The Microbiological Quality of Selected Food Products

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DEVELOPMENT of microbiological standards for the sanitary control of food products has been considered in recent years by the committee on environmental health of the California Conference of Local Health Officers. The committee concluded that standards based on bacterial counts associated with disease, although desirable, were not feasible at present. It seemed practical to proceed to establish standards based on bacterial counts in foods associated with current production practices. Such a standard might specify the permissible number of bacteria in an acceptable food product.

In order to decide whether such a microbiological standard should be selected and the range which it would set, public health workers needed some basic data. The State department of public health was asked to plan and coordinate a preliminary study to provide basic information. The health departments of Alameda, Fresno, Los Angeles, and San Diego Counties agreed to collect food samples, make necessary field observations, and do bacteriological testing.

The objectives of this study were to determine the ranges of bacterial counts in selected foods obtained from the usual commercial marketing channels and to search for any correlations between such results and selected field observations.

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Methods

The foods studied were egg salad sandwich, chicken salad sandwich, banana cream pie, and custard pie. These foods are widely distributed and are of special interest as potential carriers of foodborne disease organisms.

Food samples with accompanying field information were collected from retail establishments by four health departments which serve areas with different types of populations and climates. The four health departments agreed to collect and perform bacteriological analyses on 100 samples of each of the four foods. Detailed procedures and definitions to be employed for collection and laboratory examination of samples were prepared and discussed with participants.

Wrapped sandwiches were to be collected whole and refrigerated immediately. Collectors were to pick up the entire package of prepackaged pies, have the retail establishment package unpackaged pies, or take one serving as cut by the establishment of pies from a restaurant and place it in an unused 1-pint ice cream carton or similar container. Collectors used urethane foam refrigerated boxes, sufficient quantities of freeze gel to completely cover the food collected, and thermometers to take interior temperature of food.

All procedures conformed with the 11th edition of the American Public Health Association's "Standard Methods for the Examination of Dairy Products." The bacteriological procedures used were the standard plate count (S.P.C.) and coliform plate count (coliform count). Media used were standard methods agar (Difco 0479), violet red bile agar (Difco 0012), and phosphate buffered 99-ml. distilled water blanks.

A subsample of the food was placed in a blender. An equal weight of sterile phosphate buffered water was added, and the material was blended for 1 minute. Serial tenfold dilutions were prepared. For S.P.C., 10^{-3} to 10^{-7} dilutions were plated on standard methods agar. For coliform count, 10^{-1} to 10^{-4} dilutions were plated on violet red bile agar with an overlay of 3–4 ml. of medium to inhibit surface colony formation. All plates were incubated at 35° C. for 24 hours.

Plates with 30 to 300 colonies were selected for counting. A Quebec colony counter was used to count all colonies, including those of pinpoint size on S.P.C. plates. Dark red colonies measuring 0.5 mm. or more in diameter on uncrowded plates were considered to be coliform bacteria. When all plates had counts less than 30 colonies, the number of colonies at the lowest dilution was determined. When all plates had more than 300 colonies, the plate having the nearest to 300 colonies was selected and an estimate of the number of colonies determined from a sampling of squares.

A marginal punchcard was used to record the field and laboratory information for each sample. Distributions of S.P.C. and coliform count were prepared for each category of response to a question or each item of information recorded for a food sample. To simplify analysis, both S.P.C. and coliform count were grouped in intervals by dilution (tenfold) and resulting intervals coded 0, 1, 2, and so on. Data for all health jurisdictions were combined for the statistical analysis of counts by item.

Distributions of counts were compared for all practical combinations of categories within an item by the nonparametric Kolmogorov-Smirnov test. Statistical tests were applied at the 5 percent level of significance. Spurious significant differences are known to occur when one set of data is repeatedly subjected to statistical tests, but with this level of significance such results should occur no more than five times in 100.

Table 1. Food samples, by type of food and health jurisdiction

Type of food	Health jurisdiction				Total
	1	2	3	4	
All 4 foods	397	304	400	400	1, 501
Egg salad sandwich	99	94	100	100	393
Chicken salad sandwich	97	68	100	100	365
Banana cream pie	100	90	100	100	390
Custard pie	101	52	100	100	353

Interpretation of significant differences was made conservatively, and only those comparisons which consistently yielded significant differences (for all foods, both counts, all comparisons with a particular category, and so forth) were considered important. In addition, correlation coefficients were calculated for numerical variables.

