PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

Upper Respiratory Tract Culture Procedure

Department of Microbiology

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1.0 Purpose

Rapid antigen testing is frequently used to screen for the presence of group A beta-hemolytic strep (GAS) in throat swabs collected from patients presenting with signs of strep throat. However, collecting high quality pharyngeal specimens from uncooperative children can be difficult. For this reason, the American Academy of Pediatrics and the Infectious Disease Society of America recommend that negative rapid antigen tests be confirmed by culture. Culture permits the detection of low numbers of GAS in throat specimens.

2.0 Clinical Significance

While most cases of pharyngitis have a viral etiology, GAS is the most common bacterial pathogen. GAS accounts for 30% of pharyngitis cases in children aged 5 to 15 years but only 10% of adult cases. Serious complications, including peritonsillar abscess, acute glomerulonephritis, and acute rheumatic fever can result from infections with this organism. Antimicrobial therapy alleviates pharyngeal symptoms and prevents some of the sequelae of infection.

Throat specimens collected with a swab are heavily contaminated with resident microbiota. In addition, many microorganisms present in the nares and throat are found in both disease and carrier states. As many as 75% of healthy individuals harbor *H. influenzae* and *S. pneumoniae*, 50% harbor *M. catarrhalis*, and 90% of healthy individuals harbor *S. aureus* in the upper respiratory tract. Culturing the upper respiratory tract for these other bacteria does not produce clinically relevant information. Other bacterial causes of pharyngitis include group C and G beta-hemolytic streptococci, *Neisseria gonorrhoeae, Corynebacterium diphtheriae,* and *Arcanobacterium haemolyticum.* Clinicians that suspect bacterial pathogens other than GAS, based on clinical history and presentation, must request specific testing to rule out these pathogens. Except for specimens obtained from patients with cystic fibrosis, a request for "routine" bacterial culture or a request to culture for "all pathogens" on noninvasively collected throat specimens should not be processed. When requests are received by the laboratory, the clinician should be contacted to determine if the specimen was sent for the detection of one of the specific pathogens listed above. If the specimen is being sent to diagnose the bacterial cause of lower respiratory disease, otitis media, or sinusitis, inform the caregiver that a more invasive specimen, such as endotracheal aspirate, maxillary sinus puncture, or tympanocentesis fluid, respectively, is needed to make the diagnosis.

Other upper respiratory tract specimens, such as nasopharyngeal swabs and nasal discharge, can easily be obtained but are also contaminated with resident microbiota and should only be used to test for specific bacteria. Nasopharyngeal specimens may be used for the detection of *Bordetella pertussis*. Nasal swabs can be used to determine MRSA colonization.

3.0 Scope

This procedure is classified under CLIA as Highly Complex. It should be carried out by technical personnel familiarized and trained to identify group A beta-hemolytic strep. Testing includes but is not limited to: morphologic recognition, confirmatory testing, and Quality Control testing of media and reagents.

4.0 Safety - Personal Protective Equipment

Performance of this procedure will expose testing personnel to biohazardous material. All specimens must be handled as potentially infectious material as outlined in the Microbiology Biohazards and Safety document.

The reagents and/or chemicals that are used in this procedure may be hazardous to your health if handled incorrectly. A brief listing of precautions for each chemical hazard is included in the reagent section of this procedure.

More extensive information concerning the safe handling of the reagents and/or chemicals used in this procedure, as well as other important safety information may be obtained by consulting the Material Safety Data Sheet (MSDS). Before performing any part of this procedure, the technologist must take any and all precautions and adhere to all prescribed policies.

This procedure may expose you to:

- Airborne pathogens
- Hazardous reagents

To perform this procedure, you must use:

- Gloves – must be worn when handling specimens and concentrates.
- Laboratory Coat – must be worn when handling specimens and reagents.
- Biological Safety Cabinet – must be used when processing specimens.

Disinfectant following procedure:

- Bleach dilution sprayers can be used for on demand disinfectant.

