ELI.H.A Amoeba

Serodiagnosis of amoebiasis by indirect haelagglutination

120 Tests

(Réf. 66602)

8000100-EN-2025-07

For *in vitro* diagnostic use only, for professional use only.
Single use test.

CE

1 - AIM

ELI.HA Amoeba enables the quantitative determination of anti-*Entamoeba histolytica* serum antibodies by indirect haemagglutination.
Each kit allows 120 tests to be carried out or 20 reactions of 6 dilutions.

2 - INTRODUCTION

Amoebiasis is a parasitic infection caused by a protozoon specific to man: *Entamoeba histolytica*. It is the only pathogenic amoeba of man. Serodiagnosis enables the confirmation of hepatic and pulmonary amoeba infections.

3 - PRINCIPE

ELI.HA Amoeba is based on the indirect haemagglutination principle. The sensitized red blood cells consist of sheep red blood cells covered with an *Entamoeba histolytica* antigen.
The presence of anti-*Entamoeba histolytica* serum antibodies results in agglutination of the sensitized red blood cells resulting in a cloudy red/brown deposit coating the well. In the absence of specific antibodies, the red blood cells form a ring-like deposit at the bottom of the well.
The non-sensitized red blood cells ensure the specificity of the reaction making it possible to eliminate any interference from the natural anti-sheep agglutinins (Forssman heteroantibodies, infectious mononucleosis antibodies…).
The reaction is carried out in a U-microplate.
Handling is simple and fast, with results within 2 hours.

4 - REAGENTS AND MATERIAL

Description	Quantité
R1: Vial of 2.4 mL of sensitized red blood cells	**1**
R2: Vial of 1 mL of non-sensitized red blood cells	**1**
BUF: Vial of 55 mL of phosphate buffer pH 7.2	**1**
R3: Vial of 2 mL of adsorbent	**1**
CONTROL +: Vial of 0.2 mL of titrated positive control	**1**
CONTROL -: Vial of 0.2 mL of negative control	**1**
MICROPLATE: Microplate with a U-bottom	**2**
DROPPER: Special dropper	**2**

5 - PRECAUTIONS

- The reagents are intended for *in vitro* diagnostic use only and must be handled by authorized personnel.
- Tests are for single use only.
- All the reagents, except the **BUF** reagent, contain raw materials of animal origin and must be handled with caution.
- Patient samples are potentially infectious. They must be handled with caution, in observance of hygiene rules and the current regulations for this type of product in the country of use.
- The reagents contain sodium azide (concentration < 0.1%). The sodium azide contained in the reagents can react with the heavy metals in the pipes to form explosive compounds. It is therefore recommended not to dispose of the reagents down the sink and to follow the recommendations and regulations for waste disposal in force.
- Do not use reagents after the expiry date.
- Do not use reagents from different batch numbers.
- Prior to use, allow the serum and the reagents to reach room temperature.
- Carefully shake the **R1** and **R2** reagents before use.
- When dispensing the **R1** and **R2** reagents, make sure that the dropper is perfectly vertical. Check for the absence of air bubbles in the drops to ensure constant delivery volumes.

6 - SAMPLE COLLECTION AND TREATMENT

Use fresh serum or serum preserved at - 20°C, and not showing any sign of haemolysis, cloudiness or of contamination.
Avoid repeated freezing and defrosting.
Do not decompliment the serum.

7 - STABILITY, STORAGE AND PREPARATION OF REAGENTS

The reagents are ready-to-use.
All the reagents stored at 2-8°C, in their original packaging, are stable until the expiry date indicated on the box. Do not freeze.

8 - MATERIAL REQUIRED BUT NOT SUPPLIED

- Automatic pipette(s) with a pipetting volume adapted to the volume that will be measured;
- Contaminated waste containers;
- Centrifuge;
- Haemolysis tubes.

9 - METHOD

Allow the reagents to reach room temperature before use.

9.1 - Sample preparation

Carry out a 1:40 dilution of the serum to be tested:
- 50 µL of serum ;
- 1,95 mL of **BUF** reagent.

9.2 - Realization of the test on a microplate

- Using a multichannel micropipette, add 50 µL of **BUF** reagent to 8 wells of the microplate.

- Using a micropipette, add 50 µL of diluted serum to the 1st well.
  Mix the serum with the **BUF** reagent and carry out a serial dilution, preferably using a microdiluter, by transferring 50 µL from the 1st well into the 2nd, then 50 µL from the 2nd to the 3rd, and so on until the 6th well is reached. 50 µL from the 6th well is then discarded.
  In this way, dilutions from 1:80 to 1:2560 are obtained.

