A transport method for swab specimens submitted for aerobic and anaerobic bacteriology

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SUMMARY The need for separate swab transport methods for aerobes and anaerobes may result in inadequate transport of specimens for anaerobic bacteriology. Most microbiology laboratories in Australia rely on Stuart's transport medium to protect anaerobic bacteria. This paper presents a new, simple transport medium (Transport Deep) suitable for use with aerobes and anaerobes. Comparative evaluations demonstrate that Transport Deep is as good as Stuart's medium for the maintenance of fastidious bacteria and is far superior for the protection of even extremely oxygen-sensitive anaerobes. This medium has been used successfully in a large Sydney hospital for more than a year. It is proposed that Transport Deep be used on a routine basis for all swab specimens.

A recent significant change in microbiology laboratories has been increased emphasis on the isolation of anaerobic bacteria from clinical specimens. Major advances have been the use of prereduced anaerobically sterilised (PRAS) media and the development of techniques that allow manipulation of specimens in the complete absence of oxygen such as anaerobic glove boxes or roll tubes.¹⁻³ These methods have led to an increased ability to culture the extremely oxygen-sensitive (EOS) bacteria that normally colonise the highly reduced microenvironments of the mouth and intestinal tract. Some of these organisms may die after as little as five minutes exposure to air. However, there is considerable controversy as to the significance of these EOS bacteria in clinical specimens. Some consider them to be very important in clinical medical microbiology⁴⁻⁸ while others state that EOS anaerobes are only rarely involved in infection and the use of special techniques is not warranted.⁹⁻¹²

Irrespective of the methods used for their culture, it is agreed that special care needs to be taken in transporting to the laboratory specimens likely to contain anaerobes; exposure to oxygen must be minimised. A number of expensive transport devices are available for the transport of anaerobes.^{13 14} The cost and inconvenience of using separate specimen collection methods for aerobic and anaerobic bacteria limits the use of these devices. A survey of anaerobic procedures used in Australian

laboratories, revealed that most hospitals use Stuart's medium for transport of specimens likely to contain aerobes or anaerobes. As this medium was not designed for the maintenance of anaerobes, there is a need for a transport medium better suited to both types of organisms. This paper reports the results of a comparative evaluation of Stuart's medium and a new simple medium (Transport Deep).

Material and methods

Survey A questionnaire was sent to 103 clinical microbiology laboratories throughout Australia. The overall response rate was 64% with a good response from private pathology, hospital and government health laboratories.

The question in the survey relevant to this paper was: "How are specimens suspected of containing anaerobes transported from the patient to your laboratory? List all methods in order of usage."

ANAEROBIC CHAMBER

The anaerobic chamber used in this study was similar to that of Aranki.¹ It was constructed of flexible vinyl by the Synflex Co (Sydney). The anaerobic environment inside the chamber consisted of 10% H₂ and 90% CO₂. To maintain anaerobiosis and to absorb any water formed, the gaseous mixture was circulated by a fan over trays containing palladium catalyst and silica gel. The catalyst and silica gel were rejuvenated for three hours every week at 160°C. Plates and tubes were incubated inside the

chamber in a 37°C incubator. Platinum loops were used for subculturing and these were sterilised by means of a Bactocinerator (Sherwood). A Model EE-DO oxygen electrode (EDT) was used to monitor the concentration of oxygen inside the chamber.

BACTERIAL CULTURES

The following bacterial strains were used as stock cultures:

Peptostreptococcus anaerobius (Virginia Polytechnic Institute (VPI) stock culture 5750).

Bacteroides fragilis ss fragilis (VPI stock culture 2556-1).

Escherichia coli (Kaplan, S¹⁵ stock culture 5F₂).

Fusobacterium sp (isolated from human faeces at the University of New South Wales (Univ NSW)).

Neisseria gonorrhoeae (Prince of Wales Hospital (POW) Bacteriology Department).

Streptococcus pyogenes (POW Hospital, Bacteriology Department).

Haemophilus influenzae (POW Hospital, Bacteriology Department).

Pseudomonas aeruginosa (isolated at Univ NSW).

