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FINAL REPORT

STUDY OF METHODS FOR THE IMPROVEMENT OF BACTERIAL TRANSPORT MEDIA

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I. ABSTRACT

A series of 500 transport media recipes was tested for ability to hold pure cultures of Streptococcus equisimilus, Corynebacterium equi, Neisseria perflava, and Haemophilus parainfluenzae for 21 days. Stuart Medium Base with 0.4% agar (SMB) was used as the control medium for this and the other experiments in the investigation. At the end of the holding period inoculated transport media were quantitatively assayed, and the control media were assayed immediately after inoculation. Three vials of each medium were inoculated with an organism, and each vial's medium was diluted 10^2 and 10^4 and spread on duplicate plates. Assay media for this experiment included Brain Heart Infusion Agar (BHIA), Tryptic Soy Agar (TSA), and BHIA with 1% IsoVitaleX Enrichment.

Media 383 and 408 held all four organisms best and were selected for further study with artificial mixed cultures and throat cultures. Mixed cultures were prepared with the four organisms previously mentioned and with Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus mitis, Streptococcus salivarius, Corynebacterium pseudodiphtheriticum, and Neisseria catarrhalus. Inoculated transport media were held for two- and 12-day periods. The quantitative assay procedure was the same as that of the pure culture studies. Assay media included Chocolate Agar, Mannitol Salt Agar,

Staphylococcus Medium 110, Mitis Salivarius Agar, Meuller Hinton Agar, Mueller Tellurite Agar, BHIA, and TSA. Two vials of each transport medium were inoculated.

Duplicate calcium alginate swabs were used to obtain throat cultures from 12 subjects. One swab was immediately struck on a Chocolate Agar plate in an attempt to isolate Neisseria and Haemophilus species. The other swab was inserted into a transport medium and stored at 4 C for 21 days. At the end of the test period the swab was removed and struck on a chocolate plate. The remaining transport medium was assayed in the manner of previous experiments. This procedure facilitated qualitative identification of Haemophilus (in a vial of 383) and Neisseria (in a vial of 383 and one of 408).

The results suggest that 383 and 408 are better at holding fastidious organisms than SMB. They also raise questions about the relationship of limited growth to survival.

II. MATERIALS AND METHODS

Organisms and assay media were chosen for pure culture studies according to previous NASA investigations (F and N). A gram positive coccus (Streptococcus equisimilus, ATCC 9542), a gram positive rod (Corynebacterium equi, ATCC 6939), a gram negative coccus (Neisseria perflava, ATCC E14799), and a gram negative rod (Haemophilus parainfluenzae, ATCC 7901) were used. In addition to these four the following organisms were used for mixed culture studies; Staphylococcus aureus (ATCC 25923) Staphylococcus epidermidis (ATCC 14990, NASA #301), Streptococcus mitis (Lunar Receiving Lab #349), Streptococcus salivarius (ATCC 13419), Corynebacterium pseudodiphtheriticum (ATCC 10700), and Neisseria catarrhalus (ATCC 8176).

S. aureus, S. epidermidis, Strep. equisimilus, Strep. mitis, Strep. salivarius, C. equi, C. pseudodiphtheriticum, N. perflava, and N. catarrhalus were maintained on slants made with Brain Heart Infusion Broth (Difco) and 1.5% (w/v) Agar (Difco). Plates of Brain Heart Infusion Agar (BHIA) were streaked with bacteria from the slants, and 48-hour colonies were picked to inoculate culture media for experimental use. The backup slants were stored at 4 C and sometimes covered with oil. The organisms were also lyophilized. H. parainfluenzae was maintained on Brain Heart Infusion Agar with 1% (v/v) IsoVitaleX Enrichment (BBL) at room temperature, and C. equi was kept on Tryptic Soy Agar (TSA) (Difco) slants at 4 C.

