

# Difficulties in CDI Diagnosis: Is NAAT/PCR Alone Adequate?

Erik R. Dubberke, MD, MSPH

Professor of Medicine

Washington University School of Medicine

# Diagnosics Available

Test	Advantage(s)	Disadvantage(s)
<b>Toxin testing</b>		
Toxin Enzyme immunoassay (EIA)	Rapid, simple, inexpensive, associated with outcomes	Least sensitive method, assay variability
Tissue culture cytotoxicity	More sensitive than toxin EIA, associated with outcomes	Labor intensive; requires 24–48 hours for a final result, special equipment, inter-rater variability
<b>Organism identification</b>		
Glutamate dehydrogenase (GDH) EIA	Rapid, sensitive	Non-toxigenic and toxigenic <i>C. difficile</i> detected
Nucleic acid amplification tests (NAAT) (PCR)	Rapid, sensitive, detects presence of toxin gene	Cost, special equipment, does not detect toxin production
Stool culture	Most sensitive test available when performed appropriately	Non-toxigenic and toxigenic <i>C. difficile</i> detected; labor-intensive; requires 48–96 hours for results

# Historical Flaws in Diagnostic Literature Interpretation

- Lack of clinical data
  - Test for CDI does not exist: detect toxin or organism
    - Up to 15% of patients admitted to the hospital are colonized with toxigenic *C. difficile*
    - Other reasons for diarrhea are often present
  - Enhanced sensitivity for *C. difficile* detection will increase detection of asymptomatic *C. difficile* carriage
- Lack of appreciation not all toxin detection assays are equal
  - Original toxin EIAs: detect toxin A only
    - Some strains produce only toxin B (as many as 20%)
  - Manufacturer, target(s) and format make a difference








# Types of False Positive Tests for CDI

- Toxigenic *C. difficile* present but no CDI
  - Concern of more sensitive tests
    - GDH
    - NAAT/PCR
    - Culture
- Assay result positive but toxigenic *C. difficile* not present
  - Tests that detect non-toxigenic *C. difficile*
    - GDH alone
    - Culture alone
  - False positive test

# Enhanced Sensitivity to Detect *C. difficile* Decreases Specificity for CDI

- Including clinically significant diarrhea in gold standard:
  - No impact on sensitivity
    - NAATs 99%
    - Toxin EIAs 80%-94%
  - Specificity of NAATs decreased from ~98% to ~89% ( $p < 0.01$ )
    - Positive predictive value decreased to ~60% (25% drop)
  - No NAAT (+) / toxin (-) developed CDI-related complications

