Paper # **P-1177**

Comparison of VersaTREK® 528 and BACTEC® 9240 Continuous Monitoring Blood Culturing Systems for the Detection of Clinical Isolates in a Seeded Bottle Study

K. M. Doing[1,2] and M. W. Fogler[1]

1. Affiliated Laboratory, Inc., Eastern Maine Healthcare Systems, Bangor, ME USA1. 2. Department of Biology and Ecology, University of Maine, Orono, ME USA2

ABSTRACT (revised)

Objectives: Continuous monitoring blood culture systems are used in many clinical laboratories. We evaluated the VersaTREK 528 (VT) and BACTEC 9240 (BD) blood culture systems in a seeded study comparing time to detection (TTD) and media type for the recovery of *Staphylococcus aureus* (20 strains) *Streptococcus pneumoniae* (20 strains), beta streptococci (25 strains), *Neisseria meningitidis* (13 strains), and *Hemophilus influenzae* (17 strains). ATCC strains were included as controls.

Methods: BACTEC Plus, Lytic, and Peds Plus™ media were compared to VT universal aerobic and anaerobic media (80 and 40 mL bottles). Bottles were supplemented with 100 or 500 ul of human blood and inoculated with either 1 x 10^2 or 1 x 10^4 cfu/ml of bacteria (21 bottles per isolate); colony counts were performed from each dilution. Bottles were incubated in appropriate instruments with the TTD and media type recorded for each isolate. Subcultures were performed from positive bottles to ensure purity and from negative bottles after 5 days of incubation.

Results: Both systems recovered all challenge isolates, with minor differences between TTD and media noted. Isolates of *H. influenzae* and *N. meningitidis* were recovered from all aerobic media, but most failed to grow in anaerobic and all lytic media. Streptococci were detected in both systems but had a shorter TTD using BD lytic media. No differences were noted in the TTD of staphylococci. As expected, a higher inoculum shortened the TTD in both systems; however, volume of blood supplementation had no apparent impact.

Conclusions: We used a controlled inoculum to approximate the microbial load in bacteremic patients and compared the TTD using VT universal media to BD high volume blood culture media. Optimal performance of the BD system required use of three different media, while the VT showed equivalent recovery using their universal two bottle media formulations. Indeed, the VT system is uniquely approved in the U.S. to culture low blood volumes facilitating routine use of both aerobic and anaerobic media for adult and paediatric patients. In addition, differences in instrument and media costs are significant; BD high volume resin and lytic media cost as much as 45% more than universal VT media with comparable performance. The VT system has also been cleared for body fluids and verified protocols for platelet testing. The same instrument can also be used to culture mycobacteria, making this the most cost effective and versatile of the two instruments.

INTRODUCTION

Bacterial sepsis is a significant clinical finding making blood cultures one of the most critical specimens processed in the microbiology laboratory, therefore prompt isolation, followed with accurate identification and susceptibility studies on blood culture isolates remains a priority (1,5,14,15). Blood cultures are the primary means of determining the etiology of infective endocarditis and may also be positive in patients with pneumonia, pyelonephritis, and invasive bacterial infections. In addition, patients with infected implanted prosthetic devices or intravenous catheters typically have positive blood cultures; however, confirming the latter can be challenging (2,13).

Automated blood culture instruments allow culture bottles to be monitored “continuously” throughout the incubation process for microbial growth, signaling the user through alarms when a “positive” culture has been detected (6,8,11). Both the VersaTREK (VT) (TREK Diagnostic Systems, Cleveland, OH) and BACTEC (BD) Fluorescent Series (BD Diagnostics, Sparks, MD), blood culture instruments are continuous monitoring systems; however there are differences in the way microbial growth is detected. The VT system continuously monitors head-space gas pressure in each bottle through a pressure sensor attached to each bottle prior to placing it in the instrument. Pressure changes associated with gas consumption (decreased pressure) or gas production (increased pressure) are monitored over time and compared to growth algorithms to signal a positive culture. The BD system is based soley on CO_2 detection. As CO_2 is generated by metabolizing organisms it is released into the medium and diffuses through a membrane into a chemical sensor manufactured into each bottle. Through photocells in the instrument, each bottle sensor is interrogated every 10 minutes. Positive cultures are detected by an increase in fluorescence measured in the sensor over time which is proportional to the amount of CO_2 in the medium, correlating with microbial growth.

