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European Society of Clinical Microbiology and Infectious Diseases: update of the diagnostic guidance document for *Clostridium difficile* infection

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ABSTRACT

In 2009 the first European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guideline for diagnosing *Clostridium difficile* infection (CDI) was launched. Since then newer tests for diagnosing CDI have become available, especially nucleic acid amplification tests. The main objectives of this update of the guidance document are to summarize the currently available evidence concerning laboratory diagnosis of CDI and to formulate and revise recommendations to optimize CDI testing. This update is essential to improve the diagnosis of CDI and to improve uniformity in CDI diagnosis for surveillance purposes among Europe. An electronic search for literature concerning the laboratory diagnosis of CDI was performed. Studies evaluating a commercial laboratory test compared to a reference test were also included in a meta-analysis. The commercial tests that were evaluated included enzyme immunoassays (EIAs) detecting glutamate dehydrogenase, EIAs detecting toxins A and B and nucleic acid amplification tests. Recommendations were formulated by an executive committee, and the strength of recommendations and quality of evidence were graded using the Grades of Recommendation Assessment, Development and Evaluation (GRADE) system. No single commercial test can be used as a stand-alone test for diagnosing CDI as a result of inadequate positive predictive values at low CDI prevalence. Therefore, the use of a two-step algorithm is recommended. Samples without free toxin detected by toxins A and B EIA but with positive glutamate dehydrogenase EIA, nucleic acid amplification test or toxigenic culture results need clinical evaluation to discern CDI from asymptomatic carriage. **M.J.T. Crobach, CMI 2016;22:S63**

Introduction

The previous European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidance document for *Clostridium difficile* infection (CDI) was published in 2009 [1]. Since then many laboratories in Europe have implemented a diagnostic algorithm for diagnosing CDI. However, many new diagnostic tests have become available in the meantime, especially nucleic acid amplification tests (NAATs). Although several of these tests have been marketed, their role in the diagnosis of CDI needs to be clarified. Also, the importance of free toxin detection in stool needs to be addressed. This update of the previous guidance document is essential to improve the diagnosis of CDI; to optimize its management, prevention and control; and to improve uniformity in CDI diagnosis for surveillance purposes across Europe.

The main objectives of this guidance document are to summarize the currently available evidence concerning laboratory diagnosis of CDI and to formulate recommendations to optimize CDI testing. This guideline is intended for use among medical microbiologists, gastroenterologists, infectious disease specialists

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and infection control practitioners. The target population is diarrhoeal patients suspected of having CDI.

Material and Methods

To be able to revise our previous recommendations, an update of the 2009 meta-analysis was performed. In addition, other guidelines and recent literature concerning the diagnosis of CDI were reviewed.

Update of meta-analysis

Search strategy

Studies evaluating laboratory assays for diagnosing CDI were searched in PubMed, Embase, Web of Science, Central and the Cochrane Library. Searches were performed in June 2014 with the support of a trained librarian. The search was restricted to articles published since 2009 in the English language. Meeting abstracts were excluded. The search strategy is displayed in Supplementary Material 1.

Reference tests

A reference test is the best available test and is the standard against which other assays are compared. Cell cytotoxicity neutralization assay (CCNA) and toxigenic culture (TC) are regarded as reference tests for diagnosing CDI [2].

CCNA demonstrates the presence of free toxin B. For this test, 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). The cytopathic effect is evaluated at 24 and 48 hours. Cell lines commonly used for CCNA include Vero cells, HeLa cells, human foreskin fibroblast cells and Hep-2 cells. Neutralization of the cytopathic effect is necessary to determine the specificity of this effect and can be done by using *Clostridium sordelli* antitoxin or *C. difficile* antitoxin [3]. This reference test takes 1 to 2 days to perform and requires cell culture and laboratory expertise, so it is not routinely used in most diagnostic laboratories.

TC demonstrates the presence of *C. difficile,* which is able to produce toxins *in vitro.* Stools are incubated anaerobically for at least 48 hours on selective media. Many different culture media exist for this purpose, all aiming to enhance the recovery of *C. difficile* while inhibiting the overgrowth of other faecal flora [4]. Pretreatment with alcohol shock [5] or heat shock can also be used to decrease overgrowth of normal faecal flora [4]. Also, broth enrichment before plating onto a solid medium is sometimes used (also called enriched culture) [4]. Furthermore, a chromogenic medium (ChromID agar; bioMerieux) for the recovery of *C. difficile* has been developed which is designed to isolate and identify *C. difficile* within 24 hours. However, no consensus exists on which culture medium and/or culture method is the most appropriate to use. Colonies suspicious for *C. difficile* can be recognized by Gram staining, colony morphology, 'horse manure' odour, biochemical testing, gas–liquid chromatography, ultraviolet light fluorescence, latex agglutination and matrix-assisted desorption ionization–time of flight mass spectrometry [6]. Isolates from positive cultures are either tested for *in vitro* toxin production by the use of CCNA or toxin A/B enzyme immunoassay (EIA) or tested for the presence of toxin A/B genes by NAAT.

As outlined above, both these reference tests detect different things, and because of this they will not necessarily agree with each other in all samples. Results for each reference test will be analysed separately.

Index tests

Index tests are the tests whose performance is being evaluated compared to the reference tests. The index tests we reviewed comprise all commonly applied and commercially available laboratory tests for diagnosing CDI other than the reference tests. These include EIAs that detect glutamate dehydrogenase (GDH), EIAs that detect toxins A and B and NAAT.

GDH EIAs detect glutamate dehydrogenase, an enzyme that is produced by both toxigenic and nontoxigenic strains of *C. difficile.* GDH EIAs are available in well-type format (results are displayed as a colour change which can be detected visually or photospectrometically) or membrane-type format (results can be visually read from a membrane).

Toxin A/B EIAs detect toxins A and B and are also available in well-type or membrane-type format. Most EIAs detecting only toxin A have been replaced by EIAs detecting both toxins A and B, as strains that only produce toxin B and not toxin A are reported.

Several membrane-type tests that include both an EIA detecting GDH and an EIA detecting toxins A and B are also available (*C. diff* Quik Chek Complete, Techlab, Combo *C. difficile;* Theradiag).

NAATs include assays that use PCR, helicase-dependent amplification and loop-mediated isothermal amplification. Most assays detect conserved regions within the gene for toxin B (*tcd*B), but assays that detect a highly conserved sequence of the toxin A gene (*tcd*A) have also been developed (Illumigene, Meridian, Bioscience and Amplivue, Quidel) [7,8]. NAATs that not only detect *tcd*B but also the binary toxin genes (*cdt*) and the deletion at nucleotide 117 on *tcd*C are also available (Verigene *C. difficile* test, Nanosphere and Xpert, Cepheid) and offer the potential advantage of detecting PCR ribotype 027, although highly related PCR ribotypes may also be detected by these tests (without distinguishing them from PCR ribotype 027) [9]. NAATs that detect multiple targets at the same time, including *C. difficile* toxin genes, are also available (Seeplex Diarrhea ACE detection, Seegene, xTAG Gastrointestinal Pathogen Panel, Luminex, FilmArray Gastrointestinal Panel, BioFire Diagnostics).

Test performance

The numbers of truly positive, falsely positive, falsely negative and truly negative index test results are generally displayed in a 2 2 table (Table 1). Test performance can be derived from this 2 2 table. The sensitivity of a test is defined as the probability that the index test result will be positive in a person with disease ($a/a+c$). The specificity of a test is defined as the probability that the index test result will be negative in a person without disease ($d/b+d$). The positive predictive value (PPV) of a test is the probability that a person has the disease, given the positive test result ($a/a+b$). The negative predictive value (NPV) of a test is the probability that a person is free of disease, given the negative test result ($d/c+d$). PPV and NPV are dependent on disease prevalence in the tested population (http://training-old.cochrane.org/sites/training-old.cochrane.org/files/uploads/DTA/1.3_Introduction_to_test_accuracy/story.html).

Eligibility criteria

Studies eligible for inclusion had to: (1) describe original research, (2) compare an index test (one commercially available in

Table 1
The 2 2 table used to calculate test characteristics

	Diseased or reference test positive	Not diseased or reference test negative
Index test positive	(a) True positive	(b) False positive
Index test negative	(c) False negative	(d) True negative

Europe) with a reference test (CCNA or TC), (3) perform the tests on *C. difficile*-negative and -positive clinical human stool samples and (4) provide sufficient information to recalculate sensitivity and specificity and their confidence intervals. Culture without determining the toxigenic status was accepted as a reference test if only assays detecting GDH were evaluated.

Studies were excluded if: (1) the reference test was not performed on all samples but only on positive, negative or discordant samples (to exclude partial verification bias), (2) not all samples were tested by the same reference test, (3) the reference method was a composite of more than one test, (4) the reference method included clinical data for its interpretation, (5) the index test was partly used as reference method, (6) the index test did not follow manufacturers' instructions for testing or sample collection, (7) for CCNA, samples were not stored correctly before testing (refrigerated or frozen at 20 C and thawed only once) or neutralization to determine the specificity of the cytopathic effect was not executed and (8) only selected samples were included.

Selection process

Study eligibility was assessed in a two-step selection process by two independent investigators (MC, ET). Inconsistencies were resolved by consensus and by consultation of a third and fourth investigator (EK, TP).

Outcome measures, data extraction and quality assessment

The principal measures of outcome were the sensitivity and specificity of different index tests compared to one of the 2 reference tests. Toxin A/B EIAs, GDH EIAs and NAATs were compared to CCNA and TC. GDH EIAs were additionally compared to culture. From each study we extracted the number of true-positive, false-positive, false-negative and true-negative findings to be able to calculate the sensitivity and specificity of the index test evaluated in that study. Data were extracted by two independent investigators (MC, ET) using a data extraction form (Supplementary Material 2). Additional data that were extracted included year of publication, storage conditions of the samples, information about the study population and information about the execution of the index test and reference test.

The quality of the studies was assessed by the same two independent investigators using a quality assessment tool. This quality assessment tool (Supplementary Material 3) consisted of items from the Quality Assessment for Studies of Diagnostic Accuracy (QUADAS) tool [10], supplemented with items concerning the appropriate handling of specimens and appropriate execution of reference tests.

