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SeroPertussis™ IgG

Enzyme Linked Immunosorbent Assay (ELISA) for the semi-quantitative determination of specific IgG antibodies to ***Bordetella Pertussis*** in human serum

Instruction Manual

Test kit for 96 determinations
(Catalog No A231-01M)

For ***In Vitro*** Diagnostic Use
For Professional use only
Store at 2-8°C. **Do Not Freeze**

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Intended Use
SeroPertussis™ IgG kit is a semi-quantitative Enzyme Linked Immunosorbent assay (ELISA) for the determination of specific IgG antibodies to *Bordetella pertussis*.

For ***In Vitro*** Diagnostic Use and for Professional use only.

Introduction

Whooping Cough (Pertussis) is a highly contagious bacterial respiratory tract infection, caused by *Bordetella pertussis* – gram-negative bacilli. It is typically manifested in children with paroxysmal spasms of severe coughing; whooping and posttussive vomiting that resides for many weeks.

The disease results in high morbidity and mortality, especially of children.
Pertussis is an endemic disease, but epidemics occur every 3 – 5 years. In the USA, 5000 – 7000 cases are reported each year. The incidence of Pertussis has been greatly reduced by mass vaccination; however, even in countries with high vaccination coverage, the disease is re-merging [(1)]. Worldwide, nearly 50 million cases of pertussis are diagnosed annually and about 350,000 people die of the disease [(2)]. The incidence of pertussis has increased steadily since 1980 [(3)]. The vaccine induced immunity wanes after 5 to 10 years, making the vaccinated host vulnerable to infection. Infection in vaccinated persons causes a milder non-specific disease, without the classical clinical stages. Whooping cough is seen in only 6% of such cases; instead the illness is characterized by a non-specific, prolonged cough, lasting several weeks to months. Because of these atypical symptoms, Pertussis is under-diagnosed in adults and adolescents, who may be the reservoirs for infection of unvaccinated infants [(4)]. Children who are too young to be fully vaccinated and those who have not yet completed the primary vaccination series are at highest risk for severe illness.
The disease is highly contagious, with up to 90% of susceptible household contacts developing clinical disease following exposure.
Early anti-microbial treatment will reduce the severity of the symptoms and limit the period of communicability. Prompt identification of the cases may help to prevent un-vaccinated or under-vaccinated persons from being infected by vaccination or by anti-microbial prophylaxis.
Laboratory diagnosis of Pertussis can be either direct by culture, DFA or PCR or by indirect serological tests. Since the bacteria reside in the upper respiratory tract during the first two weeks of the infection it can be detected by direct methods during this period only. The preferred specimen for direct detection is naso-pharyngeal sample (aspirates or swabs). Serology tests are helpful in diagnosis of atypical infections with prolonged cough and for epidemiological purposes. Elevated levels of antibodies against Pertussis Toxin (PT) and Filamentous Hemagglutinin (FHA) are regarded as sensitive serological markers for the diagnosis of Pertussis in adults and non- vaccinated children [(5)]. In unvaccinated children increases in the levels of either immunoglobulin G (IgG) or immunoglobulin A (IgA) antibodies to a single or various antigens are required to meet the World Health Organization (WHO) definition of Pertussis. In vaccinated children, a single serum specimen may be diagnostic for Pertussis [(6)].
SeroPertussis™ IgG and SeroPertussis™ IgA/IgM utilize enriched fraction of PT and FHA as antigens, allowing sensitive detection of IgA and/ or IgM antibodies and the semi-quantitative determination of IgG antibodies to *Bordetella pertussis* allowing immuno-status follow-up and antibodies kinetics.

Principle of the Test

- SeroPertussis™ microtiter plates are coated with enriched fraction of *Bordetella pertussis* toxin and filamentous heamaglutinin.
- The serum to be tested is diluted 1/100 and incubated in the SeroPertussis™ plate. In this step B. pertussis specific antibodies are bound to the immobilized antigens.
- Non-specific antibodies are removed by washing.
- Anti-human IgG conjugated to horseradish peroxidase (HRP) is added. In this step the HRP-conjugate is bound to the prebound antigen-antibody complex.
- Unbound conjugate is removed by washing.
- TMB-substrate is added and is hydrolyzed by the peroxidase, yielding a blue solution of the reduced Substrate.
- Upon the addition of the stop solution, the blue color turns yellow and the absorbance should be read by an ELISA reader at a wavelength of 450/620nm.
- The absorbance is proportional to the levels of the specific antibodies that are bound to the coated antigens.

