

Effect of Different Incubation Temperatures  
and Periods on Standard Plate Count  
of Milk

BY

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CHAPTER I

INTRODUCTION

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## CHAPTER I

### INTRODUCTION

Milk occupies an unique position in the diet of most of peoples in the world. This is because of the fact that it contains all the essentials of a perfect food for any type of person. In addition to this, its facility of ingestion and comparative ease of digestion, it constitutes an important food for the sick, invalids, infants and persons with weak digestive systems.

Unfortunately, these virtues of milk act as foe and friend of human race as the milk is also an excellent medium for the growth of both pathogenic and non-pathogenic organisms. The total number of bacteria present in milk gives a rough idea about the conditions under which the milk has been obtained. Hence, the bacteriological quality of milk is better judged by determining the total number of bacteria present in it.

The standard plate count is one of the oldest and most frequently used quantitative methods for enumerating bacteria in milk. When it was originally introduced, its purpose was to count all bacteria contained in a portion of milk plated. During last few decades, many changes have been included in respect of media, incubation temperature and incubation period etc.. As a result of these changes, the present method followed is far superior to older ones

for estimating the bacterial count in milk.

Under specified conditions pertaining to apparatus, technique of plating, incubation, counting and reporting of counts etc., the number of colonies per ml of milk is reported as 'Standard Plate Count .. (SPC/ml) or (SPC/gram). In other words S.P.C. is the estimate of number of viable organisms per ml or gram of milk or any other milk products.

Although, this method is time consuming and comparatively more expensive, it has been widely used with satisfactory results and is particularly suitable where low bacterial population is expected. This method is specially useful for pasteurised milk and for line testing at various stages of processing for detecting the possible source of contamination. It is also commonly used for determining the efficiency of pasteurised milk, for examining raw milk, particularly of low bacterial densities and where premiums are paid to the producers on the basis of bacterial quality. In some countries, this method has also been adopted as standard method for the routine examination of 'designated' milks and 'certified' milks.

In order to get the real bacterial count in milk, besides the proper food and moisture, bacteria require favourable temperature for their multiplication. The optimum temperature varies over wide limits with different kinds of bacteria. Roughly, bacteria may be grouped into

three categories : the psychrophilic which can grow at temperature down to  $32^{\circ}\text{F}$ ; the mesophilic or common bacteria which are more prevalent in milk and grow best between  $70$  and  $100^{\circ}\text{F}$  and the thermophilic or heat-loving bacteria which can grow at the pasteurizing temperature of  $143^{\circ}\text{F}$ . Although, they grow best at their optimum temperatures, multiplication of bacteria takes place more slowly at either lower or at higher temperatures. It is, thus evident that different types of microorganisms are present in milk with varying temperature requirements for their growth. For this reason from time to time, temperature of incubation, incubation period and growth media have been critically studied and the results have been published in technical magazines. The investigations made during last few decades revealed that the incubation temperatures of  $30^{\circ}$  or  $32^{\circ}\text{C}$  could give higher bacterial counts in milk than at  $37^{\circ}\text{C}$  incubation temperature.

Recently, the countries like U.K., U.S.A. and Columbia have changed the incubation temperature of  $37^{\circ}\text{C}$  to  $32^{\circ}\text{C}$ . They are further suggesting to bring it down to  $30^{\circ}\text{C}$ . The Indian Standards Institute in their publication of 1962 has recommended the  $37.0 \pm 0.5^{\circ}\text{C}$  incubation temperature and 48 hour incubation period for determining the Standard Plate Count in milk. Looking to the tropical nature of climate in this country and the overall trend in different parts of

world to reduce the incubation temperature, it was felt necessary to verify these recommendations under Poona conditions.

An attempt has, therefore, been made to use three incubation temperatures namely 32<sup>o</sup>, 35<sup>o</sup> and 37<sup>o</sup>C and the incubation period of 42, 48, 60 and 72 hours by using a Tryptone Glucose Extract Agar medium suggested by Difco manual. The work undertaken in this study may prove the reliability or otherwise, of the recommendations made by I.S.I. (1962).

CHAPTER II  
REVIEW OF LITERATURE

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## CHAPTER II

### REVIEW OF LITERATURE

Of the various factors which contribute to inaccuracies in plate counts, the incubation temperature and the period of incubation have the greatest impact on Standard Plate Count of milk. The literature regarding the effect of incubation temperature and period of incubation on the Standard Plate Count of milk and milk products, have been reviewed in the foregoing pages.

In the bacteriological study of milk, water and sewage, the incubation temperature of 37 °C for 48 hours was generally followed in the past. Use of 37 °C as incubation temperature was first adopted at the 1915 (Jacksonville) meeting of American Public Health Association. It was generally assumed that incubators were quite accurate and incubating plates at 37 °C for 2 days was satisfactory.

Mudge (1927) suggested an incubation temperature of 30 °C for 48 hours and pointed out that larger colonies and higher counts could be obtained at 32 °C than at 37 °C.

Norton (1928) worked on incubation temperatures of milk and reported that higher counts were of minor importance in milk control, but he emphasized that there should be uniformity in the counts. The new proposed incubation temperature as recommended by Pederson and Yale is 32 °C. He

suggested to keep the temperature of the ordinary incubators down to 32 °C (89.6 °F) on hot days in the country.

Hiscox et al. (1933) studied the effect of time and incubation temperature on the bacterial count of milk. They incubated the plates at 37 °C for 48 hours and at 22 °C for 48 hours and reported that the count was very difficult, when the counting was made after 48 hours, particularly with pasteurised milk samples which made work troublesome due to minute size and transparency of the colonies. Method of counting at the end of 48 hours period was retained for reason of economy of time and incubator space. There was more increase in the number of colonies at 22 °C than at 37 °C. When the period of incubation was further extended by 18 hours for the plates incubated at 22 °C, there was not only a definite increase in the number of colonies but there was a marked visibility in the colonies. This was observed both on standard agar and on standard agar + milk.

Pederson and Yale (1934) made a comparative counts on 78 samples of pasteurised and raw milk, cream and ice cream after 48 hours incubation period at different temperatures viz., 21°, 25°, 30°, 32°, 35°, 37°, 39°, 45° and 55 °C. The highest count was obtained at 32 °C with pasteurized milk. Hence, they concluded that the optimum temperature for raw milk samples lay around 32 °C and that the counts at 37 °C were approximately only 50 per cent of the 32 °C. Ice-cream

and other products also gave significantly higher counts at 32 °C than at 37 °C temperature.

Bowers and Hucker (1936) observed higher counts on Standard Agar employed in milk control work, when the plates were incubated at 32 °C than at 37 °C.

Wilson et al. (1935) made a comparative study on the optimum temperature of growth of milk organisms in United Kingdom with the temperature used in U.S.A. and concluded that incubation at about 30 °C for 3 days gave higher counts than at 37 °C with raw and pasteurised milk samples.

In a subsequent study, Wilson and Co-workers (1935) reported that 37 °C was the most practical incubation temperature for pathogenic bacteria as they found it quite optimum for their growth.

McDonald and Hale (1935) studied the relation of keeping quality of milk and low bacterial counts. They found that both titrable acidity and agar plate counts were much higher in milk samples and plates incubated at 68 °F (20 °C) than at 98 °F (37 °C). They concluded that the 68 °F gave better indication of bacterial flora than the 98 °F (37 °C) temperature.

Optimum temperature of incubation for standard methods of milk analysis as influenced by the medium was studied by Yale and Pederson (1936). The authors previously

recommended an incubation temperature of 32 °C for 48 hours rather than 37 °C for standard beef extract agar. They noticed that the optimum temperature in case of raw milk was slightly below 30 °C and in the case of pasteurised milk, slightly above 31 °C. The conclusion was drawn that an incubation temperature of 32 °C or slightly lower is fully as desirable with tryptone agar as with the present standard agar because (1) it yielded at least 95 per cent on the average of the maximum 2 day count in comparison to only about 50 per cent in the case of 37 °C incubation, (2) less errors were caused by temperature variations in 32 °C than in 37 °C incubators, (3) the percentage of the maximum 48 hour count did not vary greatly between samples in the case of 32 °C incubation whereas it varied greatly in the case of 37 °C incubation.

Alec Bradfield and Ellenberger (1936) compared 2 incubation temperatures as 32 °C and 37 °C on standard medium and tryptone glucose skim milk agar. They reported that 32 °C incubation temperature with tryptone-glucose agar as a medium gave the highest counts in the greatest number of instances. The 32 °C incubation temperature gave a more marked advantage than the incubation temperature at 37 °C.

Breed (1936), in an appraisal of the work done on optimum incubation temperature, reported that a lowering of the temperature of incubation from 37 °C to 32 °C increased

counts in comparison with a change in composition of the agar. This increase in counts seemed to be due to the better growth of Streptococci in case of raw milk. In pasteurised products such as cream and ice-cream mix, this increase in count was attributed largely to the growth of bacteria that find 37 °C too warm. He commented that 37 °C temperature is poorly selected as it is too warm for many types of bacteria and too cold for the thermophilic types.

Joseph Phelan (1936) examined 1962 samples of different dairy products such as grade A, grade A pasteurised and bottled milk, grade B tank raw milk, grade B producers can milk etc., with 2 media viz., standard agar and tryptone skim milk agar and 2 incubation temperatures viz., 32 °C and 37 °C for 48 hours. With the combination of tryptone agar and 32 °C, temperature there was a tremendous increase in the bacterial count. Certified milk which showed an increase of 23 per cent where all of the other types showed serious percentage increase over 37 °C. The percentage was higher in nearly every case and sharply higher in many cases viz., in grade A milk from 45 to 68 per cent; in grade B cans from 40 to 84 per cent; in cream cans from 73 to 92 per cent and in certified milk from 21 to 40 per cent increase was observed.

