Version 14-06/25

User's Manual

Human Anti-Pertactin IgG ELISA Kit

REF **DEIA-250-PHG**

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 Human Anti-B. Pertussis Pertactin IgG ELISA Kit detects and quantifies B. pertussis pertactin-specific IgG in human serum or plasma of vaccinated or immunized hosts. This immunoassay is suitable for:

(1) Determining immune status relative to non-immune controls;

(2) Assessing efficacy of vaccines, including dosage, adjuvantcy, route of immunization and timing;

(3) Qualifying and/or standardizing vaccine batches and protocols.

This assay is for research use only (RUO), not for diagnostic use.

General Description

Pertussis, also known as Whooping Cough, is a highly contagious disease caused by Bordetella pertussis bacteria. Vaccines for pertussis, available in combination with vaccines for tetanus, diphtheria, H. influenza b, hepatitis & polio, use acellular components, primarily the inactivated pertactin. The toxin, a protein exotoxin, produced only by B. pertussis, is central to pertussis pathogenesis; vaccination with the toxoid elicits high levels of protection from the disease. Also included are two other highly immunogenic pertussis proteins: pertactin (PRN or p69), an outer membrane protein that promotes adhesion to host cells, and filamentous hemagglutinin (FHA). P.69 is produced as a large (910-aa) precursor protein. It is proteolytically processed at its N and C termini to produce P.69 and P.30, which are located at the cell surface and in the outer membrane, respectively. The Anti-B. Pertussis ELISAs quantify antibodies produced by vaccines, or from infection with the toxin-producing organisms.

Principles of Testing

The Human Anti-Pertactin IgG ELISA kit is based on the binding of antibodies in samples to pertactin immobilized on the microwells, and anti-pertactin IgG antibody is detected by antihuman IgG-HRP (horseradish peroxidase) enzyme. After a washing step, chromogenic substrate (TMB) is added and color is developed by the enzymatic reaction of HRP on the substrate, which is directly proportional to the amount of anti-pertactin IgG present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microwell reader. The activity of human IgG antibody in samples is calculated relative to anti-pertactin calibrators.

Reagents And Materials Provided

1. Pertactin Coated Strip Plate: 8-well strips(12). Coated with pertactin, and post-coated with stabilizers.
2. Anti- Pertactin IgG Calibrators: 1 U/ml, 2.5 U/ml, 5 U/ml, 10 U/ml. 0.65ml for each. Four (4) vials, each containing antipertactin; in buffer with antimicrobial.
3. Anti-Pertactin Positive Control: 0.65 ml. Anti-pertactin; diluted in buffer with protein, detergents and antimicrobial. [Value range on label]
4. Low NSB Sample Diluent (LNSD): 30 ml. Buffer with protein, detergents and antimicrobial. Use as is for sample dilution.

5. Wash Solution Concentrate (100x): 10ml.
6. Sample Diluent Concentrate (20x): 10ml.
7. Anti-Human IgG-HRP Conjugate Concentrate (100x): 0.15ml.
8. TMB Substrate: 12ml. Chromogenic substrate for HRP containing TMB and peroxide.
9. Stop Solution: 12ml. Dilute sulfuric acid.

Materials Required But Not Supplied

1. Pipettors and pipettes that deliver 100ul and 1-10ml.
2. Disposable glass or plastic 5-15ml tubes
3. Stock bottle to store diluted Wash Solution; 0.2 to 1L.
4. Distilled or deionized water to dilute reagent concentrates.
5. ELISA reader at 450 nm and ELISA plate washer

Storage

The microtiter well plate and all other reagents, if unopened, are stable at 2-8 °C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

Specimen Collection And Preparation

Serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For serum, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage.

Caution: Human serum and other bodily fluids may contain infectious material. Always wear gloves when handling human samples, including the standards and controls (which have been tested non-reactive for HbsAg and Anti-HIV), and dispose of these samples and containers as biohazard waste.

Antibody Stability & Dilution

Initial dilution of serum into Working Sample Diluent (WSD) is recommended to stabilize antibody activity. This enhances reproducible sampling, and stabilizes the antibody activity for years, stored refrigerated or frozen. Further dilution into Low NSB Sample Diluent (LNSD), which provides the lowest assay background, should be at least 10 times the initial dilution and performed the same day as the assay.

Example:

Initial (1/5): 10ul serum + 40ul WSD [or 0.1ml + 0.4ml]

Further (1/50): 10ul initial (1/5) + 90ul LNSD (1/50)

Plate Preparation

Bring all reagents to room temperature (18-30°C) equilibration (at least 30 minutes).

1. Determine the number of wells for the assay run. Duplicates are recommended, including 8 Calibrator wells

and 2 wells for each sample and control to be assayed.

2. Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
3. Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

Assay Procedure

Assay Design

Review Interpretation of Results before proceeding:

1. Select the proper sample dilutions accounting for expected potency of positives and minimizing non-specific binding (NSB) and other matrix effects; for example, net signal for non-immune samples should be lower than the 1 U/ml Calibrator. This is usually 1:200 or greater dilution for human serum with normal levels of IgG and IgM.
2. Run the Anti-Pertactin Positive Control; value range is on the label.
3. Run a Sample Diluent Blank. This signal is an indicator of proper assay performance, especially of washing efficacy, and is used for net OD calculations, if required. Blank OD should be <0.3
4. Run a set of Calibrators, which validate that the assay was performed to specifications: 10 U/ml should give a high signal (>1.5 OD); 1 U/ml should give a low signal which can be used to discriminate at the Positive/Negative threshold.

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. 1 st Incubation [100ul – 60 min; 4 washes]

(1) Add 100ul of calibrators, samples and controls each to predetermined wells.

(2) Tap the plate gently to mix reagents and incubate for 60 minutes.

(3) Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

2. 2 nd Incubation [100ul – 30 min; 5 washes]

(1) Add 100ul of diluted Anti-Human IgG HRP to each well.

(2) Incubate for 30 minutes.

(3) Wash wells 5 times as above.

3. Substrate Incubation [100ul – 15 min]

(1) Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.

(2) Incubate for 15 minutes in the dark, e.g., place in a drawer or closet. Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop Step [Stop: 100ul]

(1) Add 100ul of Stop Solution to each well.

(2) Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

(1) Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.

(2) Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

Calculation

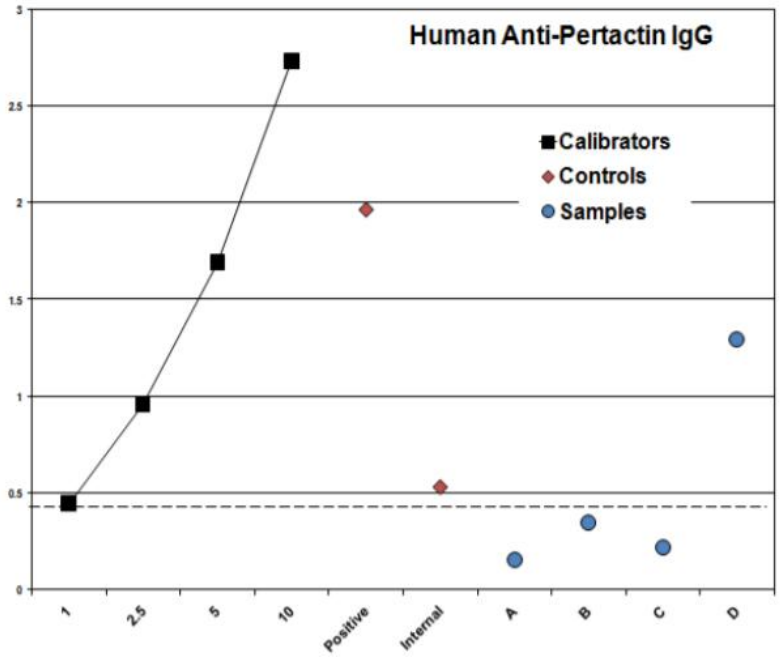
The calibrators are dilutions of antibody reactive to pertactin. Values are assigned as arbitrary anti-pertactin activity units.

Interpretation Of Results

Method A. Antibody Activity Threshold Index

Compare Samples to 1 U/ml Calibrator or Internal Control =Positive/Negative Cut-off.

Example:



Results

The sensitivity of the assay to detect anti-Pertactin IgG, from either natural infection or vaccination, is controlled so that the 1 U/ml Calibrator represents a threshold OD for most true positives in human serum diluted to 1:200 or greater. Visual inspection of the data in the above graph shows the following:

Calibrators – dilution curve of antiserum from pertactin immunization, shows the OD range of the assay; high value indicates optimal sensitivity of the assay.

1U /ml: a 'Cut-off' line has been drawn to indicate a threshold distinguishing between Positive/Negative. This is not a clearcut threshold, rather a low OD area that could represent either low positives or high background negatives.

Positive Control – a serum with reactivity to PRN; value range is on the label. This Control can be used to assess reproducibility, and to normalize between-assay variation.

Internal Control – a true low positive from an immune animal that represents the lab's experience in distinguishing low positive from negative samples (not included in the kit). This should be run in each assay to supplement the 10 U/ml Calibrator for Positive/Negative discrimination purposes.

Samples A,B,C,D – 3 samples (1:200) (A, B, C) are negative: below the threshold; 1 sample (D) is positive: clearly above the threshold.

