Evaluation of LIAISON® *C. difficile* glutamate dehydrogenase and LIAISON® *C. difficile* toxin A and B in Copan FecalSwab™ samples in a three-step algorithm for the diagnosis of *Clostridium difficile* infection

Massimo Oggioni, Alessandra Bielli, Alice Nava, Daniela Adele Pia Campisi

Chemical Clinical Analysis and Microbiology, Niguarda Ca' Granda Hospital, Milan, Italy

Summary

The presumptive laboratory diagnosis of *Clostridium difficile* infection is achieved by the means of the detection of a *common antigen* (glutamate dehydrogenase, GDH) in stool, then confirming the positives either by the detection of toxins A and B or by a molecular test for the detection of pathogenicity *locus*, encoding for the two toxins and for the binary toxin. A fully automated chemiluminescence system for the GDH antigen (LIAISON® *C. difficile* GDH) and for the detection of toxins A and B (LIAISON® *C. difficile* Toxin A and B) (DiaSorin, Gerenzano, Italy) allows for the performance of these tests on large numbers of samples in a short time, ensuring the traceability of the data.

Objective

The first phase of the study evaluated the use of LIAISON® *C. difficile* GDH test on stool samples collected using Copan FecalSwab™ (Copan Italia, Brescia, Italy), comparing them with the results obtained using stool samples collected with no transport medium.

In the second phase, using only samples collected using Copan FecalSwab™, we compared the current routine two-step algorithm (GDH + molecular), with a three-step diagnostic algorithm (GDH + Toxins A and B + molecular), assuming the molecular test being performed only on GDH-positive but negative for toxins samples.

Correspondence: Massimo Oggioni, S.C. Analisi Chimico-Cliniche e Microbiologia, Niguarda Ca' Granda Hospital, Piazza dell'Ospedale Maggiore 3, 20162 Milan, Italy.
E-mail: massimo.oggioni@ospedaleniguarda.it

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Materials and Methods

LIAISON® *C. difficile* GDH is a chemiluminescent immunoassay (CLIA) intended for use as a screening assay to detect *Clostridium difficile* antigen, glutamate dehydrogenase, in human feces from persons suspected of having *C. difficile* disease.

LIAISON® *C. difficile* Toxin A & B is an chemiluminescent immunoassay (CLIA) intended for the qualitative determination of *Clostridium difficile* toxins A and B in human feces with suspected CDAD.

EUROCLONE C. *difficile* GDH is a rapid chromatographic immunoassay for the qualitative determination of glutamate dehydrogenase (GDH) in human feces from people with suspected CDAD.

Xpert® *C. difficile* (Cepheid Inc., Sunnyvale, CA, USA) is a qualitative in-vitro diagnostic test for the rapid identification and differentiation of Toxin B, and Binary Toxin from appropriate stool specimens collected from patients suspected of having *C. difficile* infection (CDI). The test utilizes automated real-time polymerase chain reaction (PCR) to detect Toxin producing *C. difficile*, which is associated with CDI.

Results

In the first phase were tested 100 samples: 11 were positive and 89 negative by both methods (100% agreement) (Figure 1A).

Eleven GDH positive samples for both methods were tested with LIAISON® *C. difficile* Toxin A & B. 3 were positive and 6 were negative for both methods, while 2 were discordant (positive for LIAISON® *C. difficile* Toxin A&B IFU and negative for LIAISON Toxin A&B Copan fecalSwab™) (Figure 1B).

These results of the first phase confirming that the LIAISON® *C. difficile* GDH and Toxin A&B tests can be performed with stool samples collected in Copan FecalSwab™.

In the second phase 200 samples analyzed by routine laboratory immunochromatographic method for the detection of GDH were re-tested with the test LIAISON® GDH. 157 samples were negative with both methods; whereas 39 samples were GDH positive for both methods, and 4 were GDH-weakly positive with LIAISON® and negative for the routine method. (being the four negative samples for the routine method were not analyzed by the molecular method and consequently discarded) (Figure 1C).

