Journal of Agricultural Engineering and Food Technology
p-ISSN: 2350-0085; e-ISSN: 2350-0263; Volume 4, Issue 4; October-December, 2017, pp. 202-205

http://www.krishisanskriti.org/Publication.html

Rapid Assessment of Microbiological Quality of Milk: A Comparison of New Technique with Established Techniques

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Abstract—*The major aim of the present study is introduction of a New Technique for Rapid Assessment of Microbiological Quality of Milk. The microbial/sanitary quality of milk is of great importance whether it is meant for drinking or for use in other dairy products. If the water used during milking is polluted from fecal matter, it is likely to contain many intestinal pathogens and may communicate dangerous diseases like typhoid, cholera and dysentery to man.*

Although standard conventional techniques have been serving well over the years for assessing microbiological quality of milk, but there are some inherent limitations with them. Keeping in view the limitation of conventional methods, the need for development of rapid methods in the area of microbiological quality assurance is of immense importance. Therefore, a new technique has been proposed for rapid assessment of microbiological quality of milk, which utilizes chemically defined culture medium during quantitative estimation of bacteria (specially fecal coliforms). The present results show that the proposed technique may serve as an emergency test for detecting fecal contamination in milk. The developed test/techniques are sensitive, easy to use, rapid, economic and give reproducible results. The techniques can be incorporated in existing milk testing field kits.

Keywords*: Fecal Coliforms, E.coli, pathogens, indicator organisms, colonies.*

1. INTRODUCTION

Milk is single most nearly complete natural food. Today, milk and milk products have become indispensable integral component of our diet. Milk contains many essential nutrients such as carbohydrates, proteins, lipids, minerals and vitamins. However, the milk and milk products are still considered as important vehicles of disease transfer. The pathogens enter in milk from animals, humans or from the environment. The milk being nutritionally rich provides excellent medium for their growth, which can cause health hazards. The pathogens enter in milk and transfer diseases like typhoid, cholera and dysentery. Both milk and milk products are very delicate and are highly perishable, hence they are to be handled carefully.

Volk and Wheeler [4] described "Sanitary methods of handling milk" According to them milk itself is a good growth medium; even a small number of bacteria can multiply considerably if it is not timely refrigerated.

Murphy [3] suggested that cleaning and disinfection of equipment after each milking is important for reduction of contamination of milk from the equipment.

Goodefay and Molla [1] suggested methods for maintaining quality of milk.

Various standard techniques and rapid analytical techniques are available for testing and grading of milk like MTF technique, MF technique, Standard Plate Count, Methylene Blue Reduction Test (MBRT), Test for Coliform Organisms (using MacConkey medium) and One Hour Resazurin Test.

Teka [2] calculated bacterial population in raw milk using methylene blue reduction test for milk.

Marth [5] discussed about standard methods for the examination of dairy products.

Although standard conventional techniques are serving us well over the years, yet there are some inherent limitations with them. Keeping in view the limitation of conventional methods, the aim of present study is development of *a* new technique for rapid assessment of microbiological quality of milk.

2. MATERIALS AND METHODS: DESCRIPTION OF NEW TECHNIQUE (BASED ON MTFT TECHNIQUE)

The test involves following steps:

Step 1. Serial Dilutions of Sample

a. Preparation of Saline Water/ Diluent:

Diluent is 0.9% NaCl solution.

Sodium chloride	9.0 g
Water	1000 ml (1 L)

Dispense the diluent in suitable quantities (90 ml) in specified bottles. Stopper the bottles and sterilize by autoclaving at 121ºC for 15 minutes. If not used immediately, store in dark at a temperature between 0º to 5ºC for not longer than one month, in conditions which do not allow any change in its volume.

b. Preparation of Test Samples:

Agitate the sample thoroughly so that the microorganisms are distributed as evenly as possible by rapidly inverting the container 25 times. Foaming should be avoided. Prepare different dilutions using diluents as shown in diagram 1.

