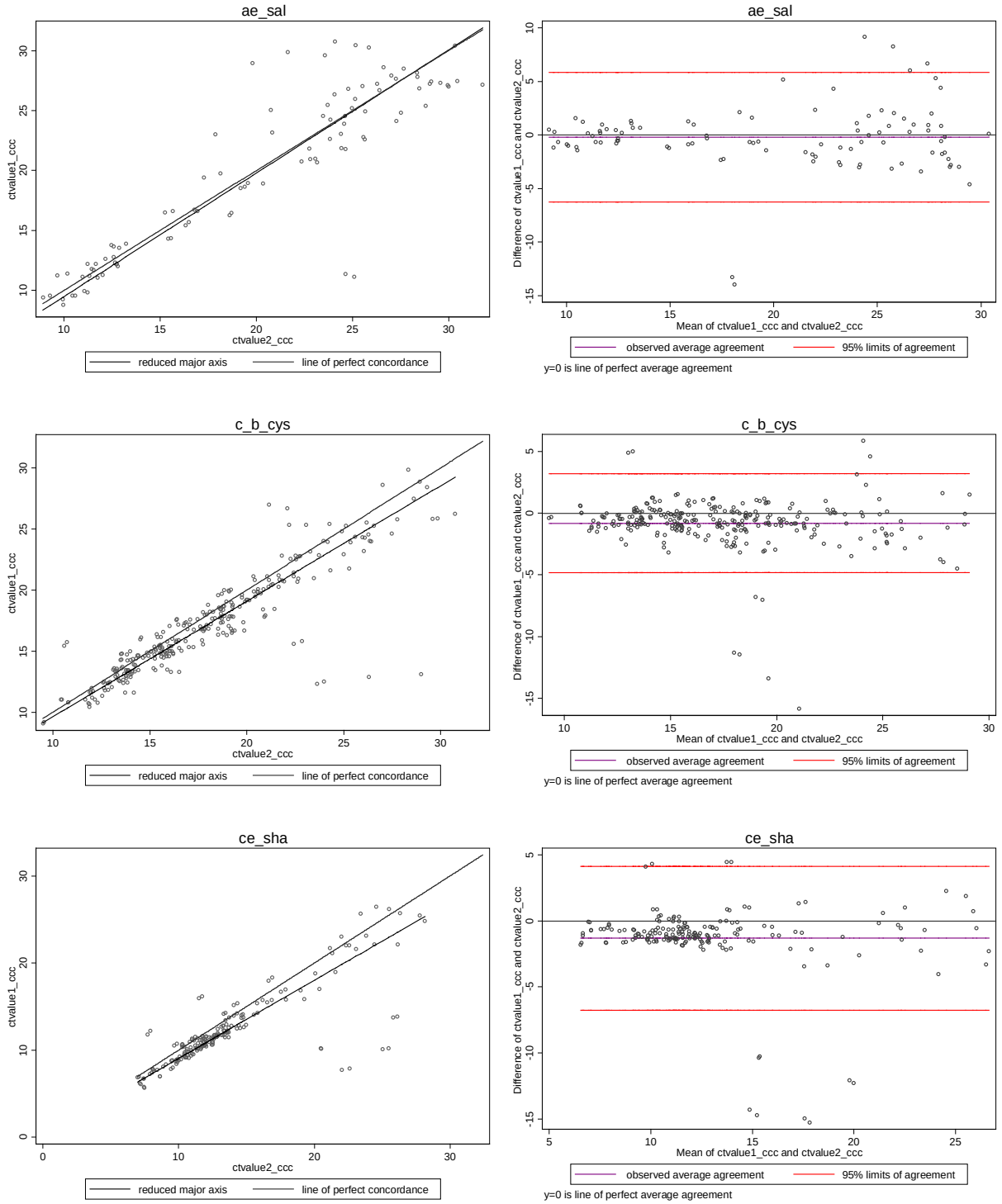
Assay	Organism	Type	Neoparamoeba perurans (Fringuelli et al. 2012)	Ichthyophthirius multifiliis (MGL)	Gyrodactylus salaris (Collins et al. 2010)	Spironucleus salmonicida (MGL)	Nanophyetus salmincola (MGL)	Facilispora margolisi (MGL)	Loma salmonae (MGL)	Nucleospora salmonis (Fritz et al 2009)	Paranucleospora theridion (Nylund et al. 2010)	Ceratomyxa shasta (Hallett et al. 2006)	Myxobolus arcticus (MGL)	Myxobolus cerebralis (Kelley et al. 2004)	Myxobolus insidiosus (MGL)	Parvicapsula kabatai (MGL)	Parvicapsula minibicornis (Hallet and Bartholomew 2009)	Parvicapsula pseudobranchicola (Borgersen et al 2011)	Tetracapsuloides bryosalmonae (Bettge et al. 2009)	Dermocystidium salmonis (MGL)	Ichthyophonus hoferi (MGL)	Sphaerothecum destruens (MGL)	Cryptobia salmositica (MGL)
		Type	Amoeba	Ciliate	Ecto-worm	Flagellate	Fluke	Microsp	Microsp	Microsp	Microsp	Myxozoan	Myxozoan	Myxozoan	Myxozoan	Myxozoan	Myxozoan	Myxozoan	Myxozoan	Protozoan	Protozoan	Protozoan	Protozoan
ne_per	*Neoparamoeba perurans*	Amoeba	1																				
ic_mul	*Colpidium camphylum* X56532.1	Ciliate		1																			
ic_mul	*Glaucoma chattoni* X56533.1	Ciliate		1																			
ic_mul	*Ichthyophthirius multifiliis*	Ciliate		1																			
ic_mul	*Lambornella* JQ723973.1	Ciliate		2																			
ic_mul	*Tetrahymena australis* M98015	Ciliate		1																			
ic_mul	*Tetrahymena thermophila* X56165.1	Ciliate		1																			
ic_mul	*Tetrahymena tropicalis* M98023	Ciliate		2																			
gy_sal	*Gyrodactylus salaris*	Ecto-worm			1																		
sp_sal	*Spironucleus barkhanaus*	Flagellate				1																	
sp_sal	*Spironucleus salmonicida*	Flagellate				1																	
x	*Spironucleus torosa*	Flagellate				1																	
x	*Deropegeus sp. "aspina"* 18 sequence strains	Fluke					18																
na_sal	*Nanophyetus salmincola*	Fluke					1																
x	*Plegioporus shawi* 7 sequence strains	Fluke					7																
fa_mar	*Facilispora margolisi*	Microsporidian						1															
fa_mar	*Kabatana sp.* FJ843105	Microsporidian						1															
lo_sal	*Loma embiotocia*	Microsporidian							1	1	1												
lo_sal	*Loma lenti*	Microsporidian							1	1	1												
lo_sal	*Loma lingcodae*	Microsporidian							1	1	1												
lo_sal	*Loma pacificodae*	Microsporidian							1	1	1												
lo_sal	*Loma salmonae*	Microsporidian							1	1	1												
lo_sal	*Loma wallae*	Microsporidian							1	1	1												
lo_sal	*Loma richardi*	Microsporidian							1	1	1												
lo_sal	*Loma branchialis*	Microsporidian							1	1	1												
x	*Microsporidia sp.* GQ868443	Microsporidian						1															
x	*Nadelspora canceri* AY958070	Microsporidian						1															
nu_sal	*Nucleospora salmonis*	Microsporidian							1	1	1												
pa_the	*Paranucleospora theridion*	Microsporidian							1	1	1												
x	*Perezia nelsoni*	Microsporidian						1															
x	*Spraguea sp.* AB623034	Microsporidian						1															
ce_sha	*Ceratomyxa shasta*	Myxozoan										1	1	1	1								
x	*Chlormyxum auratum*	Myxozoan																					
x	*Henneguya salmonicola*	Myxozoan										1	1	1	1								
my_arc	*Myxobolus arcticus*	Myxozoan										1	1	1	1								
my_cer	*Myxobolus cerebralis*	Myxozoan										1	1	1	1				1				
my_cer/my-ins/my_arc	*Myxobolus fryeri* EU346370	Myxozoan										1	1	1	1				1				
my_ins	*Myxobolus insidiosus*	Myxozoan										1	1	1	1				1				
my_cer/my-ins/my_arc	*Myxobolus kisutchi* AB469988	Myxozoan										1	1	1	1				1				
my_cer/my-ins/my_arc	*Myxobolus neurobius* AB469987	Myxozoan										2	2	2	2				1				
my_cer/my-ins/my_arc	*Myxobolus neurotropus* DQ84666.1	Myxozoan										1	1	1	1				1				
my_cer/my-ins/my_arc	*Myxobolus squamalis*	Myxozoan										2	2	2	2				1				
x	*Parvicapsula asymmetrica*	Myxozoan														1	1	1					
x	*Parvicapsula bicornis* EF429097.1	Myxozoan														1	1	1					
pa_kab	*Parvicapsula kabatai*	Myxozoan														1	1	1					
x	*Parvicapsula limandae* EF429096.1	Myxozoan														1	1	1					
pa_dup	*Parvicapsula minibicornis*	Myxozoan														1	1	1					
pa_pse	*Parvicapsula pseudobranchicola*	Myxozoan														1		1					
x	*Parvicapsula spinachiae* EF431928.1	Myxozoan														1	1	1					
x	*Parvicapsula unicornis*	Myxozoan														1	1	1					
te_bry	*Tetracapsuloides bryosalmonae*	Myxozoan												1					1				
de_sal	*Dermocystidium salmonis*	Protozoan																		1	1	1	
de_sal/ic_hof	*Dermocystidium percae*	Protozoan																		1	1	1	
de_sal	*Amoebidium parasiticum* AF2740	Protozoan																		1	1	1	
ic_hof	*Anurofeca richardsi* AF070445.1	Protozoan																		1	1	1	
ic_hof	*Diaphanoeca grandis* DQ059033.1	Protozoan																		1	1	1	
ic_hof	*Ichthyophonus hoferi*	Protozoan																		1	1	1	
ic_hof	*Ichthyophonus irregularis* AF2323	Protozoan																		1	1	1	
ic_hof	*Pseudoperkinsus tapetis* AF192386.1	Protozoan																		1	1	1	
ic_hof	*Sphaeroforma arctica* Y16260.2	Protozoan																		1	1	1	
ic_hof	*Sphaerothecum destruens*	Protozoan																		1	1	1	
cr_sal	*Cryptobia salmositica*	Protozoan																					1
cr_sal	*Trypanoplasma borrell* EF538806	Protozoan																					1

KEY:
% homology over assay region
100
>95-99
>90-95
>85-90
>80-85
<80
no alignment

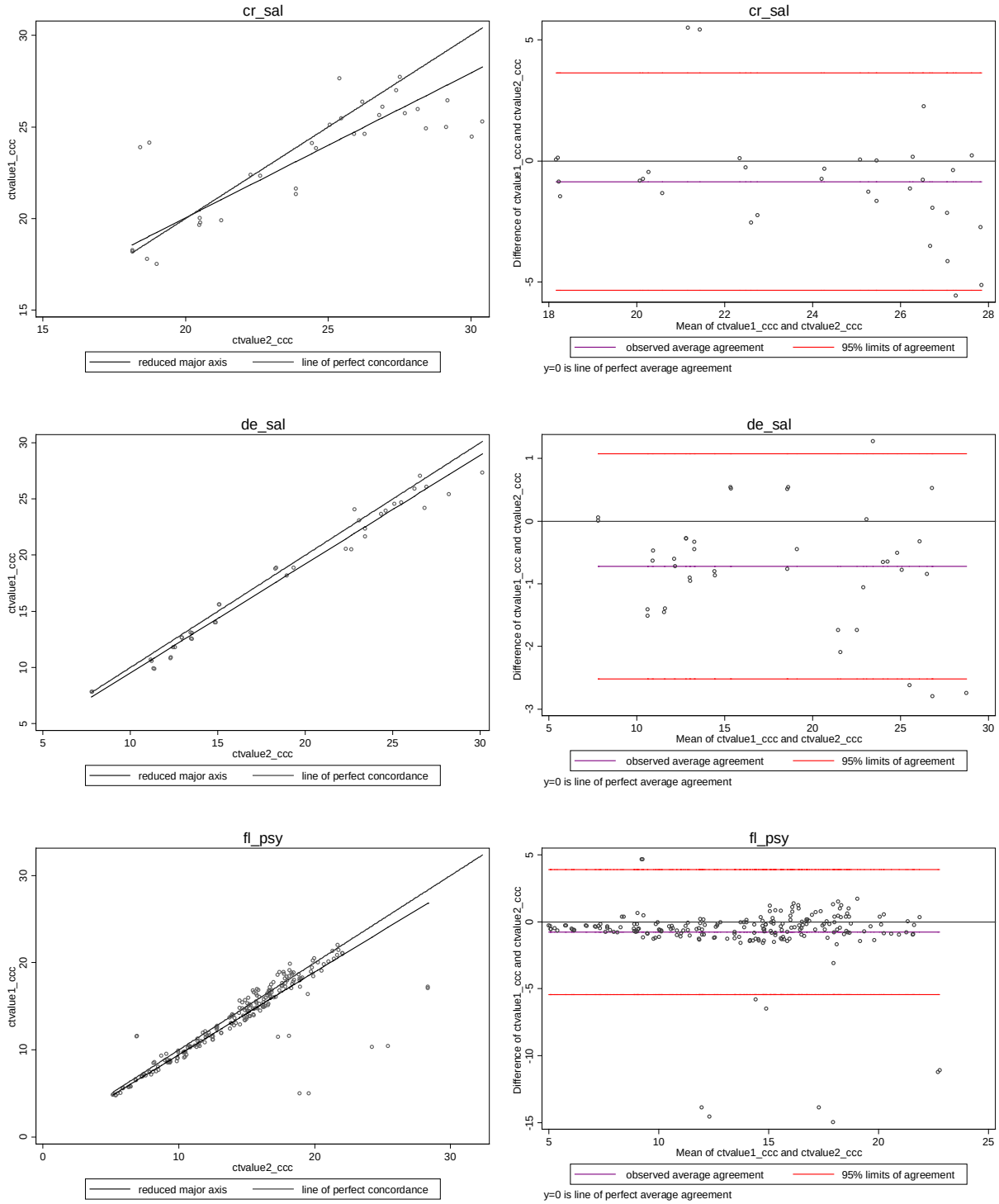
ASSAY REPEATABILITY ON THE BIOMARK



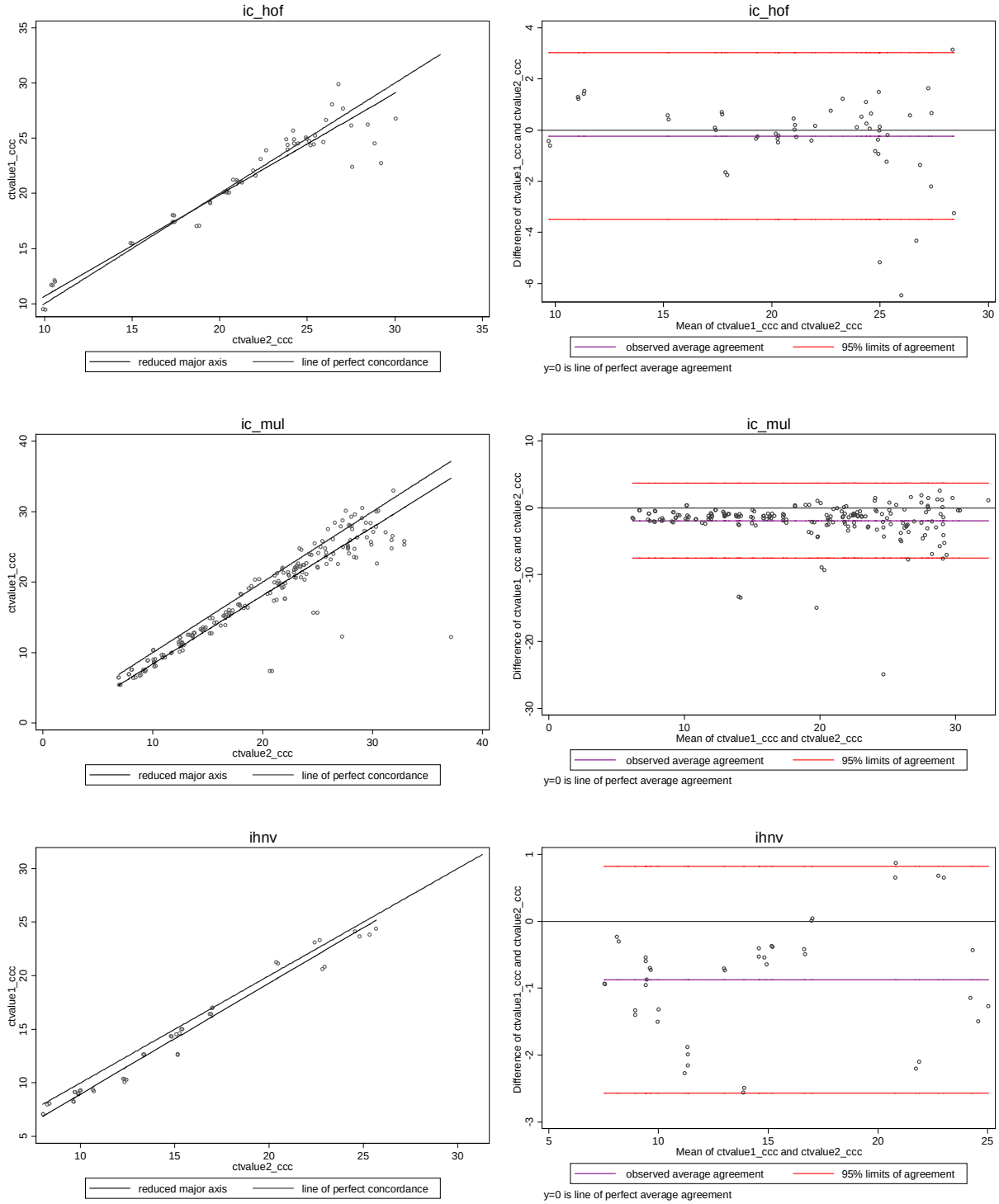
Supplemental Figure 6. Panel graph of concordance correlation coefficients (CCCs) and limit of agreement (LOA) for all assays in the repeatability study (Part 1).



Supplemental Figure 7. Panel graph of concordance correlation coefficients (CCCs) and limit of agreement (LOA) by assay (n=26) in the repeatability study (Part 1). The list of assays in these analyses include (continued over the next several pages): ae_sal, c_b_cys, ce_sha, cr_sal, de_sal, fl_psy, ic_hof, ic_mul, ihnv, ku_thy, lo_sal, my_arc, na_sal, pa_kab, pa_min, pa_pse, pa_ther, pch_sal, pmcv, prv, pspv, rlo, sch, sp_des, te_bry, and ven.



Supplemental Figure 7 (Continued)



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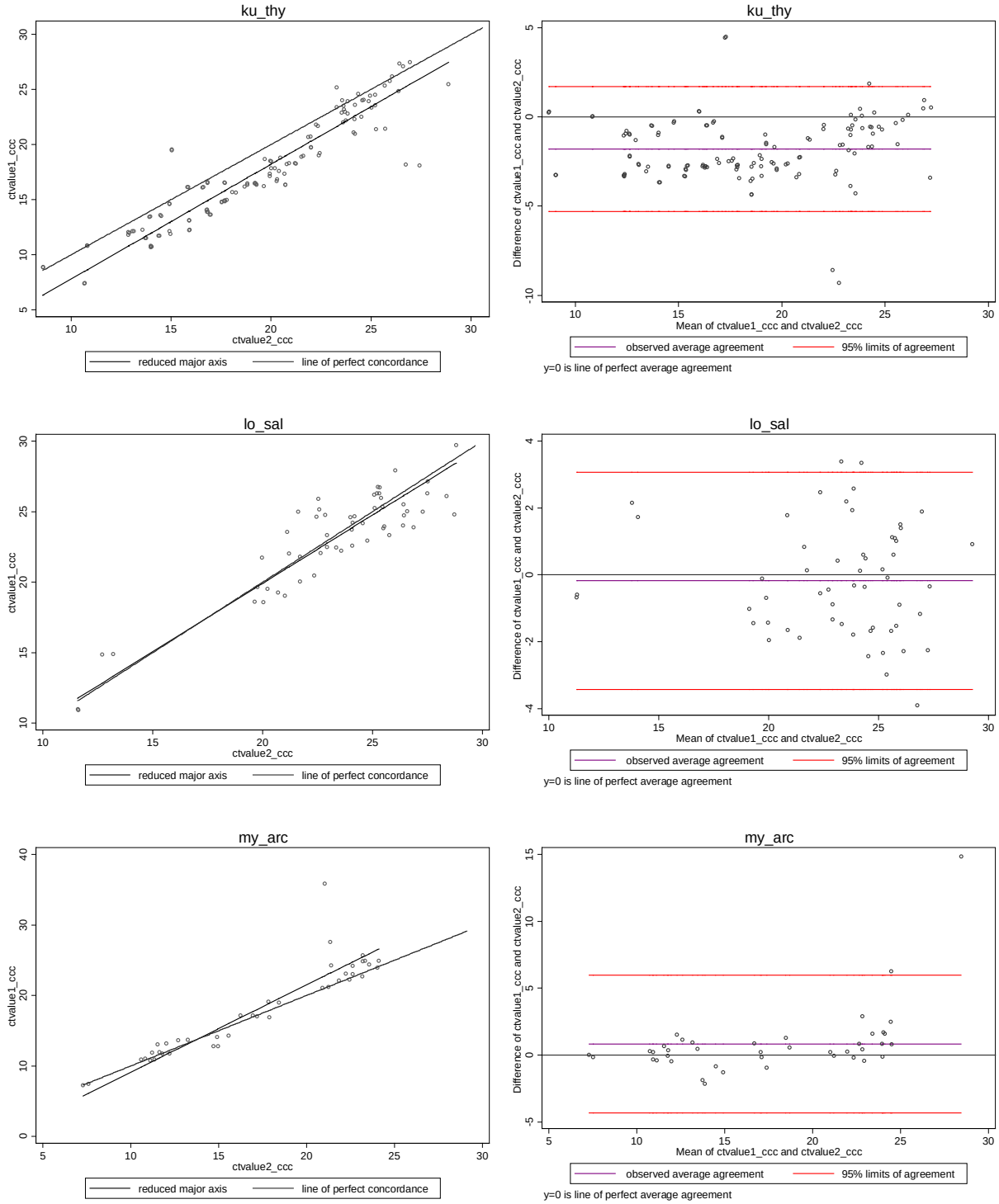

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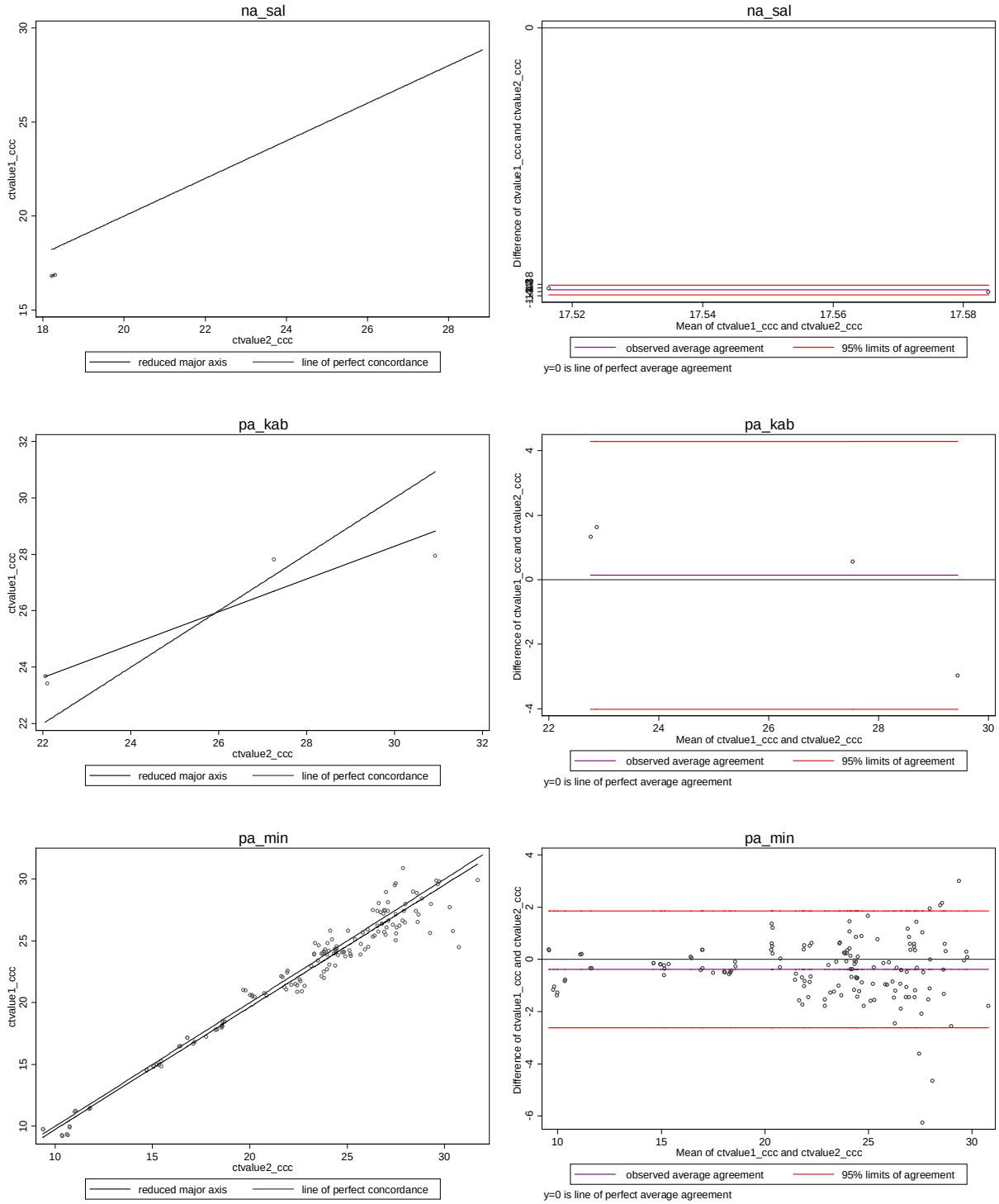

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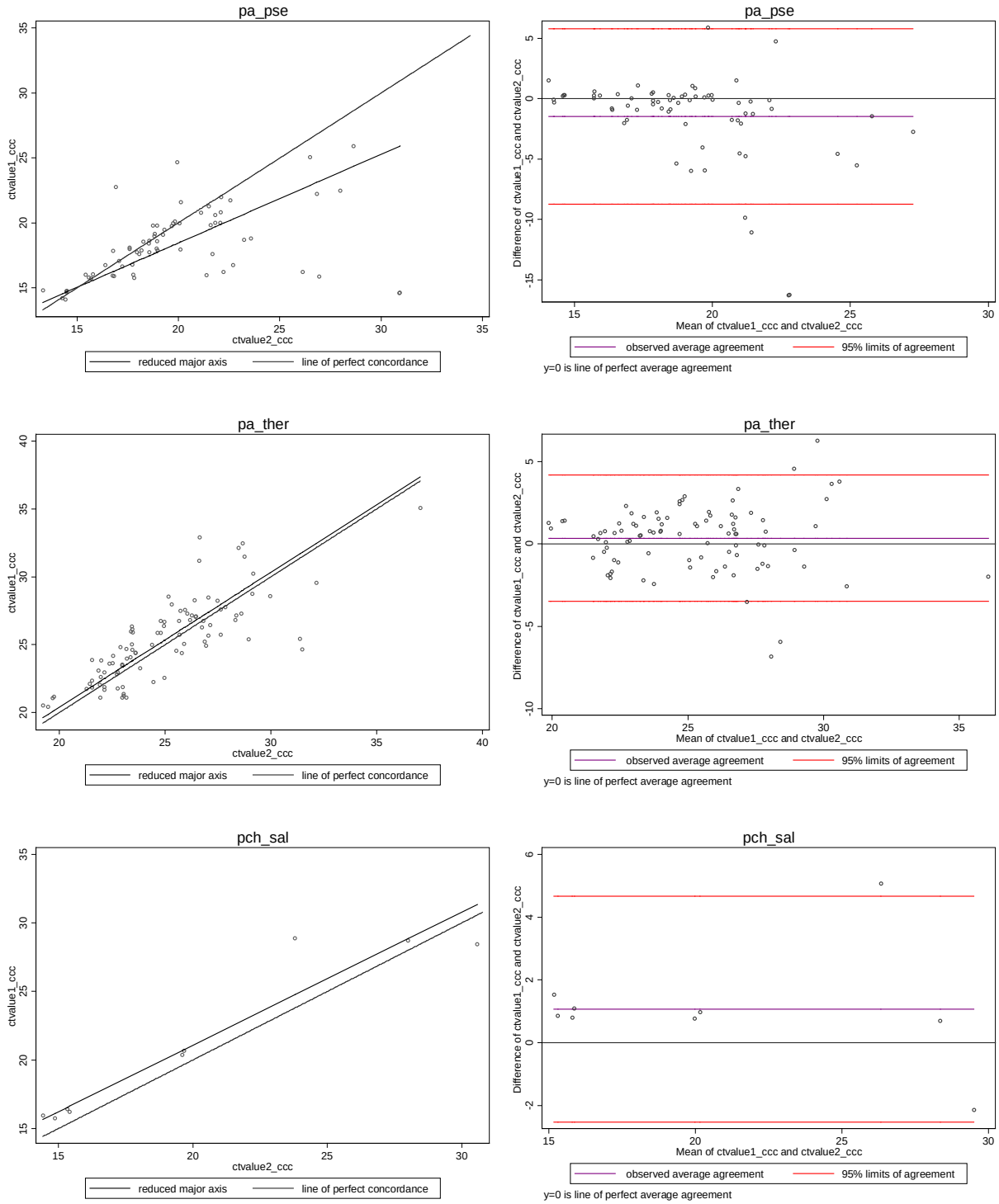

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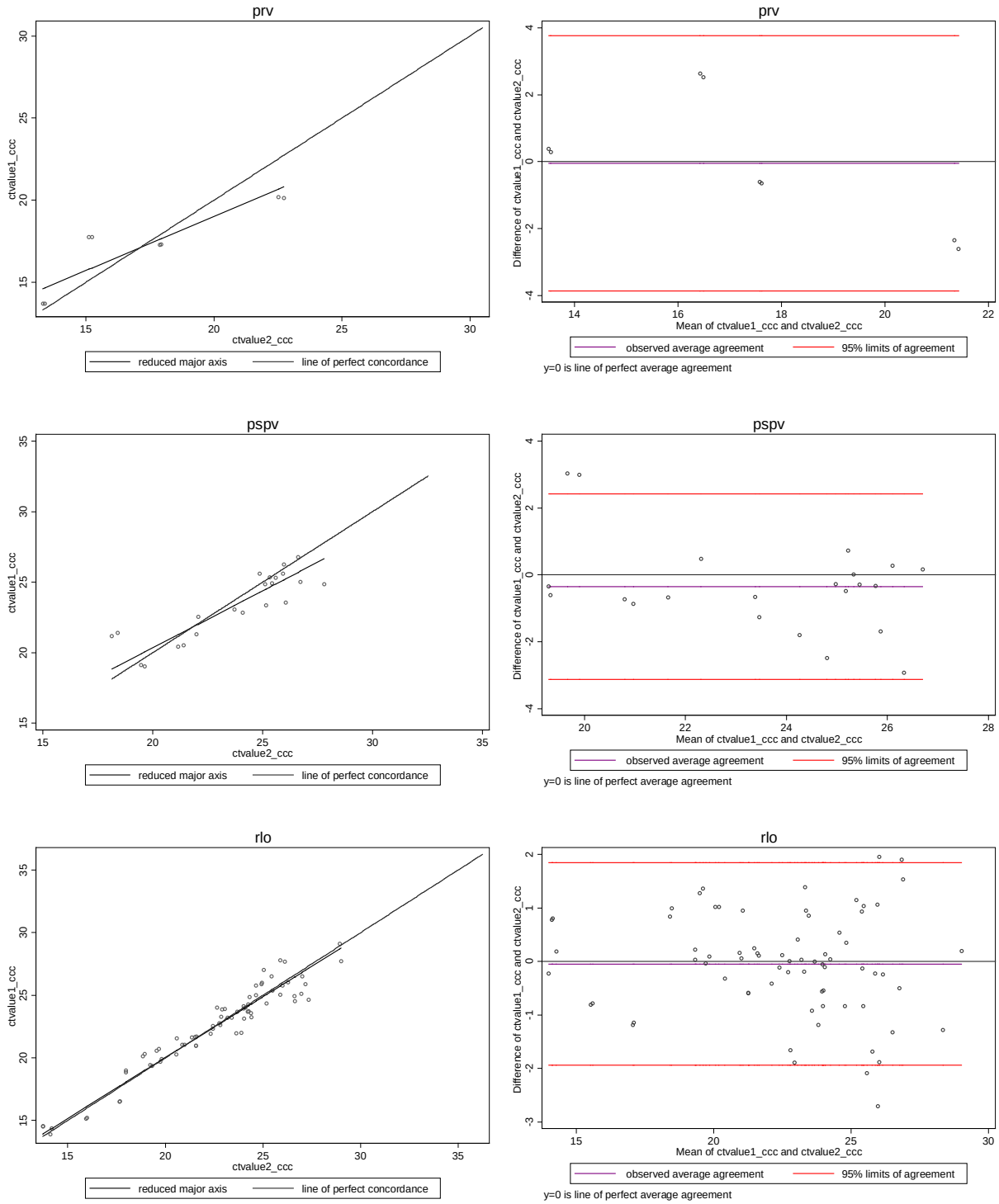

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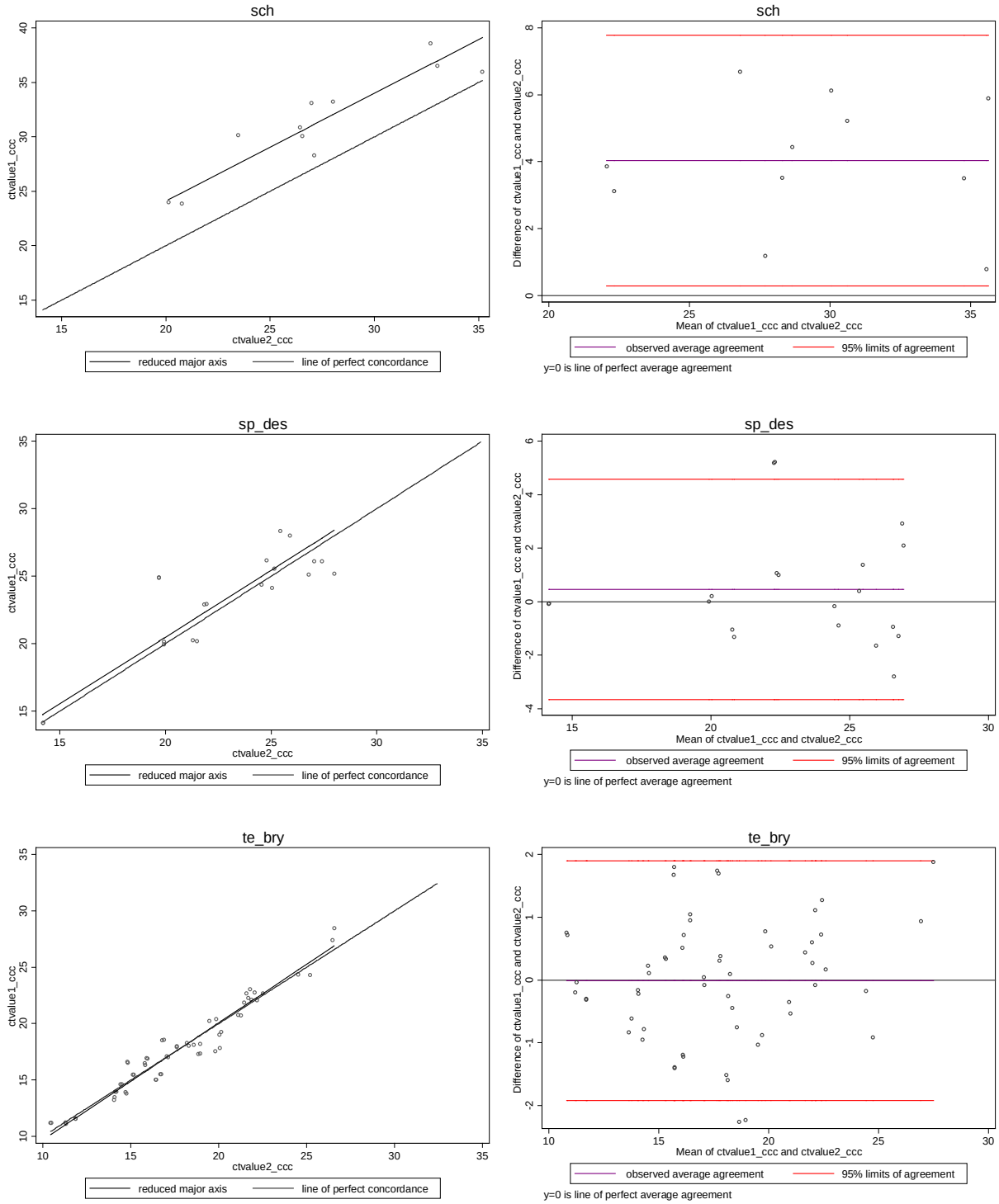

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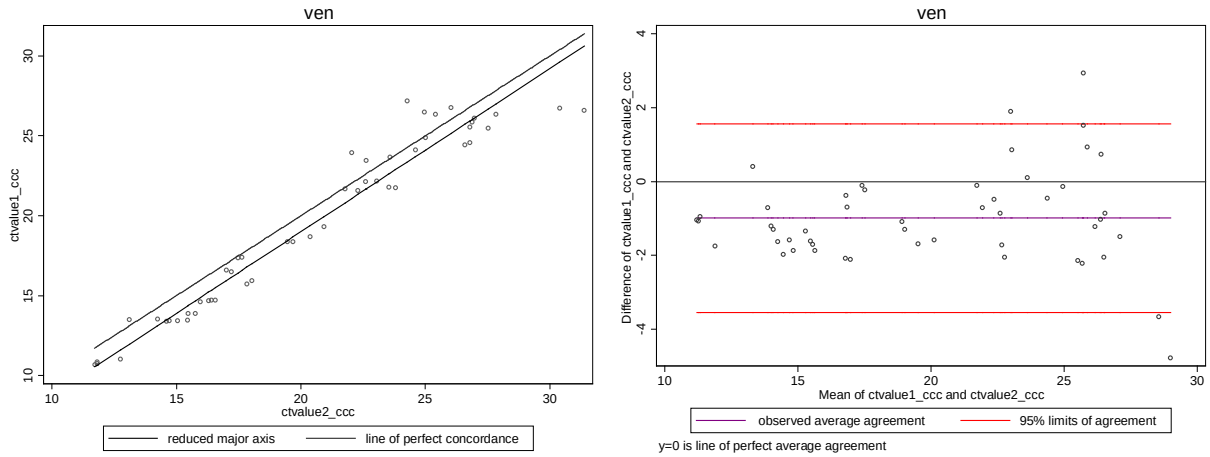

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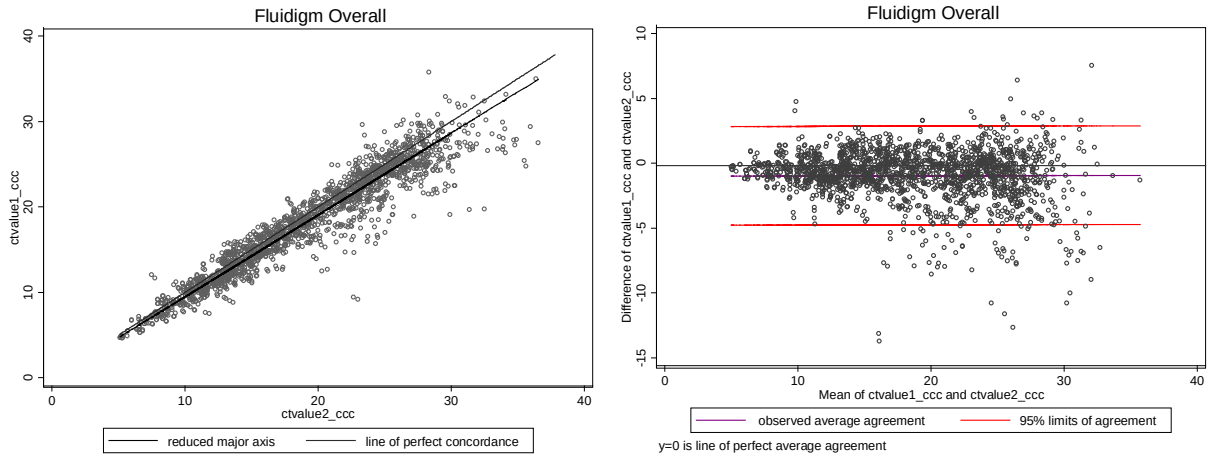
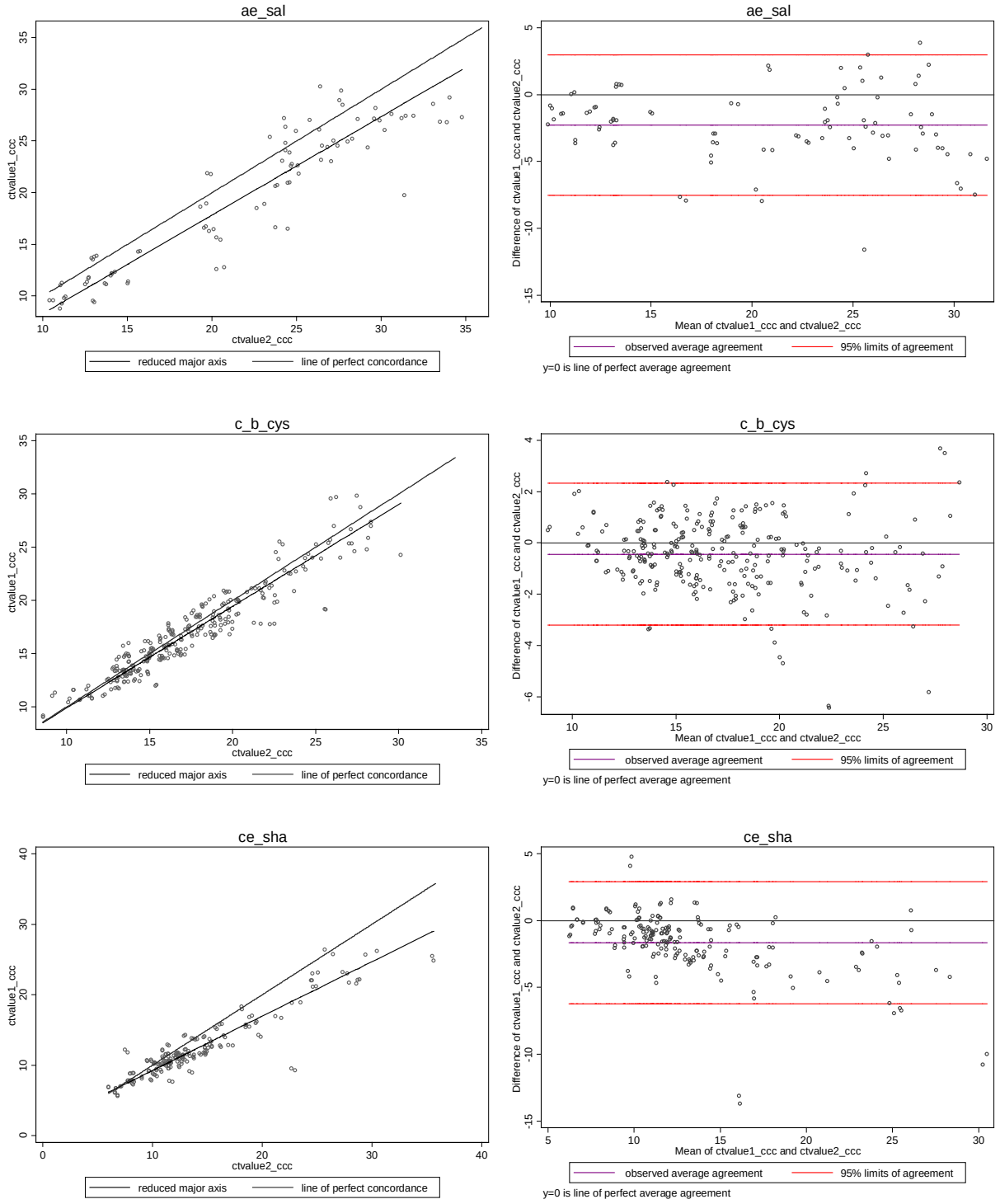
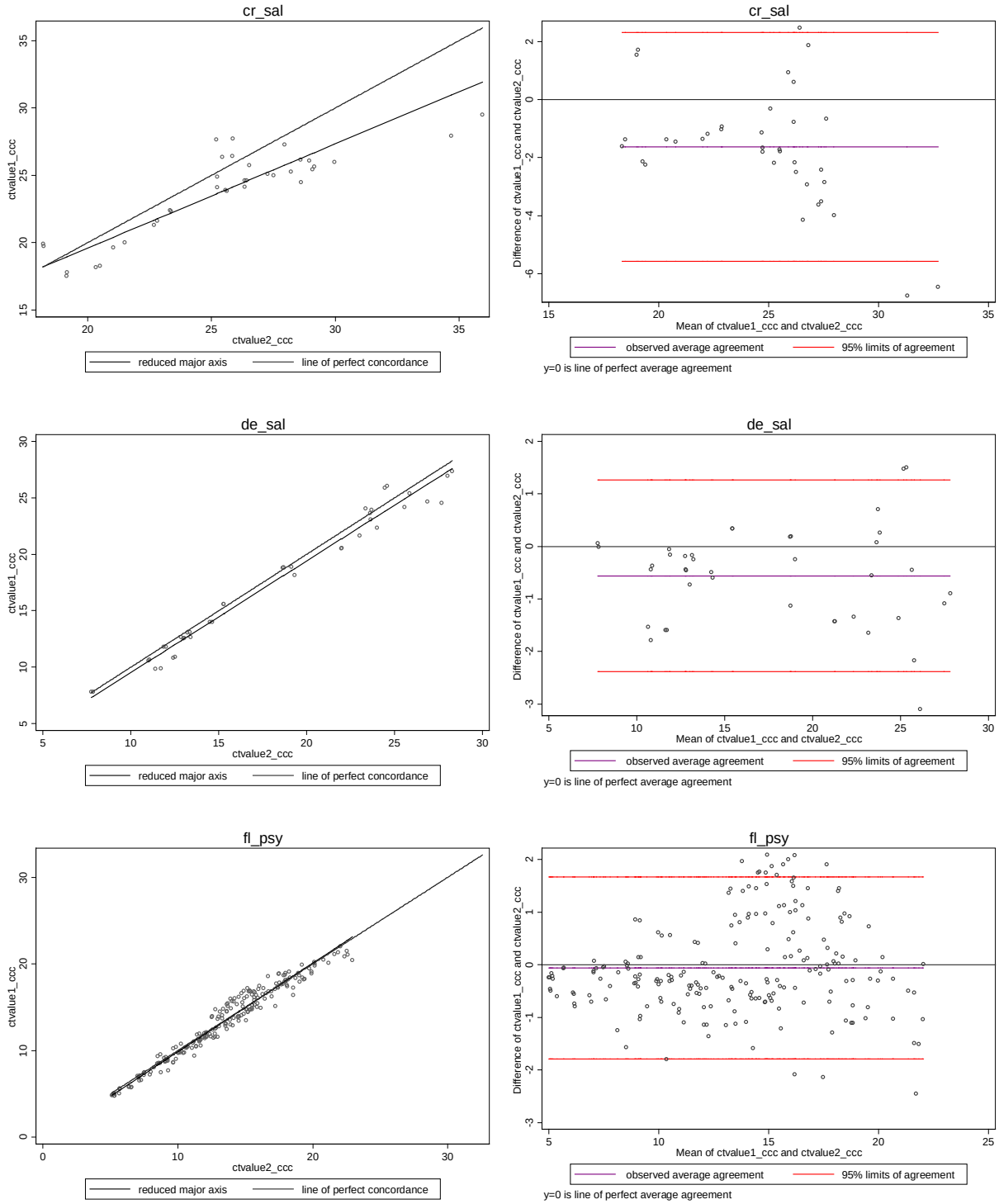


Supplemental Figure 8. Panel graph of concordance correlation coefficients (CCCs) and limit of agreement (LOA) for all assays in the repeatability study (Part 2).



Supplemental Figure 9. Panel graph of concordance correlation coefficients (CCCs) and limit of agreement (LOA) by assay (n=26) in the repeatability study (Part 1). The list of assays in these analyses include (continued over the next several pages): ae_sal, c_b_cys, ce_sha, cr_sal, de_sal, fl_psy, ic_hof, ic_mul, ihnv, ku_thy, lo_sal, my_arc, na_sal, pa_kab, pa_min, pa_pse, pa_ther, pch_sal, pmcv, prv, pspv, rlo, sch, sp_des, te_bry, and ven.



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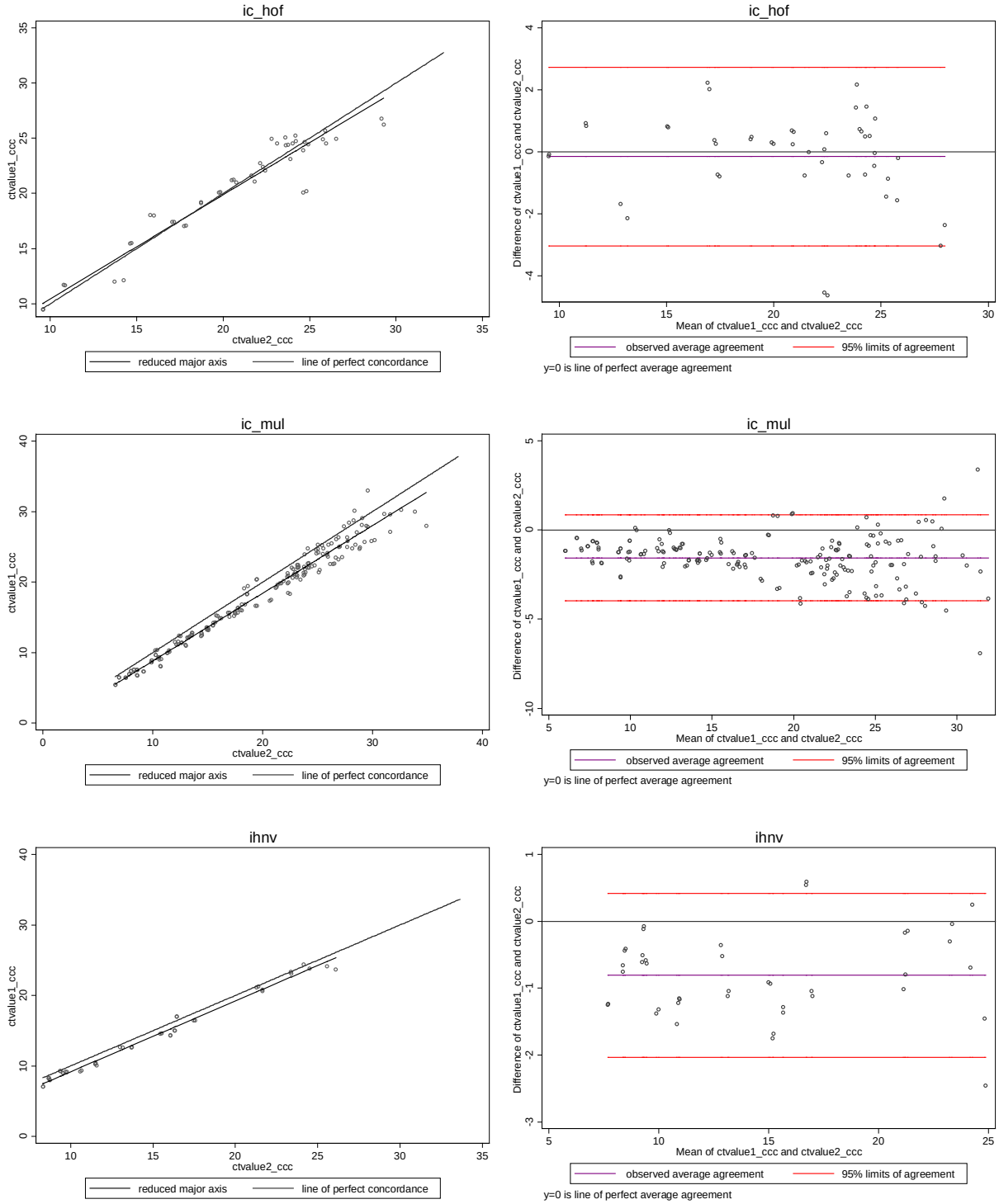

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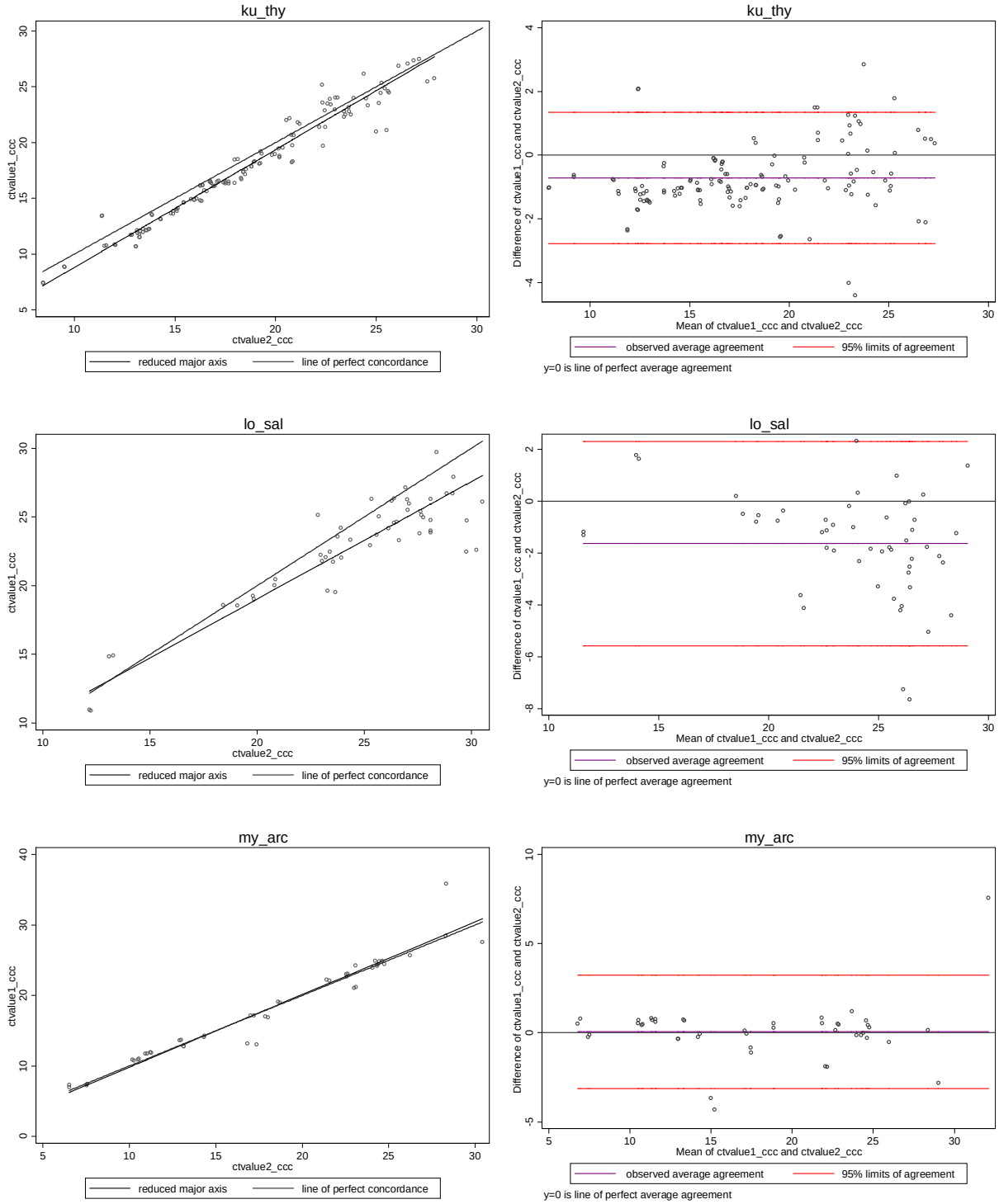

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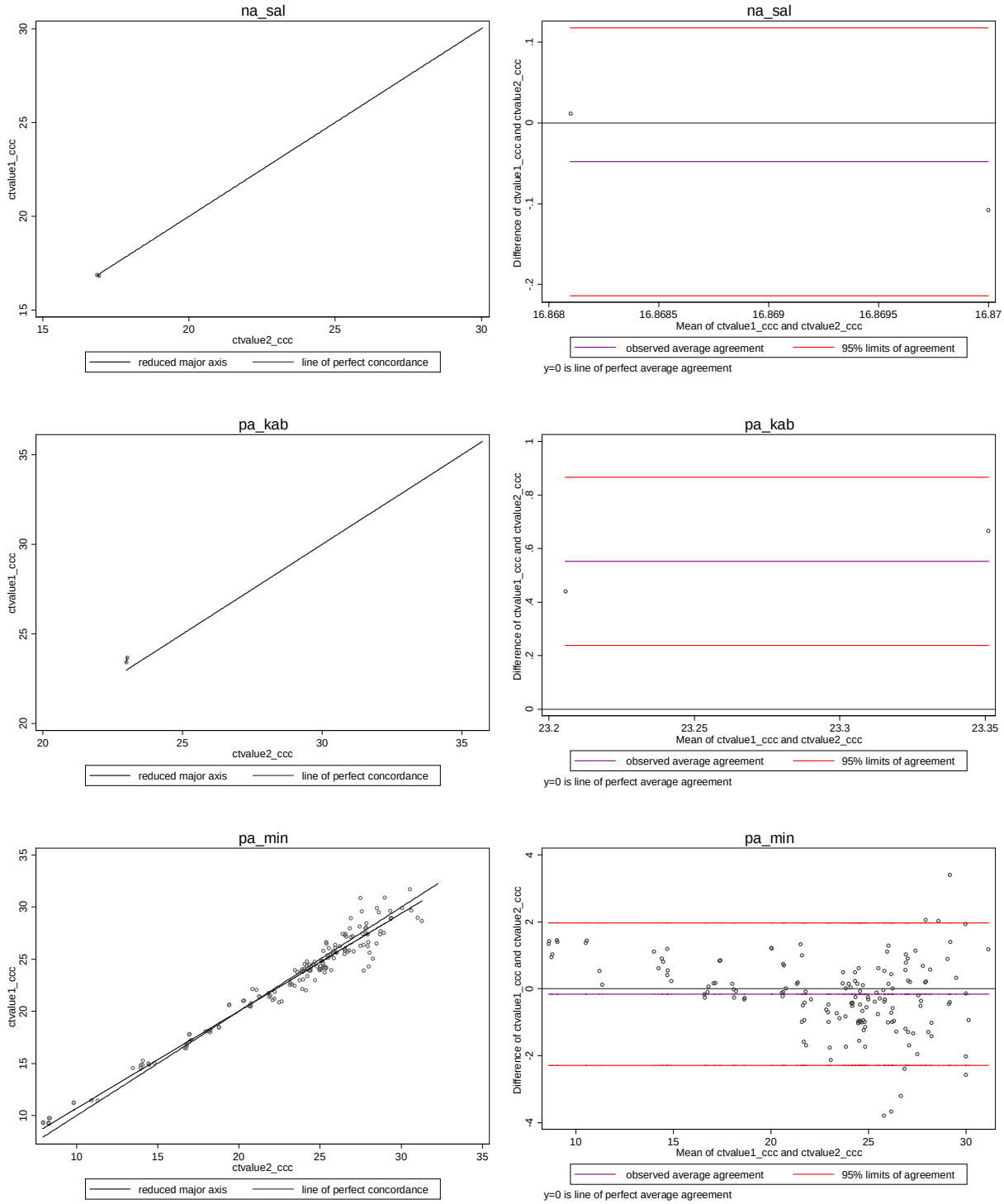

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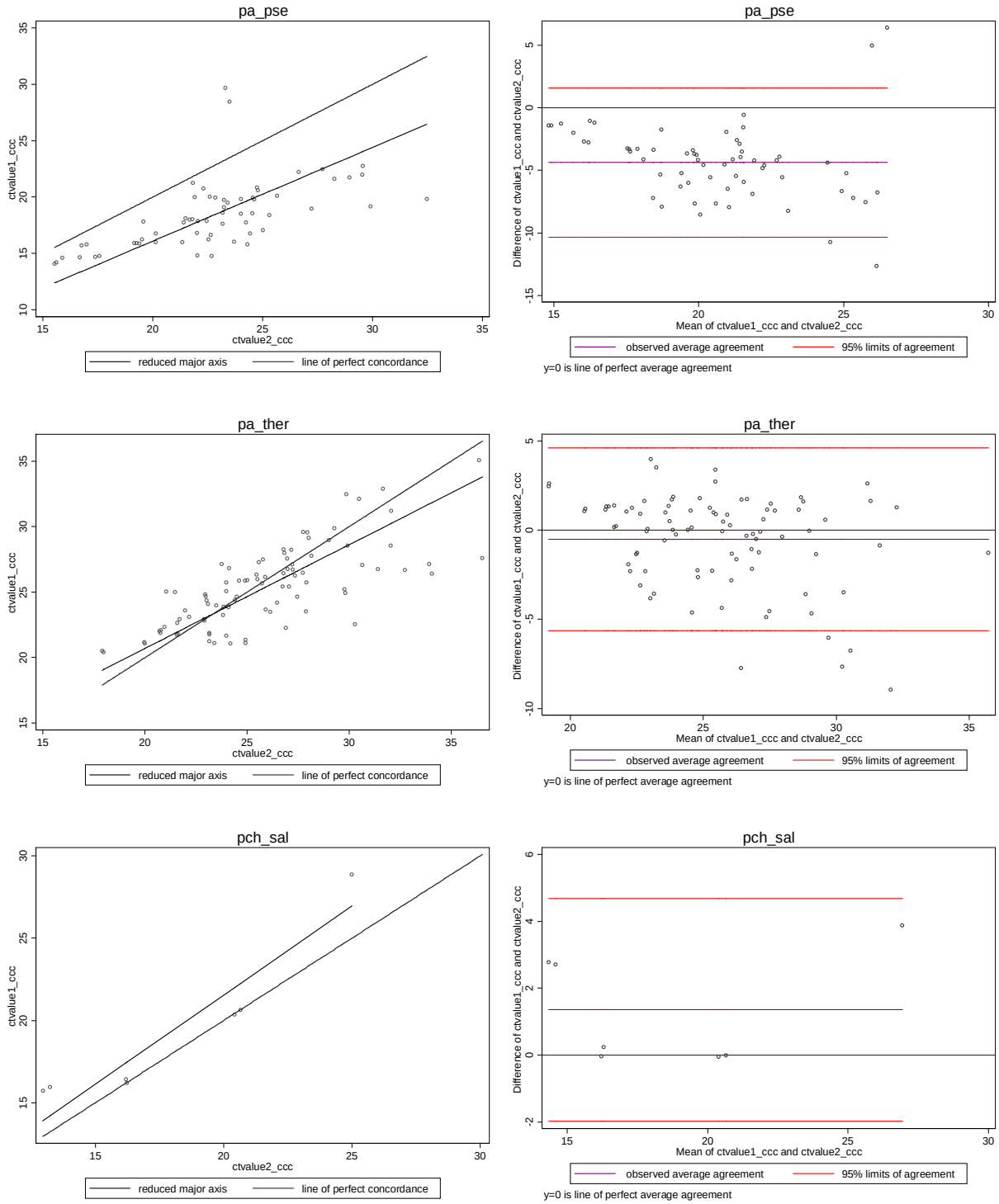

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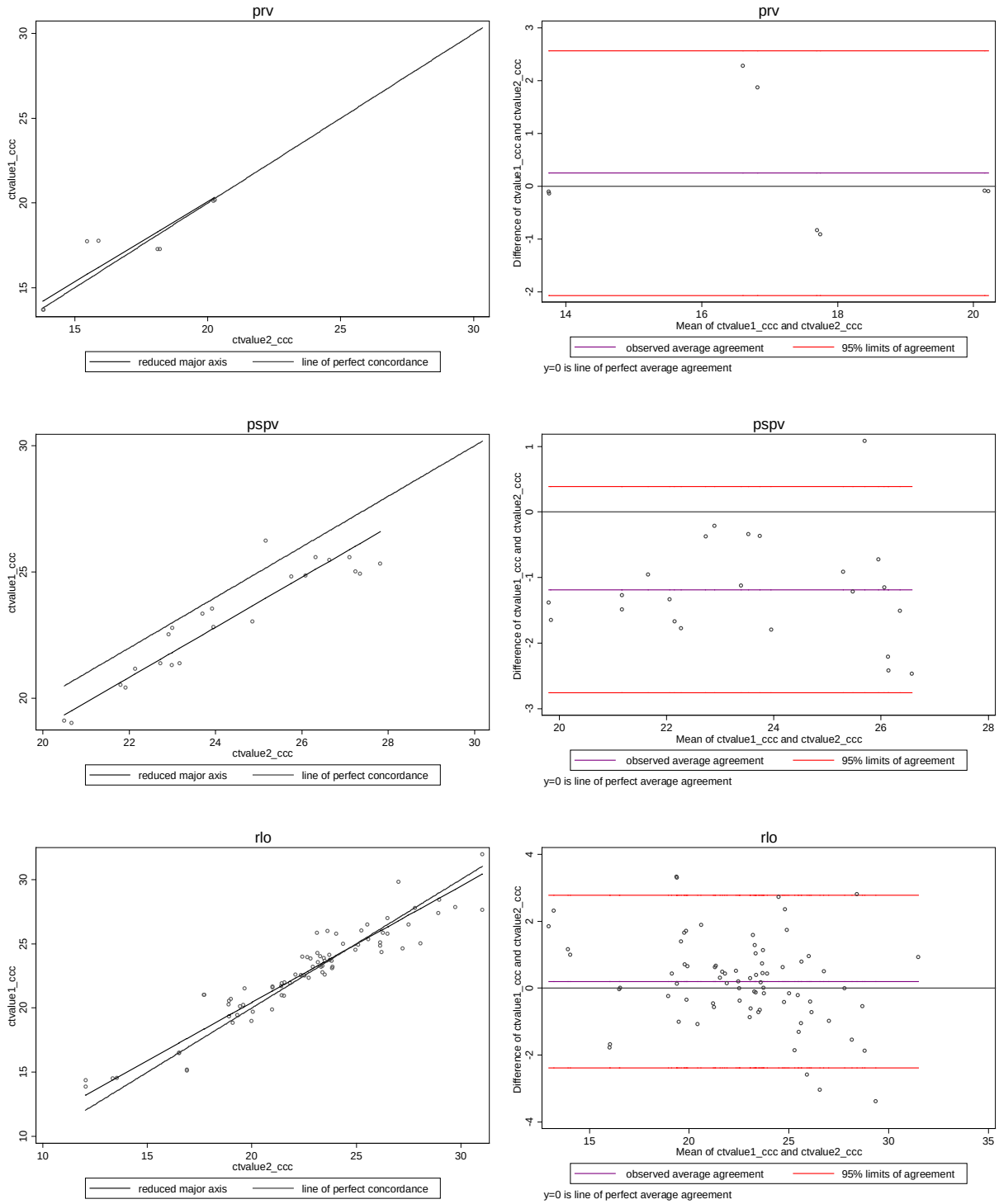

