GastroVir *K*-SeT

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Produced in BELGIUM

IFU-5816/EN/02

***In vitro* rapid diagnostic test for the detection of Rotavirus and Adenovirus gastro-enteritis (serotypes 40/41)**

FOR IN VITRO USE **EN**

FOR PROFESSIONAL USE ONLY

References: K-1516, 20 tests per kit, with collection set
K-1216, 20 tests per kit, without collection set

I. INTRODUCTION

Human gastroenteritis can be caused by viruses (Rotavirus, Adenovirus, Astrovirus, Calcivirus, etc), by bacteria such as Salmonella and by protozoan organisms such as *C. parvum* and *G. lamblia*.

In children under 4 years, virus account for 40% of cases. Among these cases, Rotavirus is the most important cause of the disease (45% of the cases). Each year, rotavirus causes approximately 111 million episodes of gastroenteritis requiring only home care, 25 million clinic visits, 2 million hospitalizations, and 440 000 deaths on average in children <5 years of age. By age 5, nearly every child will have an episode of rotavirus gastroenteritis, 1 in 5 will visit a clinic, 1 in 65 will be hospitalized, and approximately 1 in 293 will die. (CDC, May 2003)[7]

Enteric Adenovirus (EAd) is considered to be the second cause with 5% to 20% of the cases. Although non enteric Adenovirus (NEAd) are found in stool, the serotypes 40 and 41 of subgenes F (EAd) are dominant (30 to 80% of all adenovirus detected in feces) and have been shown to be the main causative agents in enteric infections. Serotypes 40 and 41 are found almost exclusively in stool of ill patients while NEAd serotypes are shed in stool of both ill and control patients. Furthermore, most of the NEAd serotypes found in stool are known to be responsible of respiratory infections (serotypes 1, 2, 3, 4, 5, 6, 7) and for some there are no evidence of being causes of gastroenteritis (serotypes 12, 18).[1-2-3-4-5-6]

Rotavirus is transmitted by faecal-oral contact. After an incubation period of about three days it triggers fever, vomiting, and diarrhea that can persist for up to ten days. Adenovirus infection occurs also by the faecal-oral route, but can also result from inhalation. The incubation period is from five to eight days and the symptoms of the stomach and intestinal inflammation are watery diarrhea, vomiting, fever, and abdominal cramps.

The GastroVir *K*-SeT detects all Rotavirus group A and Adenovirus subgroup F (serotypes 40/41).

II. PRINCIPLE OF THE TEST

This is a ready-to-use test that is based on the homogeneous membrane system technology with latex microspheres. The faecal sample must be diluted in the dilution buffer that is supplied with the test. A nitrocellulose membrane is sensitized with antibodies directed against Rotavirus and Adenovirus. The test's specificity comes from two monoclonal antibodies directed against Group A VP6 proteins of human Rotavirus and specific proteins of human Adenovirus serotype 40/41, respectively, that are conjugated to latex microspheres. These conjugates are dried on a polyester membrane.

The faecal sample must be diluted in the dilution buffer that is supplied with the test. When the liquid phase of the faecal suspension come into contact with the strip, the solubilized mixed conjugate migrates with the sample by passive diffusion and the conjugate and sample material come into contact with monoclonal antibody directed against specific Adenovirus proteins. If the sample contains Adenovirus 40/41, the conjugate-Adenovirus complex remains bound to the monoclonal antibody adsorbed to the nitrocellulose and a red line develops. The solution continues to migrate to encounter an anti-Rotavirus monoclonal antibody that is adsorbed to the nitrocellulose. If the sample contains Rotavirus, the conjugate-Rotavirus complex will remain bound to the anti-Rotavirus monoclonal antibody and a blue line will develop. The result is visible within 10 minutes.

The solution continues to migrate to encounter a third reagent (control reagent) that binds the control conjugate, thereby producing the green control line that confirms that the test is working properly.

