2-2-2023

Technology in Action: Multiplex PCR in the Tripledemic

Aditya Loganathan
George Washington University

Ishan S. Abdullah
George Washington University

Andrew C. Meltzer
George Washington University

Technology in Action: Multiplex PCR in the Tripledemic

Aditya Loganathan, Ishan S. Abdullah, Andrew C. Meltzer, MD

02/02/2023

This winter's "tripledemic" surge of RSV, the flu, and COVID-19 has strained healthcare systems nationally. COVID-19 national hospitalization rates have sharply risen to a daily average of 46,900 new cases, the highest since last March, while RSV and influenza hospitalizations remain high relative to previous years. Dr. Christopher J. Gill of Boston University associates the increased susceptibility partly to the PHSMs (public health and social measures) in place during the pandemic, which decreased the public's exposure to these viruses. Healthcare professionals recommend wearing masks, getting vaccinated, and testing regularly. While testing kits for COVID-19 are readily available, methods to quickly and accurately screen for influenza and RSV are not as easily accessible.

The current standard for COVID testing is singleplex polymerase chain reaction (PCR), which amplifies small amounts of viral material with a single primer to detect only SARS-CoV-2, the causative pathogen in COVID-19 cases. The drawback with conventional PCR is that it often requires the patient specimen to be sent to a specialized facility, and results can take up to 3 days, preventing them from playing an immediate role in clinical decisions. Alternatively, rapid antigen swabs are available for at-home use and provide results in about 15 minutes. However, these are considered less sensitive and require a higher viral load for a positive result. Additionally, both of these testing options can only detect the presence of one pathogen, which can lead providers to inefficient downstream testing, incurring greater costs for the patient while wasting valuable time. Adding to this flaw, researchers found that over 20% of

patients with COVID-19 were co-infected with another respiratory pathogen, making it more vital than ever that providers have an effective way of simultaneously screening for multiple pathogens.

A solution to this testing challenge is Multiplex PCR (mPCR), a technique that simultaneously uses multiple primers to assay for many distinct pathogens. One example of mPCR is Biomerieux's BioFire respiratory screening panel (RP2.1) which provides results with speeds comparable to rapid antigen swabs while maintaining the specificity of conventional PCR. This BioFire panel is the first FDA De Novo authorized for detecting COVID-19. The RP2.1 FilmArray uses nasopharyngeal swab samples to simultaneously detects 22 respiratory pathogens, including influenza virus types A and B (with influenza A subtyping), adenovirus, coronaviruses HKU1, NL63, 229E and OC43, human metapneumovirus, human rhinovirus/enterovirus, parainfluenza virus types 1–4 and RSV and three bacteria: Mycoplasma pneumoniae, Chlamydia pneumoniae, and Bordetella pertussis. As a CLIA-waived device, clinical staff can be trained to use the BioFire system. When discussing the value of the RP2.1, Dr. Tufik Assad, a pulmonary and critical care physician at Williamson Medical Center, says, "...It gives us actionable information right away."

In terms of accuracy, mPCRs have an overall 97.1% sensitivity and 99.3% specificity, which shows it is more accurate than singleplex PCR, which displays 86.1% sensitivity and 95.8% specificity. For a benchmark comparison, the FDA states that most at-home antigen tests can positively detect the virus in at least 80% of people with SARS-CoV-2. In addition to accuracy, the RP2.1's panel demonstrates a 45-minute test-to-result time which is more comparable to the 15-minute rapid antigen testing rather than longer conventional PCR tests. Due to this short time for results, the BioFire panel can be used onsite to provide patients with

results before they leave the clinic. The demand for faster test-to-result is showcased in a study by The Advisory Board, which stated that 77% of patients preferred onsite lab services and that 67% of patients were willing to drive up to 20 minutes to go to a clinic with onsite lab services.

Studies suggest that mPCR may lower overall costs. Rapid mPCR has been shown to reduce the length of stay (LOS) for patients in the ICU from 9.2 to 6.2 days ($P < 0.0001$). In a time of boarding and hospital overcrowding, a shorter LOS for those hospitalized during the "tripledemic" increases patient flow, therefore improving hospital productivity and patient satisfaction. A study by the American College of Emergency Physicians (ACEP) demonstrated that by reducing the average ED boarding time to under 1 hour, an additional $13,298 in hospital revenue could be captured each day from unprofitable events such as patients leaving the ED without being seen and ambulance patients being diverted. A study of infantile patients at the Children's Hospital of Michigan found that the average LOS decreased from 10.4 to 5.7 days with a negative respiratory pathogen panel assay (RPP) versus a positive RPP ($P = 0.017$).

Other studies have shown that rapid mPCR may reduce the average number of days of antibiotic treatment from 3.2 to 2.7 days ($P = 0.003$). When providers are uncertain of the course of treatment, it is common for unnecessary antibiotic prescriptions to occur. Since antibiotics are an ineffective treatment for these illnesses, supply is stretched thin during surges. Understanding the implications of prescribing unnecessary antibiotics towards developing antibiotic resistance is important. With quick identification of the pathogens in an infected patient, there is a lower chance that patients will be overprescribed antibiotics and a faster turnaround time to optimal treatment and thus discharge from the hospital. In the same study performed at the Children's Hospital of Michigan, researchers also found that the duration of antimicrobial consumption was

significantly decreased from 5.2 days to 2.8 in positive RPP patients versus negative RPP patients ($P < 0.001$).

While mPCR panels are often more costly than conventional diagnostic tests, the increased upfront costs may be justified if they are balanced by a decrease in downstream testing or a reduction in LOS. In a cost analysis study of multiple diagnostic means for respiratory viruses, it was found that mPCR was the least costly strategy and was suggested to generate significant savings for hospitals. In times of infectious disease surges, such as recently with the tripledemic, hospitals may become saturated with sick patients. The mPCR innovation allows providers to quickly make informed decisions about optimal treatment plans, which may lead to a smaller backlog of patients waiting to be seen during times of limited healthcare resources. As mPCR techniques continue to improve, they have the potential to transform the future of infectious disease management.

Dr. Andrew C. Meltzer is grant funded by Biomerieux, manufacturer of RP-EZ multiplex PCR. Authors have no other conflicts to report.