REGIONAL MEDICAL LABORATORY

COVID-19 Test Myths and Clarifications

Antigen and molecular (PCR, RT-PCR) testing are utilized to detect the presence of virus particles in a patient's respiratory tract. Antibody tests detect an immune response in the patient to the virus, either from infection or vaccination. The current antibody test available at RML only evaluates the presence of IgG antibodies to the viral Nucleocapsid protein, which will only be present in a person that has had prior infection with SARS-CoV2. We are hoping to go live with a second antibody test soon that will detect antibodies to the Spike (S) protein, and this should be present in patients after infection AND after vaccination.

Antigen tests detect the presence of viral proteins, and PCR tests detect the presence of viral RNA, the genome of the virus. Antigen tests are generally much less sensitive than molecular/PCR tests, meaning a much higher number of virus particles must be present for the test to be positive than for the PCR tests.

There are now many antigen tests offered, and most are considered "rapid" tests. They are very useful to screen a patient with symptoms, because, if a patient has symptoms, it is likely they have a sufficient viral load to be detected, but it is only recommended for patients within 5-7 days of symptom onset to have the maximal viral load. They are also useful to screen asymptomatic patients in a repetitive manner, such as for patients who live in close quarters such as nursing homes or dorms since an outbreak can be detected more quickly this way.

All antigen tests require a follow up confirmatory PCR test if they are negative in a patient with symptoms or if they are positive in an asymptomatic patient, because of the risk for false negative or false positive results. In other words... if the test result does not match the clinical impression, confirm with a PCR test.

Molecular or PCR tests on the other hand, are very sensitive and have a very low limit of detection, meaning very few virus particles are necessary to trigger a positive result. In general, however, the more rapid the test, the less sensitive it is, and the more likely it is to have false negative results.

PCR stands for polymerase chain reaction - it is indeed a chain reaction. Each reaction in the chain is a "cycle", and in general, the more cycles that it performs, the more sensitive the test and the lower the limit of detection. Generally, the lower the cycle number when it triggers a positive result, the higher the viral load, and conversely, the higher the cycle number when it becomes positive, the lower the viral load. Every test has a slightly different number of cycles that are required to trigger a positive result, and this is dictated by the manufacturer of the test and their specifications that were submitted to the FDA for emergency use authorization (EUA). Laboratories must follow these specifications in running the test - they are not permitted to increase the cycle number to increase the number of positives, or to decrease the cycle number to decrease the number of positives.

There is a new misconception that PCR tests have high false positive results, where in fact, the test is positive when virus particles are present. A positive result doesn't always have clinical implications or that a person is contagious. For example, patients commonly have a very low viral load at the beginning of infection, before symptoms start, and at the end of infection, after symptoms resolve, and even beyond as the body continues to remove dead virus from the respiratory tract. We do not know the exact number of virus particles that are necessary to make

someone symptomatic or to make that person infectious to another person. Additionally, the current tests do not differentiate between dead or dying virus particles and virus particles that are alive and infectious. This is why the CDC has discontinued the prior recommendation for negative testing before being released from quarantine. It is now based on symptom duration and severity and whether or not the patient is severely immunocompromised.

What about the new variant strains with mutations of the viral genome? Most PCR tests, particularly the “non-rapid” tests, evaluate several of the viral genes, so mutation of one gene will not cause a false negative result. Rapid tests, however, are more likely to query only a small portion of the viral genome or a single gene, and if the area that is being queried is mutated, the test may not detect it. Three rapid molecular tests have been identified by the FDA that may be impacted by the known mutations, but thus far, the impact has not been significant. RML does not perform any of these identified tests that may be impacted. The test manufacturers and the FDA continue to monitor this to be sure the available tests will detect all strains in circulation in our communities.