Reference for spill/decontamination:

- MSDS
- Chemical hygiene plan

5.0 Specimen Collection, Handling and Storage

Two swabs may be collected simultaneously, one for the antigen test and one for culture if necessary. If one swab is collected, the culture plate must be inoculated prior to using the swab for antigen testing. Samples should be collected by extending the swab(s) between the tonsillar pillars and behind the uvula. The swab(s) should be swept back and forth across the posterior pharynx, tonsillar areas, and any inflamed/exudative areas, while avoiding the tongue, buccal mucosa and uvula.

Swabs for culture should be submitted in bacterial transport medium. Specimens are stable at ambient or refrigerated temperatures for 3 d. Do not expose specimens to freezing or excessive heat.

6.0 Materials

6.1 Equipment

- Biosafety cabinet
- Incubator with ambient air at 35 ± 2°C

6.2 Media & Consumables

- GBS Detect™ Agar (Hardy Diagnostics) - store at 2-8°C away from direct light. Medium 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.
- Transfer pipettes
- Wooden applicators
- Glass test tubes and racks

6.3 Reagents

- Streptex Extraction Enzyme - store at 2-8°C.
- Streptex Latex Group A (50 tests) - store at 2-8°C.

7.0 Procedure

7.1 Specimen Processing

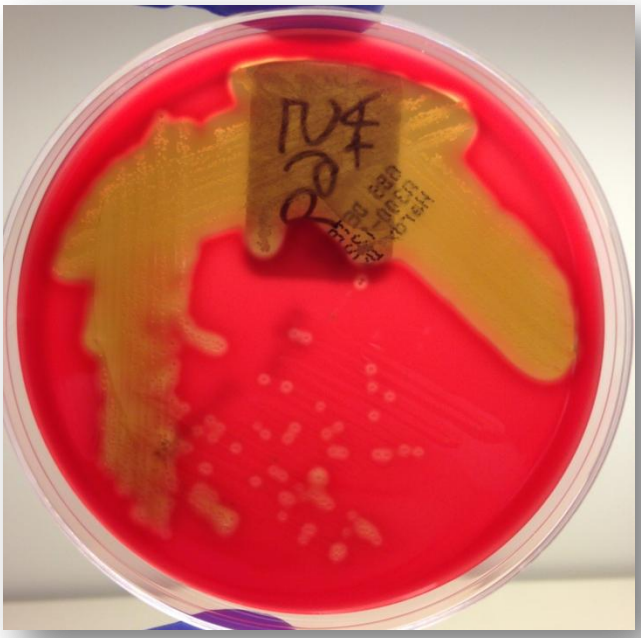
- Routine throat cultures should be ordered as a Beta Strep A Screen (CBSAS). Inoculate a small area of the GBS Detect Agar surface. Streak the plate in quadrants for isolation.
- Culture requests to rule out *Neisseria gonorrhoeae* should be ordered as a screen (CGC). Inoculate a Chocolate/MTM plate and streak for isolation.
- Culture requests to rule out *Arcanobacterium haemolyticum* – add a TSA with 5% sheep blood agar plate (BAP).
- Gram stains should not be performed on throat specimens.

7.2 Incubation

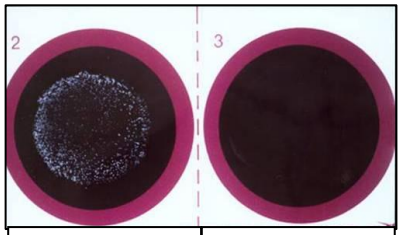
Incubate GBS Detect Agar plates in ambient air at 35 ± 2°C for 18-24 h.

7.3 Culture Workup & Reporting

After 18-24 h of incubation, examine the GBS Detect Agar plate for beta-hemolytic colonies. GAS will produce large, transparent zones of hemolysis. Ignore small, incomplete or weak zones of hemolysis.



Perform Strep Latex Agglutination Test for group A. If there are insufficient colonies to perform latex testing, subculture the isolate and perform latex testing on the following day.



