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Chlamydia trachomatis Testing: An Example of Evolving Technology

by Harvey George

Chlamydia trachomatis is a nonmotile, gram-negative, obligate intracellular bacterium that is almost exclusively a human pathogen. It is the most common sexually transmitted genital infection. It is manifested in males primarily as urethritis and in females as a cervicitis. A swab of the intraurethral or endocervical canals is collected to detect and identify an infection with the organism.

Isolation and identification of the agent initially was done in cell culture with cycloheximide-treated McCoy or other appropriate cells, followed by the use of fluorescein-conjugated monoclonal antibodies to demonstrate the *C. trachomatis* inclusion in the cell culture. Although this method was used as the "gold standard", having a specificity of 100%, its sensitivity was highly technique dependent, varying from 50 to 75%. In addition, cell culture required skills and equipment not commonly found in bacteriology laboratories.

Subsequently, antigen detection enzyme immunoassay (EIA) test methods were developed commercially. These tests possessed the advantage of speed and ease of use, but were not as sensitive and specific as cell culture. The sensitivities of the commercial kits varied from 70 to almost 100% as compared to cell culture. It should be noted however, that all of these EIA methods are considered by CLIA to be screening tests and hence need to be confirmed by another more definitive test.

Recognition of the need to improve both sensitivity and specificity of disease detection resulted in the development of nucleic acid probes for chlamydial RNA and systems for the amplification of chlamydial DNA. Available data suggests that the sensitivity of nucleic probes is comparable to the better antigen detection kits and cell culture methods, while possessing improved specificity over the EIA methods. On the other hand, the sensitivity of amplification tests is greater than either the EIA or the cell culture methods, and theoretically should approach 100%. In practice, the sensitivity probably never reaches 100% because of sampling problems, when very low numbers of chlamydial particles are present, and when there is inhibition of the amplification reactions by factors in the clinical specimens.

The number of nucleic acid amplification methods licensed for the detection of *C. trachomatis* in clinical specimens is increasing. At the present time three are in general use: ligase chain reaction (LCR), polymerase chain reaction (PCR) and transcription-mediated amplification (TMA). These assays can be used with first-voided urine samples as well as urethral and endocervical swab specimens. The ability to use urine specimens provides a major advantage to the amplification tests, because invasive collection methods are not required and specimens can be collected in settings other than traditional clinical or physician office settings.

Unfortunately, the cost of performing an amplification test is much greater than that of an EIA, so some labs have taken an indirect approach to improving their sensitivity and keeping their expense down. These labs are screening all specimens with an EIA test and then re-analyzing all "high" negative specimens with an amplification test, a less than ideal compromise.

Following our laboratory's conversion from EIA technology to nucleic acid amplification technology (LCR), we observed a two-fold increase in positivity rate from similar populations. Therefore, the State Laboratory tests all specimens, both urine and swab, directly by nucleic acid amplification to assure optimal sensitivity.

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PCB Breast Cancer Study

by Julianne Nassif

The Massachusetts Department of Public Health (MDPH) is conducting a pilot study to measure serum polychlorinated biphenyls (PCBs) and selected organochlorine pesticide levels in women newly diagnosed with breast cancer and in those with no breast disease. Data from this pilot project will be used to determine whether a full-scale study may contribute to a better understanding of the relationship between specific PCB congeners, DDE and breast cancer. Study participants complete a detailed personal, occupational and residential history questionnaire, developed and administered by the Bureau of Environmental Health Assessment, and provide four blood samples over a one year period.

The State Laboratory Institute analyzes the specimens for 33 specific PCB congeners, and two insecticides: DDE, a metabolite and breakdown product of DDT, and benzene hexachloride (BHC). These analytes were selected because they are "estrogen like" compounds and may disrupt endocrine function in the body. This dysfunction could

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PCB Breast Cancer Study

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play a role in the promotion of tumor development. Retrospective studies quantifying PCB and selected organochlorine pesticides in breast cancer patients have yielded conflicting conclusions. This prospective study considers levels over a one-year time period and includes clinical treatment regimes as a variable.

