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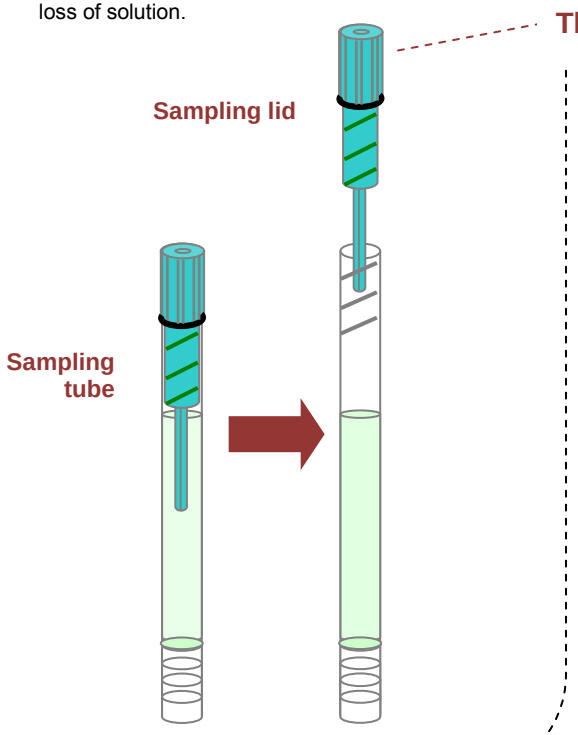
EpiTuub® Calprotectin Test Instructions for Fecal Sample Collection

Monoclonal antibody based immunoassay test for the detection of inflammatory bowel disease

Version 3

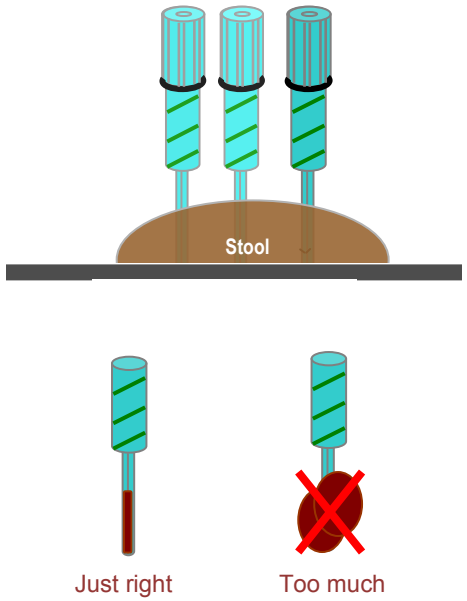
1

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.



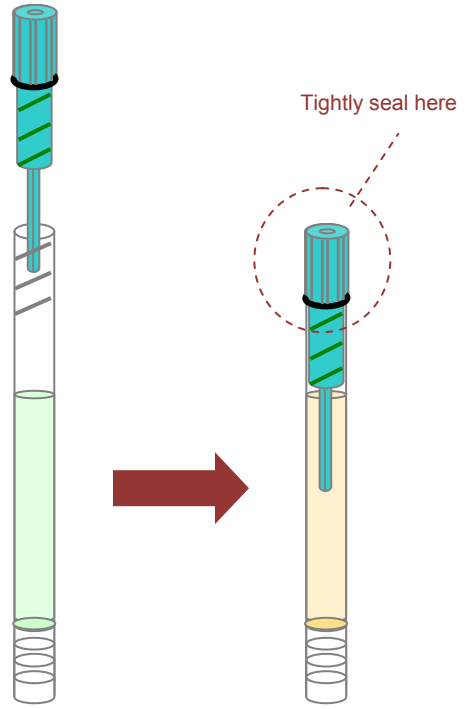
2

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.



