Advances in Rapid Testing for Bacterial, Viral, and Parasitic Infections

Written by Brian Hoyle

The traditional culture-based detection of bacteria has been bolstered by microscopy-, molecular, and antigen-based detection techniques, according to Arjana T. Andrasevic, MD, University Hospital for Infectious Diseases, Zagreb, Croatia.

Automated point-of-care testing of swab or blood samples relies on detection of pathogen-specific antigens and sequences of genetic material. Results are available within hours and can guide treatment [Little P et al. *BMJ*. 2013; Maltezou HC et al. *J Antimicrob Chemother*. 2008]. Such testing could conceivably expand to locales including local pharmacies.

Polymerase chain reaction (PCR)–based rapid diagnostics can detect bacterial and viral respiratory infection, which can progress rapidly and which remains one of the leading causes of morbidity and mortality worldwide (Table 1).

PCR testing is widely used for the diagnosis of tuberculosis, gastrointestinal infections, urogenital infections, and bacteremia. Detection is sensitive: it identifies the causal pathogen with a fidelity approaching 100%. The speed and accuracy of PCR testing have been exploited in hospital infection control programs that aim to minimize the spread of bacterial pathogens.

Other rapid diagnostic techniques, such as fluorescence in situ hybridization and matrix-assisted laser desorption ionization–time of flight (MALDI-TOF), remain more in the research realm. Whole genome sequencing holds the potential of detecting resistance-associated genes and genetic determinants of infection. Further refinements may allow sequence-based point-of-care testing.

Rapid diagnostics have potential value in the diagnosis of malaria and other parasitic infections, according to Sanjeev Krishna, ScD, St George's University, London, United Kingdom. Malaria causes 500 million infections resulting in >1 million deaths each year. Microscopy identification is ponderous and time-consuming. DNA-based speciation of *Plasmodium* is rudimentary. Refinements in PCR identification and development of rapid, accurate, and portable diagnostic tests are needed.

Several resistance-associated malaria gene mutations have been identified, and a prototype apparatus capable of detecting these mutations has been developed. Efforts to economize testing are ongoing, with the goal of real-time surveillance at sites of malaria outbreaks and in the screening of travelers returning from malaria-endemic regions.

As described by Jesse Papenburg, MD, MSc, Montreal Children's Hospital, Montréal, Quebec, Canada, accurate automated viral diagnostic testing that is easier to do and faster than the traditional approaches is under development or already in place, particularly for acute infections caused by respiratory syncytial virus (RSV) and influenza virus.

The goal is to have test results available during the clinician's examination of the patient. The benefits of rapid diagnosis include delivery of care during hospitalization, patient management following discharge, and the availability of testing at home and in the community [Pai NP et al. *PLoS Med*. 2012; Staub LP et al. *Int J Health Tech Assess*. 2012].

The majority of respiratory infections in hospitalized children are viral [Papenburg J et al. *J Infect Dis*. 2012]. Being able to rapidly identify these infections lessens the inappropriate and futile use of antibiotics and decreases ancillary testing. Rapid diagnosis helps guide antiviral therapy for influenza and improves infection control.

Rapid antigen detection tests (RADTs) for RSV are widely used in clinical laboratories and are licensed for point-of-care use. With this technique, RSV antibody specifically binds to surface-immobilized antigen and is visualized [Prendergast C, Papenburg J. *Future Microbiol*. 2013]. The test is not perfect; an as-yet-unpublished meta-analysis of RSV RADT evaluations revealed a low sensitivity in adults (29%; 95% CI, 11% to 48%). False-negative results are more

Peer-Reviewed Highlights From the

25th European Congress of Clinical Microbiology and Infectious Diseases

April 25–28, 2015
Copenhagen, Denmark

Table 1. Examples of Rapid Diagnostics for Respiratory Infections

Assay	Sample	Technology	Pathogens	Turnaround Time, h
FilmArray Respiratory Panel	Nasopharyngeal swab	Real-time PCR	*B pertussis*, *Ch pneumoniae*, *M pneumoniae* + 17 viruses	1
Anyplex II RB5/Seeplex PneumoBacter ACE	Nasopharyngeal swab/ aspirate, BAL, sputum	Real-time PCR with DPO and TOCE technology	*M pneumoniae*, *Ch pneumoniae*, *L pneumophila*, *B pertussis*, *B parapertussis*, *S pneumoniae*, *H influenzae*	3-4
Unyvero Pneumonia P50 test[a]	Sputum, BAL, protected brush, tracheal aspirate	PCR + array	16 bacteria + *P jirovecii*	4

B, Bordetella; BAL, bronchioalveolar lavage; *Ch, Chlamydophila*; *H, Haemophilus; L, Legionella; M, Mycoplasma; P, Pneumocystis*; PCR, polymerase chain reaction; *S, Streptococcus.*

[a]Resistance genes, n = 22.

Reproduced with permission from AT Andrasevic, MD.