Each 48-hour colony picked from a streak plate was inoculated into a 500-ml Delong culture flask containing 50 ml of Brain Heart Infusion Broth (Difco). Cultivation of H. parainfluenzae required the addition of 1% IsoVitaleX to the broth. After 12 to 18 hours of incubation on a shaker at 37 C the cultures were standardized with a Spectronic 20 (Bausch and Lomb) using a wavelength of 540 milimicrons. Standardized cultures had an optical density that indicated approximately 10^5 cells/ml. The ten organisms used in the mixed culture studies were standardized before mixing.

Each vial was inoculated with 0.1 ml of standardized culture with a 1.0-ml gas tight Hamilton syringe and repeating dispenser. In this way the inoculum could be reproducibly distributed throughout the medium.

Commercial transport media used in this study included Transport Medium Amies (Difco), Cary and Blair Transport Medium (BBL), Transport Medium Stuart (Difco), and Stuart Medium Base (Difco) with 0.4% agar. Stuart Medium Base (SMB) was the control transport medium in these experiments (16).

The 500 media screened in the initial phase of this investigation were modifications of commercial and experimental recipes (1, 4, 6, 8, 11-14). The two media selected for more detailed study were designated 383 and 408.

Medium 383 has the following formula (in grams per liter):

NaCl (Baker)	3.0
Sodium thioglycollate (Nutritional Biochem. Co.)	1.0
$CaCl_2$ (Baker)	0.1
Na_2HPO_4 (Baker)	8.22
KH_2PO_4 (Baker)	1.20
Agar (Difco)	4.0
Methylene blue	0.002
Amberlite CG-50 (100-200 Mesh)	1.0
L-cysteine HCl (Nutritional Biochem. Co.)	5.0
pH = 6.9	

A solution of $CaCl_2$ (1% w/v) and one containing the other components in the medium should be made separately and autoclaved at 15 pounds pressure (121 C) for 15 minutes. Allow the solutions to cool to 50 C before mixing them. Dispense 5-ml aliquots in sterile 1½-dram screw-capped sample vials (Belco). Tighten the caps and invert the vials just before cooling is completed so that the Amberlite CG50 is evenly distributed in the solidified medium.

The following formula (in grams per liter) is for medium 408:

NaCl	3.0
Sodium thioglycollate	1.0
$CaCl_2$	0.1
Na_2HPO_4	8.22
KH_2PO_4	1.20
Agar	4.0
Methylene blue	0.002
Amberlite CG-50	1.0
IsoVitaleX	10.0
Activated Charcoal (Matheson, Coleman, and Bell)	10.0

(Methylene blue is optional in this medium because the charcoal makes it useless as an Eh indicator.) Preparation of 408 is like that of 383 with the addition of IsoVitaleX after autoclaving.

Media used for the pure culture assay procedures included Brain Heart Infusion Broth (Difco) with 1.5% (w/v) of Agar (Difco), Brain Heart Infusion Agar (BHIA) with 1% (v/v) IsoVitaleX, and TSA. Media used in assaying mixed cultures included chocolate agar made from G C Medium Base (Difco) with 2% Hemoglobin (Difco) and 1% IsoVitaleX, Mannitol Salt Agar (Difco), Staphylococcus Medium 110 (Difco), Mitis Salivarius Agar (Difco) with 0.1% Chapman's Tellurite Solution (Difco), Mueller Tellurite Agar (Difco), Mueller Hinton Agar (Difco),

BHIA, and TSA.

Transport media for the pure culture experiment were assayed 21 days after their inoculation, and the SMB controls were assayed immediately. Transport media containing mixed cultures were assayed two and 12 days after their inoculation. Throat swabs were held in transport media for 21 days before analysis.

The assay procedure consisted of emptying the medium from a vial into a 25 x 150 mm screw-capped tube. Three 5-ml rinses of the vials with Tryptose Phosphate Broth (Difco) were dumped into the tube with the medium. The material in the tube was mixed on a vortex mixer for 10 to 20 seconds. The material from SMB controls were diluted 10^2 with Tryptose Phosphate Broth before they were assayed. 0.2-ml aliquotes of the mixture were spread on each of the appropriate plates. The inoculated plates were then incubated at 37 C for 24 to 48 hours before colonies were counted.

