Application

*Bordetella pertussis* IgG-PT ELISA Kit is a quantitative test for the detection of IgG antibodies against pertussis toxin in human serum samples.

Background

Pertussis (whooping cough) is a highly infectious respiratory disease caused by the bacterium *Bordetella pertussis*, an exclusively human pathogen. Classical whooping cough is most common in children, and is characterised by a paroxysmal cough followed by whooping and/or vomiting.

Clinical severity varies widely, but the most severe complications, such as apnea, encephalopathy, and pneumonia, is most common in the age group less than one year. Therefore, vaccination programs focus on an early start of vaccination (< 6 months).

The immunity after vaccination lasts for 4 - 12 years, and it has been observed internationally that pertussis is increasing in older children and adults. Furthermore, the clinical symptoms for this age-group are often mild, and the clinical picture can be characterised by prolonged cough which often lasts for up to three months. However, as adults are frequently the source of transmission of pertussis to infants, it is very important to diagnose such cases correctly.

Role of PT Serology in the Diagnosis of *Bordetella Pertussis*

Definitive diagnosis of pertussis has traditionally been made by culture of the causative organism on Bordet-Gengou medium. However, this approach may be insensitive and slow (up to 1 week). PCR demonstrated a significant improvement in diagnostic yield and speed over culture. Especially in infants and early cases real-time PCR will provide a rapid definitive diagnosis, and it is the most widespread method for diagnosis of such cases today.

Serology has proved especially useful for later diagnosis of prolonged cough in older children and adults. Pertussis toxin (PT) is specific for
Bordetella pertussis and only IgG antibodies are useful for diagnostic purposes. Older children and adults can have a milder clinical picture and will therefore often attend medical advice late in the course of the illness. Since diagnosis by means of culture and PCR are only useful in the very beginning of the illness, cases among older children and adults are frequently missed by these two methods. However, in such cases serology is known to be very useful.

Serological analysis used as a supplement to PCR clearly increases the amount of correctly diagnosed cases. Furthermore, a number of comparisons between the value of PCR and serology (IgG to PT), and serology based on a single measurement was better than PCR in a range of cases, in part due to the timing of sampling.

The Bordetella pertussis IgG-PT ELISA kit provides a method able to analyze heat-treated sera without the risk of false-positive results. Sensitivity is 81 % and specificity is 96 % based on data from Denmark using a cut-off at 75 IU/mL. Cut-off values should be determined for each country/region as the general antibody levels can vary between populations. Cut-offs in the range of 62 - 125 IU/mL are frequently used worldwide.

The kit can be used for patients experiencing pertussis-like symptoms for more than two weeks. In general, serology is not recommended for very young children or recently boosted individuals as remaining antibodies from pertussis vaccines can interfere with the results.

Limitations
• Diagnosis of recently pertussis-vaccinated individuals (< 2 years prior) is not possible.
• Use of the ELISA for evaluation of vaccine status and/or protective level is not possible.
• Detection of antibody-response to Bordetella parapertussis is not possible.
Description
The *Bordetella pertussis* IgG-PT ELISA Kit contains:

- 1 Maxisorp ELISA plate (NUNC®) (store at room temperature (RT))
- 5 plate seals (NUNC®) (store at RT)
- 50 µL of highly purified pertussis toxin (store at 2 - 8ºC) *
- 4 vials of human antiserum for construction of the standard curve containing 100 µL per vial. Each control serum is from a single individual. The sera are from recently pertussis vaccinated individuals (store at 2 - 8ºC)
- 16 mL coating buffer (store at 2 - 8 ºC)
- 100 mL wash buffer 10x concentrated (store at RT)
- 2,5 g skim milk powder (store at RT)
- 100 µL HRP labelled Rabbit-Anti-Human IgG (store at 2 - 8ºC)
- 16 mL sulphuric acid (1 M) (store at RT)
- 16 mL TMB One Substrate (store at 2 - 8ºC)

* Labelled with danger symbol. See material safety data sheets at www.ssi.dk.

Equipment Required
ELISA Reader set at 450 nm and 650 nm.

Procedure

1. ELISA procedure
The *Bordetella pertussis* IgG-PT ELISA Kit is a traditional ELISA setup. The 4 human standard antisera and patient samples should be assayed in duplicates.
Example of a setup:

To make the best possible use of the ELISA Kit we recommend performing at least 4 patient samples at the same time.

