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ABSTRACT

Normally sterile body fluids that become infected with bacteria may result in severe morbidity and mortality, but unlike CSF, the variety of bacteria recovered from infected pleural, pericardial, joint, or peritoneal fluids is more extensive. For culture on solid media, samples should be concentrated prior to plating and to improve culture sensitivity the addition of blood culture bottles has been recommended, yet only VersaTREK® and one other commercially available system make this claim in their technical inserts. In the current study we cultured 175 body fluid samples (pleural=53, pericardial=15, joint=79, and peritoneal=28) collected from 159 patients using both solid media and VersaTREK® universal blood culture bottles. Most of the peritoneal samples were collected under ultrasound guidance with 10 mL of fluid aseptically inoculated into both aerobic and anaerobic bottles at bedside; a second 50 mL aliquot was placed in a sterile tube and submitted with the bottles. The remaining samples were divided equally in the laboratory; half was centrifuged (1,500 x g for 30 min.) and the sediment plated on solid media with the remainder of the sample split between aerobic and anaerobic VersaTREK® blood culture bottles; however, in some instances sample volume (<=2 mL) permitted that only an aerobic blood culture bottle be inoculated. In addition, a cytospin Gram stain was prepared from each sample. Overall 121 cultures were sterile by both methods and 54 samples yielded positive cultures; of these, 40 were concordant, with the same organism(s) recovered on solid media and from blood culture bottles. For 14 samples, only the blood culture bottles were positive and based on the direct Gram stain, time-to-positivity of the blood culture bottle, and clinical intervention, only two of the VersaTREK® only positive samples appeared to represent contaminants. There were no instances where only the plated media was positive. *S. aureus* was the most frequent pathogen isolated, but streptococcal species, *Enterobacteriaceae*, and anaerobic organisms were also recovered. While both culture methods complemented each other, and growth on solid media allowed for a more expedited workup, 26% of the positive cultures would have been missed if VersaTREK® media had not been included.

MATERIALS AND METHODS

Samples and distribution. This study was conducted prospectively with concurrent inoculation of VersaTREK® blood culture bottles and solid or plated culture media. Body fluid samples were collected following stringent aseptic technique; 0.1 mL of fluid was required to qualify for the study (table 1). After collection, samples were held at room temperature and immediately transported to the laboratory. Samples of >=2 mL were measured using either a graduated 15 or 50 mL sterile polypropylene centrifuge tube and distributed as described in table 1.

Blood Culture Bottle Inoculation. After sample volume was measured the screw cap on each VersaTREK® bottle was decontaminated and carefully unscrewed followed by aseptic inoculation of sample into 80 mL VersaTREK® universal aerobic (Redox 1) and anaerobic (Redox 2) bottles using a sterile transfer pipette. Bottle caps were re-secured on appropriate bottles, the septums were cleaned with 70% isopropyl alcohol, a bottle connector was then attached and bottle(s) were placed into the VersaTREK® instrument for continuous monitoring. Exceptions were for peritoneal fluid samples as most of these were collected under ultrasound guidance followed by aseptic bedside inoculation of bottles: 10 mL of sample into Redox 1 (aerobic) and 10 mL into Redox 2 (anaerobic) bottles, with an additional 50 mL sent to the laboratory for Gram stain and solid media inoculation. Bottles that signaled positive by the instrument were removed from the system. A direct Gram stain was prepared and examined, followed by sub-culturing to solid media. Bottles that remained negative after 5 days of incubation in the instrument were discarded.

Solid Media Culture. Body fluids were centrifuged in sterile, conical polypropylene tubes at 1500 x g for 15 minutes at 15-20°C. Extremely purulent or viscous fluids that could not be adequately concentrated with centrifugation were plated directly. Following centrifugation, all but 1.0 mL of supernatant was decanted into a second sterile container, labeled and stored. The pellet was resuspended in remaining supernatant and was used to inoculate media (1-3 drops of sediment/plate). No growth plates were incubated at appropriate temperature and environment for 7 days if: The direct Gram revealed many PMNs with or without bacteria present and plates were not growing or, if blood culture bottles were positive and plated media remained sterile. Plates were discarded as negative after 48 hours if the direct cytospin Gram stain was negative for organisms and inflammatory cells.

Direct Gram stains were prepared from all samples of sufficient volume (table 1). These were used for patient management, but also helped with culture interpretation, notably when blood culture bottles and solid media results were discordant.

