NovaLisa®
Bordetella pertussis toxin (PT) IgA ELISA
(BPTA0610)

Performance Characteristics
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1. **Intended Use**

The NovaLisa® Bordetella pertussis toxin (PT) IgA ELISA is intended for the quantitative determination of IgA antibodies to Bordetella pertussis toxin (PT) in human serum or plasma (citrate, heparin).

2. **Principle of the Assay**

The quantitative immunoenzymatic determination of IgA-class antibodies to Bordetella pertussis toxin (PT) is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.

Microtiter strip wells are coated with B. pertussis toxin (PT) antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) labelled anti-human IgA conjugate is added. This conjugate binds to the captured B. pertussis toxin (PT)-specific antibodies. The immune complex formed by the bound conjugate is visualized by adding Tetrathon benzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of B. pertussis toxin (PT)-specific IgA antibodies in the specimen. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450 nm is read using an ELISA microwell plate reader.

3. **Performance Characteristics**

3.1. **Reproducibility**

**Material**

NovaLisa® Bordetella pertussis toxin (PT) IgA Lot: BPTA-010
Production date: 2013-08 Expiry date: 2014-08-31

NovaLisa® Bordetella pertussis toxin (PT) IgA Lot: BPTA-011
Production date: 2013-10 Expiry date: 2014-10-31

NovaLisa® Bordetella pertussis toxin (PT) IgA Lot: BPTA-012
Production date: 2013-10 Expiry date: 2014-10-31

Positive and negative samples

**Test Description**

The reproducibility of the NovaLisa® Bordetella pertussis toxin (PT) IgA ELISA kit was determined by comparing 24 (23) replicates of 3 different samples in one assay (within-run) and by comparing 3 different samples assayed in 12 different runs (between-run). In addition, the inter-batch coefficient of variation was determined by measuring three samples (performed as duplicates) with three different batches.

Acceptance Criterion: CV < 15 %
Results

Within-run, between-run precision as well as inter-batch precision were estimated by analysis of variance and are presented in Tables 1-3.

Table 1: Within-Run Precision (BPTA-011)

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean (E)</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>23</td>
<td>0.547</td>
<td>8.0</td>
</tr>
<tr>
<td>#2</td>
<td>24</td>
<td>1.954</td>
<td>2.0</td>
</tr>
<tr>
<td>#3</td>
<td>24</td>
<td>1.048</td>
<td>2.7</td>
</tr>
</tbody>
</table>

Table 2: Between-Run Precision (BPTA-010)

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean (IU/ml)</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>12</td>
<td>5.19</td>
<td>7.3</td>
</tr>
<tr>
<td>#2</td>
<td>12</td>
<td>16.29</td>
<td>4.4</td>
</tr>
<tr>
<td>#3</td>
<td>12</td>
<td>6.23</td>
<td>8.2</td>
</tr>
</tbody>
</table>

Table 3: Inter-Batch Precision (BPTA-010, BPTA-011, BPTA-012)

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean (IU/ml)</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>3</td>
<td>2.00</td>
<td>11.76</td>
</tr>
<tr>
<td>#2</td>
<td>3</td>
<td>21.19</td>
<td>4.47</td>
</tr>
<tr>
<td>#3</td>
<td>3</td>
<td>22.86</td>
<td>2.41</td>
</tr>
</tbody>
</table>

Conclusion

The acceptance criterion was met for all samples.

3.2. Diagnostic Sensitivity and Specificity (In-house Study)

Introduction

The purpose of this study was to determine the efficiency of the assay to discriminate between positive and negative clinical samples.

To evaluate the diagnostic performance of the NovaLisa® Bordetella pertussis toxin (PT) IgA ELISA, internal studies were conducted by NovaTec either with well defined samples from an External Quality Control Scheme (EQAS) or in comparison to an immunoassay already established on the market.

Samples from newborns and immunocompromised individuals were excluded from the study as in these patients serological data only have limited value.
Test Description (Part I)

The evaluation of the diagnostic performance of the NovaLisa® Bordetella pertussis toxin (PT) IgA ELISA was performed in comparison to the Euroimmun Anti-Bordetella-pertussis-Toxin-ELISA (IgA), (El 2050-9601 A). This internal study was conducted at NovaTec.

Material

NovaLisa® Bordetella pertussis toxin (PT) IgA
Lot: BPTA-010
Production date: 2013-08 Expiry date: 2014-08-31

Euroimmun Anti-Bordetella-pertussis-Toxin-ELISA (IgA)
Lot E130513BC Expired date: 2014-05-12

33 samples

Results

Table 4: Diagnostic Sensitivity and Specificity (Part I)

<table>
<thead>
<tr>
<th>Euroimmun</th>
<th>positive</th>
<th>negative</th>
<th>Σ</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NovaLisa® Bordetella pertussis toxin (PT) IgA</strong></td>
<td>positive</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>negative</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>Σ</td>
<td>9</td>
<td>24</td>
<td>33</td>
</tr>
</tbody>
</table>

Test Description (Part II)

The evaluation of the diagnostic performance of the NovaLisa® Bordetella pertussis toxin (PT) IgA ELISA was performed in comparison to well defined samples from the following External Quality Control Scheme (EQAS):

“Bakteriologische Infektionsserologie - Bordetella pertussis AK” (317), www.instandev.de

This internal study was conducted at NovaTec.

