

# Test Instructions

## IMTEC-Anti-Salmonella-Antibodies Screen (cut-off)

### Enzyme Immunoassay for the Detection of Anti-Salmonella-Antibodies (IgG, IgM and IgA)

REF : TC 40040

Please read the instructions carefully before testing.

**Procedural precautions:**

- ▶ Do not use the reagents beyond the date of expiry.
- ▶ Never mix reagents from different test kits nor 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 urogenital tract with chlamydia and after infection of the gastrointestinal tract with salmonella, yersinia, shigella or campylobacter. 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 60-80% of cases, serological antibody detection is also important because it is not yet possible to cultivate salmonella from synovial fluid.

The Widal agglutination test is most frequently used for diagnosis of salmonella infection. However, this test is not suitable for use in patients with chronic infection or antigen persistence since it preferentially detects IgM antibodies. The presence of IgG antibodies reflects a previous infection no longer active, and high titers of IgA antibodies indicate the persistence of antigens in the intestine or joint.

This ELISA for salmonella diagnosis is therefore designed to detect all three immunoglobulin classes - IgG, IgM and IgA. The ELISA is based on the binding of antibodies to salmonella lipopolysaccharide. The sensitivity of IMTEC's ELISA is greatly superior to that of the Widal test. For example, the Widal test detected only 38.5% of 130 patients with salmonella infections whereas 88.5% were detected by ELISA.

#### 2. Principle of the Test

The test is based on the absorptive immobilisation of LPS from *Salmonella typhimurium* and *S. enteritidis* to the solid phase (polystyrene) of microtitre plates, and subsequent binding of the anti-Salmonella antibodies. For the detection of the anti-Salmonella antibodies bound to the microtiter plate a horseradish peroxidase conjugated anti-human-IgG-, IgM-, IgA-antibody, is used. After addition of a peroxidase substrate solution, a color stain develops, the intensity of it is proportional to the concentration of the anti-Salmonella antibodies.

#### 3. Materials Provided

<b>MTP</b>	1 plate
LPS antigen-coated microtiter strips (1 x 8), breakable, ready to use	à 12 strips
<b>CONTROL co</b>	1 vial
cut-off control serum (co), ready to use; contains sodium azide	2 mL
<b>CONTROL -</b>	1 vial
Negative control serum (NK), ready to use; contains sodium azide	1 mL
<b>CONTROL +</b>	1 vial
Positive control serum (PK), ready to use; contains sodium azide	1 mL
<b>BUF WASH 10x</b>	1 bottle
Washing buffer concentrate (10x)	50 mL
<b>DIL SPE 5x</b>	1 bottle
Sample dilution buffer concentrate (5x) containing sodium azide	22 mL
<b>CONJ a(hum IgGAM):HRP</b>	1 bottle
Anti-human-IgG, -IgA, -IgM HRP conjugate, ready to use	12 mL
<b>SUBS TMB</b>	1 bottle
TMB solution (HRP substrate), ready to use	12 mL
<b>SOLN STOP</b>	1 bottle
Stopping 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 stored at 2-8°C.

### 4.3. HRP Conjugate, Stopping Solution, Control Sera and Substrate Solution

HRP Conjugate, stopping solution, control sera and substrate solution are ready to use. Used bottles should be closed carefully and stored at 2-8°C. **Store substrate solution also protected from light.**

### 4.4. 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).

### 4.5. Microtiter Strips

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

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## 5. Test Procedure

- **Pipette 100 µl serum dilution** or control sera [CONTROL] [+], [CONTROL] [co] and [CONTROL] [-] into each well, for blank 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 IgGAM):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 serum [CONTROL] [+] has to be distinctly higher than the absorbance of the cut-off control [CONTROL] [co]. The absorbance of the negative control [CONTROL] [-] has to be lower than the cut-off control [CONTROL] [co]. A patient serum with a measured absorbance that is higher than the absorbance of cut-off control [CONTROL] [co] 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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