JOURNAL OF CLINICAL MICROBIOLOGY, July 1992, p. 1837–1840
0095-1137/92/071837-04$02.00/0

Vol. 30, No. 7

# Comparison of the VIDAS *Clostridium difficile* Toxin A Immunoassay with *C. difficile* Culture and Cytotoxin and Latex Tests

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Received 25 November 1991/Accepted 27 April 1992

**The VIDAS *Clostridium difficile* toxin A immunoassay (CDA) is a new, automated, enzyme-linked fluorescent-antibody assay for detection of *C. difficile* toxin A antigen in stool specimens. Simultaneous, parallel testing was performed by using the VIDAS CDA, the Culturette brand CDT latex test for *C. difficile* antigens, and conventional laboratory cell culture tests for *C. difficile* cytotoxicity and *C. difficile* culture. One hundred ninety-four consecutive fresh soft or liquid stool samples submitted for *C. difficile* testing between July and September 1990 were evaluated. Of the 194 samples tested, 19 (10%) were from 16 patients who met our case definition for *C. difficile*-associated disease. The in vitro tests were evaluated in relation to two forms of a clinical case definition. In one form, a positive culture for toxin-producing *C. difficile* or a positive cytotoxin result obtained directly from the stool specimen was required as laboratory evidence of *C. difficile*. In the other, a positive result of any of the four laboratory tests was accepted for the laboratory portion of the case definition. No significant difference between the sensitivity of the VIDAS CDA and that of the Culturette brand CDT latex test was found (48 to 58% sensitivity for the CDT latex test and 52 to 63% sensitivity for the VIDAS CDA compared with 93 to 100% sensitivity for culture and 70 to 100% sensitivity for cytotoxin testing). The performance of the VIDAS CDA, however, was hampered by a high percentage of tests (19%) which gave an uninterpretable result.**

As *Clostridium difficile*-associated diarrhea and pseudomembranous colitis have been recognized and investigated in recent years (5, 6, 8), increasing attention has been given to laboratory procedures required to make the diagnosis of *C. difficile*-associated disease (CAD) in the appropriate clinical setting. The benchmark laboratory methods which have been used for this purpose are culture for isolation of the *C. difficile* organism and cell culture methods for detection of specific *C. difficile* cytotoxin in stool samples (1, 11). Culture of *C. difficile* usually requires 48 h of incubation. Tissue culture cytotoxin detection with appropriate neutralization testing requires 48 to 96 h. Colonoscopy can rapidly establish the diagnosis of pseudomembranous colitis but is positive in up to only 51% of cases of *C. difficile*-associated clinical disease even when the stool sample is positive by both cytotoxin and culture examination (5). Various newer laboratory methods to aid in the more rapid diagnosis of CAD have therefore been investigated (1, 3, 11). Recently, the VIDAS *C. difficile* toxin A immunoassay (VIDAS CDA; Vitek Systems, Inc., Hazelwood, Mo.), an automated, enzyme-linked fluorescent-antibody test for detection of *C. difficile* toxin A antigen in stool samples, became available for clinical evaluation.

The purpose of this study was to evaluate the usefulness of the VIDAS CDA as a laboratory test in the rapid diagnosis of CAD. Simultaneous parallel testing was performed by using the VIDAS CDA and a latex agglutination test for *C. difficile* antigens along with cell culture testing for *C. difficile* cytotoxin and *C. difficile* culture on 194 fresh soft or liquid stool samples submitted for *C. difficile* testing from patients at the Minneapolis VA Medical Center. Relevant clinical data were acquired for all patients with any positive test to determine which patients had a clinical course compatible with the diagnosis of CAD.

