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Rapid sequencing workflows deliver comprehensive respiratory pathogens results, faster

By Anna Maria Niewiadomska

Respiratory pathogen testing is always a challenge, from the notoriously unpredictable demand to a rapidly evolving reimbursement model surrounding a broad range of testing options. Each year, clinical laboratories must make their best guesses about testing needs for the upcoming flu and cold season and decide how much of each test to stock.

To complicate matters further, pathogen testing is also an urgent challenge, as results must be reported very quickly. For generally healthy patients, rapid results provide valuable information that can help them take reasonable precautions to avoid transmitting the microbe to others while doing what's appropriate for their own recovery. For critically ill patients in the hospital, rapid results have an even bigger impact. Some of these patients may be given broad-spectrum antibiotics as a preventive measure, and getting a clinical lab report of a positive viral pathogen test allows physicians to stop the unnecessary treatment sooner — ideally before the patient's natural microbiota have had a chance to develop resistance to the therapy. Without rapid results, patients might be treated with inappropriate antibiotics for days, increasing the risk of contributing to the growing crisis of antimicrobial resistance. Many clinical labs have adopted molecular viral tests. These are generally part of targeted panels (typically covering influenza A/B, SARS-CoV-2 and respiratory syncytial virus) or of much broader syndromic panels that cover all common respiratory infection culprits. Molecular tests have the great advantage of speed, often delivering results in a matter of hours. But they can only look for known targets, making it harder to find rare or unexpected pathogens. With targeted panels, it is also more difficult to spot cases of co-infections.

Advanced DNA sequencing technologies may overcome these challenges. While some platforms require workflows that can take multiple days, others have been demonstrated in rapid workflows that can return results within a single day. A sequencing-based approach also allows for an unbiased view, enabling discovery of co-infections and

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LEARNING OBJECTIVES

Upon completion of this article, the reader will be able to:

1. List the challenges identified in seasonal respiratory pathogen testing.
2. List the major advantages and disadvantages to molecular-based respiratory pathogen testing.
3. Discuss findings and outcomes in nanopore-based molecular testing.
4. Discuss findings and outcomes in metagenomic workflow techniques.

unexpected or unculturable pathogens. In some cases, sequencing data can be used to evaluate genotypic markers of antimicrobial resistance, making it a useful technique for ensuring appropriate treatment for patients. The following sections take a deeper dive into these latest developments in sequencing-based pathogen testing.

Rapid results

Respiratory pathogen results offer the most value in supporting needed interventions, from isolation protocols to treatment selection, when they are delivered quickly — ideally within a single day. Clinical teams have reported success with this tight time frame using a nanopore sequencing-based approach.

At Guy's & St. Thomas' NHS Foundation Trust in London, researchers developed a whole-genome analysis pipeline for influenza A virus that allowed them to report results with a 24-hour turnaround time.[1] In one study, they analyzed 128 nasopharyngeal samples known to be positive for influenza A. The samples had been collected from patients who were admitted to the hospital or who visited the emergency department during the winter of 2022–2023. In 75% of samples, all segments of the flu genome were accurately captured through the sequencing workflow, and the clinically important hemagglutinin gene was fully recovered in more than 90% of samples.

While a major goal of the study was achieved in establishing the feasibility of a 24-hour turnaround time for whole genome sequencing of influenza, the data generated also made it possible to track clusters of infections by phylogeny. Among 19 patients previously reported to have hospital-acquired infections, results showed two separate infection clusters in a single hospital ward and refuted the idea that transmission had occurred across wards. The identification of nosocomial transmission events can lead to the implementation of pharmaceutical and non-pharmaceutical interventions to prevent further spread of infections.

In Bangladesh, scientists at the International Centre for Diarrhoeal Disease Research developed a culture-free, amplicon sequencing workflow to perform whole genome sequencing of influenza A and report all flu subtypes from a clinical sample.[2] In 19 samples studied, the workflow led to 100% coverage of all genome segments and took only 24 hours to complete. The team noted that this method, based on a portable sequencing platform, is "ideal for resource-limited clinical settings, aiding in real-time surveillance, outbreak investigation, and the detection of emerging viruses and genetic reassortment events." Accessible and affordable sequencing platforms also help increase the number of genomic sequences from low- and middle-income countries, which can bias vaccine strain selection as they are often underrepresented in genomic sequence databases.

Subsequent studies have demonstrated that nanopore sequencing technology can be used to report results even faster. Members of the same team at Guy's & St. Thomas' NHS Foundation Trust deployed a metagenomic workflow that enabled same-day reporting of results for nearly all samples tested, with the median turnaround time clocking in at 6.7.[3]

Of course, getting results quickly is not helpful if they aren't accurate. Based on a broad number of clinical research studies, a team of reviewers noted that nanopore-based sequencing methods "have been shown to be as accurate as PCR-based methods with regard to common viral [respiratory tract infections], with the crucial advantage of generating real-time data whilst also being more portable and requiring less laboratory infrastructure."[4]

Metagenomic sequencing

The metagenomic study mentioned above was designed to enable broad pathogen detection, rather than a singular focus on influenza. The idea was to use a metagenomic technique — the same concept used for assessing communities of microbes living in soil or on plants — to identify all microbes found in a clinical sample. With appropriate filtering based on population thresholds, scientists reasoned that it should be possible to quickly eliminate harmless members of the patient's natural microbiome and home in on the pathogen or pathogens responsible for the respiratory infection.

