



# Neonatal Toxoplasma gondii IgM EIA

For research use only



480 tests



**Instructions for use**



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# INSTRUCTIONS FOR USE

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## NEONATAL TOXOPLASMA GONDII IgM EIA

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

Product no.:  
61 99 804 (GRADE 903) (Microplate 12x8, 480 wells)

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### INTENDED USE

This Neonatal *Toxoplasma gondii* IgM EIA kit is intended for the determination of IgM-class antibodies to *Toxoplasma gondii* in blood samples 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 samples 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

Neonatal *Toxoplasma gondii* IgM EIA kit is a solid-phase capture enzyme immunoassay 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. 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. After the second washing step an enzymatic reaction with the 3,3',5,5'-Tetramethylbenzidine (TMB) is performed. The reaction is stopped by addition of 0.45M H<sub>2</sub>SO<sub>4</sub> and the absorbance in each well is measured with microplate photometer at 450 nm.

### KIT CONTENTS

- Reagents are stored between +2°C and +8°C.
- The expiration date is printed on each component label and on the package. Do not use reagents after the expiration date.
- Avoid unnecessary exposure to light. This is merely a precaution. The light sensitive reagents are the conjugate and the TMB-chromigen, the latter one is packaged in non-transparent plastic vial for protection.

- 1 COATED MICROPLATE, 5 plates of 12 x 8 wells  
Coated Microplate.
- 2 ANTI-HUMAN IgM ANTIBODY, 90 ml  
Sheep anti-human IgM antibody in buffer containing Bronidox® as preservative.
- 3a TOXOPLASMA GONDII ANTIGEN, 3 vials   
*Toxoplasma gondii* tachyzoite preparation (RH strain) in buffer containing Kathon CG as a preservative, lyophilized. Xi (Irritant) R43, S37

- 3b ANTI-TOXOPLASMA GONDII ANTIBODY-CONJUGATE, 1 ml (200X)**  
Antibody against *Toxoplasma gondii* conjugated with horseradish peroxidase Bromonitrodioxane as a preservative.
- 4a TMB CHROMOGEN, 3 ml**   
3,3',5,5'-Tetramethylbenzidine dissolved in dimethylsulfoxide (DMSO). Xi, T (Irritant, Toxic) R25, R36/38, S26
- 4b DILUENT FOR TMB-CHROMOGEN, 250 ml**  
Solution containing Bromonitrodioxane as a preservative.
- 5 WASHING SOLUTION, 220 ml concentrate (10x)**  
Buffer solution containing Bronidox® as preservative.
- 6 CONTROLS and CALIBATOR**   
1 sheet (grade 903 filter paper)  
Ready to use controls, 5 sets.  
The value (EIU) of calibrator is lot specific. For exact value refer to the calibrator sheet included in each kit. Potential biohazardous material.  
A = Negative control  
B = Borderline control  
C = Low positive control  
D = Calibrator
- 7 STOPPING SOLUTION, 100 ml**  
0,45 M H<sub>2</sub>SO<sub>4</sub>

PLASTIC COVERS, 10 pcs  
Plastic incubation covers for microplates

REAGENT BASINS, 10pcs

SHEET WITH VALUE FOR CALIBRATOR, 1 pc

*Relevant R-phrases:*

- 25 Toxic in contact with the skin or if swallowed.  
36/38 Irritating to eyes, respiratory system and skin.  
43 May cause sensitization by skin contact.

*Relevant S-phrases:*

- 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.  
37 Wear suitable protective clothes and gloves.

**REAGENT PREPARATION**

**Table 1** Reagent preparation. Bring the reagents to room temperature (+18°C to +30°C) before use.

Reagent	Preparation	Stability of opened and diluted reagents (+2°C to +8°C)
1 Coated Microplate	Ready for use	At least 2 weeks *

