LABORATORY MEDICINE

POLYMERASE CHAIN REACTION IN CLINICAL PRACTICE

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One of the most heralded developments in basic science to reach clinical application in recent years has been the Polymerase Chain Reaction (PCR).

PCR has been applied in various areas of clinical medicine including rapid diagnosis of viral, bacterial, fungal and parasitic disease, the diagnosis and prediction of inherited disease, the detection of an association between certain viruses and specific cancers, the detection of organ transplant rejection and HLA subtyping. In basic research PCR is useful in identification of point mutation, deletion, insertions, rearrangements, amplifications and translocations.

Diagnostic PCR is a technique of DNA amplification that uses specific DNA sequences to serve as markers for the presence ofmicroorganisms and is in theory capable of detecting a single organism in a biologic specimen such as sputum, lavage fluid, cerebrospinal fluid, pleural fluid, blood or any other specimen including nail and hair[1].

Types of PCR

Numerous modifications of the standard PCR procedure have been developed since its inception[2-4].

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1. Reverse-transcriptase (RT) PCR was developed to amplify RNA targets. RT PCR has played a important role in diagnosing RNA containing virus infections, detecting viable mycobacteria species and monitoring the effectiveness of antimicrobial therapy. Commercial kits are now available for detection of HIV using single new enzyme (DNA polymerase Tth pol) technology.

2. Nested PCR designed mainly to increase sensitivity uses two sets of amplification primers[2,5]. One set of primers is used for the first round of amplification and the amplification products of the first round are subjected to a second round of amplification with another set of primers. Nested PCR has extremely high sensitivity because of dual set of amplification process.

3. Multiplex PCR in which two or more sets of primers from the same organism or even different organism are introduced into the same tube. This increases the sensitivity as well as cuts cost.

4. Broad range PCR: Another important technical modification is the development of broad-range PCR in which conserved sequences within phylogenetically informative targets are used to diagnose microbial infection. This technique has identified several fastidious or uncultivatable bacterial pathogens directly from infected human tissue or blood. There are other amplification methods like ligase chain reaction, QB replicase system and strand displacement amplification which has not been evaluated on a large scale[2].

PCR is very useful to detect the micro-organisms which are either unculturable or extremely fastidious or hazardous to laboratory personnel. PCR has made it easy to detect such organisms directly from inactivated clinical specimens without exposing the laboratory personnel to live organisms. PCR has been successfully used in clinical and epidemiological investigations and control of emerging nosocomial pathogens such as Pseudomonas aerugenosa, Staphylococcus aureus, coagulase-negative Staphylococci, Enterococci, Chlamydia pneumoniae[6-8] and Candida albicans and viruses like respiratory syncytial virus, varicella zoster virus, herpes simplex virus and legionella. The ability to rapidly and unambiguously characterise organisms suspected of causing a disease outbreak is critical to public health and hospital infection-control endeavors.

Robert Wadowsky[9] have recommended a multiplex PCR assay for the detection of Bordetella pertussis in nasopharyngeal swab specimens. Van eys[10] was first to develop PCR for detection of leptospires in urine samples. The samples containing as little as 10 leptospires give positive results. Diagnostic laboratories all around the world are increasingly faced with challenge of detecting rabies virus(RV) and rabies related (RRV) viruses. A hemi nested reverse transcriptase PCR protocol is more sensitive to florescent antibody test (FAT) which is the commonly used test because it is able to detect RV even in the decomposed brain tissue whereas FAT can detect only when the brain tissue is fresh.

A multiplex PCR procedure was developed for the simultaneous detection of Alloicoccus otitidis. Haemophilus influenza, Moraxella catarrhalis and Streptococcus pneumoniae in the middle ear effusions from patients with chronic otitis media with effusion. By conventional culture method 8 (32%) of the specimens showed growth of one of the organisms tested in contrast 21 (84%) of the specimens tested positive by the multiplex PCR[11]. The advent of DNA based methods for causative organisms may improve the diagnostic yield in cases of culture negative endocarditis. However, the sensitivity and specificity and positive predictive value of these new technologies is unknown and like serology the result should be interpreted with caution.

