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Performance of Commercial Tests for Molecular Detection of Shiga Toxin-producing Escherichia coli (STEC): A Systematic Review and Meta-analysis Protocol

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Key Words

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Abstract

Introduction

Rapid detection of Shiga toxin-producing *Escherichia coli* (STEC) enables appropriate treatment and provides clarity to often confusing cases. Numerous commercially-available molecular tests exist, but they vary in clinical performance. This systematic review aims to synthesize available evidence to compare the clinical performance of enzyme immunoassay (EIA) and nucleic acid amplification tests (NAAT) for the detection of STEC.

Methods and Analysis

The following databases will be searched employing a standardized search strategy: Medline, Embase, Cochrane CENTRAL Register of Controlled Trials, Cochrane Database of Systematic Reviews, PubMed, Scopus, and Web of Science. Grey literature will be searched under advice from a medical librarian. Independent reviewers will screen titles, abstracts, and full-texts of retrieved studies for relevant studies. Data will be extracted independently by two reviewers, using a piloted template. QUADAS-2 will be employed to assess the risk of bias of individual studies, and the quality of evidence will be assessed with the GRADE approach. Bivariate random effects and hierarchical receiver operator characteristic models will be used to meta-analyze the sensitivity and specificity of commercial STEC diagnostic tests.

Ethics and Dissemination

Ethics approval was not required for this systematic review and meta-analysis. Findings will be disseminated in conferences, through a peer-reviewed journal, and via personal interactions with relevant stakeholders.

PROSPERO Registration Number

CRD42018099119

Article Summary

Strengths and Limitations of this Study

- There is little evidence reviewing the relative clinical performance of commercially-available tests for Shiga Toxin-producing *Escherichia coli* (STEC)
- A key strength of this study is the comprehensive comparison of enzyme immunoassays (EIA) and nucleic acid amplification tests (NAAT) to inform clinical practice
- A limitation is the lack of a common gold standard for STEC identification, which may introduce heterogeneity into our analysis
- Another limitation is that the finding of a Shiga toxin (Stx) 1 producing STEC that does not also produce Stx2, especially in the absence of bloody diarrhea, is of unclear clinical and epidemiologic value.

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) cause significant disease. Although prototypical *E. coli* O157:H7 is the leading cause of hemolytic uremic syndrome (HUS), other STEC serotypes have been associated with severe disease and large outbreaks.[1-4] Multiple serotypes have now been linked to disease, and, unlike the O157 serotype, detection of non-O157 serotypes has increased significantly in the past decade, though likely because of expanding technology to detect these organisms.[5] Patients infected with STEC often seek care through emergency departments (EDs), especially if they have bloody diarrhea. Strong evidence suggests that antibiotics may increase the risk of developing HUS if administered to people infected with STEC,[6-8] and a recent meta-analysis demonstrated that the early administration of fluids is associated with improved outcomes.[9] Therefore, it is important that healthcare providers have a means of detecting STEC that is both rapid and applicable to any serotype.

Historically, STEC testing has focused on the O157 serogroup using culture on sorbitol-MacConkey agar, leveraging its inability to ferment sorbitol.[10] This attribute is not shared by other STEC

serogroups, so they are overlooked if sorbitol-MacConkey agar culture is the only detection method employed. Further, culture can take days to yield results, delaying informed management.[11] In light of the limitations of culture, enzyme immunoassay (EIA) and nucleic acid amplification tests (NAAT) have been developed to detect STEC irrespective of serogroup. Reflecting their popularity, the U.S. Council of State and Territorial Epidemiologists (CSTE) has recently revised the probable STEC case definition to include laboratory evidence from EIA and NAAT.[12]

Numerous tests to detect STEC are commercially available.[13,14] The EIAs detect Shiga toxin, and most NAAT detect the Shiga toxin genes *stx1* and *stx2*, and some additionally seek a locus that is specific to the O157 serogroup. For NAAT, STEC is often one of several enteropathogens detected by the assay. EIA has suboptimal sensitivity, particularly if a time-consuming enrichment step is not conducted.[15-18] Commercial NAAT appear to be more sensitive, but results vary by study and test.[19-21] NAAT are more costly than traditional microbiologic techniques owing to the equipment and consumables required to perform them. However, higher cost may be compensated by increased ascertainment,[21] improved patient outcomes, or decreased need to implement contact precautions.[22] As laboratories consider NAATs, it is crucial to identify the best testing strategy to support time-sensitive, cost-effective treatment decisions. Thus, we will conduct a systematic review of commercial EIA and NAAT for STEC detection to determine if and how their performance differs in terms of diagnostic test accuracy.

Methods and Analysis

This systematic review and meta-analysis will be conducted in accordance with reporting requirements for Preferred Reporting Items for Systematic Review and Meta-analysis Statement (PRISMA). This protocol was prepared according to PRISMA-P guidelines.[23]

Research Question

What is the accuracy of commercially-available EIA and NAAT for the detection of STEC and how do they differ?

Eligibility Criteria

- Population: study participants with acute diarrhea, who provide a stool specimen or rectal swab for testing; any age, geography, or sub-population
- Intervention: any commercially-available EIA or NAAT for the detection of Shiga toxin, or *stx1* and *stx2*; NAAT for the identification of the O157 serogroup, if available
- Comparisons: for NAAT, reference standards incorporating at least one of the following: enhanced protocols, real-time PCR, sequencing, and/or other NAAT; for EIA, reference standards incorporating Vero cell cytotoxicity assay and/or those accepted for NAAT comparison
- Outcomes: primary- sensitivity and specificity for the detection of STEC; secondary- 1) area under the curve (AUC) and other single diagnostic performance measures, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), and negative likelihood ratio (LR-) for the detection of STEC, 2) sensitivity and specificity for detection of specific Shiga toxin subtypes, and 3) sensitivity and specificity for detection of the O157 serogroup
- Study designs: Cross-sectional stool samples/swabs with participants may be drawn from randomized trials, cohort studies, case-control studies, cross-sectional studies, and case series

Literature Searches

The following databases will be searched from 2005: MEDLINE, Cochrane CENTRAL Register of Controlled Trials, Cochrane Database of Systematic Reviews, EMBASE, PubMED, SCOPUS, and Web of Science. Clinical trial databases (ClinicalTrials.gov), FDA applications, product materials, government/NGO reports and conference abstracts will also be searched under the advice of STEC subject experts and a medical librarian. The reference lists of included studies will be scanned to

identify additional studies of relevance to this review. The specific search strategy can be found in Appendix I.

Study Records

Data Management

Records retrieved will be uploaded into EndNote (Philadelphia, V8), and de-duplicated using EndNote (Philadelphia, V8) and Rayyan for Systematic Reviews (Qatar, 2018).

Selection Process

Two trained reviewers (GT, CYL) will independently screen all titles and abstracts in duplicate, and a third reviewer (SF) will adjudicate any disagreements. Studies will be included if the title and abstract indicate that the manuscript may contain data related to the evaluation of EIA or NAAT for the detection of STEC. The full text of all potentially relevant citations will then be obtained and reviewed by two independent reviewers (GT, CYL) using the predefined eligibility criteria outlined above, with the involvement of a third reviewer (SF) in case consensus cannot be reached. Reasons for inclusion and exclusion will be documented. A tool to document the selection process will be developed, piloted with the first 25 search results, and modified as necessary.

Data Extraction

Two trained reviewers will extract data independently and in duplicate using a structured form. The form will be piloted on the first 5 included studies and modified as necessary. Discordances will be resolved through discussions involving the investigators and subject matter experts. First and last study authors will be contacted if data necessary to calculate sensitivity or specificity are absent from the manuscript. Study characteristics and study outcomes (Table 1) will be extracted from included studies.

Risk of Bias Assessments

To assess the risk of bias in individual studies, we will employ the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2).[24] We will follow the recommended process for tailoring the QUADAS-2 to our systematic review, including iteratively tailoring the QUADAS-2 assessment tool and piloting it on at least five studies until consensus has been reached on a version of the tool.[24] As part of this process, we will review the Standards for Reporting of Diagnostic Accuracy (STARD)[25] for the addition of relevant criteria. The risk of bias in individual studies (for all outcomes reported) will be rated as Low/Medium/High.[26] Assessments will be made independently by two reviewers, and disagreements will be resolved by discussion, or where necessary, by a third reviewer. Risk of bias will be reported for all included studies.

Data Synthesis

Separate synthesis will be conducted for EIA and NAAT. For each of test type, data will be quantitatively synthesized if at least four studies have been identified. If the number of included studies for either EIA or NAAT is insufficient, point estimates and confidence intervals from the individual papers will be shown, and the comparison of EIA and NAAT will be based on the range of estimates reported in individual papers.

