1. INTENDED USE

Immunofluorescence assay for the semiquantitative determination of antibodies against Toxoplasma gondii in human serum and plasma

IgG REF MK 113 G ▼ 25 ▼▼ kit
IgG REF IF 113 G ▼ 50 ▼▼ kit
IgG REF IF 115 G ▼ 100 ▼▼ kit
IgM REF MK 113 M ▼ 25 ▼▼ kit
IgM REF IF 113 M ▼ 50 ▼▼ kit
IgM REF IF 115 M ▼ 100 ▼▼ kit
Screening REF MK 113 C ▼ 25 ▼▼ kit
Screening REF IF 113 C ▼ 50 ▼▼ kit
Screening REF IF 115 C ▼ 100 ▼▼ kit

2. PRINCIPLE OF THE ASSAY

The detection of antibodies is based on the principle of an Indirect Immunofluorescence Assay (IFA). The slides are coated with Toxoplasma gondii (inactivated). Any specific antibodies present in the patient’s sample are bound during the first incubation. After removing unbound material by washing, the presence of specific antibodies is detected using Anti-Human IgG, IgM- or Screening-conjugate during the second incubation. Excess FITC conjugate is then removed. The formation of a stable three-part complex consisting of fluorescent antibody bound to human antibody, which is bound to antigen, can be visualized with the aid of a fluorescence microscope.

3. DIAGNOSTIC RELEVANCE AND INTERPRETATION OF RESULTS

To obtain a final diagnosis the patient history and clinical symptoms should be included for the interpretation of the serological results and possible cross-reactivity should be taken into consideration.

4. PERFORMANCE CHARACTERISTICS

Specificity/ Sensitivity
209 samples were tested parallel in IFA TOXO -IgG, 251 samples were tested parallel in IFA TOXO -IgM and 294 samples were tested parallel in IFA TOXO -Screening and comparison methods (ELISA). The sensitivity and specificity are based on the results found.

- IgG 97,0% Sensitivity: IgG 99,3%
- IgM 99,5% Sensitivity: IgM 100%
- Screen. 98,2% Sensitivity: Screen. 100%

The results refer to the groups of samples investigated.

5. KIT COMPONENTS

Number and volume of the kit components are indicated on kit label.

6. SLIDES

7. STORAGE AND STABILITY

Store all reagents at ▼▼ 2-8°C. Protect them from intense light and do not freeze. The expiration date of each component is indicated on the respective vial label. Do not use reagents beyond the expiration date.

The diluted BUF/PBS is stable up to 4 weeks when stored at ▼▼ 2-8°C. Use only with an intact vacuum packaging.

8. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- distilled water
- graduated cylinder
- coplin jars or staining dishes
- plastic squeeze bottle
- test tubes for sample dilution
- cover slips (24x60 mm)
- volumetric pipette (10 – 1000µl) with pipette tips
- moist chamber
- timer
- fluorescence microscope with FITC system, (excitation wavelength 490 nm, emission wavelength 510 nm)
9. WARNINGS OR PRECAUTIONS

SAFETY PRECAUTIONS
The IFA test is for [IVD] use only.

1. Only qualified and well-trained employees should carry out the assay procedure.
2. The instruction for use describes the applicable test method. In case of modification or applications others than the intended use, or the use of automatic processors, the user has to validate the procedure and take the responsibility for it.
3. Do not mix lot specific reagents, such as SLIDES controls and CONJ|FITC from different kit lots. [BUF|PBS] (MM) [EVBL] and [SPE|DIL] can be used for all IFA tests.
4. Seal all bottles properly after use in order to avoid bacterial contamination. All samples and kit components should be considered potentially infectious. All control samples have been tested for Hepatitis Bs antigen, anti-HIV 1 and II, anti-HCV (CE/FDA) and found to be negative.
5. The coated SLIDES are inactivated. However, normal laboratory precautions should be maintained when handling with infectious material. Do not pipette by mouth.
6. Avoid contact with skin and mucous membranes when handling reagents, which contain preservatives (see kit contents). Wash thoroughly with water in case of contact.
7. Controls containing sodium azide may react with lead and copper plumbing, building up explosive metal acids. Flush with sufficient water when disposing of reagents.
8. For disposal the legal regulations have to be followed.

10. SPECIMEN COLLECTION AND STORAGE

1. Microbial contaminated specimen may cause interference.
2. Lipoaemic, haemolytic or icteric samples should only be tested with reservations although in our testing no negative influence has been found.
3. Suitable specimens are serum or plasma (heparinized, EDTA) samples.
4. The samples should not be heat-inactivated since non-specific results may occur.
5. Patient samples should be stored at 2-8°C. For long-term storage at -20°C or lower is recommended.
6. Avoid repeated freeze-thaw cycles.
7. Note: Diluted patient samples must be used on the same day.