Results

The number of samples of each type of food is reported by health jurisdiction in table 1. The percentage of each type of food sample is given by S.P.C. and coliform count in table 2, and the percentage of samples by count is shown in figure 1.

The distribution of samples in categories within an item varied greatly among the four health jurisdictions. There is no way to estimate the representativeness of the data for each health jurisdiction or for the entire set of data. Analysis was discontinued for prepackaged sandwiches, type of production plant, foods refrigerated during transport to the establishment, and foods refrigerated at the establishment. There were too few samples in some categories, the proportion of unknown responses was too large in others, and some questions had been interpreted in several ways by persons collecting information.

Comparisons of counts for categories within an item generally yielded similar data for egg salad sandwiches, chicken salad sandwiches, and banana cream pies. Fewer comparisons of counts on custard pie samples were significant than for the other three types of food.

As expected, whole banana cream pies had lower S.P.C.'s and coliform counts than did cut pieces. Similarly, S.P.C.'s were lower for whole custard pies than for pieces. However, there was no significant difference in coliform counts between whole and cut pieces of custard pie.

No difference in counts between packaged and unpackaged samples was found for banana cream pies. For custard pies no difference in coliform counts between packaged and unpackaged samples was found; significantly higher S.P.C.'s were found in prepackaged than in unpackaged samples. However, most of the prepackaged samples with higher counts were collected from unrefrigerated shelves in groceries, so refrigeration may have influenced the counts more than packaging.

Food samples were classified into seasons by collection dates—winter included December–February; spring, March–May; summer, June–August; and fall, September–November. Most food samples were collected during spring and summer, but the number of each type of food sample collected in each season differed among health jurisdictions. Therefore, all seasons are not represented equally for all health jurisdictions or types of food.

No particular season yielded consistently higher or lower counts for all foods. Only banana cream pies collected in fall had significantly higher S.P.C.'s and coliform counts than those collected in other seasons. No particular factors could be associated with these samples to explain the higher counts.

Sandwiches were collected mainly from cigar stores, industrial catering vehicles, and restaurants; pies were collected mainly from restaurants, bakeries or groceries, and industrial catering vehicles. Comparisons of counts on food samples from different types of establishments did not yield consistent differences. Only banana cream pie samples from bakeries or groceries showed significantly lower S.P.C.'s and coliform counts than pies collected elsewhere.

The names and addresses of production plants were used to group samples by plant to determine whether some plants produce foods with significantly higher counts. The S.P.C.'s and coliform counts were tabulated and plotted for each production plant with 10 or more food samples. All sandwich samples were grouped, as were all pie samples.

Obvious differences in counts between production plants within each health jurisdiction were revealed by the graphs and verified by statistical tests. For example, figure 2 shows the cumula-

Table 2. Percentage of food samples, by counts per gram

Counts per gram	Egg salad sandwich	Chicken salad sandwich	Banana cream pie	Custard pie
S.P.C.	100	100	100	100
Less than 100	(1)	0	(1)	6
100–990	(1)	(1)	8	13
1,000–9,900	2	6	12	44
10,000–99,000	14	30	13	25
100,000–990,900	44	30	23	6
1,000,000–9,900,000	14	16	18	4
10,000,000–99,000,000	16	16	17	1
100,000,000–990,000,000	9	2	8	(1)
1,000,000,000–9,900,000,000	(1)	(1)	0	0
Coliform count	100	100	100	100
Less than 100	32	27	31	84
100–990	17	24	23	13
1,000–9,900	19	21	22	2
10,000–99,000	14	17	12	1
100,000–990,000	11	8	8	1
1,000,000–9,900,000	6	2	5	0
10,000,000–99,000,000	1	0	(1)	0
100,000,000–990,000,000	(1)	0	0	0
Too numerous to count	0	1	0	0

[1] Less than 0.5 percent.

NOTE: Percentages are rounded independently and may not add to total.

