Microbial Contamination of Automated Analyzers

To the Editor:
Recently we have adapted some enzymatic methods to determine sugars, lipids, serum urea nitrogen, uric acid, and enzymes in serum. When these analyses are done with an automated analyzer that has automatic reagent dispensers, we must be wary of bacterial growth through the whole reagent-supply line: reagent bottles, transmission tubings, valves, etc.

Table 1. Sensitivity

<table>
<thead>
<tr>
<th>Organism</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas sp.</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Moraxella sp.</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Erwinia sp.</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavobacterium sp.</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Acinetobacter sp.</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>A, chloramphenicol; B, ampicillin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For example, Table 1 lists organisms that grew in the transmission tubing of a Technicon SMA 12/60, especially in its aminotransferase-containing channels. The presence of NADH and phosphate (pH 7.4) provides suitable environments for bacterial growth. If we forget to wash out the NADH line after every daily use, bacteria flourish during the night. Treatment with weak NaOH or a cleanser sometimes does not work, because the organisms may survive such treatment. If they form colonies in the glass coils or tubing, it is as if they were immobilized enzymes, and the enzymatic activity supposedly being measured may be erroneous. Table 1 also shows the sensitivities of the cultural organisms to two antibiotics.

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Rapid Micromethod for Acetaminophen Determination in Serum

To the Editor:
We have developed a high-performance liquid chromatographic method for determination of high therapeutic and toxic concentrations of acetaminophen in serum. We have adapted the procedure which Orcutt et al. (1) developed for determining serum theophylline. With the same mobile phase, C18 column, and monitoring the wavelength at 254 nm, acetaminophen elutes just before theophylline.

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More Efficient Thin-Layer Chromatography

To the Editor

In a recent Letter to the Editor [McIntyre et al., Clin Chem. 24, 171 (1978)] on more economical use of chromatographic plates for drug screening, the authors suggest a method that halves plate consumption.

The method suffers two inconveniences: the development must be watched fairly closely during the later period and the two halves must be developed separately.

These drawbacks are easily overcome by use of a method that also further decreases work time by about 1 h. This method has been used in our laboratory for about eight years, first for the semi-quantitative determination of meprobamate and during the last year for determination of the lecithin/sphingomyelin ratio. The plates are placed horizontally, and samples spotted at the end are simultaneously run towards the middle. A method is fast, requiring virtually no equilibration time, is free from distortions, and is economical of developing solution.

We have used 20 x 20 cm plates, either those of Merck or locally produced. Before samples are applied, the plate is scored across the middle. This score must penetrate the thin-layer completely and be broad enough to ensure that the solvent front will stop there. Samples are spotted along the two edges parallel to the score, about 1.5 cm from the edges. The plate is marked in one corner, to help in recognition of the spots.

The tank, made of stainless steel, is 24 cm wide, 25 cm long, and 1.5 cm high. The bottom is a 5-mm stainless-steel plate, to prevent torsions of the tank. The sides of this tank are 1 cm wide, the top surface of the sides being plane polished to fit almost perfectly against a glass plate that is used as the cover for the tank. Sealing is completed by applying water along the edges; the water disperses by the capillary effect. As wicks we use two 4-mm diameter rods, each 22 cm long, laid crosswise at each end of the tank and wrapped in one layer of filter paper. The wick-rods are placed so that the plates can be positioned upon them in a way that allows the developing liquid to pass through the application sites — i.e., the application sites are just medial to the wick-wrapped rods.

The tank must be leveled very carefully, otherwise the run becomes skewed.

Immediately before the development of the plate the developing solution is poured to cover the bottom of the tank. It is necessary to use an exact volume — experience will tell how much, as it depends on the dimensions of the tank.

The thin-layer plate is held with the fingertips — thin-layer side down and with the applied spots between the wicks — a few millimeters over the wicks for 2–3 seconds, then carefully laid down on the wicks. The glass plate cover is quickly placed on the tank and sealed by applying water along its edges.

Because the thin-layer plate rests on the wicks, the distance between its surface and the surface of the developing solution is short, and so equilibration time would be expected to be brief. That this is so is verified by the absence of distortions in the runs.

The development may now be allowed to complete without further attention. Each of the fronts can run no further than the middle of the plate. If the sealing is effective, there will be no evaporation from the surfaces and therefore no distortions, even during 6 to 8 h after the run has been finished.

To the advantages stated by McIntyre et al. may be added the savings in developing solution and more than 1 h saved in running each plate.

Significant drawbacks are that separation must be adequate in the 8 cm of solvent migration, and any problems encountered in producing the tank, although any good machine shop should be able to make it satisfactorily.

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