ASIP 2011 JMD CME Program

The Journal of Molecular Diagnostics, Vol. 13, No. 4, July 2011
Copyright © 2011 American Society for Investigative Pathology
and the Association for Molecular Pathology.
Published by Elsevier Inc. All rights reserved.
DOI: 10.1016/j.jmoldx.2011.03.004

# Comparison of Five Assays for Detection of *Clostridium difficile* Toxin

Kimberle C. Chapin,*†‡ Roberta A. Dickenson,* Fongman Wu,* and Sarah B. Andrea*

*From the Departments of Pathology,* and Medicine,† Rhode Island Hospital, Providence; and Department of Medicine, Albert Brown Medical School,‡ Providence, Rhode Island*

**Performance characteristics of five assays for detection of *Clostridium difficile* toxin were compared using fresh stool samples from patients with *C. difficile* infection (CDI). Assays were performed simultaneously and according to the manufacturers' instructions. Patients were included in the study if they exhibited clinical symptoms consistent with CDI. Nonmolecular assays included glutamate dehydrogenase antigen tests, with positive findings followed by the Premier Toxin A and B Enzyme Immunoassay (GDH/EIA), and the C. Diff Quik Chek Complete test. Molecular assays (PCR) included the BD GeneOhm Cdiff Assay, the Xpert *C. difficile* test, and the ProGastro Cd assay. Specimens were considered true positive if results were positive in two or more assays. For each method, the Youden index was calculated and cost-effectiveness was analyzed. Of 81 patients evaluated, 26 (32.1%) were positive for CDI. Sensitivity of the BD GeneOhm Cdiff assay, the Xpert *C. difficile* test, the ProGastro Cd assay, C. Diff Quik Chek Complete test, and two-step GDH/EIA was 96.2%, 96.2%, 88.5%, 61.5%, and 42.3%, respectively. Specificity of the Xpert *C. difficile test* was 96.4%, and for the other four assays was 100%. Compared with nonmolecular methods, molecular methods detected 34.7% more positive specimens. Assessment of performance characteristics and cost-effectiveness demonstrated that the BD GeneOhm Cdiff assay yielded the best results. While costly, the Xpert *C. difficile* test required limited processing and yielded rapid results. Because of discordant results, specimen processing, and extraction equipment requirements, the ProGastro Cd assay was the least favored molecular assay. The GDH/EIA method lacked sufficient sensitivity to be recommended.** ***(J Mol Diagn 2011, 13:395–400; DOI: 10.1016/j.jmoldx.2011.03.004)***

*Clostridium difficile*, an anaerobic, spore-forming, gram-positive bacillus, has been associated with antibiotic-induced diarrhea since 1974 and with pseudomembranous colitis since 1978.[1,2] Toxigenic strains are responsible for *C. difficile* infection (CDI). Transmitted via the fecal-oral route, CDI has been historically associated primarily with antibiotic therapy, however, community-acquired CDI has been reported.[3,4] Symptoms include but are not limited to diarrhea, abdominal pain, and leukocytosis.[5–8] Age 65 years or older, immunosuppression, history of gastrointestinal disease, and recent antibiotic therapy have been associated with CDI.[3,5–8] Annually, there are 9000 deaths from hospital-acquired CDI, 3000 postdischarge deaths, and 16,500 deaths from CDI acquired in nursing homes (Overview of *Clostridium difficile* Infections; http://www.cdc.gov/HAI/organisms/cdiff/Cdiff_infect.html, last accessed April 2010). Methods currently in use for detection of *C. difficile* toxin include toxigenic culture, cytotoxicity assay, initial screening with glutamate dehydrogenase (GDH) antigen tests with positive screens followed by subsequent assays to detect toxins A and B, and most recently, molecular assays to detect the *tcdB* gene.[9–11]

The microbiology laboratory of the Lifespan network, which encompasses four hospitals in Rhode Island, performs 15,000 *C. difficile* assays per year. In this study, multiple assays were performed simultaneously using fresh stool specimens from patients who fulfilled the clinical criteria for CDI. Performance characteristics including the cost of each method were compared to determine the appropriate methods for use in each institution. The methods evaluated were the two-step algorithm currently used to detect *C. difficile* toxin [GDH testing followed by an enzyme immunoassay (EIA) for toxins A and B in GDH-positive specimens (GDH/EIA)] and four other assays including a combined GDH/toxin assay performed in a single cartridge and three molecular methods approved by the US Food and Drug Administration for detection of the *tcdB* gene. At Lifespan, the microbiology laboratory is mandated to provide *C. difficile* toxin results within 24 hours, seven days a week.

