

Evaluation of real-time PCR and conventional diagnostic methods for the detection of *Clostridium difficile*-associated diarrhoea in a prospective multicentre study

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In this prospective multicentre study, an enzyme-linked fluorescent assay (VIDAS CDA2; bioMérieux), an enzyme-linked assay [Premier Toxins A and B (PTAB); Meridian] and an in-house real-time PCR amplifying the *tcdB* gene were compared with the cell cytotoxicity assay used as the 'gold standard' for diagnosis of *Clostridium difficile*-associated diarrhoea (CDAD). Faecal samples from patients with a request for *C. difficile* diagnosis and samples from patients with diarrhoea hospitalized for at least 72 h were collected for 3 consecutive months from four university medical centres in The Netherlands. In total, 547 faecal samples were obtained from 450 patients. Of 540 samples available for all of the assays, 84 (15.6%) showed a positive result in one or more assays. The cell cytotoxicity assay was positive in 31 samples (5.7%) from 28 patients. A diagnosis of CDAD was not considered by the physician in 5 (23.8%) of 21 patients with CDAD who were hospitalized for at least 72 h. Compared with the cell cytotoxicity assay, the sensitivity of VIDAS, PTAB and PCR was 83.9, 96.8 and 87.1%, respectively. The specificity of VIDAS, PTAB and PCR was 97.1, 94.3 and 96.5%, respectively. The positive and negative predictive values for VIDAS, PTAB and PCR were 63.4 and 99.0%, 50.9 and 99.8%, and 60.0 and 99.2%, respectively. Of 61 samples that were positive in one, two or three of the assays, 56 were available for discordance analysis. Discordance analysis was performed by culture of toxinogenic strains. The concordance of VIDAS, PTAB and PCR with culture was 53.6% (30/56), 55.4% (31/56) and 71.4% (40/56), respectively. It was concluded that real-time PCR had the highest concordance with toxinogenic culture and is therefore the preferred method for diagnosing CDAD in faecal samples. It was also concluded that diagnosis of patients with diarrhoea who have been hospitalized for more than 72 h should focus mainly on the detection of *C. difficile*, irrespective of the physician's request.

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INTRODUCTION

Clostridium difficile is a Gram-positive, spore-forming rod that grows anaerobically. Strains of *C. difficile* that produce the toxins A (TcdA) and B (TcdB) are known to be the

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Abbreviations: CDAD, *Clostridium difficile*-associated diarrhoea; EIA, enzyme immunoassay; NPV, negative predictive value; PMC, pseudomembranous colitis; PPV, positive predictive value; PTAB, Premier Toxins A and B; ROC, receiver operating characteristic.

causative agents of *C. difficile*-associated diarrhoea (CDAD) and pseudomembranous colitis (PMC). CDAD is an important nosocomial infection. Various predisposing factors for *C. difficile* infection have been recognized, such as antibiotic use, age, surgical procedures, tube feeding, length of hospitalization, use of chemotherapeutic agents and use of acid-suppressive therapy (Brown *et al.*, 1990; Clabots *et al.*, 1992; Kelly & LaMont, 1998). *C. difficile* is usually diagnosed by a cell cytotoxicity assay or by specific culture of toxinogenic isolates. Due to their rapid

turnaround time, enzyme immunoassays (EIAs) for the detection of TcdA and/or TcdB have been implemented in most microbiological laboratories. As a result of the increasing incidence of strains producing only TcdB (Drudy *et al.*, 2004; van den Berg *et al.*, 2004), EIAs detecting both toxins are preferred. However, despite a turnaround time of >48 h, the cell cytotoxicity assay is still considered to be the 'gold standard' (Johnson & Gerding, 1998; Oldfield, 2004).

