# 66610 – ELI.H.A TOXO

FR – La notice 8000160-FR-2012-02 a été mise à jour depuis le numéro de lot 10037A. Les changements apportés dans la nouvelle version 8000160-FR-2023-09 sont surlignés en gris.

EN – The IFU 8000160-EN-2012-02 has been updated from lot number 10037A. Changes made in new version 8000160-EN-2023-09 are highlighted in grey.

ES – La instrucción de uso 8000160-ES-2012-02 ha sido actualizada a partir del número de lote 10037A. Los cambios realizados en la nueva versión 8000160-ES-2023-09 están resaltados en gris.

DE – Die Gebrauchsanweisung 8000160-DE-2012-02 wurde ab Losnummer 10037A aktualisiert. Die in der neuen Version 8000160-DE-2023-09 vorgenommenen Änderungen sind grau unterlegt.

NL – De gebruiksaanwijzing 8000160-NL-2012-02 is bijgewerkt met lotnummer 10037A. Wijzigingen in de nieuwe versie 8000160-NL-2023-09 zijn grijs gemarkeerd.

IT - Le istruzioni per l'uso 8000160-IT-2012-02 sono state aggiornate dal lotto numero 10037A. Le modifiche apportate nella nuova versione 8000160-IT-2023-09 sono evidenziate in grigio.

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# ELI.H.A TOXO

**Serodiagnosis of Toxoplasmosis by indirect hemagglutination**

**120 tests**
(Ref. 66610)

**8000160-EN-2023-09**

CE 0459

## 1 – AIM

The **ELI.H.A TOXO** device enables the semi-quantitative determination of anti-*Toxoplasma gondii* serum antibodies by indirect hemagglutination.
The kit allows 120 tests to be carried out or 20 reactions of 6 dilutions.

## 2 – INTRODUCTION

Toxoplasmosis is a parasitic disease caused by the protozoan *Toxoplasma gondii*. Benign or asymptomatic in most of cases, it presents a serious risk for pregnant seronegative women, newborns and subjects with a weakened immune system.
Different antibodies corresponding to the variety of antigens involved in *Toxoplasma* infections are developed during the immune response. The relative proportion of these various antibodies in serum may differ from one subject to another and change in time for the same patient (1,2,3,4).

## 3 – PRINCIPLE:

**ELI.H.A TOXO** is based on the indirect hemagglutination principle. The sensitized red blood cells consist of sheep red blood cells covered with a *Toxoplasma* antigen. This technique makes it possible to detect anti-*Toxoplasma* IgG and IgM in patient serum. They can be differentiated by treating the serum with 2-mercaptoethanol (2-ME) which inhibits the agglutinating power of IgM (2).
The presence of serum antibodies against *Toxoplasma gondii* 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, *etc*).
The reaction is carried out in a U-microplate.
Handling is simple and fast. Results are available in 2 hours.

## 4 - REAGENTS AND MATERIALS

| Description | Quantity |
|---|---|
| **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:** microplates with a U-bottom | **2** |
| **DROPPER:** special dropper (18 ± 2 µL) | **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 reagents, except **BUF**, 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 **CONTROL** reagents contain sodium azide (<0.1%).
- Do not use reagents after the expiry date.
- Do not use reagents from different batches numbers.
- Do not use damaged or poorly preserved reagents.
- Allow reagents and samples to return to room temperature before performing the test.
- Carefully shake reagents **R1** and **R2** before use: vigorous manual or vortex shaking is mandatory for the homogenization of red blood cells. It should be ensured that no red blood cell deposits remain in the bottom of the vial.
- When dispensing reagents **R1** and **R2**, 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 on a dry tube (up to 7 days storage at +2°C to +8°C or -20°C for storage longer than 7 days), and not showing any sign of hemolysis, cloudiness, jaundice or contamination. It is recommended that each laboratory check the compatibility of the sampling tubes used.
Do not refreeze the sample.
Do not decompliment the serum.

