JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2003, p. 1266–1269 Vol. 41, No. 3
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.3.1266–1269.2003

Conventional and Molecular Methods for Verification of Results Obtained with BacT/Alert Nonvent Blood Culture Bottles

Elizabeth M. Marlowe,[1]* Linda Gibson,[1] James Hogan,[2] Shannon Kaplan,[2] and David A. Bruckner[1]

Gen-Probe Incorporated, San Diego,[2] and Department of Pathology and Laboratory Medicine, UCLA Medical Center, Los Angeles,[1] California

Received 27 August 2002/Returned for modification 16 November 2002/Accepted 5 December 2002

A strategy comparing molecular and conventional methods for verification of the BacT/Alert nonvent blood culture bottles (Organon Teknika, Durham, N.C.) was performed with seeded isolates. The bottles were evaluated with 12 common organisms from bloodstream infections. Overall, the bottles were equivalent as determined by conventional and molecular methods.

Blood culture is currently the only routine method for detecting bacterial bloodstream infections. Over the past 2 decades, advances in blood culture technology have resulted in significant decreases in the time to detection and identification of these etiological agents (8, 12). Consequently, one of the most challenging tasks facing the clinical microbiology laboratory is the meaningful verification of a new blood culture system. Suggested guidelines available for verification of new blood culture bottles include parallel and seeded blood culture studies (5).

In conjunction with advances in conventional blood culture methodologies, there have been several studies examining the use of nucleic acid tests for the rapid detection of positive blood cultures (4, 6, 7, 10, 11, 13, 14; D. Bruckner, L. Gibson, J. Hindler, J. Hogan, I. Andruszkiewicz, K. Clark-Dickey, and W. Weisburg, Abstr. 101st Gen. Meet. Am. Soc. Microbiol., abstr. C-1, p. 147, 2001). Specifically, the Gen-Probe Incorporated hybridization protection assay (HPA), which uses a DNA probe to detect rRNA, has been utilized to evaluate positive blood cultures. The chemiluminescent signal generated by the HPA has been found elsewhere to directly correlate with CFU (Bruckner et al., Abstr. 101st Gen. Meet. Am. Soc. Microbiol.; D. Fuller and T. Davis, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1558, 1999; J. F. Hindler, S. Kozen, and D. A. Bruckner, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1557, 1999).

Organon Teknika (Durham, N.C.) has replaced its standard vented BacT/Alert bottle with a redesigned nonvent (NV) blood culture bottle. As a result, laboratories have been confronted with performing a verification study of these new blood culture bottles. The objective of this study was to perform a seeded study to (i) evaluate the NV bottle for the ability to support growth in a timely manner by conventional methods and (ii) compare conventional methods with molecular probe detection as a possible rapid alternative method for blood culture bottle verification.

In this seeded study performed at UCLA Medical Center, BacT/Alert standard and NV (aerobic and anaerobic) bottles were evaluated. Bottles were tested in triplicate with approximately 50 organisms/bottle. The bacterial isolates used include *Enterococcus faecium* (UCLA 236), *Staphylococcus aureus* (ATCC 29213), *Streptococcus viridans* (UCLA 893 and patient sample 4457), *Streptococcus pneumoniae* (ATCC 49619 and patient sample 4452), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Bacteroides fragilis* (ATCC 25285), *Clostridium perfringens* (ATCC 13124), *Listeria monocytogenes* (UCLA 1174), *Corynebacterium jeikeium* (UCLA 1126), *Haemophilus aphrophilus* (UCLA 1146), and *Candida albicans* (ATCC 14053). Inoculum was prepared from an overnight culture, with diluted 0.5-McFarland-standard suspensions of each isolate. All bottles were incubated in a BacT/Alert3D continuous monitoring system (Organon Teknika Corp.) according to the manufacturer's recommendation. Bottles remained incubated until they flagged positive or until 7 days had elapsed. The time from loading to detection was recorded for each bottle. If isolates failed to grow, 5 to 10 ml of blood was added to bottles and retested in duplicate. Furthermore, isolates still failing to grow were replaced with a patient strain and evaluated with and without blood.

