Review Article

Molecular Diagnostics versus Traditional Culture Method for Diagnosis of Infectious Diseases

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ABSTRACT

Molecular diagnostic (MDx) techniques have revolutionized the field of clinical diagnostics, providing several advantages over traditional microbial culture methods. The ability to perform multiplex molecular panel tests is a significant advancement that allows for the simultaneous detection of multiple pathogens from a single specimen. Multiplex panels are available for various types of infections, including respiratory, gastrointestinal, urinary, and sexually transmitted infections. This versatility makes them valuable tools in different clinical scenarios. The integration of these advanced diagnostic tools into routine clinical practice holds great promise for improving patient outcomes and public health.

Keywords: *Culture, Molecular diagnostics, PCR, Infectious disease.*

INTRODUCTION

Microbial culture is traditionally the gold standard for the detection of many infectious pathogens[1-6]. However, there are limitations. Limitations of culture methods include long turnaround times, labor-intensive processes, and difficulty in identifying difficult-to-culture microorganisms. Molecular diagnostics (MDx), especially nucleic acid amplification tests (NAATs), offer several advantages over traditional culture methods. MDx technologies are faster, more sensitive, and more specific in detecting infectious pathogens. NAATs can detect organisms that may be missed by routine culture. Worldwide infectious disease experts already have acknowledged the advantages of NAAT-based diagnostics. The Infectious Diseases Society of America (IDSA) and the American Society for Microbiology recommend the use of NAATs, stating that they have largely replaced rapid antigen tests and culture for respiratory virus detection[7]. MDx, particularly NAATs, have played a pivotal role in the detection of infectious agents during the coronavirus crisis. The US Centers for Disease Control and Prevention (CDC) notes that laboratory-based NAATs are considered he most sensitive tests for detecting SARS-CoV-2, the virus responsible for COVID-19[8]. The World Health Organization (WHO) states that confirmation of monkeypox infection relies on NAAT, specifically using real-time or conventional polymerase chain reaction (PCR) to detect unique sequences of viral DNA[9].

ADVANTAGES OF MOLECULAR DIAGNOSTICS

Besides being quicker and more accurate than microbial culture, MDx has facilitated the development of multiplex molecular panels, enabling comprehensive testing for a range of potential pathogens in a single test from a single specimen. This advancement has implications for various clinical scenarios:

1. Comprehensive Testing with Multiplex Molecular Panels:
   - Multiplex molecular panels can test for a breadth of potential pathogens in a single test, providing a more comprehensive view of the microbial landscape in a given specimen.
   - In cases where the dominant symptom is non-specific, such as diarrhea, clinicians can order a single test that covers multiple

pathogens, avoiding the need for multiple individual tests.

2. Challenges in Traditional Testing:
   - Traditional testing methods, such as pathogen-specific cultures, may be less effective in identifying causative agents, especially when symptoms are ambiguous or multiple pathogens are involved.
   - Despite repeated testing using individual tests, the causative agents of certain symptoms may remain unknown, making diagnosis difficult and treatment nonspecific.
3. Applications Across Different Infections:
   - Multiplex molecular panel tests are available for various types of infections, including gastrointestinal, respiratory, urinary, and sexually transmitted infections.
   - These tests offer a streamlined approach for diagnosing a range of conditions, from upper respiratory tract infections to urinary tract infections, vaginal discharge, and fevers of unknown origin.
4. Applications Across Different Infections:
   - The faster and more accurate diagnosis provided by multiplex molecular panels allows for earlier and more specific treatments.
   - This streamlined approach has the potential to lead to better clinical outcomes by enabling targeted interventions based on accurate identification of the causative agents.
5. Reduction in Inappropriate Antibiotic Usage:
   - By facilitating a more specific diagnosis including resistance gene information, multiplex molecular panels may contribute to a reduction in inappropriate antibiotic usage.
   - Clinicians can tailor treatments based on the identified pathogens, avoiding unnecessary and broad-spectrum antibiotic prescriptions.

Gastrointestinal System

There are challenges associated with the traditional culture-based diagnosis of Campylobacter infections in the gastrointestinal system and the advantages of using molecular methods, such as PCR and quantitative PCR (qPCR), for the detection of Campylobacter and other gastrointestinal pathogens[10]. Campylobacter is a leading cause of human gastroenteritis worldwide, particularly affecting vulnerable populations. Difficulties in culturing Campylobacter contribute to its underdiagnosis. Campylobacter species are fastidious, requiring specific growth conditions, including a microaerobic environment and a temperature of 42°C. Culture-based diagnosis involves stool sample implantation into a selective medium and a lengthy incubation period (about 72 hours), followed by an additional time for bacterial identification (up to 7 days). Molecular tests are found to be superior to culture-based methods in terms of sensitivity, specificity, and positive predictive value for Campylobacter detection. In a study comparing culture and culture-independent tests, PCR identified more Campylobacter-positive specimens than culture (sensitivity of 51.2% for culture)[11]. The Global Enteric Multicenter Study (GEMS) showed higher attributable incidences of Campylobacter and other gastrointestinal pathogens with qPCR compared to traditional microbiological methods[12]. qPCR demonstrated higher sensitivity for various pathogens, including adenovirus, Shigella spp / Enteroinvasive Escherichia coli (EIEC), and heat-stable enterotoxin-producing Escherichia coli. There has been a marked increase in the use of culture-independent diagnostic tests (CIDTs) for detecting Campylobacter, Salmonella, Shigella, and other gastrointestinal pathogens[13-16]. Healthcare providers in developed countries are more likely to order CIDTs and DNA-based syndromic panels due to their speed and ease of use compared to traditional culture methods. Centers for Disease Control (CDC) and Prevention confirms the trend of increased use of CIDTs over the last decade.

