Optimization of a three-step algorithm for the diagnosis of *Clostridioides difficile* infection. The experience of ASST Brianza.

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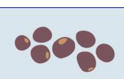
Introduction

In recent decades, *Clostridioides difficile* infection (CDI) has experienced not only an increase in cases but also in severity and associated mortality, following the spread of hypervirulent and multidrug-resistant strains. In patients with suspected CDI, international guidelines[1] recommend the use of multi-step diagnostic algorithms that involve, through immunoenzymatic (EIA) or immunofluorescence (FIA) methods, the search for the common antigen (GDH) and toxins A / B in fecal samples. In case of conflicting results between GDH and toxins, confirmatory molecular tests are performed. The aim of this study was to define a gray zone for the index of cutoff (COI) values of toxins in order to reduce the number of potential false-positive samples. In this regard, we focused on samples positive for Tox A / B by FIA, all of which were subjected to molecular confirmation (MC).

Materials and Methods

Using the Bristol Stool Chart classification criteria[2],

TYPE 1		Separate hard lumps (hard to pass).
TYPE 2		Lumpy, hard, sausage-shaped.
TYPE 3		Sausage-shaped with cracks on the surface.
TYPE 4		Sausage-shaped or snake-like; smooth and soft.
TYPE 5		Soft blobs with clear-cut edges (easy to pass).
TYPE 6		Fluffy pieces with ragged edges; mushy.
TYPE 7		Entirely liquid, watery, no solid pieces.

fecal samples (types 5, 6, and 7) were evaluated for suitability. Subsequently, a 3-step diagnostic algorithm was applied: for the detection of GDH, the STANDARD F GDH test (SD Biosensor, Suwon, South Korea) was used, while for the detection of toxins, the STANDARD F Toxin A/B test (SD Biosensor, Suwon, South Korea) was used.Molecular confirmation of toxin-positive FIA was performed using the Xpert® C. difficile BT test (Cepheid, Sunnyvale, USA).

Results

Between March and December 2023, 600 fecal samples were examined. Of these, 518 (86.5%) were GDH negative while 82 (13.5%) were positive and tested for toxins A / B.

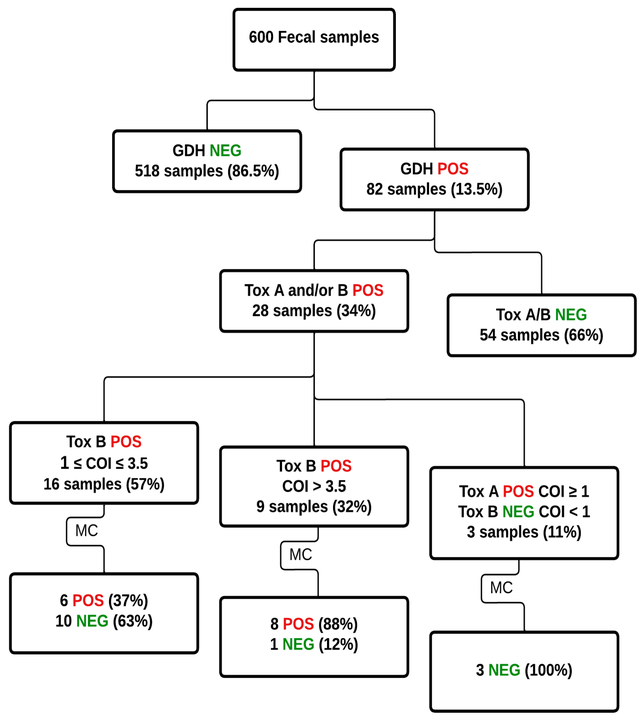
28 samples were positive for toxins according to the kit's interpretive criteria (Tox A and/or Tox B COI ≥1).

Of these, 3 were positive for Tox A only (COI ≥1) and 25 for Tox B (16 had 1≤COI≤3.5 while 9 had COI >3.5). The 3 samples positive for Tox A only were all negative by molecular testing.

