

Testing at a Glance: GC Cultures

Screening for sexually transmitted diseases is common in local health departments. Gonorrhea (GC) is caused by the bacteria, *Neisseria gonorrhoeae*, and is one of the most commonly reported bacterial infections in the United States. GC affects both men and women, especially those 15-24 years old. It is most often found in the genitals, rectum, and throat. If left untreated, complications relating to fertility and chronic pain can result. Accurate identification of a GC infection ensures appropriate treatment is administered to prevent these serious complications.

Media

Proper media practices play a very important role in accurately identifying *N. gonorrhoeae*. The bacteria require nutrient supplementation in order to grow in laboratory cultures. Selective culture media, such as Thayer-Martin and Martin-Lewis agars, contain nutrients that facilitate the growth of *Neisseria*, but also contain antibiotics that inhibit the growth of contaminating bacteria and fungi.

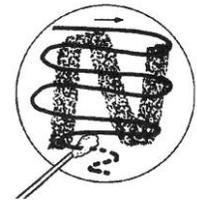
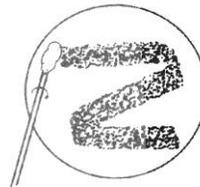
Upon receipt of the media in your laboratory, the media should be visually examined for any defects or deterioration such as cracked media or plates, excessive bubbles and obvious bacterial contamination. Damaged or contaminated media should not be used for testing. Appropriate quality control (QC) testing should be performed according to the manufacturer's directions using known organisms. Check with your distributor to see if this testing has been conducted. If it has, obtain proper documentation of these results. A sterility test should also be performed and results should be documented. One uninoculated plate from each shipment or lot number should be incubated at $36 \pm 1^\circ \text{C}$ for 72 hours to rule out microbial contamination.

The last important item concerning media is storage temperature. Media should be stored in the refrigerator, and brought to room temperature before use. If the warmed plates are not used, they

may be returned to the refrigerator one time. If brought to room temperature again and not used, the plates should be discarded. Try not to remove more plates from the refrigerator than will be needed during that day.

Specimen and Inoculation

Appropriate sites for testing depend on the patient's age, sex, sexual practices, and clinical presentation. Swabs must be made of calcium alginate or Dacron. Other types of swabs may be inhibitory for GC. Immediately after collection, swabs should be rolled over the media in a large "Z" pattern. The plate should then be cross-streaked with a sterile loop.



Incubation

N. gonorrhoeae is a relatively fragile organism, susceptible to temperature changes, drying, UV light, and other environmental stresses. Optimum conditions for growth should be obtained in a timely manner. The plate should be placed in a CO_2 environment (CO_2 incubator or candle jar) within 15 minutes and into an incubator maintained at $36 \pm 1^\circ \text{C}$ within one hour of collection.

Identification

After 24 hour incubation, GC colonies appear gray to white, opaque, raised and glistening. Colonies are often tiny. Some strains may not even appear until 48 hours. Cultures should be examined at 24 and 48 or 72 hours if needed, but not longer. The first step in identification involves the oxidase test. It is essential that you follow the manufacturer's instructions when performing this screening test.

Manufacturers cannot guarantee accurate results if the testing procedure is different than the one stated in the product insert. You must also follow the manufacturer's instructions and test the appropriate QC organisms.

If the patient's result is positive, and acceptable reactions are achieved with the QC organisms, a gram stain should follow. As with the oxidase, follow the manufacturer's instructions. The slide should be prepared and each step should be timed as stated in the product insert and slides with the correct QC organisms tested.

Details are often overlooked during the rinsing step. Some gram stain kits state to rinse with tap water and some state to rinse with sterile water or other water. Be sure your procedure follows the manufacturer's instructions. A presumptive identification of *N. gonorrhoeae* can be reported with a positive oxidase and gram negative diplococci seen on the gram stain.

The role of CLIA

Depending on the type of CLIA certificate your laboratory has, you may have the capability to perform a definitive identification. These systems are considered highly complex tests, so make sure your certificate allows you to perform high-complexity testing. Those with moderately complex certificates can only report presumptive identification based on the screening test results. Those with a Certificate of Waiver or certificate for Provider-Performed Microscopy Procedures are not allowed to perform in-house testing for GC.

Conclusion

Due to the high number of cases each year, the appropriate treatment for patients with gonorrhea is of great significance in the United States. With proper testing and diagnosis, the chances of controlling the easily spread STD are greatly improved.

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