

Instructions for use

For *in vitro* diagnostic use only

Neonatal *Toxoplasma gondii* IgM FEIA

Enzyme immunoassay for the determination of IgM-class antibodies to *Toxoplasma gondii* from blood specimens dried on filter paper.

Product no. 61 99 802 (S&S 903)
(Microstrips®, 480 wells)

CE 0537

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INTENDED USE

This Neonatal *Toxoplasma gondii* IgM FEIA kit is intended for the determination of IgM-class antibodies to *Toxoplasma gondii* in blood specimens dried on filter paper. The test is intended as a primary method for screening of newborns for congenital toxoplasmosis (CT). Because IgM-class antibodies due to their high molecular weight do not cross placenta, determination of specific antibodies in newborn specimens taken shortly after birth indicates exposure of the fetus *intra utero* to *Toxoplasma gondii*. Cases showing elevated levels of specific antibodies should be further investigated.

INTRODUCTION


Congenital toxoplasmosis (CT) is a disease caused by the intracellular parasite *Toxoplasma gondii*. Vertical transmission rate from the mother with acute acquired toxoplasmosis to the fetus is reported to vary between 20-70% [1]. Asymptomatic infants with congenital infection may later develop severe neurological and visual disabilities which could be prevented if the disease is diagnosed early and appropriately treated. Mass prenatal screening for CT has been implemented in some European countries.

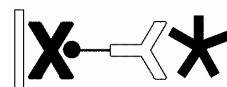
However, in countries where economic policies or the structure of the health care system does not permit prenatal screening of the mothers, screening of newborns could present a reasonable choice. This alternative is medically and ethically justified because the majority of infected cases are asymptomatic at birth and treatment shortly after birth is efficient [2-4]. Neonatal screening for CT can be easily linked to the existing screening programs for metabolic and endocrine disorders. Generally, neonatal screening for CT meets the requirements set by WHO (the disease is frequent, condition is amenable to treatment, reliable tests for screening and confirmation are available).



Reports [2-4] on the experience of screening for CT covering the period from January 1986 to June 1992 advocates the addition of screening for CT to the battery of screening tests. The method provides acceptable diagnostic sensitivity at reasonable retest/recall rates [5]. In a 3-year prospective study in Brazil, in which 62564 neonates were screened with Ani Lab systems' Neonatal *Toxoplasma gondii* IgM FEIA, the prevalence of CT was found to be 1 per 3,000. Retest rate was 0.5% and recall rate was 0.42% [15].

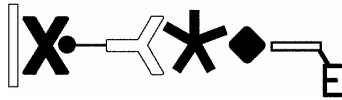
PRINCIPLE OF THE ASSAY

1. Neonatal *Toxoplasma gondii* IgM FEIA kit is a solid-phase capture enzyme immunoassay with fluorometric

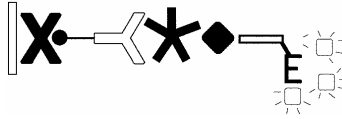
detection in which specific IgM antibodies (*) are eluted from dried blood disks and simultaneously captured by sheep polyclonal anti-human IgM antibody () immobilised to the solid phase by ligand binding technology (X⁻).



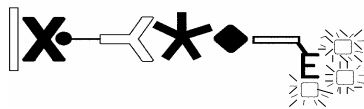
2. After the first washing step a mixture of *Toxoplasma gondii* disrupted tachyzoites as an antigen (RH strain) () and a HRP-labelled monoclonal antibody () derived against *Toxoplasma gondii* major membrane protein P30 is added and allowed to bind to the immobilised IgM.



3. After the second washing step an enzymatic reaction with the fluorogenic substrate, 3-p-hydroxyphenylpropionic acid (HPPA) is performed.



4. The reaction is stopped by addition of glycine buffer and the fluorescence in each well is measured with Fluoroskan Ascent Neonatal at 405 nm (excitation being 320 nm).



KIT CONTENTS

- For *in vitro* diagnostic use.
 - Reagents are sufficient for 480 determinations.
 - Reagents are stored between +2°C and +8°C.
 - Prewarm all reagents and Microstrips to +18°C....+30°C before use.
 - Avoid excessive exposure to light. HPPA-Fluorogen reagent and conjugate are light sensitive.
 - **Once opened, the components must be sealed tightly e.g. with parafilm or tape!**
 - Please note that the reagents are lot specific.
- 1 COATED MICROSTRIPS®, 5 plates of 12 x 8 wells
Coated Microstrips® in foil package with a desiccant.
 - 2 ANTI-HUMAN IgM ANTIBODY, 90 ml
Sheep anti-human IgM antibody in phosphate buffer, pH 7.4 containing 0.05% Bronidox® as preservative.
 - 3a TOXOPLASMA GONDII ANTIGEN, 5 x18 ml vials
Toxoplasma gondii tachyzoite preparation (RH strain) in phosphate buffer, pH 7.4, containing 0.2% Kathon CG as a preservative, lyophilized. Reconstitute with 18 ml of distilled water 10-60 minutes prior to use. R43, S37
 - 3b HRP-LABELLED ANTI-TOXOPLASMA GONDII ANTIBODY (CONJUGATE), 1 ml concentrate (100x)
Antibody (mouse, monoclonal) against *Toxoplasma gondii* conjugated with horseradish peroxidase. The antibody concentrate contains 0.05% Bromonitrodioxane as a preservative. Dilute (1+100) in reconstituted antigen (vial 3a) 10-60 minutes prior to use.

