Cross-reactivity

Cross-reactivity with Spirochaetes, particularly T. pallidum, can lead to false positive results with ELISA. Clarification of the antibody response, with a Luess specific test (e.g. TPHA and FTA-ABS) or a Western Blot, is advisable. Possible cross-reactivity may be avoided by using a spirochaete absorbent prior to testing. Fresh EBV infections may also give rise to a false positive Borrelia IgM titre. Thus an isolated positive IgM result should be checked against an EBV test.

5.


6.

KIT COMPONENTS

KIT COMPONENTS \( \varnothing 48 \text{ (kit small = S)} \)
contains half the amount of reagents as the regular test kit

1. MICROTITERSTRIPS
One microtiterplate [MTS] is supplied which contains 12 microtiterstrips [MTS] of 8 breakapart wells. The wells are coated with purified, inactive antigen. Strips are colour-coded.
12x [MTS] (EG 111 / EM 111), 5x [MTS]x12 (EG 111 B / EM 111 B)

2. PEROXIDASE CONJUGATE
Vial(s) containing ready-to-use anti-human IgG or IgM Peroxidase Conjugate.
One vial of 12 ml (EG 111 / EM 111), five vials of 12 ml (EG 111 B / EM 111 B).
Peroxidase conjugate contains 0.049% Thimerosal as preservative.

3. NEGATIVE CONTROL
One vial of 1.2 ml (EG 111 / EM 111) or one vial of 6 ml (EG 111 B / EM 111 B) containing human plasma with 0.095% sodium azide as preservative.
Ready to use.

4. CUT OFF CONTROL
One vial of 1.2 ml (EG 111 / EM 111) or one vial of 6 ml (EG 111 B / EM 111 B) containing human plasma with 0.095% sodium azide as preservative.
Ready to use.

5. POSITIVE CONTROL
One vial of 1.2 ml (EG 111 / EM 111) or one vial of 6 ml (EG 111 B / EM 111 B) containing human plasma with 0.095% sodium azide as preservative.
Ready to use. The titre is reported on the label.

6. TMB SUBSTRATE
One vial of 13 ml (EG 111 / EM 111) or one vial of 65 ml (EG 111 B / EM 111 B) containing ready-to-use tetra-methylbenzidine (TMB) substrate.

7. SAMPLE DILUENT
One bottle of 100 ml (2x50 ml) (EG 111 / EM 111) or one bottle of 500 ml (EG 111 B / EM 111 B) containing ready-to-use sample diluent buffer. The buffer includes 0.049% Thimerosal as preservative.

8. WASH SOLUTION 25X
One bottle of 80 ml (2x40 ml) (EG 111 / EM 111) or one bottle of 400 ml (EG 111 B / EM 111 B) containing wash solution concentrate.

9. STOP SOLUTION
One bottle of 15 ml (EG 111 / EM 111) or one bottle of 75 ml (EG 111 B / EM 111 B) containing 0.95N H\textsubscript{2}SO\textsubscript{4} stop solution. Ready-to-use.

10. PLASTIC BAG WITH DESICCANT
The safety data sheet (MSDS) is available upon request.

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.

After opening, MTS must be stored at 2-8°C in the plastic bag with desiccant and are stable up to 4 weeks. The diluted WASHBUF is stable up to 4 weeks when stored at 2-8°C. Use only MTS with an intact vacuum packaging.
8. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED
- Test tubes for sample dilution
- Timer
- Micropipettes, multipipettes 10-1000 µl
- One-liter graduated cylinder, distilled water
- ELISA washer or multichannel pipette
- Spectrophotometer for micro-plates (450 nm/ reference wavelength 630/620 nm)
- Paper towels, pipette tips

9. WARNINGS OR PRECAUTIONS
SAFETY PRECAUTIONS
The ELISA 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 lots of specific reagents, such as [MTS], controls and [CONJ|POD] from different kit lots. The [SUB|STM] doesn’t have to be from the original test kit, but the lot of the [SUB|STM] has to be the same as indicated on the kit label. The [SPE|DIL] (except Immunocapture assays), the [WASHBUF|25x] and the [SOLN|STOP] can be used for all ELISA 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 I and II, anti-HCV (CE/FDA) and found to be negative.
5. The [MTS] are coated with inactive antigen. However, normal laboratory precautions should be maintained when handling with infectious material.
6. Do not pipette by mouth.
7. Avoid contact with skin and mucous membranes when handling reagents, which contain preservatives (see kit contents). Wash thoroughly with water in case of contact and possibly look up a doctor.
8. Controls containing sodium azide may react with lead and copper plumbing, building up explosive metal acids. Flush with sufficient water when disposing of reagents.
9. The [SOLN|STOP] 0.95N H2SO4 contains sodium hydroxide which may irritate skin and mucous membranes.
10. For disposal the legal regulations have to be followed.