Accepted for publication March 9, 2011.

Preliminary results were presented at the 110th General Meeting of the American Society for Microbiology, San Diego, CA, May 2009 (Poster C-1094). Becton Dickinson and Co. provided poster printing and handouts.

CME Disclosure: Reagent kits for the BD GeneOhm Cdiff assay were provided by Becton, Dickinson and Co., and for the Xpert *C. difficile* test by Prodesse, Inc.

Address reprint requests to Kimberle C. Chapin, M.D., Rhode Island Hospital, APC 11, Rm 1166, 593 Eddy St, Providence, RI 02903. E-mail: Kchapin@lifespan.org.

## Materials and Methods

### Patients and Specimen Selection

From July 28, 2009, to August 28, 2009, consecutive liquid or soft stool specimens were obtained from hospitalized patients and transported refrigerated to the Lifespan network laboratory for detection of *C. difficile* toxin. Patient medical records were reviewed retrospectively. Inclusion criteria for entry into the study were the presence of one or more CDI-associated symptoms including diarrhea, abdominal pain, previous CDI infection, recent antibiotic therapy, leukocytosis, fever, loss of appetite, gastrointestinal tract bleeding, and nausea or vomiting.[5–8] Patients who tested positive for CDI less than 1 month before specimen collection were excluded. The study design was approved by the Lifespan Institutional Review Board (#2142–10).

### Assays

#### Nonmolecular Assays

All nonmolecular assays required visual interpretation of the results, and contained a positive control line.

The C. Diff Quik Chek test (TechLab, Inc., Blacksburg, VA), a membrane-bound lateral-flow immunoassay, was used to screen stool specimens for the presence of *C. difficile* GDH-specific antigens. Samples positive using the C. Diff Quik Check test underwent reflex testing using the Premier Toxin A and B Enzyme Immunoassay (Meridian Bioscience, Inc., Cincinnati, OH) to confirm the presence of *C. difficile* toxins A and B. For the second nonmolecular method, *C. difficile* GDH antigen and A and B toxins were simultaneously detected using antibodies specific to those antigens in a single cartridge (C. Diff Quik Chek Complete test; TechLab, Inc.).

#### Molecular Methods

The BD GeneOhm Cdiff Assay (Becton, Dickinson and Co., Franklin Lakes, NJ) was the first of three molecular methods assessed. If present in the stool, the *tcdB* gene was amplified using manual lysis and detected via a molecular beacon on emission of a fluorescent signal. Fluorescent emissions were monitored, and data were compiled using the SmartCycler II System (Cepheid, Sunnyvale, CA).

Testing using the second molecular method, Xpert *C. difficile test* (Cepheid, Sunnyvale, CA), was conducted using a self-contained cartridge that, along with the GeneXpert DX System (Cepheid), automated and integrated sample purification, nucleic acid amplification, and detection of the target sequence using RT-PCR for detection of the *tcdB* gene.

The final molecular assay assessed was the ProGastro Cd Assay (Prodesse, Inc., Waukesha, WI). After a manual process of stool clarification and nucleic acid extraction and purification using the NucliSENS easyMAG System (bioMérieux SA, Marcy l'Etoile, France), samples were added to the *C. difficile* master mix, which contained oligonucleotide primers and probes for the *tcdB* gene. PCR amplification and detection were performed using the SmartCycler II System (Cepheid).

### Specimen Processing

On arrival in the laboratory, stool specimens were refrigerated until tested. All five assays were performed within 24 hours. Specimens were fresh and not frozen before processing. Assays were performed simultaneously from fresh specimens to yield optimum results and enable valid comparisons between assays. All assays were performed according to the respective manufacturer's instructions.

### Laboratory Personnel

The C. Diff Quik Chek Complete test and subsequent EIAs were performed for clinical diagnostic purposes by the microbiology laboratory. After GDH/EIA was performed, two certified and licensed clinical laboratory scientists who specialize in molecular diagnostics (R.A.D. and F.W.) performed the C. Diff Quik Chek Complete test and all molecular methods.

