April 9, 2020

Dear Colleagues:

A few recent cases, in which a negative COVID-19 PCR test was followed by a positive result upon retesting of the same patient within a few days, have raised concern among providers about the sensitivity and negative predictive value of the COVID-19 test performed in the URMC laboratory. These examples understandably raise questions about our confidence in testing to rule out COVID-19. A few important facts:

- PCR is the gold standard for diagnosis of virtually every respiratory virus infection
- The viral diagnostic laboratory at URMC is among the very best in the U.S.
- The lower limit of detection for the Roche assay, the main COVID-19 assay performed in our laboratory, is approximately 0.025 – 0.050 copies of viral RNA per microliter, which is extraordinary. Most positive samples have very high viral loads on the order of 10^3 copies per microliter. In other words, if virus is present in the sample, the assay is highly sensitive to detect it.
- The overall performance of COVID-19 testing depends on the degree of virus shedding, the adequacy of the NP swab sample, and the analytical performance of the test in the laboratory. When there are discordant results upon retesting of a symptomatic patient within a short interval, it is highly likely that the initial negative test reflects either 1) an inadequate sample, or 2) sample collection early in the course of infection with very low viral load, or 3) testing on a different instrument platform (a variety of platforms are utilized base on the availability of reagents, but all have excellent detection of virus).
- ***The adequacy of the sample is our greatest concern when we see discordant results***.

Our real question, as providers, is this: *can we be confident in a negative result to rule-out COVID-19?* That is, what is the negative predictive value of the test. Remember: negative predictive value depends on the sensitivity of the test *and the pre-test probability of the disease in the population you are testing, i.e. the prevalence of the disease in the particular patient group.* Pre-test probability makes a big difference in your interpretation of a negative result, as illustrated here:

- A test with 99% sensitivity, performed in a population with 30% prevalence of the disease, will result in 3 false negative tests among 1000 patients tested, and a negative predictive value of 700/703 = 99.6% (i.e. false negative rate = 0.4%).
- The same test in a population with 90% prevalence of disease results in 9 false negatives among 1000 patients tested, a negative predictive value of 100/109 = 91.7 % (i.e. false negative rate ~ 8%).
- If you have a very, very high pre-test probability of the disease in a patient (based on the clinical presentation, prevalence of disease in the community, and possibly taking into account exposure history), you will have less confidence that a negative result rules out disease, even with a highly sensitive test.

The bottom line: no test is perfect -- providers must apply clinical judgement to interpret the result of a negative COVID-19 result, as for every other clinical test, in the context of the pre-test probability of the disease. ***If you have very, very high suspicion for COVID-19 in a particular patient, and the initial test is negative, you can order a repeat test. This is especially true if you are uncertain about the adequacy of the initial NP swab collection. For a repeat sample, make certain that a deep NP swab is collected.***

Our laboratory is reviewing each and every pair of discordant results in our patients, when the samples are collected within 3-4 days of one another, first to ascertain the PCR cycle time (a measure of viral load) in the positive samples, and secondly to reprocess the testing of the existing specimens using the most sensitive Roche test and other

techniques. The lab will make every effort to identify any issues in our processes which can be improved to optimize our test performance. Please call the laboratory with any questions about this process.

Note: Approximately 5% of positive results on the COVID-19 PCR are reported as "Presumptive Positive." These should be interpreted as true positives with a low viral load, either early or late in the course of infection.
"Presumptive Positive" does *not* require repeat testing for confirmation.

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