Marshfield Labs™
A division of Marshfield Clinic

Laboratory News

VOL. 36, NO. 16 - DECEMBER 23, 2013

Inside This Issue

NEW TEST ANNOUNCEMENT: NUCLEIC ACID TEST FOR THE DETECTION OF *SALMONELLA*, *SHIGELLA*, *CAMPYLOBACTER*, & SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* INCLUDING SEROTYPE O157:H7

Thomas Novicki, PhD, DABMM; Mary Ellen Nedd, MT(ASCP); Tara Marti, BS; Timothy Uphoff, PhD, DABMG, MLS(ASCP)CM - Division of Laboratory Medicine

Effective **01/13/2014** the current feces culture (test code FEC) will be replaced by the nucleic acid test (NAT) **Fecal Bacterial Pathogens, NAT(test code SSCSNAT)**. As with routine fecal culture, this test detects the bacterial enteropathogens *Salmonella, Shigella, Campylobacter*, and the O157:H7 serotype of Shiga Toxin-producing *Escherichia coli* (STEC). Importantly, this test also identifies Shiga toxins produced by non-O157 STEC strains not now routinely detected in our laboratory. These non-O157 strains cause approximately 60% of all STEC disease in the US. The NAT has been shown to be more sensitive than culture for detection of these pathogens. Another benefit is a substantial reduction in analytical turnaround time, from five days to twenty-four hours. To provide enhanced service and the fastest results possible, Marshfield Labs will perform NAT testing Monday through Saturday. Antimicrobial susceptibility tests (AST) will continue to be available on request. Rectal swabs will no longer be accepted.

BACKGROUND

Acute bacterial gastroenteritis, often related to contaminated foods, accounts for a significant portion of all infectious diseases, with an estimated 3.6 million cases in the US alone in 2011. While at least 31 bacterial, viral, and parasitic enteropathogens have been identified, only a subset is commonly recovered in clinical labs by routine bacterial fecal

continued on page 2

culture: *Salmonella enterica* (>2000 serotypes), *Shigella sp.* (*S. boydii, S. dysenteriae, S. flexneri,* and *S. sonnei*), *Campylobacter sp.* (*S. coli, S. jejuni*) and *E. coli* serotype O157:H7. In addition, some clinical laboratories routinely employ a commercially available enzyme immunoassay (EIA) to detect non-O157 serotypes of STEC not identifiable by culture. However, recent data suggests that these EIAs may be only 29% sensitive when compared to molecular methods[4].

The **Fecal Bacterial Pathogens, NAT** utilizes an FDA cleared *Salmonella, Shigella, Campylobacter*, Shiga toxin (SSCS) real-time PCR assay kit. Marshfield Labs was part of a multi-center clinical trial comparing the performance of the NAT with routine culture and EIA in the examination of more than 1,100 stool samples[5]. In this trial, culture for *Salmonella* species was shown to be only 67% as sensitive as the SSCS method. Conventional culture and EIA for other pathogens were inferior to the SSCS method as well (71% for *Shigella sp*., 77% for *Campylobacter sp*., and 53% for STEC). In the trial, the prevalence of these four pathogens was 5.64% using culture and EIA methods, but this prevalence increased by 32 % to 8.33% when the SSCS method was employed. Discordant findings between conventional culture/EIA and NAT methods were resolved using DNA sequence analysis of independent targets.

SAMPLE TYPES

Raw stools must be placed into Carey Blair or C&S transport medium within two hours of collection. In transport medium, samples are stable for up to 5 days if stored at 2-8°C. One gram is the minimum amount of stool necessary for testing. Swab samples are not acceptable.

ANTIMICROBIAL SUSCEPTIBILITY TESTS

A potential drawback to the use of NATs in the microbiology lab is that no culture isolate is immediately available for AST. Given the poor sensitivity of conventional culture in comparison with NAT, an isolate of the disease causing microorganism may not be recoverable. Since AST is not routinely performed on fecal isolates, this should not pose a significant limitation. Note that AST will continue to be available on request for NAT-positive fecal specimens, if the enteropathogen can be isolated in culture.

