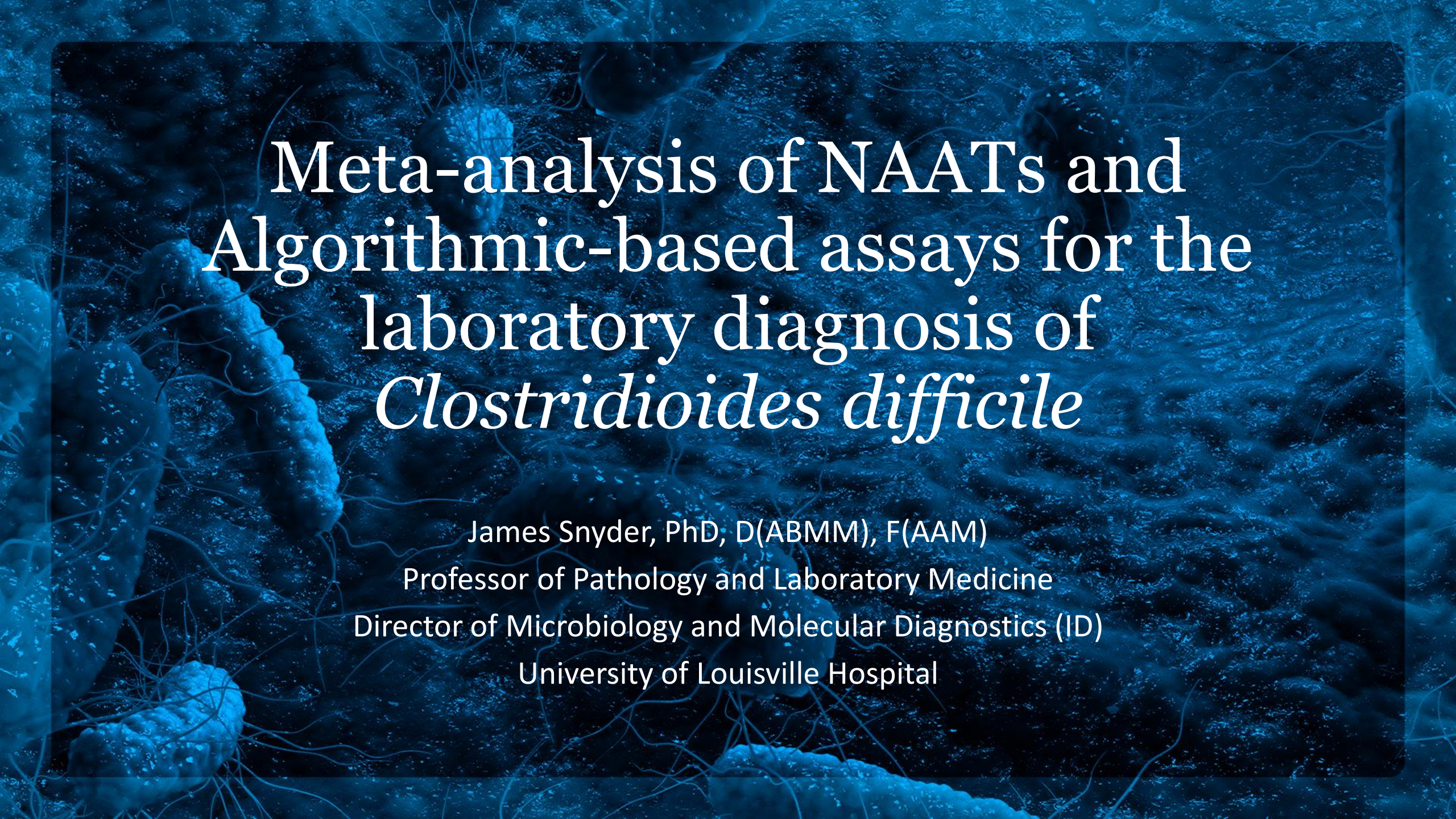

Meta-analysis of NAATs and Algorithmic-based assays for the laboratory diagnosis of *Clostridioides difficile*

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Disclosures

- Diasorin Molecular: Research Funding
- Abbott: Sponsor of Webinar

Learning Objectives

- Review the latest ASM *C. difficile* meta-analysis for NAAT testing
- Discuss IDSA guidelines and how guidelines fit into clinical diagnosis
- Review analytical detection vs. clinical diagnosis
- Identify and describe the various diagnostic test methods (including EIA, PCR and other molecular methods)

Agenda

- *Clostridioides difficile (C. difficile)* characteristics
- Overview of diagnostic assays
- Preanalytical Considerations
- Questions identified for Systematic Review
- LMBP Process
- Assays Evaluated in this Systematic Review
- Recommendations
- Alignment with IDSA Guidelines
- Summary

C. difficile

- Anaerobic, Gram – positive bacillus
- Most common healthcare-associated infection in US
  - Community- and hospital-acquired diarrheal disease globally
  - 500,000 cases annually in the US
  - $4.8 billion for acute care facilities
- Optimal method of diagnosing *C. difficile* Infection (CDI) remains controversial

Photo credit to Science Photo Library

C. difficile Testing Considerations

1. Diagnosis of CDI requires clinical and laboratory assessment
2. Testing is **Analytical** in nature and independent of the **Clinical** presentation
3. Two testing strategies: 1) Direct NAAT; 2) Algorithmic
4. Pre-test probability
5. Formal Laboratory and Clinical Definition of CDI lacking

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NAAT, Nucleic acid amplification test

Patient label

Clostridium difficile Testing Checklist

(Answer the questions below to determine if *C.difficile* testing is indicated.)

Does patient have ≥3 loose/liquid bowel movements a day?

☐ Yes ☐ No→ ⚠ *C.difficile* testing is NOT recommended.

Has patient received laxative, bowel prep, and/or enema within the past 48 hours?

☐ No ☐ Yes→ ⚠ *C.difficile* testing is NOT recommended. Hold laxative, bowel prep and/or enema for minimum of 48hrs and assess for resolution of diarrhea prior to *C.difficile* testing.

Does stool conform to the shape of the container (liquid)?

☐ Yes ☐ No→ STOP Do not send specimen, lab will reject.

Has patient had a negative *C.difficile* stool sample in the past seven (7) days?

☐ No ☐ Yes→ STOP Do not send specimen, lab will reject. Look for other cause of diarrhea.

Has patient had a positive *C.difficile* stool sample in the past 30 days?

☐ No ☐ Yes→ STOP Do not send specimen, lab will reject. Do not test for cure.

Action taken:

- ☐ *C.difficile* testing indicated and sample sent (send checklist with specimen to lab)
- ☐ *C.difficile* testing NOT indicated, prescriber notified and test order canceled (fax checklist to x6807)
- ☐ Other_____

Comments:_____

Not part of the patient's medical record. Contact Infection Prevention & Control with questions (x3794).

