SARS-CoV-2 Quality Control for Molecular and Serology assays

Questions and Answers

Quality Control

1. **So how efficiently this RNA release assay work compared to one in which RNA is extracted. What's the agreement between two different assays?**

   Dr Newton: The assay we use that has an extraction step (Abbott m2000) is approximately 5 times more analytically sensitive than the assay without extraction (Diasorin). We know that analytically the LoD for the Abbott assay is 100 copies/mL, whereas the LoD for Diasorin is 500 copies/mL. While this can likely be attributed to the enhanced recovery of nucleic acids by extraction, the input volume of the Abbott assay is 700-800 uL, while for the Diasorin assay it is only 50 uL, so the chances of recovering more target are higher from the beginning for the Abbott assay. Unfortunately, we don't really know how the assays perform clinically (how many truly infected patients are detected by each) as there is no gold standard to which they can be compared. However, we have a limited number of patients (about 500 of the >90,000 tests we've run) that were tested by both assays and the concordance was >95%.

2. **Have you been able to identify and stop any potential issues affecting your processes by using any third party control material during your common routine, which might have affected patient results?**
   a. Dr Theel: I think one of the key advantages of third party controls is their generally long stability, which allows you monitor changes of your assay across reagent or kit lots. The ability to monitor that sort of trending allows you to more quickly identify regent issues that could impact results, versus if you are relying on pooled controls, for example, which you have to make more frequently and potentially QC using affected reagent lots – you wouldn't as readily detect kit-related issues using these controls.
   b. Dr Newton: We have used them primarily with our lab developed quantitative tests and have found them to identify changes in assay controls (degradation) that might have failed runs and caused repeat testing (and potential lack of specimen available for retesting), or could have caused erroneous results to be reported had those changes not been identified

BIO-RAD

3. **So we can use either 3rd party control or pooled patient serum. Which is better? Shouldn't we be testing pooled serum for multiple times to validate them as controls and what are actually the steps for in house control formation?**
    a. Dr Theel: There are a number of advantages of using vended controls, including potentially longer shelf life and stability relative to pooled patient samples. If pooled serum will be used as a control, a mean and acceptable range has to be validated, ideally by testing the pool at least 20 times over separate days to account for as much day-to-day variability in the process as possible.
    b. Dr Newton: It is correct that you can use pooled patient samples, but as you described and as I mentioned in my talk, this requires effort by the lab to identify the correct specimens, manually pool them, then perform validations on the samples to determine their concentrations. This would be followed by dilutions to achieve the desired concentration, and verification testing to insure that concentration was obtained. The value of commercially available (3rd party controls) is that these steps are done for you with material that can be stored/maintained/used for extended periods without much less concern for degradation over time. This allows for used of the controls over extend time periods, multiple lots of reagents, and any possible equipment changes.

4. **Do you have experience using State of the Art from Interlab Comparison Programs to calculate Total Error Allowable and with this limit calculate Sigma Performance for serological tests?**

    Dr Theel: I have not used State of the Art from an interlab comparison program, so unfortunately, cannot comment.

5. **For the molecular assays - do you recommend QC testing at LOD - or at a higher level?**
    Dr Newton: For qualitative assays, positive control material should be near the LoD, approximately 2-3x the LoD, in order to identify any loss of sensitivity, but not so close to the LoD that biological variation inappropriately results in failures. For quantitative assays, the low positive would be designed similarly, with additional points (at least one additional positive within the linear range) as described in the CAP checklist referred to in the presentation.

6. **Is it common for IgM and IgG to show within a short period of time after resulting positive for RT-PCR? What about people with immunocompromise systems?**
    Dr Newton:This depends on when the sample for the RT-PCR was collected relative to days post symptom onset – if the sample for RT-PCR was collected with the first 5 or so days post symptom onset, it is fairly uncommon to see antibodies present, although some patients may be seropositive. If the sample for RT-PCR was collected during week 2 post symptom onset, a larger percentage of the patients would be seropositive for antibodies.

There are few studies looking at seropositivity in immunocompromised patients at this point. In general though, the risk of being seronegative for antibodies to SARS-CoV-2 despite prior infection would be higher in this group, similar to the situation for any other infectious disease in these patients.

7. **What is the advantage/s of having controls in liquid form over lyophilized materials?**

   Dr Theel: One of the key advantages is that there is no risk of reconstituting the lyophilized material incorrectly. It's a small risk, but present none-the-less!

The following questions were asked during the presentation. Please see the webinar recording for the responses.

8. What is the purpose of including genomic human DNA in a SARS-CoV-2 control?

9. As long as my positive QC is positive and negative is negative, why would I worry about shifts or trends for a qualitative test?

10. Is there any guidance available suggesting which targets are preferred for antibody testing? I mean in terms of: Nucleocapsid, S1, S2 or Receptor Binding Domain? If not, do you have any recommendations?

11. What's the best material to evaluate the limit of detection for my method?

12. Some third party QC vendors provide interlaboratory comparison reports for their QC. Are there any benefits from participating in such a program for Sars-CoV-2 antibody testing?