- Add 50 µL of diluted serum to the 7th well.
  Mix the serum with the **BUF** reagent and then discard 50 µL.
  This dilution (1:80) is the serum control, whose role is to detect the natural anti-sheep agglutinins that could be present in certain serum samples.

- Carefully shake the **R1** and **R2** reagents.
  - Add 1 drop of **R1** reagent to the first 6 wells.
  - Add 1 drop of **R2** reagent to the 7th well (serum control).
  - Add 1 drop of **R1** reagent to the 8th well (reagent control) whose role is to control the validity of the **BUF** and **R1** reagent.

Note**:** Only carry out one reagent control for each series of tests.

- Very carefully, shake the contents of the wells:
  - either manually, by tapping laterally the side of the microplate that has been posed flat on the bench;
  - or by using a vibrating plate shaker for microtiter plates (for example at 1300 rpm for 10 seconds). Do not use an orbital shaker.

- Now leave the plate to rest, away from any sources of vibration.

- The plate can be read after 2 hours.

9.3 - Adsorption of the natural anti-sheep agglutinins in the event of agglutination of the serum control

- Carefully shake the **R3** reagent.
- In a tube, add and mix:
  - 0,1 mL of serum;
  - 0,3 mL of **R3** reagent.
- Incubate at room temperature for 60 minutes.
- Centrifuge at 2000 rpm for 15 minutes.
- Collect the supernatant; the serum is now at a 1:4 dilution.
- Carry out a 1:10 dilution of the supernatant in **BUF** reagent to obtain an adsorbed stock dilution (1:40).
- Follow the steps described in "Realization of the test on a microplate", but replace the stock dilution by the adsorbed stock dilution.

10 - READING

Negative reaction: **Absence of haemagglutination.**
Presence of a more or less large ring at the bottom of the well.

Postive reaction: **Presence of haemagglutination.**
Presence of a cloudy red/brown deposit coating the well, sometimes there is the presence of a fine peripheral border.

Example: Serum positive at a dilution of 1:1280

11 - INTERPRETATION OF RESULTS

Titer < 1/80: **Negative reaction.**
Probable absence of amoeba tissue infection.

1/80 ≤ Titer ≤ 1/160: **Doubtful reaction.**
A control must be carried out with the electrosyneresis technique, latex agglutination technique (**ELITex Bicolor Amoeba**) and indirect immunofluorescence.

Titer ≥ 1/320: **Significant reaction in favour of visceral amoebiasis.**

12 - INTERNAL QUALITY CONTROL

The **CONTROL +** and **CONTROL -** reagents must be treated like test serums. The titer of the **CONTROL +** reagent must be the same as the titer printed on the vial label ± one dilution. There must not be any haemagglutination of the **CONTROL -**. If haemagglutination is present then the test is not valid..

13 - CAUSES OF ERROR AND TEST LIMITS

- Poor conservation of the serum.
- Poor conservation of the reagents after opening.
- Only use the droppers provided in the kit.
- Do not interchange the droppers between the **R1** and **R2** reagents.
- In the case of a positive reaction in the first 6 wells, carry out a further serial dilution in order to determine the titer limit of haemagglutination.
- The serum control must give a negative reaction (ring). In the event of haemagglutination of this control, it will be necessary to renew the test after having eliminated the natural anti-sheep agglutinins from the serum by adsorption.
- The reagent control must give a negative reaction (ring). In the event of haemagglutination of this control, the **ELI.H.A Amoeba** cannot be used.
- Certain serums, whose antibody concentration is very high, can give rise to a zone phenomenon (with disappearance of the clouding) in the initial dilutions, which disappears in the subsequent dilutions.
- The quality of the reagents makes it possible to carry out the reaction in the evening and to read the test the following morning, provided that the microplate is not moved in any way and is protected from any sources of vibration.
- In all cases, it is necessary that the clinical, epidemiologic and biological data are taken fully into consideration before establishing the final diagnosis.

14 - PERFORMANCE

ELI.H.A Amoeba consists of red blood cells sensitized with an *Entamoeba histolytica* antigen, obtained by axenic culture , that ensures the specificity and sensitivity of the reaction.
Evaluation has demonstrated that the test **ELI.H.A Amoeba** has a sensitivity of 93 % and a specificity of 97 %.

15 - WASTE ELIMINATION

Waste should be disposed of in accordance with the hygiene rules and current regulations for this kind of product in the country of use.
If the **BUF** reagent is spilled, clean the work area with absorbent paper and rinse with water.
If a serum or another reagent is spilled on the work area, clean using bleach and absorbent paper.

16 - BIBLIOGRAPHY

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The changes from the previous version are highlighted in grey.

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