REX
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UPDATES AND INFORMATION FROM REX PATHOLOGY LABORATORY

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Molecular Testing for GC/Chlamydia

In the past decade, molecular testing has moved from research laboratories in academic centers to clinical laboratories testing human specimens. We have entered this area of testing with the assistance of Dr. Ronald McGlennen and Access Genetics. Dr. McGlennen is the founder of Access Genetics and holds an associate professorship at the University of Minnesota Medical School. He has published more than 70 scientific articles and book chapters in this area and is board certified by the American Board of Medical Genetics with a specialty in clinical molecular genetics. His company has developed a turnkey approach to molecular diagnostics that employs telemedicine to provide real-time consultation for technical issues in the laboratory as well as means to aid in test interpretation. The plan is to use a single and common specimen (either cervicovaginal sampling in a SurePath® vial or urine) to perform both standard cytologic evaluation and use molecular testing to augment our diagnostic capabilities in regards to Chlamydia, Gonorrhea, HPV, and Cystic Fibrosis testing. In January 2008, we began testing for Gonorrhea and Chlamydia by polymerase chain reaction (PCR) using both urine and SurePath® Pap vial specimens.

Screening for STD's: Improvement in Patient Care with Molecular Testing

In November of 2006 Rex Hospital Laboratories began offering molecular testing for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhea* (NG) from the Surepath Pap specimen vial. The following is a synopsis of current molecular testing and will also serve as a model of how the clinical laboratory hopes expand the menu of services available.

Testing for CT/NG

An estimated three million new *CT* infections are reported in sexually active children and adolescents each year in this country. In the majority of cases, infected persons are asymptomatic, which makes the management of these cases more difficult and the likelihood of complications greater.

CT infections may cause pelvic inflammatory disease (PID) in women. The majority of patients have symptoms that are too mild or nonspecific for them to seek medical treatment. Regardless of symptoms, the consequences of PID may be severe. Of those with PID, 20% will become infertile; 18% will experience debilitating, chronic pelvic pain; and 9% will have a life-threatening tubal pregnancy. CT infections during pregnancy may lead to neonatal conjunctivitis or pneumonia and maternal postpartum endometritis. Among men, urethritis is the most common illness resulting from CT infection. Complications (e.g., epididymitis) affect a minority of infected men and rarely result in sequelae. Among men who engage in receptive anal intercourse, the rectum is a common site of CT infection. Rectal infections are usually asymptomatic, but can cause symptoms of proctitis or proctocolitis. CT can cause conjunctivitis among adults and sexually acquired reactive arthritis.

Similar to *CT*, uncomplicated *NG* infection is usually confined to the mucosa of the cervix, urethra, rectum, or throat. NG infection is often asymptomatic among

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females; and, if untreated, NG infection can also lead to PID, tubal infertility, ectopic pregnancy, and chronic pelvic pain. NG infections usually cause symptomatic urethritis in males, and occasionally progress to epididymitis. Rarely, local infection disseminates to cause an acute dermatitis tenosynovitis syndrome, which can be complicated by arthritis, meningitis, or endocarditis. Also, similar to CT, NG can be acquired at birth.

Based on the epidemiology of these infections and the potential of severe complications, the use of diagnostic screening for CT and NG has become a standard of practice across the United States. In 2001, cases of CT were the most commonly reported communicable disease. Rates were the highest in adult women and adolescents, notably in both males and females. Conventional diagnostic strategies to confirm a clinical impression of Chlamydia infection of the genital tract have relied on culture-based methodologies requiring special media and laboratory apparatus. These tests, while highly specific, are usually too insensitive for use in screening algorithms and may produce results inconsistent with the clinician's impression.

Infections of the genital tract with NG are noted to be second in incidence only to that of CT. Detection of GC by culture systems is well described, but is challenged by the fact that the GC organisms have highly fastidious growth requirements. Such assays are also known to be relatively insensitive due to the prevalence of antimicrobial resistant strains not detected by culture.

