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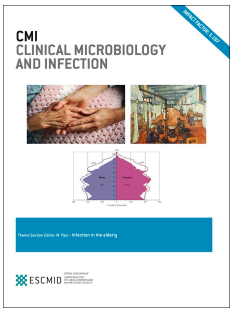

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Commentary

The pitfalls of laboratory diagnostics of *Clostridium difficile* infection

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An update of the diagnostic guidance document for *Clostridium difficile* infection (CDI) issued by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) [1] was recently reviewed by Gateau *et al.,* [2]. The aim of these publications was to optimize and improve CDI laboratory diagnostic on a global level by defining sample selection, testing algorithms and the interpretation of laboratory results [1,2]. We highlight some recently identified pitfalls that do not affect CDI laboratory diagnosis overall, but could have a significant impact on individual patients.

An unusual Clostridium difficile phenotype on chromogenic selective medium

To speed up the identification of cultured *C. difficile* strains, a chromogenic agar was developed (ChromID® agar, bioMérieux). However, as was recently shown for strains from Spain and Northern Ireland, isolates of PCR ribotype 023 may fail to produce black colonies, a presumptive identification of *C. difficile* [3,4], because *C. difficile* PCR ribotype 023 strains cannot hydrolyse esculin [3]. The *C. difficile* PCR ribotype 023 was one of the ten most frequent ribotypes identified in Eastern and Western Europe during 2012-2013 [5], which highlights the need for awareness among clinical microbiologists that PCR ribotype 023 strains produce atypical (colourless) colonies on ChromID® agar (bioMérieux).

Toxin A/B negativity in Clostridium difficile strains that are only binary toxin gene-positive

The clinical significance of *C. difficile* PCR ribotype 033 in humans was recently reported in Australia, France and Italy [6-8]. PCR ribotype 033 clusters with ribotypes 045, 066, 078, 126 and 193 in sequence type 11 [9] and encodes binary toxin genes, but also contains a truncated pathogenicity locus (part of *tcdA* gene); however, neither of the large clostridial toxins are produced, [10] and thus this ribotype cannot be detected by commercial tests for the detection of free toxins A/B in faeces. Depending on the chosen primers, nucleic acid amplification tests (NAATs) can reveal a positive result for the detection of binary toxin

gene(s) and/or for the presence of *tcdA* gene, but this information is lacking for most of the commercially available NAATs. While PCR ribotype 033 CDI did not result in diarrhoea and death in a hamster model, [11] there is evidence for its ability to cause CDI in humans [6-8]. PCR ribotype 033 has also been recovered from domestic animals and livestock [12,13]. Furthermore, a newly described four-gene insertion, which affects trehalose metabolism and may be associated with increased virulence, has been found only in a few *C. difficile* PCR ribotypes, including 033 [14]. These observations mean that further research is needed on the role of PCR ribotype 033 in human CDI.

Glutamate dehydrogenase negative and toxin A/B positive results from diagnostic assays

The simultaneous detection of glutamate dehydrogenase (GDH) and toxins A/B by enzyme immunoassays, which include both of these targets in one assay, is an alternative to a two-step CDI testing algorithm. A GDH negative and toxin A/B positive test result is considered as invalid, with a need for stool sample retesting [1,2]. We have confirmed that five stool samples, sent from different Czech hospitals, repeatedly showed such GDH negative and toxin A/B positive test results. However, when these five stool samples were anaerobically cultured, the *C. difficile* colonies tested were positive for both GDH and toxins A/B with the same combined assay. Moreover, PCR ribotyping of the cultured *C. difficile* colonies revealed five different PCR ribotypes (012, 014, 020, 070 and 176). Although there is no explanation at present for the failure to detect GDH, it suggests a third diagnostic step may be needed in order to confirm a GDH negative toxin A/B positive result.

The inaccuracy of molecular methods for detecting epidemic PCR ribotype 027

NAATs that target *tcdB* or *tcdA* of toxigenic *C. difficile,* are an alternative first step option in CDI diagnostics due to their high negative predictive value. NAATs can also be used as a third step option to distinguish toxigenic from non-toxigenic *C. difficile* strains in GDH

positive and free toxin A/B negative stool samples [1,2]. Some NAATs (e.g. Xpert® *C. difficile* (Cepheid), GenoType® Cdiff, (Hain Lifescience), Verigene *C. difficile* Test, (Nanosphere)) can also detect the binary toxin gene(s) (*cdtA/cdtB*) and the specific deletion at position 117 of the *tcdC* gene, which is assumed to indicate the presence of *C. difficile* PCR ribotype 027. However, this marker has recently been identified in other PCR ribotypes [15]. Thus, the interpretation of "presumptive ribotype 027 positive" results should be correlated with the local-CDI epidemiology. Definitive ribotype identification requires capillary electrophoresis-based PCR ribotyping.

Negative diagnostic tests for CDI in patients with endoscopically confirmed pseudomembranous colitis.

Though most frequently associated with CDI, a wide differential diagnosis should be considered when pseudomembranous colitis is diagnosed endoscopically and laboratory tests are negative. Other bacterial, viral, and parasitic pathogens have also been implicated in pseudomembranous colitis including *Escherichia coli* O157:H7, cytomegalovirus and *Entamoeba histolytica*. Additionally, the pseudomembranous colitis can occur in inflammatory bowel diseases, ischemic colitis and also can be caused by several chemicals such as cisplatin and cyclosporine A [16].

There is a wide choice of assays available to facilitate the rapid laboratory diagnosis of CDI. It is crucial, however, that microbiology laboratories select appropriate test combinations to optimise CDI diagnosis and provide clear local guidance on sample selection for CDI testing [1,2]. Furthermore, given the complexity of CDI diagnosis, the interpretation and communication of test results is at least as important as the result itself. In the case of an inconclusive test result, particularly when associated with unwell patients or if sub-optimal assay performance is suspected, the national reference or central laboratory for *C. difficile* should be contacted.

Transparency declaration:

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