Demester et al. (1937) worked on standard agar, standard agar plus lactose and tryptone-glucose skim milk

agar and showed that the incubation for 2 days at 30 °C gave more satisfactory results as compared to the incubation for 3 days.

Powers (1937) proposed to change the standard nutrient agar to tryptone-glucose skim milk agar and lower the incubation temperature from 37 °C to 32 °C.

Kelly (1937) presented a summary of extensive work done by different laboratories which compared standard nutrient agar with tryptone skim milk agar at 37 °C and 32 °C incubation temperatures. Raw milk with standard agar gave higher counts with an average increase of 44 per cent in count with plates held for 48 hours at 32 °C compared with 37 °C. Grade A pasteurised milk under the same conditions gave with an average increase of 72 per cent. He stated that lowering the temperature of incubation from 37 °C was the greatest single factor in increasing counts irrespective of culture media used.

Yale and Hickey (1937) studied the effect of using Tryptone-glucose skim milk agar and 32 °C incubation temperature on the bacterial colony count of 112 samples of ice-cream. There was an average 162 per cent increase in count in favour of tryptone agar and 32 °C incubation temperature. These results were highly significant.

Dennis and Weiser (1937) determined the bacterial counts of 37 samples of raw milk at the incubation temperatures of 20°, 30° and 37 °C on different culture media for

Fay and Howards (1940) made several series of parallel determinations of various milk grades using tryptone-glucose beef extract milk agar and incubating at 32 °C and 37 °C. Higher counts were obtained at 32 °C as compared to those at 37 °C. It was observed that the use of incubation temperature of 32 °C increased the bacterial counts and hence they suggested to adopt 32 °C temperature for incubating the samples.

Similarly, Abele (1940) made a comparative study of plate counts on 3 media viz., the old standard agar, the new standard agar and the agar recommended by the American Association Milk Committee and at 2 temperatures of incubation viz., 37 °C and 32 °C. Incubation at 32 °C in combination with the modified agars gave greater increase in plate counts than the use of any of these agars at 37 °C.

Nelson (1949) noted that except in the case of raw milk, use of the improved agar tended to lessen the increase in count commonly observed when an incubation temperature of 32 °C was used instead of 37 °C. However, the colonies developed on new agar were larger and easily countable.

Abele (1940) summarised the principal arguments for change in medium composition and temperature of incubation. He reported that incubation on modified agar at 32 °C resulted in greater uniformity in the counts of divided samples and appeared to favour the development on the plates of the

types of organisms found in incompletely cleaned milk vessels and those predominating in improperly cooled milk.

Marcus (1940) stated that using the new tryptone agar at 32 °C, it was possible to obtain low bacterial counts in commercial practice.

Hastings (1941) surveyed the studies made on relation of the old and new methods for the bacteriological analysis of milk and reported that higher bacterial counts were obtained when plates were incubated at 32 °C in place of 37 °C on the new tryptone glucose-medium.

Thomas and Jenkins (1941) compared the colony counts in milk agar of aseptically drawn udder milk, raw bulk milk, pasteurised milk, utensil rinsings, ice-cream, milk powder and water at 3 temperatures viz., 25°, 30° and 37 °C. Counting was done at the 3rd day and 5th day of incubation. The results showed that except for udder milk, incubation for 3 days at 30 °C gave a significant increase in colony count as compared with 37 °C. An increase in counts of approximately were twice for bulk raw milk, 6 times for pasteurised milk, 5 times for rinsings of dairy utensils and 11 times for dairy farm water supplies.

In the incubation for 3 days at 30 °C as compared with 37 °C, differences were more marked in pasteurised milk than in raw milk. This was attributed to the lower optimum temperature of the thermoduric organisms present in pasteurised milk.

Hileman et al. (1941) examined 100 lots of milk from each lot of about 300 gallons of raw milk. Four plates were made on each sample or 1600 plates for the 100 lots of milk. One plate with each agar was incubated at 37 °C; the other at 32 °C the incubation period being very close to 48 hours. They showed that lowering the temperature of incubation for plate counts from 37 °C to 32 °C or changing from the old standard agar to tryptone-glucose-extract milk agar, or making both changes simultaneously not only resulted in higher counts of organisms on both raw and pasteurised milk, but also in a higher percentages of organisms in the raw milk being classified as thermoduric. The percentage increase was considerably more in pasteurised milk i.e. from 2 to 5 times as great as with raw milk. This was stated to be due to much greater percentage of thermoduric bacteria in pasteurised milk than in raw milk.

Hileman and Leber (1941) noted that incubation of plates at 32 °C caused a much larger increase in count with the samples of pasteurised milk at 143 °F for 35 minutes than with those pasteurised at the higher temperature.

Elizabeth D and Elizabeth F (1943) carried out experiment on the effect of incubation temperature upon certain organisms in cream. They showed that higher counts could be obtained at 20 °C than incubating at 37 °C temperature.

Hunter (1944) concluded that Streptococci grew more rapidly at 30 °C than at 22 °C but were inhibited to a greater or less degree at 37 °C temperature.

Murray and Horwood (1944) recommended 35-37 °C temperature for the bacteriological examination on tryptone-glucose agar of fine grounded edible gelatin at 48 hours incubation period.

Abdel-Malek (1945) studied the bacterial flora of pasteurised milk. He showed that incubation of post-pasteurization plates at 30 °C for 5 - 6 days usually resulted in significantly higher counts than at 37 °C for 2 - 3 days. The Corynebacteria and many micrococci did not grow at 37 °C. He concluded that for estimating thermoduric organisms, 30 °C was definitely a better incubation temperature than 37 °C.

Crossley (1945) examined 186 samples of sweetened condensed milk and observed that colonies were eliminated by lowering the incubation temperature from 37 °C to 32 °C or lower. Majority of samples yielded slightly higher counts at 22 °C than at 32 °C when plates were incubated for 5 days.

Messner (1945) carried out comparative counts on 1,279 samples of pasteurised milk using incubation temperatures of 37 °C and 32 °C. Three samples were tested at each temperature and incubated for 48 hours. He reported that there was low count at 37 °C; as compared to 32 °C incubation temperature. Sometimes, the count was as high as 45 per cent.

Hiscox (1945) studied the effect of the method of reconstitution and the temperature of incubation on the plate count of spray-dried milk powder. He incubated plates at

temperature from 22 to 37 °C and showed that the highest counts at 30 °C, with a slight decrease at 35 °C followed by a sharp decrease between 35 and 37 °C temperature. Many organisms growing at 30 °C failed to grow at 37 °C.

Robinton et al. (1946) showed much higher counts at 20 °C than duplicate plates at 37 °C for 48 hours in milk and cream.

Pestana and DeLima (1948) made a comparative study of plate counts on samples of pasteurised milk. They compared 2 media and incubation temperature. All samples gave a higher count on tryptone-glucose milk agar than on standard agar at 32 °C than at 37 °C temperature.

Thomas et al. (1948) reported that plate counts at 37 °C were materially lower than 32 °C. A greater percentage of pasteurised milk was placed in lower grades by lowering the incubation temperature from 37 °C to 35 °C.

Verone O. (1948) suggested a count after 48 hours of incubation at 37 °C instead of 24 hours of incubation.

Keeping in view the above studies, the American Public Health Association (1948) recommended the use of 32 °C in place of 37 °C as temperature of incubation for estimating standard plate count of milk and milk products for 48 hours incubation period.

Erichsen and Golding (1949) studied 37 samples of raw milk from different producers to determine the effect of 2 incubation temperatures viz., 30 °C and 37 °C on plate counts. The former incubation temperature had a marked influence on raw milk counts.

Thomas et al. (1949) stated that thermoduric colony counts on yeastrel milk agar incubated for 4 days at 30 °C were nearly 12 times of those obtained at 37 °C.

Chandrasekhar (1950) studied the micropopulation of raw and pasteurised milk and showed optimum temperature for growth was 30 °C.

Thomas et al. (1950) suggested that large differences in 2 counts were due to the presence of microbacteria and possibly sometypes of micrococci which were unable to grow at 37 °C. They recommended that colony counts of commercially pasteurised milk, unpasteurised milk should be also obtained by incubating on yeastrel milk agar at 30 °C for 4 days.

Asthan (1950) carried out 36 experiments at the 3 temperatures of incubation and showed that plate counts of freshly pasteurised milk samples were highest at 22 °C and lowest at 37 °C. Maximum mean plate count on the raw milk samples was observed at 30 °C.

Thomas, Levine and Black (1950) carried out a direct comparison of plate counts at 37 °C, 35 °C and 32 °C and

showed that counts at 37 °C were considerably lower than at 32 °C while the counts at 35 °C were only slightly lower.

Todoroff and Assenowa (1950) suggested incubation period of 48 hours at 32 °C to 35 °C for bacterial plate counts.

Quencer (1951) showed by examining a total of 1,175 samples of raw, pasteurised unhomogenized and homogenized milk at 6 dairy laboratories for colony plate counts after incubation at 35 °C and 37 °C that the former incubation temperature gave higher counts than at the latter. However, the magnitude of the increase in count varied considerably and was stated to be dependent upon the specific flora present in milk under examination.

Nelson and Baker (1953) studied influence of time and temperature of plate incubation, upon bacterial counts of market milk and related products. Plates were incubated at 35 °C for 2 days ; 32 °C for 2 days ; 25 °C for 2 and 3 days and 21 °C for 2, 3 and 4 days et.. The shorter incubation periods gave counts much below those of the longest incubation periods and the colonies also were much more difficult to count. Samples in which considerable bacterial growth occurred at 5 °C usually gave the same counts at all temperatures of plate incubation except at 35 °C. Counts at 35 °C frequently were less than 1 per cent of the counts at other temperatures. With few -

exceptions, the shorter incubation periods gave counts essentially equal to those after maximum incubation periods. The colonies were very small which made counting more difficult in samples incubated for shorter periods.

