# Development and validation a method for determining the total bacterial count in raw cow milk by an automate instrument using flow cytometry technique

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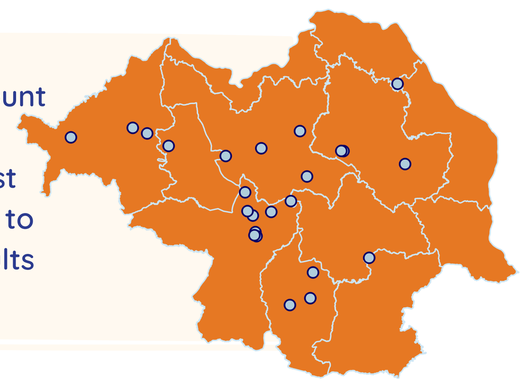
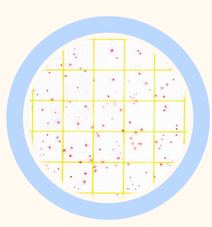
## BACKGROUND

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.



## METHODS



ESTABLISHING A CONVERSION RELATIONSHIP

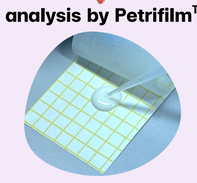
samples recieved

sub-samples preparation

sub-samples A

sub-samples B

analysis by Petrifilm™



analysis by Bactocount

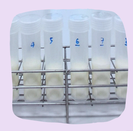
calculate the conversion relationship equation

STUDY THE RELATIVE TRUENESS AND ACCURACY PROFILE

select samples

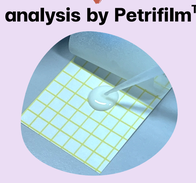
sub-samples preparation

sub-samples A



sub-samples B

analysis by Petrifilm™



analysis by Bactocount

Bland-Altman difference plot and accuracy profile plot

## RESULTS AND DISCUSSIONS

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 ($S_{y,x}$) 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 $Log_{10}$ cfu/mL = 1.1343 x $Log_{10}$IBC/mL -1.2862, with an $R^2$ value of 0.9887, which showed no statistically significant difference between the two analytical methods ($p \geq 0.05$) (Figure 1). For the relative trueness test according to the ISO 16140-2:2016, the results of the BactoCount instrument compared to the Petrifilm™ method showed a consistency of 95% confidence. This demonstrates that both methods are consistent (Figure 2 and Figure 3).
In addition, it was found that the accuracy profile of the total bacterial count from all samples ranged from 3.86 $Log_{10}$ cfu/mL to 7.2 $Log_{10}$ cfu/mL which are smaller than the acceptability limits level. Therefore, the accuracy profile of this method is acceptable.

Figure 1. A scatter plot of relationship between units from the Bactocount method are transformed into Petrifilm™ units resulting

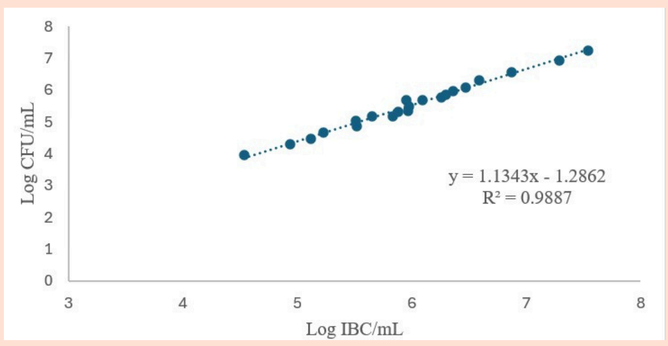



Figure 2. Bland-Altman difference plot

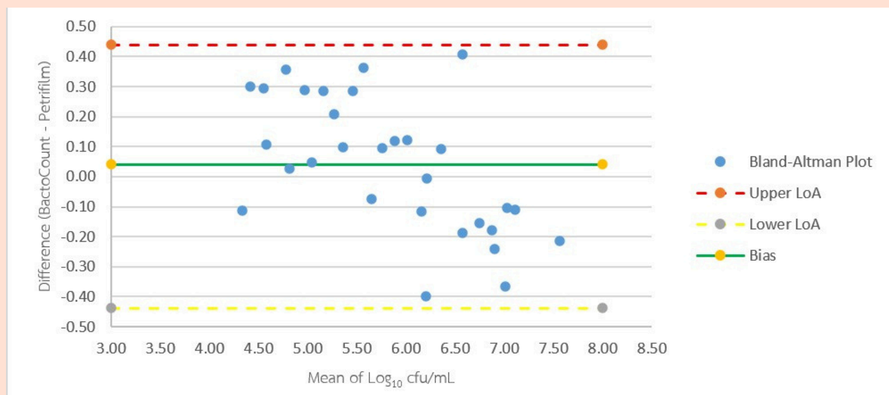



Figure 3. Accuracy profile


## CONCLUSIONS

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.

## ACKNOWLEDGEMENTS

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## REFERENCES

- Official Methods of Analysis of AOAC International. 2023.22nd Method 986.33.
- ISO 21187:2021/IDF 196, Milk- Quantitative determination of microbiological quality - Guidance for establishing and verifying a conversion relationship between results of an alternative method and anchor method results
- ISO 16140-2:2016, Microbiology of the food chain - Method validation Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method