

3/ MINNESOTA DAIRY PRODUCTS PROCESSOR



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We recently looked at a new procedure for detecting penicillin in milk. In the process, with assistance from Dairy Quality Control Institute, Inc., we also evaluated the incidence of residues as measured by three different procedures. These included the standard Disc Assay (DA) method, the Sarcina lutea Cylinder Plate method (CP), and the Delvotest-P procedure.⁽¹⁾ The latter is a faster and somewhat simpler test than either of the other two.

WHAT WE DID

Raw milk samples were collected from three different categories of milk supplies: (1) grade A, (2) manufacturing grade bulk milk, and (3) manufacturing grade can milk. Disc Assay and Delvotest-P determinations were made on all samples. Those samples found to be positive were further tested by the Cylinder Plate method. A total of 288 milk samples were tested, 25 samples from each supply type being taken and analyzed each week over a 4-week interval.

Besides raw milk samples, a number of finished product samples were also analyzed.

SOME CHARACTERISTICS OF THE TEST METHODS

One of the chief advantages of the DA method is the sensitivity to a wide range of inhibitors. It is also a fairly simple, reasonably rapid method. However, it lacks the sensitivity of the CP method. The CP method is a more complicated procedure, and slower, but considerably more sensitive to penicillin. (The DA is considered sensitive to 0.05 units of penicillin, the CP to 0.00625 units, almost a 10-fold difference).

The primary advantages of the Delvotest-P method lie in the simplicity, speed, and sensitivity. Test kits consist of seeded (Bacillus stearothermophilis var. calidolactis) agar ampules, tablets of nutrient medium and indicators, and disposable sampling syringes. Ampules are broken, a nutrient/indicator tablet added, milk sample added, and the ampule incubated in a water bath ($64^{\circ}\text{C} \pm 2^{\circ}\text{C}$) for $2\frac{1}{2}$ hours. If no color change (from the original purple) takes place, the sample is considered positive for antibiotic: a yellow color is negative, a

⁽¹⁾ Developed and marketed by Gist-Brocades NV, P.O. Box 1, Delft, Holland.

yellow/purple coloring effect is questionable. The limit of detectability is about 0.002 units.

WHAT WE FOUND

Data in table 1 show the incidence of antibiotic (confirmed in all cases as penicillin) as detected by two of the three methods for three different milk supplies. Positive samples were fairly evenly distributed among the different supplies of milk. Only one positive (0.34%) sample was identified by DA, two (0.7%) by CP, and seven (2.4%) by the Delvotest-P method. An additional sample gave a \pm reading by the latter procedure. It seems clear, then, that the Delvotest-P method is considerably more sensitive than the other commonly used procedures. As such it would appear to offer special advantage in detection of antibiotics in pooled supplies of milk. In this study it was not found to yield false positive results in rancid milk (up to an Acid Degree Value of 4.75) but, according to the manufacturer, the test might yield false negative reactions in presence of rancidity (free fatty acids). By dilution, quantitative estimates of penicillin can be made. In this study positive samples ranged from 0.002 to about 0.02 units of penicillin.

RESULTS ON FINISHED PRODUCTS

Analysis of cheddar cheese, lactose, whey powder, and whey cream made from portions of the milk supplies tested in this investigation showed no positive results. Delactosed whey was found to yield false positive results in all cases, probably due to high mineral content. While no positive samples were identified, the Delvotest-P method was (and can be) applied to nonfat dry milk.

For this study we used the following sample dilution rates: nonfat dry milk, 10 g of sample to 30 mls water; lactose, whey powder, delactosed whey powder, one g of dried product to 10 mls water; whey cream, 0.2 ml of product to 1.8 mls of water; cheddar cheese, 0.2 g to 2 mls water. Cheese samples were blended with water prior to sampling. The above dilution rates consistently proved satisfactory.

Table 1. Incidence of penicillin residues in samples of grade A bulk and manufacturing can and bulk raw milk supplies as detected by the Disc Assay and Delvotest-P methods⁽¹⁾

<u>Week no.</u>	<u>Numbers of positive samples in each class</u>					
	<u>Disc Assay</u>			<u>Delvotest-P</u>		
	<u>Grade A</u>	<u>Mfg can</u>	<u>Mfg bulk</u>	<u>Grade A</u>	<u>Mfg can</u>	<u>Mfg bulk</u>
1	0	0	0	1	1	1
2	0	0	0	1	0	1 (+/-)
3	0	1	0	0	2	0
4	0	0	0	1	0	0

(1) Twenty-four samples of each class were collected and analyzed each week, for a total of 288 samples over a month-long period.

Mention of trade names in this publication does not imply endorsement nor does failure to mention a name imply criticism by the Minnesota Agricultural Extension Service.

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