### Invalid Results

In addition to definitive positive and negative results, all analyzed molecular assays had a third test interpretation, that is, invalid results. Methods of resolution for invalid specimens varied between molecular assays; however, all were specified in each manufacturer's package insert. Specimens that initially yielded an invalid test result were reanalyzed, following the manufacturer's recommendation, and final interpretation of the specimen was determined. The rate of invalid results for each assay was determined.

### Discrepant Analysis

Specimens with discrepant molecular results were frozen at $-70°C$ after initial testing and sent to a reference laboratory for further testing. Discrepant samples underwent toxigenic culture. Culture-positive specimens were subsequently analyzed using PCR for both the *tcdC* gene and GDH, and with a toxin A and B enzyme-linked immunosorbent assay (ELISA). The reference laboratory labeled specimens positive according to results of ELISA, and both PCR targets as positive. Samples that were culture-positive, PCR positive for GDH but negative using ELISA toxin A and B, and PCR *tcdC*-negative were labeled nontoxigenic. The reference laboratory was blinded to the original individual PCR specimen results.

### Data Interpretation

Specimens were considered true positive when at least two of the three molecular methods detected the presence of the *tcdB* gene.

### Statistical Analysis

Fisher's exact test was performed to calculate the statistical significance of associated risk factors and sensitivity

and specificity for each assay using commercially available software (PRISM, version 5.00 for Windows; GraphPad Software Inc., San Diego, CA). The null hypothesis was rejected at $P < 0.05$ (two-sided). Matched sample tables and the Youden index, a single characteristic that captures the performance of a test, was applied to each molecular method to determine comparability, as previously described.[12] The Youden index was calculated as follows: (sensitivity + specificity) − 1. Method with the calculated Youden index closest to 1 exhibited comparatively superior performance.

### Cost-Effectiveness Analysis

Cost-effective analysis was performed as previously described using the formula: CE ratio = (Cost of molecular assay − Cost of nonmolecular assay) ÷ (Effect of molecular assay − Effect of nonmolecular assay).[13,14] Costs exclusive to the laboratory were included in the analysis. BD GeneOhm Cdiff assay cost was based on an all-inclusive reagent rental program. The BD GeneOhm Cdiff assay kit (48 tests) includes master mix for an additional 16 tests. Thus, unless a high number of invalid results was experienced (>30%), there was no additional cost for retesting of invalid results. Total cost for the Xpert *C. difficile* test included purchase of the instrument, service program, initial cartridges, and invalid retest cartridges. Cost of the ProGastro Cd assay included kits and service packages for both the easyMAG and SmartCycler II systems. Technologist time was not factored into this cost analysis. Marginal effect, the difference between molecular and nonmolecular assay effects, was defined as the difference between the Youden index values of the methods compared.[13]

## Results

### Patient Data

Of 89 patients who submitted specimens, 81 patients with symptoms compatible with CDI were included in the study. Their mean (median; range) age was 64.4 (71; 4 to 97) years, and 26 (32%) tested positive for CDI. Signs and symptoms of CDI in the patients evaluated are given in Table 1. The most common symptoms were diarrhea (69.1%), abdominal pain (63.0%), and leukocytosis (39.5%). Physicians requested testing for *C. difficile* toxins appropriately in 81 of 82 patients (98.8% of test requests), as determined by the presence of signs and symptoms consistent with CDI. Assessment for CDI in patients with known risk or symptoms resulted in detection of CDI 32.1% of the time. While leukocytosis was the only statistically significant independent symptom ($P = 0.02$; relative risk, 3.3; 95% confidence interval, 1.3 to 8.5), leukocytosis in conjunction with abdominal pain was statistically significant ($P = 0.047$; odds ratio, 3.2; 95% confidence interval, 1.1 to 9.1). CDI occurred in patients younger than 65 years; however, individuals 65 years or older exhibited a higher incidence of CDI (17 versus 9 cases, respectively). However, there was no statistical difference between the two age groups.

