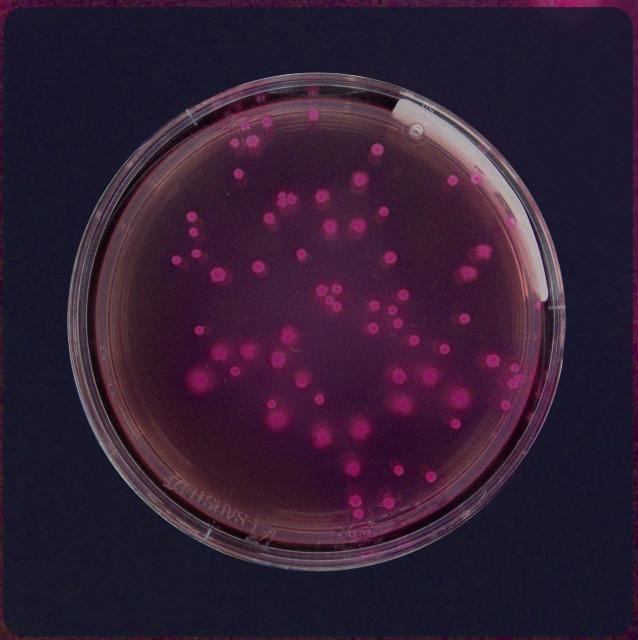
RESHAPE

# Method Validation Report

ISO 21528



Enumeration of *Enterobacteriaceae* following ISO 21528; Comparing manual and automated counts.

## Key Findings

Evaluation of the Reshape Smart Incubator for enumerating *Enterobacteriaceae* revealed that the automated counting system offers significant advantages over traditional manual assessment methods. The automated process demonstrated superior speed and accuracy, providing an efficient and reliable alternative for plate counting with **98.46%** agreement between manual and model counts. The assessment was performed using the internationally recognized ISO 21528-2 method, a quantitative culture-based technique.

The use of the automated imaging system for plate analysis significantly streamlined the workflow by providing a consistent and objective approach to colony enumeration and morphology assessment. This improved overall efficiency and supported reproducibility by reducing technician-to-technician variability, while delivering performance comparable to manual assessment. To further strengthen consistency and standardization in routine testing, QC laboratories should consider implementing automated plate assessment systems such as this, particularly where equivalent or improved performance relative to human evaluation can be demonstrated.

## Introduction & Background

The family *Enterobacteriaceae* is a large group of Gram-negative, rod-shaped bacteria commonly used as indicator-organisms in the food industry. Their presence and numbers can provide an indication of poor hygiene, inadequate heat processing, or post-processing contamination of a food product and pose a potential health hazard. While many members are harmless, the family also includes significant pathogens such as *Salmonella spp*. and *Escherichia coli*. Their general sensitivity to heat makes their presence in cooked foods a key indicator of potential safety issues.

The purpose of this study is to perform a quantitative assessment, or enumeration, of the number of viable *Enterobacteriaceae* cells following the ISO 21528-2 method done using an automatized system (the Reshape Smart Incubator) and compare it with manual counts and assessments by at least 3 trained technicians. This internationally standardized protocol provides a reliable and reproducible way to count these bacteria, which is crucial for assessing food safety and process hygiene. This assessment is necessary to provide proof of concept for our internal food safety protocols and to demonstrate compliance with relevant regulations.

## Materials, Methods & Protocols

The enumeration of Enterobacteriaceae followed the standardized ISO 21528-2:2017 protocol. This method is a multi-step process: sample preparation and dilution, surface plating on selective agar, incubation, colony counting, and biochemical confirmation.

**Sample Preparation and Dilution:** Known positive and negative strains (*Serratia marcescens, Escherichia coli* NCIMB 12805, and *Bacillus subtilis* NCIMB 13061) were pre-cultured and suspended in Brain Heart Infusion broth (BHI; Sigma-Aldrich) to prepare the initial inoculum. A decimal dilution series was then prepared from the suspension to obtain plates within a countable colony range for accurate bacterial load determination. In addition, selected cultures were combined to simulate mixed-microorganism samples representative of real-world conditions.

**Plating and Incubation:** From the selected dilutions, 100 µL was spread onto Violet Red Bile Glucose (VRBG) agar plates. Plates were incubated at 37°C for 24 hours. VRBG is selective for Enterobacteriaceae, as crystal violet and bile salts inhibit Gram-positive organisms, while glucose and a pH indicator support differentiation of fermenting colonies.

