Detection of SARS-CoV-2 Armored RNA Quant®* in Global Interlaboratory Harmonization Study

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Background

Towards the beginning of 2020 a global effort began to effectively respond to the COVID-19 pandemic by developing molecular tests that could accurately and rapidly diagnose this emerging disease. A critical component lacking was a control to harmonize the results of the myriad of tests being developed. In order to address this urgent need, a Coronavirus Standards Working Group was formed in March of 2020 to provide recommended infrastructure for COVID-19 testing and ensure reliability of test results. This international consortium was convened by the Joint Initiative for Metrology in Biology at Stanford University and included a variety of represented disciplines. The focus of this study was on molecular controls. Molecular tests were chosen initially over antibody tests for their ability to detect the virus directly at symptom onset and before antibody load was sufficiently high to detect. Additionally, molecular tests provided higher sensitivity and specificity to allow a definitive diagnosis using more easily attained synthetic sequences as controls (Figure 1). Many of the first iterations of molecular tests were problematic in terms of specificity and/or sensitivity[1,2], a result of accelerated development to meet the exponentially growing demand for testing as cases soared. The Steering Committee systematically considered different aspects of the measurement process, including standards and controls, and how they impacted various stages of the testing process. Here we describe a study planned by the consortium and executed globally by independent laboratories to assess multiple sources and types of molecular controls.

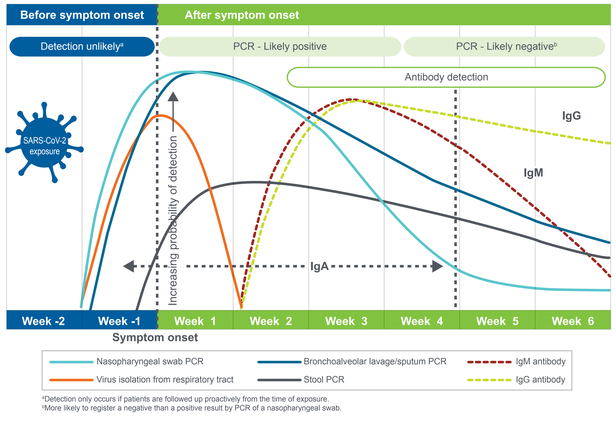

Figure 1. Estimated Variation Over Time in Diagnostic Tests for Detection of SARS-CoV-2 Infection. (Sethuraman, et al., JAMA. 2020;323(22):2249-2251)

Methods

Eight vendors provided molecular controls that fell into one of three categories: inactivated virus, recombinant viral-like particles (VLPs), or recombinant bacteriophage-encapsulated RNA (Armored RNA Quant®).

The distribution of controls to the fourteen participating laboratories is outlined in Figure 2. Each vendor provided replicate material to test at nominal concentrations ranging from 5E+03 copies/mL to 2E+10 copies/mL. Additionally, World Health Organization International Standards (WHO-IS) were prepared for each laboratory to use as calibrators. Each laboratory was instructed to construct an eight-point standard curve from 1E+08 to 0 copies/mL for normalization. The RNA extraction methods used by the 14 laboratories varied from column-based viral RNA extraction kits to magnetic bead-based extraction kits to all-in-one systems that did not require extraction ahead of processing. Platform chemistries included probe-based detection methods on digital PCR using Bio-Rad systems (QX200, T100, C1000) and real-time PCR using instruments from Roche (Cobas6800), Fluxergy, Abbott (Alina m), ThermoFisher (7500Fast and QuantStudio), and Bio-Rad (CX384). Five out of the 14 laboratories used digital PCR, while the rest used real-time PCR. Of the nine laboratories using real-time PCR, three involved platforms that required no extraction prior to loading the sample on the instrument as extraction was performed on the instrument.

Participants were instructed to run all controls in quadruplicate per run. The most common gene assayed was the nucleocapsid region. For the purpose of further analysis, these results are filtered by nucleocapsid assay data only.

Data were processed at Stanford prior to dissemination. For digital PCR results, copy numbers derived from droplet quantification were normalized to the WHO-IS standard curve run at each institution and multiplied by dilution factor (if any) used at that institution. The results were log-transformed and reported as observed log10 copies/mL. For real-time PCR results, cycle threshold (Ct) or quantification cycle (Cq) values are reported in log2. These were similarly normalized to the WHO-IS standard curve run at each institution and multiplied by the requisite dilution factor.

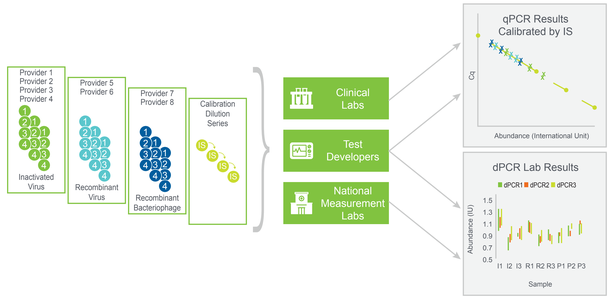

Figure 2. Molecular RNA Harmonization Study Design Outlines Sample Mix Sent and Results Expected.

Results

All vendor-submitted control material performed well compared to expected nominal genome equivalent values of the nucleocapsid gene (Figure 3, Table 1). A best fit line was generated using the actual nominal results collected from the 14 different test sites for each of the 8 control materials representing the different vendors, material categories, and concentrations. The slope of the best fit line was 0.97 and the R^2 was 0.95. Only one vendor's control showed significant difference in log10 copies/mL observed between digital and real-time PCR (data not shown).

