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## COMMENTS ON A NEW TEST FOR PENICILLIN IN MILK

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In connection with the current campaign for the elimination of penicillin from milk supplies, Arret and Kirschbaum (2) have described "a simplified and rapid method for detecting the presence of penicillin in milk in concentrations as low as 0.05 units per ml." Their method differs from the modified Difco method (1) principally in holding poured plates of seeded agar at approximately 15°C. (59°F.) for not less than three, or more than five, days. This modification is said to enable detection of penicillin in 2½ hours at 37°C.

To compare the new method with the "standard" procedure, several trials were made using both the Bacto B453 Standardized Spore Suspension of *Bacillus subtilis*, and of a spore suspension of *B. subtilis* ATCC #6633. Unfortunately, in each trial, after 3 days at 15°C., growth of the test organism was so extensive that no zones of inhibition could be expected when discs saturated with milk containing penicillin were "spotted" thereon and the plates incubated at 37°C. With two-day-old plates growth was not evident at the start, but no zone of inhibition appeared even with milk containing 0.1 unit of penicillin per ml. Thus there is a danger that negative results will be reported from milk containing amounts of penicillin detectable by other methods.

In the writer's experience, the method described by Arret and Kirschbaum is also less simple, less reliable and less sensitive than the modified Difco

method available for years. It is less simple in requiring (a) preparation of a spore suspension, when one is commercially available, (b) a low temperature incubator set at 15°C, and (c) an incubator at 37°C. (Neither of these two temperatures is usually available in dairy plant laboratories).

It is less reliable in that (a) in our hands the method, as described, failed to detect penicillin, (b) there is more likelihood of variation in sensitivity to penicillin with "home-made" spore suspension than with the commercially available ones, and (c) there is no warning that special flat-bottomed petri plates (Corning #3162) should be used, and special care taken to harden the agar layer on a perfectly level surface. It is less sensitive in that (a) it specifies the use of a 0.25" disc, whereas the 0.5" disc absorbs six times the volume of milk and will detect roughly one-fifth the concentration of antibiotic detectable by the smaller disc (4); the larger disc is also much easier to load uniformly by capillary absorption of 0.1 ml from a graduated 1 ml pipette held horizontally, and (b) it calls for the use of 10 ml of agar medium per plate, when the greater sensitivity of a thinner layer has been shown (4) and is generally recognized.

Speed in obtaining results in testing milk for antibiotics is desirable. However, no laboratory test will ever be as useful as a "marker" dye incorporated into the antibiotic preparation (3, 5) which would

permit instant recognition and rejection of such milk. Failing this, a difference of half an hour or so in obtaining results does not seem to be vital. Most workers would prefer a method simpler and more sensitive than that of Arret and Kirschbaum, even if it required an hour longer incubation. The writer's experience has been that using the modified Difco method (1) and heat-shocking the spores in the melted agar by holding at 70°C. for 15 minutes, zones of inhibition can be detected in less than 3 hours.

For many laboratories it would have been helpful if Arret and Kirschbaum had indicated that the agar medium they recommend is available in prepared form as Bacto Penassay Seed Agar or as B. B. L. Penicillin Assay Seed Agar, and that standardized spore suspensions and penicillinase-containing discs are commercially available. Time is money, and time can certainly be saved by utilizing these commercially available products.

There is an urgent need for a rapid method of detecting antibiotics in milk, so that such milk will not be used for human consumption. The suggested use of the Arret and Kirschbaum method for field testing, wherein a dairy technician would "trace the source of milk containing penicillin by carrying a portable incubator and refrigerator in a car or truck and testing a milk sample on the farm" (2) appears to be impracticable. Surely more would be accomplished

by testing milk on arrival at the plant by the modified Difco method (1) or some other simple method, and notifying offending producers that their milk would not be accepted for the next two days.

#### ADDENDUM

Since this was written, Dr. Kirschbaum, in a letter dated December 18, recommends that seeded agar plates be held under refrigeration for 3 days before using. This appears to remove any possible reason for using this test rather than the modified Difco method (1).

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