EVALUATION OF VIDAS® *CLOSTRIDIUM DIFFICILE* TOXIN A&B ON COLONIES

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Objective

Toxigenic culture (TC) is considered as a reference method for the diagnosis of *Clostridium difficile* infections (CDI). This method consists in isolating *C. difficile* on selective media and then assessing the *in vitro* toxins production from colonies. However determination of the toxigenic status of a strain using immunoenzymatic assays (IEA) on colonies is not always validated. The objective of this study was to assess the performance of VIDAS *Clostridium difficile* Toxin A&B (bioMérieux) (VIDAS) directly on colonies from chromID® *C. difficile* medium (bioMérieux) (chromID).

Materials and Methods

During a study where different selective media for *C. difficile* were compared, 36 strains were isolated on both chromID® C. difficile agar incubated anaerobically for 24 hours (chromID) and TCCA incubated anaerobically for 48 hours.
The *in vitro* determination of the cytotoxicity of isolates was performed by inoculating 2 to 5 colonies isolated on TCCA into BHI that was incubated for 5 days under anaerobic conditions. The supernatant from this culture was filtered and inoculated on MRC-5 cells. This method is referred as toxigenic culture (TC) (Figure 1A, 1B).
In parallel, the VIDAS *Clostridium difficile* Toxin A&B (VIDAS CDAB) test was performed on colonies from chromID (from primary culture or subculture to obtain sufficient colonies) (Figure 2A). Colonies were homogenized with water to reach a 3 McFarland density. VIDAS tests were then performed according to the manufacturer's instructions (Figure 2B). The VIDAS tests were repeated whenever results were equivocal (VT ≥0.13 and <0.37) or discordant (positive-TC and negative-VIDAS result or negative-TC and positive-VIDAS result).

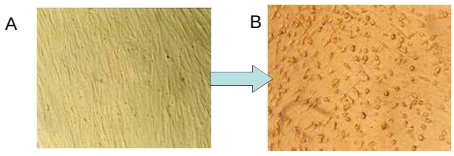

Fig.1. A. MRC5 cells. **B.** cytopathic effect on MRC5 cells due to the toxin B

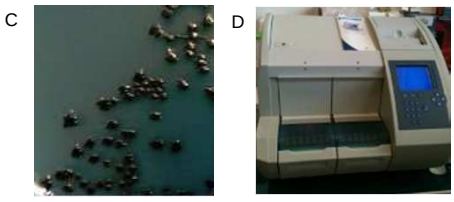

Fig 2 A. *C. difficile* colonies on chromID *C. difficile* agar. **B.** miniVIDAS (bioMérieux)

Results

Among the 36 strains studied, 27 (75%) were positive and 9 (25%) negative by TC.

There was sufficient colonies on chromID agar in 29 cases (80.6%) allowing to perform VIDAS assay on colonies from primary culture. In 7 cases (19.4%) a subculture was necessary to have sufficient colonies to reach a 3 McFarland density.

Results were equivocal in 6 cases (16.7%). After repeating the assay results remained equivocal in one case (2.8%). The sensitivity and specificity for VIDAS on colonies from chromID compared to TC were 70.4% [49.7-85.5] and 87.5% [46.7-99.3], respectively (Table 1).

Table 1. Performances of the VIDAS CDAB for the detection of toxigenic strains of *C. difficile*

	TC			
		+	-	N=
VIDAS on colonies	+	19	1	20
	-	8*	7	15
	N=	27	8	35
Equivocal result			1	

*7 Vidas assays were performed on primary culture

	VIDAS on colonies/TC
Sensitivity (%) (CI 95%)	70.4 (49.7-85.5)
Specificity (%) (CI 95%)	87.5 (46.7-99.3)

After resolving discordant results, the sensitivity and specificity for VIDAS on colonies from chromID compared to TC were 83.3% [61.8-94.5] and 100% [59.8-100], respectively (Table 2). Results were equivocal in 4 cases (11.1%).

Table 2. Performances of the VIDAS CDAB for the detection of toxigenic strains of *C. difficile* after resolving discrepant results

	TC			
		+	-	N=
VIDAS on colonies	+	20	0	20
	-	4	8	12
	N=	24	8	32
Equivocal result		3	1	

	VIDAS on colonies/TC
Sensitivity (%) (CI 95%)	83.3 (61.8-94.5)
Specificity (%) (CI 95%)	100 (59.8-100)

Conclusions

VIDAS *Clostridium difficile* Toxin A&B performed directly on colonies from chromID® C. difficile agar (24h) is a simple and rapid test to confirm the toxigenicity of the microorganism, when sufficient colonies are present on the medium. However, negative results must be confirmed by a reference test.