## MATERIALS AND METHODS

**Specimens.** One hundred ninety-four soft or liquid stool samples submitted to our laboratory for *C. difficile* testing between July and September 1990 were analyzed. Formed stool samples were rejected by the laboratory and not processed. Specimens were processed daily, Monday through Friday. Of the 194 samples tested, 19 samples were collected from 16 patients who met our case definition for CAD. There were 166 samples collected from 114 patients who did not meet our case definition when the sample was collected. The remaining nine samples consisted of three samples collected from patients during *C. difficile*-directed therapy and six samples from patients whose clinical status was not ascertained because the relevant medical records were not available. These nine samples were excluded from the analysis of the results. Most samples were tested on the day they were received. All samples were refrigerated at 4°C until tested; none were refrigerated for more than 72 h.

**Culture.** Samples were inoculated onto reduced cycloserine-cefoxitin-fructose-egg yolk agar (CCFA) base plates prepared in our laboratory according to the formulation of George et al. (4). The plates were reduced in an anaerobic atmosphere before inoculation, and inoculated plates were incubated anaerobically for 48 h and then read as previously

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described (9). We have found laboratory quality control testing of CCFA plates to be critical in the performance of *C. difficile* cultures. We define acceptable performance of CCFA medium as isolated colonies of *C. difficile* ATCC 9689 and ATCC 17858 both growing to at least 3 mm in diameter in 48 h and producing flat, yellow colonies with an appearance similar to that of ground glass, with a surrounding yellow halo in the medium. The Gram stain of these colonies must show morphology typical for *C. difficile*. Additionally, the medium must be visually normal and must inhibit the growth of *Escherichia coli* ATCC 25922.

**Cytotoxin assay.** This test was performed as previously described (5). Briefly, stool was mixed with phosphate-buffered saline and centrifuged, and then the supernatant was filtered through a 0.45-μm-pore-size filter and inoculated onto a monolayer of HEp-2 cells. A neutralization assay using *C. sordelli* antitoxin (Bureau of Biologics, Food and Drug Administration, Bethesda, Md.) was performed on samples which showed cytopathic effect at 24 or 48 h. Screening for cytotoxicity was initially performed at a stool specimen dilution of 1:40, and neutralization was performed at a final dilution of 1:200.

**Culturette brand CDT latex agglutination test.** The Culturette brand CDT test (Becton Dickinson, Cockeysville, Md.), a latex agglutination test for detection of a *C. difficile*-associated antigen (7), was performed according to the manufacturer's instructions by mixing about 0.5 g of stool with an equal volume of buffer, vortexing and centrifuging the mixture, and testing 1 drop of the resultant supernatant.

**VIDAS CDA.** This test is an automated, enzyme-linked fluorescent-antibody assay for detection of *C. difficile* toxin A antigen in stool samples. Two disposable devices are used by the system. One is a solid-phase receptacle (SPR), which is a polystyrene device resembling a pipet tip; the inside of the SPR is coated with rabbit anti-*C. difficile* toxin A antibody. The other is a dual reagent strip, which is a parallel series of sealed reagent wells ending with an optical cuvette. In the test side of the strip, the sample is mixed with diluent and then cycled in and out of the antibody-coated SPR. After unbound sample components are washed away, a mouse monoclonal anti-*C. difficile* toxin A antibody, which binds to any toxin A from the sample that has bound to the SPR wall, is introduced and cycled in and out of the SPR. After additional washes, an anti-mouse antibody conjugated to phosphatase is cycled in and out of the SPR. Following a final wash step, a fluorescent substrate, 4-methylumbelliferyl phosphate, is introduced into the SPR. Phosphatase bound to the SPR in positive samples results in the hydrolysis of this substrate to a fluorescent product, 4-methylumbelliferone. The reference side of the dual reagent strip is identical, except that rather than containing mouse anti-*C. difficile* toxin A antibody, the corresponding well of the reference side of the strip contains normal mouse serum (12). To perform the test, equal volumes of fresh stool sample and diluent were mixed and microcentrifuged at 16,000 × *g* for 5 min, and 300 μl of supernatant was pipetted into the sample well and the reference well of the dual reagent strip. Processing beyond this point was performed automatically by the instrument, which performed the testing, analyzed test data, and printed test results. In the final phase of product testing reported here, test interpretations were based on a retrospective analysis of the original data by the manufacturer after a revision of the detection thresholds. This revision was still considered investigational by the manufacturer. The VIDAS CDA required about 10 min of hands-on time, and results were available in approximately 3 h.

