Transportation of Specimens for *Neisseria gonorrhoeae* Culture

Culture plays an important role in the diagnosis and treatment of *Neisseria gonorrhoeae* infections and may be used for confirmation of suspected treatment failure, or phenotypic susceptibility testing for surveillance or clinical purposes. To obtain a *N. gonorrhoeae* isolate, efficient specimen submission and transport from the collection site to the laboratory is critical. However, there remains limited data comparing the multitude of transport systems and there are significant barriers to utilization of the various systems. This document describes the transport systems suitable for transporting primary specimens for *N. gonorrhoeae* culture and isolates for additional testing. Potential advantages and disadvantages of the different categories of transport systems are highlighted.

**BACKGROUND**

*Neisseria gonorrhoeae* (GC) is a fastidious bacterium that can be challenging to isolate and culture. Specific nutritional and environmental conditions must be provided for optimal growth and recovery of GC. Effective specimen collection and appropriate storage and transport are essential to achieve and maintain the organism's ideal growth conditions. Barriers—including but not limited to strict incubation requirements and quick turnaround times between specimen collection and specimen processing—create challenges for both clinical sites and laboratories. When barriers such as these are overcome, GC recovery can be optimized.

The US Centers for Disease Control and Prevention (CDC) published two resources which specifically address use of culture for GC in several situations beyond surveillance for antimicrobial resistance, including treatment failure, test of cure (TOC) and disseminated gonococcal infection: *Update to CDC’s Treatment Guidelines for Gonococcal Infection, 2020* and *Sexually Transmitted Infections Treatment Guidelines, 2021*.

**TRANSPORT AND CULTURE OF PRIMARY SPECIMENS**

Successful isolation of *N. gonorrhoeae* from primary specimens is dependent on many factors associated with specimen collection, transportation and storage including:

- Collection method (e.g., organism entrapment within a swab)
- Selection of media (see below for more detail)
- Transport environment (e.g., temperature, CO2) and duration of transport
- Storage environment (e.g., temperature) and duration in storage
- Overgrowth by competing organisms
- Dilution of the organism in surrounding collection medium.

Using less than ideal practices related to any of the above factors may lead to loss of viability and poor conditions for culturing *N. gonorrhoeae* and, ultimately, to “false negative” culture results. To ensure proper conditions for successful culturing, laboratories must carefully select and subsequently verify their selected transport system. Transport to the laboratory as quickly as possible is vital to the success of the culture. Depending on where primary specimens are being collected (physically near the laboratory vs. further away), laboratories may offer more than one transport system. If sample receipt is delayed compared to specimen acceptance criteria established for the system utilized, re-collection is recommended or, at the laboratory director’s discretion, a disclaimer may be added to the report that the delay in transport could significantly reduce recovery rates.
TRANSPORT CATEGORIES

Multiple microbiological transport systems, formulations and devices are available for collection and transport of specimens for *N. gonorrhoeae* culture (Table 1). Two basic formats include:

- **Nutritive transport system:** Directly inoculated agar plate placed in a container which maintains enriched CO₂ conditions during transport and incubation.
- **Non-nutritive transport system:** Liquid or semi liquid (gel) transport medium (Amies with or without charcoal) and sampling device (swab)

According to Recommendations for the Laboratory-Based Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, nutritive transport systems are generally preferable to swab-based, non-nutritive transport systems due to advantages such as an extended shelf life and better recovery.² Both formats may provide sufficient efficiency for culturing and have various factors to consider for successful test outcome.⁷⁻⁹ Table 1 provides an overview of the available microbiological transport approaches for *N. gonorrhoeae*. Characteristics, advantages, disadvantages and considerations for selecting each transport approach are outlined. While the intent of the table is to assist laboratories in selecting the most appropriate transport approach, individual jurisdictional needs should be factored into the final decision and more than one approach may be needed to serve different needs. It is important to note that not all systems are FDA-approved for transport and/or culture of *N. gonorrhoeae*.

NUTRITIVE TRANSPORT SYSTEMS

Certain transport and culture approaches rely on directly plating the collected swab prior to transportation. If you are considering one of these approaches, selecting the appropriate media becomes a key factor in specimen recovery. Commonly used media-based systems for retrieval of *N. gonorrhoeae* include:

- Modified Thayer-Martin (MTM) agar containing vancomycin, colistin, nystatin and trimethoprim in combination with a CO₂ generation system.
- In-Tray GC (Bio-Med Diagnostics).

VERIFICATION PROCEDURES

It is incumbent on the laboratory to verify the performance of any transport system considered for use with *N. gonorrhoeae* culture. The Clinical and Laboratory Standards Institute (CLSI) developed the M40-A2 Quality Control of Microbiological Transport Systems: Approved Standard¹⁰ to provide guidelines to systematically evaluate transport systems for performance effectiveness. These can be used as a guide for public health laboratories verifying a transport system. CLSI recommends that end users should subject a transport system to the extremes of temperature, pressure and mechanical forces (e.g., pneumatic tubes, airplanes, courier vehicles) prior to use. When evaluating a transport system for GC, laboratories should consider the following:

