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Detection Kinetics for Positive Blood Culture Bottles by Using the VITAL Automated System

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The VITAL system principle is based on homogeneous fluorescence technology. During an 11-month period, a total of 19,706 blood cultures from adult patients hospitalized in various establishments of the Montpellier Teaching Hospital were collected in VITAL bottles, of which 1,939 were declared positive. Only 204 bottles (1.04%) were false positives. The 1,735 true-positive bottles were collected from 130 patients. The final visual control permitted the detection of 10 falsely negative bottles (0.05%), of which 5 contained clinically significant microorganisms from four patients. The kinetics of detection for all microorganisms showed that 66.6% were detected within 24 h, 83.1% within 48 h, 95.5% within 120 h, and 100% within 150 h. No clinical episode would have been missed had a 5-day protocol been used instead of a 7-day protocol. Among the positive bottles, 65.7% were detected by the SLOPE algorithm, 20.1% by the DELTA algorithm, and 14.2% by the THRESHOLD algorithm. This retrospective study of our results shows that a 5-day protocol is sufficient for the detection of septic episodes using the VITAL system.

A retrospective study of the detection of positive blood cultures using a new automated system was conducted over a period of 11 months to determine the optimal duration of the analysis protocol (5, 6, or 7 days) for this instrument. An initial comparative study carried out over 4 months with the VITAL system (bioMérieux, Marcy-l'Etoile, France) and the Septi-Chek method (Becton Dickinson, Meylan, France) enabled the sensitivity and specificity of this system, as well as its rapidity of detection, to be validated (16, 19). During the following 7 months, the blood cultures were analyzed by the VITAL system only.

The VITAL system is a new analytical system which has been marketed since June 1993 (3). It detects positive blood cultures on the basis of variations in the pH, modifications of the oxidoreductant potential, and production of carbon dioxide, as a result of microorganism growth. The system uses an original technology, homogeneous fluorescence technology. Both VITAL AER (aerobic) and VITAL ANA (anaerobic) bottles contain a fluorescent probe in solution in the broth. The fluorescence is measured by a noninvasive, kinetic method.

MATERIALS AND METHODS

The retrospective study of the detection kinetics of positive blood cultures using the VITAL system was conducted over a period of 11 months (June 1993 to April 1994). This study was performed with 19,706 bottles containing 9,853 blood cultures from adult patients hospitalized in various establishments of the Montpellier Teaching Hospital in France (St. Charles, St. Eloi, Gui de Chauliac, Lapeyronie, and Arnaud de Villeneuve hospitals). The samples were collected at patients' bedsides by nursing staff (19). First the VITAL AER and then the VITAL ANA bottles were inoculated with 5 to 10 ml of blood (1, 3, 12).

These bottles were placed in the analysis module at the laboratory either as soon as they were inoculated or after preincubation in temporary storage incubators.

VITAL system. The apparatus consists of a modular analyzer capable of handling from 200 to 1,200 bottles simultaneously.

The probe is a fluorescent molecule, emitting light in the visible spectrum. This fluorescence disappears as a result of the metabolism of the bacteria (production of CO_2, acidification, or reduction of the medium). Measurement of the fluorescence level for each bottle is automatically performed every 15 min, 24 h a day throughout the protocol period (7 days for this study).

The measurement of the decrease in fluorescence is analyzed according to a three-branch algorithm (Fig. 1). The SLOPE algorithm calculates the slope of the fluorescence curve. Positivity is signalled when this slope is steeper than the system reference slope. This algorithm enables the detection of bottles which become positive while in the analyzer. The DELTA algorithm measures the instantaneous speed of the decrease in fluorescence at three reading points. It enables the detection of bottles in which the microorganisms are in the exponential phase of growth. The THRESHOLD algorithm counts as positive any bottle with a lower level of fluorescence than the system reference threshold. Consequently, it enables the detection of bottles which were already positive when they arrived at the laboratory.

VITAL AER and VITAL ANA blood culture media. Both VITAL AER and VITAL ANA media are based on an enriched broth containing growth factors such as NAD and vitamin B_6. They both contain a sedimentation agent, the function of which is to separate the blood cells from the broth. Furthermore, glucose and cysteine hydrochloride are added to the aerobic and anaerobic broths, respectively. Both media also include 0.025% sodium polyanethol sulfonate as an anticoagulant agent (21). VITAL AER bottles do not need venting after inoculation as they are ready-vented.