**Toxin assay of *C. difficile* isolates.** An isolated colony of *C. difficile* was inoculated into prereduced chopped-meat broth (DiMed, Inc., St. Paul, Minn.) and incubated anaerobically at 35°C for 7 days. The supernatant of this culture was assayed for both toxins A and B. Enterotoxin (toxin A) assay was performed by an enzyme-linked immunosorbent assay (TechLab, Inc., Blacksburg, Va.) based on a method previously described by Walker et al. (13). Cytotoxin (toxin B) determination was performed in the same manner as testing of stool specimens detailed above.

**CAD case definition.** Patients were defined as having CAD if (i) they had at least six watery stools over a period of 36 h; (ii) they received antimicrobial therapy within 8 weeks of the onset of diarrhea; (iii) pseudomembranes were seen in a lower gastrointestinal endoscopy, the stool sample was positive for the presence of toxin-producing *C. difficile*, or cytotoxin was detected in the stool; (iv) they responded to therapy for CAD (either withdrawal of the inciting antimicrobial agent or specific anti-CAD treatment with either metronidazole or vancomycin; and (v) they had no other recognized etiology for the diarrhea. Test performance characteristics were based on detection of CAD in relation to this case definition (10). The additional requirement that a *C. difficile* isolate needed to produce toxin A or B when isolated from a cytotoxin-negative stool is a modification of our previous criteria necessitated by the finding during this investigation of six patients with non-toxin-producing *C. difficile* who otherwise fulfilled the criteria for CAD.

**Analysis of test performance.** Results were analyzed by interpreting the laboratory-derived portion of case definition item iii in two ways. In one analysis, a culture positive for toxin-producing *C. difficile* or a positive stool cytotoxin test was required as evidence of the presence of pathogenic *C. difficile*. In this analysis, the results of the VIDAS CDA, the latex test, and culture of nontoxigenic *C. difficile* did not have a role in the case definition (Table 1). In the other analysis, any positive result for any of the four tests (culture, cytotoxin test, latex test, or VIDAS CDA) was accepted as laboratory evidence of the presence of *C. difficile* or its toxin (Table 2).

## RESULTS

Of the 194 samples tested, 185 were from patients whose clinical status could be evaluated according to the criteria of our case definition. There were 19 samples from patients who met the case definition for CAD when the sample was collected (with a positive toxigenic *C. difficile* culture or a positive cytotoxin test required as laboratory evidence) and 166 samples from patients who did not. Of the remaining nine samples, six were from patients whose records were not available and three were collected during treatment for CAD.

The results of the four tests using both forms of the case definition are summarized in Tables 1 and 2. Modifying the case definition to accept a positive result for any of the four tests (culture, cytotoxin test, latex test, or VIDAS CDA) as laboratory evidence of *C. difficile* or its toxin changed the classification of eight samples. Two specimens (from different patients) were positive by the VIDAS CDA and were negative by culture, the cytotoxin test, and the latex test. Six specimens from four patients were culture positive for nontoxigenic *C. difficile* (negative for toxin A and toxin B) and negative in the other tests indicating the possibility of CAD. These samples therefore did not meet the first case definition (toxigenic *C. difficile* culture or cytotoxin must be

TABLE 1. Test characteristics using first definition of evidence of *C. difficile*[a]

| Test | Sensitivity (%) | Specificity (%) | Predictive value (%) | | % Uninterpretable tests |
|---|---|---|---|---|---|
| | | | Positive | Negative | |
| Culture | 100 | 86 | 44 | 100 | 0 |
| Cytotoxin test | 100 | 96 | 79 | 100 | 0.5 |
| CDT latex test | 58[b] | 94[b] | 52 | 95 | 0 |
| VIDAS CDA | 63[b] | 75[b] | 50 | 99 | 19 |

[a] Either a positive test for cytotoxin in a stool sample or isolation of a toxin-producing *C. difficile* strain from a stool sample was required as laboratory evidence of the presence of *C. difficile*.

