

Transportation of Specimens for *Neisseria gonorrhoeae* Culture

There is a critical need to ensure accurate antimicrobial resistance testing for *Neisseria gonorrhoeae* to help prevent possible treatment failures. An important aspect of this testing is efficient specimen submission and transport from the clinic to the laboratory. This document describes the transport systems suitable for transporting *N. gonorrhoeae* specimens, highlights available performance data and outlines the benefits and limitations of each system. While this document intends to aid laboratories and STD programs to select the most appropriate GC culture system, it is important to recognize that the information may need to be adapted to meet individual facility or jurisdiction needs.

BACKGROUND

In August 2012, the Centers for Disease Control and Prevention (CDC) published an Update to CDC's *Sexually Transmitted Diseases Treatment Guidelines, 2010*.¹ The revised guidelines removed oral cephalosporins as a first-line treatment recommendation for gonococcal infections. The recommended treatment for uncomplicated urogenital, anorectal and oropharyngeal gonorrhea is now combination therapy with a single intramuscular dose of ceftriaxone plus either a single dose of azithromycin or a one-week course of twice-daily doxycycline.

The guidelines specifically recommend GC culture in two situations. First, for patients with suspected cephalosporin treatment failure, CDC recommends that clinicians request *N. gonorrhoeae* culture and antimicrobial susceptibility testing for specimens collected from the site of infection. Second, if an alternative treatment regimen (e.g. cefixime combination therapy with either azithromycin or doxycycline) is used, CDC recommends performance of a test-of-cure (TOC) on specimens collected from the site of infection using culture or a nucleic acid amplification test (NAAT) if culture is not readily available. A NAAT positive TOC should be followed up with the collection of a culture suitable specimen for antimicrobial-susceptibility testing.

These clinical management scenarios require effective methods to transport adequately collected specimens to laboratories for culture and susceptibility testing. Since *N. gonorrhoeae* culture methods are not available at all clinical or public health laboratory facilities, there is a need to provide guidance for transporting specimens to laboratories with this capability.

TRANSPORT AND CULTURE

Successful culturing of *N. gonorrhoeae* is dependent on many factors associated with specimen collection, transportation and storage. Some of these factors are: collection method (e.g.

organism entrapment within a swab), transport environment (e.g. temperature, CO₂), loss of viability due to transport or storage temperature, loss of viability due to duration of transport, overgrowth of *N. gonorrhoeae* by competing organisms in the sample collected, and dilution of the organism in surrounding collection medium.² The result of any of these factors may lead to poor conditions for culturing *N. gonorrhoeae* and ultimately to “false negative” culture results.

Multiple microbiological transport systems, formulations and devices are available for collection and transport of specimens for *N. gonorrhoeae* culture (Table 1). There are three basic formats of devices for GC culture; 1) transport medium in transport container 2) transport systems and 3) transport swabs. All three formats may provide sufficient efficiency for culturing, but have various factors to consider for successful test outcome.

VERIFICATION PROCEDURES

It is incumbent on the laboratory to verify the performance of any transport system considered for use for *N. gonorrhoeae* culture. The Clinical and Laboratory Standards Institute (CLSI) developed the M40-A *Quality Control of Microbiological Transport Systems: Approved Standard*,³ which provides guidelines to systematically evaluate systems for performance effectiveness. These can be used as a guide to laboratories verifying a transport system. CLSI recommends that end users must still subject a transport system to the extremes of temperature, pressure and mechanical forces (e.g. pneumatic tubes, airplanes, courier vehicles) prior to use. It is up to the end user to define acceptable levels of performance. Other limitations that end users should consider when evaluating a transport system include:

- CLSI recommends using only *N. gonorrhoeae*, ATCC strain 43069 plated to chocolate agar, incubated at 35-37C for 24 hours. Multiple research articles have suggested that gonococcal viability is strain dependent.^{4,5,6,7} Use of patient strains or isolates with resistance or decreased susceptibility to cephalosporin and azithromycin could provide a more exacting standard for evaluation of the transport system’s ability to maintain viability of *N. gonorrhoeae*.
- Quality control of combined culture media and transport devices is addressed in CLSI Document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*.
- Survivability of microorganisms on agar plates during transport is not included in the CLSI standard and should be included in the verification. The verification study should encompass the maximum time it may take for specimens to reach the laboratory. This may be 72 hours or more for some laboratories. Falsely negative cultures can result in mismanagement of the patient and lead to more serious disease. If timely delivery of specimens to the public health laboratory cannot be met, it may be prudent to collaborate with a private sector clinical laboratory for this testing. Other transport conditions that should be considered in a verification study are temperature and CO₂ environment during transportation.
- The CLSI standard does not provide criteria for refrigerated transport, but does suggest that manufacturer’s should test at both room and refrigerated temperature. Multiple studies have shown increased survivability of *N. gonorrhoeae* at refrigerated temperatures.