TEST INFORMATION

Test Name: Fecal Bacterial Pathogens, NAT, Stool
Test Code: SSCSNAT
Synonyms/Keywords: *Salmonella, Shigella, Campylobacter, Escherichia coli* O157:H7, STEC, EHEC, Shiga Toxin, Stool, Feces, Nucleic Acid Test
Clinic (Clinical Order Manager): Fecal Bacterial Pathogens, NAT, (test code SSCSNAT)
Hospital (Centricity): Fecal Bacterial Pathogens, NAT, (test code SSCSNAT)
Downtime: Write-In (Form I)
Specimen Requirements:

- **Local:** Raw stool should be placed in Cary Blair or C&S Transport Medium within 2 hours of collection. Remove cap and place approximately 1 gram of the raw stool into the transport medium or a sufficient amount to bring the liquid level up to the "fill to here" line. Send refrigerated.
- **Outreach:** Raw stool should be placed in Cary Blair or C&S Transport Medium within 2 hours of collection. Remove cap and place approximately 1 gram of the raw stool into the transport medium or a sufficient amount to bring the liquid level up to the "fill to here" line. Replace cap and tighten. Agitate

continued on page 3

the vial to permit adequate mixing of the specimen with the transport medium. Send refrigerated.

- **Minimum**: 1 gram raw stool.
- **Rejection Criteria:**
  The following are not acceptable:
  - Rectal swabs.
  - Feces at room temperature equal to or greater than 12 hours old.
  - Feces refrigerated equal to or greater than 24 hours old.
  - ParaPak C&S filled to fill line, equal to or greater than 120 hours old.
  - Feces in ParaPak Enteric Plus transport media

Storage: Refrigerate.
Available: Test is set up Monday through Saturday; analytic time of 1 day.
Qualitative Interpretation: Positive or Negative.
CPT Code: 87798 x 5

QUESTIONS

As of January 13, 2014, additional information will be available in: Marshfield Labs Test Reference Manual.
For clinical consultation, contact Dr. Thomas Novicki or Dr. Thomas Fritsche at 800-222-5835.
For technical information, contact Dr. Timothy Uphoff at 800-222-5835.

REFERENCES

1. CDC. National enteric disease surveillance: Shiga toxin-producing *Escherichia coli* (STEC) annual report, 2011. http://www.cdc.gov/ncezid/dfwed/PDFs/national-stec-surv-summ-2011-508c.pdf (accessed 08/26/2013).
2. CDC. Recommendations for diagnosis of Shiga Toxin-producing *Escherichia coli* infections by clinical laboratories. MMWR 2009 58:RR-12.
3. Scallan, E., R.M. Hoekstra, F.J. Angulo, et al. Foodborne illness acquired in the United States – major pathogens. 2011. Emerg. Inf. Dis. 17:7.
4. Vallieres E, Saint-Jean M, Rallu F: 2013. Comparison of three different methods for detection of Shiga toxin-producing Escherichia coli in a tertiary pediatric care center. J Clin Microbiol, 51(2):481-486.
5. Buchan, B.W., W.J. Olson, M. Pezewski, M. J. Marcon, T. Novicki, T. S. Uphoff, L. Chandramohan, P. Revell, and N. A. Ledeboer, et al. Clinical evaluation of a real-time PCR assay for the identification of *Salmonella, Shigella, Campylobacter* (*C. jejuni* and *C. coli*), and Shiga toxin producing *E. coli* in stool specimens. J Clin Microbiol, 2013 Sep 18. [Epub ahead of print].

TEST CHANGES IN CHEMISTRY - 24 HOUR SERVICES

Annu Khajuria, PhD, Section Head, Chemistry - 24 Hour Services

NEW CHEMILUMINESCENT IMMUNOASSAY FOR ERYTHROPOIETIN

Effective December 23, 2013, a chemiluminescent immunoassay for Erythropoietin (EPO) is replacing the immunoassay method currently used in Chemistry - 24 Hour Services.

continued on page 4

The manufacturer of the current method is no longer supporting the assay due to permanent unavailability of antibody for the assay.

