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Bordetella pertussis toxin IgA/IgG ELISA

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**For Research Use
Only – Not for Use
in Clinical
Procedures**

IBL-America Bordetella pertussis toxin IgA/IgG ELISA

Enzyme-immunoassay for determination of human antibodies

Bordetella pertussis toxin IgA ELISA

Order no.: IB05013

Bordetella pertussis toxin IgG ELISA

Order no.: IB05014

For Research Use Only – Not for Use in Clinical Procedures

1 INTENDED USE

The IBL-America ELISA Bordetella pertussis toxin IgA and IgG tests are qualitative immunoassays for the demonstration of human antibodies in serum or plasma directed against *Bordetella pertussis* toxin. These assays can be used to demonstrate contact with the pathogen and are recommended to be used in combination with the Bordetella pertussis IgA, IgG, and IgM ELISA tests (Codes IB05010, IB05011, IB05012) in various kinds of samples.

2 BACKGROUND

Bordetella pertussis belongs to the genus of *Bordetella*. It is a small coccoid, gram negative bacillus, which occurs world-wide.

The attachment of *B. pertussis* and *B. parapertussis* to the ciliated cells in the mucosa of the human respiratory tract is mediated by adhesins. An important adhesion protein and an equally important immunogen is the so-called filamentous haemagglutinin (FHA).

Colonisation of the respiratory tract and establishment of infection are facilitated by the synergistic action of several virulence factors. An important virulence factor is pertussis toxin (PT) which mediates multiple biological effects such as leukocytosis, lymphocytosis, mitogenicity, and increased sensitivity to histamine.

3 TEST PRINCIPLE

This ELISA (Enzyme Linked Immunosorbent Assay) is an immunoassay, which is particularly suited to the determination of antibodies in various kinds of samples. The reaction is based on the specific interaction of antibodies with their corresponding antigen. The test strips of the microtiter plate are coated with specific antigens of the pathogen of interest. If antibodies in the sample are present, they bind to the fixed antigen. A secondary antibody, which has been conjugated with the enzyme alkaline phosphatase, detects and binds to the immune complex. The colourless substrate p-nitrophenylphosphate is then converted into the coloured product p-nitrophenol. The signal intensity of this reaction product is proportional to the concentration of the analyte in the sample and is measured photometrically.

4 KIT COMPONENTS

Test Components	Pieces / Volume
Break apart microtiter test strips each with eight antigen coated single wells, (altogether 96) [MTP] , 1 frame. The coating material is inactivated.	12 pieces
Standard serum (ready-to-use) [STD] , Human serum in protein containing phosphate buffer; negative for anti-HIV Ab, HBs-Ag (Hepatitis B-Virus surface antigen) and anti-HCV Ab; preservative: < 0.1 % sodium azide; colouring: Amaranth O.	2 x 2 ml
Negative control serum (ready-to-use) [NEG] , Human serum in protein containing phosphate buffer; negative for anti-HIV Ab, HBs-Ag (Hepatitis B-Virus surface antigen) and anti-HCV Ab; preservative: < 0.1 % sodium azide; colouring: Lissamin Green V.	2 ml
Anti-human IgA or IgG conjugate (ready-to-use) [APC] , Anti-human IgA or IgG polyclonal antibody, conjugated to alkaline phosphatase, stabilised with protein stabilisation solution; preservative: 0.01 % methylisothiazolone, 0.01 % bromnitrodioxane.	13 ml
Washing solution concentrate (sufficient for 1000 ml) [WASH] , Sodium chloride solution with Tween 20 and 30 mM Tris/HCl, pH 7,4; preservative: < 0.1 % sodium azide.	33,3 ml
Dilution buffer (ready to use) [DILB] , Protein containing phosphate buffer with Tween 20; preservative: < 0.1 % sodium azide; colouring: 0.01 g/l Bromphenol blue.	2 x 50 ml
Stopping solution (ready to use) [STOP] , < 0.1 N sodium hydroxide, 40 mM EDTA.	15 ml
Substrate (ready to use) [pNPP] , Para-nitrophenylphosphate in solvent free buffer; preservative: < 0.1 % sodium azide	13 ml
Quality control certificate with standard curve [INFO]	1 page

