Molecular Diagnostics. The Polymerase Chain Reaction and its use in the Diagnosis of *Chlamydia Trachomatis* and *Neisseria Gonorrhoeae* *

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In the microbe magnets-the world's urban centers-research is needed to determine which aspects of city life most amplify microbial spread...Ultimately, humantly will have to change its perspective on its place in Earth's ecology if the species hopes to stave off or survive the nest plague.

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Resumen

El diagnóstico de Chlamydia trachomatis y Neisseria gonorrheae fue realizado utilizando las técnicas microbiológicas clásicas de cultivo de células de clamidia y el aislamiento de agar, respectivamente, en pacientes estudiados en Pittsburgh. La reacción en cadena de la polimerasa (RCP) fue comparada por los procedimientos estándar usados para diagnosticar estos organismos transmitidos sexualmente. Se observan diferencias estadísticamente significativas en el diagnóstico molecular versus las técnicas de aislamiento clásicas. Hubo problemas en el manejo de numerosos especímenes de Neisseria. Se discute la mayor agudeza del diagnóstico de estas entidades y se compara con lo logrado en el estudio de la inmunodeficiencia adquirida (VIH).

Palabras clave: *Diagnóstico molecular, Clamydia trachomatis, Neisseria gonorrhoeae.*

Summary

The diagnosis of Chlamydia trachomatis and Neisseria gonorrhoeae was performed using classical microbiological techniques of chlamydial cell culture and agar isolation respectively in patients studied in Pittsburgh. The polymerase chain reaction (PCR) was compared to standard procedures used for diagnosing these sexually transmitted organisms. Statistically significant differences were observed in molecular diagnostics versus classical isolation techniques. Numerous specimen handling problems were identified in the handling of Neisseria. The enhanced ability to diagnose these sexually transmitted organisms is discussed in relation to the Human Immunodeficiency Virus (HIV).

Key words: *Molecular diagnosis, Clamydia trachomatis, Neisseria gonorrhoeae.*

Nature tells a story with every infection. Science advances as knowledge and technology provide partial solutions to human problems. The only true progress occurs when the tools of science and medicine succeed in the diagnosis, treatment and alleviation of human suffering. This paper will summarize and briefly review several studies of these technological advances using the polymerase chain reaction (PCR).[1-4] The porpuse of this review is to discuss how PCR has been used to diagnose two sexually transmitted diseases, Chlamydia trachomatis and Neisseria gonorrhoeae.

Background

Molecular diagnostics to the use of collecting and manipulating deoxyribonucleic acid (DNA) for the identification of humans, human infections, or

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forensic material. One of the most recent technologies to be added to the array of test for the pathologist during the last decade came with the discovery of the Polymerase Chain Reaction (PCR) in 1985. It was recognized and adopted by molecular biologist, microbiologists, paleontologists and physicians throughout the world as being one of the most ingenious tools for making exact copies of native DNA.

The determination of whether a patient harbors a certain infectious disease has historically been determinated by the isolation of a specific organism, bacterium, virus or a parasite from the human host. It is an essential premise of infectious disease that isolation of the organism or a specific antibody to the organism is suitable for the laboratory diagnosis of a particular infection. Bacteria are isolated onto agar growth media and viruses onto suitable cell cultures. The isolation and growth of bacteria causing infectious in human, animal and plants has been the gold standard and accepted methodology since the time of Pasteur in the late 19th century. Although bacterial isolation will continue to be conducted for several decades, new molecular tools like PCR will eclipse this laboratory procedure during the next several decades.

PCR will be briefly described in order to provide a suitable understanding of how molecular diagnosis has proven to be useful in the diagnosis of sexually transmitted diseases. The polymerase chain reaction is comprised of three separate processes: denaturation, annealing and amplification. These three events represent a single PCR cycle and each is conducted at different temperatures.[5]

The key biochemical component of the PCR are taq polymerase, a thermostable DNA enzyme which can extend a short DNA template that anneals to native DNA. This short DNA template is called a primer and its specificity is determinated by the infectious organism of interest. Since each bacteria, virus or other DNA has a specific sequence of nucleotide bases (adenine, guanine, cytosine and thymidine) it is the sequence of the bases which determines the genetic character of the organism. Magnesium, buffer and the nucleotide bases are essential ingredients of the PCR reaction mixture where the organism will then be added. Heating of these components in the presence of native DNA to a temperature of 93-96C results in the denaturation of native DNA (either bacterial or viral). Cooling this mixture to 55-60C permits the short DNA primers that are complimentary in sequence to the two native DNA strands to anneal.[5]