Assay Procedure

Add 50µl of each Ready to Use calibrator P10, P50, P75, and 50µl of Negative Control, Positive Control, and specimens diluted 1/100 to microtiter plate wells coated with specific immunodominant B.pertussis proteins

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Cover plate and incubate 1h at 37ºC at 100% humidity

↓

Wash 3 times with Wash Buffer

↓

Add 50µl of 1/300 diluted HRP Conjugate

↓

Cover plate and incubate 1 h at 37ºC at 100% humidity

↓

Wash 3 times with Wash buffer

↓

Add 100µl of TMB-Substrate

↓

Cover plate and incubate 15min at room temperature

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Add 100 µl of Stop Solution

↓

Read absorbance at 450/620nm

↓

Calculate and interpret results

Kit contents

Test kit for 96 Determinations
Cat. No. A231-01M

1. ***B. pertussis* Antigen Coated Microtiter Plate:** 96 break apart wells (8x12) coated with *Bordetella pertussis* antigens, packed in an aluminum pouch containing a desiccant card.
   1 Plate
2. **Concentrated Wash Buffer (20X):** A PBS - Tween buffer. Contains less than 0.05% proclin as a preservative.
   1 bottle, 100 ml
3. **Serum Diluent–RT:** A ready-to-use buffer solution. Contains less than 0.05% proclin as a preservative.
   1 Bottle, 30 ml
4. **Conjugate Diluent:** A ready-to-use buffer solution. Contains less than 0.05% proclin as a preservative**.**
   1 Bottle, 40 ml
5. **Negative Control:** A ready-to-use *B. pertussis* IgG negative human serum. Contains less than 0.05% proclin and less than 0.1% Sodium Azide as preservatives.
   1 Vial, 2 ml
6. **Positive Control:** A ready-to-use *B.pertussis* IgG positive human serum. Contains less than 0.05% proclin and less than 0.1% Sodium Azide as preservatives.
   1 Vial, 2 ml
7. **P10 Calibrator:** A ready-to-use calibrator containing 10 BU/ml (arbitrary binding units) of human specific IgG antibodies to *B. pertussis*. Contains less than 0.1% Sodium Azide and less than 0.05% proclin as preservatives.
   1 Vial, 2 ml
8. **P50 Calibrator:** A ready-to-use calibrator containing 50 BU/ml (arbitrary binding units) of human specific IgG antibodies to *B. pertussis*. Contains less than 0.1% Sodium Azide and less than 0.05% proclin as preservatives.
   1 Vial, 2 ml
9. **P75 Calibrator:** A ready-to-use calibrator containing 75 BU/ml (arbitrary binding units) of human specific IgG antibodies to *B. pertussis*. Contains less than 0.1% Sodium Azide and less than 0.05% proclin as preservatives.
   1 Vial, 2 ml
10. **HRP-Conjugate (300X):** Horseradish peroxidase (HRP) conjugated anti-human IgG (γ chain specific). Contains less than 0.05% proclin as a preservative.
    1 Vial, 0.2 ml
11. **TMB-Substrate:** A ready-to-use solution. Contains 3, 3', 5, 5' - Tetramethylbenzidine as a chromogen and peroxide as a substrate.
    1 Bottle, 14 ml
12. **Stop Solution:** A ready-to-use solution. Contains 1M H_2SO_4.
    1 Bottle , 15 ml
13. **Plate Cover: 1 unit**
14. **Instruction Manual: 1**

Materials Required But Not Supplied

1. Clean test tubes for dilution of patients sera.
2. Disposable plastic vial for dilution of the concentrated HRP- conjugate.
3. Adjustable micropipettes and multichannel pipettes (5-50, 50-200 and 200-1000µl ranges) and disposable tips.
4. One liter volumetric flask.
5. One 50ml volumetric cylinder.
6. Wash bottle.
7. Absorbent paper.
8. Vortex mixer
9. A 37°C water bath with a lid, or a moisture chamber placed in a 37°C incubator.
10. ELISA-reader with a 450 and 620nm filters.
11. Distilled or double deionized water.

Warning and Precautions

For *In Vitro* Diagnostic Use

1. This kit contains human sera which have been tested by FDA approved techniques, and found to be negative for HBsAg and for HCV and HIV antibodies. Since no known method can offer complete assurance that products derived from human blood do not transmit infection, all human blood components supplied in this kit must be handled as potentially infectious serum or blood according to the recommendations published in the CDC/NIH manual "Biosafety in Micro Biological and Biomedical Laboratories, 1988".
2. TMB-Substrate solution is an irritant material to skin and mucous membranes. Avoid direct contact.
3. All the components of this kit have been calibrated and tested by lot. It is not recommended to mix components from different lots since it might affect the results.
4. Diluted sulfuric acid (1M H_2SO_4) is an irritant agent for the eyes and skin. In case of contact with eyes, immediately flush area with water and consult a physician.

