CME/SAM

Pathology Consultation on Detection of *Clostridium difficile*

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Upon completion of this activity you will be able to:

- list major indications for, and limitations of, *Clostridium difficile* diagnostic testing.
- recognize benefits and drawbacks of traditional and new molecular methods for laboratory detection of *C difficile*.
- discuss common and controversial test ordering practices encountered by clinical laboratories.

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Questions appear on p 156. Exam is located at www.ascp.org/ajcpcme.

Abstract

Laboratory methods for detecting Clostridium difficile *have undergone considerable evolution since the organism's etiologic association with antibiotic-associated diarrhea and colitis was established. Clearly, familiarity with the advantages and shortcomings of the various assays is essential for the laboratory director when choosing among these tests. For the consulting pathologist, furthermore, an understanding of the laboratory's role in securing a diagnosis of* C difficile *infection (CDI) is also required to identify requests for unnecessary testing that may be costly and potentially misleading. The purpose of this article is to highlight the major differences in laboratory test methods for CDI and to review a few commonly encountered provider ordering scenarios.*

Consult Questions

Many new laboratory methods have been developed recently for the detection of *Clostridium difficile*, including several commercially available molecular assays. This pathology consultation focuses on clinical laboratory testing for *C difficile* in the context of the following questions:

- How do currently available *C difficile* detection methods compare in terms of sensitivity, specificity, and cost-efficiency?
- What are some common issues related to *C difficile* test-ordering practices, and should these be monitored or mandated by clinical laboratory professionals?

Background

C difficile infection (CDI) is a major cause of health care–associated gastrointestinal infection in the United States.[1] The responsible organism is an obligate anaerobic, gram-positive bacillus initially described in 1935 as a normal inhabitant of fecal flora in neonates.[2] Like many clostridia, *C difficile* produces endospores that promote its persistence in the environment and transmission between persons. The subset of toxigenic strains contains genes that code for toxins A and B (*tcdA* and *tcdB*, respectively). These potent cytotoxins are directly responsible for the colonic epithelial damage in CDI.

CDI has been specifically linked to prior antimicrobial therapy that disrupts normal bowel microflora and health care settings (eg, nursing homes and hospital wards) where rates of colonization far exceed rates in the general population. CDI

typically manifests as diarrhea that may be accompanied by fever, leukocytosis, and hypoalbuminemia. CDI symptoms range from mild and self-limiting to severe pseudomembranous colitis with toxic megacolon and death. Severe disease is linked to advanced age, while symptomatic infection in infants and toddlers is uncommon despite very high carriage rates. Treatment consists of discontinuation of inciting antimicrobial(s) and, if indicated, a 10- to 14-day course of oral metronidazole or vancomycin along with supportive measures. Clinical improvement is usually noted within days, but more than 10% of patients treated will experience recurrent symptoms.[3,4]

In the early to mid 2000s, reports of community-acquired CDI cases without antecedent antibiotic exposure began to appear,[5] concomitant with the emergence of a quinolone-resistant, "hypervirulent" strain of *C difficile* (designated BI/NAP1/027 strain). This organism produces binary toxin and increased amounts of toxins A and B, possibly related to a truncating mutation in the *tcdC* gene, which codes for a putative repressor of toxin A/B production.[6-8] The spread of this strain has led to renewed interest in *C difficile* and to significant technical improvements in diagnostic laboratory testing for CDI.

Discussion

Issues Related to Detection Methods: Standardization, Complexity, and the Balance Between Sensitivity and Specificity

Traditional methods for detection include toxigenic culture (TC) and cell cytotoxicity neutralization assay (CCNA). TC consists of anaerobic stool culture, with in vitro confirmation of the toxigenicity of any *C difficile* isolates. TC has very high analytic sensitivity but requires several days and considerable expertise, limiting its applicability for routine use. However, TC remains absolutely essential for epidemiologic investigations. For CCNA, stool filtrate is inoculated onto confluent cell culture monolayers, which are monitored for cytopathic effect that is neutralized by antitoxin. This method requires 24 to 72 hours to complete and remained the diagnostic "gold standard" for many years owing to its high specificity (reviewed by Gerding and Brazier[9]). It is important to note that neither assay is standardized, so individual laboratory protocols vary widely.

