

Molecular Diagnosis of Infectious Diseases

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During the past 10 to 15 years, we have seen expansive growth of the use of molecular technology in the clinical laboratory for diagnosing infectious diseases. As a result, many laboratories are able to offer more sensitive testing, faster turnaround times, and ultimately improved patient care. The gold standard in bacteriology largely remains culture, primarily due to cost accounting and the potential complex nature of associated infections (ie, urine, wound, and respiratory cultures). However, in circumstances in which there may be minute quantities of a specific pathogen present, the patient may have received antibiotics prior to specimen collection, or the etiologic agent may require unusual culture conditions, molecular detection offers a great advantage to culture techniques. In many virology laboratories, molecular detection has supplanted cell culture techniques for the identification of several viral pathogens and in many cases has become the new gold standard. Though molecular techniques can offer an abundance of added benefits when used to augment current gold standards such as culture and/or serology, the optimal use of molecular methodologies in microbiology resides with specimens in which a limited number of pathogenic organisms are sought and in cases where the enhanced sensitivity and faster turnaround time of molecular methods far outweighs the increased cost.

Applications in Bacteriology

A classic example of successful nucleic acid amplification (NAA) testing in microbiology is the detection of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) from vaginal, cervical, urethral, and first-void urine specimens. Sexually transmitted infections such as those caused by CT and NG can be rapidly and accurately identified using NAA, thus improving

treatment and transmission prevention. Implementation of routine screening for CT has lowered the prevalence rates of CT and associated pelvic inflammatory disease.¹ The increased sensitivity offered by NAA detection of CT and NG is important not only for the diagnosis of symptomatic patients, but also for the asymptomatic individuals that account for more than 70% of positive cases. Until implementation of NAA testing for CT and NG, culture was the gold standard, although it has subsequently been shown to have only 60% to

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75% sensitivity compared to NAA.² A further disadvantage of culture is that organism viability must be preserved during transport. The implementation of routine confirmatory testing should be considered when using NAA for a low prevalence population that results in a positive predictive value below 90%.²

Another prime example of NAA results positively impacting patient care is the laboratory diagnosis of tuberculosis. Using

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direct detection of *Mycobacterium tuberculosis* (MTB) from respiratory samples, tuberculosis can be confirmed in less than 24 hours as opposed to 6 to 8 weeks. The sensitivity of NAA detection of MTB in smear-positive respiratory specimens is 96.9%, and the specificity is 100%, whereas the sensitivity and specificity in smear-negative specimens is 72.0% and 99.3%, respectively.³ It should be noted that NAA of MTB does not replace the need for routine mycobacterial culture and susceptibility testing. In addition to the direct detection of MTB, techniques such as probe-based technology and sequence analysis can be applied to cultured isolates to decrease the time to identification over routine biochemical analysis. Rapid identification of MTB impacts not only patient care, but also infection control. Due to the increasing frequency of isolation of mycobacterial species associated with immunocompromised hosts and the increased incidence of multi-drug resistant MTB, it has become imperative to offer accurate yet rapid diagnostic tools for the detection and identification of mycobacteria.

A debate exists regarding the gold standard for the laboratory diagnosis of *Bordetella pertussis*. Historically, culture plates collected at the patient's bedside (ie, cough plates) have been considered the reference method. Although culture is very specific, its sensitivity suffers partially due to the organism's fastidious nature, but primarily because the highest sensitivity for culture occurs before patients are symptomatic. NAA remains positive for longer after therapy than culture, and NAA is also positive for a longer period after onset of symptoms.⁴ Therefore, NAA is useful for patients presenting later in their illness. NAA testing allows for same-day results and since erythromycin-resistant *B. pertussis* is still rare, a cultured isolate is rarely needed for antimicrobial susceptibility testing. Multiple studies have demonstrated significant increased detection of *B. pertussis* when comparing NAA to culture: reported PCR-positive, culture-negative samples range from 13% to 88%.⁵ However, due to potential false positive and false negative results with *B. pertussis* NAA procedures, it is strongly recommended that results be considered in the context of patient clinical presentation, and clinically inconsistent results should be confirmed by a second method.

NAA is also being used in bacteriology to detect antimicrobial resistance. Since antimicrobial resistance can be multi-factorial, this practice is limited to organisms in which the results can be interpreted with confidence in regard to the genotypic relationship to clinical treatment and/or infection control precautions. Such examples are direct detection of vancomycin-resistant *Enterococcus* (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA) from rectal and nares surveillance cultures, respectively. Screening patients for VRE and MRSA carriage is a key strategy for preventing the spread of these organisms in health care settings. NAA technology reportedly increases VRE detection by up to 120%.⁶ In addition, enterococci that confer low-level intrinsic resistance, and thus not considered "true" VRE, are accurately ruled out preventing unnecessary contact precautions and contributing to hospital savings.⁶ NAA detection of MRSA has been shown to be equal in sensitivity to culture-based methods, but has the advantage of offering a faster turnaround

time, thus impacting hospital cost savings.⁶ However, it should be noted that direct specimen testing for MRSA comes with limitations, often including a lower positive predictive value than conventional methods.^{7,8} More recently, new strains of MRSA have appeared that are associated with skin and soft tissue infections in outpatients and are called community-associated MRSA (CA-MRSA).⁹ The increasing incidence of CA-MRSA is causing overall rates of MRSA to rise. Therefore, it has become even more important to quickly and accurately identify resistant isolates.

