Review paper

RECENT ADVANCES IN THE DIAGNOSIS OF CHLAMYDIA TRACHOMATIS AND NEISSERIA GONORRHOEAE

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SUMMARY

The diagnosis of sexually transmitted diseases (STD) can be confirmed by classical laboratory tests as well as by modern molecular-biologic methods, but even by these it is not possible to detect all the infections. The molecular biologic methods contributed significantly to the accuracy of diagnostical tests, they are however rather complicated and expensive. For this reason at the moment they are not used as routine tests in the microbiological laboratories. The methodology of polymerase chain reaction (PCR) and ligase chain reaction (LCR) is shortly explained.

KEY WORDS

Neisseria gonorrhoeae, Chlamydia trachomatis, diagnostic methods, polymerase chain reaction (PCR), ligase chain reaction (LCR).

INTRODUCTION

The World Health Organization estimates that 250 million cases of sexually transmitted diseases (STDs) occur each year worldwide indicating them as a major public health problem especially among young adults. The effort to prevent spread of genital infections requires sensitive, specific, widely available, inexpensive, and rapid tests that can be used for diagnosis of STDs as well as routine screening of STD core groups. In addition, highly sensitive and specific diagnostic procedures enable the users to better define the epidemiology and pathogenesis of genital infections.

METHODS

Culture techniques

Successful culture procedures require the presence of viable bacteria, exact specimen collection and transport as well as suitable culture medium and incubation. They are still the gold standard technique for diagnosis of a number of bacterial genital pathogens such as Chlamydia trachomatis and Neisseria gonorrhoeae (Table 1, 2). However, they will be substituted in the future by molecular-biological techniques, which show a markedly increased detection of the organism when compared to the traditional cultivation of the agent (Table 3).

Table 1

Diagnostic Methods for Diagnosis of Neisseria gonorrhoeae

1. Staining Methods
   - Methylene blue
   - Gram
2. Culture
   - Thayer-Martin, New York
3. Antigen Detection
   - Enzyme immunoassay (EIA)
4. DNA-Hybridization
   - Gen-Probe assay
5. Amplification methods
   - Polymerase Chain Reaction
   - Ligase Chain Reaction

Molecular-biological methods

Molecular-biological methods offer an additional possibility to detect organisms that are difficult or impossible to cultivate. Using the DNA-fingerprinting new aspects can be observed concerning transmission of genital pathogens or pathogenesis of latency or subclinical infections. In the beginning of molecular diagnosis by a recombinant DNA technology the nucleic acid probes were radiolabeled and used after denaturation of the double-stranded DNA to hybridize with the single-stranded DNA segments. Efforts have been undertaken to exchange radiolabeled probes with non-isotop labeled ones for more practical purposes. First results were reported using nucleic acid probes for the diagnosis of Neisseria gonorrhoeae in comparison with culture and showed a sensitivity and specificity of about 90% (1).

PACE 2 assay

Since few years a non-isotopic DNA probe assay is available as a commercial kit for the identification of Neisseria gonorrhoeae and Chlamydia trachomatis (2). The Gen-Probe PACE 2 assay (Gen-Probe, San Diego, CA) uses an oligonucleotid directed against rRNA specific to Neisseria gonorrhoeae or Chlamydia trachomatis and can be used for the diagnosis of both chlamydial and gonococcal infections performed on material collected from a single swab. Dependent on the quality conditions of culture for Neisseria gonorrhoeae the PACE 2 assay shows a high detection rate for this important genital pathogen with a sensitivity and specificity reported to be 88% and 99.4%, respectively (3). In a study of a total of 502 males and females the PACE 2 assay was

Table 2

Diagnostic Methods for Diagnosis of Chlamydia trachomatis

1. Culture
2. Antigen Detection
   - Direct Immunoassay
   - Enzyme immunoassay (EIA)
3. DNA-Hybridization
   - Gen-Probe assay
4. Amplification methods
   - Polymerase Chain Reaction
   - Ligase Chain Reaction
5. Serology

compared with gonococcal culture (4). The data reported show that it is important to confirm especially borderline results in case to avoid a false positive outcome. It has been shown that this assay is an advantage especially for laboratories processing large numbers of specimens. Disadvantages of this test are a lack of quality control of the sampling and of commercially available confirmation tests, which may be helpful to solve borderline results (4). In addition, up to now the evaluation of antibiotic resistance proof can not be performed by molecular-biological methods.

For chlamydial diagnosis, the PACE 2 shows similar results to enzyme immunoassays (EIA) with a sensitivity ranging between 70% and 92% and specificities of about 98% (6). Especially for cervical specimens the accuracy of the Gen-Probe assay has been shown to be lower than that of an EIA or can produce borderline results which have to be confirmed by retesting or by using the noncommercial

Table 3

Overview of Nucleic Acid Amplification Techniques

1. Target amplification
   - Polymerase Chain Reaction (PCR)
   - Transcription-based amplification (TAS)
   - Self-sustaining sequence replication (3SR)
   - Strand displacement amplification (SDA)
2. Probe amplification
   - Ligase Chain Reaction (LCR)
   - QB replicase-based amplification
3. Signal amplification
   - Compouned probes
   - Branched DNA probes

probe competition assay.

