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Evaluation of BacT/ALERT System for Detection of *Mycoplasma hominis* in Simulated Blood Cultures

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We used simulated blood cultures inoculated with clinical isolates of *Mycoplasma hominis* to determine whether liquid media of the BacT/ALERT (Organon Teknika, Durham, N.C.) will support growth of this fastidious organism and whether its presence can generate a positive signal with the instrument. Viability of clinical isolates of *M. hominis* was maintained for 7 days in BacT/ALERT media, and organisms were able to multiply when 1% gelatin was added to neutralize the mycoplasmastatic effects of the sodium polyanetholsulfonate anticoagulant. Without the addition of gelatin to BacT/ALERT bottles, the mycoplasmas declined in numbers or became completely nonviable. Mycoplasmal growth was further enhanced in BacT/ALERT PF both supplemented with gelatin, arginine, and DNA in comparison to broth with only gelatin added. No BacT/ALERT bottles containing *M. hominis* in simulated blood cultures were flagged positive by the instrument, despite growth of microorganisms of up to 10^7 CFU/ml after incubation for up to 7 days, suggesting that inadequate CO_2 production or some other mechanism prevents the instrument from recognizing the presence of the organism and its metabolic products. The fastidious cultivation requirements and relatively slow growth of *M. hominis* warrant that dependence on automated systems and techniques designed to detect conventional bacteria will not be reliable for recovery of *M. hominis* and that specialized media and incubation conditions designed for optimum cultivation of mycoplasmas should be employed when this organism is suspected on clinical grounds.

Mycoplasma hominis is a commensal inhabitant of the lower urogenital tract in many sexually active adult men and women. However, it may also cause localized as well as extragenital disease. *M. hominis* bacteremia occurs in neonates; women with postpartum endometritis; following renal transplantation, trauma, surgery, and genitourinary manipulations; and in various systemic infections that occur in immunosuppressed hosts (1, 4, 6, 7, 12, 14, 15, 17–19). There is no doubt that *M. hominis* bacteremia is underdiagnosed because the organism is rarely sought in blood cultures, even in clinical settings where it is known to cause bloodstream invasion, and because reliable detection requires specialized media and cultivation techniques that are rarely offered in diagnostic microbiology laboratories where personnel may be unfamiliar with this organism. Detection of *M. hominis* in systemic conditions can be of clinical importance for patient management since its identification will allow targeted antimicrobial therapy and lessen the need to search for other infectious etiologies in most cases.

Many of the published cases of *M. hominis* bacteremia unsuspected initially were eventually detected after suspicious pinpoint colonies subsequently proven to be *M. hominis* grew in routine bacteriologic media after several days of incubation or when specialized media and growth conditions designed to culture mycoplasmas in vitro were employed on specimens obtained after treatment failures with drugs inactive against mycoplasmas.

Automated blood culture instruments are commonly used in clinical laboratories to detect a wide array of microorganisms, not only in blood, but also in other normally sterile body fluids, such as pleural fluid, synovial fluid, and peritoneal fluid. Use of this technology allows more rapid detection of microbial growth while decreasing laboratory workload (20). Older systems such as the radiometric BACTEC 460 system and newer nonradiometric instruments in the BACTEC series have been evaluated for their ability to detect *M. hominis*. Results have been generally disappointing (2, 4, 7–10, 13). The inability to detect the growth of this organism using automated instruments has been attributed to a great extent to the mycoplasmastatic effects of the sodium polyanethol sulfonate (SPS) anticoagulant widely used in liquid blood culture media (2, 4, 7–10, 13).

The BacT/ALERT system (Organon Teknika Corporation, Durham, N.C.) was the first fully automated, noninvasive, continuous-monitoring blood culture system to be marketed and is widely used throughout the world. This system has now become an accepted standard method for performing blood cultures (20). In the present study, we have used simulated blood cultures containing *M. hominis* to determine whether various BacT/ALERT liquid media will support growth of this organism, whether growth is enhanced by the addition of 1% aqueous gelatin to neutralize the inhibitory effects of SPS by cell membrane stabilization or by supplentation with additional metabolic substrates, and whether the presence of *M. hominis* can generate a positive signal with the BacT/ALERT instrument.

