

# Analysis of Gram-Negative Escherichia Coli Bacteria in Dairy Experimentation

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**Abstract** - Consumers purchase pasteurized fluid milk with the belief that they are taking home a wholesome, nutritious, good quality product. Microbial contamination directly affects the quality of food material. The standard procedure for isolating bacteria from samples was followed. For each sample, bacteria colony count was calculated and also analysed that *E. coli* contamination is present or not.

**Keywords** - *E.Coli.*, SPC count, micro-organism, bacteria.

## I. INTRODUCTION

To check the quality of milk and milk product, generally conventional methods are used for microbiological analyses. These analyses are maintained for various stages of life of milk from its raw condition up to finalised stage of market.

To detect contamination and microorganisms, a number of modified tests or stress tests, have been developed. To check the quality of milk material, two essential tests have been performed at every milk industries.

1. Bacteria population counting
2. Coliform contamination testing in milk sample

### A. Bacteria Population Counting

This is a classical conception, but the different phases have not always been defined in the same way.

1. Lag phase: growth rate null;
2. Acceleration phase: growth rate increases;
3. Exponential phase: growth rate constant;
4. Retardation phase: growth rate decreases;
5. Stationary phase: growth rate null;
6. Phase of decline: growth rate negative.

This is a generalized and rather composite stage of the growth of a bacterial culture.

### B. Coliform contamination testing in milk sample

Coliform add up is performing by plating milk model on violet red Bile agar (VRBA), which is discriminating for these categories of gram-negative microorganisms. The plates are incubated at 32°C for 24 hours after which dark red colonies are counted. Since VRBA may allow growth of non-coliform gram-negative bacteria (generally smaller, light coloured colonies), any growth would indicate a PPC concern.

## II. EXPERIMENTATION

### A. Milk Sample Preparation

The experiment has been set up to record various information at different range of temperature and then to establish relationship between bacteria content and pH, and the accuracy of this relationship in different temperatures. In dilution process, 9 ml sample of each milk sample used for testing has prepared. Comparing the pH measurements for three temperatures could establish that temperature differences do not affect the

correlation between pH level and bacteria count in the milk sample. Fifty number of samples of various categorised of milk were obtained for the experimentation. Here we have considered six samples of milk.

Sample 1: Sample Amul Taja +RST Culture

Sample 2: Sample Amul Taja +STI Culture

Sample 3: Sample Amul Taja +CHN Culture

Sample 4: Sample Dinshaw Curd

Sample 5: Sample Amul Milk

Sample 6: Sample Cow Milk

### B. Measurement of pH value

The EL-20, Education Line pH probe was properly calibrated using standard reference buffers using a 2-point calibration. A reference buffer of pH 4 was used as 37 for point 1, and a buffer of pH 10 was used for point 2. The probe was then tested for accuracy on a buffer of pH 7 to confirm the calibration. At the time of experimentation, the pH of each milk sample was measured and recorded using the calibrated electrode.

### C. Agar Preparation

Agar preparation is done separately for testing of bacterial contamination and bacteria population present in sample. First Agar of VRB (8.4gm) and Tergital-7(0.2ml) are used for contamination testing in milk sample (Red solution) and SPC Agar is used for microorganisms people including (yellow solution). The solution was then autoclaved for sterilization.