Storage and Shelf -Life of Reagents

1. All the reagents supplied should be stored at 2-8°C. The unopened reagents vials are stable until the expiration date indicated on the kit pack. Exposure of originally stoppered or sealed components to ambient temperature for a few hours will not cause damage to the reagents. **DO NOT FREEZE!**
2. Once the kit is opened, its shelf life is 90 days.
3. Unused strips must be resealed in the aluminum pouch with the desiccant card, by rolling the open end and sealing tightly with tape over the entire length of the opening.
4. Crystals may form in the 20x concentrated Wash Buffer during cold storage, this is perfectly normal. Redissolve the crystals by warming the buffer to 37°C before diluting. Once diluted, the solution may be stored at 2-8°C up to twenty one days.

Serum Collection

Prepare sera from aseptically collected samples using standard techniques. Heat inactivated sera should not be used. The use of lipemic, turbid or contaminated sera is not recommended. Particulate material and precipitates in sera may cause erroneous results. Such specimens should be clarified by centrifugation or filtration prior to the test.

Specimens Storage

Specimens should be stored at 2-8°C and tested within 7 days (adding of 0.1% Sodium Azide is highly recommended). If longer storage period is anticipated, aliquot and store the specimens below -20°C. Avoid repeated thawing and freezing.

Test Procedure - Manual

Automation protocol available upon request

A. Preparation of Reagents

1. Bring all components and the clinical specimens to be tested to room temperature. Mix gently the calibrators (P10, P50, P75), Negative Control, Positive Control and the clinical specimens before use.
2. Determine the total number of specimens to be tested. In addition to the specimens, the following must be included in each test: Three wells of calibrators (P10, P50, P75), one well of Negative Control and one well of Positive Control
3. Withdraw the microtiter plate from its aluminum pouch by cutting one end near the seal. Leave the required number of strips (according to the number of specimens to be tested) in the 96 well frame.
   Unused strips must be resealed in the aluminum pouch with the desiccant card, by rolling the open end and sealing tightly with tape over the entire length of the opening.
4. Dilute the Concentrated Wash Buffer 1/20 with double-deionized or distilled water. For example, in order to prepare one liter of wash buffer, add 50ml of the Concentrated Wash Buffer to 950ml of double-deionized or distilled water.

B. Incubation of sera samples and controls

5. Dilute each patient serum 1/100 with the supplied Serum Diluent–RT as follows: Add 10 µl of patient serum to 190µl of Serum Diluent–RT (1/20), and then dilute further by adding 25µl of 1/20 dilution to 100µl of Serum Diluent–RT.
6. Dispense 50µl of each of the three calibrators (P10, P50, P75), of negative and positive controls, and of serum samples into separate wells of the test strip.
7. Cover the strips with a plate cover and incubate for 1h at 37°C in a moisture chamber.
8. Discard the liquid content of the wells.
9. **Washing step:**
   Manual Wash:
   Fill each well with wash buffer up to the end of the well and discard the liquid, repeat this step twice for a total of three washing steps.
   Automated Wash:
   Fill each well with 350ul of wash buffer and discard the liquid, repeat this step twice for a total of three washing steps.
10. Dry the strips and frame by gently tapping them over clean absorbent paper.

C. Incubation with conjugate

11. Concentrated HRP-Conjugated anti-human IgG should be diluted to working solution shortly before use. Dilute 1/300 the concentrated HRP-conjugated anti-human IgG with Conjugate Diluent. For example: for two strips prepare a minimum of 3 ml conjugate as follows:10 µl of Concentrated HRP-conjugated anti-human IgG is mixed with 3ml of Conjugate Diluent.
12. Dispense 50µl of diluted HRP- Conjugate into each well.
13. Cover the strips with a plate cover and incubate for 1h at 37°C in a moisture chamber.
14. Discard the liquid content and wash as described in steps 9-10.

D. Incubation with TMB - Substrate

15. Dispense 100µl TMB-Substrate into each well, cover the strips with a plate cover and incubate at room temperature for **15 minutes.**
16. Stop the reaction by adding 100µl of stop solution (1M H_2SO_4) to each well.

E. Determination of Results

17. Determine the absorbance at 450/620nm and record the results. Determination should not exceed 30 minutes following stopping of chromogenic reaction.

- ***Note:*** *Any air bubbles should be removed before reading. The bottom of the ELISA plate should be carefully wiped.*

Test Validation

The following criteria must be met for the test to be valid. If these criteria are not met, the test should be considered invalid and should be repeated.