Positive latex agglutination	Negative latex agglutination

7.3.1 Positive Cultures (GAS)

If the latex agglutination test is positive, report:
Positive for group A strep [GAP]

Susceptibility testing is only performed if specifically requested. The following comment should be attached to positive culture reports: **Group A streptococcus is predictably susceptible to penicillins and cephalosporins. Surveillance testing of regional isolates has shown 4% resistance to both erythromycin and clindamycin. [BSAS12]**

7.3.2 Negative Cultures (GAS)

If no beta-hemolytic colonies are seen at 18-24 h, or if the latex agglutination test for group A is negative, issue a final report:
Negative for Group A Strep [GAN]

7.3.3 *Neisseria gonorrhoeae* Cultures

Refer to the Genital Culture Procedure for culture workup.

7.3.4 *Arcanobacterium* Cultures

For cultures requesting rule out for *Arcanobacterium,* examine BAP for hemolytic colonies at 18-24 h. If no hemolytic colonies are observed, incubate for an additional 18-24 h and reexamine BAP. Gram stain any beta-hemolytic colonies. If a colony reveals gram-positive bacilli, consult Rounds.

8.0 Quality Control

Refer to the Strep Latex Agglutination Procedure and the GBS Detect Procedure for Quality Control testing details.

9.0 Validation Information

An extensive evaluation was conducted using four types of media for GAS culture. The media included BBL Trypticase Soy with 5% Sheep Blood Agar incubated anaerobically (BAP), BBL Group A Selective Strep Agar with 5% Sheep Blood incubated in 5% CO_2 (BBL), Remel Strep A Isolation Agar incubated in 5% CO_2 (REM), and Hardy GBS Detect Agar incubated in ambient air (GBSD). A total of 699 throat swabs were placed into 200 µL of Tris EDTA buffer and vortexed for 1 min. Plates were inoculated with 25 µL of eluted specimen, streaked for isolation using a BD InocuLab, incubated at 35°C, and examined at 24 and 48 h. Beta-hemolytic colonies were tested with Streptex® Latex Group A (Remel, Lenexa, KS) and occasionally with catalase reagent.

A total of 63 GAS isolates were recovered with an overall positivity rate of 9%. The recovery rates for each medium at 24/48 h were: BAP 54/55 (86/87%), BBL & REM 51/57 (81/90%), and GBSD 57/58 (90/92%). Five (8%) isolates were missed by BAP due to low numbers, and 3 (5%) GAS were overgrown. Six (10%) isolates were missed by BBL due to a failure to grow. Four (6%) isolates were missed by REM due to low numbers, and 2 (3%) GAS failed to grow. Five (8%) isolates were missed by GBSD due to low numbers. The incidence of non-GAS beta-hemolytic colonies requiring latex testing for each medium at 24/48 h were: BAP 152/220 (22/31%), BBL 34/58 (5/8%), REM 29/35 (4/5%), and GBSD 72/105 (10/15%). The number of subcultures required for each medium at 24/48 h was: BAP 37/70 (5/10%), BBL 20/24 (3/3%), REM 12/13 (2/2%), and GBSD 19/28 (3/4%).

GBSD detected the most (90%) isolates after 24 h. Incubation of GBSD and BAP beyond 24 h did little to improve the sensitivity (2%), and had a negative impact on specificity. However, the sensitivity of BBL and REM increased by nearly 10% with the full 48 h incubation. Although GBSD is the most expensive medium, it moderates the amount of latex testing required, reduces subcultures, eliminates the need for anaerobic or CO_2 incubation, and reduces labor and result turn-around-time by 50% by permitting culture completion at 24 instead of 48 h.

10.0 References

1. Package insert: Hardy Diagnostics GBS Detect™, 071708mg.
2. www.hardydiagnostics.com
3. Clinical Microbiology Procedures Handbook, 3rd ed. and 2007 update, Vol. 2. Garcia, L.S., editor in chief. ASM Press, Washington, D.C.

11.0 Document Control History

Microbiology Director Approval: Dr. Ann Robinson 03/29/2011

Microbiology Supervisor Reviews: Jerry Claridge 03/29/2011, 03/2013

Revisions: 01/27/2012 Updated susceptibility comment for group A strep based on recent surveillance testing. 01/23/2014 Updated procedure for change in media and incubation from BAP-ANA to GBS Detect in ambient air. Changed final report from 48 h to 18-24 h. Added verification study information. Added culture and workup for *Arcanobacterium*.