- 4a HPPA FLUOROGEN, 2 x 50 ml
3-(p-Hydroxyphenyl)propionic acid, in Tris buffer, pH 7.8 containing 0.2 % Kathon CG as preservative. R43, S37
 - 4b H₂O₂ SOLUTION, 45 ml
Citrate-acetate buffer, pH 6.0 containing hydrogen peroxide.
 - 5 STOPPING SOLUTION, 150 ml concentrate (2x)
Glycine buffer, pH 10.3.
Preparation: Dilute the amount needed 1+1 (1:2) with distilled water. R34; S24/25
 - 6 WASHING SOLUTION, 220 ml concentrate (10x)
Concentrated phosphate buffered saline containing Tween 20 and 0.05% Bronidox® as preservative. Dilute 1+9 in distilled water.
- Allow the bottle to reach +18 to +30°C before dissolvment with water. Prolonged storage at +4°C may lead to crystal formation.
- 7 CONTROLS AND CALIBRATOR, 1 sheet with 5 sets of 3 controls and 1 calibrator in a foil package with a desiccant
Specific IgM antibodies in dried blood spots on a filter paper S&S903.

The controls and the calibrator are prepared by mixing adult erythrocytes with human sera positive or negative in regard to *Toxoplasma gondii* IgM.

| | | |
|---|---|----------------------|
| A | = | Negative Control |
| B | = | Borderline Control |
| C | = | Low Positive Control |
| D | = | Calibrator |

NOTE It is a good laboratory practice that screening laboratories include their own positive controls for comparison and internal quality control. The Borderline Control of the kit is not a cut-off sample. Interpretation of all clinical specimens should be based on predetermined cut-off level in each laboratory.

NOTE To ensure sustained EIU values for unknowns when changing lots, EIU value of the Calibrator used for the calculation is informed in a separate sheet.

CALIBRATOR SHEET, 1 pc

DISPOSABLE REAGENT BASINS, 5 pcs
For dispensing the substrate solution

MICROSTRIP COVERS, 10 pcs
Plastic sheets to cover the Microstrips® during incubation in other than iEMS Incubator/Shaker or during overnight incubation with antibody.

REAGENT PREPARATION

Table 1 Reagent preparation

| Reagent | Preparation | Stability of opened and diluted reagents (+2°C to +8°C) |
|--|--|--|
| 1 Coated Microstrips® | Ready for use | At least 2 weeks when protected from moisture, heat and excessive light. |
| 2 Anti-Human IgM antibody | Ready for use | 6 months |
| 3a Toxoplasma gondii antigen | Lyophilized, reconstitute one vial with 18 ml of distilled water 10-60 min prior to use. | 2 weeks. |
| 3b HRP-labelled Anti-Toxoplasma antibody (Conjugate) | Ready for use in Antigen - Conjugate Solution, see below | 6 months |
| Antigen – Conjugate solution | Add 1 volume of Conjugate (vial 3b) to 100 volumes of freshly reconstituted Antigen (vial 3a) (1+100) 10-60 min prior to use and mix carefully. | Discard unused solution. |
| 4a HPPA Fluorogen | Ready for use in substrate solution. | 6 months |
| 4b H ₂ O ₂ Solution | Ready for use in substrate solution. | 6 months |
| Substrate solution | Dilute 1+5 (1:6) H ₂ O ₂ solution (vial 4b) with HPPA Fluorogen (vial 4a) just before use. Mix carefully (see also table 3): | Discard unused solution. |
| 5 Stopping solution | Dilute 1+1 (1:2) With distilled water. | 6 months |
| 6 Washing solution | Dilute 1+9 (1:10) With distilled water. | Discard if turbidity develops. |

| | | |
|---------------------------|----------------|---|
| 7 Controls and Calibrator | Ready for use. | Once opened, stable at least 6 months when protected from moisture, heat and excessive light. |
|---------------------------|----------------|---|

Table 2 Preparation of antigen - conjugate solution

| No of plates | Toxoplasma gondii antigen (vial 3a) (ml) | HRP-labelled anti-Toxoplasma antibody (conjugate) (vial 3b) (ml) |
|--------------|--|--|
| 1 | 18 | 0.18 |
| 2 | 36 | 0.36 |
| 3 | 54 | 0.54 |
| 4 | 72 | 0.72 |
| 5 | 90 | 0.90 |