5 MATERIAL REQUIRED BUT NOT SUPPLIED

- common laboratory equipment
- for the IgM detection: Rf-Absorbent, order no. IB05998 (20 ml)
- photometer for microtitre plates with filter, wavelength 405 nm, recommended reference wavelength 620 nm - 690 nm (e.g. 650 nm)
- incubator 37 °C

- moist chamber
- distilled water

Recommended but not required:

Control serum 5 x 3 ml order no.: IB05013CON for Bord.pert. toxin IgA

Control serum 5 x 3 ml order no.: IB05014CON for Bord.pert. toxin IgG

6 STORAGE AND STABILITY

Reagent	Storage	Stability
Microtiter strips (coated with antigen)	unopened after opening at 2 – 8 °C in closed aluminum bag with desiccant <i>Strips which are not used must be stored dry in the closed aluminum bag.</i>	see expiry date; minimum shelf-life: four weeks
Control sera / Standard sera	unopened / after opening at 2 – 8 °C	see expiry date
Conjugate	unopened / after opening at 2 – 8 °C	see expiry date
Dilution buffer	Unopened / after opening at 2 – 8 °C	see expiry date
Washing solution	unopened / after opening at 2 – 8 °C working dilution at 2 – 8 °C working dilution at room temperature	see expiry date 2 weeks 1 week
Substrate	unopened / after opening at 2 – 8 °C	see expiry date
Stopping solution	unopened / after opening at 2 – 8 °C	see expiry date

7 TEST PROCEDURE

7.1 Evidence of Deterioration

The components of this kit must not be exchanged for reagents of other manufacturers. Standard and control sera are defined exclusively for the test kit to be used and must not be used in other lots. Dilution buffer, washing solution, substrate and stop solution can be used for all IBL-America immunoassays coded IB05xxx irrespective of the lot and the test.

Unopened, all components of this ELISA may, if stored accordingly, be used up to the expiry dates given on the labels. Reagents may not be used after date of expiry.

Dilution or alteration of the reagents may result in a loss of sensitivity.

Avoid exposure of reagents to strong light during storage and incubation. Reagents must be tightly closed after use to avoid evaporation and contamination.

To open the aluminum bag of the microtiter plate please cut off the top of the marked side only, in order to guarantee proper reclosing. Do not use the strips if the aluminum bag is damaged or if the bag with remaining strips and desiccant was not properly reclosed.

Use aseptic techniques when removing aliquots from the reagent tubes to avoid contamination. To avoid false positive results ensure not to contact or splash the top-walls of wells while pipetting conjugate. Take care not to mix the caps of the bottles and/or vials.

Reproducibility of test results is dependent on thorough mixing of the reagents. Agitate the flasks containing control sera before use and also all samples after dilution (e.g. by using a vortex mixer).

Be sure to pipette carefully and comply with the given incubation times and temperatures. Significant time differences between pipetting the first and last well of the microtiter plate when dispensing samples and control sera, conjugate or substrate can result in different pre-incubation times, which may influence the precision and reproducibility of the results.

Optimum results can only be achieved if the instructions are strictly followed.

The results of this ELISA are only valid if the lot-specific validation criteria on the quality control certificate are fulfilled.

Adequate washing avoids test unspecificities. Therefore, the washing procedure should be carried out carefully. All of the flat bottom wells should be filled with equal volumes of washing buffer. At the end of the procedure ensure that the wells are free of all washing buffer in order to avoid uncontrolled dilution effects. Avoid foaming!

Take care not to damage the inscription (pathogen / antibody class) on the microtiter test strips during washing and aspiration to avoid confusion.

7.2 Sample Preparation and Storage

Lipaemic, hemolytic or icteric samples (serum or plasma) should only be tested with caution. Obviously contaminated samples should not be tested. Serum or plasma (EDTA, citrate, heparin) collected according to standard laboratory methods are suitable samples. Samples must not be thermally inactivated.

