The Advent of Box PCR Testing

How rapid PCR testing affects the medical laboratory

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Krystle Pardell prepares a sample for processing at the Kelowna General Hospital Laboratory. Photo courtesy Allen Lee.

In 2003, about 490,000 people[1] attended an outdoor rock concert in Toronto to hear The Rolling Stones, AC/DC and other famous bands. Nicknamed "SARS Stock," the event celebrated the end of the severe acute respiratory syndrome 1 (SARS-CoV-1) outbreak.[2] But Grant Johnson, MLT, team leader of microbiology at The Hospital for Sick Children at the time, was not at the concert.

As a key member of Toronto's SARS response team, he was testing patient samples that evening to confirm the outbreak was over. Johnson says that during the outbreak, only a few Toronto hospital laboratories had the expertise to perform polymerase chain reaction (PCR) testing, and they had to develop their own "home-brew" assays.

By contrast, the recent pandemic caused by SARS-CoV-2 created an immediate demand for rapid PCR tests to accommodate unprecedented volumes. Accordingly, Health Canada approved several in early 2020.[3] CJMLS spoke to Johnson and laboratory professionals at another Canadian hospital to learn how incorporating Cepheid's GeneXpert, a popular rapid PCR box testing platform, has affected the work of laboratory professionals and patient care and how they managed issues that arose with it during the COVID-19 pandemic.

GeneXpert Box Testing Platform

The Cepheid GeneXpert platform is a molecular diagnostic PCR testing system that automates sample extraction, amplification and detection of infectious diseases using patented, single-use cartridges. Available in various sizes ranging from two to 16 modules, the GeneXpert platform can process tests for different indications simultaneously, making "on-demand molecular testing available to everyone with unprecedented speed and ease of use," according to the company's marketing materials.[3] Preparation time is about five to seven minutes, and results are available within about 50 minutes.[4] For example, the Cepheid GeneXpert Xpert Xpress SARS-CoV-2 assay targets two genome regions on the virus, the envelope and the nucleocapsid. It returns a cycle threshold (Ct) value if the target is detected within 45 amplification cycles.[4]

Improving Speed and Productivity

Kelowna General Hospital (KGH), part of Interior Health in British Columbia, is a referral hospital that performs most of the PCR testing for hospitals in the Okanagan Valley. At the beginning of the COVID-19 pandemic, they used a Seegene STARlet to process batches of 93 samples at a time and obtained results in about six hours. It had the flexibility to run different panels but was very labour intensive. To meet growing volumes for COVID testing, they quickly expanded to a total of five platforms from various manufacturers, including another STARlet. "We had staff using every instrument, all the time," says Chandra Hauer, MLT, technical lead of PCR at KGH. "We were

Rommel Castro (left), Chandra Hauer and Krystle Pardell (right) at the Kelowna General Hospital Laboratory. Photo courtesy Allen Lee.

already using GeneXpert for MRSA, flu and *Clostridioides difficile* testing. When Cepheid introduced a COVID cartridge, GeneXpert became our stat instrument for expedited requests for COVID tests."

Angela Knight, MLT, operations supervisor of microbiology and PCR at KGH, says that while their 16-module GeneXpert provides fast turnaround with minimal labour, it is not a point-of-care test. "It's not entirely plug-and-play. We still need to make sure the curvatures are good, check controls to make sure they pass, and enter Ct values and ensure they are within acceptable ranges," she says. "There is also expertise required to understand complexities, such as interferences and what happens when late amplifications occur."

Johnson, who has been clinical director of laboratory medicine and infection prevention and control at Lakeridge Health in Durham Region, Ontario, since 2010, says they too had already been using GeneXpert for influenza and C. difficile testing, so it was an easy decision to use it for COVID testing. They added eight more modules to their original 16 for a total of 24, and have been processing COVID-19 tests in Oshawa for all five hospitals in the network, loading them in as single tests or mini-batches as specimens arrive.

GeneXpert's ease of use has allowed Johnson to reallocate human resources without needing to hire additional staff, a crucial consideration given the current shortage of medical laboratory technologists (MLTs). "We had both microbiology and cytogenetics technologists set up samples on GeneXpert so that MLTs could also focus on interpretation and microbiology. In the last year, we've also had laboratory assistants run samples," he says. "We set up automatic verification, so MLTs only review results for the relatively smaller percentage of positive samples."