## 7 – STORAGE AND PREPARATION OF REAGENTS

All reagents are ready to use.
Reagents stored at +2°C to +8°C in their original state are stable until the expiry date indicated on the box. They should not be used after.

## 8 – MATERIEL REQUIRED BUT NOT PROVIDED

- Automatic pipette(s) with a pipetting volume adapted to the volume that will be measured.
- Contaminated waste containers
- 2-mercaptoethanol (2-ME)
- Centrifuge
- Hemolysis tubes

## 9 – METHOD

Allow the reagents and serum to be analyzed to return to 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 the diluted serum to the 1st well.
  Mix the serum with the **BUF** reagent and carry out a serial dilution using a micropipette by transferring 50 µL from the 1st well to 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 the 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 serums samples.
- Vortex or vigorously shake reagents **R1** and **R2** before use.
  - 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** reagents.

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, until complete homogenization of the wells;
  - or by using a vibrating stirrer for microdilution plates (up to 1500 rpm). Do not use an orbital shaker.

- Now leave the plate to rest, away from any sources of vibration, at room temperature between +15°C and +30°C.
- The plate can be read after 2 hours. The results can be interpreted from a minimum of 1h30 reaction and up to a maximum of 24 hours.

### 9.3 – Detection of IgM: treatment with 2-mercaptoethanol

The determination of anti-*Toxoplasma* antibodies, before and after treatment of the serum with 2-ME, allows the detection of IgM type antibodies.
To test the serum, proceed as explained below:
- Prepare the 2-ME solution: 140 µL 2-ME and **BUF** reagent to make 10 mL. This solution should be kept in a brown flask. It is stable for 1 month at +2°C to +8°C.
- Mix and deliver 50 µL of this 2-ME solution into 6 wells of the microplate.
- Then follow the protocol of "Realization of the test on a microplate" from the second step.

### 9.4 – Adsorption of 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 800 g 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).
- Repeat the steps described in "Realization of the test on a microplate", but replace the stock dilution by the adsorbed stock dilution.

## 10 – INTERNAL QUALITY CONTROL

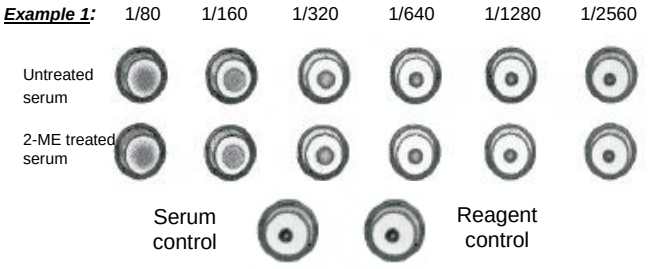
- The **CONTROL+** and **CONTROL-** reagents must be treated like test serums. The titer of the **CONTROL+** must be the same as the titer printed on the vial label ± one dilution. There must not be any hemagglutination of the **CONTROL-**. If hemagglutination is present, then the test is not valid.
- The serum control must give a negative reaction (ring). In the event of hemagglutination 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 hemagglutination of this control, **ELI.H.A TOXO** cannot be used.

## 11 – READING

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

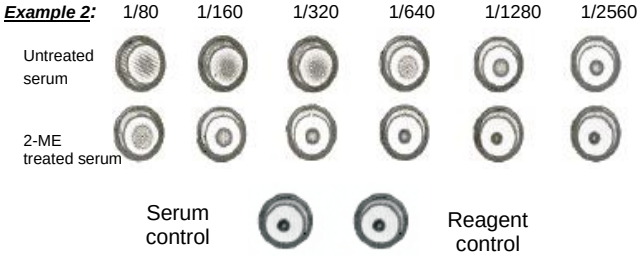
**Positive reaction:** **Presence of hemagglutination.**
Presence of a cloudy red/brown deposit coating the well; sometimes there is the presence of a fine peripheral border.

