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MENINGOCOCCAL CONTROL IN THE CANADIAN FORCES II. ASSESSMENT OF GROWTH-SUPPORTING MEDIA FOR THE TRANSPORT OF *NEISSERIA MENINGITIDIS* (U)

by

L.A. White and M.R. Spence

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ABSTRACT

The abilities of Transgrow (TG), Thayer-Martin (TM) and New York City (NYC) solid media to maintain viability of 12 strains of *Neisseria meningitidis* under various controlled conditions were assessed. The effects of charcoal impregnation of swabs, temperature and the presence of CO_2 were determined with holding for up to 21 days. Strains employed included 5 laboratory strains, 3 isolated from cases of overt disease, 3 isolated from the nasopharynx of apparently healthy carriers and one isolated from a naturally occurring aerosol. Recovery from those samples held at 35°C was in almost all instances greater than at 22 or 4°C. A strong requirement for CO_2 was demonstrated, especially at lower temperatures. No positive effect could be attributed to the use of charcoal-impregnated swabs. NYC and TM media were found to be the best overall, with the former permitting recovery from more than 75% of all samples held on slants after holding for 20 days at 4°C in the presence of 5% CO_2.

(U)

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INTRODUCTION

Neisseria meningitidis and *N. gonorrhoeae* are extremely fragile microorganisms when separated from the host or when removed from very specific laboratory and cultural conditions. Because of this fragility, the transport of infectious samples to diagnostic laboratories presents major problems. A recent review of the literature resulted in the conclusion that insufficient evidence exists to enable the designation of a single medium as superior for the transport of these pathogens (16). It was apparent, however, that the three best media were Transgrow (TG) (9), Thayer-Martin (TM) (15) and New York City (NYC) (3) agars. In this study, we investigated the efficacy of these media in maintaining the viability of pure cultures of *N. meningitidis* and *N. gonorrhoeae* at three temperatures: 37, 22 and 4°C. In addition, the effect of holding in an atmosphere of 5% CO_2 in air was determined as was the benefit, if any, of the use of swabs impregnated with charcoal. Several laboratory strains of *N. meningitidis* were employed and results with these were compared and contrasted with those obtained using a number of strains isolated at Canadian Forces

Base (CFB) Cornwallis from cases of acute disease, apparently-healthy carriers and the air. (The Canadian Forces have instituted a comprehensive program on the delineation of factors involved in the transmission of the meningococcal carrier-state and overt disease among recruits (2).)

MATERIALS AND METHODS

Neisseriae Strains and Culture Conditions

The 12 strains of *N. meningitidis* and two of *N. gonorrhoeae* employed in this study are presented in Table 1. Cultures were routinely grown, with shaking, in Neisseria Chemically-Defined Medium (NCDM) (7) for 16 hours at 35°C in an atmosphere of 5% CO_2. Each sample was inoculated from a freshly reconstituted vial of lyophilized bacteria. In the case of those strains isolated at CFB Cornwallis, all inocula consisted of cells which were no more than 5 subcultures removed from original isolation. The gonococcal cultures were fresh isolates from male urethras and were included as a control since the majority of the literature has been concerned with problems of transporting this organism.

Transport Media

Thayer-Martin medium (15) was obtained commercially (Mogul Diagnostics, Madison, Wis.) whereas TG (9) and NYC (3) media were prepared in this laboratory in accordance with directions outlined in the literature. All three media have been employed with reasonable success for the transport of *N. gonorrhoeae*. Their use is based on the principle of supporting the growth of pathogenic Neisseriae while inhibiting the growth of contaminating microorganisms through the use of antibiotic supplements.

Test Method

Two methods were employed for the inoculation of media. In the first, cotton-tipped swabs were prepared in accordance with Stuart et al. (13) by boiling in Sorensen's phosphate buffer, pH 7.4. Swabs were either impregnated with activated charcoal or not and they were

dipped into broth cultures of the test organism (approx. 10^8 Colony Forming Units (CFU)/ml) and excess inoculum was permitted to drain off. (Impregnation with charcoal has been suggested as a means of increasing survival of organisms on swabs by virtue of the ability of charcoal to bind oxygen thus reducing the role of oxidation in cell death.) The swab was then stabbed into the test medium. The excess handle was removed aseptically and the screw cap replaced. In the second method, inocula were streaked on the surface of slants of each medium. These slants were then incubated at 35°C for 24 hours in a 5% CO_2 atmosphere. Screw capped vials containing the various media were permitted to equilibrate for 1 hr in an atmosphere of 5% CO_2 prior to inoculation.

After inoculation, and inoculation plus incubation in the case of slants, samples were held at 4, 22 and 35°C both in the presence of 5% CO_2 and its absence. Samples were assessed on a growth/no-growth basis by streaking of either the swab or a loop of material from the surface of slants on plates of Columbia agar (Gibco) containing Isovitalex (BBL) and 4% sheep red blood cells (Supplemented Columbia blood agar (CBA)). All assessment plates were incubated at 35°C for 24 hours in an atmosphere of 5% CO_2. Those samples inoculated by the "swab" method were examined after 1, 2, 3, 7, 14 and 21 days. Slants were examined after 1, 2, 6, 13 and 20 days. Three separate samples were assayed for each set of conditions.

The data were analyzed by a multifactorial statistical program developed at DRES.

Retention of Viability By Freezing

As a control, the survival of 6 of the test strains of *N. meningitidis* at -60°C was studied. Tissue culture flasks (250 ml) containing CBA were inoculated from a rapidly growing broth culture (NCDM) of the strains employed. The flasks were incubated at 35°C for 16 hours in an atmosphere of 5% CO_2 in air. The growth was washed off into trypticase soy broth (TSB) with the aid of glass beads, dispersed by means of a tissue homogenizer and diluted to approximately 1×10^8 CFU/ml in TSB. One ml ali-

quots were placed in 5 ml glass lyophilization vials, sealed and frozen immediately at -60°C. At weekly intervals for one year, vials were removed, thawed quickly and assayed immediately. Assays were conducted on plates of CBA by a drop plate method (11).

RESULTS

A. "Stab" Method

Figure 1 shows average recovery rates for 12 strains of *N. meningitidis* held in the three transport media at the different temperatures in air or 5% CO_2 in air. The raw data are tabulated in Appendix A. All data were analysed statistically. Initial analysis revealed that the use of charcoal impregnated swabs had no significant effect, either positive or negative, on recovery and, therefore, these data were pooled for further analysis.

Effect of CO_2

The effects of 5% CO_2 on the survival of *N. meningitidis* are presented in Table 2. A dramatic positive effect was evident with all three media. This was most evident at 22 and 4°C, with significantly greater recovery being observed in samples held for as little as 24 hr, and became apparent at 35°C after 48 hr of holding. Lack of significance after 7 days holding at 4°C was due to overall low survival at that temperature (see Figure 1).

Effect of Holding Time

In the 5% CO_2 atmosphere, at 35 and 22°C, significantly reduced recovery was not observed until samples had been held for 7 days or longer. With all media, a temperature of 4°C appeared to be antagonistic with significantly fewer isolations being made after 3 days holding.

