JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2003, p. 667–670 Vol. 41, No. 2
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.2.667–670.2003

Six Rapid Tests for Direct Detection of *Clostridium difficile* and Its Toxins in Fecal Samples Compared with the Fibroblast Cytotoxicity Assay

David K. Turgeon,[1]† Thomas J. Novicki,[2]‡ John Quick,[1] LaDonna Carlson,[1] Pat Miller,[2]§ Bruce Ulness,[2]‡ Anne Cent,[1] Rhoda Ashley,[1] Ann Larson,[1] Marie Coyle,[1] Ajit P. Limaye,[1] Brad T. Cookson,[1] and Thomas R. Fritsche[1]*

Department of Laboratory Medicine, School of Medicine, University of Washington,[1] and Clinical Research Division, Fred Hutchinson Cancer Research Center,[2] Seattle, Washington

Received 1 April 2002/Returned for modification 28 May 2002/Accepted 11 November 2002

***Clostridium difficile* is one of the most frequent causes of nosocomial gastrointestinal disease. Risk factors include prior antibiotic therapy, bowel surgery, and the immunocompromised state. Direct fecal analysis for *C. difficile* toxin B by tissue culture cytotoxin B assay (CBA), while only 60 to 85% sensitive overall, is a common laboratory method. We have used 1,003 consecutive, nonduplicate fecal samples to compare six commercially available immunoassays (IA) for *C. difficile* detection with CBA: Prima System *Clostridium difficile* Tox A and VIDAS *Clostridium difficile* Tox A II, which detect *C. difficile* toxin A; Premier Cytoclone A/B and Techlab *Clostridium difficile* Tox A/B, which detect toxins A and B; and ImmunoCard *Clostridium difficile* and Triage Micro *C. difficile* panels, which detect toxin A and a species-specific antigen. For all tests, Triage antigen was most sensitive (89.1%; negative predictive value [NPV] = 98.7%) while ImmunoCard was most specific (99.7%; positive predictive value [PPV] = 95.0%). For toxin tests only, Prima System had the highest sensitivity (82.2%; NPV = 98.0%) while ImmunoCard had the highest specificity (99.7%; PPV = 95.0%). Hematopoietic stem cell transplant (HSCT) patients contributed 44.7% of all samples tested, and no significant differences in sensitivity or specificity were noted between HSCT and non-HSCT patients. IAs, while not as sensitive as direct fecal CBA, produce reasonable predictive values, especially when both antigen and toxin are detected. They also offer significant advantages over CBA in terms of turnaround time and ease of use.**

Clostridium difficile is one of the most frequently identified causes of nosocomial gastrointestinal disease (14). It has been implicated as a causative agent in antibiotic-associated diarrhea, antibiotic- associated colitis, and pseudomembraneous colitis (3, 4, 10, 19, 30). *C. difficile*-associated disease (CDD) is most often associated with nosocomial acquisition and prior antibiotic therapy, but the immunocompromised state, bowel surgery, and bowel stasis are also predisposing factors. CDD may also occur when no known risk factors are present (9, 11, 12, 13, 16). Many strains of *C. difficile* produce two protein exotoxins, A and B, which are thought to be the primary cause of colonic mucosal injury and inflammation (23). Toxin A exerts primarily enterotoxic effects, while toxin B is primarily cytopathic. However, not all individuals (up to 50% of infants and 32% of cystic fibrosis patients) who carry toxigenic *C. difficile* in their bowels exhibit disease (25).

Several laboratory techniques exist to aid in the diagnosis of CDD. The organism may be detected by culture, immunoassay (IA) for the *C. difficile* glutamate dehydrogenase common antigen, or metabolic end product analysis for isocaproic acid by gas-liquid chromatography (15). However, these methods are nonspecific in that both toxigenic and nontoxigenic strains of *C. difficile* are identified. A latex agglutination toxin test and *C. difficile* PCR protocols have also been described but are not in widespread use (13, 14). The current standard is the cytotoxin B assay (CBA). It is most commonly performed directly on feces, where it typically displays ≤85% sensitivity (5, 20). This sensitivity may be increased to >99% by combining CBA with toxigenic culture (i.e., *C. difficile* culture followed by CBA performed on the culture broth), but this increase comes at a cost of increased turnaround time (5, 26). However, CBA is not standardized, requires tissue culture facilities, and has a turnaround time of at least 24 h to 4 days (26, 31).