- CLSI recommends using only *N. gonorrhoeae* ATCC strain 43069 plated to chocolate agar, incubated at 35 °C +/- 2 °C with 5% CO₂ for 24 hours. However, data suggests that gonococcal viability is strain dependent.⁸,¹¹⁻¹³ Use of a variety of patient strains or isolates could provide a more robust evaluation of the transport system’s ability to maintain viability of *N. gonorrhoeae*. Currently there is an isolate panel available for this purpose in the AR Isolate Bank.
- The verification study should encompass the maximum allowable time it may take for specimens to reach the laboratory.
- Prompt processing of the specimen following receipt in the laboratory is essential to ensuring successful culture.
- CO₂ environment during transportation must be considered.
- The verification study should examine survivability of GC across a variety of starting concentrations in a biologically relevant matrix.
- The CLSI standard does not provide criteria for refrigerated transport but does suggest that manufacturers should test at both room and refrigerated temperature. Multiple studies have shown increased survivability of *N. gonorrhoeae* at refrigerated temperatures.⁹,¹⁴
Other resources that may be beneficial to laboratories evaluating transport systems for *N. gonorrhoeae* culture:

- M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*.
- *Individualized Quality Control Plan* (IQCP) for media
- *CLIA-Compliant Analytical Method Validation Plan and Template*
- *General Checklist for Establishment of Performance Specifications for Tests Not FDA-Cleared or Approved*

**LOGISTICAL CONSIDERATIONS AND COMMUNICATION**

When determining which transport system(s) to verify a laboratory should consider logistical factors including:

- Shipping mechanism utilized (i.e., local courier vs. overnight shipment)
- Likelihood of delays in transit
- Client capabilities (GC culture offered, or collection only)
- Supplies and equipment available at specimen collection sites (i.e., incubators, dry ice, refrigeration).

Laboratories must ensure that collection sites either have these transport systems on hand or know that coordination is required to obtain them. Additionally, laboratories should ensure that collection sites understand how to utilize the specialized transport systems. While communication between the laboratory and its clients is always important, it is particularly important when dealing with culture of a fastidious organism.

**ADDITIONAL CONSIDERATIONS AND REMINDERS FOR GC CULTURE**

- When multiple specimens are needed from an anatomic site, GC culture specimens should be collected first. This helps ensure that the maximum bacterial load is obtained and facilitates a successful culture.4

- Laboratories that have the capability to isolate *N. gonorrhoeae* but lack the ability to perform further workup of the organism may choose to send a clinical isolate to a reference laboratory for further testing. *N. gonorrhoeae* isolates are more stable than *N. gonorrhoeae* organisms in primary specimens. Shipping a frozen isolate on dry ice is an effective mechanism that maintains viability of GC during transport as long as the isolate remains frozen in transit. However, in addition to *N. gonorrhoeae* culture capacity, a laboratory that utilizes this mechanism of transport must also have access to personnel trained to ship dangerous goods, dry ice and/or a -80 °C freezer.

- Specimen collection devices intended for nucleic acid testing may not be appropriate for culture, please review the package insert and follow manufacturer’s recommendations.

- Due to potential biosafety concerns, laboratories working up genital and extragenital cultures including presumptive isolates of *N. gonorrhoeae* should be aware of the potential presence of *N. meningitidis* in these cultures. Laboratories should consult with a biosafety expert and/or perform a risk assessment of their testing procedures to ensure safe testing practices.
### Table 1. *N. gonorrhoeae* specimen transportation categories

<table>
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<tr>
<th>Type</th>
<th>Examples</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<td><strong>Nutritive Transport Systems</strong></td>
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| Directly inoculated agar plate placed in a container which maintains CO₂ conditions during transport and incubation. May or may not include a sampling device (swab). | • **In-Tray GC** (BioMed Diagnostics)  
  • MTM media plus  
    ○ **BD GasPak EZ Gas Generating Pouch System**  
    ○ **BD Bio-Bag Environmental Chambers**  
    ○ **Oxoid CO₂ Gen Compact**  
    ○ **Mitsubishi™ Double Zip AnaeroPouch-Bag** and **Mitsubishi™ AnaeroPouch-CO₂**  
    ○ **Candle Jar** | • Immediate inoculation enhances bacterial viability  
  • Self-contained system for production of carbon dioxide*  
  • When properly utilized, viability can be maintained up to 72 hours at room temperature (e.g., 15–25°C**) post collection and inoculation15, 16 | • Refrigerated storage (e.g., 2–8°C**) of transport systems may be required prior to use  
  • Must manually activate system to produce CO₂  
  • Optimal use may require incubators at the collection sites due to pre-incubation step  
  • Cost***  
  • Extra inoculation step following collection means more opportunities for human error |
| **Non-nutritive Transport Systems** |                                                                          |                                                                            |                                                                               |
| Liquid or semi liquid (gel) transport medium (Amies with or without charcoal) and sampling device (swab). | • **Copan Liquid Elution Swab** (Eswab)  
  • **Copan Venturi Transystem** (gel or liquid in sponge)  
  • **Puritan Opti-Swab Liquid Amies Collection and Transport System**  
  • **BD Culture Swab** (EZ Collection, MaxV and MaxV(+)) | • Ease of collection/use  
  • Transport media helps support growth of organism  
  • May be used for transport of other bacteria, not just GC  
  • Collection facilities may be more likely to regularly stock these products  
  • Charcoal containing media may enhance recovery compared to non-charcoal containing media17 | • Organism overgrowth  
  • Requires rapid processing within as little as 24 hours |

* Candle jar requires a separate apparatus to generate CO₂  
** While it is often accepted that refrigerated temperatures span 2–8°C and room temperatures fall within the range of 15–25°C, laboratories should ensure that temperature ranges are specifically defined and aligned with manufacturer’s recommendations.  
*** Candle Jar is lower cost
REFERENCES


Association of Public Health Laboratories

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This project was 100% funded with federal funds from a federal program of $150,000. This publication was supported by Cooperative Agreement #NU60OE000104 from the US Centers for Disease Control and Prevention (CDC). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of CDC.

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