Petri Dishes to PCR: Navigating the Transition from Traditional to Molecular Diagnostic Techniques in Microbiology

Moiz Ahmed Khan

Department of Clinical Laboratory, Blood Bank and Infection Control, Tabba Heart Institute, Karachi, Pakistan

ABSTRACT

Clinicians have always been concerned with the prompt and accurate diagnosis of infectious diseases. With the advent of modern technology, such as automated identification systems and molecular testing, traditional methods such as microscopy and culture, which have been useful for decades, are no longer considered sufficient. This has consequently led to the idea that traditional methods are unnecessary and should be replaced completely with molecular methods. The author wants to shed light on the value of conventional methods, which represent some of the simplest and most cost-effective ways to diagnose infectious diseases. These methods also provide valuable information regarding antimicrobial susceptibility, which is instrumental for antimicrobial stewardship initiatives. The author recommends that conventional and molecular methods should be used in conjunction wherever applicable, rather than being used exclusively, especially in developing countries with limited molecular testing facilities.

Key Words: *Molecular techniques*, *Microbiology*, *Microscopy*, *Culture*, *PCR*, *MALDI-TOF*.

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Microbiology laboratory services have been profoundly affected in recent decades by the development and application of innovative diagnostic techniques. For many years, microscopy, culture, and biochemical testing have served as the primary means for microbial identification and susceptibility testing.[1] Although most laboratories are still performing these tests, molecular approaches are becoming more common. They are either supplementing current diagnostic algorithms or replacing conventional methods. In this communication, the author intends to define an inclusive strategy for microbiology laboratories so that both conventional and molecular methods can be used effectively for diagnosing infectious diseases.

Molecular techniques have consistently demonstrated their efficacy in diagnosing infectious diseases and have become the cornerstone for identifying various viral, parasitic, and culture-negative bacterial infections.[2-4] Various molecular techniques have been developed over the decades, each serving an instrumental role in elucidating distinct aspects of infectious diseases. Many laboratories around the world have developed and routinely use a wide range of commercially available nucleic acid-based tests.[5]

Correspondence to: Dr. Moiz Ahmed Khan, Department of Clinical Laboratory, Blood Bank and Infection Control, Tabba Heart Institute, Karachi, Pakistan
E-mail: moiz.khan@tabbaheart.org

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The polymerase chain reaction (PCR), in which target DNA is amplified by heating-cooling cycles involving specific DNA primers, substrate, and DNA polymerase, is commonly employed in nucleic acid-based assays.[6] Furthermore, real-time PCR involves monitoring the PCR reaction as it proceeds, to enable the detection of the final product and quantification of the initial DNA content.[7] RNA targets can also be amplified through similar methods.

In certain clinical situations, it is desirable to test for the presence or absence of a specific organism. Other scenarios might warrant evaluation of multiple potential pathogens in a clinical specimen, which has now become possible through a variety of advanced molecular techniques. One such technique is the multiplex real-time PCR, which allows for the simultaneous analysis of multiple nucleic acid targets in a single tube by using various primers with different detection markers.

The traditional phenotypic identification method for microorganism speciation has been replaced by sequencing. Technological developments and automation have made sequencing considerably more cost-effective, enabling its ubiquitous application in reference microbiology laboratories. Nucleic acid amplification, purification, and subsequent sequencing are the general steps in the standard sequencing process. Ribosomal RNA genes are the main targets due to their high level of conservation across microbial species. In order to identify the organism, the generated sequence is subsequently compared to reference databases.

Advances in mass spectrometry technology and its application to biomolecules have been the most significant and recent development in clinical microbiology laboratories. To separate and convert molecules into gas phase ions for spectrometry, thermal vaporisation is used in traditional mass spectrometry. Large biomolecules, however, are not suited for this method due to degradation. To overcome this limitation, matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS) was developed, which can analyse larger biomolecules, such as DNA, peptides, and proteins. For spectrometric analysis, samples are initially placed on a conductive sample support for UV-laser desorption and are then embedded in the crystalline structure of a small organic compound matrix. The resulting spectrum is compared with spectra from reference databases. MALDI-TOF MS can analyse samples rapidly with minimal sample preparation, identifying organisms to the genera or species level within minutes.

Conventional microbiology testing has typically involved the use of microscopy, culture, and serological methods. Microscopic evaluation of clinical specimens has been crucial in the initial identification of bacterial, fungal, and protozoal diseases.[1] While the results usually warrant confirmation through additional testing, in many situations, microscopy serves as the gold standard for identifying pathogens, especially stool parasites and Plasmodium species. In addition, electron microscopy is necessary for identifying viruses, however, it is rarely feasible in resource-limited settings.