Table 1 is an overview of the available microbiological transport systems for *N. gonorrhoeae*. Characteristics, advantages, disadvantage and performance data (when available) are outlined for each type of transport system. While the intent of the table is to assist laboratories in selecting the most appropriate transport system, individual jurisdictional needs should be factored into the final decision. It is important to note that not all systems are FDA-approved for transporting *N. gonorrhoeae*.^{7,8,9}

Table 1: *Neisseria gonorrhoeae* specimen transportation categories

Type	Description	System	Manufacturer	Performance Data	Advantages	Disadvantages
Transport medium in transport container (nutritive transport)	Directly inoculated agar plate in a container which maintains CO ₂ conditions during transport and incubation	In-Tray GC	BioMed Diagnostics	Direct inoculation of agar should increase the sensitivity of detecting one organism, if properly handled and incubated.	Immediate inoculation enhances bacterial viability, self-contained system for production of carbon dioxide*	Refrigerated storage, must manually activate system to produce CO ₂ , requires pre-incubation prior to transport, short expiration date**, cost***
		Bio-Bag CO ₂ + MTM	BD Diagnostic Systems			
		CO ₂ Gen Compact + MTM	Oxoid (Thermo Scientific)			
		Candle Jar + MTM agar	Various manufacturers			
Transport System (non-nutritive transport system)	Liquid or semi liquid (gel) transport medium (Amies with or without charcoal) and sampling device (swab) Used for transport of other bacteria, not just GC	Copan Liquid Elution Swab (Eswab)	Copan Diagnostics, Inc.	Recoverable GC (ATCC 49226) after 24 hours at room temperature; improves slightly if refrigerated †	Room temperature storage, swab is in 1 mL liquid media, which provides material for additional testing and prevents drying of organisms.	For optimum recovery of GC, plating and incubation must be performed within 24 hours, organism overgrowth
		Eswab Collection and Transport System	BD Diagnostic Systems	None available		
		Copan Venturi System (gel or liquid in sponge)	Copan Diagnostics, Inc.	Less sensitive than Copan Eswab	Room temperature storage, all organisms including GC viable for more than 24 hours	Organism entrapment (gel), organism overgrowth
		BD Culture Swab (MaxV and MaxV(+))	BD Diagnostic Systems	Did not meet the CLSI M40 guidelines for organism recovery at room or refrigerated temperature †	Ease of use, retards penetration of air into system, charcoal helps prolong GC viability, media prevents drying of organisms	24-hour transport time for GC, organism entrapment, organism overgrowth
Transport Swab (non-nutritive transport system)	Specimen device with no transport medium	BD Culture Swab EZ	BD Diagnostic Systems	100% viability of various ATCC GC strains when transported at RT within 24-hours ‡	Ease of use, synthetic fibered swab without liquid media prevents overgrowth of bacteria, room temperature storage	24-hour transport time for GC, poor recovery due to lack of transport media, organism entrapment, organism overgrowth

* Candle jar requires a separate apparatus to generate CO₂

** In Tray GC has a longer expiration date (12 months from date of manufacture)

*** Candle Jar is lower cost

† I Siryani, R Ghneim, A Abu-Rayan, M Kanaan, M Hindiyeh. Evaluation of Eswab against BD CultureSwab MaxV(+) and Medical Wire & Equipment Transwab for the Maintenance of Fastidious Aerobic Bacteria. Poster session presented at: American Society for Microbiology 2009 May 18-20; Philadelphia, PA.

‡ Becton, Dickinson and Company. BBL CultureSwab EZ and EZ II [Package Insert]. Sparks, MD: Becton, Dickinson and Company; January 2010.

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Patricia Armour, MPA, MT(ASCP)

Kirk Bengel, MPH

George Dizikes, PhD

Sarah Guerry, MD

Celia Hagan, MPH

Kevin Karem, PhD

Sarah Kidd, MD, MPH

John Papp, PhD

Olusegun Soge, PhD

Anthony Tran, DrPH, MPH, MT(ASCP)

Susanne Zanto, MPH, MLS(ASCP)

Association of Public Health Laboratories

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8515 Georgia Avenue, Suite 700
Silver Spring, MD 20910
Phone: 240.485.2745
Fax: 240.485.2700
Web: www.aphl.org