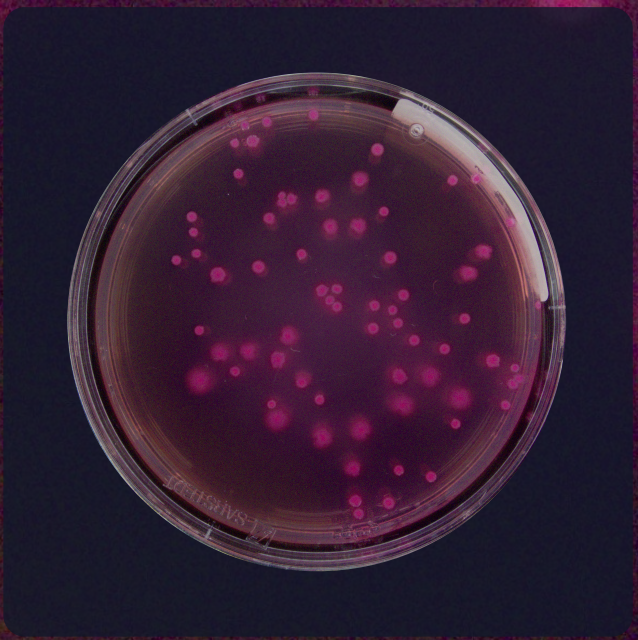
RESHAPE

Method Validation Report

ISO 21528



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

Key Findings

This section summarizes the key takeaways in a comparison between an automatized method and imaging device for the enumeration of *Enterobacteriaceae* as compared to the conventional way using manual counts & assessments. The assessment was performed using the internationally recognized ISO 21528-2 method, a quantitative culture-based technique.

The results show a 98.45% agreement between trained technicians and the automated counts and only one false negative (see figure 1 and 2).

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 and was on par with the manual assessments. For reproducibility and consistency, it is recommended that QC laboratories consider switching to assessment such as the one provided here, given they are on par or better with human assessments.

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 imaging device (RID)) 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: A specified quantity of pre-cultured known positive and negative strains, *S. marcescens* and *Escherichia coli* NCIMB 12805 as well as *B. subtilis* NCIMB 13061 were grown to create the initial suspension in brain heart infusion broth (BHI, Sigma-Aldrich). From this suspension, a decimal dilution series was prepared to ensure that the subsequent plates would yield a countable number of colonies, allowing for an accurate calculation of the bacterial load. Additionally, different cultures were also mixed to simulate real life samples with multiple different microorganisms present.

Plating and Incubation: From the selected dilutions, a 100 uL culture was transferred onto Violet Red Bile Glucose (VRBG) agar. The plates were incubated at 37°C for 24 hours. This selective medium contains crystal violet and bile salts to inhibit Gram-positive bacteria, while the presence of glucose and a pH indicator allow for the differentiation of lactose-fermenting Enterobacteriaceae.

