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# Immunoassay

REF **CMK0601 / CMK0602 / CMK0603 / CMK0605**

***50 tests*1 / 100 tests*1 / 100 tests*2 / 50 tests*2***

# Toxo IgG CLIA Microparticles

*This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination of Toxo IgG (specific IgG antibodies to Toxoplasma gondii) in human serum or plasma.*



**Key to Graphical Symbols Used**

| | | | |
|---|---|---|---|
| LOT | batch code | | use by |
| | manufacturer | Σ | contains sufficient for <n> tests |
| IVD | *in vitro* diagnostic medical device | | temperature limitation |
| REF | catalogue number | | consult instructions for use |
| EC REP | authorized representative in the European Community | | |

EC REP

OBELIS S.A.
Bd. Général Wahis, 53
1030 Brussels
Belgium

AUTOBIO DIAGNOSTICS CO., LTD.
No.87 Jingbei Yi Road
National Eco & Tech Development Area
Zhengzhou
China
450016

CE 0123 IVD

For any technical assistance please contact us in English at:
Email: customerservice@autobio.com.cn
Contact your local dealers for all product-related questions in your local language

## Introduction

Toxoplasmosis is a quite widespread infectious disease caused by an intracellular protozoan parasite, called *Toxoplasma gondii*.[1] The disease, affecting both human and warm blooded animals, can be transmitted by ingestion of food infected;[2] direct contagion from domestic animals;[3] or transplacental infection.

In the normal adult population, toxoplasmosis has a generally benign course, being largely asymptomatic; sometimes mildly symptomatic (headache, sore throat, asthenia); or in rare cases accompanied by lymphadenitis. Exceptionally, severer disorders may be present, like myocarditis, hepatitis, pneumonia, meningoencephalitis and retinochoroiditis.[4] The prevalence of positive serological tests increases with age, indicating past exposure.

If the infection occurs in pregnant women, toxoplasmosis can cause a threat to the fetus with possible spontaneous abortion, prematurity or stillbirth, as the pathogen can be transmitted to the fetus via the placenta. The fetus whose mother is exposed to Toxoplasma infection during the first trimester of pregnancy develops severe lesions to the central nervous system that generally lead to fetal demise. Toxoplasma infection acquired during the second trimester may cause hydrocephalus, mental and psychomotor retardation, blindness and cerebral calcifications. Toxoplasma infection, however, is commonest during the third trimester, causing retinochoroiditis and other ocular lesions, lesions to the central nervous system and latent asymptomatic infection which may eventually develop into full-blown disease.[5]

Specific IgM antibodies to Toxoplasma develop two to four weeks after the onset of clinical signs and gradually decline thereafter, disappearing in three to nine months.[6] Therefore, the presence of IgM and IgA in the absence of IgG or in the presence of low IgG levels is a strong evidence of acute toxoplasmosis. Conversely, the presence of IgM in the presence of decreasing or constant IgG levels indicates sub-acute infection. The differential diagnosis of acute toxoplasmosis made possible by the specific IgM assay allows adequate treatment which reduces the risks of the disease both in immune-compromised patients and in pregnant women. Specific IgG antibodies to Toxoplasma rise gradually and peak two to five months after the onset of clinical signs. Therefore, the presence of IgG is useful in distinguishing subjects having acquired the disease from those who have not.[7] This is particularly important in order to adopt suitable prophylaxis in susceptible women of child-bearing age.

## Measurement Principle

This assay is based upon the two-step indirect method. The sample, Toxo antigen coated microparticles are combined. IgG antibodies to Toxoplasma present in the sample bind to the Toxo antigens coated on the microparticles. After washing, Enzyme Conjugate is added. During the incubation, a complex is generated among the solid phase, the Toxo IgG within the sample and HRP-conjugated anti-human IgG by immunological reactions. The complex catalyzes substrate, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is proportional to the amount of Toxo IgG in the sample.

## Materials Provided

1. Calibrators

6 vials each containing 1.0 mL of calibrator A through F. The matrix is Tris-NaCl buffer containing BSA (bovine serum albumin). Contains sodium azide and ProClin 300® preservatives.

Calibrators provided ready to use.

2. Reagent pack

Reagent pack provided ready to use.

| Σ | 50*1 | 100*1 | 100*2 | 50*2 |
|---|---|---|---|---|
| Microparticles Solution | 1.2 mL*1 | 2.3 mL*1 | 2.3 mL*2 | 1.2 mL*2 |
| Enzyme Conjugate | 5.5 mL*1 | 11.0 mL*1 | 11.0 mL*2 | 5.5 mL*2 |
| Sample Diluent | 5.5 mL*1 | 11.0 mL*1 | 11.0 mL*2 | 5.5 mL*2 |

- Microparticles Solution

Recombinant Toxo antigen coated microparticles in PBS (phosphate buffered saline) buffer containing casein. Contains ProClin 300® and sodium azide preservatives.

