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## ListeriaTEST

### Human Anti-LLO IgG Immunoassay - IVD

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#### Intended use

Human Anti-LLO IgG Immunoassay – IVD is an Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of anti-LLO IgG in human serum and plasma, as an aid in the diagnosis of listerial infection and to screen people for *L. monocytogenes* exposure.

#### Introduction

*Listeria monocytogenes* is a facultative intracellular Gram-positive food-borne bacterium, increasingly recognized as being responsible for severe infections in both animals and humans. Ingestion of contaminated food causes an infection, named *listeriosis*, which affects especially immunocompromised patients, new-borns and pregnant women and is characterized by a variety of severe syndromes, such as encephalitis, meningoenzephalitis, septicemia and abortion.

Listeriolysin O (LLO), a major virulence factor produced by all pathogenic strains of *L. monocytogenes*, has been identified as a candidate antigen for a serological assay. Antibodies to LLO have been already detected in the serum of goats, sheep, lambs, cows and humans by western blot, dot blot or ELISA analysis.

Detection of anti-LLO antibodies in humans has been proved to be particularly useful for listeriosis diagnosis especially when bacteria cannot be isolated from clinical specimens, owing to the intermittent presence in blood or the inaccessible foci of bacterial replication.

The presentation of listeriosis during pregnancy includes mild flu like symptoms, backache, vomiting/diarrhoea, muscle pains and sore throat. Some women may be asymptomatic. Infection with *Listeria* does not confer any immunity.

#### Principle of the assay

96-well plate with 8-well strips on single well holding frame coated with LLO are incubated with collected samples.

During this incubation step, anti-LLO antibodies bind to the antigen forming specific complexes. Antibody excess is removed by washing and the antigen-antibody complex in each well is detected by adding an anti-human IgG HRP-conjugated antibody. Detection is performed by incubating the strips with 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) as chromogen and reading the absorbance values with a microplate reader at  $410 \pm 10\text{nm}$ .

The kit allows 96 reactions; duplicates for each sample or control are strongly recommended.

The kit does not show cross reactivity with interfering antibodies present usually in the sera of pregnant women.

It has been demonstrated that specific anti-listeriolysin O antibodies appear as early as day 8 after an oral infection and peaked by days 16-32 of infection; nevertheless, it should be noted that they may persist for months after an infection has resolved.

Moreover, antibodies to LLO are mostly constituted by immunoglobulin G (IgG), since as IgA response has never been detected and only a transient and inconstant IgM response has been observed.

#### Limitations of the procedure

- The presence of anti-listeriolysin O antibodies does not discriminate between current or prior infection.
- No drugs have been investigated for assay interference.
- Any variation in specimen diluent, operator, pipetting technique, washing technique, incubation time or temperature can cause variation in binding.
- The values obtained from this assay are intended to be an aid for diagnosis only. Each physician must interpret the results in conjunction with the patient's history, physical findings and other diagnostic procedures.

#### Kit contents

- **96-well plate (12 x 8 well strips on single well holding frame) STRIPS-Ag**: 1 microplate coated with LLO recombinant protein (Accession number: AF253320.1), with preservative
- **Samples Diluent DIL**: 2 x 50ml ready to use, with preservative
- **10X Wash Buffer concentrate 10XWASH**: 3 x 100ml, to dilute to 1X with distilled water; with preservative
- **Conjugated antibody AB-HRP**: 18ml ready to use, goat anti-human IgG-HRP conjugated
- **ABTS Solution ABTS**: 23ml ready to use
- **STOP Solution STOP**: 13ml ready to use
- **Negative control CONTROL-**: 2 x 1300µl ready to use, with preservative
- **Positive control CONTROL+**: 2 x 1300µl ready to use, with preservative
- **Calibrator CALIBRATOR**: 2 x 1300µl ready to use, with preservative
- **Instruction for Use**

#### Other Supplies Required:

- Microplate reader equipped with  $410 \pm 10\text{nm}$  filter (maximum at  $420\text{nm}$ ).
- Precision pipettes and pipette tips.
- Deionized or distilled water.
- 1000 ml graduated cylinder for preparation of Wash Buffer.
- Vortex mixer.
- Multi-channel pipette, manifold dispenser, or automated microplate washer.
- Latex gloves and other personal protective equipments.
- Glass tubes or plastic tubes (low protein retention).

#### **Storage**

The opened reagents are stable up to the expiry date stated on the label when stored between  $+2$  and  $+8^\circ\text{C}$ .

