Journal of Microbiological Methods 173 (2020) 105912

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Review

The reference method influence on the sensitivity of the *Clostridium difficile* enzyme immunoassays: A meta analysis

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ARTICLE INFO

Keywords:
Clostridium difficile
Meta-analysis
Sensitivity
Enzyme immunoassays
Bivariate model

ABSTRACT

The use of enzyme immunoassays to screen for toxins A and B produced by *Clostridium difficile* is a common procedure in algorithms designed for its detection. Moreover, the absence of a unique test capable of providing reliable results at low cost motivates a great discussion about which algorithm is the best. Thus, several studies have evaluated the performance of these enzyme immunoassays. However, all fail to provide sufficient explanations for the different behaviours observed in different studies that evaluate the same index test against a common reference method. Our main goal was to find out which factors affect the sensitivity of these assays, since the specificity is very close to 1.

In this research, we verified that sensitivity increases with the prevalence rate and with the proportion of reported cases of onset diarrhea. Therefore, its use is advisable for high prevalence rates (e.g. in an epidemic setting).

As far as reference methods are concerned, nucleic acid amplification tests can be used as a reference method, with a performance similar to the well-accepted toxigenic culture. The method chosen for toxigenicity screening in a toxigenic culture also seems to affect the evaluation performance of tests and should be better studied in the future.

1. Introduction

Clostridium difficile (CDI) is the most important cause of nosocomial diarrhea worldwide (Pancholi et al., 2012). In the United States, the now called *Clostridioides difficile* infection is the 18*th* leading cause of death among people over 65 (Rao et al., 2015).

Most pathogenic strains of CDI produce two toxins: toxin A and toxin B. Several laboratory techniques are available to detect CDI toxins or the genes that encode them in fecal samples. Screening for the presence of the glutamate dehydrogenase (GDH) enzyme is also a possibility. However, GDH enzyme is also produced by bacteria other than CDI, so the result can be misleading. The emergence of new strains related to hyper-virulent outbreaks and increased disease severity have been the seeds of much research related to rapid enzyme immunoassays (EIA) tests (Johansson et al., 2016).

Recent European guidelines on the diagnosis of CDI (Crobach et al., 2016) suggest the use of a two-step algorithm: a high sensitivity first step (a GDH EIA test or a nucleic acid amplification test (NAAT)) and a EIA test (highly specific) to detect toxins A and B as a second step. This last EIA is known for its lack of sensitivity. However, it is commonly used to confirm the infection (Goret et al., 2015).

Currently, there is no consensus on a gold standard test for the diagnosis of CDI. However, two reference tests are generally accepted in the literature: toxigenic culture (TC) for the screening for toxin-producing CDI in stool and cell cytotoxicity neutralisation assay (CCA) that detects free toxins in stool.

In TC, stool samples are first exposed to alcohol or heat shock to kill vegetative cells of CDI and other organisms. Nevertheless, CDI spores survive. Then, the samples are plated on selective agar and incubated at 37 °C anaerobically for a minimum of 48 h. If the isolates produce toxins in vitro, they must be confirmed as there may be nontoxigenic strains. Toxigenicity can be confirmed by NAAT, CCA or EIA for toxins A and B.

No standard method for TC assays with CDI has been established. A wide variety of media and differences in isolation protocols and variations in incubation time are common (Silva et al., 2014). These variations add another problem when assessing an EIA with TC as the gold standard method. In addition, (René et al., 2012) suggest that the

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https://doi.org/10.1016/j.mimet.2020.105912
Received 6 June 2019; Received in revised form 22 March 2020; Accepted 31 March 2020
Available online 09 April 2020

Table 1
Characteristics of the included primary studies (Prev: prevalence rate; Premier: Premier toxins A/B; Immuno: ImmunoCard toxins A/B (Meridian); Tox A + B: *C. difficile* toxin A + B (Diagnostic Automation); Ridascreen: Ridascreen toxins A/B (Biopharm); ProSpec T: Remel ProSpec T and Xpect (Oxoid); VIDAS: VIDAS CDAB (bioMérieux); Tox AB II: *Clostridium difficile* Tox A/B II; Quik Chek: Tox A/B Quik Chek (Techlab); Complete: Quik Chek Complete (Techlab)).

Study	Country	Reference	Index test	n	Prev. (%)
Ashraf et al. (2019)	USA	NAAT	Complete	486	30.9
Barkin et al. (2012)	USA	ETC	Premier	272	13.2
Benedek et al. (2016)	Germany	TC	VIDAS	236	17.8
Boyanton et al. (2012)	USA	NAATs	Tox AB II/Complete	139	15.1
Brown et al. (2011)	USA	CCA/NAAT	ProSpec T	357	9.8–10.4
Bruins et al. (2012)	Netherlands	TC	Premier/Immuno Complete	986	7.4
Canado et al. (2018)	Brazil	TC	Toz AB II/Complete	53	22.6
Caulfield et al. (2018)	USA	NAATs	Complete	500	12.0
Chapin et al. (2011)	USA	NAATs	Premier/Complete	31–34	73.5–80.6
Chung and Lee (2017)	South Korea	Xpert	Complete	231	35.9
de Boer et al. (2010)	Netherlands	CCA	Xpect	161	9.9
Eastwood et al. (2009)	UK	CCA/TC	all unless Tox A + B	556–600	17.2–20.9
Goldenberg et al. (2010)	UK	TC	Premier	500	7.2
Hart et al. (2014)	Australia	TC	Complete	150	30.0
Hernández-Rocha et al. (2013)	Chile	ETC/NAAT	VIDAS	230	10.0–10.4
Herrera-Cáceres et al. (2010)	Mexico	TC	Immuno/VIDAS	223–230	4.5–6.5
Hirvonen and Kaukoranta (2015)	Finland	TC/1NAAT	Quik Chek	302	23.8–25.8
Hong et al. (2014)	South Korea	TC	VIDAS	293	17.7
Humphries et al. (2013)	USA	TC	Premier	296	46.6
Jamal et al. (2014)	Kuwait	TC	Complete	409	3.2
Johansson et al. (2016)	Sweeden	NAAT	Complete	419	14.8
de Jong et al. (2012)	Netherlands	CCA/TC NAAT	Immuno	150	8.0–11.3
Kawada et al. (2011)	Japan	TC	Complete/Quik Chek	60	46.7
Kim et al. (2012)	South Korea	TC	VIDAS	125	8.8
Kim et al. (2014)	Korea	TC	Complete/VIDAS	590–608	8.3–9.0
Kosai et al. (2017)	Japan	NAAT	Complete/Xpect	118	28.8
Kvach et al. (2010)	USA	CCA/NAAT	TOX AB II	400	22.0–24.0
Kwon et al. (2017)	USA	TC	Tox AB II	111	8.1
Larson et al. (2010)	USA	CCA	Quik Chek	114	41.2
Le Guern et al. (2012)	France	TC	Quik Chek	360	12.2
Leitner et al. (2013)	Austria	TC	Premier	180	12.8
Makristathis et al. (2017)	Austria	CCA/TC	Tox AB II	300	8.3–11.7
McElgunn et al. (2014)	UK	NAAT	Ridascreen	27	92.6
Miller et al. (2013)	USA	CCA/NAAT	Complete	244	35.7–51.2
Moon et al. (2016)	South Korea	TC	VIDAS	271	20.3
Morinaga et al. (2018)	Japan	TC	Complete	231	22.9
Murad et al. (2015)	Canada	NAAT	Immuno	1592	13.3
Novak-Weekley et al. (2010)	USA	ETC	Premier	432	16.7
Ota and McGowan (2012)	USA	CCA	Premier/Complete	141	18.4
Peterson et al. (2011)	USA	TC	Premier/Complete Immuno/Tox AB II	1000	14.6
Planche et al. (2013)	UK	CCA/TC	Premier/Tox AB II	9161–12,369	5.9–8.5
Qutub et al. (2011)	Saudi Arabia	CCA	Tox AB II	150	34.7
Qutub et al. (2019)	Saudi Arabia	NAAT	Complete	103	14.6
Rajabally et al. (2016)	South Africa	TC	Immuno/VIDAS	141	21.2
Reller et al. (2010)	USA	CCA	Quik Chek	600	7.7
René et al. (2012)	Canada	CCA/TC	Immuno Quik Chek/Xpect	494	9.3–11.7
Samra et al. (2013)	Israel	NAAT	Complete/Quik Chek	223	35.4
Selvaraju et al. (2011)	USA	TC	Complete	199	23.6
Senoh et al. (2014)	Japan	TC/NAAT	Complete	44	34.0–43.2
Senok et al. (2017)	Saudi Arabia	NAAT	Complete	210	14.8
Sharp et al. (2010)	USA	1NAAT	Complete/Quik Chek	282	14.5
Shin et al. (2012)	South Korea	TC	VIDAS	245	18.4
Shin et al. (2016)	South Korea	TC/NAAT	VIDAS	329	23.7–26.4
Silva et al. (2014)	Brazil	CCA/TC	ProSpec T/Tox AB II Ridascreen	92	25.0–27.1
Strachan et al. (2013)	UK	TC	Premier	860	11.4
Swindells et al. (2010)	UK	CCA/TC NAATs	Complete/VIDAS	150	10.0–12.7
Tenover et al. (2010)	Canada/USA	ETC	Premier/Tox AB II	173–1023	11.2–19.1
Terhes et al. (2009)	Hungary	NAAT	VIDAS	557	8.8
van Prehn et al. (2015)	Netherlands	TC	VIDAS	3576	11.4
Vasoo et al. (2014)	USA	NAATs	Complete/Quik Chek	192	25.0
Vasoo et al. (2014)	UK	TC	Quik Chek	1007	8.6
Yoldaş et al. (2016)	Turkey	NAAT	Tox A + B	95	6.3

method used to the detection of toxins may be an important reason to explain the observed disparity. Enrichment TC (ETC) is also a possibility that improves the number of infected specimens detected.