Laboratory Assays for the Detection of *C. difficile*

- Toxigenic Culture (TC)
- Cell Cytotoxicity Neutralization Assay (CCNA)
- Enzyme Immunoassay (EIA)
  - Glutamate Dehydrogenase (GDH)
  - Toxin
- Polymerase Chain Reaction (PCR)
- Loop-mediated Isothermal Amplification (LAMP)

Fang, F. C., C. R. Polage, M. H. Wilcox. JCM, 55, 2017: Point-Counterpoint

Diagnostic Testing Strategy

1. Direct PCR/LAMP
2. Algorithmic
    a. GDH plus Toxin: 2-step
    b. NAAT plus Toxin: 2-step
    c. GDH plus Toxin plus NAAT (confirmatory if toxin is neg)

Questions for Systematic Review

- What is the diagnostic accuracy of NAAT only versus TC or CCNA for detection of *C. difficile* toxin gene?
- What is the diagnostic accuracy of a GDH-positive EIA followed by NAAT versus TC or CCNA for detection of the *C. difficile* organism/toxin gene?
- What is the diagnostic accuracy of a GDH-positive/toxin-negative EIA followed by NAAT versus TC or CCNA for detection of the *C. difficile* organism/toxin/toxin gene?
- What is the increased yield of repeat testing using NAAT after an initial negative result for *C. difficile* detection of the toxin gene?

Goals of Analysis

- Evaluate the effectiveness:

1. the diagnostic accuracies of NAAT-only and algorithmic (“two-step” or “three-step”) testing strategies, including detection of toxin or GDH in addition to NAAT
2. the diagnostic yield of repeat testing after an initial negative NAAT result

Seek evidence using LMBP Systematic Review Process: translate results into evidence-based recommendations.