RESULTS (con't)

Fifty-three pleural fluid samples were cultured, all from different patients. Of these, 45 were sterile by both culture methods and three were positive; two grew mixed anaerobic flora, while *Streptococcus anginosus* group was recovered from the third sample. Five samples were positive in blood culture bottles only: *Streptococcus anginosus* group was recovered from two samples and *Peptostreptococcus* sp. was recovered from the third; importantly, all three samples had positive direct Gram stains. The two remaining blood culture bottle only positives were felt to represent contamination as the direct Gram stains revealed rare PMNs, no organisms were observed, and CoNS was recovered only from the bottles.

Ultrasound guided paracentesis was used to collect 22 of the 28 abdominal fluid samples; 18 were sterile whereas 10 specimens, collected from six different patients, had positive cultures. *Acinetobacter baumannii* complex was recovered from four different samples submitted on the same patient over 4 weeks; blood culture bottles were positive in all four instances, whereas three of the solid media cultures from these samples were positive. Concordant culture results were obtained with three additional samples: *S. aureus*, *P. aeruginosa*, and *S. marcescens*, were all isolated from separate samples by both culture processes. The three remaining positive paracentesis samples were detected only in VersaTREK® bottles. These grew: *Streptococcus anginosus* group, *P. aeruginosa*, and *Citrobacter freundii* complex.

Only 15 pericardial fluid samples from 13 patients were submitted during the study period. Ten were not growth with both culture systems while five were positive using both culture methods; of these, three were submitted on the same patient; *Propionibacterium* sp. was isolated from both the bottles and solid media from two of the samples, while *Enterococcus faecalis* was isolated from the bottles and plated media on the remaining specimen; the latter also grew a nonfermenting gram negative bacillus only on the plated media that was felt to represent contamination. The final two samples were positive only in the VersaTREK® bottles. One of these was a "true" positive with Group B *Streptococcus* and *Streptococcus anginosus* group both recovered, whereas the other was consistent with contamination as the direct Gram stain was negative and *Propionibacterium* sp. was isolated.

INTRODUCTION

For descriptive and anatomic purposes one can divide the body into compartments or body cavities. These spaces are lined with thin membranes and contain a small amount of sterile fluid that serves to lubricate and reduce friction between organs and body walls. Under healthy or normal conditions the body maintains homeostasis, but if the fluid or surrounding membranes become infected through homogenous seeding of bacteria, during surgical procedures, or by trauma with damage to the surrounding tissue, an inflammatory response ensues and a subsequent exudative or purulent effusion develops. Infections of normally sterile body fluids typically have greater clinical urgency, and frequently these patients have concomitant positive blood cultures.

The collection of body fluid samples requires stringent attention to sterile procedures and is typically done by needle aspiration, often with ultrasound guidance, or during a surgical procedure. Samples should be sent to the laboratory immediately as delays can impact organism viability (6). While 1-5 mL is generally considered adequate for the isolation of most bacteria, the quantity of fluid sampled is often variable, and somewhat dependent on the site of infection. Larger volumes of 5-10 mL, or even greater, are required to recover smaller numbers of organisms. Moreover larger volumes of fluid may accumulate in pleural and peritoneal spaces, resulting in further dilution of already small numbers of bacteria, and possible "false-negative" culture results if a sufficient amount of fluid is not appropriately processed for culture. These inconsistencies support having established protocols detailing sample distribution coupled with using the most sensitive culture methods.

While the etiologic agents that may be recovered from infected body fluids span the microbial spectrum of viruses, bacteria, fungi, and parasites, this study focused on best practices for the recovery of bacteria from these sites; the diversity of which, in its own right, can be quite extensive. The addition of blood culture media, or using it exclusively, for body fluid cultures has been recommended for some time (1,5,7-9), however, only two of the commercially available systems in the U.S., VersaTREK® (Trek Diagnostic Systems, Cincinnati, OH) and MacTALERT® (bioMérieux, Durham, NC) have attained appropriate FDA clearances to make this claim in their technical inserts, albeit both systems recommend that blood or another nutritional supplement be added to the bottles for better recovery of *Haemophilus* and *N. gonorrhoeae*.

In the current study we describe our experiences in standardizing a body fluid culture protocol that combined VersaTREK® universal blood culture media with solid media for the isolation of bacteria from normally sterile body fluids.