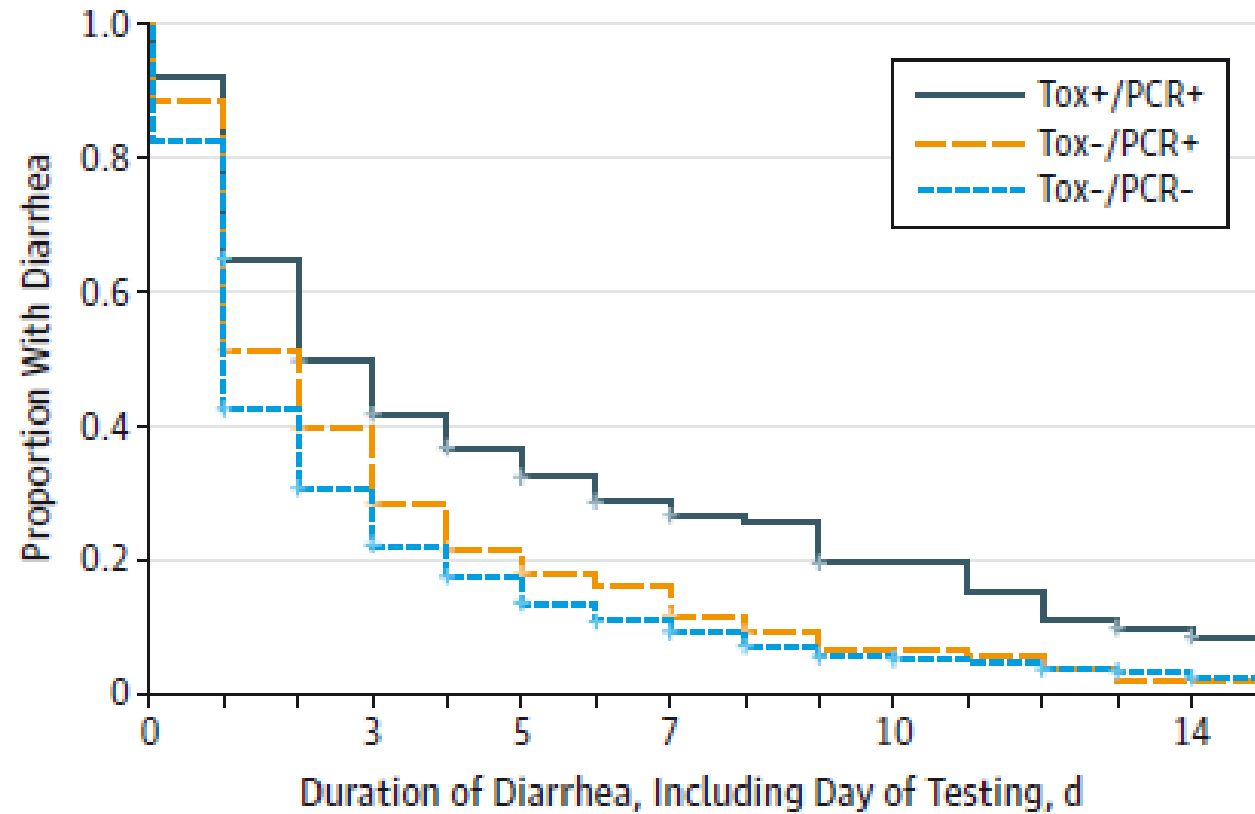
## Bristol Stool Chart

Type 1		Separate hard lumps, like nuts (hard to pass)
Type 2		Sausage-shaped but lumpy
Type 3		Like a sausage but with cracks on its surface
Type 4		Like a sausage or snake, smooth and soft
Type 5		Soft blobs with clear-cut edges (passed easily)
Type 6		Fluffy pieces with ragged edges, a mushy stool
Type 7		Watery, no solid pieces. <b>Entirely Liquid</b>

# Increase in “CDI” Incidence after Switch to NAAT from Toxin EIA

State	Switch to NAAT median ratio (95% CI)	Non-switch median ratio (95%CI)	Attributable % increase due to NAAT
California	1.52 (0.69-2.50)	1.0 (1.00-1.50)	52%
Colorado	1.43 (1.21-2.33)	1.0 (0.85-1.06)	43%
Georgia	1.67 (1.50-2.06)	1.0 (0.89-1.04)	67%

# Time to Resolution of Diarrhea



No. at risk

Tox+/PCR+	131	62	41	29	25	8
Tox-/PCR+	162	60	29	21	10	2
Tox-/PCR-	1123	328	172	99	42	23

# Largest Assay Comparison To Date

Variable	Cytotoxin (CTX) +	NAAT + alone	-/-	(CTX+ ) vs. (NAAT+ alone)	(CTX+) vs. (-/-)	(NAAT+ alone) vs. (-/-)
Number	435	311	3943			
White blood count (SD)	12.4 (8.9)	9.9 (6.6)	10.0 (12.0)	<0.001	<0.001	0.863
Died	72 (16.6%)	30 (9.7%)	349 (8.9%)	0.004	<0.001	0.606

