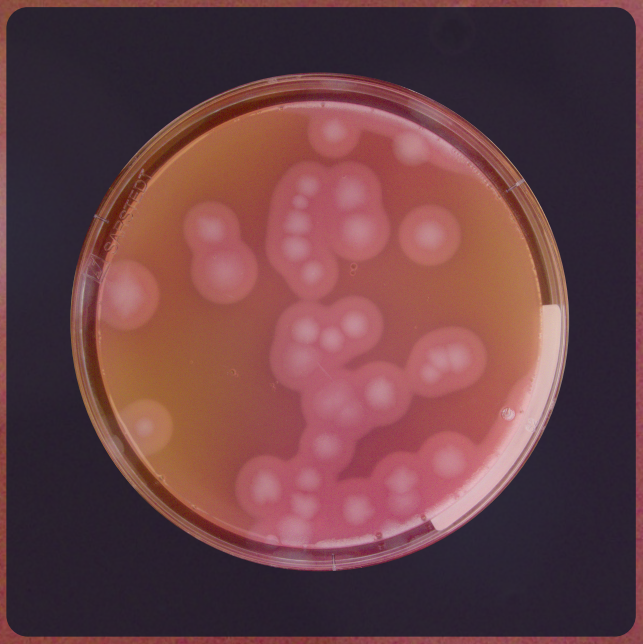
RESHAPE

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 validation study compared an automated colony counting method with manual enumeration for *Bacillus cereus,* using the internationally recognized ISO 7932 quantitative culture-based method as the reference standard.

Overall, Reshape's automated system demonstrated high accuracy and strong comparability to manual assessment, achieving 98.25% agreement in total colony counts.

In addition, the Reshape's automated imaging-based plate analysis streamlined the workflow by providing a consistent, objective approach to colony counting and morphology assessment. This will improve operational efficiency while supporting greater reliability and standardization of results.

Introduction & Background

Bacillus cereus is a genus of Gram-positive, rod-shaped bacteria belonging to the family Bacillaceae. It is a significant cause of food-borne 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: Two Bacillus species, *Bacillus cereus* NCIMB 9373 and *Bacillus subtilis* NCIMB 13061 (Sigma-Aldrich), was used to ensure the model could reliably differentiate between distinct morphologies and growth types. Both cultures were grown overnight in nutrient broth. A decimal dilution series was then prepared to obtain a range of dilutions suitable for colony counting.

Plating on Selective Agar: From each dilution, 100 uL was pipetted onto a selective Mannitol Egg Yolk Polymyxin (MYP) agar, and subsequently incubated at 30 ºC 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: Following incubation, agar plates were imaged using Reshape's automated colony counting and imaging system (Reshape Smart Incubator). This system captured high-resolution images of the plates under controlled lighting conditions, after which the integrated software automatically identified and and enumerated colonies based on predefined morphological features including size, shape, and color. On MYP agar, presumptive *Bacillus cereus* colonies typically appear 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. Automated analysis therefore enabled standardized and objective colony assessment, providing an objective and reproducible basis for calculating CFU/g.

Of the workflow described above, the study specifically focused on accurate colony enumeration using the Reshape platform, with parallel manual plate assessment performed to verify reliability.