- Enzyme Conjugate

Horseradish peroxidase labeled mouse anti-human IgG monoclonal antibodies in a Tris-HCl buffer containing bovine serum. Contains ProClin300® and Bronidox preservative.

- Sample Diluent

Tris-HCl buffer containing casein. Contains sodium azide and ProClin 300® preservatives.

## Assay Analyzers on which the kit can be used

- AutoLumo A2000 Plus
- AutoLumo A2000 Plus B
- AutoLumo A1000

The chemiluminescent microparticle immunoassay (CLIA Microparticles) is intended for use on Assay Analyzer which is AutoLumo A2000 Plus, AutoLumo A2000 Plus B or Autolumo A1000.

## Materials Required but not Provided

1. Assay Analyzer
2. Reaction vessel(s) for sample and reagent reaction
3. Sample cup(s) or tube(s) for sample containing
4. Diluent Universal
5. Chemiluminescent Substrate
6. System Wash for washing the pipetting needles
7. Wash Buffer used in the washing procedure
8. Distilled water or deionized water

## Metrological Traceability of Calibrators

The product calibrators are signal matched to our working calibrators, which are also signal matched to an international conventional calibrator purchased from WHO (The World Health Organization) International Standard Anti-Toxoplasma IgG, Human (01/600) at each concentration level.

## Warnings and Precautions

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this instruction for use.
3. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
4. Handle the potentially contaminated materials and wastes safely according to local requirement.
5. CAUTION: the calibrators contain material of human origin, which has been tested and found non-reactive for HBsAg, HIV-1 and HIV-2,

HCV and syphilis. This assay contains materials of animal origin. Bovine components originate from countries where BSE has not been reported.

6. Some reagents contain ProClin 300® may cause sensitization by skin contact, which must be avoided to contact with skin. This material and its container must be disposed in a safe way. If swallowed, seek medical advice immediately.
7. Do not smoke, drink, eat or use cosmetics in the working area.
8. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
9. Use caution when handling patient samples to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
10. Conduct the assay away from bad ambient conditions. e. g. Ambient air containing high concentration corrosive gas, such as sodium hypocritical acid, alkaline, acetaldehyde and so on, or containing dust.
11. Do not use reagents beyond the labeled expiry date.
12. Do not mix or use components from kits with different batch codes.
13. Ensure the microparticles are re-suspended before loading it on the analyzer.
14. Avoid foam formation in all reagents and sample types (samples, calibrators and controls).
15. Do not substitute any reagent in this kit from other manufacturers or other lots.
16. When any damage to the protective packaging or any change of analytical performance is observed, do not use the kit.

## Storage

1. Store the kit at 2-8 ℃. Do not freeze. Avoid strong light. When stored as directed, all reagents are stable until the expiration date.
2. Refrigerate the reagent pack at 2-10 ℃ for a minimum of 2 hours prior to use.
3. Store the unsealed reagents pack upright on the analyzer or 2-10℃ for a maximum of 28 days. After 28 days, the reagent pack must be discarded. Once they are removed from the analyzer, store them at 2-10 ℃ in an upright position.
4. Seal and return the remaining calibrators at 2-8 ℃ immediately after the experiment, under which conditions the stability will be retained for 2 months.

## Sample

1. Collect samples in accordance with correct medical practices.
2. Plasma samples collected in tubes containing EDTA, heparin or sodium citrate have been tested and may be used with this assay.
3. Do not use heat-inactivated samples. Do not use sodium azide preservative in samples. Do not use samples with obvious microbial contamination.
4. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit in creased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
5. Prior to shipment, it is recommended that samples be removed from the clot, serum separator or red blood cells.
6. Insufficient processing of the sample or disruption of the sample during transportation may cause depressed results.
7. Avoid grossly hemolytic, lipemic or turbid samples.
8. Cap and store the samples at 18-25 ℃ for no more than 8 hours, for longer use samples should be capped and stored at 2-8 ℃ up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20 ℃. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortex or by inverting 10 times. Visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.
9. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy in appearance prior to use to ensure consistency in the results.
10. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
11. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
12. For optimal results, inspect all samples for bubbles. Remove bubbles with a tip prior to analysis. Use a new tip for each sample to prevent cross contamination.

