

Evolution of the Clinical Microbiology Laboratory

S. TRAVIS ALTHEIDE, MICHELLE BUTINA, USHA CHATTERJEE, JASON EVANS

LEARNING OBJECTIVES

1. List the 3 different diagnostic approaches to infectious disease and organism identification.
2. Briefly describe each of the diagnostic approaches and identify a specific example of each one.
3. Recognize emergent biomarkers for the detection of bacterial and fungal infections and state the clinical utility of each.

ABSTRACT

Over the course of nearly 150 years, the clinical laboratory has diagnosed infectious diseases and identified their causative agents using a variety of approaches. These approaches can be broadly placed into 3 categories: biochemical or growth-based methods, molecular and genomic diagnostics, and biomarker and serologic detection of blood components. The principle of the biochemical approach is based on isolating an unknown microorganism before conducting a series of growth-based and preformed-enzyme detection tests to determine an identification and subsequent antimicrobial susceptibilities. The molecular approach is the newest diagnostic approach used by the laboratory and is based on detection of the genetic components of an unknown organism, either isolated or directly in a clinical specimen. There are a variety of molecular techniques with the polymerase chain reaction serving as the basis of most currently available methods. The detection of infection and inflammatory indicators, as well as serologic molecules, has been used for diagnostic purposes nearly as long as growth-based identification methods. The biomarker approach to infectious-disease diagnosis has primarily occurred outside of the traditional microbiology department, usually within chemistry and hematology where large scale automated instruments provide rapid results. In this focus series, a concise review and a brief history of these different approaches are presented.

S. Travis Altheide, Eastern Kentucky University

Michelle Butina, West Virginia University

Usha Chatterjee, Bellarmine University

Jason Evans, West Virginia University

Address for Correspondence: S. Travis Altheide, Eastern Kentucky University, travis.altheide@eku.edu

The underlying methods are described with advantages and disadvantages, while specific examples of each are highlighted with the internal and external factors that influence their development.

ABBREVIATIONS: MALDI-TOF - matrix-assisted laser desorption/ionization time-of-flight, MS - mass spectrometry, PCR - polymerase chain reaction.

INDEX TERMS: biochemical, biomarkers, diagnostic microbiology, molecular testing.

Clin Lab Sci 2019;32(4):146–148

Infectious diseases have plagued humanity for millennia, yet it was only 140 years ago that the link between infection and microorganism was established. Since that time, various laboratory approaches and techniques have been developed and deployed to increase sensitivity and specificity in the identification of infectious agents. These approaches can be categorized into 3 separate areas based on identification: (1) biochemical or culture-based techniques requiring organism isolation, (2) molecular and genomic methods, and (3) detection of infectious-disease indicators and microbial markers detected in blood, which include serology that are often analyzed outside the microbiology department in the “core” laboratory.

The biochemical culture-based approach was the initial identification method established in the form of plated and tubed media containing various growth substrates, such as carbohydrates and proteins.^{1,2} Later, it included the use of rapid (spot) enzyme testing,^{3,4} which further established the biochemical methods as an accurate, adaptable, and versatile form of routine bacterial identification. By the 1950s, in the face of increasing population, health care demand, and private and public insurance availability,^{5,6} the first significant advancement of the biochemical approach came in the form of miniaturized multitest media and kits,⁷⁻⁹ which simply took the existing principle of identification and shrunk it. The miniaturized multitest media and kits allowed for reduced labor, media, and waste disposal costs while providing accurate identification in a timely manner.¹⁰ The advantages of the miniaturized-kit approach were further realized with the incorporation of automated growth-based instrumentation,¹¹⁻¹³ which remains the standard for bulk identification of bacteria and many yeast today.^{14,15}

Only within the last 10–15 years has the basic biochemical principle of growth dependency been replaced

as the method for routine organism identification. Mass spectrometry (MS), an analytic chemistry technique used for decades, has been adapted for use as a powerful tool for the diagnostic microbiology laboratory.^{16,17} Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS, as it is specifically known in the diagnostic laboratory, produces a unique protein fragment “fingerprint” for many different microorganisms, bacterial and fungal alike.^{18,19} The advantages of MALDI-TOF cannot be overstated; once an organism has been isolated, MALDI-TOF provides the most accurate and cost effective identification in the shortest amount of time of any technique.²⁰⁻²² Of course, there are some limitations of current MS methods, chief among them are the initial cost of the instrument and the need to consistently update the proprietary databases.^{23,24} However, the initial cost aside, MS is likely to become more common as laboratories continue to look for ways to handle increasing test volume while providing the fastest turn-around time possible.