Quadruplicate concentration measurements of the Armored RNA Quant SARS-CoV-2 control were reported by each institution and plotted (Figure 3, 4). In general, replicates within each institution were very tight. Two notable exceptions were Lab10 and Lab11, where RNA extraction and testing were performed on the same instrument. The average log concentration for the Armored RNA Quant control across all laboratories was 10.2 compared to the expected 10.3 nominal value, with a standard deviation of 0.455 and a %CV of 4.5 (Table 1).

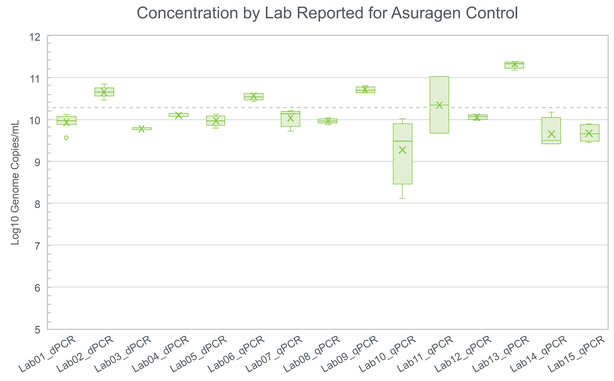

Figure 3. Observed Log10 Copies/mL as Reported Across All Laboratories That Tested the Asuragen Control. Expected value of 10.3 as determined at Asuragen using an analytic method is represented by the dotted line. Lab07 and Lab08 represent two datasets from the same institution.

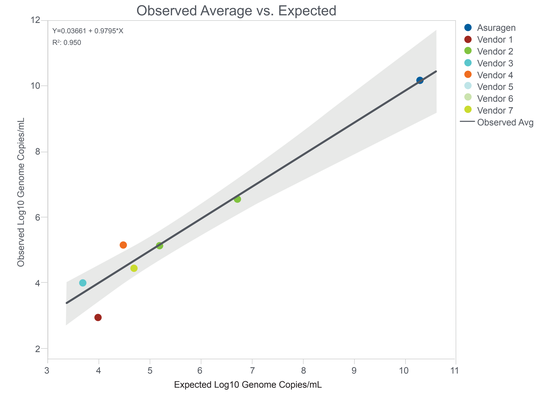

Figure 4. Observed vs Expected Log10 Copies/mL Across All Vendor-Provided Control Materials.

Table 1. Observed vs Expected Log10 Copies/mL for Each Vendor-Provided Control Material. Average Expected values were provided by the control material vendors and Average Observed are representative of all 15 data sets provided by the 14 testing labs.

Vendor	Type of Control Provided	Average Expected Log10 Copies/mL	Average Observed Log10 Copies/mL	Stdev of Observed Log10 Copies/mL	CV
Asuragen	Recombinant Bacteriophage	10.3	10.2	0.455	4.5%
Vendor 1	Recombinant Bacteriophage	4	2.9	0.710	24.2%
Vendor 2	Inactivated virus	5.2	5.1	0.267	5.2%
Vendor 3	VLP	3.7	4.0	0.249	6.3%
Vendor 4	Inactivated virus	4.5	5.1	0.441	8.6%
Vendor 5	VLP	6.73	6.5	0.748	11.5%
Vendor 6	Inactivated virus	3.7	4.0	0.337	8.5%
Vendor 7	Inactivated virus	4.7	4.4	0.355	8.0%

Conclusion

- Despite the differences in quantitation methods of starting material, dilution schema, assays used, detection chemistry involved, platform used, and laboratory location, all vendors' controls submitted to this interlaboratory global study demonstrated linearity, accuracy, and precision typically acceptable for clinical testing at all laboratories where testing was performed.
- Inactivated virus, recombinant viral-like particles, and recombinant bacteriophage-encapsulated RNA each have unique advantages as controls depending on the stage of the molecular assay being optimized[4,5]. Although having greatest utility for assessing real-world performance, inactivated virus is least available at the beginning of an outbreak and poses a significant health risk if not handled properly. VLPs and recombinant bacteriophage-encapsulated RNAs have the advantage of mimicking a virus without the risk of infectivity. Bacteriophage-encapsulated RNA controls are also resistant to nuclease degradation and safe for world-wide distribution.
- The data summary highlights that bacteriophage-encapsulated RNA controls perform as well as inactivated virus and VLPs in the hands of laboratorians. Digital and real-time PCR are extremely sensitive molecular methods, providing detection down to 5000 viral copies/mL; molecular controls such as bacteriophage-encapsulated RNAs can be reproducibly manufactured lot to lot, formulated over a wide concentration range, and are stable over time to provide an easy to use option to characterize and/or monitor assay performance at both high and very low viral levels. These types of commutable, surrogate controls can be rapidly and widely deployed as an important part of future response planning. In addition, once agreement on a consensus sequence is achieved and a corresponding control is produced, sufficient flexibility remains to allow quick design and production updates when new variants emerge.
- Regardless of which control format is utilized, agreement between control suppliers and assay developers should be made to ensure that the supply of controls and standards does not become a limiting factor when faced with an aggressive timeline for assay development and validation during emerging pandemics.

References

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Keyword: molecular diagnostics ; multicenter study
CVS Track: Track I – Respiratory Viruses (RSV, Influenza A & B, Parainfluenza Viruses 1, 2, 3, 4, Metapneumoviruses, Adenoviruses, Rhinoviruses, Coronaviruses [annual coronaviruses (OC43, 229E, HKU1, NL63), SARS-CoV, MERS and SARS-CoV-2]

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