RESHAPE

# Method Validation Report

ISO 15214

Enumeration of lactic acid bacteria following ISO 15214; Comparing manual and automated counts.

## Key Findings

Evaluation of the Reshape Smart Incubator for enumerating lactic acid bacteria revealed that the automated counting system offers significant advantages over the traditional manual assessment methods. The automated process demonstrated superior speed and accuracy, providing an efficient and reliable alternative for plate counting with **92.97%** agreement between manual and model counts. The assessment was performed using the internationally recognized ISO 15214 method, a quantitative culture-based technique.

## Introduction & Background

The enumeration of lactic acid bacteria (LAB) is a critical quality control step in the food and pharmaceutical industries. Traditionally, this process relies on manual counting of colonies on agar plates by trained technicians, a method that is both labor-intensive and susceptible to human error. Factors such as fatigue, variations in colony morphology, and subjective interpretation can impact the reproducibility and accuracy of results. To enhance efficiency and standardize microbial enumeration, new technologies such as automated colony counters have emerged. This report compares the performance of trained personnel against an automated imaging device (Reshape Smart Incubator) for the enumeration of LAB, following the established ISO 15214 standard for microbiological methods. The objective is to assess the automated system's potential as a faster and more accurate alternative to manual counting.

## Materials, Methods & Protocols

The enumeration of LAB followed the standardized ISO 15214 protocol.

**Sample Preparation and Dilution:** Three lactic acid bacterial strains, *Levilactobacillus brevis, Pediococcus pentosaceus, lactiplantibacillus plantarum* (Sigma-Aldrich) were grown overnight in Man-Rogosa Sharpe (MRS) broth, before being diluted into a countable range.

**Plating on Selective Agar:** From each dilution, pour plates were prepared with a semi-selective MRS agar, and subsequently incubated at 30 °C for 72 hours.

**Automated Imaging and Counting:** The plates were then imaged using an automated colony counting and imaging system (Reshape Smart Incubator). This system captured high-resolution images of the plates under controlled lighting conditions. The images were then analyzed by the system's software to automatically identify and count colonies based on their size, shape, and color. This automated process helped to standardize the assessment and counting of colonies, providing an objective and reproducible basis for calculating CFU/g (See Figure 1).

