UK Health Security Agency

Clostridioides difficile diagnostic test accuracy

A rapid review

Contents

Main messages

1. This rapid review (search up to 2 January 2024) identified and summarised evidence of the accuracy of new diagnostic tests for Clostridioides difficile (C. difficile) compared to the diagnostic tests recommended by the Department of Health and Social care (DHSC) (1). The existing DHSC guidance recommends using a 2-step approach to diagnose C. difficile, either a glutamate dehydrogenase (GDH) enzyme immunoassay (EIA) or a nucleic acid amplification test (NAAT), followed by a specific toxin gene EIA.
2. This review also compared the diagnostic accuracy of different combinations of tests used in the existing DHSC guidance for diagnosis of C. difficile.
3. Two studies compared the diagnostic accuracy of new tests to the DHSC recommended testing method (2, 3). One study assessed calprotectin, an indicator of intestinal inflammation (2) and the other investigated whether scent dogs can detect the presence of C. difficile toxin (3).
4. The new tests were not as accurate as the DHSC recommended 2-step approach for diagnosis of C. difficile.
5. Seven studies compared the diagnostic accuracy of different combinations of tests methods for C. difficile (4 to 10). Five studies investigated 2-step testing and 2 investigated 3-step testing combinations.
6. Two-step testing with GDH and NAAT consistently had the highest sensitivity (how well the test identifies C. difficile in samples) and high specificity (how well the test identifies samples without C. difficile) across all studies which compared the diagnostic accuracy of different C. difficile testing combinations (4, 6 to 10). Two-step testing with GDH and toxin gene EIA was reported to have lower sensitivity but high specificity across all studies (4 to 10).
7. There was no difference in the diagnostic accuracy between 2-step and 3-step testing combinations.
8. Risk of bias assessment suggested the results may have been biased by lack of blinding when interpreting results from new tests or their comparators, and the interval of time between testing methods, because most studies did not report enough information about blinding of test results and whether the compared tests were performed at similar times. Lack of detail on patient clinical information also raised possible concern about the generalisability of the evidence to this review question.
9. In summary, the evidence identified by this rapid review supports the 2-step testing approach recommended in the 2012 DHSC guidance. No new tests were identified that performed as well as the 2-step approach, and studies considering different combinations of tests consistently reported the 2-step approach of GDH and NAAT as having best balance of sensitivity and specificity.
10. The findings of this rapid review should be interpreted with caution given the risks of bias identified and potential concerns that the findings of these studies may not be generalisable to all populations.

Purpose

The purpose of this rapid review was to identify and assess the available evidence on the diagnostic accuracy of new tests to diagnose Clostridioides (C.) difficile, in comparison to the existing tests recommended by the Department of Health and Social Care (DHSC) guidance in the UK (1). As the existing DHSC 2-step testing guidance provides different test options at each step, this review also looked for evidence for the diagnostic accuracy of different combinations of currently recommended tests.

Methods

A rapid review was conducted, following streamlined systematic methods to accelerate the review process (11). A literature search was undertaken to look for relevant diagnostic studies, published or available as preprint, from 1 January 2010 up to 2 January 2024. Full details of the methods and search strategy are available in the protocol in Annexe A, which was agreed before starting this review.

There were 2 review questions:

1. Are any new diagnostic tests, or combination of tests, more accurate than the 2-step testing approach for diagnosis of C. difficile recommended in the existing DHSC guidance?
2. Is there a difference in diagnostic accuracy when performing any combination of the individual diagnostic tests included within the existing DHSC guidance?

There was one protocol deviation, toxigenic culture as a standalone test was used as a comparator to the diagnostic accuracy combinations for review question 2 (diagnostic accuracy of different testing combinations), instead of the full 2-step testing approaching recommended by the existing DHSC guidance. This was due to the lack of evidence on diagnostic accuracy of combinations compared to the DHSC recommended 2-step testing approach. Toxigenic culture was agreed as an appropriate comparator test for the second review question because it is a commonly used comparator in diagnostic studies (and may be conducted as part of the recommended DHSC 2-step testing).

Screening of title and abstracts of all studies was completed in duplicate by 5 independent reviewers for 25% of the eligible studies, with the remainder completed independently by the reviewers. Screening of full text of the potentially relevant studies was completed by one reviewer and checked by a second. Data extraction was completed by one reviewer and checked by a second.

The reference lists of relevant reviews identified during title and abstract screening were also searched (backwards citation searching) for primary studies that met the protocol inclusion criteria for this rapid review, using Citation Chaser.

Risk of bias assessment was conducted independently by 2 reviewers using the Quality Assessment Tool for Diagnostic accuracy Studies (QUADAS-2 (2)), with disagreements resolved by a discussion between reviewers or if necessary, with a third reviewer.

Context

The DHSC C. difficile diagnostic guidance was last updated in 2012 on advice from the Advisory Committee on Antimicrobial Resistance and Healthcare Associated Infection (1). The guidance recommended the use of a 2-step test for diagnosis of C. difficile, involving:

1. A highly sensitive screening test (glutamate dehydrogenase (GDH) enzyme immunoassay (EIA), or nucleic acid amplification test (NAAT), such as polymerase chain reaction (PCR)).
2. Positive samples should then proceed to a highly specific toxin gene (A and or B) EIA, cytotoxin assay or toxigenic culture.

If the first test (GDH or NAAT) is negative, the second test (toxin gene EIA) does not need to be performed (1).

As the DHSC guidance was published in 2012, this review only searched for studies published from 1 January 2010 until the 2 January 2024. The overlap in dates was agreed to allow identification of evidence that may not have been considered in the DHSC guidance.

The DHSC guidance does not specify the use of any specific laboratory methods for each test, therefore this rapid review did not compare the diagnostic accuracy of specific laboratory methods, but rather the diagnostic accuracy of the overall tests (GDH, NAAT, toxin EIA, cytotoxin assay or toxigenic culture).

This rapid review also did not compare the diagnostic accuracy of methods to confirm diagnosis in samples where step one suggested a positive diagnosis for C. difficile, but step 2 suggested C. difficile was not present (otherwise known as reflex testing).

This review reported on several measures of diagnostic accuracy, including:

- sensitivity: the proportion of samples with C. difficile correctly identified by the test
- specificity: the proportion of samples without C. difficile correctly identified by the test
- positive predictive value (PPV): the proportion of samples with a positive test result who have C. difficile
- negative predictive value (NPV): the proportion of samples with a negative test result who do not have C. difficile
- accuracy: how likely a sample tested for C. difficile is to be correctly classified by the test (a combined measure of disease prevalence, sensitivity, and specificity)

- area under curve: overall measure of accuracy of a diagnostic test across a range of thresholds

If studies did not provide summary measures of diagnostic accuracy but provided the test result data (the number of samples identified as true and false positive and negative results), sensitivity and specificity were calculated and reported. If the prevalence of C. difficile in the study population was also reported, PPV and NPV were also calculated.

Evidence

In total, 8440 primary studies were screened at title and abstract and 139 studies were screened at full text. Of these, 9 studies met the inclusion criteria (2 to 10). The full text for one study could not be retrieved. Study details and results are provided in Table 1 within Annexe B. Studies excluded during full text screening are available, with exclusion reasons, in Annexe C. Results of the risk of bias assessment can be found in Annexe D.

The studies were conducted between 2010 and 2017, although study time periods were not reported by 4 studies (2 to 4, 9). There were 7 prospective diagnostic cohort studies (4 to 10), one cross-sectional diagnostic cohort study (3) and one diagnostic case-control study (2). Three studies were conducted in the UK (2, 4, 10), 2 were conducted in the USA (8, 9), one was conducted in France (5), one was conducted in Canada (3), one was conducted in Australia (6) and one was conducted in China (7).

The results for each review question are discussed separately below.

New diagnostic tests compared to the recommended C. difficile 2-step testing approach

Two studies compared the diagnostic accuracy of new C. difficile tests to the 2-step testing approach recommended by the 2012 DHSC guidance (2, 3).

Whitehead and others (study time period not reported) conducted a diagnostic case control study in the UK on the use of faecal calprotectin, an indicator of intestinal inflammation, to diagnose C. difficile in faecal samples from patients previously tested for C. difficile (2). The accuracy of faecal calprotectin to diagnose C. difficile was compared to 2-step testing with GDH and PCR in 45 samples. The study reported that the area under curve across the range of faecal calprotectin values compared to the 2-step test was 0.80, which suggests that faecal calprotectin has good ability to diagnose C. difficile. However, at faecal calprotectin levels of 50 micrograms per g^{-1} (the predefined threshold for calprotectin), the sensitivity of faecal calprotectin was high (95%), but specificity was low (26%). The study authors determined that the optimum threshold faecal calprotectin value for diagnosis of C. difficile was 169 micrograms per g^{-1} with a sensitivity of 73% and specificity of 77% compared to the 2-step test (13).

