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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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-025950
Article Type:	Protocol
Date Submitted by the Author:	09-Aug-2018
Complete List of Authors:	Tarr, Gillian ; University of Calgary Cumming School of Medicine, Department of Pediatrics LIN, CHU YANG; University of Alberta Faculty of Medicine and Dentistry Lorenzetti, Diane; University of Calgary, Community Health Sciences Chui, Linda ; Provincial Laboratory for Public Health, Microbiology Section Tarr, Philip; Washington University in St. Louis School of Medicine, Department of Pediatrics Hartling, Lisa; University of Alberta, Pediatrics Vandermeer, Ben; University of Alberta, Department of Pediatrics Freedman, Stephen; University of Calgary Cumming School of Medicine, Department of Pediatrics
Keywords:	Shiga Toxin-producing Escherichia coli, diagnostic, testing, sensitivity, specificity

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Manuscripts

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

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 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

1
2
3 identify additional studies of relevance to this review. The specific search strategy can be found in
4
5 Appendix I.
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8 9 **Study Records**

10 *Data Management*

11 Records retrieved will be uploaded into EndNote (Philadelphia, V8), and de-duplicated using
12
13 EndNote (Philadelphia, V8) and Rayyan for Systematic Reviews (Qatar, 2018).
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17 *Selection Process*

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19 Two trained reviewers (GT, CYL) will independently screen all titles and abstracts in duplicate, and a
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21 third reviewer (SF) will adjudicate any disagreements. Studies will be included if the title and abstract
22
23 indicate that the manuscript may contain data related to the evaluation of EIA or NAAT for the
24
25 detection of STEC. The full text of all potentially relevant citations will then be obtained and reviewed
26
27 by two independent reviewers (GT, CYL) using the predefined eligibility criteria outlined above, with
28
29 the involvement of a third reviewer (SF) in case consensus cannot be reached. Reasons for
30
31 inclusion and exclusion will be documented. A tool to document the selection process will be
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33 developed, piloted with the first 25 search results, and modified as necessary.
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37 *Data Extraction*

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39 Two trained reviewers will extract data independently and in duplicate using a structured form. The
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41 form will be piloted on the first 5 included studies and modified as necessary. Discordances will be
42
43 resolved through discussions involving the investigators and subject matter experts. First and last
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45 study authors will be contacted if data necessary to calculate sensitivity or specificity are absent from
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47 the manuscript. Study characteristics and study outcomes (Table 1) will be extracted from included
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52 **Risk of Bias Assessments**

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

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24 Separate synthesis will be conducted for EIA and NAAT. For each of test type, data will be
25 quantitatively synthesized if at least four studies have been identified. If the number of included
26 studies for either EIA or NAAT is insufficient, point estimates and confidence intervals from the
27 individual papers will be shown, and the comparison of EIA and NAAT will be based on the range of
28 estimates reported in individual papers.
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35 If four or more studies are included for a given test type, a bivariate random effects model²⁷ will be
36 used to calculate summary estimates and confidence intervals of primary outcomes and secondary
37 outcomes, and a hierarchical summary receiver operating characteristic (ROC) curve²⁸ will be
38 constructed.²⁹ The summary point for sensitivity and specificity with confidence ellipse and the
39 hierarchical summary ROC curve will be graphed. These analyses take into account the correlation
40 between sensitivity and specificity and potential threshold effects (e.g. due to cycle thresholds used
41 in PCR).²⁹ To identify study characteristics that may be contributing to heterogeneity, we will conduct
42 subgroup analyses when at least four studies are available per subgroup.
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52 To compare EIA and NAAT, we will graphically compare point estimates and confidence intervals for
53 sensitivity and specificity resulting from the separate meta-analysis of each type of test. Additionally,
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3 if there is adequate consistency in gold standards used to assess validity, we will pool all EIA and
4 NAAT studies in a single meta-analysis and include test type as a covariate to test the difference in
5 accuracy between EIA and NAAT. Meta-analysis packages in R³⁰ and RevMan³¹ will be used to
6
7 conduct all analyses.
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12 We will also conduct the follow subgroup analyses:
13

- 14 • Funding (industry vs. other)
 - 15 • Data source (published vs. unpublished)
 - 16 • Age (<10 years-old and <18 years-old)
 - 17 • Location of care
 - 18 • Diarrhea duration (<7 days, ≥7 days, not specified)
 - 19 • Presence of bloody diarrhea
 - 20 • Specimen type
 - 21 • Test brand
 - 22 • Test targets
 - 23 • Comparator type (i.e. reference standard)
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36 Other subgroup analyses not pre-specified here will be identified as such in all reports.
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39 A sensitivity analysis excluding studies with a high risk of bias will be conducted. Additional
40 sensitivity analyses will be added if other potential biases become apparent during the review.
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44 **Quality of Evidence Assessment**

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46 For the body of evidence as a whole, two reviewers, one with clinical and one with methodological
47 expertise, will independently use the GRADE approach to assess the quality of evidence for the
48 primary outcomes.^{32,33} The test will be considered in the context of common diagnostic algorithms,
49 subsequent clinical management, and patient outcomes to assign importance to the consequences
50 of summary sensitivity and specificity findings (e.g. frequency of false negatives). The domains of
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3 study design, limitations/risk of bias, directness, consistency, and precision will be assessed and
4
5 combined into a summary grade for all important outcomes of the test.
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8 9 Ethics and Dissemination

11
12 Ethics approval was not required for this systematic review and meta-analysis. Findings will be
13
14 disseminated in conferences, through a peer-reviewed journal, and via personal interactions with
15
16 relevant stakeholders.
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19 20 Author Contributions

22
23
24 GT conceived the study, contributed to study design, drafted the protocol, and revised the protocol
25
26 following author comments; CYL contributed to study design, drafted the protocol, and provided
27
28 critical revisions; DL contributed to study design and provided critical revisions; LC contributed to
29
30 study design and provided critical revisions; PT contributed to study design and provided critical
31
32 revisions; LH contributed to study design and provided critical revisions; BV contributed to study
33
34 design and provided critical revisions; SF conceived the study, contributed to study design, and
35
36 provided critical revisions.
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38
39

40 41 Funding

43
44 This review is supported by a 2018 Systematic Review Grant from the Alberta Emergency Strategic
45
46 Clinical Network grant number RES0039208. The Alberta SPOR Support Unit Knowledge
47
48 Translation Platform is providing in-kind methodologic and biostatistical support for the design,
49
50 conduct, and analysis of the review.
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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).

