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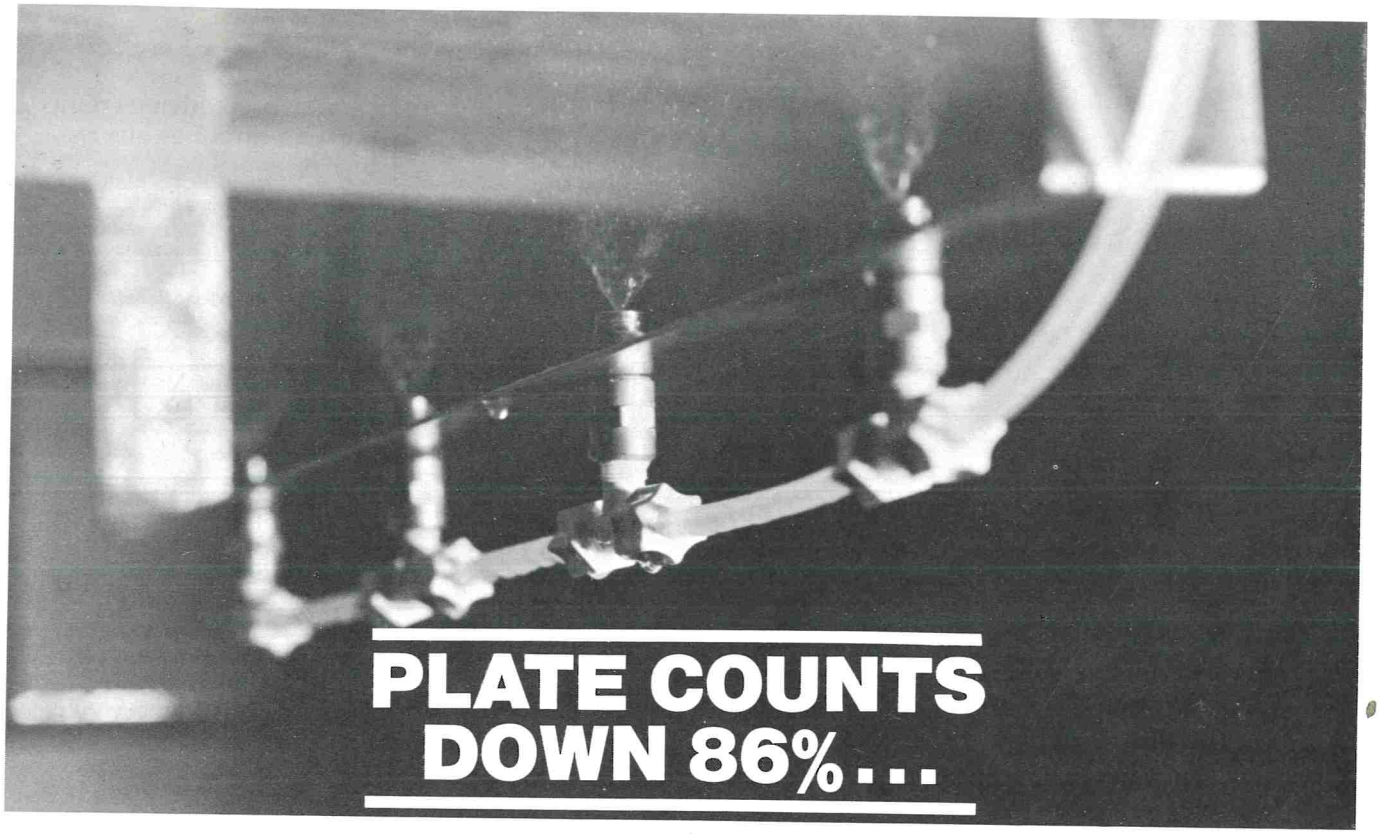

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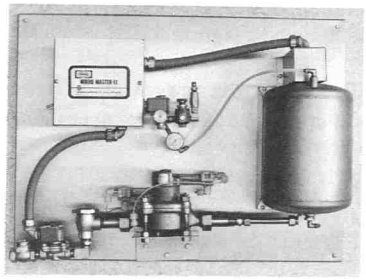

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Research Papers

General Interest

**Asterisk designates person to whom inquiries regarding this paper should be addressed*

Journal of Food Protection Vol. 41, No. 4, Pages 248-250 (April, 1978)

Survival of *Lactobacillus acidophilus* in "Sweet Acidophilus Milk" during Refrigerated Storage[1]

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(Received for publication September 14,1977)

ABSTRACT

Samples of three brands of commercial "Sweet Acidophilus Milk" were obtained directly from the processors and evaluated periodically for microbial, chemical, and flavor changes during 23-24 days of storage at 4 ± 2 C. Counts of *Lactobacillus acidophilus* decreased from 2.6×10^6-6.4×10^6 /ml initially to 5.1×10^4-3.1×10^6 /ml at the end of the study. Several samples dropped below 2×10^6 viable *L. acidophilus* before the pull date was reached. Final contaminant counts were 10^6 - 10^8 /ml in all samples, but rate of decline of *L. acidophilus* was not directly related to growth of contaminants. Titratable acidity and pH changed very little, and yeast and mold growth was minor. Notable off-flavors developed in 14-21 days.

Since 1975, "Sweet Acidophilus Milk" has been successfully marketed in some areas of the U.S. (*9,10*). This product is made by mixing a concentrate of selected strains of *Lactobacillus acidophilus* with cold, pasteurized milk and storing at 40 F or lower, thereby avoiding the cooked, high acid flavor of traditional acidophilus milk. Because at least some strains of *L. acidophilus* are capable of intestinal implantation, some feel that ingestion of large numbers of these organisms in milk may provide benefits in the control of gastrointestinal disturbances (*9,10*).

In 1931, Kulp (*5*) found that in cultured acidophilus milk the initial count of $5\text{-}10 \times 10^8$ /ml of *L. acidophilus* could be maintained at 5 C for a week if the acidity was low, but that within 12 days there would be a drastic die-off, coupled with appreciable growth of contaminants. Mikolajcik and Hamdan (*7*) found little loss in viability when acidophilus-cultured skim milk was stored at 5 C for 2 weeks.

Duggan et al. (*3*) described a method of concentrating *L. acidophilus* cells by centrifugation followed by quick-freezing in eutectic brine. This frozen concentrate could be stored up to 6 months with good viability, and could subsequently be added to fresh milk as needed for therapeutic use.

The level of inoculation in Sweet Acidophilus is 2-4 million organisms/ml (*9*). Since refrigerator temperatures virtually prevent growth of *L. acidophilus* and acid production, the milk will remain sweet (*8*), and producers should use *L. acidophilus* strains which remain viable for several weeks (*9*).

The purpose of this study was to follow the survival of *L. acidophilus* in three brands of commercial Sweet Acidophilus milk held at 4 C until spoilage occurred. Growth of contaminants and yeasts and molds was followed, as well as pH, titratable acidity, and organoleptic quality.

MATERIALS AND METHODS

Samples

Half-gallon cartons of three brands of Sweet Acidophilus milk were obtained directly from the processing plants, packed on ice at <4 C, and brought to the laboratory refrigerator. In Trial I, all samples contained 1% milkfat, while Trial II, an additional sample (Brand A2-II) was Sweet Acidophilus Vitamin D milk from the same processor as Brand A 1. The age of milk when collected varied from 0 to 3 days, as noted in Tables 1 and 2. Initial counts of all samples were made within 1 day of collection, and samples subsequently were stored at 4 ± 2 C for 23-24 days.

Lactobacillus acidophilus enumeration

Duplicate plates were made from each sample every 3-4 days (3-7 days in Brand A2-II). An approximation of Lactobacillus Selection Medium (*6*) was made by adding 10 g of sodium acetate and 15 g of agar per liter of Rogosa SL Broth (Difco Laboratories, Detroit, Mich). Plates were enclosed in Perflex 62 low gas-transmission plastic bags (Union Carbide Co., Chicago, Ill.) in an initial atmosphere of approximately 10% CO_2:90% N_2 from pressurized tanks of the two gases. Incubation was at 37 C for 72 h.

Sterile tubes of litmus milk (Difco) were fortified by adding a sterile solution of yeast extract (Difco), proteose peptone (Difco), and dextrose to give a final concentration of 1%, 0.5%, and 0.5%, respectively. Typical colonies were inoculated into fortified litmus milk and after 72 h at 37 C, tubes were checked for reduction and coagulation. Smears of litmus milk growth were stained with Newman-Lampert stain (*6*) or gram-stained, and examined microscopically for gram-positive short rods with rounded ends, occurring singly, in paris, or in short chains (*2*).

Enumeration of contaminants

Duplicate plates were made from each sample every 3-4 days (3-7 days in sample A2-II) using Plate Count Agar (Difco). Incubation was at room temperature (25 C) for 72 h. Typical colonies were gram-stained

[1] *Journal paper 2782 of the Agriculture Experiment Station.*
[2] *Department of Nutrition and Food Science*
[3] *Department of Microbiology.*

and examined microscopically.

Additional tests

Samples were checked about once a week for yeasts and molds, using Potato Dextrose Agar (Difco) and incubation at room temperature (25 C) for 5 days. pH was measured twice a week using a Fisher pH meter with combination electrode (Fisher Scientific Co., Pittsburgh, Pa.). Titratable acidity was determined twice a week using 0.1 N NaOH titration, in a method described by Goss (*4*). Taste and odor were evaluated periodically to determine onset of off-flavors.

RESULTS AND DISCUSSION

Table 1 gives the counts of *L. acidophilus* for each brand throughout the 3½-week storage interval. Most initial populations of *L. acidophilus* were slightly higher in Trial II than in Trial I, and the rate of decline was relatively the same at both sampling periods for each brand. Counts of Brand A decreased the most rapidly, particularly during the latter portion of the holding period.

L. acidophilus colonies were typically pinpoint in size, and colonies from Brand A were consistently slightly larger than those of the other two brands. The medium pH 5.4 ± 0.2) was very selective for these oganisms, and no other organism types were isolated. All isolates gave typical litmus milk reactions and microscopic appearance.

Some packages are imprinted with a legend that the product contains a minimum of 2×10^6 viable *L. acidophilus* organisms/ml. Brand A was consistently below this level at the pull date, as was Brand B in Trial I. Possibly the higher initial numbers found in Trial II were in recognition of the need for higher initial numbers to maintain the population at more desirable levels during holding. Viability was maintained considerably better in the Sweet Acidophilus milk examined here than it had been in the cultured acidophilus milk examined by Kulp (*5*).

Populations of contaminating bacteria are given in Table 2. In Brand A, these organisms decreased in numbers through day 7 and then began to grow somewhat before the pull date. In samples A1-I and A2-II, populations well into the millions were soon reached. The population of sample A1-II increased very slowly, based upon counts made at 25 C, although a marked off-flavor developed at 14 days. A count made at 21 days using 4-C plate incubation for 10 days was 1.6×10^7, while the count using 25-C incubation for 2 days was 1.5×10^5, indicating this sample developed a population of quite psychrotrophic bacteria, which unfortunately were not identified. While 25-C plate incubation can be a very useful procedure in obtaining

TABLE 1. *Count (colony-forming units/ml) of* Lactobacillus acidophilus *and percentage survival during refrigerated storage of Sweet Acidophilus milk.*

	Trial I						Trial II							
	Brand A1-I Age[1] = 0 RPD[2] = 13		Brand B-I Age = 1 RPD = 9		Brand C-I Age = 2 RPD = 10		Brand A1-II Age = 2 RPD = 11		Brand A2-II Age = 3 RPD = 10		Brand B-II Age = 3 RPD = 8		Brand C-II Age = 3 RPD = 9	
Day of study	Count (10^6)	Survival[3]	Count (10^6)	Survival	Count (10^6)	Survival	Count (10^6)	Survival	Count (10^6)	Survival	Count (10^6)	Survival	Count (10^6)	Survival
Initial	4.1	—	2.6	—	3.3	—	3.6	—	4.2	—	6.4	—	5.3	—
3	3.1	76	1.8	69	3.0	91	3.0	83			7.4	116	4.5	85
6	2.1	51	1.5	58	2.6	79	1.8	50	2.4	57	4.1	64	4.4	83
10	.79	19	1.3	50	2.2	67	1.0	28			3.2	50	3.7	70
13	.34	8	1.3	50	1.9	58	.29	8	.68	16	2.1	33	4.0	75
16	.53	13	1.2	46	1.4	42								
17							.23	6			4.7	73	3.5	66
19	.58	14	.79	30	1.2	36								
20							.075	2	.28	7	4.3	67	3.4	64
23	.58	14	.55	21	.73	22								
24							.051	1	.38	9	2.5	39	3.1	58

[1]Age of milk when obtained from processor (days)
[2]Retail pull date (day of study)
[3]Survival of *L. acidophilus* from initial count (%)

TABLE 2 *Growth of contaminants (colony-forming units/ml) during refrigerated storage of Sweet Acidophilus milk.*

	Trial I			Trial II			
Day of study	Brand A1-I Age[1] = 0 RPD[2] = 13	Brand B-I Age = 1 RPD = 9	Brand C-I Age = 2 RPD = 10	Brand A1-II Age = 2 RPD = 11	Brand A2-II Age = 3 RPD = 10	Brand B-II Age = 3 RPD = 8	Brand C-II Age = 3 RPD = 9
Initial	290	200	18	1300	90 est.	25	36
4	120	490	41	940		14	33
7	31	> 30,000	1200	230	25 est.	2900	78,000 est.
11	16,000	73,000,000	160,000	710		1,500,000	50,000,000 est.
14	26,000,000	140,000,000	5,000,000	21,000	17,000	48,000,000	120,000,000
17	30,000,000	250,000,000	62,000,000				
18				38,000		140,000,000	270,000,000
20	160,000,000	330,000,000	98,000,000				
21				150,000	14,000,000 est.	210,000,000	340,000,000
23	320,000,000	550,000,000	420,000,000				
24				1,200,000	39,000,000	370,000,000	490,000,000

[1]Age of milk when obtained from processor (days)
[2]Retail pull date (day of study)

counts of psychrotrophic organisms relatively quickly, some organisms apparently can be missed on occasion.

Brands B and C had quite low initial counts of non-acidophilus organisms, but organisms capable of quite rapid growth at 4 ± 2 C obviously were present and quickly developed to large populations, being at or well on the way to 10^6/ml by the pull date. Isolates from typical colonies from both trials were gram-negative rods in all instances.

On day 18 of Trial I all three samples had detectable off-flavors. Coagulation and pronounced off-flavors had occurred by day 23. In Trial II, sample A1-II had a fruity flavor on day 14, while the other three samples did not develop off-flavors until day 21. By day 24, protein destabilization and rancid odors were evident in all samples except A1-II, which was fruity in odor and stormy in appearance.

The Sweet Acidophilus milk tested in this study apparently deteriorated in a manner typical of plain pasteurized milk. There was no drastic decrease in *L. acidophilus* to correspond with the steep incease in contaminants after 4 or 7 days, so presumably the acidophilus organisms are little affected by progressive spoilage.

Slight yeast growth occurred in Brand C-I. Samples A1-II and A2-II contained small numbers of yeasts at the end of the study, while Brand B-II had low and declining yeast counts throughout the storage period. Molds were not encountered.

The pH of all samples remained essentially unchanged throughout both trials, and titratable acidity increased at most by only a few tenths late in the holding period.

The work of Atherton et al. (*1*) shows that psychrotrophic bacteria in milk stored at low temperatures do not appreciably change the pH or titratable acidity, which is verified in the present study. Kulp (*5*) also found no change in titratable acidity in 12 days of cold storage of cultured acidophilus milk.

The data from this experiment show that commercial Sweet Acidophilus milk can be stored 2-3 weeks in the refrigerator before notable flavor defects occur. However, the count of viable *L. acidophilus* will have declined markedly, while the level of spoilage organisms will have reached a level of 10^6-10^8/ml. Further, the level of inoculation of acidophilus initially influences the final level of viable organisms, as does the strain of *L. acidophilus* used, as shown by the rate of decline in Brand A compared to Brand C.

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Microflora Isolated from Imported Frozen Lean Beef Pieces [1]

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(Received for publication September 1, 1977)

ABSTRACT

Forty samples of frozen imported lean beef pieces from six countries were obtained from two centralized meat processing operations. The samples were analyzed for total aerobic counts (35, 20, 7 C), yeasts and molds, fecal streptococci, *Staphylococcus aureus, Clostridium perfringens,* total coliforms, fecal coliforms, *Escherichia coli,* and *Salmonella.* Characterization of the microbial flora from 20 of the samples was also done. Microbial counts were consistently low in all analyses; no *Clostridium perfringens* or *Salmonella* was recovered from any samples. The microbial flora was predominantly *Pseudomonas, Flavobacterium, Moraxella, Acinetobacter, Corynebacterium, Micrococcus, Staphylococcus* and *Lactobacillus;* the remaining isolates included *Alcaligenes, Erwinia, Citrobacter, Klebsiella, Streptococcus, Bacillus* and *Arthrobacter.*

Considerable work has been done on the microbiology of retail ground beef (*4,6,9,10,12*) and the interest in some form of bacterial standards for this product continues (*3,8*). Field et al. (*5*) reported on sources of variation at the retail level in the bacteriological condition of ground beef and concluded that there would be merit for standards for centrally packaged ground beef in terms of (*a*) elapsed time between packaging and retail sale and (*b*) temperature considerations. In centralized processing of ground beef, frozen imported lean beef pieces are often blended with domestic trimmings. Recent statistics (*11*) indicate that in excess of one billion pounds of manufacturing beef were imported into the United States in 1976. Much of this product was used in the manufacture of ground beef, although the exact amount is not known.

Our study was undertaken to determine the overall microbiological quality of frozen imported lean beef pieces and to classify the specific microflora recovered from our samples.

[1] *Florida Agricultural Experiment Stations Journal Series No. 691.*

MATERIALS AND METHODS

Forty samples of imported lean beef pieces were randomly obtained from two centralized meat processing operations in Florida over a 3-month period. Samples were derived from lots of beef imported from Nicaragua, New Zealand, Costa Rica, El Salvador, Honduras and/or Guatemala. Frozen samples were obtained immediately after hydroflaking (coarse cutting) and kept frozen until just before analysis in the laboratory.

Methods employed for microbiological analyses were essentially those in the Bacteriological Analytical Manual for Foods (BAM) with the exception that a surface plating technique was used for total aerobic counts (*1*). Samples were partially thawed for 4-6 h at 5 C just before analysis. One 25-g subsample was homogenized in 225 g of sterile phosphate buffer dilution water for 2 min at low speed (8,000 rpm) in a Waring blendor. Serial dilutions of this homogenate were used for the following microbiological analyses: total aerobic plate counts on pre-poured plates of Plate Count agar incubated at 35 C for 48 h, 20 C for 5 days or 7 C for 10 days; yeast and mold counts on Plate Count agar with added antibiotics at 20 C for 5 days; fecal streptococci counts on KF-streptococcus agar at 35 C for 48 h; and *Clostridium perfringens* on Tryptose Sulfite Cycloserine (TSC) agar with egg yolk emulsion incubated anaerobically in a GasPak^R system at 35 C for 24 h. An MPN procedure using Lauryl Sulfate Tryptose (LST) broth followed by confirmation in Brillant Green Bile broth (2%) was used to estimate total coliforms. Confirmation in EC broth at 45.5 C determined fecal coliform populations. *Escherichia coli* was quantified from positive EC tubes according to BAM procedures. *Staphylococcus aureus* was enumerated with the MPN method using 10% NaCl Tryptic Soy Broth (TSB) followed by streaking onto plates of Baird Parker agar and confirmation of typical colonies with a tube coagulase test.

For *Salmonella* analyses, a separate 25-g subsample was blended with 225 ml of sterile lactose broth and the resulting homogenate transferred to a sterile flask. After incubation at 35 C for 24 h, 1-ml aliquots of the resulting culture were added to either 10 ml of Selenite Cystine broth or 10 ml of Tetrathionate broth followed by incubation at 35 C for 24 h. Selective plating (using Brilliant Green agar, *Salmonella-Shigella* agar and Bismuth Sulfite agar) and subsequent biochemical and preliminary serological identification were also done. Dehydrated culture media were Difco products except for TSC and 10% NaCl TSB which were formulated from individual components.

Bacterial isolates were taken from total aerobic count plates (35, 20, or 7 C). Individual colonies representing different morphological properties were selected from spread plates. Such colonies (equal to the square root of the number of colonies on countable plates) were transferred to Tryptic Soy agar slants for taxonomic characterization. A total of 458 isolates were obtained in this manner from 20 of the 40 samples. Standard microbiological techniques were used in the

identification of bacterial isolates using *Bergey's Manual of Determinative Bacteriology* (2) and the *Manual of Clinical Microbiology* (7).

RESULTS AND DISCUSSION

Results of the microbiological analyses are in Tables 1 and 2. There were no apparent differences between the microbiological counts of the samples as related to country of origin, although insufficient numbers of samples were obtained from some of the countries for a valid statistical comparison. There was little variation in microbial counts among the 40 samples.

Total aerobic counts of all but four samples at 35 and 20 C were in the range of 10^3-10^4 organisms/g. Surface plating was used in an effort to minimize trauma to plated cells. Highest total aerobic counts were obtained at 20 C for all samples while lowest total aerobic counts occurred at 7 C (Table 1). There was variation in the yeast and mold counts among the samples but mean counts were low. Likewise, variability occurred in the fecal streptococci counts from sample to sample with overall means being very low.

Coliform counts were low for all of the samples as shown in Table 1. The total coliform counts varied among samples with all but two of 40 samples having counts in the range of 0-43 organisms/g. The mean fecal coliform counts and *E. coli* counts were low for the samples, with 34 of 40 samples yielding no *E. coli*. No *S. aureus* was recovered from 22 of 40 samples and all but one sample had counts less than or equal to 9.1 per g. Neither *Salmonella* nor *C. perfringens* was recovered from any of the samples.

A variety of both gram-positive and gram-negative organisms was isolated from 20 of the imported lean beef samples (Table 2). There was little variation in the predominant flora from sample to sample or from country to country. As might be expected, the microflora varied according to the incubation temperature used for the plates from which they were isolated. For example, *Staphylococcus* spp. were recovered from 75% of the samples at 35-C incubation while none were recovered from the samples at 7 C. At all temperatures, the most frequently isolated gram-negative organisms were *Pseudomonas* spp., *Flavobacterium* spp., *Moraxella* spp. and

TABLE 1. *Microbiological counts*[1] *of imported frozen lean beef pieces*[2].

Test		Geometric mean	Arithmetic mean	Range
Total aerobic	35 C	1.0×10^4	2.3×10^4	4.3×10^2 - 1.1×10^5
Plate counts:	20 C	1.7×10^4	4.1×10^4	5.6×10^2 - 4.7×10^5
	7 C	2.9×10^3	1.3×10^4	1.0×10^1 - 2.8×10^5
Yeast and molds		8.2×10^1	3.2×10^2	0 - 4.0×10^3
Fecal streptococci		9.6×10^1	2.7×10^2	5.0 - 4.1×10^3
Total coliforms		5.8	1.8×10^1	0 - 4.1×10^2
Fecal coliforms		1.3	1.4	0 - 3.5×10^1
Escherichia coli		1.3	1.3	0 - 3.5×10^1
Staphylococcus aureus		2.2	3.1	0 - 2.3×10^1
Clostridium perfringens		0	0	—
Salmonella		0	0	—

[1]Per g of sample.
[2]Forty imported lean beef samples.

TABLE 2. *Microbial flora isolated from imported frozen lean beef pieces*[1]

Isolates	35 C[2]		20 C[2]		7 C[2]		All temperatures	
	No. of Isolates	% of Samples[3]	No. of Isolates	% of Samples	No. of Isolates	% of Samples	No. of Isolates	% of Samples
Gram-negative:								
Pseudomonas spp.	31	70	22	65	33	75	86	95
Flavobacterium spp.	18	55	34	80	19	60	71	95
Moraxella spp.	9	30	18	60	27	50	54	70
Acinetobacter spp.	9	40	5	25	24	50	38	65
Alcaligenes spp.	3	15	2	10	—	—	5	25
Erwinia herbicola	1	5	2	10	1	5	4	15
Citrobacter freundii	2	10	—[4]	—	1	5	3	15
Klebsiella pneumoniae	1	5	—	—	—	—	1	5
Gram-positive								
Corynebacterium spp.	26	60	29	75	18	50	73	85
Micrococcus spp.	14	50	21	60	1	5	36	90
Staphylococcus spp.	32	75	11	35	—	—	43	85
Lactobacillus spp.	5	15	9	30	18	55	32	80
Streptococcus spp.	4	20	2	10	—	—	6	25
Bacillus spp.	2	10	1	5	1	5	4	15
Arthrobacter spp.	1	5	1	5	—	—	2	5

[1]Twenty imported lean beef samples.
[2]Incubation temperature of aerobic plate counts.
[3]Percentage of samples from which the organism was isolated.
[4]No recovery of this organism.

Acinetobacter spp., while *Corynebacterium* spp., *Micrococcus* spp., *Staphylococcus* spp. and *Lactobacillus* spp. were the most frequently isolated gram-positive organisms. With the exception of *Staphylococcus*, the predominant organisms were psychrotrophs commonly found on refrigerated fresh beef. Species of staphylococci were found on 85% of the samples and included only one coagulase-positive isolate. Few members of the *Enterobacteriaceae* family were isolated with *Erwinia herbicola* being most often recovered. Four of six streptococci isolates were *Streptococcus faecalis* (found on 25% of the samples).

Processing conditions such as initial microbial quality of fresh trimmings, method of freezing and duration of cold storage before export from the six countries were, unfortunately, not known. Factors such as these would obviously affect the microbial flora of this product in terms of both types and numbers present. With this in mind, our results indicate that the microbiological quality of the imported lean beef pieces used for ground beef at two centralized operations was good. The low total aerobic counts indicate that this product would have a neglible effect on the initial microbial quality of ground beef prepared from imported lean and domestic trimmings because suggested plate count standards for ground beef approximate 10^6 per g.

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A Simple Method to Screen Fruit Juices and Concentrates for Heat-Resistant Mold

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ABSTRACT

A simple test is described for screening fruit juices, juice concentrates, or any suspected juice products for the presence of heat resistant mold. Product in bottles heated at 77 C is plated. The plates and contents remaining in the bottles are incubated for periods up to 30 days at 30 C. Several plating and liquid media for enumerating heat resistant mold were investigated.

Mold, of the genus *Byssochlamys*, with heat resistant ascospores, has caused spoilage in canned fruits and in both canned and bottled fruit drinks and juices. It has been readily isolated from many fruits, including grapes, cherries (*13*), apples (*12*), and strawberries (*10*). It was first reported in England in the 1930's (*8*) and in the United States in 1964 (6). Recent outbreaks have also occurred in Canada, Europe, South America, and Australia (*4*).

Byssochlamys is characterized by production of ascospores contained in an 8-spored ascus. The mold has been cultured on a variety of media including Czapek Agar, Potato Agar, Potato Sucrose Agar, Potato Dextrose Agar, and Orange Serum Agar. Colony formation is dependent somewhat on the medium used. Usually they are characterized by buff-colored conidial structure.

Byssochlamys shows unusual resistance to a number of influences which are lethal to most fungi. It can grow at low oxygen tension, hence its ability to grow in cans or bottles of processed fruit products. Olliver and Smith (*8*) noted the spores to survive in absolute alcohol for 30 weeks. Murdock and Hatcher (*7*) found it to grow at temperatures as low as 1.7 C. Ito (*3*) reported 1,000 ppm chlorine solution was not sufficiently fungicidal to be effective in normal sanitizing procedures. The ascospores are also extremely heat resistant. Maunder (*6*) reported survival between 30 and 40 min at 86 C in a canned grape drink. Ascospores with this degree of heat resistance are capable of surviving the normal processing temperature for fruits and fruit drinks, with subsequent germination and growth in the finished product. The pectolytic enzyme produced by this organism can destroy the texture of canned fruits (*9*).

In recent years there has been an increase in spoilage in thermally processed fruit juices, fruit drinks, and drink bases caused by *Byssochlamys* and other heat resistant fungi. To minimize this type of spoilage it is neccessary to screen fruit juices and/or concentrates for the presence of heat resistant mold. A number of procedures used by various members of the food industry appear in the *Byssochlamys* Seminar Abstracts (*1*). Splittstoesser et al. (*11*) described a method for detection of heat resistant mold in a variety of fruit samples.

The method described herein employs a minimum of equipment. It is especially adapted for the detection of small numbers of heat resistant spores in fruit juices and/or concentrates received at a processing plant.

METHODS

Maunder and Murdock in 1968 (*5*) developed a method for detection of heat resistant mold during a survey of a grape processing plant in the Midwest. A slight modification of the procedure appeared in the Proceedings of the *Byssochlamys* Seminar 1969 (*1*). It consisted briefly of diluting 25 ml of grape concentrate in an 8-oz. prescription bottle with an equal volume of 0.05% peptone solution. The sample was then heat shocked for 20 min at 77 C, cooled, and then incubated at 30 C with the bottle placed on its side, cap loosened.

This procedure was further modified in our laboratory. It has been designed specifically for checking fruit concentrates such as grape, apple, and cherry and juice bases made from these products, for the presence of heat resistant mold. It consists of the following (Fig. 1): (*a*) place 50 g of product in a sterile 8-oz. medicine screw-capped bottle or a sterile 250-ml tissue culture bottle; (*b*) add 50 ml of sterile water, (*c*) spore test 30 min at 77 C (start timing when test bottle of product containing thermometer reaches this temperature); (*d*) cool immediately; (*e*) distribute 30 to 40 ml among 4 or 5 petri plates[1], add 2% plain agar and mix contents; (*f*) place bottle, containing remaining product, on its side with cap loosened, and incubate at 30 C; (*g*) examine plates and bottles weekly for the presence of mold growth and discard after 30 days if no growth occurs; and (*h*) check outgrowth microsopically, looking for the presence of characteristic 8-spore asci as illustrated in Fig. 2.

Above procedure may be used for single strength products having a Brix of 35° or less. When product of this type is being screened, use 100-g sample and do not dilute with sterile water.

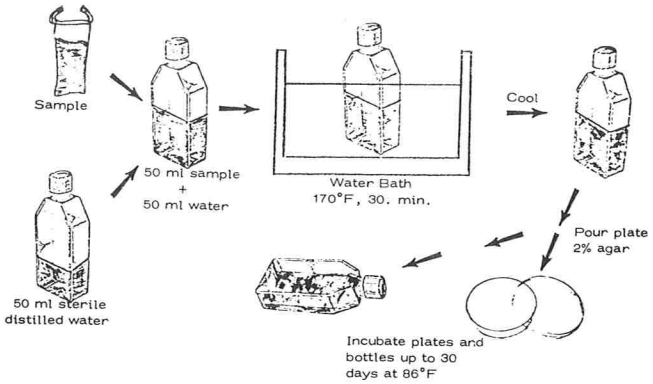

Figure 1. *Procedure for detection of heat resistant mold in fruit juice concentrates.*

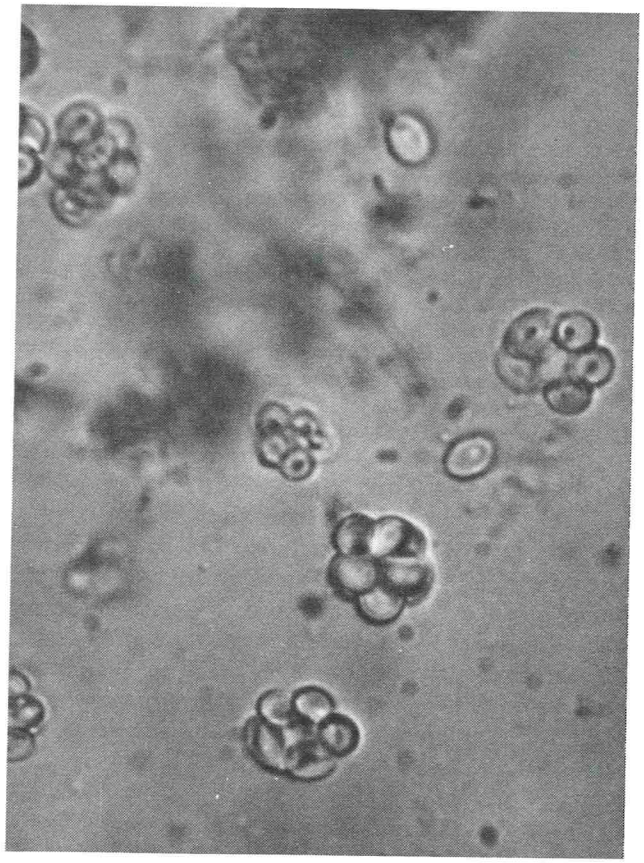

Figure 2. *Eight-spored asci, one of the differential characteristics of the genus* Byssochlamys *(1000×).*

RESULTS AND DISCUSSION

Comparison of media

The procedure described herein was developed to be used in those plants which have little or no microbiological equipment or selective media. One of the first items investigated was a comparison of various plating media for enumerating heat resistant mold. The media studied were Potato Dextrose Agar (PDA), Sabouraud Dextrose Agar (SDA), Malt Agar (MA), and Orange Serum Agar (OSA). All of the foregoing contained 2% agar. Also included was 2% plain agar (PA). Fifty ml of 68° Brix grape concentrate was diluted with an equal volume of water in an 8-oz. screw cap bottle. The contents were sterilized for 10 min in flowing steam, cooled, then inoculated so as to contain 10 spores of *B. fulva* strain M-78[2]/ml. Ten ml of the inoculated product was then distributed over five petri plates. This was repeated five times. Five plates were then poured for each test medium. The results in Table 1 show comparable counts were obtained in all media.

TABLE 1. *Comparison of plating media for enumerating heat resistant mold*[a]

Media	Total colonies per 5 plates 30 h	36 h	42 h
PDA	60	86	87
SDA	74	87	93
MA	71	104	110
OSA	76	108	109
PA	74	91	93

[a]50 ml of 68° Brix grape concentrate diluted with an equal volume of water, 10 ml poured over 5 plates for each test medium. Plates incubated at 30 C.

The growth of heat resistant mold in a liquid medium was also investigated. In this test 50 ml of sterile 68° Brix grape concentrate in an 8-oz. screw cap bottle was diluted with an equal volume of Potato Dextrose Broth (PDB), similarly with Sabouraud Broth (SB), and another with sterile water. The bottles were inoculated to contain 10 spores per ml from the same suspension previously mentioned. They were then placed on their sides, caps loosened, and incubated at 30 C. Mold growth in all bottles appeared to be about the same (Table 2). However, colony formation took longer in the liquid media than in the plates. It appears grape concentrate supplies the necessary growth factors for mold growth and that selective media are not necessary, i.e., water can be used as a diluent for grape concentrate in the bottles and

TABLE 2. *Growth of heat resistant mold in liquid media*[a]

Media	Days at 30 C 2	3	5	7
Grape conc. +PDB	NG	+	+	++
Grape conc. +SB	NG	+	+	++
Grape conc. +water	NG	+	+	++

NG = No growth; +slight growth (1 or 2 colonies); ++heavy growth (4 or more colonies).
[a]68° Brix grape concentrate diluted with an equal volume of PDB, SB, and sterile water.

2% plain agar for the plating medium. Our studies have also shown that the same arrangement can be used for checking for the presence of heat resistant mold in apple and cherry concentrate.

Heat shock

The heat shock of 30 min at 77 C has been designed to eliminate non-heat resistant fungi, restricting outgrowth to those organisms which may be able to survive the

[1]*100 × 25 mm petri dishes will prevent possible spillage of product during mixing process with 2% agar.*
[2]*The test organism was from a suspension supplied by John Folinazzo of Continental Can Co., Inc. and had been heat shocked 15 min. at 80 C.*

thermal process given the finished product. Molds of this type are able to survive several hours at this temperature. Also, Hull (2) reported optimum germination is obtained by heating the spores for 30 min, at 75 C, which is in the temperature range specified.

Screen test

Our laboratories have used this procedure since 1972 to screen incoming fruit juice concentrates for presence of heat resistant mold. Outgrowth usually occurs after 3 to 5 days of incubation, if the product contains 10 or more spores per gram. However, if concentration of spores is extremely low it may take as long as a month before colony formation appears. By incubating both plates and bottles there is a greater chance of obtaining outgrowth. If cultures are still negative after this period they should be discarded, as no further outgrowth is likely to occur. The test exhibits fairly good reproducibility. Four different laboratories checking the same samples of grape base for heat resistant mold reported positive results after 5 days incubation at 30 C. As with any other type of microbiological test, aseptic technique should be used to prevent contamination from other types of mold such as *Penicillium*.

B. fulva has been the species most frequently isolated. However, *B. nivea* and *Paecilomyces* have also been found. Colonies growing on the product medium may range in color from white to buff brown, with buff color usually being associated with this organism.

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A Research Note

Evaluating Cheese-like Emulsions from Animal Blood Proteins and Whey Solids [1]

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ABSTRACT

Animal blood globin powder and blood plasma concentrate prepared from cattle blood, were incorporated, along with modified whey solids, hydrolyzed cereal solids, butter, cellulose gum and water into heat processed cheese-like emulsions. Globin protein in the emulsion ranged from 12.2 to 16.4% while the plasma protein range was from 1.8 to 6.2%. The ingredients were blended while the pH was being raised to pH 6.8 and the mixtures were processed at 116 C for 15 min. Texture profile parameters of hardness, springiness and cohesiveness were evaluated. Increased globin protein concentration decreased springiness and cohesiveness. Hardness apparently was maximal at an approximate globin/plasma protein ratio of 5:1.

