



SeroPertussis Toxin™ IgA

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

Instruction Manual

Test kit for 96 determinations (Catalog No. M1233-01D)

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

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Intended Use

SeroPertussis Toxin™ IgA kit is a quantitative Enzyme Linked Immunosorbent assay (ELISA) for the determination of specific IgA antibodies to *Bordetella pertussis* Toxin.

For ***In Vitro*** Diagnostic Use

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 transmission. 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 during this period only by direct methods. 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⁾.

ELISA based tests for Pertussis Toxin are recommended for the specific detection of antibodies against *B.pertussis*, as they avoid detection of parapertussis infection.⁽⁷⁾ Concentrations of antibodies to *B. pertussis* antigens should be quantitatively expressed in International Units per milliliter (IU/ml) as reference preparations are available⁽⁸⁾.

SeroPertussis™ Toxin IgA utilizes purified PT as an antigen, allowing quantitative determination of IgA antibodies to Pertussis Toxin according to the first International WHO Standard (WHO International Standard Pertussis Antiserum, human, 1st IS NIBSC Code 06/140).

Principle of the Test

- SeroPertussis Toxin™ microtiter plates are coated with enriched fraction of *Bordetella pertussis* Toxin.
- The serum to be tested is diluted 1/101 and incubated in the SeroPertussis Toxin™ plate. In this step *B. pertussis* Toxin specific antibodies are bound to the immobilized antigens.
- Non-specific antibodies are removed by washing.
- Anti-human IgA 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 100µl of each Ready to Use (RTU) calibrator C10, C20, C40, and 100µl of Negative Control and specimens diluted 1/101 to microtiter plate wells coated with specific immunodominant *B. pertussis* Toxin proteins

↓
Cover plate and incubate 1h at 37°C at 100% humidity

↓
Wash 5 times with Wash Buffer

↓
Add 100µl of RTU HRP Conjugate

↓
Cover plate and incubate 1 h at 37°C at 100% humidity

↓
Wash 5 times with Wash buffer

↓
Add 100µl of TMB-Substrate

↓
Cover plate and incubate 15min at room temperature

↓
Add 100 µl of Stop Solution

↓
Read absorbance at 450/620nm

↓
Calculate and interpret results

Kit contents

Test kit for 96 Determinations
Cat. No. 1233-01

- B. pertussis* Toxin Antigen Coated Microtiter Plate:** 96 break apart wells (8x12) coated with *Bordetella pertussis* posttussive antigens, packed in an aluminum pouch containing a desiccant card.
1 Plate
- Concentrated Wash Buffer (20X):** A PBS - Tween buffer. Contains less than 0.05% proclin as a preservative.
1 bottle, 100 ml
- Serum Diluent:** A ready-to-use buffer solution. Contains less than 0.05% proclin as a preservative.
2 Bottles, 60 ml
- Negative Control:** A ready-to-use *B. pertussis* Toxin IgA negative human serum. Contains a mercury-free and azide-free preservatives.
1 Vial, 2 ml
- C10 Calibrator:** A ready-to-use calibrator containing 10 IU/ml (arbitrary international units) of human specific IgA antibodies to *B. pertussis* Toxin. Contains a mercury-free and azide-free preservatives.
1 Vial, 2 ml
- C20 Calibrator:** A ready-to-use calibrator containing 20 IU/ml (arbitrary international units) of human specific IgA antibodies to *B. pertussis* Toxin. Contains a mercury-free and azide-free preservatives.
1 Vial, 2 ml
- C40 Calibrator:** A ready-to-use calibrator containing 40 IU/ml (arbitrary international units) of human specific IgA antibodies to *B. pertussis* Toxin. Contains a mercury-free and azide-free preservatives.
1 Vial, 2 ml
- HRP-Conjugate:** A ready-to-use Horseradish peroxidase (HRP) conjugated anti-human IgA (γ chain

specific) . Contains a mercury-free and azide-free preservatives.

- TMB-Substrate:** A ready-to-use solution. Contains 3, 3', 5, 5' - Tetramethylbenzidine as a chromogen and peroxide as a substrate.
1 Bottle , 15 ml
- Stop Solution:** A ready-to-use solution. Contains 1M H₂SO₄.
1 Bottle, 16ml
- Plate Cover:**
1 unit
- Instruction Manual:**
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Materials Required But Not Supplied

- Clean test tubes for dilution of patients sera.
- Adjustable micropipettes and multichannel pipettes (5-50, 50-200 and 200-1000µl ranges) and disposable tips.
- One liter volumetric flask.
- One 50ml volumetric cylinder.
- Wash bottle.
- Absorbent paper.
- Vortex mixer
- A 37°C water bath with a lid, or a moisture chamber placed in a 37°C incubator.
- ELISA-reader with a 450 and 620nm filters.
- Distilled or double deionized water.

Warning and Precautions

For In Vitro Diagnostic Use

- This kit contains human sera, which have been tested by FDA and CE approved techniques, and found to be negative for HBV antigen, and for antibodies to HCV 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.
- TMB-Substrate solution is an irritant material to skin and mucous membranes. Avoid direct contact.
- Diluted sulfuric acid (1M H₂SO₄) is an irritant agent for the eyes and skin. In case of contact with eyes, immediately flush area with water and consult a physician.)
- 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.

Storage and Shelf -Life of Reagents

- 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!**
- Once the kit is opened, its shelf life is 90 days.
- 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.

