Evaluation of a new Glutamate dehydrogenase immuno assay in a two step algorithm for the diagnosis of Clostridium difficile infection.

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Introduction

During the first years of the 21st century, the epidemiology of C. difficile infections (CDI) dramatically changed in North America and Europe. A significant increase of incidence as well as of severity of CDI were reported on both sides of the Atlantic ocean. The rapid emergence and spread of a specific clone of C. difficile was rapidly demonstrated. The increased virulence of this clone is associated with the overproduction of toxins A and B and the production of binary toxin. Primarily detected in North America, C. difficile -027 - was rapidly identified in outbreaks that occurred in several european countries (UK, The Netherlands, Belgium and France).

All strategies should aim at a same-day diagnosis in case of suspicion of CDI. In case of a positive result, immediate treatment of the patient will improve his condition and limit the risk of room contamination. And the rapid implementation of hygiene measures will prevent further spread of the disease. With such a goal and such implications however, the accuracy of the laboratory diagnosis is of crucial importance.

False positive results may induce inadequate treatment and increase cost due to isolation procedures and false negative results may lead to outbreaks.

Since October 2011, the diagnosis scheme for Clostridium difficile associated diarrhea (CAD) in our laboratory (Fig.1a) has been based on an algorithm testing glutamate-dehydrogenase (GDH) and Tox A&B on all samples followed by a Loop-mediated toxin gene amplification (LAMP) on GDH+ Tox A&B samples. Toxicogenic Culture (TC) was performed on all stool samples as a reference method (Fig 1b).

The latter consists of culture of faeces on selective medium and detection of toxin production on colonies by enzyme immunoassay (EIA); it has demonstrated a much better sensitivity than EIA on stools alone and a better specificity than culture alone (Delmée et al. 2005).

The negative predictive value of the GDH test is crucial. Here we evaluated a new GDH detection system: Clostridium K-Set (K-SeT) (Coris BioConcept, Gembloux, Belgium).

Materials and methods

Stools: were from inpatients suffering from antimicrobial or chemotherapy associated diarrhea. 259 stools were collected between October and December 2011. The prevalence of positive culture samples was 8.5%.

Cultures: on Cytoceine cefoxitine Fructose Agar (CCFA) with twice overnight anaerobic incubation

Toxicogenic culture (TC): colonies on CCFA are picked up, mixed in the EIA sample diluent and tested for tox A&B as for faecal specimens.

illumigene Lamp Method: (Meridian, Cincinnati, OH USA) The test was performed according to the manufacturer’s instructions.

Culture was performed on all samples and Toxicogenic Culture was considered as the Gold Standard.

Two tests were evaluated:

Immunosay:

C.Diff. Quik Check Complete® (TechLab, Blacksburg VA, USA)

> GDH (+ tox) (30 minutes)

Colloidal Gold membrane technology:

Clostridium K-SeT: (K-SeT) (Coris BioConcept Gembloux, Belgium).

> GDH (15 minutes)

Diagnostic algorithm

On all diarrheal stools, GDH detection followed by illumigene on GDH positive stools

Discussion

The negative predictive value of a GDH test is crucial when a GDH algorithm is used to perform a screening test in clostridium difficile detection. With a single false negative GDH among 100 stools (1%) one could miss, in case of a prevalence of 10%, 10% of the positive stools.

The choice of a performant GDH test is, hence, of primary importance.

In this study on 259 samples, the Clostridium K-SeT GDH test from Coris BioConcept (Gembloux, Belgium) gave a negative predictive value of 100%. It detected all C. difficile toxin positive and toxigenic negative samples.

The test is easy to perform and takes only 15 minutes. All reagents can be kept at room temperature.

When we used the Clostridium K-SeT GDH test as a screening test followed by the illumigene toxin A gene detection, we needed to perform the illumigene test on 15.4% of the samples.

The new K-SeT GDH assay is an excellent new test for the detection of C. difficile in stool samples. The use of the illumigene LAMP method on GDH positive samples allows a very sensitive detection of toxigenic strains in the stool within a few hours.

Results

Table 2: K-SeT and Quik Check® results

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<thead>
<tr>
<th>N=259</th>
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<th>K-SeT +</th>
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Patients with positive GDH + illumigene were considered as positives.

Table 1: GDH activity against C. difficile

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<th>Culture -</th>
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The new K-SeT kit was evaluated with TC as gold standard. It was compared on the same cohort of the Quik Check Complete® GDH from TechLab (Blacksburg, VA USA) in a two step algorithm as follows: all stools were screened by both GDH. All positive were tested by illumigene (Igene) from Meridian (Cincinnati,OH,USA) which is a loop-mediated toxin gene amplification (LAMP) targeting the toxin A and B genes. A positive result was defined by a positive signal on both tests. The specificity of each GDH test was 100% and sensitivity for illumigene of 99.6% and 100%.

Results

From Oct to Dec 2011, 259 routine diarrheal stool samples from our University Hospital St Luc, were tested. Culture was positive in 20 cases and illumigene was positive in 22 cases (prevalence 8%).

The specificity of K-SeT was 100% (80/80) and of the illumigene 99.6% (22/22). The sensitivity of K-SeT was 100% (100/100) and of the illumigene 100% (22/22). The positive predictive value of the K-SeT and Quik Check® was respectively 100% and 99.6%.

Discussion

The two tests were complementary: the K-SeT was used as a screening test and illumigene was used for confirmatory test.

Conclusions

The K-SeT GDH assay is an excellent new test for the detection of C. difficile in stool samples. The use of the illumigene LAMP method on GDH positive samples allows a very sensitive detection of toxigenic strains in the stool within a few hours.

References


Orenzi et al. The two step algorithm for Clostridium difficile including PCR: can we replace the toxin ELISA?” Journal of Hospital Infection (2011) 1:3