INTENDED USE

The MarDx B. pertussis PT/FHA EIA Test System is intended for use in testing human serum for the presence of human IgG, IgM and IgA antibody to Bordetella pertussis virulence factors, pertussis toxin (PT) and filamentous hemagglutinin (FHA). The MarDx B. pertussis PT/FHA EIA Test System is not to be used for diagnostic purposes and is intended to be used only when actual diagnosis is based on an established method or procedure including clinical findings. The test system is for professional use only. For Export Only

SUMMARY AND PRINCIPLES

Bordetella pertussis is the etiological agent for a highly contagious, acute and chronic upper respiratory disease referred to as whooping cough or pertussis (2,6). Most overt disease are seen in infants and young children though a milder form is recognized in adults. A whole cell vaccine has greatly reduced disease incidence in many industrialized countries, however, outbreaks still occur (5,6).

After a 7 to 10 day incubation period, disease begins with the catarrhal stage which resembles a common cold (2,6,8). Patients at this stage pose the greatest risk to contacts because bacterial load is at its highest and the cause of disease is normally unrecognized. After 1 to 2 weeks of the catarrhal stage, the paroxysmal stage begins, characterized by the classic whooping cough paroxysms. Paroxysms are episodes of repetitive coughs followed by a rapid inspiration or whoop of air. The patient may have as many as 40 to 50 paroxysms per day. After 2 to 4 weeks the disease enters convalescence where paroxysms diminish in number and severity.

B. pertussis is a slow growing, gram negative cocccobacillus. Isolation is obtained by streaking nasopharyngeal samples to Bordet Gengou or Regan Lowe media usually containing antibiotic to inhibit normal flora. Isolation rates vary (20 to 83%) and are dependent on the time between disease onset and sample collection, the time between sample collection and culturing, and whether or not the patient is on antibiotics (2-4). A direct fluorescent antibody (DFA) test performed on nasopharyngeal samples provides faster but less sensitive results.

Various serological procedures have been developed for the detection of antibody to B. pertussis (2-4, 49). These include the indirect fluorescent antibody (IFA) test, agglutination, complement fixation, enzyme linked immunosorbent assay (ELISA), and Western blot (WB). Early ELISA’s measured antibody to whole cell sonicate preparations. In an effort to improve sensitivity and especially specificity, more recent ELISA’s measure antibody to B. pertussis virulence factors, especially pertussis toxin (PT) and filamentous hemagglutinin (FHA).

PT is a bacterial exotoxin and FHA has been shown to play a major role in the attachment of B. pertussis to the (epithelium of the) upper respiratory tract of the host, the target site for establishment of infection. Antibodies to both PT and FHA develop as a result of infection with B. pertussis. It is believed that such antibodies confer humoral protection against infection. For this reason, both PT and FHA are included in axenic immunization preparations now used as a replacement for the killed pertussis vaccine.

The MarDx PT/FHA EIA test is an indirect enzyme immunoassay (EIA) technique utilizing PT and FHA antigens of B. pertussis bound to polystyrene microwells. The antibody from the patient sample, which is added to the microwells in the first step of this procedure, binds with the antigen. Following a rinsing period which removes unbound antibody, a peroxidase labeled antihuman IgG, IgM and IgA is added to each microwell. The conjugate will attach during the second incubation period which removes unbound peroxidase conjugate, a color indicator solution is added to the microwells which will react only in the presence of bound peroxidase. An acid solution is added after a specified period of time in order to stop the enzymatic conversion of the indicator solution for spectrophotometric analysis.

WARNINGS

1. Do not deviate from the specified temperature and timing requirements as listed in the package insert for both incubation and washing steps. Deviations will significantly alter the results of this test.

2. If more than 48 microwells are to be used in a single assay, dilutions should be transferred from the test tubes to a nonsensitized microwell plate. All the dilutions are then simultaneously transferred using an eight channel pipette to the B. pertussis sensitized microwell plate in order to minimize the difference in the incubation time of the first sample and the last sample applied to the antigen sensitized microwells.

3. Add reagents must be brought to +20 to +25°C before performing this test procedure. Temperatures above or below the recommended range will result in substantial variation of the test results.

4. If a full plate is available, use the strips within the plate frame to secure the strips in place.

5. Do not deviate from the specified temperature and timing requirements as listed in the package insert for both incubation and washing steps. Deviations will significantly alter the results of this test.

ADDITIONAL MATERIALS REQUIRED BUT NOT SUPPLIED

1. Test tubes, 12 x 75mm.
2. Test tube rack.
3. Storage container, 1.0 L, plastic or glass.
4. Pipettes, 10 µL, 100 µL, and 1.0 mol capacity.
5. Pipettor, 100 µL capacity, 8 channel (optional).
6. Timer, 30 minute.
7. Plastic squeeze bottle, 500 mL.
8. Absorbent paper (towels).
9. EIA plate reader with 450 nm filter.
10. Transfer plate, 96 well unsensitized microwell plate.
11. Desiccant storage for open microwell strips.