Besides detection methods, there are also differences in media used with the VT and BD systems. The VT system uses two media formulations (REDOX- 1 and REDOX 2) supplied in either 40 or 80 ml bottles. These media were developed and intended to be used in paired sets for optimal performance and have also obtained FDA clearance for very low blood volumes for pediatric specimens (e.g., 0.1 ml). In contrast, BD offers multiple choices of media, including a standard media that allows only 5.0 ml of blood to be inoculated, half the recommended blood volume that should be cultured, as well as aerobic and anaerobic media accommodating 10 ml blood draws (3,4,7,9,10). In addition there are separate high volume resin media, lytic, pediatric, and fungal media, from which the laboratory must decide the best combination for their patient population (12).

Our study evaluated the time-to-detection (TTD) of both blood culture instruments using VT 40 and 80 ml universal media, REDOX 1 (R1) and REDOX 2 (R2) and BACTEC high volume resin, lytic, and pediatric media. Blood culture bottles were supplemented with either 0.1 or 0.5 ml of human blood then inoculated with different concentrations of bacteria followed by incubation in appropriate instruments. Clinical isolates of *Staphylococcus aureus* (20 strains), *Streptococcus pneumoniae* (20 strains), beta streptococci (10 Group A, 10 Group B, 2 Group C, and 3 Group G), *Haemophilus influenzae* (17 strains), and *Neisseria meningitidis* (13 strains) were used as challenge strains, with ATCC organisms included as controls.

MATERIALS AND METHODS

Packed human red blood cells collected during therapeutic phlebotomies from patients with no history of antibiotic use over the past 30 days was used to supplement blood culture bottles prior to organism inoculation. For each isolate all bottles were supplemented with 0.1 ml of blood (minimum amount of blood recommended by VT manufacturer, BD recommends up to 3.0 ml of blood to support fastidious organism growth). Additional bottles supplemented with 0.5 ml of blood were added to the challenge set for all organisms except staphylococci.

To approximate the numbers of organisms that may be present in the blood of bacteremic patients, (which can be <1 CFU/ml) test and quality control isolates were diluted as follows:

1. A suspension equal to a 0.5 McFarland (approx. organism concentration = 1-2 X 10^8 cfu/ml) was prepared in 5.0 ml of sterile water Mueller-Hinton Broth (pneumococci) (Trek Diagnostic Systems, Cleveland, OH)
2. 100-fold serial dilutions were prepared in 10 ml of Mueller-Hinton broth
3. Two challenge inoculums, 1 x 10^4 and 1 x 10^2 CFU/0.1 ml (5-30 organisms/0.1 ml) were used for each isolate and in each media type.
4. 0.1 ml of the appropriate dilution was aseptically added to each blood supplemented bottle; total of 21 bottles for each test isolate (except staphylococci) as follows:

Organism Concentration	Blood Added	VT Media	BD Media
1 x 10^2 CFU/0.1ml (0.1 ml inoculated/bottle)	0.1 ml	REDOX 1 – 80 ml REDOX 2 – 80 ml REDOX 1 – 40 ml REDOX 2 – 40 ml	BACTEC Plus w/ resin BACTEC Lytic BACTEC Peds Plus
1 x 10^2 CFU/0.1ml (0.1 ml inoculated/bottle)	0.5 ml	REDOX 1 – 80 ml REDOX 2 – 80 ml REDOX 1 – 40 ml REDOX 2 – 40 ml	BACTEC Plus w/ resin BACTEC Lytic BACTEC Peds Plus
1 x 10^4 CFU/0.1ml (0.1 ml inoculated/bottle)	0.1 ml	REDOX 1 – 80 ml REDOX 2 – 80 ml REDOX 1 – 40 ml REDOX 2 – 40 ml	BACTEC Plus w/ resin BACTEC Lytic BACTEC Peds Plus

To ensure purity and inoculum density, colony counts were performed from each dilution by plating 0.1 ml onto a trypticase agar plate with 5% sheep blood or chocolate agar (Remel, Inc. Lenexa, KS). After overnight incubation colony count was determined.

All bottles were inoculated in sequence using aseptic technique. A connector was positioned on each VT bottle and they were placed into the VersaTREK 528. Similarly, after inoculation, all BD bottles were immediately placed into the BACTEC 9240. When a bottle was “flagged” by either instrument both the time-to-positivity and media type were recorded. In addition bottles were subcultured to appropriate media to prove purity.

For reference, TTD data from patient blood cultures positive for *S. aureus* (n=29), *S. pneumoniae* (n=24), beta streptococci (n=12), *H. influenzae* (n=2), or *N. meningitidis* (n=1) during the study period were compared to data from the seeded studies.