Hemagglutination pictures, obtained with positive serum, are presented hereafter:




**Positive to 1/160 serum. Absence of IgM.**

***Example 2:***




Untreated serum positive at 1/640 – 2-ME treated serum positive to 1/80

↳ **Positive serum. Presence of IgM**

**The titer is given by the first dilution showing a wide and peripheral ring.**
**The titer in IU/mL is equal to the inverse of this limit dilution multiplied by the sensitivity threshold printed on the box.**
*Example: If a serum is positive until dilution 1/320, and if the sensitivity threshold is 0.1 IU/mL, then the titer of the serum will be 320 x 0.1 = 32 IU/mL.*

## 12 – INTERPRETATION OF RESULTS

**Titer < 1/80: Negative reaction.**
No anti-*Toxoplasma* antibodies or non-detectable amount.

- Screening test: probably non-immune patient requesting a serological follow-up for pregnant women and immunocompromised patient.
- Test carried out in case of potential acute infection for an immunocompetent patient: no *Toxoplasma* infection or very recent beginning infection. Must be controlled on a 2nd sample to detect a possible appearance of antibodies.

**Treatment with 2-mercaptoethanol (2-ME):**
Treatment of serum with 2-ME eliminates the IgM response. A decrease of at least 2 dilutions before and after treatment with 2-ME solution of the serum means that the serum contains IgM antibodies. Presumption of acute toxoplasmosis.
Otherwise: probably past infection (residual antibodies).

**1/80 ≤ Titer < 1/160: Equivocal reaction**
Presence of limit level of anti-*Toxoplasma* antibodies. A possible absence of IgM can be demonstrated by serum treatment with 2-ME.

- If IgG equivocal (1/80) and IgM negative: carry out an IgG confirmation technique of different principle ; sending the serum to an expert laboratory is recommended for carrying out additional techniques:
  → If the result of additional IgG tests is negative: conclude with the absence of IgG antibodies ; serological follow-up to be adapted (pregnancy or immunocompromised patient).
  → If the result of additional IgG tests is positive: an old infection is likely; the result is to be confirmed on a new sample taken 3 weeks apart.

**Titer ≥ 1/160: Positive reaction**
Presence of significant levels of anti-*Toxoplasma* antibodies. Treatment of the serum with 2-ME may be performed to test for the presence of IgM.

- If IgG positive (> 1/160) and IgM negative:
  - In the case of an immunocompromised patient, presence of an old infection; the serology result is to be compared with the clinic, the biology and the degree of immunosuppression.
  - In the case of an immunocompetent or pregnant patient, a serological test 3 weeks later must be carried out:
    → If the IgG titer is stable: the infection is old.
    → If the IgG titer is increasing: perform an infection dating with an IgG avidity method to be able to conclude on a recent infection or a possible reactivation of the infection.
- If IgG negative and IgM positive (>1/160): in this case, perform an IgM confirmation technique based on a different principle. If positive results then probable recent infection, perform a new serological check at 2 weeks. If negative results then natural nonspecific IgM or interferences, recent infection cannot be ruled out, perform serological monitoring at 2 weeks.
- If IgG and IgM positive (>1/160):
  - In the case of an immunocompromised patient, the serology results will have to be compared with the clinic, the biology and the degree of immunosuppression.
  - In the case of an immunocompetent or pregnant patient, carry out the dating of the infection with an IgG avidity test.

## 13 – CAUSES OF ERRORS AND TESTS LIMITS

- Poor preservation 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 to determine the titer limit of hemagglutination.
- Some 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 french nomenclature of medical biology acts specifies 2 tests must be realized simultaneously for the serological diagnostic of toxoplasmosis. Therefore, the overall interpretation of this serology must be done according to the results of the different techniques. In any case, diagnosis should be made using the results of this test together with the other clinical, epidemiological and laboratory findings.

