Isolation of Acid-Fast Organisms from Surgical Specimens

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A total of 204 surgical specimens positive for acid-fast organisms yielded 169 strains of Mycobacterium tuberculosis, 15 of which were isolated by Middlebrook 7H-9 broth medium only. Mycobacteria other than M. tuberculosis were isolated from 33 specimens, and Nocardia asteroides was isolated from 2 specimens.

A case of BCG bacteremia in a cancer patient from whom Mycobacterium bovis (BCG) was isolated by Middlebrook 7H-9 broth from a liver biopsy (7) prompted this laboratory in 1975 to review its isolation procedure for mycobacteria and to attempt to improve the recovery of Mycobacterium tuberculosis and of other mycobacteria from clinical specimens other than sputa, urines, and cerebrospinal fluids.

No information is available as to the results of histological investigations of more than 4,000 surgical specimens received by this laboratory during a 4-year period. For that reason we will report about the 204 patients from whom mycobacteria or nocardia were isolated bacteriologically. No statistical comparisons were made of different culture media or of the effect on acid-fast bacilli of specimen treatment with sodium hydroxide, because most surgical specimens were either infected or contaminated with non-acid-fast organisms and had been submitted to this laboratory only to rule out mycobacterial infections. Consequently, overgrowth of a non-selective medium, such as Middlebrook 7H-9 broth, by non-acid-fast organisms statistically favors the use of both sodium hydroxide pre-treatment and the exclusive use of a selective medium, such as Lowenstein-Jensen (L-J).

Nevertheless, a laboratory must be prepared to recover mycobacteria from the uncontaminated tuberculous surgical specimen in an optimal manner; therefore, untreated samples and nonselective media continue to be used for those instances when surgical specimens are submitted.

The specimens were divided into two groups: fluid specimens such as pleural, pericardial, synovial, and ascitic; and tissue specimens such as lung, pleura, pericardium, bone marrow, bone, lymph node, skin, liver, and kidney and tissue specimens from the genital tract.

All fluids were centrifuged in a Sorvall RC-5 superspeed refrigerated centrifuge for 30 min at 8,000 x g. The supernatant was decanted, and a portion of the sediment was inoculated onto one L-J medium slant (BBL Microbiology Systems, Cockeysville, Md.). The remainder of the sediment was treated with approximately 2 ml of an aqueous solution of 2% sodium hydroxide for 18 min. After that time, sterile distilled water was added to make a volume of 45 ml. The specimen was then centrifuged at an average speed of 3,200 x g in an IEC model UV International Centrifuge for 30 min. Subsequently, the supernatant was decanted, and the sediment was inoculated onto two L-J slants and into one homemade Middlebrook 7H-9 broth. A smear to be stained by auramine-rhodamine (9) was also made at this time. The L-J cultures were incubated in 10% carbon dioxide at 35 to 37°C for 60 days unless determined positive for mycobacteria or nocardia or totally contaminated by non-acid-fast bacilli at 21 or 40 days.

All 7H-9 broth cultures were subcultured after 20 days of incubation at 35 to 37°C in 10% CO₂ onto one L-J slant which was incubated for another 20 days in 10% CO₂ at 37°C. For more rapid results, the 7H-9 broth primary culture should be examined microscopically for the presence of mycobacteria. This was not done in this study.

Tissue specimens were ground for approximately 5 min in a Sorvall Omni-Mixer (Du Pont Co., Wilmington, Del.; no. 17105), using 5 ml of distilled water if no transport fluid was present. One L-J slant was inoculated before the specimen was decontaminated with 2% NaOH. The remainder was treated with NaOH for 18 min, washed with distilled water, centrifuged, and inoculated onto two L-J slants and into one 7H-9 broth. Attempts were made to inoculate the broth with a large portion of the ground tissue or chunks that might not have been thoroughly ground. A smear was made at this time. All cultures from tissue specimens were incubated in the same way as those from fluid specimens.