CCA is an assay based on detecting the presence of free toxins in feces. In this assay, stool samples are filtrated and then added to toxin sensitive cell lines (e.g. Vero cells). After a 24 to 48 h incubation, the cytopathic effect that can be neutralized by antiserum is examined by microscopy.

These two reference tests are usually not performed for screening CDI, since they involve longer turnaround times (24-48 h), are more expensive and require specialized staff.

The high sensitivity of GDH assays (Canado et al., 2018) (very close to 100%) opens the possibility of using subsamples of GDH positive cases to assess the performance of the EIAs for toxins A and B with

minimal risk of bias.

The meta-analysis of diagnostic test accuracy involves modelling both sensitivity and specificity since they are usually correlated. When primary studies report a wide range of values for both measures, it is recommended to estimate a bivariate model (Lee et al., 2015; Makristathis et al., 2017). It accounts both within and between study heterogeneity. In a previous work on this subject, (Crobach et al., 2016) derived pooled estimates and confidence intervals (CIs) through the use of a random effects logistic regression.

The purpose of this work is to contribute to the understanding of the different performances observed for the same EIA concerning the same reference method. In literature, there are already some studies that point out to some possible reasons to observe different performances for the similar quality assessment of the EIAs. However, they fail to provide statistical evidence. For instance, it was shown by (Guh et al., 2019; Origuen et al., 2018) that the severity of the disease is related to a higher sensitivity of the EIA. Proton pump inhibitor medication (Boyanton et al., 2012), prior CDI episodes (Hernández-Rocha et al., 2013) and the type of ribotypes (Johansson et al., 2016; Lee et al., 2014) are possible explanations for a higher sensitivity. The performance of EIAs in a pediatric setting is a problem and can result in a high number of false positives results (Luna et al., 2011; Toltzis et al., 2012).

Testing-related factors, such as pre-analytical conditions (storage and transport temperatures), can also affect performance. See, for instance, (Krutová and Nyc, 2018; Viechtbauer, 2010). Pre-sampling therapy can negatively affect the sensitivity of the assay (Bogaty et al., 2017; Zarandi et al., 2017; Nazemalhosseini-Mojarad et al., 2011).

Our work aims to find significant statistical factors that affect the sensitivity of the EIAs for toxins A and B. The specificity of these assays is not a problem, as it is recognized as being very close to 1.

2. Material and methods

The search strategy and data extraction followed the general guidelines found in other papers, e.g. (Crobach et al., 2016).

2.1. Search strategy

Studies were considered eligible for this meta-analysis if they meet the following criteria:

- describe original research;
- compare the index test to a standard reference (CCA, TC or ETC) or to a non-standard reference (NAAT or NAATs followed by TC if results are discordant);
- performed on clinical human stool samples;
- report sufficient information to allow the calculation of the number of true positives (TP), false positives (FP), false negatives (FN) and true negatives (TN);
- use index tests that are still available;
- published in the English language in 2009 or later.

As the number of publications on the subject addressed is quite high for a meta-analysis we decided to work with studies published in the last 10 years.

The use of several NAATs with CCA for discordant cases was not considered, as we used NAAT as a gold standard similar to TC. This resulted in only two reference methods: CCA and non-CCA.

We searched PubMed for studies concerning the laboratory diagnosis of CDI infections. Searches were performed in February 2019 according to the search strategy provided in the supplementary matherial. Studies from a previous work (Crobach et al., 2016) were also analysed and added when fulfilling our criteria.

2.2. Data extraction

The three authors of this paper independently assessed the study eligibility. Inconsistencies were resolved by consensus. The number of TP, FP, FN and TN was extracted according to each reference method. More than one reference could be found in the same paper. This led to the use of different sample sizes to the same study (see Table 1). We extracted additional information, including year of publication, study population and the reference method of choice. All the extracted data was imputed into Microsoft Excel 2016 and is provided in the Supplementary Material.

2.3. Statistical analysis

A graphical overview of the results is given by forest plots produced according to the reference method (CCA or non-CCA). The associated CIs for the sensitivity are based in the arcsine transformation, which is a variance stabilizing transformation (Hartung et al., 2008). All estimates were computed under a random model setting obtained using the restricted maximum likelihood (REML) methodology. Estimates of the heterogeneity τ^2 between studies were obtained via REML (Viechtbauer, 2010). I^2 statistic (the ratio between τ^2 and the total variance) was also computed. All of these procedures were performed in the R (R Core Team, 2014) package metafor (Viechtbauer, 2010). CIs were computed using the Knapp and Hartung adjustment (Hartung et al., 2008). Trim and fill method (Duval and Tweedie, 2000) was applied to screen for possible publication bias.

We used a bivariate model to jointly model the sensitivity and the false positive rate for both reference methods (Rutter and Gatsonis, 1995, 2001). This model is equivalent to the hierarchical summary receiver operator characteristic (HSROC) model (Reitsma et al., 2005) in the absence of covariates (Harbord et al., 2007). The bivariate model assumes an underlying bivariate normal distribution for the sensitivity and false positive rate. The models were obtained using R package mada (Doebler and Holling, 2017) where the variance components and the random model were estimated by REML. When any of the outcome measures was zero the value of 0.5 was added.

3. Results

3.1. Study characteristics

The search was performed in PubMed leading to 1008 different citations (see Supplementary Material). A total of 41 studies (published since 2009) from a previous meta-analysis (Crobach et al., 2016) were added, although three of those studies were not used. This gave us a total of 1018 studies after removing duplicates (one experimental study was described in two different papers). Reading the abstract allowed us to unanimously reject 899 studies for not being related to the subject addressed here. From the remaining 119 studies, 57 studies were excluded from further analysis. Thus, a total of 62 studies were included in the meta-analysis. The selection process is detailed in PRISMA flow chart (Moher et al., 2009) displayed in Fig. 1.

Sample sizes ranged from 27 to 12,369 and the number of infected individuals (according to the reference method) ranged from 6 to 1034 (prevalence rate 3.2–92.6%). In 10 studies (Benedek et al., 2016; Brown et al., 2011; Canado et al., 2018; de Boer et al., 2010; Hart et al., 2014; Johansson et al., 2016; de Jong et al., 2012; McElgunn et al., 2014; Selvaraju et al., 2011; van Prehn et al., 2015), not all tests were performed (or at least this is not clear) on unformed stool specimens.

In three studies (Hart et al., 2014; Ota and McGowan, 2012; Selvaraju et al., 2011) individuals are all under the age of 18 while 13 studies (Ashraf et al., 2019; Barkin et al., 2012; Canado et al., 2018; Hernández-Rocha et al., 2013; Humphries et al., 2013; Kawada et al., 2011; Kwon et al., 2017; Viechtbauer, 2010; Morinaga et al., 2018; Rajabally et al., 2016; Shin et al., 2016; Strachan et al., 2013;

