Anaerobe ◆ 2008

**The 9th Biennial Congress of the
Anaerobe Society of the Americas**

**Renaissance Hotel ◆ Long Beach, California USA
June 24-27, 2008**

SESSION IX—CLOSTRIDIUM DIFFICILE: DIAGNOSIS

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CLOSTRIDIUM DIFFICILE: DIAGNOSIS

CLOSTRIDIUM DIFFICILE TESTING ALGORITHMS: WHAT IS PRACTICAL AND FEASIBLE?

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The laboratory diagnosis of *C. difficile* infection is currently undergoing an evolution. For the past decade, the predominant testing strategy was the detection of toxin A + B in stool using a solid phase enzyme immunoabsorbent assay (EIA). Recent studies using immunologic detection of glutamate dehyrogenase (GDH) in stool either by solid phase EIA or immunochromatographic detection (IDT) have proven to be more sensitive but less specific than toxin A + B EIAs or IDTs. GDH screening has negative predictive value of 99%+ but has a positive predictive value of approximately 50%. Given the high negative predictive value, approximately 80% of specimens can be reported as negative after screening. The key issue in a GDH driven testing algorithm, is what should be used as the confirmatory test for the 20% that are positive by GDH screening. Four potential confirmatory tests are available, cytotoxicity neutralization, toxigenic culture, IDT for toxin A & B, or PCR. All four approaches have been used. In this presentation, the rationale for using GDH screening will be presented as well recommendations for the use of the 4 different confirmatory approaches either alone or in combination. These recommendations will be based not only on test performance but also on cost analysis, efficiency, and availability of technology.

CLOSTRIDIUM DIFFICILE: DIAGNOSIS

USE OF MOLECULAR DIAGNOSTIC TESTING FOR THE DIAGNOSIS OF *CLOSTRIDIUM DIFFICILE*-ASSOCIATED DIARRHEA (CDAD)

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Clostridium difficile-associated diarrhea (CDAD) is the major recognized cause of healthcare-associated infectious diarrhea. Current laboratory testing lacks a single assay that is sensitive, specific, and rapid. The purpose of this presentation is to describe and compare real-time polymerase chain reaction (PCR) diagnostic tests for CDAD to more commonly used testing, in particular enzyme immunoassays (EIA). Our real-time PCR assay was validated from July 2004 to April 2006 and involved tests on 1,368 stool samples. Subsequently, 350 inpatients were prospectively interviewed for clinical findings in 365 episodes of diarrheal illness as retrospective chart review could not provide the needed clinical information; a prospective process for new CDAD diagnostic tests that should be routinely followed. Test results and clinical criteria were used to assess the performance of four assays. Using clinical criteria requiring at least 3 loose stools in one day as part of the reference standard for a positive test supporting the diagnosis of CDAD, the sensitivity, specificity, positive and negative predictive value for the EIA was 73.3%, 97.6%, 73.3%, 97.6%; for real-time PCR was 93.3%, 97.4%, 75.7%, 99.4%; for cell culture cytotoxin assay was 76.7%, 99.1%, 69.7%, 97.9%; and for anaerobic culture (for toxigenic *C. difficile* strains) was 100.0%, 95.9%, 68.2%, 100.0%. The real-time PCR and anaerobic culture assays were significantly more sensitive than the EIA test ($p<0.01$ to $p<0.05$). Others have confirmed the low sensitivity of EIA testing, which is used by the majority of diagnostic laboratories (>90%) in the USA. The technologist testing time and reagent costs ranged from 1-5 minutes and \$2.69-\$6.85, suggesting all are feasible for the clinical laboratory. With an assay turn-around-time of under 4 hours, real-time PCR is a more sensitive and equally rapid test that is a feasible laboratory option to replace EIA for toxigenic *C. difficile* detection. We also found a critical need for ongoing medical education of healthcare providers to assure they are informed as to the clinical presentation of patients having illness consistent with CDAD.