Table 2. One-Step, Rapid, Commercially Developed Nucleic Acid Amplification Tests

Assay	Company	Targets	Amplification/ Detection	TAT, min	Access (Run Size)
Xpert Flu, Xpert Flu/RSV[a]	Cepheid	Flu A, flu B ± RSV	PCR/RTF	~90	Random, continuous (1-80)
BioFire FilmArray RP[b]	BioMerieux	17 viruses, 4 bacteria	PCR/EPF	60-90	Random (1/cycler)
ePlex Respiratory Pathogen Panel (RP)[c]	Genmark	22 viruses, 4 bacteria	PCR/RTF	60-90	Random, continuous (6/tower)
Cobas Liat Influenza A/B	Roche	Flu A, flu B	PCR/RTF	25	Random (1)
Simplexa Flu A/B and RSV Direct	Focus Diagnostics	Flu A, flu B, RSV	PCR/RTF	60-70	Not random (1-8/disc)
Alere Influenza A and B[d]	Alere	Flu A, flu B	Isothermal (nicking endonuclease)	< 15	Random (1)

ETF, end point fluorescence; PCR, polymerase chain reaction; RSV, respiratory syncytial virus; RTF, real-time fluorescence; TAT, turnaround time.

[a]FDA cleared in 2011 and 2014.

[b]FDA cleared in 2011.

[c]Not yet available.

[d]Clinical Laboratory Improvement Amendments waived.

Adapted with permission from J Papenburg, MD, MSc.

prevalent in older hospitalized children, those with symptom duration exceeding 5 days, and those infected with the RSV-B genotype [Papenburg J et al. *J Pediatr*. 2013]. RADTs for influenza have room for improvement, with a reported overall sensitivity of 62.3% (95% CI, 57.9% to 66.6%), with even lower sensitivity in adults and those infected with influenza B, A/H1N1/2009, H3N2v, H7N9, and H5N1 [Chartrand C et al. *Ann Intern Med*. 2012]. For both viral respiratory infections, negative RADT results are not reliable. Follow-up PCR testing may be indicated, which delays diagnosis and adds to the health care cost.

New-generation influenza RADTs that detect viral nucleoprotein have been developed. They feature improved detection sensitivity, high specificity, and an analytic time of about 15 minutes.

Nucleic acid amplification tests feature sensitivities and specificities > 90% and > 97%, respectively. Some may be almost as rapid and as easy to do as RADTs and, because of their closed system design, lessen the risk of contamination. Commercial tests capable of detecting the target virus in a 1-step process are on the clinical horizon (Table 2).

As discussed by Alex van Belkum, PhD, bioMérieux, La Balme les Grottes, France, rapid testing is moving toward antimicrobial susceptibility testing on a phenotype-specific basis. This aspect of automated rapid technology remains rooted in culture-based methods (hands-on and automated) and antibiotic broth and disc diffusion assays, which are still valuable in selection of resistance phenotypes. Rapid automation of these tried-and-true approaches has merit. These traditional approaches have been bolstered by the development of PCR techniques, MALDI-TOF, specialized microscopy methods, microarrays, and color-dependent assays of bacterial viability. Still, ever-increasing bacterial resistance and a shortening list of available effective drugs make the need for rapid identification and testing of candidate antibacterial compounds urgent [Pulido MR et al. *J Antimicrob Chemother.* 2013]. In this changing landscape, standardized guidelines of test certification and interpretation are important.

A plethora of technologies offers potential value in rapid automated susceptibility testing, according to Prof van Belkum, which include but are not limited to the following:

- Flow cytometry
- Isothermal microcalorimetrics
- Microsound
- Next-generation mass spectrometry
- Real-time video-enhanced microscopy

For example, combining fluorescence live/dead microscopic examination or absorbance-based (cell density) examination with multiwell growth-based assays could be exploited to screen for antibacterial compounds.

For the future, MALDI-TOF is being explored as a means of detecting antibiotic degradation or modification [Jung J et al. *Eur J Clin Microbiol Infect Dis.* 2014]. Next-generation mass spectrometry may enable detection of bacterial vibrations on the nanoscale, allowing detection of viability that cannot be otherwise detected [Kasas S et al. *Proc Natl Acad Sci U S A.* 2015]. The use of quartz crystal microbalance and electrochemical sensing has potential in detecting surface changes of bacteria associated with lysis [Ma F et al. *Anal Chem.* 2015].

These and other molecular-level techniques [Barczak AK et al. *Proc Natl Acad Sci U S A.* 2012] offer hope for rapid determination of antibacterial susceptibility. Issues of concern include compatibility of the next-generation approaches in the workflow of the typical laboratory, potential barriers to complete automation, certification of techniques, purchase cost, and handling/disposal of test materials.

MD CONFERENCE EXPRESS®

The editors would like to thank the many members of the 25th European Congress of Clinical Microbiology and Infectious Diseases presenting faculty who generously gave their time to ensure the accuracy and quality of the articles in this publication.