PCR has become popular in the field of tuberculosis diagnosis, because the conventional methods are less sensitive and time consuming. For example smear microscopy is less sensitive with a limitation that only when a patient excretes 25000 bacilli in sputum it can detect, and it takes 4 weeks to culture M. tuberculosis Tuberculosis is the largest killer among the infectious diseases. With the emergence of HIV epidemic and multidrug resistance there is utmost necessity to improve diagnostic methods of Mycobacterium tuberculosis using advanced molecular methods. PCR has a lot of promise to offer in the detection of extra pulmonary tuberculosis where culture, which is the gold standard, often fails to detect the organism. The great potential of PCR in diagnosing tuberculosis was revealed particularly in relation to speed and sensitivity.

The commercial PCR assay recently being evaluated (Amplicor Roche Molecular Systems, Somersille, New Jersey) is based on amplification of a 584 bp region of the gene encoding 16sRNA. This approach could permit simultaneous testing for several species of mycobacteria in a single assay using a series of species-specific oligonucleotide in separate wells. Recent publications have described the various evaluation experiments

undertaken using respiratory specimens[12-14]. As was the case with the research assays the sensitivity with smear positive specimens was excellent around 95% but with smear negative/culture positive specimens, the sensitivity was lower. However some culture negative samples are positive by PCR. Analysis of these samples suggest that they are not false positive.

The risk of false positive results due to carry over of target DNA from a positive to a negative sample is probably the single greatest hurdle that is in the way of the clinical application of PCR diagnosis. The extremely high sensitivity afforded by exponential amplification of DNA, which renders it very powerful also exposes it to the risk of contamination by minute quantities ofexogenous DNA. Contaminating DNA may come from clinical specimens containing large numbers of target molecules from bacterial cultures sometimes used as positive control in molecular tests or from plasmid DNA generated in cloning specific M. tuberculosis DNA sequences. Most frequently the problem arises from the accumulation in the laboratory of PCR amplicons. False positive results can be avoided by following a number of specific precautions including physical isolation of pre and post amplification procedures, the use of disposable materials (gloves, sterile aerosol-resistant tips) pre aliquoted reagents, and dedicated positive- displacement pipettes, together with careful choice of negative and positive controls. An enzymatic method using an uracil-N-glycosylase and substituting dUTP instead of dTTP in the PCR and photochemical method with psoralen has been advocated to reduce false positivity. Just like false positivity, PCR can report false negative results due to presence of exogenous inhibitors like anticoagulants and detergents and endogenous inhibitors in clinical specimens. DNA processing methods can be improved to remove inhibitors of PCR. The assay is relatively expensive and technically demanding and is mainly recommended for use in special laboratories[15,16].

What is the role of molecular diagnosis is the clinical management of suspect tuberculosis cases? A recent commentary of the American Journal of Respiratory and Critical Care Medicine[17,18] cautioned against the use of PCR in the clinical setting. Sensitivity, specificity and predictive power of the PCR test then contended are not significantly higher than fluorescent microscopy and cultures in selective media.

PCR testing for M. tuberculosis is a rapidly evolving research area. There are difficulties with the interpretation of available studies due to the variety of criteria used for patients selection and of procedures used for microbiology as well as molecular testing. Most importantly however the gold standard test for the diagnosis of tuberculosis is not solid gold with extrapulmonary tuberculosis cases as well as the sharp rise observed in the members of patients, most often immunocompromised with paucibacillary tuberculosis. These are the ones who may go without microbiological diagnosis until autopsy e.g. tuberculosis in HIV patients.

The evidence that PCR diagnostic testing is useful for diagnosis of tuberculosis is quite convincing. PCR testing will definitely improve the diagnosis of tuberculosis in patients with atypical presentation, extrapulmonary tuberculosis, treated with antibiotics and in immunodeficient patient.

Reliability of PCR for detection of Hepatitis C virus has been tested by performing double blind study in 31 laboratories by HCV RNA detection. HCV PCR should be

interpreted with caution. Similar evaluation done on HBV PCR also warrants careful interpretation of PCR results. Reliability of PCR for detection of M. tuberculosis have been tested by Noordheck et al., who conducted a quality control double blind study giving coded samples to 30 laboratories and analysing the results [19]. There again only 5 laboratories were perfectly correct. This clearly shows PCR cannot be ignored as a useless test. But quality control should be emphasized. Right now PCR can be performed only in specialised laboratories by trained people. PCR can be definitely advocated as an additional test for extrapulmonary tuberculosis and other paucibacillary specimens as long as the interpretation of the results are done along with the relevant clinical criteria.