CLOSTRIDIUM DIFFICILE: DIAGNOSIS

LESSONS LEARNED: SETTING UP A REFERENCE LABORATORY FOR *CLOSTRIDIUM DIFFICILE*

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During the past years, a new variant of toxigenic *Clostridium difficile*, designated North American pulsed-field type 1 or PCR ribotype 027 (NAP1/027), with a more severe outcome of *C. difficile*-associated disease (CDAD) has been experienced, first in American continent and then in western part of Europe. In Finland, nationwide knowledge about the incidence of CDAD has been scarce, thus, increasing awareness of this hypervirulent strain prompted the National Public Health Institute (KTL) with the support of the Ministry of Social Affairs and Health to put effort to clarify the situation, including changing epidemiology of CDAD and potential emergence of this hypervirulent strain in the country. In 2006, we conducted a questionnaire survey on the methods used in Finnish clinical microbiology laboratories to diagnose CDAD. According to the reply from 28 out of 32 laboratories requested, most laboratories performed diagnostic tests for *C. difficile*; however, there were some discrepancies between the criteria for investigations and diagnostic tests used in the laboratories. At the same time, based on the analysis of Finnish hospital discharge data and death certificates during 1996-2004, it was reported that the incidence of CDAD and CDAD-related deaths had significantly increased in Finland among elderly. In 2007, we started to set up typing facilities for *C. difficile* and, as the European reference method, PCR ribotyping was selected. Somewhat unexpectedly, three cases with the involvement of the PCR ribotype 027 were detected in October 2007. Since its first detection, more than 200 strains identified as 027 have been isolated from various health-care premises. So far, this specific strain seems to be disseminated only in southern and south-western Finland. Future considerations on *C. difficile* include further typing facilities, survey on antimicrobial usage in connection to fluoroquinolone-resistant strains as well as investigation on the prevalence of various ribotypes in Finnish CDAD cases.

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OPTIMIZATION OF A THREE-STEP ALGORITHM TO DETECT TOXIGENIC *CLOSTRIDIUM DIFFICILE* IN FECAL SAMPLES

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The purpose of this study was to optimize the second step of a 3-step algorithm we developed previously to detect toxigenic *C. difficile* in feces. The algorithm previously enabled completion of final reports on 79% of stools within 3 hours of specimen receipt. In Step 1, the C. DIFF CHEK™-60 (TechLab; Inverness) EIA was used to detect *C. difficile* glutamate dehydrogenase (GDH). If negative, the assay was finaled. If positive, Step 2 was performed using the ProSpecT *C. difficile* Toxin A/B (Remel) EIA. If this Remel toxin A/B test was positive, a final result of "Positive *C. difficile* assay" was reported. If the toxin A/B EIA was negative, a preliminary "indeterminate" result was issued and Step 3 was performed. In Step 3, direct stool cytotoxin testing and culture for toxigenic *C. difficile* was performed. If both were negative, the assay was reported as "Stool filtrate negative for *C. difficile* toxin and culture for toxigenic *C. difficile*." If either test was positive, a final report indicated this.

Focusing on Step 2 of the algorithm, we compared the performance of the Remel Toxin A/B (R-A/B) test against a second generation ELISA, the TechLab Tox A/B II™ (TL-A/B), on 151 fresh stool samples from patients suspected of having *C. difficile*-associated diarrhea. Both tests for toxins A and B were performed in parallel along with a stool cytotoxin assay and culture for toxigenic *C. difficile*.

A true positive specimen was defined as one from which toxigenic *C. difficile* was isolated, one in which *C. difficile* cytotoxin in stool was demonstrated, or one that was positive by any two tests. The TL-A/B was positive on 13 more stools than the R-A/B ($P < 0.01$). The sensitivity, specificity, negative (N) and positive (P) predictive values (PV) of the R-A/B were 54%, 100%, 56% and 100%, respectively. The sensitivity of the TL-A/B was 66% with specificity 98%, PPV 98% and NPV 64%. Compared to the R-A/B, the TL-A/B had a shorter (30 min) incubation time.

In conclusion, the more rapid format *C. difficile* Tox A/B II was more sensitive than the ProSpecT *C. difficile* Toxin A/B, while specificities of both tests did not differ significantly. Using the Tox A/B II in Step 2 of the algorithm enabled us to complete more final reports more rapidly on the day of specimen receipt.

