Clostridium difficile-Associated Diarrhea

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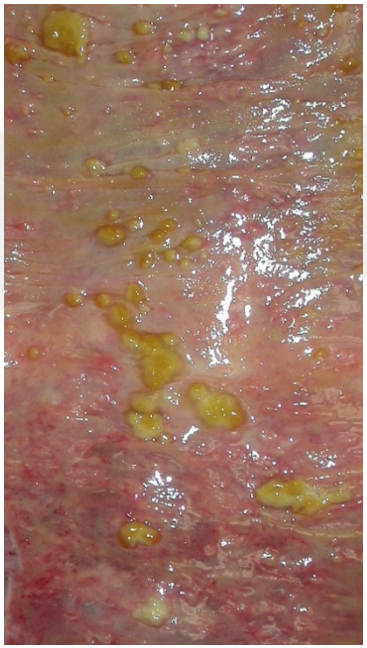
Case

- 73 year-old female, diabetes, peripheral vascular disease
- Below-knee amputation
- Post-operative day 2
  - Low-grade fever, diffuse abdominal pain, cramps
  - Leukocytosis
- Post-operative day 5
  - *C. difficile* toxin
- Antibiotic therapy
- Post-operative day 7
  - Respiratory/cardiovascular failure, mental status changes, patient expires
- Postmortem
  - Pseudomembranous colitis (entire colon and rectum)



Clostridium difficile

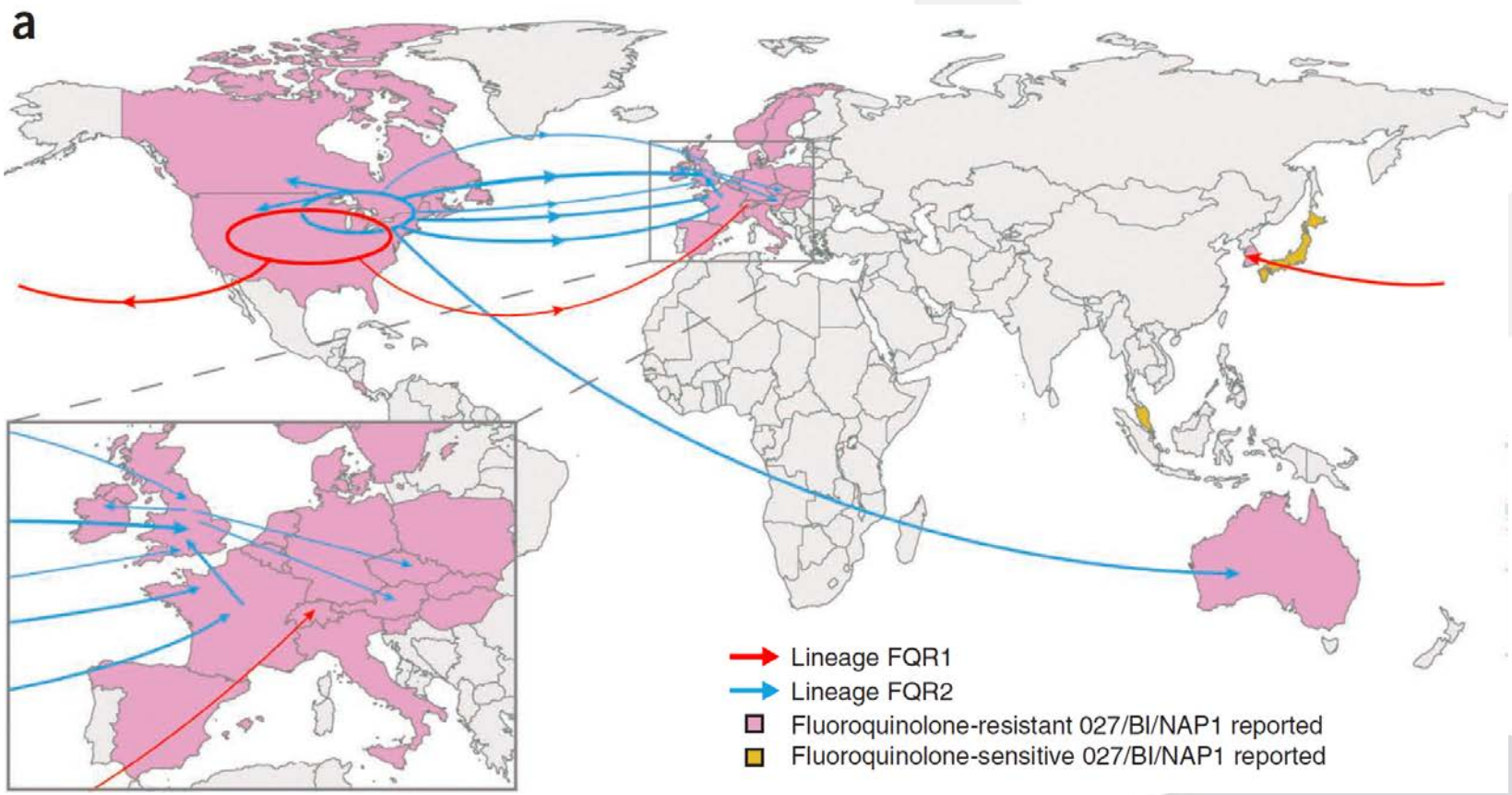
- Anaerobic, gram-positive rod
- Toxigenic vs. non-toxigenic strains
- Spores
  - Resistant to heat, acid, antibiotic
- Vegetative forms
  - Toxin producing

