Entamoeba histolytica Ag ELISA

Enzyme Immunoassay for the detection of Entamoeba histolytica antigen in faecal samples

IVD

REF DEM-DE4454

96 wells
Please use only the valid version of the package insert provided with the kit. 
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Arbeitsanleitung.
Si prega di usare la versione valida dell’inserto del pacco a disposizione con il kit.
Por favor, se usa solo la versione válida de la metodico técnico incluido aquí en el kit.

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1 INTRODUCTION

*Entamoeba histolytica* is the causative agent of amoebiasis (amoebic dysentery, amoebic liver abscess). Only trophozoites of virulent strains invade the intestinal wall and cause ulcers releasing blood and mucus into the faeces. Invasive Amoebae reach the liver via the enteral vascular system and cause extensive abscesses (1). The infectious cysts are spread via faecal-oral transmission. The vegetative trophozoites are released by excystation and multiply by bisection. Meanwhile molecular biological (PCR) and biochemical (isoenzyme analysis) methods confirm the classification of the pathogenic, invasive strains as separate species *Entamoeba histolytica*. The non-invasive strains are classified as *Entamoeba dispar*. The two species share an identical morphology and therefore a microscopic differentiation is impossible (1-6).

*Entamoeba histolytica* releases specific antigens into the intestine during its life cycle. These antigens are excreted with the faeces of infected persons. The antigen detection by enzyme immunoassay can serve as specific and easy to perform alternative to microscopy.

References / Literature:


2 INTENDED USE

The *Entamoeba histolytica Ag ELISA* is an *in-vitro*-diagnostic device for direct detection of *Entamoeba histolytica* specific antigen in faecal samples.

3 PRINCIPLE OF THE TEST

Entamoeba histolytica Ag ELISA is a fast enzymometric two-step immunoassay based on polyclonal peptide antibodies recognizing two different epitopes of the serine-rich 30 kD membrane protein (SREHP) of *Entamoeba histolytica*.

Diluted stool specimens as well as positive and negative controls react in the first incubation step of 60 minutes at room temperature with the solid phase bound antibodies. In the following washing step unbound components are removed from the wells.

In the next step horseradish-peroxidase (HRP) labelled antibodies react with solid phase bound antibody-antigen-complexes within a reaction time of 30 minutes at room temperature. Non-bound material is separated from the solid-phase immune complexes by a subsequent washing step. HRP converts the subsequently added colorless substrate solution of 3,3’,5,5’-tetramethylbenzidine (TMB) into a blue product. The enzyme reaction is terminated by sulphuric acid dispensed into the wells after 10 min incubation at room temperature turning the solution from blue to yellow.

The optical density (OD) of the solution read at 450/620 nm is directly proportional to the specifically bound amount of *Entamoeba histolytica* antigen.
4 TEST COMPONENTS

<table>
<thead>
<tr>
<th></th>
<th>Microtiteration plate coated with polyclonal anti-SREHP- antibodies (rabbit)</th>
<th>For 96 Wells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SORB MT</td>
<td>12 single breakable 8-well strips colour coding light brown vacuum-sealed with desiccant</td>
</tr>
<tr>
<td>2</td>
<td>WASH SOLN 10x Wash buffer 10-fold</td>
<td>100 mL concentrate for 1000 mL solution white cap</td>
</tr>
<tr>
<td>3</td>
<td>SAM DIL Sample diluent</td>
<td>100 mL · ready to use coloured yellow black cap</td>
</tr>
<tr>
<td>4</td>
<td>CONTROL + Positive control SREHP peptide</td>
<td>2.0 mL · ready to use coloured blue red cap</td>
</tr>
<tr>
<td>5</td>
<td>CONTROL – Negative control Entamoeba histolytica negative sample</td>
<td>2.0 mL · ready to use coloured blue green cap</td>
</tr>
<tr>
<td>6</td>
<td>ENZ CONJ HRP-conjugate HRP labelled, polyclonal anti-SREHP-antibodies (Sheep)</td>
<td>15 mL · ready to use coloured green brown cap</td>
</tr>
<tr>
<td>7</td>
<td>SUB TMB Substrate 3,3',5,5'-Tetramethylbenzidine and hydrogen peroxide</td>
<td>15 mL · ready to use blue cap</td>
</tr>
<tr>
<td>8</td>
<td>STOP SOLN Stop solution 0.25 M sulphuric acid</td>
<td>15 mL · ready to use yellow cap</td>
</tr>
</tbody>
</table>

5 PREPARATION AND STORAGE OF SAMPLES

5.1 Collection and storage

Stool samples should be stored at 2 - 8 °C immediately after collection and processed within 72 hours. Longer storage is possible at -20 °C. Repeated freezing and thawing of samples should be avoided. Stool samples already diluted with the sample diluent [SAM DIL] can be stored for 72 h at 2 - 8 °C before testing. Faecal samples that are already diluted in transportation media should not be used.