7.2.1 Dilution of Samples

Before running the test, all samples (V_1) must be diluted in dilution buffer (V_2) as follows:

Bordetella pertussis toxin IgA/IgG ELISA:

$V_1 + V_2 = 1 + 100$	add	10 μ l	sample
	each to	1000 μ l	dilution buffer

After dilution and before pipetting into the microtiter plate the samples must be mixed thoroughly to prepare a homogenous solution.

7.2.2 Sample Storage

The samples should not be stored for more than 7 days at 2 – 8 °C. Extended storage is possible at ≤ -20 °C. Avoid repeated freezing and thawing of samples. Diluted samples can be stored at 2 – 8 °C for one week.

7.3 Preparation of Kit Reagents

Bring all reagents to room temperature before testing.

7.3.1 Microtiter Test Strips

The microtiter test strips labeled with abbreviations for pathogen and immunoglobulin class are packed with a desiccant in an aluminum bag. To open the aluminum bag of the microtiter plate please cut off the top of the marked side only, in order to guarantee proper resealing. Take unrequired cavities out of the frame and put them back into the aluminum bag. Close bag carefully to ensure airtight conditions. Do not use the strips if the aluminum bag is damaged or if the bag with remaining strips and desiccant was not properly resealed.

7.3.2 Control Sera / Standard Sera (ready to use)

Control and standard sera are ready-to-use and must not be diluted any further. For each test run - independent of the number of microtiter test strips to be used - control and standard sera must be included. The standard sera should be set up in duplicate. Do not treat control sera with Rf-absorbent.

7.3.3 Anti-human IgA or IgG AP-Conjugate (ready-to-use)

Conjugates with the same concentration and of the same immunoglobulin class are interchangeable. Avoid contamination of ready-to-use conjugates e. g. by using sterile tips.

7.3.4 Washing Solution (Concentrate)

Dilute washing buffer concentrate (V_1) 1:30 with aqua dest. to a final volume of V_2 .

Example:

Buffer concentrate (V_1)	Final volume (V_2)
33,3 ml	1000 ml
1,0 ml	30 ml

7.3.5 Dilution Buffer for Samples (ready-to-use)

7.3.6 Substrate (ready-to-use)

Substrate in unopened bottle may have a slightly yellow coloring, which does not reduce the quality of the product!

7.3.7 Stopping Solution (ready-to-use)

7.4 Overview - Test Procedure

IBL-America Bordetelle pertussis toxin IgA/IgG

Sample dilution¹
1 + 100

Pipette diluted samples and ready-to-use control /
standard sera into the microtest wells (100 µl)



INCUBATION 60 min./ 37 °C
moist chamber



WASH (4 x 300 µl [DIL] [WASH])²



Pipette conjugate solution [APC] (100 µl)



INCUBATION 30 min./ 37 °C
moist chamber



WASH (4 x 300 µl [DIL] [WASH])²



Pipette substrate solution [pNPP] (100 µl)



INCUBATION 30 min./ 37 °C
moist chamber



Pipette stopping solution [STOP] (100 µl)



READ EXTINCTION at 405 nm

¹Special dilution buffers for the following IBL-America ELISA tests:
Borrelia burgdorferi IgG, IgM, EBV EA IgG and Hantavirus Puumala IgG, IgM

²For manual use:

tap plate at the end of the wash procedure on paper towel.

7.5 Manual Test Procedure

1. Place the required number of **cavities in the frame** and prepare a protocol sheet.
2. Add each **100 µl of diluted sample or ready-to-use controls** into the appropriate wells of microtiter test strips. Spare one well for substrate blank, e.g.:

IgA/IgG	
well no.	
well A1	Substrate blank
well B1	Negative control
well C1	Standard serum
well D1	Standard serum
well E1	Sample 1....

