

# What do your microbiology test results really mean?

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It is generally recognised that no measurement is perfect due to the uncertainties arising from many factors.

This is even more complex in microbiology due to the particulate nature of bacteria and their ability to reproduce by binary fission. This results in localised pockets of higher concentrations of bacteria where each individual represents a unique variable entity.

Consequently, there is an uneven distribution of microbes even in well mixed samples which creates problems not only for test methods but sampling in order to get a meaningful result for the batch or consignment. The working group of the International Laboratory Accreditation Cooperation states 'it is virtually impossible to know the exact microbial concentration in any sample, natural or artificial'.

The vagaries of microbial measurement are often conveniently forgotten resulting in unreasonable expectations of both laboratories and the methods deployed.

So what do microbiological test results actually mean? What can we expect and does it apply equally to both product and environmental samples?

Food products are generally well controlled and manufactured to a consistency where microbial specifications are established. Conversely, there are no agreed standards for microbes for environmental surface samples that are less controlled and more variable.

Each facility is expected to do 'the best it can' for monitoring cleaning processes due to the uniqueness of each manufacturing facility. Most food manufacturers strive for high hygienic standards to protect their products, consumers and brands.

## Sources of variation

The unit of measurement for the enumeration of microbes is a colony forming unit (CFU) derived from plate count methods. This technique has remained largely unchanged since the pioneering days of Pasteur and Koch in the 19th Century. It is defined as 'a rough estimate of the number of viable bacteria or fungal cells in a sample' because it relies on the false assumption that each colony is derived from a single bacterium.

Microbes exist as clumps or chains and are often difficult to separate into single cells. Hence, there is a large natural variation in CFU results from plate counts particularly if single replicate samples are used and single tests are conducted.

There are several steps in this method where additional variation can arise. To obtain the optimum number of colonies for counting (30-300), dilutions of the sample have to be prepared.

Since the distribution of microbes in the sample is not uniform, each series may produce different numbers of CFUs. More variation occurs if there are fewer than 30 colonies per plate.

The normal expected variation from plate counts is typically 0.2-0.5 Log units, hence for a target 1000 cfu (Log 3.0) this means the actual result can be anywhere between 300-3000 and still be correct.

Such variation is well known and regularly examined among accredited testing laboratories. Under the Proficiency Testing (PT) scheme laboratories using standard methods

are provided with several replicates of stable, homogenous samples.

These are examined by the participants and are expected to show a 10 fold (1 Log) variation in results between laboratories. Sometimes this variation is exceeded by >2 Logs for plate counts such as coliform and Enterobacteriaceae.

Mathematical models can be applied to gauge the quality or confidence of the results.

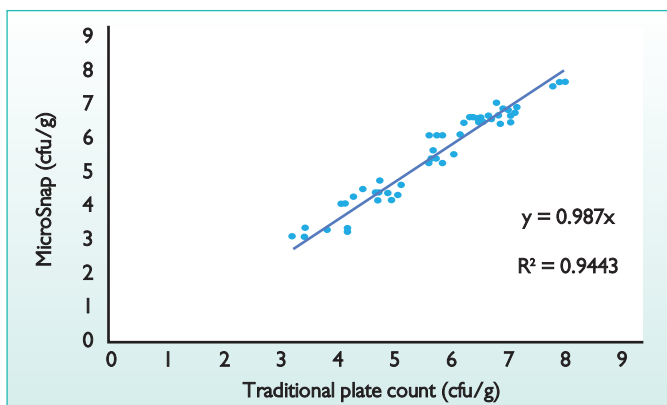
## Measurement uncertainty

Measurement uncertainty is used to calculate the dispersion of the values attributed to a measured quantity. The uncertainty reflects the doubt in the result of the measurement. In the case of a standard method for