Owing to its simplicity and rapid turnaround time, enzyme immunoassay (EIA) targeting toxin A and/or toxin B is currently the most commonly used laboratory method for *C difficile* detection.[10] Similar to CCNA, toxin-based EIAs have high specificity,[11] but recent evidence suggests their sensitivity compared with TC is quite low[12] **Table 1**.[13-27] Therefore, negative test results obtained by toxin EIA should be confirmed by an alternative method. By contrast, EIAs that detect *C difficile*–specific glutamate dehydrogenase (GDH) can achieve sensitivities approaching 100% compared with TC[28-30] (Table 1). However, GDH assays exhibit lower specificity relative to the toxin EIAs because GDH detection cannot distinguish toxigenic from nontoxigenic organisms. Hence, samples positive by GDH EIA require confirmation by an assay that targets toxigenicity per se. The high negative predictive value of GDH assays has led to numerous investigations of 2- and even 3-step testing algorithms using lower-cost GDH EIA as an initial "screening" assay, with subsequent confirmation of positive results by more laborious or expensive CCNA or polymerase chain reaction (PCR).[14,15,19,26,29,30]

The merits of algorithmic testing (eg, lower costs, more rapid reporting of negative results) vs universal molecular testing (eg, higher sensitivity and negative predictive value for some strains) are the subject of intense debate, which is exacerbated by the absence of a single laboratory diagnostic standard (see the following text) and by differences in interpretation of the strengths and limitations of our present knowledge (reviewed by Wilcox et al[31]). What is clear is that either approach will result in higher detection rates compared with toxin EIAs (Table 1). The Infectious Disease Society of America (IDSA) and the Society for Healthcare Epidemiology of America (SHEA) consensus guidelines endorse the GDH algorithmic approach but stipulate that universal molecular testing may also be considered.[32]

As of May 2011, 5 assay kits using molecular methods have received 510(k) clearance from the US Food and Drug Administration (FDA) for use as in vitro diagnostic devices for CDI detection **Table 2**. Molecular tests have demonstrated sensitivity comparable to or higher than the methods outlined earlier (Table 1). Factors that decrease the viability of organisms (eg, treatment with antibiotics) that would lower the sensitivity of the functional assays noted above do not affect molecular assays. All FDA-cleared methods are based on real-time PCR except Illumigene, which uses the isothermal loop-mediated amplification (LAMP) method.

Generally, molecular assays have a higher cost for reagents than the serologic or functional assays, although the price per assay may differ substantially between molecular platforms. Considerable investment may also be required for the acquisition of dedicated instruments. Only the Gen-Probe protocol uses a specific automated extraction instrument, whereas the other procedures include manual extraction or use of raw specimens (Table 2). It is critical to estimate the anticipated test volume and institutional turnaround time requirements to determine if batch testing is preferable over testing specimens in real time.

The Xpert *C difficile*/Epi test differentiates the B1/NAP1/027 epidemic strains from other strains. However, the

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Table 1
Published Performance Characteristics of Some Commercially Available and Laboratory-Developed Methods of Testing for *Clostridium difficile*[*]

Report	Comparator	Method	Sensitivity (%)	Specificity (%)	N	Prevalence (%)
Tenover et al[13]	TC	Xpert PCR	93.5	94.0	2,296	14.7
Novak-Weekley et al[14]	TC	Xpert PCR	94.4	96.3	428	16.8
		GDH + PCR	86.1	97.8		
		toxEIA	58.3	94.7		
Sharp et al[15]	GDH + PCR + EIA consensus or TC	GDH	100	94.2	284	14.7
		Xpert PCR	100	99.6		
		toxEIA	59.5	99.2		
Swindells et al[16]	TC	GDH	100	97	150	12.7
		Xpert PCR	100	99.2		
		GeneOhm PCR	94.4	99.2		
Stamper et al[17]	TC	GeneOhm PCR	83.6	98.2	401	14.2
Eastwood et al[18]	TC	GeneOhm PCR	88.5	95.4	600	20.8
		GDH	87.6	94.3		
Quinn et al[19]	LDT PCR + PCR + TC consensus	GDH	100	88.1	174	13.2
		GDH + toxEIA	78.3	100		
		PCRs	91-96	97-100		
		TC	91.3	98.3		
Stamper et al[20]	TC	proGastro PCR	77.3	99.2	285	15.7
		toxEIA	63.6	99.2		
Norén et al[21]	TC + CCNA	Illumigene LAMP	98.0	98.2	272	18.4
		CCNA	72.0	100		
Lalande et al[22]	TC	Illumigene LAMP	91.8	99.1	472	10.4
Sloan et al[23]	TC	LDT PCR	86	97	200	22
		toxEIAs	48	84-98		
		GDH1 + EIA	32	100		
Peterson et al[24]	Laboratory consensus + clinical diarrhea	LDT PCR	93.3	97.4	370	8.1
		toxEIA	73.3	97.6		
		CCNA	76.7	97.1		
Wren et al[25]	TC	GDH	100	98.5	500	11.4
		toxEIAs	31-45	99.8		
Larson et al[26]	LDT PCR + CCNA	CCNA	58.8	100	699	11.4
		GDH	86.3	92.7		
Barbut et al[27]	TC	LDT PCR	86.6	97.4	881	9.3