Ferraz and Silva (1953) made a comparison on plates of peptone beef extract agar at 37 °C and tryptone glucose milk agar at 35 °C giving 48 hours incubation period. Counts were nearly twice as high as on tryptone glucose milk agar at 35 °C. However, the colonies on T.G.M. agar were easier to count.

Lakshminarasim (1953) studied biochemical properties of cocci in milk. He observed that the total counts obtained at 30 °C were always greater than at 37 °C, the average increase being about 50 per cent. He reasoned that the majority of bacteria normally found in milk had a lower optimum temperature for growth e.g. optimum temperature of growth for micrococci was reported to be 25 - 30 °C, which also approximated to the average atmospheric temperature prevailing in the region during major part of the year.

Atherton et al. (1953) took observations on bacterial population. Plates incubated at 10 °C, 25 °C and 35 °C for 10, 3 and 2 days respectively. When bacterial plates were incubated at 30 °C, 26 °C and 20 °C, almost identical counts were obtained. At 35 °C incubation, the counts lagged far behind after 3 days holding. Counts at 37 °C were consistently low.

Barber and Debaun (1954) carried experiments on a new plate count media in routine plate counts on milk. A comparison was made among 3 media in which duplicate plates of each medium were incubated at 37 °C, 35 °C and 32 °C. The result summarised showed that the same media differences were obtained at each temperature.

Cuthbert et al. (1954) studied counts of thermoduric organisms obtained by employing different incubation periods at 30 °C and it was concluded that 3 days incubation at 30 °C was adequate for any routine purpose. The colonies which developed within 2 days were of the same type as those which developed later.

Tewes et al. (1954) studied the consumer's milk for colony counts. They examined the effect of incubation time on the plate count of raw and pasteurised milk and reported that the count after 48 hours incubation was greater. In pasteurised milk, the predominant thermoduric organisms were microbacteria whose slow growth rate was demonstrated.

Lewton (1955) compared incubation temperatures of 32 °C and 35 °C on 416 samples of milk and cream for the coliform count. It was concluded that there were no differences between the count obtained on a sample after incubation at 32 °C or 35 °C. It was suggested that for routine analysis, 32 °C was a suitable temperature for the incubation of coliform plates. The adoption of 32 °C as the incubation temperature would also reduce the equipment.

In Netherlands, the scientific Committee for the standardization of methods for the analysis of milk and dairy products, suggested  $30 \pm 2$  °C as an incubation temperature for the plate count of bacteria in milk.

Murray (1955) compared incubation temperatures in the presumptive coli-acrogenes test for raw and pasteurised milk. He stated that incubation at 30 °C provided a better guide to the hygienic quality of both raw and pasteurised milk. The difference between the counts was greater than 25 per cent.

Vanderzant and Moore (1955) studied the influence of time and temperature of plate incubation on bacterial counts. For plating, tryptone-glucose extract agar was used. The counts at the shorter incubation periods usually were some-what lower than at maximum plate incubation time and the colonies frequently were more difficult to count because of the small size. This was more frequently observed at the lower plate incubation temperatures. The incubation at 32 °C and 25 °C gave maximum counts. The counts at 35 °C were much lower than at the other incubation temperatures.

Babel et al. (1955) conducted trials to ascertain the reliability of 32 °C or 35 °C temperature for  $48 \pm 3$  hours of incubation in the standard plate count of milk. They also aimed to determine the time and temperature other

than the standard which would give the highest plate count of milk. Temperatures selected were 10°, 20°, 26°, 32°, 35°, 37°, 45 °C and for each temperature 4 periods of time of incubation were chosen. The timings were 4, 6, 8 and 10 days for 10 °C; 2, 4, 6 and 8 days for 20 °C; 1, 2, 3 and 5 days for 26°, 32 °C and 35 °C and 1, 1.5, 2 and 3 days for 37 °C and 45 °C. The studies were made with raw and pasteurised milk samples. It was found that the highest mean count for raw milk was obtained on plates incubated at 20 °C for 8 days and was only slightly lower at 32 °C for 5 days. The mean count at 32 °C was generally higher than that at 35 °C. The mean count at 32 °C was higher than at 26 °C after 2 days incubation. For pasteurised milk samples, the highest mean count was given by incubating at 32 °C for 5 days, although only slightly higher than at 20 °C for 8 days. The counts at 32 °C and 35 °C increased at about the same rates after the various incubation times, but the counts at 32 °C were higher. The development of colonies from pasteurised milk samples was slower than from raw milk, because bacterial flora of pasteurised milk was slow growing than the flora of raw milk. The mean count at 32 °C was generally higher for raw and pasteurised milk than at 35 °C. In using a single incubation temperature for raw and pasteurised milk samples, the 32 °C was found to be the most suitable. They suggested that use of 37 °C should definitely be discontinued. There was a marked increase in counts of the pasteurised milk between 1 and 2 days. It was decided that

the possible effect of this increase on the plus or minus -3 hour tolerance from the standard of 48 hours incubation. The pasteurised plates were counted after 42, 45, 48, 51 and 54 hours of incubation at 32 °C. A total of 24 samples of pasteurised milk were used in this phase of the study. The results were 93.8, 98.6, 100.0, 102.8 and 104.2 percentages for the incubation time 42, 45, 48, 51 and 54 hours respectively.

Although there was a gradual increase in the colony counts from 45 to 51 hours, this increase was not marked. A gradual increase in colony size generally was noted as the incubation time was increased. The plates incubated for 42 and 45 hours needed closer scrutiny to detect the small colonies present.

Galesloot (1956) studied the cause of increased plate counts at 30 °C of spray-dried milk powder. He concluded that the difference between plate counts at 37 °C and at 30 °C is caused by Microbacterium lacticum which does not grow at 37 °C.

Sainclivier (1956) determined the bacterial counts, after 8 hours incubation at 6 temperatures between 16° and 31 °C. The mean growth rate was calculated at 22.5 °C.

Jazeski (1956) reported methods for the examination of milk, cream and butter, in the agar plate count. The temperatures of incubation were recognised as standard viz., 32 ° and 37 °C for 48 hours.

Thomas et al. (1957) carried out coli-aerogenes bacteria in farm milk supplies test at 30 °C. The 5 day 30 °C test was preferred to the 2 days 37 °C test, because many of bacteria did not form gas at 37 °C.

Mallmann (1957) determined the bacterial population of milk by surface plating technique and claimed that plates incubated by surface technique method at 35 °C for 36 - 48 hours, were comparable to those obtained by pouring method. He used tryptone-glucose extract agar as a medium.

Tentoni (1957) reported that for Italian conditions, an incubation temperature of 32 °C instead of 37 °C is recommended. He concluded that an incubation period of 48 hours was too short and not less than 72 hours is required for plates incubated at 32 °C.

Heather and Vanderzant (1957) made a study to determine the effects of time and temperature of incubation on heat-treated psychophilic bacteria. Lower counts were found when the incubation time was shortened. At 32 °C, counts were comparable to those observed at 25 °C. They concluded that non-heated psychophiles grew readily on plates during the early phase of the incubation period.

Murray et al. (1958), carried out bacterial count at 37 °C and 30 °C. An increase of 50 per cent in bacterial numbers being recorded at 30 °C, on the farm milk samples. In the case of coliform tests, the lowering of the incubation

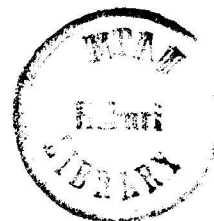
temperature to 30 °C resulted in an increase of 50 per cent.

Mossel et al. (1958) compared incubation temperature of 31 ° + 1 °C for total colony count after incubation for 18-72 hours. They found that psychophilic bacteria did not develop at 37 °C for 72 hours; but grew well when incubated at 31 ° + 1 °C for 48 hours. They also recommended incubation temperature of 31 ° + 1 °C for food spoilage bacteria except for selective enrichment and special groups of organisms.

Atherton (1958) tested the temperatures and periods of incubation at 32 °C for 48 hours; 20 °C for 48 hours; 20 °C for 72 hours and 5 °C for 10 days. He reported that counts made on milk samples were quite comparable regardless of the plating medium or temperature of incubation, except with 5 °C for 10 days or 20 °C for 3 days.

Madelung (1959) conducted research for content of thermoduric organisms in bulk milk and reported that 30 °C for 3 days was optimum temperature for thermoduric organisms.

Murray (1959) studied a temperature of incubation on 9, 497 samples of pasteurised milk, particularly for the presumptive coli-aerogene organisms. He showed that the processing plants with the better counts could maintain the bacteriological standard of absence of Coli-Aerogene organisms, if the incubation temperature in the test was lowered from 37 °C to 30 °C.



Buchanan and Nelson (1960), while carrying out comparative counts on thermoduric microorganisms present in bulk-tank farm milk, observed that when streptococci predominated, the count at 35 °C was approximately the same as the count at 32 °C. When micrococci predominated, the count at 32 °C was significantly higher than the count at 35 °C. They recommended the temperature of 32 °C as it was more satisfactory than 35 °C for incubating plates for the standard plate count.

The incubation temperature recommended for standard plate count by the American Public Health Association (1960) was 32 °C or 35 °C for 48 ± 3 hours but preferably at 32 °C while in United Kingdom, the incubation temperature of 30 °C to 32 °C for 72 hours or 37 ° for 48 hours was favoured (Davis 1959).

Vester (1962) fixed the incubation temperature at 30 °C as a standard method for analysis of milk and milk products in the Netherlands as some types of coliform bacteria producing gas at 30 °C did not produce it at 37 °C. For plate counts, the incubation temperature was also 30 °C and thus the same incubator could be used for both purposes.

