DUKE ANTIMICROBIAL STEWARDSHIP OUTREACH NETWORK (DASON)

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Diagnostic Testing for *Clostridium difficile* Infection

Background

Clostridium difficile infection (CDI) is one of the most common causes of healthcare-associated infection (HAI) in community hospitals (1). Early and accurate diagnosis of CDI is paramount to providing optimal care for patients with this infection and decreasing risk of transmission to other hospitalized patients (2-4). Many laboratory tests are available for diagnosis of CDI. Current guidelines do not recommend a single, favored diagnostic algorithm to clinicians and clinical microbiologists (5). In this newsletter, we discuss the common laboratory tests used to diagnose CDI and describe the strengths and weaknesses of established testing strategies.

CDI Laboratory Tests

Three primary laboratory tests are used alone or in combination to diagnose *Clostridium difficile* infection:

1) **Toxin AB enzyme immunoassay (EIA):** This antibody test detects presence of *Clostridium difficile* toxins A and B. Toxin EIA testing is fast, inexpensive, and easy to perform in the laboratory; however, both sensitivity and specificity of this test are lower than nucleic acid amplification tests (NAAT). Toxin AB tests are often coupled with other tests as part of diagnostic algorithms designed to improve upon performance characteristics of individual tests.
2) **Glutamate dehydrogenase (GDH) antigen test:** The GDH antigen test detects the cell wall enzyme GDH produced by *C. difficile*. Similar to Toxin AB tests, GDH tests are also rapid, inexpensive, and easy to perform. Unlike Toxin AB tests, GDH tests have very high sensitivity, and a negative result effectively rules out CDI. The very poor specificity, however, limits the interpretation of a positive result, and specimens positive for the GDH antigen require subsequent testing with a different test (6). A positive GDH test alone does not meet the NHSN definition of a *C. difficile* infection event, which requires either a positive NAAT or toxin-based assay.
3) **Nucleic acid amplification test**: Two types of molecular NAATs are FDA approved to diagnose CDI.
   a.) Polymerase chain reaction (PCR) testing detects *Clostridium difficile* toxin gene *tcdB*. PCR tests have very high sensitivity and specificity, and if a PCR test is performed first, no follow-up or repeat testing is indicated. Downsides of PCR testing include its expense and the required technical expertise. In addition, the superior sensitivity of this test may capture patients who have colonization with *C. difficile* instead of true *C. difficile* disease.

b.) Loop-mediated isothermal amplification (LAMP) testing detects toxin gene *tcdA*. The LAMP assay (Illumigene *C. difficile* [Meridian]) is similar to PCR tests but may have lower sensitivity and is less expensive (7). Like PCR tests, the LAMP assay may also detect *C. difficile* colonization in addition to true infection.

CDI Testing Strategies

Testing strategies for *C. difficile* utilize various combinations of the three primary component tests described above (8). The following are common testing approaches (Table 1):

DICON/DASON First Tier Strategies:

1) **Perform NAAT molecular testing on all clinical specimens.** If PCR or LAMP is used as the initial test on all specimens, no other testing is required. Using only a NAAT for diagnosis is usually more expensive than other approaches that selectively utilize molecular tests; however, experienced laboratory personnel may be able to integrate new-generation NAAT platforms into the laboratory workflow using fewer laboratory personnel resources than what are required by multi-step algorithms. Laboratories that perform high-volume testing may actually save money by choosing this single-test pathway. In addition, the GeneXpert PCR (Cepheid) assay identifies hypervirulent 027/NAP1/B1 strains of *C. difficile*; patients with these strains may receive different treatment than patients with less virulent strains. We favor PCR testing over LAMP testing because of the higher sensitivity of PCR and the ability of new-generation PCR assays to identify 027/NAP1/B1 strains.
2) **First perform GDH antigen/toxin AB combination test**. **Then use a NAAT only when the combination test gives discordant results.** If both components of the combination test (C. Diff Chek Complete [Alere]) give the same result, then testing is complete. If the GDH antigen and toxin AB components give discordant results or are "indeterminate," subsequent molecular testing is required. In one study, nearly 90% of combination tests were concordant (6), allowing rapid and inexpensive diagnosis. Only the remaining 10% of studies required more expensive follow-up PCR testing, but patients in this category may have slower diagnosis with delayed therapy and infection prevention interventions due to the need for a second test.

