Nebraska Public Health Laboratory Newsletter

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This edition of the NPHL newsletter highlights the breadth of activities undertaken the past six months. The accompanying articles describe the implementation of a new amplified assay for detection of gonorrhea and Chlamydia. An analysis by Peter Iwen and Phil Medina showed that use of the new assay significantly increased the detection of cases infected with Chlamydia. Although new technology provides significant advantages such as the ability to test urine from males and females, these new technologies also bring along new challenges. One example is illustrated by the article by Nate Birch and his finding of a significant number of falsified urine specimens submitted for testing for sexually transmitted diseases. Although this problem is well known in the drug testing arena it had not been previously described in the microbiology field.

Another important technology development is the recent completion of a new version of the NPHL internet based ordering and reporting system. This program has shown remarkable progress under the leadership of our state epidemiologist, Tom Safranek and in many ways this program leads the nation in the field of electronic laboratory reporting. The new version of the PHLIP software is a big step forward as it now includes an epidemiology module which vastly extends the capability of the system to include all important public health test types. The new module was made active after an extensive three year evaluation of the security capabilities of the system and a demonstrated ability to keep information confidential while at the same time taking advantage of the numerous efficiencies existing in a web based paperless system.

Other new developments are underway and we look forward to sharing these with our public health partners throughout the state.

Steven Hinrichs, M.D., Director NPHL

New NCCLS Interpretive Standards for *Streptococcus pneumoniae*

by Paul Fey, Ph.D.

The most recent recommendations (January 2002-M100-S12) from the National Committee for Clinical Laboratory Standards (NCCLS) included an important change regarding minimum inhibitory concentration (MIC) interpretations of cefepime, ceftriaxone and cefotaxime for *Streptococcus pneumoniae*. The new interpretations reflect clinical knowledge that therapeutic levels of these drugs are higher in peripheral tissues than in the central nervous system when using a standard dosing regimen. Two different interpretations will be used based on the reported source

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Testing for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

by Peter C. Iwen, Ph.D.

Sexually transmitted diseases (STDs) constitute an epidemic with an estimated 15 million persons in the United States acquiring a new STD each year. Infections with *Chlamydia trachomatis* and /or *Neisseria gonorrhoeae* (C/GC) were the first and second most common STDs in the United States reported to the Centers for Disease Control and Prevention (CDC) in the year 2000 and accounted for 80% of all notifiable diseases *(1)*.

The array of technologies now available for the laboratory diagnosis of Chlamydia and gonorrhea has expanded greatly over the last few years with the addition of molecular assays. The first

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NPHL Investigates Specimen Integrity in Urine STD Screening

by Nathan Birch, M.D.

Although the development of amplified assays has increased the detection rate in at-risk populations at least one major un-foreseen problem has appeared: the potential submission of falsified specimens. *(1,2)*. First generation amplification technologies utilized material collected on a swab from either the cervix or urethra. Such an approach necessitated appropriate examination equipment and trained medical personnel. The advent of high sensitivity amplification procedures expanded the range of specimen types to include urine *(3)*. Urine amplification testing not only offered a non-invasive means of specimen collection, but also eliminated the need for a private examination room and medical personnel. Consequently, institutions with a high prevalence of disease and limited medical facilities, such as state penitentiaries and youth correctional facilities, can provide routine STD testing with the expectation that expanded screening will reduce the number of sub-clinical infections and ultimately the number of new cases *(4)* Detection of C/GC in urine using an amplified assay was implemented in 2001 as a pilot project at the NPHL. Since introductions of the amplified assay, the overall percentage of positive results increased from 3.8 to 5.8% (see accompanying article by Peter Iwen, Ph.D.).

Between 95 and 99% of random urine specimens submitted to the clinical laboratory for urinalysis testing are yellow in color *(5)*. Interest in determining the validity of urine specimens developed when NPHL technologist noted a substantial number of colorless urine

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"Specimen Integrity"

specimens submitted for C/GC amplification.

Alteration of urine used in screening for drugs of abuse is a well-known problem. However, falsification of urine specimens submitted for C/GC amplification testing has not been previously described. Therefore, no procedures were in place for the detection of potentially manipulated specimens.

No single test is able to validate or refute a specimen as urine or not. Therefore to characterize the sample as compatible with urine or not, the criteria derived from the studies of Cook etal. were used . A specimen with a urine creatinine of < 5 mg/dL and a specific gravity of < or = to 1.001 was judged to be incompatible with urine. Approximately 8% of all specimens submitted during a six week evaluation period were determined to be inconsistent with urine.

The motivation for the submission of falsified specimens from clients who have been offered optional STD screening at no cost is unknown. Discussions with staff from submitting institutions indicate that the suspicion of random drug testing was the most likely reason for the apparent submission of water in place of urine. The consensus of the institutional staff was that patient education and reassurance would provide the most effective and cost efficient method of reducing the substitution rate.