In a pilot study, researchers analyzed 128 samples collected from 87 patients who were believed to have lower respiratory tract infections.[3] All samples were evaluated with the sequencing-based respiratory metagenomic workflow. Compared to routine testing, the technique was found to have sensitivity of 93% and specificity of 81% for detecting clinically relevant pathogens. With same-day reporting of results for almost all samples, the study had a significant impact on patient care. In nearly half of cases, the metagenomic results led to changes in treatment selection, with 26% de-escalated and 22% of cases being escalated; the latter was based on species identification or detection of genes indicating acquired resistance to certain treatments. Interestingly, the workflow enabled discovery of unexpected or fastidious organisms in 21 samples. These included a dozen anaerobes as well as *Mycobacterium tuberculosis*, cytomegalovirus, *Tropheryma whipplei*, and *Legionella pneumophila*, among others.

Building on this pilot project, the UK government recently allocated £3 million to expand access to the respiratory metagenomic approach in intensive care units across many British hospitals.[5]

Antimicrobial resistance testing

Genomic analysis of microbes — whether through whole genome sequencing or metagenomic workflows — also enables the detection of genetic mechanisms associated with resistance to certain classes of therapy.

In one example, researchers associated with the Oxford University Hospitals National Health Service (NHS) Foundation Trust used metagenomic analysis to investigate 180 respiratory samples collected during respiratory illness season, comparing a novel sequencing-based workflow to standard molecular diagnostic tools.[6] In all H1N1 and H3N2 subtypes analyzed, the team found a known mutation in the M2 protein that confers some level of resistance to the antiviral amantadine therapy. One H3N2 genome encoded a worrisome mutation associated with resistance to oseltamivir, also known as Tamiflu.

The same approach has been validated for respiratory pathogens beyond influenza. In a study of lower respiratory tract infections caused by bacteria, researchers at the University of California, San Francisco, turned to sequencing-based metagenomics to detect pathogens and identify genes associated with antimicrobial resistance.[7] As part of the study, they evaluated the combination of Cas9 enrichment with nanopore sequencing in samples from 10 patients suffering from acute respiratory failure. Compared to standard short-read sequencing workflows, the Cas9-plus-nanopore method made it possible to enrich for antimicrobial resistance genes by more than 2,500-fold using a technique known as FLASH (short for finding low abundance sequences by hybridization).

These target genes "could be identified within 10 [minutes] of real-time nanopore sequencing, suggesting a potential turnaround time of less than 6 [hours] for a single sample," the team reported. That's substantially less time than clinical antibiotic susceptibility testing, which takes more than 72 hours, and also less than standard short-read sequencing workflows, which take at least 24 hours, according to the researchers.

Surveillance and epidemiology

Going beyond individual patient results, sequencing-based analysis can also support broader public health goals such as population-scale influenza surveillance. Clinical research teams have already demonstrated the effectiveness of this approach.

At the National Institute of Health in Pakistan, scientists carried out a national flu surveillance program.[8] They used nanopore-based sequencing to study influenza A viruses circulating in the population from January 2020 to January 2023. Based on the whole genome sequences they generated for 126 virus isolates, they identified several amino acid changes in the hemagglutinin genes and in other gene segments of an H1N1 subtype and an H3N2 subtype circulating at this time. This validation study allowed them to prepare for real-time monitoring of flu viruses to support epidemiological analysis and public health responses.

Researchers across the UK used a similar approach to conduct flu surveillance in the winter of 2022-2023; this included targeted surveillance at a hospital in Oxfordshire as well as a UK-wide household study.[9] For the hospital arm of the study, the team sequenced 292 residual materials left over after molecular diagnostics had been performed on nasal or throat swab samples, with positive results for influenza A. The hemagglutinin segment was fully sequenced in 69% of samples, and that data was used as the foundation for phylogenetic comparisons. Researchers focused particularly on 88 samples flagged as infections that may have been acquired within the hospital, analyzing ward-based clusters and other potential transmission occurrences.

For the broader UK study, the scientists analyzed 130 samples that had previously tested positive for influenza A (a subset of nearly 15,000 swabs randomly selected from participants in the national household study). The flu-positive samples came from people in England, Scotland, Northern Ireland, and Wales. They were analyzed by PCR, and for 113 samples with enough material, also with sequencing. Again, they ran phylogenetic analysis to track potential chains of transmission. As expected, the circulating flu strains were very similar at the genomic level, both within the hospital and across the UK.

"While we show good concordance between circulating and vaccine strains for A/H3N2, A/H1N1, and B/Victoria and a low frequency of anti-viral resistance, our approach could also be used to provide surveillance in future seasons where this might not be the case," the UK team reported in a paper describing the study.[9]

Surveillance efforts could be even more useful when expanded to other sample types, as demonstrated in an air sampling study from scientists in Wisconsin and Minnesota. They deployed a metagenomic approach with nanopore-based sequencing for air samples collected in community settings between July 2021 and December 2022.[10] This supported the goal of routine pathogen monitoring, allowing the team to detect human respiratory viruses including influenza A and C, SARS-CoV-2, rhinovirus, and more. The approach is based on sequence-independent single-primer amplification designed to capture common and rare RNA viruses. Of the 22 samples analyzed, 19 were positive for human viruses. This unbiased method allows for non-invasive community surveillance, enhancing our ability to monitor and respond to public health threats.

Effective, comprehensive flu testing

As these studies illustrate, the use of a rapid, long-read sequencing platform can help clinical laboratory teams achieve their flu testing goals. This approach can deliver whole genome or metagenomic sequencing data in less than a day, enabling same-day reporting of results to guide clinical interventions. Genetic resistance mechanisms can also be reported, further honing treatment selection and helping to avoid the inappropriate use of antimicrobial therapies. At the institutional or population level, sequencing-based flu testing supports influenza surveillance programs as well, and at a global level, having this genomic data available has far-reaching implications such as early outbreak detection of novel pathogens, or future vaccine strain selection.

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Anna Maria Niewiadomska serves as Global Public Health Segment Manager at **Oxford Nanopore Technologies**.