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Consequences of applying two-step or three-step/multistep algorithm in the diagnosis of *Clostridioides difficile* infection

Consecuencias de la aplicación del algoritmo de 2 pasos o de 3 pasos/multipaso para el diagnóstico de la infección por Clostridioides difficile

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ABSTRACT

Introduction: Toxigenic Clostridioides difficile (CDI) is a leading cause of nosocomial infection. To optimize the diagnosis of this infection, several algorithms have been proposed: in the two-step algorithm, the first step involves the detection of glutamate dehydrogenase (GDH), while in the three-step/multistep algorithm, GDH and toxins are detected simultaneously.
Methods: Between January 2023 and April 2025, immunochromatography (ICT) was used to detect GDH and toxins A/B in stool samples from patients with suspected CDI, with PCR used as a confirmatory test. The number of CDI cases that would be reported according to each algorithm was compared.
Results: The application of the three-step/multistep algorithm generated 135 additional positive cases compared to the two-step algorithm, representing a 36.9% increase.
Conclusion: The diagnostic algorithm for CDI needs to be reviewed when using ICT as the initial test to avoid potential overdiagnosis.

Palabras clave:
Clostridioides difficile
Algoritmo
Falso positivo
Inmunocromatografía
TAAN

RESUMEN

Introducción: Clostridioides difficile (ICD) toxigénico constituye una de las principales causas de infección nosocomial. Para optimizar el diagnóstico de esta infección, se han propuesto distintos algoritmos: en el algoritmo de dos pasos, el primer nivel consiste en la detección de glutamato deshidrogenasa (GDH), mientras que en el de tres pasos/multipaso, se realiza la detección simultánea de GDH y toxinas.
Métodos: Entre enero-2023 y abril-2025 se utilizó una inmunocromatografía (ICT) para detectar GDH y toxinas A/B en heces de pacientes con sospecha de ICD, realizando PCR como prueba confirmatoria. Se comparó el número de casos de ICD que serían reportados según el algoritmo utilizado.
Resultados: La aplicación del algoritmo de tres pasos/multipaso generó 135 casos positivos adicionales respecto al de dos pasos, representando un incremento del 36,9%.
Conclusión: Es necesario revisar el algoritmo diagnóstico de la ICD cuando se utiliza la ICT como prueba inicial para evitar posibles sobrediagnósticos.

Introduction

Toxin-producing *Clostridioides difficile (C. difficile)* infection (CDI) is the leading cause of nosocomial diarrhoea in industrialised countries. In 2022–2023, it accounted for 6.2% of reported nosocomial infections in Europe and 3.4% in Spain.[1] In addition, more cases of community CDI requiring hospital admission are being reported, rising from an inciden-

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Table 1
Description of algorithms proposed by different societies.

American College of Gastroenterology (ACG)[5]	2-step algorithm: 1.° High sensitivity test: NAAT or GDH by EIA 2.° Toxin detection with EIA If both tests positive: CDI diagnosis
Infectious Diseases Society of America (IDSA)[6]	Multi-step algorithm. Perform any of the following combinations: GDH plus toxin (EIA) GDH plus toxin (EIA), mediated by NAAT NAAT plus toxin (EIA)
European Society of Clinical Microbiology and Infectious Diseases (ESCMID)[7]	Detection of GDH and toxin A/B by EIA (without the need for NAAT testing): diagnosis of CDI
Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica [Spanish Society of Infectious Diseases and Clinical Microbiology] (SEIMC)[8]	2-step algorithm: 1. GDH detection (IA) 2. If GDH positive: NAAT (toxin A or B gene) If both positive: CDI diagnosis 3-step/multi-step algorithm: Detection of GDH and Toxin A/B by IA (without the need for NAAT testing): diagnosis of CDI

ce of 0.69 cases/1,000 admissions in 2018 to 1.35/1,000 admissions in 2020.[2]

C. difficile can produce toxins A (TcdA) and B (TcdB), and binary toxin(*C. difficile* transferase), leading to disruption of the colonic epithelium and causing inflammation and tissue damage.[3] This can lead to a variety of clinical manifestations, from mild diarrhoea to pseudomembranous colitis, which can progress to toxic megacolon or intestinal perforation, complications with high mortality.[4] Therefore, the detection of this microorganism is crucial in order to prescribe appropriate antibiotic treatment and to establish contact isolation in hospitalised patients.