CTX = cytotoxin

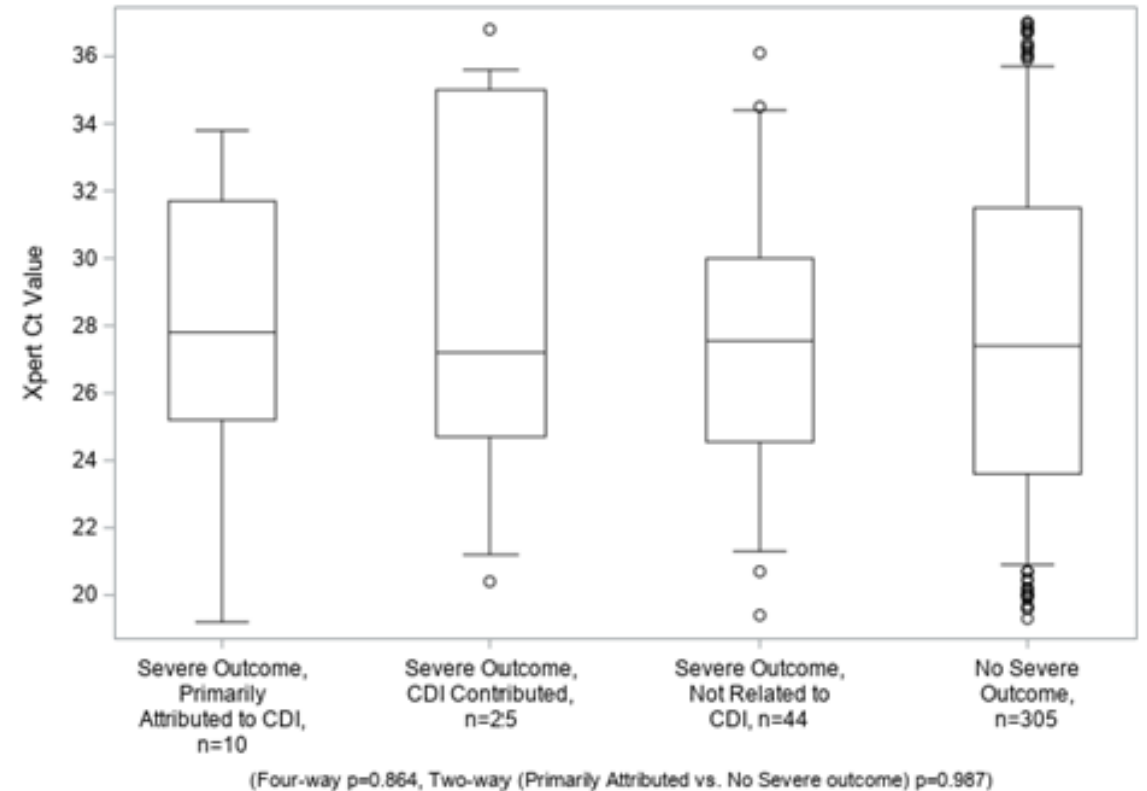
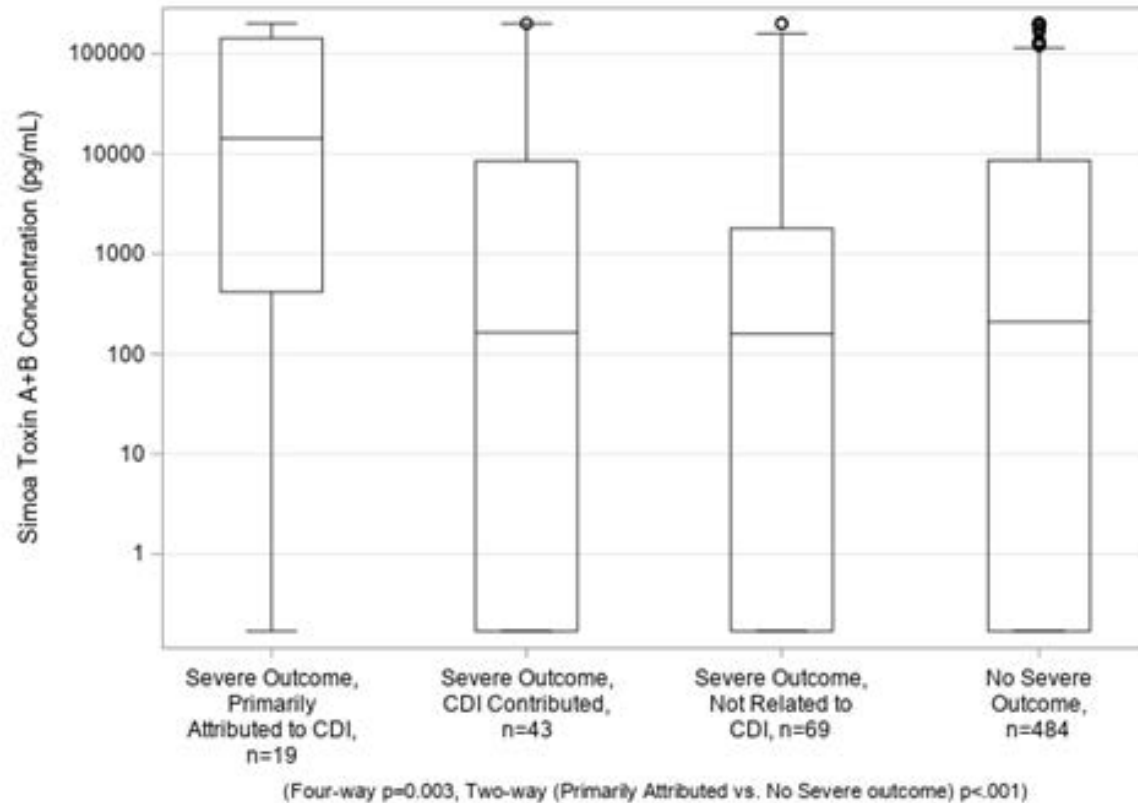
NAAT = Nucleic acid amplification test



