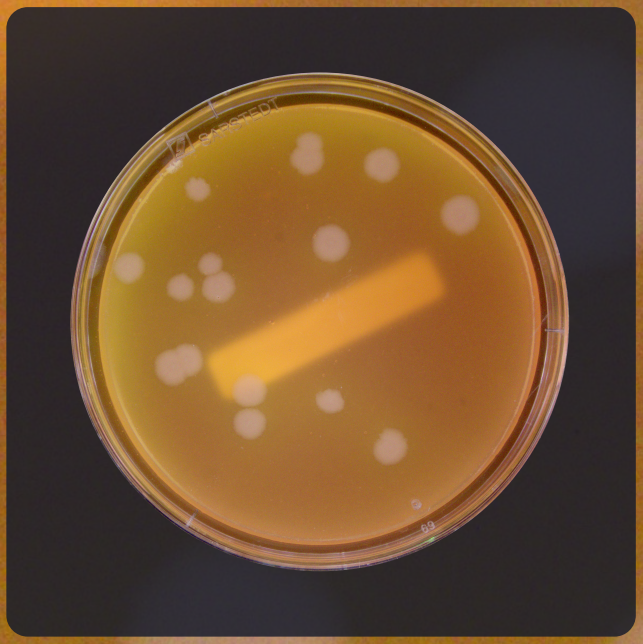
RESHAPE

Method Validation Report

ISO 7932



The aim of this validation is to demonstrate that Reshape Biotech's imaging technology and machine learning model performs in line with the ISO 7932; comparing manual and automated counts.

Key Findings

This section summarizes the key takeaways from a comparison between an automated counting method and manual assessment for the enumeration of *Bacillus cereus*. The assessment was performed using the internationally recognized ISO 7932 method, a quantitative culture-based technique.

The results show that the automated system's counts were highly accurate and comparable to the manual method, but only when using the top light imaging setting, which achieved 98.25% agreement. The bottom light setting was found to be significantly less reliable. Based on these findings, it is recommended that the automated system be adopted for routine testing, strictly using the validated top light protocol to ensure data integrity for critical food safety decisions.

The use of the automated imaging system for plate analysis significantly streamlined the process, providing a consistent and objective method for colony enumeration and morphology assessment, which improved the overall efficiency and reproducibility of the results. This work underscores the critical importance of validating and standardizing all parameters of an automated system, especially imaging conditions, before its implementation in a quality control laboratory.

Introduction & Background

Bacillus cereus is a genus of Gram-positive, rod-shaped bacteria belonging to the family Bacillaceae. It is a significant cause of foodborne illness, known for its ability to produce two distinct types of toxins: a diarrheal toxin and an emetic toxin. A key characteristic of B. cereus is its ability to form heat-resistant spores, which can survive the cooking process. If food is then improperly cooled or stored, these spores can germinate and multiply to levels sufficient to cause illness. The presence of B. cereus is particularly associated with starchy foods like rice, pasta, and potatoes, as well as meat dishes and dairy products.

The purpose of this study is to perform a quantitative assessment, or enumeration, of the number of viable *Bacillus cereus* cells following the ISO 7932 method using both conventional manual assessments and an automated counting system using the Reshape Smart Incubator. This internationally standardized protocol provides a reliable and reproducible way to count this pathogen, which is crucial for assessing food safety and public health risks. A bacterial count exceeding 105 CFU/g is generally considered a potential health risk, making enumeration necessary to demonstrate compliance with relevant regulations.

Materials, Methods & Protocols

The enumeration of *Bacillus cereus* followed the standardized ISO 7932:2004 protocol. This method is a three-step process: sample preparation and dilution, plating on selective agar, and confirmation of presumptive colonies.

Sample Preparation and Dilution: A specified quantity of the sample pre-grown culture (*B. cereus* NCIMB 9373 and *B. subtilis* NCIMB 13061 grown in nutrient broth (Sigma-Aldrich)). From this initial suspension, a decimal dilution series was to obtain a range of dilutions suitable for counting. Additionally, diluted cultures of *B. cereus* and *B. subtilis* were also mixed to ensure that the model could positively differentiate between the different types of growth.