For the initial studies each standardized culture was inoculated into three vials of a particular transport medium. The medium and rinse material mixture from each vial was spread on duplicate plates. S. equisimilus and N. perflava were plated on BHIA plates; C. equi and H. parainfluenzae were plated on TSA and BHIA with 1% IsoVitaleX respectively.

The mixture of standardized cultures was inoculated into two vials of each type of transport medium. Each vial was assayed in the same manner as those just mentioned, but the medium and rinse mixture was plated on a series of the eight different media discussed above. This procedure and knowledge of the ten organisms in the mixed culture permitted qualitative and quantitative identification of the bacteria. Identification also depended on gram stains and comparisons of microscopic and colonial morphology with those characteristics of pure cultures of the ten organisms on each of the eight media.

Throat cultures were obtained with duplicate calcium alginate swabs from 12 randomly selected subjects. One swab was immediately used to streak a chocolate agar plate. The other swab was inserted into a transport medium and held for 21 days before assaying. Four vials of each medium were inoculated with swabs. At the end of the holding period the swab was removed and struck on a chocolate agar plate. The remaining medium was assayed in the same way as media containing mixed cultures. Comparison of gram stains and microscopic and colonial morphology with pure cultures were used to aid in the identification of possible Haemophilus species and Neisseria species.

III. RESULTS

Of the 500 media screened during the initial studies only a few held the bacteria adequately. Almost every medium held pure cultures of Strep. equisimilus and C. equi for the 21 day holding period without more than a 10^3 loss in viable cell counts. N. perflava and H. parainfluenzae were unable to survive most of the storage conditions studied. Only 142 of the 500 media held N. perflava, and only 57 held H. parainfluenzae satisfactorily. The 31 media that held all four organisms with less than a 10^4 loss of viable cells provided the most significant data.

Physical conditions were just as important as media constituents to long term bacterial survival. Generally, the data support the requirement that transport media have a high pH (D and N). However, of 97 recipes having pH's between 5.5 and 6.9 there were seven media in which survival was good (Table 1). The low pH was associated with the Amberlite CG-50 which is a weakly acidic cationic exchange resin.

Temperature was another important factor. In most instances, a temperature of 25 C contributed to survival of the four organisms used in the pure culture study (Table 2). Under certain conditions, however, the 25 C temperature was detrimental to the survival of H. parainfluenzae (Compare medium 409 with 415.) and N. perflava (Compare medium 408 with 414).

Some media components other than buffers, reducing agents, salts, and agar enhanced survival significantly. Table 3 shows that the weekly acidic cationic exchange resin (Amberlite CG-50 in medium 479) is more beneficial to survival than the strongly acidic cationic exchange resin (Amberlite CG-120 in medium 481) or either of the anionic exchange resins (Amberlite CG-4B in medium 480 or Amberlite CG-400 in medium 482). Media 24, 36, 49, and 60 (Table 4) demonstrate that Amberlite CG-50 is more beneficial in medium 24 (ionic strength = 0.237) than in medium 49 (ionic strength = 0.086).

Comparisons of medium 47 with 408, medium 383 with 409, and medium 398 with 424 in Table 5 demonstrate the effect of IsoVitaleX. The data of Table 2 show that the effect of Iso-VitaleX is influenced by storage at 25 C (Compare medium 407 with 413, medium 412 with 418, medium 419 with 425, and medium 421 with 427).

The effect of L-cysteine and L-methionine on survival is shown in Table 6 (Compare medium 35 with 383, and medium 48 with 396 and 398).

Studies of the effects of ionic balance (between Ca^{+2} and Mg^{+2}, or K^{+} and Na^{+}), ionic strength, glycerol, Sephadex G-25, Sephadex G-75, Sephadex G-200, and Dextran provided inconclusive data. In general, polyethylene glycol had little effect on

survival although media 108 and 109 held the four organisms (Table 7).

