

European Society of Clinical Microbiology and Infectious Diseases (ESCMID): Data review and recommendations for diagnosing *Clostridium difficile*-infection (CDI)

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Abstract

The aim of the present systematic review was to evaluate the available evidence on laboratory diagnosis of CDI and to formulate recommendations to optimize CDI testing. In comparison with cell culture cytotoxicity assay (CCA) and toxigenic culture (TC) of stools, we analyzed the test characteristics of 13 commercial available enzyme immunoassays (EIA) detecting toxins A and/or B, 4 EIAs detecting *Clostridium difficile* glutamate dehydrogenase (GDH), and a real-time PCR for *C. difficile* toxin B gene. In comparison with CCA and TCA and assuming a prevalence of CDI of 5%, PPV and NPV varied between 0.28–0.77, 0.12–0.65 and 0.98–1.00, 0.97–1.00, respectively. Only if the tests were performed in a population with a CDI prevalence of 50 percent, would PPVs be acceptable (ranging from 0.71 to 1.00). To overcome the problem of a low PPV, we propose a two step approach, with a second test or a reference method in case of a positive first test. Further reducing the number of false negative results would require either retesting of all subjects with a negative first test, or re-testing all subjects with a negative second test, after an initially positive test. This approach resulted in non-significant improvements, and emphasizes the need for better diagnostic tests. Further studies to validate the applicability of two-step testing, including assessment of clinical features, are required.

Keywords: *Clostridium difficile*, systematic review, diagnostics, recommendations, review

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Introduction

Clostridium difficile is the primary cause of nosocomial diarrhoea in industrialized countries. Only toxigenic strains cause disease, due to the production of toxins A and/or B. The incidence and severity of *C. difficile* infections (CDI) have been increasing in recent years [1]. Therefore, the diagnosis of CDI requires renewed attention, as rapid and accurate diagnosis of CDI is not only essential to individual patient management but also to prevention of nosocomial transmission.

The diagnosis of CDI is usually based on the clinical history in combination with laboratory tests. Various laboratory tests are currently available for the detection of *C. difficile* or its toxins [2]. The diagnostic tests for *C. difficile* can be

divided into tests for (i) *C. difficile* products (glutamate dehydrogenase (GDH), aromatic fatty acids, toxins A and/or B), (ii) culture methods for the detection of toxin-producing *C. difficile* (toxigenic culture), and (iii) tests for *C. difficile* genes (PCRs for 16S RNA, toxin genes, genes for GDH). The cell culture cytotoxicity assay (CCA) [3] is still regarded as the reference standard for the detection of *C. difficile* toxins. Culture followed by *in vitro* toxin detection of the isolated strains has been adopted by some investigators as a more sensitive reference standard than CCA [4], though the clinical relevance of this so-called toxigenic culture (TC) is not entirely clear. As the above-mentioned reference standard methods are time-consuming and require specific laboratory facilities and technical expertise, many laboratories have replaced these methods by enzyme immunoassays

[5]. These are rapid and easy-to-perform assays designed to detect *C. difficile* toxins or the enzyme glutamate dehydrogenase, which is produced by both toxigenic and non-toxigenic *C. difficile* strains. Another new development is the application of real-time PCR to detect the toxin genes of *C. difficile* directly from stools [6–10].

Though these rapid and easy tests can be attractive alternatives to the time-consuming reference standards, they have been reported to have limited sensitivity and/or specificity. This has given rise to many different testing protocols, including multiple sample submission [11] or multiple testing of samples by different methods [12–16]. Therefore, we conducted a systematic review to evaluate the diagnostic performance of these tests for CDI. In order to provide a reasonable approach to the diagnosis of CDI, results from this systematic review were used to formulate recommendations for CDI testing in clinical practice.

Objective

The main aim of the present systematic review was to evaluate the available evidence concerning laboratory diagnosis of CDI and to formulate recommendations to optimize CDI testing in patients suspected of CDI.

Material and Methods

Commonly applied laboratory tests

Cell culture cytotoxicity assay (CCA) and toxigenic culture (TC) are regarded as reference standard methods for the diagnosis of CDI. For CCA, stool filtrates are inoculated onto a monolayer of a cell culture which is then observed for a toxin B induced cytopathic effect (rounding of the cells) after 24 and 48 h. Many cell lines can be used for this CCA, including Vero cells, HeLA cells, human foreskin fibroblasts and Hep-2 cells. To determine the specificity of the cytopathic effect, neutralization with an antiserum (*C. sordelli* antitoxin or *C. difficile* antitoxin) is executed. Toxigenic culture consists of culture on selective media followed by *in vitro* toxin detection (by EIA, CCA or PCR) to determine the toxigenicity of the isolated strain. Culture of *C. difficile* is performed on selective media; cycloserine cefoxitin fructose agar (CCFA) is used in most laboratories [17]. Pretreatment with alcohol shock is another alternative to decrease the normal feces flora and to prevent overgrowth [18]. Stools are incubated in an anaerobic atmosphere for at least 48 h.

Enzyme immunoassays (EIAs) available for detection of *C. difficile* or its toxins include EIAs detecting GDH, EIAs

detecting toxin A only, and EIAs detecting both toxins A and B. A panel that includes both an EIA detecting toxin A and an EIA detecting GDH is also available (Triage Panel). EIAs can be subdivided in well-type EIAs (results are displayed as a colour change which can be detected visually or photometrically) and membrane-type EIAs (results can be visually read from a membrane). The single-use membrane-type EIAs are suitable for testing solitary samples; well-type EIAs are suitable for testing samples in batches.

Real-time polymerase chain reaction (RT-PCR) is a nucleic acid amplification technique that can be used to detect the presence of toxin genes directly from stools. Targeted genes are the *tcdB* gene (regulating toxin B production) or the *tcdC* gene (the putative negative regulator of toxin A and B production). Base pair deletions in the *tcdC* gene, thought to be responsible for toxin hyperproduction, can also be detected.

Eligibility criteria

Studies evaluating diagnostic tests for CDI were eligible for inclusion. Studies had to: (i) describe original research; (ii) compare the index test to a reference standard (CCA or TC); (iii) be performed on clinical human stool samples; and (iv) report sufficient information to allow us to calculate sensitivities and specificities. If assays to detect toxin and non-toxin producing *C. difficile* were evaluated (e.g. GDH tests), culture without further characterization was also accepted as a standard.

We excluded studies if: (i) the reference test was not performed on all samples, but only on positive, negative or discordant samples (to exclude partial verification bias); (ii) the index test was partly used as a reference standard; (iii) the reference standard included clinical data or was a composite of more than one test; (iv) not all samples were tested by the same reference test; or (v) in studies using CCA as a reference test, neutralization to determine the specificity of the cytopathic effect was not executed.

Tests were included in the analysis only if they were still available. They were categorized as well-type enzyme immunoassays (EIAs) for detection of toxins A and/or B, membrane-type EIAs for detection of toxins A and/or B, well-type EIAs for detection of glutamate dehydrogenase (GDH), membrane-type EIAs for detection of GDH, and nucleic acid amplification techniques, such as Real-time PCR.

Search strategy

We searched Pubmed, EMBASE, Web of Science, CINAHL and the Cochrane Library for studies concerning the laboratory diagnosis of *C. difficile* infections. Searches were performed with the support of a librarian, using the search strategy displayed in Appendix S1. In addition, we checked

the references of relevant studies for additional articles. Searches were performed in July 2008 and in July 2009. Meeting abstracts were excluded. Only articles published in the English language were included.

Data review and outcome measures

Two independent investigators (M.J.T.C and A.G) assessed the study eligibility in a two-step selection process. Inconsistencies were resolved by consensus. Data were extracted using a prespecified data extraction form, as shown in Appendix S2.