Laboratory Medicine Best Practices (LMBP) Process

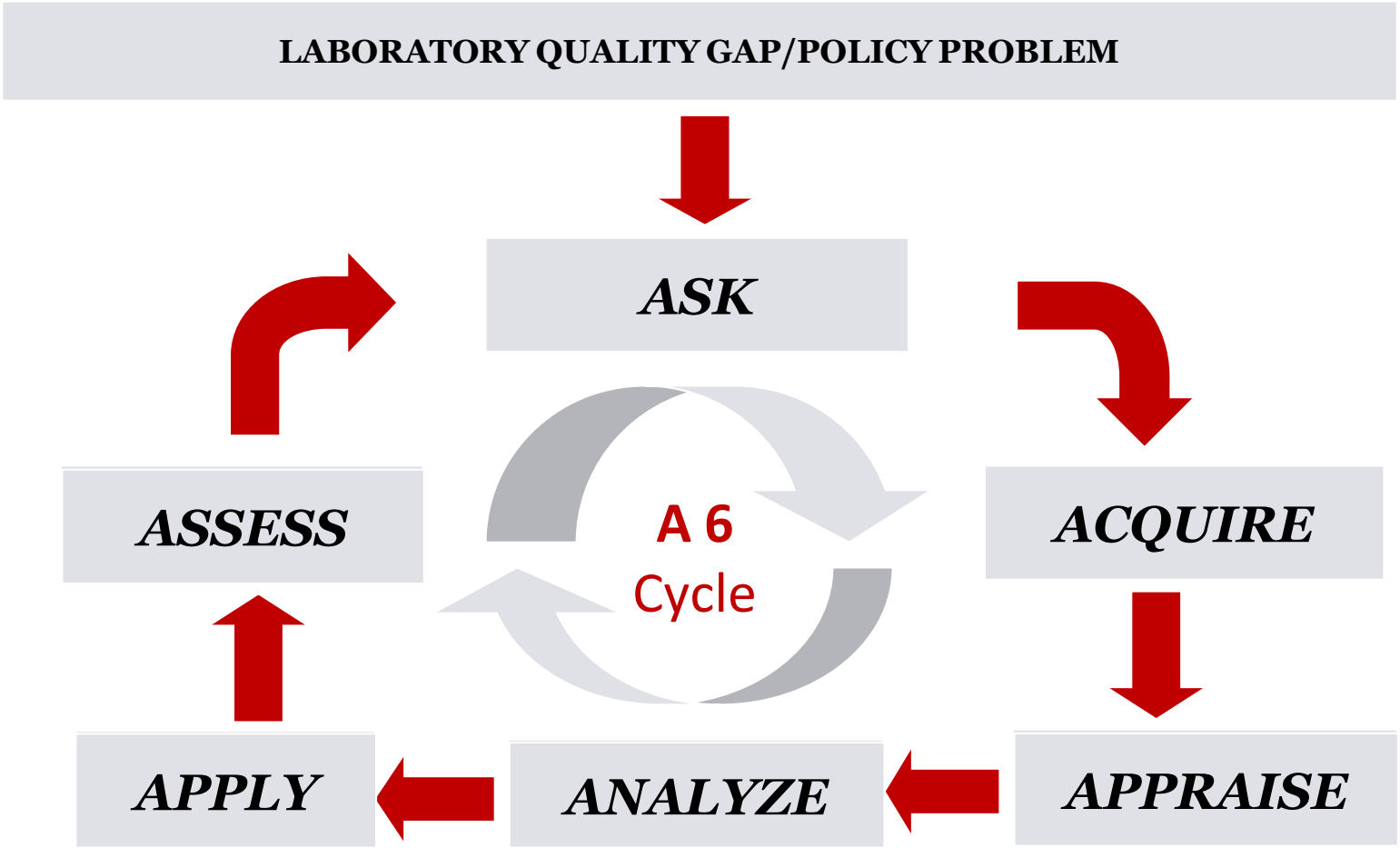
LMBP A-6 Cycle

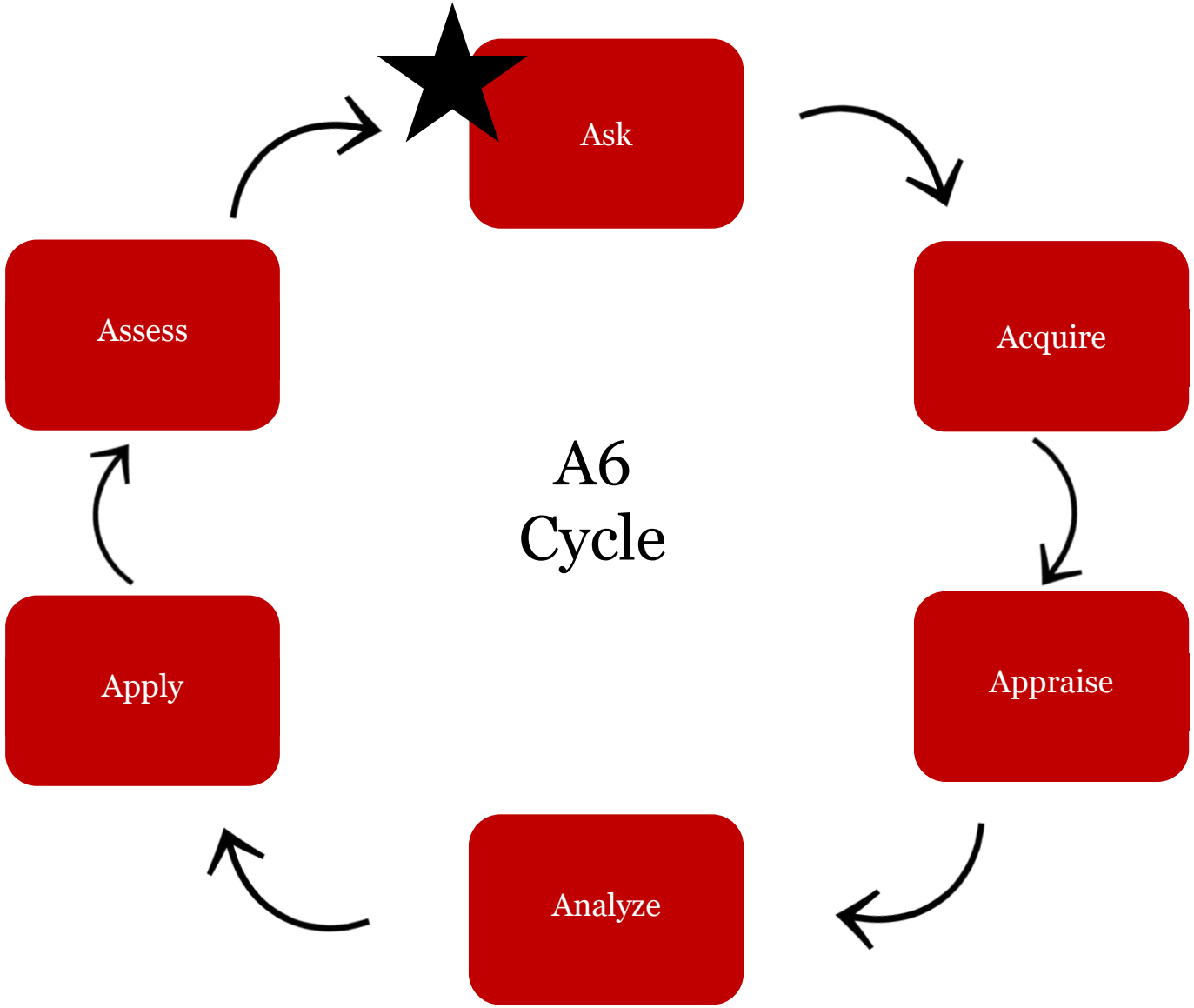
- a validated evidence review and evaluation method for quality improvement in laboratory medicine (www.cdc.gov/labbestpractices/index.html; https://www.cdc.gov/library/researchguides/systematicreviews.html)

Designed to assess the results of studies of practice effectiveness to derive evidence-based practice recommendation

Review Coordinator, Technical Coordinator, Statistician (experienced in quantitative evidence analysis), volunteer faculty (expert panel) trained in the application of the LMBP methods

Fundamentals of an Evidence-Based Approach




Ask
Acquire
Appraise
Analyze
Apply
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Analytical Framework