Paulsimonart et al. (1962) carried out series of 8 experiments on bacterial flora of milk. Total colony counts were made after incubation for 2 days at 37 °C and for 3 days

at 30 °C using plate count agar medium. They compared the colony counts at 30 °C, with those at 37 °C. There was a much higher proportion of microbacteria at 30 °C incubation temperature.

Mekenzie (1962) carried out experiments to ascertain plate counts of milk at 86 °F (30 °C) and 89.6 °F (32 °C) on 120 raw farm milk samples. He pointed out that the adoption of 89.6 °F (32 °C) for advisory and official testing purposes would avoid the additional 24 hours incubation period; required at present when incubating at 86 °F (30 °C) for 3 days.

In India the incubation temperature at present, recommended by I.S.I. (1962) is  $37 \pm 0.5$  °C for 48 hours although the reliability of this temperature need further investigation under the controlled conditions.

Thomas et al. (1963) showed that colony production by arthrobacter and micrococcus was favoured more at 32 °C and 28 °C than at 35 °C. Thermoduric count increased upon extended plate incubation at 35 and 32 °C temperatures.

Samagh and Dudani (1966) studied the effect of temperature and growth medium on standard plate count of milk. They carried out experiments at 32 °C and 37 °C for 48 hours incubation period, for pail milk samples, farm bulk tank, pasteurised and village milk samples on tryptone dextrose agar and milk agar. They noted that 32 °C temperature of

incubation gave significantly higher standard plate count than 37 °C for all the 4 categories of milk samples. Similarly, both media Tryptone dextrose agar and milk agar used, gave identical plate counts for all of the 4 categories of milk investigated. Some difficulty was experienced in use of milk agar in counting of colonies. No interaction was found between the types of milk and temperature or growth media. They reported that the increased counts in pasteurised milk samples might be due to high incidence of thermoduric organisms present which were known to have lower temperature of growth. This investigation indicated that 32 °C for 48 hours incubation period with tryptone-dextrose agar, presented better picture of the bacteriological quality of milk.

Pedraja and Mengelis (1967) made a comparative study on effect of time and temperature of incubation on the plate count of 167 dried skim-milk samples and 54 samples of dried butter milk. The plates were incubated at 35 °C for 48 hours, 32 °C for 48 or 72 hours. In all cases, the highest plate counts/g product were obtained at 32 °C for 72 hours.

Pack and Sandine (1968) reported that the population of aroma bacteria were greater at 30 °C than at 21 °C.

Hartley et al. (1969) studied the effects of medium and incubation temperature on recovery of microorganisms from manufacturing grade, Grade A and pasteurised milk. They carried out the comparative study by using 7, 21, 28 and

and 32 °C incubation temperatures for 10, 5, 4 and 2 days incubation periods respectively. The highest count was obtained at 21 °C for Grade A milk. For pasteurised milk, the highest count was obtained at 28 °C with standard agar. He reported that the optimum temperature and time combination was 28 °C for 4 days.

Mallett et al. (1969) carried out comparative experiments on counts of coliform bacteria of milk at 32 °C and 37 °C incubation temperatures. Samples of raw and pasteurised milk were plated in desoxycholate lactose agar and incubated at 32° ± 1 °C and 37° ± 1 °C for coliform bacteria. They reported that the lower temperature i.e. 32 °C produced counts comparable to those at 37 °C.

CHAPTER III  
MATERIALS AND METHODS

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## CHAPTER III

### MATERIALS AND METHODS

The present investigation was undertaken and completed at the post-graduate laboratory of the Agricultural College Dairy, Poona. The various research techniques used during the course of study are briefly described in the following paragraphs :

1) Collection of raw milk samples.

Milk samples from cows and buffaloes were collected from the Agricultural College Dairy Farm, Poona during the period of July 1969 and September 1969. The samples were drawn soon after the fresh milk was filled in the can. Before the samples were taken from the can, the milk in the cans was thoroughly mixed with the help of a stainless steel plunger having a perforated disc of 150 mm diameter and a metal rod of sufficient length. The sample was then collected aseptically with the help of one litre capacity dipper in 250 ml sterilized flask. After collection of buffalo and cow milk in stoppered sterilized flasks; then it was kept in the refrigerator and the plating for the standard plate count was completed within 2 to 3 hours.

In all, 30 raw milk samples from cows and 30 raw milk samples from buffaloes were examined for studying the effect of Incubation Temperature and Timings on the standard plate count of milk.

2) Collection of pasteurized bottled milk samples.

The Government Milk Supply Scheme, Poona supplies pasteurized bottled milk to Poona city. In this dairy plant, pasteurization of milk is done by High Temperature short time method in which milk is heated at 163 °F for 15 seconds. The pasteurized milk is filled in bottles and stored at 3 to 4 °C until it is despatched to various booths for distribution.

Sometimes, previous day pasteurized milk bottles are also sold at this booth. Thirty samples of pasteurized milk were collected from the Central Dairy Milk Scheme booth for the standard plate count.

3) Collection of milk samples from village raw milk weighing tank.

The samples of milk produced under village conditions were collected from the Central Dairy Milk Scheme, Poona-3 which collects milk from the neighbouring villages and from different towns, located at longer distances. In the morning, the milk is collected at the different centres and is chilled in ice-chambered cans which are loaded in trucks and brought to the Central Dairy Milk Scheme, Poona.

The samples were taken from the following villages and cities :

- 1) Mahur route : Mahur, Mandhar, Dawaniwadi.
- 2) Shivapur route : Kaldari, Khed-Shivapur, Nasarapur, Warve etc.
- 3) Bhima-shankar route : Ghodegaon, Ghindi, Dimbha etc.
- 4) Junnar route : Khamgaon, Paddi, Anery etc.
- 5) Cities : Junnar, Satara and Mahabaleshwar.

The reason for selecting these routes was that the milk from these routes used to arrive between 2-30 p.m. and 4-0 p.m. after which it was possible to carry out further analytical work.

The trucks are unloaded at the plat form. The cans are poured and immediately filtered in weighing tank, having a capacity of 500 litres. From this tank, the milk samples were drawn with the help of milk sampler in stoppered sterilized flasks of 250 ml. They were brought immediately to the laboratory and kept in the refrigerator.

The microbial examination of the samples was carried within half an hour after collection.

#### 4) Preparation of media.

The Tryptone Glucose Extract Agar medium suggested by 'Difco Manual of Dehydrated Culture Media and Reagents (9th Edition)' and by 'I.S.I.(1952)' was prepared from dehydrated base stock and used as a plating medium.

Composition of T-G-E-Agar (B<sub>2</sub>)

## Dehydrated

Bacto-Beef extract	...	3.0 g
Bacto-tryptone	...	5.0 g
Bacto-Dextrose (d-glucose)		1.0 g
Bacto-Agar	...	15.0 g
Water, distilled	...	1000.0 ml
Final pH	...	7.0 ± 0.1

Preparation of media, pH readings (taken with the help of Indicator papers) sterilisation and storage, diluting of samples with distilled water; measuring test portions, pouring plates, checking sterility of glass ware, dilution blanks and growth medium etc., were done as per the methods prescribed by the I.S.I. No. 1470 (Part III) 1962.

5) Incubation and counting.

The temperature of all the incubators was recorded daily at 9 a.m. and at 5 p.m. in order to check the temperature variation, if any. Incubators used maintained temperature of  $32.0^{\circ} \pm 0.5^{\circ} \text{C}$ ;  $35.0^{\circ} \pm 0.5^{\circ} \text{C}$  and  $37.0^{\circ} \pm 0.5^{\circ} \text{C}$  constantly throughout the period of study.

The petri-dishes were incubated at  $32^{\circ}$ ,  $35^{\circ}$  and  $37^{\circ} \text{C}$ , respectively for a varying durations as per the plan of work.

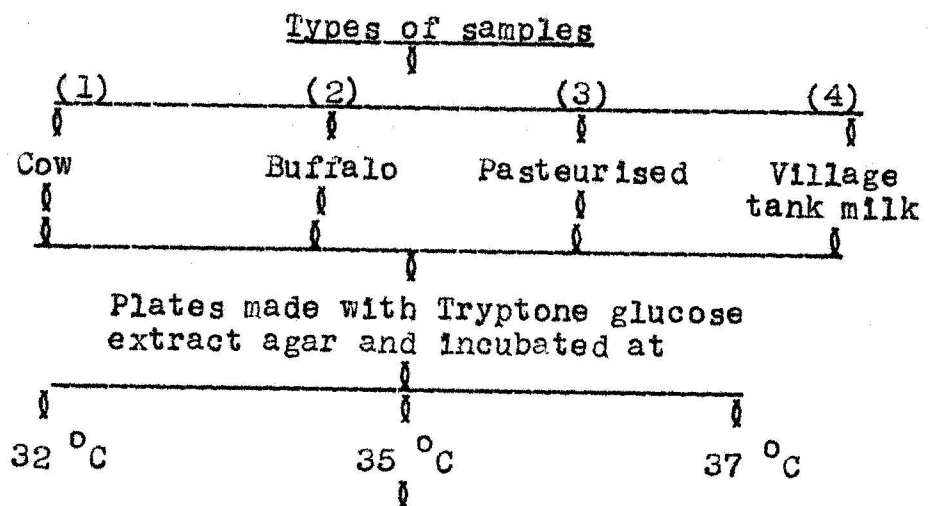
<u>Type of milk</u>	<u>Number of plates</u>
(1) Cow bulk milk	5
(2) Buffalo bulk milk	5
(3) Pasteurised milk	5
(4) Village tank milk	5

Spreader-free plates were selected with 30 to 300 colonies. All colonies were counted including those of pin-point size on selected plates. Pin-point colonies were counted with the aid of Quebec colony counter.

