

General Disclaimer

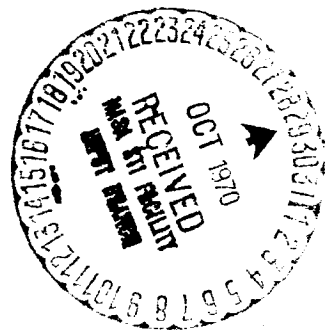
One or more of the Following Statements may affect this Document

- This document has been reproduced from the best copy furnished by the organizational source. It is being released in the interest of making available as much information as possible.
- This document may contain data, which exceeds the sheet parameters. It was furnished in this condition by the organizational source and is the best copy available.
- This document may contain tone-on-tone or color graphs, charts and/or pictures, which have been reproduced in black and white.
- This document is paginated as submitted by the original source.
- Portions of this document are not fully legible due to the historical nature of some of the material. However, it is the best reproduction available from the original submission.

THIRTEENTH SUMMARY REPORT OF PROGRESS

Services Provided in Support of the Planetary Quarantine Requirements
of the
National Aeronautics and Space Administration

REDUCTION OF MICROBIAL DISSEMINATION



by the

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Health Services and Mental Health Administration
National Communicable Disease Center
Microbiological Control Section, Bacterial Diseases Branch
Epidemiology Program

July 1969

FACILITY FORM 602

N70-42272	
(ACCESSION NUMBER)	(THRU)
9	
(PAGES)	(CODE)
CR-114263	04
(NASA CR OR TMX OR AD NUMBER)	(CATEGORY)

Reduction of Microbial Dissemination from Humans

Methods for Sampling of Airborne Clostridia -- Investigations on human shedding of Clostridium perfringens were continued. Further studies were conducted in an attempt to develop a satisfactory technique with high efficiency for recovering and culturing viable airborne particulates of Cl. perfringens. Studies described previously (Twelfth Summary Report of Progress) emphasized the inconsistent result obtained with the media and procedures examined. Reinforced clostridial agar (RCM) gave better results than other media tested in these previous studies.

Two additional culture media have been investigated: (a) Brain heart infusion agar (BHI) and (b) Anaerobic agar (AA). Suitability of these two media was determined by sampling of anaerobic Cl. perfringens nebulized into a plastic chamber.

It has been reported that nutrient agar poured into petri plates and then stored in the refrigerator until use may be inhibitory for growth of anaerobes: absorption of oxygen by the agar surface, even during refrigerated storage, results in aerobic conditions not ideal for initiation of synthesis and energy processes necessary for proliferation and development of colonies by anaerobic microorganisms. Thus, the procedure for preparation of culture plates exposed in the air samplers was changed so that only freshly poured plates would be used for recovery and culture. The candidate agar was first prepared in liter quantities and then dispensed in desired aliquots into small screw-capped bottles. The agar was sterilized, and the bottle caps were tightened immediately after the material was taken out of the sterilizer. The agar was allowed to cool and then stored in the refrigerator. Immediately prior to use, the agar

was melted in a hot-water bath and poured into petri dishes. Shortly after the petri dish had been exposed in the air sampler, an overlay of the same agar, which also had been stored and melted in small quantities, was poured over the exposed agar surface. With this procedure, the recovery layer of nutrient agar as well as the agar overlay was exposed to atmospheric gases for a minimum of time.

The initial comparison was made between brain-heart-infusion agar (BHI) and sulfite-polymixin-sulfadiazine agar (SPS), which was used as the standard in previous comparative studies (Twelfth Summary Report of Progress). Brain-heart-infusion agar was selected for tests since it is a non-selective, highly nutritious medium found to be highly favorable for cultivation of Cl. perfringens from a variety of sources. The results of a total of 61 trials in 2 series of tests are shown in Table 1.

