Clostridium *K*-SeT

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IFU-5820/TB/02

***In vitro* rapid diagnostic test for the detection of *Clostridium difficile* antigen in human stool samples**

FOR IN VITRO USE ***EN***

FOR PROFESSIONAL USE ONLY

References: K-1520, 20 tests per kit, with collection set
K-1220, 20 tests per kit, without collection set

(EN) For Instructions For Use in your language : (FR) Pour obtenir les notices dans la langue de votre choix : (ES) Para las instrucciones de uso en su idioma : (PT) Para Instruçoes de Uso na sua lingua : (IT) Per le Istruzioni di Uso nella sua lingua : (DE) Für Gebrauchsanleitungen in Ihrer Sprache : (NL) Voor Gebruiksaanwijzing in uw eigen taal :	
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I. INTRODUCTION

Clostridium difficile is an anaerobic bacteria acting as an opportunistic pathogen: it grows in the intestine when the normal flora has been altered by treatment with antibiotics. Toxinogenic strains of *Clostridium difficile* cause infections from mild-diarrhea to pseudomembranous colitis, potentially leading to death.

Disease is caused by two toxins produced by toxinogenic strains of *C.difficile*: Toxin A (tissue-damaging enterotoxin) and Toxin B (cytotoxin). Some strains produce both toxins A and B, some others produce Toxin B only. The potential role of a third (binary) toxin in pathogenicity is still debated.

The use of Glutamate Dehydrogenase (GDH) as an antigen marker of *C.difficile* proliferation has been shown to be very effective because all strains produce high amount of this enzyme.

Clostridium K-SeT allows the specific detection of *C.difficile*'s GDH in stool specimen. Samples with a positive Clostridium *K*-SeT result should be investigated further to test for toxigenicity of the bacteria.

II. PRINCIPLE OF THE TEST

This is a ready-to-use test that is based on the use of a membrane technology with colloidal gold. A nitrocellulose membrane is sensitized with antibody directed against *Clostridium difficile* antigen (GDH). The test's specificity is ensured by an antibody specific to the *Clostridium difficile* GDH that is conjugated to the colloidal gold. This conjugate is dried on a membrane.

The faecal sample must be diluted into the dilution buffer that is supplied with the test. When 4 drops of the liquid phase of the faecal suspension come into contact with the strip, the solubilised conjugate migrates with the sample by passive diffusion and the conjugate and sample material come into contact with the anti-*Clostridium* antibody adsorbed onto the nitrocellulose. If the sample contains *C.difficile* GDH, the conjugate-antigen complex will remain bound to the anti-*C.difficile* GDH reagent and a red line will develop. Solution continues to migrate to encounter a second reagent that binds the migration control conjugate, thereby producing a red control line that confirms that the test is working properly. The result is visible within 15 minutes.

III. REAGENTS AND MATERIALS

1. Clostridium *K*-SeT (20)
20 sealed pouches containing one device and one desiccant. Each device contains one sensitized strip.

2. Instruction for use (1)

3. ST-A buffer
Saline dilution buffered to pH 7.5 containing Tris, EDTA, NaN_3 (<0,1%), a detergent and blocking proteins.
- K-1220: 1 vial (15 mL)
- K-1520: 20 Faecal Sampling System (FSS) (2 mL) with a sampling screw

Materials to be ordered separately:
- Negative control (Ref.: CTR-1000)

IV. SPECIAL PRECAUTIONS

- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices (GLP).
- All reagents are for *in vitro* diagnostic use only.
- Pouch must be opened with care
- Avoid touching nitrocellulose with your fingers.
- Wear gloves when handling samples.
- Never use reagents from another kit.
- Green lines indicate immunoreagents adsorption sites. Green colour disappears during the test.
- Reagents' quality cannot be guaranteed beyond their shelf-life dates or if reagents are not stored under required conditions as indicated in the insert.

V. WASTE DISPOSAL

- Dispose of gloves, swabs, test tubes and used devices in accordance with GLP.
- Each user is responsible for the management of any waste produced, and must ensure that it is disposed of in accordance with the applicable legislation.

VI. STORAGE

- An unopened pouch may be kept at between 4 and 30°C and used until the shelf-life date indicated on the packaging. Once the pouch is opened, run the test immediately. Real time stability tests are in progress at Coris BioConcept. Please contact us if you require interim results.
- Avoid freezing devices and buffer.

VII. SPECIMEN HANDLING AND COLLECTION

The stool specimens must be tested as soon as possible after collection. If necessary, they may be stored at 2-8°C for 1 week or -20°C for longer periods of time.

Make sure that the specimens are not treated with solutions containing formaldehyde or its derivatives.

VIII. PROCEDURE

PREPARATIONS OF THE TEST:
Allow kit components, in unopened packaging, and specimens to reach room temperature (15-30°C) before performing a test.

