ENZYME IMMUNOASSAYS AND AGGLUTINATION FOR THE DIAGNOSIS OF PERTUSSIS AND PARAPERTUSSIS

Bordetella pertussis
Bordetella parapertussis

ELISA and IMMUNOBLOT kits are optimized and validated for detection of IgA, IgG and IgM antibodies in human serum and plasma.

Components for agglutination are optimized and validated for detection of all immunoglobulin classes in human serum.
B. pertussis and B. parapertussis are very closely related species, producing similar virulence factors.

*Bordetella pertussis* is considered to be the main cause of whooping cough. Before a vaccination campaign was launched, the disease had been one of the most serious diseases of infants and children.

The common symptoms of pertussis are a paroxysmal cough, inspiratory whoop, and fainting and/or vomiting after coughing. The cough from pertussis has been documented to cause subconjunctival hemorrhages, rib fractures, urinary incontinence, hernias, post-cough fainting, and vertebral artery dissection.

The incubation period is typically seven to ten days and rarely may be as long as 42 days, after which there are usually mild respiratory symptoms, mild coughing, sneezing, or runny nose. This is known as the catarrhal stage. After one to two weeks, the coughing usually develops into uncontrolled fits, each with five to ten forceful coughs, followed by a high-pitched “whoop” sound in younger children, or a gasping sound in older children, as the patient struggles to breathe in afterwards (paroxysmal stage).

Whooping cough does not induce lifelong immunity. Antibodies against pertussis toxin, filamentous hemagglutinin and fimbrial antigen can be detected in serum.

*B. parapertussis* causes milder forms of the disease. This is due to the fact that the bacteria do not produce the pertussis toxin. The infection of *B. parapertussis* can be the main cause of prolonged bronchitis.

Postvaccination immunity and immunity following *B. pertussis* infection do not protect from the disease caused by *B. parapertussis*. 

*Introduction*
Diagnosis of the disease is based on a clinical picture and laboratory tests (cultivation, PCR and serological methods - detection of IgM, IgA and IgG antibodies in the serum).

IgM antibodies are detected first; they have short half-life and endure for 2-3 months. IgA antibodies can be determined after 1-2 weeks and may persist for 6-24 months depending on age. IgG antibodies are found as early as 2-3 weeks after the onset of the disease and reach their maximum after 6-8 weeks. They can be detected until adulthood and may persist for several years.

In children, IgA antibodies are produced more slowly – they reach detectable levels 6-7 weeks after infection in infants. The detection of specific IgM antibodies is suitable for diagnosis of the acute disease in younger children while specific IgA antibodies show better diagnostic potential in older children.

IgA antibodies are not produced after vaccination. Therefore presence of IgA antibodies may be a suitable marker to distinguish between natural infection and postvaccination reaction. Natural B. pertussis infection leads to increase of IgA antibodies whereas immunization increases mainly IgM and IgG antibodies.

Serological findings should be interpreted in the context of clinical picture, epidemiologic and vaccination data and available results of other laboratory tests.
**Test Characteristics**

<table>
<thead>
<tr>
<th>ELISA</th>
<th>Diagnostic Sensitivity</th>
<th>Diagnostic Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>EIA Bordetella pertussis Toxin IgA</td>
<td>95.8%</td>
<td>99.9%</td>
</tr>
<tr>
<td>EIA Bordetella pertussis Toxin IgG</td>
<td>97.1%</td>
<td>99.9%</td>
</tr>
<tr>
<td>EIA Bordetella pertussis Toxin IgM</td>
<td>90.5%</td>
<td>92.0%</td>
</tr>
<tr>
<td>EIA Bordetella parapertussis IgA</td>
<td>93.3%</td>
<td>99.9%</td>
</tr>
<tr>
<td>EIA Bordetella parapertussis IgG</td>
<td>96.7%</td>
<td>99.9%</td>
</tr>
<tr>
<td>EIA Bordetella parapertussis IgM</td>
<td>68.8%</td>
<td>99.9%</td>
</tr>
</tbody>
</table>

**Test Principle**

**Antigens**

- **EIA Bordetella pertussis**
  - Highly purified *Bordetella pertussis* toxin
- **EIA Bordetella parapertussis**
  - Mixture of specific antigens for *Bordetella parapertussis*

**Clinical Application**

- Screening test for the detection of specific IgA, IgG and IgM antibodies in human serum or plasma
- Detection of postinfection and postvaccination antibodies
- Disease stage diagnosis

**User Comfort and Advantages**

- Ready-to-use components
- Colour-coded, interchangeable components
- Breakable colour-coded microplate strips
- Calibrators and Controls
- Quantitative evaluation of results (U/ml) - *B. pertussis*
- Semiquantitative evaluation of results (IP) - *B. parapertussis*
- Standardization according to WHO International Standard Pertussis Antiserum 06/140 and 06/142
- High reproducibility and dynamics of antibody response
- Identical assay procedure, ready for automation
- Short total assay time