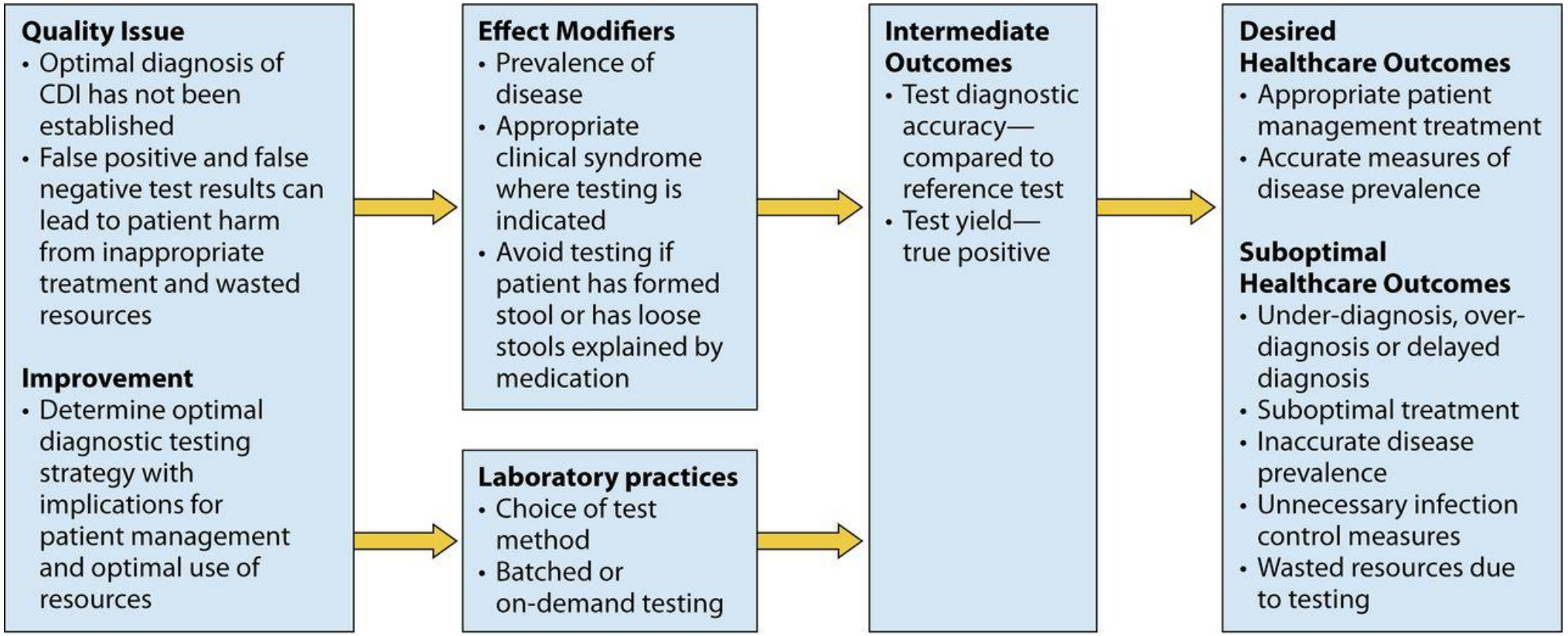


TABLE 1 Assays evaluated in this systematic review

Assay (manufacturer)[a]
NAAT only
BD GeneOhm C diff (Becton Dickinson, Sparks, MD)
Lyra Direct C diff (Quidel, San Diego, CA)
Illumigene (Meridian Bioscience, Cincinnati, OH)
Verigene (Luminex, Austin, TX)
ProGastro *C. difficile* (Gen-Probe Prodesse, Waukesha, WI)
Xpert *C. difficile* (Cepheid, Sunnyvale, CA)
Xpert *C. difficile* Epi (Cepheid, Sunnyvale, CA)
Portrait toxigenic *C. difficile* assay (Great Basin, West Valley, UT)
AdvanSure CD RT-PCR (LG Life Sciences, South Korea)
BD Max Cdiff (Becton, Dickinson, Franklin Lakes, NJ)
GDH$^+$, NAAT
C. Diff CHEK-60 EIA (GDH) (Techlab, Blacksburg, VA) → Xpert *C. difficile* Epi
C. Diff CHEK-60 EIA (GDH) → Xpert *C. difficile*
C. Diff CHEK-60 EIA (GDH) → BD GeneOhm Cdiff assay
Quick Chek GDH (Alere, Waltham, MA) → Illumigene (Meridian Bioscience, Cincinnati, OH)
C. Diff CHEK-60 EIA (GDH) → BD GeneOhm Cdiff assay
C. Diff CHEK-60 EIA (GDH) → ProGastro CD (Prodesse, Waukesha, WI)
GDH$^+$, toxin negative, NAAT
C. diff Quik Chek complete (Techlab, Blacksburg, VA) → GenomEra (Abacus Diagnostica, Turku, Finland)
C. diff Quik Chek complete → Xpert *C. difficile*
C. Diff CHEK-60 EIA (GDH) → ProSpecT *C. difficile* toxin A/B (Remel/Thermo Fisher, Lenexa, KS) → BD GeneOhm Cdiff assay
C. diff Quik Chek complete → Quik Chek direct (Techlab, Blacksburg, VA) → in-house PCR of *tcdB*
C. diff Quik Chek complete → Illumigene
Premier *C. difficile* GDH combined with ImmunoCard → Illumigene
C. diff Quik Chek complete → Prodesse ProGastro CD
C. diff Quik Chek complete → BD GeneOhm Cdiff assay

[a]→ indicates a subsequent test. RT-PCR, reverse transcription-PCR.

TABLE 2 Questions from QUADAS-2 used by the expert panel to evaluate studies[a]

Domain	Patient selection	Index test	Reference standard	Flow and timing
Description	Describe methods of patient selection; describe included patients (prior testing, presentation, intended use of index test, and setting)	Describe the index test and how it was conducted and interpreted	Describe the reference standard and how it was conducted and interpreted	Describe any patients who did not receive the index test(s) and/or reference standard or who were excluded from the 2-by-2 table[b]; describe the time interval and any interventions between index test(s) and reference standard
Signaling question (yes/no/unclear)	Was a consecutive or random sample of patients enrolled?	Were the index test results interpreted without knowledge of the results of the reference standard?	Is the reference standard likely to correctly classify the target condition?	Was there an appropriate interval between index test(s) and reference standard?
Risk of bias (high/low/unclear)	Was a case-control design avoided?	If a threshold was used, was it prespecified?	Were the reference standard results interpreted without knowledge of the results of the index test?	Did all patients receive a reference standard?
Concerns regarding applicability (high/low/unclear)	Did the study avoid inappropriate exclusions?	Are there concerns that the index test, its conduct, or its interpretation differed from the review question?	Are there concerns that the target condition as defined by the reference standard does not match the review question?	Did all patients receive the same reference standard?

[a]Adapted from reference 34 with permission of the publisher.
[b]See the flow diagram in reference 34.

Likelihood Ratio

Positive Likelihood (+LR)

Negative Likelihood (-LR)

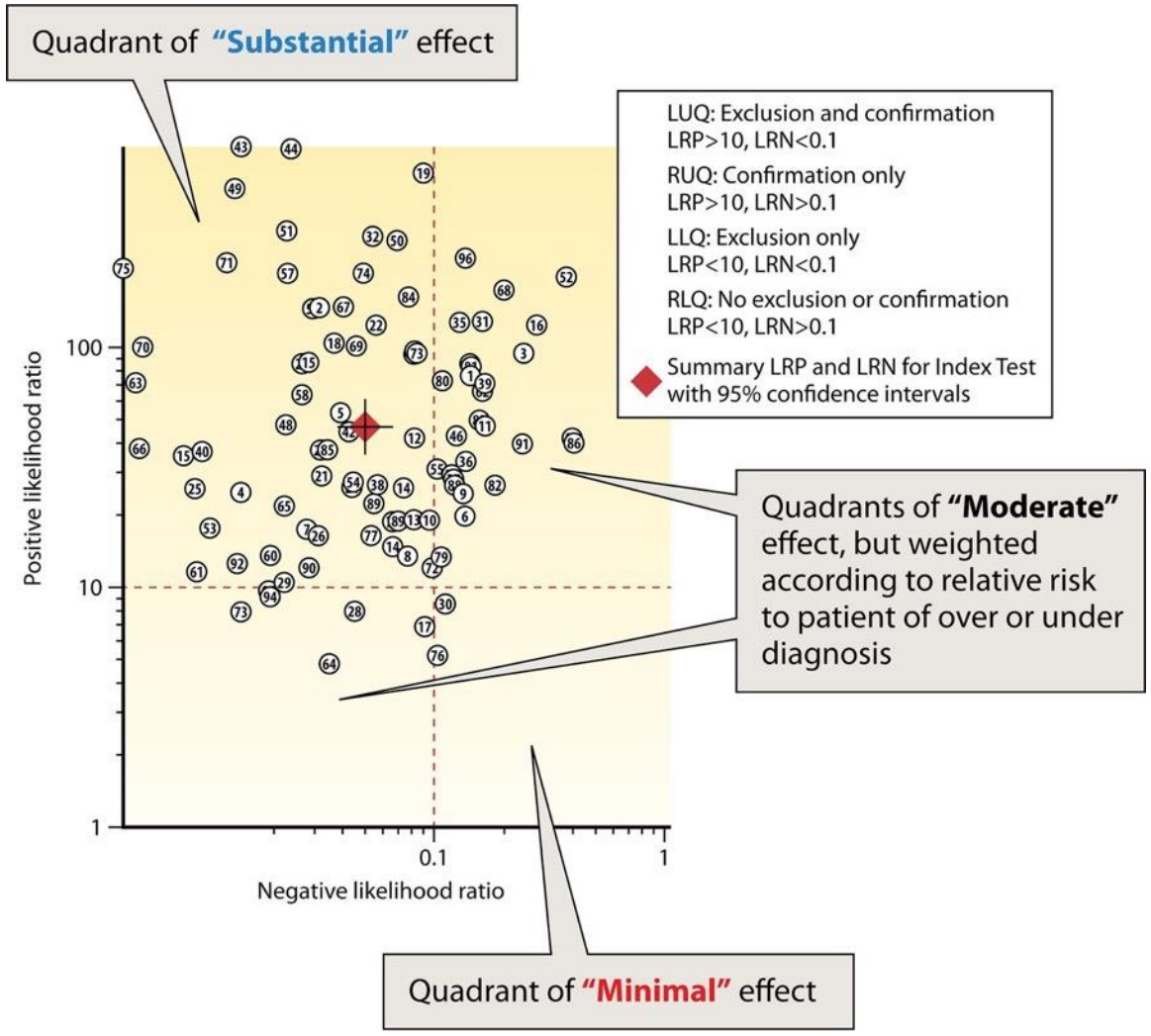
Substantial Effect Rating: if +LR is >10 and –LR is <0.1

