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# MILK and FOOD TECHNOLOGY

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# THE EFFECT UPON BACTERIAL SURVIVAL OF SANITIZER RESIDUES ON A SOILED SURFACE

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(Received for publication September 16, 1969)

# Abstract

A method is described in which bacteria are brought into contact with sanitized films of sterile skimmilk. Five different commercial sanitizers at six concentrations were used and tested against five strains of bacteria. None of the sanitizers was found to be significantly effective for rendering milk films bactericidal at the 0.01% level of application.

The iodophore and quaternary ammonium compound tried were found to be the most effective by producing bactericidal conditions at the 0.1% level of application and the hypochlorites appeared the least potent. The activity of chlorinated tri-sodium phosphate appeared to be influenced to a greater extent by the Gram type of the bacteria than the other sanitizing agents. Prolonged exposure of the bacteria enhanced the destructive influence of all five sanitizers.

A recent study (10) on the agreement between bacterial numbers in milk and the sanitary condition of equipment used to produce it brought to light some disquieting sanitary practices that appear to warrant further investigation.

In several instances, milk producers whose milking equipment was visibly very dirty but whose milk yielded satisfactory bacterial counts were following the practice of substituting a strong sanitizing treatment with chemical sanitizing agents for wash-These observations brought to mind an experience of some 15 years ago which had been dismissed as "one of those things" where a farmer admitted he had not washed his milking machines for 17 years and had not in that time experienced any trouble with high bacterial counts. He had contented himself with a "good" sanitizing using a popular brand of sodium hypochlorite. The encrustation of milk-stone upon his milker buckets bore mute but compelling testimony to his veracity but dealt an equally severe blow to the generally accepted theory concerning the effect of organic matter on the efficacy of hypochlorite.

These observations indicate that certain sanitizers in association with milk residues can control bacterial multiplication. Presumably this is accomplished by the destruction of those bacteria associated with the residue before application of the sanitizer as well as those that gain access to it afterwards.

Much work has been reported in the literature concerning the effect of organic matter upon sanitizers, particularly the hypochlorites, which can be nicely summed up in the words of Davis, 1968 (4): "Many thousands of experiments have been made on this aspect and it has been clearly established that if all soiling matter is not removed, chemical sterilization will be a failure  $\ldots \ldots$ ".

Most of the work reported concerning the effect of organic matter upon sanitizers has dealt with the addition of the organic material to the sanitizing solution and then noting the decrease in the availability of the agent. On the other hand considerable effort has been expended in studying the effects of organic films placed upon surfaces (2, 6, 8, 9).

Working with films, it is interesting to note that Johns, in 1948, (9) commented on the residual effect of hypochlorite when applied to films of inoculated milk on the walls of a vat. He stressed the fact that his results failed to show the expected reduction in the germicidal activity of both hypochlorite and quaternary ammonium compound in the presence of organic matter. Two years earlier, in 1946, Wolfe and Cousins (14) commented upon the residual germicidal effect caused by formation of chloramines from the action of hypochlorite on milk films.

Cox and Whitehead, in 1949 (3), studied the effect against *Streptococcus cremoris* of chloramino compounds formed by the interactions of hypochlorite and proteins. They considered the noted germicidal effect too weak to be of any practical significance.

The preceding studies indicate that sanitizers, when applied to organic matter, retain some of their germicidal activity. They also show that this activity may result directly from the sanitizer residue or from combination with the organic material. Any such behaviour on the part of sanitizers with organic matter could have considerable effect when bacterial counts made upon raw milk are used to estimate its hygienic quality. Further, the significance of such germicidal activity would be emphasized under conditions where bacterial multiplication in raw milk was limited by efficient cooling. In such instances a reduction, in the milk, of its initial contamination arising from soiled equipment could have a profound effect upon the final bacterial count and hence the estimate of its quality. The premise that bacterial counts of well cooled milk reflect the care and

cleanliness of its production is based on the belief that there is a considerable reduction in the activity of chemical sanitizers if applied to improperly washed equipment. The literature casts some doubts upon this belief and conjures up some questions as to whether different sanitizers behave alike toward organic matter as well as the role concentration may play in their behaviour. The following work was undertaken to provide some answers to these questions.

# MATERIALS AND METHODS

Many trials and changes in technique were made before reasonably reproducible results were obtained and a standardized method evolved.

Essentially, the method employed was to spread a sanitizing solution upon a dried sterile milk film, allow it to dry, and then spread a bacterial suspension upon the treated film which was then kept moist for an appropriate time interval before a culture medium was poured over it. Following incubation, the colonies developing in the medium were counted and compared in number with those arising from another milk film which had not been treated with sanitizer.

To make the milk film, 0.2 ml of sterile skimmilk was spread uniformily over the surface of a dry sterile glass slide  $(4 \times 8 \text{ cm})$  contained in a sterile petri dish. Skimmilk was used to avoid the complications that might arise from fat in heat-sterilized whole milk. The milk was sterilized by intermittent sterilization at 100 C for 30 min on 3 successive days. The milk film was dried on a level surface in a 45 C warming oven. When dry, a second 0.2 ml application of the same milk was added to it, spread over its surface, and dried again. When dry, an appreciable film of skimmilk was left upon the surface of the glass; more than would be encountered on even a poorly rinsed surface under practical conditions. Films prepared in this manner were ready for application of the sanitizing solution.

Five commercial sanitizing agents were used: an iodophore, sodium and calcium hypochlorite, a chlorinated tri-sodium phosphate preparation, and a quaternary ammonium compound (QAC). All of these preparations were in practical use among milk producers in the Guelph area.

A relatively wide range of concentrations was used to bracket those used in practise and to provide figures to measure the effect of concentration. Six concentrations were tried: 0.002% (20 ppm), 0.005% (50 ppm), 0.01% (100 ppm), 0.05% (500 ppm), 0.1% (1000 ppm), and 1% (10,000 ppm).

Stock solutions at a concentration of 1% of the active agent of the various sanitizers were prepared with distilled water. For the halogen-containing compounds, which were freshly prepared for each experiment, the concentration was checked by titration against sodium thiosulfate (1) and the concentration of QAC checked by the method of Harper et al. (5). Concentrations for use were prepared by the appropriate dilution of the stock solution with sterile distilled water.

The sanitizer solution was applied to the prepared milk films by adding 0.2 ml and spreading it carefully over the surface of the film which was then allowed to dry. This procedure was followed for all concentrations except for the control slides when the addition of sanitizer was omitted.

Three genera of gram-negative and two of gram-positive bacteria were studied. These organisms, which had been originally isolated from milk, were obtained from the Department of Microbiology, University of Guelph. They were

Escherichia coli, Micrococcus freudenreichii, Pseudomonas fluorescens, Sarcina lutea, and Serratia marcescens.

Suspensions of bacteria for inoculating the sanitized milk films were prepared by picking a portion of the growth from 24 hr slants into sterile phosphate-buffered distilled water (1). Care was taken to limit the final suspension to give a colony count of about 1000 per ml.

Inoculation was accomplished by adding 0.2 ml of the bacterial suspension to the sanitized milk film and spreading the inoculum carefully over it by means of a sterile bent glass rod. At the time of inoculation two moist circles of filter paper were placed in the lid of each petri dish containing the inoculated slides to prevent the inoculum from drying out.

For each concentration of a sanitizer, four sanitized milk films and four non-sanitized film controls, were inoculated.

Two minutes after the inoculation, two sanitized milk films and two controls were flooded with melted Standard Methods Agar (1) at 45 C, swirled gently, and the medium allowed to set. After the medium had gelled, the dishes were placed in an incubator, and held at the temperature appropriate for the organisms under study; 25 C for S. *lutea* and 35

TABLE 1. THE SURVIVAL RATIOS OF FIVE GENERA OF BACTERIA IN THE PRESENCE OF SIX CONCENTRATIONS OF FIVE SANITIZING AGENTS. EXPOSURE PERIOD 2 MINUTES.

		Survival	ratios of	bacteria		
Sanitizer concentration (%)	P. fluorescens	S. marces- cens	E. coli	S. lutea	M. freuden- <b>reic</b> hii	
Iodophore						
.002	0.74	1.49	1.00	1.12	1.06	
.005	1.27	0.99	0.85	0.93	1.25	
.01	1.05	0.92	1.10	0.95	1.14	
.05	0	0.68	0.97	0.41	0	
.10	0	0.02	0	0.10	0	
1.00	0	0	0	0	0	
QAC						
.002	1.08	1.52	1.12	1.46	0.77	
.005	1.55	1.02	0.79	1.04	0.92	
.01	0.97	1.22	0.94	1.17	1.00	
.05	0.45	1.35	1.02	0.03	0	
.10	0	0	0.77	0	0	
1.00	0	0	0	0	0	
Sodium hypo	chlorite					
.002	0.89	1.68	0.87	0.86	0.91	
.005	1.00	1.07	1.18	1.28	0.80	
.01	0.54	0.90	0.94	1.20	1.54	
.05	0.75	1.35	0.79	1.09	1.70	
.10	0.25	1.19	0.86	0.95	1.31	
1.00	0	0.23	0	1.24	0.96	
Calcium hyp	ochlorite					
.002	1.11	1.43	0.74	0.86	1.15	
.005	0.83	1.54	1.57	0.74	1.01	
.01	0.87	0.75	1.39	0.92	1.00	
.05	1.08	1.27	0.85	0.96	1.07	
.10	0.87	1.19	0.57	0.83	1.37	
1.00	0.10	0.49	0.55	0.77	0.02	
Chlorinated	TSP					
.002	1.25	1.16	0.87	1.05	0.81	
.005	1.85	0.67	1.23	0.83	0.72	
.01	1.25	1.46	0.97	0.84	1.07	
.05	0.04	0.68	1.00	1.29	1.91	
.10	0	0.21	0.29	1.00	0.91	
1.00	0	0	0	0.85	1.01	

TABLE 1a. The survival ratios of five genera of bacteria in the presence of six concentrations of five sanitizing agents. Exposure period 4 hours.

		Survival	ratios of	bacteria	
Sanitizer concentration (%)	P. fluorescens	S. marces- cens	E. coli	S. lutea	M. freuden- reichii
Iodophore					
.002	0.84	1.64	0.94	1.08	0.73
.005	0.72	1.05	1.35	0.92	0.81
.01	1.00	1.25	2.25	0.96	0.61
.05	0.08	0.84	1.02	0.03	0
.10	0	0.02	0	0	0
1.00	0	0	0	0	0
QAC					
.002	0.98	0.94	1.14	1.96	1.86
.005	1.52	1.03	1.27	1.16	1.23
.01	1.41	1.29	0.88	1.14	0.38
.05	0.80	0.51	0.68	0.02	0
.10	0.18	0	0.27	0	0
1.00	0	0	0	0	0
Sodium hypod	chlorite				
.002	1.46	1.44	0.66	1.30	0.60
.005	0.65	0.59	0.94	0.97	1.43
.01	0.93	1.16	0.84	1.21	0.85
.05	0.68	0.51	0.45	0.43	0.74
.10	0.87	0.59	0.47	2.04	0.65
1.00	0	0 0		1.33	0.26
Calcium hype	ochlorite				
.002	0.73	1.78	1.56	1.30	1.50
.005	0.91	0.58	1.33	1.27	0.85
.01	0.68	1.11	1.04	0.77	1.16
.05	0.56	1.04	0.85	1.08	0.90
.10	0.76	0.59	0.53	0.91	0.48
1.00	0	0	0	0.91	0
Chlorinated T	'SP				
.002	1.00	1.46	1.30	1.02	0.61
.005	0.51	1.48	1.33	0.85	0.32
.01	0.69	0.71	1.02	1.07	0.55
.05	0.04	0.36	0.33	1.16	1.07
.10	0	0	0.02	1.08	0.46
1.00	0	0	0	0.18	0

C for the others. The 2 min interval was used to measure the early response of the organism toward the sanitized film.

The remaining dishes containing inoculated films were held at the incubation temperature of the organism for 4 hr before they were flooded with medium and incubated as previously described. This interval between inoculation and adding the medium measured the effect of more prolonged exposure of the organism to the sanitized films.

All films were incubated for 3 days before the colonies developed were counted, Where few or no colonies had developed in 3 days, a further 24 hr incubation was given.

# RESULTS AND DISCUSSION

Exploratory trials performed to establish a suitable method for this study yielded somewhat chaotic results which did suggest a pattern or trend of effects that appeared promising. The final series of experiments will be reported here.

The effect of the sanitizer residue upon exposed

bacteria was measured by dividing the averaged results from the nonsanitized milk films, controls, into the averaged counts of the sanitized milk films. This ratio will be referred to as the survival ratio.

Results are tabulated according to exposure periods in Tables 1 and 1a. A trend in the effect of increasing concentration of the sanitizing agents on bacterial survival is manifest from the data in these tables. A closer scrutiny of the survival ratios shows a considerable variability of response on the part of the different organisms towards the type of sanitizer as well as to concentration. For instance, the three chlorine-bearing sanitizers show quite a marked difference in activity when compared with the iodophore or QAC. Again, a more erratic response toward the chlorine products was exhibited by the different organisms than was true with the other two materials.

An analysis of variance (12) was used to test the significance of the effect produced upon the five bacterial types by the sanitizers and their various concentrations. The results from these analyses are shown in Table 2. They confirm the impression already obtained from Tables 1 and 1a. A highly significant difference in effectiveness between the various sanitizers is shown and, too, that this difference is not changed by exposure time. The most significant difference in effectiveness was shown between the concentrations of sanitizers and here, by virtue of the heightened significance, the influence of prolonged exposure of the organisms to the sanitizers is apparent. It also will be seen there was a significant difference in response between organisms after the 4 hr exposure period which was not apparent at the shorter interval.

TABLE 2. ANALYSIS OF VARIANCE BETWEEN THE EFFECTS OF FIVE SANITIZING AGENTS AT SIX CONCENTRATIONS ON FIVE DIFFERENT TYPES OF BACTERIA AT TWO EXPOSURE TIMES. DATA FROM TABLES 1 AND 1a.