The primary aim of this multicentre study was to compare four different diagnostic methods. The performances of the rapid EIA Premier Toxins A and B (PTAB) assay, the VIDAS CDA2 assay (which detects only TcdA) and a rapid, in-house, real-time PCR assay to amplify the *tcdB* gene were evaluated in comparison with the conventional cell cytotoxicity assay. Discordant samples were analysed further by culture of toxinogenic strains. The second aim of this study was to evaluate the '3 day rule'. When patients hospitalized for at least 72 h develop diarrhoea, the advice is to test faecal samples only for *C. difficile* and not for the presence of *Salmonella*, *Campylobacter*, *Shigella* or *Yersinia* spp. (Bowman *et al.*, 1992; Fan *et al.*, 1993; Siegel *et al.*, 1990; Yannelli *et al.*, 1988). We also tested this diagnostic algorithm in the current study.

METHODS

Patient inclusion and faecal samples. Faecal samples from patients with a request for *C. difficile* diagnosis and samples from patients with diarrhoea who had been hospitalized for at least 72 h were collected for three consecutive months in the period from October 2003 to February 2004 at the Departments of Medical Microbiology of four university medical centres in The Netherlands: Erasmus Medical Centre, Rotterdam (Erasmus MC), Leiden University Medical Centre (LUMC), VU University Medical Centre, Amsterdam (VUMC) and the University Medical Centre St Radboud, Nijmegen (UMC St Radboud). A computer algorithm was developed to recognize faecal samples from patients who had been admitted to the hospital for at least 72 h. All samples were stored within 6 h of arrival at the laboratory at -20°C in two individual vials. One vial was used in the respective hospitals for their diagnostic methods and the second vial was used for subsequent testing in the reference centre at the LUMC. All faecal samples were thawed only once for a specific test.

Diagnostic assays. The enzyme-linked fluorescent assay VIDAS CDA2 (bioMérieux), the PTAB assay (Meridian) and an in-house real-time PCR for *tcdB* were used for diagnosing CDAD, and compared with the cell cytotoxicity assay, as the gold standard. All hospitals performed the PTAB assay and conventional culture for *Salmonella*, *Shigella*, *Campylobacter* and *Yersinia* spp. on the faecal samples, whereas real-time PCR was performed only in the LUMC. Erasmus MC, VUMC and LUMC performed the VIDAS assay; the cell cytotoxicity assay was performed in the LUMC. UMC St Radboud performed the cell cytotoxicity assay and their samples were subsequently tested in the LUMC by the VIDAS assay. VUMC cultured all of their samples for the presence of *C. difficile*.

The cell cytotoxicity assay was performed at LUMC using Vero cells in a 24-well format. Faecal samples were diluted 1:4 in Eagle's minimum essential medium containing 5% fetal bovine serum and centrifuged. Subsequently, the supernatant was filtered through a

0.45 μm pore-size filter. Neutralization of the cytotoxic effect was performed using specific *C. difficile* antitoxin (Techlab). At UMC St Radboud, the assay was performed using Vero cells in a microwell format. Faecal samples were diluted (1:20 to 1:10 240) after filtration through a 0.45 μm pore-size filter. Neutralization was performed using *Clostridium sordellii* antiserum (Techlab).

The VIDAS and PTAB assays were performed according to the instructions of the manufacturers. The interpretation of results of the PTAB assay was the same at Erasmus MC, VUMC and LUMC, with an optical density cut-off value of 1.00 using the spectrophotometric dual wavelength 450/630 nm. UMC St Radboud performed the interpretation visually, with a yellow colour indicating positive samples. Samples with equivocal results for the VIDAS assay (test value threshold ≥ 0.40 to < 1.0) were retested with a VIDAS blocking test (VIDAS CDB) according to the manufacturer's recommendations.