Plating on Selective Agar: From each dilution, 100 uL aliquots were transferred unto a selective agar, Mannitol Egg Yolk Polymyxin (MYP) agar, and subsequently incubated at 30 °C for 18-24 hours. The polymyxin in the agar inhibits the growth of most competing bacteria, while the mannitol and egg yolk allow for differentiation of *B. cereus*.

Automated imaging and Counting: The agar plates were then imaged using an automated colony counting and imaging system (Reshape Smart Incubator). 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. On MYP agar, presumptive *Bacillus cereus* colonies are typically pink-orange due to their inability to ferment mannitol and are surrounded by a white zone of precipitate from the egg yolk, indicating lecithinase activity. This automated process helped to standardize the assessment and counting of colonies, providing an objective and reproducible basis for calculating CFU/g.

Of the above-mentioned steps, the focus in this study was on the accurate enumeration of colonies using the Reshape Smart Incubator and manual assessment of the plates to ensure reliability.

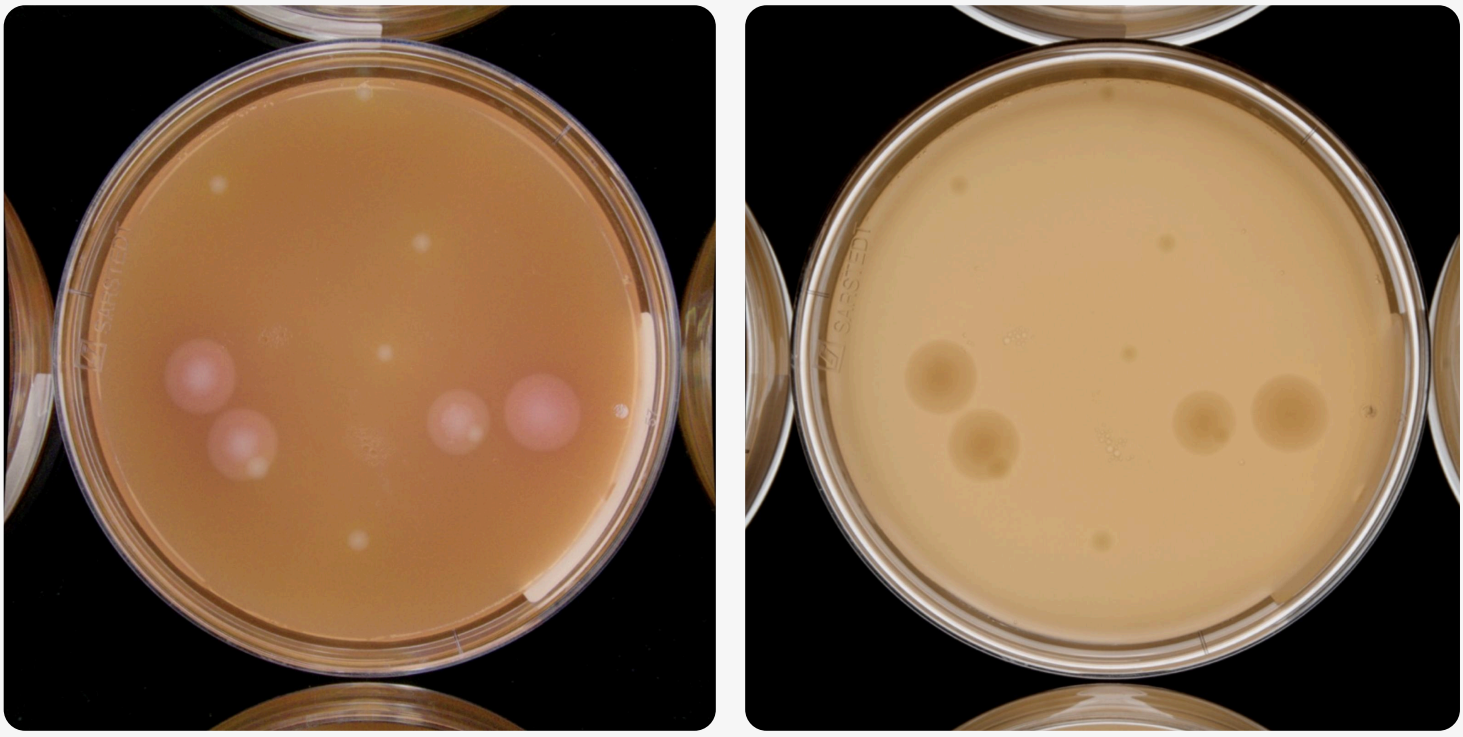


Figure 1: Image of randomly chosen MYP agar plate. Pink colonies are B. cereus, yellow are B. subtilis.
Left: Top light image | **Right**: Bottom light image

Results

A total of 114 images were analyzed. There was an overall agreement of 87.72% of the cases using the bottom-light setting. Using the top-light setting resulted in an agreement of 98.25% (See Figure 2).

