

## Comparison of Direct Inoculation and Copan Transport Systems for Isolation of *Neisseria gonorrhoeae* from Endocervical Specimens

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**Two commercial swab transport systems, Copan Amies gel agar with and without charcoal (Copan Diagnostics, Corona, Calif.), were compared to direct inoculation onto modified Thayer-Martin medium for detection of *Neisseria gonorrhoeae* in 1,490 endocervical specimens obtained from women attending a sexually transmitted disease clinic. Copan swabs were held in the transport system for 24 h at room temperature prior to inoculation onto modified Thayer-Martin medium. All cultures were incubated at 35°C in 5% CO<sub>2</sub>, and bacteria were identified on the basis of Gram stain, oxidase, and biochemical reactions. Copan Amies gel agar transport system without charcoal detected 77 of 81 (95%) direct inoculation culture-positive specimens, and Copan Amies gel agar transport system with charcoal detected 53 of 56 (95%) directly inoculated culture-positive specimens. Copan Amies gel agar without charcoal inoculated after 6 h supported growth of 56 (98%) positive cultures out of only 55 directly inoculated culture-positive specimens. This study demonstrates that Copan swabs represent a reasonable alternative, providing convenience, low cost, and ease of use while still maintaining a satisfactory recovery rate of *N. gonorrhoeae* from clinical specimens, if specimens can be inoculated onto selective media within a relatively short time period not involving overnight shipment.**

Despite declining numbers of reported cases of gonorrhea over the past several years, rates of infection remain disproportionately high in adolescents and in minorities. Accurate laboratory detection of this highly communicable disease of major public health importance is still extremely important, not only for acute urogenital infection but because of the likelihood of complications, especially in women, for whom *Neisseria gonorrhoeae* is a leading cause of ectopic pregnancy, pelvic inflammatory disease, and tubal infertility (6, 8).

*N. gonorrhoeae* is a nutritionally fastidious organism that requires an enriched medium in a moist environment of 35 to 37°C with an atmosphere of 3 to 5% CO<sub>2</sub> for growth. It does not grow well in the presence of commensal organisms, and small numbers may be present in clinical material (9, 12, 14). Optimum recovery of *N. gonorrhoeae* from endocervical specimens in women is achieved by direct inoculation of a selective gonococcal agar medium with direct incubation at 35°C in CO<sub>2</sub> (8). However, the direct plating technique is not always practical, or even feasible, in some clinical facilities. To circumvent this need, specialized transport systems have been developed.

Copan Amies gel agar transport systems with and without charcoal (Copan Diagnostics, Corona, Calif.) are available for use in clinical laboratories for collection and transport of endocervical specimens for culture of *N. gonorrhoeae*. However, no studies have compared these systems with direct inoculation of clinical specimens onto selective media. We conducted an investigation to evaluate the ability of Copan transport systems to preserve the viability of *N. gonorrhoeae* in endocervical specimens at different time points in comparison to direct inoculation at the site of collection onto culture media.

### MATERIALS AND METHODS

**Study population.** The study population consisted of 1,490 adult women who sought treatment at the Jefferson County Public Health Department Sexually Transmitted Disease Clinic in Birmingham, Ala., between June 1998 and January 1999. The Institutional Review Board for Human Use at the University of Alabama at Birmingham and the Jefferson County Department of Health approved this study, and informed consent was obtained from all participants.

**Specimen collection.** A total of 1,490 cervical specimens were obtained from women attending the Sexually Transmitted Disease Clinic. A Dacron swab and the Copan swab were separately inserted into the endocervical os and rotated 360° before being removed. The Dacron swab was then streaked onto modified Thayer-Martin (MTM) medium (Remel, Inc., Lenexa, Kans.), placed into a CO<sub>2</sub> biobag (Remel), and incubated at 35°C until transported to the University Hospital Microbiology Laboratory the same day. During the first phase of the study, the Copan transport system without charcoal was used. Swabs were held for 24 h at room temperature prior to inoculation onto MTM agar and incubated at 35°C in an atmosphere supplemented with 5% CO<sub>2</sub>. During the second phase, Copan swabs with charcoal were used and handled the same way. During the final phase, Copan swabs without charcoal were collected and held for 6 h prior to inoculation onto MTM medium. Upon arrival in the laboratory, directly inoculated plates were removed from the CO<sub>2</sub> bag and placed in a 5% CO<sub>2</sub> incubator at 35°C. Swabs from the Copan transport systems were processed as described above and incubated with the directly inoculated plates.

Specimens were picked up each afternoon by courier and transported in a styrofoam container by automobile four blocks to the University Hospital Microbiology Department. Transport time did not exceed 10 min. The order of collection of the two study swabs was randomized to eliminate bias that could occur if bacterial concentration decreased after each collection. Nurses in odd-numbered exam rooms collected the specimens with the Copan swab first and then the Dacron swab. Nurses in even-numbered exam rooms collected the specimens with the Dacron swab first, followed by the Copan swab. In all cases, two additional swabs for diagnostic purposes were collected prior to either study swab.