[b] Difference between latex test and VIDAS CDA for indicated parameter ($P > 0.1$).

positive) (Table 1) but did meet the second case definition (culture, cytotoxin test, latex test, or VIDAS CDA must be positive) (Table 2). The difference between these two case definitions did not have a significant effect on the performance characteristics for the VIDAS CDA or the CDT latex test.

The most striking difference between the various tests used for the laboratory assessment of CAD was that 19% of the samples (36 of 194) gave an uninterpretable VIDAS CDA result. Of these 36 samples, 18 gave data that the system classified as invalid and 18 produced data that the system interpreted as equivocal. Of these 36 samples, 7 were from patients classified as having CAD. Two of these were reported as invalid and five were reported as equivocal. There was a sufficient amount of sample remaining to repeat the test on only three of these samples, one of which gave a positive result and two of which gave a negative result. Twenty-nine of the 36 were from patients who did not meet the case definition for CAD. There was a sufficient amount of sample remaining to repeat the testing on nine of these samples. One gave a positive result, four gave a negative result, and four gave an uninterpretable VIDAS CDA result when retested.

One cell culture cytotoxin test (from a patient who did not meet the case definition for CAD) was uninterpretable (cytotoxic on initial screening but not neutralized by antitoxin). No culture results and no Culturette Brand CDT latex tests were uninterpretable for these 194 samples.

## DISCUSSION

When a new test is evaluated, the analysis is most often done in terms of the new test's correlation with other tests currently in use for a similar purpose. In this evaluation, several tests to aid in the diagnosis of CAD were performed in parallel; however, the basis for our evaluating the performance of the VIDAS CDA was not its correlation with one or more of the current tests but rather its usefulness in indicating whether or not a patient met a case definition for CAD which includes both clinical and laboratory criteria as necessary elements at the time the sample was collected. For a diagnosis such as CAD, in which no single laboratory test is an adequate indicator of clinical disease, comparisons of new test methods solely with current test methods in the absence of clinical data do not provide adequate information about how useful the test will be to clinicians in trying to make a diagnosis of CAD.

TABLE 2. Test characteristics using second definition of evidence of *C. difficile*[a]

| Test | Sensitivity | Specificity | Predictive value | | % Uninterpretable tests |
|---|---|---|---|---|---|
| | | | Positive | Negative | |
| Culture | 93 | 89 | 58 | 99 | 0 |
| Cytotoxin test | 70 | 96 | 79 | 95 | 0.5 |
| CDT latex test | 48[b] | 95[b] | 62 | 91 | 0 |
| VIDAS CDA | 52[b] | 75[b] | 58 | 94 | 19 |

[a] A positive result in any of the four tests was accepted as laboratory evidence of the presence of *C. difficile*.

[b] Difference between latex test and VIDAS CDA for indicated parameter ($P > 0.1$).

The test characteristics of the VIDAS CDA and the Culturette brand CDT latex test are compared in Tables 1 and 2. The test characteristics of the VIDAS CDA, however, may appear more favorable than these statistics actually are because of the large number of uninterpretable VIDAS CDA results (19% of samples tested). A similar problem of lesser magnitude was found by De Girolami et al. in their evaluation of a new enzyme immunoassay for *C. difficile* toxin (3). They found eight specimens which gave uninterpretable results (four of which were from patients determined to have CAD by other means). We disagree with their approach of excluding those patients from analysis of test performance, but specimens from those patients made up only 1.4% of their test samples, compared with 19% in our evaluation of the VIDAS CDA. Defining a range of uninterpretable results enhances the apparent performance statistics for strongly positive or strongly negative tests at the expense of not giving any results for tests that are less strongly positive or negative.

