WHITE PAPER

Diagnostic tests for *Clostridioides difficile*

The need for accurate detection of *Clostridioides difficile*

Clostridioides difficile is the major cause of nosocomial diarrhoea and pseudomembranous colitis. The bacterium produces spores and infection usually occurs when the normal gut microflora is altered or killed by antibiotics allowing the germination of spores into vegetative cells. Once *C. difficile* growth begins, toxins A and B may be produced, causing diarrhoea and colitis.

The glutamate dehydrogenase (GDH) of *C. difficile* is a good antigen marker for the organism in faeces because it is produced in high amounts by all strains, toxigenic or non-toxigenic.

C. difficile disease results from production of two main toxins; toxin A, an enterotoxin, and toxin B, a cytotoxin. Toxigenic strains of *C. difficile* produce both toxins or only toxin B.

Rapid, accurate diagnosis of diarrhoea caused by *C. difficile* is central to a patient receiving appropriate treatment in a timely manner and is necessary to inform infection prevention interventions.

Available diagnostic tests for *Clostridioides difficile*

Una Health Ltd provides a range of TECHLAB® diagnostic tests for *C. difficile* GDH and Toxin A/B detection. These tests are enzyme immunoassays in either 96-well plate or single membrane cassette formats. They provide rapid results that are simple to perform compared to cell cytotoxicity neutralisation assays and toxigenic culture.

Product	Format	Product Code	Description of test	Time to result
C. DIFF QUIK CHEK COMPLETE®	Rapid EIA	T30525C (25 tests) T30550C (50 tests)	Simultaneous detection of GDH and Toxins A&B in a single reaction well on a rapid EIA membrane cassette	30 min
C. DIFF CHEK™-60	96-well plate	TL5025	Detection of GDH antigen in 96-well EIA format	1hr
C. DIFFICILE TOX A/B II™	96-well plate	T5015	Detection of Toxins A&B in 96-well EIA format	1hr
C. DIFFICILE QUIK CHEK®	Rapid EIA	30390	GDH detection on a rapid EIA membrane cassette	30 min
TOX A/B QUIK CHEK®	Rapid EIA	30394	Detection of toxins A&B detection on a rapid EIA membrane cassette.	30 min
C. difficile Toxin/Antitoxin Kit	Tissue Culture	T5000	Toxin B detection using tissue culture format	18 hrs

Available diagnostic tests for *C. difficile*. GDH: glutamate dehydrogenase

Key Benefits

The TECHLAB® range of kits largely comprise two formats that have the following benefits:

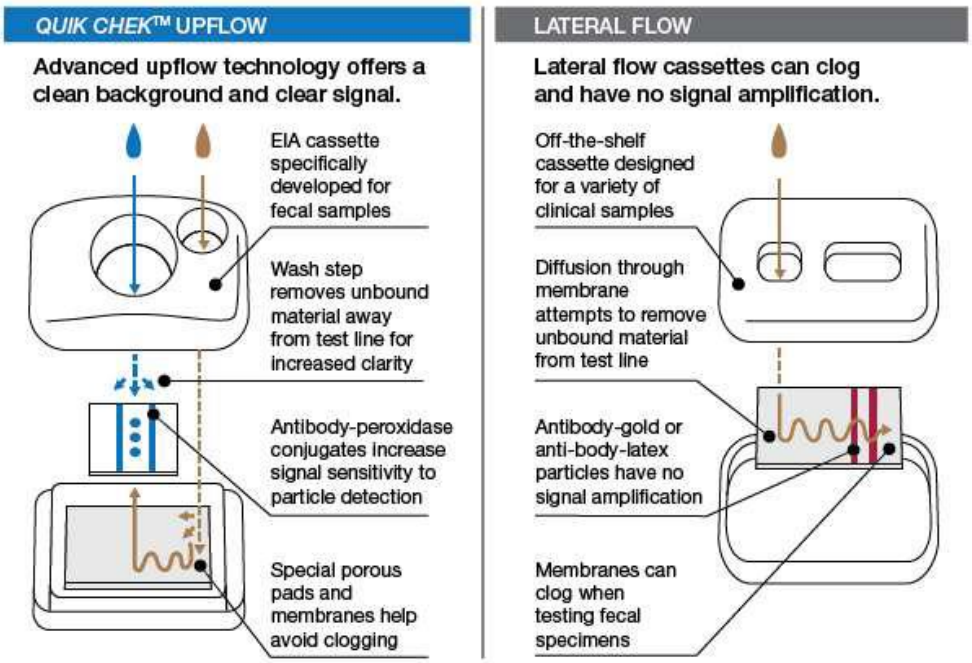
CHEK™ 96-well plate-based enzyme immunoassay (EIA)	QUIK CHEK™ Single test membrane EIA technology in a cassette
▪ ELISA-based, 96-well plate format ▪ Suitable for screening large numbers of samples ▪ Results within 2 hours ▪ Simple procedure ▪ Automatable ▪ Highly standardised	▪ Direct faecal specimen testing in a rapid assay format ▪ Individual device ▪ Membrane bound EIA technology ▪ Suitable for smaller numbers of samples or for 'out-of-workflow' testing ▪ Results within 30 minutes ▪ Easy to interpret ▪ No equipment needed ▪ Highly specific and sensitive