Laboratory analysis of the study specimens is complex and labor intensive. Proteins present in the serum are denatured with methanol and removed. PCBs are then extracted with a mixture of ethyl ether and hexane, and extraneous compounds present in the extracts are removed by silica gel adsorption chromatography. The concentrated extracts are then analyzed by capillary gas chromatography with electron capture detection (see Figure 1). Specific congeners and pesticides are separated and identified using a 60-meter DB-5 (5% phenyl methyl polysiloxane) and 60-meter DB-1701 (14% cyanopropyl-phenyl methyl polysiloxane) capillary columns. Chromatographic runs are 90 minutes each.

Quantification is accomplished using an algorithm developed at the federal Centers for Disease Control and Prevention. Target analytes must be detected using each of these dissimilar columns in order to be reported. If analyte concentrations from each column agree within 15%, then the average value is reported. However, if the measured difference is greater than 15%, then the lower concentration is reported.

Analysis of study specimens began in October and is expected to take approximately one year.

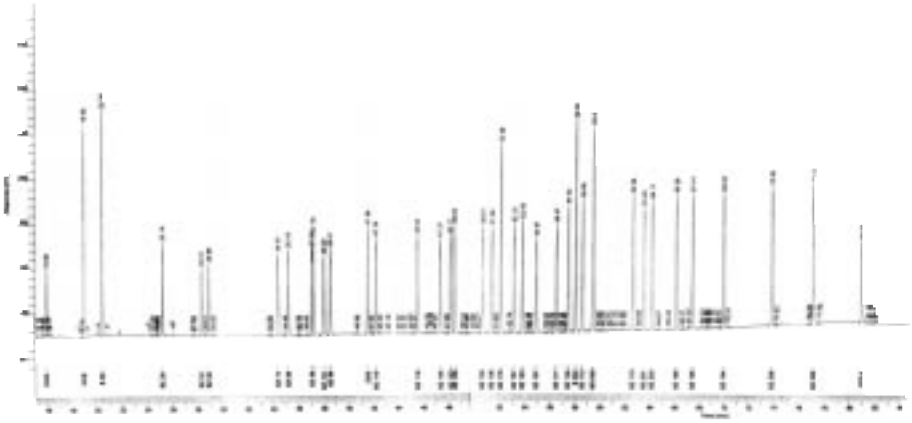

Figure 1. Chromatogram (DB-5) showing standard peaks for specific PCB congeners and insecticides that will be analyzed for the breast cancer study.

Quality Assurance & Regulations — Instrument Validation

by Dina Caloggero

Various agencies responsible for the accreditation and regulation of clinical laboratories, such as the College of American Pathologists (CAP) and the Health Care Financing Administration (HCFA), have regulations and guidelines regarding instrument validation. The validation process consists of three essential elements: performance verification, function verification, and instrument maintenance.

Performance verification, the first element, is the initial evaluation of an instrument to determine if the instrument meets manufacturer's performance specifications before reporting patient test results. This includes performing accuracy, precision, carryover, and linearity studies. Accuracy studies can be performed either by using validated reference materials or by a method comparison study using patient samples to evaluate the bias between two instruments. A precision evaluation may be carried out by using pooled patient samples, manufacturer controls, or commercial materials. Often carryover and linearity studies are performed simultaneously with precision studies.

Function verification, the second element, includes the daily activities, such as zeroing, optical alignment, and background counts, that are conducted to check the instrument's performance etc. Function checks should be conducted before patient testing and should be within laboratory or manufacturer established limits in order to verify that the instrument is working properly. Controls are also used to check instrument function. Tolerance ranges for each control level must be established, documented, and monitored for appropriateness and acceptability.

Instrument maintenance is based on the maintenance guidelines provided by the manufacturer. CLIA §493.1215 (a) requires that the laboratory "perform maintenance as defined by the manufacturer and with at least the frequency specified by the manufacturer." Instrument maintenance should include both unscheduled repairs and scheduled preventive maintenance.

