Bordetella pertussis

Bordetella pertussis Serology from medac

Competence - Quality - Continuity
**Bordetella pertussis serology from medac**

*Bordetella pertussis* (B.p.) is a gram-negative aerobic coccobacillus, with worldwide endemic circulation and belonging to the family of the *Alcaligenaceae*, genus *Bordetella* (B.). Partial antigenic identity exists between *B.p.*, *B. parapertussis*, and *B. bronchiseptica*, but without inducing cross-immunity.

The key stages in the pathogenesis of pertussis (whooping cough) are the attachment of the B.p. pathogen to the host cell, the avoidance of host-cell-specific defence mechanisms, local cell damage and the systemic manifestations of disease. These aspects are regulated by a variety of virulence factors and toxins. The genus-specific pertussis toxin (PT) regulates pertussis-associated lymphocytosis, is highly immunogenic and is an important component of acellular pertussis vaccines.

Pertussis infections occur throughout the year. Depending on the particular country, there may be winter or summer clustering of the disease. Epidemiological waves have been noted in a 3- to 5-year rhythm. Incidence is between 20 and 50 cases per 100,000 persons per year.

The illness, and immunisation to it, induces immunity for a limited period of time. Reinfection with clinical pertussis symptoms is possible after 5 – 10 years. The risk of severe, sometimes life-threatening infections is highest in unvaccinated infants (5, 11).

Since 2013 in Germany pertussis has been a notifiable disease under § 6 (1) no. 1 of the German Infectious Diseases Protection Act (IfSG). At the same time direct or indirect evidence of B.p. has been included in the list of 'Notifiable evidence of pathogens' under §7 (1) no. 1 of the IfSG.

Pertussis is transmitted via highly contagious respiratory droplets containing the pathogen. The nasopharynx is the portal of entry for B.p. The pathogen multiples on the ciliary epithelium of the respiratory tract mucosa.

B.p. infection may evolve as an uncomplicated respiratory illness or as a classic persistent whooping cough with typical staccato coughing symptoms, additional secondary clinical signs and complications such as pneumonia or otitis media.

In infants and very young children pertussis infection frequently manifests itself merely in the form of non-specific respiratory symptoms. However, it is not uncommon for it to lead to pneumonia, respiratory arrest or encephalopathy.

After pertussis contact, immunised individuals may shed the pathogens for a short while without becoming ill themselves.
Pertussis infections belong in the category of diseases that can be prevented by immunisation. Many countries, including Germany, recommend acellular pertussis vaccines consisting of purified immunogenic B.p. proteins. Compared with the inactivated bacterial cell vaccines used in the past, these are associated with markedly fewer local and systemic adverse reactions.

Together with antigens of other pathogens, pertussis antigens are administered as triple to sextuple combination vaccines.

Because B.p. may be associated with severe clinical courses in infants and young children, the Standing Committee on Vaccination (STIKO) at the Robert Koch Institute urgently recommends that basic immunisation be performed as early as possible from the age of 2 months onwards.

Because immunity lasts for only a limited time, both after immunisation and after infection, booster immunisations are indicated at intervals of 5 – 10 years using combination vaccines with reduced pertussis antigen content, not only for children but also in adults (3).

Alongside the typical clinical symptoms, direct detection of the pathogen by culture or PCR from nasopharyngeal secretions or swab is the method of choice for demonstrating acute B.p. infection. Because of their greater sensitivity and speed, molecular biology-based techniques* are the preferred choice in routine practice. Pathogens can be detected for up to about 4 weeks after the onset of illness (period of cough duration).

B.p. serology is useful for confirming pertussis infection that has been present for a long time or has been recently completed, for differential diagnosis in respiratory infections, or for immunisation control (1, 4, 8, 10).

The exclusive use of the B.p.-specific, highly-purified PT as antigen represents the state of the art for serology using immunoassay techniques (7).

Due to the high level of vaccine uptake in the general population, IgG antibodies to PT can be detected in almost all healthy subjects, usually in low concentrations.

The immune response following immunisation is indistinguishable from that after infection. Consequently, serology cannot be unequivocally assessed within a 1-year period after immunisation.

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*Bordetella pertussis/parapertussis real-time PCR from medac available (Cat.-No. BP/ISIN/xxx, BP/ISEX/xxx)
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### Interpretation of antibody diagnosis/MiQ 13b

In line with the recommendations in the Microbiology and Infection Quality Standards (MiQ 13b) issued by the German Society for Hygiene and Microbiology (9) and the case definition for the mandatory notification of cases and fatalities and evidence of pertussis pathogen (2):

- **an antibody increase > 100 %**
  - or
- **an antibody reduction > 50%**

In two consecutive samples serves to confirm the diagnosis.

For single-sample serology:

- **IgG concentrations > 100 IU/ml** constitute compelling evidence of recent B.p. contact.

- **In the range from 40 bis < 100 IU/ml** recent contact is possible. In this case the suspected diagnosis should be confirmed in a second serum sample or by further investigations such as IgA determination.

- **IgG concentrations < 40 IU/ml** do not indicate recent B.p. contact.

