

Test Instructions IMTEC-Yersinia-Antibody Screen (cut-off)

Enzyme Immunoassay for the Detection of IgG, IgM and IgA Antibodies against *Yersinia enterocolitica* (HRP-conjugate: anti-human Ig(GAM))

REF : TC 40060

Please read the instructions carefully before testing.

Procedural precautions:

- ▶ Do not use the reagents beyond the date of expiry.
- ▶ Never mix reagents from different lots.
- ▶ Store reagents at 2-8°C.

1. Clinical Use

Reactive arthritis is a joint disease that progresses to arthritis within a few days to weeks after infection. The clinically most significant forms of arthritis develop after infection of the gastrointestinal tract with yersinia, salmonella, shigella or campylobacter and after infection of the urogenital tract with chlamydia. Up to 40% of all cases of reactive arthritis, including a special form of Reiter's syndrome, are chronically progressive.

An early diagnosis based on a careful clinical case history and laboratory work-up is therefore essential.

Although HLA B27 antigen is detected in 70% of cases with proved Yersinia etiology, serological antibody detection is important. Yersinia enteritis is usually confirmed in stool, but the test often remains negative in case of post infectious complications.

The Widal agglutination test is most frequently used for diagnosis of Yersinia infection. However, this test is not suitable for use in patients with chronic infection or antigen persistence since it preferentially detects IgM antibodies typically appearing at the beginning of disease.

However the presence of IgG antibodies reflects a previous infection no longer active, and high titers of IgA antibodies indicate the persistence of antigens

12-16 months after infection with Yersinia enterocolitica 0:3 anti-Yersinia IgA antibodies are confirmed in 85% and anti-Yersinia IgG antibodies in 72% of cases with proved reactive arthritis as postinfectious complication.

This ELISA for Yersinia diagnosis is designed to detect IgG, IgM and IgA antibodies to LPS of Yersinia enterocolitica serotype 0:3 and is suitable for use in patients with antigen persistence.

2. Principle of the Test

The test is based on the absorptive immobilisation of LPS from Yersinia enterocolitica serotype 0:3 to the solid phase (polystyrene) of microtitre plates, and subsequent binding of the anti-Yersinia antibodies.

Bound antibodies are detected with a peroxidase-labeled secondary antibody directed to human IgG, IgM and IgA. After addition of substrate solution, a color develops. Its intensity is proportional to the concentration and/or the avidity of the detected anti-Yersinia antibodies

3. Materials Provided

MTP	1 plate
LPS antigen-coated microtiter strips (1 x 8), breakable, ready to use	á 12 strips
CONTROL co	1 vial
cut-off control serum (co), ready to use; contains sodium azide	2 mL
CONTROL -	1 vial
Negative control serum (NK), ready to use; contains sodium azide	1 mL
CONTROL +	1 vial
Positive control serum (PK), ready to use; contains sodium azide	1 mL
BUF WASH 10x	1 bottle
Washing buffer concentrate (10x)	50 mL
DIL SPE 5x	1 bottle
Sample dilution buffer concentrate (5x) containing sodium azide	22 mL
CONJ a(hum Ig(GAM)):HRP	1 bottle
Anti-human-Ig(GAM) HRP conjugate, ready to use	12 mL
SUBS TMB	1 bottle
TMB solution (HRP substrate), ready to use	12 mL
SOLN STOP	1 bottle
Stoping solution, ready to use (sulfuric acid, handle with care, corrosive!)	12 mL

4. Preparation of Reagents

Attention!

Allow the kit and all its components to reach room temperature completely before executing it!

Please do not use any polystyrene vessels for handling of HRP conjugates.

If the test is running automatically, it is recommended to use fresh conjugate each time. Please remove traces of old conjugate completely.

4.1. Preparation of Washing Buffer

If any salt has been crystallized inside the bottle, it must be resolved before use. Dilute 1 part washing buffer concentrate [BUF] [WASH] [10x] with 9 parts distilled water. The diluted buffer is stable for 6 weeks stored at 2 – 8 °C.

4.2. Preparation of Sample Buffer

If any salt has been crystallized inside the bottle, it must be resolved before use. Dilute 1 part sample buffer concentrate [DIL] [SPE] [5x] with 4 parts distilled water. The diluted buffer is stable for 6 weeks if stored at 2-8 °C.

4.3. Preparation of Sera

Allow the sera to reach room temperature (30 min). Dilute sera 1:100 with sample buffer (10 µL sample to 1 mL buffer).

4.4. Stopping Solution, Standards, Control Sera, HRP-Conjugate and TMB Solution

Stopping solution, standards, control sera, HRP-conjugate and TMB solution are ready to use. Used bottles should be closed carefully and stored at 4-8°C. **Store TMB solution also protected from light.**

4.5. Microtiter Strips

The strips are ready to use. Unused strips should be sealed and stored in the lockable original bag at 2-8°C.

5. Test Procedure

- **Pipette 100 µL serum dilution** or control sera ([CONTROL] [+], [CONTROL] [co] + [CONTROL] [-]), into each well, for blanks use sample buffer instead of serum dilution, seal wells with adhesive foil.
- **Incubate for 1 hour** at room temperature (RT).
- **Rinse the wells 3 x** using at least 200 µL washing buffer per well.
- **Pipette 100 µL HRP-conjugate** [CONJ] [a(hum Ig(GAM)):HRP] into each well, seal wells with adhesive foil.
- **Incubate for 30 minutes** at RT.
- **Rinse the wells 3 x** using at least 200 µL washing buffer per well.
- **Pipette 100 µL TMB solution** [SUBS] [TMB] into each well.
- **Incubate for 10 min** at RT in the dark. At room temperatures above 25 °C the substrate incubation could be shortened, but should never fall short of 5 minutes.

- **Pipette 100 µL stopping solution** [SOLN] [STOP] per well.
- **Measure at 450 nm** within the next 30 min after stopping.

6. Interpretation of Results

To prove the functionality of the test, the absorbance of the positive control has to be distinctly higher than the absorbance of the cut-off control. The absorbance of the negative control serum has to be lower than the cut-off value of the test.

A patient serum with a measured absorbance that is higher than the absorbance of the cut-off control possesses an enhanced level of specific antibodies (positive).

Precautions

For in vitro diagnostic use only. [IVD]

The human Control Sera and Standards in this kit have been prepared from blood donations which have been tested for Hepatitis B Surface Antigen, anti-HCV- and anti-HIV 1/2 antibodies and shown to be NEGATIVE.

However, as no known test can guarantee the absence of an infectious virus, all reagents and samples must be handled carefully and disposed of in accordance with local legislation.



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