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# The Advantage of Combining Resin Containing Medium with Saponin Supplemented Lytic Medium


Blood cultures represent one of the most important specimens submitted to the Microbiology Laboratory. The mortality due to sepsis remains high despite appropriate antimicrobial therapy. Therefore, it is important, that laboratories maintain rapid and reliable methods for detection of microorganisms in blood.[1] Detection and identification of anaerobic bacteria in blood cultures is a well-recognized challenge in clinical microbiology. This may be explained by the fact that these microorganisms are typically fastidious, slow growing and difficult to culture.[2] Furthermore, the anaerobic bottle may continue to provide clinically relevant information because other studies have demonstrated that 4.2 to 9.4% of significant isolates from blood were anaerobes.[1] However, the developments of BD BACTEC™ Automated Blood Culture System and anaerobic blood culture bottles have improved the detection of these microorganisms.[2]

**BACTEC™** Lytic/10 Anaerobic/F culture bottles (pre-reduced enriched Soybean-Casein Digest broth with $CO_2$) are for anaerobic blood cultures. The principle used is with the **BACTEC** fluorescent series instruments for the qualitative culture and recovery of anaerobic microorganisms from blood. The Lytic anaerobic/F bottle contains saponin as a lysing agent to lyse blood cells and release intracellular microorganisms to facilitate early pathogen detection. Hollick G.E. et al. demonstrated that the addition of saponin to anaerobic media has been shown to enhance the recovery of both anaerobic and facultative anaerobic bacteria.[3]

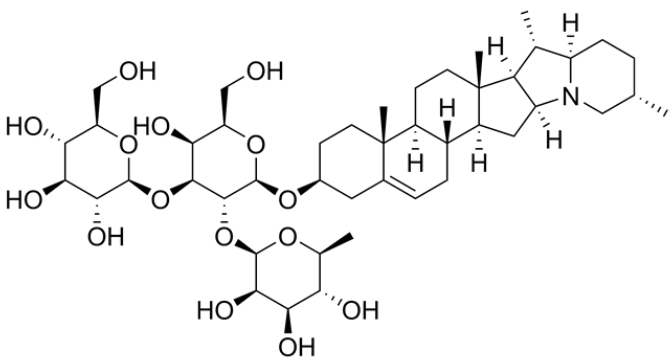


**Figure 1. Structure of the Saponin**
Lysis of blood cells which allows release of viable intracellular organisms or growth factors contained in the cells

In an early study, Rohner and colleagues have proved that combination of Plus aerobic/F and Lytic anaerobic/F was a valuable pair of blood culture media. Plus aerobic/F performs better for patients under antibiotic treatment, due to the antimicrobial-neutralizing effect of resins. For patients without antibiotic therapy, more organisms could be isolated from Lytic anaerobic/F due to cell lysis. They also reported more *S. aureus* isolates (P = 0.05), *Pseudomonas spp.* (P < 0.0001), other gram-negative bacteria (P = 0.004) and yeasts (P < 0.0001) were isolated from Plus aerobic/F medium, but more streptococci (P < 0.0001), *E. coli* (P = 0.02) strains and anaerobes (P < 0.0001) were detected with Lytic anaerobic/F medium. Lytic anaerobic/F bottles were significantly (P < 0.0001) more often positive at least 6 hours before Plus aerobic/F bottles (n = 112 vs. 52, respectively).[4]

A recent study (Rocchetti A. et al, 2016) have evaluated the positivity rate and time to detection of three BACTEC media in an emergency department of Alessandria General Hospital in Italy: Plus Aerobic/F, Plus Anaerobic/F and Anaerobic Lytic/F. During an 8– month period between July 2014 and March 2015, 1503 blood culture sets were collected from 688 adult patients (> 18 years of age).[5]