In those samples held in the absence of CO_2 at 35°C, recoveries were significantly lower on the second day and thereafter. Significantly reduced recoveries were not as evident at 22 or 4°C but this was due in large part to the generally lower recoveries, even after as little as

24 hrs, from samples held in the absence of CO_2 as compared to the recovery from samples held with CO_2 (see Figure 1).

Effect of Temperature On Survival

In the CO_2-air atmosphere, with a single exception (35°C vs 4°C), significantly reduced recovery due to holding temperature was not observed in samples held 48 hr or less. Differences were generally not apparent between samples held at 35 and 22°C, but in all cases survival was significantly reduced at 4°C in samples held 3 days and longer.

With those samples held in air, survival at 35°C was significantly higher than at 4°C at all test intervals and greater than at 22°C over the first 3 days. Greater recovery was generally observed from those samples held at 22°C than those held at 4°C.

Effect of Medium

When employed in this manner ("stab" method), all three media appeared to be equally effective in maintaining the viability of *N. meningitidis*. No distinct trends were apparent in samples held either in the presence or absence of CO_2 at any of the test temperatures.

B. Samples Streaked on Slants

Average recovery rates for 12 strains of *N. meningitidis* in the three transport media at the test temperatures are presented in Figure 2. These data were also analysed statistically and the effects of the various experimental variables determined. Raw data are presented in tabular form in Annex B.

Effect of CO_2

A statistically significant positive effect due to CO_2 was not noted until samples had been held for at least 6 days. Samples held on TG showed the greatest requirement with samples held both at 22 and 4°C benefiting from the added CO_2. Only at 22°C was a requirement demonstrated on samples held on TM. No significant positive effect was evident in samples held on NYC until 20 days and then only at 4°C.

Effect of Holding

Recovery from samples held on NYC in CO_2-air was not significantly reduced until 20 days at any of the test temperatures. In the absence of added CO_2, however, recovery was significantly reduced by day 6 at 35°C and by day 13 from samples held at 22 or 4°C. Recovery from TG, on the other hand, was significantly less by 6 days at 35 or 22°C both in the presence and absence of added CO_2. The negative effect of increased holding was less evident in samples held at 4°C. Results obtained with samples held on TM were intermediate between NYC and TG.

Effect of Temperature

Temperature was not a significant factor in subsequent recovery of viable meningococci from TM or NYC. With TG on the other hand, 4°C appeared to be the temperature of choice, particularly for samples held between 2 and 13 days, and in the presence of added CO_2. For samples held on this medium, 22°C was the most adverse temperature.

Effect of Medium

Direct statistical comparison revealed that no medium was consistently more effective. However, when the total analysis was considered it was apparent that TG suffered from some serious limitations in that samples held on this medium:

a) demonstrated the strongest requirement for added CO_2,

b) showed the earliest negative effect due to holding, and

c) exhibited reduced recovery at non-refrigerated temperatures.

Thayer-Martin and NYC were of approximately equal effectiveness.

C. Retention of Viability of *N. meningitidis* by Freezing

Freezing has been employed as a means of maintaining viability of *N. meningitidis* in liquid samples collected from the air at Canadian Forces Bases and for broth cultures of this organism (2,12). In this study, the results were obtained with 6 strains of *N. meningitidis* which were frozen in TSB without the addition of a cryoprotectant. A subsequent study has shown TSB to be a preferable freezing menstruum to

similar media containing some of the commonly used cryoprotectants (5). Three strains, DRES-06 and -18 and -2241, did not show a drop in viable numbers even after 52 weeks. For strain 1628, there was a rapid initial drop of about 50 - 70% but, thereafter, no further decrease was observed. With strain DRES-17, there was an immediate loss in viability of two logarithmic units. No further loss was noted with increased holding. Strain DRES-05, a throat isolate, yielded extremely irreproducible recovery from sample to sample, indicating this strain to be highly susceptible to some factor or factors of the experimental condition. Rates for cooling during freezing, or rewarming during thawing, may have been the major factor responsible (1) since these factors were not rigidly controlled in our experiments. Variability of a lesser magnitude was also observed with strains 1628 and DRES-17.

D. Observations with *N. gonorrhoeae*

The two *N. gonorrhoeae* strains employed did not survive as well as the meningococci and, in addition, showed a much more dramatic requirement for the 5% CO_2 atmosphere. New York City medium proved to be the medium of choice for this organism. The results obtained by the slant method indicated that NYC cultures did not have as critical a requirement for added CO_2 as did TG or TM cultures. A holding temperature of 35°C gave the best rate of recovery from all 3 media, although, in those samples held on NYC slants, good recovery was noted at the two adverse temperatures (22 and 4°C) even after 6 days of holding.

DISCUSSION

The utility of Transgrow, Thayer-Martin and New York City media for the maintenance of viability of pure cultures of *N. meningitidis* has been clearly demonstrated, thus confirming the conclusion of the earlier literature review (16). Recovery from samples maintained for up to 3 days at 35 or 22°C was essentially 90 - 100% provided that the samples were held in the presence of CO_2. This requirement for CO_2 was dramatic for meningococci with all three media and the present report is the first con-

trolled study in which this has been demonstrated. A similar requirement has been demonstrated by several workers (4, 6, 8, 10 and 14) for the survival of gonococci during transport of samples from cases of suspected gonorrhoea. With meningococci, the requirement was most evident in the case of the slant method in which samples were incubated at 35°C for 24 hr prior to being held at 4°C. Substantial recovery was observed even after 20 days of holding at this temperature provided that the samples were held in the presence of CO_2. The Biological Environment Chamber (BEC) method of Martin and Jackson (8) does not ensure a sufficient CO_2 atmosphere for transport periods in excess of 24 hr (4) and for that reason may not be adequate for Canadian Forces applications where transport delays may easily exceed this period. Evidence presented here indicates that for most isolates freezing may represent a more practical method for the Canadian Forces.

These results further indicate that *N. meningitidis* is much less susceptible to death in tranpsort media than is *N. gonorrhoeae*, but for both organisms, NYC and TM media appeared to be marginally superior to TG. This superiority was most evident at holding periods of greater than 7 days; at temperatures of 22°C and 4°C; and in the absence of CO_2.

The data presented here were obtained with pure cultures, and extrapolation to what might be expected with suspected *N. meningitidis* isolated directly from patients and transported under irregular conditions may be questioned. Results obtained with these strains, recently isolated from natural sources at CFB Cornwallis and maintained no more than 5 culture generations from initial isolation, were essentially the same as those obtained using the 5 laboratory strains. These findings are suggestive that such extrapolation may be possible but, at the same time, they do not rule out the possibility that adaption to laboratory strain status may be a rapid phenomenon and occur early during laboratory sub-culture. Relationships between laboratory strains and the organism in the disease state are being investigated further and we are hopeful that these studies will permit a more definitive answer as to pathogenic factors of this

organism and means of preserving these factors on artificial media.

In conclusion, it has been clearly shown that if a 5% CO_2 atmosphere and temperature of 35°C can be maintained, recovery of *N. meningitidis* from three of the best tranpsort media available (New York City, Thayer-Martin and Transgrow) can be essentially 100% after holding for 72 hrs and in excess of 70% even after 7 days.

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Table 1: Neisseriae strains employed.