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MATERIALS AND METHODS

Microorganisms. Low-passage clinical isolates of *M. hominis* were stored frozen at −70°C in SP4 broth (Remel Laboratories, Lenexa, Kans.). To prepare

inocula for the BacT/ALERT system, stock cultures were thawed and incubated aerobically in 5 ml of SP4 broth on a mechanical agitator at 37°C for 48 h. The organism density of each actively growing culture that was added to blood culture bottles was verified by serial dilution of 0.1-ml aliquots into 0.9 ml of sterile saline and plating 0.02 ml of each dilution on SP4 agar plates (Remel). Agar plates were incubated anaerobically in a sealed container with a GasPak catalyst (Remel) at 37°C for 72 h and then examined under a stereomicroscope for the typical fried-egg appearance of mycoplasmal colonies. Colony counts were performed on dilutions containing 30 to 300 distinct colonies for ease of enumeration.

Evaluation of FA and FN BacT/ALERT media with and without gelatin for growth of *M. hominis*. Aliquots (0.5 ml) of four *M. hominis* clinical isolates actively growing in SP4 broths were inoculated into four BacT/ALERT aerobic (FA) bottles and four anaerobic (FN) bottles, each containing 5 ml of human blood with or without 1% aqueous gelatin (wt/vol). Final mycoplasmal concentrations in inoculated bottles were 10^3 to 10^5 CFU/ml of blood culture medium. Bottles were loaded into the BacT/ALERT instrument according to the manufacturer's instructions, incubated, and checked daily for positive growth indications over a period of 7 days. Quantitative subcultures were performed on fluid from each bottle on day 7 as described above.

Evaluation of additional BacT/ALERT media supplemented with gelatin for growth of *M. hominis*. Due to concern over the possibility that the activated charcoal in FN and FA media that is incorporated to help overcome the presence of antibiotics and facilitate growth of fastidious organisms might adversely affect the growth of *M. hominis*, we evaluated additional media that included BacT/ALERT standard aerobic (SA), standard anaerobic (SN), and activated-charcoal-containing pediatric (PF) media supplemented with 1% aqueous gelatin (wt/vol) against one of the four strains of *M. hominis* tested with the FA and FN media. A volume of 0.5 ml of actively growing *M. hominis* culture was inoculated into one bottle each of SA, SN, and PF media containing 5 ml of human blood. Final mycoplasma concentrations in inoculated bottles were 10^2 to 10^3 CFU/ml of blood culture medium. Bottles were incubated and subcultured on day 7 as described above.

Evaluation of supplemental arginine and DNA on growth of *M. hominis* in BacT/ALERT PF media. We sought to determine if additional supplementation of PF medium with 1% gelatin (wt/vol), arginine (5 g/liter), and DNA (0.2 g/liter) (all from Sigma Chemical Co., St. Louis, Mo.) would enhance mycoplasmal growth and elicit a positive signal from the instrument. A 0.5-ml aliquot of an actively growing *M. hominis* culture was added to a bottle of PF medium with 5 ml of human blood. A second PF bottle containing the same inoculum, blood, and gelatin received an equivalent volume of sterile water in place of the arginine and DNA. The final mycoplasma concentration in inoculated bottles was 10^4 CFU/ml of blood culture medium. Subcultures were performed, colony counts were determined on days 3, 5, and 7 of incubation, and the pH was checked at each timepoint for each bottle. PF medium was chosen for this component of the study because of its lower concentration of SPS (0.020 versus 0.044% for other bottle types).

RESULTS

No BacT/ALERT bottles were flagged positive by the instrument in any of the experiments, and there were no discernible changes in reflectance over the 7-day time period. In the first experiment, none of the four FN bottles without gelatin contained viable organisms recoverable by culture after 7 days of incubation, but all four FN bottles supplemented with 1% gelatin had viable organisms (10^5 to 10^6 CFU/ml) recovered after incubation, with each bottle showing an increase of 1 to 3 logs per ml. Mycoplasma numbers in four FA bottles without gelatin were unchanged, decreased, or increased by $\leq$1 log, whereas in FA bottles supplemented with 1% gelatin, the organisms increased 1 to 3 logs per ml in all four bottles. Results of the second experiment demonstrated that individual bottles containing SA, SN, and PF media supplemented with 1% gelatin supported growth of *M. hominis*, with colony counts increasing by 1 to 4 logs per ml in each bottle when subcultured after 7 days of incubation in the BacT/ALERT.

