CONTAINERS, REFRIGERANTS AND INSULATION FOR SPLIT MILK SAMPLES[1]

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Methods are described for packing unfrozen split milk samples which hold their temperature within acceptable limits of 32-40°F for 24-30 hours when stored at ordinary room or higher temperature. The samples may be packed in a wrapped vacuum bottle, surrounded by a refreezant, and placed in cork or Styrofoam shippers or may be packed in nested Styrofoam boxes. Plate counts of split samples, shipped in the nested boxes to participating laboratories for examination, agreed well with each other and with the control laboratory.

Checking the performance of milk sanitation laboratories by split sample procedures normally requires that a sample of fluid milk be divided into portions for transportation to the participating laboratories under conditions that minimize bacterial or chemical change. The results reported by these laboratories are then compared with the results of concurrent examinations by one or more reference laboratories. Such a procedure is provided for in the recommendations of the National Conference on Interstate Milk Shipment and is intended to supply factual evidence of correct analysis by laboratories examining milk for interstate shipment (*3*).

Programs of split sampling have been initiated in at least 25 of the interstate milk shipping states. The procedures used by three of these states, Maryland, Missouri and Wisconsin have been described. (*1*, *2*, *4*).

In 1955 the Robert A. Taft Sanitary Engineering Center initiated a research project to develop a procedure for shipping split milk samples to all central state laboratories certified by the Public Health Service. This procedure would be an adjunct to the periodic surveys by which these laboratories are presently evaluated and would assist the Public Health Service in advising states regarding suitable equipment and procedures for split sampling programs.

Methods

Split milk samples may be frozen and shipped in dry ice, or they may be shipped in packages containing refrigerant to maintain the samples at desired temperatures for holding milk (32° - 40°F). The latter method yields samples which more closely represent those routinely examined by milk sanitation laboratories and, for this reason, may be preferable. To prevent the growth of psychrophilic bacteria, refrigerated samples should reach their destination within 24-30 hours. In many areas this is accomplished by packing the samples in regular milk sample shippers and transporting them by express or parcel post. In other areas split milk samples must be shipped by air, if they are to reach their destination in 24-30 hours, and the higher cost of air transportation limits the usefulness of the heavier types of sample shippers. As the Public Health Service would need to ship split samples by air to most of the 48 states, studies were carried out to develop a lightweight package or shipper that would maintain the temperature of the samples at 32° - 40°F for a period of 24-30 hours.

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Samples

The samples used in this study were usually prepared by dispensing homogenized milk into clean sample vials or tubes. The rate and extent of temperature changes in the samples served as the primary criterion for evaluation of the shipping containers and refrigerants. When split samples were to be examined by standard plate count as well, aseptic precautions were taken in their preparation. To avoid loss of samples due to breakage of glass tubes, the samples were initially dispensed in 10-ml. polyethylene screw-cap vials. These vials could not be strilized by heat and were replaced in later experiments by 12 - ml. centrifuge tubes (made of heat resistant polyethylene) which can be sterilized repeatedly by autoclaving at 15 lbs. for 15 minutes. Rubber-sleeved stoppers have been used as closures for these tubes. Experimentation indicates that neither the tubes nor the stoppers affect the phosphatase test on milk held in them for 40 hours at 36° - 41°F.

Insulation

Most of the insulating and refracting materials used in constructing the split sample packages are well known. Two comparatively new materials are Fiberglas and Styrofoam[2]. Insulating Fiberglas consists of laminated glass fibers in a variety of thicknesses. In this study one-inch Fiberglas, both plain and faced with aluminum foil, was used. Styrofoam is a rigid, plastic foam that can be sawed and shaped in the same manner as wood. It comes in several thicknesses and is sold by the board-foot measure. One-inch boards were used in constructing the boxes for this study.

Preliminary evaluations of packages indicated that the samples would freeze if placed in direct contact with the refreezant. To prevent freezing a double package was designed which consisted of a small insulated inner package and a larger insulated outer package. The sample tubes were placed in the inner package which was surrounded by the refreezant and then put in the outer package that acted as a barrier against the ambient temperature.

The small, inner packages consisted variously of Fiberglas envelopes, insulated Jiffy bags, Styrofoam boxes, or wide-mouthed pint vacuum jars, wrapped, in most instances, in Fiberglas and aluminum foil. The larger, outer packages were constructed of Fiberglas, both plain and faced, aluminum foil, large Jiffy bags, frozen food shippers insulated with cork or felt, Styrofoam boxes, and shipping cartons of corrugated paper.