In recent years, IAs for the detection of *C. difficile* toxins, sometimes combined with *C. difficile* common-antigen detection, have become available. These newer tests have been reported to approach the sensitivity of direct CBA, are specific, and offer significant advantages in terms of turn around time, cost, and ease of performance (14, 17, 22, 28, 29, 33). In this study, we tested over 1,000 fecal samples to evaluate the performance of the following commercial IAs in comparison with direct CBA: Prima System *Clostridium difficile* Tox A (Prima A; Bartels Inc., now Trinity Biotech, Bray, Ireland) and VIDAS *Clostridium difficile* Tox A II (VIDAS A; bioMérieux Vitek, Inc., Hazelwood, Mo.), which detect *C. difficile* toxin A; Premier Cytoclone A/B (Cytoclone A/B; Meridian Diagnostics Inc., Cincinnati, Ohio) and *Clostridium difficile* Tox A/B (Tox A/B; Techlab Inc., Blacksburg, Va.), which detect toxins A and

* Corresponding author. Mailing address: Department of Laboratory Medicine, University of Washington, Room NW120, 1959 NE Pacific St., Seattle, WA 98195-7110. Phone: (206) 598-6131. Fax: (206) 598-6189. E-mail: fritsche@u.washington.edu.

† Present address: Department of Pathology, Madigan Army Medical Center, Tacoma, WA 98431.

‡ Present address: Department of Laboratory Medicine, School of Medicine, University of Washington, Seattle, WA 98195.

§ Present address: Department of Pathology, Children's Hospital and Regional Medical Center, Seattle WA 98105.

TABLE 1. Correlation of IA and CBA performance

CBA result	Cytoclone A/B (n = 999)		ICard antigen (n = 1,003)		ICard toxin A (n = 1,003)		ICard panel[a] (n = 1,003)		Prima A (n = 1,001)		Tox A/B (n = 1,003)		Triage antigen (n = 1,002)		Triage toxin A (n = 1,002)		Triage panel[a] (n = 1,002)		VIDAS A (n = 994)	
	No. pos	No. neg	No. pos	No. neg	No. pos	No. neg	No. pos	No. neg	No. pos	No. neg	No. pos	No. neg	No. pos	No. neg	No. pos	No. neg	No. pos	No. neg	No. pos	No. neg
Pos[b]	74	27	81	20	57	44	56	45	83	18	78	23	90	11	60	41	60	41	69	30
Neg[b]	8	890	68	834	3	899	70	832	15	885	5	897	93	808	4	897	93	808	10	885

[a] ICard and Triage panels consider the respective toxin and antigen tests together.
[b] Pos, positive; Neg, negative.

B; and the ImmunoCard *Clostridium difficile* (ICard; Meridian Diagnostics Inc.) and Triage Micro *C. difficile* (Triage; Biosite Diagnostics, San Diego, Calif.) panels, which detect toxin A and the glutamate dehydrogenase common antigen.

MATERIALS AND METHODS

Test methodology. A total of 1,003 consecutive, nonduplicate, fecal specimens of any consistency from patients at Children's Hospital and Regional Medical Center, Fred Hutchinson Cancer Research Center, Harborview Medical Center, and University of Washington Medical Center submitted for CBA during a 7-month period were selected for this study. Each sample was stored at 4°C and then tested by CBA at the University of Washington Medical Center Virology Laboratory within 24 h of receipt. Aliquots were also made and stored at −20°C for IA testing.

CBA. A modification of the CBA protocol of Rifkin et al. (27) was used. Briefly, fecal specimens were diluted 1:3 in Hanks balanced salts solution with antibiotics and centrifuged, and the supernatant was collected. The supernatant was then filtered through a 0.8-μm-pore-size filter. A 100-μl portion of each supernatant was added to previously prepared confluent human diploid fibroblast cell monolayers in 96-well microtiter plates, with and without the addition of *Clostridium sordellii* antitoxin (Burroughs-Wellcome Research Laboratories, Beckenhem, England) that has been titrated against a standard made up of four known positive fecal samples. Plates were covered, incubated at 35°C in 5% CO^2, and then examined at 24 and 48 h for cytopathic effects characteristic of *C. difficile* toxin B. Samples producing cytopathic effects in the well without *C. sordellii* antitoxin but not in the *C. sordellii* antitoxin-containing well were considered positive for *C. difficile* toxin B. A positive *C. difficile* toxin B control (TechLab) was included with each run.

