FOR REFERENCE USE ONLY

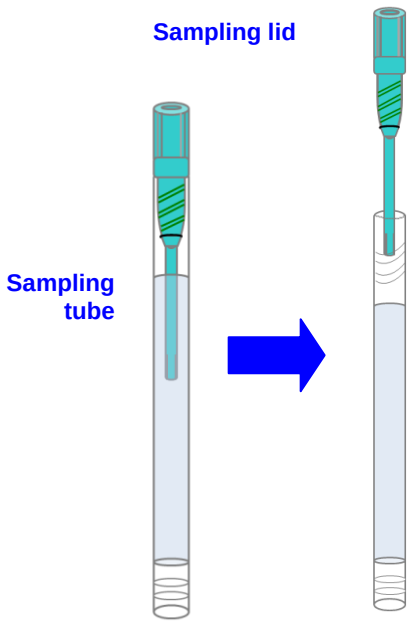
EpiTuub® Fecal Cryptosporidium *parvum* Antigen Rapid Test - Instructions for Fecal Sample Collection

Qualitative detection of Cryptosporidium parvum antigen in human feces.

Version 6

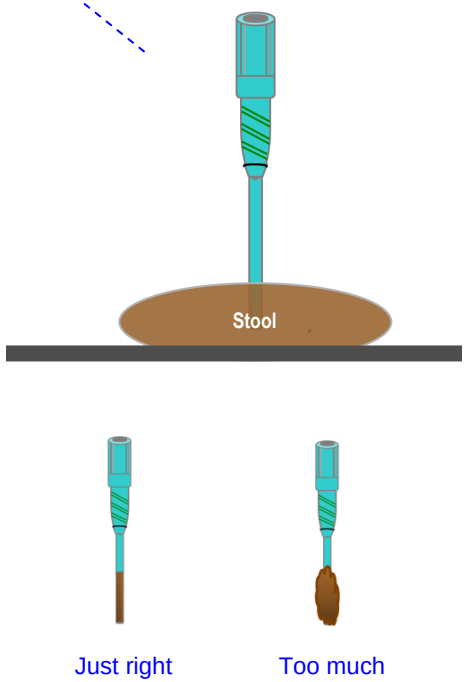
1

1. Collect a stool sample using the enclosed stool sampling paper:
   a. Clean the bowl and flush the toilet two times. Unfold and lay the Sample Collection Paper directly on the top of the water in the toilet bowl (the paper should float above the water).
   b. After bowel movement, take the sampling tube and unscrew the sampling lid, keeping the sampling tube in a **vertical position** to prevent loss of solution.



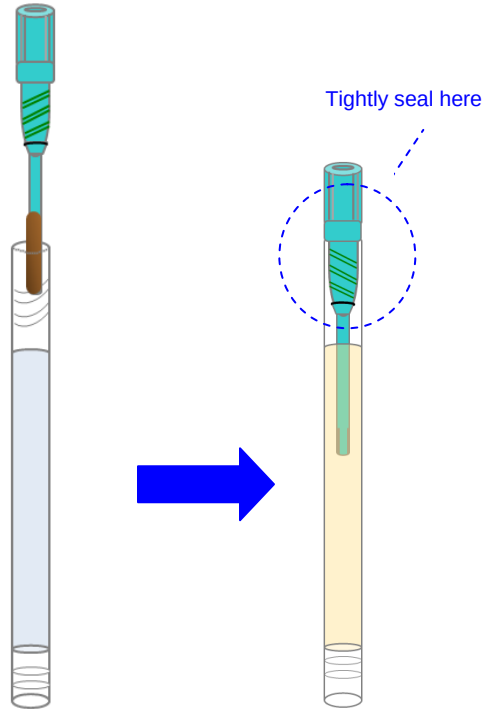
2

1. Hold the sampling lid by the **Thumb Grip**.
2. Use the tip of the sampling lid to collect a small amount of fecal sample at two or more sites. Only take the fecal sample that sticks to the sampling lid tip (never intentionally place any separate piece of fecal sample into the tube). The total amount of stool collected should be less than one grain of cooked rice. For liquid stool, collect 0.1mL into the sampling tube.



3

1. Insert and screw the sampling lid back into the sampling tube **in a vertical position**. Do not spill any solution from the tube.
2. Tightly seal the lid with the tube.
3. Flush toilet.