## Measurement Procedure

1. Check the consumable materials

- Verify adequate volume of consumable materials is present prior to running the test.
- Refer to the Assay Analyzer's operation manual.

2. Load the kit

- Mix contents of new (un-punctured) reagent packs by gently inverting pack several times before loading on the analyzer. Avoid foam formation in all reagents. Don't invert the open (punctured) packs. If necessary, shake gently to mix horizontally after the first loading.
- Read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Refer to the Assay Analyzer's operation manual.

3. Order tests

- Place the sample tubes on the sample rack, 30 μL of serum or plasma sample for each test. But consider the sample container and 150 μL of system dead volumes, which can be refer to the appropriate Assay Analyzer manuals for the minimum sample volume required.
- Load the sample rack and input the sample information on the system software interface.
- Select "run" to start the test, the analyzer automatically operates tests. It performs the following functions:
  - Moves the samples to the set point
  - Loads a reaction vessel into the process path
  - Aspirates and transfers Diluent Universal and sample into the reaction vessel and mixed well
  - Aspirates and transfers the diluted sample to the reaction vessel
  - Adds Microparticles Solution and Sample Diluent to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Enzyme Conjugate to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine the quantity of Toxo IgG in the sample

- Discards the used reaction vessel
- Calculates the result
- Refer to the Assay Analyzer's operation manual.

4. Calibrate the curve
- Analyzer can read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the calibrators into the sample cups or tubes and place them on the sample rack. Conduct duplicate detection on the system.
- Load the sample rack and input calibrators' information on the system software interface.
- Select "run" to start the test and generate the calibration curve, calibration is required every 28 days.
- Once a calibration curve is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - Controls are out of range after repeated measurements
  - A reagent kit and substrate solution with new batch code is used
  - Beyond the expiration date (28 days) of a calibration curve
  - Important parts of the analyzer are replaced or repaired
- Refer to the Assay Analyzer's operation manual.

5. Dilute the sample

Samples with a Toxo IgG value exceeding 120 IU/mL may be diluted manually. Diluent Universal is used to dilute the samples. The concentration of the sample after dilution should not be less than 1.2 IU/ml. After dilution, multiply the result by the dilution factor. Antibodies to *Toxoplasma gondii* are heterogenous. A non-linear dilution behavior is frequently observed.

## Measurement Results

The sample test results are determined automatically by the system software. The amount of Toxo IgG in the samples is determined from the measured light production by means of the stored calibration data. Refer to the Assay Analyzer's operation manual on reviewing the stored data.

## Interpretation of Results

Results obtained with the Toxo IgG CLIA Microparticles can be interpreted as follows:

Non-reactive: $<$ 0.8 IU/mL

Equivocal: 0.8-1.2 IU/mL

Reactive: $\geq$1.2 IU/mL

A non-reactive result indicates that the specific immunity has not been acquired but cannot rule out the early stage of acute infection. Patients with non-reactive results are still suspected the exposure to *Toxoplasma gondii* should be retested within 2 weeks.

A reactive result indicates either early acute infection or past exposure to the pathogen. If it is suspected to be early acute infection, a Toxo IgM test or other serological method for detection of additional *Toxoplasma gondii* markers, such as a Toxo IgG Avidity test, could be performed.

For equivocal result, a second sample should be taken and repeated Toxo IgG testing no less than one or two weeks later, or/and using a Toxo IgM test to confirm.

## Control Procedure

Controls for the various concentration ranges should be run individually when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

## Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. However, diagnosis of Toxoplasma infection should not be established on the basis of a single test result as positive or negative for the presence of Toxo IgG but should be determined in conjunction with clinical examinations, diagnostic procedures and other test results.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. If the patient is immune-compromised or is receiving immune-suppressive therapy (for example, transplant recipients, AIDS patients), the reference value of their IgG antibodies serological detection is limited, and wrong medical explanation may be obtained.
4. A negative result, however, does not always rule out the possibility of Toxoplasma infection. Because different people have different times from Toxoplasma infection to produce antibodies, maybe the infections in its very early stage and the patient may be still unable to synthesize enough *Toxoplasma gondii* specific IgG. If clinical exposure to *Toxoplasma gondii* is suspected despite a negative finding, a second sample should be collected and tested on less than one week later.
5. Samples from neonates, cord blood, pre-transplant patients or body fluids other than serum and plasma, such as urine, saliva or amniotic fluid have not been tested.
6. For the samples who have received blood transfusions or other blood products in recent months, the positive result should be given careful analysis.
7. This test measures concentrations within the range of 0.1-120 IU/mL. If Toxo IgG concentrations above the measuring range to be expected, it is recommended to dilute samples with Diluent Universal, the maximum dilution is 1:9 of this test, allowing samples to be quantitated up to approximately 1200 IU/mL.