Fluid Volume	Routine Planting	Blood Culture Bottle(s)	Direct Gram Stain
<=0.1 mL	Plant entire sample directly to chocolate and thio broth using a sterile pipette	Not used	No
0.2 – 1.9 mL	Plant 1/5 of sample directly to media (Chocolate, Brucella agar & thio broth at a minimum)	1/5 of sample into aerobic bottle	Yes – direct or cytospin, volume dependent
2.0 – 40 mL	Centrifuge 1/5 of sample and plant sediment	Divide other 1/5 of sample equally between aerobic and anaerobic bottles	Yes – cytospin
>=40 mL	Centrifuge up to 50 mL and plant sediment	10 mL in aerobic bottle 10 mL in anaerobic bottle	Yes – cytospin

Table 1

RESULTS

Over a 5 month period we cultured 175 samples (pleural fluid = 53, hip fluid = 11, pleural fluid = 53, peritoneal fluid = 28, and pericardial fluid = 15) from 159 different patients using VersaTREK® blood culture bottles in parallel with solid media. Overall 121 (69%) of the samples were sterile by both culture methods and 54 (31%), collected from 38 patients were positive. Forty produced concordant VersaTREK® and solid media culture results, whereas 14 of the 54 positive cultures were detected only in the VersaTREK® bottles. None of the samples were positive on solid media alone (Table 2).

Knee and hip fluid samples represented the most frequent specimen type with 79 samples from 58 different patients cultured. Of these, 31 (from 29 different patients) were culture positive, and not unexpectedly *S. aureus* was the most frequent pathogen noted, with 17 isolates recovered in both blood culture bottles and on solid media. *S. aureus* and Group C Beta streptococci were recovered from an additional sample on solid media and in blood culture bottles, while one sample yielded *S. aureus* only in the VersaTREK® bottles. *Nocardia* nova was isolated in blood culture bottles (time to positivity = 69 hours), with viable colonies present on solid media after 72 hours of incubation from a knee fluid submitted on a patient who suffered an open fracture of his patella in a fall.

Enterococcus faecalis was recovered from two different patients with prosthetic knee joint infections. From patient "A", three separate knee fluid samples were submitted over a 40 day period and all grew *E. faecalis*, but only in the VersaTREK® bottles; solid media cultures remained sterile on all three samples after 7 days of incubation. This patient was receiving long-term vancomycin therapy for this infection, and interestingly, the MIC to vancomycin increased from 1.0 to 4.0 µg/mL over the series of the three cultures. Patient "B" also had three consecutive cultures positive for *E. faecalis*. The VersaTREK® bottles flagged positive in the first 24 hours of incubation, while 1-10 colonies were observed on the solid media cultures after 48-72 hours of incubation. Coagulase negative staphylococci (CoNS) were recovered from the three remaining, bottle only positives. The direct Gram stains on all three of these samples showed many PMNs, and while no organisms were observed; two of these patients had knee implants and both were on vancomycin therapy at the time of sample collection. The time-to-positivity was <=36 hours for all three of these samples, and one was also accompanied with tissue from which CoNS was also recovered.

Sample Type	Total Cultured	Total Positive	VersaTREK® Only Positive
Joint Fluid	79	31	7
Pleural Fluid	53	8	5*
Peritoneal Fluid	28	10	4
Pericardial Fluid	15	5	2**

Table 2. Sample types and number positive

*Two cultures with CoNS deemed contaminants
**One culture with *Propionibacterium* sp. deemed a contaminant

Bacteria	No. Isolates	VersaTREK® Only Positive	Both Methods Positive
<i>Staphylococcus aureus</i>	20	1	19
Coagulase negative staphylococci	5	5	5
Group B Beta streptococci	1	1	1
Group C Beta streptococci	1	1	1
<i>Streptococcus anginosus</i> group	5	4	1
<i>Enterococcus faecalis</i>	7	3	4
<i>Peptostreptococcus</i> sp.	1	1	1
<i>Propionibacterium</i> sp.	3	1	2
<i>Nocardia nova</i>	1	1	1
<i>Acinetobacter baumannii</i> complex	4	1	3
<i>Citrobacter freundii</i> complex	1	1	1
<i>Serratia marcescens</i>	1	1	1
<i>Pseudomonas aeruginosa</i>	2	1	1
Mixed anaerobic flora	2	2	2

Table 3. Distribution of Isolates Recovered

DISCUSSION

The concept of using blood culture media for the recovery of bacteria or yeast from normally sterile body fluid samples is not new and a number of studies support this view and current reference texts also recommend their use (1,2,3,4,5,7,8,9). We cultured 175 body fluid samples using a standardized algorithm for sample collection, distribution, and processing. Our results are consistent with others and clearly showed the increased sensitivity afforded when VersaTREK® bottles were added to the culture battery. Indeed without them, 14 of the 54 positive cultures, or 26%, would not have been detected. Similar to other studies, the increased sensitivity was accomplished without adding blood or other nutritional supplements to the culture bottles prior to use, an observation that speaks to the enriched media formulations in use today and eliminates a potential source for culture contamination (1,2,3,5,6). While we did not observe cultures that were positive only on solid media, the two culturing processes complemented each other as most samples did yield concordant results and this enabled organism work-up to proceed without the delay of sub-culturing.

