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 Closstridium 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 CDAB (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).

Results

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

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

| BACTERIAL CULTURE | GDH | VDAS | Liaison
|-------------------|-----|------|------
| Sensitivity | 93.7% | 94.7% | 94.7%
| [86.9-97.7] | [88.1-98.3] | [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: Toxicigenicity vs Toxigenic culture results on frozen stools

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

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® C. 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.

References


Dionne et al. (2013). “Correlation between Closstridium difficile bacterial load, commercial real-time PCR cycle thresholds and results of diagnostic tests based on enzyme immunoassay and cell-culture cytotoxicity assay.” J Clin Microbiol. Aug 2013


Oredt GB et al. “A two-stage algorithm for Closstridium difficile including FOC can be replaced with the EIA.” J Med Microbiol 2011:1-3