Statistical analysis

For all index tests in all studies, the sensitivity and specificity and their respective confidence intervals were calculated from the number of true-positive, false-positive, false-negative and true-negative findings supplied in these studies. Wherever possible, the results after initial testing (instead of results after retesting of indeterminate results) were used to calculate the sensitivity and specificity. Random effects logistic regression was used to pool the mean sensitivities and specificities for the different index tests and the different types of index tests. In case of fewer than four studies, a fixed effect model was used. NPVs and PPVs were calculated using a hypothetical prevalence of CDI of 5, 10, 20 and 50% in the tested population. We used Stata 12.0 software (StataCorp) for all statistical analyses.

Guidelines and additional studies

An electronic search was performed on topics concerning laboratory diagnosis of CDI not included in our meta-analysis (e.g. repeated testing, sample selection). Published guidelines on CDI testing were also studied. These included guidelines from the Society for Healthcare Epidemiology of America/Infectious Diseases Society of America (published in 2010) [11], guidelines from the Australasian Society for Infectious Diseases (published in 2011) [12], guidelines from the American College of Gastroenterology (published in 2013) [13], guidelines from the American Academy of Pediatrics (published in 2013) [14] and guidelines from the UK National Health Service (update published in 2012) [15].

Formulation of recommendations

The guideline was developed according to the Appraisal of Guidelines for Research and Evaluation (AGREE II) instrument [16]. Findings of the literature review and meta-analysis results were discussed with the members of the executive committee, and recommendations were formulated. We slightly modified the GRADE system to grade the strength of the recommendations and the quality of evidence [17] (Table 2). A good practice statement could be made instead of a formal graded recommendation for domains where this was deemed appropriate [18]. The drafting group (consisting of experts in the field) and a patients' representative were invited to comment on the recommendations, and results from these discussions were incorporated in the final recommendations.

Results

Literature search and selection process

A total of 795 unique citations were identified by our current search. On the basis of title and abstract, 693 articles were excluded, leaving 102 full-text articles for detailed assessment. In total, 61 studies were excluded after detailed assessment. Reasons for exclusion were (some studies had more than one reason for

Table 2
Scoring system for grading quality of evidence and strength of recommendations

Quality of evidence	
High quality	Evidence from at least one properly designed cross-sectional or cohort study in patients with diagnostic uncertainty and direct comparison of all test results with an appropriate reference standard.
Moderate quality	Evidence from: (1) at least one cross-sectional or cohort study in selected patients and/or no or partial comparison of test results with an appropriate reference standard, (2) case–control studies.
Low quality	Evidence from opinions of respected authorities, based on clinical experience, descriptive case studies or reports of expert committees.
Strength of recommendation	
Strong recommendation for use	Desirable effects clearly outweigh undesirable effects.
Weak recommendation for use	Desirable and undesirable effects are closely balanced or recommendation is based on low-quality evidence.
Weak recommendation against use	Desirable and undesirable effects are closely balanced or recommendation is based on low-quality evidence.
Strong recommendation against use	Undesirable effects clearly outweigh desirable effects.
Good practice statement	Desirable effects clearly outweigh undesirable effects, but no or only indirect evidence is/will become available.

exclusion): not all samples were tested by the (same) reference method (23 studies), no or an inadequate reference test was used (16 studies), samples were selected inadequately (13 studies), not enough information was provided (seven studies), the study did not describe original research (five studies), no clinical human stool samples were included (three studies), no commercial diagnostic test was investigated (two studies) and stool samples were incorrectly collected in transport medium (one study).

From all 43 studies included in the previous meta-analysis [1], 28 were excluded. Twenty-four of these studies evaluated tests that were no longer available (mainly EIAs detecting toxin A only). Two other studies were excluded because they did not evaluate a commercial test (both studies evaluated an in-house PCR), one study was excluded because not all samples were tested by the same reference test and one study was excluded because samples were stored incorrectly for CCNA testing. A total of 56 studies (15 from the previous meta-analysis and 41 published since 2009) were included in the meta-analysis [7,8,19–72]. A summary of the selection process is shown in Fig. 1.

Study characteristics

Twenty-four different laboratory assays were evaluated: one well-type EIA for GDH, three membrane-type EIAs for GDH, five well-type EIAs for toxins A and B, four membrane-type EIAs for toxin A and B and 11 NAATs (Table 3). In total, 133 comparisons between index tests and reference tests were available, including 53 comparisons to CCNA, 69 comparisons to TC and 11 comparisons to culture. Studies were published between 1996 and 2014. The number of evaluated index tests per study ranged from one to ten, and the number of included samples ranged from 60 to 12 369. The CDI prevalence in the tested population ranged from 6 to 48%. Table 4 lists the characteristics of included studies.

Quality assessment

None of the studies fulfilled all our quality assessment criteria, mainly because required information was frequently missing (Fig. 2, Supplementary Material 4). The process used to select samples was adequately reported in 23 (41%) of 56 studies. A minority of studies (6/56, 11%) reported that they did not exclude formed samples from CDI testing. In around half of the studies, conditions of storage for the samples before testing with the index test were not (or were insufficiently) reported. Samples tested by GDH EIA, toxin A/B EIA and NAAT were reported to be stored according to manufacturer's instructions in 10 (46%) of 22, 14 (45%) of 31 and 15 (50%) of 30 studies, respectively. In the remaining 12, 16 and 15 studies, respectively, storage conditions

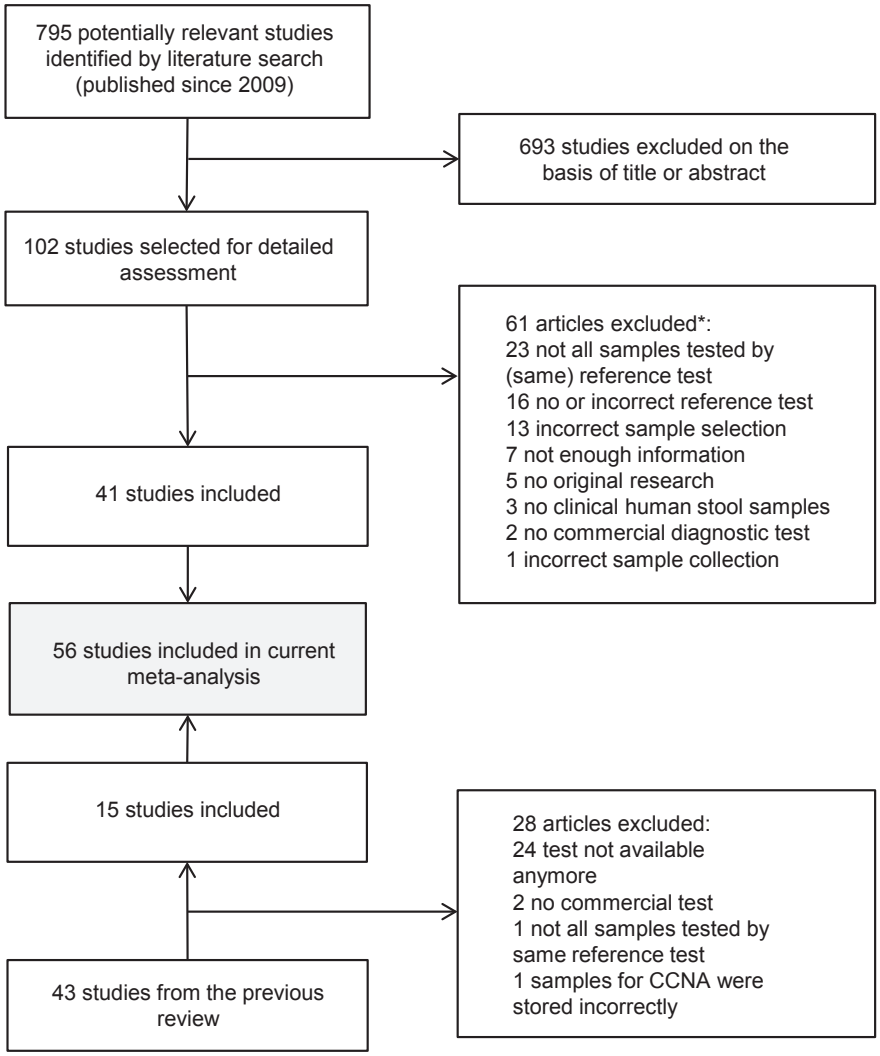

Fig. 1. Summary of selection process. *Some studies had more than one reason for exclusion.

Table 3
Index tests included in meta-analysis

Assay type	Test	Manufacturer	Target	Method
(A) Well-type EIA GDH	*C. diff* Chek-60	Techlab	GDH	Well-type EIA
(B) Membrane-type EIA GDH	*C. diff* Quik Chek	Techlab	GDH	Membrane-type EIA
	ImmunoCard *C. difficile*	Meridian	GDH	Membrane-type EIA
	Quik Chek Complete–GDH[a]	Techlab	GDH	Membrane-type EIA
(C) Well-type EIA toxins A/B	Premier toxins A/B	Meridian	Toxins A and B	Well-type EIA
	Remel ProSpecT	Oxoid	Toxins A and B	Well-type EIA
	Ridascreen toxins A/B	Biopharm	Toxins A and B	Well-type EIA
	Clostridium difficile Tox A/B II	Techlab	Toxins A and B	Well-type EIA
	Vidas CDAB	bioMerieux	Toxins A and B	Automated EIA
(D) Membrane-type EIA toxins A/B	ImmunoCard toxins A/B	Meridian	Toxins A and B	Membrane-type EIA
	Quik Chek Complete–Tox A/B[a]	Techlab	Toxins A and B	Membrane-type EIA
	Tox A/B Quik Chek	Techlab	Toxins A and B	Membrane-type EIA
	Xpect	Oxoid	Toxins A and B	Membrane-type EIA
(E) NAAT	Advansure CD	LG Life Sciences	*tcdA*, *tcdB*	RT-PCR
	Amplivue	Quidel	*tcdA*	Isothermal helicase-dependent amplification
	BD GeneOhm	Becton Dickinson	*tcdB*	RT-PCR
	BD Max Cdiff	Becton, Dickinson	*tcdB*	RT-PCR
	GenomEra	Abacus Diagnostica	*tcdB*	RT-PCR
	Illumigene	Meridian	*tcdA*	LAMP
	Portrait	Great Basin	*tcdB*	Isothermal helicase-dependent amplification
	Prodesse ProGastro Cd Assay	Hologic Gen-Probe	*tcdB*	RT-PCR
	Seeplex Diarrhea ACE Detection[c]	Seegene	*tcdB*	RT-PCR
	Verigene	Nanosphere	*tcdA*, *tcdB*, *cdt*,[b] *tcdC* deletion nt 117[b]	PCR/nanoparticle-based microarray
	Xpert *C. difficile*	Cepheid	*tcdB*, *cdt*, *tcdC* deletion nt 117	RT-PCR