The widespread use of urine C/GC testing methodology in the arena of public health with limited funding, makes reducing the number of altered specimens a priority. An understanding of the patient population served and coordination with submitting facilities will likely provide an inexpensive and timely solution. Alternatively, other approaches used by drug screening programs such as temperature monitoring, on-site specific gravity measurements, and direct observation could be instituted.

Knowledge of specimen manipulation is necessary to effectively implement a urine based C/GC screening program.

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"Interpretive Standards"

of the original specimen, i.e. cerebral spinal fluid (CSF) or non-CSF, such as sputum.

The revised interpretive standards for cefepime, ceftriaxone, and cefotaxime are:

CSF

≤ 0.5 µg/ml = susceptible	(S)
1 µg/ml = intermediate	(I)
≥ 2 µg/ml = resistant	(R)

Non-CSF

≤ 1 µg/ml = susceptible	(S)
2 µg/ml = intermediate	(I)
≥ 4 µg/ml = resistant	(R)

It is currently recommended that only the CSF interpretation be reported if the original source is from CSF. However, both interpretations should be reported if a non-CSF specimen is submitted in anticipation of the possible need to treat a secondary meningeal infection.

In areas of the state where penicillin-resistance is low (< 20% resistant), it is warranted and cost-effective to first test, by disk diffusion, for resistance to penicillin (using an oxacillin disk), erythromycin and trimethoprim/sulfamethoxazole if the isolate is from a respiratory source. If the isolate is resistant to penicillin, it is recommended that penicillin, ceftriaxone (or cefotaxime), meropenem, vancomycin, and an anti-pneumococcal fluoroquinolone (levofloxacin, gatifloxacin, or moxifloxacin) be tested by a reliable MIC method. However, in areas where penicillin resistance is high (~50% resistant), it is recommended that the oxacillin disk screen not be done, but instead, an MIC method be performed initially to test penicillin, ceftriaxone (or cefotaxime), meropenem, vancomycin and levofloxacin. Both erythromycin and trimethoprim/sulfamethoxazole can still be tested using a disk-diffusion method if desired. Only MIC values should be generated if a S. *pneumoniae* is isolated from the blood, CSF, or another sterile site. It is recommended that penicillin, ceftriaxone (or cefotaxime), meropenem, and vancomycin be reported for CSF isolates; whereas penicillin, ceftriaxone (or cefotaxime), meropenem, vancomycin, and an anti-pneumococcal fluoroquinolone be reported for isolates from blood or other sterile sites.

Table 1. Comparison between the Gen-Probe PACE 2C and the BD ProbeTec assays for the detection of *C. trachomatis* at various state program testing sites. [a]

	Family Planning clinics			CHD clinics			Other STD clinics		
Specimen type (Test)	Total tests	Pos	%	Total tests	Pos	%	Total tests	Pos	%
Endocervix									
(GP)	17772	638	3.6	1189	122	10.3	8410	361	4.3
(BD)	11433	510	4.5	1097	146	13.3	5103	284	5.6
Male urethra									
(GP)	608	70	11.5	2114	182	8.6	764	95	12.4
(BD)	423	67	15.8	1546	207	13.4	333	57	17.1

[a] Nebraska, January 2000 through September 2002.
Abbreviation: CHD, county health department (Lancaster and Douglas)

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"Testing for CT and GC"

FDA-cleared assays to detect for the presence of specific nucleotides in clinical specimens were probe-based. Prior to February 2002, the NPHL utilized the Gen-Probe PACE 2C probe-based assay (Gen-Probe, Incorporated, San Diego, CA) to detect both *C. trachomatis* and *N. gonorrhoeae* from genital specimens. Recently, the NPHL switched to a nucleic acid amplification test (NAAT) as a more sensitive approach to detection chlamydia and gonorrhea. Numerous commercial NAATs have been developed and marketed which differ substantially in amplification methodology and their target nucleic acid sequences . The assay, chosen for use at NPHL called the BD ProbeTec^{TM}ET (Becton, Dickinson, Franklin Lakes, NJ), has the ability (found among all NAATs) to amplify as few as a single copy of the target DNA or RNA specific for the organisms being detected. This leads to increased sensitivity for these tests and allows for the testing of direct specimens such as urine. One of the most important advantages of NAAT is to reduce the dependence on invasive swab procedures to collect specimens.

The NPHL has been monitoring the percentage of positive screens to compare current amplification test results to prior non-amplification testing. **Table 1** shows a comparison of the non-amplified Gen-Probe assay results to BD ProbeTec assay for the detection of chlamydia at various state program sites. The incidence of chlamydia detected in endocervical specimens by NAAT showed a 1.2% to 3% increase over non-amplified depend ing on which clinic site was tallied. The sites with the highest number of positives were the County Health Department (CHD) clinics at 10.3% and 13.3% for the non-amplified and amplified tests, respectively. In male urethral specimens, the greatest increase in chlamydia positives was also seen in the CHD clinics where the increase between non-amplified and amplified testing was 4.8%.

