

COLIFORM DENSITIES IN PASTEURIZED DAIRY PRODUCTS

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The sanitary significance of low coliform densities of pasteurized dairy products and the importance of using laboratory methods sufficiently sensitive to detect such densities are stressed. A comparative study of common solid media and brilliant green bile broth reveals frequent failure of solid media, as usually employed, to yield typical colonies in cases of gross coliform contamination and the superiority of the tube method for examining pasteurized samples with low coliform densities.

To be thoroughly safe for the consumer, milk and milk products should not only be efficiently pasteurized but they must also be free from recontamination. Although this is theoretically well understood, it is surprising that laboratory procedures, which have played a most important role in reducing bacterial counts of pasteurized products, have not been more intensely used to detect post-pasteurization recontaminations and to prevent them. The coliform test, first officially suggested, in 1920, by an A.P.H.A. Committee on Milk Supply, has been the one most generally recommended since then for this purpose. McCrady and Langevin¹, however, noted in 1932 that this suggestion was not followed by the study which reasonably might have been anticipated. Despite the numerous reports published later, recent papers show that the place of the coliform test in pasteurized milk control is still misunderstood in some quarters.

NO COLIFORMS IN SAFE MILK

We know of no instance where coliforms survived pasteurization in significant numbers, when the raw milk was of acceptable quality. The best report on the subject is probably the one published by Buchbinder and Alff² who concluded, after a rather complete study of previous reports and considerable investigation, that heat-resistant coliforms have absolutely no practical significance in the coliform test of pasteurized milk. They incubated overnight 468 flasks, of approximately 1 litre each, of pasteurized milk, all taken before bottling and usually from a sanitary line just

after the pasteurizer. All the samples, except one, yielded negative results, although the coliform counts of the raw milk used in these trials ranged from 1,000 to 56,000,000 per 100 ml. Their conclusions are in perfect agreement with our own experience. Our results on pasteurized milk taken from pasteurizers are nearly always negative in the 45.5-ml total volume we use in our routine test; when coliforms have been found, investigation has nearly always revealed something wrong. Various coliform strains obtained from positive samples taken from pasteurizers have been submitted to pasteurization tests, in the laboratory, over a long period, and none of them has been found to be of a heat-resistant type. Presence of coliforms in milk taken from pasteurizers, according to our experience, is usually due to inadequate heating of the milk, or to contamination with raw or incompletely pasteurized milk, or with drippings from defective covers, etc. In fact, coliform densities of properly pasteurized and uncontaminated milk taken from pasteurizers are normally so low (probably much less than 1 per litre) that, for all practical purposes, the organisms may be considered as absent from such milk. If they occur in significant numbers at subsequent stages, we must conclude that they have gained access to, or have multiplied in the milk, as a result of faulty operation or of poorly sanitized or defective equipment.

Neither the swab test nor the Standard Plate Count can serve as a substitute for the coliform test. It is extremely common in plants employing careless methods of sanitation to find no coliforms in the milk taken from the pasteurizer, but a high coliform content in the bottled milk with no apparent change in the plate counts. There is no over-all test available at the present time that can detect existence of opportunities for addition or multiplication of bacterial contaminants, that might be associated with



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pathogens, so readily, so rapidly, and so surely as does the coliform test.

We must usually rely upon the phosphatase test positively to detect inadequate heating or contamination with raw milk, but the coliform test may often prove much more sensitive for detecting leakage of raw milk into the pasteurized milk. Tiedeman³ and Weber⁴ have emphasized that the coliform test can reveal slight recontamination with raw milk or cream that cannot be detected by the phosphatase test. More recently, Olsen⁵ reports the interesting case of a plant in which leakage of raw milk through a fissured regenerator plate was responsible for persistent abnormally high coliform densities in stasitized milk for a period of nearly 6 months, before the fissure had become large enough to permit such

TABLE I—SUMMARY OF COLIFORM RESULTS OBTAINED ON SAMPLES OF PASTEURIZED MILK, AS DELIVERED TO CONSUMER, FROM MONTREAL SUBURBS* (PLANTS IN MONTREAL AND SUBURBS), DURING A 4 MONTH PERIOD FROM MAY TO AUGUST.