Table 3 Preparation of substrate

| No of plates | HPPA-Fluorogen (vial 4a) (ml) | H ₂ O ₂ -Solution (vial 4b) (ml) |
|--------------|-------------------------------|--|
| 1 | 12.5 | 2.5 |
| 2 | 25 | 5 |
| 3 | 37.5 | 7.5 |
| 4 | 50 | 10 |
| 5 | 62.5 | 12.5 |

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate fluorometer eg. Fluoroskan Ascent Neonatal (100-240V) cat. no. 5210530
- Microplate shaker eg. iEMS Incubator/Shaker with 9 thermal microplate holders, temp. range: RT – +40°C (cat.no. 5112 200) or iEMS Incubator/Shaker HT, with 3 thermal microplate holders, temperature range: +14°C – +69°C (cat. no. 5112250)
- Microplate washer eg. Wellwash 4 Mk 2 (cat. no. 516 0770) or 8-channel pipette with 300 µl volume
- Disk puncher with a diameter of 3 mm to cut off paper disks of dried blood controls, calibrator and samples, and water suction with eg. Pasteur pipette to remove paper disks from microtitration wells or Woodpecker disk processor to punch disks (cat. no. 5600 200)
- Disk holders for Woodpecker, disposable (100 pcs, cat. no. 9700300X)
- 8-channel pipette to pipet 100 µl and 200 µl volumes
- Liquid dispenser eg. Multidrop 384 (cat. no. 584 0150)

SPECIMEN COLLECTION AND HANDLING

A blood spot on the filter paper is obtained by one application of the filter paper onto a drop of blood from the pricked heel of the baby 3-5 days after birth. Schleicher & Schuell 903 filter paper is suitable for collection of blood spots. Make sure that the filter paper is fully covered and soaked through. The blood spot is dried for at least 2 hours. Dry spots can be stored at +4°C ... +8°C for at least 4 months.

The specimen collection technique is described in detail in NCCLS document LA4-A3 [6].

It is essential that the blood spots are collected by application of a single drop of blood. Layering of successive drops, often recognizable by a visible caking of blood on the filter paper support, will produce falsely elevated results. It is likewise important that the blood is large enough to spread over the required area, penetrating the filter paper from one side to the other; incomplete saturation of the support medium may result in underestimation of analyte content.

To enhance blood flow at the puncture site, the infant's heel can be covered with a warm, moist towel at a temperature of 42°C or less for three minutes. Clean the skin with 70 % alcohol and wipe with dry sterile gauze. To minimize the risk of injury to the bone, use a disposable lancet. Puncture the skin on the lateral side of the flat walking surface of the heel. (Avoid the arch and the posterior curvature of the heel.)

Caution: A lancet length of 2.4 - 2.5 mm as specified in NCCLS standard LA4-A may be excessive owing to the marked compression of the skin during skin puncture [7]. Recent studies suggested that blood may be obtained when depths of the lancet or puncturing device are as little as 1 mm – e.g., Simplate® (Organon Teknika Corp., Durham, NC), and Tenderfoot™ Surgicutt® (International Technidyne Corp., Edison, NJ) [7]. Wipe off the first drop of blood and encourage the formation of subsequent drops, while holding the foot in a dependent position, by applying gentle pressure. Excessive squeezing may result in hemolysis or dilution of the sample with tissue fluid.

Once a drop of blood of adequate volume has formed, touch the filter paper gently to it - do not press it against the heel - and watch from the opposite side as the blood saturates the paper. Be careful not to handle the filter paper in the region of the preprinted circles prior to collection and not to touch or smear the blood spots. Avoid contamination with water or alcohol.

Allow the blood spots to air dry in a horizontal position for 2 to 6 hours at ambient temperature. During the drying process, do not subject the spots to heat and neither stack them nor let them touch other surfaces.

Once dry, place each specimen in a separate paper envelope and mail it to the laboratory. Blood spot specimens received in the laboratory should be stored at +4°C ... +8°C protected against moisture.

PRECAUTIONS

For *in vitro* diagnostic use.

WARNING - POTENTIAL BIOHAZARDOUS MATERIAL

Each donor unit used in the preparation of the kit controls and calibrator has been tested for the presence of the antibodies to HIV (human immunodeficiency virus) as well as for hepatitis B markers and found to be negative. Because no test method can offer complete assurance that HIV, hepatitis B virus, or other infectious agents are absent, these controls, calibrator as well as specimens should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories", 1999.

Discard solutions into a waste drainage network and flush with large volumes of water. After elution disks can be disposed of as conventional laboratory wastes.

Note that components 3a (*Toxoplasma gondii* antigen) and 4a (HPPA Fluorogen) contain Kathon CG which may cause sensitization by skin contact (R43). User should wear suitable gloves (S37). Note also that component 5 (Stopping solution) contains NaOH which causes burns (R34). Avoid contact with skin and eyes (S24/25).

