A TWO STEP ALGORITHM FOR THE DIAGNOSIS OF *CLOSTRIDIUM DIFFICILE* INFECTION: SCREENING WITH A RAPID IMMUNOASSAY FOR THE DETECTION OF GLUTAMATE-DEHYDROGENASE AND TOXINS A AND B FOLLOWED BY A REAL-TIME PCR FOR *CLOSTRIDIUM DIFFICILE*

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Poster

Introduction

Since 1996, the diagnosis scheme for *Clostridium difficile* infection (CDI) in our laboratory (fig.1a) has been based on both direct faecal-cytotoxicity assay (CTA) and toxigenic culture (TC). The latter consists of culture of faeces on selective medium and detection of toxin production on colonies by enzyme immuno-assay (EIA); it has demonstrated a much better sensitivity than CTA alone and a better specificity than culture alone (Delmée et al. 2005). It is however a very slow procedure : it takes at least an overnight incubation to get a result.

Aiming at providing more rapid and accurate results, we evaluated an algorithm testing glutamate-dehydrogenase (GDH) and Tox A&B on all samples followed by a RT-PCR on GDH+ Tox A&B- samples (fig3a). We compared this algorithm with RT-PCR on all samples (fig2a).

Abstract

Aim:
The Techlab ® C. diff Quik Chek Complete ™ (Techlab Blacksburg,VA,USA) is a rapid immunoassay for the detection of Glutamatedehydrogenase and toxins A and B in fecal specimens, using specific antibodies. The GeneXpert ® C difficile (Cepheid Sunnyvale, CA 94089, USA) is a real-time PCR assay targeting the toxin B gene (TcdB) of *Clostridium difficile*, binary toxin and a deletion in the tcdC gene with a fluorogenic target-specific hybridization probe for the detection of the amplified DNA. With toxigenic culture (TC) as gold standard, we evaluated the performances of the combination of these tests for the diagnosis of *C. difficile* infections (CDI) on stool specimens.

Materials and methods:
Stools were from inpatients of the St Luc-UCL University hospital (890 beds), older than 2 years and suffering from diarrhoea. Cell cytotoxicity (CTA) was performed on MRC5 cells. Cultures were performed on CCFA. In case of positive culture and negative CTA, colonies were tested for 'in vitro' toxin production (TC). Specimens giving discordant results were repeated on CCFA with added taurocholate (TCCFA). Real-time PCR and the rapid immunoassay were performed according to the manufacturer's instructions.

Results: A total of 236 stool specimens were freshly tested during march and april 2010 , by GeneXpert. Twentythree samples were shown to contain toxigenic *C. difficile* by CTA and/or toxigenic culture (prevalence : 9,75%). Five specimens gave invalid results by GeneXpert. The sensitivity, specificity, PPV and NPV of GeneXpert were respectively : 87%, 96,7%, 74% and 98,6%. Those of C. diff Quik Chek Complete ™ were for GDH: 95,7%, 93,9%, 62,7% and 99,5%, for toxin A&B: 69,6%, 100%, 100% and 96,8%. CTA values were 69,6%, 99%, 88,9% and 96,8% . Screening with C. diff Quik Chek Complete ™ followed by GeneXpert on GDH positive, toxins A and B negative samples gave sensitivity, specificity, PPV and NPV of respectively : 82,6%, 98%, 82,6% and 98,1%.

Conclusion: The combination of theTechlab ® C. diff Quik Chek Complete ™ and GeneXpert ® Cdifficile demonstrated a very good sensitivity much superior to that of CTA and a good specificity. It is a rapid method allowing result in less than two hours.

Materials and methods

Stools: were from inpatients (>2y) suffering from antimicrobial- or chemo-therapy associated diarrhoea.

270 stools were tested : 236 (87.4%) were fresh stool samples collected in 2010 and 34 (12,6%) were stool samples collected over a 24 months period and kept at –80°C. The prevalence of positive culture samples was 9,75% for the prospective samples and 20% for all samples.

Cultures: on CCFA (twice overnight anaerobic incubation

CTA: sterile faecal filtrate on MRC-5 cells

CCFAT: CCFA + sodium taurocholate.

Toxigenic culture (TC): colonies on CCFA are picked up, mixed in the EIA sample diluent and tested for toxA&B as for faecal specimens (C. Diff Quik Chek Complete™ (TechLab, Blacksburg VA USA).

Two tests were evaluated :

-**Immunoassay**

C.Diff Quik Chek Complete™ (TechLab, Blacksburg VA USA)

→ GDH and tox A&B

-**real-time PCR**

GeneXpert ® C difficile (Cepheid Sunnyvale, CA 94089, USA)

→ toxin B gene (TcdB)

TC was considered as the Gold Standard.

Diagnostic algorithms

The performances of a two-step algorithm (GDH and Tox A&B), followed by RT-PCR on GDH (+) Tox A&B (-) stools, was compared with the one step RT-PCR algorithm and the two-step algorithm (GDH and Tox A&B), followed by TC on GDH (+) Tox A&B (-) stools with TC on all samples as Gold Standard.

