Proflow™ Rotavirus-Adenovirus Dual Test

(for *In Vitro* Diagnostic use only)

PRODUCT CODE PL.3033 **20 Tests**

INTENDED USE

The Proflow™ Rotavirus-Adenovirus Dual Test is a coloured chromatographic immunoassay for the simultaneous qualitative detection of Rotavirus and Adenovirus in human faeces samples to aid in the diagnosis of Rotavirus and Adenovirus infection. For *In Vitro* Diagnostic Use.

SUMMARY AND EXPLANATION

Rotavirus, Adenovirus and Astrovirus are most common and major causes of severe gastroenteritis in infants and young children. Pattern also observed in adults. They are transmitted by faecal-oral contact. The main symptoms of viral gastroenteritis are watery diarrhoea and vomiting. The affected person may also have headache, fever and abdominal cramps ("stomach ache"). In general, the symptoms begin 1 -2 days following infection with a virus that causes gastroenteritis and may last for 1- 10 days, depending on which virus is causing the illness (Rotavirus 3 days, Adenovirus 5-8 days and Astrovirus 3 days).

PRINCIPLE OF THE TEST

The Proflow™ Rotavirus-Adenovirus Dual Test is based on the principle of a qualitative immunochromatographic assay for the determination of Rotavirus and Adenovirus in stool samples.

Strip A consists of a nitrocellulose membrane pre-coated with mouse monoclonal antibodies on the test line (T), in the results window, against Rotavirus and with rabbit polyclonal antibodies, on the control line (C), against a specific protein. The label/ sample absorbent pad is sprayed with test label solution (mouse monoclonal antibodies anti-Rotavirus) conjugated to red polystyrene latex and control label solution (specific binding protein) conjugated to green polystyrene latex, forming two coloured conjugate complexes.

Strip B consists of a nitrocellulose membrane pre-coated with mouse monoclonal antibodies on the test line (T), in the results window, against Adenovirus and with rabbit polyclonal antibodies, on the control line (C), against a specific protein. The label/ sample absorbent pad is sprayed with test label solution (mouse monoclonal antibodies anti-Adenovirus) conjugated to red polystyrene latex and control label solution (specific binding protein) conjugated to green polystyrene latex, forming two coloured conjugate complexes.

If the sample is Rotavirus positive, the antigens of the diluted sample react with the red-coloured conjugate complex (anti-Rotavirus monoclonal antibodies-red polystyrene microspheres) in the strip A, and if the sample is Adenovirus positive, the antigens of the diluted sample react with the red-coloured conjugate complex (anti-Adenovirus monoclonal antibodies-red polystyrene microspheres) in the strip B, which were previously pre-dried on the absorbent pad. The mixture then moves upward on the membrane by capillary action. As the sample flows through the test membrane, the binding conjugate complexes migrate. The anti-Rotavirus antibodies present on the membrane of strip A (test line) and the anti-Adenovirus antibodies on the membrane of strip B (test line) capture the coloured conjugate and the red line will be visible in both strips. These bands are used to interpret the result.

If the sample is negative, there is no Rotavirus and Adenovirus presence and yet, the antigens may be present in a concentration lower than the detection limit value, for which the reaction will not take place with any red-coloured conjugate complex. The anti-Rotavirus and anti-Adenovirus antibodies present on the membranes (test lines) will not capture the antigen-red-coloured conjugate complex (not formed), for which the red lines will not appear.

Whether the sample is positive or not, in both strips, the mixture continues to move across the membranes to the immobilised specific antibodies placed in the control lines. The anti□specific protein antibodies present on both membranes will capture control green□conjugate complex and both control lines will always appear. The presence of these green lines serve as: 1) verification that sufficient volume is added, 2) that proper flow is obtained and 3) an internal control for the reagents.

MATERIALS PROVIDED

- PL.3340 Proflow™ Rotavirus-Adenovirus Dual Test Devices: 20 devices
- PL.3350 Proflow™ Rotavirus-Adenovirus Dual Sample Prep Device: 20 devices
- Package insert

MATERIALS REQUIRED BUT NOT PROVIDED

- Sample collection container
- Timer or stopwatch
- Biohazard disposal container
- Disposable gloves

STABILITY AND STORAGE

- Store all components at 2-30ºC.
- Do not freeze or overheat.
- Do not use beyond the expiration date printed on the outer package label.
- The test kits should be kept away from direct sunlight, moisture and heat.