Refrigeration

To avoid the nuisance of melting ice, a gel "refreezant" was used for refrigeration. This gel, which freezes at 30°F, can be purchased in cans or in plastic packages of various sizes. For the purpose described here, a 13-oz. plastic package was found to be most satisfactory. Originally these packages were frozen in the freezing compartment of a refrigerator but later, when more space was required, in a food freezer. Experimentation has shown that this refreezant provides effective refrigeration when it is arranged in single units in direct contact with a large portion of the surface of the small, inner box (Figure 1).

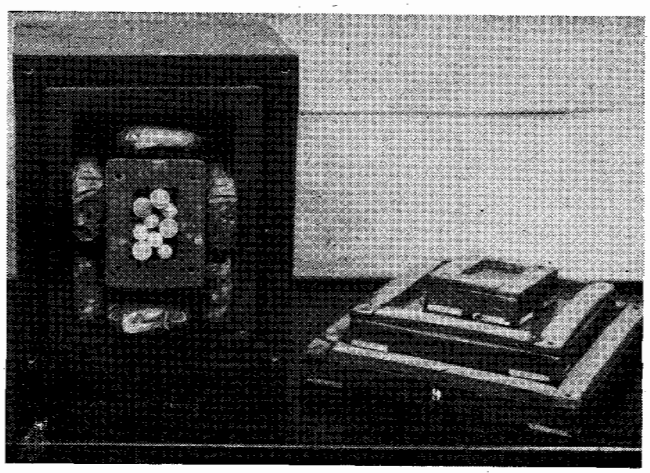

Figure 1. Assembled Styrofoam boxes containing split milk samples.

Generally the split samples at temperatures of 32° -40°F were prepared and packaged in a walk-in refrigerator. The small, inside packages had been precooled at freezer or refrigerator temperatures. Each package was surrounded by the refreezant and the whole wrapped or packed in materials which had also been precooled in the freezer or refrigerator. These packages were then placed in shipping containers, in some instances precooled, and stored at controlled temperatures during the testing period. A wire leading from a thermocouple in one of the packed samples was attached to a potentiometer, and the temperatures[3] maintained by the samples were measured at frequent intervals. Initially these measurements were made with a galvanometer-type potentiometer but in

[2]Trade names are used in the paper solely for the purpose of identifying products that cannot be adequately described by a common name.

[3]The temperatures of all samples were indicated by the temperature of the sample which contained the thermocouple.

later experiments a recording potentiometer was employed which could be operated continuously or at varying intervals.

Although a considerable number of split sample packages were designed, constructed, and evaluated, this report includes only comparative data from a few representative items. Temperatures maintained by the packaged split milk samples formed the primary basis for comparison, but standard plate counts were also used in a few instances.

EXPERIMENTAL RESULTS

Initially several split milk sample packages were constructed in the laboratory. In one of these the split sample vials were wrapped in two layers of Fiberglas, then in leadfoil, and packed in an 8½ in. x 12½ in. Jiffy bag, which was wrapped in a layer of Fiberglas faced with aluminum foil. This package was then placed in a larger Jiffy bag which was surrounded with the refreezant and the whole placed in a third Jiffy bag. The resulting package was wrapped in Fiberglas faced with aluminum foil and placed in an insulated corrugated paper carton which was closed and stored at room temperature. Temperatures between 32° and 42°F were maintained by these split samples for a period of 30 hours. These results illustrate the fact that readily available materials can be used in the laboratory to make packages suitable for shipping split samples at ordinary room temperature. The making of such packages is time consuming and quality is apt to vary with the skill of the technician. Studies were carried out to evaluate prefabricated packages that would be suitable for temperatures higher than ordinary room temperature. In these studies, two commercial frozen food shippers and an experimental Styrofoam shipper were tested.

Both commercial shippers were canvas-covered, box-bottomed bags. The larger of these, with a capacity of approximately four gallons, was insulated with 2 in. of cork and weighed 12 lbs. It was closed with one flap which fitted down into the top and with four overlapping flaps which buckled over the first one. The other shipper was insulated with 1 in. felt and had a capacity of approximately one gallon. It was closed with four overlapping, buckle-down flaps.

