AN IMPROVED PROCEDURE FOR MICROSCOPIC GRADING OF MILK INTENDED FOR PASTEURIZATION

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Sequential analysis has been applied to the microscopic grading of raw milk for three common bacterial standards. Simple grading tables have been developed which permit rapid acceptance or rejection of good and poor milk respectively but require maximum examination of borderline milk. Routine use of sequential grading requires considerable less microscopic examination and yields results of a precision equal to or greater than that expected with the present standard procedure.

In the present standard method for microscopic grading of raw milk intended for pasteurization the number of microscopic fields examined for bacterial clumps is constant within four rather wide ranges of counts[1]. The number of fields examined is essentially the same for milks in which the count approaches the grade limit set by regulation as when the count falls very much below or above the limit. A more efficient counting procedure would allow rapid acceptance of "good" milk or rejection of "poor" milk and concentrate attention on "borderline" milk.

If the present method operated ideally, all milks accepted as meeting a given grade limit would have less than the legal limit of bacterial clumps per ml and all milks rejected would have more. Because of the random variation of clumps among the microscopic fields examined this ideal is not realized. Some milks with fewer bacteria than the prescribed limit are rejected and other milks with more than the limit are accepted. The probability of these two types of errors may be referred to as the "producers' risk" and the "consumers' risk" respectively. Heretofore the magnitude of these risks has not been defined.

The authors have recently studied the distribution of bacterial clumps in standard milk films[2]. The following results were pertinent to the development of an improved grading procedure.

1. The distribution of bacterial clumps in milk films with means in the range from 0.18 to 1.05 clumps per field* agrees substantially with the Poisson series.
2. Despite this evidence of their random distribution, bacterial clumps are more concentrated in a central area of a film than in the remaining border area, indicating that fields selected in routine counting should be limited to representative portions of the film.
3. Microscopic clump counts of different films from the same sample of milk seldom differ significantly when the mean count is less than 1.05 clumps per field.

On this evidence a sequential grading procedure has been developed which is considerably more efficient that the present standard method.

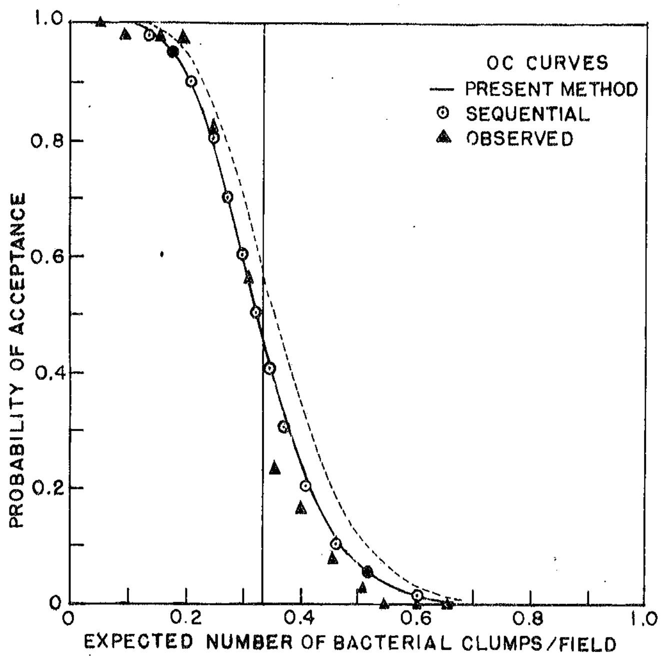
A recent survey[3] indicates that 31 states and most larger cities enforce bacterial standards of 100,000 -200,000 per ml for the best grades of raw milk intended for pasteurization. The purpose of this paper is to present in simplified form the development of the proposed sequential grading procedure for a bacterial standard of 200,000 clumps per ml and to offer additional grading plans for standards of 100,000 and 400,000 clumps per ml.

OPERATING CHARACTERISTIC CURVE OF THE PRESENT STANDARD METHOD

When using a microscopic factor of 600.000, milk meeting a standard of 200.000 bacterial clumps per ml must not average more than one-third bacterial clump per field in 30 fields. Ideally, every milk of this grade would contain fewer than 200,000 bacterial clumps per ml. This limit corresponds to the vertical line in figure 1 at 0.33 clump per field; all milk to the left of this line would be accepted and all milk with a larger bacterial content would be rejected.