Total samples examined : 927	
Proportion of samples with coliform densities (M.P.N.)	
	not exceeding 10 per 100 ml. : 73.1 %
	not exceeding 50 per 100 ml. : 87.4 %
	not exceeding 100 per 100 ml. : 89.3 %
Number of dairies represented : 20 (including all plants from which at least 8 samples have been examined)	
Proportion of plants having, in at least 75% of samples examined, coliform densities	
	not exceeding 10 per 100 ml. : 10 / 20 (50%)
	not exceeding 50 per 100 ml. : 17 / 20 (85%)
	not exceeding 100 per 100 ml. : 17 / 20 (85%)

* City of Montreal excluded (samples from Montreal are examined by the City)

leakage that positive phosphatase tests of the contaminated product were obtainable.

PRACTICABLE COLIFORM STANDARD

Since it is difficult to attain absolute perfection in a pasteurizing plant, a few coliforms may sometimes be expected to occur in the finished product, but they should be very few: certainly usually less than 100, and preferably less than 20, per 100 ml. However, in a recent study of the milk from representative plants in 8 American cities, Dahlberg, Adams, and Held⁶ reported that only one of these cities had 100 percent of freshly pasteurized samples with coliform densities lower than 0.1/ml, whereas the others had from 72 to 27 percent of samples with such densities. Claims, often expressed, that it is not possible to get the coliform densities of pasteurized milk down to low figures derive largely from the disinclination of the control officer to conduct the stage by stage bacteriological investigation which is usually necessary to discover the sources of the contamination or cultures that are being fed into the pasteurized product.

Probably one of the factors most responsible for this condition has been the lack of coliform standards in most areas. We may hope that the recent limiting standard of 10 coliforms per ml, proposed for

an initial control program by the U. S. P. H. S., although, in our opinion, too lenient, will stimulate the interest of milk workers in the test and bring conviction that, with reasonable effort, coliform densities in pasteurized milk can be maintained at low levels. As reduction of bacterial counts in pasteurized milk has been secured by adequate supervision of milk production on the farm, coliforms may be reduced to extremely low densities by proper control in the pasteurizing plants. Since coliform densities, however, are more directly related to the safety of the product, a supply with an average plate count of around 75,000/ml and extremely low coliform density is evidently safer than one with a count of around 30,000/ml and a coliform density of 1,000/100 ml, provided both give satisfactory phosphatase results. We learned dearly, from the Montreal milk-borne typhoid epidemic of 1927, the importance of keeping pasteurized products as free as possible from contamination and the possibilities of the coliform test for this purpose.

A summary of coliform results obtained by our laboratories on pasteurized milk from the Montreal suburbs is given in Table I, showing the usual achievement of our plants during summer months when the coliform densities are usually highest.

According to this summary, 89.3

percent of the samples, all representing milk as delivered to consumers, did not exceed 50/100 ml and 73.1 percent, 10/100 ml. Of the 20 dairies represented, 17 (85%) had not more than 25 percent of samples with coliform densities exceeding 50/100 ml: the best of these plants had not a single sample above this limit and only 18 percent of samples with over 10/100 ml, while the worst had 21 percent with over 50 and 37 percent with over 10 coliforms/100 ml.

INTERPRETATION OF COLIFORM COUNTS

If the pasteurized milk is kept properly refrigerated, as generally required, outside temperatures should have little practical influence on increase of its coliform density from the moment of bottling to the time of delivery, especially when the initial coliform content is practically nil as it should be. According to our data, when coliform densities are abnormally high in street samples during the warmer months, they usually are likewise high in the freshly bottled samples. Warmer temperatures, which tend to multiply causes of contamination (dust, flies, condensation water) and favor the rapid growth of contaminants on equipment, in cracks, etc., evidently contribute to increase the coliform densities of the bottled milk; but since such increases are due to a multiplica-

TABLE 2—COMPARATIVE STUDY OF BBL DESOXYCHOLATE AGAR, DIFCO VIOLET RED BILE AGAR AND DIFCO BRILLIANT GREEN BILE BROTH.

