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Clinical Laboratory Evaluation of the Fifty-Milliliter Vacutainer Blood Culture Tube

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The efficacy of the 50-ml vacutainer culture tube (Becton-Dickinson) for recovery of microorganisms from blood cultures was compared with laboratory-prepared blood culture media. The isolation of *Bacteroides* sp. was significantly higher in the 50-ml vacutainer culture tube whereas that of nonfermenting gram-negative rods and yeasts was higher in the in-house media. There was no significant difference in the recovery of all other organisms.

The clinical need for monitoring infectious diseases by the use of blood culture has increased with advances in medical science. The recognition that microorganisms representing the indigenous and hospital microbiota may complicate the recovery of patients who have undergone various surgical procedures, physical and/or pharmacological treatment for neoplastic diseases, etc., accounts for the continuing interest in establishing the identity of microorganisms which may be involved in complications of an infectious nature. At the same time, many of the clinical laboratories no longer produce their own media but use commercial sources for such supplies. The present study was undertaken to evaluate the performance of the Beckton-Dickinson 50-ml vacutainer culture tube (VCT) designed for direct phlebotomy and the recovery of clinically significant microorganisms from the blood.

MATERIALS AND METHODS

Culture media. The blood culture medium used in this laboratory consisted of two McCartney bottles, one of which contained 50 ml of Eugon broth (BioQuest)and 0.025% sodium polyanethol sulfonate. The second bottle contained 50 ml of Trypticase soy broth (BioQuest), 0.025% sodium polyanethol sulfonate, 0.1% yeast extract, and several pieces of cooked liver which were added before autoclaving.

The 50-ml VCT bottle consisted of 45 ml of modified supplemented peptone broth under vacuum and with CO_2. Two types of venting units were supplied for the bottles. The unplugged venting unit provided aerobic cultural conditions, the other contained a vaspar sealed plug to insure an anaerobic environment. The plug could be forced out by excess gas produced within the bottle by microbial activity.

Clinical blood cultures. All patients' bloods were drawn by either a house physician or a member of the phlebotomy team. The blood was collected aseptically with a sterile needle and 20-ml syringe after first cleaning the skin with Betadine and isopropyl alcohol. The blood was inoculated into each blood culture bottle (10% vol/vol) at the patient's bedside and then transported to the laboratory. The tops of the 50-ml VCT bottles were swabbed with Betadine before inserting the venting units. Each blood culture set consisted of four bottles: the two routine culture bottles and two vacutainer bottles, one with an unplugged venting unit and the other with a plugged venting unit.

The blood cultures were incubated at 35 C and were inspected daily for macroscopic growth for a period of 14 days. A blind subculture of each blood bottle was performed on days 2 and 14 of incubation, if visual evidence of growth had not occurred previously. Before subculturing, the bottles were shaken gently to distribute the blood in each bottle evenly. Blood was removed from the vacutainer bottles with a sterile needle and syringe; sterile Pasteur pipettes were employed with the routine culture bottles. The blood from each subcultured bottle was inoculated onto two blood agar plates; one was incubated aerobically, the other was incubated anaerobically in a GasPak jar (BioQuest) at 35 C. The plates were examined at 24 h for growth; if no growth was observed, the plates were incubated at 35 C for an additional 24 h. Gram stains were performed on smears of all blood cultures with evidence of growth before subculture.

Postmortem blood cultures. During autopsy, heart blood was obtained aseptically with a needle and syringe. Equal portions of the collected blood were inoculated into each of the four bottles described above. These blood cultures were treated in the same manner as mentioned previously. A 20-ml blood sample could not be procured in most instances.

Control cultures. The ability of the 50-ml VCT to support the growth of *Haemophilus influenzae*, *Neisseria meningitidis*, and *Clostridium novyi* B was examined by simulating routine blood culture procedures. Five colonies of each organism were suspended in 1 ml of broth which had been removed from a 50-ml VCT. This suspension was diluted 1:10

in this broth and 0.1 ml from this dilution was added to each of two 50-ml VCT bottles along with 5 ml of human blood. The bottles were shaken thoroughly; 0.02 of the culture medium was removed and streaked onto the appropriate media. The plates and 50-ml VCT were incubated for 24 h at 35 C after which the number of colony-forming units were determined. This procedure was repeated with the 24-h cultures from the 50-ml VCT.

