PreventID® **Entamoeba histolytica** Manual (FOR PROFESSIONAL USE)

→REF KSTIEH602G10
KSTIEH602GP

Intended Use

PreventID® Entamoeba histolytica is a rapid chromatographic immunoassay for the qualitative detection of Entamoeba histolytica antigens in human faeces.

Introduction

Entamoeba histolytica is an anaerobic parasitic amoebozoan, part of the genus Entamoeba [1]. Predominantly infecting humans and other primates causing amoebiasis, *E. histolytica* is estimated to infect about 50 million people worldwide. Previously, it was thought that 10% of the world population was infected, but these figures predate the recognition that at least 90% of these infections were due to a second species, *E. dispar* [2]. Mammals such as dogs and cats can become infected transiently, but are not thought to contribute significantly to transmission. *E. histolytica*, as its name suggests (histolytic = tissue destroying), is pathogenic; infection can be asymptomatic or can lead to amoebic dysentery or amoebic liver abscess [1, 3].

Test Principle

PreventID® Entamoeba histolytica is a qualitative, lateral flow immunoassay for the detection of *Entamoeba histolytica* antigens in human faeces. The membrane is precoated with anti-Entamoeba histolytica antibody on the test line region of the test. During testing, *E. histolytica* antigens, if present in the specimen react with *Entamoeba histolytica* antibodies conjugated coloured particles. The antigen-conjugate complex migrates upward on the membrane chromatographically by capillary action to react with anti-*Entamoeba histolytica* antibodies on the membrane and generate a coloured line. The presence of this coloured line in the test line region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a coloured line will always appear in the control line region, indicating that the proper volume of specimen has been added and membrane wicking has occurred. The test contains anti-*Entamoeba histolytica* antibody conjugated coloured particles and anti-*Entamoeba histolytica* antibodies coated on the membrane.

Materials

Materials provided

- test devices (with sample droppers), individually packed TEST
- sample collection tubes with extraction buffer TUBE
- manual

Materials Required but not Provided: timer or stop watch, specimen collection containers

Storage and Stability

Store the test between 2 °C and 30 °C; **do not freeze**. The test device is stable though the expiry date printed on the sealed pouch. The test device is sensitive to humidity as well as to heat. Perform the test immediately after removing the test device from the pouch. Do not use it beyond the expiry date.

Precautions

1. For *in vitro* diagnostic use only.
2. Do not eat or smoke while handling specimen.
3. Wear protective gloves and wash hands thoroughly after performing the test.
4. Avoid splashing or aerosol formation while handling specimen and performing the test.
5. All samples and materials used should be treated as potentially infectious and disposed in a biohazard container. Clean all contaminated objects and surfaces carefully. The used test should be discarded according to the local regulations.
6. Do not use test if the pouch is torn or if the membrane of the test device is visibly damaged. Note the expiry date. The test should remain in the sealed pouch until use.
7. Read the instruction carefully before performing the test.
8. Do not mix reagents from different lots.
9. If you have any questions please contact Preventis GmbH.

Sample Collection and Sample Preparation

1. To collect faecal specimens:
   Collect sufficient quantity of faeces (1–2 mL or 1–2 g) in a clean, dry specimen collection container to obtain maximum antigens (if present). Best results will be obtained if the assay is performed within 6 hours after collection. Specimen collected may be stored for 3 days at 2-8 °C if not tested within 6 hours. For long term storage, specimens should be kept below -20 °C.
2. To process faecal specimens:
   For Solid Specimens:
   Unscrew the cap of the sample collection tube, then randomly stab the specimen collection stick into the faecal specimen in at least 3 different sites to collect approximately 50 mg of faeces (equivalent to 1/4 of a pea). Do not scoop the faecal specimen.
   For Liquid Specimens:
   Hold the dropper vertically, aspirate faecal specimens, and then transfer 2 drops (approximately 80 µL) into the sample collection tube containing the extraction buffer.
3. Tighten the cap onto the sample collection tube, then **shake the sample collection tube vigorously** to mix the specimen and the extraction buffer. Leave the sample collection tube for reaction for 2 minutes.

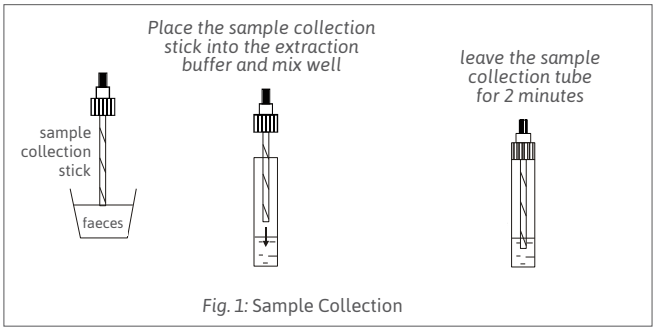

Fig. 1: Sample Collection

Test Procedure

Allow the test, specimen, buffer and/or controls to reach room temperature (15–30°C) prior to testing.

1. Bring the pouch to room temperature before opening it. Remove the test device from its pouch and place it on a flat dry surface. The oval sample application window at the one end of the test device should be at the right side.
2. Hold the sample collection tube upright and open the cap onto the sample collection tube. Invert the sammple collection tube and transfer **2 full drops of the extracted specimen (approximately 80 µL)** to the sample application window (S) of the test device, then start the timer. Avoid trapping air bubbles in the sample application window *(Fig. 2)*.