**Table 1**
Positivity rate and time to detection.

| Culture media | N.[a] | N.Tot[b] | %[c] | Average TTP[d] | Median | Sd[e] |
|---|---|---|---|---|---|---|
| Single media | | | | | | |
| Anaerobic media type | 177 | 255 | 69.4% | 21.5 | 15.4 | 17.2 |
| Aerobic media type | 195 | 255 | 76.5% | 17.6 | 14.5 | 12.3 |
| Lytic media type | 196 | 255 | 76.9% | 16.3 | 12.0 | 14.4 |
| Paired media (at least one of the two media type is positive) | | | | | | |
| Lytic + aerobic media type | 241 | 255 | 94.5% | 15.7 | 12.3 | 11.3 |
| Anaerobic + aerobic media type | 224 | 255 | 87.8% | 18.4 | 14.1 | 14.8 |
| Paired media (when the two media type are positive together) | | | | | | |
| Lytic + aerobic media type | 150 | 255 | 58.8% | 12.3 | 11.2 | 5.9 |
| Anaerobic + aerobic media type | 148 | 255 | 58.0% | 13.6 | 13.0 | 4.3 |

[a] Number of positive vials (for single media) and positive sets (for paired media).
[b] Total Number of positive sets: on a total of 1503 sets for blood cultures 255 resulted positive for microbiological growth. Sets were composed of anaerobic medium. Aerobic medium and lytic medium. A set was considered positive if at least one of the vials was positive.
[c] Percent of positive media on a total of 255 positive sets for blood culture.
[d] Average TTP (ttp): time from incubation to time to positivity.
[e] Standard deviation.

- Positivity rate (PR) was similar for Anaerobic Lytic/F bottle (76.9%) and Plus Aerobic/F bottle (76.5%) but better if compared with Plus Anaerobic/F bottle (69.4%).
- Median time to detection (TTD) was optimum with the Anaerobic Lytic/F bottle (12 h) versus Plus Aerobic/F bottle (14.5 h) and Plus Anaerobic/F bottle (15.4 h).
- The PR and median TTD for the combination of Plus Aerobic/F with Anaerobic Lytic/F (94.5% and 12.3h, respectively) was significantly better than Plus Aerobic/F with Plus Anaerobic/F (87.8 and 14.1h).

**Table 2**
Recovery rate and percentage of microorganisms identified in each media. Percentages in brackets indicate percentage of total number for that isolate (N. Tot).

| Organisms class | Organisms | N.Tot | Lytic N.P | Anaerobic_N.P | Aerobic_N.P |
|---|---|---|---|---|---|
| Anaerobic bacteria | *Bacteroides fragilis* | 3 | 2 (66.7%) | 1 (33.3%) | |
| | *Bacteroides stercoris* | 2 | 2 (100%) | | |
| | *Bacteroides caccae* | 1 | 1 (100%) | 1 (100%) | |
| Gram-negative bacilli | *Escherichia coli* | 96 | 77 (80.2%) | 62 (64.6%) | 69 (71.9%) |
| | *Klebsiella pneumoniae ssp pneumoniae* | 14 | 12 (85.7%) | 10 (71.4%) | 13 (92.9%) |
| | *Proteus mirabilis* | 13 | 12 (92.3%) | 10 (76.9%) | 8 (61.5%) |
| | *Providencia stuartii* | 9 | 9 (100%) | 9 (100%) | 8 (88.9%) |
| | *Pseudomonas aeruginosa* | 7 | 1 (14.3%) | 2 (28.6%) | 7 (100%) |
| | *Acinetobacter baumannii* | 3 | 1 (33.3%) | 1 (33.3%) | 3 (100%) |
| | *Klebsiella oxytoca* | 3 | 3 (100%) | 3 (100%) | 3 (100%) |
| | *Enterobacter aerogenes* | 2 | 2 (100%) | 2 (100%) | 2 (100%) |
| | *Enterobacter cloacae* | 2 | 1 (50%) | 1 (50%) | 1 (50%) |
| | *Salmonella enterica* | 2 | 2 (100%) | 2 (100%) | 2 (100%) |
| | *Aeromonas salmonicida* | 1 | | 1 (100%) | |
| | *Aeromonas hydrophila* | 1 | | 1 (100%) | 1 (100%) |
| | *Brevundimonas vesicularis* | 1 | | | 1 (100%) |
| Gram-positive cocci | *Staphylococcus aureus* | 41 | 36 (87.8%) | 37 (90.2%) | 36 (87.8%) |
| | *Staphylococcus epidermidis* | 15 | 11 (73.3%) | 10 (66.7%) | 12 (80%) |
| | *Enterococcus faecalis* | 13 | 12 (92.3%) | 12 (92.3%) | 12 (92.3%) |
| | *Streptococcus pneumoniae* | 8 | 6 (75%) | 8 (100%) | 8 (100%) |
| | *Staphylococcus hominis ssp. hominis* | 6 | 2 (33.3%) | 2 (33.3%) | 4 (66.7%) |
| | *Streptococcus gordonii* | 4 | 2 (50%) | 3 (75%) | |
| | *Streptococcus mitis* | 3 | 1 (33.3%) | 2 (66.7%) | |
| | *Alloiococcus otitidis* | 2 | 2 (100%) | | |
| | *Gemella morbillorum* | 2 | | 1 (50%) | 2 (100%) |
| | *Peptoniphilus asaccharolyticus* | 2 | 2 (100%) | 1 (50%) | |
| | *Streptococcus anginosus* | 2 | 2 (100%) | 2 (100%) | 2 (100%) |
| | *Staphylococcus capitis* | 1 | 1 (100%) | 1 (100%) | 1 (100%) |
| | *Streptococcus sanguinis* | 1 | | | 1 (100%) |
| Other Gram positive bacteria/yeasts | *Candida parapsilosis* | 5 | 2 (40%) | 2 (40%) | 5 (100%) |
| | *Listeria monocytogenes* | 2 | 2 (100%) | 2 (100%) | 2 (100%) |
| | *Candida albicans* | 2 | 1 (50%) | | 2 (100%) |
| | *Candida glabrata* | 1 | 1 (100%) | | 1 (100%) |
| | Recovery rate (for each media)[a] | | 76.3% | 70.0% | 77.0% |

