| KCMC Biotechnology Laboratory, Microbiology | STANDARD OPERATING PROCEDURE | Effective Date 7 Nov 2006 | SOP-Number MIC.034.01 |
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| | | Page 1 of 8 | Date 7 Nov 2006 |

**Title: DRYSPOT PNEUMO TEST**

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**Approvals/Date:**

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**This SOP has been read and understood by:**

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**Title: DRYSPOT PNEUMO TEST**

**Document History:**

| Version Number | Reason for Changes | Date |
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**Title: DRYSPOT PNEUMO TEST**

**PURPOSE**
For presumptive identification of *Streptococcus pneumoniae* from growth on solid media or blood culture..

**PRINCIPLE**
The Oxoid DrySPOT Pneumo Test uses antibody sensitized blue latex particles coated with antibody to capsular antigens of most recognized serotypes *S. pneumoniae* dried onto dots on cards. A drop of supernate from a positive blood culture or colonies suspended in saline is added and mixed with the latex reagent. The latex particles will agglutinate in the presence of sufficient antigen. A control with latex particles coated with non-reactive globulin is included with each test to check for autoagglutination.

**SCOPE**
This Standard Operating Procedure applies to the testing of positive blood cultures or colonies suspected of being *S. pneumoniae* by technical staff in the microbiology laboratory that have been trained and are competent in the use and performance of this test.

**SPECIMEN COLLECTION – NA**

**SAFETY PRECAUTIONS**
Dispose of all contaminated materials in infectious waste.

**MATERIALS/STORAGE**
- Oxoid DrySPOT Pneumo Test kit:
  - Test Reagent Cards with Test and Control areas (20 cards/3 tests per card.)
  - Positive Control Strips (10)
  - Negative Control Strips (10)
  - Phosphate Buffered Saline (PBS)
  - Mixing paddles
- Inoculating loop
- Saline

**EQUIPMENT**
- Centrifuge for preparation of blood culture aliquot.

**QUALITY CONTROL – NEW LOTS**

**Frequency:** Each shipment/lot of test kits

**Controls/Acceptable Results:**

Positive Control Strip*/Positive
Negative Control Strip*/Negative
*included in kit

**Procedure:**

1. Add a 50 ul drip of PBS to the small circle at the bottom of the oval reaction area.
2. Tear off a Positive Control Strip without touching the colored spots at the end of the strip.
3. Turn the stick over so the spots are on the bottom and place stick on the card with the spots touching the liquid.
4. Push down so the end bends at the hinge and mix in a circular manner for 10 seconds to rehydrate the dried reagent (blue spots on card).
5. Rock the card and look for agglutination.
6. Repeat the above using the Negative Control Strip.

**Acceptable Results:**

Positive Control – Agglutination within 60 seconds
Negative Control – No agglutination within 60 seconds.

**Corrective Actions for Unacceptable Results**:

1. Repeat test using fresh Control Strips of control organisms.
2. If still unsatisfactory, do not use for identification of isolates.
3. Arrange for replacement.

**Documentation:**

1. Record QC results on DRYSPOT PNEUMO TEST QC sheet.
2. Record unacceptable results and corrective actions on a QC DEVIATION form and submit for supervisor review.
3. supervisor will review QC monthly..

**QUALITY CONTROL – EACH TEST**

1. The Control oval on each card is inoculated with either blood culture supernate or the test organism to check for autoagglutination. Any reaction in the Control oval renders the test uninterpretable.

**OPENING TEST KIT**

1. Remove pouch containing test cards and allow to reach room temperature before opening.
2. Cut the pouch open just below the seal.
3. Remove the number of cards needed within 10 minutes of opening the pouch. Cards may be cut along the indicated lines if fewer tests are being performed.