Figure 1. Percentage of food samples in each count interval, by type of food

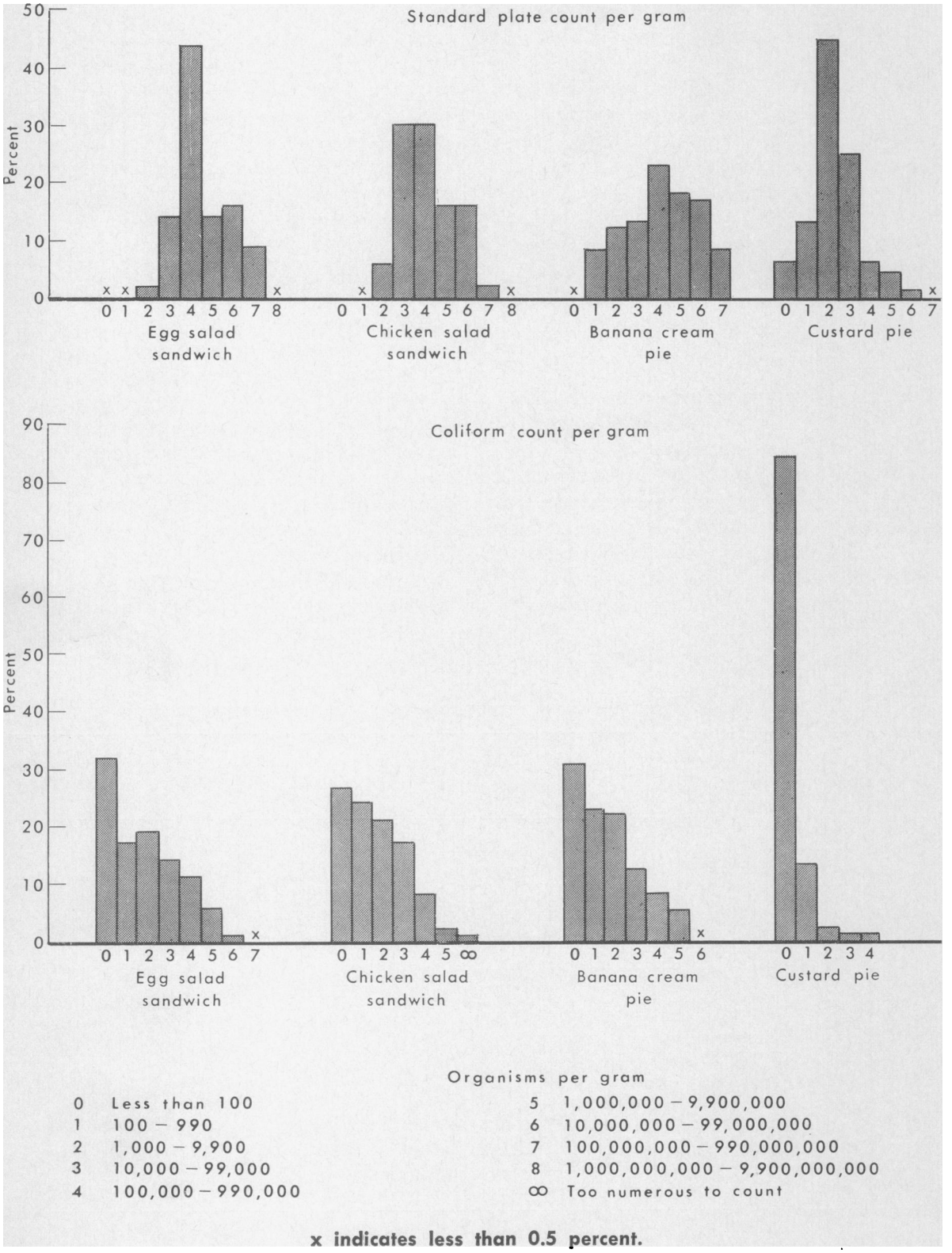
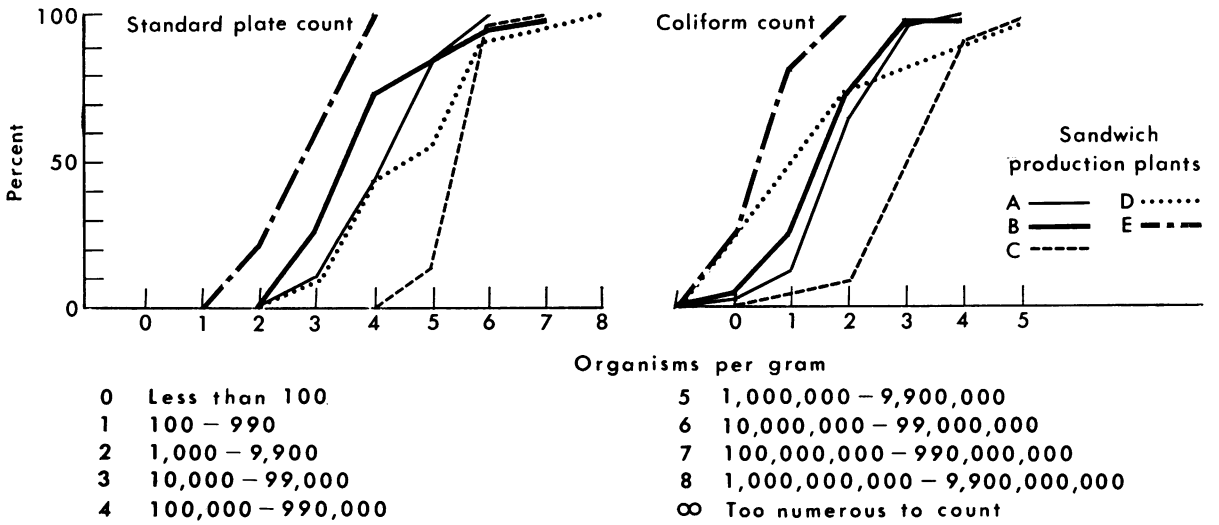


