Use Scenarios for CSWG Benchmarking Reagent Products

Plate 0 — Sample Panel for Collaborative Study

Plate 0 is the reservoir for a panel of samples that we can use to conduct the collaborative interlab study we've been discussing. This study will establish the copy concentration (or "genome copies/ml"[1]) of a panel of SARS-CoV-2 control samples, putting the concentrations all on the same scale — 'harmonized.' There are several different types of SARS-CoV-2 controls, ranging from positive control DNA used to validate a primer design for the qPCR step in the process, to RNA control materials that can act as calibrants for the analytical RT-qPCR, to inactivated viral controls that are likely the most commutable reference samples to evaluate whole-process performance (see Figure 2 of the manuscript we are developing).

The initial design we presented focused on whole-process controls, including inactivated virus and engineered "packaged" SARS-CoV-2 genomes in other viral hosts or virus-like particles. The discussion at our WG meeting indicated that there is interest in including RNA control materials in our collaborative harmonization study. The plate design is appropriate for both types of controls. We will designate the plate containing the viral and virus-like controls Plate 0.vir, and the plate containing the panel of nucleic acid controls as Plate 0.na

The study might be conducted by distributing 3 copies of the plate to each of a group of two types of lab:

- Reference Labs — National Measurement Labs or designees, who will conduct digital PCR to directly measure copy concentration without reference to external standards
- Leading Diagnostic Labs — labs participating or affiliated with the CSWG who use EUA assays

Conducting the study with both types of labs will assure that the copy concentration value assignment is "fit for purpose," that the reference procedures are compatible with the EUA procedures.

Plate 1 — Analytical Sensitivity Benchmark Plate

The Analytical Sensitivity Benchmark Plate (Plate 1, the "ASBP") is intended as a shared reagent to be used to establish the analytical sensitivity (here, Limit of Detection, or LOD) of a SARS-CoV-2 molecular assay. Users of this plate and the accompanying analysis calculation will obtain results that can be meaningfully compared with results from other assays, other labs, or other methods that use the same reagents and calculation,[2] yielding LODs that can be compared over space and time.

Plate 1 contains sufficient wells without SARS-CoV-2 genomic material ("No Template Controls") such that an experiment measuring all 96 wells will inform the lab of contamination in their environment or assay reagents.

The SARS-CoV-2 controls used in Plate 1 will be those harmonized in the collaborative study using Plate 0, assuring the comparability that is a key value of this shared reagent.

We need to decide the scope of the controls in Plate 1 — will there also be 2 versions, a Run-control and an RNA-control version? Plate 1.vir and Plate 1.na?

Plate 2 — Validation Plate

The contents of Plate 2 are identical to those in Plate 1, but randomly arrayed robotically with a key escrowed in a cloud-hosted public-facing dashboard. The measurements of each well could (would?) be uploaded to the dashboard along with metadata describing the measurements (platform, reagent kit part number & lot number, location, date & time, other metadata?).

The intent of Plate 2 is to provide the standard reagent in Plate 1 broadly available to testing labs across the US and the globe. If testing labs measure this plate once for every hundred plates in their workflow, the aggregation of analytical performance data and metadata will inform on testing performance, capacity, supply chain, and trends of all factors. This could be critical knowledge for coordination of a global testing enterprise.

The value proposition of an anonymized testing performance dashboard for the testing labs would be for their own monitoring of test performance, knowledge of their performance relative to the population of labs, foreknowledge of the state of the supply chain, a platform for optimization or management, and engagement in a fluid load balancing "Smart Testing Grid." This model could be foundational in a resilient and reliable testing enterprise.

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Footnotes

1. Pavšič, Jernej, Alison S. Devonshire, Helen Parkes, Heinz Schimmel, Carole A. Foy, Maria Karczmarczyk, Ion Gutiérrez-Aguirre, et al. "Standardization of Nucleic Acid Tests for Clinical Measurements of Bacteria and Viruses." Edited by G. V. Doern. *Journal of Clinical Microbiology* 53, no. 7 (July 2015): 2008–14. https://doi.org/10.1128/JCM.02136-14.
2. Burd, Eileen M. "Validation of Laboratory-Developed Molecular Assays for Infectious Diseases." *Clinical Microbiology Reviews* 23, no. 3 (July 2010): 550–76. https://doi.org/10.1128/CMR.00074-09.

Other notes

Notes post Steering Cmte Meeting

14 August 2020

Consider making BOTH "Run Control" and "Nucleic Acid" Plate 0's

- Run Control Candidates
  - NIBSC-WHO
  - SeraCare
  - FDA
  - BEI
  - EVA
  - Imperial?
- NA Control Candidates
  - NIST
  - Assuragen
  - Imperial?

Use Scenarios

Plate 0(s) — Nucleic Acid controls, Full-process Run Controls

- what is the unit we're looking for — copies*well or copies per*ul
- need to describe what's in the well — volume and contents

Plate 1

- need report per row and column
- need Ct at each well
- logistic LOD
- candyman

Plate 2

- this is what we imagine using widely and in high throughput

Invite all in WG to reach out if they want to dive in deeper

plan to follow-up in next week's SC meeting

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Interaction of Ct variation with copy number for Plates 1&2

- is this a real sensitivity matter?