REX

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Shiga Toxin Testing of Stool

Effective Spring 2008, the Rex Laboratory will begin testing all stool samples submitted for bacterial culture for Shiga toxins. We have been using a sorbitol MaConkey agar (SMAC) plate to look for *E. coli* 0157. The new test will increase the sensitivity in detecting *E. coli* 0157 and will also allow detection of other non-0157 serotypes of shiga toxin producing *E. coli* (STEC).

Background

Escherichia coli is a gram negative bacterial species closely related to *Shigella*. *E. coli* is the most commonly isolated bacterial species in clinical laboratories. It can be a source of infection in nearly every organ including urinary tract infections, pneumonia in hospitalized patients, meningitis in neonates, sepsis, and gastroenteritis. Serotypes of *E. coli* are commonly designated by their O (somatic) and H (flagellar) surface antigens.

Among the many subtypes of *E. coli*, several are agents of gastroenteritis. These are typically subdivided into groups based on their mechanisms of causing disease. The groups include enterotoxigenic *E. coli* (ETEC, a common cause of traveler's diarrhea), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enteroaggressive *E. coli* (EAEC), diffuse adherent *E. coli* (DAEC) and enterohemorrhagic *E. coli* (EHEC). The first five types are typically limited to the developing world, and only EHEC commonly causes diarrhea in children in the United States. EHEC, now commonly termed STEC (to confuse you), is associated with outbreaks of diarrhea, hemolytic uremic syndrome (HUS), and postdiarrheal thrombotic thrombocytopenic purpura (TTP).

Prior to 1982, STEC was not a recognized pathogen. During that year, an outbreak of hemorrhagic colitis with several cases of HUS, was linked to STEC O157:H7 (so named because it contained the 157th somatic antigen and the 7th flagellar antigen). In 1993 a large outbreak again linked with STEC O157 was traced to undercooked hamburgers at a fast food chain. This outbreak had over 500 confirmed cases and four deaths. In Washington State alone there were 30 cases of HUS with three of those patients dying. In 1993 *E. coli* 0157 became a nationally notifiable organism. In 1999 the CDC estimated it caused 73,000 illnesses, 2000 hospitalizations, and 60 deaths per year[4]. The Red Book reports an 8% rate of HUS in children with STEC O157 diarrhea. Fifty percent of those children required dialysis and the mortality rate was 3-5%[5].

In the last few years, several reports have noted a relative increase in STEC infections caused by non-O157 strains. In a report from Connecticut, the percentage of non-O157 STEC detected by toxin testing of stool cultures ranged from 41% to 69%. The most common STEC serotypes after 0157 include O26, O45, O103, O111, O121, and O145. The Connecticut study showed that the non-O157 serotypes caused less severe illness with bloody diarrhea in 56% of cases (compared to 90% with O157), less hospitalizations (12% versus 45%) and no HUS (0% versus 9%)[3]. In other studies, HUS has been linked to non-O157 strains, but O157 is considered the most virulent of the STEC.

Epidemiology

STEC O157 can be a component of the intestinal flora of cattle, sheep, deer, and other ruminants. These animals lack the receptor necessary for STEC O157 to cause disease. In studies the carriage rate in cattle has been about 1%. Fecal material transmits the organisms to humans. The infectious dose is as little as 100 organisms. STEC can be transmitted by undercooked ground beef, contaminated water or produce, or unpasteurized milk or cider. The organisms can also be transmitted directly from animals and their surroundings including petting zoos. Less is currently known about the non-O157 STEC.

REX PATHOLOGY ASSOCIATES, P.A.

John D. Benson, M.D.	(919) 784-3059	F. Catrina Reading, M.D.	(919) 784-3255
Timothy R. Carter, M.D.	(919) 784-3058	Vincent C. Smith, M.D.	(919) 784-3056
Stephen V. Chiavetta, M.D.	(919) 784-3060	John P. Sorge, M.D.	(919) 784-3062
Keith V. Nance, M.D.	(919) 784-3286	Rhonda Humphrey, Practice Manager	(919) 784-3063

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Early outbreaks led to changes by the US Food and Drug Administration concerning the "doneness" of meat, and no outbreaks from fast food hamburgers have been reported since 1995. Rules on shelf stable fermented sausages (think salami) were also changed and no outbreaks from this source have been reported in the last 10 years.

With increased safety in meat processing, there has been a relative increase in outbreaks from contaminated produce, such as the spinach associated outbreak of 2006. Unfortunately, washing produce has not been shown to prevent transmission. Other sources such as pools and drinking water can be reduced with appropriate chlorination. Once a person is infected, person to person transmission can occur, particularly in the day care setting.

It is important to accurately identify the type of outbreak to limit transmission. Patients who have recovered from a bout of STEC diarrhea can still harbor the organism for more than a week. Those that are more seriously ill should not be treated with antibiotics, as some classes of antibiotics have been reported to increase the incidence of HUS in patients with STEC O157.

