**Entamoeba Ag Rapid Test**

*Cat. No.: DTS209*

*Pkg. Size: 20T*

### Intended use

Entamoeba Ag Rapid Test chromatographic immunoassay is a procedure for measurement of Entamoeba histolytica antigens in human stool samples.

### General Description

Entamoeba is a genus of Amoebozoa found as internal parasites or commensals of animals. In 1875, Fedor Lösch described the first proven case of amoebic dysentery in St Petersburg, Russia. He referred to the amoeba he observed microscopically as 'Amoeba coli'; however it is not clear whether he was using this as a descriptive term or intended it as a formal taxonomic name. The genus Entamoeba was defined by Casagrandi and Barbagallo for the species Entamoeba coli, which is known to be a commensal organism. Lösch's organism was renamed Entamoeba histolytica by Fritz Schaudinn in 1903; he later died, in 1906, from a self-inflicted infection when studying this amoeba. For a time during the first half of the 20th century the entire genus Entamoeba was transferred to Endamoeba, a genus of amoebas infecting invertebrates about which little is known. This move was reversed by the International Commission on Zoological Nomenclature in the late 1950s, and Entamoeba has stayed 'stable' ever since.

### Principle Of The Test

Entamoeba Ag Rapid Test use monoclonal antibodies specific for Entamoeba histolytica that detect all forms of the parasite during its life cycle.

The test is based on the use of red microspheres covalently linked to an anti-Entamoeba histolytica monoclonal antibody, plus blue microspheres as test control.

The parasites present in stool samples react with the latex particles which are coated with specific monoclonal antibodies against the antigen. This latex particles/antibodies/parasite complex migrates through a chromatographic process towards the reaction area. In this area, anti-Entamoeba histolytica antibodies that react with the latex particles/antibodies/parasite complex are present. This reaction leads to the appearance of a red line. These lines are used to interpret the result after a five-minute incubation at room temperature.

### Reagents And Materials Provided

1. Entamoeba Ag Rapid Test (Stool) (Cassettes)
2. 20 Reaction devices (cassettes)
3. A vial containing dilution buffer.

The volume of dilution buffer provided is proportional to the number of strips included and is indicated on the vial label.

### Materials Required But Not Supplied

1. Suitable applicators for sample collection (solid or liquid).
2. Vortex mixer.
3. Centrifuge adapted to sample extraction tubes.
4. 96-well microplate or other suitable containers for strip-format device measurements.
5. Test tubes.
6. Variable micropipettes.
7. Pipette tips.
8. Timer.
9. Disposable gloves.

**Storage**

The kit can be stored at temperatures between 2 °C and 30 °C (it can be stored in a refrigerator). The expiration date is printed on the package.

**Specimen Collection And Preparation**

1. Stool samples should be collected in an appropriate sterile container as soon as possible after symptom onset. The sample should be representative, whenever possible.
2. The samples can be stored in the refrigerator (at approximately 4 °C) for 1-2 days before being analyzed.
3. To store samples for a longer period, store them in a freezer at -20 °C, with no further handling. In this case, the sample must be completely thawed, brought to room temperature and homogenized before analysis.
4. Avoid freezing and thawing the samples several times.
5. Do not use specimens that have been collected or stored in transport medium or preserving agents like 10 % formalin, SAF, PVA, Ecofix, etc., because they interfere with the test.

**Procedure of sample preparation**

1. **Important**: Obtain samples from three different sampling sites at least, in order to get a sample as much representative as possible.
2. Place **1.0 mL of extraction buffer** in a properly labeled testing tube.
3. **Add a solid sample** portion of approximately **50 mg**, with a swab, a wooden applicator or a bacteriology loop (homogenize the sample before collecting). For **liquid or semi-solid stools** add **100 μL** of stool using an appropriate pipette.
4. **Shake** the test tube thoroughly by using a vortex mixer to assure proper mixing.
5. **Centrifuge** 5 minutes at 700 xg (approximately 3000 rpm in a benchtop centrifuge) to settle solid particles.

**Assay Procedure**

1. Following preparation of the sample, take the reaction device out of the aluminum pouch.
2. Discard the small bag of desiccant because is not used during the test; its only purpose is to protect the test against moisture.
3. Add **125 μL** of the supernatant prepared in the "Procedure of sample preparation” section into the sample area of the reaction device (round window marked with an arrow).

**Quality Control**

The test is invalid if no blue line appears, whether because the test was not properly performed or the reagents have deteriorated. If this happens, repeat the analysis following the working protocol indicated in these instruction sheets closely.

**WARNING**: Including a control with an established result is recommended to ensure that the data obtained are correct.

**Interpretation of Results**

**NEGATIVE:**

Only a single **BLUE** line appears in the result area (in the cassette test, aligned with the letter "C" (Control) marked on the device frame). This line should always appear.

**POSITIVE:**

A **BLUE** line and a separate **RED/PINK** line appear in the result area (in the cassette test, aligned with the letter "T" (Test))
marked on the device frame). The intensity may vary according to the antigen concentration present in the sample.

**INVALID:**
If no blue line appears the test is INVALID because the procedure was not correctly followed, the reagents have deteriorated or because an incorrect amount of sample was added. Repeat the test with a new reaction device.

Interpretation code for strip images:
C: Entamoeba histolytica
N: Negative

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**Precautions**

1. Specimen samples (fecal matter) may contain infectious agents and should be treated and disposed of as potentially dangerous biological materials.
2. The buffer contains sodium azide as antimicrobial agent. Avoid direct contact with skin and mucosa. Dispose of appropriately.
3. Do not use the buffer if signs of contamination or precipitation are observed.
4. Do not store or prepare food, eat, drink or smoke in the area where the reagents and samples are handled.
5. Wear disposable gloves when handling the samples. Wash your hands thoroughly once you have finished working.
6. Do not exchange components from kits with different lot numbers.
7. All reagents are for research use exclusively.
8. Before using, let all kit components and samples reach room temperature, because cold reagents and/or samples can reduce test functionality. About 20-30 minutes are usually sufficient for reaching room temperature.
9. Do not use kit components beyond the expiration date.
10. If the package is broken, the product can still be used as long as none of the components have been damaged.
11. It is important to add the correct sample amount (see procedures). If less amount than the indicated is used, the chromatography may not occur because the sample may not reach the reaction area; if an excess amount is used, brown lines may appear instead of red and blue ones.
12. The used product should be disposed of as indicated by current legislation.
13. Do not use the test if a colored line appears in the result area before its use.
14. It is important to take the appropriate amount of stool sample: 50 mg of solid stool or 100 µL of liquid stool to be extracted in 1 mL of diluent. It is very important to keep the correct sample:buffer ratio to ensure the correct performance of the test. An excessive sample amount in regard to buffer amount prevents correct chromatography.
15. In the case of the strip product packaged in a tube, it is important to close the tube immediately after taking the reactive strip out; otherwise, high room humidity might damage the rest of the strips inside the tube.

REFERENCES

7. Paniagua, G.L. et al., Two or more enteropathogens are associated with diarrhoea in Mexican children. 28: 17 (2007).