**Table 1.** Signs and Symptoms in Population Analyzed

| Variable | Patients No. (%) | CDI-Positive No. (%) |
|---|---|---|
| Symptom | | |
| Diarrhea | 56 (69.1) | 16 (61.5) |
| Abdominal pain | 51 (63) | 17 (65.4) |
| Leukocytosis* | 32 (39.5) | 15 (57.7) |
| Nausea | 24 (29.6) | 10 (38.5) |
| Loss of appetite | 21 (25.9) | 4 (15.4) |
| Vomiting | 16 (19.8) | 8 (30.8) |
| GI bleeding | 12 (14.8) | 4 (15.4) |
| Fever | 11 (22.2) | 4 (15.4) |
| Previous CDI | 5 (6.2) | 3 (11.5) |
| Associated risk | | |
| Female sex | 34 (42.0) | 12 (46.2) |
| Age ≥65 years | 46 (56.8) | 17 (65.4) |
| History of colitis, IBS, or other GI disorder | 29 (35.8) | 10 (38.5) |
| Previous antibiotic therapy | 23 (28.4) | 7 (26.9) |
| Immunocompromise | 19 (23.5) | 5 (19.2) |
| Nursing home | 9 (11.1) | 4 (15.4) |

*Defined as >10 WBC per cubic millimeter. $P = 0.02$; relative risk, 3.3; 95% confidence interval, 1.3 to 8.5. No other symptoms were significant.
CDI, *C. difficile* infection; GI, gastrointestinal tract; IBS, irritable bowel syndrome.

### Performance Data

Performance data for each assay after invalid results and discrepant analysis testing was performed are given in Table 2. Sensitivity of the BD GeneOhm Cdiff assay, Xpert *C. difficile* test, ProGastro Cd assay, C. Diff Quik Chek Complete test, and two-step GDH/EIA were 96.2%, 96.2%, 88.5%, 61.5%, and 42.3%, respectively. Specificity of the Xpert *C. difficile* test was 96.4%, and for all other methods was 100%.

### Invalid Results

Tests with results initially determined as invalid were repeated per the manufacturer's instructions. The rates of invalid results for the BD GeneOhm Cdiff assay, Xpert C difficile test, and ProGastro Cd assay were 4.0%, 1.1%, and 0%, respectively. Repeat testing of all specimens with initially invalid results yielded a definitive negative result.

### Discrepant Results

Of the 81 stool specimens tested, six were considered discordant between molecular methods. Two of two specimens positive using the BD GeneOhm Cdiff assay and Xpert *C. difficile* test but negative using the ProGastro Cd assay were resolved as true positive. One of three specimens positive using only the Xpert *C. difficile* test was resolved as true positive. One of one specimen positive using only the ProGastro Cd assay was resolved as true positive.

### Cost-Effectiveness Analysis

Results of cost-effectiveness analysis for each assay evaluated are given in Table 3.

**Table 2.** Comparison of Methods for Detection of CDI in Patients with Symptoms

| Diagnostic method | No. true positive | No. false positive | No. false negative | No. true negative | Invalid results*, % | Sensitivity† | | Specificity | | Predictive value, % | | Youden index, % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | % | 95% CI | % | 95% CI | Positive | Negative | |
| GeneOhm Cdiff assay | 25 | 0 | 1 | 55 | 4.0 | 96.2 | 88.0–96.2 | 100 | 96.1 to 100 | 100 | 98.2 | 96.2 |
| Xpert *C. difficile* test | 25 | 2 | 1 | 53 | 1.1 | 96.2 | 86.0–99.2 | 96.4 | 91.6 to 97.8 | 92.6 | 98.1 | 92.5 |
| ProGastro Cd assay | 23 | 0 | 3 | 55 | 0 | 88.5 | 79.3–88.5 | 100 | 95.6 to 100 | 100 | 94.8 | 88.5 |
| Quik Chek Complete‡ | 16 | 0 | 10 | 55 | NA | 61.5 | 51.4–61.5 | 100 | 95.2 to 100 | 100 | 84.6 | 61.5 |
| GDH/EIA§ | 11 | 0 | 15 | 55 | NA | 42.3 | 32.4–42.3 | 100 | 95.3 to 100 | 100 | 78.6 | 57.7 |

Eighty-one specimens were analyzed using all methods.
*Xpert demonstrated an additional 5.5% "error rate." At repeat testing, 100% of invalid specimens and errors for both GeneOhm and Xpert were resolved and provided definitive results, which are included in the total.
†$P < 0.03$ for all molecular methods versus nonmolecular methods; Quik Chek Complete versus GDH/EIA was not significant.
‡GDH component was 96.2% sensitive and 81.8% specific.
§GDH component was 96.2% sensitive and 76.4% specific.
CDI, *C. difficile* infection; GI, gastrointestinal tract; IBS, irritable bowel syndrome.