2 Anti-Human IgM antibody	Ready for use	6 months
3a Toxoplasma gondii antigen	Reconstitute one vial with 36 ml of distilled water 10-60 min prior to use.	2 weeks.
3b HRP-labelled Anti-Toxoplasma antibody (Conjugate)		6 months
Antigen – Conjugate solution	Add 1 volume of Conjugate (vial 3b) to 200 volumes of freshly reconstituted Antigen (vial 3a) (1+200) 10-60 min prior to use and mix carefully.	Discard unused solution.
4a TMB Chromogen		6 months
4b Diluent for TMB chromogen		6 months
Substrate solution	Just before use dilute TMB-chromogen (vial 4a) 1+49 (1:50) with diluent (vial 4b). Mix carefully	Discard unused solution. A deep blue colour present in the substrate solution indicates that the solution has been contaminated and must be discarded.
5 Washing solution	Dilute 1+9 (1:10) With distilled water.	Discard if turbidity develops.
6 Controls/ calibrator	Ready for use.	Stable at least 6 months*
7 Stopping solution	Ready for use	6 months

\*) When air-tightly closed in the foil package with the desiccant after opening and stored properly at +2°C to +8°C.

**Table 2** Preparation of antigen - conjugate solution

No of plates	Reconstituted Toxoplasma gondii antigen, vial 3a (ml)	HRP-labelled anti-Toxoplasma antibody (conjugate), vial 3b (ml)
1	18	0.09
2	36	0.18
3	54	0.27
4	72	0.36
5	90	0.45

**Table 3** Preparation of substrate solution

No of plates	TMB-Chromogen, 4a (ml)	Diluent for TMB chromogen, 4b (ml)
1	0.4	19.6
2	0.8	39.2
3	1.2	58.8
4	1.6	78.4
5	2.0	98.0

### MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or deionized water, preferably sterile.
- Graduated cylinders for reagent dilution.
- Vials to store the diluted reagents.
- Precision pipettes (multi-channel 50-300 µl)
- Paper towels or absorbent paper.
- Timer, 60 min range.
- Microplate incubator / shaker
- Microplate photometer
- Microplate washer (not compulsory)
- Disk puncher with a diameter of 3 mm to cut off paper disks of dried blood controls, calibrators and samples or Woodpecker disk processor to punch disks (cat. no. 5600 200 ) with disk holders for Woodpecker, disposable (100 pc)
- Sodium hypochlorite solution, free available chlorine 50-500 mg/l.
- Disposable gloves.

### PRECAUTIONS

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### Warning - POTENTIAL BIOHAZARDOUS MATERIAL:

All human materials used in the preparation of the calibrators/controls in the kit have been tested for the presence of the antibodies to HIV (Human Immunodeficiency Virus) and HCV (Hepatitis C Virus) as well as Hepatitis B surface antigen (HBsAg) and found to be non-reactive. Because no test method can offer complete assurance that HIV, hepatitis B virus, HCV, or other infectious agents are absent, these calibrators and controls as well as samples should be handled at the Biosafety level 2 as recommended for any potentially infectious human serum or blood sample in the Centers for Disease Control and prevention/National Institutes for Health Manual, "Biosafety in Microbiological and Biomedical Laboratories," 1999 [14].

Discard all materials and samples as if capable of transmitting infection. The preferred method of disposal is autoclaving for a minimum of one hour at 121°C. Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 50-

500 mg/l free available chlorine. Allow 30 minutes for decontamination to be completed. Spills should be wiped off thoroughly using either an iodophor disinfectant or sodium hypochlorite solution. Materials used to wipe off spills should be added to biohazardous waste matter for proper disposal. Reusable glassware must be disinfected, washed out and rinsed free of detergents.

Avoid contact with skin and eyes when handling antigen, conjugate, antigen-conjugate solution, TMB chromogen, substrate and stopping solutions.

Wear disposable gloves while handling samples and kit reagents. Afterwards wash hands carefully. Never pipette by mouth.

### SAMPLE 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. Grade 903 filter paper is recommended for collection of blood spots. Make sure that the filter paper sample is fully covered and soaked through. The blood spot is dried for at least 3 hours. Once dry, place each sample in a separate paper envelope and mail it to the laboratory. Blood spot samples received in the laboratory should be stored at +2°C ... +8°C protected against moisture. The sample collection technique is described in detail in NCCLS document LA4-A5 [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 drop of blood is large enough to spread out 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 (eg. 1 mm) [12]. 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.)

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. Ordinarily three blood spots are collected from each infant. 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.