Moderate Effect Rating: if +LR is >10 and –LR is >0.1 or +LR is <10 and –LR is <0.1

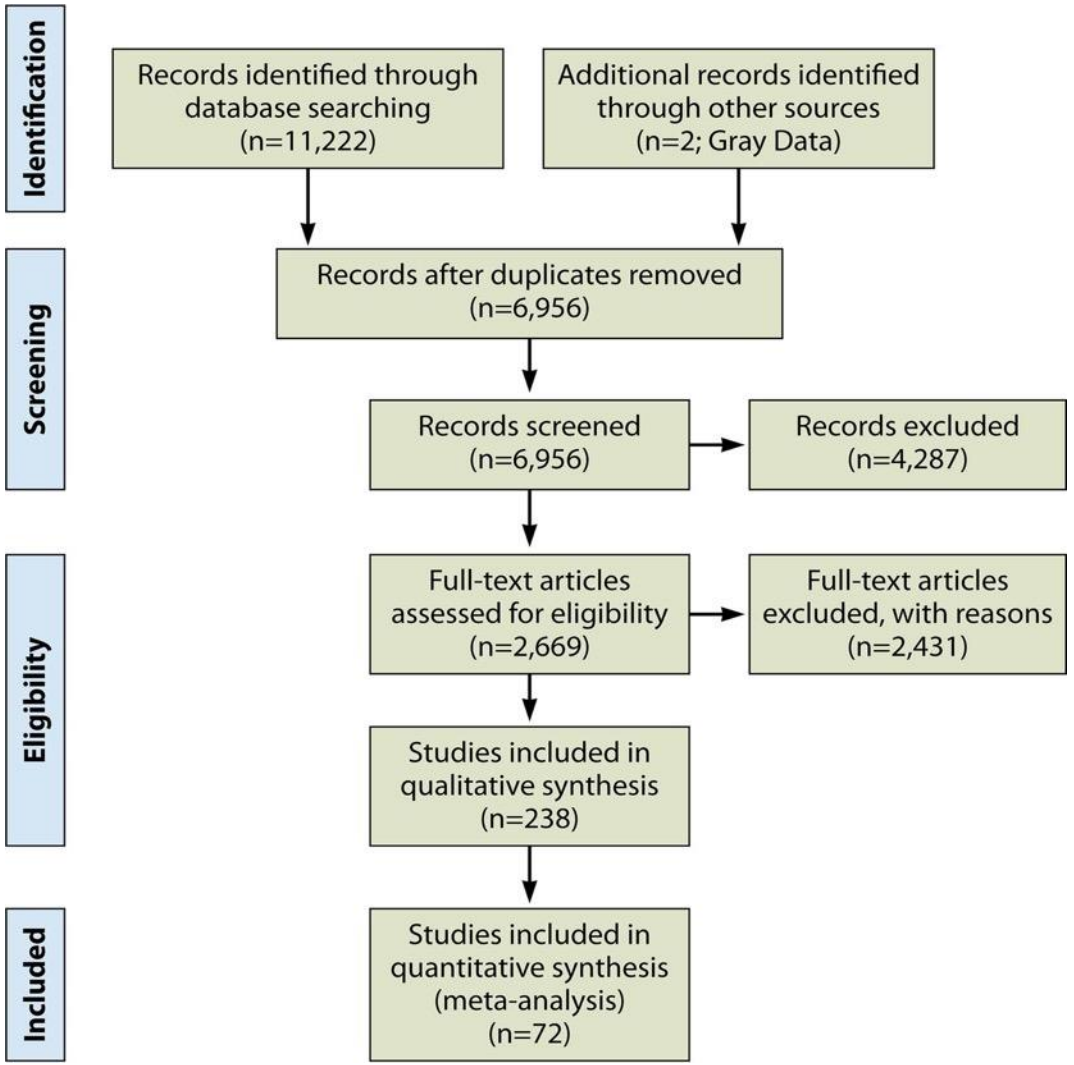
Minimal Effect Rating: if +LR is <10 and –LR is >0.1

Cutoffs represent thresholds for “high” clinical validity, or a “high” test information value (e.g., for determinations of post-test probability of disease for individual patients

Likelihood Ratio Scatter Matrix



Study Selection Flow Diagram



Diagnostic Accuracy

TABLE 6 Diagnostic accuracy statistics by number of tests

	Value for test					
	NAAT only		GDH/NAAT		GDH/toxin/NAAT	
Parameter[a]	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
No. of studies	96		12		9	
Prevalence	0.17		0.11		0.13	
Sensitivity	0.95	0.94–0.96	0.91	0.86–0.95	0.89	0.84–0.92
ICC SEN[b]	0.27	0.18–0.35	0.10	0.00–0.23	0.03	0.00–0.15
Specificity	0.98	0.97–0.98	0.99	0.98–1.0	0.99	0.98–1.00
ICC SPE[c]	0.27	0.19–0.34	0.25	0.00–0.53	0.26	0.00–0.62
Positive likelihood ratio	46.0	35.7–59.2	113.5	49.9–258.1	155.8	57.7–420.2
Negative likelihood ratio	0.05	0.04–0.06	0.09	0.06–0.14	0.11	0.08–0.16
Diagnostic odds ratio	934	652–1,338	1,282	484–3,395	1,383	436–4,388

[a]ICC, interclass correlation coefficient; SEN, sensitivity; SPE, specificity.
[b]Proportion of total variance in sensitivity explained by between-study variation.
[c]Proportion of total variance in specificity explained by between-study variation.

Accuracy of Reference Methods (TC, CCNA)

TABLE 8 Sensitivity analysis of diagnostic accuracy statistics by reference standard[a]

	Value					
	Toxigenic culture		CCNA		Combined TC/CCNA	
Parameter	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
No. of studies	74		33		10	
Prevalence	0.16		0.16		0.21	
Sensitivity	0.94	0.92, 0.95	0.93	0.93, 0.95	0.99	0.96, 1.00
ICC SEN[b]	0.22	0.13, 0.31	0.17	0.06, 0.28	0.39	0.03, 0.74
Specificity	0.99	0.98, 0.99	0.98	0.96, 0.98	0.98	0.96, 0.99
ICC SPE[c]	0.26	0.18, 0.35	0.30	0.17, 0.43	0.32	0.04, 0.60
Positive likelihood ratio	65.3	48.7, 87.8	38.5	24.9, 59.5	57.5	24.3, 135.9
Negative likelihood ratio	0.06	0.05, 0.08	0.08	0.05, 0.11	0.01	0.00, 0.04
Diagnostic odds ratio	1,079	745, 1,563	509	302, 857	5,022	1,127, 22,377