Risk of bias assessment indicated concerns regarding applicability of the faecal calprotectin test to this review's question. This is because faecal calprotectin is an indicator of general intestinal inflammation, not specific to C. difficile, and may indicate other gastrointestinal illnesses. Concerns were also raised in interpretating tests results as the faecal calprotectin results were interpreted by study authors with knowledge of the 2-step testing results. This could have resulted in overestimation of faecal calprotectin's sensitivity and specificity, as study authors may have been more likely to interpret a result as positive if they knew the sample had previously received a positive diagnosis. The C. difficile diagnosis of all faecal samples used in this study was known at the beginning of the study, before either the faecal calprotectin or 2-step test was performed. This type of study design introduces bias in patient selection, as it can result in unrepresentative samples compared to the population the test would be used for in practice. The study also did not report enough detail to tell whether samples were tested at similar times using the faecal calprotectin test and the 2-step testing, which could also lead to a risk of bias as differences in timing may affect ability to detect C. difficile in the samples.

Taylor and others (study time period not reported) conducted a diagnostic cohort study using scent dogs to detect C. difficile toxin (3). Two scent dogs were provided with 300 stool samples to 'sniff'. The samples had known C. difficile diagnosis status, 70% negative and 30% positive, however, scent dog trainers did not know which samples was positive or negative for C. difficile, and study authors were not present while the dogs identified the samples. Each detection round consisted of 10 samples with a random number of C. difficile positive samples (between 1 and 5 positive samples). Each dog did a maximum of 3 detection rounds per day.

Results for each dog were compared to 2-step testing with GDH and toxin gene EIA. Scent dog 2 had a higher sensitivity (92.6%, 95% confidence interval (CI): 84.6% to 97.2%) and specificity (84.5%, CI: 79% to 89%) than to dog one (sensitivity 77.6%, CI: 67.3% to 86%, specificity 85.1%, CI: 79.6% to 89.6%). The degree of agreement between the 2 dogs was reported to be moderate (interrater reliability assessed by Cohen's kappa: 0.52). This indicates that the results between scent dogs were not always consistent. The dogs had relatively low degree of agreement between each other, and results from only 2 dogs are unlikely to be generalisable to all scent dogs. The findings from this study did not support the use of scent dogs in place of the 2-step testing method already recommended by the 2012 DHSC guidance.

Summary

In summary, 2 studies which reported new diagnostic tests for C. difficile (faecal calprotectin and scent dogs) reported lower sensitivity and specificity compared with the diagnostic method recommended by the 2012 DHSC guidance. Noting the risks of bias identified and applicability concerns, the findings of these studies should be interpreted with caution, however, the evidence does not suggest a better test is available.

Accuracy of different combinations of the currently recommended C. difficile testing approaches

Seven studies compared different combinations of tests included within the 2012 DHSC guidance for diagnosis of C. difficile (4 to 10). Different laboratory kits and methods were used in these studies, and they were from populations with different reported C. difficile prevalence, so the results from each study are summarised individually below. Five combinations of 2-step testing and 2 combinations of 3-step testing were compared:

- 2-step: GDH and toxin A or B EIA (4 to 10)
- 2-step: toxin A or B EIA and NAAT (4, 6 to 10)
- 2-step: GDH and NAAT (4 to 10)
- 2-step: toxigenic culture and cell cytotoxin neutralisation assay (CCNA) (6)
- 2-step: GDH and CCNA (8)
- 3-step: GDH, and toxin A or B EIA, and toxigenic culture (5)
- 3-step: GDH, and NAAT, and toxin A and B EIA (4)

Bamber and others conducted a diagnostic cohort study that compared the diagnostic accuracy of 4 combinations of tests involving GDH, toxin EIA and loop-mediated isothermal amplification (LAMP, a type of NAAT), using 811 loose stool samples in the UK (study time period not reported) (4). No demographic or clinical information was reported for patients from whom the samples were taken. The different testing combinations were all compared to toxigenic culture and test results were interpreted without knowledge of the results from other testing methods. Diagnostic accuracy was reported for 3 different combinations of 2-step tests and one 3-step test, see Table 1.

The GDH and LAMP 2 or 3-step test combinations performed best, with high sensitivity and specificity (over 90%). Two-step testing with GDH and toxin AB EIA had lower sensitivity than GDH and LAMP but high specificity, as did 2-step testing with toxin AB EIA and LAMP.

Table 1. Bamber and others diagnostic accuracy estimates

Acronyms: GDH = glutamate dehydrogenase, LAMP = loop mediated isothermal amplification NPV = negative predictive value, PCR = polymerase chain reaction, PPV = positive predictive value

Test combinations	Accuracy	Sensitivity	Specificity	PPV	NPV
2-step GDH and LAMP	97.4%	91.6%	98.1%	84.4%	99%
2-step GDH and toxin AB EIA	94.7%	56.5%	99.2%	88.9%	95.1%
2-step toxin AB EIA and LAMP	95.1%	55.4%	99.6%	94%	95.1%
3-step GDH, LAMP and toxin AB EIA	97.3%	94%	97.7%	82.1%	99.3%

Hart and others conducted a diagnostic cohort study that compared 4 different test combinations for C. difficile using 150 loose stool specimens from 75 children, between October 2011 and January 2012 (6). The patients were 44% female, with a median age of 3 years (ranging from 11 days to 17 years of age). Forty percent were recruited from haematology or oncology clinics. The study reported diagnostic accuracy for 4 different combinations of 2-step tests, compared to toxigenic culture, see Table 2.

All testing combinations were reported to have good specificity, ranging from 97% to 100%, however sensitivity varied. Two-step testing with toxigenic culture and cytotoxin assays, as well as 2-step testing with GDH and toxin AB EIA, showed poor sensitivity (30% or less). It is important to note that as 40% of patients were recruited from haematology or oncology clinics, which had higher C. difficile prevalence than the whole study population (38% and 60.7% respectively, compared to 32% in non-haematology/oncology patients), the findings of this study may not be generalisable to the general population.

Table 2. Hart and others diagnostic accuracy estimates

Acronyms: GDH = glutamate dehydrogenase, LAMP = loop mediated isothermal amplification NPV = negative predictive value, PCR = polymerase chain reaction, PPV = positive predictive value

Test combinations	Sensitivity	Specificity	PPV	NPV
GDH and LAMP	85%	100%	100%	94%
GDH and reverse-transcriptase PCR	83%	99%	97%	93%
Toxigenic culture and cytotoxin assay	30%	100%	100%	76%
GDH and toxin AB EIA	28%	97%	81%	75%

Goret and others conducted a diagnostic cohort study that compared 2 different combinations of 2-step tests and one 3-step test to toxigenic culture using 468 loose stool samples, between June to September 2014 (5). No demographic or clinical information was reported for patients from whom samples were taken. As the purpose of this review was not to compare different methods of the same test, only the diagnostic accuracy of the standard GDH method was included in this review, the new chemiluminescent GDH method (which uses light based chemical reactions) analysed by the study authors has not been reported here. Absolute data for the tests was not presented and therefore pooled diagnostic accuracy measures could not be calculated, so results of both tests are included here for completeness, see Table 3.

All testing combinations had high specificity, as well as good PPV and NPV. Two-step testing with GDH and NAAT had the highest sensitivity (90%). Two-step testing with GDH and toxin AB EIA had lower sensitivity (86.7%) than GDH and NAAT. Three-step testing with GDH, toxin AB EIA, and LAMP had the lowest sensitivity (60%).

Table 3. Goret and others diagnostic accuracy estimates

Acronyms: CI = 95% confidence interval, GDH = glutamate dehydrogenase, LAMP = loop mediated isothermal amplification, NAAT = nucleic acid amplification test, NPV = negative predictive value, PPV = positive predictive value

Testing method	Sensitivity (CI)	Specificity (CI)	PPV (CI)	NPV (CI)
2-step GDH and NAAT	90% (72.3% to 97.8%)	98.9% (97.4% to 99.5%)	84.4% (66.5% to 94.1%)	99.3% (97.8% to 99.8%)
2-step GDH and toxin AB EIA	86.7% (68.4% to 95.6%)	99.1% (97.7% to 99.8%)	86.7% (68.3% to 95.6%)	98.1% (97.5% to 99.7%)
3-step GDH, toxin AB EIA and LAMP	60% (40.7% to 76.7%)	99.5% (98.1% to 99.9%)	90% (66.8% to 98.2%)	97.3% (95.2% to 98.5%)

Liu and others conducted a diagnostic cohort study that compared diagnostic accuracy in 2 C. difficile testing combinations to toxigenic culture, using 186 stool samples from 179 patients with diarrhoea, between June 2016 and May 2017 (7). Patients were 59.2% male, with an average age of 44 years. The study reported that compared to toxigenic culture 2-step testing with GDH and NAAT had better diagnostic accuracy than 2-step testing with GDH and toxin AB EIA, see Table 4.