## TEST PROCEDURE

### PRELIMINARY PREPARATIONS

- **Bring the reagents and microplates to room temperature (+18°C to +30°C) before starting the assay.**

#### STEP I

Punch out **3 mm** disks containing blood calibrator and controls into the microplate wells<sup>1)</sup>

Punch out single **3 mm** disks from patient samples into microplate wells.<sup>1)</sup>

Add **150 µl** of anti-human IgM antibody (vial 2). Make sure that the disks are properly soaked.

Cover the plate and incubate<sup>2)</sup> :

**30 min** (+/- 5 min) at **RT** with shaking speed of 650 -900 rpm

**OR**

**15 min** (+/- 5 min) at **RT** with shaking speed of 650-900 rpm and then overnight at **+ 4°C** without shaking

#### STEP II

Remove the disks **and liquid**, then wash 4 x 300 - 400 µl / well

#### STEP III

Add **150 µl** of antigen-conjugate solution (vials 3a+3b).

Cover the plate and incubate<sup>2)</sup> :

**1 hour** (+/- 5 min) at **RT** with shaking speed of 650 -900 rpm

#### STEP IV

Wash 4 x 300 - 400 µl / well

#### STEP V

Add **150 µl** of Substrate solution (vials 4a+4b)

Incubate **20 min** at **RT** in the **dark** without shaking

#### STEP V

Add **100 µl** of Stopping solution.

Measure immediately absorbance at 450 nm

### NOTES:

1. To avoid chromatographic effect punch disks symmetrically around the center of the spot. The disks can be punched manually or with the Woodpecker disc processor.
2. To avoid high background it is **important to cover the plate** with both manual punching and disc holder procedures.

Concentrated washing solution may form crystals when stored at +4°C, it is crucial to warm it to room temperature to eliminate crystal before use and further dilution.

The use of an 8-channel pipette device is recommended for improved efficiency and precision.

It is recommended to use **calibrator and controls in duplicates on each plate**. **Avoid contamination:** When removing aliquots from the reagent vials, use aseptic technique

to avoid contamination. The kit includes 10 disposable reagent basins that can be used for pipetting the reconstituted substrate.

Do not use reagents after the expiry date printed on the label. Do not mix or interchange reagents from different lots. Do not interchange vial caps.

Once the assay has been started, all subsequent steps should be performed without interruption. Do not let the wells dry once the assay has been started.

Do not reuse the microplate strips even if some wells were not used.

Use **only clean glassware** when preparing and dispensing of substrate solution. Use preferably disposable tubes to mix the substrate components and disposable reagent basin when pipetting the substrate solution.

Accurate and precise pipetting, as well as following the exact time and temperature requirements, is essential.

## RESULTS

### Calculation of the Results

1. Calculate the mean absorbance of the Negative Control (preferably 2 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 value higher than the absorbance of a sample, a negative value is produced for the difference (Abs. sample — Abs. 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 absorbance signals into enzyme immunounits (EIU) as in the formula.

$$EIU = (\text{Abs. Sample} - \text{Abs. NC}) / (\text{Abs. CAL} - \text{Abs. NC}) \times RVC$$

where

Abs. sample = absorbance of the sample

Abs. NC = mean absorbance of the Negative Control (if CV% <15%)

Abs. CAL = mean absorbance 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 absorbance signals may slightly vary from run to run.

Example of Calculation Control / Calibrator	Absorbance values	EIU
Negative Control (2 replicates)	0,120 0,112	
Mean	<b>0,116</b>	
CV%	4,9	

<b>Borderline Control</b> (Mean of 2 replicates)	<b>0,443</b>	$(0,443-0,116)/(2,225-0,116) \times 124 = 19,2$
<b>Low Positive Control</b> (Mean of 2 replicates)	<b>1,177</b>	$(1,177-0,116)/(2,225-0,116) \times 124 = 62,4$
<b>Calibrator (RVC)</b> (Mean of 2 replicates)	<b>2,225</b>	<b>124</b> (lot specific value)

**Quality Control Values**

The expected value for the calibrator is given for each lot on a separate sheet included in the kit. The CV% of the replicates calculated from the raw absorbance should be below 15%. The mean of the Borderline Control absorbance values is at least 2-fold of the mean of the Negative Control.