For real-time PCR, primers 398CLDs (5'-GAAAGTCCAAGTTTAC-GCTCAAT-3') and 399CLDs (5'-GCTGCACCTAACTTACACCA-3') were designed to amplify 177 bp of the non-repeat region of the *tcdB* gene (van den Berg *et al.*, 2006). A specific 6-carboxyfluorescein (FAM)-labelled Taqman probe (5'-ACAGATGCAGCCAAAGTTGT-TGAATT-3') was used as an internal probe. DNA isolation from faecal samples was performed using STAR (stool transport and recovery) buffer pre-treatment and subsequent automated isolation using a MagnaPure LC DNA isolation kit III (Roche) in a MagnaPure system, according to the manufacturer's instructions. Phocid herpesvirus was included as an internal control for detection of inhibition in the PCR.

Discordance analysis. All samples positive for *C. difficile* in one or more of the assays were cultured for the presence of toxinogenic isolates. Culture was performed as described previously (van den Berg *et al.*, 2005a). Briefly, faecal samples were treated with an ethanol shock pre-treatment prior to inoculation onto Columbia agar containing colistin and nalidixic acid and onto *C. difficile*-selective agar with cefoxitin, amphotericin B and cycloserine (CLO; bioMérieux) and incubated in an anaerobic environment at 37°C for 2 days. CLO medium was also used to inoculate faecal samples that were not pre-treated with ethanol. DNA was isolated from Gram-positive rods with subterminal spores and a positive proline aminopeptidase reaction (García *et al.*, 1997) using QiaAmp DNA isolation columns (Qiagen) according to the manufacturer's recommendations, including a 10 min incubation at 55°C with proteinase K (Qiagen). These isolated strains were subsequently tested by PCR for the presence of *tcdA* and *tcdB*, as described by Kato *et al.* (1998, 1999).

Statistical analysis. The statistical software SPSS 11.0 was used. A χ^2 test and a *t*-test for independent samples were used to compare all characteristics between patients hospitalized for at least 72 h with and without a request for CDAD diagnosis. The area under the receiver operating characteristic (ROC) curve, a measurement of the accuracy of a test independent of the cut-off values used, was also calculated using the statistical software.

RESULTS

Patients

In total, 547 samples were included from 450 patients: 202 samples from 149 patients from Erasmus MC, 142 from 106 patients from LUMC, 116 from 116 patients from VUMC and 87 from 79 patients from UMC St Radboud. Of these 450 patients, 382 had only one sample, 45 had two samples and 23 patients had three or more samples included in this study.

Diagnostic assays

Only samples with results in all four assays were included in our analysis. Of the total of 547 faecal samples collected, 7 (1.3 %) were excluded due to the absence of sufficient material for testing in all assays. Of the remaining 540 samples, 456 samples were negative in all assays. A total of 84 (15.6 %) of the 540 samples were positive in one or more assays and 31 (5.7 %) samples from 28 patients were positive by the cell cytotoxicity assay (Table 1). The highest percentage of positive cell cytotoxicity tests (9.4 %) was found at LUMC, followed by UMC St Radboud (7.6 %), EMCR (7.4 %) and VUMC (3.4 %). Using the cell cytotoxicity assay as the gold standard, the highest sensitivity was observed for the PTAB assay (96.8 %), compared with 83.9 % for the VIDAS assay and 87.1 % for the real-time PCR assay. The specificity and positive predictive value (PPV) were comparable for both the VIDAS (97.1 and 63.4 %, respectively) and real-time PCR (96.5 and 60 %, respectively) assays and were slightly higher than for the PTAB assay (94.3 and 50.9 %, respectively). The negative predictive value (NPV) was comparable for all three assays (99–100 %; Table 1). Correlation of the VIDAS, PTAB and real-time PCR assays with the cell cytotoxicity assay was 96.3, 94.4 and 95.9 %, respectively. No significant differences in sensitivity, specificity, NPV and PPV for any of the assays were observed between Erasmus MC, VUMC and LUMC (data not shown), compared with Table 1. However, UMC St Radboud showed 100 % specificity and PPV for the PTAB assay, with a sensitivity of 83 %.

The area under the ROC curve was 0.957 (SEM = 0.016) for VIDAS and 0.993 (SEM = 0.003) for PTAB compared with the cell cytotoxicity assay.