III. REAGENTS AND MATERIALS

1. **GastroVir *K*-SeT (20)**

20 sealed pouches containing one device and one desiccant. Each device contains one sensitized strip.

2. **Instruction for use (1)**
3. **HC dilution buffer**

Saline solution buffered to pH 7.5 containing Tris, EDTA, NaN_3 (<0,1%), a detergent and blocking proteins.

- K-1216: 1 vial (15 mL)
- K-1516: 20 Faecal Sampling System (FSS) (1 mL) with a sampling screw

Materials to be ordered separately:

- Rotavirus positive control (Ref.: C-1081)
- Adenovirus positive control (Ref.: C-1082)
- 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.
- 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.

Kit test is an acute-phase screening test. Specimens that are collected after this phase may contain antigen titres below the reagent's sensitivity threshold.

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.

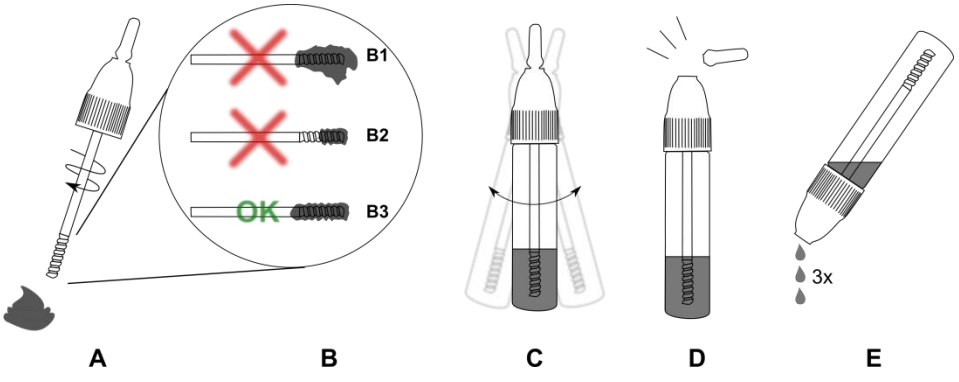
Open the pouch and remove the device. Once opened, run the test immediately. Indicate the patient's name or specimen number on the device (one device per sample).

SPECIMEN PREPARATION PROCEDURE WITH FAECAL SAMPLING SYSTEM (K-1516):

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 **3** drops of diluted sample into the sample well of the device as illustrated below (E).

SPECIMEN PREPARATION PROCEDURE (K-1216):

1. Add **14** 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.



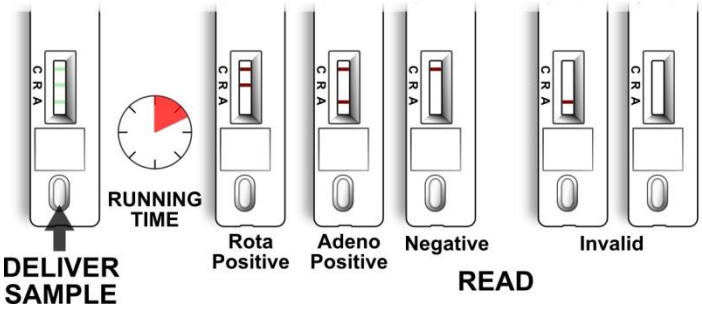
Leave to react for 10 minutes. The results are observed in the reading window. Positive results may be reported sooner the moment the test and control lines become visible.

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 dark-green 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 dark-green band at the Control line (C), a visible blue band appears at the Rotavirus test line position (R) or a visible red band appears at the Adenovirus test line (A). 3 lines (C-R-A) will appear in the case of an infection by both Rotavirus and Adenovirus 40/41. Intensity of the test line may vary according to the quantity of antigens found in the sample. Any test line (R or A), 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. For the test, dispense 100 µL of prepared control (see CTR-1000, C-1081 or C-1082 instructions for use) into the sample well of the device.