[a] On a total of 270 isolates.

- Improved recovery of strict anaerobes, facultative anaerobes (gram-negative rods) and yeasts was observed with the Anaerobic Lytic/ F bottle vs. the Plus Anaerobic/ F bottle
- Recovery of gram-positive cocci was observed to be equivalent in the Anaerobic Lytic/ F bottle and the Plus Anaerobic bottle

- A total of 40 isolates were obtained from patients receiving antibiotics at the time the blood cultures were collected.
- Overall recovery of isolates was equivalent for the individual bottles as well as the combination bottles.
- The median TTD was optimum with the Anaerobic Lytic/F bottle (15.1 h).

**Table 3**
Positivity rate and time to detection for patients receiving antibiotic therapy.

| Culture media | N.[a] | N.Tot[b] | %[c] | Average TTP[d] | Median | Sd[e] |
|---|---|---|---|---|---|---|
| Single media | | | | | | |
| Anaerobic media type | 28 | 40 | 70% | 27.6 | 23.3 | 18.3 |
| Aerobic media type | 29 | 40 | 72.5% | 24.1 | 18.4 | 18.5 |
| Lytic media type | 30 | 40 | 75% | 18.0 | 15.1 | 8.1 |
| Paired media (at least one of the two media type is positive) | | | | | | |
| Lytic + aerobic media type | 37 | 40 | 92.5% | 19.7 | 17.2 | 14.7 |
| Anaerobic + aerobic media type | 36 | 40 | 90% | 24.6 | 18.5 | 19.4 |
| Paired media (when the two media type are positive together) | | | | | | |
| No data available | | | | | | |

[a] Number of positive vials (for single media) and positive sets (for paired media).
[b] Total number of positive sets: on a total of 1503 sets for blood cultures 255 resulted positive for microbiological growth. Sets were composed of anaerobic medium. Aerobic medium and lytic medium. A set was considered positive if at least one of the vials was positive.
[c] Percent of positive media on a total of 255 positive sets for blood culture.
[d] Average TTP (ttp): time from incubation to time to positivity.
[e] Standard deviation.

**Conclusion:**

i. The use of resin containing medium with a saponin-supplemented lytic media further improve overall recovery and earlier detection of bacteria and yeasts in the blood of septic patients.
ii. Differences in performance were evident when comparing Anaerobic Lytic/F media vs. Plus Anaerobic/F media: significantly better recovery of strict anaerobes, strict aerobic & facultative anaerobic gram-negative rods and yeasts was observed with the Anaerobic Lytic/F media.
iii. Median TTD was significantly decreased with Anaerobic Lytic/F (2.6 h faster for all patients and 3.3 h faster for patients receiving antibiotics). This is because the lysing ability of the Anaerobic Lytic/F media, which reduces background signal due to host cell metabolic activity.

**References**


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