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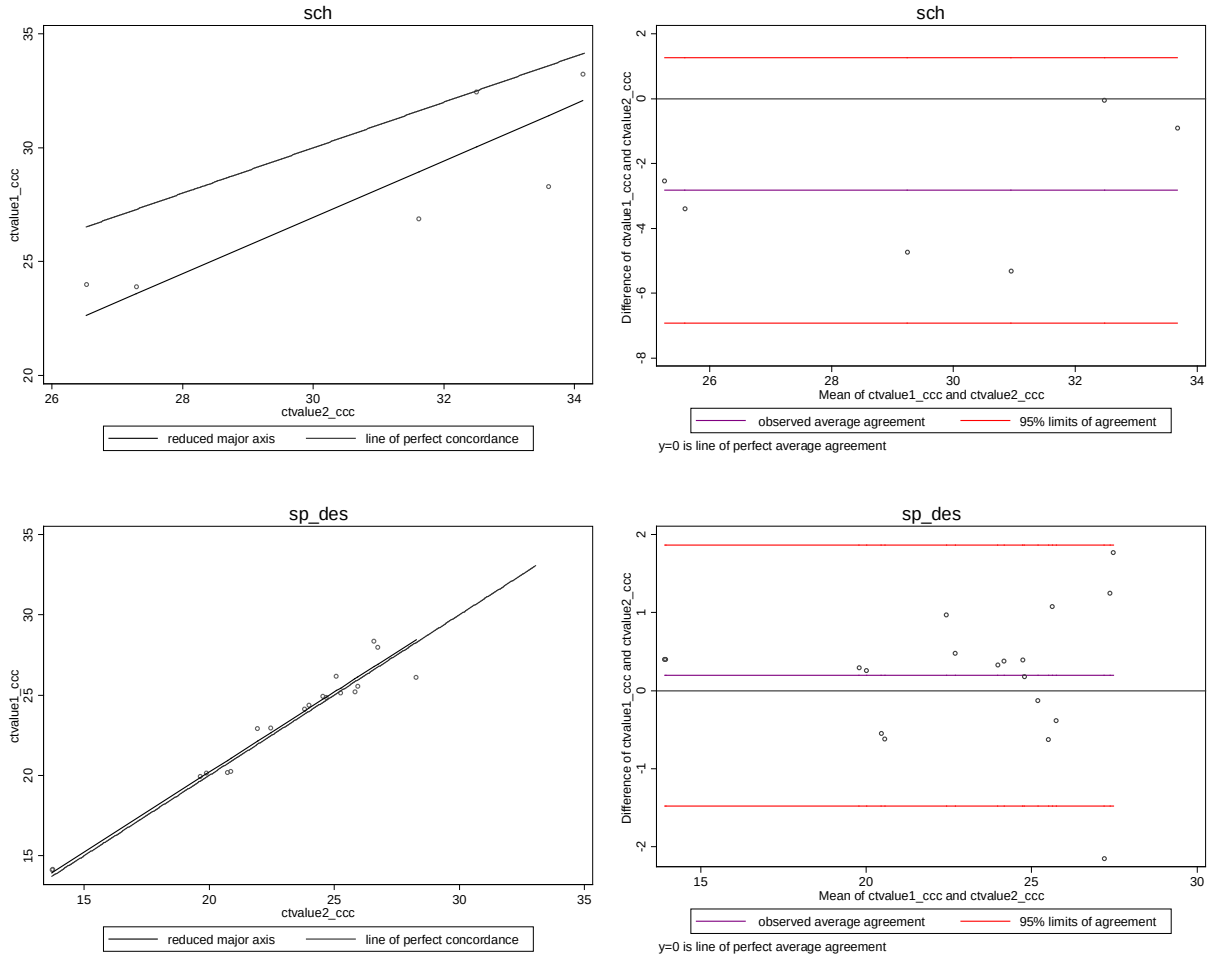

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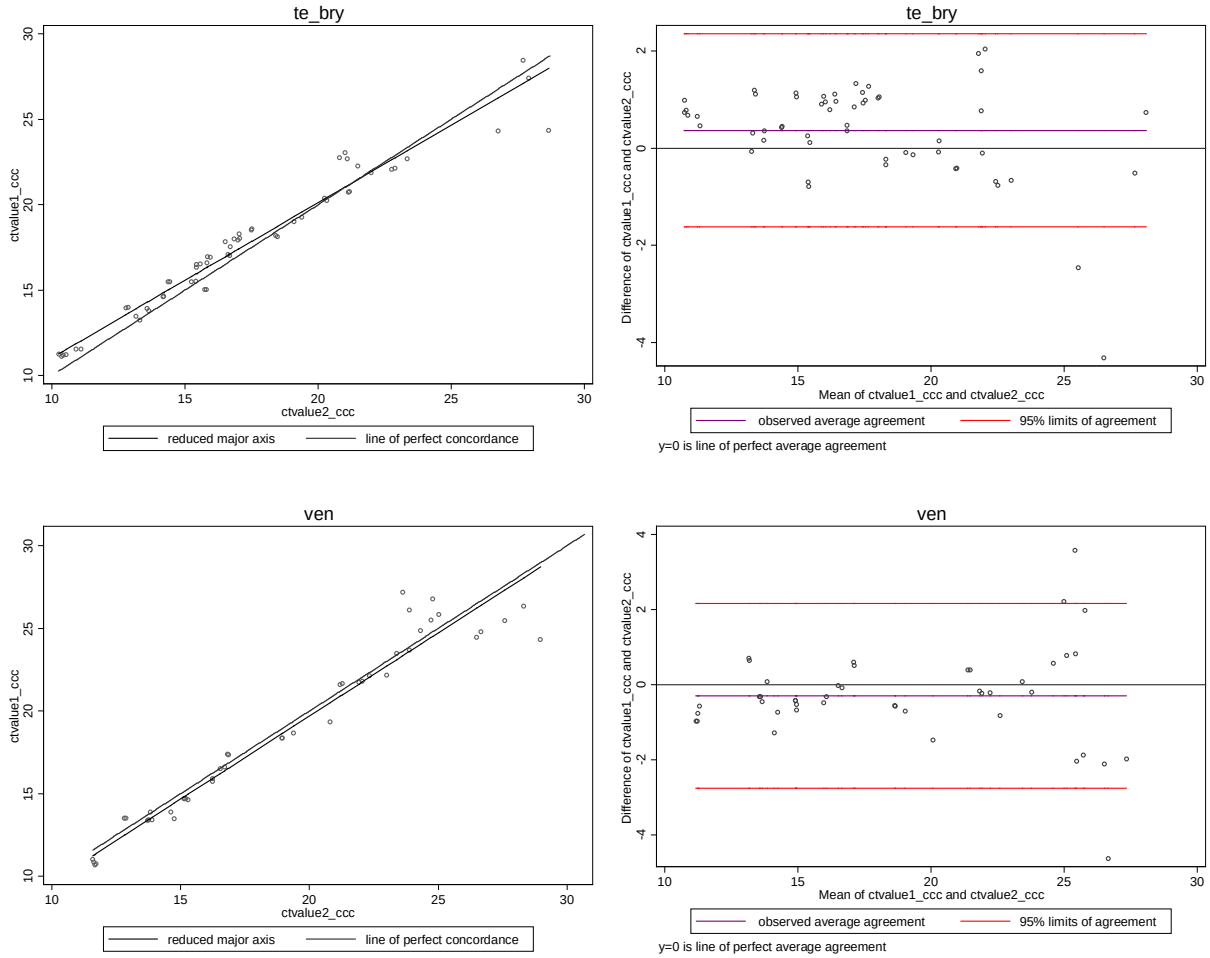

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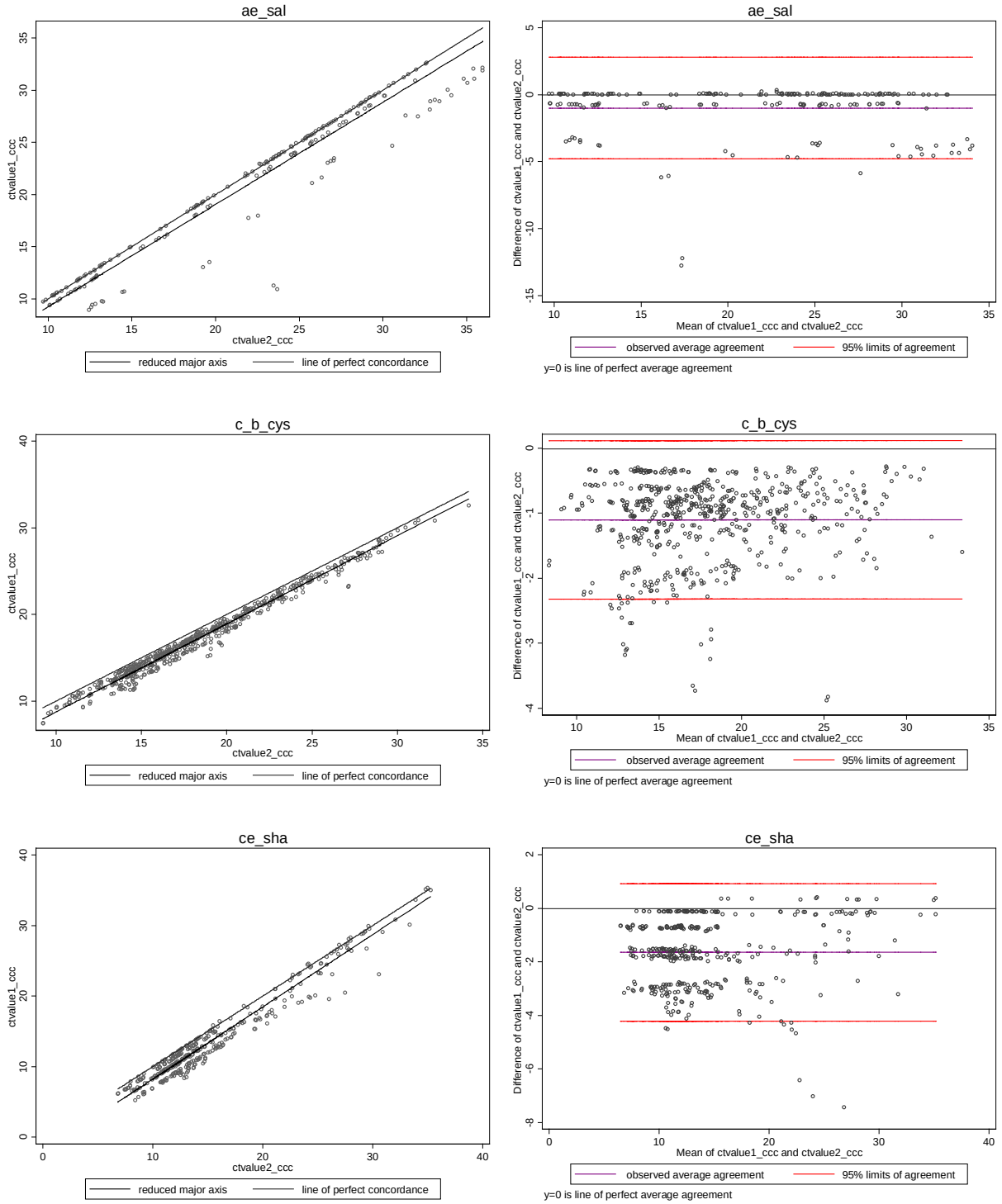
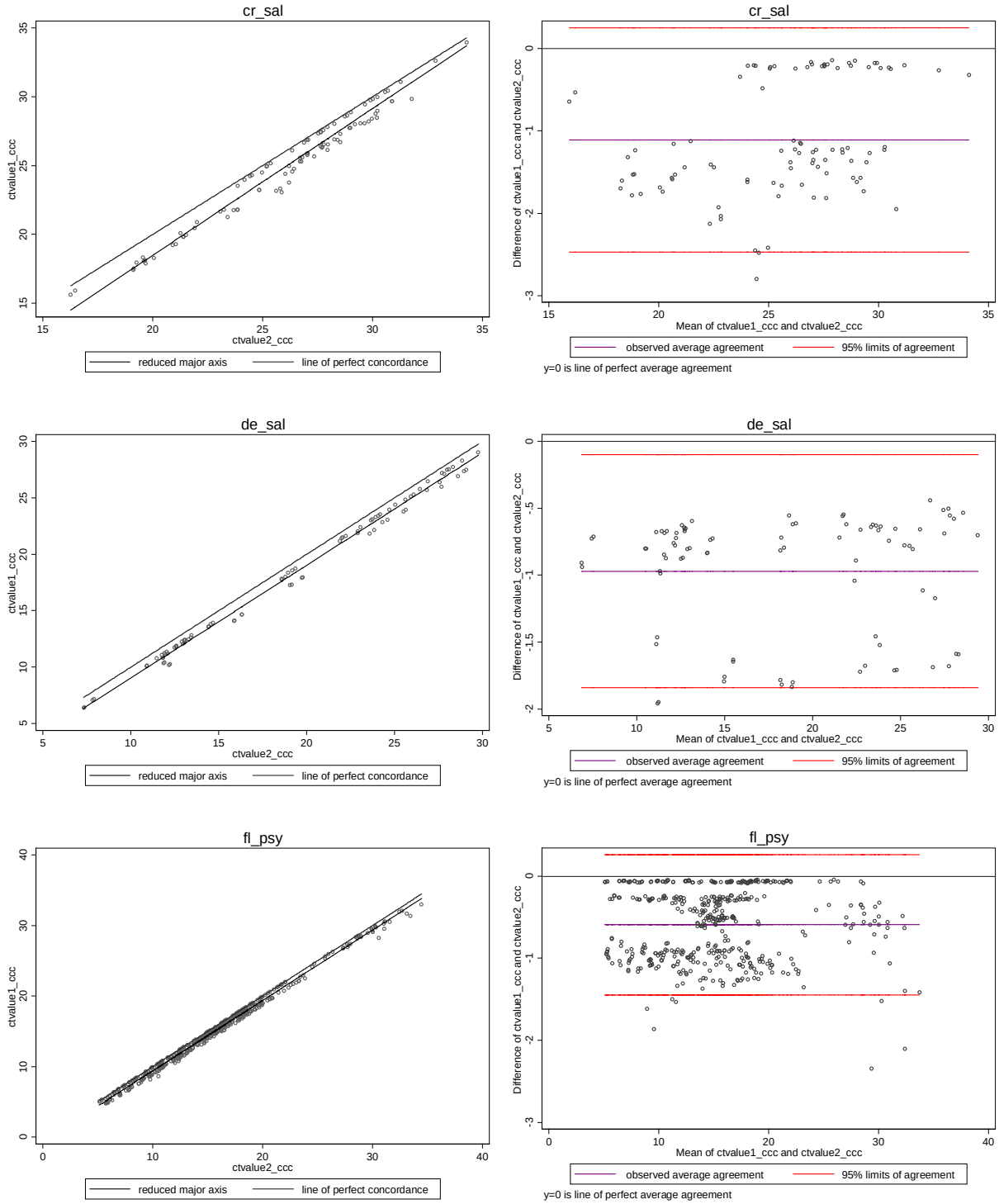
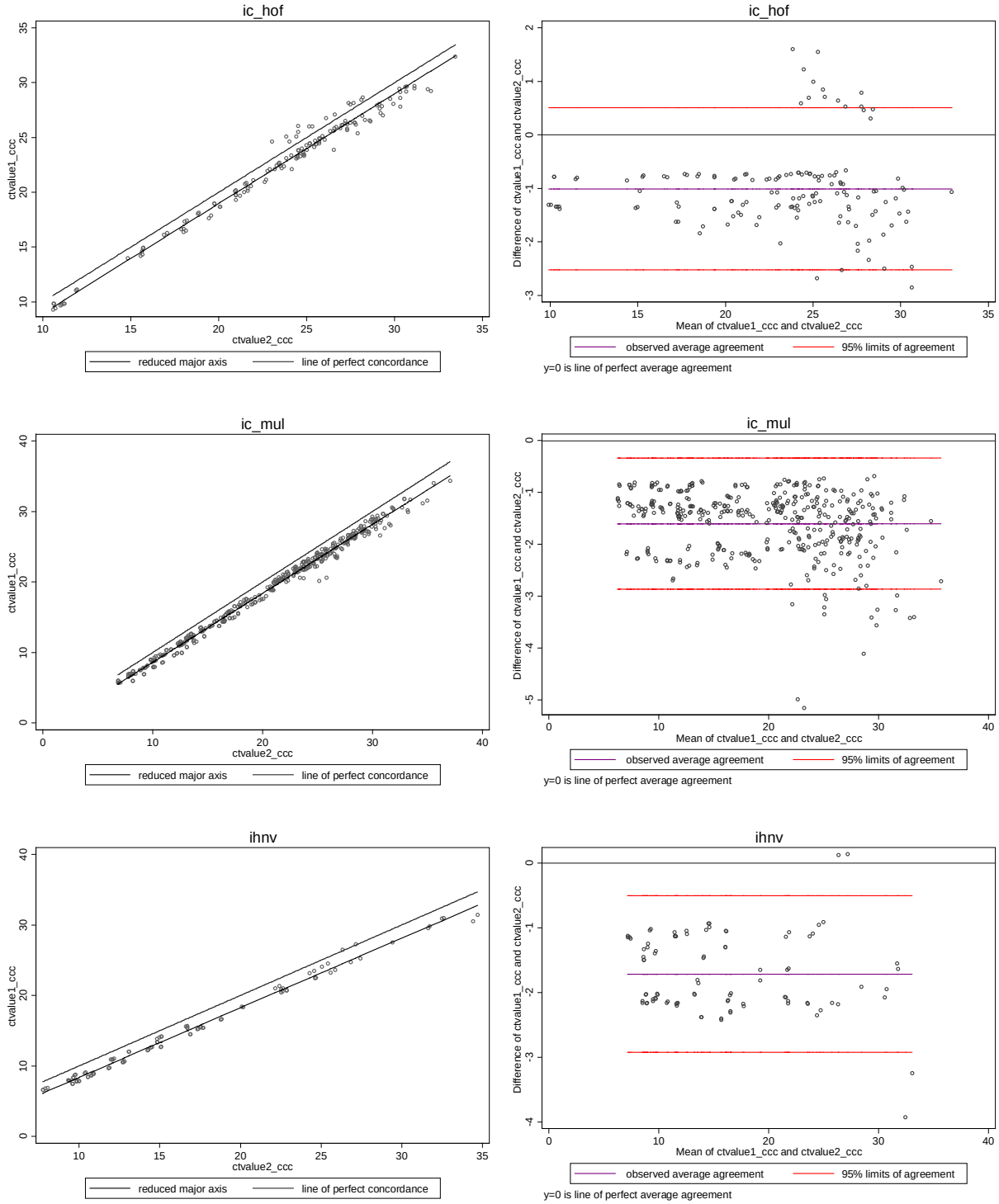


Supplemental Figure 10. Panel graph of concordance correlation coefficients (CCCs) and limit of agreement (LOA) by assay (n=26) in the repeatability study (Part 3) comparing technician 1 to technician 2. The list of assays in these analyses include (continued over the next several pages): ae_sal, c_b_cys, ce_sha, cr_sal, de_sal, fl_psy, ic_hof, ic_mul, ihnv, ku_thy, lo_sal, my_arc, na_sal, pa_kab, pa_min, pa_pse, pa_ther, pch_sal, pmcv, prv, pspv, rlo, sch, sp_des, te_bry, and ven.



Supplemental Figure 10 (Continued)



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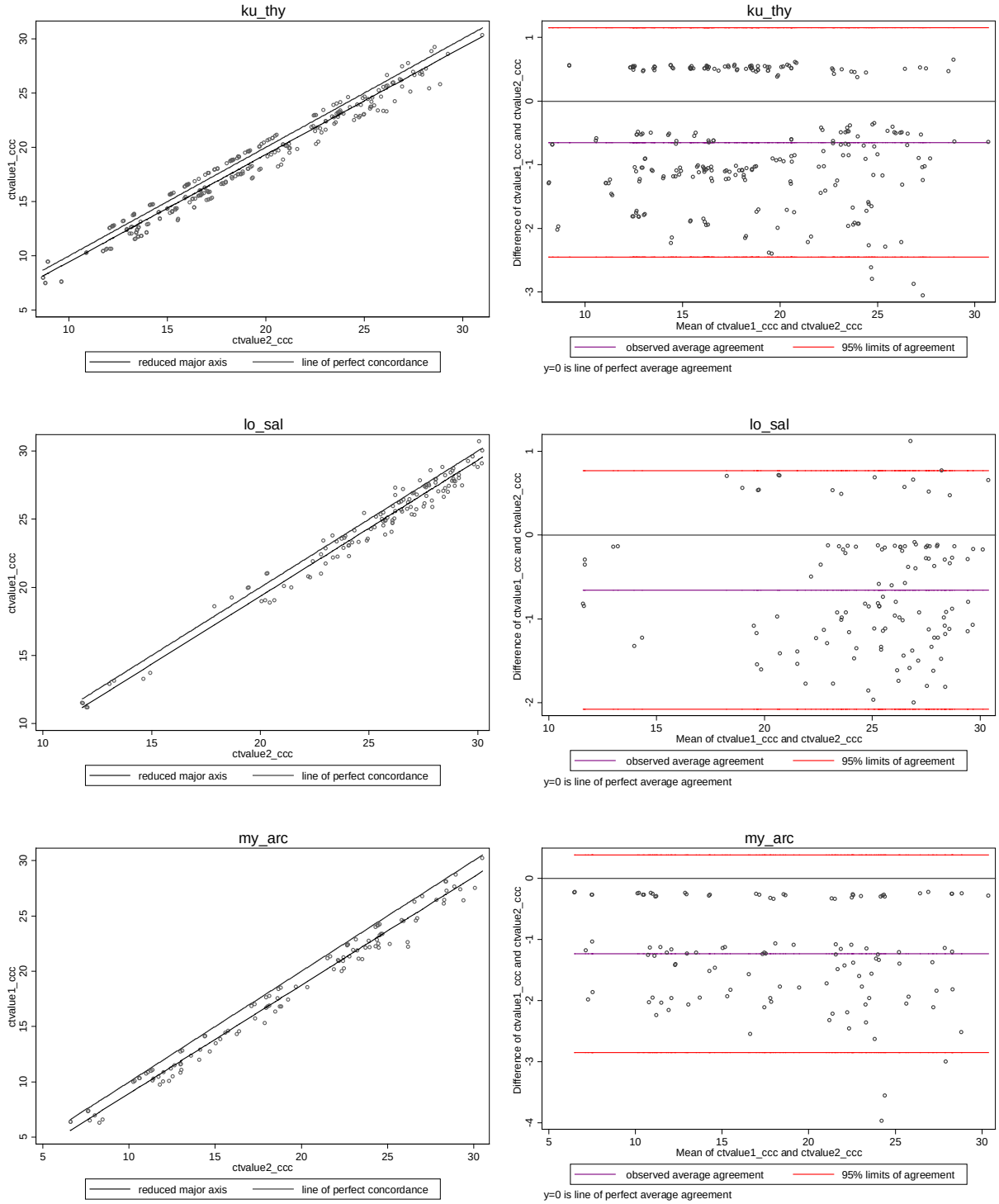

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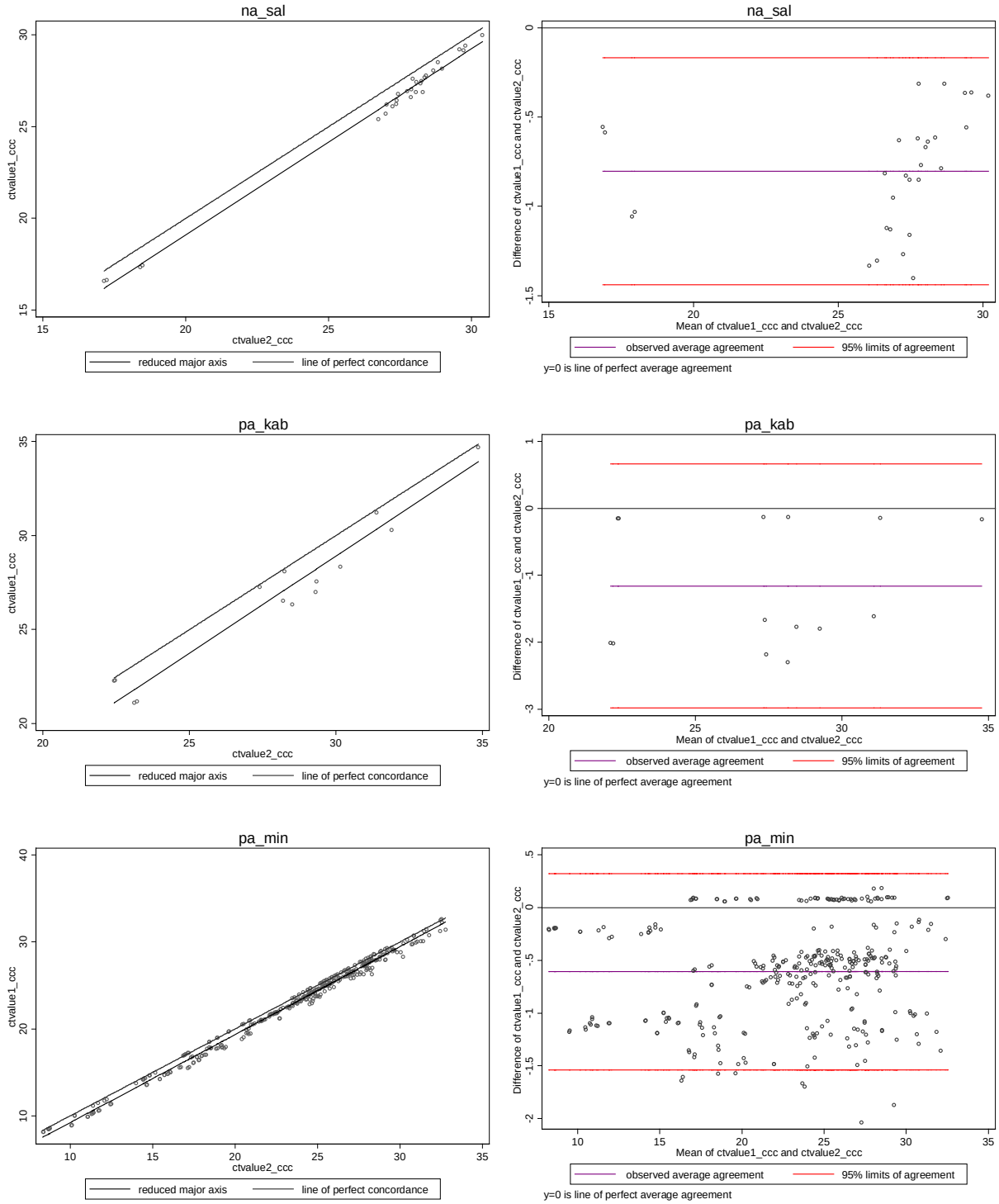

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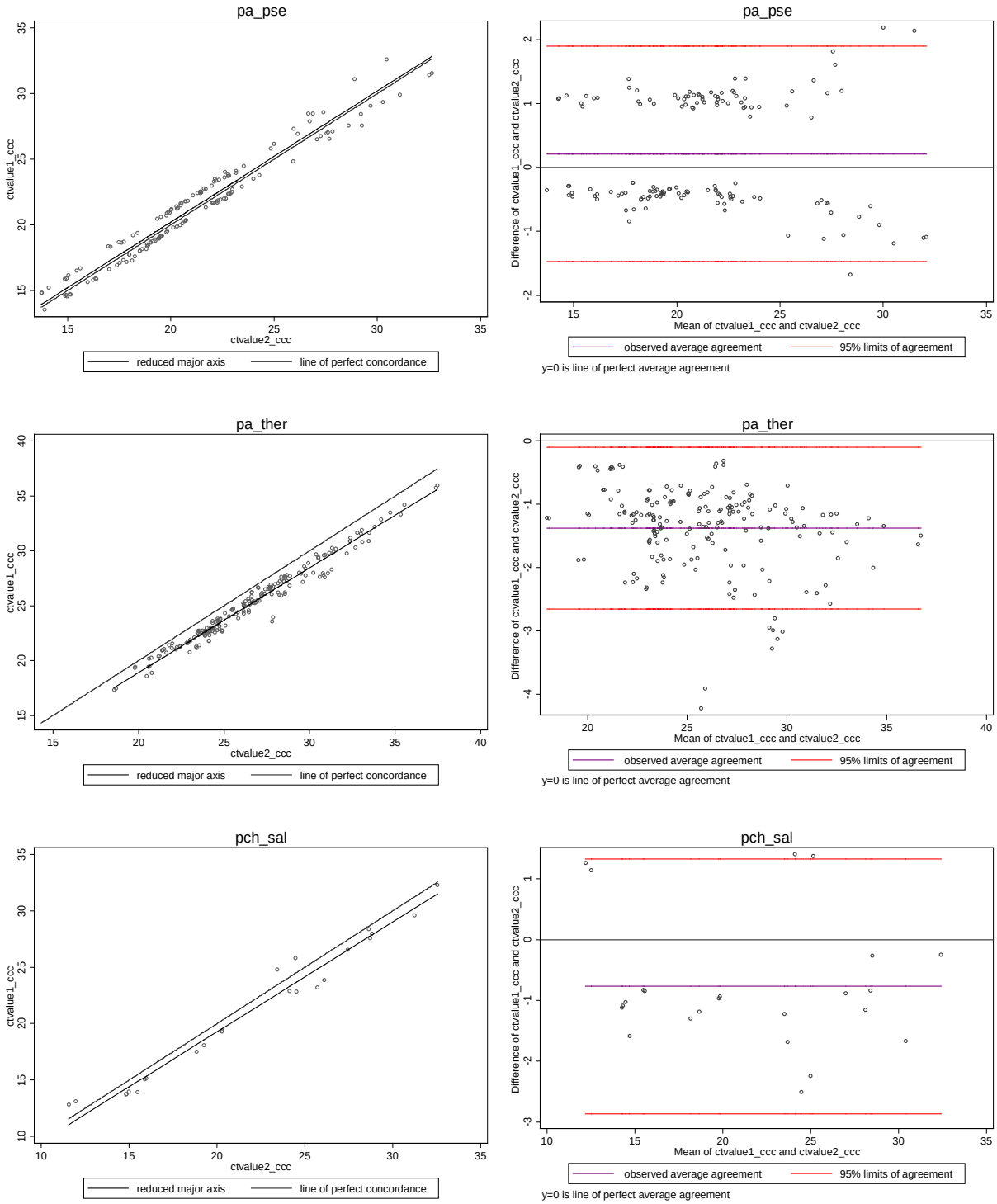

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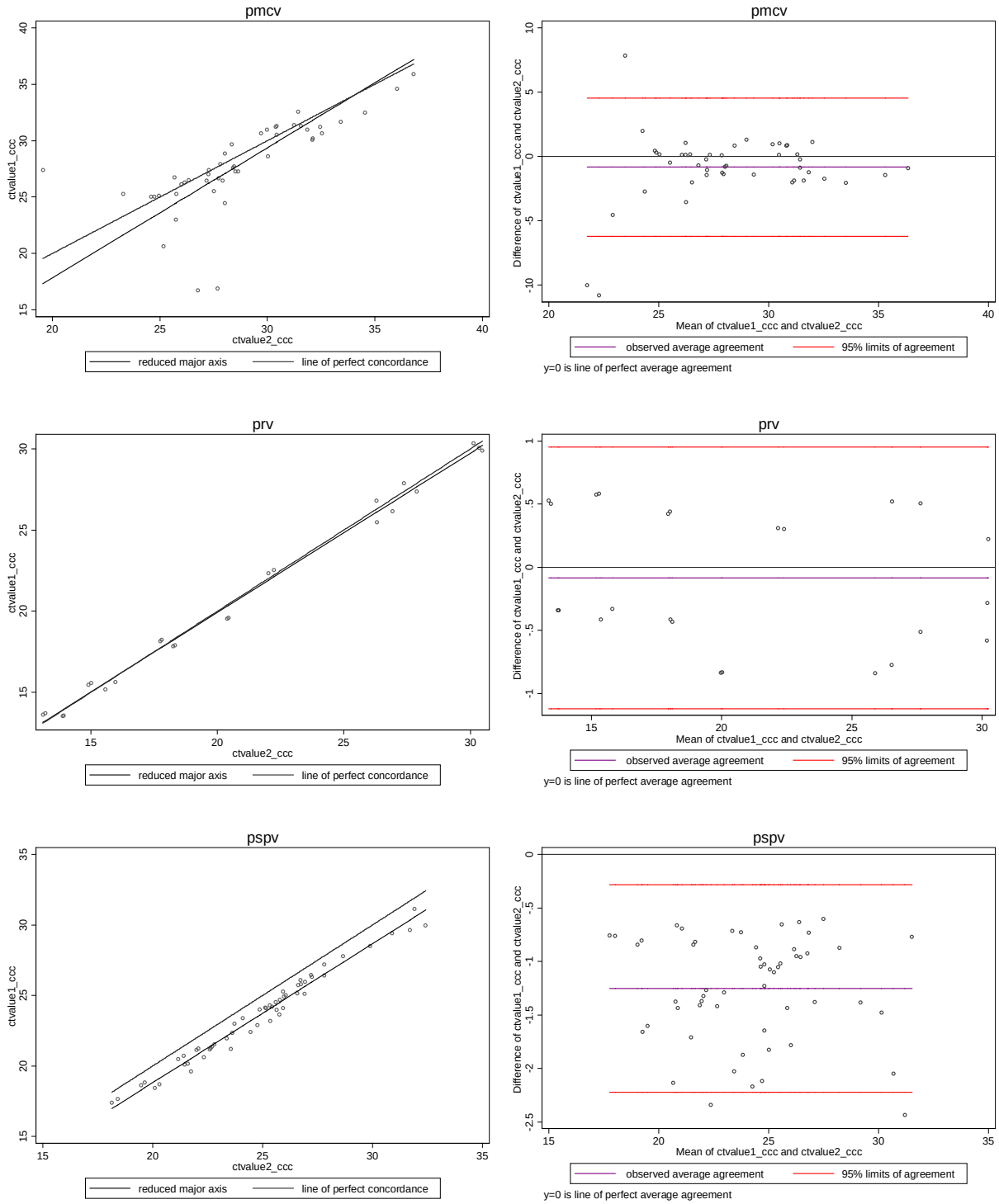

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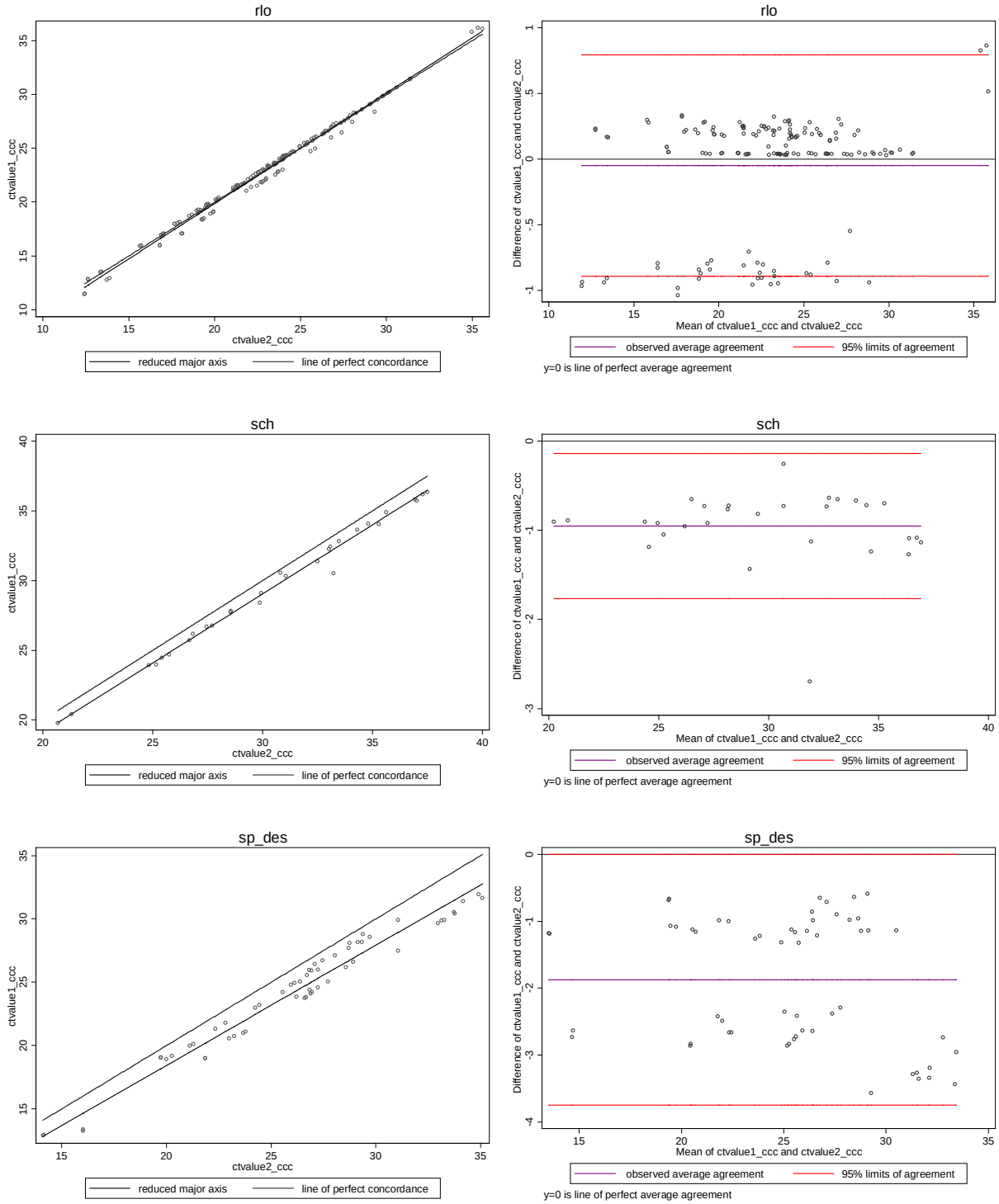

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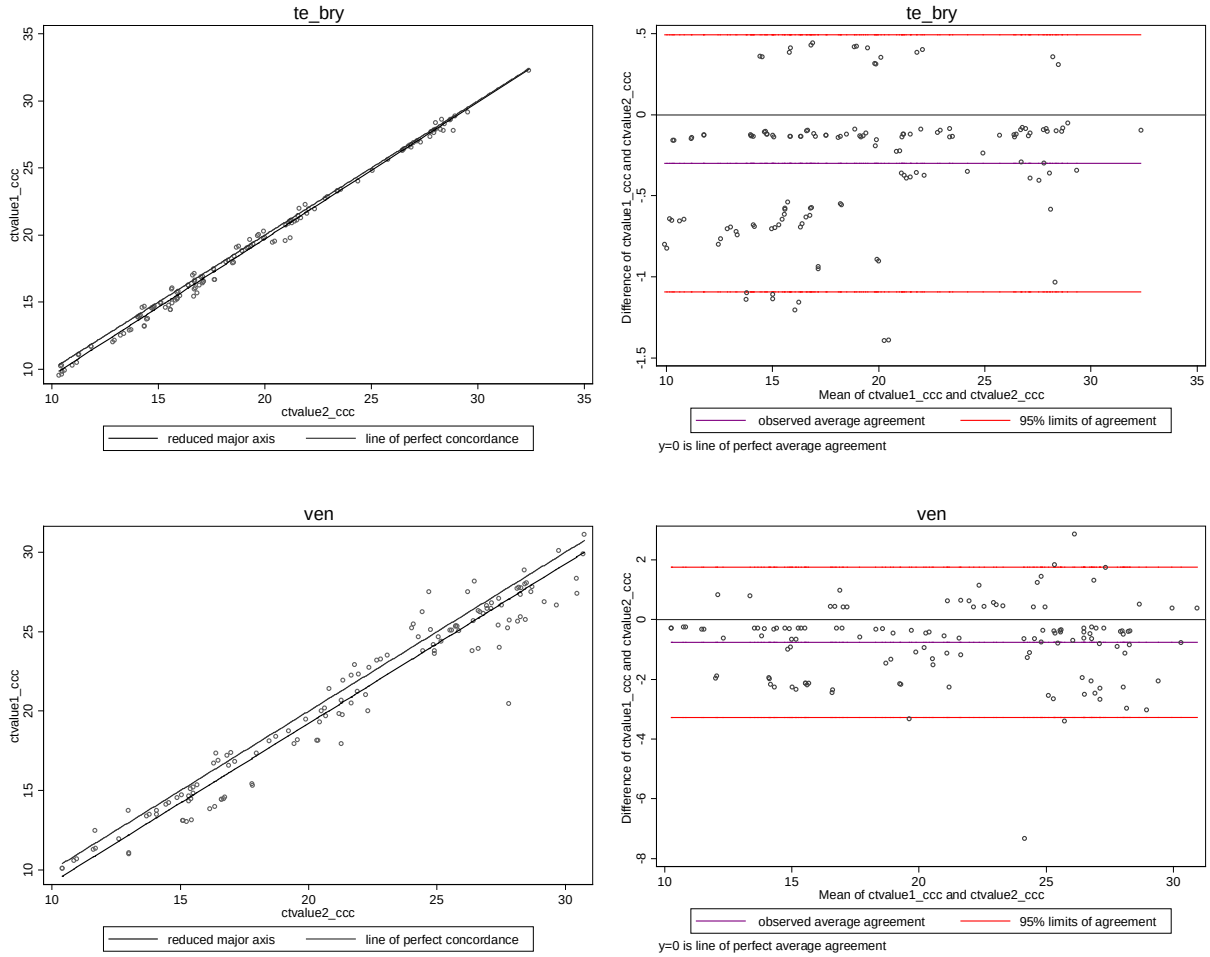

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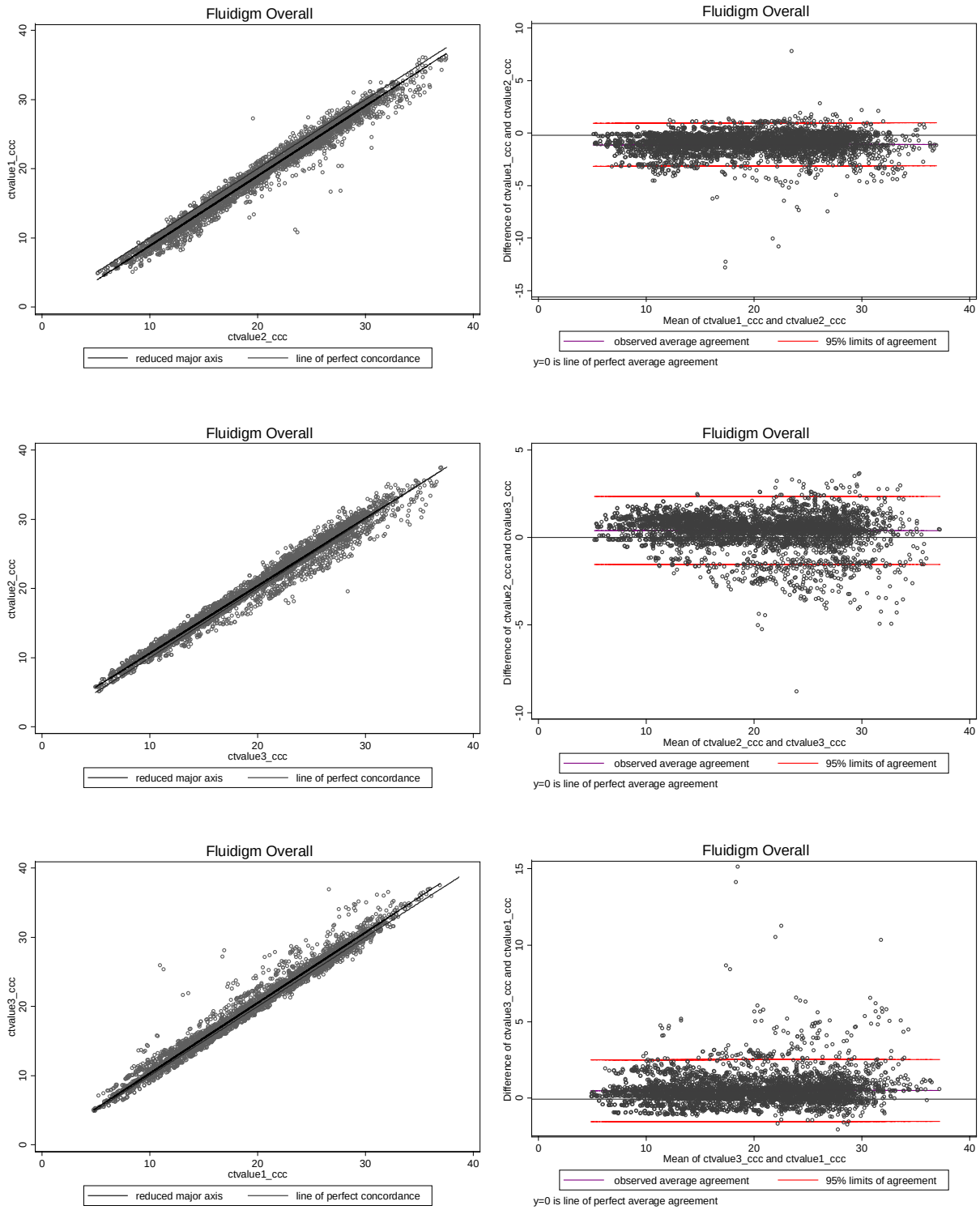
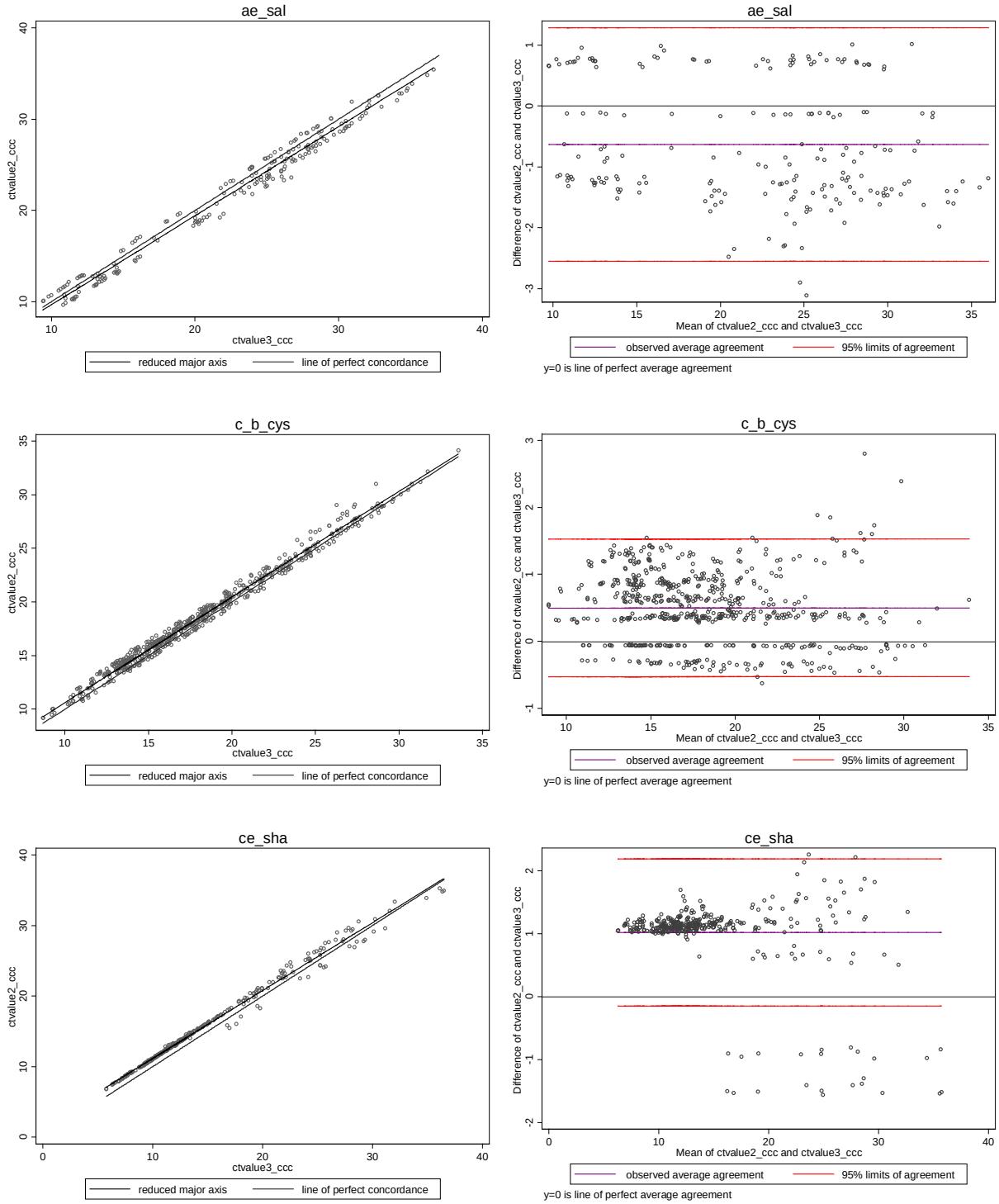
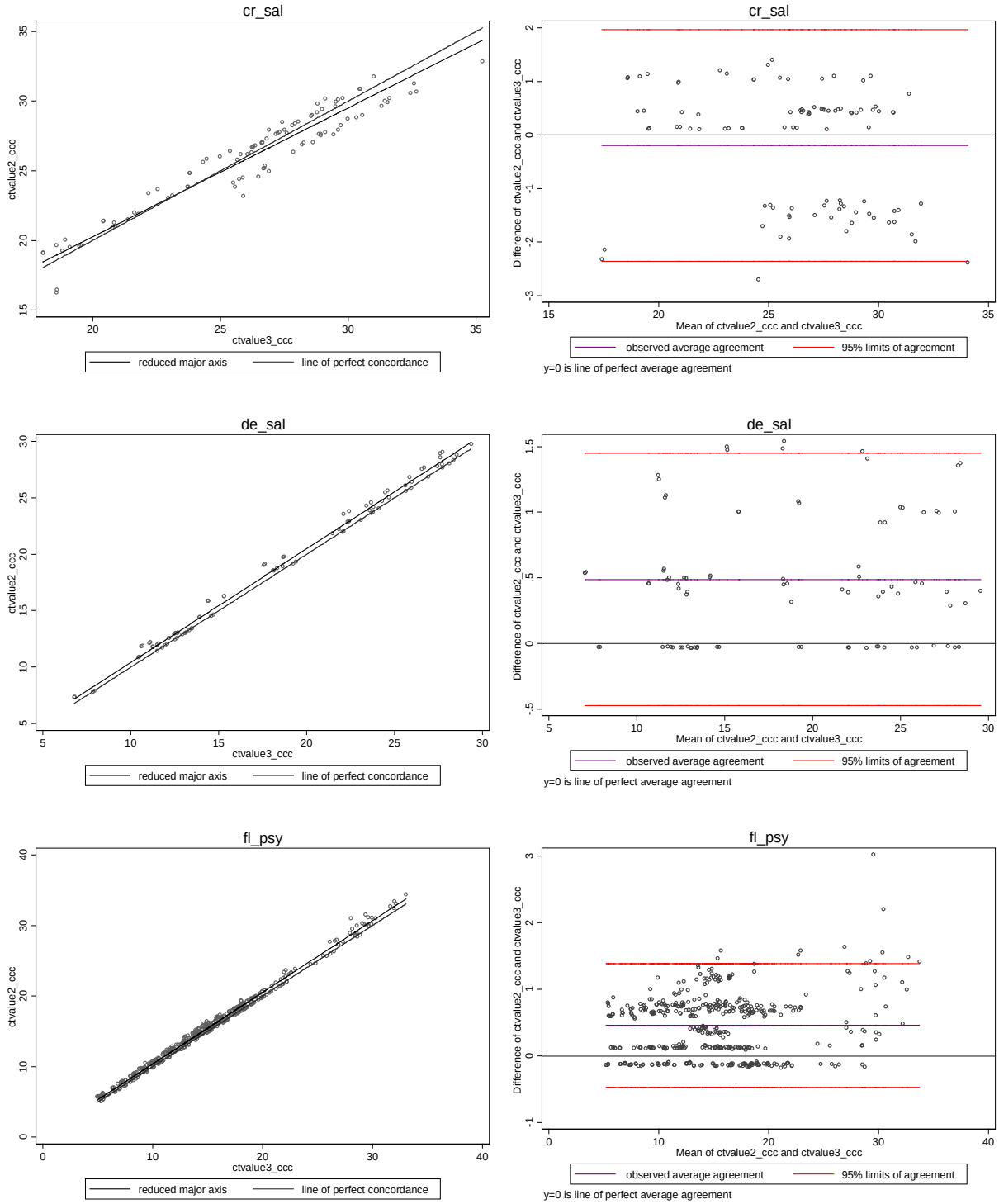


Supplemental Figure 11. Panel graph of concordance correlation coefficients (CCCs) and limit of agreement (LOA) for all assays in the repeatability study (Part 3) comparing technician 1 to 2 (top), 2 to 3 (middle) and 1 to 3 (bottom).



Supplemental Figure 12. Panel graph of concordance correlation coefficients (CCCs) and limit of agreement (LOA) by assay (n=26) in the repeatability study (Part 3) comparing technician 2 to technician 3. The list of assays in these analyses include (continued over the next several pages): ae_sal, c_b_cys, ce_sha, cr_sal, de_sal, fl_psy, ic_hof, ic_mul, ihnv, ku_thy, lo_sal, my_arc, na_sal, pa_kab, pa_min, pa_pse, pa_ther, pch_sal, pmcv, prv, pspv, rlo, sch, sp_des, te_bry, and ven.



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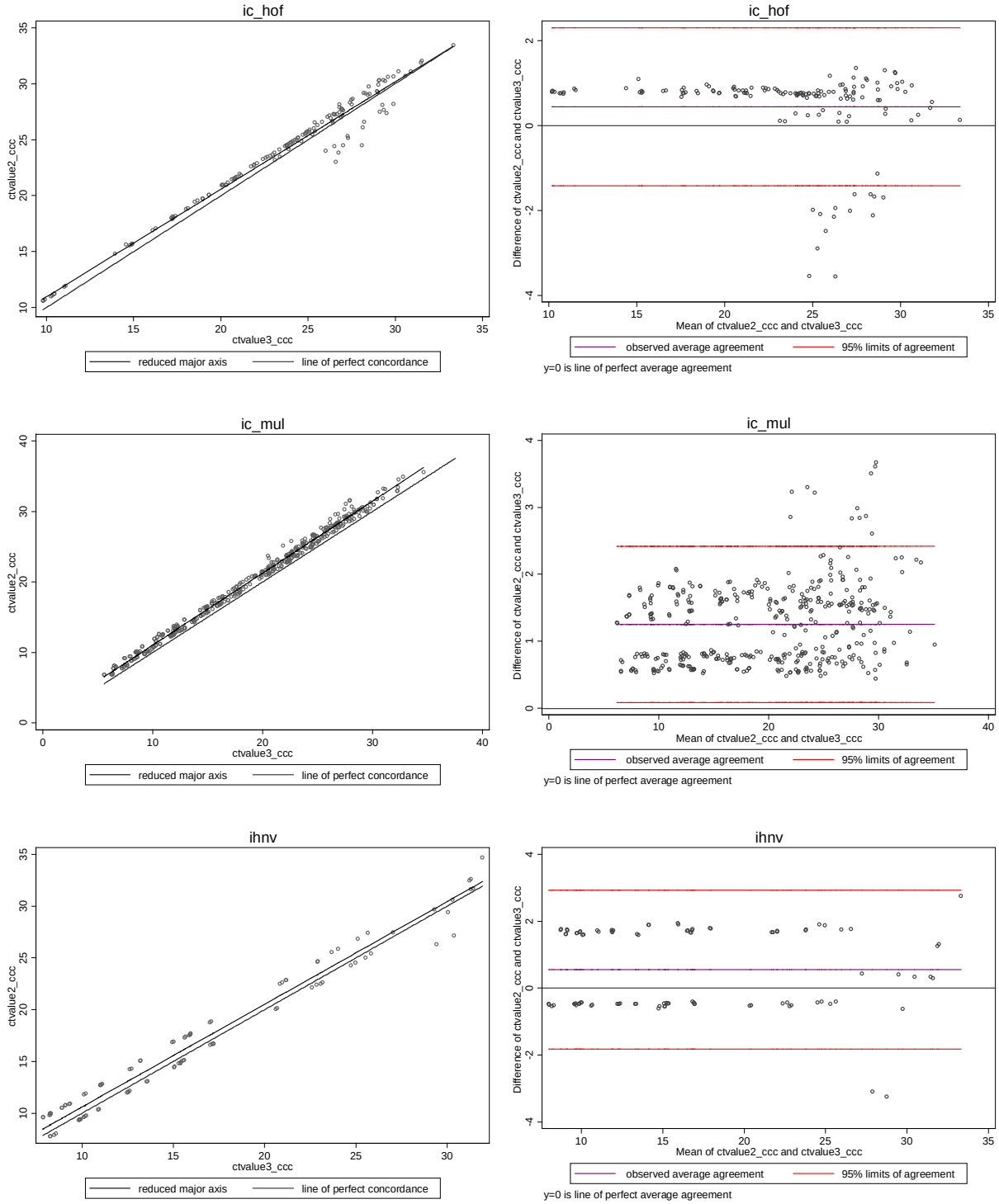
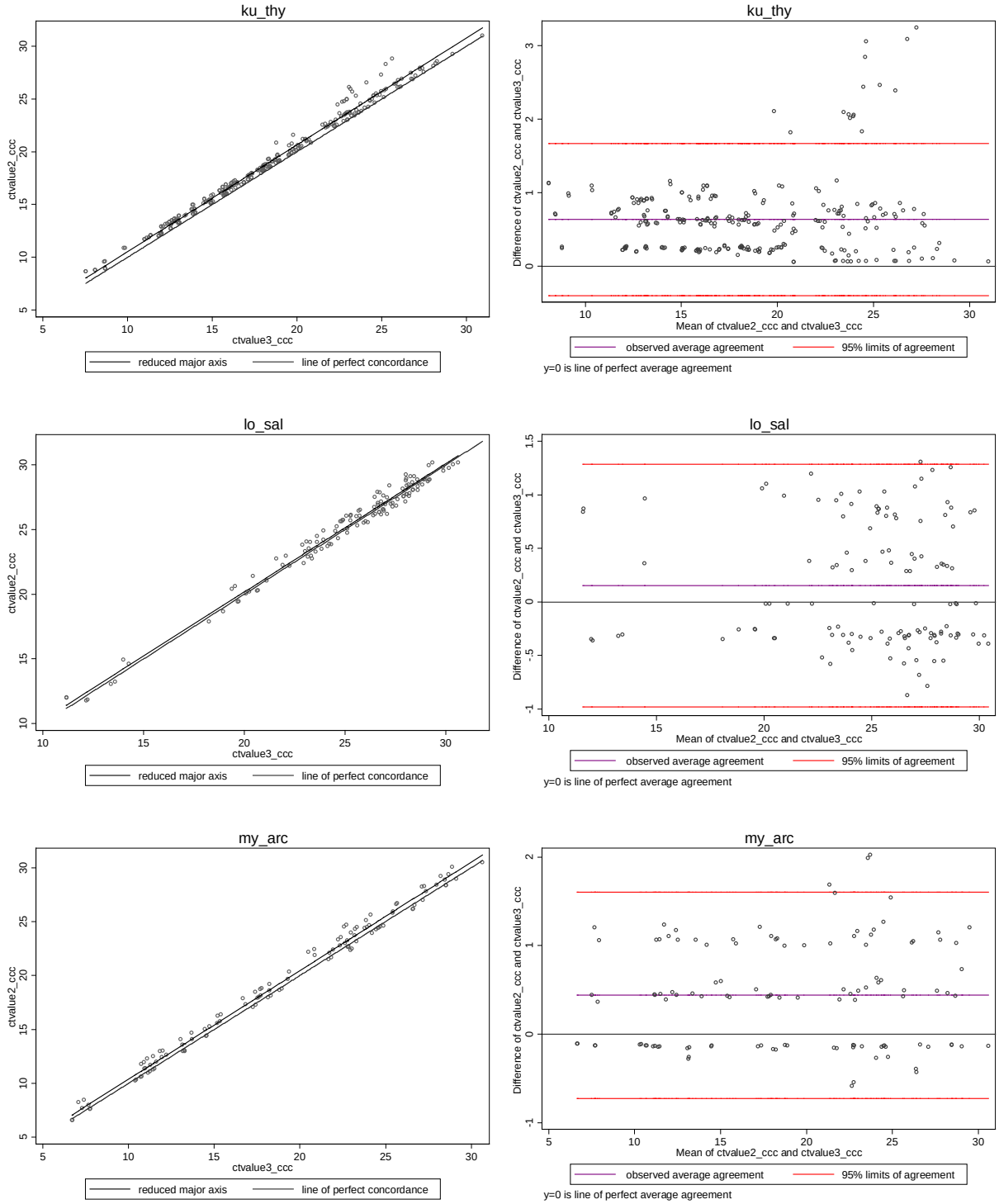


Supplemental Figure 12 (Continued)



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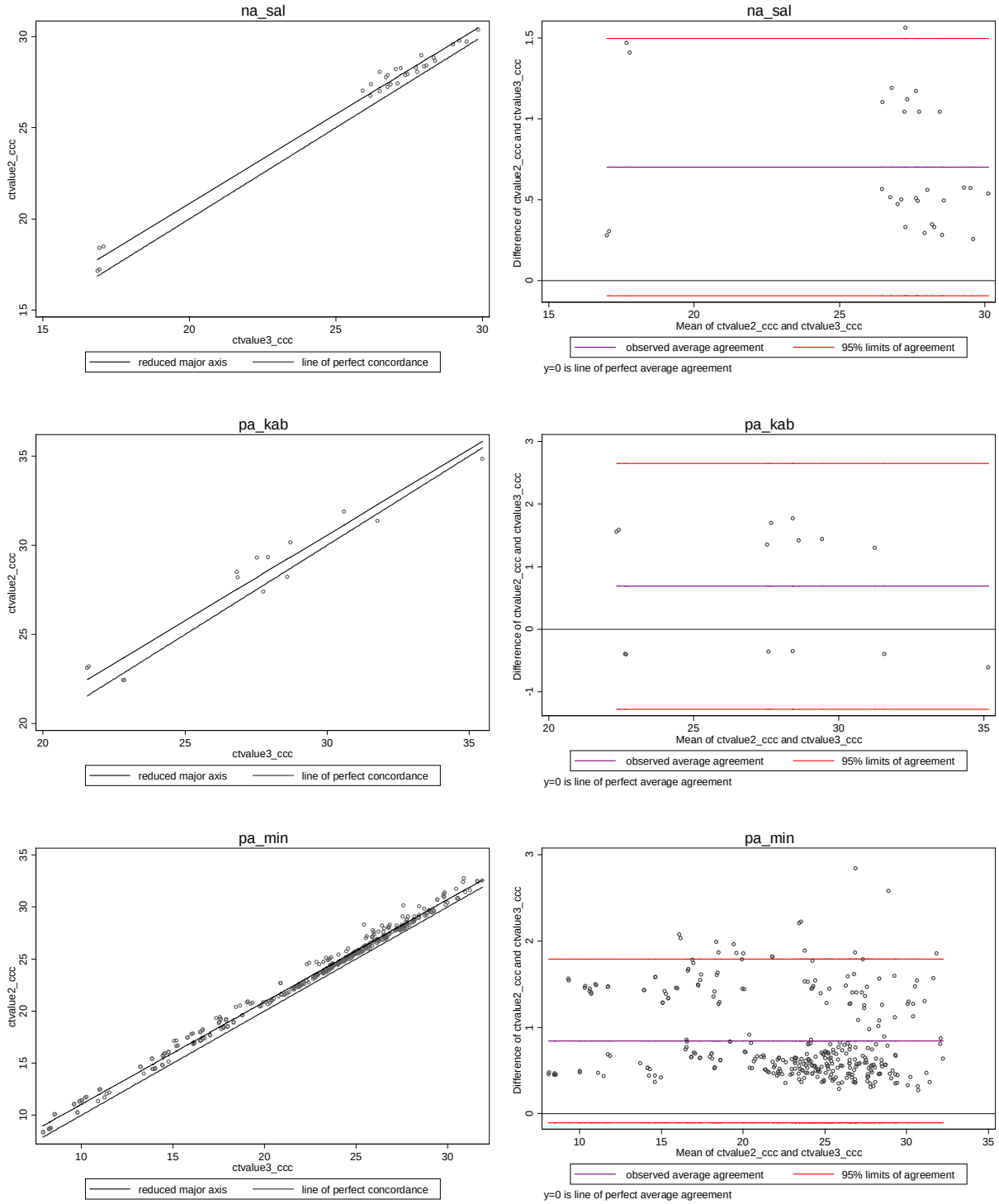

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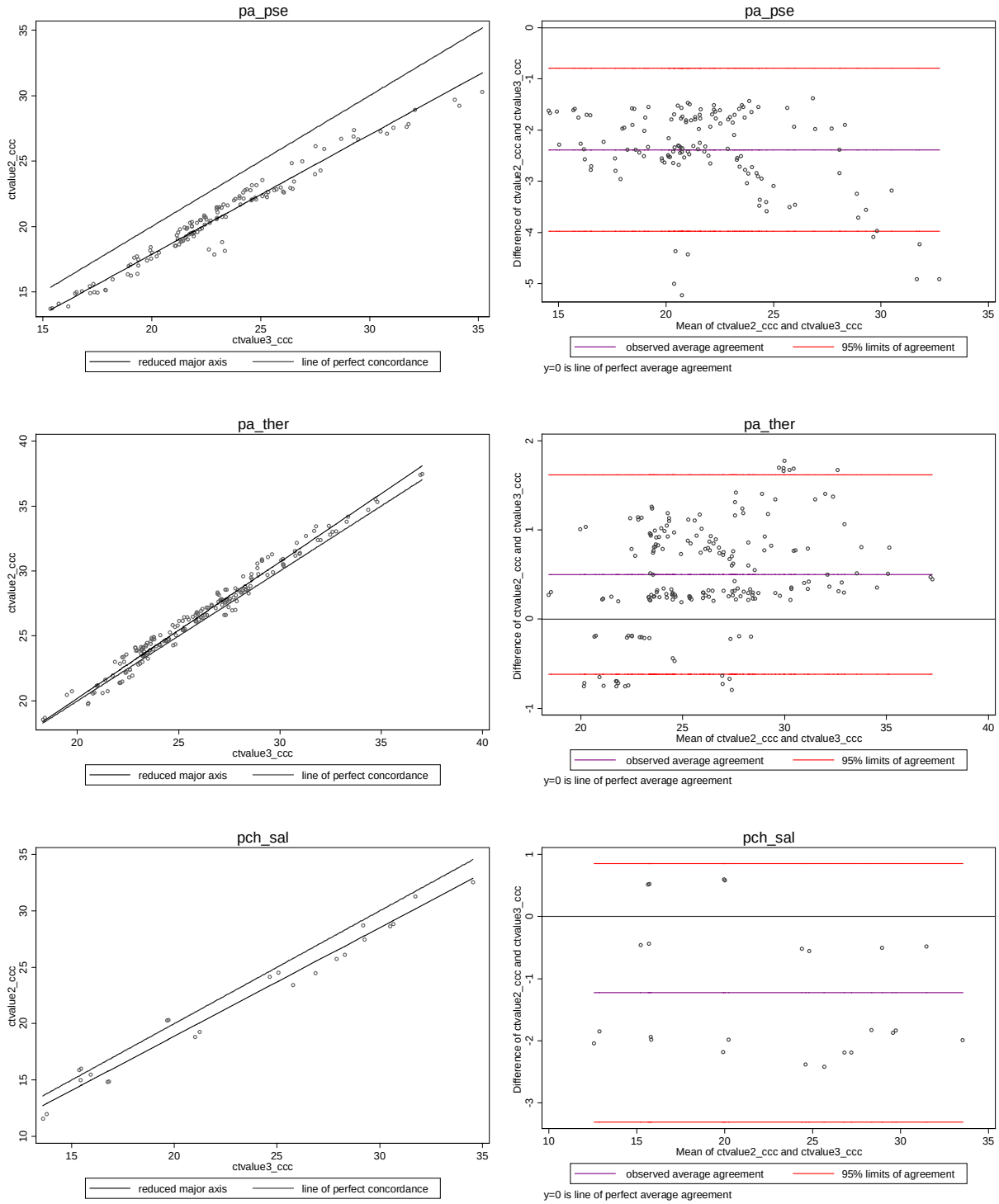