Molecular diagnostics made its debut as polymerase chain reaction (PCR)-based testing, which sought to identify single specific organisms from patient samples.²⁵ The area of the microbiology laboratory that benefitted most from these early tests was the virology section, as virology testing typically required mammalian cultures to propagate the virus and took up to 2 weeks before identification was possible.²⁶ Since this first implementation of molecular diagnostics, new testing systems and modifications to PCR technologies have been developed. The technologies reduce hands-on time with test setup, decrease cost, decrease the time to obtain results, and enhance the sensitivity and specificity toward the target organism.²⁷ An example of a current advancement implemented in the microbiology laboratory is syndromic-panel testing, which may concomitantly detect several common infectious organisms associated with particular body system afflictions, such as the upper respiratory and gastrointestinal tract.²⁸

Traditional culture-based identification methods are still a cornerstone of clinical laboratory testing and will most likely remain so in the near future; however, molecular-based testing is becoming increasingly prevalent in microbiology laboratories. The prevalence is evident in the increasing number of molecular tests that receive and seek approval from the Food and Drug Administration for clinical use,²⁹ including next-generation sequencing and whole-genome sequencing of organisms found in patient samples.³⁰ With this potentially forthcoming paradigm shift in organism identification, comes numerous advantages and caveats that must be thoroughly considered before implementation of any of these systems. These considerations will need to be assessed based on an individual laboratory's needs because there is not yet a “one size fits all” scheme for implementation. Nevertheless, molecular testing will continue to improve and remain a staple in microorganism identification and diagnostics.

Parallel to the biochemical culture-based and molecular-technology developments in the microbiology laboratory, there is a quest to find biomarkers with increased sensitivity and specificity for early identification of infection, especially for the diagnosis of sepsis. Traditional laboratory markers, such as the white blood cell count and erythrocyte sedimentation rate, are longstanding biomarkers of infection and inflammation.^{31,32} Research efforts to identify emergent biomarkers for sepsis led to the inclusion of lactate and procalcitonin as effective biomarkers.³³ Several promising emergent biomarkers are being studied for sepsis, including pentraxin 3 and presepsin, which offer quicker diagnosis and prognosis.

Biomarker research expands beyond sepsis and includes promising emergent biomarkers for other bacterial and fungal infections. For example, the human neutrophil elastase and cathepsin G are being investigated as biomarkers for chronic wound infections, as early detection can prevent progression to systemic infection.^{34,35} Timely treatment is critical in the case of bacterial pneumonia, and interleukin 6—a cytokine in the acute phase response—is being studied as a potential biomarker.³⁶ Currently, detection of galactomannan allows for rapid testing of infections caused by pathogenic species of *Aspergillus* while other potential biomarkers for invasive fungal infections are being investigated.³⁷

The technological advances seen throughout the history of diagnostic microbiology are spurred on by the necessity of immediate quality health care services for a growing population, and this will continue to factor in future developments. Yet, each laboratory must consider additional factors unique to their situation—such as their budget, patient population of service, and volume of testing—before deciding whether to implement the newest molecular syndromic testing panel, replace their current automated biochemical analyzer with MALDI-TOF, or validate a new biomarker for disease detection. Nevertheless, newer approaches and technology are becoming more commonly used and are likely to continue to be used as laboratories consolidate into fewer, but larger, facilities that look to maximize efficiency through automation.

This focus series presents a concise review with a brief history of these different approaches by describing their underlying methods, advantages, and disadvantages, while highlighting specific examples of each with the internal and external factors that influenced their development. This focus series is intended to be a basic review of the topic and is written for a general clinical audience.

REFERENCES

1. Muir R, Ritchie J. *Manual of Bacteriology*. YJ Pentland; 1899.
2. Macconkey A. Lactose-fermenting bacteria in faeces. *J Hyg (Lond)*. 1905;5(3):333–379. doi: [10.1017/S002217240000259X](https://doi.org/10.1017/S002217240000259X)
3. Ljutov V. Technique of indole test. *Acta Pathol Microbiol Scand*. 1959;46(4):349–360. doi: [10.1111/j.1699-0463.1959.tb01106.x](https://doi.org/10.1111/j.1699-0463.1959.tb01106.x)

