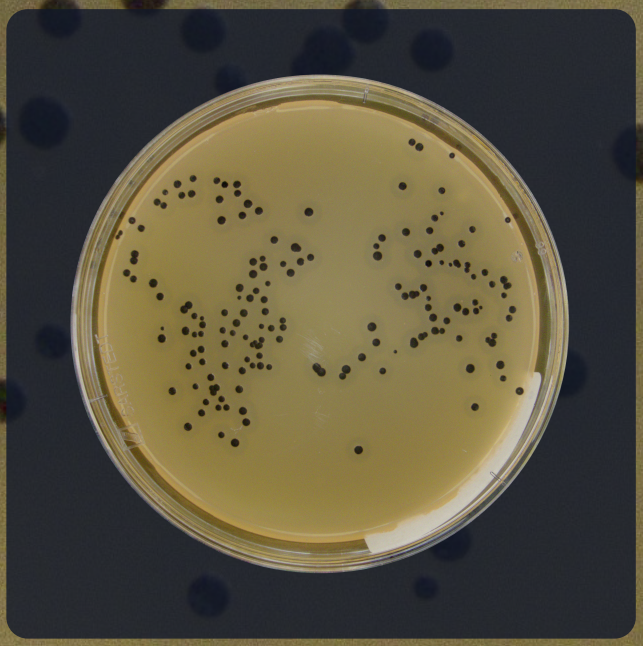
RESHAPE

Method Validation Report

ISO 6888



Comparison of manual and automated counts generated according to ISO 6888; Enumeration of coagulase-positive *Staphylococci*

Key Findings

This section summarizes the key takeaways from a comparison between the manual counts by trained personnel and an automated imaging system (Reshape Smart Incubator). The focus here was on enumeration of coagulase-positive *Staphylococci*. The assessment was performed using the internationally recognized ISO 6888 method, a quantitative culture-based technique.

The results show 100% agreement using bottom light setting, whereas top light resulted in a 98.85% agreement.

Based on these findings, it is recommended that laboratories consider switching to automated imaging systems, as it allows for saving time as well as money, whilst not compromising the safety of the consumers or quality standard of the data. 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

Coagulase-positive *Staphylococci* are a group of Gram-positive, spherical bacteria that are a major cause of food poisoning. The most significant species within this group is *Staphylococcus aureus*. These bacteria are a particular concern in food safety because they produce heat-stable enterotoxins, which, even if the bacteria themselves are killed by cooking, can remain in the food and cause illness. Symptoms of staphylococcal food poisoning can include nausea, vomiting, and abdominal cramps. Sources of contamination are often linked to human contact, as these bacteria are commonly found on the skin, in nasal passages, and on open wounds. Contamination typically occurs through improper hygiene during food handling.

The purpose of this study is to perform a quantitative assessment, or enumeration, of the number of viable coagulase-positive *Staphylococci* following the ISO 6888 method. 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 10^4 CFU/g is generally considered a potential health risk, making enumeration necessary to demonstrate compliance with relevant regulations.