References

1. Preussel K, Hohle M, Stark K, Werber D. Shiga toxin-producing *Escherichia coli* O157 is more likely to lead to hospitalization and death than non-O157 serogroups--except O104. *PLoS One*. 2013;8(11):e78180.
2. Gould LH, Mody RK, Ong KL, et al. Increased recognition of non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States during 2000-2010: epidemiologic features and comparison with *E. coli* O157 infections. *Foodborne pathogens and disease*. 2013;10(5):453-460.
3. Kuehne A, Bouwknegt M, Havelaar A, et al. Estimating true incidence of O157 and non-O157 Shiga toxin-producing *Escherichia coli* illness in Germany based on notification data of haemolytic uraemic syndrome. *Epidemiology and infection*. 2016;144(15):3305-3315.
4. Luna-Gierke RE, Griffin PM, Gould LH, et al. Outbreaks of non-O157 Shiga toxin-producing *Escherichia coli* infection: USA. *Epidemiology and infection*. 2014;142(11):2270-2280.
5. Tseng M, Sha Q, Rudrik JT, et al. Increasing incidence of non-O157 Shiga toxin-producing *Escherichia coli* (STEC) in Michigan and association with clinical illness. *Epidemiology and infection*. 2016;144(7):1394-1405.
6. Wong CS, Mooney JC, Brandt JR, et al. Risk factors for the hemolytic uremic syndrome in children infected with *Escherichia coli* O157:H7: a multivariable analysis. *Clin Infect Dis*. 2012;55(1):33-41.
7. Smith KE, Wilker PR, Reiter PL, Hedican EB, Bender JB, Hedberg CW. Antibiotic treatment of *Escherichia coli* O157 infection and the risk of hemolytic uremic syndrome, Minnesota. *Pediatr Infect Dis J*. 2012;31(1):37-41.
8. Freedman SB, Xie J, Neufeld MS, et al. Shiga Toxin-Producing *Escherichia coli* Infection, Antibiotics, and Risk of Developing Hemolytic Uremic Syndrome: A Meta-analysis. *Clin Infect Dis*. 2016;62(10):1251-1258.
9. Grisar S, Xie J, Samuel S, et al. Associations Between Hydration Status, Intravenous Fluid Administration, and Outcomes of Patients Infected With Shiga Toxin-Producing *Escherichia coli*: A Systematic Review and Meta-analysis. *JAMA Pediatr*. 2017;171(1):68-76.
10. Tarr PI, Gordon CA, Chandler WL. Shiga-toxin-producing *Escherichia coli* and haemolytic uraemic syndrome. *Lancet*. 2005;365(9464):1073-1086.
11. Freedman SB, Vandermeer B, Milne A, Hartling L, Pediatric Emergency Research Canada Gastroenteritis Study G. Diagnosing clinically significant dehydration in children with acute gastroenteritis using noninvasive methods: a meta-analysis. *The Journal of pediatrics*. 2015;166(4):908-916 e901-906.
12. Council of State and Territorial Epidemiologists. Public Health Reporting and National Notification for Shiga Toxin-Producing *Escherichia coli* (STEC). In. Vol 17-ID-10. Atlanta, Georgia2017.

13. Health Canada. Medical devices active licenses search. <https://health-products.canada.ca/mdall-limh/prepareSearch-preparerRecherche.do?type=active>. Accessed December 20, 2017.
14. U.S. Food & Drug Administration. Nucleic Acid Based Tests: List of Microbial Tests. 2018; <https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm330711.htm#microbial>. Accessed April 9, 2018.
15. Grys TE, Sloan LM, Rosenblatt JE, Patel R. Rapid and sensitive detection of Shiga toxin-producing *Escherichia coli* from nonenriched stool specimens by real-time PCR in comparison to enzyme immunoassay and culture. *Journal of clinical microbiology*. 2009;47(7):2008-2012.
16. Qin X, Klein EJ, Galanakis E, et al. Real-Time PCR Assay for Detection and Differentiation of Shiga Toxin-Producing *Escherichia coli* from Clinical Samples. *Journal of clinical microbiology*. 2015;53(7):2148-2153.
17. Chui L, Patterson-Fortin L, Kuo J, Li V, Boras V. Evaluation of enzyme immunoassays and real-time PCR for detecting Shiga toxin-producing *Escherichia coli* in Southern Alberta, Canada. *Journal of clinical microbiology*. 2015;53(3):1019-1023.
18. Gerritzen A, Wittke JW, Wolff D. Rapid and Sensitive Detection of Shiga Toxin-Producing *Escherichia coli* Directly from Stool Samples by Real-Time PCR in Comparison to Culture, Enzyme Immunoassay and Vero Cell Cytotoxicity Assay. *Clinical Laboratory*. 2011;57(11-12):993-998.
19. Buss SN, Leber A, Chapin K, et al. Multicenter Evaluation of the BioFire FilmArray Gastrointestinal Panel for Etiologic Diagnosis of Infectious Gastroenteritis. *Journal of clinical microbiology*. 2015;53(3):915-925.
20. Duong VT, Phat VV, Tuyen HT, et al. Evaluation of Luminex xTAG Gastrointestinal Pathogen Panel Assay for Detection of Multiple Diarrheal Pathogens in Fecal Samples in Vietnam. *Journal of clinical microbiology*. 2016;54(4):1094-1100.
21. Faron ML, Ledebor NA, Connolly J, et al. Clinical Evaluation and Cost Analysis of Great Basin Shiga Toxin Direct Molecular Assay for Detection of Shiga Toxin-Producing *Escherichia coli* in Diarrheal Stool Specimens. *Journal of clinical microbiology*. 2017;55(2):519-525.
22. Goldenberg SD, Bacelar M, Brazier P, Bisnauthsing K, Edgeworth JD. A cost benefit analysis of the Luminex xTAG Gastrointestinal Pathogen Panel for detection of infectious gastroenteritis in hospitalised patients. *The Journal of infection*. 2015;70(5):504-511.
23. Moher D, Shamseer L, Clarke M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev*. 2015;4:1.
24. Whiting PF, Rutjes AWS, Westwood ME, et al. QUADAS-2: A Revised Tool for the Quality Assessment of Diagnostic Accuracy Studies. *Annals of Internal Medicine*. 2011;155:529-536.
25. Bossuyt PM, Reitsma JB, Bruns DE, et al. Towards complete and accurate reporting of studies of diagnostic accuracy: The STARD Initiative. *Ann Intern Med*. 2003;138(1):40-44.
26. Santaguida PL, Riley CR, Matchar DB. Assessing risk of bias as a domain of quality in medical test studies. AHRQ Publication No. 12-EHC077-EF. In: *Methods Guide for Medical Test Reviews (AHRQ Publication No. 12-EHC017)*. Rockville, MD: Agency for Healthcare Research and Quality; 2012.
27. Reitsma JB, Glas AS, Rutjes AW, Scholten RJ, Bossuyt PM, Zwinderman AH. Bivariate analysis of sensitivity and specificity produces informative summary measures in diagnostic reviews. *J Clin Epidemiol*. 2005;58(10):982-990.
28. Rutter CM, Gatsonis CA. A hierarchical regression approach to meta-analysis of diagnostic test accuracy evaluations. *Stat Med*. 2001;20(19):2865-2884.
29. Leeflang MM. Systematic reviews and meta-analyses of diagnostic test accuracy. *Clin Microbiol Infect*. 2014;20(2):105-113.
30. *R: A Language and Environment for Statistical Computing* [computer program]. Vienna, Austria: R Foundation for Statistical Computing; 2017.
31. *Review Manager (RevMan)* [computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration; 2014.
32. Schunemann HJ, Oxman AD, Brozek J, et al. GRADE: assessing the quality of evidence for diagnostic recommendations. *Evid Based Med*. 2008;13(6):162-163.
33. Schunemann HJ, Oxman AD, Brozek J, et al. Grading quality of evidence and strength of recommendations for diagnostic tests and strategies. *BMJ*. 2008;336(7653):1106-1110.