Table 4. Liu and others diagnostic accuracy estimates

Acronyms: CI = 95% confidence interval, GDH = glutamate dehydrogenase, NAAT = nucleic acid amplification test, NPV = negative predictive value, PPV = positive predictive value

Test combinations	Sensitivity (CI)	Specificity (CI)	PPV (CI)	NPV (CI)
GDH and NAAT	74.4% (60.7% to 88.1%)	100%	100%	93.6% (89.8% to 97.5%)
GDH and toxin AB EIA	48.7% (33% to 64.4%)	97.3% (94.7% to 99.9%)	82.6% (67.1% to 98.1%)	87.7% (82.7% to 92.8%)

Miller and others conducted a diagnostic cohort study that compared 4 different test combinations for C. difficile, using 381 stool samples between January and June 2010 (8). Three different test combinations were compared to toxigenic culture, and one was compared to GDH and CCNA, see Table 5. No demographic or clinical information was reported for patients from whom the samples were taken.

Two different GDH commercial kits (C. Diff Quik Chek and C. Diff CHEK-60) were used in the 2-step GDH and PCR and GDH and toxin B test combinations assessed by the study. As these are still the same type of test (GDH) the comparison of accuracy between the 2 was not relevant to this review protocol and will not be discussed in the interpretation. It was not possible to calculate pooled diagnostic accuracy measures for GDH as absolute data was not provided by the study, therefore the results of both have been included.

Three testing combinations were compared to toxigenic culture, and one was compared to 2-step testing with GDH and CCNA. All testing methods compared to toxigenic culture had high specificity, (reported to be 100% for all except the 2-step testing method of GDH [C. Diff Quik Chek] and toxin B). The test combinations of GDH and PCR (performed using 2 different commercial GDH kits), displayed the highest sensitivity.

One testing combination (GDH and PCR) was compared to 2-step testing with GDH and CCNA. Two-step testing with GDH and PCR had high sensitivity (99%) but low specificity (74%).

Table 5. Miller and others diagnostic accuracy estimates

Acronyms: CCNA = cell cytotoxin neutralisation assay, GDH = glutamate dehydrogenase, NPV = negative predictive value, PCR = polymerase chain reaction, PPV = positive predictive value

Test combination	Reference standard	Sensitivity	Specificity
GDH (C. Diff Quik Chek) and PCR	Toxigenic culture	96.1%	100%
GDH (C. Diff Chek-60) and PCR	Toxigenic culture	92.9%	100%
GDH (C. Diff Quik Chek) and CCNA	Toxigenic culture	67.7%	100%
GDH (C. Diff Chek-60) and CCNA	Toxigenic culture	66.1%	100%
GDH (C. Diff Quik Chek) and toxin B	Toxigenic culture	44.9%	99.6%
GDH (C. Diff Quik Chek) and PCR	GDH (C. Diff CHEK-60) and CCNA	99%	74%

Novak and others conducted a diagnostic cohort study that compared different C. difficile testing methods, 2 of which were relevant to this review question 2-step testing with GDH and NAAT and with GDH and toxin AB EIA both compared to toxigenic culture, using 432 stool specimens (study time period not reported (9)). No demographic or clinical information was reported for patients from whom the samples were taken. Two-step testing with GDH and NAAT had the highest measures of diagnostic accuracy, whilst 2-step testing with GDH and toxin AB EIA performed similarly for specificity but had poor sensitivity (see Table 6).

Table 6. Novak and others diagnostic accuracy estimates

Acronyms: CI = 95% confidence interval, GDH = glutamate dehydrogenase, NAAT = nucleic acid amplification test, NPV = negative predictive value, PPV = positive predictive value

Test combination	Accuracy	Sensitivity (CI)	Specificity (CI)	PPV (CI)	NPV (CI)
GDH and NAAT	95.8%	86.1%	97.8%	88.6%	97.2%
GDH and toxin AB EIA	91.2%	55.6%	98.3%	87%	91.7%

Planche and others conducted a diagnostic cohort study investigating accuracy of 3 combinations of 2-step tests (GDH and NAAT, toxin EIA and NAAT, GDH and toxin EIA) compared to toxigenic culture and also to cytotoxin assay to diagnose C. difficile using 12,420 stool samples from 10,186 patients in the UK between October 2010 and September 2011 (10). The patients were 54% female with an average age of 61 years (standard deviation: 21 years of age), see Table 7. Two-step testing with GDH and NAAT had the best sensitivity, high specificity and NPV, but lower PPV regardless of the comparator test. The 2-step testing methods with toxin AB EIA assay and NAAT, and with GDH and toxin AB EIA both had lower sensitivity (particularly when compared to cytotoxigenic culture), but specificity, PPV, and NPV remained high.

Table 7. Planche and others diagnostic accuracy estimates

Acronyms: CI = 95% confidence interval, GDH = glutamate dehydrogenase, NAAT = nucleic acid amplification test, NPV = negative predictive value, PPV = positive predictive value

Test combination	Reference standard	Sensitivity (CI)	Specificity (CI)	PPV (CI)	NPV (CI)
GDH and NAAT	Toxigenic culture	91.5% (89.6% to 93.1%	98% (97.7% to 98.3%)	80.7% (78.3% to 82.9%)	99.2% (99% to 99.4%)
Toxin AB EIA and NAAT	Toxigenic culture	57.8% (54.8% to 60.9%)	99.5% (99.3% to 99.6%)	90.7% (88.3% to 92.8%)	96.3% (95.9% to 96.6%)
GDH and toxin AB EIA	Toxigenic culture	57% (53.9% to 60%)	99.4% (99.3% to 99.6%)	90.1% (87.5% to 92.2%)	96.2% (95.8% to 96.5%)
GDH and NAAT	Cytotoxin assay	95.6% (93.9% to 97%)	95.9% (95.6% to 96.3%)	59.7% (56.8% to 62.5%)	99.7% (99.6% to 99.8%)
Toxin AB EIA and NAAT	Cytotoxin assay	82.9% (80% to 85.6%	99.6% (99.4% to 99.7%)	92.1% (89.8% to 94%)	98.9% (98.7% to 99.1%)
GDH and toxin AB EIA	Cytotoxin assay	81.8% (78.8% to 84.5%)	99.5% (99.4% to 99.6%)	91.6% (89.2% to 93.6%)	98.9% (98.7% to 99%)

Risk of bias assessment

Most studies did not report if the results of the tests or combination of tests were interpreted without knowledge of the comparator (4 to 10). Having knowledge of the results of one test when performing the other can introduce bias when interpreting the test by inadvertently favouring one outcome over another. Furthermore, some of the studies used samples with known C. difficile diagnostic status. In these cases, bias is again introduced if the person interpreting the tests knows the status, but it can also lead to concerns in generalisability of the results to the general population because participants in the study may not represent most individuals in the population that the test would be used in.

It was also unclear in all studies whether there was an appropriate time interval between testing with the test combinations and the comparator tests. As tests assess diagnostic status at a particular point in time, the tests being compared should be performed as close to the same time point as possible. Lack of information on the time interval between compared tests means it was not possible to determine if bias could have been introduced which could affect assessment of diagnostic accuracy.

No applicability concerns were identified in any of the studies.

Summary

Seven studies compared the diagnostic accuracy of different testing combinations for C. difficile. Most studies compared diagnostic accuracy of different test combinations to toxigenic culture, although some studies compared to cytotoxin assays. Although toxigenic culture alone is not the DHSC recommended test, it was agreed as an appropriate comparator test for the second review question because it is commonly used as a comparator in diagnostic test accuracy studies and may be conducted as part of the recommended DHSC 2-step testing.

The evidence demonstrated that in studies assessing test combinations with 2-step GDH and NAAT, this combination consistently was reported to have higher sensitivity than other combinations assessed in the studies, as well as high specificity. GDH and toxin AB EIA consistently had lower sensitivity than other tests, though specificity was often higher. Three-step testing did not appear to offer improvement in diagnostic accuracy compared to 2-step testing.

Risk of bias assessment was impacted by poor reporting in most studies. It was often unclear whether the test results that were being compared were interpreted without knowledge of each other, or if the tests were conducted at similar times, which may have meant bias was introduced that could lead to over or underestimation of diagnostic accuracy. Many studies also did not report on patient clinical information, which may have relevance to the assessment of the diagnostic tests. Therefore, the findings of this review should be interpreted with caution, as these factors may impact diagnostic accuracy and the applicability of these findings.

Health inequalities

The majority of included studies did not report patient demographic information, however, when agreeing the protocol for this review it was agreed that it would not be expected that C. difficile diagnostic tests would perform differently in various population groups including inclusion health groups and no group was identified as being at particular risk of inequality.

Limitations

This rapid review used streamlined systematic methods to accelerate the review process. Sources of evidence searched included databases of peer-reviewed and preprint research, but it is possible relevant evidence may have been missed.

Many of the studies did not clearly report on elements critical to risk of bias assessment meaning it was not possible to assess if there was a risk of bias introduced by selection of patients or samples and interpretation of the index or reference tests. Risk of bias assessments for each study are provided in Annexe D, which shows where studies have been rated as having high risks of bias or concerns about applicability. Findings of this review may therefore be influenced by potential bias.

