A Comparative Study of Commonly Used Staining Procedures for the Direct Microscopic Examination of Milk*

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This study of direct microscopic methods for determination of the sanitary quality of milk was based (1) on a general review of the literature; (2) on personal interviews with workers directly responsible for the introduction and development of this method; (3) on observation of the accuracy and proficiency attained by laboratory workers, these observations being independent and in addition to those reported by one of the authors; and, (4) on results of laboratory studies carried out on an experimental basis.

Procedure and Techniques in the Field

Observations in various laboratories indicated that most workers making direct microscopic examinations generally stay within the broad limits of procedure-conformity suggested in *Standard Methods for the Examination of Dairy Products*. On the other hand, and as was previously indicated by Black,[1] many take advantage of the fact that statements in Standard Methods are in the form of suggestions, and employ procedures or steps not included therein. Thus, in making the milk smears, either the 10 x 10 mm. square, the 4 x 8 mm. rectangle, or the circular areas are used. Milk may be measured by a standard pipette, or by platinum loops of different diameters, despite the fact that Standard Methods does not permit the use of the loop for official control purposes.[2]

The desired area is established by a scratched or etched groove only in the few places where circular areas are used. With square or rectangular areas, guide plates are placed under the slides, with little attempt made to prevent slipping of the slide. Occasionally the pipette was used in spreading the milk. Where milk was deposited upon the slide by means of a loop, the latter was occasionally used as the spreader. The resulting film, after drying and staining, frequently appeared irregularly between a square and a circle. The prescribed bent needle was used in spreading milk over the slide in only a few instances. Even then this was commonly done in two rapid strokes, without apparent regard to slippage of the slide over the guide plate. From field observations it would appear that one could prepare a film by eye approximation, omitting use of the guide plate or the etched circle, without exceeding errors due to other inaccuracies observed in preparing films.

The methods used in defatting, fixing, and staining milk smears were noted. Some used xylene, 95 percent ethyl alcohol and either the aqueous-alcoholic solution or the carbolated aqueous solution of methylene blue. Others used a single dip combined defatting-fixing-staining solution, containing methylene blue, a strong acidifying agent, such as sulfuric, hydrochloric, or acetic acid, and a fat solvent, usually tetrachlorethane. Still others used a two-stain, single dip preparation of methylene blue and basic fuchsin, containing sulfuric acid

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as the acidifying agent and tetrachlorethane as the defatting agent. Such staining procedures were adopted by many workers without previously making any critical investigation of the suitability of the methods to their laboratory needs. Occasionally, a heretofore unknown and undescribed procedure was found in use by a laboratory worker, of which the head of the laboratory had no knowledge.

The microscopes in nearly all cases observed were properly standardized to varying field magnifications, resulting in factors of 600,000, 500,000, and 300,000. In this connection it should be noted that in reporting on his survey of the direct microscopic method of examining milk and cream samples in approved and registered laboratories in Connecticut, Eglinton[3] lists "Microscope not standardized to a workable field diameter" among the deviations he found in practise.

Time spent in examining the slides varied with each laboratory worker, and it appeared that no careful account was taken of the number of fields examined. In view of the limited time spent on the examination of each stained milk smear, regardless of the number of bacteria and other organized elements present, it did not appear feasible that even the minimum number of microscopic fields required for the particular grade of milk could be examined, unless the observation of each field was very cursory. Reports were made in general terms, such as less than a certain number of bacteria, or in terms qualifying the milk as good, fair, or bad. Notations were entered in most cases regarding the presence of streptococci, unusual numbers of leucocytes, and large numbers of what the workers seemed to think were thermoduric bacteria.

In summarizing the field observations, it appeared that application of the direct microscopic method presented a state of mild confusion, primarily because of the lack of adherence to rigid standard requirements, and it would seem that this situation should be remedied as soon as possible. However, before any group of qualified workers can be asked to discard their habits or favorite techniques and adhere to the practise of prescribed new ones, evidence must be presented in favor of the changes offered. A restudy of the manifold variations now practised in laboratories engaged in the control of milk sanitation, therefore, appeared of primary and urgent importance.