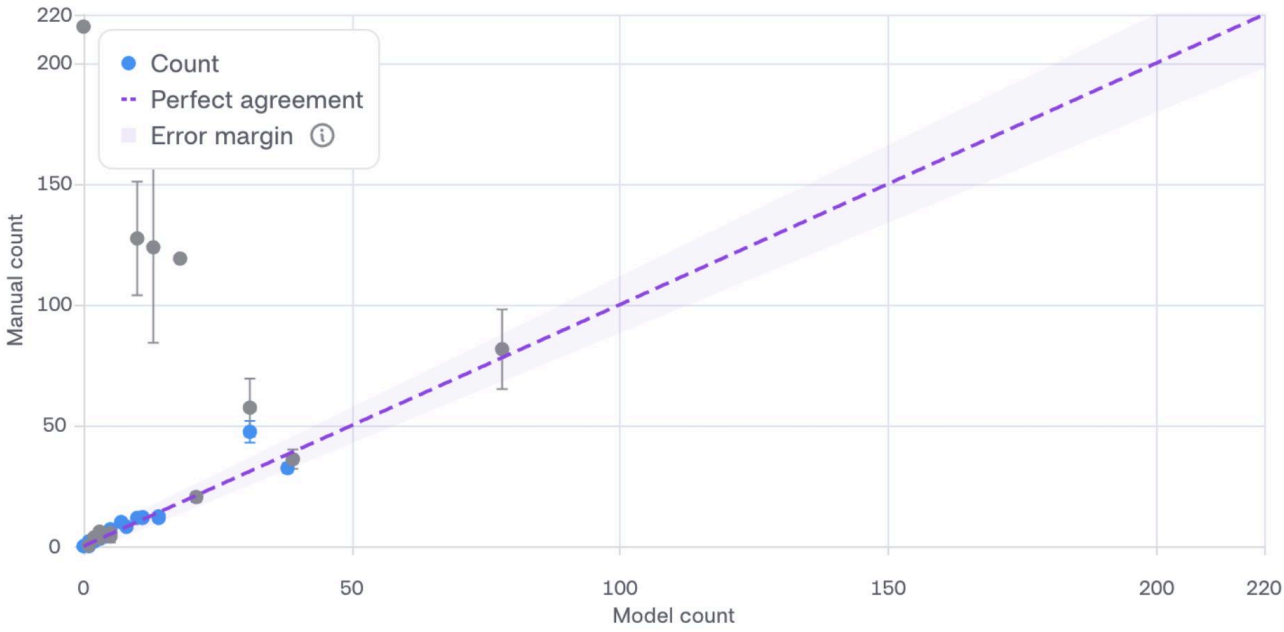


Figure 2: Count graph showing agreement between manual counts and model counts. The purple line indicates perfect agreement, and the purple area shows the error margin.