After 42 hours of incubation period, colonies were counted, marked with ink and the result was recorded as a 'SPC/ ml at temperature of 32 °C, 35 °C and 37 °C for 42 hours incubation period'. Then the same plates which were marked with the ink for 42 hours incubation period reading were kept in the incubators for 48, 60 and 72 hours incubation period.

At 48 hours incubation period, the count was taken and the colonies which were developed between 42 and 48 hours incubation period were marked with the ink and recorded in the similar manner. The same plates were further incubated for 60 and 72 hours and new developed colonies were recorded.

Standard Plate Count



Counts taken at the interval of

1 42 hrs.

ii 48 hrs.

iii 60 hrs.

iv 72 hrs.

Statistical Design of the Experiment.

Thirty samples of buffalo and cow milk were divided in 3 groups and 30 milk samples of pasteurised and village milk were divided in 2 groups. The statistical design adopted for the experiment was Factorial design.

Replication : One milk sample was taken as one replication.

Treatments : (A) Different milk samples -

(1) Buffalo milk sample.

(2) Cow milk sample.

(3) Pasteurised milk sample.

(4) Village milk sample.

(B) Incubation temperatures -

32° , 35° and 37 °C.

(C) Incubation periods : 42, 48, 60 and 72 hrs.

CHAPTER IV  
RESULTS AND DISCUSSION

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## CHAPTER IV

### RESULTS AND DISCUSSION

The results of the investigation are given in the following tables :

TABLE 1  
ANALYSIS OF VARIANCE OF BUFFALO AND COW MILK  
SAMPLES FOR JULY 1969

Source of variation	D.F.	S.S.	M.S.	Calculated F.
Block	9	210500	23388.88	33.36
Type of milk	1	89745	89745.00	128.04**
Temperature	2	86614	43307.00	61.78**
Time of Incub.	3	3667	1222.33	1.74
Type X temp.	2	3312	1656.00	2.36
Type X Time	3	107	35.66	0.05
Temp. X Time	6	188	31.33	0.04
Type X Temp. X Time	6	267	44.50	0.06
Error	207	145090	700.91	-
Total :	239	539490	-	-

D.F. = Degrees of freedom.

S.S. = Sum of squares.

M.S. = Mean square.

\*\* = Significant at 1 % level.

TABLE 2  
SUMMARY OF TABLES

MEAN STANDARD PLATE COUNTS OF BUFFALO AND COW  
MILK SAMPLES AT DIFFERENT INCUBATION TEMPERATURES

Temp. °C	T y p e		Mean S.P.C.
	Buff. milk	Cow milk	
32	92.50	105.50	99.00
35	67.50	140.50	104.00
37	56.00	86.00	71.00
Mean	72.00	111.00	-

S.E. for body of the table = 4.18

TABLE 3

MEAN STANDARD PLATE COUNTS OF BUFFALO AND COW  
MILK SAMPLE AT DIFFERENT INCUBATION PERIODS

Time (hrs)	T y p e		Mean S.P.C.
	Buff. milk	Cow milk	
42	67	104	85.50
48	71	110	90.50
60	74	113	93.50
72	76	116	96.00
Mean	72	111	-

S.E. for body of the table = 4.83

TABLE 4

MEAN STANDARD PLATE COUNTS OF BUFFALO AND COW  
MILK SAMPLES AT DIFFERENT TEMPERATURES AND  
INCUBATION PERIODS

Time hrs.	Temperature			Mean S.P.C.
	32 °C	35 °C	37 °C	
42	111.60	82.35	62.90	85.61
48	115.30	85.35	70.90	90.51
60	118.75	88.75	73.80	93.76
72	121.20	91.00	75.90	96.03
Mean	114.71	86.86	70.87	-

S.E. for body of the table = 5.92

It is seen from the data of analysis of variance of Table 1 that the temperatures of incubation differ significantly at 1% level. It is observed that incubation temperatures of 32 °C and 35 °C have given higher bacterial counts than the 37 °C. The 'F' test shown in the table indicates that the effect of incubation temperature on buffalo and cow milk samples is significant but the time of incubation is not significant.

Summary of tables given in tables 2, 3, 4 show that interactions are not significant indicating that the data do not reveal any definite evidence of response of milk samples to the incubation temperatures and periods.

TABLE 5

ANALYSIS OF VARIANCE FOR BUFFALO AND COW MILK  
SAMPLES FOR AUGUST 1959

Source of variation	D.F.	S.S.	M.S.	Calculated F.
Block	9	171954	19106.00	8.92
Type	1	30487	30487.00	14.24 **
Temp.	2	86541	43270.50	20.21 **
Time	3	2949	983.00	0.45
Type X Temp.	2	354	177.00	0.08
Type X Time	3	9	3.00	0.001
Temp X time	6	10	1.66	0.0007
Type X Temp X Time	6	21	3.50	0.001
Error	207	442987	2140.03	-
Total	239	-	-	-

D.F. = Degrees of freedom.

S.S. = Sum of squares.

M.S. = Mean square.

\*\* = Significant at 1% level.

TABLE 6

## SUMMARY OF TABLES

MEAN STANDARD PLATE COUNTS OF BUFFALO AND COW MILK  
SAMPLES AT DIFFERENT INCUBATION TEMPERATURES

Temp. C	Type		Mean S.P.C.
	Buff. milk	Cow milk	
32	122.70	101.77	112.23
35	98.37	77.65	88.01
37	78.72	52.75	65.73
Tot Mean	99.93	77.39	

S.E. for body of the table = 7.31

TABLE 7

MEAN STANDARD PLATE COUNTS OF BUFFALO AND COW  
MILK SAMPLES AT DIFFERENT INCUBATION PERIODS

Time hrs.	T y p e		Mean S.P.C.
	Buff. milk	Cow milk	
42	94.86	73.00	83.93
48	98.50	75.80	87.15
60	101.60	78.80	90.20
72	104.76	81.96	93.36
Mean	99.93	77.39	

S.E. for body of the table = 8.44

TABLE 8

MEAN STANDARD PLATE COUNTS OF BUFFALO AND COW MILK  
SAMPLES AT DIFFERENT TEMPERATURES AND INCUBATION PERIODS

Time hrs.	T e m p e r a t u r e			Mean S.P.C.
	32 °C	35 °C	37 °C	
42	107.50	83.35	60.95	83.93
48	110.70	86.45	64.30	87.15
60	114.15	89.55	66.90	90.20
72	116.60	92.70	70.80	93.36
Mean	112.23	88.26	65.73	-

S.E. for body of the table = 10.35

Analysis of variance given in table 5 for the month of August shows that the temperature of incubation is significant at 1 per cent level for buffalo and cow milk samples but the time of incubation is not significant.

Summary of tables given in tables 6, 7 and 8 reveal that there is no interaction between types of milk and temperature of incubation; types of milk and incubation time; incubation temperature and time and types of milk; incubation temperature and time.

TABLE 9  
ANALYSIS OF VARIANCE OF BUFFALO AND COW MILK  
SAMPLES FOR SEPTEMBER 1969

Source of variation	D.F.	S.S.	M.S.	Calculated F.
Block	9	133959	14884.33	9.72
Type of milk	1	4310	4310.00	2.81 **
Temperature	2	97674	48837.00	31.91 **
Time of Incb.	3	2271	757.00	0.49
Type X Temp.	2	1062	531.00	0.34
Type X time	3	0	0.00	0.00
Temp X Time	6	20	3.33	0.0021
Type X Temp X Time	6	8	1.33	0.0008
Error	207	316771	1530.29	-
Total	230			

D.F. = Degrees of freedom.

S.S. = Sum of squares.

M.S. = Mean square.

\*\* = Significant 1% level

TABLE 10

## SUMMARY OF THE TABLES

MEAN STANDARD PLATE COUNTS OF BUFFALO AND COW MILK  
SAMPLES AT DIFFERENT INCUBATION TEMPERATURES

Temp. °C	T y p e		Mean S.P.C.
	Buff. milk	Cow milk	
32	121.47	118.25	119.86
35	94.65	85.97	90.31
37	77.55	64.02	70.78
Mean	97.89	89.41	

S.E. for body of the table = 6.18

TABLE 11

MEAN STANDARD PLATE COUNTS OF BUFFALO AND COW MILK  
SAMPLES AT DIFFERENT INCUBATION PERIODS

Time hrs.	T y p e		Mean S.P.C.
	Buff. milk	Cow milk	
42	93.70	85.06	89.38
48	96.60	88.13	92.36
60	99.43	91.20	95.31
72	101.83	93.26	97.55
Mean	97.89	89.41	-

S.E. for body of the table = 7.14

TABLE 12

MEAN STANDARD PLATE COUNTS OF BUFFALO AND COW MILK  
SAMPLES AT DIFFERENT TEMPERATURES AND INCUBATION PERIODS

Time hrs	T e m p e r a t u r e			Mean S.P.C.
	32 °C	35 °C	37 °C	
42	115.35	86.00	66.80	89.38
48	118.40	88.80	69.90	92.36
60	121.55	92.00	72.40	95.31
72	124.15	94.45	74.05	97.55
Mean	119.86	90.31	70.78	-

S.E. for body of the table = 8.75

The analysis of variance given in Table 9 for the month of September show that the temperature of incubation for the buffalo and cow milk samples is significant at 1 per cent level but the time of incubation is not significant.

Summary of Tables given in Tables 10, 11 and 12 show that the interactions in between type of milk and incubation temperatures; type of milk and incubation time; incubation temperature and time and type of milk, incubation temperature and time are not significant.