IA. All IAs are qualitative and were performed as recommended by the manufacturer. Cytoclone A/B and Tox A/B use a 96-microwell IA format to simultaneously detect *C. difficile* toxins A and B, while Prima A uses an 8-microwell IA strip format to detect toxin A. The Cytoclone A/B and Tox A/B assays were performed by manually washing the plates between steps, while a semiautomatic plate washer was used for Prima A. All three used a semiautomated plate reader to determine the final optical densities. ICard and Triage utilize single-use cards with membrane IA technologies. Triage combines *C. difficile* common antigen and toxin A detection on a single card, while ICard uses separate cards to detect the common antigen and toxin A. VIDAS A is a semiautomated enzyme- linked fluorescence immunoassay for the detection of toxin A and is performed on the VIDAS (bioMérieux), a proprietary, multiple-analyte analyzer.

Statistical analysis. Each sample was initially tested by all methods, with the exception of two samples that were not tested by Cytoclone A/B, one that was not tested by Triage, and one that was not tested by Prima A. Any indeterminate result led to a single retest by the same method; a second indeterminate result led to disqualification of that sample from further evaluation by that method, while definitive answers by other methods were accepted.

Calculations of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) have been previously described (21). For the ICard panel and Triage panel statistics, true- positive and -negative results were considered to be obtained from samples that yielded all-positive or all-negative results, respectively, for the CBA, common-antigen, and toxin tests; false-positive results were obtained from samples that yielded either a positive common-antigen result, a positive toxin result, or both, but were CBA negative; false-negative results were obtained from CBA-positive samples that gave a negative common-antigen result, a negative toxin result, or both. The interpopulation comparison analysis was done using generalized estimating equation modeling (18).

RESULTS

Reference method results. A total of 101 (10%) of 1,003 fecal specimens were positive for *C. difficile* toxin B by CBA.

IA results. The performance of all IAs in comparison to CBA is displayed in Table 1. For the samples that were tested by all methods (n = 920), CBA and all toxin/*C. difficile* common-antigen tests were in complete agreement for 813 samples (88.4%) (766 negative and 47 positive); CBA and all toxin tests alone were in complete agreement for 903 samples (98.2%) (855 negative and 48 positive).

The sensitivity, specificity, and predictive values of each IA compared to CBA are given in Table 2 for all patients. For all tests (i.e., toxin and antigen tests), the sensitivities ranged from 55.4% (NPV = 94.9%) for the ICard panel to 89.1% (NPV = 98.7%) for the Triage antigen while specificities ranged from 89.7% (PPV = 39.2%) for the Triage panel to 99.7% (NPV = 95.0%) for ICard toxin A. For toxin tests only, sensitivities ranged from 56.4% (NPV = 95.3%) for ICard toxin A to 82.2% (NPV = 98.0%) for Prima A while specificities ranged from 98.3% (PPV = 84.7%) for Prima A to 99.7% (PPV = 95.0%) for ICard toxin A.

Of 1,003 samples, 448 (44.7%) were derived from hematopoietic stem cell transplant (HSCT) patients at one institution (Fred Hutchinson Cancer Research Center). No significant differences in sensitivity (P = 0.55) or specificity (P = 0.23) associated with HSCT versus non-HSCT patients were identi-

TABLE 2. Comparison of IAs with the CBA for all patients

IA	Sensitivity (%)[a]	Specificity (%)[a]	PPV (%)[a]	NPV (%)[a]
Cytoclone A/B	73.3	99.1	90.2	97.1
ICard antigen	80.2	92.5	54.4	97.7
ICard toxin A	56.4	99.7	95.0	95.3
ICard panel[b]	55.4	92.2	44.4	94.9
Prima A	82.2	98.3	84.7	98.0
Tox A/B	77.2	99.4	94.0	97.5
Triage antigen	89.1	89.7	49.2	98.7
Triage toxin A	59.4	99.6	93.8	95.6
Triage panel[b]	59.4	89.7	39.2	95.2
VIDAS A	70.3	98.9	87.7	96.8

[a] Results are relative to CBA.
[b] The ICard and Triage panels consider the respective antigen and toxin tests together.

fied when controlling for the effects of test type by generalized estimating equation modeling (data not shown).