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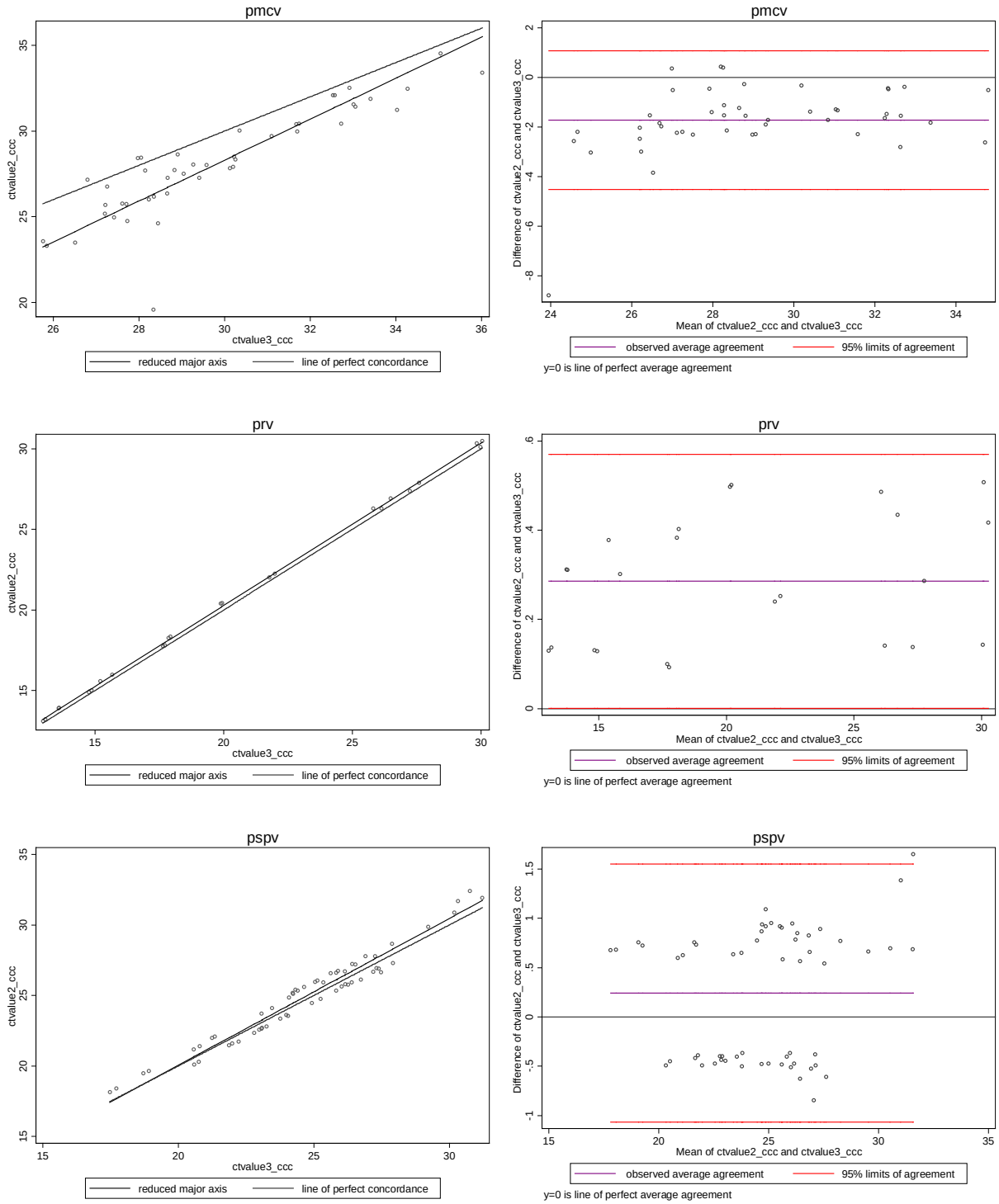

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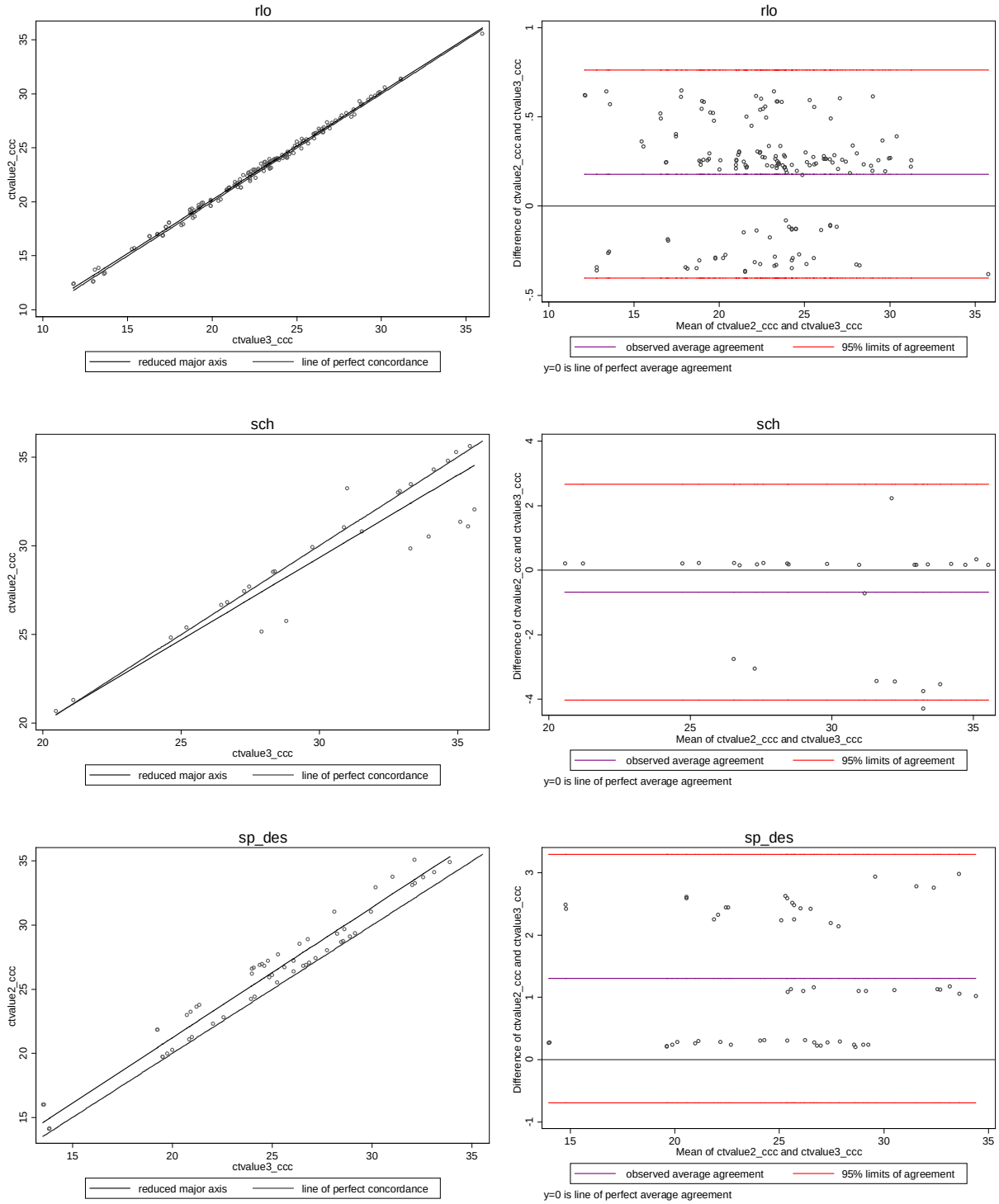

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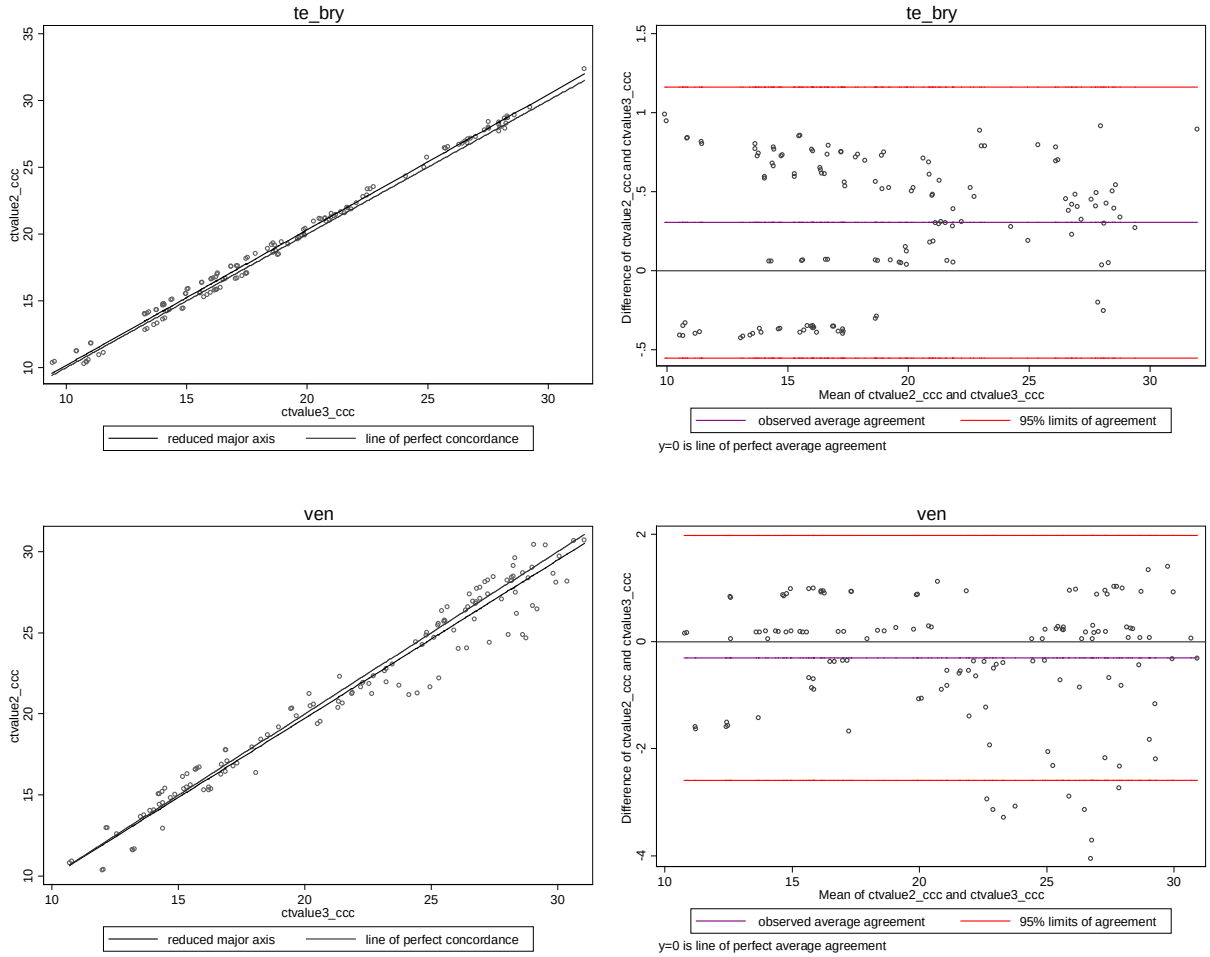

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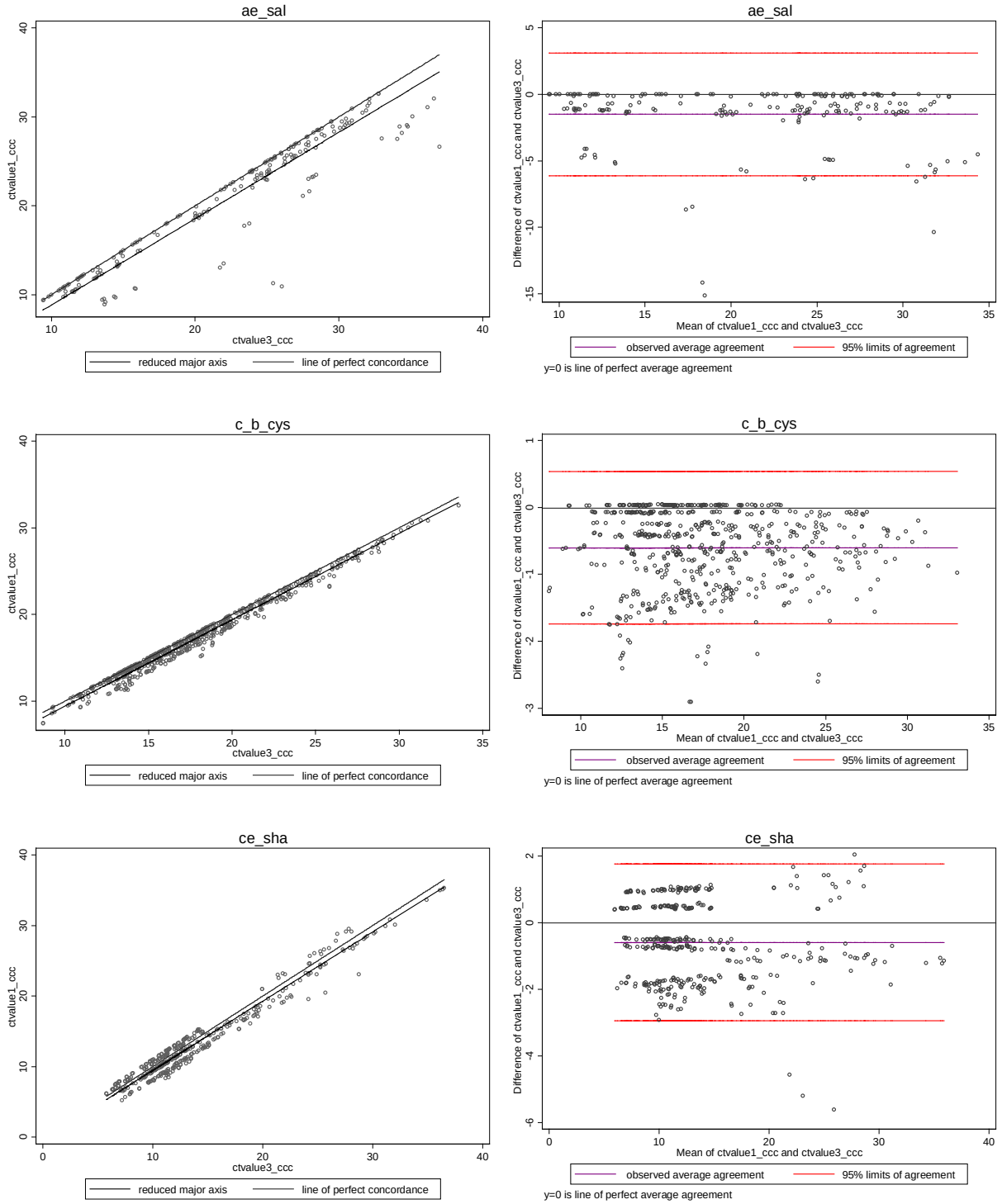
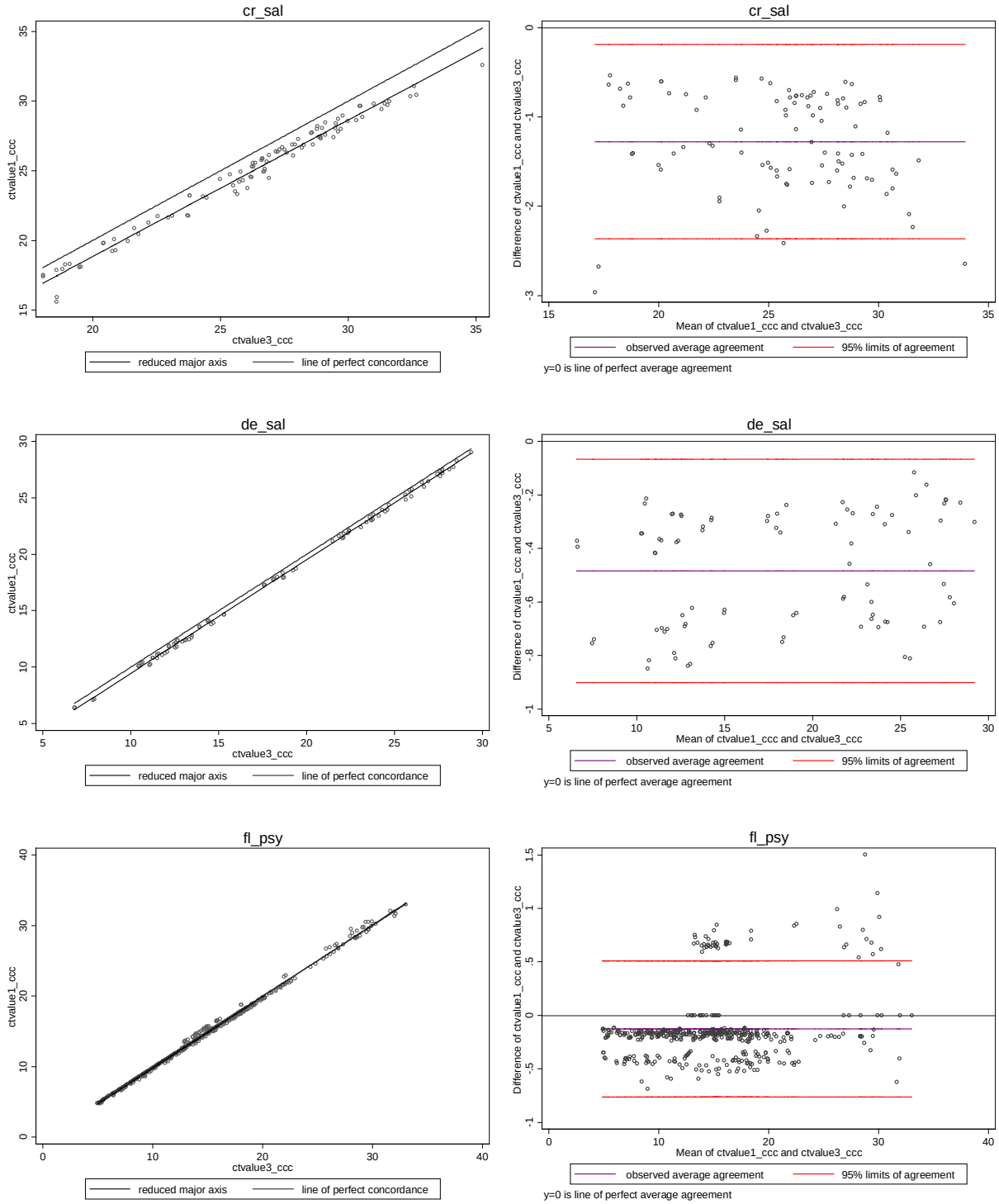


Supplemental Figure 13. Panel graph of concordance correlation coefficients (CCCs) and limit of agreement (LOA) by assay (n=26) in the repeatability study (Part 3) comparing technician 1 to technician 3. The list of assays in these analyses include (continued over the next several pages): ae_sal, c_b_cys, ce_sha, cr_sal, de_sal, fl_psy, ic_hof, ic_mul, ihnv, ku_thy, lo_sal, my_arc, na_sal, pa_kab, pa_min, pa_pse, pa_ther, pch_sal, pmcv, prv, pspv, rlo, sch, sp_des, te_bry, and ven.



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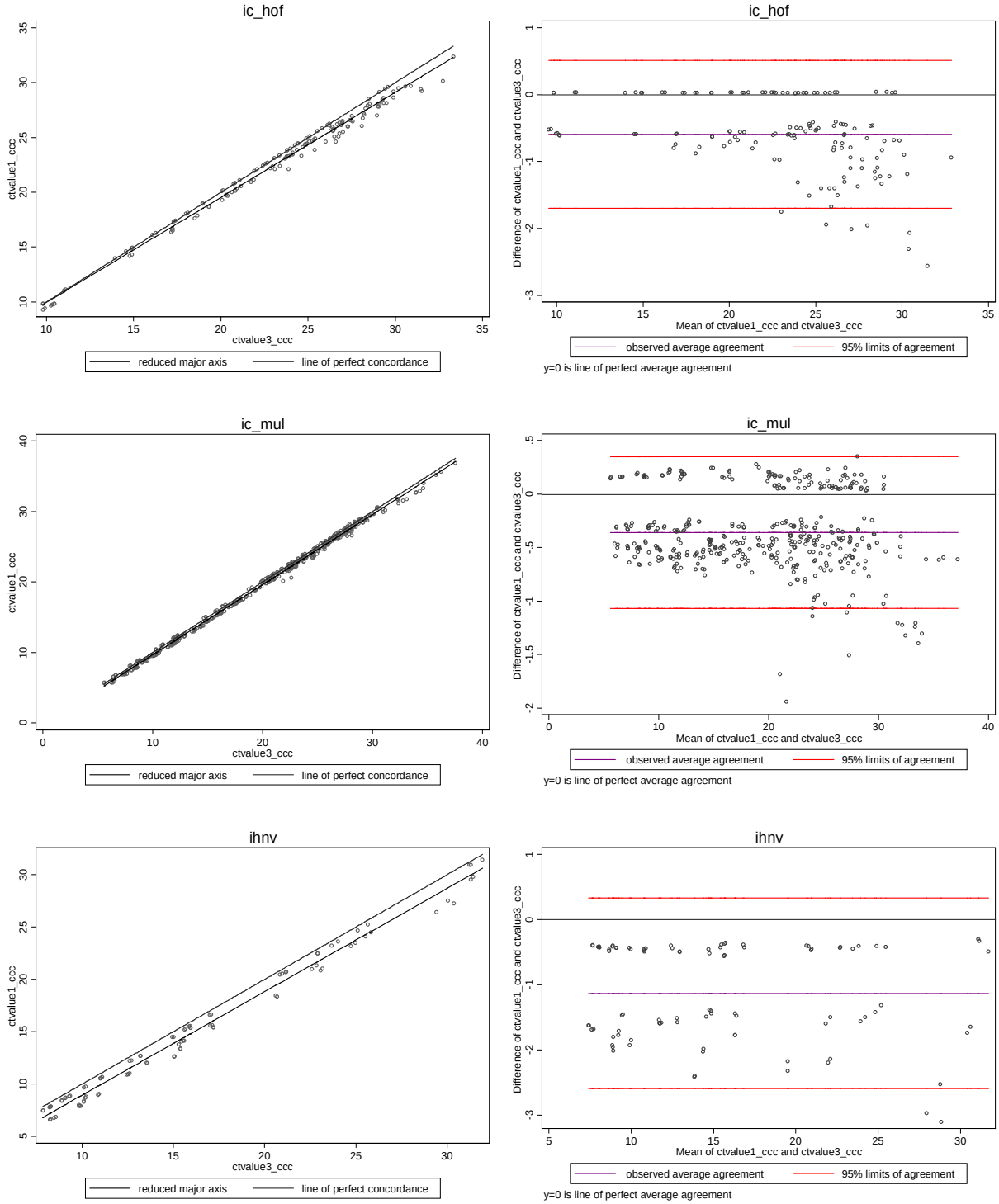

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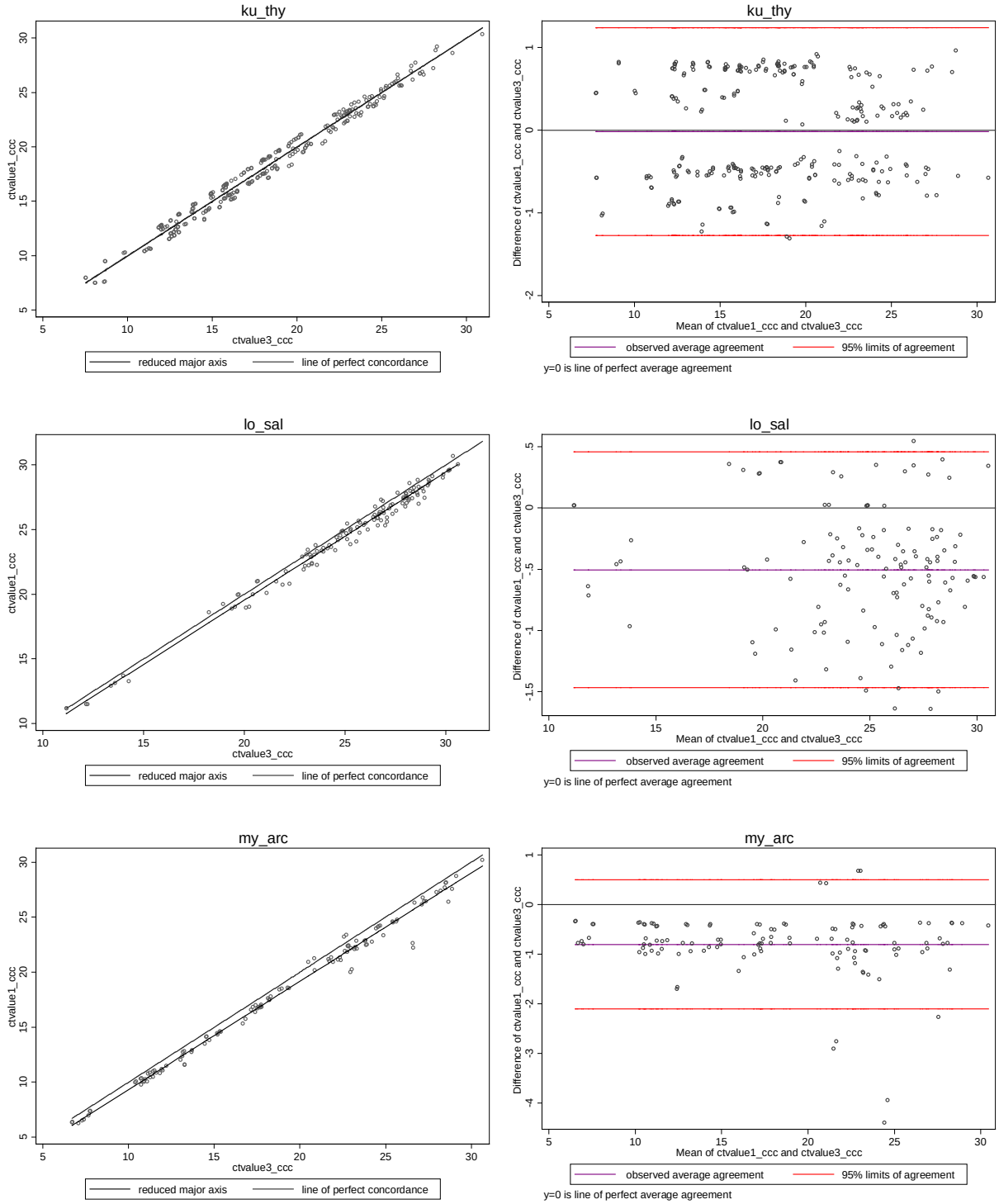

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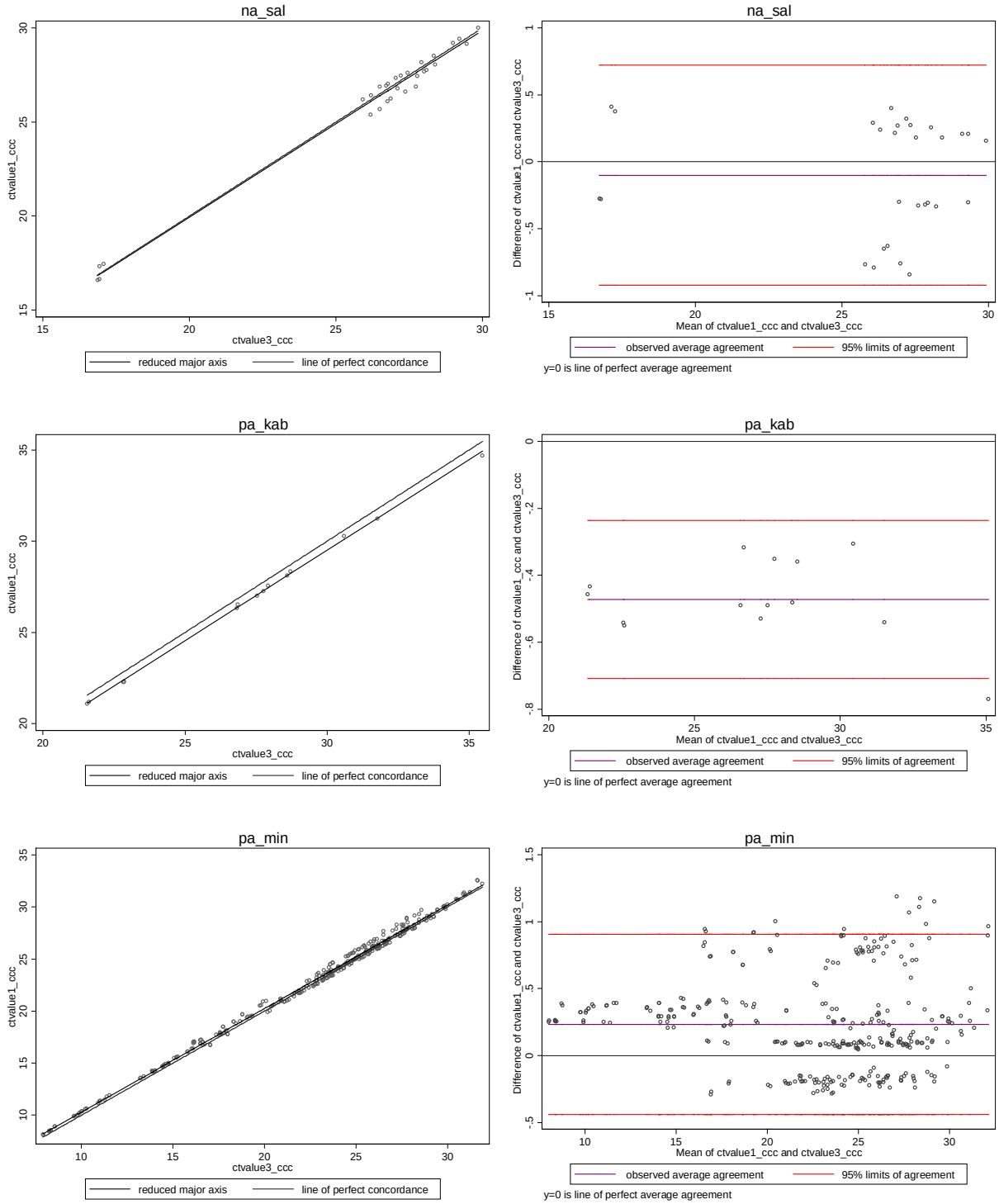

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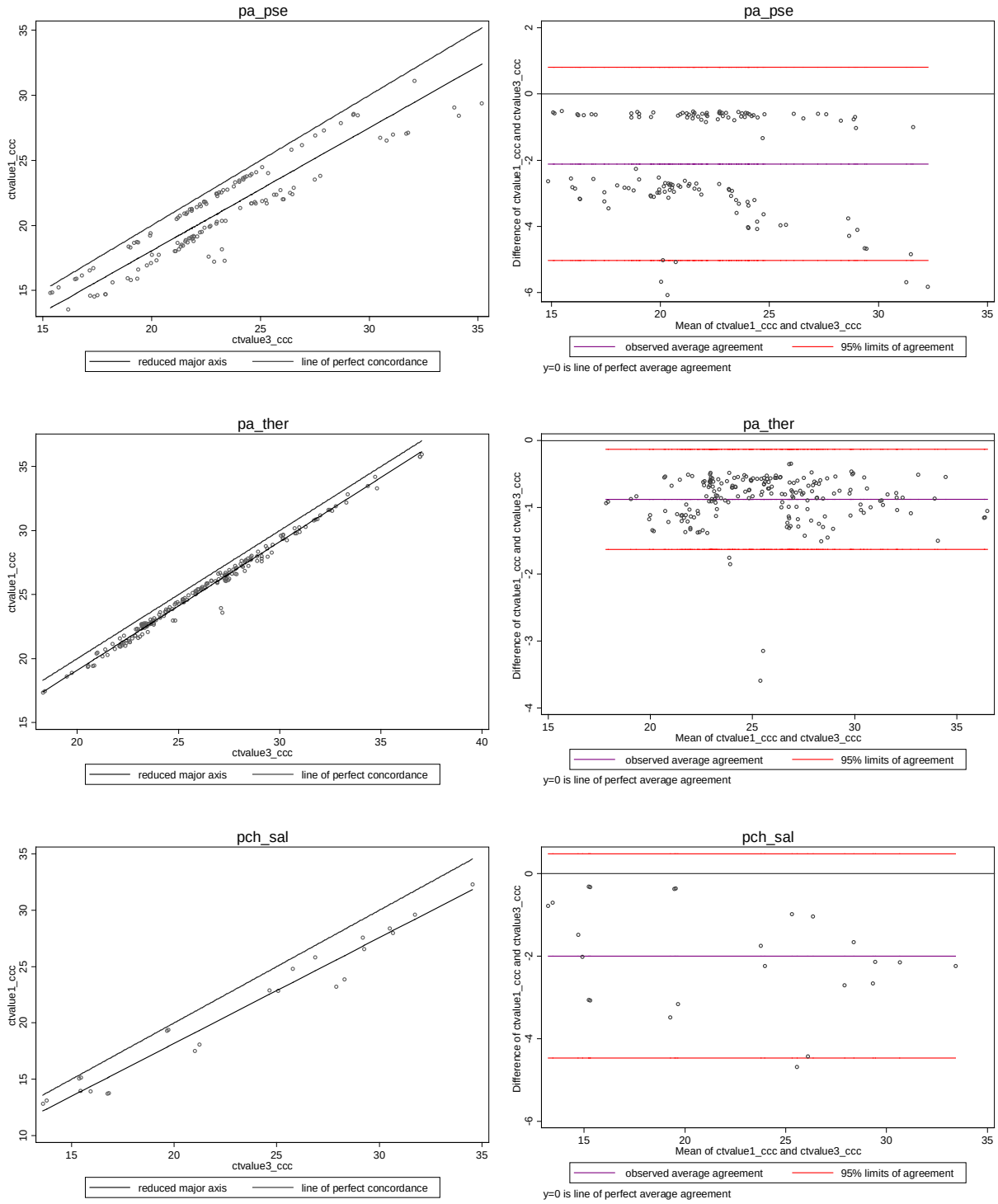

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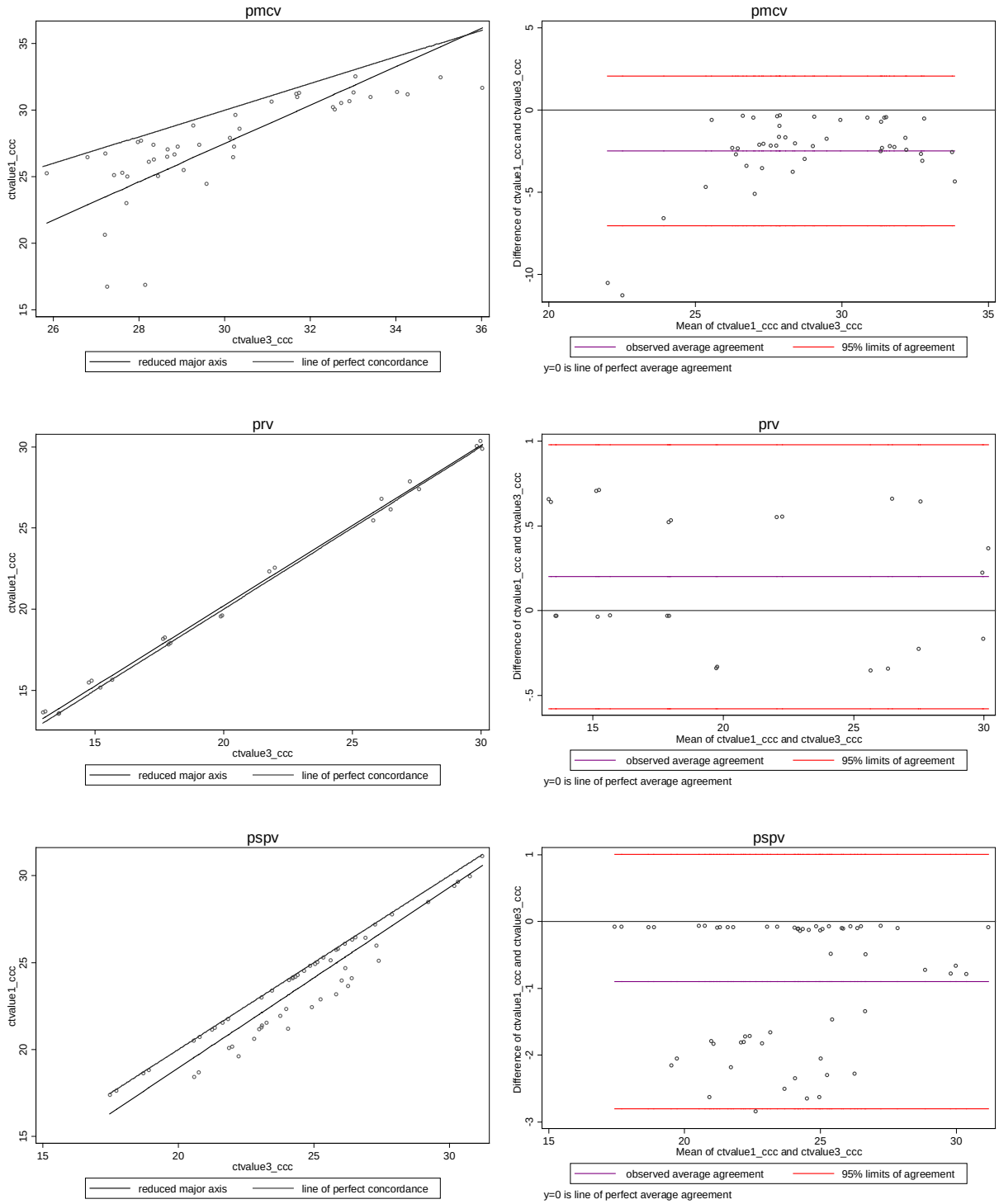

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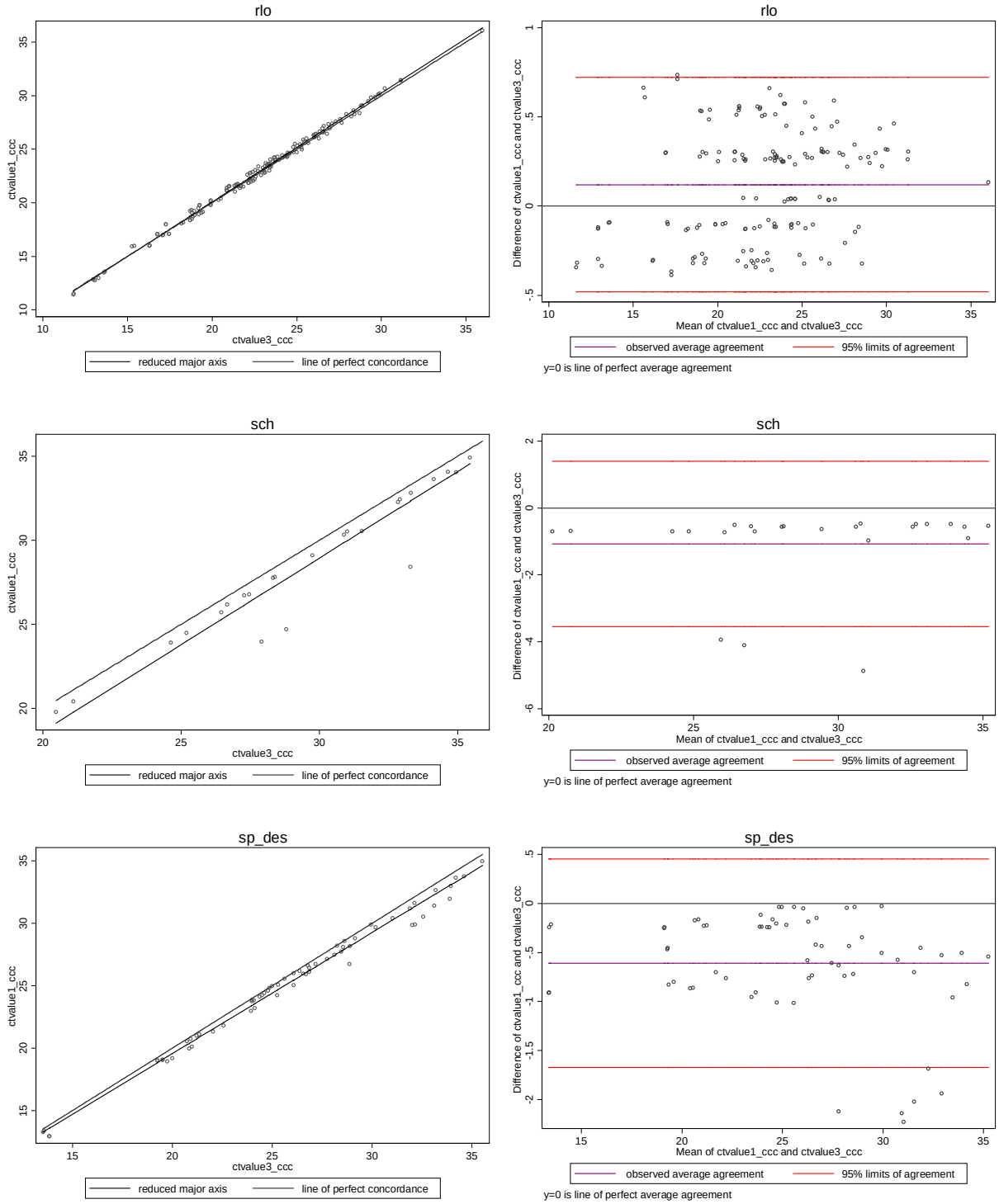

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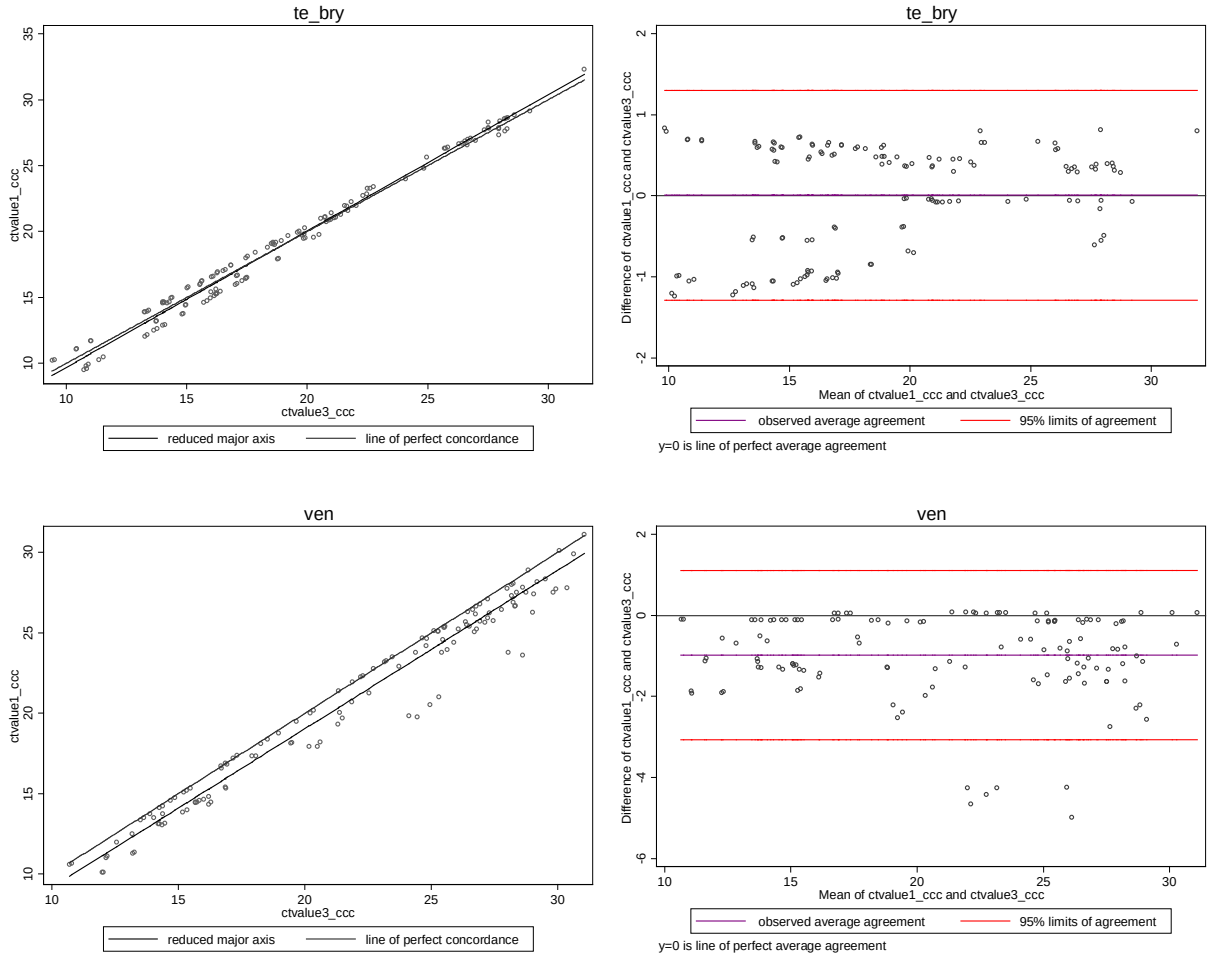

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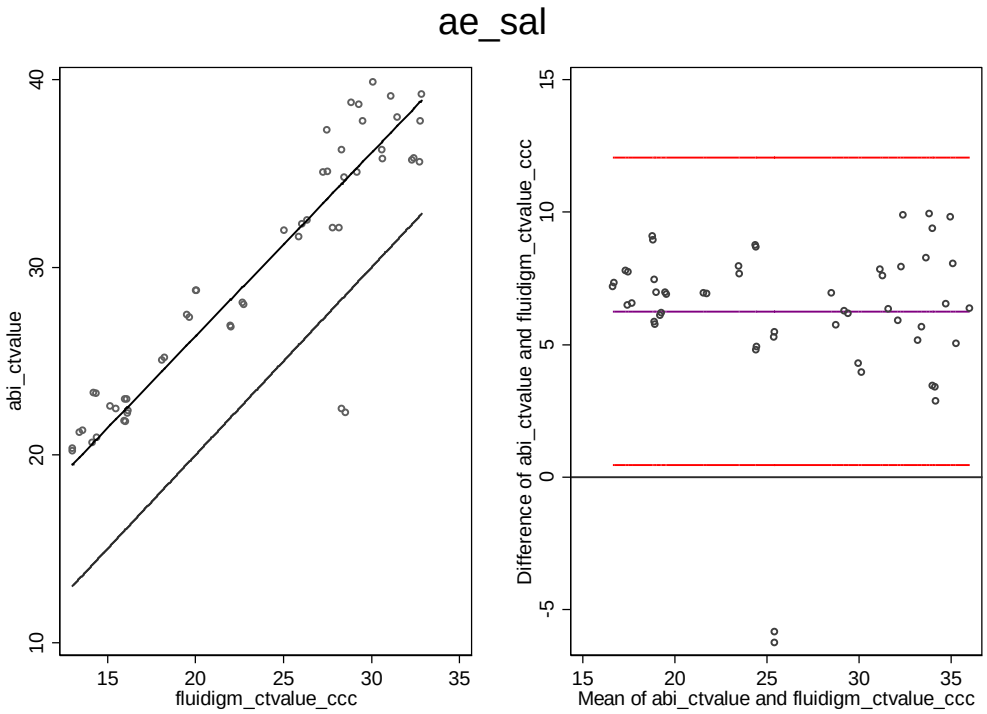
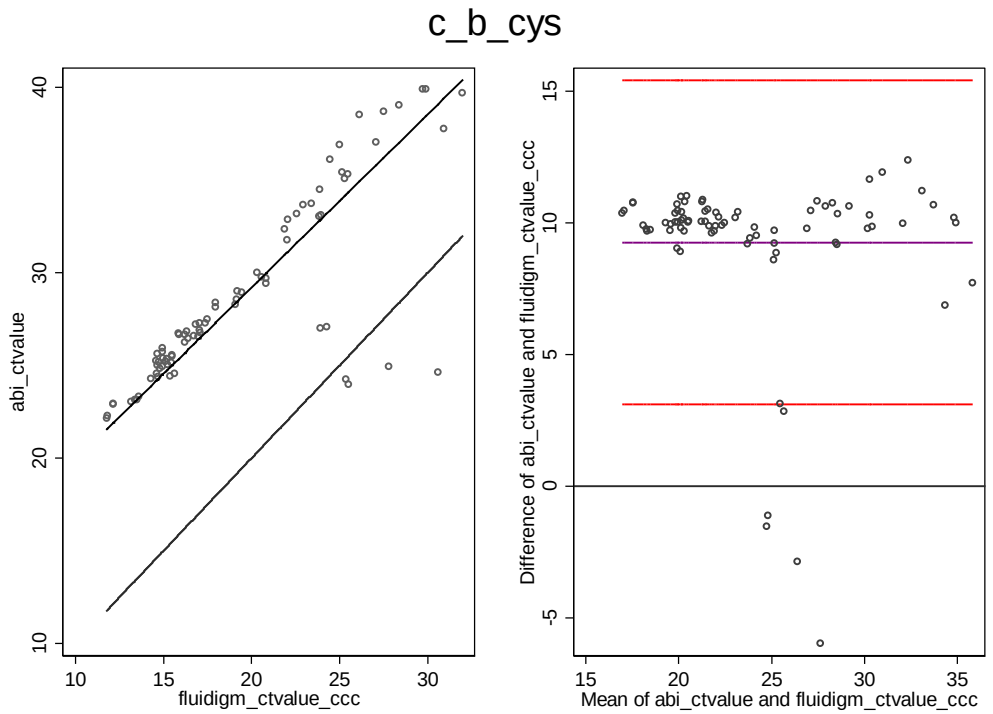
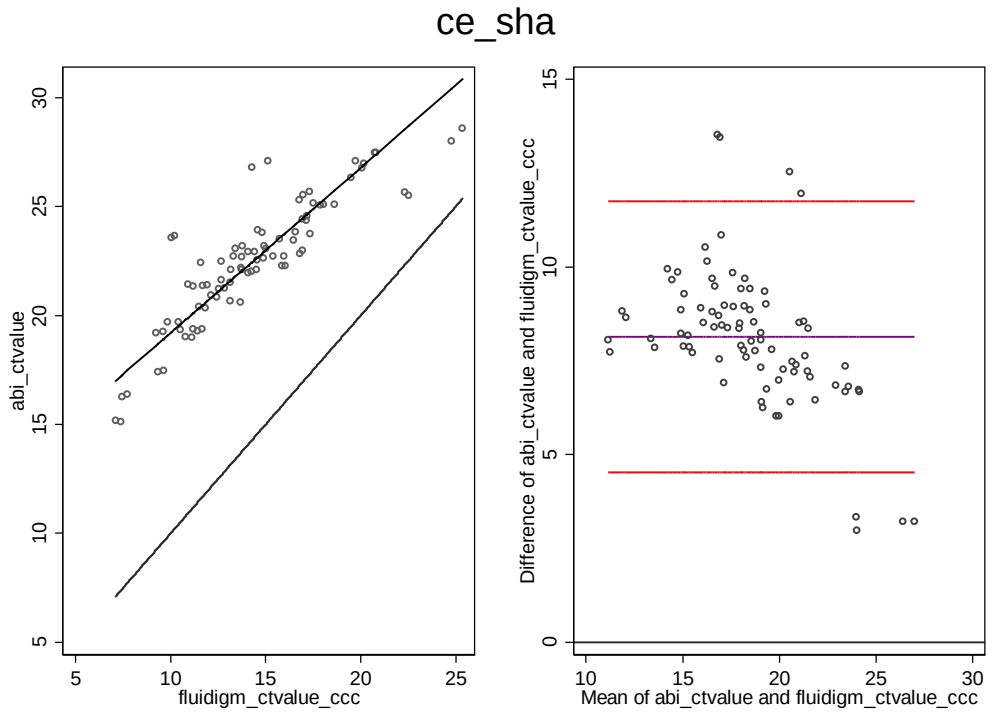


Supplemental Figure 14. Panel graph of concordance correlation coefficients (CCCs) and limit of agreement (LOA) by assay (n=26) comparing ABI 7900 and Fluidigm systems, using unadjusted Ct values. The list of assays in these analyses include (continued over the next several pages): ae_sal, c_b_cys, ce_sha, cr_sal, de_sal, fl_psy, ic_hof, ic_mul, ihnv, ku_thy, lo_sal, my_arc, pa_min, pa_pse, pa_ther, pspv, rlo, sch, sp_des, te_bry, and ven.





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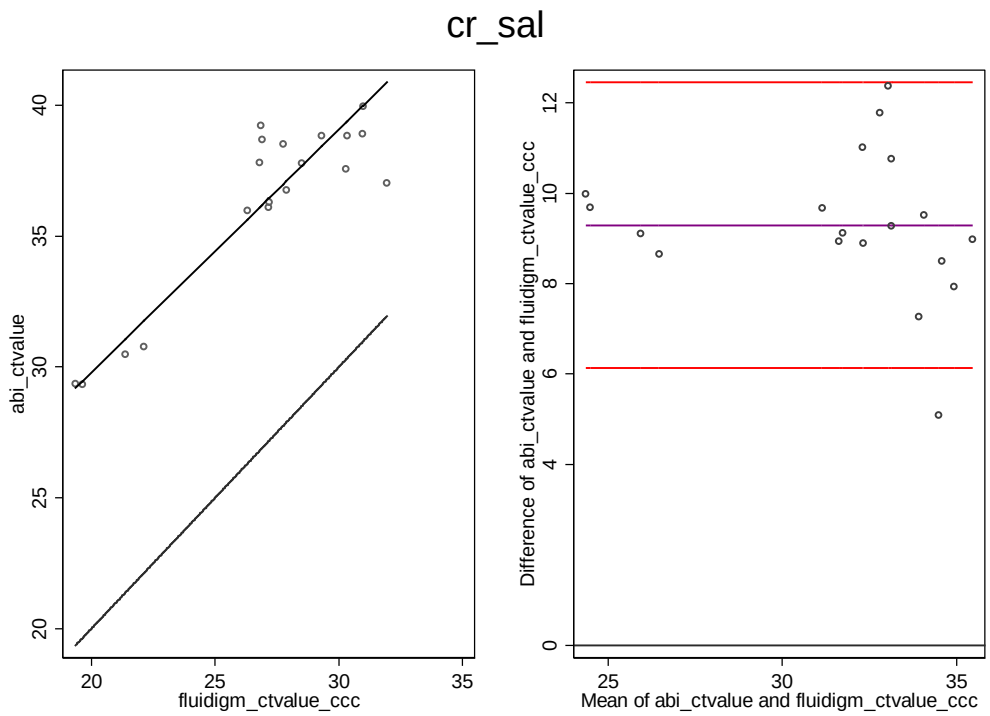

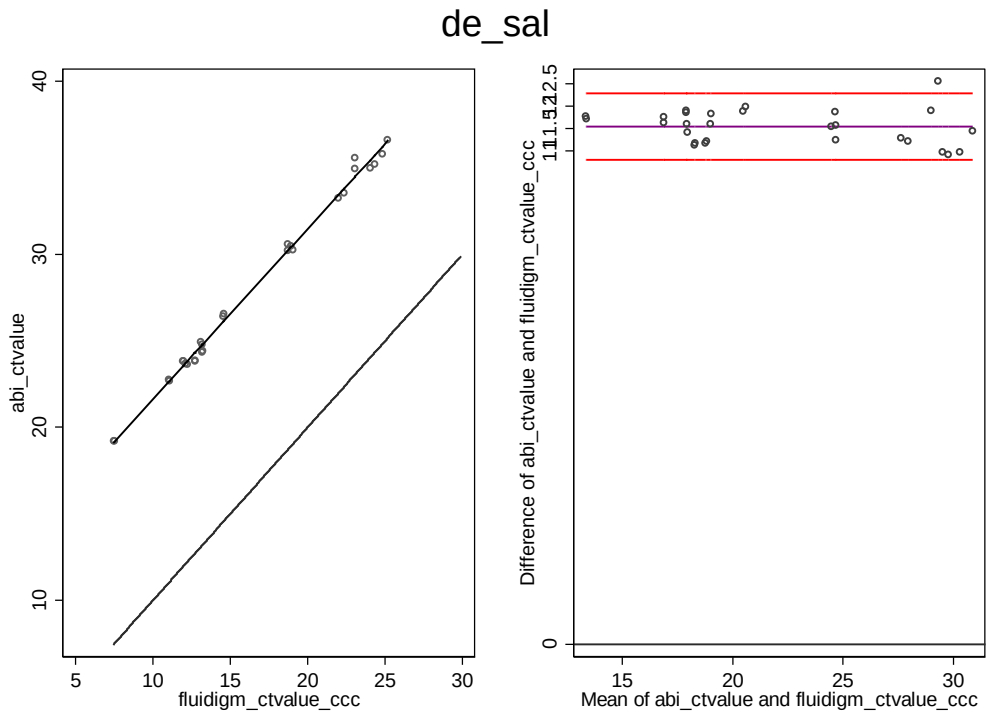

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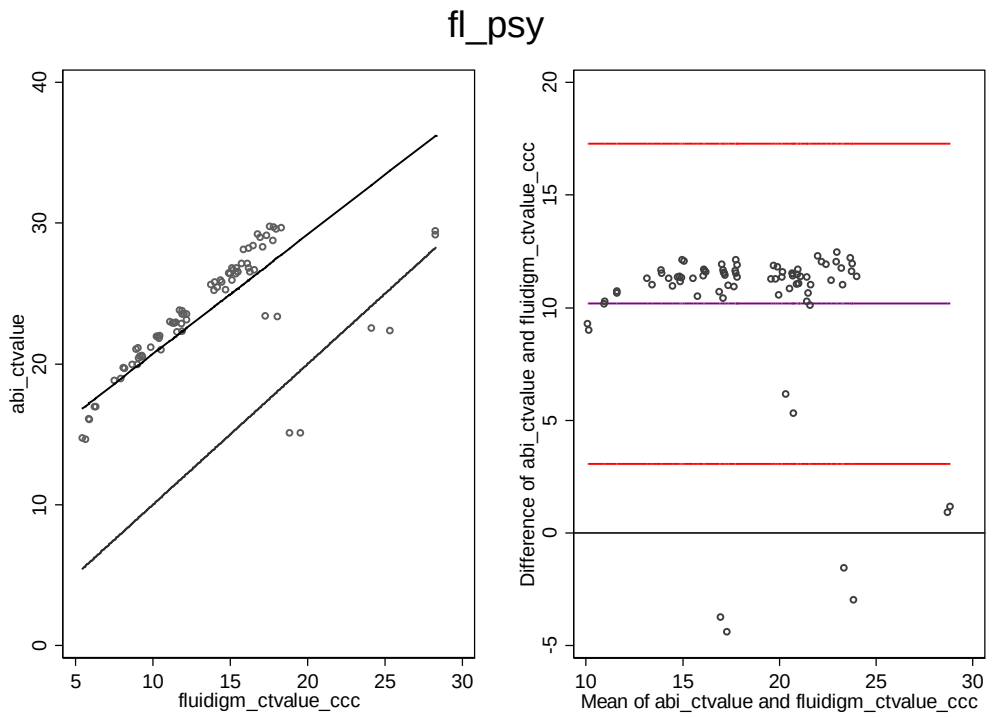

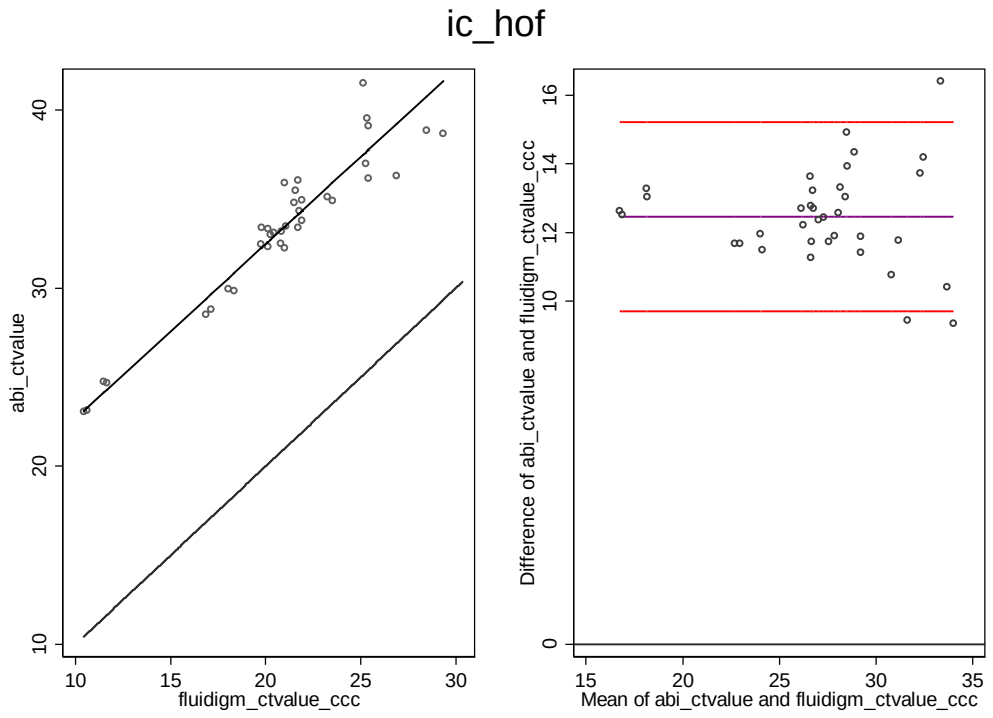

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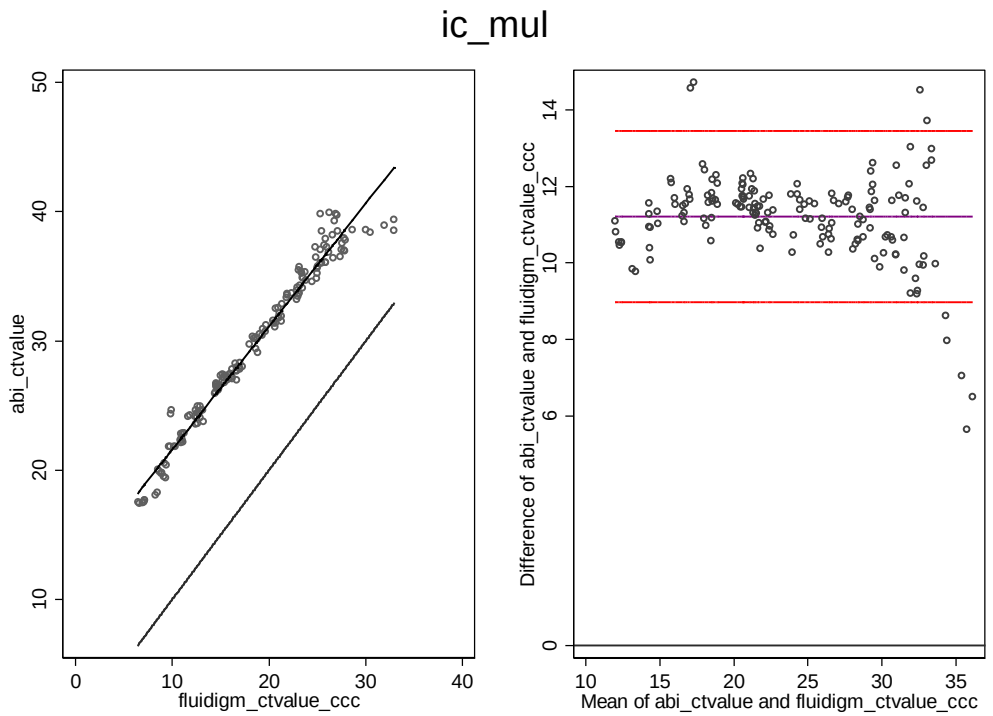

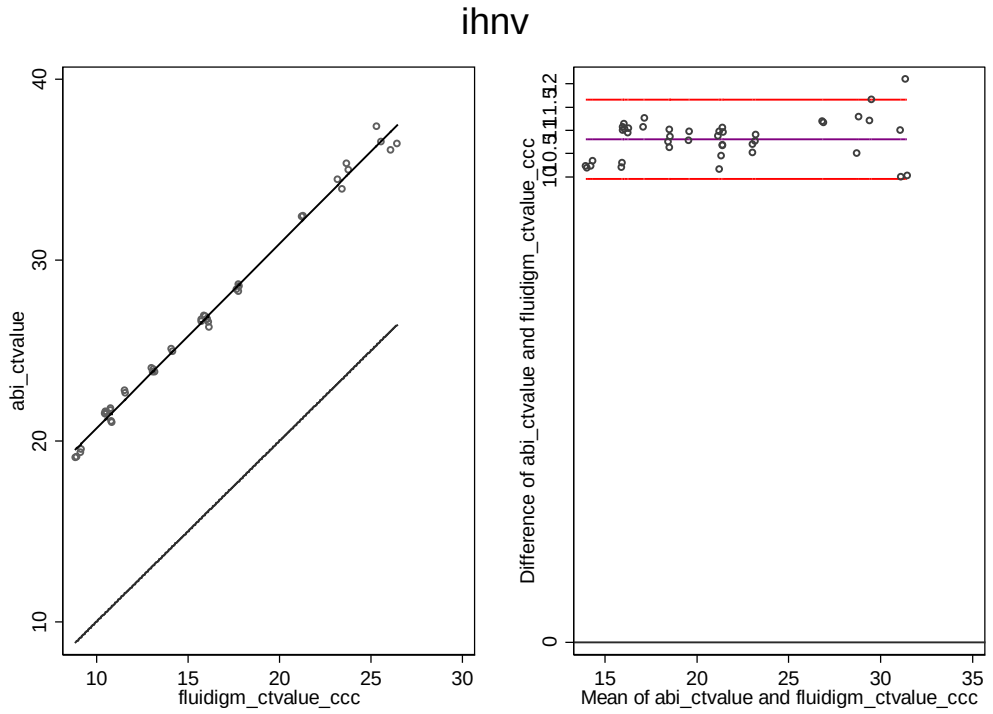

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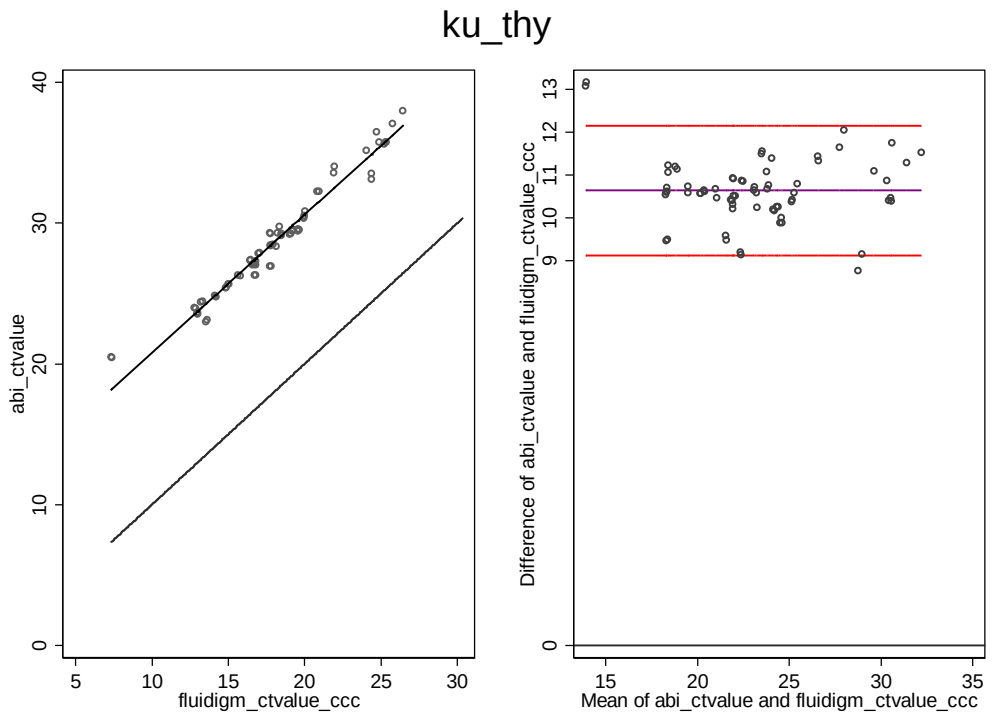