EIA, enzyme immunoassay; GDH, glutamate dehydrogenase; LAMP, loop-mediated isothermal DNA amplification; RT-PCR, real-time PCR.
[a] Part of an EIA that detects both toxins A/B and GDH.
[b] Only for epidemiologic purposes.
[c] Multiplex PCR system.

did not or not completely comply with manufacturer's instructions. In 18 (72%) of 25 studies using CCNA as the reference test, samples were stored according to our predefined storage requirements: samples were either refrigerated and tested within 5 days (15 studies) [8,25,27,36,45–48,58–61,63,65,68] or were frozen at 20 C and thawed no more than once (three studies) [44,66,67]. In the remaining seven studies (28%), storage conditions for CCNA were not or incompletely described. Storage conditions for samples tested by TC were reported in 23 (68%) of 34 studies, but no specific requirements for storage of samples tested by TC were set. The execution of the reference test was described in sufficient detail in 44 (79%) of 56 studies. In 2 (8%) of 26 studies using CCNA as reference test, the incubation period was only 24 hours [61,63]. In studies using TC as reference test, ethanol shock was reported to be performed in 18 of 35 studies [19,21,23,32,35,37,38,47,51–55,57,61,69–71], and heat shock was performed in three of 35 studies [22,49,58]. Eight studies (23%) used an enrichment broth before plating onto a solid agar [19,22–24,32,43,58,62]. Toxigenicity was confirmed by PCR (15/32, 47%) [21,23,29,33–35,37,51–57,70], CCNA (9/32, 28%) [7,8,22,24,43,47,58,61,62], toxin EIA (7/32, 22%) [19,30,32,38,40,69,71] or both PCR and CCNA (1/32, 3%) [26]. Blinding (index test interpreted without knowledge of reference test or *vice versa*) was reported in 8 (14%) of 56 studies. Thirty-one studies (55%) reported if any indeterminate results (i.e. invalid, 'no call' or difficult-to-interpret results) were found. Indeterminate results actually occurred in 28 studies and were reported for one membrane-type GDH EIA (ImmunoCard *C. difficile*), three membrane-type toxin A/B EIAs (Tox A/B Quik Chek, ImmunoCard Tox A/B, Xpect), one automated EIA (Vidas) and nine NAATs. The amount of indeterminate results ranged from 0.3 to 6.8% of tested samples. Repeat testing of samples after an initial indeterminate result was done in 24 (86%) of these 28 studies. Of these, 22 presented results only after repeat testing [7,8,20–22,24,29,30,34,35,37,38,43,46,47,54,58,59,62,65,69,70], and two presented results of both initial and repeat testing [27,63].

Test performances

Sensitivity and specificity of the index tests were calculated on the basis of the numbers provided in the articles. Discrepancies between calculated sensitivity or specificity and published data were found in two articles; the correct data were provided by both authors upon request [38,39]. In Table 5, sensitivity and specificity of index tests are compared to CCNA. Reported estimates of sensitivity ranged from 0.80 to 1.00 for GDH EIAs, from 0.44 to 0.99 for toxin A/B EIAs and from 0.83 to 1.00 for NAATs. Reported estimates of specificity ranged from 0.82 to 0.95 for GDH EIAs, from 0.87 to 1.00 for toxin A/B EIAs and from 0.87 to 0.98 for NAATs. Table 6 lists sensitivity and specificity compared to TC. Sensitivities ranged from 0.83 to 1.00, 0.29 to 0.86 and 0.77 to 1.0 for GDH EIAs, toxin A/B EIAs and NAATs, respectively. Specificities ranged from 0.88 to 1.00, 0.91 to 1.00 and 0.83 to 1.00, respectively. In Table 7, sensitivity and specificity of GDH EIAs are compared to culture. Sensitivities ranged from 0.71 to 1.00, and specificities ranged from 0.67 to 1.00. In Table 8, estimates of pooled sensitivity and pooled specificity for the different categories of index tests are shown. The estimated pooled sensitivities and specificities compared to CCNA were used to compute PPVs and NPVs of the categories of index tests at different hypothetical CDI prevalences (Table 9, Supplementary Material 5). At a CDI prevalence of 5%, PPVs ranged from 34 to 81%, and NPVs ranged from 99 to 100%. At a CDI prevalence of 50%, PPVs ranged from 91 to 99%, while NPVs ranged from 83 to 98%.

Discussion

In the present meta-analysis, we evaluated the diagnostic accuracy of various commercial laboratory assays for diagnosing CDI.

Table 4
Characteristics of included studies

Study	Year	Country	Reference test	Index test	Total no. samples	Study population	Consistency of stool samples	Prevalence CDI (CCNA)	Prevalence CDI (TC)
Barkin [19]	2012	USA	TC	Premier toxins A/B, ImmunoCard *C. difficile*, Illumigene	272	Adult inpatients of large community teaching hospital with diarrhoea, risk factors for CDI and for whom CDI test was requested by their physician	Unformed		13.1
Berg, van den [66]	2005	Netherlands	CCNA	ImmunoCard toxins A/B	367	Unformed stools of adults with specific request for CDI testing or hospitalized >72 hours that were submitted to laboratories of three university hospitals	Unformed	6.3	
Berg, van den [67]	2007	Netherlands	CCNA	Premier toxins A/B	540	Unformed stools of patients suspected of having CDI or hospitalized >72 hours in four university medical centres	Unformed	5.7	
Berry [20]	2014	UK	CCNA	Xpert	1034	Inpatients in two acute-care hospitals aged >15 years with suspected CDI for whom CDI testing was requested by treating physician	Unformed	6.0	
Boer, de [25]	2010	Netherlands	CCNA	Xpect	161	Clinical stool specimens from patients for whom request for CDI testing was issued, prospectively collected at laboratory for infectious diseases	Unclear	9.9	
Bruins [21]	2012	Netherlands	TC	ImmunoCard toxins A/B, Quik Chek Complete, Premier toxins A/B, Illumigene	986	Hospitalized and nonhospitalized patients with diarrhoea who had stool sample sent to laboratory of major hospital, preferably from those patients known to have CDI-associated symptoms or risk factors	Unformed		7.4
Buchan [22]	2012	USA	TC	Portrait, GeneXpert, GeneOhm, Illumigene	540/275/169/96	Stool specimens from patients >2 years old suspected of having CDI collected at four institutions	Unformed		22.5
Calderaro [23]	2013	Italy	TC	Illumigene, Quik Chek Complete	306	Patients attending university hospital with suspicion of CDI	Unclear		19.6
Carroll [24]	2013	USA	TC	Verigene	1875	Leftover stool samples submitted specifically for CDI testing according to institution's routine practice to five geographically diverse clinical microbiology laboratories	Formed and unformed		8.4 (direct), 14.7 (enriched)
Eastwood [27]	2009	UK	CCNA	Premier toxins A/B, Xpect, Tox A/B Quik Chek, Ridascreen toxins A/B, Tox A/B II, ProSpecT, VIDAS CDAB, ImmunoCard toxins A/B, *C. diff* Chek-60, BD GeneOhm	488	Stool specimens submitted for CCNA testing at laboratory of teaching hospital; ten samples were randomly chosen each day	Unformed	18.1	

Eckert [8]	2014	France	CCNA, TC	Amplivue, *C. diff* Quik Chek	308	Inpatients in four university-affiliated hospitals >2 years old with suspected CDI for whom CDI testing was requested by treating physician or if diarrhoea occurred after day 3 of hospitalization	Unformed	7.5	11.7
Fenner [28]	2008	Switzerland	Culture	*C. diff* Chek-60	1468	Stools of adults patients suspected of having CDI at university hospital	Unclear		12.7 culture positive
Hart [29]	2014	Australia	Culture, TC	Illumigene, BD GeneOhm, Quik Chek Complete	150	Stools of children collected at laboratory of paediatric hospital fulfilling criteria for CDI testing in this hospital[a]	Formed (4%) and unformed (96%)		30.0
Hirvonen [30]	2013	Finland	TC	GenomEra	310	Stool specimens from inpatients (7–95 years old), collected prospectively according to routine hospital practice for antibiotic-associated diarrhoea at large teaching hospital	Unformed		24.9
Huang [31]	2009	Sweden	CCNA	Xpert	220	Consecutive stool specimens from patients >2 years old who were symptomatic and had request for CDI testing at university hospital	Unformed	10.5	
Jacobs [32]	1996	Israel	Culture, TC	ImmunoCard *C. difficile*	258	Stool samples from patients who developed diarrhoea during hospitalization in community teaching hospital and control samples from 24 patients without diarrhoea	Formed and unformed		7.0
Jong [26]	2012	Netherlands	TC	ImmunoCard toxins A/B, VIDAS CDAB	150	Hospitalized adult patients in tertiary teaching hospital who had stool specimens submitted for CDI testing	Unclear		9.7
Kawada [33]	2011	Japan	Culture, TC	Quik Chek Complete, ImmunoCard *C. difficile*, Tox A/B Quik Chek	60	Patients hospitalized at geriatric hospital and diagnosed as having antibiotic-associated diarrhoea	Unformed		46.7
Kim [35]	2014	Korea	TC	Quik Chek Complete, VIDAS CDAB	608	Suspected CDI patients in tertiary-care teaching hospital	Unformed		9.0
Kim [34]	2012	Korea	TC	AdvanSure, VIDAS CDAB	127	Diarrhoeal stool specimens submitted to hospital laboratory for *C. difficile* culture	Unformed		8.8
Lalande [7]	2011	France	TC	Illumigene	472	Consecutive stools from patients suspected of having CDI	Unformed		10.4
Larson [36]	2010	USA	CCNA	*C. diff* Quik Chek	699	Stool samples submitted for CDI testing from adult patients at university hospital	Unformed	6.7	
Le Guern [37]	2012	France	TC	BD Max Cdiff, BD GeneOhm, Tox A/B Quik Chek	360	Diarrhoeal stool specimens collected from inpatients at university hospital	Unformed		12.2

(*continued on next page*)

Table 4 (*continued*)