Medium 383 and 408 were chosen for further studies because they offered optimum conditions for long term survival. Tables 8 and 9 show how 383 and 408 compare with Stuart Medium Base for holding mixed cultures. Both 383 and 408 lost Strep. mitis during the 12 day holding period (Table 9). Medium 383 also lost C. pseudodiphtheriticum and Neisseria species during the same holding period. The survival of H. parainfluenzae can only be speculated because the assay procedure provided only presumptive evidence of its presence in media 383 and 408.

Qualitative results from the holding of throat cultures for 21 days at 4 C in Stuart Medium Base and media 383 and 408 were also obtained. A Haemophilus species was isolated from a vial of medium 383, and a Neisseria species was isolated from a vial of 383 and a vial of 408.

IV. DISCUSSION

Cary and Blair (D) described four requirements that a transport medium must satisfy, and one of these is high pH. As mentioned earlier a few media with low pH's (between 5.5 and 6.9) effectively held the bacteria for the 21 day test period. The data of Table 1 suggest that pH decreases as

the Amberlite CG-50 concentration increases. Medium 383 with a pH of 6.9 contains 1.0 g/l of the exchange resin whereas the other media listed in the table have 10.0 g/l with a lower pH. Attempts to adjust the low pH showed that Amberlite CG-50 acts as a powerful buffer which is a disadvantage for the transport medium containing large amounts of the exchange resin. The low pH is too difficult to change.

A second requirement for a good transport medium is low toxicity. Previous investigators suggested that agar (A, B, D, G, K-N) and swabs (C, E, H-J) were sources of toxicity, and they suggested that purified agar or charcoal protect bacteria from the sources of toxicity. Some of the media studied in this investigation contained these agents, but they were beneficial to survival only in the presence of other components such as L-cysteine or L-methionine (Table 6), IsoVitaleX or Amberlite CG-50. Calcium alginate swabs which are relatively harmless to bacteria (E and H) were used for the throat culture studies. In view of these considerations it is tempting to suggest that toxicity is associated with cotton swabs, but further study is necessary to prove such a suggestion.

The requirement of low oxidation reduction potential (Eh) was loosely satisfied in this investigation. Some media needed agitation to suspend charcoal or ion exchange resins evenly in them as they solidified. The agitation oxidized the media to

some extent so that the lowest possible Eh was not present. This condition and the condition of low pH discussed earlier in this section demonstrate the ability of these transport media to maintain survival under less than optimum conditions.

The fourth requirement is that transport media contain as little nutrient material as possible. The media investigated in this study had a minimum content. However, data from Tables 2, 3, 5, and 6 suggest that growth occurred especially in media containing Amberlite CG-50, IsoVitaleX, and amino acids. Storage of inoculated transport media at 25 C enhances the effects of these reagents on long term survival. Mixed culture results further indicate the possibility of growth during long term storage especially with respect to staphylococci (see Tables 8 and 9). These observations raise questions about the relationship of limited growth to detectable survival. Is this limited growth necessary for reliable isolation of pathogens from transport media stored for long periods ?

The most significant component of transport media studied here is Amberlite CG-50. In addition to its possible role as substrate supporting limited growth, there is its relationship to ionic strength (Table 4). This relationship brings up the question of the importance of ionic strength to long term survival. Such considerations require further study before they can be applied toward improved bacteriological transport

media.

Media 383 and 408 held H. parainfluenzae in pure culture for 21 days while Stuart Medium Base did not. The identification of Haemophilus and Neisseria species from throat cultures held for 21 days in these media suggests their ability to hold fastidious organisms whereas SMB could not.

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Table 1.

