even three neighbouring laboratories investi-
gate 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

K A Kitson, K C Wright

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 homi-
is septicaemia.


Keywords: Mycoplasma hominis, BACTEC 9000 series
automated system.

Mycoplasma hominis may be an under-reported
cause of septicaemia, particularly in patients
who have undergone some form of genitouri-
nary manipulation. Most frequently, the
organism has been isolated from the blood of
patients with postpartum pyrexia. There may
also be an increased incidence in immuno-
suppressed patients and in neonates.

Previous studies have shown the failure of
some blood culture media to support the
growth of M hominis. In many cases this
failure has been attributed to the presence of
sodium polyanethol sulphonate (SPS), a sub-
stance to which mycoplasma and some other
bacteria are known to be susceptible.

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 microb-
ial growth. Positive cultures are flagged when the
appropriate changes in the continuously moni-
tored 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 organ-
isms 10 ml of blood should be added to their
Plus/F blood culture media.

The aim of this study was to assess the abil-
ity of the medium used in the BACTEC 9000
series system to support the growth of M homi-
is 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. 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. 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 subcul-
ture. 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
**Table 1  Isolation of M. hominis from BACTEC Plus/F media**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Inoculum (cfu/10 ml)</th>
<th>BHI broth</th>
<th>BACTEC (aerobic)</th>
<th>BACTEC (anaerobic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>70</td>
<td>1</td>
<td>No growth</td>
<td>7</td>
</tr>
<tr>
<td>B</td>
<td>260</td>
<td>1</td>
<td>No growth</td>
<td>7</td>
</tr>
<tr>
<td>C</td>
<td>150</td>
<td>1</td>
<td>No growth</td>
<td>6</td>
</tr>
<tr>
<td>D</td>
<td>30</td>
<td>1</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td>E</td>
<td>40</td>
<td>1</td>
<td>No growth</td>
<td>No growth</td>
</tr>
</tbody>
</table>

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 investigators1 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 Eggington4 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.

The authors would like to thank Becton Dickinson UK Limited for the supply of BACTEC media used in this study.


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**Heat tolerance of vancomycin resistant Enterococcus faecium**

S Panagea, P R Chadwick

**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°C, 65°C, 71°C, and 80°C for one, three, 10, and 30 minutes and the log₁₀ reductions in bacterial counts were determined. Exposure at 71°C 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, animal, or food origin. *(J Clin Pathol* 1996;49:687–689)

Keywords: Enterococcus faecium, heat, antibiotics, glycopeptide, disinfection methods.
Isolation of Mycoplasma hominis using the BACTEC 9000 series blood culture system.  
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