Study	Year	Country	Reference test	Index test	Total no. samples	Study population	Consistency of stool samples	Prevalence CDI (CCNA)	Prevalence CDI (TC)
Leitner [38]	2013	Austria	TC	BD Max Cdiff, Premier toxins A/B	180	Stool specimens from adults and children with specified request for CDI testing at medical university	Unformed		16.7
Massey [39]	2003	Candada	CCNA	Tox A/B II	557	Stool samples of adult hospitalized patients suspected of having CDI at large teaching hospital	Unformed	25.7	
Mattner [40]	2012	Germany	TC	Ridascreen	254	All liquid stool samples sent to university microbiology laboratory	Unformed		16.4
Musher [41]	2007	USA	CCNA	Premier toxins A/B, ImmunoCard toxins A/B, Tox A/B II, ProSpecT	446/131	Consecutive stool samples submitted to laboratory of medical centre for CDI testing	Unclear	17.0/41.2	
Noren [42]	2011	Sweden	CCNA	Illumigene	272	Consecutive stool specimens from adults and children submitted for CDI testing from hospitals and communities	Unclear	13.2	
Novak-Weekley [43]	2010	USA	TC	Xpert, Premier A/B	432	Leftover stool samples from patients >2 years old with suspected CDI for whom toxin enzyme immunoassays were ordered according to institution's standard practices at regional reference laboratories serving hospitals and associated medical clinics	Unformed		16.8
O'Connor [44]	2001	Ireland	CCNA	Tox A/B II, Premier toxins A/B	200	Consecutive stools of adult patients suspected of having CDI submitted to laboratories of university hospitals	Formed and unformed	30.5	
Ota [45]	2012	USA	CCNA	*C. diff* Quik Chek Complete, Premier toxins A/B, Illumigene	141	Consecutive stool specimens prospectively collected at children's hospital from patients 1 −18 years of age and submitted for CDI testing	Unformed	18.4	
Pancholi [46]	2012	USA	CCNA	Illumigene, Xpert	200	Consecutive and prospectively collected stools from adult patients submitted to university medical centre laboratory for routine CDI testing	Unformed	11.6	
Planche [47]	2013	UK	CCNA, TC	Xpert, *C. diff* Chek-60, Premier toxins A/B, Tox A/B II	8827/12 365/ 9192/12 369	Faecal samples from hospital and community patients submitted for routine CDI testing according to routine protocol[b] submitted to four hospital diagnostic laboratories serving major teaching hospitals and their communities	Unformed	5.9	8.4
Qutub [48]	2011	Saudi Arabia	CCNA	*C. diff* Chek-60, Tox A/B II	150	Stool samples from consecutive inpatients with suspected CDI	Unformed	34.7	

Reller [49]	2007	USA	Culture	*C. diff* Chek-60	439	Stool samples from hospitalized adults and children suspected of having CDI	Unclear		36.7 culture positive
Reller [50]	2010	USA	CCNA	*C. diff* Chek-60, *C. diff* Quik Chek, Tox A/B Quik Chek	600	Sequential weekday stool samples submitted to university hospital microbiology laboratory for CDI testing	Unformed	7.7	
Shin [52]	2009	Korea	TC	Vidas CDAB	1596	Stool samples from patients admitted to tertiary teaching hospital with clinical signs compatible with CDI			19.6
Shin [51]	2009	Korea	TC	Vidas CDAB	555	Patients >2 years old with suspected CDI from two hospitals	Formed (51%) and unformed		20.3
Shin [53]	2012	Korea	TC	Seegene, BD GeneOhm	243	Fresh stool specimens from patients with clinical signs compatible with CDI who were hospitalized in 3 teaching hospitals	Unclear		28.8
Shin [54]	2012	Korea	TC	Xpert/epi, Vidas CDAB	253	Consecutive stool specimens from suspected CDI patients in tertiary hospital	Unformed		18.4
Sloan [55]	2008	USA	TC	Premier toxins A/B, Xpect, ImmunoCard A/B	200	Stools of patients suspected of having CDI submitted to clinical microbiology laboratory of large tertiary-care teaching hospital	Unformed		22.0
Snell [56]	2004	Canada	Culture, TC	*C. diff* Chek-60, Tox A/B II	497	Stools of inpatients suspected of having CDI at large teaching hospital	Unformed		10.5
Soh [57]	2014	Korea	TC	AdvanSure CD, Illumigene	203	Stool samples collected at tertiary university teaching hospital	Unformed		12.8
Stamper [59]	2009	USA	CCNA	BD GeneOhm	401	Symptomatic adult patients who had stool sample submitted for routine CDI testing in tertiary-care university medical centre	Unformed	11.0	
Stamper [58]	2009	USA	CCNA, TC	ProGastro CD	280	Stool samples submitted for routine CDI testing from symptomatic patients >2 years old at tertiary-care university medical institution	Unformed	11.0	15.7
Staneck [60]	1996	USA	CCNA	ImmunoCard *C. difficile*	906	Stool samples submitted to three hospital microbiology laboratories	Unclear	14.1	
Swindells [61]	2010	UK	Culture, CCNA, TC	*C. diff* Quik Chek Complete, Vidas CDAB, Xpert, GeneOhm	150	Consecutive stool specimens from inpatients >65 years old who developed diarrhoea at least 48 hours after admission	Unformed	10.0	12.0
Tenover [62]	2010	USA/Canada	TC	Xpert	2296	Leftover stool specimens from patients >2 years old from seven health care organizations (six USA, one Canada) for whom CDI testing was ordered according to institution's practices	Unformed		10.8 (direct), 14.7 (enriched)

(*continued on next page*)

Table 4 (*continued*)

Study	Year	Country	Reference test	Index test	Total no. samples	Study population	Consistency of stool samples	Prevalence CDI (CCNA)	Prevalence CDI (TC)
Terhes [63]	2009	Hungary	CCNA	BD GeneOhm	600	Inpatients and outpatients at local university hospital who had diarrhoeal stool sample sent to laboratory for CDI testing	Unformed	6.4	
Ticehurst [64]	2006	USA	CCNA	*C. diff* Chek-60	266	Stools of patients suspected of having CDI submitted to laboratories of two acute-care hospitals	Unclear	9.0	
Turgeon [65]	2003	USA	CCNA	ImmunoCard *C. difficile*	1003	Consecutive stools of adults and children suspected of having CDI at five major hospitals	Unformed and formed	10.1	
Vanpoucke [68]	2001	Belgium	CCNA	Ridascreen	156	Stool specimens submitted to laboratory of university hospital with request for CDI testing	Unformed	31.8	
Viala [69]	2012	France	TC	BD GeneOhm, Xpert, Illumigene	94	Fresh stool specimens from symptomatic patients collected at university hospital, 45 TC positive and 49 TC negative were selected	Unformed		47.8
Walkty [70]	2013	Canada	TC	Illumigene, *C. diff* Quik Chek	428	All diarrhoeal stool specimens from patients >1 year old submitted for CDI testing to three microbiological laboratories serving major hospitals and surrounding communities	Unformed		14.7
Wren [71]	2009	UK	Culture, TC	*C. diff* Quik Chek, Tox A/B Quik Chek	1007	Stool samples submitted for CDI testing from patients who developed diarrhoea after being admitted to major university hospitals	Unformed		8.6
Zheng [72]	2004	USA	Culture	*C. diff* Chek-60	992	Stool samples submitted for routine CDI testing because of antibiotic-associated diarrhoea collected from hospital laboratories and supplied to TechLab, a large medical centre and reference laboratory	Unclear	13.8	

CCNA, cell cytotoxicity neutralization assay; CDI, *Clostridium difficile* infection; TC, toxigenic culture.

[a] Criteria were: oncology/haematology patient, specific request for CDI testing by treating physician, history of diarrhoea developed while receiving antibiotics, or pseudomembranous colitis.

[b] Criteria were: all unformed faecal samples not clearly attributable to an underlying disease, or treatment from all hospital patients >2 years and from individuals in the community >65 years irrespective of *C. difficile* or other testing requests.

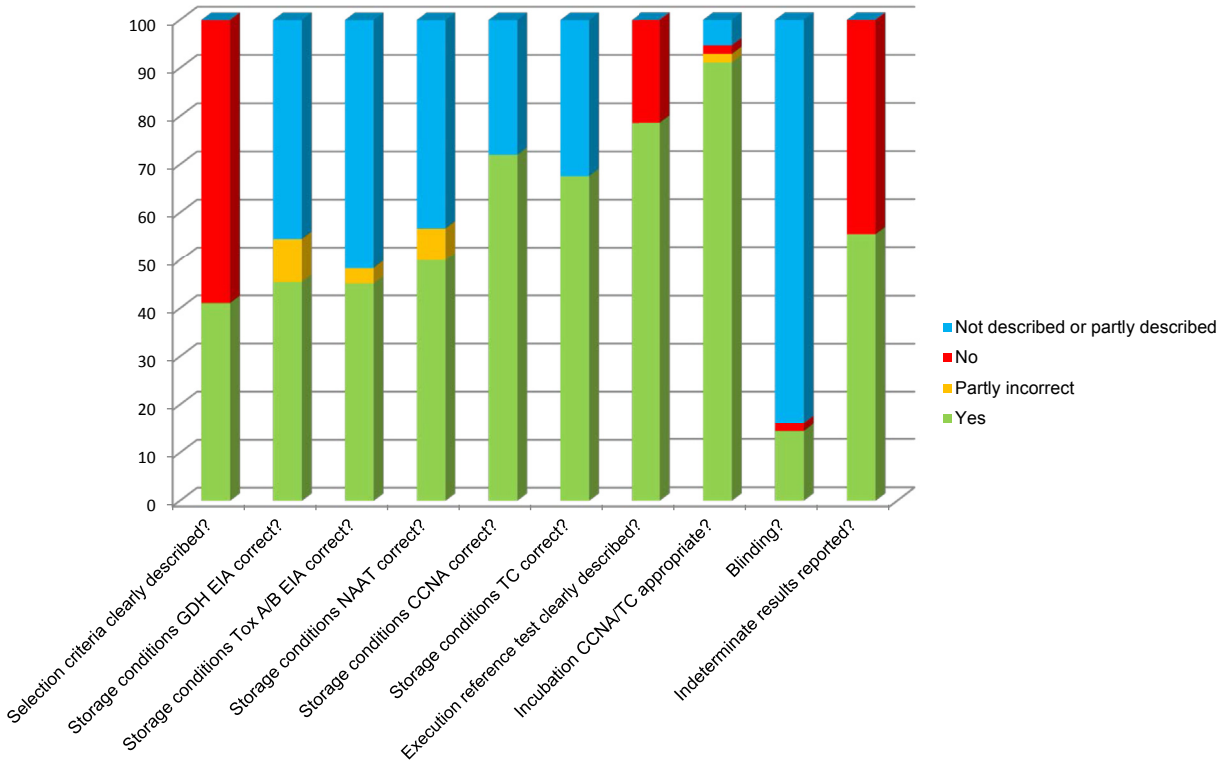

Fig. 2. Quality assessment of included studies.

