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Comparison of Commercial Amies Transport Systems with In-House Amies Medium for Recovery of *Neisseria gonorrhoeae*

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Microbiologists are still encumbered by the variable performance of Amies charcoal transport medium in recovery of *Neisseria gonorrhoeae*. The objective of this study was to evaluate and select a good quality commercial system to replace our in-house preparation. We adsorbed 0.1 ml of a suspension from 30 gonococcal isolates onto each swab type and replaced the swab into the transport medium. We plated the swabs to New York City medium at 0, 24, 48, 72, and 96 h. We compared the survival of each isolate in the commercial Amies transport systems with that in our in-house Amies transport medium. The best recovery was observed with Copan transport systems. Some systems are inadequate and unacceptable for culture of gonococci.

The incidence of gonorrhea in Canada was 5,500 cases in 1995. Despite the declining rates since 1981, infections with gonococci (GC) still cost the Canadian health care system in excess of $43 million a year (4). GC are still technically difficult to preserve and recover from clinical specimens. An internet search (2a) for publications regarding GC transport and specimen handling from 1966 to 1998 revealed 6 publications from 1966 to 1974, 10 for 1975 to 1979, none in the period 1980 to 1984, 2 for 1985 to 1989, none from 1990 to 1994, and 3 for 1995 to 1998. Previous work was done principally from 1966 to 1979. Despite that work, we continue to struggle with the quality of transport media.

In 1967, C. R. Amies, medical microbiologist for the Ontario Public Health Laboratories (PHL) published his modification of Stuart's transport medium (1) for improved recovery of GC from clinical specimens. We (the PHL) have been evaluating commercial systems for 5 years to replace the in-house preparation. This study reports recent evaluations of commercial Amies-like transport swab systems.

(Previous work was reported at the 65th Conjoint Meeting on Infectious Diseases of the Canadian Association for Clinical Microbiology and Infectious Diseases, St. John's, Newfoundland, 26 to 29 October 1997 [5].)

We used fresh GC isolates, rather than stock cultures, which are poor predictors of medium performance characteristics. Thirty isolates recovered from clinical specimens on New York City (NYC) medium with antibiotics (colistin, lincomycin, amphotericin B, and trimethoprim) (2) were used for each system. Five were tested each time, until a total of 30 isolates were tested in each transport system. Suspensions were prepared to match a McFarland no. 1 nephelometer standard in Sorensen's phosphate-buffered saline (pH 7.6) from subcultures grown for 18 to 24 h on NYC medium without antibiotics. The inoculum was estimated by plating 20 μl to NYC medium for a spread plate as soon as the inoculum was prepared. The suspension was adsorbed onto each of the eight commercial swab types (0.1 ml per swab). The following swab systems were tested: PHL in-house lots 1390 (6 May 1997), 2058 (16 June 1997), 5028 (15 December 1997); Transystem lots 7029, 7073, and 7323 (Copan Italia, Bovezzo, Italy); CultureSwab lot 100660TA (manufactured by Copan for Difco Laboratories, Detroit, Mich.); Starswab lot 7G17A (Starplex Scientific, Etobicoke, Ontario, Canada); NCS lot 7F26A (Starplex Scientific); Transwab lot 97G28 (Medical Wire and Equipment Co., Ltd., Corsham, England); and Culturette lot N7KA020 (Becton Dickinson and Co., Cockeysville, Md.). All contained charcoal, except for Transystem lot 7323.

Tests were set up in triplicate so that three of each swab type could be plated at 0, 24, 48, 72, and 96 h. Inoculated swab transport media were left at room temperature (20 to 22°C) before plating. We compared commercial systems with our in-house Amies medium (PHL). Swabs were plated to NYC medium with antibiotics at 0, 24, 48, 72, and 96 h. NYC medium was incubated at 36°C in 5% CO_2 for 36 to 48 h. Growth was graded from 1 to 5 (1 to 50 colonies in swabbed area, >50 colonies in swabbed area, growth in swabbed area and primary streaking, growth into secondary streaked area, or growth in all streaked areas, respectively).

The inoculum determined by the spread plate technique ranged from 10^6 to 10^8 colonies per ml (average of 5.5×10^7) for the 30 GC suspensions used to inoculate swabs. Results with Culturette lot N7KA020 include only 29 isolates. (We missed pipetting the sixth isolate into the tubes used to adsorb the GC suspensions onto swabs.) Transwab lot 97G28 was not plated at 96 h.

