Original article

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LABORATORY EXPERIENCE WITH THE LIAISON ANALYZER IN THE DIAGNOSIS OF *CLOSTRIDIUM DIFFICILE*-ASSOCIATED DIARRHEA

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Background: Chemiluminescent or enzyme-linked fluorescent immunoassays are commonly used to diagnose *Clostridium difficile*-associated diarrhea. *Methods:* The LIAISON analyzer (DiaSorin, Italy) was compared to miniVIDAS (bioMérieux, France) and, furthermore, to culture of toxigenic strains. In total, 249 native stool samples were analyzed. Sensitivities, specificities, and positive and negative predictive values were investigated. Furthermore, performance under routine conditions was assessed. *Results:* The glutamate dehydrogenase chemiluminescent immunoassay (GDH-CLIA) assay revealed a high sensitivity and negative predictive value. The toxins A&B assays exhibited approximately the same low sensitivity and high specificity. Technical drawbacks experienced with the LIAISON analyzer in 48% of the analyses considerably delayed the time to the first diagnostic report and interfered with laboratory routine workflow. *Conclusion:* The analytical performance of the investigated platforms should be reflected in the context of implementation into the laboratory workflow.

Keywords: *Clostridium difficile,* GDH, TcdA, TcdB, LIAISON, miniVIDAS

Introduction

The rising incidence and severity of *Clostridium difficile*-associated infection (CDI) require a rapid and accurate laboratory diagnosis supporting the therapy and prevention of this important nosocomial infection. Several algorithms have been developed to optimize the diagnostic procedure [1]. A two-step approach incorporating the detection of glutamate dehydrogenase (GDH) for screening and the detection of the exotoxins TcdA and TcdB (toxins A&B) as a confirmatory test belongs to the most widely accepted techniques recommended by American [2] and European [3] guidelines.

Among the several devices available for this two-step diagnostic procedure, we evaluated the performance of the LIAISON analyzer (DiaSorin, Italy), employing a chemiluminescent immunoassay (CLIA) to identify GDH and the toxins A&B, and compared it to the miniVIDAS testing system (bioMérieux, France), relying on an enzyme-linked fluorescent immunoassay (ELFA) to detect toxins A&B and, as a gold standard test, to stool cultures on a *C. difficile*-selective medium (CLO Agar, bioMérieux, France).

Within this study, sensitivities, specificities, and positive and negative predictive values were investigated. Furthermore, performance under routine conditions was assessed.

Materials and methods

Stool samples

A total of 249 native fecal specimens were collected from patients with a suspected CDI from September to December 2014 at a tertiary care hospital.

Stool culture

The samples were inoculated onto *C. difficile* selective agar (CLO, BioMérieux, France) and incubated at 37 °C for 48 h in an anaerobic pouch (GENBag, bioMérieux, France). The involved bacterial species from morphologically characteristic colonies was identified via latex agglutination (*C. difficile* Test Kit, Oxoid, UK).

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Identification of GDH, TcdA, and TcdB

All fecal specimens were tested with the miniVIDAS system, employing a one-step fluorescent sandwich immunoassay for the presence of toxins A&B according to the manufacturer's instructions. *C. difficile* strains isolated from toxin-negative stool samples were analyzed for toxin production with the miniVIDAS system as described previously [4]. In parallel, all samples were examined with the LIAISON platform for the presence of GDH and toxins A&B according to the manufacturer's instruction with the aim to compare its analytical performance to the miniVIDAS system. The LIAISON analyzer uses a two-step chemiluminescent sandwich immunoassay with one monoclonal antibody on paramagnetic beads for capture and polyclonal antibodies for detection of the test molecules. Gas-freed Millipore water was used to operate the system. *C. difficile* strains isolated from toxin-negative stool samples were tested for toxin production not only with miniVIDAS but also with LIAISON.

Statistics

Test results are presented as positive, negative, or equivocal. Sensitivities, specificities, and positive and negative predictive values with 95% confidence intervals were determined. The upper boundary was stated as 100% in the case of calculated values exceeding 100% *(Table 1)*.

Ethics

In our study we compared the technical performance of two CE-marked instruments by running parallel tests from noninvasively sampled material for one parameter specifically ordered by the attending physician. The set-up had the function of a quality control. Therefore, according to the German Medical Product Law our work did not require ethical approval or consent.

Results

Analytical performance

A total of 249 stool samples were examined in 46 analytical runs both with the miniVIDAS-ELFA *C. difficile* toxins A&B assay (VIDAS CDAB) and with the LIAISON CLIA *C. difficile* GDH and toxins A&B tests in comparison to toxigenic culture as a reference test. Sensitivities, specificities, and positive and negative predictive values were determined *(Table 1)*. The GDH-CLIA assay revealed a high sensitivity (98.0%; CI: 88.2–99.9) and negative predictive value (99.5%; CI: 98.4–100). The toxins A&B assays exhibited approximately the same low sensitivity and high specificity, i.e., 66.7% (CI: 52.0–78.9) with miniVIDAS versus 68.6% (CI: 54.0–80.1) with LIAISON, and 99.5% (CI: 96.8–100) with miniVIDAS versus 100% (CI: 97.6–100) with LIAISON, respectively *(Table 1)*. The two systems did not have a high positive (77%) and negative (93%) concordance since the equivocal test values of the ELFA match approximately the same number of positive and negative CLIA results. Detecting toxin production in *C. difficile* cultures of toxin-negative stool samples via CLIA and ELFA showed a 100% concordance.