TEST PROCEDURE (Manual Punch)

Outline of the Procedure

| | |
|-----------------|---|
| STEP I | Place 3-mm disks from controls, calibrator and specimens into the wells Add 150 µl of anti-human IgM antibody A) Incubate 30 min, room temperature, shaking or B) 15 min, room temperature, shaking and overnight at 4°C without shaking Remove disks, wash 4 x 300-400 µl |
| STEP II | Add 150 µl of antigen - conjugate solution Incubate 1 h, room temperature, shaking Wash 4 x 300-400 µl |
| STEP III | Add 150 µl of substrate solution Incubate 30 min, room temperature, shaking |
| STEP IV | Add 50 µl stopping solution Measure fluorescence at ex. 320, em. 405 nm |

Bring all reagents and Microstrips® to room temperature (+18°C to +30°C) before starting the assay.

1. Punch in duplicates 3-mm disks from controls, calibrator and specimens, and put them into the wells (1 disk/well) of the coated Microstrips®. Alternatively, the disks can be prepunched into the coated Microstrips® and stored at +4°C overnight in a microplate with cover.
2. Add 150 µl of Anti-human IgM antibody (vial 2).
3. Incubate according to the chosen procedure:
 - A: 30 min at room temperature (up to +30°C) on a shaker, speed 650–900 rpm. To ensure constant temperature during cold and warm seasons set the Incubator/Shaker at +27°C.
 - B: 15 minutes at room temperature on a shaker and then cover the plate and incubate overnight without shaking at +4°C .
4. Remove the solution and the paper disks with water suction connected to Pasteur pipette.
5. Manual washing:
 - Add 300 µl of washing solution into each well.
 - Empty the wells by shaking out the liquid.
 - Perform the washing four times in total.
 - After the fourth washing, tap the inverted Microstrips® a few times and leave them upside down on a paper towel for a moment.
 Automated washing:
 - Use only serviced washers with appropriate aspiration.
6. Add 150 µl of antigen - conjugate solution (vials 3a and 3b).
7. Incubate for 1 hour as in item 3, procedure A.
8. Wash the plate as in item 5.
9. Add 150 µl of Substrate solution (vials 4a and 4b). Use disposable reagent basins for dispensing the substrate solution.
10. Incubate for 30 min as in item 3, procedure A.
11. Add 50 µl of Stopping solution (vial 5).
12. Read the fluorescence using Fluoroskan Ascent Neonatal excitation 320 nm/emission 405 nm within 60 min.

TEST PROCEDURE (Woodpecker system)

Outline of the Procedure

| | |
|---------------|---------------------------------------|
| STEP I | Add 150 µl of anti-human IgM antibody |
|---------------|---------------------------------------|

Place disk holders with 3-mm disks from controls, calibrator and specimens into the wells

- A) Incubate 30 min, room temperature, shaking
- or
- B) 15 min, room temperature, shaking and overnight at 4°C without shaking

Remove the disk holders, wash 4 x 300-400 µl

STEP II Add 150 µl of antigen - conjugate solution

Incubate 1 h, room temperature, shaking

Wash 4 x 300-400 µl

STEP III Add 150 µl of substrate solution

Incubate 30 min, room temperature, shaking

STEP IV Add 50 µl stopping solution

Measure fluorescence at ex. 320, em. 405 nm

Bring all reagents and Microstrips® to room temperature (+18°C to +30°C) before starting the assay.

1. Add 150 µl of Anti-human IgM antibody (vial 2).
2. Punch in duplicates 3-mm disks from controls, calibrator and specimens. Insert disk holders with disks into wells of coated Microstrips®.
3. Incubate according to the chosen procedure:
 - A: 30 min at room temperature (up to +30°C) on a shaker, speed 650-900 rpm. To ensure constant temperature during cold and warm seasons set the Incubator/Shaker at +27°C.
 - B: 15 minutes at room temperature on a shaker and then cover the plate and incubate overnight without shaking at +4°C .
4. Remove the disk holders.
5. Manual washing:
 - Add 300 µl of washing solution into each well.
 - Empty the wells by shaking out the liquid.
 - Perform the washing four times in total.
 - After the fourth washing, tap the inverted Microstrips® a few times and leave them upside down on a paper towel for a moment.
 Automated washing:
 - Use only serviced washers with appropriate aspiration.
6. Add 150 µl of antigen - conjugate solution (vials 3a and 3b).
7. Incubate for 1 hour as in item 3, procedure A.
8. Wash the plate as in item 5.

9. Add 150 μ l of Substrate solution (vials 4a and 4b). Use disposable reagent basins for dispensing the substrate solution.
10. Incubate for 30 min as in item 3, procedure A.
11. Add 50 μ l of Stopping solution (vial 5).
12. Read the fluorescence using Fluoroskan Ascent Neonatal excitation 320 nm/emission 405 nm within 60 min.

up to 90 min (due to processing of multiple plates) it is expected to have higher fluorescence values in the last plates, however discrimination between negatives and borderline/positives will not be compromised.

