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BORDETELLA PERTUSSIS

IGG CLASS

96 WELL ELISA KIT

PACKAGE INSERT
INSTRUCTIONS AND INFORMATION

Store at 2-8°C



Product Code: 300 0060
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1. INTENDED USE

This kit is an *in-vitro* laboratory assay for the determination of IgG class antibodies to Bordetella pertussis and Bordetella pertussis toxin in human serum.

2. INTRODUCTION

Bordetella species are non-spore-forming encapsulated bipolar gram negative coccoid-bacilli approx 0.3-0.5um thick and 1um long. B.pertussis and B. parapertussis are the human parasites with B. bronchiseptica and B. avium causing enzootic infections in some wild and domestic animal species. B. pertussis is the classical exciter of pertussis and exists only in ill people. Humans are the reservoir of B. pertussis. B parapertussis causes 5-20% of a milder and often clinical unapparent form of pertussis. P.bronchiseptica may occur as opportunistic secondary infecter in mixed infections.

Pertussis, also known as whooping cough, is a bacterial infection of the respiratory tract. The B. pertussis stick to the cilia of the epidermal cells of the respiratory system. Fragments of the bacterial cell wall inhibit the movement of the cilia in the tracheal mucosa. It most commonly appears in children, especially newborn to 6 months, and less commonly in adults. It is a highly contagious childhood disease transmitted by respiratory contact. The disease incubation time is 1-3 weeks with the disease course taking upto 4 months. The distribution of the disease is worldwide.

Clinical pertussis can provide an immunity that is long lasting but not life long. Immunisation can provide up to 90% protection for up to 12 years.

3. PRINCIPLE OF THE TEST

The Bordetella pertussis IgG assay is an indirect solid-phase enzyme immunoassay that detects B. pertussis specific IgG in human serum.

Bordetella pertussis/toxin antigens (coated onto microwells) bind corresponding antibodies in patient's serum, forming an IgG-Ag complex. Anti-human IgG conjugated with the enzyme horseradish peroxidase (HRP) is added and binds to immobilized IgG.

This complex is then reacted with a specific substrate, Tetramethylbenzidine (TMB), to yield a blue colour. The intensity of the colour is proportional to the amount of Bordetella pertussis/toxin specific IgG antibodies in the serum. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour.

Following termination of colour development, the absorbance is measured at 450nm and reference at 620nm.

4. KIT COMPONENTS

1. Bordetella pertussis Coated Wells (IgG): 12 breakapart 8-well snap-off strips coated with Bordetella pertussis antigen; vacuum sealed, in resealable aluminium foil.
2. Bordetella pertussis IgG Positive Control***: 1 bottle containing 2 ml; coloured yellow; ready to use; red cap.
3. Bordetella pertussis IgG Cut-off Control***: 2 bottle containing 1.5 ml; coloured yellow; ready to use; green cap.
4. Bordetella pertussis IgG Negative Control***: 1 bottle containing 2 ml; coloured yellow; ready to use; blue cap.
5. IgG Sample Diluent ***: 2 bottles containing 50 ml of buffer for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; amber cap.
6. Bordetella pertussis anti-IgG conjugate** : 2 bottles containing 10 ml of peroxidase labelled rabbit antibody to human IgG; coloured blue, ready to use; blue cap.
7. Washing Solution (20x conc.)*: 1 bottle containing 50 ml of a 20-fold concentrated buffer (pH 7.2 ± 0.2) for washing the wells; white cap.
8. TMB Substrate Solution: 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB); ready to use; amber cap. Colourless solution.
9. Stop Solution: 1 bottle containing 15 ml sulphuric acid, 0.2 mol/l; ready to use; clear cap

* contains 0.01 % Kathon after dilution

** contains 0.2 % Bronidox L

*** contains 0.1 % Kathon

10. 1 Strip holder

11. 1 Package Insert

5. OTHER EQUIPMENT REQUIRED BUT NOT SUPPLIED

This protocol has been validated for the Triturus™ Analyser

1. MICROWELL PLATE READER (capable of reading at 450 / 620 nm).
2. WASH BOTTLE - 500mL or suitable MICROWELL PLATE WASHER.
3. MEASURING CYLINDERS
4. MULTICHANNEL PIPETTE
5. REAGENT TROUGHS
6. VARIABLE VOLUME PIPETTORS (10µL – 2mL) AND DISPOSABLE TIPS