11. REAGENT PREPARATION

Bring all reagents to room temperature prior to use!

Prepare [BUF|PBS]
Completely dissolve one vial of [BUF|PBS] in 1 litre of distilled water.
Reconstituted buffer solution should have a pH of 7.3 to 7.6.

Dilution of samples: Dilute patient sample with [SPE|DIL] according to test demands (screening titre).

For the IgM specific antibody determination an absorption of IgG / RF with IgG RF-Sorbent must be performed in order to avoid interferences with high IgG titres and rheumatoid factors (RF) (IFA Sorb [REF] ISB 100).
By using a Rheumatoid factor absorption it is necessary to regard the final serum dilution. Please follow the instruction for the use of the IgG-RF-Sorbent used in the test.

Controls are ready to use.

12. PIPTETTING AND INCUBATION STEPS

Screening Titre TOXO IgG: 1:50
Screening Titre TOXO IgM: 1:50
Screening Titre TOXO Screening: 1:50

Dilute samples with [SPE|DIL]

Note! Do not allow wells to dry at any time during the test procedure!

1. Take required SLIDES out of the foil packets shortly before use and identify slides using a permanent marking pen. Do not touch the wells.
2. First incubation:
   Pipette 1 drop of each control and 20 – 50 µl of each diluted sample onto the respective wells (covered completely) being careful not to touch cell substrate with pipette tip. The controls should be carried out for every test run. Place SLIDES into a well-closed moist chamber to prevent drying.
   Incubate SLIDES for the detection of
   - IgG- Antibodies: 30 minutes at room temperature
   - IgM- Antibodies: 45 minutes at room temperature
   - Screening- Antibodies: 45 minutes at room temperature
   - Controls are ready to use.

3. First wash step:
   Rinse the SLIDES gently with [BUF|PBS], using a squeeze wash bottle. Do not focus the buffer stream directly onto the wells. To prevent cross contaminations avoid rinsing from one well across other wells. For ten-well slides run PBS-stream from the midline of the slides successive along both rows to the edge of the SLIDES. Soak the SLIDES three times for 5 minutes with [BUF|PBS] in a coplin jar or staining dish with [BUF|PBS].

4. Second incubation:
   After the washing procedure, shake off excess [BUF|PBS] (if necessary use blotting paper to draw off excess fluid but do not touch the wells directly) and place SLIDES back into moist chamber. Immediately add 1 drop of CONJ|FITC to each well.

Incorporate SLIDES for the detection of
- IgG- Antibodies: 30 minutes at room temperature in the dark
- IgM- Antibodies: 30 minutes at room temperature in the dark
- Screening- Antibodies: 30 minutes at room temperature in the dark

5. Second wash step: see First wash step

6. Counter stain:
   Add 5 drops [EVBL] to 100 ml [BUF|PBS] solution in a staining dish, mix well and stain SLIDES for max. 5 minutes [EVBL] covers unspecific background fluorescence.

7. Remove SLIDES from the staining dish, rinse briefly with [BUF|PBS], shake off the excess [BUF|PBS] and apply 2-3 drops of [MM] across the slides. Gently lower the cover slip from the bottom to the top of the slides, avoid air bubbles.

9. Read SLIDES within 30 minutes at 400-800x total magnification, using a fluorescence microscope (FITC- filter combination). Avoid longer exposition of one field of vision to minimize bleaching of FITC fluorescence.

13. SUGGESTIONS FOR TROUBLESHOOTING

In case that the IFA instructions are followed strictly, the reagents are handled with care and the samples and reagents are pipetted carefully, the following kinds of errors can be avoided to a large extent.