The 39 GDH-positive samples were evaluated using either the LIAISON® *C. difficile* Toxin A & B and routine molecular method

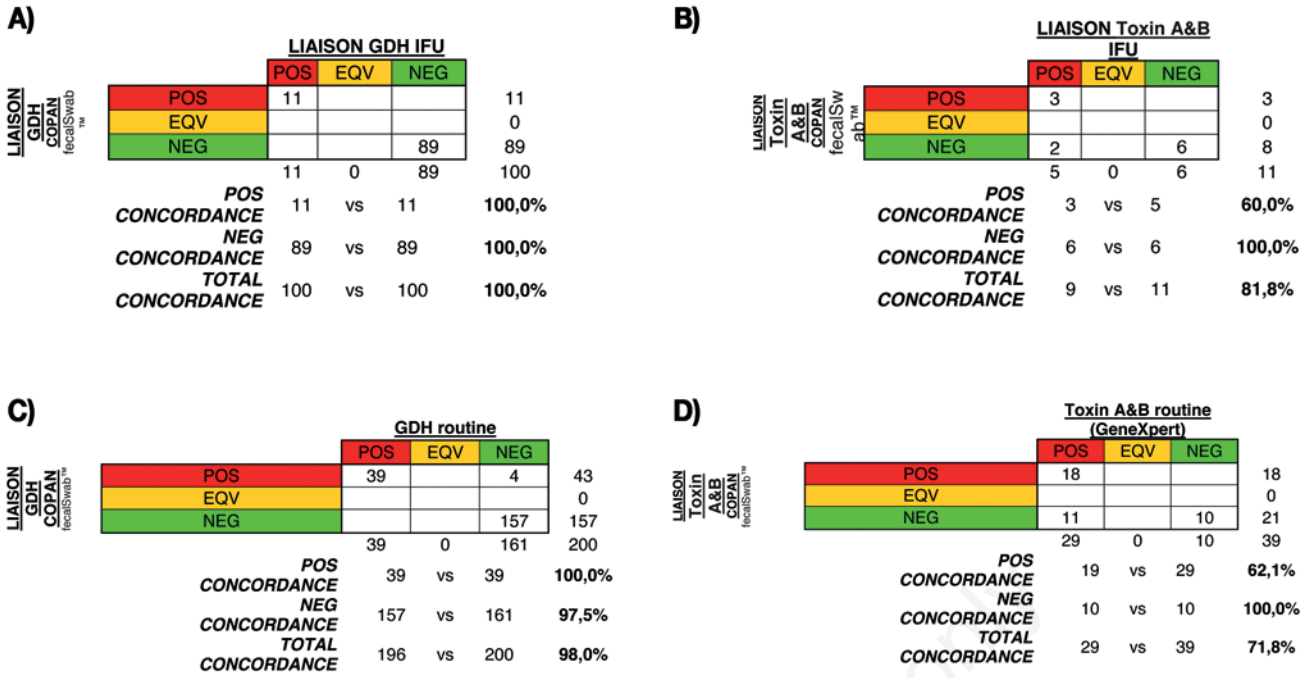

Figure 1. A) First phase of the study: 100 samples were tested for both methods; B) 11 GDH positive samples for both methods were tested with LIAISON® *C. difficile* Toxin A&B. C) In the second phase, 200 samples analyzed by routine laboratory immunochromatographic method for the detection of GDH were re-tested with the test LIAISON® GDH. D) The 39 GDH-positive samples were evaluated using either the LIAISON® *C. difficile* Toxin A & B and routine molecular method.

(GeneXpert® *C. difficile* test, Cepheid). 18 samples were positive and 10 negative by either method, whereas 11 samples were discordant (negative LIAISON® Toxin A & B and positive for the molecular test) (Figure 1D).

Conclusions

The LIAISON® test is a reliable method for detecting *C. difficile* toxins. The use of a molecular test allows for an increase in sensitivity but is significantly more expensive. Using a three-steps algorithm and performing the molecular test only if the test LIAISON® Toxin A & B was negative, only 21 samples would have been tested by the molecular method (11 discordant + 10 Toxins A and B negative), rather than 39, with a 46.2% reduction of molecular tests.

Finally, the three-step algorithm makes it possible to achieve the same results of well the two-step algorithm (GDH + molecular), however with significant time- and cost-savings by optimizing the workflow, thanks to the complete automation of the LIAISON® system.