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TABLES

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

Item	Rationale
<i>Study Characteristics</i>	
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
<i>Clinical Data</i>	
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
<i>Test</i>	
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
<i>Outcomes</i>	
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

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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

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3 BDM-EBP[Title/Abstract] OR BioFire FilmArray GI Panel*[Title/Abstract] OR
4 Commercial molecular test*[Title/Abstract] OR EIA[Title/Abstract] OR Enzyme
5 immunoassay*[Title/Abstract] OR FilmArray Gastrointestinal panel*[Title/Abstract] OR
6 FilmArray GI panel*[Title/Abstract] OR Gastrointestinal pathogen panel*[Title/Abstract]
7 OR GPP[Title/Abstract] OR ImmunoCard STAT*[Title/Abstract] OR LD-
8 PCR[Title/Abstract] OR Liaison EHEC Toxins[Title/Abstract] OR Luminex xTAG
9 multiplex assay*[Title/Abstract] OR Multiplex real-time PCR[Title/Abstract] OR
10 NAAT[Title/Abstract] OR Nucleic acid amplification test*[Title/Abstract] OR Premier
11 EHEC[Title/Abstract] OR Prodesse ProGastro SSCS Assay*[Title/Abstract] OR Prolisa
12 EHEC EIA[Title/Abstract] OR RAPID-B*[Title/Abstract] OR real-time PCR
13 assay*[Title/Abstract] OR Shiga Toxin Chek[Title/Abstract] OR Shiga Toxin
14 Direct[Title/Abstract] OR ST Direct[Title/Abstract] OR Shiga Toxin Quik
15 Chek[Title/Abstract] OR Stool Bacterial Pathogens Panel[Title/Abstract] OR Verigene
16 Enteric Pathogens Nucleic Acid Test[Title/Abstract] OR xTAG Gastrointestinal Pathogen
17 Panel[Title/Abstract] OR xTAG GPP[Title/Abstract])
18
19 2. Search (((Diarrhea [Diagnosis]) OR Escherichia coli O157 [Isolation & Purification])
20 OR FECES [Microbiology]) OR Shiga-Toxigenic Escherichia coli [Isolation &
21 Purification]) OR ((Diarrhea[Title/Abstract] OR E coli O157[Title/Abstract] OR E coli
22 serotype O157[Title/Abstract] OR Escherichia coli O157[Title/Abstract] OR
23 ehec[Title/Abstract] OR "enteric bacterial[Title/Abstract] AND viral
24 pathogen*" [Title/Abstract] OR enteric pathogen* [Title/Abstract] OR infectious
25 gastroenteritis[Title/Abstract] OR O157[Title/Abstract] OR rfbEO157[Title/Abstract] OR
26 shiga* [Title/Abstract] OR shigella[Title/Abstract] OR shigellosis[Title/Abstract] OR
27 STEC[Title/Abstract] OR stool[Title/Abstract] OR Stx1[Title/Abstract] OR
28 stx?2[Title/Abstract] OR Stx2)[Title/Abstract])
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30 3. 1 and 2
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32 4. Search (((("Sensitivity and Specificity"[MeSH Terms])) OR False Negative
33 Reactions[MeSH Terms]) OR False Positive Reactions[MeSH Terms]) OR Reference
34 Values[MeSH Terms]) OR (accuracy[Title/Abstract] OR detect[Title/Abstract] OR
35 detecting[Title/Abstract] OR detection[Title/Abstract] OR diagnosis[Title/Abstract] OR
36 diagnosing[Title/Abstract] OR false negative*[Title/Abstract] OR false
37 positive*[Title/Abstract] OR predictive value*[Title/Abstract] OR
38 sensitive[Title/Abstract] OR sensitivity[Title/Abstract] OR specificity[Title/Abstract] OR
39 validat*[Title/Abstract])
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41 5. 3 and 4
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43 6. Filters: Publication date from 2005/01/01 to 2018/12/31
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52 SCOPUS (Elsevier)