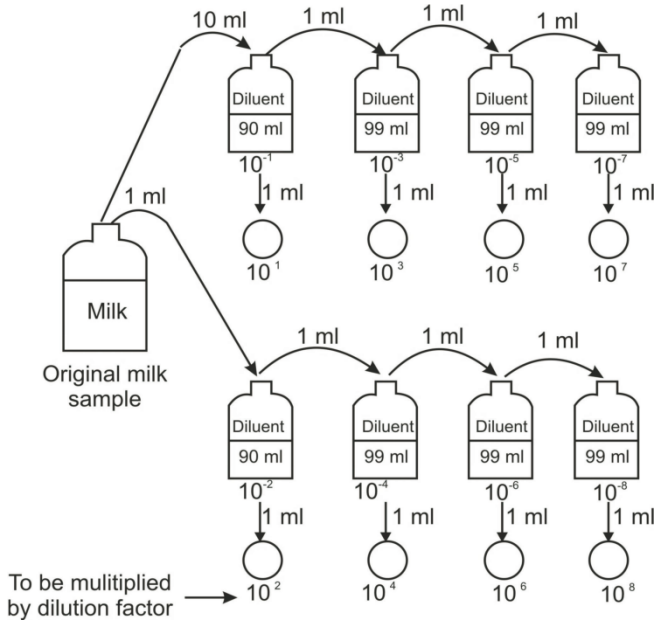


Diagram 1

The technique employs newly formulated MS culture medium and it completes in two stages.

Step 2. Estimation of Bacterial Density (Especially Fecal Coliforms)

Estimation completes in two phases

I) Presumptive phase: MS Broth is Used

Composition of MS broth

Lactose	15.0 g
L-lysine	5.0 g
Sodium desoxycholate	1.0 g
Urea	1.0 g
Sodium Citrate	1.0 g
Na_2HPO_4	2.0 g
Sodium lauryl sulphate	0.01 g
Bromothymol blue	0.01 g
Distilled water	1.00 L

Procedure

About 2.5 g of above medium is dissolved in 10 ml diluted milk sample in a specially designed sterilized inoculating tube (graduated at 10 ml and 110 ml) by gentle heating using spirit lamp/ burner. The contents are cooled and volume is made up to 110 ml by the inoculum (diluted milk sample under test). It is then covered with a loose lid as shown and incubated at 44±0.5ºC in incubator with occasional stirring by a pointed glass rod provided inside the tube. The positive test is indicated by change of color from blue-green to yellow with simultaneous liberation of acid and gas and appearance of turbidity in the fermented broth. This shows presence of fecal coliforms (E. coli) and also possible presence of pathogens.

Note*:

i) In case of raw milk the original sample should be diluted to minimum 1:1000 and then it can be used as inoculum. If MPN value exceeds 1100, then onward dilutions can be used.

ii) In case of pasteurized milk the original sample should be diluted to minimum 1:10 and then it can be used as inoculum. If MPN value exceeds 1100 then samples of onward dilutions can be used.

iii) MPN value can be recorded after multiplication with appropriate factor.

II) Completed Phase: (MS agar is used)

Composition of MS agar	
Lactose ...	1.5 g
L-lysine .	0.5 g
Sodium desoxycholate	0.1 g
Urea	0.1 g
Sodium Citrate .	0.1 g
Na_2HPO_4	0.2 g
Sodium lauryl sulphate	0.01 g
Agar	1.5 g
Eosin 10B + methylene blue	0.004g + 0.001g
Distilled water	100 ml

Procedure

About 200 mg MS agar medium is used for making standard plate using 5 ml water and the plate is streaked by fermented broth (presumptive phase) with the help of pointed glass rod. It is kept in incubator in inverted position for 8-10 h at 40ºC under facultative anaerobic condition. The temperature must not exceed 40ºC because then the agar medium usually gets dried up.

Typical and discrete colonies (2-3mm dia.) with dark centers are seen as shown in Figure 2. This confirms the presence of fecal coliforms in milk sample under test.

3. RESULT AND DISCUSSION:

Selection of indicator organism: For our study, E. coli is the proposed indicator organism. For the New Rapid Technique, studies were carried out in following steps

I) Formulation and Selection of New Chemically Defined Culture Medium: Various chemically defined culture media have been formulated and tried for assessment of bacteriological quality of water, keeping in mind that they should be stable, cheap and give the results within shortest period of time. Based on their response, finally MS culture medium has been selected for use in new proposed technique.

II) Selection of Suitable Dye to be used with Newly Formulated BR Culture Medium

i) For Presumptive Phase: Studies were carried out using eight different dyes and results were recorded. Finally, Bromothymol Blue dye was selected because it shows sharp color change with early bacterial growth as shown in Figure 1. So this was finally incorporated with MS medium for further studies.

ii) For Completed Phase: Studies were carried out using three different dyes and results were recorded. Finally Eosin10B+Methylene Blue (mixed dye) was selected. Colonies about 2-3 mm in diameter with clear and dark centers are shown in Figure 2.