Table 1

Comparison of Recovery of Airborne Cl. perfringens on Brain-heart-infusion Agar (BHI) and Sulfite-polymixin-sulfadiazine Agar (SPS) Exposed in TDL Slit Samplers

Series	Number of Trials	Number of Trials Positive for <u>Cl. perfringens</u>		Number of Trials with Higher Count		Colony-count Ratio, BHI/SPS
		SPS	BHI	SPS	BHI	
A	30	9	30	0	30	6.3
B	31	21	31	0	31	8.7

The number of plates on which Cl. perfringens developed was much less on the selective (SPS) medium than on the brain-heart-infusion agar. All of the exposed plates with BHI agar revealed the presence of Cl. perfringens in the

aerosol, while only 30 of the total 61 trials of SPS agar demonstrated anaerobic particles. In addition, BHI agar showed a much higher recovery rate in terms of the number of anaerobic particles recovered. The colony count on this medium was approximately 7 to 8 times higher than that observed on SPS agar. The results demonstrate that the non-selective medium was much superior for primary isolation of airborne Cl. perfringens. Although recognition of isolates of Cl. perfringens is readily made on SPS agar due to characteristic colonies, failure of this medium to show the presences of Cl. perfringens particles in approximately one-half of the samples precludes its use in shedding studies, even those with qualitative objectives. As with other organisms, the use of a highly sensitive, non-selective media appears necessary for primary isolation of Cl. perfringens.

Anaerobic agar (AA), which has been specifically designed for culturing of anaerobes and members of the genus Clostridium, was also examined for suitability in recovering airborne particles of Cl. perfringens. Although reducing agents and an indicator dye are incorporated in anaerobic agar, the medium does not contain inhibitory materials usually found in selective-type culture media. The efficiency of recovery on anaerobic agar was compared with that on SPS agar. The results of 114 trials in 4 series are given in Table 2.

Table 2

Comparison of Recovery of Airborne Cl. perfringens on Anaerobic Agar (AA) and Sulfite-polymixin-sulfadiazine Agar (SPS) Exposed in TDL Slit Samplers

Series	Number of Trials	Number of Trials Positive for <u>Cl. perfringens</u>		Number of Trials with Higher Count		Colony-count Ratio, AA/SPS
		SPS	AA	SPS	AA	
A	32	29	32	0	32	9.5
B	32	3	19	2	19	6.9
C	32	30	32	0	32	13.8
D	20	17	20	0	20	39.7

The performance of anaerobic agar was much superior to that of SPS agar. Cl. perfringens was detected in 103 out of the 114 trials by the anaerobic agar whereas SPS agar was successful in only 79 of the trials. A higher count was obtained on anaerobic agar in 103 of 105 trials. For all series, the colony ratio was also much higher for anaerobic agar. The selective agar again demonstrated an inhibitory action against airborne Cl. perfringens. Of the two media, anaerobic agar was found to be more suitable for primary isolation than the SPS agar.

The next studies were carried out to determine which of the three most favorable media -- anaerobic agar, brain-heart-infusion agar, or reinforced clostridial agar (RCM) -- would recover the highest number of airborne clostridial particles. In the initial experiments, anaerobic agar was compared with brain-heart-infusion agar. The results of 84 trials in 3 series are given in Table 3.

Table 3

Comparison of Recovery of Airborne Cl. perfringens on Anaerobic Agar (AA) and Brain-heart-infusion Agar (BHI) Exposed in TDL Slit Samplers

Series	Number of Trials	Number of Trials Positive for <u>Cl. perfringens</u>		Number of Trials with Higher Count		Colony-count Ratio, AA/BHI
		AA	BHI	AA	BHI	
A	30	30	30	19	11	1.4
B	30	28	30	1	28	0.3
C	24	21	22	12	12	1.4

Both media were about equal in the number of trials in which Cl. perfringens was detected. In series A and C, anaerobic agar had equal or more trials with higher counts than did BHI agar, and the colony-count ratio was higher on anaerobic agar in these two series. In trials for series B, however, BHI agar showed a markedly better performance. There is no readily evident explanation for this variation in results. Nonetheless, on the basis of this comparison, there appears to be little difference in the efficiency of anaerobic agar and BHI agar for determining the presence of airborne Cl. perfringens in the plastic chamber.

