VARIATIONS IN VOLUME OF MILK DELIVERED BY A STANDARD 0.01 ML LOOP

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SUMMARY

Milk smears were prepared and the weights of milk delivered were determined following use of the syringe and use of the 0.01 ml loop. Large and significant increases in the volume of milk delivered resulted when the angle of the loop with the milk was 20° rather than 90° or when the flat surface of the loop was brought out of the milk in the manner of a dipper. Slow withdrawal of the loop resulted in loss of milk and delivery of small volumes. Delivery of 0.01 ml of milk required that the loop be held in a vertical position and withdrawn quickly, but not with forced rapidity.

The platinum loop, calibrated to deliver 0.01 ml of milk, has been used extensively for counting leukocytes in milk *(1, 3, 5)*. Some limitations have been encountered, particularly the tendency for the loop to pick up an excessive volume of cell-laden fat from the suface of poorly mixed milk *(1, 6)*. With well-mixed milk, leukocyte counts from smears made with the loop using carefully standardized techniques were found not to differ significantly from those made with the syringe[1] *(6)*.

Standard procedure calls for the loop to be withdrawn vertically from the milk *(1, 3)*. It has been observed, however, that workers from different milk quality control laboratories may withdraw the loop in differing ways so that the volumes of milk removed may vary substantially *(2)*. During the course of these studies, it also became evident that the speed with which the loop was withdrawn influenced the volume of milk delivered.

When the milk container is level, the loop is easily withdrawn vertically at an angle of about 90° to the surface of the milk, or it may be withdrawn at angles considerably smaller. When the milk container is tipped, it becomes convenient to withdraw the loop at an angle of about 20°. With the container tipped, the loop may also be used as a "dipper" by bringing it out with the broad surface almost horizontal with that of the milk. Tendencies toward use of each of these procedures have been noted in general laboratory practice.

Because leukocyte counts have become a vital part of many milk quality control programs, any causes of error inherent in use of the loop should be understood. The following observations were made in order to evaluate the nature and magnitude of differences in volume of milk delivered by the loop as a consequence of variations in the manner of withdrawal of the loop from the milk.

METHODS

Milk samples contained in two ounce bottles about two-thirds full were mixed by shaking back and forth 25 times. The milk was undisturbed for three to five minutes prior to each sampling.

Observations on the volume of milk delivered by each procedure were made on 31 smears prepared as follows:

1. After sampling the milk with a 0.01 ml syringe.
2. After withdrawal of the loop at an angle of 20° from the surface of the milk.
3. After bringing the flat surface of the loop upward through the milk while the shank of the loop is held at an angle of 20° from the surface of the milk (dipping).
4. After withdrawal of the loop in a position vertical to the milk surface at a slow leisurely pace.
5. After withdrawal of the loop vertically with care at medium speed.
6. After withdrawal of the loop vertically at a very rapid rate.
7. After withdrawal of the loop vertically, quickly, but without forced rapidity.

Round smears equal to one cm^2 surface area were made, with the loop on edge, on clean glass slides of known weight. Each slide was immediately reweighed, and the weight of the milk delivered to the smear determined by difference. Counts were made utilizing a working factor of 5,000 *(7)*.

Smears were made, weighed and counted in the same fashion after using the syringe to withdraw the milk and make the smears *(1, 4)*.

RESULTS

The syringe used was found to deliver 0.0101 ± 0.00018 g of milk which is slightly below the optimum value of 0.0103 g *(1, 4)*. The weight of milk delivered by the loop with each of the technics used was found to differ significantly from that obtained with the syringe (Table 1). The actual differences in volume were, however, small in the case of the loop when it was withdrawn quickly or even when it was withdrawn at a very rapid rate. When comparisons were made with a hypothetical syringe delivering 0.0103 ± 0.00018 g, the volume delivered by the loop when quickly withdrawn in a vertical position was not significantly different ($Z = -1.22$).

The volume of milk delivered was markedly and significantly reduced when the speed of withdrawal was reduced to a deliberate (medium) pace or to a slow leisurely pace. On the other hand, the volume of milk withdrawn was greatly and significantly increased when the angle of the loop with reference to the milk surface was reduced to 20° or when the flat surface of the loop was brought through the surface of the milk in the manner of a dipper (Table 1).

Leukocyte counts on these smears varied in a manner apparently proportional to the difference in weights. Because different milk samples of varying cell count were used, the data on counts were not suitable for statistical analysis.