In summary, the shift towards molecular methods, particularly PCR and qPCR, for the diagnosis of

Campylobacter and other gastrointestinal pathogens is driven by their faster turnaround time, higher sensitivity, and ease of use compared to traditional culture-based methods. This transition has implications for improving the accuracy and efficiency of diagnosing and managing gastrointestinal infections.

Respiratory System

The utility of multiplex molecular panels, particularly in the context of detecting causative agents in atypical pneumonia is underscored. Atypical pneumonias historically posed challenges due to their varied symptoms, distinct chest X-ray appearances, and poor response to standard antibiotics. The term "atypical" now refers to pneumonias that are difficult to detect through traditional bacterial methods like culture[17]. The findings from a study highlight the effectiveness of multiplex molecular panels, specifically multiplex reverse transcription polymerase chain reaction (RT-PCR), in identifying bacterial pathogens causing atypical pneumonia[18]. Historically, atypical pneumonias were characterized by different symptoms, distinctive chest X-ray appearances, and poor response to standard antibiotics. Currently, the term "atypical" is associated with pneumonias that are challenging to detect through standard bacterial methods like culture. The study[18] focused on patients with symptoms of atypical pneumonia who tested negative for typical pneumonia agents through both culture and viral PCR. Researchers used multiplex RT-PCR to detect key bacterial pathogens causing atypical pneumonia in these cases. Among 368 samples that were culture- and viral PCR-negative, multiplex RT-PCR identified specific bacterial pathogens. Positive results were observed for Bordetella parapertussis, Bordetella pertussis, Chlamydia pneumoniae, Chlamydia psittaci, Legionella pneumophila, Legionella spp., and Mycoplasma pneumoniae. Co-infection of Bordetella pertussis and Bordetella parapertussis was observed in two patients. Multiplex molecular panels, by accurately identifying causative pathogens, have the potential to improve the diagnosis of atypical pneumonia. Accurate identification of pathogens is crucial for guiding appropriate patient management and treatment strategies. This technology enables the identification of specific bacterial pathogens that may go undetected using traditional culture and viral PCR methods, leading to improved diagnostic accuracy and subsequent patient management.

Urinary System

Culture and MDx differ in their ability to detect co-infection or polymicrobial infection. A study involving 582 patients with lower urinary tract infections, where PCR demonstrated significantly higher sensitivity compared to urine culture in detecting polymicrobial infections[19]. Among the 175 patients with polymicrobial infections, PCR reported 95% of cases, whereas culture only reported 22%. PCR revealed polymicrobial infections in an additional 67 patients who had negative culture results. This indicates that PCR has the ability to detect infections that may be missed by traditional culture methods. Simultaneous detection of various pathogens through PCR can have implications for clinical management, enabling more specific treatments and reducing the likelihood of recurrent infections resulting from inadequate or inappropriate treatments. Additionally, the combination of multiplex PCR with pooled antibiotic sensitivity testing enhances the microbiological data obtained from standard urine culture methods[20].

Sexually Transmitted Diseases and Other Infections

The emphasis on NAATs and MDx in the recommendations by CDC, and WHO highlighted already the importance of these advanced diagnostic methods in the detection, confirmation, and monitoring of infectious diseases, particularly those with implications for public health, such as sexually transmitted diseases such as Chlamydia trachomatis and Neisseria gonorrhoeae and viral infections like HIV and Hepatitis[7, 21]. The third WHO Model List of Essential In Vitro Diagnostics includes PCR for Pneumocystis jirovecii, the fungus causing Pneumocystis pneumonia, and a

human measles reverse transcription-PCR (RT-PCR) test, which accurately confirms cases and helps prevent outbreaks. The ability to provide rapid and accurate results, along with the noninvasive nature of specimen collection, makes these methods invaluable in diverse clinical settings.

BENEFITS OF CULTURE

Here are the key points:

1. Role of Microbial Culture: Microbial culture serves as a benchmark for diagnosing many infectious diseases. This method is particularly relevant in areas where MDx technologies are underdeveloped, costly, require skilled personnel, or may yield results that are clinically insignificant.
2. Antimicrobial Susceptibility Testing: Culture is crucial for determining antimicrobial susceptibility, which is essential for tailoring effective treatment plans. Examples include assessing susceptibility in patients with Mycobacterium tuberculosis infections and pregnant women with penicillin allergy who have Group B Streptococcus Colonization[22-23].
3. Molecular Subtyping: In addition to antimicrobial susceptibility data, culture may be necessary for molecular subtyping. This information is vital for identifying antibiotic resistance, tracking outbreaks, and monitoring disease trends.
4. Public Health Surveillance: The CDC encourages the use of reflex culture in laboratories for bacteria of public health importance. Reflex culture involves culturing specimens that have tested positive using CIDT (Culture-Independent Diagnostic Tests). This is particularly relevant for bacteria like Campylobacter, Salmonella, Shigella, Shiga toxin-producing Escherichia coli (STEC), Vibrio, and Yersinia infections [13-16].
5. Importance of Information for Surveillance: The information obtained through culture is crucial for public health surveillance. It aids in identifying and monitoring the prevalence of specific pathogens, tracking antibiotic resistance patterns, and responding to outbreaks effectively.

CONCLUSION

The decision to supplement culture with Molecular Diagnostics (MDx) for infectious diseases indeed involves various factors such as, technology performance, regulatory considerations, financial aspects, accessibility, and awareness. Laboratories that have embraced MDx infrastructure can leverage their capabilities not only for the recent pandemic but also for a broader range of infectious diseases, contributing to more effective disease management and public health responses.

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