Fig. 4 Age and gender distribution

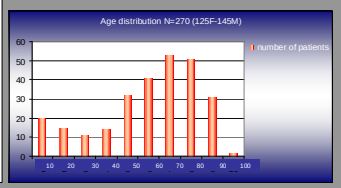


Fig. 1a
Usual scheme for the diagnosis of CDI, toxigenic culture

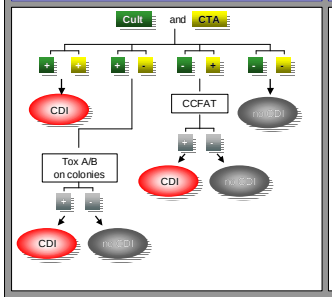


Fig. 2a
One-step algorithm RT-PCR on all samples

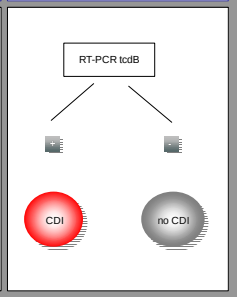


Fig. 3a
Proposed Two-step algorithm: GDH and Tox A&B on all samples followed by RT-PCR on samples GDH + Tox A&B -

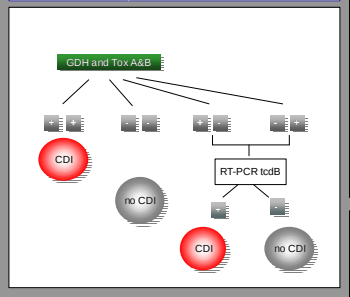


Table 1a
Results of prospective samples for cellcytotoxicity

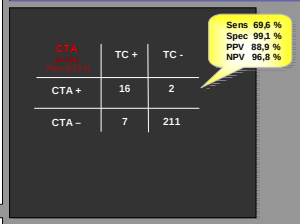


Table 2a
Results of prospective samples for RT-PCR

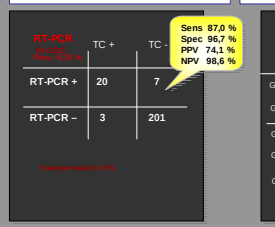


Table 3a
Results of prospective samples for Two-step algorithm: GDH and Tox A&B on all samples followed by RT-PCR on samples GDH + Tox A&B -

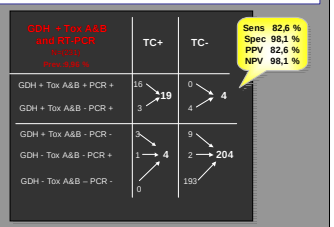


Table 1b
Results of retrospective and prospective samples for cellcytotoxicity

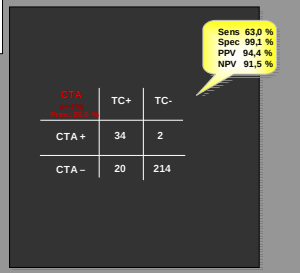


Table 2b
Results of retrospective and prospective samples for RT-PCR

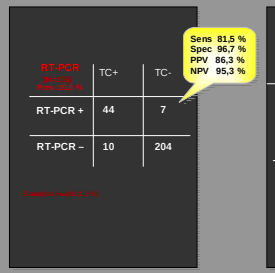
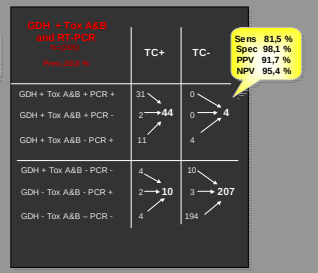


Table 3b
Results of retrospective and prospective samples for Two-step algorithm: GDH and Tox A&B on all samples followed by RT-PCR on samples GDH + Tox A&B -



Results

Table 1a shows results of cellcytotoxicity on prospective stool samples whereas table 1b shows results of cellcytotoxicity on prospective and retrospective stool samples. Table 2a shows the performances of RT-PCR on prospective stool samples and table 2b on all samples. Table 3a shows the results of the two-step algorithm (GDH and Tox A&B), followed by RT-PCR on GDH+ Tox A&B- stools, on prospective stool samples. Table 3b gives the results of the two-step algorithm (GDH and Tox A&B), followed by RT-PCR on all samples.

Discussion

- Screening fresh stools with GDH and ToxA&B, followed by an RT-PCR on GDH + and Tox A&B - samples, shows a good NPV (98,1%). With a sensitivity of 82,6%, against 86,96% for RT-PCR on all samples and 69,6% for CTA, this algorithm can be envisaged as a good method for the detection of *C. difficile* in faecal specimens, in less than 2 hours.
- With such algorithm, one missed 4/23 (17%) positive specimens, 1 due to the GDH and Tox A&B screening and 3 due to the RT-PCR. We obtained 4 false positive results, all 4 became positive on CCFA plates aditionned with sodiumtaurocholate.
- The algorithm required less RT-PCRs (19) than performing RT-PCR on all samples (236), which is financially reasonable, although 236 rapid tests, (GDH and Tox A&B), need to be performed.
- In frozen (-80°C) stool samples, the sensitivity of both GDH+Tox A&B and RT-PCR was lower, whereas toxigenic culture gave the best results.

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