## *Discussion*

This is the first study to evaluate simultaneously three Food and Drug Administration–approved molecular methods, the Xpert *C. difficile* test, the BD GeneOhm Cdiff assay, and the ProGastro Cd assay. Compared with nonmolecular methods, these assays detected 35% to 54% more specimens positive for *C. difficile*. The better performance of molecular versus nonmolecular methods was significant at $P < 0.03$ for all methods evaluated. This is consistent with previous studies that suggested that single independent molecular methods are 8.5% to 51.3% more sensitive than nonmolecular methods.[9,10,15–18] However, the data presented herein are in contrast to those of other investigators, who have demonstrated high sensitivity with GDH screening (90% to 100%).[19–21] The differences in GDH sensitivity in these studies relative to molecular assay performance could be due to geographic and genetically varied strains, timeliness of testing, sample condition, and inclusion of patients who recently tested positive. The present study eliminated potential bias related to testing timeliness, duplicate specimens, and patients who previously tested positive.

When comparing all assays using their calculated Youden index values, the BD GeneOhm Cdiff assay was the most efficient (96.2%), followed by the Xpert *C. difficile* test (92.5%), the ProGastro Cd assay (88.5%), the C. Diff Quik Chek Complete test (61.5%), and the GDH/EIA two-step algorithm (57.7%). Whereas molecular assays were similarly efficient, a significant difference in cost-effectiveness was observed due to great variance in associated laboratory costs.

Although the sample size was not sufficient to support statistical analysis for noninferiority, the results produced using the molecular methods were comparable. In our laboratory, the BD GeneOhm Cdiff assay demonstrated superior performance and cost-effectiveness when compared with both the Xpert *C. difficile* test and the ProGastro Cd assay. Invalid results occurred more often on initial testing with the BD GeneOhm Cdiff assay (4.0%); however, repeat testing of invalid results could be performed during the same shift from the frozen specimen lysate with no additional cost because each kit contained master mix overage of 33%. Repeat testing provided results 100% of the time, and all results were available within the required 24-hour turnaround time. Compared with the Xpert *C. difficile* test, the BD GeneOhm Cdiff assay required additional hands-on extraction and setup; however, it was also considerably less expensive. In addition, cost-effectiveness analysis revealed that despite the increased cost of the molecular assay, greater savings were attained with the BD GeneOhm Cdiff assay compared with the C. Diff Quik Chek Complete test and the two-step GDH/EIA method because of greatly enhanced performance. Testing the batch once a day worked well at our high-volume site because of less hands-on time; however, this benefit may not be realized in a laboratory with a smaller volume.

Turnaround time for the Xpert *C. difficile* test was quick, approximately 50 minutes, and the processing procedure was limited. However, the laboratory cost for reagents was twice that for the other molecular assays. In addition, the

**Table 3.** Yearly Cost-Effectiveness of Methods

| Diagnostic method | Total cost, $ | Effect, %* | CE ratio versus GDH/EIA |
|---|---|---|---|
| BD GeneOhm Cdiff assay | 367,650.00† | 96.2 | $5894.81 |
| Xpert *C. difficile* test | 649,662.50‡ | 92.5 | $14,625.36 |
| ProGastro Cd assay | 456,004.20§ | 88.5 | $10,237.15 |
| C.Diff Quik Chek Complete test | 175,950.00 | 61.5 | $9276.32 |
| GDH/EIA | 140,700.00 | 57.7 | NA |

Assuming 15,000 assays are performed in 1 year.
*For this analysis, effect was defined as the Youden index.
†All-inclusive reagent rental program. No additional charge for invalid retesting. Includes cost of SmartCycler II System.
‡Includes purchase of instrument, service program, initial cartridges, and invalid retest cartridges.
§Includes purchase of easyMag, SmartCycler II System, service packages, and kits.
CE, cost-effectiveness; EIA, toxins A and B enzyme immunoassay; GDH, glutamate dehydrogenase; NA, not applicable.

hands-on time at our large-volume site would have required a dedicated technologist to perform testing throughout the day, with the 16-bay instrument available. The rate of invalid results was1.1%, and the error rate was 5.5%, secondary to syringe pressure and probe check errors. Careful attention to modules with repeated errors is necessary to differentiate between specimen issues or module malfunction. Of all specimens, 6.6% were determined to be invalid or erroneous. Repeated testing was successful 100% of the time; however, the cost of the test doubled because a new cartridge was necessary for each test.

