Diagnostic Molecular Microbiology: A 2013 Snapshot

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KEYWORDS

- Molecular microbiology • PCR • Probe tests
- Rapid molecular diagnosis of infections • Multiplex PCR panels • MALDI TOF
- Nuclear magnetic resonance

KEY POINTS

- In 2013, diagnostic molecular testing has a large and increasing role in the diagnosis of infectious diseases.
- It has evolved significantly since the first probe tests were FDA approved in the early 1990s.
- It has evolved beyond PCR or even RT-PCR to include highly multiplexed PCR carried out in microfluidic pouch systems, matrix-assisted laser desorption/ionization time of flight, and nuclear magnetic resonance.

INTRODUCTION

When Kary Mullis developed the polymerase chain reaction (PCR) in 1983, its potential benefits were obvious to clinical microbiologists: faster, cheaper, more accurate detection and enumeration of all organisms in a specimen, without waiting for culture. We also wanted simultaneous antimicrobial susceptibility testing. Our dreams are now coming true. Multiplex arrays are approved or in development for the diagnosis of respiratory and gastrointestinal infections direct from patient specimens within less than an hour. An array was FDA cleared in August, 2013, that can detect common bacterial and fungal agents of bloodstream infections, as well as several important antibiotic-resistant genes, within about an hour after the culture bottle turns positive. Microbiology lines are available, starting with automated plate streakers and ending with molecular identification of organisms grown on solid media. Humans must still view the culture plates, perhaps on a television screen, and select colonies to analyze.

"Cheaper" is an ambiguous target. Microbiology laboratories are diagnostic facilities that drive subsequent therapy. Increased laboratory costs for more rapid

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microbial identification have been shown to result in earlier use of appropriate antibiotics, shorter lengths of hospital stay, and better outcomes, decreasing overall health care costs.[1–3] Diagnosis of persistent human papilloma virus infections followed by appropriate therapeutic interventions should reduce the incidence of cervical carcinomas, the cost of treatment, and the attributable morbidity and mortality.

New technologies have enabled microbiologic investigations that were not included in our original dreams. NextGen sequencing can detect and quantify populations of organisms in patient specimens. This raises the possibility of distinguishing pathogenic organisms, present in high numbers, from colonizers that are generally presumed to be present in lower numbers. Certain colonic organism profiles appear to correlate with the development of cardiovascular disease.[4] A patient's colonic flora could be analyzed, and if the profile were unfavorable, the bacteria could be eradicated and replaced.

Tests in use in 2013 have evolved significantly and will continue to do so. Thus, this article is a snapshot of rapidly changing diagnostic microbiology laboratory techniques. Because of space constraints, emphasis has been placed on tests with high market share in diagnostic microbiology and on those with technologies that are personally regarded by the authors as particularly interesting. The role of specimen processing in concentrating nucleic acid targets and removing inhibitors of amplification is largely neglected, despite its important role in the sensitivity of the assay. Most techniques mentioned here involve RT-PCR, unless otherwise specified. RT-PCR lowers the incidence of amplicon contamination in the laboratory, and has allowed many nucleic acid amplification techniques to come "out of the closet," but does not prevent specimen contamination. The authors have also attempted to select only one current citation to support most points, and these selections are arbitrary. Failure to mention a publication, technique, or trade name should not be construed as denigrating that article, technique, or manufacturer.

PROBE TECHNIQUES

The first molecular diagnostic tests approved by the Food and Drug Administration (FDA) were probe techniques. The probes were synthesized by molecular techniques, but the clinical laboratory performed only hybridization and detection. Many probe tests are still in wide use today because they fill important niches. Some involve novel detection methodologies.

Hybridization-Protection Assays

Among the first FDA-approved molecular tests were the Gen-Probe ([San Diego, CA], which became a wholly owned subsidiary of Hologic [Bedford, MA] in 2012). Pace 2 probe hybridization protection techniques for the diagnosis of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) from patient specimens. They have been largely been replaced by more sensitive amplification tests. A number of their AccuProbe culture confirmation tests remain available. Among them are *Mycobacterium tuberculosis* (TB) complex, *Mycobacterium avium, Mycobacterium intracellulare* (separately or together), *Mycobacterium kansasi*i, *and Mycobacterium gordoni*. In addition, there are 3 tests for dimorphic fungi: *Histoplasma capsulatum* (which also detects *H capsulatum var. dubosi*), *Blastomyces dermatididis* (which also detects *Paracoccidioides brasiliensis*), and *Coccidioides immitis*. These and other tests available from the same manufacturer all use most of the same reagents and instrumentation, which facilitates the use of multiple assays in the same laboratory.