In addition, the capability of the in-house as well as the commercially prepared media to support medically significant microorganisms was ascertained with representative species routinely used in this laboratory for quality control purposes before the use of the media. These organisms included *Streptococcus pyogenes, S. pneumoniae, Pseudomonas aeruginosa,* and *Acinetobacter calcoaceticus.*

RESULTS

During a 9-month period a total of 4,046 blood culture sets were processed in the laboratory. Of these, 3,182 did not meet the basic requirements for inclusion in the series, namely, not all four constituent bottles were inoculated. Only 864 sets were suitable for this evaluation. There were 73 positive cultures in this group. One or more, but not necessarily all four bottles, manifested positive growth. To be considered a clinically significant culture, communication with a physician of record was initiated immediately upon noting the positive culture and a causal relationship between the laboratory findings and the clinical symptoms could be established clearly. An additional 93 sets yielded organisms, none of which were deemed significant in light of the patient's history. These latter cultures consisted of *Staphylococcus epidermidis, Corynebacterium* sp., *Propionibacterium* sp., viridans streptococci, and *Bacillus* sp. The positive cultures in the study were obtained from 54 patients. Twenty-three different microorganisms were isolated from these cultures. Among the 3,182 cultures not included in the study, 189 were positive with clinically significant organisms. The overall rate of positive blood cultures for the 9-months period was 6.5%. For the group included in the study and positive yields were 8.4%, for the excluded group, 5.9%.

Positive heart blood cultures were obtained in one or more bottles of the four bottle set from 132 postmortem specimens; 15 cultures were negative at the end of 14 days. Twenty-nine different microorganisms were isolated from these specimens.

The blood bottle or combination of bottles from which the organisms were isolated in clinically significant positive blood cultures and positive postmortem cultures are presented in Tables 1 and 2, respectively. In most instances, the routine culture bottles and the 50-ml VCT bottles showed no significant differences in the recovery of the organisms isolated. Members of the family *Enterobacteriaceae* comprised 55% of the total organisms isolated; 149 of 176 were recovered in both the routine and the 50-ml VCT bottles. No significant differences were noted in the recovery of the streptococci, the staphylococci, and acinetobacters. The pseudomonads, however, were recovered in only 10 of the 50-ml VCT sets. On the other hand, 24 of the 28 *Pseudomonas* sp. were isolated from the routine blood culture sets.

Differences in the recovery of yeasts became evident also. These microorganisms were isolated from the 50-ml VCT sets on three occasions; they were recovered from eight routine cultures. Conversely, a total of 11 cultures yielded *Bacteroides;* the 50-ml VCT bottles led to the recovery of these organisms nine times, while the hospital routine bottles yielded these bacteria on five occasions.

Statistical chi-square analysis was performed on these findings; $P < 0.05$ was considered the critical value for significant differences in isolation rates between the commercial and laboratory prepared sets. The statistical evaluation was performed on the combined results of clinical and postmortem specimens in view of the relatively small number of positive findings for each microbial category. These analyses indicated that isolation of *B. fragilis* was significantly higher in the 50-ml VCT ($P < 0.05$). No significant differences could be established for all other microorganisms at the species level. When the various species of microorganisms recovered from all sources were combined into broad categories, the anaerobic gram-negative rods were recovered significantly better from the 50-ml VCT ($P < 0.05$) as shown in Table 3. The nonfermenting gram-negative rods and yeasts, however, were favored by the in-house media at a significant level ($P < 0.05$).

As presented in Table 4, the 50-ml VCT supported the proliferation of the *N. meningitidis, H. influenzae,* and *C. novyi* B. The numbers of organisms introduced into this medium increased to greater than 1×10^6 colony-forming units/ml in each instance.