Toxins

- Potent exotoxins
  - Receptors on intestinal epithelial cells
  - Mucosal injury, fluid secretion, inflammation -> colitis + watery diarrhea
  - Toxin A ("enterotoxin")
  - Toxin B ("cytotoxin")
- Stool toxin levels ~ disease severity
- PaLoc
  - Includes *tcdA*, *tdcB*
- Most strains: toxins A and B
  - Variant toxin expression

Nature. 2010 Oct 7;467:711
Nature 2009; 458:1176.
J Clin Microbiol 2000; 38:1696
J Clin Microbiol 2002;40:2079
Ann Intern Med 2001;135:434

NAP1/BI/027 Strain



Nat Genet;45(1):109-13

Brief History of CDI

- **1935**
  - Anaerobic, GPR, 'normal flora of neonates'
- **1978**
  - *C. difficile* as common cause of antibiotic-associated colitis
  - Clindamycin
- **1989-1992**
  - Outbreaks with **J strain**, highly **clindamycin R**
- Association with other antibiotics
  - Penicillins, cephalosporins, fluoroquinolones...
- **Since ~2002**
  - **NAP1/BI/027** (increased incidence/severity)
  - **Fluoroquinolone R**
  - Global, community associated, younger patients
- Any antimicrobial

Gastroenterology;75(5):778-82
Am J Clin Pathol;137(1):10-5
CMAJ;171(5):466-72
Clin Infect Dis;53(7):e81-90

Pathogenesis

- Fecal-oral colonization (spores)
- Antimicrobial therapy
- Disruption of normal intestinal flora
- Expansion of *C. difficile*
- Exotoxins A and B
- Intestinal epithelial disruption, ulcer
- Release: serum proteins, mucus, inflammatory cells
- Pseudomembranes
- Antibiotic-associated pseudomembranous colitis

Risk Factors

- Antibiotic use
- Hospitalization
- Advanced age
- Severe illness
  - Gastrointestinal surgery
  - Cancer chemotherapy
  - Hematopoietic stem cell transplantation
- Gastric acid suppression (PPI)
- Enteral feeding
- None (children, postpartum women)

Curr Opin Infect Dis, 25, 405-411
N Engl J Med;365(18):1693-703

Symptoms

- **Case definition of CDI**
  - Symptoms (diarrhea x3/24h)
  - Stool test or pseudomembranous colitis
- **Colitis** with **watery diarrhea**
  - Asymptomatic, pseudomembranous colitis, toxic megacolon
  - Abdominal pain, low-grade fever, leukocytosis (~15,000)
- Onset classically during/after antibiotic therapy
- Median onset 2-3 days after colonization
- Nosocomial vs. community acquired
- Recurrence in 10-25% (relapse > re-infection)