DISCUSSION

Smears satisfactory for counting leukocytes can be made with the loop providing procedures are standardized. The

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TABLE 1. WEIGHTS OF MILK SMEARS MADE BY DIFFERENT METHODS

Method	Weight (g) range	Weight (g) mean	S.D.	Z
Syringe	0.0098-0.0105	0.0101	0.00018	
Loop 20°	0.0101-0.0151	0.0124	0.00151	— 8.64
Loop 20°-flat	0.0135-0.0203	0.0169	0.00185	—19.8
Loop 90°-slow	0.0061-0.0089	0.0074	0.00064	22.7
Loop 90°-medium	0.0081-0.0113	0.0094	0.00100	3.8
Loop 90°-rapid	0.0100-0.0124	0.0106	0.00064	— 4.2
Loop 90°-quickly	0.0096-0.0116	0.0104	0.00042	— 3.65

correct volume can be very nearly approximated when the loop is withdrawn directly upward with the shank at a 90° angle from the surface of the milk. As the angle between the loop and the surface of the milk narrows a greater volume is obtained. Volume is greatest when the flat surface of the loop is brought through the surface of the milk in a nearly horizontal position in the manner of a dipper. In the presence of a concentration of fat at the milk surface, the resulting discrepancy would be enhanced *(1, 6)*.

The variation induced by the speed of withdrawal is also important. Slow withdrawal allows milk to drain away while the loop is in contact with the milk surface so that less milk is retained. Even a moderately slow rate of withdrawal can be responsible for a major error. A medium rate of speed, which was inadvertantly somewhat slower than our routine rate because of extra care being taken, proved to be too slow since the volume removed was too small. When care was taken to withdraw the loop quickly, without hesitation, satisfactory volumes were attained. By forcing rapid withdrawal, a small error on the high side resulted. Error, which can be quite large, is most likely to occur from withdrawal of the loop at a rate of speed which is too slow since one is not naturally likely to withdraw the loop too fast for good results.

Errors which result from small variations in the quick or rapid withdrawal of the loop are small ($<$ than 5%) even though statistically significant. Error of this magnitude can be tolerated in most circumstances where cells in milk are counted, but the errors resulting from slower withdrawal or withdrawal with the loop at the wrong angle or in a wrong position are much too large to be tolerated.

Extreme differences in volume of milk held in the loop can be visualized. After proper withdrawal, the surface of the milk within the loop is flat. After withdrawal in a flat position, the milk surface tends to be convex. After slow withdrawal, the film of milk within the loop becomes very thin or may even break. Wide differences in volume also become evident when the milk is transferred to a glass slide.

Although the syringe is less subject to error in delivering a uniform volume of milk, and is to be recommended for routine use, *(1)* some workers believe the loop is easier or quicker to handle in making smears. Those who prefer the loop should be especially careful that it is used properly in order to assure delivery of a standard volume of milk. Actual standardization of technic by quantitative methods is recommended when the loop is used routinely. Those who prefer the syringe should maintain it properly and check the calibration biennially or oftener, if needed.

CONCLUSIONS

1. The loop should be withdrawn from the milk vertically with the shank of the loop perpendicular to the surface of the milk.
2. As the angle between the loop and the milk narrows, an increased volume of milk is removed.
3. When the flat surface of the loop is brought upward through the surface of the milk while in a nearly horizontal position, the volume of milk may be increased about 1.8 times the standard volume.
4. Slow withdrawal allows milk to drain away, greatly reducing the volume retained.
5. Very rapid withdrawal allows retention of an excessive amount of milk within the loop.
6. Proper technic requires that the loop be removed in a vertical position from the milk. The speed of removal must be quick and without hesitation, avoiding a motion which is either too slow or too fast.
7. Use of the syringe, when properly calibrated and maintained, will avoid several errors inherent in use of the loop. The syringe is, therefore, generally to be preferrd for withdrawing 0.01 samples of milk for cell counts.

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BRING THE LADIES

The ladies' activities committee has planned exciting items for the ladies during the annual "Sanitarians" meeting. On Tuesday, the 16th, they will tour Minneapolis-St. Paul scenic spots by bus. The day will be highlighted by a luncheon at Diamond Jim's! There will be time to browse (and shop!) the unusual wares of this unique Minneapolis establishment.

On Wednesday, the 17th, Betty Crocker Kitchens will host the gals in the morning. Samples of new products are to be given away. And recipe books! The afternoon will be left free—just in case a shopping might be in order. So come! Bring the ladies—53rd Annual Meeting!