Media with low pH

Medium No.	pH	Haemophilus		Streptococcus		Corynebacterium		Neisseria	
		Control	Test	Control	Test	Control	Test	Control	Test
36	6.3	9.5×10^5	6.9×10^2	3.0×10^5	2.9×10^3	1.3×10^6	8.7×10^4	3.2×10^5	3.1×10^3
383	6.9	7.25×10^5	4.93×10^3	4.6×10^5	$>10^5$	3.78×10^5	$>10^5$	2.8×10^6	5.5×10^2
396	5.5	9.45×10^5	5.08×10^5	6.35×10^5	$>10^5$	3.0×10^5	$>10^5$	1.24×10^6	9.83×10^2
398	.3	9.45×10^5	1.1×10^3	6.35×10^5	$>10^5$	3.0×10^5	$>10^5$	1.24×10^6	5.36×10^5
409	6.4	6.2×10^5	3.07×10^3	1.88×10^6	$>10^5$	3.1×10^5	$>10^5$	8.1×10^5	2.95×10^4
424	6.45	6.01×10^5	3.0×10^2	4.23×10^4	—	4.63×10^5	$>10^5$	1.16×10^5	5×10^2
499	6.6	$>10^7$	2.64×10^4	3.34×10^6	$>10^5$	9.45×10^5	$>10^5$	3.43×10^6	1.78×10^4

Table 2

Effects of temperature*

Medium No.	Temp.	Haemophilus		Streptococcus		Corynebacterium		Neisseria	
		Control	Test	Control	Test	Control	Test	Control	Test
407	4 C	6.2×10^5	0	1.88×10^6	$>10^5$	3.1×10^5	$>10^5$	8.1×10^5	0
413	25 C	3.8×10^5	$>10^5$	1.92×10^6	$>10^5$	1.39×10^6	$>10^5$	1.47×10^6	$>10^5$
408	4 C	6.2×10^5	$>10^5$	1.88×10^6	$>10^5$	3.1×10^5	$>10^5$	8.1×10^5	$>10^5$
414	25 C	3.8×10^5	$>10^5$	1.92×10^6	2.18×10^4	1.39×10^6	$>10^5$	1.47×10^6	0
409	4 C	6.2×10^5	3.07×10^3	1.88×10^6	$>10^5$	3.1×10^5	$>10^5$	8.1×10^5	2.95×10^4
415	25 C	3.8×10^5	0	1.92×10^6	$>10^5$	1.39×10^6	$>10^5$	1.47×10^6	$>10^5$
412	4 C	6.2×10^5	3×10^2	1.88×10^6	$>10^5$	3.1×10^5	$>10^5$	8.1×10^5	0
418	25 C	3.8×10^5	$>10^5$	1.92×10^6	$>10^5$	1.39×10^6	$>10^5$	1.47×10^6	$>10^5$
33	4 C	9.5×10^5	0	3.0×10^5	6.8×10^3	1.3×10^6	$>10^5$	3.2×10^5	0.16×10^2
343	25 C	5.2×10^5	$>10^5$	7.2×10^5	$>10^5$	7.8×10^5	$>10^5$	4.6×10^6	5.1×10^4
35	4 C	9.5×10^5	0.16×10^2	3.0×10^5	3.2×10^2	1.3×10^6	9.5×10^4	3.2×10^5	5.8×10^3
344	25 C	5.2×10^5	$>10^5$	7.2×10^5	$>10^5$	7.8×10^5	$>10^5$	4.6×10^6	$>10^5$
36	4 C	9.5×10^5	6.9×10^2	3.0×10^5	2.9×10^3	1.3×10^6	8.7×10^4	3.2×10^5	3.1×10^3
345	25 C	5.2×10^5	1.6×10^2	7.2×10^5	$>10^5$	7.8×10^5	—	4.6×10^6	3.1×10^4

Table 2

Effects of temperature*

* Media are listed in pairs made up of media with the same basic formula. Each member of the pair differs only in the storage temperature listed in the second column of the table. For example, medium 33 and 343 are a pair. One is stored at 4 C (Medium 33) and the other is stored at 25 C (Medium 343).

Table 3.