#### **Precautions**

- Do not interchange components between different kits.
- Check buffers for precipitate and re-dissolve at room temperature by mixing before use.
- Avoid cross-contamination between serum specimens.
- Do not use the kit after the expiration date.
- Treat all samples as potentially infectious.

#### **Procedure**

##### **SAMPLE COLLECTION**

Serum – Collect blood in Serum-Use pyrogen/endotoxin free collecting tubes. After blood clotting centrifuge it at approximately  $1\ 000 \times g$  for 10 min and separate serum from the red cells.

Plasma - Collect blood in Serum-Use pyrogen/endotoxin free collecting tubes preferentially in EDTA (avoid heparin) and centrifuge it at  $1\ 000 \times g$  for 10 min. Remove plasma rapidly and carefully.

Storage – Samples can be stored at  $2-8^\circ\text{C}$  for up to 24 hours after collection. For longer periods samples should be stored frozen. Avoid freezing and thawing cycles.

Recommendation - Before use thaw completely samples at room temperature. Vortex the samples before testing.

##### **REAGENT PREPARATION**

Prepare 1X Wash Buffer by diluting the provided **10X Wash Buffer Concentrate** with an appropriate volume of distilled water. Eg.: add 900ml distilled water to 100ml of **10X Wash Buffer Concentrate**.

In case of salt crystallization in the **10X Wash Buffer Concentrate** and/or in the **STOP Solution** dissolve the crystals by mixing and careful warming up to  $37^\circ\text{C}$  in a water bath. Diluted Wash Buffer is stable for one month at room temperature and for several months if stored at  $+4 \pm 2^\circ\text{C}$ .

**10X Wash Buffer Concentrate** and **STOP Solution** may be stored at room temperature ( $18-25^\circ\text{C}$ ) to avoid salt crystallization.

Please note that **Positive Control**, **Negative Control** and **Calibrator** are provided ready to use. Mix well in order to ensure homogeneity.

##### **ASSAY METHOD**

- 1 Bring all reagents and the required number of wells to room temperature immediately before use and mix well. Unnecessary wells can be simply removed from the tray and stored between  $+2$  and  $+8^\circ\text{C}$  in the provided sealable bag with desiccant.
- 2 Dilute sera/plasma samples **1:100** (v/v) in separated marked glass vials with **Samples Diluent** and mix thoroughly (es.  $5\mu\text{l}$  of sample +  $495\mu\text{l}$  of **Samples Diluent**). Do not dilute positive, negative controls and calibrator.
- 3 Dispense  $100\mu\text{l}$ /well of provided **Positive Control**, **Negative Control** and **Calibrator**. Dispense  $100\mu\text{l}$ /well of **Samples Diluent** as *No Sample Control* (Background).
- 4 Dispense diluted samples in duplicates.  
Two replicates are strictly recommended for samples, Calibrator and Background.
- 5 Cover the microtiter strips and incubate at  $37^\circ\text{C}$  for  $60 (\pm 5)$  minutes.
- 6 Wash the microtiter strips five times with 1X wash buffer.
- 7 Dispense  $100\mu\text{l}$  of provided **Conjugated Antibody** in each well, including *No Sample Control* wells.
- 8 Cover the microtiter strips and incubate at  $37^\circ\text{C}$  for  $60 (\pm 5)$  minutes.
- 9 Wash the microtiter strips five times with 1X wash buffer.
- 10 Add  $100\mu\text{l}$  of provided **ABTS Solution** to each well, including *No Sample Control* wells.
- 11 Cover the microtiter strips and incubate at room temperature for **20 minutes**.
- 12 Stop the reaction by adding  $50\mu\text{l}$ /well of the provided **STOP Solution**. Use the same pipetting order as with the ABTS Solution to ensure the same reaction time in all the wells. Visually ensure that no bubbles are remaining in the wells. Cover gently and maintain in the dark until you are ready for reading the plate.
- 13 Read the absorbance at  $410 \pm 10\text{nm}$  using a microplate reader within 30 minutes.

## Quality Control

Calculate the mean absorbance values for positive and negative controls and for background. To ensure that the ELISA test has worked properly, the following criteria must be met:

Controls	Acceptability ranges (Abs)
Positive control	≥ 1.2
Negative control	≤ 0.35
Background	≤ 0.12

If these criteria are not met, the test is not valid and must be repeated.

