

Evaluation of Fastidious Anaerobe Broth as a blood culture medium

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SUMMARY Three commercial blood culture media were compared with a freshly prepared cooked meat medium in tests to simulate the recovery of small inocula of anaerobic and aerobic bacteria in routine blood cultures. The cooked meat medium gave the most reliable recovery and supported continued viability, whilst Fastidious Anaerobe Broth (LAB M) was a good alternative. Results with Southern Group thioglycollate and Difco Thiol were less satisfactory as delays in recovery and loss of viability occurred on continued incubation with some of the test strains.

Increasing clinical awareness of the importance of anaerobic infections has resulted in the continuing need for the development of better recovery media, both for purulent material and blood cultures. The superiority of freshly prepared cooked meat medium over various commercially available media for routine blood cultures has previously been reported, but the use of this medium is an extra burden on busy clinical laboratories. The present study was undertaken to compare a new commercial blood culture medium Fastidious Anaerobe Broth (FAB) from LAB M, Ford Lane, Salford, with a freshly prepared cooked meat medium and two other commercial media.

Material and methods

ORGANISMS

The following standard strains were used: *Bacteroides melaninogenicus* ss *intermedius*, NCTC 9336; *Peptostreptococcus anaerobius* ATCC 27337; *Pseudomonas aeruginosa* NCTC 10662; *Staphylococcus aureus* NCTC 6571 and laboratory clinical isolates of *Bacteroides fragilis*; *Fusobacterium necrophorum*, *Streptococcus milleri* and *Streptococcus pneumoniae*.

MEDIA

The following media were bought as prepared blood culture bottles and used according to the manufacturer's instructions. Freshly prepared cooked meat medium was prepared in the laboratory.

(i) Freshly cooked meat medium (CM) was prepared according to Holdeman, Cato and Moore.²

Ground, fat-free, beef heart in a ratio of 1 part meat to 4-5 parts of fluid was distributed in 75 ml volumes in 100 ml bottles.

(ii) Fastidious Anaerobe Broth (FAB) from LAB M, Ford Lane, Salford (no product code number available). Bottles contained 75 ml broth.

(iii) Brewer's thioglycollate medium (05-86 CX) from Southern Group Laboratories (SG), Hither Green Hospital, Hither Green Lane, London. Bottles contained 80 ml broth.

(iv) Bacto-Thiol broth (0355-74-2) from Difco Laboratories (Thiol), Central Avenue, West Molesey, Surrey. Bottles contained 100 ml volume with added CO₂ under vacuum.

Subcultures were inoculated on to blood agar made with Columbia agar base (LAB M) with 5% defibrinated horse blood (CBA) medium. Heated blood agar had the same constituents and was heated after the addition of blood. Brain Heart Infusion broth from LAB M (BHI) was presteamed for 10 min and cooled before being used to prepare the bacterial inoculum.

INOCULUM

CBA plates were spread with each organism to give confluent growth. These plates were incubated overnight (48 h for *B melaninogenicus*). The growth was removed with a swab moistened in BHI and emulsified into 9 ml of BHI. Previous studies showed that this emulsion contained approximately 10¹⁰ colony forming units per ml (CFU/ml). Serial dilutions were made in BHI and 0.1 ml of the three lowest dilutions—that is, 10⁻⁶, 10⁻⁷, 10⁻⁸, were used to seed the blood culture bottles. Defibrinated horse blood was added to these bottles to give a final

concentration of 5%.

Each organism was used at three dilutions and each dilution was also put up in triplicates of the blood culture medium; nine bottles of medium were thus used in tests with each organism. To estimate the variation in inoculum size, 0.1 ml of each diluted culture was also placed on to three CBA plates to give a surface count by the spread plate method.

INCUBATION

All bottles were incubated at 36°C on a trolley in a hot room.

SUBCULTURES

Subculture procedure followed the normal routine in the laboratory, at approximately six hours, then three and seven days. Additional subcultures were done for this project at 24 h and five days. A sterile 10 µl disposable plastic loop (Nunc, Denmark) was used for each subculture except from Thiol broth

when a syringe was used according to the manufacturer's specifications.

Subcultures were made by seeding CBA plates which were incubated aerobically and anaerobically (GasPaks in Don Whitley jars). Heated blood agar was also seeded and incubated in 10% CO₂. Growth on these media was recorded subjectively as ranging from a few colonies (+) to heavy confluent growth (+++).