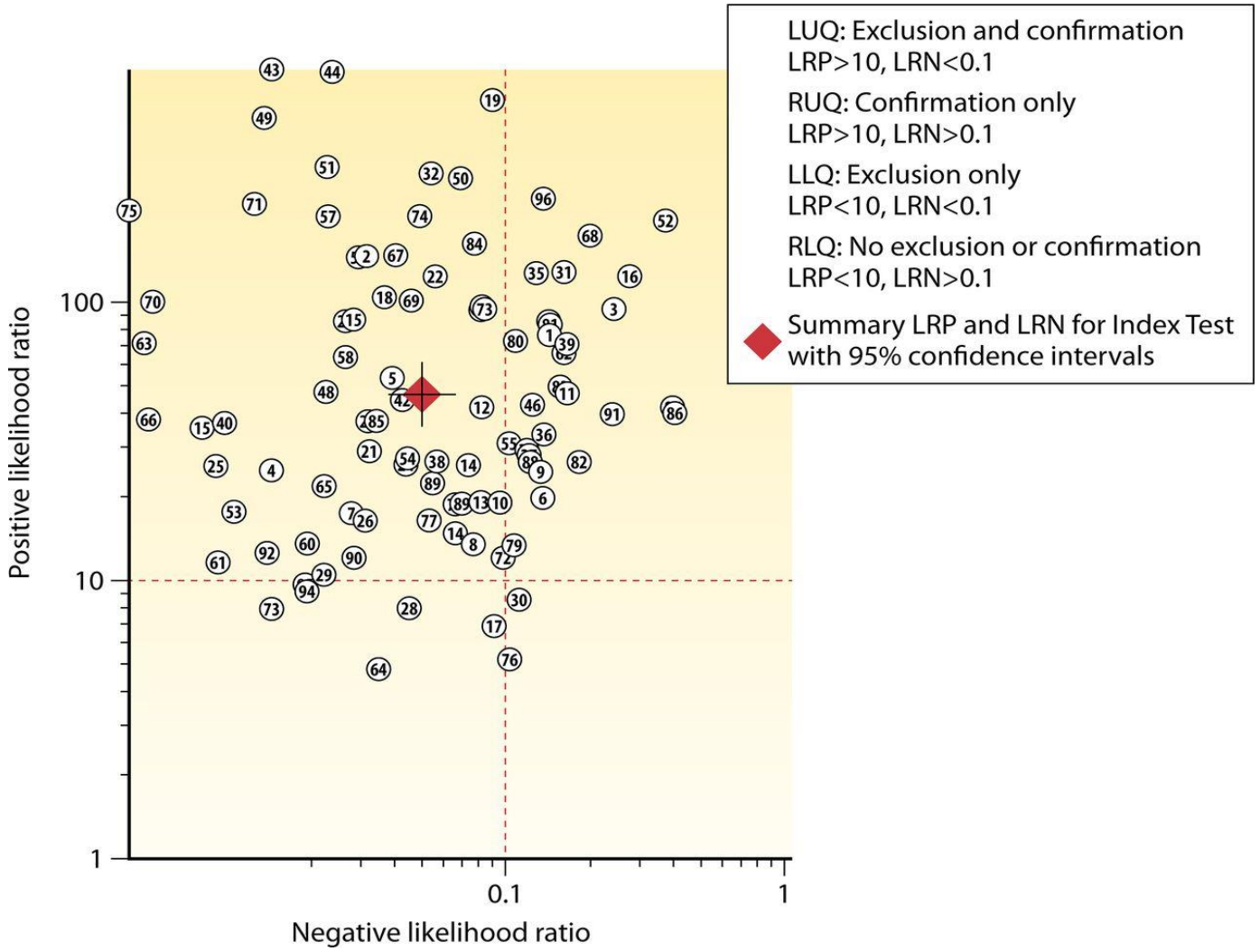
[a]CCNA, cell cytotoxicity neutralization assay; TC, toxigenic culture; ICC, interclass correlation coefficient.
[b]Proportion of total variance in sensitivity explained by between-study variation.
[c]Proportion of total variance in specificity explained by between-study variation.

TABLE 4 (Continued)

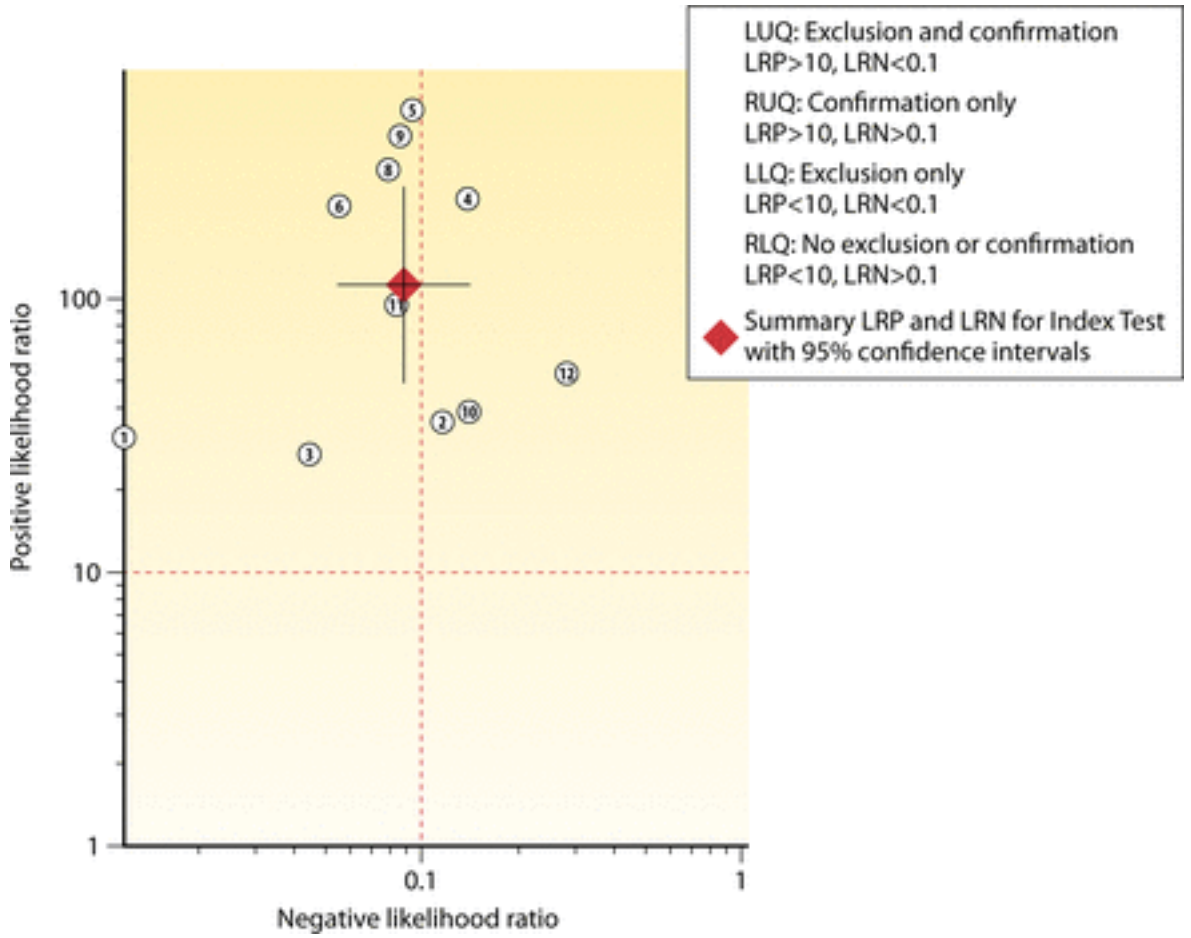
	Risk of Bias				Applicability of Concerns			LMBP Quality Rating	LMBP Effect Size Rating
	Patient Selection	Index Test	Reference Standard	Flow and Timing	Patient Selection	Index Test	Reference Standard		
Peterson 2011 (86)	Low	Low	Low	Low	Low	Low	Low	Good	Moderate
Putsathit 2015 (87)	High	Low	High	Low	Low	Low	Low	Fair	Substantial
Shin 2012 (88)	Low	Low	Low	Low	Low	Low	Low	Good	Substantial
Silva 2014 (89)	Low	Unclear	High	High	Low	High	High	Poor	Moderate
Soh 2014 (90)	Low	Low	Low	Low	Low	Low	Low	Good	Moderate
Stamper 2009 (91)	Low	Low	Low	Low	Low	Low	Low	Good	Moderate
Swindells 2010 (92)	Low	Low	Low	Low	Low	Low	Low	Good	Substantial
Terhes 2009 (93)	Low	Low	Unclear	Low	Low	Low	Low	Good	Substantial
Tojo 2014 (94)	Low	Low	Low	Unclear	Low	Low	Low	Good	Substantial
Van Broeck 2010 (95)	Unclear	Low	Low	Low	Unclear	Low	Low	Good	Substantial
Van Broeck 2012 (96)	Unclear	Low	Low	Low	Low	Low	Low	Good	Moderate
Vasoo 2014 (97)	Unclear	Low	Unclear	Low	Low	Low	Low	Good	Substantial
van den Berg 2005 (98)	Unclear	Low	High	Low	Low	Low	Low	Fair	Moderate
van den Berg 2006 (99)	Low	Low	Low	Low	Low	Low	Low	Good	Substantial
van den Berg 2007 (100)	Unclear	Low	Unclear	Low	Unclear	Unclear	Unclear	Fair	Substantial
Viala 2012 (101)	Unclear	Low	Low	Low	Low	Low	Low	Good	Moderate
Walkty 2013 (102)	Low	Low	Low	Low	Low	Low	Low	Good	Moderate
Yisiurua 2013 (103)	Unclear	Low	Low	Low	Low	Low	Low	Good	Substantial
Zidaric 2011 (104)	Low	Low	Low	Low	Low	Low	Low	Good	Moderate