Sensitivity and specificity of different tests for detection of CDI were chosen as the principal measures of outcome. To be able to calculate these test characteristics, we extracted the number of true positives, true negatives, false positives and false negatives for each test under investigation, thereby comparing the index test to the reference standard.

We created a quality assessment tool (Appendix S3) which included items based on the 'quality assessment for studies of diagnostic accuracy' (QUADAS) tool [19] and items concerning the execution of the reference standard. Diagnostic odds ratios were not calculated [20]. We extracted additional information including year of publication and information about the study population, storage conditions of stool samples, and execution of the reference standard. In cases of incomplete data, the original authors were contacted if possible.

The levels of evidence and grades of recommendation were determined using the criteria in Appendix S5, which is the standard of the Dutch Institute for Healthcare Improvement CBO in The Netherlands.

Statistical analysis

We analyzed the test characteristics of EIAs detecting toxins A and/or B and RT-PCR in comparison with CCA and TC. We analyzed the test characteristics of EIAs detecting GDH both in comparison with both CCA and TC (considering GDH as a screening test for toxigenic *C. difficile*) as well as in comparison with culture (C) only (considering GDH as an alternative for culture). For all index tests, sensitivity, specificity, and their confidence intervals were calculated from the data supplied in each study. Sensitivity and specificity were pooled in a fixed effects model. The proportions were weighted according to the inverse of the squared standard error. Only data from the initial test results were used, unless data were presented after repeated testing (eight studies). Intermediate results were not included in calculations of sensitivity and specificity. We calculated positive and negative predictive values (PPVs and NPVs), using hypothetical CDI prevalences of 5%, 10%, 20% and 50% in the tested

population. We used the weighted mean of the NPVs and PPVs by combining individual studies assessing a specific index test. For data processing and statistical analysis, we used REVIEW MANAGER 5.0 software (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2008).

Results

Literature search

We identified 847 unique citations by search in Pubmed, EMBASE, Web of Science, CINAHL and the Cochrane Library. One additional study was identified by an expert. A summary of the selection process is shown in Fig. 1. We excluded 777 papers on the basis of title and abstract. Of the 71 potentially relevant papers retrieved for more detailed assessment, 28 were excluded from further analysis: 15 studies investigated tests that were no longer commercially available; three studies did not test all samples with the reference test; three studies used an inappropriate reference standard; two studies comprised non-clinical human stool samples; two studies did not report sufficient information to allow us to calculate sensitivity and specificity; one study using CCA did not report if neutralization of the cytopathic effect was executed; and for two studies, full text articles were not available. Therefore, a total of 43 studies was included in this systematic review [6–10,12–15,21–23,23–54].

Study characteristics

In total, 18 different diagnostic assays were investigated (Table 1): 13 commercially available EIAs for detection of toxins A and/or B (34 studies); four commercially available EIAs for detection of GDH (14 studies); and one RT-PCR (four studies). Details of the 43 included studies are summarized in Table 2. The studies were published between 1991

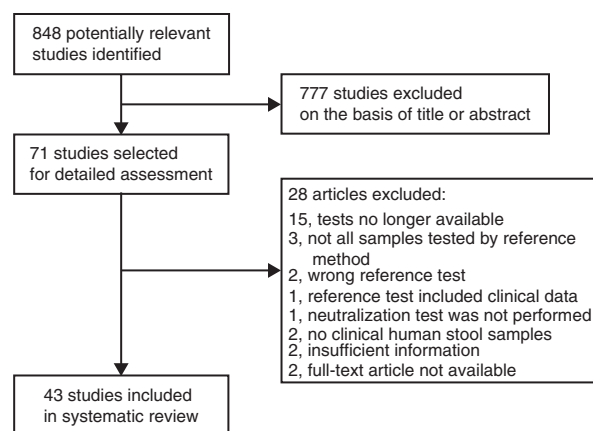


FIG. 1. Summary of selection process.

and 2008 and the number of included samples ranged from 56 to 1468. Cytotoxicity assay was the reference test of choice in the majority of studies (85%).

Quality assessment

For each study, the items of the quality assessment form were scored (Appendix S4).

In 22/43 studies, one or more selection criteria (consistency of samples, age of patients, suspicion of CDI) were not described. Twenty-five of 43 studies (58%) included unformed stool samples only, none of 43 studies (21%) included both unformed and formed stool samples, and nine studies (21%) did not report the consistency of tested samples. Blinding of investigators was reported in 12/43 studies. Intermediate test results (i.e. not interpretable, difficult to interpret, invalid or weak positive results) were reported in 17/43 studies (40%), including four membrane-type EIAs (six studies using the Triage assay [13,14,30,31], the ColorPac Toxin A assay [31], and/or the Immunocard *C. difficile* assay [39,54]). Repeated testing of samples with an initial intermediate result was performed in nine studies. Of these, eight studies reported results only after repeated testing [31,33,43–46,53,54] and one study reported the results of both initial and repeated testing [48]. In one study, samples with intermediate results after repeated testing were called negative [53].

In 10/37 studies using CCA as the reference test, storage conditions of the samples before testing with CCA were not reported or insufficiently reported. In the remaining 27 studies, samples were refrigerated at 2–8°C (16/26 studies), frozen at –20 till –80°C (8/26 studies) or partly refrigerated and partly frozen (2/26 studies). All samples stored at 4°C were tested within 72 h. Studies in which (part of) the samples were frozen (6/10) reported that samples were thawed

only once; one study reported that samples were thawed no more than twice. Commercial CCAs were used in 9/37 studies; in-house CCAs were used in 30/37 studies. The most frequently used cell lines were Vero cells (12 studies), MRC-5 cells (eight studies), and HFF cells (six studies).

Test performances

Calculated sensitivities and specificities of index tests are presented in Tables 3A–C. Discrepancies between the published data and the recalculated data were observed in one study; the specificity was incorrectly calculated as confirmed by the original author [12]. In Fig. 3a, index tests are compared with CCA as a reference standard. Sensitivities ranged from 0.31 to 0.99 for EIAs detecting toxins A and/or B, from 0.80 to 0.97 for EIAs detecting GDH and from 0.87 to 1.00 for RT-PCR. Specificities ranged from 0.65 to 1.00 for EIAs detecting toxins A and/or B, from 0.75 to 0.97 for EIAs detecting GDH, and from 0.94 to 1.00 for RT-PCR. No differences were observed among studies in which samples were stored at 4°C or frozen, or in which storage conditions were not described (data not shown). It is therefore tempting to speculate that samples in these studies were appropriately stored. In Fig. 3b, index tests are compared with toxigenic culture as the reference standard. Sensitivities ranged from 0.32 to 0.79 for EIAs detecting toxins A and/or B. The sensitivity of Immunocard *C. difficile* was 0.60; the sensitivity of RT-PCR was 0.86. Specificities ranged from 0.84 to 1.00 for EIAs detecting toxin A and/or B. The specificity of Immunocard *C. difficile* was 0.76; the specificity of RT-PCR was 0.97. In Fig. 3c, GDH EIAs are compared with culture as a reference standard. Sensitivities ranged from 0.71 to 1.00. Specificities ranged from 0.67 to 0.99.