Results

INOCULUM SIZE

The mean numbers of test bacteria (CFU) inoculated per bottle are shown in Figs 1 and 2. The triplicate plate counts obtained for the minimal inocula were: *B fragilis* 27, 32, 31; *B melaninogenicus* 16, 12, 17; *F necrophorum* 41, 25, 24; *Peptostrep anaerobius* 5, 4, 9; *Staph aureus* 6, 9, 9; *Ps aeruginosa* 2, 3, 10; *Str pneumoniae* 2, 4, 0; and *Str milleri* 132, 148, 140.

ORGANISM	Mean no. organisms inoculated per bottle	Replicate	6 HRS				24 HRS				3 DAYS				5 DAYS				7 DAYS			
			CM	FAB	SG	THI	CM	FAB	SG	THI	CM	FAB	SG	THI	CM	FAB	SG	THI	CM	FAB	SG	THI
BACTEROIDES FRAGILIS SS FRAGILIS (Laboratory Strain)	30	1																				
		2																				
		3																				
	400	4																				
		5																				
		6																				
	10,000	7																				
		8																				
		9																				
BACTEROIDES MELANINOGENICUS SS INTERMEDIUS NCTC 9336	15	1																				
		2																				
		3																				
	200	4																				
		5																				
		6																				
	2,666	7																				
		8																				
		9																				
FUSOBACTERIUM NECROPHORUM (Laboratory Strain)	30	1																				
		2																				
		3																				
	40	4																				
		5																				
		6																				
	1,300	7																				
		8																				
		9																				
PEPTO-STREPTOCOCCUS ANAEROBIUS ATCC 27337	6	1																				
		2																				
		3																				
	40	4																				
		5																				
		6																				
	200	7																				
		8																				
		9																				

CM – Fresh cooked meat
 FAB – Fastidious anaerobe broth (lab M)
 SG – Southern group brewers thioglycollate
 THI – Thiol (DIFCO)

☐ + growth ▨ ++ growth ▩ +++ growth

Fig. 1 Recovery of anaerobic test strains from four blood culture media.

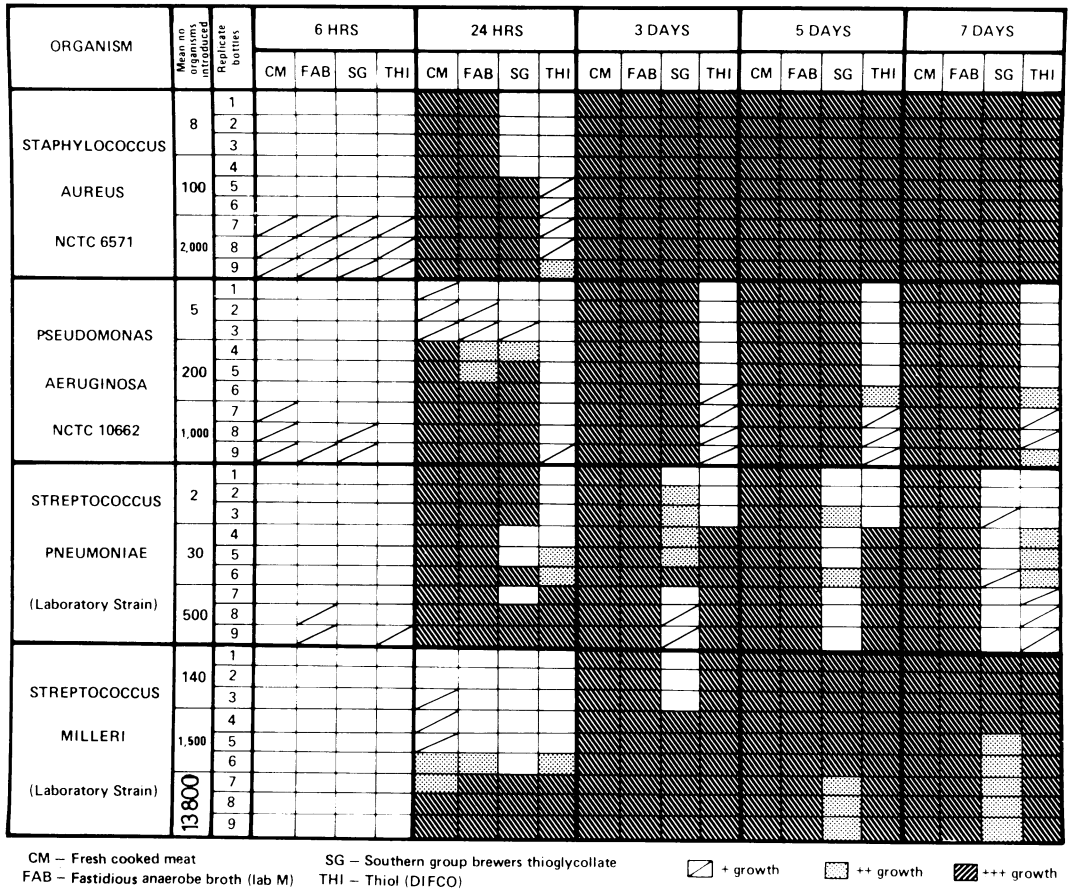


Fig. 2 Recovery of aerobic test strains from four blood culture media.

