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| Strep Group A Culture |
| **Purpose** | This procedure provides instruction for STREP GROUP A CULTURE for the Microbiology laboratory. |
| **Policy Statements** | This procedure applies to Microbiologists who perform culture set-up and plate reading. |
| Principle and Clinical Significance | *Streptococcus pyogenes* (group A beta-hemolytic streptococcus) can be isolated from rectal and vaginal swabs from self inoculation of pathogenic oral flora and can cause peri-rectal cellulitis. The primary cause is *Streptococcus pyogenes* (group A beta-hemolytic streptococcus). Treatment is important because infections with *S. pyogenes* can lead to poststreptococcal sequelae such as acute rheumatic fever, glomerulonephritis, and toxic shock syndrome. Selective Streptococcus Agar incorporates neomycin and polymixin B that suppresses normal flora for improved recovery of *S. pyogenes.*  |
| **Test Code** | TCS |
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|  | **Reagents** | **Supplies** | **Equipment** | **Media** |
| **Materials** | * 3% hydrogen peroxide
* Gram Stain reagents
* PathoDx™ strep grouping
 | * Glass slides (plain)
 | * CO2 incubator
* Incinerator
* Inoculating loop
* Microscope
* MALDI
 | Refer to the Sunquest specimen label for media information.* Selective streptococcus agar (SSBA)
* CNA agar (CNA)
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| Sample |  |  |
|  | 1. Acceptable specimens
* Dacron or rayon tipped swab
1. SDES codes/Specimen type
* RS –rectal swab
* VAG – vaginal
1. Refer to the [*Lab Test Directory - Strep group A culture*](http://www.childrensmn.org/Manuals/Lab/MicroBioViral/034448.asp) for Specimen Collection, Transport and Assessment
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| **Special Safety Precautions** | Microbiologists/virologists are subject to occupational risks associated with specimen handling. Refer to the safety policies**:**1. Biohazard Containment
2. Safety in the Microbiology/Virology Laboratory
* Biohazardous Spills
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| **Procedure** | InoculationWarm SSBA and CNA before inoculation.Label plate properly with the patient’s name, accession number and date. Specimen processing1. Roll swab across the upper quadrant of the agars, (in the same order as the Sunquest label), touching all surfaces of the swab.
2. Streak plates semi-quantitatively for primary isolation.
3. Sterilize the inoculating loop in the incinerator for 5 s to 10 s. Allow the loop to cool.
4. Pass the loop through the edge of the first quadrant approximately 4 times while streaking into the second quadrant. Continue streaking in the second quadrant without going back into the first quadrant 3-4 times.
5. Flame loop again, turn the plate another quarter of a turn, and pass the loop through the edge of the second quadrant approximately four times while streaking into the third quadrant. Continue streaking in the third quadrant without going back into the second quadrant 3-4 times.
6. Make small cuts in the primary area of the SSBA and CNA for hemolysis.

1. Incubation
2. Incubate SSBA and CNA in 4-10% CO2 at 35ºC
3. Culture examination
4. Day 1
5. After 18-24 hours of incubation, examine plates for colonies showing beta-hemolysis. *S. pyogenes* will appear as white to gray colonies surrounded by a zone of beta hemolysis.
6. If beta-hemolytic colonies are present, gram stain each suspicious colony type and perform catalase testing.
7. If large colony size >0.5 mm, catalase negative and a gram positive cocci, perform Patho DX™ Strep Latex Typing.
8. If colony size is <0.5 mm, catalase negative and a gram positive cocci, subculture to SB and perform MALDI for identification the next day.
9. Subculture organisms that are not well isolated to appropriate media for further work-up.
10. Report preliminary results.
11. Re-incubate primary plates and subcultures for an additional day.
12. Day 2
13. Examine primary plates from the previous day for colonies showing beta-hemolysis.
14. Perform MALDI for identification from subculture or PathoDX™ Strep grouping on large colony >0.5 mm.
15. Send updated report and finalize.
16. Save primary plate if positive at room temperature for 7 days in case a physician calls for further studies.
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| **Limitations** | 1. Some strains of group A streptococci are inhibited on SSBA.
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| **Result Reporting** | Culture Results: Record culture results and culture work-ups in Sunquest MRE *Culture Entry* tab in Observations or Workups by using customized keyboards or by entering a code in the result box. Report results semi-quantitatively, i.e., 1+, 2+, 3+ or 4+.