When bottles were triggered as positive, cell mass was measured by viable plate counts and probe detection was performed with the Gen-Probe Incorporated all-bacterial and all-fungal DNA probes directed toward rRNA targets. Bottles not triggered as positive were terminally subcultured and probed after 7 days. The all-bacterial and all-fungal probes were derived from the 23S and 18S regions of the rRNA genes, respectively. For probing a 1-ml sample was centrifuged to pellet the organism, washed twice with a saponin-based cocktail, lysed at 100°C in a succinate-buffered detergent cocktail, and frozen for further analysis. Frozen pellets were diluted when necessary to obtain relative light unit (RLU) levels within the linear range of the luminometer when run in an HPA (1, 9).

Overall, the BacT/Alert NV bottles were equivalent to the standard BacT/Alert bottles as determined by both conventional and molecular methods. These results agree with others with clinical samples (2, 12). The comparative mean times to detection are summarized in Table 1. In the bottles that did not require blood, the standard and NV bottles were equivalent in

* Corresponding author. Present address: Gen-Probe Incorporated, 10210 Genetic Center Dr., San Diego, CA 92121-4362. Phone: (858) 410-8692. Fax: (858) 410-8870. E-mail: bethm@gen-probe.com.

TABLE 1. Mean time to positive blood bottle detection

Organism and bottle[e]	Avg time (h)	
	Standard	NV
Gram-positive cocci		
S. aureus		
A	13.9 ± 0.92	14.1 ± 0.38
AN	21.3 ± 5.51	25.5 ± 5.44
S. pneumoniae		
A	16.3 ± 0.95	22.9 ± 0.84
AN	15.9 ± 7.35	35.4[a]
S. pneumoniae with blood		
A	14.0 ± 0.29	14.2 ± 0.69
AN	14.9 ± 0.56	14.7 ± 0.38
S. viridans		
A	30.2 ± 20.7	39.5 ± 25.7
AN	64.7 ± 21.3	51.3 ± 9.4
S. viridans with blood		
A	18.6 ± 0.59	18.4 ± 1.16
AN	17.9 ± 0.39[b]	19.1 ± 0.17
E. faecium		
A	17.3 ± 0.20	17.5 ± 1.38
AN	19.4 ± 0.25	18.8 ± 0.64
Enteric		
E. coli		
A	13.3 ± 0.51	12.9 ± 0.36
AN	12 ± 0.48	12.8 ± 0.15
Nonfermenting bacillus		
P. aeruginosa		
A	18.1 ± 1.22	19.3 ± 2.23
AN	N/F[f]	N/F
Gram-positive bacilli		
L. monocytogenes		
A	18.8 ± 0.20	19.6 ± 0.17
AN	21.5 ± 0.05	25.8 ± 1.79
C. jeikeium		
A	N/F	N/F
AN	N/F	N/F
C. jeikeium with blood		
A	58.8 ± 15.27	57.5 ± 24.89
AN	56.8 ± 23.90	81.6[c]
Fastidious bacilli		
H. aphrophilus		
A	28.7 ± 1.02	33.0 ± 4.05
AN	26.0 ± 2.43	27.0 ± 1.28
H. aphrophilus with blood		
A	26.7 ± 1.05	26.3 ± 1.45
AN	25.7 ± 0.35	25.9 ± 0.10
Anaerobes		
C. perfringens		
A	N/F	N/F
AN	15.2 ± 0.59	19.3 ± 0.52
B. fragilis		
A	N/F	N/F
AN	30.1 ± 2.11	34.3 ± 3.44
Yeast		
C. albicans		
A	25.2 ± 2.75[d]	24.0 ± 0.34
AN	N/F	N/F

[a] One of six bottles positive, suspected autolysis.
[b] Three of four bottles positive.
[c] One of two bottles positive.
[d] Two of four bottles positive.
[e] A, aerobic; AN, anaerobic.
[f] N/F, not flagged positive after 7 days of incubation.

their mean times to positivity. Isolates failing to grow without blood were equivalent in their mean times to positivity for the standard and NV bottles after blood was added to the bottles (Table 1), excluding *C. jeikeium*.

The equivalent mean times for detection of the isolates are comparable to those reported by Snyder et al. (12) and within the range of performance characteristics reported in the package insert. The manufacturer states in the package insert that organisms may require added blood or other supplements to support growth. Consequently, *S. pneumoniae*, *S. viridans*, and *C. jeikeium* required blood in some of the bottles to support growth. This was necessary for *S. pneumoniae* and *S. viridans* only in the anaerobe bottles. It is important to keep in mind that any nonblood clinical specimen, such as joint fluids and peritoneal dialysates, cultured in these bottles may require supplements for adequate growth. Those isolates that required blood because they failed to grow well were also retested with a patient strain, with and without blood. The original isolates and the patient strains showed no difference in growth performance.