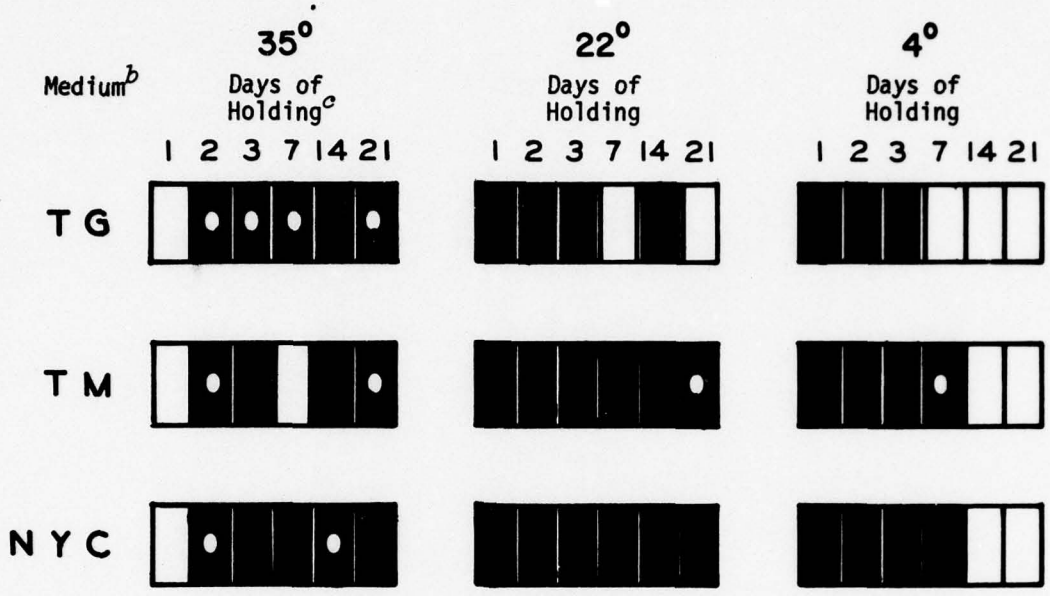
SPECIES	STRAIN	SEROGROUP	SOURCE
N. meningitidis	604	A	LCDC[b]
	608	B	LCDC
	2241	C	LCDC
	1628	C	LCDC
	547	Y	LCDC
	DRES-01[a]	B	Disease Case[c]
	DRES-02	C	Disease Case
	DRES-04	C	Throat Swab
	DRES-05	C	Throat Swab
	DRES-06	C	Disease Case
	DRES-17	B	Air Sample
	DRES-18	B	Sputum Plate
N. gonorrhoeae	73-049		LCDC
	73-278		LCDC

[a]DRES: Defence Research Establishment Suffield.

[b]LCDC: Laboratory Centre for Disease Control, Dept. National Health and Welfare, Ottawa, Ontario.

[c]All DRES strains were isolated at Canadian Forces Base Cornwallis.

Table 2: Effect of CO_2 on the survival of *N. meningitidis*[a].



[a]Survival on swabs inserted into the transport medium.

[b]TG (Transgrow), TM (Thayer-Martin) and NYC (New York City).

[c]Recovery observed in the presence of CO_2 was compared with that obtained from swabs held under identical conditions in the absence of CO_2.

■ - Significantly greater recovery at >99th percentile.

▪ - Significantly greater recovery at >95th percentile.

□ - No significant difference.

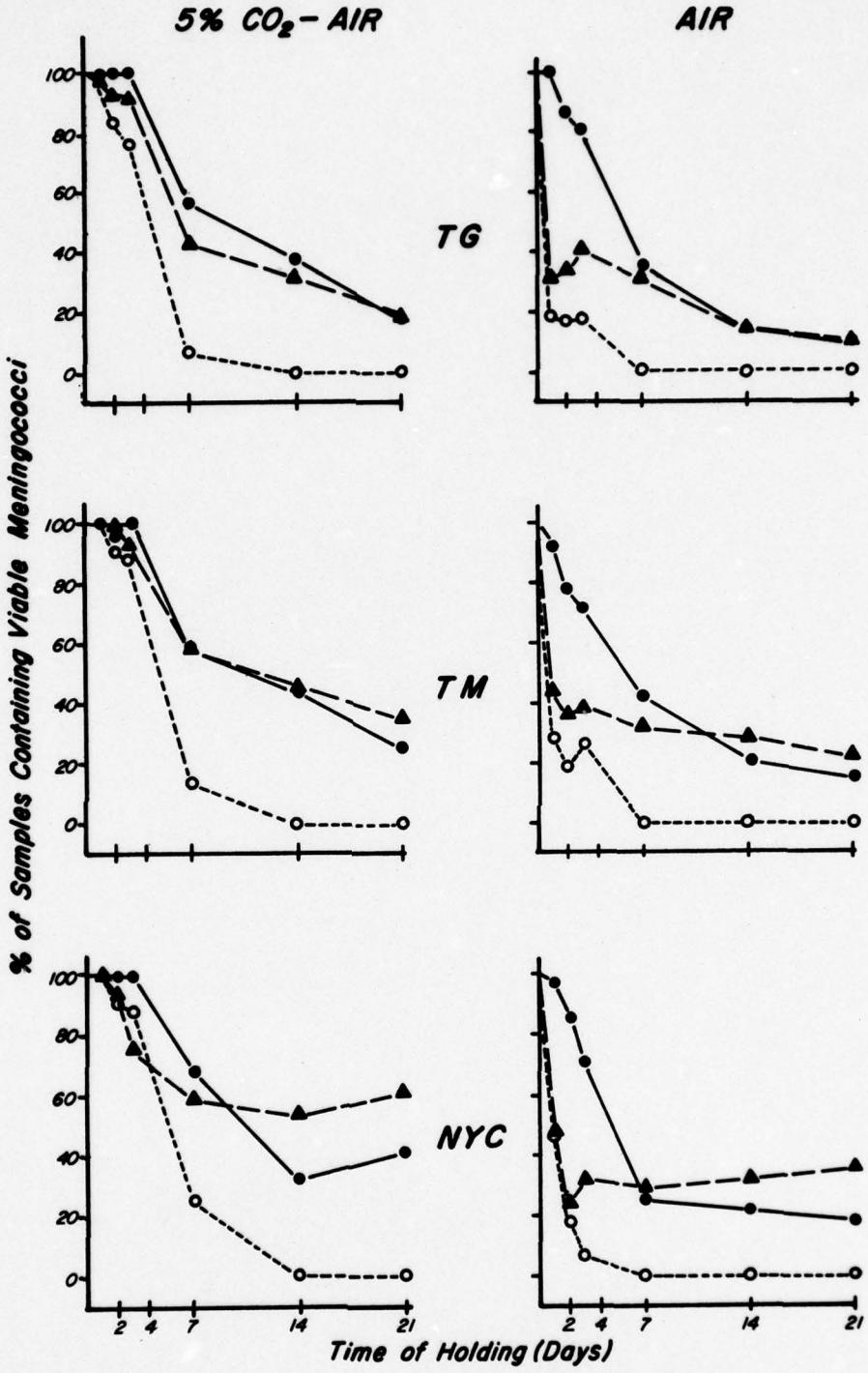


Figure 1. Survival of *N. meningitidis* on cotton swabs in transport media held in air and in an atmosphere of 5% CO_2 in air at 35 (●), 22 (▲) and 4°C (o). Media employed were Transgrow (TG), Thayer-Martin (TM) and New York City (NYC).