**Summary of EIA Protocol**

<table>
<thead>
<tr>
<th>Step No.</th>
<th>Test steps</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dilute samples serum/plasma 1:101 (10 μl + 1 ml)</td>
</tr>
<tr>
<td>2</td>
<td>Pipette Controls (Calibrators) and diluted samples – 100 μl Blank = empty well</td>
</tr>
<tr>
<td>3</td>
<td>Incubate at 37°C for 30 min</td>
</tr>
<tr>
<td>4</td>
<td>Aspirate and wash the wells 5x</td>
</tr>
<tr>
<td>5</td>
<td>Pipette Conjugate – 100 μl Blank = empty well</td>
</tr>
<tr>
<td>6</td>
<td>Incubate at 37°C for 30 min</td>
</tr>
<tr>
<td>7</td>
<td>Aspirate and wash the wells 5x</td>
</tr>
<tr>
<td>8</td>
<td>Pipette Substrate (TMB-Complete) – 100 μl Including blank</td>
</tr>
<tr>
<td>9</td>
<td>Incubate at 37°C for 30 min</td>
</tr>
<tr>
<td>10</td>
<td>Pipette Stop Solution – 100 μl Including blank</td>
</tr>
<tr>
<td>11</td>
<td>Read colour intensity at 450 nm</td>
</tr>
</tbody>
</table>
Recombinant antigens are transferred to a nitrocellulose membrane using a micro-dispensing method.

**Clinical Application**
- Method for differentiation of postinfection and postvaccination antibodies
- Method for proof of acute infection
- Method for differential diagnostics of *B. pertussis* and *B. parapertussis*
- Method for detailed determination of the presence of anti-*Bordetella* specific antibodies
- Method for confirmation of ELISA and/or agglutination tests

**Test Principle**
Recombinant antigens are transferred to a nitrocellulose membrane using a micro-dispensing method.

**Antigens**

- **B. pertussis**
  - PT – Pertussis toxin (45 kDa) – basic virulence factor, specific only for *B. pertussis*; the most important pertussis antigen
  - FHA – *B. pertussis* filamentous hemagglutinin – adhesive protein, important immunogen; selected part of the sequence with high specificity
  - ACT – Adenylate cyclase toxin (CyaA) – important virulence factor of *B. pertussis*; antiphagocytic factor during infection
  - TCF – Tracheal colonization factor – protein produced only by *B. pertussis* strain, not by *B. parapertussis*; protein adhesin, that binds to ciliated epithelial cells of respiratory tract

- **B. parapertussis**
  - Pertactin – Outer membrane protein (75 kDa) of virulent *B. parapertussis* strains
  - FimN – Fimbriae N – protein adhesin; it is not produced by *B. pertussis*
  - EntA – Entericidin A – membrane lipoprotein

**Test Characteristics**

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Diagnostic Sensitivity</th>
<th>Diagnostic Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bordetella pertussis IgA</td>
<td>95.45%</td>
<td>95.59%</td>
</tr>
<tr>
<td>Bordetella pertussis IgG</td>
<td>99.00%</td>
<td>95.56%</td>
</tr>
<tr>
<td>Bordetella parapertussis IgA</td>
<td>99.00%</td>
<td>87.10%</td>
</tr>
<tr>
<td>Bordetella parapertussis IgG</td>
<td>88.89%</td>
<td>96.36%</td>
</tr>
</tbody>
</table>

**User Comfort**
- Ready-to-use components
- Colour-coded strips
- Positive and Negative controls
- Control of reaction course and Conjugate control are present on the strip
- Interchangeable components
- Easy assay procedure
**Interpretation results**

**Interpretation of BLOT-LINE B. pertussis/B. parapertussis results**

<table>
<thead>
<tr>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Presence of IgA, IgG or IgM antibodies – recent or current natural infection</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>–</td>
<td>Presence of IgG and IgM antibodies in the absence of IgA antibodies – state after recent vaccination (B. pertussis) or an early infection stage without IgA antibodies production</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>+</td>
<td>Presence of IgA antibodies or parallel presence of IgM antibodies – early infection stage</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>–</td>
<td>Presence only of IgM antibodies – early infection stage</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>+</td>
<td>Presence only of IgG antibodies – recent infection or postvaccination state (B. pertussis)</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>–</td>
<td>No presence of anti B. pertussis or anti B. parapertussis antibodies – in the case of a suspected infection it is recommended to test a new sample in 2-3 weeks</td>
</tr>
</tbody>
</table>