Ion exchange resin effect*

Medium No.	Exchange resin	Haemophilus		Streptococcus		Corynebacterium		Neisseria	
		control	test	control	test	control	test	control	test
479	Amberlite CG-50	6.93×10^5	0.16×10^2	2.48×10^6	$>10^5$	7.0×10^5	$>10^5$	3.25×10^5	10^2
480	Amberlite CG-4B	6.93×10^5	0.33×10^2	2.48×10^6	$>10^5$	7.0×10^5	$>10^5$	3.25×10^5	0
481	Amberlite CG-120	6.93×10^5	0	2.48×10^6	$>10^5$	7.0×10^5	$>10^5$	3.25×10^5	0
482	Amberlite CG-400	6.93×10^5	2.16×10^2	2.48×10^6	$>10^5$	7.0×10^5	$>10^5$	3.25×10^5	0.5×10^2

* These four media have the same basic formula. The Amberlites are the distinguishing reagents.

Table 4.

Amberlite CG-50 and its relationship to ionic strength and survival.*

edium o.	Ionic Strength	Haemophilus		Streptococcus		Corynebacterium		Neisseria	
		Control	Test	Control	Test	Control	Test	Control	Test
4	0.237	4.2×10^5	0	6.2×10^4	2.1×10^2	3.5×10^5	1.7×10^4	2.8×10^3	4.1×10^2
5**	—	9.5×10^2	6.9×10^2	3.0×10^5	2.9×10^3	1.3×10^6	8.7×10^4	3.2×10^4	3.1×10^3
9	0.086	5.2×10^5	0	9.2×10^4	7.5×10^4	1.3×10^4	4.0×10^2	8.8×10^4	0
0**	—	3.3×10^5	0	2.2×10^4	$.83 \times 10^2$	1.1×10^5	5.5×10^4	3.8×10^5	0

Media are arranged in pairs. Each pair contains a basic medium (24 or 49) and the basic medium containing Amberlite CG-50 (10.0 g/ℓ). For example, media 24 and 36 have the same basic recipe. Medium 36 contains 1% Amberlite CG-50.

* Media 36 and 60 contain 1% Amberlite CG-50.

Table 5.

Effects of IsoVitaleX*

Medium No.	Haemophilus		Streptococcus		Corynebacterium		Neisseria	
	Control	Test	Control	Test	Control	Test	Control	Test
47	3.5×10^6	0	6.1×10^3	1.8×10^3	1.5×10^5	8.2×10^4	2.0×10^6	0
408	6.2×10^5	$>10^5$	1.88×10^6	$>10^5$	3.1×10^5	$>10^5$	8.1×10^5	$>10^5$
383	9.25×10^5	4.93×10^3	4.6×10^5	$>10^5$	3.78×10^5	$>10^5$	2.8×10^6	5.5×10^2
409	6.2×10^5	3.07×10^3	1.88×10^6	$>10^5$	3.1×10^5	$>10^5$	8.1×10^5	2.95×10^4
398	7.45×10^5	1.1×10^3	6.35×10^5	$>10^5$	3.0×10^5	$>10^5$	1.24×10^6	5.36×10^5
424	6.01×10^5	3.0×10^2	4.23×10^4	—	4.63×10^5	$>10^5$	1.16×10^5	5×10^2

* Media are listed in pairs. Each medium of a pair has the same basic formula. The second medium listed in each pair contains 1% IsoVitalex. For example, medium 47 and 408 have the same basic formula. 408 contains 1% IsoVitaleX.

Table 6.

Effects of Amino Acids*

dium .	Amino Acid	Haemophilus Control	Haemophilus Test	Streptococcus Control	Streptococcus Test	Corynebacterium Control	Corynebacterium Test	Neisseria Control	Neisseria Test
	none	9.5×10^5	0.16×10^2	3.0×10^5	3.2×10^3	1.3×10^6	9.5×10^4	3.2×10^5	5.8×10^3
3	L-cysteine	7.25×10^5	4.93×10^3	4.6×10^5	$>10^5$	3.78×10^5	$>10^5$	2.8×10^6	5.5×10^2
	none	5.2×10^5	2.3×10^2	9.2×10^4	6.5×10^2	1.3×10^4	8.7×10^4	8.8×10^4	0
6	L-cysteine	9.45×10^5	5.08×10^4	6.35×10^5	$>10^5$	3.0×10^5	$>10^5$	1.24×10^6	9.83×10^3
8	L-methio-nine	9.45×10^5	1.1×10^3	6.35×10^5	$>10^5$	3.0×10^5	$>10^5$	1.24×10^6	5.36×10^5

Media 35 and 383 have the same basic recipe and media 48, 396, and 398 all have another basic recipe. The difference between each medium of a set is the amino acid it contains.