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

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- 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)

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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

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5. Limit to 2005-2018

For peer review only

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	
1			
2	Sources	#5a Indicate sources of financial or other support for the review	9
3			
4	Sponsor	#5b Provide name for the review funder and / or sponsor	9
5			
6			
7	Role of sponsor or	#5c Describe roles of funder(s), sponsor(s), and / or institution(s),	9
8	funder	if any, in developing the protocol	
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11	Rationale	#6 Describe the rationale for the review in the context of what is	3
12		already known	
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15	Objectives	#7 Provide an explicit statement of the question(s) the review will	4
16		address with reference to participants, interventions,	
17		comparators, and outcomes (PICO)	
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20	Eligibility criteria	#8 Specify the study characteristics (such as PICO, study design,	5
21		setting, time frame) and report characteristics (such as years	
22		considered, language, publication status) to be used as	
23		criteria for eligibility for the review	
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27	Information	#9 Describe all intended information sources (such as electronic	5
28	sources	databases, contact with study authors, trial registers or other	
29		grey literature sources) with planned dates of coverage	
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32	Search strategy	#10 Present draft of search strategy to be used for at least one	6
33		electronic database, including planned limits, such that it	
34		could be repeated	
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37	Study records -	#11a Describe the mechanism(s) that will be used to manage	6
38	data management	records and data throughout the review	
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41	Study records -	#11b State the process that will be used for selecting studies (such	6
42	selection process	as two independent reviewers) through each phase of the	
43		review (that is, screening, eligibility and inclusion in meta-	
44		analysis)	
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48	Study records -	#11c Describe planned method of extracting data from reports	6
49	data collection	(such as piloting forms, done independently, in duplicate), any	
50	process	processes for obtaining and confirming data from investigators	
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53	Data items	#12 List and define all variables for which data will be sought	13
54		(such as PICO items, funding sources), any pre-planned data	
55		assumptions and simplifications	
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1	Outcomes and	#13	List and define all outcomes for which data will be sought,	13
2	prioritization		including prioritization of main and additional outcomes, with	
3			rationale	
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6	Risk of bias in	#14	Describe anticipated methods for assessing risk of bias of	6
7	individual studies		individual studies, including whether this will be done at the	
8			outcome or study level, or both; state how this information will	
9			be used in data synthesis	
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13	Data synthesis	#15a	Describe criteria under which study data will be quantitatively	7
14			synthesised	
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17		#15b	If data are appropriate for quantitative synthesis, describe	7
18			planned summary measures, methods of handling data and	
19			methods of combining data from studies, including any	
20			planned exploration of consistency (such as I ² , Kendall's τ)	
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24		#15c	Describe any proposed additional analyses (such as	8
25			sensitivity or subgroup analyses, meta-regression)	
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28		#15d	If quantitative synthesis is not appropriate, describe the type	N/A
29			of summary planned	
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31	Meta-bias(es)	#16	Specify any planned assessment of meta-bias(es) (such as	N/A
32			publication bias across studies, selective reporting within	
33			studies)	
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37	Confidence in	#17	Describe how the strength of the body of evidence will be	8
38	cumulative		assessed (such as GRADE)	
39	evidence			
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42 The PRISMA-P checklist is distributed under the terms of the Creative Commons Attribution License
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 44 by the [EQUATOR Network](#) in collaboration with [Penelope.ai](#)
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BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-025950.R1
Article Type:	Protocol
Date Submitted by the Author:	22-Dec-2018
Complete List of Authors:	Tarr, Gillian ; University of Calgary Cumming School of Medicine, Department of Pediatrics LIN, CHU YANG; University of Alberta Faculty of Medicine and Dentistry Lorenzetti, Diane; University of Calgary, Community Health Sciences Chui, Linda ; Provincial Laboratory for Public Health, Microbiology Section Tarr, Philip; Washington University in St. Louis School of Medicine, Department of Pediatrics Hartling, Lisa; University of Alberta, Pediatrics Vandermeer, Ben; University of Alberta, Department of Pediatrics Freedman, Stephen; University of Calgary Cumming School of Medicine, Department of Pediatrics
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Epidemiology, Gastroenterology and hepatology
Keywords:	diagnostic, testing, sensitivity, specificity, Shiga toxin-producing Escherichia coli

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Manuscripts

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3 1 Performance of Commercial Tests for Molecular Detection of Shiga Toxin-producing
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5 2 Escherichia coli (STEC): A Systematic Review and Meta-analysis Protocol
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39 18 On behalf of the Alberta Provincial Pediatric Enteric Infection TEam (APPETITE)
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43 20 Corresponding author: Gillian A.M. Tarr, Alberta Children's Hospital, 28 Oki Drive NW, Office

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50 23 Key words: Shiga Toxin-producing Escherichia coli, diagnostic, testing, sensitivity, specificity
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54 25 Word count: 1772
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26 Abstract

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

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

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

47 PROSPERO Registration Number

48 CRD42018099119

49 Article Summary

50 Strengths and Limitations of this Study

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

60

61 Introduction

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

73
74 Historically, STEC testing has focused on the O157 serogroup using culture on sorbitol-
75 MacConkey agar, leveraging its inability to ferment sorbitol.¹⁰ This attribute is not shared by
76 other STEC serogroups, so they are overlooked if sorbitol-MacConkey agar culture is the only
77 detection method employed. Further, culture can take days to yield results, delaying informed
78 management.¹¹ In light of the limitations of culture, enzyme immunoassay (EIA) and nucleic acid
79 amplification tests (NAAT) have been developed to detect STEC irrespective of serogroup.
80 Reflecting their popularity, the U.S. Council of State and Territorial Epidemiologists (CSTE) has
81 recently revised the probable STEC case definition to include laboratory evidence from EIA and
82 NAAT.¹²