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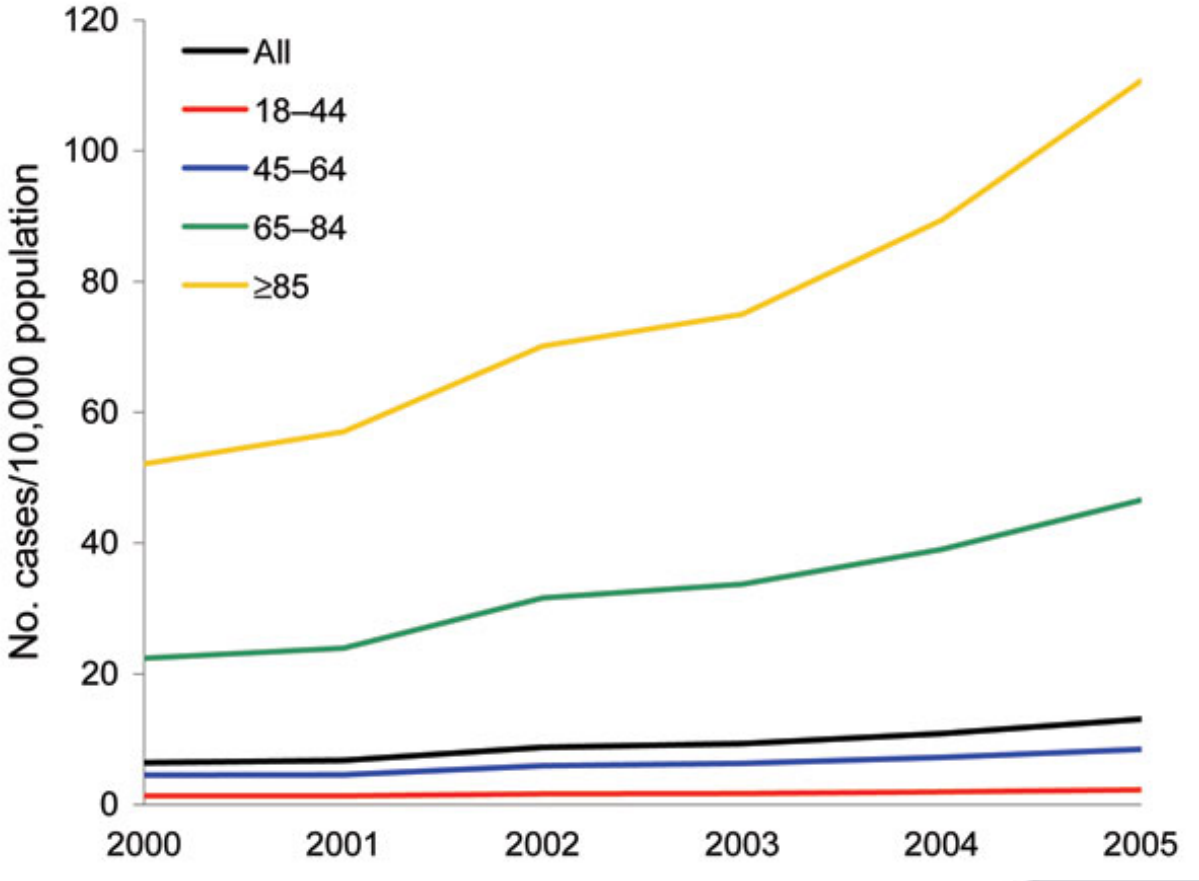
Adult *C. difficile*–related Hospitalizations



Emerging Infectious Diseases;14:949

Nosocomial

- Common healthcare-associated infection (HAI)
  - ~1% of hospitalized patients
- Increasing incidence, severity
- Carrier rate
  - Low in healthy adults
  - Up to 20% (hospitalized adults), 50% (long-term care)
  - Up to 50% in infants
  - Asymptomatic shedding
- Highly transmissible
  - Fomites (hands, clothing, stethoscopes)
  - Can be aerosolized