CCNA, cell cytotoxicity neutralization assay; EIA, enzyme immunoassay; GDH, glutamate dehydrogenase EIA; LAMP, loop-mediated isothermal amplification; LDT, laboratory-developed test; PCR, polymerase chain reaction; TC, toxigenic culture.
[*] GeneOhm PCR, Becton Dickinson Diagnostics, San Diego, CA; Illumigene LAMP, Meridian Bioscience, Cincinnati, OH; proGastro PCR, Prodesse, Waukesha, WI; Xpert PCR, GeneXpert, Cepheid, Sunnyvale, CA.

Table 2
Commercially Available, US Food and Drug Administration–Cleared Molecular Assays for Testing for *Clostridium difficile*

Assay/Manufacturer	Method/Target	Instruments Required	Platform[*]	Separate Sample Prep/Extraction Step[†]	Time to Results (h)
Prodesse ProGastro Dc Gen-Probe/Gen-Probe, San Diego, CA	Real-time PCR/tcdB	NucliSENS easyMAG (bioMerieux, Durham NC); Smartcycler II (Cepheid)	Open	Stool clarification + automated extraction	3
Xpert C. difficile/Cepheid, Sunnyvale, CA	Real-time PCR/tcdB, binary toxin, *tcdC* gene deletion in 027/NAP/BI	GeneXpert System (Cepheid)	Closed	No	1
Xpert C. difficile/Epi/Cepheid	Real-time PCR/tcdB, binary toxin, *tcdC* gene deletion in 027/NAP/BI	GeneXpert System (Cepheid)	Closed	No	1
GeneOhm Cdiff assay/ BD Diagnostics–GeneOhm, San Diego, CA	Real-time PCR/tcdB	Smartcycler II (Cepheid)	Open	Manual extraction	2
Illumigene *Clostridium difficile* assay/Meridian Bioscience, Cincinnati, OH	LAMP (loop-mediated isothermal amplification)/ tcdA	Illumipro-10 incubator/reader	Closed	Manual extraction	1

[*] Open platforms can be used for assays from other manufacturers or for laboratory developed tests, while closed platforms are specifically dedicated to tests from one manufacturer.
[†] According to the manufacturers' technical services departments, the preparation time for 1 sample is about 15 minutes for the GeneOhm and Illumigene assays. For the Illumigene assay, Meridian estimates a sample preparation time of 30 minutes for 16 samples; this would extend the total time to results to about 70 minutes. For the Prodesse ProGastro assay, the stool clarification step requires 5 to 10 minutes for a single sample and up to 30 minutes for a batch of 16 samples. The EasyMAG extraction takes about 45 minutes. The total time for extraction of 16 samples would be about 1 hour and 15 minutes, of which approximately 30 minutes is hands-on time. The amount of training required to perform the assays is related to the complexity of the assay protocol. The Xpert and Illumigene assays are labeled as moderately complex and, thus, can be performed by technicians.

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assay does not specifically distinguish other strain types that may be of epidemiologic interest.[33] Furthermore, the immediate clinical usefulness of distinguishing the BI/NAP1/027 strain is unclear at present since the therapeutic regimen and isolation precautions are largely based on the presence and severity of symptoms rather than the specific strain.[32]

Currently, the absence of a consensus gold standard assay for laboratory confirmation of CDI limits direct comparison between many published studies and presents a quandary to clinical pathologists. Methods that require toxin production—the sine qua non of CDI—for positivity (eg, toxin EIAs and CCNA) are less sensitive than assays that detect the organism itself (eg, GDH EIAs and TC) or its genes (eg, PCR and LAMP). Conversely, because colonization by toxigenic *C difficile* commonly occurs in the absence of disease,[34,35] the latter, more-sensitive methods can be considered less specific for the presence of CDI. For these reasons, it is incumbent on laboratories, especially those using more analytically sensitive methods, to ensure that CDI testing is performed on the appropriate patient populations and in the correct clinical context.

