ENHANCED SENSIBILITY AND OBJECTIVITY BY LASER READING OF RAPID TESTS FOR *Clostridium difficile* GDH DETECTION

Van Broeck Johan [1], Ochoa Theysa[1] and Delmée Michel [1]

[1] *Belgian Reference Centre for Clostridium difficile, Pôle de microbiologie médicale, Université Catholique de Louvain, Brussels, Belgium (johan.vanbroeck@uclouvain.be)*

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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 (2). Since October 2011, the diagnosis scheme for *Clostridium difficile* associated diarrhea (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 EIA on stools alone and a better specificity than culture alone (4). One of the 'pitfalls' in using immuno-rapid tests is the reading. Since each human eye is different this compromises an objective reading. This study had three objectives: first to estimate the performance of the Clostridium *K*-SeT and C diff-strip test against three other methods (Culture, QCC and Liaison GDH test) used routinely in the reference laboratory, secondly to evaluate the use of two Laser readers the Skan-Smart (Skannex) and the aLf (Qiagen) and finaly test the usability of the readers in the laboratory.

Diagnostic algorithm

On all diarrheal stools: GDH detection followed by PCR on GDH positive stools	On all diarrheal stools: culture followed by toxin or toxin gene detection on colonies in case of discordant results

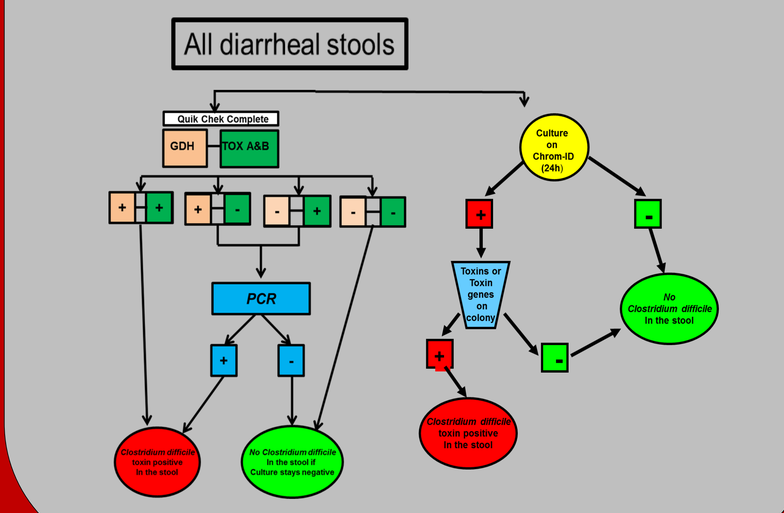
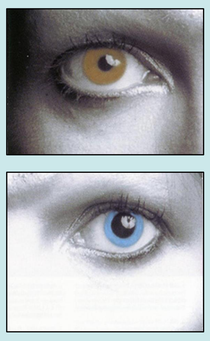


Fig. 1: diagnostic algorithm

Results



			SE: 88,6 %
N=206	cult POS	cult NEG	SP: 87,7 %
GDH K-Set operator 1 POS	39	20	PPV: 66,1 %
GDH K-Set operator 1 NEG	5	142	NPV: 96,6 %
			Reliability: 87,9 %

			SE: 88,6 %
N=206	cult POS	cult NEG	SP: 85,2 %
GDH K-Set operator 2 POS	39	24	PPV: 61,9 %
GDH K-Set operator 2 NEG	5	138	NPV: 96,5 %
			Reliability: 85,9 %

Visual reading

Visual reading by two persons independently at the same time reveals 3,9% discordant results (results not shown). Operator 2 reads 4 more 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.

N=206	cult POS	cult NEG	SE: 95,5 %
cut-off test line 2.0			SP: 84,6 %
GDH K-Set Skansmart POS	42	25	PPV: 62,7 %
GDH K-Set Skansmart NEG	2	137	NPV: 98,6 %
			Reliability: 86,8 %

N=206	cult POS	cult NEG	SE: 95,3 %
cut-off test line 2.3			SP: 86,3 %
GDH K-Set Skansmart POS	41	22	PPV: 65,1 %
GDH K-Set Skansmart NEG	2	139	NPV: 98,6 %
			Reliability: 88,2 %

SkanSmart laser reading

The laser lecture with the Skansmart was performed using a cut-off value 2.0 This enhanced the 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%.

N=200 (6 invalid)	cult POS	cult NEG	SE: 90,7 %
cut-off test line 23			SP: 78,3 %
GDH K-Set aLf reader POS	39	34	PPV: 53,4 %
GDH K-Set aLf reader NEG	4	123	NPV: 96,9 %
			Reliability: 81 %

N=200 (6 invalid)	cult POS	cult NEG	SE: 88,6 %
cut-off test line 44			SP: 87,7 %
GDH K-Set aLf reader POS	39	20	PPV: 66,1 %
GDH K-Set aLf reader NEG	5	142	NPV: 96,6 %
			Reliability: 87,9 %

Qiagen aLf laser reading

The laser lecture with the aLf (Qiagen) reader was performed using a cut-off value 23 This enhanced the sensitivity for GDH from 88,6% to 90,7%. Afterwards we recalculated the readings with a cut-off value of 44 which lead to a sensitivity loss of 2,1% but a gain in specificity of 9,4%.

	VISUAL READING		
N=205	POS	NEG	
cut-off test line 2,3			Reliability: 95,6 %
Skansmart POS	57	7	
Skansmart NEG	2	139	

	VISUAL READING		
N=204	POS	NEG	
cut-off test line 44			
aLf reader POS	56	2	Reliability: 98 %
aLf reader NEG	2	144	

Comparison of the visual reading of Coris tests with that of the two readers

The cut-off values of readers are those defined above. The agreement between both readers and the visual reading are calculated.