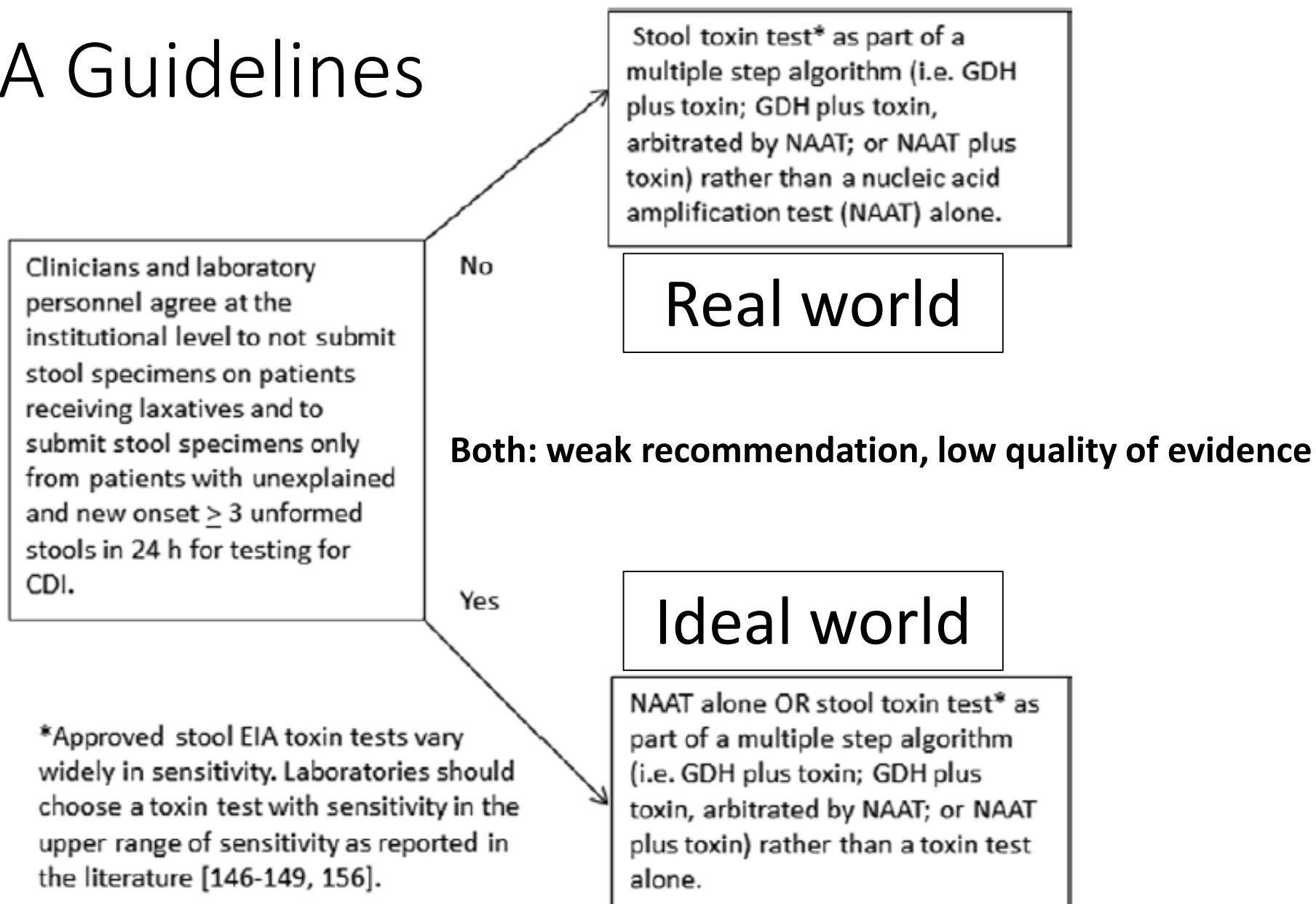
# Toxin+ with More Severe Presentation and Recurrences than NAAT+ alone