Skin specimens were inoculated onto an additional set of two L-J slants and into one 7H-9.
broth for incubation at 32°C.

Microscopically positive specimens were inoculated onto a set of homemade drug-containing and control Middlebrook 7H-10 agar plates. The use of this agar medium for primary isolation of mycobacteria from microscopically negative specimens offered, in our experience, no advantage over L-J medium, provided 10% CO₂ is used for the latter (3). Once mycobacterial growth was obtained on solid medium, the organism was identified according to the procedures of the U.S. Department of Health, Education, and Welfare (9). Nocardia was identified according to Gordon’s recommendations (2).

We isolated 169 strains of *M. tuberculosis* (154 in 7H-9 broth or L-J medium or both and 15 in 7H-9 broth only), 25 of *Mycobacterium intracellulare*, 2 each of *Mycobacterium marinum*, *Mycobacterium bovis*, *Mycobacterium gordonae*, and *Nocardia asteroides*, and 1 each of *Mycobacterium scrofulaceum* and *Mycobacterium vaccae* (Table 1) from 204 clinical specimens obtained from different patients. Additionally, 24 pulmonary or extrapulmonary specimens were positive for *M. tuberculosis* in 22 cases in which generalized tuberculosis was proven by positive cultures of the genital tract (7 cases); the joints, bone marrow, and cervical lymph nodes (3 cases each); the kidneys (2 cases); and the liver, pericardium, bone, and epiglottis (1 case each). With the exception of two strains of *M. gordonae*, one of *M. vaccae*, and one of *M. bovis* (BCG) from a vaccination site, there is little doubt about the clinical significance of the nocardia and mycobacteria isolated from lung and pleural tissue, lymph nodes, skin, and abscesses. However, none of the "atypical" isolates showed additional involvement of other body sites, ostensibly because of the lower virulence of these organisms for humans.

In this study 15 *M. tuberculosis* strains from four lung and five pleural biopsies and 1 strain each from biopsies of the cervical lymph node, synovium, bone, disk, bone marrow, and uterus were grown only in 7H-9 primary broth culture after the specimens had been decontaminated. Although not done, microscopic investigation of such primary broth cultures is indicated after 1 to 2 weeks of incubation since all exclusive broth isolates in this study were *M. tuberculosis*.

Kirchner (5) used a 10% serum-containing liquid medium approximately 50 years ago for the primary isolation of *M. tuberculosis* from pathological material containing only few acid-fast organisms. He reported that 13 of his 86 specimens, consisting mainly of lymph nodes and pleural fluids, were positive for *M. tuberculosis* in his liquid medium only.

**Table 1. Isolation of acid-fast organisms from tissues and body fluids**

<table>
<thead>
<tr>
<th>No. and source of specimens</th>
<th>No. of strains isolated</th>
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</thead>
<tbody>
<tr>
<td></td>
<td><em>M. tuberculosis</em></td>
</tr>
<tr>
<td></td>
<td>7H-9 only</td>
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<tr>
<td></td>
<td>7H-9 or L-J or both</td>
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<td></td>
<td>7H-9 or L-J or both</td>
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<td>-----------------------------</td>
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</tr>
<tr>
<td>Lung</td>
<td>4</td>
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<tr>
<td>Pleural</td>
<td>5</td>
</tr>
<tr>
<td>Cervical nodes</td>
<td>1</td>
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<tr>
<td>Skin and abscesses</td>
<td>12</td>
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<tr>
<td>Bone and disks</td>
<td>10</td>
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<tr>
<td>Genital</td>
<td>8</td>
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<tr>
<td>Bone marrow</td>
<td>5</td>
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<tr>
<td>Pericardium</td>
<td>3</td>
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<tr>
<td>Liver/kidney</td>
<td>3</td>
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<tr>
<td>Gastrointestinal and epiglottis</td>
<td>3</td>
</tr>
</tbody>
</table>

* 7H-9 = Middlebrook 7H-9 broth medium (homemade); L-J = L-J alans (BBL Microbiology Systems).