4. Steel KJ. Microbial identification. *J Gen Microbiol.* 1965;40(1):143–148. doi: [10.1099/00221287-40-1-143](https://doi.org/10.1099/00221287-40-1-143)
5. Arestad FH. Hospital service in the United States: Twenty-Fifth Annual Presentation of Hospital Data by the Council on Medical Education and Hospitals of the American Medical Association. *J Am Med Assoc.* 1946;130(16):1073–1161. doi: [10.1001/jama.1946.02870160019009](https://doi.org/10.1001/jama.1946.02870160019009)
6. Andersen R, Hull JT. Hospital utilization and cost trends in Canada and the United States. *Health Serv Res.* 1969;4(3):198–222.
7. Kohn J, ed. A 2-tube technique for the identification of organisms of the enterobacteriaceae group. *J Pathol Bacteriol.* 1954.
8. Buissonière J, Nardon P, Nardon P. Microméthode d'identification des bactéries. I. Intérêt de la quantification des caractères biochimiques. *Ann Inst Pasteur (Paris).* 1968;115(2):218–231.
9. Washington JA II, Yu PKW, Martin WJ. Evaluation of accuracy of multitest micromethod system for identification of Enterobacteriaceae. *Appl Microbiol.* 1971;22(3):267–269. doi: [10.1128/am.22.3.267-269.1971](https://doi.org/10.1128/am.22.3.267-269.1971)
10. Bartlett RC, Kohan TS, Rutz C. Comparative costs of microbial identification employing conventional and prepackaged commercial systems. *Am J Clin Pathol.* 1979;71(2):194–200. doi: [10.1093/ajcp/71.2.194](https://doi.org/10.1093/ajcp/71.2.194)
11. Staneck JL, Vincelette J, Lamothe F, Polk EA. Evaluation of the Sensititre system for identification of Enterobacteriaceae. *J Clin Microbiol.* 1983;17(4):647–654. doi: [10.1128/jcm.17.4.647-654.1983](https://doi.org/10.1128/jcm.17.4.647-654.1983)
12. Truant AL, Starr E, Nevel CA, Tsolakis M, Fiss EF. Comparison of AMS-Vitek, MicroScan, and Autobac Series II for the identification of gram-negative bacilli. *Diagn Microbiol Infect Dis.* 1989;12(3):211–215. doi: [10.1016/0732-8893\(89\)90017-5](https://doi.org/10.1016/0732-8893(89)90017-5)
13. Stager CE, Davis JR. Automated systems for identification of microorganisms. *Clin Microbiol Rev.* 1992;5(3):302–327. doi: [10.1128/CMR.5.3.302](https://doi.org/10.1128/CMR.5.3.302)
14. Eigner U, Schmid A, Wild U, Bertsch D, Fahr A-M. Analysis of the comparative workflow and performance characteristics of the VITEK 2 and Phoenix systems. *J Clin Microbiol.* 2005;43(8):3829–3834. doi: [10.1128/JCM.43.8.3829-3834.2005](https://doi.org/10.1128/JCM.43.8.3829-3834.2005)
15. Jin W-Y, Jang S-J, Lee M-J, et al. Evaluation of VITEK 2, MicroScan, and Phoenix for identification of clinical isolates and reference strains. *Diagn Microbiol Infect Dis.* 2011;70(4):442–447. doi: [10.1016/j.diagmicrobio.2011.04.013](https://doi.org/10.1016/j.diagmicrobio.2011.04.013)
16. Anhalt JP, Fenselau C. Identification of bacteria using mass spectrometry. *Anal Chem.* 1975;47(2):219–225. doi: [10.1021/ac60352a007](https://doi.org/10.1021/ac60352a007)
17. Krishnamurthy T, Ross PL. Rapid identification of bacteria by direct matrix-assisted laser desorption/ionization mass spectrometric analysis of whole cells. *Rapid Commun Mass Spectrom.* 1996;10(15):1992–1996. doi: [10.1002/\(SICI\)1097-0231\(199612\)10:15<1992::AID-RCM789>3.0.CO;2-V](https://doi.org/10.1002/(SICI)1097-0231(199612)10:15<1992::AID-RCM789>3.0.CO;2-V)
18. Neville SA, Lecordier A, Ziochos H, et al. Utility of matrix-assisted laser desorption ionization-time of flight mass spectrometry following introduction for routine laboratory bacterial identification. *J Clin Microbiol.* 2011;49(8):2980–2984. doi: [10.1128/JCM.00431-11](https://doi.org/10.1128/JCM.00431-11)
19. De Carolis E, Posteraro B, Lass-Flörl C, et al. Species identification of Aspergillus, Fusarium and Mucorales with direct surface analysis by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Clin Microbiol Infect.* 2012;18(5):475–484. doi: [10.1111/j.1469-0691.2011.03599.x](https://doi.org/10.1111/j.1469-0691.2011.03599.x)
20. Seng P, Drancourt M, Gouriet F, et al. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Clin Infect Dis.* 2009;49(4):543–551. doi: [10.1086/600885](https://doi.org/10.1086/600885)