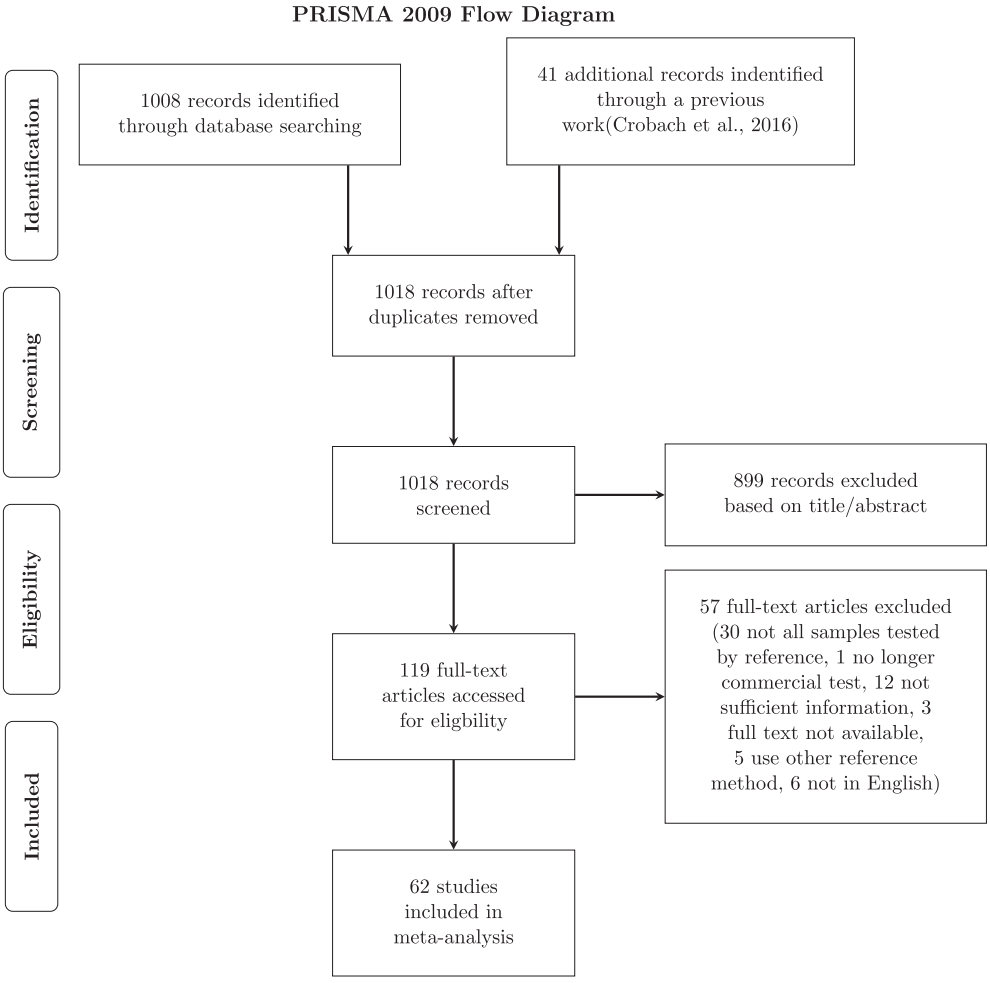


Fig. 1. Flow-chart showing the process of identification of studies for inclusion in the systematic review.

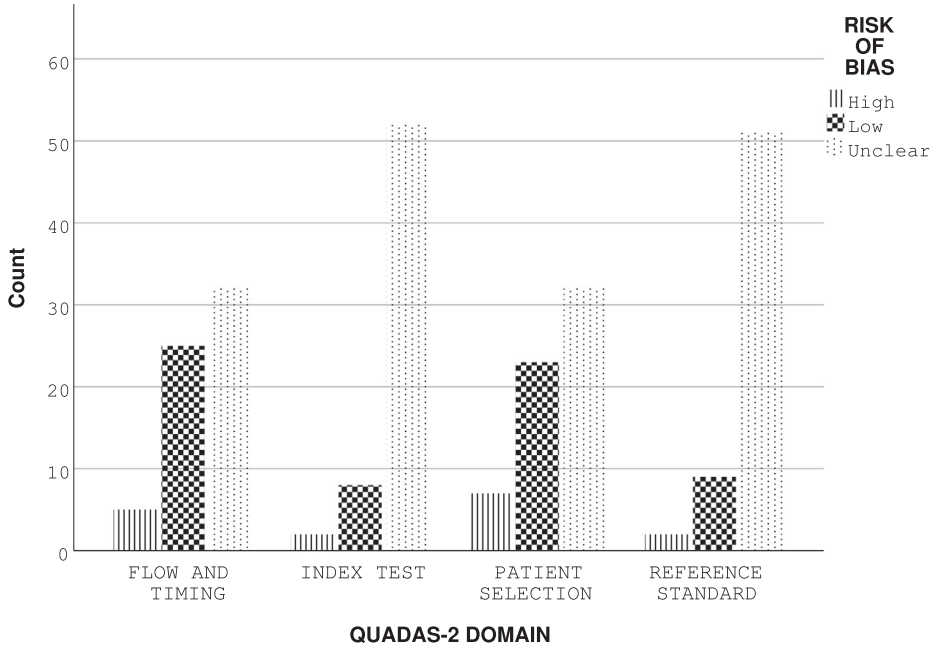
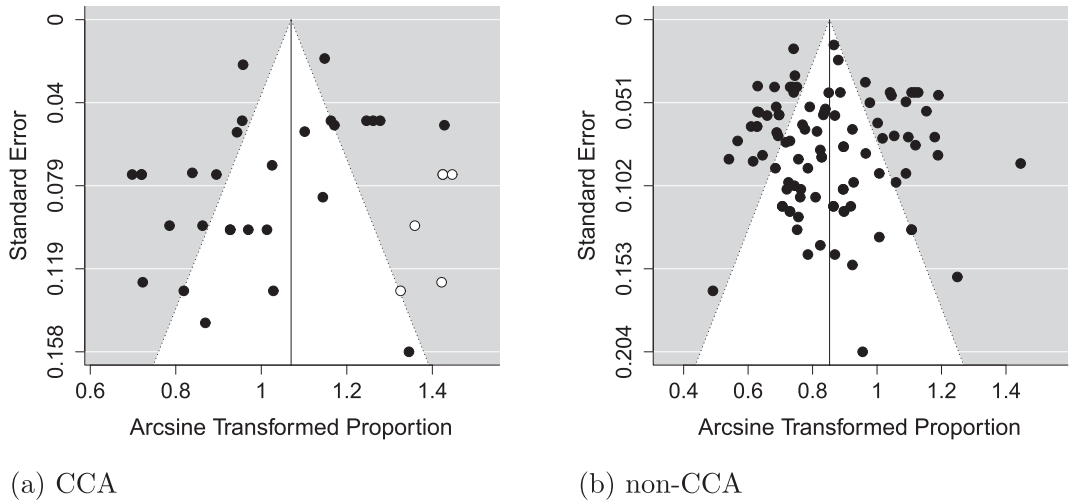


Fig. 2. Proportion of studies with low, high or unclear risk of bias (QUADAS-2 assessment).



(a) CCA (b) non-CCA

Fig. 3. Reading from the left to the right, it is displayed funnel plot when CCA and non-CCA are reference. Black and white dots represent the observed and missing studies.

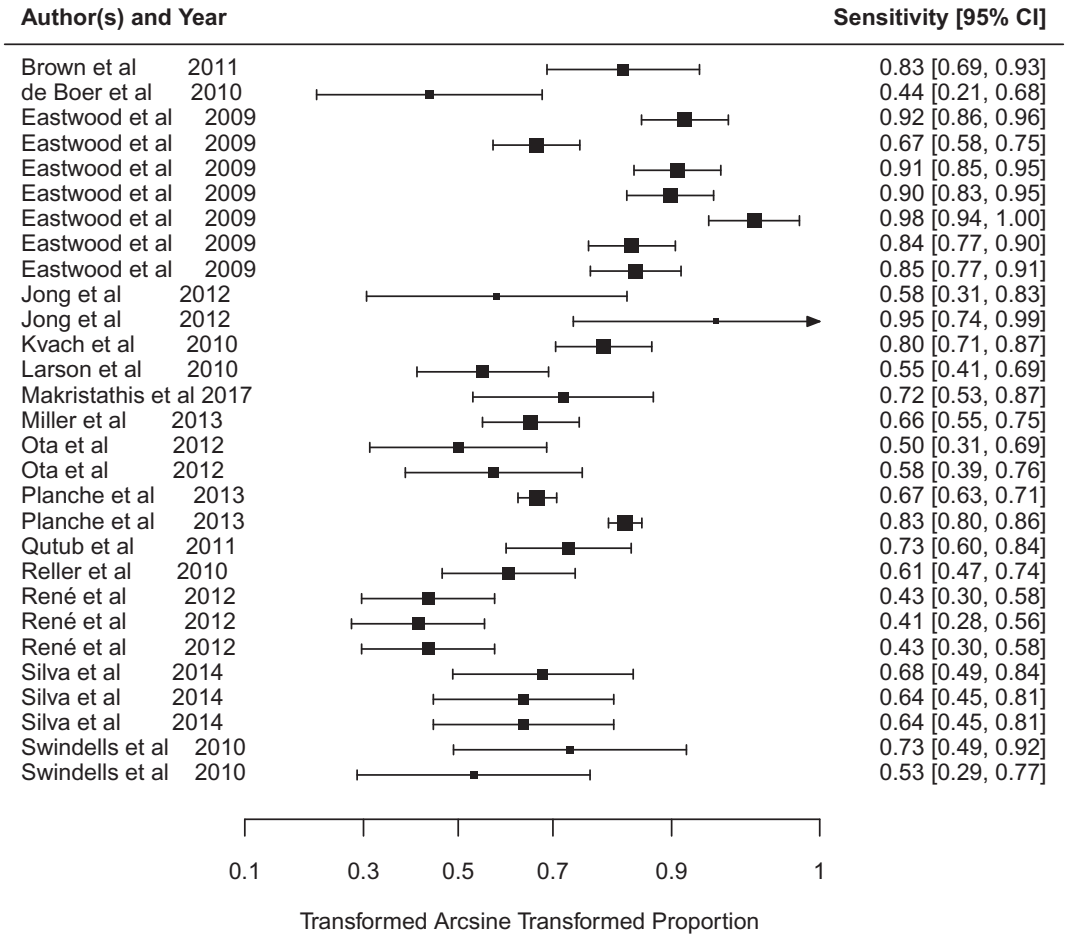


Fig. 4. Forest plot for sensitivity when CCA is the reference. Sensitivities are represented by individual squares proportional to the precision of the estimates and the horizontal lines represent the 95% CIs for each included study. Snsitivities are ordered updown with an increasing sample size.

Viechtbauer, 2010) considered adults only. All of these studies used unformed stools. Ten studies (Boyanton et al., 2012; Chapin et al., 2011; Goldenberg et al., 2010; Hirvonen and Kaukoranta, 2015; Jamal et al., 2014; Novak-Weekley et al., 2010; Planche et al., 2013; Tenover et al., 2010; Wren et al., 2009; Yoldaş et al., 2016) considered samples only with individuals older than 2 years (infants rarely develop a clinical infection). The other 36 studies do not report any information about the age of individuals.

