Throat Cultures for *Streptococcus*

This LabFacts will serve as a ready reference for the proper performance and the related requirements of testing for the presence of group A beta-hemolytic *Streptococcus,* commonly known as beta-Strep or group A Strep, in throat specimens via culture and rapid kit tests. It will explain the various testing methods, proficiency testing requirements, and quality control requirements for each method.

TECHNIQUE FOR SWABBING THE THROAT FOR RAPID ANTIGEN KITS AND THROAT CULTURES

The technique for swabbing the throat is critical to an accurate recovery of the specimen:

1. Depress the patient's tongue completely with a sterile tongue blade. It helps to ask the patient (especially a child) to "pant like a dog". Adults can be asked to say "EHH" (as in "less").
2. Using the appropriate sterile swab, start at one side of the throat. Vigorously swab the tonsillar fissa, go across the back of the throat and finish by swabbing the other tonsillar fissa. Be sure to swab any obvious pus. Withdraw the swab and take care to avoid touching lips, teeth, palate, cheeks, or tongue.

Note: The swabbing should be done very quickly by rotating the swab in a circular motion while swabbing the throat. Rotating the swab assures that all areas of the swab contain the specimen. The patient may gag; however, a "gentle" swabbing does not provide a satisfactory specimen. This is especially important when rapid antigen detection test kits are used, since there is no way to gauge the quality of the specimen. With a culture, a result of "no growth" indicates that the specimen was not satisfactory, since the growth of normal flora is expected.

Selecting the test method to use should be based on cost effectiveness, complexity desired, personnel education requirements, and turn-around time.

RAPID ANTIGEN DETECTION OF GROUP A *STREPTOCOCCUS* TESTS:

COMPLEXITY

The use of a rapid antigen detection test for presumptive identification of Group A *Streptococcus* from a throat swab is considered moderate complexity. However, if a rapid strep kit test is used to confirm a suspicious colony grown on any media, then it is considered high complexity.

SPECIMEN COLLECTION

Most kit tests are packaged with swabs included. If not, sterile Dacron swabs are usually preferred; read the package insert for the type of swab required. Rapid *Streptococcus* antigen tests do not require living organisms; therefore, do not use preservation vials because the preserving fluid dilutes the amount of organism on the swab and could cause false negative results. The kit tests are intended to be run immediately upon collection, so do not hold the swab for a lengthy period. However, many laboratories prefer to culture patients who test negative by rapid antigen detection methods to rule out false negatives. In this case, two swabs should be collected.

PROCEDURE

When using any rapid antigen detection test system, follow the manufacturer's directions for proper test performance. Refer to the package insert for the particular test system you are using.

QUALITY CONTROL

Quality Control is a key element in ensuring the accuracy of whatever test system you choose. Most rapid antigen detection test systems have built-in procedural controls. If your kit has built-in controls then no other external controls are necessary, unless required by the manufacturer.

Observe the control reaction each time you perform this test to verify the performance of each individual test pack. Document the procedural control reactivity at least once per day. If the kit does not have built-in procedural controls, you must test external positive and negative controls upon receipt of a kit and once each day of testing. Check your package insert for these requirements, and document those control reactions as well.

THROAT CULTURE FOR GROUP A *STREPTOCOCCUS:*

COMPLEXITY

The use of selective *Streptococcus* agar plates in conjunction with a bacitracin disk is considered moderate complexity. These plates contain inhibitors to limit the growth of most normal flora.

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All other methods for initial (presumptive) or definitive identification of Group A *Streptococcus* are considered high complexity. This includes the use of 5 percent sheep blood agar plates with a bacitracin disk, or when identifying suspicious colonies grown on any media, and/or confirming them by using a rapid strep test system.

SPECIMEN COLLECTION

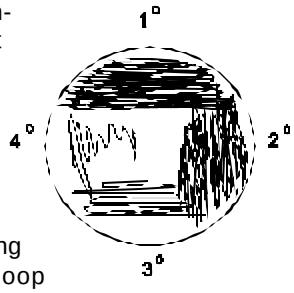
A plain sterile cotton swab can be used if the specimen is to be plated immediately, before the swab dries out. When the swab is not to be plated immediately, the best collection system is a swab/transport medium combination. This combination will ensure that the organisms remain living until the culture is plated. If your laboratory chooses to plate all cultures at the end of the work day, the swab/transport medium combination should be your system of choice. Follow the manufacturer's instructions for the proper use of the swab/transport medium collection system.

PROCEDURE

For all throat culture methods, the testing procedure is as follows:

1. Label the bottom of all plates with proper patient identification and the date of inoculation. It is recommended to use one plate per patient.
2. Roll the specimen swab over 1/4 of the plate. (*See Figure 1,1º*)
3. Discard the swab in a biohazard bag.
4. Using a sterile inoculating loop, streak the second of four quadrants by moving from one edge of the primary area across 1/4 of the media. The streaks should be as close together as possible. Streak into the primary inoculation area a few times to further spread the primary inoculum. (*See Figure 1, 2º)*
5. Rotate the plate 1/4 turn counter clockwise, (the primary inoculation area should be along the bottom of the plate). Stab the loop one to two times through the agar in an area above the primary inoculum. This stabbing will inoculate some organisms below the surface in order to view hemolysis when there is scant growth. Hemolysis is the lysing of the red blood cells within the agar. There are two types of hemolysis; alpha or beta hemolysis. Alpha, or partial, hemolysis leads to a "greening" of the agar around the organism. Beta hemolysis is the complete lysing of the red blood cells contained in the 5 percent sheep blood agar. The agar surrounding an organism that causes beta hemolysis will have a clear appearance.