Analysis of the bottles. Visual control of the negative bottles was performed before their elimination at the end of the 7-day protocol. This visual control consisted of checking that there was no hemolysis, turbidity, or color change in the broth. It was made easier owing to the presence of the sedimentation agent which keeps the broth clear. Systematic subculturing of the negative bottles proved to be unnecessary, considering the low frequency of false negatives and the fact that the bottles were detected by this simple visual control (3, 16).

The positive bottles were subcultured after the sterile sampling of 2 ml of broth, which was examined in its fresh state and after Gram staining. The microorganisms were isolated and identified by conventional techniques.

RESULTS

A total of 19,706 bottles representing 9,853 aerobic and anaerobic blood culture pairs were analyzed. Among these bottles, 1,939 bottles were declared positive by the system (990 aerobic bottles and 949 anaerobic bottles), i.e., 9.84% of the total number of bottles. Of these 1,939 positive bottles, 1,735 grew microorganisms and 204 (1.0%) proved to be false positives (negative direct examination and subculture). Among the 17,767 negative bottles, 10 bottles contained microorganisms. These bottles were detected owing to the visual control performed when the negative bottles were eliminated at the end of the protocol.

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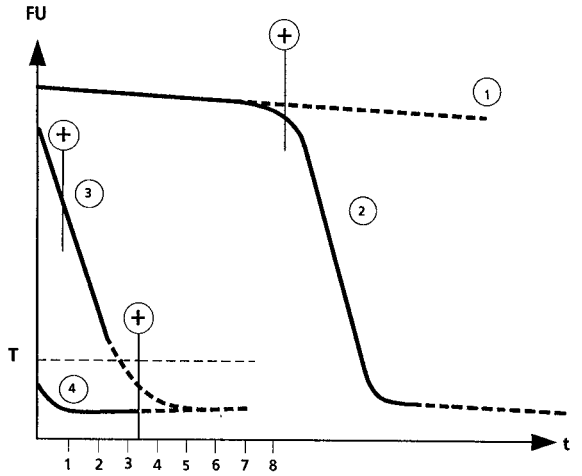

FIG. 1. VITAL detection of positivity by a three-branch algorithm. Samples: 1, positive; 2 to 4, positive by the SLOPE, DELTA, or THRESHOLD algorithm, respectively. FU, fluorescence units; t, time in hours; T, threshold.

The 204 bottles in which no microorganism was detected, either upon direct examination or after subculture, were considered to be false positives (12). These bottles represent 1.04% of the total number of bottles analyzed. Consequently, 1,735 bottles from 638 patients were true positives and permitted the isolation of 1,808 microorganisms. It should be noted that 66 bottles (3.8% of the true positives) were polymicrobial; of these, 59 bottles contained two microorganisms and 7 bottles contained three microorganisms (Table 1).

TABLE 1. Microorganisms isolated from positive VITAL bottles[a]

Microorganism(s)	*n* [1,808] (%)	No. of patients [638]
Staphylococcus aureus	269 (14.9)	60
Coagulase-negative *Staphylococcus* sp.	655 (36.2)	331
Beta-hemolytic *Streptococcus* sp.	22 (1.2)	7
Deficient *Streptococcus* sp.	16 (0.9)	1
Streptococcus pneumoniae	57 (3.15)	15
Other streptococci	64 (3.55)	16
Enterococcus spp.	44 (2.4)	16
Listeria monocytogenes	6 (0.35)	1
Bacillus spp.	10 (0.55)	5
Corynebacterium spp.	11 (0.6)	7
Micrococcus sp.	11 (0.6)	8
Pedicoccus sp.	1 (0.05)	1
Escherichia coli	219 (12.1)	74
Salmonella spp.	17 (0.95)	5
Other enterobacteria	206 (11.4)	57
Pseudomonas aeruginosa	83 (4.6)	17
Pseudomonas spp.	11 (0.6)	3
Acinetobacter spp.	9 (0.5)	5
Neisseria meningitidis	8 (0.45)	2
Cardiobacterium hominis	11 (0.6)	1
Haemophilus parainfluenzae	8 (0.45)	1
Flavobacterium indologenes	1 (0.05)	1
Campylobacter jejuni	1 (0.05)	1
Candida albicans	12 (0.7)	4
Candida spp.	12 (0.7)	5
Clostridium spp.	6 (0.35)	5
Lactobacillus spp.	24 (1.3)	3
Bacteroides fragilis group sp.	4 (0.2)	2
Propionibacterium spp.	10 (0.55)	10

[a] 3.8% of blood cultures were polymicrobial. Totals are given in brackets.