2. Preparation of reagents

Solution A: Antigen solution for coating the ELISA plate
• PT is mixed with the cold coating buffer to a final concentration of 0.2 µg/mL. The concentration is stated on the label. (example: 10 µL 274 µg/mL PT is mixed with 13.7 mL coating buffer)

Solution B: Wash buffer
• 100 mL 10x wash buffer is sufficient for 1 liter of wash buffer (100 mL 10x wash buffer is mixed with 900 mL ion-exchanged water)
  Automated washing is recommended. Wash by hand can result in lower OD values but the standard curve will adjust to this.
Solution C: Blocking buffer with 1 % milk
- 0.5 g skim milk powder is weighed and mixed with 50 mL wash buffer. Should be stirred for at least 30 min.
- Should be prepared and used only on the day of the analysis

Solution D: Dilution buffer with 0.1 % milk
- 20 mL of blocking buffer is mixed with 180 mL of wash buffer. For better visual inspection phenolred can be added to a final concentration of 0.1 %.
- Should be prepared and used only on the day of the analysis

Solution E: Secondary antibody
- HRP labelled rabbit anti-human IgG is diluted 1:2500 in wash buffer (ex. 10 µL HRP is added to 25 mL wash buffer)
- Should be prepared and used only on the day of the analysis

Solution F: Patient samples
- Each sample should be diluted 1:1000 in the dilution buffer (minimum 5 µL patient sample)

Solution G: Standard serum
- Each standard should be diluted 1:1000 in the dilution buffer
3. Flow-sheet

**STEP 1** Add 100 µL diluted antigen (solution A) to each well

Incubate 1h at 37 °C sealed with plate seal in a plastic bag; no shaking

Wash 3 times 1 min with 250 µL wash buffer (solution B).

**STEP 2** Add 200 µL blocking buffer (solution C) to each well

Incubate 30 min at RT, no shaking

Wash 3 times 1 min with 250 µL wash buffer (solution B).

**STEP 3** Add 100 µL of diluted sample or standard serum (solution F,G)

Incubate 1h at RT, no shaking

Wash 3 times 1 min with 250 µL wash buffer (solution B).

**STEP 4** Add 100 µL of diluted HRP to each well (solution E)

Incubate 30 min at RT, no shaking

Wash 3 times 1 min with 250 µL wash buffer (solution B).

**STEP 5** Add 100 µL of TMB-One Substrate to each well

Incubate exactly 15 min at RT; no seal/shaking

**STEP 6** Add 100 µL of 1 M sulphuric acid to each well

Read the absorbance within 10 min using an ELISA reader set at 450 nm and 650 nm. The result is obtained by subtracting OD_{650} from OD_{450}

* Step 1: Incubation can also be done at 37 °C or room temperature (RT) overnight. Incubation for 1 hour at RT is not recommended.
Calculation of Results
The 4 standard sera represent the four points in the log-log linear standard curve. The regression equation from the standard curve is used to calculate the concentration of pertussis toxin IgG antibodies in patient samples based on ΔOD values obtained by subtracting OD$_{650}$ from OD$_{450}$. A cut-off value of 75 IU/mL can be used if no country-specific cut-off has been determined.

Construction of the standard curve (example):
An example of ΔOD values of the standards and the corresponding concentration (IU/mL) could be as follows:

<table>
<thead>
<tr>
<th>Standard serum</th>
<th>Concentration (IU/mL)</th>
<th>ΔOD (example automated washing)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) 1:1000</td>
<td>15</td>
<td>0,128</td>
</tr>
<tr>
<td>2) 1:1000</td>
<td>56</td>
<td>0,432</td>
</tr>
<tr>
<td>3) 1:1000</td>
<td>148</td>
<td>1,052</td>
</tr>
<tr>
<td>4) 1:1000</td>
<td>289</td>
<td>1,749</td>
</tr>
</tbody>
</table>

The standard curve is drawn as shown below with the concentration as a function of the OD. The regression line of the points must be linear, which is achieved using a power trendline (IU/mL = A * OD^B). Concentrations should be calculated using the regression equation rather than a graphical standard curve.
Interpretation of Results

Results are valid if the CV % (coefficient of variance) for duplicate samples is lower than 20 %. Higher CV % is allowed if the difference between the duplicate samples is less than 0.05 OD. Results with OD readings above 2.0 or below 0.1 are outside the log-log linear range of the standard curve and should be interpreted as “higher than” or “lower than” the corresponding IU/mL values from the standard curve of these two OD values.

Cut-off values used in Denmark are

- $\geq 75$ IU/mL: positive for *B. pertussis* infection
- $\geq 50$ and $< 75$ IU/mL: indeterminate
- $< 50$ IU/mL: negative
Storage Shelf Life
The Rabbit-Anti-Human IgG HRP, TMB-One Substrate, coating buffer, serum controls and pertussis toxin are stored at 2 - 8 °C. All other reagents can be stored at room temperature. Expiry date of the kit is printed on the package.

IMPORTANT NOTICE: If the kit has been subjected to freezing, it should be discarded.

References