Type	Test	Target	Manufacturer
A: well-type EIA toxins A and/or B	<i>Clostridium difficile</i> tox A/B II	Toxins A and B	Techlab
	Premier tox A	Toxin A	Meridian
	Premier tox A/B	Toxins A and B	Meridian
	ProSpecT A/B	Toxins A and B	Remel
	Ridascreen A/B	Toxins A and B	R-Biopharm
B: membrane-type EIA toxins A/B	<i>C. difficile</i> tox A test	Toxin A	Oxoid
	Clearview tox A	Toxin A	Oxoid
	ColorPac toxin A	Toxin A	Becton Dickinson
	Immunocard tox A/B	Toxins A and B	Meridian
	Immunocard tox A	Toxin A	Meridian
	Triage tox A	Toxin A	Biosite
	Tox A/B Quik Chek	Toxins A and B	Techlab
	Xpect A/B	Toxins A and B	Remel
	<i>C. difficile</i> chek-60	GDH	Techlab
C: well-type EIAs GDH D: membrane-type EIAs GDH	Immunocard <i>C. difficile</i>	GDH	Meridian
	<i>C. difficile</i> quik chek	GDH	Techlab
E: RT-PCR	Triage GDH	GDH	Biosite
	–	tcdB or tcdC	–

EIA, enzyme immunoassay; RT-PCR, real-time polymerase chain reaction.

TABLE 1. Tests included in the analysis

TABLE 2. Characteristics of included studies

Author	Year	Reference test	Index test(s)	Total no. Samples	Study population	Prev. CDI (%)
Miendje Deyi et al.	2008	CCA	Immunocard tox A/B, Xpect CD toxin A/B, CD toxin A test, Tox A/B Quik Chek	100	unformed stools suspected of CDI, age >65 yrs	23
Sloan et al.	2008	TC	Premier tox A/B, Xpect <i>C. difficile</i> toxin A/B, Immunocard A/B, Triage tox A, RT-PCR	200	unformed stools suspected of CDI	22
Fenner et al.	2008	C	<i>C. difficile</i> chek-60	1468	all stools suspected of CDI, adults	5
		TC	Tox A/B Quik Chek	172	all GDH-positive stools, adults	60
Reyes et al.	2007	C	Triage GDH, <i>C. difficile</i> quik chek	401	unformed stools suspected of CDI	17
Reller et al.	2007	C	<i>C. difficile</i> chek-60	439	stools suspect of CDI, all ages	26
Musher et al.	2007	CCA	Premier tox A/B, Immunocard A/B	446	all stools suspected of CDI, adults	17
		CCA	<i>C. difficile</i> tox A/B II, ProSpecT A/B	131	all stools suspected of CDI, adults	41
Van den Berg et al.	2007	CCA	Premier tox A/B, RT-PCR	540	unformed stools suspected of CDI, all unformed stools after >72h admission, adults	6
Ticehurst et al.	2006	CCA	<i>C. difficile</i> chek-60	266	stools suspected of CDI	9
Van den Berg et al.	2006	CCA	RT-PCR	85	all unformed stools after >72h admission, adults	7
Van den Berg et al.	2005	CCA	Immunocard tox A/B	367	unformed stools suspected of CDI, all unformed stools after >72h admission, adults	6
Snell et al.	2004	C	<i>C. difficile</i> chek-60, Triage-GDH	497	unformed stools suspect of CDI	19
		TC	Triage-Tox A, <i>C. difficile</i> tox A/B II			
Zheng et al.	2004	C, CCA	<i>C. difficile</i> chek-60	992	all stools suspected of CDI, all ages	14
Anderson et al.	2003	CCA	Clearview tox A	166	unformed stools suspected of CDI, all unformed stools after >72h admission	8
Bélangier et al.	2003	CCA	RT-PCR	56	unformed stools suspect of CDI	52
Turgeon et al.	2003	CCA	Immunocard <i>C. difficile</i> , Immunocard tox A, Triage panel	1003	all stools suspected of CDI, all ages	10
Massey et al.	2003	CCA	Triage panel, <i>C. difficile</i> tox A/B II	557	unfomed stools suspected of CDI, adults	26
Alfa et al.	2002	CCA	Triage panel	400	unfomed stools suspected of CDI	15
O'Connor et al.	2001	CCA	Immunocard tox A, CD toxin A test, <i>C. difficile</i> tox A/B I I, Premier tox A/B	200	stools suspected of CDI, adults	31
Landry et al.	2001	CCA	Triage panel	90	unfomed stools suspected of CDI, adults	18
Vanpoucke et al.	2001	CCA	Ridascreen tox A/B, Clearview tox A, Colorpac, Triage panel	156	unfomed stools suspected of CDI	32
Patel et al.	2001	CCA	Clearview tox A, Immunocard tox A	537	unfomed stools suspected CDI, adults	9
Barbut et al.	2000	C	Triage-GDH	304	unformed stools suspected of CDI, adults	25
		CCA	Triage panel			
Fedorko et al.	1999	CCA, TC	Immunocard tox A	654	all stools suspected of CDI	10
Fille et al.	1998	CCA	CD toxin A test	105	all stools suspected of CDI, all ages	21
Bentley et al.	1998	CCA	Clearview tox A	407	unformed stools suspected of CDI	29
Barbut et al.	1997	CCA, TC	Immunocard tox A	236	stool suspected of CDI	14
Jacobs et al.	1996	TC, C	Immunocard <i>C. difficile</i>	150	unformed stools suspected of CDI, all ages	12
		TC	Premier tox A	258	unformed stools suspected of CDI, all ages	8
Staneck et al.	1996	CCA	Immunocard <i>C. difficile</i>	906	unclear	14
Siarakas et al.	1996	CCA	Premier tox A	184	unformed stools suspected of CDI, all unformed stools after >72h admission	20
Langley et al.	1995	CCA	Premier tox A	200	unformed stools suspected of CDI	20
Arrow et al.	1994	CCA	Premier tox A	160	unclear	33
Bowman et al.	1994	CCA	Premier tox A	314	unclear	21
Merz et al.	1994	CCA	Premier tox A	699	stools suspected of CDI	8
Altaie et al.	1994	CCA	Premier tox A	410	unformed stools suspected of CDI, age 2-28 yrs	19
Whittier et al.	1993	CCA	Premier tox A	326	unformed stools suspected of CDI, all ages	10
Knapp et al.	1993	CCA	Premier tox A	301	formed and unformed stools	16
Gilligan et al.	1993	CCA	Premier tox A	559	stools suspected of CDI	6
Barbut et al.	1993	CCA, TC	Premier tox A	284	stools suspected of CDI	18
Doern et al.	1992	CCA	Premier tox A	315	stools suspected of CDI	22
DeGirolami	1992	CCA	Premier tox A	496	stools suspected of CDI, all ages	16
Borriello et al.	1992	CCA	Premier tox A	101	unformed stools suspected of CDI	68
Delmée et al.	1992	CCA, TC	Premier tox A	228	unformed stools suspected of CDI, all ages	29
DiPersio et al.	1991	CCA, TC	Premier tox A	313	stools suspected of CDI	14

CCA, cell cytotoxicity assay; TC, toxigenic culture; C, culture; CDI, *Clostridium difficile* infection; Prev., prevalence; RT-PCR, real-time polymerase chain reaction.

No important differences in test characteristics were observed among studies that tested unformed samples only or studies that tested both formed and unformed stool samples.

Table 4 shows calculated mean sensitivities and specificities for all types of index tests compared with CCA (Table 4A), toxigenic culture (Table 4B), and culture

(Table 4C). Sensitivities ranged from 0.72 to 0.93 in comparison with CCA, from 0.52 to 0.86 in comparison with toxigenic culture, and from 0.88 to 0.89 in comparison with culture. Specificities ranged from 0.89 to 0.98 in comparison with CCA, from 0.76 to 0.98 in comparison with toxigenic culture, and from 0.91 to 0.97 in comparison with culture.