The strain of *E coli* was obtained from Samuel Kaplan.¹⁵ *E coli* (5F₂) is a mutant of a K12 strain of *E coli* which is temperature-sensitive for glutamyl-tRNA synthetase.¹⁵ It will grow readily at 31°C but not at 37°C.

MEDIA

Subcultures and viable counts of all anaerobic stock cultures were made on Brain Heart Infusion agar (Oxoid) (BHIA suppl) which was supplemented as described in the Virginia Polytechnic Institute *Anaerobe laboratory manual*.³ Supplemented Brain Heart Infusion broth (BHIB suppl) was used for broth subcultures of all bacteria. Plate subcultures and viable counts of the facultative anaerobe *E coli* were done on (BHIA). Nutrient broth was used for serial dilution. *N gonorrhoeae* and *H influenzae* were grown on chocolate agar and 5% horse blood agar was used to grow *S pyogenes*.

TRANSPORT METHODS TESTED

(i) Aerobic tube

Cultures were inoculated on to swabs and held in a sterile tube with a loose fitting lid.

(ii) Stuart's medium

This medium was prepared according to the manufacturer's instructions (Oxoid), and dispensed in 5 ml lots into Bijou bottles.

(iii) Alternative transport medium

Composition:

CaCl ₂ (anhydrous)	0.1 g
MgSO ₄ .7H ₂ O	0.1 g
NaHCO ₃	5.0 g
NaCl	1.0 g
K ₂ HPO ₄	0.5 g
KH ₂ PO ₄	0.5 g
Resazurin (22 mg/ml)	8.0 ml
Cysteine	0.5 g
Purified agar (Oxoid)	6.0 g

CaCl₂ and MgSO₄ were dissolved in 300 ml of distilled water then 500 ml of distilled water and NaHCO₃ and NaCl were added. K₂HPO₄ and KH₂PO₄ were mixed together in a separate 200 ml of distilled water. The two solutions were then combined. This order of mixing prevented precipitation of the phosphates.

The remaining components were added and the pH was adjusted to 7.8. The solution was steamed to dissolve the agar. Fifteen millilitres of the hot medium were dispensed into tubes which were autoclaved at 121°C for 10 min. The tubes were taken into the anaerobic chamber while still hot and allowed to cool. Screw caps were tightly fitted to the tubes which were taken from the chamber, stored at room temperature and used as required. If during storage the resazurin turned pink, the tube was not used.

INOCULATION OF SWABS

All culture media and broths were allowed to reduce inside the anaerobic chamber for at least 24 h before use. All preparative procedures for evaluations involving anaerobes were carried out inside the chamber.

Single colonies were picked from plate cultures and inoculated into BHIB suppl, which was then incubated at 37°C (30°C for *E coli*) overnight. Three drops from each broth culture to be used were inoculated into a tube containing 10 ml of BHIB suppl. This stock suspension was then used to inoculate the various transport devices being tested. Zero time viable counts were done for a set of three swabs for each experiment so there was no need to estimate the number of bacteria in the original broth. Due to difficulties in growing *N gonorrhoeae* in broth, a suspension was prepared by washing organisms off a chocolate agar slope which had been inoculated with the organisms and incubated overnight.

The swabs were inoculated by pipetting 0.01 ml of the stock suspension into a well of a sterile microtitre plate, then placing a sterile swab in the well and allowing it to absorb the fluid. Every swab was inoculated in this way so that each carried the same number of bacteria.

TEST METHODS

Mixture of anaerobes

When all swabs had been inoculated, a "zero time" count was made using three swabs to determine the number of bacteria which could be recovered from each swab. Three other inoculated swabs were sealed in sterile tubes and were kept inside the chamber to be used as the 24-hour control swabs. The rest of the swabs were removed from the chamber and immediately inserted into aerobic tubes, Stuart's media and Transport Deep. Viable counts were done for sets of three transport devices three, six and 24 h after the zero time count. Therefore the viable count for each time was the average of three counts.

Quantification

Swabs were vortexed in 0.9 ml of nutrient broth and the resulting suspension was used for serial tenfold dilutions. Each dilution was plated on to BHIA plate and a BHIA suppl plate. These were then incubated at 30°C aerobically and 37°C anaerobically respectively. The *E coli* grew only at 30°C, while the anaerobes could clearly be distinguished on the 37°C plates.