A subsample with only positive results for GDH was used in 4 studies (Chapin et al., 2011; Goldenberg et al., 2010; Larson et al., 2010; Miller et al., 2013).

A total of 83 index tests comparisons to gold reference methods (TC

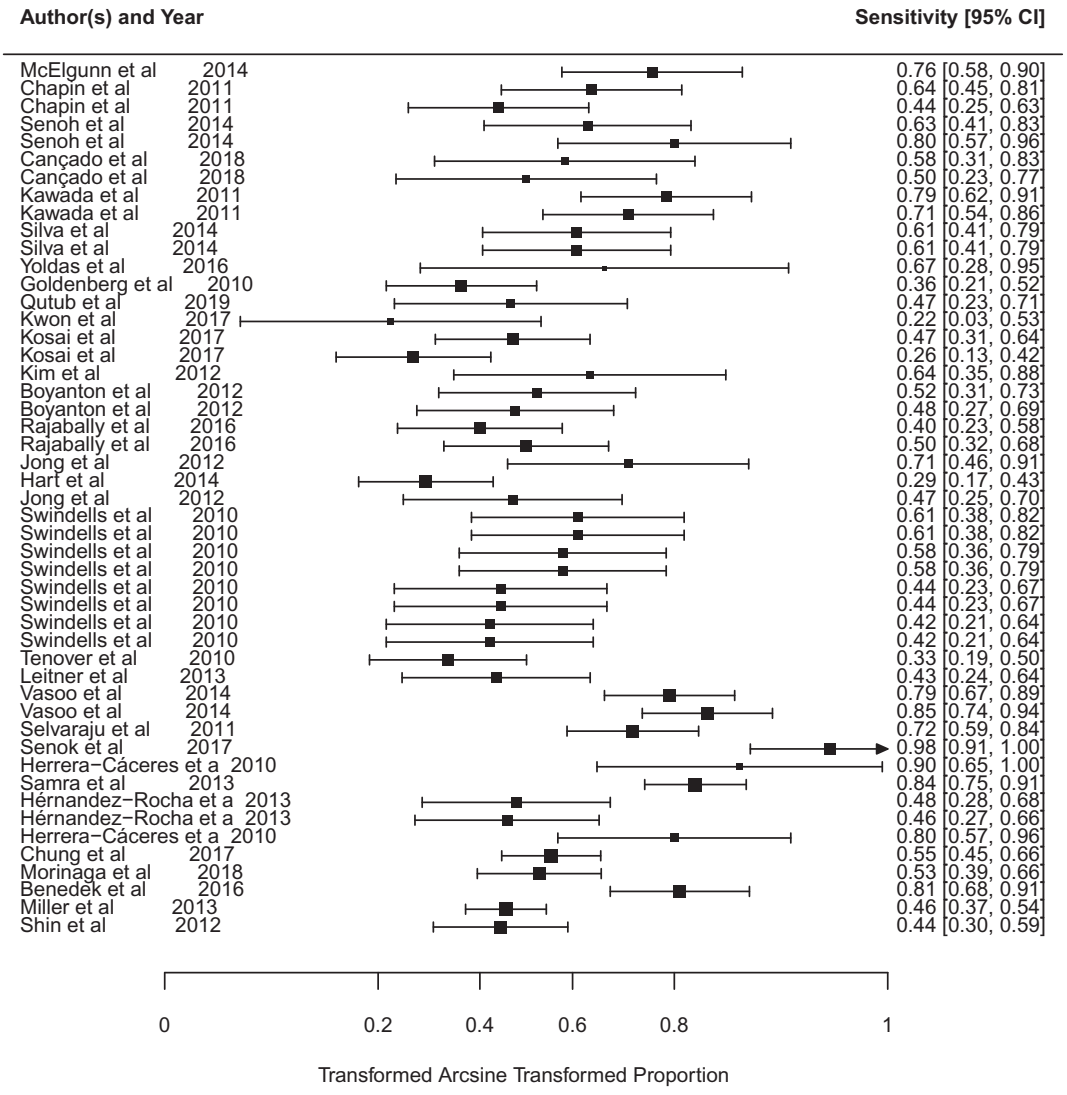


Fig. 5. Forest plot for sensitivity when reference is not CCA (the 49 studies with the lowest sample sizes). Sensitivities are represented by individual squares proportional to the precision of the estimates and the horizontal lines represent the 95% CIs for each included study. Snsitivities are ordered updown with an increasing sample size.

or CCA) were performed. The reference method TC was used more (54 times) than CCA (29 times). Seven of the studies compared the index tests to both reference methods.

A NAAT (1NAAT) was used as reference in 15 studies (27 times). Two or more NAAT with TC for discordant cases (2NAAT) were used in 4 studies (9 times).

The EIAs found in all studies were Premier toxins A/B and ImmunoCard toxins A/B (Meridian, USA), *C. difficile* toxin A + B (Diagnostic Automation, USA), Ridascreen toxins A/B (Biopharm, Germany), Remel ProSpec T and Xpect (Oxoid, UK), VIDAS CDAB (bioMérieux, France), *Clostridium difficile* Tox A/B II, Tox A/B Quik Chek and Quik Chek Complete (Techlab, USA). The last EIA screens for both CDI's toxins and GDH, although the result for the presence of GDH was not used to assess the performance of this assay.

Table 1 summarizes some information about the selected (primary) studies.

3.2. Quality assessment

The QUADAS 2 tool was used (Whiting et al., 2011) to assess the risk of bias and the applicability of the primary studies. In the domain of patient selection, a low risk of bias was characterized by the use of unformed stools and patients over the age of 2. This was found in 23 studies. At least one of the conditions was not fulfilled in at least 7 studies. The risk of bias associated to QUADAS-2's index test domain was determined by blindness and the performance of the assay according to manufacturer's instructions. Regarding to the reference test domain, blindness was required along with a minimum of 48 h culture under anaerobic conditions for stool samples submitted to TC. This last requirement was fulfilled by all studies reporting incubation time. However, for both index test and reference domains a large majority of studies were unclear regarding blindness. The flow and timing domain required both index and reference test to be performed in less than 72 h. This was the case in 25 of the 62 studies. Concerning applicability, no problems were found. The results are summarized in Fig. 2.

Stool samples after transport to the laboratory were refrigerated or frozen in at least 41 cases for reference performance and in at least 36 cases for EIA performance.

3.3. Tests performance across all index tests

Studies where the reference method was TC, ETC or a combination of both were considered as having a similar reference in the analysis that follows, since the between study variance decreases (I^2 decreases