The experimental Styrofoam shipper (which weighed only 5 lbs.) was constructed of 1 in. Styrofoam covered with Fiberglas and coated with a polyester resin. The outside dimensions were 11 in. x 13 in. x 9 in.; the inside dimensions, 9 in. x 11 in. x 8 in. It was closed with a detachable lid which was held in place by a screw on each of two sides. A rubber gasket between the lid and the box was designed to provide a tight closure.

To evaluate these shippers, polyethylene tubes containing milk were packed in a wide-mouthed, pint vacuum jar which was wrapped in aluminum foil, then in Fiberglas, and again in aluminum foil. This package was placed in an 8½ in. x 12 in. Jiffy bag and another Jiffy bag was pulled down over the open end of the first one. This package was then wrapped in aluminum foil and surrounded with six packages of the refreezant, previously frozen in a refrigerator, and the whole wrapped in Fiberglas and aluminum foil. Samples packed in this manner were placed in cork, felt, and Styrofoam shippers, and held for 30 hours, at room temperature (81°F) in the first experiment and at 92°F. in a second experiment. In a later experiment the cork and Styrofoam shippers were evaluated at 99°F.

Temperatures observed in the milk in these tests are recorded in Table 1, which shows that the felt shipper was unsatisfactory because the milk rose above 40°F in less than 24 hours. Split samples packed in the cork shipper maintained satisfactory temperatures for 30 hours when stored in ambient temperatures of 81°, 92° or 99°F. Split samples packed in the Styrofoam shipper maintained Satisfactory temperatures for the 30-hour period when stored at 81° and 92°F, but when stored at 99°F the sample temperatures were less satisfactory for they rose above 40°F by the end of 24 hours.

TABLE 1 — TEMPERATURES OBSERVED IN SPLIT MILK SAMPLES STORED AT DIFFERENT AMBIENT TEMPERATURES IN CORK, FELT AND STYROFOAM SHIPPERS

	Temperature in °F							
	Average Ambient Temperature							
	81°F			92°F			99°F	
Storage time in hours	Cork	Felt	Styro-foam	Cork	Felt	Styro-foam	Cork	Styro-foam
0	40.6	40.1	39.9	40.5	40.5	40.5	40.8	40.8
2	38.7	38.3	37.2	37.8	37.8	37.2	37.2	37.6
4	37.0	36.1	35.6	35.4	36.1	34.0	34.2	35.4
6	35.6	35.6	34.7	34.3	35.6	34.2	33.4	34.2
8	34.9	35.2	34.0	33.6	35.2	33.4	32.9	34.0
10	34.3	34.7	33.8	33.4	35.2	33.3	32.4	33.8
12	34.0	35.2	33.6	32.7	35.2	32.7	32.4	33.8
14	34.0	35.2	33.6	32.5	35.6	32.5	32.4	33.8
16	33.8	35.6	33.6	32.5	36.1	32.4	32.9	34.0
18	33.8	36.5	33.6	33.4	37.9	32.5	33.6	35.4
20	33.8	37.0	33.4	33.6	39.7	33.3	33.6	36.0
22	33.8	40.1	33.6	34.2	42.8	33.6	34.0	37.6
24	34.0	43.0	34.0	35.2	45.3	34.2	34.7	40.6
26	34.3	46.4	34.5	35.8	48.9	35.4	35.2	43.7
28	34.5	51.4	35.2	37.2	52.7	36.5	—	—
30	35.2	53.4	36.0	39.2	57.0	38.5	36.5	51.8

These results show that the cork shipper provided better insulation against high temperatures than did the Styrofoam shipper. However, the data also indicated that the Styrofoam shipper would be satisfactory for shipping split milk samples at the temperatures usually maintained in heated aircraft compartments.

TABLE 2 — COMPARATIVE PLATE COUNTS OF SPLIT MILK SAMPLES IN GLASS AND POLYETHYLENE TUBES REFRIGERATED OR STORED AT ROOM TEMPERATURE IN A STYROFOAM SHIPPER

Sample	Tubes	Replicate number	Initial plate count[a]	Plate count[a] after 30 37 hrs. Refrigerated 35°F	Styrofoam 29.6-45.4[b]
Pasteurized	Pyrex	1	144	143	149
		2	115	137	133
		3	119	132	132
		Average	126	137	138
	Polyethylene	4		123	151
		5		137	133
		6		136	143
		Average		132	142
Pasteurized + raw	Pyrex	1	245	234	234
		2	243	239	189
		3	—	250	220
		Average	244	241	214
	Polyethylene	4	226	247	231
		5	227	280	271
		6	—	204	215
		Average	227	244	239

[a]Average of duplicate plates, 1:100 dilution
[b]Temperature range of samples held in Styrofoam shipper

Standard Plate Counts

A further evaluation of Styrofoam shippers was made by determining the standard plate counts of split milk samples which, when held in the shipper at room temperature (76°F) for 30-36 hours, had maintained temperatures in a range of 29.6°-45.4°F. These counts were compared with the initial count of the split samples and with the counts of refrigerated controls.

