# THE USE OF THE 0.01 ML LOOP IN THE PLATE LOOP METHOD FOR MAKING VIABLE COUNTS OF MILK[1]

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SUMMARY

A plate loop count method for determining viable counts of pasteurized milk products is described. The technique uses a 0.01 ml calibrated platinum alloy loop attached to a continuous pipetting syringe for rinsing the 0.01 ml of sample into a petri dish with 1 ml of sterile buffered dilution water prior to pouring with agar. Results with this method indicate that accuracy within ± 15 percent is attainable on samples of skim, 2 percent, homogenized, whole milk, and half and half. Measurements of chocolate milk were not accurate.

The plate loop count (PLC) method using a 0.001 ml calibrated loop has been used for determining viable counts of raw milk since it was introduced by Thompson, Donnelly and Black *(7)* in 1960. Approved milk laboratories in at least half of the states are now using the PLC on raw milk samples.

The principle of using a loop for measurement was reported by Burri *(2)* in 1928 for inoculating agar slants in making viable counts of milk. Robertson *(4)* found wide variations in making replicate measurements of the same sample with the same loop.

It now seems possible to attain acceptable accuracy and uniformity in measuring milk volumes with a calibrated loop. The authors *(7)* showed close comparisons between the standard plate count (SPC) and PLC methods on Grade A raw milk when a 0.001 ml loop was used. Tatini, Dabbah and Olson *(6)* found good agreement between the SPC and PLC only when the counts were equal to or less than 100,000 per ml when the 0.001 ml loop was used on manufacturing grade raw milk.

Experience by the authors in Cincinnati, Ohio, and in Wisconsin in evaluating milk laboratories has shown that the speed of loop withdrawal is critical in obtaining accuracy with the PLC. Jasper and Dellinger *(3)* showed that a 0.01 ml loop can be used accurately for making milk smears if the loop is removed vertically from the sample at the correct speed.

In this report, the use of a 0.01 ml calibrated loop attached to a continuous pipetting syringe is described for making viable counts of pasteurized milk. The accuracy of this method is determined by comparing replicate PLC platings with replicate platings of the Standard Plate Count (SPC) on specially prepared test samples as well as by weighings of samples adhering to the loop.

## MATERIALS AND EQUIPMENT

The equipment used for the PLC (0.01 ml) differs from the original PLC method in several ways other than loop size: The cannula is 14 gauge with a longer barrel. The wire shank attached to the loop is inserted into the barrel of the cannula with the loop extending straight out from the end of the barrel. No bend is placed in the exposed portion of the shank. This design permits a more forceful rinsing of the loop.

*(a)* Loop, true circle of No. 19 B & S gauge welded platinum-rhodium wire 4 mm inside diameter attached to a 50 mm wire shank (Arthur H. Thomas Co., Cat. No. 7433-A[3] or equivalent). A slight bend is placed in the wire shank to hold it in place when inserted in barrel of cannula. It is essential that the loop have a smooth weld and be free rinsing.

*(b)* Cannula, 14 gauge Becton-Dickinson No. 1250 NR sawed off about 60 mm from point where barrel enters hub.

*(c)* Cornwall continuous pipetting outfit, adjustable, 2 ml size, Becton-Dickinson No. 1251.

The above parts are assembled in the following manner: The wire shank of the loop is inserted into the barrel of the cannula to a point where the center of the loop extends about 18 mm from the end of the barrel as shown in Figure 1. The luer-lok hub is attached to the luer-lok fitting on the Cornwall continuous pipetting outfit. This apparatus is sterilized by autoclaving for 10-15 minutes at 121 C or by submerging parts in boiling water for 10 minutes. The loop and shank may be flame sterilized.

Optional equipment: metronome, Seth Thomas or equivalent.

[1]Work on this project was initiated while the senior author was on active duty as an army reserve officer at the Robert A. Taft Sanitary Engineering Center. The experiments were completed at the Wisconsin State Laboratory of Hygiene, Madison.

[2]Official laboratory for the Wisconsin State Board of Health.

[3]Mention of commercial products does not imply endorsement by the Public Health Service.

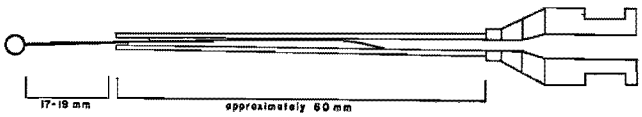


Figure 1. Loop and wire shank attached to the sawed off cannula.