References

1. Einstein B. The polymerase chain reaction. A new diagnostic method of using molecular genetics for medical diagnosis. N Engl J Med 1990; 322: 178-183.
2. Elrich HA, Gelfard D, Srinsky JJ. Recent advances in the polymerase chain reaction (Review). Science 1991,252: 1643-1651.
3. Persing DH. Polymerase chain reaction: trenches to benches (Review). J Clin Microbiol 1991, 29 : 1281-1285.
4. Wagar EA. Direct hybridisation and amplification applications for the diagnosis of infectious diseases (Review). J Clin Lab Anal 1996, 10 : 312-325.
5. Haqqi TM, Sarkar G, David CS, Somer SS. Specific amplification of a refractory segment of genomic DNA. Nucleic Acids Res 1988, 16 : 1184.
6. Banerjee SN, Emori TG, Culver DH, Gaynes RP, Jarvis WR, Horan T. et al. Secular trends in nosocomial primary blood stream infections in the United States, 1980-1989. Am J Med 1991, 91 : 86S-98S.
7. Pfaller MA, Wenzel RP. Impact of the changing epidemiology of fungal infections in the 1990s. Eur J Clin Microbiol Infect Dis 1992, 11 : 289.
8. Troy CJ, PeelingRW, Ellis AG, Hockin JC, Bennett DA, Murphy MR, Spika JS. Chalmydia pneumoniae as a new source of infectious outbreaks in nursing homes. JAMA 1997, 277 : 1214-1218.
9. Robert M, Wadowsky, Richard H, Michaels, Therese Libert, Laowrence A, Kingsley and Garth D. Ehrlich. Multiplex PCR-based assay for detection of Bordetella pertussis in Nasopharyngeal swab specimens. J Clin Microbiol 1996, 34 : 2645-2649.
10. Van Eys J Clin Microbiol 1989, 27: 2254-2258.
11. Panu H, Hendolin, Aila Markkanen, Jukka Ylikoski, J Jarmo Wahlfors. Use of multiplex PCR for simultaneous detection of 4 bacterial species in middle ear effusions. J Clin Microbiol 1997, 35 : 11, 2854-2858.
12. Beavis KG, Lichty MB, Jungkind DL, Giger O. Evaluation of Amplicor PCR for direct detection of M. tuberculosis from sputum specimens. J Clin Microbiol 1995, 33 : 2582-2586.
13. Moore DF, Curry JL. Detection and identification of M. tuberculosis directly from sputum sediments by Amplicor PCR. J Clin Microbiol 1995; 33 : 2686-2691.

14. Granato PA, Franz MR. Use of the Gen-probe PACE system for the detection of Neisseria gonorrhoeae in urogenital samples. Diag Microbiol Infect Dis 1990, 3 : 217-221.

15. Longo MC, Berminger MS, Hartley JC. Use or uracil DNA glycosylase to control carry over contamination on polymerase chain reaction. Gene 1990, 93 : 125-128.

16. Cimino GD, Metchette KC, Tessman JW, Hearst JE, Isaacs ST. Post PCR sterilisation, a method to control carry over contamination for the polymerase chain reaction. Nucleic Acids Res 1991, 19 : 99-107.

17. Schluger WW, Rom WN. Current approaches to the diagnosis of active pulmonary tuberculosis. Am J Respir Crit Care Med 1994, 149 : 264-267.

18. Clarridge III JE, Shawar RM, Shermich TM, Plikayte BB. Large scale use of polymerase chain reaction for detection of M. tuberculosis in a routine mycobacteriology laboratory. J Clin Microbiol 1993, 31L 2049-2056.

19. Noordhoek GT, Kolk AHJ, Bjune G, Catty D, Dale JW, Fine PEM Sensitivity and specificity of PCR for detection of M. tuberculosis: A blind comparison study among 30 laboratories. J Clin Microbiol 1994, 32 : 277-284.