RESULTS

Overall both the VersaTREK and BACTEC continuous monitoring blood culture systems performed well and were able to detect the seeded cultures as positive. For all isolates except meningococci, at least one media type in each challenge set signalled positive within the first 24 hours of incubation. However, as expected slight differences in the TTD were noted and ranged from 2-5 hours between the two systems with comparative media (e.g. VT R1 vs. BD AER PLUS) (fig 1-5).

S. aureus was recovered in all media and on both systems within the first 18 hours of incubation. Bottles challenged with a higher inoculum on average became positive 3 hours sooner and, not surprisingly, TTD in VT R2 (anaerobic) media consistently lagged behind the aerobic (R1) counterpart by 5 hours.

Culture bottles seeded with *S. pneumoniae* and beta streptococci were detected by both instruments as well, and in all media types. For VT media and pneumococci specifically, growth was detected on average 5 hours faster in R2 anaerobic media compared to the paired aerobic (R1) bottle. The BD lytic media demonstrated the fastest overall recovery of both pneumococci and beta streptococci, with positive bottles detected on average 4 and 6 hours sooner respectively (fig. 2 & 3).

H. influenzae isolates were recovered more efficiently in the aerobic media of both systems, and if the mated anaerobic bottle(s) was identified as positive, it took on average twice as long for either instrument to detect growth in these media, again demonstrating the need to use two media formulations for optimal performance (fig. 4). A significant exception was *N. meningitidis* as not all strains were recovered or detected by either system. Ten of 13 strains were detected in BACTEC PLUS, 11 of 13 in VT R1 media, and 12 of 13 in BACTEC Peds Plus media. Only one strain inoculated to VT R2 (anaerobic) media was detected by the instrument while meningococci were recovered from 45 of 77 blind subcultures of VT anaerobic media after completing 5 days of incubation in the instrument and never being signalled positive. For BD, the lytic media proved lethal to all meningococcal strains tested. None of the lytic bottles were signalled positive, nor were viable organisms recovered from any of the blind subcultures. Not unexpectedly, *N. meningitidis* appeared the most fastidious organism, with TTD impacted the greatest by the amount of blood added to the bottle; those bottles supplemented with 0.5 ml, in general, demonstrated a more rapid time to positivity (fig. 5).

TTD data of seeded cultures compared to patient blood cultures that were also incubated in the VersaTREK system during the study period revealed the latter were detected in a shorter time, likely reflecting a higher level of bacteremia and possibly because of higher blood supplementation (fig 6). For example, patient cultures positive for beta streptococci or pneumococci were detected in as little as 5-8 hours using the VersaTREK system, with a mean TTD of 10 and 12 hours respectively, equalling the performance of the BD Lytic bottle observed with similar challenge isolates. Interestingly, and opposite of study data, the aerobic bottle (R1) signalled first in all patient cultures positive for either pneumococci or beta streptococci. *S. aureus* was the lone exception with a mean time to positivity in seeded VT bottles of 11-13 hours, compared to an average time to positivity of 16 hours for patient cultures (fig. 1 & 6). We speculate on the cause, but possibilities include concurrent antibiotic therapy and/or low bioburden. Indeed, seven of these cultures suggested possible contamination as only one bottle in two sets was positive, and the mean TTD in these cultures averaged 10 hours longer, a likely reflection of lower organism load associated with contaminated cultures (6,7). However, even if these are removed from consideration, seeded cultures were still detected positive, on average, 3 hours faster.

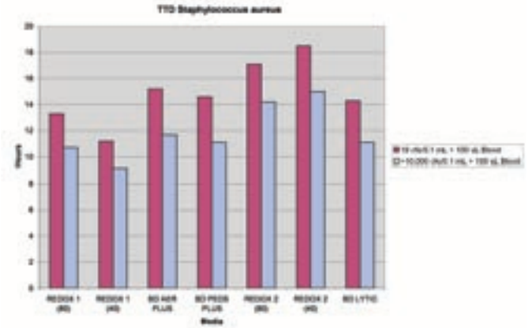

Fig. 1.

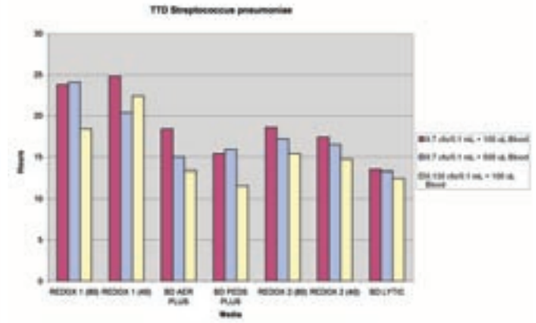

Fig. 2.