READ ALL THE INFORMATION IN THIS LEAFLET BEFORE SAMPLING

Store at 2-8°C. Do not freeze. Keep out of reach of children. For in-vitro diagnostic use. Not to be taken internally. Not to be sampled directly from anus.

If you have any questions, please contact your physician or laboratory staff or call Epitope Diagnostics at 858-693-7877 from 8:00 a.m. to 5:00 p.m. PST

EU: IVD CE +2 +8 °C

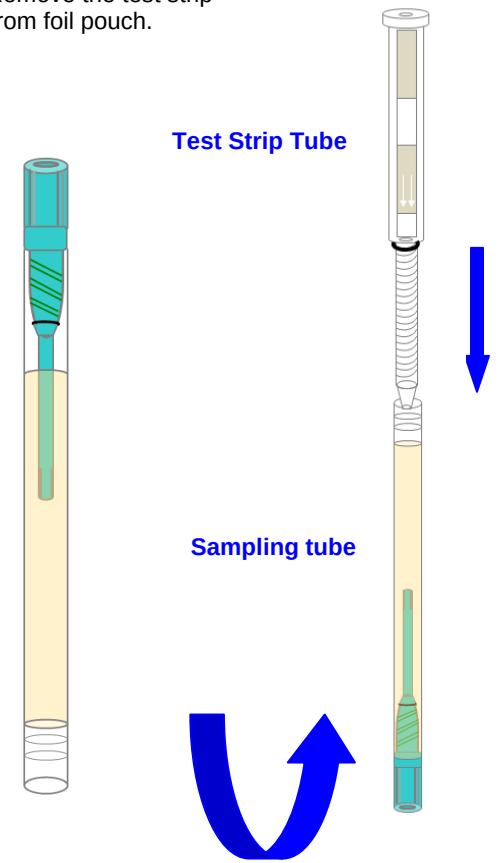
EC REP MDSS GmbH Schiffgraben 41 30175 Hannover Germany

EpiTuub® Fecal Cryptosporidium parvum Antigen Rapid Test Kit - Instructions for Test Procedures

Qualitative detection of Cryptosporidium parvum antigen in human feces.

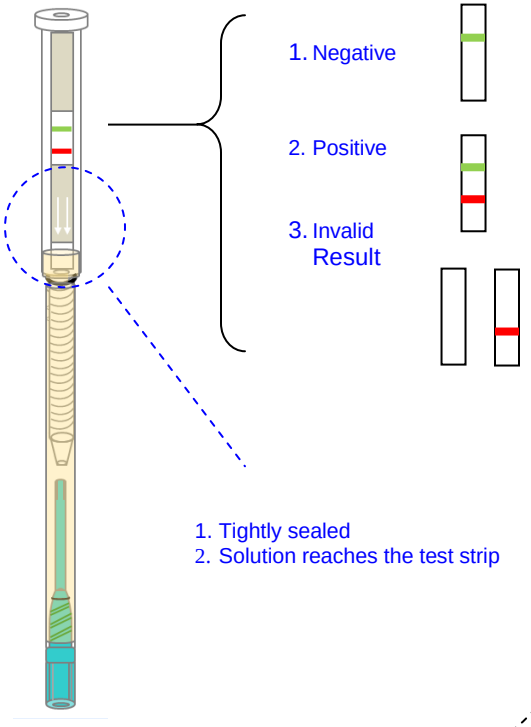
A

1. Shake to dissolve the stool into solution.
2. Turn the sampling tube **upside down vertically.**
3. Remove the test strip from foil pouch.



B

1. Insert and screw the test strip **in a vertical position** into the sampling tube by breaking the bottom seal of the sampling tube.
2. Allow the solution to flow into the bottom space of test strip, keeping the device **in a vertical position**.
3. You may soon see a red fluid moving across the white area of the test strip. Read test result after 5 minutes.



For In-Vitro Diagnostic Use

Catalog Number: KT928 (30T/Kit)
KT928.10 (10T/Kit)

INTENDED USE

This EpiTuub® Cryptosporidium *parvum* antigen rapid immunochromatographic test kit is intended for the direct qualitative detection of the presence of Cryptosporidium parvum antigen in a fecal sample. The test might be used as an aid for detecting patients or animals with acute and chronic cryptosporidiosis infected with Cryptosporidium *parvum*. It is for professional use only.