3. **Sample incubation** for 60 minutes (+/- 5 min) at 37 °C (+/- 1°C) in moist chamber
4. After incubation **wash** all wells with washing solution (by automated washer or manually):
 - aspirate or shake out the incubation solution
 - fill each well with 300 µl washing solution
 - aspirate or shake out the washing buffer
 - repeat the washing procedure 3 times (altogether 4 times!)
 - dry by tapping the microtiter plate on a paper towel
5. **Addition of conjugate**
Add 100 µl of the ready-to-use IgA/IgG conjugate to the appropriate wells (except substrate blank)
6. **Conjugate incubation** for 30 minutes (+/- 1 min) at 37 °C (+/- 1 °C) in moist chamber.
7. After incubation **wash** all wells with washing solution (see above)
8. **Addition of substrate**
Add 100 µl of ready-to-use substrate solution to each well (including well for substrate blank!)
9. **Substrate incubation** for 30 minutes (+/- 1 min) at 37 °C (+/- 1 °C) in moist chamber.
10. **Stopping of the reaction**
Add 100 µl stopping solution to each well, shake microtiter plate gently to mix.
11. **Read extinction**
Read optical density (OD) within 60 minutes at 405 nm against substrate blank, reference wave length between 620 nm and 690 nm (e.g. 650 nm).

7.6 Automated Test Procedure

This ELISA also is suited for processing on automats and evaluated for use with Immunomat™ and Gemini as well as with DYNEX DSX® and DS2®. The automated processing is performed analogous to manual use. Please note, that under special working-conditions internal laboratory adaptations of the incubation times may be necessary.

7.7 Positive Control / Accuracy Control

For the periodic verification of the test method, in order to fulfil the requirements of laboratory internal quality management systems, we recommend using IBL-America ELISA controls (cat.-no. IB05xxxCON, see also chapter 5) to determine precision and accuracy of the test runs. The use of IBL-America ELISA controls is described in specific instruction manuals.

8 TEST EVALUATION

For qualitative interpretation of serum samples a lot specific correction factor as well as a lot specific grey zone is calculated by manufacturer for each kit lot. These values can be found on the lot specific quality certificate included in each test kit.

For test run control a standard serum is used in each individual test run. For this control serum a reference value with a validity range is determined by the quality control of the manufacturer. Within this range a correct cut-off interpretation is ensured.

8.1 Criteria of Validity

The substrate blank must be < 0.25 OD

The negative control must produce a negative test result.

The mean OD-value (after subtraction of the substrate blank!) of the standard serum must be within the validity range, which is given on the lot specific qualitycontrol certificate.

The variation of OD-values of the standard serum may not be higher than 20%.

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

8.2 Cut-off Calculation

A lot specific quality control certificate is included in the test kit so that the obtained OD values can be interpreted qualitatively. The substrate blank must be subtracted from all OD values prior to evaluation.

To fix the cut-off ranges multiply the mean value of the measured standard OD with the lot specific correction factor from the quality certificate. Then add and subtract the lot specific grey zone percentage mentioned on the quality certificate to obtain the upper and lower

cut-off. The following numbers are an example only, the valid data you will find in the lot-specific QC certificate which comes with each kit.

Lot specific correction factor: 0,805

Lot specific grey zone: 15%

If the measured mean absorbance value of the standard serum is 0.84 OD, the range of the cut-off is:

Lower cut-off: $(0,84 * 0,805) - 15\% = \text{OD } 0,575$

Upper cut-off: $(0,84 * 0,805) + 15\% = \text{OD } 0,778$

8.3 Borderline Ranges

The borderline range indicates the range for borderline test results. Values obtained, when testing a sample, which fall below this range indicate a negative test result; values above the borderline range are interpreted positive. In cases where the results are within the borderline range a definitive interpretation of the result is not possible. In such cases, the test should be repeated in parallel with a follow-up sample taken one to two weeks later (serum pair).

9. PERFORMANCE CHARACTERISTICS

9.1 Sensitivity and Specificity

IBL-America Bordetella pertussis toxin IgA and IgG

The IBL-America Bordetella pertussis toxin IgA and IgG ELISA was verified in an internal study. The sensitivity and specificity in both cases exceeded 99%.

