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New diagnostic tools

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Medical microbiology is a clinical discipline that evolves slowly and the majority of diagnoses relies still upon demonstrable growth of an infectious microorganism. However, various new diagnostic technologies have been introduced into laboratory practice over the past few decades.[1] As recently stated by Isenberg,[2] molecular biology has the potential to revolutionize the diagnosis of infectious disease in order to optimize the care of infected patients, whether they are in hospital or in the community. Molecular tests promise to be extremely useful also in therapy, epidemiological investigations, and infection control. However, many laboratories have been reluctant to introduce these new methods, so the vast majority of clinical laboratories do not currently use any molecular diagnostics. By contrast, such technology is becoming more widespread in specialized regional laboratories, as well as in national reference laboratories.[3]

Currently, the most practical and useful application of molecular methods is in detecting and identifying infectious agents for which routine growth-based culture and microscopy methods may not be adequate.[4,5] The commonly adopted nucleic-based tests use standard methods for isolating nucleic acids from organisms and clinical material and restriction endonuclease enzymes, gel electrophoresis, and nucleic hybridization techniques to analyze DNA or RNA.[6] Because the target DNA or RNA may be present in very small amounts in clinical specimens, various signal amplification and target amplification techniques have been used to detect infectious agents in clinical microbiology laboratories.[4,6] Although mainly a research tool, nucleic acid sequence analysis coupled with target amplification is an useful method for detecting and identifying previously uncultivable organisms and characterizing antimicrobial resistance gene mutations.[4,7] Automation and high-density oligonucleotide probe arrays (DNA chips) also hold great promise for characterizing microbial pathogens.[6]

In this report, we present the current status of molecular diagnostics with regard to the application in the area of medical microbiology, with the overall aim to provide an appreciation of the role that molecular tests may play in routine clinical microbiology.

Applications of molecular methods in the clinical microbiology laboratory

Commercial kits are available for the most prevalent infectious agents and they all provided a degree of standardization and ease to use (Table 1). The use of nucleic acid probes for identifying cultured organisms and for direct detection of organisms in clinical material was the first exposure that most laboratories had to commercially available molecular tests. In spite of the fact that these probe tests are still widely used, amplification-based methods are increasingly employed for detection, identification and quantitation of pathogens, and characterization of antimicrobial-drug resistance genes. Commercial amplification kits are available for some pathogens (Table 1), but "home brew" methods have been developed for some clinically important microorganisms (Table 2).

Molecular detection and identification of pathogens

Commercial kits containing non-isotopically labeled nucleic acid probes are available for direct detection of pathogens in clinical material and identification of organisms after isolation in culture (Table 1). Direct detection of organisms in clinical specimens by nucleic acid probes require at least 104 copies of nucleic acid per microliter, a requirement rarely met in clinical samples without any form of amplification. Amplification of the detection signal after probe hybridization improves sensitivity to as low as 500 gene copies, but does not match the analytical sensitivity of target-amplification based methods, such as polymerase chain reaction (PCR), for detecting organisms. Probe hybridization is useful for

Table 1. FDA-approved molecular diagnostic tests for infectious diseases.

Test	Method	Company
Chlamydia trachomatis detection	PCR LCR TMA Hybrid capture	Roche Abbott Gen-Probe Digene
Neisseria gonorrhoeae detection	LCR Hybrid capture	Abbott Gen-Probe
C. trachomatis/N. gonorrhoeae screening/detection	Hybridization SDR	Gen-Probe Becton-Dickinson
Mycobacterium tuberculosis detection	PCR TMA	Roche Gen-Probe
HPV screening	Hybrid capture	Digene
CMV	Hybrid capture NASBA	Digene Organon Teknika
Group A strep detection	Hybridization	Gen-Probe
HIV quantitation	PCR	Roche
Gardnerella, Trichomonas vaginalis and Candida	Hybridization	Becton-Dickinson
Culture confirmation for bacteria and fungi	Hybridization	Gen-Probe

identifying slow-growing organisms after isolation in culture using either liquid or solid media. Identification of mycobacteria and other slow-growing organisms such as the dimorphic fungi (*Histoplasma capsulatum, Coccidioides immitis*, and *Blastomyces dermatitidis*) has certainly been facilitated by commercially available probes. Due to the ability to selectively amplify specific targets, amplification-based methods offer superior performance, in terms of sensitivity, over the direct (non-amplified) probe-based tests. PCR was the first such technique to be developed and because of its flexibility and ease of performance remains the most widely used molecular diagnostic technique in both research and clinical laboratories. To this regard, several different strategies have been developed and are available commercially (Table 2). Given the adaptability of PCR, numerous additional infectious pathogens have been detected by investigator-designed or "home brew" PCR assays (Table 2). Amplification-based methods are also valuable for identifying cultured and uncultivable organisms. To this purpose, amplification reactions may be designed to amplify a genus-specific or "universal target", which then is characterized by using restriction enzyme digestion, hybridization with multiple probes, or sequence determination to provide species delineation.[8]

