DIAGNOSIS OF *CLOSTRIDIUM DIFFICILE* INFECTIONS: WHEN and HOW?

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Paris, France
# Burden of CDI in Europe and US

<table>
<thead>
<tr>
<th>US</th>
<th>Europe³</th>
</tr>
</thead>
<tbody>
<tr>
<td>- 453,000 CDI/year¹</td>
<td>- 124,000 CDI/year</td>
</tr>
<tr>
<td>- 29,500 deaths</td>
<td>- 9% mortality (direct or indirect)</td>
</tr>
<tr>
<td>- 1st agent responsible for HAI (12.5%)²</td>
<td>- 8th agent responsible for HAI (5.4%)</td>
</tr>
<tr>
<td>- Urgent threat (CDC)</td>
<td></td>
</tr>
</tbody>
</table>

The changing epidemiology of CDI

- The incidence of CDI has increased in many countries worldwide 1–4, 6-7
  - Dissemination of the hypervirulent NAP1/027/BI clone
  - Better awareness of CDI from physicians
  - More sensitive methods for the diagnosis (PCR)
  - In some countries (e.g. the UK) the incidence of CDI and prevalence of the hypervirulent strain NAP1/027BI have decreased over the last few years5

5. Health Protection Agency. 2011;
Association between testing rates and CDI incidence or prevalence of 027 across Europe

48-fold variation was noted in country specific testing rates

An inverse correlation was noted between the rate of testing and prevalence of ribotype 027

Davies, Lancet Inf. Dis 2014, 14, 1208-19
Underdiagnosis of *C. difficile* across Europe: the EUCLID study

- Prospective point prevalence study
- 482 HCF from 20 countries
- 7297 stool samples
  - Standardized testing of CDI: GDH+ Toxins A/B (Alere)
  - Comparison with local testing
- 641 (8.7%) positive samples
- Underdiagnosis: 148 (23%) of samples positive for CD were not diagnosed by participating labs due to a lack of clinical suspicion
- Misdiagnosis: 68 (1.5%) of false negative results

Davies K. Lancet Inf. Dis 2014, 14, 1208-19
Molecular epidemiology of CD

Adapted from Lessa, NEJM 2015, 372, 825

Adapted from Davies KA, Lancet Infect Dis 2014;14: 1208–19
Davies KA, eurosurveillance, 2016
Cases of community-acquired (CA)-CDI are increasingly common

- 2541 diarrheal stools (prescribed by GP)\(^1\)
  - 1.81 % positive for toxins
  - 3.27 % positive for toxigenic strains
  - 12.9% were requested by GP \(\rightarrow\) 56% CDI detected

- Performance of algorithm for testing diarrheal samples from patients in general practice

<table>
<thead>
<tr>
<th>Test algorithm in diarrheal samples from the community</th>
<th>Setting</th>
<th>Patients tested (% of all unformed stools)</th>
<th>Positive results (% of all tested samples)</th>
<th>Detection of CDI (% of all tested positives)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 65 y, after AB use or hospitalization</td>
<td>UK 2012</td>
<td>31</td>
<td>3.5</td>
<td>72</td>
</tr>
<tr>
<td>≥ 65 years OR previous AB OR previous hospitalization</td>
<td>France</td>
<td>29</td>
<td>2.6</td>
<td>70</td>
</tr>
</tbody>
</table>

2. Barbut, CMI 2019 in press
Current challenges for CDI diagnosis

• Diagnosis has changed over the last 10 years
• Rapid and accurate diagnosis is crucial for:
  – Patient management
  – Prevention of nosocomial transmission
  – Epidemiology of the disease
• Ideal criteria for a diagnostic method are:
  – Specificity
  – Sensitivity
  – Rapid turnaround time
  – Cost-effectiveness
  – Technical simplicity (minimum hands on time)
Guidelines

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


Clinical Practice Guidelines for *Clostridium difficile* Infection in Adults and Children: 2017 Update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA)

L. Clifford McDonald, Dale N. Gerding, Stuart Johnson, Johan S. Bakken, Karen C. Carroll, Susan E. Coffin, Erik R. Dubberke, Kevin W. Garey, Carolyn V. Gould, Ciaran Kelly, Vivian Loo, Julia Shaklee Sammons, Thomas J. Sandora, and Mark H. Wilcox
General considerations

1. Only diarrheic stools should be processed
   - Laboratory definition:
     - Stool taking the shape of the container
     - Aspect 5, 6, 7 on Bristol scale