Testing

Prior to the CDC recommendation of 2006, the Rex Laboratory only tested stools for *E. coli* O157 if ordered by the physician or if the stool specimen was grossly bloody. The testing method consisted of plating the stool on a SMAC agar. Typical enteric bacteria ferment sorbitol and therefore turn pink on this type of media. *E. coli* O157 is unusual in that it does not ferment sorbitol and thus remains colorless. Colorless colonies were then tested against O157 sera and positive results were sent to the North Carolina State Public Health Laboratory (NCPHL). In 2006, after the large outbreak of STEC 0157 linked to spinach, the CDC recommended all stool cultures be tested for at least *E. coli* O157, and preferably for all STEC by shiga toxin testing. In 2007 there were only two cases of *E. coli* O157 detected at in the Rex Microbiology laboratory. The most cases identified were in 2004, associated with the North Carolina State fair petting zoo, when eleven stools grew *E. coli* O157.

The traditional SMAC culture for *E. coli* O157 has been shown to be between 50 and 80% sensitive and does not detect any non-O157 strains, since these ferment sorbitol like the rest of the common enteric bacteria. The virulence factor that all STEC strains do share is production of a shiga toxin (also called shiga-like toxin or verotoxin). These shiga toxins are divided into two main groups, shiga toxin 1 (ST1) and shiga toxin 2 (ST2). STEC strains can make one or both types. Those making only ST2 are most often linked with HUS, while those making only ST1 are least likely to cause HUS. STEC that produce ST1 and ST2 have an intermediate risk of HUS.

Rex will begin testing all stool cultures with the Meridian ImmunoCardSTAT!™ EHEC qualitative immunoassay. This test is performed on growing broth cultures. The test can only be performed when growth is detected. In cases of no growth, the stool specimen can be applied to a new broth. If growth is still not attained an additional stool sample would be required. Note, this test is not a direct stool test, as shiga toxin production has been shown to be much higher in growing cultures, rather than in the stool itself[1].

Cultures with a positive result for ST1, ST2, or both will be called to the clinician and then forwarded to the NCPHL where the STEC will be serotyped and investigated to look for an evolving outbreak. The sensitivity of the STAT! EHEC™ immunoassay for STEC O157 is 40% higher than the traditional SMAC plate. The new test will also allow for the detection of STEC non-O157 strains, previously undetectable by the SMAC plate method.

Since all stool cultures will be tested for shiga toxin production, a specific order is not needed. Stool that cannot be transported to the lab within two hours should be placed in a C and S Para-Pak container (Modified Cary-Blair) and refrigerated. Specimens can be refrigerated for up to three days prior to testing.

Vincent C. Smith MD

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Cytologic Changes of Intrauterine Contraceptive Devices Observed on Pap Test

Over recent years, intrauterine contraceptive devices (IUDs) such as Mirena® (levonorgestrel-releasing device) and ParaGard® (copper device) have gained in popularity with women of child-bearing age as a safe and effective long-term reversible method of contraception. Accordingly, one would expect cellular changes associated with such devices to be observed in higher frequency in both liquid-based and conventional Pap tests. As these changes can mimic both squamous and glandular neoplasia, it is important for the clinician to provide the clinical history of IUD use, and imperative for the pathologist to correlate such information with the cytomorphologic findings to arrive at an accurate diagnostic conclusion.

The variable reactive/regenerative and degenerative cytologic changes associated with IUDs are thought to result from irritation of the endometrial and endocervical epithelium by the mechanical effect of the intrauterine portion of the device as well as the attached filament[1]. Such changes can be observed from the time of insertion, though most commonly appearing after three months of use, and may persist for months beyond removal of the device[2].

Changes in Endometrial Cells

Immediately after insertion of an IUD, endometrial cells may acquire reactive changes, with an increase in attendant neutrophils and histiocytes. With an IUD in place, endometrial cells may be shed throughout the menstrual cycle. Without one, endometrial cells observed 12 days beyond the date of the patient's last menstrual period ("out of phase") would raise concern for possible endometrial pathology. The endometrial cells may be shed as single cells, or in clusters, usually with uniform nuclear features and scant cytoplasm. Acquired cytologic changes may be reactive or degenerative with cytoplasmic vacuolization and nuclear irregularities, mimicking adenocarcinoma. (Image 1, 2) Features that should not be found in IUD effect, and when identified should raise the diagnosis of adenocarcinoma, include nuclear pleomorphism and enlargement, abnormal chromatin, prominent nucleoli, and background watery tumor diathesis. Atypical endometrial cells shed singly with changes that mimic CIN 3 have been termed "IUD cells"[2]. The cytologic changes include increased N:C ratios, nuclear hyperchromasia, and irregularly folded nuclear membranes, though usually to a lesser degree than seen in cells of true CIN 3. In addition, the spectrum of squamous dysplasia is absent in the background of IUD cells.