Discordance analysis

Discordance analysis was performed by culture for *C. difficile* of samples that were positive in one, two or three of the diagnostic assays. *C. difficile* isolates were subsequently tested for the presence of *tcdA* and *tcdB* to determine the

capacity of the isolates to produce TcdA and TcdB. The results of the discordance analysis are presented in Table 2. A total of 56 out of 61 samples were available for specific culture of toxinogenic *C. difficile*; 22 of these showed a positive culture of a toxinogenic strain. Of 12 samples that were only positive by PCR, 5 were culture positive. The nine samples that were only positive by the VIDAS assay were culture negative. Of the 19 samples that were only positive by the PTAB assay, 3 were culture positive. One of these three samples was positive for a TcdA⁻/TcdB⁺ strain. The VIDAS assay showed a concordance with culture of 53.6 % (30/56) and the PTAB assay had a concordance of 55.4 % (31/56). The real-time PCR assay showed a higher concordance with culture of 71.4 % (40/56), similar to the concordance of the cell cytotoxicity assay (75 %, 42/56). The sensitivity of the cell cytotoxicity assay compared with toxinogenic culture in our discrepancy analysis was 36.4 %, although the specificity was 100 %.

Determining patient group

Of the 450 patients, 372 had been hospitalized for at least 72 h when they developed diarrhoea. Of these 372 patients, 251 had a request for *C. difficile* diagnosis, whereas 121 patients had no request (Table 3). The mean age for patients with a request was significantly higher than for the other patients ($P=0.005$, Table 3). Significant differences were observed for the Departments of Internal Medicine, Surgery, Neurology and Paediatrics between samples where a CDAD diagnosis was requested and for samples without such a request. No significant differences were observed for gender, the number of hospitalized days before onset of diarrhoea or patients with a previous episode of CDAD in the last 3 months. The data observed in the four participating centres did not differ significantly from each other (data not shown). For 5 (23.8 %) of the 21 patients with diarrhoea and at least 72 h of hospitalization who were positive by the cell cytotoxicity assay, the diagnosis of CDAD was not considered by the physician. This distribution was not significantly different (Table 3).

Table 1. Results of three different diagnostic assays for diagnosis of CDAD, compared with the cell cytotoxicity assay, on 540 faecal samples

Only samples tested in all four assays were included.

Assay	Result	No. of cell cytotoxicity assay results ($n=540$)		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Correlation (%)
		Positive ($n=31$)	Negative ($n=509$)					
VIDAS	Positive	26	15	83.9	97.1	63.4	99.0	96.3
	Negative	5	494					
PTAB	Positive	30	29	96.8	94.3	50.9	99.8	94.4
	Negative	1	480					
Real-time PCR	Positive	27	18	87.1	96.5	60.0	99.2	95.9
	Negative	4	491					

Table 2. Discordance analysis by culture of toxinogenic *C. difficile* of 56 faecal samples positive in one to three of the assays. The toxinogenicity of cultured *C. difficile* strains was determined by PCR for the presence of *tcdA* and *tcdB*.

No. of samples	Assay result				Culture positive
	VIDAS	PTAB	Real-time PCR	Cytotoxicity assay	
12	–	–	+	–	5
19	–	+	–	–	3*
4	–	+	+	–	3
9	+	–	–	–	0
1	+	–	+	–	1
2	+	+	–	–	1
1	+	+	+	–	1
1	–	–	+	+	1
1	–	+	–	+	1
3	–	+	+	+	3
3	+	+	–	+	3
Total no. positive (<i>n</i> =56)	16	33	22	8	22

*One strain was TcdA[–]/TcdB⁺.

DISCUSSION

This present study was undertaken to ascertain the diagnostic values of four different assays for the diagnosis of CDAD and to investigate whether patients with diarrhoea hospitalized for at least 72 h should be investigated for CDAD, irrespective of the physician's request.