Toxin A/B EIAs tended to be the most specific assays, while GDH EIAs and NAATs were more sensitive tests. Although many toxin A/B EIAs belong to the least sensitive tests, the sensitivity of this category of assays is not as low as reported earlier [1]. This is because only currently available tests were included in the present analysis, and the newer generation of toxin A/B EIAs turns out to be more sensitive than the earlier toxin A EIAs.

We compared all categories of the index tests (GDH EIAs, toxin A/B EIAs and NAATs) to both of the reference tests, CCNA and TC. However, not only are the targets of these three categories of index tests somewhat different, but also the targets of the two reference tests differ: CCNA detects *in vivo* toxin production, while TC detects the presence of a toxigenic *C. difficile* strain.

This explains why sensitivities and specificities were different for each reference test that was used as a comparator. For example, toxin A/B EIAs were less sensitive compared to TC instead of CCNA: toxin EIAs will not (like the TC) detect all samples containing toxigenic *C. difficile* strains but only (some of) those with free toxin present. It also explains why NAATs were less specific compared to CCNA instead of TC: NAATs are not able (like CCNA) to discern samples with *in vivo* toxin production from samples with *in vitro* toxin production.

We included both CCNA and TC as reference tests, as there has always been debate which of these tests best defines CDI cases. Recently a large study reported that CCNA positivity (i.e. demonstration of free toxin) but not TC positivity (i.e. demonstration of toxin-producing capacity) correlated with clinical outcome. Therefore, at least all samples with a positive CCNA can be considered to represent true CDI cases [47]. However, samples with a positive TC but negative CCNA are difficult to interpret. These samples could either belong to *C. difficile* carriers (harbouring a toxigenic *C. difficile* strain not producing detectable toxins at that moment) or to patients with CDI with toxin levels below the threshold of detection.

To guarantee a certain level of uniformity and quality, only studies that met our eligibility criteria were included in the meta-analysis. Still, studies differed from one another in many aspects. For CCNA, diverse dilutions of faecal filtrate and diverse cell lines were used. For TC, diverse culture media and diverse methods to demonstrate toxigenicity were applied. Also, none of the studies satisfied all our quality assessment criteria. Notwithstanding these differences, all included studies met the minimal—quite strict—requirements we set. We therefore think that it is justifiable that we calculated summary estimates of sensitivity and specificity, especially because we intended to provide a general overview of test performances of different categories of laboratory assays instead of pointing out one 'best' assay. It is, however, important to realize that test performances of individual assays may have been influenced by the design of included studies analysing these tests. Besides, test characteristics presented here should not be considered unchanging over time and should not be considered fixed characteristics. This is because procedures of commercial assays are sometimes revised to enhance test performance, and also because assays may perform differently among different populations (e.g. high- vs. low-risk patients). Also, in all categories, new assays were marketed. The introduction of newer toxin A/B EIAs leading to a better sensitivity of this category of assays is a good example of the latter.

On the basis of the review results, PPVs and NPVs were calculated at different hypothetical prevalences of CDI in the tested population. The prevalence of CDI can be seen as the pretest probability of having CDI and would typically be around 5–10% in an endemic setting [73]. At a CDI prevalence of 5%, even the most specific tests (toxin A/B EIAs) would have PPVs of only 69–81%. On the contrary, NPVs would be very high for all index tests. If the prevalence of CDI would rise to 50% among the tested patients, the PPV would consequently raise to 98.8% for the most specific test, but the NPV would drop to 82.5% for the least sensitive tests. Both

Table 5
Sensitivity and specificity of index tests compared to CCNA

Type	Index test	Study	Sensitivity (95% CI)	Specificity (95% CI)
(A) Well-type EIA GDH	*C. diff* Chek-60	Eastwood [27]	0.90 (0.82–0.95)	0.93 (0.90–0.95)
	C. diff Chek-60	Planche [47]	0.96 (0.95–0.98)	0.92 (0.92–0.93)
	C. diff Chek-60	Qutub [48]	0.94 (0.84–0.99)	0.88 (0.80–0.94)
	C. diff Chek-60	Reller [50]	0.91 (0.79–0.98)	0.90 (0.87–0.92)
	C. diff Chek-60	Ticehurst [64]	0.96 (0.79–1.00)	0.90 (0.86–0.94)
(B) Membrane-type EIA GDH	*C. diff* Quik Chek	Eckert [8]	1.00 (0.85–1.00)	0.92 (0.88–0.94)
	C. diff Quik Chek	Larson [36]	1.00 (0.92–1.00)	0.90 (0.87–0.92)
	C. diff Quik Chek	Reller [50]	1.00 (0.92–1.00)	0.83 (0.79–0.86)
	ImmunoCard *C. difficile*	Staneck [60]	0.84 (0.77–0.90)	0.92 (0.90–0.94)
	ImmunoCard *C. difficile*	Turgeon [65]	0.80 (0.71–0.87)	0.92 (0.91–0.94)
	Quik Chek Complete-GDH	Ota [45]	0.81 (0.61–0.93)	0.82 (0.73–0.88)
	Quik Chek Complete-GDH	Swindells [61]	1.00 (0.78–1.00)	0.95 (0.90–0.98)
(C) Well-type EIA toxins A/B	*Clostridium difficile* Tox A/B II	Eastwood [27]	0.91 (0.84–0.95)	0.96 (0.93–0.97)
	Clostridium difficile Tox A/B II	Massey [39]	0.75 (0.67–0.82)	0.98 (0.96–0.99)
	Clostridium difficile Tox A/B II	Musher [41]	0.96 (0.87–1.00)	0.87 (0.77–0.94)
	Clostridium difficile Tox A/B II	O'Connor [44]	0.80 (0.68–0.89)	0.99 (0.96–1.00)
	Clostridium difficile Tox A/B II	Planche [47]	0.83 (0.80–0.86)	0.99 (0.99–0.99)
	Clostridium difficile Tox A/B II	Qutub [48]	0.73 (0.59–0.84)	1.00 (0.96–1.00)
	Premier toxins A/B	Berg, van den 2007 [67]	0.97 (0.83–1.00)	0.94 (0.92–0.96)
	Premier toxins A/B	Eastwood [27]	0.92 (0.85–0.96)	0.97 (0.95–0.98)
	Premier toxins A/B	Musher [41]	0.99 (0.93–1.00)	0.97 (0.95–0.99)
	Premier toxins A/B	O'Connor [44]	0.82 (0.70–0.91)	0.99 (0.96–1.00)
	Premier toxins A/B	Ota [45]	0.58 (0.37–0.77)	1.00 (0.97–1.00)
	Premier toxins A/B	Planche [47]	0.67 (0.63–0.71)	0.99 (0.99–0.99)
	Remel ProSpecT	Eastwood [27]	0.90 (0.83–0.95)	0.93 (0.90–0.95)
	Remel ProSpecT	Musher [41]	0.91 (0.80–0.97)	0.97 (0.91–1.00)
	Ridascreen toxins A/B	Eastwood [27]	0.67 (0.57–0.75)	0.95 (0.93–0.97)
	Ridascreen toxins A/B	Vanpoucke [68]	0.57 (0.43–0.70)	0.97 (0.92–0.99)
(D) Membrane-type EIA toxins A/B	ImmunoCard toxins A/B	Berg, van den (2005) [66]	0.91 (0.72–0.99)	0.97 (0.95–0.99)
	ImmunoCard toxins A/B	Eastwood [27]	0.85 (0.76–0.91)	0.99 (0.98–1.00)
	ImmunoCard toxins A/B	Musher [41]	0.96 (0.89–0.99)	0.99 (0.97–1.00)
	Quik Chek Complete-Tox A/B	Ota [45]	0.50 (0.30–0.70)	1.00 (0.97–1.00)
	Quik Chek Complete-Tox A/B	Swindells [61]	0.73 (0.45–0.92)	1.00 (0.97–1.00)
	Tox A/B Quik Chek	Eastwood [27]	0.84 (0.76–0.91)	0.99 (0.98–1.00)
	Tox A/B Quik Chek	Reller [50]	0.61 (0.45–0.75)	0.99 (0.98–1.00)
	Xpect	Boer, de [25]	0.44 (0.20–0.70)	1.00 (0.97–1.00)
	Xpect	Eastwood [27]	0.83 (0.74–0.90)	0.99 (0.98–1.00)
(E) Automated EIA toxins A/B	VIDAS CDAB	Eastwood [27]	0.98 (0.93–1.00)	0.99 (0.98–1.00)
	VIDAS CDAB	Swindells [61]	0.53 (0.27–0.79)	1.00 (0.97–1.00)
(F) NAAT	Amplivue	Eckert [8]	0.96 (0.78–1.00)	0.95 (0.91–0.97)
	BD GeneOhm	Eastwood [27]	0.92 (0.85–0.97)	0.95 (0.93–0.97)
	BD GeneOhm	Stamper (2009–1) [59]	0.91 (0.78–0.97)	0.95 (0.92–0.97)
	BD GeneOhm	Swindells [61]	1.00 (0.78–1.00)	0.98 (0.94–1.00)
	BD GeneOhm	Terhes [63]	0.95 (0.82–0.99)	0.96 (0.94–0.98)
	Illumigene	Noren [42]	1.00 (0.90–1.00)	0.93 (0.89–0.96)
	Illumigene	Ota [45]	0.88 (0.70–0.98)	0.97 (0.93–0.99)
	Illumigene	Pancholi [46]	0.87 (0.66–0.97)	0.91 (0.86–0.95)
	Prodesse ProGastro Cd assay	Stamper (2009–2) [58]	0.83 (0.65–0.94)	0.96 (0.92–0.98)
	Xpert *C. difficile*	Berry [20]	1.00 (0.94–1.00)	0.94 (0.92–0.95)
	Xpert *C. difficile*	Huang [31]	0.96 (0.78–1.00)	0.87 (0.82–0.92)
	Xpert *C. difficile*	Pancholi [46]	1.00 (0.85–1.00)	0.89 (0.83–0.93)
	Xpert *C. difficile*	Planche [47]	0.98 (0.96–0.99)	0.93 (0.92–0.94)
	Xpert *C. difficile*	Swindells [61]	1.00 (0.78–1.00)	0.97 (0.93–0.99)

CI, confidence interval; CCNA, cell cytotoxicity neutralization assay; EIA, enzyme immunoassay; GDH, glutamate dehydrogenase; NAAT, nucleic acid amplification test.

suboptimal PPV and NPV have implications. A low PPV will result in many patients with false-positive results. These noninfected patients may receive unnecessary treatment for CDI, and unnecessary isolation precautions may be taken. A low NPV will result in many undetected cases, which may not only have implications for individual patients but also for further transmission of *C. difficile*. It is therefore important to be aware not only of the sensitivity and specificity of an assay but also of the CDI prevalence in the tested population, as the predictive values and hence the clinical utility of the assays depend on them.