Furthermore, microscopy provides significant benefits in terms of antimicrobial stewardship. A recent trial found that gram stain-based treatment was non-inferior to guidelines-based treatment for ventilator-associated pneumonia.[8] Additionally, it proved effective in limiting the need for broad-spectrum antibiotics in these cases. Since various studies have demonstrated the usefulness of microscopy, it is crucial to emphasise the importance of initial microscopy followed by a specified workflow when processing samples in a microbiology laboratory.[9]

Serological methods typically involve the detection of monoclonal antibodies in response to specific antigens. Though these tests are highly specific, and a positive result is usually diagnostic, their sensitivity is frequently low, and a negative result is often unhelpful. The detection of host humoral response to infection serves as the foundation for serological testing and hence, certain serological methods exhibit a high false positive rate due to nonspecific antibodies and cross-reactivity. Another significant limitation of these methods is the fact that the host must seroconvert in response to the infectious agent. As a result, serological methods are frequently useful in hindsight and are rarely applicable in the diagnosis of acute illness.

The main tool used in traditional testing is culture. Clinical specimens for fungi and bacteria are incubated after being inoculated on a variety of media. By using selective and differential media, a presumptive diagnosis can be made. Species identification is then typically achieved by biochemical testing, and antimicrobial susceptibilities are ascertained through several susceptibility testing methods. Cultures when positive, are considered as the gold standard in the diagnosis of infectious diseases.[10] Nevertheless, phenotypic testing when used exclusively can sometimes result in a difficult diagnosis at the species level and depending solely on incubation means that results may usually take days or even weeks to months, especially in cases of fungal and mycobacterial infections. In this regard, treatment delays of even a few hours can have a major negative impact on the course of a severe illness.

In addition, culturing pathogens in a microbiology laboratory also allows for antibiotic susceptibility testing of the isolated organisms. While modern genotypic techniques are promising in determining antimicrobial resistance, phenotypic methods are still preferred for antimicrobial susceptibility testing due to various reasons. Firstly, resistance to antimicrobials can be caused by various genetic determinants that may exceed the diagnostic capacities of current molecular techniques. Secondly, molecular approaches are costly and susceptible to contamination through carryover of genetic material in targeted molecular assays. Thirdly, the normal flora in clinical samples may also contain resistance determinants. In such situations, molecular techniques have limited discriminatory power and require caution when interpreting data.[11] Additional scenarios where culture methods play an instrumental role include determining the minimum inhibitory concentration of antimicrobial agents against specific isolates and preparing hospital antibiograms, both of which are vital in the clinical practice of infectious diseases.

Furthermore, the Ziehl-Neelsen staining remains the primary method for early detection and treatment of tuberculosis (TB) in resource-limited settings. In rural areas, in the absence of relevant culture facilities, microscopically performed sputum smear conversion is an important predictor for anti-tubercular treatment response among TB patients.[12] Also, microscopy can detect organisms in sterile body fluids, such as cerebrospinal fluid and blood, allowing for early diagnosis and treatment. Lastly, the importance of microscopy in the diagnosis of parasitic infections cannot be over-emphasised. Microscopy is the preferred method for detecting medically important parasites, such as plasmodium species, microfilaria, amoebae and helminths, in peripheral blood smears, and wet mount preparations of stool specimens, due to labour-intensive culture methods, stringent biosafety measures, and costly and technologically demanding molecular techniques.

The ultimate goal of pathogen detection in a diagnostic microbiology laboratory should be achieved by utilising both conventional and molecular techniques. Each of the techniques has its limitations when employed exclusively. For instance, selecting the right medium, incubation conditions, and distinct cultivable clones are essential for assuring optimal culture yield.[13] Furthermore, for ensuring accurate culture results, it is necessary that specimens with a high load of the suspected pathogen are inoculated in addition to sampling an optimal volume of specimens from anatomic sites that are comparatively free of commensals or colonizers.[13] On the other hand, recent molecular techniques may be able to identify most of the culturable, not-

yet-culturable, and previously uncultured microbes from mixed microbial populations in clinical specimens.

The application of conventional methods appears to be beneficial for certain reasons, even though molecular methods generally outperform traditional techniques such as culture. While traditional methods are often less expensive, molecular diagnostic testing necessitates expensive reagents, sophisticated automated equipment, and expertise. Screening for important pathogens from a mixed microbial population using selective culture media tends to produce faster results than metagenomic analysis. Furthermore, the distinction between viable and non-viable organisms cannot be made using molecular techniques.

Research studies in past have demonstrated the ability of molecular techniques for supplementing culture-based methods in identifying organisms that are missed by culture.[14,15] There have also been suggestions that, although rapid diagnostic tests such as automated molecular assays are able to make an early diagnosis, enabling prompt treatment initiation, they should not be used in place of conventional methods as they are less thorough and may not be feasible in resource-limited settings.