5.2 Preparation

Warm samples to room temperature and mix well. Pipette 1000 µL of sample diluent into a clean tube. Using a disposable stirring rod transfer about 200 mg (diameter about 4-6 mm) of faeces if solid or 200 µL if liquid into the tube and suspend thoroughly. If necessary, sediment floating particles by a centrifugation step with a micro centrifuge for one min at maximum speed.

6 MATERIALS REQUIRED BUT NOT PROVIDED

- micropipettes
- multi-channel pipette or multi-pipette
- reagent container for multi-channel pipette
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with optical filters for 450 nm for measurement and ≥ 620 nm for reference
- distilled or de-ionized water
- glassware
- tubes (2 mL) for sample preparation
7 PREPARATION AND STORAGE OF REAGENTS

7.1 Kit size and expiry
One kit is designed for 96 determinations. The expiry date of each component is reported on its re-
spective label, that of the complete kit on the outer box label. Upon receipt, all test components have
to be kept at 2 - 8 °C, preferably in the original kit box. After opening all kit components are stable for
at least 2 months, provided proper storage. The ready to use wash buffer solution is stable for at least
one month when stored at 2 - 8 °C.

7.2 Reagent preparation
Allow all components to reach room temperature prior to use in the assay. The microtitration plate is
vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells.
Allow the sealed plate to reach room temperature before opening. Unused wells should be stored
refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the 10-fold concentrated wash buffer 1 + 9
with distilled or de-ionized water.

For Example:
10 mL wash buffer concentrate (2) + 90 mL distilled or deionized water.

8 ASSAY PROCEDURE

o Dilute samples with sample diluent (3) 1:6,
  e.g. 200 mg or 200 µL faeces + 1.0 mL sample diluent (3)
  o Avoid any time shift during dispensing of reagents and samples.
  o Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle and
that residual fluid is completely drained in every single wash cycle.
  o Avoid light exposure of the TMB substrate solution.

8.1 Working Steps
1. Warm all reagents to room temperature (RT) before use. Mix gently without causing foam.
2. Pipette:
   100 µL CONTROL + positive control (4)
   100 µL CONTROL - negative control (5)
   100 µL diluted sample
3. Cover plate and incubate for 60 min at RT.
4. Decant, then wash each well 5x with 300 µL wash solution (diluted from (2)) and tap dry onto
   absorbent paper.
5. Dispense 3 drops (or 100 µL) ENZ CONJ (6) per well
6. Cover plate and incubate for 30 min at RT.
7. Decant, then wash each well 5x with 300 µL wash solution (diluted from (2)) and tap dry onto
   absorbent paper.
8. Dispense 3 drops (or 100 µL) SUB TMB substrate (7) per well.
9. Incubate for 10 min at RT protected from light.
10. Dispense 3 drops (or 100 µL) STOP SOLN stop solution (8), mix gently.
11. Read OD at 450 nm / ≥ 620 nm with a microplate reader within 30 min after reaction stop.

9 RESULT INTERPRETATION

9.1 Qualitative evaluation
Cut-off determination: OD negative control + 0.20
Samples with absorbances equal to or higher than the cut-off value are considered positive, samples
with absorbances below the cut-off value are considered negative for Entamoeba histolytica antigen.
10 REFERENCE VALUES

<table>
<thead>
<tr>
<th>Entamoeba histolytica Ag ELISA</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>≥ Cut-off</td>
</tr>
<tr>
<td>Negative</td>
<td>&lt; Cut-off</td>
</tr>
</tbody>
</table>

It is recommended that each laboratory establishes its own normal and pathological reference ranges as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

10.1 Test validity
The test run is valid, if:
- the mean OD of the negative control is:
  - ≤ 0.20 (manual performance)
  - ≤ 0.30 (automatic performance)
- the mean OD of the positive control is:
  - ≥ 0.80

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

10.2 Limitations of the procedure
There is no correlation between measured absorbance and seriousness of the infection.
It is also not allowed to correlate absorbencies of the samples with that of the positive control.
Cross contamination of reagents and samples can produce false positive results. Incorrect dilutions and not sufficiently homogenized samples can cause false results. A negative ELISA result does not exclude an Entamoeba histolytica infection, because the number of excreted cysts can decrease below the detection limit of the assay in invasive amoebiasis. Thus additional investigations (e.g., detection of specific antibodies or ultrasound) should be performed in case of a negative ELISA result but clinical suspect. Clinical findings have to be considered for a final result interpretation.