TABLE 13

ANALYSIS OF VARIANCE FOR PASTEURIZED AND VILLAGE  
MILK SAMPLES FOR NOVEMBER 1969

Source of variation	D.F.	S.S.	M.S.	Calculated F
Block	14	68553	4896.64	49.84
Type	1	942081	942081.00	9590.50**
Temp.	2	153122	76561.00	779.40**
Time	3	28125	9375.00	95.43**
Type X Temp.	2	1820	910.00	9.26**
Type X Time	3	13782	4594.00	46.76**
Temp X Time	6	372	62.00	0.63
Type X Time X Temp.	6	286	47.66	0.48
Error	322	31632	98.23	-
Total	359	1239773	-	-

D.F. = Degrees of freedom

S.S. = Sum of Squares.

M.S. = Mean square.

\*\* = Significant at 1% level.

TABLE 14

## SUMMARY OF THE TABLES

MEAN STANDARD PLATE COUNTS OF PASTEURIZED AND VILLAGE  
MILK SAMPLES AT DIFFERENT INCUBATION TEMPERATURES

Temp. °C	Type		Mean S.P.C.
	Pasteurized milk	Village milk	
32	121.08	227.88	174.48
35	96.28	192.45	144.36
37	72.31	176.28	124.29
Mean	96.55	198.87	-

S.E. for body of the table = 1.28

C.D. = 3.53

TABLE 15

MEAN STANDARD PLATE COUNTS OF PASTEURIZED AND VILLAGE MILK SAMPLES AT DIFFERENT INCUBATION PERIODS

Time hrs.	T y p e		Mean S.P.C.
	Past. milk	Village milk	
42	77.15	195.02	136.08
48	87.84	197.95	142.89
60	105.40	200.33	152.86
72	115.84	202.17	159.00
Mean	96.55	198.87	-

S.E. for body of the table = 1.47 C.D. = 4.06

TABLE 16

MEAN STANDARD PLATE COUNTS OF PASTEURIZED AND VILLAGE MILK SAMPLES AT DIFFERENT TEMPERATURES AND INCUBATION PERIODS

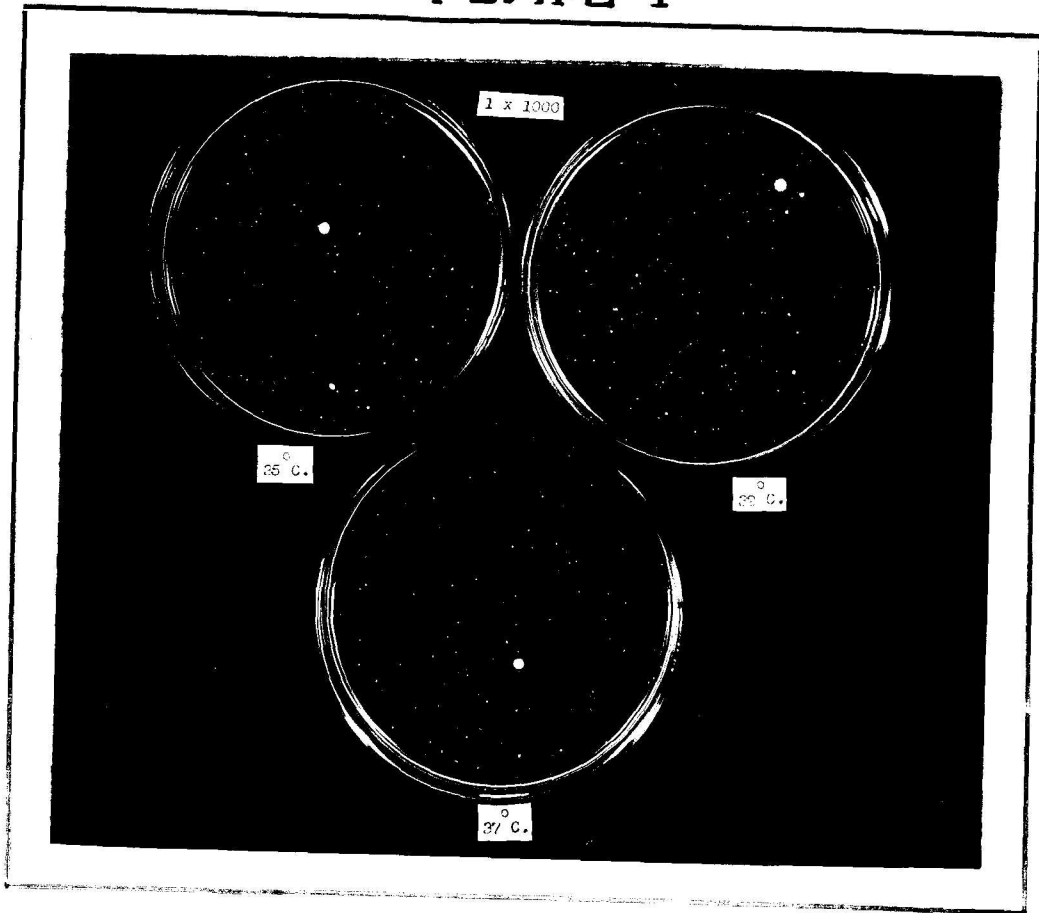
Time hrs.	T e m p e r a t u r e			Mean S.P.C.
	32 °C	35 °C	37 °C	
42	161.10	132.60	114.56	136.08
48	169.26	139.50	119.93	142.89
60	180.50	149.53	128.56	152.86
72	187.06	155.83	134.13	159.00
Mean	174.48	144.36	124.29	-

S.E. for body of the table = 1.81

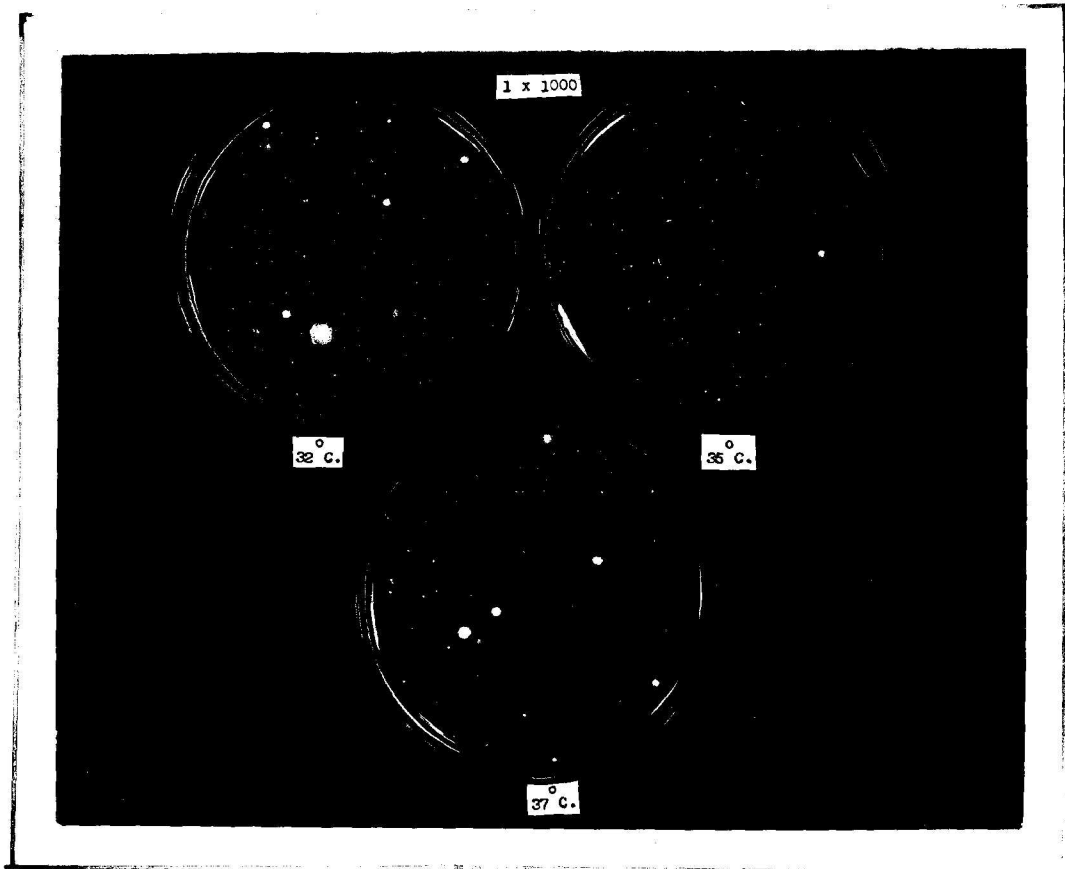
It is seen from the analysis of variance Table 13 for the month of November that incubation temperature and time for the pasteurized and village raw milk samples are significant at 1 per cent level. It is observed that



# PLATE-I



# PLATE-II



32 °C and 35 °C incubation temperatures for 48, 60 and 72 hour incubation periods have given higher counts than 37 °C temperature for 42 hour incubation period.

Summary of Tables presented in Tables 14, 15 and 16 show that the interactions in between type of milk and temperature of incubation and type of milk and incubation periods are significant. But the interactions in between incubation temperature and time and type of milk incubation temperature and time are not significant. Summary of tables also show that as incubation period increases, there is a definite increase in the bacterial counts.

TABLE 17  
ANALYSIS OF VARIANCE FOR PASTEURIZED AND VILLAGE  
MILK SAMPLES FOR DECEMBER 1969

Source of variation	D.F.	S.S.	M.S.	Calculated F
Block	14	201672	14405.14	20.83
Type	1	696344	696344.00	1007.20**
Temp.	2	99277	49638.50	71.80**
Time	3	20184	6728.00	9.73**
Type X Temp.	2	1453	726.50	1.05
Type X Time	3	11519	3839.66	5.55**
Temp X Time	6	519	86.50	0.12
Type X Temp X time	6	369	61.50	0.08
Error	322	222602	691.31	-
Total	359			

D.F. = Degrees of Freedom

S.S. = Sum of square

M.S. = Mean square

\*\* = Significant at 1 % level.