Variable	Toxin + (n=2160)	NAAT+ alone (n=2718)
Pseudomembranous colitis	9/89 (10.5%)	2/130 (1.5%)
WBC $\geq$ 15,000 cells/ul	483/1539 (31.4%)	423/1978 (21.4%)
CDI recurrence	445 (20.6%)	311 (11.4%)

# Toxin Levels Correlate with Outcomes but NAAT Cycle Threshold Does Not



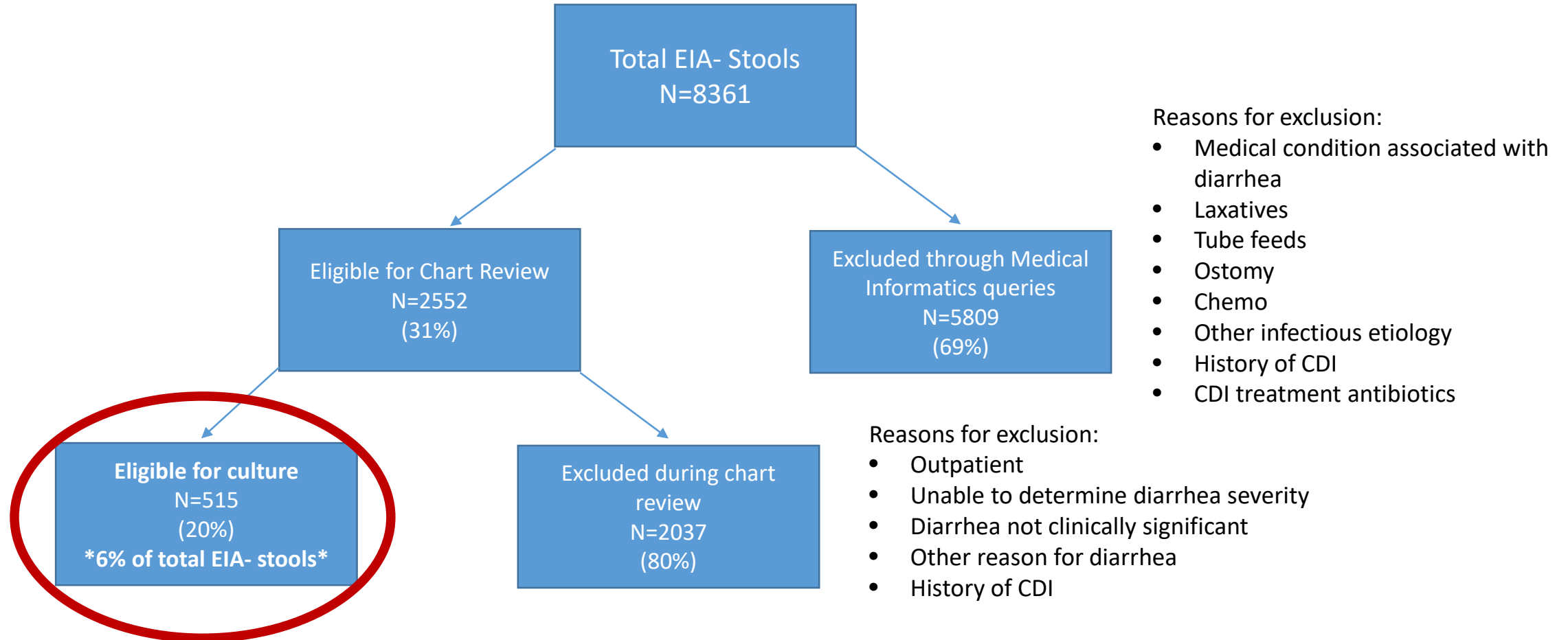
# IDSA/SHEA Guidelines



# Will Limiting Testing to the “Ideal” World Limit False Positive NAATs for CDI?

- 2 years of data: 8,931 testing episodes
  - 8,361 toxin EIA-
  - 570 toxin EIA+
- Patients with
  - Clinically significant diarrhea ( $\geq 3$  diarrheal BM/d or diarrhea plus abd pain)
  - No alternate explanation for diarrhea (e.g. laxatives, tube feeds, colostomy, etc)
  - No recent CDI
  - For toxin EIA-, no treatment for CDI
  - Inpatient

