Field Evaluation of New York City Medium in the Biological Environment-CO₂ Chamber in Recovery of *Neisseria gonorrhoeae* and Urogenital Mycoplasmas

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The biological environment-CO₂ chamber containing New York City (NYC) medium was evaluated in a clinical field trial in comparison with Transgrow and NYC medium in plates for the recovery of *Neisseria gonorrhoeae* and with NYC medium for urogenital mycoplasmas. The results of the study indicated that the biological environment chamber-CO₂ culturing system with NYC medium is an effective method for the handling, transport, and culture of *N. gonorrhoeae*, large-colony mycoplasmas, and T-mycoplasmas if the delay in transport and incubation does not exceed 24 h.

With the introduction of Thayer-Martin medium (23), as well as other selective formulations (3, 4, 14), the laboratory can readily recover gonococci (GC) when it receives specimens promptly. However, the underlying problem for many screening facilities and private physicians is the varying delay between collection of a specimen and its transport to a competent central laboratory. This delay may range from several hours to several days in environments that can vary in extremes of cold or heat. Prolonged transport and suboptimal ambient temperatures play a major role in failures to recover *Neisseria gonorrhoeae* from clinical specimens.

The ideal transport system would provide the necessary enrichment and environmental conditions to maintain viability of even small numbers of GC for as long as it takes to get the specimen to a laboratory. As yet, such a transport system has not been devised (10).

As early as the turn of the century, Chapin recognized the need for CO₂ in the primary isolation of *N. gonorrhoeae* (2). Subsequent research in developing transportation mechanisms has shown that CO₂ is one of the critical factors in maintaining viability for reasonable time periods. Current transport systems are based on supplying CO₂ directly, either by gassing the medium container, as in Transgrow (T-G) (12, 16), or by the use of a CO₂ tablet composed of a mixture of sodium bicarbonate and citric acid, which is activated by moisture contained in the medium. A CO₂ tablet is provided in a number of current systems such as Clinicult (18), Gono-Pak (9), and Gonax (21). Its most recent use is in the biological environmental chamber (BEC) developed by Martin and

Jackson (15). In this study, the chamber provided adequate CO₂ and eliminated the disadvantages normally encountered with the T-G bottle.

The purpose of this investigation was to evaluate the effectiveness of New York City (NYC) medium as a transport medium when used in the BEC for the recovery of *N. gonorrhoeae* as well as urogenital *Mycoplasma* sp. NYC medium is a selective enriched agar for the isolation of pathogenic *Neisseria* sp. (3, 4). It also readily supports the growth of large-colony mycoplasmas and T-mycoplasmas from urogenital specimens (6). At the time of its original formulation, NYC medium, with the addition of yeast dialysate filtrate, was evaluated as a transport system by using it in closed vessels such as screw-capped plates and screw-capped bottles (5). Although exogenous CO₂ was not added, growth was observed in these sealed containers with a number of strains. Similar findings have been reported with the use of yeast extract (8). This transport system was not fully comparable to NYC medium incorporated in petri plates.

MATERIALS AND METHODS

Media. NYC medium consists of proteose-peptone no. 3-corn starch agar, to which is added a supplemental nutrient mixture containing lysed horse erythrocytes, citrated horse plasma, yeast dialysate, dextrose, and the following antimicrobial agents: vancomycin hydrochloride, colistin, amphotericin B, and trimethoprim lactate. It was prepared as described previously (3, 4), except that the concentration of vancomycin was reduced from 3 to 2 µg/ml based on reports in the literature on the occurrence of vancomycin-sensitive strains (17, 19),

and the concentration of amphotericin B was increased to 1.5 $\mu\text{g/ml}$.

BEC-CO₂ system. The complete system is composed of the following components: (i) the BEC, which is a rectangular styrene medium dish with a 100-mm surface, a removable cover, and an inner compartment for a CO₂ tablet; (ii) a CO₂-generating tablet, to be placed in the well of the dish after inoculation of the medium; and (iii) a polyethylene "zip-locked" plastic bag in which the inoculated dish is transported and incubated. (BEC, CO₂ tablets, and incubation bags were supplied as TABCO₂ by the Ames Co., Div. Miles Laboratories, Inc., Elkhart, Ind.).