Am J Infect Control. 2009 May;37(4):263-70
Curr Opin Infect Dis, 25, 405-411
Infect Control Hosp Epidemiol;31(5):431-55

Prevention of HAI with *C. difficile*

- Effective
  - 20% reduction over ~21 months in 71 hospitals
- Contact isolation
  - Gloves, gowns
  - Hand hygiene
  - Soap, water during ourbreaks/increased prevalence
  - Individual rooms or cohorting
- Environmental cleaning (sporocidal)
- Antimicrobial stewardship
- Screening for carriers not recommended

Infect Control Hosp Epidemiol;31(5):431-55
MMWR / March 9, 2012 / Vol. 61 / No. 9

Community-Associated

- Increasing incidence
- Younger, healthier, less likely on antibiotics
- Less common severe infections
- Emerging sources
  - Food products
  - Domestic animals

Clinical Infectious Diseases 2010; 51(5):577

Treatment

- **Antibiotic**
  - Metronidazole (PO, IV)
  - Vancomycin (PO)
  - Fidaxomycin (PO)
- **Under investigation**
  - IVIG, monoclonal anti-toxin antibodies
  - Intestinal microbiota transplantation
  - Vaccination

Curr Opin Infect Dis, 25, 405-411
Clin Infect Dis. 2011 Nov;53(10):994-1002

Diagnosis

- Indication for testing
  - 2 days of significant **diarrhea** (3+ stools/d)
  - 1 day of 10-15 stools/d, fever/nocturnal diarrhea
  - Exception: ileus
- Recurrence: same as initial episode
- No indication
  - Asymptomatic, i.e. **formed stool specimens**, unless **ileus** is suspected
  - No test of cure

Infect Control Hosp Epidemiol 2010; 31(5)

Laboratory Testing

- **Toxigenic culture**
  - Stool -> culture -> isolate -> cytotoxin detection
- **Cytotoxin assay**
  - Stool -> cytotoxin detection
- **Toxin A/B EIA**
  - Stool -> toxin EIA
- **GDH + toxin detection**
  - Stool -> GDH -> if positive: toxin detection
- **NAAT**
  - Stool -> NAAT

Cytotoxin Assay

- Fresh stool sample
  - Dilute, buffer, filter
- Inoculation of cultured cells
  - Human (foreskin) fibroblasts
- Incubate
- Cytotoxic effect (rounding)
  - Filtered sample (cytotoxic)
  - Preincubated with neutralizing antibody (normal)
- Sensitivity 67%-100%
- TAT ~ 24-48h

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Toxigenic Culture

- Anaerobic stool culture
  - CCFA agar (cycloserine, cefoxitin, fructose)
  - No distinction: toxigenic vs. non-toxigenic strains
- Testing of *C. difficile* isolates for toxin production
  - Isolate suspension
  - Cytotoxicity assay
- Most sensitive
- TAT ~ 2-3 days, up to 9 days
- Surveillance (provides isolates)

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Toxin A/B EIA

- Direct detection of toxin A/B
  - Filtered stool sample
- Toxin A-only assays not recommended
  - Variant and/or toxin A-non-expressing strains
- Rapid TAT
- **Insufficient sensitivity**
  - No significant improvement with early repeat testing
  - Performance varies between kits
- Sensitivity 63%–94%

J Clin Microbiol; 46:3686
Clin Infect Dis 2011; 52:1451–7
Infect Control Hosp Epidemiol.;31(5):431-55

GDH Screen + Toxin Detection

- EIA for glutamate dehydrogenase (GDH)
  - Produced by all *C. difficile* strains
  - Sensitivity 85%-95%
- Screening as part of multi-step algorithms
  - Detects toxigenic and non-toxigenic *C. difficile*
  - Separate conformation of toxin production
  - E.g. cytotoxicity assay, NAAT, toxin A/B EIA…