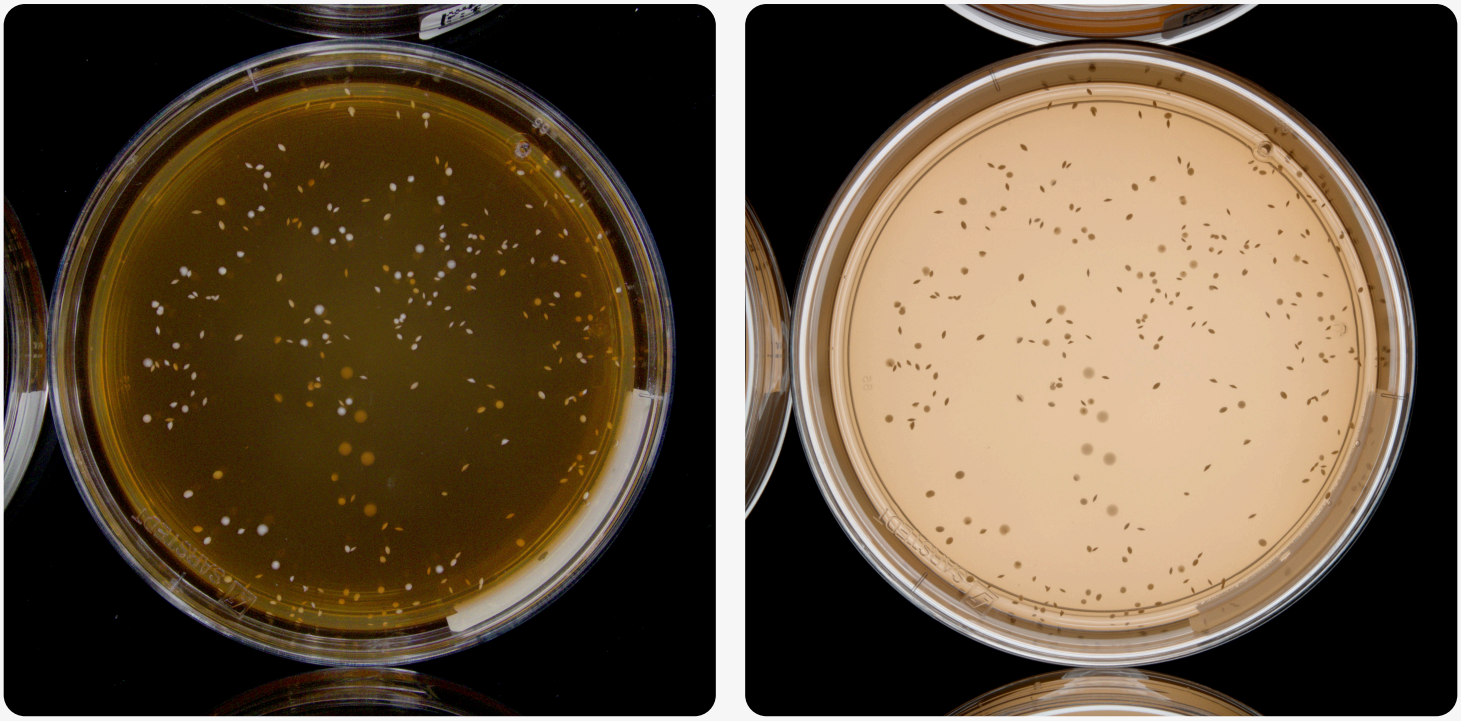

**Figure 1:** (Left) Picture of a petri dish with LAB counts on top light setting. (Right) Picture of the same petri-dish on bottom light setting. Media is MRS-agar.

Manual counting was performed by a group of trained personnel. Each plate was independently counted by a minimum of three people to minimize and account for inter-observer variability. Prior to manual assessment, all plates were incubated and processed using the Reshape Smart Incubator, which captures high-resolution images of the plates and uses proprietary software to identify and count colonies, resulting in an automated count. The total count from each plate was recorded for both the manual and automated methods.

## Results

Based on 122 images, there was an overall agreement in 92.97% between manual and model counts. (See figure 2). Only 6 images were classified as TNTC (too numerous to count) by the trained personnel, where the model characterized them as not TNTC (see figure 3).

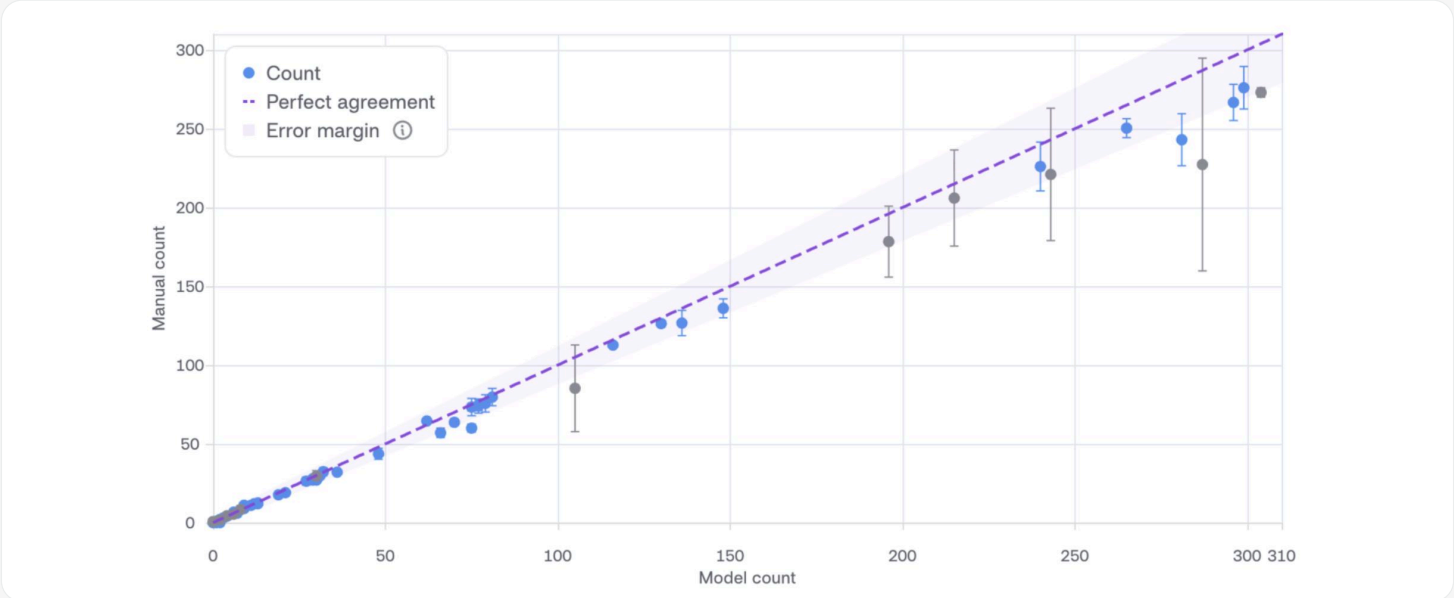


**Figure 2**: A correlation plot between based on 122 images comparing manual and model CFU counts. The purple line indicates perfect agreement, and the purple area shows the error margin.