The CO₂ tablets are activated by moisture, and the CO₂ produced is maintained during transport and incubation in the tightly closed system.

NYC medium was poured into BEC chambers as well as standard petri plates; after cooling, chambers and plates were placed in closed plastic bags and stored at 4°C.

T-G bottles were purchased from Flow Laboratories, Inc., Rockville, Md.

Laboratory evaluation of the CO₂ culture system. The selective ability of NYC medium in the CO₂ culture-transport system (NYC-BEC) to support the growth of freshly isolated strains of pathogenic *Neisseria*, large-colony mycoplasmas, and T-mycoplasmas was evaluated as previously described (6). Large-colony mycoplasmas and T-strain mycoplasmas were transferred from isolation plates to NYC-BEC by the agar block method.

Clinical evaluation of the CO₂ culture system. NYC-BEC was tested against NYC medium in plates (NYC-Pl) and T-G using clinical specimens obtained from patients seen in a Social Hygiene Clinic of the New York City Department of Health (Bureau of Venereal Disease Control).

Specimens were collected on swabs, which were placed in tubes containing 0.5 ml of a sterile 0.3% gelatin solution in distilled water. NYC-BEC, NYC-Pl, and T-G kept for 1 h at room temperature (22°C) were inoculated with swabs twirled in the gelatin suspension, the sequence of inoculation being varied from specimen to specimen. After inoculation, a carbon dioxide-generating tablet was placed in the special compartment of each BEC chamber; the chamber was closed and then sealed in a plastic incubation bag. NYC-Pl were placed in candle jars.

Direct incubation. All media were incubated at the clinic for 18 to 24 h at 35°C, and then transported to our laboratory where they were examined upon arrival for growth of *N. gonorrhoeae*. NYC-Pl, the BEC chambers, and T-G bottles were placed in candle extinction jars and incubated for another 16 to 24 h. After this period, all media were reexamined for the presence of *N. gonorrhoeae*. NYC-BEC and NYC-Pl were also screened for the growth of large-colony mycoplasmas and T-mycoplasmas.

Delayed incubation. The ability of the NYC-BEC system to maintain the viability of GC, large-colony mycoplasmas, and T-mycoplasmas was tested by holding NYC-BEC and T-G bottles for 24, 48, and 72 h at room temperature (22°C) before incubation.

Identification of isolated organisms. *N. gonor-*

rhoeae was identified by colonial morphology, Kovacs oxidase test, Gram stain, and sugar fermentation (7, 11, 13).

Large-colony mycoplasmas were identified by colonial morphology, using the low-power (10 $\times$) magnification of the light microscope; T-strains of mycoplasmas were detected with the direct test for urease (20).

Contamination of media by growth of organisms other than *N. gonorrhoeae* was recorded as follows: 1+, light growth; 2+, moderate growth; and 3+, heavy growth of contaminant(s).

RESULTS

In laboratory trials, NYC-BEC supported the growth of all pathogenic *Neisseria*, large-colony mycoplasmas, and T-mycoplasmas strains tested. The colonial morphology, number of colonies, and speed of growth compared well with that on NYC-Pl.

The results of the clinical evaluation are presented in Tables 1 to 4. In the first comparative study (Table 1), which included 178 specimens, NYC-BEC, T-G, and NYC-Pl were incubated directly after inoculation. Under these conditions, NYC-BEC and NYC-Pl each recovered a total of 36 (20.2%) GC strains, whereas a total of 31 strains (17.4%) were recovered on T-G.

When NYC-BEC and T-G were kept for 24 h at room temperature before incubation (Table 2), a total of 38 (21%) of 181 specimens were positive for *N. gonorrhoeae* on NYC-BEC and 32 (17.6%) on T-G, whereas on NYC-Pl, which were incubated directly, a total of 41 (22.6%) GC strains were isolated.