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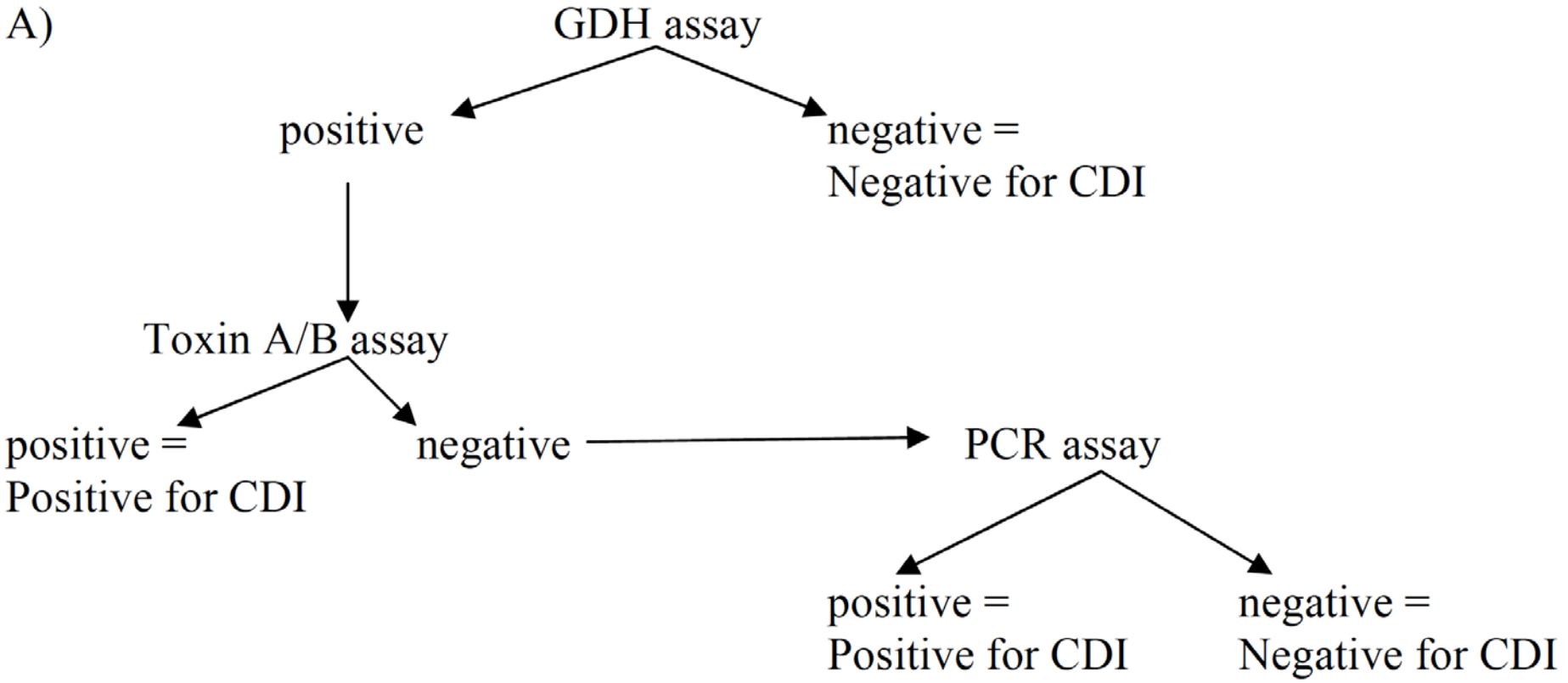
Example Algorithm



ASM: A Practical Guidance Document for C. difficile Toxin Laboratory Testing, 8/24/2010

Nucleic Acid Amplification Tests

- Methodologies
  - Real-time PCR
  - Isothermal amplification (LAMP and others)
- High sensitivity
- High specificity
- Rapid
  - Treatment, infection control
- More expensive, less experience
- Limited PPV with prevalence <10%