2. Do not test stool samples from neonates < 3 y.
   - Asymptomatic colonisation is frequent in neonates (6 m - 1 y).
   - The carriage rate drops progressively
   - With a physician’s request only

General considerations

3- Repeated diagnostic testing is not useful

- Repeat testing is a frequent and costly practice
- Stool samples tested twice (<7 days) following 13.7% of EIA, 12.4% of PCR tests\(^1\)
- The frequency of test results converted from negative to positive (diagnostic gain) following repeat testing is low, whatever the method used

<table>
<thead>
<tr>
<th>Authors</th>
<th>Technique</th>
<th>Patients (n)</th>
<th>Diagnostic gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aichinger et al. 2008(^1)</td>
<td>EIA A + B, PCR</td>
<td>5,788</td>
<td>1.9% (7 days)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2,827</td>
<td>1.7% (7 days)</td>
</tr>
<tr>
<td>Renshaw et al. 1996(^2)</td>
<td>CTA</td>
<td>2,009</td>
<td>1%</td>
</tr>
</tbody>
</table>

- Repeat testing may lead to false-positive result (lack of specificity)\(^3\)


EIA, enzyme immunoassay; CTA, cytotoxicity assay; PCR, polymerase chain reaction
4. Test-of-cure is not recommended\(^1\)

- Spores detectable in 7% (2/28) of patients at the end of treatment for CDI\(^2\)
- Positive cultures found in 56% (15/27) of patients 1–4 weeks after therapy\(^2\)

**Percentage of stool cultures positive for *C. difficile* among 52 patients with CDI\(^2\)**

5. Stool samples should be taken prior initiation of CDI treatment

- Prospective study to determine the time to conversion of CDI test result
- 51 patients with CDI
- For PCR, 14%, 35%, and 45% of positive CDI tests converted to negative after 1, 2, and 3 days of treatment, respectively
- Increased age and infection with NAP1 strains were associated with persistent positive PCR results.

Sunkesula, CID 2013, 57, 494-500
Reducing inappropriate testing and treatment

- Inappropriate testing may lead to treat asymptomatic carriers
- Dubberke *et al.* (2011)
  - 36% of patients tested for CD did not meet criteria for testing because they did not have clinically significant diarrhea
- Kundrapu *et al.* (Idweek 2014)
  - 18% did not have clinically significant diarrhea or had a clear alternative explanation for diarrhea (eg laxatives)
- Ongoing education of physicians and nurses and feedback are successful to reduce inappropriate testing

Testing methods: an overview

Stools

Clinical history

Detection of C. difficile
- Glutamate Dehydrogenase (GDH)
- Culture

Detection of ‘free’ toxins
- Cell cytotoxicity Neutralization assay (CCNA)

Detection of toxigenic C. difficile
- EIA for Toxins A+B
- Toxigenic culture
- NAAT (tcdA, tcdB)

EIA, enzyme immunoassay;
GDH, glutamate dehydrogenase;
NAAT: nucleic acid amplification tests
Reference methods: current issues

- The two reference methods are the stool cell cytotoxicity neutralization assay and the toxigenic culture.
- They detect different targets:
  - Free toxin or
  - Presence of a strain with the potential to produce toxins
  - Results are not directly comparable
- Not frequently used in routine practice
- Not standardised
- Slow turnaround time (>48 hours) and time consuming
- Used as ‘gold standard’ for evaluation of other methods

Different methods, different targets

<table>
<thead>
<tr>
<th>Method</th>
<th>Target</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTA</td>
<td>Free toxins in stools</td>
<td>CDI</td>
</tr>
<tr>
<td>EIA for toxins A and B</td>
<td>Presence of C. difficile in stools</td>
<td>Toxigenic strain or not?</td>
</tr>
<tr>
<td>EIA for GDH</td>
<td>Presence of a toxigenic C. difficile strain</td>
<td>CDI or carriage of a toxigenic strain?</td>
</tr>
<tr>
<td>Culture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toxigenic culture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molecular methods (NAAT)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