The final visual control permitted the detection of 10 falsely negative bottles (0.05%). These bottles contained five contaminants and five clinically significant microorganisms from four patients (two *Escherichia coli* strains, one *Staphylococcus aureus*, one *Lactobacillus acidophilus*, and one *Candida albicans*), which furthermore were detected earlier in several other VITAL bottles from these four patients.

The growth rates of the microorganisms were compared according to the type of medium in which they developed, by using the chi-square test. Only the strict anaerobic bacteria grew significantly better in the VITAL ANA bottles ($P = 0.047$): 28 grew in the VITAL ANA bottles, and 16 grew in the VITAL AER bottles.

For the other microorganisms isolated, there was no significant difference in growth between the different types of medium.

Study of the detection kinetics. The kinetics of detection of gram-positive and gram-negative microorganisms showed that 66.6% were detected during the first 24 h, 83.1% during the first 48 h, 95.5% within 120 h (5 days), and 100% within 150 h (Fig. 2).

The kinetics of detection of gram-positive microorganisms showed that 60.5% were detected during the first 24 h, 80.6% during the first 48 h, 95.5% within 120 h (5 days), and 100% within 150 h. The study of these results according to the type of bottle (aerobic or anaerobic) showed that 96.0% of the VITAL AER bottles and 93.4% of the VITAL ANA bottles were positive within 120 h, i.e., 5 days (Table 2).

The kinetics of detection of gram-negative microorganisms showed that 79.4% were detected during the first 24 h, 88.3% during the first 48 h, 95.5% within 120 h (5 days), and 100% within 150 h. The study of these results according to the type of bottle (aerobic or anaerobic) showed that 96.6% of the aerobic broths and 97.6% of the anaerobic broths were positive within 120 h, i.e., 5 days (Table 2).

Analysis of the bottles which became positive after the 5th day of the protocol. A total of 78 bottles were declared positive by the system after the 5th day of incubation. For 53 of these bottles, bacteria considered to be skin contaminants (coagulase-negative *Staphylococcus* spp., *Propionibacterium* spp., *Corynebacterium* spp., and *Bacillus* spp.) (2, 20) were isolated from one or both bottles corresponding to the same collection. The other 25 bottles declared positive after the 5th day of the protocol were from 15 patients, each with a true septic episode. For these 15 clinical cases, several positive bottles (ranging from 2 to 24) had already permitted detection of the microorganism responsible for the morbidity. The bottle was positive during the 7th day of incubation for only five of these patients (Table 3).

Detection algorithms. Among the positive bottles, 65.7% were detected by the SLOPE algorithm, 20.1% by the DELTA algorithm, and 14.2% by the THRESHOLD algorithm. This underlines the importance of the latter algorithm for the detection of samples which become positive while in the temporary storage incubators.

DISCUSSION

The sensitivity, specificity, and rapidity of detection of the VITAL system were validated in a preliminary evaluation. In the present study, the system permitted the isolation of 1,808 microorganisms over a period of 11 months. The percentages of the different species detected are comparable to those found in international or French prevalence studies (5, 17, 20).

Owing to the diversity of the species discovered, it can be concluded that the broths are well adapted to the growth of the