Amplifying methods

A new advantage in diagnosis of STDs are methods using the artificial amplification of nucleic acid or signals after hybridization (Table 3) (7).

Polymerase chain reaction

The most common amplification technique is PCR, which has become a standard technique in many laboratories. It uses the replication of target nucleic acid sequences which are - dependent on the temperature - separated and hybridized in about 30 cycles with specific oligonucleotide primers and a taq-polymerase. A specific DNA sequence information is required to design the amplification process. The advantages of PCR in diagnostic laboratories are the high sensitivity and the detection of pathogens without radioactive isotopes and without culture procedures. Furthermore, one of the major advantages of molecular amplification is the ability to detect the organisms also in urine samples, which is not possible by culture methods (8,9).

There are several reports describing the use of PCR to detect Chlamydia trachomatis in clinical specimens. In these studies targeted MOMP, ribosomal RNA gene, or cryptic plasmid DNA sequences are used for amplification. PCR, largely evaluated in the detection of chlamydial infections, has been shown to identify true positive specimens that culture failed to detect. Comparing culture, Gen-Probe and PCR, the sensitivity was highest for PCR (95%), while culture reached 86% and the non-isotopic hybridization assay PACE 2 65%. In a study on genital pathogens causing epididymitis PCR has been shown to detect more chlamydial infections when compared to culture. The organisms could also be detected by PCR in cryopreserved donor semen (10). The transmission rate of chlamydia among partners was again determined by using the PCR assay in comparison to culture. Male partners of infected females yielded a positive result in 75% by PCR testing, compared with 45% by culture. Female partners of infected males yielded a positive result in 58% by PCR testing compared to 56% by culture. This increase of the detection rate especially in male partners indicates that PCR may detect also low numbers of the organisms in asymptomatic males difficult to recover in culture (11).

With Amplicor™ (Roche Molecular Systems, Branchburg, NJ), the first commercially available PCR test for Chlamydia trachomatis is on the market. In several clinical studies it has been compared with culture, antigen detection methods and with the PACE 2 assay. The sensitivity of this assay ranged from 76.7% to 98.3%, and was increased from 78.8% to 98.6% after resolving discrepant results by duplicate repeat testing, by additional MOMP-PCR and by the DFA. In a comparison study of PCR and the PACE 2 assay in a total of 474 urogenital male and female samples the sensitivity of the Amplicor assay has been shown to be higher than that of the PACE 2 test after resolving discrepant results. Altogether, the results of comparison studies of different detection methods for Chlamydia trachomatis demonstrate that PCR is more sensitive than culture or antigen detection methods.

One of the major difficulties of highly sensitive tests is the analysis of results discrepant to culture, which is still defined as the goldstandard technique. Enlarged gold standards are therefore established, where 2 positive results from different techniques are accepted even if culture is negative. PCR or another amplifying method should be included as one of the techniques for determining gold standard results.

Problems associated with PCR assays include contaminants of postamplified products into pre-amplified specimens decreasing the specificity. From results published up to now, the discussion arose whether inhibitors may decrease the sensitivity of Amplicor especially in female samples. This appears to be a special problem in the specimen processing system and has to be solved. A disadvantage of the Amplicor is that the test procedure is still labor intensive. Up to now the high costs of the PCR performance prohibit an extensive use of this technique for diagnostic and screening purposes of different STDs. Efforts have to go in the direction of automation of the test procedure to decrease the cost of the commercially available test.

Ligase chain reaction (LCR)

The ligase chain reaction (LCR) is an alternative amplifying method for the diagnosis of Chlamydia trachomatis as well as Neisseria gonorrhoeae. It is based on the ligation of oligonucleotide probes which serve as a copy of the original target sequence and are immediately adjacent to each other. For the cyclic amplification method a thermostable ligase is necessary. This method is able to detect as few as 10 nucleic acid targets and appears to be better suited than PCR for diagnostic work. The ligase reaction makes use of known sequences for the sole

purpose of diagnosis. In studies reported up to now the LCR is a highly sensitive and specific technique for the diagnosis of Chlamydia trachomatis (12). It has been shown to detect more chlamydia infections in the urogenital samples or in urine than culture methods do. The success to diagnose chlamydial infections by testing first void urine also in females represents a major step forward in chlamydial diagnosis (13). In a study in military recruits, the LCR has been shown to be the most sensitive test when compared to other techniques.

CONCLUSIONS

Advances in diagnosis of STDs are encouraging to better define the pathogenicity and epidemiologic pattern of the genital pathogens and to decrease the infection rate of STDs. Including molecular-biological tests the sensitivity and specificity can be increased. However, the ultimate goal to make them worldwide available as rapid, easy processed, and cost-effective tests based on reliable results is not yet reached.

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