21. Gaillot O, Blondiaux N, Loïez C, et al. Cost-effectiveness of switch to matrix-assisted laser desorption ionization-time of flight mass spectrometry for routine bacterial identification. *J Clin Microbiol.* 2011;49(12):4412. doi: [10.1128/JCM.05429-11](https://doi.org/10.1128/JCM.05429-11)
22. Tan KE, Ellis BC, Lee R, Stamper PD, Zhang SX, Carroll KC. Prospective evaluation of a matrix-assisted laser desorption ionization-time of flight mass spectrometry system in a hospital clinical microbiology laboratory for identification of bacteria and yeasts: a bench-by-bench study for assessing the impact on time to identification and cost-effectiveness. *J Clin Microbiol.* 2012;50(10):3301–3308. doi: [10.1128/JCM.01405-12](https://doi.org/10.1128/JCM.01405-12)
23. Carroll KC, Patel R. Systems for identification of bacteria and fungi. In: Jorgensen JH, Carroll KC, Funke G, et al, eds. *Manual of Clinical Microbiology.* 11th ed. John Wiley & Sons Inc; 2015:29–43.
24. Wieser A, Schneider L, Jung J, Schubert S. MALDI-TOF MS in microbiological diagnostics-identification of microorganisms and beyond (mini review). *Appl Microbiol Biotechnol.* 2012;93(3):965–974. doi: [10.1007/s00253-011-3783-4](https://doi.org/10.1007/s00253-011-3783-4)
25. Das S, Shibib DR, Vernon MO. The new frontier of diagnostics: molecular assays and their role in infection prevention and control. *Am J Infect Control.* 2017;45(2):158–169. doi: [10.1016/j.ajic.2016.08.005](https://doi.org/10.1016/j.ajic.2016.08.005)
26. Josko D. Molecular virology in the clinical laboratory. *Clin Lab Sci.* 2010;23(4):231–236. doi: [10.29074/ascls.23.4.231](https://doi.org/10.29074/ascls.23.4.231)
27. Fairfax MR, Bluth MH, Salimnia H. Diagnostic molecular microbiology: a 2018 snapshot. *Clin Lab Med.* 2018;38(2):253–276. doi: [10.1016/j.cll.2018.02.004](https://doi.org/10.1016/j.cll.2018.02.004)
28. Ramanan P, Bryson AL, Binnicker MJ, Pritt BS, Patel R. Syndromic panel-based testing in clinical microbiology. *Clin Microbiol Rev.* 2017;31(1):e00024–e17. doi: [10.1128/CMR.00024-17](https://doi.org/10.1128/CMR.00024-17)
29. US Food and Drug Administration. Nucleic acid based tests: microbial tests. FDA.gov. 2019. Accessed July 12, 2019. <https://www.fda.gov/medical-devices/vitro-diagnostics/nucleic-acid-based-tests>.
30. Deurenberg RH, Bathoorn E, Chlebowicz MA, et al. Application of next generation sequencing in clinical microbiology and infection prevention. *J Biotechnol.* 2017;243:16–24. doi: [10.1016/j.jbiotec.2016.12.022](https://doi.org/10.1016/j.jbiotec.2016.12.022)
31. Smock K, Perkins S. Examination of the blood and bone marrow. In: *Wintrobe's Clinical Hematology.* Williams and Wilkins; 2014:1–64.
32. Bray C, Bell LN, Liang H, et al. Erythrocyte sedimentation rate and C-reactive protein measurements and their relevance in clinical medicine. *WMJ.* 2016;115(6):317–321.
33. Surviving Sepsis Campaign: Society of Critical Care Medicine. SCCM.org. 2019. Accessed May 1, 2019. www.survivingsepsis.org.
34. Blokhuis-Arkes MH, Haalboom M, van der Palen J, et al. Rapid enzyme analysis as a diagnostic tool for wound infection: comparison between clinical judgment, microbiological analysis, and enzyme analysis. *Wound Repair Regen.* 2015;23(3):345–352. doi: [10.1111/wrr.12282](https://doi.org/10.1111/wrr.12282)
35. Tegl G, Schiffer D, Sigl E, Heinzele A, Guebitz GM. Biomarkers for infection: enzymes, microbes, and metabolites. *Appl Microbiol Biotechnol.* 2015;99(11):4595–4614. doi: [10.1007/s00253-015-6637-7](https://doi.org/10.1007/s00253-015-6637-7)
36. Karakioulaki M, Stolz D. Biomarkers in pneumonia-beyond procalcitonin. *Int J Mol Sci.* 2019;20(8):2004. doi: [10.3390/ijms20082004](https://doi.org/10.3390/ijms20082004)
37. Ramanan P, Wengenack NL, Theel ES. Laboratory diagnostics for fungal infections: a review of current and future diagnostic assays. *Clin Chest Med.* 2017;38(3):535–554. doi: [10.1016/j.ccm.2017.04.013](https://doi.org/10.1016/j.ccm.2017.04.013)