Presumptive Identification of *Neisseria gonorrhoeae*

Requirements for good laboratory practice and COLA Laboratory Accreditation programs are underlined.

This LabFacts is designed to assist laboratories in the presumptive identification of *Neisseria gonorrhoeae (*gonococcus or GC). Identification of GC can be accomplished by several methods. The most popular method, as outlined here, uses growth on selected media, oxidase and Gram stain reactions to provide presumptive identification of GC and is classified as moderate complexity.

SPECIMEN SOURCE

Urogenital or rectal specimens ONLY can be tested for GC and remain moderate complexity. If a different source is tested, such as the throat, this same procedure can be followed, however it becomes high complexity.

SPECIMEN COLLECTION

N. gonorrhoeae has very special growth requirements and is very sensitive to its culture environment. Therefore, it is important to take special care in collecting and culturing the specimen.

- Synthetic (calcium alginate or dacron) swabs rather than cotton swabs are required for specimen collection. Cotton contains fatty acids that are toxic to the GC organism. Cotton swabs may only be used if the culture is to be plated immediately.

- Culture plates must be at room temperature when the specimen is plated because GC is very sensitive to cold.

- The specimen should be plated as soon as possible. This is frequently done in the examining room immediately after collection, rather than transporting the specimen to the lab. Several GC plates can be available at room temperature in the exam rooms or at the nurses station for this purpose. They can be re-refrigerated if not used.

- If the swab cannot be plated immediately, it may be refrigerated at 4-6°C for no longer than 3 hours before inoculation. Swab specimens should never be left at room temperature or incubated at 35°C. In these instances, GC either die off or are overgrown by other bacteria.

Male Urogenital Specimen

Obvious discharge:
- Milk the shaft of the penis until a drop of discharge forms. Collect the drop on a sterile calcium alginate or dacron swab. If a Gram stain is desired, collect two separate swabs.

No obvious discharge:
- Insert a thin sterile urethrogenital calcium alginate or dacron swab 2 cm into the urethra, rotate it and then remove.

Female Urogenital Specimen

The endocervical canal is the preferred site for specimen collection in women. In prepubescent females, a vaginal, not endocervical, specimen is required.

- Moisten a speculum with warm water. Do not use a lubricant as it may be toxic to the organism.
- Remove excess mucus from the cervical os.
- Swab the area with a calcium alginate or dacron swab.

The specimen site of choice for women with urinary frequency, dysuria AND a negative urine culture or for women who have had a hysterectomy is the female urethra.

- Insert a thin calcium alginate or dacron urethrogenital swab (may be moistened with sterile water) 2 cm into the urethra, gently rotating the swab.

Rectal Specimens

Asymptomatic patients:
- Insert a regular size swab about 2-3 cm into the rectum and gently rotate for 10 seconds.
- Use lateral pressure to minimize placing the swab into fecal material.
- If the swab is grossly contaminated with feces, discard and repeat.

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Symptomatic patients:

- Use a disposable anoscope and expose the rectal mucosa.
- Wipe the mucosa with the proper swab.

Pharyngeal Specimens (High Complexity)

- Depress the patient's tongue completely with a sterile tongue blade. It helps to ask the patient to "pant like a dog" or asked to say "EHH" (as in "less").
- 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.

CULTURE MEDIA

The culture media used for the isolation of GC must selectively inhibit other bacterial flora normally found in the urogenital and rectal areas while providing nutrients for the growth of GC. Nonselective media, such as chocolate agar, may be used but the procedure is classified as high complexity.

- Selective media for isolation of GC include Modified Thayer-Martin, New York City media and Martin-Lewis media. The media may be packaged in the standard petri dish or may be incorporated in an environmental system, such as the Jembec plate or Transgrow media.

INOCULATION OF MEDIA

Inoculate the specimen onto culture media at room temperature (do not use media directly from the refrigerator) as soon as possible after collection. The inoculated media must be incubated at 35 degrees Centigrade in a 5-10 percent CO_2 environment. The CO_2 can be supplied by a candle jar, CO_2 incubator, or a biochamber. If a Transgrow or Jembec system is used, the CO_2 is supplied in the system. Follow the instructions provided by the manufacturer when using these systems.

INCUBATION TIME

Plates should be examined for growth (colony formation) within 18 to 24 hours. If no growth is detected, the plates should be incubated for an additional 24 hours, and then reexamined. Temperature of the incubators, refrigerators and ambient air must be monitored daily to assure proper growth environment and preservation of media.

To ensure that the temperature is accurate, check your thermometer against a standardized thermometer at least once a year unless your thermometer is a referenced thermometer.

PRESUMPTIVE IDENTIFICATION PROCEDURE

Growth On Selective Media

Neisseria gonorrhoeae will usually appear as glistening, colorless to grey, transparent to translucent, shiny to mucoid colonies.

Oxidase Test

Colonies observed should be tested with 1 percent oxidase reagent. Oxidase reagent is available as a liquid or in paper strips or discs. Refer to the manufacturer's instructions for the proper use of the oxidase reagent in the format you choose. Colonies that turn purple to black within the manufacturer's prescribed timeframe or that turn the oxidase pad purple when in contact with the reagent are oxidase positive. Oxidase positive colonies should then be Gram stained.

Gram Stain

Gram negative diplococci in a characteristic configuration, flattened on the ends that join each other like coffee beans, are suggestive of *Neisseria gonorrhoeae*.

REPORTING RESULTS

Colonies growing on the selective media must be BOTH oxidase positive AND show characteristic Gram stain morphology for presumptive identification as GC. Report as: "Presumptive positive for *Neisseria gonnorrhoeae*". The organism may be sent to the state or reference lab for confirmation by carbohydrate fermentation if desired. Or an in-office test may be used but the confirmation of Neisseria gonorrhoeae is classified as high complexity.

Before cultures are reported as "no growth", as an additional precaution, some laboratories choose to flood the plate with oxidase reagent. Any previously indiscernible pinpoint colonies of GC will appear dark purple.

QUALITY CONTROL

Culture Media

Once before initial use, each lot or shipment of GC culture media should be checked to see that it can support the growth of *Neisseria gonorrhoeae* and inhibit non-*Neisseria* organisms. (Specific ATCC strains are not required.)

- Inoculate a plate with a known *Neisseria* species and *E. coli*, incubate for 24 hours and check for characteristic growth of the *Neisseria* species. There should be minimal growth of *E. coli*, however pinpoint colonies may be observed.

Oxidase Reagent

Each day of use, the oxidase reagent should be checked with a known oxidase positive organism, such as *Pseudomonas aeruginosa* or *Neisseria* species and an oxidase negative organism such as *E. coli*.

Gram Stain

Each week of use, the Gram stain reagents should be checked against a known gram positive organism such as *Staphylococcus aureus* and a known Gram negative organism such as *E. coli*.

Document all quality control results.

PROFICIENCY TESTING

The isolation and presumptive identification of GC is regulated, therefore proficiency testing (PT) is required. Report results of PT to the same level of identification that patients are reported. For example, if you report patients as "No growth" and "Growth" referred for ID, then report the same for your PT specimens. If you presumptively identify *N. gonorrhoeae,* then indicate that result on your PT response sheet.

CONCLUSION

Remember that *Neisseria gonorrhoeae* is a fragile organism whose growth is easily affected by variation in temperature, CO_2 levels, and the improper use of cotton swabs. Once again, the specimen collection process is critical in the recovery of this organism. A "no growth" culture for GC does not necessarily mean the patient was not infected.

BIBLIOGRAPHY

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