Preservation of Tissue Specimens During Transport to Mycobacteriology Laboratories

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Chloramine-T and sodium borate solutions were evaluated for their effectiveness in preserving Mycobacterium bovis and controlling the growth of non-mycobacterial contaminants on tissue specimens during transport to laboratories. The number of culturable M. bovis cells in suspension was reduced by $5.1 \log_{10}$ upon exposure to chloramine-T solution and by $<1 \log_{10}$ upon exposure to sodium borate solution for 7 days. Reinoculation of laboratory media (because of overgrowth by non-mycobacterial contaminants) was required for 52.6% of 190 routine bovine tissue specimens shipped refrigerated in chloramine-T solution and for 6.1% of 520 specimens shipped unrefrigerated in sodium borate solution. M. bovis was isolated from bovine tissue stored in sodium borate solution at 23°C for 17 weeks and at 4°C for 25 weeks. Unrefrigerated sodium borate solution has been used successfully to ship tissue specimens to our laboratory for the past 11 years.

The isolation and identification of mycobacteria from tissue specimens from domestic animals are important factors in the eradication of bovine tuberculosis in the United States, and proper preservation of specimens during shipping to laboratories is essential for the successful isolation of mycobacteria. A solution of chloramine-T (CT) was used by the U.S. Department of Agriculture from 1964 to 1971, when the CT solution was replaced by a saturated sodium borate (SB) solution.

The CT solution was an effective preservative only when the specimen was refrigerated (1). This required the use of a can of frozen water and a large, expanded, polystyrene shipping container (12 by 12 by 15 in. [30.48 by 30.48 by 38.10 cm]) to provide sufficient insulation. The use of SB has eliminated the need for refrigeration, thus permitting the use of a smaller shipping container (3.25 by 5.37 by 7.75 in. [8.25 by 13.64 by 19.68 cm]) which fits in a standard U.S. mailbox.

The purpose of this paper is to describe the comparative effectiveness of CT and SB in preserving mycobacteria and controlling the growth of non-mycobacterial contaminants (NMC) on tissue specimens during transport to laboratories. Comparisons were made with suspensions of bacterial cultures and tissues of infected cattle.

MATERIALS AND METHODS

Test microorganisms. Mycobacterium bovis 878 was acquired from the Bureau of Animal Industry Laboratories, Beltsville, Md. It was used in the in vitro evaluation of preservatives in direct contact with culture suspensions.

Media. Media routinely used for the isolation of mycobacteria from animal tissues were used in this investigation: Lowenstein-Jensen, Stonebrink, Herrold, and Middlebrook 7H-10 (7H-10) (4). Nutrient agar with 0.5% NaCl was used to propagate naturally occurring NMC recovered from tissue specimens, and 7H-10 agar was used to culture M. bovis surviving the direct in vitro exposure to preservatives.

Preservatives. CT (sodium p-toluenesulfonchloramide; Mallinckrodt Inc., St. Louis, Mo. was used at 90 mg per 100 ml [wt/vol] of distilled water. Sodium tetraborate decahydrate (Borax; powder form: U.S. Borax and Chemical Corp., Los Angeles, Calif.) was used at 16.6 g in 100 ml of distilled water to obtain a saturated solution. Approximately 8 mm of undissolved Na$_2$B$_4$O$_7$ remained in the bottom of each 4-oz. (120-ml) specimen jar. (The pentahydrate form should not be used because the crystal of undissolved Na$_2$B$_4$O$_7$·5H$_2$O frequently breaks the glass jars as it expands during hydration to the decahydrate form.) Other manufactured products such as Borateem and Boraxo (both from U.S. Borax and Chemical Corp.) were not used because of additives which may be harmful to mycobacteria.

Biometrical analysis. The results of tests involving culture suspensions were evaluated by the analysis of variance. The other test results were evaluated by a simple analysis of the data.

Contact of culture and preservative. M. bovis 878 grown on 7H-10 agar in petri dishes for 4 weeks at 37°C was harvested with Butterfield buffer (4) to obtain a smooth, dense bacterial suspension. A 1-ml amount of the suspension was mixed with 9.0 ml of SB, 9.0 ml of CT, or 9.0 ml of sterile distilled water. A portion of the distilled water suspension was serially diluted and
plated immediately on 7H-10 agar in glass petri dishes (15 by 100 mm) by a surface plating method (3). Serial 10-fold dilutions of the three test suspensions were plated after 3 and 7 days exposure at room temperature (RT), approximately 23°C. The inoculated media were incubated at 37°C for 2 weeks. All tests were performed in duplicate. Plates with 3 to 50 colonies per 0.01-ml drop were counted.