If four or more studies are included for a given test type, a bivariate random effects model[27] will be used to calculate summary estimates and confidence intervals of primary outcomes and secondary outcomes, and a hierarchical summary receiver operating characteristic (ROC) curve[28] will be constructed.[29] The summary point for sensitivity and specificity with confidence ellipse and the hierarchical summary ROC curve will be graphed. These analyses take into account the correlation between sensitivity and specificity and potential threshold effects (e.g. due to cycle thresholds used in PCR).[29] To identify study characteristics that may be contributing to heterogeneity, we will conduct subgroup analyses when at least four studies are available per subgroup.

To compare EIA and NAAT, we will graphically compare point estimates and confidence intervals for sensitivity and specificity resulting from the separate meta-analysis of each type of test. Additionally,

if there is adequate consistency in gold standards used to assess validity, we will pool all EIA and NAAT studies in a single meta-analysis and include test type as a covariate to test the difference in accuracy between EIA and NAAT. Meta-analysis packages in R[30] and RevMan[31] will be used to conduct all analyses.

We will also conduct the follow subgroup analyses:

- Funding (industry vs. other)
- Data source (published vs. unpublished)
- Age (<10 years-old and <18 years-old)
- Location of care
- Diarrhea duration (<7 days, ≥7 days, not specified)
- Presence of bloody diarrhea
- Specimen type
- Test brand
- Test targets
- Comparator type (i.e. reference standard)

Other subgroup analyses not pre-specified here will be identified as such in all reports.

A sensitivity analysis excluding studies with a high risk of bias will be conducted. Additional sensitivity analyses will be added if other potential biases become apparent during the review.

Quality of Evidence Assessment

For the body of evidence as a whole, two reviewers, one with clinical and one with methodological expertise, will independently use the GRADE approach to assess the quality of evidence for the primary outcomes.[32,33] The test will be considered in the context of common diagnostic algorithms, subsequent clinical management, and patient outcomes to assign importance to the consequences of summary sensitivity and specificity findings (e.g. frequency of false negatives). The domains of

study design, limitations/risk of bias, directness, consistency, and precision will be assessed and combined into a summary grade for all important outcomes of the test.

Ethics and Dissemination

Ethics approval was not required for this systematic review and meta-analysis. Findings will be disseminated in conferences, through a peer-reviewed journal, and via personal interactions with relevant stakeholders.

Author Contributions

GT conceived the study, contributed to study design, drafted the protocol, and revised the protocol following author comments; CYL contributed to study design, drafted the protocol, and provided critical revisions; DL contributed to study design and provided critical revisions; LC contributed to study design and provided critical revisions; PT contributed to study design and provided critical revisions; LH contributed to study design and provided critical revisions; BV contributed to study design and provided critical revisions; SF conceived the study, contributed to study design, and provided critical revisions.

Funding

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Competing Interests

Dr. Stephen Freedman has previously received in-kind grant support from BioMerieux and Luminex Corporation. Dr. Chui received funding from TechLab for a previous study on SHIGA TOXIN QUICK CHEK and SHIGA TOXIN CHEK. Dr. Phillip Tarr has served as a consultant to BioRad.

Data Statement

All data can be accessed upon request to the corresponding author (GT).

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TABLES

Table 1. Data to be extracted from each included study.

Item	Rationale
Study Characteristics	
Data source	Peer-reviewed studies will be distinguished from non-peer-reviewed data for potential subgroup analysis
Funding source	Studies funded by diagnostic test companies may be subject to additional bias; potential subgroup analysis
Study design	Cross-sectional studies are expected; other study designs will be noted for potential subgroup analysis
Population	Population restrictions within the study (e.g. by age, HUS status, etc.) will be noted for potential subgroup analysis
Setting	Country or region; potential subgroup analysis
Clinical Data	
Location of care	Primary care vs. ED vs. hospital, and potentially other; potential subgroup analysis
Diarrhea definition	Study definition for diarrhea (e.g. ≥3 episodes in 24 hours) will facilitate comparability assessment and interpretation
Diarrhea duration	Mean/median or restrictions on illness duration at the time of sampling; facilitate comparability assessment and interpretation
Specimen type	Stool specimen or rectal swab; potential subgroup analysis
Bloody diarrhea	Frequency of bloody diarrhea; potential subgroup analysis
Test	
Brand name	Ease of reference
Type	EIA or NAAT for main comparison
Enrichment	For EIA tests; potential subgroup analysis
Targets	Toxin vs. DNA, STEC-only vs. multianalyte; interpretation and potential subgroup analysis
Cycle threshold	Cycle cutoff for positivity; facilitate comparability assessment and interpretation
Comparator/reference standard	Composite standard with component tests, discrepant analysis with confirmatory tests; interpretation and potential source of bias
Specimen comparability	Specimens tested by index and comparator from the same point in time, of the same type, etc.; potential source of bias
Outcomes	
Outcome type	For STEC generally, Shiga toxin 1 vs. 2, or O157 vs. non-O157; distinguish primary and secondary outcomes
Number tested	Outcome calculation and interpretation
Number confirmatory tested	Outcome calculation and interpretation
Number of true positives	Outcome calculation
Number of false positives	Outcome calculation
Number of true negatives	Outcome calculation
Number of false negatives	Outcome calculation
Sensitivity	Primary outcome
Specificity	Primary outcome
Single accuracy measures	e.g. AUC, diagnostic accuracy, diagnostic OR; secondary outcome
PPV	Secondary outcome
NPV	Secondary outcome

LR+	Secondary outcome
LR-	Secondary outcome

Appendix I

Shiga Toxin Detection Review
Search Strategies

MEDLINE

1. *Molecular Diagnostic Techniques/mt [Methods]
2. *Real-Time Polymerase Chain Reaction/
3. exp *Immunoenzyme Techniques/mt [Methods]
4. (Allplex Gastrointestinal Panel Assay* or BD MAX Enteric Bacterial Panel* or BD MAXTM Enteric Bacterial Panel* or BDM-EBP or BioFire FilmArray GI Panel* or Commercial molecular test* or EIA or Enzyme immunoassay* or FilmArray Gastrointestinal panel* or FilmArray GI panel* or Gastrointestinal pathogen panel* or GPP or ImmunoCard STAT* or LD-PCR or Liaison EHEC Toxins or Luminex xTAG multiplex assay* or Multiplex real-time PCR or NAAT or Nucleic acid amplification test* or Premier EHEC or Prodesse ProGastro SSCS Assay* or Prolisa EHEC EIA or RAPID-B* or real-time PCR assay* or Shiga Toxin Chek or Shiga Toxin Direct or ST Direct or Shiga Toxin Quik Chek or Stool Bacterial Pathogens Panel or Verigene Enteric Pathogens Nucleic Acid Test or xTAG Gastrointestinal Pathogen Panel or xTAG GPP).tw.
5. 1 or 2 or 3 or 4
6. Diarrhea/di [Diagnosis]
7. Escherichia coli O157/ip [Isolation & Purification]
8. FECES/mi [Microbiology]
9. exp Shiga-Toxigenic Escherichia coli/ip [Isolation & Purification]
10. (Diarrhea or E coli O157 or E coli serotype O157 or Escherichia coli O157 or ehec or "enteric bacterial and viral pathogen*" or enteric pathogen* or infectious gastroenteritis or O157 or rfbEO157 or shiga* or shigella or shigellosis or STEC or stool or Stx1 or stx?2 or Stx2).tw.
11. 6 or 7 or 8 or 9 or 10
12. 5 and 11
13. limit 12 to yr="2005 -Current"
14. limit 13 to animals
15. limit 13 to (animals and humans)
16. 14 not 15
17. 13 not 16
18. limit 17 to (editorial or letter)
19. 17 not 18
20. limit 19 to "review"
21. 19 not 20
22. ((systematic or critical or scoping) adj (review or overview or synthesis)).tw.
23. 19 and 22

24. limit 19 to systematic reviews

25. 21 or 23 or 24

26. exp "Sensitivity and Specificity"/

27. False Negative Reactions/ or False Positive Reactions/ or Reference Values/

28. (accuracy or detect or detecting or detection or diagnosis or diagnosing or false negative* or false positive* or predictive value* or sensitive or sensitivity or specificity or validat*).tw.

