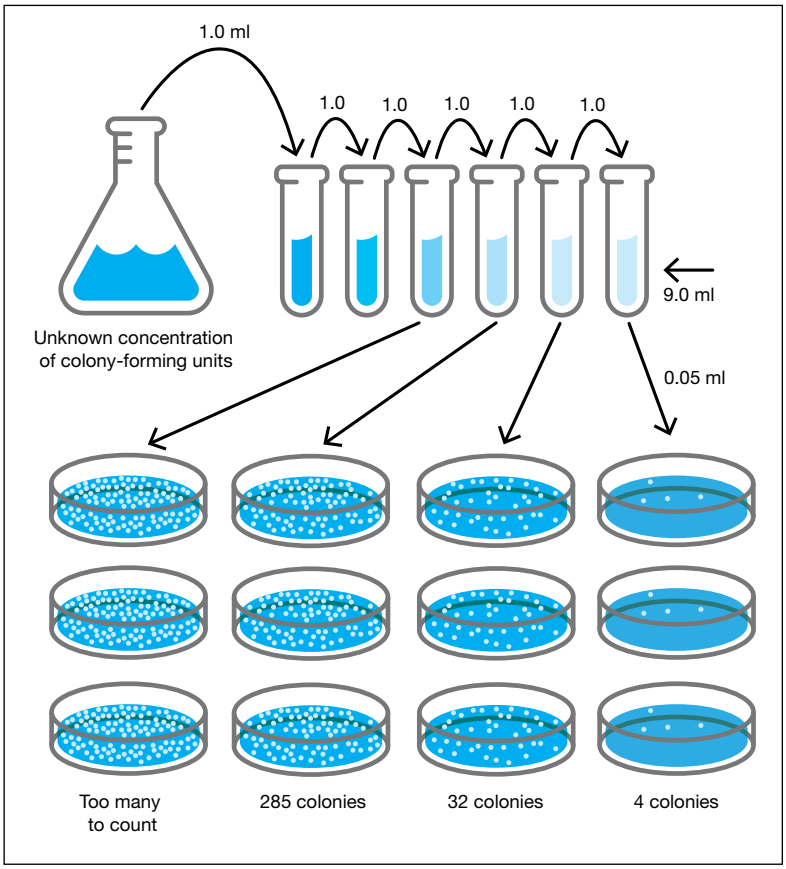
Comparing Total Viable Counts from Traditional Plates with MicroSnap™ Values

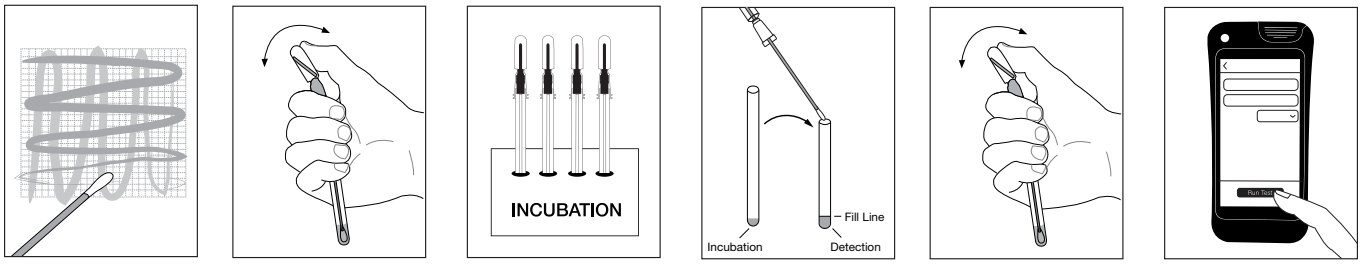
Introduction

Culture-based methods involve a test sample that is placed on a nutrient agar and incubated for several days. The result is expressed as a colony forming unit (CFU), defined as an "estimate of viable cells." This uniquely variable CFU is neither precise nor accurate and often results in an unfair comparison of novel methods to the plate count and unreasonable expectations of alternative enumeration methods. Ease of use of the methods has been improved by pre-packing (for example, pre-poured agar plates and dip slides), convenience dry packaging (self-diffusing dry media in film), and all-in-one swab devices with chromogenic enrichment broth (for example, InSite™ *Listeria* and InSite™ *L. mono* Glo with fluorescent indication of *L. monocytogenes*). Nevertheless, there are several steps in these methods where additional variation can arise. First, to obtain the optimum number of colonies for counting (30-300), dilutions of the initial sample must be made. The distribution of microbes is not uniform within any samples, so each series of dilutions can lead to different CFU counts. Using mathematical models to measure the confidence of these results, there is a wide range of "true values" which can be seen, even when the confidence limit is set at 95%.



