ABSTRACT
Objective: To find the effect of syringe filter for the sterilization of different milk samples. Raw cow milk, commercial Pasteurized milk and goat milk was taken as the samples for the analysis. Method: The sterility testing was done for the three samples with direct inoculation method using pour plate method and spread plate method. The filtration sterilization was performed by using single use syringe filter. Result: Highlighted spread plate method as it gives maximum number of colonies. 437 number of colonies were observed in the goat milk, 184, 326 number of colonies were found in commercial pasteurized milk and raw cow milk respectively. Conclusion: We decided to find the effectiveness of the sterilization of the above milk samples by using syringe filter (minisart single use syringe filter Non-pyrogenic 0.2 micrometer). After sterilization the number of colonies were nil in both commercial pasteurized milk and goat milk but 21 number of colonies were still observed in raw cow milk. Hence concluded that the use of syringe filter shall be for commercial and goat milk but not effective for raw cow milk.

KEY WORDS: Milk analysis, test for sterility, direct inoculation method, Syringe filter.

1. INTRODUCTION
Milk is an important and popular source of nutrition that is widely consumed around the world. Milk for human consumption can be obtained from a number of domesticated animals including sheep, goat buffalo and cow, whose milk is by far the most consumed. Fresh cow
milk contains approximately 3.5% protein, 80% casein, 15% whey protein, as well as vitamins, and lipids, all of which provide necessary ingredients for growth. The number of bacterioids present in colon was huge as much in milk. [1-4] It is well documented the composition of milk changes between breeds, feed and of course species. [5-7]. Because they make up a significant proportion of the content, milk proteins have been a focus of research in order to better understand its makeup, quality, and health-related properties.

A typical dairy case at a major grocery store today contains numerous choices for the customer. There is milk labeled with different levels of fat content, and where retail raw milk sales are allowed, the consumer may choose between conventional, organic, and raw milk products, as well as homogenized or non-homogenized. In addition to fluid milk, other dairy products include butter, cheese, cream, ice cream, colostrum, yogurt, kefir, and other fermented dairy products. [8-12]

Below are some basic definitions of raw and pasteurized milk. “Raw” or “unpasteurized” refers to a dairy product that has received no heat treatment to destroy pathogens or spoilage organisms. WAPF promotes a more refined definition for raw milk, termed “real milk,” that also includes organic, non-homogenized, “grass fed,” and produced from certain breeds of cattle as criteria. [13-15]

1.1 Sources and Causes of High Bacteria Counts in Raw Milk
Milk is synthesized in specialized cells of the mammary gland and is virtually sterile when secreted into the alveoli of the udder. Beyond this stage of milk production, microbial contamination can generally occur from three main sources from within the udder, from the exterior of the udder and from the surface of milk handling and storage equipment. The health and hygiene of the cow, the environment in which the cow is housed and milked, and the procedures used in cleaning and sanitizing the milking and storage equipment are all key factors in influencing the level of microbial contamination of raw milk. [16-18] equally important are the temperature and length of time of storage, which allow microbial contaminants to multiply and increase in numbers. All these factors will influence the total bacteria count and the types of bacteria present in bulk raw milk.

1.2 Milk Storage Temperature and Time
Refrigeration storage, while preventing the growth of non-psychrotrophic bacteria, will select for psychrotrophic microorganisms that enter the milk from soiled cows, dirty equipment and
the environment. Minimizing the level of milk contamination from these sources will help prevent psychrotrophs from growing to significant levels in the bulk tank during the storage period on the farm or at the dairy plant. In general these organisms are mostly not thermoduric and will not survive pasteurization. The longer raw milk is held before processing (potential up to 5 days; 2 days on the farm, 3 at the plant), the greater the chance that psychrotrophs will increase in numbers. Holding milk near the legal limit of 7.2°C (45°F) allows much quicker growth than milk held below 4.4°C (40°F). Although milk produced under ideal conditions may have an initial psychrotroph population of less than 10% of the total bulk tank count, psychrotrophic bacteria can become the dominant microflora after 2-3 days at 4.4°C (40°F).

