Enrichment Methodology to Increase the Positivity of Cultures from Body Fluids

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Isolation and identification of etiological agents found in body fluids can be of critical importance for the recovery of patients suffering from potentially-severe infections, which are often followed by serious sequels. Eighty-two samples of different body fluids were analyzed using two different methods: (1) the conventional culture method (agar plating) and (2) the enrichment culture technique, using the Bact/Alert® blood culture bottle. The number of positive cultures increased on average from 9.7% to 23.1% with the enrichment culture technique. *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* were the most frequently isolated bacteria. The enrichment method could provide a more accurate means the identifying etiological agents.

Key Words: Body fluids, etiological agents, enrichment.

Biological body fluids, such as pleural, synovial, peritoneal and pericardial liquids, are usually sterile but may be invaded and infected with various types of microrganisms, including bacteria [1]. These infections are generally serious and leave sequels. The isolation and identification of the etiological agent can be a critical factor for patient healing [2].

Among all isolation methods currently in use, agar plating or inoculation of samples into agar-based culture media (conventional method) is the most common. However, the risk of false negative results is high because only a small number of microorganisms may be present in the specimens [3,4]. In an attempt to overcome this drawback, innovative techniques using enrichment media and newly-developed laboratory devices and instruments, such as blood culture bottles, have been adopted [5]. We evaluated the positivity of various biological body fluids, comparing the results obtained with the conventional method with those produced with the enrichment method.

Eighty-two samples received at the Clinical Laboratory of the Nossa Senhora das Graças Hospital from November 2002 to April 2004 were inoculated (100 µL) into the following culture media: 5% sheep blood, MacConkey and chocolate agars. The samples inoculated onto chocolate agar were incubated in a 5% to 10% carbon dioxide-supplemented atmosphere. After inoculation, the plates were incubated at 37ºC for one to two days [6]. At the same time, 5 to 10 mL of each sample was inoculated in a BacTAlert blood culture bottle (Organon Teknika Corporation, Durham, NC) containing TSB (Tryptic Soy Broth) and subsequently incubated in an automated system for up to seven days [7]. Once bacterial growth was detected, either on the agar plates or in the broth bottles, the microrganisms were further isolated and identified using biochemical, serological and standard sensitivity techniques [8,9].

Pseudomonas aeruginosa, Escherichia coli and *Staphylococcus aureus* were the bacterial species most frequently isolated from the specimens analyzed. Diaz et al. [2] reported similar findings. *Streptococcus pneumoniae*, *Streptococcus anginosus*, *Streptococcus viridans*, *Enterococcus faecalis*, coagulase-negative *Staphylococcus*, *Acinetobacter baumannii*, and *Stenotrophomonas maltophilia*, as well as other *Enterobacteriaceae*, were also isolated, although in smaller percentages.

The positive scores produced by the body fluid cultures analyzed with the conventional and enrichment methods are shown in Figure 1.

Although positive scores varied considerably among the different types of specimens (body fluid types), the blood culture bottles gave more (23.1%) positive results than did the agar plates (9.7%). Similar results were reported by Simor et al., who observed 11.2% and 19.8%, for agar plates and blood culture bottles, respectively [10]. This finding could be explained by (a) the use of a large sample volume, with a corresponding increase in the initial bacterial load inoculated into the culture media; (b) the liquid culture media may favor initial growth of the microrganisms and (c) a longer period of incubation allows the isolation of bacteria that are characterized by a slow growth rate, such as fastidious bacteria [11].

According to Silva [11], the standard manual incubation system gives the same rate of positive scores as the automated system, but the period of time necessary for the detection of microbial growth is greatly reduced in the automated system due to the constant agitation.

We found that the enrichment method produced a significant and consistent increase in the isolation rate for biological body fluid cultures when compared to conventional culture systems. The enrichment method is a valid alternative

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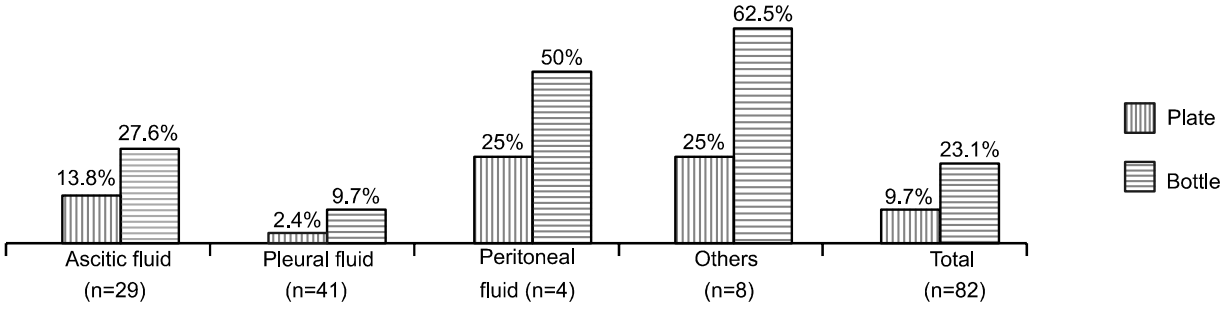
Figure 1. Percentage of positive results produced on agar plates and in blood culture bottles.



that can be used as a routine procedure, allowing more accurate detection of etiological agents, thereby enabling more adequate and efficient treatment for the patient.

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