	Significance						
Source	D. <b>F</b> .	S.S.	M.S.	F.	5%	1%	
Total	149	36.9535					
Organisms	4 4	0.9009	0.2252	2.08	2.44	$3.47 \\ 3.47 \\ 3.17$	
Sanitizers		6.2252	1.5563	14.40	2.44 2.29		
Concentrations	5	15.1233	3.0247	27.98			
Within	136	14.7041	0.1081	1	4 2		
		4 hr	-		Signific	ance	
Source	D.F.	<b>S.S</b> .	M.S.	F.	5%	1%	
Total	149	43.3797					
Organisms	4	1.5597	0.39	3.85	2.44	3.47	
Sanitizers	4	5.9230	1.4808	14.04	2.44	3.47	
Concentrations	5	22.1060	4.4212	43.60	2.29	3.17	
Within	136	13.7910	0.1014				





Gram-negative bacteria



Figure 2. Effect of QAC at various concentrations upon gram-negative and -positive bacteria.

It is surprising that a greater variation between the resistance of organisms toward the sanitizers was not encountered because such differences have been noted by Johns (9), McCulloch et al. (11), and others. Again the relationship of the Gram reactions of the microflora of milk to the sanitary state of the milk-handling equipment shown by Jackson (7) and by Thomas (13) apparently indicates an influence upon bacterial types resulting from the presence of organic

### matter.

However, when the data from this study were analyzed for the variance caused by the Gram reactions of the bacteria, it was found to be insignificant. Graphical representations of the data were made for each sanitizer by plotting its concentration against the mean survival ratios of both gram-negative and -positive bacteria and these will be found in Fig. 1 through 5.

Figure 1, illustrating the effect of iodophore on bacteria, indicates that this agent started to exert its action at the 0.01% level and apparently stopped all multiplication at 0.1%. Essentially the same pattern of effect prevailed for both types of organisms. A similar picture is presented by QAC, Fig. 2; here it is noteworthy, that despite a common belief to the contrary, very little difference is shown in its activity toward either gram-negative or -positive organisms. The effect of time of exposure is shown for these two germicides by their increased effectiveness at somewhat lower concentrations with increased time.

The two hypochlorites, Fig. 3 and 4, showed a marked erratic response particularly with the grampositive organisms and, though lengthened time of exposure showed some improvement in effectiveness, it was not as pronounced as recorded in Fig. 1 and 2. Results shown here for hypochlorite confirm the findings of Cox and Whitehead (3) regarding the feeble activity of this material in association with milk proteins. The weak effect that they noted would be even more apparent because they were



Figure 3. Effect of sodium hypochlorite at various concentrations upon gram-negative and -positive bacteria.



Figure 4. Effect of calcium hypochlorite at various concentrations upon gram-negative and -positive bacteria.





using gram-positive organisms in their study.

The chlorinated TSP showed the most pronounced specificity towards type of organism as will be seen in Fig. 5.

As was to be expected, not all sanitizers were equally effective against bacteria when associated with organic matter. Accordingly, of those tried, the iodophore, QAC, chlorinated TSP, and sodium and calcium hypochlorite compare in that order of activity. From these results it is apparent that there is little chance of a dairy farmer getting low bacterial counts in his milk if he uses chlorine compounds at the recommended levels of 100 to 200 ppm to sanitize soiled equipment. However, one who is reckless with an iodophore might be able to substitute sanitizing for washing as was noted in the introduction to this work.

Finally, these results suggest a study to find whether milk residues can accumulate the active agent from repeated applications of sanitizer in which instance soil treated with hypochlorite may become germicidally potent.

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# Abstract

The U. S. Public Health Service and the National Conference on Interstate Milk Shipments have agreed to require that producers meet standards for the abnormal milk program. Effective July, 1, 1970, all raw milk shall contain less than 1,500,000 leucocytes per milliliter. The regulations of various states now require that qualified personnel in approved laboratories make total bacterial plate counts on all raw supplies at least once a month. A proposed modified direct microscopic somatic cell count can effectively be used as a rapid screening test by the same personnel, eliminating the need for other screening tests. This procedure also utilizes low power "spotting" to detect clustered leucocytes with enmeshed bacteria to establish the presence of bacterial mastitis. When these clusters are found, the need for counting leucocytes is eliminated. Photographs of clustered leucocytes with enmeshed bacteria under low and oil immersion are shown. Typical epithelial cells are photographed. The proposed procedure is advocated because it is accurate, not timeconsuming, and can establish the presence of bacterial mastitis

# Public Health Service and National Conference on Interstate Milk Shipment Regulations

The National Conference on Interstate Milk Shipments (NCIMS) has decided to limit considerations of abnormal milk to only mastitis and not to include other contaminants such as pesticides, radioactive materials, etc. The NCIMS and the National Mastitis Council have agreed that mastitis is present if the somatic cell count of a milk is 1,500,000 or more per milliliter. The recorded indicator tests for this determination are detailed in U.S. Public Health Service Publication No. 1306. Laboratory examinaitons or screening procedures of the milk for the presence of unwholesome, altered mammary secretionswhether of an inflammatory, infectious, physiological, or environmental origin, should be made at the same frequency as specified for bacterial tests in milk. When a herd milk contains 1,500,000 leucocytes per milliliter, the following procedures should be followed:

(a) A warning letter shall be sent to the producer notifying him of the high leucocyte count. The letter shall also list the principal causes of excess leucocyte counts. (b) Following the second consecutive test indicating a raw milk count of 1,500,000 or more leucocytes per milliliter, an inspection shall be made by an official sanitarian or a person designated by him. (c) A third herd milk sample shall be taken. If this sample also indicates a leucocyte count of 1,500,000 or more per milliliter, the milk regulatory authority shall, if he deems it necessary, require the producer to: (aa) have milking equipment analyzed by a milking equipment serviceman, and (bb) have individual animals examined by a veterinarian. Cows producing abnormal milk shall be milked separately and the milk shall be withheld from the milk supply.

Effective July 1, 1970, milk supplies containing 1,500,000 or more leucocytes per milliliter on three out of five of the last tests shall have their permit suspended and/or court action shall be taken in accordance with the recommendations of the USPHS Grade A Pasteurized Milk Ordinance, "provided that leucocyte counts of 1,500,000 or more per milliliter shall not have been officially recorded nor penalty applied unless corroborated by the direct microscopic leucocyte count or the equivalent as published by the U.S.P.H.S. and subjected to the State Laboratory Certification Program."

Presently 47 of the States utilize the NCIMS Program. There are slight variations in regulations of the States: for example, New Jersey lists as unsatisfactory a leucocyte count of 2,000,000 per milliliter or more. For milks produced in New York state, the only acceptable test is the Whiteside; for milks produced out of the State for import there, other screening tests are accepted, with qualifications.



Figure 1. Low-power photomicrograph (100X) of stained milk smear showing appearance of leucocyte cluster in center of field. In observation through microscope, individual leucocytes in cluster are better delineated as field is taken in and out of focus.

<sup>&</sup>lt;sup>1</sup>Presented at the 30th Annual Meeting of the Pennsylvania Approved Dairy Laboratory Directors' Association, the Pennsylvania State University, April 22, 1969.

# MASTITIS

There is widespread disagreement as to the definition of "mastitis." The dictionary definition and, in fact, the nation-wide dairy-industry-accepted definition is "inflammation of the mammary gland . . . whether of an inflammatory, infectious, physiological, or environmental origin." It seems to me that regulatory enforcement agencies should be concerned, from a public health hazard standpoint. *only with bacterial mastitis*. Certainly, the ingestion of somatic cells has not and will not make anyone ill. The existing regulations of the various states nevertheless detail screening tests which use only the leucocyte count to determine if milk is "abnormal." It is my expectation that these regulations will be changed, but in the meantime we must live with them.

The screening test of choice should be one which will most accurately indicate the presence of bacterial infection. In many, many cases the mere presence of high somatic cell counts does not establish the presence of bacterial mastitis. The California Mastitis Test, the Catalase Test, the Modified Whiteside



Figure 2. Same cluster as in Fig. 1 under oil immersion objective (970X). Only some bacterial cells can be seen photographically; many more can be seen under microscope when it is focused to different depths.



Figure 3. Low-power magnification similar to Fig. 1.



Figure 4. Oil immersion objective magnification of cluster in Fig. 3.



Figure 5. Oil immersion objective magnification of stained epithelial cells. Note that most cells are part-moon shaped.

Test, and the Wisconsin Mastitis Test all indirectly estimate the somatic cell count. There has been a tremendous diversity of opinion as to the accuracy of these tests and much of the published work has established their unreliability.

# DIRECT MICROSCOPIC SOMATIC CELL COUNT

The direct microscopic somatic cell count in milk reported by the Subcommittee on Screening Tests of the National Mastitis Council, in the November, 1968 *Journal of Milk and Food Technology*, details quite comprehensively the procedure for determining the nucleated somatic cell count. It is unfortunate that nucleated epithelial cells (Fig. 5) are also included in the count. The presence of these cells very frequently is not associated with bacterial infections. The direct microscopic somatic cell count test as detailed is quite elaborate and consumes a considerable amount of time per test, but this report does establish the microscopic count to be reproducible and reliable.

Let's go back to the regulations which will go into effect July 1, 1970: "Penalties will be applied only if the leucocyte count has been corroborated by the direct microscopic leucocyte count."

A modified direct microscopic somatic cell count can effectively be used as a rapid screening test. Ten fields spaced out over the length of a strip are adequate to establish that the leucocyte count is significantly below or above 1,500,000 per milliliter. In these instances there is no point in making full strip counts. If the field count yields a figure close to the 1,500,000 cutoff, then full strip counts should be made. This modification is rapid for the majority of the milks encountered and we now have a definite, accurate procedure for determining the leucocyte level on which action must be taken. On this basis, there is no logic for the continued use of the indirect screening tests in connection with the *official* Abnormal Milk Program.

Although better qualified personnel are needed to perform the direct microscopic somatic cell count than any of the indirect screening tests, (and I might say that there has been much controversy as to whether technicians are properly reading the indirect screening tests) the reglations of the various states now require that qualified personnel in approved laboratories make total bacterial plate counts on all of the raw supplies at least once a month. I know from experience that the same qualified technicians can quickly learn how to make microscopic leucocyte counts and thereby eliminate the use of these controversial indirect screening tests.

# LOW-POWER "SPOTTING"

For over 30 years at our laboratory we have auto-

matically made microscopic smears for type analysis of all milks subjected to plate counts. It is important for the field man to know the source of the bacterial count found, whether it be from utensils, from udder infections, or whether it is complicated by inadequate cooling. These same smears are available for leucocyte counting. One thing that we have always done, and which I propose be included as an integral part of the microscopic method for determining abnormal milk, is to examine these smears for positive evidence of the presence of bacterial infection. When bacterial mastitis exists there is a clustering of leucocytes with bacteria enmeshed in these clusters. These clusters can be readily spotted under low-power magnification (see Fig. 1 and 3). Oil immersion observation of this grouping then will confirm the actual mastitic condition (see Fig. 2 and 4). There are only about five low-power strips in an entire square centimeter smear. The length of time required to examine this area for evidence of clustered leucocytes # is minimal. Keep in mind that if you do locate clustered leucocytes with enmeshed bacteria, there is no need to make a leucocyte count because you have already established the presence of bacterial infection. If it is the intent, and I think it should be, of the Abnormal Milk Program to locate and then eradicate the presence of bacterial infections in the milk supply, the combination of leucocyte counts and lowpower examination for detecting mastitic clusters is essential. Routine examination has disclosed a large percentage of herd milks showing the presence of infection, even though the leucocyte count was significantly below 1.500,000 per milliliter.

# 3-A SANITARY STANDARDS FOR MILK AND MILK PRODUCTS EVAPORATORS AND VACUUM PANS, REVISED

Serial #1604

Formulated by International Association of Milk, Food and Environmental Sanitarians United States Public Health Service The Dairy Industry Committee

It is the purpose of the IAMFES, USPHS, and DIC in connection with the development of the 3-A Sanitary Standards program to allow and encourage full freedom for inventive genius or new developments. Milk and milk products evaporator and vaccum pan specifications heretofore and hereafter developed which so differ in design, material, fabrication, or otherwise as not to conform with the following standards, but which, in the fabricator's opinion are equivalent or better, may be submitted for the joint consideration of the IAMFES, USPHS, and DIC at any time.

# Α.

# SCOPE

# A.1

These standards cover the sanitary aspects of evaporators and vacuum pans used for milk and milk products.

# A.2

In order to conform with these 3-A Sanitary Standards, evaporators and vacuum pans shall comply with the following design, material, and fabrication criteria.

### B.

# DEFINITIONS

**B.1** 

Product: Shall mean the milk or milk product which is processed in this equipment.

# **B.2**

Evaporators and Vacuum Pans: Shall mean equipment in which products may be concentrated in vacuo. Product heating surfaces and vapor condensing units are considered to be essential auxiliary equipment and are, therefore, included.

# **B.3**

Vacuum Pan: Shall mean equipment in which the heat exchange surface is that of a steam jacket and/or a series of steam coils within the vacuum chamber.

# **B.4**

Evaporators: Shall mean equipment in which the heat exchange surface, either tubular or plate type, is located outside of the vacuum chamber.

**B.5** 

Product Contact Surface: Shall mean all surfaces that are exposed to the product, or from which liquid may drain, drop, or be drawn into the product.

Non-Product Contact Surface: Shall mean all other exposed surfaces.

# **B.7**

**B.6** 

Mechanical Cleaning or Mechanically Cleaning: Shall denote cleaning, solely by circulation and/or flowing chemical detergent solutions and water rinses onto and over the surfaces to be cleaned, by mechanical means.

# C.

# MATERIALS

C.1.

All product contact surfaces shall be of stainless steel of the AISI 300 series1 or corresponding ACI2 types (See Appendix, Section E.), or equally corrosion resistant metal that is non-toxic and nonabsorbent, except that:

C.1.1

Rubber and rubber-like materials may be used for gaskets, seals, and parts used in similar applications. These materials shall comply with the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Rubber and Rubber-Like Materials Used as Product Contact Surfaces in Dairy Equipment, Serial #1800."

C.1.2

Plastic materials may be used in sight and/or light openings and for gaskets, seals and parts used in similar applications. These materials shall com-

<sup>&#</sup>x27;The data for this series are contained in the following reference: AISI Steel Products Manual, Stainless & Heat Resisting Steels, April 1963, Table 2-1, pp. 16-17. Available from: American Iron & Steel Institute, 633 Third Avenue New York, N. Y. 10017.

<sup>&</sup>lt;sup>2</sup>Alloy Casting Institute, 300 Madison Avenue, New York, N. Y. 10017.

ply with the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, Serial #2000." as amended.

# C.1.3

Glass may be used in sight and/or light openings and when used shall be of a clear heat resistant type.