Although an uninterpretable Culturette brand CDT latex test is possible (if nonspecific agglutination occurs in the negative control well for a sample), and we have seen this result at a rate of 3.3% in the past (9), this result did not occur with any of the samples tested in the current study. In our earlier evaluation of the Culturette brand CDT latex test (10), we had found a sensitivity of 68% (98 positives from 144 patients with CAD) for the Culturette brand CDT latex test, as compared with 58% (11 positives of 19 samples from patients with CAD) in the current evaluation ($P \geq 0.1$).

There is currently no consensus about which reference tests should be used for comparison of new methods in the diagnosis of *C. difficile*-related disease. Our belief is that suitably performed culture using medium that adequately supports the growth of *C. difficile* must be included as one of these laboratory tests (11). We have previously shown that 11% of culture-positive, cytotoxin-negative stools can be from patients found to have pseudomembranes by lower gastrointestinal endoscopy (5), presumably because of either insensitivity of cytotoxin testing or sampling problems when dealing with a nonhomogeneous body fluid such as stool. In the same study, 51% of specimens that were both culture and cytotoxin positive were found to have pseudomembranes on endoscopy. Extrapolation of these data to a setting in which a positive cytotoxin test is used as the sole diagnostic criterion for the presence of CAD could imply that specimens that are culture positive only (because of insensitivity of stool cytotoxin testing) should be anticipated in 22% of the true cases with a diagnosis of CAD. Additionally, using our clinical criteria as described above, we have consistently found that approximately 30% of our patients with CAD have stool specimens that are culture positive and cytotoxin negative. We have found that the majority of culture-positive, cytotoxin-negative stool specimens have been associ-

ated with a toxigenic *C. difficile* isolate. This continued to be true in our most recent epidemiologic evaluation, in which only 3% (2 of 63) of *C. difficile* strains isolated from patients whose clinical course was consistent with CAD were found to be nontoxigenic (2). Similar results were also found in a recent evaluation of a new enzyme immunoassay for toxin A (3). That study found seven patients whose stool specimen was negative in an enzyme immunoassay but who had positive cultures for toxigenic *C. difficile* and a clinical picture compatible with CAD. This number comprised 9% of the 82 patients considered to have CAD (3). Small numbers of patients who have diarrhea and whose stools harbor nontoxigenic *C. difficile* (i.e., whose disease should be attributable to another cause) generally would not affect the interpretation of a new laboratory test method. However, our finding that 6 of 25 specimens harbored nontoxigenic strains as the only laboratory evidence for CAD caused us to modify the criteria we previously had used and to test all isolates from cytotoxin-negative stools for the presence of toxins A and B. Using a toxin assay (cytotoxin) as the only reference test or "gold standard" can artificially increase the sensitivity of all comparative tests by eliminating any false-negative category, since the cases of CAD which are *C. difficile* culture positive and stool cytotoxin negative will be missed. The difficulty arises with the use of culture alone as a laboratory criterion for the diagnosis of CAD in a setting in which there is a high prevalence of nontoxigenic *C. difficile* strains as potential colonizers of hospitalized patients. When this occurs, an approach similar to that which we employed in the current study should be taken, i.e., all *C. difficile* isolates from toxin-negative stools should be tested for toxin production, and an isolate should be counted as part of a case definition only if it is found to be toxigenic.

Although the VIDAS CDA is a relatively rapid, easy-to-perform, automated assay with test performance characteristics roughly equivalent to those of the Culturette brand CDT latex test, the usefulness of the VIDAS CDA is diminished by its generation of uninterpretable results for nearly one-fifth of the samples tested. In addition, neither the investigational version of the VIDAS CDA which we evaluated nor the Culturette brand CDT latex test was sufficiently sensitive, in our study, to be relied on as a single test for laboratory detection of *C. difficile*.

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