Growth of *M. hominis* was 1 log higher in PF broth supplemented with gelatin, arginine, and DNA than in broth with only gelatin added when subcultured on day 5 of incubation in the BacT/ALERT system. However, growth declined slightly in both bottles by day 7 from the peak values obtained on day 5. The pH increased from 7.1 at the time of inoculation to 7.6 on day 7 in bottles containing additional arginine and DNA. Conversely, by day 7, the pH had decreased to 6.8 in the bottle inoculated with bacteria alone and decreased to 6.5 in an uninoculated bottle.

DISCUSSION

This is the first evaluation of the ability of the BacT/ALERT system to detect growth of *M. hominis*. Using simulated blood cultures in which BacT/ALERT bottles containing human blood were inoculated with actively growing cultures of *M. hominis*, we have demonstrated that the viability of this organism can be maintained for a 7-day period in multiple BacT/ALERT media. Some replication occurred, allowing organisms to increase their numbers by multiple logs per milliliter to achieve titers as high as 10^7 CFU/ml, primarily when 1% gelatin was added. Without the addition of gelatin to BacT/ALERT bottles, the mycoplasmas did not grow as well, declined in numbers, or became completely nonviable.

Inhibitory effects of SPS on growth of *M. hominis* are well known, and previous studies have found a similar beneficial effect on mycoplasmal growth when gelatin was incorporated into blood culture bottles from other automated systems (2, 3, 7, 8, 10, 13). The concentrations of SPS used in BacT/ALERT media (0.020 to 0.044%) are similar to those used in other systems, such as the BACTEC series (8, 10, 13). One study (3) has reported that 30% of *M. hominis* strains could be cultivated successfully in broth media containing 0.025% SPS but not in media with 0.05% SPS. Another study (13) reported a positive growth index with the radiometric BACTEC 460 system with *M. hominis* when the SPS concentration was 0.006%. Carski et al. (2) reported that five of nine simulated blood cultures containing *M. hominis* could be detected radiometrically with the BACTEC 460 system using SPS-free bottles, versus two of nine when SPS was added.

Detection of bacterial growth by the BacT/ALERT system is based on indirect measurement of the CO_2 released as bacteria grow. CO_2 permeates a membrane in the bottom of the bottle and interacts with water to produce hydrogen ions that acidify a sensor, causing it to change from green to yellow. A light-emitting diode shines on the sensor every 10 min, and a photodiode generates a voltage signal proportional to the amount of light reflected from the sensor, which changes in relation to CO_2 concentration. The microcomputer analyzes the curve of CO_2 reflectance units against time and flags bottles as positive based on (i) an initial reading that exceeds an arbitrary threshold to detect growth before incubation; (ii) a sustained linear increase in CO_2 production, or (iii) an increased rate of CO_2 production (20).

If the unique metabolic properties of *M. hominis* are considered, there are several possible explanations why the BacT/ALERT instrument failed to detect growth. Schimke and Barile (11) proposed that *M. hominis* generates ATP by hydrolysis of arginine, a process that utilizes a three-enzyme pathway with end products of CO_2 and NH_3. Arginine deiminiase, the first enzyme in the pathway, is inducible by arginine in *M. hominis*,

suggesting that this enzyme may not become operative until some other energy-yielding metabolite is exhausted (5, 16). This could be affected by medium composition. An alternative mechanism for energy production involves phosphate acetyltransferase and acetate kinase. These enzymes catalyze the reactions to make ATP, using acetyl phosphate as the substrate without use of arginine or liberation of CO_2 (16). Thus, under acceptable growth conditions in which appropriate substrates are available, *M. hominis* may multiply without producing the CO_2 necessary for generating a BacT/ALERT signal.