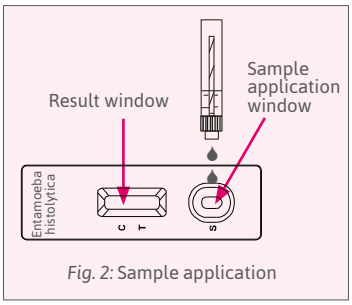

Fig. 2: Sample application

3. Read results at **5 minutes** after dispensing the specimen. Do not read results after 10 minutes.

Note: If the specimen does not migrate, centrifuge the extracted specimens contained in the extraction buffer vial. Collect 80µL of supernatant, dispense into the sample application window (S) of a new test device and start afresh following the instructions mentioned above.

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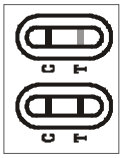
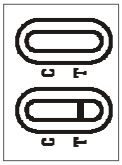
Test Interpretation

Positive: Two lines appear. One coloured line should be in the control line region (C) and another apparent coloured line should be in the test line region (T). **Note:** The intensity of the colour in the test line region (T) will vary depending on the concentration of *Entamoeba histolytica* antigen present in the specimen. Therefore, any shade of colour in the test line region (T) should be considered positive.



Negative: One coloured line appears in the control line region (C). No line appears in the test line region (T).

Invalid: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test.
If the problem persists, discontinue using the test kit immediately and contact Preventis.



Caution: In order to prevent incorrect results the test should not be interpreted later than after 10 minutes.

Test Characteristics

Clinical Sensitivity, Specificity and Accuracy

The performance of the **PreventID® Entamoeba histolytica** rapid test has been evaluated with 142 clinical specimens collected from the patient symptomatic and asymptomatic in comparison with other rapid test method. The results show that the relative sensitivity of the **PreventID® Entamoeba histolytica** rapid test is 95.7% and the relative specificity is 99.2%.

		Other rapid test		
PreventID® Entamoeba histolytica	Results	Postive	Negative	**Total Result**
	Positive	22	1	23
	Negative	1	118	119
Total Result		23	119	142

Relative sensitivity: 95.7% (95% CI*: 78.1% ~ 99.9%)
Relative specificity: 99.2% (95% CI*: 95.4% ~ 99.9%)
Accuracy: 98.6% (95%CI*: 95.0% ~ 99.8%) *Confidence Intervals

Reproducibility

Intra-Assay:
Within-run precision has been determined by using three replicates of these specimens: negative, low positive, middle positive and high positive specimens. The specimens were correctly identified >99% of the time.

Inter-Assay:
Between-run precision has been determined by three independent assays on the same specimens: negative, low positive, middle positive and high positive specimens. Three different lots of the **PreventID® Entamoeba histolytica** rapid test have been tested using these specimens. The specimens were correctly identified >99% of the time.

Cross-reactivity

An evaluation was performed to determine the cross reactivity of **PreventID® Entamoeba histolytica** rapid test. No cross reactivity against gastrointestinal pathogens occasionally present as following:

Campylobacter coli
Colstridium difficile
Campylobacter jejuni
E.coli O157:H7
H. pylori
Listeria monocytogenes
Salmonella enteritidis
Salmonella paratyphi
Salmonella typhimurium
Salmonella typhi
Staphylococcus aureus
Yersinia enterocolitica

Quality Control

A procedural control is included in the test. A coloured line appearing in the control line region (C) is considered an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique. Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

Test Limitations

The **PreventID® Entamoeba histolytica** rapid test is for in vitro diagnostic use only.
The **PreventID® Entamoeba histolytica** rapid test will only indicate the presence of Entamoeba histolytica antigen in the faeces specimen, the detail concentration of *Entamoeba histolytica* antigen was not confirmed with the rapid test.
As with all diagnostic tests, all results must be considered with other clinical information available to the physician. Other clinically available tests are required if questionable results are obtained.

References:
1. Ryan KJ, Ray CG, eds. (2004).Sherris Medical Microbiology(4th ed.). McGraw Hill. pp.733–8.
2. Weekly Epidemiological Record.72(14): 97–9. April 1997.
3. Nespola, Benoît; Betz, Valérie; Brunet, Julie; Gagnard, Jean-Charles; Krummel, Yves; Hansmann, Yves; Hannedouche, Thierry; Christmann, Daniel; Pfaff, Alexander W.; Filisetti, Denis; Pesson, Bernard; Abou-Bacar, Ahmed; Candolfi, Ermanno (2015)."First case of amebic liver abscess 22 years after the first occurrence"

Short Instruction PreventID® **Entamoeba histolytica**

1. Collect a faecal sample with the aid of the sample collection tube and the sample collection stick as described in the instruction.
2. Shake the solution in the sample collection tube very thoroughly. Unpack the test device.
3. Open the cap of the sample collection tube carefully.
4. Transfer **2 full drops of the extracted sample** into the oval sample application window.
5. Interpret the test after **5 minutes.**

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Status: 2019-12-09

US: all products: Research Use Only. Not for use in diagnostic procedures.

Temperature limitation	Manufacturer
In vitro diagnostic device	Lot number
Catalogue number	Expiry date
To be used with	Do not reuse
Read user instructions	Contains sufficient for <n> tests
Keep away from sunlight	

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