To determine whether the polyethylene tubes affected bacterial survival, standard plate counts were made on replicate split samples of pasteurized milk stored in sterile pyrex glass and polyethylene tubes. Three tubes of each type were packed in the Styrofoam shipper, which was stored at room temperature (76°F) for approximately 37 hours. As controls, a similar set of split samples was refrigerated at 35°F. Duplicate plates of each sample, both packed and refrigerated, were prepared and examined by standard methods. A second series of comparative tests was made using split samples prepared from pasteurized milk to which a little raw milk had been added.

The results summarized in Table 2 show no significant differences among the variables tested. In other words, the polyethylene tubes can be substituted for glass containers without altering the bacterial count, and storage in either the refrigerator or in the Styrofoam shipper at room temperature allows little, if any, increase over the initial count.

Table 3 gives the results of a similar evaluation in which the initial counts on split samples of pasteurized and raw milk are compared with counts obtained after 30 hours storage at an average room temperature of 81°F in the Styrofoam, cork, or felt shippers and in the refrigerator. The counts of stored pasteurized milk showed only minor increase over the initial counts, but the counts of samples containing raw milk increased more noticeably when the samples were held in the felt shipper and in the refrigerator. The increases occurring in the felt shipper are probably due to multiplication of psychrophilic bacteria allowed by the relatively rapid rise in temperature typical of this shipper (Table 1). The increased counts of raw milk samples held in the refrigerator as contrasted with the samples held in the styrofoam and cork shippers, may be attributed to the fact that an average temperature of 35°F or below was maintained in these shippers while the refrigerator was approximately 38° -40° throughout the holding period. Apparently the slightly higher temperature of the refrigerated controls allowed a more rapid multiplication of psychrophilic bacteria.

Thermocouple Studies of Styrofoam Boxes

The Styrofoam shipper was subsequently improved by using a thicker and softer rubber sealing gasket and by using four screws (one at each corner) to tighten the lid. This improved shipper provided in-

TABLE 3 — COMPARATIVE PLATE COUNTS OF SPLIT MILK SAMPLES REFRIGERATED OR STORED AT ROOM TEMPERATURE IN CORK, FELT AND STYROFOAM SHIPPERS

Samples	Replicate number	Initial plate count	Plate count[a] after 30 hrs. Refrigerated 39.2°F	Cork 33.8 to 40.6[b]	Felt 34.7 to 52.4[b]	Styrofoam 33.6 to 39.9[b]
Pasteurized	1	77	87	107	98	92
	2	83	94	97	86	69
	3	69	88	87	91	79
	Average	76	90	97	92	80
Raw	1	59	159	110	194	91
	2	59	176	78	182	85
	3	68	153	74	193	85
	Average	62	163	87	190	87

[a]Average of duplicate plates, 1:1000 dilution
[b]Temperature range of samples held in shippers (°F)

sulation which compared favorably with that provided by the cork shipper.

The method of packing the sample tubes in a wrapped vacuum bottle (before packing them in shippers), while providing satisfactory insulation, was too cumbersome and time consuming to be practical. Studies were made to determine whether nested Styrofoam boxes, one small and one intermediate size, could be substituted, respectively, for the wrapped vacuum bottle and the outer wrappings of Fiberglas and aluminum foil. These boxes were of similar design and were constructed of the same materials as the Styrofoam shipper. The smaller box held 9 split samples tubes, It was packed in the intermediate box and was surrounded by six packages of refreezant (Figure 1). The intermediate box was inverted in the shipper. The lid of each box was fastened tightly with screws.