Materials, Methods & Protocols

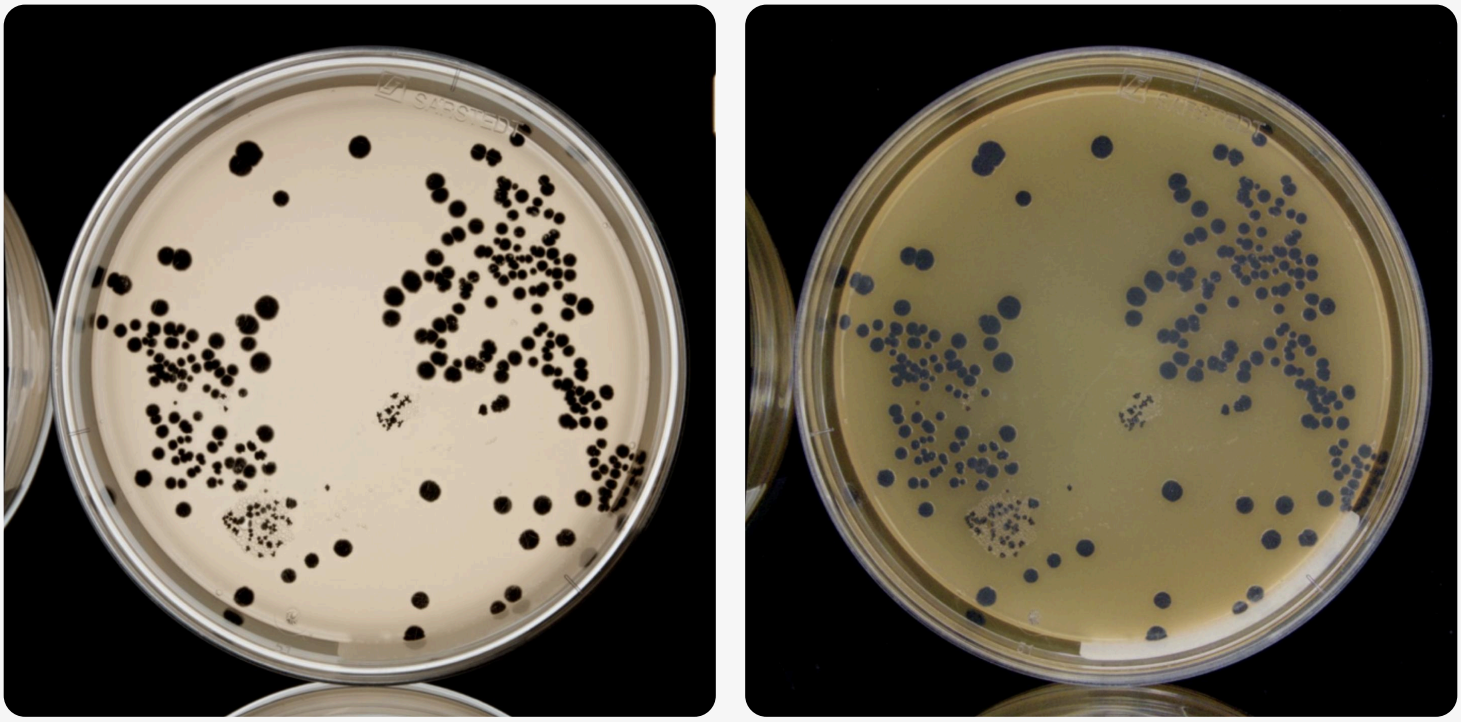
The enumeration of coagulase-positive *staphylococci* followed the standardized ISO 6888:2021 protocol. This method is a three-step process: sample preparation and dilution, plating on selective agar, and confirmation of presumptive colonies.

Sample Preparation and Dilution: A specified quantity of the sample pre-cultured *Staphylococcus aureus* DSM799 and *S. aureus* NCIMB 13062 (grown in brain heart infusion broth, Sigma-Aldrich) was diluted into the countable range.

Plating on Selective Agar: From each dilution, 100 uL aliquots were transferred unto a selective and differential agar, Baird-Parker agar, and then incubated at 37 °C for 24-48 hours. The agar contains tellurite and egg yolk, which help inhibit competing bacteria and aid in the identification of presumptive colonies.

		Model	
		TNTC	Not TNTC
Manual	TNTC	10	0
	Not TNTC	0	77

- **Figure 2**: Confusion matrix for bottom light setting.



- Figure 3: Photo of *staphylococcus aureus* positive plate. Black colonies indicate S. aureus.
  Left: using bottom light setting | **Right**: using top light setting

Automated imaging and Counting: 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 and count colonies based on their size, shape, and color. On Baird-Parker agar, presumptive coagulase-positive *Staphylococci* colonies typically appear black or dark grey with a shiny surface, often surrounded by a light-colored halo or clear zone. This automated process helped to standardize the assessment and counting of colonies, providing an objective and reproducible basis for calculating CFU/g. Trained personnel counted plates, resulting in a count or a too numerous to count (TNTC) (>250 colonies) marker being added to each individual plate.

Of the above-mentioned steps, the focus in this study was on the accurate enumeration of colonies using the Reshape Smart Incubator and manual assessment of the plates to ensure reliability.

Results

Based on a total of 87 plates, with 3 individual manual evaluations, there was a total agreement between the model and the manual counters of 98.85% using the top light setting and 100% from the bottom light setting (see Figure 1 and 3). Confusion matrix for the bottom light setting can be seen in Figure 2, with no false positives or false negatives for this given setting.

