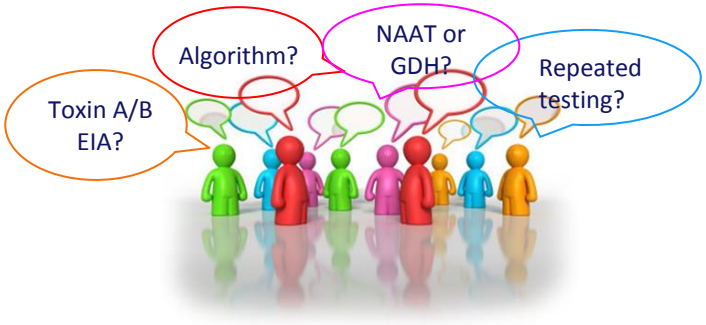
LEIDS UNIVERSITAIR MEDISCH CENTRUM

Diagnostics of *Clostridium difficile* infection (CDI)



Clostridioides difficile **(Paul Lawson et al, Anaerobe 2016: August)**

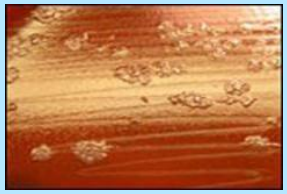
M.J.T. Crobach and E.J. Kuijper

ESCMID EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

Clostridium difficile infection (CDI): a diagnostic problem

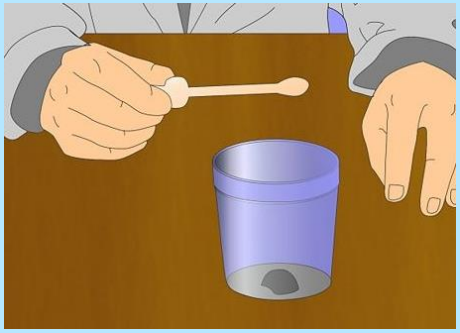
Reference test: cell cytotoxicity assay (CCNA)

Reference test: toxigenic culture (TC)



toxin detection

Rapid test: glutamate dehydrogenase enzyme immunoassay (GDH EIA)



Rapid test: toxin A/B enzyme immunoassay (tox A/B EIA)

Rapid test: nucleic acid amplification test (NAAT)

Alternative: a beagle's superior olfactory sensitivity

CDI Diagnostics

	Advantages	Drawbacks
Reference methods CCNA TC	• High positive predictive value (PPV)	• Slow • Laborious • Expensive
Rapid tests NAAT GDH EIA Tox A/B EIA	• Rapid • Easily performed • Inexpensive • High negative predictive value (NPV)	• Low PPV

$$\text{PPV} = \frac{\text{true positives}}{\text{true positives} + \text{false positives}}$$

$$\text{NPV} = \frac{\text{true negatives}}{\text{true negatives} + \text{false negatives}}$$

Methods

1. Meta-analysis of commercially available laboratory tests
   - Studies from the previous meta-analysis and studies published since 2009 in the English language
   - Included studies had to:
     - Compare GDH EIA, Tox A/B EIA or NAAT to CCNA or TC
     - Test all samples with the reference test
   - Numbers of true positives, true negatives, false negatives and false positives were extracted from each study
   - Logistic regression was used to calculated pooled estimates of sensitivity and specificity
2. Review of recent literature and guidelines
3. Based on (1) and (2), recommendations were formulated

Included assays

Assay type	Assay	Manufacturer	Target(s)
Well-type EIA GDH	C. diff Chek-60	Techlab	GDH
Membrane-type EIA GDH	C. diff Quik Chek	Techlab	GDH
	Immunocard C. difficile	Meridian	GDH
	Quik Chek Complete- GDH*	Techlab	GDH
Well-type EIA toxins A&B	Premier tox A/B	Meridian	Toxins A and B
	Remel ProSpecT	Oxoid	Toxins A and B
	Ridascreen tox A/B	Biopharm	Toxins A and B
	Clostridium diffiicle Tox A/B II	Techlab	Toxins A and B
	Vidas CDAB	Biomérieux	Toxins A and B
Membrane-type EIA toxins A&B	Immunocard tox A/B	Meridian	Toxins A and B
	Quik Chek Complete- tox A/B*	Techlab	Toxins A and B
	Tox A/B Quik Chek	Techlab	Toxins A and B
	Xpect	Oxoid	Toxins A and B
NAAT	Advansure CD	LG Life Sciences	tcdA. tcdB
	Amplivue	Quidel	tcdA
	BD GeneOhm	Becton, Dickinson	tcdB
	BD Max Cdiff	Becton, Dickinson	tcdB
	GenomEra	Abacus Diagnostica	tcdB
	Illumigene	Meridian	tcdA
	Portrait	Great Basin	tcdB
	Prodesse ProGastro Cd Assay	Hologic Gen-Probe	tcdB
	Seeplex Diarrhea ACE Detection	Seegene	tcdB
	Verigene	Nanosphere	tcdA, tcdB, cdt*, tcdC deletion
	Xpert C. difficile	Cepheid	tcdB, cdt, tcdC deletion nt 117