Figure 1



6. Use the loop to streak the third quadrant by streaking into or dragging the loop through the second quadrant a few times to further spread the inoculum. (*See Figure 1, 3º)* There is no need to flame the loop between streaks. Remember to keep your streak lines close together.
7. Turn the plate counter clockwise one last time, so the previous quadrant is on the left. Streak again, this time in a loose fish-tail like manner for colony isolation. (*See Figure 1, 4º*)
8. Place the bacitracin disk where there will be a high concentration of organisms to demonstrate an area of inhibition around the disk. If using selective strep agar, the disk should be placed in the primary inoculation area. You may select either the primary or secondary streak areas, if blood agar is used.
9. Invert the plate and incubate at 35°C for 18-24 hours. Note: Most manufacturers of selective Group A *Streptococcus* media, such as SXT media, recommend incubation of their plates in a CO_2 environment. Check the package insert for the incubation requirements of your media. When CO_2 is required, you may choose a candle jar, an anaerobic jar, or a CO_2 incubator to achieve the necessary conditions. Check with your local distributor when selecting the system that is best for your laboratory.

IDENTIFICATION

After the incubation period, examine the plates for evidence of beta hemolytic colonies. Group A *Streptococcus* will appear as small, pinpoint clear colonies surrounded by a clear area of beta hemolysis. A clear area around the stabs made into the agar may also be observed. Large white or yellow beta hemolytic colonies may be present but they are not Group A *Streptococcus*. Also look for a red ring around the bacitracin disk where growth of this same organism was inhibited. Refer to Table 1 to help you report findings.

Scant growth of beta hemolytic colonies that did not display a definitive area of inhibited growth around the bacitracin disk should be confirmed by sending the culture to a reference laboratory or by performing a subculture, or using a direct antigen test system.

Be aware that confirmation by subculturing or using a direct antigen test system is high complexity. When subculturing, take one or two similar colonies suspicious for Group A *Streptococcus* and streak in a small area of agar. Place a disk in the center of the streaked area, incubate and read within eight hours. Up to four restreaks may be placed on one plate. The streaks will demonstrate a definitive zone of inhibition very quickly.

QUALITY CONTROL

For throat cultures, quality control is needed for the media as well as the bacitracin disk. If the manufacturer of your media provides a National Committee for Clinical Laboratory Standards (NCCLS) statement of quality control performance, then you are not required to perform any additional quality control on this media. Maintain these statements as part of your record keeping.

If you make the media used in your laboratory, or if the media you purchase has not been tested to meet the NCCLS quality control guidelines, you will need to confirm that the media meets these standards. Refer to the NCCLS document M22-A, NCCLS Standards on Quality Assurance for Commercially Prepared Media. Document these control reactivities in your records.

Test the bacitracin disk against a known positive and known negative organism upon receipt of a new batch or lot of disks and/or media and weekly thereafter. The positive control can be a confirmed beta hemolytic Group A *Streptococcus* from a patient. The negative can be any organism that shows a negative reaction with the bacitracin disk.

NOTE: If you are using selective streptococcus media, your choice for a negative control should be a Non-Group A *Streptococcus*, since the media is designed to inhibit all other types of organisms. Using American Type Culture Control (ATCC) strains of organisms for these controls is not required.

PROFICIENCY TESTING

Regardless of the method you choose, a throat culture is considered a regulated analyte; therefore, enrollment in a COLA-approved proficiency testing (PT) program for this test is required. PT samples will be sent three times a year. You will need to report results back to your PT company, using the same test system and procedure that you use for your general patient population. For further details on PT, see COLA LabFacts 8 *Proficiency Testing,* or call the COLA Information Resource Center at (800) 981-9883.

BIBLIOGRAPHY

1. Baron, E., Peterson, L., Finegold, S. *Bailey and Scott's Diagnostic Microbiology*, 9th ed. St. Louis, C.V. Mosby. 1994.

2. Finegold, S., Martin. W., Scott, E. *Bailey and Scott's Diagnostic Microbiology,* 5th ed. St. Louis. C.V. Mosby. 1978.

3. National Committee for Clinical Laboratory Standards. *Quality Assurance for Commercially Prepared Microbiological Culture Media.* NCCLS Document M22-A, 1990.

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Table 1

Beta Hemolysis Present?	Growth Inhibited by Bacitracin Disk?	Report As:
Yes	Yes	Presumptive Positive for Group A beta *Streptococcus*
No	Yes/No	No Group A *Streptococcus* isolated
Yes	No	Beta Hemolytic colonies isolated, not Group A *Streptococcus*