10. SPECIMEN COLLECTION AND STORAGE
1. Microbiologically contaminated specimens may cause interference.
2. Lipaemic, hemolytic 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 obtained by standard laboratory techniques.
4. The samples should not be heat-inactivated since non-specific results may occur.
5. For ethical reasons, the use of CSF samples was only evaluated by testing pairs of CSF and serum samples (only if available), distributed by the WHO Collaborating Centre for Quality Assurance and Standardization in Laboratory Medicine” in Germany. Therefore the use of CSF samples should be proved by testing a sufficient number of samples.
6. Patient samples should be stored at 1-8°C. For long term storage -20°C or lower is recommended. Avoid repeated freeze-thaw cycles.
7. Note: Diluted patient samples must be used on the same day.
8. For the IgM specific antibody determination absorption of IgG / RF with IgG-RF-Sorbent must be performed in order to avoid interferences with high IgG titres and rheumatoid factors (RF).

11. ASSAY PROCEDURE
REAGENT PREPARATION
Bring all reagents to room temperature prior to use!
[WASHBUF]: Dilute the [WASHBUF|25x] 1:25 with distilled water e.g. add 40ml of [WASHBUF|25x] to 960 ml distilled water and mix well.
Dilution of samples IgG: Dilute patient samples 1:101 with [SPE|DIL] e.g. 10µl sample = 1ml [SPE|DIL] mix thoroughly.
For the IgM specific antibody determination the final dilution of 1:101 must be considered when absorption with IgG-RF-Sorbent is performed. Please follow the instruction for the use of the IgG-RF-Sorbent used in the test.
Controls are ready to use.
Note: To take into consideration pipetting time, it is recommended that the [CUTOFF] is repeated after every 4 [MTS] resp. after a pipetting time of >=5 min.) to evaluate the following patient’s tests with the new calculated cut-off value. In case of a semiquantitative determination the [CONTROL] should also be repeated the same way the [CUTOFF] was dispensed.

Take the required [MTS] out of the foil packets and place them in the holder. Possibly remaining wells of a [MTS] have to be stored at 2-8°C tightly sealed in the plastic bag provided, with the desiccant inside.

12. PIPETTING AND INCUBATION STEPS
A. Pipette 100µl of the controls or diluted patient sample into the wells. Pipette 100 µl of sample diluted into well A1 (Blank).
B. Incubate the wells at room temperature (21-25°C) for 30 minutes, protected from intense light.
C. Wash the wells four times as described in section k. WASHING PROCEDURE.
D. Add 100µl of ready-to-use peroxidase conjugate to each well.
E. Incubate the wells at room temperature (21-25°C) for 30 minutes, protected from intense light.
F. Repeat washing as in section C above.
G. Add 100µl of ready-to-use TMB substrate to each well.
H. Incubate the wells at room temperature (21-25°C), in the dark for 10 minutes.
I. Add 100µl of stop solution to each well. Tap gently to ensure homogenous color distribution and read within 10 minutes.
J. To read the plate, make sure that the bottom is free from moisture and that no air bubbles are in the wells. Read the absorbance of the well contents at 450nm on a suitable plate reader. On readers equipped with a dual wavelength facility set the reference filter to 620/630 nm.

ATTENTION:
The absorbance (OD) of the Blank must be always substracted from the OD values of the controls and samples.

PROCEDURAL NOTES
Do not allow the wells to dry out between incubations. Comply with the given incubation temperatures and times.
k. WASHING PROCEDURE
The washing procedure can be done manually with a multichannel pipette or on an automatic plate washer. Empty the wells, invert and tap dry on paper towel. Wash four times with a soaking time of approx. 30 seconds (300 µl).