10.3 Automatic Processing
Performing the Entamoeba histolytica Ag ELISA on fully automated microplate processors (e.g. DS2, DSX) may cause elevated absorbencies in comparison to the manual procedure due to individual differences concerning wash procedures and general technical specifications of the equipment. In these cases a maximum value of 0.3 absorbance units is permissible for the negative control. It is recommended to use a wash procedure including 10 seconds soak time per strip and wash step followed by a wash step with distilled or de-ionized water with 10 seconds of soak time after the final wash step of each wash cycle. If necessary the number of washing steps can be enhanced from 5x to 7x-8x.

Correlation: Manual vs. automatic processing
A panel of 140 stool specimens was investigated in parallel by manual and automatic processing method (DS2, Dynex Technologies) resp. The correlation was calculated with $r = 0.998$. 

![Graph showing correlation between manual and automatic processing](image)
11 PERFORMANCE CHARACTERISTICS

Precision
Intra-assay coefficient of variation (CV) in the *Entamoeba histolytica Ag ELISA* calculated from 12 fold determinations of samples:

<table>
<thead>
<tr>
<th>sample</th>
<th>mean OD</th>
<th>standard deviation</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.841</td>
<td>0.137</td>
<td>7.5</td>
</tr>
<tr>
<td>2</td>
<td>1.208</td>
<td>0.078</td>
<td>6.5</td>
</tr>
<tr>
<td>3</td>
<td>0.620</td>
<td>0.040</td>
<td>6.4</td>
</tr>
<tr>
<td>4</td>
<td>0.463</td>
<td>0.024</td>
<td>5.3</td>
</tr>
</tbody>
</table>

Inter-assay coefficient of variation (CV) in the *Entamoeba histolytica Ag ELISA* from 11 different test runs from 3 fold determination of samples:

<table>
<thead>
<tr>
<th>sample</th>
<th>mean OD</th>
<th>standard deviation</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.720</td>
<td>0.128</td>
<td>4.7</td>
</tr>
<tr>
<td>2</td>
<td>1.647</td>
<td>0.122</td>
<td>7.4</td>
</tr>
<tr>
<td>3</td>
<td>0.968</td>
<td>0.074</td>
<td>7.7</td>
</tr>
<tr>
<td>4</td>
<td>0.409</td>
<td>0.019</td>
<td>4.6</td>
</tr>
</tbody>
</table>

11.1 Lower detection limit
The lower detection limit was determined by titration of faecal samples spiked with trophozoites from culture. The lower detection limit was determined 5x10³ - 6x10³ trophozoites per mL stool suspension.

11.2 Specificity
A sample panel of in all n= 160 faecal specimens from a routine microbiological laboratory was tested in the *Entamoeba histolytica Ag ELISA*: All samples were tested negative (OD < Cut-off) corresponding to a specificity of 100% for this sample panel.

11.3 Cross reactivity
Faecal samples positive for one of the following intestinal parasites did not show any cross reaction in the *Entamoeba histolytica Ag ELISA*: *Ancylostoma duodenale, Ascaris lumbricoides, Blastocystis hominis, Cryptosporidium parvum, Dientamoeba fragilis, Entamoeba coli, Entamoeba dispar, Entamoeba hartmanni, Giardia lamblia*.

Negative stool specimens have been spiked with ≥ 10⁸ colony forming units of the following microorganisms and tested negative with the *Entamoeba histolytica Ag ELISA* (OD 450/620 nm < Cut-off):

<table>
<thead>
<tr>
<th>Aeromonas hydrophila  (ATCC 7966)</th>
<th>Klebsiella pneumoniae (ATCC 10231)</th>
<th>Proteus vulgaris (ATCC 60015)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus cereus  (ATCC 11778)</td>
<td>Peptostreptococcus anaerobius (ATCC 27337)</td>
<td></td>
</tr>
<tr>
<td>Bacillus subtilis  (ATCC 6633)</td>
<td>Proteus vulgaris (ATCC 8427)</td>
<td></td>
</tr>
<tr>
<td>Bacteroides fragilis  (ATCC 25285)</td>
<td>Pseudomonas aeruginosa (ATCC 10145)</td>
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<tr>
<td>Candida albicans  (ATCC 10231)</td>
<td>Salmonella enterica Serovar enteritidis (ATCC 13076)</td>
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<tr>
<td>Campylobacter coli  (ATCC 33559)</td>
<td>Salmonella enterica Serovar typhimurium (ATCC 14028)</td>
<td></td>
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<tr>
<td>Campylobacter jejuni (ATCC 33291)</td>
<td>Shigella flexneri (ATCC 12022)</td>
<td></td>
</tr>
<tr>
<td>Citrobacter freundii (ATCC 8090)</td>
<td>Shigella sonnei (ATCC 25931)</td>
<td></td>
</tr>
<tr>
<td>Clostridium sordellii (ATCC 9714)</td>
<td>Staphylococcus aureus (ATCC 25923)</td>
<td></td>
</tr>
<tr>
<td>Enterobacter aerogenes (ATCC 13048)</td>
<td>Staphylococcus epidermidis (ATCC 12228)</td>
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</tr>
<tr>
<td>Enterobacter cloacae (ATCC 13047)</td>
<td>Vibrio parahaemolyticus (ATCC 17802)</td>
<td></td>
</tr>
<tr>
<td>Enterococcus faecalis (ATCC 29212)</td>
<td>Vibrio cholerae (Clinical isolates)</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli  (ATCC 25922)</td>
<td>Yersinia enterocolitica (O3, O9)</td>
<td>(Clinical isolates)</td>
</tr>
</tbody>
</table>

