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# Improved Enrichment Broth for Cultivation of Fastidious Organisms

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**An enrichment broth developed in our laboratory, fastidious broth (FB), was compared with two commercially available broth media, supplemented thioglycolate broth and enriched eugonic broth. FB supported the growth of a number of organisms that were not cultivatable in either of the other two media, including *Corynebacterium jeikeium*, *Haemophilus influenzae*, *Neisseria gonorrhoeae*, and *Streptococcus pneumoniae*. In addition, for several organisms that were able to grow in all three broths, including *Neisseria meningitidis*, *Nocardia asteroides*, and *Actinomyces* spp., both the time of incubation and the starting inoculum necessary to enable detection of growth were decreased significantly by using FB.**

The majority of clinical microbiology texts recommend utilization of an enrichment broth medium to improve the sensitivity of cultures for specimens that may contain small numbers of organisms (2). Little or no data, however, have been published on the relative efficacies of different broth media for this purpose. Experience in our laboratory suggested that for many organisms, the use of commercially available broth media, including enriched brain heart infusion (containing hemin and vitamin K), Columbia broth, supplemented thioglycolate broth (TG; containing hemin and vitamin K), and enriched eugonic broth (EB; containing pyridoxal), was a relatively insensitive culture technique. In an attempt to address this problem, we developed a new liquid enrichment medium, fastidious broth (FB), formulated to support the growth of a wide spectrum of microorganisms and to permit easy visual detection of growth. This paper reports the results of a study comparing FB with supplemented TG and enriched EB, the two commercially available broth media that in our experience supported growth of the widest range of organisms.

The formulation of FB was as follows. A total of 35 g of Columbia broth base, 5 g of glucose, 5 g of yeast extract, 2 g of neopeptone, and 0.75 g of agarose were dissolved in 960 ml of distilled water. A 30-ml portion of hematin (0.05% [wt/vol] in 0.1 M NaOH) and a 5-ml portion of Tween 80 (10% [vol/vol]) were then added, and the resultant broth was sterilized by autoclaving. Following aseptic addition of 6 ml of pyridoxal (0.1% [wt/vol]) and 1.5 ml of NAD (1% [wt/vol]), 10-ml aliquots of FB were dispensed into 15-ml culture tubes and stored at 4°C. Once this formulation was established, FB was prepared commercially by Remel Microbiology Products, Lenexa, Kans. To compare this medium with supplemented TG and enriched EB (both obtained from Remel Microbiology Products), known inocula of clinical isolates (approximately $10^4$, $10^3$, and $10^2$ organisms $ml^{-1}$) were prepared by serial dilution of standardized 0.5 McFarland suspensions of organisms (inoculum density was verified by subculturing dilutions onto chocolate agar and performing colony counts) and added to each of the three media. All tubes were then loosely capped and incubated at 35°C in the presence of 5% $CO_2$. Broths were examined daily for visual signs of growth. The presence of growth in visually positive broths was confirmed by Gram stain. All broths showing no visual signs of growth after 5 days of incubation were subcultured to chocolate agar plates, and the plates were held for an additional 48 h.

FB effectively supported the growth of many clinically important microorganisms (Table 1). A number of organisms grew exclusively in FB, and, as might be expected, these were predominantly nutritionally fastidious bacteria, for example, *Haemophilus influenzae* and *Neisseria gonorrhoeae*. Of perhaps greater significance is our finding that although a large number of organisms were able to grow in all three media, for many the time of incubation, the initial inoculum required to permit detectable growth, or both were decreased significantly by using FB (Table 1). In addition, the use of agarose rather than agar to decrease the $O_2$ tension in the medium greatly improved the ease with which growth could be visualized yet did not adversely affect the ability of FB to support the growth of oxygen-sensitive organisms. Indeed, FB proved to be superior to supplemented TG for the isolation of most anaerobic bacteria tested, including those *Actinomyces* spp. for which TG is a recommended medium (3). A secondary and entirely serendipitous finding was the lack of utility of terminal blind subculturing of liquid media. All terminal subcultures of visually negative broths remained negative after a further 48-h incubation, strongly indicating the adequacy of visual detection of growth, at least for nonpurulent specimens (for example, cerebrospinal fluid [CSF]). It should be noted that several organisms, namely *Helicobacter pylori*, *Legionella pneumophila*, and *Rochalimaeae henselae*, failed to grow in any of the broths, even after 14- to 21-day incubation periods. One would not, therefore, advocate using FB to recover such extremely fastidious organisms.

