

# ELI.H.A *Echinococcus*

## Serodiagnosis of hydatidosis by indirect haemagglutination

102 tests  
(Réf. 66604)

8000140-EN-2026-01\_V2

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



### 1 – AIM

ELI.H.A *Echinococcus* enables the quantitative determination of anti-*Echinococcus granulosus* serum antibodies by indirect haemagglutination. The target population includes anyone suspected of having hydatidosis. Each kit allows 102 tests to be carried out or 17 reactions of 6 dilutions.

### 2 – INTRODUCTION

Hydatidosis or cystic echinococcosis is a parasitic disease caused by the larvae (hydatid) of a cestode of the *Echinococcus* genus. The life-cycle of *Echinococcus granulosus* requires both definitive and intermediate hosts. In general, the dog is the definitive host, with sheep and in rare cases humans being the intermediate host.

The hydatids are most frequently found in the liver (50 to 70%) and then the lungs (25 to 40%).

*E. granulosus* hydatidosis infection is characterized by a slow and insidious disease progression. Infection is relatively asymptomatic, with biological diagnosis resting primarily upon the detection of antibodies.

### 3 – PRINCIPLE

ELI.H.A *Echinococcus* is based on the indirect haemagglutination principle.

The sensitized red blood cells consist of sheep red blood cells covered with an *Echinococcus granulosus* antigen.

The presence of anti-*Echinococcus granulosus* 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	Quantity
<b>R1</b> : vial of 2,2 mL of sensitized red blood cells	1
<b>R2</b> : vial of 2,2 mL of non-sensitized red blood cells	1
<b>BUF</b> : vial of 55 mL of phosphate buffer pH 7,2	1
<b>R3</b> : vial of 2 mL of adsorbent	1
<b>CONTROL +</b> : vial of 0,2 mL of titrated positive control	1
<b>CONTROL -</b> : vial of 0,2 mL of negative control	1
<b>MICROPLATE</b> : microplate with a U-bottom	2
<b>DROPPER</b> : special dropper	2

### 5 – PRECAUTIONS

The reagents are intended for *in vitro* diagnostic use only and must be handled by authorized personnel.

The reagents and microplate can be used for up to the number of tests indicated on the box. Each well of the microplate is 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.

Do not use damaged or incorrectly stored reagents before use.

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 obtained after blood collection in dry tubes (up to 7 days of storage at 2-8°C or at -20°C for storage longer than 7 days), and showing no hemolysis, cloudiness, icterus, or contamination. Each laboratory is advised to verify the compatibility of the collection tubes used.

Avoid repeated freezing and defrosting.

Do not decompartmentalize the serum.

### 7 – REAGENTS STORAGE AND PREPARATION

The reagents are ready for use.

The reagents stored at 2-8°C in their original condition and after opening are stable until the expiry date indicated on the box. They must not be frozen.

### 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.

- Then leave the plate undisturbed, protected from vibration, at room temperature between 15 and 30°C.

- Read the reaction after 2 hours. The results can be interpreted after a minimum of 1.5 hours and up to a maximum of 24 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 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.**

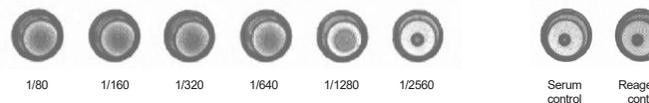
Presence of a more or less large ring at the bottom of the well.

**Positive reaction:**

**Présence of haemagglutination.**

Presence of a cloudy red/brown deposit coating the well, sometimes there is the presence of a fine peripheral border

Example: Positive serum at a dilution of 1/1280



### 11 – INTERPRETATION OF RESULTS

**Titre < 1/160:**

**Non-significant reaction.**

Probable absence of hydatidosis.

Repeat the test 2 to 3 weeks later and combine with an immunoblotting assay.

**Titre = 1/160:**

**Doubtful reaction.**

Repeat the test 2 to 3 weeks later and combine with an immunoblotting assay.

**Titre ≥ 1/320:**

**Significant reaction in favour of progressive hydatidosis.**

### 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 ERRORS AND TEST LIMITS

- Poor conservation of the serum.
- Poor conservation of the reagents after opening.
- Only use the droppers provided in the kit
- Only use the plates provided in the kit with wells that have a U-shaped bottom.
- 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 Echinococcus** kit 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 source of vibration.
- Do not reuse a well that has already been used to perform a test.
- The French nomenclature for medical biology procedures specifies that, for serological screening for echinococcosis, the search for anti-*Echinococcus* antibodies must be performed using two different techniques. This is so that any unknown interferents can be eliminated. The overall interpretation of this serology must therefore be based on the results of the different techniques used. In all cases, it is necessary that the clinical, epidemiologic and biological data are taken fully into consideration before establishing the final diagnosis.
- Without moving or vibrating the microplate, the test can be performed in the evening and read the next morning (i.e., after a maximum reaction time of 24 hours).

### 14 – PERFORMANCES

#### 14.1 – ANALYTICAL PERFORMANCE

##### 14.1.1 – Repeatability

On positive serum, the device has 100% repeatability with an acceptable tolerance of one well difference.  
On negative serum, the device has 100% repeatability with no tolerance for positivity in the result.  
The serum control and reagent control wells have 100% repeatability.