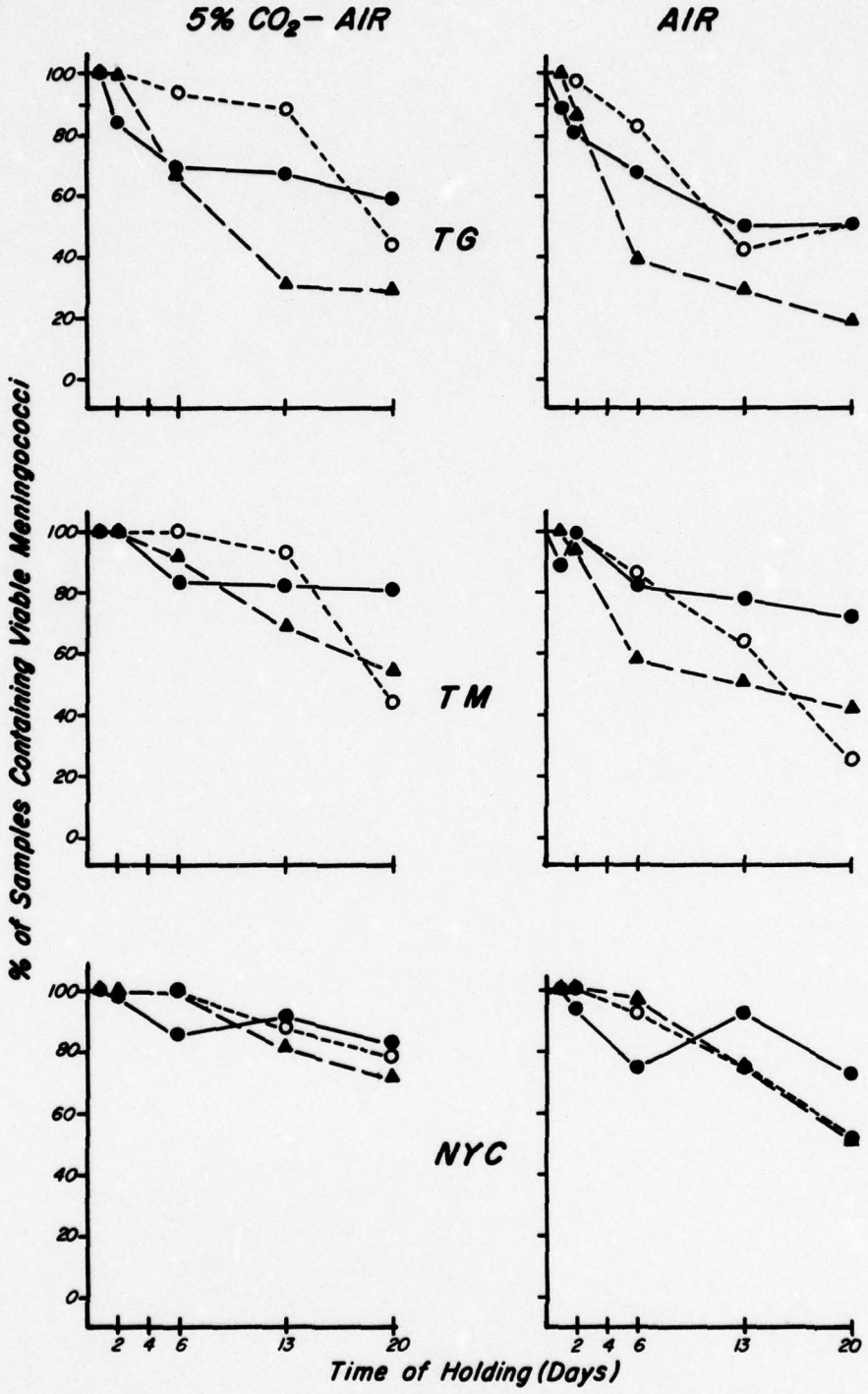


Figure 2. Survival of *N. meningitidis* on slants of transport media held in air and in an atmosphere of 5% CO_2 in air at 35 (●), 22 (▲) and 4°C (○). Media employed were Transgrow (TG), Thayer-Martin (TM) and New York City (NYC).

Appendix A

Tabulated Data Obtained By The "Stab" Method

TABLE A-1

SURVIVAL OF NEISSERIAE STRAINS UNDER VARIOUS CONDITIONS ON SWABS INSERTED INTO TRANSGROW MEDIUM

35°C

Strain	Day 1				Day 2				Day 3				Day 7				Day 14				Day 21			
	$+CO_2$[a]		$-CO_2$		$+CO_2$		$-CO_2$		$+CO_2$		$-CO_2$		$+CO_2$		$-CO_2$		$+CO_2$		$-CO_2$		$+CO_2$		$-CO_2$	
	P[b]	C[c]	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C
604	3[d]	3	3	3	3	3	3	3	3	3	3	3	3	1	1	3	1	0	1	0	1	0	0	0
608	3	3	3	3	3	3	3	3	3	3	3	3	1	0	2	0	1	1	0	0	1	0	0	0
2241	3	3	3	3	3	3	3	3	3	3	3	3	3	3	1	1	3	1	0	1	0	0	0	0
1628	3	3	3	3	3	3	3	3	3	3	2	3	2	1	2	1	0	0	0	0	0	0	0	0
547	3	3	3	3	3	3	3	1	3	3	3	3	3	2	0	0	0	1	0	0	0	1	0	0
DRES-01	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	2	3	2	2	2	2	2	2	2
DRES-02	3	3	3	3	3	3	3	3	3	3	1	1	1	1	0	1	1	1	0	0	0	1	0	1
DRES-04	3	3	3	3	2	3	0	2	3	3	2	0	1	2	0	0	1	1	0	0	0	0	0	0
DRES-05	3	3	3	3	3	3	3	1	3	3	3	3	2	1	2	1	1	0	2	0	1	0	0	0
DRES-06	3	3	3	3	3	3	2	3	3	3	0	1	1	1	0	1	3	2	0	0	1	1	1	0
DRES-17	3	3	3	3	3	3	3	3	3	3	3	3	1	2	0	2	1	1	0	1	1	0	1	0
DRES-18	3	3	3	3	3	3	3	2	3	3	3	3	2	2	2	1	2	1	1	1	1	0	1	0
NG[e]73-049	3	3	3	3	3	3	3	1	2	3	0	2	0	0	0	0	0	0	0	0	0	0	0	0
NG 73-278	3	3	1	2	3	3	1	2	3	3	0	0	0	2	0	0	0	2	0	0	2	2	0	0

22°C

604	3	3	3	3	2	3	1	3	2	3	1	3	0	2	0	1	1	0	0	1	0	1	0	1
608	3	3	3	2	3	3	3	2	3	3	3	3	3	3	3	3	3	3	1	2	1	3	0	0
2241	3	3	0	0	3	3	3	3	3	3	0	1	2	3	1	3	2	1	0	0	0	0	0	0
1628	3	2	0	0	2	3	0	0	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
547	3	3	0	0	3	3	0	0	3	3	0	0	1	2	0	0	0	2	0	0	0	0	0	0
DRES-01	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	3	3	3
DRES-02	3	3	0	0	1	3	0	0	1	2	0	0	0	2	0	0	0	1	0	0	0	1	0	0