Table 7

Effect of polyethylene glycol*

	Haemophilus		Streptococcus		Corynebacterium		Neisseria	
Medium No.	Control	Test	Control	Test	Control	Test	Control	Test
62	3.3×10^5	0	2.2×10^4	0.83×10^2	1.1×10^5	7.5×10^5	3.8×10^5	1.4×10^3
108	4.6×10^5	0.16×10^2	2.9×10^5	6.8×10^3	2.5×10^5	4.4×10^4	6.6×10^5	0.5×10^2
63	3.3×10^5	0	2.2×10^4	0.83×10^2	1.1×10^5	5.9×10^5	3.8×10^5	4.5×10^2
109	4.6×10^5	0.16×10^2	2.9×10^5	1.1×10^4	2.5×10^5	$>10^5$	6.6×10^5	0.5×10^5

Media 62 and 108 have the same basic recipe except that 108 contains polythylene glycol. The same relationship exists between media 63 and 109.

Table 8

Artificially mixed cultures held for two days.

(Media were stored under nitrogen at 4 C for 30 days before their inoculation. Results are expressed as cells/ml.)

Organism	Control	Stuart Medium Base	Medium 383	Medium 408
Staphylococcus aureus	2×10^2	1.4×10^3	9×10^3	9×10^3
S. epidermidis	2.2×10^3	$\sim 10^3$	$\sim 10^3$	9×10^3
Streptococcus mitis	4.4×10^5	2.04×10^3	1.88×10^3	1.47×10^3
Strep. salivarius	8.9×10^5	1.42×10^4	2×10^3	1.58×10^3
Strep. equisimilus	7.25×10^5	6.9×10^3	7.0×10^3	8.3×10^3
Corynebacterium pseudodiphtheriticum	6.15×10^4	2.9×10^5	1.5×10^5	5×10^3
C. equi	1.44×10^5	1.1×10^5	1.4×10^5	1.11×10^5
Neisseria sp.*	2.6×10^4	1.15×10^5	6.6×10^4	2.5×10^4
Haemophilus parainfluenzae	**	——	**	**

* N. perflava and N. catarrhalus were the species used, but the assay procedure was insufficient for their separate identification.

** The presence of H. parainfluenzae was suggested only by colonial morphology. Gram stains were inconclusive.

Table 9

Artificially mixed cultures held for 12 days.

(Transport media were stored for 20 days before inoculation. Results are expressed as cells/ml.)

Organism	Control	Stuart Medium Base	Medium 383	Medium 408
S. aureus	5.0×10^6	4×10^3	2.5×10^3	$\sim 8 \times 10^3$
S. epidermidis	5.5×10^3	3×10^3	5×10^3	$\sim 10^3$
Streptococcus mitis	4.5×10^5	3.36×10^5	0	0
Strep. salivarius	1.55×10^6	2.5×10^2	6.9×10^2	3.64×10^3
Strep. equisimilus	2.25×10^6	7.0×10^6	3.1×10^2	6.5×10^2
Corynebacterium pseudodiphtheriticum	1.95×10^4	8×10^4	0	3.0×10^3
C. equi	8.5×10^3	9.4×10^5	1.35×10^4	1.26×10^5
Neisseria sp.	3.2×10^5	5.7×10^4	0	1.2×10^5
Haemophilus parainfluenzae	*	—	*	*

* The presence of H. parainfluenzae was only suggested by colonial morphology. Gram stains were inconclusive.