# Development and Validation a Method for Determining the Total Bacterial Count in Raw Cow Milk by an Automate Instrument Using Flow Cytometry Technique

Sunantha Rumgmee[1,*], Chonlada Thongdee[1] and Siriprapha KhamKhote[1]

**Abstract**

**Background:** Total bacterial count contamination is recognized as an indicator of raw milk quality, hygienic conditions during production, packaging and storage. According to Thai agricultural standard TAS 6003-2010, the regulation required for microbiological criteria of standard plate count in raw cow milk shall not exceed $5 \times 10^5$ cfu/mL. Currently, Upper Northeastern Veterinary Research and Development Center (VRDNE) laboratory analyze the total bacterial count in raw cow milk using the standard AOAC 986.33 method with Petrifilm™, which requires an incubation period of 48±3 hours. In contrast, automated instruments for testing total bacterial count by the flow cytometry technique can rapidly analyze at least fifty samples per hour. Therefore, an automated raw milk bacterial count analyzer utilizing flow cytometry has been developed to optimize both the accuracy and efficiency of microbial analysis, particularly in high-volume testing operations, where rapid results are required.

**Objectives:** The study aimed to develop and validate a method using an automated instrument using flow cytometry technique to determine the total bacterial count in raw cow milk.

**Methods:** A total of 237 raw cow milk samples with natural microbial contamination were collected from twenty-five milk collection centers and submitted to the VRDNE laboratory for analysis of milk quality. All samples are subject to testing for bacterial enumeration by using an automated total bacterial count instrument (BactoCount IBCm3.0) compared with the Petrifilm™ method. After that, all the obtained datasets were then used to establish a scatter plot graph and an equation relationship between the two methods. Subsequently, An adjusted equation was used to replace the original equation in the BactoCount machine to convert the test results from IBC/mL to cfu/mL in accordance with ISO 21187/IDF 196:2021. Then, the validity of BactoCount instrument method is verified according to ISO 16140-2:2016/Amd 1:2024.

**Results:** The analysis of 237 raw cow's milk samples to determine the correlation between the BactoCount IBCm3.0 method and the AOAC 986.33 standard method revealed a standard error (Sy,x) of 0.2245, which complies with the requirement of ISO 16297/IDF 161:2020, which specifies a maximum error of 0.4. When the relationship was analyzed using a linear regression test, the equation correlation obtained from the BactoCount instrument and the standard AOAC 986.33 Petrifilm™ method was Log10 cfu/mL = 1.1343 x Log10IBC/mL - 1.2862, with an $R^2$ value of 0.9887, which showed no statistically significant difference between the two analytical methods ($p \geq 0.05$). For the relative trueness test according to the ISO 16140-2:2016/Amd 1:2024, the results of the BactoCount instrument compared to the Petrifilm™ method showed a consistency of 95% confidence. This demonstrates that both methods are consistent. In addition, it was found that the accuracy profile of the total bacterial count from all samples ranged from 3.86 Log10 cfu/mL to 7.2 Log10 cfu/mL which are smaller than the acceptability limits level. Therefore, the accuracy profile of this method is acceptable.

**Conclusion:** The BactoCount instrument, using the flow cytometry technique, was developed and validated to determine the total bacterial count in raw cow milk. It indicated reliability and equivalence to the Petrifilm™ method.

**Keywords:** Raw cow milk, Total bacterial count, Validation method, Flow cytometry technique

[1] Upper Northeastern Veterinary Research and Development Center.

[*] Corresponding author; E-mail: sunantha_ru@hotmail.com