# C.2

All non-product contact surfaces shall be of corrosion-resistant material or material that is rendered corrosion-resistant. If coated, the coating used shall adhere. All non-product contact surfaces shall be relatively non-absorbent, durable and cleanable. Parts removable for cleaning having both product contact and non-product contact surfaces shall not be painted.

# D.

# FABRICATION

# D.1

All product contact surfaces shall be at least as smooth as a No. 4 mill finish on stainless steel sheets. (See Appendix, Section F.).

# D.2

All permanent joints in metallic product contact surfaces shall be welded, except that tubes may be either expanded and rolled or welded into the tube sheets. All welded areas of product contact surfaces shall be at least as smooth as the adjoining surfaces. When tubes are expanded and rolled into a tube sheet, the resulting joint shall be completely rigid and without pockets or crevices. All joints shall be flush.

# D.3

All appurtenances having product contact surfaces shall be easily removable for cleaning, or shall be readily cleanable in place.

# **D.4**

All product contact surfaces shall be easily accessible for cleaning, either when in an assembled position or when removed. Removable parts shall be readily demountable.

A vacuum pan, to comply with the requirements of this section, shall have spaces between coils at least as great as the following:

Between coils2-1/2 inchesBetween coils and vacuum pan walls3 inchesBetween coil banks3-1/2 inches

A product heater designed to be mechanically cleaned in which the heat exchange surface is one continuous tube shall be considered to be in compliance with the requirements of this section.

# D.5

All product contact surfaces shall be self draining except for normal clingage.

# D.6

All internal angles of  $135^{\circ}$  or less on product contact surfaces shall have minimum radii of 1/4 inch, except that:

# D.6.1

Minimum radii for fillets of welds in product contact surfaces may be 1/8 inch where the thickness of one or both parts joined is less than 3/16inch.

# D.6.2

The minimum radius of any internal angle in a gasket groove or a gasket retaining groove shall be at least 1/8 inch (See D.15.4).

# D.7

The minimum inside diameter of product heat exchange tubing shall be that of nominal 1 inch O.D., 16 gauge tubing, except that smaller tubing may be used in a supplement product heater designed to be mechanically cleaned in which the heat exchange surface is one continuous tube.

# D.8

Where the heat exchanger surface is of the plate type, the plate equipment shall comply with the applicable provisions of the "3-A Sanitary Standards, of Plate Type Heat Exchangers for Milk and Milk Products," Serial #1100, as amended, except the provision of paragraph B.5 of the aforementioned standard. (The 3-A Sanitary Standards of Plate Type Heat Exchangers for Milk and Milk Products was published in the January-February, 1952 issue of the Journal of Milk and Food Technology).

# D.9

Connections in product contact surfaces, vacuum breakers and sampling valves shall conform to the applicable provisions of the 3-A "Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products," Serial #0800 as amended and Supplements thereto and/or the applicable provisions for welded sanitary productpipelines found in the "3-A Accepted Practices for Permanently Installed Sanitary Product-Pipelines and Cleaning Systems," effective June 9, 1966, as amended.

# D.10

Sight and light openings, when provided, shall be of such design and construction that the inner surfaces drain inwardly, and if the evaporator or vacuum pan is designed for mechanical cleaning, the inner surface of the glass (or plastic) shall be relatively flush with the inner surface of the evaporator or vacuum pan. The exterior flare shall be pitched so that liquids cannot accumulate. The glass (or plastic) shall be readily removable. The inside diameter of the opening shall be at least 3-3/4 inches.

# D.11

Thermometer connections, when provided, shall conform to 3-A "Sanitary Standards for Thermometer Fittings and Connections Used on Milk and Milk Products Equipment," Serial #0900, as amended and Supplements thereto.

# D.12

The minimum inside diameter of a manhole shall be 16 inches.

# D.13

The cover for a manhole shall be of the outside swing type.

# D.14

Openings, when provided, for vacuum breakers and sampling valves shall be in a product contact surface.

# D.15

Gaskets having any surface in contact with the product and grooves in metallic product contact surfaces in which gaskets are inserted or which hold gaskets in place (retaining grooves) shall comply with the applicable portions of the following:

# D.15.1

These gaskets shall be removable.

# D.15.2

In grooved gaskets, the length of the shorter leg shall not exceed twice the width of the groove.

# D.15.3

A gasket groove or gasket retaining groove shall not have a depth greater than its width; the depth shall not exceed 1/2 inch and the width shall be at least 1/4 inch.

# D.15.4

The minimum radius of any internal angle in a gasket groove or gasket retaining groove shall be not less than 1/8 inch.

# D.15.5

All gasket grooves and gasket retaining grooves shall be readily cleanable.

# D.16

There shall be no threads on product contact surfaces.

# D.17

All exhaust vapor lines beyond the product contact surface shall drain away from the product contact surface. A pitch of at least 3/8 inch per foot to the first vertical drop shall be provided.

# D.18

Vacuum gauge connections shall be placed in the vapor line beyond the product contact surface.

D.19

Non-product contact surfaces shall be readily cleanable and those to be coated shall be effectively prepared for coating.

# APPENDIX

# Ε.

# STAINLESS STEEL MATERIALS

Stainless steel conforming to the applicable composition ranges established by AISI for wrought products, or by ACI for cast products, should be considered in compliance with the requirements of Section C.1 herein. Where welding is involved the carbon content of the stainless steel should not exceed 0.08%. The first reference cited in C.1 sets forth the chemical ranges and limits of acceptable stainless steels of the 300 series. Cast grades of stainless steel equivalent to types 303, 304, and 316 are designated CF-16F, CF-8, and CF8M, respectively. These cast grades are covered by ASTM<sup>3</sup> specifications A296-67 and A351-65.

# F.

# PRODUCT CONTACT SURFACE FINISH

Surface finish equivalent to 150 grit or better as obtained with silicon carbide, is considered in compliance with the requirements of Section D.1 herein.

# G.

# SPECIAL CONSIDERATIONS

G.1

In tube chests, vapor heaters and surface condensers of the tubular type, in which vapors of the product and/or the condensate of these vapors enter the space between the exterior of the tubes and the interior of the shell, means shall be provided for mechanically cleaning the exterior surfaces of the tubes and the interior surface of the shell.

1.

# ACCESSORIES

<sup>3</sup>Available from American Society for Testing and Materials, 1916 Race Street, Philadelphia, Pa. 19103.

# Н.1

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An automatic condenser water level control for preventing water from entering the product shall be provided by one of the following means:

### H.1.1

A barometric leg extending at least 35 feet vertically from the maximum safe water level in the condenser system to the free water level at which the leg discharges, less 1 foot vertical height for each 1200 feet of elevation above sea level.

# H.1.2

A surface condenser, in which the vapor and the condensing water are separated by metal walls and do not come into contact with each other.

H.1.3

A safety shut-off valve, located in the water feed

line to the condenser, automatically actuated by a control which will shut off the inflowing water when the water level rises above a predetermined point in the condenser. This valve may be actuated by water, air or electricity, and shall be so designed that failure of the primary motivating power will automatically stop the flow of water.

These standards shall become effective July 2, 1970, at which time the "3-A Sanitary Standards for Milk and Milk Products Evaporators and Vaccum Pans" dated March 1, 1957, published in the July, 1957, Journal of Milk and Food Technology, and amendments thereto, are rescinded and become null and void.

# AMENDMENT TO 3-A SANITARY STANDARDS FOR STAINLESS STEEL AUTOMOTIVE MILK TRANSPORTATION TANKS FOR BULK DELIVERY AND/OR FARM PICK-UP SERVICE

# Serial #0507

Formulated by International Association of Milk, Food and Environmental Sanitarians United States Public Health Service The Dairy Industry Committee

The "3-A Sanitary Standards for Stainless Steel Automotive Milk Transportation Tanks for Bulk Delivery and/or Farm Pick-Up Service, amended April 28, 1954," Serial #0501, are hereby further amended in the sections indicated below:

The following sentence is added to the DEFINI-TION:

Both may have more than one tank or compartment.

Substitue the following for subsection D.1:

1. Outlet: A milk outlet is defined as the opening in the inside lining of a tank or compartment and the outlet passage for milk to the exterior of the tank or compartment. The outlet passage starts at the opening in the inside lining and terminates at the connection for the outlet valve. Each tank or compartment shall have a separate outlet passage. The outlet shall be of all welded construction (except that a rolled-on flange may be used on the terminal end) and shall have an inside diameter at least as large as that of a 2 inch 3-A Sanitary Fitting. The top of the terminal end of the outlet passage shall be lower than the low point of the bottom of the inside lining at the outlet. The terminal end shall have a welded or rolled-on flanged connection for the outlet valve. The terminal end of the outlet passage shall not extend more than 6 inches beyond the inside lining of the tank or compartment(s). The outlet passage may be increased in length provided that:

- a. The outlet passage is straight and the outlet and outlet passage may be adequately cleaned manually or the tank or compartment with the increased outlet passage is provided with a fixed spray device(s) so that the outlet and outlet passage may be mechanically cleaned.
- b. The outlet passage is insulated sufficiently that the temperature rise of the water in the outlet passage does not exceed the allowable average temperature rise of the tank full of water  $(2^{\circ}F)$  specified in subparagraph A.4.
- c. The outlet passage is protected against damage (denting) and is braced and sloped.

This amendment shall become effective July 2, 1970.

# AMENDMENT TO 3-A SANITARY STANDARDS FOR INLET AND OUTLET LEAK PROTECTOR PLUG VALVES FOR BATCH PASTEURIZERS

Serial #1401

Formulated by International Association of Milk, Food and Environmental Sanitarians United States Public Health Service The Dairy Industry Committee

The "3-A Sanitary Standards for Inlet and Outlet Leak Protector Plug Valves for Batch Pasteurizers" dated October 8, 1954, Serial #1400, are hereby amended by changing the captions on two drawings and adding three drawings for acceptable inlet and outlet valves for applications in which the product flow will be in a vertical direction.

Change the captions on the drawings listed below to the following:

3A-100-16 Two-Way Horizontal Plug-Type Inlet Valve (Design A)

3A-100-17 Two-Way Horizontal Plug-Type Inlet Valve (Design B)

and the star

The following three drawings are added:

3A-100-27 Two-Way Vertical Plug-Type Inlet Valve 3A-100-28 Plug-Type Bottom Outlet Valve 3A-100-29 Close Coupled Bottom Outlet Valve

This amendment shall become effective July 20, 1970.

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TWO-WAY VERTICAL PLUG-TYPE INLET VALVE

NOTES: A, B, C and D are leak-protector grooves. A extends part way in the plug and mates with B (in upper and lower shell) in all closed positions. Grooves C and D are diagonally opposite A and B.

 $C^{1}$  is an air-relief groove for the outlet. Upper or lower half of  $C^{1}$  may be omitted.

Leak-protector grooves must begin to function within 5/64 of an inch after the "just closed position" is passed in closing.

The configuration of leak-protector grooves D (the drain) shall be such that there will be no pocket to trap product and all product will be drained.

A 90° stop shall be provided. The positioning of the stop shall be such that the product in the passage in the plug will drain completely.

SANITARY FITTINGS 3-A STANDARD 3A-100-27



NOTES: A, B, C and D are leak-protector grooves. A extends part way in the plug and mates with B (in upper and lower shell) in all closed positions. Grooves C and D are diagonally opposite A and B.

Cl is an air-relief groove for the outlet. Upper or lower half of C<sup>1</sup> may be omitted. Leak-protector grooves must begin to function within 5/64 of an inch after the "just closed position" is passed in closing.

The configuration of leak-protector groove D (the drain) shall be such that there will be no pocket to trap product and all product will be drained.

A 90° stop shall be provided. The positioning of the stop shall be such that the product in the passage in the plug will drain completely.

SANITARY FITTINGS 3-A STANDARD 3A-100-28

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# VERTICAL SECTION

NOTE: Close coupled bottom outlet valve connected to holder showing design requirements.

d = inside diameter of outlet valve.

a = greatest distance from valve seat to end of holder portion of outlet having same diameter as the passage in the valve.

a shall not be more than d.

CLOSE-COUPLED BOTTOM-OUTLET VALVE

SANITARY FITTINGS 3-A STANDARD 3-A-100-29

# 3-A SANITARY STANDARDS FOR FITTINGS USED ON MILK AND MILK PRODUCTS EQUIPMENT AND USED ON SANITARY LINES CONDUCTING MILK AND MILK PRODUCTS, REVISED

# Serial #0809

Formulated by

# International Association of Milk, Food and Environmental Sanitarians United States Public Health Service

The Dairy Industry Committee

It is the purpose of the IAMFES, USPHS, and DIC in connection with the development of the 3-A Sanitary Standards program to allow and encourage full freedom for inventive genius or new developments. Sanitary fittings which are developed and which so differ in design, material, construction, or otherwise so as not to conform with the following standards, but which in the opinion of the manufacturer or fabricator are equivalent or better, may be submitted at any time for the joint consideration of IAMFES, USPHS, and DIC.

SCOPE

A.1 These standards cover the sanitary aspects of gaskets, fittings, valves and tubing used on equipment and lines which hold or convey milk or milk products.

# A.2

0

In order to conform with these 3-A Standards, fittings, valves and tubing shall comply with the folowing in design, material and fabrication.

# B. DEFINITIONS

# B.1

Product: Shall mean the milk and milk products which are held or conveyed in these fittings, valves or tubing.

# **B.2**

Surfaces:

# B.2.1

Product Contact Surfaces: Shall mean all surfaces that are exposed to the product or from which liquid may drain, drop or be drawn into the product.

# B.2.2

Non-Product Contact Surfaces: Shall mean all other exposed surfaces.

# MATERIALS

# C.1

C.

All product contact surfaces shall be constructed of stainless steel of the AISI 300 series1 or corresponding ACI<sup>2</sup> types (see APPENDIX, Section A.) or metal which is equal in cleanability to stainless steel of the foregoing types, and which under conditions of intended use is equally corrosion resistant, non-toxic and non-absorbent, or heat resistant glass piping except that:

C.1.1

Optional metal alloy may be used but only in applications requiring disassembly and manual clean-(See APPENDIX, Section I for the coming. position of an acceptable optional alloy metal.)

# C.1.2

Plug-Type valves, other than leak protector valves, may have plugs covered with rubber or rubberlike materials or plastic materials. Rubber and rubber-like materials and plastic materials used

<sup>&#</sup>x27;The data for this series are contained in the following reference: AISI Steel Products Manual, Stainless & Heat Resisting Steels, April 1963, Table 2-1, pp. 16-17. Available from American Iron & Steel Institute, 633 3rd Ave., New York, N. Y. 10017.

