

## Comparison of Bactec NR-660 and Signal systems

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**SUMMARY** Bactec NR-660, a computerised blood culture system using infrared analysis of microbial generated carbon dioxide, was compared with the Signal system, which detects gaseous pressure (due to bacterial metabolism) by a manometer. Four trials were undertaken: an *in vitro* evaluation of 99 bacteria in simulated blood cultures, and three prospective comparisons of a total of 2588 paired patient samples.

Combined results for bacteria in simulated blood cultures showed a highly significant difference ( $p < 0.001$ ) between Bactec NR-660 aerobic medium (6A) and any other phial under test. Detection rates for most bacteria by Signal were on average three times slower than the first Bactec phial (mean delay 58.3 hours).

Overall, the systems were not comparable.

Bactec NR-660 (Johnston Laboratories, Baltimore, Maryland) an automated blood culture system that detects microbial generated carbon dioxide in separate aerobic and anaerobic phials by infrared spectroscopy, was compared with Signal (Oxoid Ltd, Basingstoke, Hampshire), a single phial system that detects gaseous pressure by simple manometry. Due to continual protocol changes recommended by Oxoid Ltd, Signal was compared with Bactec as follows:

- (i) An *in vitro* evaluation of 99 bacteria in simulated blood cultures (trial 1).
- (ii) A prospective comparison of 2000 paired patient samples (trial 2).
- (iii) A prospective comparison of 433 paired patient samples (trial 3).
- (iv) A prospective comparison of 155 paired patient samples (trial 4).

### Material and methods

During trials 1 and 2, Signal media were provided in total head space gas evacuated bottles, and once inoculated, were shaken twice a day for 72 hours and once a day thereafter to day 7.

In trial 3 Signal media were provided in partially evacuated bottles, and once inoculated, were shaken continuously for 24 hours and then once a day thereafter. The manometer had also been increased in volume from 10 ml to 30 ml.

Trial 4 used Signal media that had been modified to reduce the number of false positive cultures.

Throughout the course of the four investigations, the formulation of both Bactec NR-6A (aerobic) and Bactec NR-7A (anaerobic) media remained constant.

Simulated blood cultures were prepared using isolates from septicaemic episodes and were inoculated at 10 colony forming units (cfu)/ml or less to each phial and supplemented with 10% v/v of sterile defibrinated horse blood. Manometers were fitted to Signal phials immediately after inoculation. All phials were incubated for a minimum of eight hours and then examined at regular intervals to determine the mean time to detection of positive cultures. All positive findings were confirmed by microscopy and culture.

Patient samples were tested according to laboratory testing schedules,<sup>1</sup> manometers being fitted to Signal phials on arrival at the laboratory.

### Results

#### TRIAL 1 (SIMULATED BLOOD CULTURES)

Mean time to detection (in hours) for major bacterial groups is summarised in table 1. Isolation times for most of the 49 Gram positive bacteria were much improved in Bactec NR-6A compared with Bactec NR-7A or Signal, and showed a highly significant difference ( $p < 0.001$ ) using a paired *t* test.<sup>2</sup> The delay in mean detection time from Signal was more than 30 hours for staphylococci, more than 22 hours for streptococci, and 119 hours for yeasts when compared with results by Bactec NR-6A. This may have been due to the poor aeration of both Bactec NR-7A and Signal phials as there was little or no agitation.

Similar disparity was shown for 50 Gram negative bacteria when Bactec NR-6A was compared with Signal ( $p < 0.001$ ). Mean detection time in Signal was delayed by 61 hours for fastidious aerobes, 55.5 hours for pseudomonads, and 120.5 hours for miscellaneous

Table 1 Mean time to detection for major bacterial groups

Bacteria	No of strains	Mean time (hours)		
		NR-6A	NR-7A	Signal
Staphylococci	26	16.1	36.3	47.4
Enterobacteriaceae	25	14.2	17.6	19.5
Streptococci	15	22.6	40.4	45.4
Fastidious aerobes	12	25.1	38.4	86.1
Anaerobes	8	20.8*	65.6	71.7
Pseudomonads	6	57.2	106	112.7
Miscellaneous Gram negative bacilli	4	38.5	No growth	159
Yeasts	3	40.0	135.5	159
Total	99	29.3	62.8	87.6

\*Aerotolerant species isolated

Gram negative bacilli. Again this was probably due to poor aeration, resulting in a reduced Eh value maintained when phials remained static. No significant difference ( $p > 0.3$ ) was shown between Bactec NR-7A and Signal.