<table>
<thead>
<tr>
<th>ERROR</th>
<th>POSSIBLE CAUSES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross contamination</td>
<td>Too much test material</td>
</tr>
<tr>
<td></td>
<td>Fluid remaining between the wells, should be carefully dried around outside edges if necessary</td>
</tr>
<tr>
<td>Too few cells or substrate</td>
<td>Cell lysis following prolonged contact with deionised water (observe the wash procedure)</td>
</tr>
<tr>
<td></td>
<td>Buffer squirited directly on the substrate in the well (observe the wash procedure)</td>
</tr>
<tr>
<td></td>
<td>Proteolytic enzymes have attacked the substrate</td>
</tr>
<tr>
<td></td>
<td>While placing the cover slip on SLIDES , the monolayer was disturbed</td>
</tr>
<tr>
<td>Inhomogeneous fluorescence</td>
<td>Sample dried in the well, fluorescence stronger at the edge (moist environment)</td>
</tr>
<tr>
<td></td>
<td>Sample does not cover the test well (air bubbles/ completely cover well with sample)</td>
</tr>
<tr>
<td></td>
<td>Buffer crystals on the SLIDES (wash)</td>
</tr>
<tr>
<td></td>
<td>Microscope incorrectly adjusted (check the adjustment of the microscope)</td>
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<tr>
<td></td>
<td>Unsuitable immersion oil</td>
</tr>
<tr>
<td>Unclear picture</td>
<td>Too much MM or air pockets</td>
</tr>
<tr>
<td></td>
<td>Microscope is dirty (cleaning)</td>
</tr>
<tr>
<td>Little or no fluorescence</td>
<td>Microbial contamination of the sample or CONJ</td>
</tr>
<tr>
<td></td>
<td>Microscope not adjusted</td>
</tr>
<tr>
<td></td>
<td>pH-value (7.3 – 7.6) of [BUF</td>
</tr>
<tr>
<td></td>
<td>CONJ</td>
</tr>
<tr>
<td>Background fluorescence</td>
<td>SLIDES dried in a hot air stream (do not use a hairdryer and do not let the wells dry)</td>
</tr>
<tr>
<td></td>
<td>Microbial contaminated specimen</td>
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<tr>
<td></td>
<td>Marking the SLIDES with a wax pencil produces a film on the SLIDES (use a waterproof glass marker)</td>
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</tbody>
</table>

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14. VALIDITY OF THE ASSAYS
All controls should be carried out with every test run. The fluorescence intensity of the [CONTROL] is shown in the Quality Control Certificate. If controls give invalid levels then results from test samples are invalid too and retesting is required.

15. READING OF THE RESULTS
The fluorescence intensity may be semi-quantified following our introduction:
3+ - 4+ = maximal fluorescence, brilliant yellow-green
2+ = less brilliant yellow-green fluorescence
1+ = definite but dull yellow-green fluorescence
Equivocal = (+) = very dim yellow-green fluorescence

The degree of intensity is not of clinically relevance and has only limited value as an indicator of titre. Differences in microscope optics, filters and light source may result in differences of +1 or more in intensity.

<table>
<thead>
<tr>
<th>Interpretation</th>
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</thead>
<tbody>
<tr>
<td><strong>TOXO</strong></td>
</tr>
<tr>
<td>IgG:</td>
</tr>
<tr>
<td>IgM:</td>
</tr>
<tr>
<td>Scr.:</td>
</tr>
</tbody>
</table>

**Fluorescence pattern of the Toxoplasma gondii**

<table>
<thead>
<tr>
<th>&gt;70%</th>
<th>Cell nucleus</th>
<th>Cell membrane</th>
<th>Whole cell</th>
<th>Pole</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>+</td>
<td>*</td>
<td>+</td>
<td>+</td>
<td>positive</td>
</tr>
<tr>
<td>IgM</td>
<td>+</td>
<td>*</td>
<td>+</td>
<td>+</td>
<td>positive</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>*</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>negative</td>
</tr>
</tbody>
</table>

Equivocal results found at the screening dilution of the samples should be retested. Following the confirmation of the equivocal result the monitoring of the patient’s antibodies is recommended in order to exclude unspecific reactions resp. cross-reactivity, which may also cause equivocal results.

**Note:** By the detection of IgG- antibodies unspecific cell fluorescence can occur caused through autoantibodies (ANA, AMA etc.). By the detection of IgM antibodies unspecific cell fluorescence can appear caused through non-immune fluorochromic proteins.

**A. SEMIQUANTITATIVE TITRE**

For the semi-quantification of the test results the [CONTROL] must be tested with every test run. The fluorescence intensity of the sample can then be compared visually with the fluorescence intensity of the [CONTROL].

**B. QUANTITATIVE TITRE**

For quantification of the test results, the samples must be diluted to the endpoint titre. The endpoint titre is determined as the last dilution in which an equivocal – (+) reaction is visible.

Sample titration:

If the fluorescence intensity of the sample is < the fluorescence intensity of the Positive control, please dilute the sample to the endpoint titre of the Positive control (the titre is reported on the label).

If the fluorescence intensity of the sample is > the fluorescence intensity of the Positive control, please dilute the sample at least two dilutions higher than the endpoint titre of the Positive control (the titre is reported on the label).

For further information please visit our website: [http://www.viro-immun.de/](http://www.viro-immun.de/)