Two important reservoirs of protein which are not fully used at present are blood from slaughtered animals and whey from cheese manufacture. While many people in this country have an aversion to eating foods containing blood, progress is being made in producing animal blood protein isolates suitable for incorporation into foods (*5,7,10*). Even more progress has been made in recovering and utilizing cheese whey (*8,9*) especially in the forms of whey solids and whey proteins.

A variety of cheese analogs have been investigated based on hydrolyzed cereal solids, protein and vegetables fat (*3*). Use of whey solids in cheese-like products has been sparsely investigated (*1,11*).

The purpose of this research was to produce and evaluate cheese-like products formulated from animal blood protein/whey blends.

MATERIALS AND METHODS

Whole beef blood collected during slaughter with salt and citrate to prevent hemolysis and coagulation was separated into plasma and cell fractions with a cream separator. Plasma was desalted and concentrated to 17% protein by circulation through cellulose hollow fibers (Bio-Rad Laboratories, Richmond, CA).

After removal of heme from hemoglobin by acidified acetone, the precipitated globin hydrochloride was collected on a Buchner funnel, dissolved in water, precipitated at pH 6.8, washed at that pH to remove residual acetone, redissolved at low pH and spray-dried. The resulting white fluffy powder contained 85% protein (by Kjeldahl), 6% moisture (vacuum oven) and 8% ash. The blood proteins were mixed with modified whey solids (ENR-RO™, Stauffer Chemical Co.), hydrolyzed cereal solids (MOR-REX™, CPC International), and other ingredients at low blender speed while the pH was adjusted to 6.8 with 12 N NaOH, then high speed blended for 10 min. The emulsions (100 g) were poured into 250-ml beakers, heat processed (116 C, 15 min) in a pressure cooker to set the structure and to develop color. Compositional data are in Table 1.

TABLE 1. *Formulation and composition of cheese-like emulsions.*

Formulation	*Percent*
Globin protein	12.2-17.0
Plasma proteins	2.8-6.2
ENR-RO™ whey solids	5.0
MOR-REX hydrolyzed cereal solids	8.5
Butter	8.5
Cellulose gum	0.8
Composition of processed products	
Protein	14.5-18.1
Fat	5.9- 7.5
Ash	4.1- 6.7
Moisture	54.7-64.3

The General Foods Texture Profile Analysis (*2*) was done using the Instron Universal Testing Instrument Model TM-M, Instron Corp., Canton, MA) on replicated 1 cm × 1 cm cylindrical portions of the finished product. Shama and Sherman (*6*) have demonstrated a high correlation between sensory panel scores for the texture parameter "hardness" of cheese and data from this instrument. The parameters of springiness and cohesiveness were also considered applicable to this type of product.

RESULTS AND DISCUSSION

It was apparent from preliminary investigations (*4*) that a combination of plasma and globin was necessary for the formation of cheese-like textures with heating. Emulsions formulated at high plasma levels in the absence of globin were extremely tough and rubbery while emulsions containing globin without plasma protein were more liquid. Increased levels of hydrolyzed cereal solids tended to decrease hardness while increased levels of whey solids decreased springiness and cohesiveness.

Graduated levels of globin and of plasma proteins

[1] *Florida Agricultural Experiment Stations Journal Series No. 9067.*

were used with fixed levels of other ingredients. The effect of the ratio of globin/plasma protein on the texture of the heat processed products is presented in Fig. 1. Data presented are the means of duplicate trials. The

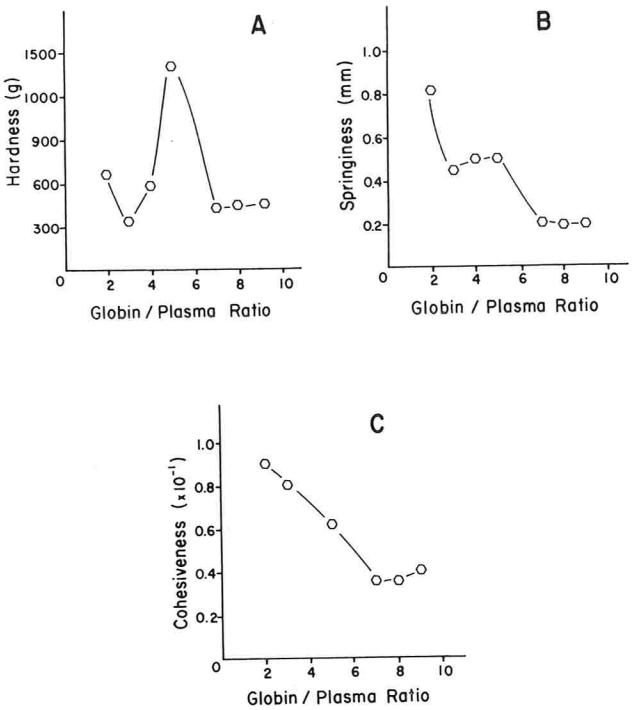

Figure 1. *The effect of the globin/plasma protein ratio on the texture of heat processed emulsions.*
A. Effect on hardness; B. Effect on springiness; C. Effect on cohesiveness.

hardness value reached a maximum at an approximate globin/plasma ratio of 5:1. Higher levels of globin in the blend drastically lowered the hardness rating. Springiness and cohesiveness values generally decreased with an increase in the globin/plasma protein ratio to greater than 4:1.

The texture of the cheese-like products did not correlate well to commercial cheese examined in the laboratory. For example, the processed emulsion formulated at 5:1 globin/plasma ratio was similar in hardness to processed cheese while the springiness was similar to cream cheese. The cohesiveness of this product was intermediate to processed and cream cheese. Interactions between globin and plasma protein with respect to moisture and texture parameters need to be investigated further before cheese-like characteristics can be predicted with confidence.

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Microbiological Studies on Aging of Intact and Excised Beef Muscle [1]

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ABSTRACT

Beef cattle from the University herd were used for these studies; aging treatments after slaughter were as follows: (*a*) sides were held at room temperature (21-23 C), (*b*) sides were held at 2 C, (*c*) sides were kept for 6 h at room temperature and then the round was removed and placed at 2 C for 18 h (*d*) sides were held for 3 days at 2 C, then the excised round was kept for 4 days at 2 C for a total of 1 week of low temperature aging. After aging by procedures described, steaks were cut from the round, packaged and stored in a display case at about 5 C. Similar treatment was given to ground beef prepared from the same round muscles. Holding an entire side of beef at high temperature for 24 h promoted bacterial growth on the surface with subsequent proliferation on retail cuts. Shortening the aging treatment at high temperature resulted in reduced bacterial populations on packaged items. Highest bacterial loads and most rapid spoilage resulted from excising the muscle after low temperature holding and then continuing to hold the muscle in the cooler. This treatment was even more conducive to spoilage than was holding at room temperature for 24 h, and is not to be recommended.

Trends in the meat industry indicate that consumer preferences are more closely related to tenderness than to grade or other attributes of meat such as juiciness and flavor (*7*). A usual procedure in the meat processing industry is to hold meat at low temperature to allow tenderization to occur. State of contraction of muscle is a primary factor related to tenderness of beef (*4, 8, 9*), and the post-mortem contraction during rigor is more pronounced in excised muscles than in muscles remaining attached to the carcass (*6*). However, Busch et al. (*2*) reported improved tenderization of excised muscles by holding at 16 C instead of at lower temperatures. Also, since tenderization time varies with different muscles, aging of beef cuts instead of carcasses or sides might provide for better control and more rapid turnover of meat in a centralized system (*10*). We previously reported that sides aged at elevated temperature (7, 15, 21 C) followed by 2-C aging differed little in tenderness from companion sides aged continuously at 2 C for 24 or 48 h (*11*). Also, when aging of beef sides at low temperature was prolonged to allow for tenderization equivalent to that at high temperature, spoilage was more rapid than with high temperature-short time aging (*12*).

The general objectives of the present work were to evaluate effects of high temperature aging of muscles intact in beef sides and similar excised muscles on subsequent growth of microorganisms on the meat. Comparisons were also made with beef aged conventionally at low temperature.

MATERIALS AND METHODS

Twenty-one cattle from the university beef cattle herd were used for these studies. Slaughtering and preparation of steaks and ground meat were done at the University Meat Laboratory. Carcasses weighed about 600 lbs (270 kg) and graded U.S. Choice.

Aging treatments

The animals were assigned randomly to four different aging treatments. Each side of the same animal received a different treatment; the four treatments were replicated five times.

Aging was accomplished as follows: (*a*) holding the sides for 24 h at room temperature (21-23 C) (*b*) holding the sides for 24 h at 2 C; (*c*) holding the sides for 6 h at room temperature, removing the round and placing the excised muscle for 18 h in the cooler at 2 C; (*d*) holding the side for 3 days in the cooler, removing the round and storing the excised round for 4 days in the cooler, amounting to 1 week of low temperature aging. Room temperature storage was in an unrefrigerated walk-in room.

Preparation of retail cuts

After aging by any of the producers described above, steaks about 1-inch (2.5 cm) thick were cut from the top round, placed in plastic trays and packaged with 195 MSAD-80 cellophane (E.I. DuPont de Nemours and Co., Inc., Wilmington, Del.). Ground meat with fat from the trimmings after boning was also prepared from each round and packaged in 1-lb. portions as described for the steaks. The packages of hamburger and steaks were stored in a display case at 5 C under white fluorescent light to be analyzed at intervals. The initial quality was tested by analyzing replicate samples of the retail cuts right after preparation.

Sampling procedures

Sterile aluminum templates having an area of 2 cm^2 were used for sampling the steaks by a wetted swab method similar to that described by Ayres (*1*). Bacterial counts were then related to surface area. For detection of *Salmonella* and *Staphylococcus*, 10-12 sq inches

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[1]*Journal Paper No. J-8570 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 2012.*

[2]*Present address: Stokely-Van Camp, Inc., Central Laboratories, 6815 E. 34 st., Indianapolis, Indiana 46226.*

(65-77 cm^2) were sampled by swabbing the top surface of the steaks, transferring the cotton swab into the appropriate broths for culturing.

Ground meat samples were weighed and blended for 2 min with distilled water. Serial dilutions were made from the homogenate to obtain bacterial counts per gram.

Five replications were determined per treatment. For each replication, two steaks and one portion of ground beef were sampled at each time interval (0, 1,2,5 days).

Bacteriological procedures

The methods employed for bacteriological determinations are summarized in Table 1. Abbreviations used in the table are: TSA for trypticase soy agar, BBL (BBL Division of BioQuest, Cockeysville, MD.); VRB for Violet red bile agar, Difco (Difco Laboratories, Detroit, Mich.); KF for KF Streptococcus agar (Difco); BGS for Brilliant green agar (BBL) with 0.08 mg of sodium sulfadiazine per ml; and TSI for Triple sugar iron agar (Difco).

TABLE 1. *Bacteriological procedures*

Quantitative determinations	Growth media	Plating technique	Incubation
Total aerobes	TSA	Pour plates	15 C, 5 days
Coliforms	VRB	Pour plates	37 C,24 h
Enterococci	KF	Pour plates	37 C,48 h
Qualitative determinations	**Enrichment**	**Isolation**	**Confirmation**
Salmonella	CDC Procedure for meats (*3*)	BGS (*3*)	TSI and agglutination (*3*)
Coagulase-positive *Staphylococcus*	Procedure of Wilson et al. (*13*)	Procedure of Herman and Morelli (*5*)	Tube coagulase test

RESULTS AND DISCUSSION

Numbers of total aerobic bacteria recovered from steaks are plotted in Fig. 1. The initial load of total aerobes was rather low on all steaks. The highest initial contamination was detected on steaks from meat aged at room temperature for 24 h with slight differences in the initial loads of total aerobes among steaks from all other treatments.

During storage at 5 C, numbers of total aerobes on steaks aged for 24 h at low temperature remained at the lowest levels of all aging treatments. A very slow rate of multiplication was observed on steaks from meat aged for 1 week in the cooler. On the fifth day of storage at 5 C, the loads of total aerobes on steaks aged for 1 week in the cooler reached the highest levels among all treatments and the meat had a definite off-odor, indicating the fastest bacteriological spoilage of all the samples.

Total aerobic counts on ground meat are presented in Fig. 2. The initial load of total aerobes was 2 to 3 logs higher on ground meat than on steaks for every aging treatment. Keeping time of the ground meat was considerably shorter than that of the steaks. Handling and equipment can be a great source of contamination for meat. Preparation of ground meat required more handling and contact with equipment than preparation of steaks. Also, residual cuts, usually from the surface of the rounds, were used for preparation of hamburger, which is similar to commercial practices. The surface of the rounds was the most exposed to contamination during

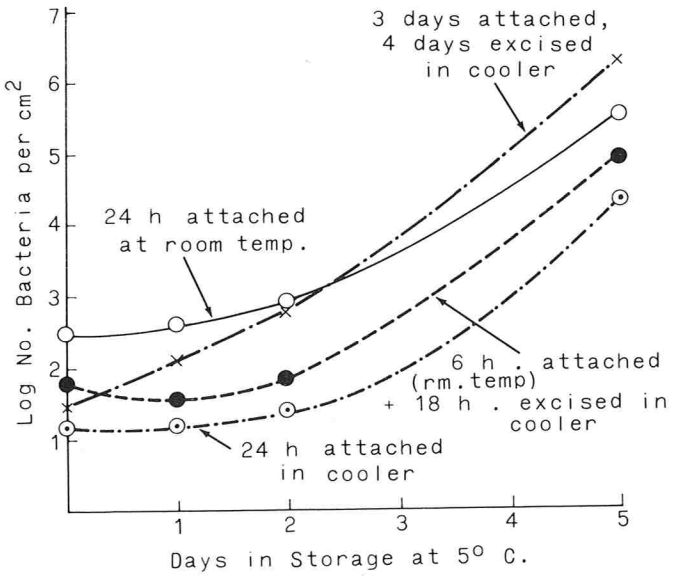

Figure 1. *Total aerobes on steaks from various aging treatments during storage at 5 C.*

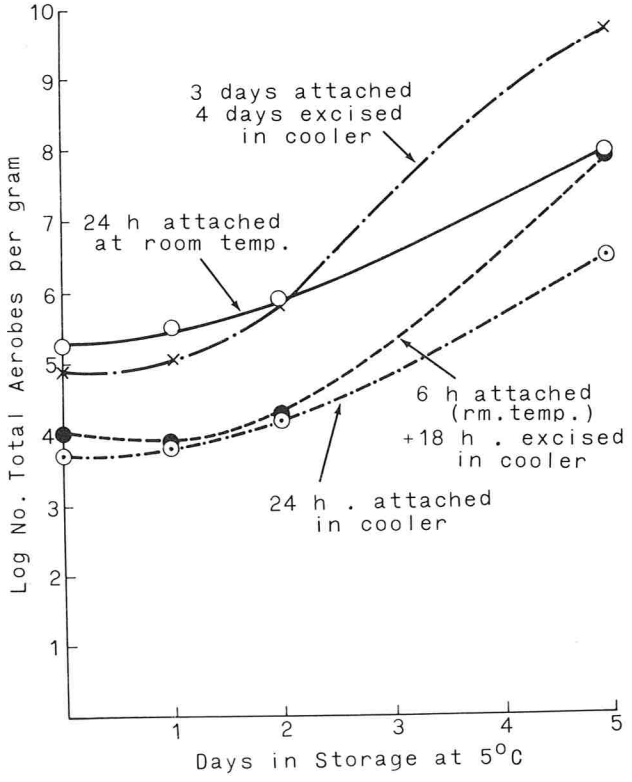

Figure 2. *Total aerobes in ground meat from various aging treatments during storage at 5 C.*

aging. Therefore, selection of the cuts for grinding also contributed to the increased loads of bacteria observed on the ground meat. Equipment for grinding was cleaned between use for different aging treatments.

Results presented in Fig. 1 and 2 demonstrated that even when the highest initial contamination for both types of retail cuts was recorded for meat aged at room temperature for 24 h, bacteriological spoilage progressed most rapidly on meat held for 1 week in the cooler. In contrast, the lowest bacterial counts corresponded to samples from sides held for 24 h in the cooler which combines the least amount of handling with low-temperature-short-time holding. Ground meat demonstrated trends similar to those of steaks for bacterial growth after different treatment of the rounds.

Coliforms and enterococci were occasionally found and only in low numbers on steaks from rounds aged at low temperature, but they were always present after preparation of steaks from sides aged at room temperature for 24 h (Fig. 3). Higher aging temperature was conducive to growth of these indicator bacteria. The average density of enterococci and coliforms comprised

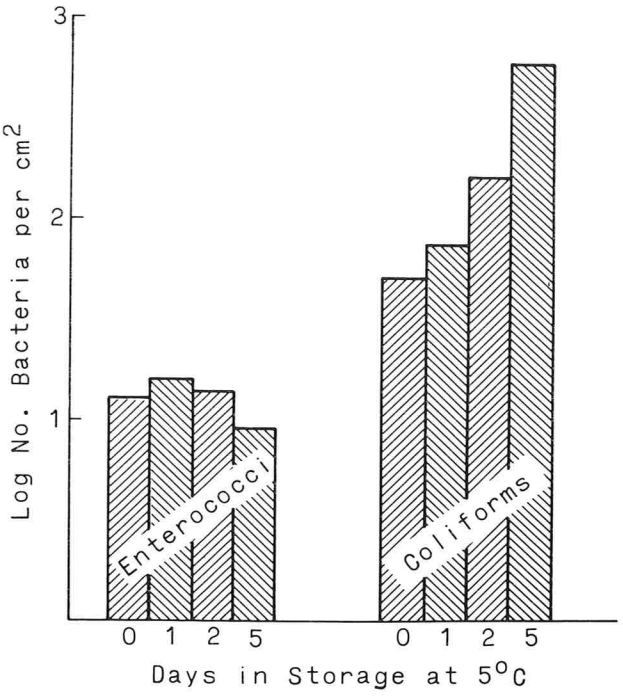

Figure 3. *Coliforms and enterococci on steaks aged at room temperature for 24 hours.*

about 10% and 20%, respectively, of the total aerobic flora initially present on these steaks. In general, whenever present on the steaks, the numbers of enterococci remained fairly constant while the coliform density increased during storage at 5 C. This is similar to our earlier results with beef held at low temperature (*12*) and is typical of growth patterns of the bacteria on refrigerated meat.

Salmonellae were isolated only from one of the 178 steaks analyzed. The steak yielding salmonellae was prepared from a side aged at room temperature for 24 h, and had been stored for 5 days at 5 C. The initial numbers of coliforms (more than 10,000 per cm^2) on steaks from this side were the highest of all the samples analyzed. There was no attempt to isolate salmonellae from ground meat. In previous work, salmonellae were not recovered from ground meat (*12*).

Coagulase-positive *Staphylococcus* were recovered from 16 out of 64 steaks prepared from sides aged at room temperature for 24 h, from all 16 steaks prepared from the companion sides which were aged at 2 C for 24 h, and also from 8 of 32 steaks prepared from meat aged for 1 week in the cooler. All steaks yielding the organism were prepared on only two different dates. The steaks prepared then had a high incidence of coagulase-positive staphylococci during later storage regardless of animal, slaughtering date, or aging treatment.

From these observations it was concluded that contamination with coagulase-positive *Staphylococcus* was more closely associated with handling during cutting and preparation of the steaks than with effect of aging treatment.

Some advantage was gained by holding the muscle for 6 h instead of 24 h at room temperature; differences were observed in rate of bacterial growth and total numbers of organisms (Fig.1). Although only incipient spoilage was noted for steaks prepared from sides aged at room temperature for 24 h, it appeared likely that shortening the aging time to 6 h might result in extending the keeping time of subsequent packaged cuts by one day. With ground beef, counts were lower from sides aged for only 6 h as compared with 24 h at room temperature, but only for the first 3 or 4 days of display case storage. After 5 days, all ground beef from high-temperature aged sides demonstrated high counts (10^7 to 10^8 bacteria per gram) and onset of spoilage (Fig. 2) as demonstrated by off-odor. Development of the psychrotrophic flora over the storage period tended to produce similar counts.

Examination of the bacterial growth curves in Fig. 1 and 2 for storage of retail cuts prepared from muscles held at 2 C for 1 week indicates that the predominant types of contaminants with this type of aging demonstrated more rapid growth than that of organisms on meat aged at higher temperature and shorter time. Similar observations were reported for previous work (*12*) when carcasses aged at 2 C for 11 days produced retail cuts that spoiled faster than those from carcasses given accelerated aging at temperatures between 16 and 22 C. Evidently a psychrotrophic spoilage flora had already become established on the beef at low temperature for 7 days before retail cuts were prepared.

Excising the muscle also appeared to produce different patterns for bacterial growth curves than those for attached muscle. Removal of the muscle with further storage in the cooler produced more rapid proliferation of psychrotrophic aerobes on steaks or in ground meat than observed when the retail items were prepared from

intact sides (Fig. 1 and 2). Increased handling and exposure of cut meat surfaces by removal of the round, and continued cooler storage, may have provided conditions conducive to rapid development of low temperature spoilage organisms on the packaged meat later stored in the display case at 5 C.

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Comparison of Methods for Estimation of Coliforms, Fecal Coliforms and Enterococci in Retail Ground Beef

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ABSTRACT

Seven recommended methods for estimation of coliforms, three for fecal coliforms and four for enterococci were tested on 30 retail ground beef samples. Lauryl sulfate tryptose broth gave higher coliform counts than did MacConkey's broth or brilliant green lactose bile broth 2% in 3-tube Most Probable Number (MPN) methods. With all MPN broths, coliforms counts were significantly ($P \leq 0.05$) higher after 48 than after 24 h of incubation. Presumptive coliform counts were higher with surface-overlay plating on violet red bile agar than with pour plating on the same agar or with the MPN broths. However, presence of *Escherichia coli* Type I was not confirmed as often from the agar medium as the broths. For estimation of fecal coliforms, counts did not differ significantly ($P \leq 0.05$) between EC broth (45.5 C) and brilliant green lactose bile broth 2% (44 C). Enterococci counts varied significantly ($P \leq 0.05$) among the four methods.

The microbial quality of meat products has come under close scrutiny with the current interest in microbiological standards. Methods for enumeration of the indicator organisms (coliforms, fecal coliforms, *Escherichia coli* and enterococci) are recommended in many manuals (*1,4,6,7,9*). Thus, a quality control supervisor in the meats industry may be faced with a choice of methods. Time, space personnel available for microbiological testing will affect the choice of methods. For this study, we have chosen recommended methods for coliform, fecal coliform and enterococcus enumeration and compared their performance on retail ground beef samples.

MATERIALS AND METHODS

Sample source and preparation

Thirty packages (about 500 g each) of ground beef were purchased in 20 local retail markets, transported to the laboratory, held at 5 C and sampled within 24 h after purchase. From each package, 25 g of ground beef were weighed into a sterile Mason jar containing 225 ml of sterile Butterfield's phosphate diluent (*9*) and blended 2 min for the initial 10^{-1} dilution. This initial dilution and serial dilutions to 10^{-6} were used to inoculate the media evaluated. All dilutions were made and pipeted within 20 min of the initial blending.

Coliform methods

The incubation times and temperature and reference for each method are shown in Table 1. Briefly, these methods are:

Presumptive coliform (LST). A 3-tube Most Probable Number (MPN) technique with lauryl sulfate tryptose broth (Baltimore Biological Laboratory, Cockeysville, Maryland).

Confirmed coliforms (LST-BGLB). Brilliant green lactose bile broth 2% (Difco Laboratories, Detroit, Michigan) was inoculated with a loopful of broth from positive LST tubes.

LST (APHIS). A 1-tube MPN (one-tube limiting dilution) using the first presumptive coliform (LST) tube of each dilution.

Coliforms (MAC). A 3-tube MPN technique with MacConkey's broth (Difco).

Coliforms (BGLB). A 3-tube MPN technique with brilliant green lactose bile broth 2%. Inoculum from positive BGLB tubes was streaked onto Levine's eosin methylene blue agar (L-EMB, Difco) and Endo agar (Difco), incubated for 24 h at 35 C and examined for the presence of typical colonies.

VRB agar pour plates. Violet red bile agar (Difco) pour plates were inoculated in duplicate; plates were overlaid before incubation.

VRB agar surface-overlay plates. Fifteen to 18 ml of VRB agar was poured and allowed to dry overnight in sterile Petri dishes. On duplicate plates, 0.1 ml of inoculum was spread over the surface of the agar with a sterile glass "hockey stick." The plates remained at room temperature for 1 h before an overlay was poured.

Fecal coliform methods

Incubation times and temperatures are shown in Table 1. Briefly, these methods are:

LST-EC. For enumeration of fecal coliforms (LST-EC), tubes of EC broth (BBL) were inoculated with a loopful of broth from each positive LST tube. Inocula from EC tubes producing gas were streaked onto L-EMB and Endo agars. Two typical colonies from each sample were picked for IMViC testing.

APHIS-E. coli (LST-EC). Positive LST (APHIS) tubes were used to inoculate EC broth tubes.

MAC-BGLB. Fecal coliforms (MAC-BGLB) were determined by inoculating a tube of BGLB and a tube of peptone water (to test for indole production) from each positive coliform (MAC) tube. BGLB and peptone water tubes were incubated at 44 ± 0.05 C for 24 h. Peptone water tubes paired with BGLB tubes producing gas were tested for production of indole with Kovac's reagent. All remaining tubes were incubated an additional 24 h. Inocula from positive BGLB tubes were streaked onto L-EMB and Endo agars. Two typical colonies were picked for IMViC testing.

IMViC tests. All L-EMB and Endo agar plates were incubated for 24 h at 35 C and examined for dark nucleated colonies with green metallic sheen and for pink to red colonies with or without green metallic sheen,

TABLE 1. *Media, methods and incubation conditions used to estimate populations of coliforms, fecal coliforms and enterococci in 30 retail samples of ground beef.*

Medium[1]	Method	Incubation time (h)	Incubation temperature (C)	References
Coliforms				
LST (Presumptive)	3-tube MPN	24 & 48	35	*4, 6*
LST-BGLB (Confirmed)	3-tube MPN	24 & 48	35	*4,6*
LST (APHIS)	1-tube MPN	24 & 48	35	*9*
MAC	3-tube MPN	24 & 48	37	*6*
BGLB	3-tube MPN	24 & 48	37	*6*
VRB	Pour plate	24	35	*1*
VRB	Surface-overlay plate	24	35	*8*
Fecal Coliforms				
LST-EC	3-tube MPN	24 & 48	45.5	*4*
LST-EC (APHIS *E. coli*)	1-tube MPN	24 & 48	45.5	*9*
MAC-BGLB + peptone	3-tube MPN	24 & 48	44	*6*
Enterococci				
AD	3-tube MPN	48	35	*4*
AD-EVA	3-tube MPN	48	35	*1*
KF	Pour plate	48	35	*4*
m-Enterococcus	Pour plate	48	35	*2*

[1]LST = lauryl sulfate tryptose broth, BGLB = brilliant green lactose bile broth 2%, MAC = MacConkey's broth, VRB = violet red bile agar, EC = EC broth, AD = azide dextrose broth, EVA = ethyl violet azide broth, KF = streptococcal agar.

respectively.

Typical colonies from L-EMB and Endo agar plates and from VRB agar surface-overlay and pour plates were picked and streaked onto plate count agar (PC, Difco) slants, incubated 24 h at 35 C and refrigerated for 1 to 4 days until IMViC testing was begun. Growth from PC agar slants was used to inoculate LST broth, MR-VP medium (Difco), Koser citrate medium (BBL) and tryptone broth (Difco). Colonies were classified according to their biochemical reactions (*4*); IMViC for *E. coli* Type I was classified as ++− −, lactose positive.

Enterococcus methods

The incubation time and temperature and references are shown in Table 1. These methods, briefly summarized, are:

AD. A 3-tube MPN technique with azide dextrose broth (BBL).

AD-EVA. A loopful of broth from positive AD tubes was inoculated into ethyl violet azide broth (BBL) tubes.

KF agar pour plate. Each dilution was plated in duplicate with KF streptococcal agar (BBL). Pink or red colonies were counted.

m-Enterococcus agar pour plate. Duplicate m-Enterococcus agar pour plates were inoculated. Pink to maroon colonies were counted.

Statistical analysis

All counts were converted to logarithms (base 10) and treated by analysis of variance and the mean separation technique of Duncan (*3*).

RESULTS AND DISCUSSION

Although all the methods we tested are recommended methods, we found significant ($P \leq 0.05$) differences among them. Counts were higher from the VRB agar surface-overlay and pour plates than from MPN procedures (Table 2). VRB agar surface-overlay or pour plates give a rapid estimate of the coliform density and may be the method of choice if only coliform numbers are required. In our study, means of counts were 0.45 log higher ($P \leq 0.05$) from the VRB agar surface-overlay than from the VRB agar pour plates. That comparison indicates that some cells might have been stressed by the temperature (45 C) of the melted VRB agar when the plates were poured. Ray and Speck (*8*) reported that with pure cultures VRB agar pour plates tended to underestimate coliform counts. Surface plating does eliminate some stress to cells.

TABLE 2. *Variability of coliform counts (log_{10}) in retail ground beef determined by various methods.*

Method	Incubation Temperature (C)	Incubation Time (h)	Mean[1]	Range
APHIS coliform (LST)	35	24	2.00gh[2]	1.00-3.00
		48	2.90c	1.00-5.00
Presumptive coliform (LST)	35	24	2.28ef	.96-4.18
		48	3.27b	1.36-4.97
Confirmed coliform (LST-BGLB)	35	24	2.26efg	.96-4.18
		48	2.98c	1.36-4.97
Coliform (MAC)	37	24	1.89h	0-3.63
		48	2.40de	.96-4.36
Coliform (BGLB)	37	24	2.08fgh	.61-3.63
		48	2.57d	.85-4.63
VRB surface-overlay plate	35	24	3.97a	2.18-5.61
VRB pour plate	35	24	3.52b	2.18-5.01

[1]Mean of 30 determinations.
[2]Means followed by different letters are significantly different ($P \leq 0.05$) by Duncan's multiple range test (3).

In our study less than 10% of the colonies isolated from VRB agar plates were *E. coli* Type I. A total of only two colonies from each plate were selected and thus represented a low percentage of the colonies present. Hall (*7*) reported that *Proteus* species may interfere with coliform counts either by forming small atypical colonies or by inhibiting precipitation of bile by coliforms. He found that those situations resulted in overestimation if all red colonies were counted but underestimation of coliforms if only typical colonies were included.

Comparison of the liquid media used in the coliform MPN techniques showed that LST broth provided the highest numbers (Table 2). The presumptive coliform (LST) counts were significantly higher than confirmed

coliform (LST-BGLB) counts because many LST tubes which were negative after 24 but positive after 48 h did not confirm in BGLB. The confirmed coliform (LST-BGLB) count was also significantly higher than counts of either coliforms (MAC) or coliforms (BGLB). The difference between the 24- and 48-h incubations was significant ($P \leq 0.05$) for all liquid media used for the MPN method.

The Animal and Plant Health Inspection Service (APHIS;*9*) recommends a 1-tube rather than a 3-tube MPN technique; results are read after 24 h. That method saves media but for the samples we tested gave mean coliform counts that were from 1 to 2 logs lower than the VRB agar counts (Table 2).

The 3-tube MPN methods for fecal coliforms at 48 h were not significantly different (Table 3) even though the inoculum was derived from coliform methods that did differ significantly (Table 2). Increasing the incubation time from 24 to 48 h did not significantly increase the fecal coliform counts except in the APHIS *E. coli* (LST-EC) method. However, since only 1 tube per dilution was used, each positive tube resulted in an increase of one log while in 3-tube methods single values had smaller effects. Goepfert (*5*) also noted that increasing incubation from 24 to 48 h had only a slight effect on fecal coliform counts. Although we found a slight increase in fecal coliform counts after 48 h of incubation, it is questionable whether incubation for 48 h is necessary, especially for a perishable product such as ground beef. Time of incubation is less important for fecal coliforms than for coliforms because the inoculum is a young culture grown at optimum temperature (35-37 C) in a medium that is selective for coliforms.

TABLE 3. *Variability of fecal coliform counts (log_{10}) in retail ground beef determined by various methods.*

Method	Incubation Temperature (C)	Incubation Time (h)	Mean[1]	Range
APHIS *E. coli* (LST-EC)	45.5	24	1.07c[2]	0-3.00
		48	1.37b	0-3.00
Fecal coliform (LST-EC)	45.5	24	1.57ab	0-4.18
		48	1.81a	0-4.18
Fecal coliform (MAC-BGLB)	44	24	1.48b	0-3.63
		48	1.65ab	0-3.63

[1]Mean of 30 determinations.
[2]Means followed by different letters are significantly different ($P \leq 0.05$) by Duncan's multiple range test (3).

Incubation at 44 or 45.5 C eliminated many of the non-fecal coliforms; less than 3% of the positive fecal coliform tubes yielded atypical colonies on either L-EMB or Endo agar plates. Isolation of *E. coli* Types I and II from fecal coliform (LST-EC) were 92% and 4%, respectively, and from fecal coliform (MAC-BGLB) were 89% and 9%. Goepfert (*5*) also was usually able to confirm the presence of *E. coli* in positive EC broth tubes.

Of 30 meat samples evaluated in this study, 13 (43%) would have been considered adulterated under an arbitrary standard of less than 50 *E. coli*/g for fresh meat. Both 1-tube and 3-tube MPN LST-EC methods would have justified rejection of the 13 samples after 48 h of incubation. With only 24 h of incubation for the 3-tube MPN technique, 11 samples had more than 50 *E. coli*/g. The other fecal coliform method with MAC-BGLB broth would have rejected 13 samples after 24 h and 14 after 48 h of incubation.

The enterococcus counts were highly variable; each method gave results significantly ($P \leq 0.05$) different from the others (Table 4). Counts were almost two logs (base 10) higher for the unconfirmed AD 3-tube MPN than for the KF agar pour plates. Azide dextrose broth was the least inhibitory medium used to determine enterococci. Confirmation of counts from AD broth in EVA broth reduced enterococcus estimates by one log. With the solid media used in this study, further confirmation is generally not considered necessary (*2,4*).

TABLE 4. *Enterococci counts (log_{10}) in retail ground beef.*

Method[1]	Mean[2]	Range
AD 3-tube MPN	4.53a[3]	3.36-5.66
AD-EVA 3-tube MPN	3.18c	2.32-4.63
KF agar pour plate	2.69d	0-3.70
m-Enterococcus agar pour plate	3.72b	2.70-5.22

[1]AD = azide dextrose broth, EVA = ethyl violet azide broth, KF = KF streptococcal agar.
[2]Mean of 30 determinations.
[3]Means followed by different letters are significantly different ($P \leq 0.05$) by Duncan's multiple range test (3).

Any of the methods we tested should give relative estimates of contamination levels. The VRB agar surface-overlay plates gave the highest coliform counts within 24 h, but this method has some disadvantages. Surface plating takes longer than pour plating and the 0.1-ml inoculum may be subject to more sampling error than the 1.0 ml used in pour plating. The time required for counting VRB agar plates also should be considered; meat particles make counting difficult. Possible interference of species other than coliforms may cause over or underestimation. Suspect colonies can be confirmed in BGLB broth; however, that step would obviate the time-saving aspect of this solid media method.

Although the broad range of confidence limits has caused criticism of the MPN technique, 3-tube MPN with LST broth should be used whenever regulatory action is involved. The 48-h incubation period is a major disadvantage. Reading and recording the pattern of positive and negative tubes is more rapid than counting VRB agar plates. Another advantage of LST 3-tube MPN is that positive tubes can easily be used to inoculate EC broth to determine fecal coliforms. The 1-tube MPN method (*9*) requires only one-third as much media as the 3-tube method; this is an advantage for a broad range of dilutions.

The EC broth was the best medium for evaluation of fecal coliforms. The 24-rather than 48-h incubation may be adequate for EC broth tubes, but further studies would be required before recommendations could be made. Methods for enumeration of enterococci also should be tested further before recommendations could be made for ground beef.

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A Research Note

Effect of Mold Growth on the pH of Tomato Juice

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ABSTRACT

Fifty-eight species of 21 genera of molds were grown on tomato juice for 35 days to determine the effect on pH. The molds included members of the class *Phycomycetes* and the families *Moniliaceae, Dematiaceae,* and *Tuberculariaceae.* All molds except two raised the pH from the initial pH 4.1 to a range from 4.9 to greater than 9.0. Thirty-three of the *Fungi Imperfecti* (53%) raised the pH to values above 7.0. None of the *Phycomycetes* tested raised the pH above 7.0.

Huhtanen et al. (*2*) observed that *Cladosporium* sp. raised the pH of tomato juice from 4.2 to 7.8 in 19 days of incubation at room temperature and that *Clostridium botulinum* grew in association with the mold. The alkalinizing ability of molds growing in tomatoes or tomato juice has not been recorded. Such a study is of value, since the lower limit for the growth of *C. botulinum* in foods is reported to be pH 4.7 (*5*), and in tomatoes, pH 4.9 (*4*).