For all bacteria studied, a highly significant difference was shown between Bactec NR-6A and all other phials tested ( $p < 0.001$ ), but no significant difference between Bactec NR-7A and Signal ( $p > 0.5$ ) was established. On no occasion did terminal subcultures yield bacteria which had failed to initiate a positive response.

Total numbers of positive blood culture sets and isolates detected by each system are shown in table 2.

#### TRIAL 2 (2000 PAIRED PATIENT SAMPLES)

Analysis of the total number of bacteria isolated in vivo by both systems showed that Signal failed to isolate 35 of 189 (18.5%) Gram positive, 36 of 100 (36%) Gram negative, but only one of 12 (8.3%) anaerobic bacteria. Bactec failed to isolate five of 189 (2.6%) Gram positive, nine of 100 (9%) Gram negative, and six of 12 (50%) anaerobic bacteria.

Signal detected the presence of micro-organisms before Bactec on the following occasions: 12 of 189 (6.3%) Gram positive, 12 of 100 (12%) Gram negative, and eight of 12 (66.6%) anaerobic bacteria. Bactec detected the presence of micro-organisms before Signal on the following occasions: 132 of 189 (69.8%) Gram positive, 56 of 100 (56%) Gram negative, and only one of 12 (8.3%) anaerobic bacteria. Equivalence was found in 23.8% of Gram positive, 32% of Gram negative, and 25% of anaerobic bacteria.

Speed of detection by specific laboratory schedule is shown in table 3. At 24 hours' incubation 74.1% of positive results were detected by Bactec NR-660 and 51.5% by Signal. Of the 45 polymicrobial isolations (two or more bacteria present), 95% of the bacteria were detected by Bactec NR-660 and 76% by Signal. Highly significant ( $p < 0.001$ ) differences (McNemar

analysis)' were found between the two systems for both absolute numbers of bacteria recovered and speed of isolation.

Overall contamination of the two systems (isolation of either coagulase negative staphylococci or coryneforms from only one of the phials under test) remained low, Bactec NR-660 yielding 1.7% and Signal 1.2%.

Numbers of false positive results highlighted in the Bactec NR-660 system by potential positive growth values and in the Signal system by a rise in the level of the blood-broth mixture in the manometer were also evaluated: Bactec NR-6A (1.7%), Bactec NR-7A (2.25%), and Signal (0.75%).

On three separate occasions Signal gave false negative culture results when manometer levels, microscopy, and Bactec phials were positive. The isolates missed were *Streptococcus pneumoniae*, *Enterobacter cloacae*, and a mixed growth of *Staphylococcus aureus* and *Streptococcus pneumoniae*.

#### TRIAL 3 (433 PAIRED PATIENT SAMPLES)

A range of bacteria similar to those found in trial 2 were isolated, but there was, however, a difference in the speed of detection within the first 24 hours. Bactec detected 75% of all bacteria and Signal 63.2% of all isolates. Presumably, this was due to the improved aeration which continuous shaking for 24 hours provides.

Contamination rates for both systems remained at less than 1.0%, but there was a significant increase in the number of false positive results highlighted by Signal (11.3%), Bactec NR-6A (1.1%), and Bactec NR-7A (2.1%). This would suggest that while aeration improves isolation rates it has a detrimental effect on false positive rates.

#### TRIAL 4 (155 PAIRED PATIENT SAMPLES)

Obviously with a small sample of 155 paired blood culture sets, the numbers of positive results and bacterial isolations obtained is of limited value, but a continuing trend of higher numbers of false positive

Table 2 Cumulative in vivo results

	Trial 2 No (%)	Trial 3 No (%)	Trial 4 No (%)
Total No of infected sets (not infective episodes)*	246	52	14
Bactec NR-660 sets positive	231 (93.9)	49 (94.2)	14 (100)
Signal sets positive	198 (80.5)	44 (84.6)	10 (71.4)
Total No of isolates	301	61	15
Total No of isolates detected by Bactec NR-660	281 (93.4)	56 (91.8)	15 (100)
Total No of isolates detected by Signal	229 (76.1)	49 (80.3)	11 (72.0)

\*This also excludes contaminants such as coagulase negative staphylococci or coryneforms isolated from only one bottle.