Such a critical study was undertaken. Since, however, the number of variations is comparatively great and the study of each both exacting and time-consuming, so far only a few factors have been investigated. This paper is limited to reporting upon the results obtained in the study of staining procedures.

STAINING PROCEDURES STUDIED

Of the stains proposed for use in the direct microscopic examination of milk, the five most commonly known to laboratory workers and sanitary inspectors and most frequently used are the following: (1) The stain suggested in Standard Methods prepared by adding 0.3 gm. of certified methylene blue powder to 30 ml. of 95 percent ethyl alcohol, and adding this to 100 ml. of distilled water.[2] (2) Also suggested in Standard Methods is the stain prepared by adding 10 ml. of a saturated aqueous solution of methylene blue powder to 90 ml. of a 2.5 percent phenol solution.[2] (3) The Newman-Lampert Formula No. 2, likewise mentioned in the 8th edition of Standard Methods, and which is made up of 1.0–1.2 gm. certified methylene blue powder added to a solvent mixture consisting of 54 ml. ethyl alcohol and 40 ml. technical tetrachlorethane, and which is then strongly acidified by adding to the above 6 ml. of glacial acetic acid.[2, 4] (4) The Mallman Acid Stain Procedure, not suggested in Standard Methods, is prepared by adding 1.0 gm.

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of methylene blue powder to 500 ml. of 95 percent ethyl alcohol, and acidified by adding up to 5 ml. of concentrated hydrochloric acid.[5] (5) The Broadhurst-Paley stain, not suggested in Standard Methods, consists of a mixture of 54 ml. of 95 percent ethyl alcohol and 40 ml. of technical tetrachlorethane acidified with 0.4 ml. of concentrated CP sulfuric acid, to which after heating to 55° C. are added 1.2 gm. certified methylene blue powder and 8 ml. of 1.0 percent alcoholic solution of basic fuchsin.[6]

In addition to the above, we included in our studies the following staining procedures found in the literature: (6) the North Aniline-Methylene Blue Stain [2] suggested in Standard Methods for use in the bacteriological study of powdered milk; and (7) Gray's two-stain method for direct bacterial counts.[7]

With the two Standard Methods methylene blue stains, defatting in xylene or other suitable fat solvent and then fixing the milk smear in 95 percent ethyl alcohol precede the staining of the smear. The procedure thereby becomes a three-step method. With the Mallmann acid stain the need for a separate fixation is eliminated, since the dye is dissolved in 95 percent ethyl alcohol, and the procedure is a two-step technique. In the Newman-Lampert and the Broadhurst-Paley stains the defatting, fixing, and staining are accomplished in one step, and the procedures are commonly referred to as single dip stains. Single dip stains, if satisfactory from every other angle, possess the merits of simplicity and time economy.

BASIC REQUISITES FOR GOOD MILK STAINS

On the basis of observations in the field and in the laboratory, it appeared that the most suitable staining procedure for the direct microscopic count of bacteria in milk must be characterized by the following qualities: 1. It must be simple. 2. It must show the presence of a maximal number of bacteria, as well as of leucocytes and other organized matter capable of living and proliferating in milk. 3. It must show the least possible tendency to overstaining. 4. It must produce a light background with the bacteria stained in different degrees of intensity corresponding to the degree of affinity various types of bacteria or bacteria of different ages have for the dye. 5. The resulting preparation must not be fatiguing to the eye. 6. The stain must not be conducive to bacterial growth. 7. The stain must not deteriorate with time.

The Mallmann Stain. In the Mallmann stain the hydrochloric acid apparently effects a denaturation of the milk proteins causing the film to lose its property to adhere to the surface of the slide. Upon washing, no matter how carefully done, the milk film frequently lifted off the slide and remained floating in the wash-water. Even with the greatest caution exercised, we lost as high as 50 percent of our smears stained by this method.