83
84 Numerous tests to detect STEC are commercially available.^{13,14} The EIAs detect Shiga toxin,
85 and most NAAT detect the Shiga toxin genes *stx1* and *stx2*, and some additionally seek a locus

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3 86 that is specific to the O157 serogroup. For NAAT, STEC is often one of several
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5 87 enteropathogens detected by the assay. EIA has suboptimal sensitivity, particularly if a time-
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7 88 consuming enrichment step is not conducted.¹⁵⁻¹⁸ Commercial NAAT appear to be more
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9 89 sensitive, but results vary by study and test.¹⁹⁻²¹ NAAT are more costly than traditional
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11 90 microbiologic techniques owing to the equipment and consumables required to perform them.
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13 91 However, higher cost may be compensated by increased ascertainment,²¹ improved patient
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15 92 outcomes, or decreased need to implement contact precautions.²² As laboratories consider
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17 93 NAATs, it is crucial to identify the best testing strategy to support time-sensitive, cost-effective
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19 94 treatment decisions. Thus, we will conduct a systematic review of commercial EIA and NAAT for
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21 95 STEC detection to determine if and how their performance differs in terms of diagnostic test
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23 96 accuracy.
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98 Methods and Analysis

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

104 Research Question

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

108 Eligibility Criteria

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

- 1
2
3 121 • Study designs: Cross-sectional diagnostic accuracy studies, encompassing all studies
4
5 122 with both index and reference tests conducted on stool samples/swabs collected at a
6
7 123 single point of time during the acute diarrhea illness, including both single test and
8
9 124 comparative accuracy studies.
10
11 • Report characteristics: years 2005 to present (2015 to present for conference abstracts),
12
13 published or unpublished, in any language.
14
15
16
17

18 128 Literature Searches

19
20
21 129 The following databases will be searched from 2005: MEDLINE, Cochrane CENTRAL Register
22
23 130 of Controlled Trials, Cochrane Database of Systematic Reviews, EMBASE, PubMed, SCOPUS,
24
25 131 and Web of Science. Clinical trial databases (ClinicalTrials.gov), FDA applications, package
26
27 132 inserts for commercial assays, company product websites and literature, government/NGO
28
29 133 reports and conference abstracts will also be searched under the advice of STEC subject
30
31 134 experts and a medical librarian. The reference lists of included studies will be scanned to
32
33 135 identify additional studies of relevance to this review. The specific search strategy can be found
34
35 136 in Appendix I.
36
37
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39

40 138 Study Records

41 139 *Data Management*

42
43 140 Records retrieved will be uploaded into EndNote (Philadelphia, V8), and de-duplicated using
44
45 141 EndNote (Philadelphia, V8) and Rayyan for Systematic Reviews (Qatar, 2018).
46
47
48

49 142

50 143 *Selection Process*

51
52 144 Two reviewers (GT, CYL) will independently screen all titles and abstracts in duplicate, and a
53
54 145 third reviewer (SF) will adjudicate any disagreements. Studies will be included if the title and
55
56
57
58
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60

1
2
3 146 abstract indicate that the manuscript may contain data related to the evaluation of EIA and/or
4
5 147 NAAT for the detection of STEC. The full text of all potentially relevant citations will then be
6
7 148 obtained and reviewed by two independent reviewers (GT, CYL) using the predefined eligibility
8
9 149 criteria outlined above, with the involvement of a third reviewer (SF) in case consensus cannot
10
11 150 be reached. Reasons for inclusion and exclusion will be documented. A tool to document the
12
13 151 selection process will be developed, piloted with the first 25 search results, and modified as
14
15 152 necessary.
16
17
18 153

19 20 154 *Data Extraction*

21
22 155 Two reviewers will extract data independently and in duplicate using a structured form. The form
23
24 156 will be piloted on the first 5 included studies and modified as necessary. Discordances will be
25
26 157 resolved through discussions involving the reviewers and subject matter experts. First and last
27
28 158 study authors will be contacted if data necessary to calculate sensitivity or specificity are absent
29
30 159 from the manuscript. Study characteristics and study outcomes (Table 1) will be extracted from
31
32 160 included studies.
33
34
35 161

36 37 162 *Risk of Bias Assessments*

38
39
40 163 To assess the risk of bias in individual studies, we will employ the Quality Assessment of
41
42 164 Diagnostic Accuracy Studies-2 (QUADAS-2).²⁵ We will follow the recommended process for
43
44 165 tailoring the QUADAS-2 to our systematic review, including iteratively tailoring the QUADAS-2
45
46 166 assessment tool and piloting it on at least five studies until consensus has been reached on a
47
48 167 version of the tool.²⁵ As part of this process, we will review the Standards for Reporting of
49
50 168 Diagnostic Accuracy (STARD)²⁶ and prior QUADAS-2 modifications for comparative accuracy
51
52 169 studies²⁷ for relevant criteria. For comparative accuracy studies, we will add a signaling question
53
54 170 regarding the assessment of EIA and NAAT tests in the same group of patients. The risk of bias
55
56
57
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1
2
3 171 in individual studies (for all outcomes reported) will be rated as Low/Unclear/High.²⁸
4
5 172 Assessments will be made independently by two reviewers, and disagreements will be resolved
6
7 173 by discussion, or where necessary, by a third reviewer. Risk of bias will be reported for all
8
9 174 included studies.

10 175

14 176 Data Synthesis

16 177 Separate synthesis will be conducted for EIA and NAAT. For each of test type, data will be
17
18 178 quantitatively synthesized if at least four studies have been identified. If the number of included
19
20 179 studies for either EIA or NAAT is insufficient, point estimates and confidence intervals from the
21
22 180 individual papers will be shown, and the comparison of EIA and NAAT will be based on the
23
24 181 range of estimates reported in individual papers.

25
26 182
27
28 183 If four or more studies are included for a given test type, a bivariate random effects model²⁹ will
29
30 184 be used to calculate summary estimates and confidence intervals of primary outcomes and
31
32 185 secondary outcomes, and a hierarchical summary receiver operating characteristic (ROC)
33
34 186 curve³⁰ will be constructed.³¹ The summary point for sensitivity and specificity with confidence
35
36 187 ellipse and the hierarchical summary ROC curve will be graphed. These analyses take into
37
38 188 account the correlation between sensitivity and specificity and potential threshold effects (e.g.
39
40 189 due to cycle thresholds used in PCR).³¹ Meta-analysis packages in R³² and RevMan³³ will be
41
42 190 used to conduct all analyses.

