

Method Validation Report

ISO 15214



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

Key Findings

The evaluation of the Reshape imaging device 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.

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 the Reshape automated imaging device (RID) 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: A specified quantity of the sample pre-grown culture (*Levilactobacillus brevis*, *Pediococcus pentosaceus*, *Lactiplantibacillus plantarum* grown in Man-Rogosa sharpe (MRS) broth (Sigma-Aldrich)). From this initial suspension, a decimal dilution series was to obtain a range of dilutions suitable for counting.

Plating on Selective Agar: From each dilution, 100 uL aliquots were transferred unto a sterile petridish and pour plates were made with semi-selective agar, MRS agar, and subsequently incubated at 30 °C for 72 hours.

Automated Imaging and Counting: The agar plates were then imaged using an automated colony counting and imaging system (Reshape imaging device (RID)). This machine captured high-resolution photographs of the plates under controlled lighting conditions. The images were then analyzed by the machine'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).

		Model	
		TNTC	Not TNTC
Manual	TNTC	46	6
	Not TNTC	0	70

■ Figure 3: Confusion matrix based of the manual and model counts. Numbers are images.

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.

One notable difference is the performance difference between the top- and bottom-light settings, clearly illustrating the need to consider each case and which setting allows for the best detection and model performance.

Additionally, even for cases where the accuracy is not on par with counting, adding more training data should 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 automated imaging device 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 research should focus on a broader application of this technology to other types of microbes and a larger dataset to further validate its performance across different matrices and conditions.

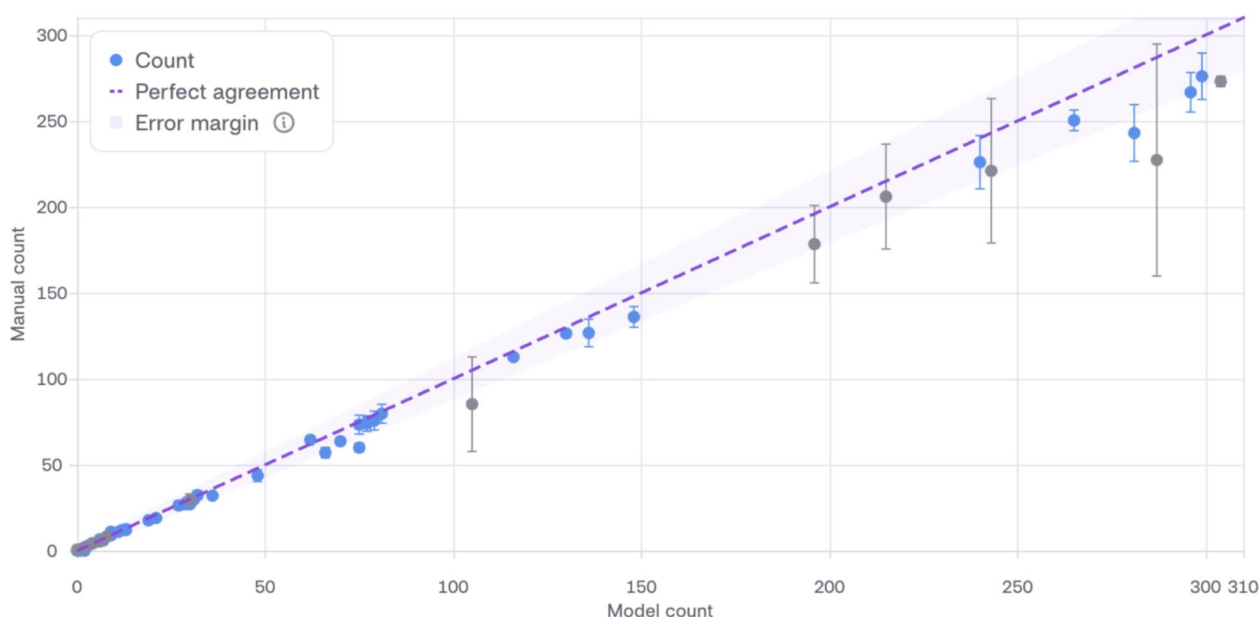


■ Figure 1 left: Picture of a petri dish with LAB counts on top light setting, Right: picture of the same petri-dish on bottom light setting. Media is MRS-agar

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 automated imaging device (RID), 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

There was an overall agreement in 92.62% of the cases using the bottom light setting, based on 122 images total. Using the top-light setting, the agreement was slightly lower, resulting in a total agreement of 90.16% on the CFU count (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: The count graph for the 122 images. On the X-axis is the value for the manual counts, on the Y-axis is the model count. The purple line indicates perfect agreement, and the purple area shows the error margin.