Extremely oxygen sensitive anaerobes

The method used was the same as that for the mixture of organisms, except that the dilutions were only plated on to BHIA suppl.

Aerobes and facultative aerobes

Stuart's medium and Transport Deep were tested using fresh clinical isolates of *N gonorrhoeae*, *H influenzae* and *S pyogenes* which were inoculated onto charcoal, buffered and plain swabs. The method used was the same as that described for the mixture of organisms except that a different method of measurement was used.

Quantification

A semiquantitative method was used to measure the survival of these organisms. A standard method of streaking plates was used and the amount of growth was scored 1-5 depending on the location of colonies on the plate. Thus, a rating of five was given when heavy growth was seen on all the inoculated areas of the plate, while a score of one indicated growth on the primary inoculum only. Where intermediate growth occurred, a qualitative intermediate value of 0.5 was added.

Results

TRANSPORT METHODS USED IN AUSTRALIA

Table 1 lists the types of devices most commonly

Table 1 *Methods most commonly used by clinical microbiology laboratories in Australia for the transport of specimens suspected of containing anaerobes*

Transport method	Number of laboratories using this method*
Swabs:	
Stuart's medium	36
Dry	6
Cary-Blair transport medium	1
Port-A-Cul tube	1
Anaerobic Vacutainer	1
Transcul	7
Amies' transport medium	2
Swab total:	54
Volumes (exudate or body fluid)	
Needle	4
Staff go to bedside	3
Volume total:	7

*Total number of replies to this question: 61.

used to transport specimens which are routinely cultured for anaerobes in Australia. Swabs are the commonest specimen type submitted for culture. Fifty-four of the 61 laboratories received mostly swabs, while only seven laboratories received volumes of exudate more frequently and none of the laboratories routinely received tissue specimens.

Only three of 54 laboratories customarily received their swabs in anaerobic transport devices (Anaerobic Vacutainer, Port-A-Cul and Cary-Blair deeps) whereas 36 of these laboratories commonly received most swabs in Stuart's medium. Surprisingly, in six laboratories, dry swabs were most commonly received. The results of this questionnaire provided the stimulus for the development of a cheap alternative to Stuart's medium which could also be made up simply in the hospital media sections but would better support the viability of anaerobes. The basic salts composition of this medium is the same as in the basic salts solution described as a diluent in the VPI *Anaerobe laboratory manual*.³ Results of an evaluation of this Transport Deep compared to other transport methods are reported in the next section.

EVALUATION OF THE SURVIVAL OF ANAEROBIC BACTERIA ON SWABS IN VARIOUS TRANSPORT DEVICES

Mixed cultures

Aerobic tubes, Stuart's medium and Transport Deep were tested using a mixture of *E coli*, *B fragilis* and *P anaerobius* which is a combination of bacteria commonly associated with clinical infections. The results of this evaluation are shown in Fig. 1. All three methods maintained significant numbers of the three organisms up to six hours; after 24 hours,

Fig. 1 Survival of the mixture of *P. anaerobius*, *B. fragilis* and *E. coli* held on swabs in various devices for 24 h.

however, *P. anaerobius* was only recovered from the Transport Deep. The 24-hour anaerobic control counts are shown in Table 2.

Table 2 Twenty-four hour anaerobic control viability counts for the experiment shown in Fig. 1

Transport device	$\log_{10}$ CFU/swab		
	<i>P. anaerobius</i>	<i>E. coli</i>	<i>B. fragilis</i>
(a) Stuart's medium	7.0	9.2	8.9
(b) Aerobic tube	7.3	7.6	8.4
(c) Transport Deep	7.0	9.2	8.9

CFU = colony-forming units.

Extremely oxygen sensitive (EOS) anaerobes

The protection offered by the Transport Deep was more dramatically shown in experiments which used an EOS species of *Fusobacterium* that had been isolated from human faeces. On plate media, this organism dies after only five minutes exposure to air. In Fig. 2 it is seen that the *Fusobacterium* could be recovered on swabs after 24 h in the Transport

Fig. 2 Survival of *Fusobacterium* sp. held on swabs in various devices for 24 h.