All elements of the instrument validation process must be documented and reviewed. A written preventive maintenance schedule, as well as a system for recording these activities, must be developed and implemented by the laboratory.

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Program Report — Drug Analysis Laboratories

by Kevin McCarthy

The Drug Analysis Laboratory has been a part of the Massachusetts Department of Public Health (MDPH) since the first half of this century. At that time and years after, it was the only agency of the state government engaged in this kind of analysis. For many years drug analysis was only a part time job for a single chemist, but during the 1960's the demand from law enforcement for the analysis of drugs to support criminal prosecutions increased dramatically. There are now four drug analysis laboratories operated by state agencies; two laboratories are operated by DPH, one in Amherst and one at the State Laboratory in Jamaica Plain, with a total combined staff of twenty-two, including eighteen chemists. A third laboratory is operated by the Department of Public Safety and a fourth by the University of Massachusetts Medical School for Worcester County.

The Jamaica Plain laboratory provides services for Boston and all city and town police departments in the counties east of Worcester County; the Amherst laboratory provides services for local police departments west of Worcester County. The State Police Laboratory in Sudbury provides service for the Department of Public Safety, and the University of Massachusetts Medical School Drug Analysis Laboratory in Worcester provides service for Worcester County.

Testing provided by the two DPH laboratories is not limited to city and town police departments. Submissions from college and university police departments, the environmental police and the MBTA are regularly received for analysis. Occasional submissions are received from various federal agencies, including the National Park Service and the U.S. Drug Enforcement Agency, as well as a significant number of cases from the MDPH Division of Food and Drugs. The latter usually involve drug tampering at hospitals and nursing homes, most commonly the diversion and adulteration of painkillers such as demerol and morphine.

Today, drug analysis, and indeed analytical chemistry as a whole, is primarily based on instrumental methodology. The Drug Analysis Laboratories employ a wide range of analytical methods in their procedures. These include gas chromatography (GC), ultra-violet spectroscopy (UV), infrared spectroscopy (IR), and gas chromatography / mass spectrometry (GC / MS). GC and UV are used for drug screening and quantifying drug strength. IR and GC / MS provide structural identification of drugs. The net weight for drugs received is also determined, as prison sentences in both state and federal courts are frequently based on the amount of a drug in a defendant's possession. Approximately three thousand submissions, or cases, are analyzed per month, many containing multiple numbers of specimens for testing. Because of the high volume of testing, auto-samplers are required on many of our instruments to permit tests to be run twenty-four hours a day.

Legal considerations are very important in any type of forensic work, including the analysis of illicit drugs. Many times in the course of a month, subpoenas are received from state and federal courts requiring complete case records. Documents must be provided that include records of submission and return of specimens, as well as the analyst's documentation and printouts of instrumental results. The Drug Analysis Laboratory staff members are also frequently subpoenaed to appear in court to testify as expert witnesses in Superior and District Courts in Massachusetts and in Federal Courts.

In addition to the work for criminal trials, analysts perform high priority analyses in cases of drug overdose and compile information regarding the varieties, strengths and distribution of illicitly used drugs throughout Massachusetts. Currently, the laboratory is undertaking a wide ranging study of heroin purity in relation to brand names (heroin is most often sold with a "brand" or "logo" imprinted on the packaging) and packet type. It is also beginning a varieties / logo study on methylendioxymethamphetamine (MDMA), commonly known as "ecstacy", submissions, which have increased dramatically over the past year. Future plans call for a review of abused legitimate pharmaceuticals that are submitted in large amounts, and compilation of information on the many counterfeit anabolic steroids. Information from drug analyses is compiled and provided to the MDPH Bureau of Substance Abuse Services to support their prevention efforts.

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One problem, common to all nucleic amplification procedures, is the inability to distinguish between active infection and residual nucleic acid that may be remaining from ruptured cells after successful treatment. This renders the practice of "test of cure" testing impractical in most clinical settings. However, the ability to extend screening services to such underserved populations as juveniles, for whom invasive sample collection would be inappropriate, or prisoners where screening STD examinations would be financially prohibitive, more than compensates for this apparent shortcoming.

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