Figure 1: Presumptive phase

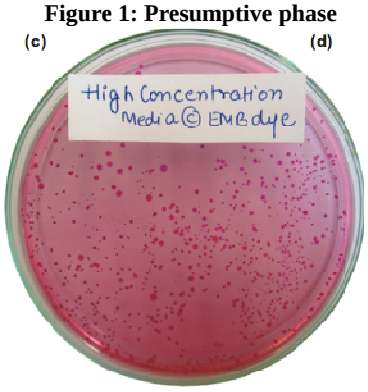


Figure 2: Completed phase

Typical colonies of E.coli with EMB Dye

III) Selection of Optimum Incubation Temperature (during presumptive phase): Studies were also carried out in laboratory to find out the behavior of bacteria (especially fecal coliforms) in different temperatures (using MS culture medium). Results have been are summarized as follows;

Table 1: Time-Temperature Relationship with respect to Bacterial Coun

E. coli MPN*/100 ml	Incubation period (hours)			
	at 37+0.5ºC	at 40+0.5ºC	at 42+0.5ºC	at 44+0.5ºC
> 1100	8.0	6.0	5.0	4.0
1100	10.0	8.5	6.0	5.0
460	14.0	9.5	7.5	6.0
240	17.0	10.0	8.0	7.0
150	20.0	11.0	9.0	8.0
43	23.0	13.0	10.0	9.0
23	24.0	14.0	13.0	10.0
9	28.0	17.0	15.0	12.0
4	36.0	21.0	17.0	14.0
2	40.0	25.0	20.0	15.0

* MPN values were calculated using MTFT technique simultaneously.

The table reveals that at temperature 44±0.5ºC, minimum incubation time is required (even for lower MPN value) so further studies were carried out at this temperature.

After all the above observations, results were as follows;

i) Presumptive Phase: The positive test is indicated by change of color from blue-green to yellow incubated at 44 ± 0.5°C with simultaneous liberation of gas and appearance of turbidity in the fermented broth within 15 hours. This shows the presence of fecal coliforms (E. coli) and also possible presence of pathogens.

ii) Completed Phase: Typical and discrete colonies (2-3 mm diameter) with dark red centers within 8-10 hours at 40 ± 0.5°C under facultative anaerobic conditions confirm the presence of fecal coliforms, especially E. coil in water.

Comparative study on effect of temperature and bacterial concentration on incubation period for MacConkey's media (commonly used in Dairy Industries) and MS media is also carried out (refer table 4.8). The characteristic colonies and their numbers on both the media were also evaluated.

Anderson [6] discussed the microbial content of unexpired pasteurized milk from selected supermarkets in a developing country using MacConkey Medium.

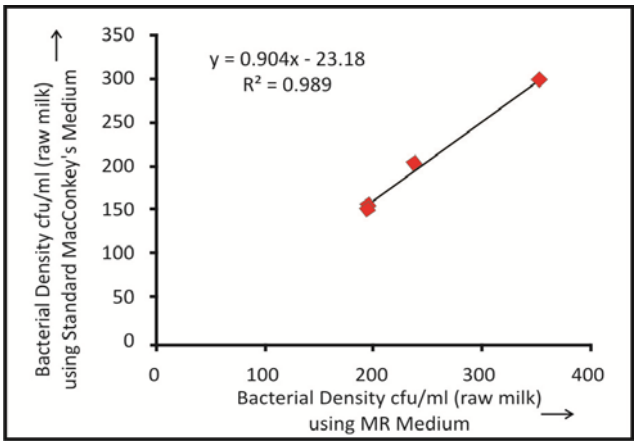
Table 2: Comparison Between MS and MacConkey's Medium (using same test milk sample)

Media (agar)	Temperature (°C)	Time (h)	No. of colonies with dia mm.				Total counts	Ratio of counts
			0.5-0.1	1.2	2-3	3 and above		
MS MacConkey	37	24	90 76	99 83	31 28	20 17	240 204	117:100
MS MacConkey	40	24	98 88	123 110	74 53	60 44	355 295	120:100
MS MacConkey	42	24	93 97	86 58	20 0	0 0	199 155	128:100
MS MacConkey	44	24	85 102	88 48	25 0	0 0	198 150	132:100

Results show that MS agar helps to grow the colonies bigger in size and more rapidly.

Perusal of Table 2 suggests that the proposed culture media / technique appears to be of greater value than any other standard technique for rapid and reliable detection of fecal coliforms / bacterial contamination by pathogens in milk. The technique can be incorporated in dairy industries / Food testing laboratories for field test analysis of milk.

The Graph 1 indicates Linear Correlation between Standard MacConkey and Proposed MS Medium.



Graph 1: Linear Correlation between Standard MacConkey and Proposed MS Medium.

4. CONCLUSION

The present results show that the proposed technique may serve as an emergency test for detecting fecal contamination in milk. The developed test/techniques are sensitive, easy to use, rapid, economic and give reproducible results. The techniques can be incorporated in existing milk testing field kits.

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