Taken together, CT and NG infections are a significant health threat, the most common form of STDs, and a challenge to the diagnostic lab. With the advent of nucleic acid amplification testing (NAAT), many of the problems with assay insensitivity have been solved. In 2002 the Centers for Disease Control revised its recommendations for the preferred methods for the detection of CT and NG from culture to NAAT based methods[1]. From that publication the research then demonstrated that "NAATs for *C. trachomatis* are substantially more sensitive than previous tests." And because of the improvements in sensitivity, "When using a NAAT, any sacrifice in performance when urine is substituted for a traditional swab specimen is limited, thus reducing dependence on invasive procedures and expanding the venues where specimens can be obtained. NAATs can also detect both *C. trachomatis* and N. gonorrhoeae organisms in the same specimen[2]. As a result of these guidelines, the use of molecular methods for the laboratory screening of CT and NG infections has become the standard of care.

Molecular Detection of CT and NG: Design of the Test

Rex Pathology has implemented a diagnostic test for the specific detection of CT and NG by methods involving the polymerase chain reaction or PCR. PCR based testing is significantly less invasive, useable from a variety of sample types, and improves sensitivity and specificity.

The application of PCR in the detection of CT and NG are well understood. Scientific publications on the subject date back to the late 1980's where the characterization of the so-called pjDC cryptic plasmid, an endogenous segment of DNA with an unknown function, provides a highly specific locus for the design of the oligonucleotide primers used in the PCR reaction to detect CT infections[3].

Similarly, PCR based tests designed to detect NG could be directed at any of several plasmids which can be divided into three classes; a large, -36- kilobase (kb), plasmid which is involved in conjugal transfer of plasmids, two genetically related R-factors (5.6 and 7.1 kb) that encode a B-lactamase, and a 4.2-kb cryptic plasmid. The latter plasmid is found in greater than 96% of clinical isolates of *NG*, and is the most commonly used target for the design of diagnostic tests[3].

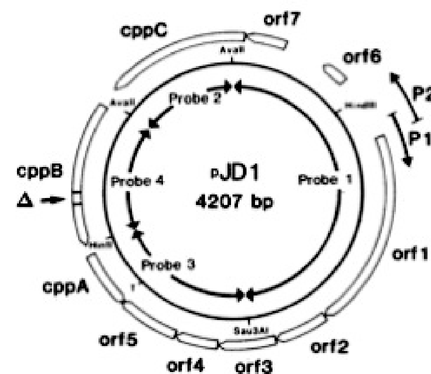

Figure 2: Genomic organization off the pJD1 cryptic plasmid and the position of the DNA primers used in the PCR test to detect CT.

How the Test is Performed

In recent months, the Rex laboratory staff have implemented a method for detection of CT and NG based on PCR combined with direct detection of fluorescence by melt curve analysis. In brief, if present, the regions of the cryptic plasmids found in CT or NG are amplified by PCR to a level where the insertion of the fluorochrome Cyto9 into the resulting DNA double helix can then be detected by a photomultiplier tube. After a prescribed level of amplification, the DNA product is heated until the double helix separates into single strands, thereby releasing the fluorescent molecule. This process of "melt" occurs predictably and precisely at an empirically anticipated temperature. The rate of the melt process can be plotted as function of temperature. The first derivative of the resulting plot then reveals a peak at the temperature where there is specific melt of the DNA fragment(s) of interest. Interpretation of the diagnostic test involves recording the melt profile of each DNA sample and comparing the resulting peaks to a series of known controls. Importantly, the interpretation of these tests requires a validated "electronic" profile, followed by a series of double blinded reviewers to ensure clinical utility of these very sensitive and accurate tests.

The Rex laboratory validated its experience in testing for CT and NG based on a series of criteria to demonstrate the sensitivity and specificity of their assay to accurately diagnose CT and NG infections. The process of validation was applied to a variety of sample types used for these tests including Surepath liquid Pap collection samples and urine. One of the clear advantages of using PCR methods is the utility of such tests for DNA derived from a wide variety of sample types.

