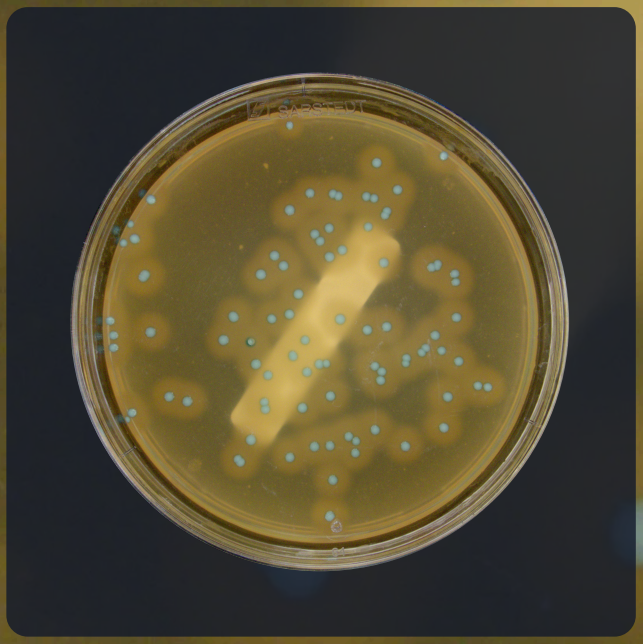
RESHAPE

Method Validation Report

ISO 11290-1



The aim of this validation is to demonstrate that Reshape Biotech's imaging technology and machine learning model performs in line with the ISO 11290-1 standard.

Key Findings

This validation study compared manual plate observations with the performance of Reshape's platform for detecting the presence of *Listeria spp*. Testing was conducted in accordance with the internationally recognized ISO 11290-1 method, a qualitative culture-based reference method, to benchmark assessments performed by trained personnel against an automated imaging and analysis system.

Overall, the results demonstrated a 95.83% agreement between Reshape's platform and manual evaluation. Use of the automated imaging system significantly streamlined plate reading and interpretation by providing a consistent, objective, and traceable approach to colony detection and morphology assessment. This improved workflow efficiency and supported reproducibility by reducing operator-to-operator variability in plate interpretation.

Introduction & Background

Listeria is a genus of Gram-positive, rod-shaped bacteria belonging to the family Listeriaceae. *Listeria monocytogenes* is the primary pathogenic species and is a major concern in food safety due to its ability to cause listeriosis, a serious and often fatal food-borne illness. The World Health Organization (WHO) estimates that listeriosis has a high hospitalization and mortality rate, making its detection critical for public health. Sources of contamination are varied but often include ready-to-eat foods, unpasteurized dairy products, and contaminated raw vegetables. *Listeria* is particularly resilient, able to grow in cold temperatures (refrigeration) and tolerating high salt concentrations, making it a persistent threat in food processing environments.

The purpose of this study is to perform a qualitative assessment of the presence or absence of *Listeria spp*. following the ISO 11290-1 method using an automated incubation and imaging system (Reshape Smart Incubator) and compare it to the manual performance of trained technicians and scientists. This internationally standardized protocol provides a reliable and reproducible way to detect this pathogen, which is crucial for ensuring food safety and public health. This assessment is necessary to provide a proof of concept for our internal food safety protocols and to demonstrate compliance with relevant regulations.

Materials, Methods & Protocols

The qualitative detection of *Listeria spp.* followed the standardized ISO 11290-1:2017 protocol. This method is a five-step process: non-selective pre-enrichment, selective enrichment, plating on selective agar, biochemical confirmation, and serological confirmation. Of the above-mentioned steps, the focus in this study was on plating on selective agar as well as a manual assessment of the plates to ensure reliability of the Reshape Smart Incubator.