C. DIFF CHEK™- 60

C. DIFF QUIK CHEK COMPLETE®

How TECHLAB® QUIK CHEK™ kits differ from lateral flow assays

TechLab's QUIK CHEK™ products are based on enzyme immunoassay (EIA) technology in a membrane cassette format. Following addition of sample, a wash step removes unbound material away from the test line for a clearer signal. Specific antibody-peroxidase conjugates amplify the signal to the target antigen, increasing sensitivity compared to traditional lateral flow tests. In addition, the membrane cassette itself is specifically designed for faecal samples and specially designed porous pads and membranes avoid clogging with such clinical samples.



Comparison of QUICK CHEK™ technology compared to lateral flow

Supporting evidence and guidelines

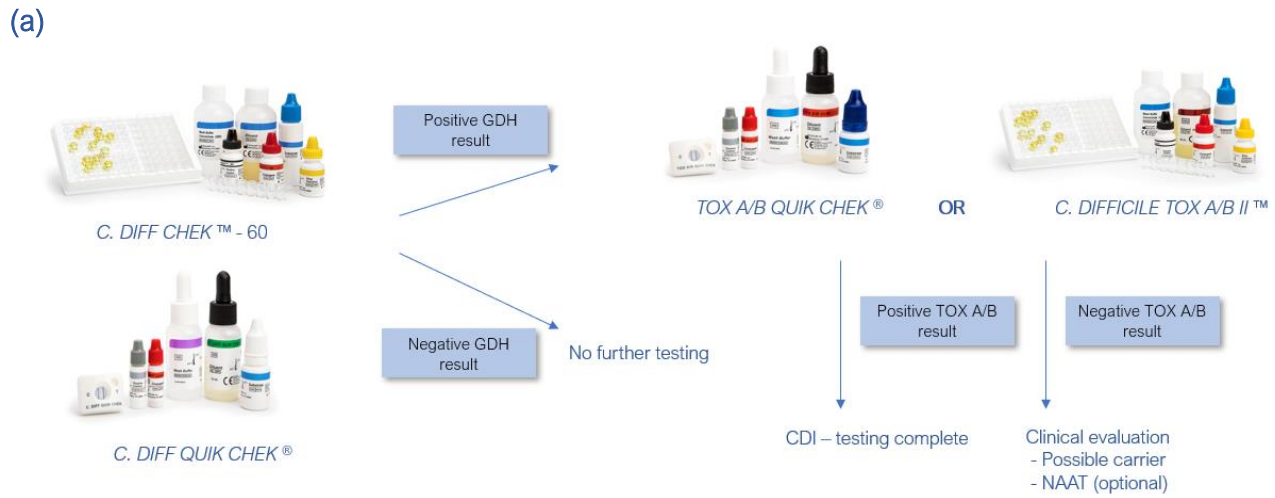
Not all strains of *C. difficile* produce toxins (non-toxigenic), therefore current UK guidelines advise that organisations adhere to a two-stage testing approach which consists of a test to screen for glutamate dehydrogenase (using EIA, NAAT, or PCR), followed by a toxin test.

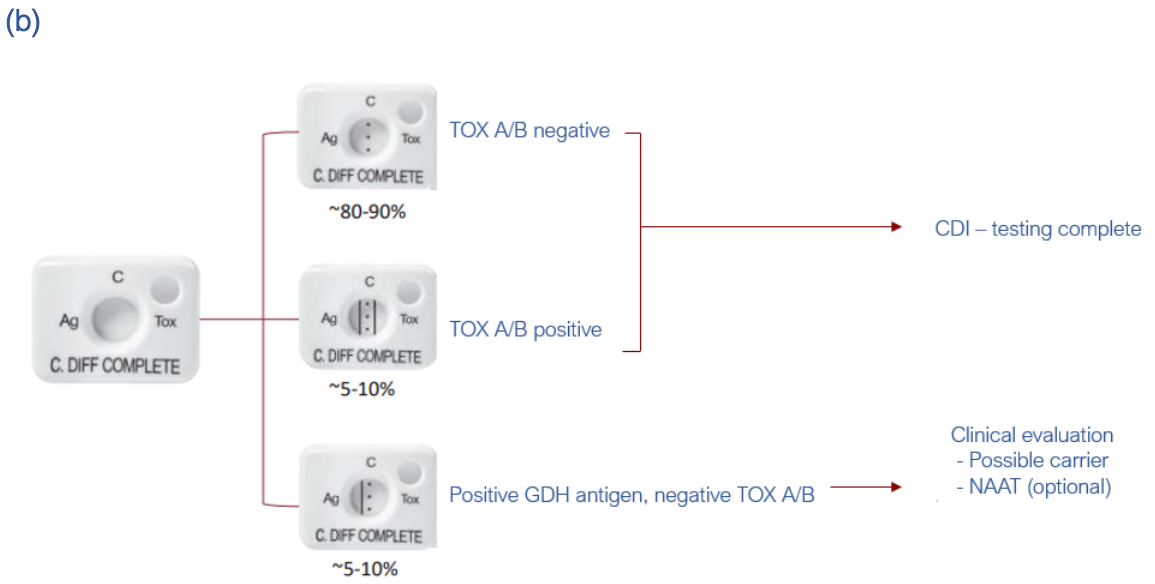
This guidance is reflected by that of ESCMID, who revised their guidance for *C. difficile* infection (CDI) diagnosis in 2016. Available evidence for *C. difficile* diagnostics was summarised in this publication (see table below).