9.2 Reproducibility

Intraassay reproducibility was determined by testing samples of different reactivities 20 times in one test run. Interassay reproducibility was determined by testing samples of different reactivities 10 times in 10 independent assays performed on 5 different days.

$$\text{Coefficient of Variation (CV \%)} = \frac{\text{Standard deviation}}{\text{Mean value}} \times 100$$

Bordetella pertussis toxin IgA:

Sample	Mean Value (OD)	Intraassay (CV %)	Mean Value (OD)	Interassay (CV %)
Sample 1	0.309	9.1	0.412	12.1
Sample 2	0.997	3.6	0.991	6.5
Sample 3	1.579	2.1	1.623	7.0

Bordetella pertussis toxin IgG:


Sample	Mean Value (OD)	Intraassay (CV %)	Mean Value (OD)	Interassay (CV %)
Sample 1	0.229	9.9	0.156	16.3
Sample 2	0.846	8.5	0.954	13.1
Sample 3	1.334	7.6	1.372	10.7

10 SAFETY MEASURES

10.1 Statements of Warning

The IBL-America ELISA test kits are designed for use by qualified personnel who are familiar with good laboratory practice.

All kit reagents and specimens should be handled carefully, using established good laboratory practice.

- This kit contains human blood components. Although all control- and cut-off sera have been tested and found negative for anti-HIV-ab, HBs-Ag (*Hepatitis B-Virus-surface Antigen*) and anti-HCV-ab, they should be considered potentially infectious.
- Do not pipette by mouth.
- Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
- Wear disposable gloves, laboratory coat and safety glasses while handling kit reagents or specimens. Wash hands thoroughly afterwards.
- Sample material should be decontaminated after the test run.
- Reagents should be stored safely and be inaccessible to unauthorized access e.g. children.
- Stopping solution:  corrosive (C); causes acid burn (R34)
Use safety glasses, gloves and laboratory coat while handling!

10.2 Disposal

Please observe the relevant statutory requirements!

11 REFERENCES

- [1] Aase A. *et al.* (2007) Opsonophagocytic Activity and Other Serological Indications of *Bordetella pertussis* Infection in Military Recruits in Norway. *Clin. Vac. Immunol.* 14, 855-62.
- [2] Baughman *et al.* (2004) Establishment of Diagnostic Cut off Points for Levels of Serum Antibodies to Pertussis Toxin, Filamentous Hemagglutinin, and Fimbriae in Adolescents and Adults in the United States. *Clin. Diagn. Lab. Immunol.* 11, 1045-53.
- [3] de Melker, H. E., Versteegh, F. G., Conyn-Van Spaendonck, M. A., Elvers, L. H., Berbers G. A., van Der Zee, A., Schellekens, J. F. (2000) Specificity and sensitivity of high levels of immunoglobulin G antibodies against pertussis toxin in a single serum sample for diagnosis of infection with *Bordetella pertussis*. *J. Clin. Microbiol.* 38, 800-6.
- [4] Granström, G., Wretling, B., Salenstedt, C.-R., Granström, M. (1988) Evaluation of serologic assay for diagnosis of whooping cough. *J. Clin. Microbiol.* 26, 1818-23.
- [5] Guiso, N., Berbers, G., Fry, N.K, He, Q., Riffelmann, M., Wirsing von König, C.H., EU Pertstrain group (2010) What to do and what not to do in serological diagnosis of pertussis: recommendations from EU reference laboratories. *Eur. J. Clin. Microbiol. Infect. Dis.* 30, 307-12.
- [6] Kösters, K., Riffelmann, M., Dohrn, B., Wirsing von König, C.-H. (2000) Comparison of five commercial enzyme-linked immunosorbent assays for detection of antibodies to *Bordetella pertussis*. *Clin. Diagn. Lab. Immunol.* 7, 422-6.
- [7] Pebody R.G. *et al.* (2005) The seroepidemiology of *Bordetella pertussis* infection in Western Europe. *Epidemiol. Infect.* 133, 159-71.
- [8] Riffelmann, M., Thiel, K., Schmetz, J., Wirsing von Koenig, C.H. (2010) Performance of commercial enzyme-linked immunosorbent assays for detection of antibodies to *Bordetella pertussis*. *J. Clin. Microbiol.* 48, 4459-63.
- [9] Xing D. *et al.* (2009) Characterization of reference materials for human antiserum to pertussis antigens by an international collaborative study. *Clin Vac. Immunol.* 16, 303-11.

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