- 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

A. Preparation of Reagents

- Bring all components and the clinical specimens to be tested to room temperature. Mix gently the calibrators (C10, C20, C40), Negative Control and the clinical specimens before use.
- 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 (C10, C20, C40) and one well of Negative Control.
- 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.
- 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

- Dilute each patient serum 1/101 with the supplied Serum Diluent as follows: Add 10 µl of patient serum to 1000µl of Serum Diluent.
- Dispense 100µl of each of the three calibrators C10, C20, C40), Negative Control and of serum samples into separate wells of the test strip.
- Cover the strips with a plate cover and incubate for 1h at 37°C in a moisture chamber.
- Discard the liquid content of the wells.
- 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 5 washing steps.

Automated Wash:

Fill each well with 350µl of wash buffer and discard the liquid, repeat this step twice for a total of 5 washing steps.

- Dry the strips and frame by gently tapping them over clean absorbent paper.

C. Incubation with conjugate

- Dispense 100µl of ready-to-use HRP- Conjugate into each well.
- Cover the strips with a plate cover and incubate for 1h at 37°C in a moisture chamber.
- Discard the liquid content and wash as described in steps 9-10.

D. Incubation with TMB - Substrate

- Dispense 100µl TMB-Substrate into each well, cover the strips with a plate cover and incubate at room temperature for **15 minutes**.
- Stop the reaction by adding 100µl of stop solution (1M H₂SO₄) to each well.

E. Determination of Results

- 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.

- C40: OD ≥ 0.8
C20: OD ≥ 0.4
C10: OD ≥ 0.2
- Ratio OD C40 / OD C20 : >1.3
- Ratio OD C20 / OD C10 : >1.3
- Negative Control: <10 IU/ml (See chapter – Calculation and interpretation of Test Results)

Calculation of Test Results

1. Manual method, using a squared graph paper:

- Plot the absorbance values (OD) of the 3 calibrators (C10, C20 and C40) on X axis versus their concentration (IU/ml) on Y axis.
- Using the standard curve, interpolate the concentration of the tested sample values (in IU/ml) from each absorbance measured (see example 1).

2. Computerized method using MS-Excel:

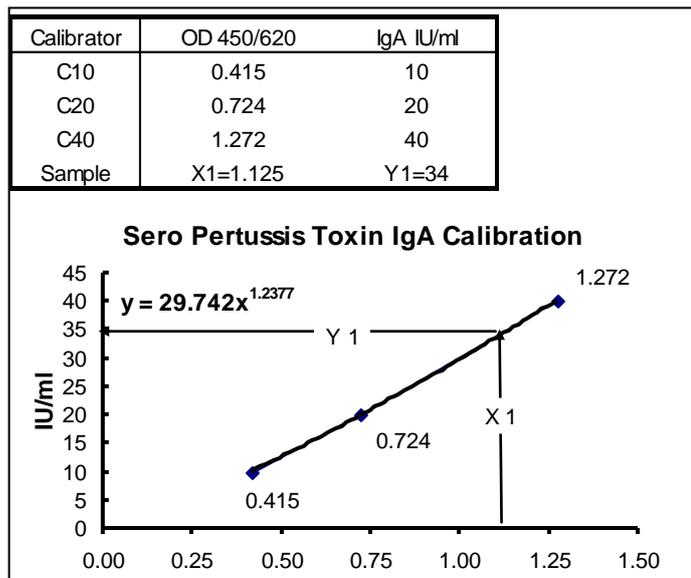
- Open an MS-Excel spreadsheet
- In raw B, mark down the values of the three calibrators (10, 20, 40) and in raw A add the corresponding OD values.
- Click the plot icon () and choose scatter plot. Plot raw B against raw A.
- Right click on one of the plotted symbols and choose "Add Trendline...".
- Under the "Type" tab" choose "Power" and under the "Options" tab choose "Display equation on chart". Click the "OK" button.

- vi. An equation in the form of $Y = aX^b$ will be displayed where Y corresponds to the PT concentration in IU/mL and X to the OD_{450/620}. Use this equation to calculate the PT concentration according to the OD values for each given sample (see example 1).

Example 1. Interpolation of results:

Read the absorbance value of the sample (X1) and mark it on the X axis. Draw a vertical line from this point to the calibration curve, and from the intercept draw a horizontal line to the Y-axis.

Read the concentration in IU/ml of the sample.



Excel: (Y) =29.742*1.125^1.2377

Interpretation of Results

- Validity of sample results:** If OD_{450/620} of a certain sample is ≥ 3.0 , it is recommended that the sample will be re-tested at a dilution of 1:404. The result in IU/ml of this particular sample should be then multiplied by a factor of 4.
- Cut-off:** According to recent literature and recommendations from reference laboratories across the EU (Guiso et al, 2010), Savyon Diagnostics recommends the following interpretation of results:

IgA IU/ml	Result
<10 IU/ml	Negative
≥ 10 IU/ml <15 IU/ml	Borderline
≥ 15 IU/ml	Positive

- IgG/IgA antibody profiling:** In order to achieve a comprehensive antibodies profile, Savyon Diagnostics recommends to also to test IgA levels:

IgG	IgA	Interpretation
Negative	Negative	No indication of <i>B.pertussis</i> infection (see test limitations)
Positive	Positive	Indication of current infection
Positive or Negative	Positive	Indication of recent infection
Positive	Negative or Positive	Indication of recent or past infection or past immunization

Test Limitations

- No single serological test should be used for final diagnosis. All clinical and laboratory data should be taken into account.
- 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.
- 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%
40IU/ml	10	42	3.6
10IU/ml	10	11	6.8

Inter-assay (between-run) precision:

Sample	No of Replicates	Mean OD Value	CV%
40IU/ml	10	43	8.7
10IU/ml	10	10	12.4

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	Temperature Limitation
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	In Vitro Diagnostic Medical Device
	Manufacturer
	Authorized European Representative