Figure 2. Cumulative percentage distribution of sandwich samples count for selected production plants, health jurisdiction 1



tive distribution of sandwich samples prepared in different production plants in health jurisdiction 1. Samples prepared in plant E had lower S.P.C.'s and coliform counts while plant C produced samples with high counts. Similar differences in counts were seen for samples of both pies and sandwiches prepared in plants in other health jurisdictions. A sanitary survey of plants was not included in this study, but these data suggest such a survey as a future study.

Data on hours since food left the production plant were difficult to obtain. When the food samples with "unknown" responses to this item were eliminated, 82 percent of the remaining samples were less than 8 hours old upon reaching the retail establishment. Only 10 percent were more than 24 hours old. Thus, the time intervals used in the analysis were 0–2 hours, 3–4 hours, 5–8 hours, and more than 8 hours.

No significant differences were seen in counts between time intervals for custard pies. For the other three foods, the 5–8 hour samples yielded consistently higher S.P.C.'s and coliform counts than all other age categories. The relationship of the distributions of counts for the categories 0–2, 3–4, and more than 8 hours varied by food.

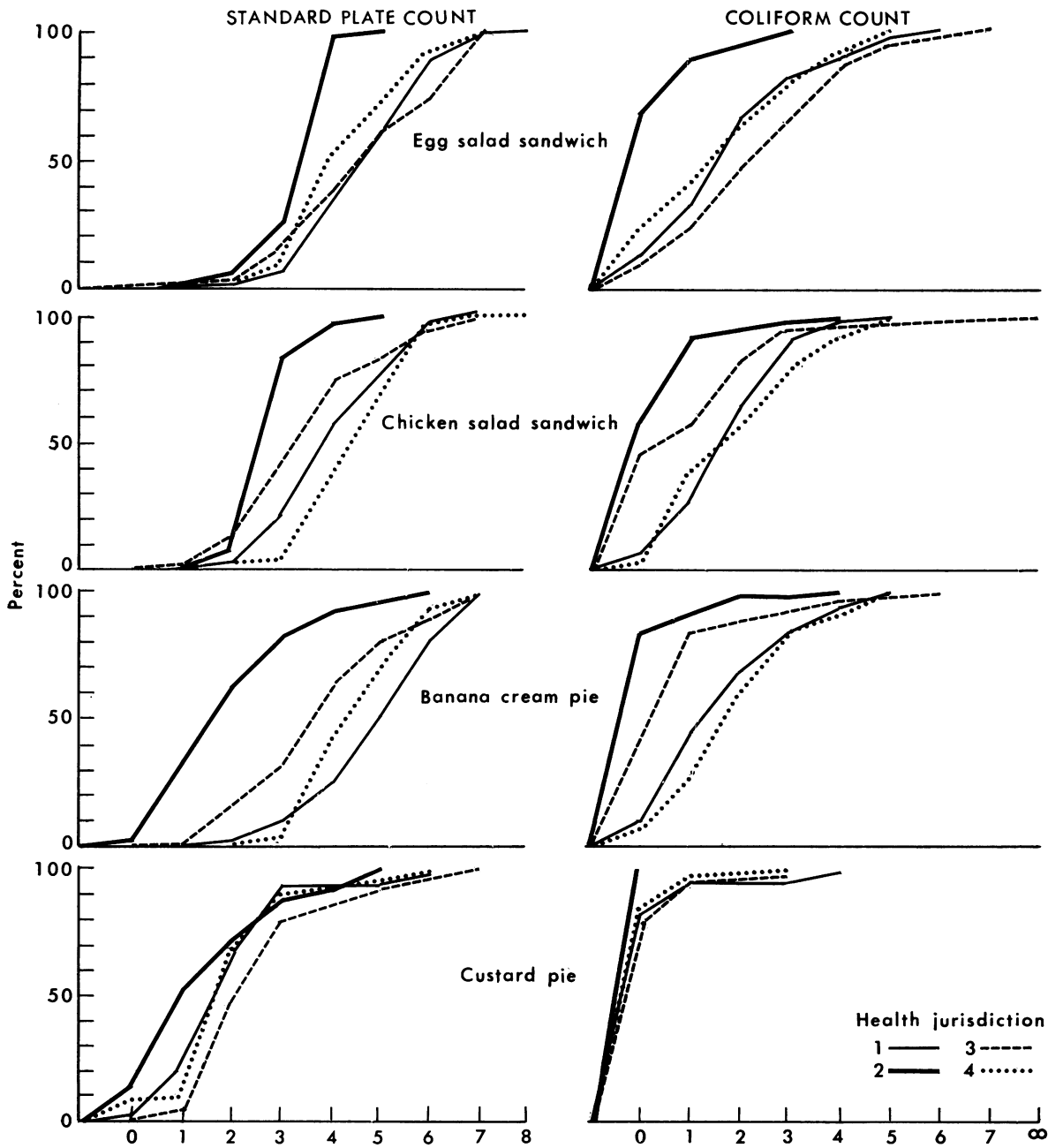
Correlation coefficients were calculated for hours by S.P.C. and hours by coliform count. All values were low, indicating no strong association of counts with the time between preparation of food and when it reached the retail establishment. Perhaps interactions of other factors obscured this association.

For the statistical analysis, temperatures were grouped by 10-degree categories. No significant differences in counts by temperature were found for custard pies. For all other foods, S.P.C.'s and coliform counts for samples with temperatures 51°–60° F. were significantly higher than counts for foods 41°–50° F. However, comparisons with other temperatures did not consistently yield significant differences in counts. Although foods with lower temperatures seemed to have lower counts, the correlation coefficients for temperature and count indicated no strong association of these two variables. The coefficients ranged from 0.04 for banana cream pie S.P.C.'s and custard pie coliform counts to 0.26 for chicken salad sandwich S.P.C.'s.

S.P.C. and coliform count distributions were prepared for each health jurisdiction by type of food (fig. 3). Many significant differences were seen among health jurisdictions. Jurisdiction 2 had consistently lower S.P.C.'s and coliform counts in comparison with the other three health jurisdictions except for custard pie coliform counts. No other jurisdiction had consistently higher or lower counts for all types of food.

