NOTES

SIMPLE METHOD FOR ESTIMATING SLIDE CULTURE SURVIVAL

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Postgate et al. (J. Gen. Microbiol. 24:15, 1961) described a slide culture method requiring a special metal annulus on a slide for measuring directly the percentage of survival of bacteria. Sufficient agar was added so that a small air chamber remained over the inoculum when a round coverslip was placed on top. Since annuli of metal, plastic, or glass were found to be of limited value, the following method was developed with good results.

Approximate hot-agar medium was passed through a membrane filter or centrifuged to remove debris and then autoclaved in small amounts for storage. A clean, alcohol-flamed no. 1 coverslip (22 by 50 mm) was placed on a disinfected level surface under a sterile petri dish lid. Approximately 0.05 ml of melted cell-free agar was rapidly pipetted in a smooth thin film to cover about 10 by 25 mm on the coverslip and was allowed to harden. If many cultures were to be examined, a piece of wet blotting paper under the lid kept the film from becoming too dry. From a 0.1-ml pipette, approximately 0.003 ml of a bacterial suspension (2 × 10^7 organisms/ml) was placed near one end of the agar film. The coverslip was held vertically for a few seconds to allow the droplet to flow down the agar surface and then was replaced under the petri dish lid to absorb.

The observation chamber was prepared from a piece of thick blotting paper (25 by 45 mm), such as those supplied as membrane filter pads. A center section (15 by 30 mm) was cut out with a sharp razor blade, leaving a flat, rectangular "doughnut." Holes may be cut to fit any shape of coverslip. The blotter chamber was fastened near one end of a clean slide by the capillary action of six drops of water. After the inoculum had been absorbed into the agar, the coverslip was inverted over the rectangular hole in the blotter chamber (Fig. 1). The agar film must be less than the thickness of the blotter, and the film plus coverslip should not exceed the working distance of the "high-dry" microscope objective. The slides were incubated in petri dishes at 37 C for 2 to 6 hr, depending on the medium and the prior treatment of the cells. Moribund populations require longer times for outgrowth. Divided (viable) and single (dead) cells in each field were counted separately with a 40 × phase-contrast objective, 12.5 × oculars with a Whipple eyepiece micrometer, and a green filter. A total of 300 clones per slide was counted in adjacent fields, avoiding the edges of the film.

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FIG. 1. Simple blotter chamber for slide culture.

Postgate et al. (J. Gen. Microbiol. 24:15, 1961) could not evaluate Escherichia coli because of "long ramifying Escherichia coli" because of "long ramifying Escherichia coli" because of "long ramifying Escherichia coli." but our culture, grown on HYT agar (Brettz, Can. J. Microbiol. 7:293, 1961) without dextrose or with 2% glycerol prior to washing, standardization, and frozen storage did not form filaments on HYT agar slide culture; cells from HYT plus 1% dextrose agar produced filaments both before and after frozen storage. In moribund populations, some cells swell only during the time required for most viable cells to divide; these cells

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eventually produce microcolonies and should be counted as viable. The substage condenser may need refocusing when changing from one mount to another, and, if moisture condenses on the bottom of the chamber during incubation, the coverslip can be moved easily to a dry blotter chamber taped to another slide for counting. Preparations not counted immediately after incubation may be placed in a refrigerator overnight without impairing the survival estimate.

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**EFFECT OF EXPOSURE OF ESCHERICHIA COLI TO STREPTOMYCIN ON ABILITY TO UNDERGO CELL DIVISION**

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In a previous report, the effect of streptomycin on the viability of sensitive *Escherichia coli* cells was described (Hurwitz, Rosano, and Landau, *J. Bacteriol.* 83:1210, 1962). Loss of viability was measured as a decline in the percentage of cells capable of forming macrocolonies on nutrient agar after streptomycin was removed by dilution of the cells in 0.85% NaCl. Such a measurement does not differentiate between the two possibilities: that the cells were incapable of undergoing any cell division at the time of plating, or that the cells could still undergo a number of cell divisions but stopped dividing before the colonies became large enough to be observed as macrocolonies. The present study was undertaken to distinguish between these two possibilities.

Cells growing in nutrient broth were exposed to 1 μg/ml of streptomycin for various periods of time. The cells were then immediately washed with saline on a Millipore filter, resuspended in saline, and a loopful was placed on a glass slide covered with a 1-mm film of nutrient agar. Microscopic observations were made in an Emedco incubation chamber kept at 37 C. A representative field was found under oil immersion, using phase optics, and was photographed at 30-min intervals. The percentage of cells unable to yield microcolonies could then be related to the percentage of cells unable to yield macrocolonies.

The following data show that the percentage of cells incapable of forming macrocolonies is the same as the percentage of cells unable to undergo even a single cell division.

In the first series, cells were momentarily exposed to 1 μg of streptomycin per ml. Figure 1a shows the field just after plating. Figure 1b shows the field after 90 min of incubation. All but one of the cells had undergone about three to four cell divisions.

In the second series, cells were exposed to 1 μg/ml of streptomycin for 17.5 min before washing and plating. After this exposure, only about 50% of the cells were capable of forming macrocolonies. Figure 2a shows the field just after plating, while Fig. 2b shows the field after 90 min of incubation. Two of the cells have formed microcolonies while two have been unable to undergo a single cell division. This field was representative of the rest of the bacteria on the slide.

As can be seen from Fig. 2a, the surviving cells had undergone only about two to three cell divisions during the 90-min incubation period, as compared with three to four cell divisions of the momentarily exposed cells. It would appear that exposure to streptomycin also increases the length of the lag time of surviving cells before the first cell division begins. Studies with other times of exposure to streptomycin are in accord with this observation.

In the third series, the cells were exposed to 1 μg/ml of streptomycin for 35 min. Less than 1% of the cells were capable of forming macrocolonies after this exposure (Hurwitz et al., 1962). Figure 3 shows the field after 90 min of incubation. None of the cells had undergone cell division. A search of the entire slide showed a few cells that did produce microcolonies.

Intermediate exposures, e.g., for 15 and 25