29. 26 or 27 or 28

30. 25 and 29

Cochrane CENTRAL Register

1. *Molecular Diagnostic Techniques/mt [Methods]

2. *Real-Time Polymerase Chain Reaction/

3. exp *Immunoenzyme Techniques/mt [Methods]

4. (Allplex Gastrointestinal Panel Assay* or BD MAX Enteric Bacterial Panel* or BD MAXTM Enteric Bacterial Panel* or BDM-EBP or BioFire FilmArray GI Panel* or Commercial molecular test* or EIA or Enzyme immunoassay* or FilmArray Gastrointestinal panel* or FilmArray GI panel* or Gastrointestinal pathogen panel* or GPP or ImmunoCard STAT* or LD-PCR or Liaison EHEC Toxins or Luminex xTAG multiplex assay* or Multiplex real-time PCR or NAAT or Nucleic acid amplification test* or Premier EHEC or Prodesse ProGastro SSCS Assay* or Prolisa EHEC EIA or RAPID-B* or real-time PCR assay* or Shiga Toxin Chek or Shiga Toxin Direct or ST Direct or Shiga Toxin Quik Chek or Stool Bacterial Pathogens Panel or Verigene Enteric Pathogens Nucleic Acid Test or xTAG Gastrointestinal Pathogen Panel or xTAG GPP).tw.

5. 1 or 2 or 3 or 4

6. Diarrhea/di [Diagnosis]

7. Escherichia coli O157/ip [Isolation & Purification]

8. FECES/mi [Microbiology]

9. exp Shiga-Toxigenic Escherichia coli/ip [Isolation & Purification]

10. (Diarrhea or E coli O157 or E coli serotype O157 or Escherichia coli O157 or ehec or "enteric bacterial and viral pathogen*" or enteric pathogen* or infectious gastroenteritis or O157 or rfbEO157 or shiga* or shigella or shigellosis or STEC or stool or Stx1 or stx?2 or Stx2).tw.

11. 6 or 7 or 8 or 9 or 10

12. 5 and 11

13. limit 12 to yr="2005 -Current"

14. limit 13 to animals

15. limit 13 to (animals and humans)

16. 14 not 15

17. 13 not 16

18. ((systematic or critical or scoping) adj (review or overview or synthesis)).tw.

19. exp "Sensitivity and Specificity"/

20. False Negative Reactions/ or False Positive Reactions/ or Reference Values/

21. (accuracy or detect or detecting or detection or diagnosis or diagnosing or false negative* or false positive* or predictive value* or sensitive or sensitivity or specificity or validat*).tw.

22. 19 or 20 or 21

23. 17 and 22

Cochrane Database of Systematic Reviews

1. (Allplex Gastrointestinal Panel Assay* or BD MAX Enteric Bacterial Panel* or BD MAXTM Enteric Bacterial Panel* or BDM-EBP or BioFire FilmArray GI Panel* or Commercial molecular test* or EIA or Enzyme immunoassay* or FilmArray Gastrointestinal panel* or FilmArray GI panel* or Gastrointestinal pathogen panel* or GPP or ImmunoCard STAT* or LD-PCR or Liaison EHEC Toxins or Luminex xTAG multiplex assay* or Multiplex real-time PCR or NAAT or Nucleic acid amplification test* or Premier EHEC or Prodesse ProGastro SSCS Assay* or Prolisa EHEC EIA or RAPID-B* or real-time PCR assay* or Shiga Toxin Chek or Shiga Toxin Direct or ST Direct or Shiga Toxin Quik Chek or Stool Bacterial Pathogens Panel or Verigene Enteric Pathogens Nucleic Acid Test or xTAG Gastrointestinal Pathogen Panel or xTAG GPP).tw.

2. (Diarrhea or E coli O157 or E coli serotype O157 or Escherichia coli O157 or ehec or "enteric bacterial and viral pathogen*" or enteric pathogen* or infectious gastroenteritis or O157 or rfbEO157 or shiga* or shigella or shigellosis or STEC or stool or Stx1 or stx?2 or Stx2).tw.

3. 1 and 2

4. limit 3 to yr="2005 -Current"

5. (accuracy or detect or detecting or detection or diagnosis or diagnosing or false negative* or false positive* or predictive value* or sensitive or sensitivity or specificity or validat*).tw.

6. 4 and 5

EMBASE

1. *molecular diagnosis/

2. *real time polymerase chain reaction/

3. *enzyme immunoassay/

4. (Allplex Gastrointestinal Panel Assay* or BD MAX Enteric Bacterial Panel* or BD MAXTM Enteric Bacterial Panel* or BDM-EBP or BioFire FilmArray GI Panel* or Commercial molecular test* or EIA or Enzyme immunoassay* or FilmArray Gastrointestinal panel* or FilmArray GI panel* or Gastrointestinal pathogen panel* or GPP or ImmunoCard STAT* or LD-PCR or Liaison EHEC Toxins or Luminex xTAG multiplex assay* or Multiplex real-time PCR or NAAT or Nucleic acid amplification test* or Premier EHEC or Prodesse ProGastro SSCS Assay* or Prolisa EHEC EIA or RAPID-B* or real-time PCR assay* or Shiga Toxin Chek or Shiga Toxin Direct or ST Direct or Shiga Toxin Quik Chek or Stool Bacterial Pathogens Panel or Verigene Enteric Pathogens Nucleic Acid Test or xTAG Gastrointestinal Pathogen Panel or xTAG GPP).tw.

5. 1 or 2 or 3 or 4

6. exp diarrhea/di [Diagnosis]

7. escherichia coli o157/
8. feces/an [Drug Analysis]
9. feces analysis/
10. shiga toxin producing escherichia coli/
11. (Diarrhea or E coli O157 or E coli serotype O157 or Escherichia coli O157 or ehec or "enteric bacterial and viral pathogen*" or enteric pathogen* or infectious gastroenteritis or O157 or rfbEO157 or shiga* or shigella or shigellosis or STEC or stool or Stx1 or stx?2 or Stx2).tw.
12. 6 or 7 or 8 or 9 or 10 or 11
13. 5 and 12
14. limit 13 to yr="2005 -Current"
15. limit 14 to animals
16. limit 14 to (human and animals)
17. 15 not 16
18. 14 not 17
19. limit 18 to ("book review" or editorial or letter)
20. 18 not 19
21. limit 20 to "review"
22. 20 not 21
23. limit 20 to "systematic review"
24. ((systematic or critical or scoping) adj (review or overview or synthesis)).tw.
25. 20 and 24
26. 22 or 23 or 25
27. "sensitivity and specificity"/
28. exp reference value/
29. diagnostic error/ or false negative result/ or false positive result/
30. validity/ or predictive validity/
31. predictive value/
32. diagnostic test accuracy study/
33. (accuracy or detect or detecting or detection or diagnosis or diagnosing or false negative* or false positive* or predictive value* or sensitive or sensitivity or specificity or validat*).tw.
34. diagnostic accuracy/
35. 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34
36. 26 and 35

PubMED

1. Search (((Molecular Diagnostic Techniques [Methods]) OR Real-Time Polymerase Chain Reaction[MeSH Major Topic]) AND Immunoenzyme Techniques/mt [Methods]) OR (Allplex Gastrointestinal Panel Assay*[Title/Abstract] OR BD MAX Enteric Bacterial Panel*[Title/Abstract] OR BD MAXTM Enteric Bacterial Panel*[Title/Abstract] OR

BDM-EBP[Title/Abstract] OR BioFire FilmArray GI Panel*[Title/Abstract] OR Commercial molecular test*[Title/Abstract] OR EIA[Title/Abstract] OR Enzyme immunoassay*[Title/Abstract] OR FilmArray Gastrointestinal panel*[Title/Abstract] OR FilmArray GI panel*[Title/Abstract] OR Gastrointestinal pathogen panel*[Title/Abstract] OR GPP[Title/Abstract] OR ImmunoCard STAT*[Title/Abstract] OR LD-PCR[Title/Abstract] OR Liaison EHEC Toxins[Title/Abstract] OR Luminex xTAG multiplex assay*[Title/Abstract] OR Multiplex real-time PCR[Title/Abstract] OR NAAT[Title/Abstract] OR Nucleic acid amplification test*[Title/Abstract] OR Premier EHEC[Title/Abstract] OR Prodesse ProGastro SSCS Assay*[Title/Abstract] OR Prolisa EHEC EIA[Title/Abstract] OR RAPID-B*[Title/Abstract] OR real-time PCR assay*[Title/Abstract] OR Shiga Toxin Chek[Title/Abstract] OR Shiga Toxin Direct[Title/Abstract] OR ST Direct[Title/Abstract] OR Shiga Toxin Quik Chek[Title/Abstract] OR Stool Bacterial Pathogens Panel[Title/Abstract] OR Verigene Enteric Pathogens Nucleic Acid Test[Title/Abstract] OR xTAG Gastrointestinal Pathogen Panel[Title/Abstract] OR xTAG GPP[Title/Abstract])