A group of 195 inoculated NYC-BEC and T-G bottles were then left at room temperature for 48 h before incubation. In this experiment, 52 (26.7%) GC strains were isolated on NYC-BEC, 47 (24.1%) on T-G, as compared with 63 (32.3%) strains recovered on NYC-Pl incubated without delay (Table 3).

Finally, 162 specimens inoculated on NYC-BEC and T-G were kept for 72 h at room temperature before incubation. Under these conditions, only 20 (12.3%) GC strains were isolated on NYC-BEC and 18 (11.1%) on T-G, whereas a total of 39 (24%) cultures were positive on NYC-Pl incubated directly (Table 4).

The rate of gross contamination (3+) varied from 1.5 to 5.5% on NYC-BEC and NYC-Pl (Tables 2 and 4) and from 18 to 35.4% on T-G.

Large-colony mycoplasma and T-mycoplasma recoveries. When NYC-BECs were incubated directly or kept for 24 h at room temperature, the rate of recovery and the amount of growth of large-colony mycoplasmas and T-mycoplasmas paralleled that obtained on NYC-Pl. However, when NYC-BECs were kept for 48

TABLE 1. Comparative data for recovery of *N. gonorrhoeae* after direct incubation^a

Determination	NYC-Pl ^b		NYC-BEC		T-G	
	No.	%	No.	%	No.	%
GC recovered on:						
Each medium ^c	36	20.2 (100)	36	20.2 (100)	31	17.4 (86)
NYC-Pl only	0					
NYC-BEC only			0			
T-G only					0	
NYC-Pl and NYC-BEC	5	3.0	5	3.0		
NYC-Pl and T-G	0				0	
Contamination						
3+	0		9	5.1	63	35.4
2+	5	3.0	4	2.2	30	16.9
1+	8	4.5	9	5.1	8	4.5
GC positives contaminated	0		2	6.5	21	58.3

^a Total of 178 cultures; total GC recovery, 36 (20.2%).^b Directly incubated in candle jar (reference medium).^c All percentages are based on total specimens except those in parentheses, which are based on total positives recovered on NYC-Pl.TABLE 2. Comparative data for recovery of *N. gonorrhoeae* after delay for 24 h before incubation^a

Determination	NYC-Pl ^b		NYC-BEC		T-G	
	No.	%	No.	%	No.	%
GC recovered on:						
Each medium ^c	41	22.6 (100)	38	21.0 (93)	32	17.6 (78)
NYC-Pl only	3	7.3				
NYC-BEC only			0			
T-G only					0	
NYC-Pl and NYC-BEC	6	14.6	6	14.6		
NYC-Pl and T-G	0					
Contamination						
3+	10	5.5	8	4.4	35	19.3
2+	5	2.8	8	4.4	35	19.3
1+	2	1.1	4	2.2	3	1.7
GC positives contaminated	0		1	0.6	2	1.1

^a Total of 181 cultures; total GC recovery, 41 (22.6%).^b Directly incubated in candle jar (reference medium).^c All percentages are based on total specimens except those in parentheses, which are based on total positives recovered on NYC-Pl.

or 72 h at room temperature before incubation, there was a steady decline in the rate of recoveries for both mycoplasmas (Table 5).

DISCUSSION

The results of these studies indicate that the "BEC-CO₂ culturing system" with NYC medium is an effective system for the handling and the transport of *N. gonorrhoeae*, large-colony mycoplasmas, and T-mycoplasmas, and also a satisfactory culture system if the delay in transportation and incubation does not exceed 24 h. A recent study by Symington (22) using

NYC medium with lincomycin reported comparable results for the recovery of *N. gonorrhoeae*.

Compared with T-G, the BECs are more convenient for the handling of a large volume of cultures and for the visual identification of pathogenic *Neisseria*. The large, exposed inoculation surface of the chambers can be examined and processed with the same ease as that of standard petri plates; the time necessary to process such cultures compared well with that of NYC plates, whereas T-G bottles required considerably longer.