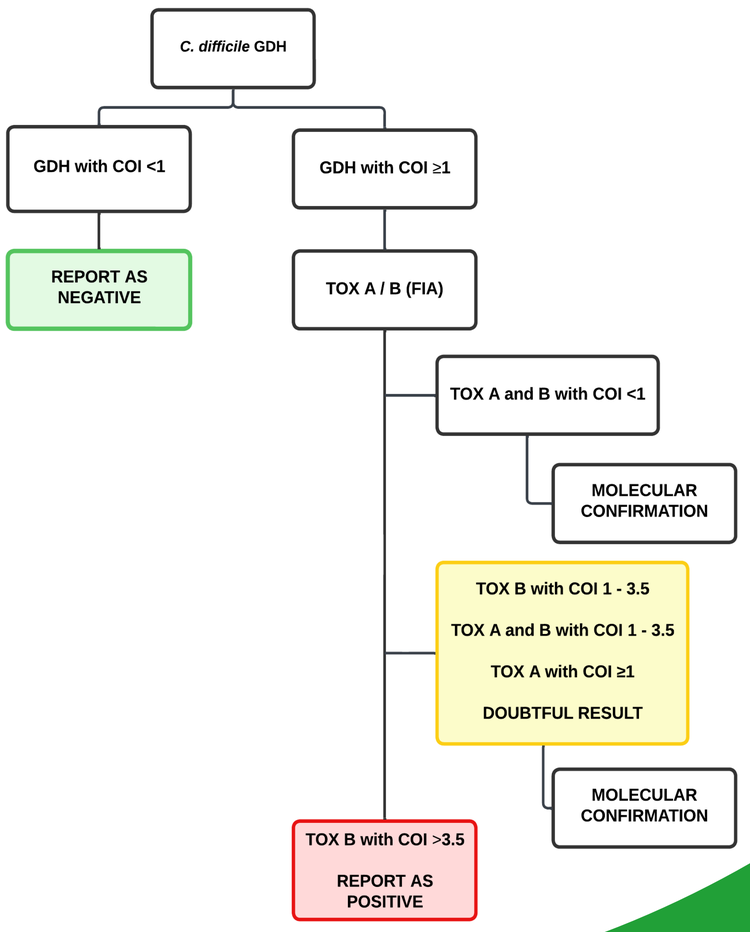
Of the 16 samples that had Tox B with 1≤COI≤3.5, only 6 were confirmed positive by molecular method, while 10 were negative. All samples positive for Tox B with COI >3.5, except one, were confirmed positive by molecular test.

In summary, of the samples positive for Tox B with COI between 1 and 3.5, 63% turned out to be false positive, while for those with a COI >3.5, only one false positive was detected (1 out of 10) represented by a heavily bloody sample (possible interference).

Finally, the samples with Tox A only (COI ≥1) were all false positives. A summary chart follows.



Flowchart



Discussion and Conclusions

Preliminary data indicate that a COI ≥1 cannot be considered definitive for the diagnosis of CDI. In fact, an evaluation of the COI index of Tox A / B with the introduction of a gray zone (1≤COI Tox A / B≤3.5) in the diagnostic algorithm could allow for a reduction in potential false positive results, thus improving antibiotic stewardship and the management of patients with suspected CDI.

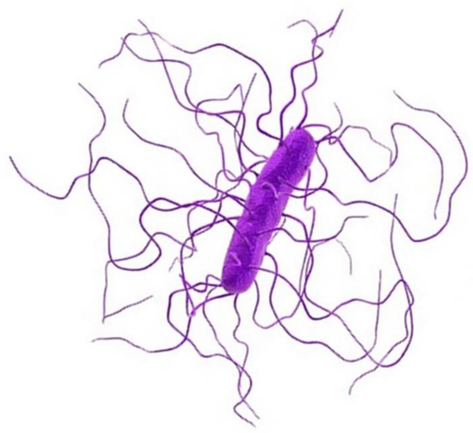
This evaluation will continue in the coming months with the aim of increasing the sample size.

References

1. McDonald LC *et al*. 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). Clin Infect Dis 2018;**66**(7): e1-e48. doi: 10.1093/cid/cix 1085
2. R.A. Corrigan, *et al*. Usefulness of the Bristol Stool Form Chart scoring system for the laboratory processing of faecal samples in suspected Clostridioides difficile cases. Journal of Hospital Infection 2020;**105**:95-97

Key Words

CDI, EIA, FIA, GDH, COI, MC, gray zone, algorithm.



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