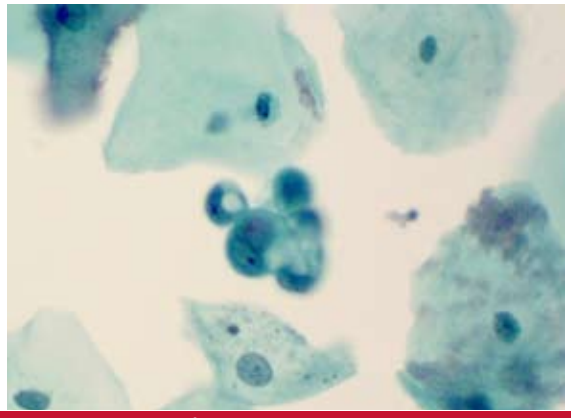

Image 1: Small cluster of reactive endometrial cells with cytoplasmic vacuolization and nucleoli

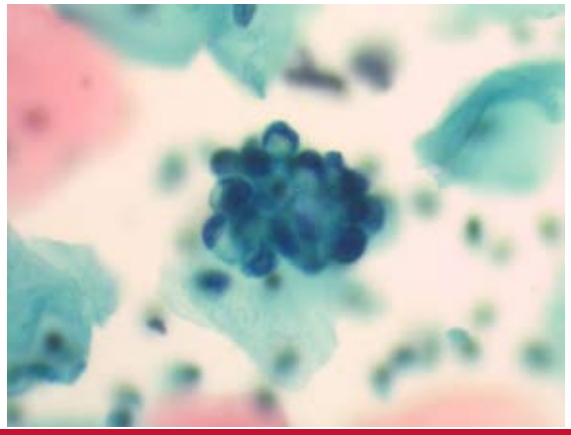

Image 2: Irregular cluster of vacuolated signet-ring cell-like endometrial cells mimicking ovarian carcinoma

Changes in Endocervical Cells

The mechanical effect of the IUD filament on endocervical epithelium can lead to shedding of clusters or papillary aggregates of endocervical cells with exuberant reactive changes. These changes include cytoplasmic distention by large clear vacuoles, which in turn may be infiltrated by neutrophils. (Image 3) In addition, enlargement of the nuclei with prominent nucleoli, and nuclear hyperchromasia may be observed[2]. Mitoses and psammoma bodies may be rarely observed in association with the reactive groups[3]. Without a clinical history of IUD use, these reactive changes may be misinterpreted as diagnostic of endometrial or ovarian adenocarcinoma.

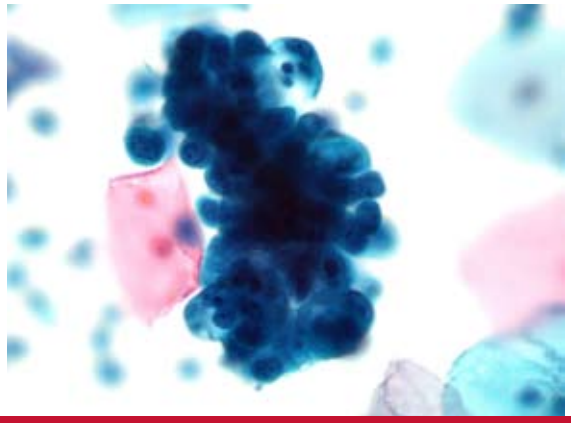

Image 3: Papillary fragment of reactive endocervical cells with cytoplasmic vacuoles and pyknotic debris

Actinomyces

The presence of Actinomyces in a Pap test is associated with plastic IUDs in place for three or more consecutive years without replacement[1]. Actinomyces appears as tangled clumps of radially-distributed filamentous organisms, imparting a "cotton ball" or "woolly body"

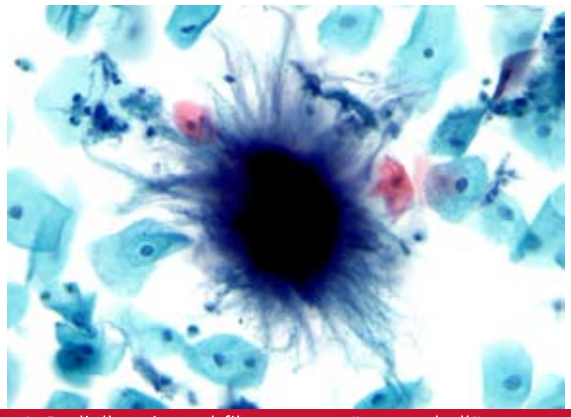

Image 4: Radially-oriented filamentous "cotton ball" arrangement of Actinomyces

appearance from low-power[3]. (Image 4) In most instances, Actinomyces is not a pathogenic infection, and is generally harmless to the patient[1].

In summary, the IUDs create cellular changes that may cause "false positive" interpretation of cervicovaginal smears. Thus, clinical history of IUD use should be provided on the cytology requisition at the time the smear is submitted for review.

F. Catrina Reading, M.D.

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