Applications in Virology

Monitoring the viral load (quantified determinations of virus using NAA) in patients infected with human immunodeficiency virus (HIV), hepatitis C virus (HCV), or hepatitis B virus (HBV) is useful for tracking therapeutic response to antivirals and potential antiviral resistance. In addition, viral load monitoring for cytomegalovirus (CMV) in transplant recipients has allowed clinicians the benefits of identifying patients most at risk for developing clinical CMV disease, monitoring antiviral therapy response, and optimizing pre-emptive treatment.¹⁰ Analogously, Epstein-Barr virus (EBV) viral loads can be monitored in the posttransplant setting to identify patients at risk for developing posttransplant lymphoproliferative disorder.

Molecular detection of viruses has extended beyond the standard therapeutic monitoring of viral loads in specific patient populations. For example, NAA testing for the laboratory diagnosis of herpes simplex virus (HSV) encephalitis and enterovirus (EV) meningitis has become the standard of care. Cell culture techniques are insensitive due to the low viral burden typically found associated with encephalitis and perhaps also the presence of host neutralizing antibodies.

HSV is the most common cause of nonepidemic encephalitis in the US, accounting for up to 20% of cases. CSF culture for HSV detects less than 2% of clinically determined adult HSV encephalitis cases and 40% of neonatal central nervous system (CNS) disease.¹¹ In contrast, HSV NAA is positive in most adult cases resulting in sensitivity and specificity > 95%¹¹ and is 75% sensitive and 100% specific for neonatal meningitis.¹² The rapid diagnosis of HSV encephalitis can prevent a brain biopsy and rapidly determine the need for acyclovir therapy.

Enterovirus is the most common cause of aseptic meningitis in the summer and fall months in temperate climates and accounts for 10% to 20% of encephalitis cases. A wide array of cell lines must be utilized to recover the majority of EV types by culture, and culture sensitivity still remains approximately 70%.¹³ The sensitivity and specificity of CSF NAA for EV are estimated to both be > 95%.¹²

Nucleic acid amplification has also been successfully applied to other etiologies of viral CNS disease, such as CMV and varicella-zoster virus (VZV), but these assays have not been implemented as broadly as those for HSV and EV, so are still transitioning to becoming the method of choice. It should be noted that not all encephalitis viruses are readily detected by NAA. For example, due to the short period of viremia in many

arboviral infections (ie, West Nile Virus), CSF NAA has low sensitivity, and the gold standard remains serology.¹⁴ CSF NAA false negative results can occur due to collection of CSF very early or very late in illness, rapid viral clearance in immunocompetent hosts, and NAA inhibitors.¹² False positive CSF NAA results also occur primarily due to lack of data to suggest the detection of certain viral nucleic acids correlates with clinical CNS disease, but can also be caused by the presence of peripheral blood in the CSF.¹² While CSF NAA is considered by many the diagnostic standard of care as discussed above, the lack of standardized FDA-approved assays has made implementation of CSF NAA difficult in nonacademic settings. While most laboratories offering CSF NAA use qualitative methods, data indicate a role for quantitative CSF NAA in differentiating nonspecific presence of virus and virus-associated disease, to aid in prognosis for improved patient management, and in monitoring antiviral therapy.¹⁴

Challenges and Opportunities

The field of molecular infectious disease testing has grown so rapidly that the diagnostic industry has not kept up. To fill this void, independent investigators have turned to the development of user-defined, or “homebrew,” molecular detection methods in the clinical laboratory. The implementation of user-defined NAA testing has revolutionized clinical molecular infectious disease testing. In addition, commercially-available non-FDA-approved NAA assays are increasingly becoming available as analyte specific reagents (ASRs). Though all reagents necessary for the amplification reaction can be purchased commercially, assay development and verification studies must be performed by individual laboratories. In many cases, there are no comparative studies between user-defined NAA procedures, including ASRs, limiting the comparative value of assays between institutions (particularly in viral load monitoring) and restricting the application of such procedures to more experienced laboratories.

It is not without considerable cost that a molecular infectious disease diagnostic lab is developed. It represents an institutional commitment because the costs may only be offset when analysis of hospital-wide cost savings is employed (ie, shorter hospital stays, decreased use of unnecessary antibiotics). The costs incurred not only stem from instrumentation purchases, but also from the dedicated, expert staff required for such testing. Since many academic medical centers have resorted to implementing user-defined assays, verification and validation studies are substantial and require extensive resources, including time, staff, and expertise. These studies are crucial to defining the performance

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of the assay and determining appropriate clinical utilization. Most laboratory directors view the implementation of user-defined assays and ASRs as a temporary fix until FDA-approved assays are available. However, many diagnostic companies are opting not to seek FDA-clearance to replace current ASRs or “research use only” tests. The FDA, diagnostic companies, and major molecular infectious disease laboratories need to work together to resolve the poor standardization that exists between laboratories using user-defined assays or ASRs. Further, in the absence of FDA-approved tests, many nonacademic medical centers will not have the opportunity to enter the field of molecular infectious disease diagnostics.

Conclusion

The applications of molecular technology in clinical microbiology are endless, but challenges also abound. We are still learning what many NAA results mean in terms of infectious etiology. With the use of molecular technology to detect potential etiologic agents of disease, we need to remember Koch’s postulates.¹⁵ Is the mere presence of an organism’s nucleic acid convincing evidence of disease causation? Undoubtedly, additional clinical scientific evidence is needed to make such a claim, and such evidence or lack thereof should be considered when interpreting molecular infectious disease results. Though there is still much to be learned regarding the appropriate application and interpretation of molecular infectious disease testing, there are numerous exciting opportunities on the horizon. User-defined assays and ASRs have allowed experienced laboratories to offer critical diagnostic services that have yet to become available with FDA clearance. As investigators refine molecular applications for infectious disease testing, diagnostic companies market such applications, quality control and government organizations standardize results, and as costs associated with implementation decrease and reimbursement increases, molecular infectious disease testing will not only be available in academic medical centers and reference laboratories, but will also transition to community hospitals, thus more globally impacting patient care. **NCMJ**

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