A second possibility is that CO_2 is generated but that the amounts are below the threshold for the instrument to detect. This explanation seems reasonable in view of the fact that these organisms, which are the smallest free-living forms, have a cell mass so tiny that they fail to produce turbidity in liquid medium even when present in high titers. There seems to be no doubt that at least some CO_2 is generated by growth of *M. hominis* in blood culture bottles, based on previous reports in which radiometric BACTEC systems had positive indices (2, 4, 10, 13). However, the growth index, when described, was typically very low, suggestive of small CO_2 concentrations. Pratt (10) speculated the radiometric systems may be more sensitive for detection of low levels of CO_2 than the nonradiometric instruments. However, the apparent advantage held by radiometric blood culture systems over nonradiometric systems for detection of *M. hominis* is now irrelevant, since the former have been replaced in most diagnostic laboratories by the newer-generation noninvasive, nonradiometric continuously monitoring systems and the radiometric medium for bacterial blood cultures is no longer manufactured.

A third reason for failure of the BacT/ALERT system to detect growth of *M. hominis* could be that the amount of the arginine substrate in the medium is inadequate for the organism to generate sufficient CO_2 to elicit a positive signal. Although the precise amount of arginine in BacT/ALERT medium is not known, we detected a 10-fold increase in the number of mycoplasmas growing in PF medium when arginine and DNA were added versus that in unsupplemented media. Pratt (10) also speculated that the inability of radiometric systems to generate a positive signal with *M. hominis* may have been due to insufficient ^{14}C substrate in arginine in the media used. However, the BacT/ALERT system still did not produce a positive signal after addition of excess arginine, meaning this is probably not the primary explanation. Fenske and Kenny (5) noted that the rate of $^{14}CO_2$ evolution from [*guanido*-^{14}C]arginine in *M. hominis* supplemented with arginine was not altered compared with that in organisms grown in low arginine and that CO_2 production did not parallel increased arginine deiminase activity. These findings further suggest that alternative metabolic pathways may be operative in this organism.

A final problem could be that the elevated pH produced by *M. hominis* as a result of the NH_3 by-products of arginine metabolism may affect the CO_2 equilibrium at the membrane interface in BacT/ALERT bottles. For conventional bacteria, the pH of the broth will decrease as bacteria grow and produce organic acids. We observed a pH increase in the PF bottles when supplemental arginine and DNA were added but observed a very gradual pH decrease in unsupplemented PF media that was not too different from what was observed in an uninoculated bottle. The gradual continuous decrease in pH in unsupplemented PF medium also suggests that arginine hydrolysis is not sufficient to cause the expected increased pH under these growth conditions or with the amount of substrate available.

Limited reports of naturally occurring bacteremias due to *M. hominis* and studies that performed simulated blood cultures (2, 3, 7–10) suggest that the inability of the BacT/ALERT system to detect mycoplasmal growth is not unexpected. To date, no automated systems other than the older radiometric BACTECs have been shown to produce a positive signal in the presence of *M. hominis*, and there have been many inconsistencies in their abilities to produce a positive growth index (2, 4, 7–10, 13).

Use of a standard blood culture medium that supports viability of *M. hominis* would allow subculture to specific mycoplasmal media upon request for patients at risk for mycoplasma septicemia, even if not identified at the time of original inoculation, or if evidence of microbial growth was present based on acridine orange staining of fluid from a blood culture bottle. Based on our findings, unsupplemented BacT/ALERT media cannot be relied upon to cultivate the organism. Even though the addition of gelatin enhanced growth and helped preserve viability, allowing organism recovery on suitable agar medium upon subculture, this is probably not practical for application in a high-volume diagnostic laboratory, where the possibility of *M. hominis* bacteremia might be of concern in a very small minority of specimens; the manufacturer of the BacT/Alert does not provide a supplement that would fit this need. Therefore, techniques such as those described in the *Manual of Clinical Microbiology* (17) in which specialized media such as SP4 broth and agar designed for cultivation of mycoplasmas are inoculated with the clinical specimen, free of anticoagulant, and incubated for at least 7 days should be employed if infection with this organism is suspected. Some investigators have used Columbia agar for cultivation of *M. hominis* (8, 9, 13). However, we found that this medium is not reliable for subculture of *M. hominis* in simulated cultures when compared directly to SP4 agar, and many isolates may not grow at all (data not shown). The availability of commercially prepared liquid mycoplasma media in lyophilized vials from suppliers such as Remel Laboratories makes it possible for clinical laboratories that have only an occasional request for mycoplasmal cultures to be able to provide a more appropriate method capable of detecting this organism. The fastidious cultivation requirements and relatively slow growth of *M. hominis* warrant that dependence on growth media and techniques designed to detect conventional bacteria will not be reliable for its recovery in all instances.

