INSTRUCTION MANUAL

REF 6101

April 01, 2014

Rotavirus Antigen Quick

- 25 determinations -

CE

IVD *In vitro* diagnostic device

Rapid immunochromatographic test for the detection of Rotavirus antigen in fecal specimens

REF	Catalogue number	LOT	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction		Biological risk

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INTENDED USE

Rotavirus Antigen Quick is used for the qualitative determination of Rotavirus antigen in fecal specimens.

Rotaviruses are major causative agents of infectious gastroenteritides. They are transmitted by fecal-oral contact. The main symptoms of viral gastroenteritis are watery diarrhea and vomiting. The affected person may also have headache, fever, and abdominal cramps ("stomach ache"). In general, the symptoms begin 1 to 2 days following infection with a virus that causes gastroenteritis and may last for 3 days.

Virus detection from fecal samples should be carried out within the first days after onset of symptoms and is successful in more than 90% of the cases. A negative test result does not necessarily exclude a viral infection.

The golden standard for the diagnosis of virus infections is direct virus detection by electron microscopy. Meanwhile antigen detection methods based on immunological techniques using polyclonal or monoclonal antibodies have been developed. Direct detection of antigen in stool samples by rapid one-step assay is an inexpensive, easy to handle sensitive test with no need of invasive examination and specialized instrumentation.

Cukor G et al.: Detection of Rotavirus in Human Stools by Using Monoclonal Antibody. J. Clin. Microbiol. 1984, 19, 888-892

PRINCIPLE OF THE TEST

Rotavirus Antigen Quick is a fast one-step immunochromatographic assay for the detection of Rotavirus antigen in fecal samples.

The membrane is pre-coated with monoclonal antibodies to Rotavirus. During testing, the antigen in the sample is allowed to react with the colored conjugates (anti-Rotavirus antibodies - red microspheres) which were pre-dried on the test. The complex formed of virus antigen and respective conjugate then migrates upward on the membrane by capillarity. In case of a positive result the specific antibodies present on the membrane will capture the colored conjugate forming a RED band.

The access conjugate mixture continues to migrate across the membrane to the immobilized antibodies placed in the control band region forming a green colored band. The presence of this GREEN band serves as 1) verification that sufficient volume is added, 2) that proper flow is obtained and 3) as an internal control for the reagents.

PATIENT SAMPLES

Specimen collection and storage

Stool samples should be collected in clean containers and the assay should be done right after collection. The samples can be stored at 2-8 ºC for 1-2 days prior to testing. For longer storage, maximum 1 year, the specimen must be kept frozen at –20ºC. In this case, the samples have to be totally thawed and brought to room temperature before testing. Repeated freezing and thawing of samples should be avoided.

Preparation before use

Allow frozen or refrigerated fecal samples to reach room temperature prior to assay. Take care to agitate samples gently in order to ensure homogeneity. Shake the test tube with a vortex in order to assure good sample dispersion.

TEST COMPONENTS for 25 determinations

A Ab 25	**Test strips**, 25 test strips containing mouse monoclonal antibodies to Rotavirus	1 container vacuum sealed with desiccant
B DIL	**Sample diluent**	3 x 10 ml ready for use dropper bottle, capped black

Materials required but not provided

- specimen collection container
- swabs
- micropipettes
- tubes or vials
- vortex / shaker
- disposable gloves
- timer
- centrifuge

Size and storage

Rotavirus Antigen Quick has been designed for 25 determinations.

The expiry date of each component is reported on its respective label that of the complete kit on the box labels.

Upon receipt, all components of the Rotavirus Antigen Quick have to be kept at 2 - 8 °C, preferably in the original kit box.

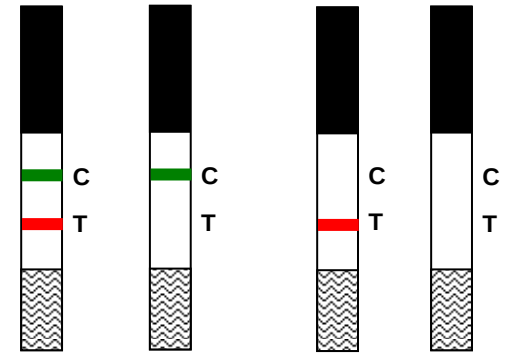
After opening all kit components are stable for at least 3 months, provided proper storage.

ASSAY PROCEDURE

1. Allow the test strips and samples to reach room temperature (15-30°C) prior to testing. Do not open the package until ready to perform the assay.
2. Place 1 ml (approximately 20 drops) of the sample diluent in a test tube.
3. Add a sample portion of approximately 5 mm diameter with a swab, and shake gently in order to unstick and facilitate the sample dispersion. In cases where the stool sample is liquid, just use it directly.
4. Shake the test tube (e.g. with a vortex) in order to assure good sample dispersion.
5. Let the tube rest for at least 5 min for sedimentation. For optimal results, centrifugation of the sample tube for 5 minutes at 1000/min is recommended.
6. Using a pipette, extract some liquid from the upper layer and dispense into a small tube or vial, enough to get a depth of max. 1 cm.
7. Immerse the test strip in the liquid prepared in step 5. Do not exceed the line shown on the strip.

   Read the result **10 minutes** after the immersion of the strip.

EVALUATION OF RESULTS



NEGATIVE: Only one GREEN band (control line) appears in the white central zone of the test (control region).

POSITIVE: In addition to the GREEN control band, a distinguishable RED band also appears in the white central zone of the test (result region).

INVALID: A total absence of the control band (GREEN) regardless of the appearance or not of the result lines (RED). Insufficient specimen volume, incorrect procedural techniques or deterioration of the reagents are the most likely reasons for control line failure. Review the procedure and repeat the test performance using a new test. If the problem persists please contact you local distributor.

Test validity

Internal procedural controls are included in the test. A green line appearing in the control region is an internal control. It confirms sufficient specimen volume and correct procedural technique.

Limitations of the method

The test must be carried out within 2 hours after removing the strips from the container.

An excess of stool sample could cause wrong results (brown bands appear).

The intensity of the result band (RED) varies depending on the concentration of antigens present in the specimen. However, neither the quantitative value, nor the rate of increase in antigens can be detemined by this qualitative test.

After one week of infection, the number of viruses in feces is decreasing, making the sample less reactive. Stool samples should be collected within one week of the onset of symptoms.

A negative test result not necessarily excludes a viral infection. Inhomogeneous antigen distribution in the sample can cause false negative results.

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

ANALYTICAL PERFORMANCE

Sensitivity

The minimum detectable unit of Rotavirus is about 10 ng/ml.

Comparison to reference methods

The comparison of Rotavirus Antigen Quick with commercial ELISAs assays shows a concordance level of > 90%.

Diagnostic specificity

The monoclonal antibodies used in the manufacturing of Rotavirus Antigen Quick recognise epitopes present in the antigen found in stool of patients as well as in preparations from cultures in vitro.

SAFETY PRECAUTIONS

- **This kit is for in vitro use only**. Follow the working instructions carefully. GA GENERIC ASSAYS GmbH and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The kit should be performed by trained technical staff only.
- The expiration dates stated on the respective labels are to be observed.
- Do not use or mix reagents from different lots. Do not use reagents from other manufacturers.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts of Sodium azide (0.095%) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Sample materials must be handled as infectious material. Wear disposable gloves while handling clinical specimens. Dispose clinical specimens in accordance with local legislation.
- Since the kit contains potentially hazardous materials, the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.