Evaluation of the impact of freezing on positive *Clostridium difficile* stools using VIDAS® GDH & VIDAS® CD toxins A/B, Liaison GDH, Liaison toxins A&B and C. DIF QUIK CHEK Complete®.

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Introduction

- During the early years of the 21st century, the incidence and severity of *C. difficile* infections (CDI) increase rapidly in North America and Europe.
- The rapid emergence and spread of a specific clone of *C. difficile* was rapidly demonstrated and is associated with the overproduction of toxins A and B and the production of binary toxin.
- Diagnostic strategies should aim at a same-day diagnosis in case of suspicion of CDI to support immediate treatment of the patient and limit the risk of cross-contamination.
- Since October 2011, the scheme for diagnosis of *Clostridium difficile* infection (CDI) in our laboratory (fig.1) has been based on an algorithm testing glutamate-dehydrogenase (GDH) and Toxins A & B on all samples followed by toxin gene amplification on GDH +ve Toxins A & B -ve. Toxigenic culture (TC) was performed on all stool samples.
- This approach has demonstrated a much better sensitivity than EIA on stool alone and a better specificity than culture alone (Delmée et al. 2005).

Objectives

- Investigation on CDI is limited by the unknown impact of freezing on the stools. The objective of this study was to evaluate the impact of freezing (-70°C) fresh positive *C.difficile* stools on the detection of GDH and Toxins A & B, using two automated immunoanalysers and a rapid test for the detection.

Methods

- **Stools** from inpatients (>2 years old) within symptoms of antimicrobial- or chemotherapy-associated diarrhoea. 100 culture positive stools collected over an 8 month period (between April and November 2013) were tested and frozen at -70°C.
- **Sample preparation:** a single stool suspension was made using a minimum of physiological saline solution.
- **Methods** Fresh positive stools were characterized with chromID® *C.difficile* culture media (bioMérieux S.A., Marcy L'Etoile, France), C. DIF QUIK CHEK Complete® (QCC) from Techlab (Blacksburg, VA,USA) and toxigenic culture.
- After diagnosis, the stools were frozen and stored at -70°C until testing. After thawing, stools were tested with ChromID® C.difficile culture media and a single stool suspension was tested on QCC, VIDAS® *C.difficile* GDH and CDA/B (bioMérieux S.A., Marcy L'Etoile, France), Liaison® *C.difficile* GDH and Tox A&B assays (Diasorin, Stillwater, MN USA).
- Toxigenic culture, used as GS, was performed by testing *C.difficile* colonies for toxin production. Discordant results were analysed by XPert *C.difficile* (Cepheid, Sunnyvale, CA 94089, USA).

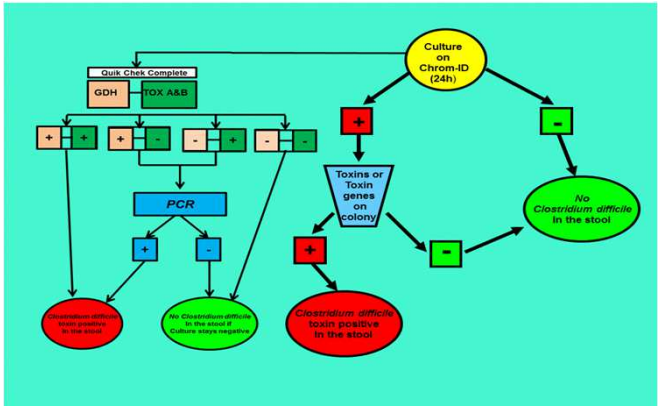

Fig. 1: Current diagnostic algorithm at the National Reference laboratory

Results

- Out of 100 routine diarrheal stool samples that were positive for *C. difficile*, 95 were positive after thawing

Table 1: Perfromance of GDH EIA vs culture results on chromID after 24h reading on frozen stools

	BACTERIAL CULTURE		
	QCC GDH	VIDAS GDH	LIAISON GDH
Sensitivity	93.7% [86.8-97.7]	94.7% [88.1-98.3]	94.7% [88.1-98.3]*

*For Liaison, 3 samples were equivocal and after restesting, 1 became positive and 2 negative

- Out of 87 positive fresh sample on toxigenic culture, 82 samples found positive after thawing

Table 2: Toxigenicity vs Toxigenic culture results on frozen stools

	TOXIGENIC CULTURE		
	QCC GDH+ and A&B or PCR +	VIDAS GDH+ and A&B or PCR +	LIAISON GDH+ and A&B or PCR +
Sensitivity	85.4% [75.8-92.2]	84.2% [74.4-91.3]	85.4% [75.8-92.2]

Results

- From April to Nov 2013, 100 routine diarrheal stool samples that were positive for *C. difficile* were obtained.
- 87 stools were toxigenic culture positive & 13 stools were toxigenic culture negative.
- All were stored at -70°C.
- After thawing 95/100 samples were positive on culture and 82/87 positive on toxigenic culture.
- From the 5 negative stools with bacterial culture after thawing, 3 were positive for VIDAS GDH, 1 for Liaison GDH and 1 for QCC GDH.
- Three stools gave an equivocal result for the Liaison® *C.difficile* GDH test. In this case, the flex-system automatically performs the Liaison® *C.difficile* Tox A&B test and all three remained negative. One stool gave an equivocal result for the Liaison® *C.difficile* Tox A&B, and, when repeated, the result was negative.
- There were no equivocal results with VIDAS® GDH.
- 15 stools gave an equivocal result with VIDAS® *Clostridium difficile* TOX A&B, and when repeated two became positive and 13 remained equivocal.

Conclusion

- In this study, 5% of positive *C. difficile* stool samples on culture became negative after freezing and 6% of positive toxigenic stool samples became negative after freezing.
- **VIDAS® GDH shows the most robust performance after the freezing step**.
- Sensitivities of either the 3-step algorithms including VIDAS® *Clostridium difficile* GDH and Toxins A&B or Liaison® *C.difficile* GDH and Toxins A & B are comparable.
- Both VIDAS and Liaison EIA methods can be part of a 3-step algorithm allowing easier interpretation and traceability of results.
- According to European (ESCMID) guidelines, immuno-enzymatic tests that detect toxins lack sensitivity and cannot be used as stand-alone tests for the diagnosis of CDI.

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