When considering the diagnostic accuracy of different testing combinations, it should be noted that measures of diagnostic accuracy of tests are interpreted in comparison to a reference standard test, which is assumed to be the 'true' diagnosis. If the reference standard is not completely accurate, some true positives may be incorrectly classified as negatives (false negatives) or vice versa.

Evidence gaps

One aim of this review was to identify whether there were any new tests compared to the 2-step testing method recommended in the 2012 DHSC guidance. Only 2 studies were identified that met the inclusion criteria for this question. Many studies were excluded as they used alternative comparator tests rather than the recommended 2-step testing method, including toxigenic culture as a standalone test. The use of this reference standard to determine accuracy of any new tests was agreed as necessary to ensure comparison to the existing DHSC guidance. For the second review question a protocol deviation to include toxigenic culture alone as a reference test was agreed as acceptable, to enable consideration of any combinations of the existing DHSC testing approach.

Conclusion

This review identified no new C. difficile diagnostic tests with equivalent diagnostic accuracy compared to the 2-step testing method recommended by the 2012 DHSC guidance.

Seven studies compared the diagnostic accuracy of different testing methods for C. difficile, usually in comparison to toxigenic culture. 2-step testing with GDH and NAAT consistently displayed the highest sensitivity compared to 2-step testing with GDH and toxin gene EIA, with good specificity. Two-step testing appeared to offer similar diagnostic accuracies to 3-step testing. The results of these studies therefore do not suggest any evidence for a different combination of tests to that recommended by the 2012 DHSC guidance.

It should be noted that accuracy, PPV and NPV are affected by the prevalence of C. difficile and should therefore not be compared between studies with differing, or unknown prevalence values. This is also true for diagnostic accuracy across studies that have used the same comparator tests with different threshold cut offs (for example, cycle thresholds for a PCR test) which should not be considered comparable.

Risk of bias in the included studies generally resulted from a lack of blinding of the results from the different testing methods, although reporting on this (as well as patient clinical information and timing interval between the reference standard and index test) was poor across most studies. The evidence should therefore be interpreted with some caution.

Acknowledgment

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7. Liu C and others. 'A 2-step algorithm combining glutamate dehydrogenase and nucleic acid amplification tests for the detection of Clostridioides difficile in stool specimens'. European Journal of Clinical Microbiology & Infectious Diseases 2021: volume 40, issue 2, pages 345 to 351
8. Miller S and others. 'Evaluation of glutamate dehydrogenase immunoassay screening with toxin confirmation for the diagnosis of clostridium difficile infection' Laboratory Medicine 2013: volume 44, pages e65 to e71
9. Novak-Weekley SM and others. 'Clostridium difficile testing in the clinical laboratory by use of multiple testing algorithms' Journal of Clinical Microbiology 2010: volume 48, issue 3, pages 889 to 893
10. Planche TD and others. 'Differences in outcome according to Clostridium difficile testing method: a prospective multicentre diagnostic validation study of C difficile infection' The Lancet Infectious Diseases 2013: volume 13, issue 11, pages 936 to 945
11. Tricco AC and others. Rapid reviews to strengthen health policy and systems: A practical guide World Health Organization 2017
12. Haddaway NR and others. 'Citationchaser: A tool for transparent and efficient forward and backward citation chasing in systematic searching' Research Synthesis Methods 2022: volume 13, issue 4, pages 533 to 545
13. de Hond AAH and others. 'Interpreting area under the receiver operating characteristic curve' The Lancet Digital Health 2022: volume 4, issue 12, pages e853 to e855
14. Zangiabadian M and others. 'Accuracy of diagnostic assays for the detection of Clostridioides difficile: A systematic review and meta-analysis' Journal of Microbiological Methods 2023: volume 204, page 106,657

Annexe A. Protocol

Review question

There were 2 review questions:

1. Are any new diagnostic tests, or combination of tests, more accurate than the 2-step testing approach for diagnosis of C. difficile recommended in the existing DHSC guidance?
2. Is there a difference in diagnostic accuracy when performing any combination of the individual diagnostic tests included within the existing DSHC guidance?

A search for evidence to answer this review question will be conducted from 1 January 2010 up to 2 January 2024.

Eligibility criteria

	Included	**Excluded**
Population	All	Animals
Index tests	Clostridioides (C.) difficile diagnostic tests which are not part of the reference standard diagnostic algorithm	
Reference standard	C. difficile diagnostic tests which comprise the current 2-step diagnostic algorithm [note 1]: 1. glutamate dehydrogenase enzyme immunoassay (GDH) or nucleic acid amplification tests 2. toxin gene EIA, toxigenic culture, cytotoxin assay	
Target condition	C. difficile	
Outcomes	Measures of diagnostic accuracy: • sensitivity and specificity (either reported directly in an included study or able to be calculated from the study's raw data) • likelihood ratios • positive and negative predictive values If multiple thresholds of a particular type of index test are reported (such as different cycle thresholds for polymerase	

	Included	Excluded
	chain reaction [PCR]), we will extract all reported outcomes for each threshold.	
Language	English	Any other language
Date of publication	Published from 2010 up to 2 January 2024	Published before 2010
Study design	• diagnostic cohort studies • cross-sectional studies • case-control studies	• ecological studies • guidelines • modelling studies • opinion pieces • qualitative studies • reviews
Publication type	• peer-reviewed published research • preprints	• editorials • letters • news articles

Note 1: see Context section for further details on the diagnostic algorithm.

Context

Guidance published in 2012 from the Advisory Committee on Antimicrobial Resistance and Healthcare Associated Infection recommends the use of a 2-step testing system for the diagnosis of C. difficile in the United Kingdom (1), which is referred to as diagnostic algorithm. The diagnostic algorithm involves:

1. A highly sensitive screening test (glutamate dehydrogenase enzyme immunoassay (GDH), or nucleic acid amplification test (NAAT) such as polymerase chain reaction (PCR)).
2. Positive samples should then proceed to a highly specific toxin gene EIA. A cytotoxin assay or toxigenic culture may be performed instead of a toxin gene EIA, but these tests are slower and therefore are often not used (1).

If the first test (GDH or NAAT) is negative, the second test (toxin gene EIA) does not need to be performed.

As this diagnostic algorithm was recommended in 2012, this review will only include studies published from 1 January 2010.

Identification of studies

The following databases and be searched for studies published up to 02 January 2024: Medline, Embase, Web of Science (Science Citation Index and Preprints Citation Index). The search strategy is presented below: Search strategy. The search strategy will be checked by another information specialist. Duplicate references will be removed using Deduklick.

Screening

Screening on title and abstract will be undertaken in duplicate by 2 reviewers for at least 20% of the eligible studies, with the remainder completed by one reviewer. Disagreement will be resolved by discussion.

Screening on full text will be undertaken by one reviewer and checked by a second.

The reference lists of relevant reviews, identified in the title and abstract screening stage, will also be screened by one reviewer for relevant studies during full text screening.

Data extraction

Summary information for each study will be extracted and reported in tabular form. Information to be extracted will include country, participants, study design, index test, reference standard, details of how each diagnostic test is performed, time frame between index test and reference standard, results, and any relevant contextual data. This will be undertaken by one reviewer and checked by a second.

Risk of bias assessment

Two reviewers will independently complete a risk of bias assessment of included studies using a quality assessment tool for diagnostic accuracy studies (QUADAS-2 (14)), with disagreements resolved by discussion or with a third reviewer.

Synthesis

If data is presented in a consistent format between studies, a narrative synthesis will be produced to describe the evidence as a whole. Alternatively, if data are too heterogeneous, a narrative summary of each study will be provided.

Health inequalities

Variations across populations and subgroups, for example cultural variations or differences between ethnic or social groups will be considered, where evidence is available.

Search strategy

Database: Ovid MEDLINE(R) ALL <1946 to December 29, 2023>

Date of search: 2 January 2024

1. C* difficile.tw,kf. (19354)
2. Cdifficile.tw,kf. (97)
3. Clostridioides difficile/ (11564)
4. Clostridium Infections/ (10877)
5. Enterocolitis, Pseudomembranous/ (7633)
6. Peptoclostridium difficile.tw,kf. (12)
7. clostridium infection*.tw,kf. (167)
8. or/1-7 (27336)
9. Glutamate Dehydrogenase/ (6294)
10. glutamate dehydrogenase*.tw,kf. (6519)
11. ("Toxin* A" or "Toxin* B").tw,kf. (12028)
12. exp *Nucleic Acid Amplification Techniques/ (63769)
13. Nucleic Acid Amplification.tw,kf. (5683)
14. nucleic acid test*.tw,kf. (2652)
15. NAAT.tw,kf. (1114)
16. exp *Polymerase Chain Reaction/ (56557)
17. Polymerase Chain Reaction*.tw,kf. (294378)
18. (qPCR or PCR).tw,kf. (687494)
19. exp Immunoenzyme Techniques/ (220434)
20. immunoassay*.tw,kf. (80480)
21. immune assay*.tw,kf. (978)
22. Immunospot.tw,kf. (2371)
23. Immunoenzyme.tw,kf. (1602)
24. immunosorbent assay*.tw,kf. (108546)
25. membrane bound assay*.tw,kf. (0)
26. isothermal amplification assay*.tw,kf. (776)
27. assay*.ab. /freq=3 or assay*.ti,kf. (267064)
28. enzyme-linked immunosorb*.tw,kf. (109632)
29. ELISA.tw,kf. (207317)
30. Loop-mediated isothermal amplification.tw,kf. (4578)
31. LAMP.tw,kf. (25709)
32. (diagnos* adj3 (test* or technique*)).tw,kf. (136471)
33. Molecular Diagnostic Techniques/ (13821)