Mold appears not to have been observed in acid canned foods associated with outbreaks of botulism. Meyer and Gunnison (*3*) obtained growth of *C. botulinum* in Bartlett pears which were also inoculated with a *Lactobacillus* and a yeast. More recently, a yeast-like organism, a diplococcus and *Enterobacter agglomerans* were isolated from home-canned tomato juice which was incriminated in a single case of botulism (*1*); however, the observation was not pursued to determine a possible synergistic effect. The pH of the juice was 4.2.

The pH of a few jars of molded, canned tomatoes brought into this laboratory has ranged from 5 to 8.65. Few molds from these specimens were identified. It would appear from these observations that molds generally have an alkalinizing ability when growing in canned tomatoes or tomato juice, and this study was conducted to test this observation. The study has further merit because of questions asked occasionally by the thrifty housewife who has sought assurance on the use of molded tomatoes from which the surface growth has been removed.

MATERIALS AND METHODS

The molds were taken from the Food Microbiology collection of the University of Tennessee. They represent 24 genera and 58 species of the filamentous fungi. Many had been isolated from foods. The molds were grown on slants of mycophil agar from which spore inocula were taken with a moist needle.

Canned tomato juice was tubed in 10-ml volumes in tubes measuring 25 mm outside diameter, capped with plastic overcaps and sterilized at 121 C for 15 min. The pH after sterilization was 4.1. After inoculation the tubes were incubated at room temperature (22 C) for 35 days. Tubes were then heated for 20 min in flowing steam to destroy the molds. The contents were transferred to another container to ensure mixing, and the pH was measured with a Corning pH meter.

RESULTS AND DISCUSSION

The molds produced the characteristic pellicle on the surface of the tomato juice. All molds except *Nigrospora* and *Spicaria* raised the pH above the value of the control tubes (Table 1). The change in pH ranged from only slight to marked, with some values reaching above 9 following incubation. The pH values may reflect the relative rates of change rather than the ultimate attainable values, since the activity of the fungi was interrupted on the 35th day. Thirty three molds (53%) raised the pH to values above 7.0. Several species in the genera *Aspergillus* and *Penicillium* raised the pH to the range of 5 to 6, while other species of these genera raised the pH to values between 8 to 9 and above 9.0. Six species, including three of *Aspergillus,* raised the pH above 9.

The ability to raise the pH is a species attribute. None of the *Phycomycetes* raised the pH to a value above 7 (Table 2). Twenty six (56.6%) of the *Moniliaceae* raised the pH to values between 7.1 to more than 9. Members of the genera *Aspergillus* and *Penicillium* are found in several columns of Table 1. Variability in the ability to alter the pH was found among each of the three families *Moniliaceae, Dematiaceae,* and *Tuberculariaceae.*

Huhtanen et al. (*2*) showed that alkalinization is not restricted to the portion of tomato juice immediately beneath the mat, but the increased pH extends as a

gradient downward into the tube. Presumably a similar gradient exists in jars of home-canned tomatoes and tomato juice. Since nearly all molds appear to have the ability to raise the pH above the point which permits growth of *C. botulinum,* one can only emphasize the inherent risk associated with the use of canned tomatoes or juice from which the surface growth of mold has been removed.

TABLE 1. *Molds and pH of inoculated tomato juice after 35 days at 22 C.*[1,2]

pH Range					
4.9-5.0	5.1-6.0	6.1-7.0	7.1-8.0	8.1-9.0	Above 9.0
Acrophialophora	*A. flavus*	*A. candidus*	*Aerospeira*	*A. allahabadii*	*Alternaria*
Absidia	*A. funigatus*	*A. clavatus*	*A. sulfureus*	*A. fisheri*	*A. microviride-citreus*
A. oryzae[3]	*A. tamari*	*A. parasiticus*	*A. sydowi*	*A. janus*	*A. nidulans*
A. wentii	*Choanephora*	*Cunninghamella*	*A. terreus*	*A. sulfureus*	*A. ochraceus*
Syncephalastrum	*Circinella*	*Epicoccum*	*A. terricola*	*F. lateriticum*	*Cylindrocarpon*
	M. rouxii		*P. chrysogenum*	*Gliocladium*	*Ulocladium*
	P. brevicompactum	*P. notatum*	*P. commune*	*Helminthosporium*	
	P. lanosum	*P. restrictum*	*P. oxalicum*	*P. aculeatum*	
	T. koningii	*P. waksmannii*	*P. steckii*	*P. canadense*	
	Torula sp.		*Pleichochaeta*[4]	*P. claviforme*	
				P. decumbens	
				P. funiculosum	
				P. granulatum	
				P. lanosum	
				P. lapidum	
				P. solitum	
				Scopulariopsis	

[1]Initial pH 4.1.
[2]No change: *Nigrospora, Spicaria.*
[3]Abbreviations: A.- *Aspergillus;* F. - *Fusarium;* M. - *Mucor;* P. - *Penicillium;* T. - *Trichoderma.*
[4]Tentative identification.

TABLE 2. *Increase in pH of tomato juice due to growth of molds.*[1]

pH Range	Number	Class or family of molds			
		Phycomycetes	*Moniliaceae*	*Dematiaceae*	*Tuberculariaceae*
No change	2		1	1	
4.9-5.0	5	2	3	0	0
5.1-6.0	10	3	6	1	0
6.1-7.0	9	1	7	0	1
7.1-8.0	10	0	8	2	0
8.1-9.0	17	0	15	1	1
Above 9.0	6	0	3	2	1
Totals	59	6	43	7	3

[1]Initial pH 4.1.

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Evaluation of Quick Bacterial Count Methods for Assessment of Food Plant Sanitation

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ABSTRACT

Four methods to detect microbial contamination on food plant equipment surfaces were compared to determine the accuracy, precision, cost and time required to do each test. A standard method for swabbing; a simplified swab test (Millipore Corporation); a contact-transfer method (Con-Tact-It, Birko Chemical Corporation) and a direct method using Rodac plates (BBL) were evaluated. The equipment surfaces were found to be highly contaminated indicating the necessity for regular microbial testing. Under conditions of the test, there was good agreement in enumeration of bacteria between the standard swab test and both the Millipore swab method and the Con-Tact-It system; the standard swab method and Rodac plates showed the best precision. The contact methods were by far the quickest tests to do, and the Con-Tact-It system was the least expensive.

Equipment used in any food processing operation plays a major role in control of contamination in final products. Many small plants have neither the facilities to conduct chemical and microbiological tests nor any provisions to have these done routinely. This makes some form of quality control difficult, and as a result, many small establishments are blind to the sanitation of their facility, equipment and subsequent safety of their product. As an alternative, simplified testing procedures, that do not require a sophisticated laboratory and highly qualified personnel, are available for the small food processor. While these methods may not be definitive, they are reported to be indicative of plant sanitation (*2,3,5,8,9*). Our objective was to compare three tests — Millipore Swab Test Kit, Rodac Procedure of Surface Sampling and Con-Tact-It System Bacteria Detection Unit with the standard swabbing procedure (*1*).

MATERIALS AND METHODS

The deboning table tops, hamburger pattie and steaking line conveyors of a local meat cutting plant were selected as surfaces for the work. Locations for each test on the surface were randomized, as were days and times for sampling. However, samplings were mostly done during night after washing the equipment or in the morning before start up. Each test was conducted in duplicated on 20 different occasions.

Falcon brand swabs (Swube, in a 17 × 100-mm tube) were obtained from Fisher Scientific Company. The swab test kit for total bacterial count (Total-Count Sampler) was obtained from Millipore Ltd., Mississauga, Ontario, Canada. Rodac plates were the product of BBL (Division of Becton, Dickinson and Company). The Con-Tact-It System Bacteria Test Unit was obtained from Birko Chemical Corporation, Denver, Colorado.

Standard microbiological procedures were followed for enumeration of microorganisms on equipment surfaces by the swab method (*1*). A sterile 4-inch^2 glass template was used as a guide to swab the area. For the other procedures, manufacturers' instructions were followed. Incubation was at 35 C for 2 days.

RESULTS AND DISCUSSION

The results from the trials are presented as ranges in Table 1. The counts are high but could be considered typical of a food operation where there are no provisions for microbial testing. Table 2 shows the relative precision of the four testing methods, and correlation coefficients between the Swab test and each of the Millipore, Rodac and Con-Tact-It tests. The calculated values for relative precision, as described by Kramer and Twigg (*6*), indicates that the order of precision for the test methods would be Rodac > Swab > Millipore > Con-Tact-It. Correlation coefficients between both the Millipore and Swab test and, the Con-Tact-It system and Swab method were significant, while the r value calculated from the results of Rodac plates and the Swab test was not significant. If the Swab test were considered to be standard, then these results indicate that, under the conditions of our trials, the Millipore and Con-Tact-It tests are more accurate than is the use of Rodac plates. A possible explanation for the failure of the Rodac results to agree with those of the Swab test might be in the population numbers on the equipment surfaces tested. Very often the Rodac plates were covered by colonies, which severely diminished their accurate enumeration. Favero et al. (*4*) and Baldock (*3*) also pointed out that agar contact procedures are quantitative only if the level of contamination is low. A recent report (*7*) suggested that accuracy was lower above 200 colonies per cm^2 using the Con-Tact-It procedure.

TABLE 1. *Microbial counts per sq in area from two replicates on food plant equipment surfaces using four methods of testing.*

Test no.	Swab Method (×100)		Millipore (×100)		Rodac (×10)		Con-Tact-it (×10)	
1	250	570	270	540	2.5	6	—	—
2	1.5	8.7	0.4	16	6	14	4	7.2
3	850	1100	180	220	20	37	72	150
4	2.2	16	0.6	12	1.2	13	8.6	15
5	21	32	0.4	0.7	9	22	6.7	13
6	400	500	190	220	120	150	83	110
7	150	320	130	160	90	120	45	54
8	170	270	45	130	45	50	31	34
9	25	35	13	27	20	25	14	20
10	72	95	58	150	45	55	54	94
11	5	20	8.1	12	15	15	5	20
12	16	95	53	65	26	74	70	72
13	1.2	2	0.1	0.5	1.5	7	0	1.4
14	19	47	11	19	28	33	4	10
15	7	11	1.1	1.4	4.5	22	17	41
16	2.7	3	1	5.4	13	21	4.7	5.8
17	2.5	3.2	0.4	0.5	15	19	16	23
18	2.2	2.7	1.8	2.9	0.7	2.7	0	0.4
19	0.5	7	0.2	0.3	0.7	1.2	0.2	2
20	13	23	4.5	19	16	37	13	14

TABLE 2. *Correlation coefficient (r) and relative precision[1] of Swab, Millipore, Rodac and Con-Tact-It procedures.*

	Swab	Millipore	Rodac	Con-Tact-It
r^2	—	0.75* *	0.37	0.81* *
Rp (%)	9.1	15.3	8.6	17.4

[1]Relative precision, Rp = sd/Rs where sd = standard deviation of difference between two duplicates and Rs = range of means of the duplicates, a smaller value indicates greater precision.
[2]r Calculated with the swab test as the independent variable.
* * $p \leq 0.01$

A control experiment in which a laboratory bench top area was spread with about 100 *Pseudomonas aeruginosa* colony forming units per inch[2] was used to assess the variability of each testing method. The standard deviations and coefficients of variability calculated from this trial (Table 3) show that the Swab test was the least variable and Con-Tact-It was the most, the order being Swab > Rodac > Millipore > Con-Tact-It.

TABLE 3. *Estimation of microbial populations by the Swab, Millipore, Rodac and Con-Tact-It procedures on a prepared surface[1].*

Swab	Millipore	Rodac	Con-Tact-It
550	30	110	5
550	27	126	2
620	18	83	9
820	23	74	1
750	14	124	6
121.9	6.5	23.8	3.2
C^SV^2(%)18.5	29.0	23.0	69.8

[1]Surface swabbed with approximately 100 *Pseudomonas aeruginosa* colony forming units per inch[2].

[2]$CV = \frac{100s}{\bar{X}}$ where s = standard deviation
CV = coefficient of variability

The time or labor required to do each of the tests in this study, and cost per test are given in Table 4. As expected, the Swab test was the most labor-intensive; Millipore, which follows similar principles, was slightly faster and easier to do, but considerably more expensive.

TABLE 4. *Time, in minutes, taken to conduct the Swab, Millipore, Rodac and Con-Tact-It tests, and the cost of materials per test.*

Test	Time Required (min)	Const/Test ($)
Swab	7	0.25
Millipore	5	2.95
Rodac	1.5	0.72
Con-Tact-It	1.5	0.10[1]

[1]This cost is based on the utilization of all 9 spaces/plate. If less than 9 tests were done at one time, the cost/test would increase $0.09/space left.

The number of contaminants detected per unit area of the surface using Con-Tact-It and Rodac procedures were consistently low, indicating that these methods recovered fewer contaminants than swabbing methods. Consequently, agar contact methods should be used only as quick indicators of bacterial numbers. The Con-Tact-It system was slightly faster to count as a smaller contact area was involved. With respect to skill required to carry out the tests, they could be ranked as follows: Swab > Millipore > Con-Tact-It > Rodac.

The finding that the meat processing plant under study consistently showed heavily contaminated food contact surfaces stresses the need for monitoring the equipment for sanitation. This will indicate to management if sanitation is adequate, and areas that require attention. Since these plants need only some indication of relative numbers of contaminants present, they could adopt a method which is inexpensive and does not require highly skilled workers or elaborate laboratory facilities. It was our observation that except for the standard swab procedure, the methods studied during this investigation were relatively simple and could be conducted by a technician with little training.

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Activities of Five Acid Phosphatases in Purple, Green, and White Eggplants

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ABSTRACT

Tissues of purple, green, and white varieties of eggplant, *Solanum melongena*, were analyzed for relative activity of phenyl phosphatase, fructose-1, 6-diphosphatase, glucose-1-phosphatase, glucose-6-phosphatase, and ATPase. Activities of all phosphatases were highest in the purple variety and lowest in the white. Relative rates of activity decreased in the order: ATPase, phenyl phosphatase, fructose-1, 6-diphosphatase, glucose-6-phosphatase, and glucose-1-phosphatase (only found in the purple variety).

Phosphatases include a broad group of enzymes that catalyze the hydrolysis of mon-, di-, and triesters of phosphate bound to sugars, lipids, or nucleic acids. Another role suggested is related to the onset and development of senescence (*2*). Although phosphate esters and phosphatases play a major role in virtually all aspects of carbohydrate metabolism in plant tissues, no information is available on phosphatase activity in different varieties of eggplants. Eggplants (*Solanum melongena*) are not grown extensively in northern climates because they need a warm growing season of 14 to 16 weeks for good yields (*4*). Although the purple is the most popular variety, others differing in size, shape and color are known. A white variety has been grown in Europe for many years, but apparently for ornamental purposes only (*6*). A green eggplant, grown in India for several years (*12*), is now appearing in home gardens in the southern United States. Pink and black eggplants have also been cultivated in India (*11*).

Constantin et al. (*5*) compared the processing properties of purple and green eggplants, but not their composition. Flick et al. (*9*) examined proximate compositions of purple, green, and white eggplants, and found more fiber in white (22.3% dry white basis) than in purple (10.8%) or green (11.9%). Also differences in four enzyme activities (polyphenoloxidase, lipoxygenase, alcohol dehydrogenase, and catalase) were reported (*8,9*) between the three cultivars. Some of these enzymes have been correlated with flavor or organoleptic qualities in fruits and vegetables. The purpose of this research was to determine whether the three eggplant varieties differed in phosphatase activities and to correlate the activities with the fiber differences previously reported. The five acid phosphatases compared were: phenyl phosphatase, EC 3.1.3.2 (Ø-Pase); ATPase, EC 3.6.1.3; fructose-1, 6-diphosphatase, EC 3.1.3.11 (F-1, 6-di-Pase); glucose-1-phosphatase, EC 3.1.3.10 (G-1-Pase); and glucose-6-phosphatase, EC 3.1.3.9 (G-6-Pase).

MATERIALS AND METHODS

All eggplants were grown under identical conditions in the same outdoor plot in Chalmette, La., and were harvested at about the same stage of maturity (same age of fruit after flowering). The fruit were stored in a refrigerator at 4 C for 1 to 2 days until used. Peeled fruit were rapidly cut into 1-cm^3 pieces and immediately homogenized (50 g/250 ml cold deionized water) for 1 min in a food blender. Homogenates were centrifuged at 15,000 × *g* at 5-9 C for 15 min and the clear supernatant fluids wre decanted into test tubes placed in crushed ice. Nitrogen contents of tissue extracts for comparing enzyme and analyses were determined by the macroKjeldahl method. Triplicate analyses were repeated eight times on 2-4 fruit of each variety. Buffer salts and reagents were purchased commercially. Acid phosphatase activity was measured as described in the Worthington Enzyme Manual (*13*). Each cuvette contained 0.1 ml 0.15 M acetate buffer, pH 5.0, 0.05 ml 0.01 M substrate (disodium salt of phenyl phosphate, fructose-1, 6-diP, ATP, glucose-1-P, or glucose-6-P), 0.05 ml 0.01 M $MnCl_2$, 0.02 ml water, and 0.5 ml eggplant extract (or water in the blank control). Inorganic phosphorus released was determined by the Fiske-Subbarow (*7*) method at 710 nm in a spectrophometer from specific substrates after 45 min, pH 5.0, room temperature (25-26 C).

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RESULTS AND DISCUSSION

Figure 1 shows a photograph of the three varieties of eggplants used in these experiments. The purple eggplant is slightly larger and is pear-shaped whereas the green and white varieties are more round. Figure 2 shows the relative activites of the five acid phosphatases in all three varieties. The release of phosphate from ATP by ATPase activity appears to be inversely proportional to the high fiber contents reported earlier (*9*). In white eggplants, the ATPase activity was the lowest, whereas the fiber content was the highest. The opposite was observed for the purple variety. This suggests that ATPase activity in eggplants is not primarily associated with fiber formation. The high ATPase activity in all three varieties also indicates that the reactions requiring high energy phosphate are significantly greater than those involving hydrolysis of hexose mono- and diester phosphates. ATPase activity in purple eggplants was also eight times higher than Ø-Pase. Since Ø-Phosphate is not considered as a natural substrate in plants, it is possible that some of the observed Ø-Pase activity may be due to nonspecific esterases, as was reported in peanuts (*3*).

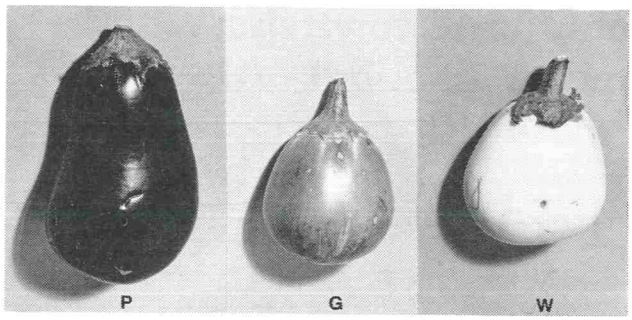

Figure 1. *Purple, green, and white eggplants picked at the same stage of maturity.*

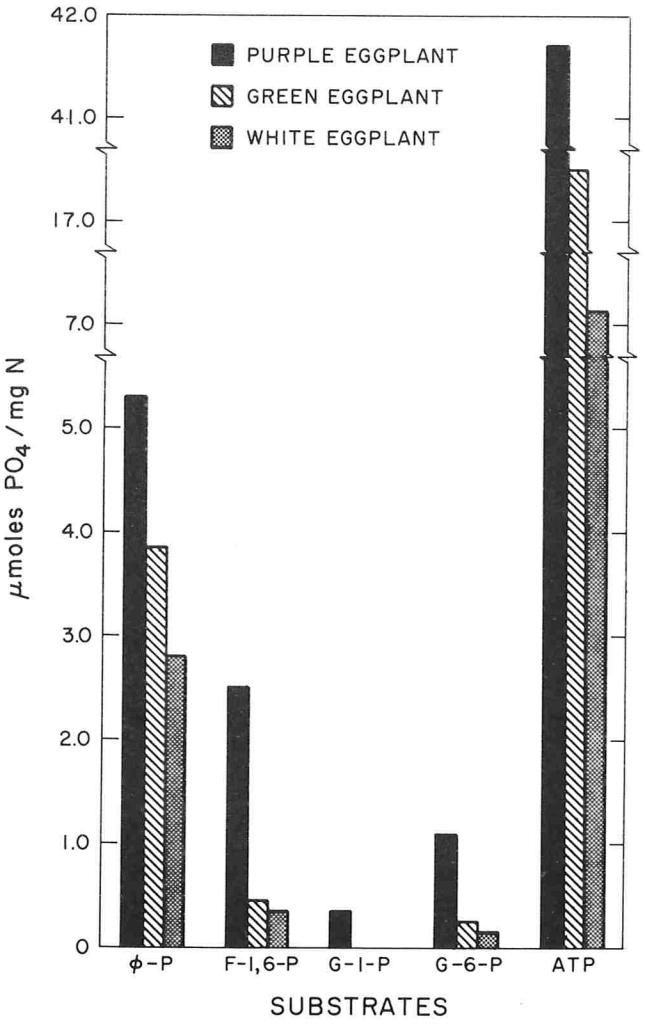

Figure 2. *Activities of acid phosphatases in purple, green, and white eggplants. Conditions as described in text. Substrates: ØP, phenyl phosphate; F-1 6-P, fructose-1, 6-diphosphate; G-1 Pase, glucose-1-phosphate; G-6-P, glucose-6-phosphate; and ATPase.*

F-1, 6-diPase (2.4) and G-6-Pase (1.0) activities were both higher in purple eggplants, with only trace amounts found in green and white. A small amount of G-1-Pase was found only in purple eggplants. This phosphatase is not common in fresh tissue as is G-6-Pase and F-1, 6-diPase. In a study of ungerminated barley grains (*10*), G-1-Pase was not present but significant activity was measured after 4 to 6 days germination (*1*), suggesting that G-1-Pase may not be a normal constituent of fresh seeds and vegetables. Some seeds were present in the pieces of tissue used to prepare eggplant extracts, but not enough to affect the measurements of G-1-Pase activity. G-1-Pase activity is present in the fleshy tissue of purple eggplants, but not in the other two varieties.

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A Research Note

Activity of *Streptococcus diacetilactis* and its UV- Induced Mutant in Dahi Prepared from Different Types of Milk[1]

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ABSTRACT

Streptococcus diacetilactis and one of its UV-induced mutants were individually used to prepare dahi from cow's and buffaloe's milk and reconstituted full-cream dried cow's milk. Dahi samples were analysed for titratable and volatile acidities, diacetyl and proteolytic activity. Each sample was evaluated organoleptically and acceptability of the product was measured by the 9-point hedonic scale. The dahi prepared with the mutant scored better in all the types of milk used, as compared to the parent culture.

Dahi is a fermented milk product consumed by a large section of the population of the Indian subcontinent. The role of lactic streptococci in production of acid and flavor in fermented milk products like dahi is now well recognized (*1,3,7,8*). Some workers (*2,10,11*) have reported variations in the biochemical performance of cultures in milk of different species. Earlier studies in this laboratory (*6*) have dealt with isolation of mutants of *Streptococcus diacetilactis* on citrate agar after exposure to ultra-violet rays. The present communication reports on comparison of activity of *S. diacetilactis* and one of its UV-induced mutants in different types of milk.

MATERIALS AND METHODS

A strain of *S. diacetilactis* (S-I) and its UV-induced mutant (PM-1), which was stable through several hundred sub-cultures for a period of 5 years, were used. Reconstituted full-cream dried cow's milk prepared at the Experimental Dairy, National Dairy Research Institute, Karnal, fresh cow's milk and buffaloe's milk were steamed for 30 min and cooled to 30 C. Milk samples were individually inoculated at the 1% level with 16-h-old cultures and then aseptically poured into dahi bottles, which were then incubated at 30 C. After 18 h of incubation, one set of dahi bottles was examined for titratable and volatile acidities, diacetyl and proteolytic activity. Another set was transferred to the refrigerator (6 C) for 4 h and then the dahi was graded for appearance, consistency, taste and flavor.

Titratable acidity was determined as percent lactic acid by titrating a known aliquot of the sample against 0.1 N NaOH, and volatile acidity was estimated by the method of Hempenius and Liska (*4*). Diacetyl and proteolytic activity of the cultures were determined by the methods of Pack et al. (*9*) and Hull (*5*), respectively.

The overall acceptability of dahi samples was measured by the 9-point hedonic scale (9 =Liked extremely; 8 =Liked very much; 7 =Liked moderately; 6 =Liked slightly; 5 =Neither liked nor disliked; 4 = Disliked slightly; 3 = Disliked moderately; 2 = Disliked very much; 1 =Disliked extremely).

RESULTS AND DISCUSSION

From data in Table 1, it may be seen that the mutant PM-1 produced greater titratable and volatile acidities, more diacetyl and greater proteolysis than the parent, irrespective of the type of milk used. It is also noteworthy that the mutant in comparison to its parent culture, exhibited approximately 31, 41, and 109% increases in total acidity, volatile acidity and diacetyl, respectively. Further, both cultures produced more acidity in buffaloe's milk, more diacetyl and volatile acids in cow's milk and greater proteolytic activity in reconstituted full cream dried cow's milk. Similar differences had been reported by Thomas et al. (*11*) and Dutta et al. (*2*) for acid production and proteolytic activity by *S. diacetilactis* in different types of milk. The greater acidities (both titratable and volatile) found with the mutant is quite significant in view of the important role this organism plays in preparation of fermented milk such as dahi.

On the basis of organoleptic evaluation (Table 2), dahi prepared from cow's milk using the mutant scored maximum points and was accepted on the hedonic scale as "Liked moderately" to "very much." With buffaloe's milk dahi, similar ratings were noted and acceptability of the product was as "Liked slightly" to "moderately."

[1] *N.D.R.I. Publication No. 77-99.*
[2] *Haryana Dairy Development Corporation, Bhiwani, India.*

TABLE 1. *Activity of* S. diacetilactis *(S-1) and its mutant (PM-1) in different types of milk.*[a]

Test	Cow's milk		Buffaloe's milk		Reconstituted full-cream dried cow's milk	
	S-1	PM-1	S-1	PM-1	S-1	PM-1
Titratable acidity (Percent lactic)	0.70	0.92	0.84	1.05	0.65	0.80
Volatile acidity (ml of 0.01 N NaOH/50 g of curd)	20.3	28.6	16.5	19.0	18.5	24.0
Diacetyl (ppm)	15.0	31.4	8.5	11.8	12.0	14.9
Proteolytic activity (mg of tyrosine liberated/1 g of curd)	0.30	0.36	0.27	0.33	0.35	0.39

[a]Cultures were examined after 18 h of incubation at 30 C. Results represent an average of three trials.

TABLE 2. *Score card*[a] *for dahi prepared from* S. diacetilactis *S-1 and its mutant PM-1.*

Detail of scoring	Maximum Points	Cow's milk		Buffaloe's milk		Reconstituted full-cream dried cow's milk	
		S-1	PM-1	S-1	PM-1	S-1	PM-1
Appearance	3	2	3	2	3	2	2
Consistency	4	3	3	2	3	2	3
Taste	6	4	5	4	4	3	4
Flavor	7	4	6	4	4	4	4
	20	13	17	12	14	11	13

[a]Excellent =14-17
Very good = 10-13
Good = 8-10

However, dahi prepared with the mutant scored better as compared to the parent strain in all the three types of milk used in this investigation.

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Automated Impedance Measurements for Rapid Screening of Milk Microbial Content

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ABSTRACT

The electrical impedance of media is altered with chemical changes brought about by microbial metabolism and growth. Time required to bring about readily detectable change (detection time — DT) is a function of the initial levels of microorganisms in the sample. DTs were compared to Standard Plate Counts for 407 milk samples — homogenized, low fat, skim and raw. Using the criterion that a sample of pasteurized milk with a DT of 7 h or less was indicative of a plate count of 10,000/ml or greater, 323 of 380 samples were correctly classified. For raw milk, the DT was 10 h to resolve samples into greater or less than 10,000 organisms per ml. Results of a preliminary study on estimation of psychrotrophs in pasteurized milk showed that impedance monitoring at 21 C provided a 22-h screen correctly classifying 88% of the samples into categories of more than or less than 1,000 organisms per ml. Better agreement (91%) in a shorter time (13.7 h) was obtained with a screen for 10,000 organisms. Finally, for the first 22 samples analyzed, keeping quality data on pasteurized milk have correlated better with post-pasteurization impedance measurements than with either post-pasteurization total counts or psychrotrophic counts.

The dairy industry has long been interested in the bacterial populations found in milk. In addition to the need to meet State regulatory standards, bacterial spoilage or line contamination caused by high microbial concentrations can be very expensive for milk producers, processing plants, and distributors, since it relates to the milk's keeping quality and consumer brand preferences.

Since the effect of spoilage is so important, the dairy industry would certainly be interested in a rapid method for determining the microbial population in milk products. Present microbiological techniques are not very practical as test results are usually not available until several days after products have been shipped to consumers (*3,11,13*). Plate count tests to determine total counts of organisms present in milk take 48 h. Methods for measuring psychrotrophs (organisms able to grow at refrigeration temperatures), as presently practiced, take 5-10 days. Furthermore, present keeping quality tests, which try to predict spoilage based on presence of psychrotrophic organisms, have two severe limitations. First, spoilage is not always directly related to the number of organisms present (*20*). Second, it appears that psychrotrophs are only part of the milk spoilage problem. Poor flavor and keeping quality can also be attributed to the presence of microbial enzymes and metabolic products (*6,17,18,19*) from organisms present before pasteurization even though the organisms themselves may be killed by pasteurization. Thus, present methods, although offering some useful information, are too slow and often too inaccurate to meet the needs of milk producers and processors.

An optimal microbiological test would provide counting and keeping quality estimates within a time period allowing for effectual corrective measures. This would enable raw milk to be rejected before accepting delivery. Line contamination could be detected and corrected quickly; poor quality final products could be shifted into other products, thus preventing marginal products from reaching the consumer and reducing spoilage costs. In addition, an optimal method should be easy to use and should cost no more than present methods. Keeping these objectives in mind, we investigated impedance techniques, which provide rapid microbiological results for other food products (*10*). It was hoped that development of rapid methods would be of use to the dairy industry.

The impedance method is based on the observation that organisms growing in a liquid culture medium produce chemical changes which alter the electrical resistance (impedance in an AC circuit) of the solution. With a sensitive impedance monitor, the impedance changes caused by the growing organisms can be detected as the organisms reach the instrument's threshold. The time of detection can then be used to roughly estimate the concentration of organisms initially present in the milk sample. Furthermore, since impedance measurements may detect activity not only from organisms present in the milk but also from enzymes remaining from bacteria killed by pasteurization, impedance monitoring may provide a new means of predicting keeping quality. The results of our work to date are summarized in this paper.

MATERIALS AND METHODS

Impedance measurements

The impedance monitoring instrument used in all experiments was the Bactometer[1] 32 Microbial Monitoring System (Fig. 1) described elsewhere (5). The system was operated at 2 KHz, Gain 9 and all data were displayed via a strip chart recorder. Detection time was defined as the time required to produce an accelerating impedance change of 0.8%.

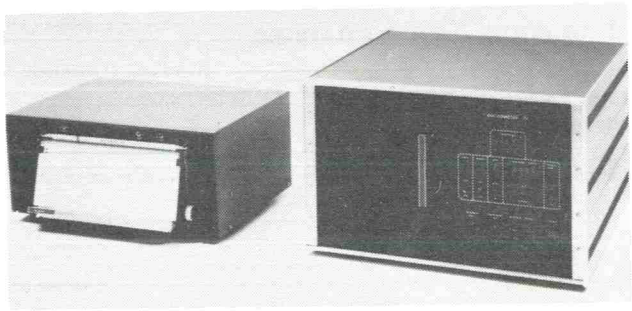

Figure 1. *Bactometer[1] 32 Microbial Monitoring System and strip chart recorder.*

Samples and media were aseptically added to 20-ml vials equipped with stainless steel electrodes descending from the cap. These vials were placed in a basket (Fig. 2) with electrical connection to the instrument. The basket of vials was then put in a standard incubator. Samples incubated at 21 C were monitored in modules (Fig. 3), containing sample chambers with electrodes for eight samples, and plugged directly into the instrument's incubator section. All samples, whether in vials or modules, had a corresponding reference of uninoculated medium.

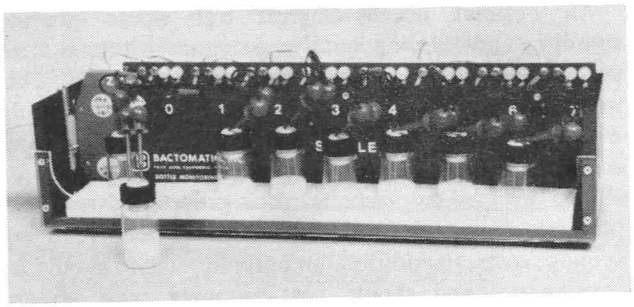

Figure 2. *Vials with vertical stainless steel electrodes in a rack which can be placed in a standard incubator and connected to the Bactometer 32 Microbial Monitoring System with an extension cable.*

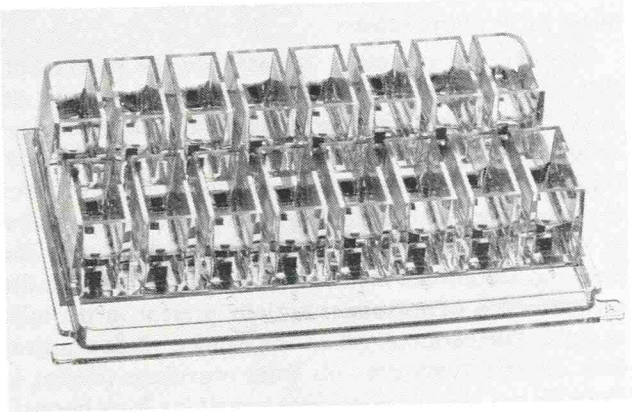

Figure 3. *Disposable module with stainless steel electrodes.*

[1]*Trademark, Bactomatic, Inc.*

The theory relating impedance detection times to initial microorganism concentration has been described by Hardy et al. (*10*) and is breifly summarized in Fig. 4. The upper half of the figure illustrates typical bacterial growth curves starting at two different concentrations (10^5 and 10^3 organisms/ml). The horizontal dashed line indicates the level of organisms where significant impedance changes are detectable. The lower half of the figure shows the impedance changes resulting from these two cultures. The response to the smaller initial concentration occurs later than the response to the larger initial concentration. In general, as long as the microbial growth rate is roughly the same from sample to sample, samples with high microbial numbers produce impedance changes before those with low numbers of organisms. Thus, for any prescribed concentration of organisms, a cutoff time can be defined such that an impedance change before the cutoff time indicates microbial numbers above the prescribed concentration and an impedance change after the cutoff time indicates microbial numbers below the prescribed concentration. This method was applied to impedance-based screens for both total mesophilic organisms and psychrotrophic organisms.

Microbiological methodology

Figure 5 illustrates a schematic diagram comparing the conventional method and the impedance method of estimating the number of organisms per ml of milk. In the conventional method 1 ml of milk was

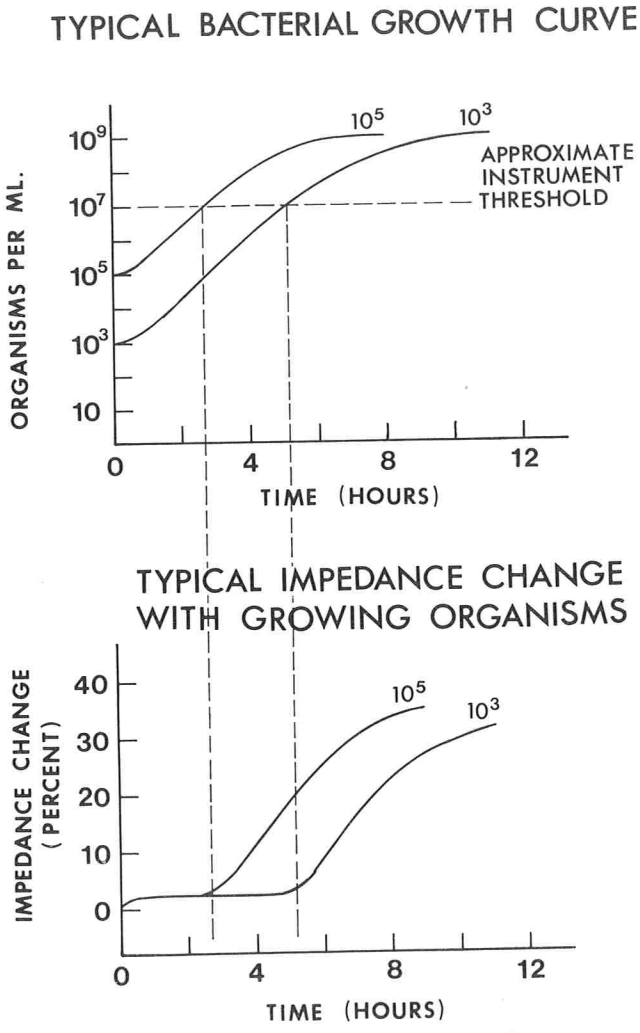

Figure 4. *Relationship between microorganism growth (top) and impedance response (bottom) which makes possible the method of estimating initial microorganism concentration from impedance response detection times.*

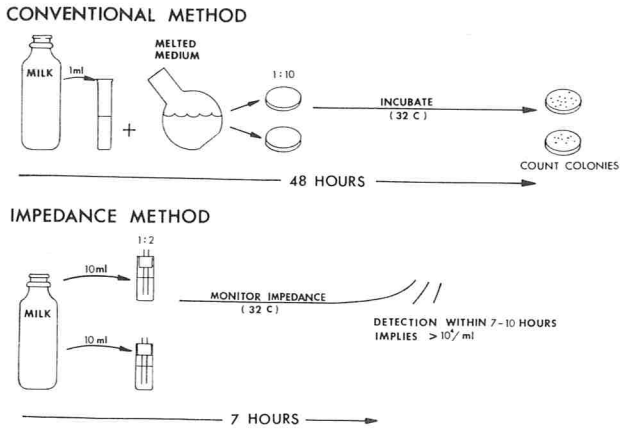

Figure 5. *Schematic representations of the conventional plate count and impedance methods for screening a milk sample.*

added to 9 ml of phosphate buffer, and 1 ml of this dilution was mixed with 10-15 ml of Standard Methods Agar to make a pour plate (*1*). For samples incubated at 32 C (but not at 7 C or 21 C), the standard method was modified by adding a 3-5 ml agar overlay to the pour plates. This eliminated spreading colonies at the expense of a slight reduction in surface colonies. Plates were done in duplicate and incubated under each of the following conditions: 32 C for 48 h (*1*), 21 C for 25 h (*16*), and 7 C for 10 days (*1*).

