

## Directigen Meningitis Combo Test

For the detection of *Haemophilus influenzae* type B, *Streptococcus pneumoniae*, *Escherichia coli* K1, Group B *Streptococcus* and *Neisseria meningitidis* Groups A, B, C, Y and W135

\*\*\*See package insert for more information\*\*\*

### Specimen Collection and Handling:

Specimens should be tested as soon as possible. Serum must be separated from whole blood prior to testing or storage.

### SPECIMEN PREPARATION:

#### Procedure for Use with CSF:

1. Heat specimens for 3 min at 100°C and allow to cool to room temperature before use. For optimal sensitivity, *N. meningitidis* Group B and *E. coli* should be performed on UNHEATED specimens.
2. For CSF specimens showing turbidity, centrifuge after heating for 10 min at 1400 x g prior to testing. The supernatant fluid is to be used as the test specimen.
3. Test specimens as described in “Procedures”.

#### Procedure for Use with Serum:

1. Dilute serum specimens of at least 0.6mL 1:1 with **Directigen** Specimen Buffer and mix.
2. Heat specimens for 5 min at 100°C and allow to cool to room temperature before use.
3. Using a wooden applicator stick, break up the protein “clot” formed, and vortex vigorously.
4. Centrifuge at a minimum of 1400 x g for 15 min.
5. Test supernatant fluid as described in “Procedures”.

#### Procedure for Use with Unconcentrated Urine:

1. Dilute urine specimens of at least 0.4mL 1:1 with **Directigen** Specimen Buffer and mix.
2. Heat specimens for 5 min at 100°C and allow to cool to room temperature before use.
3. Centrifuge at a minimum of 1400 x g for 10 min.
4. Test supernatant fluid as described in “Procedures”.

#### Procedure for Use with Concentrated Urine:

1. Urine samples that are turbid or have particulate material should be centrifuged at 1400 x g for 10 min before concentrating.
2. Urine samples may be concentrated 25-fold with a Minicon B-15 concentrator.
3. Dilute at least 200µL of urine concentrate 1:1 with **Directigen** Specimen Buffer and mix.
4. Heat specimens for 5 min at 100°C and allow to cool to room temperature before use.
5. Centrifuge at a minimum of 1400 x g for 10 min.
6. Test supernatant fluid as described in “Procedures”.

#### Procedure for Confirmation of Colonies from Culture:

1. Locate suspected colonies on the agar surface from 18-24 h cultures that meet morphological and Gram stain characteristics of organisms that are appropriate for testing with **Directigen** latex reagents.
2. Pipette 0.5mL (approx. 10 drops) of Negative Control reagent into a small glass tube.
3. Select 2-3 isolated colonies of similar morphology from the original or subculture plate using a sterile loop and suspend into the above tube to achieve a suspension equal to a McFarland #1 turbidity standard.
4. Heat the suspension for 3 min at 100°C and allow to cool to room temperature before testing.\
5. Centrifuge at minimum of 1400 x g for 10 min.
6. Test supernatant as described under “Procedures”.

## **PROCEDURES**

### **User Quality Control:**

Include Positive and Negative Controls with each batch of specimens.

### **Performance of Test:**

1. Dispense one drop of **Control +** onto circles 1 through 6 of row **Control +**. Place one drop of **Control -** onto circles 1 through 6 of **Control -**.
2. Micropipette 50µL of test sample onto circles 1 through 6, row **S** and in circles labeled “Control Latex A” and “Control Latex B.” Rows + and - are used for controls.
3. Holding the dispensing bottle by the cap, vigorously swing (without inverting) to thoroughly mix **Reagents 1-6** and **Reagents A** and **B**. Before uncapping each bottle, gently tap base on counter to ensure that no latex reagent remains in the tip.
4. Dispense one drop of **Reagent A** onto the Control Latex A circle. Repeat the procedure dispensing **Reagent B** onto the Control Latex B circle.
5. Dispense one drop of **Reagent 1** onto the circles in column 1, rows +, -, and **S**. Repeat the procedure for the remaining Latex Suspensions (**Reagents 2-6**) in rows +, -, and **S**, columns 2 through 6.
6. Mix the specimen sample and Latex Suspension in each circle with a plastic stirrer, alternately using first one end, and then the opposite end for the next circle. Discard the stirrer.
7. Place the card or glass slide on a mechanical rotator and rotate at a speed of  $100 \pm 2$  rpm for *10 min*. Use a moistened humidifying cover to prevent evaporation.
8. Immediately at the end of 10 min, read the test results macroscopically under a high intensity incandescent light.

### **Interpretation of Test Results:**

Record Positive and Negative Control test results first: The positive controls should yield strong agglutination within 10 min. The negative controls should show no agglutination. Agglutination in any of the circles containing Negative Antigen Control renders the reaction uninterpretable.

Record patient test results:

**Positive Test** – Should show agglutination. Any degree of agglutination present in one of the latex reagents indicates the presence of the corresponding antigen. *Agglutination in two or more latex reagents or the corresponding **Directigen** Control Latex renders the reaction uninterpretable.*

**Negative Test** – Should show no agglutination.