34. Clinical Laboratory Techniques/ (23942)
35. Microbiological Techniques/ (7275)
36. Bacteriological Techniques/ (34432)
37. exp Bacterial Typing Techniques/ (70244)
38. Ribotyping/ (2424)
39. ribotyp*.tw,kf. (3960)
40. riboprint*.tw,kf. (183)
41. bacterial typ*.tw,kf. (2131)
42. Enterotoxins/an (1789)
43. Bacterial Toxins/an (2314)
44. Bacterial Proteins/an (9830)
45. (cytotox* adj5 (culture* or test* or assay*)).tw,kf. (39066)
46. (toxigenic adj5 (culture* or assay* or test*)).tw,kf. (642)
47. (toxin* adj5 (EIA or assay* or enzyme* or gene* or test* or detec* or analy*)).tw,kf. (21736)
48. or/9-47 (1718123)
49. exp "Sensitivity and Specificity"/ (651616)
50. sensitivity.tw,kf. (1010051)
51. specificity.tw,kf. (570610)
52. ((pre-test or pretest) adj probability).tw,kf. (2963)
53. post-test probability.tw,kf. (756)
54. predictive value$.tw,kf. (140823)
55. likelihood ratio$.tw,kf. (20334)
56. diagnos*.tw,kf. (3159038)
57. Diagnosis/ (17541)
58. Diagnosis, Differential/ (468999)
59. diagnosis.fs. (2983068)
60. false positive*.tw,kf. (70083)
61. false negative*.tw,kf. (39406)
62. true positive*.tw,kf. (10265)
63. true negative*.tw,kf. (4242)
64. marker*.tw,kf. (948315)
65. or/49-64 (6982859)
66. Premier Toxin.tw,kf. (7)
67. (Vidas adj3 toxin).tw,kf. (16)
68. GA Clostridium difficile Antigen.tw,kf. (0)
69. Ridascreen.tw,kf. (176)
70. Techlab Toxin.tw,kf. (0)
71. Remel ProSpec.tw,kf. (0)
72. Remel Xpect.tw,kf. (7)
73. Quik Chek.tw,kf. (90)
74. Techlab tox*.tw,kf. (7)
75. Premier Immunocard.tw,kf. (0)
76. Chek-60.tw,kf. (9)
77. GeneOhm.tw,kf. (115)

78. Wampole.tw,kf. (87)
79. LEUKO EZ VUE.tw,kf. (0)
80. BD Diagnostics.tw,kf. (75)
81. Xpert.tw,kf. (3322)
82. Prodesse TaqMan.tw,kf. (0)
83. Illumigene.tw,kf. (55)
84. Meridian.tw,kf. (4902)
85. Bioconnections.tw,kf. (0)
86. Techlab.tw,kf. (115)
87. Biopharm.tw,kf. (265)
88. Oxoid.tw,kf. (533)
89. The Binding Site.tw,kf. (110150)
90. Vidas.tw,kf. (876)
91. Remel.tw,kf. (139)
92. or/66-91 (120579)
93. 8 and 92 (349)
94. 8 and 48 and 65 (3058)
95. 93 or 94 (3123)
96. (C* difficile adj2 (test or tests)).tw,kf. (351)
97. clostridium infections/di or Enterocolitis, Pseudomembranous/di (3665)
98. 96 or 97 (3860)
99. 95 or 98 (5435)
100. limit 99 to yr="2010 -Current" (3198)
101. Animals/ not (Animals/ and Humans/) (5147995)
102. 100 not 101 (3033)

Database: Embase <1974 to 2023 December 29>

Date of search: 2 January 2024

1. C* difficile.tw,kf. (29383)
2. Cdifficile.tw,kf. (476)
3. clostridioides difficile/ (5354)
4. Clostridium difficile infection/ (20070)
5. pseudomembranous colitis/ (5295)
6. Peptoclostridium difficile.tw,kf. (14)
7. clostridium infection*.tw,kf. (177)
8. clostridium difficile toxin a/ or clostridium difficile toxin b/ or clostridium toxin/ (3969)
9. or/1-8 (41309)
10. glutamate dehydrogenase/ (8473)
11. glutamate dehydrogenase*.tw,kf. (6528)
12. ("Toxin* A" or "Toxin* B").tw,kf. (16232)
13. exp nucleic acid amplification techniques/ (1251043)
14. Nucleic Acid Amplification.tw,kf. (7723)
15. nucleic acid test*.tw,kf. (3793)

16. NAAT.tw,kf. (2024)
17. exp polymerase chain reaction/ (1245689)
18. Polymerase Chain Reaction*.tw,kf. (339129)
19. (qPCR or PCR).tw,kf. (1003880)
20. exp immunoassay/ (750719)
21. immunoassay*.tw,kf. (109792)
22. immune assay*.tw,kf. (1520)
23. Immunospot.tw,kf. (2870)
24. Immunoenzyme.tw,kf. (1809)
25. immunosorbent assay*.tw,kf. (127286)
26. membrane bound assay*.tw,kf. (2)
27. isothermal amplification assay*.tw,kf. (805)
28. assay*.ab. /freq=3 or assay*.ti,kf. (360639)
29. enzyme-linked immunosorb*.tw,kf. (127515)
30. ELISA.tw,kf. (332162)
31. Loop-mediated isothermal amplification.tw,kf. (4808)
32. LAMP.tw,kf. (31336)
33. (diagnos* adj3 (test* or technique*)).tw,kf. (191792)
34. molecular diagnosis/ (30220)
35. exp laboratory technique/ (216815)
36. microbiological examination/ (44349)
37. bacterium identification/ (64449)
38. exp bacterium examination/ (280912)
39. Ribotyping/ (2847)
40. ribotyp*.tw,kf. (4562)
41. riboprint*.tw,kf. (206)
42. bacterial typ*.tw,kf. (2400)
43. enterotoxin/ (8830)
44. Bacterial Toxins/an (612)
45. bacterial protein/ (92225)
46. (cytotox* adj5 (culture* or test* or assay*)).tw,kf. (52197)
47. (toxigenic adj5 (culture* or assay* or test*)).tw,kf. (833)
48. (toxin* adj5 (EIA or assay* or enzyme* or gene* or test* or detec* or analy*)).tw,kf. (25345)
49. or/10-48 (3265144)
50. "sensitivity and specificity"/ (499182)
51. sensitivity.tw,kf. (1314374)
52. specificity.tw,kf. (747223)
53. ((pre-test or pretest) adj probability).tw,kf. (5290)
54. post-test probability.tw,kf. (1088)
55. predictive value$.tw,kf. (211798)
56. likelihood ratio$.tw,kf. (27797)
57. diagnos*.tw,kf. (4556424)
58. diagnosis/ (1440666)
59. differential diagnosis/ (377501)

60. diagnosis.fx. (3698286)
61. false positive*.tw,kf. (95787)
62. false negative*.tw,kf. (56225)
63. true positive*.tw,kf. (15438)
64. true negative*.tw,kf. (6774)
65. marker*.tw,kf. (1370563)
66. or/50-65 (9648762)
67. Premier Toxin.tw,kf. (11)
68. (Vidas adj3 toxin).tw,kf. (43)
69. GA Clostridium difficile Antigen.tw,kf. (0)
70. Ridascreen.tw,kf. (286)
71. Techlab Toxin.tw,kf. (1)
72. Remel ProSpec.tw,kf. (0)
73. Remel Xpect.tw,kf. (10)
74. Quik Chek.tw,kf. (174)
75. Techlab tox*.tw,kf. (11)
76. Premier Immunocard.tw,kf. (0)
77. Chek-60.tw,kf. (14)
78. GeneOhm.tw,kf. (186)
79. Wampole.tw,kf. (116)
80. LEUKO EZ VUE.tw,kf. (2)
81. BD Diagnostics.tw,kf. (145)
82. Xpert.tw,kf. (4740)
83. Prodesse TaqMan.tw,kf. (0)
84. Illumigene.tw,kf. (119)
85. Meridian.tw,kf. (6410)
86. Bioconnections.tw,kf. (5)
87. Techlab.tw,kf. (236)
88. Biopharm.tw,kf. (915)
89. Oxoid.tw,kf. (923)
90. The Binding Site.tw,kf. (122746)
91. Vidas.tw,kf. (1380)
92. Remel.tw,kf. (216)
93. or/67-92 (137965)
94. 9 and 93 (678)
95. 9 and 49 and 66 (6430)
96. 94 or 95 (6596)
97. (C* difficile adj2 (test or tests)).tw,kf. (668)
98. Clostridium difficile infection/di [Diagnosis] (1791)
99. pseudomembranous colitis/di [Diagnosis] (943)
100. 97 or 98 or 99 (3249)
101. 96 or 100 (8039)
102. limit 101 to yr="2010 -Current" (6054)
103. Animal experiment/ not (human experiment/ or human/) (2594766)