Open the pouch and remove the device. Once opened, run the test immediately. Indicate the patient's name or specimen number on the device (one device per sample).

SPECIMEN PREPARATION PROCEDURE WITH FAECAL SAMPLING SYSTEM (K-1520):

1. Open the FSS tube and use the screw to collect the stool sample (A). **The dilution ratio must be about 4% w/v**. Take care not to take too much (B1) or too little specimen (B2). For liquid or semi-liquid samples, pipette 80 µL of sample using a micropipette (not provided) into the FSS vial.
2. Insert the screw into the FSS and tighten the cap. Vortex the preparation to homogenize (C). The entire stool sample must be suspended into the solution.
3. Break off the point of the cap (D) and dispense **4** drops of diluted sample into the sample well of the device as illustrated below. To assure proper delivery, FSS vial must be held vertically (E).

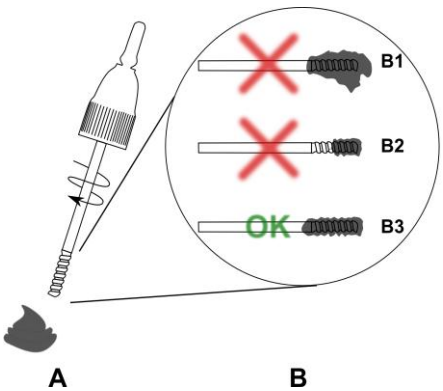
SPECIMEN PREPARATION PROCEDURE (K-1220):

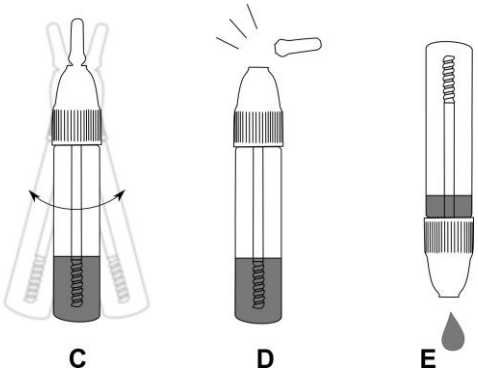
1. Add 0.5 mL or 15 drops of the dilution buffer solution into a tube.
2. Dip a loop containing the stool sample into the tube. **The dilution ratio must be about 4% w/v**. For liquid samples, take 2 loops of 10 µL; for solid samples, take 1 loop.
3. Discard the sampling loop.
4. Vortex the preparation to homogenize. The entire stool sample must be suspended into the solution.
5. Slowly dispense 100µL of diluted sample into the sample well of the device.

Leave to react for 15 minutes. The results are observed in the reading window. Positive results may be reported sooner the moment the test and control lines become visible.

Do not take the appearance of new lines into account after the reaction time is passed.

The results must be read on still wet strips.





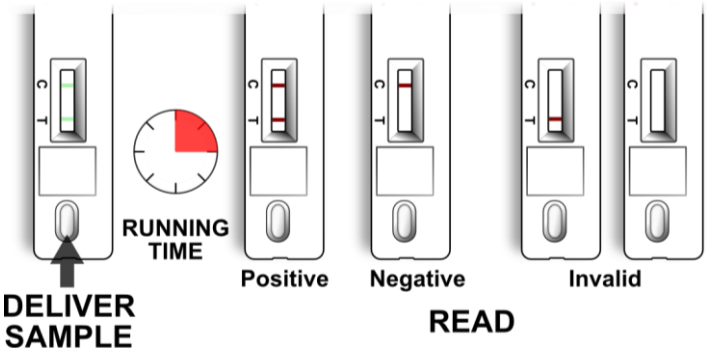
IX. INTERPRETING RESULTS

The results are to be interpreted as follows:

Negative test result: a reddish-purple line appears across the central reading window at the Control line (C) position. No other band is present.

Positive test result: in addition to a reddish-purple band at the Control line (C), a visible reddish-purple band appears at the Test line position (T). Intensity of the test line may vary according to the quantity of antigens found in the sample. Any reddish-purple line (T), even weak, should be considered as a positive result.

Invalid test result: The absence of a Control line indicates a failure in the test procedure. Repeat invalid tests with a new test device.



Note: during the drying process, a very faint shadow may appear at the Test line position. It should not be regarded as a positive result.

X. QUALITY CONTROL

In accordance with Good Laboratory Practices, we recommend checking the test's performance regularly according to the laboratory's requirements. Dispense 100 µL of prepared control (see CTR-1000 instructions for use) into the sample well of the device.

XI. PERFORMANCE

A. Detection Limit

The detection limit was evaluated by diluting a purified GDH preparation and the results show that the concentration of protein detected is 1 ng/mL.

B. Sensitivity - Specificity (Correlation):

1°) An evaluation was conducted on 318 human stool samples. The results were compared with those obtained by a conventional culture method in a National Reference Laboratory (Belgium).