2. Search ((((Diarrhea [Diagnosis]) OR Escherichia coli O157 [Isolation & Purification]) OR FECES [Microbiology]) OR Shiga-Toxigenic Escherichia coli [Isolation & Purification]) OR ((Diarrhea[Title/Abstract] OR E coli O157[Title/Abstract] OR E coli serotype O157[Title/Abstract] OR Escherichia coli O157[Title/Abstract] OR ehec[Title/Abstract] OR "enteric bacterial[Title/Abstract] AND viral pathogen*"[Title/Abstract] OR enteric pathogen*[Title/Abstract] OR infectious gastroenteritis[Title/Abstract] OR O157[Title/Abstract] OR rfbEO157[Title/Abstract] OR shiga*[Title/Abstract] OR shigella[Title/Abstract] OR shigellosis[Title/Abstract] OR STEC[Title/Abstract] OR stool[Title/Abstract] OR Stx1[Title/Abstract] OR stx?2[Title/Abstract] OR Stx2)[Title/Abstract])
3. 1 and 2
4. Search (((((("Sensitivity and Specificity"[MeSH Terms])) OR False Negative Reactions[MeSH Terms]) OR False Positive Reactions[MeSH Terms]) OR Reference Values[MeSH Terms]) OR (accuracy[Title/Abstract] OR detect[Title/Abstract] OR detecting[Title/Abstract] OR detection[Title/Abstract] OR diagnosis[Title/Abstract] OR diagnosing[Title/Abstract] OR false negative*[Title/Abstract] OR false positive*[Title/Abstract] OR predictive value*[Title/Abstract] OR sensitive[Title/Abstract] OR sensitivity[Title/Abstract] OR specificity[Title/Abstract] OR validat*[Title/Abstract])
5. 3 and 4
6. Filters: Publication date from 2005/01/01 to 2018/12/31

SCOPUS (Elsevier)

1. (*{Allplex Gastrointestinal Panel Assay*}* OR *{BD MAX Enteric Bacterial Panel*}* OR *{BD MAXTM Enteric Bacterial Panel*}* OR *{BDM-EBP}* OR *{BioFire*

FilmArray GI Panel}* OR *{Commercial molecular test*}* OR *eia* OR *{Enzyme immunoassay*}* OR *{FilmArray Gastrointestinal panel*}* OR *{FilmArray GI panel*}* OR *{Gastrointestinal pathogen panel*}* OR *gpp* OR *{ImmunoCard STAT*}* OR *{LD-PCR}* OR *{Liaison EHEC Toxins}* OR *{Luminex xTAG multiplex assay*}* OR *{Multiplex real-time PCR}* OR *naat* OR *{Nucleic acid amplification test*}* OR *{Premier EHEC}* OR *{Prodesse ProGastro SSCS Assay*}* OR *{Prolisa EHEC EIA}* OR *rapid-b** OR *{real-time PCR assay*}* OR *{Shiga Toxin Chek}* OR *{Shiga Toxin Direct}* OR *{ST Direct}* OR *{Shiga Toxin Quik Chek}* OR *{Stool Bacterial Pathogens Panel}* OR *{Verigene Enteric Pathogens Nucleic Acid Test}* OR *{xTAG Gastrointestinal Pathogen Panel}* OR *{xTAG GPP}*))) [TITLE/ABSTRACT/KEYWORD]

2. (*diarrhea* OR *{E coli O157}* OR *{E coli serotype O157}* OR *{Escherichia coli O157}* OR *ehec* OR *{enteric bacterial and viral pathogen*}* OR *{enteric pathogen*}* OR *{infectious gastroenteritis}* OR *o157* OR *rfbeo157* OR *shiga** OR *shigella* OR *shigellosis* OR *stec* OR *stool* OR *stx1* OR *stx?2* OR *stx2*)))) [TITLE/ABSTRACT/KEYWORD]
3. ((*accuracy* OR *detect* OR *detecting* OR *detection* OR *diagnosis* OR *diagnosing* OR *{false negative*}* OR *{false positive*}* OR *{predictive value}* OR *sensitive* OR *sensitivity* OR *specificity* OR *validat**))) [TITLE/ABSTRACT/KEYWORD]
4. 1 and 2 and 3
5. Limit to 2005-2018

Web of Science (SCI-Expanded)

1. (Allplex Gastrointestinal Panel Assay* or BD MAX Enteric Bacterial Panel* or BD MAXTM Enteric Bacterial Panel* or BDM-EBP or BioFire FilmArray GI Panel* or Commercial molecular test* or EIA or Enzyme immunoassay* or FilmArray Gastrointestinal panel* or FilmArray GI panel* or Gastrointestinal pathogen panel* or GPP or ImmunoCard STAT* or LD-PCR or Liaison EHEC Toxins or Luminex xTAG multiplex assay* or Multiplex real-time PCR or NAAT or Nucleic acid amplification test* or Premier EHEC or Prodesse ProGastro SSCS Assay* or Prolisa EHEC EIA or RAPID-B* or real-time PCR assay* or Shiga Toxin Chek or Shiga Toxin Direct or ST Direct or Shiga Toxin Quik Chek or Stool Bacterial Pathogens Panel or Verigene Enteric Pathogens Nucleic Acid Test or xTAG Gastrointestinal Pathogen Panel or xTAG GPP) [TOPIC/TITLE]
2. (Diarrhea or E coli O157 or E coli serotype O157 or Escherichia coli O157 or ehec or "enteric bacterial and viral pathogen*" or enteric pathogen* or infectious gastroenteritis or O157 or rfbEO157 or shiga* or shigella or shigellosis or STEC or stool or Stx1 or stx?2 or Stx2) [TOPIC/TITLE]
3. (accuracy or detect or detecting or detection or diagnosis or diagnosing or false negative* or false positive* or predictive value* or sensitive or sensitivity or specificity or validat*) [TITLE]
4. 1 and 2 and 3

5. Limit to 2005-2018

Reporting checklist for protocol of a systematic review.

Based on the PRISMA-P guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the PRISMA-P reporting guidelines, and cite them as:

Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA. Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4(1):1.

		Reporting Item	Page Number
Identification	#1a	Identify the report as a protocol of a systematic review	1
Update	#1b	If the protocol is for an update of a previous systematic review, identify as such	N/A
	#2	If registered, provide the name of the registry (such as PROSPERO) and registration number	2
Contact	#3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contribution	#3b	Describe contributions of protocol authors and identify the guarantor of the review	9
	#4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important	N/A

		protocol amendments	
Sources	#5a	Indicate sources of financial or other support for the review	9
Sponsor	#5b	Provide name for the review funder and / or sponsor	9
Role of sponsor or funder	#5c	Describe roles of funder(s), sponsor(s), and / or institution(s), if any, in developing the protocol	9
Rationale	#6	Describe the rationale for the review in the context of what is already known	3
Objectives	#7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	4
Eligibility criteria	#8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	5
Information sources	#9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	5
Search strategy	#10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	6
Study records - data management	#11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	6
Study records - selection process	#11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	6
Study records - data collection process	#11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	6
Data items	#12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	13

Outcomes and prioritization	#13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	13
Risk of bias in individual studies	#14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	6
Data synthesis	#15a	Describe criteria under which study data will be quantitatively synthesised	7
	#15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I2, Kendall's τ)	7
	#15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	8
	#15d	If quantitative synthesis is not appropriate, describe the type of summary planned	N/A
Meta-bias(es)	#16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	N/A
Confidence in cumulative evidence	#17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	8

BMJ Open

Performance of Commercial Tests for Molecular Detection of Shiga Toxin-producing Escherichia coli (STEC): A Systematic Review and Meta-analysis Protocol

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Performance of Commercial Tests for Molecular Detection of Shiga Toxin-producing Escherichia coli (STEC): A Systematic Review and Meta-analysis Protocol

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On behalf of the Alberta Provincial Pediatric EnTeric Infection TEam (APPETITE)

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Key words: Shiga Toxin-producing Escherichia coli, diagnostic, testing, sensitivity, specificity

Word count: 1772

Abstract

Introduction: Rapid detection of Shiga toxin-producing *Escherichia coli* (STEC) enables appropriate treatment. Numerous commercially available molecular tests exist, but they vary in clinical performance. This systematic review aims to synthesize available evidence to compare the clinical performance of enzyme immunoassay (EIA) and nucleic acid amplification tests (NAAT) for the detection of STEC.