METHOD

The new Erythropoietin (EPO) assay is a two-site immunoenzymatic paramagnetic particle, chemiluminescent immunoassay, traceable to WHO 2nd IRP 67/343.

The precision observed as 4 to 6% is well within the acceptable performance limits. A significant difference was observed between the previous method and the new method, which signifies different antibody specificity between the methods. The reference intervals have therefore, been evaluated for the new EPO chemiluminescent immunoassay with normal subjects.

TEST INFORMATION

Test Name: Erythropoietin
Text Code: EPOT
Specimen Requirements: Serum or heparinized plasma; minimum volume 0.5 mL.
Morning values are higher than afternoon values because of diurnal rhythm of secretion. For optimal results in serial patient monitoring, all specimens should be collected at the same time of day.
Storage: Refrigerate.
Available: Monday through Sunday, 24 hours.
New Reference Interval: 3 – 19 mIU/mL (Reference interval applies to all ages).
It is recommended that any serial testing performed on the same patient over time should be performed with the same EPO assay. EPO results on different methods are not interchangeable.
CPT Code: 82668

TESTOSTERONE RE-STANDARDIZATION

Effective December 23, 2013, a re-standardized serum Testosterone assay is replacing the current serum Testosterone assay.

The manufacturer has made changes to the Testosterone calibrators using a new source for primary calibrators. The new calibrators however, continue to be traceable to USP reference material based on EN ISO 17511. When a calibration standard is changed, the accepted laboratory practice is to establish a new baseline for patient monitoring.

METHOD

The testosterone assay was re-evaluated for its precision and accuracy with the new calibrators. The precision observed as 3% is well within the acceptable performance limits. Patient comparisons between the current and re-standardized testosterone shows a downward shift of approximately 8% (6% to 9% CI) which is consistent and within the bias proposed by the manufacturer.

The reference intervals have been re-evaluated for normal adult males & females.

continued on page 5

TEST INFORMATION

Test Name: Testosterone, Total
Test Code: TEST
Specimen Requirements: Serum; minimum volume 0.5 mL.
Testosterone secretion is episodic. Serum testosterone levels in adult males peak in the early morning (7 a.m.), decreasing about 25% to the evening (minimum at about 8 p.m.). Levels increase after exercise and decrease after immobilization and after glucose load.
Storage: Refrigerate <48 hours; freeze >48 hours.
Available: Monday through Sunday, 24 hours.
New Reference Intervals:

Adult Males 18 to 49 years:	200 – 700 ηg/dL
Adult Males >50 years:	185 – 540 ηg/dL
Adult Females >18 years:	<10 – 47 ηg/dL

Reference intervals for the pediatric population have not been established and are adopted from the literature. Serum and plasma values should not be used interchangeably. Values obtained from different immunoassay methods may differ and cannot be used interchangeably.
CPT Code: 84403

Results obtained by liquid chromatography/tandem mass spectrometry are lower than results by immunoassays. Testosterone levels can fluctuate substantially between different days. Assessment of androgen status should be based on more than a single measurement.

The Endocrine Society and American Society of Andrology recommends using total testosterone measurement preferably obtained on more than one morning sample as a screening test for hypogonadism in men. Most direct immunoassays are able to accurately distinguish between concentrations found in classic hypogonadism and normal levels and allow fast turnaround time. Direct immunoassays have been found to be adequate for identifying but not accurately quantifying elevated testosterone in women. Testosterone determinations in children should be assessed using assays with sufficient sensitivity and in conjunction with appropriate reference intervals (J Clin Endocrinol Metab. 2007; 92: 405).

For the diagnosis of androgen dysfunction in females and children as well as monitoring hypogonadal men, it is recommended that high sensitivity and specificity assays able to measure very low levels of testosterone concentrations be used. Direct immunoassays are not able to accurately measure at very low levels.

QUESTIONS

For queries or for additional information, refer to Marshfield Labs Test Reference Manual, or contact Dr. Annu Khajuria at 800-222-5835.