The comparative mean CFU per milliliter in the positive blood culture bottles for the various representative organisms and bottles are summarized in Table 2. For bacteria, the positive bottles were detected in the late log phase of growth, within a range of 1.53×10^7 to 7.41×10^9 CFU/ml, excluding *H. aphrophilus*. For yeast, positive bottles were detected with approximately 4.5×10^6 CFU/ml. Interestingly, molecular probe detection was proportionally equivalent to conventional culture methods for verification of the positive bottles. Results of the mean comparative RLU/ml are summarized in Table 3. The CFU-per-milliliter numbers for *H. aphrophilus* may be underrepresented due to the organism's granular growth in broth. Despite clumping of cells, the RLU-per-milliliter signals for *H. aphrophilus* had substantially lower standard deviations than did the CFU per milliliter from culture. This phenomenon demonstrates that measurement of total amount of rRNA is not dependent on the organisms being monodispersed. This suggests that a nucleic acid detection system is a better measure of organisms when used for quality control of growth media. When bottles failed to flag as positive after 7 days, they were terminally subcultured and probed.

Both molecular and conventional methods were able to detect organisms below the limit of detection of the BacT/Alert3D instrument (1.53×10^7 to 7.41×10^9 CFU/ml). The limit of detection was approximately 10^4 CFU/ml for the HPA probe system, while culture displayed a sensitivity of 300 to 3,000 CFU/ml. For example, CFU and RLU probe signals were detected in the 7-day subcultures of *P. aeruginosa* in all anaerobic bottles even though the Bact/Alert3D instrument did not detect them. This reflects a 10,000-fold-greater sensitivity of the probe assay than of the Bact/Alert3D for detecting positive blood cultures.

The 7-day subcultures of *C. albicans* from the standard aerobic bottles had a mean of 35 CFU/ml, while the 7-day subcultures of the standard and new NV anaerobic bottles had means of <30 and 2.3×10^3 CFU/ml, respectively. Either no probe or low probe signals were detected for the 7-day subcultures of *C. albicans*.

The ratio of the RLU to CFU for a given organism is dependent upon a number of variables. The growth state, plating

TABLE 2. Mean CFU/per milliliter in positive blood culture bottles

Organism and bottle[e]	Avg CFU/ml (1.0×10^8)	
	Standard	NV
Gram-positive cocci		
S. aureus		
A	1.24 ± 1.87	2.81 ± 3.89
AN	0.82 ± 9.39	0.30 ± 0.10
S. pneumoniae		
A	0.64 ± 0.90	2.98 ± 48.3
AN	46.2 ± 2.32	3.48 ± 0.08[a]
S. pneumoniae with blood		
A	2.89 ± 1.02	5.87 ± 2.67
AN	2.68 ± 1.17	3.00 ± 0.65
S. viridans		
A	0.19 ± 1.02	0.15 ± 0.18
AN	0.25 ± 0.23	1.02 ± 1.06
S. viridans with blood		
A	3.03 ± 4.08	1.11 ± 2.37
AN	2.53 ± 1.14[b]	2.30 ± 2.65
E. faecium		
A	74.1 ± 47.9	6.81 ± 4.63
AN	51.2 ± 34.5	51.4 ± 38.8
Enteric		
E. coli		
A	17.4 ± 2.16	13.3 ± 2.80
AN	16.8 ± 1.38	14.2 ± 6.69
Nonfermenting bacillus		
P. aeruginosa		
A	2.58 ± 0.16	3.14 ± 0.33
AN	N/F[f]	N/F
Gram-positive bacilli		
L. monocytogenes		
A	0.13 ± 1.40	8.78 ± 0.73
AN	20.9 ± 1.78	15.3 ± 2.25
C. jeikeium		
A	N/F	N/F
AN	N/F	N/F
C. jeikeium with blood		
A	0.59 ± 0.81	1.74 ± 5.11
AN	3.80 ± 0.37	5.90 ± 0.07[c]
Fastidious bacilli		
H. aphrophilus[g]		
A	0.004 ± 0.0002	0.089
AN	0.217 ± 0.12	0.72 ± 3.19
H. aphrophilus with blood		
A	0.01 ± 0.005	3.10 ± 2.49
AN	0.07 ± 0.02	0.15 ± 0.06
Anaerobes		
C. perfringens		
A	N/F	N/F
AN	3.67 ± 0.52	2.99 ± 0.80
B. fragilis		
A	N/F	N/F
AN	0.19 ± 3.37	11.2 ± 5.34
Yeast		
C. albicans		
A	0.04 ± 0.04[d]	0.05 ± 0.009
AN	N/F	N/F

[a] One of six bottles positive.
[b] Three of four bottles positive.
[c] One of two bottles positive.
[d] Two of four bottles positive.
[e] A, aerobic; AN, anaerobic.
[f] N/F, not flagged as positive after 7 days.
[g] *H. aphrophilus* clumps in broth; thus, accurate CFU were not available.