**TEST PROCEDURE - POSITIVE BLOOD CULTURE**

1. Remove a 1-2 ml aliquot from positive blood culture into a sterile capped test tube.
2. Centrifuge at 1000g/3000 rpm for 5 minutes to pellet the RBCs.
3. Dispense 1 drip of supernatant to the small ring at the bottom of each oval of both the Test and Control Reaction Areas. DO not allow the liquid to mix with dried latex reagents.
4. Using a loop or mixing paddle provided mix the supernatant into the dry control latex spot until completely suspended and spread to cover the reaction area.
5. Discard loop or paddle.
6. Using a new loop or paddle, proceed in the same way with the Test Latex.
7. Rock the card for up to 2 minutes and look for agglutination under normal lighting conditions. Do not use a magnifying glass.
8. Dispose of card into infectious waste container.

**TEST PROCEDURE - CULTURE**

1. Dispense 1 drip of saline inside the small ring at the bottom of each oval of both the Test and Control Reaction Areas. DO NOT allow the liquid to mix with dried latex reagents.
2. Using a sterile loop or mixing paddle provided remove several colonies from the culture plate and apply to the saline in the Control reaction area. Mix colonies with the saline until a smooth suspension is achieved.

3. Using the loop or paddle provided mix the organism suspension into the dried latex spots until completely suspended and spread to cover the reaction area.
4. Using a separate loop or paddle, repeat the procedure in the same way with the Test Latex.
5. Rock the card for 60 seconds and look for agglutination under normal lighting conditions. Do not use a magnifying glass.
6. Dispose of card into infectious waste container.

**TEST INTERPRETATION**

| RESULT | INTERPRETATION |
|---|---|
| Agglutination on the Control area. | TEST IS UNINTERPRETABLE |
| Agglutination of latex particles in Test oval within 60 seconds for culture confirmation, no agglutination in Control oval. | POSITIVE[a] |
| Agglutination of latex particles in Test oval within 2 minutes for blood cultures; no agglutination in Control oval. | POSITIVE |
| No agglutination in the Test Reaction area, smooth, blue suspension remains; no agglutination on the Control oval. | NEGATIVE |
| Granular/stringy reaction with greater clearing of the blue background in the Test Reagent compared with that of the Control Reagent | POSITIVE |
| Granular/stringy reaction with no difference between clearing of the blue background in both Test and Control areas. | NEGATIVE |

[a] Presumptive presence of *S. pneumoniae*

**RECORDING RESULTS**

Enter results on POSITIVE CULTURE Form.

**REPORTING RESULTS**

1. A report may be issued when a blood culture aliquot is positive identifying the organism as "presumptive *S. pneumoniae*, confirmation in progress" and the microscopic morphology is compatible with *S. pneumoniae* (i.e. elongated Gram positive cocci in pairs and chains)
2. Colonies with morphology compatible with *S. pneumoniae* (alpha hemolytic, smooth or mucoid) and optochin susceptible that test positive may be reported as confirmed *S. pneumoniae.*

**TEST LIMITATIONS**

1. If strain of pneumococcus being tested does not possess capsular antigen, the test will be not be positive.
2. Cross reactions with Group C streptococci, some strains of Strep. viridans group and with Gram negative organisms have been reported.

**REFERENCES**


Package Insert, October 2003. DRYSPOT Pneumo Test, Oxoid Ltd. Basingstoke, Hampshire, UK.

Kristensen, B, etal. 2001. Rapid immunodiagnosis of streptococci and enterococci in blood cultures. APMIS 109:284.


**APPENDIX A – DRYSPOT Pneumo Test QC sheet**

**Title: DRYSPOT PNEUMO TEST**

**APPENDIX A**

**KCMC Biotechnology Laboratory** **Quality Control**
**Microbiology** **DRY SPOT PNEUMO**
**TEST**
**Controls/Acceptable Results*:***
**Positive Control Strip (POS)/Positive**
**NegativeControl Strip (NEG)/Negative** **YEAR: _____**

| QC DATE | QC BY | LOT# | POS(+) | NEG(-) | A/NA* |
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***A/NA = Acceptable/Not acceptable DOCUMENT ALL CORRECTIVE ACTION ON QC DEVIATION FORM**

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| **Supervisor Review :** | | | | | | |
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**RY SPOT PNEUMO QC/QC SHEETS**