DISCUSSION

Current laboratory diagnosis of CDD is based on either the detection of *C. difficile* in feces by culture on selective media or on the detection of a *C. difficile* surrogate marker antigen or its toxins (the latter by CBA until recently). Organism identification by culture or antigen detection is, however, nonspecific in that these methods detect both toxigenic and nontoxigenic strains. While sensitive and specific, the widely accepted CBA has several limitations in that it is not standardized, has a relatively long turnaround time (TAT) of >48 h, and requires tissue culture facilities. Although lack of standardization is perhaps not a practical limitation in that interlaboratory comparison of data is not common outside of proficiency testing, the other two factors must be considered. Numerous commercial IA methods are now available that, while not being the equal of CBA in terms of sensitivity, offer important advantages in terms of decreased TAT and ease of use. Previous studies comparing these IAs to CBA have been hampered by the fact that they have evaluated one or at most several of the former in a given study. We have attempted to address this by comparing a large selection of commercial *C. difficile* IAs to the direct detection of fecal toxin B by CBA.

Regarding *C. difficile* toxin detection alone, we found a wide range of performance among the various IA methods. In general, the toxin A portions of the ICard and Triage panels (the two assays using a single-use card format) were notably inferior in terms of sensitivity but, conversely, had the highest specificities compared to the other assays, with differences in sensitivity being much more apparent. When adjusted for prevalence, as determined by PPV and NPV, these differences remained. These data support the findings of others that the single-use card format is inferior to traditional enzyme-linked immunosorbent assay from the perspective of its ability to detect its analyte (24). In contrast, other workers have found that the single-use card IA format can be equal to or better than microwell IA (8, 32). Overall, the Tox A/B test had the best performance among the toxin-only tests, having the highest PPV and the second highest NPV. In addition, the Tox A/B test performs assays for both *C. difficile* toxins A and B: while strains producing only one of the two toxins do not appear to be common, they have been implicated in human disease (2, 19).

The ICard and Triage common-antigen tests had some of the highest sensitivities of all IAs, but both suffer from the fact that they are unable to distinguish toxigenic from nontoxigenic infections. Of these two tests, the Triage was found to be superior. Again, these differences were maintained in their predictive values. Some consideration may, however, be given to the use of these tests in their panel form (i.e., the detection of antigen and toxin A). The value of these panels appears to lie in the individual strengths of each component: while the antigen portions of these tests provide very good NPVs and are therefore useful in ruling out disease in the vast majority of cases, the toxin portions of these tests have reasonable PPVs compared to direct CBA and therefore would be expected to detect many cases of CDD. However, it must be remembered that the CBA, when performed directly on feces, is expected to only detect 60 to 85% of all cases. By extrapolation, any of these IAs will therefore have an ultimate sensitivity of between 34 and 76%, with the ICard and Triage toxin tests at the bottom of this range.

To attain maximal sensitivity, any of these tests, including direct CBA, must be supplemented by an additional method to confirm negative results. While nucleic acid amplification of *C. difficile* toxin genes in stool remains an option, toxigenic culture is by far the most commonly recommended confirmatory method, and the Society for Healthcare Epidemiology of America in fact recommends culture in addition to direct fecal toxin testing (5, 8, 13, 20, 26). Toxigenic culture and one of the rapid methods evaluated in this study could be combined in a single algorithm as follows: (i) all fecal samples would be tested with an IA test; (ii) samples displaying a positive result would be signed out appropriately; (iii) negative samples would be sent on for confirmatory testing by toxigenic culture. If Triage were used as the initial test, the algorithm might be modified as follows to make use of the high NPV of the antigen portion of the panel: (i) all fecal samples would be tested with Triage; (ii) samples displaying concordant antigen and toxin results (i.e., negative/negative or positive/positive) would be so reported; (iii) samples giving discordant results would be sent on for confirmatory testing by toxigenic culture. A limitation of this algorithm is that it is not clear that the sensitivity of the Triage test is sufficiently high to report antigen-negative samples as being truly negative for *C. difficile.* Further analysis is required to resolve this issue.