CLOSTRIDIUM DIFFICILE: DIAGNOSIS

CLINICAL COMPARISON OF THE MOLECULAR-BASED BD GENEOHM CDIFF ASSAY TO THE CYTOTOXICITY TISSUE CULTURE ASSAY FOR THE DIRECT DETECTION OF TOXIN B GENE FROM TOXIGENIC *CLOSTRIDIUM DIFFICILE* STRAINS IN FECAL SPECIMENS

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Objectives: The primary objective of this prospective evaluation was to demonstrate the use of the BD GeneOhm™ Cdiff (BD Diagnostics, San Diego, CA) real-time Polymerase Chain Reaction (PCR) assay as a diagnostic test for the detection of toxigenic *Clostridium difficile* (Cdiff) strains from fecal specimens. The performance of PCR was compared to a cytotoxicity reference standard (TechLab®, Blacksburg, VA). The BD GeneOhm™ Cdiff assay is a qualitative *in vitro* diagnostic test performed on the Cepheid SmartCycler® (Cepheid, Sunnyvale, CA), a random-access real-time PCR instrument. The assay utilizes PCR for the amplification of the toxin B gene (tcdB) and fluorogenic target-specific hybridization probes for the detection of the amplified DNA. The amplification, detection and interpretation of the signals are done automatically by the SmartCycler® software.

Methods: Liquid to soft stools received in the clinical laboratory for Cdiff testing were included in the evaluation. Briefly, stools were tested with the TechLab Cdiff chek™-60 enzyme immunoassay (EIA) for detection of the "common antigen", glutamate dehydrogenase (GDH), and positive results were confirmed with the Tox A/B assay. If the Tox A/B was negative, a cytotoxicity neutralization assay was also performed. Concurrently, the BD GeneOhm™ PCR assay was also performed on each stool specimen. Additional test methods (culture, cytotoxin/neutralization testing) were also performed on discordant specimens to aid in resolving discrepancies. Each stool was collected, processed, and tested according to the institution's standard of care and each assay was performed according to manufacturer's investigational use package insert.

Results: Of the 300 specimens included in this study, 248 (83%) tested negative with both PCR and cytotoxicity while 29 (10%) tested positive with both assays yielding 90.6% sensitivity and 92.9% specificity. After resolution of discordant results, the sensitivity and specificity was 93.6% and 98.0% respectively with a prevalence of nearly 15%.

Conclusions: The diagnosis of toxigenic *C. difficile* is usually done by a combination of cytotoxicity assay, culture, and EIA, all of which are either labor intensive and time-consuming or lack sensitivity or specificity. The BD GeneOhm™ Cdiff assay (performed directly on stool specimens) offers sensitivity and specificity that is comparable to the cytotoxicity reference standard and produces results in about one hour.

CLOSTRIDIUM DIFFICILE: DIAGNOSIS

COMPARISON OF BD GENEOHMTM CDIFF ASSAY (BD GENEOHM, SAN DIEGO, CA) TO *CLOSTRIDIUM DIFFICILE* TOXIN B TEST (TECHLAB,® BLACKSBURG, VA)

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Purpose: Rapid detection of toxin producing strains of *Clostridium difficile* is essential for management of patients with *C. difficile* associated disease (CDAD) during an era of increasingly virulent infection. PCR shows promise for rapid detection (2-3 hrs) and enhanced sensitivity. We compared the BD GeneOhm™ Cdiff Assay (Cdiff) (BD GeneOhm, San Diego, CA), a real time PCR that amplifies *tcdB*, to the *C. difficile* Toxin B Test (TOX-B) (TechLab,® Blacksburg, VA) for a 510K FDA clinical submission.

Methods: Liquid (n=273) or soft (n=131) stool specimens from 377 patients were tested for *C. difficile*. Set up on the same day by independent staff, Cdiff Assay and TOX-B Test were performed according to the manufacturers' package inserts. Bacterial culture on selective media followed by toxin and PCR testing of recovered isolates was done for discrepant analysis.

