Brief communication: Rapid culture of tubercle bacilli*

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One of the biggest obstacles to the correct diagnosis and efficient treatment of tuberculosis is the absence of a rapid technique for culturing tubercle bacilli and for testing their susceptibility to antituberculosis drugs. Current procedures typically take 6–10 weeks to perform. This article describes a simple, rapid, reliable and cheap method of culturing tubercle bacilli using a liquid medium consisting of a mixture of coconut water, horse serum, glycerol and benzylpenicillin. Addition of specific concentrations of antituberculosis drugs to the medium, permits information on the drug susceptibility of tubercle bacilli to be obtained in only 6 days. The procedure requires no special instruments or technical skill and can therefore be carried out routinely in the average laboratory in developing countries.

**Introduction**

One of the main reasons for the failure of antituberculosis drugs to control tuberculosis in recent years is the growing resistance of *Mycobacterium* spp. to these drugs. Since use of X-rays or smear microscopy does not provide information on the drug susceptibility of tubercle bacilli, these methods are no longer considered adequate for use in the diagnosis and treatment of tuberculosis. Culture of tubercle bacilli and testing them for susceptibility to drugs have therefore become extremely important, but current procedures for this take 6–10 weeks to perform.

Numerous attempts have been made to develop a rapid diagnostic procedure for tubercle bacilli based on immunological and molecular biological techniques, to date without complete success; however, even if such a test were available, it is doubtful whether laboratories in developing countries would have the expertise and financial resources to use it. Thus, microscopy and culture techniques remain the primary methods of laboratory diagnosis and are likely to remain so for several years (7). There is therefore a need for a rapid culture technique for tubercle bacilli so that their susceptibility to antituberculosis drugs can be tested as rapidly as possible.

In this article we report a simple, reliable and cheap method of culturing tubercle bacilli in 6 days in a liquid medium consisting of a mixture of coconut water, horse serum, glycerol, and benzylpenicillin.

**Materials and methods**

A total of 1250 sputum specimens were obtained from tuberculosis patients, both newly diagnosed and undergoing treatment, and processed with the single-step culture technique using trisodium phosphate as the decontaminant (2). Smears were made from the decontaminated sputum deposits and stained using the cold staining method for acid-fast bacilli (AFB) (3). The deposits were inoculated into both the above-mentioned liquid medium and Löwenstein–Jensen (LJ) medium and the results obtained compared.

**Preparation of the liquid medium**

Water from a tender coconut was filtered first through a cotton gauze pad and then through Whatman No. 1 filter-paper. To 80ml of the filtrate were added 20ml horse serum and 5ml glycerol. Benzylpenicillin was added to this mixture to make a final concentration of 100IU/ml of the medium. The mixture was then filtered through a 0.2-µm cellulose acetate membrane and 2.5-ml aliquots of the filtrate were transferred to sterile, screw-capped test-tubes.

**Culture in the liquid medium**

Samples of each decontaminated and washed sputum deposit were inoculated into two test-tubes containing the culture medium. The amount of inoculum depended on the number of AFB seen in the smear by light microscopy. For example, if >10 AFB were

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identified in one microscope field, 0.05ml of deposit was added to the medium using a 50-μl automatic pipette; if 1–10 AFB/field were seen, 0.1ml of deposit was added; if 10–99 AFB/field were seen, 0.2ml was added; and if <10 AFB/100 fields were seen, 0.3ml was added using a 100-μl automatic pipette.

One set of test-tubes was centrifuged at 3000rpm for 10min on the same day without incubation and kept as a control. Smears were made from the sediments and these were stained and examined for AFB. The other set was incubated at 37°C for 5 days, whereupon the supernatant was removed by pipette and 5ml of sterile distilled water was added to the sediment. The tubes were then shaken well and left overnight in a refrigerator. The following morning the supernatant was removed by pipette and the sediment shaken thoroughly. One drop of sediment was then placed on a clean microscope slide, allowed to dry, and fixed by heating before being stained and examined for AFB as described above and observed by light microscopy using a ×100 oil immersion objective. The findings were compared with those for the controls. The presence of clumps and cords in the test smears was taken as evidence of bacterial multiplication (4).

**Culture in Löwenstein–Jensen medium**

A loopful of sputum deposit was inoculated on to each of two Löwenstein–Jensen medium slopes and incubated at 37°C for 6 weeks. The cultures were examined at the end of every week.