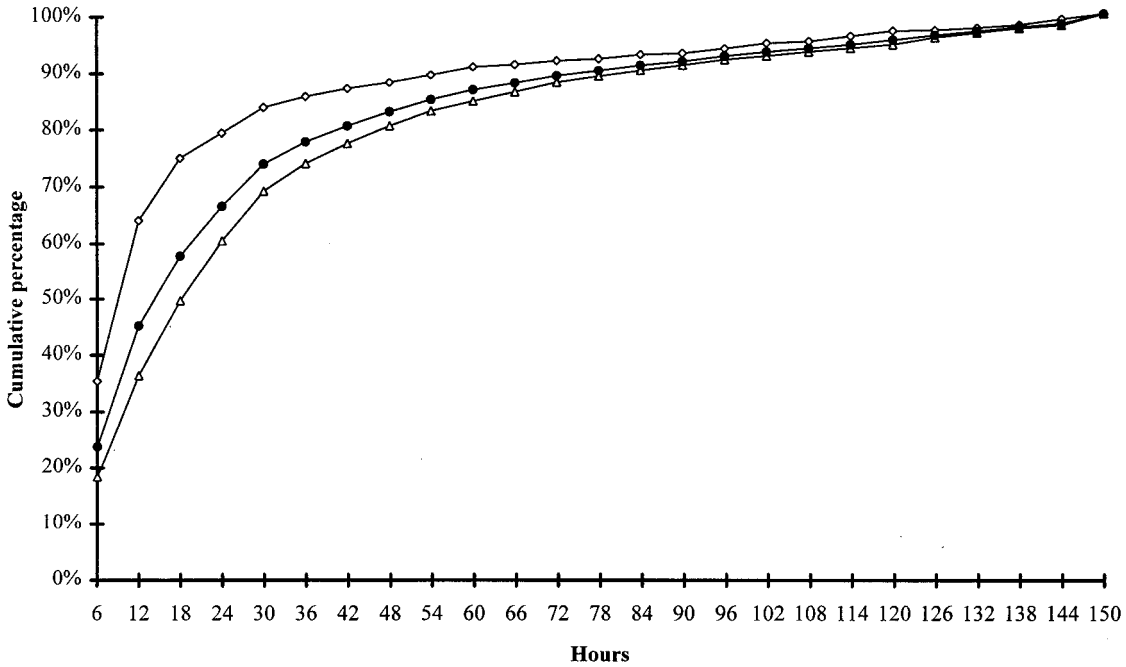

FIG. 2. Cumulative percentage of samples analyzed by VITAL as a function of time. △, gram-positive microorganisms; ◇, gram-negative microorganisms; ●, total microorganisms.

microorganisms encountered in human pathology. This is illustrated by the fact that fastidious bacteria or bacteria requiring growth factors (deficient *Streptococcus* bacteria, *Haemophilus parainfluenzae*, *Neisseria meningitidis*, *Cardiobacterium hominis*, and *Campylobacter jejuni*) were isolated, as well as the more common microorganisms.

The percentage of anaerobic bacteria (0.22% of the total number of bottles) can be considered significant insofar as, for several years now, prevalence studies have shown a gradual decrease in the number of positive blood cultures involving anaerobic bacteria (5, 8, 11, 13, 18, 23).

In a retrospective study (1974 to 1988) conducted by the Mayo Clinic, the anaerobic bacteria represented 0.77% of the total number of blood cultures for the period 1974 to 1978 and 0.41% for the period 1984 to 1988 (8). These percentages correspond to 118,019 and 164,222 blood cultures, respectively. A minimum of 100,000 blood cultures would therefore be required to validate the ability of the VITAL system to detect the various species of anaerobic bacteria encountered in human pathology.

Among the positive bottles, 1.4% were positive for *Candida* spp. The percentages given in literature vary between <1 and 5% (9, 14). These variations are due to several factors (for example, the different wards included in the study).

Yeasts tend to grow better and more rapidly in the VITAL AER media (54 h compared with 77 h for the anaerobic broth on average), thus validating the reasons for including a glucose supplement and confirming the advantage of using ready-vented bottles.

TABLE 2. Detection of gram-positive and gram-negative microorganisms from VITAL AER and VITAL ANA bottles

Time to positivity [h (days)]	No. of bottles (%)			
	Gram-positive microorganisms		Gram-negative microorganisms	
	AER	ANA	AER	ANA
6	118 (19.0)	108 (17.8)	107 (36.9)	98 (33.9)
12	235 (37.8)	212 (34.9)	185 (63.8)	186 (64.4)
24 (1)	382 (61.4)	362 (59.6)	229 (79.0)	231 (79.9)
48 (2)	511 (82.2)	480 (79.1)	251 (86.6)	260 (90.0)
72 (3)	561 (90.2)	523 (86.2)	264 (91.0)	269 (93.1)
96 (4)	582 (93.6)	551 (90.8)	270 (93.1)	275 (95.2)
120 (5)	597 (96.0)	567 (93.4)	280 (96.6)	282 (97.6)
144 (6)	614 (98.7)	590 (97.2)	286 (98.6)	288 (99.7)
150[a]	622 (100)	607 (100)	290 (100)	289 (100)

[a] A 7-day protocol has a duration of 168 h.