TABLE 18

## SUMMARY OF TABLES

MEAN STANDARD PLATE COUNTS OF PASTEURIZED AND VILLAGE MILK SAMPLES AT DIFFERENT INCUBATION TEMPERATURES

Temp. °C <del>°C</del>	Type		Mean S.P.C.
	Past. milk	Village milk	
32	121.16	203.81	162.48
35	98.06	186.91	142.48
37	75.63	168.00	121.81
Mean	98.28	186.24	-

S.E. for body of the table = 3.40

TABLE 19

MEAN STANDARD PLATE COUNTS OF PASTEURIZED AND VILLAGE MILK SAMPLES AT DIFFERENT INCUBATION PERIODS

Time hrs.	Type		Mean S.P.C.
	Past. milk	Village milk	
42	82.77	183.42	133.09
48	89.60	185.75	137.67
60	104.03	187.28	145.65
72	116.75	188.53	152.63
Mean	98.28	186.24	-

S.E. for body of the table = 3.92

C.D. = 10.82



PLATE - III

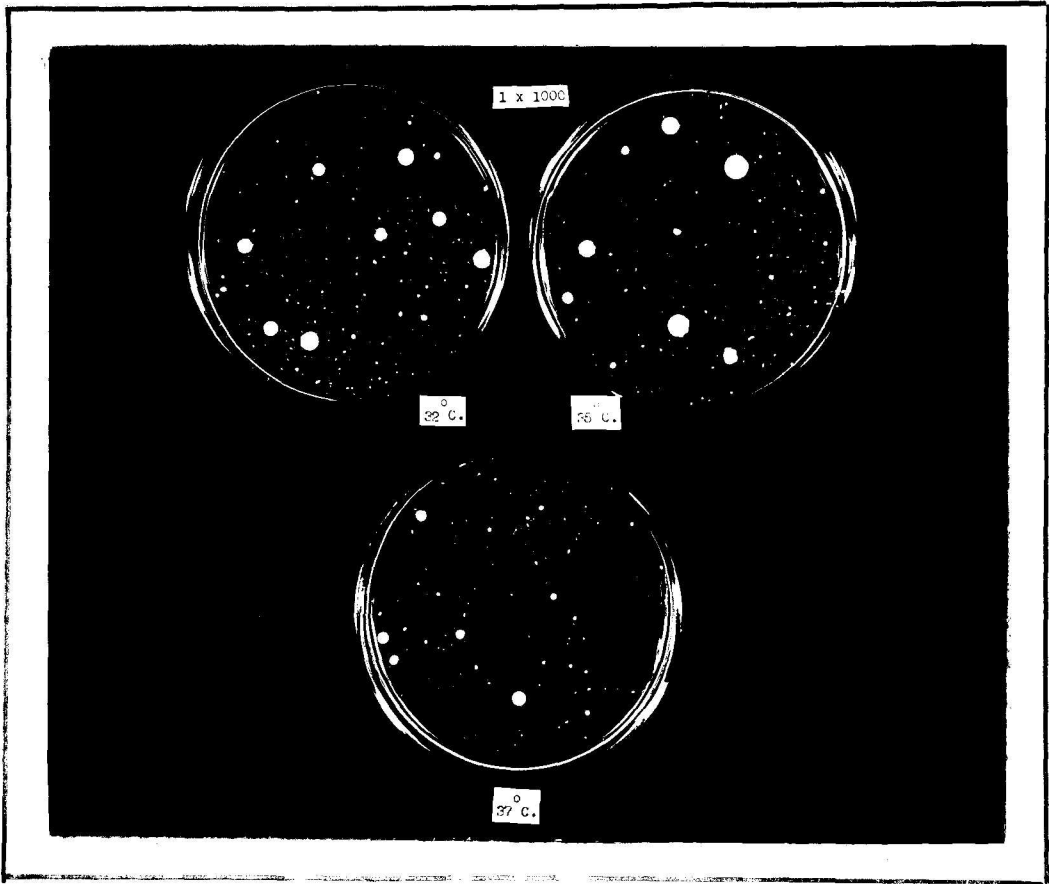


PLATE - IV

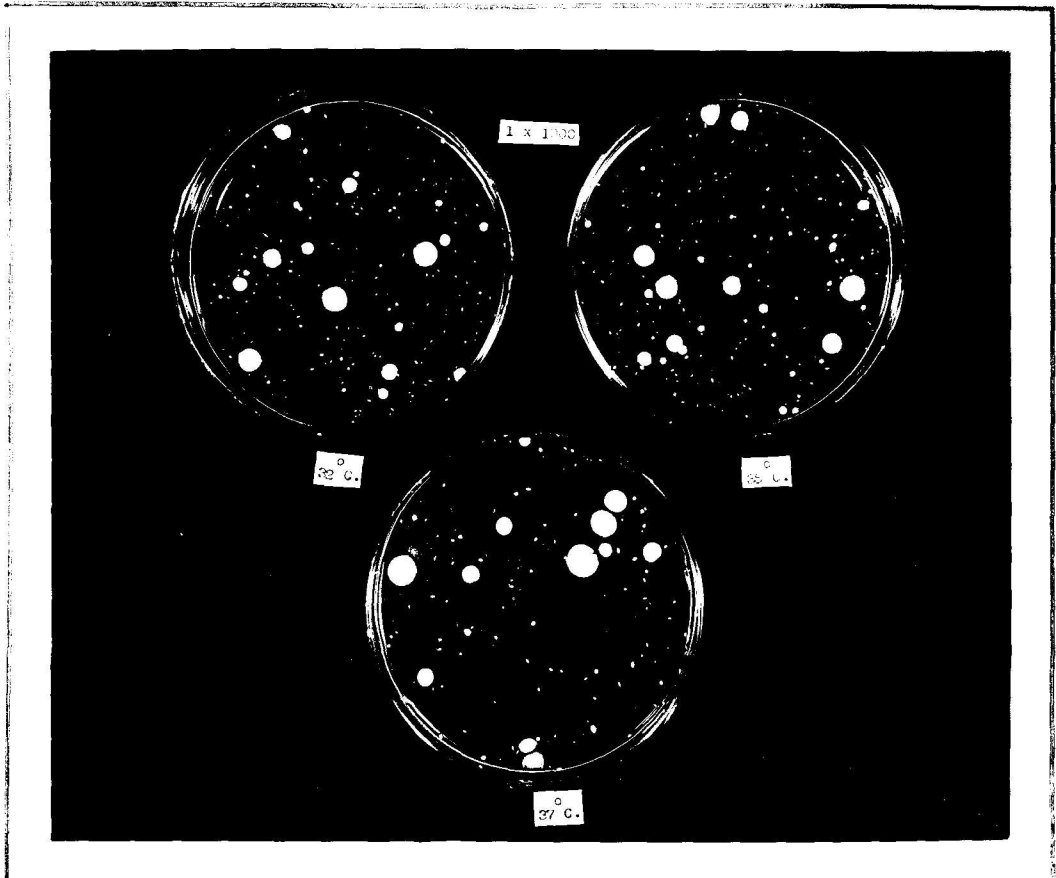


TABLE 20

MEAN STANDARD PLATE COUNTS OF PASTEURIZED AND VILLAGE MILK SAMPLES AT DIFFERENT TEMPERATURES AND INCUBATION PERIODS

Time hrs	T e m p e r a t u r e			Mean S.P.C.
	32 °C	35 °C	37 °C	
42	151.56	133.10	114.63	133.09
48	157.20	137.73	118.10	137.67
60	166.53	145.93	124.50	145.65
72	174.66	153.23	130.33	152.74
Mean	162.48	142.49	121.89	-

S.E. for body of the table = 4.80

The 'F' test shown in the analysis of variance table of pasteurized and village raw milk samples for the month of December presented in Table 17 indicates that the incubation temperatures and periods are significant at 1 per cent level.

The interaction in between types of milk and incubation period is significant. The interactions in between types of milk and incubation temperature; incubation temperature and time and types of milk, incubation temperature and time are not significant. While the trend of the count obtained is similar i.e. as incubation temperature decreases and incubation period increases, there is an increase in the bacterial counts.

TABLE 21

THE EFFECT OF -6, + 12, +24 HOURS TOLERANCE FROM THE 48 HOURS INCUBATION PERIOD AT 32 °C, 35 °C AND 37°C INCUBATION TEMPERATURES, ON PLATE COUNTS OF FOUR DIFFERENT TYPES OF MILK SAMPLES

( EXPRESSED AS AVERAGE PERCENTAGE )

Incub. Temp.	Timings	Buff. milk	Cow milk	Past. milk	Village milk
°C	hrs				
32	42	96.93	97.28	90.09	98.60
	48	100.00	100.00	100.00	100.00
	60	103.10	102.75	117.12	100.93
	72	105.35	104.85	128.83	101.86
35	42	74.16	72.08	71.17	86.51
	48	77.13	74.30	79.28	87.91
	60	80.23	76.85	94.59	88.34
	72	82.31	79.29	105.10	89.77
37	42	59.96	57.35	54.05	78.60
	48	62.81	60.16	59.46	79.53
	60	65.01	62.43	72.07	80.46
	72	67.60	64.62	80.18	81.39

TABLE 22

AVERAGE PLATE COUNTS OF FOUR DIFFERENT TYPES OF MILK SAMPLES, WITH CONFIDENCE INTERVAL (EXPRESSED IN ORIGINAL NUMBER IN THOUSANDS) AT DIFFERENT INCUBATION TEMPERATURES AND PERIODS