Meta-analysis results

Type	Compared to CCNA			Compared to TC		
	N studies	Sensitivity (95% CI)	Specificity (95% CI)	N studies	Sensitivity (95% CI)	Specificity (95% CI)
EIA GDH						
total	13	0.94 (0.89-0.97)	0.90 (0.89-0.92)	8	0.96 (0.86-0.99)	0.96 (0.91-0.98)
well-type	*6*	*0.94 (0.91-0.96)*	*0.91 (0.89-0.92)*	*1*	*0.94 (0.93-0.96)*	*0.94 (0.94-0.95)*
membrane-type	*7*	*0.98 (0.78-1.00)*	*0.90 (0.87-0.93)*	*7*	*0.97 (0.84-1.00)*	*0.96 (0.90-0.99)*
EIA Tox A/B						
total	27	0.83 (0.76-0.88)	0.99 (0.98-0.99)	29	0.57 (0.51-0.63)	0.99 (0.98-0.99)
well-type	*18*	*0.85 (0.77-0.91)*	*0.98 (0.96-0.99)*	*16*	*0.60 (0.52-0.68)*	*0.98 (0.97-0.99)*
membane-type	*9*	*0.79 (0.66-0.88)*	*0.99 (0.98-0.99)*	*13*	*0.53 (0.45-0.61)*	*0.99 (0.97-1.00)*
NAAT	14	0.96 (0.93-0.98)	0.94 (0.93-0.95)	32	0.95 (0.92-0.97)	0.98 (0.97-0.99)

Sensitivity of Tox A/B EIAs not as low as reported earlier!

Arriving at recommendations…

- Which rapid test reliably detects true CDI cases?
  - Free toxin detection indicates a true or more severe CDI case
  - Test has to have adequate positive and negative predictive value in the tested population

	GDH EIA	Tox A/B EIA	NAAT
Positive predictive value (%)	33.1	81.4	45.7
Negative predictive value (%)	99.7	99.1	99.8
Free toxin detection	No	Yes	No

Endemic situation, CDI prevalence 5% in tested population

- No single test!

NAAT – Tox A/B EIA algorithm

N=10.000
Prevalence CDI 5%

NAAT: sensitivity 96% , specificity 94%

Negative test
CDI -
N= 8930

TN

Positive test
N= 1050

Negative test
CDI +
N= 20

FN

Tox A/B EIA: sensitivity 83%, specificity 99%

Negative test
CDI -
N= 564

TN

Positive test
CDI +
N= 398

TP

Positive test
CDI -
N= 6

FP

Negative test
CDI +
N= 82

FN

TP= 4.0%, TN= 94.9%, FP= 0.06%, FN= 1.0%

GDH EIA – Tox A/B EIA algorithm

N=10.000
Prevalence CDI 5%

GDH EIA: sensitivity 94%, specificity 90%

TN — Negative test CDI - N= 8550

Positive test N= 1420

Negative test CDI + N= 30 — FN

Tox A/B EIA: sensitivity 83%, specificity 99%

TN — Negative test CDI - N= 940

Positive test CDI + N= 390 — TP

Positive test CDI - N= 10 — FP

Negative test CDI + N= 80 — FN

TP= 3.9%, TN= 94.9%, FP= 0.1%, FN= 1.1%

ESCMID Guidelines 2016

European Society of Clinical Microbiology and Infectious Diseases: update of the diagnostic guidance document for *Clostridium difficile* infection