A total of 84 (15.6 %) of 540 samples were positive in one or more assays and 31 samples (5.7 %) of 28 patients were positive by the cell cytotoxicity assay. Using the cell cytotoxicity assay as the gold standard, the PTAB assay showed the highest sensitivity (96.8 %), although the PPV (50.9 %) was about 10 % lower than for the real-time PCR and VIDAS assays. Turgeon *et al.* (2003) compared six

Table 3. Characteristics of patients (*n*=372) with diarrhoea, hospitalized for at least 72 h, included in this study

No. of patients	Total (<i>n</i> =372)	CDAD requested (<i>n</i> =251)	No request for CDAD (<i>n</i> =121)	<i>P</i> value*
Mean age (±SD)	52.5 (±22.6)	54.8 (±21.0)	47.7 (±25.0)	0.005†
Male gender (%)	179/371 (48.2 %)	128/250 (51.2 %)	51/121 (42.1 %)	≤0.20
Department				
Internal Medicine	210	158 (75.2 %)	52 (24.8 %)	≤0.001
Surgery	52	26 (50 %)	26 (50 %)	≤0.01
Paediatrics	28	14 (50 %)	14 (50 %)	≤0.05
Neurology	9	3 (33.3 %)	6 (66.6 %)	≤0.05
Intensive Care	38	29 (76.3 %)	9 (23.7 %)	≤1
Other	35	21 (60 %)	14 (40 %)	≤1
Days of admission before onset mean (SD)	15.5 (15.5)	15.7 (15.4)	14.8 (15.8)	0.603†
Previous CDAD in last 3 months	6/270 (2.2 %)	6/191 (3.1 %)	0/79 (0 %)	≤0.20
Number of positive cell cytotoxicity tests	21 (5.6 %)	16 (6.4 %)	5 (4.1 %)	≤1
Request for culture of other enteropathogens	177/370 (47.8 %)	86/249 (34.5 %)	91 (75.2 %)	≤0.001
<i>Salmonella</i> culture positive	0	0	0	1
<i>Shigella</i> culture positive	1 (0.3 %)	0	1 (0.8 %)	≤0.20
<i>Campylobacter</i> culture positive	0	0	0	1
<i>Yersinia</i> culture positive	0	0	0	1

*Analysed by χ^2 test, unless noted otherwise.

†Analysed by *t*-test with independent samples.

different immunoassays with the cell cytotoxicity assay and found PPVs for PTAB and VIDAS of 90.2 and 87.7%, respectively. These data contrast with our results, but may be due to differences in the design of the study. The low PPV in all tests, compared with the cell cytotoxicity assay, probably reflects the low sensitivity of the cell cytotoxicity assay. An explanation could be the storage procedures used in this study. Turgeon *et al.* (2003) tested all faecal samples for cytotoxicity within 24 h of receipt, whereas the multicentre approach of our study did not allow such a procedure. Faecal samples included in our study were stored within 6 h of arrival at the laboratories and were thawed once. It has been demonstrated that storage at -20°C and repeated freezing and thawing will decrease the cytotoxic activity of faecal samples containing *C. difficile* TcdB, although this has only been tested with artificially contaminated faecal samples (Freeman & Wilcox, 2003). Another study that evaluated the VIDAS assay for the diagnosis of CDAD tested a total of 38 consecutive cell cytotoxicity-positive samples and 33 negative samples (Lipson *et al.*, 2003). The authors also applied a discordance analysis by toxinogenic culture and found sensitivity, specificity, PPV and NPV for the VIDAS assay of 80.6, 96.8, 96.7 and 81.1%, respectively. Concordance with culture was 83%. Although the VIDAS assay displayed a reduced sensitivity compared with the cytotoxicity test, the authors recommended the VIDAS assay because of the rapid results. In contrast, we found that the VIDAS assay had a concordance with culture of only 53.6% and we therefore prefer the PTAB or real-time PCR assay as a rapid diagnostic test. O'Connor *et al.* (2001) compared four rapid immunoassays (Oxoid Toxin A test, ImmunoCard Toxin A test, Techlab Toxin A/B II test and the PTAB assay) with toxinogenic culture and the cell cytotoxicity assay. When the final diagnosis of CDAD based on clinical criteria was taken as the gold standard, the cell cytotoxicity assay had the highest sensitivity (98%) and specificity (99%), whereas the sensitivity and specificity of the Techlab and PTAB assays were, respectively, 79 and 98% for the Techlab test, and 80 and 98% for the PTAB. Using the cytotoxicity assay as the gold standard, the PTAB test had the best performance. This result is in agreement with our observation, except for the PPV of the PTAB assay, as elucidated above. In contrast to the results of O'Connor *et al.* (2001), we did not perform a retrospective chart review for patients with a positive test for CDAD. All faecal samples submitted to the laboratories participating in our study were derived from patients with diarrhoea. It is therefore impossible to rule out a positive diagnostic test as a false positive. A second discrepancy of our results compared with the results of O'Connor *et al.* (2001) was the low sensitivity (57%) of the culture method; however, the authors mentioned a number of factors that may have contributed to the relatively poor performance of culture. There are reports indicating that lysozyme incorporation into culture media enhances germination of *C. difficile* spores (Verity *et al.*, 2001; Wilcox *et al.*, 2000). Although this results in an increase in isolation of *C. difficile* from the environment, it is unlikely that more patients will be diagnosed, as vegetative