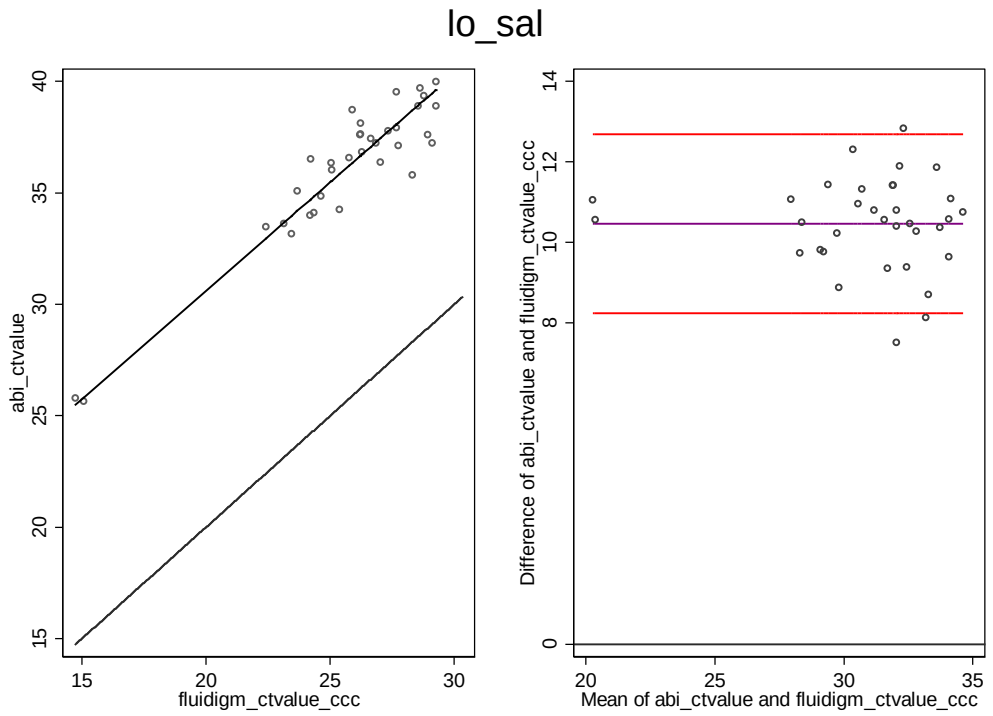

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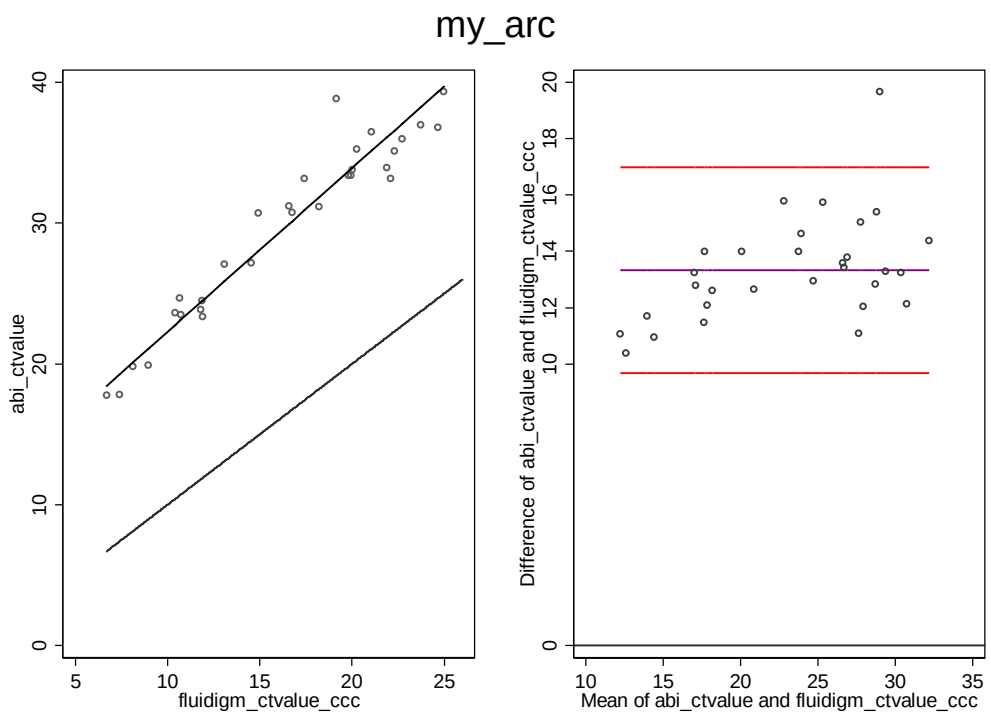

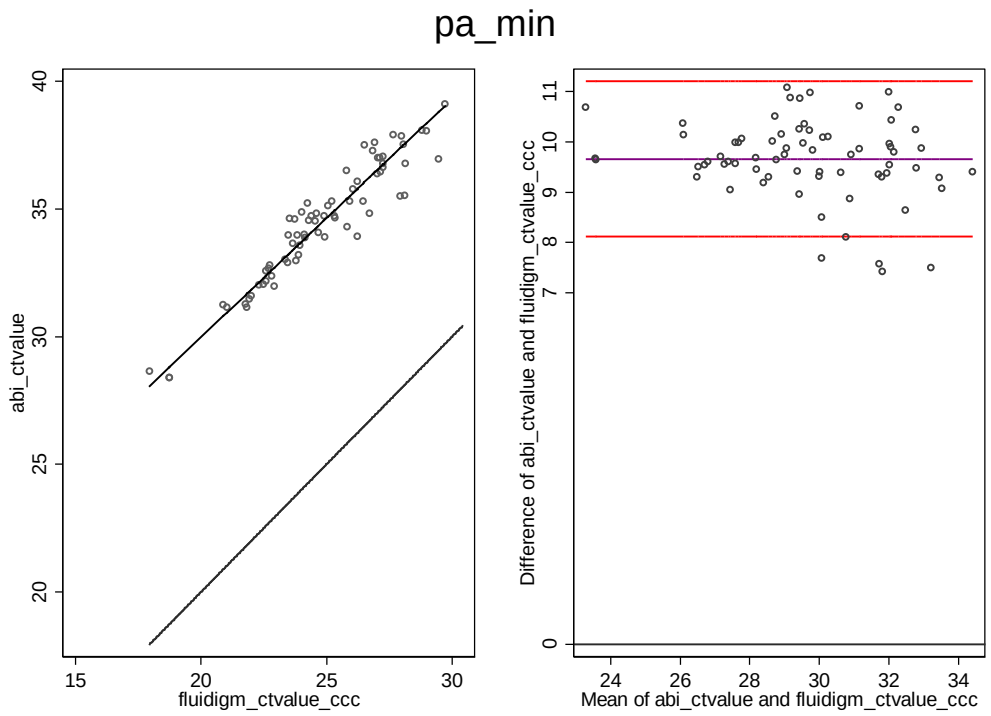

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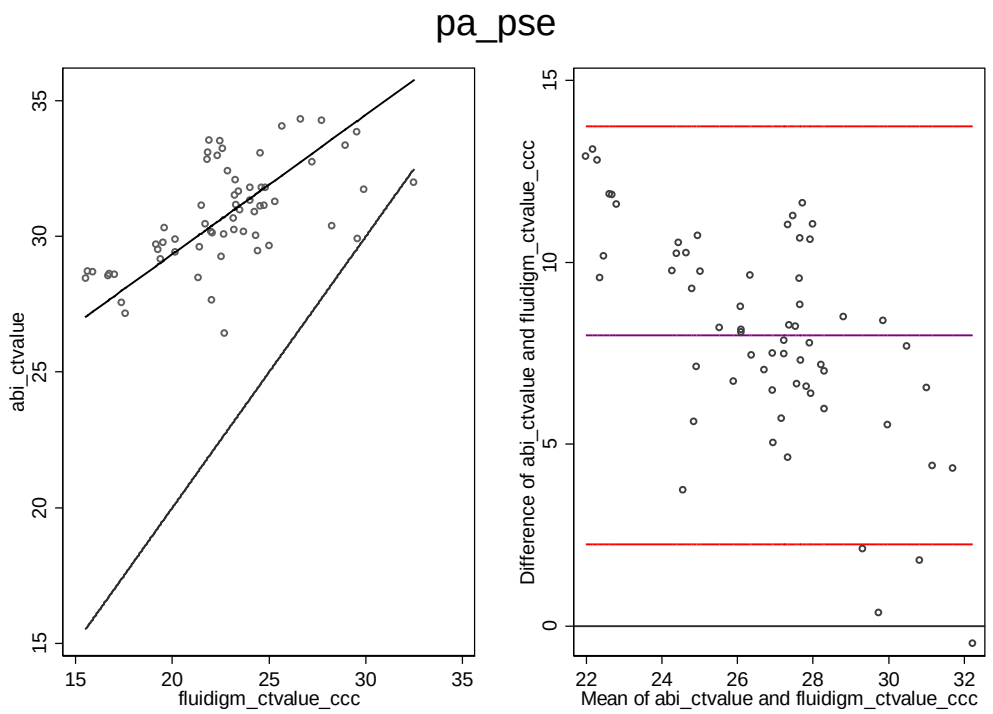

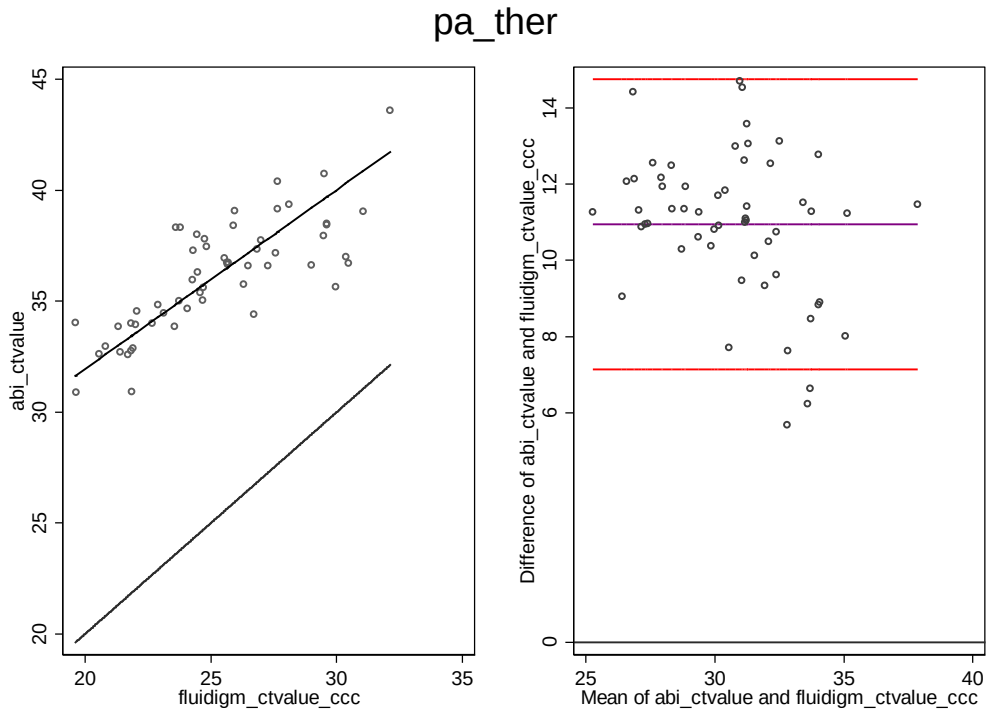

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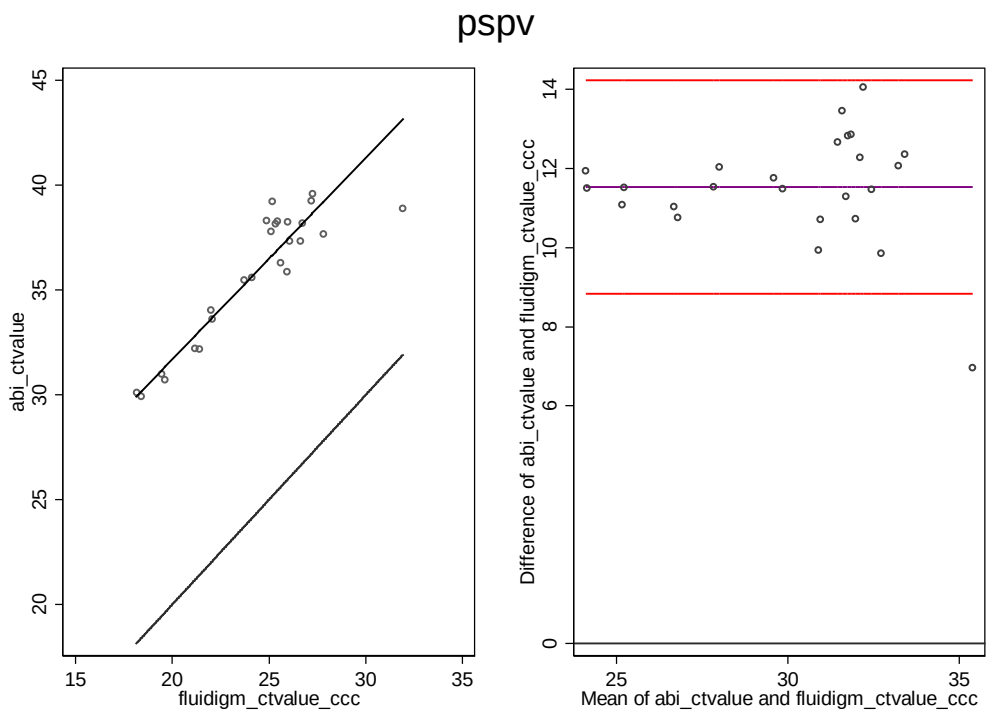

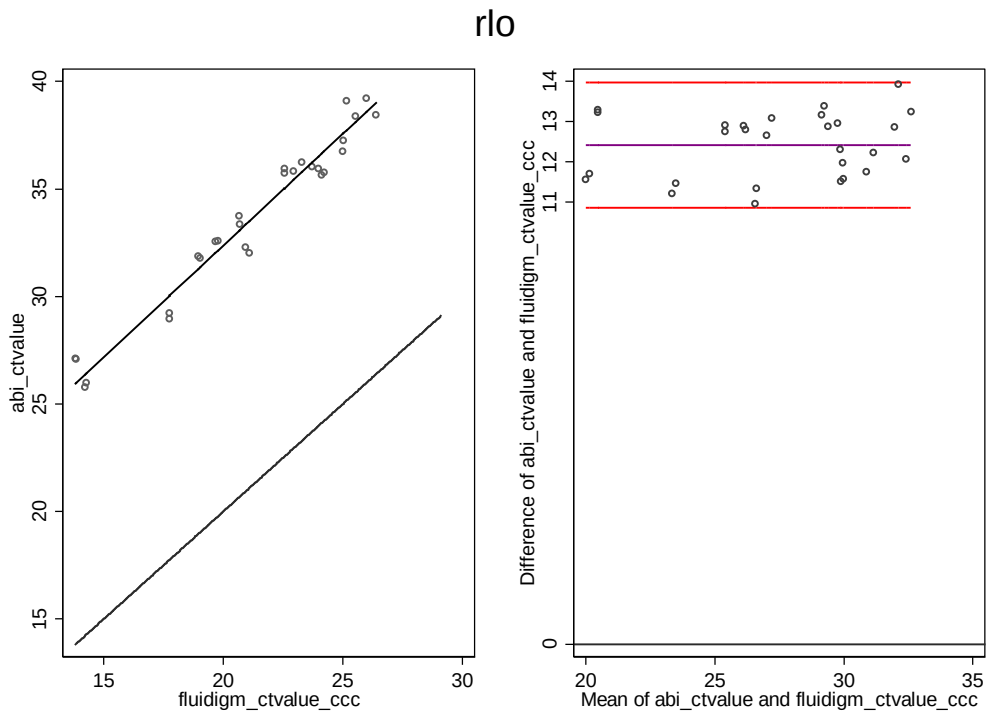

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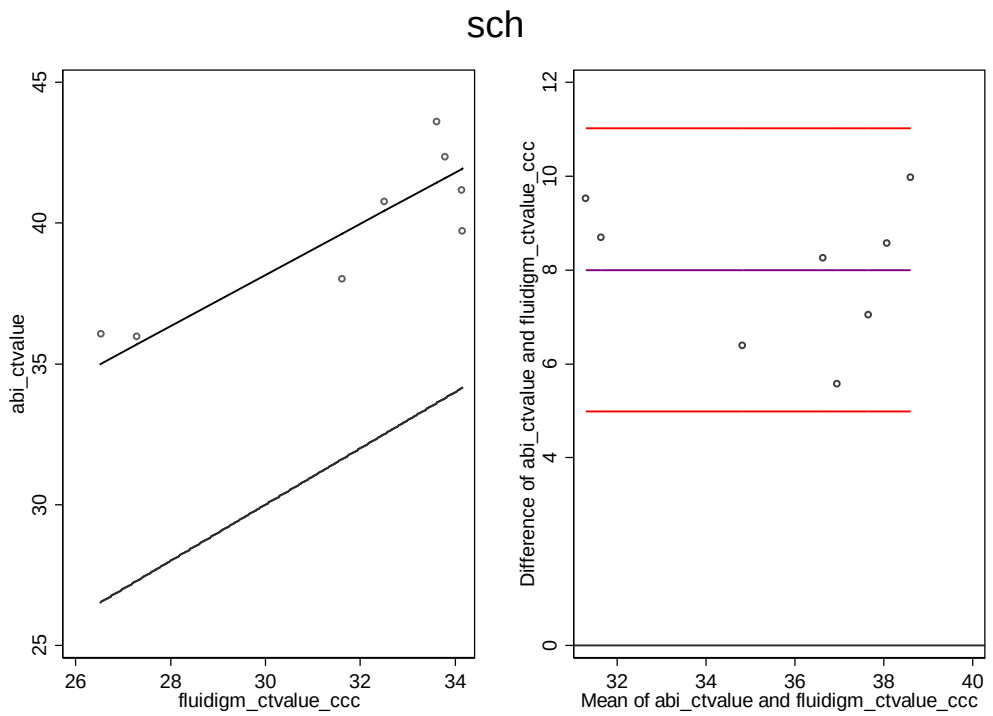

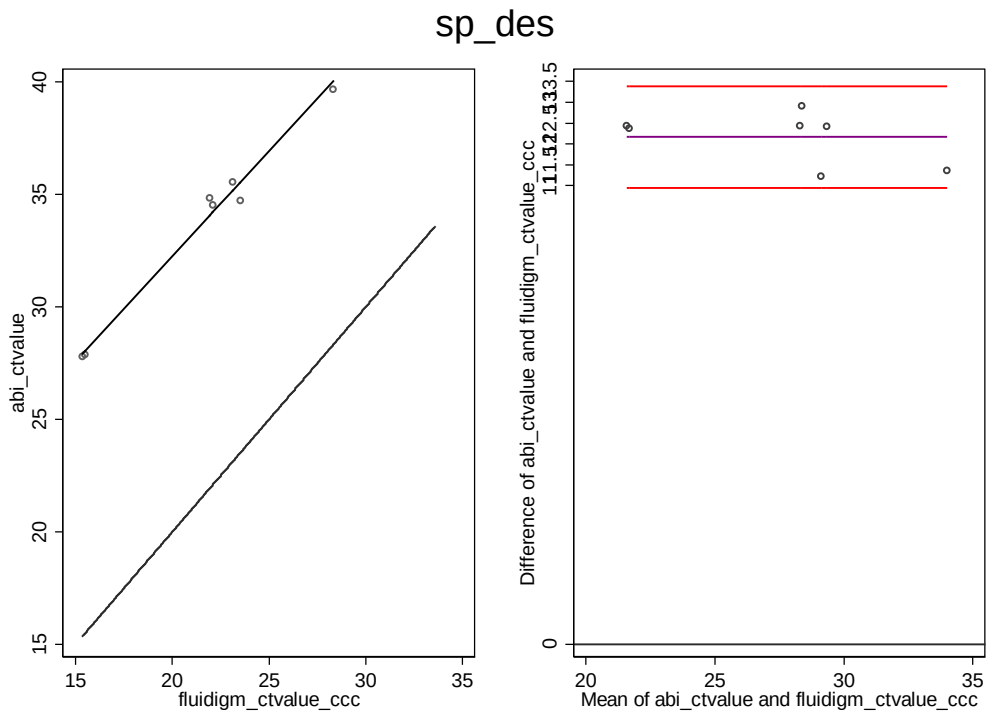

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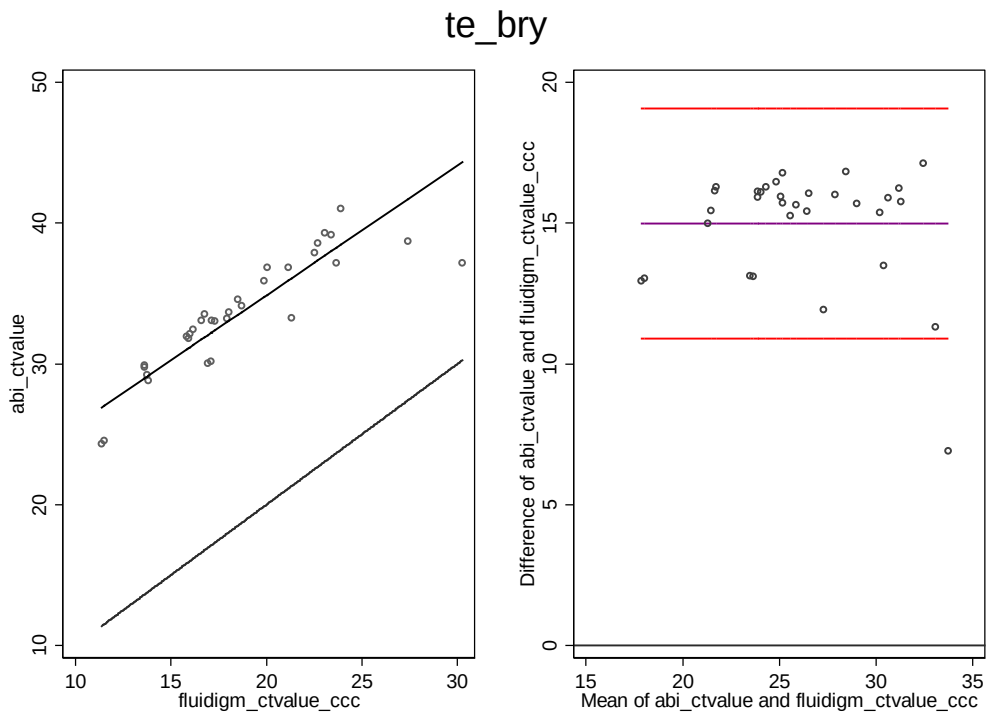

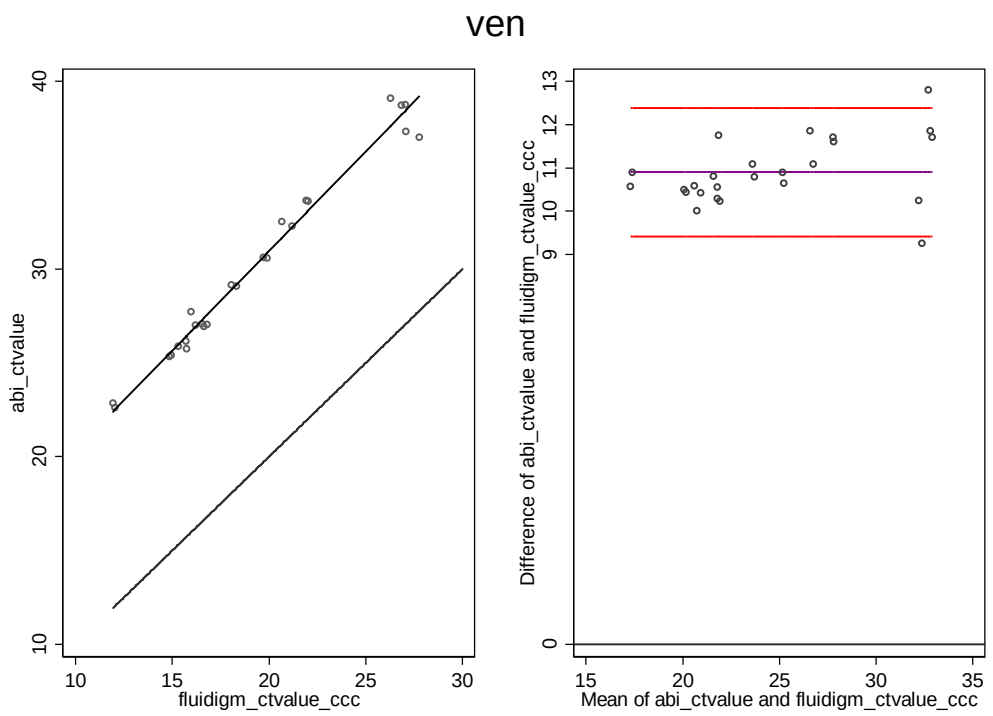

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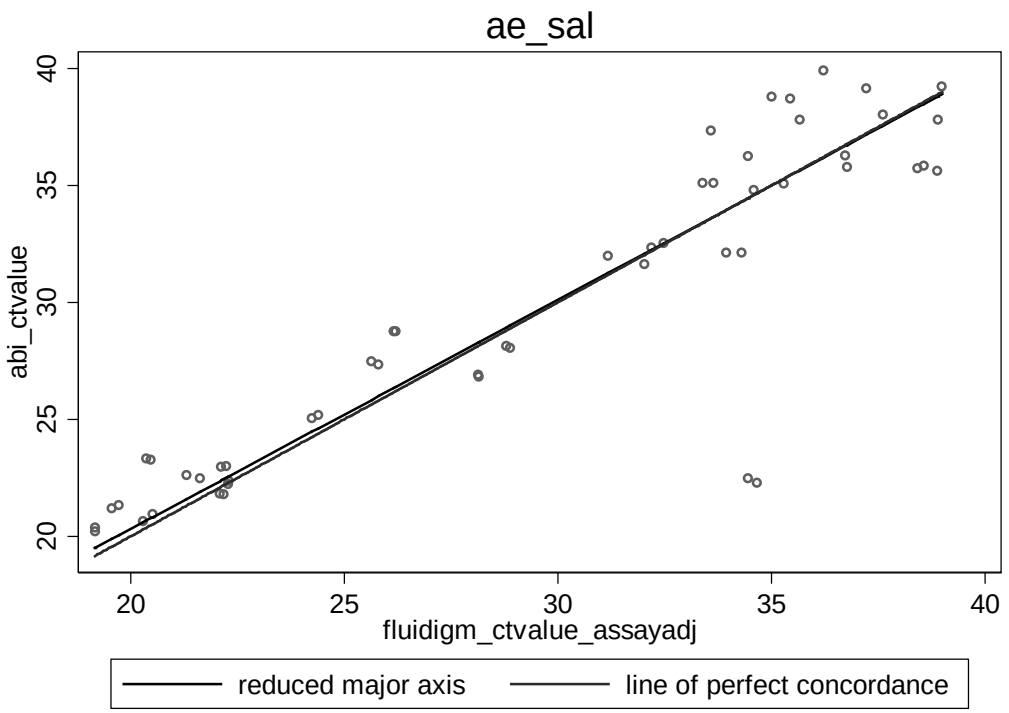
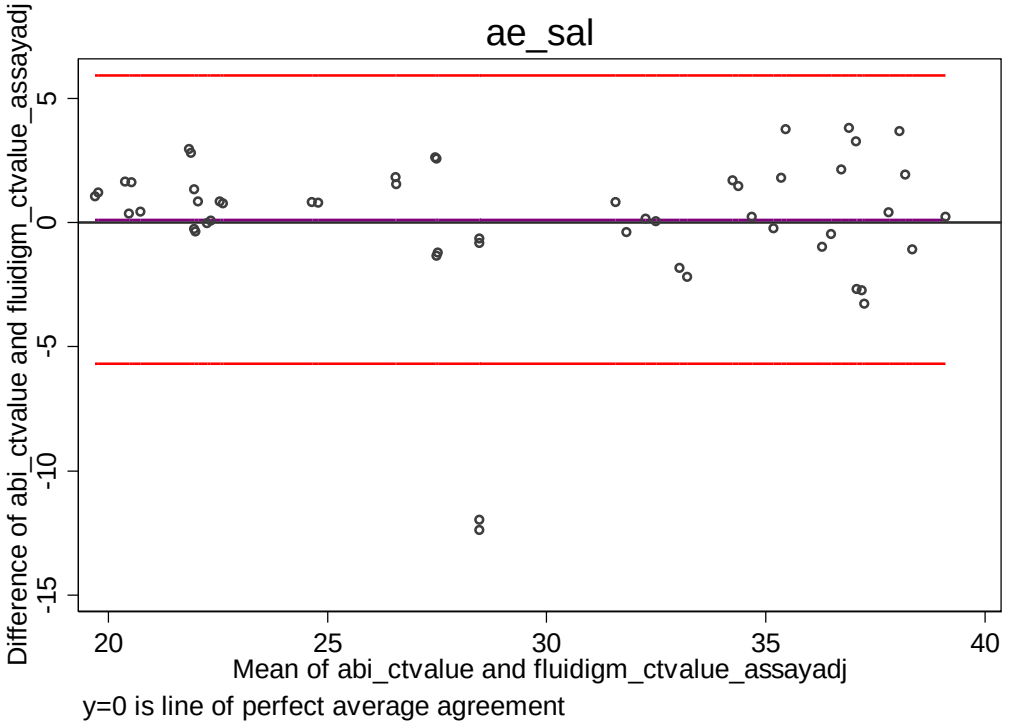
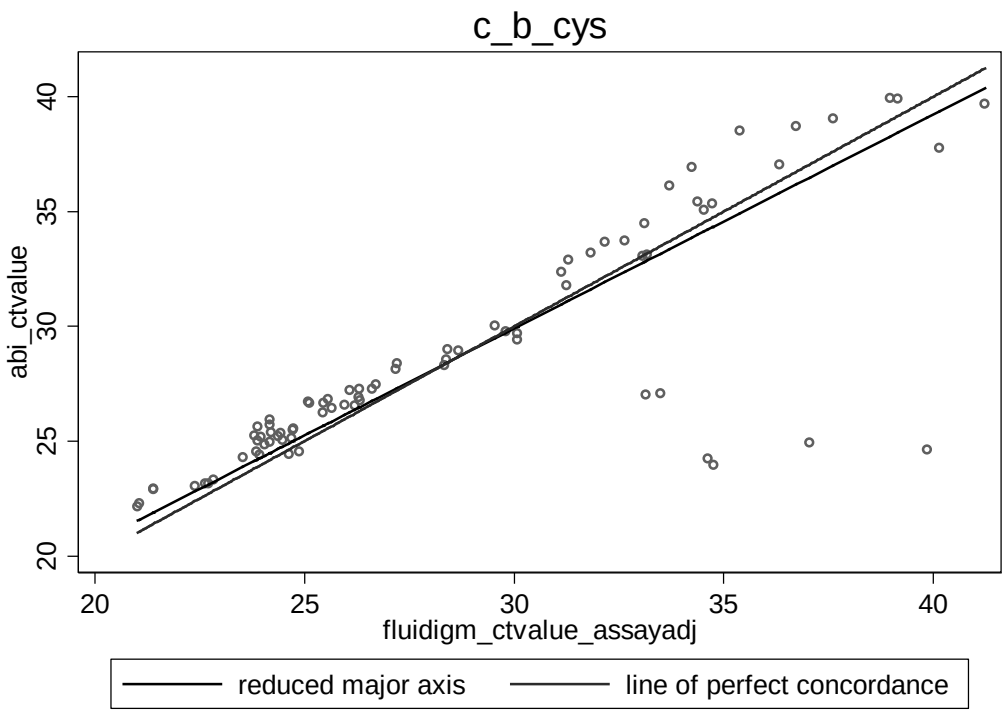
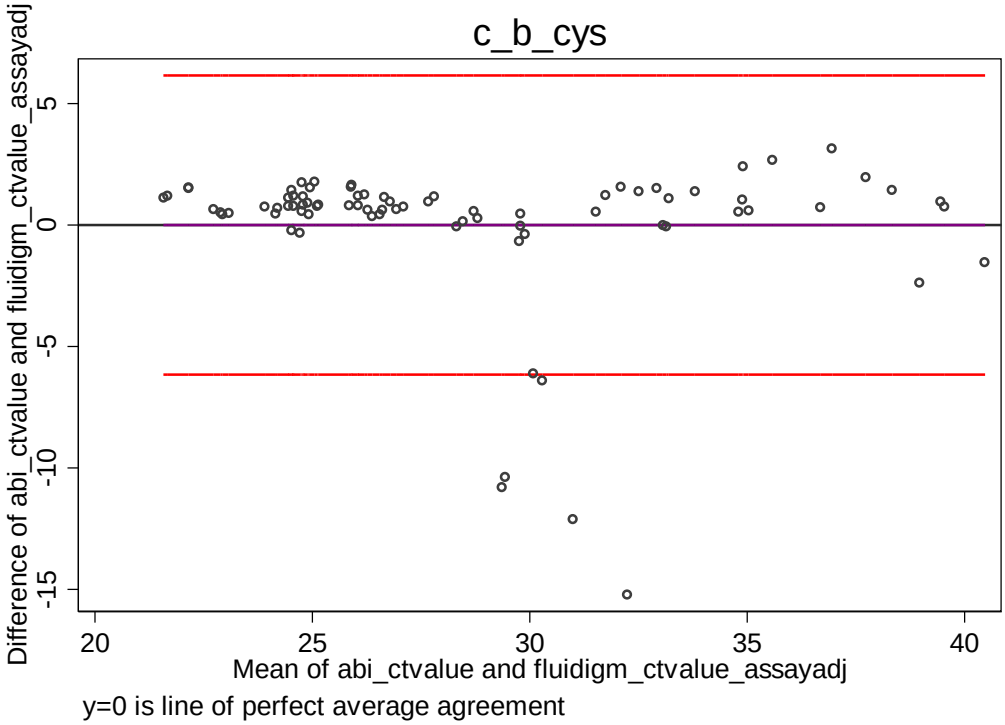




Supplemental Figure 15. Panel graph of concordance correlation coefficients (CCCs) and limit of agreement (LOA) by assay (n=26) comparing ABI 7900 and Fluidigm systems, using assay-specific Ct adjusted values. The list of assays in these analyses include (continued over the next several pages): ae_sal, c_b_cys, ce_sha, cr_sal, de_sal, fl_psy, ic_hof, ic_mul, ihnv, ku_thy, lo_sal, my_arc, pa_min, pa_pse, pa_ther, pspv, rlo, sch, sp_des, te_bry, and ven.





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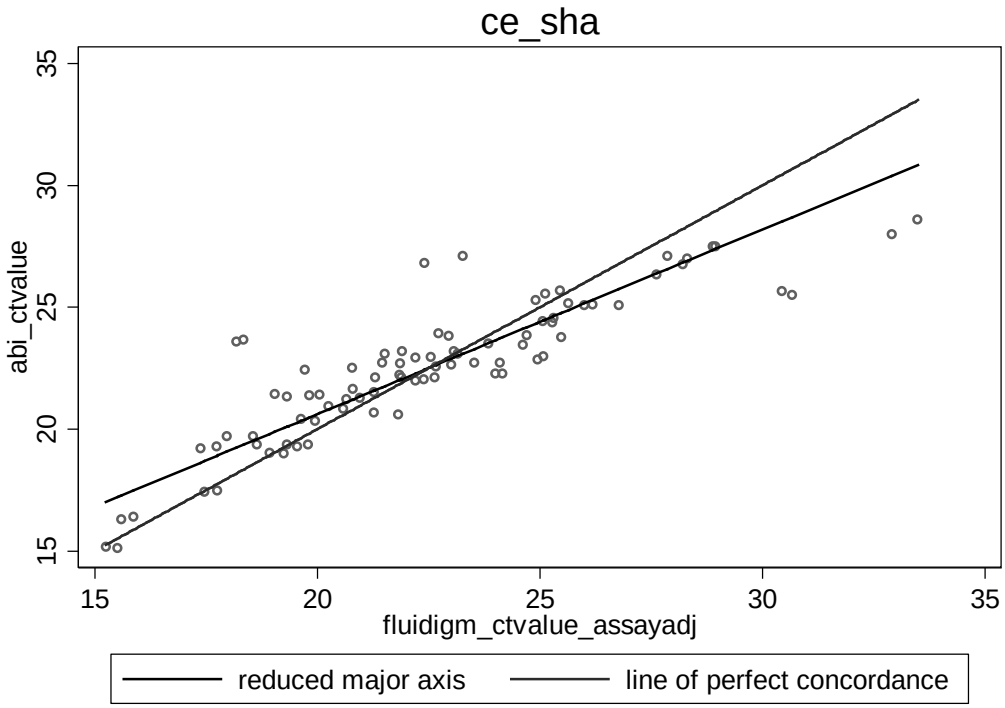

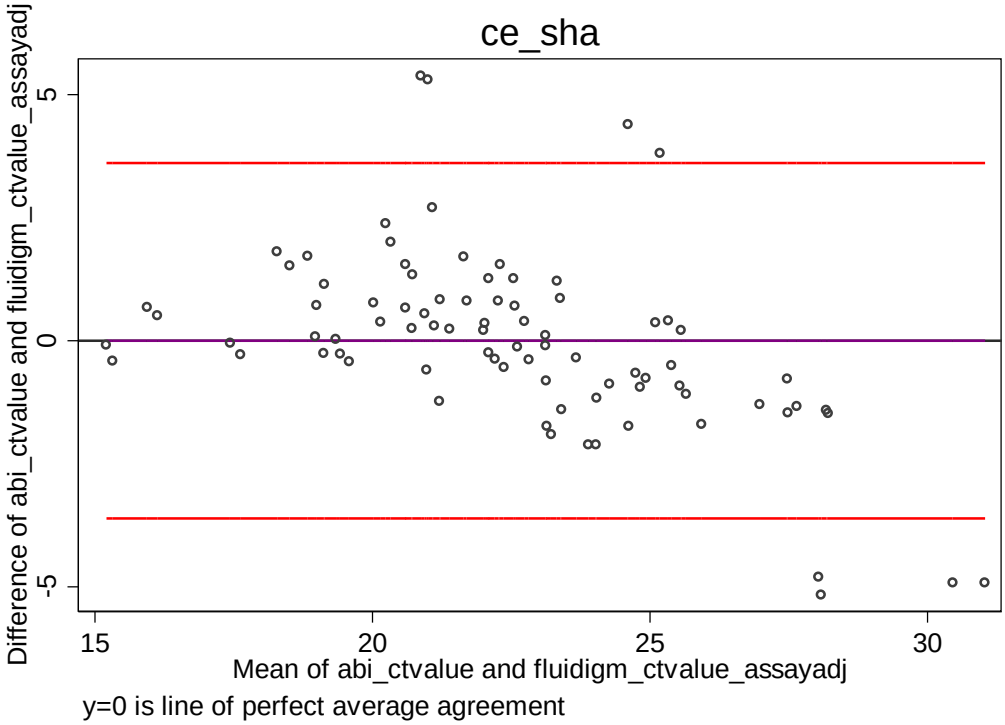

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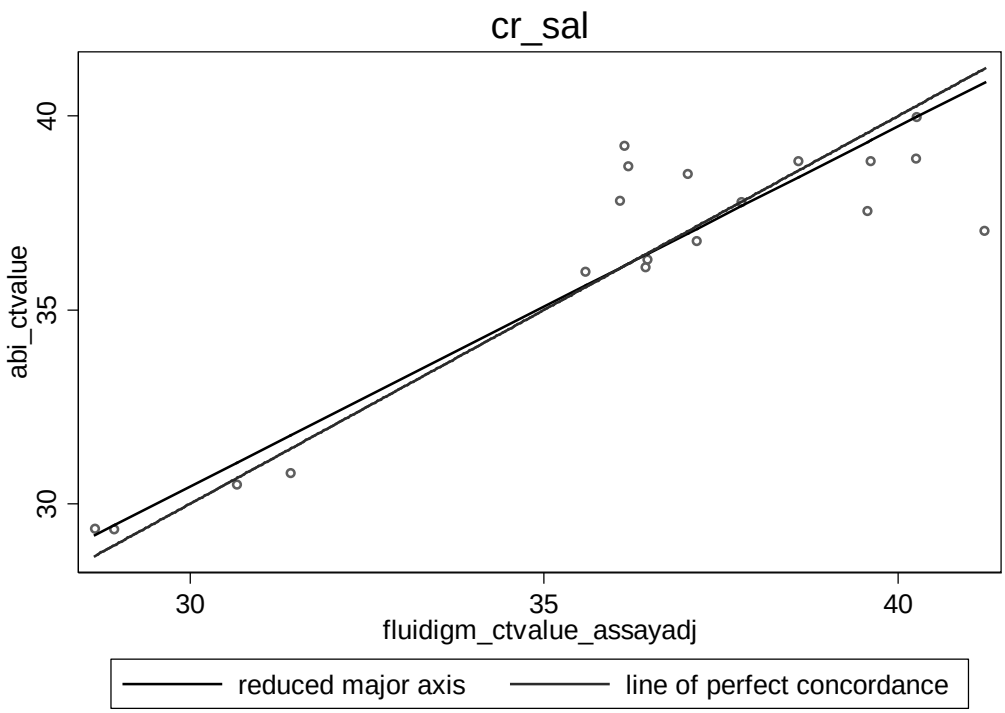

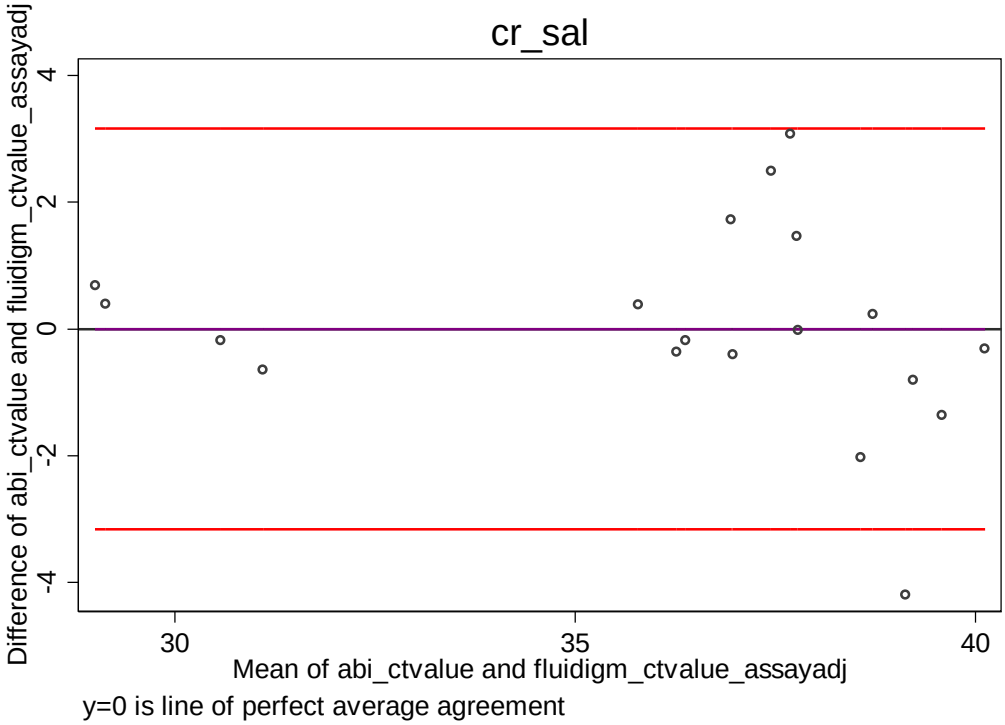

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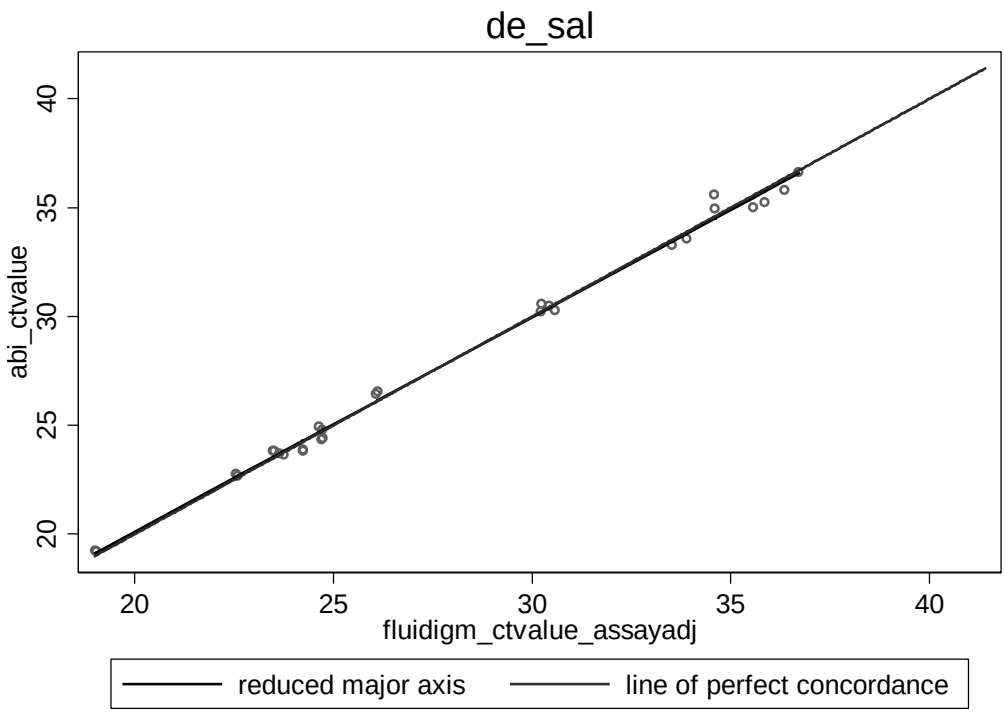

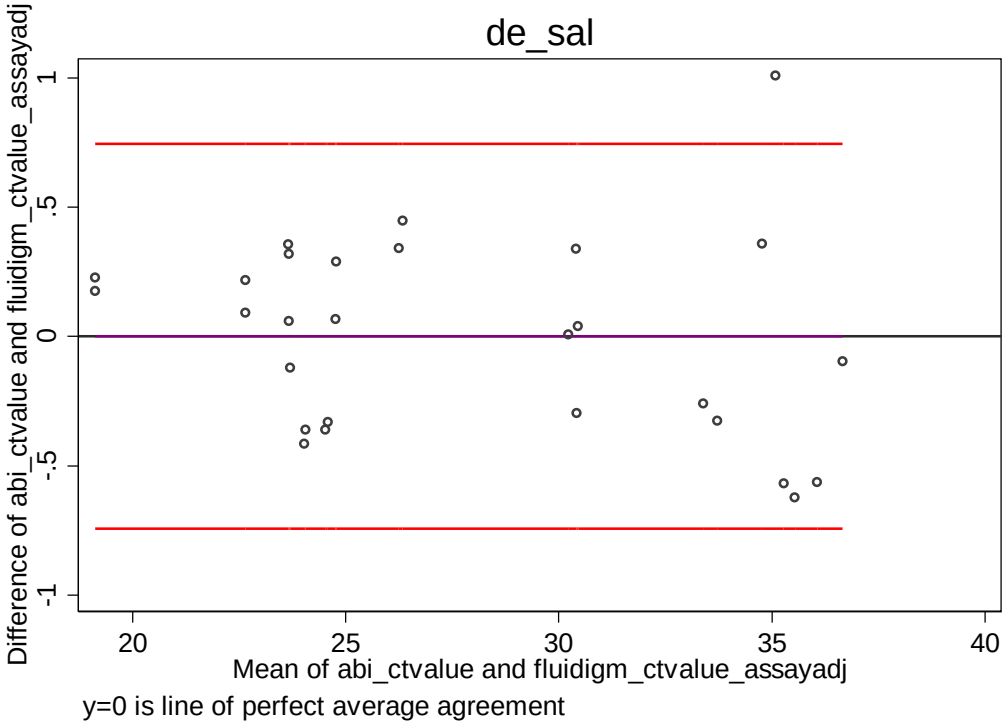

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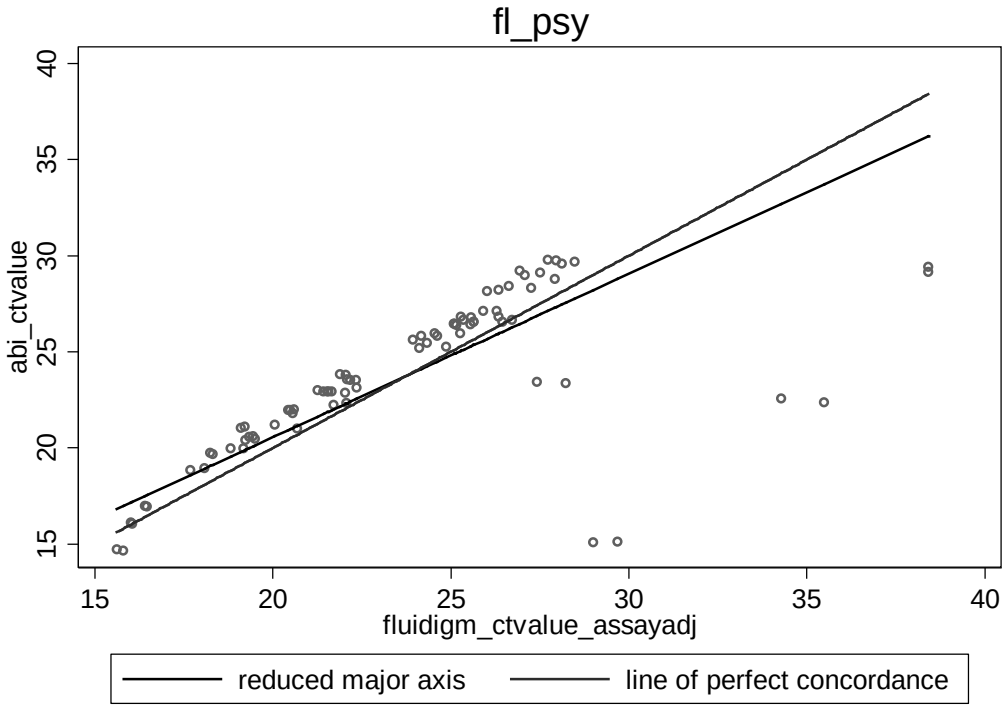

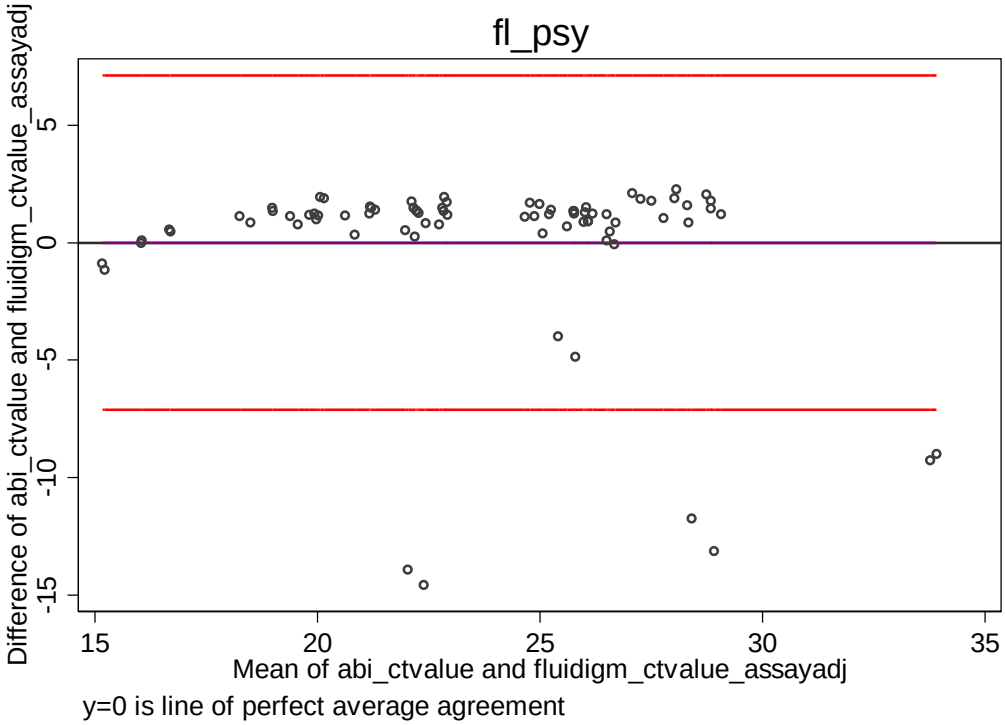

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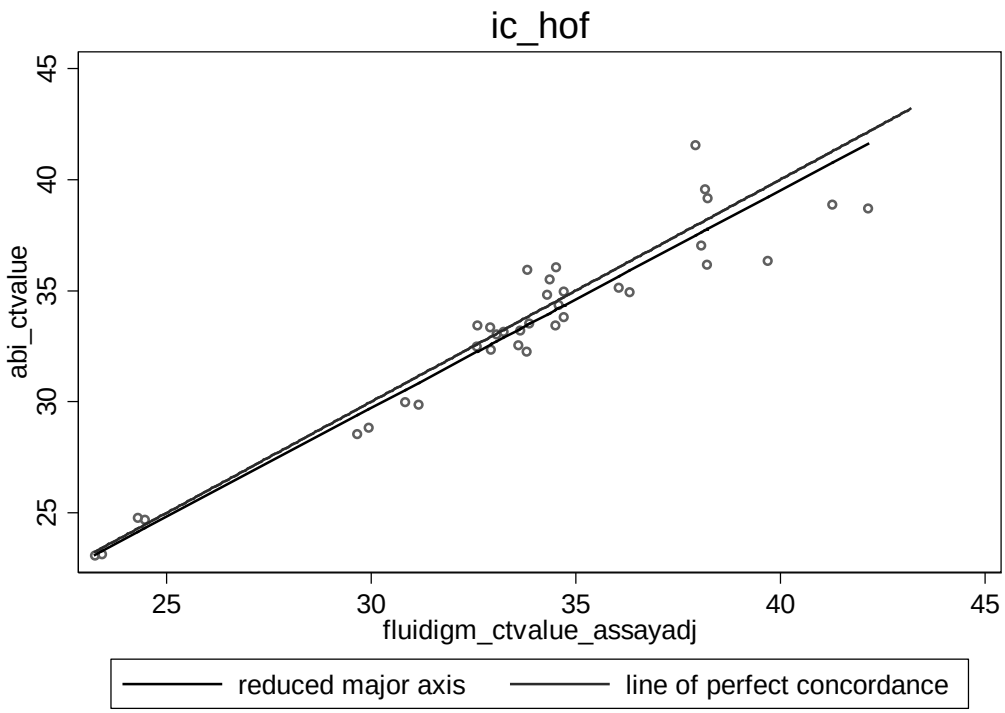

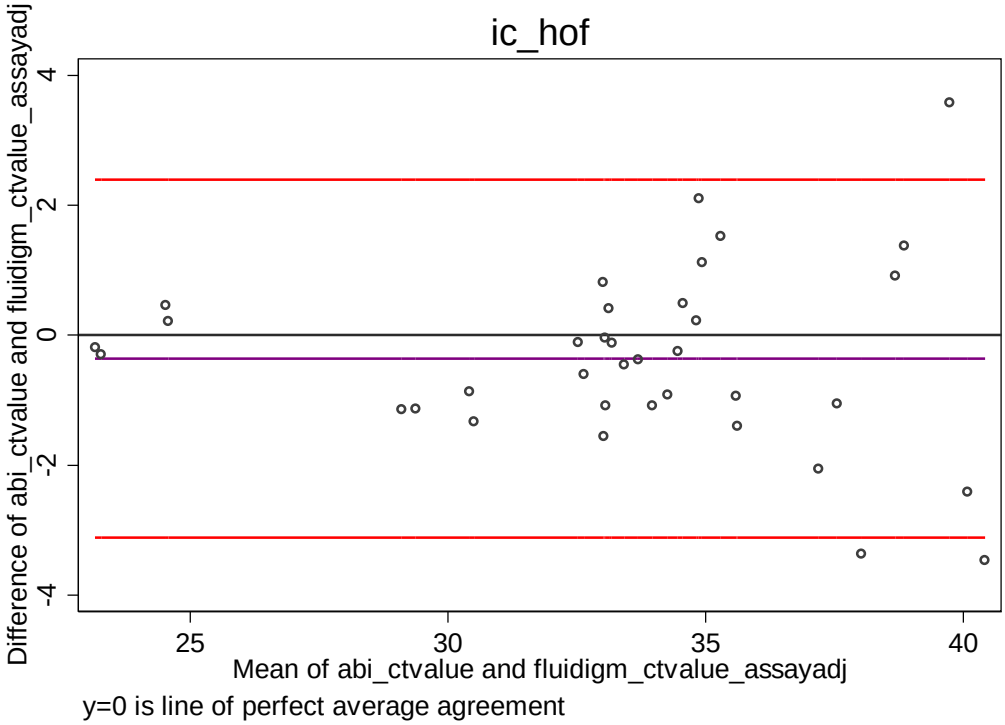

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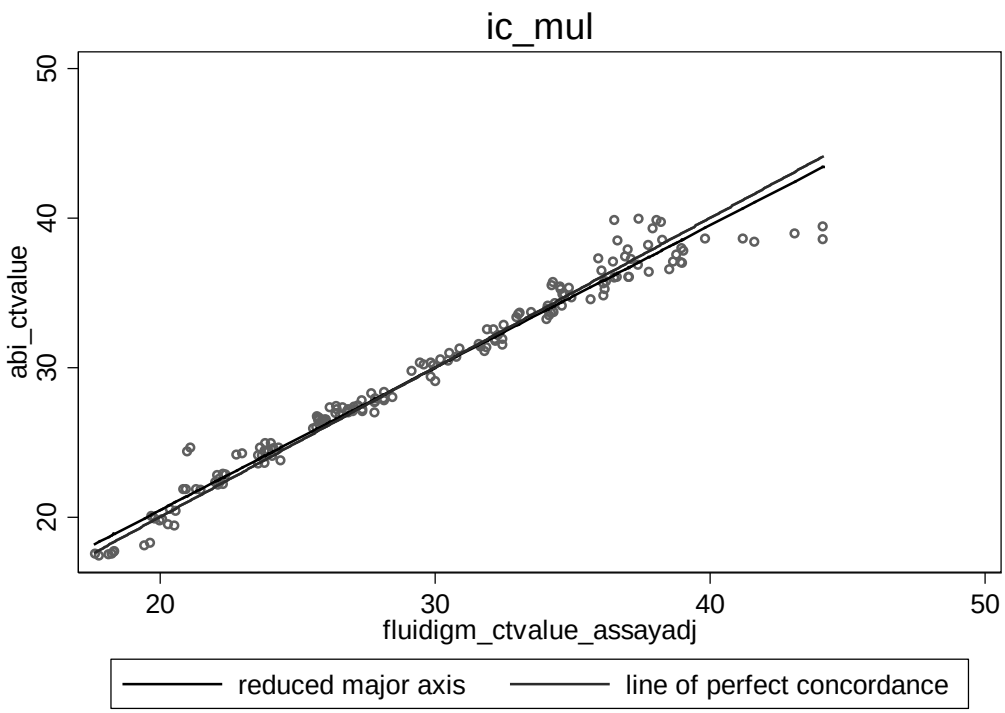
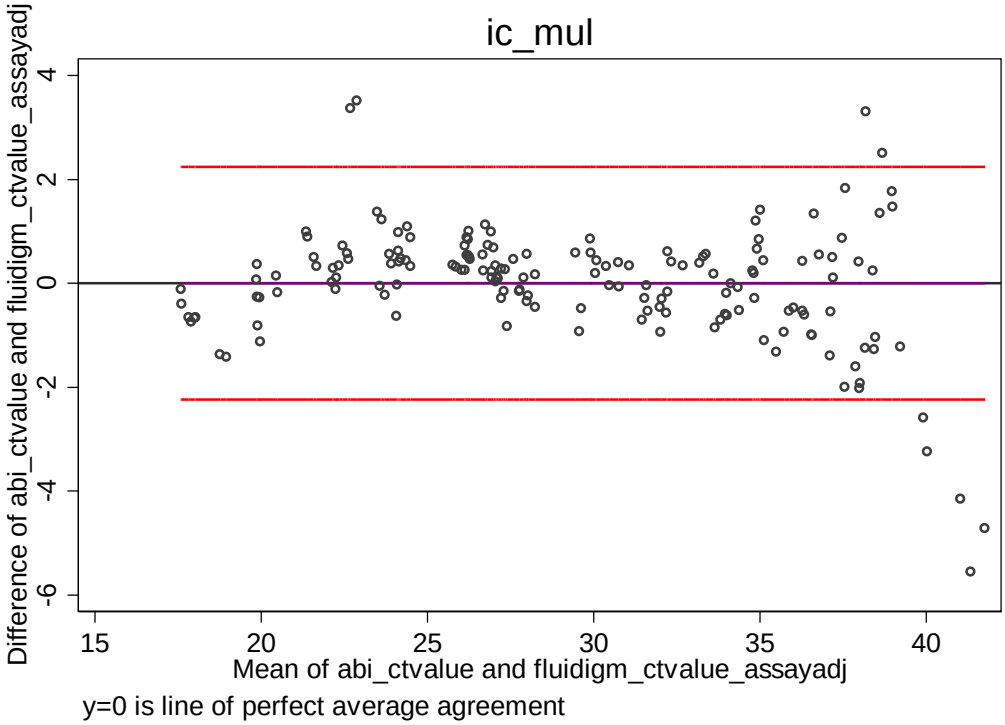
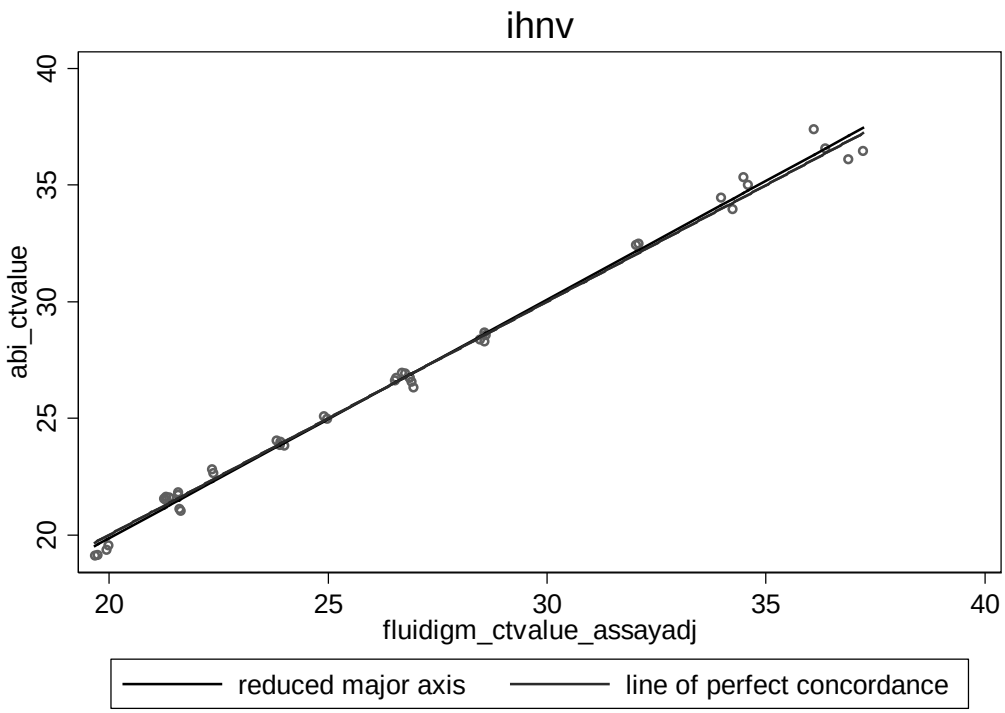
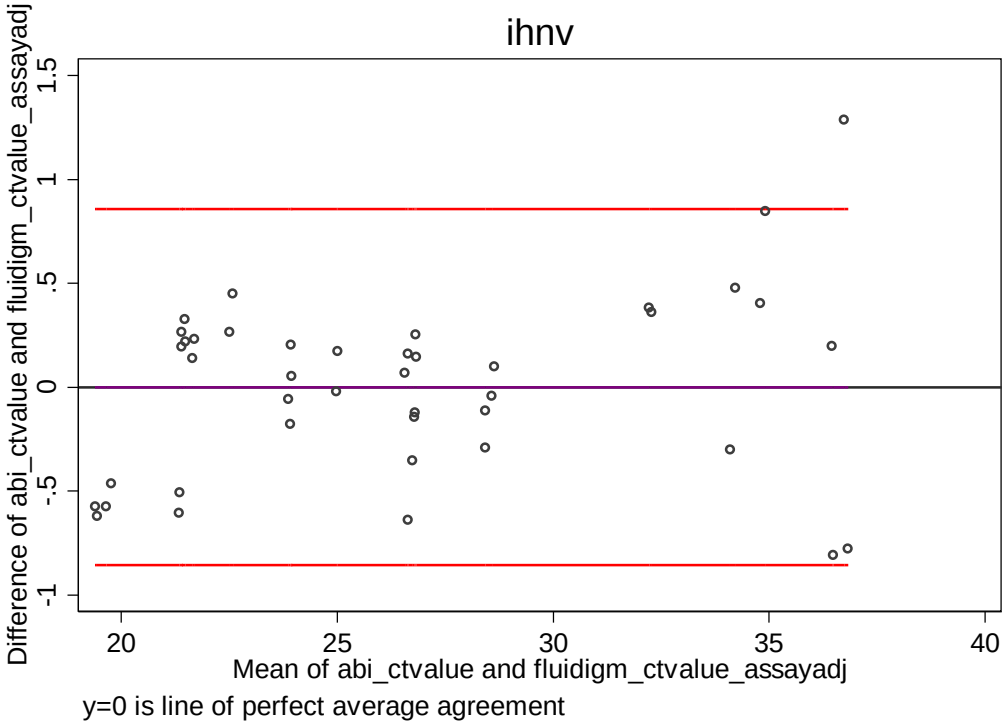