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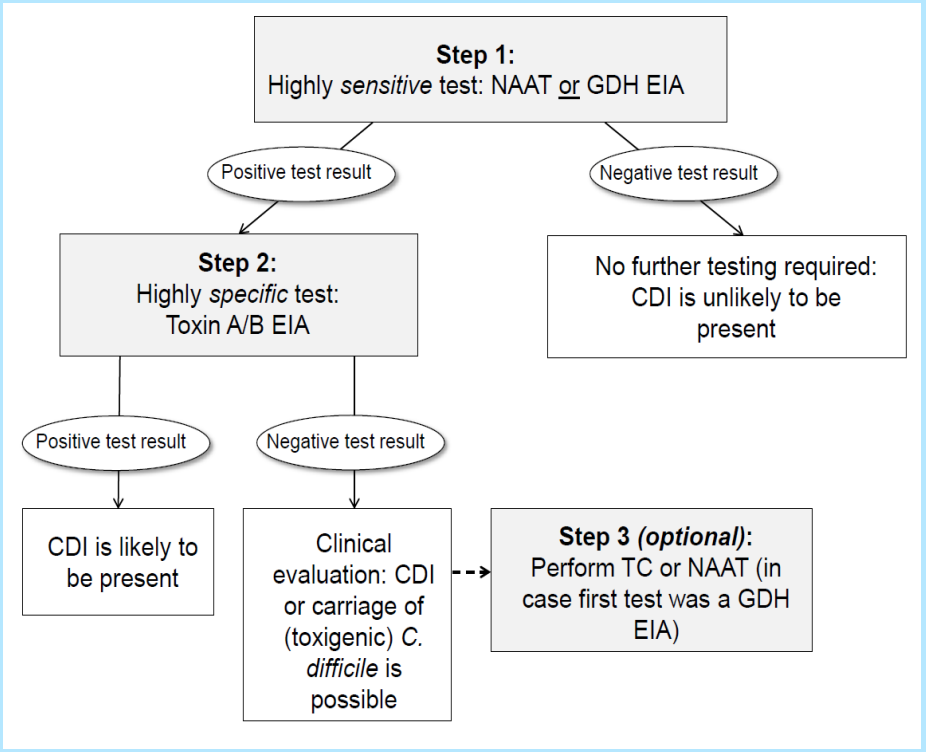
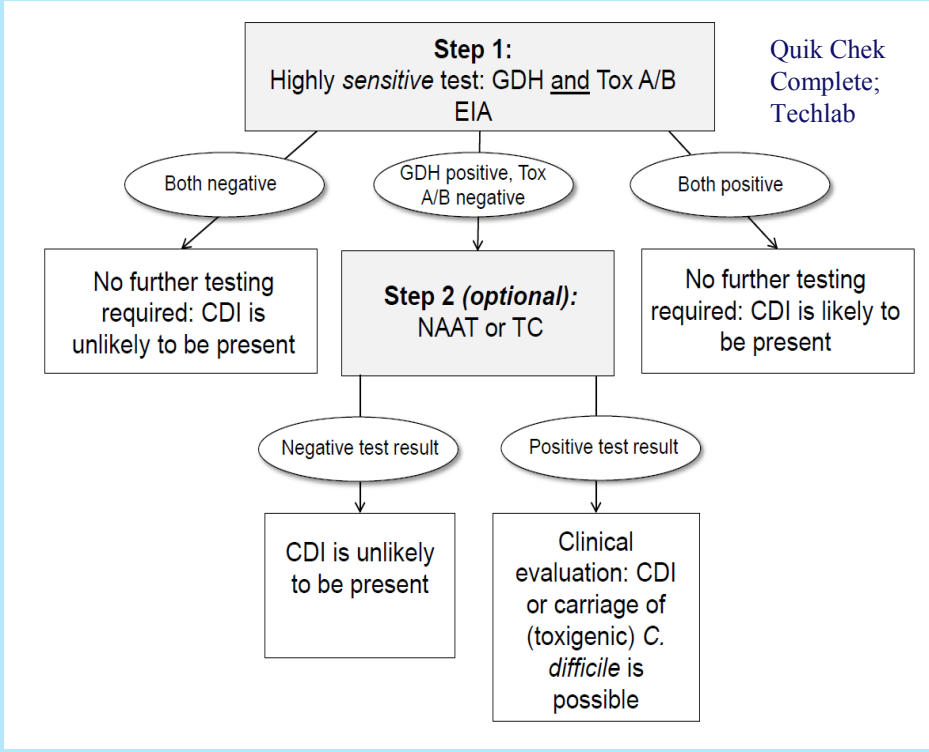
"Two step algorithm with free toxin detection in stools"





Additional recommendations

- CDI testing should not be limited to samples with a physician's request
- Repeated testing during the same diarrheal episode is not recommended in an *endemic* situation
- Repeated testing after a first negative sample during the same diarrheal episode may be useful in cases with ongoing clinical suspicion during an *epidemic* situation
- In case of outbreak situations, we recommend to perform toxigenic culture and molecular typing of recovered isolates

Optimal diagnostics

Categorization of CDI diagnostics	CDI diagnostic algorithm		
	First test	Second test	Optional third test
ESCMID-recommended	NAAT	Toxin A/B EIA	N/A
	GDH EIA	Toxin A/B EIA	NAAT or toxigenic culture
	GDH and Tox A/B EIA	NAAT or toxigenic culture*	N/A
Not recommended	All other algorithms		