Deep, while no growth was found on swabs in aerobic tubes or Stuart's medium after three hours. The 24-hour anaerobic control counts (Table 3) show that this organism could survive 24 h on a swab if not exposed to the air.

Table 3 Twenty-four hour anaerobic control viability counts for the experiment shown in Fig. 2

Transport device	$\log_{10}$ CFU/swab <i>Fusobacterium</i> sp
(a) Stuart's medium	6.2
(b) Aerobic tube	5.3
(c) Transport Deep	4.0

EVALUATION OF THE SURVIVAL OF FASTIDIOUS AEROBIC AND FACULTATIVELY ANAEROBIC BACTERIA ON SWABS IN VARIOUS TRANSPORT DEVICES

If Transport Deep is to be a feasible alternative to Stuart's medium, it must offer protection for fastidious aerobic and facultative anaerobic bacteria likely to be found in clinical specimens. Figs 3 (Transport Deep) and 4 (Stuart's medium) show the results of an evaluation using *Neisseria gonorrhoeae*, *Haemophilus influenzae* and *Streptococcus pyogenes*. All of these cultures were fresh clinical isolates. The only type of organism which would routinely be transported on charcoal swabs is *N*

Fig. 3 The survival of *H influenzae*, *S pyogenes* and *N gonorrhoeae* held on a variety of swabs in Transport Deep

Fig. 4 The survival of *H influenzae*, *S pyogenes* and *N gonorrhoeae* held on a variety of swabs in Stuart's medium.

gonorrhoeae, therefore charcoal swabs were not included in the evaluations using *H influenzae* or *S pyogenes*. There were no major detectable differences between Stuart's medium and Transport Deep in the maintenance of these fastidious bacteria. The well established importance of charcoal swabs for the protection of *Neisseria* sp is also illustrated in these results. The survival of the strict aerobe *Pseudomonas aeruginosa* in both these media is shown in Fig. 5.

Fig. 5 *Pseudomonas aeruginosa* transported on plain swabs and held in Stuart's medium and Transport Deep for 48 h.

Discussion

There are a number of commercially available transport devices that are as good as Transport Deep in maintaining the viability of oxygen-sensitive bacteria.^{13 16 17} However, these devices are too expensive for general use and have met with consumer resistance.

Only in certain cases do medical and nursing staff think it necessary to use a specialised transport device and, there are many specimens that are inadequately protected during transport. Use of a general transport medium for all specimens removes the need for such a decision.

The transport medium described here is extremely simple and cheap. It is also effective in maintaining the viability of fastidious bacteria such as *N gonorrhoeae*, *S pyogenes* and *H influenzae*. It can therefore

be considered as a feasible substitute for Stuart's medium which offers poor protection from oxygen for anaerobic bacteria.

Not all laboratories use specialised anaerobic culture methods such as glove boxes or roll tubes. There is often considerable oxygen exposure of specimens during culture. If the specimen has been adequately protected before culture, then total oxygen exposure of the organisms is less, resulting in a greater likelihood of isolation, in other words, strict protection during specimen transport enhances the usefulness of conventional anaerobic culture methods.

Results of this study on the transport medium were sufficiently encouraging for trial use in St Vincent's Hospital, Sydney, thereafter it has been adopted as the routine transport medium for all specimens for more than a year; it is considered to be superior to Stuart's medium. In this hospital, 16 ml bottles ($\frac{1}{2}$ g) are filled with the medium, autoclaved, caps closed, and tops dipped in paraffin wax; an anaerotic chamber is not used.

A disadvantage of the Transport Deep is one common to all semisolid preparations used for swab transport. The material from the swab does not allow the preparation of a good Gram-stain of the exudate. As the stained direct smear can be a valuable source of information in clinical microbiology,¹⁸ St Vincent's Hospital supplies a sterile slide in a glass bottle with each Transport Deep kit.

The significance of certain EOS bacteria in clinical specimens is yet to be evaluated. However, some species of anaerobes involved in infection—for example, *P. anaerobius*, clearly would not survive the oxygen exposure routinely experienced in many Australian laboratories. We believe that use of a simple general transport method for all swabs will lead to increased isolations of anaerobes and allow assessment of their clinical significance.

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