TABLE 3. Mean RLU/per milliliter in positive blood culture bottles

Organism and bottle[e]	Avg RLU/ml (10^8)	
	Standard	NV
Gram-positive cocci		
S. aureus		
A	42.1 ± 14.3	26.0 ± 3.54
AN	11.1 ± 7.03	13.6 ± 7.63
S. pneumoniae		
A	9.10 ± 6.59	11.1 ± 2.87
AN	75.5 ± 38.1	27.7[a]
S. pneumoniae with blood		
A	58.4 ± 17.4	45.2 ± 6.98
AN	57.6 ± 28.2	61.1 ± 26.1
S. viridans		
A	21.7 ± 15.0	14.8 ± 13.3
AN	33.5 ± 25.5	37.3 ± 9.16
S. viridans with blood		
A	41.5 ± 29.8	44.3 ± 21.2
AN	69.4 ± 6.47[b]	48.9 ± 28.6
E. faecium		
A	55.0 ± 25.4	5.34 ± 0.78
AN	43.1 ± 10.9	23.3 ± 6.81
Enteric		
E. coli		
A	62.9 ± 18.5	33.7 ± 21.4
AN	51.5 ± 8.72	49.4 ± 4.34
Nonfermenting bacilli		
P. aeruginosa		
A	23.0 ± 7.41	14.3 ± 3.85
AN	N/F[f]	N/F
Gram-positive bacilli		
L. monocytogenes		
A	2.64 ± 0.49	1.75 ± 1.04
AN	1.95 ± 0.72	2.69 ± 0.60
C. jeikeium		
A	N/F	N/F
AN	N/F	N/F
C. jeikeium with blood		
A	0.033 ± 0.022	0.021 ± 0.015
AN	0.005 ± 0.003	0.005[c]
Fastidious bacilli		
H. aphrophilus		
A	4.90 ± 4.10	1.66 ± 2.06
AN	11.8 ± 7.46	8.91 ± 3.08
H. aphrophilus with blood		
A	3.70 ± 1.68	8.02 ± 8.08
AN	2.19 ± 0.41	5.57 ± 1.91
Anaerobes		
C. perfringens		
A	N/F	N/F
AN	113.0 ± 24.7	123.0 ± 12.9
B. fragilis		
A	N/F	N/F
AN	63.1 ± 15.1	36.3 ± 14.1
Yeast		
C. albicans		
A	4.23 ± 4.39[d]	4.73 ± 18.9
AN	N/F	N/F

[a] One of six bottles positive.
[b] Three of four bottles positive.
[c] One of two bottles positive.
[d] Two of four bottles positive.
[e] A, aerobic; AN, anaerobic.
[f] N/F, not flagged as positive after 7 days.

efficiency, and maximum growth rate will affect this ratio for a given organism. Organisms such as *E. coli* with very rapid doubling times have much higher numbers of ribosomes per cell than do organisms with low growth rates, such as mycobacteria. Size is a factor in some mycoplasma organisms, which are so small that only a very limited number of ribosomes can be accommodated within the volume of the cell. For most organisms from bloodstream infections detected in routine blood culture systems, the ratios of RLU to CFU were found to be consistent for a variety of targets (Bruckner et al., Abstr. 101st Gen. Meet. Am. Soc. Microbiol.; Fuller and Davis, 39th ICAAC; Hindler et al., 39th ICAAC).

HPA probe detection requires less specimen processing than do conventional methods and provides an answer within 1 to 2 h of the bottle triggering as positive. It has the capability of detecting positive blood cultures much earlier than the automated systems due to a 10^3-CFU/ml difference in sensitivity. The HPA probe matrix methodologies have provided early, rapid, and precise detection of positive blood cultures (Bruckner et al., Abstr. 101st Gen. Meet. Am. Soc. Microbiol.; Fuller and Davis, 39th ICAAC; Hindler et al., 39th ICAAC). It has also been applied to blood components as a rapid means of screening for transfusion-associated bacterial contamination (3). HPA provided quick and accurate verification of blood culture bottle performance, thus further demonstrating the versatility of this technology.