Less significant improvement was seen in the detection of gonorrhea, between the Gen-Probe and BD ProbeTec tests. The highest incidence of gonorrhea was observed in the CHD clinics for female endovervical testing at 6.4% using the amplified test and for the testing of male urethra at 10.7% using the non-amplified test (**Table 2**). The number of males tested reflects only those associated with known exposure to an infected female partner or those who present to the clinic with symptoms of disease. Therefore, as expected, the number of screens performed in males is lower.

Table 2. Comparison between the Gen-Probe PACE 2C and the BD ProbeTec assays for the detection of *N. gonorrhoeae* at various state program testing sites.[a]

	Family Planning clinics			CHD clinics			Other STD clinic		
Specimen type (Test)	Total tests	Pos	%	Total tests	Pos	%	Total tests	Pos	%
Endocervix									
(GP)	17772	98	0.6	1311	70	5.3	8349	60	0.7
(BD)	11433	51	0.4	1097	70	6.4	5103	34	0.7
Male Urethra									
(GP)	678	25	3.7	2114	226	10.7	704	60	8.5
(BD)	423	20	4.7	1546	142	9.2	333	30	9.0

[a]Nebraska, January 2000 through September 2002.
Abbreviation: CHD, county health department (Lancaster and Douglas)

The testing of urine specimens has only recently been developed as an alternative to invasive specimen collection techniques. Generally, the testing of urine is performed as a screening tool for clinics associated with the correctional facilities and the youth detention centers. The increase in chlamydia positivity between the Gen-Probe assay and the BDProbeTec assay when testing urine was 4.2% and 8.2% in males and females, respectively (**Table 3**). It is unknown why a difference was seen between these assays however, increased training for collection and more selective testing may have played a role in this difference. An FDA-cleared test for urine was not available for use from Gen-Probe to detect for *N. gonorrhoeae*, therefore no comparison was possible.

As expected, a substantial increase in the number of specimens positive for chlamydia and gonorrhea resulted from changing to an amplified method. Although amplification testing requires more attention to procedural details than the non-amplified nucleic acid hybridization probe assay, the cost per positive result is actually lower due to the increase in the number of positive patients. Selective testing of high-risk target groups will add to the cost effectiveness of amplification assays.

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Table 3. Comparison between the amplified Gen-Probe PACE 2C and the BD ProbeTec assays for the detection of *C. trachomatis* and *N. gonorrhoeae* from urine.[a]

	Chlamydia			Gonorrhea		
Gender (Test)	Total tests	Pos	%	Total tests	Pos	%
Female						
(AGP)	122	8	6.6	*	*	*
(BD)	485	69	14.8	485	24	4.9
Male						
(AGP)	2155	53	2.5	*	*	*
(BD)	2233	150	6.7	2233	25	1.1

[a]Nebraska, January 2000 through September 2002.
[b]Gen-Probe amplification test used at the NPHL was not FDA-cleared to detect for *N. gonorrhoeae*.

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by Jeff Gehring,M.T. (ASCP)

The Department of Health and Human Services System and the Nebraska Public Health Laboratory have completed the development of a new version of the web-based information system called the Public Health Information Program (PHLIP). The first version of PHLIP was implemented statewide for use by the Nebraska Childhood Lead Poisoning and Prevention Program in 1999. Experience gained with version 1 allowed the development of multiple new features and capabilities. PHLIP is web base computer link that allows program users across the state to order tests online. Further, it allows authorized personnel to view the individual results. The ability to order and view results online provides numerous opportunities and efficiencies for public health users. More importantly, the systems stores individual client demographics and allows this information to be automatically recalled and entered into any new test orders. This function allows for the elimination of many labor intensive steps that are typical of paper order systems. The new version also includes the capability to assist in the preparation of mailing specimens to the laboratory and the replacement of hand-written requisitions. A number of the users who have worked with the beta version of the new PHLIP have also noted the value of being able to track the location of the specimen as it moves through the system. Therefore, programs will be able to follow test specimens and know when results will be available and subsequently print out these results online without having to wait for a printed report to appear in the mail. The availability of results online also ensures that no results will be missing when the client returns for an office visit. Program administrators will have greater functionality for data analysis such as ability to create specialized reports.

The NPHL and all the people involved in the development of the new version extend their thanks to our partners within NHHSS as well as various private and public health program users across the state. Without their help, we would not have been able to optimize all of the various features of the new PHLIP system through development, testing and implementation. Together, Nebraska is able to play a leading role in developing web base public health communication systems.