

Combi-Strip, Combi-CIT, Combi Uni-Strip

EN



In vitro Rapid Diagnostic Test for the Detection of Rotavirus and Adenovirus in human stool sample.

FOR IN VITRO USE

FOR PROFESSIONAL USE ONLY

Reference :C-1004, 25 tests per kit

C-1504, 10 tests individually packed, sampling devices

C-1204, 20 tests individually packed

I. INTRODUCTION

Diarrhoea and gastro-enteritis in human beings can be caused by viruses (Rotavirus, Adenovirus, Astrovirus, calicivirus, etc), bacteria such as Salmonella and E. coli, and protozoa such as Cryptosporidium and Giardia. Viruses cause 45% of the diarrhoea in children under 1 year old and 40% of the diarrhoea in children under four years.

Rotavirus is the leading cause of gastro-enteritis in children under five years. Adenovirus's prevalence is 4-12%, which puts it in second place as a cause of viral enteritis in children under two years.

Rotavirus is transmitted by faecal-oral contact. After an incubation period of about three days it triggers fever, vomiting, and diarrhoea that can persist for up to ten days. It is responsible for 140 million cases of diarrhoea per year, with 870,000 deaths mainly due to dehydration in developing countries (WHO 1997). It is thus a major cause of mortality in Third World countries. However, it remains a severe infection even in the developed world. In the United States, 75 to 125 children are estimated to die from rotavirus infections each year. As it is highly contagious, it spreads very rapidly in paediatric populations, which are risk groups for such infections.

Adenovirus infection occurs 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 diarrhoea, vomiting, fever, and abdominal cramps.

The Adenoviruses are divided into six subgroups labelled A to F. Subgroup F is the most frequently involved in paediatric gastro-enteritis. The Combi-Strip detects all Rotavirus and Adenovirus group viruses.

II. PRINCIPLE OF THE TEST

This is a ready-to-use test that is based on the use of a homogeneous membrane system with colloidal gold particles. 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 (test lines). The test's specificity is due to two monoclonal antibodies directed against Rotavirus and specific proteins of human Adenovirus (Hexon antigen), respectively, that are conjugated to the colloidal gold. These conjugates are insolubilized on a polyester membrane.

When the strip is dipped into the liquid phase of the faecal suspension, the solubilized conjugates migrate 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, 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 the anti-Rotavirus polyserum that is adsorbed to the nitrocellulose. If the sample contains Rotavirus, the conjugate-Rotavirus complex will remain bound to the anti-Rotavirus polyserum and a red line will develop. The result is visible within ten minutes.

The solution continues to migrate to encounter a third reagent (an anti-chicken IgY polyclonal antibody) that binds the migration control conjugate, thereby producing the red control line that confirms that the test is working properly.

III. REAGENTS AND MATERIALS

Each kit contains Combi-Strips, HC dilution buffer and optimal components (for C-1504)

1. Combi-Strip

Each strip is sensitized with a mouse monoclonal antibody directed against the VP6 Rotavirus antigen, a mouse monoclonal antibody directed against the Hexon antigens of Adenovirus groups A to F, and a goat anti-chicken IgY polyserum.

The anti-Rotavirus conjugate is produced with a mouse monoclonal antibody and the anti-Adenovirus conjugate is produced with a mouse monoclonal antibody that recognizes the antigens of Adenovirus groups A, B, C, D, E, and F. The reagents are purified and coupled to colloidal gold particles.

These strips come in a bottle or a pouch with a desiccant.

2. HC Dilution Buffer (15 mL)

Saline solution buffered to pH 7.5 with Tris and containing EDTA, NaN₃ (<0.1%), a detergent, and blocking proteins.

3. Instruction for use (1)

4. Required materials (supplied with C-1504)

- 3 or 5 mL test tubes
- sampling loops for taking the faecal samples.
- cardboard rack

IV. SPECIAL PRECAUTIONS.

- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices (GLP).
- Combi-Strips are for in vitro diagnostic only.
- Avoid touching the nitrocellulose with your fingers.
- Wear gloves when handling the samples.
- Dispose of gloves, swabs, test tubes, and sensitized strips in accordance with GLP.
- Never use reagents from another kit.
- If strips are stored in container, the container must be resealed as soon as the necessary number of strips for the operation has been removed for the strips are sensitive to humidity. Make sure that the desiccant bag is present.
- If strips are stored in individual pouches, pouch must be opened with care to avoid damaging the strip.
- Three green lines indicate the antibody adsorption sites. They disappear in the course of the test.
- Discard the buffer solution if it is contaminated with bacteria or mould.
- The reagents' quality cannot be guaranteed beyond their expiration dates or if the reagents are stored under inappropriate conditions.

To avoid diluting the colloidal gold conjugate in the solution, take care not to immerse the strip above the line placed under the arrows.

V. WASTE DISPOSAL

Each user is responsible for the management of any waste produced, and must ensure that it is disposed of in accordance with applicable legislation.

VI. STORAGE

An unopened Combi-Strip kit may be kept at between 4 and 30°C and used until the expiration date indicated on the packaging.

The strips remain stable for 15 weeks (in the closed container) after the bottle is opened if they are kept at between 4 and 30°C and in a dry environment.