In the validation process several key findings were noted:

1. As compared to alternative methods, the PCR “rectified” several false negative cases (samples called negative by other testing platforms).
2. There was a near perfect correlation between the old and new versions of the PCR based test.
3. The high throughput-melt curve assay has an improved sensitivity as compared to the gel based assay
4. Early experience promotes the high throughput aspect of the newer version of the test.

Current activity of CT/NG Testing at Rex Hospital

The following tables detail the current clinical performance of the CT/NG test at Rex Hospital.

1. Test volume and positivity rates: Month to month monitoring of test volumes and positivity rate are independent metrics of test utilization and assay accuracy:

	Sep 2007	Oct 2007	Nov 2007	Dec 2007	Total
Total Samples	416	606	536	519	2077
Total Tests Run	832	1212	1072	1038	4154
C. Trachomatis					
Total CT Tests	416	606	536	519	2077
Positive Samples	12	11	13	7	43
Positivity Rate	2.88%	1.82%	2.43%	1.35%	2.07%
G. Neisseria					
Total NG Tests	416	606	536	519	2077
Positive Samples	2	2	1	0	5
Positivity Rate	0.48%	0.33%	0.19%	0.00%	0.24%

2. Repeat rates: Monitoring of repeat rates are both a function of the integrity of the assay as well as the quality of the laboratory personnel

	Sep 2007	Oct 2007	Nov 2007	Dec 2007	Total
Repeat Information					
Total Repeats	35	15	17	9	76
Repeat Rate	6.01%	0.17%	1.12%	0.39%	2.07%
Repeat Chemistry	25	0	2	0	27
Repeat Extraction	1	0	0	0	1
Repeat 2x Template	0	1	0	0	1
Repeat 2x Hydration	0	0	0	0	0
Repeat for Confirmation	0	0	0	0	0
CT Repeat for Confirmation	10	11	11	7	39
NG Repeat for Confirmation	0	2	0	0	2
EXT-Increased Dilution	0	0	4	2	6
EXT-Decreased Dilution	0	0	0	0	0
Repeats by Specimen					
Swab Repeats	0	0	0	0	0
Pap Repeats	35	15	17	9	76
Urine Repeats	0	0	0	0	0
Other Repeats	0	0	0	0	0
QNS	0	0	0	0	0

Among the concerns from clinicians in the care of patients tested for CT or NG is the adequacy a “routinely” collected sample. Because of the sensitivity of the PCR based test, far fewer “Quantity not sufficient, QNS” or failed runs occur. This reduced repeat and QNS rate in turn reduces patient wait for results and correspondingly less anxiety over repeat collections.

Who Should be Tested for CT and NG

Initial screening should be performed on all clinically suspicious patients or those at “high risk of STD”. Additionally, the American College of Obstetricians and Gynecologists recommends testing on all women under the age of 25 in concert with their annual Pap smear test[4].

Follow-up should be conducted as recommended for the infections for which a woman is treated. If symptoms persist, women should be instructed to return for reevaluation. Management of sex partners of women treated for cervicitis should be appropriate for the identified or suspected STD. Partners should be notified and examined if chlamydia, gonorrhea, or if another STD was identified or suspected in the index patient.

CT urogenital infection in women can be diagnosed by testing urine or liquid based Surepath Pap specimens. Diagnosis of CT urethral infection in men can be made by testing a urine specimen. Urine collection instructions are summarized:

Before Collection:

Mark the 20 and 30 ml levels on the container to be provided for collection with a felt tip marker and properly label the container.

Collection Instructions:

Remind the patient that the genital area should not be cleansed prior to urine collection. This is not a clean-catch collection.

Instruct the patient to collect the initial stream of urine to a volume between the marked lines on the urine container and tightly cap the container upon completion of the urine collection.