[a]See references 39–104.

NAAT-only Detection of *C. difficile*



GDH/NAAT Detection of *C. difficile*



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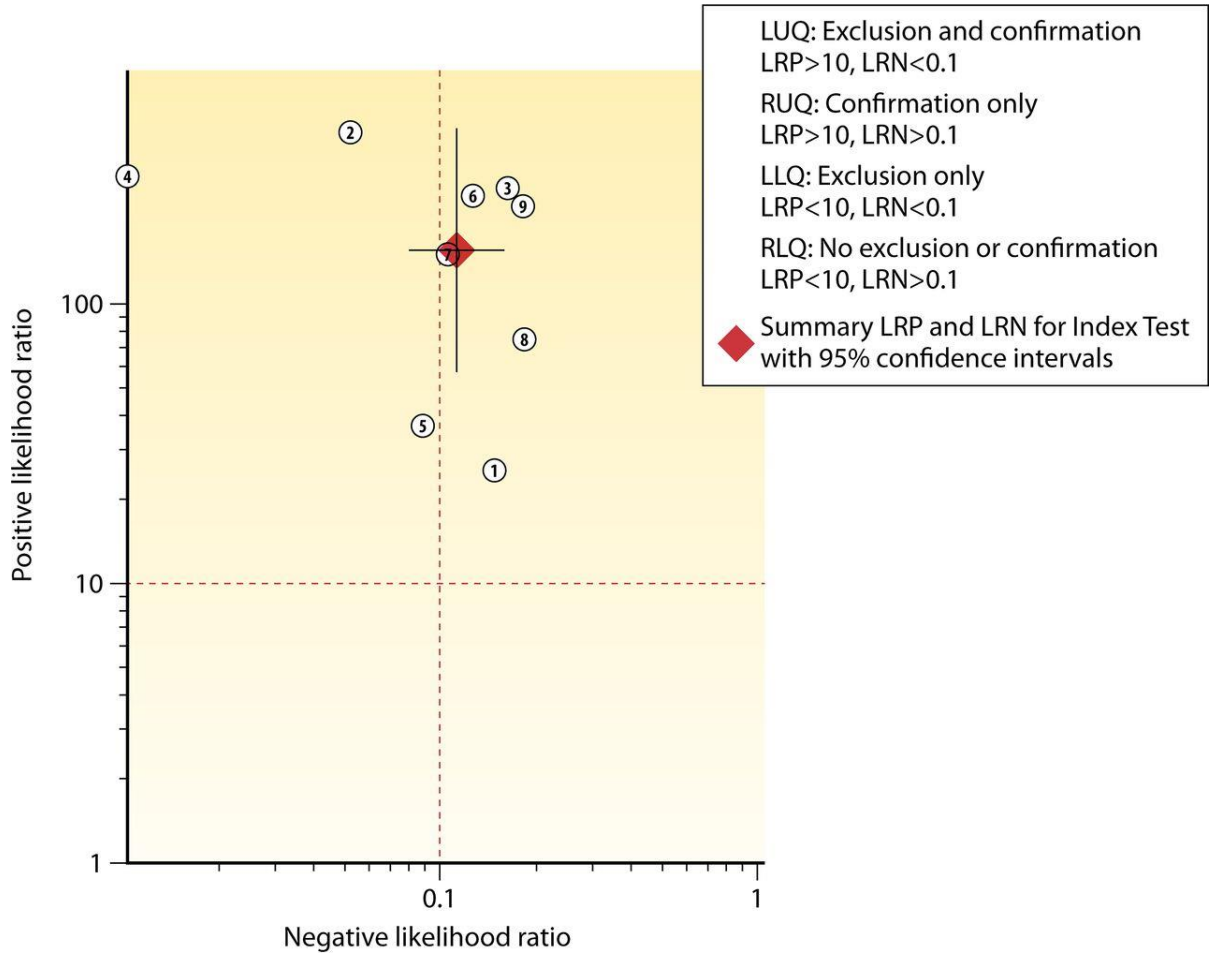
GDH/Toxin/NAAT Algorithm



NAAT Alone vs Algorithmic Testing

TABLE 10 Comparison of sensitivities and specificities by whether authors reported that the stool conforms to the container[a]

Categorization of whether stool meets criteria reported	No. of studies in arm	Sensitivity		*P* value for sensitivity	Specificity		*P* value for specificity
		Estimate	95% CI		Estimate	95% CI	
NAAT only							
Yes	48	0.94	0.92–0.96	<0.001	0.97	0.96–0.98	<0.001
No	49	0.96	0.94–0.97		0.99	0.98–0.99	
GDH/NAAT							
Yes	7	0.91	0.86–0.96	0.02	0.99	0.98–1.00	0.16
No	5	0.92	0.86–0.98		0.99	0.98–1.00	
GDH/toxin/NAAT							
Yes	4	0.86	0.79–0.92	<0.001	1.00	0.99–1.00	0.58
No	5	0.89	0.85–0.93		0.99	0.98–1.00	

[a]In those studies where the stool had to meet the criteria before being tested, only the samples that met the preanalytic requirement were tested.