TABLE 3A. Calculated sensitivities and specificities compared to cell cytotoxicity assay

Type	Index test	Study	Sensitivity (95% CI)	Specificity (95% CI)	
Well-type EIA toxin A/B	<i>Clostridium difficile</i> tox A/B II	Massey 2003	0.75 (0.67, 0.82)	0.98 (0.96, 0.99)	
	<i>C. difficile</i> tox A/B II	Musher 2007	0.96 (0.87, 1.00)	0.87 (0.77, 0.94)	
	Premier tox A	Knapp 1993	0.65 (0.50, 0.78)	0.95 (0.92, 0.98)	
	Premier tox A	Doern 1992	0.69 (0.56, 0.79)	1.00 (0.99, 1.00)	
	Premier tox A	Borriello 1992	0.71 (0.59, 0.81)	1.00 (0.89, 1.00)	
	Premier tox A	Siarakas 1996	0.72 (0.55, 0.86)	0.87 (0.81, 0.92)	
	Premier tox A	Barbut 1993	0.73 (0.58, 0.84)	1.00 (0.98, 1.00)	
	Premier tox A	Bowman 1994	0.78 (0.66, 0.87)	1.00 (0.98, 1.00)	
	Premier tox A	DiPersio 1991	0.84 (0.70, 0.93)	0.99 (0.97, 1.00)	
	Premier tox A	Whittier 1993	0.84 (0.67, 0.95)	0.97 (0.94, 0.99)	
	Premier tox A	DeGirolami 1992	0.85 (0.75, 0.92)	0.98 (0.96, 0.99)	
	Premier tox A	Arrow 1994	0.85 (0.72, 0.93)	0.99 (0.95, 1.00)	
	Premier tox A	Langley 1995	0.87 (0.73, 0.96)	0.96 (0.92, 0.99)	
	Premier tox A	Merz 1994	0.87 (0.75, 0.95)	0.98 (0.96, 0.99)	
	Premier tox A	Delmee 1992	0.88 (0.77, 0.95)	0.96 (0.91, 0.99)	
	Premier tox A	Gilligan 1993	0.89 (0.78, 0.96)	0.97 (0.95, 0.98)	
	Premier tox A	Altaie 1994	0.93 (0.85, 0.98)	1.00 (0.99, 1.00)	
	Premier tox A/B	O'Connor 2001	0.82 (0.70, 0.91)	0.99 (0.96, 1.00)	
	Premier tox A/B	Van den Berg 2007	0.97 (0.83, 1.00)	0.94 (0.92, 0.96)	
	Premier tox A/B	Musher 2007	0.99 (0.93, 1.00)	0.97 (0.95, 0.99)	
	ProSpecT A/B	Musher 2007	0.91 (0.80, 0.97)	0.97 (0.91, 1.00)	
	Ridascreen A/B	Vanpoucke 2001	0.57 (0.43, 0.70)	0.97 (0.92, 0.99)	
	Membrane-type EIA toxin A/B	<i>C. difficile</i> tox A test	O'Connor 2001	0.49 (0.36, 0.62)	0.99 (0.96, 1.00)
		<i>C. difficile</i> tox A test	Fills 1998	0.68 (0.45, 0.86)	0.84 (0.75, 0.91)
		<i>C. difficile</i> tox A test	Miendje Deyi 2008	0.87 (0.66, 0.97)	0.99 (0.93, 1.00)
		Clearview tox A	Patel 2001	0.70 (0.55, 0.83)	0.99 (0.97, 1.00)
		Clearview tox A	Bentley 1998	0.83 (0.75, 0.89)	0.97 (0.94, 0.99)
		Clearview tox A	Anderson 2003	0.85 (0.55, 0.98)	0.65 (0.57, 0.73)
		Clearview tox A	Vanpoucke 2001	0.89 (0.78, 0.96)	0.83 (0.75, 0.90)
		ColorPac toxin A	Vanpoucke 2001	0.89 (0.78, 0.96)	0.89 (0.82, 0.94)
Immunocard tox A/B		Van den Berg 2005	0.91 (0.72, 0.99)	0.97 (0.95, 0.99)	
Immunocard tox A/B		Miendje Deyi 2008	0.91 (0.72, 0.99)	1.00 (0.95, 1.00)	
Immunocard tox A/B		Musher 2007	0.96 (0.89, 0.99)	0.99 (0.97, 1.00)	
Immunocard tox A		O'Connor 2001	0.52 (0.39, 0.65)	1.00 (0.97, 1.00)	
Immunocard tox A		Turgeon 2003	0.56 (0.46, 0.66)	1.00 (0.99, 1.00)	
Immunocard tox A		Fedorko 1999	0.58 (0.46, 0.70)	0.99 (0.97, 0.99)	
Immunocard tox A		Patel 2001	0.74 (0.60, 0.86)	0.99 (0.98, 1.00)	
Immunocard tox A		Barbut 1997	0.82 (0.65, 0.93)	1.00 (0.98, 1.00)	
Triage tox A		Landry 2001	0.31 (0.11, 0.59)	1.00 (0.95, 1.00)	
Triage tox A		Alfa 2002	0.53 (0.40, 0.67)	1.00 (0.99, 1.00)	
Triage tox A		Turgeon 2003	0.59 (0.49, 0.69)	1.00 (0.99, 1.00)	
Triage tox A		Massey 2003	0.70 (0.62, 0.77)	0.99 (0.98, 1.00)	
Triage tox A		Vanpoucke 2001	0.77 (0.64, 0.87)	0.97 (0.91, 0.99)	
Triage tox A		Barbut 2000	0.79 (0.62, 0.91)	1.00 (0.98, 1.00)	
Tox A/B QuikChek		Miendje Deyi 2008	0.96 (0.78, 1.00)	1.00 (0.95, 1.00)	
XpexT A/B		Miendje Deyi 2008	0.91 (0.72, 0.99)	1.00 (0.95, 1.00)	
Well-type EIA GDH		<i>C. difficile</i> chek-60	Zheng 2004	0.93 (0.87, 0.96)	0.89 (0.87, 0.91)
		<i>C. difficile</i> chek-60	Tichehurst 2006	0.96 (0.79, 1.00)	0.90 (0.86, 0.94)
		Immunocard <i>C. difficile</i>	Turgeon 2003	0.80 (0.71, 0.87)	0.92 (0.91, 0.94)
		Immunocard <i>C. difficile</i>	Staneck 1996	0.84 (0.77, 0.90)	0.92 (0.90, 0.94)
		Triage GDH	Turgeon 2003	0.89 (0.81, 0.94)	0.90 (0.88, 0.92)
		Triage GDH	Alfa 2002	0.91 (0.81, 0.97)	0.89 (0.85, 0.92)
	Triage GDH	Vanpoucke 2001	0.93 (0.83, 0.98)	0.75 (0.65, 0.83)	
	Triage GDH	Landry 2001	0.94 (0.70, 1.00)	0.82 (0.72, 0.90)	
	Triage GDH	Barbut 2000	0.97 (0.85, 1.00)	0.86 (0.81, 0.90)	
	Triage GDH	Massey 2003	0.97 (0.93, 0.99)	0.86 (0.83, 0.89)	
Real-time PCR	RT-PCR	Van den Berg 2007	0.87 (0.70, 0.96)	0.96 (0.94, 0.98)	
	RT-PCR	Belanger 2003	0.97 (0.82, 1.00)	1.00 (0.87, 1.00)	
	RT-PCR	Van den Berg 2006	1.00 (0.54, 1.00)	0.94 (0.86, 0.98)	

RT-PCR, real-time polymerase chain reaction.

Negative and positive predictive values

In Table 5, the calculated mean sensitivities, specificities, PPVs and NPVs of the index tests are compared with reference standards CCA (Table 5A), toxigenic culture (Table 5B) and culture (Table 5C). PPVs ranged from 0.12 to 1.00 at a prevalence of 5%. At a prevalence of 50%, PPVs ranged from 0.71 to 1.00. NPVs ranged from 0.97 to 1.00 at a prevalence of 5%. At a prevalence of 50%, NPVs ranged from 0.62 to 0.96.