In the presence of taq polymerase, these primers are then extended at the rate of 150-200 bases per second whereupon reheating of the DNA causes the newly synthesized strands to disassociate. Cooling to 55C then permits another round of annealing and amplification. At the completion of thirty of these PCR cycles, millions of copies of bacterial DNA are produced for subsequent detection.[5]

Material and methods

PCR was used to study a group of male and female patients visiting the Allegheny County Health Depattment in Pittsburgh, Pennsylvania and compare the results of an Enzyme-linked immunosorbent assay (ELISA) for chlamydia detection.[1-4] In the evaluation of women, 328 patients provided endocervical specimens for both ELISA and PCR tests by randomly collected paired samples. Urethral swabs were obtained from 256 of these women in an effort to assess whether any of these women were infected with urogenital infections not being routinely tested by cervical specimens.[1]

To evaluate the PCR amplification of N. gonorrhoeae in women a separate group of 199 endocervical specimens were tested.[3] Endocervical specimens were collected by nurses and physicians using routine standards of care where swabs of the endocervix were streaked across JEMBEC agar plates, incubated and then transported to the Allegheny County Public Health Laboratory by courier. The swabs for PCR analysis were refrigerated and sent to the testing lab where they were frozen and batched for subsequent processing and testing.

The 474 young men participating in this study provided urethral specimens and of this group 362 of these patients also supplied a urine specimen for chlamydia PCR testing.[2] Routine swabs for ELISA testing were randomly collected with the PCR swabs from these patients in order to prevent a depletion of the available material for analysis.

The specific laboratory procedures followed for the Chlamydiazyme ELISA and the PCR for chlamydia along with the discrepant analysis have been detailed elsewhere.[1-3,4,5] Chlamydiazyme test used were manufactured by Abbott Diagnostics and the PCR test provided by Roche Molecular Systems.

Results.

The diagnosis of urogenital infections in women provided the following results. Twenty-five patients (7.6%) tested positive for C. trachomatis by ELISA and 35 patients (10.7%) were positive by PCR. The sensitivity of the Chlamydiazyme was 57.1% and the specificity was 98.3% prior to the resolution of discrepant specimens. The positive predictive value was 805 and the negative predictive value was 95.0%.

After discrepant analysis, the PCR of endocervical infections had a sensitivity of 89.7% and a specificity of 100%. The positive predictive value was 100% and the negative predictive value was 98.6%. Twenty-one of 26 patients (80.8%) of women with cervical chlamydial infections had concomitant urethral infections. Similarly, of the 35 patients with positive urehtral infections determined by PCR analysis 21 (60%) were positive in the cervix. Chalamydia was identified in both sites in 21 patients (52.5%) while 14 (35%) were positive by PCR for chalamydial DNA in the urethra. Five (12.5%) were positive solely in the cervix.[1]

Among the young men participating in this study, 27 patients were positive (5.7%) by the Chalamydiazyme whereas 65 (13.7%) were positive by PCR.[2] After discrepant analysis, Chalamydiazyme has a sensitivity of 43.5% and a specificity of 100%. PCR assay sensitivity was 98.4% and the specificity 99% for the males participating in this study. Comparison of the urethral swab PCR to the 362 urine PCR specimens showed that 60 patients (16.6%) had positive urine specimen results for Chalamydial DNA amplification versus 57 (15.7%) for swabs. The sensitivity of urine PCR after discrepant analysis was 87.1% and the specificity was 98.0%.[2]

The analysis of specimens from 199 women for N. gonorrhoeae culture and PCR provided the following results.[3] The overall population had a prevallence of gonorrhea of 3.5% as determined by PCR. Although the specificities of both culture and PCR was 100%, the sensitivity for culture was only 28.6% while PCR sensitivity was 110%.

Discussion

The diagnosis of chalamydia infections in women clearly demonstrated the superior sensitivity of PCR testing over the ELISA assay commonly in use throughout the public health laboratories of the United States. The sensitivity of PCR was 89.7% while the ELISA (chalamydiazyme) sensitivity was 61.5% after discrepant specimen analysis. Fifteen additional patients were diagnosed with chalamydia not diagnosed using routine test. Chlamydiazyme cervicitis was found to be 10.7% and urethritis 13.7% while the prevalece of urogenital infection in this population of women was 15.6%. Since 35% of these infections would have been missed by only cervical screening, the swabbing of cervix and urethra are necessary to diagnose all chlamydial urogenital infections by PCR.