TABLE 3. Analysis of the bottles which became positive after the 5th day of the protocol[a]

Microorganism	No. of bottles positive	
	Only after the 5th day[b]	Before the 5th day
Staphylococcus aureus	1	2
Staphylococcus aureus	1 (1)	4
Klebsiella oxytoca	1	5
Candida albicans	2 (1)	5
Candida albicans	1	2
Proteus mirabilis	1	5
Pseudomonas aeruginosa	1	5
Staphylococcus aureus	1	8
Staphylococcus schleiferi	1	8
Coagulase-negative *Staphylococcus* sp.	1	8
Klebsiella pneumoniae	1	10
Staphylococcus aureus	1	24
Staphylococcus lugdunensis	1 (1)	7
Cardiobacterium hominis	4 (1)	7
Lactobacillus acidophilus	7 (1)	15

[a] Blood cultures were obtained from 15 patients with a septic episode.
[b] The numbers of bottles positive only after 144 h (6 days) are given in parentheses.

The percentage of false positives (1.04% of the total number of bottles) is totally acceptable. These false positives may be encountered in four main situations.

(i) A false-positive bottle detected early in a series of negative bottles. This may be due to an artifact or a suicidal microorganism (early autolysis of the microorganism after its metabolism has been detected by the system, as can be seen from the fluorescence curve). This phenomenon, which is not revealed by the manual methods, is detected as a result of the sensitivity of the new-generation automated systems.

(ii) A false-positive bottle detected in a series of true positives. This is likely due to autolysis of a microorganism which was actually present (4).

(iii) A false-positive bottle detected at the end of the protocol in a series of negative bottles. This may be caused by an alteration of the physicochemical characteristics of the broth.

(iv) Detection of a series of false-positive bottles from the same patient. No obvious reasons could be determined from the study of these files except for a possible acidometabolic disorder in this patient.

At the end of the preliminary evaluation which showed that systematic final subculturing is unnecessary (16), it was judged that rapid visual control of the bottles before their elimination is sufficient, since the 10 false-negative bottles (5 contaminants and 5 clinically significant microorganisms), i.e., 0.05%, were detected in this way. This fact is in keeping with the findings of the evaluations conducted with other automated systems (6, 7, 10, 15, 22, 23, 24).

Since this evaluation was performed, a more sensitive threshold algorithm has been produced. The new threshold has increased from 500 to 730 fluorescence units. Had this improved algorithm been used in the evaluation, most of these false-negative results would have been judged positive within 2 to 3 h by the VITAL system, as 6 of the 10 false-negative bottles were positive before being loaded into the instrument.

During the 11-month evaluation period, we found the system to be technically reliable and easy to use.

The principle of decreasing fluorescence of the probe guarantees a reduced risk of false negatives, without increasing the rate of false positives (1.04% only).

The ratio of the different algorithms in the detection of positive bottles permits the time taken for the bottles to be transported from the patient's bedside to the analyzer to be controlled. According to hospital practice, the bottles may be preincubated before delivery to the laboratory. The percentage of positives detected by the threshold algorithm will therefore be as high as the delay between sample collection and introduction of the bottle into the analyzer is long. The time which elapses between the moment when the bottle becomes positive in the temporary storage incubator and the moment when the bottle is actually counted as positive by the instrument can be only detrimental to the patient. It is therefore of the utmost importance that the samples are transmitted to the laboratory as rapidly as possible, even if the laboratory is equipped with an instrument which correctly detects bottles which were positive before being introduced for analysis.

In conclusion, this retrospective analysis shows that 83.1% of positive blood cultures were detected within 48 h and 95.5% were detected within 5 days. The bottles judged positive after the 5th day contained either clinically significant microorganisms detected early in other bottles from the same patient or contaminants. Had the duration of the protocol been shortened from 7 to 5 days, the number of infectious episodes detected would not be reduced but the number of unnecessary subcultures due to non-clinically significant microorganisms that were detected on days 6 and 7 would (3).

If an episode involving a fastidious or slowly growing microorganism is suspected, or as soon as such a microorganism has been isolated from other bottles, it is recommended to extend the duration of the analysis to 10 days for the remaining bottles from the same patient. The VITAL software permits this modification. Our experience has shown that fastidious or slowly growing microorganisms (*C. hominis*, *L. acidophilus*, *C. jejuni*, or yeasts) reveal their positivity after a mean detection time of 86 h (21 to 162 h). The retrospective study of our results shows that a 5-day protocol by default is sufficient for the detection of septic episodes using the VITAL system.

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