Questioning of laboratory workers in the field and associates in our laboratory indicated that others have had similar experience with this stain. Johns and Berard [8] reported that they tried the Mallmann acid stain in their work with dried whole eggs and experienced trouble with films washing off. Probably for the purpose of counteracting this difficulty in the fixing of the milk film to the slide, in a later publication Bryan, Mallmann, and Turney [9] suggested that the smear be allowed to air-dry, then fixed by heating in a flame until the slide is just too hot to touch, then cooled, repeating three times, or placed in an incubator for at least one hour.

Such modification in the procedure would increase the complexity of the staining procedure, lengthen the time required for the preparation of the slide, and is not in accord with the

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requisites generally considered essential to an appropriate staining procedure. We did not try these additional steps in our comparative studies, and are not able to state whether or not they obviate the supposed denaturation of the milk proteins by the hydrochloric acid. Anderson [10] reports that he was able to obtain better stained smears and easier and higher counts by the Mallmann acid stain than by the standard methylene blue or the Broadhurst-Paley procedures.

The Broadhurst-Paley Stain. In the Broadhurst-Paley stain, the adhesive properties of the milk smears remain unimpaired, and upon macroscopic examination the film appears as a rather even and pleasant reddish pink color. Under the microscope, however, the picture is different. The red background proved fatiguing to the eye, and, apparently due to the formation of insoluble sulfates, there appeared on the slides crystalline and granular material which was not present in the milk originally, as judged by the study of smears of the same milks stained by other procedures. Apparently due to this formation of insoluble sulfates, the stain itself formed a precipitate and deteriorated in a comparatively short time.

The addition of acid to the stain where only methylene blue is used, as suggested in Standard Methods, lightens the background and renders the smear less liable to overstaining. Where basic fuchsin is used in conjunction with methylene blue, the addition of acid brings other influences into action. Due to the acid, a modification in the affinities of the protein constituents of the milk, bacteria, and other organized matter apparently takes place. The affinity of the bacteria and other cells for the methylene blue which varies with the species and age of the cells in the first place, is lessened or completely lost, and the affinity for the basic fuchsin is enhanced.

As a result, many of the bacterial cells stain a reddish pink and cannot be distinguished from the background. This appeared to be equally true, not only of the bacteria but of the leucocytes as well. Some of the cells take on both dyes to a varying degree and make judgment of the nature of the observed material far from easy. Some of the cells which stain distinctly blue, present what appears under the lens as distortions in the original shape. This may be due to sulfate compounds precipitating within the cells.

A summary of a comparative series of counts by the carbolated methylene blue, the Newman-Lampert No. 2, and the Broadhurst-Paley techniques is presented:

Triplicate sets of smears were made of fifty raw milks. One set of smears was stained by each of the techniques mentioned. All slides stained by the standard methylene blue and the Newman-Lampert procedures produced readable stains. In twenty of the fifty slides stained by the Broadhurst-Paley procedure we could see no leucocytes or bacteria stained blue. In the remaining 30 slides the numbers of bacteria and leucocytes fell far short of the number shown by either of the other two stains. The results of the leucocytes counts are summarized in Table 1.

TABLE 1

SUMMARY OF COMPARATIVE LEUCOCYTE COUNTS OBTAINED BY VARIOUS STAINS

	Carbolated Methylene Blue	*Newman-Lampert No. 2*	*Broadhurst-Paley*
High	17,000,000	14,000,000	3,000,000
Low	150,000	425,000	10,000
Average	5,000,000	4,000,000	600,000

This average number of leucocytes counted by the Broadhurst-Paley stain comprised only 12 percent of the average number obtained by the standard

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methylene blue, and only 15 percent of that obtained by the Newman-Lampert stains in this set.