**Expected Values and 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 performed with the fluorometric version of the test (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.

**PERFORMANCE CHARACTERISTICS**

**Reproducibility**

Within-run reproducibility was calculated from 10 replicates.

**Table 4** Within-run reproducibility

Sample	EIU	Abs mean n=10	CV%
Negative 1	0	0.153	0.64
Positive 1	75	1.144	4.81
Positive 2	36	0.632	2.33

Between-run and between-lot reproducibility was calculated from 12 successive runs.

**Table 5** Between-run reproducibility

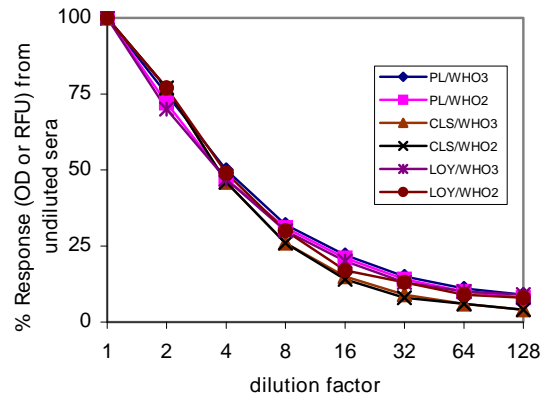
Sample	EIU values, n=12	CV%
Negative 1	1	57
Negative 2	6	18
Positive 1	77	9.3
Positive 2	82	6.8
Positive 3	88	8.0
Positive 4	39	10.6

**Analytical Recovery and Accuracy**

**Sensitivity**

Comparison of the analytical sensitivity was performed using the fluorometric version of the test, Ani Labsystems Neonatal *Toxoplasma gondii* IgM FEIA kit (Cat. no. 61 99 802).

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 EVALUATION**

The comparison of the Neonatal *Toxoplasma gondii* IgM EIA with the respective FEIA showed good agreement in interpretation of the samples.

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 samples 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 ABSORBANCES	
Cause/Error	Remedy
1. Reagents are deteriorated * due to contamination * due to improper storage	1. To prevent deterioration use aseptic technique when pipetting reagents repeatedly from the same vial. 2. To avoid deterioration, see instructions for reagent storage.
2. Reagents are not warmed up to room temperature	The reagents should be brought to room temperature at least 30 minutes /2 hours before the assay, depending on the reagent bottle size.

3. Incubation time for immunological reaction is too short	Incubate according to the instructions
4. Photometer wavelength setting is not correct	photometer wavelength is 450 nm

<b>HIGH ABSORBANCE (whole plate)</b>	
<b>Cause/Error</b>	<b>Remedy</b>
1. Insufficient washing due to poor aspiration	1. Adjust the tip of the washing head 2. Check that the washing tips are not stucked with paper matter
2. Incubation time is too long	Incubate according to the kit instructions
3. Incubation temperature is too high	Choose 15 minutes incubation at room temperature, and then overnight at +4°C for the immunological reaction
4. Impure glassware for the substrate solution (residuals of detergent)	Use only clean glassware, possibly remove detergent by acid treatment.
5. Degraded antigen	Do not use reconstituted antigen stored a +4C beyond 2 weeks

<b>POOR PRECISION</b>	
<b>Cause/Error</b>	<b>Remedy</b>
<b>Calibrator and/or controls</b>	
1. Calibrators and controls are deteriorated due to improper storage	Protect calibrators and controls from excessive light and moisture by resealing the foil package tightly with dessicant
<b>Whole plate (including calibrator/controls)</b>	
1. Liquid handling devices are not properly calibrated	Check calibration of your pipetting device
2. Improper washing due to blocking of washing tips by the filter paper dust	Clean regularly tips of the washing head. Empty properly the plate before placing it in the washer.
3. The plate is allowed to stay too long after washing (drying of the plate)	Follow the test instructions
<b>Only patient samples</b>	
1. Uneven distribution of blood in the sample	When possible, use samples fully impregnated with blood

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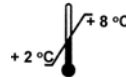
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