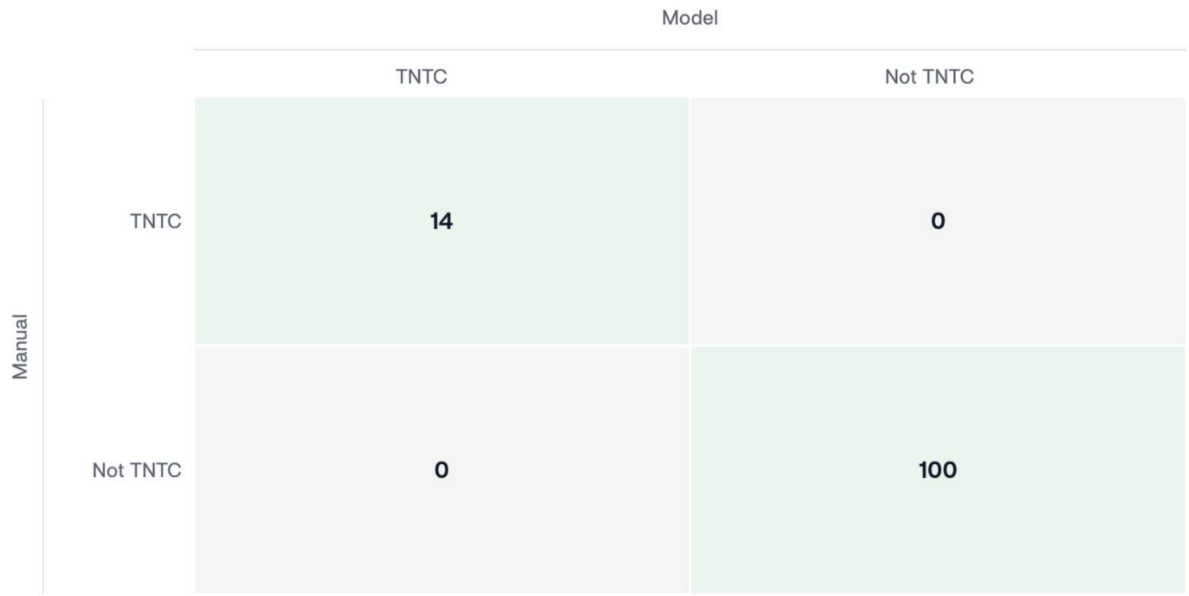


Figure 3: The confusion matrix of between manual and model counts. Numbers are images.

Discussion

The analysis of 114 images reveals that the model's accuracy in microbiological counting is critically dependent on the lighting conditions used during imaging. The top light setting proved to be exceptionally effective, yielding a 98.25% agreement with manual counts. This high level of accuracy indicates that the model is a robust and reliable tool for automating colony enumeration under these conditions, suggesting it can increase efficiency and reduce subjective error. In contrast, the bottom light setting resulted in a notably lower agreement of 87.72%. This performance drop suggests that bottom lighting likely introduces visual artifacts, such as shadows or inconsistent contrast, that challenge the model's algorithms.
Therefore, while the model shows strong potential, these findings underscore that standardizing on the top light setting is essential to achieve the highest accuracy and ensure its successful implementation as an alternative to manual counting.

The exceptional 98.25% accuracy achieved with the top light setting establishes this model as a superior alternative for scientists, particularly in food safety applications like monitoring Bacillus cereus. For scientists, this automation offers a dramatic increase in efficiency and throughput, allowing more samples to be processed in less time. Crucially, it eliminates the subjectivity and potential fatigue-related errors associated with manual counting, ensuring a higher degree of consistency and objectivity across all tests.

This enhancement does not compromise food safety. For a pathogen like B. cereus, accurate enumeration is non-negotiable to ensure consumer protection. Because the model's performance (when using top lighting) is statistically equivalent to a trained professional, it can reliably determine if bacterial counts exceed established safety limits. By standardizing the validated imaging protocol, labs can adopt this technology to conduct faster, more consistent safety checks while upholding the rigorous standards required to protect public health. This frees up highly trained microbiologists from repetitive tasks to focus on more complex analyses, ultimately strengthening the overall food safety system.

Conclusions

This quantitative assessment successfully applied the ISO 7932 method for the enumeration of *Bacillus cereus* and compared it to the assessment of plates using the Reshape imaging device. The results of the study indicate that the automated imaging device demonstrates a high degree of correlation with the manual ISO 7932 method, but its accuracy is critically dependent on the lighting conditions. Specifically, the model achieved an exceptional 98.25% agreement with manual counts using a top light setting, whereas the agreement dropped to 87.72% with a bottom light setting.

This information is critical for validating the automated method for routine use in quality control. It confirms that when the standardized top light protocol is used, the automated system provides reliable data equivalent to the standard method, suitable for making critical decisions such as product release or initiating a food safety investigation. Conversely, it highlights the risk of generating inaccurate data if improper imaging settings are used, which could compromise consumer safety.
The use of an automated colony imaging system proved highly effective in standardizing the observation of agar plates. This automation improved the efficiency and accuracy of the analysis, providing an objective, reproducible method for enumerating presumptive *B. cereus* colonies, thereby reducing the chance of human error and improving the overall integrity of the results.