Agar plates were examined daily for up to 3 days after initial plating of the specimen. The criteria for identification of *N. gonorrhoeae* were the presence of typical oxidase-positive, gram-negative diplococci and utilization of only glucose when tested in the QuadFERM System (bioMérieux Vitek, Inc., Hazelwood, Mo.).

**Statistical analysis.** Prior to study initiation, a power analysis was performed to determine the minimal sample size. A total of 326 subjects were needed for each phase in order to detect an increase in the kappa coefficient from 0.5 (moderate agreement) to 0.75 (strong agreement), assuming a 10% prevalence rate, a type I error rate of 5%, and a power of 90%. Sensitivity to detect a positive culture and kappa coefficient of variation were calculated to determine the degree of agreement between direct inoculation and Copan transport systems with and without charcoal independently (1, 4). A 95% confidence interval for

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TABLE 1. Comparison of direct inoculation with transport in Copan Amies gel agar for recovery of *N. gonorrhoeae* in endocervical specimens

Detection method	No. of specimens tested	No. (%) of positive specimens		Sensitivity (%) <sup>a</sup>	Kappa coefficient of variation (%)	95% Confidence intervals
		Direct	Copan			
Copan without charcoal held 24 h	697	81 (12)	77 (11)	95	97.14	89.7–100
Copan with charcoal held 24 h	466	56 (12)	53 (11) <sup>b</sup>	95	96.86	87.8–100
Copan without charcoal held 6 h	329	55 (17)	56 (17)	98	96.75	85.9–100

<sup>a</sup> Calculations were based on the definition of a true positive being a positive culture for *N. gonorrhoeae* by either direct inoculation or by Copan transport systems.

<sup>b</sup>  $P = 0.97$ , Copan transport system with charcoal versus that without charcoal.

the kappa coefficient of variation was calculated to assess the strength of agreement for each transport system with direct inoculation. The chi-square test was utilized to assess whether statistically significant differences existed between the results for each type of Copan swab transport system relative to direct inoculation (4). A  $P$  value of 0.05 was used to establish statistical significance.

## RESULTS

Of 697 specimens, 81 (12%) were culturally positive for *N. gonorrhoeae* by direct inoculation versus 77 (11%) after transport in the Copan system without charcoal in the first phase of the study (Table 1). This resulted in a sensitivity for detection of a positive culture of 95% for the Copan transport systems. The kappa coefficient showed a 97.14% agreement between direct inoculation and the Copan transport system without charcoal. All matched patient samples negative for *N. gonorrhoeae* by direct inoculation were concordantly negative after being held at room temperature in the Copan system for 24 h prior to inoculation of selective media.

Of 466 specimens, 56 (12%) were culturally positive by direct inoculation versus 53 (11%) by the Copan system with charcoal in phase 2, resulting in a Copan sensitivity for detection of a positive culture of 95%. The kappa coefficient showed a coordinated agreement of 96.86%. All matched patient samples negative for *N. gonorrhoeae* by direct inoculation cultures were concordantly negative after being held at room temperature in the Copan system for 24 h before inoculation of selective media. There was no significant difference ( $P = 0.97$ ) in recovery of *N. gonorrhoeae* with Copan Amies gel agar without charcoal or Copan Amies gel agar with charcoal relative to that with direct inoculation in phases 1 and 2.

To determine whether further improvement in recovery of *N. gonorrhoeae* could be achieved if swabs in the Copan transport system were held for a shorter duration, more realistic for diagnostic laboratories providing service to on-site or nearby clinical facilities, a third phase of the study compared Copan swabs held for 6 h prior to inoculation of selective media with direct inoculation. Since the Copan system with charcoal showed no advantage in preserving organism viability, this phase of the investigation utilized only the swab system without charcoal. Of 329 specimens, 55 (17%) were culturally positive for *N. gonorrhoeae* by direct inoculation without charcoal compared with 56 (17%) after transport in the Copan system without charcoal. This resulted in a sensitivity of 98% for the Copan transport system compared to direct inoculation for the detection of a positive culture. The kappa coefficient was 96.75%. Two samples that were positive for *N. gonorrhoeae* by the Copan transport system in phase 3 showed no growth on direct inoculation samples at 72 h. One sample positive by direct inoculation was negative by the Copan transport system.

## DISCUSSION

Even though newer non-culture-based molecular methods such as gene amplification and other techniques such as nucleic

acid probes are becoming more widely used in diagnostic laboratories, culture is still the most commonly used method of identifying females with gonorrhea. Therefore, attention must still be paid to proper specimen collection and transport to maintain organism viability, thereby assuring accurate laboratory detection of infection.

A new transport system, Copan Amies gel agar, available both with and without activated charcoal, has been developed. Several studies have indicated that the Copan transport systems currently in use are reliable for transport of a variety of clinically important microorganisms (3, 7, 11, 12, 16, 17). However, this is the first in vivo study that assessed Copan transport systems as a method of collection and transport of patient samples for detection of *N. gonorrhoeae*.