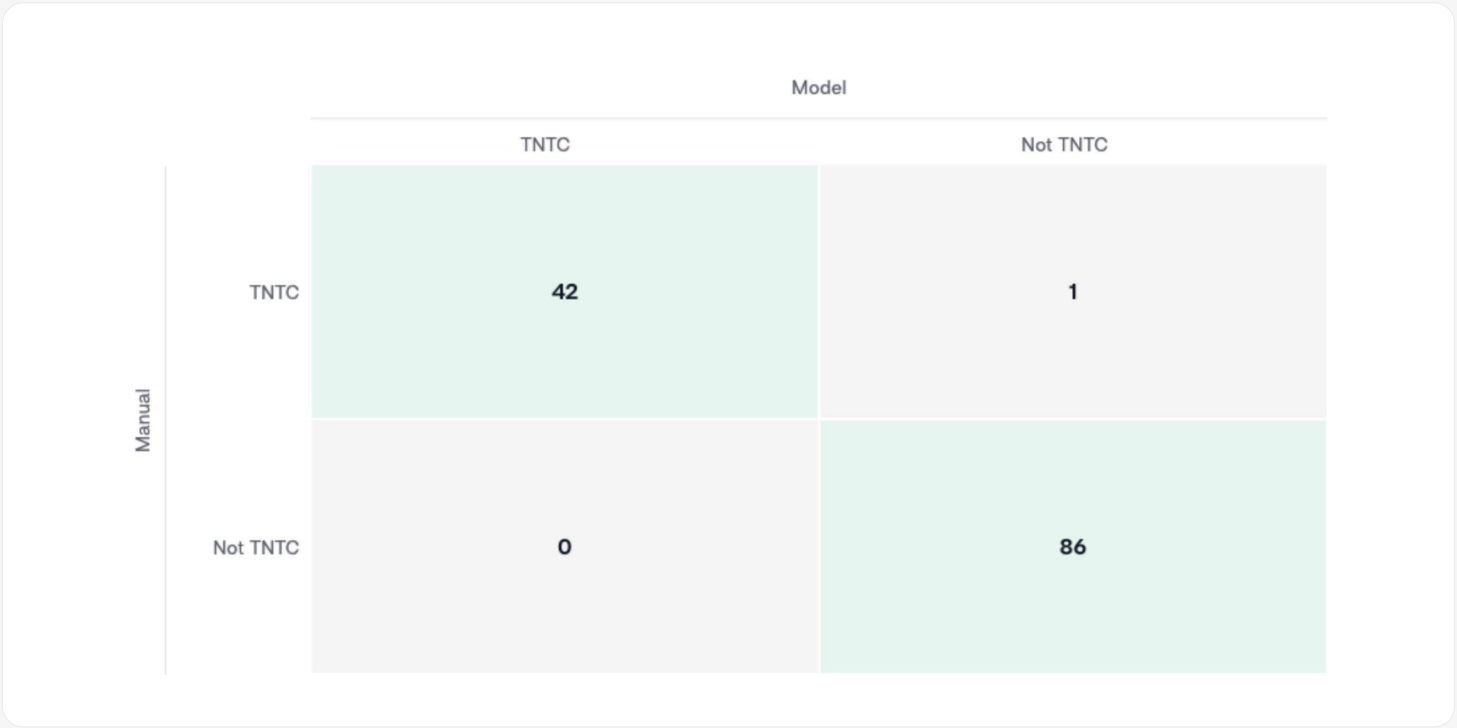


Figure 2: Confusion matrix of the model (automated counting) and the manual counts. TNTC: Too numerous to count (above the given ISO threshold of 300 colonies).

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 compared it to the assessment of plates using an automated imaging device (RID) and manual counts. The results of the study indicate that better performance can be obtained using the automated imaging. This information is critical for ensuring high quality of the data in food safety testing and ensure proper batch release and quick as well as correct recalls.
The use of an automated colony imaging system proved highly effective in standardizing the enumeration and assessment of agar plates. This automation improved the efficiency of the analysis and provided an objective, reproducible method for counting characteristic colonies, thereby reducing the chance of human error and improving the overall integrity of the results contributing to upholding a high standard for future food microbiological safety assessments.

Automated Imaging and Colony Counting: Following incubation, the agar plates were imaged using an automated colony counting and imaging system. 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. 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.

In this study the focus was on the plating and incubation as well as the automated imaging and colony counting, to compare the manual and automatized colony enumeration. The automated counts were compared to at least 3 counts done by trained personnel.

Results

Automated imaging and colony counts compared to the manual counted by trained technicians resulted in figure 1. The total agreement of the CFU counts was at 98.45%, based on a total of 129 images based on top-light images.

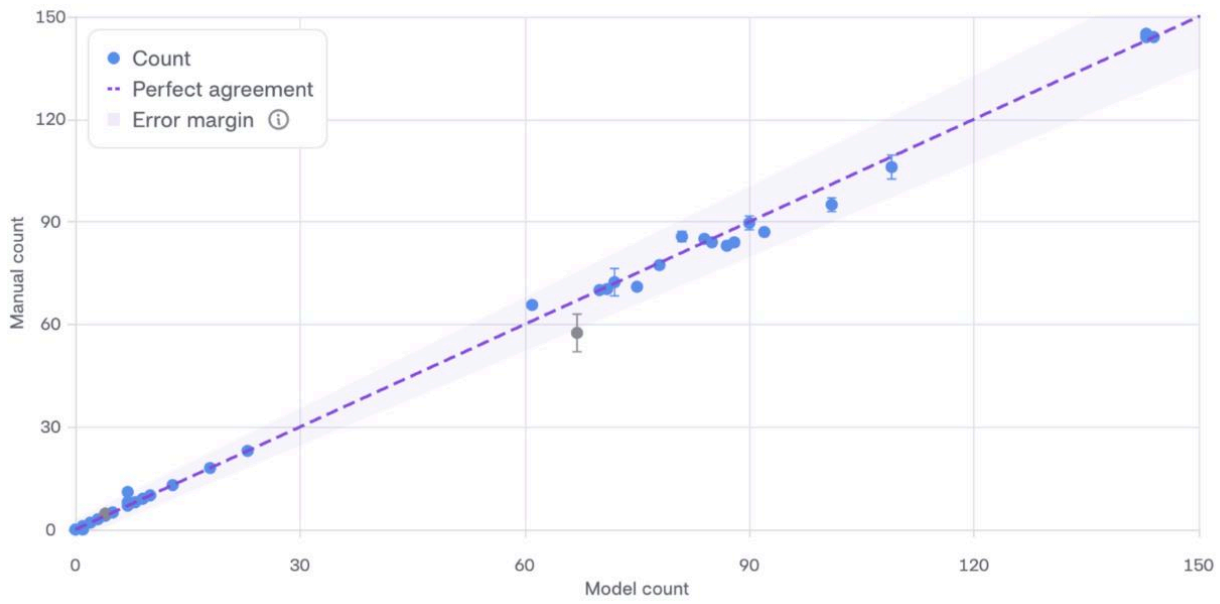


Figure 1: The manual count and the model count: Each blue point shows a CFU count for a given plate. The agreement between the model and manual count.