Transport Instructions:

Refrigerate urine specimen until it is transported to Rex Hospital Laboratory.
Insert the test requisition into the side pocket of the specimen Biohazard transport bag and send to Rex Hospital Laboratory.

Contact Information:

Cytology Department: (919) 784-3050
Molecular Pathology: (919) 784-7513.

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4. *Koumans E.H., Black C.M., Markowitz L.E., et al. Comparison of methods for detection of Chlamydia trachomatis and Neisseria gonorrhoeae using commercially available nucleic acid amplification tests and a liquid pap smear medium. J. Clin Microbiol. 2003;41(4):1507-11.*

April E. Kemper, MHS, Joins Rex Pathology Associates

We are pleased to announce the association of April E. Kemper with the Rex pathologists beginning April 15, 2008. Ms. Kemper received her B.S. in Animal Science at N.C. State University in 1989, followed by an M.S. in Nutritional Biochemistry in 1992. After working several years as a research associate at Embrex Inc. and a research analyst in the Neuromuscular Genetics Laboratory at UNC, she obtained a masters degree in health science at the Duke University pathology assistant program in 2004. From 2004 to the present she worked as a pathology assistant in the Surgical Pathology program at Wake Forest University Baptist Medical Center where her duties included grossing surgical specimens, supervising and training pathology residents. Ms. Kemper was co-captain of the 1988 ACC champion and NCAA finalist NCSU women's soccer team. She was also a member of the 1998 W-League Champion Raleigh Wings semi-professional women's soccer team. She is an avid runner and enjoys participating in triathlons, 10k's and half marathons and loves to read. She recently spent her 40th birthday hiking for eight days from hut to hut in the Dolomites of northern Italy.

Ms. Kemper will join Emily Sundlof, MHS to assist the pathologists with gross examination of surgical and autopsy specimens, providing remedial training where indicated, and perhaps kicking a few keisters when necessary.

JDB

2008 Antibiogram

This issue of the Lab Bulletin contains an insert with the 2008 Rex Antibiogram. Thanks to Susan Tricas, MA(ASCP) and Myra Hawkins, PharmD for compiling the data. The 2008 Antibiogram may also be accessed via RexWeb at the following link:
http://rexweb/division/pcs/pharmacy/2008%20 antibiogram.pdf

Vincent C. Smith, MD
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Rex Healthcare 2008 ANTIBIOGRAM
January-December 2007 Results

Gram-Negative Organisms

	PCNs				Cephalosporins				Quin	Aminoglycides			Miscellaneous			URINES			
					1st	3rd		4th											
	# Isolates	Ampicillin	Amp/Sulbactam	Piperacillin/Tazo	Cefazolin	Ceftazidime	Ceftriaxone	Cefepime	Levofloxacin	Amikacin	Gentamicin	Tobramycin	Aztreonam	Imipenem	TMP/SMX	***# Isolates***	Levofloxacin	Nitrofurantoin	TMP/SMX
Acinetobacter baumannii	8	*	**67**	*	*	**38**	0	**50**	**50**	**75**	**50**	100	*	**62**	**62**	2	**50**	*	100
Citrobacter freundii	4	*	*	*	*	**75**	**75**	100	**75**	100	**75**	**67**	**75**	100	100	26	**88**	96	82
Citrobacter koseri	3	*	*	*	*	100	**100**	100	100	100	100	100	**100**	100	100	26	100	85	100
Enterob. aerogenes	14	*	*	**83**	*	**86**	**86**	100	100	100	100	100	**86**	100	100	15	93	**13**	**100**
Enterob. cloacae	28	*	*	100	*	**100**	**100**	100	90	100	97	100	100	100	93	31	88	**38**	84
E. coli	209	**49**	57	95	87	95	96	97	**68**	100	85	85	96	100	76	817	**58**	96	72
Klebsiella oxytoca	19	0	88	94	**74**	89	89	89	**84**	100	95	94	89	100	95	20	85	65	90
Klebsiella pneumoniae	70	0	85	100	88	90	90	90	92	97	93	93	90	100	88	261	91	38	94
Proteus mirablis	69	**80**	94	100	93	99	94	96	**61**	100	90	93	96	*	83	158	56	0	75
Pseudomonas aeruginosa	115	*	*	90	*	**76**	*	78	**57**	95	81	92	*	82	*	119	47	*	*
Serratia marcescens	19	*	*	100	0	100	100	100	95	100	100	100	100	100	95	8	88	0	**88**
Stenotrophomonas maltophilia	13														**100**	3			100