13. Use **only clean glassware** when preparing and dispensing substrate solution. The **presence of detergent residues** may result in low signal level.

PROCEDURAL NOTES

1. Read kit instructions carefully.
2. Improper storage of the kit will result in decreased fluorescence response, decreased assay sensitivity and thus can lead to compromised results.
3. Do not use reagents after the expiry date printed on the label.
4. Do not mix or interchange reagents from different lots.
5. Bring all reagents to room temperature (+18°C to +30°C) before starting the assay.
6. Do not interchange vial caps.
7. When removing aliquots from the reagent vials, **use aseptic technique** to avoid contamination, or incorrect results may occur.
8. Once the assay has been started, all subsequent steps should be performed without interruption.
9. Do not touch the wells with pipette tips while pipetting. Be especially careful while pipetting the Stopping solution to avoid contamination of neighbouring wells.
10. Do not reuse the Microstrips®.
11. Optimal results are obtained by strict adherence to the test protocol. Accurate and precise pipetting, as well as careful washing of the plate, are essential.
12. Slight variation in incubation time is allowed. Ideally, time tolerances for all incubation steps are ± 5 min. It is noteworthy, however, that increase of incubation time or overnight incubation at +4°C during the first incubation step should not change fluorescence values. On the contrary, deviation in incubation time in the second incubation step will result in decreased (shorter incubation time) or increased (longer incubation time) fluorescence values (see figure 2 below **Trouble shooting**). If incubation time during the second step is extended

RESULTS

Quality Control

The CV% of the replicates calculated from the raw fluorescence should be below 15%. The mean of the Borderline Control fluorescence values is at least 2-fold of the mean of the Negative Control.

Calculation of the Results

1. Calculate the mean fluorescence of the Negative Control (preferably 3 replicates) for each plate. In case the CV% is >15% disregard the possible outlier and calculate the mean again. Subtract the value of the mean Negative Control from each well. If the mean of the Negative Control gives fluorescence value higher than the fluorescence of a sample, a negative value is produced for the difference ($\text{Fluor.}_{\text{sample}} - \text{Fluor.}_{\text{NC}}$). In this situation, replace the negative difference by 1. Perform this calculation for each plate separately using the plate respective mean of the Negative Control.
2. Convert net fluorescence signals into enzyme immunounits (EIU) as in the formula.

$$\text{EIU} = (\text{Fluor.}_{\text{sample}} - \text{Fluor.}_{\text{NC}}) / (\text{Fluor.}_{\text{CAL}} - \text{Fluor.}_{\text{NC}}) \times \text{RVC}$$

where

- $\text{Fluor.}_{\text{sample}}$ = fluorescence of the specimen
 $\text{Fluor.}_{\text{NC}}$ = mean fluorescence of the Negative Control (if CV% < 15%)
 $\text{Fluor.}_{\text{CAL}}$ = mean fluorescence of the Calibrator
 RVC = Relative Value of the Calibrator. This value can vary from lot to lot depending on the Calibrator sera used. The value is given on a separate sheet included in the kit.

Expression of results in EIUs allows better between-run reproducibility, whereas fluorescence signals may slightly vary from run to run.

Example of Calculation

| Control / Calibrator | Fluorescence values | EIU |
|---|-------------------------|---|
| Negative Control (3 replicates) | 104.4 109.4 129.4 | |
| Mean | 114.4 | |
| SD | 13.2 | |
| CV% | 11.5 | |
| Borderline Control (Mean of 2 replicates) | 390 | $(390-114.4)/(3945-114.4) \times 98 = 7.1$ |
| Low Positive Control (Mean of 2 replicates) | 850 | $(850-114.4)/(3945-114.4) \times 98 = 18.8$ |
| Calibrator (Mean of 2 replicates) | 3945 | 98 (lot specific value) |

Interpretation of the Results

It is recommended that each laboratory establishes its own cut-off value. On the basis of a preliminary multicenter clinical evaluation (5), the proposed cut-off for the IgM test varied from 4 (in USA) to 5 EIU's (in Denmark). In a larger clinical evaluation at the New England Regional Newborn Screening Laboratory, USA, the cut-off was between 3 - 4 EIU's (see chapter CLINICAL EVALUATIONS).

PERFORMANCE CHARACTERISTICS

Reproducibility

Between-run reproducibility was calculated from 8 successive runs with alternating long and short sample incubation procedures.