Because the number of climps varies at random from field to field, this ideal is not realized in practice. Some samples of milk with fewer bacteria than the legal requirement are unavoidably rejected and other samples with more than the requirement are accepted. The precision of the present method of grading is expressed quantitatively by its "operating characteristic (OC) curve", as show in figure 1. The probability of acceptance of a sample of milk is plotted against its theoretical true bacterial content, expressed as clumps per field, when any milk is rejected which has a total count of 10 or more clumps in 30 fields. This means that borderline milk, which contains an estimated 200,000 clumps per ml would be rejected, although it would be accepted under the usual interpretation of the present regulations. If counts of 10 clumps or less are accepted the OC curve is represented by the dotted line in figure 1. Use of this curve would reduce the producers' risk by increasing the probability of accepting samples which should be rejected. Conversely the consumers' risk is increased. Since the solid curve in figure

* Microscopic factor = 600,000.



1. Operating characteristic curves for a grading limit of 200,000 clumps per ml.

1 is nearer to 50 percent acceptance at the grade limit, it is believed to express better the intent of of the regulations.

As interpreted from these curves 20 percent of the milks now passed as meeting a limit of 200,000 clumps per ml would be expected to have counts of 250,000 clumps per ml or more. Conversely, more than 28 percent of the milks having only 180,000 clumps per ml would be rejected.

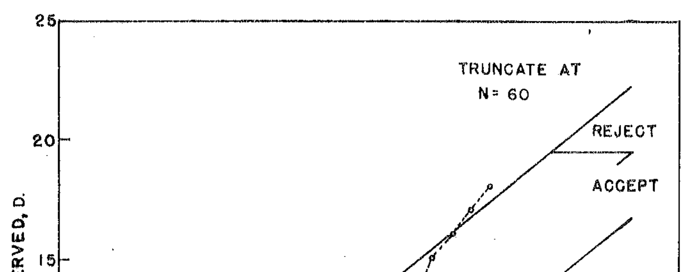
Design of the Sequential Grading Procedure

Sequential inspection is a statistical method in which the number of items to be inspected from a lot is not predetermined. The decision to accept or reject the lot depends at each stage of the inspection on the cumulative result of the previous observations[4]. In applying this technique to the microscopic grading of milk, after each field is counted, the milk is (1) accepted as meeting the grade standard, (2) rejected as not meeting the grade standard, or (3) the examination is continued.

In designing a sequential grading plan for a standard of 200,000 clumps per ml, two points were selected on the present OC curve at which 5 percent of the samples would be rejected and 5 percent accepted. The sequential curve was then made to coincide with the present OC curve at these points, representing theortical counts of 0.18 or less and of 0.52 or more clumps per field respectively. These counts correspond to 108,000 and 312,000 clumps per ml and are indicated in figure 1 as solid circles.

Limiting the number of samples which would be accepted or rejected incorrectly to 5 percent at expected clump densities of 0.18 and 0.52 per field and the assumption that the distribution of clumps in standard milk films follow a Poisson series, completely determined the sequential plan. The sequential grading plan is defined graphically by the two parallel lines in figure 2. The abscissa N is the number of fields counted and the ordinate D is the total number of clumps observed at a given number of fields. The calculation of the equations for the slopes of the two lines is described in full in reference[5]. The sequential grading of two samples of milk is illustrated in the figure by dotted lines. As soon as the cumulative count of the upper sample crossed the rejection line the count was terminated and the sample was rejected as not meeting the standard. When the count of the second sample crossed the acceptance line, it was acceped without further counting.

To check the agreement of the proposed sequential plan with the present standard grading procedure, values for the expected sequential OC curve were calculated as described in reference[5]. These points are plotted in figure 1 as