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Improving Patient Care

Rapid PCR testing has been essential for managing patient care during the COVID-19 pandemic. Test results for individual patients confirmed whether they needed to isolate to prevent infecting others or if it was safe for them to visit loved ones, such as those in long-term care facilities with a higher risk of severe disease and mortality from SARS-CoV-2 infection. "Patients deserve to know as soon as possible if they test positive or negative for an infectious disease," says Kendra Soukeroff, MLT, professional practice leader, laboratory at KGH. "Labs should embrace new technologies that provide faster turnaround times than traditional methods. Those that do not could cause harm to patients because they're delaying diagnoses."

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Hospitals also depend on rapid infectious disease results for managing patient care. "GeneXpert continues to be invaluable for our traditional microbiology testing, such as for methicillin-resistant *Staphylococcus aureus* infections, allowing us to turn around results in about an hour so doctors can prescribe the correct antibiotic," says Hauer. "For COVID-19 testing, we received tons of requests on expedited turnaround, and GeneXpert became our stat instrument. Physicians needed to decide quickly whether patients needed to be isolated in the COVID ward or could go to other areas of the hospital."

Johnson concurs: "Throughout the pandemic, we've depended on rapid turnaround time for managing bed capacity and determining optimal patient flow. For example, on some days in July 2022, we were running more than 80 tests on GeneXpert." He adds that rapid testing was also critical for laboratory staff. "Fast results mattered for patients, but also for staff who were sick," says Johnson. "Rapid testing cleared them to return to work sooner, so we could keep processing patients' tests in a timely manner."

The Only Constant is Change

Johnson says that the GeneXpert SARS-CoV-2 assay was highly sensitive in the early days. "The test was returning positives for Ct values over 40. Some other hospitals decided not to call anything over 38 or said the results were indeterminate and recommended repeating the test. From our perspective, a positive was a positive," says Johnson. "We worked through the algorithms with our microbiologist, infectious disease physician and public health to ensure we were aligned on interpretation. Our solution was to report all positives and provide Ct values to clinicians so they could determine if the results were clinically relevant or if they wanted to repeat the test."

Laboratories at KGH and Lakeridge Health have experienced a few equipment malfunctions with GeneXpert. For example, a few modules stopped working, but they were still able to run tests on unaffected modules while waiting for the vendor to fix the issue. Lakeridge Health noticed a rise in errors with a particular lot of cartridges, a bit higher than the usual one per cent, and worked with the vendor's technical support team to resolve the problem. "It's essential to monitor what happens with rapid testing solutions and not assume they're always perfect," says Johnson. "Even though GeneXpert is easy to use, laboratories still need to monitor what they're doing, do the analysis and contact the vendor when problems arise."

Grant Johnson in front of the GeneXpert machine at Lakeland Health, Oshawa, Ontario. Photo courtesy Grant Johnson.

As Cepheid continues to innovate and introduce new assays, each one must be validated before it can be incorporated into the laboratory. For example, in 2021, Cepheid expanded the XpertFlu/Respiratory Syncytial Virus (RSV) assay to include targets for SARS-CoV-2, expecting the newer multiplex test, Xpert SARS-CoV-2/Flu/RSV, would replace the separate Xpert SARS-CoV-2 and Xpert Flu/RSV assays in wide use across North America.[3] Johnson and his team at Lakeridge Health collaborated with the National Microbiology Laboratory (NML) to validate the new multiplex assay and found it had a high agreement with the Xpert SARS-CoV-2 and the Xpert Flu/RSV tests, as well as the BioFire FilmArray RP2.1, another rapid PCR testing platform.[3] "We process a lot of GeneXpert tests here," says Johnson. "The NML prepared a smaller validation set from remote sites in Northern Ontario. The collaboration allowed the smaller labs to complete their validation." KGH and Lakeridge Health have both been using the combination Xpert SARS-CoV-2/Flu/RSV assay since February 2021.

PCR testing technology has come a long way since the SARS outbreak in 2003 and will continue to evolve as manufacturers introduce new products in various combinations. As funding and budgets become more challenging, it's essential to consider the costs of new technologies to determine the best solution for your organization.

"The days of collecting specimens at a rock concert and processing them with lab-developed tests are long past," Johnson says. "Today, our focus remains on validating and incorporating promising new technologies as they become available and staying flexible as needs change." J

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