For the impedance method, 10-ml milk samples were added to an equal amount of trypticase soy broth containing 0.1% yeast extract (TSBY) in vials containing electrodes; or 1-ml milk samples were added to an equal amount of TSBY or Standard Methods Broth (SMB) in modules. The sample containers were connected to the instrument and monitored at 32 C for comparison with mesophilic organism counts and keeping quality and at 21 C for comparison with psychrotrophic counts.

Flavor scoring

Milk flavor was judged according to the method used by Hankin and Dillman (*8*), by a panel of four trained milk tasters consisting at any one time, of at least three persons uninformed as to the identity of the sample being tasted. A flavor score of 40 was deemed excellent, while 35 or less was considered unsatisfactory. Intertester reliability was high, with an average standard deviation of less than 0.7 unit. Testing was done every other day until day 8 and then daily until the sample spoiled.

Samples

For psychrotrophic counts and keeping quality testing, homogenized milk samples were obtained from local dairies, and plate counts and impedance monitoring were begun within 6 h of pasteurization. For total mesophilic organism screening, refrigerated samples of raw, skim, low fat and homogenized milk were obtained from 24 milk processing plants across the United States.

RESULTS AND DISCUSSION

Raw milk screen

Figure 6 shows a scattergram of impedance response detection times graphed against initial microbial concentration for 27 raw milk samples. Note that the shorter the detection time, the greater is the initial concentration. (These data have a correlation coefficient of –0.8 between detection time and the logarithm of the initial concentration.) The solid diagonal line on the left side of the figure is the least squares linear fit to the data (regression line). The slope of this line indicates that the doubling time for the total population of microorganisms

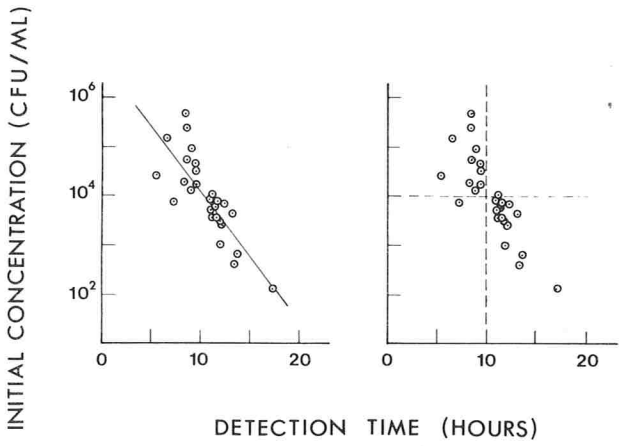

Figure 6. *Scattergram of impedance response detection times (averages of duplicate channels) for 27 raw milk samples graphed against initial microorganism concentration as determined by plate count at 32 C. The solid line on the left side is the least squares linear fit to the data. The broken lines on the right side illustrate a scheme by which samples could be classified as having more than or less than 10^4 organisms/ml.*

in the mixture of raw milk and TSBY during impedance monitoring is approximately 70 min.

The broken lines on the right side of the figure show a classification by which these raw milk samples could be classified as having more or less than 10,000 organisms/ml. The horizontal dashed line represents the 10,000 organism/ml level. The vertical dashed line at 10 h represents the cutoff time that best distinguishes samples containing greater than 10,000 organisms/ml from those with fewer than 10,000 organisms/ml. Therefore any sample with detection time before 10 h would be classified as having over 10,000 organisms/ml and any sample with detection after 10 h would be classified as having less than 10,000 organisms/ml. The two broken lines separate the samples into four quadrants. For samples in the upper left and lower right quadrants, the impedance and plate count classifications agree. For these data, 25 out of 27 samples, or 92.6%, yielded agreement between the impedance and plate count classifications. Samples in the upper right quadrant were classified above 10,000 organisms/ml by plate count but below 10,000 organisms/ml by the impedance technique (false negatives). Samples in the lower left quadrant were classified below 10,000 organisms/ml by plate count but above 10,000 organisms/ml by impedance (false positives). Moving the vertical cutoff line forward or backward in time will reduce false negatives at the expense of increasing false positives and vice versa.

Because the number of samples is small, this agreement may be fortuitously high; however, it compares favorably with the work of Gnan and Luedecke, who reported 99% agreement between impedance and plate count classifications for raw milk using similar methods and a larger number of samples (*7*).

Total mesophile screen

Figure 7 shows similar scattergrams for various types of pasteurized milk. Shown with their regression lines are

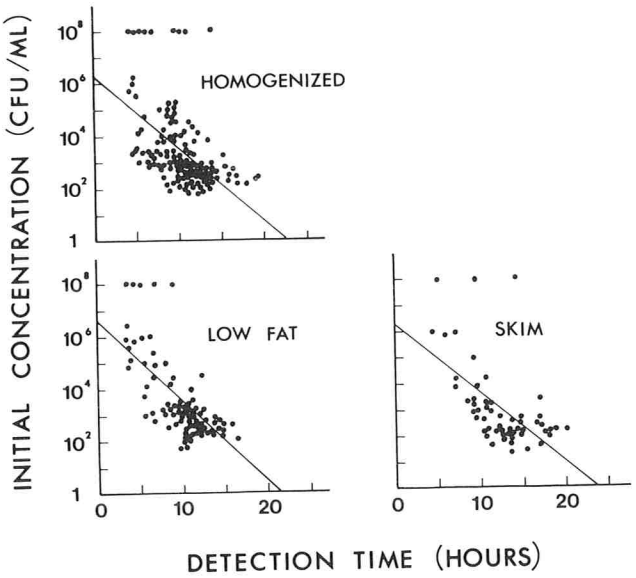

Figure 7. *Scattergrams of impedance response detection times (averages of duplicate channels) graphed against initial microorganism concentration as determined by plate count at 32 C for 191 homogenized, 119 low fat, and 70 skim milk samples. The solid lines are the least squares linear fits to the data.*

the data from 191 samples of homogenized milk, 119 samples of low-fat milk and 70 samples of skim milk. Among these were samples held at refrigeration temperatures from a few hours to as long as 12 days after pasteurization. All of the samples with more than 100,000 organisms/ml were of this latter category.

Although the slopes of the regression lines differ slightly between homogenized, low fat, and skim milk, they do not differ significantly. Hence, these data have been combined and the 380 data points are displayed together in Fig. 8. The correlation coefficient for these data is –0.60, indicating a good deal more spread in these pasteurized milk data than with, for example, frozen vegetable data, which showed a correlation coefficient of –0.85. The coefficient of determination (0.36) indicates that only 36% of the variance of the

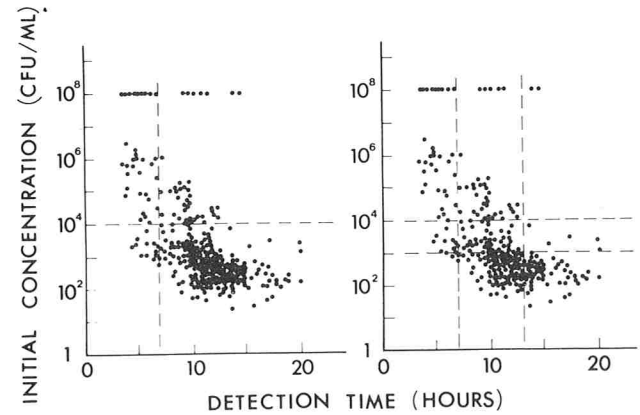

Figure 8. *Scattergram of impedance response detection times (averages of duplicate channels) graphed against initial microorganism concentration as determined by plate count at 32 C for 380 samples of pasteurized milk. The dashed lines indicate two-way (left side) and three-way (right side) classification schemes applied to these data.*

detection time is associated with variability in the log of the plate count. Nevertheless, the correlation between log plate count and detection time is significantly different from zero ($p < .001$).

The left side of this figure shows a two-way classification similar to that shown for raw milk in Fig. 6. For the pasteurized milk shown here, the best cutoff time to classify samples as having more than or less than 10,000 organisms/ml is 7 h. On 323 (85%) of the 380 samples the impedance and plate count methods agreed. There were 4% false positives and 11% false negatives.

The data points at the 10^8 organisms/ml level in Fig. 7 and 8 correspond to samples where the plates were too numerous to count, thus indicating an initial concentration above 10^6 organisms/ml. The fact that many of these very high count samples were not detected until after 10 h indicates one of the necessary precautions needed with impedance screening. When initial concentrations of microorganisms exceed the instrument's threshold level of 10^7/ml, the initial accelerating impedance change is lost or obscured by the impedance changes resulting from the initial thermal equilibration. If the initial response is missed, a secondary response about 6 h later is frequently detected. Presumably, concentrations in excess of 10^7 organisms/ml would be rare in fresh milk, and were they to occur, there would be a good chance that such high-count milk could be caught by inspection when setting up the samples for testing.

On the right side of Fig. 8 is an example of the three-way screen. By selecting two cutoff times, one can have classifications for low, intermediate, and high count product. For example, in Fig. 8 cutoff times of 7 h and 13 h may be used to divide all samples into those containing greater than 10,000 organisms/ml (with detection times under 7 h), those containing greater than 1,000 organisms/ml but fewer than 10,000 organisms/ml (with detection times greater than 7 but under 13 h), and those containing fewer than 1,000 organisms/ml (detection times greater than 13 h). The agreement between impedance and plate count classification is 50, 80 and 32% for samples over 10,000 organisms/ml, between 10,000 and 1,000 organisms/ml and below 1,000 organisms/ml, respectively. Although the agreement for each classification is low using this scheme, the chance of a serious misclassification is surprisingly low. Thus 98% of all samples are either classified correctly or into the neighboring category. Bray et al. (*4,12*) have proposed a three-way classification scheme where a number of subsamples of a batch is tested and the batch is rejected if any one subsample is high count or if a high proportion of them are intermediate. In this particular illustration, the screen yields a much higher percentage of intermediate samples than might normally be expected, probably because milk samples of various ages and origins have been included.

The impedance-based screen provides a rough estimate of total mesophilic count in pasteurized milk samples within about 7 h (or within 13 h if a lower

organism demarcation level or a three-way screen is used). Even though this is considerably faster than the total mesophilic plate count, requiring 48 h of incubation, it probably is not fast enough to allow a milk processor to test his milk before shipment. Most milk processors will have already sent their milk out before 7 h have elapsed from pasteurization. The value of the 7-h screen, however, is that potential problems will be detected almost 2 days earlier, thus saving large quantities of milk from being processed under less than ideal conditions.

Psychrotroph screen

The number of psychrotrophic organisms in a milk sample is a frequently used predictor of the sample's keeping quality. The conventional procedure for psychrotrophic counts requires 10 days of incubation at 7 C. Last year, Oliveria and Parmelee (*16*) reported that milk psychrotrophs grow well at 21 C whereas the mesophiles grow very slowly at this temperature. They found that incubation at 21 C for 25 h was equivalent to incubation at 7 C for 10 days. Our investigation utilizing 21 milk samples (some of which were incubated various periods to provide 74 sets of plates counted by each method) has supported their findings (see Fig. 9). These observations suggest that a rapid test for psychrotrophs at 21 C could be done with impedance measurements.

Figure 10 presents a scattergram of detection times for milk samples incubated at 21 C graphed against psychrotrophic counts as determined by the conventional 10-day method. The 69 points shown on this scattergram were obtained from 21 milk samples analyzed after various periods of refrigeration for some samples to provide a wider range of psychrotrophic counts.

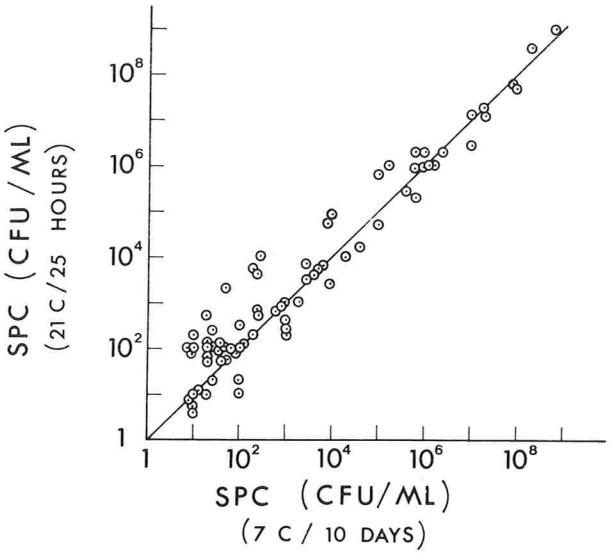

Figure 9. *Scattergram of psychrotrophic counts obtained from incubating plates at 21 C for 25 h graphed against counts obtained from the same samples when plates were incubated at 7 C for 10 days. The solid line represents the locus of points where equal counts are achieved. The 74 sets of plate counts were obtained from 21 milk samples after varying periods of refrigeration. The correleation coefficient is 0.97.*

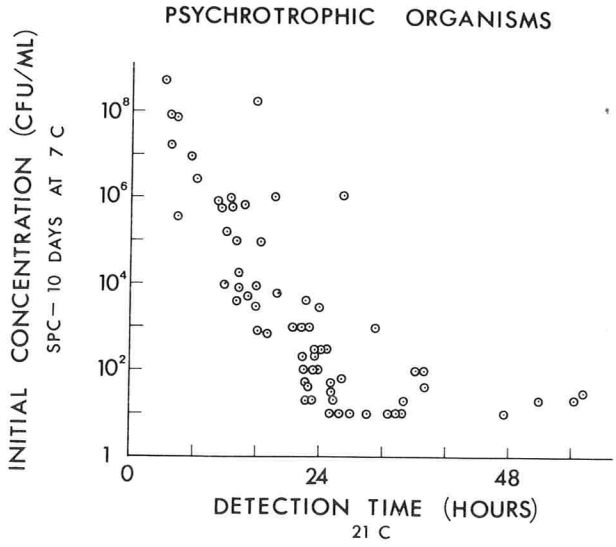

Figure 10. *Scattergram of impedance response detection times (earliest of duplicate channels) graphed against initial psychrotrophic count as determined by incubating plates 10 days at 7 C. Impedance monitoring was performed with SMB in modules at 21 C.*

The correlation coefficient for these psychrotrophic counts is –0.73. One source of spread in these data is the total population growth rate, which at 21 C (data shown in Fig. 10) is slower than the mesophilic organisms grown at 32 C (data shown in Fig. 8). The slopes of the regression lines for these two sets of data indicate a doubling time of about 2 h for the former and about 1 h for the latter. This results in a greater variation in detection time for the same relative variation in growth rate. In addition, the impedance changes were much more gradual, leading to less well-determined detection times.

For classification above or below 1,000 psychrotrophs/ml, a cutoff time of 21.3 h produced the maximum agreement between the impedance and plate count classifications (61 out of 69 samples or 88%). For classification above or below 10,000 psychrotrophs/ml, a cutoff time of 13.7 h was best (63 out of 69 samples or 91% agreement). A screening test for these higher concentrations of psychrotrophic organisms may find use in conjunction with an initial period of preincubation. Compared with the 10-day conventional test, these impedance-based screens (14 or 21 h) offer a considerable reduction in the time required to get test results.

Shelf life prediction

It is widely assumed that the keeping quality of milk is influenced by a great number of factors, many bearing upon milk's microbial content and the conditions that impede or further growth of the milk's endogenous flora. In spite of our knowledge of the microbiology of milk and of milk products, keeping quality is difficult to predict on the basis of laboratory estimates of the microbial content of freshly pasteurized milk (*9*).

Some probable reasons for this difficulty stem from the complexity of those factors leading to poor keeping

quality. The microbial flora of raw milk can influence the keeping quality of the pasteurized product in at least four ways: First, the microbial flora can contain thermoduric psychrotrophic organisms which survive pasteurization and then go on to cause spoilage even under the best of storage conditions. It has been estimated that as few as 10 thermoduric psychrotrophs in a quart of milk can cause spoilage within a few days (*3*). Second, microbial flora can be a source of enzymes surviving pasteurization and going on to cause continuing biochemical change. Third, there is some evidence to show that the microbial flora of raw milk might influence the rate of growth of organisms either surviving pasteurization or appearing as post-pasteurization contaminants (*18*). Presumably, metabolites produced by the raw milk flora can either augment or inhibit the post-pasteurization flora. Finally, certain metabolites in the raw milk, not destroyed by pasteurization, contribute directly to off-flavors and poor quality.

In addition to these factors are those contributing to errors in estimating microbial content. The population that will be seen by microbiological testing will depend very greatly upon the diluent used, the medium in which the sample is grown (*14,15,21*) and the temperature and length of time of incubation (*2*). Add to this the degree to which psychrotrophs form dense clumps, resistant to breaking up (*21*), and it is not surprising to find errors of up to two orders of magnitude in psychrotroph estimations.

An investigation of impedance response parameters (detection time, response strength, etc.) and their relationship to milk keeping quality has just begun. So far, 22 milk samples have been analyzed, and these samples have shown only a 6-day variation in shelf life. (Shelf life has been defined as the number of days from pasteurization until an unsatisfactory flavor score [≤ 35] occurs.) In Table 1 are shown the mesophilic plate count, psychrotrophic count, and impedance response detection times for 10 samples whose shelf lives were the shortest or the longest of the samples analyzed. The detection times presented in this table result from incubation at 32 C. In general, these detection times appear to reflect the values of the shelf life, the second sample being the graphic exception. The detection times, in fact, seem to correlate better with the shelf life than do the standard plate count and psychrotrophic count, at least for these few samples. Confirmation of this correlation will require the analysis of a much larger number of samples with a much larger spread in shelf life duration. These early data, however, show promise of a 9-14-h impedance-based keeping quality prediction.

TABLE 1. *Comparison of shelf life, impedance response detection time, standard plate count and psychrotrophic count for 10 milk samples.*

Shelf Life (days)	Detection time[1] (hours)	Mesophilic plate count[2] (cfu/ml)	Psychrotrophic count[3] (cfu/ml)
9	9.4	400	20
9	12.2	7000	30
10	9.6	400	10
10	9.4	200	100
10	10.4	200	—
14	11.1	300	10
14	10.9	400	100
14	11.5	100	30
15	11.4	100	10
15	10.3	200	100

[1]Earliest detection of duplicate vials with TSBY at 32 C.
[2]Incubation at 32 C for 48 h.
[3]Incubation at 7 C for 10 days.

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Antimicrobial Efficacy of a Potassium Sorbate Dip on Freshly Processed Poultry

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ABSTRACT

Fresh poultry breasts were dipped in a solution containing either 0, 2.5, 5.0 or 10.0% potassium sorbate for 1 min. Another lot of fresh poultry breasts was dipped in a suspension of either 10^3 or 10^5 cells of three strains of *Salmonella*/ml before being dipped in one of the potassium sorbate solutions. Use of a potassium sorbate dip significantly reduced the total number of viable bacteria on the poultry parts when compared to the untreated control parts after 7 days of storage at 10 C and after 8 days at 6 C. Use of a 10.0% sorbate dip significantly reduced the total plate count as related to the total plate count of the control parts after 5 days of storage at 22 C. Application of a 10.0% sorbate dip resulted in a significantly lower *Salmonella* count than of the untreated parts after 7 days of storage at 10 C and a 5.0% or greater sorbate dip markedly reduced the growth rate of the *Salmonella* at 10 and 22 C when compared to the growth rate of the *Salmonella* on control parts.

The short shelf-life of fresh, unfrozen poultry is a major problem within the poultry industry. Fresh broilers on the retail shelf normally contain between 10^4 and 10^5 microorganisms per cm^2 (*11,12*) and can only be stored for 1 or 2 days in a refrigerator at 3-5 C and still maintain their quality (*6*).

The incidence of salmonellae on fresh poultry is of concern to consumers, processors, and public health officials. In outbreaks of foodborne disease traced to poultry in 1972-1974 in which a causative agent was identified, *Salmonella* was implicated in 44% of the cases (*4*). Mountney (*6*) stated that the most important group of bacteria in poultry that can cause human illness is *Salmonella*. There have been numerous studies that have reported on the incidence of salmonellae on fresh poultry ranging from 2.5% (*2*) to 34.8% (*1*). Although fresh poultry is normally cooked in such a manner that salmonellae are destroyed, their presence on the raw meat is a source of contamination for other foods, and offers the possibility of recontamination of the cooked product through kitchen equipment and surfaces.

Sorbic acid has been reported to be an effective antimicrobial agent when applied to poultry as a 7.5% solution in a 70:20:10 propylene glycol, water and glycerine mixture at 140 F (*9*). Kaloyereas et al. (*5*) reported that ice containing sorbic acid and glycol diformate was effective in preserving poultry. Perry et al. (*9*) stated that sorbic acid used alone had limited value due to its low water solubility; use of water-soluble potassium sorbate can overcome this problem.

Sorbic acid has also been reported to inactivate *Salmonella typhimurium* in media, milk and cheese (*7,8*). Potassium sorbate has been reported to retard growth of salmonellae in cooked, uncured sausage (*10*). Preliminary studies in this laboratory indicated that potassium sorbate was effective in inhibiting growth of *S. typhimurium* 13311 in laboratory media and on retail chicken parts.

This investigation was designed to evaluate the efficiency of a potassium sorbate dip in controlling growth of the normal flora of freshly processed broiler parts, thereby extending shelf-life, and in controlling growth of *Salmonella* inoculated on the surface of broiler parts and stored at various temperatures.

MATERIALS AND METHODS

Poultry

Chicken breasts were obtained on the day of processing from a local slaughterhouse. The breasts had an average weight of 232 g.

Test organisms

Three species of *Salmonella* (*S. typhimurium*) 13311, *Salmonella heidelberg* 8326, and *Salmonella montevideo* 8387) were grown overnight in trypticase soy broth (Difco), mixed in a 1:1:1 ratio, and were diluted in 3 liters of sterile, 0.005 M phosphate buffer (pH 7.2) to a final concentration of 10^5 cells/ml for the large inoculum study, and to a final concentration of 10^3 cells/ml for the small inoculum study.

Inoculation of poultry

Freshly processed chicken breasts were inoculated with *Salmonella* by dipping them into either the concentrated or diluted suspension of *Salmonella* for 1 min. Breasts were then removed and drained for 5 min on a wire rack.

Chicken breasts used for the total plate count studies were not inoculated with *Salmonella*.

Sorbate dip

Both inoculated and uninoculated chicken breasts were dipped for 1 min in 5-liters of tap water, 2.5 (wt/vol), 5.0, or 10.0% potassium

sorbate, drained for 10 min, and packaged in sterile, polyethylene plastic bags.

Sorbate analysis

Ten grams of sample were carefully weighed into a Waring blendor and blended for 5 min with 100 ml of 0.5 N KOH and 0.02 g of benzyl alcohol. A portion of the slurry was centrifuged at 15,000 rpm for 10 min. Ten ml of supernatant fluid were pipetted into a 250-ml separatory funnel and acidified to a methyl orange end point with concentrated phosphoric acid. The supernatant fluid was extracted with 100 ml of chloroform by shaking vigorously for 1 min and then the chloroform fraction was collected through a sodium filter. The chloroform was evaporated at 70 C under nitrogen to about 5 ml and then transferred to a graduated 10-ml centrifuge tube and evaporated to 0.05 ml. Three μl of concentrate were injected onto a gas chromatograph. The chromatographic column used was a 1.83 m/6.4 mm i.d. glass column of 10% SP 1200/1% H_3PO_4 on 80-100 mesh Chromasorb WAW. The oven temperature was 175 C, injection port 250 C and FID temperature 250 C. The flow rate through the column was 35 ml of helium per minute. Under these conditions, benzyl alcohol eluted at 3.4 min and sorbic acid eluted at 5.2 min. Using benzyl alcohol as an internal standard, the amount of sorbic acid was determined for each sample. Recovery of sorbate from chicken treated with the chemical only was 95% or greater.

Storage studies

All samples were stored at 6, 10, or 22 C. Four breasts were analyzed per variable in the uninocluated study, and six breasts per variable were analyzed in the *Salmonella* study.

Organisms were enumerated by adding 100 ml of the sterile 0.005 M phosphate buffer (pH 7.2) to the packaged chicken, shaking for 1 min and diluting further before plating with Standard Plate Count agar (Difco) for the uninoculated samples or spread plating onto Salmonella-Shigella agar (S-S, Difco) for *Salmonella* counts. Total plate counts were determined after 48 h of incubation at 29 C. Enumeration of *Salmonella* was done after 24 h of incubation at 37 C. Confirmation of typical *Salmonella* colonies from the S-S agar was done by picking colonies to Triple Sugar Iron agar (Difco) and Lysine Iron agar (Difco), and examining for typical reactions after 24 h at 37 C. All counts are reported on a per cm² basis, according to the method of Goresline and Haugh (*3*).

RESULTS AND DISCUSSION

Results from determination of sorbic acid in chicken parts are in Table 1. The 2.5% dip left an average residue of 0.05% sorbic acid, the 5.0% dip left an average residue of 0.13% sorbic acid, and the residue from a 10.0% dip was 0.32% sorbic acid.

TABLE 1. *Determination of potassium sorbate (as sorbic acid) from fresh poultry breasts.*

% Dip level	% Sorbic acid	No. samples tested
0	0	3
2.5	0.05	9
5.0	0.13	6
10.0	0.32	9

The data obtained on the effect of a potassium sorbate dip on the total plate count per cm² of chicken are presented in Fig. 1, 2, and 3. Use of a potassium sorbate dip significantly reduced the total number of viable bacteria per part as related to the control parts after 4 and 8 days of storage at 6 C (Fig. 1). After 4 days at 6 C objectionable off-ordors were apparent in the control

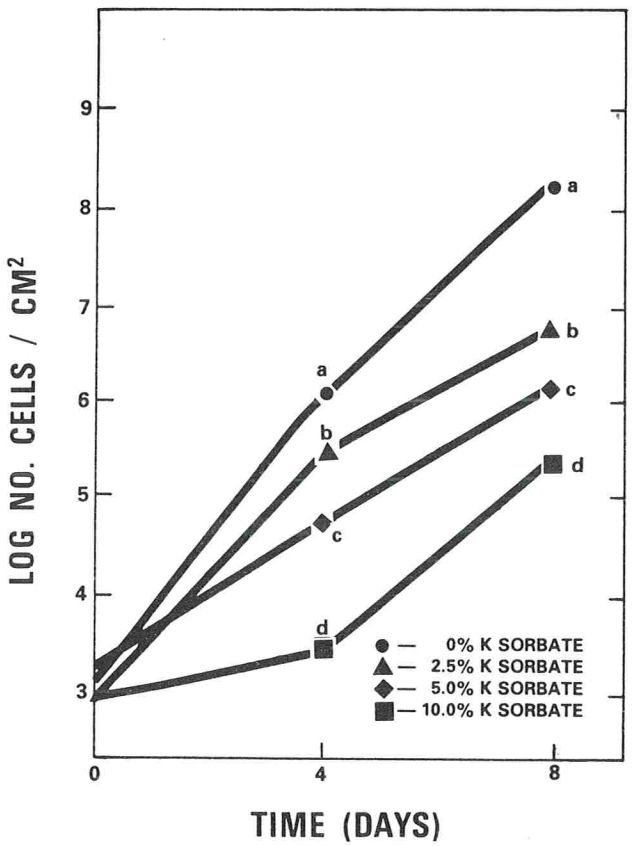

Figure 1. *Effect of a 1-min potassium sorbate dip on growth of the microflora of freshly-processed chicken breasts stored at 6 C. a,b,c,d — points at each time interval which have the same letter are not significantly different at the 0.05% level by the Duncan New Multiple Range Test.*

parts, and after 8 days slime formation also was evident. Off-odors were observed after 8 days at 6 C from the 2.5% treated parts, and slight, astringent odors were noticed from the 5.0% treated parts. The 10.0% parts exhibited no evidence of spoilage throughout the 8-day storage period at 6 C.

When breasts were stored at 10 C, sorbate dips significantly reduced the number of viable bacteria per cm² when compared to the control count throughout the storage period (Fig. 2). After 2 days of storage at 10 C. control parts exhibited a putrid off-odor, the 2.5% treated parts exhibited a slight odor, while the 5% and 10% treated parts had no off-odor. After 5 days of storage slime formation was apparent on the control parts and the 2.5% treated parts displayed putrid off-odors. The 5.0% parts had a slight off-odor after 5 days of storage, and putrid off-odors after 7 days at 10 C. The 10% parts showed no signs of deterioration throughout the storage period.

Breasts receiving a 10% dip had a significantly lower plate count than the untreated breasts after 96 h at 22 C. (Fig. 3). The control parts were obviously spoiled after 24 h of incubation, exhibiting both slime formation and putrid off-odors. The 2.5% parts displayed putrid off-odors after 24 h and slime after 48 h of storage. The 5.0% treated parts had a slight off-odor after 24 h and

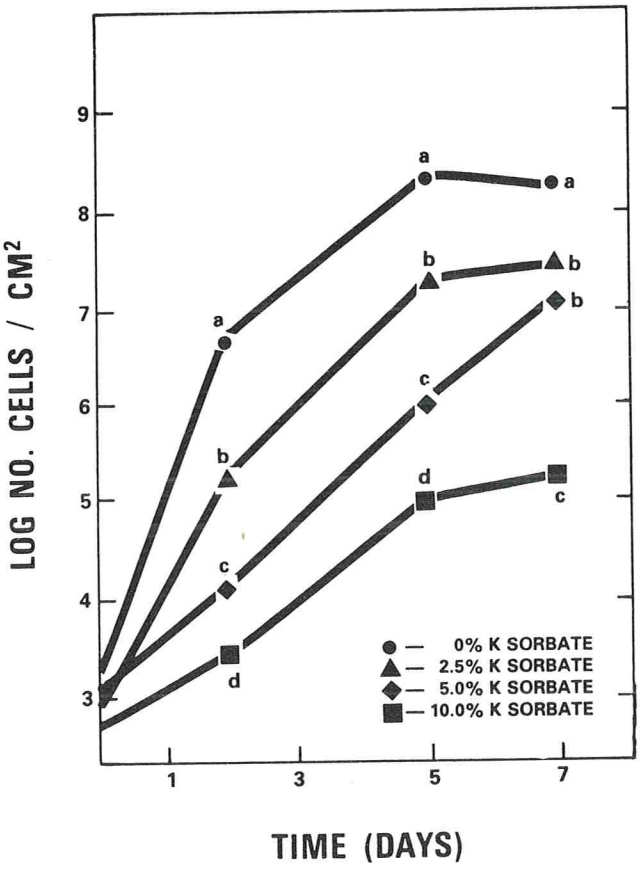

Figure 2. *Effect of a 1-min potassium sorbate dip on growth of the microflora of freshly-processed chicken breasts stored at 10 C. a,b,c,d — defined in legend for Fig. 1.*

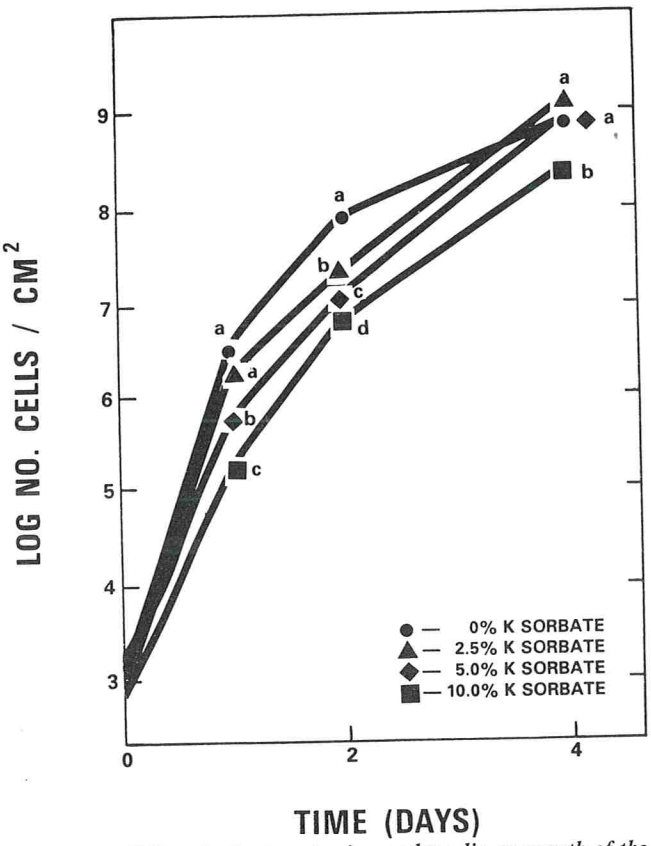

Figure 3. *Effect of a 1-min potassium sorbate dip on growth of the microflora of freshly-processed chicken breasts stored at 22 C. a,b,c,d — defined in legend for Fig. 1.*

were slimy after 96 h of storage at 22 C. The 10.0% treated parts had off-odors after 48 h and slime formation after 96 h of storage.

Results obtained on the effect of a potassium sorbate dip on growth of *Salmonella* on freshly processed poultry are in Fig. 4, 5, 6 and 7. When the initial *Salmonella* count was approximately 3×10^3 cells/cm², a 5% sorbate dip significantly reduced the number of viable *Salmonella* per cm² when compared to the control count after 2 days of storage at 10 C (Fig. 4). When the high inoculum parts were stored at 22 C, a 5.0% sorbate dip significantly reduced the number of viable *Salmonella* as related to the control count after 24 and 48 h of storage (Fig. 5). A 10% sorbate dip significantly reduced the viable *Salmonella* count as related to the control count after 5 days of storage at 10 C (Fig. 6) and after 24 h at 22 C (Fig. 7). A 5% dip had a *Salmonella* count significantly lower than the 2.5% dip counts after 2 days at 10 C (Fig. 6), and after 72 h at 22 C (Fig. 7). The 10% dip resulted in *Salmonella* counts significantly lower than the 5% dip counts after 7 days of storage at 10 C (Fig. 6), and after 72 h of storage at 22 C (Fig. 7).