Comparative tests of Styrofoam boxes were conducted to define with greater precision the conditions necessary for maintaining sample temperatures within acceptable limits of the prefered range of 32° -40° F. In certain of these tests, the packages of gel (refreezant) were arranged in the liners and frozen around the sample box before the samples were packed. In other tests samples which were at refrigerator temperatures were placed in a sample box that had been precooled in the refrigerator. The sample box was then placed in a liner precooled in the freezer, and was surrounded by packages of the gel which had been frozen directly on the freezer shelves[4]. This method was more satisfactory and was used in obtaining the data presented in Figure 2. These data, which are fairly typical of several tests, show that samples packed by this latter method and stored at 75°F held temperatures in a range of 31.1°-37°F for 30 hours; when stored at 98°F the samples held temperatures of 31.8°-42.4°F for 24 hours.

It should be noted that in some of these tests, during the initial hours of storage, the temperature of the samples fell below the accepted freezing point of milk (31.01°F) and remained in the range of 30° -31°F for one to four hours. In one instance, when the temperature had fallen to 30°F, the package was opened and two of the samples were examined, neither of which appeared to be frozen. In other instances, temperatures as low as 27°-30°F were observed in samples of milk stored in the freezer or the refrigerator, yet most of them were still liquid. For example, samples which had held temperatures in a range of 28°-30°F for 24 hours were opened (when at an indicated temperature of 28.2°F) and were apparently unfrozen. These observations indicate that the temperatures of the samples may be slightly below the freezing point of milk for several hours without ice crystals forming in the milk. They are not intended to imply that the nested boxes will prevent samples from freezing if exposed to low temperatures, and it is necessary to emphasize that samples shipped in the boxes be protected from prolonged exposure below ordinary room temperature.

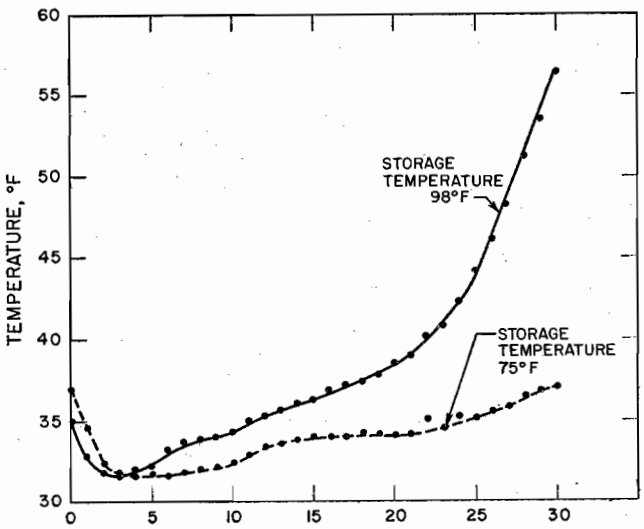

Figure 2. Temperatures observed in split milk samples packed in nested Styrofoam boxes and stored at different temperatures.

Pilot Study

A pilot study was arranged to determine whether the actual shipment of split samples in Styrofoam boxes would be satisfactory for comparative laboratory evaluations. Samples were sent by air to several milk sanitation laboratories located at varying distances (300 to 2200 miles) from Cincinnati.

The day before the samples were shipped, raw milk (received at the plant in the morning) and pasteurized milk (regular and homogenized) and cream (processed in the morning) were obtained from a dairy plant. These products were plated for the initial count and each was divided into several samples which were dispensed into sterile polyethylene tubes and refrigerated until the next day.

[4]Figure 2 is based on the temperatures held by samples which were packed in refreezant that had been frozen for 23-25 hours to 5°-9°F. Certain data show the gel frozen for 12 hours to 5°-14°F provide refrigeration equal to that of the gel frozen for the longer period.

The split samples were packed in the improved Styrofoam boxes and shipped by air parcel post to the participating laboratories. Each shippment consisted of duplicate samples (one tube 2/3 full and one completely filled) of the raw, pasteurized and homogenized milk and pasteurized cream. Each laboratory was requested to record the date and time it received the samples and to note, on opening, the temperature as well as the extent of fat separation, especially in the partly filled tubes. The laboratories were also requested to examine the samples by Standard Plate Count. Two sets of samples, packed for shipment, were held in the laboratory at room temperature as controls. One of these sets was plated after 26 hours; the other after 46 hours. The temperatures of these samples when examined were, respectively, 34° and 47°F.