Clin Infect Dis. 2011 Oct;53(7):e81-90

FDA-Cleared NAAT

Assay	Manufacturer
BD GeneOhm C. diff Assay	BD Diagnostics, Inc.
Illumigene C. difficile Assay	Meridian Bioscience, Inc.
Portrait Toxigenic C. difficile Assay	Great Basin Scientific, Inc.
ProGastro Cd Assay	Gen-Probe, Inc.
Simplexa C. difficile Universal Direct Assay	Quest Diagnostics
Verigene C. Difficile Nucleic acid Test	Nanosphere, Inc.
Xpert C. difficile	Cepheid
Xpert C. difficile/Epi	Cepheid

http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm330711.htm#microbial
FDA-Cleared/Approved Molecular Diagnostic Tests (AMP, July 5th, www.amp.org/FDATable/FDATable.doc)

Considerations

- Assay comparison is difficult
  - No single accepted reference test
  - Lack of standardized protocols for reference tests
  - Regional differences in strain types
- Repeat testing
  - Not indicates (7 days commonly used)
- Test of cure
  - Not indicated (2-4 weeks commonly used)

Infect Control Hosp Epidemiol. 2010 May;31(5):431-55

Laboratory Testing - Summary

- **Optimal strategy has not been determined**
- **Reference methods**
  - Cytotoxicity (tissue culture) assay: specific, slow, requires cell culture, not standardized
  - Toxigenic culture: most sensitive, provides isolate, slow, requires cell culture, not standardized
- **Frequently used**
  - Toxin A/B EIA: rapid, inexpensive, insensitive
  - **GDH + toxin A/B EIA**: rapid, high negative predictive value, sensitivity variable (GDH) and limited by toxin A/B EIA
  - **NAAT**: rapid, sensitive, more data needed

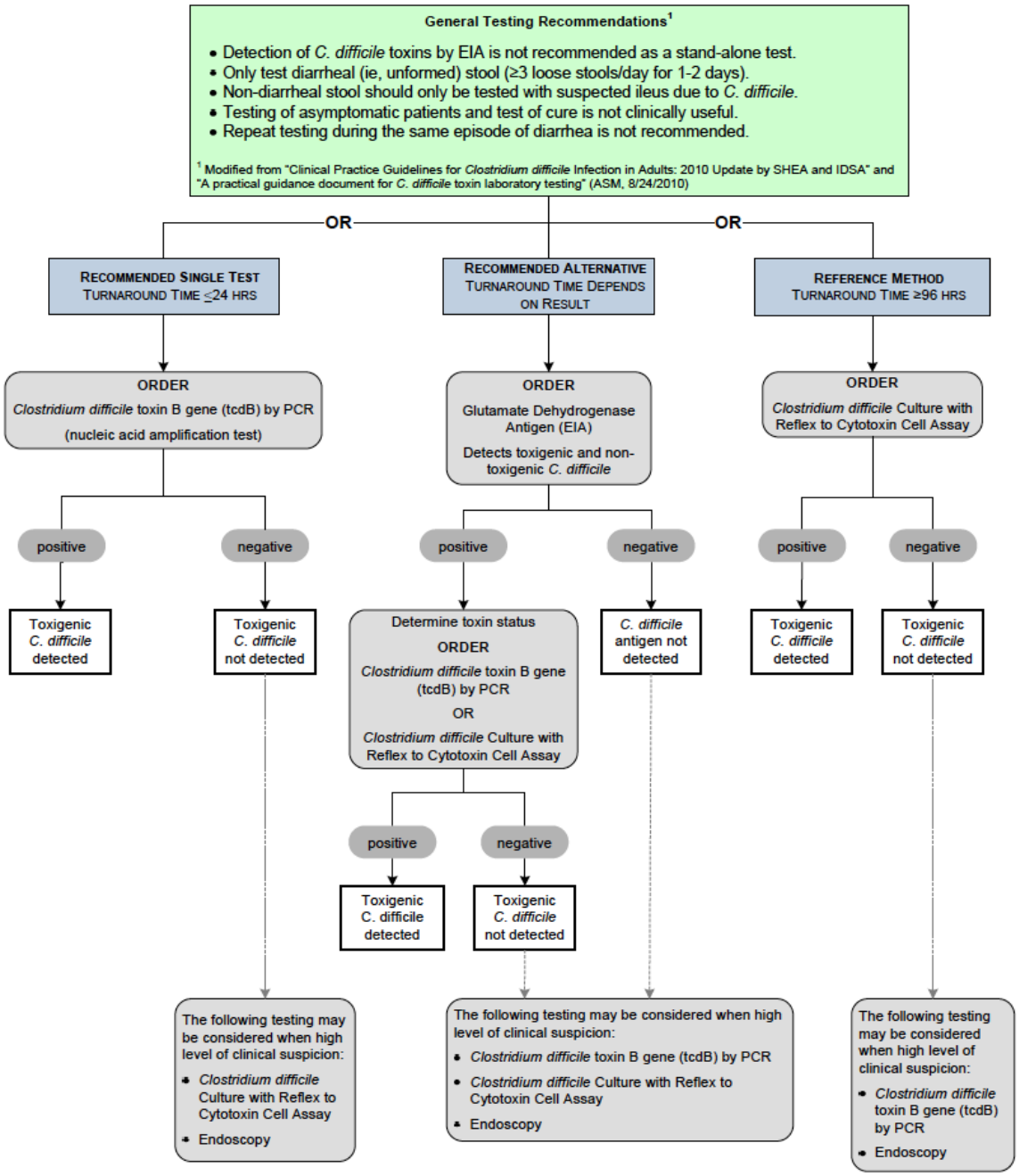
Infect Control Hosp Epidemiol. 2010 May;31(5):431-55