**Results**

Of the 1250 sputum samples cultured, 340 were positive and 910 were negative for AFB when examined as smears. Of the 340 positive samples (Table 1), 291 were also positive following culture in the liquid medium, 36 were negative, and 13 had become contaminated during culture. Following culture in Löwenstein–Jensen medium, 289 of the 340 were positive, 33 negative, and 18 had become contaminated. A total of 284 samples were positive using both methods; similarly, 32 samples were negative and 11 contaminated using both methods. The agreement between the two methods was statistically highly significant (κ = 0.853).

Of the 910 samples that were negative for AFB when examined as smears (Table 2), 30 were positive following culture in the liquid medium, 812 were negative and 68 had become contaminated during culture. Following culture in Löwenstein–Jensen medium, 92 of the 910 were positive, 758 negative and 60 had become contaminated. A total of 23 samples were positive using both methods; 732 samples were negative and 42 contaminated using both methods. Statistically, there was only moderate agreement between the two methods (κ = 0.50).

**Discussion**

In addition to carbohydrates and fats, coconut water contains a number of amino acids, vitamins, and minerals (5). The medium that we have described here was found to be very suitable for culturing tubercle bacilli; clumps and cords appeared progressively over a period of 6 days following inoculation of the liquid medium with a positive sputum sample from a newly diagnosed tuberculosis patient. Cord formation, considered important in the identification of tubercle bacilli, became increasingly marked from the fifth day onwards. The liquid medium was as efficient as Löwenstein–Jensen medium for culturing tubercle bacilli from smear-positive samples, though it was less efficient for culturing them from smear-negative samples.

The technique described is simple to perform and requires no special instruments or technical skill; it is also cheap, the cost per test being less than US$ 0.1. By spiking the medium with known concentrations of antituberculosis drugs, information on the susceptibility of tubercle bacilli to these drugs can be obtained in 6 days.

**Table 1:** Comparison of the results of culturing 340 smear-positive samples in the liquid medium and in Löwenstein–Jensen medium

<table>
<thead>
<tr>
<th>Liquid medium</th>
<th>Löwenstein–Jensen medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. +ve</td>
</tr>
<tr>
<td>No. +ve</td>
<td>284</td>
</tr>
<tr>
<td>No. -ve</td>
<td>3</td>
</tr>
<tr>
<td>No. contaminated</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>289</td>
</tr>
</tbody>
</table>
Résumé

Culture rapide du bacille tuberculeux

La progression récente de la résistance de *Mycobacterium* spp. aux antituberculeux a rendu nécessaire la mise en culture du bacille tuberculeux afin de tester sa pharmacosensibilité. Toutefois, les techniques de culture actuellement utilisées demandent 6 à 10 semaines et il est donc urgent de mettre au point une nouvelle méthode simple, rapide, fiable et bon marché.

On décrit ici une technique qui répond à toutes ces exigences et fournit des informations sur la croissance du bacille tuberculeux et sa pharmacosensibilité en 6 jours. Elle fait appel à un milieu liquide constitué de lait de coco, de sérum équin, de glycérol et de benzylo pénicilline. Des échantillons de crachats décontaminés sont inoculés dans 2,5 ml de ce milieu et incubés à 37°C pendant 5 jours. Au cinquième jour, on élimine le surnageant et on le remplace par 5 ml d’eau distillée; le mélange est ensuite agité puis laissé au réfrigérateur toute la nuit. Le lendemain matin, on élimine le surnageant et on agite à nouveau le dépôt soigneusement. On dépose ensuite une goutte de dépot sur une lame propre et on la laisse sécher; on effectue ensuite une fixation à la chaleur, une coloration de Ziehl-Nelssen et on examine la lame au microscope optique. La formation d’amas et de cordons de bacilles signe la multiplication bactérienne.

Mille deux cent cinquante échantillons de crachats ont été cultivés au total dans ce milieu liquide et dans du milieu de Löwenstein-Jensen et l’on a ensuite comparé les résultats. Le milieu liquide a été aussi efficace que le milieu de Löwenstein-Jensen pour cultiver le bacille tuberculeux à partir d’échantillons à frottis positifs, mais s’est révélé moins efficace pour le cultiver à partir d’échantillons à frottis négatifs. En ajoutant des concentrations précises de divers antituberculeux au milieu liquide, il est possible d’obtenir des informations sur la croissance et la pharmacosensibilité des cultures en 6 jours. Comme cette méthode n’exige aucun instrument particulier ni compétence technique précise et qu’elle fait appel à des produits bon marché disponibles au niveau local, son coût par test est inférieur à US $0,1. Elle convient donc tout à fait à la moyenne des laboratoires des pays en développement et peut être employée comme technique diagnostique de routine.

References