Material

NovaLisa® Bordetella pertussis toxin (PT) IgA
Lot: BPTA-012
Production date: 2013-10 Expiry date: 2014-10-31

6 EQAS samples

Results

Table 5: Diagnostic Sensitivity and Specificity (Part II)

<table>
<thead>
<tr>
<th>Target</th>
<th>positive</th>
<th>negative</th>
<th>Σ</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NovaLisa® Bordetella pertussis toxin (PT) IgA</strong></td>
<td>positive</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>negative</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Σ</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>
Conclusion (Parts I and II combined)

Table 6: Diagnostic Sensitivity and Specificity

<table>
<thead>
<tr>
<th>Target</th>
<th>positive</th>
<th>negative</th>
<th>Σ</th>
</tr>
</thead>
<tbody>
<tr>
<td>NovaLisa® Bordetella pertussis toxin (PT) IgA</td>
<td>positive</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>negative</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>Σ</td>
<td>12</td>
<td>27</td>
<td>39</td>
</tr>
</tbody>
</table>

Diagnostic Sensitivity: 100 % (12/12)
Diagnostic Specificity: 88.9 % (24/27)
Agreement: 92.3 % (36/39)

Note: In the instructions for use, the diagnostic sensitivity and the diagnostic specificity are stated as > 98 % and 88.9 %, respectively.

3.3. Analytical Specificity

3.3.1. Interference from Hemoglobin, Bilirubin and Triglycerides

Introduction

The aim of the study was to evaluate the impact of possible interference materials found in blood, such as, bilirubin, hemoglobin, and triglycerides on the NovaLisa® Bordetella pertussis toxin (PT) IgA ELISA.

Material

NovaLisa® Bordetella pertussis toxin (PT) IgA ELISA Lot: BPTA-010
Production date: 2013-08 Expiry date: 2014-08-31

10 defined positive and negative samples

Test Description

Pathological concentrations of hemoglobin, bilirubin, or triglyceride were added into the specimen to simulate hemolytic, icteric, or lipemic samples. The samples were then measured with the NovaLisa® Bordetella pertussis toxin (PT) IgA ELISA and the change of the analyte concentrations was determined.

Interference was defined as more than 40 % deviation from the initial value measured.
Results

Table 7: Interferences

<table>
<thead>
<tr>
<th>Sample</th>
<th>without interfering substance IU/ml (%)</th>
<th>Bilirubin (0.5 mg/ml) IU/ml (%)</th>
<th>Hemoglobin (10 mg/ml) IU/ml (%)</th>
<th>Triglycerides (5 mg/ml) IU/ml (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.11 (100)</td>
<td>9.29 (91.9)</td>
<td>11.65 (115.2)</td>
<td>10.34 (102.3)</td>
</tr>
<tr>
<td>2</td>
<td>9.52 (100)</td>
<td>9.10 (95.6)</td>
<td>9.31 (97.8)</td>
<td>9.43 (99.1)</td>
</tr>
<tr>
<td>3</td>
<td>6.42 (100)</td>
<td>5.76 (89.7)</td>
<td>6.50 (101.2)</td>
<td>8.23 (128.2)</td>
</tr>
<tr>
<td>4</td>
<td>9.04 (100)</td>
<td>8.62 (95.4)</td>
<td>9.41 (104.1)</td>
<td>11.14 (123.2)</td>
</tr>
<tr>
<td>5</td>
<td>16.80 (100)</td>
<td>15.44 (91.9)</td>
<td>17.70 (105.4)</td>
<td>19.24 (114.5)</td>
</tr>
<tr>
<td>6</td>
<td>13.15 (100)</td>
<td>13.32 (101.3)</td>
<td>13.96 (106.2)</td>
<td>14.25 (108.4)</td>
</tr>
<tr>
<td>7</td>
<td>3.15 (100)</td>
<td>2.36 (74.9)</td>
<td>2.61 (82.9)</td>
<td>3.44 (109.2)</td>
</tr>
<tr>
<td>8</td>
<td>7.94 (100)</td>
<td>5.84 (73.6)</td>
<td>6.09 (76.7)</td>
<td>8.40 (105.8)</td>
</tr>
<tr>
<td>9</td>
<td>22.99 (100)</td>
<td>16.32 (71.0)</td>
<td>18.80 (81.8)</td>
<td>19.27 (83.8)</td>
</tr>
<tr>
<td>10</td>
<td>27.50 (100)</td>
<td>22.56 (82.0)</td>
<td>35.10 (127.6)</td>
<td>29.92 (108.8)</td>
</tr>
</tbody>
</table>

Samples with added potential interference substance displayed IU/ml values ranging from 71.0 to 128.2 % of the untreated sample.

