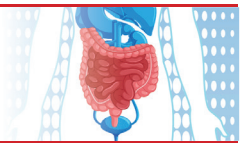
THE PATHCARE NEWS

OPTIMISING CLOSTRIDIOIDES DIFFICILE DIAGNOSIS:
IMPLEMENTATION AND BENEFITS OF ROUTINE TWO-STEP TESTING



Clostridioides difficile infection (CDI) remains a leading cause of healthcare-associated diarrhoea and antibiotic-associated colitis. *C. difficile* produces 2 types of toxins; toxins A and B (encoded by TcdA & TcdB genes, respectively), which are virulence factors causing cytotoxicity and cellular detachment from intestinal epithelium and are responsible for CDI symptomatology. Many laboratories routinely perform singleplex or multiplex PCR testing, which detects *C. difficile* toxin genes (tcdA/tcdB) with/without the detection of the hypervirulent ribotype O27/NAP1/B1 strain.

Local PathCare data confirm a rise in *C. difficile* detections, with increasing multiplex test positivity rates and a corresponding increase in absolute detection numbers. Although PCR testing is highly sensitive, it cannot distinguish between *C. difficile* colonisation and true infection driven by toxin production and may remain positive despite successful CDI therapy. In the context of rising *C. difficile* transmission, this limitation may contribute to overdiagnosis and unnecessary CDI treatment.

South African and international guidelines emphasize a two-step testing algorithm for *C. difficile* detection and testing for free toxin detection to improve diagnostic accuracy of CDI and improve antimicrobial stewardship. In keeping with local and international guidelines, stool samples sent for *C. difficile* testing at the reference laboratory in Cape Town, PathCare will perform routine two-step testing following a positive *C. difficile* detection on stool PCR testing.

What is Two-Step testing in our setting?

The two-step approach combines:

1. Initial molecular test: A highly sensitive PCR test that detects the presence of *C. difficile* and toxin-encoding genes of a toxigenic strain of *C. difficile*. Strains may be toxin-producing or non-toxin-producing (toxin genes are not expressed – most likely a colonising strain). A negative test excludes the diagnosis of CDI.
2. Confirmatory toxin-production test: Detection of *C. difficile* **free toxin** in stool using enzyme immunoassay (EIA). At PathCare, the EIA test for the detection of free Toxin A/B in stool is a highly specific test that indicates toxin production. *C. difficile* EIA testing is not appropriate as stand-alone tests due to lower sensitivity.

Stool samples positive for both the presence of the organism and its toxin are considered consistent with active *C. difficile* infection.

Figure 1: SASCM C difficile initial PCR (NAAT)-guided two-step testing guideline recommendation

Result interpretation

C difficile PCR result	Toxin A/B EIA result	Interpretation
Negative	N/A	*C. difficile* infection highly unlikely.
Positive	Positive	Active *C. difficile* toxin-mediated infection (CDI)
Positive	Negative	Colonisation most likely: CDI treatment not recommended, first excl. alternative causes for diarrhoea. Consider repeat toxin EIA test if no alternative aetiology is identified. Treatment may be considered in consultation with a clinical microbiologist and/or gastroenterologist.

Evidence in support of reflex C. difficile EIA testing on *C. difficile* positive PCR patients:

1. Clinical outcomes correlate with toxin presence and show that patients with **free toxin detected** have more severe disease, higher WCC, and increased mortality.
2. **PCR-only** testing results in **overdiagnosis** since PCR-only testing detects both infection and colonisation, inflating CDI rates, and risks unnecessary antibiotic patient treatment.
3. **PCR positive,** *C difficile* **toxin negative** patients often behave like C difficile test-negatives with clinical outcomes including recurrence and mortality similar to those with negative results, suggesting colonisation rather than active disease.

Advantages of the Two-Step Algorithm

- Improved clinical specificity: Distinguishes between colonisation and true toxin-mediated disease.
- Better alignment with disease severity: Studies show that toxin-positive (EIA) cases are more likely to have clinically severe CDI.
- Supports antimicrobial stewardship: Reduces unnecessary CDI treatment in colonised patients, preventing disruption of gut microbiota and minimising selective pressure for resistance.
- Prevents inflated hospital *C difficile* infection rates and optimizes resources for appropriate infection control hospital interventions.
- Aids clinician assessment to more accurately interpret results in context of symptoms, antibiotic exposure, and risk factors
- Strengthens diagnostic accuracy, aligns with SASCM guidance, and promotes responsible antibiotic use.

What's the clinical implication of reflex toxin immunoassay testing in those with PCR positive toxigenic C difficile patients?

Given rising *C. difficile* colonisation rates and evidence of more severe outcomes in toxin-positive cases, a two-step algorithm provides a balanced approach wherein we offer higher clinical relevance while maintaining diagnostic confidence. Clinicians are encouraged to interpret results in conjunction with clinical presentation, risk factors and antibiotic exposure history. Infection prevention control precautions should be maintained to prevent ongoing transmission within healthcare units.

For further information or diagnostic interpretation support, contact your local PathCare microbiologist.

References

1. Nana T, Moore C, Boyles T, et al. South African Society of Clinical Microbiology Clostridioides difficile infection diagnosis, management and infection prevention and control guideline. SAJID. 2020;35(1),a219. https://doi.org/10.4102/ sajid.v35i1.219.
2. Kelly C, Fischer M, Allegretti J, et al. ACG Clinical Guidelines: Prevention, Diagnosis, and Treatment of Clostridioides difficile Infecttions. AJG 2021;116:1124–1147. https://doi.org/10.14309/ajg.0000000000001278/
3. Van Prehn J, Reigadas E, Vogelzang E, et al. ESCMID: 2021 update on the treatment guidance document for Clostridioides difficile infection in adults. Clinical Microbiology and Infection, Vol 27, S1 - S21.
4. Johnson S, Lavergne V, Skinner AM, et al. Clinical Practice Guideline by the IDSA and SHEA: 2021 Focused Update Guidelines on Management of Clostridioides difficile Infection in Adults. Clin Infect Dis, 2021; 73(5), e1029-e1044. doi: 10.1093/cid/ciab549.
5. Kociolek LK, Gerding DN, Carrico R, et al. (2023). Strategies to prevent Clostridioides difficile infections in acute-care hospitals: 2022 Update. ICH Epidemiology, 44: 527–549, doi: 10.1017/ice.2023.18
6. Planche TD, Davies KA, Coen PG, et al. Differences in outcome according to Clostridium difficile testing method: a prospective multicentre diagnostic validation study of C difficile infection. Lancet ID. 2013 Nov;13(11):936-45. doi: 10.1016/S1473-3099(13)70200-7.
7. Polage CR, Gyorke CE, Kennedy MA, et al. Overdiagnosis of Clostridium difficile Infection in the Molecular Test Era. JAMA Intern Med. 2015;175(11):1792–1801. doi:10.1001/jamainternmed.2015.4114
8. Hogan CA, Hitchcock MM, Frost S, et al. Clinical Outcomes of Treated and Untreated C. difficile PCR-Positive/Toxin-Negative Adult Hospitalized Patients: a Quasi-Experimental Noninferiority Study. JCM 2022 Jun 15;60(6):e0218721. doi: 10.1128/jcm.02187-21. Erratum in: J Clin Microbiol. 2022 Dec 21;60(12):e0151822. doi: 10.1128/jcm.01518-22. PMID: 35611653; PMCID: PMC9199396.

Prepared by:
Dr Dawood da Costa, Clinical microbiologist, PathCare Reference Laboratory
Dawood.dacosta@pathcare.net
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