The results of the attempted bacterial counts can be summarized as follows: In two milk smears, counts by the standard methylene blue and the Newman-Lampert staining techniques averaged 300 evenly spread individual bacteria per field. Our counts in smears of the same raw milks, stained by the double stain solution averaged as low as 10. In twenty-eight smears we experienced considerable difficulty in deciding whether the blue observed represented bacteria distorted by the acid of the stain or adventitious matter. In the remaining twenty smears we could see no blue-stained material resembling bacteria. We tried the suggestions made by Cohen [11] to obtain better results, but found that nothing in the way of basic improvement in the final counts was assured.

The Addition of Acids to Milk-Smears Staining Solutions. As a result of our experience with the Mallmann hydrochloric acid and the Broadhurst-Paley sulfuric acid double-stain solutions, we are of the opinion that the use of inorganic acids in the preparation of milk smear stains should be discouraged. If for some reason a double-stain solution containing basic fuchsin is considered desirable, the solution will have to be strongly acidified to produce the double-stain effect. Such an effect can be adequately produced by adding some of the basic fuchsin dye to the Newman-Lampert stain. We did that on several occasions, and have found that with acetic acid the solution does not deteriorate upon standing, does not form crystalline or granular material on the smear, and does not distort the bacteria or the leucocytes. However, a loss of affinity for the blue dye and an augmentation in the affinity for the red dye is manifest even with the acetic acid. As the amount of glacial acetic acid added is reduced, this effect becomes less marked. We found that with less than 1 ml. of the acid per 100 ml. of stain, a balance in the affinities of the two dyes begins to make itself apparent. Violet, blue, and red colors appeared not only in the organized material, but in the milk protein background as well, and the stained smear became blotchy and not readable. With about 2.5 to 3.0 ml. of acetic acid per 100 ml. of the solution, a maximal number of blue-stained cells can be obtanied, without apparent distortion of the morphology of the cells. Such number, however, still remained considerably below the number obtained by other appropriate single-stain solutions, and the red background in our judgment still remained fatiguing to the eye.

The Standard Methods Methylene Blue Stains. Two methylene blue staining solutions are incorporated in the 8th Edition of *Standard Methods for the Examination of Dairy Products.* Both give up the dye to the milk proteins of smears with equal ease and rapidity. Therefore, both solutions easily and quickly overstain the milk smears, if they are not removed from the solution and washed in just the proper time. Both stain the background intensely enough to conceal from visibility many of the minute and faintly staining bacteria which do not form clumps or chains but which are evenly distributed through the smear as individual bacteria. Both staining solutions in most smears produce strong contrasts between the background and leucocytes and those of the bacteria which they stain clearly, but both lack the desired quality of producing a picture of the maximal number of bacteria differentially stained in correspondence with the difference in their affinities to the blue dye.

The ease and rapidity with which methylene blue dye is absorbed by milk proteins from its aqueous solution and the resulting intensely stained background of milk smears may offer the basic explanation to the fact that other

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newly proposed staining solutions discussed in another paper[12] constantly counting smears of the same milks, as is shown in Table 2.

TABLE 2

COMPARATIVE COUNTS OF BACTERIA AND OF LEUCOCYTES IN RAW MILKS STAINED BY CARBOLATED METHYLENE BLUE AND NEWMAN-LAMPERT PROCEDURES

	Bacterial Counts		*Leucocyte Counts*	
Series	*Carbolated Methylene Blue*	*Newman-Lampert*	*Standard Methylene Blue*	*Newman-Lampert*
1	435,000	960,000	1,185,000	1,350,000
2	648,000	612,000	1,968,000	1,752,000
3	852,000	996,000	3,408,000	2,904,000
4	1,224,000	1,116,000	3,900,000	3,744,000
5	1,452,000	1,584,000	1,452,000	1,584,000
6	2,625,000	3,075,000	7,215,000	6,130,000
7	2,925,000	3,150,000	3,030,000	3,150,000
8	3,132,000	2,892,000	2,880,000	1,632,000
9	5,415,000	4,140,000	14,985,000	13,920,000
10	26,400,000	16,290,000	1,770,000	1,080,000
Average	4,510,000	3,482,000	4,404,000	3,481,000
Count Ratios	1.0	0.78	1.0	0.90
Average exclusive of milk No. 10	2,074,000	2,053,000		
Count Ratio	1.0	1.0		