DISCUSSION

The major goal of this investigation was the assessment of the nutrient and environmental performance of the 50-ml VCT culture set. Our findings indicated that for the organisms usually encountered in the clinical laboratory this commercially prepared medium performed as

TABLE 1. *Microorganisms recovered from clinically significant blood cultures*

Organisms	EMOP[a]	E	M	P	O	EM	OP	EMP	MOP	EMO	EOP	MO	EO	EP	MP	OP/Total	EM/Total
Escherichia coli	5		3		1	1					1				1	8/12	11/12
Proteus mirabilis	4							2					1			7/7	7/7
P. morganii			1										1			1/2	2/2
Klebsiella pneumoniae	2				1					1						4/4	3/4
Enterobacter cloacae												1				1/1	1/1
E. aerogenes			1													0/1	1/1
Serratia marcescens	3				1											4/4	3/4
Pseudomonas aeruginosa	1	2		1							1					3/5	4/5
P. alcaligenes		1														0/1	1/1
Acinetobacter anitratus[b]		3			1											1/4	3/4
A. lwoffi[b]			1													0/1	1/1
Staphylococcus aureus	1	3	1													1/5	4/5
Streptococcus faecalis	2		3	1			1				1	1				6/9	7/9
S. mitis group			2							1						1/3	3/3
Streptococcus group B[c]	2															2/2	2/2
Listeria monocytogenes								2								2/2	2/2
Clostridium perfringens	1															1/1	1/1
Bacteroides fragilis					1									1		2/2	1/2
Bacteroides sp.				1					1							2/2	1/2
Candida albicans						1							1			1/2	2/2
C. parapsilosis															1	1/1	1/1
Torulopsis glabrata						1										0/1	1/1

[a] E, Eugon broth; M, chopped meat broth; O, 50-ml VCT with open venting unit; P, 50-ml VCT with plugged venting unit.

[b] Variety of species *Calcoaceticus*.

[c] Lancefield serogroup B.

well as our in-house prepared media. Although not all of the yeasts and pseudomonads grew in the 50-ml VCT bottles, the better recovery of *Bacteroides* sp. must be noted. It is of interest that the recovery of quality control organisms representing species not isolated in the clinical and postmortem trials such as *N. meningitidis*, *H. influenzae*, and *C. novyi* B was efficient. The discrepancies which became manifest between the two approaches to the recovery of organisms from blood cultures, therefore, might have been a reflection of the distribution of the organisms in the blood drawn. It must be pointed out that 20-ml samples had to be obtained, from patients who were split randomly, in 5-ml aliquots among the four bottles. This greater than usual volume of blood drawn at one time may account for the slightly increased positive percentage obtained with the study sets as compared to those which were excluded from this investigation. It may also account for the differences in the recovery of microorganisms by a chance distribution of these bacteria into each of the bottles comprising the two sets (1). As pointed out previously (3, 4, 6), a variety of techniques are required for the rapid diagnosis of bacteremia. In these studies by Finegold and associates it was reported that 42.6% of the cultures in a limited series yielded 1 or less colony-forming units/ml of blood, while 25.9% harbored organisms between 1 to 10/ml. Thus, a total of 68.5% of the bloods in the various Finegold studies (3, 4, 6) contained 10 organisms or less per ml of blood. Certainly the conclusions of Hall et al. (5) pertain to any study directed at setting the efficacy of blood culture techniques and media. These authors listed as important factors for consideration beside the composition of media, the volume of blood sampled, atmosphere and duration of incubation, frequency and types of routine "blind" subcultures, and manner of processing of recognized positive cultures. Although the statistical methods of Cochran (2) as used by Washington (7) represent a mathematical approach to the interpretation of findings, the data gathered during this present investigation with quality control studies indicate that the nutrient qualities, as well as the atmospheric conditions with a small inoculum, could not explain the slightly different yields between the commercial and laboratory manufactured media employed. Since all of the manipulations accorded to the two different sets were identical, the best explanation appears to