cells are in the majority in faecal specimens. The application of enrichment media for culturing *C. difficile* from faecal samples is considered unnecessary for the diagnosis of CDAD (Brazier, 1998).

The performance of the diagnostic tests for CDAD for the individual laboratories did not differ from the overall performance of the assays, except for the PTAB assay. At UMC St Radboud, the PPV for the PTAB assay was 100% compared with 55, 63 and 40% at LUMC, VUMC and Erasmus MC, respectively. UMC St Radboud used visual interpretation of results and all other hospitals applied the procedure as recommended by the manufacturer and used an EIA reader at 450 and 630 nm. From this, we conclude that the cut-off values of the PTAB assay need re-evaluation to improve the PPV and NPV.

In some cases, toxinogenic culture is used as the gold standard, instead of the cell cytotoxicity assay (Delmée *et al.*, 2005; Zheng *et al.*, 2004). Therefore, toxinogenic culture was used for discordance analysis on all faecal samples that were positive in one to three assays. The sensitivity of the real-time PCR was 87.1% and this assay showed good concordance (71.4%) with toxinogenic culture. In contrast, the PTAB assay had a concordance of 55.4% with culture. Interestingly, all samples positive by the cell cytotoxicity assay were positive by culture and at least one other assay.

A remarkable finding of our study was the large number of samples positive by immunoassay and negative by cytotoxicity, culture and real-time PCR. Of 19 samples positive only using the PTAB assay in the discordance analysis, 16 were negative by toxinogenic culture. Additionally, nine samples positive only by the VIDAS assay were negative by culture. The results of these immunoassays were therefore considered to be false positives and to contribute to the low PPVs. As mentioned previously, another factor responsible for the low PPV of the immunoassays could be the cut-off values used. Compared with the cell cytotoxicity assay, the area under the ROC curve was very good for the VIDAS assay (0.957) and excellent for the PTAB assay (0.993). This means that both tests, independent of the cut-off values used, correlated well with the gold standard. However, raising the cut-off values to increase the specificity would result in an unacceptable decrease in sensitivity and would therefore not be helpful to increase the PPV.

For 68 (15.1%) of the 450 patients, more than one sample was tested in this study. Of these 68 patients, 45 had two samples and 23 patients had three or more samples included. In total, 97 of 540 samples (18%) were repeat samples. This is considerably lower than the findings of Renshaw *et al.* (1996) and O'Connor *et al.* (2001), who observed 36 and 34% repeat samples, respectively. In two cases, a negative cell cytotoxicity assay was followed by a positive result, and in two other cases, a negative result followed a positive one. For these four cases, the switch was detected by all four assays. The time between these switching results was

9–17 days. It is therefore appropriate to reject repeat specimens from patients who have already been tested on a recent specimen within a 7 day time frame (O'Connor *et al.*, 2001; Renshaw *et al.*, 1996).