The ProGastro Cd assay was the least favored of the three molecular assays for the following reasons. According to the Food and Drug Administration–approved product package insert, stool specimens must be kept on ice during processing, and an easyMAG extraction instrument (bioMérieux SA) must be used for specimen processing. While many laboratories have this equipment, the coordination of workflow with other molecular assays that require use of this extraction instrument could be a factor in high-volume sites. In addition, this assay produced the greatest number of discordant results.

Compared with the molecular assays evaluated, both the antigenic C. Diff Quik Chek Complete test and the two-step GDH/EIA assay performed poorly. However, testing using nonmolecular assays may be the only option in certain settings, and has performed adequately as a two-step algorithm with GDH antigen as the preliminary test followed by either PCR or the C. Diff Quik Chek Complete test.[15,18,19,21] Because the C. Diff Quik Chek Complete test detected nearly 20% more specimens correctly in this evaluation, as well as having both the GDH and toxin assay performed simultaneously, this assay is preferred over the two-step GDH/EIA method. Because of the potential severity of CDI and the implications for infection control, institutions that provide nonmolecular test results should alert physicians to performance characteristics of nonmolecular assays and the continued need to consider treatment in patients in whom there is high suspicion of CDI positivity despite negative test results.

While only laboratory costs were considered in this analysis, a turnaround time of results more than once a day may have a positive effect on nursing and housekeeping issues related to patient care, initial placement, transfer, and discharge, and a more rapid test would be appropriate in some settings such as institutions with shared rooms or a limited number of available beds.[22–25] However, more rapid laboratory turnaround time does not always indicate rapid physician response. Delays in reviewing test results are common,[26] and delayed response is consistent with findings at our institution when traditional methods were replaced with more rapid procedures.[27] To realize the full benefit of rapid molecular techniques, variables other than laboratory turnaround time and physician response are critical to cost-effective implementation.

Based on our large test volume and mandated daily toxin reporting, the BD GeneOhm Cdiff assay was selected for use in operations performed on Monday through Friday, when manual processing and testing can be done within an 8-hour shift. The Xpert *C. difficile* test was chosen for late Friday afternoon and weekend processing to enable maximum workflow efficiency with limited personnel on weekend shifts while still being able to address the daily toxin result turnaround time requirement. The molecular methods selected for use in the Lifespan laboratory yielded a statistically higher number of positive CDI results. In addition, and important for purposes of instituting the new molecular test, the BD GeneOhm Cdiff assay was more cost-effective than the previously used GDH/EIA method.