Methods and Analysis: The following databases will be searched employing a standardized search strategy: Medline, Embase, Cochrane CENTRAL Register of Controlled Trials, Cochrane Database of Systematic Reviews, PubMed, Scopus, and Web of Science. Grey literature will be searched under advice from a medical librarian. Independent reviewers will screen titles, abstracts, and full-texts of retrieved studies for relevant studies. Data will be extracted independently by two reviewers, using a piloted template. QUADAS-2 will be employed to assess the risk of bias of individual studies, and the quality of evidence will be assessed with the GRADE approach. A bivariate random effects model will be used to meta-analyze the sensitivity and specificity of commercial STEC diagnostic tests, and a hierarchical summary receiver operator characteristic curve will be constructed. Studies of single test accuracy of EIA and NAAT tests and studies of comparative accuracy will be analyzed separately.

Ethics and Dissemination: Ethics approval was not required for this systematic review and meta-analysis. Findings will be disseminated in conferences, through a peer-reviewed journal, and via personal interactions with relevant stakeholders.

PROSPERO Registration Number

CRD42018099119

Article Summary

Strengths and Limitations of this Study

- There is little evidence reviewing the relative clinical performance of commercially available tests for Shiga Toxin-producing *Escherichia coli* (STEC)
- A key strength of this study is the comprehensive comparison of enzyme immunoassays (EIA) and nucleic acid amplification tests (NAAT) to inform clinical practice
- A limitation is the lack of a common gold standard for STEC identification, which may introduce heterogeneity into our analysis
- Another limitation is that the finding of a Shiga toxin (Stx) 1 producing STEC that does not also produce Stx2, especially in the absence of bloody diarrhea, is of unclear clinical and epidemiologic value.

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) cause significant disease. Although prototypical *E. coli* O157:H7 is the leading cause of hemolytic uremic syndrome (HUS), other STEC serotypes have been associated with severe disease and large outbreaks.[1-4] Multiple serotypes have now been linked to disease, and, unlike the O157 serotype, detection of non-O157 serotypes has increased significantly in the past decade, though likely because of expanding technology to detect these organisms.[5] Patients infected with STEC often seek care through emergency departments (EDs), especially if they have bloody diarrhea. Strong evidence suggests that antibiotics may increase the risk of developing HUS if administered to people infected with STEC,[6-8] and a recent meta-analysis demonstrated that the early administration of fluids is associated with improved outcomes.[9] Therefore, it is important that healthcare providers have a means of detecting STEC that is both rapid and applicable to any serotype.

Historically, STEC testing has focused on the O157 serogroup using culture on sorbitol-MacConkey agar, leveraging its inability to ferment sorbitol.[10] This attribute is not shared by other STEC serogroups, so they are overlooked if sorbitol-MacConkey agar culture is the only detection method employed. Further, culture can take days to yield results, delaying informed management.[11] In light of the limitations of culture, enzyme immunoassay (EIA) and nucleic acid amplification tests (NAAT) have been developed to detect STEC irrespective of serogroup. Reflecting their popularity, the U.S. Council of State and Territorial Epidemiologists (CSTE) has recently revised the probable STEC case definition to include laboratory evidence from EIA and NAAT.[12]

Numerous tests to detect STEC are commercially available.[13,14] The EIAs detect Shiga toxin, and most NAAT detect the Shiga toxin genes *stx1* and *stx2*, and some additionally seek a locus

that is specific to the O157 serogroup. For NAAT, STEC is often one of several enteropathogens detected by the assay. EIA has suboptimal sensitivity, particularly if a time-consuming enrichment step is not conducted.[15-18] Commercial NAAT appear to be more sensitive, but results vary by study and test.[19-21] NAAT are more costly than traditional microbiologic techniques owing to the equipment and consumables required to perform them. However, higher cost may be compensated by increased ascertainment,[21] improved patient outcomes, or decreased need to implement contact precautions.[22] As laboratories consider NAATs, it is crucial to identify the best testing strategy to support time-sensitive, cost-effective treatment decisions. Thus, we will conduct a systematic review of commercial EIA and NAAT for STEC detection to determine if and how their performance differs in terms of diagnostic test accuracy.

Methods and Analysis

This systematic review and meta-analysis will be conducted in accordance with reporting requirements for Preferred Reporting Items for Systematic Review and Meta-analysis Statement (PRISMA). This protocol was prepared according to PRISMA-P and PRISMA-DTA guidelines.[23,24]

Research Question

What is the accuracy of commercially available EIA and NAAT for the detection of STEC and how do they differ?

Eligibility Criteria

- Participants: study participants with acute diarrhea, who provide a stool specimen or rectal swab for diagnostic testing; any age or sub-population.
- Setting: health care systems or medical facilities, including outpatient clinics, emergency departments, hospitals, long-term care centers, and similar, without geographic limitation.
- Index tests: any commercially available EIA or NAAT for the detection of Shiga toxin, or *stx1* and *stx2*; NAAT for the identification of the O157 serogroup, if available. Included studies may assess the accuracy of commercially available EIA, NAAT, or both, including comparative accuracy studies.
- Reference standard: at least one of the following: enhanced protocols, real-time PCR, sequencing, and/or other NAAT.
- Target condition: acute diarrhea associated with STEC infection.

- Study designs: Cross-sectional diagnostic accuracy studies, encompassing all studies with both index and reference tests conducted on stool samples/swabs collected at a single point of time during the acute diarrhea illness, including both single test and comparative accuracy studies.
- Report characteristics: years 2005 to present (2015 to present for conference abstracts), published or unpublished, in any language.

Literature Searches

The following databases will be searched from 2005: MEDLINE, Cochrane CENTRAL Register of Controlled Trials, Cochrane Database of Systematic Reviews, EMBASE, PubMED, SCOPUS, and Web of Science. Clinical trial databases (ClinicalTrials.gov), FDA applications, package inserts for commercial assays, company product websites and literature, government/NGO reports and conference abstracts will also be searched under the advice of STEC subject experts and a medical librarian. The reference lists of included studies will be scanned to identify additional studies of relevance to this review. The specific search strategy can be found in Appendix I.

Study Records

Data Management

Records retrieved will be uploaded into EndNote (Philadelphia, V8), and de-duplicated using EndNote (Philadelphia, V8) and Rayyan for Systematic Reviews (Qatar, 2018).

Selection Process

Two reviewers (GT, CYL) will independently screen all titles and abstracts in duplicate, and a third reviewer (SF) will adjudicate any disagreements. Studies will be included if the title and

abstract indicate that the manuscript may contain data related to the evaluation of EIA and/or NAAT for the detection of STEC. The full text of all potentially relevant citations will then be obtained and reviewed by two independent reviewers (GT, CYL) using the predefined eligibility criteria outlined above, with the involvement of a third reviewer (SF) in case consensus cannot be reached. Reasons for inclusion and exclusion will be documented. A tool to document the selection process will be developed, piloted with the first 25 search results, and modified as necessary.

Data Extraction

Two reviewers will extract data independently and in duplicate using a structured form. The form will be piloted on the first 5 included studies and modified as necessary. Discordances will be resolved through discussions involving the reviewers and subject matter experts. First and last study authors will be contacted if data necessary to calculate sensitivity or specificity are absent from the manuscript. Study characteristics and study outcomes (Table 1) will be extracted from included studies.

Risk of Bias Assessments

To assess the risk of bias in individual studies, we will employ the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2).[25] We will follow the recommended process for tailoring the QUADAS-2 to our systematic review, including iteratively tailoring the QUADAS-2 assessment tool and piloting it on at least five studies until consensus has been reached on a version of the tool.[25] As part of this process, we will review the Standards for Reporting of Diagnostic Accuracy (STARD)[26] and prior QUADAS-2 modifications for comparative accuracy studies[27] for relevant criteria. For comparative accuracy studies, we will add a signaling question regarding the assessment of EIA and NAAT tests in the same group of patients. The risk of bias

in individual studies (for all outcomes reported) will be rated as Low/Unclear/High.[28] Assessments will be made independently by two reviewers, and disagreements will be resolved by discussion, or where necessary, by a third reviewer. Risk of bias will be reported for all included studies.

Data Synthesis

Separate synthesis will be conducted for EIA and NAAT. For each of test type, data will be quantitatively synthesized if at least four studies have been identified. If the number of included studies for either EIA or NAAT is insufficient, point estimates and confidence intervals from the individual papers will be shown, and the comparison of EIA and NAAT will be based on the range of estimates reported in individual papers.

If four or more studies are included for a given test type, a bivariate random effects model[29] will be used to calculate summary estimates and confidence intervals of primary outcomes and secondary outcomes, and a hierarchical summary receiver operating characteristic (ROC) curve[30] will be constructed.[31] The summary point for sensitivity and specificity with confidence ellipse and the hierarchical summary ROC curve will be graphed. These analyses take into account the correlation between sensitivity and specificity and potential threshold effects (e.g. due to cycle thresholds used in PCR).[31] Meta-analysis packages in R[32] and RevMan[33] will be used to conduct all analyses.