In this study, one faecal sample contained a *C. difficile* strain that lacked part of the toxin A gene (TcdA⁻) but contained the gene for toxin B (TcdB⁺). All other cultured isolates from faecal samples were TcdA⁺/TcdB⁺. The faecal sample containing the TcdA⁻/TcdB⁺ strain was only positive by the PTAB assay and by culture of toxinogenic strains. As the VIDAS assay only detects TcdA, this result is explainable. The cell cytotoxicity assay and the real-time PCR should have been able to detect this strain, but the load of this bacterium was apparently under the detection level of the cell cytotoxicity and PCR assays. An increasing number of reports mention TcdA⁻/TcdB⁺ strains associated with diarrhoea (van den Berg *et al.*, 2004). In some hospitals, TcdA⁻/TcdB⁺ strains have completely replaced other types and are now the most prevalent strain (Drudy *et al.*, 2004). Of all *C. difficile* isolates in an Argentinean hospital, the percentage of TcdA⁻/TcdB⁺ isolates increased from 12.5 % in 2000, 58.1 % in 2001 and 87.9 % in 2002 to 96 % in 2003 (van den Berg, *et al.*, 2005b).

Of 251 patients with diarrhoea and hospitalized for at least 72 h, and with a request for CDAD diagnosis, 6.4 % had a positive cell cytotoxicity assay. Of the 121 patients without such a request, 4.1 % were positive. This indicates that routine testing for CDAD in patients with diarrhoea hospitalized for at least 72 h will greatly improve the diagnosis of CDAD. In 75.2 % of patients at the Department of Internal Medicine with diarrhoea, the physician requested a diagnosis of CDAD. Further comparison of the two groups revealed that physicians from the Departments of Surgery, Neurology and Paediatrics considered CDAD less frequently in patients with diarrhoea hospitalized for at least 72 h than diarrhoea due to common community-acquired enteropathogens. This is not unexpected, but emphasizes that more education should be given to physicians of these departments to recognize CDAD in order to treat and prevent the spread of *C. difficile*. Only 1 (0.8 %) of the 121 patients without a request for CDAD diagnosis was positive for *Shigella* spp. This patient was admitted with diarrhoea and the diagnosis of shigellosis was made on a faecal sample submitted on the day of admission. In conclusion, our observation strengthens the suggestion that samples from patients hospitalized for at least 72 h should not be cultured routinely for *Salmonella*, *Shigella* or *Campylobacter* spp. unless there are specific indications (Bowman *et al.*, 1992; Fan *et al.*, 1993; Siegel *et al.*, 1990; Yannelli *et al.*, 1988). Two patients with a request for CDAD were positive for *Campylobacter* spp. They both also had a request for culture of the other enteropathogens and had been admitted to hospital for less than 72 h. This also strengthens the suggestions made. Implementation of this rule in hospitals will significantly decrease the costs of culturing faecal samples for community-acquired pathogens.

Comparing the different rapid assays in this study, PTAB showed the highest sensitivity and NPV, whilst the real-time PCR assay showed the highest concordance with toxinogenic culture in the discordance analysis. The VIDAS assay was outperformed by both PTAB and real-time PCR. Due to the long turnaround time of the cell cytotoxicity assay and toxinogenic culture, rapid assays are advisable in addition. We conclude that real-time PCR is the preferred rapid method for diagnosing CDAD in faecal samples. Additionally, pre-screening using the PTAB method is suggested. Diagnosis of patients with diarrhoea who are hospitalized for more than 72 h should be focused mainly on *C. difficile* detection, irrespective of the physician's request.

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