The easiest way to diagnose CDI would be to use a single rapid laboratory test that is able to reliably predict disease status. A rapid CDI diagnosis is associated with more prompt CDI treatment and less unnecessarily treated patients [74]. However, two problems arise if the rapid assays are used as stand-alone test for diagnosing CDI. First, as described above, the PPVs of even the most specific tests are inadequate at low disease prevalence. If toxin EIAs were to be used in an endemic situation (CDI prevalence of 5% in the tested population, PPV 81%), an unacceptably high percentage (19%) of patients with a positive test result would not actually have CDI. Second, as the targets identified by the index tests are (just like the targets of the reference test) different from each other, a positive index test does not necessarily indicate a real CDI case. Two of the three categories of index test are not able to differentiate carriers from CDI patients: both GDH EIAs and NAATs do not detect free toxins. Using NAAT as a stand-alone test and relying on clinical symptoms to discern patients with CDI from asymptomatic carriers is not an optimal approach: patients colonized by a toxigenic *C. difficile* strain may very well develop diarrhoea due to other causes, and no specific clinical symptoms exist to differentiate CDI

Table 6
Sensitivity and specificity of index tests compared to TC

Type	Index test	Study	Sensitivity (95% CI)	Specificity (95% CI)
(A) Well-type EIA GDH	*C. diff* Chek-60	Planche [47]	0.94 (0.93–0.96)	0.94 (0.94–0.95)
(B) Membrane-type EIA GDH	*C. diff* Quik Chek	Eckert [8]	0.97 (0.85–1.00)	0.95 (0.92–0.97)
	C. diff Quik Chek	Walkty [70]	0.83 (0.71–0.91)	0.97 (0.95–0.98)
	ImmunoCard *C. difficile*	Barkin [19]	1.00 (0.90–1.00)	1.00 (0.98–1.00)
	ImmunoCard *C. difficile*	Jacobs [32]	0.60 (0.32–0.84)	0.76 (0.68–0.83)
	Quik Chek Complete—GDH	Bruins [21]	0.97 (0.90–1.00)	0.98 (0.96–0.98)
	Quik Chek Complete—GDH	Kawada [33]	1.00 (0.88–1.00)	0.88 (0.71–0.96)
	Quik Chek Complete—GDH	Swindells [61]	1.00 (0.81–1.00)	0.97 (0.92–0.99)
(C) Well-type EIA toxins A/B	*Clostridium difficile* Tox A/B II	Planche [47]	0.58 (0.55–0.61)	0.99 (0.98–0.99)
	Clostridium difficile Tox A/B II	Snell [56]	0.85 (0.72–0.93)	0.98 (0.96–0.99)
	Premier toxins A/B	Barkin [19]	0.86 (0.71–0.95)	0.91 (0.86–0.94)
	Premier toxins A/B	Bruins [21]	0.41 (0.30–0.53)	0.99 (0.98–0.99)
	Premier toxins A/B	Leitner [38]	0.40 (0.21–0.61)	1.00 (0.98–1.00)
	Premier toxins A/B	Novak-Weekley [43]	0.58 (0.46–0.70)	0.95 (0.92–0.97)
	Premier toxins A/B	Planche [47]	0.46 (0.42–0.49)	0.99 (0.99–0.99)
	Premier toxins A/B	Sloan [55]	0.48 (0.32–0.63)	0.98 (0.94–1.00)
	Ridascreen toxins A/B	Mattner [40]	0.52 (0.36–0.68)	0.98 (0.95–0.99)
(D) Membrane-type EIA toxins A/B	ImmunoCard toxins A/B	Bruins [21]	0.41 (0.30–0.53)	0.99 (0.98–1.00)
	ImmunoCard toxins A/B	de Jong [26]	0.47 (0.23–0.72)	0.99 (0.96–1.00)
	ImmunoCard toxins A/B	Sloan [55]	0.48 (0.32–0.63)	0.99 (0.95–1.00)
	Quik Chek Complete—Tox A/B	Bruins [21]	0.55 (0.43–0.66)	1.00 (1.00–1.00)
	Quik Chek Complete—Tox A/B	Calderaro [23]	0.68 (0.55–0.80)	0.89 (0.84–0.92)
	Quik Chek Complete—Tox A/B	Hart [29]	0.29 (0.16–0.44)	1.00 (0.97–1.00)
	Quik Chek Complete—Tox A/B	Kawada [33]	0.79 (0.59–0.92)	0.97 (0.84–1.00)
	Quik Chek Complete—Tox A/B	Kim (2014) [35]	0.64 (0.50–0.76)	0.98 (0.96–0.99)
	Quik Chek Complete—Tox A/B	Swindells [61]	0.61 (0.36–0.83)	1.00 (0.97–1.00)
	Tox A/B Quik Chek	Kawada [33]	0.71 (0.51–0.87)	0.94 (0.79–0.99)
	Tox A/B Quik Chek	Le Guern [37]	0.43 (0.28–0.59)	1.00 (0.98–1.00)
	Tox A/B Quik Chek	Wren [71]	0.40 (0.30–0.51)	1.00 (1.00–1.00)
	Xpect	Sloan [55]	0.48 (0.32–0.63)	0.84 (0.77–0.89)
(E) Automated EIA toxins A/B	VIDAS CDAB	Jong, de [26]	0.71 (0.42–0.92)	0.95 (0.90–0.98)
	VIDAS CDAB	Kim (2012) [34]	0.64 (0.31–0.89)	1.00 (0.97–1.00)
	VIDAS CDAB	Kim (2014) [35]	0.76 (0.61–0.87)	0.97 (0.96–0.99)
	VIDAS CDAB	Shin (2009–1) [52]	0.68 (0.62–0.73)	0.96 (0.95–0.97)
	VIDAS CDAB	Shin (2009–2) [51]	0.69 (0.59–0.78)	0.97 (0.94–0.98)
	VIDAS CDAB	Shin (2012–2) [54]	0.44 (0.30–0.60)	1.00 (0.98–1.00)
	VIDAS CDAB	Swindells [61]	0.44 (0.22–0.69)	1.00 (0.97–1.00)
(F) NAAT	Advansure CD	Kim (2012) [34]	1.00 (0.72–1.00)	0.98 (0.94–1.00)
	Advansure CD	Soh [57]	0.85 (0.65–0.96)	0.98 (0.95–1.00)
	Amplivue	Eckert [8]	0.86 (0.71–0.95)	0.98 (0.95–0.99)
	BD GeneOhm	Buchan [22]	0.97 (0.86–1.00)	0.98 (0.95–1.00)
	BD GeneOhm	Hart [29]	0.89 (0.76–0.96)	0.99 (0.95–1.00)
	BD GeneOhm	Le Guern [37]	0.95 (0.85–0.99)	1.00 (0.98–1.00)
	BD GeneOhm	Shin (2012–1) [53]	0.96 (0.88–0.99)	0.97 (0.93–0.99)
	BD GeneOhm	Swindells [61]	0.94 (0.73–1.00)	0.99 (0.96–1.00)
	BD GeneOhm	Viala [69]	0.96 (0.85–0.99)	0.98 (0.89–1.00)
	BD Max Cdiff	Le Guern [37]	0.98 (0.88–1.00)	1.00 (0.98–1.00)
	BD Max Cdiff	Leitner [38]	0.96 (0.80–1.00)	0.99 (0.96–1.00)
	GenomEra	Hirvonen [30]	1.00 (0.95–1.00)	0.99 (0.96–1.00)
	Illumigene	Barkin [19]	1.00 (0.90–1.00)	1.00 (0.98–1.00)
	Illumigene	Bruins [21]	0.93 (0.85–0.98)	1.00 (0.99–1.00)
	Illumigene	Buchan [22]	0.93 (0.68–1.00)	0.95 (0.88–0.99)
	Illumigene	Calderaro [23]	1.00 (0.94–1.00)	0.83 (0.78–0.87)
	Illumigene	Hart [29]	0.89 (0.76–0.96)	1.00 (0.97–1.00)
	Illumigene	Lalande [7]	0.92 (0.80–0.98)	0.99 (0.98–1.00)
	Illumigene	Soh [57]	0.92 (0.75–0.99)	0.99 (0.97–1.00)
	Illumigene	Viala [69]	0.87 (0.73–0.95)	1.00 (0.93–1.00)
	Illumigene	Walkty [70]	0.73 (0.60–0.83)	1.00 (0.98–1.00)
	Portrait	Buchan [22]	0.98 (0.94–1.00)	0.93 (0.90–0.95)
	Prodesse ProGastro Cd assay	Stamper (2009–2) [58]	0.77 (0.62–0.89)	0.99 (0.97–1.00)
	Seeplex ACE	Shin (2012–1) [53]	0.90 (0.80–0.96)	0.97 (0.93–0.99)
	Verigene	Caroll [24]	0.91 (0.87–0.94)	0.93 (0.91–0.94)
	Xpert *C. difficile*	Buchan [22]	1.00 (0.94–1.00)	0.92 (0.87–0.95)
	Xpert *C. difficile*	Novak-Weekley [43]	0.94 (0.86–0.98)	0.96 (0.94–0.98)
	Xpert *C. difficile*	Planche [47]	0.95 (0.93–0.96)	0.96 (0.96–0.97)
	Xpert *C. difficile*	Shin (2012–2) [54]	1.00 (0.93–1.00)	0.95 (0.91–0.98)
	Xpert *C. difficile*	Swindells [61]	1.00 (0.81–1.00)	0.99 (0.96–1.00)
	Xpert *C. difficile*	Tenover [62]	0.93 (0.90–0.96)	0.94 (0.93–0.95)
	Xpert *C. difficile*	Viala [69]	0.98 (0.88–1.00)	0.98 (0.89–1.00)

CI, confidence interval; EIA, enzyme immunoassay; GDH, glutamate dehydrogenase; NAAT, nucleic acid amplification test; TC, toxigenic culture.