## Performance Characteristics

1. Measurement Precision

3 pooled human serum-based panel members (1, 2 and 3) were assayed, using 3 batches of reagents, in replicates of 2, twice per day across 20 testing days. Data from this study are summarized in the following table.

| Batch | Panel | n | Mean (IU/mL) | SD | Within-run CV% | Total CV% |
|---|---|---|---|---|---|---|
| 1 | 1 | 80 | 5.12 | 0.23 | 0.23 | 4.56 |
| | 2 | 80 | 19.98 | 0.89 | 0.89 | 4.44 |
| | 3 | 80 | 93.84 | 9.59 | 9.59 | 10.22 |
| 2 | 1 | 80 | 4.54 | 0.25 | 0.25 | 5.42 |
| | 2 | 80 | 19.03 | 0.71 | 0.71 | 3.71 |
| | 3 | 80 | 85.50 | 6.26 | 6.26 | 7.32 |
| 3 | 1 | 80 | 4.82 | 0.25 | 0.25 | 5.24 |
| | 2 | 80 | 22.53 | 0.93 | 0.93 | 4.14 |
| | 3 | 80 | 93.47 | 5.87 | 5.87 | 6.28 |

*Representative data; results in individual laboratories may vary from these data.

2. Analytical Sensitivity

Analytical sensitivity represents lowest measurable analyte level that can be distinguished from zero, is 0.1 IU/mL.

The study was conducted on 3 reagent batches using 5 human serum-based panels which were prepared at target concentrations. The panel were assayed in replicates of 3 over 4 days for a total of 60 replicates per batch.

3. Analytical Specificity

**Cross reaction:** This assay is tested to have no cross reactivity with the HSV-1 IgG, HSV-2 IgG, CMV IgG, Rubella IgG, HEV IgG, MP IgG, CP IgG as well as HIV, TP, HCV and HBs antibodies, RF and ANA.

| Potentially Cross-reactive Samples | Number of Samples | Number of Reactive Samples |
|---|---|---|
| HSV-1 IgG | 5 | 0 |
| HSV-2 IgG | 5 | 0 |
| CMV IgG | 5 | 0 |
| Rubella IgG | 10 | 0 |
| HEV IgG | 9 | 0 |
| MP IgG | 5 | 0 |
| CP IgG | 5 | 0 |
| HIV antibodies | 10 | 0 |
| TP antibodies | 10 | 0 |
| HCV antibodies | 9 | 0 |
| HBs antibodies | 10 | 0 |
| RF | 15 | 0 |
| ANA | 12 | 0 |
| Total | 110 | 0 |

**Interference:** Controlled studies of potentially interfering substances or conditions showed that the assay performance was not affected by anticoagulants (sodium citrate, EDTA, heparin), bilirubin (up to 20 mg/dL), hemoglobin (up to 1000 mg/dL), triglyceride (up to 3000 mg/dL).

4. Clinical Study

**Relative Sensitivity and Relative Specificity:** A study was performed where samples were tested using this assay and a reference Toxo IgG assay which was already available on the market. Equivocal or inconsistent results after comparison shall be tested by two other reference assays, the result can be determined by at least 3 assays with the same results. Data for relative sensitivity and relative specificity are summarized in the following table.

| Toxo IgG CLIA Microparticles | | | | | |
|---|---|---|---|---|---|
| Site | Number of Sample | Relative Sensitivity | Lower 95% CI limit | Relative Specificity | Lower 95% CI limit |
| Site 1 | 813 | 100% | 99.45% | 100% | 99.92% |
| Site 2 | 411 | 100% | 99.70% | 100% | 99.88% |
| Site 3 | 235 | 100% | 99.40% | 99.54% | 98.65% |
| Total | 1459 | 100% | 99.75% | 99.93% | 99.78% |

* CI denotes Confidence Interval

**NOTE:** A total of 34 samples giving unconfirmed results determined by abovementioned rules were not included in the calculation of relative sensitivity and relative specificity.

**CDC Panel:** The CDC Toxoplasma 1998 Human Serum Panel is comprised of 100 frozen blind specimens (70 Toxoplasma IgG reactive samples and 30 Toxoplasma IgG non-reactive samples). Toxo IgG CLIA Microparticles correctly detected the 70 Toxoplasma IgG reactive samples (100% agreement) and 30 Toxoplasma IgG non-reactive samples (100% agreement).

## Literature References


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