DICON/DASON Second Tier Strategies:

3) **First perform GDH antigen test. Then perform NAAT only if GDH test is positive.** Initially using the GDH antigen test alone (C. Diff Chek 60 [Alere]) without the toxin AB test requires that more NAAT studies are performed as compared with strategy #2 above. The added expense of increased molecular testing likely negates the money saved by using the GDH single-component test in the first step. Increased reliance on NAAT follow-up testing could further delay accurate diagnosis and therapeutic interventions.
4) **Perform GDH antigen/toxin AB combination test. For discordant results, repeat the combination test.** This approach is similar to algorithm #2, but in this pathway, when the result is indeterminate, the same test is repeated (rather than using a NAAT) on a second patient specimen. Laboratories using this algorithm report two indeterminate results as a positive test. Indeterminate results can cause diagnostic delays because when the same test is repeated, a new clinical specimen is required. This approach may also be associated with an increased number of false-positive results.
5) **Perform only toxin AB EIA on all clinical specimens.** This testing method is fast, inexpensive, and simple to perform; however, sensitivity and specificity are lower than for other testing algorithms.

Some clinicians send a second test if a first test is negative, but discordant results are difficult to interpret. Definitive diagnosis may be delayed, depending upon the laboratory batching protocol. Further, clinicians who are aware of decreased sensitivity of the EIA tests will be hesitant to discontinue empiric therapy or "believe" a negative test.

Clinical Presentation

The clinical presentation of patients chosen for *Clostridium difficile* testing will affect the performance of any testing algorithm used by your hospital. All clinical microbiology laboratories should have strictly enforced specimen rejection rules and train personnel to avoid inappropriate testing (Table 2). We support the following recommendations, which avoid testing patients who are unlikely to have CDI and reduce false-positive results (5):

- Do not test asymptomatic patients for CDI.
- Diagnostic tests for *C. difficile* should be performed only on diarrheal (unformed) stool that takes the shape of the specimen container. We recommend that the laboratory reject formed stool or swab specimens with very rare exceptions (e.g., there is high clinical suspicion for ileus due to CDI). The NHSN also requires this rule for *C. difficile* LabID event reporting.
- Do not perform a "test of cure" after a patient diagnosed with CDI has clinically improved.
- Do not routinely test multiple stool specimens from the same patient during the same episode of diarrhea. Many labs that utilize "tier 1" testing strategies will reject specimens from the same patient sent within 7 days of a prior negative CDI test or within 14 days of a prior positive test.

DICON/DASON Recommendations

Several diagnostic testing pathways for CDI are available to microbiology laboratories. No one strategy is superior for all hospitals, and clinicians and microbiologists alike continue to debate the benefits and drawbacks of each approach. The best CDI diagnostic pathway for your hospital depends upon many factors, including volume of testing, prevalence of CDI in your specific patient population, testing practices among clinicians, expertise of laboratory personnel, and other laboratory resource factors that affect turnaround time and reporting.

For most hospitals, we recommend one of the two "first tier" approaches described above. Both of these approaches include NAAT molecular testing, either as a single test performed on all specimens (algorithm #1), or as a follow-up test performed only when a GDH antigen/toxin AB combination test gives discordant results (algorithm #2).

Importantly, infection preventionists and antimicrobial stewards must be partners with the microbiology laboratory to educate providers and reinforce best practices in use and interpretation of these tests. The type of test used will impact how tests are interpreted clinically, the accuracy of CDI diagnoses and surveillance rates, and strategies for infection control and antimicrobial stewardship. Your DICON/DASON team can help you evaluate your current diagnostic protocol for CDI and answer questions about other available tests and associated algorithms.