While the value of including blood culture bottles when culturing sterile body fluids is not disputed, how the bottles are used and inoculated can still be problematic. While laboratories tend to rigorously control the use and inoculation of blood culture bottles used for "blood cultures", we do not always employ the same level of control when the bottles are used in random fashion by clinicians for other sample types. For example, it is still important to maintain an appropriate sample to broth ratio for optimal performance (e.g., 1:8), use of both aerobic and anaerobic media is preferred, and bedside inoculation of samples has proven to be beneficial (6,7). Far too often our laboratory has received "over-filled" blood culture bottles from nursing units, typically submitted without extra fluid, so a direct Gram stain could not be performed and solid media was not added. To address these concerns, and not unlike routine blood cultures, we worked to establish, and then aggressively inscribed a protocol for consistent handling of sterile body fluid samples. The protocol addressed large volume samples such as ascites fluid where dilution of small numbers of organisms may prevent recovery, and enforced bedside inoculation of blood culture bottles and fluids collected under ultra-sound guidance, with a second 50 mL aliquot collected and sent for direct Gram stain and solid media culture. Low volume samples were also considered with a focus of including at least one VersaTREK® bottle with all body fluid cultures (table 1). This was possible in part, because VersaTREK® 80 mL universal media are FDA cleared for sample volumes ranging from 0.1 to 10.0 mL.

Joint fluids were the most common sample type submitted, and *S. aureus* the predominant pathogen recovered with both culture methods. For the seven instances where only the VersaTREK® bottles were positive, antibiotic therapy, specifically vancomycin, appeared to negatively impact the recovery of both enterococci and CoNS on solid media, yet these organisms grew nicely in the VersaTREK® bottles, with a mean time-to-positivity of <=36 hours. While easy to assume CoNS recovered only from blood culture bottles represented contamination, we found the direct Gram stain provided useful information in resolving these discordant cultures as high numbers of inflammatory cells, even in the absence of bacteria, coupled with clinical symptoms and presence of an artificial joint were all consistent with infection.

Overall only 3 of the 54 positive cultures (5.6%) were felt to represent contaminants. This is lower than others have reported (2,3). We feel this was attributed to work done in standardizing the pre-analytical processes of sample collection and standardizing bottle inoculation. That said low level contamination can be useful in assessing the sensitivity of the culture method employed, especially when attempting to recover small numbers of organisms. However, it should also be mentioned that concomitant use of solid media was helpful in determining the clinical significance of an isolate.

We conclude that using the VersaTREK® for sterile body fluid cultures in conjunction with solid media and a well defined and inscribed protocol does improve culture sensitivity. We also found the VersaTREK® to be very versatile with the ability to perform blood and sterile body fluid cultures, along with cultures for *Mycobacteria* in the same cabinet.

REFERENCES

- Boudabia, M., et al. 1988. Improved method for bacteriological diagnosis of spontaneous bacterial peritonitis. J. Clin. Microbiol. 27:2145-2147.
- Bourbeau, P., et al. 1998. Use of the BacT/Alert blood culture system for culture of sterile body fluids other than blood. J. Clin. Microbiol. 36:3273-3277.
- Fuller, D. D., et al. 1994. Comparison of BACTEC Plus 26 and 27 media with and without fastidious organism supplement with conventional methods of culture of sterile body fluids. J. Clin. Microbiol. 32:1488-1491.
- Hay, E. E., et al. 1996. Clinical comparison of Isolator, Septi-Check, nonvented tryptic soy broth, and direct agar plating combined with thiocyclate broth for diagnosing spontaneous bacterial peritonitis. J. Clin. Microbiol. 34:34-37.
- Hughes, J. C., et al. 2001. Culture with BACTEC Peds Plus/F bottle compared with conventional methods for detection of bacteria in synovial fluid. J. Clin. Microbiol. 39:4468-4471.
- Runyon, B. A., et al. 1990. Bedside inoculation of blood culture bottles with ascites fluid is superior to delayed inoculation in the detection of spontaneous bacterial peritonitis. J. Clin. Microbiol. 28:2811-2812.
- Siersma, P. D., et al. 1992. Blood culture bottles are superior to lysis-centrifugation tubes for bacteriological diagnosis of spontaneous bacterial peritonitis. J. Clin. Microbiol. 30:867-869.
- Thomson Jr., R. B. 2007. In Manual Clinical Microbiology, 9th ed., Ch. 20. ASM, Washington DC.
- Wong, C. L., et al. 2008. Does this patient have bacterial peritonitis or portal hypertension? How do I perform a paracentesis and analyze the results? JAMA. 299:1166-1178.