## References

1. Tedesco F, Stanley R, Alpar D: Diagnostic features of clindamycin-associated pseudomembranous colitis. N Engl J Med 1974, 290: 841–843
2. Bartlett J: Narrative review: the new epidemic of Clostridium difficile–associated enteric disease. Ann Intern Med 2006, 145:758–764
3. Fellmeth G, Yarlagadda S, Iyer S: Epidemiology of community-onset Clostridium difficile infection in a community in the South of England. J Infect Public Health 2010, 3:118–123
4. Durai R: Epidemiology, pathogenesis, and management of Clostridium difficile infection. Dig Dis Sci 2007, 52:2958–2962
5. Curry S: Clostridium difficile. Clin Lab Med 2010, 30:329–342
6. Yoo J, Lightner AL: Clostridium difficile infections: what every clinician should know. Permanente J 2010, 14:35–40
7. Salkind AR: Clostridium difficile: an update for the primary care clinician. South Med J 2010, 103:896–900
8. Bobo L, Dubberke E: Recognition and prevention of hospital-associated enteric infections in the intensive care unit. Crit Care Med 2010, 38:S324–S334
9. Eastwood K, Else P, Charlett A, Wilcox M: Comparison of nine commercially available Clostridium difficile toxin detection assays, a real-time PCR assay for C. difficile tcdB, and a glutamate dehydrogenase detection assay to cytotoxin testing and cytotoxigenic culture methods. J Clin Microbiol 2009, 47:3211–3217
10. Kvach EJ, Ferguson D, Riska PF, Landry ML: Comparison of BD GeneOhm Cdiff Real-Time PCR Assay with a two-step algorithm and a toxin A/B enzyme-linked immunosorbent assay for diagnosis of toxigenic Clostridium difficile infection. J Clin Microbiol 2010, 48:109–114
11. Terhes G, Urban E, Soki J, Nacsa E, Nagy E: Comparison of a rapid molecular method, the BD GeneOhm Cdiff assay, to the most frequently used laboratory tests for detection of toxin-producing Clostridium difficile in diarrheal feces. J Clin Microbiol 2009, 47:3478–3481
12. Hawass N: Comparing the sensitivities and specificities of two diagnostic procedures performed on the same group of patients. Br J Radiol 1997, 70:360–366
13. American College of Physicians: Primer on cost-effectiveness analysis. Effective Clin Pract 2000, 5:253–255
14. Pisapia J: Cost Analysis and Learning Technologies: How to conduct a cost-effectiveness analysis. Richmond, VA: Virginia Commonwealth University, School of Education, Metropolitan Education Research Consortium; 1994
15. Larson A, Fung A, Fang F: Evaluation of tcdB real-time PCR in a three-step diagnostic algorithm for detection of toxigenic Clostridium difficile. J Clin Microbiol 2010, 48:124–130
16. Novak-Weekley S, Marlowe EM, Miller JM, Cumpio J, Nomura JH, Vance PH, Weissfeld A: Clostridium difficile testing in the clinical laboratory by use of multiple testing algorithms. J Clin Microbiol 2010, 48:889–893
17. Stamper P, Alcabasa R, Aird D, Babiker W, Wehrlin J, Ikpeama I, Carroll KC: Comparison of a commercial real-time PCR assay for tcdB detection to a cell culture cytotoxicity assay and toxigenic culture for direct detection of toxin-producing Clostridium difficile in clinical samples. J Clin Microbiol 2009, 47:373–378
18. Swindells J, Brenwald N, Reading N, Oppenheim B: Evaluation of diagnostic tests for Clostridium difficile infection. J Clin Microbiol 2010, 48:606–608
19. Sharp S, Ruden L, Pohl JC, Hatcher PA, Jayne LM, Ivie WM: Evaluation of the C. Diff Quik Chek Complete Assay, a new glutamate

dehydrogenase and A/B toxin combination lateral flow assay for use in rapid, simple diagnosis of Clostridium difficile disease. J Clin Microbiol 2010, 48:2082–2086
20. Schmidt M, Gilligan P: Clostridium difficile testing algorithms: what is practical and feasible? . Anaerobe 2009, 15:270–273
21. Reller M, Alcabasa R, Lema C, Carroll KC: Comparison of two rapid assays for Clostridium difficile common antigen and a C difficile toxin A/B assay with the cell culture neutralization assay. Am J Clin Pathol 2010, 133:107–109
22. Dubberke ER, Wertheimer A: Review of current literature on the economic burden of Clostridium difficile infection. Infect Control Hospital Epidemiol 2009, 30:57–66
23. Nguyen G, Kaplan G, Harris ML, Brant SR: A national survey of the prevalence and impact of Clostridium difficile infection among hospitalized inflammatory bowel disease patients. Am J Gastroenterol 2008, 103:1443–1450
24. O'Brien JA, Lahue BJ, Caro JJ, Davidson DM: The emerging infectious challenge of Clostridium difficile–associated disease in Massachusetts hospitals: clinical and economic consequences. Infect Control Hosp Epidemiol 2007, 28:1219–1227
25. Cherifi S, Delmee M, Van Broeck J, Beyer I, Byl B, Mascart G: Management of an outbreak of Clostridium difficile–associated disease among geriatric patients. Infect Control Hosp Epidemiol 2006, 27:1200–1205
26. Poon EG, Gandhi TK, Sequist TD, Murff HJ, Karson HJ, Bates DW: "I wish I had seen this test result earlier!": dissatisfaction with test result management systems in primary care. Arch Intern Med 2004, 164: 2223–2228
27. Koster M, Daigneault L, Dickenson R, et al: Utilization of Rapid PCR Testing for Enterovirus and Impact on Physician Practice. Boston, MA: 2008;C-238