The Microbiology laboratory has several rapid techniques available for the detection of toxigenic *C. difficile* (TCD), among them immunoassays (IA), including enzyme immunoassays (EIA) and immunochromatography (ICT); as well as nucleic acid amplification techniques (NAAT). Different scientific societies have proposed diagnostic algorithms aimed at standardising the testing strategy and optimising the use of available resources (Table 1).

All these societies argue for the development of the described algorithms in order to avoid false negatives in CDI detection. However, none of them question the possibility that following any of these algorithms may lead to over-reporting of CDI cases.

The aim of our study was to perform a comparison between the 2-step diagnostic algorithm and the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC) 3-step/multi-step diagnostic algorithm, to assess whether there is any difference in the number of CDIs detected using one or the other procedure when an ICT test is used for screening.

Methods

Between January 2023 and April 2025, diarrhoeal stool samples (Bristol scale 5–7) from patients over 2 years of age with suspected CDI received at the Microbiology laboratory of the Hospital Universitario Virgen Macarena (Seville) were analysed using ICT that simultaneously detects glutamate dehydrogenase (GDH) and toxins A and B. From January to December 2023, the *C. difficile* GDH + Toxin A + B combo card test (CerTest, Zaragoza, Spain) and from January 2024 to April 2025, CoproStrip *C. difficile* GDH + ToxA + ToxB (Sayvon Diagnostics, Israel) were used. Samples were anotated internally for pathological products (blood, mucus or pus).

In samples testing negative for GDH, no further testing was performed and it was assumed that no CDI was present. In GDH-positive samples (irrespective of the toxin result by ICT), PCR was performed (Xpert *C. difficile* BT, Cepheid, USA), which detects the targets *cdtB*, binary toxin *(cdtA* and *cdtB*) and *tcdC* deletion.

We recorded the number of samples that were positive for GDH, toxin A or toxin B, as well as how many of the samples that underwent PCR were positive. Next, we computed how many samples would be reported as CDI following the 2-step algorithm (first perform GDH immunodetection and, if positive, perform NAAT for toxin A or B) and how many by the 3-step/multi-step algorithm (simultaneously perform immunodetection of GDH, toxin A and B. If GDH and any toxin is positive, report CDI directly).

Statistical analysis was performed with the software program SPSS® Statistics (Version 21.0.1.0). The χ^2 test was performed to compare whether there were statistically significant differences between the number of positives reported using one algorithm or the other, and the possible causes of discrepancies between the two algorithms were analysed.

Results

Some 4223 stool samples were processed; 3593 (85.1%) were reported as negative as neither GDH nor toxins were detected by ICT.

The ICT and PCR results of the 630 GDH-positive samples are shown in Fig. 1:

If the 2-step algorithm was used for CDI diagnosis, 365/630 samples would have been reported as positive, while applying the 3-step/multi-step diagnostic algorithm, 500/630 samples would have been reported as positive. This would mean a difference in the reporting of positive cases of 135 patients, an increase of 36.9% ($p < 0.001$).

With respect to the ICT tests used, with the CerTest, 14.4% more positives would be reported with the 3-step algorithm (27/188), while with the Sayvon Diagnostics, this figure was 22.17% (98/442); this also indicates an increase in the reporting of false positives ($p = 0.025$).

Considering the interferences described by the manufacturers, in particular the possibility of false positives (FP) due to the presence of blood in the stool, a macroscopic evaluation of the 135 discrepant samples was performed. Haematic debris was observed in 32 of them (25.6%).