Table 3 Comparison of in vivo detection times for Bactec NR-660 and Signal systems

	No (%) of isolates detected by system					
	Trial 2		Trial 3		Trial 4	
	Bactec	Signal	Bactec	Signal	Bactec	Signal
Early (less than 16 hours)	21 (7.5)	4 (1.7)	1 (1.8)	1 (2.0)	1 (6.7)	6 (9.1)
AM 24 hours	144 (51.3)	69 (30.1)	33 (58.9)	25 (51.0)	7 (46.7)	6 (54.5)
PM 24 hours	43 (15.3)	45 (19.7)	8 (14.3)	5 (10.2)	0	1 (9.1)
48 hours	56 (19.9)	67 (29.3)	7 (12.5)	14 (28.6)	5 (33.3)	2 (18.2)
Day 3	10 (3.6)	18 (7.9)	1 (1.8)	0	0	1 (9.1)
Day 5	3 (1.1)	20 (8.7)	4 (7.1)	2 (4.1)	0	0
Day 7	4 (1.4)	6 (2.6)	2 (3.6)	2 (4.1)	2 (13.3)	0
	281 (93.4)	229 (76.1)	56 (91.8)	49 (80.3)	15 (100)	11 (72.0)

results with Signal (13.5%) compared with Bactec (3.9%) was found.

### Discussion

Bactec NR-660 is a technologically advanced detection system which requires considerable capital outlay with associated maintenance and running costs. Signal, a manual method, requires only visual inspection and no capital costs.

The in vivo trial was designed to determine the sensitivity of each system under controlled conditions. Although artificial, as bacteria were not subject to targeting from antibiotics or host defence mechanisms, the absolute time to detection can be established from the moment of inoculation.

Analysis of findings for major bacterial groups (table 1) in simulated blood cultures showed a highly significant difference ( $p < 0.001$ ) between mean detection times for the Bactec NR-6A phial and any other under test. Comparable detection times were shown only for the isolation of members of Enterobacteriaceae.

The Signal system is designed for 0.1 ml–10 ml volumes (unlike Bactec NR-660 phials which require the addition of 3 ml–5 ml) of drawn blood, which means that it can be used in children's hospitals. A disturbing feature, however, was the apparent delay in detection of the fastidious aerobic pathogens *Haemophilus* and *Neisseria*; Signal was 61 hours slower than Bactec NR-6A. This finding was further substantiated in trial 2 where Signal failed to isolate three strains of *Haemophilus influenzae* and two *Neisseria* sp. Therefore, children's hospitals should recommend that clinicians use blood volumes well in excess of 0.1 ml whenever possible.

Results from trial 2 showed that Signal failed to isolate 15.9% of Gram positive, 27.0% of Gram negative, but only 8.3% of anaerobic bacteria. Although there were only 12 anaerobic isolates, probably due to the use of metronidazole within the

hospital, Signal produced a positive culture before Bactec NR-660.

Results from trial 3, using partially evacuated blood culture phials, continuous shaking for 24 hours, and improved manometer volumes, did improve isolation rates within the first 24 hours to 63.2% for Signal. Staff time is wasted, however, because of the need to deal with the increased numbers of false positive results generated by Signal under these conditions.

The media modification used in trial 4 did not correct the problem of false positive cultures, but increased them slightly. Both systems isolated 53.3% of all bacteria within 24 hours. A recent report (Roberts and Kaczmarek)<sup>4</sup> also commented on the numbers of false positive results and isolation failures of the Signal system.

In conclusion, the in vitro study of 99 bacteria in simulated blood cultures showed a highly significant difference ( $p < 0.001$ ) in recovery rates for all bacteria between Bactec NR-6A and any other phial under test. The results of the prospective trial of 2000 paired patient samples also showed highly significant differences between the Bactec NR-660 and Signal for both absolute numbers of bacteria isolated and speed of detection. Improved aeration through shaking did improve isolation rates for Signal but increased the number of false positive cultures. Under these conditions Signal was not comparable with Bactec NR-660.

### References

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