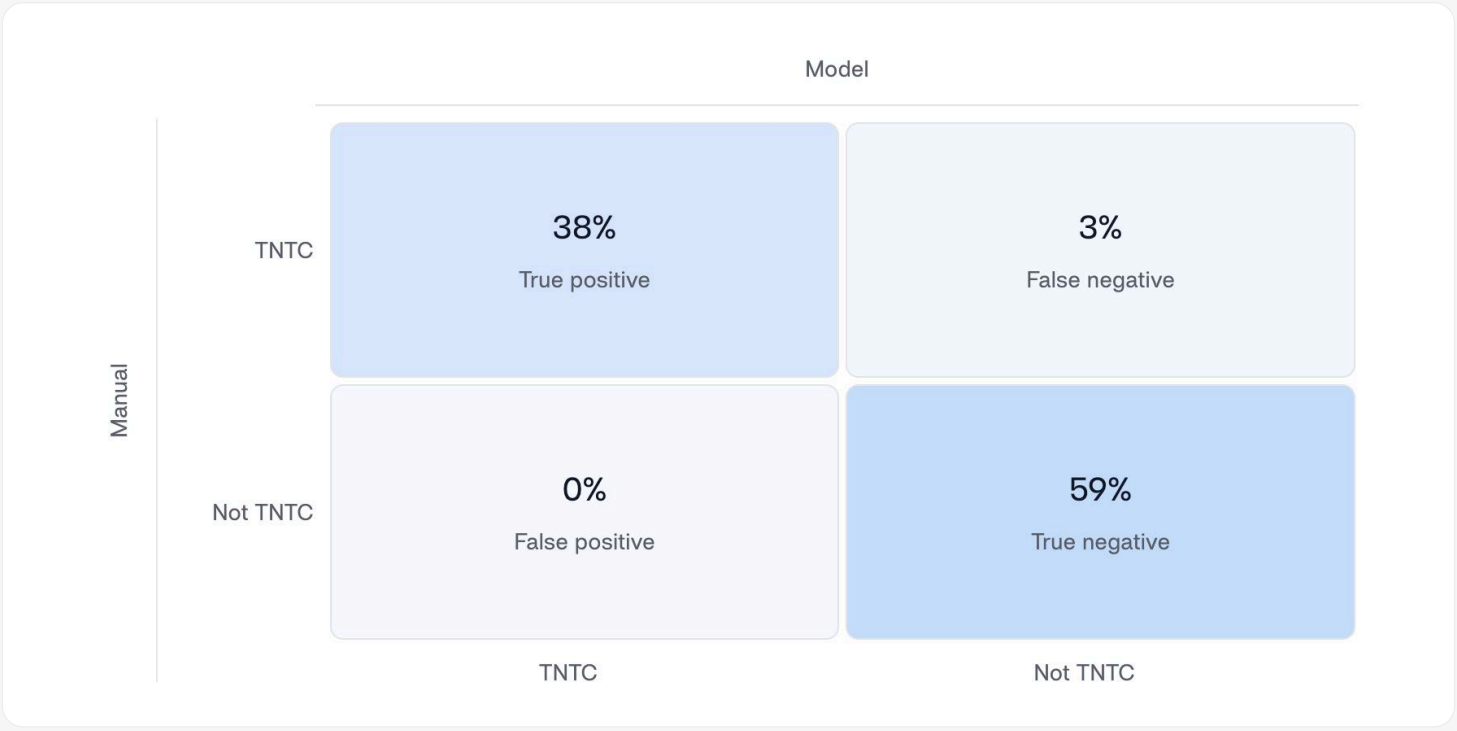


**Figure 3**: Confusion matrix based of the manual and model counts. TNTC = too numerous to count

## Discussion

The preliminary findings indicate that the Reshape automated counting system offers a significant improvement in both speed and accuracy over the manual counting method. For cases where the accuracy is not on par with counting, adding more training data will alleviate this over time, resulting in even better performance. The reduced time required for plate enumeration suggests that the automated system can substantially increase laboratory throughput and productivity, while not compensating on quality; The improved accuracy reduces the risk of human-induced errors, which is particularly critical in quality control and regulatory compliance.

The consistent and objective nature of automated counting is expected to enhance the reproducibility of results; a key challenge associated with manual techniques. While the specific data is pending, these results strongly support the implementation of automated systems as a more reliable and efficient standard for microbiological enumeration. The findings underscore the potential for the Reshape device to standardize procedures and improve overall data quality in a laboratory setting.

## Conclusions

In conclusion, the Reshape's platform holds great promise as a superior method for enumerating lactic acid bacteria. The system's demonstrated ability to provide fast and more accurate counts positions it as a valuable tool for replacing traditional manual counting. The adoption of such technology can lead to increased laboratory efficiency, improved data reliability, and enhanced compliance with microbiological standards. Future optimization should focus on a larger dataset to further validate its performance across different matrices and conditions.