Table 1. Selected Algorithms for Diagnosis of *Clostridium difficile* Infection.

DICON/ DASON Category	Algorithm Description	Pros	Cons
Tier 1	NAAT (PCR or LAMP) molecular testing for all specimens (DICON/DASON recommends PCR over LAMP because it has better sensitivity and detects 027/NAP1/B1 strains.)	- Fast - Excellent sensitivity and specificity - Only 1 test required	- Expensive - Requires technical expertise - May identify colonized patients if specimen rejection rules are not adequately practiced.
Tier 1	Step 1: GDH antigen/toxin AB combination test Step 2: NAAT testing for discordant results only	- Fast for most specimens - Few NAAT tests required - Cost effective	- Two tests required for some specimens - Possible delayed diagnosis when both steps required
Tier 2	Step 1: GDH antigen test Step 2: NAAT testing for positive results only	- Fast for most specimens - Moderate number of NAAT tests required - Cost effective	- Two tests required for some specimens - Possible delayed diagnosis when both steps required
Tier 2	Step 1: GDH antigen/toxin AB combination test Step 2: Repeat combination test for discordant results (discordant results on Step 2 are reported as positive)	- Fast for most specimens - Cost effective	- Second test requires new stool sample - May increase number of false-positive results
Tier 2	Toxin AB EIA for all specimens	- Fast - Inexpensive - Only 1 test required	- Lower sensitivity and specificity than other algorithms

Table 2. Example Rejection Rules for Stool Specimens Sent for *C. difficile* Nucleic Acid Amplification Test (NAAT) Molecular Testing.

1. Specimen not labeled with patient's name, medical record number, date/time of collection, and collector's initials.
2. Container leaking.
3. Received >2 hours after collection unless on ice/cold pack or stored in refrigerator prior to transport.
4. Formed/hard/swab specimen.
5. Received in Cary-Blair transport, Para-Pak (formalin/PVA), or diaper.
7. Patient has negative NAAT within last 7 days.
8. Patient has positive NAAT within last 14 days.

References

1. Lewis SS, Moehring RW, Chen LF, Sexton DJ, Anderson DJ. Assessing the relative burden of hospital-acquired infections in a network of community hospitals. Infection control and hospital epidemiology : the official journal of the Society of Hospital Epidemiologists of America 2013;34:1229-30.
2. Wilcox MH. Overcoming barriers to effective recognition and diagnosis of Clostridium difficile infection. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 2012;18 Suppl 6:13-20.
3. Barbut F, Surgers L, Eckert C et al. Does a rapid diagnosis of Clostridium difficile infection impact on quality of patient management? Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 2014;20:136-44.
4. Bartlett JG, Gerding DN. Clinical recognition and diagnosis of Clostridium difficile infection. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2008;46 Suppl 1:S12-8.
5. Cohen SH, Gerding DN, Johnson S et al. Clinical practice guidelines for Clostridium difficile infection in adults: 2010 update by the society for healthcare epidemiology of America (SHEA) and the infectious diseases society of America (IDSA). Infection control and hospital epidemiology : the official journal of the Society of Hospital Epidemiologists of America 2010;31:431-55.
6. Goldenberg SD, Cliff PR, French GL. Glutamate dehydrogenase for laboratory diagnosis of Clostridium difficile infection. J Clin Microbiol 2010;48:3050-1; author reply 3051.
7. Pancholi P, Kelly C, Raczkowski M, Balada-Llasat JM. Detection of toxigenic Clostridium difficile: comparison of the cell culture neutralization, Xpert C. difficile, Xpert C. difficile/Epi, and Illumigene C. difficile assays. J Clin Microbiol 2012;50:1331-5.
8. Bartsch SM, Umscheid CA, Nachamkin I, Hamilton K, Lee BY. Comparing the economic and health benefits of different approaches to diagnosing Clostridium difficile infection. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 2015;21:77 e1-9.