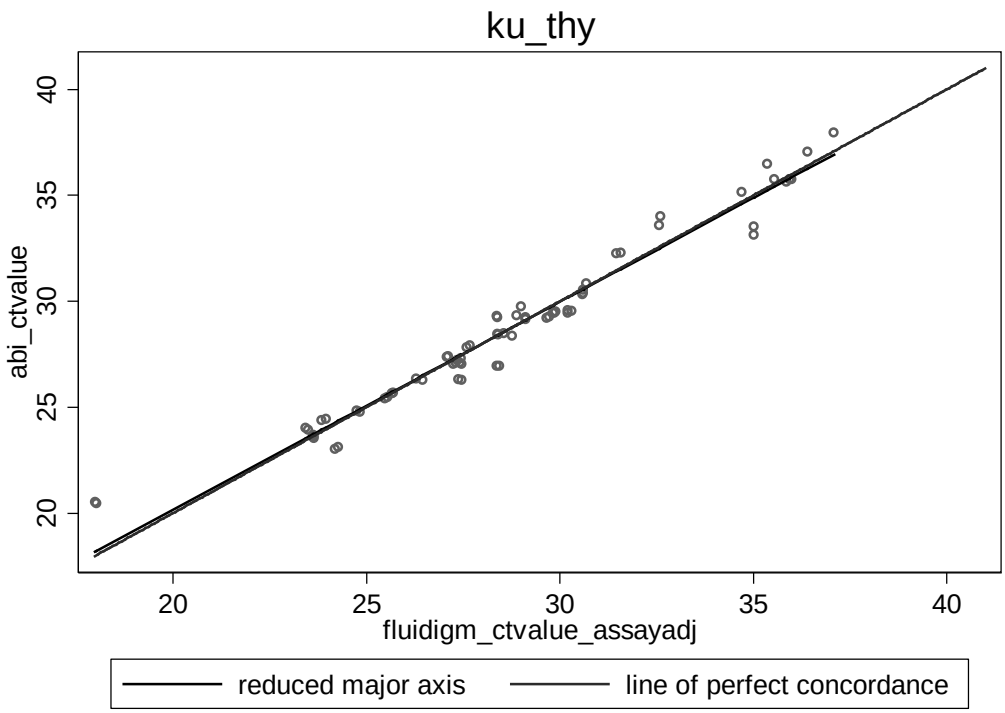


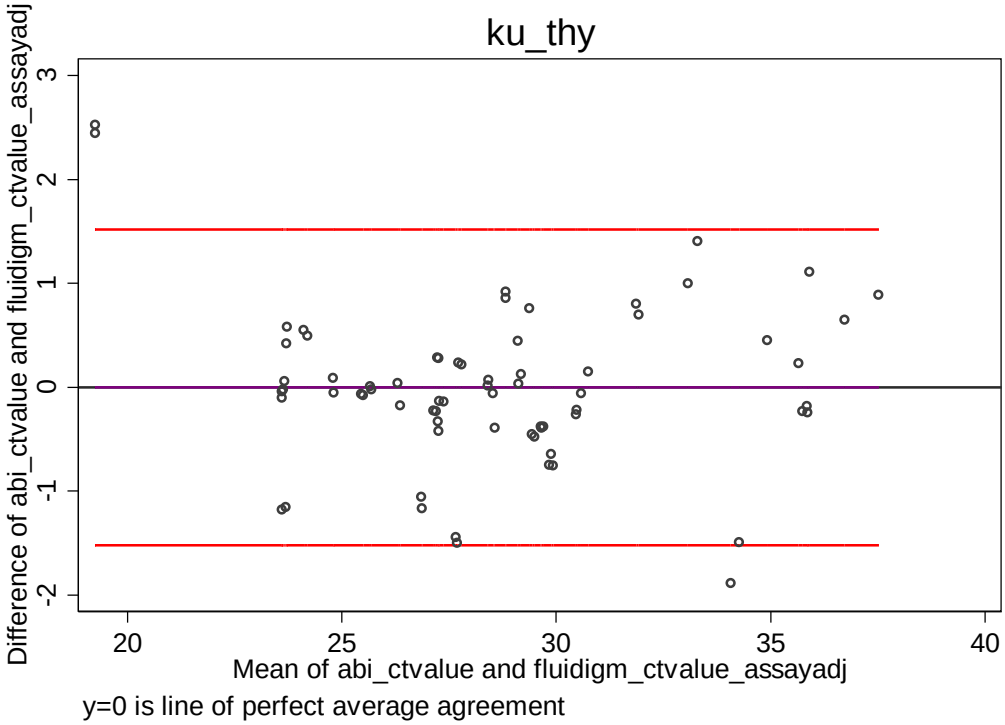
Supplemental Figure 15 (Continued)





Supplemental Figure 15 (Continued)





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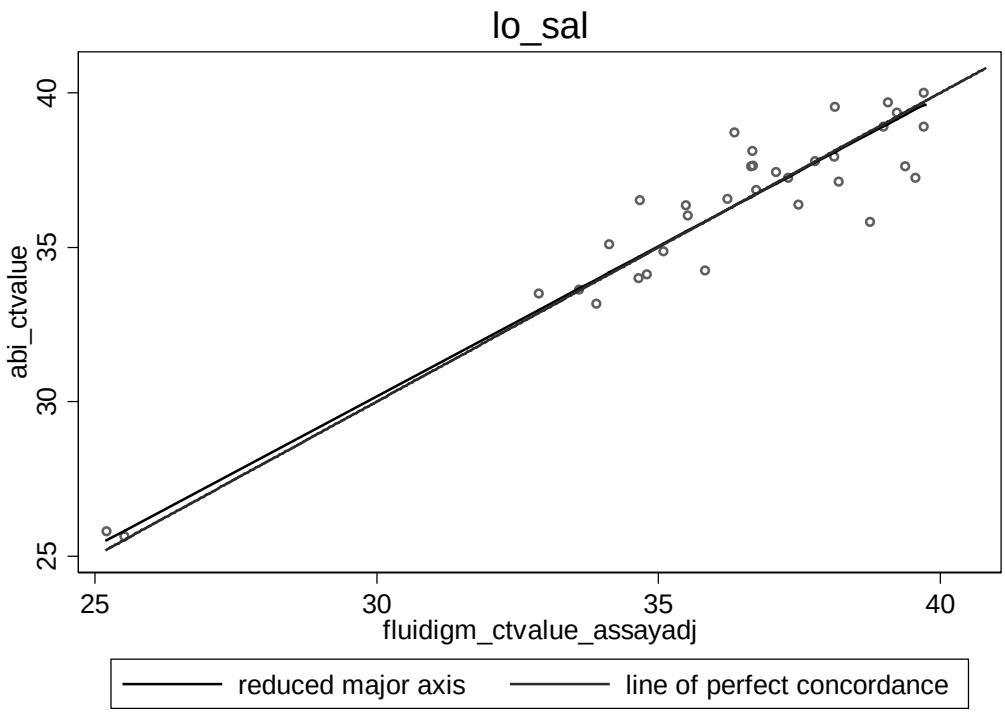

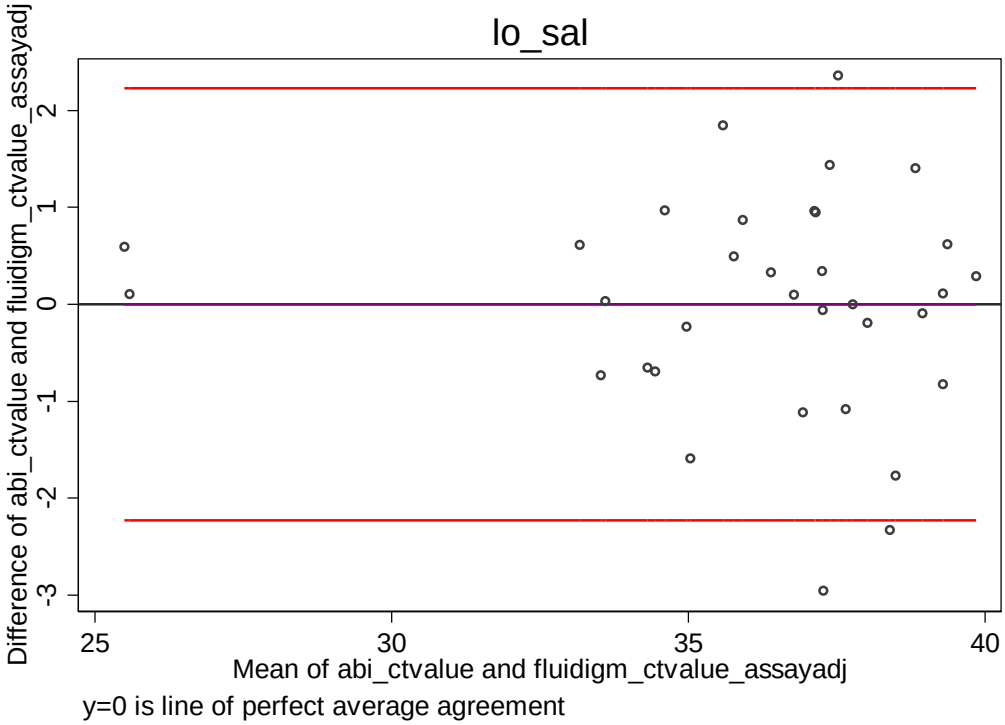

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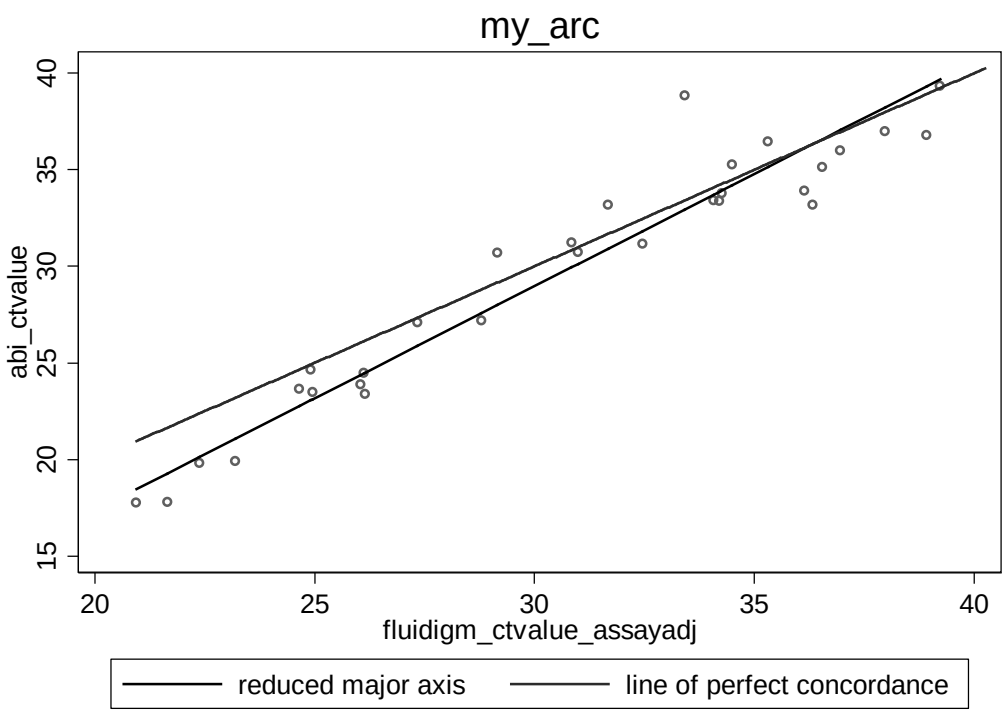

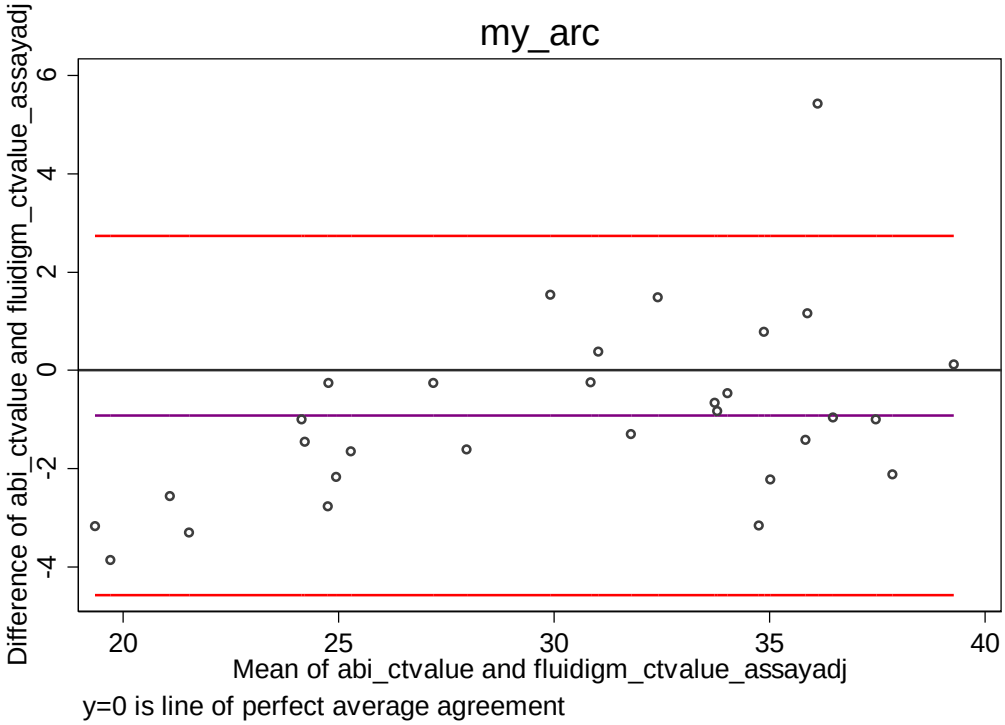

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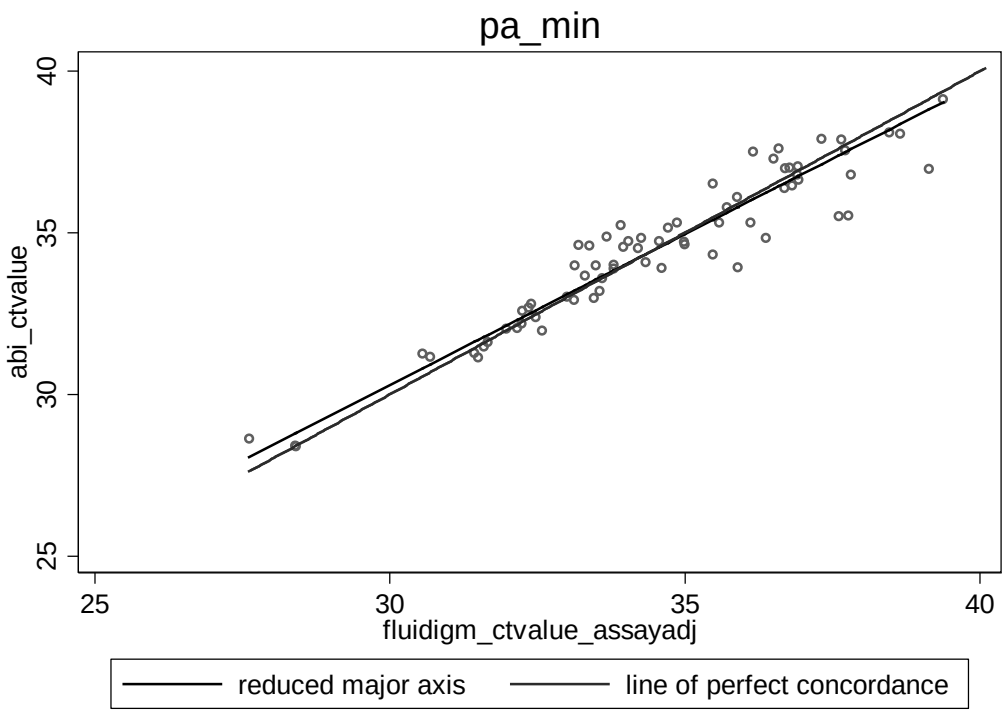

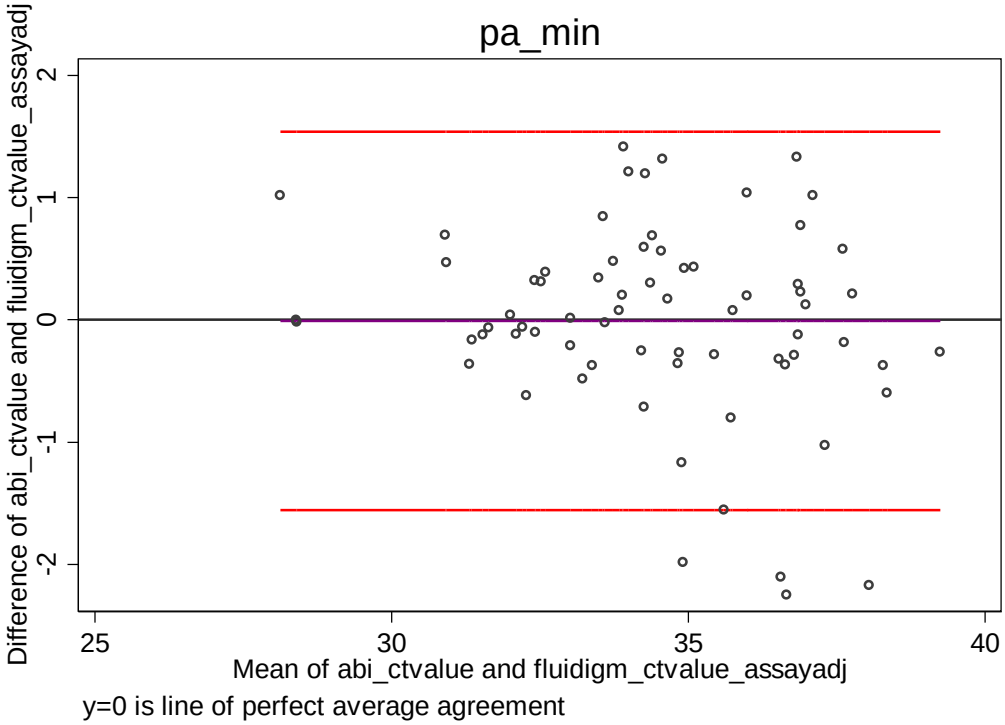

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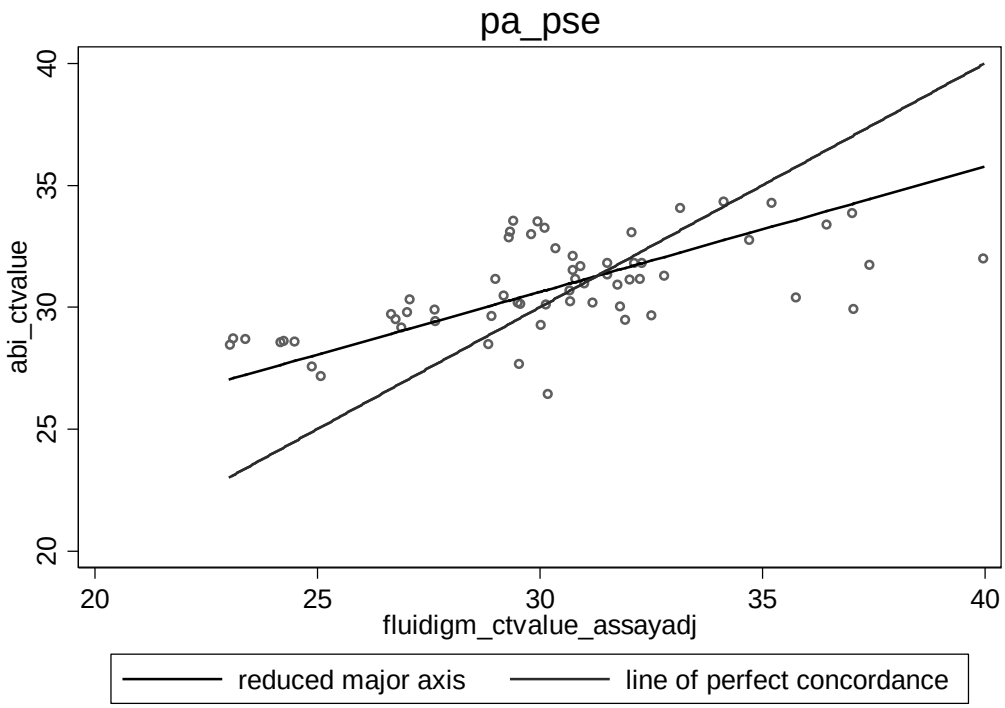

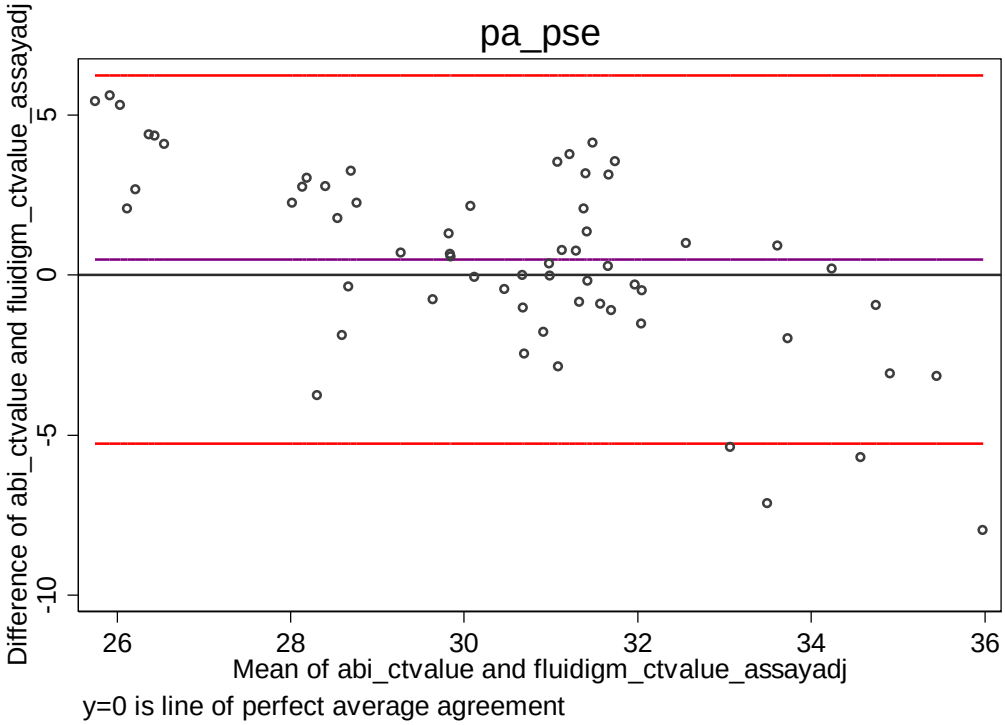

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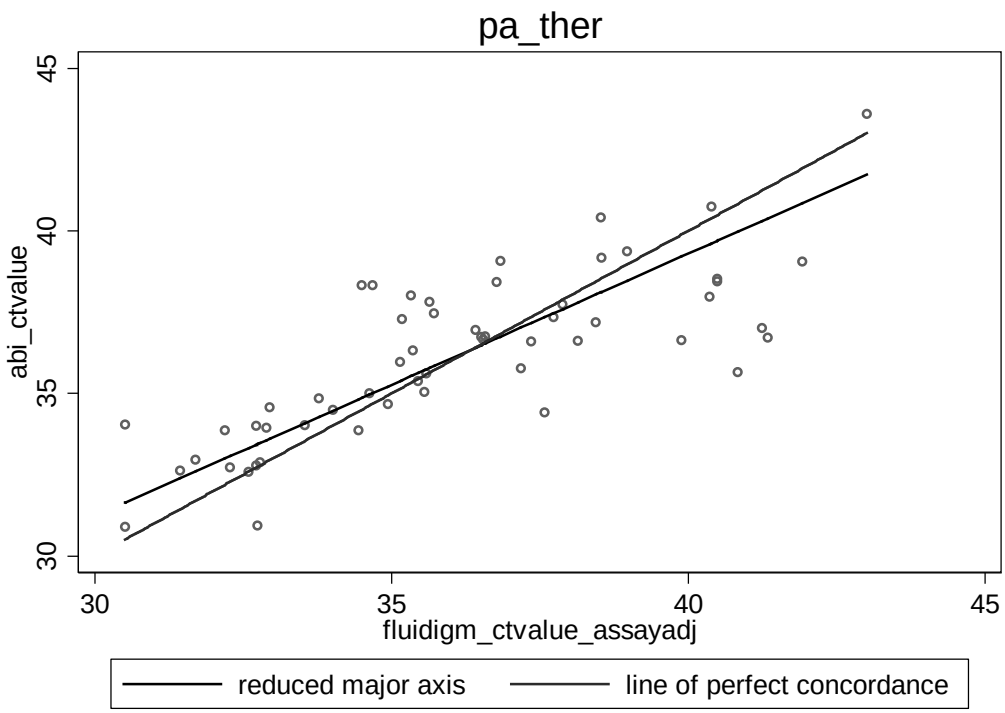
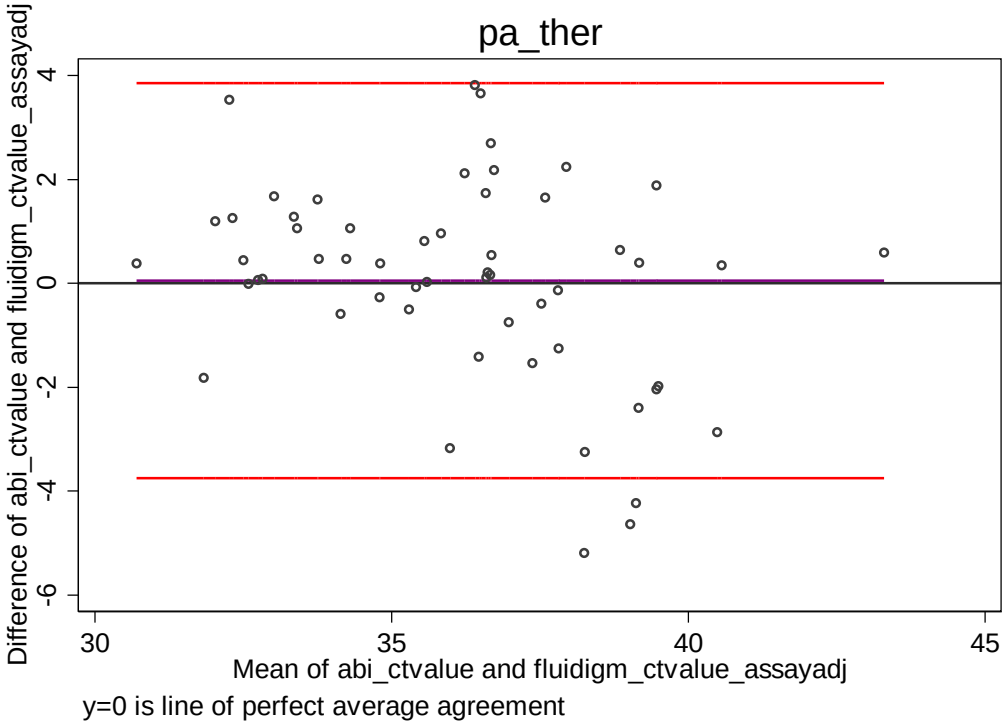
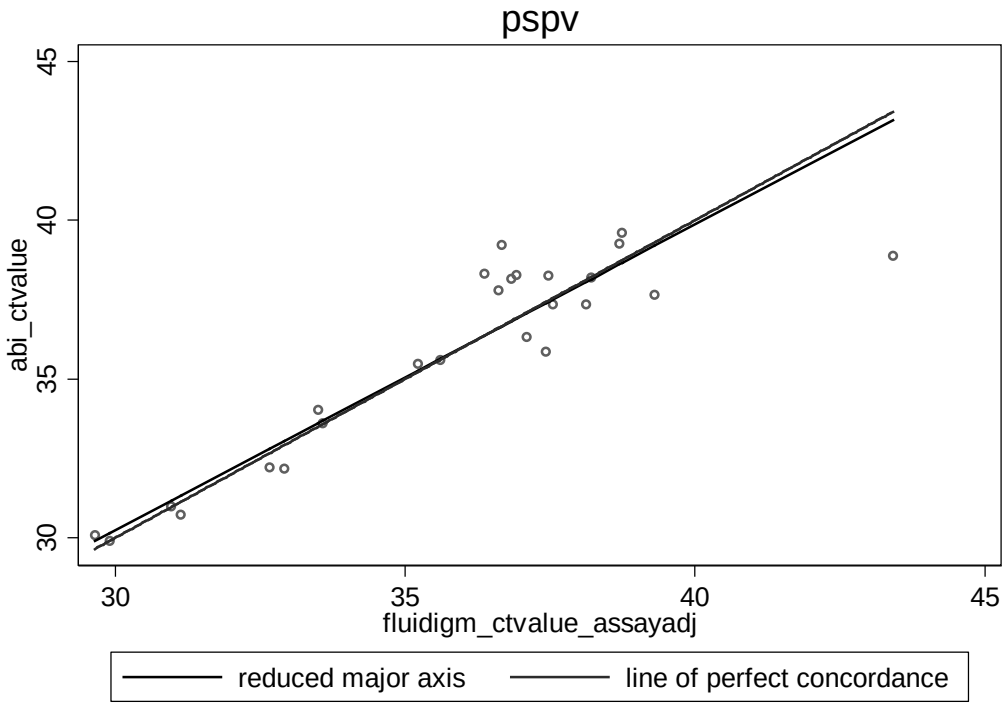
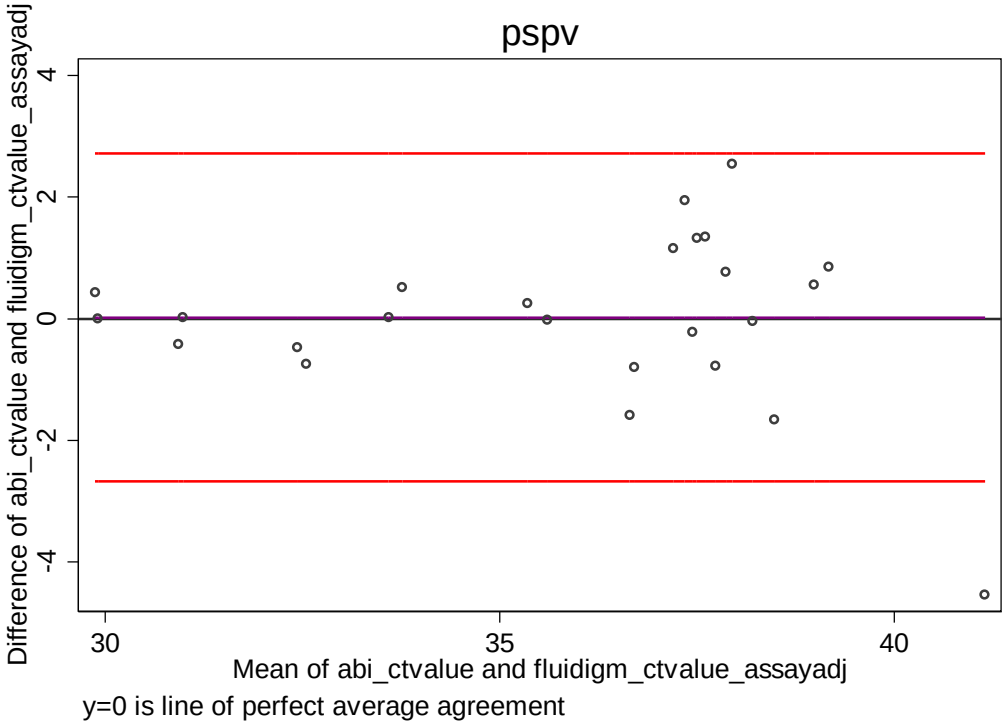


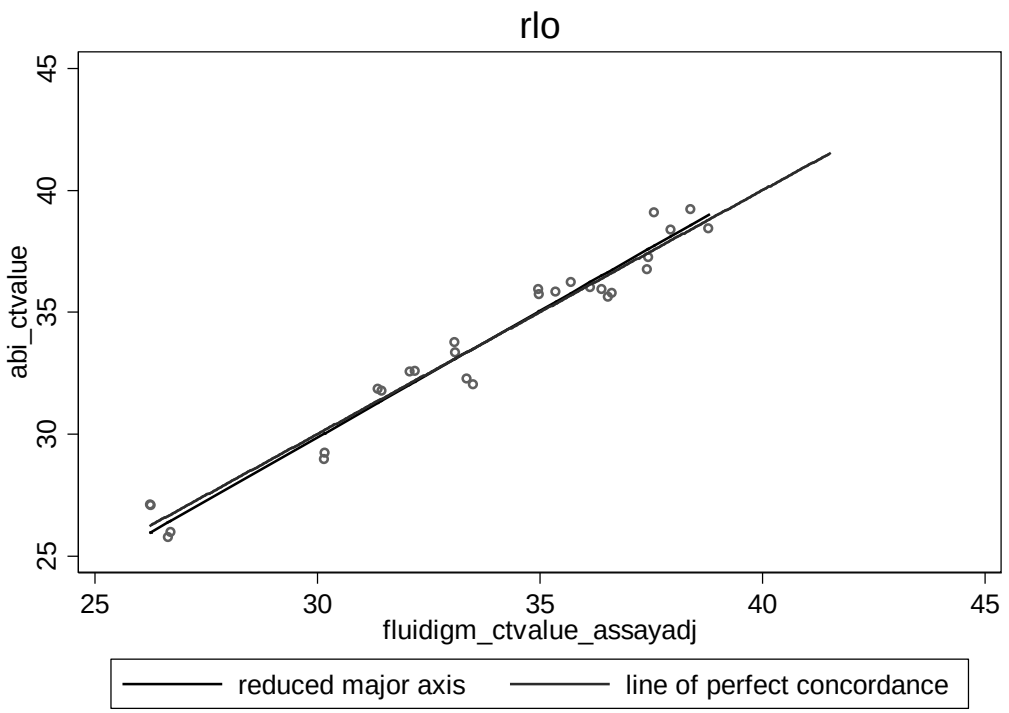


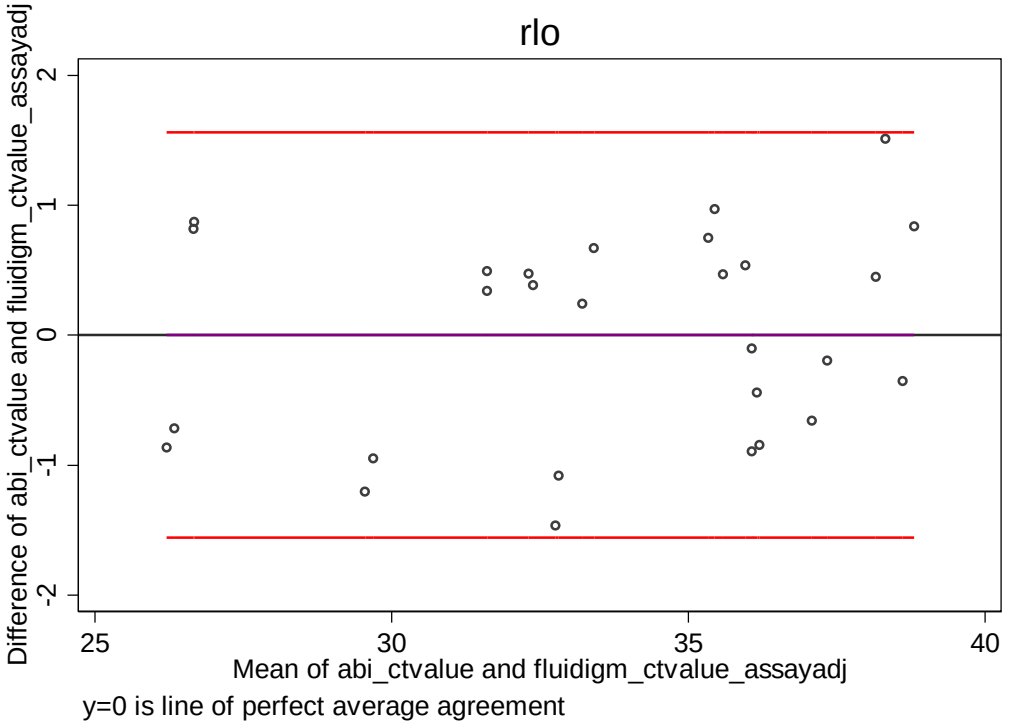
Supplemental Figure 15 (Continued)



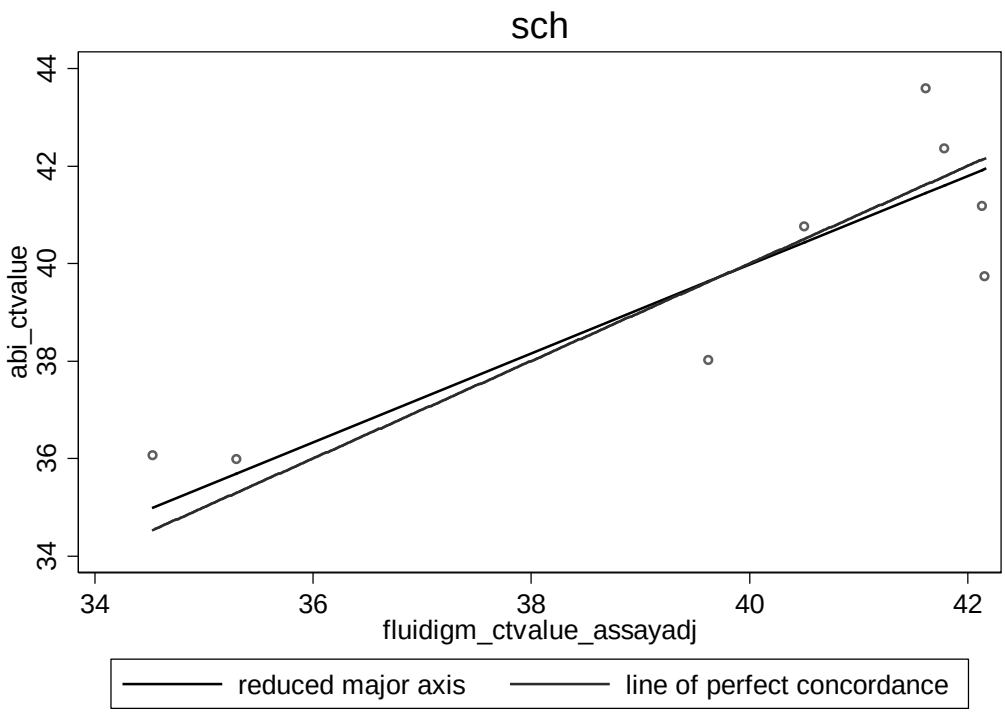


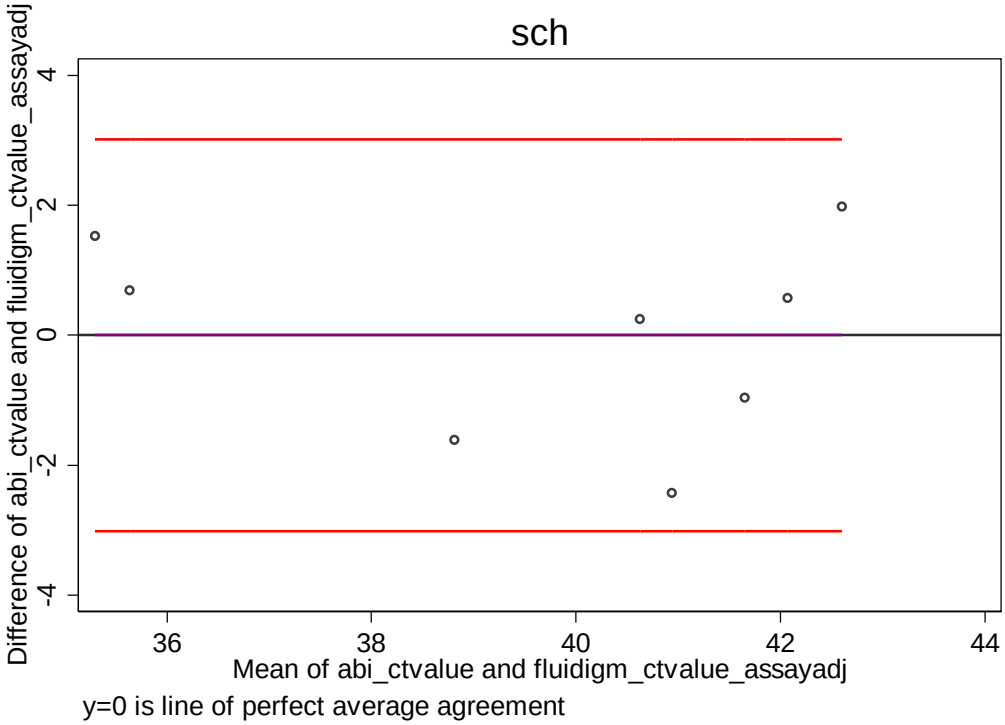
Supplemental Figure 15 (Continued)



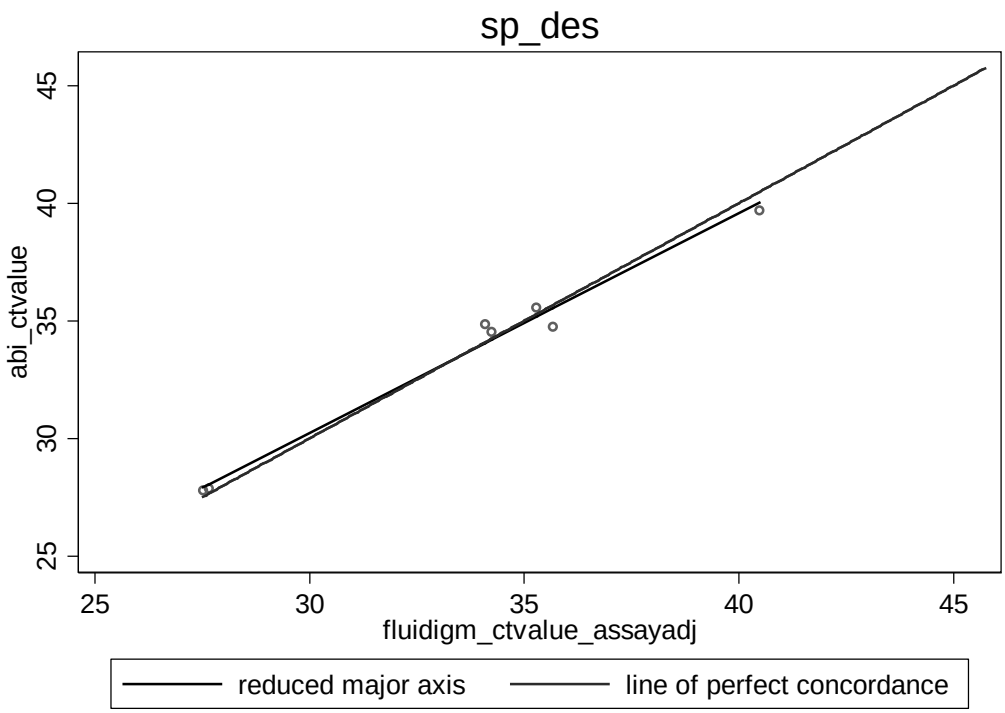


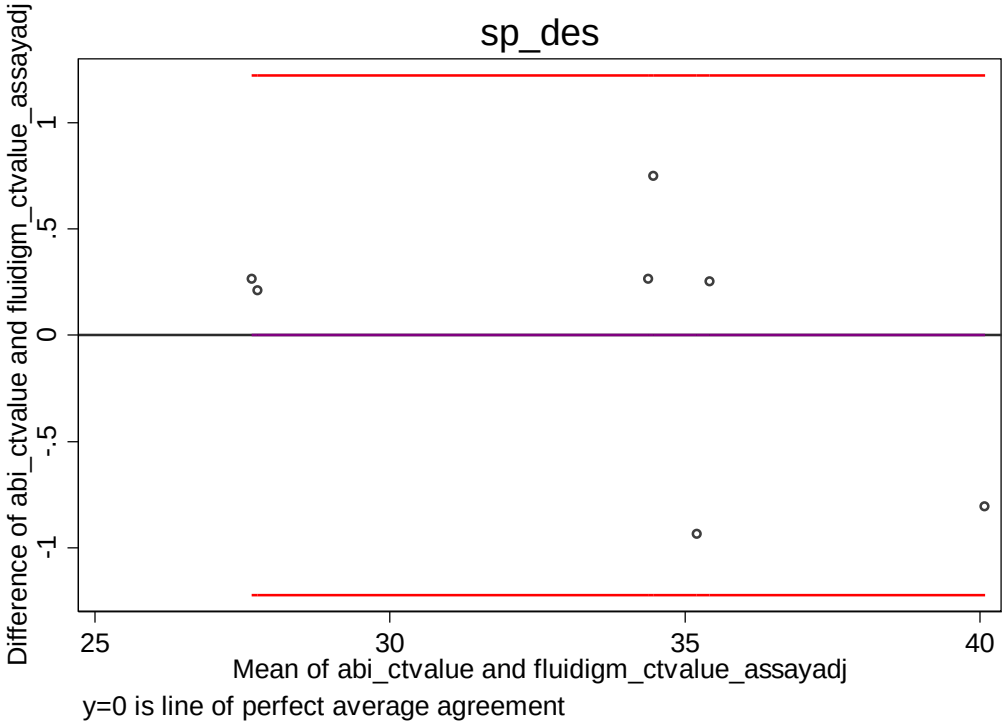
Supplemental Figure 15 (Continued)



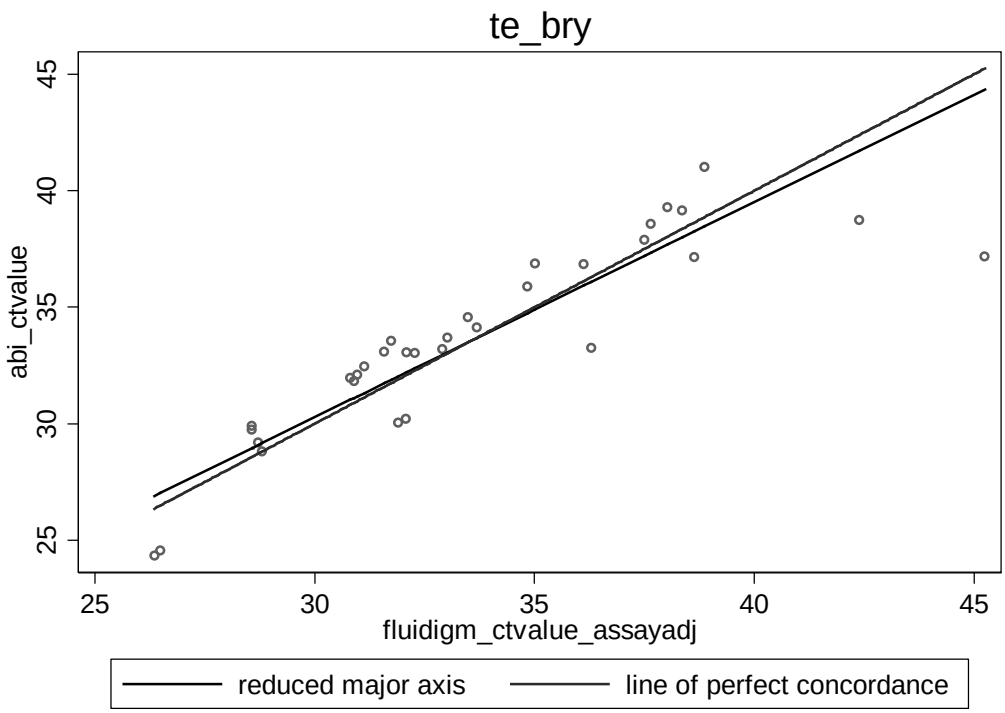


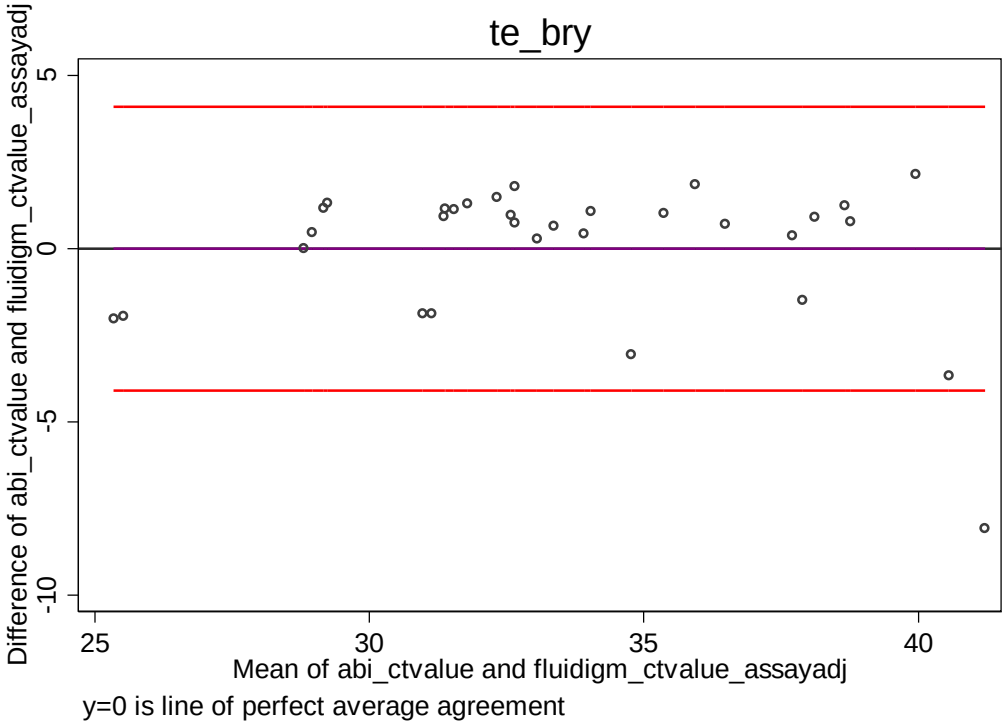
Supplemental Figure 15 (Continued)





Supplemental Figure 15 (Continued)





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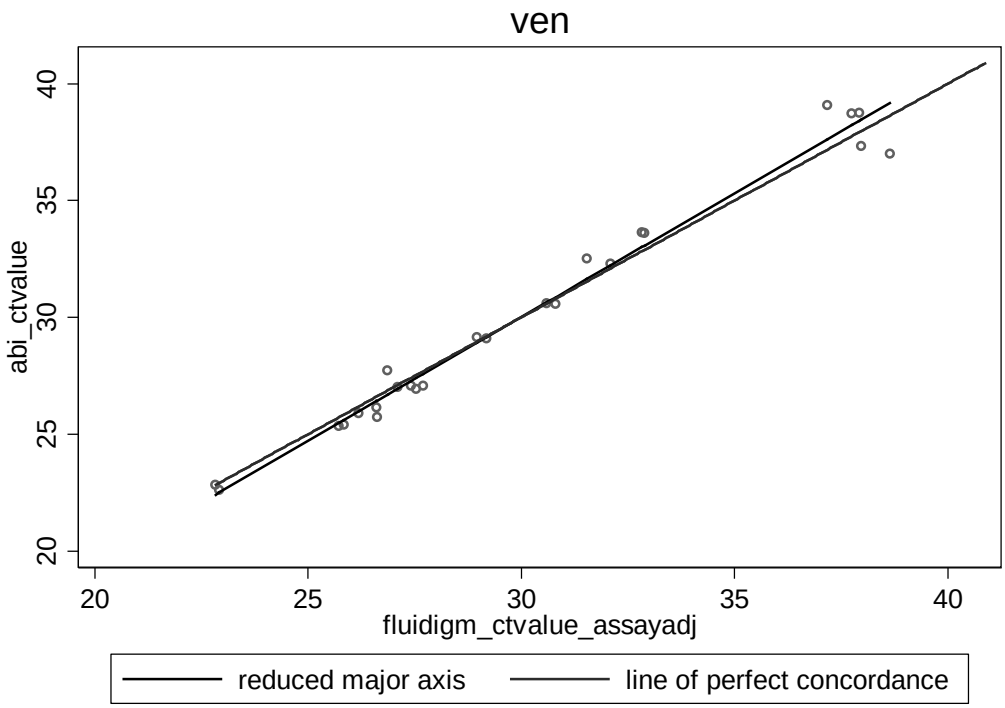

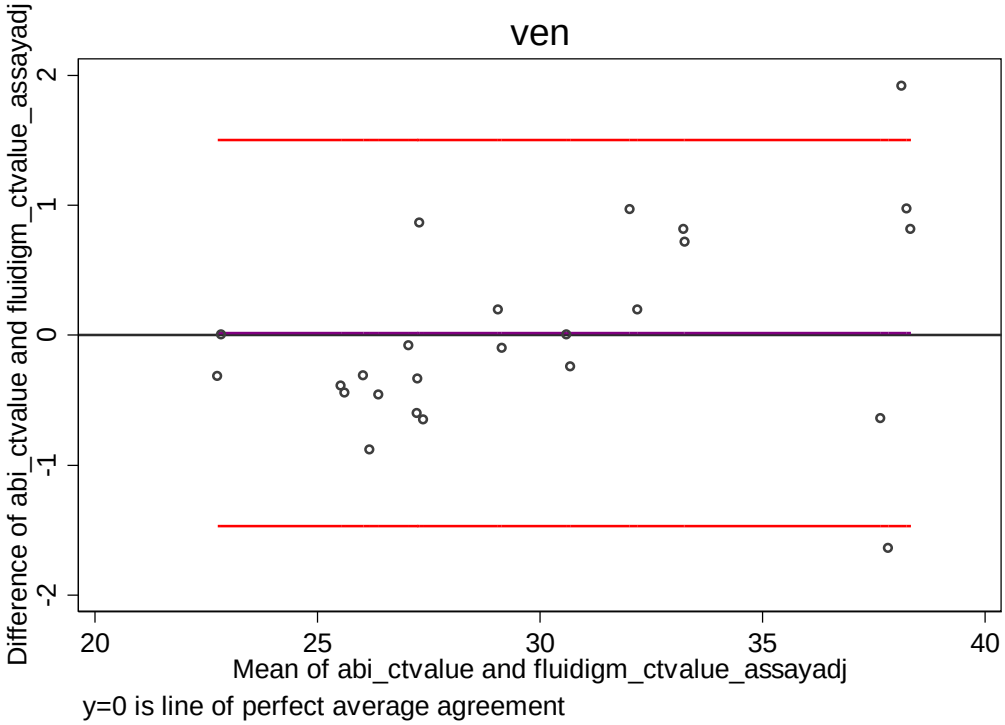

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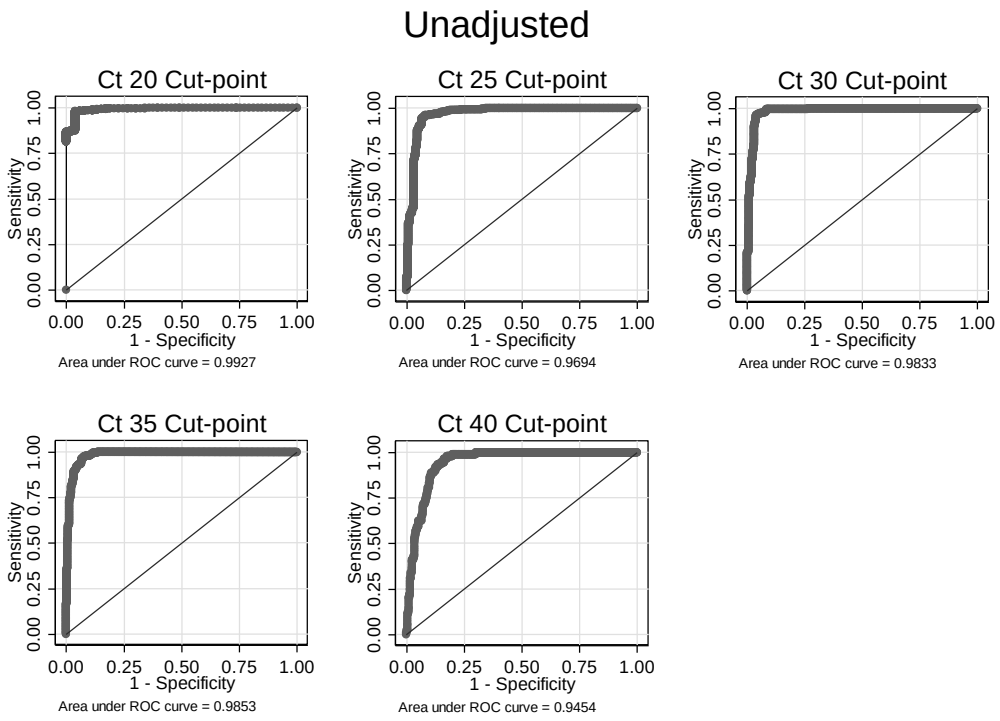
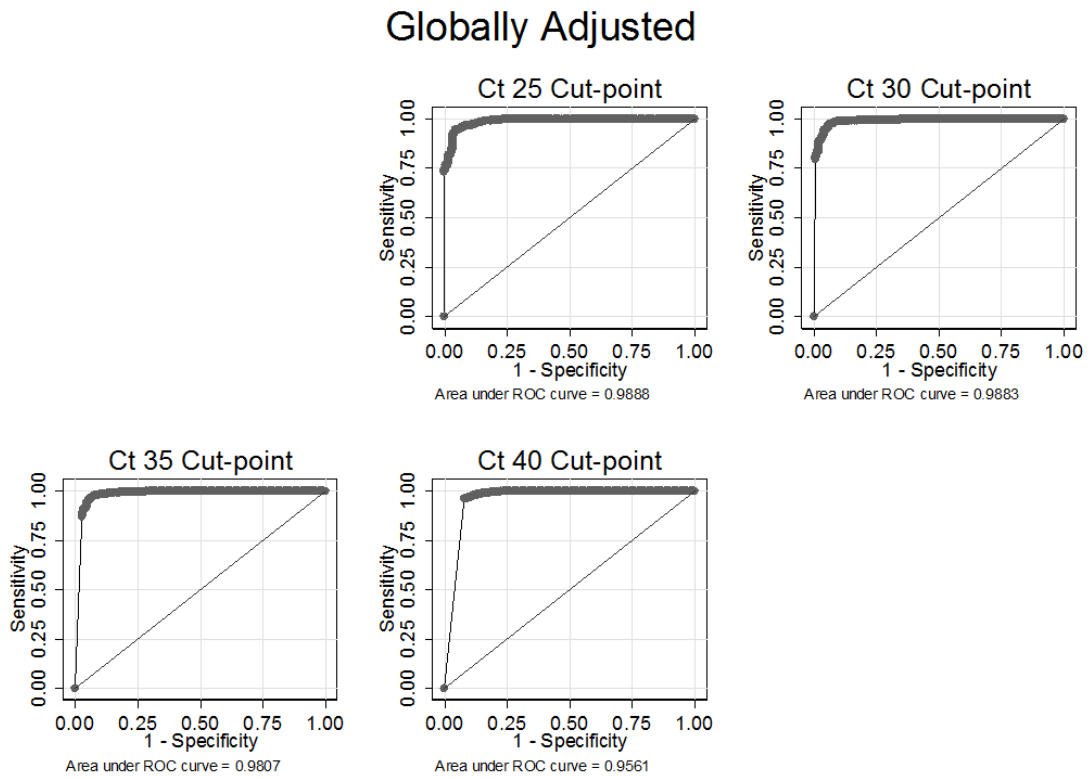
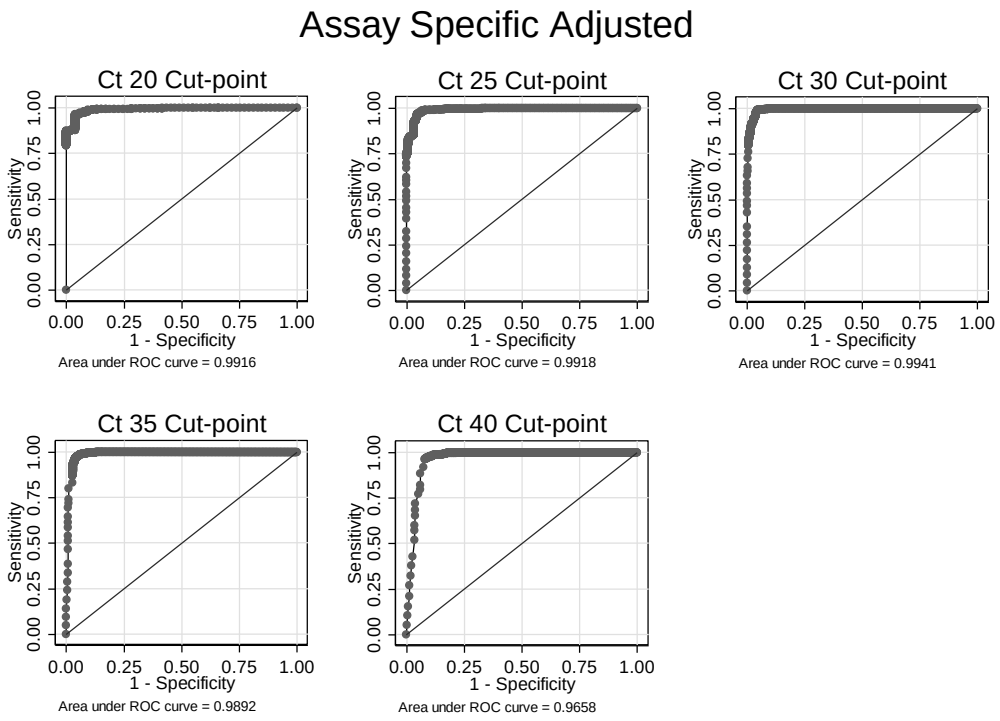


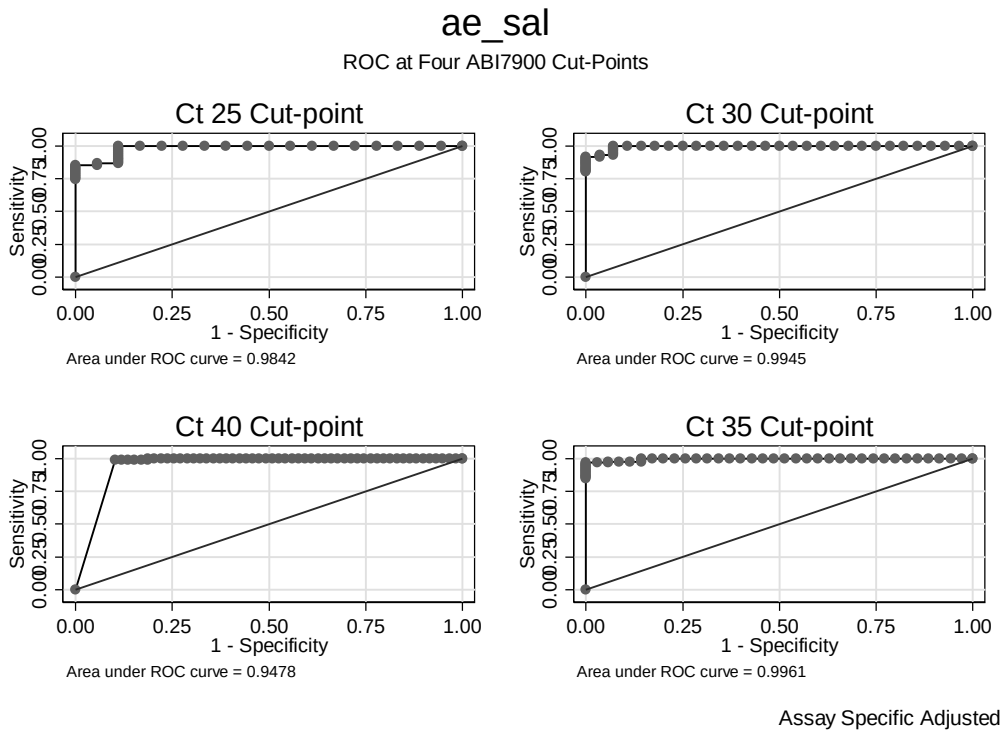
Supplemental Figure 16. Panel graph of receiver operating characteristic (ROC) curves for unadjusted Fluidigm Ct values, based on five chosen ABI7900 cutpoints (20, 25, 30, 35, and 40) for all assays.

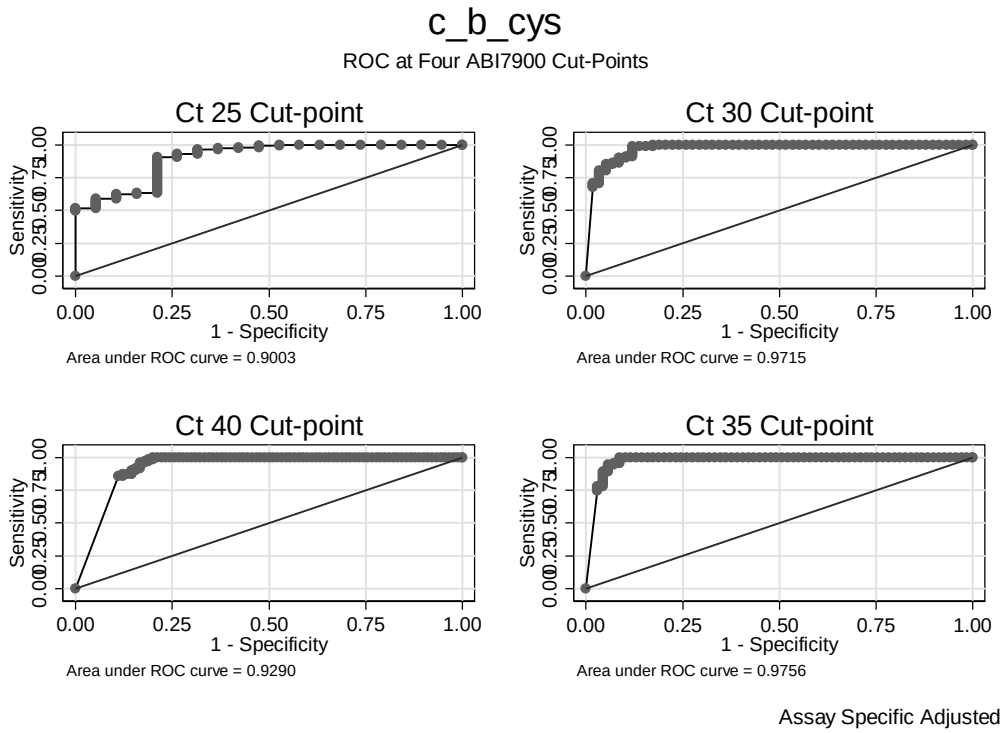


Supplemental Figure 17. Panel graph of receiver operating characteristic (ROC) curves, for globally adjusted Fluidigm Ct values, based on five chosen ABI7900 cutpoints (20, 25, 30, 35, and 40) for all assays. There was no variation at Ct 20 (missing).