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| Quantity | 1st quadrant# colonies | 2nd quadrant# colonies | 3rd quadrant# colonies |
| 1+ | <10 |  |  |
| 2+ | >10 | <5 |  |
| 3+ | >10 | >5 | <5 |
| 4+ | >10 | >5 | >5 |

1. **Negative culture**: Report “NO BETA STREPTOCOCCI, GROUP A ISOLATED”, Sunquest code **NBS.**
2. **Positive culture:** Sunquest MO code **BSA**

 Observations: 1. 4+ STREPTOCOCCUS PYOGENES, GROUP A  Workups: Wkup # 1 Workup Components Med: SB MSID: 1  Desc: BH GMS : STR Id: BHS CAT : NEG Review **Culture Summary** for accuracy before filing report.If a culture requires a correction, the code **CORR** (corrected report) must be reported on an observation line in the *Direct Exam* or *Culture Entry* tab. Refer to policy MC 5.1 LABELINGERRORS/SPECIMEN MIXUPSfor Sunquest report entry information.If growth should occur or additional testing should be requested after the culture has been finalized, remove the final status and send out a supplementary report. The code SRPT (supplementary report) must be used in SREQ or *Culture Observations* as follows:Updated or new culture information: In the *Culture Entry* tab, enter SRPT on an observation line followed by new results.Requests for additional testing: In the *Misc. Updates* tab, enter **SRPT** in SREQ followed by the request.* Re-final the culture when identifications and/or testing are complete.
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| **References** | 1. York, M., Section 3, Aerobic Bacteriology, 3.11.2, 3.11.3, *Garcia, Lynne*  (ed) *Clinical Microbiology Procedures Handbook*, 2010, American Society for Microbiology, Washington, D.C.
2. Forbes, B.A., et al., Bailey & Scott’s *Diagnostic Microbiology*, eleventh edition, 2007. Mosby, Inc., St. Louis, MO., pg. 899-905.
3. Versalovic, James., et al, *Manual of Clinical Microbiology*, 2011, ASM press, American Society for Microbiology, Washington, D.C., pg. 318-319.
4. Leber, A  *Clinical Microbiology Procedures Handbook*, 4th edition, 2016, Vol. 1, American Society for Microbiology, Washington, D.C.
5. Weinberg, Arnold N. MD, Group C and Group G streptococcal Infection. UpToDate®. Wolters Kluwer Health. 10/2/2013.
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| **Appendices** | WORKLABEL MEDIA DEFINITION  BATTERY SPEC FORM MEDIA TCS ALL UNDF MCSTDMCL-WLB SSBA, CNA, CUT3 RS MCSTDMCL-WLB SSBA, CNA, CUT3 VAG MCSTDMCL-WLB SSBA, CNA, CUT3 |
| **Training Plan/ Competency Assessment** | **Training Plan** | **Initial Competency Assessment** |
| 1. Employee must read the procedure
2. Employee will observe trainer performing the procedure.
3. Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer.
 | 1. Direct observation
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1.0 | Pat Ackerman | 1978 | Initial Version |
| 1.1 | Pat Ackerman | 06/1985 |  |
| 1.2 | Pat Ackerman | 02/03/1992 |  |
|  | 1.3 | Pat Ackerman | 08/10/2003 |  |  |  |
| 1.4 | Pat Ackerman | 01/11/2008 | Updated Sunquest 6.2 reporting information. Revised SRPT and CORR statements. Added Hyper-link to Labeling policy. Added worklabel definition. |
| 1.5 | Becky Carlson | 08/25/2010 | Re-named and revised to include rectal and vaginal sources. Media section updated. |
| 1.6 | Becky Carlson | 07/14/2013 | Removed strep FA testing. Reagent no longer available. |
| 1.7  | Becky Carlson | 12/22/2013 | Added Direct Access notification of positive results. |
| 1.8 | Becky Carlson | 02/26/2014 | Added large and small colony definition. |
| 1.9 | Tina Gronquist | 06/16/2014 | Updated into online format. |
| 2.0 | Becky Carlson | 4/18/2015 | Removed THROAT as acceptable source : Method for Throat specimens is Group A Strep DNA PCR. Removed: South Lake Pediatrics Scant Growth Beta Hemolytic Organism IDRe-numbered from MC 428 for CMS load |
| 3 | Susan DeMeyere | 12/14/2018 | Removed reporting of Group C and G. Removed testing only large colony organisms. Removed finalizing of culture after 24 hours. |
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| **Archived by:** |  | **Archived Date:** |  |