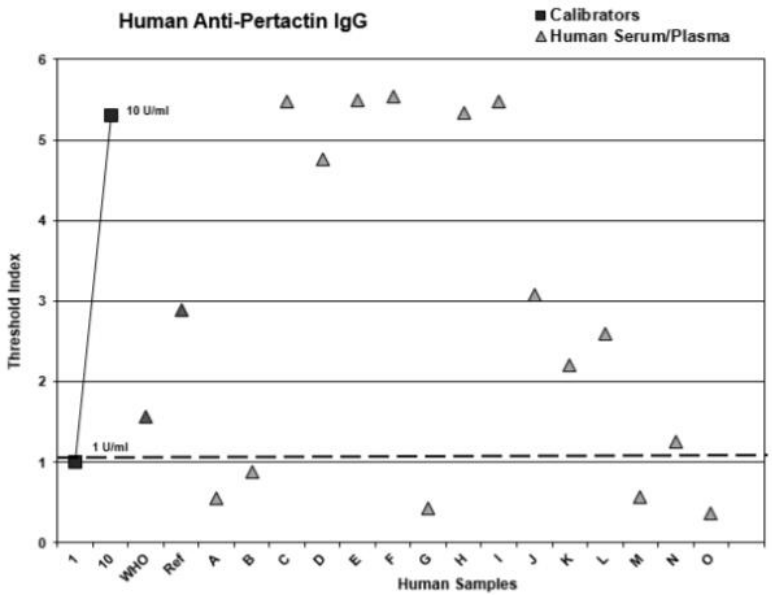
The 1U/ml Calibrator can be used to calculate a Threshold Index that numerically discriminates Positive/Negative:

❖ Divide each Sample net OD by the 1 U/ml Calibrator net OD. Values above 1.0 are a measure of Positive Antibody Activity; below 1.0 are Negative for antibody.

Example:

Human Serum/Plasma IgG

A panel of human serum/plasma of unknown history, and several reference sera, were tested for anti-pertactin IgG (1:200 dilution). Threshold Index was calculated using the 1 U/ml Cal.



Results

Human Anti-Pertactin IgG: ten (10) of the NHS/P were positive (Threshold Index>1.0) at 1:200 dilution. When a significant portion of the positives are>4 Index, it may be more useful to run dilution curves to calculate titers.

WHO Reference 1 st RR: preparation 06/142, human antipertussis (pertactin: 39 IU/ml) established as immunoassay reference. Index: 1.57 for 26 mIU/ml prep [1.0 U/ml = 16.5 mIU/ml]

Non-WHO Reference: human anti-pertussis sera from infection or vaccination, established as immunoassay reference. Index: 2.9 for 1:5k dilution.

Notes:

1. Positives may be due to prior infection and/or vaccination with B. pertussis.

2. The sensitivity of the assay may be adjusted by changing the sample dilutions: a) increase dilution (e.g., 1:400) to lower the signals of borderline positives to negative, b) decrease dilution (e.g., 1:100) to convert borderline samples to positive. With the latter, the values of negatives may increase, so an alternative threshold should be considered using known negatives to develop a Positive Index (see below) or use an Internal Control.

B. Positive Index

Experimental sample values may be expressed relative to the values of Control or Non-immune samples, by calculation of a Positive Index. One typical method is as follows:

1. Calculate the net OD mean + 2 SD of the Control/Nonimmune samples = Positive Index.
2. Divide each sample net OD by the Positive Index. Values above 1.0 are a measure of Positive Antibody Activity; below 1.0 are Negative for antibody.

A sample value would be Positive if significantly above the value of the pre-immune serum sample or a suitably determined non-immune panel or pool of samples, tested at the same sample dilution.

This calculation also quantifies the positive Antibody Activity level, assigning a higher value to samples with higher Antibody Activity, and vice versa.

Method C. Titers from Sample Dilution Curves

The titer of elevated antibody activity calculated from a dilution curve of each sample is recommended as the most accurate quantitative method. Best precision can be obtained using the following guidelines:

1. Use an OD value Index in the mid-range of the assay (2.0 – 0.5 OD); this provides the best sensitivity and reproducibility for comparing experimental groups and replicates. An arbitrary 1.0 OD is commonly used.
2. Prepare serial dilutions of each sample to provide a series that will produce signals higher and lower than the selected index. With accurate diluting, duplicates may not be required if at least 4 dilutions are run per sample.
3. A 5-fold dilution scheme is useful to efficiently cover a wide range which produces ODs both above and below 1.0 OD. The dilution scheme can be tightened to 3-fold or 2-fold for more precise comparative data.
4. The Positive and Sensitivity Control values can be used to normalize inter-assay values.

Calculations

1. On a log scale of inverse of Sample Dilution as the x-axis, plot the OD values of the two dilutions of each positive sample having ODs above and below the OD value of the Index (arbitrary or selected Calibrator).
2. From a point-to-point line drawn between the two sample ODs, read the dilution value (x-axis) corresponding to the OD of the selected Index = IgG Antibody Activity Units

Sensitivity

The pertactin coating level, HRP conjugate concentration and Low NSB Sample Diluent are optimized to differentiate antipertactin IgG from background (non-antibody) signal with human serum samples diluted 1:100.

Specificity

Purified full length recombinant pertussis pertactin is used to coat the microwells; thus the assay is specific

for antibodies directed to pertussis pertactin. The anti-human IgG HRP conjugate reacts with human IgG antibodies that bind to pertactin on the plate. IgA, IgM and IgE class antibodies would not be measured above background signals.

Precautions

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

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