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

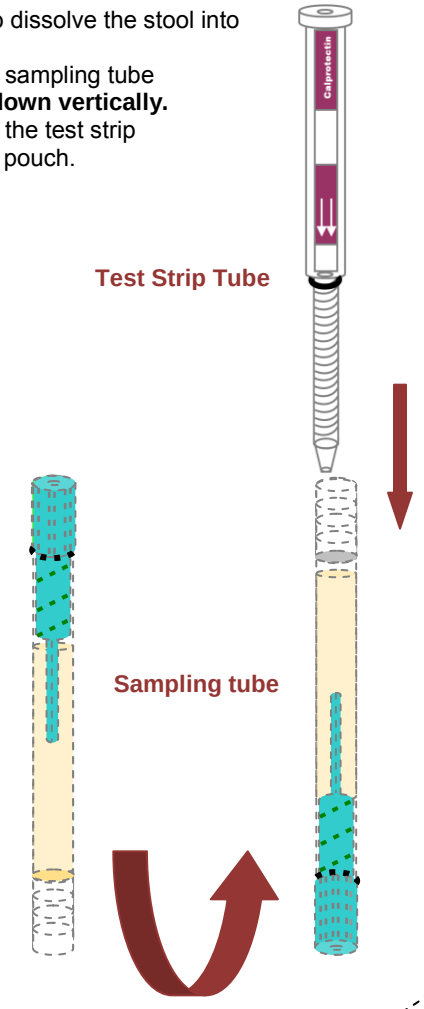
Store below 86°F (30°C). Do not freeze. Keep out of reach of children. For in-vitro diagnostic use. Not to be taken internally. Not to be sampled direct from anus.

EpiTuub® Calprotectin Test — Instructions for Test Procedures

Monoclonal antibody based immunoassay test for the detection of inflammatory bowel disease

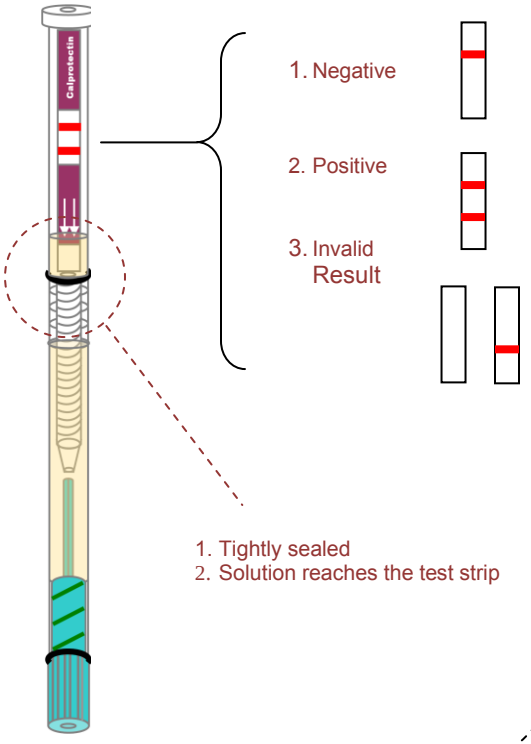
A

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
CLIA Complexity: Waived

Catalog Number: EPI-KT920 (30 Tests/ Kit)
EPI-KT921 (10 Tests/ Kit)

INTENDED USE

This Epituub Calprotectin Test Device is a rapid immunological test intended for the qualitative detection of Calprotectin in feces by professional laboratories and physician office laboratories. The test is used as an aid for the diagnosis of inflammatory bowel disease. Fecal Calprotectin levels correlate significantly with histological and endoscopic assessment of disease activity in ulcerative collitis, as well as with fecal -antitrypsin levels and fecal excretion of indium labeled white blood cells in patients with Crohn disease.

SUMMARY OF PHYSIOLOGY

Calprotectin (MRP 8/14 is a heterodimer of two calcium-binding proteins present in the cytoplasm of neutrophils and expressed by the membranes of monocytes. It constitutes nearly 60% of the soluble cytosol proteins in neutrophils and plays a central role in neutrophils defense. upon neutrophil activation or endothelial adhesion of monocytes, Calprotectin is release and may be detected in serum, body fluids or stool as a potentially useful clinical inflammatory marker.