EIA, enzyme immunoassay; CTA, cytotoxicity assay; TAT, turnaround time

Performances of individual tests compared to gold standards

<table>
<thead>
<tr>
<th>Type</th>
<th>Compared with CCNA</th>
<th>Compared with TC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N studie(s)</td>
<td>Sensitivity (95% CI)</td>
</tr>
<tr>
<td>EIA GDH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>13</td>
<td>0.94 (0.89-0.97)</td>
</tr>
<tr>
<td>well-type</td>
<td>6</td>
<td>0.94 (0.91-0.96)</td>
</tr>
<tr>
<td>membrane-type</td>
<td>7</td>
<td>0.98 (0.78-1.00)</td>
</tr>
<tr>
<td>EIA Tox A/B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>27</td>
<td>0.83 (0.76-0.88)</td>
</tr>
<tr>
<td>well-type</td>
<td>18</td>
<td>0.85 (0.77-0.91)</td>
</tr>
<tr>
<td>membrane-type</td>
<td>9</td>
<td>0.79 (0.66-0.88)</td>
</tr>
<tr>
<td>NAAT</td>
<td>14</td>
<td>0.96 (0.93-0.98)</td>
</tr>
</tbody>
</table>

Crobach M., et al., CMI 2016
Patients with free toxins best correlate with severe outcome

- Prospective cohort study during which stools were tested in parallel by a commercial PCR assay (*tcdB*), and a three-step algorithm GDH and toxins A and B by enzyme immunoassay and cell culture cytotoxicity assay (EIA/CCA)

<table>
<thead>
<tr>
<th>Complications</th>
<th>CDI Cases detected by PCR, but not by EIA/CCA (n = 29)</th>
<th>CDI Cases detected by both PCR and EIA/CCA (n = 56)</th>
<th>( P )-value ( ^a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-day mortality (%)</td>
<td>1 (3)</td>
<td>10 (18)</td>
<td>0.09</td>
</tr>
<tr>
<td>Colectomy (%)</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Admission to intensive care unit (%)</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Readmission for CDI (%)</td>
<td>0 (0)</td>
<td>11 (20)</td>
<td>0.01</td>
</tr>
<tr>
<td>Occurrence of ( \geq 1 ) complication (%)</td>
<td>1 (3)</td>
<td>22 (39)( ^b )</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

 Patients with free toxins best correlate with severe outcome

- Prospective study on 12,420 samples analysed from 10,691 patients
- Presence of toxins was significantly associated with poor clinical outcomes
- The presence of toxigenic *C. difficile* in faeces in the absence of a positive toxin assay was not associated with any significant clinical outcome worse than that of negative samples

<table>
<thead>
<tr>
<th>Group 1: CTA+ (n=435)</th>
<th>Group 2: TC+ CTA− (n=207)</th>
<th>Group 3: CTA− TC− (n=5,880)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mortality</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.6%</td>
<td>9.7%</td>
<td>8.6%</td>
</tr>
<tr>
<td>(p=0.022 vs Gp. 1)</td>
<td>(p&lt;0.001 vs Gp. 1; p=0.53 vs Gp. 2)</td>
<td></td>
</tr>
<tr>
<td><strong>WBC count</strong> (× 10⁹/L)</td>
<td>12.4 ± 8.9</td>
<td>9.9 ± 10.7</td>
</tr>
<tr>
<td></td>
<td>(p&lt;0.001 vs Gp. 1)</td>
<td>(p&lt;0.01 vs Gp. 1 P=0.69 vs Gr2)</td>
</tr>
<tr>
<td><strong>Mean LOS after sample (days)</strong></td>
<td>19.4 ± 25</td>
<td>14.2 ± 22</td>
</tr>
<tr>
<td></td>
<td>(p=0.94 vs Gr1)</td>
<td>(p&lt;0.0001 vs Gr 1 P=0.002 vs Gr2)</td>
</tr>
</tbody>
</table>

CTA, cytotoxicity assay; TC, toxigenic culture; WBC, white blood cell

Patients with free toxins best correlate with severe outcome - US data

- Prospective observational study
- 1416 adults tested for *C. difficile* > 72 h after admission
- Stools were tested by PCR (not reported)) and by EIA for toxins (reported to physicians)

<table>
<thead>
<tr>
<th></th>
<th>PCR+/tox+ (n=131)</th>
<th>PCR+/tox- (n=162)</th>
<th>C. difficile negative (n=1123)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>N stools J1 (median)</td>
<td>5 (3-6)</td>
<td>3 (2-5)</td>
<td>3 (2-5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Complications (%) or death within 30 d</td>
<td>18 (13.7%)</td>
<td>1 (0.6%)</td>
<td>3 (0.3%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD testing between 15 and 30d Positive test for toxins</td>
<td>26 (19.8%)</td>
<td>18 (11.1%)</td>
<td>1076 (9.4%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Treatment within 14 d</td>
<td>131 (100%)</td>
<td>66 (40.7%)</td>
<td>361 (32.1%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leucocytosis &gt; 15 000/µl</td>
<td>54/129 (41.9%)</td>
<td>50/154 (32.5%)</td>
<td>323/1101 (29.3%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Fecal lactoferin (median)</td>
<td>37.7 (8-261)</td>
<td>20.1 (5-50)</td>
<td>7.8 (05-32)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Polage et al., JAMA Inter Med 2015
ESCMID recommended diagnostic algorithm for CDI (2016)