The Copan transport systems have a plastic-laminated film pouch that is flushed with nitrogen gas to expel atmospheric air. Swabs are inserted into an agar gel in a polypropylene tube that contains active scavenging compounds that neutralize oxygen, superoxide, and free radicals. Thus, specimens are maintained in a reduced environment, preventing oxidation. Another attractive feature of the Copan transport systems is the nonbreakable tube with no glass ampoule or seal to break, thus minimizing biosafety risks for health care workers and further reducing the likelihood of desiccation resulting in nonviable organisms because someone forgot to crush an ampoule prior to specimen transport.

This study was designed to test the ability of Copan swabs to maintain viability of *N. gonorrhoeae* for up to 24 h after collection to determine whether these transport systems are sufficient to preserve the organism for overnight shipment. Although direct inoculation of specimens is considered the gold standard, isolation of *N. gonorrhoeae* from specimens held in Copan transport systems for 24 h prior to inoculation of selective media were generally good and arguably comparable in that 95% of cultures positive by direct inoculation were detected. However, if greater than 95% accuracy and efficiency is to be achieved, then the Copan transport systems are not optimum when 24 h elapses before plating. Swabs maintained in the Copan transport system without charcoal performed as well as direct inoculation when cultures were plated at the 6-h time point, making them an acceptable, practical, and cost-effective method for transport of *N. gonorrhoeae* cultures for most hospital laboratories since the time elapsed from specimen collection until inoculation of selective medium should usually fall within this time period.

Addition of activated charcoal to transport systems for *N. gonorrhoeae* has been advocated for many years to neutralize bactericidal unsaturated fatty acids present in transport medium (8, 15). While this appears to be advantageous and has gained widespread acceptance in many settings, the presence of charcoal in transport media makes Gram stains much more difficult to interpret, thus requiring two different types of swabs to be maintained in inventory. Our findings were somewhat

unexpected in that there was no apparent advantage of Copan swabs with charcoal for recovery of *N. gonorrhoeae*. Due to the satisfactory performance of the Copan transport system without charcoal in recovering *N. gonorrhoeae* after 24 h of storage compared to that with charcoal at 24 h, we determined that there was no need to stock swabs with charcoal specifically for isolation of this organism. This study was limited to evaluation of one particular type of transport system. Similar performance with other products cannot be automatically assumed, and laboratories should not eliminate usage of charcoal-containing swab systems without documentation of adequate performance in maintaining viability of *N. gonorrhoeae*.

There were a total of seven false-negative cultures from specimens transported in Copan swabs and held for 24 h before plating. As opposed to mere loss of viability of *N. gonorrhoeae* in the Copan transport system during the holding period, other factors were likely to be of equal or greater significance. Overgrowth of competing organisms, including commensal bacteria and yeasts, has been implicated in other circumstances (5, 9, 13, 18). The 24-h holding period before plating provided an opportunity for commensal yeast to multiply at room temperature, overwhelming the nystatin in the MTM medium. Five of these seven false-negative cultures were overgrown by yeast.

The reasons for failure to isolate *N. gonorrhoeae* from the other two Copan transport specimens are only speculative. Collection of samples for diagnostic purposes prior to obtaining swabs for research could have reduced the numbers of organisms. Furthermore, all of the Copan false-negative cultures from phases 1 and 2 were from specimens that were collected with the Dacron swabs for direct inoculation first. Discordance rates of 1 to 18% for organism recovery have been reported when multiple swab specimens are obtained from a single body site (10).

Among specimens collected in phase 3, the two cultures positive by the Copan systems and negative by direct inoculation had only 1 to 5 colonies visible on MTM agar. In the reverse situation, the discrepant specimen positive after direct inoculation had only 5 colonies on the MTM agar plate. In contrast to specimens held in the Copan transport systems for 24 h prior to inoculation, those held for only 6 h were less likely to grow yeast in large amounts, and this was not a factor in the three discrepancies.

The concentration of *N. gonorrhoeae* in the endocervix can be as low as  $10^2$  CFU/ml, whereas other commensal bacteria and yeast may be present in excess of  $10^6$  CFU/ml (12). Carlin et al. (2) reported that one-fourth of 350 directly inoculated culture plates for detection of *N. gonorrhoeae* had inadequately plated samples and 85% of them were obtained by the same staff members who were either inexperienced or well-distanced from their last in-house training. These examples emphasize the importance of proper specimen collection and handling technique on recovery of fastidious organisms such as *N. gonorrhoeae*.

While direct inoculation has been considered technically superior for recovery of *N. gonorrhoeae* in clinical specimens, particularly if shipment by courier to a reference laboratory is required, there are numerous disadvantages that make this procedure increasingly less attractive in a busy, cost-driven, clinical environment. Direct inoculation requires agar plates to be purchased, maintained, refrigerated, and kept in-date on-

site in clinical facilities that may have limited storage space. Medical and nursing staff must have additional training and skills to ensure that samples are collected and inoculated onto selective medium correctly. Facilities must also have an incubator on-site and either CO<sub>2</sub> bags or a candle jar for transport to a clinical laboratory. Thus, transport systems such as Copan swabs represent a reasonable alternative for convenience, low-cost, and ease of use, while still maintaining a satisfactory recovery rate of *N. gonorrhoeae* from clinical specimens, providing that specimens can be inoculated onto selective media within a relatively short time period not involving overnight shipment.

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