**NOTE:** If the test results not valid, check the procedures for the preparation of reagents, the kit expiration date, the conditions of its storage and the efficiency of the washing and reading systems, before repeating the assay.

## Data analysis

### CUT-OFF CALCULATION

A cut-off value has been determined by the manufacturer and correlated to the CALIBRATOR. The correction factor (CF) will allow you to determine the cut-off value and to correct for slight day-to-day variations in test results.

Calculate the cut-off value by multiplying the CALIBRATOR mean value by the CORRECTION FACTOR. The correction factor may vary lot-to-lot and is indicated on the top of the first page of the product information provided with the kit.

*Example of cut-off calculation:*

CALIBRATION Absorbance values: 0.512; 0.498

CALIBRATION Mean: 0.505

Correction Factor (provided): 0.950

**Cut-off value:**  $0.505 \times 0.950 = 0.48$

### CALCULATION OF RESULTS

Calculate the mean absorbance values for all duplicates and obtain the INDEX value for each analyzed sample as follows:

$$\text{Sample mean absorbance value} / \text{Cut-off value} = \text{INDEX}$$

*Example of result calculation:*

Sample #1 absorbance values: 0.355; 0.325

Sample #1 Mean: 0.34

Cut-off value: 0.48

**Sample #1 INDEX** =  $0.34/0.48 = 0.71$

**NOTE:** absorbance values obtained from duplicate tests should fall within 20% of the mean of the two values. Samples whose results are outside of this limit should be re-tested.

### INTERPRETATION OF RESULTS

Consider as negative samples with INDEX lower than 0.85 and as positive samples with INDEX higher than 1.15.

Specimens with INDEX comprised between 0.85 and 1.15 fall into the equivocal range. It's suggested to repeat the test after 15 days on a fresh serum sample; if the result of the second sample becomes positive, the individual may be infected with *Listeria monocytogenes*.

The final results have to be interpreted as follow:

INDEX	RESULT	INTERPRETATION
≤ 0.85	<b>Negative</b>	No antibodies to listeriolysin O are detected by the ELISA test. Such individuals are presumed to be uninfected with <i>Listeria monocytogenes</i> .
0.86 – 1.14	<b>Equivocal</b>	Antibodies against the antigen could not be clearly detected. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as <b>negative</b> .
≥ 1.15	<b>Positive*</b>	Antibodies to listeriolysin O are present in the sample. There has been a contact with the antigen and the individual may be infected with <i>Listeria monocytogenes</i> .

\* Positive observed index range from 1.15 and 5.20.

**Performance characteristics****Intra-assay precision**

Intra-assay precision was determined using 4 different serum samples. Each sample has been tested for 24 times in one test run. The calculated coefficients of variation (CV) of the samples were < 20%.

**Inter-assay precision**

Inter-assay precision was determined by testing serum samples from four pregnant women in 10 independent test runs. The calculated CVs for each samples were shown in the table below:

<b>Intra-assay variation, n=24x4</b>		
<b>SERUM</b>	<b>MEAN INDEX</b>	<b>CV%</b>
<b>1</b>	0,81	10%
<b>2</b>	1,1	19%
<b>3</b>	1,93	8%
<b>4</b>	2,06	10%

<b>Inter-assay variation, n=5x4</b>		
<b>SERUM</b>	<b>MEAN INDEX</b>	<b>CV%</b>
<b>G4</b>	0,73	10%
<b>G6</b>	0,71	13%
<b>G10</b>	0,27	15%
<b>GV</b>	0,59	8%

**Diagnostic specificity and diagnostic sensitivity**

Specificity and sensitivity of the test have been evaluated by comparing "Human anti-LLO IgG Immunoassay" with the reference culture method starting from blood samples.

The specificity of the test is 100% and the sensitivity is 86%.

NOTE: These results refer to the group of samples investigated; these are not guaranteed specifications.

<b><i>Listeria monocytogenes</i> status</b>	<b>Negative</b>	<b>Equivocal</b>	<b>Positive</b>	<b>Sum</b>
<b>Seronegative</b>	22	0	0	22
<b>Seropositive</b>	9	13	56	78

**Anti-listeriolysin O antibodies prevalence (Reference range)**

Levels of anti-listeriolysin O antibodies were analysed in a group of 133 healthy blood donors constituted by 64 males and 69 females and in 86 pregnant healthy women. 11% of the samples resulted positive for the presence of antibodies anti-LLO.

**References**

Available on library section: <http://www.diatheva.com/library.htm>