604	3	3	3	3	2	3	1	3	2	3	1	3	0	2	0	1	1	0	0	1	0	1	0	1
608	3	3	3	2	3	3	3	2	3	3	3	3	3	3	3	3	3	3	1	2	1	3	0	0
2241	3	3	0	0	3	3	3	3	3	3	0	1	2	3	1	3	2	1	0	0	0	0	0	0
1628	3	2	0	0	2	3	0	0	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
547	3	3	0	0	3	3	0	0	3	3	0	0	1	2	0	0	0	2	0	0	0	0	0	0
DRES-01	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	3	3	3
DRES-02	3	3	0	0	1	3	0	0	1	2	0	0	0	2	0	0	0	1	0	0	0	1	0	0
DRES-04	3	3	0	0	3	2	0	0	3	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0
DRES-05	3	3	0	3	3	3	0	1	3	3	3	3	0	3	0	3	0	2	0	1	0	0	0	0
DRES-06	3	3	0	0	3	3	0	0	3	3	0	0	0	0	0	0	0	0	0	0	2	0	1	0
DRES-17	3	3	0	1	3	3	1	0	3	3	0	0	0	0	0	0	1	1	0	1	0	0	0	0
DRES-18	3	3	0	2	3	3	0	1	3	3	1	3	1	2	1	3	0	1	0	0	0	0	0	0
NG 73-049	3	3	1	0	3	3	0	0	3	3	0	0	0	0	0	0	1	1	0	0	0	0	0	0
NG 73-278	3	3	0	0	2	2	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

4°C

604	3	3	3	3	1	3	2	3	1	3	2	3	0	0	0	0	0	0	0	0	0	0
608	3	3	0	0	1	3	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
2241	2	3	0	0	3	3	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1628	3	3	0	0	0	0	0	0	1	3	0	0	0	0	0	0	0	0	0	0	0	0
547	3	3	0	0	3	3	0	0	3	3	0	0	0	1	0	0	0	0	0	0	0	0
DRES-01	3	3	3	3	3	3	1	0	3	3	0	0	0	2	0	0	0	0	0	0	0	0
DRES-02	3	3	0	0	1	3	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
DRES-04	3	3	0	0	3	3	0	0	3	3	0	0	0	0	0	0	0	0	0	0	0	0
DRES-05	3	3	0	1	3	3	0	0	3	3	3	3	0	0	0	0	0	0	0	0	0	0
DRES-06	3	3	0	0	3	3	0	0	3	3	0	0	0	0	0	0	0	0	0	0	0	0
DRES-17	3	3	0	1	3	3	0	0	3	3	0	0	0	1	0	0	0	0	0	0	0	0
DRES-18	3	3	0	0	3	3	0	0	3	3	0	2	0	0	0	0	0	0	0	0	0	0
NG 73-049	3	3	0	0	3	3	0	0	3	3	0	0	3	3	0	0	2	0	0	0	0	0
NG 73-278	3	3	0	0	3	0	0	0	3	2	0	0	0	0	0	0	0	0	0	0	0	0

[a] Atmosphere of 5% CO_2 in air.

[b] Cotton swabs, plain.

[c] Cotton swabs, impregnated with charcoal.

[d] Number of samples (out of 3) yielding recovery of organism.

[e] *Neisseria gonorrhoeae*

TABLE A-2

SURVIVAL OF NEISSERIAE STRAINS UNDER VARIOUS CONDITIONS
ON SWABS INSERTED INTO THAYER-MARTIN MEDIUM

35°C

Strain	Day 1				Day 2				Day 3				Day 7				Day 14				Day 21			
	$+CO_2$[a]		$-CO_2$		$+CO_2$		$-CO_2$		$+CO_2$		$-CO_2$		$+CO_2$		$-CO_2$		$+CO_2$		$-CO_2$		$+CO_2$		$-CO_2$	
	P[b]	C[c]	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C
604	3[d]	3	3	3	3	3	3	3	3	3	3	3	1	2	3	3	1	1	1	0	1	0	1	0
608	3	3	3	3	3	3	3	3	3	3	3	3	3	1	3	1	3	2	3	2	2	0	2	0
2241	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	2	0	3	0	0	0	0	0	0
1628	3	3	3	1	3	3	3	0	3	3	3	0	1	2	1	1	1	1	0	1	1	0	0	0
547	3	3	3	3	3	3	3	1	3	3	3	1	0	1	1	2	1	0	0	0	0	0	0	0
DRES-01	3	3	3	3	2	3	3	3	3	3	2	2	3	2	3	2	3	2	3	1	2	2	2	2
DRES-02	3	3	3	3	3	3	2	3	3	3	2	0	3	1	0	0	1	2	1	0	1	2	0	0
DRES-04	3	3	2	0	3	1	0	0	3	3	2	0	3	1	0	0	1	0	1	0	1	0	1	0
DRES-05	3	3	3	3	3	3	3	2	3	3	3	3	2	0	2	0	1	0	0	0	0	0	0	0
DRES-06	3	3	3	3	3	3	2	3	3	3	0	2	2	2	2	0	2	0	0	0	2	2	0	1
DRES-17	3	3	3	3	3	3	3	2	3	3	2	3	1	2	0	1	1	0	0	0	0	0	0	0
DRES-18	3	3	3	3	3	3	3	3	3	3	3	3	2	1	1	0	2	2	1	1	1	1	1	1
NG 73-049	3	3	3	3	3	3	1	3	2	3	1	0	0	1	0	0	0	0	0	0	0	1	0	1
NG 73-278	3	3	2	1	3	3	1	2	3	3	0	0	1	2	0	0	0	1	0	0	0	1	0	0

22°C

604	3	3	3	3	3	2	1	3	3	2	1	3	0	3	0	1	1	1	0	1	1	0	1	0
608	3	3	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	2	0
2241	3	3	0	3	3	3	3	3	3	3	0	2	3	3	2	2	3	2	1	1	0	0	0	0
1628	3	3	0	0	3	3	0	0	1	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0
547	3	3	0	0	3	3	0	0	3	3	0	0	0	2	0	0	1	1	0	0	1	0	1	0
DRES-01	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
DRES-02	3	3	0	0	3	3	0	0	3	3	0	0	3	3	0	0	2	0	1	0	3	1	2	0
DRES-04	3	3	0	0	3	3	0	0	2	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0
DRES-05	3	3	1	2	3	3	0	2	3	2	2	3	0	3	0	3	1	2	1	0	0	0	0	0