**Automated Imaging and Colony Counting:** Following incubation, the agar plates were imaged using Reshape's automated colony counting and imaging system. 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. Characteristic colonies of Enterobacteriaceae on VRBG agar typically appear pink to dark red, sometimes surrounded by a halo of precipitated bile salts. This automated process helped to standardize the assessment of colony morphology and provided an objective basis for the selection of colonies for further testing, minimizing potential human error and improving traceability.

This study focused on the plating and incubation workflow and subsequent colony enumeration to compare manual counting with automated imaging and colony counting. Automated results were benchmarked against manual colony counts performed independently by at least three trained personnel.

## Results

Automated imaging and colony counts were benchmarked against manual counts performed by trained technicians (Figure 1). Based on 130 images, the automated system achieved an overall CFU count agreement of 98.46%. The confusion matrix revealed 0% false positives and only 1% false negatives (correlating to 1 miscount).

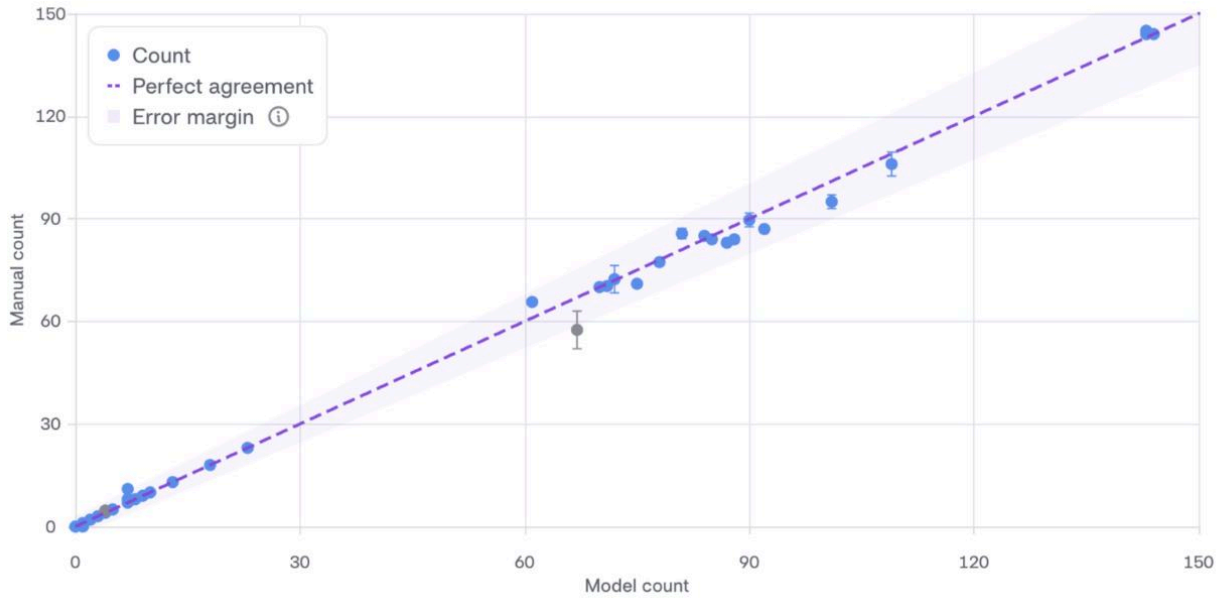



**Figure 1**: Correlation plot of the manual count and model counts: each blue point shows a CFU count for a given plate. The purple line indicates perfect agreement, and the purple area shows the error margin.


**Figure 2**: Confusion matrix of the model (automated counting) and the manual counts. TNTC = too numerous to count (above the given ISO threshold of 300 colonies).

## Discussion

The model demonstrated strong performance with minimal discrepancies (See figure 1 and 2). Validating the model on the same plates enabled a reliable, repeatable assessment of its performance. The model consistently outperformed the human manual count in cases of high inter-rater variability, which highlights the need for automated quality control methods.

## Conclusions

This quantitative assessment successfully applied the ISO 21528-2 method for the enumeration of Enterobacteriaceae and benchmarked manual plate counting against automated plate assessment using the Reshape Smart Incubator. The results demonstrate that automated imaging and colony enumeration can deliver performance comparable to, and in this dataset exceeding, manual counting. This is highly relevant for food microbiology QC, where accurate and consistent enumeration supports reliable batch release decisions, timely identification of deviations, and rapid initiation of corrective actions, including product holds and recalls when required.

The automated colony imaging system proved highly effective in standardizing plate enumeration and interpretation. By providing an objective, reproducible, and traceable approach to counting characteristic colonies, automation reduced technician-to-technician variability and the risk of human error while improving overall workflow efficiency. Collectively, these improvements strengthen data integrity and support a high standard of ISO-aligned food safety testing.