Technical performance

Frequent difficulties were experienced when setting up daily controls of the LIAISON system during the test period *(Table 2),* leading to repeated error calls. Analyses were run on 46 distinct days, and on almost half of them (22 days = 48%) a total of 32 control errors were detected *(Table 2)*. Most of the errors (56%) were connected to the Light Check solution *(Table 2)*. This fluid was used for

Table 1. Comparison of the analytical performance of VIDAS CDAB and the LIAISON *C. difficile* toxins A&B and GDH tests

Test method		Toxigenic culture		Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Positive predictive value (%) (95% CI)	Negative predictive value (%) (95% CI)
		Positive	Negative				
VIDAS CDAB	Positive	34	1	66.7 (52.0–78.9)	99.5 (96.8–100)	97.1 (88.3–100)	92.1 (87.3–95.1)
	Equivocal	9	4				
	Negative	8	193				
LIAISON *C. difficile* toxins A&B	Positive	35	0	68.6 (54.0–80.1)	100 (97.6–100)	100 (87.7–100)	92.5 (87.9–95.5)
	Equivocal	0	0				
	Negative	16	198				
LIAISON *C. difficile* GDH	Positive	50	14	98.0 (88.2–99.9)	92.9 (88.2–96.0)	78.1 (65.7–87.1)	99.5 (98.4–100)
	Equivocal	0	0				
	Negative	1	184				

The analytical performance of the applied immunological test systems was determined by calculation of sensitivities, specificities, and positive and negative predictive values using the cultural detection of toxigenic *C. difficile* strains as a gold standard. For statistical analyses, equivocal results were regarded as negative

Table 2. Types of and time loss through control errors with LIAISON analyzer

Type of control error	Absolute number of control errors	Percentage of control errors (%)	Total time loss during the testing period to reporting (min)	Average daily time loss during the testing period (min)
Light check	18	56	360	8
Positive control GDH	7	22	315	7
Negative control GDH	1	3	45	1
Positive control toxins A&B	5	16	225	5
Negative control toxins A&B	1	3	45	1
Total	32	100	990	22

The average time loss through control errors of the LIAISON analyzer was calculated based on the time loss through a single error type, the absolute number of errors, and the number of days with analytical runs

functionality control of the photomultiplier and for accuracy control of pipetting. The positive controls of the toxins A&B and GDH were linked 22% and 16% of the error messages, respectively *(Table 2)*. On 8 days, double error rates occurred and even a triple error rate was observed on a single day during the testing period. For troubleshooting, all of the control tests had to be rechecked consecutively, resulting in a longer turnover time to the first diagnostic report. Repeating the Light Check added 20 min every time, and repeating the positive controls added 45 min per control to the whole test procedure. Both delays severely interfered with the daily routine workflow.

Discussion

The LIAISON analyzer is a popular chemiluminescent platform for testing clinical biochemical and serological parameters with excellent analytical performance and a complex and sensitive operative system [5]. The LIAISON test kits *C. difficile* GDH and *C. difficile* toxins A&B were employed in a two-step approach to diagnose CDI. The GDH-CLIA test showed a high sensitivity and negative predictive value; hence, it is well suited as a screening test in the two-step diagnostic approach in CDI in accordance to previous reports [6, 7]. The toxins A&B-CLIA along with the comparator ELFA on the miniVIDAS device excel with low sensitivity and high specificity in agreement with the previous reports as well [4, 8], indicating that both assays perform well as confirmatory tests. In the case of CLIA, the specificity was 100%, pointing that the LIAISON *C. difficile* toxins A&B test could be the confirmatory test of choice.

Previous reports about *C. difficile* diagnostics on the LIAISON analyzer focused only on the analytical performance but did not evaluate the technical handling of the device. With error messages on 48% of the days when test runs were performed, working with LIAISON is cumbersome and time-consuming. Test errors result in a loss of time by an average of 22 min, therefore, interfering with the daily routine workflow.

During the implementation and verification of new diagnostic methods, it is crucial to ensure that drawbacks, due to internal systematic errors, are rapidly and smoothly eliminated through appropriate preparatory training of the personnel. Our technicians were thoroughly and specially trained by the manufacturer before starting to work with the LIAISON analyzer; therefore, typical sources of errors like, e.g., the utilization of not properly gas-freed Millipore water, could have been excluded. However, dispatch of the above mentioned errors was not possible, causing a constant daily hindrance in the diagnostics of *C. difficile*-associated infection.

Conclusion

The LIAISON analyzer and the miniVIDAS system revealed a comparable analytical performance in the laboratory diagnosis of *Clostridium difficile*-associated enterocolitis. Nonetheless, due to numerous technical drawbacks experienced with the LIAISON analyzer, the time to the first diagnostic report was delayed and the laboratory workflow was consecutively hampered. Especially, these two latter issues should individually be considered when thinking about establishing a two-stage diagnostic approach to diagnose *Clostridium difficile* enterocolitis.

Conflict of interest

The authors declare that no conflicts of interests exist.

Financial disclosure

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