13. SUGGESTIONS FOR TROUBLESHOOTING
In case that the ELISA 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 extend.

<table>
<thead>
<tr>
<th>ERROR</th>
<th>POSSIBLE CAUSES</th>
</tr>
</thead>
<tbody>
<tr>
<td>No colourimetric reaction after addition TMB substrate</td>
<td>No Peroxidase conjugate pipetted, contamination of Peroxidase conjugate (possibly with control sera during pipetting) may cause an inactivation.</td>
</tr>
<tr>
<td>Generally too high reaction</td>
<td>Incorrect Peroxidase conjugate (i.e. not from original test kit), incubation time too long or incubation temperature too high, water quality for Washing Solution insufficient (low grade of deionization)</td>
</tr>
<tr>
<td>Generally too weak reaction</td>
<td>Incorrect Peroxidase conjugate (i.e. not from original test kit), incubation time too short, incubation temperature too low</td>
</tr>
<tr>
<td>Reagent blank too high</td>
<td>Incorrect pipetting of sample diluted, contaminated reagents, reagents expired, exceeding of incubation time and temperature, external contamination of the bottom of microtiterstrips, (clean carefully!)</td>
</tr>
<tr>
<td>False positive / negative samples</td>
<td>Incorrect dilution of samples, microbially contaminated specimen</td>
</tr>
<tr>
<td>Unexplainable outliers</td>
<td>Contamination of pipettes, tips or containers or with metals (iron, copper etc.), insufficient washing</td>
</tr>
<tr>
<td>High variation (within a series)</td>
<td>Reagents (including microtiterstrips) not pre-warmed to room temperature prior to use. Washer is not washing correctly!</td>
</tr>
<tr>
<td>High variation (from series to series)</td>
<td>Incubation conditions not constant (time, temperature) high variation of incubation temperature, controls and samples are not carried out at same time (same intervals) check pipetting order, person related variation, strips dried out after washing (unreproducible results)</td>
</tr>
</tbody>
</table>
14. VALIDITY OF THE ASSAY
All controls should be carried out with every test run. The test must comply with the following validation criteria:

- OD-value of Negative Control should be < 0.100.
- OD-value of Cut-off Control should be >0.200.
- Ratio of Positive Control: Cut-off value should be ≥ 1.5
- OD-value of the Blank should not be higher than 0.100.

If controls give invalid results then results from the test samples are invalid too and retesting is required.

15. CALCULATION OF RESULTS
A QUALITATIVE CALCULATION
Calculation of “Cut-off Value”
The Cut-off value is calculated from the absorbance of the Negative Control and the absorbance of the Cut-off Control and defines the Cut-off range.

\[ \text{Cut-off Value} = \text{OD of the Negative Control} + \text{OD of the Cut-off Control} \]

**CUT-OFF RANGE = CUT-OFF VALUE ± 10%**

Interpretation of sample results:

<table>
<thead>
<tr>
<th>RESULT</th>
<th>DEFINITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>negative [-]</td>
<td>OD value sample &lt; Cut-off value -10%</td>
</tr>
<tr>
<td>equivocal</td>
<td>OD value sample ≥ Cut-off value -10% and OD value sample ≤ Cut-off value +10%</td>
</tr>
<tr>
<td>positive [+</td>
<td>OD value sample &gt; Cut-off value +10%</td>
</tr>
</tbody>
</table>

Equivocal results 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.

B CALCULATION OF RATIO (CUT-OFF INDEX, COI):
Patient samples may also be quantified and interpreted by means of the calculation of the ratio (Cut-off Index, COI):

\[ \text{COI} = \frac{\text{OD value of sample}}{\text{Cut-off value}} \]

whereby a ratio of 1.000 is equivalent to the Cut-off value.

Interpretation of sample results:

- Ratio < 0.9 negative result
- Ratio 0.9-1.1 equivocal result
- Ratio > 1.1 positive result

C SEMI-QUANTITATIVE TITRE CALCULATION
A semi-quantitative diagram is enclosed. The first point on the curve is obtained from the Cut-off value (y-axis) and the cut-off titre 1:100 (x-axis). The second point of the curve is obtained from the absorbance of the Negative Control and their titre (x-axis) as indicated on the label. Drawing a straight line between the two points produces the semi-quantitative curve. The titre of the patient samples may be read from the curve. The graph is linear up to the titre of the Negative Control.

Samples with titres higher than the titre of the Negative Control should be diluted further with SPE/DIL according to the expected titre. For calculation of results, the dilution factor should be taken into consideration.

The calculated titres of the patient samples may also be indicated as IU (VIRO-Units), e.g. a titre of 1:250 is equivalent to 250 IU.

Regarding diagnostic relevance and interpretation of results see page 1.

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