XI. PERFORMANCE

A. Sensitivity - Specificity:

A multi-centre (China) validation has been performed on a total of 1201 faecal samples in comparison with a PAGE analysis (Rotavirus) and with PCR method (Adenovirus).

CDC (China)	Rota PAGE analysis			40/41 Adeno PCR method		
Coris BioConcept	**+**	**-**	**Total**	**+**	**-**	**Total**
+	113	6	119	29	10	39
-	3	278	281	1	360	361
Total	116	284	400	30	370	400

Sensitivity	97.4 % (92.1 to 99.3 %)	96.7 % (80.1 to 99.8 %)
Specificity	97.9 % (95.2 to 99.1 %)	97.3 % (94.9 to 98.6 %)
PPV	95.0 % (88.9 to 97.9 %)	74.4 % (57.6 to 86.4 %)
NPV	98.9 % (96.7 to 99.7 %)	99.7 % (98.2 to 100 %)
Agreement	97.8 % (391/400)	97.3 % (389/400)

Capital Institute of pediatrics (China)	Rota PAGE analysis			40/41 Adeno PCR method		
Coris BioConcept	**+**	**-**	**Total**	**+**	**-**	**Total**
+	251	1	252	43	2	45
-	1	147	148	4	351	355
Total	252	148	400	47	353	400

Sensitivity	99.6 % (97.5 % to 100 %)	91.5 % (78.7 % to 97.2 %)
Specificity	99.3 % (95.7 % to 100 %)	99.4 % (97.7 % to 99.9 %)
PPV	99.6 % (97.5 % to 100 %)	95.6 % (83.6 % to 99.2 %)
NPV	99.3 % (95.7 % to 100 %)	98.9 % (96.9 % to 99.6 %)
Agreement	99.5 % (398/400)	98.5 % (394/400)

Beijing children hospital (China)	Rota PAGE analysis			40/41 Adeno PCR method		
Coris BioConcept	**+**	**-**	**Total**	**+**	**-**	**Total**
+	151	3	154	67	3	70
-	2	245	247	11	320	331
Total	153	248	401	78	323	401

Sensitivity	98.7 % (94.9 % to 99.8 %)	85.9 % (75.7 % to 92.4 %)
Specificity	98.8 % (96.2 % to 99.7 %)	99.1 % (97.1 % to 99.8 %)
PPV	98.1 % (94 % to 99.5 %)	95.7 % (87.2 % to 98.9 %)
NPV	99.2 % (96.8 % to 99.9 %)	96.7 % (94 % to 98.2 %)
Agreement	98.8 % (396/401)	96.5 % (387/401)

PPV: Positive Predictive Value
NPV: Negative Predictive Value
(Values in parenthesis are the 95% Confidence interval[1])

B. Repeatability and reproducibility

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

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

C. Interference

Cross-reactivity to samples positive for the following pathogens was tested and found to be negative: Enterovirus, Rhinovirus, HSV, *Candida albicans, Aspergillus niger, Haemophila influenza, H.pylori, Cryptosporidium parvum, Giardia lamblia, E. coli K99, E.coli* (ATCC25922, ATCC35150), Coronavirus.

Tests for cross-reactivity has been tested on *Staphylococcus aureus* and found positive at high bacteria concentrations (approx. 10^9 CFU/mL).

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.

Kit test is an acute-phase screening test. Specimens that are collected after this phase may contain antigen titres below the reagent's sensitivity threshold. If a sample is given a negative result despite the observed symptoms, any other relevant test should be run to check the sample.

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 the kit batch number
2. If possible, keep the clinical sample in the freezer during the complaint management
3. Contact Coris BioConcept (client.care@corisbio.com) or your local distributor

XIV. BIBLIOGRAPHIC REFERENCES

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REF	Catalogue number		Manufactured by
IVD	In vitro diagnostic medical device		Temperature limits
	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

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