I. GENERAL PRINCIPLE

Anaerobic bacteria cause a wide variety of human infections, but they are overlooked or missed unless the specimen is properly collected, identified, and transported to the laboratory. The specimen must then be processed appropriately for isolation. Anaerobes vary in their sensitivity to oxygen and nutritional requirements. A brief exposure of 10 minutes is enough to kill some organisms. So, proper collection, media, and incubation are all vital to the recovery of anaerobic bacteria from a clinical specimen.

Knowing which anaerobes have been isolated helps a physician diagnose disease, decide on treatment, determine clinical significance, and select proper antimicrobial therapy. Isolation of anaerobes from clinical specimens, determining the number of anaerobes present, and establishing the clinical significance of the species are all dependent on proper collection and transport of specimens.

II. SPECIMEN COLLECTION

A. The best specimen for anaerobic culture is an aspirate obtained by needle and syringe.

1. Clean the needle puncture site with alcohol and then disinfect with an iodine solution (usually a 10% solution of povidone-iodine). Allow the disinfectant to dry prior to collection.

2. Aseptically perform the aspiration from the deepest part of the lesion with 3-5 ml syringe and a 22- to 23-gauge needle. If aspirating a vesicle, collect fluid from the base of the lesion. EXPRESS EXCESS AIR FROM THE SYRINGE. Remove needle and discard in sharps container. Replace the sterile cap on the syringe if necessary to transport specimen in this manner.

a. If the initial aspiration fails to yield material, sterile nonbacteriostatic 0.85% saline may be injected subcutaneously and the aspiration attempt repeated.

b. If no material is obtained, the needle and syringe may be rinsed in the laboratory by pulling liquid culture media through the needle into the syringe. This is the only time the needle may be included in the transport of the specimen.

3. Using the same aseptic technique, the physician may also perform a percutaneous aspiration to obtain pleural, pericardial, peritoneal, or synovial fluid for anaerobic culture.

4. Transfer small volume specimens from the syringe to an anaerobic culturette. See following section for detailed instructions on use of the anaerobic transport culturette. Large volumes of specimen should be placed in a sterile screw cap tube.

B. Tissue samples and biopsies are also very good specimens for anaerobic culture.

1. Punch biopsies, bone, or any other tissue specimen may be collected surgically.

2. Place specimen in a sterile container without formalin.
C. When a swab must be used to collect a specimen, use a commercially prepared anaerobic culturette system and take special care to sample the active site of infection.

1. Clean the site of surface debris and disinfect as above.

2. Remove anaerobic culturette from package.

3. Remove white plastic plunger with swab attached. Sample only the infected area. Carefully avoid surrounding surface areas that may be inhabited by normal anaerobic flora.

4. Replace swab through hole in gray stopper and guide into the inner small glass tube. Hold tube at 45° angle and press down on the disc portion of the plastic plunger with gentle pressure until it rests flat against the stopper. The inner tube will be forced into the outer tube.

5. Rotate the tube with a swirling motion to facilitate the mixing of air in the inner tube with the hydrogen atmosphere in the outer tube.

III. TRANSPORT

A. Rapid transport of specimens to the laboratory is always the best policy.

B. Very small volume specimens (<1.0 ml) should be transported in the syringe, without the needle. Delivery to the lab should be within 10 minutes.

C. Small volume specimens (1.0-2.0 ml) should be transferred to the anaerobic culturette immediately after collection at the bedside. Discard the needle and syringe in the sharps container at the collection site. Transport time can be up to 2-3 hrs.

D. Large volume specimens (>2.0 ml) should be transferred to a sterile screw cap container at the bedside, as well as tissue or bone samples. Transport should be as soon as possible, but can be delayed up to 2 hrs.

E. The anaerobic culturette can maintain organism viability up to 72 hrs, as long as the indicator in the bottom of the large glass tube is not pink, but prolonged delays must be documented on the report.

F. Transport all specimens at room temperature and avoid extremes of heat or cold.

G. As always, transport specimens to lab in a biohazard bag.
IV. NOTES AND PRECAUTIONS

A. Do not use the anaerobic culturette if:
   1. The package is damaged.
   2. The indicator disc in the bottom of the large glass tube is pink.

B. **DO NOT EVER REMOVE THE GRAY STOPPER OF THE ANAEROBIC CULTURETTE DURING COLLECTION.**

C. Although aerobic organisms may survive in the anaerobic culturette, the system is designed for the collection and transportation of anaerobic specimens. If routine culture for aerobes is also desired, it is best to collect a separate regular culturette.

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**APPROVED BY:** Martin F. Belli, M.D. **DATE:** 4-99, 5-2000

See original policy in the Laboratory for all documented biennial reviews.

**REFERENCES:**