The results are summarized in Table 1. The number of GC isolates surviving room-temperature incubation in the eight commercial Amies transport systems at 24-h intervals from 0 to 96 h was compared with that in our in-house Amies medium. All isolates were viable in each transport medium at time zero. All 30 isolates remained viable for 24 h in Copan Transystem 7073, Copan Cultureswab 100660TA, Copan Transystem 7029, Copan Transystem 7323, Medical Wire Transwab 97G28, and PHL. The Culturette system only maintained 20 of 29 isolates at 24 h. The Starswab and NCS systems maintained 29 of 30 and 28 of 30 isolates, respectively. At 48 h, we recovered 30 GC isolates from Copan Transystem 7323; 29 each from Copan Transystem 7073, Copan Cultureswab 100660TA, and Copan Transystem 7029; and 28 from Transwab 97G28 and PHL. The numbers recovered from Culturette, Starswab, and NCS were considerably less at 48 h: 3 of 29, 14 of 30, and 14 of 30, respectively. None was recovered from NCS, Starswab, or Culturette after 72 h. The best recovery overall was from Copan Transystem 7029, with 26 of 30 isolates still viable after 96 h in transport medium. Copan Transystem 7073 and Copan Tran-

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TABLE 1. Survival of 30 GC isolates in Amies transport systems

Swab system	No. of isolates surviving at plating interval of:				
	0 h	24 h	48 h	72 h	96 h
Transystem 7073[a]	30	30	29	27	15
CultureSwab 100660TA[b]	30	30	29	22	Not tested
Starswab 7G17A[c]	30	29	14	0	0
Transystem 7029[d]	30	30	29	28	26
Transystem 7323[e]	30	30	30	27	15
Transwab 97G28[f]	30	30	28	16	4
Culturette N7KA020[g]	29	20	3	0	0
NCS 7F26A[h]	30	28	14	0	0
PHL 1390/2058/5028[i]	30	30	28	20	13

[a] Transystem (Copan Italia, Bovezzo, Italy); standard medical peel pouch with bottom layer of paper and top plastic film.
[b] CultureSwab (manufactured by Copan for Difco Laboratories, Detroit, Mich.); standard medical peel pouch with bottom layer of paper and top plastic film.
[c] Starswab (Starplex Scientific, Etobicoke, Ontario, Canada).
[d] Transystem (Copan Italia); completely plastic peel pouch.
[e] Transystem (Copan Italia); without charcoal packaged with completely plastic peel pouch.
[f] Transwab (Medical Wire and Equipment Co., Ltd., Corsham, England).
[g] Culturette (Becton Dickinson and Co., Cockeysville, Md.); only 29 cultures tested.
[h] NCS (Starplex Scientific).
[i] In-house lots. (In-house lots yielded about 500 transport vials, which required us to use three different lots over the period of evaluation.)

system 7323 were next best, both with 15 of 30 isolates still viable at 96 h. PHL followed next in order of performance, with 13 of 30 isolates recovered.

Copan Transystem lots 7073 and 7029 had been tested in a previous study (12 May 1997 to 15 September 1997) along with other lots and were found to give the best performance. Copan lots 7029 and 7073 showed no decline in performance in this study compared with the study done in 1997. In the 1997 study, we recovered 18 of 20 GC isolates from lot 7029 and 16 of 20 from lot 7073 at 72 h. In this study, we recovered 27 of 30 isolates from 7073 and 28 of 30 from lot 7029 at 72 h.

Until 1996, we had been unable to find a commercial transport system as good as our own in-house preparation, but saw promise in early trials with Difco CultureSwab, manufactured by Copan for Difco Laboratories. Copan indicated they were collaborating with Difco and using new innovations to improve the performance of their transport medium. Copan modified their product by using oxygen-neutralizing and -scavenging agents to counteract superoxide radicals. Copan lots 7029 and 7323 had a modified plastic barrier packaging that allowed for nitrogen gassing at the time of production. This modification was intended to retard oxygen penetration into the medium for improved shelf life. The best performance overall was with the Copan product (lots 7029, 7073, and 7323). Copan lot 7323 contained no charcoal and was formulated for customers preferring to prepare smears from transport media. These lots were superior to our in-house preparation. Lot 7029 preserved 26 of 30 cultures for 96 h compared with lots 7073 and 7323, which both sustained 15 of 30 cultures. The Starplex, NCS, and Culturette transport swabs performed poorly, with a major decline in viability of isolates between 24 and 48 h. Our results support the data by Perry (3) who compared the Copan Transystem without charcoal to the Culturette system. He was unable to recover GC at 24 or 48 h in the Culturette system from 0.1 ml of a 10^6-CFU/ml inoculum compared with 23% of the inoculum at 24 h and 6% at 48 h recovered with the Copan system.

An important feature of culture medium performance is the age of the medium. Shelf life depends largely on the age of the medium. Copan Diagnostics and Starplex decoded each product, giving us manufacturing dates. We tested Copan lot 7073 manufactured in March 1997 and lot 7029 manufactured in November 1996 twice and found no deterioration in performance within 14- and 18-month periods of manufacture, respectively. The difference in performance of the three Copan lots at 96 h may be related to better packaging in lot 7029 (plastic barrier) and the absence of charcoal in lot 7323.

On 1 October 1998, the PHL adopted the use of Copan Transystem with charcoal and plastic barrier film packaging. Our decision was based on our evaluation of performance in this study.

The variability in performance seen in this study reinforces the need to do quality assurance before using Amies transport medium for transport of specimens for recovery of fastidious organisms like *Neisseria gonorrhoeae*.

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