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REFERENCES

1. **Bøe, Ø., O. E. Iversen, and A. Mehl.** 1983. Septicemia due to *Mycoplasma hominis*. Scand. J. Infect. Dis. **15:**87–90.
2. **Carski, T. R., R. L. Broman, and R. A DelGiudice.** 1981. A note on positive BACTEC readings produced by mycoplasma. J. Appl. Bacteriol. **51:**87–90.
3. **Davies, S., and R. Eggington.** 1991. Recovery of *Mycoplasma hominis* from blood culture media. Med. Lab. Sci. **48:**110–113.
4. **Degirolami, P. C., and S. Madoff.** 1982. *Mycoplasma hominis* septicemia. J. Clin. Microbiol. **16:**566–567.

5. **Fenske, J. D., and G. E. Kenny.** 1976. Role of arginine deiminase in growth of *Mycoplasma hominis*. J. Bacteriol. **126:**501–510.
6. **Friis, H., A. Plesner, J. Scheibel, T. Justesen, and K. Lind.** 1983. *Mycoplasma hominis* septicemia. Br. Med. J. **286:**2013–2014.
7. **Kelly, V. N., S. M. Garland, and G. L. Gilbert.** 1987. Isolation of genital mycoplasmas from the blood of neonates and women with pelvic infection using conventional SPS-free blood culture media. Pathology **19:**277–280.
8. **Kitson, K., and K. C. Wright.** 1998. The *in vitro* simulation of *Mycoplasma hominis* isolation using supplemented culture media in the BACTEC 9000 series blood culture system. Br. J. Biomed. Sci. **55:**9–12.
9. **Kitson, K., and K. C. Wright.** 1996. Isolation of *Mycoplasma hominis* using the BACTEC 9000 series blood culture system. J. Clin. Pathol. **49:**686–687.
10. **Pratt, B.** 1990. Automated blood culture systems: detection of *Mycoplasma hominis* in SPS-containing media. Zentbl. Bakteriol. **1990**(Suppl. 20)**:**778–781.
11. **Schimke, R. T., and M. F. Barile.** 1963. Arginine metabolism of pleuropneumonia-like organisms isolated from mammalian cell culture. J. Bacteriol. **86:**195–206.
12. **Simberkoff, M. S., and B. Toharsky.** 1976. Mycoplasmemia in adult male patients. JAMA **236:**2522–2524.
13. **Smaron, M. E., S. Boonlayangoor, and C. H. Zierdt.** 1985. Detection of *Mycoplasma hominis* septicemia by radiometric blood culture. J. Clin. Microbiol. **21:**298–301.
14. **Ti, T. Y., M. Dan, G. W. Stemke, and G. Goldsand.** 1982. Isolation *of Mycoplasma hominis* from blood of men with multiple trauma and fever. JAMA **247:**60–61.
15. **Tully, J. G., and L. G. Smith.** 1968. Postpartum septicemia with *Mycoplasma hominis*. JAMA **204:**827–828.
16. **Vinther, O.** 1983 Biochemistry of *Mycoplasma hominis*. Sex. Transm. Dis. **10**(Suppl.)**:**244–245.
17. **Waites, K. B., and D. Taylor-Robinson.** 1999. Mycoplasma and ureaplasma, p. 782–794. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.) Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
18. **Waites, K. B., P. T. Rudd, D. T. Crouse, K. C. Canupp, K. G. Nelson, C. Ramsey, and G. H. Cassell.** 1988. Chronic *Ureaplasma urealyticum* and *Mycoplasma hominis* infections of central nervous system in preterm infants. Lancet **ii:**17–21.
19. **Wallace, R. J., S. Alpert, K. Browne, J. S. L. Lin, and W. M. McCormack.** 1978. Isolation of *Mycoplasma hominis* from blood cultures in patients with postpartum fever. Obstet. Gynecol. **51:**181–184.
20. **Wilson, M. L., M. P. Weinstein, and L. B. Reller.** 1994. Automated blood culture systems, p. 149–169. *In* M. L. Wilson (ed.), Clinics in laboratory medicine, vol. 14. W. B. Saunders Co., Philadelphia, Pa.