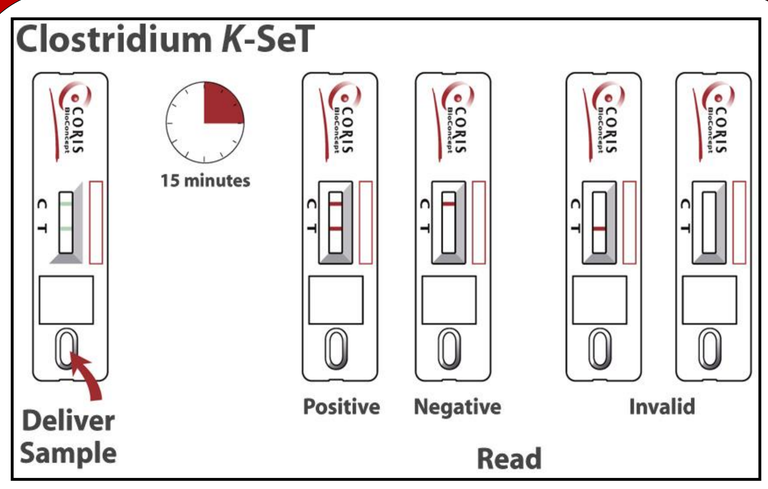


Fig. 4 : *Clostridium K*-Set possible results

Performances off the different GDH tests

			SE: 96,3 %
N=128	cult POS	cult NEG	SP: 96 %
GDH Liaison XL POS	26	4	PPV: 86,7 %
GDH Liaison XL NEG	1	97	NPV: 86,7 %
			Reliability: 96,9 %

			SE: 94,1 %
N=78	cult POS	cult NEG	SP: 98,4 %
GDH QCC POS	16	1	PPV: 94,1 %
GDH QCC NEG	1	60	NPV: 98,4 %
			Reliability: 97,4 %

			SE: 90,9 %
N=206	cult POS	cult NEG	SP: 87 %
GDH C diff-Strip POS	40	21	PPV: 65,6 %
GDH C diff-strip NEG	4	141	NPV: 97,2 %
			Reliability: 87,9 %

Materials and methods

Stools: From March to April 2018, 206 routine diarrheal stool samples from our St Luc University Hospital were tested following the algorithm here beside (Fig.1)
Hospital patients and outpatients (147/32)
Culture: on chromID® *C.difficile* (bioMérieux, Lyon, France) overnight anaerobic incubation (4).
Toxigenic culture (TC): 48 hours culture colonies supernatants 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,MN USA) and the Clostridium *K*-SeT (Coris BioConcept, Gembloux, Belgium).
Lazer reading : The Clostridium *K*-SeT was read with the aLF scanner (Qiagen, Hilden, Germany) and the Skan-Smart (Skannex, Oslo, Norway).
EIA Screening of Toxin A&B: C.Diff Quik Chek Complete™ (QCC) (TechLab, Blacksburg VA USA) or Liaison® *C.difficile* (Diasorin, Stillwater,MN USA).
RT-PCR toxin B gene (*tcd*B): LIAISON®MDX (MDX) (Diasorin Molecular LLC, Cypress , CA, USA) detects toxin B gene (*tcd*B).

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

Fig.2: Skan-Smart

Fig.3: the aLF scanner

Abstract

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 diarrhea. Between March 2018 and April 2018, 206 stools were tested for GDH using the Liaison® *C. difficile* GDH assay (Diasorin, Stillwater, USA), the Quik Chek Complete (Techlab® Blacksburg, USA) and the Clostridium *K*-SeT (Coris BioConcept, Gembloux, Belgium). Cultures were performed on ChromID® C. *diff* (bioMérieux). 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 aLF (Qiagen, Hilden, Germany) and the Skan-Smart (Skannex, Oslo, Norway).

Results: Visual reading by two persons gave discordant results in 3.9%. Liaison GDH gave a sensitivity of 96.3%. Laserscan reading with Skansmart enhanced sensitivity for GDH from 88.6% to 95.5% towards visual reading. Laserscan reading with the aLF enhanced sensitivity for GDH from 88.6% to 90.7% towards visual reading. Skansmart reading generated less false negative GDH samples (N=2) than aLF reading (N=4) or visual reading (N=5).

Discussion and conclusion:

Laserscan reading gives not only an objective traceable reading but it enhances sensitivity of GDH detection in the Coris BioConcept rapid Clostridium *K*-SeT.

References

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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 choise of a performant GDH test is important.

Visual reading by two persons gave discordant results in 3.9%. Liaison GDH gave a sensitivity of 96.3%. Laserscan reading with Skansmart enhanced sensitivity for GDH from 88.6% to 95.5% towards visual reading. Laserscan reading with the aLF enhanced sensitivity for GDH from 88.6% to 90.7% towards visual reading. Skansmart reading generated less false negative GDH samples (N=2) than aLF reading (N=4) or visual reading (N=5). Adapting cut-off values leads to less sensitivity but higher specificity.

Laserscan reading gives not only an objective traceable reading but it also enhances sensitivity of GDH detection in the Coris BioConcept rapid Clostridium *K*-SeT.

The study was done on a small number of samples (N=206) which explains the slightly altered performances of the Clostridium *K*-SeT. In small numbers the variability of weak or strong positive samples is predominant.