**Field trials.** Veterinary meat inspectors at slaughter plants collected granulomatous lymph nodes (predominately bronchial and mediastinal) from 100 cattle removed from tuberculous herds. Each specimen was equally divided. One half was placed in a 4-oz. (120-ml) jar of SB, and the other half was placed in a 4-oz. (120-ml) jar of CT. The CT-preserved specimens were shipped to the laboratory in refrigerated, expanded, polystyrene containers. The SB-preserved specimens were shipped in the same type of containers but without refrigeration. All specimens were in transport for 4 days (containers which arrived in less time were held unopened at RT until 4 days had elapsed). Each specimen was cultured in the routine manner for the isolation of mycobacteria.

**Reinoculation.** Medium slants were reinoculated with reserve tissue suspension when four or more of the eight medium slants were overgrown by NMC. The reserve tissue suspension was retreated as in the routine method for the isolation of mycobacteria (4) with 0.5 N NaOH or 0.2% Zephiran (Winthrop Laboratories, New York, N.Y.) for 10 min. A record was kept of the reinoculations required for the 100 condemned cattle and 711 routine cattle submissions received during the 7-month transition period (CT to SB).

**Extended storage of bovine tissues.** Tissues for extended storage were selected from bovine specimens submitted for routine culturing during a 42-week period. Specimens with lesions and of a volume of 10 to 40 ml were divided for routine processing and extended storage at RT in SB. Histopathological findings were compatible for mycobacteriosis, and *M. bovis* had been previously isolated from all tissues used in this test.

**RESULTS**

**Direct contact of culture and disinfectant.** The number of viable *M. bovis* cells per milliliter was reduced by <1 log₁₀ upon exposure to SB or water for 3 and 7 days at RT. Exposure to CT for 3 days caused a reduction of *M. bovis* counts of 5.3 log₁₀, and exposure for 7 days caused a 5.1 log₁₀ reduction (Table 1).

![Table 1](https://jcm.asm.org/)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CFU of <em>M. bovis</em> per ml at:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 days</td>
</tr>
<tr>
<td>Water</td>
<td>1.8 × 10⁶</td>
</tr>
<tr>
<td></td>
<td>1.9 × 10⁶</td>
</tr>
<tr>
<td>SB</td>
<td>1.0 × 10⁶</td>
</tr>
<tr>
<td></td>
<td>1.0 × 10⁶</td>
</tr>
<tr>
<td>CT</td>
<td>9.8 × 10⁴</td>
</tr>
<tr>
<td></td>
<td>5.0 × 10⁴</td>
</tr>
</tbody>
</table>

* M. bovis CFU per ml, initial count (before treatment) 3.9 × 10⁶ CFU.
* A duplicate test was made of each treatment.

The media was necessary for 26 of the condemned cattle specimens shipped refrigerated in CT but for only 15 specimens shipped unrefrigerated in SB. Media inoculated with 32 (6.1%) of 521 unrefrigerated, SB-preserved specimens required reinoculation, whereas media inoculated with 100 (52.6%) of 190 specimens shipped refrigerated in CT required reinoculation.

**Extended storage of field specimens.** *M. bovis* was isolated from all nine specimens stored in SB at RT for 8 weeks or less. No isolation was made from the single specimen stored for 14 weeks, but *M. bovis* was isolated from the single specimen stored for 17 weeks. No isolation was made from the 10 remaining specimens stored for random periods of time ranging from 24 to 42 weeks.

In a subsequent (and similar) test, *M. bovis* was isolated from bovine bronchial lymph nodes after storage at 4°C for 25 weeks.

**DISCUSSION**

The chemical agent used to preserve specimens from tuberculous animals during shipment to laboratories must minimize the multiplication of NMC without significantly reducing the culturability of *M. bovis*. These criteria were met by SB but not by CT.

The term reinoculation used here refers to the supplemental treatment of the contaminated inoculum with 0.5 N NaOH or 0.2% Zephiran before the inoculation of additional media. Both agents are effective in controlling NMC but can also reduce the culturability of *M. bovis* (2). Therefore, the superiority of SB over CT is extended to include a potentially greater *M. bovis* isolation rate because fewer specimens shipped in SB require supplemental chemical treatment.

Based on the data presented here and on 11 years of satisfactory use in our laboratory, we recommend SB for preserving tissue specimens...
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during transport to laboratories for mycobacteriological examination. The maximum recommended storage time in SB at RT is 8 weeks.

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LITERATURE CITED