Culture / Coris BioConcept	Positive	Negative	Total
Positive	75	17	92
Negative	0	226	226
Total	75	243	318

95% Confidence Interval[1]

Sensitivity:	100%	(93.9 to 100%)
Specificity:	93%	(88.8 to 95.7%)
Positive Predictive value:	81.5%	(71.8 to 88.6%)
Negative predictive value:	100%	(97.9 to 100%)
Accuracy:	94.7% (301/318)	

2°) The kit was also validated in-house by comparison with an EIA on 65 human stool samples. The following results were obtained:

EIA competitor / Coris BioConcept	Positive	Negative	Total
Positive	41	0	41
Negative	1	23	24
Total	42	23	65

95% Confidence Interval[1]

Sensitivity:	97.6%	(85.9 to 99.9%)
Specificity:	100%	(82.2 to 100%)
Positive Predictive value:	100%	(89.3 to 100%)
Negative predictive value:	95.8%	(76.9 to 99.8%)
Accuracy:	98.5% (64/65)	

3°) An evaluation was conducted on 100 human stool samples. The results were compared with those of an ICT competitor test.

ICT competitor / Coris BioConcept	Positive	Negative	Total
Positive	7	1	8
Negative	0	92	92
Total	7	93	100

95% Confidence Interval[1]

Sensitivity:	100%	(56.1 to 100%)
Specificity:	98.9%	(93.3 to 99.9%)
Positive Predictive value:	87.5%	(46.7 to 99.3%)
Negative predictive value:	100%	(95 to 100%)
Accuracy:	99% (99/100)	

[1] Newcombe, Robert G. "Two-Sided Confidence Intervals for the Single Proportion: Comparison of Seven Methods," *Statistics in Medicine,* **17**, 857-872 (1998).

C. Accuracy

To check intra-batch accuracy, the same positive samples and a buffer solution were processed 15 times on kits of the same production batch in the same experimental conditions. All observed results were confirmed as expected.

To check inter-batch accuracy, some samples (positive and buffer) were processed on kits from three different production batches. All results were confirmed as expected.

D. Interference

Cross-reactivity to samples positive for the following pathogens was tested and found to be negative: *Campylobacter coli, Campylobacter jejuni, Enterobacter cloacae, Enterococcus faecalis, Escherichia coli, Escherichia hermanii, Haemophilus influenzae, Helicobacter pylori, Klebsiella pneumoniae, Legionella bozemanii (sg1), Legionella lonbeachae, Legionella pneumophila (sg1), Moraxella catarrhalis, Mycobacterium avium, Mycobacterium intracellulare, Mycobacterium tuberculosis, Mycoplasma hominis, Neisseria meningitides (sg B & C), Neisseria sicca, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella enteritidis, Salmonella typhimurium, Serratia marcescens, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermis, Streptococcus (Gr B, C, F, G), Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahemolyticus, Yersinia enterocolitica* (type1, 3, 9).

XII. LIMITS OF THE KIT

The test is qualitative and cannot predict the quantity of antigens present in the sample. Clinical presentation and other test results must be taken into consideration to establish diagnosis.

A positive test does not rule out the possibility that other pathogens may be present.

XIII. TECHNICAL PROBLEMS / COMPLAINTS

If you encounter a technical problem or if performances do not correspond with those indicated in this package insert:

1. Record batch number of incriminated kit
2. If possible, keep the problematic sample in the freezer for the time lapse of complaint management
3. Contact Coris BioConcept (client.care@corisbio.com) or your local distributor

XIV. BIBLIOGRAPHIC REFERENCES

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D. **Ramsey L. et al:** *Fulminant Clostridium difficile: an underappreciated and increasing cause of death and complications,* Annals of Surgery 235 (3) p. 363-372: Mar. 2002

E. **Wren MW., Kinson R., Sivapalan M., Shemko M., Shetty NR.:** *Detection of Clostridium difficile infection: a suggested laboratory diagnostic algorithm,* British Journal of Biomedical Sciences, 66(4) p. 175-179, 2009.

F. **Willis DH. And JA Kraft:** *Confirmation that the latex-reactive protein of Clostridium difficile is a Glutamate Dehydrogenase.* Journal of clinical microbiology, 30, p. 1363-1364, May 1992

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H. **Shetty N., Wren MW., Coen PG.:** *The role of glutamete dehydrogenase for the detection of Clostridium difficile in faecal samples: a meta-analysis,* Journal of Hospital Infections, 77(1), p. 1-6, Jan. 2001.

Last update: SEPTEMBER 2012

REF	Catalogue number		Manufactured by
IVD	In vitro diagnostic medical device		Temperature limitation
	Contains sufficient for <n> tests	DIL SPE	Diluent specimen
	Consult instructions for use		Do not reuse
	Keep dry		Use by
DIL AS	Diluent assay	CONT NaN_3	Contains Sodium azide