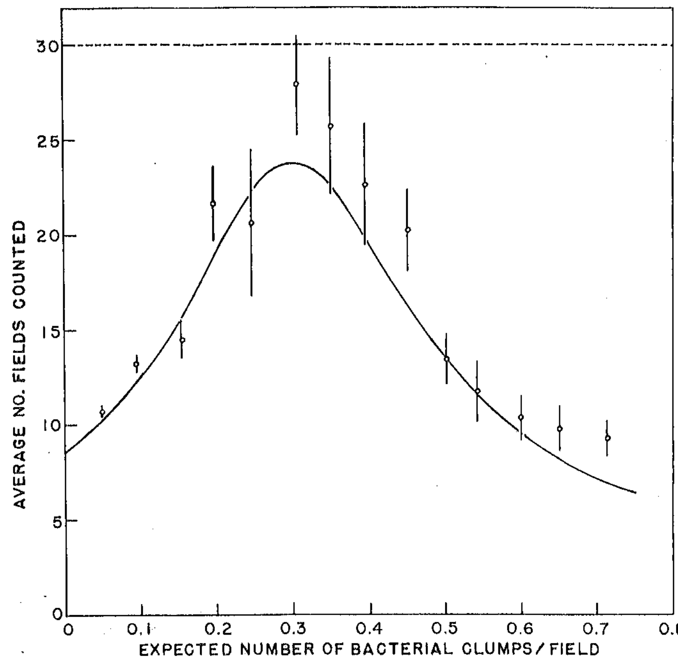


2. Graphic presentation of the sequential grading plan for a 200,000 clump limit and the results for two films.

open circles. Although the curves were forced to agree at the 5 and 95 percent points, the sequential curve was free to diverge at other levels. The points calculated for the sequential plan agree satisfactorily with the present OC curve and where they diverge the sequential plan lessens both the producers' and consumers' risk.

Sequential sampling is known to reduce substantially the number of units which must be examined as compared with a fixed sample size. The curve in figure 3 shows the average sample number (ASN) for different expected clump densities with the sequential grading procedure, as determined theoretically for a Poisson distribution[5]. The number of fields is largest when the expected number of bacterial clumps per field is slightly less than 0.33, the grade limit. This number decreases as samples are either more or less contaminated. The uniform number of fields specified in the standard method for a grade limit of 200,000 clumps per ml is indicated by the horizontal dotted line at 30 fields. The expected saving in microscopic examination through use of the sequential procedure is considerable.

For any individual milk the number of fields required to reach a decision may be larger or smaller than the expected ASN. While it is unlikely to exceed two or three times the ASN for a given film, the process may be terminated at some agreed upper limit. Wald[4] states than truncation at two and one-half times the maximum ASN has but little affect on the producers' and consumers' risks. Since the maximum ASN is 24 fields the count may be truncated at 60 fields on borderline milks. A milk that had not been accepted or rejected would be accepted if after counting this number of fields it fell in the lower half of the intermediate zone and rejected if it fell in the upper half. As shown in figure 2 a count of 19 or fewer bacterial clumps in 60 fields would be accepted and one reaching a count of 20 or more would be rejected.



3. Expected and observed average number of microscopic fields examined in reaching a decision to accept or reject a sample with the sequential plan for a 200,000 clump limit.

PROPOSED GRADING PLAN

Whether to accept or reject milk for a legal limit of 200,000 clumps per ml may be determined as each field is counted from table 1. This table has been based upon the equations for the acceptance and rejection lines in figure 2.

Bias due to the concentration of bacterial clumps in the central area of a film[2] can be minimized by restricting the selection of fields for counting. A count should be started about one-quarter of the way down from the upper edge of the film and a few fields inside the left margin. Five fields are counted across the film, selecting fields at random with the eyes removed from the oculars. The number of clumps is recorded after examining each field and the cumulated number compared with the acceptance and rejection limits in table 1. Further 5-field transits of the film are made at intervals of 2 to 5 fields down the film until a decision can be reached, terminating after not more than 60 fields or 12 transits.

TABLE 1

SEQUENTIAL GRADING TABLE FOR GRADE LIMIT OF 200,000 CLUMPS PER ML

A milk in which the total number of bacterial clumps does not exceed the acceptance number d^1 in a given number of fields is accepted; one in which it equals or exceeds the rejection number d^2 is rejected.

Field no.	d^1	d^2	Field no.	d^1	d^2	Field no.	d^1	d^2	Field no.	d^1	d^2
1	....	4	16	2	8	31	7	13	46	12	18
2	....	4	17	2	9	32	7	14	47	12	18
3	....	4	18	3	9	33	7	14	48	12	19
4	....	5	19	3	9	34	8	14	49	13	19
5	....	5	20	3	10	35	8	15	50	13	19
6	....	5	21	4	10	36	8	15	51	13	20
7	....	6	22	4	10	37	9	15	52	14	20
8	....	6	23	4	11	38	9	16	53	14	20
9	0	6	24	4	11	39	9	16	54	14	20
10	0	6	25	5	11	40	10	16	55	14	20
11	0	7	26	5	12	41	10	17	56	15	20
12	1	7	27	5	12	42	10	17	57	15	20
13	1	7	28	6	12	43	10	17	58	15	20
14	1	8	29	6	13	44	11	17	59	16	20
15	2	8	30	6	13	45	11	18	60	19	20

Experimental Tests with the Sequential Grading Procedure

The proposed sequential grading procedure has been tested on data collected in studying the distribution of bacterial clumps in milk films[2]. Clump counts of at least 100 fields were available from each film. The selection of field counts for testing the sequential procedure approximated the method proposed above. In the low count films as many as 11 sequential counts could be obtained from the record of 100 fields. Each sequential test was given equal weight, whether one or many counts were taken from the same film. The number of such tests totaled 571.