104. 102 not 103 (5948)

Web of Science Core Collection (Science Citation Index 1970-current)

Date of search: 2 January 2024

Search 1

(TS=(“C* difficile” OR Cdifficile OR “Peptoclostridium difficile” OR “clostridium infection*”)

And

TS=(“glutamate dehydrogenase*” OR "Toxin* A” OR “Toxin* B" OR “Nucleic Acid Amplification” OR “nucleic acid test*” OR NAAT OR “Polymerase Chain Reaction*” OR qPCR or PCR) OR TS=(immunoassay* OR “immune assay*” OR Immunospot OR Immunoenzyme OR “immunosorb* assay*” OR “membrane bound assay*” OR “isothermal amplification assay*”) OR TS=(“enzyme-linked immunosorbent” OR ELISA OR “Loop-mediated isothermal amplification” OR LAMP) OR TI=(assay*) OR KP=(assay*) OR TS=((diagnos* NEAR/3 (test* or technique*)) OR ribotyp* OR riboprint* OR “bacterial typ*” OR (cytotox* NEAR/5 (culture* or test* or assay*)) OR (toxigenic NEAR/5 (culture* or assay* or test*)) OR (toxin* NEAR/5 (EIA or assay* or enzyme* or gene* or test* or detec* or analy*)))

And

TS=(sensitivity OR specificity OR ((“pre-test” or pretest) NEAR/0 probability) OR “post-test probability” OR “predictive value*” OR “likelihood ratio*” OR diagnos* OR “false positive*” OR “false negative*” OR “true positive*” OR “true negative*” OR marker*))
OR TS=(“C* difficile” NEAR/2 (test OR tests))

Search 2

TS=(“C* difficile” OR Cdifficile OR “Peptoclostridium difficile” OR “clostridium infection*”)

And

TS=(“Premier Toxin” OR (Vidas NEAR/3 toxin) OR “GA Clostridium difficile Antigen” OR Ridascreen OR “Techlab Toxin” OR “Remel ProSpec” OR “Remel Xpect” OR “Quik Chek” OR “Techlab tox*” OR “Premier Immunocard” OR “Chek-60” OR GeneOhm OR Wampole OR “LEUKO EZ VUE” OR “BD Diagnostics” OR Xpert OR “Prodesse TaqMan” OR Illumigene OR Meridian OR Bioconnections OR Techlab OR Biopharm OR Oxoid OR “The Binding Site” OR Vidas OR Remel)

Search 3

Search 1 OR search 2,979 results

Web of Science Preprint Citation Index (1991-current)

Date of search: 02/01/2024

Search 1

(TS=(“C* difficile” OR Cdifficile OR “Peptoclostridium difficile” OR “clostridium infection*”)

And

TS=(“glutamate dehydrogenase*” OR "Toxin* A” OR “Toxin* B” OR “Nucleic Acid Amplification” OR “nucleic acid test*” OR NAAT OR “Polymerase Chain Reaction*” OR qPCR or PCR) OR TS=(immunoassay* OR “immune assay*” OR Immunospot OR Immunoenzyme OR “immunosorb* assay*” OR “membrane bound assay*” OR “isothermal amplification assay*”) OR TS=(“enzyme-linked immunosorbent” OR ELISA OR “Loop-mediated isothermal amplification” OR LAMP) OR TI=(assay*) OR KP=(assay*) OR TS=((diagnos* NEAR/3 (test* or technique*)) OR ribotyp* OR riboprint* OR “bacterial typ*” OR (cytotox* NEAR/5 (culture* or test* or assay*)) OR (toxigenic NEAR/5 (culture* or assay* or test*)) OR (toxin* NEAR/5 (EIA or assay* or enzyme* or gene* or test* or detec* or analy*)))

And

TS=(sensitivity OR specificity OR ((“pre-test” or pretest) NEAR/0 probability) OR “post-test probability” OR “predictive value*” OR “likelihood ratio*” OR diagnos* OR “false positive*” OR “false negative*” OR “true positive*” OR “true negative*” OR marker*))
OR TS=(“C* difficile” NEAR/2 (test OR tests))

Search 2

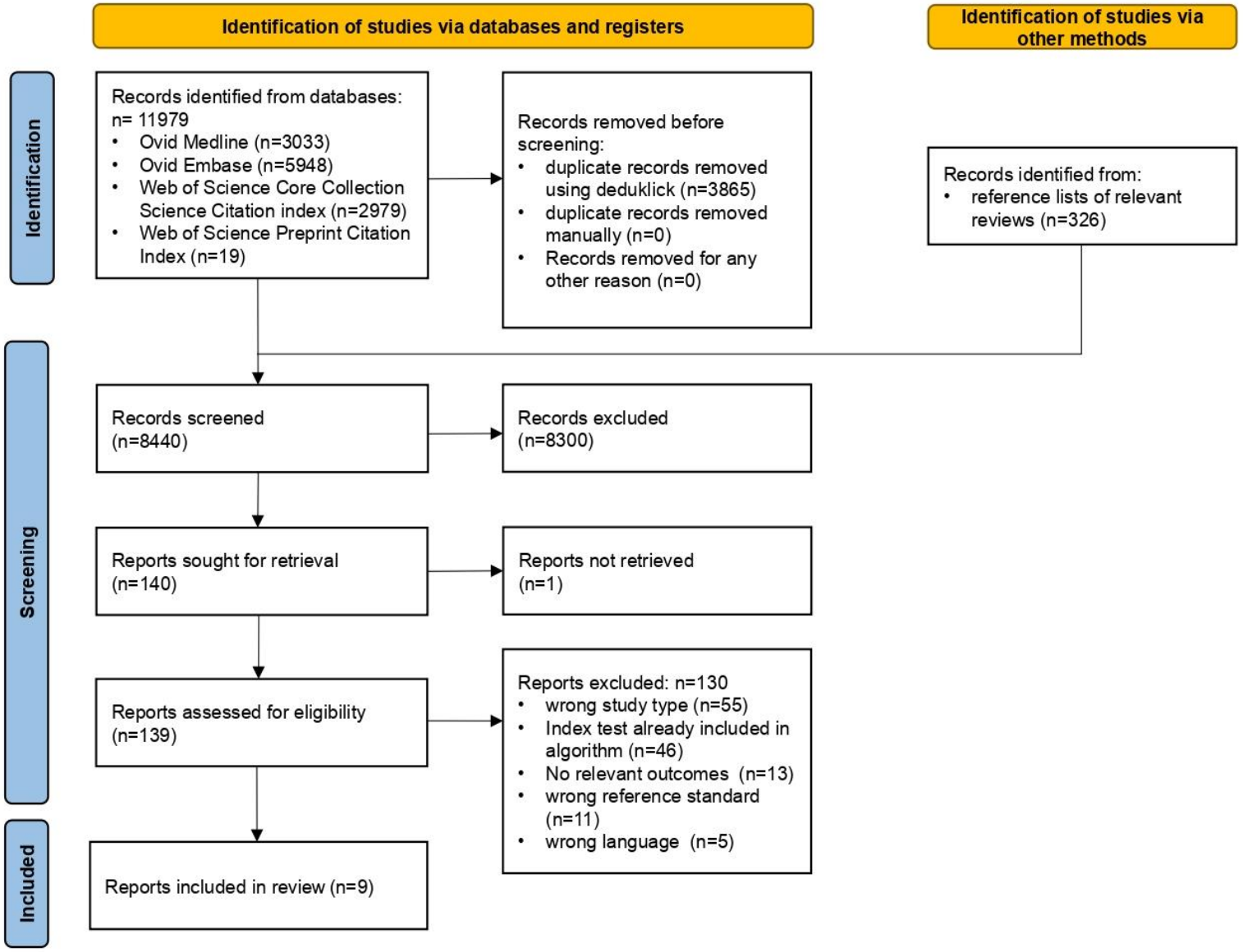
TS=(“C* difficile” OR Cdifficile OR “Peptoclostridium difficile” OR “clostridium infection*”)

And
TS=(“Premier Toxin” OR (Vidas NEAR/3 toxin) OR “GA Clostridium difficile Antigen” OR Ridascreen OR “Techlab Toxin” OR “Remel ProSpec” OR “Remel Xpect” OR “Quik Chek” OR “Techlab tox*” OR “Premier Immunocard” OR “Chek-60” OR GeneOhm OR Wampole OR “LEUKO EZ VUE” OR “BD Diagnostics” OR Xpert OR “Prodesse TaqMan” OR Illumigene OR Meridian OR Bioconnections OR Techlab OR Biopharm OR Oxoid OR “The Binding Site” OR Vidas OR Remel)

Search 3

Search 1 OR search 2 = 19 result

Figure A.1. PRISMA diagram



Text version of Figure A.1. PRISMA diagram

A PRISMA diagram showing the flow of studies through this review, ultimately including 9 studies.