Test and Algorithm Comparison

Parameter[a]	Test(s)				
	EIA only	GDH + EIA	GDH + EIA + cytotoxin[b]	GDH + Xpert[c]	Xpert only[d]
No. of specimens	432	432	431	432	428
Sensitivity	58.3 (42/72)	55.6 (40/72)	83.1 (59/71)	86.1 (62/72)	94.4% (68/72)
Specificity	94.7 (341/360)	98.3 (354/360)	96.7 (348/360)	97.8 (352/360)	96.3 (343/356)
Accuracy	88.7 (383/432)	91.2 (394/432)	94.4 (407/431)	95.8 (414/432)	96.0 (411/428)
PPV	68.9 (42/61)	87.0 (40/46)	83.1 (59/71)	88.6 (62/70)	84.0 (68/81)
NPV	91.9 (341/371)	91.7 (354/386)	96.7 (348/360)	97.2 (352/362)	98.8 (343/347)

Assay[a]	Result	Comparison to CYT and DPCR results[b]					
		No. of specimens		% Sensitivity (95% CI)	% Specificity (95% CI)	% PPV (95% CI)	% NPV (95% CI)
		Either positive[c]	Negative				
CYT (tissue culture)	Positive	47	0	58.8 (47.8–68.9)	100	100	94.9 (93.0–96.4)
	Negative	33	619				
GDH-Q	Positive	69	45	86.3 (76.9–92.3)	92.7 (90.4–94.5)	60.5 (51.3–69.0)	98.1 (96.6–99.0)
	Negative	11	574				
Two-step GDH-Q/AB-Q	Positive	26	2[d]	32.5 (23.2–43.4)	99.7 (98.8–100)	92.9 (76.3–99.1)	92.0 (89.6–93.8)
	Negative	54	617				
Three-step GDH-Q/AB-Q/DPCR	Positive	67	2[d]	83.8 (74.0–90.4)	99.7 (98.8–100)	97.1 (89.4–99.8)	97.9 (96.5–98.8)
	Negative	13	617				

J Clin Microbiol; 48:124
J Clin Microbiol; 48:889



http://www.arupconsult.com/Algorithms/CDAD.pdf

SHEA, IDSA Guidelines

- Test only diarrheal stool (exception: ileus)
- Don’t test if asymptomatic (test of cure)
- Culture most sensitive, not clinically practical
  - Reference test if performed by experienced lab
- Toxin A/B EIA suboptimal (rapid, less sensitive)
- GDH screening + cytotoxicity/culture
  - Sensitivity varies by kit, interim recommendation
- NAAT rapid, sensitive, specific
  - More data on utility necessary
- No repeat testing during same episode

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Thank you for your attention