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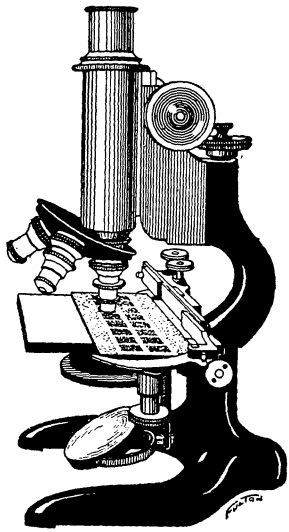

USING THE MICROSCOPE IN MILK GRADING.

SUMMARIZED BY
F. H. HALL
FROM BULLETIN BY
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*Connected with Grape Culture Investigations. †On leave.

POPULAR EDITION*

OF

BULLETIN NO. 443.

USING THE MICROSCOPE IN MILK GRADING.

F. H. HALL.

A rapid, reliable method.

The number of bacteria in milk has been used for more than a decade by certain milk distributing companies as a means of judging the commercial quality of the milk they received. The abundance of bacteria does not alone determine the quality of milk, by any means, but does furnish a guide by which we may judge of the keeping quality of the milk and make an approximate estimate of the time limit of its usefulness in fresh condition. Until recently this application of the bacterial count to the milk industry has been made by a method which could not give the counts until after the milk had been used or had soured; that is, the agar plate method reported conditions which were already *several days* past. It was useful in showing what kind of milk, from the bacterial standpoint, a producer had brought to market some time before; but it could not give notice, in time to be of much use, of any transitory change in the quality of the milk of a herd or any particular cow in it. By this method the bacteria in milk diluted with germ-free water were allowed to grow for from two to five days in a shallow glass dish containing a " culture medium," when the separate bacteria, or collected groups of them, developed " colonies " or masses large enough to be seen and counted. By the microscopic method, whose practical application we are to discuss, a small portion of the milk is spread on a small rectangular piece of glass and dried, then prepared by immersion in liquids and staining solutions in such a way as to make the bacteria visible and the whole suitable for examination under the high magnification of a compound microscope. This slide, which forms a permanent record of the bacterial condition of the milk, can be prepared in ten to fifteen minutes and the preparation examined at once, or it can be kept until some convenient

* This is a review of Bulletin No. 443 of this Station on The Control of Bacteria in Market Milk by Direct Microscopic Examination, by Robert S. Breed and James D. Brew. Anyone interested in the details of the investigations will be furnished, on request, with a copy of the complete bulletin. Names of those who so desire will be placed on the Station mailing list to receive bulletins as issued, popular or complete edition as requested.

time. The rapidity and simplicity of the method are sufficient to permit examination of the bacterial quality to be made before the milk is consumed or placed on sale. By this method the individual bacteria are seen and counted, whether each is separated from all others or many are united in a clump. These counts are therefore greater than those obtained by the ordinary methods in which clumps of bacteria are counted as if they were single bacteria. All of these facts give the microscopic method many advantages over the generally used plating method for judging the quality of milk produced on farms.

Practical tests made.

Preliminary tests of this method made in 1913 gave very promising results and it was decided to try it out under commercial conditions and in comparison with the plating method. This test was made at Hobart, N. Y., in 1914 and proved the new method at least as valuable and as reliable, if not more so, than the older one; so it was used alone for more than two years to grade the milk of the City of Geneva. For two full years of the time it was accepted by both dairymen and milk distributors as a guide to the payment of premiums for good quality milk.

Tests at Hobart.

The preliminary tests were made in a laboratory operated by the Lederle Laboratories for one of the largest milk distributing companies of New York City. This Laboratory tests the milk received at Hobart for butter making, and that brought to three pasteurizing plants at Bloomville, South Cortright and Cobleskill for shipment to the city. At the shipping stations premiums are paid for milk with low bacteria counts, but at Hobart no such stimulus to the production of milk with a good bacterial quality was offered, and only 50 per ct. of the milk brought in there graded A, while the supply at the shipping stations averaged 80 per ct. Grade A.

The grading of the milk was based on the agar-plate counts made by an assistant of the Lederle Laboratories, and the microscopic slides were made at the same time, from the same milk samples, by an assistant bacteriologist of the Station. The gradings were made independently.