Incub. Temp.	Timings	Buff. milk	Cow milk	Past milk	Village milk
°C	hrs				
32	42	107	115	100	212,0000
	48	111	119	111	215,0000
	60	114	122	130	217,0000
	72	116	124	143	219,0000
35	42	82	85	79	186,0000
	48	85	88	88	189,0000
	60	89	91	105	191,0000
	72	91	92	117	193,0000
37	42	66	68	60	169,0000
	48	70	71	66	171,0000
	60	72	74	80	173,0000
	72	74	77	89	175,0000

TABLE 23

AVERAGE MEAN PLATE COUNTS (EXPRESSED IN ORIGINAL  
NUMBER AS LOGARITHMS) AT DIFFERENT INCUBATION  
TEMPERATURES AND PERIODS

Incub. Temp.	Inc. Time	Buff. milk	Cow milk	Past milk	Village milk
<sup>o</sup> C	hrs.				
32	42	5.03	5.06	5.00	9.33
	48	5.04	5.07	5.04	9.33
	60	5.06	5.08	5.11	9.34
	72	5.07	5.09	5.15	9.34
-----					
35	42	4.91	4.93	4.90	9.27
	48	4.93	4.94	4.94	9.28
	60	4.95	4.96	5.02	9.28
	72	4.96	4.97	5.07	9.29
-----					
37	42	4.82	4.83	4.78	9.23
	48	4.84	4.85	4.82	9.23
	60	4.86	4.87	4.90	9.24
	72	4.87	4.89	4.95	9.24

TABLE 24

AVERAGE MEAN PLATE COUNT (EXPRESSED IN ORIGINAL NUMBER IN THOUSANDS) AT DIFFERENT INCUBATION TEMPERATURES AND PERIODS

Incub. Temp. °C	Incub. time hrs	Mean	Buff. milk	Cow milk	Past. milk	Village raw milk
32	42	Mean	176	140	114	191,0000
		U. limit	299	229	142	272,0000
		L. limit	53	52	86	110,0000
	48	Mean	180	144	123	194,0000
		U. limit	304	234	154	275,0000
		L. limit	56	55	92	114,0000
	60	Mean	183	147	137	196,0000
		U. limit	308	237	163	277,0000
		L. limit	58	58	111	116,0000
	72	Mean	184	149	152	198,0000
		U. limit	310	239	174	278,0000
		L. limit	59	60	130	118,0000
35	42	Mean	130	112	88	169,0000
		U. limit	222	183	125	241,0000
		L. limit	39	42	51	97,0000
	48	Mean	134	115	94	171,0000
		U. limit	225	187	133	243,0000
		L. limit	43	44	55	100,0000
	60	Mean	137	118	106	173,0000
		U. limit	228	190	140	245,0000
		L. limit	46	46	72	102,0000
	72	Mean	138	119	119	175,0000
		U. limit	230	192	150	246,0000
		L. limit	47	47	89	104,0000
37	42	Mean	105	91	63	155,0000
		U. limit	179	150	88	210,0000
		L. limit	32	33	38	101,0000
	48	Mean	109	95	68	158,0000
		U. limit	183	155	94	212,0000
		L. limit	35	36	42	104,0000
	60	Mean	111	97	78	160,0000
		U. limit	185	157	100	214,0000
		L. limit	37	38	56	106,0000
	72	Mean	112	99	86	161,0000
		U. limit	185	159	110	215,0000
		L. limit	38	39	63	107,0000

U. limit = Upper limit,

L. limit = Lower limit.

CHAPTER V  
GENERAL DISCUSSION

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## CHAPTER V

### GENERAL DISCUSSION

The exact picture of the real bacterial count in milk is required to be known for establishing a package of practices in the hygienic production of milk. While doing so, our methods should be accurate, easy for practical execution and at the same time, they should be less expensive.

The results obtained in this work have been discussed under the result chapter along with the statistical data. In the following paragraphs, the results are discussed in general manner :

It is observed from the analysis of variance Tables 1, 5, 9, 13 and 17 that three incubation temperatures gave varying standard plate counts of the same milk sample. 32 °C incubation temperature gave significantly higher counts than 37 °C while the counts at 35 °C were more or less close to those at 32 °C.

The counts at 37 °C were considerably below those at 32 ° and 35 °C. Hence 32 °C incubation temperature for 48 hours incubation period presents a better picture of the hygienic quality of milk than at 35 °C or 37 °C. These results are in confirmity with the number of earlier workers namely, Pederson and Yale (1934); Wilson et al. (1935); Alec

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Bradfield and Ellenberger (1936); Breed (1936); Abele (1939); Fay and Howard (1940); Thomas and Jenkins (1941); Messner (1945); Hiscox (1945); Quencer (1951); Nelson and Baker (1953); Babel et al (1955); Samagh and Dudani (1966) and Hartley et al (1969) etc..

The data presented in summary Tables i.e. 2, 3, 4, 6, 7, 8, 10, 11 and 12 show that there was no interaction between the types of milk and incubation temperature; types of milk and incubation period and types of milk, temperature of incubation and incubation period. It was revealed from this data that the standard plate counts of cow and buffalo milk samples did not differ significantly due to these three interactions. However, the temperature of incubation was highly significant for both types of milk. It was observed that 32 ° and 35 °C incubation temperatures gave higher plate counts than 37 °C temperature. 35 °C incubation temperature was significant over 37 °C and 32 °C temperature was significant over both the incubation temperatures. This showed that the incubation temperature of 32 °C was more favourable for the multiplication of bacteria in milk than either 35 ° or 37 °C. The same observations could be seen from the Graph-1A to 1-D and plates I to IV.

Analysis of variance for pasteurised and village milk samples given in Tables 13 and 17 show that 32 ° and 35 °C incubation temperatures were highly significant over

37 °C. The difference, however, was more in pasteurised milk than in village milk. The percentage increase was far greater in pasteurised milk which is 2 to 3 times more than the village milk samples. These results are in agreement with the findings of Pederson and Yale (1934); Breed (1936); Thomas and Jenkins (1941); Hileman et al. (1955) and Samagh and Dudani (1966). This increase in bacterial counts might be due to more sensitivity of the bacterial flora of pasteurised milk to the higher incubation temperatures and hence the incubation temperature of 32 °C might have been more favourable to them for rapid multiplication. However, the data from Tables 14, 15, 16, 18, 19 and 20 showed that there was no interaction effects between incubation temperature and period of incubation and type of milk, incubation temperature and period of incubation.

The results presented in Tables 15 and 19 revealed that in case of pasteurized milk when the period of incubation is increased, there is a significant increase in bacterial counts. The observations made in Table 21 showed that there was a marked increase in counts of pasteurised milk between 48 and 72 hours incubation period. The plate counts at 42, 48, 60 and 72 hours were 90, 100, 117 and 129 per cent, respectively. The count of 72 hours of incubation period at 32 °C was 29 per cent more than the count obtained at 48 hours incubation period at the same temperature of incubation. This might be due to the predominance of thermophilic bacteria whose rate of growth is generally slow.

These results are in agreement with Hiscox et al. (1933); Nelson and Baker (1953); Tewas et al (1954); Murray (1955) and Babel et al (1955). The bacterial colonies at 42 hours incubation period at 32 °C incubation temperature was difficult to count as the colonies were not clearly visible. But at 48 hours incubation period, these colonies could be clearly counted. Hence, the incubation period of 48 hours is preferred as it is also convenient for work and at the same time, it is economical. This view has also been expressed by Hiscox et al (1933). The data presented in Table 24 showed that the bacterial counts of pasteurised and village milk samples were mor or less uniform at 32 °C for 48 hour incubation period than the other incubation temperatures. Also, it showed that the bacterial counts of cow and buffalo milk samples were not uniform at any temperature or incubation period used in this study. It is seen from the data presented in Tables 25 and 26 that there was a variation in plate counts from month to month. However, the higher counts were obtained in rainy season i.e. in August and September as compared to other months. This might be due to unhygienic conditions created due to rains, mud etc. It is revealed from Tables 27 and 28 that the original flora of microorganisms in village milk was more than the pasteurised milk.

Eventhough, the incubation temperature of 32 °C is more favourable for the bacterial growth, there is a diffi-

-culty of maintaining this temperature on account of high atmospheric temperatures in India. It will not, therefore, be possible to maintain this temperature unless the present incubators are replaced by those with proper cooling arrangements. This difficulty can also be overcome by air-conditioning the bacteriological laboratories. This view has also been expressed by Wilso et al (1935).

CHAPTER VI  
SUMMARY AND CONCLUSIONS

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## CHAPTER VI

### SUMMARY AND CONCLUSIONS

The present investigation was undertaken to compare the Standard Plate Count of four types of milk when three incubation temperatures viz., 32, 35 and 37 °C were used with four incubation periods viz., 42, 48, 60 and 72 hours.

The results obtained are summarised and concluded as under :

- 1) The incubation temperature of 32 °C gave a significantly higher Standard Plate Count than the incubation temperature of 35° and 37 °C for all four types of milk samples i.e. cow raw milk, buffalo raw milk, village raw milk and pasteurized milk.
- 2) The incubation period of 48 hours gave a comparatively more clear picture of bacterial count in milk samples on Tryptone Glucose Agar medium than other incubation periods used in this study namely, 42, 60 and 72 hours.
- 3) In case of cow, buffalo and village raw milk samples, no interactions were observed between types of milk and temperature of incubation; types of milk and time of incubation; temperature and time of incubation and types of milk; time and temperature of incubation. However, in case of pasteurized milk, interactions were observed in milk sample and the incubation temperature and milk sample and the incubation period.

It may, therefore, be concluded that the incubation temperature of 37 °C recommended by the Indian Standards Institute needs to be reconsidered in the light of these results and the possibility of recommending 32 °C incubation temperature in place of 37 °C should be explored. In case of incubation period of 48 hours for the Standard Plate Count, there is no need of any change.

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\* Originals seen.