Many variables could have contributed to the differences in counts between health jurisdictions which had different types of populations

Figure 3. Cumulative percentage distribution of food samples for each type of food by S.P.C., coliform count, and health jurisdiction



and climates. Obvious differences between jurisdictions were seen in the schedules and regularity of sample collection, the consistency in interpretation of definitions, the proportion of unknowns reported, and the completeness of information. Although a standard laboratory protocol was employed, no split-sample study was conducted to determine the comparability of test results between health jurisdictions.

Correlation coefficients of S.P.C. to coliform count were calculated for each type of food. The correlation for custard pie counts was low (0.3), but was high (0.7) for all other types of food. Although the association between the two kinds of counts differed by type of food, future researchers should be able to conduct studies using only one of the counts after preliminary testing establishes the correlation for the particular food under study.

The 50th, 75th, 90th, and 95th percentiles of S.P.C.'s and coliform counts were estimated (table 3). S.P.C.'s were consistently higher than coliform counts by at least two dilutions (a factor of 100). Counts for egg salad sandwiches and banana cream pies were similar; those for chicken salad sandwiches were slightly lower, and those for custard pies were lowest.

Percentiles were also calculated for each food by health jurisdiction. For egg salad sandwiches and banana cream pies, percentiles varied as much of three dilutions (a factor of 1,000) between jurisdictions; percentiles for banana cream pies varied by two dilutions, and those for custard pies varied by one dilution. Health jurisdiction 2 had noticeably lower S.P.C.'s and coliform counts for all four types of food.

Discussion

There has been a growing interest in developing and applying microbiological criteria to the sanitary control of food. Ideally such standards should be based on bacterial counts associated with disease, but this is not feasible. Instead, a standard related to sanitation practices might specify the permissible number of bacteria in an acceptable food product derived from percentile counts obtained in a large-scale study on representative types of foods.

Usefulness of these study data is limited since the study was designed to survey bacterial counts in only selected foods. No attempt was made to insure representative sampling—the four health departments volunteered to participate in the study, foods surveyed were selected because of their potential as carriers of foodborne disease, and no schedule of sample collection was specified to include representative numbers of types of establishments or production plants.

Furthermore, this study has revealed several other factors for consideration in future studies in the development of microbiological standards. The number of bacteria in different types of food samples varied greatly, from less than 10 to more than 1 billion organisms per gram. This suggests that different bacteriological cri-

Table 3. Selected percentiles for S.P.C.'s and coliform counts, by type of food

Type of food	Percentile			
	50	75	90	95
S.P.C.:				
Egg salad sandwich	100, 000–990, 000	1, 000, 000–9, 900, 000	10, 000, 000–99, 000, 000	100, 000, 000–990, 000, 000
Chicken salad sandwich	10, 000–99, 000	1, 000, 000–9, 900, 000	10, 000, 000–99, 000, 000	10, 000, 000–99, 000, 000
Banana cream pie	100, 000–990, 000	1, 000, 000–9, 900, 000	10, 000, 000–99, 000, 000	10, 000, 000–99, 000, 000
Custard pie	1, 000–9, 900	1, 000–9, 900	10, 000–99, 000	100, 000–990, 000
Coliform count:				
Egg salad sandwich	100–990	10, 000–99, 000	100, 000–990, 000	100, 000–990, 000
Chicken salad sandwich	100–990	1, 000–9, 900	10, 000–99, 000	100, 000–990, 000
Banana cream pie	100–990	1, 000–9, 900	10, 000–99, 000	100, 000–990, 000
Custard pie	<10	<10	10–99	100–990

teria may have to be set for different food products. For example, 60 percent of the egg salad sandwich samples had S.P.C.'s less than 1 million per gram, whereas 94 percent of the custard pie samples had counts below this level (table 2). Similarly, 68 percent of the egg salad sandwiches and 99 percent of the custard pie samples had coliform counts below 10,000 per gram.

The cumulative results reported by the different jurisdictions for the same food raise the question of whether a bacteriological criterion for a given food could apply to all areas of a State. For example, more than 90 percent of the samples of banana cream pie in one jurisdiction had an S.P.C. of 990,000 or less; but in the other three jurisdictions only about 65 percent, 40 percent, and 25 percent of the samples did not exceed 990,000 (fig. 3).

