Version 24-04/24

User's Manual

Human Bordetella pertussis IgG ELISA Kit

REF **DEIA315**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The ELISA test kit provides a quantitative in vitro assay for human antibodies of the immunoglobulin class IgG against Bordetella pertussis toxin in serum or plasma for the diagnosis of Bordetella pertussis infections and whooping cough.

General Description

The Anti-Bordetella pertussis toxin ELISA (IgG) is based on species-specific pertussis toxin (PT). The test is calibrated using the international WHO standard serum and is in accordance with the latest guidelines for the serological diagnosis of B. pertussis infections. Increased anti-PT IgG titers (≥100 IU/ml) are considered as proof of an acute B. pertussis infection. Titers below 40 IU/ml should be further investigated. Recent vaccinations (<1 year) should be taken into consideration, since these can also cause high anti-PT IgG titers.

Principles of Testing

The test kit contains microtiter strips each with 8 break-off reagent wells coated with Bordetella pertussis toxin. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies (also IgA and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

Reagents And Materials Provided

1. Microplate wells coated with antigens, 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use, 12 x 8 **STRIPS**
2. Calibrator 1, 200 IU/ml (human IgG), ready for use, 1 x 2.0 ml **CAL 1**
3. Calibrator 2, 100 IU/ml (human IgG), ready for use, 1 x 2.0 ml **CAL 2**
4. Calibrator 3, 25 IU/ml (human IgG), ready for use, 1 x 2.0 ml **CAL 3**
5. Calibrator 4, 5 IU/ml (human IgG), ready for use, 1 x 2.0 ml **CAL 4**
6. Positive control, (IgG, human), ready for use, 1 x 2.0 ml **POS CONTROL**
7. Negative control, (IgG, human), ready for use, 1 x 2.0 ml **NEG CONTROL**
8. Enzyme conjugate, peroxidase-labelled anti-human IgG (rabbit), ready for use, 1 x 12 ml **CONJUGATE**
9. Sample buffer, ready for use, 1 x 100 ml **SAMPLE BUFFER**
10. Wash buffer, 10x concentrate, 1 x 100 ml **WASH BUFFER 10x**
11. Chromogen/substrate solution, TMB/H_2O_2, ready for use, 1 x 12 ml **SUBSTRATE**
12. Stop solution, 0.5 M sulphuric acid, ready for use, 1 x 12 ml **STOP SOLUTION**
13. Protective foil --- 2 pieces **FOIL**
14. Test instruction --- 1 booklet

15. Quality control certificate --- 1 protocol

Storage

The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Specimen Collection And Preparation

Samples: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: Patient samples are diluted 1:101 sample buffer. For example: dilute 10 µl sample in 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

NOTE: The calibrators and controls are prediluted and ready for use, do not dilute them.

Plate Preparation

	1	2	3	4	5	6	7	8	9	10	11	12
A	C 1	P 3	P 11	P 19								
B	C 2	P 4	P 12	P 20								
C	C 3	P 5	P 13	P 21								
D	C 4	P 6	P 14	P 22								
E	pos.	P 7	P 15	P 23								
F	neg.	P 8	P 16	P 24								
G	P 1	P 9	P 17									
H	P 2	P 10	P 18									

The pipetting protocol for microtiter strips 1 to 4 is an example for the **quantitative analysis** of 24 patient sample (P 1 to P 24).

The calibrators (C 1 to C 4), the positive (pos.) and negative (neg.) controls, and the patient samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample.

The wells can be broken off individually from the strips. Therefore, the number of tests performed can be matched to the number of samples, minimising reagent wastage.

Both positive and negative controls serve as internal controls for the reliability of the test procedure.

They should be assayed with each test run.

Reagent Preparation

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use.

After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below. The thermostat adjusted ELISA incubator must be set at +37°C ± 1°C.

- Coated wells: Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag). Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.

- Calibrators and controls: Ready for use. The reagents must be mixed thoroughly before use.

- Enzyme conjugate: Ready for use. The enzyme conjugate must be mixed thoroughly before use.

- Sample buffer: Ready for use. - Wash buffer: The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to +37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionised or distilled water (1 part reagent plus 9 parts distilled water).

For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water. The working strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

- Chromogen/substrate solution: Ready for use. Close the bottle immediately after use, as the contents are sensitive to light . The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

- Stop solution: Ready for use.

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Warning: The calibrators and controls of human origin have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the agent sodium azide in a nondeclarable concentration. Avoid skin contact.

Assay Procedure

For quantitative analysis incubate calibrators 1 to 4 along with the positive and negative controls and patient samples.

Sample incubation: (1st step)

Transfer 100 µl of the calibrators, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol. For manual processing of microplate wells, cover the finished test plate with the protective foil. When using an automated microplate processor for incubation follow the recommendations of the instrument manufacturer. Incubate for 60 minutes at +37°C ± 1°C.

Washing:

Manual: Remove the protective foil, empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.

Automatic: Remove the protective foil and wash the reagent wells 3 times with 450 µl of working strength

wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Mode"). Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing, thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (>10 µl) in the reagent wells after washing can interfere with the substrate and lead to false low extinction values.

Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction values.

Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)

Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells. Incubate for 30 minutes at room temperature (+18°C to +25°C).