The PCR diagnosis of chlamydia in men in this study was shown to be more than twice as sensitive (98.4% versus 43.5% as ELISA Chlamydiazyme)) testing for this bacterial infection. Likewise, urine sensitivity by PCR analysis was 87.1% and this was superior to the sensitivity by ELISA. It must noted that the collection of male urethral specimens by swabbing represents an invasive and sometimes painful procedure which could limit the adequate collection of suitable amounts of antigen for the ELISA assay. Along with this fact, the enhanced sensitivity of the DNA obtained from the urethral swab. Since the laboratory analysis of any sample is only as reliable as the sample itself, inadequate specimen collection for male chlamydial infections to date may be circumvented and improved upon by the collection of small amounts of chlamidial DNA for PCR testing.

The detection of chlamydia infections in both men and women have important and serious public health consequences.[6] Early detection of this bacterial infection in either men or women permit earlier treatment and eradication and thereby limits the numbers of infected human carriers in the community.

Clearly, the most significant observation from these tree studies was provided by the low N.gonorrhoeae culture sensitivity in women. An evaluation of the clinical operations in use to collect, process and store these culture specimens was then conducted in order to determine exactly why PCR testing found almost four times more infections versus the gold standard. The following problems were identified and documented on numerous, separate days in the clinics providing outpatient care to these women:

1. Endocervical specimens obtained by swabs showed these swabs were physically present in the cervix for only 2-3 seconds. Certains public health authorities (New York) mandate 10 seconds as a minimum time for the swab to remain in the endocervix in order for an adequate number of bacterialo be obtained for culture.
2. The manufacter's recommendation for JEMBEC agar plates used initially isolating and culturing N.gonorrhoeae mandate the plates be warmed to room temperature priortoapplying specimens to the plate surface for isolation. This was not being performed by clinicians.
3. The carbon dioxide pellet the accornpanies JEMBEC plates provides an environment of 5% carbon dioxide atmosphere to the organisrnduringitsinitialgrowth. Thesepellets were found no to be added to the plate correctly in the clinics and were occasionally not added at all.
4. Incubation of JEMBEC plates at 35-36C immediately upon inoculation is mandated by the manufacturer's instructions. These plates wereobservedto beon lab and cliniccounters for hours prior to incubation.
5. Incubators were found to vary from a temperature of 30-33C.
6. JEMBEC plates must be held overnight at 35-36C prior to transport to the lab and it was documented these agar plates were routinely taken by the courier prior to any incubation. These plates were found to occasionally rernain in the transport van all day at various ambient temperatures (20-25C) before they reached the public health laboratory.

The significantly low culture sensitivity for N. gonorrhoeae suggests that the numerous and consistent failure to follow standard operating procedures by the manufacturer of these agar plates was responsible for not diagnosing gonorrhea in many patients. The lackof any quality control program to monitor the clinical procedures necessary for rnaintaining a reliable standard of care represents a failurein operations disclosed by PCR diagnosis.

The presence of gonorrhea infections have been shown to enhance the transmission of the Human Immunodeficiency Virus by 3-5 fold.[7-9] These gonorrhea infections in men and women occur since inflammation of the tissues lining the male urethra and fernale urethra and cervix result in increased lymphoid cell traffic to the site of bacterial infection and this results in increased concentration of susceptible T4 cells and macrophages as sites for HIV infection.

This work along with nurnerous other studies document that significantly more sensitive diagnosis tools are currently available to the molecular epidemiologist to provide a truer estimate and records of the infectious diseases of a population [1-4,10] it is now estimated that 20 million people are currently infected by HIV throughout the world. Enlightened and aggressive rneasures must be taken to limit and prevent the transmission of HIV in susceptible populations of men, wornen and infants in the developing countries of the world if control of HIV is to occur.[7,11,12,13] The use of molecular diagnostics represents one of the best methods of diagnosis both chlamydiaandgonorrheainfections for public health authorities to use treatment of these bacterial infections are available today, bacterial infections from enhancing HIV transmission should be pursued as a national strategy to control HIV in Mexico and South Arnerica.

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