Product	Format	Sensitivity GDH	Sensitivity Tox A/B	Specificity GDH	Specificity Tox A/B	Reference
C. DIFF QUIK CHEK COMPLETE®	Rapid EIA	95.7% 94.4% 100% 100% 97%	60.0% 92.3% 61% 79% 55%	92.5% 97.7% 94.8% 88% 98%	94.4% 100% 100% 97% 100%	[1]Yoo [2] Yazisiz [7]Swindells [8]Kawada [12]Bruins
C. DIFF CHEK™-60	96-well plate	94·5% 93.5%	N/A	94·5% 98%	N/A	[3]Planche [4]Snell
C. DIFFICILE TOX A/B II™	96-well plate	N/A	58·0% 84.6%	N/A	98·7% 98.2%	[3]Planche [4]Snell
C. DIFFICILE QUIK CHEK®	Rapid EIA	97% 83%	N/A	95% 97%	N/A	[5]Eckert [6]Walkty
TOX A/B QUIK CHEK®	Rapid EIA	N/A	71% 43% 40%	N/A	94% 100% 100%	[8]Kawada [9]Le Guern [10]Wren

Compared to cytotoxigenic culture. Mainly taken from Crocach et al.[11], with more recent publications added.

No single test is suitable as a stand-alone test for CDI, but it is recommended to adopt a two test algorithm. The first test should be one that has high NPV (highly sensitive), which includes GDH EIA (C. DIFF CHEK™-60 or C. DIFF QUICK CHEK). The second reflex test should have high PPV (highly specific) so that all samples with a positive second test can be diagnosed as CDI. Toxin EIAs are recommended for this purpose as they detect free toxin and not just the gene. Samples with a first positive test but negative toxin A/B EIA need to be evaluated further (e.g. for low toxin producers or *C. difficile* carriage).





Recommended use of TECHLAB C. difficile tests based on algorithms by ESCMID for CDI testing. (a) GDH or NAAT – Tox A/B algorithm. (b) GDH *and* Tox A/B algorithm[11].

References

1. Yoo et al. (2019) Simultaneous Detection of *Clostridioides difficile* Glutamate Dehydrogenase and Toxin A/B: Comparison of the C. DIFF QUIK CHEK COMPLETE and RIDASCREEN Assays
2. Yazisiz et al. (2020) The Evaluation of the Performance of C. Diff Quik Chek Complete and Toxin A + B (Clostridium difficile) DUO Diagnostic Tests Compared with Toxigenic Culture in the Diagnosis of Clostridium difficile Infection - PubMed (nih.gov)
3. Planche et al. (2013) https://linkinghub.elsevier.com/retrieve/pii/S1473309913702007
4. Snell et al. (2004) Performance of the TechLab C. DIFF CHEK-60 Enzyme Immunoassay (EIA) in Combination with the C. difficile Tox A/B II EIA Kit, the Triage C. difficile Panel Immunoassay, and a Cytotoxin Assay for Diagnosis of Clostridium difficile-Associated Diarrhea (nih.gov)
5. Eckert et al. (2014) Molecular Test Based on Isothermal Helicase-Dependent Amplification for Detection of the Clostridium difficile Toxin A Gene (nih.gov)
6. Walkty et al (2013) Evaluation of an Algorithmic Approach in Comparison with the Illumigene Assay for Laboratory Diagnosis of Clostridium difficile Infection (nih.gov)
7. Swindells et al (2010) Evaluation of Diagnostic Tests for Clostridium difficile Infection - PMC (nih.gov)
8. Kawada et al. (2011) Evaluation of a simultaneous detection kit for the glutamate dehydrogenase antigen and toxin A/B in feces for diagnosis of Clostridium difficile infection - PubMed (nih.gov)
9. Le Guern et al. (2012) Evaluation of a New Molecular Test, the BD Max Cdiff, for Detection of Toxigenic Clostridium difficile in Fecal Samples (nih.gov)
10. Wren et al. (2009) Detection of Clostridium difficile infection: a suggested laboratory diagnostic algorithm: British Journal of Biomedical Science: Vol 66, No 4 (tandfonline.com)
11. Crobach et al (2016) European Society of Clinical Microbiology and Infectious Diseases: update of the diagnostic guidance document for Clostridium difficile infection - Clinical Microbiology and Infection

Pricing and ordering

For all order enquiries, please email enquires@unahealth.co.uk