During February, March and April, 1504 samples of milk from the three Grade A pasteurizing stations were examined by both methods. The ratings made by the microscopic method were found to agree with those made by the plate method on 1339 samples, over 89 per ct. When the two methods disagreed the microscopic method gave too low counts in a little more than half the cases, and the plate count gave too low counts in the others. In the following July, 426 additional samples of milk from the same stations were graded by both methods, with an agreement in 85 per ct. of the comparisons. Many of the discrepancies in both winter and summer work were due to slight variations above or below the

number fixed as the limit for Grade A milk; so that most of the error in grading really falls within limits that would be considered reasonable in any experimental work; but there were occasional wide variations in both directions.

These variations, small or great, so offset each other that the totals of samples placed in the two grades by either method agreed very closely. Thus out of 1504 samples examined in cold weather, 1280 were graded A by the microscopic method and 1270 by the agar plate method; and of the summer samples 212 were graded A by the microscopic method and 234 by the plate method. This is a surprisingly close agreement and is probably as good as could have been secured if duplicate analyses of the same samples had been made by the agar plate method.

Microscopic method used at Geneva

So satisfactory was the agreement between the two methods as used at Hobart, it was thought there could be no unfairness to anyone in using the microscopic method alone in grading the milk received at two milk stations in Geneva, especially as slight changes in the details of the method promised even better results than were secured in the comparison tests. After a preliminary period of two months, the two milk distributing companies of Geneva and the dairymen who sold the milk agreed to accept the grades as determined by the new method. These grades were made to conform as nearly as possible to the three grades established by the State Sanitary Code designated by the letters A, B and C. To prevent confusion with these, the ratings given by the microscopic examination were called "good," "medium" and "poor." Because of the fact that the individual bacteria are seen under the microscope, the numbers used as the limits between the grades given microscopically are necessarily much higher than those used in the Sanitary Code for milk of similar quality. Thus the milk with less than 1,000,000 bacteria per c. c. was graded as "good," that with 1,000,000 to 10,000,000 "medium" and that with more than 10,000,000 as "poor." Actual counts were not made unless the sample appeared to be close to the limits between the grades. Usually an examination of 30 "fields" of the microscope was sufficient to place the milk in its proper grade, without counting.

The samples were collected at least once weekly from each can of milk delivered at the milk stations, temperatures taken, and records kept to show which samples were taken from night milk and which morning milk. As a check upon the accuracy of such weekly sampling, each can of milk brought in was tested every day during two periods of one week each. Reports showing the grading of each dairyman's milk were sent after each examination, and if anything of special interest developed in the milk of any patron at any time, he was informed of it by telephone. Many of the

dairymen visited the Station laboratory in response to such messages, often coming when it was possible to show them the entire process of making the microscopic preparations, and to have them study the milk thru the microscope. The permanence of the slides also made it possible to show any skeptic why his milk had been given a low grade. The rapidity and simplicity of the method, the visibility of the tiny germs themselves under the microscope, and the permanence of the preparations make such grading of the milk easier to understand than that by the older, indirect, and slower agar plate method.

As payments for the milk were based on the monthly grades, and as some cans from a producer would at times vary in quality from the majority of them, 25 per ct. of poorer milk was allowed in each grade without changing the rating; but in computing the 25 per ct. allowance each can of "poor" milk was considered equivalent to three cans of "medium" milk. Thus a producer who marketed during the month, 16 cans of "good" milk, 3 cans of "medium" milk and one can of "poor" milk would receive a "medium" rating. In this case the percentage was computed as if he had delivered 22 cans of milk, 16 of which were "good" and six "medium" in quality.

In this work at Geneva, nearly 12,000 cans of milk were examined, of which about 86 per ct, were found to contain less than 1,000,000 bacteria per c. c. and were rated as "good," 13 per ct. were graded "medium" and a little over one per ct. contained more than 10,000,000 bacteria per c. c. and so were rated "poor." Lest anyone should be surprised that milk containing as many bacteria as 1,000,000 per c. c. should be rated as "good," attention is again called to the fact that these are counts of the bacteria themselves, and not counts of groups of bacteria such as are obtained in the officially recognized agar plate count. The "good" milk was all of such a grade that it would have satisfied the standards ordinarily set for milk to be pasteurized and sold under the Grade A label, and therefore really deserved this rating.