### D. Preparation of SPC Dish

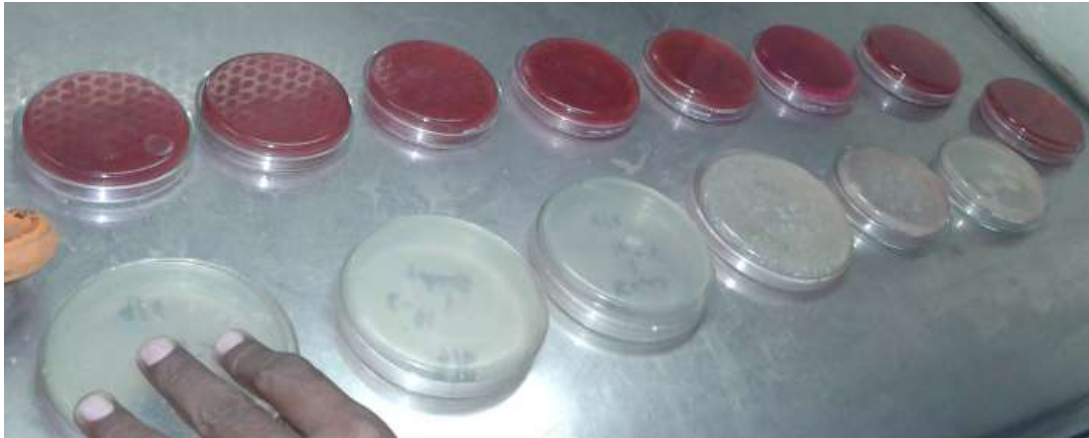
Petri dishes are used in laboratory of milk industry to measure the standard plate count which is an estimate of the total number of bacteria present in a sample that are capable to grow on SPC media when incubated at 32°C for 48 hours. Two types of sample plates were prepared to test the contamination and bacteria population for sample under test.



Photo 1: Prepared Samples

### III. ANALYSIS OF MILK SAMPLE

Prepared sample kept in the laboratory for 48 hours and after that results were collected and recorded for the comparative testing of the various samples with IR system response.



**Photo 2: Tested Samples**

Temperature is an important factor affecting the occurrence and multiplication of bacteria in the milk material. To obtain the co-relation between growth of bacteria and storage condition of milk sample, the milk sample kept at different atmospheric condition i.e. 30<sup>0</sup>C, 40<sup>0</sup>C and 50<sup>0</sup>C for number of days. For each sample, all the above test were performed and analysed the quality of milk sample either milk was contaminated or not. Also generation and regeneration of bacteria was studied by calculating the bacteria count on Petri dishes. The bacteria have been found in milk in temperatures ranging from 6<sup>0</sup>-55<sup>0</sup>C, but do not proliferate below 20<sup>0</sup>C and die above 55<sup>0</sup>C. ). It has also been noted that optimum temperatures for growth lie between 30<sup>0</sup>C and 40<sup>0</sup>C.

#### **Sample 1: Sample Amul Taja Milk+RST**



**Photo 3: Sample 1 SPC**

**Photo 4: Sample 1 SPC Two types of agar solution were prepared for contamination testing and bacteria population counting.**

**(Contamination not present)**

**(132\*10<sup>3</sup> CFU/ml)**

In order to identify, the generation of bacteria, culture RST was added to sample milk in the laboratory. Photo 5.2.3 shows the colonies of the LAB on the Agar dish. The cells form colonies on the agar surface after at least 2 days. The colour and appearance of the cell can help to differentiate colonies in samples containing different species of bacteria.

**Sample 2: Sample Amul Taja +STI**

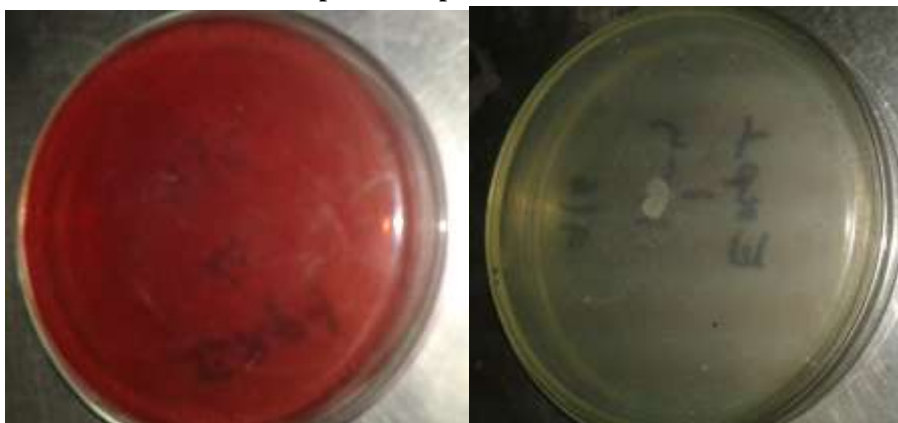
Two types of agar solution were prepared for contamination testing and bacteria population counting.