Conclusion

Possible interference materials found in blood, such as, bilirubin, hemoglobin, and triglycerides were tested in the NovaLisa® Bordetella pertussis toxin (PT) IgA ELISA at pathological concentrations (10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.5 mg/ml bilirubin).

These substances did not alter the test results of the NovaLisa® Bordetella pertussis toxin (PT) IgA ELISA.

3.3.2. Cross-Reactivity

Material

NovaLisa® Bordetella pertussis toxin (PT) IgA Lot: BPTA-010
Production date: 2013-08 Expiry date: 2014-08-31

84 potentially cross-reactive samples

A panel of 84 specimens from patients with confirmed diseases other than Pertussis was tested to establish the analytical specificity of the NovaLisa® Bordetella pertussis toxin (PT) IgA ELISA. The specimens were from patients infected with pathogens that may cause similar signs and symptoms to those observed for Bordetella pertussis or from individuals with diseases or conditions that have the potential for cross-reactivity.
Results

Table 8: Cross-Reactivity

<table>
<thead>
<tr>
<th>Disease Type</th>
<th>Total Specimens</th>
<th>Positive Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>10</td>
<td>0/10</td>
</tr>
<tr>
<td>Chlamydia trachomatis</td>
<td>10</td>
<td>0/10</td>
</tr>
<tr>
<td>Chlamydophila pneumoniae</td>
<td>10</td>
<td>0/10</td>
</tr>
<tr>
<td>Epstein-Barr virus (HHV-4)</td>
<td>7</td>
<td>0/7</td>
</tr>
<tr>
<td>Helicobacter pylori</td>
<td>10</td>
<td>0/10</td>
</tr>
<tr>
<td>Influenzavirus A</td>
<td>3</td>
<td>0/3</td>
</tr>
<tr>
<td>Legionella pneumophila</td>
<td>10</td>
<td>0/10</td>
</tr>
<tr>
<td>Mycoplasma pneumoniae</td>
<td>8</td>
<td>0/8</td>
</tr>
<tr>
<td>Toxoplasma gondii</td>
<td>10</td>
<td>0/10</td>
</tr>
<tr>
<td>Varicella zoster virus (HHV-3)</td>
<td>6</td>
<td>0/6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>84</strong></td>
<td><strong>0/84</strong></td>
</tr>
</tbody>
</table>

Conclusion

Investigation of a specimen panel with antibody activities to potentially cross-reacting parameters (including several respiratory pathogens) did not reveal evidence of false-positive results due to cross-reactions.

3.4. Analytical Sensitivity (Limit of Detection)

Introduction

The terms Limit of Blank (LoB) and Limit of Detection (LoD) are used to describe the smallest concentration of a measurand that can be reliably measured by an analytical procedure. The LoB is the highest apparent analyte concentration expected to be found when replicates of a blank sample containing no analyte are tested. The LoD is the lowest analyte concentration likely to be reliably distinguished from the LoB and at which detection is feasible.

Method

The LoD or analytical sensitivity was determined according to the approved guideline CLSI EP17-A „Protocols for Determination of Limits of Detection and Limits of Quantitation“.

For this the zero standard, Standard A (= blank) and one low concentration sample were determined 60-fold on the Bordetella pertussis toxin (PT) IgA ELISA.

The LoD was calculated according to the following formula:

\[
\text{LoD} = \text{LoB} + 1.64 \times \text{SD}_{\text{low concentration sample}} = \text{mean}_{\text{blank}} + 1.645 \times \text{SD}_{\text{blank}} + 1.645 \times \text{SD}_{\text{low concentration sample}}
\]

Specification

Acceptance Criterion: \( \text{LoD} \leq 5 \text{ IU/ml} \)
Material
NovaLisa® Bordetella pertussis toxin (PT) IgA Lot: BPTA-010
Production date: 2013-08 Expiry date: 2014-08-31
Zero calibrator (Standard A)
Low concentration sample

Results and Conclusion
The Limit of Detection (LoD) was estimated at 1.56 IU/ml.
However, it cannot be excluded that the Limit of Detection shows some variance. Therefore, the
value stated in the instructions for use is < 3 IU/ml, which is far below the Standard B (10 IU/ml).

3.5. Summary
The Performance Characteristics of the NovaLisa® Bordetella pertussis toxin (PT) IgA ELISA
can be summarized as follows:

Reproducibility
Within-run, between-run as well as inter-batch precision were estimated by analysis of variance.
The acceptance criterion (CV < 15 %) was met.

Diagnostic Performance
Diagnostic Sensitivity: 100 % (12/12)
Diagnostic Specificity: 88.9 % (24/27)
Agreement: 92.3 % (36/39)

Analytical Specificity
Interference from high levels of bilirubin, hemoglobin, and lipid was not detected.
No cross-reactivity with other (respiratory) pathogens included in the panel was detected.

Analytical Sensitivity
The Limit of Detection (LoD) was estimated to be < 3 IU/ml.