Comparative Accuracy

To compare EIA and NAAT, we will meta-analyze only comparative accuracy studies that evaluate both types of test against the same reference standard. If no comparative accuracy studies are identified, we will graphically compare point estimates and confidence intervals for

sensitivity and specificity resulting from the separate meta-analysis of each type of test. If there is adequate consistency in reference standards used to assess single test accuracy, we will pool EIA and NAAT studies in a single meta-analysis and include test type as a covariate to test the difference in accuracy between EIA and NAAT.

Subgroup Analysis

To identify study characteristics that may be contributing to heterogeneity, we will conduct subgroup analyses when at least four studies are available per subgroup:

- Funding (industry vs. other)
- Data source (published vs. unpublished)
- Age (<10 years-old and <18 years-old)
- Location of care
- Diarrhea duration (<7 days, ≥7 days, not specified)
- Presence of bloody diarrhea
- Specimen type
- Test brand
- Test targets
- Reference standard

Other subgroup analyses not pre-specified here will be identified as such in all reports. Subgroup analyses will illustrate the magnitude of differences in accuracy, and thus allow readers to interpret whether they are clinically meaningful. We will obtain statistical evidence of whether these factors contribute to heterogeneity in the primary analysis by adding each to the bivariate random effects model as a predictor.

A sensitivity analysis excluding studies with a high risk of bias will be conducted. Additional sensitivity analyses will be added if other potential biases become apparent during the review.

Quality of Evidence Assessment

For the quality of evidence for each test type, two reviewers, one with clinical and one with methodological expertise, will independently use the GRADE approach to assess the quality of evidence for sensitivity and specificity.[34,35] The test will be considered in the context of how it relates to patient-important outcomes to assign importance to the consequences of summary sensitivity and specificity findings (e.g. frequency of false negatives). The domains of study design, limitations/risk of bias, directness, consistency, precision, and publication bias will be assessed and combined into a summary grade for all important outcomes of the test. Publication bias will be assessed based on differences in accuracy reported in industry-funded vs. non-industry-funded studies.

For the comparison of EIA and NAAT, we will use a similar approach to grade the quality of evidence, with the same domains as for single test accuracy. Risk of bias will reflect the modifications we make to QUADAS-2 for comparative accuracy studies. Indirectness will be affected by the number of comparative accuracy studies including both EIA and NAAT; if few comparative accuracy studies are identified and the comparison is based on single test accuracy from different studies, quality will be downgraded due to indirectness.

Study results will be reported according to the PRISMA-DTA guidelines.[24]

Patient and Public Involvement

This protocol was designed without patient involvement. Patients were not invited to comment on the systematic review design and were not consulted to develop patient relevant outcomes. Patients were not invited to contribute to the writing or editing of this protocol for readability or accuracy.

Ethics and Dissemination

Ethics approval was not required for this systematic review and meta-analysis. Findings will be disseminated in conferences, through a peer-reviewed journal, and via personal interactions with relevant stakeholders.

Notes

Author Contributions

GT conceived the study, contributed to study design, drafted the protocol, and revised the protocol following author comments; CYL contributed to study design, drafted the protocol, and provided critical revisions; DL contributed to study design and provided critical revisions; LC contributed to study design and provided critical revisions; PT contributed to study design and provided critical revisions; LH contributed to study design and provided critical revisions; BV contributed to study design and provided critical revisions; SF conceived the study, contributed to study design, and provided critical revisions.

Funding

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Competing Interests

Dr. Stephen Freedman has previously received in-kind grant support from BioMerieux and Luminex Corporation. Dr. Chui received funding from TechLab for a previous study on SHIGA TOXIN QUICK CHEK and SHIGA TOXIN CHEK. Dr. Phillip Tarr has served as a consultant to BioRad.

Data Statement

All data can be accessed upon request to the corresponding author (GT).

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TABLES

Table 1. Data to be extracted from each included study.

Item	Rationale
Study Characteristics	
Data source	Peer-reviewed studies will be distinguished from non-peer-reviewed data for potential subgroup analysis
Funding source	Studies funded by diagnostic test companies may be subject to additional bias; potential subgroup analysis
Study design	Cross-sectional studies are expected; other study designs will be noted for potential subgroup analysis
Population	Population restrictions within the study (e.g. by age, HUS status, etc.) will be noted for potential subgroup analysis
Setting	Country or region; potential subgroup analysis
Clinical Data	
Location of care	Primary care vs. ED vs. hospital, and potentially other; potential subgroup analysis
Diarrhea definition	Study definition for diarrhea (e.g. ≥3 episodes in 24 hours) will facilitate comparability assessment and interpretation
Diarrhea duration	Mean/median or restrictions on illness duration at the time of sampling; facilitate comparability assessment and interpretation
Specimen type	Stool specimen or rectal swab; potential subgroup analysis
Bloody diarrhea	Frequency of bloody diarrhea; potential subgroup analysis
Test	
Brand name	Ease of reference

Type	EIA or NAAT for main comparison
Enrichment	For EIA tests; potential subgroup analysis
Targets	Toxin vs. DNA, STEC-only vs. multianalyte; interpretation and potential subgroup analysis
Cycle threshold	Cycle cutoff for positivity; facilitate comparability assessment and interpretation
Comparator/reference standard	Composite standard with component tests, discrepant analysis with confirmatory tests; interpretation and potential source of bias
Specimen comparability	Specimens tested by index and comparator from the same point in time, of the same type, etc.; potential source of bias
Outcomes	
Outcome type	For STEC generally, Shiga toxin 1 vs. 2, or O157 vs. non-O157; distinguish primary and secondary outcomes
Number tested	Outcome calculation and interpretation
Number confirmatory tested	Outcome calculation and interpretation
Number of true positives	Outcome calculation
Number of false positives	Outcome calculation
Number of true negatives	Outcome calculation
Number of false negatives	Outcome calculation
Sensitivity	Primary outcome
Specificity	Primary outcome
Single accuracy measures	e.g. AUC, diagnostic accuracy, diagnostic OR; secondary outcome

PPV	Secondary outcome
NPV	Secondary outcome
LR+	Secondary outcome
LR-	Secondary outcome

Appendix I

Shiga Toxin Detection Review
Search Strategies

MEDLINE

1. *Molecular Diagnostic Techniques/mt [Methods]
2. *Real-Time Polymerase Chain Reaction/
3. exp *Immunoenzyme Techniques/mt [Methods]
4. (Allplex Gastrointestinal Panel Assay* or BD MAX Enteric Bacterial Panel* or BD MAXTM Enteric Bacterial Panel* or BDM-EBP or BioFire FilmArray GI Panel* or Commercial molecular test* or EIA or Enzyme immunoassay* or FilmArray Gastrointestinal panel* or FilmArray GI panel* or Gastrointestinal pathogen panel* or GPP or ImmunoCard STAT* or LD-PCR or Liaison EHEC Toxins or Luminex xTAG multiplex assay* or Multiplex real-time PCR or NAAT or Nucleic acid amplification test* or Premier EHEC or Prodesse ProGastro SSCS Assay* or Prolisa EHEC EIA or RAPID-B* or real-time PCR assay* or Shiga Toxin Chek or Shiga Toxin Direct or ST Direct or Shiga Toxin Quik Chek or Stool Bacterial Pathogens Panel or Verigene Enteric Pathogens Nucleic Acid Test or xTAG Gastrointestinal Pathogen Panel or xTAG GPP).tw.
5. 1 or 2 or 3 or 4
6. Diarrhea/di [Diagnosis]
7. Escherichia coli O157/ip [Isolation & Purification]
8. FECES/mi [Microbiology]
9. exp Shiga-Toxigenic Escherichia coli/ip [Isolation & Purification]
10. (Diarrhea or E coli O157 or E coli serotype O157 or Escherichia coli O157 or ehec or "enteric bacterial and viral pathogen*" or enteric pathogen* or infectious gastroenteritis or O157 or rfbEO157 or shiga* or shigella or shigellosis or STEC or stool or Stx1 or stx?2 or Stx2).tw.
11. 6 or 7 or 8 or 9 or 10
12. 5 and 11
13. limit 12 to yr="2005 -Current"
14. limit 13 to animals
15. limit 13 to (animals and humans)
16. 14 not 15
17. 13 not 16
18. limit 17 to (editorial or letter)
19. 17 not 18
20. limit 19 to "review"
21. 19 not 20
22. ((systematic or critical or scoping) adj (review or overview or synthesis)).tw.