<sup>&</sup>lt;sup>2</sup>Alloy Casting Institute, 300 Madison Avenue, New York, N. Y. 10017.

shall be of such composition as to retain their surface and conformation characteristics when exposed to conditions encountered in the environment of intended use and in cleaning and bactericidal treatment. Valve plug cores, if completely covered, may be made of metal, plastic or rubber or rubber-like materials.

# C.1.3

Rubber and rubber-like materials may be used for gaskets (either bonded or removable), compression-type valve plugs, diaphragms, seals and parts used in similar applications.

# C.1.4

Rubber and rubber-like materials when used shall conform to the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Rubber and Rubber-Like Materials Used as Product Contact Surfaces in Dairy Equipment, Serial #1800."

# C.1.5

Plastic materials may be used for covering the interior surface of the bonnet of diaphragm-type valves, gaskets, (either bonded or removable), valve plugs, valve body liners, diaphragms, seals and parts used in similar applications.

# C.1.6

All plastic materials, when used, shall conform to the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, Serial #2000," as amended.

# C.2

All non-product contact surfaces shall be of corrosion-resistant material or material that is rendered corrosion-resistant. If coated, the coating used shall adhere. All non-product contact surfaces shall be relatively non-absorbent, durable and cleanable. Parts removable for cleaning having both product contact and non-product contact surfaces shall not be painted.

# D.

# FABRICATION

# D.1

All product contact surfaces shall be at least as smooth as a No. 4 mill finish on stainless steel sheets (see APPENDIX, Section H).

# D.2

All product contact surfaces of demountable fittings, valves or tubing shall be readily accessible, visible and readily cleanable either when in an assembled position or when removed. Removable parts shall be readily disassembled. All product contact surfaces shall be self-draining when properly installed.

# D.4

All internal angles on product contact surfaces shall have minimum radii of 1/16 inch, except gasket recesses and grooves in which all sharp corners shall be avoided.

# D.5

There shall be no threads in contact with the product.

# D.6

The rubber or rubber-like covering or the plastic covering of valve parts shall be bonded in such a manner that the bond is continuous and mechanically sound, and so that in the environment of its intended use the rubber or rubber-like material or the plastic material does not separate from the base material.

# D.7

Non-product contact surfaces shall have a smooth finish, be free of pockets and crevices, and be readily cleanable and those to be coated shall be effectively prepared for coating.

# D.8

Valves shall not have stuffing boxes.

# D.9

Stainless steel sanitary tubing shall conform to the applicable provisions of the American Society for Testing and Materials "Standard Specifications for Seamless and Welded Austenitic Stainless Steel Sanitary Tubing, #A-270-64,"<sup>3</sup> and the finish of product contact surfaces shall be as provided for in D.1 above.

# D.10

Removable fittings may be used with or without gaskets and shall be of such design as to form flush interior joints.

# D.11

Fittings shall conform to the dimensions in the drawings.

# D.11.1

The dimensions and angles of fittings shall not vary from those in the drawings more than the following tolerances:

<sup>3</sup>Available from American Society for Testing and Materials, 1916 Race Street, Philadelphia, Pa. 19103.

# D.3

# Fitting Name

Tees, Crosses & 90° Bends Tees, Crosses & 90° Bends Three-Way Plug Valves Three-Way Plug Valves Straight Way Plug Valves Straight Way Plug Valves All 3-A fittings-Large Diameter of Bevel Seat-Dimension H Plain Ferrules-U Dimension of the Shoulder Bends-Angularity of Bends-Angularity of Bevel-Angularity of Bevel-Angularity of 45 Degree Bends-2K and 2P

### 1" thru 2" 2 1/2" thru 4" 1″ thru 2" 2 1/2" thru 4" 1″ thru 2" 2 1/2" thru 4"

all sizes

all sizes 1" thru 2" 2 1/2" thru 4" 1" thru 2" 2 1/2" thru 4"

# Maximum Tolerance

### Centerline-to-face plus or minus 3/64" plus or minus 1/32" plus or minus 1/16" plus or minus 3/64" plus or minus .024" plus or minus .024" .032" plus or minus plus or minus .032" plus or minus .024" \_\_\_\_\_ plus or minus 0.32" \_\_\_\_\_

# plus or minus .010"

present dimension minimum plus or minus 1/2-degree plus or minus 1 -degree plus or minus 1/2-degree plus or minus 1 -degree

all sizes

# SPECIAL CONSIDERATIONS

E.1

E.

Special sanitary fittings may be used where interchangeability is not required. These special fittings must conform to the provisions of this standard with respect to material, finish, construction, thread dimensions (if used) and use of gaskets but do not have to conform to the face-to-face or center line-to-face dimensions in the drawings. All product contact surfaces of such fittings shall be accessible for cleaning and inspection. All internal angles on product contact surfaces shall have radii of not less than 1/16 inch except gasket recesses and grooves, in which all sharp corners shall be avoided.

# E.2

The inside diameter of the tubing in the following table is the same as the inside diameter of sanitary fittings in the drawings of this standard.

SIZE	GAGE
(O.D.)	(B.W.G.)
1″	18
1 1/2"	18
2″	16
2 1/2"	16
3‴	16
4‴	14

E.3

Plain end fittings for use in welded sanitary pipelines must conform to the provisions of this standard with respect to material, finish and construct-The outside diameter of the butt welding ion. ends shall conform to the sanitary tubing sizes given in E.2 and the inside diameter shall be the same as that of the part to which it is to be welded.

From a practical angle, due to the relative impossi-

bility of measurement means, the right is reserved to hold 45 degree bends as close to the tolerances

for 90 degree bends as can be attained.

# E.4

Compression-type valves (reference 3-A Drawings No. 3A-100-24 and 3A-100-25) shall comply with the following:

# E.4.1

Valves may be metal to metal or rubber or rubberlike material to metal seat. The rubber or rubberlike material either removable or bonded, if used, shall comply with C.1.4.

# E.4.2

Grooves for removable rubber or rubber-like parts shall be readily cleanable.

# E.4.3

Outlet valves shall be close coupled to minimize the distance between the mounting face and the shut off point.

# E.4.4

The valve bore of outlet valves and in-line-valves shall be of such design that the valves will be selfdraining in their installed positions.

Face-to-face

# E.5

Diaphragm-type valves (reference: 3-A Drawing No. 3A-100-26) shall consist of a bonnet assembly [handwheel or power (air) operated], a diaphragm and a suitable body and shall comply with the following:

# E.5.1

The bonnet shall be secured to the body with a minimum number of clamps or nuts. The diaphragm shall separate the product from the working assembly in the bonnet.

# E.5.2

The valve shall be of such design as to be selfdraining in its installed position. Diaphragm valves shall be permanently marked with a descriptive stainless steel mounting instruction plate on the bonnet, to show and describe positively the self-draining angle, when the valve is placed in service. The mounting instruction plate shall also have a statement that only sterile food grade lubricant shall be used on the valve stem. Lubricants complying with the Food, Drug and Cosmetic Act as amended, regulation 121.2553 (Lubricants with incidental food contact) are considered meeting the requirements of this section.

# E.5.3

The chamber on the exterior side of the diaphragm (herein defined as the bonnet) shall have one or more 3/32 inch holes just above the bonnet flange in a suitable bossed area(s) located so that one hole will be at the lowest point in the installed position for the detection of leakage.

# E.5.4

Surfaces which may become product contact surfaces if the diaphragm fails in service shall be readily accessible for cleaning and inspection.

# E.5.5

The valve bonnet, if not made of corrosion-resistant material, shall have the interior surface (1) plated with a corrosion-resistant, non-toxic, nonabsorbent metal or (2) covered with a plastic material complying with C.1.6. The interior of the valve bonnet shall be at least as smooth as ACI Surface Indicator Scale SIS-3<sup>4</sup>.

# E.5.6

Retaining grooves for the positioning of removable rubber or rubber-like parts and/or plastic parts shall be readily cleanable.

# E.5.7

All internal angles except those connecting removable parts shall have radii of not less than 1/16 of an inch.

# E.5.8

The valve bonnet and all bonnet parts shall be readily demountable.

# F.

# 3-A STANDARD SANITARY FITTINGS

# F.1

The following shall be classified as 3-A Standard Sanitary Fittings:

Summary Trems.	~	
	Page	3A
Fitting Name	No.	Drawing No.
2C Bend	6	3A-100-01
2CG Bend	7	3A-100-02
2E Bend	19	3A-100-22
2EG Bend	20	3A-100-23
2F Bend	8	3A-100-03 🦸
2FG Bend	8	3A-100-03
2K Bend	9	3A-100-04
2KG Bend	9	3A-100-04
2P Bend	9	3A-100-04
2PG Bend	9	3A-100-04
7 Tee	6	3A-100-01
7G Tee	7	3A-100-02
7X Tee	19	3A-100-22
7XG Tee	20	3A-100-23
7A Tee	10	3A-100-05
7AG Tee	11	3A-100-06
7AX Tee	19	3A-100-22
7AXG Tee	20	3A-100-23
7B Tee	10	3A-100-05
7BG Tee	11	3A-100-06
7BX Tee	19	3A-100-22
7BXG Tee	20	3A-100-23
9 Cross	6	3A-100-01
9G Cross	7	3A-100-02
10BF Valve	12	3A-100-07
10BFG Valve	13	3A-100-08
10C Valve	6	3A-100-01
10CG Valve	7	3A-100-02
11C Valve	6	3A-100-01
11CG Valve	7	3A-100-02
12R Union	17	3A-100-12
12RG Union	17	3A-100-12
13H Nut	8	3A-100-03
13SH Nut	18	3A-100-13
14R Ferrule	16	3A-100-11
14RG Ferrule	16	3A-100-11
15R Ferrule	16	3A-100-11
15RG Ferrule	16	3A-100-11
16A Cap	16	3A-100-11
16AG Cap	16	3A-100-11

<sup>&</sup>lt;sup>4</sup>Specifications for Visual Inspection of Cast Surface Finish as well as the Surface Indicator Scale may be obtained from the Alloy Casting Institute, 300 Madison Avenue, New York, N. Y. 10017.

30F Angle Valve	12	3A-100-07
30FG Angle Valve	13	3A-100-08
31-15 Reducer	14	3A-100-09
31-15G Reducer	14	3A-100-09
32-15 Reducer	15	3A-100-10
32-15G Reducer	15	3A-100-10
33F Can Filler	12	3A-100-07
33FG Can Filler	13	3A-100-08
60 C Compression Valve	22	3A-100-25
60 CG Compresson Valve	22	3A-100-25
60CF Compression Valve	21	3A-100-24
60CFG Compression Valve	21	3A-100-24
60T Compression Valve	22	3A-100-25
60TG Compression Valve	22	3A-100-25
60TF Compression Valve	21	3A-100-24
60TFG Compression Valve	21	3A-100-24
Diaphragm Valve	23	3A-100-26
Thread Dimensions	24	3A-100-30

Additional fittings may be added to the list of 3-A Standard Sanitary Fittings as the demand arises. In each case the new fitting shall be assigned a 3-A drawing number and shall be considered to be incorporated into this standard after acceptance by IAMFES, USPHS and DIC.

# APPENDIX

G.

The series or types of stainless steel specified should comply with the applicable composition ranges of AISI for wrought products and of ACI for cast products. For AISI chemical composition ranges and limits see Table 2-1 (pp. 16-17) in AISI Steel Products Manual, "Stainless and Heat Resisting Steels," April, 1963. ACI designations for cast grades equivalent to the AISI types 302, 303, 304, and 316 are designated CF-20, CF-16F, CF-8, and CF8M, respectively. (See ASTM Specs. A-296-67 and A-351-65).

Н.

١.

Surface finish equivalent to 150 grit or better as obtained with silicon carbide, is considered in compliance with the requirements of D.1 herein.

# OPTIONAL METAL ALLOY

An optional alloy metal having the folowing minimum and maximum composition is deemed to be in compliance with C.1.1:

Zinc—8% maximum Nickel—19 1/2% minimum Tin—3 1/2% minimum Lead—5% Maximum Iron—1 1/2% maximum Copper—the balance

An alloy of the composition given above is properly designated "nickel silver," or, acording to ASTM #B 149-52, may be entitled, "leaded nickel bronze."

These standards shall become effective July 2, 1970, at which time the 3-A "Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products" approved in and dated March, 1950, and amendments and supplements thereto, are rescinded and become null and void.









GMAX

36

16

76

132

9/16

1/16

J

.062

.062

.062

.062

.062

.062

Ν

.902

1.402

1.870

2.370

2.870

3.834

0 + 001

1.219

1.750

2.250

2.750

3.312 "

4.312"

Ρ

1.317

1.849

2.381

2.913

3.445

4.509

Q

1.462

1.994

2.526

3.058"

3.590"

4.695

#2-CG BEND

TUBE ACME THREADS GAGE PER INCH

8

8

8

8

8

6

SIZE

1"

1/2"

2"

21/2

3"

4 "

18

18

16

16

16

14

· / · · · · · · · · · · ·





#10-CG VALVE

в

1/2"

2 1/4"

4"

5

7

A

17/8

234

3% 3"

42×32

523 2

7%

С

3%

412

5%"

6"

6%

8 5/16

D

Ε

4% 11/16

5 % 214

61/2 25/8

3"

3%

4 32

7%

9%

12/16

F

2 32

225/

3'1

313/6

4132

6 32

	-
3A STANDARD	
SANITARY FITTINGS	
3A-100-02	

FORMERLY EX-10807-2



SUPERSEDES DRWG. EX-11180 FORMERLY EX-13190





No. 7-B TEE



No. 7-A TEE

Revised 4-14-45 + 9-19-48

SIZE	Acme Thds. Per In.	с	E	v	G	EI	н	N	Р	Q	R	S	υ	z
1	8	3 8	1 13	18	9	2 16	1 16	902	1.317	1.462	1.160	1.316	<u>3</u> 16	4 1/2
1 1/2	8	4 34	2 3	5 32	<u>9</u> 16	3 32	1 32	1.402	1.849	1994	1.676	1.848	<u>3</u> 16	5 <u>25</u> 32
2	8	5 16	232	5 32	19	3 13	2 <del>1</del>	1.870	2381	2.526	2.192	2.380	<u> </u>	6 <u>19</u> 32
21/2	8	6 3	3 16	3	23	4 4	2 34	2.370	2 913	3.058	2 708	2.912	<u> </u>	7 16
3	8	7	31/2	3	34	4 5/8	3 16	2.870	3 4 4 5	3 5 9 0	3.224	3.444	9 32	8 =
4	6	9 16	432	14	78	6 18	44	3.834	4.509	4.695	4.256	4.508	5 16	10 32

Dimensions in Above Table are Shown in Inches.