From identification of studies via databases and registers, n=11979 records identified from databases:

- Ovid Medline (n=3033)
- Ovid Embase (n=5948)
- Web of Science Core Collection Science Citation Index (n=2979)
- Web of Science Preprint Citation Index (n=19)

From these, records removed before screening:

- duplicate records removed using Deduklick (n=3865)
- duplicate records removed manually (n=0)
- records marked as ineligible by automation tools (n=0)
- records removed for other reasons (n=0)

n=326 further studies were identified from the reference lists of relevant reviews.

n=8440 records screened, of which n=8300 were excluded, leaving n=140 papers sought for retrieval, of which n=1 was not retrieved.

Of the n=139 papers assessed for eligibility, n=130 reports were excluded:

- wrong study type (n=55)
- Index test already included in algorithm (n=46)
- wrong relevant outcomes (n=13)
- wrong reference standard (n=11)
- wrong language (n=5)

n=9 papers included in the review.

Annexe B. Data extraction table

Table B.1. Characteristics of relevant reviews

Acronyms: CI: 95% confidence intervals, C. difficile: Clostridioides difficile, CCNA: cytotoxin neutralisation assay, EIA: enzyme immunoassay, GDH: glutamate dehydrogenase, LAMP: loop-mediated isothermal amplification, PCR: polymerase chain reaction, PPV: positive predictive value, NPV: negative predictive value

Reference	Country and time period	Population	Study type	Reference standard	Index test (or diagnostic test combination)	Results
Bamber 2012 (4)	UK, time period not reported	811 stool samples from patients with type 6 or 7 stools (Bristol stool chart, mushy or entirely liquid stool) and one or more of the following criteria: • requested by GP, ward or infection control • specimens from post-operative patients • specimens from patients previously treated with antibiotics • visibly bloodstained faeces • microscopic presence of pus cells and culture-negative for Salmonella sp. Shigella sp, Camplyobacter sp, E. coli 0157 • specimens from patients with crohn's disease or other inflammatory bowel disease • specimens from patients 65 years or old No demographic or clinical information was reported for patients from whom the samples were taken.	Prospective diagnostic cohort	Toxigenic culture (C. difficile prevalence: 10.5%)	GDH (Launch Premier) followed by toxin AB EIA assay (Launch Premier)	• sensitivity: 56.5% • specificity: 99.2% • PPV: 88.9% • NPV: 95.1% • accuracy: 94.7%
					Toxin AB EIA assay (Launch Premier) followed by Illumigene LAMP assay (Meridian Bioscience)	• sensitivity: 55.4% • specificity: 99.6% • PPV: 94% • NPV: 95.1% • accuracy: 95.1%
					GDH (Launch Premier) followed by Illumigene LAMP assay (Meridian Bioscience)	• sensitivity: 91.6% • specificity: 98.1% • PPV: 84.4% • NPV: 99% • accuracy: 97.4%
					GDH (Launch Premier) followed by Illumigene LAMP assay (Meridian Bioscience) followed by Toxin AB EIA assay (Launch Premier)	• sensitivity: 94% • specificity: 97.7% • PPV: 82.1% • NPV: 99.3% • accuracy: 97.3%
Goret 2015 (5)	France, June to September 2013	468 stool samples (diarrhoea or loose stool) either submitted by a physician for C. difficile testing or submitted for testing systematically where patients had diarrhoea 3 days after hospitalisation. No demographic or clinical information was	Prospective diagnostic cohort	Toxigenic culture (C. difficile prevalence not reported)	DiaSorin chemiluminescence test: GDH and toxin AB EIA assay	• sensitivity: 86.7% (CI: 68.4% to 95.6%) • specificity: 99.1% (CI: 97.7% to 99.8%) • PPV: 86.7% (CI: 68.3% to 95.6%) • NPV: 98.1% (CI: 97.5% to 99.7%)
					Meridian test: GDH followed by NAAT for toxin AB EIA detection	• sensitivity: 90% (CI: 72.3% to 97.8%) • specificity: 98.9% (CI: 97.4% to 99.5%) • PPV: 84.4% (CI: 66.5% to 94.1%)

Reference	Country and time period	Population	Study type	Reference standard	Index test (or diagnostic test combination)	Results
		reported for patients from whom the samples were taken.				• NPV: 99.3% (CI: 97.8% to 99.8%)
					2-step Alere test: GDH followed by toxin AB EIA assay	• sensitivity: 50% (CI: 31.7% to 68.3%) • specificity: 99.5% (CI: 98.1% to 99.9%) • PPV: 88.2% (CI: 62.2% to 97.9%) • NPV: 96.7% (CI: 94.5% to 98.1%)
					3-step Alere test: GDH, followed by toxins A and B EIA, followed by toxigenic culture	• sensitivity: 60% (CI: 40.7% to 76.7%) • specificity: 99.5% (CI: 98.1% to 99.9%) • PPV: 90% (CI: 66.8% to 98.2%) • NPV: 97.3% (CI: 95.2% to 98.5%)
Hart 2014 (6)	Australia, October 2011 to January 2012	150 stool specimens from 75 patients (specimens were loose, liquid, watery or semi-formed). The patients were 44% female, median age 3 years (range 11 days to 17 years). 40% were recruited from haematology/oncology.	Prospective diagnostic cohort	Toxigenic culture (C. difficile prevalence: 36%)	GDH (Quik Chek complete) and toxin AB EIA assay	• true positives: 13 • true negatives: 101 • false positives: 3 • false negatives: 33 • sensitivity: 28% • specificity: 97% • PPV: 81% • NPV: 75%
					GDH and NAAT (specifically, LAMP, Illumigene)	• true positives: 39 • true negatives: 104 • false positives: 0 • false negatives: 7 • sensitivity: 85% • specificity: 100% • PPV: 100% • NPV: 94%
					GDH plus NAAT (specifically reverse transcriptase-PCR, GeneOhm)	• true positives: 38 • true negatives: 103 • false positives: 1 • false negatives: 8 • sensitivity: 83% • specificity: 99% • PPV: 97% • NPV: 93%

Reference	Country and time period	Population	Study type	Reference standard	Index test (or diagnostic test combination)	Results
					Toxigenic culture plus cytotoxin assay (CCNA)	• true positives: 14 • true negatives: 103 • false positives: 0 • false negatives: 33 • sensitivity: 30% • specificity: 100% • PPV: 100% • NPV: 76%
Liu 2021 (7)	China, June 2016 to May 2017	186 stool samples from patients with diarrhoea and clinical symptoms compatible with C. difficile, including n=117 hospital inpatients and n=62 outpatients. Average age: 44 years (no measure of variance reported), 59.2% male. Samples from children under 2 years of age and duplicate samples from the same patient were excluded.	Prospective diagnostic cohort	Toxigenic culture	GDH (VIDAS) followed by toxin AB EIA assay (VIDAS)	• sensitivity: 48.7% (CI: 33% to 64.4%) • specificity: 97.3% (CI: 94.7% to 99.9%) • PPV: 82.6% (CI: 67.1% to 98.1%) • NPV: 87.7% (CI: 82.7% to 92.8%)
					GDH (VIDAS) followed by NAAT (in-house PCR)	• sensitivity: 74.4% (CI: 60.7 to 88.1%) • specificity: 100% (CI: NA) • PPV: 100% (CI: NA) • NPV: 93.6% (CI: 89.8 to 97.5%)
Miller 2013 (8)	USA, January to June 2010	381 stool specimens submitted for C. difficile testing. No demographic or clinical information was reported for patients from whom the samples were taken.	Prospective diagnostic cohort	1. Toxigenic culture (prevalence not reported)	GDH (C. Diff Quik Chek) followed by toxin B assay (C. Diff Quik Chek)	• sensitivity: 44.9% • specificity: 99.6 Raw data not reported to calculate other measures of accuracy.
					GDH (C. DIFF CHEK-60 EIA) followed by CCNA	• sensitivity: 66.1% • specificity: 100% Raw data not reported to calculate other measures of accuracy.
					GDH (C. DIFF QUIK CHEK) followed by CCNA	• sensitivity: 67.7% • specificity: 100% Raw data not reported to calculate other measures of accuracy.
					GDH (C. DIFF CHEK-60 EIA) followed by PCR	• sensitivity: 92.9% • specificity: 100% Raw data not reported to calculate other measures of accuracy.