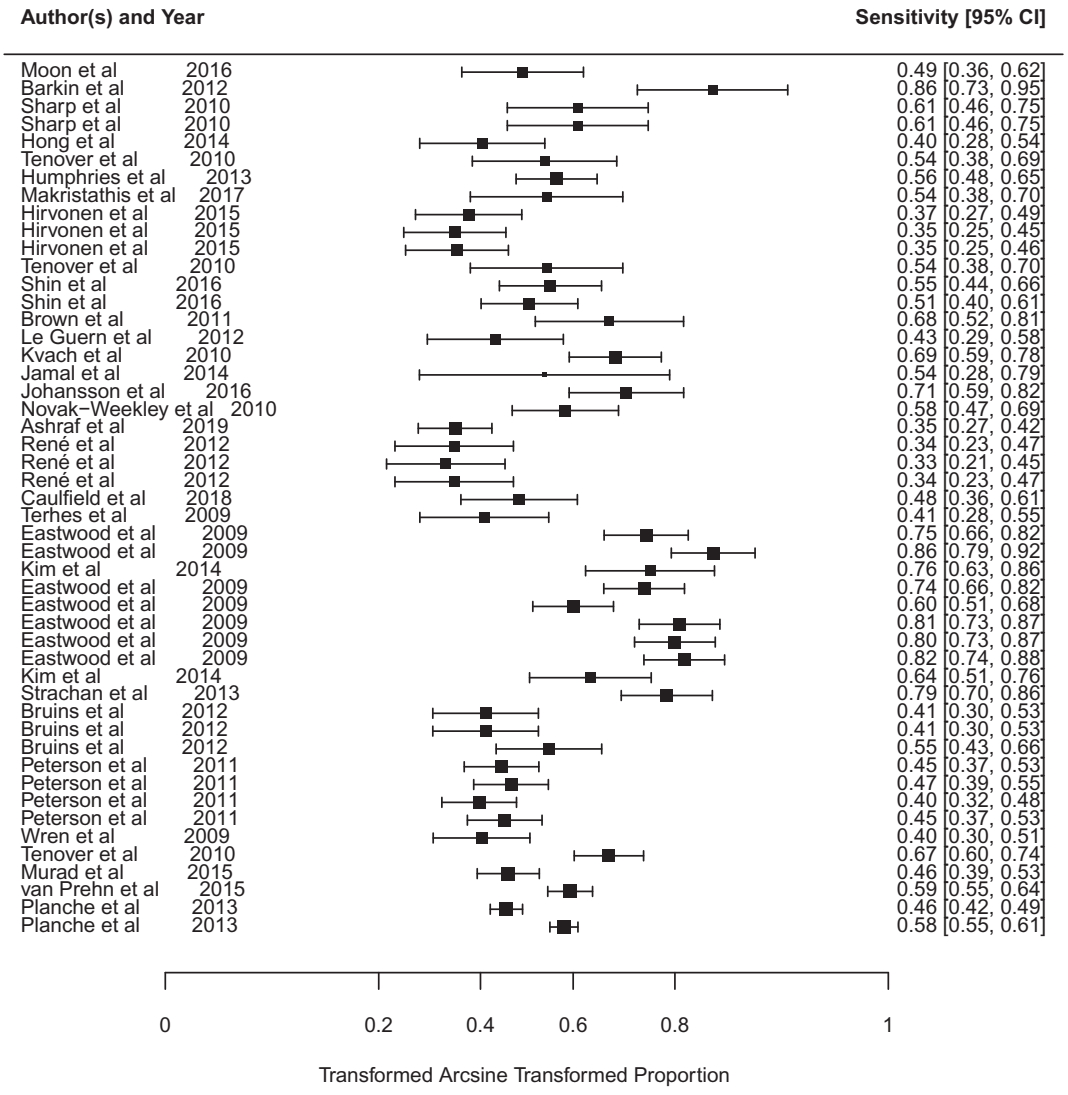


Fig. 6. Forest plot for sensitivity when reference is not CCA (the 49 studies with the highest sample sizes). Sensitivities are represented by individual squares proportional to the precision of the estimates and the horizontal lines represent the 95% CIs for each included study. Snsitivities are ordered updown with an increasing sample size.

from 90.25% to 89.64%). Moreover, even studies in which the reference method were NAAT or several NAATs with TC for discordant cases, were grouped with the previous studies because the variance I^2 decreases even more (88.53%). No evidence of publication bias is found in the funnel plot for this set of studies (see Fig. 3). Hence, all non-CCA references were dealt as the same, leading to a total of 98 comparisons.

CCA is dealt separately. In this case, evidence of publication bias is found (6 studies missing when trim and fill method is applied).

In Figs. 4, 5 and 6 forest plots for the estimated sensitivity, in relation to the two previously established references (CCA or non-CCA), are presented. The studies are displayed in ascending order, according to the sample size (reading updown). The reported sensitivities ranged from 0.41 to 1 (compared to CCA) and from 0.22 to 1 (compared to other reference) whereas the reported specificities ranged from 0.88 to 1 (compared to CCA) and from 0.91 to 1 (compared to non-CCA).

Two bivariate models were adjusted according to the two references considered. The SROC curves are displayed in Fig. 7. The area under the curve is greater than 0.91 in both cases. The normalized partial area under the curve restricted to the observed false positive rates is 0.860/0.744 for the CCA/non-CCA reference. This suggests a very good overall diagnostic accuracy.

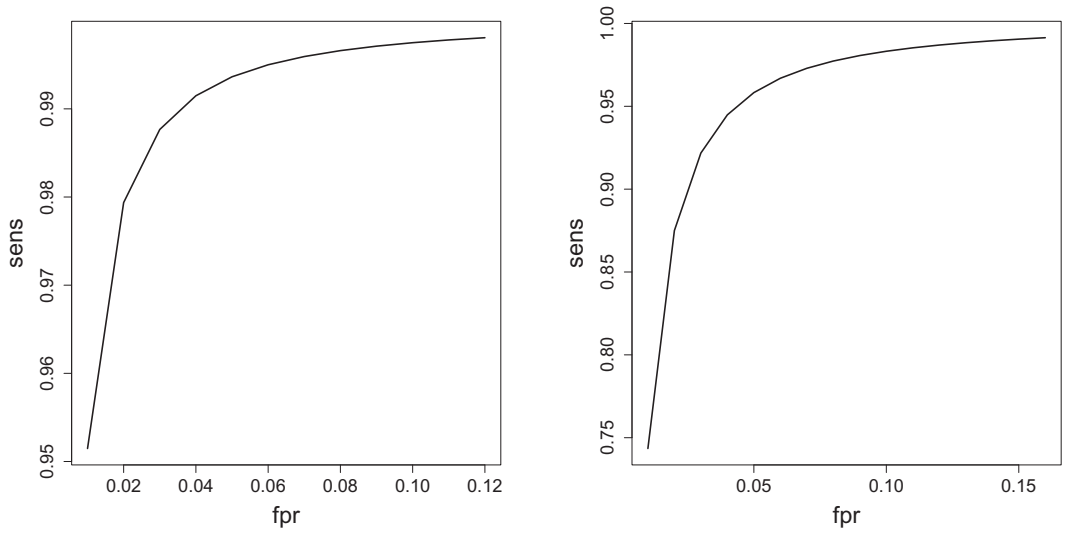
To compare the effect of the prevalence rate on the EIA's performance, we used the threshold 0.10 and 0.15 for CCA and non-CCA reference. These thresholds allowed us to obtain samples of approximately equal size. Regardless of the reference, the sensitivity of an EIA is higher when the prevalence rate is higher. Comparing the use of unformed stools in adults with the performance of the EIA in individuals older than two years, no significant relation was found. Samples with all diarrhea stools have a higher pooled sensitivity. All of these data are displayed in Table 2.

Table 3 presents the results under different TC protocols. The comparison between studies that state (or not) the performance of an ethanol shock before incubation and the time of incubation does not reveal any significant differences. Toxigenicity assessment by CCA in toxigenic culture is associated with a significantly lower estimated sensitivity.

4. Discussion

EIAs for screening the toxins associated to CDI are widely used. The specificity of these EIAs does not generate a great discussion since for golden (or less golden) references values are always close to one.

However, sensitivity values have an opposite performance. Studies report sensitivities as low as 0.33 or as high as 1. Note that the



(a) CCA (b) non-CCA

Fig. 7. Reading from the left to the right, it is displayed the SROC curve for the bivariate model when CCA and non-CCA are reference. Sens: sensitivity; fpr: false positive rate.

Table 2
Test performance under several conditions related to the sample (in each condition the top/below line displays the results for CCA/non-CCA reference). A *p*-value lower than 0.05 or 0.01 is identified with * or **. ND: not done.

	Sensitivity(yes/no)	p-value
Low prevalence	0.641/0.768	0.027*
	0.535/0.591	0.049*
Unformed stools and only adults	ND	
	0.545/0.492	0.104
All diarrhea	0.801/0.658	0.012*
	0.607/0.537	0.026*

differences observed in sensitivity when CCA and non-CCA are the chosen reference are not a surprise. In a previous meta-analysis described in (Crobach et al., 2016), the pooled sensitivity estimate was 0.57 (0.51–0.63) and 0.83 (0.76–0-98) according to TC and CCA, respectively (the 95% confidence intervals are displayed in brackets). Our estimates show a smaller gap: 0.571 and 0.714 for TC and CCA references. The sensitivity estimate is similar even when using all non CCA-references together: 0.566 (0.531–0.599). When the reference is CCA, the estimate for sensitivity presented in (Crobach et al., 2016) is quite different from ours. This is even more relevant, since a large number of studies are common. The number of studies used is also similar and the biggest difference is related to the age of the studies, since we only considered studies published between 2009 and 2019, instead of all studies published until 2016. However, as trim and fill method pointed out for a possible publication bias, it is important to carefully analyse the results associated to this reference.

Sensitivity seems to increase with the prevalence rate. This conclusion does not depend on the chosen reference. Onset diarrhea is a classic symptom of CDI infection. The observed relation to a higher EIA sensitivity probably share an underlying effect with the prevalence rate. Moreover, the lowest *p*-values are found in both cases when CCA is the reference. Some other risk factors associated to CDI infection (e.g. use of antibiotics, proton pump inhibitor (PPI) medication or cancer chemotherapy) were found in very few studies which did not allow us to test for further associations.

Only two studies were performed in a pediatric population using unformed stools. Hence, it was not possible to directly compare the EIA performance in an adult population versus a population aged no more than 18 years old. No significant relation was found when comparing individuals older than two years with adults.

In TC, toxigenicity may be confirmed by PCR, CCA or EIA. We found a significantly lower performance when CCA is the chosen procedure. In (René et al., 2012), the possible influence of this choice was already suggested. It would be interesting in the future to design an experiment for a direct assessment of these differences and how they can/are affecting the assessment of the EIAs.

NAAT and TC have the same target (Wilcox, 2012) and NAAT is highly sensitive and specific, as shown in a recent review study. NAAT detects a gene that encodes toxin and not the toxin itself (Carroll, 2011). In addition to the two traditional references (TC and CCA), we showed that NAAT can be used as a reference method. In this context of evaluation of EIAs that screen for toxins A and B, TC and NAAT are similar reference methods.

5. Conclusions

The guidelines for CDI are based on pooled means established for sensitivity and specificity. Under those estimates, predictions about the performance of some algorithms for screening toxigenic CDI is easily

Table 3
Test performance under several conditions when TC is the reference. A p-value lower than 0.05 or 0.01 is identified with * or **.

