**MICRO.CULT.10.0 CULTURE, GROUP A STREPTOCOCCUS**

**PRINCIPLE**

Group A beta-hemolytic streptococcus (GABHS) is an important pathogen that causes pharyngitis, cellulitis, and bacteremia. Serious sequelae, including scarlet fever, acute glomerulonephritis, toxic shock syndrome, and acute rheumatic fever, can result from infections with this organism. GABHS accounts for 30% of pharyngitis cases in children aged 5 to 15 years but only 10% of adult cases. GABHS are important causes of bactermia and skin and soft tissue infections as well. Recently appreciated is the role of GABHS as an etiologic agent of perianal dermatitis in children following swallowing or direct inoculation of infectious respiratory secretions. This procedure describes the detection and identification of this organism by culture.

**OWNERS**

Manager, Regional Microbiology

**SPECIMEN**

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| **Collection and Transport** | |
| Specimen type | Pharyngeal swab  Perianal swab  Inoculated blood agar plate (BAP) |
| Collection containers | Culturette swab  Culturette swab with amies gel |
| Stability and Storage Requirements | Store swab specimens at room temperature for up to 48 hours. Inoculated plates should be incubated at the appropriate temperature prior to submission. |
| Unacceptable specimens | Dry swabs |
| Test codes | QLS: Culture, Group A Streptococcus (4485)  Sunquest: Throat Culture (THC) |

**MATERIALS**

1. Group A Beta Strep Agar, Hardy Diagnostics, store at 2-8°C until use (Regional)
2. Selective Strep agar by BBL, store at 2-8°C until use (Evansville)
3. Trypticase soy agar with 5% Sheep Blood (BAP), store at 2-8°C until use
4. Antibiotic disk impregnated with SXT
5. Inoculating loop
6. Bacti-cinerator
7. Incubator, 35°C ambient air

**QUALITY CONTROL**

1. BAP, SSA (Selective Strep agar), and Group A Beta Strep Agar are exempt from QC beyond that performed by the manufacturer. Each new shipment is checked for appearance. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), hemolysis, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.
2. SXT discs are QC’d upon receipt and weekly thereafter to verify potency.

**PROCEDURE**

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| **If** | | **Then** |
| Inoculated plate is submitted | | Examine plate for evidence of growth. If there is no visible growth on the plate, proceed to step three. If the plate is growing, proceed to step four. |
| Swab specimen | | Proceed to step one. |
| **Step** | **Action** | |
| 1. | Firmly roll swab over one-sixth of the agar surface, and streak carefully for isolation in four quadrants.   * For Regional Micro, inoculate Group A Beta Strep Agar only. * For SVEV, inoculate both BAP and SSA agars.   Note: When Group A Beta Strep Agar or SSA IS not available, it is acceptable to inoculate BAP only as an alternative method. After BAP is streaked, place one SXT disc in the primary quadrant of the inoculated BAP plate. | |
| 2. | Carefully stab the agar several times with the same loop both in an area that has been streaked and in an area that has not been streaked, in order to improve detection of beta-hemolysis. | |
| 4. | Incubate inoculated plate at 35 to 37°C in ambient air.  Note: Plates may also be incubated in 5-10% CO 2 or anaerobically for better development of hemolytic reactions. | |
| 5. | Examine culture media after 24 hours for small translucent or transparent colonies that are dome shaped, have an entire edge, and are surrounded by a relatively wide zone of complete (beta-) hemolysis.  Note: For the alternative method, the growth of normal floral will be inhibited around the SXT disc, making it easier to isolate and detect beta strep A, which is SXT resistant. | |

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| **If** | **Then** |
| No suspicious colonies are observed after 24 hours | Issue a preliminary report and reincubate the plate for an additional 24 hours. |
| No suspicious colonies are observed after 48 hours | Issue a final report and discard the plate. |
| Suspicious colonies are observed | Proceed to step 6. |

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| 6. | Perform Catalase test. |

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| **If** | **Then** |
| Catalase positive | Organism is part of normal flora. No further workup is required. |
| Catalase negative | Proceed to step 7. |

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| 7. | Perform latex agglutination for Group A streptococcus. |

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| **If** | **Then** |
| Agglutination positive for group A streptococcus | Report “[quantity] Group A Beta Streptococcus.” Final the culture and save a representative plate. |
| Agglutination negative for group A streptococcus | Report “No Beta Strep Group A Isolated.” Final the culture and discard the plate. |

**REPORTING RESULTS**

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| **If** | **Then** | **Preliminary** | | **Final** | |
| **Sunquest** | **QLS** | **Sunquest** | **QLS** |
| Negative for Group A *Streptococcus* | Report “No Beta *Streptococcus* Group A Isolated to date” or Culture in progress if Group A is suspected. | English Text Codes:  “NBSA” and “TDATE” | Report PR NGAS in STREPA workcard. File, prelim and verify results. | English Text Code:  “NBSA” | Report FR NGAS in STREPA workcard.  File, final and verify results. |
| Positive for Group A *Streptococcus* | Report “[quantity] Beta Strep Group A. | Not applicable | Not applicable | English Text Codes:  [quantity] “BSAI” and “PENS” | Workup panel WGAS,  Quantitate  File, final and verify results. |
| Positive for A. haemolyticum (see NOTE) | Report  “[quantity] A. haemlyticum” and  “No Beta *Streptococcus* Group A Isolated” | Not applicable | Not applicable | [quantity], “ARCH”  And  “NBSA” | Report NF NGAS in STREPA workcard  Isolate 1:  Report biochem reactions, morphology  and report FR ARHA |

NOTE: If the beta isolate does not type with latex agglutination, perform identification if the organism is GPR to rule in or out of *Arcanobacterium haemolyticum*.

**PROCEDURE NOTES**

1. A positive pharyngeal culture for GABHS indicates the presence of S. pyogenes but does not distinguish between infection and colonization.
2. All GABHS are susceptible to penicillin (the antimicrobial agent of choice for treating infection), negating the need for susceptibility testing. The comment “PENS” will be attached to all isolates of GABHS to inform the physician why routine susceptibility testing is not performed. For penicillin-allergic patients, erythromycin is the therapeutic agent of choice.
3. GABHS can be resistant to erythromycin and clindamycin. Susceptibility testing may be indicated when penicillin therapy is contraindicated.
4. GABHS can be alternatively identified using the following criteria: beta hemolytic colony demonstrating gram positive cocci in pairs and chains that is catalase negative and PYR positive.
5. In additional to Group A, SVEV reports Group C, G beta-hemolytic *Streptococcus* and *Streptococcus pneumonia* if quantity is pure or predominant.

**LIMITATIONS**

1. Falsely negative pharyngeal cultures can result from overgrowth of cultures by normal oral microorganisms or from the lack of beta-hemolysis in cultures incubated aerobically.
2. Group C and G beta-hemolytic streptococci can cause pharyngitis and fever, but they do not place patients at risk of acute rheumatic fever.
3. Culture of the mouth or areas in the oral cavity other than the tonsil or pharynx when bacteria pharyngitis is suspected may yield false negative culture results.
4. SXT discs, used directly with clinical specimens or sources containing mixed flora, enhance in the ability to isolate group A beta-hemolytic strep. More specific physiological and/or serological tests must be used for definitive identification of group A strep.

**REFERENCES**

1. Forbes, B.A., et. Al., Bailey *& Scott’s Diagnostic Microbiology*, Mosby Elsevier, pp 814-821, 2007.
2. Isenberg, H.D., ed., *Clinical Microbiology Procedures Handbook*, 3.11.8, American Society for Microbiology, 2007