##### 14.1.2 – Reproducibility

On positive serum, the device has 100% reproducibility with an acceptable tolerance of one well difference.  
On negative serum, the device has 100% reproducibility with no tolerance for positivity in the result.  
The serum control and reagent control wells have 100% reproducibility.

##### 14.1.3 – Interference

Due to the high genetic homology between the two species of *Echinococcus*: *E. multilocularis* and *E. granulosus*, secondary tests are necessary to rule out cross-reactivity (9).

Cross-reactions have been observed with positive samples for anti-*Fasciola* antibodies or anti-*S. mansoni* antibodies (2).

Cross-reactions have been observed with samples from patients with toxoplasmosis, HIV, EBV, aspergillosis, filariasis, and clonorchiasis (1,3).

Potential interference with hemoglobin, lipids, and bilirubin was studied according to CLSI EP07 recommendations (4,5,6,7,8).

No significant interference was detected up to the following maximum concentrations:

Substances tested	Maximum concentrations
Bilirubin	800 mg/L
Lipids	15 g/L
Hemoglobin	10 g/L

An in-depth literature review allowed us to rule out potential drug interactions, while reducing, as far as possible, the risk associated with any as-yet unidentified interferents.

### 14.2 – CLINICAL PERFORMANCE

**ELI.H.A Echinococcus** consists of red blood cells sensitized by an *Echinococcus granulosus* antigen. It ensures sensitivity and specificity in the reaction. A study of 221 human serum demonstrated a sensitivity of 93.0% (regardless of cyst location) and a specificity of 94.9%. Comparisons with IFI and ELISA tests showed remarkable complementarity between these different reactions (1).

The clinical performance of the **ELI.H.A Echinococcus** kit was evaluated using a review of scientific literature based on five publications between 2009 and 2023 (2,10,11,12,13).

The following results were obtained:

Table 1: Clinical performance for a threshold of 1/160

Study	Study n°4 [12]
Year of publication	2023
Population	23 serum from patients with suspected cystic echinococcosis
Comparative method (reference standard)	Histopathological examination
Sensitivity 1/160	94% <sup>a</sup>
Specificity 1/160	100% <sup>a</sup>
PPV* 1/160	100% <sup>a</sup>
NPV* 1/160	85,7% <sup>a</sup>

Table 2 et 3: Clinical performance for a threshold of 1/320

Study	Study n°1 [10]			Study n°2 [11]	Study n°3 [2]		
Year of publication	2009			2024	2010		
Population	19 serum from patients with pulmonary hydatid cysts	40 serum from patients with parasitosis and other pulmonary diseases	20 serum from healthy subjects	74 serum from patients with suspected cystic echinococcosis (51 women and 23 men aged between 3 and 86 years)	30 serum from patients with cystic echinococcosis and no other parasitological infection	30 serum from patients with other parasitosis, <i>S. mansoni</i> (n=6), <i>Fasciola</i> (n=6), <i>H. nana</i> (n=6), <i>E. histolytica</i> (n=6) and <i>Giardia lamblia</i> (n=6).	10 serum from parasitosis-free patients
Comparative method (reference standard)	Operating results	NA	NA	Results of clinical and radiological reports	Clinical analysis and surgery	NA	NA
Sensitivity 1/320	73,6%			70,8%	86,7%		
Specificity 1/320	98,3%			96,2%	95%		
PPV* 1/320	93,3%			97,1%	92,9%		
NPV* 1/320	92,1%			64,1%	90,5%		

Study	Study n°4 [12]	Study n°5 [13]		
Year of publication	2023	2016		
Population	23 serum from patients with suspected cystic echinococcosis	18 serum from children with cystic echinococcosis	27 serum from adults with cystic echinococcosis	20 serum from healthy subjects
Comparative method (reference standard)	Histopathological examination	Clinical examination and medical imaging		
Sensitivity 1/320	82,4% <sup>a</sup>	88,9% <sup>a</sup>		
Specificity 1/320	100% <sup>a</sup>	100% <sup>a</sup>		
PPV* 1/320	100% <sup>a</sup>	100% <sup>a</sup>		
NPV* 1/320	66,7% <sup>a</sup>	80% <sup>a</sup>		

\*: PPV: Positive Predictive Value

\*: NPV: Negative Predictive Value

\*: Value calculated by ELITech Microbio

In scientific literature, clinical sensitivity and specificity for a significance threshold of 1/160 are 94% and 100%, respectively.

For a significance threshold of 1/320, clinical sensitivity and specificity range from 73.6% to 88.9% and from 95% to 100%, respectively.

For a significance threshold of 1/160, the PPV is 100% and the NPV is 85.7%.

For a significance threshold of 1/320, the PPV ranges from 92.86% to 100% and the NPV ranges from 66.67% to 92.1%.

### 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 of another reagent is spilled on the work area, clean using bleach and absorbent paper.

### 16 – BIBLIOGRAPHY

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Any serious incident related to the device shall be reported to the manufacturer and to the Competent Authority of the Member State in which the user is established.

The changes from the previous version are highlighted in gray.