A salient feature of the sequential method is that the number of fields counted depends upon the true mean clump count per field. Our best estimate of the true value for each film was the mean of all 100 fields. The results obtained sequentially were grouped in terms of this mean by intervals of 0.05 clump per field. Within each grouping interval the record showed the number of films accepted, rejected and truncated, and the frequency distribution of the observed number of fields required for a decision. From the latter distribution the mean sample number and its standard error was determined for each interval.

The proportion of tests accepted by the sequential procedure has been plotted as solid triangles against the mean clump count per field in figure 1. The observed proportions were based upon varying numbers of tests with a mean of about 36 per point. The experimental values agreed satisfactorily with the expected OC curve for the present standard method and the sequential plan. Where they differed, the divergence usually improved the OC curve, so as to reduce the number of milk samples classified incorrectly.

The observed ASN exceeded that expected in a majority of the sampling intervals. In individual cases the difference between the observed and the expected values was not statistically significant. This is clear from the standard errors plotted in figure 3. With few exceptions, however, the ratio of the observed to the expected mean was larger than 1. In the clump range for 5 and 95 percent acceptance, the ratio of the observed to the expected ASN was $1.101 \pm .040$.

The larger number of counts may be due not only to an occasional non-random distribution of clumps in the film but also to the fact that the number of fields counted is nessessarily discrete. The calculated number of clumps at the acceptance and rejection levels was never a whole number. This necessitates continuing the count beyond the number expected theoretically in computing the observed ASN and may account for much of the discrepancy. It is doubtful that truncation contributed to the larger sample number. Only three sequential counts were truncated, representing 5 percent or less of the tests in their respective grouping intervals.

Although more counts were needed in practice than the number expected, many fewer fields were examined by the sequential plan than with a uniform sample number. Within a clump range determined by 98 and 2 percent acceptance levels on the OC curve, 5518 fields were counted sequentially in 289 tests. If 30 fields had been counted routinely, as recommended in the present standard method for a grade limit of 200,000 clumps per ml, 8670 fields would have been required. This represented a saving of 36 percent in the number of fields examined.

Grading Plans for Limits of 100,000 and 400,000 Clumps per ml

The acceptance or rejection of milk for legal limits of 100,000 and 400,000 clumps per ml may be determined from tables 2 and 3. These grading plans were developed in the same manner as that for the 200,000 clump limit. Expected sequential OC curves were constructed so that they agreed with the present OC curves at acceptance levels of 95 and 5 percent of milk samples. Here again the expected sequential curves agreed well with the present OC curves and divergent points tended to lessen the producers' and consumers' risks.

The equations for the acceptance and rejection curves comparable to those in figure 2 were calculated as $d^1 = -1.917 + 0.1559N$ and $d^2 = 1.917 + 0.1559N$ for the 100,000 clump limit and $d^1 = -2.763 + 0.6458N$ and $d^2 = 2.763 + 0.6458N$ for the 400,000 clump limit.

The maximum expected average sample numbers for the two grades were 24 and 12 respectively as compared to the present recommended constant sample numbers of 30 and 15. In setting up the grading tables (tables 2 and 3) the number of fields to be examined was limited to two and one-half times the maximum ASN or 60 and 30 fields respectively. These sequential plans were then tested in the same manner as that for a limit of 200,000 clumps. Totals of 479 and 1080 sequential tests of the two plans were made on available film count data.

Sequential Grading Table for Grade Limit of 100,000 Clumps per ml

TABLE 2

Clumps observed *Total No.*	Accept at *Fields*	Reject at *Fields*
0	13-18	—
1	19-25	—
2	26-31	—
3	32-37	1-6
4	38-44	7-13
5	45-50	14-19
6	51-57	20-26
7	58-59	27-32
8	60	33-39
9	60	39-45
10	60	46-60

TABLE 3

Sequential Grading Table for Grade Limit of 400,000 Clumps per ML

Field no.	d1*	d2*	Field no.	d1*	d2*	Field no.	d1*	d2*
1		4	11	4	10	21	10	17
2		5	12	4	11	22	11	17
3		5	13	5	12	23	12	18
4		6	14	6	12	24	12	19
5	0	6	15	6	13	25	13	19
6	1	7	16	7	14	26	14	20
7	1	8	17	8	14	27	14	20
8	2	8	18	8	15	28	15	20
9	3	9	19	9	16	29	15	20
10	3	10	20	10	16	30	19	20

*Interpret as in table 1

The resulting observed OC curves compared satisfactorily with those expected for the two grades. Any divergence tended to reduce the number of samples graded incorrectly.