TEST PRINCIPLE

The Epituub Calprotectin test is a “sandwich” immunoassay utilizing two monoclonal antibodies to specifically detect the presence of Calprotectin in feces. It consists of two units, a fecal sampling device and a test strip. A stool specimen is collected into the sampling tube containing extraction solution. After mixing the stool sample, a test strip is screwed into the sampling tube by breaking the bottom seal of the sampling tube while maintaining a vertical position. The extracted fecal solution flows into the bottom space of the test strip and triggers the start of the Calprotectin immunoassay. If the Calprotectin level is greater than 50 µg/g in a fecal sample extract, an immuno-complex of “labeled monoclonal anti-calprotectin antibody – membrane coated monoclonal anti-human calprotectin antibody” is formed. A red colored band appears in the test region, which is located in the lower half of the test membrane. a similar colored band must appear in the control region located in the upper-half of the test membrane, indicating the test strip is functioning properly and the result is valid.

REAGENTS AND MATERIALS PROVIDED

1. Fecal specimen collection device (30436): containing sampling tube, sampling lid and pre-added extraction solution in the sampling tube. This device should be stored at 2 to 8ºC. Do not freeze.

READ ALL THE INFORMATION IN THIS INSERT BEFORE TESTING
Store below 46°F (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 direct from anal.

EpiTuub® Calprotectin Test — Instructions for Test Procedures

Monoclonal antibody based immunoassay test for the detection of inflammatory bowel disease

2. Test strip tube (30437): one dipstick for the Calprotectin 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 30ºC. Do not freeze.
3. 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, we should see a minimum 5 mm extraction buffer liquid in the bottom of the strip tube.
2. We should see liquid migrating across the membrane area right after the screw in process. If not, we should 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 colored bands are visible within 5 minutes, the test result is positive and valid (Figure B).

- **Negative:**

If test area has no colored band and the control area displays a 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).

QUALITY CONTROL

Good laboratory practices recommend the use of appropriate controls. There are two types of controls for the Epituub Calprotectin test, the internal procedural control and external controls.

1. **Internal procedural control:** Each Epituub Calprotectin test consists 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 occult blood 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. Urine and excessive dilution of fecal samples with water from toilet bowl may cause erroneous results.
2. Intermittent tumor bleeding and irregular distribution of blood in the feces also contribute to false negative results.
3. Urine and excessive dilution of fecal samples with water from toilet bowl may cause erroneous results.
4. Epituub Calprotectin test is not for use in testing urine, gastric specimens or other body fluids.
5. 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 Calprotectin test is designed for the preliminary screening for IBD and should not replace other diagnostic procedures.

PERFORMANCE CHARACTERISTICS

The performance characteristics are under investigation

REFERENCES

1. Damms, A., Bischoff., S.C. Validation and clinical significance of a new Calprotectin rapid test for the diagnosis of gastrointestinal diseases. Int. J. colorectal Dis. 23:985-992, 2008.
2. Sidler, Marc, A., Leach, S. T., and Day, A. S. Fecal SI00A12 and fecal Calprotectin as noninvasive markers for inflammatory bowel disease in children. Inflamm. Bowel Dis. 14:359-366, 2008.
3. Voganatsi A., Panutich A. Miyasaki, K.T., and Murthy R.K. Mechanism of extracellular release of human neutrophil Calprotectin complex. J. Leuk. Biol. 70:130-134.
4. Roseth A.G., Aadland E., Jahnsen J., et al. Assessment of disease activity in ulcerative colitis by fecal Calprotectin, a novel granulocyte marker protein. Digestion, 58:176-180, 1997.
5. Silberer, H., Kuppers, B., Mickisch O., et al. Fecal leukocyte protein in inflammatory bowel disease and irritable bowel syndrome. Clin. Lab 51:117-126, 2005.
6. Bunn S.K., Bisset W.M., Main M.J., et al. Fecal Calprotectin: validation as a noninvasive measure of bowel inflammation I childhood inflammatory bowel disease. J. Pediatr. Gastroenterol Nutr. 33:14-22, 2001.
7. Roseth A.G., Kristinsson J., Fagerrhol M.K. et al. Faecal Calprotectin; a novel test for the diagnosis of colorectal cancer? Scand. J. Gastroenerol. 28:1073-1076, 1993.