The Combi-Strips and the buffer must not be frozen.

VII. SAMPLES.

The stool specimens must be tested as soon as possible after they are collected. 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 :

If the Combi-Strip kit was kept at 4°C, let all the reagents warm up to room temperature before proceeding with the test.

Write the patient's name or specimen number on the test tube (prepare one test tube per sample).

Place the marked test tubes in a rack

SPECIMEN PREPARATION PROCEDURE:

1. Add 0.5 ml or 15 drops of the dilution buffer solution to each tube.
2. Dip the inoculating loop containing the stool sample into the tube. **The dilution ratio must be at most 4% w/v. For liquid samples, take 2 loops of 10 µL, for solid samples, take 1 loop.**
3. Stir to homogenize the solution and let stand for 1-2 minutes.
4. Discard the sampling loop and dip the sensitized strip in the direction indicated by the red arrow.
5. Let react for 10 minutes.

Results must be read on wet strips after 10 minutes incubation.

IX. INTERPRETING THE RESULTS

Results are to be interpreted as follows:

- 1 line = negative or invalid****
- 2 or 3 lines = positive ***

0 line = invalid**

* 3 lines will appear in the case of an infection by both Rotavirus and Adenovirus.

* The absence of the control line, which is the upper line, makes the result invalid. In this case, the sample must be retested.

The intensity of the test lines may vary according to the quantity of antigens found in the sample. Note that a weak signal on the test line must be regarded as a positive result. Nevertheless, the test is qualitative and cannot predict the quantity of antigens present in the sample. The clinical presentation and other test results must be taken into consideration to establish the diagnosis.

Variations in intensity of the control line are acceptable.

After 10 minutes, or after the strip has dried, do not take the appearance of new lines into account.

To store the results, let the strip dry after removing the absorbent material at its base. After drying, a very faint shadow may appear along the test line. **Do not take this into account when interpreting results.**

X. PERFORMANCE.

A. Detection limit :

For Adenovirus, the analytical sensitivity has been realised with a purified adenovirus-5 antigen and has been evaluated at about 3.9×10^8 vp/mL.

B. Sensitivity- Specificity (Correlation) :

An evaluation has been carried out in which the results obtained with the Combi-Strip kit were compared with those of two marketed ELISA tests (for Rotavirus and for the Adenovirus group).

The rotavirus and adenovirus Combi-Strip kit's sensitivities and specificities were tested on 214 and 130 stool samples. The following results were obtained:

Combi-Strip	ELISA Rota	Positive	Negative	Total
Positive		105	0	105
Negative		1	108	109
Total		106	108	214

Sensitivity for Rotavirus: 99.1 % (105/106) Reliability (Concordance): 99.5%
Specificity for Rotavirus : 100 % (108/108) (213/214)

Combi-Strip	ELISA Adenovirus	Positive	Negative	Total
Positive		12	0	12
Negative		0	118	118
Total		12	118	130

Sensitivity for Adenovirus : 100 % reliability (concordance) : 100% (130/130)
Specificity for Adenovirus : 100% (N=130)

C. Accuracy

Intra-batch:

One Rotavirus- and Adenovirus-positive viral culture sample was tested fifteen times by the same batch of Combi-Strip kits.

The dilution buffer was tested fifteen times in parallel with the Rotavirus- and Adenovirus-positive viral culture sample.

The results were correct in 100% of the cases.

All fifteen tests carried out on the Rotavirus- and Adenovirus-positive viral culture samples were positive, with the development of two coloured lines.

All fifteen tests carried out on the dilution buffer were negative, with the development of a single coloured line (control line).

Inter-Batch:

One Rotavirus- and Adenovirus-positive viral culture sample was tested three times by nine different batches of Combi-Strip kits.

The dilution buffer was tested three times in parallel with the viral culture.








The results were correct in 100% of the cases.

The nine batches gave positive results for the Rotavirus- and Adenovirus-positive viral culture sample and negative results for the dilution buffer for every test.

D. Interference

Cross-reactivity with samples positive for the following pathogens was tested and found to be negative:

- *Giardia lamblia*
- *Escherichia coli* 0157: H7
- *Salmonella typhimurium*
- *Salmonella enteritidis*
- *Escherichia coli* K99
- Coronavirus
- *Entamoeba histolytica*
- *Entamoeba dispar*
- *Cryptosporidium parvum*

	Consult instructions for use		Do not reuse	REF	Catalogue number		Manufactured by
	Keep dry		Use by	IVD	In vitro diagnostic medical device		Temperature limitation
DIL AS	Diluent assay	CONT NaN3	Contains Sodium azide		Contains sufficient for <n> tests	DIL SPE	Diluent specimen

XI. LIMITS OF THE KIT

Combi-Strip kit results must be compared with all other available clinical and laboratory information.

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

The Combi-Strip is an acute-phase screening test. Stool specimens that are collected after this phase may contain antigen titres below the reagent's sensitivity threshold.

XII. 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 lot No of the kit in question
2. If necessary, store the problematic sample in the freezer as soon as possible
3. Contact Coris BioConcept or your local distributor

XIII. BIBLIOGRAPHIC REFERENCES

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Package insert last updated: October 2007

IFU-C -5704/EN

Rev : 06

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