Issues Related to Provider Test-Ordering Practices: Repeated Testing, Testing of Infants and Children, Testing for Cure

Repeated Testing

For many years, the relatively low sensitivity of toxin EIAs compelled providers to initiate empiric therapy for suspected CDI in the absence of laboratory confirmation or to repeat testing until a positive result was obtained or symptoms subsided. For repeated testing with low-sensitivity assays, improved detection is anticipated with sequential samples from a single patient,[36] but this may be accompanied by a successive decrease in positive predictive value.[37] Several studies have failed to demonstrate any significant impact on management or outcomes when repeated toxin testing is performed.[38-40]

Having replaced toxin EIAs with higher sensitivity methods (eg, a molecular assay as a single method or a 2-step GDH algorithm), many laboratories will no longer accept a repeated stool sample from patients who tested negative for *C difficile* within a prescribed period (usually ~7 days). Based on review of all CDI testing performed during 3 years at our institution, which accepts any degree of repeated testing, we found that more than 25% of positive results by toxin EIA but only 5.4% of positive results by a 2-step, GDH/PCR algorithm would have been missed if repeated testing were not allowed within 7 days of a negative result.[41] Similar findings have been noted by others,[42] further affirming consensus guidelines that recommend against repeated testing for *C difficile* following a prior negative result, no matter which assay method is used.[32]

Testing of Infants and Children

C difficile is detected in the stool of 10% to 50% of children younger than 2 years.[43,44] Despite this prevalence, CDI is rarely seen in young children. Because the clinical specificity and usefulness of diagnostic assays performed in this pediatric population is uncertain, CDI testing should be undertaken judiciously.[45] Furthermore, if a clinical laboratory uses an FDA-cleared test that is not validated by the manufacturer for testing in this young population, the laboratory should perform validation studies to demonstrate clinical usefulness for this age group. Currently, the Illumigene assay is the only molecular test that is cleared by the FDA for use in children younger than 2 years.

Testing for Cure

The high recurrence rate of CDI following treatment has prompted some providers to request laboratory confirmation of organism eradication. Because laboratory evidence of organism persistence is not a predictor of CDI relapse[46] and treatment of asymptomatic colonized patients may actually increase the likelihood of subsequent CDI,[47] repeated testing of asymptomatic patients has no clinical validity. A "test for cure" is not endorsed by IDSA/SHEA,[32] and many laboratories require a 2- to 4-week interval between a prior positive result and the acceptance of subsequent samples for CDI testing.

Some providers or even health care facilities have requested that negative test results be documented before discontinuing patient contact isolation precautions. Given that organism shedding occurs for several weeks following successful therapy,[4,48] such a practice could prove costly. Since most nosocomial transmission is believed to occur chiefly as a result of shedding by symptomatic patients, infection control guidelines indicate that discontinuing isolation should be considered on, or within a few days of, symptom resolution, irrespective of laboratory results.[49] Still, the unclear contribution to nosocomial transmission by asymptomatic colonized patients remains a concern,[50] and additional studies will be required to determine whether recognition of carrier status by stool testing can ultimately reduce transmission rates.[48]

Conclusions

The answers to the "Consult" questions posed in the beginning of this article are as follows:

- Currently available molecular assays and a comparison of published performance characteristics for different assays are outlined in Tables 1 and 2. While comparison of methods between studies is limited by the lack of standardized protocols, a gold-standard comparator assay, and regional differences in strain types, some

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general observations are possible: (1) Testing by toxin EIA is an insensitive, albeit specific method. It is not considered reliable as a sole means of excluding disease. (2) The commercially available and laboratory-developed molecular assays have very high sensitivities compared with TC. Published data do not demonstrate significant analytic superiority of one particular method or manufacturer over another. (3) Algorithmic testing approaches that use GDH EIA as a primary screening assay also have high sensitivity compared with TC but require secondary testing to confirm toxigenicity.

- As with all laboratory tests, results must be interpreted within the context of a patient's history, signs, and symptoms. Laboratories that perform *C difficile* testing should make efforts to optimize pretest probability. This may include (1) accepting only loose or liquid stool specimens for analysis and (2) monitoring and/or regulating particular test-ordering practices such as repeated requests following negative results, testing for cure, and/or testing of infants and children. Yet even as consensus guidelines do not support these ordering practices in general, there exist valid reasons for permitting testing in each of these scenarios, so some degree of flexibility must be maintained. Consultation with infectious disease specialists should be sought in these situations.

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