Dimensions Not Specified Are Not Standardized Since They Bear No Relation To Interchangeability.





-

17-18- MA



No. 7AG-TEE

No.7BG-TEE

Revised	4-14-45
	8-27-45
	9-19-48

SIZE	Acme Thds. Per In.	с	E	v	GMAX	ΕI	J	N	0	Ρ	Q	R	S	υ	w	z
1	8	38	116	3 32	7 16	2 39 64	.062	.902	1.219	1.317	1.462	1.160	1.316	9 64	1.187	4 64
1 1/2	8	4 1/2	2 4	3 32	<u>7</u> 16	3 1 <u>9</u> 64	.062	1.402	1 750	1.849	1.994	1 676	1.848	9 64	1.718	5 64
2	8	5 <del>1</del>	2 5	3 32	1 16	3 23 32	.062	1.870	2250	2381	2 526	2.192	2 380	<u>7</u> 32	2.218	6 <del>11</del> 32
21/2	8	6	3	3 32	17 32	41/8	.062	2.370	2.750	2 913	3.058	2 708	2.912	7 32	2.718	7 <del> </del>
3	8	6 5	3 16	3 32	9	4 1/2	.062	2.870	3312	3 4 4 5	3.590	3.224	3.440	<u> </u>	3.280	7 1 <u>3</u>
4	6	8 16	432	7 64	Ш 16	5 61 64	.062	3.834	4.312	4.509	4.695	4.256	4.508	9 32	4280	10 27

Dimensions in Above Table are Shown in Inches.

Dimensions Not Specified Are Not Standardized Since They Bear No Relation To Interchangeability.

3A STANDARD SANITARY FITTINGS 3A-100-06

147

FORMERLY EX-13233







150



#31-15 REDUCER

Revised-4-14-45 " 9-19-48

Corrected 1-31-49

	SIZE	Acme Thds. Per. In.	AB	۷	GMAX	н	N	Р	Q	R	s	U
	1 <u>1</u> "×1"	8	21	<u>5</u> " 32	2	1 3	.902	1.317"	1.462	1.676	1.848	3
	2"× I"	8	21	5" 32	916	1 10	.902"	1.317"	1.462	2.192"	2.380	4
	2"×11"	8	21	· <u>5</u> "	9 16	123	1.402	1.849	1.994	2.192	2.380	<u> </u> ".
*	2 <sup>1</sup> / <sub>2</sub> <sup>x</sup> 1"	8	21	3" 16	9	1 3	.902"	1.317"	1.462	2.708	2.912"	1 <sup>n</sup>
	2 <sup>1</sup> / <sub>2</sub> <sup>x</sup> 1 <sup>1</sup> / <sub>2</sub>	8	2 <u>1</u> "	3° 16	916	1 32	1.402	1.849	1.994	2.708	2.912	<u> </u>
	2 1 <sup>°</sup> x2"	8	2 <sup>19</sup> / <sub>32</sub>	3° 16	19 32	24	1.870	2.381"	2.526	2.708	2.912"	ŧ
*	3°¤ 1"	8	21	3" 16	9	13"	.902	1.317	1.462	3.224	3.444	9° 32
	3×11	8	21	3" 16	9° 16	132	1.402	1.849	1.994	3.224	3.444	9" 32
	3"x 2"	8	2 <sup>19</sup> 32	3" 16	19 32	24	1.870	2.381	2 526	3.224	3.444	<u>9</u> " 32
	3x21	8	233	3	23° 32	2 <del>3</del> "	2.370	2.913"	3.058	3.224	3.444	<u>9</u> " 32
*	4"x 1"	8	$2\frac{1}{2}^{"}$	<u> </u>	<u>9</u>	1 3	.902"	1.317"	1.462	4.256	4.508	5
*	4×12	8	21	1"	<u>9</u> 16	123	1.402	1.849	1.994	4.256	4.508	5
	4"x 2"	8	2 19	<u> </u>	19 32	24	1.870"	2.381	2.526	4.256	4.508	<u>5</u> " 16
	4x212	8	2 <sup>23</sup>	1 <u>4</u> .	23" 32	2 <sup>:3</sup> *	2.370	2.913	3.058	4.256	4.508	5
	4"x 3"	8	232	<u> </u> "	<u>3</u> "	3 <u>5</u> 3 <u>16</u>	2.870	3.445	3.590	4.256	4.508	<u>5</u> "





			1-	AC	,	1.							*м	inimum	IG Rodius
	SIZE	Acme Thds. Per. In.	AB	v	G	J	N	0	Ρ	Q	R	S	υ	w	
	1 <u>2</u> ×1"	8	2 <mark>17</mark> 264	3" 32	<u></u> Ζ.	.062	.902"	1.219"	1.317"	1.462	1.676	1.848	å	1.718*	
	2"× I"	. 8	2932	32	7" 16	.062	.902"	1.219"	1.317"	1.462	2.192"	2.380	<u>7</u> " 32	2.218"	
	2"×1	8	232	3132	Z"	.062	1.402"	1.750	1.849	1.994	2.192	2.380	7" 32	2.218"	
•	2 <sup>5</sup> ×۱	8	24"	<u>3</u> " 32	Z" 16	.062	.902"	1.219"	1.317"	1.462	2.708	2.912	7	2.718"	
	2242	8	24	3 32	7° 16	.062	1.402	1.750	1.849	1.994	2.708	2.912"	32	2.718"	
	2 <u>1</u> "x2"	8	2 5	3" 32	7 16	.062	1.870"	2.250	2.381	2.526	2.708	2.912	7" 32	2.718"	
*	3"×1"	8	24	<u>3</u> " 32	7" 16	:062"	.902"	1.219"	1.317"	1.462	3.224	3.444	-	3.280"	
	3×11	8	24	332	7° 16	.062	1.402°	1.750"	1.849	1.994	3.224	3.444	<u> </u>	3.280"	
	3"x 2"	8	2 <u>5</u>	3" 32	7"	.062	1.870 <sup>°</sup>	2.250	2.381	2.526	3.224	3.444	-4	3.280"	
	3 k 2 1	8	2 <u>13</u> "	3 32	17" 32	.062	2.370	2.750	2.913"	3.058	3.224	3.444	4	3.280"	
#	4"×  "	8	2 <u>13</u> 64	<u>7</u> " 64	7 16	.062	.902"	1.219"	1.317"	1.462	4.256	4.508	54	4.280"	
*	4%I	8	264	7 <sup>°</sup>	7°	.062	1.402	1:750"	1.849	1.994	4.256	4.508	9" 32	4.280"	
	4"x 2"	8	2 <u>17</u> 64	7" 64	7" 16	.062	1.870	2.250	2.381	2.526	4.256	4.508		4.280"	
	4x2 1	8	2 <u>23</u> "	7" 64	17" 32	.062*	2.370	2.750	2.913	3.058	4.256	4.508	32	4.280"	
	4"x 3"	8	2 <u>27</u> "	7" 64	9	.062"	2.870	3.312"	3.445	3.590	4.256	4.508	9" 32	4.280"	

\*31-15G REDUCER

\* Special Order Only



FORMERLY EX-13237

.


							<sup>♥</sup> 1/16	" Mini	mum				
	SIZE	Acme Thds. Per. In.	AB	v	G	н	к	N	P	Q	R	s	υ
	1 <u>1</u> "×1"	8	21	<u>5</u> 32	16	16	.250"	.902"	1.317"	1.462	1.676	1.848	<u>3</u> " 16
	2"× I"	8	21	<u>5</u> " 32	9" 16	1 3"	.484"	.902"	i.317"	1.462	2.192"	2.380	<u> </u> "
	2×11	8	2 <u>1</u> "	<u>5</u> " 32	9	1 32	.234"	1.402	1.849	1.994	2.192	2.380	<u> </u> "
*	2 <sup>1</sup> / <sub>2</sub> ×1"	8	212	3" 16	916	1 3"	.734"	.902	1.317"	1.462	2.708	2.912"	<u> </u> "
U	2 <sup>1</sup> / <sub>2</sub> ×1 <sup>1</sup> / <sub>2</sub>	8	2 <u>1</u> "	<u>3</u> " 16	916	123"	.484"	1.402	1.849	1.994	2.708	2.912"	<u> </u> "
	2 <sup>1</sup> / <sub>2</sub> ×2	8	2 <sup>19</sup> 32	<u>3</u> " 16	19 32	$2\frac{1}{4}$	.250"	1.870	2.381	2.526	2.708	2.912*	<u> </u>
*	3"× I"	8	21	$\frac{3}{16}$	9 0	1 3"	.984"	.902	1.317"	1.462	3.224	3.444	9° 32
	3×11	8	212	$\frac{3}{16}^{"}$	<u>9</u> "	135	.734"	1.402	1.849	1.994	3 224	3.444	9" 32
	3"× 2"	8	2 19 32	3" 16	19 32	$2\frac{1}{4}$	.500"	1.870	2.381	2.526	3.224	3.444	<u>9</u> " 32
	3×21	8	2 <sup>23</sup> 32	3	23 32	2 3"	.250"	2.370	2.913"	3.058	3.224	3.444	<u>9</u> " 32
*	4"x  "	8	2 <u>1</u>		<u>9</u> "	1 3"	1.466"	.902"	1.317"	1.462	4.256	4.508	5" 16
*	4×1-2	8	21	-14	9 16	123	1.216	1.402	1.849	1.994	4.256	4.508	5"
	4"x 2"	8	2 32	<u> </u>	<u>19</u> " 32	24	.982"	1.870"	2.381	2.526	4.256	4.508	5" 16
	4x21	8	2 <u>23</u> "	<u> </u>	23 32	2 3"	.732"	2.370	2.913	3.058	4.256	4.508	5"
	4*x3*	8	232	<u> </u>	<u>3</u> "	3 16	.482	2.870	3.445	3.590	4.256	4.508	5"

NHP

Q



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1 as the as

	SIZE	Per. In.	AB	V	G	J	ĸ	N	0	Ρ	Q	R	S	υ	w
	1 12 ×1"	8	2 64	32	<u>7</u> " 16	.062"	.250"	.902"	1.219"	1.317"	1.462	1.676	1.848	9 64	1.718*
	2"× I"	8	2.9	3"	7"	.062	.484"	.902"	1.219"	1.317"	1.462	2.192"	2.380	<u>7</u> " 32	2.219"
	2"×1	8	232	3 32	7	.062"	.234"	1.402"	1.750	1.849	1.994	2.192	2.380	7"	2.218*
*	2 <sup>1</sup> / <sub>2</sub> ×1"	8	24	212	<u>7</u> "	.062"	.734"	.902"	1.219"	1.317"	1.462	2.708	2.912	<u>7</u> " 32	2.718"
	2242	8	24	3	7° 16	.062"	.484"	1.402	1.750	1.849	1.994	2.708	2.9i2"	<u>7</u> " 32	2.718"
	2 <u>1</u> x2"	8	2 <u>5</u> "	3	7" 16	.062"	.250"	1.870	2.250	2.381	2.526	2.708	2.912	<u>7</u> 32	2.718"
*	3"×1"	8	2 <u>1</u>	3" 32	Z" 16	.062"	.964"	.902"	1.219"	1.317"	1.462	3.224	3.444	<u> </u> "	3.280"
	3 x 1 1 2	8	24	3	7" 16	.062*	.734"	1.402"	1.750"	1.849	1.994	3.224	3.444	<u> </u> "	3.280"
	3"x 2"	8	2 16	3" 32	7" 16	.062"	.500"	1,870	2.250	2.381	2.526	3.224	3.444	4	3.280"
	3 % 2 <u>1</u>	8	2 <u>13</u> " 32	3" 32	17" 32	.062"	.250"	2.370	2.750	2.913"	3.058	3.224	3.444	<u> </u> "	3.280"
*	4"× l"	8	2 <u>1</u> 3 64	<del>7</del> " 64	7	.062"	1.466"	.902"	1.219"	1.317"	1.462	4.256	4.508	<u>9</u> " 32	4.280"
*	4%1 1	8	2 <mark>13</mark> 264	7" 64	7" 16	.062"	1.216	1.402"	1.750	1.849	1.994	4 256	4.508	9"	4.280"
	4"x 2"	8	2 <u>17</u> 64	7" 64	7" 16	.062"	.982"	1.870	2.250	2.381	2.526	4.256	4.508		4.280"
	4x21	8	2 <u>23</u>	<del>7</del> 64	17" 32	.062"	.732"	2.370	2.750	2.913	3.058	4.256	4.508	- "	4.280"
	4"x 3"	8	227	7" 64	9 <sup>°</sup>	.062"	.482"	2.870	3.312"	3.445	3.590	4.256	4.508	0"	4.280"

# 32-15G REDUCER

**3A STANDARD** SANITARY FITTINGS 3A-100-10

SUPERSEDES DRWG. EXIII78

Revised 4-14-45 · 9-18-48 Corrected 1-31 - 49

# 32-15 REDUCER

FORMERLY EX-13191

151

\*Special Order Only



9-19-48

**3A STANDARD** 

SANITARY FITTINGS

3A-100-11

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Ground Gasket Seat Type U U ACME THD'S PER IN Ground Seat Type P R Т 0 Q S Size BL CL DL AL I G 9' 64 3 3 16 1.187 5" 9 3 7 .062 1.219 1.317 1.462 1.160 1.316 1.005 43 13 11 34 1" 8 8 <u>9</u>" 3" 3 .718 1-1" 1 32 7 .062" 1.750 1.849 1.994 1.676 1.848 1.505 7" 5 32 9 1<u>↓</u> 64 1 1/2 1 15 8 7" 3 7 4 .062 2.250 2.381 2.526 2.192 2.380 2.005 2.218 1 16 5 32 19 24 1 32 13 1 32 7" 2 8 17 23 3" 1 2.718 3 23 .062 2.750 2.913 3.058 2.708 2.912 2.505 13 1-16 32 21 1-4 8 12 32 1 20 .062 3.312 3.445 3.590 3.224 3.444 3.005 3 34 316 3" 9 5 1 16 1 3.280 17 5 3 8 1 7 <u>9</u>" 32 1" 7 5 21 15 14 3 16 .062 4.312 4.509 4.695 4.256 4.508 4.005 4.230 4" 112 44 6

Gasket Type

Dimensions Not Specified Are Not Standardized Since They Bear No Relation To Interchangeability.