# Toxin EIA- Stools



Toxigenic culture positive: N=63 (12.2%)

# False Positives in Ideal World Testing Scenario

- Same process for EIA+ specimens
  - 107 (20%) met criteria
- 170 total that were EIA+ (107) or EIA- / toxigenic culture+ (63)
  - Most EIA- / toxigenic culture+ would be NAAT+
- If NAAT used:  $63/170 = 37\%$  false positives for CDI
  - Similar to what is seen in real world

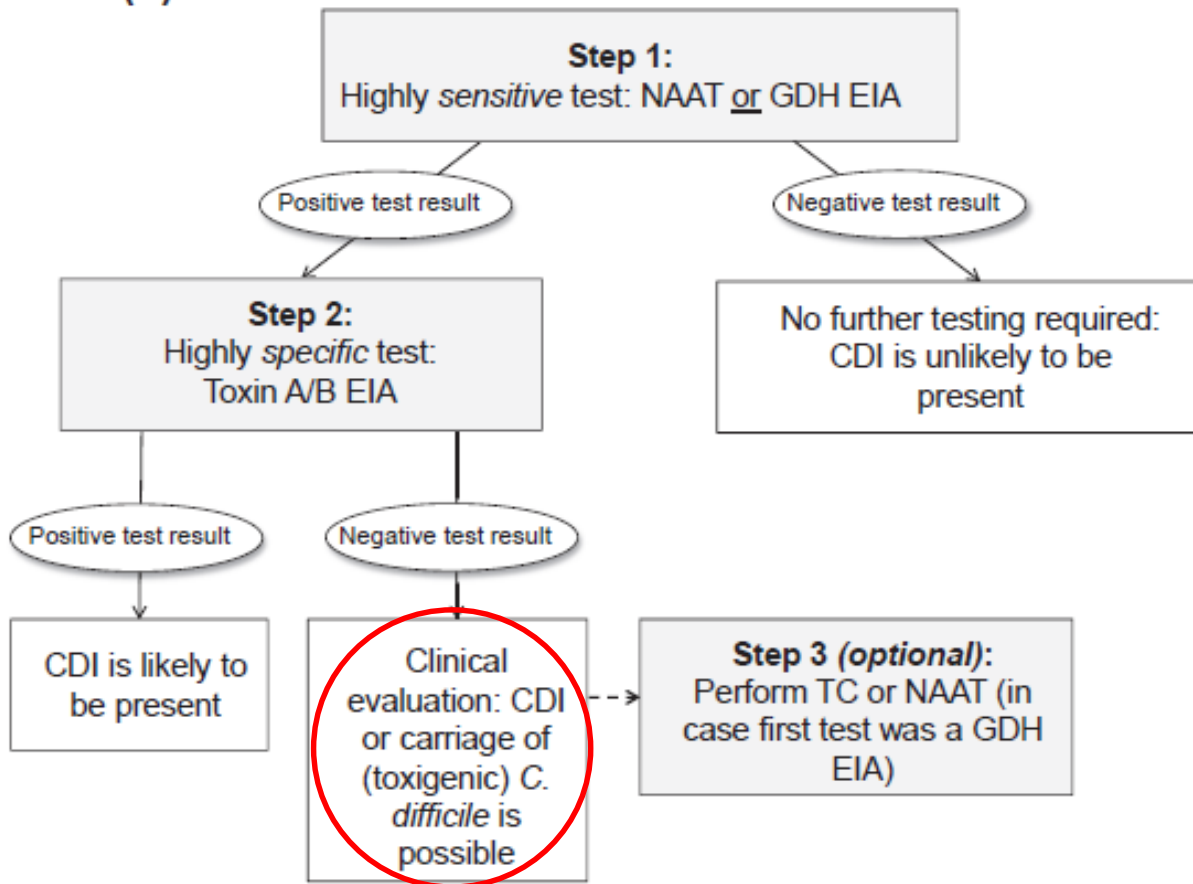
# Different Testing Strategies and False Positives and False Negatives

