Determining the infectious potential of individuals with positive RT-PCR SARS-CoV-2 tests

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Efforts to control spread of contagious diseases have historically focused on separating sick (symptomatic) from not sick persons. The logic behind this approach was straightforward: sick people carried infectious potential while those not sick did not or did so less. With the advent of germ theory, the twentieth century witnessed laboratory tests becoming increasingly leveraged to provide additional insight into the dynamics of infectious diseases and tracking of epidemics through the culturing of live organisms from potentially infectious individuals.

Today, multiple new forms of testing other than culture are in widespread use. One particular type of test for SARS-CoV-2, the now ubiquitous RT-PCR, is its presumed ability to identify current SARS-CoV-2 infection (i.e. the presence of pathogenic organisms with a host response). Testing is seen as essential to identify people who are infected but not showing symptoms at the time of testing, and amongst those who are symptomatic, to identify Covid-19 patients from those with other etiologies (e.g. RSV, rhinoviruses, influenza viruses, adenoviruses, parainfluenza viruses, etc.).

Positive test results are interpreted as indicating active infection with replicating virus (and therefore the identification of infectious individuals who have potential to transmit that live virus to others), while negative results are used to rule this out. So long as tests are used in ways to reduce false negatives and false positives, the role of RT-PCR tests has seemed essential to mitigating epidemic spread of SARS-CoV-2.

Any test is useful only when used in the right persons at the right time and with information on how to interpret results in that context. Yet clinicians have always encountered challenges with using tests properly. The Institute of Medicine found that inappropriate use of diagnostics tests could cause patient harm from unneeded interventions and was a major driver of increased health care costs.[1]

In this issue of *Clinical Infectious Diseases*, Jefferson et al. present a systematic review providing information on interpreting RT-PCR results in relation to viral cultures. Their report highlights the necessity to understand RT-PCR in the context of the distinct concepts of infection versus infectiousness, and viable viruses versus non-infectious RNA fragments. In their systematic review of studies, all case series in which investigators evaluated RT-PCR positive results and compared to viral culture, Jefferson et al. found many specimens, despite being PCR-positive, failed to grow SARS-CoV-2 in culture.

Culture medium presents ideal conditions for a virus to grow and may detect virus that is not present in the quantities required to initiate infection in a human host.[2] Logically, if a specimen is culture negative, one might conclude it does not contain viable virus, which might indicate decreased infectious potential to others. On the other hand, the presence of genetic material

detected in RT-PCR might be able to reflect live virus below the level of detection of culture – or it may represent a false positive test (i.e. no viral RNA[3]) or non-infectious viral RNA from a SARS-CoV-2 infection of the recent past.

The existence of people who are PCR-positive for SARS-CoV-2 yet not infectious disrupts the alluring logic of simple interpretations of test results. In the early days of the pandemic, there were explanations everywhere reminding us that there were two types of tests: those testing for "active infection" (with molecular tests like RT-PCR and rapid, antigen tests fitting this category) and those used for diagnosing "past infection" (serological tests for antibodies the body produces with time).[4] This view is widespread. Yet the studies Jefferson et al. review suggest the facts are more complicated. As they write, PCR tests for the presence of specific RNA sequences that reflect SARS-CoV-2; it does not test for whole viruses, and indeed cannot distinguish between live virus (capable of causing infection in a susceptible host) and RNA fragments with no infectious potential.

Yet this does not mean the test is worthless. Indeed, across the studies, Jefferson et al. identify repeating patterns with respect to what patient and test characteristics relate to potential for culturable (i.e. theoretically infectious) virus. First, generally the longer after symptom onset a specimen was taken, the less likely it was to contain viable virus. In particular, Jefferson et al. noted that infectious potential declined after day 8 (following symptom onset), "even among cases with ongoing high viral loads." Specimens were also less likely to culture from patients with milder symptoms versus more severe symptoms. This is consistent with the interpretations of other authors who reviewed some of the same studies.[5]

An inverse relationship was also observed between ability to grow virus in culture and cycle threshold, a finding that deserves careful unpicking. RT-PCR tests, noted for their ability to detect genetic target sequences even when only present in miniscule amounts, is enabled by the core feature of the technology, which amplifies genetic target sequences through repeated "cycles." There is an inverse relationship between cycle threshold (Ct) and amount of genetic material in the specimen: the fewer the number of cycles needed to detect genetic material, the greater the amount of genetic material present in the specimen. The findings from Jefferson et al. therefore indicate that cycle threshold--a value that may not be reported to clinicians or patients--could have utility in determining infectious potential of the individual, a point that has been previously made in this journal.[6]

Important questions remain and will need future research to answer. The question is not whether people who are PCR-positive yet culture negative ever transmit virus but how often this occurs and in whom? How "early" is early infection? How "late" is so late one need not worry about transmission? What environmental characteristics (indoor vs outdoor) impact transmission even when tests are positive. Do "asymptomatic" people really have no symptoms or just fewer symptoms missed because we do not evaluate symptoms systematically? Even more importantly

what are the consequences for themselves and for others? There is still far more to learn about the context of testing, whom to test and what it all means.

There is a larger important takeaway from this story: we need to become more careful with the language we use. RT-PCR tests do not detect the virus, they detect the presence of known genetic sequences from which inferences are drawn. And the common phrase "viral shedding" does not measure how much virus (or even non-infectious RNA fragments) is being actively dispersed by a person. The phrase that only indicates PCR-positivity. We have allowed seemingly benign short-hands, like "detecting the virus," to obscure what is actually being measured, leading to potentially erroneous conclusions with serious consequences: quarantining non-infectious persons and its attendant aspects on other parts of people's lives and health.

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