SUMMARY OF PHYSIOLOGY

Cryptosporidium is a protozoan pathogen of the Phylum Apicomplexa and causes a diarrheal illness called cryptosporidiosis.

Cryptosporidiosis is typically an acute short-term infection but can become severe and non-resolving in children and immunocompromised individuals. In humans, it remains in the lower intestine and may remain for up to five weeks. The parasite is transmitted by environmentally hardy microbial cysts (oocysts) that, once ingested, exist in the small intestine and result in an infection of intestinal epithelial tissue.
There are many diagnostic tests for *Cryptosporidium*. They include microscopy, staining, and detection of antibodies. Detecting antigens is yet a way to diagnose the disease. This can be done with direct fluorescent antibody (DFA) techniques, indirect immunofluorescence assay, Enzyme-Linked ImmunoSorbent Assay (ELISA) and immunochromatographic rapid test.

ASSAY PRINCIPLE

The Cryptosporidium parvum Antigen Rapid Test Strip employs dye-conjugated monoclonal antibody against Cryptosporidium parvum antigen, and solid-phase/membrane coated specific anti- Cryptosporidium parvum monoclonal antibody. In this test the specimen is first treated with an extraction solution to extract Cryptosporidium parvum antigens from the feces. Following extraction, the only step required is to screw the Cryptosporidium parvum test strip tube into the sample collection tube. As the sample extraction upward flows through chamber and reach the test strip, the colored particles migrate. In the case of a positive result the specific antibody present on the membrane will capture the colored particles. Different colored lines will be visible, depending upon the bacteria content of the sample. These lines, after 10 minutes of incubation at room temperature, are used to interpret the result.

REAGENTS: Preparation and Storage

1. Fecal specimen collection device (30499): containing sampling tube, sampling lid and pre-added extraction solution in the sampling tube. This device should be stored at 2-8°C. Do not freeze.

READ ALL THE INFORMATION IN THIS INSERT BEFORE TESTING

Store at 2-8°C. Do not freeze. Keep out of reach of children. For in-vitro diagnostic use. Not to be taken internally. Not to be sampled directly from anus.
If you have any questions, call customer information staff of Epitope Diagnostics at 1-858-693-7877, 8:00 a.m. to 5:00 p.m. PST.

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MDSS GmbH
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2. Test strip tube (30498): one dipstick for the Cryptosporidium parvum test is assembled in a transparent housing and sealed in a foil pouch with desiccant. It should remain in its original sealed pouch until ready for use. The test strip should be stored at 2 to 8ºC. Do not freeze.
3. Patient label for sample collection tube.
4. Sample collection paper.
5. Instruction for use.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Timer or clock

PRECAUTIONS

1. For in-vitro diagnostic use only. Not to be taken internally.
2. Do not use product beyond the expiration date.
3. Handle all specimens as potentially infectious.
4. Do not reuse the test.

PATIENT PREPARATION

1. Dietary restrictions are not necessary.

SPECIMEN COLLECTION

1. Stool specimens can be collected at any time of the day.
2. Collect a random sample of feces in a clean, dry cup or toilet paper or as indicated in the Figure 1.
3. Unscrew the sampling lid and keep the sampling tube in a vertical position to prevent the loss of any extraction solution.
4. Insert and twist the tip of the sampling lid into the stool specimen at two or more different sites (Figure 2).
5. Collect fecal sample that is stuck to the surface of the sampling lid. The total amount of stool sample should be less than one grain of cooked rice. Do not intentionally collect any separate and large pieces of fecal sample into the tube.
6. Replace the sampling lid into the tube and secure tightly (Figure 3).
7. The specimen is ready for testing, transportation or storage. It can be stored at 2-8ºC for up to 21 days and at room temperature for up to 14 days.

TEST PROCEDURE

1. Bring the sealed foil pouch test strips and collected specimens to room temperature.
2. Shake the sampling tube vigorously to ensure a good liquid suspension.
3. Position the sampling tube upside down vertically and let it settle for about 1 minute.
4. Remove the test strip from the sealed foil pouch.
5. Screw the test strip tube into the sampling tube by **breaking** the bottom seal of the sampling tube. Secure tightly! (Figure A)
6. Allow the solution to flow into the bottom space of the test strip and keeping the device **in a vertical position**.
7. Read test result at 5 minutes. Do not interpret test result after 10 minutes.