PRECAUTIONS

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3. If less than a full plate is to be used, center the strips within the plate frame to secure the strips in place.

4. Do not deviate from the specified temperature and timing requirements as listed in the package insert for both incubation and washing steps. Deviations will significantly alter the results of this test.

5. Water desionized with equipment utilizing polyester resin beads may inadverently the peroxidase conjugate.

6. All unused microwells must be stored in desiccation at +2 to +8°C between use.

The safety data sheet is available upon request.

WARNING

Some components of this kit contain 0.1% ProClin 300®, a biocidal preservative that may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.

H317: May cause an allergic skin reaction.

H335: May cause respiratory irritation.

P280: Wear protective gloves / protective clothing / eye protection / face protection.

P302 + P352: IF ON SKIN: Wash with plenty of soap and water.

P333 + P313: If skin irritation or rash occurs: Get medical advice/attention.

P501: Dispose of contents and container in accordance to local, regional, national and international regulations.

WARNING

Some components of this kit contain < 0.1% sodium azide.

H332: Harmful if swallowed.

P264: Wash thoroughly with plenty of soap and water after handling.

P270: Do not eat, drink or smoke when using this product.

P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.

P330: If swallowed, rinse mouth.

P501: Dispose of contents/container to in accordance to local, regional, national and international regulations.
MATERIALS PROVIDED

<table>
<thead>
<tr>
<th>Prod #</th>
<th>Description</th>
<th>Quantity</th>
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<tbody>
<tr>
<td>40-6008</td>
<td>B. pertussis PT/PH/A Microwell Full Plate</td>
<td>96 wells</td>
</tr>
<tr>
<td>40-6009</td>
<td>B. pertussis PT/PH/A EIA High Positive Control</td>
<td>250 μL</td>
</tr>
<tr>
<td>40-6010</td>
<td>B. pertussis PT/PH/A EIA Low Positive Control</td>
<td>250 μL</td>
</tr>
<tr>
<td>40-6001</td>
<td>B. pertussis EIA Negative Control</td>
<td>250 μL</td>
</tr>
<tr>
<td>40-6010P</td>
<td>Peroxidase Conjugate, Anti-Human (Goat) IgG,M,A</td>
<td>13.5 mL</td>
</tr>
<tr>
<td>40-1006</td>
<td>EIA Color Developer</td>
<td>13.0 mL</td>
</tr>
<tr>
<td>40-1004</td>
<td>Stop Solution</td>
<td>15.0 mL</td>
</tr>
<tr>
<td>40-9012</td>
<td>Serum Diluent</td>
<td>100 mL</td>
</tr>
<tr>
<td>40-1013</td>
<td>10x Wash Solution</td>
<td>100 mL</td>
</tr>
</tbody>
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REAGENT PREPARATION

1. Required Volumes of Prepared Reagents

   **TABLE I**
   
   **REAGENT REQUIREMENTS**

   *Wells Required* | Strips Required | EIA Color Developer | Conjugate |
<table>
<thead>
<tr>
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<tr>
<td>1-16</td>
<td>1-2</td>
<td>2.0 mL</td>
<td>2.0 mL</td>
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<tr>
<td>17-32</td>
<td>3-4</td>
<td>4.0 mL</td>
<td>4.0 mL</td>
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<tr>
<td>33-48</td>
<td>5-6</td>
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<td>49-64</td>
<td>7-8</td>
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<tr>
<td>65-80</td>
<td>9-10</td>
<td>10.0 mL</td>
<td>10.0 mL</td>
</tr>
<tr>
<td>90-112</td>
<td>11-12</td>
<td>12.0 mL</td>
<td>12.0 mL</td>
</tr>
</tbody>
</table>

   * 5 Control wells and 1 reagent blank are needed for each run.

2. Prepare 1X wash solution from the 10X Wash Solution provided. On removal of the 10X Wash Solution from refrigeration, undissolved salts may be present. Allow the reagent to reach room temperature and shake the bottle to dissolve the salts. Do not dispense from the reagent until all salts are dissolved. In order to prepare the 10X Wash Solution, dilute 1 part of concentrate with 9 parts of distilled or deionized water in a clean plastic squeeze bottle. For each 16 well strip to be used, prepare a minimum of 150 mL. The final pH should be 7.5 +/- 0.2 pH units.

3. The EIA Color Developer is ready to use.
4. The Serum Diluent is ready to use.
5. The Conjugate is ready to use.
6. Stop solution is ready to use.

PREPARATION OF CONTROLS

A High Positive, three Low Positive, a Negative, and a Reagent Blank are required for each assay run. All control sera are ready for use.