604	3	3	3	3	3	2	1	3	3	2	1	3	0	3	0	1	1	1	0	1	1	0	1	0
608	3	3	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	2	0
2241	3	3	0	3	3	3	3	3	3	3	0	2	3	3	2	2	3	2	1	1	0	0	0	0
1628	3	3	0	0	3	3	0	0	1	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0
547	3	3	0	0	3	3	0	0	3	3	0	0	0	2	0	0	1	1	0	0	1	0	1	0
DRES-01	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
DRES-02	3	3	0	0	3	3	0	0	3	3	0	0	3	3	0	0	2	0	1	0	3	1	2	0
DRES-04	3	3	0	0	3	3	0	0	2	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0
DRES-05	3	3	1	2	3	3	0	2	3	2	2	3	0	3	0	3	1	2	1	0	0	0	0	0
DRES-06	3	3	0	0	3	3	0	0	3	3	0	0	2	1	0	0	2	2	0	0	3	3	1	2
DRES-17	3	3	2	2	3	3	0	1	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DRES-18	3	3	3	3	3	3	0	1	3	3	2	3	2	3	0	3	1	2	1	2	0	2	0	1
NG 73-049	3	3	1	0	3	3	0	0	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NG 73-278	3	3	0	0	1	2	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

4°C

604	3	3	3	3	3	3	3	3	3	3	3	3	0	0	0	0	0	0	0	0	0	0	0	0
608	3	3	0	0	0	2	0	0	1	3	0	1	0	1	0	0	0	0	0	1	0	0	0	0
2241	3	3	0	0	3	3	3	3	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1628	3	3	0	0	0	3	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
547	3	3	0	0	3	3	0	0	3	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0
DRES-01	3	3	3	3	3	3	0	0	3	3	0	0	3	3	0	0	0	0	0	0	1	0	1	0
DRES-02	3	3	0	0	3	3	0	0	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DRES-04	3	3	0	0	3	3	0	0	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DRES-05	3	3	0	0	3	3	0	2	3	3	3	3	0	1	0	0	0	0	0	0	0	0	0	0
DRES-06	3	3	0	0	3	3	0	0	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DRES-17	3	3	2	0	3	3	0	0	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DRES-18	3	3	3	3	3	3	0	0	3	3	3	3	0	0	0	0	0	0	0	0	0	0	0	0
NG 73-049	3	3	0	0	3	3	0	0	3	3	0	1	3	3	0	0	3	2	0	0	3	0	0	0
NG 73-278	3	3	0	0	2	3	0	0	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a] Atmosphere of 5% CO_2 in air.

[b] Cotton swabs, plain.

[c] Cotton swabs, impregnated with charcoal.

[d] Number of samples (out of 3) yielding recovery of organism.

[e] *Neisseria gonorrhoeae*

TABLE A-3

SURVIVAL OF NEISSERIAE STRAINS UNDER VARIOUS CONDITIONS ON SWABS INSERTED INTO NEW YORK CITY (NYC) MEDIUM

35°C

Strain	Day 1				Day 2				Day 3				Day 7				Day 14				Day 21			
	$+CO_2$[a]		$-CO_2$		$+CO_2$		$-CO_2$		$+CO_2$		$-CO_2$		$+CO_2$		$-CO_2$		$+CO_2$		$-CO_2$		$+CO_2$		$-CO_2$	
	P[b]	C[c]	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C
604	3[d]	3	3	3	3	3	3	3	3	3	3	3	3	3	0	0	1	1	1	1	0	1	0	1
608	3	3	3	3	3	3	3	3	3	3	3	2	2	3	1	2	0	3	0	2	1	1	0	0
2241	3	3	3	3	3	3	0	2	3	3	1	1	3	2	0	0	1	2	1	2	1	0	0	0
1628	3	3	3	3	3	3	3	3	3	3	3	3	3	3	1	0	0	0	0	0	2	0	1	0
547	3	3	3	3	3	3	3	3	3	3	3	3	2	3	0	0	0	1	0	1	0	1	0	1
DRES-01	3	3	3	3	3	3	3	3	3	3	3	3	2	2	2	1	0	3	0	3	2	1	2	1
DRES-02	3	3	3	3	3	3	2	3	3	3	1	0	0	0	0	0	1	0	1	0	3	2	1	0
DRES-04	3	3	3	1	3	3	1	0	3	3	1	0	0	0	0	0	1	1	1	0	2	1	0	0
DRES-05	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	1	0	1	0	3	1	0	0
DRES-06	3	3	3	3	3	3	2	3	3	3	0	3	1	3	1	2	2	2	0	0	2	1	1	1
DRES-17	3	3	3	3	3	3	3	3	3	3	3	3	2	1	2	0	0	0	0	0	1	1	1	1
DRES-18	3	3	3	3	3	3	3	3	3	3	2	1	3	3	1	0	2	2	1	1	0	2	0	1
NG 73-049	3	3	3	3	3	3	1	0	3	3	0	1	1	1	0	0	0	1	0	0	0	1	0	0
NG 73-278	3	3	0	1	3	3	0	0	3	3	0	0	2	1	0	0	2	0	0	0	0	1	0	0

22°C

604	3	3	1	3	3	3	0	0	3	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0
608	3	3	2	0	3	3	1	3	3	3	3	3	3	3	1	3	2	2	2	0	3	2	1	2
2241	3	3	0	0	3	3	0	0	3	3	0	0	3	3	0	1	3	3	2	2	3	3	2	0
1628	3	3	3	3	3	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
547	3	3	3	3	3	3	1	0	3	3	0	3	3	3	0	1	3	3	3	3	3	3	3	3
DRES-01	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	2
DRES-02	3	3	0	0	3	3	1	0	3	3	1	0	3	2	1	0	2	1	1	0	3	0	1	0
DRES-04	3	3	0	0	3	3	0	0	3	2	0	0	2	1	0	0	2	2	0	0	3	1	0	0
DRES-05	3	3	3	3	3	3	0	3	3	3	2	2	2	3	3	3	3	1	3	1	3	3	3	3

604	3	3	1	3	3	3	0	0	3	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0
608	3	3	2	0	3	3	1	3	3	3	3	3	3	3	1	3	2	2	2	0	3	2	1	2
2241	3	3	0	0	3	3	0	0	3	3	0	0	3	3	0	1	3	3	2	2	3	3	2	0
1628	3	3	3	3	3	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
547	3	3	3	3	3	3	1	0	3	3	0	3	3	3	0	1	3	3	3	3	3	3	3	3
DRES-01	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	2
DRES-02	3	3	0	0	3	3	1	0	3	3	1	0	3	2	1	0	2	1	1	0	3	0	1	0
DRES-04	3	3	0	0	3	3	0	0	3	2	0	0	2	1	0	0	2	2	0	0	3	1	0	0
DRES-05	3	3	3	3	3	3	0	3	3	3	2	2	2	3	3	3	3	1	3	1	3	3	3	3
DRES-06	3	3	1	0	3	3	1	0	3	3	0	0	3	2	2	0	3	1	0	0	3	2	2	1
DRES-17	3	3	1	2	1	2	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
DRES-18	3	3	0	0	2	3	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	0	0	0
NG 73-049	3	3	0	0	3	3	0	0	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NG 73-278	3	3	0	0	1	2	0	0	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0

4°C

604	3	3	3	3	3	3	0	1	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
608	3	3	0	0	3	3	0	0	3	3	3	2	0	0	0	0	0	0	0	0	0	0	0	0
2241	3	3	0	0	3	0	0	0	2	3	0	0	0	3	0	0	0	0	0	0	0	0	0	0
1628	3	3	0	3	1	3	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
547	3	3	3	3	3	3	0	1	3	3	0	0	3	3	0	0	0	1	0	0	0	0	0	0
DRES-01	3	3	3	3	3	3	1	0	3	3	0	0	1	3	0	0	0	0	0	0	0	0	0	0
DRES-02	3	3	0	0	3	3	0	0	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DRES-04	3	3	0	0	3	3	0	0	3	3	0	0	2	2	0	0	0	0	0	0	0	0	0	0
DRES-05	3	3	3	3	3	3	3	2	3	3	0	0	1	0	0	0	0	0	0	0	0	0	0	0
DRES-06	3	3	0	0	3	3	0	0	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DRES-17	3	3	3	3	3	2	2	3	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DRES-18	3	3	0	0	3	3	0	0	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NG 73-049	3	3	1	0	3	3	0	0	3	3	0	0	1	2	0	0	0	0	0	0	0	0	0	0
NG 73-278	3	3	0	0	1	1	0	0	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a] Atmosphere of 5% CO_2 in air.