produced counts far above those obtained by the officially recognized methylene blue solutions. They may also adequately account for differences and inconsistencies in counts obtained in smears of the same milks stained at different times with the same or different batches of the standard methylene blue staining solutions.

The Newman-Lampert No. 2 Stain. In the Newman-Lampert stain the background is generally considerably lighter than in the standard methylene blue stained smears. However, and as previously pointed out, the strong acidity caused by the addition of 6 ml. of glacial acetic acid to 100 ml. of the solution reduces the affinity of practically all the otherwise lightly staining bacteria, so that either they do not take up any blue dye or take it up to such a slight degree that the normal eye cannot see them under the microscope as it is usually used in the routine laboratory. In most instances they are the same bacteria which are concealed by the intensely stained background of milk smears. This may explain the remarkable closeness in the counts of cells which one can obtain in carefully

The data presented in this table demonstrate several of the points brought out in the preceding discussion of stains. It will be observed, for instance, that the average of the first nine counts in the slides stained by the standard methylene blue and by the Newman-Lampert procedures are practically identical. However, when the count of slide No. 10 is included, the average of the Newman-Lampert counts becomes reduced by about 22 percent. A careful study of slide No. 10 showed that the greater part of the bacteria, averaging about 90 per field in the standard methylene blue slide, consisted of very minute faintly staining bacteria, mostly of coccus morphology. The stained smear in this case happened to be exceptionally good, and an average count of 90 bacteria could be made with ease. When stained by the highly acidified Newman-Lampert stain, a large number of the bacteria lost just enough of the difference in their affinity for the blue dye to render them unrecognizable after staining the slide.

Since the Newman-Lampert staining solution produces counts not lower

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than those by the standard methylene blue solutions, it offers the additional inducement that it is a single dip staining solution and can be advantageously used for rapid screening purposes. The advisability of its removal in the 9th edition of Standard Methods from the official position it held in the 8th edition may be questioned by some competent workers who had extensive experience with this staining solution.

North's Aniline Oil Methylene Blue Stain. This stain is included in the 8th edition of Standard Methods as an alternative stain for microscopic counts of dry milk. It was recommended by W. R. North, Jr. of the Division of Bacteriology, U. S. Food and Drug Administration, in 1938, by personal communication to the committee on Standard Methods for Analyzing Frozen Desserts and Ingredients. In a footnote on page 175 of the 8th edition of Standard Methods the following statement appears: "This stain is very lightly absorbed by the milk proteins in the smear, while the microorganisms in the preparation take a deep blue color, thus facilitating recognition of the organisms. Significantly higher counts have been reported for smears stained with this solution than for duplicate smears stained with plain methylene blue stain." This statement points to several desirable properties any good milk stain should possess, and induced us to test the stain for possible use in the bacteriological study of raw milk.

It is appropriate to state here that in giving the outline for the preparation of this staining solution, the 8th edition of Standard Methods does not sufficiently emphasize the importance of caution to be used in its preparation. Not infrequently it will be found that upon filtering the solution, a great part of the dye remains behind, leaving the solution weaker than it was intended to be. The aniline oil may not go into complete solution and may remain suspended as minute fatty globules even after the solution is filtered through Whatman No. 50 filter paper. Upon staining the slide, these globules adhere to the milk smears permanently and seriously interfere with proper staining and reading of the preparation. These difficulties can be very substantially lessened, if the staining solution is prepared step by step with extreme slowness, all the while vigorously shaking the flask.