Discussion

In the present systematic review, we evaluated the diagnostic accuracy of various tests for the diagnosis of CDI. We observed no important differences in sensitivities and specificities between well-type or membrane-type EIAs, although EIAs detecting toxins A and/or B tended to be less sensitive than the other types of tests. When EIAs for detection of

TABLE 3B. Calculated sensitivities and specificities for index tests compared with toxigenic culture

Type	Index test	Study	Sensitivity (95% CI)	Specificity (95% CI)
Well-type EIA toxin A/B	<i>Clostridium difficile</i> tox A/B II	Snell 2004	0.60 (0.48, 0.72)	0.98 (0.96, 0.99)
	Premier tox A	Jacobs 1996	0.61 (0.36, 0.83)	0.98 (0.96, 1.00)
	Premier tox A	Barbut 1993	0.65 (0.51, 0.78)	0.99 (0.97, 1.00)
	Premier tox A	Gilligan 1993	0.71 (0.59, 0.81)	0.97 (0.94, 0.98)
	Premier tox A	Delmee 1992	0.72 (0.61, 0.81)	1.00 (0.97, 1.00)
	Premier tox A	DiPersio 1991	0.79 (0.64, 0.90)	0.98 (0.95, 0.99)
	Premier tox A/B	Sloan 2008	0.48 (0.32, 0.63)	0.98 (0.94, 1.00)
Membrane-type EIA toxin A/B	Immunocard tox A	Fedorko 1999	0.56 (0.45, 0.68)	0.99 (0.98, 1.00)
	Immunocard tox A	Barbut 1997	0.77 (0.60, 0.90)	1.00 (0.98, 1.00)
	Immunocard tox A/B	Sloan 2008	0.48 (0.32, 0.63)	0.99 (0.95, 1.00)
	Tox A/B Quik Chek	Fenner 2008	0.53 (0.43, 0.63)	0.97 (0.90, 1.00)
	Triage tox A	Sloan 2008	0.32 (0.19, 0.48)	1.00 (0.98, 1.00)
	Triage tox A	Snell 2004	0.49 (0.37, 0.61)	1.00 (0.99, 1.00)
	XpecT A/B	Sloan 2008	0.48 (0.32, 0.63)	0.84 (0.77, 0.89)
	Immunocard <i>C. difficile</i>	Jacobs 1996	0.60 (0.32, 0.84)	0.76 (0.68, 0.83)
Well-type EIA GDH	Immunocard <i>C. difficile</i>	Jacobs 1996	0.60 (0.32, 0.84)	0.76 (0.68, 0.83)
Real-time PCR	RT-PCR	Sloan 2008	0.86 (0.73, 0.95)	0.97 (0.94, 0.99)

RT-PCR, real-time polymerase chain reaction.

TABLE 3C. Calculated sensitivities and specificities for index tests compared with culture of *Clostridium difficile*

Type	Index test	Study	Sensitivity (95% CI)	Specificity (95% CI)
Well-type EIA GDH	<i>C. difficile</i> chek-60	Zheng 2004	0.71 (0.63, 0.78)	0.88 (0.85, 0.90)
	<i>C. difficile</i> chek-60	Fenner 2008	0.93 (0.88, 0.97)	0.97 (0.95, 0.97)
	<i>C. difficile</i> chek-60	Snell 2004	0.94 (0.86, 0.98)	0.98 (0.96, 0.99)
	<i>C. difficile</i> chek-60	Reller 2007	1.00 (0.98, 1.00)	0.67 (0.61, 0.72)
	<i>C. difficile</i> quik chek	Reyes 2007	0.93 (0.84, 0.98)	0.95 (0.92, 0.97)
Membrane-type EIA GDH	Immunocard <i>C. difficile</i>	Jacobs 1996	0.75 (0.59, 0.87)	0.90 (0.83, 0.95)
	Triage GDH	Snell 2004	0.85 (0.76, 0.92)	0.99 (0.97, 1.00)
	Triage GDH	Barbut 2000	0.91 (0.82, 0.96)	0.99 (0.96, 1.00)
	Triage GDH	Reyes 2007	0.93 (0.83, 0.98)	0.98 (0.95, 0.99)

TABLE 4A. Mean sensitivities and specificities for the different types of index tests compared with cytotoxicity assay

Type (no. studies)	Sensitivity (95% CI)	Specificity (95% CI)
Well-type EIA toxin A/B (22)	0.82 (0.79, 0.84)	0.97 (0.97, 0.98)
Membrane-type EIA toxin A/B (24)	0.72 (0.69, 0.74)	0.98 (0.97, 0.98)
Well-type EIA GDH (2)	0.93 (0.88, 0.97)	0.89 (0.87, 0.91)
Membrane-type EIA GDH (8)	0.90 (0.87, 0.92)	0.90 (0.88, 0.90)
Real-time PCR (3)	0.92 (0.83, 0.97)	0.96 (0.94, 0.98)

TABLE 4B. Mean sensitivities and specificities for the different types of index tests compared with toxigenic culture

Type (no. studies)	Sensitivity (95% CI)	Specificity (95% CI)
Well-type EIA toxin A/B (7)	0.66 (0.61, 0.71)	0.98 (0.97, 0.99)
Membrane-type EIA toxin A/B (7)	0.52 (0.47, 0.57)	0.98 (0.97, 0.99)
Membrane-type EIA GDH (1)	0.60 (0.32, 0.83)	0.76 (0.68, 0.83)
Real-time PCR (1)	0.86 (0.73, 0.95)	0.97 (0.94, 0.99)

GDH were compared with tests for the presence of toxigenic *C. difficile*, they were less specific than EIAs detecting toxins A and/or B or real-time PCR; this can be explained by

TABLE 4C. Mean sensitivities and specificities for the different types of index tests compared with culture of *Clostridium difficile*

Type (no. studies)	Sensitivity (95% CI)	Specificity (95% CI)
Well-type EIA GDH (4)	0.89 (0.86, 0.91)	0.91 (0.90, 0.92)
Membrane-type EIA GDH (5)	0.88 (0.84, 0.91)	0.97 (0.96, 0.98)

the fact that EIAs for GDH also detect non-toxigenic strains of *C. difficile*. The specificity of EIA for the detection of GDH increased when they were compared with culture including non-toxigenic strains.

As sensitivity and specificity are test characteristics but do not take into account the different prevalences in the tested population, we used different hypothetical prevalences for calculations of negative predictive values (NPVs) and positive predictive values (PPVs). The prevalence of a disease, for example the prevalence of CDI among tested patients, can be seen as the prior probability that a tested patient actually has the disease. After having obtained a test result, knowledge of sensitivity and specificity, as well as prior probability, can be used to determine the posterior probability of

a test result. The posterior probability of a positive test result (PPV) is the probability that a person has the disease, given the positive result. In a tested population with a low prior probability for the disease, a positive test result will be only a proof of the disease only if the test is free of false positives (specificity of 100%). Otherwise the posterior probability (PPV) will be only 50%, even in the case of a high sensitivity, as can be seen in Table 5. A low PPV is associated with unnecessary treatment of non-infected patients. On the other hand, the NPV indicates the proportion of true negatives among patients with a negative test result. A low NPV will mean that many cases are not detected. This will lead to insufficient treatment and spread of infection as isolation measures may not be imposed. To overcome the problem of a low PPV, we propose a two-step approach, with a second test in case of a positive first test (Figs 2 and 3). Laboratories can choose to use a toxin test or an assay directed to a bacterial gene or enzyme. We performed a calculation to assess the effect of a two-step approach with the following assumptions: a sample of 10 000 tested patients and a prevalence of 5%. Two tests were performed: (i) Triage GDH (sensitivity 0.94, specificity 0.87); and (ii) in case of a positive test, Premier Tox A/B was performed (sensitivity 0.92, specificity 0.96). This would lead to true negative test results in 9451 patients, true positive test results in 432, false negative results in 68, and false positive results in 49. In total, among all tested 10 000 patients, the test result will be incorrect in c. 1% of the population. While this is a low overall error rate, the consequences for individual patients could be severe. Notably, false positive diagnoses could result in a

patient without true CDI being managed alongside real cases, potentially resulting in cross-infection.