Discussion

CDI is a major problem in the hospital setting, due to the ease of transmission by healthcare workers and high mortality.[9] In addition, the incidence of community-associated CDI is increasing in younger patients with fewer risk factors than inpatients.[10] Rapid diagnosis is necessary to enable treatment and contact isolation of hospitalised patients in the shortest possible time, but accurate diagnosis of cases is also very important. Reporting of FPs leads to unnecessary isolation and antibiotic treatment, with a subsequent increase in antimicrobial resistance and side effects. In addition, contact isolation can lead to poorer patient care and increased costs for the health care system.[11,12]

While these algorithms can be very useful, the limitations of the techniques used need to be taken into account. It is well known that ICT tests have specificity limitations with respect to other IAs, such as EIAs.[13] Of the ICT tests currently marketed in Spain, only four (Operon, Monlab, CerTest and Sayvon Diagnostics) specify in their instructions possible interferences with the presence of blood or mucus in the stool.

Pathological products in the stool are not uncommon in this context: they may occur in gastrointestinal infections with *Campylobacter* spp; *Salmonella* spp. or *Shigella* spp; haemorrhoids or ulcerative colitis. Therefore, macroscopic examination of the sample prior to CDT identification

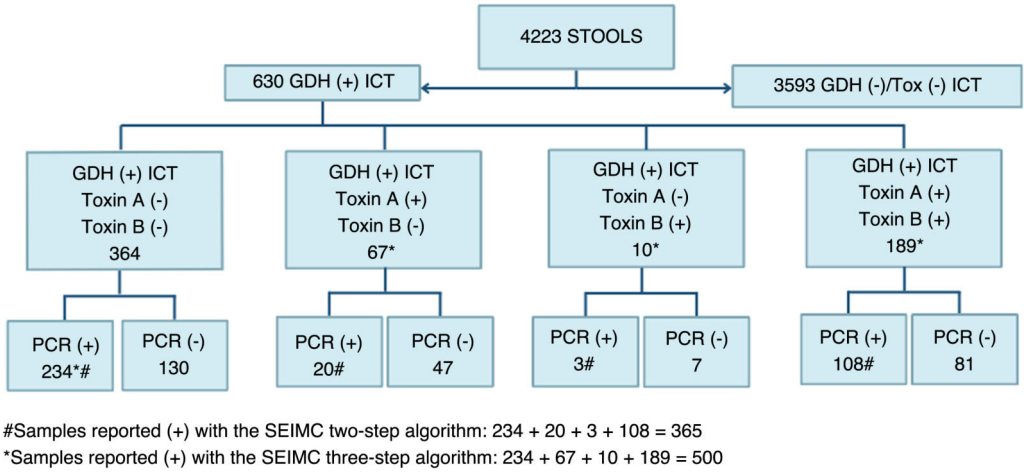

Fig. 1. Results of immunochromatographic tests for GDH and *C. difficile* toxins A and B and *C. difficile* PCR. GDH: Glutamate dehydrogenase; ICT: Immunochromatography; Tox: Toxins A and B. gr1

would be advisable to avoid FPs, or at least taking this information into account in order to proceed with caution if declared. However, on many occasions, the presence of blood may not be detected macroscopically, so it is essential to compare the positive result with the patient's clinical manifestations and predisposing factors (previous antibiotic therapy, taking proton pump inhibitors, long hospital stay, etc.).

Other studies have noted increased reporting of CDI cases when ICT and the 3-step/multi-step algorithm are used, with a 10% rate of FPs reported in the study by Yoo et al[14] and up to 35% in the study by Recio-López.[15] These results are in line with those obtained in our analysis.

The lack of specificity associated with ICT toxin detection limits the application of the 3-step/multi-step algorithm proposed by the SEIMC. In our study, the high false positive rate observed with this algorithm suggests the need to revise this proposed diagnostic scheme in order to improve accuracy and reduce false positive reporting.

Funding

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Ethical considerations

This study does not in itself involve any changes or additional actions to the working procedure approved by our laboratory, and therefore approval of the study by the ethics committee is not required.

Informed consent

This study does not in itself involve any changes or additional actions to the working procedure approved by our laboratory, and therefore no informed consent is required.

Use of artificial intelligence

No artificial intelligence has been used in the drafting or proofreading of this article.

Author contributions

MCDJ contributed to the conceptualisation of the manuscript. VVG and MCDJ contributed equally to the research and analysis of the data obtained, as well as to the drafting and revision of the article. VVG carried out the statistical analysis. AP contributed to the revision of the article.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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