	BBL ⁽¹⁾ and VRB ⁽²⁾			BBL ⁽¹⁾ and BGB ⁽³⁾		
	SAMPLES	Results higher with		SAMPLES	Results higher with	
		BBL	VRB		BBL	BGB
Raw milk	63	45 (71.4%)	16 (25.4%)	63	—	—
Past. milk	95	17 (17.9%)	17 (17.9%)	95	18 (19.0%)	17 (17.9%)
Past. homogenized milk	17	6 (35.3%)	2 (11.8%)	46	9 (19.6%)	4 (8.7%)
Past. chocolate milk	24	2 (8.3%)	8 (33.3%)	41	6 (14.6%)	17 (41.5%)
Past. cream	32	8 (25.0%)	9 (28.1%)	52	10 (19.2%)	18 (34.6%)
TOTAL Pasteurized	168	33 (19.6%)	36 (21.4%)	234	43 (18.4%)	56 (23.9%)

(1) = BBL desoxycholate agar (2) = Difco violet red bile agar (3) = Difco brilliant green bile broth.

tion of potential hazards, they really indicate the necessity of improving sanitizing procedures or of taking more care to detect and eliminate sources of contamination, rather than the need of relaxing standards.

Since bacteriological standards are essentially arbitrary limits, often set at different levels in different areas, they should not be interpreted as a sharp dividing line between safe and unsafe products. This is especially true of coliform standards, since coliforms in pasteurized milk seem always to derive from insufficient processing or recontamination and, consequently, when they are present even in small numbers the possibility of the presence of some pathogens also cannot be entirely excluded. Similarly, no complete guarantee may be had that pathogens are not present on the equipment surfaces with which milk comes in contact, if sanitizing procedures employed do not usually leave such surfaces free or almost free from coliforms. Fortunately,

coliforms are not necessarily accompanied by pathogens; otherwise, the only logical standard would be one requiring total absence of such organisms in the final product. Densities tolerated, however, should be as low as practicable since even slight contaminations involve some potential health hazard, which is the more threatening in pasteurized milk in which, according to Olsen, because of the loss of a bactericidal factor, *Salmonella* and *Shigella* grow far better than in raw milk.

When determining where the limit is to be fixed, therefore, official authorities should be guided by the usual achievement of the best plants rather than by the desire of assuring an almost general compliance without improving present faulty procedures. A standard of not more than 100 coliforms per 100 ml may usually be met by Certified-Pasteurized milk; why should a 10 times higher limit be tolerated for ordinary pasteurized milk, when the significance of the presence of coliforms is exactly the same in both cases? At least three cities in

Quebec Province have been enforcing with no great difficulty, for several years, a standard (recently adopted as a Provincial legal standard) of not more than 50/100 ml in 3 of the last 4 samples collected. Accumulated data indicate that a limit of 20/100 ml could be met nearly as easily, provided the same tolerance, allowing for the odd "accident" which may always occur, is applied.

Compliance with standards, however, should not be considered as a perfect achievement. Even lower densities may be significant, especially if they are found persistently, and procedures to detect them should be employed. The normal dilution of cultures of organisms, which may be expressed from the pocket formed by a broken joint or otherwise fed into the pasteurized product, often results in coliform densities of the order of 50, 20, or even fewer per 100 ml and, unless such densities can be properly determined, efforts to detect the sources of recontamination by sampling at various stages of processing may fail. In many areas

where competence of plant operators and control officials cannot be denied, failure to succeed in reducing coliforms to reasonably low levels is probably due, to a large extent, to the lack of sensitivity of the laboratory tests used and to the consequent lack of significance of negative results.

TECHNIQUE FOR MAKING COLIFORM COUNTS

Both the plate and the tube methods are recommended by the Standard Methods for the coliform test. Tiedeman and Smith¹, comparing the formate ricinoleate broth tube method (with 3 x 1 ml) and the desoxycholate agar plate method (with 1 ml), observed a good correlation between results obtained on 1630 pasteurized samples. Similarly, plating 836 samples in both violet red bile agar and desoxycholate agar, they found that these two media were equally satisfactory. Buchbinder and Fertig², studying 2,250 samples with desoxycholate agar and brilliant green bile broth, concluded that the productivities of the media were essentially the same. In another study for determining the influence of the milk volume plated on the productivity of the desoxycholate agar, they found that volumes of 1 to 4 ml gave comparable findings but that plating decimal dilutions often resulted in a reduction of the productivity, due to greater inhibition of sodium desoxycholate in the absence of considerable proportions of milk.