Many laboratories have implemented EIAs detecting toxins A and B, as stand-alone tests for the diagnosis of CDI [5]. We found that these EIAs were quite specific, but less sensitive in detecting CDI. Only when the tests were performed in a population with a CDI prevalence of 50%, PPVs would be acceptable (ranging from 0.75 to 1.00) due to their high specificity. However, in an endemic situation, the prevalence of CDI is expected to range between 5% and 10%. Due to this low prevalence of disease, PPVs of most of these tests would be unacceptably low. Only the most specific tests (XpecT A/B and Tox A/B Quik Chek) would have acceptable PPVs at these low prevalences, but these tests have been evaluated in one study each and more data are required before the recommendation obtains a grade of I. As EIAs detecting GDH were less specific than most EIAs detecting toxins A and/or B and RT-PCR, their PPVs would be even lower, both in high-risk and endemic situations.

We conclude that all currently available types are not suitable as stand-alone tests to diagnose CDI in endemic populations because of their low PPVs at these prevalences. Since the NPVs of the tests were very acceptable at low prevalences, these tests (i.e. EIAs detecting toxins A and/or B, EIAs detecting GDH, and real-time PCR) can be used as screening tests in an endemic situation, with the emphasis on a negative test result. When a negative test result is obtained, CDI can be very reliably excluded. However, when a positive test result is obtained, a confirmatory test must be performed to recognize a truly positive sample. There

TABLE 5A. Calculated PPVs and NPVs for the index tests compared with cell culture cytotoxicity assay at prevalence rates of 5, 10, 20 and 50% in the tested population

Type	Index test (no. studies)	Sensitivity	Specificity	Prevalence 5%		Prevalence 10%		Prevalence 20%		Prevalence 50%	
				PPV	NPV	PPV	NPV	PPV	NPV	PPV	NPV
Well-type EIA toxin A/B	<i>Clostridium difficile</i> tox A/B II (2)	0.81	0.96	0.52	0.99	0.69	0.98	0.84	0.95	0.95	0.83
	Premier tox A (15)	0.81	0.98	0.68	0.99	0.82	0.98	0.91	0.95	0.98	0.84
	Premier tox A/B (3)	0.92	0.96	0.55	1.00	0.72	0.99	0.85	0.98	0.96	0.92
	ProSpecT A/B (1)	0.91	0.97	0.61	1.00	0.77	0.99	0.88	0.98	0.97	0.92
	Ridascreen <i>C. difficile</i> tox A/B (1)	0.57	0.97	0.50	0.98	0.68	0.95	0.83	0.90	0.95	0.69
Membrane-type EIA toxin A/B	<i>C. difficile</i> tox A test (3)	0.61	0.95	0.39	0.98	0.58	0.96	0.75	0.91	0.92	0.71
	Clearview tox A (4)	0.82	0.92	0.35	0.99	0.53	0.98	0.72	0.95	0.91	0.84
	ColorPac toxin A(1)	0.89	0.89	0.30	0.99	0.47	0.99	0.67	0.97	0.89	0.89
	Immunocard tox A/B (3)	0.94	0.98	0.71	1.00	0.84	0.99	0.92	0.98	0.98	0.94
	Immunocard tox A (5)	0.61	0.99	0.76	0.98	0.87	0.96	0.94	0.91	0.98	0.72
	Triage tox A (6)	0.65	0.99	0.77	0.98	0.88	0.96	0.94	0.92	0.98	0.74
	Tox A/B Quik Chek(1)	0.96	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	0.96
	XpecT A/B(1)	0.91	1.00	1.00	1.00	1.00	0.99	1.00	0.98	1.00	0.92
Well-type EIA GDH	<i>C. difficile</i> Chek-60 (2)	0.93	0.89	0.31	1.00	0.48	0.99	0.68	0.98	0.89	0.93
Membrane-type EIA GDH	Immunocard <i>C. difficile</i> (2)	0.83	0.92	0.35	0.99	0.54	0.98	0.72	0.96	0.91	0.84
	Triage GDH (6)	0.94	0.87	0.28	1.00	0.45	0.99	0.64	0.98	0.88	0.94
Real-time PCR	RT-PCR (3)	0.92	0.96	0.55	1.00	0.72	0.99	0.85	0.98	0.96	0.92

RT-PCR, real-time polymerase chain reaction.

TABLE 5B. Calculated PPVs and NPVs for the index tests compared with toxigenic culture at prevalence rates of 5, 10, 20 and 50% in the tested population

Type	Index test (no. studies)	Sensitivity	Specificity	Prevalence 5%		Prevalence 10%		Prevalence 20%		Prevalence 50%	
				PPV	NPV	PPV	NPV	PPV	NPV	PPV	NPV
Well-type EIA toxin A/B	<i>Clostridium difficile</i> tox A/B 11(1)	0.60	0.98	0.61	0.98	0.77	0.96	0.88	0.91	0.97	0.71
	Premier tox A (5)	0.71	0.98	0.65	0.98	0.80	0.97	0.90	0.93	0.97	0.77
	Premier tox A/B (1)	0.48	0.98	0.56	0.97	0.73	0.94	0.86	0.88	0.96	0.65
Membrane-type EIA toxin A/B	Immunocard tox A (2)	0.63	1.00	1.00	0.98	1.00	0.96	1.00	0.92	1.00	0.73
	Immunocard A/B (1)	0.48	0.99	0.72	0.97	0.84	0.95	0.92	0.88	0.98	0.66
	Tox A/B Quik Chek(1)	0.53	0.97	0.48	0.98	0.66	0.95	0.82	0.89	0.95	0.67
	Triage tox A (2)	0.43	1.00	1.00	0.97	1.00	0.94	1.00	0.88	1.00	0.64
	Xpect A/B(1)	0.48	0.84	0.14	0.97	0.25	0.94	0.43	0.87	0.75	0.62
Membrane-type EIA GDH	Immunocard <i>C. difficile</i> (1)	0.60	0.76	0.12	0.97	0.22	0.94	0.38	0.88	0.71	0.66
Real-time PCR	RT-PCR(1)	0.86	0.97	0.60	0.99	0.76	0.98	0.88	0.97	0.97	0.87

RT-PCR, real-time polymerase chain reaction.