In a suspected diagnosis with IgG results in the range from 40 to 100 IU/ml, Riffelmann et al. (10) recommend the following assessment of PT-IgA results:

- **IgA concentrations > 12 IU/ml** confirm the suspicion of recent B.p. contact.
  - The IgA results should in principle be assessed in conjunction with the clinical data and the IgG results.

### Advantages of medac assays

- No cross-reactivity with other *Bordetella* species
- No cross-reactivity with *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*
- Calibration against the WHO First International Standard
- Results in IU/ml
- Single-point quantification
**Bordetella pertussis serology from medac**

- Simple handling – standardised processing conditions
- Ready-to-use reagents
- Break-off microtitre strips (individual wells)
- Suitable for use on automated open microtitre plate systems
- Colour-coded reagents to eliminate pipetting errors

To determine the sensitivity and specificity of the medac assays, samples were measured in comparison with a competitor assay. Samples with discrepant results were tested in the German reference laboratory for Bordetella pertussis in order to establish their predefined status.

<table>
<thead>
<tr>
<th>Predefined status (n = 312)</th>
<th>&lt; 40 IU/ml</th>
<th>40 - &lt; 100 IU/ml</th>
<th>≥ 100 IU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>medac &lt; 40 IU/ml</td>
<td>157</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>40 - &lt; 100 IU/ml</td>
<td>8</td>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td>≥ 100 IU/ml</td>
<td>0</td>
<td>6</td>
<td>93</td>
</tr>
</tbody>
</table>

**Sensitivity:** 100 % **Concordance:** 95.5 % **Specificity:** 95.2%

<table>
<thead>
<tr>
<th>Predefined status (n = 314)</th>
<th>&lt; 12 IU/ml</th>
<th>≥ 12 IU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>medac &lt; 12 IU/ml</td>
<td>215</td>
<td>0</td>
</tr>
<tr>
<td>≥ 12 IU/ml</td>
<td>25</td>
<td>74</td>
</tr>
</tbody>
</table>

**Sensitivity:** 100 % **Concordance:** 92 % **Specificity:** 89.6%

Perform photometric evaluation at a measurement wavelength of 450 nm (with 620 – 650 nm as reference wavelength).

Subtract the OD of the blank from all OD measurements.

Evaluate the measured results quantitatively in international units (IU/ml).

The mean OD of the **negative control must be <0.100**.

The OD of the **calibrator** must be above the **batch-specific cut-off**.

The unit value of the **positive control** must lie within the **batch-specific theoretical range**.
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**Correction of measured results:**

\[
\text{OD}_{\text{corrected}} = \frac{\text{theoretical OD of calibrator}}{\text{measured OD of calibrator}} \times \text{OD}_{\text{measured}}
\]

**Quantification of measured results:**

\[
\text{Concentration [IU/ml]} = b / \left( \frac{a}{\text{OD}_{\text{corrected}}} \right)^{-1}
\]

Algorithm modified according to Riffelmann et al. (10) for medac single sample serology

- Anti-PT IgG
  - < 40 IU/ml: No evidence of recent contact
  - 40 - < 100 IU/ml: Anti-PT IgA < 12 IU/ml
    - Evidence of recent contact not confirmed
  - 40 - < 100 IU/ml: Anti-PT IgA ≥ 12 IU/ml
    - Evidence of recent contact
  - ≥ 100 IU/ml: Compelling evidence of recent contact

Taking into account the interpretation algorithm according to MiQ 13b and Riffelmann et al. (10) for single-sample serology, the diagnostic performance data for assessing recent B.p. contact with Bordetella-PT-ELISA medac are as follows:

<table>
<thead>
<tr>
<th>Predefined status (n = 312)</th>
<th>medac</th>
<th>No evidence</th>
<th>Evidence</th>
<th>Compelling evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>No evidence</td>
<td>157</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Evidence</td>
<td>8</td>
<td>36</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Compelling evidence</td>
<td>0</td>
<td>9</td>
<td>102</td>
<td></td>
</tr>
</tbody>
</table>

**Sensitivity:** 100 %  
**Concordance:** 95.2 %  
**Specificity:** 94.6 %
Instructions in brief for:

**Bordetella-PT-IgG-ELISA medac**
Cat.-No. 380

**Bordetella-PT-IgA-ELISA medac**
Cat.-No. 381

### Preparatory steps
- **Wash buffer**: 1:10
  - 100 ml
  
  **Deionised Water**: 900 ml

### Assay steps
1. **Controls, calibrator, diluted samples**
   - 50 µl each

2. **Incubation at 37°C, humid chamber**
   - 60 min
   - 50 µl each

3. **TMB substrate**
   - 200 µl each
   - 50 µl each
   - Incubation at 37°C, in the dark, humid chamber
   - 30 min

4. **Wash plate x 3**
   - Empty and tap out

5. **IgG: Conjugate**
   - 50 µl each
   - 60 µl for automated processing

6. **IgA: Conjugate**
   - 50 µl each

7. **Incubation at 37°C, humid chamber**
   - 60 min

8. **Sample dilution**
   - 1:100

9. **Stop solution (0.5M H₂SO₄)**
   - 100 µl each

10. **Photometric reading**
    - O.D.
    - 450 nm
    - Reference 620 - 650 nm