A comparison between these findings and a series of analyses made from 20,000 samples taken by the New York City Department of Health in 1912 at 432 milk stations supplying milk for the city, indicates that the quality of the milk received at Geneva was as good as, if not better than, the milk received at that time for New York. The Geneva milk, on the other hand, did not average quite as good in quality as that received at the three Grade A pasteurizing stations and tested at Hobart; but was better in bacterial quality than that brought to Hobart for making butter.

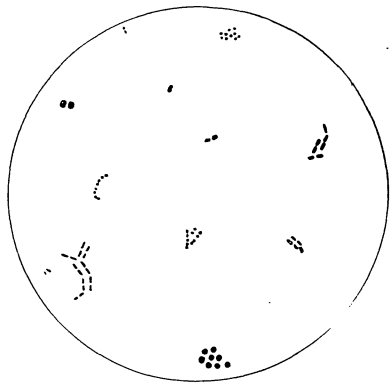
Some high-count milk was found at Geneva in winter as well as in summer, and some low-count milk in summer as well as in winter. The smallest percentages of "medium" ratings were found in May, 1915, and March, 1917; but the percentages for August, 1916, and

January, 1917, were not much higher. The milk as a whole showed the poorest condition in July, 1916, and September, 1915.

Treatment of milk cans very important.

Two companies handle practically all of the milk sold in Geneva, and the examinations showed marked differences in the bacterial counts of the milk received at the two companies. Not all of the factors responsible for this difference could be located, but some of them were, quite definitely, and correction of the poor conditions brought immediate improvement in the milk.

Insufficient steaming of the milk cans over a steam jet, with improper care given the cans after steaming, was a leading cause of high counts in the milk; or at least a marked decrease in the counts followed simple changes in the manner of handling the cans. At first, the cans at one receiving station were washed, rinsed with a jet of water, steamed for five to ten seconds, covered and allowed to stand upright, in which position the steam condensed leaving the moisture in the cans. As shown by investigations made elsewhere, cans handled in this way, when left standing for 24 hours or longer at temperatures favoring bacterial growth, may develop millions and frequently billions of bacteria. By inverting the cans and leaving the covers off, conditions were improved considerably altho the cans did not dry perfectly. Later, some cases of "medium" and "poor" milk were investigated, and these drained cans were found still to contain millions of bacteria that could be washed out with sterile water. Another change in the manner of handling the cans was made, by which the cans were steamed for twenty to thirty seconds, instead of five or ten, making them so hot that they dried out quickly after draining out any condensed steam. They were then found to be in good condition bacteriologically, and the amount of "poor" and "medium" milk brought in by the producers again decreased. However, other changes in conditions made at the same time may have aided in this reduction of bacteria.



GROUPS OF BACTERIA IN POOR QUALITY MILK AS THEY APPEAR UNDER A MICROSCOPE.

Milking machines need care.

Several of the herd-owners selling milk to this company used milking machines at some time during the two years. Most of these herds were large and the operation and care of the machines were left to hired help who did not appreciate the necessity of following directions in regard to the care of the machines.

These directions involve the use of mild germicides with whose action on bacteria they were unfamiliar. As experience at the Station has shown conclusively, milk with low bacteria counts can be drawn by machines under practical conditions by using simple and inexpensive precautions, but inattention to these precautions is fatal to success.

On one farm, where the owner of the herd did most of the work himself, the introduction of a milking machine caused no decrease in the grade of the milk, and bacteriological examination of the machines indicated that they were kept in good condition, sterile water milked thru the machines showing very few bacteria. On the other hand, milking water thru a machine used on another farm showed millions of bacteria in the water first passed thru, and almost as great a number in the second " milking " of sterile water.

On all of the six farms where machines were used except that operated by the owner, and possibly one other, the bacterial quality of the milk was lowered after machine milking was introduced, or was improved after the use of the machines was abandoned. The bacterial quality of the milk from the six farms was about the same as that of an equal quantity of milk produced on the eleven farms which supplied the poorest quality of hand-drawn milk.

These facts must not be misinterpreted. They indicate that the machines were not given proper care, not that they themselves were at fault.

However, the improper care of the machines, all but one of which (the one given the best care) happened to be owned on farms sending milk to one of the two companies, is one very good reason why the milk received by that company did not grade as high as that of the other company.

Detection of garget.