## EXPERIMENTAL PROCEDURE

Figure 2 shows the assembled 0.01 ml transfer and measuring instrument ready for use. The end of the rubber supply tube attached to the syringe is placed in a bottle of sterile, phosphate-buffered dilution water *(5)*. The syringe plunger is depressed rapidly several times to pump the water into the glass syringe. Squeezing the rubber tubing near the valve between the thumb and forefinger intermittently while pumping the plunger hastens priming. The syringe is adjusted to deliver 1 ml of sterile buffered dilution water with each depression of the plunger. Care is taken to have the loop positioned in the barrel of the cannula so the water flows rapidly directly across its center.

In examining a series of samples and before initial transfer is made, the loop is briefly flamed, preferably in a clean, high temperature gas flame and cooled with a stream of sterile dilution water by depressing the plunger. The loop is carefully dipped into the milk sample 3 times (avoiding foam) to 1-2 mm above the loop. The loop is withdrawn *vertically* from the surface of the sample (see Figure 3). The water droplet that comes off the loop during the initial dip is avoided by moving the loop to a different location on the surface of the sample for the final withdrawal. The movement is a *uniform* up and down movement of about 1.5 inches.

The speed of the final withdrawal governs the amount of product adhering to the loop. Each upward movement is made at the speed of 55-65 beats per minute. Removing the

TABLE 1. REPLICATE WEIGHTS (MILLIGRAMS) OF VARIOUS MILK PRODUCTS ADHERING TO A 0.01 ML CALIBRATED PLATINUM ALLOY LOOP

| | Skim | 2% | Homo | ½ & ½ | Chocolate milk | Lab. past. whole milk |
|---|---|---|---|---|---|---|
| Brand A | 11.2 | 8.9 | 9.8 | 12.3 | 13.7 | 9.9 |
| | 10.2 | 9.7 | 8.7 | 10.1 | 12.4 | 11.1 |
| | 11.0 | 10.2 | 8.9 | 10.8 | 14.5 | 12.6 |
| | 8.9 | 10.2 | 10.5 | 11.0 | 15.7 | 10.3 |
| | 10.3 | 11.3 | 9.8 | 11.1 | 15.7 | 11.4 |
| | 10.4 | 10.1 | 12.4 | 10.4 | 15.2 | 12.0 |
| | 10.0 | 10.2 | 13.2 | 9.9 | 15.5 | 9.3 |
| | 10.5 | 11.7 | 10.4 | 9.9 | 14.5 | 10.9 |
| Mean | 10.3 | 10.3 | 10.5 | 10.7 | 14.7 | 10.9 |
| Coefficient of Variation | 6.8% | 8.5% | 15.1% | 7.5% | 7.8% | 10.0% |
| Brand B | 12.3 | 9.9 | 8.4 | 11.4 | 16.5 | 9.7 |
| | 10.7 | 10.8 | 9.2 | 9.3 | 16.4 | 11.1 |
| | 10.7 | 9.6 | 10.1 | 11.5 | 15.1 | 8.6 |
| | 9.8 | 10.3 | 9.3 | 10.4 | 16.5 | 10.7 |
| | 11.7 | 10.6 | 9.6 | 11.2 | 16.6 | 11.0 |
| | 11.4 | 10.0 | 10.3 | 9.0 | 17.2 | 10.9 |
| | 10.5 | 12.2 | 10.5 | 10.8 | 16.8 | 9.1 |
| | 10.2 | 10.5 | 9.8 | 10.0 | 15.4 | 9.8 |
| Mean | 10.9 | 10.5 | 9.7 | 10.5 | 16.3 | 10.1 |
| Coefficient of Variation | 7.6% | 7.6% | 7.0% | 9.0% | 4.3% | 9.4% |
| Brand C | 10.3 | 10.1 | 8.8 | 11.4 | 13.5 | 9.9 |
| | 10.7 | 9.8 | 9.0 | 10.2 | 11.0 | 11.3 |
| | 12.1 | 9.6 | 10.4 | 12.1 | 11.3 | 10.5 |
| | 11.3 | 9.1 | 9.6 | 10.2 | 12.5 | 10.2 |
| | 11.9 | 10.4 | 11.6 | 11.4 | 12.4 | 9.2 |
| | 12.3 | 11.7 | 11.2 | 10.9 | 16.1 | 9.6 |
| | 10.4 | 10.8 | 11.1 | 12.1 | 15.5 | 9.0 |
| | 12.6 | 10.7 | 9.7 | 10.9 | 15.1 | 10.9 |
| Mean | 11.5 | 10.3 | 10.2 | 11.2 | 13.4 | 10.1 |
| Coefficient of Variation | 7.8% | 7.9% | 10.4% | 6.7% | 14.6% | 8.0% |

