

Modified Sabouraud Medium Containing Neomycin and Polymyxin

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A commercially available antibiotic mixture effectively decreased overgrowth of Sabouraud medium by gram-negative flora frequently found in the sputum of hospitalized patients.

Isolation of fungi on Sabouraud medium is largely dependent upon the inability of the accompanying bacteria to develop in the presence of acid production from the breakdown of the medium's large amount of glucose, an acidity which the fungi tolerate relatively well. Unfortunately, various species of bacteria, particularly the enterobacteria and the pseudomonads, tolerate acidity to different degrees, and in the presence of heavy bacterial contamination the sought-after fungus may not grow. Because of these inadequacies, Robinson and Kotcher (5) suggested the addition of penicillin and streptomycin to the usual formula, and Cooke (1) recommended the routine incorporation of chloramphenicol in Sabouraud medium. Chloramphenicol and cycloheximide are responsible for the selective properties of the popular selective medium developed by McDonough et al. (3) and marketed commercially as Mycobiotic (Difco) or Mycosel (BBL). Given the marked increase in the number of infections with gram-negative organisms occurring in hospitals today (2) and the concomitant increase in interest in culturing for the possibility of fungal infections, particularly pneumonias, in the same debilitated patients who are bearing the brunt of this shift in the ecology of hospital infection, many specimens submitted to the diagnostic laboratory for fungal culture contain heavy growth of competing bacterial flora which neither plain Sabouraud medium nor the medium supplemented with the above-mentioned antibiotics can suppress. Such failure is not surprising in view of the widespread microbial resistance to antibiotics. For example, Maiztegui et al. (4) noted that, of 100 consecutive gram-negative organisms isolated from the blood stream of patients, only 13% were inhibited by streptomycin and only 49% were inhibited by chloramphenicol. Thus, cultural evidence of fungal infection is made more difficult to obtain, some

cultures being negative for fungi and others so overgrown as to greatly increase the work of the mycologist in obtaining isolation in pure culture.

The work of Maiztegui et al. (4) and of others has demonstrated that the only commonly used antibiotics still retaining a very high degree of efficacy against the usual gram-negative flora are kanamycin and polymyxin B. In their series, only 18 and 14%, respectively, of the organisms were resistant to these two drugs, whereas only 4% were resistant to both drugs. The ready commercial availability of an inexpensive mixture of neomycin (which has an antibacterial spectrum which is virtually identical with that of kanamycin) and polymyxin B suggested a simple modification of the usual Sabouraud formulation.

Sabouraud Dextrose Agar (Difco) and Sabouraud agar with chloramphenicol and cycloheximide (Mycobiotic Agar, Difco) were prepared in the usual manner, with the addition of one ampoule of a mixture of 40 mg of neomycin base activity and 20 mg of polymyxin B base activity (Neosporin GU-Irrigant, Burroughs, Wellcome and Co.) per liter of the melted medium, which was then distributed into screw-capped tubes and autoclaved. Previous experience has shown that these antibiotics do not lose activity with autoclaving. Materials submitted for fungal culture to the diagnostic laboratory at the University of California San Francisco Medical Center were subsequently inoculated onto slants of each medium, in addition to the other media routinely used, and the recovery of fungi was compared.

One-hundred consecutively submitted specimens were compared (Table 1). Only *Candida* species were recovered, and the Sabouraud-NP (Sabouraud Dextrose Agar with added neomycin-polymyxin B) had the highest rate of recovery, 71 of the isolates being *C. albicans* and 2 being *C. parakrusei*. The *C. parakrusei* strains were not detected on either of the other Sabouraud media,

and no fungi were detected in the media with poorer recovery rates that were not also found on those media with better rates. Not only did the Sabouraud-NP yield fewer cultures with bacteria, but bacterial growth in the contaminated speci-

mens was scant in all cases and isolation of suspicious colonies was done with ease, whereas heavy overgrowth regularly occurred in the other media.

It is concluded that the addition of a neomycin-polymyxin B mixture to Sabouraud Dextrose Agar is a useful adjunct in the isolation of fungi and a definite improvement over earlier modifications containing penicillin and streptomycin or chloramphenicol. It is suggested that a similar modification of cycloheximide-containing Sabouraud agar would be expected to improve the performance of that medium.

TABLE 1. Comparison of performance of three modifications of Sabouraud Dextrose Agar medium (100 consecutive cultures)^a

| Organisms recovered | Sab-NP | Sab-CC | Sab |
|-----------------------------------|--------|--------|-----|
| Fungi | | | |
| <i>Candida albicans</i> | 71 | 67 | 61 |
| <i>C. parakrusei</i> | 2 | 0 | 0 |
| Total..... | 73 | 67 | 61 |
| Contaminants | | | |
| <i>Klebsiella pneumoniae</i> | 5 | 8 | 20 |
| <i>Pseudomonas aeruginosa</i> ... | 9 | 15 | 19 |
| <i>Escherichia coli</i> | 1 | 4 | 17 |
| <i>Citrobacter</i> | 0 | 3 | 10 |
| <i>Enterobacter cloacae</i> | 0 | 1 | 6 |
| <i>Herellea</i> | 0 | 1 | 3 |
| <i>E. aerogenes</i> | 0 | 0 | 1 |
| Total..... | 15 | 32 | 76 |

^a Sab-NP = Sabouraud Dextrose Agar with added neomycin-polymyxin B; Sab-CC = Sabouraud Dextrose Agar with added chloramphenicol and cycloheximide (Mycobiotic, Difco); Sab = Sabouraud Dextrose Agar (Difco) unmodified.

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