Table 7
Sensitivity and specificity of index tests compared to culture

Type	Index test	Study	Sensitivity (95% CI)	Specificity (95% CI)
(A) Well-type EIA GDH	*C. diff* Chek-60	Fenner [28]	0.93 (0.88–0.97)	0.97 (0.95–0.97)
	C. diff Chek-60	Reller (2007) [49]	1.00 (0.98–1.00)	0.67 (0.61–0.72)
	C. diff Chek-60	Snell [56]	0.94 (0.86–0.98)	0.98 (0.96–0.99)
	C. diff Chek-60	Zheng [72]	0.71 (0.63–0.78)	0.88 (0.85–0.90)
(B) Membrane-type EIA GDH	*C. diff* Quik Chek	Wren [71]	0.95 (0.90–0.98)	0.99 (0.98–1.00)
	Quik Chek Complete—GDH	Bruins [21]	0.95 (0.89–0.99)	0.99 (0.98–0.99)
	Quik Chek Complete—GDH	Hart [29]	0.87 (0.75–0.95)	0.97 (0.91–0.99)
	Quik Chek Complete—GDH	Kawada [33]	1.00 (0.88–1.00)	0.93 (0.78–0.99)
	Quik Chek Complete—GDH	Swindells [61]	1.00 (0.82–1.00)	0.98 (0.93–1.00)
	ImmunoCard *C. difficile*	Jacobs [32]	0.75 (0.59–0.87)	0.90 (0.83–0.95)
	ImmunoCard *C. difficile*	Kawada [33]	0.80 (0.61–0.92)	1.00 (0.88–1.00)

CI, confidence interval; EIA, enzyme immunoassay; GDH, glutamate dehydrogenase; NAAT, nucleic acid amplification test.

Table 8
Pooled sensitivities and specificities of categories of tests

Type	Test	Compared to CCNA			Compared to TC			Compared to culture		
		No. of studies	Sensitivity (95% CI)	Specificity (95% CI)	No. of studies	Sensitivity (95% CI)	Specificity (95% CI)	No. of studies	Sensitivity (95% CI)	Specificity (95% CI)
EIA GDH	Total	12	0.94 (0.89–0.97)	0.90 (0.88–0.92)	8	0.96 (0.86–0.99)	0.96 (0.91–0.98)	11	0.94 (0.86–0.97)	0.96 (0.92–0.98)
	Well type	5	0.94 (0.91–0.97)	0.92 (0.92–0.93)	1	0.94 (0.93–0.96)	0.94 (0.94–0.95)	4	0.89 (0.86–0.91)	0.91 (0.90–0.92)
	Membrane type	7	0.98 (0.78–1.00)	0.90 (0.87–0.93)	7	0.97 (0.84–1.00)	0.96 (0.90–0.99)	7	0.93 (0.84–0.97)	0.98 (0.95–0.99)
EIA toxins A/B	Total	27	0.83 (0.76–0.88)	0.99 (0.98–0.99)	29	0.57 (0.51–0.63)	0.99 (0.98–0.99)			
	Well type	18	0.85 (0.77–0.91)	0.98 (0.96–0.99)	16	0.60 (0.52–0.68)	0.98 (0.97–0.99)			
	Membrane type	9	0.79 (0.66–0.88)	0.99 (0.98–0.99)	13	0.53 (0.45–0.61)	0.99 (0.97–1.00)			
NAAT		14	0.96 (0.93–0.98)	0.94 (0.93–0.95)	32	0.95 (0.92–0.97)	0.98 (0.97–0.99)			

CI, confidence interval; CCNA, cell cytotoxicity neutralization assay; EIA, enzyme immunoassay; GDH, glutamate dehydrogenase; NAAT, nucleic acid amplification test; TC, toxigenic culture.

Table 9
PPV and NPV for different categories of index tests at hypothetical CDI prevalences of 5, 10, 20 and 50%

Test type	CDI prevalence 5%		CDI prevalence 10%		CDI prevalence 20%		CDI prevalence 50%	
	PPV	NPV	PPV	NPV	PPV	NPV	PPV	NPV
Well-type EIA GDH	38	100	54	99	72	98	91	94
Membrane-type EIA GDH	34	100	52	100	71	99	91	98
Well-type EIA toxins A/B	69	99	83	98	91	96	98	87
Membrane-type EIA toxins A/B	81	99	90	98	95	95	99	83
NAAT	46	100	64	100	80	99	94	96

Pooled estimates of sensitivity and specificity compared to cell cytotoxicity neutralization assay were used to calculate the predictive values.
CDI, *Clostridium difficile* infection; EIA, enzyme immunoassay; GDH, glutamate dehydrogenase; NAAT, nucleic acid amplification test; NPV, negative predictive value; PPV, positive predictive value.

from other causes of diarrhoea. From the above, we conclude that neither GDH EIA nor toxin A/B EIA or NAAT can reliably be used as a stand-alone test to diagnose CDI.

Because no single test is suitable to be used as a stand-alone test, it is best to combine two tests in an algorithm in order to optimize the diagnosis of CDI. The advantage of an algorithm is that tests can be combined in such a way that the percentage of false-positive results can be decreased. This can be done by testing all samples with a first test, then performing reflex testing on samples with a positive first test result only. The first test should be a test that reliably classifies samples with a negative test result as non-CDI; these samples will not be tested further. This first test should therefore be a test with a high NPV (i.e. a highly sensitive test). Thus, in our case, this first test can either be a GDH EIA or NAAT. The choice between these two categories of assays can be made by each individual laboratory. The second test should be a test with a high PPV (i.e. a highly specific test), so that all samples with a positive second test result can reliably be classified as CDI. Toxin A/B EIAs can very well be used for this purpose, because besides being the most specific tests, these tests also have the advantage of detecting free toxin. Thus, after application of a first sensitive test (GDH EIA or NAAT), the toxin A/B EIA can then be performed as a second step on all samples that tested positive by NAAT or GDH EIA (Fig. 3(a)). Samples with a positive second test result can be classified as CDI likely to be present. However, samples with a first positive test result but a negative toxin A/B EIA need to be clinically evaluated. Among these samples, CDI (with toxin levels below the threshold of detection or a false-negative toxin A/B EIA result) or *C. difficile* carriage is possible.

A recent large study tried to establish the optimum diagnostic algorithm for CDI [47]. In this study, 12 420 faecal samples were tested by diverse commercial assays, TC and CCNA. The overall performance of combined tests was superior to individual tests. The combination of a NAAT (Xpert) and toxin A/B EIA (Techlab Tox A/B II) was the optimal algorithm compared to the CCNA test, but the GDH EIA (*C. diff* Chek-60)–toxin A/B EIA algorithm performed almost identically [47]. These findings can be seen as a validation of our more theoretical approach to establish the best testing strategy,

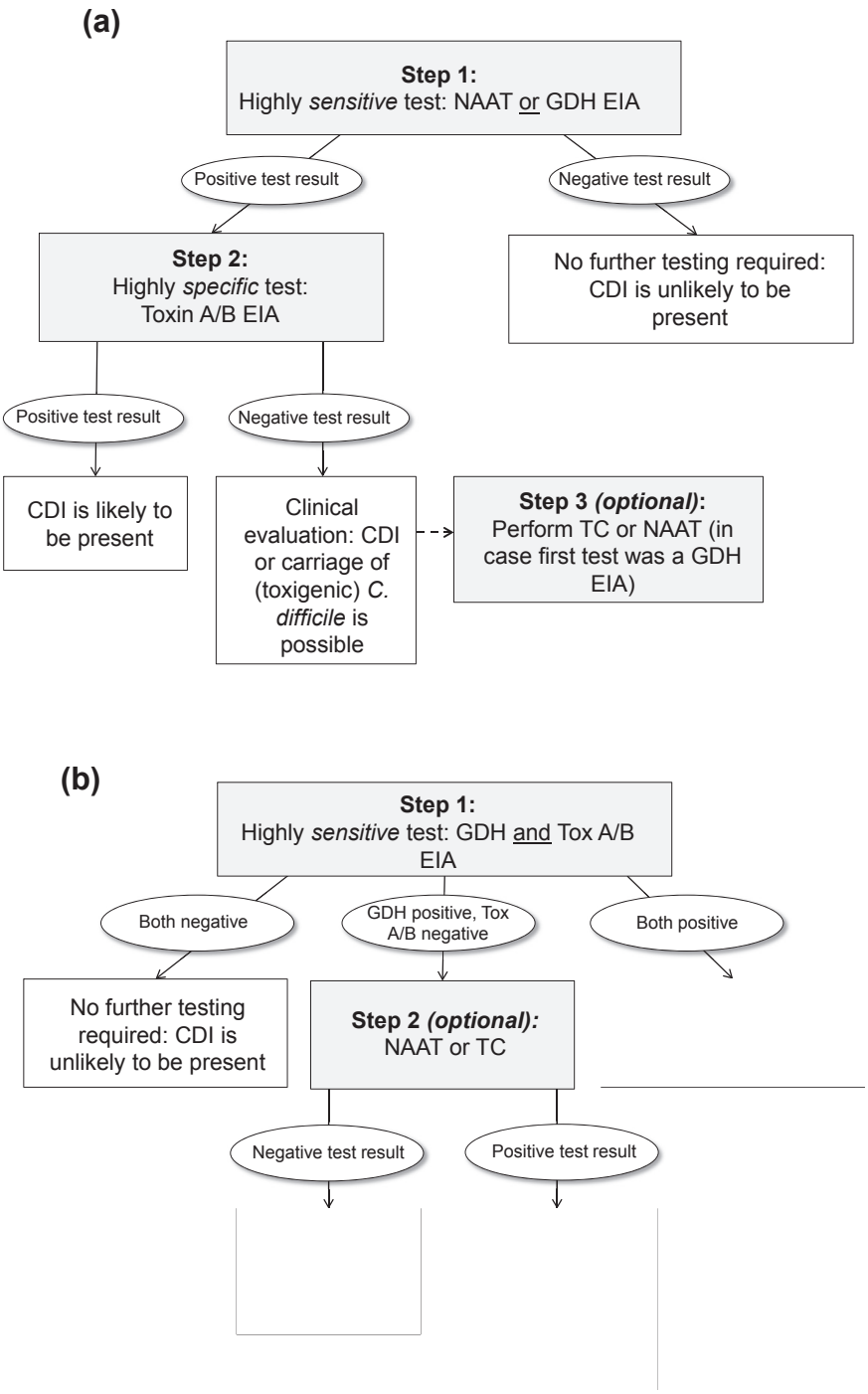

Fig. 3. Recommended algorithms for CDI testing. (a) GDH or NAAT–Tox A/B algorithm. (b) GDH and Tox A/B–NAAT/TC algorithm. CDI, *Clostridium difficile* infection; GDH, glutamate dehydrogenase; NAAT, nucleic acid amplification test; TC, toxigenic culture; Tox A/B, toxin A/B; EIA, enzyme immunoassay.

and they endorse the conclusion that NAAT–toxin A/B EIA, or alternatively GDH EIA–toxin A/B EIA, are two of the best algorithms to diagnose CDI (Fig. 3(a)).

