

REVISED ABSTRACT

**Objectives:** The performance of current continuously monitoring blood systems for the isolation of fastidious microorganisms that infrequently cause bloodstream infections is poorly documented. Therefore, we did *in-vitro* simulations in blood culture bottles seeded with fastidious and other microorganisms.

**Methods:** Aerobic and anaerobic bottles with and without fresh human blood were inoculated and monitored in both the VersaTREK and BacT/ALERT continuously monitoring blood culture systems. A total of 37 bacterial strains (16 species) were tested. Three isolates each of fastidious bacteria previously isolated from patients were used. These included *Neisseria catarrhalis*, *N. gonorrhoeae* (1 isolate only), and *N. meningitidis*; the HACEK group; *Bordetella bronchiseptica*; and *Campylobacter jejuni*. Additionally, two isolates each of group B beta-hemolytic streptococci, *Enterococcus faecalis*, and *E. faecium* were tested as were ATCC® control strains of *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and *Bacteroides fragilis*.

**Results:** The overall recovery of the VersaTREK system for aerobic (A) and anaerobic (N) bottles with (+) and without (-) blood was 35 (A+), 34 (A-), 20 (N+), and 17 (N-), respectively; the corresponding recovery for BacT/ALERT was 26 (A+), 18 (A-), 31 (N+), and 27 (N-). The overall recovery of each two-bottle (aerobic and anaerobic) system was 37/37 (+) and 35/37 (-) for VersaTREK and 36/37 (+) and 32/37 (-) for BacT/ALERT. Blood was required for the isolation of 2 of 3 strains of *Eikenella* in both systems in aerobic bottles. With blood supplementation all strains were detected by the VersaTREK system, but one strain of *N. meningitidis* was missed by the BacT/ALERT system.

**Conclusion:** We conclude that both aerobic and anaerobic media are required in both the VersaTREK and BacT/ALERT blood culture systems to achieve optimal recovery of the spectrum of fastidious microorganisms studied.

BACKGROUND

VersaTREK® (TREK Diagnostic Systems, Cleveland, Ohio, USA) and BacT/ALERT® (bioMérieux, Durham, North Carolina, USA) are common blood culture systems used in the United States. The VersaTREK system (VTI) provides increased volume of broth and a pressure sensor to detect gas production and consumption, whereas the BacT/ALERT® (3D) uses a colorimetric sensor for CO<sub>2</sub> detection. A clinical evaluation in adults has been presented that showed the two systems with the media used in this study to be comparable (ECCMID 2005, P 1703). However, there were very few fastidious microorganisms detected during that trial. Therefore, we compared the two systems further by seeding isolates of fastidious microorganisms into each bottle type.

MATERIALS AND METHODS

1. A total of 37 bacterial strains (16 species) were tested. Three isolates each of fastidious bacteria previously isolated from patients were used. These included *Neisseria catarrhalis*, *N. gonorrhoeae* (1 isolate only), *N. meningitidis*; *Haemophilus aphrophilus*, *Actinobacillus actinomycetemcomitans*, *Cardiobacterium spp.*, *Eikenella spp.*, *Kingella spp.*, *Bordetella bronchiseptica*, and *Campylobacter jejuni*. Additionally, two isolates each of group B beta-hemolytic streptococci, *Enterococcus faecalis*, and *E. faecium* were tested as were ATCC® control strains of *Streptococcus pneumoniae* (27853), *Pseudomonas aeruginosa* (6303), and *Bacteroides fragilis* (25285).
2. Each isolate was subcultured for 24-72 hours depending on the time needed to obtain adequate growth to prepare a suspension comparable to a 0.8 McFarland standard. This standard was diluted to achieve an inoculum of 2-172 CFU per bottle.
3. One each of TREK REDOX 1® and REDOX 2®, and BacT/ALERT® standard aerobic and anaerobic bottles were inoculated with the standardized inoculum.
4. A second set of identical bottles was prepared with 1-2 mL of outdated human blood then inoculated as in #3 above.
5. All bottles were incubated in their respective instruments until flagged as positive or for a total of 5 days if negative.

Table 1. Mean Time to Detection (hours)