TABLE 2. COMPARISON OF SPC AND *PLC* REPLICATE PLATINGS OF THREE SPECIALLY PREPARED TEST SAMPLES (COLONIES PER PLATE)

| | Sample 1 | | Sample 2 | | Sample 3 | |
|---|---|---|---|---|---|---|
| | SPC 1:100 | PLC | SPC 1:100 | PLC | SPC 1:100 | PLC |
| | 102 | 109 | 143 | 89 | 102 | 103 |
| | 100 | 91 | 104 | 113 | 117 | 129 |
| | 111 | 93 | 103 | 109 | 130 | 115 |
| | 102 | 110 | 100 | 95 | 130 | 117 |
| | 97 | 92 | 110 | 84 | 114 | 108 |
| | 110 | 91 | 118 | 102 | 120 | 115 |
| | 116 | 109 | 117 | 96 | 114 | 101 |
| | 112 | 94 | 102 | 98 | 133 | 138 |
| Mean | 106 | 97 | 112 | 98 | 120 | 116 |
| Variation from SPC | | −8.5% | | −12.5% | | −3.3% |
| Coefficient of Variation | 6.0% | 9.0% | 12.8% | 9.9% | 8.8% | 10.8% |

loop slowly causes less than 0.01 ml to adhere. Jerking the loop out rapidly causes more than 0.01 ml to adhere. A metronome may be used to establish uniform timing. Samples are plated by raising the cover of the petri dish and rinsing the sample into the dish by depressing the plunger of the syringe causing water to flow rapidly across the center of the loop (see Figure 4).

The accuracy and uniformity of the loop measurement was determined by use of a Mettler (Type B) balance. The weight of the dry loop was subtracted from the weight of the loop dipped in the various milk products. The dipping technique described previously was followed carefully. Replicate weighings were made for each product.

The accuracy and uniformity of the loop was also checked by making replicate PLC and SPC plates on specially prepared test samples. Fresh samples of raw whole milk were collected on separate days from large storage tanks and pasteurized in the laboratory. These samples were inoculated with a pure culture of bacteria to obtain approximately 100-140 colonies on the 1:100 dilution.

Control plates were made to test whether or not the loop was free rinsing. These were made by discharging a loopful of various milk products from the loop followed by flushing the discharged loop again into a second petri dish. The plates were poured with Standard Methods agar and incubated 48 hours at 32 C.

## RESULTS

Eighteen samples of pasteurized milk products were collected over a period of several weeks. The products were from major dairy companies, plus three laboratory-pasteurized whole milk samples. Eight replicate weighings were made of each sample for a total of 144 weighings. The mean weight and coefficient of variation for each sample was determined. The approximate weight of 0.01 ml of the non-chocolate products was calculated to be 10.3 mg. Chocolate milk was estimated to weigh 10.6 mg per 0.01 ml. The results of actual weight determinations are shown in Table 1. The means of all of the non-

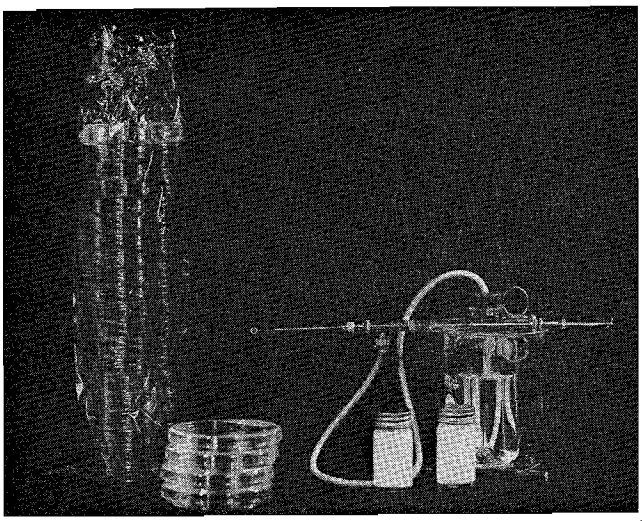

Figure 2. Transfer and measuring instrument assembled and ready for use.

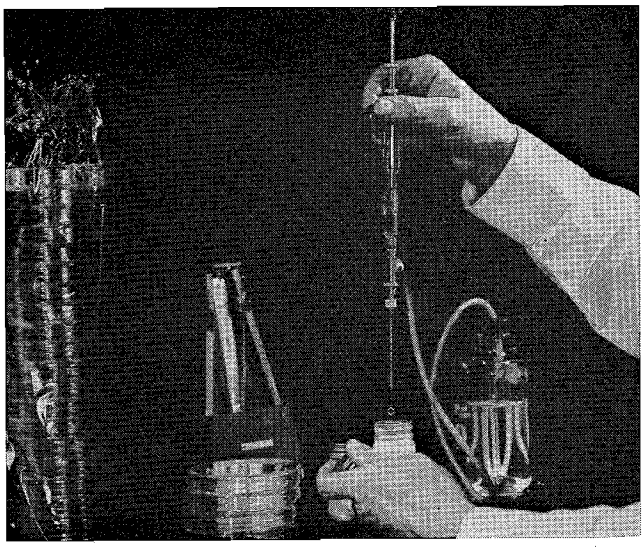

Figure 3. Loop being dipped into milk vertically. A metronome may be used to establish accurate technique. Good illumination is essential.