**Summary Protocol**

**Step No.** | **Test steps**
--- | ---
1 | Pipette Universal solution 2.5 ml
2 | Strips soaking 10 min. at room temperature
   | Shaker
3 | Aspirate
4 | Dilute samples
   | serum/plasma (30 µl + 1.5 ml)
5 | Pipette Controls and diluted samples 1.5 ml
6 | Incubate 30 min. at room temperature
   | Shaker
7 | Aspirate samples and wash strips with 1.5 ml
   | of Universal solution 3-times for 5 min.
   | Shaker
8 | Pipette Conjugate 1.5 ml
9 | Incubate 30 min. at room temperature
   | Shaker
10 | Aspirate Conjugate and wash strips with 1.5 ml
   | of Universal solution 3-times for 5 min.
   | Shaker
11 | Pipette Substrate solution (BCIP/NBT) 1.5 ml
12 | Incubate 15 min. at room temperature
   | Shaker
13 | Aspirate Substrate solution and wash strips with
   | 2 ml of distilled water 2-times for 5 min.
   | Shaker
14 | Sticking and evaluation of strips
Diagnosis of infection using determination of antibodies in the paired samples

Test Principle

Agglutination is a process which is based on the reaction of agglutinogens (antigens) and agglutinins (antibodies) giving rise to visible clumped masses – agglutinates. The test detects antibodies of all immunoglobulin classes (a single class, e.g. IgM, cannot be identified).

Clinical Application

Step No. | Test steps
--- | ---
1 | Pipette 50 μl of saline solution
2 | Add 50 μl of tested sera in column 1 (wells A1 to G1) to obtain a 1:2 dilution
3 | Transfer 50 μl of the sera from column 1 to column 2 using an 8-channel pipette to obtain a 1:4 dilution
4 | Mix the contents of the wells
5 | Continue analogously up to the 1:4096 dilution in column 12
6 | Take 50 μl of the solution from each well in column 12
7 | Add 50 μl of Bordetella parapertussis-AR-Agglutinogen or Bordetella pertussis-AR-Agglutinogen to all wells of the plate at the working dilution
8 | Shake the microplate on the shaker for 15 seconds
9 | Incubate in the humid chamber at 37°C for 2.5 hours
10 | Incubate at room temperature for 18 to 20 hours
11 | Evaluate the results

Advantages

- Easy procedure
- Cheap serological test
- Individual test for B. parapertussis

Clinical Data

Laboratory detection of acute infection in a group of patients with clinical diagnosis of pertussis (n= 25 paired samples)
# Ordering Information

## ELISA

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Product</th>
<th>No. of Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>BpAT96</td>
<td>EIA Bordetella pertussis Toxin IgA</td>
<td>96</td>
</tr>
<tr>
<td>BpGT96</td>
<td>EIA Bordetella pertussis Toxin IgG</td>
<td>96</td>
</tr>
<tr>
<td>BpMT96</td>
<td>EIA Bordetella pertussis Toxin IgM</td>
<td>96</td>
</tr>
<tr>
<td>BppA96</td>
<td>EIA Bordetella parapertussis IgA</td>
<td>96</td>
</tr>
<tr>
<td>BppG96</td>
<td>EIA Bordetella parapertussis IgG</td>
<td>96</td>
</tr>
<tr>
<td>BppM96</td>
<td>EIA Bordetella parapertussis IgM</td>
<td>96</td>
</tr>
</tbody>
</table>

## IMMUNOBLOT

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Product</th>
<th>No. of Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>BpAL20</td>
<td>BLOT-LINE Bordetella IgA</td>
<td>20</td>
</tr>
<tr>
<td>BpGL20</td>
<td>BLOT-LINE Bordetella IgG</td>
<td>20</td>
</tr>
<tr>
<td>BpML20</td>
<td>BLOT-LINE Bordetella IgM</td>
<td>20</td>
</tr>
<tr>
<td>Swim03</td>
<td>Immunoblot Software</td>
<td>1 pc</td>
</tr>
</tbody>
</table>

## AGGLUTINATION

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Product</th>
<th>No. of Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>BpeAg5</td>
<td>Bordetella pertussis – AR – Aglutinogen</td>
<td>5 ml</td>
</tr>
<tr>
<td>BppAg5</td>
<td>Bordetella parapertussis – AR – Aglutinogen</td>
<td>5 ml</td>
</tr>
<tr>
<td>BpeP01</td>
<td>Bordetella pertussis – AR – Positive Control lyophil.</td>
<td>1 ml</td>
</tr>
<tr>
<td>BppP01</td>
<td>Bordetella parapertussis – AR – Positive Control lyophil.</td>
<td>1 ml</td>
</tr>
</tbody>
</table>

## Contact

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E-mail: trade@testlinecd.com

www.testlinecd.com

Company is certified to the quality management system standards ISO 9001 and ISO 13485 for in vitro diagnostics.