Reference	Country and time period	Population	Study type	Reference standard	Index test (or diagnostic test combination)	Results
					GDH (C. DIFF QUIK CHEK) followed by PCR	• sensitivity: 96.1% • specificity: 100% Raw data not reported to calculate other measures of accuracy.
				2. GDH (C. DIFF CHEK-60 EIA) followed by CCNA (prevalence not reported) This study did not explicitly say this was the reference standard, we have assumed from their presentation of results.	GDH (C. DIFF QUIK CHEK) followed by PCR	• true positives: 84 • true negatives: 118 • false negatives: 1 • false positives: 41 • sensitivity: 99% • specificity: 74%
Novak-Weekley 2010 (9)	USA, time period not reported	432 stool specimens from patients with suspected C. difficile. No demographic or clinical information was reported for patients from whom the samples were taken.	Prospective diagnostic cohort	Toxigenic culture	GDH (C. DIFF CHEK-60 EIA) followed by toxin AB EIA assay (Premier Toxins A and B microwell EIA)	• true positives: 40 • true negatives: 354 • false positives: 6 • false negatives: 32 • sensitivity: 55.6% • specificity: 98.3% • accuracy: 91.2% • PPV: 87% • NPV: 91.7%
					GDH (C. DIFF CHEK-60 EIA) followed by NAAT (Xpert C. difficile PCR assay)	• true positives: 62 • true negatives: 352 • false positives: 8 • false negatives: 10 • sensitivity: 86.1% • specificity: 97.8% • accuracy: 95.8% • PPV: 88.6% • NPV: 97.2%

Reference	Country and time period	Population	Study type	Reference standard	Index test (or diagnostic test combination)	Results
Planche 2013 (10)	UK, October 2010 to September 2011	Multicentre study conducted in 4 UK hospital laboratories. 12,420 stool samples analysed from 10,186 patients. 8,026 results were obtained from 6,665 episodes of diarrhoea (defined as a diarrhoeal sample received more than 28 days after a previous sample). Clinical outcomes and reference assay data was were available for 6,522 inpatient episodes (from 6,283 patients). The patients were 54% female, average age 64 years (standard deviation: 21 years).	Prospective diagnostic cohort	Cytotoxigenic culture (referred to as toxigenic culture in other studies, n=12,366)	GDH and NAAT	• sensitivity: 91.5% (CI: 89.6% to 93.1%) • specificity: 98% (CI: 97.7% to 98.3%) • PPV: 80.7% (CI: 78.3% to 82.9%) • NPV: 99.2% (99% to 99.4%)
					Toxin EIA 2 and NAAT	• sensitivity: 57.8% (CI: 54.8% to 60.9%) • specificity: 99.5% (CI: 99.3% to 99.6%) • PPV: 90.7% (CI: 88.3% to 92.8%) • NPV: 96.3% (CI: 95.9% to 96.6%)
					GDH and toxin EIA 2	• sensitivity: 57% (CI: 53.9% to 60%) • specificity: 99.4% (CI: 99.3% to 99.6%) • PPV: 90.1% (CI: 87.5% to 92.2%) • NPV: 96.2% (CI: 95.8% to 96.5%)
				Cytotoxin assay (n=12,402)	GDH and NAAT	• sensitivity: 95.6% (CI: 93.9% to 97%) • specificity: 95.9% (CI: 95.6% to 96.3%) • PPV: 59.7% (CI: 56.8% to 62.5%) • NPV: 99.7% (CI: 99.6% to 99.8%)
					Toxin EIA 2 NAAT	• sensitivity: 82.9% (CI: 80% to 85.6%) • specificity: 99.6% (CI: 99.4% to 99.7%) • PPV: 92.1% (CI: 89.8% to 94%) • NPV: 98.9% (CI: 98.7% to 99.1%)
					GDH and toxin EIA 2	• sensitivity: 81.8% (CI: 78.8% to 84.5%) • specificity: 99.5% (CI: 99.4% to 99.6%) • PPV: 91.6% (CI: 89.2% to 93.6%) • NPV: 98.9% (CI: 98.7% to 99
Taylor 2018 (3)	Canada, time period not reported	300 samples (30% positive, 70% negative for C. difficile). Each detection round consisted of 10 samples with a randomised number of positives (1 to 5). Scent dog trainer was blinded to which samples were positive. Investigator was isolated from the trainer and dog during the trial. Scent dogs indicated result by ‘sit’ (positive), or ‘no sit’ (negative). Correct identification of positive samples was positively reinforced (food	Cross-sectional diagnostic cohort	GDH and Illumigene toxin assay	Scent dog 1 (3-year-old German Shepherd, trained to detect the specific odour of toxin gene positive C. difficile in stool samples)	• sensitivity: 77.6% (CI: 67.3% to 86%) • specificity: 85.1% (CI: 79.6% to 89.6%) Raw data not reported to calculate other measures of accuracy.
					Scent dog 2 (3-year-old Border Collie Pointer, trained to detect the specific odour of toxin gene	• sensitivity: 92.6% (CI: 84.6% to 97.2%) • specificity: 84.5% (CI: 79% to 89%)

Reference	Country and time period	Population	Study type	Reference standard	Index test (or diagnostic test combination)	Results
		reward), with no positive reward for correct negative or incorrect responses.			positive C. difficile in stool samples)	Cohen's kappa interrater reliability between scent dogs was moderate (0.52).
Whitehead 2014 (2)	UK, time period not reported	120 C. difficile-positive and 99 C. difficile-negative stool samples (hospital acquired diarrhoea). A 2-step diagnostic test was only used for phase 2 of the study (previously a one-step test), on 45 samples, this is reported as the reference standard (only results for this reference standard were relevant to this review and reported). Patients were aged 21 or over in with type 6 or 7 stools (Bristol Stool Chart).	Diagnostic case control study	GDH (TECHLAB C. diff Chek ELISA) followed by toxin gene assay (Cepheid Xpert reverse transcriptase-PCR)	Faecal calprotectin (Immunodiagnostik PhiCal ELISA)	Faecal calprotectin levels of 50 micrograms per g^{-1} were considered positive for C. difficile presence. Receiver operator characteristic curve analysis gave an area under the curve was 0.80 for C. difficile positivity. The optimum faecal calprotectin value was determined to be 169 µg per g^{-1}, with a sensitivity of 73% and a specificity of 77% against the reference standard. Raw data not reported to calculate other measures of accuracy.

Annexe C. Excluded full texts

Wrong study type (n=55)

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Wrong reference standard (n=11)

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Wrong language (n=5)

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Annexe D. Risk of bias assessment

Table D:1 Results of risk of bias assessment using QUADAS-2

Study	Risk of bias				Concerns regarding applicability			Note any risks of bias	Note any concerns about applicability
	Domain 1: Patient selection	Domain 2: Index tests	Domain 3: Reference tests	Domain 4: Flow and timing	Domain 1: Patient selection	Domain 2: Index tests	Domain 3: Reference tests		
Novak-Weekley and others 2010	Unclear	Unclear	Low risk	Low risk	Unclear	Low risk	Low risk		
Bamber 2012	Low risk	Unclear	Low risk	Low risk	Unclear	Low risk	Low risk		
Miller 2013	Unclear	Unclear	Unclear	Unclear	Unclear	Low risk	Low risk		
Goret 2015	Unclear	Unclear	Unclear	Unclear	Unclear	Low risk	Low risk		
Hart 2014	Low risk	Unclear	Unclear	Unclear	High Risk	Low risk	Low risk		• samples collected from patients with Haematology or Oncology background
Planche 2013	Unclear	Unclear	High risk	Low risk	Low Risk	Low risk	Low risk	• index test results not interpreted without knowledge of reference standard results, and vice versa	
Whitehead 2014	High risk	High risk	Low risk	Unclear	Unclear	High risk	Low risk	• index test results not interpreted without knowledge of reference standard results	• index test (faecal calprotectin) unlikely to be useful as a standalone diagnostic test
Liu 2021	Unclear	Unclear	Unclear	Unclear	Unclear	Low risk	Low risk		
Taylor 2018	Low risk	Unclear	Low risk	Low risk	Unclear	High risk	Low risk		• only 2 scent dogs studied, with relatively low interrater reliability, therefore unlikely to be generalisable to all sniffer dogs

QUADAS-2 questions:

Domain 1: Patient selection
Was a consecutive or random sample of patients enrolled?
Was a case-control design avoided?
Did the study avoid inappropriate exclusions?
Could the selection of patients have introduced bias?
Is there concern that the included patients do not match the review question?

Domain 2: Index tests
Were the index test results interpreted without knowledge of the results of the reference standard?

If a threshold was used, was it pre-specified?
Could the conduct or interpretation of the index test have introduced bias?
Is there concern that the index test, its conduct, or interpretation differ from the review question?

Domain 3: Reference standard

Is the reference standard likely to correctly classify the target condition?
Were the reference standard results interpreted without knowledge of the results of the index test?
Could the reference standard, its conduct, or its interpretation have introduced bias?
Is there concern that the target condition as defined by the reference standard does not match the review question?

Domain 4: Flow and timing

Was there an appropriate interval between index tests and reference standard?
Did all patients receive a reference standard?
Did patients receive the same reference standard?
Were all patients included in the analysis?
Could the patient flow have introduced bias?

About the UK Health Security Agency

UKHSA is responsible for protecting every member of every community from the impact of infectious diseases, chemical, biological, radiological and nuclear incidents and other health threats. We provide intellectual, scientific and operational leadership at national and local level, as well as on the global stage, to make the nation heath secure.

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