43 191

48 192 Comparative Accuracy

50
51 193 To compare EIA and NAAT, we will meta-analyze only comparative accuracy studies that
52
53 194 evaluate both types of test against the same reference standard. If no comparative accuracy
54
55 195 studies are identified, we will graphically compare point estimates and confidence intervals for

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

200

201

202 Subgroup Analysis

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

- 205 • Funding (industry vs. other)
- 206 • Data source (published vs. unpublished)
- 207 • Age (<10 years-old and <18 years-old)
- 208 • Location of care
- 209 • Diarrhea duration (<7 days, ≥7 days, not specified)
- 210 • Presence of bloody diarrhea
- 211 • Specimen type
- 212 • Test brand
- 213 • Test targets
- 214 • Reference standard

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

216 Subgroup analyses will illustrate the magnitude of differences in accuracy, and thus allow
217 readers to interpret whether they are clinically meaningful. We will obtain statistical evidence of
218 whether these factors contribute to heterogeneity in the primary analysis by adding each to the
219 bivariate random effects model as a predictor.

220

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

223

224 Quality of Evidence Assessment

225 For the quality of evidence for each test type, two reviewers, one with clinical and one with
226 methodological expertise, will independently use the GRADE approach to assess the quality of
227 evidence for sensitivity and specificity.^{34,35} The test will be considered in the context of how it
228 relates to patient-important outcomes to assign importance to the consequences of summary
229 sensitivity and specificity findings (e.g. frequency of false negatives). The domains of study
230 design, limitations/risk of bias, directness, consistency, precision, and publication bias will be
231 assessed and combined into a summary grade for all important outcomes of the test.

232 Publication bias will be assessed based on differences in accuracy reported in industry-funded
233 vs. non-industry-funded studies.

234

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

241

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

243

244 Patient and Public Involvement

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

249

250 Ethics and Dissemination

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

254

255

256 Notes

257 Author Contributions

258 GT conceived the study, contributed to study design, drafted the protocol, and revised the
259 protocol following author comments; CYL contributed to study design, drafted the protocol, and
260 provided critical revisions; DL contributed to study design and provided critical revisions; LC
261 contributed to study design and provided critical revisions; PT contributed to study design and
262 provided critical revisions; LH contributed to study design and provided critical revisions; BV
263 contributed to study design and provided critical revisions; SF conceived the study, contributed
264 to study design, and provided critical revisions.

266 Funding

267 This review is supported by a 2018 Systematic Review Grant from the Alberta Emergency
268 Strategic Clinical Network grant number RES0039208. The Alberta SPOR Support Unit
269 Knowledge Translation Platform is providing in-kind methodologic and biostatistical support for
270 the design, conduct, and analysis of the review.

272 Competing Interests

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

278 Data Statement

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

280 References

- 281 1. Preussel K, Hohle M, Stark K, Werber D. Shiga toxin-producing *Escherichia coli* O157 is more
282 likely to lead to hospitalization and death than non-O157 serogroups--except O104. *PLoS One*.
283 2013;8(11):e78180.
- 284 2. Gould LH, Mody RK, Ong KL, et al. Increased recognition of non-O157 Shiga toxin-producing
285 *Escherichia coli* infections in the United States during 2000-2010: epidemiologic features and
286 comparison with *E. coli* O157 infections. *Foodborne Pathog Dis*. 2013;10(5):453-460.
- 287 3. Kuehne A, Bouwknegt M, Havelaar A, et al. Estimating true incidence of O157 and non-O157
288 Shiga toxin-producing *Escherichia coli* illness in Germany based on notification data of
289 haemolytic uraemic syndrome. *Epidemiol Infect*. 2016;144(15):3305-3315.
- 290 4. Luna-Gierke RE, Griffin PM, Gould LH, et al. Outbreaks of non-O157 Shiga toxin-producing
291 *Escherichia coli* infection: USA. *Epidemiol Infect*. 2014;142(11):2270-2280.
- 292 5. Tseng M, Sha Q, Rudrik JT, et al. Increasing incidence of non-O157 Shiga toxin-producing
293 *Escherichia coli* (STEC) in Michigan and association with clinical illness. *Epidemiol Infect*.
294 2016;144(7):1394-1405.
- 295 6. Wong CS, Mooney JC, Brandt JR, et al. Risk factors for the hemolytic uremic syndrome in
296 children infected with *Escherichia coli* O157:H7: a multivariable analysis. *Clin Infect Dis*.
297 2012;55(1):33-41.
- 298 7. Smith KE, Wilker PR, Reiter PL, Hedican EB, Bender JB, Hedberg CW. Antibiotic treatment of
299 *Escherichia coli* O157 infection and the risk of hemolytic uremic syndrome, Minnesota. *Pediatr*
300 *Infect Dis J*. 2012;31(1):37-41.
- 301 8. Freedman SB, Xie J, Neufeld MS, et al. Shiga Toxin-Producing *Escherichia coli* Infection,
302 Antibiotics, and Risk of Developing Hemolytic Uremic Syndrome: A Meta-analysis. *Clin Infect Dis*.
303 2016;62(10):1251-1258.
- 304 9. Grisar S, Xie J, Samuel S, et al. Associations Between Hydration Status, Intravenous Fluid
305 Administration, and Outcomes of Patients Infected With Shiga Toxin-Producing *Escherichia coli*:
306 A Systematic Review and Meta-analysis. *JAMA Pediatr*. 2017;171(1):68-76.
- 307 10. Tarr PI, Gordon CA, Chandler WL. Shiga-toxin-producing *Escherichia coli* and haemolytic
308 uraemic syndrome. *Lancet*. 2005;365(9464):1073-1086.
- 309 11. Freedman SB, Vandermeer B, Milne A, Hartling L, Pediatric Emergency Research Canada
310 Gastroenteritis Study G. Diagnosing clinically significant dehydration in children with acute
311 gastroenteritis using noninvasive methods: a meta-analysis. *J Pediatr*. 2015;166(4):908-916
312 e901-906.
- 313 12. Council of State and Territorial Epidemiologists. Public Health Reporting and National Notification
314 for Shiga Toxin-Producing *Escherichia coli* (STEC). In. Vol 17-ID-10. Atlanta, Georgia 2017.
- 315 13. Health Canada. Medical devices active licenses search. [https://health-products.canada.ca/mdall-](https://health-products.canada.ca/mdall-limh/prepareSearch-preparerRecherche.do?type=active)
316 [limh/prepareSearch-preparerRecherche.do?type=active](https://health-products.canada.ca/mdall-limh/prepareSearch-preparerRecherche.do?type=active). Accessed December 20, 2017.
- 317 14. U.S. Food & Drug Administration. Nucleic Acid Based Tests: List of Microbial Tests. 2018;
318 [https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm3307](https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm330711.htm#microbial)
319 [11.htm#microbial](https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm330711.htm#microbial). Accessed April 9, 2018.
- 320 15. Grys TE, Sloan LM, Rosenblatt JE, Patel R. Rapid and sensitive detection of Shiga toxin-
321 producing *Escherichia coli* from nonenriched stool specimens by real-time PCR in comparison to
322 enzyme immunoassay and culture. *J Clin Microbiol*. 2009;47(7):2008-2012.
- 323 16. Qin X, Klein EJ, Galanakis E, et al. Real-Time PCR Assay for Detection and Differentiation of
324 Shiga Toxin-Producing *Escherichia coli* from Clinical Samples. *J Clin Microbiol*. 2015;53(7):2148-
325 2153.
- 326 17. Chui L, Patterson-Fortin L, Kuo J, Li V, Boras V. Evaluation of enzyme immunoassays and real-
327 time PCR for detecting Shiga toxin-producing *Escherichia coli* in Southern Alberta, Canada. *J Clin*
328 *Microbiol*. 2015;53(3):1019-1023.
- 329 18. Gerritzen A, Wittke JW, Wolff D. Rapid and Sensitive Detection of Shiga Toxin-Producing
330 *Escherichia coli* Directly from Stool Samples by Real-Time PCR in Comparison to Culture,
331 Enzyme Immunoassay and Vero Cell Cytotoxicity Assay. *Clinical Laboratory*. 2011;57(11-
332 12):993-998.