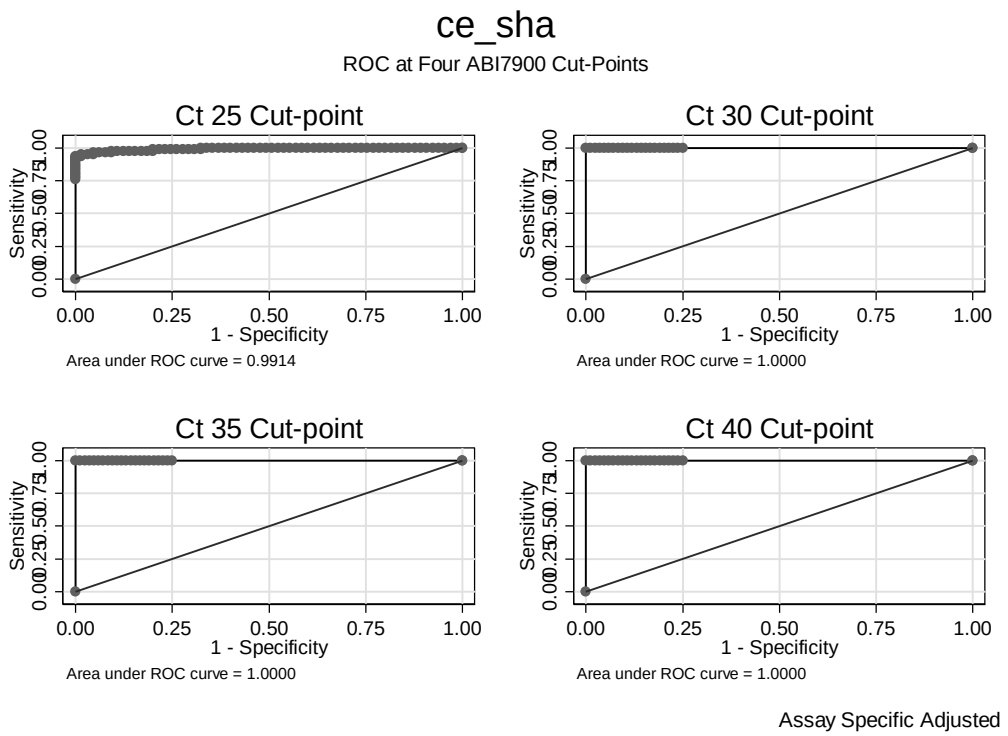


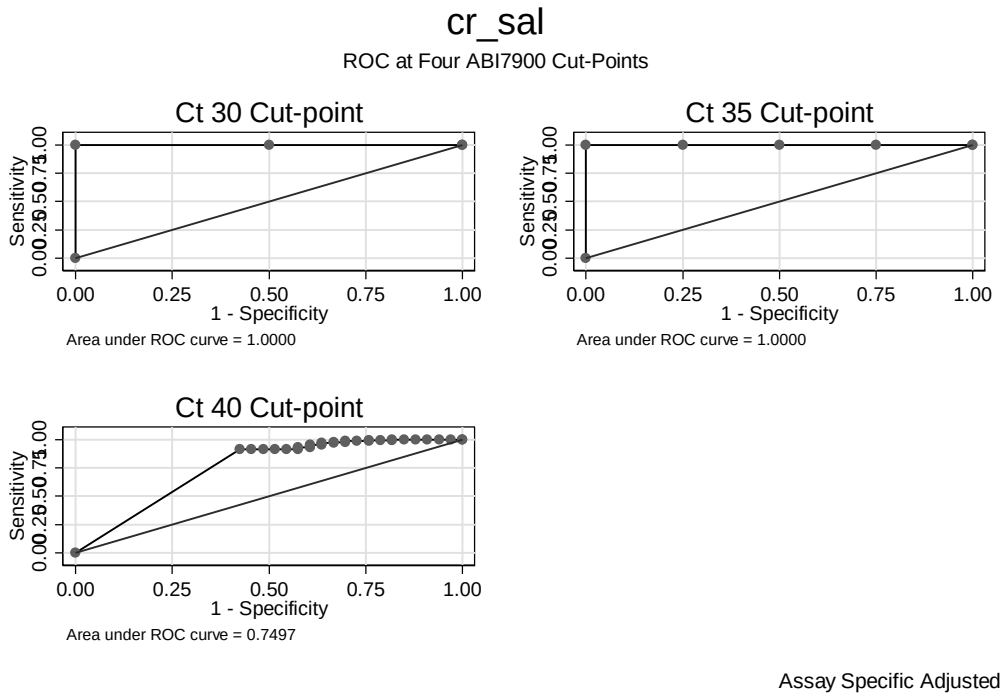
Supplemental Figure 18. Panel graph of receiver operating characteristic (ROC) curves, for assay-specific adjusted Fluidigm Ct values, based on five chosen ABI7900 cutpoints (20, 25, 30, 35, and 40) for all assays.





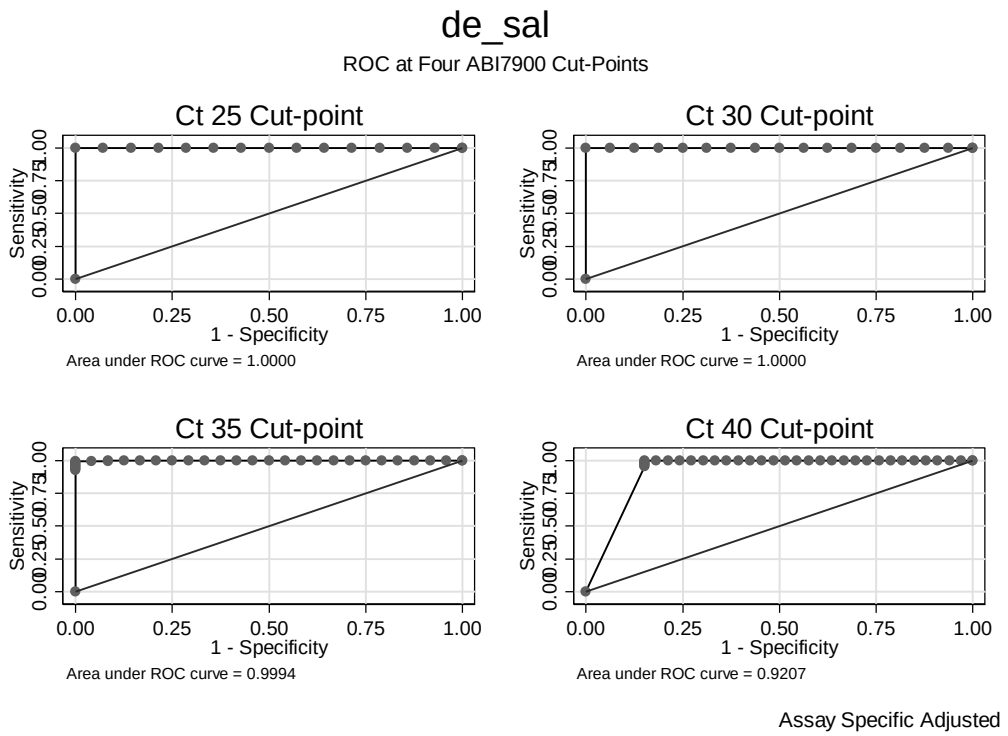
Supplemental Figure 19. Panel graph of receiver operating characteristic (ROC) curves, for unadjusted Fluidigm Ct values, based on five chosen ABI7900 cutpoints (20, 25, 30, 35, and 40), stratified by assay. The list of assays in these analyses include (continued over the next several pages): ae_sal, c_b_cys, ce_sha, cr_sal, de_sal, fl_psy, ic_hof, ic_mul, ihnv, ku_thy, lo_sal, my_arc, pa_min, pa_pse, pa_ther, pspv, rlo, sch, sp_des, te_bry, and ven.

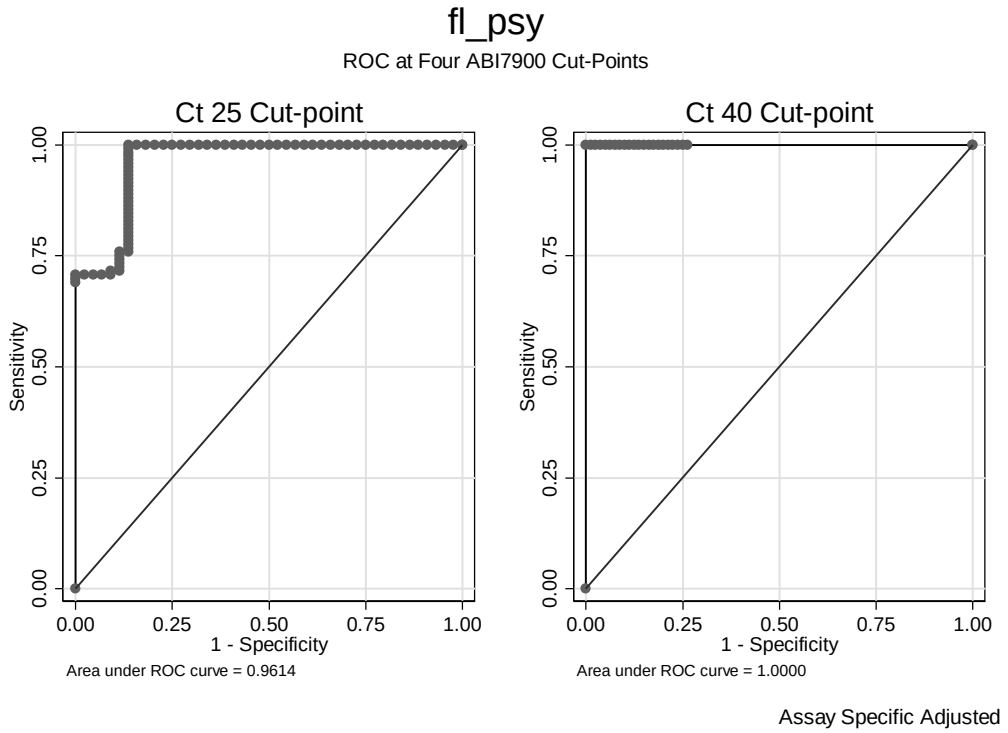




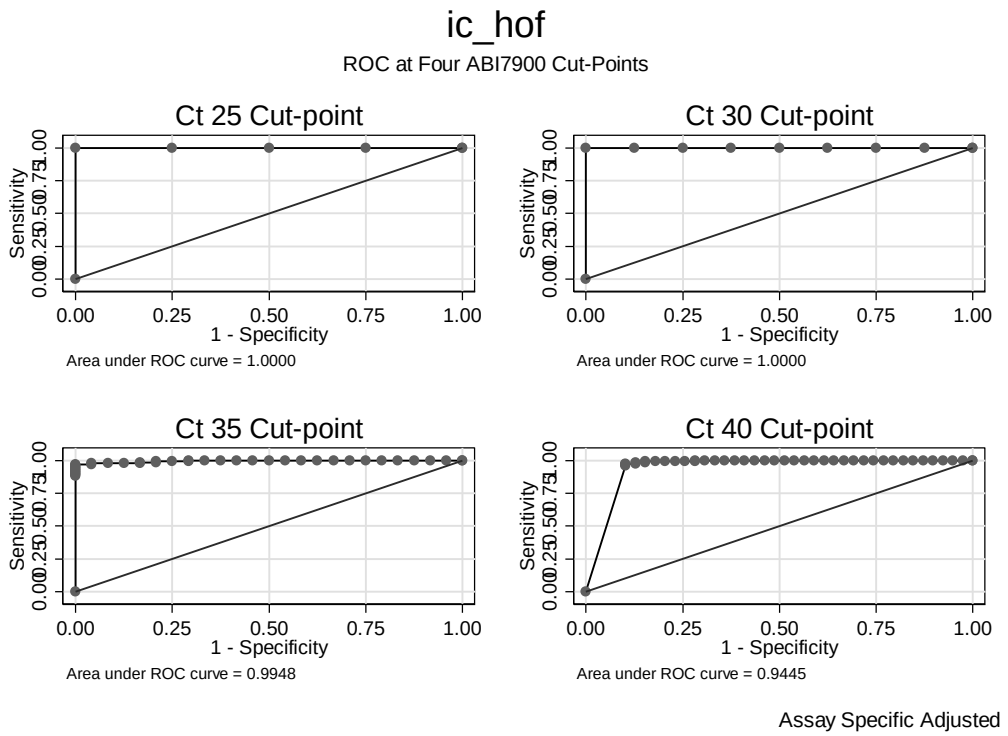
outcome does not vary at ct_25

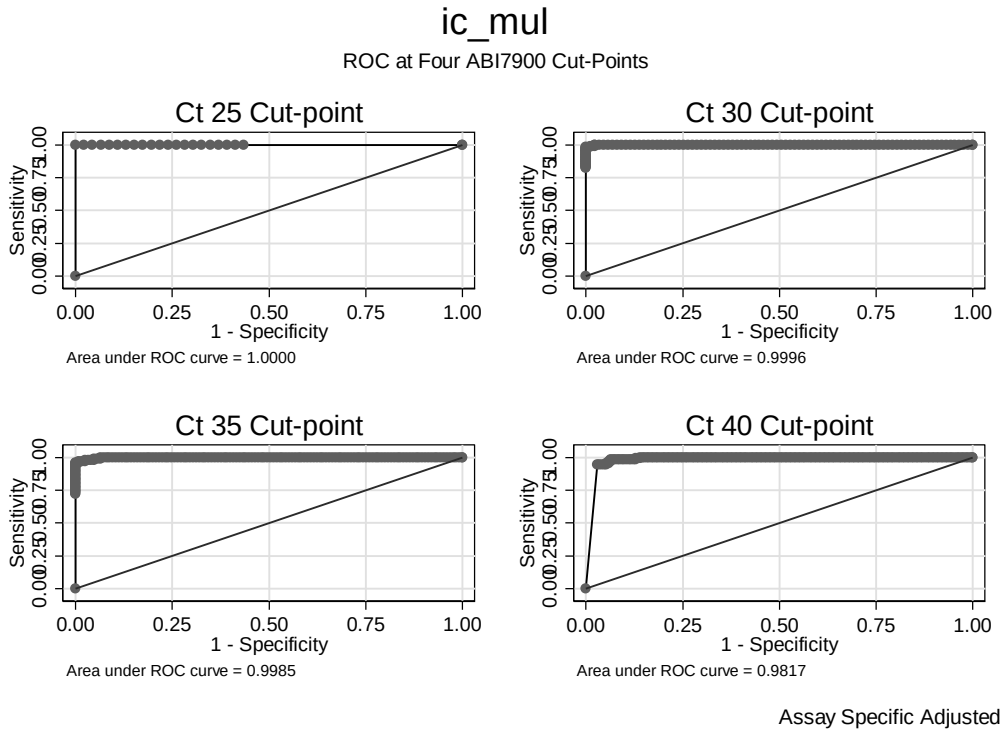
Supplemental Figure 19 (Continued)



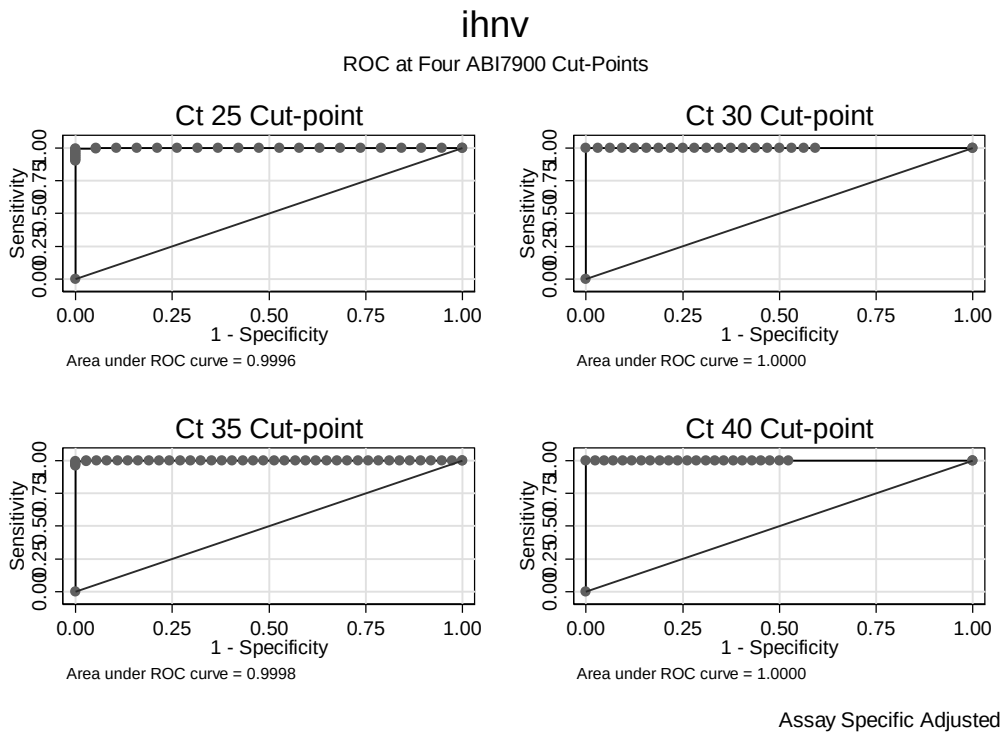


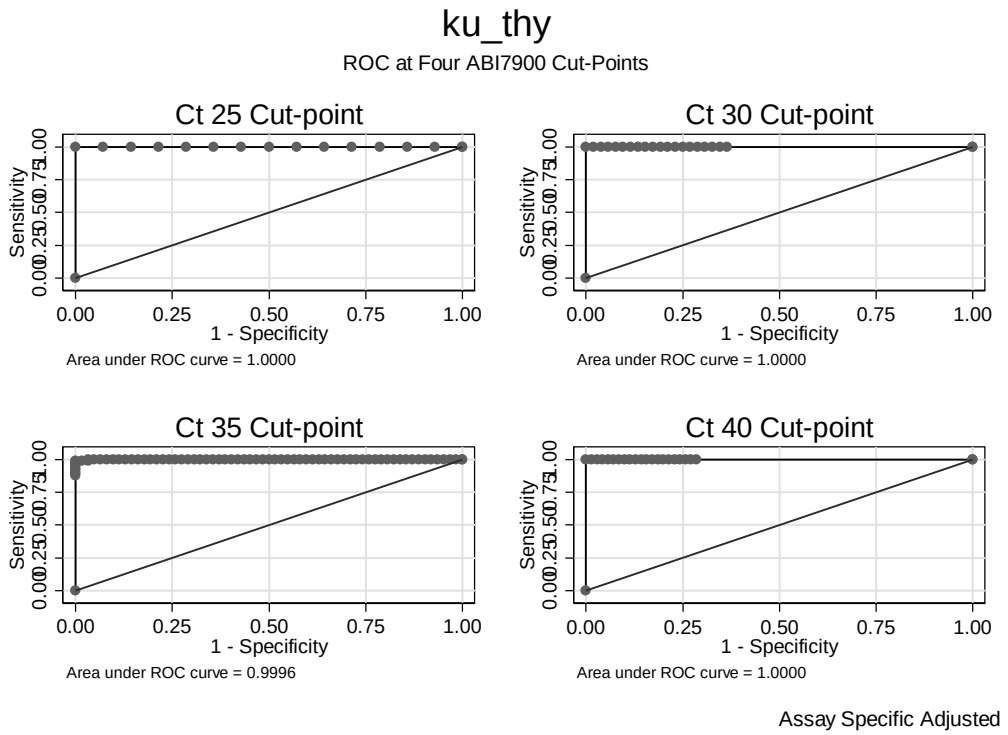
Supplemental Figure 19 (Continued)



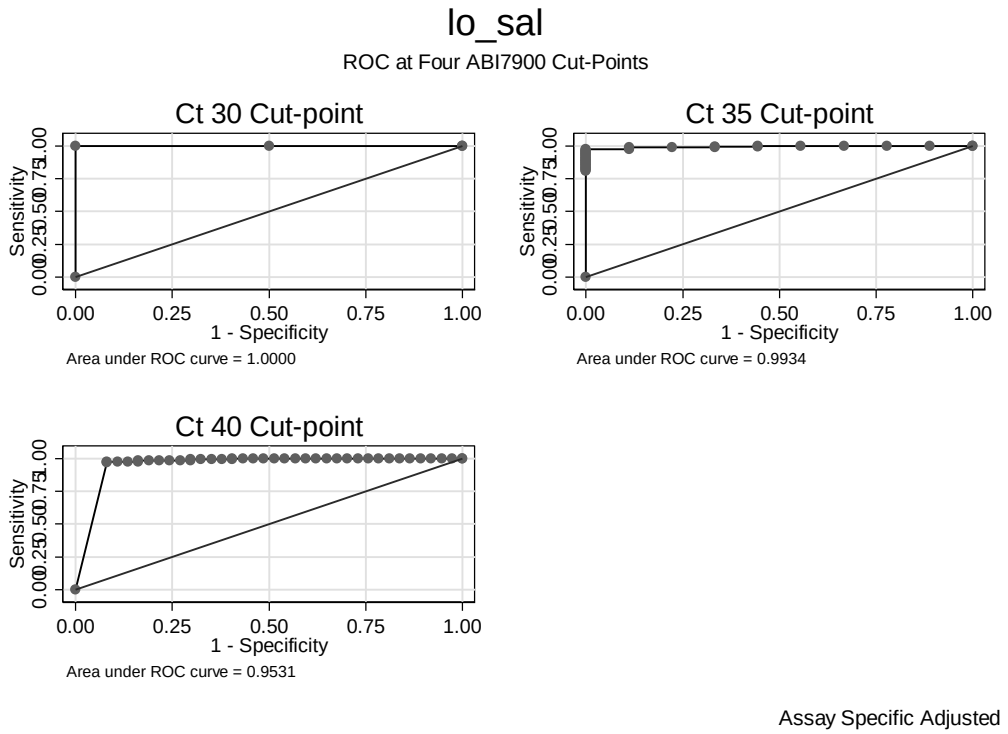


Supplemental Figure 19 (Continued)

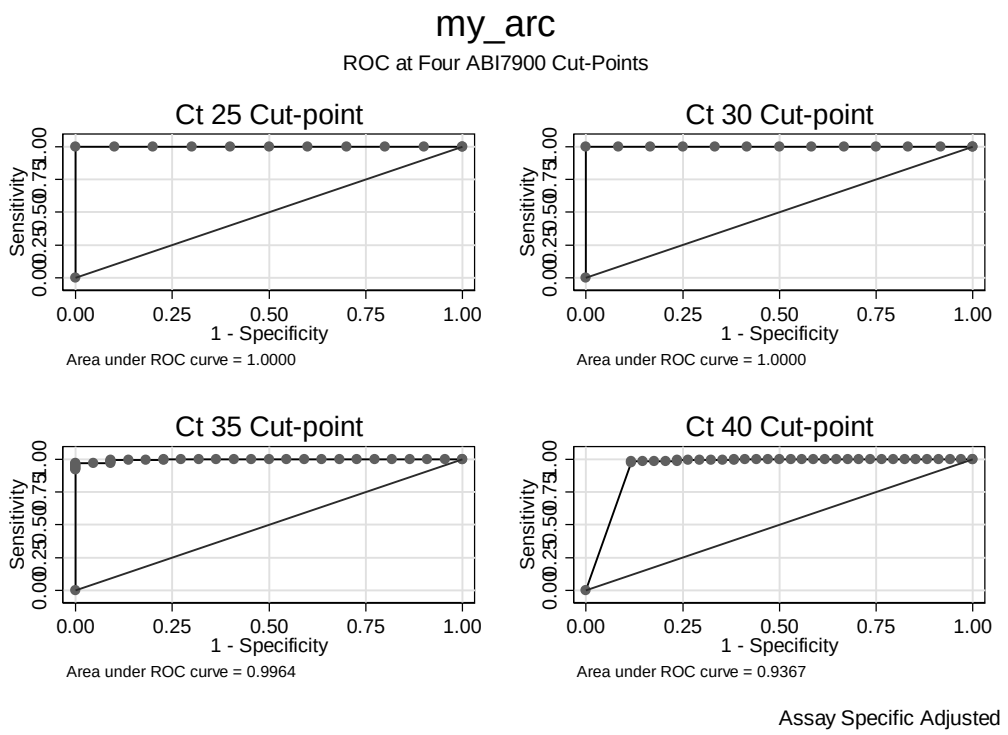




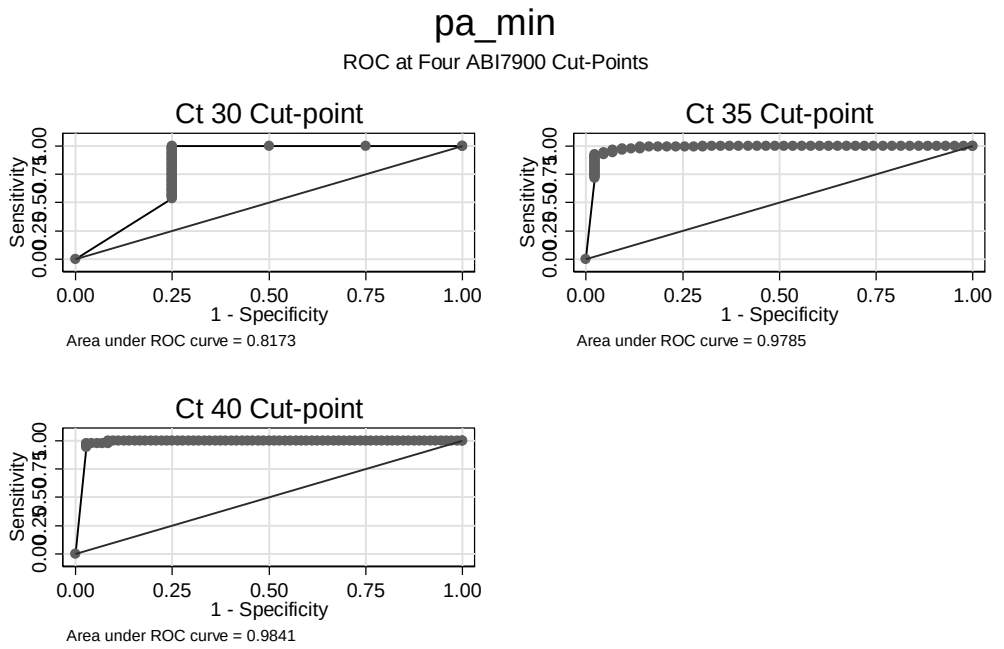
Supplemental Figure 19 (Continued)



outcome does not vary at Ct 25

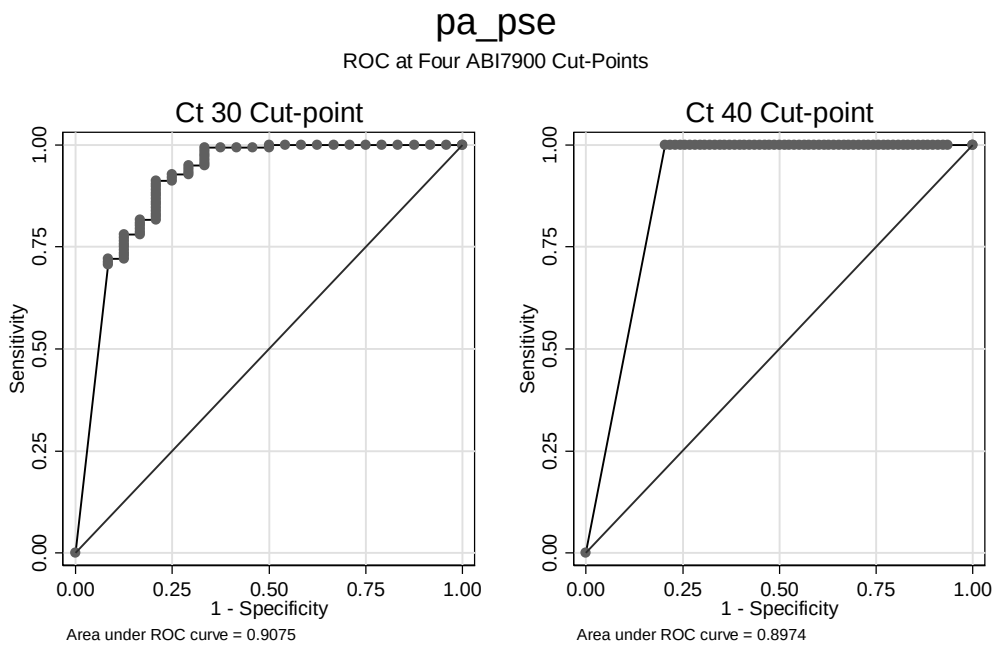


Supplemental Figure 19 (Continued)



Assay Specific Adjusted

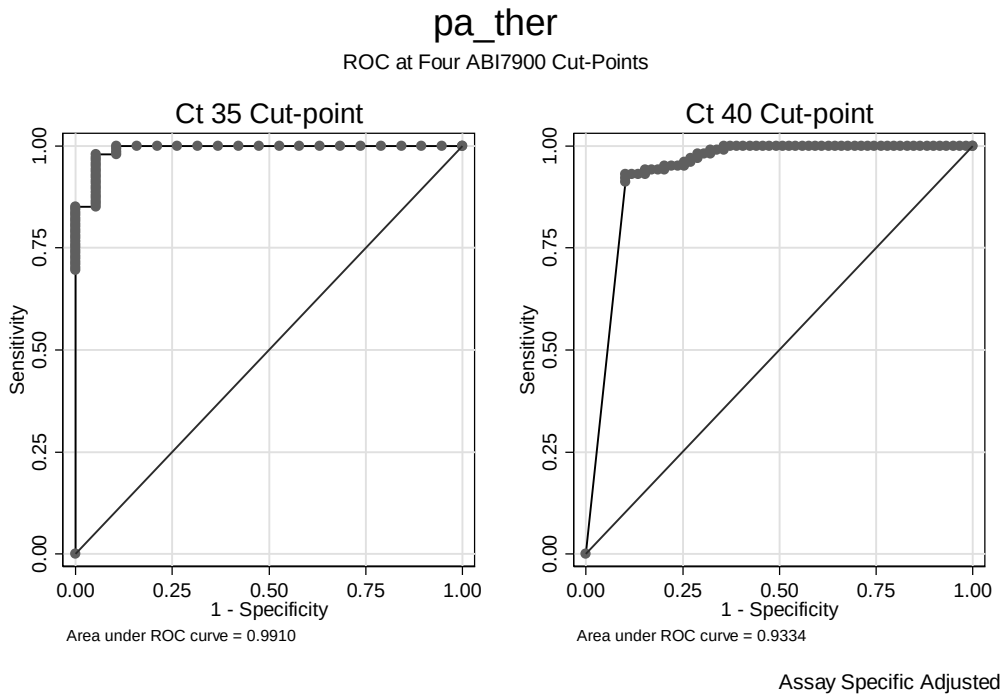
outcome does not vary at ct_25



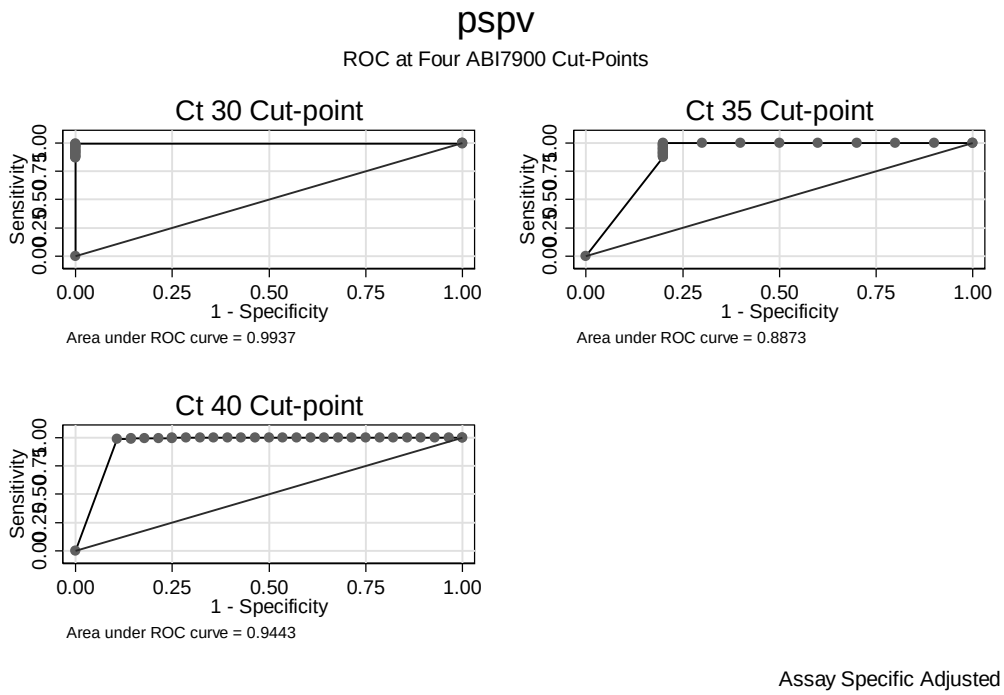
Assay Specific Adjusted

outcome does not vary at ct_25 and ct_35

Supplemental Figure 19 (Continued)

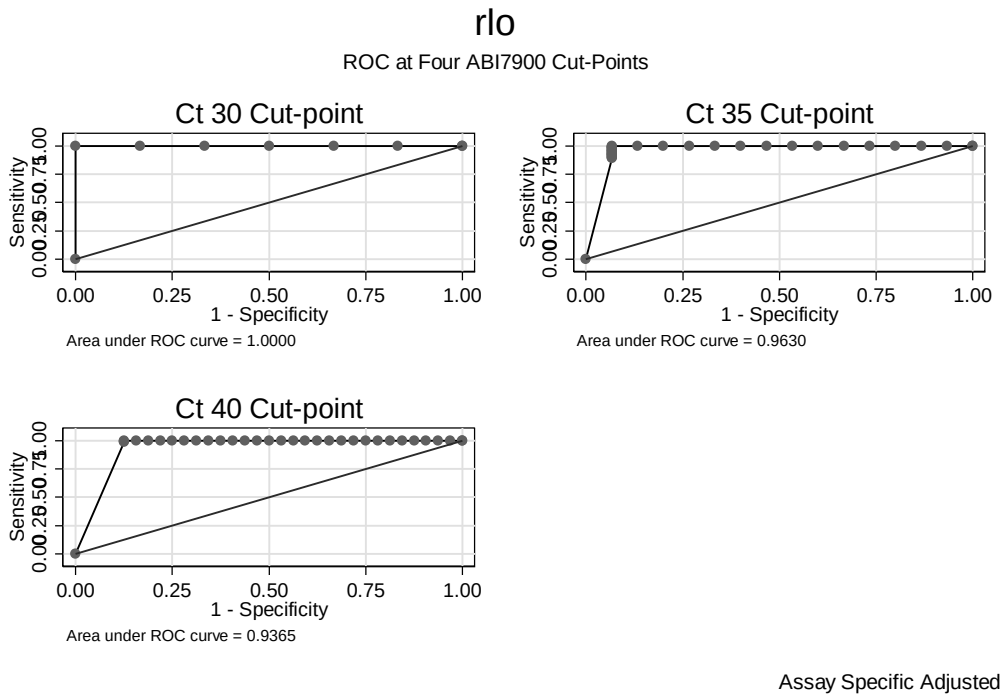


outcome does not vary at ct_25 and ct_35

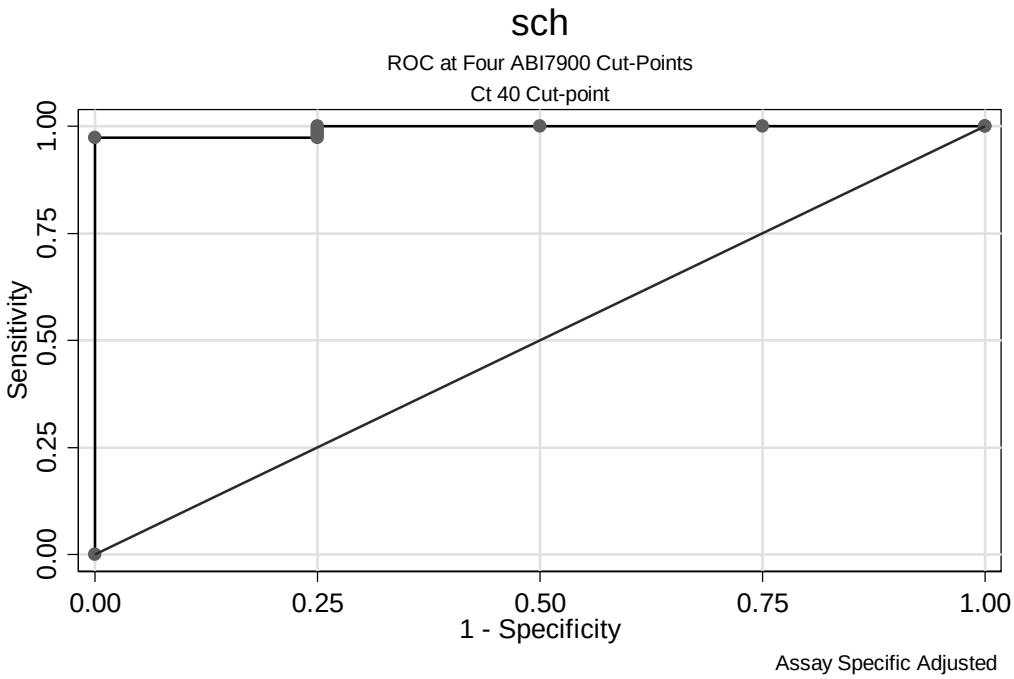


outcome does not vary at ct_25

Supplemental Figure 19 (Continued)

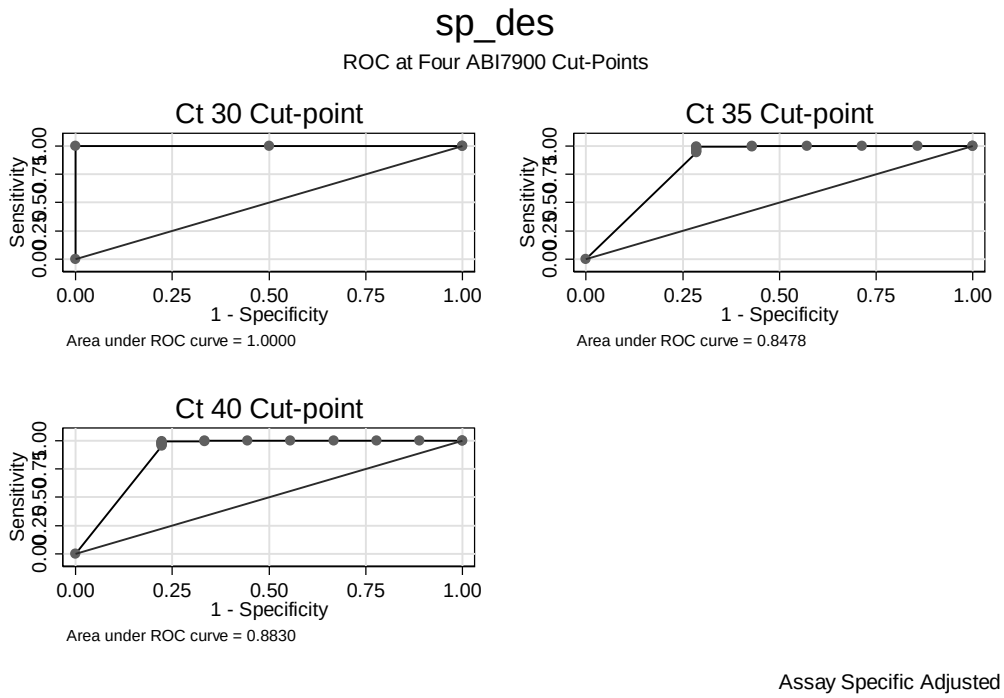


outcome does not vary at ct_25

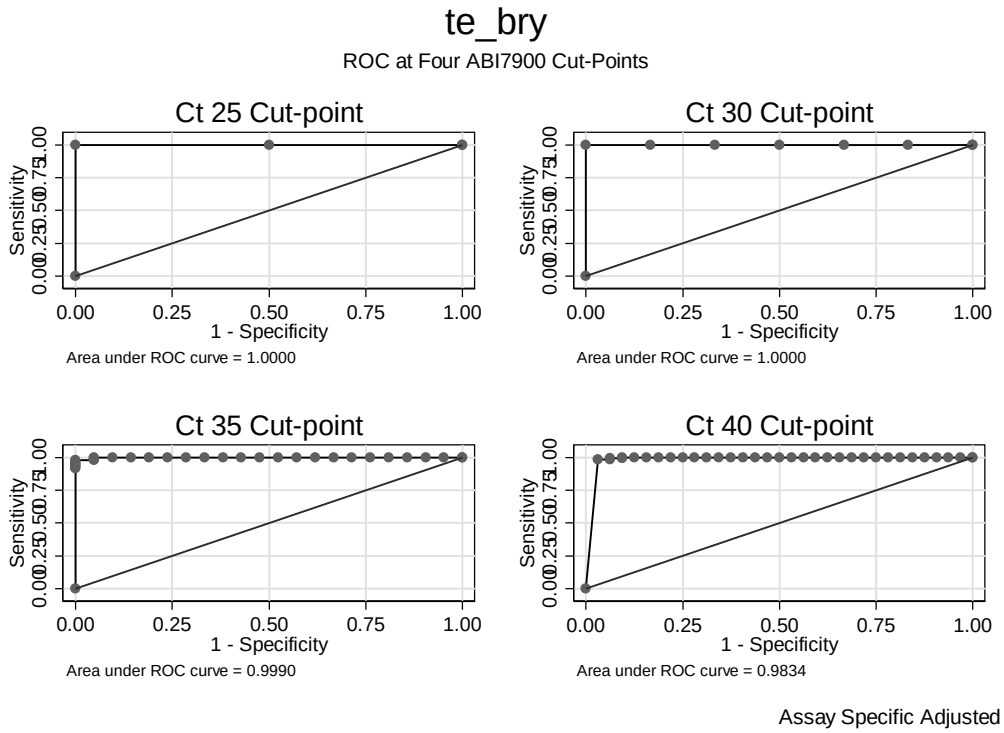


outcome does not vary at ct_25, ct_30, and ct_35

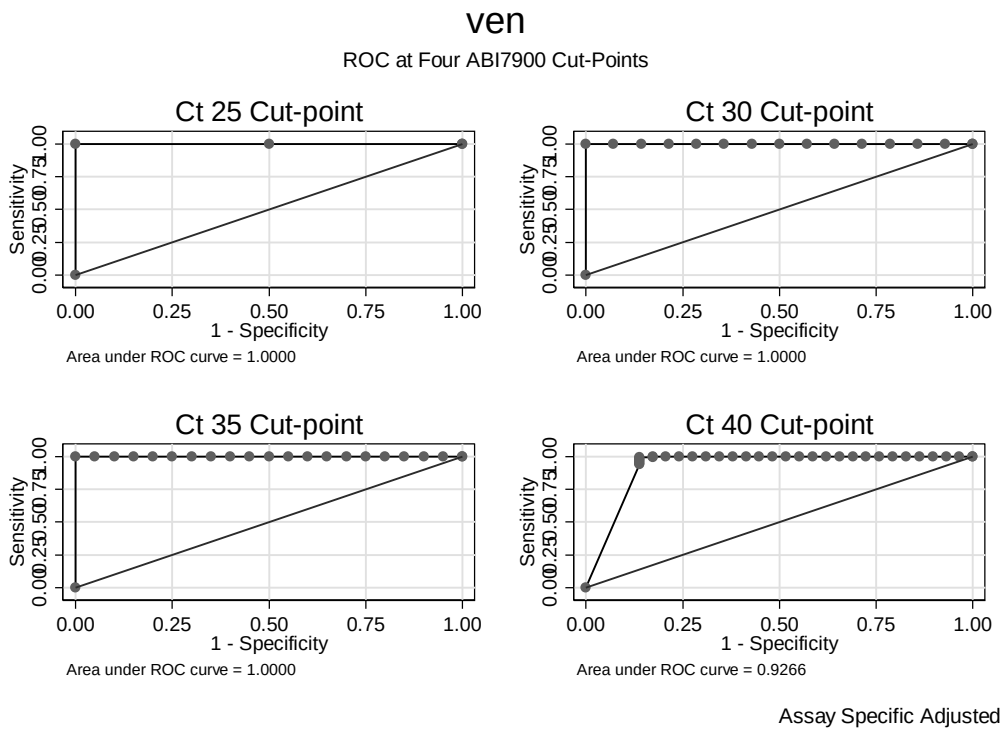
Supplemental Figure 19 (Continued)



outcome does not vary at ct_25



Supplemental Figure 19 (Continued)



Supplemental Figure 19 (Continued)

*Supplemental Table 6. Number of dynamic arrays, qPCR reactions, data processed, samples in analysis and controls/water within the dataset. Each dynamic array generates 96 X 96 (9,216) qPCR reactions with each data set generating processed data; the repeatability part 3 project employed dynamic array runs from part 1 and 2 and blind scoring of the qPCR reactions de novo, noted by the *. Statistical analysis was run on samples, while the controls were omitted from the final analysis. In the consistency across platforms study, 12 X 384 well plates were run on the ABI 7900 and the processed data from repeatability Parts 1 and 2 were used ***. Embedded within both the control sets and sample sets were water-only samples, both controls and water/blanks were omitted from the data analysis.*

	Dynamic Arrays Run	**qPCR reactions**	**Sample Rxns**	**Controls (Pos + Neg) Rxns**	**NTC Rxns**	**DA's Analyzed**	**Rxns in Analysis (samples only)**
Limits of Detection	7	64,512	54,720	9,792	4,032	7	**53,040**
Specificity	9	82,944	63,840	19,200	11,328	9	**54,960**
Repeatability (1,2,3)	9	82,944	69,120	13,824	4,320	30	**124,800**
Comparability	4 + (11 x 384well)	8,448	7,040	1,408	264	4 + (11 x 384well)	**7,040**
Sensitivity	12	110,592	92,640	17,952	4,800	12	**55,934**
Access Array	2	4,608	4,278	330	138	2	**3,999**
Total	43	354,048	291,638	62,506	24,882	60	299,773

Supplemental Table 7. Sequence of all G-blocks used in performance evaluation. Each microbe sequence is identified by classification and species with the 5' to 3' sequences listed and the total G-block length noted.

Type of Microbe	Classification of Microbe	Specificity Sample Run Name	Species	PEI Assay Name	Asp_Test or CRS	Sequence (5'-3')	Length (bp)
Bacteria	proteobacteria_gamma	A._hydrophila_aha1_gblock_APC	Aeromonas hydrophila AY165026	ae_hyd	T	TGTGTGATAAATCACCAATTCGGACGAATTTTGCCAGCGGT TATCGCTGTAAACGTTTTCCCATGGCGTGCAAACAATGTGG GATTCAGGTCACAATTTTTCCGCTGTGACTATGCTTTTCGTA AAAAGTTCCAAGTTTTTTCATTGCGGATTGGAAAACCCGGTG CTAGTCTCGGCGCCATAGTGATGCAAAGTACATCGCTAACA CAGGGAATAACAACGACTTAGTGTTTAATTACAGTAGGCATT GGAAACTATGAAAAAGACAATTCTGGCTATTGCTATCCCGG CTCTGTTTGCATCCGCCGCTAACGCTGCAGTGGTTTACGAC AAAGACGGTACCACTTTTGACGTATACGGCCGTGTTCAGGC TAACTACTACGGTGACCACAACAAATCTGTAGCTGCTACCG ATGGTTCCTGGGGCTTCAGCGGAACTGGTACCCCGGAATAT ACTCCTGGTACCGCTGCTCATTACTCTGATGTTGATGGTGA GCTGGTTGGACCGTCTAGCATCCAGTTTCTTCCCGTCTGGG TTGGTCCGGTAAGATTGCCCTGAACAACACCTGGTCCGGTA TCGCCAAGACTGAGTGGCAAGTTTCTGCTGAAAACTCCGCC AACAAGTTCGATTCCCGTCACATCTACGTTGGTTTCGACGG CACCCAGTACGGTAAGATCATCTTCGGTCAGACCGATACCG CGTTCTATGACGTGCTGGAACCGACCGATATCTTCAACGAG TGGGGCGACGTAGGTAACTTCTATGACGGTCGTCAAGAAG GTCAGATCATCTACTCCAACACCTACGGTGGCTTCAAAGGC AAACTGTCCTATCAAACCAACGACGACAAGGCCGTCAAGGT TACTGACGTAGGTCAGGGCATCAAAGAAAACGCAGTGTACG GCAAGGATGTTAAGCGTAACTACGGTTATGCCGCGGCTGC CGGTTATGACTTCGACTTCGGTCTGGGTCTGAACGCAGGTT ACTCCTACTCCGATC	1000
Bacteria	CFB group	F._branchiophilum_16S_gblock_APC	Flavobacterium branchiophilum NR_104713	fl_psy	CRS	GATGAACGCTAGCGGCAGGCTTAACACATGCAAGTCGAGG GGTATAGTAGCAATACTAGAGACCGGCGCACGGGTGCGTA ACGCGTATGCAATCTACCTTTTACATAGGGATAGCCCAGAG AAATTTGGATTAATACCTGATAGTATGTGATGATCGCATGGT TTTCACATTAAAGTACCAACGGTAAAAGATGAGCATGCGTC CCATTAGCTAGATGGTAAGGTAACGGCTTACCATGGCTACG ATGGGTAGGGGTCCTGAGAGGGAGATCCCCCACACTGGTA CTGAGACACGGACCAGACTCCTACGGGAGGCAGCAGTGAG GAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCC GCGTGCAGGATGACGGTCCTATGGATTGTAAACTGCTTTTG TACAGGAAGAAACACTTCTACGAGTAGAAACCGTCTAGCAT CCAGTGCTTGACGGTACTGTAAGAATAAGGATCGGCTAACT CCGTGCCAGCAGCCGCGGTAATACGGAGGATCCAAGCGTT ATCCGGAATCATTGGGTTTAAAGGGTCCGTAGGCGGTTTGA TAAGTCAGTGGTGAAAGCCCATCGCTCAACGATGGAACGG CCATTGATACTGTTAGACTTGAATTATTAGGAAGTAACTAGA ATATGTAGTGTAGCGGTGAAATGCTTAGAGATTACATGGAAT ACCAATTGCGAAGGCAGGTTACTACTAATGTATTGACGCTG ATGGACGAAAGCGTGGGTAGCGAACAGGATTAGATACCCT GGTAGTCCACGCTGTAAACGATGGATACTAGCTGTTGGGG GCAACTTCAGTGGCTAAGCGAAAGTGATAAGTATCCCACCT GGGGAGTACGTTCGCAAGAATGAAACTCAAAGGAATTGACG GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAT GATACGCGAGGAACCTTACCAAGGCTTAAATGTAGTTTGAC CGATTTGGAAACAGATCTTTCGC	1000
Bacteria	CFB group	F._columnare_16S_gblock_APC	Flavobacterium columnare AY747592	fl_psy	CRS	GATGAACGCTAGCGGCAGGCTTAACACATGCAAGTCGAGG GGTATGTTTCTTCGGAAGCAGAGACCGGCGCACGGGTGCG TAACGCGTATGCAATCTACCTTTTACAGAGGGATAGCCCAG AGAAATTTGGATTAATACCTCATAGTATTATAGCATGGCATC ATTTTATTATTAAAGTCACAACGGTAAAAGATGAGCATGCGT CCCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCTAC GATGGGTAGGGGTCCTGAGAGGGAGATCCCCCACACTGGT ACTGAGACACGGACCAGACTCCTACGGGAGGCAGCAGTGA GGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATGC CGCGTGCAGGATGACGGTCCTATGGATTGTAAACTGCTTTT GTACAGGAAGAAACACTGGTTCGTGAACCAACCGTCTAGCA TCCAGTGCTTGACGGTACTGTAAGAATAAGGATCGGCTAAC TCCGTGCCAGCAGCCGCGGTAATACGGAGGATCCAAGCGT TATCCGGAATCATTGGGTTTAAAGGGTCCGTAGGCGGTTTA ATAAGTCAGTGGTGAAAGCCCATCGCTCAACGGTGGAACG GCCATTGATACTGTTAGACTTGAATTATTAGGAAGTAACTAG AATATGTAGTGTAGCGGTGAAATGCTTAGAGATTACATGGA ATACCAATTGCGAAGGCAGGTTACTACTAATGGATTGACGC TGATGGACGAAAGCGTGGGTAGCGAACAGGATTAGATACC CTGGTAGTCCACGCCGTAAACGATGGATACTAGCTGTTGGA AGCAATTTCAGTGGCTAAGCGAAAGTGATAAGTATCCCACC TGGGGAGTACGTTCGCAAGAATGAAACTCAAAGGAATTGAC GGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGA TGATACGCGAGGAACCTTACCAAGGCTTAAATGTAGATTGA CCGGTTTGGAAACAGATCTTTC	1000

Type of Microbe	Classification of Microbe	Specificity Sample Run Name	Species	PEI Assay Name	Asp_Test or CRS	Sequence (5'-3')	Length (bp)
Bacteria	Chlamydiae/ Verrucomicrobia group	Sch_gblock	gill chlamydia (Sch) EU326493	sch	T	CATGCAAGTCGAACGAAGTAGCTTGCTACTTAGTGGCGAAA GGGTTAGTAATACATGAGTAACGTACCTCTTTCCTGGGGAT AACGGTTGGAAACGACCGCTAATACCGAATGAGGAGATCC GGGGTAGCCCGATATCTTCAAAGTAGGACTGGATGCTAGAC GGTGGATCCTTCGGGACCTTACGGAGAGAGAGCGGCTCAT GGGATATCAGCTTGTTGGTGTGGTAAAAGCGCACCAAGGCT AAGACGTCTAGGCGGACTGAGAGGTTGACCGCCAACACTG GGACTGAGACACTGCCCAGACTCCTATGGGAGGCTGCAGT CGAGAATCATTCGCAATGGGCGAAAGCCTGACGATGCGAC GCCGTGTGGACGATGAAGGCCTTCGGGTTGTAAAGTCCTTT CGCCAGGGAACAAGAGAAAGTAACTAATAATTGCTTGATTT GAGCGTACCTGGTAAAGAAGCACCGGCTAACTCCGTGCCA GCAGCTGCGGTAATACGGAGGGTGCAAGCATTAATCGGATT TATTGGGTGTAAAGAGCATGTAGGCGGATAAGTAAGTCAGA TGTGAAATTTCGGAGCTCAACTTCGAAGCTGCATTTGAAACT ATTTGTCTTGAGGGTAGACGGAGAAAACGGAATTCCAAGTG TAGCGGTGAAATGCGTAGATATTTGGAAGAACACCGGTGGC GAAAGCGGTTTTCTAGTTTATTCCTGACGCTGAGATGCGAG AGCAAGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCTT GCCGTAAACGATGCATACTTGGTGTAGTTGGACTCAACCCC GACTGTGCCGTAGCTAACGCGTTAAGTATGCCACCTGGGG AGTACGCCCGCAAGGGTGAAACTCAAAAGAATTGACGGGG GCCCGCACAAGCAGTGGAGCATGTGGTTTAATTCGATGCAA CGCGAAGAACCTTACCCAGGCTTGACATATAGAGGCCCGCT CTAGAGATAGAGTTTCCCGCAAG	1000
Bacteria	Chlamydiae/ Verrucomicrobia group	Chlamydiales_gp1_16S	Chlamydiales sp.	pch_sal/sch	CRS	CATGCAAGTCGAACGAAAGTAAGCTGCTGTGACTATTTAGT GGCGGAAGGGTTAGTAATACATGGGTAACTTACCTCTAACC TGGGGATAACGGTTGGAAACGGCCGCTAATACCGAATGAG GTGGCGAGAGGCATCTTATTTTCATCAAAGCGGGACTGGAT GCTAGACGGTGGACTCGAAGGACCTTGCGGTTAGAGAGAG GCTCATGGGATATCAGCTAGTTGGTGTGGTAATGGCGCACC AAGGCTAAGACGTCTAGGCGGATTGAGAGATTGACCGCCA ACACTGGGACTGAGACACTGCCCAGACTCCTACGGGAGGC TGCAGTCGAGAATCTTTCGCAATGGGCGAAAGCCTGACGAA GCGACGCCGTGTGAGCGATGAAGGCCTTCGGGTTGTAAAG CTCTTTCGCCAGGGAACAAGAGAAGCTGGATAATAACTTAC TAATTTGAGGGTACTAGGTAAAGAAGCACCGGCTAACTCCG TGCCAGCAGCTGCGGTAATACGGAGGGTGCAAGCATTAAT CGGATTTACTGGGCGTAAAGGGCGCGTAGGCGGCAAGTAA GTCAGATGTGAAATTCCGGGGCTCAACCCCGGAGCTGCATT TGAAACTGCTTGTCTAGAGGATAGGCGGGGAAAACGGAATT CCACGTGTAGCGGTGAAATGCGTAGATATGTGGAAGAACAC CGGTGGCGAAGGCGGTTTTCTAGGTTATTCCTGACGCTGAG GCGCGAAAGCAAGGGGAGCAAACAGGATTAGATACCCTGG TAGTCCTTGCCGTAAACGATGCATACTTGGTGTAGTTGGGC TCAACCCCGACTGTGTCGGAGCTAACGCGATAAGTATGCCG CCTGGGGAGTACGCTCGCAAGGGTGAAACTCAAAAGAATT GACGGGGGCCCGCACAAGCAGTGGAGCATGTGGTTTAATT CGATGCAACGCGAAGAACCTTACCCAGGCTTGACATGTAGA GGACAACATCAGAGATGATGTTTCCC	1000
Bacteria	Chlamydiae/ Verrucomicrobia group	Chlamydiales_gp2_16S	Chlamydiales sp.	sch	CRS	CATGCAAGTCGAACGGAATAATGGCTTCGGTTATTATTTAGT GGCGGAAGGGTTAGTAATACATGGATAATTTATTCTTAACTT GGGAATAACGACTGGAAACGGTCGCTAATACCGAATGTGGT ATGTTTAGGCATCTAAATTATATTAAAGAAGGGGATCTTCGG ACCTTTCGGTTGTGAGAAAGTCTATGGGATATCAGCTTGTT GGTGAGGTAATGGCTCACCAAGGCTATGACGTCTAGGCGG ATTGAGAGATTGACCGCCAACACTGGGACTGAGAACTGCCC AGACTCCTACGGGAGGCTGCAGTCGAGAATTTTTCGCAATG GGCACAAGCCTGACGAAGCGACGCCGTGTGTGTGATGAAG GTCTTAGGATCGTAAAGCACTTTTGCCTGGGAGCAAACTGG AGAGAATAATATACTCTTCAATTGAGAGTACCTGGAGAATAA GCGCCGGCTAACTGCGTGCCAGCAGCTGCGGTAATACGTG GGGCGCTAGCATTAATCGGATTTACTGGGTGTAAAGGGTGC GTAGGCTGTTTAGTAAGTTGGACGTTAAATCTTGGGGCTCA CCCCCAGGCTGCTTCCAAAACTGCTAGACTAGAGTTACCGT CTAGCATCCAGTCAAGAGGGGGAAATGGAATTCCACAAGTA GCGGTGAAATGCGTAGATATGTGGAGGAACACCGGTGGCG AAGGCGGTTTCCTAGATTGATACTGACGCTGAGGCACGAAA GCAAGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCTTG CTGTAAACGATGCACACTTGATGTAGCTAGTCTCAACCCTA GCTGTGTCGAAGCTAACGCGATAAGTGTGCCACCTGGGGA GTACGCCTGCAAGGGTGAAACTCAAAAGAATTGACGGGGA CCCGCACAAGCAGTGGAGCATGTGGTTTAATTCGATGATAC GCGAAGAACCTTACCTGGGCTTGACATGTAGTTGAATAAAT CAGAGACGATTTAGTCCG	1000
Virus	ssRNA (-) Paramyxovirus	ASPV_2_gblock	Atlantic salmon paramyxovirus EU156171/EF646380	aspv	T	GTTTATGTCACTTGTCAATGGATTAAAGAAAACTTAGGATCA AAGCAATTGATATCTACTCTAGTTGATACCGACTACTTTGAT CTTTTTCCCATATTAGCAAATGAGCTCTATCTTAGACAAAAT CAAAAGATGGCAGAACAAAAGGGCTACCCAAACCGTCTAGC ATCCAGTACATCAAGCTCAATGATGAGTTCCTTAACGAAGGT CTCAACATCCTCGGGTGTTTCCTCAGAGGAAATGCCCAAAC TGCCCCAACCCAAGAAGCACAGGGACACAAGGGTGGTGAA AATGATACCGGTGCCTCTCAAAAGGCCCCAACTCCCACCAG GAGAAGGAACAGTCTTAGTGGTCCATCCGATAGGGAAATGC GATCCCAGAGAGAAGCGGATGTCGACAAGTGGGATCATAG TGGCGTATATAGCCACGGACTCCGGGGCCAACTCGATAAG AGCACTTGTGAAGAGTTTGGAAGAGGTCAAGATGGAAACAC CGGAGGACTCAATCTTTGTGGACCAGATGTTGTGCTCAACC AGGGTCCTCCTCATAAGGATAGAGCATTGGGTGGAATTGCT GACCCGGGGAAGAAGACCCACCTGGAACCTGCTGATCTCG AGGATGGCGCTGATGAAGAGAGTCTCCCAAGGGAAACCGG TGGAAGAGCTCCTGCTGGACGAAGCATGGAAGGTGGTTTTA CCCTGGATAGAAGGGGAAGTCCGTGCAAGCCTGCCCGCAA AGCAGATTTCAGAGAAGGGACAGACAACCCCGACACCTGG TCAGACCCAGGAACGACTTTTATGAGAAAGGCCAGAGCAAT GAACCCTCAGTCTAGTGCTTCCCCTTATCTGGAGGAACAAC TCAAAGCCGAGAAGGCCCAGCTCTTGTATCAGGCAGAGGA CAACAACGAGGACAAATCAGAAGGATCGGCACCCGAGGCA GAAGAGGATCAAGAAGAAGAGGAGGAGGAAGACGAGATAG TTGAGGATCCAGACAGTGACGG	1000

Type of Microbe	Classification of Microbe	Specificity Sample Run Name	Species	PEI Assay Name	Asp_Test or CRS	Sequence (5'-3')	Length (bp)
Virus	ssRNA (-) Rhabdovirus	VHSV type Ib_N_gblock	Viral hemorrhagic septicemia virus AB672614	vhsv	T	GCTTCTCTCCTATGTACTCCAAGGGAATACACAGGAGGACC TAGGAATGAAGTGCAAGGTCCTCACAGACATGGGCTTCAAG GTGACACAGGCAGCCAGGGCTACGAGCATCGAGGCAGGAA TCATGATGCCCATGAGAGAACTGGCCCTGACTGTCAATGAC GACAACCTCATGGAAATCGTTAAGGGGACCTTGATGACATG CTCCCTTCTGACCAAGTACTCGGTGGACAAGATGATCAAGT ACATCACCAAGAAACTCGGGGAGCTGGCAGACACTCAGGG AGTTGGGGAACTGCAGTACTTCACCGCTGACAAGGCAGCC ATCAGGAAACTCGCAGGGTGTGTGCGTCCCGGGCAGAAGA TCACCAAGGCCCTCTATGCATTCATCTTGACTGAGATCGCA GACCCCACCACCCAGTCGAGGGCCCGAGCCATGGGGGCG TTGAGGCTCAACGGGACAGGAATGACCATGATTGGGTTGTT CACCCAGGCCGCCAACAACTTGGGCATTGCCCCGGCAAAG CTGCTGGAGGACCTCTGCATGGAGTCCCTGGTTGAGTCAG CCAGACGGATCATCCAGTTGATGAGACAGGTGTCAGAGGC GAAGTCCAACCGTCTAGCATCCAGTTTCAAGAGCGATACGC CATCATGATGAGTCGGATGCTGGGAGAGTCCTACTACAAGT CGTATGGACTCAATGACAACTCCAAGATCTCTTACATTCTGT CACAGATCAGTGGGAAGTACGCAGTGGACTCCCTGGAAGG CCTGGAGGGGATCAAGGTGACAGAGAAGTTCCGCGAGTTC GCTGAGCTTGTTGCAGAAGTCCTGGTGGACAAGTACGAGA GGATTGGAGAGGACAGCACAGAGGTCTCAGATGTCATCAG GGAGGCGGCCAGACAGCACGCGCGCAGGACATCTGCCAG GCCAGAGCCAAAGGCCCGCAACTTCAGGAGCTCCACCGGA AGGGGGAAGGAGCAGGAGACGGAGGGATCCG	1000
Virus	ssRNA (-) Rhabdovirus	VHSV type IIIa_N_gblock	Viral hemorrhagic septicemia virus	vhsv	T	GCTTCTCTCCTATGTACTCCAAGGGAACACACAGGAGGACC TAGAAACAAAGTGCAAGGTCCTCACAGACATGGGCTTCAAG GTGACACAGGCAGTCAGGGCCACTAGCATCGAGGCGGGAA TCATGATGCCCATGAGAGAACTGGCCCTGACTGTCAATGAC GACAACCTCATGGAAATCGTCAAGGGGACCTTGATGACATG CTCCCTTCTTACCAAGTACTCGGTGGACAAGATGATCAAGT ACATCACCAAGAAACTCGGGGAGCTGGCAGACACCCAGGG AGTTGGGGAACTGCAGCACTTCACCGCTGACAAGGCAGCC ATCAGGAAACTCGCAGGGTGTGTGCGTCCCGGGCAGAAGA TCACCAAGGCCCTCTATGCGTTCATCCTGACCGAGATCGCA GACCCCACCACCCAGTCGAGGGCCCGAGCCATGGGGGCG TTGAGGCTCAACGGGACAGGAATGACCATGATTGGGTTGTT CACCCAGGCCGCCAACAACTTGGGCATTGCCCCGGCAAAG CTGCTGGAGGACCTCTGCATGGAGTCCCTGGTTGAGTCAG CCAGACGGATCATCCAGTTGATGAGACAGGTGTCAGAGGC GAAGTCCAACCGTCTAGCATCCAGTTCCAAGAGCGCTACGC CATCATGATGAGTCGGATGCTGGGAGAGTCCTACTACAAGT CGTATGGACTCAATGACAACTCCAAGATCTCTTACATTCTGT CACAGATCAGTGGGAAGTACGCAGTGGACTCCCTGGAAGG CCTGGAGGGGATCAAGGTGACAGAGAAGTTCCGCGAGTTC GCTGAGCTTGTTGCAGAAGTCCTGGTGGACAAGTACGAGA GGATTGGAGAGGACAGCACGGAGGTCTCAGATGTCATCAG GGAGGCGGCCAGACAGCACGCGCGCAGGACATCTGCCAA GCCAGAGCCAAAGGCCCGCAACTTCAGGAGCTCCACCGGA AGGGGGAAGGAGCAGGAGACGGGGGGGTCCG	1000
Virus	ssRNA (-) Rhabdovirus	VHSV type IIIb_N_gblock	Viral hemorrhagic septicemia virus EU481506/FJ362510	vhsv	T	TCTTCTCTCCTATGTACTTCAAGGGAACAAACAGGAGGACC TGGAGGCAAAGTGCAAGGTGCTCACAGACATGGGCTTCAG GGTGACACAGTCAACCAGGGCGACGGGCATCGATGCAGGA ATCCTGATGCCTATGAGAGAGCTGGCCCTGGCCGTCAATAA CGACAGCCTCATGGACATCGTTAAGGGGACCCTGATGACAT GTTCCCTTCTGACCAAGTACTCGGTGGACAAGATGATCAAG TACATCACCAAGAAGCTTGGGGACCTGGCAGACACTCAGG GAATTGGGGAACTGCAGCACTTCACCGCCGACAAGGCAGC CATCAGGAAACTCGCAGGGTGTGTGCGTCCCGGGCAGAAG ATCACCAAGGCCCTCTATGCATTCATCCTGACCGAGATCGC AGATCCCACCACCCAATCAAGGGCCCGAGCCATGGGGGCA TTGAGGCTCAACGGGACAGGAATGACCATGATCGGGTTGTT CACCCAGGCCGCCAACAACCTGGGCATTGCCCCGGCGAAG CTGCTGGAGGACCTCTGCATGGAGTCTCTGGTTGAGTCAG CCAGACGGATCATCCAGCTGATGAGACAGGTGTCAGAGGC AAAGTCCAACCGTCTAGCATCCAGTTCCAAGAGCGCTACGC CATCATGATGAGTCGGATGCTGGGGGAATCCTACTACAAGT CGTACGGACTAAATGACAACTCCAAGATCTCCTACATTCTGT CGCAGATCAGTGGGAAGTACGCAGTGGACTCCCTGGAAGG CCTGGAGGGGATCAAGGTGACAGAGAAGTTCCGCGAGTTC GCTGAACTTGTTGCCGAGGTCCTGGTGGACAAGTACGAGA AGATTGGGGAGGACAGTACTGAGGTTTCAGATGTCATCAGG GAGGCGGCTAGACAGCACGCGCGCAGGACATCCGCCAAG CCAGAGCCCAAGGCCCGCAACTTCAGGAGCCCCACCGGAA GAGGGAAGGAGCAGGATACAGAGGAGTCTG	1000
Virus	ssRNA (-) Rhabdovirus	VHSV type IV_N_gblock	Viral hemorrhagic septicemia virus	vhsv	T	ACTTCTCTCCTATGTACTCCAAGGAAGTGCACAAGGAGATC TGGAGGCAAAGTGCAAGATCCTCACTGACATGGGCTTCAAG GTGACACAGTCACCCCGGGCAACTGGTATCGAGGCCGGAA TCCTTATGCCGATGAGGGAACTGGCCCAGACTGTCAACAAC GACAACCTCATGGACATCGTCAAGGGGGCCCTGATGACGT GTTCCCTTTTGACCAAGTACTCGGTGGACAAAATGATCAAG TACATCACCAAGAAGCTTGGGGAGCTGGCAGACACCCAGG GGGTTGGAGAACTGCAGCACTTCACCGCCGACAAAGCAGC CATCAGAAAGCTCGCAGGATGTGTGCGTCCTGGACAGAAG ATCACCAAGGCCCTCTACGCCTTCATCCTGACCGAGATCGC GGACCCCACCACCCAGTCAAGGGCCAGAGCCATGGGGGC GTTGAGGCTCAATGGGACAGGAATGACCATGATCGGACTGT TCACCCAAGCCGCCAACAACCTGGGCATCGCCCCGGCGAA GCTGCTGGAGGATCTGTGCATGGAATCTCTTGTTGAGTCAG CCAGGCGGATCATCCAGCTGATGAGGCAGGTGTCGGAGGC GCGGTCCAACCGTCTAGCATCCAGTTCCAAGAGCGATACG CCATCATGATGAGTCGGATGCTGGGAGAGTCCTACTACAAG TCGTACGGACTCAATGACAACTCCAAGATCTCCTACATTCTG TCTCAGATCAGTGGGAAGTACGCAGTGGACTCCTTGGAGG GCCTGGAGGGGATCAAGGTGACAGAGAAGTTCCGCGAGTT CGCCGAACTTGTCGCTGAGGTCCTGGTGGACAAGTATGAG AGGATCGGAGAGGACAGCACAGAGGTCTCAGATGTCATCA GAGAGGCAGCCAGACAGCATGCACGCAAGGCATCAGACAA GCCAGAGACCAAAGCCCGCAACTTCAGGAGCTCCACCGGA AGGGGGAAGGAGCAGGAGAAGGAGGAGTCCG	1000