- Hypothetical scenarios
  - Toxin EIA: sensitivity 85%, specificity 97%
  - NAAT: sensitivity 99%, specificity 89% (CDI)
  - GDH: sensitivity 99% (ignore specificity)
  - Test 1,000 patients, 100 with CDI (10% prevalence)

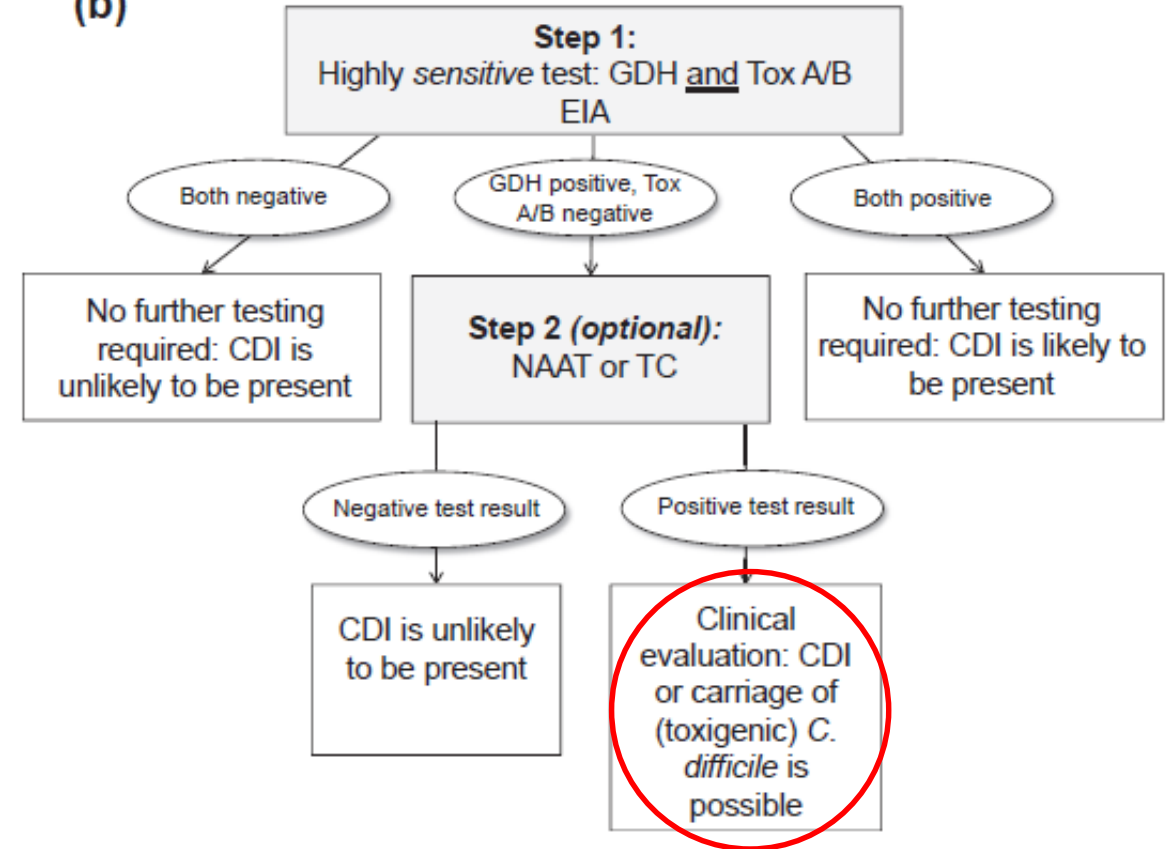
Testing strategy	True positives	False positives	False negatives
Toxin EIA only	85	27	15
NAAT only	99	99	1
NAAT or GDH (+) then Toxin EIA	84	3	14

# European Recommendations: Importance of Toxin Detection and Clinical Evaluation

(a)



(b)





# Conclusions

- NAAT+ alone poorly predictive of CDI
  - 40%-60% more positive tests
  - NAAT+ alone more similar to NAAT- than toxin+
    - Presentation and outcomes
- Most hospitalized patients have potential alternate explanation for diarrhea
  - <10% meet IDSA/SHEA criteria for NAAT alone testing
    - Asymptomatic colonization still occurs among those who meet criteria
- Toxin testing remains important, but room for improvement
  - Clinical judgement for NAAT+ / toxin EIA-
  - Future: ultrasensitive toxin testing, metabolomics, immune profiling