Research & Reviews: Journal of Veterinary Sciences e-ISSN: 2581-3897

# Future Trends of Molecular Diagnostics in Clinical Bacteriology

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Commentary

**Received:** 08-Nov-2021, Manuscript No. JVS-21-46918**; Editor assigned:** 10-Nov-2021, Pre QC No. JVS-21-46918(PQ); **Reviewed:** 24-Nov-2021, QC No. JVS-21-46918; **Revised:** 28-Feb-2022, Manuscript No. JVS-21-46918(R); **Published:** 08-Mar-2022, DOI: 10.4172/2581- 3897.6.3.e002.

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## ABOUT THE STUDY

Molecular diagnostics of infectious diseases, especially nucleic acid-based methods, are the fastest growing areas of laboratory diagnostics. These applications are gradually replacing or supplementing culture-based biochemical and immunological assays in the Institute of Microbiology. First-generation nucleic acid assays, like conventional tests, were single parameter and determined only a single parameter. Technology improvements and new approaches open up the possibility of developing multi-parameter assays using microarrays, multiplex nucleic acid amplification techniques, or mass spectrometry, and the introduction of closed-tube systems for rapid microbial diagnostics with a subsequently reduced contamination risk.

## DESCRIPTION

Although the initial assay focused on the detection and identification of microbial pathogens, these new technologies paved the way for parallel determination of multiple antibiotic resistance determinants or microbial epidemiology and monitoring at the genetic level. Detection, identification, and drug susceptibility testing of microbial pathogens represent is a key duty of microbial diagnosis in medicine. Antibiotic susceptibility testing, in particular, provides important information for making appropriate treatment decisions. Epidemiological genotyping of isolated microorganisms for transmission route monitoring is also very important. This is an essential task in developing strategies to prevent or treat infections in both communities and healthcare facilities. Recent advances, extensive research on microorganisms, and the development of new nucleic acid-based methods have increased the use of molecular assays in clinical laboratories, making several commercial tests available.

## Phenotype-based methods

One of the oldest, but still very important, methods in clinical bacteriology is the detection of human pathogens by direct microscopy of samples. Many different staining methods are available, allowing the first rough classification of detected organisms. However, the final characterization and identification depends on the phenotypic characteristics of the organism, even after culturing in the appropriate medium. It improves quality and efficiency and meets high standardization requirements.

The development of the immunoassay has enabled rapid detection and identification of microorganisms without culturing for the first time. The assay detects the presence of a specific antibody produced in response to a pathogenic antigen, or the antigen itself. This technology is available in a variety of formats, including enzyme immunoassays, immunofluorescence assays, latex agglutination assays, line immunoassays, and immunoflow immunoassays. Although direct antigen testing on clinical samples provides rapid and specific identification results, antigen detection still suffers from insensitivity and requires a relatively large amount of each antigen.

## Genotype-based methods

Culture confirmation or direct detection of microorganisms can be performed by direct hybridization assay using labeled oligonucleotide probes. Probe hybridization helps identify slow-growing organisms after isolation in culture using liquid or solid media. Using strict reaction conditions, these probe-based assays show high specificity. Direct hybridization assays to identify bacteria require a large number of target cells, which leads to a specific lack of sensitivity. This drawback can be partially avoided by targeting rRNA molecules with high copy counts per cell.

Fluorescence *in Situ* Hybridization (FISH) is an attractive method for the rapid detection and identification of bacteria or fungi directly from slide smears. This technique combines the speed and ease of use of traditional staining methods with the peculiarities of molecular methods. Hybridization with fluorescently labeled probes targeting rRNA is performed on smears using a fluorescence microscope for detection.