Of particular note is our finding that FB enabled enhanced recovery of a number of species implicated in acute and chronic meningitis, including *H. influenzae*, *Listeria monocytogenes*, *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Cryptococcus neoformans*, and *Nocardia asteroides* (Table 1). The possibility of having a small number of these organisms present in the CSF of patients with meningitis is a generally accepted rationale for including an enrichment broth, usually TG, as part of the routine culture setup for such specimens (1). Our data demonstrate that TG and EB are comparatively poor media for cultivating many of the etiologic agents of meningitis, and they do not indicate that use of these media is likely to improve the laboratory's chances of recovering clinically sig-

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TABLE 1. Comparison of FB, TG, and EB

| Organism | Growth of inocula (organisms $ml^{-1}$) in[a]: | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | FB | | | TG | | | EB | | |
| | $10^4$ | $10^3$ | $10^2$ | $10^4$ | $10^3$ | $10^2$ | $10^4$ | $10^3$ | $10^2$ |
| *Actinomyces israelii* | +(3) | +(4) | +(4) | +(5) | – | – | – | – | – |
| *Actinomyces naeslundii* | +(2) | +(3) | +(4) | +(3) | +(5) | – | – | – | – |
| *Actinomyces odontolyticus* | +(1) | +(2) | +(2) | +(2) | +(2) | +(3) | – | – | – |
| *Bacteroides fragilis* | +(1) | +(2) | +(4) | +(2) | +(4) | +(5) | – | – | – |
| *Campylobacter jejuni* | +(3) | +(5) | – | – | – | – | – | – | – |
| *Capnocytophaga canimorsus* | +(2) | +(4) | – | – | – | – | – | – | – |
| *Corynebacterium jeikeium* | +(1) | +(1) | +(3) | – | – | – | – | – | – |
| *Enterococcus faecalis* | +(1) | +(1) | +(1) | +(1) | +(1) | +(1) | +(1) | +(1) | +(1) |
| *H. influenzae* | +(1) | +(1) | +(2) | – | – | – | – | – | – |
| *L. monocytogenes* | +(1) | +(1) | +(1) | +(1) | +(2) | +(3) | +(2) | +(2) | +(3) |
| *Mycobacterium chelonae* | +(2) | +(2) | +(3) | +(3) | +(3) | – | +(3) | +(3) | +(3) |
| *N. gonorrhoeae* | +(2) | +(2) | +(4) | – | – | – | – | – | – |
| *N. meningitidis* | +(1) | +(2) | +(2) | +(3) | +(3) | +(4) | – | – | – |
| *N. asteroides* | +(2) | +(2) | +(2) | +(4) | – | – | +(2) | +(2) | – |
| *Peptostreptococcus magnus* | +(2) | +(4) | – | +(3) | – | – | – | – | – |
| *Prevotella melaninogenicus* | +(2) | +(3) | +(4) | +(3) | +(4) | – | – | – | – |
| *S. epidermidis* | +(1) | +(1) | +(1) | +(1) | +(1) | +(1) | +(1) | +(1) | +(1) |
| *Streptococcus adjacens* | +(1) | +(1) | +(2) | – | – | – | – | – | – |
| *S. pneumoniae* | +(1) | +(1) | +(1) | – | – | – | – | – | – |
| Beta-hemolytic *Streptococcus* group A | +(1) | +(1) | +(1) | +(1) | +(1) | – | +(1) | – | – |
| Beta-hemolytic *Streptococcus* group B | +(1) | +(1) | +(1) | +(1) | +(1) | +(1) | – | – | – |
| *Vibrio parahemolyticus* | +(1) | +(1) | +(2) | – | – | – | – | – | – |
| *Candida albicans* | +(1) | +(2) | +(2) | +(2) | +(4) | +(4) | +(2) | +(2) | +(4) |
| *C. neoformans* | +(2) | +(2) | +(2) | – | – | – | – | – | – |
| *Torulopsis glabrata* | +(1) | +(1) | +(1) | +(1) | +(1) | +(1) | +(1) | +(1) | +(1) |

[a] Numbers in parentheses represent the earliest times (in days) at which growth (+) was visually detectable. Minus signs indicate that no growth was visible after 5 days of incubation.

nificant organisms from CSF. To further document the potential utility of FB for recovering organisms from CSF, FB cultures containing approximately 10 organisms of *H. influenzae*, *L. monocytogenes*, *N. meningitidis*, *N. asteroides*, *S. pneumoniae*, and *C. neoformans* $ml^{-1}$ were prepared. Growth of all organisms tested was detectable after 48 h of incubation. The expediency and sensitivity with which these organisms were detected suggest that use of FB could result in a meaningful improvement in the diagnostic sensitivity of CSF culture. Enriched EB, FB, and supplemented TG were equally effective in supporting the growth of *Staphylococcus epidermidis*, the most common contaminant of CSF cultures. This result suggests that switching from TG or EB to FB probably would not appreciably increase the frequency of isolation of clinically insignificant organisms from CSF specimens.

In conclusion, our data demonstrate that FB is capable of supporting the growth of organisms with diverse nutritional and atmospheric requirements, and they indicate that use of this medium could increase the efficiency of recovery of many clinically significant organisms from specimens for which broth media are recommended. Furthermore, since this medium has been successfully made for us by a commercial company, it could be utilized in laboratories lacking the resources necessary for in-house medium production.

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