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2
3 333 19. Buss SN, Leber A, Chapin K, et al. Multicenter Evaluation of the BioFire FilmArray
4 334 Gastrointestinal Panel for Etiologic Diagnosis of Infectious Gastroenteritis. *J Clin Microbiol.*
5 335 2015;53(3):915-925.
6 336 20. Duong VT, Phat VV, Tuyen HT, et al. Evaluation of Luminex xTAG Gastrointestinal Pathogen
7 337 Panel Assay for Detection of Multiple Diarrheal Pathogens in Fecal Samples in Vietnam. *J Clin*
8 338 *Microbiol.* 2016;54(4):1094-1100.
9 339 21. Faron ML, Ledebor NA, Connolly J, et al. Clinical Evaluation and Cost Analysis of Great Basin
10 340 Shiga Toxin Direct Molecular Assay for Detection of Shiga Toxin-Producing *Escherichia coli* in
11 341 Diarrheal Stool Specimens. *J Clin Microbiol.* 2017;55(2):519-525.
12 342 22. Goldenberg SD, Bacelar M, Brazier P, Bisnauthsing K, Edgeworth JD. A cost benefit analysis of
13 343 the Luminex xTAG Gastrointestinal Pathogen Panel for detection of infectious gastroenteritis in
14 344 hospitalised patients. *J Infect.* 2015;70(5):504-511.
15 345 23. Moher D, Shamseer L, Clarke M, et al. Preferred reporting items for systematic review and meta-
16 346 analysis protocols (PRISMA-P) 2015 statement. *Syst Rev.* 2015;4:1.
17 347 24. McInnes MDF, Moher D, Thombs BD, et al. Preferred Reporting Items for a Systematic Review
18 348 and Meta-analysis of Diagnostic Test Accuracy Studies: The PRISMA-DTA Statement. *JAMA.*
19 349 2018;319(4):388-396.
20 350 25. Whiting PF, Rutjes AWS, Westwood ME, et al. QUADAS-2: A Revised Tool for the Quality
21 351 Assessment of Diagnostic Accuracy Studies. *Ann Intern Med.* 2011;155:529-536.
22 352 26. Bossuyt PM, Reitsma JB, Bruns DE, et al. Towards complete and accurate reporting of studies of
23 353 diagnostic accuracy: The STARD Initiative. *Ann Intern Med.* 2003;138(1):40-44.
24 354 27. Wade R, Corbett M, Eastwood A. Quality assessment of comparative diagnostic accuracy
25 355 studies: our experience using a modified version of the QUADAS-2 tool. *Res Synth Methods.*
26 356 2013;4(3):280-286.
27 357 28. Santaguida PL, Riley CR, Matchar DB. Assessing risk of bias as a domain of quality in medical
28 358 test studies. AHRQ Publication No. 12-EHC077-EF. In: *Methods Guide for Medical Test Reviews*
29 359 *(AHRQ Publication No. 12-EHC017)*. Rockville, MD: Agency for Healthcare Research and
30 360 Quality; 2012.
31 361 29. Reitsma JB, Glas AS, Rutjes AW, Scholten RJ, Bossuyt PM, Zwinderman AH. Bivariate analysis
32 362 of sensitivity and specificity produces informative summary measures in diagnostic reviews. *J Clin*
33 363 *Epidemiol.* 2005;58(10):982-990.
34 364 30. Rutter CM, Gatsonis CA. A hierarchical regression approach to meta-analysis of diagnostic test
35 365 accuracy evaluations. *Stat Med.* 2001;20(19):2865-2884.
36 366 31. Leeflang MM. Systematic reviews and meta-analyses of diagnostic test accuracy. *Clin Microbiol*
37 367 *Infect.* 2014;20(2):105-113.
38 368 32. *R: A Language and Environment for Statistical Computing* [computer program]. Vienna, Austria:
39 369 R Foundation for Statistical Computing; 2017.
40 370 33. *Review Manager (RevMan)* [computer program]. Version 5.3. Copenhagen: The Nordic Cochrane
41 371 Centre, The Cochrane Collaboration; 2014.
42 372 34. Schunemann HJ, Oxman AD, Brozek J, et al. GRADE: assessing the quality of evidence for
43 373 diagnostic recommendations. *Evid Based Med.* 2008;13(6):162-163.
44 374 35. Schunemann HJ, Oxman AD, Brozek J, et al. Grading quality of evidence and strength of
45 375 recommendations for diagnostic tests and strategies. *BMJ.* 2008;336(7653):1106-1110.
46 376
47 377