MicroSnap™ can detect a variety of organisms using convenient disposable devices and a smartphone-like multifunctional instrument that can be used to test products, liquids, and surfaces. Trending the results from screening methods enables many more samples to be tested thus increasing the scope and confidence of the surveillance activity and management of quality and risk. Monitoring and trending of results in their own unit of measurements is more meaningful and useful than converting them back to a variable CFU equivalent.

Simple, Rapid Method - Swab, Incubate, Detect



Additional Benefits

Reduce Materials

No serial dilutions to make

Eliminate Lab Costs

Reduce lab fees

Reduce Inventory Costs

Shorter product hold time

Fewer replicates required

No expensive overnight shipping

Less inventory in warehouse

In addition, MicroSnap™ utilizes a broth for growth and recovery, providing many advantages over culture plate methods. Advantages include rapid exponential log phase growth and improved injured-cell recovery. Agar-mediated growth has a slower transition from lag to log phase and requires additional energy expenditure for cells to grow in an atypical environment. What follows is a comparison study demonstrating the accuracy of MicroSnap™ devices compared to standard plate counts.

Equipment, Supplies & Reagents

- Culture plates (TSA) or film
- Plate spreader
- Pipette tips & pipettor
- MicroSnap™ Total
- Incubator (30 +/- 0.5°C)
- Hygiena™ Digital Dry Block Incubator
- EnSURE™ (or EnSURE™ Touch)
- Bacterial culture (*E. coli*)
- Dilution buffer (TSB, for serial dilutions)
- Sample diluent (for food homogenates)
- Stomacher bags (for food homogenates)

Methods

Sample Collection/Preparation

First, at least three bacterial levels (CFU/mL) should be used when evaluating any system. The three levels should be logs apart – the recommended levels (CFU/mL) are:

Low = 10^2 Medium = 10^3 High = 10^4

For smaller, routine testing, only one level is typically used: 10^3 CFU/mL.

Cultures were prepared in TSB and serially diluted (10-fold serial dilutions) to achieve the approximate concentrations noted above to generate low, medium, and high-level sample load.

MicroSnap™ Incubation

Culture samples were diluted to the appropriate CFU levels in TSB buffer and 1 mL aliquots were placed in the MicroSnap™ Total Incubation Devices (MS1-TOTAL). The Incubation Device was activated by holding the swab tube firmly and using a thumb and forefinger to break the Snap-Valve by bending the bulb forward and backward. The bulb was then separated from the tube to relieve internal pressure, and the bulb was squeezed to flush all media into the bottom of the device tube. After reattaching the bulb to the tube, the device was shaken to gently mix the sample with the broth. The device was incubated at 30 ± 0.5°C for 7 hours ± 10 minutes.

MicroSnap™ Detection

Following incubation, the enriched sample was transferred from the Incubation Device to the MicroSnap™ Detection Device. The Detection Device was activated by holding the swab tube firmly and using the thumb and forefinger to break the Snap-Valve by bending the bulb forward and backward, followed by squeezing the bulb three times to release all liquid to the bottom of the swab tube. After gentle shaking, the device was inserted into the EnSURE™ instrument and results were captured after 15 seconds. Results, in RLUs, were captured on the instrument for later analysis and reporting. (For EnSURE™ Touch, results can be read in 10 seconds with no need to convert results - CFUs are calculated in the device and displayed.) In addition, results are stored for later access via the instrument or SureTrend™ Cloud software for analysis.

Standard Culture Plating/Incubation

At T=0, the same culture samples (dilutions) as above were used for inoculating TSA plates; 100 µl aliquots were rapidly removed and spread onto TSA plates. Plating was done in triplicate to ensure accuracy of colony counts. Note: To cover variation in the sample, a minimum of five (5) replicates is recommended by BAM, FDA and AOAC for equivalence testing. Plates were incubated at 30-32°C for 24 hours.