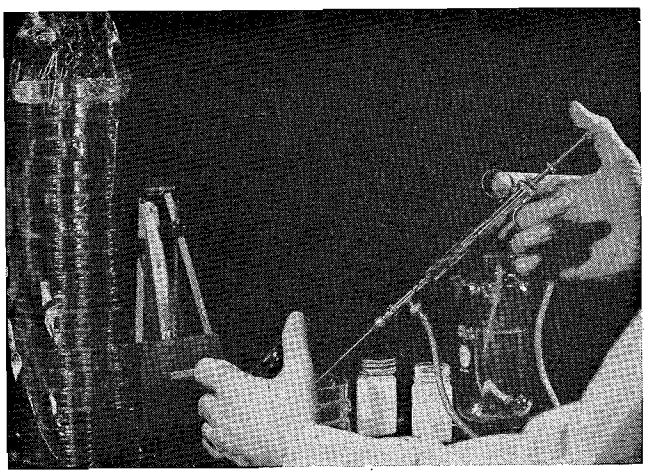

Figure 4. Washing the 0.01 ml milk sample into the petri dish.

chocolate products fell well within ± 15 percent of the 10.3 mg expected. Only sixteen of the 120 individual weighings of non-chocolate products fell outside the ± 15 percent range. The coefficients of variation for all products were less than 10 percent in all but three of the eighteen samples. The weights of chocolate milk indicate that excessive amounts of this product adhere to the loop.

Three test samples were used to compare the PLC with the SPC. Eight replicate plates were made with the loop method and eight replicate plates were made with the 1:100 dilution. The mean count and coefficients of variation were determined. These results are shown in Table 2. The means of each method were well within ± 15 percent of each other in all three trials. The coefficients of variation indicated acceptable uniformity.

Control plates (rinse) were made after discharging relatively more viscous chocolate milk as well as non-chocolate products from the loop. The samples contained at least 100 colony-producing bacteria per 0.01 ml. None of the total of 50 plates contained more than one colony.

## DISCUSSION

One of the items of concern in adapting the 0.01 ml loop to the PLC was the rinsing characteristic of the loop. Control plates indicate that smoothly-welded loops are free rinsing, and flaming between samples should not be necessary.

Analysts may check their proficiency by making replicate weighings on an analytical balance or by making replicate PLC and SPC plates on test samples. Mean weights within ± 15 percent of 10.3 mg are attainable. PLC means within ± 15 percent of SPC means are likewise attainable. The coefficients of variation should not exceed 15 percent.

If inaccurate results are experienced, several causes of error may be checked: The correct speed of withdrawal is of major importance and may be established by use of a metronome set at about 55-65 beats per minute. The angle of withdrawal should be 90° to the liquid surface and the distance of the up and down movements should be uniform (about 1.5 inches). The depth to which the loop is submerged should be 1-2 mm.

Further work will be needed to determine the suitability of using the 0.01 ml loop adaptation of the PLC or similar measuring devices (1) for making official viable counts of milk. The results of the 0.01 ml loop measurements warrant consideration by future APHA committees for inclusion of this method in Standard Methods for the Examination of Dairy Products.

Industry laboratories may find the method described here useful for routine control purposes.

## REFERENCES

1. Berridge, N. J. 1966. Reproducible Volume Delivered by an 0.01 ml "Loop". J. Milk and Food Technol. 29:371.

2. Burri, R. 1928. The Quantitative Smear–Culture: A Simple Means for the Bacteriological Examination of Milk. Proc. World's Dairy Congress, London, p. 690.

3. Jasper, D. E. and Dellinger, J. 1966. Variations in Volume of Milk Delivered by a Standard 0.01 ml Loop. J. Milk and Food Technol. 29:199-200.

4. Robertson, A. H. 1941. Bacterial Counts of Milk and Cream, Re: Loop Transfers. New York Dept. Agr. and Markets, Ann. Rep., p. 71.

5. Standard Methods for the Examininations of Dairy Products, 11th Ed. 1960. Am. Public Health Assoc., Inc., New York, N. Y.

6. Tatini, S. R., Dabbah, R., and Olson, J. C., Jr. 1967. Comparison of Plate Loop and Agar Plate Methods for Bacteriological Examination of Manufacturing Grade Raw Milk. J. Milk and Food Technol. 30:112-115.

7. Thompson, D. I., Donnelly, C. B., and Black, L. A. 1960. A Plate Loop Method for Determining Viable Counts of Raw Milk. J. Milk and Food Technol. 23:167-171.