Washing:

Empty the wells. Wash as described above.

Substrate incubation: (3rd step)

Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for 15 minutes at room temperature (+18°C to +25°C) (protect from direct sunlight).

Stopping:

Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:

Photometric measurement of the colour intensity should be made at a wavelength of 450 nm and a reference wavelength between 620 nm and 650 nm within 30 minutes of adding the stop solution. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

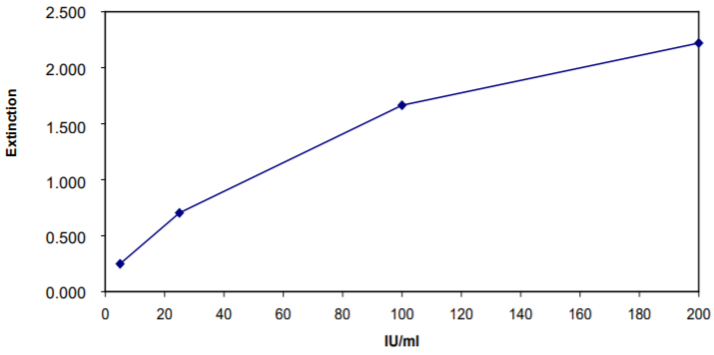
Calculation

Quantitative: The standard curve from which the concentration of antibodies in the serum samples can be taken is obtained by point-to-point plotting of the extinction values measured for the 4 calibration sera against the corresponding units (linear/linear). Use "point-to-point" plotting for calculation of the standard curve by computer.

If the extinction for a patient sample lies above the value of calibrator 1 (200 IU/ml = Cal. 1), the result should be reported as ">200 IU/ml". It is recommended that the sample be retested in a new test run at a dilution of e.g. 1:404. The result in IU/ml read from the calibration curve for this sample must then be multiplied by a factor of 4.

Typical Standard Curve

The following plot is an example of a typical calibration curve. Please do not use this curve for the determination of antibody concentrations in patient samples.



Reference Values

The levels of the anti-Bordetella pertussis toxin antibodies (IgG) were analysed with this CD ELISA in a panel of 500 healthy blood donors. 14.8% of the blood donors showed values about 38 IU/ml, wich reflects the known percentage of infections in adults.

In recent publications the following age-dependent standard value ranges have been recommended:

Antibodies	Age-dependent reference ranges in IU/ml			
	<1 year	1 - 4 years	5 - 10 years	from 11 years of age
Anti-PT IgA	<2	<2	<6	<12
Anti-PT IgG	<38	<26	<22	<38
Anti-FHA IgA	<2	<2	<18	<42
Anti-FHA IgG	<38	<30	<56	<86

Furthermore, for antibodies against Bordetella pertussis toxin of class IgG, current literature references recommend to consider interpretation according to the following scheme for all patient groups:

Anti-PT IgG ≥100 IU/ml: Indication for an acute infection or recent vaccination.

Anti-PT IgG <40 IU/ml: No indication for an acute infection.

Anti-PT-IgG ≥40 - <100 IU/ml: The results should be verified by further examination or analysis of a second blood sample taken after 7 to 10 days.

Independent of the recommendation given in the above-cited literature to analyse a second sample if the test result is between 40 - 100 IU/ml, this procedure can also help to clarify other cases.

The interval between blood withdrawals should be at least seven days. The results of both samples allow proper evaluation of titer changes.

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another, CD recommends retesting the samples.

For diagnosis the clinical picture of the patient always needs to be taken into account along with the serological findings.

Detection Limit

The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable antibody titer. The lower detection limit of the Anti-Bordetella pertussis toxin ELISA (IgG) is 0.2 IU/ml.

Specificity

The quality of the antigen used ensures a high specificity of the ELISA. Sera from patients with infections caused by various agents were investigated with the Anti-Bordetella pertussis toxin ELISA (IgG).

Antibodies against	n	Anti-Bordetella pertussis toxin ELISA (IgG) positive
Adenovirus	12	0%
Chlamydia pneumoniae	12	0%
CMV	12	0%
EBV-CA	12	0%
Helicobacter pylori	12	0%
HSV-1	12	0%
Influenza virus A	12	0%
Influenza virus B	12	0%
Measles	12	0%
Mumps	12	0%
Mycoplasma pneumoniae	12	0%
Parainfluenza virus Pool	12	0%
Parvovirus B19	12	0%
RSV	12	0%
Rubella virus	12	0%
Toxoplasma gondii	12	0%
VZV	12	0%
Yersinia enterocolitica	12	0%

Linearity

The linearity of the Anti-Bordetella pertussis toxin ELISA (IgG) was determined by assaying at least 4 serial dilutions of different patient samples. The Anti-Bordetella pertussis toxin ELISA (IgG) is linear at least in the tested concentration range (5 IU/ml to 174 IU/ml).

Reproducibility

The reproducibility of the test was investigated by determining the intra- and interassay coefficients of variation (CV) using 3 samples. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

Intra-assay variation (n = 20): CV 1.8-3.3%

Inter-assay variation (n= 4x6): CV 5.4-6.9%

Interferences

Haemolytic, lipaemic and icteric samples showed no influence on the result up to a concentration of 10 mg/ml for haemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

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