Table 3 Within-run reproducibility

| Sample | EIU values, n=8 | CV% |
|--------------|-----------------|-----|
| Borderline | 6 | 11 |
| Low Positive | 33 | 5 |
| Positive 1 | 70 | 7 |
| Positive 2 | 85 | 5 |
| Calibrator | 100 | 1 |

Table 4 Between-run reproducibility

| Sample | EIU values, n=8 | CV% |
|--------------|-----------------|-----|
| Borderline | 8 | 13 |
| Low Positive | 31 | 17 |
| Positive 1 | 58 | 11 |
| Positive 2 | 90 | 10 |
| Calibrator | 100 | |

Analytical Sensitivity

To assess analytical sensitivity serial dilution of the 2nd (1980) and the 3rd (1994) International Standard Preparations for the anti-Toxoplasma human serum from

the WHO International Laboratory for Biological Standards was performed and assayed as 5 µl/well. The proportion of response of each dilution to the response of the neat sera was calculated. Comparison was done to the respective results by two other commercial IgM-capture methods. The percentage response as a function of dilution factor is presented in the Fig. 1.

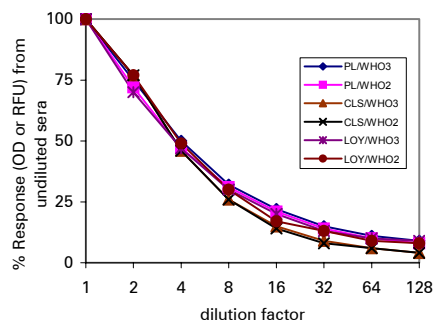


Figure 1: Comparison of dilution curves of 3 capture Toxo IgM kits

Designation:

PL/WHO3 - dilution curve of the WHO 3rd International Standard for the Anti-Toxoplasma serum with Platelia Toxo IgM (Sanofi Diagnostics, Pasteur, France)

PL/WHO2 - dilution curve of the WHO 2nd International Standard, Platelia Toxo IgM

CLS/WHO3 - dilution curve of the WHO 3rd International Standard for the Anti-Toxoplasma serum with EIAGEN Toxoplasmosis IgM (IFCI CloneSystems S.p.A., Italy)

CLS/WHO2 - dilution curve of the WHO 2nd International Standard for the Anti-Toxoplasma serum with EIAGEN Toxoplasmosis IgM

LOY/WHO3 - dilution curve of the WHO 3rd International Standard for the Anti-Toxoplasma serum with Ani Labsystems Neonatal Toxoplasma gondii IgM FEIA

LOY/WHO2 - dilution curve of the WHO 2nd International Standard for the Anti-Toxoplasma serum with Ani Labsystems Neonatal Toxoplasma gondii IgM FEIA

It is evident from the figure that the titration curves were identical in the three methods. The analytical sensitivity of the method is thus dependent on the aimed cut-off level.

CLINICAL EVALUATIONS

A clinical evaluation was performed at the New England Regional Newborn Screening Laboratory, Jamaica Plain, MA, USA. A total of 2133 normal samples were analysed with Ani Labsystems' Neonatal Toxoplasma gondii IgM FEIA. These samples were original filter paper samples

submitted to the New England Regional Newborn Screening Program for routine screening. The cut off, as determined as the 99.5 percentile, was between 3 and 4 EIU's .

In addition to these 2133 normal samples, 21 original newborn filter paper samples from congenitally infected babies were retrieved from frozen storage and tested retrospectively. These babies were diagnosed with congenital toxoplasmosis by a variety of serologic and clinical evaluations, as appropriate for each case. In situations where Ani LabSystems' FEIA was negative or borderline, a standard EIA assay was re-run to be certain the IgM survived storage. See results in table 5.

Table 5. Retrospective testing of 21 newborn samples from infants diagnosed with congenital toxoplasmosis

| Sample ID | FEIA (EIU) | Standard EIA (OD) |
|---------------|------------|-------------------|
| 012397-2167 | 79.9 | |
| 080994-0728 | 56.2 | |
| 092794-0444 | 46.5 | |
| 031194-0401 | 32 | |
| 061395-0445 | 31 | |
| 051396-0328 | 19.9 | |
| 051994-0255 | 18.6 | |
| 110894-1024 | 12.4 | |
| 103194-0541 | 8.4 | |
| 121694-0237 | 7.7 | |
| 091395-0156 | 7.6 | |
| 051094-0647 | 6.8 | |
| 090695-0231 | 4.1 | |
| 100196-0815 | 2.9 | 0.167 |
| 121994-0552# | 1.7 | 0.083 |
| 061794-0132* | 1.4 | 0.173 |
| 081094-0427* | 1.3 | 0.141 |
| 013194-0152* | 1.2 | 0.136 |
| 110496-0221 ^ | 1.1 | |
| 090696-0317 ^ | 0.7 | |
| 081696-0421# | 0.1 | 0.039 |

13 of the 21 samples were clearly positive by Ani LabSystems Neonatal *Toxoplasma gondii* IgM FEIA by the cut off 4.0 EIU, and an additional sample had an EIU of 2.9. Of the remaining 7 samples, two (marked with #) were no longer positive by the standard EIA assay, and so may have lost toxoplasma-specific IgM upon storage. Two samples (marked with ^) did not have sufficient quantity for re-testing by the standard EIA assay. The remaining 3 samples (marked with *) were borderline positive by the standard EIA assay. All 3 were more than two years old and possibly difficult to elute. Ani LabSystems FEIA was repeated for these 3 samples using the optional overnight elution procedure. The EIU results after overnight elution for these 3 samples were 7.1, 5.7 and 3.5 respectively.