Microorganism (n)	TREK O2 No Blood	BacT/ALERT O2 No Blood	TREK AnO2 No Blood	BacT/ALERT AnO2 No Blood	TREK O2 Blood	BacT/ALERT O2 Blood	TREK AnO2 Blood	BacT/ALERT AnO2 Blood
Group B β-streptococci (2)	11.2	12.2	14.5	11.0	10.6	10.8	14.0	10.6
<i>E. faecalis</i> (2)	10.9	38.9	12.9	11.1	10.0	11.5	12.9	10.8
<i>E. faecium</i> (2)	14.8	16.5	16.2	14.9	14.3	15.2	15.5	14.1
<i>M. catarrhalis</i> (3)	16.4	19.5	0.0	40.0	15.8	18.2	0.0	16.9
<i>N. gonorrhoeae</i> (1)	38.4	0.0	0.0	0.0	33.5	0.0	0.0	33.0
<i>N. meningitidis</i> (3)	20.6	42.0	0.0	20.3	18.0	23.5	0.0	18.6
<i>H. aphrophilus</i> (3)	24.2	18.0	41.6	20.4	24.7	32.2	20.7	19.9
<i>A. actinomycetemcomitans</i> (3)	43.3	62.4	63.8	39.1	29.4	48.2	53.0	32.4
<i>C. hominis</i> (3)	34.4	0.0	0.0	56.3	41.2	0.0	88.6	39.4
<i>Eikenella</i> sp. (3)	15.6	0.0	0.0	21.5	39.1	50.4	44.3	21.1
<i>Kingella</i> sp. (3)	23.8	0.0	0.0	27.5	23.7	60.8	0.0	18.6
<i>B. bronchiseptica</i> (3)	21.3	24.1	19.1	16.6	19.4	22.4	19.2	15.3
<i>C. jejuni</i> (3)	55.7	0.0	0.0	57.6	35.2	0.0	0.0	32.9
<b>Controls</b>								
<i>P. aeruginosa</i> -ATCC 27853 (1)	14.4	17.4	22.4	0.0	14.5	15.9	24.8	15.9
<i>B. fragilis</i> -ATCC 25285 (1)	0.0	0.0	26.8	0.0	0.0	0.0	28.0	55.2
<i>S. pneumoniae</i> -ATCC 6305 (1)	14.3	16.3	18.7	14.5	14.5	15.1	17.9	14.0

O2 = Aerobic; AnO2 = Anaerobic

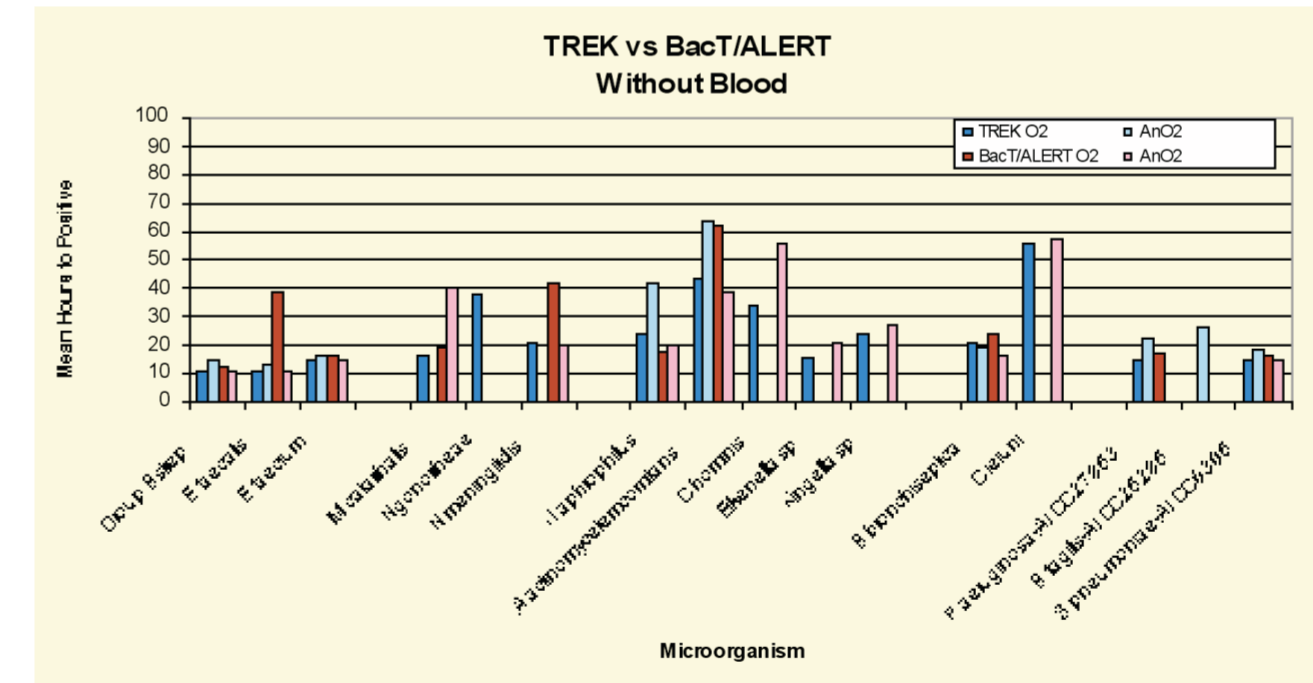
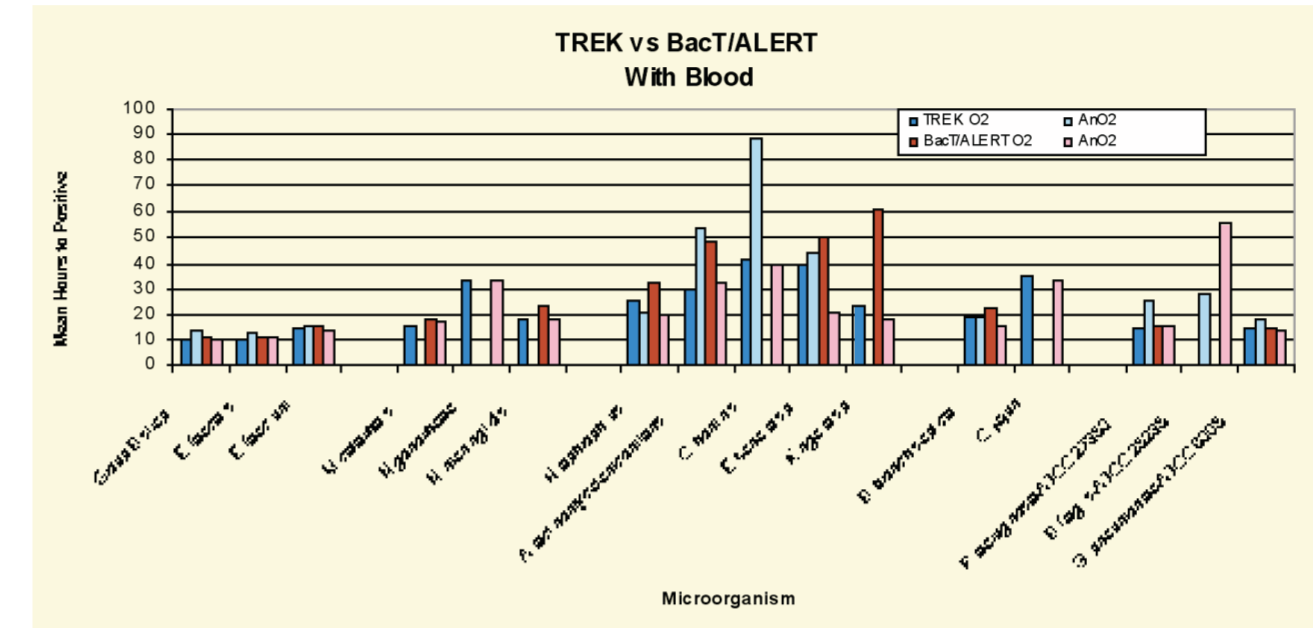