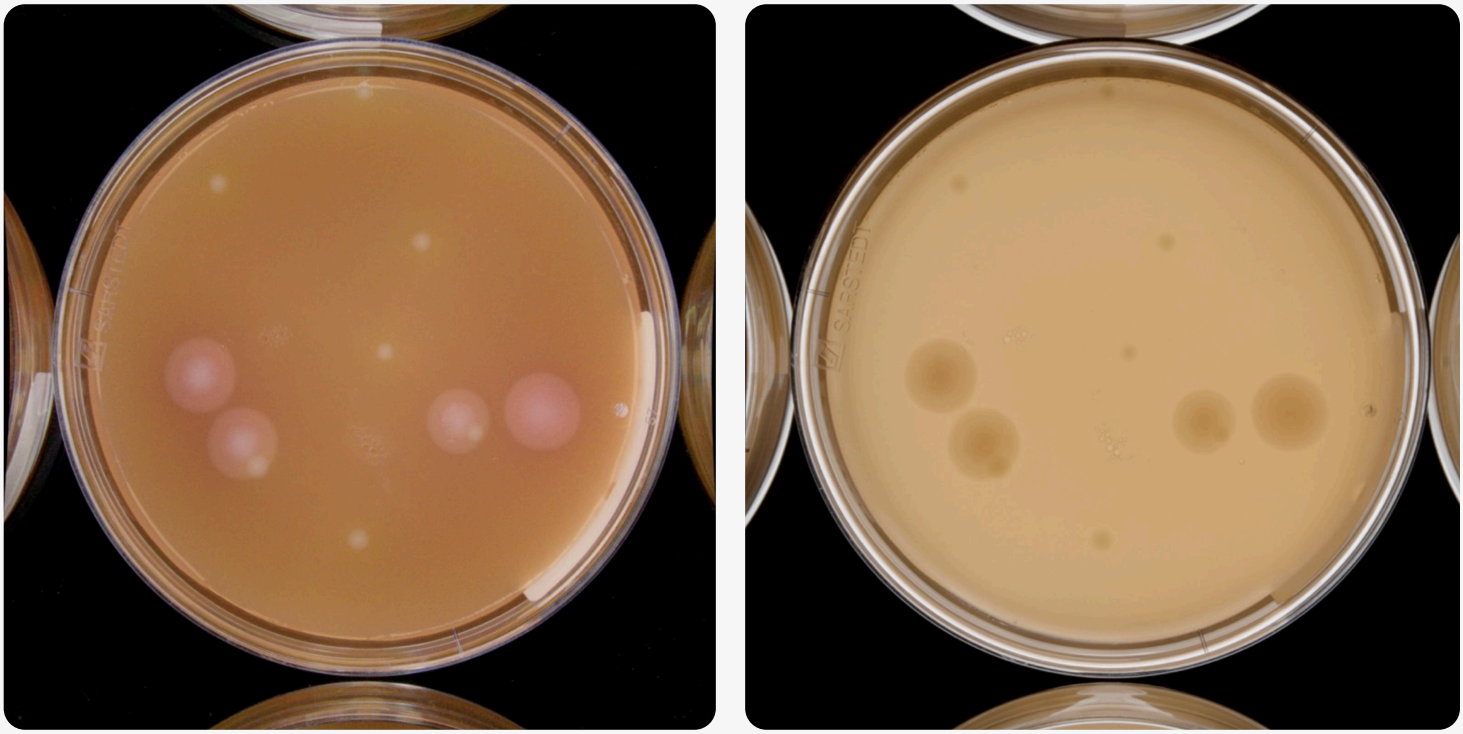
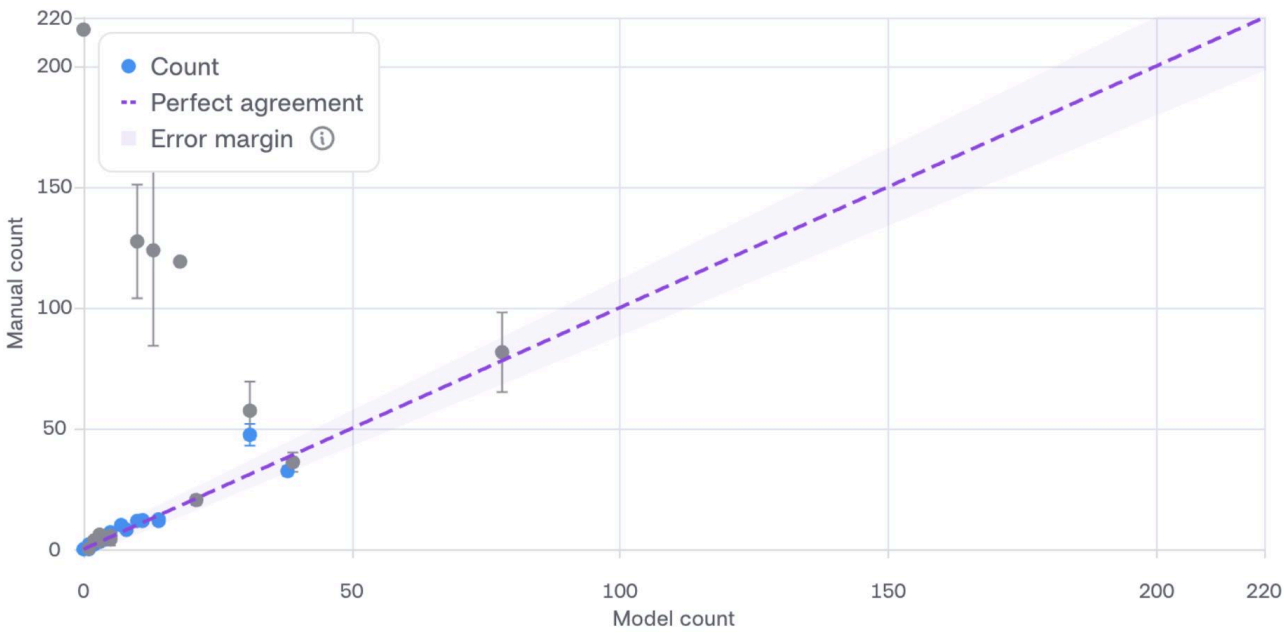


Figure 1: Image of randomly chosen MYP agar plate. Pink colonies are *B. cereus*, yellow are *B. subtilis*.
Left: Top light image | **Right**: Bottom light image

Results

A total of 114 images were analyzed with an overall agreement of 98.25% between manual and model colony counts (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.

		Model	
		TNTC	Not TNTC
Manual	TNTC	14	0
	Not TNTC	0	100

- **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 proved to be exceptionally effective, yielding a 98.25% agreement with manual counts. This high level of accuracy can be attributed to the top-light mode of the Reshape Smart Incubator - whereas the agreement was slightly lower when only bottom-light was used to compare counts. Overall, this 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.

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 Smart Incubator platform. The results of the study indicate that Reshape's platform demonstrates a high degree of correlation with the manual ISO 7932 method, with a 98.25% agreement score.

These findings are critical for supporting the validation of automated colony imaging as a routine tool in quality control laboratories. They indicate that automated analysis can provide reliable, consistent data suitable for decision-making in high-stakes workflows, including product release and food safety investigations. Importantly, automation also addresses several limitations of manual colony counting by standardizing plate observation and reducing dependence on subjective interpretation. By providing objective and reproducible colony enumeration, the Reshape's platform improves efficiency, minimizes inter-operator variability, and reduces the risk of human error, ultimately strengthening the integrity and traceability of ISO 7932 testing results.