Hobby and co-workers (4) found that a 9-week preliminary incubation period of pulmonary lesions in either a liquid albumin or a liquid Tween-albumin medium was required to demonstrate growth of *M. tuberculosis* in 9 out of 25 positive specimens.

The difficulty in recovering living organisms from surgical lymph nodes was described also by Wilmot et al. (11). More than 80% of histologically positive lymph nodes did not yield growth, and the authors reported the finding of a bacteriostatic substance in nodes of infected animals. More recently, Wichelhaus and co-workers (10) recommended liquid medium for the isolation of organisms that may be present in small numbers in uncontaminated clinical specimens. They suggested that whenever the diagnosis of tuberculosis is considered, Dubos liquid medium be used for such specimens as it will support the growth not only of mycobacteria but also of other microorganisms and pathogenic fungi. It will penetrate the specimens and will allow subsequent growth of organisms from the tissue into the surrounding fluid.

The overwhelming majority of our more than 4,000 surgical specimens were not tuberculous, but infected or contaminated with non-acid-fast organisms. The specimens had been collected for a variety of laboratory investigations, one of them to rule out mycobacterial disease. In con-
contrast to L-J medium, 7H-9 broth is particularly prone to overgrowth with non-acid-fast organisms and has not been suitable, in our hands, for the primary isolation of mycobacteria from untreated specimens as recommended by Kubica and Dye (6) and Wichelhausen and coworkers (10).

However, surgeons are encouraged to inoculate L-J slants or 7H-9 broth or both with tissue or fluid immediately after surgical intervention if mycobacterial disease is suspected.

The large portion of 16 M. intracellulare isolates from lung biopsy specimens confirms a report from this laboratory in 1973 (8) in which this mycobacterial species was described as the most prevalent acid-fast pathogen after M. tuberculosis in the greater Boston area.

Our predominantly adult patient population might explain the rather low number of atypical mycobacterial isolates from cervical lymph nodes. M. tuberculosis appears to be the cause of most cases of cervical lymphadenitis in adults in contrast to that disease in children (1, 11). Three adult patients with cervical lymphadenitis also had sputum cultures positive for M. tuberculosis.

Half of the mycobacteria isolates from skin and abscesses were mycobacteria other than M. tuberculosis: two M. marinum, two M. intracellulare, one M. bovis, and one M. bovis (BCG). In contrast, almost all isolates from synovium, bone and disk, the genital tract, bone marrow, pericardium, liver, kidney, the gastrointestinal tract, and epiglottis were strains of M. tuberculosis.

M. vaccae and M. gordonae have not been associated, to our knowledge, with disease in humans. However, M. vaccae (in pleural fluid) and two strains of M. gordonae (one each in pleural fluid and bone) were isolated and identified by their biochemical characteristics, colony morphology, and yellow pigmentation (9). They were probably introduced as contaminants, but we hasten to exclude the possibility of laboratory contamination. The laboratory staff scrupulously conducts all transfers or manipulations of organisms with cotton-plugged Pasteur pipettes with individual pipetting aids or individual loops for every specimen or culture, respectively.

Furthermore, the isolation of 25 strains of M. intracellulare from surgical specimens has no connection with the appearance of a particular pigmented strain of M. intracellulare in the Boston drinking water in 1977 and 1978 which confused the laboratory diagnosis of mycobacterial disease when it was grown numerous times from sputum and urine specimens (G. C. du Moulin, G. Friedland, K. Indorato, and K. D. Stottmeier, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, Q70, p. 206).

In conclusion, the addition of Middlebrook 7H-9 broth to sets of L-J medium slants enhances the recovery rate of M. tuberculosis from surgical tissue and fluid specimens.

LITERATURE CITED