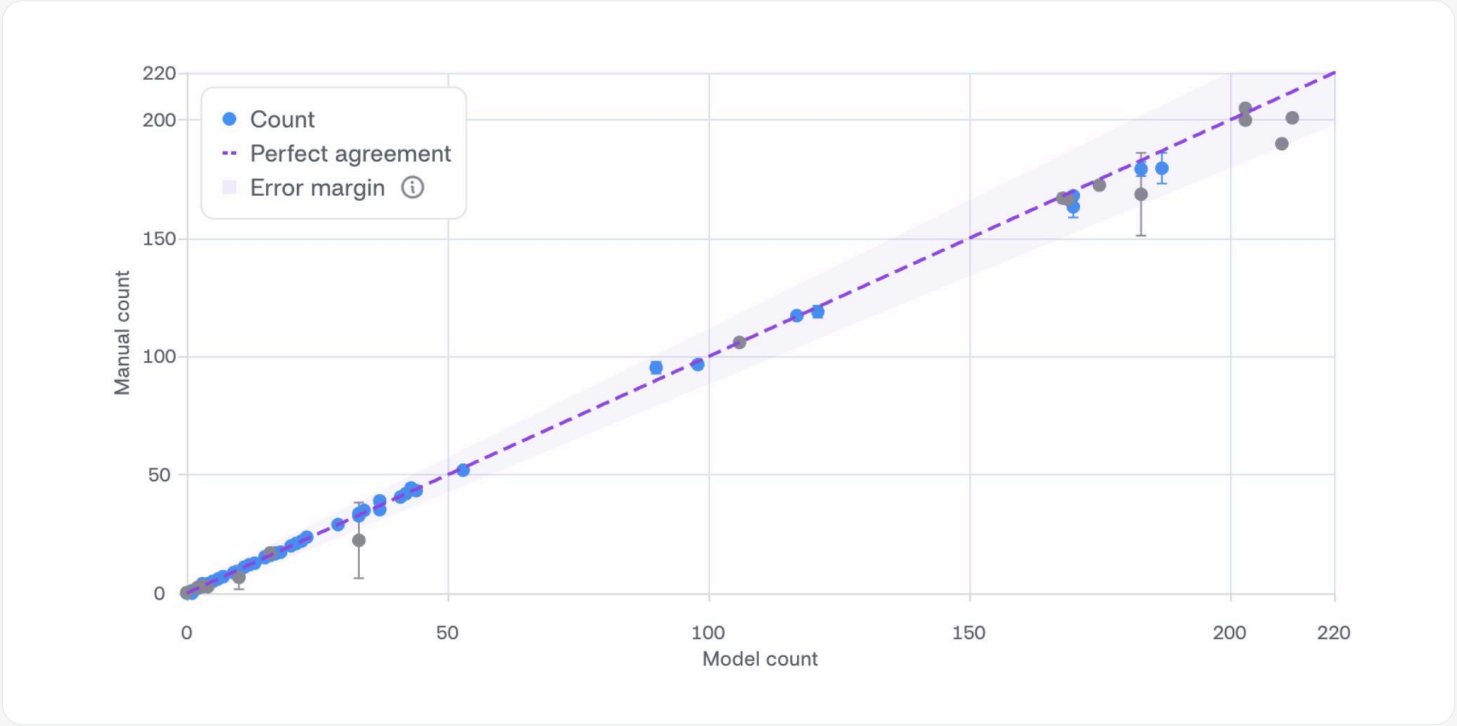


Figure 1: The alignment plot between manual counts and the model counts, with standard error indicated as the purple area. Grey data points indicate a manual disagreement large enough to warrant removal from statistics.

Discussion

The difference in agreement between bottom and top light setting illustrate that there is a lot of options for optimization when choosing which setting to use for each method/model. Overall, the model performed very well using both bottom and top light, but bottom light slightly better than that of top light potentially due to the colony morphology and color using the Baird-parker agar medium. Although further testing will continue to reaffirm the quality of automated colony counts, especially those with very hard to assess plates and “edge”-cases, this is a very promising initial performance.

Conclusions

This quantitative assessment successfully applied the ISO 6888 method for the enumeration of coagulase-positive *staphylococci* and compared it to the assessment of plates using the Reshape imaging device. The results of the study indicate that automated colony counts can perform as well, if not even better, then humans for this given scenario. This information is critical for ensuring product safety, release and recalls.

The use of an automated colony imaging system proved highly effective in standardizing the observation of agar plates. This automation improved the efficiency and accuracy of the analysis, providing an objective, reproducible method for enumerating presumptive *staphylococci* colonies, thereby reducing the chance of human error, improving the overall integrity of the results and releasing time of scientist to conduct other studies.