23. 19 and 22
24. limit 19 to systematic reviews
25. 21 or 23 or 24
26. exp "Sensitivity and Specificity"/
27. False Negative Reactions/ or False Positive Reactions/ or Reference Values/
28. (accuracy or detect or detecting or detection or diagnosis or diagnosing or false negative* or false positive* or predictive value* or sensitive or sensitivity or specificity or validat*).tw.
29. 26 or 27 or 28
30. 25 and 29

Cochrane CENTRAL Register

1. *Molecular Diagnostic Techniques/mt [Methods]
2. *Real-Time Polymerase Chain Reaction/
3. exp *Immunoenzyme Techniques/mt [Methods]
4. (Allplex Gastrointestinal Panel Assay* or BD MAX Enteric Bacterial Panel* or BD MAXTM Enteric Bacterial Panel* or BDM-EBP or BioFire FilmArray GI Panel* or Commercial molecular test* or EIA or Enzyme immunoassay* or FilmArray Gastrointestinal panel* or FilmArray GI panel* or Gastrointestinal pathogen panel* or GPP or ImmunoCard STAT* or LD-PCR or Liaison EHEC Toxins or Luminex xTAG multiplex assay* or Multiplex real-time PCR or NAAT or Nucleic acid amplification test* or Premier EHEC or Prodesse ProGastro SSCS Assay* or Prolisa EHEC EIA or RAPID-B* or real-time PCR assay* or Shiga Toxin Chek or Shiga Toxin Direct or ST Direct or Shiga Toxin Quik Chek or Stool Bacterial Pathogens Panel or Verigene Enteric Pathogens Nucleic Acid Test or xTAG Gastrointestinal Pathogen Panel or xTAG GPP).tw.
5. 1 or 2 or 3 or 4
6. Diarrhea/di [Diagnosis]
7. Escherichia coli O157/ip [Isolation & Purification]
8. FECES/mi [Microbiology]
9. exp Shiga-Toxigenic Escherichia coli/ip [Isolation & Purification]
10. (Diarrhea or E coli O157 or E coli serotype O157 or Escherichia coli O157 or ehec or "enteric bacterial and viral pathogen*" or enteric pathogen* or infectious gastroenteritis or O157 or rfbEO157 or shiga* or shigella or shigellosis or STEC or stool or Stx1 or stx?2 or Stx2).tw.
11. 6 or 7 or 8 or 9 or 10
12. 5 and 11
13. limit 12 to yr="2005 -Current"
14. limit 13 to animals
15. limit 13 to (animals and humans)
16. 14 not 15
17. 13 not 16

18. ((systematic or critical or scoping) adj (review or overview or synthesis)).tw.
19. exp "Sensitivity and Specificity"/
20. False Negative Reactions/ or False Positive Reactions/ or Reference Values/
21. (accuracy or detect or detecting or detection or diagnosis or diagnosing or false negative* or false positive* or predictive value* or sensitive or sensitivity or specificity or validat*).tw.
22. 19 or 20 or 21
23. 17 and 22

Cochrane Database of Systematic Reviews

1. (Allplex Gastrointestinal Panel Assay* or BD MAX Enteric Bacterial Panel* or BD MAXTM Enteric Bacterial Panel* or BDM-EBP or BioFire FilmArray GI Panel* or Commercial molecular test* or EIA or Enzyme immunoassay* or FilmArray Gastrointestinal panel* or FilmArray GI panel* or Gastrointestinal pathogen panel* or GPP or ImmunoCard STAT* or LD-PCR or Liaison EHEC Toxins or Luminex xTAG multiplex assay* or Multiplex real-time PCR or NAAT or Nucleic acid amplification test* or Premier EHEC or Prodesse ProGastro SSCS Assay* or Prolisa EHEC EIA or RAPID-B* or real-time PCR assay* or Shiga Toxin Chek or Shiga Toxin Direct or ST Direct or Shiga Toxin Quik Chek or Stool Bacterial Pathogens Panel or Verigene Enteric Pathogens Nucleic Acid Test or xTAG Gastrointestinal Pathogen Panel or xTAG GPP).tw.
2. (Diarrhea or E coli O157 or E coli serotype O157 or Escherichia coli O157 or ehec or "enteric bacterial and viral pathogen*" or enteric pathogen* or infectious gastroenteritis or O157 or rfbEO157 or shiga* or shigella or shigellosis or STEC or stool or Stx1 or stx?2 or Stx2).tw.
3. 1 and 2
4. limit 3 to yr="2005 -Current"
5. (accuracy or detect or detecting or detection or diagnosis or diagnosing or false negative* or false positive* or predictive value* or sensitive or sensitivity or specificity or validat*).tw.
6. 4 and 5

EMBASE

1. *molecular diagnosis/
2. *real time polymerase chain reaction/
3. *enzyme immunoassay/
4. (Allplex Gastrointestinal Panel Assay* or BD MAX Enteric Bacterial Panel* or BD MAXTM Enteric Bacterial Panel* or BDM-EBP or BioFire FilmArray GI Panel* or Commercial molecular test* or EIA or Enzyme immunoassay* or FilmArray Gastrointestinal panel* or FilmArray GI panel* or Gastrointestinal pathogen panel* or GPP or ImmunoCard STAT* or LD-PCR or Liaison EHEC Toxins or Luminex xTAG multiplex assay* or Multiplex real-time PCR or NAAT or Nucleic acid amplification test* or Premier EHEC or Prodesse ProGastro SSCS Assay* or Prolisa EHEC EIA or RAPID-B* or real-time PCR assay* or Shiga Toxin Chek or Shiga Toxin Direct or ST Direct or Shiga Toxin Quik Chek or Stool Bacterial

Pathogens Panel or Verigene Enteric Pathogens Nucleic Acid Test or xTAG Gastrointestinal Pathogen Panel or xTAG GPP).tw.

5. 1 or 2 or 3 or 4

6. exp diarrhea/di [Diagnosis]

7. escherichia coli o157/

8. feces/an [Drug Analysis]

9. feces analysis/

10. shiga toxin producing escherichia coli/

11. (Diarrhea or E coli O157 or E coli serotype O157 or Escherichia coli O157 or ehec or "enteric bacterial and viral pathogen*" or enteric pathogen* or infectious gastroenteritis or O157 or rfbEO157 or shiga* or shigella or shigellosis or STEC or stool or Stx1 or stx?2 or Stx2).tw.

12. 6 or 7 or 8 or 9 or 10 or 11

13. 5 and 12

14. limit 13 to yr="2005 -Current"

15. limit 14 to animals

16. limit 14 to (human and animals)

17. 15 not 16

18. 14 not 17

19. limit 18 to ("book review" or editorial or letter)

20. 18 not 19

21. limit 20 to "review"

22. 20 not 21

23. limit 20 to "systematic review"

24. ((systematic or critical or scoping) adj (review or overview or synthesis)).tw.

25. 20 and 24

26. 22 or 23 or 25

27. "sensitivity and specificity"/

28. exp reference value/

29. diagnostic error/ or false negative result/ or false positive result/

30. validity/ or predictive validity/

31. predictive value/

32. diagnostic test accuracy study/

33. (accuracy or detect or detecting or detection or diagnosis or diagnosing or false negative* or false positive* or predictive value* or sensitive or sensitivity or specificity or validat*).tw.