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JS
Updated 150818
11.4 Interference
None of the following substances added to negative stool samples showed a significant impact on the test result: Bariumsulfat (5%), Buscopan® (2 mg/mL), Cyclamat (5%), Diclofenac (2 mg/mL), Hämoglobin (5 mg/mL), Hylak® N (5%), Immodium® akut duo (0,2/12,5 mg/mL), Iberogast® (5%), Loperamid (0,2 mg/mL), Metronidazol (2 mg/mL), Mucin (5 mg/mL), Nexium® (2 mg/mL), Palmitinsäure (20%), Pentofuryl® (2 mg/mL), Pepto-Bismol (1 mg/mL), Perenterol (2,5 mg/mL), Rennie® (8 mg/mL), Simagel® (2 mg/mL), Stearinsäure (20%).

12 COMMON ADVICES AND PRECAUTIONS
This kit is for in-vitro use only. Follow the working instructions carefully. The kit should be performed by trained technical staff only. Do not use reagents from damaged packages or bottles. The expiration dates stated on the respective labels are to be observed. Do not use or mix reagents from different lots except for sample diluent, wash buffer, TMB/substrate solution and stop solution. Do not use reagents from other manufacturers. Avoid time shift during dispensing of reagents. All reagents should be kept at 2 - 8 °C before use. Some of the reagents (2, 3, 4, 5, 6, 7) contain small amounts of Thimerosal (0.01% w/v) and Kathon (1.0% v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucous membranes. Handle all components and all patient samples as if potentially hazardous. Since the kit contains potentially hazardous materials, the following precautions should generally be observed:
− Do not smoke, eat or drink while handling kit material,
− Always use protective gloves,
− Never pipette material by mouth,
− Note safety precautions of the single test components.
<table>
<thead>
<tr>
<th>Symbol</th>
<th>English</th>
<th>Deutsch</th>
<th>Français</th>
<th>Español</th>
<th>Italiano</th>
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<tbody>
<tr>
<td>CE</td>
<td>European Conformity</td>
<td>CE-Konformitäts-kennzeichnung</td>
<td>Conforme aux normes européennes</td>
<td>Conformidad europea</td>
<td>Conformità europea</td>
</tr>
<tr>
<td>i</td>
<td>Consult instructions for use</td>
<td>Gebrauchsanweisung beachten</td>
<td>Consultez les instructions d'utilisation</td>
<td>Consulte las Instrucciones</td>
<td>Consultare le istruzioni per l'uso</td>
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<tr>
<td>IVD</td>
<td>In vitro diagnostic device</td>
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<td>Diagnóstico in vitro</td>
<td>Per uso Diagnostica in vitro</td>
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<td>Sólo para uso en investigación</td>
<td>Solo a scopo di ricerca</td>
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<td>Katalog-Nr.</td>
<td>Référence</td>
<td>Número de catálogo</td>
<td>No. di Cat.</td>
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<tr>
<td></td>
<td>Contains sufficient for</td>
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<td>Contenu suffisant pour</td>
<td>Contenido suficiente</td>
<td>Contenuto sufficiente</td>
</tr>
<tr>
<td></td>
<td>&lt;n&gt; tests/</td>
<td>&quot;n&quot; tests</td>
<td>para &lt;n&gt; ensayos</td>
<td>per &quot;n&quot; saggi</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Note warnings and precautions</td>
<td>Warnhinweise und Vorsichtsmaßnahmen beachten</td>
<td>Avertissements et mesures de précaution</td>
<td>Tiene en cuenta advertencias</td>
<td>Annoti avvisi e le precauzioni</td>
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<td>Legal Manufacturer</td>
<td>Hersteller</td>
<td>Fabricant</td>
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<td>Fabbricante</td>
</tr>
</tbody>
</table>

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Distribuito in ITALIA da

**Li StarFish S.r.l.**
Via Cavour, 35
20063 Cernusco S/N (MI)
telefono 02-92150794
fax 02-92157285
info@listarfish.it
www.listarfish.it