An alternative algorithm is to test simultaneously with both a GDH and toxin A/B EIA. An assay is available that includes both these targets in one system (*C. diff* Quik Chek Complete; Techlab), but the sensitivity of the toxin component is unclear and may not be as a high as some individual toxin EIAs (Tables 5–7). Samples that test negative for both GDH and toxin A/B can reliably be classified as non-CDI, while samples that test positive for both GDH and toxin A/B can be classified as CDI likely to be present. Samples with a GDH-positive result but that are negative for toxin could undergo reflex testing by NAAT to determine if a toxigenic *C. difficile* strain is present (Fig. 3(b)). Samples with a negative GDH result but that are positive for toxin need to be retested, as this is an invalid result. Only one study evaluating this kind of algorithm and comparing it to a reference test was identified in the literature [45]. In this specific study, samples were screened by *C. diff* Quik Chek Complete, and inconclusive results underwent reflex testing by Illumigene. The overall sensitivity for this algorithm compared to

CCNA was 81%, while specificity was reported to be 100%. The overall sensitivity and specificity of this and the aforementioned algorithm depend, however, on the individual assays that are included.

Although we recommend the use of an algorithm for CDI testing based on two rapid assays, every laboratory should also be able to isolate *C. difficile,* ideally via TC from selected samples, for two reasons. First, TC offers the ability to perform molecular typing and susceptibility testing on recovered isolates from positive samples and can be used for outbreak investigations [75]. Second, samples with a positive GDH EIA and/or NAAT but a negative toxin A/B EIA may either be samples that tested falsely positive on GDH EIA/NAAT or samples containing *C. difficile,* but without detectable free toxin. To be able to discern between these two conditions, a third-stage reflex test to either a TC or NAAT or GDH (if not yet performed) can be performed on samples with discordant results. For patients with evidence of *C. difficile* but negative toxin A/B EIA, clinical evaluation is needed, and clinical considerations come into play to determine a case as either positive or negative; these patients can either be CDI patients with undetectable toxin levels, or false-negative toxin A/B EIA results or potential carriers of toxigenic *C. difficile.* Although *C. difficile* carriers may play an important role in the spread of the disease [76,77], the indication for treating these patients for CDI remains controversial. In addition, the need for isolation precautions for these patients remains to be clarified. Therefore, performing TCs on these samples can be of importance for epidemiologic purposes, but it is not yet a prerequisite for patient management.

The decision to treat CDI is ultimately a clinical decision, guided by laboratory results. No tests are infallible, so it may be clinically justified to treat a patient for CDI despite negative test results; treatment should not be withheld on the basis of laboratory tests alone. However, patients with toxin-negative specimens should have alternative diagnoses considered and excluded; provided an adequate testing strategy is followed, most patients with negative results for CDI will truly not have this infection, and thus treatment will be unnecessary.

Besides the question which assay or algorithm should be used for CDI detection, another issue is the number of specimens per patient that should be submitted for testing. Before the introduction of algorithms to diagnose CDI, lack of confidence in the tests for CDI detection (mainly toxin EIAs) led to the practice of multiple sample submission. However, the diagnostic gain of repeat testing within a 7-day period with both toxin A/B EIA and PCR was demonstrated to be very low [78]. If one of the above proposed algorithms is used, then the adequate NPV at low disease prevalence is based on original studies which did not test samples repeatedly by index test and only once by reference test. This adequate NPV indicates that routine submission of multiple samples after a first negative test round has to be discouraged; these samples can reliably be classified as non-CDI.

However, in cases of ongoing clinical suspicion during an endemic situation, the submission of a repeat sample may be justified, as these specific algorithms will have adequate PPVs even in a low-prevalence situation.

In outbreak situations with a higher CDI prevalence in the tested population, the NPV of the algorithm will fall. In such an outbreak situation, submitting a repeat sample in case of ongoing clinical suspicion will be of value, as has been shown for toxin A/B EIA [79].

Testing for cure is not recommended, as patients can shed spores and even toxins of *C. difficile* for a prolonged time after resolution of diarrhoea [80,81]. The infection can be considered resolved when symptoms of diarrhoea have resolved.

Selection of which of submitted stool samples should be tested for CDI is also important. Recognition of potential CDI cases may be burdensome, as it is increasingly being recognized that CDI is not only acquired in healthcare facilities by patients with well-known risk factors for the disease. In the Netherlands, *C. difficile* was relatively frequent among patients with diarrhoeal complaints in general practice [82]. Community-onset CDI can affect all age groups, and many patients do not have known risk factors [83,84]. A recent study showed that on a single day in Spain, two of every three CDI episodes were underdiagnosed or misdiagnosed owing to nonsensitive tests (19.%) but more importantly to lack of suspicion and request (47.6%) [85]. Especially for nonhospitalized patients and younger patients, CDI tests were not requested [85]. This trend was also seen in a study involving almost 500 hospitals in 20 countries across Europe: on two sampling days, 23% of samples with a positive CDI test result were initially missed due to lack of suspicion [73]. Hence, restricting testing to samples with a physician's request for CDI testing will lead to underdiagnosis.

Empirical testing of all unformed stool samples submitted to the laboratory was shown to increase the diagnostic yield [73,86]. We recommend testing all unformed faecal samples submitted to the laboratory (except samples from children under age 3). In infants, high rates of asymptomatic colonization with both toxigenic and nontoxigenic strains have been described [87]. Even in the case of toxin production, infants rarely develop clinical disease. However, CDI can occur in infants and young children [88]. A recently released policy statement from the American Academy of Pediatrics recommends to test for CDI only if age-specific clinical criteria are met [14]. According to their statement, searching for alternative aetiologies should be performed even in the case of a positive CDI test for children under 3 years of age. Concerning the problematic interpretation of positive test results in this population, we indeed recommend to limit testing of samples from children under age 3 to samples with a physician's request only. Unformed stool samples of children 3 years and older can be managed in the same way as described above.

Clinical signs and symptoms are essential to CDI diagnosis. Therefore, formed stool samples should not be tested for CDI, as these do not meet the clinical criteria of CDI. However, sometimes only solid parts of diarrhoeal faeces may be collected and submitted for *C. difficile* testing. Local protocols therefore need to enable *C. difficile* testing on specific samples to take place. Also, an exception has to be made for patients suspected of CDI who have ileus. In these patients, a rectal swab can be used with adequate sensitivity and specificity for (toxigenic) culture, NAAT or GDH EIA [89,90]. The use of perirectal swabs for NAAT or GDH EIA testing might also be an alternative in selected patient populations but may depend on the presence of faecal staining of the swab [89–91]. However, the use of (peri)rectal swabs has not been evaluated for toxin EIA, and therefore clinical judgement remains essential in these cases to discern colonized patients from patients with CDI.

Recommendations

Sample selection

We recommend that CDI testing should not be limited to samples with a specific physician's request. (Strong recommendation, high-quality evidence)

We suggest that at least all submitted unformed stool samples from patients 3 years or older should be tested for CDI. (Weak recommendation, low-quality evidence)

We suggest to limit testing of samples from children under age 3 to samples with a physician's request only. (Weak recommendation, low-quality evidence)

Formed stool samples should not be tested for CDI (except in case of paralytic ileus). (Good practice statement)

In patients suspected of ileus, a rectal swab can be used for (toxigenic) culture, NAAT or GDH EIA. (Strong recommendation, moderate-quality evidence)

Testing protocol

The diagnosis of CDI should be based on clinical signs and symptoms in combination with laboratory tests. Decision for treatment for CDI is a clinical decision and may be justified even if all laboratory tests are negative. (Good practice statement)
We recommend against the use of a single rapid test as a stand-alone test due to inadequate PPV in an endemic situation. (Strong recommendation, moderate-quality evidence)
We recommend the use of a 2-step algorithm (Fig. 3(A)). (Strong recommendation, moderate-quality evidence)
This algorithm should start with either NAAT or GDH EIA. Samples with a negative first test result can be reported as negative. (Strong recommendation, moderate-quality evidence)
Samples with a positive first test result should be tested further with a toxin A/B EIA. Samples with a positive second test results can be reported as CDI-positive. (Strong recommendation, moderate-quality evidence)
An alternative algorithm is to screen samples with both a GDH and toxin A/B EIA (Fig. 3(B)). Samples with concordant positive or negative results can be reported as such. Samples with a negative GDH result but positive for toxin need to be retested as this is an invalid result. (Strong recommendation, moderate-quality evidence)
Samples with a positive first test result and negative second test result (Fig. 3(A)) and samples with a GDH-positive test result but negative toxin A/B test result (Fig. 3(B)) may represent samples with CDI or *C. difficile* carriage and may optionally be tested with TC or NAAT (if not performed yet). (Weak recommendation, moderate-quality evidence)
We recommend to perform TC and molecular typing of recovered isolates in case of outbreak situations. (Good practice statement)

Repeated testing

Repeated testing after a first positive sample during the same diarrhoeal episode is not recommended in an endemic situation. (Strong recommendation, moderate-quality evidence)
Repeated testing after a first negative sample during the same diarrhoeal episode may be useful in selected cases with ongoing clinical suspicion during an epidemic situation or in cases with high clinical suspicion during endemic situations. (Strong recommendation, moderate-quality evidence)
A test of cure is not recommended. (Good practice statement)

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2016.03.010.

Transparency Declaration

All authors report no conflicts of interest relevant to this article.

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