A second clinical evaluation was performed in Danish Statens Serum Institut between January 2000 – January 2001. A total of 72,531 normal dried filter paper samples from the routine neonatal screening program were

analyzed with Ani LabSystems' Neonatal *Toxoplasma gondii* IgM FEIA. A low cut-off of approximately 2 EIU was used to avoid false negative results. A total of 238 samples were identified positive by the proposed cut off. These samples, with a preliminarily positive result with FEIA method, were tested with a modified Immuno Sorbent Agglutination Assay, ISAGA (bioMérieux, France), for *Toxoplasma gondii*-specific IgM antibodies. 214 samples of these were tested negative by ISAGA IgM test. The remaining 24 samples were found to be positive with ISAGA test and a blood sample from these newborn children was requested. Serum samples from suspected children showed that finally 13 children out of the 24 were truly confirmed to be infected with Congenital Toxoplasmosis. Retest rate was (238 / 72531 =) 0.33 % and recall rate is (24 / 72531 =) 0.033 %.

Another limited clinical evaluation was performed at the manufacturer's site. For the study 600 dried blood spot specimens from Moscow newborns were analyzed with alternating long and short procedures (see Test procedures A and B). The samples were stored at +4°C for 9 months and analyzed together with freshly prepared controls and those stored for 1.5 years. Out of the 600 samples one sample proved to be repeatedly reactive in the method, while no reactivity was observed after withdrawal of the antigen. The dried blood spot sample showed also specific IgA- and IgG-class reactivity. The sample was from a girl who was 2.5 years old at the time of blood collection and who was retested in the Moscow Phenylketonuria Center for congenital hypothyroidism. The girl was referred to Morosov's Children's Infection Hospital (Moscow, Russia), and the diagnosis of congenital toxoplasmosis was confirmed (8).

LIMITATIONS OF THE PROCEDURE

When cord blood is used for analysis, occasional contamination with maternal blood may give erroneous interpretation of the fetus serological status.

Neonatal screening for CT based on the detection of IgM-class antibodies alone might miss subjects with very early intra-uterine infection due to the decline of specific IgM-antibodies pre partum. Cases can be missed also when infection occurs shortly before birth and sufficient antibody response has not yet been evolved. However, in 90-95% of congenitally infected babies specific *Toxoplasma gondii* IgM-class antibodies are detectable (9-13). Theoretically, neonatal screening for congenital *Toxoplasma gondii* infection may miss about 10% of infected babies. This is well comparable with the diagnostic sensitivity of other screening programs e.g. neonatal screening for congenital hypothyroidism which is estimated to miss 10% cases (14).

It is recommended that the assay is performed by qualified and trained laboratory technician.

TROUBLE SHOOTING

| LOW FLUORESCENCE VALUES (especially of the Borderline and Low Positive Controls and Calibrator) | |
|---|---|
| Cause/Error | Remedy |
| 1. Reagents are deteriorated <ul style="list-style-type: none"> • due to contamination • due to improper storage (inactivation of HRP in the conjugate) | Use unopened reagent bottles See instructions for reagent storage Protect kit controls and calibrator from excessive light and moisture |
| 2. Substrate solution is not mixed as in the instructions | See instructions for substrate preparation |
| 3. Reagents are not warmed up to room temperature before starting the assay | The reagent should be brought to room temperature at least 30 minutes before the assay |
| 4. Incubation time for the antigen-conjugate reaction is too short | Incubate according to the instructions |
| 5. Fluoroskan Ascent Neonatal wavelength settings are not correct | Fluoroskan Ascent Neonatal filter pair should be 320/405 |
| 6. Fluoroskan Ascent Neonatal is programmed for blanking | Reprogram Fluoroskan Ascent Neonatal |
| HIGH FLUORESCENCE VALUES (especially of the Borderline and Low Positive Controls and Calibrator) | |
| Cause/Error | Remedy |
| 1. Insufficient washing due to poor aspiration | If possible, adjust the tips of the washing head Remember regular maintenance of the washer |
| 2. Incubation temperature is too high | Check the programmed temperature on iEMS Incubator/Shaker |
| 3. Impure glassware for the substrate solution (residuals of detergent) Exposure of fluorogen to excessive light | Use only clean glassware Protect fluorogen from light |
| 4. The use of reconstituted antigen stored at +4°C beyond 2 weeks | Do not use reconstituted antigen stored beyond 2 weeks |
| POOR PRECISION | |
| Cause/Error | Remedy |
| 1. Liquid handling devices are not properly calibrated | Check calibration of the pipetting device |
| 2. Improper washing due to contamination of washing tips | Clean regularly tips of the washing head |
| 3. The plate is allowed to stay too long after washing (drying of the plate) | Follow strictly the kit instructions |
| 4. Touching of liquid surface when pipetting stopping solution | Avoid touching the liquid surface when pipetting stopping solution |