**Photo 5: Sample 2 SPC****(Contamination not present)****Photo 6: Sample 2 SPC****( $10 \times 10^4$  CFU/ml)**

It is observed that growth of bacteria varies depending on fermentation agent. Culture STI was added to sample to compare the generation of LAB (Lactic acid bacteria).

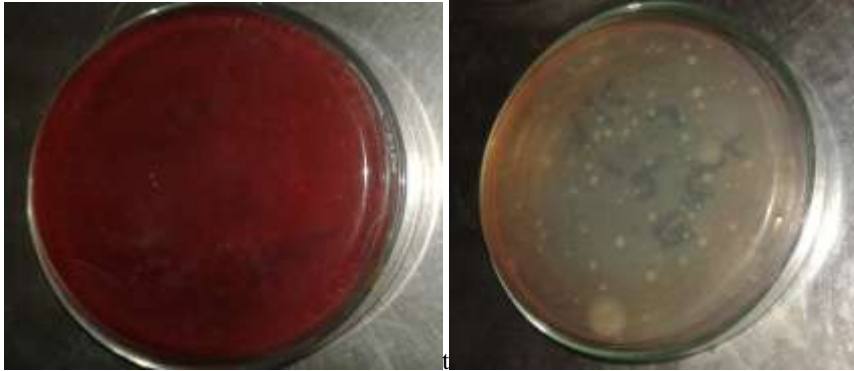
**Sample 3: Sample Amul Taja +CHN****Photo 7: Sample 3 SPC****(Contamination not present)****Photo 8: Sample 31 SPC****( $1000 \times 10^4$  CFU/ml)**

In curd preparation, culture CHN is used to provide the flavor to curd. This culture also used to increase bacteria population in milk.

**Sample 4: Sample Dinshaw Curd****Photo 9: Sample 4 SPC****(Contamination not present)****Photo 10: Sample 4 SPC****( $10 \times 10^4$  CFU/ml)**

It is observed that in fresh curd sample, there was a found approximate  $10 \times 10^4$  colony/gm lactic acid bacterium. If this sample kept for number of days, then colony count was increase as there is reproduction and multiplication of bacteria can occurs.

#### Sample 5: Sample Amul Milk



**Photo 11: Sample 5 SPC**  
(Contamination not present)

**Photo 12: Sample 5 SPC**  
( $70 \times 10^4$  CFU/ml)

It is observed that in pasteurised fresh milk, bacteria count was occurred in acceptable range. In experimentation, it was noticed that approx  $70 \times 10^4$  CFU/ml was present in prepared sample and no any contamination was occurred shown in photo 5.2.11 and photo 5.2.10 respectively.

#### Sample 6: Sample Cow Milk



**Photo 13: Sample 6 SPC**  
(Contamination present)  
(Coliform bacteria)

**Photo 14: Sample 6 SPC**  
( $40 \times 10^4$  CFU/ml)

Raw cow milk was processed through number of pasteurization steps in dairy farm. At initial condition, it is observed that Lab count was more as compare to pasteurized milk and this sample was contaminated. In contamination test, the colour of SPC changes to pink which is the indication of E. coli contamination.

#### IV. CONCLUSIONS

In order to identify the contamination it must be isolated in the laboratory. The standard procedure for isolating bacteria from the samples is detailed in the above section. The bacterial colonies have been identified by its appearance under stereomicroscope.

Under the experimentation, Sample 6 got infected by coliform bacterium. The colourised scanning electron micrograph (SEM) depicted a number of gram-negative Escherichia coli bacteria of the strain O157:H7 in this sample, which is one of hundreds of strains of this bacterium. This contamination or its most strains are although harmless, but after certain times this strains have been produces powerful toxins which can cause severe diseases in human body depending on the strength of human body.

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