TABLE 3. Comparative data for recovery of *N. gonorrhoeae* after delay of 48 h before incubation^a

Determination	NYC-Pl ^b		NYC-BEC		T-G	
	No.	%	No.	%	No.	%
GC recovered on:						
Each medium ^c	63	32.3 (100)	52	26.7 (83)	47	24.1 (75)
NYC-Pl only	11	17.4				
NYC-BEC only			0			
T-G only					0	
NYC-Pl and NYC-BEC	5	8.0	5	8.0		
NYC-Pl and T-G	0				0	
Contamination						
3+	0		3	1.5	35	18.0
2+	2	1.0	4	2.0	39	20.0
1+	3	1.5	6	3.0	5	2.6
GC positives contaminated	0		0		16	25.40

^a Total of 195 cultures; total GC recovery, 63 (32.3%).^b Directly incubated in candle jar (reference medium).^c All percentages are based on total specimens except those in parentheses, which are based on total positives recovered on NYC-Pl.TABLE 4. Comparative data for recovery of *N. gonorrhoeae* after delay of 72 h before incubation^a

Determination	NYC-Pl ^b		NYC-BEC		T-G	
	No.	%	No.	%	No.	%
GC recovered on:						
Each medium ^c	39	24.0 (100)	20	12.3 (51)	18	11.1 (46)
NYC-Pl only	17	43.6				
NYC-BEC only			0			
T-G only					0	
NYC-Pl and NYC-BEC	4	10.2	4	10.2		
NYC-Pl and T-G	2	5.1			2	5.1
Contamination						
3+	3	1.9	6	3.7	31	19.1
2+	1	0.6	1	0.6	23	14.2
1+	3	1.9	0		6	3.7
GC positives contaminated	0		0		3	7.7

^a Total of 162 cultures; total GC recovery, 39 (24.0%).^b Directly incubated in candle jar (reference medium).^c All percentages are based on total specimens except those in parentheses, which are based on total positives recovered on NYC-Pl.

TABLE 5. Recovery of mycoplasmas on NYC-Pl and NYC-BEC (total of 108 cultures)

Mycoplasma type	NYC-Pl ^a		NYC-BEC							
			Direct incubation		24-h delay		48-h delay		72-h delay	
	No.	%	No.	%	No.	%	No.	%	No.	%
Large colony ^b	42	38.8	42	38.8 (100)	42	38.8 (100)	33	30.5 (78.6)	27	25 (64.3)
T ^b	69	63.9	69	63.9 (100)	66	61.1 (95.7)	39	36.1 (5.5)	30	27.7 (43.5)

^a Directly incubated in candle jar (reference medium).^b Percentages are based on total specimens except those in parentheses, which are based on total positives recovered on NYC-Pl.

The CO₂-generating tablets and the closed environment created by the tightly sealed chamber and the "zip-locked" bag provided the cultures with the necessary amount of CO₂ needed for the initiation of growth when incubated directly or delayed for not longer than 24 h. Under our experimental conditions, when NYC-BECs and NYC-PI were immediately incubated, an equal number of *N. gonorrhoeae*, large-colony mycoplasma, and T-mycoplasma strains were recovered on both systems. A delay of 24 h before incubation of the NYC-BEC had a slight negative effect on the recovery of the organisms but, as the delay was increased, the percentage of recoveries for all three organisms declined and, after 72 h of delay, only 51.3% of GC strains isolated on NYC-PI were recovered on NYC-BEC. The same progressive loss in GC isolates was found when T-G flasks were kept under similar conditions; only 46.2% of the total positives were recoverable after 72 h of delay before incubation. Similar findings for T-G were reported by Holston et al. (9) and Bonin and Tronca (1).

The effect of extreme temperatures during transportation was not explored in these experiments, but it may play an important role in the rate of recovery of these organisms.

Assuring the viability of pathogenic *Neisseria* and mycoplasmas during transport remains a multifaceted problem of which the availability of an adequate CO₂ concentration is but one factor. There have been few studies in which other aspects of the problem have been explored. Some of the variables that must be considered in dealing with a transport system include ambient temperature and, with respect to the medium, moisture content, pH, and the role of stabilizing factors, as well as the effect of supplemental metabolites.

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