One of the advantages of the microscopic method is that it makes possible prompt detection of " gargety " milk, none of the other tests which have been used in sanitary milk control work giving results which are as satisfactory for this purpose. The prompt reports given on this type of milk enabled the owner to withhold the product of an affected cow and so maintain his grade. Accompanying gargety conditions of the udder is a type of bacterium which grows in long chains like a string of tiny beads that do not readily separate, and can easily be detected in microscopic preparations. The presence of this germ in the milk, when abundant, would be reported to the owner at once, and, if necessary, samples of the milk of the different cows in the herd be taken to locate the guilty one. In nine instances these individual samples were taken and the offending animals located at once; and in several other cases the owner was asked to look for " gargety " cows and was able to locate them without further bacteriological examinations.

No connection could be found between milking machines and garget in the herds, extensive infections with garget appearing at times in both hand-milked and machine-milked herds. All but 8 of the 36 men whose milk was tested continuously thruout the period of 26 months brought in, at some time, milk showing large numbers of this streptococcus or chain-forming bacterium, such milk comprising more than one-fifth of all that graded "medium" or "poor."

Comparison of methods. During the progress of this work, 643 samples of milk were examined by the agar-plate method as well as microscopically, and much better agreement between the results of the two methods was obtained than at Hobart. It is somewhat difficult to adjust the relation between the A, B and C grades established on the plate count basis, and the good, medium and poor milk as determined by the higher counts shown under the microscope; but making the comparisons as closely as possible, the two methods gave results which agreed in more than 91 per ct. of the cases. This, compared with the 85-89 per ct. of agreement obtained at Hobart, where but two grades were used, is a very great improvement. The discrepancies were again equally due to too high and too low readings by either method.

Daily or weekly testing. In order to learn, if possible, whether one test a week of each producer's milk gave a just basis for grading, a sample was taken from each can of milk delivered to one of the companies during one week in August, 1915, and one week in February, 1916. This extensive testing involved taking and examining from 30 to 50 samples daily; but the work was all easily done by one man and the results for each day were known before or shortly after noon.

Decided differences were shown in the bacterial quality of different cans of milk from the same herd, even on the same day; so that the records secured were not regarded as extensive enough or detailed enough to decide definitely how frequent sampling should be to give reasonably accurate grades. It is important that further studies be made along this line if milk is to be bought and sold extensively according to its bacterial count.

In the case of 17 men whose milk was tested during these two seven-day periods, frequent sampling gave no different grading from that of the month, based on weekly testing. The work did prove, however, that both morning and night milk should be tested. As the night milk, delivered in the morning, is 12 hours older than the morning milk, the bacteria in it have had time to increase and the added number may be enough to lower the grade of the can. In the winter week only three cans of "medium" milk were found in the morning milk against ten cans in the night milk. In the August week, when summer temperatures as well as the longer time of hold-

ing promoted development of bacteria in the night milk, there were four times as many cans of lower grade milk in night milk as in morning milk. Evidently if the samples had been taken only from the night milk, injustice would have been done the producer; if from the morning milk alone, to the purchaser.

Conclusions. The work at Hobart proved that the agar plate and microscopic methods agreed closely in results when used simply to divide milk into two grades, and the variations between the two methods were about equally balanced in direction. This agreement is probably about as close as would have been obtained in making duplicate tests of the same number of samples by the older method. As there is no means at present available by which we can know the exact number of bacteria in a given quantity of milk, it seems fair to assume that use of the microscope in grading gives results which are at least as accurate as those secured by the agar plate method.

The more carefully controlled experiment at Geneva, continued for twenty-six months, shows that it is entirely feasible and practicable to use the microscopic method for dividing milk into the three grades recognized in the Sanitary Code in use in the State. The labor involved and the expense of equipment are much less than that for the plate method, the results are available within a few minutes or hours as desired, and the slides can be preserved indefinitely. This permits the checking of fraudulent practices in grading in a very satisfactory and simple way and leaves a wealth of material for detailed study at any time.

Microscopic examination of the milk was found to be very helpful in detecting some causes of excessively high germ counts, especially in locating cows with garget. Other causes were not so easily identified. Two other factors were held to be responsible for high counts in the Geneva milk: (1) Improperly steamed and poorly drained cans that stood with their covers on where warmth promoted the growth of bacteria; and (2) milking machines that were not kept free from bacterial growth.

The whole series of studies proves the microscopic method worthy of consideration by all who in any way use the bacterial content of milk as a guide in commercial grading.