TABLE 2. *Microorganisms recovered from postmortem cultures*

Organisms	EMOP[a]	E	M	P	O	EM	OP	EMP	MPO	EMO	EPO	MO	EO	EP	MP	OP/Total	EM/Total
Escherichia coli	46	2	1	3	1	4	3	1	2	1		1			1	59/66	59/66
Proteus mirabilis	6						1					1				8/8	7/8
P. morganii			1				1								1	2/3	2/3
Klebsiella pneumoniae	11	2	1	2	1	4	1	1	1		2					19/26	22/26
Enterobacter cloacae	4	1				1	1									5/7	6/7
E. aerogenes	5			4	1	1	1	1		1			1	1		15/16	10/16
E. liquefaciens	1			1												2/2	1/2
E. agglomerans	4				1					1			1			7/7	6/7
Serratia marcescens	1					2										1/3	3/3
Citrobacter freundii	3					1	2								1	6/7	5/7
Pseudomonas aeruginosa	2	2	1		1		1		1							5/8	6/8
P. fluorescens						1						1				1/2	2/2
P. maltophilia		2														0/2	2/2
P. putida		1	3													0/4	4/4
P. stutzeri		1														0/1	1/1
Acinetobacter anitratus[b]	1				2	2							1			4/6	4/6
A. lwoffi[b]			1			1										0/2	2/2
Staphylococcus aureus	1	1	1		2	2	1		1							5/9	6/9
Streptococcus faecalis	25		1	1	3	2	5		4	3					1	42/45	36/45
S. group B[c]	3															3/3	3/3
Streptococcus pneumoniae											1					1/1	1/1
Clostridium perfringens	2	1						1	1			1				5/6	5/6
Bacteroides fragilis			1		2		2									4/5	1/5
Bacteroides sp.	1		1													1/2	2/2
Fusobacterium girans					1											1/1	0/1
Candida albicans	1					1										1/2	2/2
Candida sp.		1														0/1	1/1
Torulopsis glabrata		1														0/1	1/1
Lactobacillus sp.										1						1/1	1/1

[a] E, Eugon broth; M, chopped meat broth; O, 50-ml VCT with open venting unit; P, 50-ml VCT with plugged venting unit.
[b] Variety of species *Calcoaceticus*.
[c] Lancefield serogroup B.

TABLE 3. *Taxonomic grouping of total clinical and postmortem isolates*

Group	OP[a]	EM[b]	P[c]
Gram-positive cocci	61/77	62/77	NS[d]
Enterobacteriaceae	149/176	149/176	NS
Anaerobic gram-negative rods	10/12	5/12	<0.050
Nonfermenting gram-negative rods	9/23	20/23	<0.050
Yeasts	3/8	8/8	<0.050

[a] O, 50-ml VCT with open venting unit; P, 50-ml VCT with plugged venting unit.
[b] E, Eugon broth; M, meat broth.
[c] For difference in the media.
[d] Not significant.

TABLE 4. *Quantitative results of control cultures*

Organism	0 h		24 h	
	O[a]	P	O	P
Neisseria meningitidis	100[b]	160	$>10^6$	$>10^6$
Haemophilus influenzae	288	290	$>10^6$	$>10^6$
Clostridium novyi B	NG[c]	NG[c]	$>10^6$	$>10^6$

[a] O, 50-ml VCT with open venting unit; P, 50-ml VCT with plugged venting unit.
[b] Expressed in colony-forming units per milliliter.
[c] NG, No growth.

be the very low number of organisms present in the blood samples. It may very well be the nature of the bacteremia as well as the stage of the bacteremia which determines the ease of recovering organisms for diagnostic purposes. If the number of bacteria is in the neighborhood of one organism per 5 ml, the chance of an equal distribution into each of four bottles would seem to be very small. To our knowledge, no statistical methods for establishing this probability have been applied to such a model.

The failure of the nonfermenting gram-negative rods and yeasts in the 50-ml VCT may have been caused by the improper placement of the nonplugged venting unit into the rubber stop-

per. The manufacturers have since devised a unit with a longer venting needle which protrudes visibly below the neck of the stopper. This will obviate the needle of the venting unit from being imbedded in the stopper rather than in the open space of the bottle neck and permit the proper aeration needed by these organisms.

The postmortem heart bloods were used in an attempt to approach the conditions that pertain during bacteremia. This material was thought to be preferable to the addition of bacteria to sterile bloods which would then be used in the evaluation of the supportive capabilities of the various media, and as shown in Table 2, the postmortem bloods were quite suitable for this purpose. The recovery of most organisms was of the same order of magnitude. However certain differences became manifest here as well, indicating the influence of sampling and the distribution of organisms within the assayed material.

The 50-ml VCT blood culture media, packaged in novel bottles which may be used directly for phlebotomy and provided with capability of admitting or excluding air, performed at the same level as did the in-house prepared media in the support of clinically significant microorganisms. The advantages of such a system obviating separate phlebotomy modalities or delay in culturing blood are quite obvious.

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