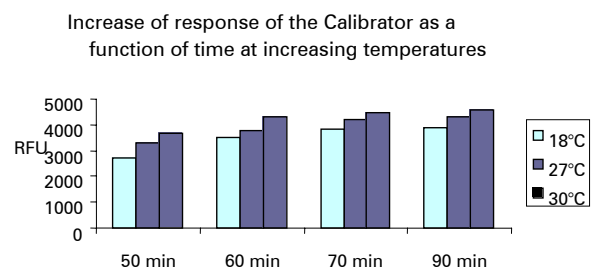
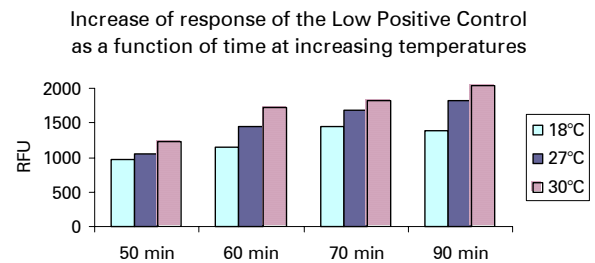
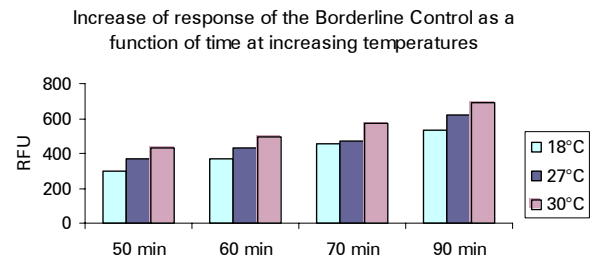
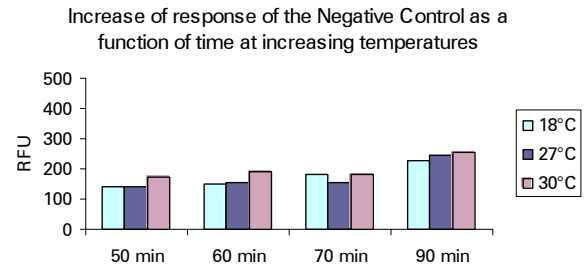
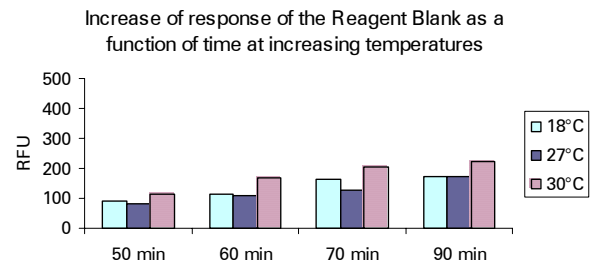


Figure 2: Dependence of fluorescence values of the reagent blank, kit controls and calibrator on temperature and reaction time with a conjugate-antigen solution

| | |
|--|---|
| 5. Air bubbles when pipetting | Pipette carefully |
| 6. Air contamination of microwells by fluorescent dust particles | Perform assay in clean environment |
| 7. Uneven warming of the plate | Service iEMS Incubator/Shaker |
| 8. In rare instances dust particles or air bubbles might cause light scattering and poor precision of the duplicates | Slightly shake the plate and measure again, the dust particles will sediment and the bubble could be broken |

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RELATED PRODUCTS AND ORDER INFORMATION:

| Product no | Product description | Kit size |
|------------|--|--------------------|
| 61 99 896 | Neonatal Phenylalanine | 960 wells |
| 61 99 897 | Neonatal Phenylalanine | 4800 wells |
| 61 90 930 | Neonatal Phenylalanine Controls | 5 sets of 3 levels |
| 61 90 940 | Neonatal Phenylalanine Calibrators | 5 sets of 5 levels |
| 6190 900 | Neonatal Phenylalanine Reference Panel | 5 sets of 5 levels |
| 61 99 880 | Neonatal hTSH FEIA Plus | 960 wells |
| 61 99 881 | Neonatal hTSH FEIA Plus | 4800 wells |
| 61 99 892 | Neonatal hTSH EIA | 960 wells |
| 61 99 8923 | Neonatal hTSH EIA | 4800 wells |
| 61 90 905 | Neonatal hTSH reference panel | 5 sets of 5 levels |
| 61 99 802 | Neonatal Toxoplasma gondii IgM FEIA | 480 wells |
| 61 99 804 | Neonatal Toxoplasma gondii IgM EIA | 480 wells |

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