TABLE 5C. Calculated PPVs and NPVs for the index tests compared with culture at prevalence rates of 5, 10, 20 and 50% in the tested population

Type	Index test (no. studies)	Sensitivity	Specificity	Prevalence 5%		Prevalence 10%		Prevalence 20%		Prevalence 50%	
				PPV	NPV	PPV	NPV	PPV	NPV	PPV	NPV
Well-type EIA GDH	<i>C. difficile</i> Chek-60 (4)	0.89	0.91	0.34	0.99	0.52	0.99	0.71	0.97	0.91	0.89
Membrane-type EIA GDH	<i>C. difficile</i> quik chek(1)	0.93	0.95	0.50	1.00	0.67	0.99	0.82	0.98	0.95	0.93
	Immunocard <i>C. difficile</i> (1)	0.75	0.90	0.28	0.99	0.45	0.97	0.65	0.94	0.88	0.78
	Triage GDH (3)	0.89	0.98	0.70	0.99	0.83	0.99	0.92	0.97	0.98	0.90

RT-PCR, real-time polymerase chain reaction.

are two options to confirm positive results. Firstly, all positive samples can be tested by a reference test. This approach was described by Ticehurst and colleagues [15]. They applied a two-step algorithm in which specimens were first tested for the presence of GDH antigen by an EIA and the positive results were confirmed by CCA. Because only GDH-positive samples were tested by CCA, this approach resulted in a reduced CCA workload (by 75–80%). Also, their laboratories' expenses were significantly less than if CCA alone had been performed on all samples of patients suspected of having CDI. Gilligan demonstrated that this two-step algorithm had an enhanced ability to detect *C. difficile* toxin-positive specimens (by 40%) compared with the results of an EIA detecting toxins A and B [16]. Secondly, all positive samples can be retested with a rapid test. As the tested population will now have a higher CDI prevalence, PPVs will be acceptable, especially if the more specific EIAs detecting toxins A and/or B or real-time PCR are used instead of EIAs detecting GDH. However, due to the higher CDI prevalence, NPVs will be less acceptable. Therefore, samples with an initial positive test result, but a negative second test result, require testing with a reference method as a third step. The group of Hussain has described such a three-step algorithm in

which specimens were first tested by an EIA detecting GDH and, if positive, the specimens were tested with an EIA detecting toxins A and/or B. The GDH-positive, but toxin-negative, samples were further tested with CCA. With this three-step approach, results of c. 85% of samples were available on the day specimens were received and the need for CCA testing was even further reduced to 15% [12–14]. Fenner and colleagues have also applied this three-step approach. In their laboratory, the results of c. 92% of samples were available within a turnaround time of 4 h; only 8% of samples had to be tested by CCA [22].

The lack of confidence in the tests for CDI detection has motivated some clinicians to submit multiple samples per patient [11]. The value of this repeated testing can also be deduced from the above-mentioned NPVs. In an endemic situation, all the above-mentioned rapid tests have high NPVs. Therefore, submission of multiple samples for testing by one of these tests will not increase the rate of detection of CDI in an endemic setting. This was also observed by Mohan et al. and Drees et al. [55,56]. However, in an epidemic setting, NPVs of these tests will be significantly lower due to the higher prevalence of disease. For that reason, repeated testing of stools will be of value to detect additional cases in

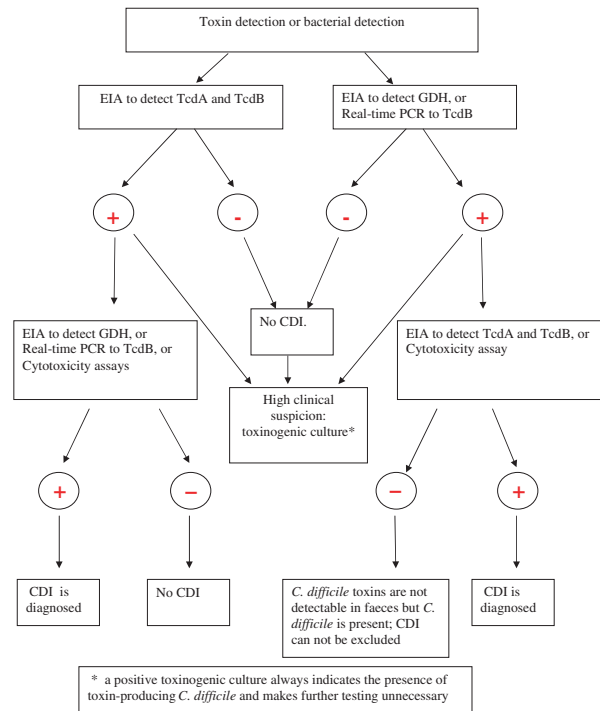


FIG. 2. Algorithm to diagnose CDI

an epidemic setting, as was demonstrated by Debast *et al.* [57] One of the strengths of the two- or three-step algorithms is the high NPV of the first test on the basis of which

samples with a negative test result are considered negative. If such an algorithm would be used in an epidemic situation, the NPV of this first test, would be lower and, therefore, there would be a possibility of missing cases. Consequently, multiple sample submission would also be of benefit if an algorithm is used in an epidemic setting, but not if it is used in an endemic setting. It should be emphasized that multiple sample testing will increase the likelihood of obtaining false-positive results, notably when low specificity tests are used. In practice this creates a real dilemma, as it is difficult to ignore a positive result even when accompanied by several other negative results.

As NPVs of rapid tests for CDI are dependent on the prevalence of disease in the tested population, one might consider using a more stringent sample selection strategy to increase the prevalence of CDI in the tested population. Restricting CDI testing to unformed samples, as is usually done, does not seem to cause the diagnosis of CDI to be missed [58]. On the contrary, restricting CDI testing to patients with characteristic risk factors may lead to missing cases. It is known that routine CDI testing in patients without a request for such testing, but with diarrhoea and hospitalization for at least 72 h, improved the rate of CDI diagnosis [6]. Moreover, the recent emergence of new *C. difficile* strains may result in a greater than before spread of the bacterium in the community and an increase in CDI outside

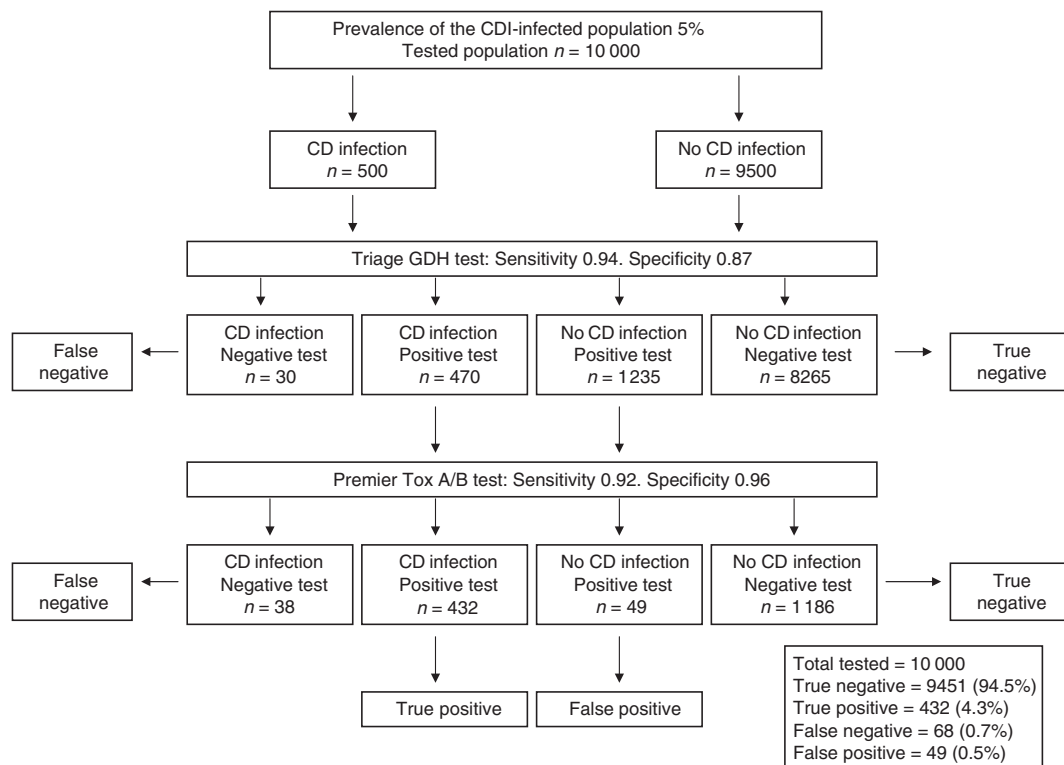


FIG. 3. Application of two-step algorithm in a population of 1000 patients with a CDI prevalence 5%.