	Sensitivity(yes/no)	p-value
Ethanol shock performed	0.580/0.561	0.32
Incubation more than 48 h	0.580/0.566	0.382
Toxin detection by CCA	0.474/−	0.001**/0.015* (compared to PCR/EIA)
Toxin detection by PCR	0.617/−	0.001**/0.300 (compared to CCA/EIA)
Toxin detection by EIA	0.588/−	0.015*/0.300 (compared to CCA/PCR)

derived. However, we have shown that some prior information about the sample, reference performance or even about the EIA choice can result in values for sensitivity quite different from the overall pooled results. In this way, this work sets the importance of establishing more individual guidelines rather than general guidelines. For instance, in a case of onset diarrhea the use of a EIA (low cost) may be more advisable.

Author contributions

All authors equally contributed to this paper.

Funding

Funded by FCT - Fundação para a Ciência e a Tecnologia, Portugal, through the project UIDB/MAT/00006/2020.

Declaration of Competing Interest

The authors declare no potential conflict of interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mimet.2020.105912.

References

Ashraf, Z., Rahmati, E., Bender, J., Nanda, N., She, R., 2019. Gdh and toxin immunoassay for the diagnosis of *Clostridioides (Clostridium) difficile* infection is not a one size fit all screening test. Diagn Micr Infec Dis 94 (2), 109–112.

Barkin, J., Miller, E., Grace, A., 2012. J.S Barkin Sussman D. superiority of the dna amplification assay for the diagnosis of *C. difficile* infection:a clinical comparison of fecal tests. Dig. Dis. Sci. 57 (10), 2592–2599.

Benedek, O., Podbielski, A., Warnke, P., 2016. Laboratory experience with the liaison analyzer in the diagnosis of *Clostridium difficile*-associated diarrhea. Eur. J. Microbiol. Immunol. 6 (3), 215–218.

de Boer, R., Wijma, J., Schuurman, T., Moedt, J., Dijk-Alberts, B., Ott, A., 2010. Evaluation of a rapid molecular screening approach for the detection of toxigenic *Clostridium difficile* in general and subsequent identification of the tcdc delta117 mutation in human stools. J. Microbiol. Methods 83 (1), 59–65.

Bogaty, C., Lévesque, S., Garenc, C., Frenette, C., Bolduc, D., Galarneau, L., Lalancette, C., Loo, V., Tremblay, C., Trudeau, M., Vachon, J., Dionne, M., Villeneuve, J., Longtin, J., Longtin, Y., 2017. Trends in the use of laboratory tests for the diagnosis of *Clostridium difficile* infection and association with incidence rates in Quebec, Canada, 2010-2014. Am. J. Infect. Control 45 (9), 964–968.

Boyanton, B., Sural, P., Loomis, C., Pesta, C., Gonzalez-Krellwitz, L., Robinson-Dunn, B., Riska, P., 2012. Loop-mediated isothermal amplification compared to real-time pcr and enzyme immunoassay for toxigenic clostridium difficile detection. J. Clin. Microbiol. 50 (3), 640–645.

Brown, N., Lebar, W., Young, C., Hankerd, R., Newton, D., 2011. Diagnosis of *Clostridium difficile infection*:comparison of four methods on specimens collected in cary-blair transport medium and tcdb pcr on fresh versus frozen samples. Infect. Dis. Rep. 3 (e5), 15–19.

Bruins, M., Verbeek, E., Wallinga, J., Bruijnesteijn van Coppenraet, L., Kuijper, E., Bloembergen, P., 2012. Evaluation of three enzyme immunoassays and a loop-mediated isothermal amplification test for the laboratory diagnosis of *Clostridium difficile* infection. Clin. Microbiol. Infect. 31 (11), 3035–3039.

Canado, G., Silva, R., Nader, A., Lobato, F., Vilela, E., 2018. Impact of simultaneous glutamate dehydrogenase and toxin a/b rapid immunoassay on *Clostridium difficile* diagnosis and treatment in hospitalized patients with antibiotic-associated diarrhea in a university hospital of Brazil. J. Gastroen. Hepatol. 33 (2), 393–396.

Carroll, K., 2011. Tests for the diagnosis of *Clostridium difficile* infection:the next generation. Anaerobe 17 (4), 170–174.

Caulfield, A., LaSalle, C., Chang, Y., Grys, T., 2018. Evaluation of 4 molecular assays as part of a 2-step algorithm for the detection of *Clostridium difficile* in stool specimens. Diagn. Micr. Infec. Dis. 91 (1), 1–5.

Chapin, K., Dickenson, R., Wu, F., Andrea, S., 2011. Comparison of five assays for detection of *Clostridium difficile* toxin. J. Mol. Diagn. 13 (4), 395–400.

Chung, H., Lee, M., 2017. Evaluation of the performance of c. diff quik chek complete and its usefulness in a hospital setting with a high prevalence of *Clostridium difficile* infection. J. Investig. Med. 65 (1), 88–92.

Crobach, M., Planche, T., Eckert, C., Barbut, F., Terveer, E., Dekkers, O., Wilcox, M., Kuijper, E., 2016. European society of clinical microbiology and infectious diseases:update of the diagnostic guidance document for *Clostridium difficile* infection. Clin. Microbiol. Infect. 22 (Suppl. 4), 563–581.

Doebler P, Holling H. Meta-analysis of diagnostic accuracy with mada. 2017. https://cran.r-project.org/web/packages/mada/index.html the R Project. (Accessed November 7, 2017).

Duval, S., Tweedie, R., 2000. Trim and fill:a simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. Biometrics 56 (2), 455–463.

Eastwood, K., Else, P., Charlett, A., Wilcox, M., 2009. Comparison of nine commercially available *Clostridium difficile* toxin detection assays, a real-time pcr assay for *C. difficile* tcdb, and a glutamate dehydrogenase detection assay to cytotoxin testing and cytotoxigenic culture methods. J. Clin. Microbiol. 47 (10), 3211–3217.

Goldenberg, S., Cliff, P., Smith, S., Milner, M., French, G., 2010. Two-step glutamate dehydrogenase antigen real-time polymerase chain reaction assay for detection of toxigenic*Clostridium difficile*. J. Hosp. Infect. 74 (1), 48–54.

Goret, J., Blanchi, J., Eckert, C., Lacome, S., Petit, A., Barbut, F., Bébéar, C., Mégraud, F., 2015. Comparison of a novel chemiluminescent based algorithm to three algorithmic approaches for the laboratory diagnosis of *Clostridium difficile* infection. Gut Pathog. 7 (33), 1–3.

Guh, A., Hatfield, K., Winston, L., Martin, B., Johnston, H., Brousseau, G., Farley, M., Wilson, L., Perlmutter, R., Phipps, E., Dumyati, G., Nelson, D., Hatwar, T., Kainer, M., Paulick, A., Karlsson, M., Gerding, D., McDonald, L., 2019. Toxin enzyme immunoassays detect *Clostridioides difficile* infection with greater severity and higher recurrence rates. Clin. Infect. Dis. 69 (10), 1667–1674.

Harbord, R., Deeks, J., Egger, M., Whiting, P., Sterne, J., 2007. Uni fication of models for meta-analysis of diagnostic accuracy studies. Biostat. 8 (2), 239–251.

Hart, J., Putsathit, P., Knight, D., Sammels, L., Riley, T., Keil, A., 2014. *Clostridium difficile* infection diagnosis in a paediatric population:comparison of methodologies. Eur. J. Clin. Microbiol. Infect. Dis. 33 (9), 1555–1564.

Hartung, J., Knapp, G., Sinha, B., 2008. Statistical Meta-Analysis with Applications. Wiley, Hoboken, New Jersey (ISBN 978-0-470-29089-7).

Hernández-Rocha, C., Barra-Carrasco, J., Álvarez Lobos, M., Paredes-Sabja, D., Guzmán-Durán, A., 2013. Prospective comparison of a commercial multiplex real-time polymerase chain reaction and an enzyme immunoassay with toxigenic culture in the diagnosis of *Clostridium difficile*-associated infections. Diag. Microbiol. Infect. Dis. 75 (4), 361–365.

Herrera-Cáceres, J., Camacho-Ortiz, A., Galindo-Fraga, A., Hernández-Durán, M., Cordero-Rangel, A., Hernández-Cruz, A., Bobadilla-del Valle, M., Ponce-de Léon, A., Sifuentes-Osornio, J., 2010. Concordance between two enzyme immunoassays for the detection of *Clostridium difficile* toxins. Arch. Med. Res. 41 (2), 92–96.

Hirvonen, J., Kaukoranta, S., 2015. Comparison of bd max cdiff and genomera *C. difficile* molecular assays for detection of toxigenic *Clostridium difficile* from stools in conventional sample containers and in fecalswabs. Eur. J. Clin. Microbiol. Infect. Dis. 34 (5), 1005–1009.

Hong, G., Park, K., Ki, C., Lee, N., 2014. Evaluation of the illumigene *C. difficile* assay for toxigenic *Clostridium difficile* detection:a prospective study of 302 consecutive clinical fecal samples. Diagn. Micr. Infec. Dis. 80 (3), 177–180.

Humphries, R., Uslan, D., Rubin, Z., 2013. Performance of *Clostridium difficile* toxin enzyme immunoassay and nucleic acid amplification tests stratified by patient disease severity. J. Clin. Microbiol. 51 (3), 869–873.

Jamal, W., Pauline, E., Rotimi, V., 2014. Comparative performance of the genexpert c. difficile pcr assay and c. diff quik chek complete kit assay for detection of *Clostridium difficile* antigen and toxins in symptomatic community-onset infections. Int. J. Infect. Dis. 29 (1), 244–248.