1. To prepare the High Positive Control, pipette 1.0 mL of Serum Diluent to a clean test tube. Add 10μL of the High Positive Control to the test tube and mix. This constitutes a 1:100 dilution. Place tube in appropriate holding rack.
2. To prepare the Low Positive Control, three pre-dilution tubes should be marked. Three separate and individual dilutions are required for the Low Positive Control. To each of the tubes, add 1.0 mL of Serum Diluent. Pipette 10 μL of the Low Positive Control to each tube and mix. This constitutes a 1:100 dilution. Place tubes in an appropriate holding rack.
3. To prepare the negative control, add 10 μL of Negative Control to a marked tube containing 1.0 mL of Serum Diluent. This constitutes a 1:100 dilution. Place tube in an appropriate holding rack.
4. The Reagent Blank is prepared from Serum Diluent without the addition of human serum.

PREPARATION OF SPECIMENS

1. Mark pre-dilution tubes for all samples.
2. To each of the tubes, add 1.0 mL of Serum Diluent.
3. Pipette 10 μL of each sample to the appropriately marked tube.
4. Mix each tube thoroughly.
5. Samples are now diluted 1:100 and are ready for application to the B. pertussis sensitized microwell plates.

TEST PROCEDURE

1. Remove the full plate from its foil pouch. If less than a full plate is used, strips must be centered within the plate frame to secure the strips during the wash steps. Place unused strips back into the foil pouch and seal tightly. Unused strips must be placed in desiccation at +2 to +8°C.
2. Add 100 μL of each prepared control and sample into wells and record on EIA worksheet. A High Positive, three Low Positive, a Negative, and a reagent blank are required for each assay run.
3. Incubate at (20-25°C) for 30 minutes.
4. Shake out contents of the wells. Riffle each well with 1X wash solution.
5. Repeat Step 4 five times and blot strips dry on absorbent paper.
6. Add 100 μL of conjugate into each well.
7. Incubate at (20-25°C) for 30 minutes.
8. Repeat Steps 4 and 5.
9. Add 100 μL of Color Developer into each well. Positive wells will develop a blue color.
10. Incubate at (20-25°C) for 10 minutes.
11. Add 100 μL of Stop Solution to each well. Positive wells will develop a yellow color.
12. Incubate for 2 minutes.
13. Read the test strips by an EIA plate reader at 450nm within 30 minutes after addition of stop solution.

QUALITY CONTROL

A spectrophotometer or EIA plate reader with a 450 nm filter is needed for the analysis of the color intensity.

1. Zero reader on blank well. Read plate.
2. After the plate has been read, calculate the mean optical density (OD) of the three Low Positive Control wells. If any of the three wells has an OD more than 10% OD units from the mean, disregard that value and recalculate the mean from the remaining two wells. If the OD from the remaining 2 wells are not within 10% OD units, assay results are invalid and must be repeated.
3. Calculate the index values for the High Positive Control and the Negative Control (seebelow).

   The assay is considered valid and reportable when:
   a) The High Positive Control has a B. pertussis Index Value greater than 1.5.
   b) The Negative Control has a B. pertussis Index Value less than 0.6.

INTERPRETATION OF RESULTS

To calculate a B. pertussis Index Value (IV) for the High Positive Control (HPC), Negative Control (NC) and test sera, first multiply the mean OD of the Low Positive Controls (LPC) by the correction factor on the LPC vial. The corrected mean OD is then divided into the OD’s of the HPC, NC and test sera to obtain the respective Biv’s.

   HPC and NC BIV = Control OD / (Mean LPC OD X LPC Correction Factor)

   Test Serum BIV = Test Serum OD/ (Mean LPC OD X LPC Correction Factor)

REPORTING

Positive - Biv of greater than or equal to 1.2
   Presence of antibodies to B. pertussis PT and/or FHA.

Negative - BIV of less than 0.8
   Absence of antibodies to B. pertussis PT and FHA.

   If pertussis is still suspected request a second sample to be taken 2 to 4 weeks after the first and retest.

   Equivocal - BIV of greater than or equal to 0.8 and less than 1.2
   For laboratories wishing to use an equivocal zone, Index Values between 0.8 and 1.19 can be reported as equivocal, suggesting the patient be retested in two to four weeks.

LIMITATIONS OF PROCEDURE

All test results must consider the clinical history presented by the patient and other test result.

EXPECTED VALUES

In patients without present or past infection with B. pertussis, test results should be negative except in immunized patients or patients with cross-reacting antibodies.

REFERENCES


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Consult Instructions for Use

Product Number
Lot Number
In Vitro Diagnostic Medical Device
Authorized Representative in the European Community

Use By

Caution, consult accompanying documents

Temperature limitation

Manufacturer

WARNING

Negative Control
Conjugate
EIA Microwell Full Plate
EIA Color Developer
Stop Solution
EIA Serum Diluent
EIA 10X Wash Solution

High Positive Control
Low Positive Control
Correction factor

EC REP - Trinity Biotech plc.
IDA Business Park
Bray, County Wicklow, Ireland
Phone: +353-1-276-9800
Fax: +353-1-276-9888
Web: www.trinitybiotech.com

Manufactured By
MarDx Diagnostics, Inc.
A Trinity Biotech Company
5919 Farnsworth Court
Carlsbad, CA 92008
Phone: 800-325-3424
Fax: 760-929-9124
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