HSCT patients frequently develop diarrhea due to both *C. difficile* and intestinal graft-versus-host disease during the first 100 days posttransplantation (7). Treatments for CDD and graft-versus-host disease differ greatly, and incorrect treatment can lead to exacerbation of disease. Since 45% of our study samples were drawn from an HSCT population, we decided to stratify the performance of these IAs with respect to HSCT. No significant differences in sensitivity or specificity were detected.

Several limitations were inherent to this study. First, no independent method of discrepancy analysis was used. Four CBA-negative samples tested toxin positive by two or more IAs (data not shown), suggesting true positivity. Whether these were in fact CBA false negatives is unknown. Likewise, 12 samples were positive by CBA only (data not shown). Given the superior analytical sensitivity of CBA, the majority of these can be expected to be true positives. However, since our version of the CBA uses *C. sordellii* antitoxin instead of *C. difficile* antitoxin B, some of these "false negatives" may in fact be due to the presence of *C. sordellii. C. sordellii* produces two exotoxins, LT and HT, and is occasionally associated with nondiarrheal illnesses (1). Diarrheal disease due to toxigenic *C. sordellii* has not been reported, but this species can be part of the human colonic flora (1). While the incidence of *C. sordellii* in our population is unknown, it may have been responsible for some positive CBA/negative IA results. The second limitation lies in the choice of our reference method (i.e., direct fecal analysis for *C. difficile* toxin B by CBA). While this method is thought to be the best direct method of detection, it is not 100% sensitive, as noted above. Thus, any determination of sensitivity when using the direct CBA as a reference method

must take this into consideration. Studies relying on clinical case criteria may more appropriately address this question. Finally, a word must be said about the effects of disease prevalence on calculated predictive values. The PPV of a test is directly related to prevalence, while the NPV is inversely related to prevalence (6). Therefore, whether a test is appropriately applied (i.e., only where a strong clinical index of suspicion exists) will have an effect on predictive values. This study was laboratory based in that it relied only on stool samples received for *C. difficile* toxin testing and made no effort to correlate test results with clinical impressions. We can therefore make no statements regarding pretest probability of disease, but we think that physician-ordering practices of other institutions are similar to ours.

In conclusion, while none of the rapid IAs evaluated here equaled the performance of direct fecal CBA, several did approach it and in fact gave useful predictive values. These tests, particularly the single use Triage and ICard tests, offer significant advantages over CBA and may therefore be considered for use in clinical microbiology laboratories, particularly those that do not have tissue culture facilities. Additionally, any of these tests have the potential to be used in conjunction with toxigenic culture to attain maximal sensitivity.

ACKNOWLEDGMENTS

We thank the manufacturers who generously contributed their products. We are also grateful to Rachel Carter and Wendy Leisenring (Fred Hutchinson Cancer Research Center Clinical Statistics/Computing section, Clinical Division) and to Troy Patient (Department of Clinical Investigations, Madigan Army Medical Center, Tacoma, Wash.) for their assistance with the statistical analyses. Finally, we thank the microbiology laboratory staff of the participating sites, without whom this study would not have been possible.