SUPERSEDES DRWG.EXIIIBI

W

FORMERLY EX-13192





13 SH HEX NUT

SANITARY FITTINGS 3A-100-13









SIZE	ACME THD. PER INCH	A	AA	в	G	н	N	Р	Q	R	S	U	V
1"	8	2"	4"	1/2"	9/16	13/16	.902"	1.317 "	1.462"	1.160"	1.316"	3/16	1/8
11	8	21/8	534	24	9/16	123	1.402"	1.849"	1.994"	1.676"	1.848"	3/16	5/32
2"	8	323	77/16	3"					2.526"				5/32
21	8	427	91/16	4"	23	23/4	2.370"	2.913"	3.058"	2.708"	2.912	1/4	3/18
3."	8	529"	1113,6	5"	3/4	35/16	2.870"	3.445"	3.590"	3.224"	3.444"	9/32	3/18
4"	6	8/16	16/8	7"	1/8	414	3.834"	4.509"	4.695"	4.256"	4.508"	5/16	1/4



7X TEE









7XG TEE



AVOID SHARP CORNERS 13H NUT-MIN. RADIUS 000 QPON AG -G → AAG

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7AXG TEE

7BXG TEE

SITE	ACME THDS	AG	AAG	AGY	в	G	J	N	0	Р	Q	R	S	U	V	W	Y
1	PER INCH	178	33/	351/	11/2	7/16	.062"	.902"	1.219"	1.317"	1.462"	1.160"	1.316"	9/64	3/32	1.187 "	15%4
11/2	8	-	51/2	533/64	21/2	7/16	.062	1.402"	1.750"	1.849"	1.994"	1.676"					24%4
2"	8	39/16		73/16	3"	7/10	.062"	1.870"	2.250"	2.381"	2.526"	2.192"	2.380"	1/32	3/32	2.218"	3 5/8
21/2	8		95/16	93/8	4"	17/32	.062"	2.370*	2.750"	2.913"	3.058	2 708"	2.912"	1/32	3/32	2.718"	423/32
3"	8	523/32		11/2"		9/16	062"	2 870"	3.312"	3.445"	3.590"	3.224"	3.444"	1/4"	3/32	3.280"	5232
4	6			154964		11/16		3.834"	4.312"	4.509"	4.695"	4.256"	4.508"	9/32	764	4.280"	75%4





SIZE	PER INCH	С	CG	D	E	EG	GMAX	GGMA	н	J	N	0	Р	Q
<b>1</b>	8	35/8	33/8	121/32	1 13/16	11/16	9/16	7/16	13/16	.062"	.902"	1.219"	1.317"	1 462"
11/2	8	43/4	41/2	23/16	23/8	21/4	9/16	7/16	123/32	.062"	1.402"	1.750*	1.849"	1.994"
2"	8	5%	51/4"	219/32	225	25/8	19/32	7/16	21/4	.062"	1.870"	2.250"	2.381	2.526"
21/2	8	63/8	6"	3"	33/16	. 3"	23/32	17/32	23/4	.062"	2.370"	2.750"	2.913"	3.058"
3"	8	7"	65/8	3%32	31/2	35/16	3/4	9/16	35/16	.062"	2.870*	3.312*	3.445"	3.590"
4"	6	95/16	815/16	413/32	42/32	415	7/8	11/16	41/4	.0,62"	3.834"	4.312"	4.509"	4.695"





63/8

7

95/16

21/2"

3"

4"

8

8

6

33/16

421

6

815/16

65/8 31/2

3'

35/16

415

23/1

3/4

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4 3

2.370

2.870"

.062" 3.834"

2.750

3.312

4.312

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3.445

4.509

3.058

3.590

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.062

.062

SANITARY FITTINGS 3A-100-25

6-23-55



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### DIAPHRAGM-TYPE VALVE

The internal design shown is intended to demonstrate general principles only, and is not intended to limit individual ingenuity. The design used shall conform with the general sanitary requirements set forth in this 3-A Sanitary Standard and specific requirements for diaphragm-type valves.

The connections may be threaded or be of a design to be used with non-threaded sanitary connections (flanged, clamp type, etc.) or may have plain ends for use in welded sanitary pipelines. Valves made with threaded connections shall conform with the dimensions for (1) threads and (2) those at the connections (including the inside diameter) shown on the drawings for other fittings included in this 3-A Sanitary Standard. Center to face dimensions will vary with valves made by different manufacturers.

The mounting instruction plate is to (1) show and describe positively the self draining angle when the value is put in service and (2) have a statement that only food grade lubricant shall be used on the value stem.

> 3A STANDARD SANITARY FITTINGS 3A-100-26



## EXTERNAL THREAD

SIZE	ACME THD'S PER IN	Р	Q	PITCH DIA	TOLERANCE P. Q & P.D.
r	8	1.317	1,462	1.3995	+.000 018
1 1/2	8	1.849	1.994	1.9315	+.000
2	8	2.381	2.526	2.4635	+.0 00
21/2	8	2.913	3.058	2.9955	+.000
3	8	3.445	3.590	3.5275	+.000
4	6	4.509	4.695	4.6120	+.000



INTERNAL THREAD

SIZE	ACME THDS PERIN	Р	Q	PIT.CH DIA	TOLERANCE
1	8	1.352	1.497	1,4145	+.018 000
1 1/2	8	1.884	2.029	1.9465	+.019
2	8	2.416	2.561	2.4785	+.020 000
2 1/2	8	2.948	3.093	3.0105	+ .021
3	8	3.480	3.625	3.5425	+ .022
4	6	4.544	4.730	4.6270	+ .025

THREAD DIMENSIONS

3A STANDARD SANITARY FITTINGS 3A-100-30

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~ 54 5- 1

THESE ARE GENERAL PURPOSE ACME THREADS CLASS 2G.

## A Research Note

## ISOLATION OF VIBRIO PARAHEMOLYTICUS FROM GULF COAST SHRIMP<sup>1, 2</sup>

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and

J. C. PARKER Agricultural Extension Service Texas A&M University College Station 77843

(Received for publication December 11, 1969)

#### Abstract

Vibrio parahemolyticus, a major cause of gastroenteritis in Japan, was isolated from white shrimp (*Penaeus setiferus*). The organism was pathogenic for brown shrimp (*Penaeus aztecus*) from the Gulf of Mexico.

Vibrio parahemolyticus is the causative agent of an infectious food poisoning syndrome associated with warm summer months and the consumption of raw sea food (7). The majority of isolates have come from Japan where V. parahemolyticus causes 70% of the gastroenteritis in that country (6). The organism has been more widely studied in the United States in recent years. It has been isolated in the northwest Pacific from Puget Sound water, sediment, and oyster samples (2); from human infections related to marine bathing (8); from Gulf of Mexico and Southern Atlantic coast sediments (10); and from lethargic and moribund blue crabs in Chesapeake Bay (5).

#### EXPERIMENTAL METHODS

This study was prompted by high mortality among brown shrimp which were kept for nutritional experiments in laboratory aquaria. Nutritional trials were in conjunction with shrimp farming operations carried out by the Texas Agricultural Extension Service under the Sea Grant Program. The brown shrimp were caught in Bastrop Bayou of West Galveston Bay and were placed 6-7 in each of ten 4-liter aquaria containing water of the Gulf of Mexico. High mortalities were observed when the brown shrimp were fed bits of frozen white shrimp. The white shrimp were caught in Oyster Lake on West Galveston Bay. They were iced immediately, stored under refrigeration for 24 hr and then frozen. The water in the aquaria became gradually turbid and a peculiar foul odor, was noticeable. The degree of mortality was highest in the aquaria with the greatest turbidity. Within 24 hr, all but 4 of the 65 shrimp died.

Samples of dead brown shrimp, white (food) shrimp, and aquarium water were streaked on blood agar (BA) plates (trypticase soy agar, BBL, with 10% defibrinated sheep blood) and Standard Methods Agar (SMA, Difco) prepared with either distilled water or water from the Gulf of Mexico. Sampling of shrimp was carried out by swabbing the exposed interior of the cephalothorax region with sterile cotton swabs. After an initial streak with a cotton swab, further streaking of the plates was done with a streaking needle. The plates were incubated at 32 C for 24 hr. Isolated colonies were then placed on brain heart infusion (BHI, Difco) slants for further diagnostic tests (1, 3, 8, 9).

#### RESULTS AND DISCUSSION

All samples streaked on BA and SMA plates prepared with seawater yielded predominantly circular, moist, light-brown colonies with entire edges. This colony type was not present on the plates prepared with distilled water. Preliminary examination showed that this isolate was a gram-negative, pleomorphic rod with a single polar flagellum. Addition of 3% inoculum (24 hr culture in BHI broth) to an aquarium caused death of brown shrimp in a few hours.

Additional characteristics of the isolate are listed in Table 1. Identification of the isolate as *V. parahemolyticus* was further confirmed by deoxyribonucleic acid (DNA) base composition and serological analysis. Base composition was determined by melting point measurement of purified DNA. The DNA base composition was 45.4 mole per cent of guanine plus cytosine. This is within the range reported for *V. parahemolyticus* (4). The isolate reacted with polyvalent 3 antisera and specifically with K30 antiserum. The organism also was pathogenic for mice.

In other experiments an 18-24 hr BHI culture of the isolate was added to aquaria with brown shrimp to yield a concentration of approximately  $10^4$  and  $10^5$ cells per ml of water. Within 0.5-3 hr, depending somewhat upon the size of shrimp and level of inoculation, the shrimp became uneasy and jumped hitting the cover of the aquarium. They then would drop to the bottom of the aquarium, lay on their side and then jump again. This was followed by death within 3 hr. V. parahemolyticus was recovered almost in pure culture from the interior of the cephalothorax region. Control shrimp (without added cul-

<sup>&</sup>lt;sup>1</sup>Technical article no. 8216 of the Texas Agricultural Experiment Station, College Station.

<sup>&</sup>lt;sup>2</sup>This work was funded in part by the NSF Sea Grant Program Institutional Grant GH-59 made to Texas A&M University.

	G
Morphology	Gram negative pleomorphic rod
Flagella	Single polar
Growth with 7.5% NaCl	+
Growth at 45 C	+
Glucose	Acid, no gas
Mannitol	Acid, no gas
Arabinose	Acid, no gas
Dulcitol	-
Lactose	
Sucrose	_
$H_2S$	Slight in TSI
Oxidase	+
MR	+
V-P	_
Citrate utilization	+
Nitrate reduction	+
Indole production	+
Catalase production	+
Urease production	_
Decarboxylases, lysine	-+
arginine	
ornithine	
Gelatin hydrolysis	+
NH <sub>3</sub> from arginine (anaer.)	-
Hemolysis	Beta
Malonate	_
Sensitivity to pteridine 0/129	+
Swarming (1% agar)	+
Luminescence	_
-	

 TABLE 1. MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS

 OF V. parahemolyticus isolated from shrimp

ture) remained alive during the experimental period.

The predominant type of colony on the BA and SMA plates prepared with distilled water consisted of small white circular umbonate colonies with entire edges. Subsequent analysis of this flora indicated that "coryneforms" predominated. These isolates were not pathogenic to shrimp.

The speed with which the culture of *V. parahemolyticus* acted on shrimp would indicate that an exotoxin might be present. However, it is reported (7) that enteropathogenicity with human volunteers was established only by administering live cultures.

The isolation of *V. parahemolyticus* from Gulf Coast shrimp further demonstrates the ubiquity of this microorganism in marine waters and seafoods. Its demonstrated pathogenicity for brown shrimp might cause reductions in shrimp yields both from marine waters or from pond operations. Seafood contaminated with this organism could become a source of food poisoning, similar to the situation existing in Japan. In addition, handling of infected shrimp could become a mode of transmission for human tissue infections. Further studies on the distribution of *V. parahemolyticus* in seafoods and the pathogenicity of these isolates for humans and various types of shellfish seem highly warranted.

#### Acknowledgment

The authors express their great appreciation to Dr. Rita R. Colwell (Dept. of Biology, Georgetown Univ., Washington, D. C.) for the DNA base composition and serological analysis.

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## SELECTIVE CULTURE OF SPOILAGE AND INDICATOR ORGANISMS<sup>1, 2</sup>

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#### Abstract

Methodology for detection and enumeration of microorganisms has involved an increasing awareness of the role of cellular injury on analytical microbiology. Injury has been shown to result from heat, freezing, chemicals, irradiation, and similar treatments. All of these treatments are commonly used in the processing and preservation of foods. Since injured microorganisms can recover and cause food spoilage or since injured pathogens can recover and become infective, the detection of injured pathogens or of injured index microorganisms is imperative in order to assure the safety of foods. The detection of injured microorganisms becomes more difficult with the use of selective media. Environmental conditions or added inhibitors which are used to allow only the development of the types being sought may exert unsuspected inhibition. This shortcoming in our methodology appears to occur in the use of selective media in quantitative determinations, as well as in enrichment media that are designed to promote the growth and easier qualitative detection of a specific pathogen or indicator organism. One of the most pressing problems facing the food microbiologist at this time appears to be means to resuscitate an injured microorganism so that it can be detected selectively in the quantitative or qualitative examination of foods.

Detection of certain index microorganisms, pathogens, and spoilage types of microorganisms is essential to the successful surveillance of safe and economic food supplies. Many food products must comply with certain minimum standards regarding the presence of indicator microorganisms, and there is a zero tolerance for microorganisms such as sal-Methodology and standards are based monellae. on the premise that approved enrichment and selective media permit the unimpaired growth of given types of microorganisms to the exclusion of others. This presumes that the sought after microorganisms possess rather sharp demarcation of tolerances to environmental conditions that are used to exclude the growth of unwanted types.

The foregoing assumptions obviously contain shortcomings. Not all strains of a given indicator microorganism, or pathogen, are able to grow optimally in the presence of inhibitors used in selective media. These variations in response are expressions of individuality among strains. The productivity of a given selective medium for the detection of a given microorganism also is affected by the contributions from the menstruum being examined for certain bacteria. For example, food ingredients may dilute the inhibitory qualities of the medium, or certain other ingredients may have a synergistic or antagonistic interaction with microorganisms being sought. Finally, the detection of survivors of different environmental stresses may be further impeded by the presence of restrictive compounds present in selective media.