Table 2. Microorganisms Missed by Each Bottle Type

Microorganism	TREK O2 No Blood	BacT/ALERT O2 No Blood	TREK AnO2 No Blood	BacT/ALERT AnO2 No Blood	TREK O2 Blood	BacT/ALERT O2 Blood	TREK AnO2 Blood	BacT/ALERT AnO2 Blood
Group B β-streptococci	0	0	0	0	0	0	0	0
<i>E. faecalis</i>	0	0	0	0	0	0	0	0
<i>E. faecium</i>	0	0	0	0	0	0	0	0
<i>M. catarrhalis</i>	0	0	3	0	0	0	3	1
<i>N. gonorrhoeae</i>	0	1	1	1	0	1	1	0
<i>N. meningitidis</i>	0	2	3	2	0	1	3	1
<i>H. aphrophilus</i>	0	1	0	0	0	0	0	0
<i>A. actinomycetemcomitans</i>	0	2	0	0	1	1	0	0
<i>C. hominis</i>	0	3	3	1	0	3	0	0
<i>Eikenella</i> sp.	2	3	3	0	0	1	2	0
<i>Kingella</i> sp.	0	3	3	1	0	0	3	2
<i>B. bronchiseptica</i>	0	0	1	2	0	0	2	2
<i>C. jejuni</i>	0	3	3	1	0	3	3	0
<i>P. aeruginosa</i> -ATCC 27853	0	0	0	1	0	0	0	0
<i>B. fragilis</i> -ATCC 25285	1	1	0	1	1	1	0	0
<i>S. pneumoniae</i> -ATCC 6305	0	0	0	0	0	0	0	0
<b>Total</b>	<b>3</b>	<b>19</b>	<b>20</b>	<b>10</b>	<b>2</b>	<b>11</b>	<b>17</b>	<b>6</b>

O2 = Aerobic; AnO2 = Anaerobic

RESULTS

- Table 1 and the charts show the mean time to positive with and without blood.
- Table 2 shows the number of microorganisms missed by each bottle type. The TREK REDOX 1 bottle was the most productive single bottle with or without blood.
- The overall recovery of the VersaTREK system for aerobic (A) and anaerobic (N) bottles with (+) and without (-) blood was 35 (A+), 34 (A-), 20 (N+), and 17 (N-), respectively.
- The corresponding recovery for BacT/ALERT was 26 (A+), 18 (A-), 31 (N+), and 27 (N-).
- The overall recovery of each two-bottle (aerobic and anaerobic) system was 37/37 with and 35/37 without blood for VersaTREK and 36/37 with and 32/37 without blood for BacT/ALERT.
- Blood was required for the isolation of 2 of 3 strains of *Eikenella* in aerobic bottles in both systems.
- With blood supplementation all strains were detected by the VersaTREK system, but one strain of *N. meningitidis* was missed by the BacT/ALERT system.

CONCLUSIONS

We conclude that both aerobic and anaerobic media are required in both the VersaTREK and BacT/ALERT blood culture systems to achieve optimal recovery of the spectrum of fastidious microorganisms studied.