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Appendix

The protocol of BACT/ALERT bottle culture method

The BACT/ALERT 3D 120 blood culture system were used for pathogen culture in all three culture methods in order to eliminate the influence of this variable. The BACT/ALERT FA/FN bottles were used and the specific ingredients of the culture media are in the following. The duration of flask culture was from 5 to 14 days. The bottles that remained negative for growth after 14 days of culture were terminated and considered as negative results. If a positive result presented in a bottle, Gram’s staining was then performed. Thereafter, the 0.5-mL turbid broth from aerobic bottles was smeared onto Sabouraud’s and Columbia blood agar (bioMérieux) plates and incubated for 24-72 h at 35°C in a 5% CO₂ incubator. Similarly, The CDC 5% sheep blood agar (BD Biosciences, USA) plates were selected for anaerobic culture. If there was no propagation on subculture and no identifiable microbiological morphology with Gram’s staining, the results were recorded as negative. The time to detection (TTD) of *Staphylococcus aureus* and coagulase-negative *staphylococcus* (CoNS) were recorded to evaluate the influence of different culturing on the TTD.
The specific ingredients of the culture media in BACT/ALERT FA/FN bottles:

**BACT/ALERT® FA FAN® Aerobic:** 4ml 1.0125g/ml activated charcoal and 16 ml peptone-enriched TSB, which contains Soy-casein digestive solution (2.0% w/v), Brain Heart Infusion (BHI) solids (0.1% w/v), polyfenel sodium sulfonate (0.025% w/v), pyridoxine hydrochloride (0.001% w/v), menadione (0.000625% w/v), hemin (0.000625% w/v), L-cysteine(0.025% w/v).

**BACT/ALERT® FN FAN® Anaerobic:** 8ml 8.5% activated charcoal and 32ml peptone-enriched TSB, which contains Soy-casein digestive solution (2.0% w/v), Brain Heart Infusion (BHI) solids (0.1% w/v), polyfenel sodium sulfonate (0.044% w/v), pyridoxine hydrochloride (0.001% w/v), menadione (0.000625% w/v), hemin (0.000625% w/v), L-cysteine(0.025% w/v).

The strategy of antibiotic selection for patients with inconsonant culture results

A consultation with clinical microbiologists and pharmacologists to determine antibiotic selection would be performed if patient obtained inconsonant culture results by different culturing techniques. The antibiotic which is sensitive to different pathogens was selected preferentially according to the results of susceptibility tests in order to reduce the complications caused by the use of multiple antimicrobial agents. If
the antibiotic is unavailable in the situation that two types of specimens obtain the same pathogen, and remaining specimen gets another pathogen, antibiotic selection according to the following work-flow:

- Patients with inconsonant culture results
  - The risks of polymicrobial infections:
    - Immunocompromised status
    - Chronic sinus tract
    - Inconsonant pathogen from sonicated implant or soft tissue
    - It matches the hospital's bacterial spectrum
    - Malnutrition
  - Yes
  - Bacteria (G+ and G-)
  - A fungus accompanied by other bacteria

- Yes
  - Combination therapy with antifungal and anti-bacterial agent

- No
  - Monotherapy for the pathogen that is from two types of specimens

- Yes
  - Contamination bacteria is eliminated

- No
  - Polymicrobial infection is confirmed
  - Combination therapy that covers all pathogenic bacteria