Reinforced clostridial agar (RCM) also was compared with brain-heart-infusion agar. The results of 86 trials in 3 series are given in Table 4.

Table 4

Comparison of Recovery of Airborne Cl. perfringens on Brain-heart-infusion Agar (BHI) and Reinforced Clostridial Agar (RCM) Exposed in TDL Slit Samplers

Series	Number of Trials	Number of Trials Positive for <u>Cl. perfringens</u>		Number of Trials with Higher Count		Colony-count Ratio, RCM/BHI
		RCM	BHI	RCM	BHI	
A	26	26	26	18	18	1.0
B	30	30	30	25	5	1.6
C	30	30	30	16	14	0.7

Both media detected Cl. perfringens in the aerosols in all trials. The reinforced clostridial agar did yield higher counts in more trials than did BHI agar; however, the difference was not large. In series A, the colony-count ratio was the same; in series B, RCM agar recovered more clostridial particles than did BHI; however, BHI gave a higher colony-count ratio in series C. On the basis of these results, it appears that there is very little difference between these two media. Again, as with the comparison between brain-heart-infusion and anaerobic agar (Table 3), there was a lack of consistency in the results between the different series of trials.

Since Cl. perfringens produces a characteristic colony on sulfite-polymixin-sulfadiazine agar, it would certainly be expeditious and more convenient to use this medium for primary isolation. However, the failure of this agar to equal the performance of brain-heart-infusion agar, anaerobic agar, or reinforced clostridial agar precludes its use for determining the shedding of Cl. perfringens. A large number of plates with sulfite-polymixin-sulfadiazine agar failed to show the presence of Cl. perfringens.

particles in aerosols known to contain viable particles as demonstrated by colony formation on other media.

The three non-selective media -- brain-heart-infusion agar, anaerobic agar, and reinforced clostridial agar -- do not produce characteristic colonies of Cl. perfringens. The use of these media in shedding studies will necessitate subculturing of a large amount of colonial growth to ascertain the shedding of anaerobic clostridia by humans. Although time consuming, this appears to be the only method for obtaining satisfactory results.

Shedding of Clostridia from Humans -- An attempt was made to determine whether clostridial shedding could be detected from two humans. The individuals, wearing street clothes, were screened in a static, plastic-isolator shedding chamber (Seventh Summary Report of Progress). A total of 60 cubic feet of air was sampled during a period of about one hour while each individual was confined in the shedding chamber. The particulate matter was recovered on both SPS agar and RCM agar. The methods used for preparation; pouring; and overlaying of the agar plates after exposure are given on pp. 1 - 2 of this report. Following exposure and overlaying, the plates were incubated under anaerobic conditions for 48 hours. Colonies resembling clostridial growth were transferred to cooked meat medium with dextrose and incubated anaerobically. Final identification of suspect colonies was made on the basis of growth on SPS agar and reactions with appropriate biochemical media.

A total of 587 bacterial particulates were recovered from individual A from both types of agar. These particulates were examined, but no Cl. perfringens were found. Individual B shed a much larger number of viable

particulates. Most of the exposed plates were overgrown, and quantitation was not practical. Examination of predominant colonial growth failed to reveal any Cl. perfringens; however, the large amount of extraneous organisms developing on the plates may have masked colonies of Cl. perfringens. With the use of non-selective media, it will be necessary to expose plates for only a short period of time in air samplers so that differential growth will develop and be recognizable after incubation. Although these initial tests failed to recover Cl. perfringens, this finding cannot be considered definitive. More refinement of the procedures will be necessary before satisfactory results are obtained.

Microbiological Control Section, Bacterial Diseases Branch
Epidemiology Program, National Communicable Disease Center
September 2, 1969