Johansson, K., Karlsson, H., Norén, T., 2016. *Clostridium difficile* infection diagnostics - evaluation of the c. diff quik chek complete assay, a rapid enzyme immunoassay for detection of toxigenic *C. difficile* in clinical stool samples. APMIS 124 (11), 1016–1020.

de Jong, E., de Jong, A., Bartels, C., van der Rijt-van den Biggelaar, C., Melchers, W., Sturm, P., 2012. Clinical and laboratory evaluation of a real-time pcr for *Clostridium difficile* toxin a and b genes. Eur. J. Clin. Microbiol. Infect. Dis. 31 (9), 2219–2225.

Kawada, M., Annaka, M., Kato, H., Shibasaki, S., Hikosaka, K., Mizuno, H., Masuda, Y., Inamatsu, T., 2011. Evaluation of a simultaneous detection kit for the glutamate dehydrogenase antigen and toxin a/b in feces for diagnosis of *Clostridium difficile* infection. J. Infect. Chemother. 17 (6), 807–811.

Kim, H., Jeong, S., Kim, M., Lee, Y., Lee, K., 2012. Detection of *Clostridium difficile* toxin a/b genes by multiplex real-time pcr for the diagnosis of *C. difficile* infection. J. Med. Microbiol. 61 (Pt 2), 274–277.

Kim, H., Kim, W., Kim, M., Jeong, S., Lee, K., 2014. Evaluation of a rapid membrane enzyme immunoassay for the simultaneous detection of glutamate dehydrogenase and toxin for the diagnosis of *Clostridium difficile* infection. Ann. Lab. Med. 34 (3), 235–239.

Kosai, K., Iwanaga, Y., Akamatsu, N., Okada, Y., Kaku, N., Uno, N., Morinaga, Y., Hasegawa, H., Miyazaki, T., Izumikawa, K., Mukae, H., Yanagihara, K., 2017. Performance evaluation of the verigene *Clostridium difficile* nucleic acid test, an automated multiplex molecular testing system for detection of *C. difficile* toxin. J. Infect. Chemother. 23 (10), 674–677.

Krutová, M., Nyc, O., 2018. Updated czech guidelines for the laboratory diagnosis of *Clostridium difficile* infections. Epidemiol. Mikrobiol. Imunol. 67 (2), 92–95.

Kvach, E., Ferguson, D., Riska, P., Landry, M., 2010. Comparison of bd geneohm cdiff real-time pcr assay with a two-step algorithm and a toxin a/b enzyme-linked immunosorbent assay for diagnosis of toxigenic *Clostridium difficile* infection. J. Clin. Microbiol. 48 (1), 109–114.

Kwon, J., Reske, K., Hink, T., Burnham, C., Dubberkea, E., 2017. Evaluation of correlation between pretest probability for *Clostridium difficile* infection and *Clostridium difficile* enzyme immunoassay results. J. Clin. Microbiol. 55 (2), 596–605.

Larson, A., Fung, A., Fang, F., 2010. Evaluation of *tcdB* real-time pcr in a three-step diagnostic algorithm for detection of toxigenic *Clostridium difficile*. J. Clin. Microbiol. 48 (1), 124–130.

Le Guern, R., Herwegh, S., Grandbastien, B., Courcol, R., Wallet, F., 2012. Evaluation of a