1.3 Syringe filter technique
This syringe filter is well suited for maximum sample recovery such as tissue culture media preparation, sterile or non sterile filtration and clarification of biological fluids, protein and enzyme filtrations, hybridization buffers, and other aqueous solutions. Its mainly recommended to filter probe solutions. Syringe filters are designed to sterilize and remove particulates from proteinaceous samples. Due to the extremely low holdup volume the negligible protein-binding characteristics, these syringe filter units are ideal for applications requiring maximum sample recovery from a standard or presterilized syringe filter.

2. MATERIALS AND METHODS
2.1 Materials: Raw cow Milk, Pasteurized Milk, Goat Milk collected from nearby areas at Sulur, Coimbatore. Nutrient agar medium, sterile disc paper, cork borer, Syringe filters, digital Colony Counter singhla make, chemicals are supplied from S.D Fine chemicals Mumbai.

2.2 Methods: Nutrient media was prepared and sterilized by autocloaving at 121°C, 15 lb/Sq.inch for 15 minutes. The Glasswares required for the work was sterilized by using hot air oven at 180°C for 45 minutes. The media and other materials after sterilization were been taken to the aseptic area under laminar air flow.

The milk samples were tested for its sterility by direct inoculation technique with pour plate method and spread plate method. Excluded streak late method as it gives isolated colonies. Upon completing the direct inoculation technique, the labeled, inoculated petriplates were kept under incubation condition at 37°C for 18-24 hrs. The obtained results were recorded.
The effectiveness of the use of syringe filter for the sterilization of above milk samples were performed with minisart single use syringe filter, Non-pyrogenic, 0.2 micrometer. Test for the sterility was performed after sterilizing with syringe filter. The results obtained were recorded by digital colony counter.

3. RESULTS AND DISCUSSION

The effectiveness of the sterilization of milk samples before and after use of minisart single use filter, Non-pyrogenic, 0.2 micrometer was compared and analysed. Before sterilization, maximum 437 number of colonies were obtained in goat milk. Also 326 and 184 number of colonies was obtained in raw milk and commercial pasteurized milk respectively (table.1). Even after sterilization, we found around 21 number of colonies in the raw milk where as other two milk samples have became sterile.

Table.1 Number of colonies observed by direct count method

<table>
<thead>
<tr>
<th>S. No</th>
<th>SAMPLE</th>
<th>METHODS</th>
<th>NUMBER OF COLONIES Before Sterilization</th>
<th>After Sterilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Raw Cow milk</td>
<td>spread plate</td>
<td>326</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pour plate</td>
<td>318</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>Commercial Pasteurized milk</td>
<td>spread plate</td>
<td>184</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pour plate</td>
<td>178</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Goat milk</td>
<td>spread plate</td>
<td>437</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pour plate</td>
<td>425</td>
<td>-</td>
</tr>
</tbody>
</table>

3.1 Before sterilization

![Figure 1 - Raw Cow milk](image_url)
3.2 After sterilization

Figure 2 - Pasteurized commercial milk

Figure 3 - Goat milk

Figure 4 - Raw cow milk

Figure 5 - Pasteurized commercial milk
Hence the present investigation suggests use the disposable syringe filter is applicable only for commercial pasteurized milk and goat milk and also not to use syringe filter for raw cow milk as the effectiveness is not much in restricting all kind of microbes present in the raw milk samples.

4. CONCLUSION
Coxiella burnetii, E-coli, Mycobacterium bovis or tuberculosis, Mycobacterium para tuberculosis, Salmonella species, Yersini enterocolitica, lacto bacillus, enterobacterogens were found to be present in all the milk samples. The above bacterial species were identified and confirmed by biochemical reactions. The current investigation suggests, that the use of minisart single use syringe filter, Non-pyrogenic, 0.2 micrometer for the sterilization was found to be effective with commercial pasteurized milk and goat milk sample. The number of colonies obtained by direct inoculation technique of test for sterility has become nil after sterilization. At the same time we are not ready to suggest the same syringe filter for the purpose of sterilizing raw milk where it fails to restrict many number of microbes which may cause serious hazards to the human being.

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6. REFERENCES


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