Highly sensitive test
NAAT or GDH EIA

-/+ Highly specific test Toxin A/B EIA

CDI is likely to be absent

GROUND-TOXIN A/B

-/+ CDI is likely to be present

NAAT

CDI is likely to be absent

Clinical evaluation: CDI or carriage of (toxigenic) C. difficile is possible

Clinical evaluation: CDI or carriage of (toxigenic) C. difficile is possible

NAAT or TC (in case first test was a GDH)

CDI is likely to be absent

CDI is likely to be present

Crobach M., et al., CMI 2016
Clinicians and laboratory personnel agree at the institutional level to not submit stool specimens on patients receiving laxatives and to submit stool specimens only from patients with unexplained and new onset ≥ 3 unformed stools in 24 h for testing for CDI.

Yes

NAAT alone OR stool toxin test* as part of a multiple step algorithm (i.e. GDH plus toxin; GDH plus toxin, arbitrated by NAAT; or NAAT plus toxin) rather than a nucleic acid amplification test (NAAT) alone.

No

Stool toxin test* as part of a multiple step algorithm (i.e. GDH plus toxin; GDH plus toxin, arbitrated by NAAT; or NAAT plus toxin) rather than a nucleic acid amplification test (NAAT) alone.

*Approved stool EIA toxin tests vary widely in sensitivity. Laboratories should choose a toxin test with sensitivity in the upper range of sensitivity as reported in the literature [146-149, 156].
Other recent documents

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Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

ESGCD
2018

Narrative review

How to: diagnose infection caused by Clostridium difficile

C. Gateau 1, J. Couturier 1, 2, J. Coia 3, 4, F. Barbut 1, 2, 4, *

1) National Reference Laboratory for C. difficile, Assistance Publique-Hôpitaux de Paris, Hôpital Saint-Antoine, Paris, France
2) EA4065, Université Paris Descartes, Paris, France
3) Scottish Microbiology Reference Laboratory, Glasgow, UK

Commentary

The pitfalls of laboratory diagnostics of Clostridium difficile infection

M. Krutova 1, 4, *, M.H. Wilcox 2, 4, E.J. Kuijper 3, 4

1) Department of Medical Microbiology, 2nd Faculty of Medicine, Charles University in Prague and Motol University Hospital, Prague, Czech Republic
2) Leeds Teaching Hospitals NHS Trust & University of Leeds, Leeds, United Kingdom
3) Department of Medical Microbiology, Leiden University Medical Centre, Leiden, The Netherlands
4) European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group for Clostridium difficile (ESGCD)
Culture on chromogenic selective agar

- A chromogenic selective agar was developed (Eckert, JCM 2013)
  - Sensitivity = 87%

- Some PCR ribotypes fail to produce black colonies (PCR ribotypes 023) because they lack the ability to hydrolyse esculine (Connor, JCM 2016; Reigadas et al., Anaerobe 2017)

- It highlights the need for awareness for atypical colourless colonies
Strains of RT 033

- Toxinotype XI, clade 5, ST11

- The clinical significance in humans remains uncertain

- Isolation of the RT in 5 patients with AAD/PMC in France and one patient in Italy (Eckert, NMNI, 2014; Grandesso New Microbiol, 2016)

- This strain is isolated in domestic animals and livestock (Janezic, BMC Microbiol, 2014)

- Difficult to diagnose:
  - Phenotype A⁻B⁻ (EIA assays)
  - Some NAAT can detect cdtB
NAAT assays for detecting epidemic RT 027 may lack specificity

- Some tests (Xpert, Cepheid; Genotype Cdiff, Hines; Verigene, Nanosphere) are designed to give a **presumptive** identification of the 027 by detecting the binary toxin gene and the specific deletion in position 117 in *tcdC*.

- These markers are observed in other RT (deletion in 117 present in RTs 016, 080, 176, 328, (Krutova et al., J Microb Immuno Infect 2018) and may lead to misidentification of RT.