known risk groups [59]. Community-acquired CDI has been diagnosed in patients without predisposing factors [60,61]. For 3 months in 2007–2008, three laboratories in The Netherlands tested all unformed stool samples for *C. difficile* submitted by general practitioners (GPs), irrespective of whether GPs specifically requested this [60]. Of 2423 patients, 37 patients (1.5%) were positive. Age varied from 1 to 92 years and 18% were under age 20. Of 31 CDI patients, 20 (65%) had not been admitted to a healthcare institution in the year before, 13 (42%) had not used antibiotics during the prior 6 months, and eight (26%) had neither risk factor. This is similar to the results of Wilcox et al. [61] who found the proportion of positive samples to be 2.1% and the absence of specific risk factors to be 25%. Since it appears that CA-CDI is not a syndrome associated with specific patient characteristics, we recommend testing for CDI in patients with potential infective diarrhoea and with negative tests for common enteropathogens, irrespective of age, prior antibiotic use, comorbidity, co-medication, and onset of diarrhoea (community-acquired or nosocomial).

We suggest that CDI testing should be performed using unformed stool samples of all patients with infectious diarrhoeae who have been negative tests for common enteropathogens. Secondly, all patients with diarrhoea who have been hospitalized more than 72 h should be tested for CDI, irrespective of the physicians' request.

In the present systematic review, we analyzed the comparison of index tests with the two reference tests for toxigenic *C. difficile* (i.e. CCA and TC) separately. Many controversies exist concerning these two reference tests. CCA was the first test described [3] and is still the most widely used reference test, but it has many drawbacks. Besides being technically demanding and time-consuming, it is prone to altered results due to toxin degradation during transport or storage of the sample. It has been reported that storage at 4°C during 56 days had no discernible effect on *C. difficile* toxins, but that multiple cycles of freezing and thawing during this time period may adversely affect toxin titres [62]. Toxigenic culture is time-consuming but suffers less from inadequate storage or transport conditions as *in vitro* toxin production is measured. Because of this, it is reported as a more sensitive test [4], but the clinical relevance of *in vitro* toxin production is not entirely clear. Based on our results, we conclude that toxigenic culture was indeed a more sensitive reference standard, as calculated sensitivities of index tests compared with toxigenic culture were somewhat lower than compared with CCA. In addition to being more sensitive and being affected less by storage and transport, toxigenic culture also allows the recovery of *C. difficile* isolates which can be used for molecular typing and susceptibility testing. Toxigenic culture

could be replaced by molecular tests, provided that the sensitivity of these assays is comparable with culture.

None of the currently available tests is suitable as a stand-alone test in endemic populations to diagnose CDI because of their low PPVs at these prevalences. This conclusion is similar to that drawn recently from a systematic review on diagnosis of *C. difficile* infection using toxin detection kits, but our analysis differed on a few points [63]. Firstly, we excluded meeting abstracts in our analysis since these are in general not peer-reviewed. Secondly, we recalculated the NPV and PPV from the rough data in the manuscript. Thirdly, Planche et al. combined studies irrespective of the reference method used. We analyzed studies using toxigenic culture or cell cytotoxicity assay separately, since toxigenic culture is considered to be a more sensitive method to detect *C. difficile*. We acknowledge, however, that the clinical significance of detection of a toxigenic strain, but not free toxin, remains debatable. Finally, in the review by Planche et al., no distinction was made between well-type EIAs and the membrane-type EIAs. We analysed these groups separately, since the implementation of these tests in daily practice is very different.

In summary, if use of a reference standard test is not considered practical (e.g. because of access to cytotoxin testing and/or culture) we recommend a two-step approach to increase the PPV. The NPV is more difficult to alter. As can be seen from Fig. 3, using a two-test approach will lead to 68 false negative subjects (0.7%) among 10 000 tested. Further reducing the number of false negative results would require either re-testing of all subjects with a negative first test, or re-testing all subjects with a negative second test, after an initially positive test. The first scenario would require retesting a total of 8,295 subjects, which is 83% of the tested population. Assuming a sensitivity of a third test of 0.92, in this scenario the 30 false negative results from the first test would be reduced to only two. The second scenario would require a re-test in 1224 subjects and would reduce the 38 false negative results after the second test to 35, again assuming a sensitivity of 0.92. These improvements are, however, not significant.

Further studies to validate the applicability of two-step testing, including assessment of clinical features with patient follow up, are required. We note that two-step testing will increase the time needed to achieve a confirmed positive result. This emphasizes the need to manage patients with suspected CDI on clinical grounds, notably isolating these individuals as soon as is practicable in order to limit the dissemination of *C. difficile* in the hospital setting. A two-step testing approach to CDI diagnosis will generate discrepant results. This underscores the value of access to reference tests, and the need for improved diagnostic methods in gen-

eral, preferably molecular tests that can replace toxigenic culture.

Recommendations

Sample selection

- CDI testing should only be performed on unformed stools (level 3).

CDI testing should be performed on unformed stool samples of all patients with potential infective diarrhoea and negative tests for common enteropathogens, irrespective of age, prior antibiotic use, co-morbidity, co-medication, and onset of diarrhoea (community-acquired or nosocomial) (level 3).

All patients with diarrhoea who have been hospitalized more than 72 h should be tested for CDI, irrespective of the physicians' request (level 3). This does not mean that CDI testing should not also be performed on samples submitted within the first 72 h of hospitalization

Patients with diarrhoeae who have been admitted in a health-care facility within a period of 3 months prior to the development of diarrhoea should also be tested for CDI.

CDI testing in an endemic setting and epidemic setting

- The diagnosis of *C. difficile* infection should be based on clinical signs and symptoms in combination with laboratory tests.

The interpretation of *C. difficile* laboratory results should be done in the clinical context, taking into account the background prevalence of *C. difficile* in the institution.

We recommend testing patients with a two-step protocol. In the first step, faeces samples could be investigated with an EIA detecting GDH, an EIA detecting toxins A and B, or molecular test detecting TcdB. Samples with a negative test result can be reported as negative. Faeces samples with a positive first test result should be re-tested with a method to detect free faeces toxins, or with a method to detect GDH or toxin genes, dependent on the assay applied as first screening test (level 1).

If free faeces toxins are absent but *C. difficile*, TcdB or GDH is present, CDI cannot be differentiated from asymptomatic colonization.

Repeated sample submission during the same episode is not recommended in an endemic situation (level 2), but may be useful in an epidemic situation (level 3).

Performances of CDI tests

- According to data published to date, the test performances of well-type assays and membrane-type assays to detect toxins A and B of *C. difficile* do not differ significantly

when compared with cytotoxicity as a reference standard (level 1).

Using toxigenic culture as a reference standard, all analysed assays had an unacceptable low sensitivity, with the exception of molecular tests (level 1).

Well-type assays and membrane-type assays with GDH as a target perform equally well and have a good correlation with faeces culture of *C. difficile* (level 1).

Using cell cytotoxicity assay as a reference standard and a prevalence of CDI between 5% and 10%, the best PPV values were observed with Tox A/B Quick Chek and Xpect A/B, but only one study of each was included. The PPVs of the tests decreased to unacceptably low rates when the toxigenic culture was used as reference standard (level 2).

The overall best performances were observed for Immuno-card toxA/B using cytotoxicity or toxigenic culture as reference standard (Level 1) but only three studies have been performed.

Transparency Declaration

No conflict of interest reported.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Search strategy.

Appendix S2. Data extraction form.

Appendix S3. Quality assessment form.

Appendix S4. Quality assessment.

Appendix S5. Levels of evidence and Grades of recommendation.

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