Table 4 shows that, with one exception, the several sets of split samples reached their destinations and were examined within 30 hours from the time they were mailed, and that temperatures of the samples, on receipt, ranged from 37° to 45°F. The plate counts reported for the various samples (except for the count of the raw milk samples reported by Laboratory B) agree fairly well with each other and with the counts of the reference laboratory. The high counts of the raw milk samples reported by Laboratory B probably were due to psyhrophilic bacteria which developed at refrigerator temperatures in the three-day period that elapsed between the receipt[5] and examination of the samples. It is interesting to note that the counts of the samples of pasteurized milk and cream (packed with the raw milk for which high counts were reported) did not appear to be affected by this extended period of storage at refrigerator temperatures.

DISCUSSION

When split milk samples were packed in a wrapped vacuum bottle and stored at approximately 100°F, they held temperatures nearer the desired range (32° -40°F) than did similar samples packed in the Styrofoam boxes. However, the vacuum bottle was subject to breakage during shipment and the need for hand wrapping proved time consuming. No wrapping was required when packing samples in the Styrofoam boxes, and the complete package could be prepared for shipment in approximately 10 minutes.

There were also advantages and disadvantages in using the cork or Styrofoam shipper. The cork shipper was 5 to 10 pounds heavier, when packed with samples, than the Styrofoam shipper containing sam-

[5]Due to failure of communications the samples remained packaged and were refrigerated from the time of arrival (June 12) until they were examined (June 15)

TABLE 4 — PLATE COUNTS ON STORED AND SHIPPED SPLIT SAMPLES OF RAW MILK AND PASTEURIZED MILK AND CREAM

		Laboratories participating in pilot study						
		Controls held at 75°F		A	B	C	D	E
Shipping time (hrs.)		0	0	25	?	22	23	29
Distance (miles)		0	0	1200	800	300	600	2200
Temperature on receipt (°F)		34°[a]	47°[a]	45°	42°[a]	37°	43°	39°
Analyzed within (hrs.)		26	46	29	96	28	29	30
Samples[b]	Initial count	Plate counts 35°C (average of duplicate plates, 1:100 dilution)						
1 PHM	39	29	25	32	40	40	26	26
5 PHM	39	42	34	23	31	35	20	26
2 PM	59	47	59	44	47	53	48	58
6 PM	59	49	60	42	43	59	49	88
3 RM	74	61	73	44	TNTC	67	61	75
7 RM	74	78	56	43	TNTC	54	64	64
4 PC	5	12	10	8	6	15	9	11
8 PC	5	10	10	6	9	9	10	11

[a]Temperature of samples at time of analysis rather than when received.
[b]R: raw, P: pasteurized, H: homogenized, M: milk, C: cream

ples in either the vacuum bottle or the nested boxes. Thus the latter was more economical for air shipment. However, the cork shipper was of sturdier and more durable construction and could be shipped unwrapped, whereas the Styrofoam shipper required the protection of a corrugated paper carton.

The studies described in this paper are intended to serve only as guides for selection of appropriate materials and procedures for the shipping of split milk samples. Depending on conditions of use, a number of modifications in packaging might prove useful. For example, the experimetal Styrofoam box could be wrapped in the same manner as the vacuum bottle and be used with the cork shipper, thus avoiding breakage of bottles and loss of samples. The sample box could be constructed of 1½ in.—or possibly 2 in.—Styrofoam to provide more effective insulation. Relatively inexpensive prefabricated plastic bags, insulated with Fiberglas, could be used to eliminate the time-consuming procedure of wrapping the vacuum bottle. Vacuum bottles of metal, provided they are again available, could be used instead of glass vacuum jars. In some areas, particularly in the cooler seasons, the amount of refreezant could be reduced. These and other modifications of the methods described could be made depending on the needs of the laboratory shipping the samples. Such modifications should, of course, be checked to determine whether the split milk samples are held within an acceptable temperature range. Insertion in the shipper of a minimal-maximal registering thermometer adjacent to the samples would serve to determine this point.

SUMMARY

1. Methods are described for packing unfrozen split milk samples in a gel refreezant which refrigerates them during shipment.

2. The samples, in heat resistant polyethylene tubes, may be packed in a wrapped vacuum bottle and shipped in a Styrofoam or cork shipper, or they may be packed in nested Styrofoam boxes. The vacuum bottle appeared to provide better insulation but the nested boxes were more convenient.

3. Temperatures of split milk samples packed by these methods remained within acceptable limits of 32-40°F for 24-30 hours when stored at ordinary room or higher temperatures.

4. Pilot samples were shipped in the Styrofoam boxes to milk sanitation laboratories in various sections of the United States for examination by Standard Plate Count. The counts reported by these laboratories agree well with each other and with the reference counts.

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