IJMS
Vol 37, No 3, September 2012

Commentary

Introducing New Laboratory-Developed Molecular Methods in the Clinical Microbiology Laboratories

The verification of laboratory-developed tests aims to characterize and compare the diagnostic accuracy of a new method to that of a reference one accepted by the laboratory community as the standard of care for a particular analyte or disease.[1] The article entitled "Evaluation of a PCR assay to detect *Enterococcus faecalis* in blood and determine glycopeptides resistance genes: Van A and Van B" published in the current issue of the Iranian Journal of Medical Sciences (page 194-199), seeks to verify a laboratory-developed multiplex PCR assay.

Over the past two decades, molecular methods such as polymerase chain reactions (PCR) have been in use in the areas of infectious diseases including diagnostic work-ups of bloodstream infections.[2] Clinical pathogens can be detected and identified earlier and more accurately by PCR methods. As reported, such methods with lower detection limit of three colony-forming units of bacteria/ml, could identify organisms missed by blood culture.[2] Moreover, direct detection of resistant organisms in clinical samples by PCR methods are already available in many clinical laboratories.[2]

The enterococci are usually recognized as the second or third most frequent nosocomial pathogens, predominantly inhabit in the gastrointestinal tract, and act as opportunistic agents. Among the least 20 species isolated from human sources, *E. faecalis* and *E. faecium* are the most frequent ones accounting for at least 85% of the isolates. Enterococci, as already reported, have exhibited resistance to glycopeptide, vancomycin and teicoplanin, by seven types of respective genes. Van A and Van B are considered the most clinically relevant phenotypes and are usually associated with *E. faecalis* and *E. faecium* isolates. Other types of glycopeptide resistance encoded by the Van D, Van E, Van G, and Van L genes are also reported in *E. faecalis*.[3]

In the above-mentioned study, the identifications of *E. faecalis* and the two glycopeptide resistance genotypes described for enterococci has been based on specific amplification of fragments intragenic to *ddl E. faecalis* gene and specific amplification of portions of the V*an A* and V*an B* genes, respectively. Three pairs of oligodeoxynucleotides, developed by Dutka-Malen and colleagues,[4] to prime the amplifications of these fragments, were used. They,[4] developed a PCR assay that allows simultaneous detection of four glycopeptide resistance genotypes (*Van A*, *Van B*, *Van C-1*, and *Van C-2*) and identification of the species level of clinically relevant enterococci (*Enterococcus faecium*, *E. faecalis*, *E. gallinarum*, and *E. casseliflavus*). The amplification of portions of the 16S rRNA (*rrs*) gene, present in almost all the bacteria, was included as an internal PCR control to promote the reliability of the assay.[5]

According to the Clinical Laboratory Improvement Act (CLIA), to verify a new assay it is critical to statistically determine if the assay performance specifications are acceptable compared to a defined standard reference method.[1] These specifications include analytical sensitivity and specificity, accuracy, precision, and any other characteristics required to test the performance and interpretation of results. The performance of clinical test can be evaluated using clinical sensitivity and specificity, receiver operating characteristic (ROC) analysis, and positive predictive and negative predictive values of the tests.[6] In the above-mentioned study, the authors tried to verify the PCR assay by determining its analytical sensitivity and specificity. The major limitation of the study is that for the determination of analytical specificity, the assay should have been tested with closely-related or genetically similar organisms to assess any cross-reactivity with other organisms. However, only two standard strains of vancomycin-resistant enterococci were used to evaluate the test. Typically, for an analytical sensitivity, one should perform minimum 12 repetitions of each target level (low, middle and high) and one level at least 1 log below the expected limit of detection.[6] Therefore, the reported analytical specificity and sensitivity need further studies to be confirmed, and clinical specificity and sensitivity remained to be determined.

Conflict of interest: none declared

Please cite this article as: Pouladfar GhR. Introducing New Laboratory-Developed Molecular Methods in the Clinical Microbiology Laboratories. Iran J Med Sci. 2012;37(3):200-201.

Gholamreza Pouladfar
Professor Alborzi Clinical Microbiology Research Center, Nemazee Hospital, Shiraz University of Medical Sciences, Shiraz, Iran.

Correspondence:
Gholamreza Pouladfar MD,
Professor Alborzi Clinical Microbiology Research Center,

Nemazee Hospital,
Nemazee Square,
Shiraz, Iran.
Tel: +98 711 6474304
Fax: +98 711 6474303
Email: pooladfar@sums.ac.ir
pooladfar@yahoo.com
Received: 3 May 2012
Revised: 28 June 2012
Accepted: 1 July 2012

References

1 Subpart K. Centers for Disease Control and Prevention. Clinical laboratory Improvement Advisory Committee, 493.2004, (cited: 4 June 2012) Available from: http://wwwn.cdc.gov/clia/regs/toc.aspx
2 Ecker DJ, Sampath R, Li H, Massire C, Matthews HE, Toleno D, et al. New technology for rapid molecular diagnosis of bloodstream infections. Expert Rev Mol Diagn. 2010;10:399-415. doi: 10.1586/erm.10.24. PubMed PMID: 20465496.
3 Arias CA, Murray BE. The rise of the Enterococcus: beyond vancomycin resistance. Nat Rev Microbiol. 2012;10:266-78. doi: 10.1038/nrmicro2761. PubMed PMID: 22421879.
4 Dutka-Malen S, Evers S, Courvalin P. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. J Clin Microbiol. 1995;33:24-7. PubMed PMID: 7699051.
5 Kariyama R, Mitsuhata R, Chow JW, Clewell DB, Kumon H. Simple and reliable multiplex PCR assay for surveillance isolates of vancomycin-resistant enterococci. J Clin Microbiol. 2000;38:3092-5. PubMed PMID: 10921985; PubMed Central PMCID: PMC87194.
6 Wolk DM, Marlowe EM. Molecular method verification. In: Persing DH, Tenover FC, Tang YW, Nolte FS, Hayden RT, Belkum AV, editors. Molecular microbiology: diagnostic principles and practice. 2nd ed. Washington, DC: ASM Press; 2011. p. 861-83.