

# Importance of Molecular Diagnostic Methods for Infectious Diseases

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## COMMENTARY

Molecular methods are no longer available only to specialized centers and now play an important role in the diagnosis of common infections seen in ambulatory practice. It is especially for the detection of pathogens that are difficult to cultivate, especially viruses that PCR has become the reference method. While it has replaced traditional methods for some indications, PCR is not applicable in all cases, and it is not foolproof. It is therefore important to know when to use molecular methods, what are their strengths and weaknesses, in order to be able to prescribe them rationally. This article reviews the characteristics of molecular tests and their main applications in outpatient practice.

The direct detection of the microbe by microscopy or by characterization of its molecular structures (proteins or nucleic acids) and the measurement of humoral immune response (specific antibodies) are the main tools of the laboratory diagnosis of infectious diseases. The molecular diagnostics methods consist to detect and analyze the genome of an organism even if antibodies and antigens are molecular structures. The first methods of DNA analysis already existed in the 1970s [1] but the development of Polymerase Chain Reaction (PCR) appeared in the mid-1980s [2,3]. Right after, the possibility of amplifying DNA and RNA (ribonucleic acid) was used for the detection of pathogens and PCR was considered as a central method of the microbiology laboratory thirty years later.

Molecular methods allow diagnosis in the acute phase of a disease, before the appearance of antibodies exactly like antigen detection. However, few days or even a few weeks are necessary to detect the presence of antibodies in the serum [4]. In the acute phase, a positive PCR demonstrates most often an infection, whereas a negative serology must be repeated within a suitable time to demonstrate seroconversion [4,5].

By allowing the detection of pathogens not or difficult to cultivate, molecular methods have revolutionized medical microbiology, mainly for the diagnosis of viral diseases. Indeed, virus culture requires immortalized human or animal cell lines and may take several weeks. It is less and less used and has been largely supplanted by PCR, which has also replaced serology for many indications, such as acute herpes or varicella zoster infection or shingles [5,6].

Contrary to viruses, molecular techniques have not replaced cultures for bacteria or fungi. However, the detection of certain slow-growing bacteria requiring very specific culture conditions (mycobacteria, Chlamydia) has also been improved and simplified by the development of specific PCRs. PCR detection is available for many parasitic infections such as toxoplasmosis, leishmaniasis, amebiasis or malaria [7]. For these diseases, traditional methods (microscopy or serology) are still widely used.

In addition to simple detection, modern molecular methods can also provide quantitative indications, for example to monitor the evolution of HIV viral load or hepatitis C under therapy, and thus detect the emergence of resistant strains. This quantification is also useful for the diagnosis of latent virus reactivations such as polyomavirus BK or CMV (cytomegalovirus) in immunosuppressed patients [6].

Although DNA sequencing was developed before PCR, all modern methods are based on it. Its most common application in microbiology is the detection and identification of bacteria by sequencing ribosomal DNA. The sequence of this gene being specific to a species or a family, identification is made possible, also when the bacterium is not or more. The detection of mutations resulting from resistance to therapies is another application of sequencing, used, for example, for the characterization of HIV.

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