PRECAUTIONS

- For *In Vitro* Diagnostic Use only
- For professional use only
- Directions should be read and followed carefully.
- Tests are for single use only. Do not reuse.
- Reagents are provided at the necessary working strength. Do not dilute reagents.
- Do not interchange reagents between kits with different lot numbers.
- Do not use kits or reagents beyond the stated expiration dates.
- Microbial contamination of reagents may decrease the accuracy of the assay.
- Treat all materials as if they were infectious and dispose of all material in accordance with local regulation

SAMPLE STORAGE AND COLLECTION

- Collect sufficient quantity of faeces (1-2g or mL for liquid samples)
- Faecal samples should be collected in clean and dry containers (no preservatives or transport media).
- Samples may be stored at 2-8°C for 1-2 days or at -20°C for longer periods before testing.
- Allow all samples to equilibrate to room temperature before testing.
- Freezing and thawing cycles are not recommended.

TEST PROCEDURE

Use a separate sample collection vial and test for each sample or control. Allow the tests, faeces samples and buffer to reach room temperature prior to testing.

1. Introduce the swab or stick four times into the faeces up to the thread of the stick to pick up approx. 50 mg, and put back into the sample preparation device with buffer. For liquid faeces samples, aspirate with a dropper and add 50 µL into the sample preparation device.
2. Shake the sample preparation device to ensure good sample dispersion.
3. Remove the Proflow™ Rotavirus-Adenovirus test from its sealed pouch.
4. Break off the top of the vial on the sample preparation device.
5. Dispense 3 drops (100 uL) into the sample well on the test (S).
6. Read the result at 10 minutes.

QUALITY CONTROL PROCEDURE

It is recommended that a positive and negative control be used to ensure that the test is performing as expected.

The control lines (C) are a procedural control and will show that the test has been performed correctly; proper flow occurred and that the test reagents functioned as expected. When a green line appears at the control line position this indicates the test has been performed correctly. The control line will appear on all valid tests, whether or not the sample is reactive or non-reactive (refer to the Interpretation of Results section).

INTERPRETATION OF RESULTS

Positive Rotavirus

One pink/red line will appear at the left test line; a green line will appear at the control line on both sides of the test. This indicates a reactive result that is interpreted as positive for Rotavirus antigen.

Positive Adenovirus

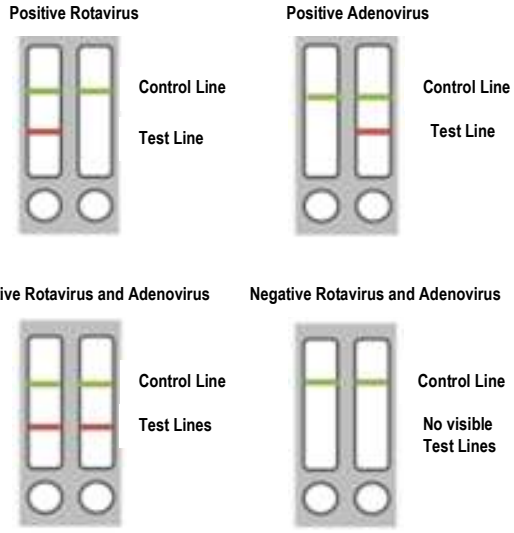
One pink/red line will appear at the right test line; a green line will appear at the control line on both sides of the test. This indicates a reactive result that is interpreted as positive for Adenovirus antigen.

Positive Rotavirus and Adenovirus

A pink/red line will appear on the test line on both sides of the test; a green line will appear at the control line on both sides of the test. This indicates a reactive result that is interpreted as positive for both Rotavirus and Adenovirus antigens.

Negative

A green line of any intensity appears in both test windows at the control line positions. There are no lines at either test line position. This indicates a non-reactive result that is interpreted as negative for both Rotavirus and Adenovirus antigens.