new molecular test, the bd max cdiff, for detection of toxigenic *Clostridium difficile* in fecal samples. J. Clin. Microbiol. 50 (9), 3089–3090.
Lee, J., Kim, K., Choi, S., Huh, J., Park, S., 2015. Systematic review and meta-analysis of studies evaluating diagnostic test accuracy:a pratical review for clinical researchers-part ii. Statistical methods of meta-analysis. Korean J. Radiol. 16 (6), 1188–1196.
Lee, Y., Kim, M., Kim, H., Lee, K., 2014. Comparison of sensitivity of enzyme immunoassays for toxin a and b in different *C. difficile* pcr ribotypes. Ann. Clin. Lab. Sci. 44 (1), 38–41.
Leitner, E., Einetter, M., Grisold, A., Marth, E., Feierl, G., 2013. Evaluation of the bd max cdiff assay for the detection of the toxin b gene of *Clostridium difficile* out of faecal specimens. Diag. Microbiol. Infect. Dis. 76 (3), 390–391.
Luna, R., Boyanton, B., Mehta, S., Courtney, E., Webb, C., Revell, P., Versalovic, J., 2011. Rapid stool-based diagnosis of *Clostridium difficile* infection by real-time pcr in a children's hospital. J. Clin. Microbiol. 49 (3), 851–857.
Makristathis, A., Zeller, I., Mitteregger, D., Kundi, M., Hirschl, A., 2017. Comprehensive evaluation of chemiluminescent immunoassays for the laboratory diagnosis of *Clostridium difficile* infection. Eur. J. Clin. Microbiol. Infect. Dis. 36 (7), 1253–1259.
McElgunn, C., Pereira, C., Parham, N., Smythe, J., Wigglesworth, M., Smielewska, A., Parmar, S., Gandelman, O., Brown, N., Tisi, L., Curran, M., 2014. A low complexity rapid molecular method for detection of *Clostridium difficile* in stool. PLoS One 9 (1), e83808.
Miller, S., Wiita, A., Wright, C., Reyes, H., Liu, C., 2013. Evaluation of glutamate dehydrogenase immunoassay screening with toxin confirmation for the diagnosis of *Clostridium difficile* infection. Lab. Med. 44 (3), 65–71.
Moher, D., Liberati, A., Tetzlaff, J., Altman, D., PRISMA Group T, 2009. Preferred reporting items for systematic reviews and meta-analyses:the prisma statement. PLoS Med. 6 (7), e1000097.
Moon, H., Kim, H., Hur, M., Shim, H., Kim, H., Yun, Y., 2016. Comparison of diagnostic algorithms for detecting toxigenic *Clostridium difficile* in routine practice at a tertiary referral hospital in korea. PLoS One 11 (8) e0161139.
Morinaga, Y., Akamatsu, N., Matsuda, J., Tateno, H., Tomaru, T., Tanaka, A., Morita, S., Nakamura, M., Kato, H., Annaka, M., Masuda, Y., Itakura, Y., Inamatsu, T., Yanagihara, K., 2018. Diagnostic utilities of a fully automated molecular test for toxigenic *Clostridium difficile*. J. Infect. Chemother. 24 (2), 88–91.
Murad, Y., Perez, J., Nokhbeh, R., Ybazeta, G., Dewar, B., Lefebvre, S., Diaz-Mitoma, F., 2015. Impact of polymerase chain reaction testing on *Clostridium difficile* infection rates in an acute health care facility. Am. J. Infect. Control 43 (4), 383–386.
Nazemalhosseini-Mojarad, E., Azimirad, M., Razaghi, M., Torabi, P., Moosavi, A., Alebouyeh, M., Aslani, M., Zali, M., 2011. Frequency of *Clostridium difficile* among patients with gastrointestinal complaints. Gastroenterol. Hepatol. Bed Bench 4 (4), 210–213.
Novak-Weekley, S., Marlowe, E., Miller, J., Cumpio, J., Nomura, J., Vance, P., Weissfeld, A., 2010. *Clostridium difficile* testing in the clinical laboratory by use of multiple testing algorithms. J. Clin. Microbiol. 48 (3), 889–893.
Origuen, J., Corbella, L., Orellana, M., Fernndez-Ruiz, M., Lpez-Medrano, F., Juan, R.S., Lizasoain, M., Ruiz-Merlo, T., Morales-Cartagena, A., Maestro, G., Parra, P., Villa, J., Delgado, R., Aguado, J., 2018. Comparison of the clinical course of *Clostridium difficile* infection in glutamate dehydrogenase-positive toxin-negative patients diagnosed by pcr to those with a positive toxin test. Clin. Microbiol. Infect. 24 (4), 414–421.
Ota, K., McGowan, K., 2012. *Clostridium difficile* testing algorithms using glutamate dehydrogenase antigen and *C. difficile* toxin enzyme immunoassays with *C. difficile* nucleic acid amplification testing increase diagnostic yield in a tertiary pediatric population. J. Clin. Microbiol. 50 (4), 1185–1188.
Pancholi, P., Kelly, C., Raczkowski, M., Ballada-Llasat, J.M., 2012. Detection of toxigenic *Clostridium difficile*: comparison of the cell culture neutralization, Xpert *C. difficile*, Xpert *C. difficile*/epi, and illumigene *C. difficile* assays. J. Clin. Microbiol. 50 (4), 1331–1335.
Peterson, L., Mehta, M., Patel, P., Hacek, D., Harazin, M., Nagwekar, P., Thomson, R., Robicsek, A., 2011. Laboratory testing for *Clostridium difficile* infection:light at the end of the tunnel. Am. J. Clin. Pathol. 136 (3), 372–380.
Planche, T., Davies, K., Coen, P., Finney, J., Monahan, I., Morris, K., O'Connor, L., Oakley, S., Pope, C., Wren, M., Shetty, N., Crook, D., Wilcox, M., 2013. Differences in outcome according to *Clostridium difficile* testing method:a prospective multicentre diagnostic validation study of *C difficile* infection. Lancet Infect. Dis. 13 (11), 936–945.
Qutub, M., AlBaz, N., Hawken, P., Anoos, A., 2011. Comparison between the two-step and the three-step algorithms for the detection of toxigenic *Clostridium difficile*. Indian J. Med. Microbiol. 29 (3), 293–296.
Qutub, M., Govindan, P., Vattappillil, A., 2019. Effectiveness of a two-step testing algorithm for reliable and cost-effective detection of *Clostridium difficile* infection in a tertiary care hospital in Saudi Arabia. Med. Sci. 7 (1), e6.
R Core Team R, 2014. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing;Vienna, Austria. http://www.R-project.org/.
Rajabally, N., Kullin, B., Ebrahim, K., Brock, T., Weintraub, A., Whitelaw, A., Bamford, C., Watermeyer, G., Thomson, S., Abratt, V., Reid, S., 2016. A comparison of *Clostridium difficile* diagnostic methods for identification of local strains in a south african Centre. J. Med. Microbiol. 65 (4), 320–327.
Rao, K., Micic, D., Natarajan, M., Winters, S., Kiel, M., Walk, S., Santhosh, K., Mogle, J., Galecki, A., LeBar, W., Higgins, P., Young, V., Aronoff, D., 2015. *Clostridium difficile* ribotype 027:relationship to age, detectability of toxins a or b in stool with rapid testing, severe infection, and mortality. Clin. Infect. Dis. 61 (2), 233–241.
Reitsma, J., Glas, A., Rutjes, A., Scholten, R., Bossuyt, P., Zwinderman, A., 2005. Bivariate analysis of sensitivity and specificity procedures informative summary measures in diagnostic reviews. J. Clin. Epidem. 58 (10), 982–990.
Reller, M., Alcabasa, R., Lema, C., Carroll, K., 2010. Comparison of two rapid assays for *Clostridium difficile* common antigen and a *C. difficile* toxin a/b assay with the cell culture neutralization assay. Am. J. Clin. Pathol. 133 (1), 107–110.
René, P., Frenette, C., Schiller, I., Dendukuri, N., Brassard, P., Fenn, S., Loo, V., 2012. Comparison of eight commercial enzyme immunoassays for the detection of *Clostridium difficile* from stool samples and effect of strain type. Diagn. Micr. Infec. Dis. 73 (1), 94–96.
Rutter, C., Gatsonis, C., 1995. Regression methods for meta-analysis of diagnostic test data. Acad. Radiol. 2 (Suppl. 1), 48–56.
Rutter, C., Gatsonis, C., 2001. A hierarchical regression approach to meta-analysis of diagnostic test accuracy evaluations. Stat. Med. 20 (19), 2865–2884.
Samra, Z., Madar-Shapiro, L., Aziz, M., Bishara, J., 2013. Evaluation of a new immunochromatography test for rapid and simultaneous detection of *Clostridium difficile* antigen and toxins. Isr. Med. Assoc. J. 15 (7), 373–376.
Selvaraju, S., Gripka, M., Estes, K., Nguyen, A., Jackson, M., Selvarangan, R., 2011. Detection of toxigenic *Clostridium difficile* in pediatric stool samples:an evaluation of quik check complete antigen assay, bd geneohm cdiff pcr, and progastro cd pcr assays. Diagn. Microbiol. Infect. Dis. 71 (3), 224–229.
Senoh, M., Kato, H., Murase, T., Hagiya, H., Tagashira, Y., Fukuda, T., Iwaki, M., Yamamoto, A., Shibayama, K., 2014. Reverse transcription polymerase chain reaction-based method for selectively detecting vegetative cells of toxigenic *Clostridium difficile*. Microbiol. Immunol. 58 (11), 615–620.
Senok, A., Aldosari, K., Alowaisheq, R., Abid, O., Alsuhaibani, K., Khan, M., Somily, A., 2017. Detection of *Clostridium difficile* antigen and toxin in stool specimens: comparison of the *C. difficile* quik chek complete enzyme immunoassay and genexpert *C. difficile* polymerase chain reaction assay. Saudi J. Gastroenterol. 23 (4), 259–262.
Sharp, S., Ruden, L., Pohl, J., Hatcher, P., Jayne, L., Ivie, W., 2010. Evaluation of the c.diff quik chek complete assay, a new glutamate dehydrogenase and a/b toxin combination lateral flow assay for use in rapid, simple diagnosis of *Clostridium difficile* disease. J. Clin. Microbiol. 48 (6), 2082–2086.
Shin, B., Lee, E., Moon, J., Lee, S., 2016. Evaluation of the vidas glutamate dehydrogenase assay for the detection of *Clostridium difficile*. Anaerobe 40 (1), 68–72.
Shin, S., Kim, M., Kim, M., Lim, H., Kim, H., Lee, K., Chong, Y., 2012. Evaluation of the xpert *Clostridium difficile* assay for the diagnosis of *Clostridium difficile* infection. Ann. Lab. Med. 32 (5), 355–358.
Silva, R., Vilela, E., Neves, M., Lobato, F., 2014. Evaluation of three enzyme immunoassays and a nucleic acid amplifi cation test for the diagnosis of *Clostridium difficile*-associated diarrhea at a university hospital in Brazil. Rev. Soc. Bras. Med. Trop. 47, 447–450.
Strachan, A., Evans, N., Williams, O., Spencer, R., Greenwood, R., Probert, C., 2013. Comparison of a frozen human foreskin fibroblast cell assay to an enzyme immunoassay and toxigenic culture for the detection of toxigenic *Clostridium difficile*. Diag. Microbiol. Infect. Dis. 75 (1), 42–45.
Swindells, J., Brenwald, N., Reading, N., Oppenheim, B., 2010. Comparison of a frozen human foreskin fibroblast cell assay to an enzyme immunoassay and toxigenic culture for the detection of toxigenic *Clostridium difficile*. J. Clin. Microbiol. 48 (2), 606–608.
Tenover, F., Novak-Weekley, S., Woods, C., Peterson, L., Davis, T., Schreckenberger, P., Fang, F., Dascal, A., Gerding, D., Nomura, J., Goering, R., Akerlund, T., Weissfeld, A., Baron, E., Wong, E., Marlowe, E., Whitmore, J., Persing, D., 2010. Impact of strain type on detection of toxigenic *Clostridium difficile*:comparison of molecular diagnostic and enzyme immunoassay approaches. J. Clin. Microbiol. 48 (10), 3719–3724.
Terhes, G., Urbán, E., Sóki, J., Nacsa, E., Nagy, E., 2009. Comparison of a rapid molecular method, the bd geneohm cdiff assay, to the most frequently used laboratory tests for detection of toxin-producing *Clostridium difficile* in diarrheal feces. J. Clin. Microbiol. 47, 3478–3481.
Toltzis, P., Nerandzic, M., Saade, E., O'Riordan, M., Smathers, S., Zaoutis, T., Kim, J., Donskey, C., 2012. High proportion of false-positive *Clostridium difficile* enzyme immunoassays for toxin a and b in pediatric patients. Infect. Control Hosp. Epidemiol. 33 (2), 175–179.
van Prehn, J., Vandenbroucke-Grauls, C.M., van Beurden, Y., van Houdt, R., Vainio, S., Ang, C., 2015. Diagnostic yield of repeat sampling with immunoassay, real-time pcr, and toxigenic culture for the detection of toxigenic *Clostridium difficile* in an epidemic and a non-epidemic setting. Eur. J. Clin. Microbiol. Infect. Dis. 34 (12), 2325–2330.
Vasoo, S., Stevens, J., Portillo, L., Barza, R., Schejbal, D., Wu, M., Chancey, C., Singh, K., 2014. Cost-effectiveness of a modified two-step algorithm using a combined glutamate dehydrogenase/toxin enzyme immunoassay and real-time pcr for the diagnosis of *Clostridium difficile* infection. J. Microbiol. Immunol. Infect. 47 (1), 75–78.
Viechtbauer, W., 2010. Conducting meta-analyses in r with the metafor package. J. Stat. Soft. 36(3). http://www.jstatsoft.org/.
Whiting, P., Rutjes, A., Westwood, M., Mallett, S., Deeks, J., Reitsma, J., Leeflang, M., Sterne, J., Bossuyt, P., 2011. QUADAS-2 G. Quadas-2:a revised tool for the quality assessment of diagnostic accuracy studies. Ann. Intern. Med. 155 (8), 529–536.
Wilcox, M., 2012. Overcoming barriers to effective recognition and diagnosis of *Clostridium difficile* infection. Clin. Microbiol. Infect. 18 (Suppl. 6), 13–20.
Wren, M., Kinson, R., Sivapalan, M., Shemko, M., Shetty, N., 2009. Detection of *Clostridium difficile* infection:a suggested laboratory diagnostic algorithm. Br. J. Biomed. Sci. 66 (4), 175–179.
Yoldaş, O., Altndiş, M., Cufal, D., Aşk, G., Keşli, R., 2016. A diagnostic algorithm for the detection of *Clostridium difficile*-associated diarrhea. Balkan Med. J. 33 (1), 80–86.
Zarandi, E., Mansouri, S., Nakhaee, N., Sarafzadeh, F., Moradi, M., 2017. Toxin production of *Clostridium difficile* in sub-mic of vancomycin and clindamycin alone and in combination with ceftazidime. Microb. Pathog. 107, 249–253.