Type of Microbe	Classification of Microbe	Specificity Sample Run Name	Species	PEI Assay Name	Asp_Test or CRS	Sequence (5'-3')	Length (bp)
Virus	ssRNA (+) Togavirus	SAV_1_2_3 nSP_gblock	Salmon alphavirus AJ316244/AJ316246/JQ799139	sav	T	TTAAATCCAGAAGCATACATATATCAATGATGCAAAATCTCA CAGCTAACCCCTCCGCCGGCGCTACAGTTACTGTAAATCTG CCCGCGGACCACCCGGCCCTGAACCAGTTCAAGACTGCGT TTCCAGGGTTCGAAGTGGTGGCCAGCAATAGGTCGTCTAAC GACACCGTCTAGCATCCAGTCATGCCGCCGCCAGAGCTTTC TCCCACTTGGCTACCAAGTGGATTGAGCGCGACATCGGTG GCCGTCAGGTCATCGTTGCCGACATAGGGAGCGCACCAGC GAGAAGAATCGGGGCACCCGATAACGTCACATACCACAGT GTGTGTCCTCGCAAATGCGCTGAAGACCCTGAGAGGCTGG CTTCTTATGCCAGGAAACTCGTCAGGGCTGTGGAGAGAGG AGATGGACACCTCGTCAATGAAAAAATCACTGACTTGAAGG ATGTTCTGGAGAACCCGGACACGTCACTGGAAACCACCAGT ATCTGCCTGAACGACGACGTGAGTTGTAAAGTAAAGGCCGA TATTGCAGTGTACCAGGATGTGTACGCAGTCGATGCTCCGT CTACCATCTACGCGCAGGCCGACAAAGGTACGCGAGTGGT ATACTGGATCGGGTTCGAGCCGTTCGTGTTCCACACGGAC GCTATGGCGGGTAGCTTCCCGCTATATGACGCCAACTGGA GCGACTCGGCGGTACTGGCAGCTAAAAACCTGCCACTGTG CTACTCAGGGTTGTCGGAGGATTCCATTAAATGGAGATTCC GTTTCCGTGACAAGCCACTGGTACCGTCTGGAGAAATCCAC TACTCGGTGGGTAGCACCCACTACGTTGAGGACCGTGACA AGCTGAAAAGCTGGCACCTGCCTTCTACCTTCCACTTCGTG GCACCCAACAAGTATACATGCCGTTGCGATACGGTGGTAAG CTGTGGCGGCTACGTCGTAAAGAAGATCACGATCTGTGAG GGTATCGTCGGTATTCCCGCGAAGGAG	1000
Virus	ssRNA (+) Nodavirus	VER gp1 Cp_gblock	Viral encephalopathy and retinopathy virus	ver	T	TCACAATGGTACGCAAAGGTGAGAAGAAATTGGCAAAACCC GCGACCACCAAGGCCGCGAATCCGCAACCCCGCCGACGTG CTAACAATCGTCGGCGTAGTAACCGCACTGACGCACCTGTG TCTAAGGCCTCGACTGTAACTGGATTTGGACGTGGGACCAA TGACGTCCATCTCTCAGGTATGTCGAGAATCTCCCAGGCCG TCCTCCCAGCCGGGACAGGAACAGACGGATACGTTGTTGTT GACGCAACCATCGTCCCCGACCTCCTGCCACGACTGGGAC ACGCTGCTAGAATCTTCCAGCGATACGCTGTTGAAACACCG TCTAGCATCCAGTACTGGAGTTTGAAATTCAGCCAATGTGC CCCGCAAACACGGGCGGTGGTTACGTTGCTGGCTTCCTGC CTGATCCAACTGACAACGATCACACCTTCGACGCGCTTCAA GCAACTCGTGGTGCAGTCGTTGCCAAATGGTGGGAAAGCA GAACAGTCCGACCTCAGTACACCCGCACGCTCCTCTGGAC CTCGTCGGGAAAGGAGCAGCGTCTCACGTCACCTGGTCGG CTGATACTCCTGTGTGTCGGCAACAACACTGATGTGGTCAA CGTGTCAGTGCTGTGTCGCTGGAGTGTTCGACTGAGCGTTC CATCTCTTGAGACACCTGAAGAGACCACCGCTCCCATCATG ACACAAGGTTCCCTGTACAACGATTCCCTTTCCACAAATGAC TTCAAGTCCATCCTCCTAGGATCCACACCACTGGACATTGC CCCTGATGGAGCAGTCTTCCAGCTGGACCGTCCGCTGTCC ATTGACTACAGCCTTGGAACTGGAGATGTTGACCGTGCTGT TTATTGGCACCTCAAGAAGTTTGCTGGAAATGCTGGCACAC CTGCAGGCTGGTTTCGCTGGGGCATCTGGGACAACTTCAA CAAGACGTTCACAGATGGCGTTGCTTACTACTCTGATGAGC AGCCTCGTCAAATCCTGCTGCCT	1000
Virus	ssRNA (+) Nodavirus	VER gp2 Cp_gblock	Viral encephalopathy and retinopathy virus AY547549	ver	T	AATGGTACGCAAAGGTGATAAGAAATTGGCTAAACAAACGA CCACAAAGGCCGTCAATCCACAACCCCGTCGGCGCAACAA CAGTCGCCGGCGTGGAATGAGAGCGGATGCACCTTTGGCT AAGGCCTCGACAGTCACGGGATTTGGACGTGGGACCAATG ACGTCCATCTCACGGGTATGTCGAGAATCGCCCAAGCGGTT ATCCCAGCTGGCACCGGCACGGACGGATACATCGTGGTCG ATGAGACCATCGTCCCCGAGCTCTTGCCAAGATTGGGATAC GCTGCTAGAATCTTCCAGCGATACGCTGTTGAAACACCGTC TAGCATCCAGTACTGGAGTTCGAAATTCAGCCAATGTGCCC CGCAAACACGGGCGGTGGTTACGTGGCTGGCTTCCTGCCT GATCCAACTGACAGCGACCACACCTTCGACGCAATACAAGC GACTCGTGGTGCGGTCGTTGCCAAATGGTGGGAAAGCAGA ACTATCCGGCCCCAGTATGCCCGCGCACTCCTCTGGACCT CGGTCGGGAAGGAGCAGCGTCTGACATCCCCGGGCCGGTT GATACTCCTGTGTGTCGGCAATAACACTGACGTGGTCAACG TGTCGGTGCTGTGTCGCTGGAGTGTACGTCTCAGTGTGCCA TCCCTTGAGACGCCCGAAGATGCAACCGCTCCAATCCTAAC CCTCGGACCACTCTACAACGATTCCCTCGCAGCCAACGATT TTAAATCGATCCTCCTTGGCGCTACTCAACTCGACATCGCT CCCGAAGGAGCCGTCTACACATTAGACCGGCCGCTTTCCAT CGATTACAATCTGGGAACTGGTGATGTTGACCGTGCCACCT ATTGGCATGTGAAGAAAGTTGCTGGGAATACGGGAACACCC GCGGGGTGGTTTCACTGGGGGCTATGGGACAATTTCAACA AAACATTTACACAGGGCGTTGCTTACTATTCTGACCAGCAG CCAAGACAGATCTTGCTGCCAGTGG	1000
Virus	ssRNA (+) Nodavirus	VER gp3 Cp_gblock	Viral encephalopathy and retinopathy virus	ver	T	TAATCCAACACTGCTTTGCCATCACAATGGTACGCAAAGGG AATAAGAAATTGGCTAAACCAGCGACCACAAAGGCCGTTAA TCCCCAACCCCGTCGACGCAACAACAACCGTCGGCGTGGC ATGAGAGCGGATGCACCTTTAGCTAAGGCCTCGACTATCAC GGGATTTGGACGTGGGACCAATGACGTCCATCTCACGGGT ATGTCGAGAATCGCCCAAGCGGTTATCCCAGCTGGCACCG GCACGGACGGATACATCGTGGTTGACGAAACCATCGTCCC CGAGCTCTTGCCAAGACTGGGATTTGCTGCTAGAATCTTCC AGCGATACGCTGTTGAGACACCGTCTAGCATCCAGTACTGG AGTTCGAAATTCAGCCAATGTGCCCCGCAAACACGGGCGG TGGTTACGTGGCTGGCTTCCTGCCTGATCCAACTGACAGCG ACCACACCTTCGACGCAATTCAAGCGACTCGTGGTGCGGTC GTTGCCAAATGGTGGGAAAGCAGAACAATCCGACCCCAGC ATGCCCGCGCACTCCTCTGGACCTCGGTCGGGAAGGAGCA GCGTTTGACATCCCCGGGCCGGTTGGTACTCCTGTGTGTC GGCAACAACACTGACGTCGTCAACGTGTCAGTGCTGTGTCG CTGGAGTGTGCGTCTCAGTGTTCCATCTCTCGAGACACCTG AAGATACATTCGCTCCAATCCTAACCCTGGGACCACTCTAC AACGACTCCCTTGCACCCAACGATTTCAAATCAATACTTCTT GGCTCTACCCAGCTTGACATCGCCCCTGAAGGAGCCGTCT ATTCATTAGATCGGCCGCTGTCCATTGACTACAGTCTGGGC ACTGGTGATGTCGACCGTGCCGTTTACTGGCATGTGAAGAA AGTTGCTGGCAATGCGGGAACACCTGCGGGGTGGTTCCAC TGGGGGCTATGGGATAATTTCAACAAAACATTCACACAGGG CGCTGCCTACTATTCTGATGCGCAG	1000

Type of Microbe	Classification of Microbe	Specificity Sample Run Name	Species	PEI Assay Name	Asp_Test or CRS	Sequence (5'-3')	Length (bp)
Virus	ssRNA (+) Nodavirus	VER gp4 Cp_gblock	Viral encephalopathy and retinopathy virus	ver	T	ATGGTACGCAAGGGTGAGAAGAAATTGGCAAAACCCGCGA CCACCAAGGCCGCAAACCCACAACCGCGTCGACGTGCCAA CAATCGCCGGCGTAGTAATCGCACTGATGCGCCTGTGTCTA AGGCCTCGACTGTGACTGGATTTGGACGCGGGACCAATGA TGTCCATCTCTCAGGTATGTCGAGAATCTCCCAGGCCGTCC TCCCAGCCGGGACAGGAACTGACGGATACGTCGTTGTTGA TGCAACTATCGTCCCCGACCTCCTGCCACGACTGGGACAC GCTGCTAGAATCTTCCAGCGATACGCTGTTGAAACACCGTC TAGCATCCAGTACTGGAGTTCGAAATTCAGCCAATGTGCCC CGCAAACACGGGCGGTGGTTACGTTGCTGGCTTCCTGCCT GATCCAACTGACAACGACCACACCTTCGACGCACTTCAAGC AACTCGTGGTGCGGTCGTGGCCAAGTGGTGGGAAAGCAGA ACAGTCCGACCACAGTACACCCGCACGCTCCTCTGGACCT CGACGGGAAAGGAGCAGCGCCTCACATCACCTGGTCGGCT GATACTCCTGTGTGTCGGCAACAACACTGACGTGGTCAACG TGTCAGTTCTGTGTCGCTGGAGTGTTCGGCTGAGCGTTCCG TCTCTTGAAACACCTGAAGAGACGACCGCTCCCATCATGAC ACAAGGTCCCCTGTACAACGATTCCCTCGCTACCAGCGACT TCAAGTCCATCCTCCTGGGGTCCACGCAGTTGGACATTGCT CCTGATGGGGCAATCTTTCAATTGGACCGACCATTGTCCAT TGATTACAGCCTAGGAACTGGTGATGTTGACCGTGCCGTCT ACTGGCACCTCAAGAAGTTCGCTGGCACATCTGCCACACCT GCGGGCTGGTTTCGCTGGGGCATCTGGGACAACTTCAACA AAACGTTCACAGATGGCGTGGCTTACTACTCTGACGCGCAG CCTCGTCAAATTCTCCTGCCCGTTGG	1000
Virus	ssRNA (+) Nodavirus	VER gp5 Cp_gblock	Viral encephalopathy and retinopathy virus AY547548	ver	T	AATGGTACGCAAAGGTGATAAGAAATTGGCTAAACAAACGA CCACAAAGGCCGTCAATCCACAACCCCGTCGGCGCAACAA CAGTCGCCGGCGTGGAATGAGAGCGGATGCACCTTTGGCT AAGGCCTCGACAGTCACGGGATTTGGACGTGGGACCAATG ACGTCCATCTCACGGGTATGTCGAGAATCGCCCAAGCGGTT ATCCCAGCTGGCACCGGCACGGACGGATACATCGTGGTCG ATGAGACCATCGTCCCCGAGCTCTTGCCAAGATTGGGATAC GCTGCTAGAATCTTCCAGCGATACGCTGTTGAAACACCGTC TAGCATCCAGTACTGGAGTTCGAAATTCAGCCAATGTGCCC CGCAAACACGGGCGGTGGTTACGTGGCTGGCTTCCTGCCT GATCCAACTGACAGCGACCACACCTTCGACGCAATACAAGC GACTCGTGGTGCGGTCGTTGCCAAATGGTGGGAAAGCAGA ACTATCCGGCCCCAGTATGCCCGCGCACTCCTCTGGACCT CGGTCGGGAAGGAGCAGCGTCTGACATCCCCGGGCCGGC TGATACTCCTGTGTGTCGGCAACAACACTGACGTGGTCAAC GTGTCGGTGCTGTGTCGCTGGAGTGTACGTCTCAGTGTGC CAACCCTTGAGACGCCCGAAGATACAACCGCTCCGATCCTA ACCCTCGGACCACTCTACAACGATTCCCTCGCAGCCAACGA TTTTAAATCGATCCTCCTTGGCGCTACTCAACTCGACATCGC TCCCGAAGGAGCCGTCTACACATTAGATCGGCCGCTTTCCA TCGATTACAATCTGGGAACTGGTGATGTTGACCGTGCCACC TATTGGCATGTGAAGAAAGTAGCTGGGAATACGGGAACACC TGCGGGGTGGTTTCACTGGGGGCTATGGGACAATTTCAACA AAACATTTACACAGGGCGTTGCTTACTATTCTGACCAGCAG CCAAGACAGATCTTGCTGCCAGTGG	1000
Virus	ssRNA (+) Nodavirus	VER gp6 Cp_gblock	Viral encephalopathy and retinopathy virus AY547547	ver	T	ACCATCGTCCCCGAGCTCTTGCCAAGACTGGGATATGCTGC TAGAATCTTCCAGCGATACGCTGTTGAAACACCGTCTAGCA TCCAGTACTGGAGTTCGAAATTCAGCCAATGTGCCCCGCAA ACACGGGCGGTGGTTACGTGGCTGGCTTCCTGCCTGATCC AACTGACAGCGACCACACCTTCGACGCAATTCAAGCGACTC GTGGTGCGGTCGTTGCCAAATGGTGGGAAAGCAGAACAAT CCGGCCCCAGTATGCCCGCGCACTCCTCTGGACCTCGGTC GGGAAGGAGCAGCGTCTGACATCCCCGGGCCGGTTGATAC TCCTGTGTGTCGGCAACAACACTGACGTGGTCAACGTGTCG GTGCTATGTCGCTGGAGTGTACGTCTCAGTGTACCATCCCT CGAGACGCCCGAAGATACATTCGCTCCAATTCTATCCCTCG GACCACTCTACAATGATTCCCTCGCCGTCAACGATTTCAAAT CGATCCTCCTTGGCGCTACTCAGCTCGACATCGCTCCTGAA GGAGCCGTCTACACATTAGATCGACCTCTTTCCATCGATTA CAATCTGGGAACTGGTGATGTCGACCGTGCCGTCTATTGGC ATGTGAAGAAAGTTGCTGGGAATGCGGGAACACCTGCGGG GTGGTTTCACTGGGGGCTATGGGACAATTTCAACAAAACAT TCACACAGGGCGTTGCTTATTATTCTGACCAGCAGCCAAGA CAGATCTTGCTGCCAGTGGGCACGCTCTTCACCCGTGCTGA CTCGGGAAACTAACCGGGTCATCCGGTTCCCTGGTGCGTAT CGTTGATGACCAATTTGACAAATTGATTCAAGCACTAAGCTC CATTAATGCCGAAATTCTAACAAACAAAACAGAAATTCAAAG AAACAAAAGCGACATTGAAGCTATCGCTAACAAATTAGACAA CAAAGCACCCAAGGAAGGTACAATTGCTATTGTTGGTACCA TTGACGGCGTACCTGGT	1000
Virus	dsRNA Totivirus	PMCV_RdRp_gblock	Piscine myocarditis virus NC_015639/HQ339954	pmcv	T	GATAAATAGGGCATTAGAATCAATTTTAGGGCCACTAAGGG GTACATGCACGGTACCGTGCTCACAAAGGGAATTTTTATTAT GTAGGGATAAATGGGCCAAAAACACGTCCGGGTACATAGG GTTAGGGCAAAATTTAGGGCGACAGAAGATAGACGTAGCAC TTAATTCAGACATCAAGGCACTGGAAAGGATGATGGAGGGA GAATTCGAGAACAGACCATTTATTAAAAGTGAACCAGGGAA AAGCAGGCCGGTAGTTAATAGTAATATATCGTGTTACCTAAA TTTAGAGTATGGGTGGGTAACCATTAAAAAGACACTGAAAAA ATACTTAGGCAAGAGGACAACAATTTTCGACGACGCAAGCC AAAAGGTACTGTTATGGCAAGAAATGATAATTGGAGCAGAT AACCATAGGGAGATTAAAGTGCCACTAGACTACTCGCGGTT CGATTCAACAATTGGGAAAACGCAAATCATGAAGTGTGTTA GACTGCTATTAGACATGGTGCGAGGTAACGACAATTGGAAA AGGTCAGTTACTGAAAGATTCGAGAGACAAACGGTGTACAT TGACGGTTACGGAAAACTCAGGTGGGAAAACAGCGTACTTA GCGGATGGAGATGGACAAGTATAATCACATCTGTAATAAATT TGGCCATTCTTAAGGCGACCGGAGCCGATGCGAGCGGAGT AGGGATTAAAGTCCAGGGAGATGATGTTAAAATCAGCTTCC AAACAATTCGAGAAGCGGAAAGGTGTGTCGAAGAAATTAAC GCTTTAGGGTATGAAATTAACCCGGGTAAAGTATTTGCGTC AAGGAAAAGGGACGAACCGTCTAGCATCCAGTGTATCTCAG GATGGTAGCGGAAGAGGGGAAAATGGCAGGTTATGTAATTA GGTGTCTACCGAAAATTGTATTTACATCACCAACAGAAGAG GTTACCACATGGGAAGAACGAATTAGGGGAACAGTGTCAAA ATGGATGAGGGTGT	1000

Type of Microbe	Classification of Microbe	Specificity Sample Run Name	Species	PEI Assay Name	Asp_Test or CRS	Sequence (5'-3')	Length (bp)
Virus	dsRNA Reovirus	PRV_L1_gblock	Piscine reovirus GU994013	prv	T	TCGCCCCTCATCAAAGAGCCGTCGTCGTCAGTGAAGCTTAC TCCCTAGTACGCACCATCATCTCACAGATTTCAGACACGCG TGGACCGCAACTCAATCCTCTGGCTTGGATCCCGGCTCCAA ACGCATCATCTCCTGTCTCAGCCGAAGTAGCCAGACTCGTC AATGACATGATCAAGGAAGCTTTTGACATGCCTGGTGAACT ACTTGAAGGATTGATCGGATATGGTGACCCTAGATACACTC AAGTCGAGATTGTTGCACAGAGGTGTCGCGCGGCTCCACTT CGGTTCGAACCACTAATTCCTCCGTCTGTTCTGGCTCAAGA GCTTCAACTTGTTGAGAACGTGATCACTGCTGAACCTAATCT GTTTGGATTGGCTACTGGAGACTTATATCTCGAGCGCATTG ACACTTCAGCCGGGTTCTCTGGTCTCAACGTTATCGGCTGG GAGCAATGGGATGCTAACACTCCAGGAGTCATTGACTGGAT GCTAGACGGTTCGCCGGTAGCTCTCTACTCATCTGCAGCG GATTCAACGGAGTAGACCCAATGATCATGGACGCTGATGGG GTGGAACGGCCGATTACCGGTAGATGGGTTGTCACACTGG AAGCTTGGCGTAGCAGCGTGGTCACCGTCCAGAAGTTGCT GTTACCAAGGATCAGAGCAGGAAAGTTGGCTGTAAGGATAC TGGTTGGTATTTTTCCATACACCATTAACTACTATGAACCCG CTGTTGGTATTGACGAGTGGAAGCTGTTGTCCGACTGGGC GTCCATGTGTGAACCTACAGGAATACCGGCCATACCTTTCA CTGCTCCAGTTCCATCTGATGTGTCCGTTGTCACCGCTGCG TGCGTGAGGTATCTGAGGTGCTCCACCTTCAACGAAGGCTC ATTGATGGCTACTAACGCAGGATCACCTCGCACCGTGTTTG GGCAATCAGTAGAGTTTGACATCGGCAGATGGATGCAGCTA TGTGATTTGAACACTGGA	1000
Parasite	Amoeba	01Neo_gblock_CHSE	Neoparamoeba perurans GU574794	ne_per	T	ATCTTGACTGGTTCTTTCGGGAGCTGGGAGGGATGTATaccg tctagcatccagtTTATTAGATTCAAAAGCCAATGCCATTCTTTTCG GAGAGTGGAAATTTAACATATGGTGAATCATGATAACTTTAC GAATCGCATGGTCTTTTGTGCCGGCGATAGTTCATTCAAATA TCTGCCCTATCAACTTTCGATGGTAGTATAGAGGACTACCAT GGTGTTAACGGGTAACGGAGAATCAGGGTTTGATTCCGGA GAAGGAGCCTGAGAAACGGCTACTACTTCCAAGGAAGGCA GCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAG TGACAAAAAATAACAATACCGGTCTTATTTAAGTCTGGTAAT TGGAATGAGTACAATCTAAATCCTTTAACGAGTATCAATTGG AGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTC CAATAGCATATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAG TTGGATTTTGAAGTTTACATATTATGACCCACTTTGTATTAAG TGTGTTTTGTTGTGATATGTACTTCTCCTTACTAGACTTTCAC TATTACACTTTATTGAGTGATAGTTTCATTTTAATTTATTAGA ATAAGTGATAAGCAGACCTAT	651
Parasite	Ciliate	02IMF_gblock_CHSE	Ichthyophthirius multifiliis	ic_mul	T	CCTTCGGGCGATTGTGATGATTCAAAGTAACTGATCGAATC GCGGCTTGCTGCGATAAATCATCTAAGTTTCTGCCCTATCA GCTCTCGATGGTAGTGTATTGGACTACCATGGCGCTCACGG GTAACGGAGAATTAGGGTTCGATTCCGGAGAAGGAGCCTG AGAAACGGCTACTACACCTACGGTAGGCAGCAGGGACGTA AATTACCCAATCCTAATTCAGGGAGGTAGTGACAAGAAATA GCAAGCCAGGAGATTCTTCTCTACGGCATTGAAATGAGAAC AGTGTAAATCTCTTAGCGAGGAACAATTGGAGGGCAAGTCA GGTGCCAGCAGCCGCGGTAATTCCTGCTCCAATAGCGTATA TTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACTTCTGC CTATTTAGCTGGGTTCTCTTAGGCTAAAATGGGCATACGTTT GCAAACCAAACTCGGCCTTCACTGGTTCGAaccgtctagcatccag tCTTGGGGAGTAAACATTTTACTGTGAAAAAATTAGAGTGTTT CAGGCAGGTTATAGCCCGGATACATCAGCATGGAATAATGG AATAGGACTGAGTCCATTTTATTGGTTATTGGATTTGGTAAT GATTAATAGGGACAGTTGGGGGCATTAGTATTTAATAGTCA GAGGTGAAATTCTTGGATTTATTAAGGACTAACTAATGCGAA AGCATTTGCCAAAGATGTTTTCATTAATCAAGAACGAAAGTT AGGGGATCAAAGACGATCAGATACCGTCGTAGTCTTAAGCT ATAAACTATACCGACTACGGGATCGGCTGGAATACTATGTC CAGTCGGCACCGTATGAGAAATCAAAGTCTTTGGGTTCTGG GGGAAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGACG GAACAGCACACCAGAAGTGGAACCTGCGGCTTAATTTGACT CAACACGGGGAAACTCACGTAGCGCAAGACAGAGAAGGGA TTGACAG	1000
Parasite	Ciliate	02IMF_Gchattoni_CHSE	Glaucoma chattoni X56533	ic_mul	CRS	CGGAACGTATTTATTAGATATTAAACCAATCGCAGCAATGTG ATTGAGATGAATCAAAGTAACTGATCGAATCGTAGCTTGCTA CGATAAATCATCTAAGTTTCTGCCCTATCAGCTCTCGATGGT AGTGTATTGGACTACCATGGCAGTCACGGGTAACGGAGAAT TAGGGTTCGATTCCGGAGAAGGAGCCTGAGAAACGGCTAC TACAACTACGGTTTGGCAGCAGGGAAGAAAATTGGCCAATC CTAATTCAGGGAGCCAGTGACAAGAAATAGCAGACCGGGA AACTACGTTTCTACGGTACTGAAATGAGAACAGTGTTAATCT CTTAGCGAGAAACAATTGAGGCAAGCCTGCTGCCAGCAGC CGCGGTAATTCCAGCTCCAATAGCGTACATTAAAGGGCAGT TAAAAAGCTCGTAGTTGAACTTCAGTGTCCAGGTTCGTCTC GGCTCGCCGAGGCAACTCTGGACATACGTCTGCAAGCTAA AATCGGCCTTTAACCGTCTAGCATCCAGTCTGGTTCGACTT AGTGAGTAGACATTTTACTGTGCAAAAAATTAGAGTGTTTCA GGCAGGTTTTAGCCCGTATACATTAGCATGGAATAATGGAA TAGGACTAAGTCCATTTTATTGGTTATTGGATTTGGTAATGA TTAATAGGGACAATTGGGGGCATTAGTATTTAATAGTCAGAG GTGAAATTCTTGGATTTATTAAGGAACTAATGCGAAAGCATT TGCCAAAGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGA TCAAAGACGATCAGATACCGTCGTAGTCTTAACTATAAACTA TACCGACTCGGGATCGGCTGGAATATATGTCCAGTCGGCAC CGTATGAGAAATCAAAGTCTTTGGGTTCTGGGGGAAGTATG GTACGCAAGTCTGAAACTTAAAGGAATTGACGGAAGAGCAC ACCAGAAGTGGAACCTGCGGCTTAATTTGACTCAACACGGG GAAACTCACG	1000

Type of Microbe	Classification of Microbe	Specificity Sample Run Name	Species	PEI Assay Name	Asp_Test or CRS	Sequence (5'-3')	Length (bp)
Parasite	Ciliate	02IMF_Lambornella_CHSE	Lambornella AF364043	ic_mul	CRS	CGATTCCGGAGAAGGAGCCTGAGAAACGGCTACTACAACTA CGGTTCGGCAGCAGGGAAGAAAATTGGCCAATCCTAATTCA GGGAGCCAGTGACAAGAAATAGCAAGCTGGGAAACTTAGTT TCTACGGCATTGAAATGAGAAAAGTGTTAATCTCTTAGCGAG GAACAATTGGAGGGCAAGTCATGGTGCCAGCAGCCGCGGT AATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAA AAAGCTCGTAGTTGAACTTCTGTTCAGATTCATAACGACTCG TCGTGTGAAGCTGGGCATACGTTTGCAAACTAAAATCGTCC TTCAACCGTCTAGCATCCAGTCTGGCACGACTTAGGGAGTA GACATTTTACTGTGAAAAAATTAGAGTGTTCCAGGCAGGTTT TAGCCCGAATACATTAGCATGGAATAATGGAATAGGACTAA GTCCATTTTATTGGTTCTTGGATTTGGTAATGATTAATAGGG ACAGTTGGGGGCATTAGTATTTAATAGTCAGAGGTGAAATT CTTGGATTTATTAAGGACTAACTAATGCGAAAGCATTTGCCG AAGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCAAA GACGGTCAGATACCGTCGTAGTCTTAACTATAAACTATACC GACTCGGGATCGGCTGGAATACATGTCCAGTCGGCACCGT ATGAGAAATCAAAGTCTTTGGGTTCTGGGGGAAGTATGGTA CGCAAGTCTGAAACTTAAAGGAATTGACGGAACAGCACACC AGAAGTGGAACCTGCGGCTTAATTTGACTCAACACGGGGAA ACTCACGAGCGCAAGACAGAGAAGGGATTGACAGATTGAG AGCTCTTTCTTGATTCTTTGGGTGGTGGTGCATGGCCGTTC TTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAA CGAGACCTTAACCTGCTAACTAGTCTGCTTGTGAACAACAG GCTGTACTTC	999
Parasite	Ciliate	02IMF_Taustralis_CHSE	Tetrahymena australis M98015	ic_mul	CRS	TTATTAGATATTAGACCAATCGCAGTAATGTGATTGAGATGA ATCAAAGTAACTGATCGAATCGTAGCTTGCTACGATAAATCA TCTAAGTTTCTGCCCTATCAGCTCTCGATGGTAGTGTATTGG ACTACCATGGCAGTCACGGGTAACGGAGAATTAGGGTTCGA TTCCGGAGAAGGAGCCTGAGAAACGGCTACTACAACTACG GTTCGGCAGCAGGGAAGAAAATTGGCCAATCCTAATTCAGG GAGCCAGTGACAAGAAATAGCAAGCTGGGAAACTAAGTTTC TACGGCATTGAAATGAGAAAAGTGTTAATCTCTTAGCGAGG AACAATTGGAGGGCAAGTCATGGTGCCAGCAGCCGCGGTA ATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAA AAGCTCGTAGTTGAACTTCTGTTCAGGTTCATTTCGACTCGT CGAGTGAAACTGGGCATACGTCTGCAAACTAAAATCGGCCT TCACCGTCTAGCATCCAGTACTGGTTCGACTTAGGGAGTAG GCATTTTACTGTGAAAAAATTAGAGTGTTTCAGGCAGGTTTT AGCCCGAATACATTAGCATGGAATAATGGAATAGGACTAAG TCCATTTTATTGGTTCTTGGATTTGGTAATGATTAATAGGGA CAGTTGGGGGCATTAGTATTTAATAGTCAGAGGTGAAATTCT TGGATTTATTAAGGACTAACTAATGCGAAAGCATTTGCCAAA GATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCAAAGA CGATCAGATACCGTCGTAGTCTTAACTATAAACTATACCGAC TCGGGATCGGCTGGAATATATGTCCAGTCGGCACCGTATGA GAAATCAAAGTCTTTGGGTTCTGGGGGAAGTATGGTACGCA AGTCTGAAACTTAAAGGAATTGACGGAACAGCACACCAGAA GTGGAACCTGCGGCTTAATTTGACTCAACACGGGGAAACTC ACGAGCG	1000
Parasite	Ciliate	02IMF_Tthermophila_CHSE	Tetrahymena thermophila X56165	ic_mul	CRS	TATTAGATATTAGACCAATCGCAGCAATGTGATTGAGATGAA TCAAAGTAACTGATCGAATCGAGGCTTGCTTCGATAAATCAT CTAAGTTTCTGCCCTATCAGCTCTCGATGGTAGTGTATTGGA CTACCATGGCAGTCACGGGTAACGGAGAATTAGGGTTCGAT TCCGGAGAAGGAGCCTGAGAAACGGCTACTACAACTACGG TTCGGCAGCAGGGAAGAAAATTGGCCAATCCTAATTCAGGG AGCCAGTGACAAGAAATAGCAAGCTGGGAAACCTAGTTTCT ACGGCATTGAAATGAGAAAAGTGTTAATCTCTTAGCGAGGA ACAATTGGAGGGCAAGTCATGGTGCCAGCAGCCGCGGTAA TTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAA AGCTCGTAGTTGAACTTCTGTTCAGGTTTATAACGACTCGTC GTGTAAAACTGGACATACGTTTGCAAACTAAAATCGGCCTTC AACCGTCTAGCATCCAGTCTGGTTCGACTTAGGGAGTAAAC ATTTTACTGTGAAAAAATTAGAGTGTTCCAGGCAGGTTTTAG CCCGAATACATTAGCATGGAATAATGGAATAGGACTAAGTC CATTTTATTGGTTCTTGGATTTGGTAATGATTAATAGGGACA GTTGGGGGCATTAGTATTTAATAGTCAGAGGTGAAATTCTTG GATTTATTAAGGACTAACTAATGCGAAAGCATTTGCCAAAGA TGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCAAAGACG ATCAGATACCGTCGTAGTCTTAACTATAAACTATACCGACTC GGGATCGGCTGGAATAAATGTCCAGTCGGCACCGTATGAG AAATCAAAGTCTTTGGGTTCTGGGGGAAGTATGGTACGCAA GTCTGAAACTTAAAGGAATTGACGGAACAGCACACCAGAAG TGGAACCTGCGGCTTAATTTGACTCAACACGGGGAAACTCA CGAGCGC	1000
Parasite	Ciliate	02IMF_Ttropicalis_CHSE	Tetrahymena tropicalis M98023	ic_mul	CRS	TATTTATTAGATATTAGACCAATCGCAGCAATGTGATTGAGA TGAATCAAAGTAACTGATCGGATCGAGGTTTACCTCGATAAA TCATCTAAGTTTCTGCCCTATCAGCTCTCGATGGTAGTGTAT TGGACTACCATGGCAGTCACGGGTAACGGAGAATTAGGGTT CGATTCCGGAGAAGGAGCCTGAGAAACGGCTACTACAACTA CGGTTCGGCAGCAGGGAAGAAAATTGGCCAATCCTAATTCA GGGAGCCAGTGACAAGAAATAGCAAGCTGGGAAACTTACG TTTCTACGGCATTGAAATGAGAACAGTGTAAATCTCTTAGCG AGGAACAATTGGAGGGCAAGTCATGGTGCCAGCAGCCGCG GTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTT AAAAAGCTCGTAGTTGAACTTCTGTTCAGGTTCATTTCGATT CGTCGTGTGAAACTGGACATACGTTTGCAAACTAAAATCGG CCTTCAACCGTCTAGCATCCAGTCTGGTTCGACTTAGGGAG TAAACATTTTACTGTGAAAAAATTAGAGTGTTCCAGGCAGGT TTTAGCCCGAATACATTAGCATGGAATAATGGAATAGGACTA AGTCCATTTTATTGGTTCTTGGATTTGGTAATGATTAATAGG GACAGTTGGGGGCATTAGTATTTAATAGTCAGAGGTGAAAT TCTTGGATTTATTAAGGACTAACTAATGCGAAAGCATTTGCC AAAGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCAA AGACGATCAGATACCGTCGTAGTCTTAACTATAAACTATACC GACTCGGGATCGGCTGGAATAAATGTCCAGTCGGCACCGT ATGAGAAATCAAAGTCTTTGGGTTCTGGGGGAAGTATGGTA CGCAAGTCTGAAACTTAAAGGAATTGACGGAACAGCACACC AGAAGTGGAACCTGCGGCTTAATTTGACTCAACACGGGGAA ACTCACG	1000

Type of Microbe	Classification of Microbe	Specificity Sample Run Name	Species	PEI Assay Name	Asp_Test or CRS	Sequence (5'-3')	Length (bp)
Parasite	Ecto-worm	03Gysa_Gsal_CHSE	Gyrodactylus salaris GU187353	gy_sal	T	GCGGAAGGATCATTAAACATCGTTTCCTTACTTTGTGGTGAC TCAGTGGTGTCATTGCCTAAAATCAAAGGATTTTAAAGAATT AAAAGCAGTTAATGGTGATTCGTTTGTTATTGCATGGTTACG GTATAATGATATACCTTGAAGAATAAAGAATAAGGGTGGTG GCGCACCTATTCTACAAGCAGAACTGGTTAATAAGAaccgtcta gcatccagtTCGATTCCGAGTGACGATCGTGGGGCAAAATAAAT CCAGCTTGGGGAACTGGTTAACCATGGCATTATACGAGCAA GATGATTCCGAACGAGATTCTTTTAACATAGCAATGAACACA CGCTGTTTCATGCGCAACCAATCTGCCCTATAAAATTGGAG AGTGATTAGATTGCTCACCCACCGTCGTTTAGATGGTTGAC ATTAAAACGCTCATTGGAGTGAACTGGTAGTCTTCCGAGCT AAAATGGTAATGGCTAGTCTCGGTAAGGTCTGACTATCGGT TCGGCTACGGCCAGCTCAATGTAGTATCCGCTATTACCGAA ACATACACTACAGTGGTTCGATAGAGTTCCACACTCACTGC CTCTGCACCTTCGGGTGAACAGTCCGTAGTGCTTAGCGCCC CGTCAAAAGGGAAGAAGCTTTGGTTTATTACAACTCCATGT GGTGGATCACTCGGCTCACGTGACGATGAAGAGTGCAGCA AACTGTGTTAACCAATGTGAAACGCAAACTGCTTCGATCATC GGTCTCTCGAACGCAAATGGCGGCTAAGGGCTTGCTCTTAG CCACGTTCGATCGAGTGTCGGCTTTTACCTATCGTAACGCT TAATTAGTTACGGATTGGGAAGTATACCATGGCTATGCGATT AACTTGTTGTTGAAAGTTGAAACACGGGGTATTACACGGCC TTTACGGTTTGCCCTGTGGTGTTCTGATTCTGGTATTACACG GACTTTACGGTTTGCTAGATGAAGTTCACATTCGATGAGTAT GC	999
Parasite	Ecto-worm	03GysaAS_Gsal_CHSE	Gyrodactylus salaris GU187353	gy_sal	T	GCGGAAGGATCATTAAACATCGTTTCCTTACTTTGTGGTGAC TCAGTGGTGTCATTGCCTAAAATCAAAGGATTTTAAAGAATT AAAAGCAGTTAATGGTGATTCGTTTGTTATTGCATGGTTACG GTATAATGATATACCTTGAAGAATAAAGAATAAGGGTGGTG GCGCACCTATTCTACAAGCAGAACTGGTTAATAAGAaccgtcta gcatccagtTCGATTCCGAGTGACGATCGTGGGGCAAAATAAAT CCAGCTTGGGGAACTGGTTAACCATGGCATTATACGAGCAA GATGATTCCGAACGAGATTCTTTTAACATAGCAATGAACACA CGCTGTTTCATGCGCAACCAATCTGCCCTATAAAATTGGAG AGTGATTAGATTGCTCACCCACCGTCGTTTAGATGGTTGAC ATTAAAACGCTCATTGGAGTGAACTGGTAGTCTTCCGAGCT AAAATGGTAATGGCTAGTCTCGGTAAGGTCTGACTATCGGT TCGGCTACGGCCAGCTCAATGTAGTATCCGCTATTACCGAA ACATACACTACAGTGGTTCGATAGAGTTCCACACTCACTGC CTCTGCACCTTCGGGTGAACAGTCCGTAGTGCTTAGCGCCC CGTCAAAAGGGAAGAAGCTTTGGTTTATTACAACTCCATGT GGTGGATCACTCGGCTCACGTGACGATGAAGAGTGCAGCA AACTGTGTTAACCAATGTGAAACGCAAACTGCTTCGATCATC GGTCTCTCGAACGCAAATGGCGGCTAAGGGCTTGCTCTTAG CCACGTTCGATCGAGTGTCGGCTTTTACCTATCGTAACGCT TAATTAGTTACGGATTGGGAAGTATACCATGGCTATGCGATT AACTTGTTGTTGAAAGTTGAAACACGGGGTATTACACGGCC TTTACGGTTTGCCCTGTGGTGTTCTGATTCTGGTATTACACG GACTTTACGGTTTGCTAGATGAAGTTCACATTCGATGAGTAT GC	999
Parasite	Flagellate	04SpiroSal_gblock_CHSE	Spironucleus salmonicida AY677182	sp_sal	T	AGCCATGCATGCCTATGTGTAGACAGTTATATTCATTATTGT GGAGCGAAGACGGCGAACAGCTCATTTATCAGTGGTTAGTA CATGCAATGTATTTCGTTGGATAGTAACGGAAAATCTGTTAG TAATACATGAACTATGTTTAGTATTCTACTAAAGATAATAGTA AGTACGTTTGCATATCTGCCACTGCAGCATCATCTTACGTTG GTGGGGTATTTGCCTACCAAGGATTCGACGCTTACGGGGAA TTAGGGTTTGACTCCGGAGAATGAGCATGAGAAACAGCTCA TACATCTAAGGAAGGCAGCAGGCGCGGAAATTGCCCAATGT ATTCGTTACGAGGCAGTGACAAGAAATGAAGGGTACTTTTG TACACCATCTAGGGCCTGTTATATCTAAGCTAACCGGTTATT CGTGGGAAAGCTCGGTGCCAGCAGCCGCGGTAATTCCGAC ACGGAGAGTATTCTATaccgtctagcatccagtTGTGTTGCTGCAGT TAAAAAGTTCGTAGTTTATTTTCTTTTTCACTATAAGTAAATC TAAGTGTTTATAACTACTTAACTGTTTTTATAGTATGAAATTA TAATGCGGTATTTGGCGTAGTTTGGGGTACTCGATAGGGAC AGGTGAAATAGGATGATCTATCGAAGACCAACGGTAGCGGA GGCTCCCAACGCAGTCTAAGTATCACGATCAAGAACTAAAG TCAGGGGATAGACGACGATTAGAAACCGTTTTATTCCTGAC CCTAAACGATGTCGCCTAGCTGATGGGGTTTTTACTTCATTT GCCAAGAGAAATCGTAAGGTTTCAGACTCTGGGGGAAGTAT GATCGCAAGTTGAAACTTGAAGGGATTGACGGAGAGGTACC ACCAGACGTGGAGTCTGCGGCTCAATTTGACTCAACACGCA AATCTTACTAGGCCCAGAAGCTTTGAGGATTGACAGATGAG TGATCTTTCATGATTAAGTTGTTGGTGGTGCATGGCCGTTC CCATGCATGCCTATGTGTAGACAGTTATATTCATTATTGTGG AGCAAAAACGGCGAACAGCTCATTTATCAGTGGTAAGTGCA TACAATGTATTTCGTTGGATAGTAACGGAAAATCTGTTAGTA ATACATGAACTGTTTTTAGCATTATGTTAAAAATAATAGTAAG TGCGATTGTATATCTGCCACTGCAGCATCATCTTACGTTGGT GGGATATTTGCCTACCAAGGATTCGACGCTTACGGGGAATT AGGGTTTGACTCCGGAGAATGAGCATGAGAAACAGCTCATA CATCTAAGGAAGGCAGCAGGCGCGGAAATTGCCCAATGTAT CTTTTATACGAGGCAGTGACAAGAAATGGTAGGCACTTTTG TGCACTATCGAGGGTTAGTGGTATCTTTGCTAACCGTGACT CGTGGGCAAGCTCGGTGCCAGCAGCCGCGGTAATTCCGAC ACAGGGAGTTTTCCATACCGTCTAGCATCCAGTTTGGTTGC TGCAGTTAAAAAGTTCGTAGTTCACTGACTCTTTCACTATAA GCAAAGCCGAATGCTCCAAGTTTTTTAGCAGTATTTATAGTA TGAAATTATAGCGCGGCATTGAACGTAGTTTGGGGTACTCG ATAGGGACAGGTGAAATAGGATGATCTATCGAAGACCCACG GTAGCGGAGGCTCCCAACGAAGTCCAAGTGTCACGATCAA GAACTAAAGTCAGGGGATAGACGACGATTAGACACCGTTTT ATTCCTGACCCTAAACGATGTCGCCTAGCTGATGGGATTTTT TTCATTTGCCAAGAGAAATCGTAAGGTTTCAGACTCTGGGG GAAGTATGATCGCAAGGTTGAAACTTGAAGGGATTGACGGA GAGGTACCACCAGACGTGGAGTCTGCGGCTCAATTTGACTC AACACGCAAACATTACTAGGCCCAGAAGCTTTGAGGATTGA	1000
Parasite	Flagellate	04SpiroSal_Spirobark_CHSE	Spironucleus barkhanaus DQ186583	sp_sal	CRS	CAGATGAGTGATCTTTCATGATTAAGTTGTTGGTGGTGCATG GCCGTTCTT	1000

Type of Microbe	Classification of Microbe	Specificity Sample Run Name	Species	PEI Assay Name	Asp_Test or CRS	Sequence (5'-3')	Length (bp)
Parasite	Flagellate	04SpiroSal_Storosa_CHSE	Spironucleus torosa EF050055	sp_sal	CRS	TTAGCGCACGCGATGTATTTCGTCGGATAGCGGCGGAAAAT CTGCCGGTAATACGTGAACCGCCTTTGGGGTCTCTCCAAAA GCAACAGTAAGTACACTCGCGTACCTGCCATTGCGGCATCA TCTAAGGTTGGTAGGGTATTGGCCTACCAAGGACTCAACGC CTACGGGGAATTAGGGTTTGACTCCGGAGACTGGGCATGA GAAACAGCCCAGACTTTTAAGAAAGGCAGCAGGCGCGGAA ATTACCCAATGCTCTTGAGTGAGGTAGTGACCAGCAATGAG ATGCCCTTCGGGGCTATCTCTAAGGGATCTTGTATCTACAG GTCCTTGTTATTCGTGGGAAAGCACGGTGCCAGCAGCCGC GGTAATTCCGTCACGGAGAGTTTTCTATACCGTCTAGCATC CAGTTGTGTTGCTGTGGTTAAAAAGTTCGTAGTTTGTTTTGC GCCACTGTGAGAAAATCAAAGCGTTCATCGCTTGTTACCAG ATCATCACAGAATGGCCGTAAGCGGTATTGGACGTAGTTAA AGGTACTTGCTGGGGACAGGTGAAATAGGATGATCCAGCAA AGACCAACGGTAGCGGAGGCGTTTAACGCAGTCCACGTAT CACGATCAAGAACTAAAGTCAGGGGATAGACGACGATTAGA AACCGTTTTATTCCTGACCCTAAACGATGACGCCTAGCTGAT GGGTTCGCACTCATCTGCTAAGAGAAATCGGAAGCGTTCGG TCTCCTGGGGGATTACGCTTGCAAGACTGAAACTTTAAAGT ATTGACGGAGACTTACTACCAGAAATGGAACTTGCGGCTTA ATTTGACTCAACACGCAAATCTTACCAGGCCCAGATGCTTTA AGGATTGACCGACGAGTGATCGTTCATGATTAGGTAATTGG TGGTGCATGGCCGTTTTCAGTCCGTGTCTTAAAATGTCTGC TTTATTGCGATAACGAGCGAGACCTCCGCTATGACTACGTC ATAGGACAGCTAGCGAT	1000
Parasite	Microsporidian	07Loma_Lembiotocia_CHSE	Loma embiotocia AF3203210	lo_sal	CRS*	CTCAACGCGGGACAACTTACCGGGCCCGACGACCGTAAGA GTGTAACACACGATAGGTCGAAGAGTGGTGCATGGCCGTTA ACGACGAGTGGAGTGATCTTTGGGTTAAGTCCGTAAATTAG TGAGACCTCAGCCGTAAGGGACAGGTGCGCAAAGCACAGG AAGGATGGGTCAAGGACAGGTCAGTGATGCCCTTAGATGG TCCGGGCTGCACGCGCAATACAGTGGTCGCCGGAATTACC TGATAATTATAAAGGCGATCGAGAGGGAATGAGCTTTGTAA GAGGCTCAGGAACGAGGAATTGCTAGTAATCGCGGACTCAT TAAGACGCGATGAATACGTCCCTGTTCTTTGTACACACCGC CCGTCGTTATCGAAGATGAAGACAGGCGCGAACGATCTACC AGAAAGTGAGCGCAGGTCTTTAGATCTGATACAAGTCGTAA CAAGGTAGCTGTAGGAGAACCTGTAGCTGGATCAGACCGA CAAAAAGAAACTCATGTATATTATATGAATGTAATCTCTGCG CAAGGGATCTTTTGGTTCGCTAGACGAAGAAGGGCGCAGC GGAATGCGAAATGTGCAGGAGTCGCAGCGAAGACAGCACA TGCCTGAAATCACGAGAGTGAGACTACCCCTTACCGTCTAG CATCCAGTTGAATTAAGCATATGAGTAAAGGGAGGAAAAAA AACTAACAAGGATTCCTGTAGTAGCGGCGAGCGAAGAAGG AACAGCCCCGAGTGTAATCACAAATATGTGAGATGTCATTAA TCTATAGAACGATGTTGAAACTTCGGCCTGAGAGGGTGATA GCCCCGTAAAATAGATGTAACAAGGGAGTAGCAGTGTTTGG TATTGCACTGTGAACGGGTGGCAGCGCCCATCCAAGGCTA AATACAAAGCGAGACCGATAGTGAATAAGTAGAGCGATCGA AAAACGAATAGACTGAAAAAGTGAAATTGCGTAGGGAGCCT AGAGGCTA	985
Parasite	Microsporidian	07Loma_Llingcod_CHSE	Loma lingcodae HQ157503	lo_sal	CRS*	CGACCGTATGAGTGTAACACACGATAGGTCGAAGAGTGGT GCATGGCCGTTAACGACGAGTGGAGTGATCTTTGGGTTAAG TCCGTAAATTAGTGAGACCTCAGCCGTAAGGGACAGGTGC GCAAAGCACAGGAAGGATGGGTCAAGGACAGGTCAGTGAT GCCCTTAGATGGTCCGGGCTGCACGCGCACTATAGTGGTC GCCGGAATTACCTGATAATTTTAAAGGCGATCGAGAGGAAA TGAGCTTTGTAAGAGGCTCAGGAACGAGGAATTGCTAGTAA TCGCGGACTCATTAAGACGCGATGAATACGTCCCTGTTCTT TGTACACACCGCCCGTCGTTATCGAAGATGAAGATAGGCGC GAACGATCTACCAGAAAGTGAGCGCAGGTCTTTAGATCTGA TACAAGTCGTAACAAGGTAGCTGTAGGAGAACCTGTAGCTG GATCAGACCGATTTATATAATCTTTGTATGAATGTAATCTCT GCGCAAGGGATCTTTTGGTTCGCTAGACGAAGAAGGGCGC AGCGGAATGCGAAATGTGCAGGAGTCGCAGCGAAGATAGC ACATGCCTGAAATCACGAGAGTGTGAGACTACCCCTTACCG TCTAGCATCCAGTTGAATTAAGCATATGAGTAAAGGGAGGA AAAGAAACTAACAAGGATTCCTTAGTAGCGGCGAGCGAAGA AGGAACAGCCCCGAGTGTAATCACAAATATGTGAGATGTCA TTAATCTATAGAACGATGTTGAAACTTCGGCCTGAGAGGGT GATAGCCCCGTAAAATAGATGTAACAAGGGAGTAGCAGTGT TTGGTATTGCACTGTGAACGGGTGGCAGCGCCCATCCAAG GCTAATACAAAGCGAGACCGATAGTGAATAAGTAGAGCGAT CGAAAAACGAATAGACAGAAAAAGTGAAATTGCGTAGGGAG CCTAGAGGCTAGGCC	952
Parasite	Microsporidian	07Loma_Lrichardi_CHSE	Loma richardi HQ157488	lo_sal	CRS*	CGGCTTAATTTGACTCAACGCGGGACAACTTACCAGGCCCG ACGACCGTATGAGTGTAACACACGATAGGTCGAAGAGTGGT GCATGGCCGTTAACGACGAGTGGAGTGATCTTTGGGTTAAG TCCGTAAATTAGTGAGACCTCAGCCGTAAGGGACAGGTGC GCAAAGCACAGGAAGGATGGGTCAAGGACAGGTCAGTGAT GCCCTTAGATGGTCCGGGCTGCACGCGCACTACAGTGGTC GCCGAAATTACCTGATAATTTTAAAGGCGATCGAGAGGAAA TGAGCTTTGTAAGAGGCTCAGGAACGAGGAATTGCTAGTAA TCGCGGACTCATTAAGACGCGATGAATACGTCCCTGTTCTT TGTACACACCGCCCGTCGTTATCGAAGATGAAGATAGGCGC GAACGATCTACCAGAAAGTGAGCGCAGGTCTTTAGATCTGA TACAAGTCGTAACAAGGTAGCTGTAGGAGAACCTGTAGCTG GATCAGACCGATTTATATAATCTTTGTATGAATGTAATCTCT GCGCAAGGGATCTTTTGGTTCGCTAGACGAAGAAGGGCGC AGCGGAATGCGAAATGTGCAGGAGTCGCAGCGAAGATAGC ACATGCCTGAAATCACGAGAGTGTGAGACTACCCCTTACCG TCTAGCATCCAGTTGAATTAAGCATATGAGTAAAGGGAGGA AAAGAAACTAACAAGGATTCCTTAGTAGCGGCGAGCGAAGA AGGAACAGCCCCGAGTGTAATCACAAATATGTGAGATGTCA TTAATCTATAGAACGATGTTGAAACTTCGGCCTGAGAGGGT GATAGCCCCGTAAAATAGATGTAACAAGGGAGTAGCAGTGT TTGGTATTGCACTGTGAACGGGTGGCAGCGCCCATCCAAG ACTAAATACAAAGCGAGACCGATAGTGAATAAGTAGAGCGA TCGAAAAACGAATAGACTGAAAAAGTGAAATTGCGTAGGGA GCCTAGAGGCTAGGCC	995

Type of Microbe	Classification of Microbe	Specificity Sample Run Name	Species	PEI Assay Name	Asp_Test or CRS	Sequence (5'-3')	Length (bp)
Parasite	Microsporidian	08Nsp_gblock_CHSE	Nucleospora salmonis	nu_sal	T	TGACTCAACGCGGGAAAACTTACCAGGGTCAAGTTCTCCGTAGATCGAGTACGTGAGGGGAACGAGAGTGGTGCATGGCCGTTGGAAATTGATGGGATGACTTTTAGCTTAAATGCTGAAACCAGTGAGATCTTCTAGACAGGTGCCATCCGGCACAGGAGGGCGAAGGCGATAACAGGTCCGTGATGCCCTTAGATATCCTGGGCTGCAAGTGCAATACAATATTCATGTCGATCAAGAAAGGTGATTATGGATGAGCAGGATTAGCTCTTGTAAATGAGCTATGAATGAGGAATTCCTAGTAACAGTGTCTCACTAAGGCATTGTGAATGCGTCCCTGTTCTTTGTACACACCGCCCGTCACTATTTCAGATGGCCATGAAGATGAAGAGCTTCGGTTCTGAATATTCATGTCTAGATAAAGTACAAGTCGTAACAAGGTTGCGGTCGGTGAACCAGCCGCAGATCATTACTAAAAACCTTTTTTCCCCCCGCGCATCCAGAAATACGCAGACCGTCTAGCATCCAGTGCTGTTTAGATGCGGCGATCGACAGAGAAGCCCGTCAAAATCATAATTACTGAGGAGCAGGGTACTGCTGCTTAGATACAAATTATAAGAACCCCTGGTACGGGATCAATTGACTCTGGCAGCGATGACGGACGCATAAGAGTGCGAAAAGAGAAATTCTCTGTCCGAATTTACTATCGACGATAGTAGACTACGCCCCGAACTTAAGCATATCATTAAGGGCAGGAAAAGAAACCAACTGGGATTCCACTAGTAGCGGCGAGTGAACATGGATGAGCTCCGATGGTAAAGTGTGCGCACTGTTGTAAAGAGCGTCGAGACAGAGACCCGATGGAAAGCGGGGCTTCCACGAGTGAGAGCCTCGGAACTGTCTGCAGCAAATAGGTAGAGTAGCGTTGCTTGGTAGTGCAATGTGGAACGGGAGGTAGTAACTTCCAAAGCTAAATATACCCAGAGATCGATAGCGAATA	1000
Parasite	Microsporidian	08NspAS_gblock_CHSE	Nucleospora salmonis	nu_sal	T	TGACTCAACGCGGGAAAACTTACCAGGGTCAAGTTCTCCGTAGATCGAGTACGTGAGGGGAACGAGAGTGGTGCATGGCCGTTGGAAATTGATGGGATGACTTTTAGCTTAAATGCTGAAACCAGTGAGATCTTCTAGACAGGTGCCATCCGGCACAGGAGGGCGAAGGCGATAACAGGTCCGTGATGCCCTTAGATATCCTGGGCTGCAAGTGCAATACAATATTCATGTCGATCAAGAAAGGTGATTATGGATGAGCAGGATTAGCTCTTGTAAATGAGCTATGAATGAGGAATTCCTAGTAACAGTGTCTCACTAAGGCATTGTGAATGCGTCCCTGTTCTTTGTACACACCGCCCGTCACTATTTCAGATGGCCATGAAGATGAAGAGCTTCGGTTCTGAATATTCATGTCTAGATAAAGTACAAGTCGTAACAAGGTTGCGGTCGGTGAACCAGCCGCAGATCATTACTAAAAACCTTTTTTCCCCCCGCGCATCCAGAAATACGCAGACCGTCTAGCATCCAGTGCTGTTTAGATGCGGCGATCGACAGAGAAGCCCGTCAAAATCATAATTACTGAGGAGCAGGGTACTGCTGCTTAGATACAAATTATAAGAACCCCTGGTACGGGATCAATTGACTCTGGCAGCGATGACGGACGCATAAGAGTGCGAAAAGAGAAATTCTCTGTCCGAATTTACTATCGACGATAGTAGACTACGCCCCGAACTTAAGCATATCATTAAGGGCAGGAAAAGAAACCAACTGGGATTCCACTAGTAGCGGCGAGTGAACATGGATGAGCTCCGATGGTAAAGTGTGCGCACTGTTGTAAAGAGCGTCGAGACAGAGACCCGATGGAAAGCGGGGCTTCCACGAGTGAGAGCCTCGGAACTGTCTGCAGCAAATAGGTAGAGTAGCGTTGCTTGGTAGTGCAATGTGGAACGGGAGGTAGTAACTTCCAAAGCTAAATATACCCAGAGATCGATAGCGAATA	1000
Parasite	Myxozoan	12MA_Mfry_CHSE	Myxobolus fryeri EU346370	my_arc	CRS	GCGAGAGGTGAAATTCTTGGACCGGCCAAGGACTAACAAATGCGAAGGCAATTGCCAAGATCGTTTCCATTAATCAAGAACGACAGTCCGAGGTTCGAAGTCGATCAGATACCGACTTAGTTCGGACCGTAAACTATGCCGACCCGAGATCAGTTTTGAGCTAATTAAACGCTCGAGGTTGCTCTCCCTGGGAAACCTTAAGTTTTTAGGTTACGGGGGGAGTATGGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGGGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGAAAACTCACCTGGTCCGGACATCGAAAGGATAGACAGACTGATAGATCTTTCTTGATACGTTGGTTGGTGGTGCATGGCCGTTCTTAGTTCGTGGAGTGATCTGTCAGGTTGATTCCGGTAACGGACGAGACCATATTCTCCATTTAAGGAATGCAAAAACTCAGTCAATGTGTGTTGGTTCGCTAACATGTGTTGACTGTAGCTGGTTGTCACTGAATATCCGGATAGAATTTGACAGTATGAGTGAGAGTGTCAACCGTCTAGCATCCAGTAAACTTGAGCAAGTATTGTTGGAAACCATTCGGGGATGCTGTACGCCTTTGCAATAAACGGTGGCAGGCAACTGTTCACTGTCTTATGGAGAGACAAAGCAGAATACGTCAAAGTGCTAGGAGGTGTGGCGATAACAGGTCTGTGATGCCCTAAGATGTTCAGGGCCGCACGCGCGCTACAATGATAACGTCAGCGAGTATCTGGGTTGTCAAACTTGGGTAATCTTGTCAATCGTTATCGTGATGGGGATTGATCGTTGTAATTGTCGGTCATGAAAGAGGAATCCCTAGTATGTGCACTTCATTAGAGTGTACAGATTTAGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGAGTGAATGGTGTCATGATGCCTTGGGACTGGACGTCGTTTGTACTTATGGTG	994
Parasite	Myxozoan	12MA_Minsid_CHSE	Myxobolus insidiosus EU346373	my_arc	CRS	TATTTGGCCGCGAGAGGTGAAATTCTTGGACCGGCCAAGGACTAACAAATGCGAAGGCAATTGCCAAGATCGTTTCCATTAATCAAGAACGACAGTCCGAGGTTCGAAGTCGATCAGATACCGACTTAGTTCGGACCGTAAACTATGCCGACCCGAGATCAGTTTTGAGCTAATTAAACGCTCGAGGTTGCTCTCCCTGGGAAACCTTAAGTTTTTAGGTTACGGGGGGAGTATGGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGGGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGAAAACTCACCTGGTCCGGACATCGAAAGGATAGACAGACTGATAGATCTTTCTTGATACGTTGGTTGGTGGTGCATGGCCGTTCTTAGTTCGTGGAGTGATCTGTCAGGTTGATTCCGGTAACGGACGAGACCATATTCTCCATTTAAGGAATGCAAAAACTCAGTCAATGTGTGTTGGTTCGCTAACATGTGTTGACTGTAGCTGGTTGTCACTGAATATCCGGGTTGAATTTGACAGTATGAGTGAGAGTGTCAACCGTCTAGCATCCAGTAAACTTGATCAAATATTGTTGGAAACAATTCGGGGATGCTGTACGCCTTTGCAATAAACGGTGGCAGGCAACTGTTCACTGTCTTATGGAGAGACAAAGCAGAATACGTCAAAGTGCTAGGAGGTGTGGCGATAACAGGTCTGTGATGCCCTAAGATGTTCAGGGCCGCACGCGCGCTACAATGATAACGTCAGCGAGTATCTGGGTTGTCAAACTTGGGTAATCTTGTCAATCGTTATCGTGATGGGGATTGATCGTTGTAATTGTCGGTCATGAAAGAGGAATCCCTAGTATGTGCACTTCATTAGAGTGTACAGATTTAGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGAGTGAATGGTGTCATGATGCCTTGGGACTGGACGTCGTTTGTACT	996

