

Thesis Title Rapid isolation and identification of bacteria from blood - culture bottle using lysis-centrifugation technique.

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Date of Awarding the Degree June 19, 1986.

ABSTRACT

The early concentrate subculture technique was developed on the basis of the lysis-centrifugation technique. Saponin, the lysing agent, was tested, and the optimal concentration in lysing 10% (v/v) blood-broth was 0.04%. At the speed of 4000 rpm/20 minutes of the centrifugation, the early concentrate subculture gave the advantage over the early blind subculture technique in detecting the representative organisms from the simulated blood culture. The early concentrate subculture technique was applied to the blood culture bottles collected from the suspected

bacteremic or septicemic patients of the Maharaj Nakorn Chiangmai hospital. Of 1782 blood cultures performed by the conventional method, 227 or 12.74% organisms were detected. These included 193 or 10.83% of significant organisms and 34 or 1.91% of contaminants. About 80% of positive blood cultures or 89% of all significant organisms were detected within day 1.

The detection rates performed by the early blind subcultures in 0-6 hr, 7-12 hr, and 13-24 hr were 12.41%, 60.53% and 55.77% respectively. For the early concentrate subcultures, the yields were 35.77%, 86.48% and 61.54% respectively. This method was significantly much better than the early blind subculture performed between 0 and 6 hr and between 6 and 12 hr after blood collection ($p < 0.001$ and $p < 0.005$ respectively). Detection by gram-stained smear from the early concentrated lysate was an advantage over the early blind broth smear from the 7-12-hr blood culture ($p < 0.025$). For anaerobes detection, the blood-broth bottle recovered one more organism in comparison with the thioglycollate subculture from the concentrate, besides, the detection time was also getting shorter. The sensitivity of the early concentrated lysate of blood-broth method was about 50%. Besides, its specificity was 100% when compared with the conventional method.

The 4-hr identification method gave 100% correct presumptive identification for gram-positive bacteria, and

98.41% for gram-negative bacilli, when compared with conventional method.

The 4-hr antibiotic susceptibility test of 448 comparisons gave 75.45% agreement with the standard disk diffusion method. The vast discrepancies was 21.65% of the minor discrepancies. For major discrepancies was only 2.68% which occurred with 12 rapid growers of fermentative gram-negative bacilli. Very major discrepancies occurred only one with gentamicin tested with the slow grower of nonfermentative gram-negative bacilli. This showed the good results of the sensitivity reported at 4-hours readings.