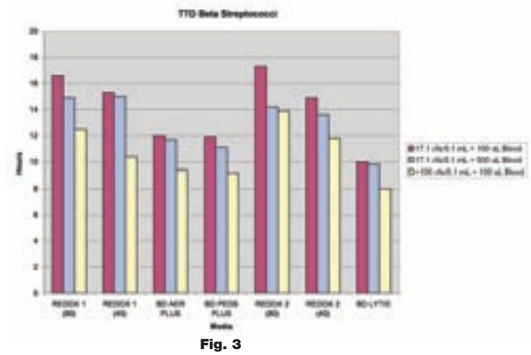

Fig. 3

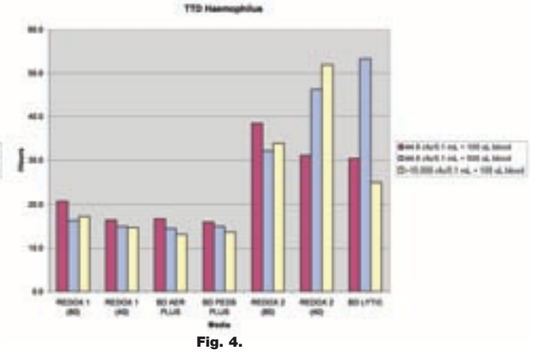

Fig. 4.

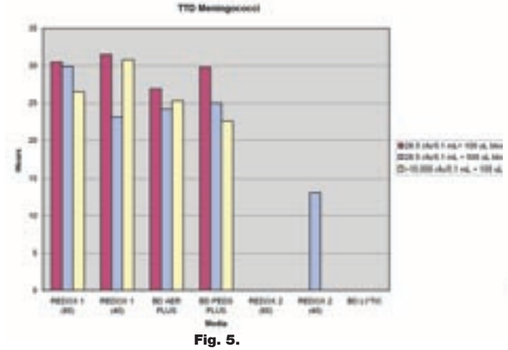

Fig. 5.

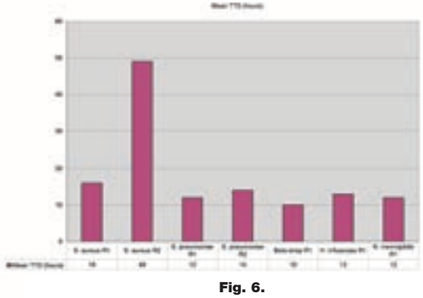

Fig. 6.

CONCLUSION

Blood cultures continue to be a high volume and high priority sample in the clinical microbiology laboratory. In this study 95 clinical isolates representing eight different species were used in a seeded study to compare the TTD of two continuous monitoring blood culture systems. The test isolates were selected based on frequency of isolation from patient cultures as well as the fastidious nature, and lethality of these organisms (1,15). A controlled inoculum of each isolate, designed to approximate the microbial load in bacteremic patients, was added to multiple blood culture bottles supplemented with human blood followed by incubation within appropriate instruments. While both systems were able to detect the various bacteria using low initial inocula and instrument incubation, optimal performance of both systems evaluated required the use of both aerobic and anaerobic medium. This choice was easier made for the VT system as their 80 ml universal media (R1 and R2) address the most important blood culturing concepts: aerobic and anaerobic media, ability to culture appropriate volume (10 ml per bottle) of blood, and dilution of sample (1:9) to limit effects of antibiotics and natural bactericidal potential of serum (1,5,11). To achieve similar benefits with the BD system high volume aerobic resin medium coupled with either high volume anaerobic or lytic media must be used; adding, on average, $2.70 in media costs to each culture set compared to universal VT media. While BD offers less expensive options, most of these do not allow for higher volumes of blood to be cultured, limiting the most important premise in detecting bacteremia (4,7,10).

While the volume of blood cultured remains the most important aspect of detecting bacteremia in patients, with the exception of *N. meningitidis*, blood supplementation as a growth factor was not apparent in the current study. The 0.1 ml blood supplementation volume was selected based on FDA clearances for VT culture bottles, and is lower than clearances for BD media; however, organism recovery was not impacted, suggesting the media used with either system is able to support the growth of fastidious organisms and blood volume primarily increases the chance of obtaining bacteria in the draw (1,3,7).

Continuous monitoring blood culture systems improve the quality of this important culture by providing a more rapid time to positivity over manual systems, and at the same time offer significant savings in technologist time as only “positive” cultures are handled manually. Additional uses of this technology include confirmation of line-related infection as vascular devices are now the most common source of hospital acquired bacteremia. Reporting positive blood cultures from patients with vascular lines often creates a clinical dilemma to leave, or remove the catheter, and clearly, contamination of cultures collected from line draws is very high and rarely diagnostic. The TTD capability of continuous monitoring systems allows an indirect comparison of the microbial load between line and peripheral draws and is quite specific in proving or disproving line-related bacteremia (2,13).