The data verify earlier work that sorbates are effective in controlling growth of spoilage organisms associated with fresh poultry (*4,7*) and that sorbates are inhibitory to salmonellae (*7,8,10*). The 5 and 10% dips extended the

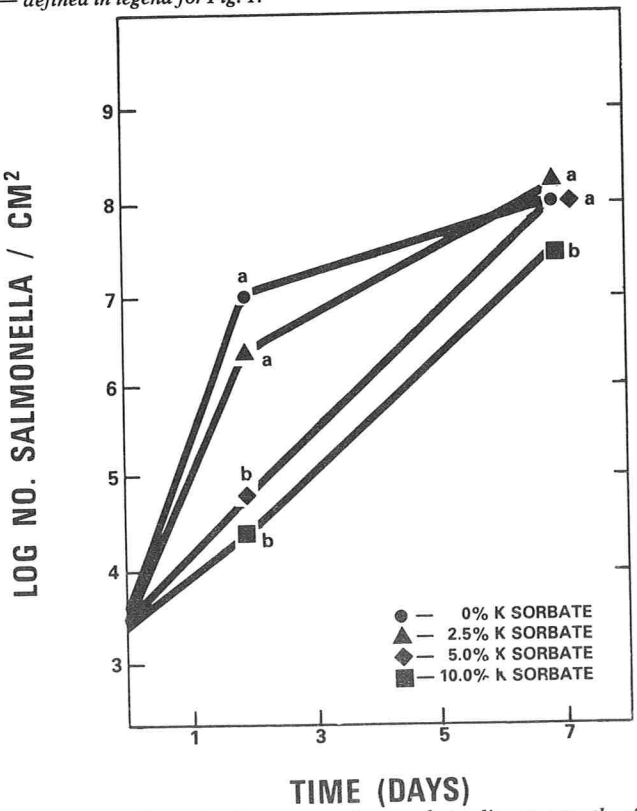

Figure 4. *Effect of a 1-min potassium sorbate dip on growth of* Salmonella *on freshly-processed chicken breasts inoculated with approximately* 3×10^3 Salmonella/cm² *and stored at 10 C. a,b,c,d — defined in legend for Fig. 1.*

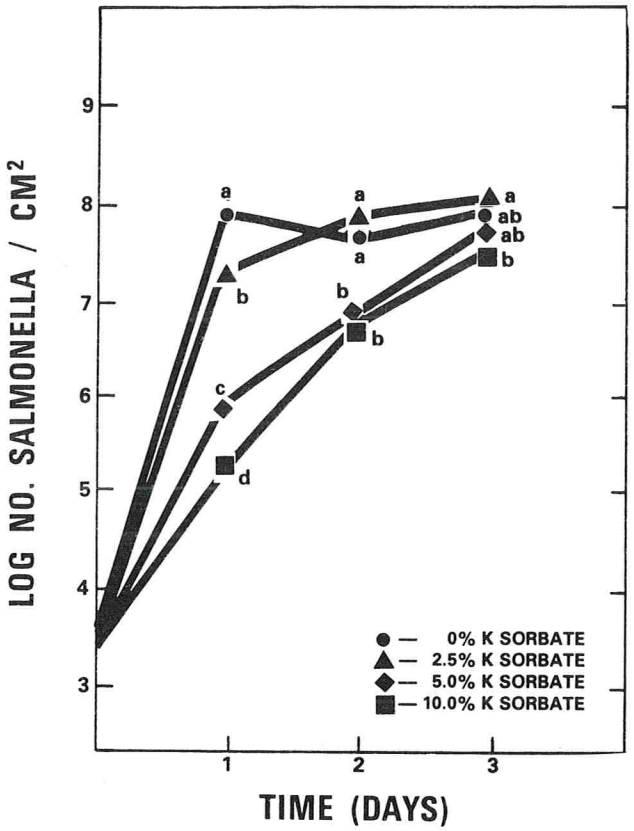

Figure 5. *Effect of a 1-min potassium sorbate dip on growth of* Salmonella *on freshly-processed chicken breasts inoculated with approximately* 3×10^3 Salmonella/cm^2 *and stored at 22 C. a,b,c,d, — defined in legend for Fig. 1.*

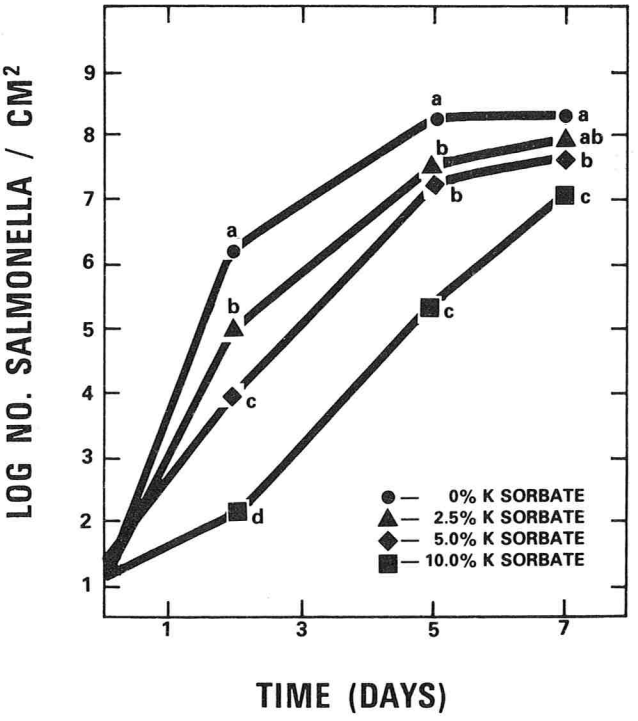

Figure 6. *Effect of a 1-min potassium sorbate dip on growth of* Salmonella *on freshly-processed chicken breasts inoculated with approximately 14* Salmonella/cm^2 *and stored at 10 C. a,b,c,d, — defined in legend for Fig. 1.*

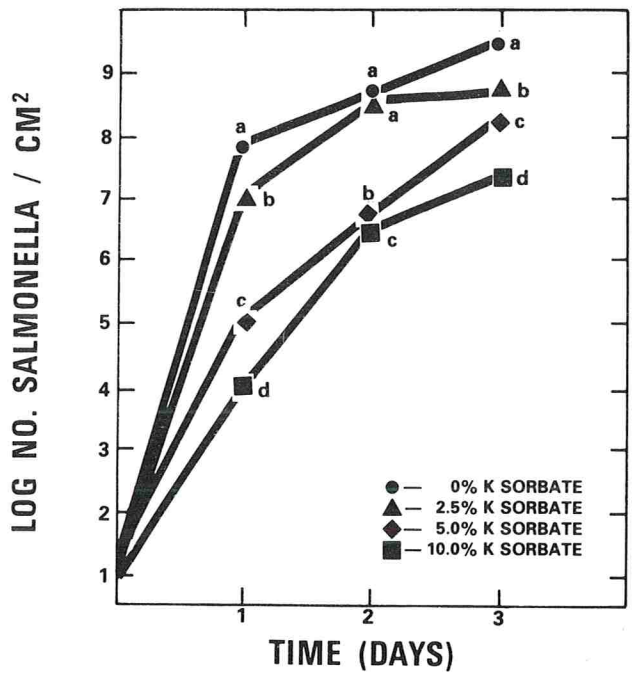

Figure 7. *Effect of a 1-min potassium sorbate dip on growth of* Salmonella *on freshly-processed chicken breasts inoculated with approximately 14* Salmonella/cm^2 *and stored at 22 C. a,b,c,d, — defined in legend for Fig. 1.*

time before poultry was organoleptically unacceptable from 4 days for control parts, to over 8 days, at which time counts were below the level at which the product becomes organoleptically unacceptable (*6*). Use of a potassium sorbate dip on fresh poultry would not only extend the shelf-life of the product both in the market and in the refrigerator, but it would also aid in inhibition of any salmonellae that may be present on the raw meat.

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A Research Note

Effect of Acids on Selenite Inhibition of *Salmonella typhimurium* and *Salmonella dublin*

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ABSTRACT

The minimum pH for growth (MPG) in selenite-cystine enrichment medium was determined for a number of acids. The MPG for lactic, citric, hydrochloric, aspartic, malic, succinic, pyruvic, and tartaric acids was 5.80; acetic acid was considerably more inhibitory, giving a MPG of 6.30-6.40; the MPG's for fumaric and pyruvic acids were 6.00 and 5.70, respectively. The least inhibitory acid was *a*-ketoglutaric acid which gave a MPG lower than or equal to 5.1. Inoculum size had little or no effect on the MPG.

Selenite as a selective agent for salmonellae was first used by Guth (*3*) in a 1% concentration in agar media. Leifson (*5*) showed this concentration to be very toxic to many salmonellae and devised a liquid medium with 0.4% selenite (Leifson's selenite-F medium). North and Bartram (*6*) reported that incorporation of 0.01% cystine in the selenite-F medium considerably enhanced salmonella recovery. This was confirmed by Byrne et al. (*2*).

Salmonella survival experiments (unpublished) in Lebanon bologna gave anomalous results in our laboratory. The Lebanon bologna was inoculated before fermentation with either *Salmonella dublin* or *Salmonella typhimurium* and aliquots were cultured after fermentation. The original technique for the most probable number was to add three parts of selenite enrichment to one part of the fermented bologna, blend, and remove aliquots representing 100, 10, and 1 g. This blended material was then diluted 10- or 100-fold to give 0.1- and 0.01-g portions. The diluted portions sometimes gave positive salmonella results whereas the 100-, 10- and 1-g aliquots gave negative results. It was postulated that lactic acid inhibited the organisms in the selenite media. Evaluation of this theory prompted the investigation reported here.

MATERIALS AND METHODS

Cultures and media

Selenite Cystine Medium (Difco) in 5-ml amounts in 13 × 150 mm tubes was used in these studies with incubation at 37 C for 5 days. The salmonella cultures were laboratory strains of *S. typhimurium* and *S. dublin* and were maintained in Tryptic Soy Broth (TSB). Twenty-hour TSB cultures were used as inocula. The optical density was standardized against Tryptic Soy Agar plate counts and the broth diluted to give the requisite number of cells in 0.1 ml that was added to the selenite media. The salmonella count of the diluted TSB was determined by surface plating on Brilliant Green Agar. (BGA).

Acids used

Solutions of 1.0 N lactic, acetic, aspartic, malic, succinic, tartaric, fumaric, *a*-ketoglutaric and pyruvic acids were used to adjust the pH values of the selenite media. The media were made up to 90% of the final concentrations (10/9×) adjusted to required pH values with the acids and then made to volume.

Determination of minimum pH for growth (MPG)

The minimum pH for growth (MPG) was defined as the lowest pH of tubes that showed visual evidence of growth (turbidity and/or selenite reduction) or that gave cultural evidence of cell proliferation when plated on BGA. The former was termed visual MPG; the latter was cultural MPG. All tubes not showing visual growth were plated on BGA. Adjustments of pH were made to the second decimal point.

RESULTS AND DISCUSSION

The effect of lactic, acetic, citric, and hydrochloric acids on the growth of *S. typhimurium* in selenite medium inoculated with 15, 150, or 1500 cells/ml indicated that inoculum size was not significant in causing growth inhibition. Acetic acid inhibited at pH 6.30 while the other acids inhibited at 5.80.

A previous study (*4*) indicated that *S. dublin* was less acid-resistant in TSB than was *S. typhimurium.* These organisms were compared for MPG in selenite medium, with acetic, citric, and hydrochloric acids used to adjust the pH (Table 1). Acetic acid inhibited both organisms at pH 6.30, both were inhibited at pH 5.70 with citric acid while hydrochloric acid inhibited *S. dublin* at pH 5.50 and *S. typhimurium* at pH 5.70. Thus, *S. dublin* was not more acid resistant in the selenite than was *S. typhimurium.*

Although acetic acid gave a MPG of 6.30 with both

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organisms, there was some cell proliferation at pH 6.30 and 6.20 but not enough to show visible evidence of growth (Table 1). Hydrochloric acid, on the other hand, allowed no increase in cells at the visual MPG. Citric acid permitted an increase in cells with *S. dublin* at the visual MPG. Incubation for 5 days did not produce visible evidence of growth in tubes showing cell proliferation.

The effect on MPG of several organic acids is shown in Table 2. Acetic acid was the most inhibitory. The others were all similar except *a*-ketoglutaric acid which was not inhibitory at the lowest pH tested, 5.10.

Well-fermented Lebanon bologna had a pH of 5.20 when blended with three parts of selenite cystine medium, a pH of 5.90 with five parts of the medium, and a pH of 6.50 with 10 parts.

The observation that the MPG of lactic acid in the selenite medium was 5.80 indicates that this is the source of the inhibition of salmonellae noted in Lebanon bologna experiments. Salmonellae are generally quite acid tolerant (*4*) with most strains growing well at pH 5.1-5.3; however, this report indicates that in the presence of selenite, the acids become much more inhibitory. One method of insuring against acid-inhibiton would be to adjust the pH of the selenite medium after blending with the fermented product to 6.40 to 7.0. Such pH adjustment is recommended for all foods by the FDA in its compilation of microbiological methods (*1*).

TABLE 1. *Inhibition of* S. dublin *and* S. typhimurium *in a selenite medium by acetic, citric, and hydrochloric acids.*

	S. dublin						*S. typhimurium*					
	Acetic		Citric		HCl		Acetic		Citric		HCl	
pH	Visible[a] growth	Cell[b] conc	Visible growth	Cell conc	Visible growth	Cell conc	Visible growth	Cell conc	Visible growth	Cell conc	Visible growth	Cell conc
6.50	+		+		+		+		+		+	
6.30	+		+		+		+		+		+	
6.10	0	2400	+		+		0	1400	+		+	
5.90	0	2000	+		+		0	200	+		+	
5.70	0	0	+		+		0	0	+		+	
5.50	0	0	0	1200	+		0	0	0	0	0	0
5.30	0	0	0	0	0	0	0	0	0	0	0	0

Incubation was for 5 days at 37 C

[a]Turbidity and/or selenite reduction.
[b]Determined by surface plating 0.01 ml on BGA. Only those tubes showing no visible evidence of growth were plated. 0 = no colonies. Original cell concentration was 160/ml for *S. dublin* and 240/ml for *S. typhimurium*.

TABLE 2. *MPG*[a] *of organic acids for* S. typhimurium[b] *in selenite medium.*

	0.05 N[c]				0.1 N			
	Selenite		Tryptic soy		Selenite		Tryptic soy	
Acid	Visual[d]	Cultural[e]	Visual	Cultural	Visual	Cultural	Visual	Cultural
Acetic	6.40	6.40	—	—	6.60	6.50	6.00	6.00
Aspartic	5.80	5.80	—	—	5.80	5.50	≤5.50	≤5.50
Malic	5.80	5.80	—	—	5.80	5.70	≤5.50	≤5.50
Succinic	5.80	5.80	—	—	5.80	5.80	≤5.50	≤5.50
Tartaric	5.80	5.70	—	—	5.80	5.80	≤5.50	≤5.50
Lactic	5.80	5.80	≤5.50	≤5.50	5.80	5.80	6.00	6.00
Fumaric	—	—	—	—	6.00	6.00	≤5.10	≤5.10
a-Ketoglutaric	—	—	—	—	≤5.10	≤5.10	≤5.10	≤5.10
Pyruvic	—	—	—	—	5.70	5.70	≤5.10	≤5.10
Citric	—	—	—	—	5.80	5.80	≤5.50	≤5.50

[a]pHs adjusted with HCl or NaOH in 10/9 × medium, then made to volume.
[b]Cell concentration was 12/ml.
[c]Final concentration of acid.
[d]Based on turbidity and/or visual selenite reduction.
[e]Based on presence of colonies in 0.01 ml medium streaked on Brilliant Green Agar.

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Concerns, Experiences, Attitudes and Practices of Executives of Chain Foodservice Firms Regarding Quality Control Procedures

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ABSTRACT

Top management of 44 chain foodservice firms with a combined total of 36,875 units answered a questionnaire about quality control procedures. Most indicated a concern for quality control laboratory tests but 22 representing firms with more than 350 units showed greatest concern. Pressures for increased quality control procedures came primarily from internal staff and then government agencies. It was felt that use of sanitation procedures would aid in dealing with customers and government agencies equally. Suppliers' products were generally considered reliable. There was widespread use of commercial laboratories but four times more of the larger firms than smaller firms had company-operated laboratories. Most felt laboratory tests were helpful. Advertising of quality control procedures was felt to be beneficial to the firm's public image and to employee pride. Top management considered sanitary instruction of employees and microbiological testing to be the most useful procedures for the foodservice industry.

A study of *Institutions/Volume Feeding Magazine* and *The Nation's Restaurant News* left the impression that quality control procedures, including sanitation inspection, laboratory testing, sanitation instruction of employees and similar efforts to produce a wholesome product was alluded to infrequently. It is generally accepted, however, that restaurants are a common source of food poisoning (*3*), the degree of which is not firmly established, likely because many persons who experience a gastrointestinal upset, frequently mild in nature, do not necessarily associate it with a restaurant meal or it is not reported to health authorities. On the other hand, some persons may have a significant intestinal upset caused by certain newly characterized viruses (*6*) and mistakenly assume a restaurant meal to be the cause. In all cases, only a limited number of gastrointestinal upsets are reported to public health authorities (*1*). In that there seems some insecurity regarding the foodborne disease problem, and foodservice firms' attempts to solve it, a study was undertaken in the Spring of 1975 in an attempt to gain some insight into the opinions, practices and experiences regarding quality control procedures as viewed by the executives of chain foodservice firms. What follows is the result of these studies.

METHODS

Eighty chain foodservice firms were selected from business reference volumes based on the availability of the names of their corporate officers (*2,5*). All firms had units in many states and it was felt their representatives could provide a variety of management experiences. A single page questionnaire, constructed in such a manner that the 11 multiple choice questions dealt with broad generalities of quality control procedures, was sent to the president of the firm or another administrative officer responsible for the firm's foodservice division. Quality control procedures were described in some questions as laboratory testing, sanitation instruction of employees and sanitation consultation while other questions referred to quality control or quality control procedures without elaboration. It was felt that a lengthy and more detailed questionnaire might result in only a few repsonses. Several of the questions had spaces for additional comments and such comments were encouraged. The questionnaire also contained a space for recording the number of units under the firm's management and the number of states in which these units were located. The questionnaire was accompanied by a letter indicating the purpose of the study and giving substantial assurances that all data would be in strict confidence and no data that could be related to any specific firm would ever be made public. Those who did not respond received at least two follow-up letters and questionnaires over a period of 3 months. Forty-four questionnaires were returned representing a spectrum from the smallest firm (less than 10 units) to a large firm with over 400 units. Only two of the 36 who did not return the questionnaire sent a specific answer declining to cooperate. All respondents were assured that the results of this study would be provided to them.

For the purpose of this report the returned questionnaires were arbitrarily divided in half into 22 firms with less than 350 units and 22 with more than 350 units, as determined from the questionnaire or published information (*4*) (there were none with exactly 350 units), in an attempt to discover if there were marked differences in those answers given by the "smaller" firms when compared to "larger" firms. Of the original 80 firms selected, 59% of the smaller firms' representatives and 24% of the larger firms' representatives did not return the questionnaire. Of the 80 firms, 54(67.5%) had less than 350 units. Those answering the questionnaire represented firms with a total of 36,875 units with gross sales of 8.11 billion dollars in 1974 (*6*) and represented a range from soft ice cream units to full-menu, "three meals per day", restaurants. The data in Table 1 are presented to give a general idea of the corporate officers reporting and the size of their firms.

TABLE 1. *Corporate titles of respondents.*

Title	Firms by the number of units				
	<50	51-200	201-500	501-2000	>2000
President	4	6	5	2	
Vice President	1		2	2	1
Director of quality control	1	1		2	
Other administrative Officers		4	6	3	4

RESULTS

The questionnaires reveal that 84% of those responding had a moderate to substantial concern for quality control laboratory tests (Table 2). Respondents from larger firms indicated greater concern for testing procedures than did those from smaller firms and this may reflect their present practices since a greater number of larger firms have company operated laboratories (Table 7). The remaining data presented here should, of course, be viewed as those obtained from a self-selected group.

TABLE 2. *Degree of concern in the foodservice industry for quality control laboratory tests.*

Concern	Firms	
	< 350 units	> 350 units
Substantial	4	16
Moderate	13	4
Little	4	1
No answer	1	1

In recent years there has been substantial rising concern for the quality of air, water, and the environment in general, including foods, drugs and cosmetics. Seemingly this has produced pressures on the management of firms dealing in products that could directly affect the health of the public. It seemed interesting, therefore, to find that leading the list of sources of pressures to increase quality control procedures were the firm's internal staff (Table 3) with government agencies and consumer groups a close second. Customers and competition seem to have their influence but external employees and managers, those located outside the corporate headquarters, have only a small effect.

TABLE 3. *Source of pressures for increased quality control procedures.*[a]

Source	Firms	
	< 350 units	> 350 units
Internal staff[b]	7	16
Government agencies	12	10
Consumer groups	8	11
Customers	6	10
Competitors	5	7
External employees and managers[c]	0	4
Advertising firms	0	3
No pressures	4	0

[a]Multiple answers.
[b]Corporate term meaning staff associated with corporate headquarters.
[c]Corporate term referring to management of individual units.

The question was posed that if in fact a firm did have comprehensive laboratory, inspection and sanitary consultation services, how would management view the value of these services in dealing with a variety of individuals and agencies? They apparently felt this would aid most in dealing with customers and government agencies (Table 4).

TABLE 4. *Agencies, organizations, and individuals best dealt with by laboratory data, sanitary inspection and consultation.*[a]

Information used for	Firms	
	< 350 units	> 350 units
Customers	14	14
Government	14	14
Suppliers	7	15
Employees, managers	8	12
Legal	10	10
No answer	0	1

[a]Multiple answers.

Agencies, at all levels of government, play a significant role in overseeing sanitation in the foodservice industry. It was thought the attitudes toward such agencies among foodservice management should be gently probed. The question was made very general so that even the Internal Revenue Service might be included but it was hoped that the nature of the questionnaire would limit answers to regulatory agencies involved with enforcement of sanitary conditions of foodservice establishments. The answers reflect some ambivalence. A number of respondents indicated both "harassing" and "very helpful" without clear differences between larger and smaller firms (Table 5). The answers do suggest that government agencies are at times helpful. The type of such help was not probed, however. It is apparent that there is also some unhappiness with government agencies, perhaps not unusual among businessmen or even the public at large.

TABLE 5. *Management attitudes toward government agencies.*[a]

Attitude	Firms	
	< 350 units	> 350 units
Harassing	6	9
Troublesome	9	6
Somewhat helpful	7	8
Of little help	4	7
Very helpful	5	4
No answer	0	1

[a]Multiple answers.

When preparing this questionnaire it was thought that the reliability of supplier's products would be a continuing concern of the management of foodservice firms and that this concern might result in a desire to monitor these products as part of a quality control program. Respondents were asked, therefore, to indicate the reliability of their suppliers' products and only a small number indicated such products were sometimes unreliable (Table 6). What criteria each respondent used was not probed in this short questionnaire but it is interesting to note that 66% of those who rated suppliers' products as "sometimes unreliable" had company-

TABLE 6. *Degree of reliability of supplier's products.*

Reliability	Firms	
	< 350 units	> 350 units
Relatively reliable	14	14
Reliable	4	3
Sometimes unreliable	2	4
No answer	2	1

operated laboratories.

It seems justified to expect that a foodservice organization sometimes needs laboratory tests done, perhaps to determine the quality of a questionable shipment from a supplier or to resolve a customer's or health official's allegation of food poisoning. It was found that very few firms had not used some type of laboratory (Table 7). Clearly commercial laboratories and a firm's own laboratory were those used by most of the firms represented. The number of firms using government laboratories, those of universities and those of suppliers was rather limited. The larger firms have their own quality control laboratories four times more often than smaller firms. Further, just as many larger firms utilize commercial laboratories as do smaller firms.

TABLE 7. *Laboratory facilities used by the foodservice industry.*

Facilities used	Firms	
	< 350 units	> 350 units
Firm's laboratory only	1	5
Commercial laboratory only	10	2
Firm's and commercial laboratories	2	7
Firm's, commercial and university laboratories	1	2
Commercial and university laboratories	2	1
Firm's, commercial and suppliers' laboratories	0	2
University or government or suppliers' laboratories	2	1
Firm's, commercial and government laboratories	0	1
None used	2	1
No answer	2	0

Once a laboratory has done certain tests, were they helpful? There seemed little dissatisfaction with the work done (Table 8). Despite this satisfaction, it was thought perhaps the foodservice industry might still have some reservations regarding who should do the laboratory tests, sanitary inspection and sanitation consultation. Respondents from larger firms certainly felt these programs should most often be within the firm while respondents from smaller firms felt local commercial organizations might better handle this program and this attitude reflects their present practices (Table 9). Those from three of the four smaller firms who chose their own firm as the likely base for such programs already had such programs. Answers from the representatives of larger firms indicate that only a little more than half choosing this internal organizational approach already had such programs. Government operated programs are not a very popular alternative and may reflect the fact that so many government agencies involved in sanitation programs are also associated with legal enforcement. In that chain foodservice firms have many widely dispersed units, a mobile commercial laboratory and teaching unit might

TABLE 8. *Degree of satisfaction with results of laboratory testing.*

Satisfaction	Firms	
	< 350 units	> 350 units
Helpful	15	17
Somewhat helpful	3	5
Not helpful	1	0
No answer	3	0

TABLE 9. *Best organizational approach for performing laboratory tests, sanitary inspection and sanitary consultation services in the foodservice industry.*[a]

Organizational approach	Firms	
	< 350 units	> 350 units
Within firm's own organization	4(3)[b]	18(10)[b]
Local external commercial organization	9	4
Industry-wide organization	4	3
Mobile commercial organization visiting each unit	3	3
Government organization	1	2
No answer	1	0

[a]Multiple answers only with firms having >350 units.
[b]Figures in parenthesis are the number of firms answering this affirmatively that already have such an organization within the corporate structure.

best fit their needs and there was support for this. The mobile unit concept has been used by some few firms and might have seemed more attractive if an additional question had indicated it might be part of the firm's corporate structure.

Quality control programs can be viewed as an expense with no direct profit to a firm. Some attempt was made to find, therefore, if management of foodservice firms thought that advertising the fact that quality control procedures were performed might have some practical impact. No one thought such a practice would injure sales (Table 10) and only a few indicated this practice would have little effect. Most thought such a practice would be worthwhile. Representatives of smaller firms seemed somewhat less convinced that customers would be affected by advertising quality control procedures.

In a somewhat similar question it was found that establishment of quality control programs might satisfy a number of areas of concern starting with customers' complaints and a concern for "sanitary image" (Table 11). Concerns for government regulations had some impact but these were not as high on the list as in Table 3. The answers listed in Table 11, however, reflect the respondents' interpretation of the entire industry's concerns for government regulations rather than just their own concerns as reflected in Table 3. Consumer

TABLE 10. *Impact of well advertised quality control procedures.*[a]

Impact	Firms	
	< 350 units	> 350 units
Enhanced public image	13	13
Enhanced employee pride and productivity	12	10
Increased customer acceptance	5	11
Have little effect	4	3
Injure sales	0	0
Not sure	0	1

[a]Multiple answers.

groups, despite their attacks on "fast-food" quality, did not seem of major significance. The variety of additional answers were not categorized, but most emphasized "Whatever was good for the firm".

In an attempt to produce somewhat more specific answers, the question was asked about those areas the firms' management felt might be useful in a quality control program (Table 12). There was little doubt that sanitation instruction of employees and microbiological testing would be emphasized, with sanitation consultation also of some importance. Despite the fact there was very limited number of choices in this question, virtually no respondent made use of the space provided to add any additional quality control procedures. None added "sanitation inspection," the most common procedure used by public health departments.

TABLE 11. *Those sources of industry-wide concerns giving rise to establishment of quality control programs.*[a]

Sources	Firms	
	< 350 units	> 350 units
Customers' complaints	11	7
Firm's desire for sanitary establishments	7	7
Adverse public image and loss of sales	7	6
Government regulations	5	4
Consumer groups	2	5
Employee cooperation	0	3
Suppliers	2	3
Various additional answers	0	6
No answer	0	1

[a]Multiple answers.

TABLE 12. *Quality control procedures deemed the most useful in the foodservice industry.*[a]

Procedures	Firms	
	< 350 units	> 350 units
Sanitation instruction for employees	18	16
Microbiological testing of establishments and products	12	14
Sanitation consultation	7	8
Water quality testing	2	6
Sewage and disposal testing	2	5
No answer	0	2

[a]Multiple answers.

DISCUSSION

The data from this survey can be faulted in a number of ways and perhaps could not be called highly scientific or comprehensive. Certainly the answers were from a selected group, yet the responses were from a sizable segment of the chain foodservice industry and suggest some tendencies. There are some clear distinctions between large and small firms although many small firms are indeed not so small. Some are part of an even larger corporate structure, yet many do not have their own internal quality control laboratories. Perhaps some might feel that larger firms are larger because they tend to emphasize quality control procedures. More likely is the alternate possibility that the larger firms have the economic ability to launch such programs. Perhaps also in having more units they have more problems. It is pertinent to add that many smaller firms seemingly continue to function and apparently are profitable without internal quality control laboratories. Perhaps this is done by using external quality control facilities.

Generally, and especially among the top management of larger foodservice firms, there seems a significant concern for quality control programs. Many firms have established quality control organizations and it would appear that government regulations are not the primary inducers for establishing these organization. It seems that the primary motivation is customer acceptance.

It is unlikely that specific data can be obtained for the quality, quantity, expenditures and effectiveness of the quality control measures in each foodservice firm. The best that can be said is that top management considers employee education and microbiological testing the areas to emphasize in a quality control programs, and believe such programs are best provided within their own organization. In addition, almost half of the firms represented in this survey have laboratories, a tangible commitment to quality control testing.

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The Need for Standards in Foodservice Sanitation Education

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ABSTRACT

At present there is only one guideline for determining what information should be taught in foodservice sanitation courses. This basic document is the Food Service Sanitation Manual, including a model foodservice sanitation ordinance, 1976 revision, by the U.S. Department of Health, Education and Welfare, Public Health Service, and Food and Drug Administration. However, this manual is not written in terms of educational outcome and is not truly based on sound educational principles. This paper approaches the problem of prevention of foodborne illness from the educator's point of view. It discusses the instructional problem in sanitation, why people must learn, who the students are, the values and benefits of this educational program, and what should be taught if the educational outcomes are to be attained.

What is the instructional problem in sanitation? Why must people learn, and what will happen if they don't learn? Foodborne illness can be caused or potentiated by a wide range of people associated with the foodservice business. If foodborne illness is to be prevented, then all such people must be educated as to their role in foodborne illness prevention, especially if this waste of resources in the United States is to be reduced. It has been demonstrated and reiterated by the Food and Drug Administration, that it is not cost-effective to prevent foodborne illness in foodservice through inspection. A foodborne illness can be caused by a worker in a foodservice establishment at any moment of the day. It's not economically feasible to have inspectors on duty in all foodservice establishments at all times. There is also the question of who really is the "best" inspector. The only cost-effective inspector in the system is the worker who is performing the duty and who, if he makes a mistake, can cause foodborne illness. That is the person who must truly understand and be educated. In addition to having the knowledge for prevention, all the people associated with the industry must also want to prevent illness. Since education includes motivation to learn, it is appropriate to also keep motivation in the classroom.

It's also important to recognize that if clean and sanitary surroundings are not provided in food service establishments, customers will not be attracted, and eventually that operation will be so unprofitable, that it will go out of business. So for the economic viability of the industry, customers must have knowledge and be able to prevent foodborne illness.

What will happen if people don't learn? Customers will get sick and, statistics tell us, a few will die. There will be a waste of the personal, financial, and material resources of the United States. Finally, of course, the business will cease to exist.

Who are the learners? What is their educational level? What are the required prerequisites for learning, and what is the self-motivation needed by these people? Figure 1 shows the people hazards in the food service system and the people who needed to be educated. It is evident that there is a wide range of educational backgrounds associated with this group of people, and the educational system must be able to cope with all levels of understanding as well as provide the motivation for these people to learn. The first and major persons who must be taught about sanitation are the customers. The customers really represent the ultimate quality control element in the foodservice system. This is the

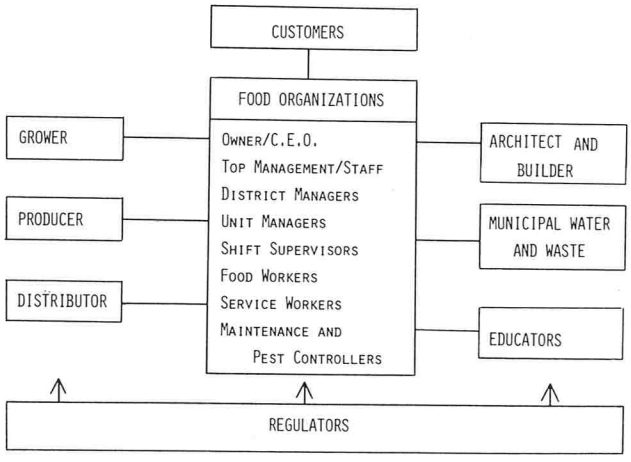

Figure 1. *People needing education in foodborne illness prevention.*

group who, if satisfied, will return and thereby help the business to prosper. Anything short of their satisfaction and the business will cease to exist. Today the average American is especially perceptive as to quality and sanitation in food service operations. This group represents the first educational opportunity and it will be necessary to teach the American public how to differentiate between a quality foodservice operation; one that is safe, where they won't be given foodborne illness and one that is run in a hazardous way, one which they should not patronize. On the left side of the figure is shown the people who put food into our system, the grower, producer, and distributor. The regulatory people of the United States, those at the local, state and national level, presently exert a reasonable level of control over these potential sources of foodborne illness. A special problem does exist though, when a food organization buys directly from a grower, for example, a farmer who is growing fruit in the community. The food organization must be able to ask pertinent questions in regard to such hazards as pesticides to insure that the grower is producing safe food. But generally speaking, the regulatory people also have control over these three people hazards.

On the other hand, there are the architect and the builder, who today have no requirement and who are not truly regulated in their duties in foodborne illness prevention. The architect and the builder of course have critical responsibilities. If the facility is not put together properly, it may be difficult to operate safely. The municipal water and waste people are relatively well-regulated through the local governments. But again, there is need for constant surveillance to ensure that the system maintains its standards. There is no regulation of educators, though, and anybody can teach, providing they can get students to come to their classes. Unless the educator knows precisely what to teach, there is no foundation on which to build the educational system to teach the prevention of foodborne illness. Of course, the regulators themselves need to be educated. They need to be educated not only in the structural aspects, but also in food technology aspects of disease prevention, and personal and public health aspects.

Finally, there are the members of the food organization. At the top, of course, is the owner or chief executive officer. This person must make policy, set the standards; otherwise inspection cannot be done because there are no standards to inspect against. Following the owner come top management, district managers, unit managers, and shift supervisors. All of these people must know how to enforce policy. Then come food workers and the service workers, followed by the maintenance and pest controllers. Every member of the food organization must be taught (*a*) how to perform his specific duties in such a way as to prevent foodborne illness, and (*b*) how to inspect work to be sure it was done correctly.

THE EDUCATIONAL CYCLE AND ELEMENTS

Figure 2 shows the basic educational cycle. The first step in education is to determine the objective, which, in this instance, is prevention of foodborne illness. Next, it

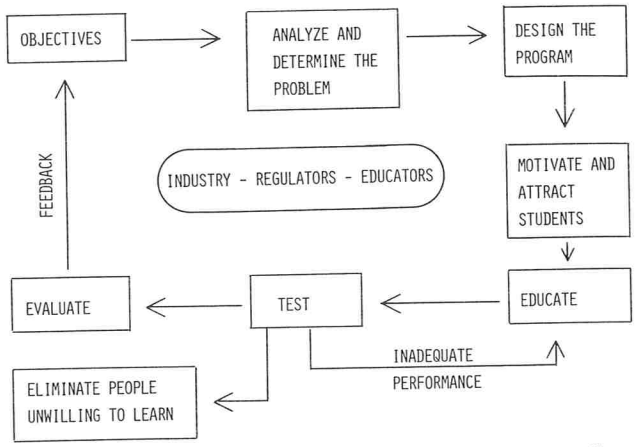

Figure 2. *The basic educational cycle for foodborne illness prevention.*

is necessary to analyze and determine precisely what the problems are so that behavioral outcomes can be determined. It is not effective to educate somebody in an area that has no payoff. When it is clearly agreed upon by the educator, regulators, and the industry that these are the problems that people must be taught to solve, then the educational program can be designed. When the program is designed, or at the same time it is being designed, students can be motivated and attracted. It may be necessary to write mandatory educational requirements into the law because as is often true, there maybe a small group of people who do not voluntarily want to be educated. The only way to deal with this problem is through mandatory education. Once designed the classes established, the people should be educated and tested for their understanding. Those that test out properly would then be allowed to go to work, where on-the-job evaluating could take place. If a person is motivated but doesn't learn the material and cannot perform properly, he would be sent back for additional education. Those that are not willing or unable to learn, would have to be eliminated from the foodservice system. It must be accepted, that not everyone will be motivated to learn and perform his duties to the degree necessary for prevention of foodborne illness. Therefore, an elimination system is essential to the educational cycle. During the evaluation, the education is evaluated to see how well those who have passed through the system do in fact perform, and as necessary the objectives of the educational program may be modified through a feed-back channel before the educational cycle repeats. It is important that this feedback be incorporated in educational programming because there is always change occurring in the world, and the educational system must keep up with it, particularly in the dynamic area of foodborne illness.

What are instructional objectives? Instructional objectives are the learning outcomes of thinking skills, doing skills, attitudes, and so forth. Some of the key words that are used in describing instructional objectives are that at the completion of the education, the student will be able to:

identify	know	analyze
describe	understand	synthesize
list	apply	evaluate
demonstrate	use	
comprehend	think critically	

These are not only words that are used to describe instructional objectives, but they represent important key words that are used very frequently. These are the words that management needs to think about when the educational instructional objectives are being prescribed.

The education system must be of value to all of the people who will be educated, or the educational system simply will not be used. Figure 3 is a simple diagram of the value analysis in the foodservice system. It says that

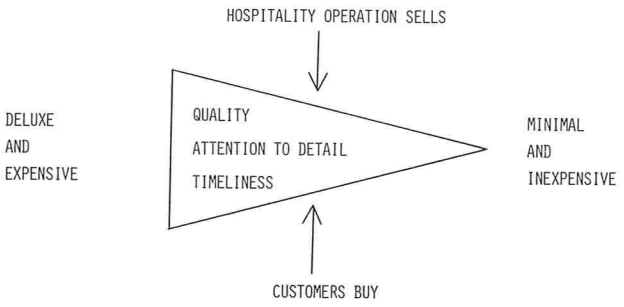

Figure 3. *Value analysis in food service operations.*

the hospitality of operation sells three things: quality, attention to detail, and timeliness in the service which is performed. This is what customers buy. Customers buy everything from deluxe and expensive services down to minimal and inexpensive services. Because there is a tremendous economic spread in our population between the very rich and the very poor, services must range from the very expensive all the way down to very inexpensive to satisfy the need of this wide range of economic population. Nevertheless, all foodservices have in common three elements: quality, attention to detail, and timeliness. If these three elements do not match in value, what was paid by the customer for the service, the customer generally will not return. It's appropriate, then, that management must prescribe the basic three elements: (*a*) the kind of quality it expects to sell; (*b*) the attention to detail or quality control intended for their system; and (*c*) the timeliness of their service to customers. This also becomes one of the elements of learning for top management, i.e. how to set policy in these three critical areas of business.