In an attempt to determine if this partial neutralization of the inhibiting agent by the milk proteins might indicate the use of a higher concentration of desoxycholate, we compared the B.B.L. (No. 114) agar and the Difco (No. 273) agar which contains double the amount of desoxycholate, plating 0.1-, 0.01-, and 0.001-ml portions of raw milk samples, and 1- and 4-ml portions of pasteurized samples. The greater inhibitory action of the higher desoxycholate content of the Difco agar seems to be very striking, since markedly higher counts were obtained with B.B.L. agar from 9 of 10 raw milk samples examined. With the relatively large volumes of 30 pasteurized samples plated, however, comparable findings were obtained: 7 (23.3%) yielded higher results with B.B.L. agar and

5 (16.6%) with Difco. The differences observed, moreover, were not excessive; but two pasteurized cream samples which, according to our routine test with brilliant green bile broth, contained 900 and more than 2400 coliforms per 100 ml respectively, gave no typical colonies at all on either agar medium. The plates with 4 ml were so opaque that no colonies could be seen and plates with 1 ml showed only atypical colonies. These particularly serious discrepancies incited us to proceed with our comparative study, using Difco violet red bile agar, B.B.L. desoxycholate agar and brilliant green bile broth with further samples of raw and pasteurized milk and other pasteurized products. The volumes plated were as indicated above except that an additional plate with 0.1 ml was also used with each medium in the case of pasteurized cream. For the fermentation test in the bile broth, five 1 ml and five 0.1 ml portions have been used, with higher dilutions of raw samples, and with four 10 ml portions of pasteurized milk and cream when the volume of the sample permitted.

A comparison of the results obtained is summarized in Table 2. In this summary, the B.B.L. agar appears to be markedly more productive than violet red bile agar with lower than 1 ml volumes of raw milk, but both media usually gave quite comparable findings with larger volumes of pasteurized milk or cream. Violet red bile agar, however, proved to be even more satisfactory than B.B.L. agar for plating chocolate milk.

In order to make as fair as possible the comparison between brilliant green bile broth and B.B.L. desoxycholate agar by taking into account the mathematical limitations of both techniques, in the case of pasteurized samples absence of coliform colonies in the 5 ml total volume plated and densities lower than 20/100 ml by the tube method were considered as concordant results, as were production of gas in all the fermentation tubes and coliform counts exceeding 2400/100 ml on B.B.L. agar. Results generally show a satisfactory concordance, but with a marked advantage in favor of the tube method with chocolate milk and cream samples.

In the case of raw milk, a satisfactory correlation has also been obtained, although no significant comparative figures can be given because of an excessive frequency of gas production in all tubes used. The tendency of the B.B.L. agar to give higher results with homogenized milk more often than the violet red bile agar and the bile broth is rather puzzling. A reverse tendency is evident with chocolate milk.

The serious failure of both solid media to reveal typical coliform colonies when numerous bacteria grew on the plate has been frequently observed, although the violet red bile agar, recently reported by Morris³ to inhibit development of typical colonies frequently when coliform contamination is heavy, proved to be superior in this respect. Counts secured from the plate, especially with desoxycholate agar frequently giving colonies of all shades from dark red to pink or white, were often most unsatisfactory on account of the very great difficulty in identifying typical colonies; the number of such colonies sometimes decreased in proportion, and often were entirely absent, in larger volumes of sample. In fact, many samples containing from 1000 to more than 2400 coliforms/100 ml, according to the tube method, gave no typical colonies at all with the various volumes plated. Such gross discrepancies were observed more frequently in tests of homogenized milk and, especially, chocolate milk and cream. With cream samples, typical coliform colonies often appeared on the 0.1-ml plates only. The frequency of this serious failure, aggravated by an objectionable opacity of the medium when 4 ml of homogenized milk, chocolate milk, or cream are plated, greatly reduces the significance of negative results, restricts the supposedly superior accuracy of coliform counts on plates, and limits the effectiveness of the plate method for determining densities lower than 100/100 ml.