The blood culture results are presented for anaerobic organisms (Fig. 1) and aerobic organisms (Fig. 2).

An interesting point was that *B melaninogenicus* was detected on subculture before either visible turbidity or haemolysis was observed in any medium.

Discussion

Overall, the cooked meat medium (CM) was the best medium both for initial recovery and sustained viability of all test organisms. FAB was seen to be a reliable medium only slightly inferior to CM under these test conditions. FAB was superior to CM in that *B fragilis* and *Str pneumoniae* were recovered after incubation for only six hours. Results with Brewer's thioglycollate (SG) and Bacto-Thiol were less satisfactory under these test conditions.

Our CM results agree with those reported by Collee *et al*¹ and Barr³ using this medium. As can be seen

from Figs 1 and 2, all organisms can be readily recovered and remained viable throughout the tests. Disadvantages associated with CM are that its preparation is both time and space-consuming, adding an extra burden to a routine laboratory. The lack of clarity of the sterile medium also presents problems in judging visible evidence of growth.

Brewer's thioglycollate medium (SG) was recommended in *ACP Broadsheet 81* for anaerobic blood culture.⁴ A survey in 1972 of media used in Britain showed SG thioglycollate to be the best available.⁵ In the present study the recovery of aerobic and anaerobic streptococci in thioglycollate broth (SG) was delayed and the viability of these strains was markedly decreased. Szawatowski⁶ also reported the delayed recovery and loss of viability of *B fragilis* strains in Brewer's thioglycollate, although the medium manufacturer was not mentioned.

Results with Thiol broth (Difco) were poor; in particular rapid recovery of anaerobes and *Str*

pneumoniae were not possible and also *B melaninogenicus* was only recovered from heavy inocula. The ability of this medium to recover *Ps aeruginosa* is unreliable, but Difco make no claims as to the effectiveness of Thiol broth to support the growth of aerobes. Szawatkowski⁶ reported Difco Thiol to be superior to thioglycollate broth and commercial cooked meat broth for the rapid isolation of various *Bacteroides* spp and *Fusobacterium* spp. Shanson and Barnicoat⁷ also found this medium to be a satisfactory alternative to SG thioglycollate for the isolation of anaerobes. Our results agree with those of Collee *et al*¹ and Barr³ who found it less reliable for the isolation of anaerobic bacteria.

Initial studies were done to determine the recovery of small inocula of *B melaninogenicus* from various anaerobic media (Turton and Ganguli, unpublished observations). The media tested included various thioglycollates—for example, United States Pharmacopeia (USP), Brewer's, and a fluid thioglycollate medium—all supplied by LAB M—and Brewer's thioglycollate from Baltimore Biological Laboratories, Division of Becton, Dickinson and Co, Cockeysville, Maryland 21030, USA and a cooked meat medium with BHI overlay (CM/BHI) from LAB M. The best medium for the recovery and viability of the test organism was CM/BHI. USP thioglycollate gave rapid recovery of small inocula of *B melaninogenicus*, but loss of viability occurred within five days of inoculation. Thus, to prevent loss of viability, USP thioglycollate was modified in collaboration with LAB M to develop Fastidious Anaerobe Broth (Porter and Hyde, unpublished observations). The relative costs for 100 bottles of the media tested are as follows (January 1981):

Thiol (Difco) 0355-74-2, £52.80 + VAT + carriage, bottles disposable; Southern Group Brewer's thioglycollate 0586CX, £33.00 + carriage, bottles returnable; FAB (LAB M), no code number available, £31.00 + VAT + carriage, bottles disposable. The cost of freshly prepared cooked meat

was £22.29 which includes £6.46 for reusable bottles; however, this price makes no allowance for technician time or laboratory overheads.

It is widely recommended that at least two media, one for anaerobes and another for aerobes, are used in order to recover the majority of organisms from infected blood. It is of additional benefit to use an anaerobic isolation medium which can also support the growth of aerobes. It is frustrating that cooked meat medium is still superior, but FAB appears to be a good medium for the recovery of both anaerobic and aerobic bacteria from blood cultures.

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References

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