PROCEDURAL NOTES

1. After the test strip tube is screwed completely into the sampling tube, you should see a minimum 5 mm extraction buffer liquid in the bottom of the strip tube.
2. You should see liquid migrating across the membrane area right after the screw in process. If not, take the tube and tap against the table several times, and the migration of the liquid should be observed.

INTERPRETATION OF RESULTS

- **Positive:**
  If two green/red colored bands are visible within 5 minutes, the test result is positive and valid (Figure B).
- **Negative:**
  If test area has no red colored band and the control area displays a green colored band, the test result is negative (Figure B).
- **Invalid:**
  If a colored band does not form in the control area regardless of there being any band in the test area, the test result is invalid (Figure B) and needs to be retested.

QUALITY CONTROL

Good laboratory practices recommend the use of appropriate controls. There are two types of controls for the EpiTuub® Cryptosporidium *parvum* test, the internal procedural control and external controls.

1. **Internal procedural control:** Each EpiTuub® Cryptosporidium *parvum* test has a built-in procedural control. It will appear if the test has been performed correctly, sample wicking has occurred and the reagents are reactive. It does not ensure that the test line antibody is accurately detecting the presence or absence of Cryptosporidium *parvum* in the test fecal sample.
2. **External controls:** It is recommended to use external positive controls. The external positive controls are not provided with this kit, but are commercially available from Epitope Diagnostics. External controls are used to assure that the test line antibody is reactive. However, external controls will not detect an error in performing the patient sample test procedure. It is recommended that the external control be tested once per kit.

Follow local, state, and federal guidelines for running quality control.

LIMITATION OF THE PROCEDURE

1. The test should be used only for the detection of Cryptosporidium *parvum* antigen in fecal samples.
2. The test is qualitative, and no quantitative interpretation should be made with respect to the intensity of the positive line, when reporting the result.
3. Two hundred samples were evaluated to assure the correct performance of the test. The correlation of the results with other techniques (ELISA) was satisfactory. However, interferences in the performance of the tests should not be excluded.
4. As with all diagnostic tests, the definitive clinical diagnosis must not be based on the result of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated. *EpiTuub*™ Fecal Cryptosporidium *parvum* antigen test is designed for the aid of clinical diagnosis and should not replace other diagnostic procedures.

PERFORMANCE CHARACTERISTICS

Sensitivity

Detection limit: purified Cryptosporidium *parvum* oocyst were sonicated, centrifuged and its protein concentration was determined. This reference antigen preparation of Cryptosporidium *parvum* was diluted in 0.01M PBS-BSA buffer and tested with this kit according to the above described test procedures. The detection limit of Cryptosporidium *parvum* is about 5 – 10 ng/ml.

Specificity

The evaluation was performed by comparison this rapid test with an commercial Cryptosporidium *parvum* antigen ELISA kit. The detection of Cryptosporidium *parvum* showed over 96% of concordance with the ELISA.

The possibility for interference of human anti-mouse antibodies (HAMA) or high levels of RF in the stool sample have not been evaluated.

REFERENCES

1. Chen XM, Keithly JS, Paya CV, LaRusso NF (May 2002). "Cryptosporidiosis". N. Engl. J. Med. 346 (22): 1723–31.
2. Carreno RA, Martin DS, Barta JR (1999) Cryptosporidium is more closely related to the gregarines than to coccidia as shown by phylogenetic analysis of apicomplexan parasites inferred using small-subunit ribosomal RNA gene sequences. Parasitol Res. 85(11):899-904
3. Abrahamsen, M. S.; Templeton, TJ; Enomoto, S; Abrahante, JE; Zhu, G; Lancto, CA; Deng, M; Liu, C et al (2004). "Complete Genome Sequence of the Apicomplexan, Cryptosporidium parvum".Science (Science/AAAS) **304** (5669): 441–5

	Manufacturer	IVD	For *in vitro* diagnostic use only
EC REP	Authorized representative		Consult instructions for use
Σ n	Contains sufficient for <n> tests		Keep dry
REF	Catalogue Code		Temperature limitation
LOT	Lot Number		Use by
DIL	Sample diluent		