Bolded numbers reflect a greater than or equal to 10% change in susceptibility relative to the prior year. Blue = Improved/ **Red** = Worsened.

Numbers reflect the percent susceptible based on achievable blood levels of antimicrobials.

A blank = not tested: an asterisk=not reportable or alternative testing method recommended.

Levofloxacin is the preferred fluoroquinolone at REX.

For Pseudomonas aeruginosa pneumonia, Piperacillin/Tazobactam requires high dosing.

ESBL (Extended Spectrum Beta-Lactamase data)

E.coli	41 isolates, 4% of all E.coli isolates
K. oxytoca	3 isolates, 3% of all K.oxytoca isolates
K. pneumoniae	27 isolates, 8% of all K. pneumoniae isolates

Rex Healthcare 2008 ANTIBIOGRAM
January-December 2007 Results

Gram-Positive Organisms

	NON-URINE SOURCES												URINES			
		Penicillins			Miscellaneous											
Gram-Positives	***# Isolates***	Ampicillin	Oxacillin	Penicillin	Clindamycin	Cefotaxime *non-CSF	Ceftriaxone *non-CSF	Erythromycin	Levofloxacin	Vancomycin	Tetracycline	TMP/SMX	***# Isolates***	Levofloxacin	Nitrofurantoin	Tetracycline
Enterococcus faecalis	123	98	*	98	*			*	*	98	*		240	*	96	
Enterococcus faecium	30	20	*	**17**	*			*	*	**30**	*		44	*	**11**	*
Staph. aureus	676	*	38	2	*			30	**40**	100	93	95	82	**26**	99	95
Staph aureus (MSSA)	252	*	100	**6**	*			63	**77**	100	94	96			99	
Staph aureus (MRSA)	424	*	0	0	**74**			**7**	**13**	100	95	96			99	
Coagulase negative Staph	17	*	**28**	**17**	*			44	**33**	100	**94**		3	**0**	100	**100**
Staph. epidermidis	99	*	26	4	*			38	30	100	89		93	**21**	95	79
Staph lugdunensis	16	*	**81**	**0**	*			**94**	94	100	94		0			
Strep. agalactiae (Group B)	109		*	100*	58			**42**			*		42	91	*	*
Strep. pneumoniae	41			**72**		100	100	**76**	100	100		**81**	0			

Bolded numbers reflect a greater than or equal to 10% change in susceptibility relative to the prior year. **Blue** = Improved/ **Red** = Worsened.

Numbers reflect the percent susceptible based on achievable blood levels of antimicrobials.

A blank = not tested; an asterisk = not reportable or alternative testing method recommended.

For Enterococcus, the designation "susceptible" implies the need for combined therapy (Penicillin or Vancomycin plus an Aminoglycoside) in endocarditis or other serious invasive infections to achieve bactericidal action and an improved response.

Nitrofurantoin is ineffective for the treatment of UTI in patients with CrCl less than 60mL/min.

Strep agalactiae (Group B) data is for both inpatients and outpatients.

* 100% susceptible based on no reported cases of resistance

All MRSA and Group B streptococci requiring susceptibilities are screened for inducible resistance to Clindamycin using the "D-test".

Levofloxacin is the preferred fluoroquinolone at REX.

25% of Strep. pneumoniae tested intermediate to Penicillin.