The food microbiologist is increasingly being concerned with microorganisms in foods that have received some type of processing or preservation treatment. Survivors in such foods have been subjected to a stress which may have left them in a state of injury. Unfortunately, injury in microorganisms may persist for some period of time before the cells expire and some injured cells apparently persist during the entire life-time of the food product. There is always the possibility that injured cells may recover in the food product, particularly if the food is diluted with other foods. Furthermore, there is increasing evidence that food-borne pathogens may still maintain pathogenicity even though they are Consequently, it has become more and injured. more important to detect injured as well as uninjured microorganisms that may exist in foods.

#### SUBLETHAL HEATING

Nelson (16) noted that microorganisms which had survived heat treatment comparable to that used for pasteurization of milk often exhibited increased demands for nutrients in order to form colonies on agar media. However, after the organisms recovered on a richer medium, they then resumed their ability to grow on a less nutritious one. Later, Lawton and Nelson (12) observed that when cultures of psychrophiles were treated by sublethal heat, the survivors had a greatly increased lag phase, particurlarly when held at 5 or 10 C. Also, immediately after the heating a larger number of survivors of the sublethal treatment were enumerated on plate count agar than

<sup>&</sup>lt;sup>1</sup>Paper number 2997 of the Journal Series of the North Carolina State University Agricultural Experiment Station, Raleigh, North Carolina.

<sup>&</sup>lt;sup>2</sup>Presented at a Round Table on Current Developments in Detection of Microorganisms in Foods at the 69th Annual Meeting of the American Society for Microbiology, Miami Beach, Florida, May 4-9, 1969.

on the older tryptone glucose meat extract agar; but, after 24 hr of storage the organisms were able to grow comparably on the two media, indicating repair of the injury.

It was observed by Busta and Jezeski (3) that Staphylococcus aureus after sublethal heat treatment lost its ability to multiply and form colonies on S-110 medium containing the normal concentration of 7.5% sodium chloride. The organisms, however, were viable as evidenced by their ability to multiply in milk and to form colonies on plate count agar. It was necessary to allow the injured staphylococci to recover from their injury before comparable counts could be obtained on the S-110 and plate count agar media. Similar results have been reported by Beuchat and Lechowich (2) on heat injured Streptococcus fecalis. This organism also was found to become sensitive to increased salt concentrations after sublethal heating.

It has been observed by various workers that bacterial spores were more exacting in requirements for growth after sublethal heating. Edwards et al. (6) examined the survival in milk of spores of *Bacillus subtilis* after various ultrahigh temperature treatments. The injury was expressed by an increased requirement in nutrients for colony formation, by the greater number of survivors enumerated by a lower incubation temperature, and by an increased number of survivors enumerated on the less nutritious medium after low temperature storage. Obviously, an inability to detect injured spores may lead to false interpretation of thermal processes designed to sterilize foods.

While heat may cause injury to cells and complicate their detection, it should be noted that heat, in some instances, can also have the reverse effects. For example, it is well known that certain bacterial spores germinate more successfully after heat shock treatment. A similar phenomenon occurs with nonsporulating microorganisms, particularly in spraydried milk powders. It was observed by Hiscox (10) that spray dried milk powders gave consistently much higher bacterial counts when reconstituted in Ringer's solution at 50 C rather than at 18 to 20 C. It was essential that the powder be reconstituted at 50 C rather than reconstituted at the lower temperature and then warmed to 50 C in order to obtain the increased count. Speck and Myers (20) reported similar results for spray-dried milk cultures of Lactobacillus bulgaricus. The many fold increase in colony count of the culture when reconstitution was at 50 C was substantiated by the increased rate of acid produced in milk by the Lactobacillus bulgaricus. Although Standard Methods for the Examination of Dairy Products recommends the reconstitution of spray dried powders at 45 or 50 C, the

importance of this procedure on the detection of microbial contaminants in spray-dried milk powders seems not to be widely recognized or understood.

#### FREEZING OF MICROORGANISMS

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Gunderson and Rose (7) noted that Escherichia coli and Aerobacter aerogenes inoculated into chow mein and stored at -14 F became increasingly less able to form colonies on violet red bile agar. This medium was able to detect only 12 to 25% of the cells that were able to form colonies on tryptone glucose meat extract agar after extended periods of frozen storage. Similar results have been recorded by Hartsell (8) on the detection of various pathogenic microorganisms present in frozen foods. Straka and Stokes (21) introduced the concept of metabolic injury for those cells which, after freezing, exhibited an increased dependency on organic nitrogen in order to grow and produce colonies. Similar results were reported for Shigella sonnei (15). Thus, certain gramnegative rods appear to require amino and peptide nitrogen rather than inorganic nitrogen after being stored in the frozen state. Freezing and frozen storage caused lactic streptococci to show an increased dependency on nitrogeneous compounds present in enzymatic hydrolysates for colony formation (13). Nevertheless, the effects of freezing appear to be more marked on gram-negative rods than on gram-positive microorganisms. It was noted by Baird-Parker and Davenport (1) that S. aureus was relatively insensitive to freezing, and that the influence of the recovery medium on its isolation after freezing did not appear to be pronounced. The medium developed by these workers did, however, show better recovery of frozen staphylococci than did medium No. 110. The recovery of Achromobacter from frozen food was found to be hampered when a selective medium was used, although the same medium had no effect on non-treated cultures (22).

The increased demand by injured cells for certain preformed nutrients has indicated damage to certain enzymes important in synthesizing essential cellular constituents. Concommitant with injury is leakage from cells of UV absorbing compounds, and much evidence suggests that chilling and freezing alter membrane permeability. Peptides leaking from frozen E. coli, and certain peptides in trypticase, allow repair of the injury. The role of intracellular ice formation on the activity of essential molecular components of the cell remains to be clarified. Storage of a streptococcal proteinase at -20 C has shown that its structure was altered and a lowered biological activity resulted. Loss in ability to produce acid in milk was associated with aggregation of the enzyme from monomer to polymer (4). At the present we have not explored further the relation of such alterations in cellular components to injury.

#### INJURY BY CHEMICALS

Certain chemicals are used in the food industry as a permissible ingredient to assist in the preservation of foods, while others are used for sanitization of equipment in the processing of foods. Heinmets, et al. (9) reported that suspensions of *E. coli* which had been treated with chlorine, a quaternary ammonium compound, and ethyl alcohol appeared to be sterile when incubated in buffer or in nutrient broth. When certain metabolites of the tricarboxylic acid cycle were added, survivors could be detected after each of the treatments. It was postulated that reactivation of injured cells was made possible by the presence of suitable metabolites which were immediately utilized as energy sources and thereby permitted growth of survivors.

Scheusner (17) reported that a large percentage of E. coli surviving treatment with a quaternary ammonium compound and hypochlorite sanitizers were not detected by the use of violet red bile agar. While the detection of chemically treated cells is reduced, it also should be noted that the detection of untreated E. coli by violet red bile agar is dependent on the physiological state of the cell. Cells in the late lag and early logarithmic phases gave noticably reduced counts but comparable counts were obtained as growth proceeded into the stationary phase. The inability of the E. coli to grow on violet red bile agar after treatment with 30 ppm of a quaternary ammonium compound was not associated with metabolic injury; exposure up to 120 sec gave comparable counts on trypticase soy agar and a minimal agar containing ammonium salt as a nitrogen source. However, with increased exposure time an increasing number of the survivors failed to grow on violet red bile agar. These data would indicate some difference in the mechanism of injury by freezing and by a surface active sanitizer.

Yeast and mold counts often are made with potato dextrose agar (PDA) acidified to pH 3.5 with tartaric acid. It was observed (11) that significantly higher counts, mostly of yeasts, were recovered when Cook's 'rose bengal agar was used instead of the PDA. The low pH appeared to be particularly inhibitory to the microorganisms suffering prior injury in foods.

#### <sup>\*</sup> Recovery of Injured Microorganisms

Our lack of information on the cellular lesions causing metabolic injury of microbial cells is accentuated by efforts that have been directed to effect recovery of these organisms. The data of Heinmets et al. (9) on the beneficial effects of metabolites from the tricarboxylic acid cycle on recovery of sublethally injured *E. coli* suggest the involvement of energy related systems. This has been observed by other workers for other microorganisms surviving certain environmental stresses. Baird-Parker and Davenport (1) reported that recovery of heated or dried cells of *S. aureus* was best on media containing blood or sodium pyruvate. It was postulated that some of the dried cells had at least a decreased catalase activity and that the pyruvate and blood were effective owing to their ability to eliminate hydrogen peroxide.

Efforts have been made to permit the recovery of microorganisms on non-selective media prior to using selective media (14). In detecting members of the family *Enterobacteriaceae*, the high toxicity of brilliant green could be overcome by first inoculating food samples into infusion broth with subsequent inoculation into brilliant green bile broth for detecting *Enterobacteriaceae*. Also, samples were spread onto a rich infusion agar which after incubation was replicated onto violet red bile glucose agar. This recovery procedure permitted an increase of up to 1000-fold of the *Enterobacteriaceae*.

The use of enrichment procedures to allow recovery of injured cells may have definite limitations, however, particularly when samples of food products are examined. For example, Dack and Lupitz (5) found the natural flora of frozen pot pies to exert an inhibitory effect upon the growth of Salmonella typhimurium, E. coli, and an enterotoxigenic strain of Staphylococcus. Similar results were reported by Mossel, et al. (14) when samples were placed in infusion broth for enrichment prior to the examination for members of the Enterobacteriaceae on selective media. Therefore, some natural antagonistic interactions that may occur among microorganisms may impose strict limitations on the use of enrichment techniques for the subsequent detection of food-borne pathogens by selective media.

#### EFFECT OF METABOLIC INJURY ON PATHOGENICITY

Schlamm (18) noted that it was not possible always to correlate the viability of *Pasteurella tularensis* with virulence. Two growth media were able to detect viable *P. tularensis* when virulence was determined by intraperitoneal injection of mice. However, after respiratory challenge with aged cells the parallelism was lost. A number of cells capable of growth and colony formation on the agar media apparently was no longer able to initiate successful infection in the host animal.

Sorrells (19) observed that Salmonella gallinarum

showed metabolic injury after freezing much in the same manner as do other gram-negative bacteria. Chicks were inoculated to evaluate the pathogenicity of cells injured metabolically by freezing. The study showed no significant difference at the 95% confidence limit between the pathogenicity of injured and uninjured cells. Therefore, in establishing whether or not foods are free from pathogens, it becomes important to ascertain that organisms being sought are not present even in an injured state that may escape detection by certain routine analytical procedures currently used in microbiology.

#### CURRENT SITUATION

The selective detection of spoilage indicator and pathogenic microorganisms in foods is complicated by certain inherent inadequacies in current methodology.

Different strains of a given microorganism appear to vary in their ability to grow on a given selective medium or under given selective cultural conditions. The age of the bacterial cell contributes to such variation since young cells are more susceptible to the selective agents than are mature cells.

Microorganisms which survive various environmental stresses placed upon them by the processing of foods may be in a state of injury which increases their susceptibility to inhibitory conditions present in selective media. The detection of injured cells must be accomplished, since injured cells of spoilage types can recover in foods and injured pathogens appear to be equally as infective as non-pathogens.

The dilution of selective culturing conditions by the amount or types of components within a given food can be a further factor contributing to selective typing of cultures.

Enrichment procedures designed for recovery of injured cells or the development of low populations of specific types, prior to their detection on selective media, must consider the possible antagonistic effects of the food microflora.

#### Acknowledgement

This investigation was supported (in part) by Public Health Service Research Grant No. UI-00154 from the National Center for Urban and Industrial Health.

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#### RECENT U. S. GOVERNMENT AND UNIVERSITY PUBLICATIONS OF INTEREST TO THE FOOD INDUSTRY DECEMBER 1969

#### Compiled by M. Kroger, Ph.D., Assistant Professor of Food Science, The Pennsylvania State University

Slaughtering, cutting, and processing beef on farm. Farmers' Bulletin 2209, revised Aug. 1969, 32 p. Agricultural Research Service, U. S. Dept. of Agric. \$.25\*.

Food and your weight. Home and Garden Bulletin 74, revised Aug. 1969, 30 p. Agricultural Research Service, U. S. Dept. of Agric. \$.15\*.

Pantothenic acid, vitamin B6, and vitamin B12 in foods. Home Economics Research Report 36, Aug. 1969, 53 p. Agricultural Research Service, U. S. Dept. of Agric. \$.55<sup>\*</sup>.

Dietary levels of households, in United States, Spring 1965. Household Food Consumption Survey, Report 6, July 1969, 117 p. Agricultural Research Service, U. S. Dept. of Agric. \$1.00°.

Packers and stockyards act, what it is, how it operates. Program Aid Series, No. 399, Aug. 1969, 8 p., Div of Information, U. S. Dept. of Agric., Washington 20250.

Your livestock claims, packers and stockyard act can help you recover losses. Program Aid Series, No. 591, revised July 1969, 6 p. Div. of Information, U. S. Dept. of Agric., Washington 20250.

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\*Note-Available from Superintendent of Documents, U. S. Government Printing Office, Washington, D. C. 20402.

## PREDICTION OF STANDARD PLATE COUNT OF MANUFACTURING-GRADE RAW MILK FROM THE PLATE LOOP COUNT

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(Received for publication September 24, 1969)

#### Abstract

Plate Loop Counts and Standard Plate Counts of manufacturing grade raw milk samples were compared. With bacterial counts of 200,000/ml and less, the Plate Loop Count approximated the Standard Plate Count very closely. With counts greater than 200,000, the agreement was not as close. Differences between counts from the two methods, however, were not statistically significant (P<0.05) within either count range.

An adjustment factor was determined to enable the Plate Loop Count to more accurately predict the Standard Plate Count of milk with a Standard Plate Count >200,000/ml. The Standard Plate Count can be reasonably predicted from the Plate Loop Count by the formula:

Standard Plate Count (predicted) = (Plate Loop Count).<sup>1.04</sup>

Conversion from can cooling to bulk cooling of milk on the farm has caused quality problems in the milk supply. LaGrange and Nelson (2) pointed out the inadequacy of direct microscopic counts and reduction tests to measure the quality of bulk-cooled milk. These tests are not accurate for grading bulk-tank milk. The Standard Plate Count, while relatively accurate, is time consuming and more expensive.

