# Microbiological Quality Assessment in Raw Milk Evaluation using Soleris System as a Rapid Alternative Method

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**Abstract**
Defined as the mammary glands/udder inflammation, mastitis caused by various infectious etiological agents, is still considered a debilitating condition in dairy cows, influencing both animal welfare and the dairy industry through decreased production performance and increased culling rates. The consumption of raw milk or other milk products is related to the microbiological quality of raw milk. The main disadvantages in applying laboratory microbiological culture are related to logistical limitations and the expense of shipping samples, as well as the time required for analysis to receive interpretations, which can range from three to five days. The aim of this study is to validate the Soleris System as a rapid alternative method to the plate-count method in order to assess the microbiological quality of raw milk. Thus, this study establishes the reliability of this alternative method for determining the total viable count in raw milk in samples from cows. In conclusion, raw milk evaluation using the Soleris System demonstrates its promise as a valid tool for accurate testing in the dairy industry.

**Keywords**: mastitis, raw milk, Soleris System, total viable count.

## 1. Introduction

According to the definition stated in 1909, by the International Congress on Prevention of Fraud in Paris, France, and cited by Chouinard P.Y. et al. in 2014[1], milk is characterized as *"The integral product of the full and uninterrupted milking of a healthy, well-nourished and not overworked milk-producing female. It should be collected under hygienic settings and should not contain colostrum"* [2].

Humans consume milk due to its high nutritional value, all of its nutrients being beneficial to human health. Excluding water, milk is an excellent source of complete nutrition, including proteins, carbs, fats, vitamins, and minerals. It also contains immunoglobulins, hormones, cytokines, and nucleotides, among other bioactive components [3].

Since ancient times, humans have consumed the milk of goats, sheep, and cows, and nowadays, the term "milk" has become associated with cow's milk. As a marketed product, milk belonging to other animal species is mentioned clearly, such as sheep or goat milk [4].

Besides that, milk is a highly nutritious food source, it is also an excellent growth medium for a wide variety of microorganisms. They are derived from numerous sources, including the mammary gland and teat canal, udder skin, the milking equipment, and the farm environment [3]. Bacteria, yeast, and molds are all part of the microbiota in milk. Their metabolisms modify and develop molecules within their habitat. Their presence in milk can be favorable for the fermentation of dairy

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products, but it can be also detrimental because of foodborne diseases or spoiling [3].

Raw milk production and the processing of dairy products is an important sector of the global food industry, and a variety of hygiene requirements must be strictly maintained from milking to processing. Despite standards to ensure the quality of milk and dairy products, the prevalence of diseases associated with milk or dairy product consumption is inherent due to gaps along the processing chain. Similar to somatic cells, bacteria produce enzymes that degrade proteins, lipids, and other components of raw milk, resulting in a reduced quality of the product when the number of germs in milk is high.

Environmentally and financially, milk spoilage by microorganisms is a major concern for dairy industry. This activity has a significant impact on the shelf life of milk and dairy products and degrades milk fat and casein, causing milk to become rancid and develop unpleasant off-flavors [3]. Although pasteurization can destroy the majority of bacteria, heat-tolerant enzymes and their enzymatic activity persist, making them, along with spore-forming bacteria that tolerate heat treatment, one of the leading causes of microbial contamination in milk [5].

Thus, in order to assess the microbiological quality of milk, determining the total viable count is one of the best practices for herd production and management and is essential to identify the potential risks before they have a significant economic impact.

Standard plate count has been validated as a reference method for determining total bacterial counts based on ISO 4833-1: 2013 [6], which governs almost all of the analytical standard procedures used for hygienic evaluation of raw milk. This method indicates the total number of aerobic bacteria found in raw milk after collecting it. To promote bacterial growth, milk samples are plated on a semi-solid culture medium and incubated at 32°C for 48 hours. Single colonies or dense clusters (such as chains or clumps) develop into visible colonies, which are subsequently counted. Finally, all bacterial plate counts are reported in colony forming units (CFU) per milliliter (ml). According to Regulation (EC) No. 853/2004 of the European Parliament and of the Council, the total bacterial count expressed as a colony forming unit (CFU) limit for raw milk is 100 000 CFU/ml.

Compared to other methods used in the dairy industry, standard plate count is a time-consuming method that requires a lot of labor and materials. Taking all this into consideration, rapid methods are recommended when we refer to a high number of milk samples. Thus, excepting the standard method mentioned below, immunological/ impedimetric methods [7,8], flow cytometry [9,10], ATP bioluminescence [8,11,12], and methods based on detection of oxygen levels [13], are some of the most frequently used in the milk industry.

The main purpose of this study was to validate the Soleris System as a rapid alternative method to the plate-count method in order to evaluate the microbiological quality of naturally contaminated samples of raw milk.

## 2. Materials and methods

The milk samples were analyzed for the total viable count during two assessments to evaluate the udder's health. Thus, a total of 330 lactating Holstein Friesian cows on a dairy farm were enrolled in this study. The cows were housed in a free-stall system and fed twice daily with a total mixed ration (TMR) and ad libitum water.