Type of Microbe	Classification of Microbe	Specificity Sample Run Name	Species	PEI Assay Name	Asp_Test or CRS	Sequence (5'-3')	Length (bp)
Parasite	Myxozoan	12MA_Mkis_CHSE	Myxobolus kisutchi AB469988	my_arc	CRS	GCGATTGGGGGCATTGGTATTTGGCCGCGAGAGGTGAAAT TCTTGGACCGGCCAAGGACTAACAAATGCGAAGGCAATTGC CAAGATCGTTTCCATTAATCAAGAACGACAGTCCGAGGTTC GAAGTCGATCAGATACCGACTTAGTTCGGACCGTAAACTAT GCCGACCCGAGATCAGTTTTGAGCTAATTAAACGCTCGAGG TTGCTCTCCCTGGGAAACCTCAAGTTTTTAGGTTACGGGGG GAGTATGGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAA GGGCACCACCAGGGGTGGAGCCTGCGGCTTAATTTGACTC AACACGGGAAAACTCACCTGGTCCGGACATCGAAAGGATA GACAGACTGATAGATCTTTCTTGATACGTTGGTTGGTGGTG CATGGCCGTTCTTAGTTCGTGGAGTGATCTGTCAGGTTGAT TCCGGTAACGGACGAGACCATATTCTCCATTTGAGGAATGC AAAAACTCAGTCAGGGTGTGTTGGTTCGCCAATACATTTTGT CTGTAGCTGGTAGTTACTGAATAACTGAATTTGGTCTTTTAT TGTAAAGTTAAAGTACCGTCTAGCATCCAGTCCAACTTTGGC CGAGCAGTTTGGGATTGAATTTGGGGATGCTGTACTCCTTT GCAATAAACGGTGGCAGGAAACTGTTCACTGTCTTATGGAG AGACAACGGTGAATACGTCAAAGACCGAGGACGTGTGGCG ATAACAGGTCTGTGATGCCCTAAGATGTTCAGGGCCGCACG CGCGCTACAATGATAACGTCAGCGAGTATCTGGGTTGTCAA ACCTGGGTAATCTTGTCAATCGTTATCGTGATGGGGATTGA TCGTTGTAATTGTCGATCATGAAAGAGGAATCCCTAGTATGT GCACTTCATTAGAGTGTACAGATTAAGTCCCTGCCCTTTGTA CACACCGCCCGTCGCTACTACCGAGTGAATGGTGTCATGAT GCTTTGGGACT	996
Parasite	Myxozoan	12MA_Mneurobius_CHSE	Myxobolus neurobius AB469987	my_arc	CRS	TGAAATTCTTGGACCGGCCAAGGACTAACAAATGCGAAGGC AATTGCCAAGATCGTTTCCATTAATCAAGAACGACAGTCCGA GGTTCGAAGTCGATCAGATACCGACTTAGTTCGGACCGTAA ACTATGCCGACCCGAGATCAGTTTTGAGCTAATTAAACGCT CGAGGTTGCTCTCCCTGGGAAACCTTAAGTTTTTAGGTTAC GGGGGGAGTATGGTTGCAAAGCTGAAACTTAAAGGAATTGA CGGAAGGGCACCACCAGGGGTGGAGCCTGCGGCTTAATTT GACTCAACACGGGAAAACTCACCTGGTCCGGACATCGAAA GGATAGACAGACTGATAGATCTTTCTTGATACGTTGGTTGGT GGTGCATGGTCGTTCTTAGTTCGTGGAGTGATCTGTCAGGT TGATTCCGGTAACGGACGAGACCATATTCTCCATTTGAGGA ATGCAAAAACTCAGTCAGGGTGTGCTGGTTCGCCAGTACGT TCTTTCTGTAGCTGGTAGTTGCTGAATATCCGAGTTTGAGCA TGAATATGAGGAAGAGGACCGTCTAGCATCCAGTGTAACTT TGACCAAGTATTTGTGACCGGATTTCGGGGATGCTGTACTC CTTTGCAATAAACGGTGGCAGGAAACTGTTCACTGTCTTAT GGAGAGACAACGGTGTATACGTCAAAGACCGAGGACGTGT GGCGATAACAGGTCTGTGATGCCCTAAGATGTTCAGGGCC GCACGCGCGCTACAATGATAACGTACAGCGAGTATCTGGGT TGTCAAACTTGGGTAATCTTGTCAATCGTTATCGTGATGGG GATTGATCGTTGTAATTGTCGGTCATGAAAGAGGAATCCCT AGTATGTGCACTTCATTAGAGTGTACAGATTAAGTCCCTGCC CTTTGTACACACCGCCCGTCGCTACTACCGAGTGAATGGTG TCATGATGCT	953
Parasite	Myxozoan	12MA_Mneurotropus_CHSE	Myxobolus neurotropus DQ84666	my_arc	CRS	GCGATTGGGGGCATTGGTATTTGGCCGCGAGAGGTGAAAT TCTTGGACCGGCCAAGGACTAACAAATGCGAAGGCAATTGC CAAGATCGTTTCCATTAATCAAGAACGACAGTCCGAGGTTC GAAGTCGATCAGATACCGACTTAGTTCGGACCGTAAACTAT GCCGACCCGAGATCAGTTTTGAGCTAATTAAACGCTCGAGG TTGCTCTCCCTGGGAAACCTCAAGTTTTTAGGTTACGGGGG GAGTATGGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAA GGGCACCACCAGGGGTGGAGCCTGCGGCTTAATTTGACTC AACACGGGAAAACTCACCTGGTCCGGACATCGAAAGGATA GACAGACTGATAGATCTTTCTTGATACGTTGGTTGGTGGTG CATGGCCGTTCTTAGTTCGTGGAGTGATCTGTCAGGTTGAT TCCGGTAACGGACGAGACCATATTCTCCATTTGAGGAATGC AAAAACTCAGTCAGGGTGTGTTAGTTCGCTAATATGTTCTGT CTGTAGCTGGTAGTTACTGAATAACTGGATTTTTTCTTTTATT GTGACGTTGGAGACCGTCTAGCATCCAGTGCAACTTTGACC GATCAGTAGGAGATTAAATTTGGAGATGCTGTACTCCTTTGC AATAAACGGTGGCGGGAAACTGTCCACTGTCTTATGGAGAG ACAACGGTGAATACGTCAAAGACCGAGGACGTGTGGCGAT AACAGGTCTGTGATGCCCTAAGATGTTCAGGGCCGCACGC GCGCTACAATGATAACGTCAGCGAGTATCTGGGTTGTCAAA CTTGGGTAATCTTGTCAATCGTTATCGTGATGGGGATTGATC GTTGTAATTGTCGATCATGAAAGAGGAATCCCTAGTATGTG CACTTCATTAGAGTGTACAGATTAAGTCCCTGCCCTTTGTAC ACACCGCCCGTCGCTACTACCGAGTGAATGGTGTCATGATG CTTTGGGACTGGACGTT	1002
Parasite	Myxozoan	12MA_Msquam_CHSE	Myxobolus squamalis MSU96495	my_arc	CRS	ACATCACCAACCGCCAATAACGGATGTTGGTTTCCGTATTG GGGTGATGATTAAAAGGAGCGATTGGGGGCATTGGTATTTG NCCGCGAGAGGTGAAATTTTTGGACCGGCCAAGGACTAAC AAATGCGAAGGCAATTGCCAAGATCGTTTCCATTAATCAAGA ACGACAGTCCGAGGTTCGAAGTCGATCAGATACCGACTTAG TTCGGACCGTAAACTATGCCGACCCGAGATCAGTTTTGAGC TAATTAAACGCTCGAGGTTGCTCTCCCCTGGGAAACCTTAA GTTTTTAGGTTACGGGGGGAGTATGGTTGCAAAGCTGAAAC TTAAAGGAATTGACGGAAGGGCACCACCAGGGGTGGAGCC TGCGGCTTAATTTGACTCAACACGGGAAAACTCACCTGGTC CGGACATCGAAAGGATAGACAGACTGATAGTCTTTCTTGAT ACGTTGGTTGGTGGTGCATGGCCGTTCTTAGTTCGTGGAGT GATCTGTCAGGTTGATTCCGGTAACGGACGAAACCATATTC TCCATTTAAGGAATGCAAAAACTCAGTCAATGTGTGTTGGC GCTAACATGTGTTGACTGTAGCTGGTTGTCACTGAATATCC GGGTGAATTTGACAGTATGAGTGAGAGTGTCaccgtctagcatcca gtAAAACTTGATCAAATATTGTTGGAAACAATTCGGGGATGCT GTTCGCCTTTGCAATAAACGGTGG	727

Type of Microbe	Classification of Microbe	Specificity Sample Run Name	Species	PEI Assay Name	Asp_Test or CRS	Sequence (5'-3')	Length (bp)
Parasite	Myxozoan	13Myx-SP_gblock_CHSE	Myxobolus cerebralis AY275708	my_cer	T	CAATGCCAGGTGTGTCTGGAGACGAGCCAAAGAAACGAGTTGCACCAGATATACCATCAGGGCCAGTCGAAGTTGTTGGGCTCATTGCATTGACTTTATTAAATACATCCGTACTAGAAAAGGGAGCCATAAGATCAGGAAATTTGTCAGAGAAACAGATTCAAGAAGACTGAAGAGACATAAGTTGAATCTCAGAGGGCACATTCCAGAGAGAATTATAGTCGACGAGCAATCTCCTCGGACGAGCATGTACACTTTGTATTGTTCCGATTTTTGGGTTATGAGTTCAAAAGCCCGTGCAGGACTTGATTTAGCCAAGGAAATTGAGTATGATTGGGCCATTGAATTTGACTTTGGATTATTCGAAGCCTTGACCATCTTTTGGCCAACCGTCTAGCATCCAGTAGTTCGGGCTTACATGAATGGTCGTACGACAGGAAAGGCCGAGTCTTTGAGAGCTCTTTTTTCGCTGAACGCTTCCTAGCCTTACGTTTGTGGAATGAGAGGACTGGATTGGACCTTGAGACTGACAGGAGCGACTCTACTGCCAAAACCGACGTTTCCCAGCTACCAAAAGTTCTTTCGAGAAACCTCGTAAGAGCAGAGTCAAGATCCTTTGAGTCGATGGTTGATAGAGAAGCCAGAGAGAAGATGAGAGCGGACCTTGAGGCTGACGAACTTGACACTGCGCTTTTCTGGCATTTAAGTGGAATATTAGAAGGGAAAAATGGATCGGAGACTGTGATCAACGGGCCCCTGAGAGGGGACCTGAGAATTCCATATGTGTTTTGGAGTTTGATAGATTCGCAGCTATTAGACCGACGATTAGAAGATGAAACTTCTTTTACTCTGGGTGCGATAATCTCAAGTGGCAGTGACGAGCGTGAAAGGGCTCCAGAACTTGAAACCGACTCGTCCGATGATTCATTATCCAGAGGAAGTGGAACGACACGCGACAGCGCTTGGATTTTTTCTGGAGTAGAGACTATAATTGGACGTG	1000
Parasite	Myxozoan	14Mi2_Minisid_CHSE	Myxobolus insidiosus EU346373	my_ins	T	ATATATGCTCTTTTCTAAGATTAAGCCATGCACGTTTCAGTTCATACATTTTAACTGTGAGACTGCGGAAGGCTCAGTAAATCAGTTATAATCTATTTGATTGTACAGCCCAATGGATAACCGTGGGAAATCTAGAGCTAATACATGCACACCAATTTGGGAGCGTCAAAACTCTCAAGGCATTTATTAGACTAATACCAACTGTATTTTCAAGGGTAAAACCTTGGAGGTACAAACCGTCTAGCATCCAGTGGTGAATCTAGATAACTTTGCCGATCGTATGGCTTCGGGCCGGCGACGTTTCAATTGAATTTCTGCCCTATCAACTTGTTGGTAAGGTAGTTGCTTACCAAGGTTGCGACGGGTAACGGGGGATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAATGGCTACCACATCCAAGGACGGCAGCAGGCGCGCAAATTACCCAATCCAGACAATGGGAGGTGGTGACGAGAAATACTAGTCAGTGTCTCAAAAGGACGCTGATCTGGAATGGACGCAAATTTAAGCAATTCGATGAGTAACAACTGGAGGGCAAGTCTAGTGCCAGCAGCCGCGGTAATTCTAGCTCCAGTGGTGTATTTTAATGTTGCTGCGTTTAAAACGCTCGTAGTTGGATTACGCAGTGCGAGTCGGTCAATTAGCAGAGCTTCACATAAAAACTAAGGCGTTTGTTTTGTGAACATGGATTCCTTGAACGCTAAACAACGTCTTGGTGTGTTGCATGTTGATTATTCGCCGGCCCGCACTACTTGTACGTAAGGATGGCGGTTGACCTTTAGTGCGTTGACTGATCGTGTCTTACGGAGTGTGCCTTGAATAAATCAGAGTGCTCAAGCAGGCTTACGCTTGAATGTTAATAGCATGGAACGAACAATTGTGTAAAAGTGACTGTACTTACAATTTGGTGGATTTATTTGTCAGGTTGTGGGTGCAGTTCACATCACCAACCGCCAATAACGGATGTTGGT	997
Parasite	Myxozoan	14Mi2_Mfry_CHSE	Myxobolus fryeri EU346370	my_ins	CRS	AAATATATGCTCTTTTCTAAGATTAAGCCATGCACGTTTCAGTTCATACATTTTAACTGTGAGACTGCGGAAGGCTCAGTAAATCAGTTATAATCTATTTGATTGTACAGCCCAATGGATAACCGTGGGAAATCTAGAGCTAATACATGCACACCAATTTGGGAGCGTCAAAACTCTCAAGGCATTTATTAGACTAATACCAACTGTATTTTCAAGGGTAAAACTTTGGAGGTACAAGACCGTCTAGCATCCAGTGTGAATCTAGATAACTTTGCCGATCGTATGGCTTCGGGCCGGCGACGTTTCAATTGAATTTCTGCCCTATCAACTTGTTGGTAAGGTAGTTGCTTACCAAGGTTGCGACGGGTAACGGGGGATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAATGGCTACCACATCCAAGGACGGCAGCAGGCGCGCAAATTACCCAATCCAGACAATGGGAGGTGGTGACGAGAAATACTAGTCAGTGTCTCAAAAGGACGCTGATCTGGAATGGACGCAATTTAAGCAATTCGATGAGTAACAACTGGAGGGCAAGTCTAGTGCCAGCAGCCGCGGTAATTCTAGCTCCAGTGGTGTATTTTAATGTTGCTGCGTTTAAAACGCTCGTAGTTGGATTACGCAGTGCGAGTCGGTCAATTAGCAGAGCTTCACATAAAAACTAAGGCGTTTGTTTTGTGAACATGGATTCCTTGAACGCAAAACAACGTCTTGGTGTGTTGCATGTTGATTATTCGCCGGCCCGCACTACTTGTACGTAAGGATGGCGGTTGACCTTTAGTGCGTTGACTGATCGTGTCTTACGGAGTGTGCCTTGAATAAATCAGAGTGCTCAAAGCAGGCTTACGCTTGAATGTTAATAGCATGGAACGAACAATTTGTGTAAAAGTGTCTGTACTTACAATTTGGTGGATTTATCTGTCAAGTTGTGGGTGCAGTTCACATCACCAACCGCCAATAACGGA	992
Parasite	Myxozoan	14Mi2_Mkis_CHSE	Myxobolus kisutchi AB469988	my_ins	CRS	GCCAGTAAATATATGCTCTTTTCTAAGATTAAGCCATGCACGTTTCAGTTCATACATTTCAACTGTGAGACTGCGGAAGGCTCAGTATATCAGTTATAATCTATTTGATTGTACAGCCCAATGGATAACCGTGGGAAATCTAGAGCTAATACATGCACACCTATTTGATAGGCCTTAAACCTATCAAGGCATTTATTAGACTAATACCAACTGTATGTTAGTGGTAAACCCATCAACGTACAAACCGTCTAGCATCCAGTGGTGAATCTAGATAACTTTGCCGATCGCATGGCTTCGGGCCGGCGACGTTTCAATTGAATTTCTGCCCTATCAACTGGTTGGTAAGGTTGAGGCTTACCAAGGTGGCGACGGGTAACGGGGGATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAATGGCTACCACATCCAAGGACGGCAGCAGGCGCGCAAATTACCCAATCCAGACAATGGGAGGTGGTGACGAGAAATACTAGTCAGTGTCTCAAAAGGACGCTGATCTGGAATGGACGCAATTTAAGCAATTCGATGAGTAACAACTGGAGGGCAAGTCTAGTGCCAGCAGCCGCGGTAATTCTAGCTCCAGTGGTGTATTTTAATGTTGCTGCGTTTAAAACGCTCGTAGTTGGATTACGCAGTGTGAGTCGGTCAATTAGCAGAGCTTCACATCAAAACCGAGGCGTTGCTATTACGAACATGGTTTCCTTGAACGTGATACAGCGTCTCGGTGTGTTGCATGTTGATTATCAGCCGGCCCACACTACTTGTACGTAAGGATGGCGGTTGACCTTTGGTGCGTTGACTGTTCGTGTCTTACGGAGTGTGCCTTGAATAAATCAGAGTGCTCAAAGCAGGCTTACGCTTGAATGTTAATAGCATGGAACGAACAATTGTGTTATACGTGTCTATGCTTACTGTTGGATGGATTTATTTTGTTTAATAGTAAGTGTAGTTCACATCACCAACCGCCAATA	995

Type of Microbe	Classification of Microbe	Specificity Sample Run Name	Species	PEI Assay Name	Asp_Test or CRS	Sequence (5'-3')	Length (bp)
Parasite	Myxozoan	14Mi2_Mneurobius_CHSE	Myxobolus neurobius AB469987	my_ins	CRS	CTAAGATTAAGCCATGCACGTTTCAGTTCATACATTTCAACTGTGAGACTGCGGAAGGCTCAGTATATCAGTTATAATCTATTTGATTGTACAGCCCAATGGATAACCGTGGGAAATCTAGAGCTAATACATGCACACTTATTTGCAAGGCGTCAAAACCTTGCAAGGCATTTATTGGACCAATACCAACTGTCGATCTGGGGTTAACGCCTCGGACGTACAAGACCGTCTAGCATCCAGTGTGAATCTAGATAACTTTGCCGATCGCATGGCTTCGGGCCGGCGACGTTTCAATTGAATTTCTGCCCTATCAACTTGTTGGTAAGATAGATGCTTACCAAGGTGGCGACGGGTAACGGGGGATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAATGGCTACCACATCCAAGGACGGCAGCAGGCGCGCAAATTACCCAATCCAGACAATGGGAGGTGGTGACGAGAAATACTAGTCAGTGTCTCAAAAGGACGCTGATCTGGAATGGACGCAATTTAAGCAATTCGATGAGTAACAACTGGAGGGCAAGTCTAGTGCCAGCAGCCGCGGTAATTCTAGCTCCAGTAGTGTATTTTAATGTTGCTGCGTTTAAAACGCTCGTAGTTGGATTACGCAGTGTGAGTTGGTCAATTAGCAGAGCTTCACATTCAAACCGAGGCGTTGTGGCTATGAACCGGGTTTCCTTGAACATGACGCAGCGTCTCGGTGTGTTGCATGTTGATTATCAGCCGGCCCACACTACTTGTACGTGAGGATGGCGGTTGACCTTTAGTGCGTTGACTGATCGTGTCTCACGGAGTGTGCCTTGAATAAATCAGAGTGCTCAAAGCAGGCTTACGCTTGAATGTTAATAGCATGGAACGAACAATTGTGTTATACGTGTCTGTGCCTGCAGTTGGATGGATTTATTTTGTCCAGTTGTAGGTGCAGTTCACATCACCAACCGCCAATAACGG	979
Parasite	Myxozoan	14Mi2_Mneurotropus_CHSE	Myxobolus neurotropus DQ84666	my_ins	CRS	CTAAGACTAAGCCATGCACGTTTCAGTTCATACATTTCGACTGTGAGACTGCGGAAGGCTCAGTATATCAGTTATAATCTATTTGATTGTACAGCCCAATGGATAACCGTGGGAAATCTAGAGCTAATACATGCACACTATTTGAATGGCGTCAAAACCATTCAAGGCATTTATTAGACCAATACCAACTGTACGTTTGTGGTTAACGCCTCTTATGTACAAGACCGTCTAGCATCCAGTGTGAATCTAGATAACTTTGCCGATCGCATGGCTTCGGGCCGGCGACGTTTCAATTGAATTTCTGCCCTATCAACTAGTTGGTAAGGTAGATGCTTACCAAGGTGGCGACGGGTAACGGGGGATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAATGGCTACCACATCCAAGGACGGCAGCAGGCGCGCAAATTACCCAATCCAGACAATGGGAGGTGGTGACGAGAAATACTAGTCAGTGTCTCAAAAGGACGCTGATCTGGAATGGACGCAATTTAAGCAATTCGATGAGTAACAACTGGAGGGCAAGTCTAGTGCCAGCAGCCGCGGTAATTCTAGCTCCAGTGGTGTATTTTAATGTTGCTGCGTTTAAAACGCTCGTAGTTGGATTACGCAGTGTGAGTCGGTCAATTAGCAGAGCTTCACATTCAAACCGAGGCGTTGTAGCCATGAACCGGGTTTCCTTGAACGTGATGCAGCGTCTCGGTGTGTTGCATGTTGATTATCAGCCGGCCCACACTACTTGTACGTGAGGATGGCGGTTGACCTTTAGTGCGTTGACTGTTCGTGTCTCACGGAGTGTGCCTTGAATAAATCAGAGTGCTCAAAGCAGGCTTACGCTTGAATGTTAATAGCATGGAACGAACAATTGTGTTATACGTGTCTGCAGTTGGATGGATTTATTTTGTCCAGTTGCAGGTGCAGTTCACATCACCAACCGCCAATAACGG	978
Parasite	Myxozoan	14Mi2_Msquam_CHSE	Myxobolus squamalis MSU96495	my_ins	CRS	ACATCACCAACCGCCAATAACGGATGTTGGTTTCCGTATTGGGGTGATGATTAAAAGGAGCGATTGGGGGCATTGGTATTTGGCCGCGAGAGGTGAAATTTTTGGACCGGCCAAGGACTAACAAATGCGAAGGCAATTGCCAAGATCGTTTCCATTAATCAAGAACGACAGTCCGAGGTTCGAAGTCGATCAGATACCGACTTAGTTCGGACCGTAAACTATGCCGACCCGAGATCAGTTTTGAGCTAATTAAACGCTCGAGGTTGCTCTCCCCTGGGAAACCTTAAGTTTTTAGGTTACGGGGGGAGTATGGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGGGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGAAAACTCACCTGGTCCGGACATCGAAAGGATAGACAGACTGATAGTCTTTCTTGATACGTTGGTTGGTGGTGCATGGCCGTTCTTAGTTCGTGGAGTGATCTGTCAGGTTGATTCCGGTAACGGACGAAACCATATTCTCCATTTAAGGAATGCAAAAACTCAGTCAATGTGTGTTGGCGCTAACATGTGTTGACTGTAGCTGGTTGTCACTGAATATCCGGGTGAATTTGACAGTATGAGTGAGAGTGTCaccgtctagcatccagtAAAACTTGATCAAATATTGTTGGAAACAATTCGGGGATGCTGTTCGCCTTTGCAATAAACGGTGG	727
Parasite	Myxozoan	15Pkab2_gblock_CHSE	Parvicapsula kabatai	pa_kab	T	AGAAATACCAAGAGCAGCCTTTTGGTTGCTTATTGGAATGGACATAATGTAAATACTTTGATGAGTAACAACTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAGTAGTGTATATCAAAATTGTTGCGTTTAAAAAGCTCGTAGTCGGATGATAAGTGCATCTGATTTTGCGACCATCTGCACGGTACTGCCTTCGGGTAGGTCCGGGTaccgtctagcatccagtGTTGGAAGGCAGAGTTGTGGTGTACTTTTATCGCAAACTCCAGTTGTGGGATTAATTTCTTGTGGCTGGTCGGTTGCGAGGCGTGCCTTGAATAAAGCAGAGTGCTCAAAGCAAGCGTAACGCTGGAATGTAATAGCATGGAACGATGAGAAAAGAGTGTCACCTTGGCTGTGTGGGTTGAGCAATCAATCTGCGCAGTTGTATTCAGTTGGTTGGTGCGGTCGGTAACGCGGGCGTGTGCTGTTAACGCAGTGCATGTTCGAGGTCGCAGGCTAACACTGCGGTGCAGTCTTTCGGGGCTGTGCTGTAGTGGTTCTGAAGTGGGTGATGGTTAAAAGGGACATTTGAGGGCGTTAGTACTTGCTGGCGAGAGGTGAAATTCTTAGACCCAGCAAAGACTGACTAATGCGAAAGCATTCGCCAAGAGTGTTTTCATTGATCAAGAACGAAAGTTGGAGGTTCGAAGACGATCAGATACCGTCCTAGTTCCATACAGTAAACTATGCCGACTCGGGATCAGTTCGAAACAGATTCAGGTTGGTCCCACAGTGAAAACTAAAGTTTTCAGGTTATGGGGAGAGTACGCTACGCAAGTTTGAAATTTAAAAGAATTGACGGAATGGCACCACAAGGAGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGCAACTTACCAGGTCCGGACATCGGAAGGATTGACAGACTGACAGATCTTTCATGATTTGATGATTGGTGGTGCATGGCCGTTCTTAGTTGGT	1000

Type of Microbe	Classification of Microbe	Specificity Sample Run Name	Species	PEI Assay Name	Asp_Test or CRS	Sequence (5'-3')	Length (bp)
Parasite	Myxozoan	15Pkab2_Pspinach_CHSE	Parvicapsula spinachiae EF431928	pa_kab	CRS	AGAAATACCAAGGACAGTCTTTTGATTGTTTATTGGAATGGA CATAATGTAAATACTTTGATGAGTAACAACTGGAGGGCAAGT CTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAGTAGTGT ATATCAAAATTGTTGCGTTTAAAACGCTCGTAGTCGGATGAC AAGTATGCCCGATGTTGCCGTTGAGTTTGTCCTGCCCTAAA GCAGGCATACCGTCTAGCATCCAGTCGAAAGCGGTGATGTT ATGGTTACTTTAATCGCAAACTCCAGTTATGGGCTTAACTGC TTGTGGCTGGTCGGTTGCGAGGCGTGCCTTGAATAAAGCA GAGTGCTCAAAGCAAGCGTAACGCTGGTATGTAATAGCATG GAACGATGAGTAAAGAGTGTCACCTTAGCTGCATGTACCGG GCAACCGGTATGTGTGGTTCTATTCAGTTGGTTGGTGCGGT TGGTGGTCTACTAATGCACTTGTGTATTTGTAGAATGCCGAC TAACGCGTAAAAAGTTGGATTTATTCAACTTTTGACGCGGTC CTGAAGTGGGTGATGGTTAAAAGGGACATTTGAGGGCGTTA GTACTTGCTGGCGAGAGGTGAAATTCTTAGACCCAGCAAAG ACTGACTAATGCGAAAGCATTCGCCAAGAGTGTTTTCATTGA TCAAGAACGAAAGTTGGAGGTTCGAAGACGATCAGATACCG TCCTAGTTCCAACAGTAAACGATGCCAACACGGGATCAGGT CGAAACAGATTCAGGTTGGTCCCCCAGTGAAAACTCAAGTC TTCTGGTTATGGGGAGAGTACGGACGCAAGTTTGAAATTTA AAAGAATTGACGGAATGGCACCACAAGGAGTGGAGCCTGC GGCTTAATTTGACTCAACACGGGGCAACTTACCAGGTCCGG ACATCGGAAGGATTGACAGACTGATAGATCTTTCATGATTTG ATGATTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGAT CTGTCAGGTTG	1000
Parasite	Myxozoan	23Kud_18S_unicapsula_CHSE	Kudoa unicapsula AM490334	ku_thy	CRS*	TGTTGAATCAAATTAAGCCGCAGGCTCCACTCCTGGTGGTG CCATTCCGTCAATTTCTTTAAATTTAGCACTTGCGTGCGCAC TCTCCCCGGAACCGAAACACTGATTTTCACCGAGGGCCCAA CCTGGATTAAACCGGGCTAATCCCATGTTGGCATAGTTTAC TGTATGGAACTAGGACGGTATCTGATCGTCTTCGAACCTCC AACTTTCGTTCTTGATTAATGAAAACACTCTTGGCGAATGCT TTCGCATTAGTTAGTCTTTGATGGGTCTAAGGATTTCACCCC TCGCCACCAAGTACTAACGCCCTCAAATGTCCCTTTTAATCG GGACCACTGCCAACAACCAACAGTCGACAAGGTCAACATAA TCGTTCCATGCTATAACATTCAAGCTAACGCCTGCTTTGAGC ACTGTGCTTTATTCAAGGCACACCTCGCAAGTGACCACACA CAAGAATTTAATCCCACGTGTGGCTCTCGCGATAAAAAAAC ACCAACTGGATGCTAGACGGTCGGCGACCAAACCTAGATTT GGCCACCAAAGAGCTTTTGTAATCCAACTACGAGCGTTTTA ACCGCAACAATTTTGATATACACTACTGGAGCTGGAATTACC GCGGCTGCTGGCACCAGACTTGCCCTCCAGTAGCTACTCAT CGAAGGTATTAGATTACGTTCATTCCGATAGTGAACCAATTA ACGGTCTACTCCGGTATTTCTCGTCACTACCTCCCAAAGTCT GGATTGGGTAATTTGCGCGCCTGCTGCCTTCCTTAGATGTG GTAGCCGTTTCTCAGGCTCCCTCTCCGGAATCGAACCCTGA TCCCCCGTTACCCGTCACTACCTTGGTGAGCCACTACCTCA CCAACTAGTTGATAGGGCAGAAATTCGATCGGACTATCGAC AAATTAATGTTCGATATGCTCAGTTATTAGGATTCATCAAAT GGCCTTGGGCCAGTTGGTTGAGTCTAATAAATGCTCCCGCA CAAGTAC	1000
Parasite	Myxozoan	23Kud_18S_miniauriculata_CHSE	Kudoa miniauriculatus AF034639	ku_thy	CRS*	AGGCTCCACTCCTGGTGGTGCCATTCCGTCAATTTCTTTAAA TTTACCACTTGCGTGCGCACTCTCCCCGGAACCGAAACACT GGTTTTCACTGAGGGCCCAACCTGGATTAAACCGGGCTAAT CCCATGTTGGCATAGTTTACTGTATGGAACTAGGACGGTAT CTGATCGTCTTCGAACCTCCAACTTTCGTTCTTGATTAATGA AAACATTCTTGGCGAATGCTTTCGCATTAGTTAGTCTTTGAT GGGTCTAAGGATTTCACCCCTCGCCACCAAGTACTAACGCC CTCAAATGTCCCTTTTAATCGGGACCACTGTCAACAACCAAC AGTCAACAAGGTCAACATAATCGTTCCATGCTATAACATTCG ACGTAACGCCTGCTTTGAGCACTGTGCTTTATTTAAGGCAC ACCTTGCAAGTGACCGCACACAAGAATTTAATCCCACATAC GGCTCTCGCGATAAAAAAACCCCAACTGGATGCTAGACGGT CAATAACCAAACCTTGATTTGGCCATCAAAGAGCTTTTGTAA TCCAACTACGAGCGTTTTAACCGCAACAATTTTGATATACAC TACTGGAGCTGGAATTACGCGGCTGCTGGCACCAGACTTG CCCTCCAGTAGCTACTCATCGAAGGTATTAGATTACGTTCAT TCCGATAGTGAACCAATTAAAGGTCTACTCCGGTATTTCTCG TCACTACCTCCCAAAGTCTGGATTGGGTAATTTGCGCGCCT GCTGCCTTCCTTAGATGTGGTAGCCGTTTCTCAGGCTCCCT CTCCGGAATCGAACCCTGATCCCCCGTTACCCGTCACAACC TTGGTGAGCCACTACCTCACCAACTAGTTGATAGGGGTCAA GAAATTCGATCGGACTATCGACAAATTAATGTTCGATATGCA CAGTTATTAGGATTCATCAAATGACCAAAGTCAGTTGGTTGA GTCTAATAAATGCTCCCGTACAAGTACGAGATTTGCTATGTA TTAGC	1000
Parasite	Myxozoan	23Kud_18S_neurophila_CHSE	Kudoa neurophila GU808768	ku_thy	CRS*	GGCTCCACTCCTGGTGGTGCCATTCCGTCAATTTCTTTAAAT TTGTCACTTGCGTGAGCACTCTCCCCAAAGCCGAAACACTA GGTTTTCACTGAGGGCCCAACCTGGATTAAACCGGGCTAAT CCCATGTTGGCATAGTTTACTGTATGGAACTAGGACGGTAT CTGATCGTCTTCGAACCTCCAACTTTCGTTCTTGATTAATGA AAACACTCTTGGCGAATGCTTTCGCATTAGTGAGTCTTTGAT GGGTCAAAGGATTTCACCCCTCGCCACCAAGTACTAACGCC CTCAAATGTCCCTTTTAATCGAGACCAATGCCAACAACCAAC AGTCGACAAGATCAACATAATCGTTCCATGCTATAACATTCA AGCTTTCGCCTGCTTTGAGCACTGTGCTTTATTCAAGGCAC ACCTCGCAAGTGACCACACACAAGAAATTAATCCCACGAGT GGCTCTCGCGATAAAAAAACCCCAACTGGATGCTAGACGGT CAACGACCAAACCTAGATTCGGCCGCCAAAGAGCTTTTGTA ATCCAACTACGAGCGTTTTAACCGCAACAATTTTGATATACA CTACTGGAGCTGGAATTACCGCGGCTGCTGGCACCAGACT TGCCCTCCAGTAGCTACTCATCGAAGGTATTAAATTACGTTC ATTCCGATAGTGAACCAATTAACGGTCTACTCCGGTATTTCT CGTCACTACCTCCCAAAGTCTGGATTGGGTAATTTGCGCGC CTGCTGCCTTCCTTAGATGTGGTAGCCGTTTCTCAGGCTCC CTCTCCGGAATCGAACCCTGATCCCCCGTTACCCGTCACAA CCTTGGTGAGCCACTACCTCACCAACTAGTTGATAGGGCAG AAATTCGATCGGACTATCGACAAATAAATGTTCGATATGCTC AGTTATTAGGATTCGTCAAATGGCGTCGAAGCCAGTTGGTT GAGTCTAACAAATGCTCCCGCACAAGTGCGAGATTTGCTAT GTATTAGC	1000

Type of Microbe	Classification of Microbe	Specificity Sample Run Name	Species	PEI Assay Name	Asp_Test or CRS	Sequence (5'-3')	Length (bp)
Parasite	Myxozoan	23Kud_18S_thalassomi_CHSE	Kudoa thalassomi AY302738	ku_thy	CRS*	CAGGCTCCACTCCTGGTGGTGCCATTCCGTCAATTTCTTTA AATTTGTCACTTGCGTGAGCACTCTCCCCAAAGCCGAAACA CTAGGTTTTCACTGAGGGCCCAACCTGGATTAAACCGGGCT AATCCCATGTTGGCATAGTTTACTGTATGGAACTAGGACGG TATCTGATCGTCTTCGAACCTCCAACTTTCGTTCTTGATTAA TGAAAACACTCTTGGCGAATGCTTTCGCATTAGTGAGTCTTT GATGGGTCAAAGGATTTCACCCCTCGCCACCAAGTACTAAC GCCCTCAAATGTCCCTTTTAATCGAGACCACTGTCAACAAC CAACAGTCGACAAGATCAACATAATCGTTCCATGCTATAACA TTCAAGCATACGCCTGCTTTGAGCACTGTGCTTTATTCAAGG CACACCTCGCAAGTGACCACACACAAGAAATTAATCCCACA TGCGGCTCTCGCGATAAAAAAACCCCAACTGGATGCTAGAC GGTCAACGACCAAACCTAGATTTGGCCGCCAAAGAGCTTTT GTAATCCAACTACGAGCGTTTTAACCGCAACAATTTTGATAT ACACTACTGGAGCTGGAATTACCGCGGCTGCTGGCACCAG ACTTGCCCTCCAGTAGCTACTCATCGAAGGTATTAGATTAC GTTCATTCCGATAGTGAACCAAATAACGGTCCACTCCGGTA TTTCTCGTCACTACCTCCCAAAGTCTGGATTGGGTAATTTGC GCGCCTGCTGCCTTCCTTAGATGTGGTAGCCGTTTCTCAGG CTCCCTCTCCGGAATCGAACCCTGATCCCCCGTTACCCGTC ACAACCTTGGTGAGCCACTACCTCACCAACTAGTTGATAGG GCAGAAATTCGATCGGACTATCGACAAATTAATGTTCGATAT GCTCAGTTATTAGGATTCATCAAATGGCCGAAGCCAGTTGG TTGAGTCTAATAAATGCCCCCGCACAAGTACGAGGTTTGCT ATGTATTAGC	1000
Parasite	Protozoan	19Dsal_gblock_CHSE	Dermocystidium salmonis	de_sal	T	GAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCT CCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTA GTTGGATTTTGGGATTTTGGTCGGTTGGTCCGCCGCAAGGT GTTGTACCGAGCCAGCCAATCCTTTCGCTTCTCGAAAGCGG CGTGTGCCCTTCaccgtctagcatccagtACTGTGGTGTGCGTCCG TCTTATTTCGGGACTTTTACTGTGAAAAAATTAGAGTGTTCA AAGCAGGCGTTTGCTTGAATATTTCAGCATGGAATAATGGA ATAGGACATTGGTTCTATTTTGTTGGTTTCTAGGACCAAAGT AATGATTAATAGGGATAGTTGGGGGCATTAGTATTTAATTGT CAGAGGTGAAATTCTTGGATTTATGAAAGACTAACTTCTGCG AAAGCATTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAG TTAGGGGATCGAAGATGATCAGATACCGTCGTAGTCCTAAC TATAAACTATGCCGACTAGGGATTGGTAGATGTTACTTTTAT GACTCTATCAGCACCTTATGAGAAATCAAAGTCTTTGGGTTC CGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATT GACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAAT TTGACTCAACACGGGGAAACTCACCAGGTCCAGACATGGTA AGGATTGACAGATTGAGAGCTCTTTCTTGATTCTATGGGTG GTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTG GTTAATTCCGTTAACGAACGAGACCTTAACCTGCTAAATAGT TGCGTGATTTTCGAATCACTTGGTAACTTCTTAGAGGGACTA TTGGTGTTTAACCAATGGAAGTTTGAGGCAATAACAGGTCT GTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACAC TGATAAAGGCAACGAGGGGATAGATCTTTGCAATT	989
Parasite	Protozoan	19DsalAS_gblock_CHSE	Dermocystidium salmonis	de_sal	T	GAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCT CCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTA GTTGGATTTTGGGATTTTGGTCGGTTGGTCCGCCGCAAGGT GTTGTACCGAGCCAGCCAATCCTTTCGCTTCTCGAAAGCGG CGTGTGCCCTTCaccgtctagcatccagtACTGTGGTGTGCGTCCG TCTTATTTCGGGACTTTTACTGTGAAAAAATTAGAGTGTTCA AAGCAGGCGTTTGCTTGAATATTTCAGCATGGAATAATGGA ATAGGACATTGGTTCTATTTTGTTGGTTTCTAGGACCAAAGT AATGATTAATAGGGATAGTTGGGGGCATTAGTATTTAATTGT CAGAGGTGAAATTCTTGGATTTATGAAAGACTAACTTCTGCG AAAGCATTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAG TTAGGGGATCGAAGATGATCAGATACCGTCGTAGTCCTAAC TATAAACTATGCCGACTAGGGATTGGTAGATGTTACTTTTAT GACTCTATCAGCACCTTATGAGAAATCAAAGTCTTTGGGTTC CGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATT GACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAAT TTGACTCAACACGGGGAAACTCACCAGGTCCAGACATGGTA AGGATTGACAGATTGAGAGCTCTTTCTTGATTCTATGGGTG GTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTG GTTAATTCCGTTAACGAACGAGACCTTAACCTGCTAAATAGT TGCGTGATTTTCGAATCACTTGGTAACTTCTTAGAGGGACTA TTGGTGTTTAACCAATGGAAGTTTGAGGCAATAACAGGTCT GTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACAC TGATAAAGGCAACGAGGGGATAGATCTTTGCAATT	989
Parasite	Protozoan	19Dsal_Aparasiticum_CHSE	Amoebidium parasiticum AF2740	de_sal	CRS	TGGCTTACCATGGTTACAACGGGTGACGGAGAATTAGGGTT CGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACTTCC AAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACAC AGGGAGGTAGTGACCAAAAATAACAATACAGGATCTCAAAA GGTCTTGTAATTGGAATGAGTACAATTTAAACCCTTAACGAG GAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTA ATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAA AAGCTCGTAGTTGGATTTTGGGTTGTGTTTACTGGTCGGCC GCAAGGTTCGTACTGGTATGACACTTCCTTCTTCTCGAAGG CTGTGCGTGCTCTTAACCGTCTAGCATCCAGTACTGAGTGT GTGCAGAGTTCGGGACTTTTACTGTGAGAAAATTAGAGTGT TCAAAGCAGGCTTATCTTGAATATTGCAGCATGGAATAATGG AATAGGATCACGATCTTATTTTGTTGGTTTCTAAGGTCGAGA TAATGATTAATAGGGATAGTTGGGGGCATTAATATTTTTGTC AGAGGTGAAATTCTTGGATTTATGAAAGATTAACTTCTGCGA AAGCATTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGT TAGGGGATCGAAGGCGATCAGATACCGTCGTAGTCTTAACC ATAAACTATGCCGACTAGGGATTGGTGGACGTTATTCATAT GACTCCATCAGCACCTTATGAGAAATCAAAGTTTTTGGGTTC CGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATT GACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAAT TTGACTCAACACGGGAAAACTCACCAGGTCCGGACATAGGA AGGATTGACAGATTGAGAGCTCTTTCTTGATTCTATGGGTG GTGGTGCATGGCCGTTCTTAGTTGGTGAGTGATTTGTCTGG TTAATTCCGTTA	999

Type of Microbe	Classification of Microbe	Specificity Sample Run Name	Species	PEI Assay Name	Asp_Test or CRS	Sequence (5'-3')	Length (bp)
Parasite	Protozoan	19Dsal_Sphaer_CHSE	Sphaerothecum destructens	de_sal	CRS	ATTTTGTCGACGATGATTCATTCAAATTTCTGCCCTATCAACTTTCGATGGTAAGGTAGTGGCTTACCATGGTTGCAACGGGTAACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCTAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACACAGGGAGGTAGTGACAAAAAATAACAATGCAGGGCTTTTTAAGTCTTGTAATTGGAATGAGTACAATGTAAAAACCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCCCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTTGGGATAAGCTTGTTGGGCCGCCGCGAGGTGTTTGCCCCGACGAGGGTATCCTTCCTCTCGAAATTGGCGTGTGCGCTTAAACCGTCTAGCATCCAGTTTGAGTGTGCGTCGAGTTTGGGACTTTTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCTATCGCTTGAATATTTCAGCATGGAATAATGGAATAGGACGTTGGTTCTATTTTGTTGGTTTCTAGGACCGAAGTAATGATTAATAGGGATAGTTGGGGGCATTAGTATTTAATTGTCAGAGGTGAAATTCTTGGATTTATGAAAGACTAACTTCTGCGAAAGCATTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCGAAGATGATCAGATACCGTCGTAGTCCTAACCATAAACTATGCCGACTAGGGATTGGTGGATGTTAATTCTATGACTCCATCAGCACCTTATGAGAAATCAAAGTCTTTGGGTTCCGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACATAGTAAGGATTGACA	978
Parasite	Protozoan	20White_Ich_Anurofeca_CHSE	Anurofeca richardsi AF070445	ic_hof	CRS	CCCGGTTCTTTGGTGATTCATAATAACTTCGCGAATCGCATGGCCTTGTGCCGGCGATGATTCATTCAAATTTCTGCCCTATCAACTTTCGATGGTAAGGTATTGGCTTACCATGGTTACAACGGGTAACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACTTCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACACAGGGAGGTGGTGACAAAAAATAACAATGCAGGACTTTTCGAAGCCTTGCAATTGGAATGAGAACAATCTAAACCCCTTATCGAGTATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTTGGATTGCGTCTAACGGTCGGCCGTGAGGTTCGTACTACCGTCTAGCATCCAGTGTTGGGTGCTTTCTTCTTCTCGAAGACCACGTGTGCCCTTAACTGAGTGTGCGTGGGGTTCGGGACTTTTACTGTGAGAAATTAGAGTGTTCAAAGCAGGCATTTGCTTGAATATTGCAGCATGGAATAATGGAATAGGATCACGATCTTATTTTGTTGGTTTCTAGGATCGAGATAATGATTAATAGGGATAGTGGGGCATTAATATTTAATTGTCAGAGGTGAAATTCTTGGATTTATGAAAGATTAACTTCTGCGAAAGCATTTGCCAAGGATGTTTTCGTTAATCAAGAACAAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTATGCCGACTAGGGATTGGCGGGCGATTATTATATGTCCCCGTCAGCACCTTATGAGAAATCAAAGTTTTTGGGTTCCGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGAAAACTCACC	992
Parasite	Protozoan	20White_Ich_Dgrandis_CHSE	Diaphanoeca grandis DQ059033	ic_hof	CRS	TTTCTTGGTGAATCATAATAACTTGGCGAATCGCATGGTCTTGTGCCGGCGATGAATCATTCAAATTTCTGCCCTATCAACTTTCGATGGTAAGGTAGTGGCTTACCATGGTTGCAACGGGTAACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACACAGGGAGGTAGTGACAATAAATAACAATACAGGGCCTTTTAGGTTTTGTAATTGGAATGAGTACAATCTAAATCTCTTAACGAGTAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCTGTTACTGGAGGGGTGGTCCGCCTCACGGCGAGCACTGCTATCCTCTGTGACATCCTCTCAAAGTTTTAGCGGGTGCACTTAACCGTCTAGCATCCAGTGTTGTGTGGCTGGTGAATTTGGGATCTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGCTATTTGCTTGAATATATTAGCATGGAATAATGGAATAGGACTTTGGTTCTATTTTGTTGGTTTCTAGGACTGAAGTAATGATTAATAGGGACAGTTGGGGGCATTAGTATTTAATTGTCAGAGGTGAAATTCTTGGATTTATGAAAGACTAACTACTGCGAAAGCATTTGCCAAGGATGTTTTCGTTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTATGCCGACTCGGGATCGGTGTGGTGAAATTAGCCGCATCGGCACCGTATGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCC	997
Parasite	Protozoan	20White_Ich_Dpercae_CHSE	Dermocystidium percae	ic_hof	CRS	CATGCGAAAAATCCCGACTGCCCTCAAGCGGAAGGGATGTATTTATTAGATAAAAAACCAATGCGGCTCCTTCGGGACCGGTTTTCCCTGGTGATTCGTAATAACTTTGCGAATCGTACGGCCTCATGCCAACGATGATTCATTCAAATTTCTGCCCTATCAACTTTCGATGGTAAGGTAGTGGCTTACCATGGTCGCAACGGGTAACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCTAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACACAGGGAGGTAGTGACAAAAAATAACAATACAGGGCTTTACAAGTCTTGTAATTGGAATGAGAACAATTTAAATCCCTTAAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTTGGGTGATCAGGTGGTTGGGTCCGCCGCAAGGTGTTTTTGTACCGAGCCCGCCGGTCCCATTTCGTTTCCCGAGAACGGCCTCGGGCGCCCTTTAACCGTCTAGCATCCAGTCTGTGGTGCCCGGCCGATTTTTCATTTTCGGGACAGTTTACTGTGAAAAAATTAGGGTGTTCAAAGCAGGCAATTTCGCTTGAATAGTTCAGCATGGAATAATGGAATAGGACATTGGTTCTATTTTGTTGGTTTCTAGGACCAAAGTAATGATTAATAGGGTCAGTTGGGGGCATTAGTATTTAATTGTTAGAGGTGAAATTCTTGGATTTATGAAAGACTAACTTCTGCGAAAGCATTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTGGGGGATCGAAGATGATCAGATACCGTCGTAGTCCCAACCATAAACTATGCCGACTAGGGATTGGTGGATGTTTGAATTTACATGACTCCATCAGCACCTTATGAGAAATCAAAGTCTTTGGGTTC	1000

Type of Microbe	Classification of Microbe	Specificity Sample Run Name	Species	PEI Assay Name	Asp_Test or CRS	Sequence (5'-3')	Length (bp)
Parasite	Protozoan	20White_Ich_Ich-irreg_CHSE	Ichthyophonus irregularis AF2323	ic_hof	CRS*	TATTTATTAGATACAGAACCAATGCAGGGCAACCTGGTTACTTGGTGATTCATAATAACTTCGCGAATCGAATGGCCTTGTGCCGTCGATGATTCATTCAAATTTCTGCCCTATCAACTTTCGATGGTAAGGTATTGGCTTACCATGGTCGCAACGGGTAACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACTTCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACACAGGGAGGTAGTGACAAAAAATAACAATACAGGATCTTTAAAGGTCTTGTAATTGGAATGAGTACAATTTAAACCCCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTTGGAAATTGCTTACTGGTCGGCCGCAAGGTCTGTACTGGTACGGCAGTTTCTTCTTCTCGAAGGCAGTGGGTGCTCTTAAaccgtctagcatccagtTTGAGTGTCTACTGAGTTCGGGACTTTTACTGTGAGAAAATTAGAGTGTTCAAAGCAGGCTTATGCTTGAATATTGCAGCATGGAATAATGGAATAGGATCACGATCTTATTTTGTTGGTTTCTAAGGTCGAGATAATGATTAATAGGGACGGTTGGGGGCATTAATATTTAATTGTCAGAGGTGAAATTCTTGGATTTATGAAAGATTAACTTCTGCGAAAGCATTTGCCAAGGATGTTTTCGTTAATCAAGAACGAAAGTTAGGGGATCGAAGATGATCAGATACCATCGTAGTCTTAACCATAAACTATGCCGACTAGGGATTAGTGGACGTTTAATAATGACACCATTAGCACCTTATGAGAAATCAAAGTTTTTGGGTTCCGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTT	999
Parasite	Protozoan	20White_Ich_Pseud-tap_CHSE	Pseudoperkinsus tapetis AF192386	ic_hof	CRS	TTCGGATCGCATGGCCTTGTGCCGGCGACGTTTCATTCAAATTTCTGCCCTATCAACTTTCGATGGTAAGGTATTGGCTTACCATGGTTACAACGGGTAACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACTTCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACACAGGGAGGTAGTGACAAAAAATAACAATACAGGACTTTTAAAAGCCTTGTAATTGGAATGAGTACAATTTAAACCCCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTTGGAATTGTGTTTAATGGTCTGCCGTGAGGTATGCACTGTTAGGCGCTTTCTTCTTGTCAAAGACTGTGTGTGCTCTTAAACCGTCTAGCATCCAGTTTGAGTGTGCTCAGGGTTTGGCACTTTTACTGTGAGAAAATTAGAGTGTTTAAAGCAGGCGTTTGCTTGAATATTGCAGCATGGAATAATGGAATAGGATCACGATCTTATTTTGTTGGTTTCTAGGATCGAGATAATGATTAATAGGGATAGTTGGGGACATTAATATTTAATTGTCAGAGGTGAAATTCTTGGATTTATGAAAGATTAACTTCTGCGAAAGCATTTGTCAAGGATGTTTTCGTTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTATGCCGACTAGGGATTGGGTGGCAGTTAATTATATGACTTCATCAGCACCTTATGAGAAATCAAAGTTTTTGGGTTCCGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGAAAACTCACCAGGTCCGGACATAGTGAGGATTGACAGATTGAG	1000
Parasite	Protozoan	21Sphaer2_gblock_CHSE	Sphaerothecum destructens	sp_des	T	TCAAATTTCTGCCCTATCAACTTTCGATGGTAAGGTAGTGGCTTACCATGGTTGCAACGGGTAACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCTAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACACAGGGAGGTAGTGACAAAAAATAACAATGCAGGGCTTTTTAAGTCTTGTAATTGGAATGAGTACAATGTAAAAACCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCCCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTTGGGATAAGCTTGTTGGGCCGCCGCGAGGTGTTTGCCCCGACGAGGGTATCCTTCCTCTCGAAATTGGCGTGTGCGCTTAATTaccgtctagcatccagtGAGTGTGCGTCGAGTTTGGGACTTTTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCTATCGCTTGAATATTTCAGCATGGAATAATGGAATAGGACGTTGGTTCTATTTTGTTGGTTTCTAGGACCGAAGTAATGATTAATAGGGATAGTTGGGGGCATTAGTATTTAATTGTCAGAGGTGAAATTCTTGGATTTATGAAAGACTAACTTCTGCGAAAGCATTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCGAAGATGATCAGATACCGTCGTAGTCCTAACCATAAACTATGCCGACTAGGGATTGGTGGATGTTAATTCTATGACTCCATCAGCACCTTATGAGAAATCAAAGTCTTTGGGTTCCGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACATAGTAAGGATTGACAGATTGATAGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGC	1000
Parasite	Protozoan	21Sphaer2_Anurofeca_CHSE	Anurofeca richardsi AF070445	sp_des	CRS	CCCGGTTCTTTGGTGATTCATAATAACTTCGCGAATCGCATGGCCTTGTGCCGGCGATGATTCATTCAAATTTCTGCCCTATCAACTTTCGATGGTAAGGTATTGGCTTACCATGGTTACAACGGGTAACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACTTCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACACAGGGAGGTGGTGACAAAAAATAACAATGCAGGACTTTTCGAAGCCTTGCAATTGGAATGAGAACAATCTAAACCCCTTATCGAGTATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTTGGATTGCGTCTAACGGTCGGCCGTGAGGTTCGTACTGTTGGGTGCTTTCTTCTTCTCGAAGACCACGTGTGCCCTTAACTACCGTCTAGCATCCAGTGAGTGTGCGTGGGGTTCGGGACTTTTACTGTGAGAAATTAGAGTGTTCAAAGCAGGCATTTGCTTGAATATTGCAGCATGGAATAATGGAATAGGATCACGATCTTATTTTGTTGGTTTCTAGGATCGAGATAATGATTAATAGGGATAGTGGGGCATTAATATTTAATTGTCAGAGGTGAAATTCTTGGATTTATGAAAGATTAACTTCTGCGAAAGCATTTGCCAAGGATGTTTTCGTTAATCAAGAACAAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTATGCCGACTAGGGATTGGCGGGCGATTATTATATGTCCCCGTCAGCACCTTATGAGAAATCAAAGTTTTTGGGTTCCGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGAAAACTCACCAGGTCCGG	1000

Type of Microbe	Classification of Microbe	Specificity Sample Run Name	Species	PEI Assay Name	Asp_Test or CRS	Sequence (5'-3')	Length (bp)
Parasite	Protozoan	21Sphaer2_Aparasiticum_CHSE	Amoebidium parasiticum AF274051	sp_des	CRS	ATTTCTGCCCTATCAACTTTCGATGGTAAGGTATTGGCTTAC CATGGTTACAACGGGTGACGGAGAATTAGGGTTCGATTCCG GAGAGGGAGCCTGAGAAACGGCTACCACTTCCAAGGAAGG CAGCAGGCGCGCAAATTACCCAATCCTGACACAGGGAGGT AGTGACCAAAAATAACAATACAGGATCTCAAAAGGTCTTGTA ATTGGAATGAGTACAATTTAAACCCTTAACGAGGAACAATTG GAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCT CCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTA GTTGGATTTTGGGTTGTGTTTACTGGTCGGCCGCAAGGTTC GTACTGGTATGACACTTCCTTCTTCTCGAAGGCTGTGCGTG CTCTTAACTACCGTCTAGCATCCAGTGAGTGTGTGCAGAGT TCGGGACTTTTACTGTGAGAAAATTAGAGTGTTCAAAGCAG GCTTATCTTGAATATTGCAGCATGGAATAATGGAATAGGATC ACGATCTTATTTTGTTGGTTTCTAAGGTCGAGATAATGATTA ATAGGGATAGTTGGGGGCATTAATATTTTTGTCAGAGGTGA AATTCTTGGATTTATGAAAGATTAACTTCTGCGAAAGCATTT GCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGA TCGAAGGCGATCAGATACCGTCGTAGTCTTAACCATAAACT ATGCCGACTAGGGATTGGTGGACGTTATTCATATGACTCCA TCAGCACCTTATGAGAAATCAAAGTTTTTGGGTTCCGGGGG GAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGACGGAA GGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTC AACACGGGAAAACTCACCAGGTCCGGACATAGGAAGGATT GACAGATTGAGAGCTCTTTCTTGATTCTATGGGTGGTGGTG CATGGCCGTTCTTA	1000
Parasite	Protozoan	21Sphaer2_Demo-salmonis_CHSE	Dermocystidium salmonis	sp_des	CRS	TCATTCAAATTTCTGCCCTATCAACTTTCGATGGTAAGGTAG TGGCTTACCATGGTTACAACGGGTAACGGAGAATTAGGGTT CGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCT AAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACAC AGGGAGGTAGTGACAAAAAATAACAATACAGGGCTTTTAAA GTCTTGTAATTGGAATGAGAACAATTTAAACCCCTTAACGAG GAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTA ATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAA AAGCTCGTAGTTGGATTTTGGGATTTTGGTAGGTTGGTCCG CCGCAAGGTGTTGTACCGAGCCAGCCAATCCTTTCGCTTCT CGAAAGCGGCGTGTGCCCTTCACTACCGTCTAGCATCCAGT GTGGTGTGCGTCCGCTTATTTCGGGACTTTTACTGTGAAAA AATTAGAGTGTTCAAAGCAGGCGTTTGCTTGAATATTTCAGC ATGGAATAATGGAATAGGACATTGGTTCTATTTTGTTGGTTT CTAGGACCAAAGTAATGATTAATAGGGATAGTTGGGGGCAT TAGTATTTAATTGTCAGAGGTGAAATTCTTGGATTTATGAAA GACTAACTTCTGCGAAAGCATTTGCCAAGGATGTTTTCATTA ATCAAGAACGAAAGTTAGGGGATCGAAGATGATCAGATACC GTCGTAGTCCTAACTATAAACTATGCCGACTAGGGATTGGT AGATGTTACTTTTATGACTCTATCAGCACCTTATGAGAAATC AAAGTCTTTGGGTTCCGGGGGGAGTATGGTCGCAAGGCTG AAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGG AGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCA GGTCCAGACATGGTAAGGATTGACAGATTGAGAGCTCTTTC TTGATTCTATGGG	1000
Parasite	Protozoan	21Sphaer2_Dgrandis_CHSE	Diaphanoeca grandis DQ059033	sp_des	CRS	GCCGGCGATGAATCATTCAAATTTCTGCCCTATCAACTTTCG ATGGTAAGGTAGTGGCTTACCATGGTTGCAACGGGTAACGG AGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACG GCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACC CAATCCTGACACAGGGAGGTAGTGACAATAAATAACAATAC AGGGCCTTTTAGGTTTTGTAATTGGAATGAGTACAATCTAAA TCTCTTAACGAGTAACAATTGGAGGGCAAGTCTGGTGCCAG CAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTT GTTGCAGTTAAAAAGCTCGTAGTTGGATTTCTGTTACTGGAG GGGTGGTCCGCCTCACGGCGAGCACTGCTATCCTCTGTGA CATCCTCTCAAAGTTTTAGCGGGTGCACTTAGTTACCGTCTA GCATCCAGTGTGTGGCTGGTGAATTTGGGATCTTTACTTTG AAAAAATTAGAGTGTTCAAAGCAGGCTATTTGCTTGAATATA TTAGCATGGAATAATGGAATAGGACTTTGGTTCTATTTTGTT GGTTTCTAGGACTGAAGTAATGATTAATAGGGACAGTTGGG GGCATTAGTATTTAATTGTCAGAGGTGAAATTCTTGGATTTA TGAAAGACTAACTACTGCGAAAGCATTTGCCAAGGATGTTTT CGTTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAG ATACCGTCGTAGTCTTAACCATAAACTATGCCGACTCGGGA TCGGTGTGGTGAAATTAGCCGCATCGGCACCGTATGAGAAA TCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCT GAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTG GAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACC AGGTCCAGACATAGTAAGGATTGACAGATTGAGAGCTCTTT CTTGATTCTATG	1000
Parasite	Protozoan	21Sphaer2_Dpercae_CHSE	Dermocystidium percae	sp_des	CRS	TACATGCGAAAAATCCCGACTGCCCTCAAGCGGAAGGGAT GTATTTATTAGATAAAAAACCAATGCGGCTCCTTCGGGACC GGTTTTCCCTGGTGATTCGTAATAACTTTGCGAATCGTACG GCCTCATGCCAACGATGATTCATTCAAATTTCTGCCCTATCA ACTTTCGATGGTAAGGTAGTGGCTTACCATGGTCGCAACGG GTAACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTG AGAAACGGCTACCACATCTAAGGAAGGCAGCAGGCGCGCA AATTACCCAATCCTGACACAGGGAGGTAGTGACAAAAAATA ACAATACAGGGCTTTACAAGTCTTGTAATTGGAATGAGAACA ATTTAAATCCCTTAAACGAGGAACAATTGGAGGGCAAGTCT GGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATA TTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTTGGG TGATCAGGTGGTTGGGTCCGCCGCAAGGTGTTTTTGTACCG AGCCCGCCGGTCCCATTTCGTTTCCCGAGAACGGCCTCGG GCACCGTCTAGCATCCAGTGCCCTTTACTGTGGTGCCCGG CCGATTTTTCATTTTCGGGACAGTTTACTGTGAAAAAATTAG GGTGTTCAAAGCAGGCAATTTCGCTTGAATAGTTCAGCATG GAATAATGGAATAGGACATTGGTTCTATTTTGTTGGTTTCTA GGACCAAAGTAATGATTAATAGGGTCAGTTGGGGGCATTAG TATTTAATTGTTAGAGGTGAAATTCTTGGATTTATGAAAGACT AACTTCTGCGAAAGCATTTGCCAAGGATGTTTTCATTAATCA AGAACGAAAGTTGGGGGATCGAAGATGATCAGATACCGTC GTAGTCCCAACCATAAACTATGCCGACTAGGGATTGGTGGA TGTTTGAATTTACATGACTCCATCAGCACCTTATGAGAAATC AAAGTCTTTGGGT	1000

Type of Microbe	Classification of Microbe	Specificity Sample Run Name	Species	PEI Assay Name	Asp_Test or CRS	Sequence (5'-3')	Length (bp)
Parasite	Protozoan	21Sphaer2_Ich-hof_CHSE	Ichthyophonus hoferi	sp_des	CRS	TGTATTTATTAGATACAGAACCACTGCAGGGCAACCTGGTTA CTTGGTGATTCATAATAACTTCGCGAATCGAATGGCTTGTGC CGTCGATGATTCATTCAAATCTCTGCCCTATCAACTTTCGGT GGTAAGGTATTGGCTTACCATGGTTCCAACGGGTAACGGAG AATTAGGGTTCGATTCCCGAGAGGGAGCCTGAGAAACGGC TACCACTTCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAA TCCTGACACAGGGAGGTAGTGACAAAAAATAACAATACAGG ATCTTTAAAGGTCTTGTAATTGGAATGAGTACAATTTAAACC CCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGC AGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTG TTGCAGTTAAAAAGCTCGTAGTTGGATTTTGGAAATTGCTTA CTGGTCGGCCGCAAGGTCTGTACTGGTACGGCAGTTTCTTC TTCTCGAAGGCAGTGGGTGCTCTTAATTACCGTCTAGCATC CAGTAGTGTCTACTGAGTTCGGGACTTTTACTGTGAGAAAAT TAGAGTGTTCAAAGCAGGCTTATGCTTGAATATTGCAGCAT GGAATAATGGAATAGGATCCCGATCTTATTTTGTTGGTTTCT AAGATCGAGATAATGATTAATAGGGACGGTTGGGGGCATTA ATATTTAATTGTCAGAGGTGGAATTCTTGGATTTATGAAAGA TTAACTTCTGCGAAAGCATTTGCCAAGGATGTTTTCGTTAAT CAAGAACGAAAGTTAGGGGATCGAAGATGATCAGATACCAT CGTAGTCTTAACCATAAACTATGCCGACTAGGGATTAGTGG ACGTTTAATAATGACACCATTAGCACCTTATGAGAAATCAAA GTTTTTGGGTTCCGGGGGGAGTATGGTCCCAAGGCTGAAA CTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGC CTGCGGCTTA	1000
Parasite	Protozoan	21Sphaer2_Ich-irreg_CHSE	Ichthyophonus irregularis AF2323	sp_des	CRS	TATTAGATACAGAACCAATGCAGGGCAACCTGGTTACTTGG TGATTCATAATAACTTCGCGAATCGAATGGCCTTGTGCCGT CGATGATTCATTCAAATTTCTGCCCTATCAACTTTCGATGGT AAGGTATTGGCTTACCATGGTCGCAACGGGTAACGGAGAAT TAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTAC CACTTCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATC CTGACACAGGGAGGTAGTGACAAAAAATAACAATACAGGAT CTTTAAAGGTCTTGTAATTGGAATGAGTACAATTTAAACCCC TTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAG CCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTG CAGTTAAAAAGCTCGTAGTTGGATTTTGGAAATTGCTTACTG GTCGGCCGCAAGGTCTGTACTGGTACGGCAGTTTCTTCTTC TCGAAGGCAGTGGGTGCTCTTAATTACCGTCTAGCATCCAG TGAGTGTCTACTGAGTTCGGGACTTTTACTGTGAGAAAATTA GAGTGTTCAAAGCAGGCTTATGCTTGAATATTGCAGCATGG AATAATGGAATAGGATCACGATCTTATTTTGTTGGTTTCTAA GGTCGAGATAATGATTAATAGGGACGGTTGGGGGCATTAAT ATTTAATTGTCAGAGGTGAAATTCTTGGATTTATGAAAGATT AACTTCTGCGAAAGCATTTGCCAAGGATGTTTTCGTTAATCA AGAACGAAAGTTAGGGGATCGAAGATGATCAGATACCATCG TAGTCTTAACCATAAACTATGCCGACTAGGGATTAGTGGAC GTTTAATAATGACACCATTAGCACCTTATGAGAAATCAAAGT TTTTGGGTTCCGGGGGGAGTATGGTCGCAAGGCTGAAACTT AAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTG CGGCTTAATTT	1000
Parasite	Protozoan	21Sphaer2_Pseud-tap_CHSE	Pseudoperkinsus tapetis AF192386	sp_des	CRS	GCGCTTCGGCGGTTTCTTGGTGATTCATAATAACTTTTCGGA TCGCATGGCCTTGTGCCGGCGACGTTTCATTCAAATTTCTG CCCTATCAACTTTCGATGGTAAGGTATTGGCTTACCATGGTT ACAACGGGTAACGGAGAATTAGGGTTCGATTCCGGAGAGG GAGCCTGAGAAACGGCTACCACTTCCAAGGAAGGCAGCAG GCGCGCAAATTACCCAATCCTGACACAGGGAGGTAGTGAC AAAAAATAACAATACAGGACTTTTAAAAGCCTTGTAATTGGA ATGAGTACAATTTAAACCCCTTAACGAGGAACAATTGGAGG GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAA TAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTG GATTTTGGATTGTGTTTAATGGTCTGCCGTGAGGTATGCACT GTTAGGCGCTTTCTTCTTGTCAAAGACTGTGTGTGCTCTTAA TTACCGTCTAGCATCCAGTGAGTGTGCTCAGGGTTTGGCAC TTTTACTGTGAGAAAATTAGAGTGTTTAAAGCAGCGTTTGCT TGAATATTGCAGCATGGAATAATGGAATAGGATCACGATCTT ATTTTGTTGGTTTCTAGGATCGAGATAATGATTAATAGGGAT AGTTGGGGACATTAATATTTAATTGTCAGAGGTGAAATTCTT GGATTTATGAAAGATTAACTTCTGCGAAAGCATTTGTCAAGG ATGTTTTCGTTAATCAAGAACGAAAGTTAGGGGATCGAAGA CGATCAGATACCGTCGTAGTCTTAACCATAAACTATGCCGA CTAGGGATTGGGTGGCAGTTAATTATATGACTTCATCAGCA CCTTATGAGAAATCAAAGTTTTTGGGTTCCGGGGGGAGTAT GGTCGCAAGGCTGAAACTTAAAGGAATTGACGGAAGGGCA CCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCAACACG GGAAAACTCA	1000
Parasite	Protozoan	22Csal_CYS4_gblock_CHSE	Cryptobia salmositica	cr_sal	T	ATGGGAGCCCCAACAACCGAGGTCCTGTTCGGCAACTTCAA GGCCGCACATGCCCGTAACTACGCGTCTCCTGACGAGGAG CGCAAGCGCTTTGAGATATTCGCCGGGAACATGAAGAAGG CCGCAGTGCTGAACCGCAAAAACCCCATGGCCACCTTCGG CCCCAACGAGTTTGCCGACATGACTTCCGAGGAGTTCCAGA CCCGCCACAACGCCGCTCGCCACTACGCTGCTGCGAAGGC CCGCCCACCCAAGAACACCAAGACATTTACTGCAGAGGAGA TTAAGGCCGCGGTCGGCCAGCAGATCGACTGGCGTTTGAA AGGAGCCGTGACCCCAGTGAAGAATCAAGGCGCTTGCGGT TCCTGCTGGAGCTTCTCTACAACTGGAAACATCGAAGGACA GCACGCCATTGCCACCGGTCAGCTAGTGGCTGTTTCTGAG CAGGAACTTGTTTCCTGTGACCCCATTGACGACGGGTGCAA TGGCGGTCTCATGGACAACGCCTTCGGATGGCTAATCTCTG CCCACAAGGGCCAAATCGCAACCGAGGCAAACTACCCATA CGTCAGCGGCAACGGCATTGTCCCCGCGTGCTCATCCAGC CCTGAGTCCAAGCCCGTCGGAGCCACCATCAGTGCCTTTCA GGACATCGCCAGAACCGAGGAGGACATGGCAGCCTTTGTA TTTAAGCAaccgtctagcatccagtTGGACCACTGTCTATTGGAGTG GATGCCTCGACCTGGCAGTCCTACGCGGGAGGCATCATGT CCTACTGCCCCCAAGATCAGATCGACCACGGTGTCCTAATC GTGGGCTTCGATGACACCGCCTCCACCCCTTACTGGATCAT AAAGAACTCCTGGACAGCCAACTGGGGAGAGGAGGGCTAC ATCCGCGTTGCTAAGGGCAGCAACCAGTGTGGTCTGACGA GCCACCCCAGCTCTTCCGTTGTGGGTAACAGTCCCAGTCCA ACCCCAGCCCCCACCACCCCCGG	1000

Key: We expect the assay to work in this CRS as the homology between the species and assay is >95%