34. diagnostic accuracy/

35. 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34

36. 26 and 35

PubMED

1. Search (((Molecular Diagnostic Techniques [Methods]) OR Real-Time Polymerase Chain Reaction[MeSH Major Topic]) AND Immunoenzyme Techniques/mt [Methods]) OR (Allplex Gastrointestinal Panel Assay*[Title/Abstract] OR BD MAX Enteric Bacterial Panel*[Title/Abstract] OR BD MAXTM Enteric Bacterial Panel*[Title/Abstract] OR BDM-EBP[Title/Abstract] OR BioFire FilmArray GI Panel*[Title/Abstract] OR Commercial molecular test*[Title/Abstract] OR EIA[Title/Abstract] OR Enzyme immunoassay*[Title/Abstract] OR FilmArray Gastrointestinal panel*[Title/Abstract] OR FilmArray GI panel*[Title/Abstract] OR Gastrointestinal pathogen panel*[Title/Abstract] OR GPP[Title/Abstract] OR ImmunoCard STAT*[Title/Abstract] OR LD-PCR[Title/Abstract] OR Liaison EHEC Toxins[Title/Abstract] OR Luminex xTAG multiplex assay*[Title/Abstract] OR Multiplex real-time PCR[Title/Abstract] OR NAAT[Title/Abstract] OR Nucleic acid amplification test*[Title/Abstract] OR Premier EHEC[Title/Abstract] OR Prodesse ProGastro SSCS Assay*[Title/Abstract] OR Prolisa EHEC EIA[Title/Abstract] OR RAPID-B*[Title/Abstract] OR real-time PCR assay*[Title/Abstract] OR Shiga Toxin Chek[Title/Abstract] OR Shiga Toxin Direct[Title/Abstract] OR ST Direct[Title/Abstract] OR Shiga Toxin Quik Chek[Title/Abstract] OR Stool Bacterial Pathogens Panel[Title/Abstract] OR Verigene Enteric Pathogens Nucleic Acid Test[Title/Abstract] OR xTAG Gastrointestinal Pathogen Panel[Title/Abstract] OR xTAG GPP[Title/Abstract])
2. Search ((((Diarrhea [Diagnosis]) OR Escherichia coli O157 [Isolation & Purification]) OR FECES [Microbiology]) OR Shiga-Toxigenic Escherichia coli [Isolation & Purification]) OR ((Diarrhea[Title/Abstract] OR E coli O157[Title/Abstract] OR E coli serotype O157[Title/Abstract] OR Escherichia coli O157[Title/Abstract] OR ehec[Title/Abstract] OR "enteric bacterial[Title/Abstract] AND viral pathogen*"[Title/Abstract] OR enteric pathogen*[Title/Abstract] OR infectious gastroenteritis[Title/Abstract] OR O157[Title/Abstract] OR rfbEO157[Title/Abstract] OR shiga*[Title/Abstract] OR shigella[Title/Abstract] OR shigellosis[Title/Abstract] OR STEC[Title/Abstract] OR stool[Title/Abstract] OR Stx1[Title/Abstract] OR stx?2[Title/Abstract] OR Stx2)[Title/Abstract])
3. 1 and 2
4. Search (((((("Sensitivity and Specificity"[MeSH Terms])) OR False Negative Reactions[MeSH Terms]) OR False Positive Reactions[MeSH Terms]) OR Reference Values[MeSH Terms]) OR (accuracy[Title/Abstract] OR detect[Title/Abstract] OR detecting[Title/Abstract] OR detection[Title/Abstract] OR diagnosis[Title/Abstract] OR diagnosing[Title/Abstract] OR false negative*[Title/Abstract] OR false positive*[Title/Abstract] OR predictive value*[Title/Abstract] OR sensitive[Title/Abstract] OR sensitivity[Title/Abstract] OR specificity[Title/Abstract] OR validat*[Title/Abstract])
5. 3 and 4
6. Filters: Publication date from 2005/01/01 to 2018/12/31

PUBMED

1. ((((((((((Faecal Pathogens B) OR Faecal Pathogens M) OR Faecal Pathogens X) OR GenMark's Gastrointestinal Pathogen Panel) OR BioCode MDx3000) OR Amplidiag Bacterial GE) OR Real-Time Gastro Panel I) OR RIDA®GENE EHEC/EPEC) OR RidaQuick Verotoxin/O157 Combi) OR ImmunoCard STAT! O157 Plus) OR ProSpecT Shiga Toxin E. coli) OR Seeplex Diarrhea B2 assay) OR CLART EnteroBac

SCOPUS (Elsevier)

1. (*{Allplex Gastrointestinal Panel Assay*}* OR *{BD MAX Enteric Bacterial Panel*}* OR *{BD MAXTM Enteric Bacterial Panel*}* OR *{BDM-EBP}* OR *{BioFire FilmArray GI Panel*}* OR *{Commercial molecular test*}* OR *eia* OR *{Enzyme immunoassay*}* OR *{FilmArray Gastrointestinal panel*}* OR *{FilmArray GI panel*}* OR *{Gastrointestinal pathogen panel*}* OR *gpp* OR *{ImmunoCard STAT*}* OR *{LD-PCR}* OR *{Liaison EHEC Toxins}* OR *{Luminex xTAG multiplex assay*}* OR *{Multiplex real-time PCR}* OR *naat* OR *{Nucleic acid amplification test*}* OR *{Premier EHEC}* OR *{Prodesse ProGastro SSCS Assay*}* OR *{Prolisa EHEC EIA}* OR *rapid-b** OR *{real-time PCR assay*}* OR *{Shiga Toxin Chek}* OR *{Shiga Toxin Direct}* OR *{ST Direct}* OR *{Shiga Toxin Quik Chek}* OR *{Stool Bacterial Pathogens Panel}* OR *{Verigene Enteric Pathogens Nucleic Acid Test}* OR *{xTAG Gastrointestinal Pathogen Panel}* OR *{xTAG GPP}*))) [TITLE/ABSTRACT/KEYWORD]
2. (*diarrhea* OR *{E coli O157}* OR *{E coli serotype O157}* OR *{Escherichia coli O157}* OR *ehec* OR *{enteric bacterial and viral pathogen*}* OR *{enteric pathogen*}* OR *{infectious gastroenteritis}* OR *o157* OR *rfbeo157* OR *shiga** OR *shigella* OR *shigellosis* OR *stec* OR *stool* OR *stx1* OR *stx?2* OR *stx2*)))) [TITLE/ABSTRACT/KEYWORD]
3. ((*accuracy* OR *detect* OR *detecting* OR *detection* OR *diagnosis* OR *diagnosing* OR *{false negative*}* OR *{false positive*}* OR *{predictive value}* OR *sensitive* OR *sensitivity* OR *specificity* OR *validat**))) [TITLE/ABSTRACT/KEYWORD]
4. 1 and 2 and 3
5. Limit to 2005-2018

Web of Science (SCI-Expanded)

1. (Allplex Gastrointestinal Panel Assay* or BD MAX Enteric Bacterial Panel* or BD MAXTM Enteric Bacterial Panel* or BDM-EBP or BioFire FilmArray GI Panel* or Commercial molecular test* or EIA or Enzyme immunoassay* or FilmArray Gastrointestinal panel* or FilmArray GI panel* or Gastrointestinal pathogen panel* or GPP or ImmunoCard STAT* or LD-PCR or Liaison EHEC Toxins or Luminex xTAG multiplex assay* or Multiplex real-time PCR or NAAT or Nucleic acid amplification test* or Premier EHEC or Prodesse ProGastro SSCS Assay* or Prolisa EHEC EIA or

RAPID-B* or real-time PCR assay* or Shiga Toxin Chek or Shiga Toxin Direct or ST Direct or Shiga Toxin Quik Chek or Stool Bacterial Pathogens Panel or Verigene Enteric Pathogens Nucleic Acid Test or xTAG Gastrointestinal Pathogen Panel or xTAG GPP) [TOPIC/TITLE]

2. (Diarrhea or E coli O157 or E coli serotype O157 or Escherichia coli O157 or ehec or "enteric bacterial and viral pathogen*" or enteric pathogen* or infectious gastroenteritis or O157 or rfbEO157 or shiga* or shigella or shigellosis or STEC or stool or Stx1 or stx?2 or Stx2) [TOPIC/TITLE]
3. (accuracy or detect or detecting or detection or diagnosis or diagnosing or false negative* or false positive* or predictive value* or sensitive or sensitivity or specificity or validat*) [TITLE]
4. 1 and 2 and 3
5. Limit to 2005-2018

Reporting checklist for protocol of a systematic review.

Based on the PRISMA-P guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the PRISMA-P reporting guidelines, and cite them as:

Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA. Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4(1):1.

		Reporting Item	Page Number
Identification	#1a	Identify the report as a protocol of a systematic review	1
Update	#1b	If the protocol is for an update of a previous systematic review, identify as such	N/A
	#2	If registered, provide the name of the registry (such as PROSPERO) and registration number	2
Contact	#3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contribution	#3b	Describe contributions of protocol authors and identify the guarantor of the review	13
	#4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list	N/A

		changes; otherwise, state plan for documenting important protocol amendments	
Sources	#5a	Indicate sources of financial or other support for the review	13
Sponsor	#5b	Provide name for the review funder and / or sponsor	13
Role of sponsor or funder	#5c	Describe roles of funder(s), sponsor(s), and / or institution(s), if any, in developing the protocol	13
Rationale	#6	Describe the rationale for the review in the context of what is already known	4
Objectives	#7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	6
Eligibility criteria	#8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	6-7
Information sources	#9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	7
Search strategy	#10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	7/Appendix
Study records - data management	#11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	7
Study records - selection process	#11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	7-8
Study records - data collection process	#11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	8

Data items	#12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	16-17
Outcomes and prioritization	#13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	16-17
Risk of bias in individual studies	#14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	8-9
Data synthesis	#15a	Describe criteria under which study data will be quantitatively synthesised	9-10
	#15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I2, Kendall's τ)	9-10
	#15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	10-11
	#15d	If quantitative synthesis is not appropriate, describe the type of summary planned	N/A
Meta-bias(es)	#16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	11
Confidence in cumulative evidence	#17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	11