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 section summarizes the key takeaways from the analysis of agar plates for the presence of *Listeria spp*. The assessment was performed using the internationally recognized ISO 11290-1 method, a qualitative culture-based technique and done to compare manual counting by trained personnel with an automized imaging system.

The results show a 95.83% agreement between the model and manual counts. 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.

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 in 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, a loopful from the Fraser broth 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. Suspect colonies of *Listeria spp.* on the agar plates were identified based on their characteristic appearance. On CLA typical colonies are greenish blue with or without an opaque halo.

Automated imaging: 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 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 120 images, 7 jobs and using 3 different individual manual assessments of plates, the following confusion matrix was obtained (see Figure 1).

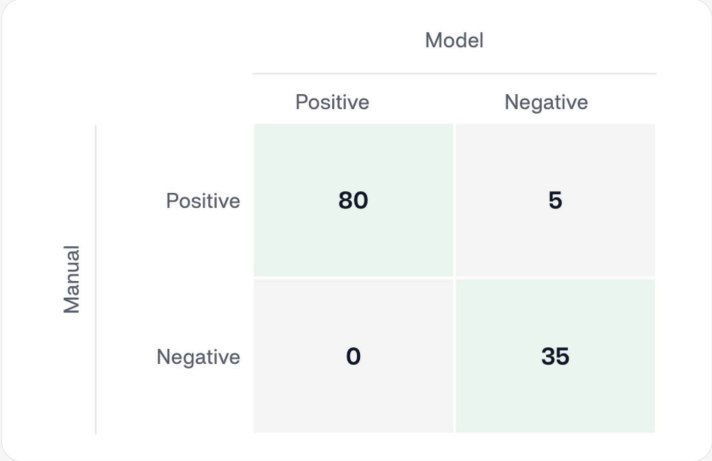

Figure 1: Confusion matrix of the model (automated counting) and the manual counts. Positive indicates presence of a blue/green colony and presence of *Listeria spp*. Negative indicates no presence of such colonies.

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 for the model will improve this 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 compared it to the assessment of plates using the Reshape Smart Incubator. The results of the study indicate that automized plate assessment has the potential to replace manual assessment of plates, as soon as the amount of false negatives are lowered. This information is critical for [explain the implications of your findings, e.g., product release, a food safety recall, or a process improvement initiative].

The use of an automated colony imaging system proved highly effective in standardizing the observation of agar plates. This automation improved the efficiency of the analysis and provided an objective, reproducible method for identifying presumptive *Listeria* colonies, thereby reducing the chance of human error and improving the overall integrity of the results.

Ongoing improvement of the model is being done with the long term goal to cover both ISO-11290-1 and ISO-11290-2 (quantification of *Listeria spp*. in samples).