Diagnostic Molecular Microbiology: A 2018 Snapshot

Marilynn Ransom Fairfax, MD, PhD[a,b,*], Martin H. Bluth, MD, PhD[a,c], Hossein Salimnia, PhD, D(ABMM)[a,b]

KEYWORDS

- Molecular microbiology • PCR • Probe tests
- Rapid molecular diagnosis of infections • MALDI-TOF • Nuclear magnetic resonance
- Next gen sequencing • Time lapsed imaging

KEY POINTS

- Molecular biological techniques have evolved expeditiously and in turn have been applied to the detection of infectious disease.
- Maturation of these technologies and their coupling with technological advancements have afforded clinical medicine additional tools toward expedient identification of infectious organisms at concentrations and sensitivities previously unattainable.
- These advancements have been adapted in select settings toward addressing clinical demands for more timely and effective patient management.

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 a culture. The discipline of infectious disease also sought the opportunity for simultaneous antimicrobial susceptibility testing. These dreams have slowly matured into realities. Multiplex arrays are approved or in development for the diagnosis of respiratory and gastrointestinal infections direct from patient specimens with results obtained in under an hour. An array was cleared by the US Food and Drug Administration (FDA) 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. Approaches are being made to organism identification and susceptibility testing directly from a blood sample without the

[a] Department of Pathology, Wayne State University School of Medicine, 540 East Canfield Street, Detroit, MI 48201, USA; [b] Clinical Microbiology Laboratories, DMC University Laboratories, 4201 St. Antoine Street, Detroit, MI 48201, USA; [c] Pathology Laboratories, Michigan Surgical Hospital, 21230 Dequindre Road, Warren, MI 48091, USA
* Corresponding author.
E-mail address: mfairfax@dmc.org

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necessity for culture. Microbiology lines are available, starting with automated plate streakers and ending with molecular identification of organisms grown on solid media. Despite these molecular and technological advancements, humans must still view the culture plates, perhaps on a television screen, and select colonies to analyze.

Furthermore, although cost containment is of paramount importance in today's medical marketplace, "cheaper" is an ambiguous target. Microbiology laboratories are diagnostic facilities that drive subsequent therapy. Increased laboratory costs for more rapid microbial identification have been shown to result in the earlier use of appropriate antibiotics, shorter durations of hospital stay, better outcomes, and decreasing overall health care costs.[1–3]

The diagnosis of persistent human papilloma virus (HPV) infections followed by appropriate therapeutic interventions should decrease 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 diagnostic approaches. Next-generation sequencing (NGS) 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 seem 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 2018 have evolved significantly from those cited in our 2013 review[5] and will continue to do so. Thus, this article is a snapshot of rapidly changing diagnostic microbiology laboratory techniques and its clinical applications. 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. However, many new procedures are automated and include specimen processing as part of a hands-off procedure. Most techniques mentioned here involve real-time PCR (RT-PCR), unless otherwise specified. Because most RT-PCR platforms are closed systems, they decrease the incidence of amplicon contamination in the laboratory, and have allowed many nucleic acid amplification techniques to become commercially available. The authors have also attempted to select current citations to support salient 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 FDA were probe techniques. 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 are used for the diagnosis of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* from patient specimens. They have been largely replaced by more sensitive amplification tests. A number of their AccuProbe culture confirmation tests remain available. Among the most useful are *Mycobacterium*