The observed ASN exceeded that expected in the majority of sampling intervals for clump limits of both 100,000 and 400,000. In the clump range from 5 to 95 percent acceptance, the ratios were 1.061 ± .036 and 1.211 ± .049 respectively. Only the latter ratio was significantly greater than 1. Within the clump count range for acceptance levels of 2 and 98 percent, 7854 fields were counted sequentially in 429 tests for the 100,000 clump grade. If 30 fields had been counted uniformly as recommended in the present standard method for this grade, 12,870 fields would have been required. In the corresponding range for the 400,000 clump grade, 6856 fields were counted sequentially in 612 tests, and 9180 fields would have been required if the recommended 15 fields had been examined in each test. These represent savings of 39 and 25 percent respectively in the number of fields examined for the two grades.

Discussion

The initial OC curves calculated in this study measure the relative precision of the current standard microscopic procedure for grading milk. To insure acceptance of 95 percent of milk for grade limits of 100,000, 200,000 and 400,000 clumps per ml, producers cannot permit the true clump count of their milk to exceed 39,400, 108,000 and 217,000 clumps per ml respectively. Conversely, 5 percent of the milk passed as meeting these grade limits exceed counts of 183,000, 315,000 and 630,000 clumps per ml respectively. These estimates are based upon rejecting milk with a count exactly at the grade limit. Except for this modification, which adjusts the producer and consumer risks more equitably, the present standards of acceptance and rejection have been retained in the present study.

The primary objective has been to develop a grading procedure of defined precision which would distribute the microscopic work more efficiently than the present method. In applying sequential analysis to the problem, the proposed grading plans have been based upon levels of contamination at which 95 and 5 percent of milk samples are now passed in each grade. This has reduced the producer and consumer risk in the intervening zone. The applicability of the sequential procedure does not depend upon the levels which have been selected here and made to coincide with the present levels of acceptance and rejection. If the curves agreed at 99 and 1 percent, for example, the sequential OC curves would be steeper but a larger number of fields would have to be counted. More stringent limits could be adopted for the consumers' than for the producers' risk or vice versa. Given the greater efficiency of the sequential procedure more rigid adherence to grade limits may be both practicable and desirable.

Summary

Simple sequential tables have been developed for three grades of raw milk which permit making decisions to accept or reject the milk or to continue the examination as each microscopic field is counted. Use of these tables permits rapid acceptance and rejection of good and poor milk respectively but increases the inspection of borderline milk. The major advantage of sequential grading is that an examination of fewer microscopic fields yields results of a precision equal to or greater than that expected with the present standard procedure.

References

1. *Standards Methods for the Examination of Dairy Products,* 9th ed. American Public Health Association, New York. (1948).

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4. Wald, Abraham. *Sequential Analysis.* John Wiley and Sons, Inc. New York. (1947).

5. Statistical Research Group, Columbia University, *Sequential Analysis when Quality is Measured by the Number of Defects per Unit.* SRG Report 255, Section 7, (1946).

Milk and Milk Products, by C. H. Eckles, W. B. Combs and H. Macy. Fourth edition. Published by Mc Graw-Hill Book Company, New York. 1951. 454 pages. 95 figures. $5.00.

This book has been slightly enlarged over the previous edition (published in 1943). Some new material has been added, especially in the introduction, and the chapters on microorganisms, new equipment, butter, ice cream, dry milk and tests.

An Introduction to Foods and Nutrition, by H. C. Sherman and C. S. Landford. Third Edition. Published by the Macmillian Company, New York. 1951. 454 pages. 36 figures.

Much new material has been added, chapters rewritten, illustrations added, and new references, some of which are as late as 1950.

A B C's of CMP – U. S. Department of Commerce National Production Authority. Published by U. S. Government Printing Office, 1951.

This booklet is intended to serve as background information and a short refresher course in the principles and operating procedures of the controlled Materials Plan. Copies of the complete regulations, and detailed operating instructions for those directly affected by CMP, will be available from NPA in Washington or the nearest Field Office of the Department of Commerce.