1. P75: OD ≥1.0
2. Ratio OD P50 / OD P10 is >1.6
3. Ratio OD P75 / OD P10 is >2.2
4. Negative Control: <10BU/ml (See chapter – Calculation and interpretation of Test Results)

5. Positive Control: ≥ 30 BU/ml (See chapter – Calculation of Test Results)

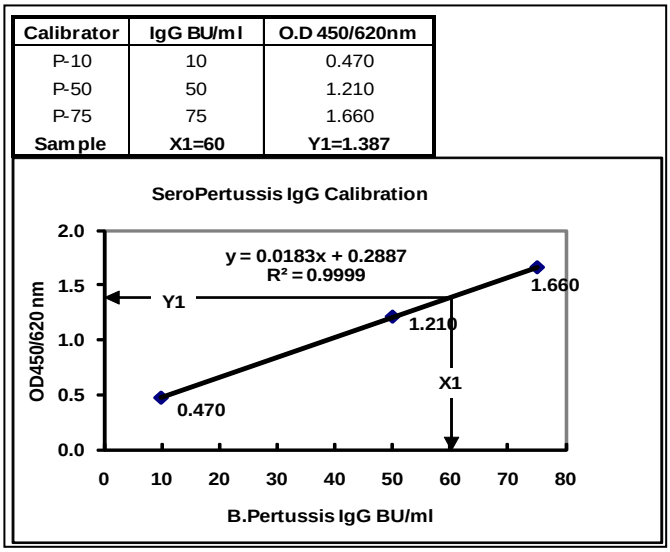
Calculation of Test Results

Manual method, using a squared graph paper:

1. Plot the absorbance values (OD) of the 3 calibrators (P10, P50 and P75) on Y axis versus their concentration (BU/ml) on X axis.
2. Draw the best fitted linear curve through the points.
3. Using the standard curve, interpolate the concentration of the Positive Control and Negative Control in BU/ml.
   Negative Control should be <10BU/ml
   Positive Control should be: ≥ 30 BU/ml. If Negative and/or Positive Control are not within specification test should be repeated.
4. Using the standard curve, interpolate the concentration of the tested sample values (in BU/ml) from each absorbance measured (see example 1).

Example 1. Interpolation of results:
On the Y-axis read the absorbency value of the sample (Y1) and draw a horizontal line to the calibration curve.
From the intercept (X1), draw a vertical line to the X-axis. Read the concentration in BU/ml of the sample.

Calibrator	IgG BU/ml	O.D 450/620nm
P-10	10	0.470
P-50	50	1.210
P-75	75	1.660
Sample	**X1=60**	**Y1=1.387**



Interpretation of Results

IgG BU/ml	Result	Interpretation
<10 BU/ml	**Negative** No detectable IgG antibodies	**No indication of *B.pertussis* Infection** (see test limitations)
≥10 BU/ml <50 BU/ml	**Positive** Detectable level of IgG antibodies	**Indication of recent, past infection[1] or immunization against *B.pertussis***
≥50 BU/ml	**High Positive** High level of IgG antibodies	**Indication recent or current *B.pertussis* infection**

[1] In order to differentiate between past and current infection, it is recommended to take a second sample after 2-4 weeks. If the BU/ml value of the second sample significantly increases current infection is indicated.

In order to evaluate if the difference between the 2 measurements is significant, the ratio between the sera should be calculated as follows:

$$R = \frac{BU2 + 15}{BU1 + 15}$$

BU1 = Concentration in BU/ml of the 1st sample
BU2 = Concentration in BU/ml of the 2nd sample
If **R ≥1.7**, the difference is statistically significant (p=0.005). This equation is not valid when both samples are less than 10 BU/ml. If one of the samples has a value of less than -13 BU/ml then 1 BU/ml should be introduced for the final calculation to achieve comprehensive results.

In order to achieve a comprehensive antibodies profile, IgM and IgA should also be tested.

Interpretation of results based on the detection of IgM, IgA and IgG antibodies detection.

Bordetella Pertussis			
IgG	**IgM**	**IgA**	
Negative	Negative	Negative	No indication of B.pertussis infection (see test limitations)
Negative or Positive	Positive	Negative or Positive	Indication of current infection
Positive or Negative	Negative	Positive	Indication of recent infection
Positive	Negative	Negative or Positive	Indication of recent or past infection or past immunization

Test Limitations

1. No single serological test should be used for final diagnosis. All clinical and laboratory data should be taken into account.
2. Samples obtained too early during primary infection may not contain detectable antibodies. If *B.pertussis* is suspected, a second sample should be obtained 2-4 weeks later and tested in parallel with the original sample.
3. When infection is suspected in infants under the age of 6 months, another test should be performed (culture, PCR) since children younger than 6 months rarely develop antibodies.

Performance Characteristics

Precision
Intra-assay (within-run) precision:

Sample	No of Replicates	Mean OD Value	CV%
50BU/ml	10	48	5.2
15BU/ml	10	15	2.5

Inter-assay (between-run) precision:

Sample	No of Replicates	Mean OD Value	CV%
50BU/ml	10	48	7
15BU/ml	10	15	13

Bibliography

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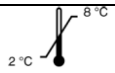	Temperature Limitation
	Consult instructions for use
IVD	In Vitro Diagnostic Medical Device
	Manufacturer
EC REP	Authorized European Representative