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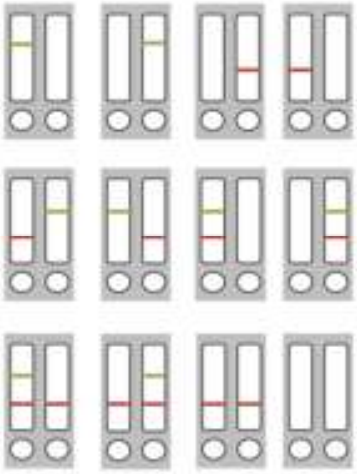
Invalid
No line appears in the test window at the control line position. This is an invalid result and cannot be interpreted. This is irrespective of whether or not a coloured line appears in the test window at the test line position. If any of the conditions below occur, the test should be repeated with a new test.



LIMITATIONS OF THE PROCEDURE

- The Proflow™ Rotavirus-Adenovirus test will only indicate the presence of Rotavirus and Adenovirus in the sample (qualitative detection) and should be used for the detection of Rotavirus and Adenovirus antigens in faeces samples only. Neither the quantitative value nor the rate of increase in antigen concentration can be determined by this test.
- An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the buffer and repeat the test.
- Some faeces samples can decrease the intensity of the control line.
- The test must be carried out within 2 hours of opening the sealed bag.
- Avoid Antimicrobials, Proton pump inhibitors and Bismuth for 10 days prior to testing.
- If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of Rotavirus and Adenovirus infection.
- This test provides a presumptive diagnosis of Rotavirus and Adenovirus infections. All results must be interpreted together with other clinical information and laboratory findings available to the clinician.

EXPECTED VALUES

Acute gastroenteritis is a common disorder in young children; moreover, the associated dehydration is a leading cause of admission to hospital in industrialized countries. Acute diarrhoea is a major health problem throughout the world and a main source of mortality in developing countries. Enteric viruses have been recognized as the most significant etiological agents of the disease and yet, four categories of viruses are being considered clinically relevant: group A Rotavirus (family Reoviridae), Norovirus (family Caliciviridae), Adenovirus and Astrovirus. Several studies proved co-infections in infants 46% with acute watery diarrhoea.

PERFORMANCE CHARACTERISTICS

SENSITIVITY AND SPECIFICITY
An evaluation with faecal samples, was performed using an immunochromatographic test (Proflow™ Rotavirus-Adenovirus Dual Test) and these results were compared with a commercially available test (Ridascreen® Rotavirus ELISA test, r-Biopharm) for strip A and the restuls for strip B were confirmed using PCR. The results were as follows:

		Ridascreen® Rotavirus ELISA test		
		+	-	Total
Proflow™ Rotavirus-Adenovirus Dual test (Rotavirus)	+	18	1	19
	-	0	43	43
	Total	18	44	62

Proflow™ Rotavirus-Adenovirus Dual Test (Rotavirus)

Sensitivity	Specificity	PPV	NPV
>99%	98%	>94%	>99%

		PCR		
		+	-	Total
Proflow™ Rotavirus-Adenovirus Dual Test (Adenovirus)	+	7	0	7
	-	0	52	52
	Total	7	52	59

Proflow™ Rotavirus-Adenovirus Dual Test (Adenovirus)

Sensitivity	Specificity	PPV	NPV
>99%	>99%	>99%	>99%

The results showed a high sensitivity and specificity to detect Rotavirus and Adenovirus using Proflow™ Rotavirus-Adenovirus Dual Test

CROSS-REACTIVITY

An evaluation was performed to determine the cross-reactivity of the Proflow™ Rotavirus-Adenovirus Dual Test; no cross-reactivity against gastrointestinal pathogens occasionally present in faeces:

Adenovirus (strip A)	Escherichia coli O157:H7	Salmonella typhimurium
Astrovirus	Giardia lamblia	Salmonella typhi
Campylobacter coli	Helicobacter pylori	Shigella boydii
Campylobacter jejuni	Listeria monocytogenes	Shigella dystenteriae
Clostridium difficile	Norovirus	Shigella flexneri
Cryptosporidium parvum	Rotavirus (strip B)	Shigella sonnei
Enterovirus	Salmonella enteritidis	Staphylococcus aureus
Entamoeba hystolitica	Salmonella paratyphi	Yersinia enterocolitica

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	= Use by
LOT	= Lot number
REF	= Catalogue number
	= Manufacturer
EC REP	= Authorized Representative in the European Community
Σ	= Contains sufficient for <n> tests
IVD	= In vitro diagnostic medical device
	= Temperature limitation
i	= Consult instructions for use

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