On the contrary, only three samples, two of chocolate milk and one of cream, gave negative results with the fermentation tubes and counts higher than 1000/100 ml with the plates. Upon investigation, in the case of the cream

sample, the organisms isolated from the fermentation tubes formed no gas in lactose broth but produced typical red colonies on both B.B.L. agar and violet red bile medium, provided the plate did not contain many more than about 100 colonies.

The tube method, consequently, appears to be the method of choice for examining pasteurized samples, the coliform content of which usually should be very low. Since there is nearly no practical limit to the volumes that can be used, the potential sensitivity of the test is practically unlimited. It offers much less risk of missing gross recontamination, or of yielding grossly discordant results in the hands of different workers and with various pasteurized products, in-

cluding chocolate milk and cream. If the plating method be used, two or more portions of different volumes should preferably be plated, including sufficiently small volumes to reduce the risk of failing to detect typical colonies on overcrowded plates.

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DAIRY INDUSTRY INITIATES REMEMBRANCE FUND

The establishment of Dairy Remembrance Fund, Inc. — an organization devoted to further the progress of the dairy industry—has been announced by Madison H. Lewis, president.

The Fund will serve as the central agency through which contributions may be made in honor of important milestones and other significant events in the lives of dairy personnel. Through delegates from eight national and international dairy groups it also will administer memorial trust funds which perpetuate great names within the dairy industry.

Headquarters of the new organization will be located at 111 North Canal street, Chicago.

Charter sponsors include American Dairy Science Association, Dairy Industry Committee of the United States, Dairy Industries Society International, Dairy Industries Supply Association, International Association of Ice Cream Manufacturers, Milk Industry Foundation, National Association of Retail Ice Cream Manufacturers and National Dairy Council.

Mr. Lewis, former president of the International Association of Ice Cream Manufacturers and grandson of the founder of the Horton Ice Cream Company, was elected as the first president of Dairy Remembrance Fund. Mr. Lewis said: "The establishment of this Fund is further evidence of the

determination of the dairy industry to solve the problems of the present temporary surplus and to find ways and means for the efficient disposal of what should rightfully be an even greater production. We plan to allocate our income for research projects which may develop an improved knowledge of the production, processing or distribution of all forms of dairy products; projects which may promote a better understanding of the nutritive value and the better utilization of all forms of dairy products; projects which may promote the general welfare of any phase of the dairy and allied fields and any other projects which may advance the good name of the dairy and allied fields."

Robert Rosenbaum, former president of D.I.S.A. and chairman of Dairy Industries Society, which sponsored the recently held First World Congress for Milk Utilization, was elected secretary and executive director of the Fund. Mr. Rosenbaum stated that the projects undertaken would be coordinated with the existing research programs of the diverse branches of the dairy field. In addition, he explained that the projects, as outlined by Mr. Lewis, could include the stimulation of students' judging contests both here and on an international basis and the exchange of dairy personnel with other nations.

E. J. Mather, director of International Association of Ice Cream Manufacturers, became the Vice President of the Fund and chairman

of its executive committee. E. B. Lehrach of Chicago serves as Treasurer both of the Fund and of National Dairy Council whose offices in Chicago will serve as headquarters for the period just ahead.

Other members of the executive committee are: B. F. Castle, Executive Vice President of the Milk Industry Foundation. V. K. Shuttleworth, board of directors, National Dairy Council.

A CAREER FOR YOU

Dairy science students at Cornell have published "A Career for You," a 16-page booklet outlining facilities for dairy science majors in the College of Agriculture and describing opportunities in the field.

Changes in the past two decades have brought about a need for more qualified personnel to meet existing competitive conditions, C. G. Kazolas, Jamaica senior and member of the Dairy Products Judging Team, points out in an article. He traces innovations from 1851, when Jesse Williams, a Rome, N. Y. farmer started the first cheese factory in the country.

Dairy graduates with a thorough background in accounting, quality control, production, labor relations, and marketing have the best chance for a successful career, Kazolas says.

"In Percentages and Possibilities," Paul R. Seymour of West Seneca reports more than 90 percent of former dairy science students have remained in the milk industry.