REFERENCES

1. **Abdulla, A., and L. Yee.** 2000. The clinical spectrum of *Clostridium sordellii* bacteraemia: two case reports and an review of the literature. J. Clin. Pathol. **53:**709–712.
2. **Alfa, M. J., A. Kabani, D. Lyerly, S. Moncrief, L. M. Neville, A. Al-Barrak, G. K. Harding, B. Dyck, K. Olekson, and J. M. Embil.** 2000. Characterization of a toxin A-negative, toxin B-positive strain of *Clostridium difficile* responsible for a nosocomial outbreak of *Clostridium difficile*-associated diarrhea. J. Clin. Microbiol. **38:**2706–2714.
3. **Bartlett, J. G.** 1984. Antibiotic-associated colitis. Dis. Mon. **30:**1–54.
4. **Bartlett, J. G.** 1994. *Clostridium difficile:* history of its role as an enteric pathogen and the current state of knowledge about the organism. Clin. Infect. Dis. **18:**S265–S272.
5. **Bouza, E., T. Pelaez, R. Alonso, P. Catalan, P. Munoz, and M. R. Creixems.** 2001. "Second- look" cytotoxicity: an evaluation of culture plus cytotoxin assay of *Clostridium difficile* isolates in the laboratory diagnosis of CDAD. J. Hosp. Infect. **48:**233–237.
6. **Burke, M. D.** 1981. Cost-effective laboratory testing. Postgrad. Med. **69:**191–202.
7. **Cox, G. J., S. M. Matsui, R. S. Lo, M. Hinds, R. A. Bowden, R. C. Hackman, W. G. Meyer, M. Mori, P. I. Tarr, L. S. Oshiro, J. E. Ludert, J. D. Meyers, and G. B. McDonald.** 1994. Etiology and outcome of diarrhea after marrow transplantation: a prospective study. Gastroenterology **107:**1398–1407.
8. **Fedorko, D. P., H. D. Engler, E. M. O'Shaughnessy, E. C. Williams, C. J. Reichelderfer, and W. I. Smith, Jr.** 1999. Evaluation of two rapid assays for detection of *Clostridium difficile* toxin A in stool specimens. J. Clin. Microbiol. **37:**3044–3047.
9. **Fekety, R. A., and B. Shah.** 1993. Diagnosis and treatment of *Clostridium difficile* colitis. JAMA **269:**71–75.
10. **George, W. L., R. D. Rolfe, and S. M. Finegold.** 1982. *Clostridium difficile* and its cytotoxin in feces of patients with antimicrobial agent-associated diarrhea and miscellaneous conditions. J. Clin. Microbiol. **15:**1049–1053.
11. **Gerding, D. N., R. L. Gebhard, H. W. Sumner, and L. R. Peterson.** 1988. Pathology and diagnosis of *Clostridium difficile* disease, p. 259–286. *In* R. D. Rolfe and S. M. Findegold (ed.), *Clostridium difficile*: its role in intestinal disease. Academic Press, Inc., New York, N.Y.
12. **Gerding, D. N., M. M. Olson, L. R. Peterson, D. G. Teasley, R. L. Gebhard, M. L. Schwartz, and J. T. Lee, Jr.** 1986. *Clostridium difficile*-associated diarrhea and colitis in adults. A prospective case-controlled epidemiologic study. Arch. Intern. Med. **146:**95–100.
13. **Gerding, D. N., S. Johnson, L. R. Peterson, M. E. Mulligan, and J. Silva, Jr.** 1995. *Clostridium difficile*-associated diarrhea and colitis. Infect. Control Hosp. Epidemiol. **16:**459–477.
14. **Groschel, D. H.** 1996. *Clostridium difficile* infection. Crit. Rev. Clin. Lab. Sci. **33:**203–245.
15. **Johnson, L. L., L. V. McFarland, P. Dearing, V. Raisys, and F. D. Schoenknecht.** 1989. Identification of *Clostridium difficile* in stool specimens by culture-enhanced gas-liquid chromatography. J. Clin. Microbiol. **27:**2218–2221.
16. **Johnson, S., and D. N. Gerding.** 1998. *Clostridium difficile*-associated diarrhea. Clin. Infect. Dis. **26:**1027–1036.
17. **Laughton, B. E., R. P. Viscidi, S. L. Gdovin, R. H. Yoken, and J. G. Bartlett.** 1984. Enzyme immunoassays for detection of *Clostridium difficile* toxins A and B in fecal specimens. J. Infect. Dis. **67:**129–143.
