Glutamatedehydrogenase (GDH) screening with toxin detection followed by a nucleic acid amplification test (NAAT) is accepted by the European guidelines as a good two or three step algorithm for the detection of toxigenic Clostridium difficile in stool (2). Since October 2011, the diagnosis scheme for Clostridium difficile associated diarrea (CAD) in our laboratory (Fig.1) has been based on an algorithm testing glutamate-dehydrogenase (GDH) and Tox A&B on all samples followed by a toxin gene amplification on GDH+ Tox A&B. Toxigenic Culture (TC) was performed on all stool samples as a reference method (Fig 1). The latter consists of culture of faeces on selective medium and detection of toxin production on colonies by enzyme immunoassay (EIA) and cytopathogenic effect (CPE); it has demonstrated a much better sensitivity than the ELISA in using immune rapid tests is the reading. Since each human eye is different this comprises an objective reading. This study had three objectives: first to estimate the performance of the Clostridium K-Set and GDH-Strip test against three other methods (Culture, CUG and Liaison GDH test) used routinely in the reference laboratory, secondly to evaluate the use of two Laser readers the Skan-Smart (Skanneex) and the aLfi (Qiagen) and finally test the usability of the readers in the laboratory.

**Introduction**

Glutamatedehydrogenase (GDH) screening with toxin detection followed by a nucleic acid amplification test (NAAT) is accepted by the European guidelines as a good two or three step algorithm for the detection of toxigenic Clostridium difficile in stool.

**Methods**

Stools were collected from inpatients at the University Hospital St.-Luc - UCL suffering from diarrea. Between March 2018 and April 2018, 208 stools were tested for GDH using the Liaison® C dificile GDH assay (DiaSorin, Stillwater, USA), the Quik Chek Complete (TechLab, Blacksburg, USA), the Quick Chek® toxigenic stool antigen (Diasorin, Stillwater, USA) and the Skansmart® GDH (Skanneex, Oslo, Norway).

Cultures were performed on ChromID® C. diff (bioMeBiux); NAAT was performed using the C. difficile LIAISON® MDX. The rapid GDH test was read visually by two different persons and two different laser scanners the aLfi (Qiagen, Hilden, Germany) and the Skan-Smart (Skanneex, Oslo, Norway).

**Results**

Visual reading: Visual reading by two persons independently at the same time reveals 3.9% discordant results (results not shown). Operator 2 reads 4 out of 23 this false positive results, which leads to more molecular biology tests to perform in our algorithm.

Traceability after visual reading is nonexistent unless you make a picture of the cassette.

**Abstract**

7 Dehncke M, et al. "Emergence of Clostridium difficile in hospitals in the USA."
8 Kugler E, et al. "Epidemiology and clinical characteristics of Clostridium difficile infection."
11 Dehncke M, et al. "Emergence of Clostridium difficile in hospitals in the USA."

**Discussion and conclusion**

The sensitivity of a GDH test is crucial when a GDH algorithm is used to perform a screening test in Clostridium difficile detection. One false negative sample on 100 stools for GDH (1%), could lead to - in case of a prevalence of 10% - 10 false negative results! The choice of a performing GDH test is important. Visual reading by two persons gave discordant results in 3.9%. Liaison GDH gave a sensitivity of 96.3%. Lasercan reading with Skansmart enhanced sensitivity for GDH from 88.6% to 95.5%. Afterwards we recalculated the readings with a cut-off value of 2.3 which leads to a sensitivity loss of 0.2% but a gain in specificity of 1.7%.

**Materials and methods**

**Methods**

Stools: From March to April 2018, 206 routine diarrheal stool samples from the St.-Luc University Hospital were tested following the algorithm here below (Fig 1).

Hospital patients and outpatients (141/262)
Culture: on chromID® C difficile (bioMeBiux, Lyon, France) overnight anaerobic incubation (4).

Toxigenic culture: (EUCLID). 48 hours culture colonies supumants were tested by cell-cytotoxicity on MRC-5 cells.

EIA Screening of GDH: C.Diff Quik Chek Complete® (QCC) (Techlab, Blacksburg VA USA) or Liaison® C difficile (DiaSorin, Stillwater, USA) or the Clostridium K-Set® (Cos Biocorpus, Camillus, Belgium).

Laser reading: The Clostridium K-Set was read with the aLfi scanner (Qiagen, Hilden, Germany) and the Skan-Smart (Skanneex, Oslo, Norway).

EIA Screening of Toxin A&B: C.Diff Quik Chek Complete® (QCC) (Techlab, Blacksburg VA USA) or Liaison® C difficile (DiaSorin, Stillwater, USA).

RT-PCR toxin B gene (codB): LIASON®MDX (DiaSorin Molecular LLC, Cypress, CA, USA) detects toxin B gene (codB).

All tests were performed according to the manufacturer’s instructions.

**Discussion and conclusion**

The sensitivity of a GDH test is crucial when a GDH algorithm is used to perform a screening test in Clostridium difficile detection. One false negative sample on 100 stools for GDH (1%), could lead to - in case of a prevalence of 10% - 10 false negative results! The choice of a performing GDH test is important. Visual reading by two persons gave discordant results in 3.9%. Liaison GDH gave a sensitivity of 96.3%. Lasercan reading with Skansmart enhanced sensitivity for GDH from 88.6% to 95.5%. Afterwards visual reading Lasercan reading with the aLfi enhanced sensitivity for GDH from 88.6% to 90.7% towards visual reading. Skan-Smart reading generated less false negative GDH samples (N=2) than aLfi reading (N=4) or visual reading (N=5). Adapting cut-off values leads to less sensitivity but higher specificity.

Lasercan reading gives not only an objective traceable reading but it also enhances sensitivity of GDH detection in the Clostridium K-Set. In small numbers the variability of weak or strong positive samples is predominat.