The educational system must also lead to satisfaction on the part of the three major elements of the food service system — the customer, the foodservice owner or chief executive officer, and the manager, and workers within a system. First, the customer must feel satisfied as to the atmosphere, the service, and the food which is purchased. He must feel that there was value received for services performed. On the other hand, the chief executive officer and owner of a business may feel differently about what their major satisfaction objectives are. They normally will want business prosperity. They would also likely prefer ethical operations. They want quality food to be served, and timely service, and they need systems discipline and reward if they are to be satisfied with a smooth running, effective operation. The managers and workers, on the other hand, do not have business prosperity as a prime objective, unless they, of course, are involved very heavily in participatory profit-sharing. Generally, these salaried people want such personal gains as achievement, recognition, challenging work, responsibility, advancement, security, and a salary which is commensurate with their technical competencies.

BASIC ELEMENTS IN FOODSERVICE MANAGEMENT

A systematic approach should always be taken in specifying management requirements. Figure 4 shows the basic foodservice management cycle. It begins with

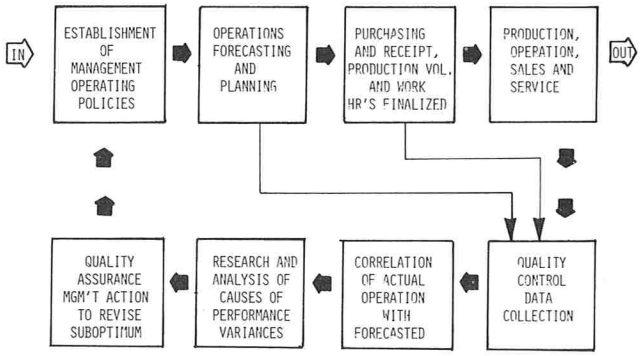

Figure 4. *The food service management cycle.*

the establishment of management operating policy. This is a critical element which must be included in the cycle. Management must prescribe its quality, its service, its level of quality control or attention to detail, and the timeliness which it intends to provide before an operation is specified. Otherwise the operation has no base on which to build. Once this is established, management can forecast and plan, purchase and receive goods, and prepare and carry out its operation. At the same time that operations are taking place though, there must be quality control data collection which is correlated with the forecasted performance. Variances from the forecasted performances may be analyzed, and the quality assurance function then takes place.

Basic to the stability of the management cycle is quality control. Quality control is a system of inspection, analysis and action designed to achieve and maintain a level of quality and a degree of excellence. The quality control department does the inspection and analysis. But

it does not take action. The line organization takes action. The reason for this system is that if the line organization is not forced to take action, people rely on inspectors to do so when the system fails; this is clearly wrong. Inspection is the process of comparing a product or service with specifications or standards. Implicit in this definition is the fact that there must be specifications and standards. These are properly designated by top management for the operators. Who are the inspectors? The inspectors are the government agencies, both local, state and national, management, the customers, and most important of all, it is the individual worker himself doing his job in the work center. The worker is the most critical inspector of all, and he is the person who requires the best education.

While quality control is the determination of a variance with standards, another element, quality assurance, is necessary if there is to be an improvement in the system. Quality assurance is the top management process of reviewing quality control trends, and prescribing more efficient and effective operating policy. It results in educating and motivating employees to follow the modified policy.

In establishing policy, and sanitation, it is also crucial that the principles of hazard analysis and critical control points be employed to be sure that inspection efforts and specifications deal with the actual sanitation hazards. Hazard analysis and critical control points (HACCP) is a systems approach to quality assurance that finds all the possible hazards in producing a food product, specifies procedures for eliminating the avoidable risks, and sets acceptable limits for unavoidable risks. HACCP is a system for prevention and pre-control. Again, the inspection elements of quality control would see to it that HACCP was working properly, and that new hazards had not been introduced to the system. HACCP also recognizes that the foodservice operation is not a sterile environment, and could not be a sterile environment for economical reasons. It is perfectly acceptable to have low levels of microbiological contaminants because these will not affect the customer's health. The alternative of providing absolutely sterile food in fact, might be very hazardous to the long range survival of the population. If the population does not have an antibody system which can fight and take care of normal low levels of contamination, the population could become seriously vulnerable to all forms of disease and illness. Management has a series of roles that it must play in quality and quality assurance. These are as follows: (*a*) definition of the business and its direction to meet planned goals and objectives, (*b*) policy that quality is to be controlled, (*c*) clearly defined standards of quality, (*d*) sufficient inspection coverage for proper process control, (*e*) correct inspection tools and well-trained inspectors, (*f*) adequate records to show trends in business operations and quality control, and (*g*) feedback to the workers for learning and improvement. The system which does not improve worker performance through feedback learning will not be very cost effective.

SANITATION TRAINING REQUIREMENTS

Figure 5 is a diagram of the elements of a sanitation quality control program for foodservice establishments. It identifies the three forms of contamination: microbiological, chemical, and material or particulate. It

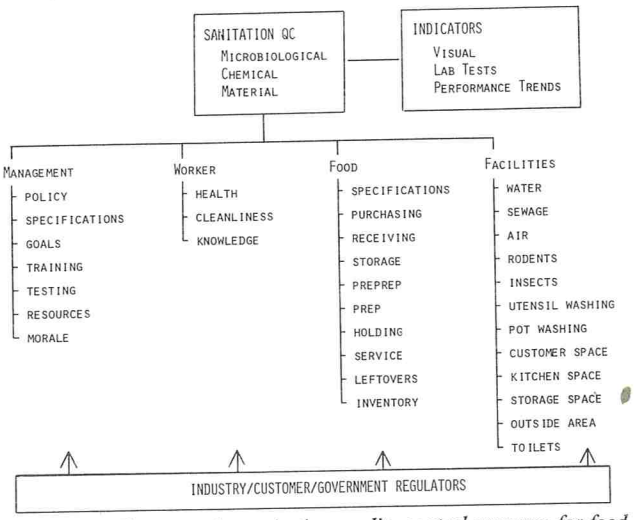

Figure 5. *Elements of a sanitation quality control program for food service establishments.*

shows that there are three indicators that can be used for sanitation quality; the first being visual, the second being laboratory tests, and the third, performance trends in quality control indicators. There are four major elements that need to be inspected by the industry itself, the customer, and the government regulators. First, is management: policy, specifications, goals training, testing, resources, and morale. If any of these elements are missing, then the organization is in an unstable operating condition and prone to quality failure. These are not rapidly changing indicators, however. They change slowly and if a business is healthy, that business will generally stay healthy as long as the same people remain in management positions.

Another element in the quality control area is that of the facilities, as shown in Fig. 5. These also are slow to vary, and if the restaurant is attached to a reliable utility system, then the facilities should need a minimum of inspection other than simply saying they have not changed. In addition, facilities must be constructed properly when the building is put up, or there will always be inherent problems in the operation of the foodservice establishment. On the other hand, there are two elements in food quality control that are very dynamic and change essentially minute by minute — they are the workers and the food handling. The workers' health and cleanliness can change with one visit to the bathroom. The food can be mishandled at any time during the operation of the foodservice facility. These two elements of sanitation quality control are the most important. It is also interesting to note that on the only common inspection report that exists in the United States today — the

recommended Food and Drug Administration Inspection Report — the worker and food preparation receive minimal coverage, and those elements which are not identified with the cause of foodborne illness receive a great deal of attention. This leads to one element in both education and inspection that quality control must be concerned with, and that is the actual causes of problems. Otherwise persons will be educated to solve problems that are not important, and inspections will be made to solve problems that are not causing foodborne illness. The major cause of foodborne illness is unquestionably bacterial growth reflected in times and temperatures of food handling in the kitchen. These problems arise at least 4 h before the time the customer sees the food, and the only person who can control them is the worker.

The customer has been identified as one of the principle inspectors of a foodservice system. If the customer is to be effective as an inspector, then the customer needs to be trained in what to look for as quality indicators. Appendix A lists a series of indicators which customers should be trained to check for, to differentiate between well-operated and poorly-operated foodservice establishments. The first series of indicators have been titled "Environmental or External," and these are the indicators (as shown in the appendix) which the customer sees before entering the establishment. These are indicators of management's and the workers' attention to detail. They are not related truly to the cause of the foodborne illness. In the second phase, the customer enters the restaurant. Again, the indicators are those of attention to detail, and not of prevention of foodborne illness. In the third area, that of service, the customer sees some indirect indicators that the operation is being run in an attentive way, but are still no clear-cut indicators that would tell him that the food has been carefully handled before service. In the fourth step, the customer consumes the food, and here the customer has the greatest opportunity to tell whether the food was properly handled. The foodservice industry has always known that cold food is served cold, and hot food is served hot. If customers look for these key indicators, they are probably among the better indicators (along with hot or cold plates) that there has been attention to detail in the preparation of the food. Also, dry, crusty food is an indication that the food has been held for extended periods, and could indicate a serious potential problem due to the growth of microorganisms. In the fifth area, after eating, the customer gets final proof as to whether the food was safe or not. If there was not foodborne illness hours or days after they've eaten in the restaurant, then the customers had a safe meal. This is certainly a very unsatisfactory indicator of quality.

There are also certain indicators of management quality as shown in Appendix A. Normally, the customer will not be aware of these indicators, but management or regulatory inspectors can look for these. If they are positive, they are strong signs that the foodservice establishment is stable, and that there is little chance of foodborne illness occurring in that establishment.

What are the learning outcomes then, if foodborne illness is to be prevented in the foodservice industry? These are shown in Appendix B. It is actually a pyramid of learning outcomes, beginning with the simplest and least complicated for the service and cleaning personnel, and gradually becoming more complex as the food worker is educated, and then, at the third level, management. The service and cleaning personnel have a few critical indicators that they are not taught today and that they should be taught. Item 1 identifies the fact that personnel must know what health symptoms need to be reported so that they can be excused from food handling. The regulations require that people who have illness will not handle food. The worker is the only one who can really identify this fact to management. It says that, that is an important fact which needs to be taught in grade school, and in high school, because of the large employment of teenagers in the foodservice industry. The last element for service and cleaning personnel is, that they know and understand the role of the sanitarian. The sanitarian is an important quality control check for the establishment and should be looked upon as a person who can teach, coach, and counsel the restaurant operators into even high standards of quality. Not only must the service worker understand that, but of course, the food worker and management should know this too.

The food worker, then, must know everything that the service worker knows plus all of the elements of the safe handling of food. From the food worker, then, we arrive at the level of management which must be able to prescribe policy in all of the areas that the workers must be able to perform. Management has a very important educational need; these are complex subjects to be discussed in the classroom. It also says that the educator who teaches management must not only understand operations, food handling, microbiology and so forth, but must also understand management policies and detail, so that he can help them learn how to write a sanitation safety policy.

SUMMARY

Unquestionably the quality control and educational system to achieve quality control which has been described will be expensive, and if there are no benefits to be derived, the system will never be implemented nor be effective. There are many benefits, though, to quality control and management. These include increased production, lower unit costs, improved employee morale, better quality, more customers, and fewer inspections (because the regulatory people can now concentrate their efforts on foodservice establishments that have low quality and low quality control). There has been a great deal said about sanitation certification. One of the best definitions of what ought to be included in certification was provided by the Single Services Institute as a result

of its 1975 sanitation conference on quality control in foodservice management. The definition that arose from this meeting is that sanitation certification is official, documented assurance (given under industry or regulatory auspices, or both) that an individual does his foodservice job proficiently and in full observance of approved food sanitation standards. As this group would define certification, and as educators would define certification, it is measured performance on the job, not the result of a simple examination in a classroom.

Certification will have different meanings, whether you're talking about the service worker, the food worker, or management. It also will have different meanings whether you're speaking about one of the suppliers of food to the foodservice operation, or about the regulator who is supposed to have knowledge and should be certified that his is competent to perform an inspection of the foodservice establishment. In each instance, certification should only come after verification by an appropriately trained person that that person can do his work satisfactorily on the job.

The key to long range quality is self-inspection by the worker and self-inspection can be effective, when the customers and the industry are educated in foodservice quality, and take action to achieve that quality. Customers must be willing to send food back that does not meet the quality standards that they expect of a restaurant. Workers must take their ideas to management to ensure that management achieves the standards that the workers have been trained to expect in a properly operated foodservice establishment. And, finally, management must educate workers until they achieve the standards that management demands in those workers' daily performance. The regulatory people in industry, and government, and customers can certify that the elements for quality control are present, and are used through the process of quality assurance. The value of quality assurance to the foodservice industry is profit, clear and simple. It is profit through maximum customer satisfaction and minimum wasted resource. It is time that industry, regulators, and educators got together and established common behavioral learning objectives so that these may become effective in providing the kinds of people that the food industry needs to provide long range service to the population of the United States.

ACKNOWLEDGMENT

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APPENDIX A

CUSTOMER FOOD SERVICE QUALITY INDICATORS

(Rank 1-9) (Will Vary With Economic Status of Customer)

1. *Environmental — (External)*
   a) Personal recommendations from former customers
   b) Clean neat outside appearance
   c) Good buildings and grounds maintenance
   d) Properly lighted
   e) Adequate signs
   f) Accessible parking facilities

2. *Entrance*
   a) Clean and maintained
   b) No rowdy people (loitering)
   c) Sanitation certification
   d) Reasonable number of other customers of same class
   e) Absence of flies and insects
   f) Odor pleasant
   g) Smoke control of smokers
   h) Light sufficient to prevent tripping and accidents
   i) Place to put belongings
   j) Pleasant greeting
   k) Prompt (or expected promptness) seating
   l) Acceptable waiting area
   m) Honesty in handling reservations and waiting customers
   n) Control of noise from operations and proper traffic
   o) Should have appearance of businesslike efficiency
   p) Proper dress of host/hostess and all other personnel
   q) No loitering of help, as well as no smoking, eating, drinking and so forth
   r) Comfortable temperature of rooms
   s) No rodents, animals
   t) No apparent employee illness

3. *Service*
   a) Sit at clean table and in clean, comfortable chairs
   b) Order taken promptly - attentive but not hovering personalized service
   c) Clean service ware and table cloths
   d) Waitress pleasant and informal
   e) Glass of water, clean, cold and clear
   f) Waitress clean - hands, hair, body, clothes
   g) Food covered and served in sanitary manner
   h) Sanitary control of salt, pepper, sugar, and condiments
   i) Food utensils handled in sanitary manner and well maintained, such as knives sharp
   j) Comfortable seating - desired personalized space - decor pleasing
   k) Little children and physically handicapped handled o.k. in seating
   l) Clean menu
   m) Accurate menu - adequate range of menu items
   n) Refuse containers adequate and clean
   o) Noise level appropriate for style of operation
   p) Enough sound isolation so that conversations can be private

4. *Food*
   a) Meets value image
   b) Portions are according to value
   c) Hot food is hotter than 140 F and cold food cooler than 45 F
   d) Plates are hot or cold
   e) Food and service look pretty - are neat, colorful, visually appealing
   f) Food texture is as specified and taste has proper flavor notes
   g) Food doneness meets qualifications
   h) Garnish is good
   i) Not swimming in juice, nutrition conservation
   j) No foreign objects in the food
   k) Truth in menu
   l) Nutrition information available
   m) Salt and sugar mildly used

5. *After eating*
   a) Attention from waitress until departure (refills of coffee, tea, water)
   b) Dishes bussed in a sanitary manner
   c) Table cleaned properly, rag, servers' fingers
   d) Check presented promptly
   e) Feeling of satisfaction with food
   f) No indigestion
   g) Kitchen and facilities open for inspection

h) Bathrooms clean
i) Good exit path
j) No foodborne illness
k) Customer grievance system
l) Liability insurance

6. *Indicators of management quality*
   a) Reasonable profit or operating cost
   b) Indications of Owner/Manager visits and caring
   c) Policies on/and the procedures are effective
      Training
      Standard HACCP recipes
      Cleaning schedule
      Specifications for food and supplies
      Inventory turnover
      Replacement of equipment
      Standards for employment
      Appearance and health of employees
   d) Management people are all trained in sanitation QC/QA
   e) There is a QA plan for the food service
   f) Knowledge of and compliance with state/federal law
   g) Workers demonstrate knowledge
   h) Proof that Management takes action to keep operation high quality
   i) There is a QC information system
   j) Inspections for rodents and insects
   k) Maintenance contracts for equipment
   l) Preventive maintenance system

LEARNING OUTCOMES FOR FOODBORNE ILLNESS PREVENTION IN THE FOODSERVICE INDUSTRY

Cleaning personnel

1. Knows how to clean himself preparatory to work and follows proper personal hygiene work habits.
2. Can describe how to wash, sanitize and dry a work surface or piece of equipment with cleaner and sanitizer.
3. Knows how to perform hot water cleaning, sanitization and drying.
4. Knows how to work out concentrations of cleaners and sanitizers and test for concentration levels.
5. Knows how to judge if a utensil or piece of equipment is sanitary and safe to use.
6. Knows how to clean facilities and knows what is adequately clean.
7. Knows how to keep serving utensils and areas clean and sanitized.
8. Can describe ways to prevent cross contamination in the service, ware and pot washing, and garbage areas.
9. Understands how food can become contaminated with hazardous chemicals and knows how to deal with pesticides and chemicals.
10. Knows how to dispose of rubbish and garbage properly.
11. Knows the major causes of foodborne illness and can relate this to their own operation.
12. Understands how chemical poisoning can occur and how it can be prevented.
13. Knows and understands the role of the sanitarian.

Service personnel

1. Knows how to conduct a hazard analysis and inspect critical control points in holding and serving food.
2. Can describe personal health symptoms that are cause to stay out of the food, food utensil, and food storage area.
3. Understands and can apply the regulations regarding use of food served to customers.
4. Knows how to measure food temperatures.
5. Can describe safe storage methods for foods in the service area.
6. Knows the importance of proper operation of food service equipment and can describe proper operation of the equipment for which they are responsible.
7. Understands the requirements for microbial growth and the relation of time and temperature for major illness microorganisms.
8. Can differentiate the hazard risk of different foods.

Food handling personnel

1. Can specify how to produce food and keep hands sanitarily safe.
2. Can describe how to look for rodents and insects and what action to take.
3. Can specify safe food preparation processes.
4. Can specify how to inspect incoming food products for wholesomeness.
5. Can describe how to measure process temperatures and prescribe safe time-temperature procedures.
6. Can conduct a Hazard Analysis Critical Control Point Analysis of food handling and kitchen cleaning.
7. Can describe safe storage methods for raw and cooked foods and food service materials.
8. Knows how to set up a Quality Control (QC) system in his/her own work center.
9. Knows how to conduct a QC check of his/her own procedures and measure QC indicators.
10. Knows what to do in an emergency when a utility has failed.
11. Can specify how to keep food hot or cold for service.
12. Can prescribe how to handle leftovers from kitchen operations.
13. Understands advantages to investigating sources when ordering for quality and safety.
14. Can specify how to operate a temporary food service.

Management

1. Can prepare a sanitation creed or policy for the organization.
2. Understands that some levels of food contamination are unavoidable and can prescribe acceptable limits posing no real risk to the public.
3. Knows what is needed for sanitary design of a food facility and is able to specify design policy.
4. Is able to specify policy on purchasing of supplies.
5. Can specify and establish a self-inspection system/quality control system with adequate record keeping and action feedback for operating components not within control limits.
6. Can prescribe accept-reject policy for marginal food products.
7. Can perform an accurate hazard analysis and critical control point inspection of the establishment.
8. Can specify a training program policy in sanitation and qualifications for certification.
9. Can prescribe a personal hygiene policy.
10. Is able to specify a cleaning and sanitizing policy.
11. Knows the cost-benefits of sanitation.
12. Can prescribe a policy of reward and punishment for good-bad employee performance.
13. Can prescribe emergency operations policy in case of utility failure.
14. Can prescribe an organizational policy to be followed when notified that the organization is suspected of being a source of foodborne illness.

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Microbiological Standards for Water: Introduction

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Within the last 5 years, a rapid interest in the "Standards Methods" type of manual for water examination has occurred among professional societies, governmental organizations and interdisciplinary industrial groups. To illustrate this involvement, the following groups are among those that are concerned with standardization of methods:

1. *Professional societies*
   - — American Public Health Association
   - — American Water Works Association
   - — Water Pollution Control Federation
   - — Association of Official Analytical Chemists
2. *Governmental organizations*
   - — Environmental Protection Agency
   - — U.S. Geological Survey
   - — British Ministry of Health
   - — Pan American Health Organization
   - — World Health Organization
   - — International Standards Organization
4. *Interdisciplinary industrial groups*
   - — National Council of the Paper Industry for Air and Stream Improvement
   - — American Society for Testing and Materials
   - — American National Standards Institute, Inc.

Undoubtedly confusion will ensue in the near future over the choice of methods and the relative sensitivity and specificity of each method for a selected bacterial indicator. Comparative testing of media and procedures is in progress in the European community with the hope of establishing a selection of procedures which will yield essentially equivalent monitoring data on international river systems. Agreement on selection of reference media and procedures has created some difficult problems, particularly where national pride and status of the experts are involved.

There is concern not only over proliferation of methods appearing in all of these Standard Manuals but also for creation of redefined bacterial indicator systems that may not have been fully evaluated. One example of this problem relates to the search for fecal contamination in water. Some of the "Standard Manuals" are being very liberal with the use of species names when in truth the procedures are not refined or selective enough in an operational test to justify such taxonomic specificity. Nomenclature for indicators should avoid taxonomic designation for specific organisms where species identification does not conform to tests prescribed by *Bergy's manual.*

From a legal point of view, data developed from any new methods and/or from redefined bacterial indicator systems will be subject to challenge. This is especially true wherever results from such tests are used in enforcement actions related to several public laws recently enacted to protect the water environment for a variety of uses. The Federal Drinking Water Standards and the public laws dealing with public water supplies and quality of sewage effluents specify the use of procedures as described either in *Standard Methods for Examination of Water and Wastewater* or the EPA Manual, *Microbiological Methods for Monitoring the Environment.* Other "Standard Manual" concepts supported by special professional societies or special interest groups will have to apply for EPA acceptance of individual tests or resort to a court test for establishment of their legality in these matters.

Another problem which may soon surface involves acceptance and certification of laboratories (their facilities, personnel, methods, etc.) under a new national certification program directed toward all laboratories that examine public water supplies. These laboratories must meet minimum standards of acceptance or be excluded from participation in the official monitoring of any public water supplies. Part of the certification

requirements relate to the use of specific procedures defined either in *Standards Methods* or in the EPA Manual, *Microbiological Methods.* Attempts to use methods from other "Standard Manuals" that differ in methodology from these references will be subject to challenge.

With these facts in mind, the stage is now set, and experts involved in several aspects of Microbiological Standards for Water Use are ready to discuss the philosophy of quality criteria, and microbiological standards for waters, including viewpoints from Federal agencies on the application of Standard Methods to monitoring and enforcement activities.

ACKNOWLEDGMENT

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Microbiology Standards for Waters

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ABSTRACT

This is a discussion of the kinds of microbial standards that apply to various kinds of water and the circumstances under which the standards might be used. Standards are universal language. Appropriate standards depend upon proper personnel, materials and procedures to be effective. Comments are made on some possible situations where the concept of standards may restrain good scientific and technical development. Historically, there is circumstantial evidence enforcement of microbial standards has aided our great advances in water hygiene.

In first looking at the title of the paper to be presented for this Seminar, my reaction was that an enumeration and justification of the various numerical standards that are used in water quality bacterial monitoring was the appropriate and obvious approach. A short period of meditation made it very apparent that it would be dull and fruitless to attempt to document the many numerical standards that are prescribed or adhered to for the many types of waters, the several bacterial tests possible and, above all, the multiplicity of standards established by the nations around the world and the States and political subdivisions within the United States.

Among the waters that need monitoring controls are: sewage and industrial wastewaters; raw, recycled and finished water for potable use; recreational water in streams, lakes, ocean beaches, swimming pools, baths, etc.; water for medical uses as infusion solutions and dialysis systems; agricultural water, especially irrigation supplies and return flows; water for food processing, preparation and manufacturing; fish and shellfish rearing waters; and special industrial uses where there are needs to control organisms that produce slimes or change the forms of such elements as iron and sulfur to detrimental end products.

STANDARDS FOR SPECIALIZED USE OF WATER

For each of these specialized uses, standards for viable bacteria and bacterial products exist or should exist, well beyond the obvious coliform standard that many people feel is the only tool of the water control microbiologist. While coliform standards have been and will continue to be used as a measure of quality of several kinds of waters, we must also look to many other bacterial measures of quality. A few selected examples of number standards that have or could have important applications are the following: (*a*) fecal coliforms in wastewater discharges, bathing waters and farm animal runoff; (*b*) the fecal streptococci, often recommended as an adjunct to coliform or fecal coliform testing; (*c*) total or standard plate counts, a misnamed, underused, misused and abused bacteriological tool that provides a great deal of information when used properly in controlling water plant operations, wastewater and swimming pool disinfection efficiency, food processing waters, and industrial water applications; (*d*) modified plate count tests, done at selected temperatures for psychrotrophs, thermophiles and other organisms with optima outside of the 32-35 C range; or at various pH values above and below the usual 7.0; or at oxygen tensions lower than ambient; or in selected substrates to indicate specific enzyme activities such as gelatin degradation, lipid utilization, DNA-ase production, etc.; or modifying the incubation time to enlarge the array of organisms that will grow; (*e*) determination of bacterial spore counts for food industries such as sugar manufacture, canning and bottling; (*f*) the *Clostridium perfringens* counts, not commonly used in this country, but fairly well accepted as a drinking water test in Europe; and (*g*) enumeration of levels of *Pseudomonas, Aeromonas* and *Flavobacterium* which are of interest in controlling fish-rearing water, in medical applications of water, especially the pyrogens of *Pseudomonas,* and in swimming waters.

Some additional standards are often discussed as needed and are in various stages of development. These include tests for detection of animal viruses of public health significance and bacteriophages in the fermentation and food industries; direct detection of pathogenic bacteria in waters; enumeration of staphylococci and micrococci which may be significant in swimming water and in manufacture of such foods as beer; detection of bacterial endotoxin in drinking water and standards for

parasites such as *Giardia*, which is of current concern in drinking water.

While enumeration of the several kinds of standard tests in the many uses of water has hopefully introduced the breadth of the problem that this symposium might cover, further comments on standards will be limited to the areas of greatest current concern, drinking water and water pollution control from a public health standpoint.

STANDARDS FOR WATER – PUBLIC HEALTH VIEW

Bacterial standards for water, initially drinking water and later wastewater discharges, evolved in the late 19th century at the community level. Cities assumed primary responsibility for imposing bacterial testing, in its early crude forms, and developing allowable limits of viable organisms. Soon the States assumed an important role in establishing standards for bacteria and in developing the appropriate laboratory methods. The professional societies, the universities and the Public Health Service of the U.S. Government each made important contributions.

For the past few years we have been moving to a new governmental philosophy. From the era of somewhat uneven provision of health protection services for the people of the country, we are now attempting to provide near-zero risk to all people in the hazards from wastewater and drinking water. The Federal Government is establishing standards for bacteria, as well as several other parameters, in these waters as minimal standards to be enforced by each of the States. Establishing higher standards in any particular situation is the prerogative of the States. Some, because of their geography, industry, or local needs are establishing or adopting stricter bacterial standards than mandated nationally. Examples of this are the enforcement of fecal coliform standards for municipal wastewater effluents, stricter fecal coliform or standard plate count standards than required by EPA for land disposal of wastewater, or self-imposition of more stringent standards than required at a metropolitan drinking water utility.

There may be special needs, at the state or local level, to protect an estuary or beaches, to maintain a sports fishery or a body contact recreational lake or reservoir. Standard quality must be viewed then as the minimal requirement that is deemed essential to reduce the risk of detrimental effects to a prescribed level. It should allow for and encourage imposition of more demanding standards in terms of allowable numbers of organisms, organisms to be tested for or number or frequency of tests run.

ADEQUACY OF TESTING

In discussing standards for bacterial testing we normally think of the kind and frequency of tests to be done or the allowable limits of the results. It is easy to overlook the fact that the test results are no better than the quality of the sample, the sampler, the laboratory and its materials and equipment and, obviously, of the person running the tests. Very important aspects of microbiological standards for waters are the training of personnel, quality control of laboratory methods and materials, standardization of equipment and collection and transport of samples. In all too many water or sewage system operations, personnel doing the sampling or operating the laboratory are in the lowest pay categories with additional duties in the community. Adequate funding for the large hardware in the system is made available, but chipped pipettes, faulty pH meters, water baths and incubators that do not hold temperature, autoclaves that are undersized or do not sterilize and many, many other items not meeting standards are in use. Laboratories are often inaccessible to small communities and samples are agitated in the mail or other delivery systems for days or incubated without cooling in the trunk of a car. It is important that standards be applied to all water laboratories, be they publicly or privately operated, large or small. Recently a committee of the American Water Works Association under the chairmanship of W. Ginsburg (*4*) attempted to gain some insight into laboratory operations by a questionnaire. The results indicated a rather deplorable situation. Geldreich, who visits many of the laboratories in his assignment to initiate a required program in laboratory certification, an objective almost synonymous with standardization, has often related some of the non-standard items he has observed. In 1975 Geldreich (*3*) authored a second edition of an EPA *Handbook for Evaluating Water Bacteriological Laboratories* that attempted to establish a standard basis for laboratory evaluation. In looking at the glossary I found that the definition for standard to read:

> "A measurement limit set by authority. Having qualities or attributes required by law and defined by minimum or maximum limits of acceptability in terms of established criteria or measurable indices."

This definition is probably a good legal one; however, my dictionary provides a number of other definitions which, I believe, justify my broader use of the term to cover the many facets that go into making the microbiological standards meaningful.

It should be added that there also are some excellent laboratories in many parts of the country that have imposed appropriate standards on their work. Several years ago some major strides were taken in improving the quality of laboratory chemicals and dye materials. If the proper grade is purchased and handled properly they do provide consistency among laboratories. More recently, with some reluctance, the manufacturers of media have made strides in standardizing their products. Competition and a broadened market have been conducive to availability of better quality laboratory equipment for water microbiology. Maintenance and repair is still a major problem. Progress has been made in another area, improvement in filter membranes.

STANDARDS AND QUALITY

One important aspect of microbial standards for water is that they provide a common base or language for relating numbers of organisms to the level of quality that is desired. This standardized numerical language is important to the legal people who are responsible for enforcement of water quality compliance regulations. They often treat (or mistreat) these numbers as though they were cast in bronze, probably one of the occupational hazards of their profession. Since we as microbiologists, in cooperation with scientists of the related technical disciplines in water quality, must supply the criteria upon which standards are set, it is important that we provide the best of advice. Providing information for standard setting by best guess, by consensus or with overly conservative safety margins is not good science nor will these standards hold up in the courts. The costs currently involved in water monitoring are high and there is always someone willing to challenge the requirements in the courts.

Abel Wolman, a great contributor to water hygiene, has written some cogent and provocative papers on the subject of standards. A few comments are worth repeating because they remind us that establishing microbial standards must not be our ultimate goal, nor by themselves will they lead to clean water.

The first quote is from a 1960 paper entitled "Concepts of Policy in the Formulation of So-Called Standards of Health and Safety" (*9a*). Although it was written on the subject of radiation standards, it could just as well have been microbial standards.

> "From its beginning, society by one means or another, has surrounded itself with restraints. These have had, for the most part, empiric origins — moral, ethical, economic, or spiritual. All the restraints have had the common basis of an assumed benefit to the particular society establishing them. As societies became more complex and more sophisticated, efforts towards both standardization and restraint became more frequent, more necessary, and presumably less empiric, although examples of the last are not as numerous as one might expect.
>
> There are all kinds of standards. Rigid definitions should preclude the loose application of the term 'standards' in discussions of standards for radiation control. The procedures often used to establish standards may roughly be classified as:
>
> 1. Regularization of techniques of measurement
> 2. Establishment of limits of concentration or density of biologic life and physical and chemical constituents
> 3. Regularization of administrative practice
> 4. Regularization of legislative fiat
> 5. Specification of materials."

Some interesting statements from a paper entitled, "Bacterial Standards for Natural Waters" (*9*) follow:

> ".....Standardization for all features of natural waters has become one of the major sanitary engineering indoor sports.
>
> Historically, ample support may be summoned for the thesis that standards of judgement are dangerous, fallacious, and inappropriate to scientific workers. These warnings range all the way from the sharp but cogent comment of the late Professor Sedgwick, in describing standards of sanitation as 'devices to save lazy minds the trouble of thinking', to equally significant but more refined warnings by Phelps,...."
>
> "*Reasons for growth of stream standards*
>
> In the intervening decade, however, the search for and the introduction into law and regulation of stream criteria has proceeded at a fast pace. Part of the process has been engendered by the literal intimidation of many workers in the field by the imaginative demands of certain militant organizations. In other instances, the appeal of the convenient handbook has been overwhelming. In still others, the subtle attractiveness of 'zoning' has given the necessary fillip to this standardization technique. In this pursuit toward the quantitative millennium for qualitative matters of judgement of a number of underlying philosophies have found their full play. At the one end of the spectrum are found the criteria established to preserve original quality and concomitantly, therefore, to avoid original sin. In this particular philosophy the studied and judicial comment of the late Professor Whipple that a regulatory edict, both in law and in philosophy, should establish the minimum for safety rather than the maximum of hope, is ignored. The reemphasis on this dictum was equally cogently and intelligently set forth by Frost and others, in sharpening the distinction between standard methods and so-called standards of judgement."
>
> "*Standards* vs. *judgement*
>
> What can one say about criteria of stream quality, solely from the standpoint of bacterial content, when one state alone offers five ways of avoiding original sin, depending on the economic status of the bather? And what can one say, furthermore, of the state of affairs in which, as was pointed out 10 years ago, one state insists that it is unsafe to swim in a body of fresh water which exceeds 5 coli per 100 ml and an adjacent state insists with equal fervor that equal safety is afforded to the swimmer by a bacterial density of 500 times that amount? What can be said about a regulatory agency's desire when it established a standard of bacterial density that virtually rules out 95% of the available surface streams within its territorial limits, provided, of course, it rains on occasions and the agricultural terrain is in part washed into the surface streams?"

These statements and problems sound quite current, but they were presented orally in 1949 and printed a year later.

Our work in helping to set standards must be based on the best possible information available, and where it is not available we must strive to design our data collection and experiments and our search for better methods to answer the questions properly. An example of one of these needs was mentioned earlier, that microbiological standards should bear a direct relationship to risk to human health, to fish life, to aesthetics or similar criteria. We seem to be having difficulty arriving at the answers. We use two methods for coliform testing, the multiple tube MPN test and the newer membrane filter procedures. The tests measure different organisms and yield different kinds of results, one statistical and the other closer to a direct enumeration, and it is very difficult to justify the different standards that may be established on these tests to the lawyer or judge or to the financial officer who must pay for laboratory results.

HISTORICAL ASPECTS

As a student of the history of science, microbiology in particular, I am often concerned that we in our modern, sophisticated, complex scientific community forget some of the sage observations of the recent past. Before publication of the first edition of *Standard Methods of Water Analysis* (*2*), a committee of the American Public Health Association was charged with studying means of extending the standard procedures of an earlier 1897 committee, to all methods involved in the analysis of water. This committee's work culminated in the 1905 edition of *Standard Methods* (*1*) and a transmittal letter stated the following:

> "The methods of analysis presented in this report as 'Standard Methods' are believed to represent the best current practice of American water analysis, and to be generally applicable in connection with the ordinary problems of water purification, sewage disposal and sanitary investigations. Analysts working on widely different problems manifestly cannot use methods which are identical and special problems obviously require the methods best adapted to them; but, while recognizing these facts, it yet remains true that sound progress in analytical work will advance in proportion to the general adoption of methods which are reliable, uniform and adequate.
>
> It is said by some that standard methods within the field of applied science tend to stifle investigations and that they retard true progress. If such standards are used in the proper spirit, this ought not to be so. The Committee strongly desires that every effort shall be continued to improve the techniques of water analysis and especially to compare current methods with those herein recommended, where different, so that the results obtained may be still more accurate and reliable than they are at present."

This historic move to establish standard laboratory procedures had a direct bearing on the development of microbial standards for waters, a process still evolving.

In the area of drinking water two paragraphs from Prescott et al. (*5*) are quite appropriate.