7. INCUBATOR 37°C
8. DISTILLED WATER
9. VORTEX TUBE MIXER
10. TIMER
11. DISPOSABLE TUBES

6. ASSAY PROCEDURE

- Review all instructions thoroughly before testing.
- Room temperature incubations should be performed at 20 – 24°C.
- All reagents, samples and controls should be brought to room temperature before use.
- Unused strips are to be returned to the foil pouch along with the desiccant and resealed.
- The kit components should not be left at room temperature for longer than the procedure requires. Store kits and kit components at 2 – 8°C when not in use.
- Kit components from different lot numbers should not be interchanged.
- The WASH BUFFER CONCENTRATE (20x) may crystallise at 4°C. Incubate in a 37°C water bath until crystals have dissolved.
- To ensure accurate quantification, it is essential that all pipettes used in the assay are calibrated and a fresh tip is used for cut-off, controls and samples.
- Specimens can be stored at 2-8°C for 5 days before testing. If the assay is not completed within this time then aliquot and freeze (-20 to -70°C). Avoid repeated freezing and thawing.
- This assay requires

| |
|-----------------------------|
| 1 well for substrate blank |
| 1 well for negative control |
| 2 wells for cutoff |
| 1 well for positive control |
- Once started, finish the assay without interruption.

1. Perform a 1/101 dilution on the test sera.

NB: CONTROLS ARE READY TO USE

2. Remove the required number of microwells from the sealed bag and place them in the holder. Ensure unused wells are resealed and stored at 4°C.

3. Pipette 100µl of diluted samples and ready-to-use controls to the appropriate wells. Remember to leave a substrate blank.
4. Incubate for 60 MINUTES ± 5 MINUTES AT 37±1°C
5. Dilute the WASH BUFFER CONCENTRATE (20x) 1/20 with distilled water. Place in either a wash bottle or plate washer reservoir.
6. Wash Procedure: Aspirate the samples from the wells and then fill all wells with diluted WASH BUFFER (350µL/well) soak for 20 seconds on the first wash. Repeat the above for 4 more cycles but with no soak time.
NB: The washing protocol needs to be programmed for a STRIP WASH not a plate wash.
7. Add 100µL of Bordetella pertussis anti-IgG CONJUGATE to all microwells except the substrate blank.
8. Incubate the plate for 30 MINUTES ± 5 MINUTES at ROOM TEMPERATURE.
9. Wash the plate as in step 6 using a plate wash. NB no soak time is required in this wash step.
10. Add 100µL/well of TMB SUBSTRATE to all microwells. Incubate the plate at ROOM TEMPERATURE for 15 MINUTES.
11. Stop the reaction by adding 100µL/well of STOP SOLUTION to the microwells.
12. Read the plate at 450/620nm within 15mins of stopping the reaction
13. Calculate the corrected optical densities (O.D.) by subtracting the Blank O.D. from the mean O.D. of the controls and samples.

7. CALCULATION AND INTERPRETATION OF RESULTS

The CUT-OFF is the mean absorbance value of the Cut-off control OD's.

Samples are considered DETECTED if the absorbance value is higher than 10% over the cut-off.

Samples are considered NOT DETECTED if the absorbance value is lower than 10% below the cut-off.

Samples with an absorbance value 10% above or below the cut-off should not be considered as clearly DETECTED or NOT DETECTED but in a grey zone.

It is recommended to repeat the test again 2 -4 weeks later with a fresh sample. If results in the second test are again in the grey zone the sample is to be considered NOT DETECTED.