## 14 – PERFORMANCES

### 14.1 – Sensitivity / Specificity / Diagnosis

The sensitivity threshold of the reagent, expressed in IU/mL, is indicated on the box.
The sensitivity of the techniques proposed for the serodiagnosis of toxoplasmosis to each of these antibodies varies according to the nature of the antigen used in the reaction.
In addition, the international standard may contain a relative percentage of each of the antibodies different from that of the serum to be tested. The titer expressed in IU/mL can therefore vary significantly depending on the methods used.
**This is why it is essential to specify the technique used in international units to render the results of the diagnosis.**
The quality of the reagents allows the reaction to be carried out in the evening and the reading to be carried out the next morning (after a maximum of 24 hours of reaction), provided that the microplate does not undergo any displacement and is protected from vibrations.
Reagent **R1** consists of red blood cells sensitized by a mixed total *Toxoplasma* antigen that includes both endogenous and membrane constituents of the *Toxoplasma*. It ensures sensitivity and specificity to the reaction.
The sensitivity and specificity results were synthesized based on two studies (5,6).
A first study comprising 1710 *anti-Toxoplasma* IgG/IgM positive sera and 1290 negative sera (5), and a second study comprising 342 anti-*Toxoplasma* IgG/IgM positive sera and 247 negative sera (6), were analyzed using the Meta Analysis model ("meta" package) of the R software (https://www.r-project.org/).
Thus, the results of this meta-analysis show a diagnostic sensitivity of 99% 95% CI [99%-99%] and a diagnostic specificity of 98% 95% CI [96%-99%]. Positive Predictive Values (PPV) are between 98.3% and 99.5% and Negative Predictive Values (NPV) are between 98% and 98.8%.

### 14.2 – Quantification limit

The limit of quantification of the ELI.H.A TOXO test is defined by the sensitivity threshold and corresponds to the dilution of the first well of the test. Its value is equal to the inverse of this dilution multiplied by the sensitivity threshold.

### 14.3 – Repeatability

On positive sera with titers greater than 16 IU/mL, the ELI.H.A TOXO device has 100% repeatability with an acceptable tolerance of +/- one dilution.
On negative sera strictly below 8 IU/mL, the ELI.H.A TOXO device has 100% repeatability with no positivity tolerance in the result.

### 14.4 – Reproducibility

On positive sera with titers greater than 16 IU/mL, the ELI.H.A TOXO device has 100% reproducibility with an acceptable tolerance of +/- one dilution.
On negative sera strictly below 8 IU/mL, the ELI.H.A TOXO device has 100% reproducibility with no positivity tolerance in the result.

### 14.5 – Interference

Cross-reactions were observed with anti-nuclear antibody or anti-syphilis antibody positive samples.
No cross-reactions were observed with scleroderma antibody, IgM HA (hepatitis A), IgM CMV (cytomegalovirus) or Lyme antibody positive samples (6).
Potential interferences with hemoglobin, lipids and bilirubin were studied according to CLSI EP07 recommendations (7,8,9,10,11).
No significant interference was detected up to the following maximum concentrations:

| Test substance | Peak concentrations. |
|---|---|
| Bilirubin | 800 mg/L |
| Lipids | 15 g/L |
| Hemoglobin | 10 g/L |

### 14.6 – Linearity / Accuracy

The ELI.H.A TOXO test makes it possible to obtain titration results directly proportional to the amount of analyte (anti-*Toxoplasma gondii* antibody) present in the sample, in the measurement range of 8 to 256 IU/mL.
The accuracy bias of the ELI.H.A TOXO method is:

| | |
|---|---|
| serum < 8 IU/mL: | 0% |
| serum at 10.4 IU/mL: | 15.38% |
| serum at 213.5 IU/mL: | -10.07% |

Any serious incident related to this device must be notified to the manufacturer and to the competent authority of the Member State in which the user and / or the patient is established.

## 15 – WASTE ELIMINATION

Waste must be disposed of in accordance with the hygiene rules and current regulations for this type 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, clean using bleach and absorbent paper.

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All modifications to the previous revision are highlighted in gray.


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