

even three neighbouring laboratories investigate routine sputum samples, and highlights the difficulties which may be faced with the potential introduction of "standardised" SOPs.

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Isolation of *Mycoplasma hominis* using the BACTEC 9000 series blood culture system

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Abstract

***Mycoplasma hominis* has been implicated as an important cause of septicaemia. There have been reported variances in the ability of blood culture systems to support the growth of this organism. In this study the ability of the BACTEC 9000 series automated system to grow and detect *M hominis* was assessed. Three of five wild *M hominis* strains grew in the BACTEC Anaerobic Plus/F medium but growth was not flagged by the detection mechanism of the system. It is recommended that users of the BACTEC 9000 series should use a seven day protocol and perform terminal subculture for suspected cases of *M hominis* septicaemia.**

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Keywords: *Mycoplasma hominis*, BACTEC 9000 series automated system.

Mycoplasma hominis may be an under-reported cause of septicaemia,^{1,2} particularly in patients who have undergone some form of genitourinary manipulation.³⁻⁵ Most frequently, the organism has been isolated from the blood of patients with postpartum pyrexia.² There may also be an increased incidence in immunosuppressed patients³ and in neonates.⁴

Previous studies have shown the failure of some blood culture media to support the growth of *M hominis*.^{1,2,4,6} In many cases this failure has been attributed to the presence of sodium polyanethyl sulphonate (SPS), a substance to which mycoplasma and some other bacteria are known to be susceptible.^{2,5}

The Becton Dickinson (Cowley, Oxford, UK) BACTEC 9000 automated system uses culture media that include 0.05% SPS in their formulation along with a mixture of resins intended to neutralise the effects of antibiotics in the patient's blood. A fluorescence sensor incorporated into the bottle detects carbon dioxide production as an indicator of microbial growth. Positive cultures are flagged when the

appropriate changes in the continuously monitored samples are detected by the indicator system.

It is recommended by Becton Dickinson that to counteract any inhibitory effect of SPS and to optimise the isolation of susceptible organisms 10 ml of blood should be added to their Plus/F blood culture media.

The aim of this study was to assess the ability of the medium used in the BACTEC 9000 series system to support the growth of *M hominis* and to assess the effectiveness of the system for detecting this organism from blood culture.

Methods

The methods used were similar to those used in a study by Davies and Spencer.¹ Five strains of *M hominis* were isolated from female genital specimens. Colonies were subcultured for purity onto Columbia blood agar (Becton Dickinson) and identified by morphology, resistance to erythromycin, ability to utilise arginine, and inability to ferment glucose or hydrolyse urea.² Cultures were incubated in anaerobic jars using Anaerogen sachets (Oxoid, Basingstoke, UK).

A 1 cm² block of Columbia blood agar with pure confluent growth of *M hominis* was removed from the plate. This block was agitated in 10 ml sterile isotonic saline, and 1 ml of the supernatant was removed and diluted in another 9 ml sterile saline. Finally, 1 ml of this dilution was pipetted into 99 ml sterile, defibrinated horse blood. Surface viable counts were performed on all dilutions to ascertain the inoculum size.

Duplicate BACTEC Aerobic Plus/F and Anaerobic Plus/F bottles were inoculated with 10 ml of the prepared blood. One pair from each set remained in the machine undisturbed while the other was removed daily for subculture. Brain heart infusion (BHI) broth was inoculated as a viability control (Technical Service Consultants, Heywood, Lancs, UK).

Daily subcultures were carried out for seven days on the control bottles and one of the sets

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Table 1 Isolation of *M. hominis* from BACTEC Plus/F media

Isolate	Inoculum (cfu/10 ml)	Days to isolation		
		BHI broth	BACTEC (aerobic)	BACTEC (anaerobic)
A	70	1	No growth	7
B	260	1	No growth	7
C	150	1	No growth	6
D	30	1	No growth	No growth
E	40	1	No growth	No growth

BACTEC (aerobic) = BACTEC Plus Aerobic/F; BACTEC (anaerobic) = BACTEC Plus Anaerobic/F.

of BACTEC bottles. The subcultures, taken by needle and syringe, were plated onto Columbia blood agar and were incubated anaerobically for 48 hours before examination. The undisturbed BACTEC pairs were terminally subcultured on the seventh day of incubation.

Results

The five test strains of *M. hominis* grew after incubation for one day in the BHI broth. None of the BACTEC bottles gave a positive growth response—that is, growth was not detected by the fluorescence system during the seven day incubation period. Growth was not detected by daily subculture of the BACTEC Aerobic Plus/F bottles during the incubation period. However, three of the strains grew in the BACTEC Anaerobic Plus/F medium. One strain was detected by subculture after six days' incubation and a further two after seven days' incubation (table 1).

Discussion

The results of this study are similar to the findings of other investigators^{1 2 4 6} in that *M. hominis* may grow poorly on some blood culture media and this may be attributable to the presence of SPS. Even at sub-bactericidal levels SPS may retard the speed of growth.¹

Davies and Spencer suggested that the minimum bactericidal concentrations of SPS for *M. hominis* in BHI broth varied between 0.006 and 0.025% (w/v). Davis and Eggington⁶ found that 30% of their *M. hominis* strains were able to grow in the presence of 0.025% SPS but that no strains were able to grow at 0.05%.

Previous studies relating to *M. hominis* using a variety of BACTEC media and detection systems have shown variable success rates for the isolation of this organism.⁴⁻⁶ This present study shows that the BACTEC Aerobic Plus/F medium does not support the growth of this organism. However, the BACTEC Anaerobic Plus/F medium will support the growth of some strains of *M. hominis* when inoculated into 10 ml blood and incubated for seven days.

Becton Dickinson state that a terminal subculture may be necessary to obtain maximum yield of isolates. Our findings support this recommendation and we would suggest that users of the BACTEC 9000 system use a seven day protocol and subculture using appropriate techniques for *M. hominis*, particularly for certain classes of patient—for example, those with postpartum pyrexia.

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Heat tolerance of vancomycin resistant *Enterococcus faecium*

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Abstract

The heat tolerance of 27 *Enterococcus faecium* isolates in water was studied. Stationary phase cultures including vancomycin resistant and sensitive clinical and food isolates were exposed to heat at 60°, 65°, 71°, and 80°C for one, three, 10, and 30 minutes and the log₁₀ reductions in bacterial counts were determined. Exposure at 71° and 80°C resulted in >6 log₁₀ reduction in viable counts for all isolates. Seven

(24%) isolates survived (<5 log₁₀ reduction) heat at 65°C for 10 minutes. The *E. faecium* isolates were more resistant to heat than the two *E. faecalis* reference strains. No differences in heat tolerance were observed between vancomycin sensitive and resistant strains or between isolates of human or food origin.

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Keywords: *Enterococcus faecium*, heat, antibiotics, glycopeptide, disinfection methods.