- **Definitive RT identification requires** capillary electrophoresis-based PCR ribotype.
Patients with endoscopically proven PMC may have negative diagnostic test for CDI

- PMC is not systematically associated to CDI

- Pseudomembranes can be seen with other infectious agents (E. coli 0157:H7; CMV, E. histolytica) or after some treatments such as Cisplatin or cyclosporin (Faroq et al., Dis Mon, 2015)

- Some *C. difficile* fulminant colitis may appear negative for toxins

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Fulminant *Clostridium difficile*: An Underappreciated and Increasing Cause of Death and Complications

Ramsey M. Dallal, MD,* Brian G. Hartrecht, MD,* Arthur J. Boujoukas, MD,† Carl A. Sirio, MD,† Linda M. Farkas, MD,* Kenneth K. Lee, MD, and * Richard L. Simmons, MD*

*From the Departments of *Surgery and †Anesthesiology/Critical Care Medicine, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania*
Correlation between CT, bacterial load, test results, and severity

- Canadian study, 203 stools TC+  
- Correlation between bacterial load, GDH tests, EIA for toxins and PCR cycle thresholds and quantitative culture

- A low Ct (<25 Cepheid) is associated to  
  - Presence of free toxin
  - Higher mortality
  - Presence of epidemic 027

The limited specificity of CT for predicting presence of toxin or outcome means that PCR cannot be used as a stand alone test

1. Dionne et al JCM 2013
2. Davies et al, PlosOne 2018
Nucleic Acid Amplification Test Quantitation as Predictor of Toxin Presence in C. difficile Infection

- Hypothesis: a preliminary diagnosis could be made on the basis of the quantitative results (Cq value) of the first test in algorithmic testing
- Retrospective analysis of 2 large collections of samples (n=2,669 and n=1,718)
- 208 and 113 samples, respectively, tested positive by NAAT
- Prediction of the eventual toxin A/B EIA results was accurate for 78.9% and 80.5% of samples, respectively.

"Cq values can serve as predictors of toxin status but, due to the suboptimal correlation between the two tests, additional toxin testing is still needed."
DETECTION of CLOSTRIDIUM DIFFICILE TOXINS by SIMOA HAS A BETTER SENSITIVITY THAN THE CYTOTOXICITY ASSAY.

- **SIMOA**: SIMOA consists of toxin capture by specific anti-toxin A and B antibodies coated on paramagnetic beads (Quanterix Corporation), followed by detection with specific antibody conjugated to an enzyme β-galactosidase. Beads are then incubated with enzyme substrate and individually isolated in microwells of an array for digital imaging.

- The threshold of positive result with SIMOA was set up at 22 and 18.8 pg/ml, for toxins A and B detection, respectively.

<table>
<thead>
<tr>
<th>100 patients TC+</th>
<th>38 controls C. difficile Culture-negative and TCA-negative</th>
<th>32 controls with a non toxigenic C. difficile strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>67 CTA+</td>
<td>36 (94.8%)</td>
<td>32 (100%)</td>
</tr>
<tr>
<td>33 CTA-</td>
<td>2* (5.2%)</td>
<td></td>
</tr>
</tbody>
</table>

| A-B- | 3 (4.5%) | 17 (51.5%) |
| A+B+ | 59 (88.0%) | 9 (27.3%) |
| A-B+ | 5 (7.5%) | 2 (6.1%) |
| A+B- | 0 | 5 (15.1%) |

* Low titers: 40 and 43 pg/ml

Gateau C. et al., ICDS 2018
New perspectives in diagnostic testing

- Singulex Clarity C. diff toxins A/B
- Frozen stool samples from 311 patients with suspected CDI
- Singulex was compared to 2-steps algorithm (Xpert C. difficile +EIA A and B+ CCNA).
- The limits of detection for TcdA and TcdB were 2.0 and 0.7 pg/ml in stool, respectively
  - 97.7% sensitivity and 100% specificity
  - This ultrasensitive toxin assay, which is automated and rapid (i.e., 32 min), has the potential to be a standalone test to replace the multistep testing algorithms

Sandlund, JCM 2018
Conclusion

- Appropriate selection of samples is essential for an accurate diagnosis.
- CD testing should be systematically performed in case of:
  - Healthcare associated diarrhea.
  - Potentially infectious diarrhea and negative tests for common enteropathogens.
- Standardisation of diagnostic methods may be indicated to improve inter-hospital comparisons\(^1,2\):
  - Two step algorithms are currently recommended.
  - Positive result should be always interpreted in conjunction the clinical assessment.
- Reducing delays in testing improves the quality of patient management\(^2\).
- Be aware of pitfalls in the laboratory diagnosis of CDI.

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2- Barbut et al. CMI 2014, 20, 136-144.