The increasing interest in the development and use of microbiological criteria for foods in California and other States and countries is apparent from the growing number of publications on this subject. In 1956 Dack (*1*) discussed microbiological standards in general terms and presented also some detailed information relevant to his evaluation of the applicability of such standards. A report on microbiological processes by Elliott and Michener (*2*) summarized advantages and disadvantages of standards. As chairman of an international meeting of specialists in food microbiology and hygiene, Thatcher (*3*) reported the discussion and recommendations of the group relating to the establishment of microbial limits for foods, especially certain groups of frozen products. Mossel (*4*) listed principles essential to standards development in his paper dealing with assessment of the sanitary condition of processing plants and products.

The literature also contains reports of studies which, like this report, were aimed primarily at bacteriological characterization of a class of products; the work of McCroan and associates (*5*) on commercial, wrapped sandwiches and that of Verma and co-workers (*6*) on precooked, frozen desserts are examples. Abrahamson (*7*) reported the experience gained in New York City with administrative microbiological criteria applied to frozen foods.

Of special interest are the views on the development and use of microbiological criteria for foods expressed by an ad hoc subcommittee on food microbiology in a report prepared for the food protection committee of the Food and Nutrition Board, National Academy of Sciences-National Research Council (*8*). The section dealing with microbiological criteria amounts to a treatise on the subject and includes sections on definitions, fields of application, bacterial species and groups, types of foods, attainability, methodology, and basic principles for setting microbiological criteria.

Summary

Commercially produced egg salad sandwiches, chicken salad sandwiches, banana cream pies, and custard pies were subjected to bacteriological testing by selected local health departments in California. Bacteriological procedures used were the standard plate count (S.P.C.) and coliform plate count (coliform count).

The correlation between S.P.C. and coliform count was high for three of the four foods studied. This indicates that one type of count might suffice for similar work in the future. The S.P.C. was consistently higher than the coliform count, usually by a factor of 100. Counts for egg salad sandwiches and banana cream pies were slightly higher than chicken salad sandwich counts. Custard pie counts were lowest. The number of organisms per gram of food varied widely—the S.P.C. range was from less than 100 to more than 1 billion per gram; the range for the coliform count was from less than 10 to more than 100 million.

Significant differences in bacteriological counts were apparent among the four geographic areas studied. There are many variables which might account for the differences, but it was not possible to extricate and identify them. Although there was a tendency for foods with lower temperatures at time of collection to show lower counts, there was no strong association of these two variables. The data from this survey do not show that the numbers of organisms vary in all instances with such factors as prepackaging, season, type of retail establishment, or time between preparation of food and delivery to the retail establishment.

Counts differed significantly among the various production plants. Further work is needed

to ascertain the bacteriological quality which could be expected if all producers and distributors of a given food were employing maximum sanitary control. Thorough sanitary surveys, coupled with bacteriological studies, are required to define what constitutes good commercial practice.

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Five-Year Study of Mania and Depression

A 5-year study of mania and depression was launched by the award of a grant from the National Institute of Mental Health, Public Health Service, to Washington University School of Medicine, St. Louis.

Seeking a better understanding of and improved treatment for the affective disorders is one of the primary missions of the NIMH. In addition to supporting many studies pertaining to these disorders, the Institute conducts an intensive intramural research program on this important mental health problem.

The general aims of this new program are to reformulate the clinical classifications and to develop laboratory measures of the affective disorders (mania and depression). These disorders will be studied in relation to a number of factors such as social effectiveness, premenstrual tension, violence, and normal grief reactions.

Biochemical studies will include investigation of brain, lactate, and carbohydrate metabolism and their relationship, if any, to depression and the study of the brains of persons who have committed suicide. The effectiveness of drug-modified electroconvulsive therapy in the treatment of depression will also be evaluated. Already underway is a study of blood factors in families of patients with affective disorders to determine if these disorders have a genetic base.

Subjects will be patients, with and without affective disorders, selected from the inpatient wards of Renard Hospital, the psychiatric unit of Barnes Hospital, and Washington University School of Medicine, all in St. Louis. A 12-year followup of patients previously hospitalized with mania or depression will be continued. The families of patients will be interviewed.

The NIMH grant supporting the first year of the proposed 5-year project totals $89,689.