18. **Leisenring, W., M. S. Pepe, and G. Longton.** 1997. A marginal regression modelling framework for evaluating medical diagnostic tests. Stat. Med. **16:**1263–1281.
19. **Limaye, A. P., D. K. Turgeon, B. T. Cookson, and T. R. Fritsche.** 2000. Pseudomembranous colitis caused by a toxin A(−) B(+) strain of *Clostridium difficile.* J. Clin. Microbiol. **38:**696–697.
20. **Lozniewski, A., C. Rabaud, E. Dotto, M. Weber, and F. Mory.** 2001. Laboratory diagnosis of *Clostridium difficile*-associated diarrhea and colitis: usefulness of Premier Cytoclone A+B enzyme immunoassay for combined detection of stool toxins and toxigenic *C. difficile* strains. J. Clin. Microbiol. **39:**1996–1998.
21. **Mahony, J. B., and M. A. Chernesky.** 1999. Immunoassays for the diagnosis of infectious diseases, p. 211. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
22. **Merz, C. S., C. Kramer, M. Forman, L. Gluck, K. Mills, K. Senft, I. Steiman, N. Wallace, and P. Charache.** 1994. Comparison of four commercially available rapid enzyme immunoassays with cytotoxin assay for detection of *Clostridium difficile* toxin(s) from stool specimens. J. Clin. Microbiol. **32:**1142–1147.
23. **Moncrief, J. S., D. M. Lyerly, and T. D. Wilkins.** 1997. Molecular biology of the *Clostridium difficile* toxins, p. 369–392. *In* J. I. Rood, B. A. McClane, J. G. Songer, and R. W. Titball (ed.), The clostridia: molecular biology and pathogenesis. Academic Press, Inc., New York, N.Y.
24. **O'Connor, D., P. Hynes, M. Cormican, E. Collins, G. Corbett-Feeney, and M. Cassidy.** 2001. Evaluation of methods for detection of toxins in specimens of feces submitted for diagnosis of *Clostridium difficile*-associated diarrhea. J. Clin. Microbiol. **39:**2846–2849.
25. **Peterson, L. R., and P. J. Kelly.** 1993. The role of the clinical microbiology laboratory in the management of *Clostridium difficile*-associated diarrhea. Infect. Dis. Clin. North Am. **7:**277–293.
26. **Peterson, L. R., P. J. Kelly, and H. A. Nordbrock.** 1996. Role of culture and toxin detection in laboratory testing for diagnosis of *Clostridium difficile*-associated diarrhea. Eur. J. Clin. Microbiol. Infect. Dis. **15:**330–336.
27. **Rifkin, G. D., F. R. Fekety, J. Silva, and R. B. Sack.** 1977. Antibiotic-induced colitis. Implication of a toxin neutralized by *Clostridium sordelli* antitoxin. Lancet **ii:**1103–1106.
28. **Shanholtzer, C. H., K. E. Willard, J. J. Holter, M. M. Olson, D. N. Gerding, and L. R. Peterson.** 1992. Comparison of the VIDAS *Clostridium difficile* toxin A immunoassay with *C. difficile* culture and cytotoxin and latex tests. J. Clin. Microbiol. **30:**1837–1840.
29. **Staneck, J. L., L. S. Weckbach, S. D. Allen, J. A. Siders, P. H. Gilligan, G. Coppitt, J. A. Kraft, and D. H. Willis.** 1996. Multicenter evaluation of four methods for *Clostridium difficile* detection: ImmunoCard *C. difficile*, cytotoxin assay, culture, and latex agglutination. J. Clin. Microbiol. **34:**2718–2721.
30. **Surawicz, C. M.** 1998. *Clostridium difficile* disease: diagnosis and treatment. Gastroenterologist **6:**60–65.
31. **Sutter, V. L., D. M. Citron, M. A. C. Edelstein, and S. M. Finegold.** 1985. Wadsworth anaerobic bacteriology manual, 4th ed. Star Publishing Co, Belmont, Calif.
32. **Vanpoucke, H., T. De Baere, G. Claeys, M. Vaneechoutte, and G. Verschraegen.** 2001. Evaluation of six commercial assays for the rapid detection of *Clostridium difficile* toxin and/or antigen in stool specimens. Clin. Microbiol. Infect. **7:**55–64.
33. **Vargas, S. O., D. Horensky, and A. B. Onderdonk.** 1997. Evaluation of a new enzyme immunoassay for *Clostridium difficile* toxin A. J. Clin. Pathol. **50:**996–1000.