378 **TABLES**379 **Table 1. Data to be extracted from each included study.**

380

Item	Rationale
<i>Study Characteristics</i>	
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
<i>Clinical Data</i>	
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
<i>Test</i>	
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
<i>Outcomes</i>	
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

381

382

For peer review only

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

1
2
3 Pathogens Panel or Verigene Enteric Pathogens Nucleic Acid Test or xTAG Gastrointestinal
4 Pathogen Panel or xTAG GPP).tw.

5
6 5. 1 or 2 or 3 or 4

7 6. exp diarrhea/di [Diagnosis]

8
9 7. escherichia coli o157/

10 8. feces/an [Drug Analysis]

11
12 9. feces analysis/

13 10. shiga toxin producing escherichia coli/

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

18
19 12. 6 or 7 or 8 or 9 or 10 or 11

20
21 13. 5 and 12

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

23
24 15. limit 14 to animals

25 16. limit 14 to (human and animals)

26
27 17. 15 not 16

28 18. 14 not 17

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

30
31 20. 18 not 19

32 21. limit 20 to "review"

33
34 22. 20 not 21

35 23. limit 20 to "systematic review"

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

37
38 25. 20 and 24

39 26. 22 or 23 or 25

40 27. "sensitivity and specificity"/

41
42 28. exp reference value/

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

44
45 30. validity/ or predictive validity/

46 31. predictive value/

47 32. diagnostic test accuracy study/

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

50
51 34. diagnostic accuracy/

52
53 35. 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34

54
55 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 Amplidag 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 *ehc* 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

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3 RAPID-B* or real-time PCR assay* or Shiga Toxin Chek or Shiga Toxin Direct or ST
4 Direct or Shiga Toxin Quik Chek or Stool Bacterial Pathogens Panel or Verigene Enteric
5 Pathogens Nucleic Acid Test or xTAG Gastrointestinal Pathogen Panel or xTAG GPP)
6 [TOPIC/TITLE]
7

- 8 2. (Diarrhea or E coli O157 or E coli serotype O157 or Escherichia coli O157 or ehec or
9 "enteric bacterial and viral pathogen*" or enteric pathogen* or infectious gastroenteritis
10 or O157 or rfbE O157 or shiga* or shigella or shigellosis or STEC or stool or Stx1 or
11 stx2 or Stx2) [TOPIC/TITLE]
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13 3. (accuracy or detect or detecting or detection or diagnosis or diagnosing or false negative*
14 or false positive* or predictive value* or sensitive or sensitivity or specificity or validat*)
15 [TITLE]
16
17 4. 1 and 2 and 3
18 5. Limit to 2005-2018
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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

1		changes; otherwise, state plan for documenting important	
2		protocol amendments	
3			
4	Sources	#5a Indicate sources of financial or other support for the	13
5		review	
6			
7			
8	Sponsor	#5b Provide name for the review funder and / or sponsor	13
9			
10	Role of sponsor	#5c Describe roles of funder(s), sponsor(s), and / or	13
11	or funder	institution(s), if any, in developing the protocol	
12			
13			
14	Rationale	#6 Describe the rationale for the review in the context of what	4
15		is already known	
16			
17			
18	Objectives	#7 Provide an explicit statement of the question(s) the review	6
19		will address with reference to participants, interventions,	
20		comparators, and outcomes (PICO)	
21			
22			
23	Eligibility criteria	#8 Specify the study characteristics (such as PICO, study	6-7
24		design, setting, time frame) and report characteristics	
25		(such as years considered, language, publication status)	
26		to be used as criteria for eligibility for the review	
27			
28			
29			
30	Information	#9 Describe all intended information sources (such as	7
31	sources	electronic databases, contact with study authors, trial	
32		registers or other grey literature sources) with planned	
33		dates of coverage	
34			
35			
36			
37	Search strategy	#10 Present draft of search strategy to be used for at least one	7/Appendix
38		electronic database, including planned limits, such that it	
39		could be repeated	
40			
41			
42	Study records -	#11a Describe the mechanism(s) that will be used to manage	7
43	data management	records and data throughout the review	
44			
45			
46	Study records -	#11b State the process that will be used for selecting studies	7-8
47	selection process	(such as two independent reviewers) through each phase	
48		of the review (that is, screening, eligibility and inclusion in	
49		meta-analysis)	
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53	Study records -	#11c Describe planned method of extracting data from reports	8
54	data collection	(such as piloting forms, done independently, in duplicate),	
55	process	any processes for obtaining and confirming data from	
56		investigators	
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1	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
2				
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6	Outcomes and	#13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	16-17
7	prioritization			
8				
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10				
11	Risk of bias in	#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
12	individual studies			
13				
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18	Data synthesis	#15a	Describe criteria under which study data will be quantitatively synthesised	9-10
19				
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22		#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 I ² , Kendall's τ)	9-10
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30		#15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	10-11
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34		#15d	If quantitative synthesis is not appropriate, describe the type of summary planned	N/A
35				
36				
37				
38	Meta-bias(es)	#16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	11
39				
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43	Confidence in	#17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	11
44	cumulative			
45	evidence			
46				
47				
48				

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 51 by the [EQUATOR Network](#) in collaboration with [Penelope.ai](#)
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