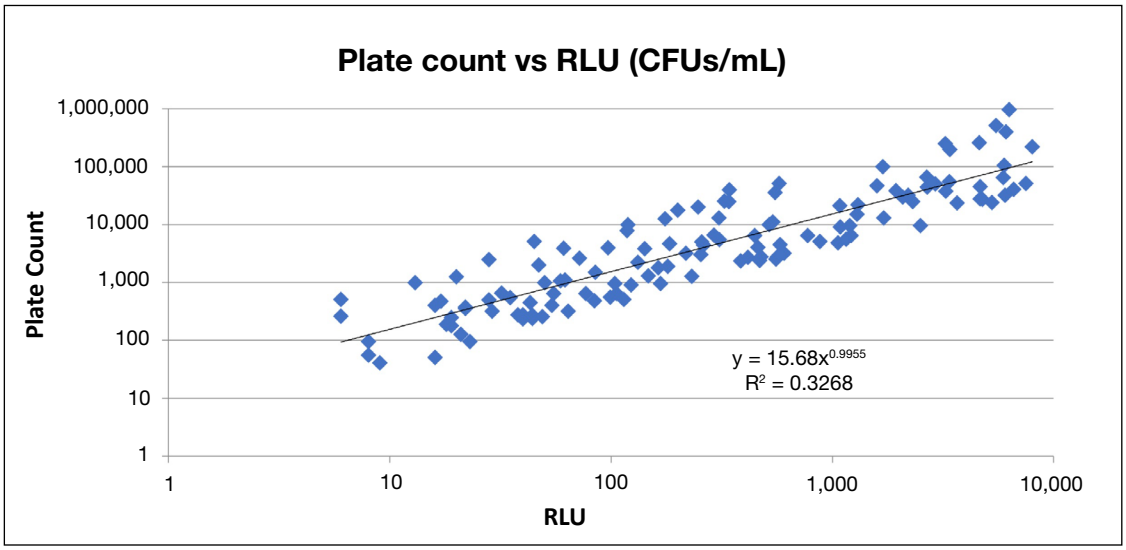
Culture Plate Detection

Following incubation for 24 hours, colonies on TSA plates were counted for each sample dilution. Triplicates were averaged to determine the best overall count for each dilution. Results were manually captured in a spreadsheet for analysis and converted to CFUs based on culture dilutions/concentrations.

Results & Discussion

Comparison

Data for plate CFUs and MicroSnap™ results were graphed to compare linearity and consistency (Figure 1). Results demonstrate linearity of data when comparing the values with a normal expected distribution pattern. In addition, a correction factor to convert RLU to CFU was calculated. This value will be unique for each matrix tested, but in this case, it was 15.68. This correction factor means that RLUs can be used as a measure of bacterial counts in any sample analyzed when using the EnSURE™ system. (For EnSURE™ Touch, no RLU to CFU conversion is needed - results, in CFUs, can be used directly for analysis.)



Conclusions

These results indicate that MicroSnap™ devices can be used to calculate microorganism load in a sample without the need to plate and calculate estimated CFUs. Based on growth time, recovery, and results analysis, MicroSnap™ provides a number of advantages including only 7 hours to results (versus at least 24-48 hours). Additionally, it provides accurate, linear values spanning more than 3 logs, which is more accurate than plating where counts are often too many to count at high concentrations and inaccurate at very low levels due to statistical variation.

In conclusion, MicroSnap™ offers a simple, rapid, and cost-effective alternative to traditional plate counts. Tests are easy to perform - just swab, incubate, and then detect using EnSURE™ (or EnSURE™ Touch). In addition, MicroSnap™ provided results in a single shift versus plating methods which take a minimum of 24-48 hours for results. Plates must also be counted and averaged to provide an estimate of the CFUs present. To ensure accuracy, multiple plates must be tested for each serial dilution, incurring extra material and labor costs. There is also the potential for human counting error while MicroSnap™ provides an easy to read value that is also saved on the system for later analysis. In conclusion, MicroSnap™ results are rapid, more accurate and represent true bacterial counts, without having to conduct physical counting from multiple plates.