Besides the total viable count, the following physicochemical parameters of raw milk were determined using the standard methods: density, acidity, pH, total lipids (fat), dry matter, trace minerals, lactose, casein, and somatic cell counts.

Total viable count testing was performed on a total number of 98 raw milk samples, using the Soleris optical system, in order to validate it as a rapid alternative method to the standard method (plate-count method), which is firstly, more time-consuming.

One mL of sample to be analyzed was inoculated into the Soleris vial containing a selective growth medium and a pH indicator (NB-100, Total Viable Count Medium-TVC), followed by incubation for 18–24 hours at 35°C (figure 1). The system consists of a single incubator with 32 independently monitored and temperature-controllable locations, with a range between 15°C and 60°C. During testing, in less than 24 hours, the Soleris software reported positive test findings.

The absence of detection within 24 hours was considered a negative result. In the case of positive samples, the growth curves were examined, and the visual validation of medium color change was also

performed. Also, randomly, for some of the positive results, standard methods were used for confirmation.

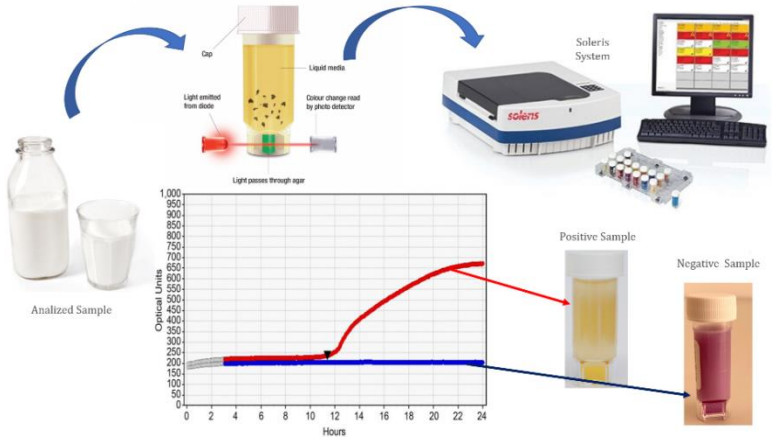


**Figure 1.** Schematic illustration of the Soleris optical system's operating


**Figure 2.** Illustration of raw milk samples in progress


**Figure 3.** Illustration of the total viable count detection curve in Real-time

## 3. Results and discussion

Currently, numerous rapid methods are used to determine the microbiological quality of milk. Therefore, although the number of samples taken into account is a small one, the findings of the present study indicate that the Soleris System can be successfully used as an alternative method for assessing the quality of raw milk.

In the five cases of milk samples in which the amount of CFU exceeded 100.000, the traditional plate count method was used to confirm the fast

approach's results, and confirmed the initial findings.

As shown in table No. 1, the Soleris System detected samples with a total viable count higher than 100.000 in less than 9 hours, which reduces costs for materials used in conventional methods, reduces labor costs, reduces processing time, incubation of samples and interpretation of results, simplifying thus the workflow as well.

Compared to the conventional method, which is time-consuming, this rapid method has the advantage of reducing economic losses by quickly identifying raw milk with a total number of germs/mL > 100.000 and quickly alerting to problems, ensuring the quality of raw milk.

**Table 1.** Comparison of Soleris System with conventional methods

| Test type | Specification levels | Conventional methods (time to results) | Total testing time to negative results (Soleris System) | Early alert time for positive results (Soleris System) |
|---|---|---|---|---|
| **Total viable count** | **< 100.000** | **48 h** | **18 h** | **6–8 h** |

**Table 2.** The obtained results for raw milk samples using the Soleris System

| Matrix | Soleris System | | <100.000 | | >100.000 | |
|---|---|---|---|---|---|---|
| **Raw milk** | **No.** | **%** | **No.** | **%** | **No.** | **%** |
| | **98** | **100** | **93** | **94.9** | **5** | **5.1** |

The comparison between this rapid and the standard method reveals the accuracy of the alternative method for determining the total viable bacterial count in raw milk samples from dairy farms. Although these results are preliminary and limited to raw milk, this highlights the promise of the Soleris System as a trustworthy microbiological analysis tool for the dairy industry and manufacturers of raw milk and derived products. Its characteristics, which include simplicity (by adding raw milk to the vial, with no sample preparation, and no need for expert technicians or a microbiological laboratory) [14], accuracy (as accurate as classical method) [14,15], and rapidity (faster than classical method), make it suitable for use in small and medium-sized dairy companies / herds for routine analysis and to improve the quality of their products.

The advantages of using this rapid detection system are that it dramatically simplifies the test flow, reduces personnel expenses compared to conventional methods of counting colonies on plates, and may provide the dairy industry with accurate data in a matter of hours, as opposed to the traditional method (up to 48 - 72 hours).

## 4. Conclusions

The Soleris System should be considered as an extra / rapid alternative method for improving the safety and quality of milk and milk products, rather than a replacement for officially designated analytical methods that must be performed in line with applicable legislation.

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