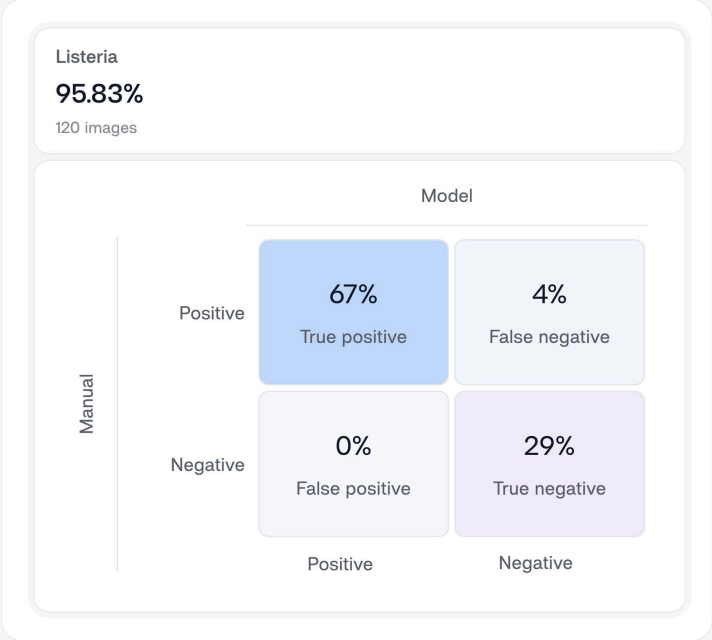
Prepping cultures: *Listeria monocytogenes* was inoculated into brain heart infusion broth and grown overnight at 37°C. It was then diluted into the countable range and plated in combination or alone with other known microorganisms to try and simulate background.

Plating on selective agar: After selective enrichment, the strain was streaked onto a chromogenic *Listeria* agar (CLA). For this study, chromogenic *listeria* agar was used and was incubated at 37 °C for 24-48 hours. Suspected colonies of *Listeria spp.* were identified based on their characteristic appearance. On CLA, colonies are typically a greenish-blue, with or without an opaque halo.

Automated imaging: The plates were then imaged using an automated colony counting and imaging system (Reshape Smart Incubator). This system captured high-resolution images of the plates under controlled lighting conditions. The images were then analyzed by the machine's software to automatically identify presence and absence of colonies based on their size, shape, and color. 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.

Results

Based on a total of 120 images, over 7 individual assays, and using 3 different manual counters, there was a total performance score of 95.83%. Furthermore, the model classified 0 false positives, 5 false negatives, 80 true positives (and countable plates) and 29% true negative plates (FP=0%, FN=4%, TP=80%, TN=29%). (see Figure 1).



- **Figure 1 (right)**: Confusion matrix of the model (automated counting) and the manual counts. Positive indicates presence of a blue/green colony and presence of *Listeria spp*. Negative indicates no presence of such colonies.

Discussion

Although the model resulted in 5 cases of false negatives, it generally showed very good performance, with agreement in 95.83% of the cases. Undoubtedly, adding more training data will improve the model's performance even further, resulting in fewer or no false negatives. Automizing this in the quality control sector of food industry will ensure reproducible, reliable results, ensuring the safety for consumers as well as saving time and economic gain for larger enterprises.

Conclusions

This qualitative assessment successfully applied the ISO 11290-1 method for the detection of *Listeria spp*. and benchmarked manual plate interpretation against automated assessment using the Reshape Smart Incubator. The results demonstrate that automated plate analysis can provide a reliable and standardized alternative to manual reading, with strong potential to support routine QC workflows. From a food safety perspective, accurate and consistent detection of *Listeria spp*. is critical for timely product release decisions, early identification of contamination events, and rapid initiation of corrective actions, including product holds or recalls when required.

The use of an automated colony imaging system proved highly effective in standardizing agar plate observations. Automation improved workflow efficiency and provided an objective, reproducible, and traceable method for identifying presumptive *Listeria* colonies. This reduces operator-to-operator variability and the risk of human error, thereby strengthening overall data integrity and confidence in results.

Reshape continues to optimize model performance with a particular focus on minimizing false negatives, which is essential in pathogen detection workflows. Long-term development aims to extend automated analysis across both ISO 11290-1 (detection) and ISO 11290-2 (enumeration), enabling broader coverage of *Listeria* testing requirements within QC laboratories.