[b] Cotton swabs, plain.

[c] Cotton swabs, impregnated with charcoal.

[d] Number of samples (out of 3) yielding recovery of organism.

[e] *Neisseria gonorrhoeae*

Appendix B

Tabulated Data Obtained By The Use Of Streaked Slants Of Neisseriae Strains On The Various Transport Media

TABLE B-1

SURVIVAL UNDER VARIOUS CONDITIONS OF NEISSERIAE STRAINS GROWN AND HELD ON TRANSGROW MEDIUM

35°C

Strain	Day 1		Day 2		Day 3		Day 6		Day 13		Day 20	
	CO_2[a]	-	CO_2	-	CO_2	-	CO_2	-	CO_2	-	CO_2	-
604	3[b]	3	3	3	3	2	2	2	1	1	0	0
608	3	3	3	3			3	3	3	3	3	3
2241	3	1	0	0			1	1	2	0	3	0
1628	3	3	3	3			1	1	0	0	0	0
547	3	3	3	3			3	2	3	2	0	3
DRES-01	3	3	2	2	2	2	3	3	3	3	3	3
DRES-02	3	3	3	3	3	3	3	3	3	2	3	2
DRES-04	3	1	1	0			0	0	0	0	0	0
DRES-05	3	3	3	3			3	3	3	3	3	3
DRES-06	3	3	3	3	3	3	3	3	3	2	2	1
DRES-17	3	3	3	3	3	3	3	3	1	1	2	2
DRES-18	3	3	3	3			0	0	2	1	2	1
NG[c] 73-049	3	3	3	3	3	1	2	2	2	1	0	0
NG 73-278	3	3	3	0	3	0	1	0	0	0	0	0

22°C

604	3	3	3	3	3	3	1	1	0	0	0	0
608	3	3	3	3			3	3	3	3	3	3
2241	3	3	3	3			3	2	0	0	0	0
1628	3	3	3	1			0	0	0	0	0	0
547	3	3	3	3			3	0	1	0	3	0
DRES-01	3	3	3	3	3	2	3	1	1	1	1	1
DRES-02	3	3	3	3	3	3	3	1	2	1	1	1

22°C

604	3	3	3	3	3	3	1	1	0	0	0	0
608	3	3	3	3			3	3	3	3	3	3
2241	3	3	3	3			3	2	0	0	0	0
1628	3	3	3	1			0	0	0	0	0	0
547	3	3	3	3			3	0	1	0	3	0
DRES-01	3	3	3	3	3	2	3	1	1	1	1	1
DRES-02	3	3	3	3	3	3	3	1	2	1	1	1
DRES-04	3	3	3	0			0	0	0	0	0	0
DRES-05	3	3	3	3			2	2	1	2	0	0
DRES-06	3	3	3	3	3	3	3	3	3	3	2	2
DRES-17	3	3	3	3	3	3	3	1	0	0	0	0
DRES-18	3	3	3	3			0	0	0	0	0	0
NG 73-049	3	3	3	1	2	1	2	0	0	0	0	0
NG 73-278	3	3	3	1	3	3	3	0	0	0	0	0

4°C

604	3	3	3	3	3	3	3	3	3	3	3	2
608	3	3	3	3			3	3	3	3	1	1
2241	3	3	3	3			3	3	3	1	0	2
1628	3	3	3	3			2	1	2	0	0	3
547	3	3	3	2			3	2	3	0	1	0
DRES-01	3	3	3	3	3	3	3	3	3	2	2	0
DRES-02	3	3	3	3	3	3	3	2	3	0	2	3
DRES-04	3	3	3	3			2	1	1	0	1	0
DRES-05	3	3	3	3			3	3	3	2	0	2
DRES-06	3	3	3	3	3	2	3	3	3	1	3	1
DRES-17	3	3	3	3	3	3	3	3	3	3	3	2
DRES-18	3	3	3	3			3	3	2	0	0	2
NG 73-049	3	3	3	0	3	1	3	0	0	0	0	0
NG 73-278	3	3	3	0	3	0	3	0	3	0	0	0

[a] Atmosphere of 5% CO_2 in air.

[b] Number of samples (out of 3) yielding recovery of organism.

[c] *Neisseria gonorrhoeae*

TABLE B-2

SURVIVAL UNDER VARIOUS CONDITIONS OF NEISSERIAE STRAINS GROWN AND HELD ON THAYER-MARTIN MEDIUM

35°C

Strain	Day 1		Day 2		Day 3		Day 6		Day 13		Day 20	
	CO_2[a]	-	CO_2	-	CO_2	-	CO_2	-	CO_2	-	CO_2	-
604	3[b]	3	3	3	3	3	3	3	3	3	3	3
608	3	3	3	3			1	3	2	2	2	1
2241	3	2	3	3	3	2	3	3	3	3	3	3
1628	3	1	3	3			1	1	1	1	1	1
547	3	3	3	3			3	2	3	1	3	2
DRES-01	3	3	3	3	3	3	3	3	3	3	3	3
DRES-02	3	3	3	3	3	3	3	2	3	3	3	2
DRES-04	3	2	3	3	3	3	3	3	3	3	3	3
DRES-05	3	3	3	3			3	3	1	2	0	0
DRES-06	3	3	3	3	3	2	3	3	3	3	3	3
DRES-17	3	3	3	3	3	3	3	3	3	3	3	3
DRES-18	3	3	3	3			1	1	2	1	2	2
NG[c]73-049	3	3	3	3	3	2	3	3	3	2	2	2
NG 73-278	3	3	3	0	3	1	3	0	0	0	0	0

22°C

604	3	3	3	3	3	3	2	0	0	0	0	0
608	3	3	3	3			3	3	3	3	3	3
2241	3	3	3	3	3	3	3	3	3	1	1	0
1628	3	3	3	3			3	2	3	1	2	2
547	3	3	3	3			3	3	3	3	2	3
DRES-01	3	3	3	3	3	3	3	3	3	3	2	2
DRES-02	3	3	3	3	3	3	3	1	3	1	2	2
DRES-04	3	3	3	1	3	2	2	0	0	0	0	0