The Plate Loop Count (PLC), more rapid and less expensive than the SPC, is a standard procedure in Standard Methods for the Examination of Dairy Products (1). It was developed by Thompson, Donnelly, and Black (5).4 They reported a high correlation between the PLC and SPC on samples with bacterial counts as high as 630,000/ml. Tatini, Dabbah, and Olson (4) noted that with farm bulk-tank milk samples, significant differences between the average bacterial count by SPC and PLC were not obtained when the counts were equal to or less than 100,000/ml; when the counts exceeded 100,000/ml, significant differences were observed. They concluded that, since bacterial counts of manufacturing-grade milk are likely to exceed 100,000/ml in many instances, this procedure should not be used to grade such milk.

This study was undertaken to find a method of using the PLC on high-count raw milk (>200,000/ml).

#### Procedure

The Iowa Department of Agriculture dairy inspectors collected 83 samples of herd-composite milk from manufacturing dairy plants within the state. Samples were collected and handled as specified in *Standard Methods for the Examination* of *Dairy Products (1)*. The counts were determined by the Dairy Division Bacteriological Laboratory of the Iowa Department of Agriculture. Procedures used for determining the SPC and PLC are described in *Standard Methods for the Examination of Dairy Products (1)*.

#### RESULTS AND DISCUSSION

Primary interest was in the development of a functional relationship between the two counts from samples having a bacterial count between 200,000 and 3,000,000/ml. After determing the PLC, a technician could calculate an estimate of the SPC. Milk with bacterial counts >3,000,000 is unacceptable by current standards. The agreement of the two methods for counts <200,000/ml was verified by a regression of SPC on the PLC base on 40 pairs of observations. The slope was 1.17 with a standard deviation of 0.066, which was not significantly different (3) (P<0.01) from a slope of 1.00.

Examination of higher-count milks indicated that such an exact relation between SPC and PLC did not exist. The degree of difference between the two methods of determination was indicated by an analysis of variance (3) for low-count (<200,000/ml) and relatively high-count (200,000 - 3,000,000/ml) milk. For the determinations of nine samples for both methods with bacterial counts <200,000/ml, there was no significant difference between methods (Table 1). These samples are different from the 83 previously mentioned that were used to calculate the derived relation. A greater difference in the two methods is indicated by the larger F value for 25 samples containing >200,000/ml (Table 2), but the F value was not significant. Again 5 determina-

<sup>&</sup>lt;sup>1</sup>Journal Paper No. J-6379 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 1050.

TABLE 1. ANALYSIS OF VARIANCE FOR MILK SAMPLES WITH BACTERIAL COUNTS LESS THAN 200,000/ML.

TABLE 3. A COMPARISON OF THE ACTUAL STANDARD PLATE (

predicted Counts	AND

Source	df	SS .	$\mathbf{MS}$	F
Methods	1	0.2122	0.2122	0.22
Samples within methods	16	15.2433	0.9527	
Determinations within samples	72	0.2514	0.0035	
Total	89	15.7069		

TABLE 2. ANALYSIS OF VARIANCE FOR MILK SAMPLES WITH BACTERIAL COUNTS GREATER THAN 200,000/ML.

Source	df	ss	MS	F
Methods	1	0.4831	0.4831	1.41
Samples within methods	48	16.4891	0.3435	
Determinations within samples	200	0.8474	0.0042	
Total	249	17.8196	e.	
		×		

Estimated log SPC



log PLC

tions were made per sample. These analyses were done on logs of the original bacterial counts. Therefore, any differences indicated would be much greater on the original scale. Furthermore, the possible differences for high-count milk are much more marked than those for the low-count milk.

To satisfy underlying statistical assumptions it was necessary to transform the bacterial counts to the log scale to stablize the variance. Several approaches were considered as possible prediction equations. These included functional forms such as  $SPC' = a + b \times PLC'$  and  $SPC' = b \times PLC'$ , where the prime' indicates the log scale. Also considered was the possibility of dividing the interval of interest into subintervals and estimating the SPC as a linear function of the PLC in each interval separately. This would present a prediction equation that would appear as a broken line (Fig. 1).

Flate loop counts x 10 <sup>4</sup>	Predicted standard count x 10 <sup>4</sup>	plate	Standard x	plate 10 <sup>4</sup>	count
52 53 54 54 54 57	$67 \\ 70 \\ 70 \\ 70 \\ 74$			$67 \\ 40 \\ 54 \\ 60 \\ 70$	
58 61 62 62 62	75 79 80 80 80			$51 \\ 120 \\ 100 \\ 90 \\ 70$	
		<b>J</b> .		68 100 97 72 80	
67 68 70 70 73	87 88 91 91 95			$78\\110\\110\\80\\100$	
73 74 78 78 78	$95 \\ 96 \\ 100 \\ 100 \\ 100 \\ 100 $			$81 \\ 100 \\ 100 \\ 92 \\ 100$	
78 83 84 87 89	$100 \\ 110 \\ 110 \\ 110 \\ 110 \\ 120$			$92 \\ 100 \\ 150 \\ 120 \\ 100$	
$91 \\ 93 \\ 97 \\ 100 \\ 110$	$120 \\ 120 \\ 130 \\ 130 \\ 150$			$130 \\ 120 \\ 140 \\ 120 \\ 110$	
110 120 120 120 120	$150 \\ 160 \\ 100 $	÷		$100 \\ 190 \\ 120 \\ 220 \\ 120 \\ 120 \\$	
120 130 130 140 140	$160 \\ 170 \\ 170 \\ 190 \\ 190$			110 200 150 200 180	
150 150 170 170 190	200 200 230 230 260			320 200 210 190 310	
230 240	$\frac{310}{330}$			$\begin{array}{c} 410\\ 350 \end{array}$	

Statistical evaluation of these approaches indicated that the best prediction equation was SPC' = 1.04x PLC'. With the PLC, this functional relation is expressed as SPC =  $PLC^{1.04}$ . In high-bacterial-count milk, the PLC determination becomes increasingly less than the corresponding SPC determination. This evidently results from overcrowding of developing TABLE 4. CHART FOR CONVERTING PLC TO PREDICTED SPC

PLC x 10 <sup>4</sup>	Predicted SPC x $10^4$
21 - 27	30
28 - 35	40
36 - 43	50
44 - 50	60
51 - 58	70
59 - 65	80
66 - 72	90
73 - 80	100
81 - 87	110
88 - 94	120
95 -100	130
110	150
120	160
130	170
140	190
150	200
160	210
170	230
180	240
190	260
200	270
210	290
220	300
230	310

colonies and consequent difficulty in counting on the agar plate. Therefore, that the concluded relation is exponentially inflationary seems reasonable.

The PLC, estimated SPC, and SPC of high-count raw-milk samples are presented in Table 3. Note that the difference between the PLC and SPC increased as the count became larger. Note also the agreement between the SPC and predicted SPC. The total variation in the SPC determinations can be partitioned into two parts, one of which may be attributed to the strength of the proposed relation. This is approximately 0.32, a significant portion (P < 0.01) of the total variation. Although it is con<sup>2</sup> ceivable that one could construct a more complicated prediction equation, we believe the simplicity of the proposed function relation is appealing and adequate for the proposed purposes.

A chart for converting the PLC to the predicted SPC is presented in Table 4.

#### Acknowledgment

The authors thank Mr. Homer Hansen and his staff for assisting in conducting tests on milk samples for this experiment. Mr. Hansen is Supervisor of Milk and Food Laboratories, Iowa Department of Agriculture.

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#### LOOK AFTER IT YOURSELF

A farmer once asked a wise man for help in improving an unprofitable farm. The wise man wrote a charm on a piece of paper and sealed it in a box which he gave to the farmer. "Carry this box into every part of your farm three times a year," he told him.

The farmer did so, in the morning he carried it into the fields and found a hired man asleep instead of working. At noon when he carried it into the barn, he found the cows deep in filth, the horses unfed. At night he carried it into the kitchen and found the cook wasting food. Every day, as he took the box from place to place, he found things to correct. At the end of the year he returned to the wise man. "Let me keep that magic charm another year," he begged. "My farm has been a hundred times more successful than ever before."

The wise man smiled and took the box. "I will give you the charm itself," he said. He broke the seal, lifted out the paper, and handed it to the farmer. "If you want things done proper to prosper, look after them constantly your self."

## USDA-UNIVERSITY OF MARYLAND CONFERENCE ON WHEY UTILIZATION TO BE HELD AT COLLEGE PARK AND BELTSVILLE, MD., JUNE 2-3

Olny about a third of the 22 billion pounds of fluid whey produced annually by American cheese manufacturers is utilized. This is used as animal feed and as an ingredient of baked goods and other foods. Must the rest be subjected to costly treatment for safe disposal into streams, or can it also be converted to profitable byproducts of the dairy industry?

A two-day conference is being planed jointly by the U. S. Department of Agriculture and the University of Maryland on the possibilities for the profitable utilization of whey as alternatives to its disposal as waste. The conference will be June 2 and 3 at the university's Adult Education Center at College Park, Maryland, and at USDA's Dairy Products Laboratory at Beltsville, Maryland.

The first day's discussion will be devoted to Federal pollution policies as they relate to whey and a review of current knowledge of whey utilization. Today's industrial processing of whey will be outlined, and new methods of processing whey will be discussed, including concentration and fractionation by reverse osmosis, fermentation, and drying.

The morning session of the second day of the conference will be devoted to uses for whey, including discussions of its nutritional value, chemical problems in its utilization, and the extent to which it can be used in foods and feeds. This session will conclude with a panel discussion that will summarize the findings of the conference and consider some of the problems of small and large processors.

On the afternoon of the second day, the conferees will leave College Park and assemble at USDA's Dairy Products Laboratory at the Agricultural Research Center in Beltsville, Md., for demonstrations of new methods for processing whey. They will include new methods for handling the wash water from cottage cheese whey, and the fractionation of whey by reverse osmosis and by gel filtration.

The conference will be open to all interested persons. Advance registration forms for the conference will be sent to industry representatives after March 1. Inquiries should be addressed to Dr. Byron H. Webb, Chief, Dairy Products Laboratory, Eastern Utilization Research and Development Division, Agricultural Research Service, USDA, Washington, D. C. 20250.

## 2ND SYMPOSIUM ON METAL CATALYZED LIPID OXIDATION

The second symposium on METAL CATALYZED LIPID OXIDATION is being organized during the joint ISF-AOCS World Fat Congress in Chicago from September 27 to October 1, 1970.

The first symposium was held in 1967 in Goteborg, Sweden at the Swedish Institute for Food Preservation Research (SIK). Fifty scientists from 15 countries discussed 29 presented papers. These have subsequently been published by SIK as SIK-Report nr. 240. The papers that were presented described analytical techniques, effect of metals and metal protides, chlorophyll- and haem-catalysis, effects in animal fats, in vegetable oils and other related subjects.

The second symposium is being organized by Dr. Reinhard Marcuse of SIK (Postbox, S-400 21 Goteborg 16, Sweden). Research workers interested in presenting a paper or other contribution reporting results of basic or applied research relating to the subject of the symposium are requested to contact Dr. Marcuse as soon as possible. A preliminary program and other details will be distributed shortly.

Selected speakers for the symposium from overseas can expect some financial support.

### SURGE "TRU-TEST" MILK METER

A new compact milk mater is being introduced by Babson Bros. Co. The Surge Tru-Test Milk Meter was widely tested in the field. It has been approved by the Dairy Herd Improvement Association and meets their 1.4% tolerance.

This new meter offers dairymen the opportunity to measure milk production accurately and more easily then ever before. Its simple meter design includes no moving parts for easy cleaning-in-place and consistent accuracy.

The meter can be used in stanchion barns or parlors with high or low milk lines and is designed to be cleaned-in-place. It is made entirely of supertough poly-carbonate plastic and stainless steel. The unit is 27 inches high and needs a space only 4 inches wide and 8 inches deep. Clear plastic lets the operator see immediately when milk flow begins and when milking is completed.

Measuring milk production is essential to good herd management. Surveys show the value of testing. In Ohio, for example, cows on an official testing program produced over 50 per cent milk than

their counterparts in herds without a testing program. It's the only sure way to know which cows are low producers and should be culled from the herd.

#### SANITATION FILMS

Three 8-minute training motion pictures demonstrating basic sanitation rules and practices are offered by National Educational Media, Hollywood. "Sanitation: Why All The Fuss?" and "Sanitation: Rules Make Sense" deal with bacterial contamination and the importance of carefully following all sanitation rules. "Dining Room Sanitation" is designed specifically for personnel who handle food served directly to the public.

Emphasis in the first two films is placed upon an understanding of how germs live and grow, how they are vectored from place to place, and how cleanliness, refrigeration, and proper cooking temperatures help control bacterial growth.

Available on either 16mm reels or 8mm automatic-loading cartridges for use on a new portable projector for individualized training, these films may be purchased, rented or leased from National Educational Media, Inc., 3518 West Cahuenga Blvd., Hollywood, California 90028.

#### JAMES J. HOUSTON JOINS DFISA STAFF

James J. Houston has joined the staff of the Dairy & Food Industries Supply Association. He will be Exhibits Manager for the organization's mammoth Exposition, which will be held in the Houston, Texas, Astrohall, November 1-5, 1970.

He was formerly with the National Machine Tool Builders Association and the National Machine Tool Builders Show, Inc. He was also associated with a division of W. R. Grace & Company, and, for the past year-and-a-half, headed his own national consulting firm that specialized in conventions and trade show management.

A native of Scranton, Pa., Mr. Houston was graduated from the University of Scranton with a B.S. in business administration. He holds an M.B.A. in marketing, economics and management from the George Washington University.

He is president of the Washington chapter of the American Marketing Association, and has been active in the Washington Junior Chamber of Commerce, United Givers Fund, International Toastmasters, American Society of Association Executives and National Association of Executives Club.

## PUBLICATION ANNOUNCEMENT

Maricopa County: An Interim Report on a Solid Waste Demonstration Project includes 1) organized data on solid wastes haul and disposal within the county and its cities and towns; 2) estimates of population growth to the year 2000; and 3) proposed regional programs for effective county-wide solid waste management. Although these proposed programs apply to specific conditions in the area, the methods and data may be of interest to others concerned with similar studies.

Single copies of this 318-page, illustrated report are available without charge, as supplies permit, from the Office of Information, Bureau of Solid Waste Management, 5555 Ridge Avenue, Cincinnati, Ohio 45213.

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## **CLASSIFIED ADS**

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\*International Association of Milk, Food and Environmental Sanitarians; U.S. Public Health Service; The Dairy Industry Committee.

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