Overall the performance of both the VersaTREK and BACTEC Fluorescent blood culture systems were equivalent in their ability to detect positive cultures in the first 24 hours of incubation when initially seeded with low levels of bacteria. However, this was not a clinical study, so the impact on patient care of the small differences noted in TTD between instruments and organisms cannot be determined, and is likely insignificant. With performance equal, cost and instrument versatility become an important part of decision making. In addition to less costly media, the VT system offers additional flexibility with verified protocols for culturing platelets, FDA clearance for body fluids, and the ability to co-incubate mycobacterial cultures within the same instrument. When this comparison was made we found the VersaTREK the most cost effective system for our laboratory.

REFERENCES

1. Aronson, M. D. and D. H. Bor. 1987. Blood cultures. Ann. Intern. Med. 106:246-253.
2. Blot. F., G. Nitenberg, E. Chachaty, B. Raynard, N. Germann, S. Antoun, A. Laplanche, C. Brun-Buisson, and C. Tancrède. 1999. Diagnosis of catheter-related bacteraemia: a prospective comparison of the time to positivity of hub-blood versus peripheral-blood cultures. Lancet. 354:1071-1077.
3. Bouza, E., D. Sousa, M. Rodriguez-Créixems, J. Lechuz, and P. Mu_oz. 2007. Is the volume of blood cultured still a significant factor in the diagnosis of bloodstream infections? J. Clin. Microbiol. 45:2765-2769.
4. Brown, D. F., and R. E. Warren. 1990. Effect of sample volume on yield of positive blood cultures from adult patients with hematologic malignancy. 43:777-779.
5. Bryan, C. S. 1989. Clinical implications of positive blood cultures. Clin. Microbiol. Rev. 2:329-353.
6. DiPersio, L. P. DiPersio, and J. A. Beach. 2008. Recovery of *Staphylococcus aureus* from BACTEC Plus Aerobic/F and VersaTREK REDOX 1 blood culture medium with increasing concentrations of vancomycin, daptomycin, or linezolid. Abst. D-306. IDSA/ICACC Annual Meeting. Washington, D. C.
7. Hall, M. M., D. M. Hstrup, and J. A. Washington II. 1976. Effect of volume of blood cultured on detection of bacteremia. J. Clin. Microbiol. 3:643-645.
8. Kirkley, B. A., K. A. Easley, and J. A. Washington. 1994. Controlled clinical evaluation of Isolator and ESP aerobic blood culture systems for detection of blood stream infections. J. Clin. Microbiol. 32:1547-1549.
9. Li, J., J. Plorde, and L. G. Carlson. 1994. Effects of volume and periodicity on blood cultures. J. Clin. Microbiol. 32:2829-2831.
10. Mermel, L. A. and D. J. Maki. 1993. Detection of bacteremia in adults: consequences of culturing an inadequate volume of blood. Ann. Intern. Med. 119:270-272.
11. Mirrett, S., K. E. Hanson, and L. B. Reller. 2007. Controlled clinical comparison of VersaTREK and BacT/ALERT blood culture system. J. Clin. Microbiol. 45:299-302.
12. Murray, P. R., P. Traynor, and D. Hopson. 1992. Critical assessment of blood culture techniques: analysis of recovery of obligate and facultative anaerobes, strict aerobic bacteria, and fungi in aerobic and anaerobic blood culture bottles. J. Clin. Microbiol. 30:1462-1468.
13. Raad, I., H. A. Hanna, B. Alakech, I. Chatzinikolaou, M. M. Johnson, and J. Tarrand. 2004. Differential time to positivity: a useful method for diagnosing catheter-related bloodstream infections. Ann. Intern. Med. 140:18-26.
14. Trenholme, G. M., R. L. Kaplan, P. H. Karakusis, T. Stine, J. Fuhrer, W. Landau, and S. Levin. 1989. Clinical impact of rapid identification and susceptibility testing of bacterial blood culture isolates. J. Clin. Microbiol. 27:1342-1345.
15. Weinstein, M. P., M. L. Towns, S. M. Quartey, S. Mirrett, L. G. Reimer, G. Parmigiani, and L. N\b. Reller. 1997. The clinical significance of positive blood cultures in the 1990's: a prospective comprehensive evaluation of the microbiology, epidemiology, and outcome of bacteremia and fungemia in adults. Clin. Infect. Dis. 24:584-602.