22°C

604	3	3	3	3	3	3	2	0	0	0	0	0
608	3	3	3	3			3	3	3	3	3	3
2241	3	3	3	3	3	3	3	3	3	1	1	0
1628	3	3	3	3			3	2	3	1	2	2
547	3	3	3	3			3	3	3	3	2	3
DRES-01	3	3	3	3	3	3	3	3	3	3	2	2
DRES-02	3	3	3	3	3	3	3	1	3	1	2	2
DRES-04	3	3	3	1	3	2	2	0	0	0	0	0
DRES-05	3	3	3	3			3	3	3	3	2	1
DRES-06	3	3	3	3	3	3	3	2	3	3	3	1
DRES-17	3	3	3	3	3	3	2	0	0	0	0	0
DRES-18	3	3	3	3			3	1	1	0	2	1
NG 73-049	3	3	2	2	3	0	2	0	0	0	0	0
NG 73-278	3	3	3	0	3	0	3	0	0	0	0	0

4°C

604	3	3	3	3	3	3	3	3	3	3	3	3
608	3	3	3	3			3	3	3	3	2	1
2241	3	3	3	3	3	2	3	3	3	3	1	0
1628	3	3	3	3			3	3	3	0	0	0
547	3	3	3	3			3	3	3	0	3	0
DRES-01	3	3	3	3	3	3	3	3	3	2	3	1
DRES-02	3	3	3	3	3	3	3	1	3	0	0	0
DRES-04	3	3	3	3	3	2	3	1	1	3	1	2
DRES-05	3	3	3	3			3	3	3	3	0	0
DRES-06	3	3	3	3	3	3	3	3	3	2	1	0
DRES-17	3	3	3	3	3	2	3	3	3	3	0	0
DRES-18	3	3	3	3			3	2	3	1	2	2
NG 73-049	3	2	3	2	3	1	3	1	2	0	0	0
NG 73-278	3	3	3	0	3	3	3	0	3	0	1	0

[a] Atmosphere of 5% CO_2 in air.

[b] Number of samples (out of 3) yielding recovery of organism.

[c] *Neisseria gonorrhoeae*

TABLE B-3

SURVIVAL UNDER VARIOUS CONDITIONS OF NEISSERIAE
STRAINS GROWN AND HELD ON NYC MEDIUM

35°C

Strain	Day 1		Day 2		Day 3		Day 6		Day 13		Day 20	
	CO_2[a]	-	CO_2	-	CO_2	-	CO_2	-	CO_2	-	CO_2	-
604	3[b]	3	3	3	3	2	2	1	3	3	2	2
608	3	3	3	3	3	3	3	3	3	3	3	3
2241	3	3	3	3	3	3	3	3	3	3	2	2
1628	3	3	3	3	3	3	3	3	3	3	3	3
547	3	3	3	3	3	3	3	3	3	3	3	3
DRES-01	3	3	3	3	3	2	0	0	3	3	3	2
DRES-02	3	3	3	3	3	3	3	3	3	3	3	1
DRES-04	3	3	3	3	3	3	3	1	3	3	3	2
DRES-05	3	3	3	2	2	2	3	3	3	3	1	1
DRES-06	3	3	3	3	3	3	3	2	3	3	3	1
DRES-17	3	3	3	3	3	3	3	3	3	3	3	3
DRES-18	3	3	2	2	3	0	2	2	1	1	1	1
NG[c] 73-049	3	3	3	3	3	3	3	3	3	2	3	1
NG 73-278	3	3	3	0	3	2	0	0	0	0	0	0

22°C

604	3	3	3	3	3	3	3	3	3	3	1	2
608	3	3	3	3	3	3	3	3	3	3	3	2
2241	3	3	3	3	3	3	3	3	3	3	2	2
1628	3	3	3	3	3	3	3	2	2	2	2	2
547	3	3	3	3	3	3	3	3	3	3	3	2
DRES-01	3	3	3	3	3	3	3	3	3	3	3	3
DRES-02	3	3	3	3	3	3	3	3	3	2	3	2
DRES-04	3	3	3	3	3	3	3	3	3	3	3	0
[illegible]	3	3	3	3	3	3	3	3	0	0	0	0

22°C

604	3	3	3	3	3	3	3	3	3	3	1	2
608	3	3	3	3	3	3	3	3	3	3	3	2
2241	3	3	3	3	3	3	3	3	3	3	2	2
1628	3	3	3	3	3	3	3	2	2	2	2	2
547	3	3	3	3	3	3	3	3	3	3	3	2
DRES-01	3	3	3	3	3	3	3	3	3	3	3	3
DRES-02	3	3	3	3	3	3	3	3	3	2	3	2
DRES-04	3	3	3	3	3	3	3	3	3	3	3	0
DRES-05	3	3	3	3	3	3	3	3	0	0	0	0
DRES-06	3	3	3	3	3	3	3	3	3	3	3	3
DRES-17	3	3	3	3	3	3	3	3	1	1	1	0
DRES-18	3	3	3	3	3	3	3	3	2	1	2	0
NG 73-049	3	3	3	3	3	3	3	0	0	0	0	0
NG 73-278	3	3	3	2	3	3	3	0	2	0	0	0

4°C

604	3	3	3	3	3	3	3	3	3	3	3	3
608	3	3	3	3	3	3	3	1	0	0	0	0
2241	3	3	3	3	3	3	3	3	3	3	3	0
1628	3	3	3	3	3	3	3	3	1	2	2	2
547	3	3	3	3	3	3	3	3	3	3	3	2
DRES-01	3	3	3	3	3	3	3	3	3	3	3	2
DRES-02	3	3	3	3	3	3	3	3	3	2	3	2
DRES-04	3	3	3	3	3	3	3	3	3	3	3	2
DRES-05	3	3	3	3	3	3	3	3	3	3	2	1
DRES-06	3	3	3	3	3	3	3	3	3	1	2	2
DRES-17	3	3	3	3	3	3	3	3	3	3	1	1
DRES-18	3	3	3	3	3	3	3	3	3	1	3	1
NG 73-049	3	3	3	2	3	1	3	2	1	1	2	2
NG 73-278	3	3	3	2	3	2	3	0	3	0	3	0

[a] Atmosphere of 5% CO_2 in air.

[b] Number of samples (out of 3) yielding recovery of organism.

[c] *Neisseria gonorrhoeae*

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The abilities of Transgrow (TG), Thayer-Martin (TM) and New York City (NYC) solid media to maintain viability of 12 strains of *Neisseria meningitidis* under various controlled conditions were assessed. The effects of charcoal impregnation of swabs, temperature and the presence of CO_2 were determined with holding for up to 21 days. Strains employed included 5 laboratory strains, 3 isolated from cases of overt disease, 3 isolated from the nasopharynx of apparently-healthy carriers and one isolated from a naturally-occurring aerosol. Recovery from those samples held at 35°C was in almost all instances greater than at 22 or 4°C. A strong requirement for CO_2 was demonstrated, especially at lower temperatures. No positive effect could be attributed to the use of charcoal-impregnated swabs. NYC and TM media were found to be the best overall, with the former permitting recovery from more than 75% of all samples held on slants after holding for 20 days at 4°C in the presence of 5% CO_2.

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KEY WORDS

Neisseria meningitidis

Meningococci

Transport media

CO_2

New York City Medium

Thayer-Martin Medium

Transgrow Medium

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