If a unit result is required apply the following formula

$$\frac{\text{Patient (mean) OD}}{\text{Cut-off}} \times 10 = ?? \text{ units}$$

| | |
|---------------|------------|
| Cut-off : | 10 units |
| Grey Zone: | 9-11 units |
| NOT DETECTED: | <9 units |
| DETECTED: | >11 units |

8. PRECAUTIONS

1. In compliance with article 1 paragraph 2b European directive 98/79/EC, the use of this in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the test kits with analysers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
2. All components containing material of human origin have been tested and found negative for HBsAg, anti-HCV and antibodies to HIV. They should still, however, be treated as though they were potentially infectious.
3. Do not substitute any component for the ones supplied with the kit.
4. All components that contain preservatives must have care exercised in handling/disposing of these products.
5. Heat inactivation or repeated freezing and thawing of serum samples may cause erroneous results.
6. Performing the assay at temperatures other than those stated may cause erroneous results.
7. Do not use components after the expiry date stated on the label.
8. Care should be taken to reduce microbial contamination of reusable kit components.
9. To avoid cross-contamination do not interchange screw caps of components.
10. It is recommended that external or "in-house" controls be included with each assay.

WARNING: In the used concentration Bronidox L has hardly any toxicological risk upon contact with skin and mucous membranes!

WARNING: Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!

9. REAGENT STORAGE AND SHELF LIFE

- All kit components must be stored at 2 – 8°C. All reagents are stable until labelled expiration date when stored at 2 – 8°C.

10. SPECIMEN COLLECTION

- Blood should be collected by venepuncture.
 - Specimens can be stored at 2-8°C for 5 days before testing. If the assay is not completed within this time then aliquot and freeze (-20 to -70°C). Avoid repeated freezing and thawing.

11. QUALITY CONTROL

- The Substrate Blank, Cutoff, Positive Control and Negative Control should be run each time the assay is performed.
- Substrate blank: Absorbance value lower than 0.300.
- Negative control: Absorbance value lower than 0.250.
- Cut-off control: Absorbance value between 0.250 and 0.900.
- Positive control: Absorbance value equal to or greater than the cut-off value.

12. LIMITATIONS

- As with other diagnostic test procedures, the results obtained serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves.
- Data from immunocompromised patients and newborns have only restricted value.
- Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values
- Interferences with haemolytic, lipemic or icteric sera are not observed up to a concentration of 10 mg/ml haemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

13. TECHNICAL PERFORMANCE

A. INTRA-ASSAY VARIATION

| Sample No. | n | Mean | %CV |
|------------|----|-------|-----|
| Pos Serum | 20 | 0.55 | 3.9 |
| Pos Serum | 24 | 0.148 | 5.2 |

B. INTER-ASSAY VARIATION

| Sample No. | n | Mean | %CV |
|------------|----|------|-----|
| Pos Serum | 12 | 1.5 | 4.9 |
| Pos Serum | 12 | 3.5 | 6.1 |

- The diagnostic specificity is defined as the probability of the assay scoring a negative in the absence of the specific analyte.
It is 72.7 %.
- The diagnostic sensitivity is defined as the probability of the assay scoring a positive in the presence of the specific analyte.
It is 98.8 %.

14. REFERENCES

Wong,K.H., and S.K. Skelton.1989. Preparation of filamentous hemagglutinin from Bordetella pertussis and assay for serum antibodies to filamentous hemagglutinin and pertussis toxin for clinical and public health lab.J.Clin.Microbiol.27:2805-2810

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15. LIMITED EXPRESS LIABILITY

The manufacturer makes no express warranty other than the diagnostic kit will measure IgG antibodies against Bordetella pertussis when used in accordance with the manufacturer's instructions. The use of the diagnostic kit for any other purpose or for the clinical diagnosis of a disease state is outside the intended use of this product.

The manufacturer disclaims any and all implied merchantability, fitness for use or implied utility for any other purpose. Any or all damages for failure of the diagnostic kit to perform according to its instructions are limited to the replacement value of the kit.

In some jurisdictions the law makes these disclaimers unenforceable and, accordingly all or part of the disclaimer may not apply to all users.



Manufactured by:
Vital Diagnostics Pty Ltd
5/18 Lexington Drive
Bella Vista NSW 2153
Australia
P +61 (0)2 9894 6988
F +61 (0)2 9899 6303
ACN 003 153 286



Vital Diagnostics S.r.l.
Via Balzella 41/G/4
47122 Forlì Italy