Ten raw milk smears were stained by the North and by the carbolated methylene blue stains and counted microscopically. The results are presented in Table 3.

TABLE 3

COMPARATIVE COUNTS OF RAW MILK STAINED BY THE NORTH * AND CARBOLATED METHYLENE BLUE STAINS

Sample	*Carbolated Methylene Blue*	*North Aniline Oil*
1	9,500,000	3,800,000
2	6,700,000	3,200,000
3	3,900,000	2,000,000
4	3,700,000	1,300,000
5	1,900,000	1,400,000
6	1,600,000	800,000
7	600,000	280,000
8	290,000	160,000
9	230,000	220,000
10	320,000	120,000
Averages	2,900,000	1,300,000

* Incorrect formula as published in *Standard Methods for the Examination of Dairy Products,* 8th edition.

The results indicate that the bacterial counts by the North stain, pre-

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pared as outlined in the 8th edition of Standard Methods, fell short of those obtained by the carbolayted methylene blue by more than 50 percent. This was primarily due to the fact that in raw milk the North stain as used by us produced a background so light that observing and reading the stained smears under the microscope proved a fatiguing and extremely annoying procedure. In the absence of a well defined background it was frequently impossible to decide whether the stained spots were bacteria, microscopic oil globules, or some other extraneous material. When all the observed stained formations suspected of being bacteria were counted, unbelievably high counts were obtained in some milk smears. On the other hand, when those stained formations were counted which the observer felt sure were bacteria, counts similar to those presented in Table 3 became the rule.

North [13] called attention to the fact that in the 8th edition of Standard Methods the statement: "Add distilled water to produce a total volume of 55.5 ml." is in error. The statement should have read: "Add 55.5 ml. of distilled water," which brings the formula up to a volume of 100 ml. Later Watrous and Doan [14] likewise called attention to this error in the 8th edition of Standard Methods. Nevertheless, we included the results of our study as described for the information of readers who may have used the North staining solution, as given in the 8th edition of Standard Methods. Later we received a quantity of the correctly prepared solution, as actually used in the laboratories of the Food and Drug Administration. We stained a number of raw milk smears and compared the counts with those obtained with carbolated methylene blue stain. We found that in no instance were the counts by the North stain lower than those by the carbolated methylene blue, and that in the majority of the smears counts were higher, ranging from 50 to over 100 percent. The correctly prepared stain produces a light background with relatively intensely stained leucocytes and less intensely stained bacteria. The background, however, is not clearly defined and appears as a greyish blue decidedly foggy screen.

We then stained a number of milk smears with the solution, leaving out the aniline oil. Upon washing, smears so stained rapidly gave up the stain and became almost completely decolorized, due to the effect of the hydrochloric acid. Apparently, the aniline oil in the North staining solution serves primarily as a buffer to the acid, and to a lesser degree as a mordant to the material of the smears. In solutions of methylene blue in alcohol or a 50–50 percent solution of alcohol and a suitable fat solvent, the buffer and mordant effects of the aniline proved in our experience to be considerably reduced.

The results of our observations in the application of the North aniline methylene blue staining solution to raw milk smears led us to believe that if the formula were further improved so that the milk proteins would stain more definitely, and the fogginess be completely eliminated, it would constitute an improvement over the plain aqueous methylene blue staining solutions.

The P. H. H. Gray Staining Solution. This two-stain solution [7] is prepared as follows: A—1.0 percent aqueous methylene blue—50.0 ml; methyl hydrate—50.0 ml. B—1.0 percent aqueous basic fuchsin—25.0 ml.; methyl hydrate—25.0 ml. Mix solutions A and B. The milk films are treated with xylene, washed with methyl alcohol, again dried and stained for a few seconds.