> "Standards for Potable Water. The information furnished by quantitative bacteriology regarding the antecedents of a water is in the nature of circumstantial evidence and required judicial interpretation. No absolute standards of purity can be established which rigidly separate the good from the bad. In this respect the terms 'test' and 'analysis' so universally used are in a sense inappropriate. Some scientific problems are so simple that they can be definitely settled by a test. The tensile strength of a given steel bar, for example, is a property which can be determined. In sanitary water examination, however, the factors involved are so complex, and the evidence necessarily so indirect, that the process of reasoning much more resembles a doctor's diagnosis than an engineering test.
>
> The older experimenters attempted to establish arbitrary standards, by which the sanitary quality of a water could be fixed automatically by the number of germs alone. Thus Miquel (1891) published a table according to which water with less than 10 bacteria per milliliter was 'excessively pure', with 10 to 100 bacteria 'very pure', with 100 to 1,000 bacteria 'mediocre', with 10,000 to 100,000 bacteria 'impure', and with over 100,000 bacteria 'very impure'. Few sanitarians would care to dispute the appropriateness of the designations applied to waters of the last two classes, but many bacteriologists have placed the standard of purity much higher."

Sternberg (*7*), in 1892, proposed numerical criteria as follows: (*a*) up to 100 bacteria/ml is good water, (*b*) up to 500/ml would be marginal but acceptable and (*c*) over 1000/ml indicative of sewage contamination. Twenty-two years later the idea of bacteria/ml was adopted as the first United States microbiological standard for drinking water by the Treasury Department (*8*). It was applicable to interstate common carriers, but was one of the precedents by which the United States Government, through EPA, can now impose a minimal microbiological standard to all of the country.

Microbial standards, by themselves, do not reduce the disease risk from drinking or body contact waters, but they do provide the monitoring goals and objectives that are needed by the engineers and the health officials to perform their proper roles in the maintenance and improvement of water systems of all types. The graphic presentation of the incidence of typhoid in Philadelphia from the 1880's to 1945 (*6*) is a typical portrayal of the point that the value of establishing standards may often be overlooked. Without detracting from the tremendous value to public health of the technological development and application of water filtration in 1906 and of chlorination in 1913 in the Philadelphia system, the role of microbiological testing and the application of standards underlies the success (Fig. 1). It must be more than just chance that the 1905 *Standards Methods* date

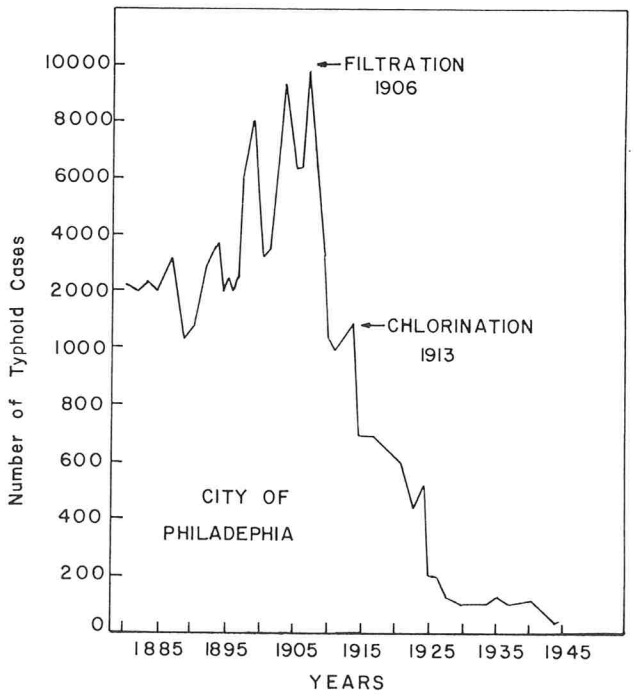

Figure 1. *Reduction of typhoid fever in Philadelphia following treatment of the water supply. (from Smillie and Kilbourne, 1962)*

and the 1914 *Treasury Standards* date, previously mentioned, coincide so well with this decline in typhoid ascribed to filtration and disinfection. Microbiological standards must have played some role.

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Microbiological Monitoring for Water-Quality Assessment

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ABSTRACT

The weakest link in the chain of events leading to production of reliable microbiological-monitoring data is a poor or indequate sample. This results primarily from diversity of environmental conditions from which a sample must be collected. In surface waters, affinity of microbiological organisms for suspended particles necessitates that sampling procedures be designed to collect a representative sample of the water-sediment mixture. The key problem and the challenge to microbiological monitoring is production of a sterilizable, depth-integrating sampler that will accommodate the disparity of sediment distribution as related to variations in depth and cross-section and the changes in streamflow. Until such a sampler has been designed, tested, and made readily available, the data produced in microbiological-monitoring programs involving surface waters can be considered of questionable accuracy, regardless of the notable advances that are taking place in the state-of-the-art of analytical procedures.

Today there are about 1,000 properties that are routinely monitored or measured in the field of water quality. The analytical coverage includes a tremendous variety of physical, chemical, and biological variables. Of importance to this paper are the microbiological variables, chiefly indicator bacteria, that are monitored for assessment of water quality.

It is appropriate to define, or at least to restrict, the meaning of the broadly applied term, "monitoring." Cairns (*5*) defined monitoring as "the regular or continuous assessment of one or more parameters and may be used to detect harmful conditions." His definition was supported by Ladd (*15*). Pickering and Ficke (*21*) referred to monitoring as successive measurements over a period for detecting change, or lack of it. The U.S. Environmental Protection Agency (*31*) recognized four types of monitoring: (*a*) ambient trend monitoring, for measuring conditions and trends in relation to standards and guidelines; (*b*) source monitoring, to locate and measure toxicity of effluents and to assess compliance of pollution sources; (*c*) case preparation monitoring, to gather evidence for enforcement actions; and (*d*) research monitoring, to support research activities.

Implicit in these definitions is measurement of some environmental factor over time. For the purpose of this paper, "monitoring" is defined as repetitive measurement or sampling for whatever the intended purpose. Excluded from this definition is the concept of biotic monitoring, the term applied to a wide variety of techniques that use the response of organisms to environmental conditions (*18*).

NEED FOR STANDARDIZATION

Because of the involvement of many persons representing many groups and agencies, any program or venture that involves microbiological monitoring requires standardization so the data obtained from all areas and sources will be comparable. The necessity for standardization for both sample collection and sample analysis is emphasized by enactment of several public laws, existence of national water-quality networks, and presence of centralized storage and retieval data systems.

There currently exists the National Water Data System (*16*) consisting of all generally available water data including those collected by both Federal and non-federal entities (*27,28*). According to the 1976 Catalog File of the U. S. Geological Survey's Office of Water Data Coordination, 33,408 water-monitoring stations were operated in the United States during the 10-year period of 1966-1976. Of these stations, bacteria were monitored at 12,902 stations by 132 Federal and State agencies. Principal Federal agencies included the U.S. Geological Survey with 2,233 stations, Environmental Protection Agency with 609 stations, U.S. Army Corps of Engineers with 468 stations, and the U.S. Forest Service with 264 stations. Currently bacteria are being monitored at 9,964 stations; 7,531 stations are on surface waters and 2,433 stations are on ground waters.

Principal national networks involved with the monitoring of water quality, including microbiological quality, are the National Stream-Quality Accounting Network (NASQAN) (*10,20*); the Benchmark Network

(*17,20*), both operated by the U.S. Geological Survey; and the National Water-Quality Surveillance System (NWQSS) (*27,28*) operated by the Environmental Protection Agency. NASQAN, presently consisting of 345 stations and an anticipated size of 540 stations, is designed to provide information on year-to-year variations in water quantity and quality and to document changes with time in water quality throughout the Nation. The Benchmark Network consists of 56 measuring sites in small basins where the hydrology is relatively unaffected by man and thus is not likely to change over the years. It is designed to document the range of "natural" streamflow and water-quality conditions and to provide a basis for understanding the natural forces controlling them.

NWQSS is designed to monitor the progress in the Nations's effort to abate water pollution. The approximately 120 stations of the network are situated in paired configurations to observe changes in quality of water passing through municipal-industrial and agricultural-rural areas.

The Environmental Protection Agency recently has proposed a basic water-monitoring program for the United States (*7,26*) which includes the three forementioned networks. The new program will emphasize those activities that aid (*a*) development of national trend assessments, (*b*) control of toxic substances, (*c*) waste treatment facilities process, (*d*) compliance assurance within the National Pollutant Discharge Elimination System, (*e*) assessment of non-point source pollution, (*f*) State water-quality management planning, and (*g*) State monitoring programs (*7*).

The national water-quality data systems receive information from a multitude of individuals and agencies. Well-known systems include the storage and retrieval system or STORET of the Environmental Protection Agency and the water storage and retrieval system or WATSTORE of the U.S. Geological Survey. In addition, the National Water Data Exchange, called NAWDEX, recently has been established under the auspices of the U.S. Geological Survey. NAWDEX is an interagency program to assist users of water information in the identification, location, and acquisition of needed data (*8*).

The reasons for standardization need not be dwelled upon in that it is paramount in the minds of all individuals involved with the collection and analysis of water-quality samples. Results of several attempts at standardization in aquatic microbiology are available.

In 1969, on the recommendation of the Coordinating Council of the International Hydrological Decade, an Inter-Agency Panel on Standardization in Hydrology was established. The most well-known product from the Inter-Agency Panel was the *International Standards of Drinking Water* (*32*). Despite international attempts at standardization, the World Meteorological Organization (*33*) recently commented, "A lot of work toward international standardization in hydrology and its related fields has been carried out already, but much remains to be done, even for simple basic measurements."

In 1972, a report entitled, *Recommended Methods of Water Data Acquistion* (*27*) was released by the Federal Inter-Agency Work Group on Designation of Standards of Water Data Acquisition. The interagency activity and the document were endorsed by the Federal Advisory Committee on Water Data. The manual currently is being rewritten as the *National Handbook for Water Data Acquistion* with extensive review by the non-federal sector.

Other notable attempts at standardization include authoritative, widely used references such as *Standard Methods for the Examination of Water and Wastewater,* published jointly by the American Public Health Association, the American Water Works Association, and the Water Pollution Control Federation (*1*); and the *Book of ASTM Standards. Part 31 Water* revised and published annually by the American Society for Testing and Materials (*2*). In addition, several publications by Federal agencies are in widespread use. The list includes *Methods for Collection and Analysis of Aquatic Biological and Microbiological Samples* (*12*) by the U.S. Geological Survey and the *Handbook for Evaluating Water Bacteriological Laboratories* (*11*) by the Environmental Protection Agency. Soon to be released will be EPA's *Microbiological Methods for Monitoring the Environment. I. Water and Wastes* (*4*).

The most recent, and perhaps most notable, move for standardization in the United States was the recently published *Water Programs—Guidelines Establishing Test Procedures for the Analysis of Pollutants—Amendments* (*25*). It recommends the specific procedures by which measurements of 115 physical, chemical, and biological characteristics (including five for microbiological determinations) of water will be made. The document was prepared pursuant to section 304(g) of the Federal Water Pollution Control Act Amendments of 1972 (Public Law 92-500, October 18, 1972).

The standardization of sampling and analytical techniques is necessary for acquiring the valid and interrelated data needed for meaningful assessments of the occurrence, distribution, and fate of water quality constituents. Although the precision, reproducibility, and quality control used in doing laboratory analyses is of a high degree, the reported data are no better than the confidence that can be placed in the representativeness of the sampling (*9*).

Use of adequate microbiological samplers and sampling techniques has not been emphasized in the technical literature. We are all familiar with the common statement that appears in methods manuals, "Take a representative sample." Undoubtedly, the weakest link in the chain of events leading to the production of microbiological data is an inadequate sample.

The problem is complex and has many pitfalls. This results primarily from the diversity of environmental

conditions from which a sample must be collected. A sampling procedure used in a flowing stream is not suitable for sampling a well; a sampling procedure used in lakes and reservoirs is not suitable for sampling a treated public water supply or industrial and municipal wastes effluents.

Even if it were technically possible to define or set minimum standards for collection of a representative sample from all possible hydrological situations, it is beyond the constraints of this paper to discuss the many fine points. Rather, it is the intention to convey some general guidelines and basic understandings necessary for developing the proper procedures for the collection of representative microbiological samples.

MICROBIOLOGICAL SAMPLING

Microbiological sampling can be defined as all the acts and procedures that must be done before delivery of a water sample to the analyzing laboratory or all the methods by which data are generated under field conditions. This includes site selection, field instrumentation, sample collectors, sample collection, and preservation.

Underlying and paramount to all the approaches to microbiological sampling is the intended use of the data after they are acquired. For example, data obtained for defining quality of a mass of water generally are not suitable for documenting spatial and temporal variations. In addition, data collected for documenting trends in a body of water are usually unsuitable for determining conformance of waste discharges with pollution control standards.

The initial step, then, begins with a statement of the problem or intent. After the objective has been clearly stated, the data needed to fulfill the objective can be determined. Determination of the data needs is followed by a designed approach to include such items as the number of required samples, sampling locations, sampling frequency, and sampling techniques. Realistically, the final step in this orderly process is a consideration of cost constraints.

When a new water quality station is established, its general location and the frequency of sample collection is set by the data needs, type of investigation, purpose of the study, and anticipated variation in microbiological characterisitics. Selection of the exact sampling site will probably depend upon a combination of factors including accessibility, availability of other information, and uniformity of water quality at the site. Even though an individual station is established to meet a specific need for information, the possibility of placing and operating it to supply data for other studies should not be overlooked.

Water samples are collected and analyzed to ascertain characteristics of a body or mass of water. The sample is usually only an infinitesimal part of the total volume and, therefore, is representative of the total mass only to the degree that uniformity of composition exists within the total mass. Uniformity of composition can be assumed for many sources of water including public water supplies and municipal and industrial wastes effluents. Methods for sampling of these water are sufficiently documented by the American Public Health Association and others (*1*), and Greeson et al. (*12*).

In their natural state, surface waters are subjected to forces that promote mixing and homogeneity. The fact that such tendencies exist, however, is not sufficient cause for assuming that a body of water is so well mixed that no attention to sampling techniques is required. In most instances, a body of water may not have uniform composition because of local conditions.

Customarily, surface waters have been sampled by filling a container held just beneath the surface of the body of water. The sample is commonly referred to as a dip sample or grab sample. If the microbiological characteristics are homogeneous throughout the cross-section of a stream, one dip sample taken anywhere in the cross section will adequately define water quality.

If the microbiological characteristics are not uniform throughout the cross section, a sample representing the average composition of the stream must be taken. In addition, and in further emphasis of this procedure, it is known that many microbiological constituents are transported in streams attached to suspended particles. Jannaseh (*14*) found that in the Nile River only 0.02-0.04% of the contained bacteria were truly planktonic or freeliving. Similar findings were observed by Wuhrmann (*34*) who found that only 0.005% of the bacteria in an experimental artificial stream were free-living, and when sewage was introduced into the stream, only 0.9% of the bacteria were free-living. Bardtke (*3*) observed that 10-30% of the bacteria were free-living in the lakes of the Stuttgart region.

A number of the devices constructed and described for microbiological sampling were reviewed by Rodina (*22*). Some samplers have mechanical devices for removing a stopper at a desired depth and replacing it when the vessel has filled with water. Other samplers make use of capillary tubes, which are broken at a desired depth by a messenger, thus permitting the sample to be drawn into a sterile collecting device. A microbiological sampler designed by Niskin (*19*) consisted of a large metal hinge fitted with a sterile plastic bag and tube. When the sampler was tripped by a messenger, the tube opened aseptically as the hinge flipped open, and the water sample was drawn in.

Most commercially available microbiological samplers are based on the original design principle of ZoBell (*35*) as modified by Sieburth (*23*); that is, a sterile collapsed rubber bulb is lowered to a desired depth and triped with a messenger. Water is drawn through a tube by the action of the rubber bulb.

The aforementioned samplers, while innovative in design, will collect a sample of water from a point at a predetermined depth. However, a point sample of this type is a grab sample and will not represent the average

composition of a stream. The data obtained from such a sample can be misleading and erroneous as to the true bacterial density in a body of water.

Theoretically, a sample representing the average composition of a stream can be obtained by compositing several depth-integrated samples. Each sample should be of equal volume and should be collected at transects of equal flow in the cross-section.

For sampling throughout the vertical profile in streams, depth-integrating samplers are used. The simplest depth-integrating sampler may consist only of a mechanism for holding and submerging the container. When the container is lowered at a uniform rate, water is admitted throughout the vertical profile. One such device is simply a weighted glass bottle that can be lowered by a nylon rope.

If the person taking the sample could be assured that the bottle was lowered to the bottom and raised to the surface at a uniform rate, he would have roughly approached collection of what is known as a depth-integrated sample (*9*).

A true depth-integrated sample, however, is collected by means of a sampler which integrates discharge as a function of depth (*13,24*). The velocity of flow in a stream, as well as the size and distribution of sediment particles, vary both vertically and horizontally (*6,30*). Depth integration is used to collect a water-sediment sample that is weighted according to the velocity at each increment of depth.

One of the best sampling techniques currently accepted by hydrologists for use in such situations is the equal-transit rate (ETR) method (*13*). In this method, the standard suspended-sediment sampler is used to collect a discharge-weighted sample. Samples are taken at a number of equally spaced verticals in the cross-section. The transit rate of the sampler, which is the rate of movement of the sampler from the water surface to the streambed and back to the surface, should be the same at all verticals. Samples collected in each vertical are composited into a single sample that is representative of the entire flow in the cross-section. According to Feltz and Culbertson (*9*), the composite sample of the water-sediment mixture collected in this manner is a representative sample that is velocity- and discharge-weighted.

Several depth-integrating samplers are in widespread use in water-quality studies and have been described by the Subcommittee on Sedimentation (*24*). However, paramount problems exist with the use of currently available depth-integrating samplers for collection of microbiological samples. First, the samplers cannot be adequately sterilized and, second, equal-volume samples collected for centroids of equal flow in a cross section cannot be composited under aseptic conditions. The latter problem can be rectified by determining the density of bacteria in the individual samples and compositing the results. Though technically acceptable, the costs and manpower constraints would make this approach prohibitive or difficult at best.

The key problem and the challenge to microbiological monitoring is the production of a sterilizable, depth-integrating sampler designed to accommodate the disparity of sediment distribution as related to variations in depth and cross section and to changes in streamflow. Until such a sampler has been designed, tested, and made readily available, the data produced in microbiological-monitoring programs involving surface waters can be considered of questionable accuracy, regardless of the notable advances that are taking place in the state-of-the-art of analytical procedures.

CONCLUSIONS

As stated in *Standard Methods* (*1*), it is impossible to give directions for sampling techniques to cover all conditions which will be encountered, and the choice of the technique must be left to the sample collector. There is not now, nor is there ever likely to be, a single method of sampling which can be used to describe all microbiological aspects of the hydrologic environment. There are many different approaches to evaluating microbiological quality and each is dependent on the intended use of the data after they are collected.

By carefully following a few simple guidelines such as (*a*) defining the intended use of data before they are collected (*b*) evaluating the hydrologic situation from which the sample is to be collected (for example, homogeneity of flow and suspended sediment concentrations), (*c*) giving consideration to measurement of significant properties, and (*d*) using common sense, it will be possible to obtain a sample that is truly representative of the whole.

The state-of-the-art technology is a changing scene, in that many of the methods that are is use today may become obsolete tomorrow. In all instances, the quality of any data is no better than the method and representativeness of the sample.

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Standardization of Microbiological Methods for Analysis of Water

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ABSTRACT

This paper discusses characteristics and advantages of standardized methods for microbiological analysis of water and their use in enforcement and monitoring programs of the Environmental Protection Agency. The increasing numbers of other methods-standardizing organizations are documented. The present standardization process is reviewed and recommendations are made for improved approaches to standardizing methods.

To protect water quality and abate pollution, agencies responsible for water quality must assess present conditions, determine effectiveness of control measures, and plan future actions to improve our environment. In part, these decisions require sound quantitative and qualitative data on microbiological indicators and pathogens. Uniform test procedures are urgently needed to measure these microorganisms and to provide reliable, precise and comparable information on water quality.

Because biological sciences are less exact than chemistry or the physical sciences, it is even more important that microbiological methods be standardized and rigidly followed.

NEED FOR STANDARDIZATION

Standardization is necessary to secure uniformity, assure valid data and provide reference procedures for methods comparisons. A major advantage is the uniform application of methods which permits data comparison among analysts in the same laboratory, in joint field surveys, between laboratories, and among federal, state and private agencies. Standardized methods become more important when data from different sources are compiled in monitoring networks or are entered in common data banks such as EPA's *STORET*, a computerized information storage and retrieval system with capability for data analysis. It is imperative that standardized methods be used in monitoring and enforcement programs.

Some workers object to standardized methods on the grounds that the analyst will be locked in on one method. They are concerned that research will be stifled and development of new or improved methods will be discouraged. This need not happen. Standardization is not intended to prevent methods research. Modifications and additions of new methods can be made by building into the standardization system a mechanism for periodic review and change. With this mechanism, standardization can further research on improved procedures for water analysis by providing a reference method against which new methods can be compared.

CHARACTERISTICS OF STANDARDIZED METHODS

Standardization should include the uniform application of sampling procedures, analytical methodology, statistical computation and reporting of results. The selected procedures must be: (*a*) generally applicable to varied types of waters and wastewaters; (*b*) proven by use in many laboratories or validated in a formal study; (*c*) sufficiently accurate, precise, selective and sensitive for users needs; (*d*) judged best by the consensus of experts in aquatic microbiology; and (*e*) practical, with consideration for rapidity, cost, ease of use and limited special training of the analyst.

EPA'S ENFORCEMENT ROLE

As a regulatory agency, EPA plays a leading role in guiding the states, local authorities and other agencies in abatement and control of environmental pollution. A recent example of potentially hazardous water pollution was the diversion of 100 million gallons per day of raw sewage into the Ohio River in March, 1977 because toxic organic chemicals had contaminated the Louisville, Kentucky waste treatment plant (*5*). From 1971-1974 a total of 99 waterborne disease outbreaks affecting 16,950 people were reported in the United States (*4*). The largest incident in 1974 was symptomatic giardiasis contacted by an estimated 4,800 persons in Rome, New York; the municipal water supply source was reported to be surface water with chlorination as the only treatment (*3*). In the

same year approximately 1,200 cases of acute gastroenteritis, later confirmed as shigellosis, occurred in Dade County, Florida because of a contaminated well source and a temporary failure in the chlorination of the community water supply (*14*). Such incidents demonstrate the need for a vigorous monitoring and enforcement program.

The legislative bases for EPA's water pollution control and water supply protection activities are three Acts: the Federal Pollution Control Act Amendments of 1972; the Marine Protection, Research, and Sanctuaries Act of 1972; and the Safe Drinking Water Act of 1974. These Acts direct the Agency to protect and improve the quality of our water resources and to control pollution by setting, enforcing and monitoring standards for water supplies, ambient waters and wastewater discharges.

WATER QUALITY STANDARDS

To facilitate abatement and control of water pollution, microbiological water quality criteria were established for different water uses by the Office of Water Planning and Standards, EPA, in *Water Quality Criteria* (*12*) 1968 and revised in 1972 (*13*). A second revision, *Quality Criteria for Water* (*9*), is scheduled for publication. These criteria are summarized in Table 1.

Microbiological water quality standards developed from these criteria established contaminant limits for several water uses. The microbiological standards are summarized in Table 2.

Present EPA enforcement and monitoring activities are based on these standards. Laboratory and field analyses are done to determine if the water quality standards are met and whether the water can be safely used for a specific intended purpose. If the data indicate a violation of the standard, some regulatory action is required. Because far-reaching decisions are based on these laboratory data, sound, legally-defensible microbiological methodologies are required which must be standardized and include quality control over their use.

TABLE 1. *Water quality criteria**

Water or wastewater	Microbiological criteria Coliforms/100 ml Total	Fecal	Reference source
Drinking water source	20,000	2,000	A
Recreational water:			
Primary contact $\bar{x}$		200	B
max		400	B
General Contact $\bar{x}$		1,000	B
max		2,000	B
Agricultural water	5,000	1,000	B
Shellfish-raising waters			C, D, E
Daily $\bar{x}$	70	14	
10% Daily	230	43	

A. Water Quality Criteria 1972. National Academy of Science and National Academy of Engineers for the Environmental Protection Agency, Washington, D.C.

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C. Quality Criteria for Water. Office of Water Planning and Standards, U.S. EPA. Washington, D.C. In press.

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* From Microbiological Methods for Monitoring the Environment, Part I. Water and Wastes. Environmental Monitoring and Support Laboratory — Cincinnati, Ohio (In Preparation).

TABLE 2. *Water quality standards*[a]

Water or wastewater	Microbiological standards Coliforms/100 ml Total	Fecal	Reference source
Potable water	< 4		PL[b] 93-523
Chlorinated effluents		200-400	PL 92-500
2° Treatment wastes		200-400	40 CFR[c] Part 133
Selected industrial wastes		200-400	PL 92-500
Leather and tanning		400	40 CFR Part 425
Feed lots		400	40 CFR Part 412
Meat products		400	40 CFR Part 432
Beet sugar		400	40 CFR Part 409
Canned fruits and vegetables		400	40 CFR Part 407
Textiles		400	40 CFR part 410
Effluents from marine sanitation devices with discharges Type I		1000	40 CFR Part 140 and amendments
Type II		200	40 CFR Part 140 and amendments

[a]From Microbiological Methods for Monitoring the Environment Part I. Water and Wastes. Environmental Monitoring and Support Laboratory — Cincinnati, Ohio (In Preparation).
[b]PL Public Law
[c]CFR Code of Federal Regulations

STANDARDIZED METHODS IN EPA

In EPA, the Environmental Monitoring and Support Laboratory (EMSL - Cincinnati) is responsible for selecting, validating and standardizing analytical methods and establishing quality control procedures for water and waste analyses. As a part of this responsibility EMSL has prepared for publication the first EPA manual for microbiology, *Microbiological Methods for Monitoring the Environment, Part I, Water and Wastes,* (*2*). This manual provides the basic methods that the Agency needs to carry out its water quality and pollution abatement activities. The manual is not a replacement for, or in competition with, *Standard Methods for the Examination of Water and Wastewater* (*1*). It cites *Standard Methods* and EPA's *Handbook for Evaluating Water Bacteriological Laboratories* (*7*) as valuable sources of detail and background information.

EPA's *Microbiological Methods for Monitoring the Environment* is a basic reference for monitoring water and wastes in compliance with applicable water and effluent standards established by the Agency. The methods apply to drinking water, ambient waters and wastewater effluents. The legal authority for the methods used to analyse effluents in the National Pollution Discharge Elimination System (NPDES) is established in the Code of Federal Regulations (CFR) Title 40, Part 136

(*8*). This publication lists the procedures required for analysis of pollutants in Section 304 (*g*) of the Federal Water Pollution Control Act. (*6*). Table 3, an excerpt from Part 136 of the Federal Register, shows the approved test procedures for bacterial parameters, referenced to *Standard Methods for the Examination of Water and Wastewater* (*1*) and the methods of the U.S. Geological Survey (*11*). The new EPA manual will be referenced in another column provided in the Table.

TABLE 3. *List of Approved Test Procedures*[a]

Parameter and units	Method	References (Page Nos.) 14th Ed standard Methods	PT.31 1975 ASTM	USGS Methods
Coliform (Fecal), Number per 100 ml	MPN;[b]	922		
	MF	937		45
Coliform (Fecal), in presence of chlorine, number per 100 ml	MPN;[b]	922		
	MF[c]	928,937		
Coliform (Total), number per 100 ml	MPN;[b]	916		
	MF	928		35
Coliform (Total), in presence of chlorine, number per 100 ml	MPN;[b]	916		
	MF	933		
	Enrichment			
Fecal Streptococci, number per 100 ml	MPN;[b]	943		
	MF	944		50
	Plate Count	947		

[a]Federal Register, Vol. 41, 232-40 CFR 136, December 1, 1976

[b]The 5-tube dulution MPN is used.

[c]Since the membrane filter technique usually yields low and variable recovery from chlorinated wastewaters, the MPN methods will be required to resolve any controversies.

The amendment mechanism also provides procedures for approval of alternate methods. National approval for test methods is obtained by application to EMSL - Cincinnati. Case by case approval is obtained by application through the EPA Regional Offices.

The procedures in the EPA manual also apply to potable water. For compliance with the National Primary Drinking Water Standards in the Safe Drinking Water Act (*10*), monitoring data must be provided by approved laboratories. A national certification program is being developed in which the EPA Regional Offices or the States will certify water supply laboratories at the State and local level, using specific minimal criteria. If not certified, the utility must obtain analyses from another certified laboratory. Further, maximum contaminant levels are set for 17 parameters. These must be met or notice must be made of public hazard. The certification criteria and the National Interim Primary Drinking Water Regulations will require use of the methods described in the EPA methods manual and in *Standard Methods.*

METHODS-STANDARDIZING ORGANIZATIONS

The increasing activity in standards-setting and methods-standardizing organizations is evidenced by the significant number of methods manuals and guides for the microbiological analysis of water that have been produced in recent years. The following organizations and their publications are examples of methods standardization or proposed standardization for water analyses:

Organization	Publication
Government Agencies:	
U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, EPA, Cincinnati, OH	Microbiological Methods for Monitoring the Environment Part I, Water and Wastes
U.S. Geological Survey, Office Department of the Interior, Reston, VA	Methods for Collection and Analysis of Aquatic Biological and Microbiological Samples
States:	
Kansas State Dept of Health and Environment Office of Laboratories and Research Topeka, KA	Procedure Protocols for the Bacteriological Examination of Water
Minnesota State Health Dept. Division of Environmental Health Minneapolis, MN	Water Bacteriology Laboratory Protocol
Florida State Board of Health Bureau of Sanitary Engineering Division of Water Supply Jacksonville, FL	Manual of Practice on Sampling, Reports and Procedures for the Bacteriological Examination of Water
Industries:	
National Council of the Paper Industry for Air and Stream Improvement, Inc. New York, NY	A Guide to the Conduct of Indicator Organism Tests Used to Study the Sanitary Quality of Effluents and Receiving Waters
Gelman Instrument Company Ann Arbor, MI	Basic Membrane Filter (MF) Procedure, in Gelman Membrane Filters (MF) for Water Testing, and Suspended Solids Testing
Millipore Corporation Bedford, MA	Microbiological Analysis of Water

Professional Societies:	
American Public Health Assn. American Water Works Assn. Water Pollution Control Federation Washington, D.C.	Standard Methods for the Examination of Water and Wastewater, 14th Edition
The American Public health Assn. Washington, D.C.	Recommended Procedures for the Examination of Sea Water and Shellfish, 4th Edition
Producer/User Organization:	
American Society for Testing and Materials Philadelphia, PA	Annual Book of ASTM Standards, Part 31 Water
Foreign Countries:	
Department of Health and Social Security, Ministry of Housing and Local Government London, England	The Bacteriological Examination of Water Supplies
Inland Waters Directorate, Scientific Operations Division, Canada Centre for Inland Waters Burlington, Ontario	Methods for Microbiological Analysis of Waters, Wastewaters and Sediments
International Organizations:	
World Health Organization Regional Office for Europe Copenhagen, Denmark	Draft: Manual on Analysis for Water Pollution Control
International Organization for Standardization Budapest, Hungary	Draft: Water Quality; Microbiological Methods
*Academia**	
Department of Environment Sciences, Rutgers University New Brunswick, NJ	Pollution Microbiology, A Laboratory manual
Department of Microbiology University of Maryland University Park Press Baltimore, MD	Marine and Estuartine Microbiology Laboratory Manual

* Examples of methods in specialized areas of aquatic microbiology where proposed methods are needed but have not yet been standardized.

The proliferation of microbiological methods manuals indicates an increasing need for the standardization of methods to achieve valid data comparisons in the single laboratory, between laboratories and among agencies, States and countries. A good example of this need is the organization of health and environmental scientists from many European countries with contiguous borders or sharing the same river and coastlines to discuss methodology and prepare manuals. The Pan American Health Organization conducts studies and exchanges information on methodology among North, Central and South American countries.

The recent rapid production of microbiological methods manuals is not necessarily detrimental, or without reason. It is generally a response to specific agency, industrial and other user needs for standard methods to measure pollution and conduct enforcement activities to clean up the environment.

PRESENT STANDARDIZATION PROCESS

Methods standardization currently begins in the single laboratory where a method is developed to satisfy a specific analytical need. The microbiologist prepares a written description of the method, evaluates the method with a variety of samples, summarizes the supporting data and submits them to a methods-standardization group for consideration. The standardization group may be an industry, government agency, professional society, or interdiscliplinary organization.

The formal acceptance by the standardization group usually includes the following steps:

1. The written method and supporting data are accepted for consideration as a candidate method study.
2. A preliminary study is done in more than one laboratory.
3. After preliminary testing, the method is considered by a review group, usually a committee of recognized experts responsible for approval. The method is accepted or rejected. Sometimes the acceptance process is simply by edict or informal agreement among committee members. Ideally the approval should be by vote and consensus action by the committee.
4. At this point the method is usually assigned a "tentative" or "provisional" status.
5. The method advances to "standard" or accepted" status after an appropriate period of use and evaluation by a wider range of laboratories.
6. The method is presented to the scientific community by publication in a technical journal or methods manual.
7. Usually some provision is made for modificaton or change.

RECOMMENDATIONS

This review of current practices in methods standardization prompts the following recommendations for improvements:

Methods development

Improved methods or modifications of methods must be tested, documented and made available to other users for evaluation. The widespread use of an analytical method supports its reliability whereas use of unknown or private techniques, based on recent research or personnel preference, weakens the validity of the test results and forces the analyst to defend his methodology.

The analyst or laboratory that develops a method must clearly define the species or group of organisms that are detected, isolated and enumerated. Morphological, biochemical and serological identification should be included in the definition. The developer should specify exact test conditions, types of samples to which the method applies, and any limitations of the procedure.

Development of a method should include performance characteristics such as specificity, selectivity, sensitivity, counting range, precision and accuracy. Specificity is the ability of a method to recover a bacterial parameter as identified by a selective or differential characteristic and verified by additional tests. A procedure is judged specific if the recovered microorganisms verify as the desired test organism, and the "other microorganisms" and false positives do not verify when picked and tested. The acceptable level of specificity for a method cannot be set absolutely, but must be established for standard procedures or for new parameters by comparisons with the accepted methods. Selectivity is the ability of a method to encourage growth of the desired organism while inhibiting or reducing the numbers of other organisms by some arbitrary degree, compared with growth and recovery on non-selective media. Sensitivity is the ability of a method to detect small differences in numbers for the bacterial parameter measured. This detection ability varies with the concentrations of microorganisms in the sample; sensitivity is reduced with each increase in dilution.

The counting range indicates the minimum and maximum numbers of colonies that can be counted reliably on a membrane, pour plate or spread plate. The counting range is related to the sample volume, selectivity of the medium, and the crowding effect that may result from high bacterial densities.

Precision and accuracy should be determined by the method developer. Precision is a measure of reproducibility, the deviation among multiple measurements of a single quantity; it requires that adequate replicate analyses be done. Accuracy is a measure of correctness, the closeness of observed values to a known true density. The magnitude of the difference between the results by the test method and the true value provides the systematic error or bias. One approach to determining accuracy is to add the test organism to natural waters that are relatively free of that organism and compare the recovery by the proposed procedure to that from a rich, nonselective medium. The seeded sample may be treated to simulate environmental stress, for example by holding under selected environmental conditions at a given temperature for a specified time before analysis. Counts must be based on colony verification.

Methods standardization

The method selected by the standardization group must be the best available procedure, based on established acceptance criteria. Workers are reluctant to part with traditional or favorite methods and to adopt new techniques without convincing proof of improved results in their geographical areas.

Collaborative studies and methods comparisons should include the testing of various types of water and wastewater from different geographical areas by several participating laboratories. Seasonal effects should be taken into account, such as temperature, pH, turbidity and chlorine residual should be noted and correlated. A detailed test protocol must be conscientiously followed by the participating laboratories.

Such methods comparisons must include acceptable measures of precision, accuracy and comparative recovery, utilizing verified counts to determine the best method. Statistical evaluations must be done by commonly accepted procedures on the collaborative test data and the results should be published. Such studies are in progress or planned by the members of the Microbiology Subcommittee on Water, D19:24, American Society for Testing and Materials, as part of their standardization activities.

Standardization must be based on true consensus as a result of full committee action in which diverse opinions are aired and reconciled into a group decision. The committee should include members within and outside the government with expertise in the parameters under consideration. The members must participate actively in group discussions and decisions. There are dangers in committee action; methods may be accepted because they are proposed by the most influential committee members rather than because they are based on group experience and judgement.

Role of professional microbiology societies

Above the laboratory level where the methods are developed and applied, and in addition to the evaluation activities of the methods standardizing groups, professional societies should be involved in methods standardization. They can provide a forum for reporting methods development and comparison studies, liaison among the methods standardization groups, and guidance in the methods standardizing process.

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A Field Topic

Trouble Shooting A Mastitis Problem

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ABSTRACT

Mastitis is a very costly problem causing the farmer financial loss because of: milk lost through decreased production, discarded milk, medicine cost, animals culled from the herd, and veterinarian fees. Mastitis is a luxury the farmer cannot afford, yet there are many that feel it is something they must live with. There are three areas of concern when confronted with a mastitis problem: (*a*) management of the herd, (*b*) adequate milking system, properly installed, and (*c*) sub-clinical or infectious mastitis. To be successful in correcting a mastitis problem requires use of all resources. A team approach is used and the team consists of the dairy plant fieldman and laboratory, milking machine serviceman, area extension dairy specialist, veterinarian and the farmer who desires to correct the problem. Every person on this team has an important part to play in solving the problem. The result of solving the problem is a financial gain for the farmer.