Strength of Evidence of Selected Papers

TABLE 11 LMBP strength of body of evidence for all questions

Question	No. of studies	No. of comparisons	Effect	Quality
NAAT only, high strength of body of evidence	60	96	Substantial	Good
GDH/NAAT, high strength of body of evidence	9	12	Substantial	Good
GDH/toxin/NAAT, moderate strength of body of evidence	7	9	Moderate	Good
Repeat testing using NAAT, insufficient strength of body of evidence	5	6	Minimal	Good

ASM Recommendations

TABLE 12 Summary of ASM practice recommendations for *C. difficile* testing

Practice category	Practice recommendation
NAAT only	Use of NAAT-only testing is recommended as a best practice for the detection of the *C. difficile* toxin gene
GDH/NAAT algorithm	Use of a GDH/NAAT algorithm is recommended as a best practice for the detection of the *C. difficile* organism/toxin gene
GDH/toxin/NAAT algorithm	Use of a GDH/toxin/NAAT algorithm is recommended as a best practice for the detection of the *C. difficile* organism, toxin, or toxin gene
Repeated testing using NAAT	A recommendation for or against repeated testing for *C. difficile* using a NAAT as a best practice cannot be made due to insufficient evidence

IDSA Guidelines,CID 2018:66

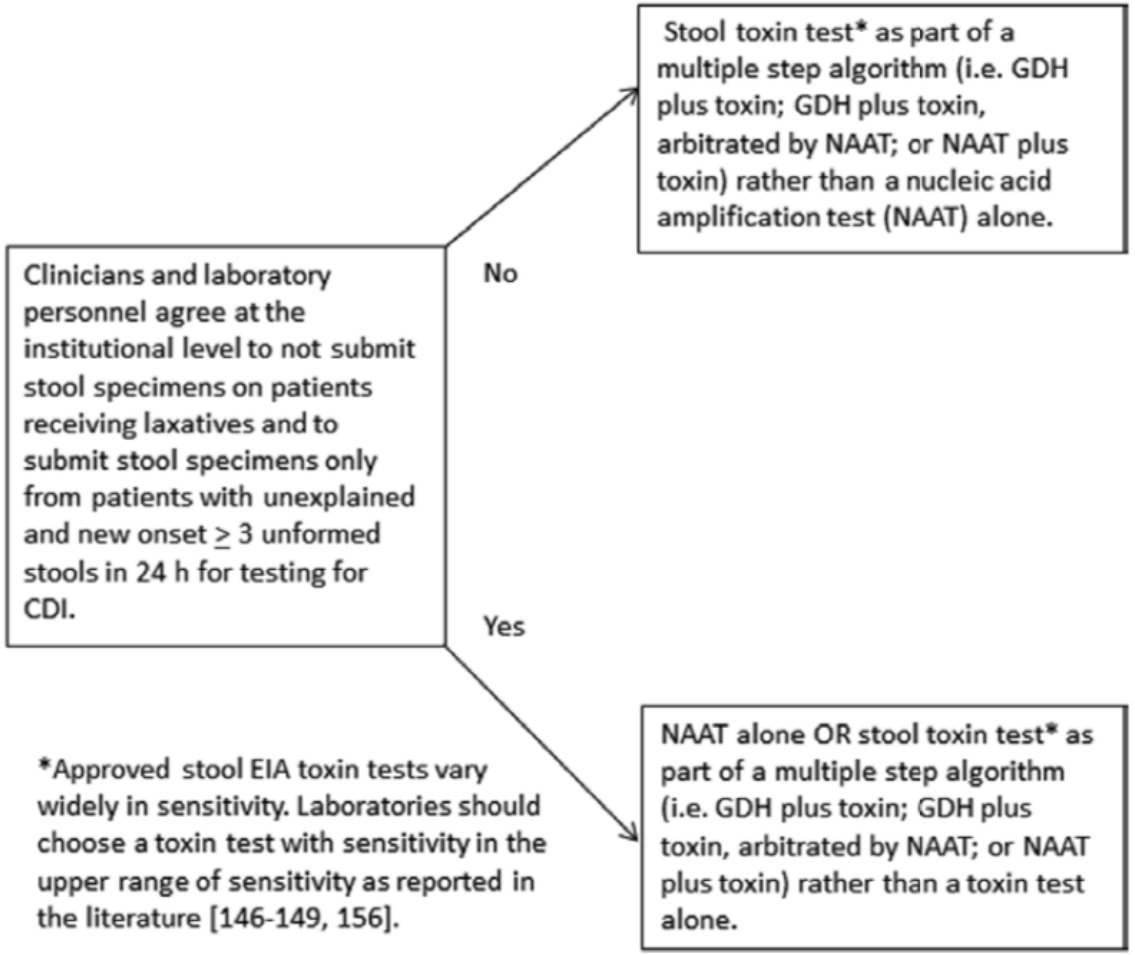


Figure 2. *Clostridium difficile* infection laboratory test recommendations based on preagreed institutional criteria for patient stool submission. Abbreviations: CDI, *Clostridium difficile* infection; EIA, enzyme immunoassay; GDH, glutamate dehydrogenase; NAAT, nucleic acid amplification test.

Diagnosis: What is the Best Testing Strategy to Diagnose CDI in the Clinical Laboratory?

1. Tests for *C. difficile* or its toxins should be performed ONLY diarrheal (unformed) stool, unless ileus due to *C. difficile* is suspected

2. Do not test stool from asymptomatic patients

3. Do not perform “test of cure” testing

4. Repeat testing during same episode of diarrhea is of limited value and should be discouraged…..one week following initial testing

Cohen, S. H., et al., 2018. Infect Control and Hospital Epidemol, 31: 431-455 (SHEA – IDSA Guidelines)

Summary and Conclusions

- LMBP process targeted diagnostic accuracy, not clinical specificity
- Recommendations are Evidenced-based (Meta-analysis)
- NAAT-only, GDH/NAAT algorithmic testing, and GDH/toxin/NAAT algorithmic testing are recommended practices for detection of *C. difficile* organism/toxin/toxin gene
- Insufficient evidence regarding value of repeat testing
- Value of diagnostic tests dependent on probability or likelihood of the patient having CDI: clinical assessment is critical

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