Theoretical considerations based on the knowledge of the principles underlying milk staining which previous studies placed at our disposal, led us to anticipate the following: 1. Due to the fact that the solution has a 50 percent water content, and to some extent that methyl instead of ethyl alcohol is

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used, the solution would very readily give up its stains to the proteins of the milk smear, and cause rapid overstaining. 2. Due to the total absence of acid in the solution, the milk proteins as well as the bacteria and other cells would absorb both dyes simultaneously, and in rough correspondence with their concentrations. This should result in an homogeneously stained smear of a bluish-violet color, and no differential staining effect should appear.

We stained three sets of slides, one set being immersed in the solution for ten seconds, the second set for five seconds, and the third set for barely a second, the slides one by one having been rapidly dipped into the solution and immediately rinsed in tap water. Microscopic examination indicated that slides stained for ten seconds absorbed so much of the two dyes that only the fat vacuoles could be seen under the microscope and no cellular elements or granulation of the milk proteins were discernible. The smears stained for five seconds took on somewhat less of the dyes, but still not cellular elements and no granulation of the milk proteins could be seen. The slides stained for one second appeared much as do smears slightly overstained by the aqueous methylene blue. All showed the anticipated blue-violet color to the complete exclusion of the red.

Some of the slides stained as described were immersed for one minute into ethyl alcohol containing 3.0 percent of glacial acetic acid. The reddish color of the fuchsin began to appear. Such treatment, however, failed to render the slides usable for the purpose of counting bacteria.

Fifty milliliters of the Gray two-stain solution were then acidified with 3.0 percent of glacial acetic acid, and several milk smears were stained. After washing and drying the stained smears appeared as readily transparent evenly stained red films. Examined under the microscope, they showed an evenly stained red background and clearly defined blue-stained leucocytes and bacteria. The results were similar to those produced by us with the Newman-Lampert solution, in which the acid was reduced to 3.0 percent and to which an appropriate concentration of basic fuchsin was added.

The results of our experiments with the Gray two-stain solution led us to conclude that, as originally described, this solution fails to impart to raw milk smears the differential staining qualities for which it was primarily proposed, and that its readiness to overstain milk smears in a very few seconds far exceeds that of the two methylene blue solutions stipulated in the 8th edition of Standard Methods.

SUMMARY

We are in general agreement with opinions prevailing regarding the direct microscopic examination of raw milk, as expressed by the following statements: The procedure is characterized by a high degree of versatility, in that it can show with some accuracy the number of clumps or of individual bacteria per milliliter of milk; the types of bacteria predominating the number and types of leucocytes present, thereby calling attention to the possible existence of certain abnormal conditions in the cow; and the presence of extraneous material such as yeasts or molds, and grossly suspended material commonly classed as dirt. This method requires only a short time for its completion, it can be carried out wherever table space and electricity are available; the slides, after drying can be taken to a central laboratory for accurate examination; it can be applied with equal rapidity to various steps of milk processing; and it can tolerate to a variable degree, differences in technique, and still indicate whether the milk examined is poor. fair, or good, from a sanitary viewpoint.

Our studies with methylene blue and basic fuchsin dyes as applied to milk

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smears may be summarized as follows: (1) aqueous solutions of methylene blue readily give up the dye to the milk proteins of the background, and cause frequent overstaining; (2) strong contrasts are attained at the expense of delicacy in color shades, which in turn results in the loss of visibility of numerous bacteria whose affinity for the dyes is only slightly greater than of the milk proteins forming the background of the smear; (3) sulfuric acid causes precipitates to appear and breaks down or distorts the organized cells; (4) hydrocloric acid causes an apparent denaturation of the milk proteins resulting in a loss of its adhesive properties; (5) all acids tried, including acetic acid, lighten the background of the stained smears by changing the original staining properties of the material in the milk smears, making many of the lightly staining bacteria imperceptible to the eye; (6) proper application of solutions containing both methylene blue and basic fuchsin requires a high acidification and results in the disadvantages previously mentioned, and the red background is fatiguing to the eye; (7) based on the studies reported, we believe that improved procedures for staining milk smears can be developed.

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