Mastitis can be a very troublesome problem, causing financial loss due to decreased milk production, discarded milk, treatment costs, and loss of cattle from the herd. Mastitis is a luxury the farmer cannot afford, yet very often rather than admit there is a problem it is given the hush-hush treatment.

How do we recognize that a farmer has a mastitis problem in his herd? If the farmer is conscientious he will withhold milk from cows with clinical mastitis and his quality record will not reveal there is a problem if it is clinical mastitis. However, subclinical mastitis will be detected by the routine testing of milk for somatic cell count, as required by the Interstate Milk Shippers Code. The legal limit for somatic cells under the code is 1,500,000 cells/ml of milk. This number is totally unrealistic from the economic stand-point; we feel that any producer that has a commingled milk sample from his farm bulk tank that exceeds 300,000 cells/ml has a definite mastitis problem and he should look for the cause of the cell count.

FINDING THE PROBLEM

What approach do we use to seek out the cause of the problem? For the most part, mastitis is management oriented; therefore it is extremely important to visit the dairyman at milking time. We like to plan our visit so there are at least 30 min to check over the basic components of the milking system and discuss our observations with the herd owner.

What do we check? What are we looking for? First we evaluate the vacuum supply pump to see if it is adequate for the work load it has been assigned. If it is of adequate size, then we check the actual out-put of the pump to verify the volume of air it can move. This is done by using a flow-meter placed directly on the intake of the pump. Be sure all fittings are tight and all hose connections have proper clamps. If it is a belt-driven pump be sure belts are in good condition and tightened properly.

Next we check the vacuum controllers to be sure they are working and that there is adequate controller capacity. The controller capacity should at least equal pump capacity, but better yet, exceed pump capacity. There are several different types of controllers available; some need field engineering to operate successfully.

After checking pump and controller capacity we check the pulsator vacuum supply line for proper size and installation. The pulsator vacuum supply line should be at least 1¼ inches in diameter for up to four units, 1½ inches in diameter for five to seven units, 2 inches in diameter for eight to ten units, and 3 inches in diameter for more than 10 units. It is very important to have the pulsator vacuum supply line looped to a distribution tank or a header line and if a header line is used it should have twice as much capacity as the pulsator vacuum supply line. Also the pulsator vacuum supply line should be used for pulsators only. Weigh jars, vacuum operated doors, etc. should be on a separate line.

After we have done this we check the pulsators to be sure they are working properly. Many dairymen fail to keep pulsators in good condition. We have found pulsators that have been in use for as long as 3 years without any service. Pulsators need regular service; the diaphram and rubber parts need changing and cleaning on a routine basis. Short air tubes are inspected for defects such as cracks and cuts that let air into the system. Check milk inlets for damage; many times units have been dropped or kicked by a cow and the tip is bent, restricting milk flow. If any damage is here it must be fixed.

OBSERVE MILKING PRACTICES

The next step is to spend at least one milking time with the dairyman. During the complete milking you should be able to detect any weakness in the milking procedure.

Is the udder preparation adequate? Massage adequate for stimulation and let-down should be at least 20 sec with an additional delay of 60 sec before the milker unit is attached. Just before the milker is attached we sanitize the teat end with alcohol and collect a sample of milk from each teat in a sterile sample bag. These samples are iced and transported to the central laboratory for testing. They are tested for *Streptococcus agalactiae*

using a special medium, TKT/FC; *Staphylococcus aureus,* using telurite glycine agar; and somatic cells, using the Wisconsin Mastitis Test.

The milker cluster should be positioned so teats are perpendicular to the udder at all times; this can be done with a mechanical attachment that is designed to keep the proper alignment.

Timing of milker unit take-off is very important. Over-milking must be avoided, if teat ends are to stay healthy. Most dairymen over-milk their cows either as a result of trying to operate too many units or doing other chores while milking.

INTERESTED PERSONS MEET

When we have completed our observation and received test results on individual cow samples, we are ready for a group meeting with the farmer, veterinarian, extension dairyman, and milking machine serviceman. Each of these men play an important role in a successful program.

Cows that carry *S. agalactiae* infection are treated, sampled two weeks later, and any infection is treated again; on the third culture cows that still are infected are culled from the herd.

Using this approach we have been able to keep the mastitis in the herd under control and increase milk production as much as 40%. Milking time calls are a must in a good quality assistance program.

ACKNOWLEDGMENT

Presented at the 64th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Sioux City, Iowa, August 14-18, 1977.

One Milk Price Seen As Grade B Is Phased Out

Grade B milk producers are switching to Grade A milk production in increasing numbers. This will increase Grade A milk surpluses—and lower Grade A prices, says Truman Graf, University of Wisconsin-Madison agricultural economist, in a recently released study.

Graf feels that national or interregional federal milk marketing orders will be needed to cope with the differences in milk prices in different regions.

"There will eventually be only one farm price for Grade A milk as Grade B milk production declines—and eventually disappears. Grade B milk production dropped 12 percent during the past decade in the U.S. and declined by 22 percent in Wisconsin. Just over a third of Wisconsin's milk is presently produced as Grade B milk.

"Many small dairy farmers and dairy plants probably won't survive the switch to all Grade A milk production," Graf says.

The Chicago Regional federal milk marketing order used less than one-third of the 1976 milk produced for Class I fluid purposes. If the 6.9 billion pounds of Grade B milk produced in Wisconsin last year had been converted to Grade A milk, Graf says, the average blend prices would have declined by 11 cents per hundredweight (cwt.).

Gross income per producer would have declined by an average of $617, and the Class I utilization rate would have fallen to 19 percent.

"Most Grade B conversion to Grade A is occurring in the upper Midwest where Grade B milk supplies are heavily concentrated. Class I utilization rates and the Grade A blend prices are already relatively low in this area. More Grade A milk production in these areas will further lower the Class I utilization rates—and Grade A blend prices," Graf explains.

"With blend prices already low in the upper Midwest region, dairy farmers just won't accept the further declines in Grade A prices that will result from Grade B milk conversion. Extremely chaotic marketing conditions could result as milk from the upper Midwest either moves—or threatens to move—into other regions to bolster blend prices," Graf warns.

Graf sees the need for federal milk marketing order mergers and broader based pooling-pricing procedures to stabilize interorder blend prices and preserve orderly milk marketing.

The problems caused by Grade B conversion in the upper Midwest won't be just a regional problem. Milk can easily be moved long distances, and the surpluses can be exported to other regions where Grade A milk prices are higher.

Producers are switching from Grade B to Grade A milk production because of increasing Grade A-Grade B price differentials, larger dairy herds, and larger dairy plants.

The national difference between Grade A and Grade B milk prices increased by 16 cents per cwt. during the past decade. Graf expects increases in these differentials, which average 48 cents per cwt. in Wisconsin last year, while the sanitary standards between the two grades of milk decrease.

Farmers battling to lower production costs per cwt. are increasing the size of their dairy herds. Herd size increased 25 percent in Wisconsin and 42 percent nationally during the past seven years. This herd size increase encourages producers to switch to Grade A production to get the higher Grade A prices, Graf says.

Dairy plants too are increasing in size—and declining in numbers. While output per manufacturing plant has almost tripled nationally during the last 25 years, the number of plants has declined by almost two-thirds.

Larger plants are encouraging dairy farmers to convert from using cans to using bulk milk tanks,

(Con't. on page 323)

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(Con't. from page 321)

for plants can reduce costs if they can use only one form of delivery. More than nine out of 10 Wisconsin dairy farmers now have bulk milk tanks; Grade B producers with tanks can meet Grade A standards at little or no additional expense. Larger plants are also encouraging farmers to switch from Grade B to Grade A—costs can be further reduced with only one Grade of milk.

"As a result, Grade B producers are faced with a choice—either convert to Grade A milk, or face losing a market for their Grade B milk," Graf adds.

The increasing conversion to Grade A milk production will paradoxically add more Grade A milk to the market at the same time the demand for Grade A fluid milk is declining—and the volume of milk used to manufacture dairy products is increasing. Grade B conversion isn't needed to satisfy fluid needs. Instead, Grade A milk is being used to manufacture dairy products because it's underutilized for fluid purposes, Graf says.

"During the past decade, the volume of milk in the U.S. utilized for fluid purposes dropped by almost 4 billion pounds while the volume of milk used in manufacturing dairy products increased by almost 7 billion pounds. This will be recognized in farm milk pricing as conversion to Grade A continues," Graf adds.

Almost half of all dairy farms in the U.S. had fewer than 20 dairy cows in 1973, and many small Grade B milk producers may not be able to afford to convert to Grade A. If Grade B conversion leads to dairy plants that accept only Grade A milk, Graf says, it could mean the end of dairying for many farmers.

Complete conversion to Grade A milk could also force small unregulated manufacturing dairy plants out of business. They would have to pay Grade A prices to get milk for manufacturing purposes, but they can't afford to pay these higher prices unless they are able to develop "fluid outlets."

Plants that can't develop fluid outlets might not survive because farmers selling to these plants would be dissatisfied with the lower prices they receive. Neighboring farmers selling milk to plants with fluid outlets would receive high milk prices.

"Dairy farmers will eventually receive one price for milk. But the changes that lead up to this single price will bring many other problems. Dairy farmers should be thinking about the adjustments they're going to have to make when Grade B milk no longer exists," Graf concludes.

Copies of Graf's study can be obtained from the UW-Madison Department of Agricultural Economics, 316 Agriculture Hall, Madison WI 53706.

Coming Events

May 1-4, 1978. ADVANCE FOOD SANITATION WORKSHOP. Minneapolis, MN. Contact: Environmental Management Assn., 1701 Drew St., Clearwater, FL 33515.

May 7-12, 1978 SCHOOL OF ENVIRONMENTAL SANITATION MANAGEMENT. University of Illinois, Champaign, IL. Contact: Environmental Management Assn., 1701 Drew St., Clearwater, FL 33515.

May 9-11, 1978. 33rd ANNUAL PURDUE INDUSTRIAL WASTE CONFERENCE. Stewart Center, Purdue University, West Lafayette, IN. Contact: J. E. Etzel, Purdue Industrial Waste Conference, Civil Engineering Bldg., Purdue University, West Lafayette, IN 47907.

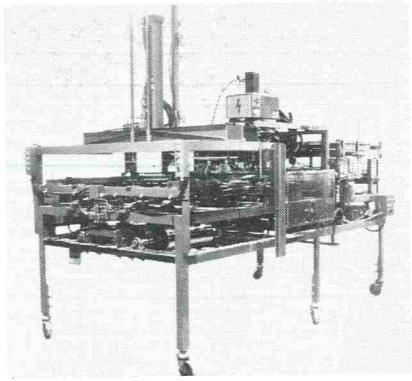
June 4-7, 1978. 1978 ANNUAL MEETING OF THE INSTITUTE OF FOOD TECHNOLOGISTS, Dallas, Texas. Contact: Dan Weber, Director of Convention Services, IFT, 221 N. LaSalle St., Chicago, IL 60601.

July 17-21, 1978. ADVANCES IN FOOD AND APPLIED MICROBIOLOGY. Massachusetts Institute of Technology, Cambridge, MA. Contact: Director of Summer Session, Rm. E19-356, Massachusetts Institute of Technology, Cambridge, MA 02139.

July 24-28, 1978. FOOD PROCESSORS BASIC MICROBIOLOGY SHORT COURSE. Cruess Hall, University of California, Davis. Contact: Dr. Robert J. Price, Food Science and Tech. Dept., University of California, Davis, CA 95616.

June 25-28, 1978. CANADIAN INSTITUTE OF FOOD SCIENCE AND TECHNOLOGY 21st ANNUAL CONFERENCE, Edmonton, Alberta. Contact: P. Jelen, Dept. of Food Science, University of Alberta, Edmonton, Alta. T6G 2N2.

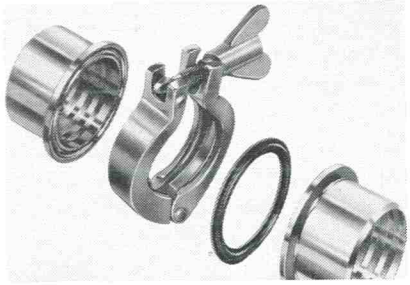
•*The new Model H-104 Double-Wall Trayformer* from R. A. Pearson Co. produces strong, heavy boxes with double-side walls and double-end walls. This new large frame trayformer turns out boxes to contain 80 to 120 lbs, of fresh meat . . . and, at the other end of the scale, produces small, strong boxes for items such as fresh



cherries. The H-104 has a capacity of up to 25 trays per minute. For complete information write: R. A. Pearson Co., W. 8120 Sunset Highway, Rt. 4, Box 65, Spokane, WA 99204.

•*The Bell-Mark Corporation* has recently introduced to the manufacturers and users of high speed packaging equipment, a revolutionary new concept for coding, dating, price marking and printing. The new UC-200-2 EP-M was designed for use in conjunction with any high speed, intermittant or constant motion, overwrap, form, fill and seal, bag making machine or slitter. It delivers registered imprints on any material web at speeds up to 350 imprints per minute. The UC-200-2 EP-M has its own variable speed motor, so no assistance from the parent machine is necessary. This means it will work well on very thin films, on wax covered films and sensitive packaging machines too. It also utilizes Bell-Mark's patented 150-2 cartridge ink system. That means prints are dry instantly on any surface and all ink mess is completely eliminated. Bolt on bracket kits are available for packaging machines manufactured by Sig, Hayssen, FMC, Cloud, Bartelt, Packaging Machine and Sheldahl. There are a number of installations now in the candy, bakery, seafood and frozen food industries.

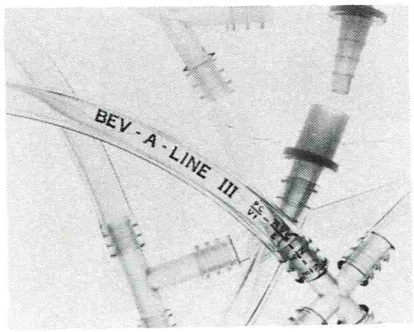
•*Quick-Connect*[tm] *stainless steel klamps* permit fast, dependable connections in 1" to 6" sanitary process tubing systems. Available in type 304 or 316 stainless, these easily assembled or disassembled klamps and fittings can be installed in new systems or adapted to existing systems with welded, flanged or threaded bevel seat components. Lightweight and heavy-duty models feature a jewel-like finish, comply with 3-A and U.S.P.H. standards and are adjustable without tools. Quick-Connect



klamps are also available for industrial applications in a variety of polishes to meet process requirements. Contact: Superior Stainless, Inc., P.O. Box 622, Delavan WI 53115.

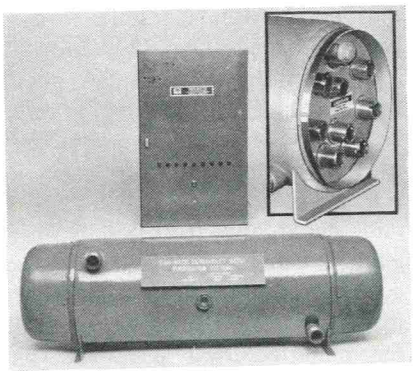
Product Potpourri

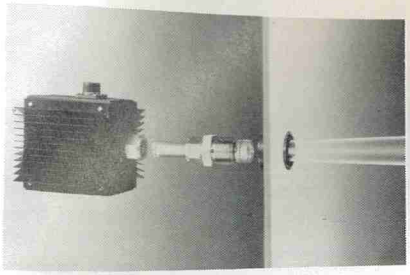
•*An economical line of translucent polypropylene* connectors, valves and adaptors, called Prop-A-Fit, is now available from Thermoplastic Scientifics, Inc., Warren, NJ, developers of patented BEV-A-LINE[R] lined tubing. Range of fittings includes "T", "Y", elbow and 4-way connectors as well as straight, reducer and "quick-disconnect" unions, check valves and Dura-Clamp flow valves. Prop-A-Fit connectors increase versatility and speed of set-up in laboratory and industrial tubing systems. Recommended for use with flexible tubing from 1/8"



to 5/8" ID, fitting ends are serrated and tapered to provide a tight grip on several different tubing sizes. Reusable and autoclavable, Prop-A-Fit connectors have excellent chemical and temperature resistance. They meet USP XIX. Class VI criteria, National Formulary, Vol. 14, standards, FDA standards for food and beverage industries and are accepted by USDA for use in meat and poultry processes. For free literature, write Thermoplastic Scientifics, Inc., 57 Stirling Road, Warren, NJ 07060.

•*Sani-Matic offers its Ultraviolet Water Purification System* as the finest, most acceptable system money can buy for controlled water quality and purity. Effective against bacteria, viruses, protozoans and spores, Sani-Matic water purification units are available from 2 GPM laboratory units to 200 GPM models. For volume applications higher than 200 GPM, two or more Sani-Matic purifiers may be manifolded together. There are no moving parts in the purification units. No special tools or training are required for installation. In-place-cleaning systems can be adapted to the Sani-Matic water purifiers, especially where water/solutions tend to coat quartz liners. 30,000 gallons of water can be purified for as little as a few cents depending upon local electricity rates. Sani-Matic units operate on common 110 volts-to 60 Hz power. Standard purifiers are manufactured with all water contact surfaces of stainless steel. For complete information write or call: The Schlueter Company, P.O. Box 548, Janesville, WI 53545 (608) 756-1266.





•*The HYL-80 Pressure Chamber Light* is a unit specially designed to transmit light across a wall/barrier through a small opening (1" pipe thread) in pressure vessels, chambers, autoclaves, etc., illuminating difficult, confined, hazardous gas or fire areas without encroachment on inside space. Easy accessibility permits bulb changing without opening the vessel or entering the room or area. The lighting system exposed to the process can be completely gas sterilized. Also, the light can be used in fluid flow piping since the light pipe does not protrude into the chamber or pipe. The HYL-80 is recommended by the manufacturer for lighting sterile rooms, silos, fermenting tanks, filters, holding tanks, sterilizers and filtration systems. For a free catalog write: Catalog No. HYL-80, J. M. Canty Assoc., Dept. 29, 117 Cornwall Ave., Tonawanda, NY 14150.

•*Accessory thremocouple probes* are now available from Transmation, Inc., for extending the temperature-measuring versatility of a plant's hand-held digital temperature indicators. A universal handle assembly, 12" long plus 36" flexible armored cable, connects the indicator Model 1060 or 1061 to a choice of four types of plug-in probes: 1) a needle probe for highly sensitive measurement of semi-fluid or fluid temperatures; 2) a bare wire probe for general use with liquids, ovens, furnaces, etc; 3) a filament probe for determining surface temperatures with extreme sensitivity and fast response; and 4) a bow type probe for fast, accurate sensing of temperatures at convex surfaces. Contact: Transmation, Inc., 977 Mt. Read Blvd., Rochester, N.Y. 14606.

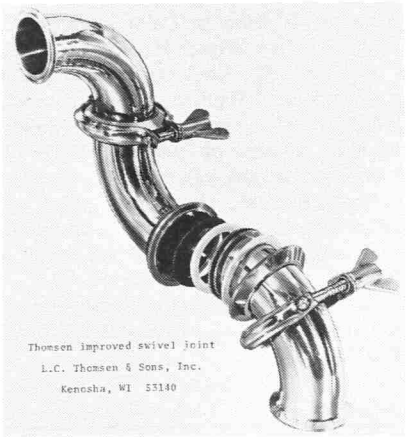
•*Automatic Controls & Systems, Inc.,* has acquired exlusive U.S. marketing rights for the new energy, money saving Hoval Recovery Unit. The HR unit is designed for easy installation on any building that exhausts heated or cooled air. Efficiencies are 55% to 90% heat recovery and 55% to 70% cooling recovery. To achieve these remarkable percentages while keeping the product cost and its operation cost low the unit features a unique lightweight, air cross flow exchanger element and a diversity of unit sizes. Additional savings may be realized because simple installation requirements allow building maintenance crews to easily and quickly install the units themselves. Units may be installed over existing ventillation openings. This system is used to advantage in factories, warehouses, stores, production bays, machine shops, laundaries, dye works, garages, bowling alleys and other sports building-any building that exhausts heated or cooled air. Descriptive literature and technical data available from Automated Controls & Systems, Inc., 500 East Higgins Road, Elk Grove, IL 60007.

•*Chromatography adsorbents,* accessories and biochemical kits for today's chromatography procedures are described and illustrated in new 36-page catalog. Often-asked questions about ICN aluminas and silicas are answered. Various chromatographic techniques are reviewed, from open (dry) column and thin-layer to high pressure and high performance liquid chromatography. Affinity (Liquid Exclusion) chromatography for analytical and preparative LC is also discussed. Applicable adsorbents, their specifications and prices are included. TLC and column chromatography accessories, plus biochemical kits are catalogued and priced. Bulk applications for aluminas and silicas in batch processing are reviewed. And a handy list of available reference abstracts are listed. This catalog is available from ICN Pharmaceuticals, Inc., 26201 Miles Road, Cleveland, Ohio 44128.

•*Aqua-Purometer II ph/L-1p,* a new process-fluid pH system, designed to consistently provide control and alarm action when preset limits are reached, was announced by McNab, Inc. According to McNab, the Aqua-Purometer II has an exclusive L-1p fitting which allows the pH probe to be quickly inserted into, or removed from, the process stream without plant shutdown. The solid state, integrated circuit, 3-range indicator AP II system is useful in a wide variety of acid and base concentrations commonly encountered on manufacturing lines. For information contact McNab, Inc., 20 North MacQuesten Parkway, Mt. Vernon, NY 10550.

•*Shipping and storage tanks* built of tough, thick polyethylene to take the roughest use and abuse are now available from United Utensils Co., Inc. When empty, the tanks are designed for nesting inside each other, thereby saving floor space. When full, a special high-strength cover enables the tanks to be stacked. Floor clearance permits forklift entry from all four sides. For additional information contact: R. Malkin, United Utensils Co., Inc., Yennicock Ave., Port Washington, NY 11050.

•*A line of improved swivel joints* is being marketed by L. C. Thomsen and Sons. The joints assure smooth, flawless, leakproof filling operations when transferring product from one container to another in food, beverage and chemical plants. Made of sanitary, polished stainless steel construction, the joints have ultra smooth inside and outside surfaces. They are easy to disassemble for cleaning, and have 100% CIP capability. The swivel joints have teflon bearing surfaces for smooth action. They are available with Acme thread, clamp or butt weld ends for welding into existing assemblies. For more information contact: L. C. Thomsen and Sons, Inc., 1303 Forty Third St., Kenosha, WI 53140.



•*Tri-Cell H.E.P.A. Filters* are now available from Tri-Dim Filter Corporation. Each filter is constructed from one continuous sheet of highly efficient glass microfiber paper in four different DOP efficiencies . . 95%, 99.97%, 99.99% and 99.999%, with a single corrugated aluminum separator placed between each pleated sheet of media to prevent squeezing together. This allows the largest possible filtering surface. Tri-Cell H.E.P.A. filters will withstand temperatures up to 250°F (custom ordered may be increased to 800°F) and 100% RH. Tri-Cell filtering applications are many and diverse depending on the contamination control required such as factory, office and hospital air systems, as well as assembly areas including aerospace, microelectronics, research laboratories, food and beverage processing plants and pharmaceutical packaging systems. For information write: Tri-Dim Filter Corp., P.O. Box 437, 85 Wagaraw Rd., Hawthorne, NJ 07506.

Applied Laboratory Methods Committee

Committee Objectives:

To review the efficacy of microbiological and chemical methods for the examination of milk, food, water and other environmental samples, conduct comparative and collaborative studies to determine the precision and accuracy of new and/or modified methods and make recommendations for the use of such methods which have a relationship to standards of public health significance. Publish results of studies in the Journal of Food Protection.

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Kenneth W. Whaley, State of Tennessee, Department of Public Health, Nashville, TN 37219.

Journal Management Committee

Committee Objectives

(1) Make a continuing review and evaluation of the mechanical makeup of the Journal of Food Protection. *Make recommendations for changes as appropriate. (2) Evaluate the needs of the membership and their desires about Journal content. Make recommendations as to how these needs could be better satisfied. (3) In case of dispute over publication of a paper, the Committee will provide the editor with recommendations as to the suitability of the paper for publication in the Journal.*

Committee Members

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Mr. Donald Raffel, Wisconsin Department of Agriculture, 4702 University Avenue, Madison, Wisconsin 53705.

Mr. Robert L. Sanders, HFF-415, Food and Drug Administration, 200 "C" Street, S.W., Washington, D.C. 20204.

Committee on Communicable Diseases

Committee Objectives:

To prepare and revise manuals on procedures to investigate foodborne- and waterborne-diseases outbreaks, including step-by-step investigative procedures, investigational forms, and a listing of foodborne diseases of contemporary importance.

To study problems related to those diseases communicable to man through the consumption of foods, including milk and milk products, meat poultry, and shellfish, and to recommend specific measures that can be taken by the sanitarian to control such diseases.

Committee Members

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Dr. E. C. Todd, Food Reseach Laboratory, Health Protection Branch, Health and Welfare, Canada, Ottawa, Ontario K1A OL2 Canada.

Journal Foodservice Food Protection Committee

Committee Objectives:

To explore newsworthy situations and events in the foodservice industry and to make recommendations as to the publication of appropriate articles in the Journal of Food Protection; *providing practical information and insight into areas of concern to the foodservice industry.*

Committee Members:

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K. J. Baker, FDA, 200 C. St. S.W., Washington, D.C. 20204.

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Fred Hegele, General Mills, Inc., Minneapolis, MN 55440.

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James C. White, Cornell University, Ithaca, NY 14853.

Dr. Oscar Snyder, University of Minnesota, St. Paul, MN 55108.

Baking Industry Sanitary Standards Committee

Committee Objectives

To participate in the revising and updating of the BISSC standards, by serving as public health representatives on task committees assigned to present drafts of revision to the general BISSC committee and serve as consultants to industry groups regarding the public health aspects of equipment, design, construction and installation.

Committee Members

Martyn A. Ronge, Chairman (Ill. Assoc.) Harold Wainess & Assoc., 464 Central Ave., Northfield, Ill. 60093.

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Tom Rolfes, Director of Sanitation (N.Y. Assoc) Continental Baking Co., P.O. Box 731, Rye, N.Y. 10580.

Sanitary Procedures Committee

Committee Objectives:

To participate jointly with the Sanitary Standards Sub-committee of the Dairy Industry Committee and the Milk and Food Branch, U.S. Public Health Service, in the formulation of 3-A Sanitary Standards for dairy equipment. Specifically the functions of this committee are: (1) to receive, consider, and comment on proposed sanitation standards for dairy equipment submitted by the Dairy Standards Sub-committee; (2) to bring to the attention of the Sanitary. Standards Sub-committee items of dairy industry equipment and methods for which formulation of sanitary standards appear desirable; and (3) to cooperate with the Dairy Industry Committee, the U.S. Public Health Service, and health officials in attaining universal acceptance of the sanitary standards upon which mutual agreement has been reached.

Committee Members

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Carl Kroppman, Division of Dairy Industry, Florida Dept. of Agriculture and Consumer Services, Tallahasse, FL 32304.

Food Equipment Sanitary Standards Committee

Committee objectives

(1) To Cooperate with other interested health organizations and related industries in the formulation of sanitary standards and educational materials for the fabrication, installation, and operation of food equipment and food vending machines. (2) To aid the food and vending industry in improving the design, construction and installation of food equipment so that it will lead to easy cleaning and proper functioning when it is placed into service. (3) To cooperate with the food industry in the preparation of standards or guidelines which public health agencies will accept, thereby securing uniformity in the manufacture and nationwide acceptance of such equipment. (4) To present to the IAMFES membership those standards and educational materials which the Committee recommends be endorsed by the Association.

Committee Members

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David J. Hodgson, Chief, Div. of Food Service Sanitation, Michigan Dept. of Public Health, 3500 N. Logan, Box 30033, Lansing, MI 48909.

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Harold Wainess, Harold Wainess & Associates, 464 Central Ave., Northfield, IL 60093.

Professional and Educational Development

Committee Objectives:

(1) To development plans to devise methods whereby the Sanitarian can more fully gain recognition as a professional worker in public health, and (2) to recommend standards of education, training and experience designed to establish desirable professional qualifications to the end that the title Sanitarian will denote adequate preparation for professional work and attainment.

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Charles White, Dean Foods Co., 1126 Kilburn Ave., Rockford, IL 61101.

Farm Methods Committee

Committee Objectives

To study dairy farm methods and procedures, to determine the sanitary problems involved, and to make recommendations for the solution of such sanitary problems, and for the improvement of dairy farm methods which have a relationship to the sanitary quality of milk.

Committee Members

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Cleaning and Sanitizing of Farm Milk Equipment Subcommittee

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Standardization of Procedures for Uniform Inspection and Recommendations for Mastitis Prevention and Control Subcommittee

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Vice-Pres., Terry Brandenberg ---------- Eau Claire
Treas., Marge Uebele ---------- Eau Claire
Sec'y., Linda Rott, 457 Summit Ave., Eau Claire, WI 54701

Notice to Affiliate Representatives

Check to make sure the names in your affiliate listing are current. If they are not, please send the proper names to: Journal of Food Protection, P.O. Box 701, Ames, IA 50010.

Affiliate Meetings

Arizona Association of Milk, Food and Environmental Sanitarians, June 10, 1978, Prescott, AZ. Joint with NEHA & NSPS affiliates.

California Association of Dairy and Milk Sanitarians, November 7-9, 1978 at Griswolds in Claremont, CA.

Connecticut Association of Dairy and Food Sanitarians. January 25, 1979*

Associated Illinois Milk, Food and Environmental Sanitarians. December 4, 1978* Blue Moon Restaurant, Elgin, IL. "Our New Environment"

Kansas Association of Sanitarians. August 13-17, 1978. Kansas City, MO (in conjunction with IAMFES Annual Meeting).

Minnesota Sanitarians Association. September 14-15, 1978, Sheraton Inn Northwest, Minneapolis, MN

Missouri Association of Milk and Food Sanitarians. August 13-17, 1978. Kansas City, MO (in conjunction with IAMFES Annual Meeting)

New York Association of Milk and Food Sanitarians. September 20-22, 1978. Stevensville Country Club, Swan Lake, NY

Oregon Association of Milk and Food Sanitarians. December 5, 1978.* Salem Oregon

South Dakota Environmental Health Association. May 17-19, 1978. Brookings, SD, Holiday Inn

*tentative

CLASSIFIED AD

For Sale

Single Service milk sample tubes. For further information and a catalogue please write, Dairy Technology, Inc., P.O. Box 101, Eugene, Oregon 97401.

Index to Advertisers

THE ONLY *Approved*
SANITARY METHOD OF APPLYING
A U. S. P. LUBRICANT
TO DAIRY & FOOD
PROCESSING EQUIPMENT

Haynes Spray

U. S. P. LIQUID PETROLATUM SPRAY
U.S.P. UNITED STATES PHARMACEUTICAL STANDARDS

CONTAINS NO ANIMAL OR VEGETABLE FATS. ABSOLUTELY NEUTRAL. WILL NOT TURN RANCID — CONTAMINATE OR TAINT WHEN IN CONTACT WITH FOOD PRODUCTS.

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ODORLESS—TASTELESS
NON-TOXIC

This Fine Mist-like
HAYNES-SPRAY
should be used to lubricate:

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HOMOGENIZER PISTONS — RINGS
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CAPPER SLIDES & PARTS
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and for ALL OTHER SANITARY MACHINE PARTS which are cleaned daily.

The Modern HAYNES-SPRAY Method of Lubrication Conforms with the Milk Ordinance and Code Recommended by the U. S. Public Health Service

The Haynes-Spray eliminates the danger of contamination which is possible by old fashioned lubricating methods. Spreading lubricants by the use of the finger method may entirely destroy previous bactericidal treatment of equipment.

PACKED 6-12 oz. CANS PER CARTON
SHIPPING WEIGHT—7 LBS.

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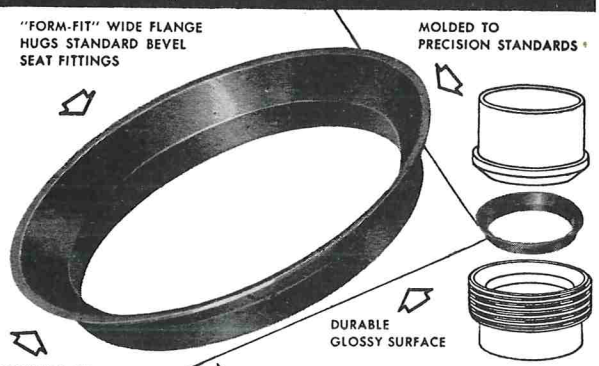

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Check these SNAP-TITE *Advantages*

- Tight joints, no leaks, no shrinkage
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- Help overcome line vibrations
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SIZES 1" - 1½" 2" - 2½" - 3" - 4"

*MADE FROM **TEFLON®**

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THE IDEAL UNION SEAL FOR BOTH VACUUM AND PRESSURE LINES

Gasket Color . . . slightly off-white

SNAP-TITE self-centering gaskets of TEFLON are designed for all standard bevel seat sanitary fittings. They SNAP into place providing self-alignment and ease of assembly and disassembly.
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FOR A FITTING GASKET THAT WILL OUT-PERFORM ALL OTHERS...

Specify . . . HAYNES SNAP-TITES of TEFLON

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* Gaskets made of DuPont TEFLON® TFE-FLUOROCARBON RESINS

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All Lubri-Film ingredients are approved additives and can be safely utilized as a lubricant for food processing equipment when used in compliance with existing food additive regulations.

ESPECIALLY DEVELOPED FOR LUBRICATION OF FOOD PROCESSING AND PACKAGING EQUIPMENT

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SPRAY — PACKED 6 — 16 OZ. CANS PER CARTON
TUBES — PACKED 12 — 4 OZ. TUBES PER CARTON

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CLEVELAND, OHIO 44113

Make Your Cows Worth More.

Dr. Allan Bringe
Professor, Dairy Science
University of Wisconsin
Madison, Wisconsin

Efficient production of clean, natural-tasting milk which will be in demand by consumers, should be every dairyman's goal. The milking operation and care of your herd should have the highest priority because a full harvest of quality milk will mean more income to you. Dairymen can take advantage of current knowledge and technology to achieve this goal, and make better use of their time while earning more profit.

DHI Production Records

These tools are essential for measurement of production to make feeding, breeding, and culling decisions. Use records to detect and correct weaknesses in herd management. You won't know which cows are worth more unless production is measured. Ideally, your milking equipment should have provisions for obtaining DHI milk weights and samples.

Identify Cows With Hidden (Sub-Clinical) Mastitis

The invisible loss of milk for each infected quarter is more than a thousand pounds per year. You need some routine method of identifying infected cows early – before you can see clinical mastitis. Each cow can be monitored for mammary infection by:

1. Somatic cell report in DHI programs.
2. California mastitis test.
3. Bacteriological culturing.

Once infected cows are identified, you and your veterinarian can make management decisions regarding proper handling and treatment. When cows become infected with sub-clinical mastitis you should also play the role of a detective to determine the cause and correct the situation that caused the new infection. Mastitis can be kept under control with the following measures:

1. A strict sanitation program.
2. Proper installation, maintenance, and use of milking equipment.
3. Using recommended procedures including teat dipping.
4. Proper treatment of infected quarters. (Select antibiotics for treatment of infected quarters based on previous culturing and sensitivity testing.)
5. Culling.

Dairymen attempting to control mastitis by treatment alone will always be in trouble.

Routine Milking Machine Service

You are milking 1977 model cows, bred for high milk production. That means your milking equipment needs to be up to 1977 operating standards. Just because your milking machine starts running when you hit the switch, doesn't mean that it's operating properly. Schedule your equipment for routine service by a competent milking machine serviceman. Make sure that pulsation, vacuum control, vacuum pump, inflations, and other essential parts are functioning correctly. Don't guess. Check equipment performance when all units are milking the highest producing cows. Remember, your milking equipment operates more hours than any other piece of farm equipment, and it's the only equipment that operates on living tissue.

Provide The Environment For Healthy Calves

Proper environment, care and attention is essential to raising healthy calves. Poor calf care allows scours and pneumonia to pre-cull many genetic assets from your herd and its future productivity. Genetically superior calves, raised in a healthy manner, give the dairyman an opportunity to cull more selectively and eliminate mastitis problem cows.

This, in turn, helps prevent the spread of pathogenic organisms throughout the herd.

Managed Milking Procedure

Plan the best sequence for proper cow milking. Even the best milking system cannot achieve maximum production and avoid udder irritations unless proper milking procedures are followed. The milker's attitude and desire to consistently milk properly is essential. Handle cows gently so they associate milking with a pleasant experience. The preparation and stimulation of the udder is important to saving milking time, obtaining more milk, and reducing teat and udder irritation. Attention to the important routine of sequencing stimulation, time of machine attachment, and proper machine removal will pay big dividends. The challenge is to control procedures so they are properly performed when milking each cow in the herd, regardless of her characteristics. This can be accomplished equally well in a stanchion barn or fully automated milking parlor. The rewards will be better use of your time, better herd health and more profit. You can make your cows worth more!!