Molecular identification of new pathogens

Molecular diagnostics can be also used for the detection of novel pathogens.[1] This requires the application of so called broad-spectrum DNA amplification methods. Using universally conserved priming sites on rRNA genes or other structurally well-conserved genes, DNA from previously uncharacterized pathogens can still be amplified. This technology, recently reviewed by Relman,[9] has facilitated, and will continue to facilitate, the detection of novel pathogens and novel members of the human flora that have not yet been associated with any disease type. This approach, especially in combination with DNA sequencing, is particularly effective.[10] The clinical relevance of this approach is unquestionable. When applied to the detection of causative agents of meningitis, for example, broad-range PCR proved to be 100% sensitive and 98.2% specific, with a positive predictive value of 94.0% and a negative predictive value of 100%.[11]

Detecting antimicrobial-drug resistance

Antibiotic resistance in microbial pathogens has become an important topic both nationally and internationally. Some scientists are forecasting the emergence of the "post-antibiotic era", where it will be difficult to control common infections, owing to the emergence of high-level multi-drug resistance in most clinically important pathogens. Consequently, there has been great interest in rapid detection of antimicrobial-drug resistance, particularly of methicillin resistance in staphylococci, which may be expressed in a very heterogeneous fashion, making phenotypic characterization of resistance very difficult.[7] Currently, molecular detection of the resistance gene, mecA, is the standard against which phenotypic methods for detection of methicillin resistance are judged.[7] Molecular methods may be used to detect specific antimicrobial-drug resistance genes in many organisms (Table 3) and applied directly to the clinical specimen, providing simultaneous detection and identification of the pathogen plus resistance characterization.[7]

Table 2. Noncommercial nucleic acid-based tests for clinically important viral and bacterial pathogens.

Organism	Specimen type	Clinical indication
Epstein-Barr virus (EBV)	Cerebrospinal fluid (CSF)	EBV lymphoproliferative disorder
Herpes simplex virus (HSV) 1 and 2	CSF Vitreous humor	Encephalitis
Varicella-zoster virus (VZV)	Various tissues	VZV reactivation
JCV	CSF	Progressive multifocal leukoencephalopathy
Enterovirus	CSF	Aseptic meningitis
Parvovirus B19	Amniotic fluid Serum	Hydrops fetalis Anemia
Adenovirus	Urine Tissues Blood	Immunocompromised patients, transplant recipients
Ehlichia	Blood	Human granulocytic and monocytic ehrichiosis
Bordetella pertussis	Nasopharyngeal aspirate	Whooping cough
Legionella pneumophila	Respiratory	Atypical pneumonia
Mycoplasma pneumoniae	Respiratory	Atypical pneumonia
Helicobacter pylori	Gastric fluid Stool	Peptic ulcer disease

Appraisal of molecular diagnostics in clinical microbiology

Although most clinicians and microbiologists enthusiastically welcome to the new molecular tests for diagnosing infectious disease, the adoption of molecular diagnostics in routine clinical laboratory has advantages and disadvantages. It is often assumed that in addition to improved patient care, major financial benefits may accrue from molecular testing because the tests reduce the use of less sensitive and specific tests, unnecessary diagnostic procedures and therapies, and nosocomial infections.[12] However, the inherent costs of molecular testing methods have limited the introduction of these tests into clinical diagnostic laboratory. Their use is, at present, largely confined to specialized or reference laboratories, but various technologies, including PCR, real-time PCR, and pulsed-field gel electrophoresis, may eventually be adopted in regional and even in district diagnostic laboratories.

Not all molecular diagnostic tests are extremely expensive. Direct costs vary widely, depending on the test's complexity and sophistication. Inexpensive

Table 3. Molecular methods for detecting antimicrobial resistance.

Organism(s)	Antimicrobial agent(s)	Gene	Detection method
Staphylococci	Methicillin Oxacillin	*mec* A	Standard DNA probe Branched chain DNA probe PCR
Enterococci	Vancomycin	*van A, B, C, D*	Standard DNA probe PCR, Real time PCR
Enterobacteriaceae *Haemophilus influenzae* *Neisseria gonorrhoeae*	Beta-lactams	bla_{TEM} and bla_{SHV}	Standard probe PCR and RFLP PCR and sequencing
Enterobacteriaceae and gram-positive cocci	Quinolones	Point mutations in *gyr A*, *gyr B*, *par C* and *par E*	PCR and sequencing
M. tuberculosis	Rifampin Isoniazid Ethambutol Streptomycin	Point mutations in *rpo* B Point mutations in *kat G*, *inh A,* and *ahp C* Point mutations in *emb B* Point mutations in *rps L* and *rrs*	PCR and SSCP PCR and sequencing Real-Time PCR PCR-DHPLC analysis

molecular tests are generally kit based and used methods that require little instrumentation or technologist experience. The more complex molecular tests, such as resistance genotyping, often have high labor costs because they require experienced, well trained technologists. However, advances in automation and the production of less expensive reagents promise to decrease these costs as well as technician time.

Conclusions

It is known that at least 13 novel microbial pathogens, including Campylobacter jejuni, Helicobacter pylori and Tropheryma whippelii, have been identified over the past 30 years and several other infectious agents now show a steep resurgence. Altogether, infections lead to an estimated 14 million human deaths per year.[13] This implies that improvement in diagnostic testing is mandatory over the coming years and also highlights that the continued search for novel microbial pathogens must have the relentless attention of the infectious disease research community. Quantitative amplification tests in combination with genomics, transcriptomics proteomics, and related methodologies will further substantiate and broaden the diagnostic armamentarium and will pave the way to further enhancement of innovative microbial detection and identification.

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