REFERENCES

1. **Arnold, L. J., P. W. Hammond, W. A. Wiese, and N. C. Nelson.** 1989. Assay formats involving acridinium-ester-labeled DNA probes. Clin. Chem. **35:** 1588–1594.
2. **Brecher, M. E., D. G. Heath, S. N. Hay, S. J. Rothenberg, and L. C. Stutzman.** 2002. Evaluation of a new generation of culture bottle using an automated bacterial culture system for detecting nine common contaminating organisms found in platelet components. Transfusion **42:**774–779.
3. **Brecher, M. E., J. J. Hogan, G. Boothe, A. Kerr, L. McClannan, M. R. Jacobs, R. Yomtovian, V. Chongokolwatana, G. Tegtmeier, S. Henderson, A. Pineda, V. Halling, M. Kemper, K. Kuramato, P. V. Holland, and M. Longiaru.** 1994. Platelet bacterial contamination and the use of a chemiluminescence-linked universal bacterial ribosomal RNA gene probe. Transfusion **34:**750–755.
4. **Einsele, H., H. Hebart, G. Roller, J. Loffler, I. Rothenhofer, C. A. Muller, R. A. Bowden, J. van Brik, D. Engelhard, L. Kanz, and U. Schumacher.** 1997. Detection and identification of fungal pathogens in blood by using molecular probes. J. Clin. Microbiol. **35:**1353–1360.
5. **Elder, B. L., S. A. Hansen, J. A. Kellogg, F. J. Marsik, and R. J. Zanbury.** 1997. Cumitech 31, Verification and validation of procedures in the clinical microbiology laboratory. Coordinating ed., B. W. McCurdy. American Society for Microbiology, Washington, D.C.
6. **Hertz, D., D. Fuller, T. Davis, L. Papile, D. Stevenson, and J. Lemmon.** 1999. Comparison of DNA probe technology and automated continuous-monitoring blood culture systems in the detection of neonatal bacteremia. J. Perinatol. **19:**290–293.
7. **Jansen, G. J., M. Mooibroek, J. Idema, H. J. M. Harmsen, G. W. Welling, and J. E. Degener.** 2000. Rapid identification of bacteria in blood cultures by using fluorescently labeled oligonucleotide probes. J. Clin. Microbiol. **38:** 814–817.
8. **Mylotte, J. M., and A. Tayara.** 2000. Blood cultures: clinical aspects and controversies. Eur. J. Clin. Microbiol. Infect. Dis. **19:**157–163.
9. **Nelson, N. C., M. A. Reynolds, and L. J. Arnold, Jr.** 1995. Detection of acridinium esters by chemiluminescence, p. 392–429. *In* L. J. Kricka (ed.), Nonisotropic probing, blotting and sequencing. Academic Press, New York, N.Y.
10. **Oliveira, K., G. W. Procop, D. Wilson, J. Coull, and H. Stender.** 2002. Rapid identification of *Staphylococcus aureus* directly from blood cultures by fluorescence in situ hybridization with peptide nucleic acid probes. J. Clin. Microbiol. **40:**247–251.
11. **Sen, Y., and D. M. Asher.** 2001. Multiplex PCR for detection of Enterobacteriaceae in blood. Transfusion **41:**1356–1364.
12. **Snyder, J. W., K. S. Benzing, G. K. Munier, G. D. Bostic, P. S. Bozigar, R. Hanna, and M. J. Zervos.** 2000. Clinical comparison of nonvented aerobic BacT/Alert blood culture bottle and standard aerobic bottle for detection of microorganisms in blood. J. Clin. Microbiol. **38:**3864–3866.
13. **Tan, T. Y., S. Corden, R. Barnes, and B. Cookson.** 2001. Rapid identification of methicillin-resistant *Staphylococcus aureus* from positive blood cultures by real-time fluorescence PCR. J. Clin. Microbiol. **39:**4529–4531.
14. **Volkhard, A., J. Kempf, K. Trebesius, and I. B. Autenrienth.** 2000. Fluorescent in situ hybridization allows rapid identification of microorganisms in blood cultures. J. Clin. Microbiol. **38:**830–838.