Comprehensive research is required to guide clinical microbiologists regarding the most appropriate strategy for diagnosing infectious diseases, incorporating both molecular and conventional methods. Until such an inclusive strategy is available, microbiology laboratories in developing countries with limited resources must rely more on traditional microbiological techniques for detecting infectious diseases.

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The author declared no conflict of interest.

AUTHOR'S CONTRIBUTION:
MAK: Conceptualisation, literature review, and writing the original draft.

REFERENCES

1. Austrian R. The gram stain and the etiology of lobar pneumonia, an historical note. *Bacteriol Rev* 1960; **24(3)**: 261-5. doi: 10.1128/br.24.3.261-265.1960.
2. Cobo F. Application of molecular diagnostic techniques for viral testing. *Open Virol J* 2012; **6**:104. doi: 10.2174/1874357901206010104.
3. Grijalva M, Horvath R, Dendis M, Enry J, Benedik J. Molecular diagnosis of culture negative infective endocarditis: Clinical validation in a group of surgically treated patients. *Heart J* 2003; **89(3)**:263-8. doi: 10.1136/heart.89.3.263.
4. Wong SSY, Fung KSC, Chau S, Poon RWS, Wong SCY, Yuen KY. Molecular diagnosis in clinical parasitology: When and why? *Exp Biol Med* 2014; **239(11)**:1443-60. doi: 10.1177/1535370214523880.
5. Lawn SD, Mwaba P, Bates M, Piatek A, Alexander H, Marais BJ, *et al*. Advances in tuberculosis diagnostics: The xpert MTB/RIF assay and future prospects for a point-of-care test. *Lancet Infect Dis* 2013; **13(4)**:349-61. doi: 10.1016/s1473-3099(13)70008-2.
6. Mullis KB, Faloona FA. Specific synthesis of DNA in vitro *via* a polymerase-catalyzed chain reaction. *Methods Enzy-mology* 1987; **155**:335-50. doi: 10.1016/0076-6879(87)55023-6.
7. Yu ACH, Vatcher G, Yue X, Dong Y, Li MH, Tam PHK, *et al*. Nucleic acid-based diagnostics for infectious diseases in public health affairs. *Front Med* 2012; **6(2)**:173-86. doi: 10.1007/s11684-012-0195-5.
8. Yoshimura J, Yamakawa K, Ohta Y, Nakamura K, Hashimoto H, Kawada M, *et al*. Effect of gram stain-guided initial antibiotic therapy on clinical response in patients with ventilator-associated pneumonia: The GRACE-VAP randomized clinical trial: The GRACE-VAP randomized clinical trial. *JAMA Netw Open* 2022; **5(4)**:e226136. doi: 10.1001/jamanetworkopen.2022.6136.
9. Jaggi N, Pandey A, Jain A. Abnormal morphology of Klebsiella pneumoniae in respiratory samples: A microscopic observation. *Indian J Applied Microbiol* 2019; **22(1)**:51-4. doi: 10.46798/ijam.2019.v22i01.006.
10. Basu S, Shetty A. Laboratory diagnosis of tropical infections. *Indian J Crit Care Med* 2021; **25(S2)**:S122-6. doi: 10.5005/jp-journals-10071-23813.
11. Louie M, Cockerill FR 3rd. Susceptibility testing. Phenotypic and genotypic tests for bacteria and mycobacteria. *Infect Dis Clin North Am* 2001; **15(4)**:1205-26. doi: 10.1016/s0891-5520(05)70191-4.
12. Asemahagn MA. Sputum smear conversion and associated factors among smear-positive pulmonary tuberculosis patients in East Gojjam Zone, Northwest Ethiopia: A longitudinal study. *BMC Pulm Med* 2021; **21(1)**:118. doi: 10.1186/s12890-021-01483-w.
13. Vidyarthi A, Das A, Gupta A. Revisiting conventional microbiology techniques in the era of molecular testing. *Curr Med Res Pract* 2022; **12(6)**:274. doi: 10.4103/cmrp.cmrp_60_22.
14. Rudkjobing VB, Thomsen TR, Xu Y, Kreft RM, Ahmed A, Eickhardt S, *et al*. Comparing culture and molecular methods for the identification of microorganisms involved in necrotizing soft tissue infections. *BMC Infect Dis* 2016; **16(1)**:652. doi: 10.1186/s12879-016-1976-2.
15. Banerjee T, Das A, Singh A, Bansal R, Basu S. The microflora of chronic diabetic foot ulcers based on culture and molecular examination: A descriptive study. *Wound Manag Prev* 2019; **65(5)**:16-23. doi: 10.25270/wmp.2019.5.1623.