Results: 404 stool specimens tested; 340 samples were concordantly negative between both assays. 40 specimens were PCR positive were also cytotoxin positive (10.0%). The overall agreement between Cdiff Assay and TOX-B Test was 94.8% (380/401). Initially, PCR for 3 samples were inhibited; 1 resolved upon retesting. One sample produced non-specific cytotoxin results. Of the PCR positive cytotoxin negative samples (n=17), 13 *C. difficile* isolates were recovered (11 isolates were Cdiff and TOX-B positive). Of the PCR negative cytotoxin positive samples (n=4), 1 isolate was recovered (Cdiff and TOX-B positive). Compared to the TOX-B test, initial sensitivity, specificity, positive and negative predictive values of the Cdiff Assay were 90.1% (40/44) and 95.5% (341/357), 71.4%, and 98.8%, respectively. After use of *C. difficile* culture to resolve 21 samples where Cdiff and TOX-B disagreed, the sensitivity, specificity, PPV, and NPV of the Cdiff assay improved to 98.1% (51/52), 98.3% (343/349); 89.5% (51/57); 99.7% (343/344), respectively.

Conclusion: The BD GeneOhm™ Cdiff Assay compares favorably with the TechLab® *C. difficile* Toxin B Test. The high negative predictive value conveys that the Cdiff Assay is dependable for excluding CDAD.

CLOSTRIDIUM DIFFICILE: DIAGNOSIS

IMPROVED TOXIN DETECTION RATES OF VIDAS CDAB COMPARED WITH CDA2, FOR DIAGNOSIS OF TOXIN PRODUCING *CLOSTRIDIUM DIFFICILE* IN *C. DIFFICILE* VARIANT PREVALENT AREA

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Variant strains of *Clostridium difficile* have been described with increasing prevalence in worldwide. TcdA⁻TcdB⁺ strains have caused wide disease spectrums ranging from uncomplicated diarrhea to fatal pseudomembranous colitis. Commercial toxin A immunoassay used by many clinical laboratories might lead to false negative results in TcdA⁻TcdB⁺ *C. difficile* strains. Therefore, we evaluated the newly developed *C. difficile* toxin immunoassay, VIDAS CDAB (BioMerieux, France), for both toxins, TcdA and TcdB.

From April to December 2007, a total 555 Fecal samples were collected from patients suspected to have *C. difficile* infection were cultured anaerobically using CCFA media. Bacterial isolates were identified according to colony morphology on CCFA, spore stain and biochemical assay with the ANA identification kits (BioMerieux, France). Toxin A immunoassay, VIDAS CDA2, was done concurrently with the same specimen to evaluate how much VIDAS CDAB could improve the detection rates in *C. difficile* variant prevalent area.

C. difficile was isolated from 150 specimens. *C. tetani* was isolated from 65 specimens, and 450 specimens showed culture negative results.

PCR assays for *tcdA* and *tcdB* gene were done with 150 *C. difficile* isolates according to the method of Koto et al.

The numbers of *tcdA⁺tcdB⁺*, *tcdA⁻tcdB⁺*, *tcdA⁺tcdB⁻* and *tcdA⁻tcdB⁻* strains among 150 strains were 75(50%), 41(27.4%), 2(1.3%) and 32(21.3%), respectively. Therefore, sensitivity and specificity of VIDAS CDAB were 62.7%(74/118) and 92.9%(406/437), respectively. Compared to the VIDAS CDAB, CDA2 positive rate was 28.8% (34/118). The specificity of CDA2 was 94.1%(411/437). If we considered equivalent results as positive, the sensitivity of VIDAS CDAB was increased up to 71.2%(84/118). One of the noticeable facts was that the false positive rate of VIDAS CDAB (10.8%) was higher than that of CDA2 (1.5%) in *C. tetani* cases.

Therefore, newly developed CDAB improved the detection rates more than double and the specificity was almost same as CDA2. We concluded that VIDAS CDAB is an effective and useful diagnostic tool for diagnosis of toxin producing *C. difficile* in *C. dfficile* variant prevalent area.