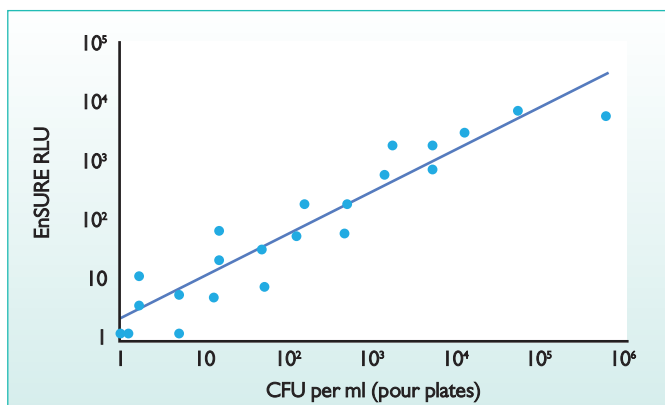
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**Fig. 1. Comparison of MicroSnap Total with traditional plate counting method.**



**Fig. 2. The detection of low level Enterobacteriaceae using MicroSnap EB.**



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TVC in milk this has been calculated as 39.6% i.e. the 'true value' of the obtained result (within 95% confidence limits) can be expected in a range  $\pm 39.6\%$  of the result.

This means that the actual value is not known for certain, and for a sample expected to contain 10,000 CFU the value lies somewhere within the range 6000-14000 CFU on 95% of occasions but can also be outside this range 5% of the time.

Microbial stress and survival also affects test results. In dry, nutrient poor environments microbial viability declines rapidly in a matter of hours such that there is a large variation in observed contamination levels. The literature shows examples of total counts  $< 2.5.0 \times 10^5$  CFU/cm<sup>2</sup> with *E. coli* detected on 15.8% of the samples with a range of 0.2-12 CFU/cm<sup>2</sup>.

Inoculating surfaces is known to result in large losses of viability with hugely variable residual contamination levels.

Inoculating surfaces with a suspension containing one million bacteria can give a final residual contamination of 10-100 bacteria with 100-500 fold variation between five replicates from the same inoculum.

Resuspending and recovering contaminants from the surface swab into a diluent prior to testing also introduces another source of varia-

tion. Therefore, great care needs to be exercised when assessing the results of environmental tests and also when comparing methods for the assessment of environmental contamination.

Accordingly, the enumeration of microbes in environmental samples yields little meaningful information. A qualitative approach is more appropriate.

General guidelines have been suggested by some authors and auditors, for example acceptable  $\leq 80$  CFU/cm<sup>2</sup> and unacceptable  $\geq 1000$  CFU/cm<sup>2</sup>.

Trend analysis is more suitable and gives better management information about risks and emerging problems. The benefits of regular testing, preferably with a simple method giving rapid results for prompt corrective actions, are well established.

### Alternative methods

MicroSnap is a simple two-step test procedure with a total time to result of seven hours and 15 seconds. The sample can be a surface swab, a 1ml liquid sample or a food suspension that is mixed with a proprietary enrichment broth in an all-in-one device.

After incubation for seven hours at 37°C a 0.1ml aliquot is transferred (using the device itself) to a specific

end detection device. Using a rapid bioluminescence method for measurement together with the EnSURE luminometer, results are available in 15 seconds. MicroSnap is formulated in different ways to measure a variety of bacteria. The test devices are currently available for Total Counts, Enterobacteriaceae, coliforms and *E. coli*.

The output of MicroSnap is directly related to inoculum size, that is the greater the number of bacteria the shorter the time to detection.

Typical results for Total and Enterobacteriaceae (Figs. 1 and 2) show excellent agreement and a high coefficient of correlation ( $> 0.90$ ) when compared with traditional plate counts.

The dynamic range of the single test device is 10-10,000 bacteria per ml (or swab) thus negating the need for serial dilutions saving labour, materials and time.

Conversely, a shorter detection time can be set according to the desired specification. For example, 100 Enterobacteriaceae can be detected in five hours.

MicroSnap's major advantage is that all viable bacteria collected on the swab are cultured and detected within the system. This permits maximum recovery and minimal losses.

A study of 300 surface samples showed an 89% agreement with the

traditional plate count methods for both Total Counts and Enterobacteriaceae and the limit of detection was calculated as 50-100 CFU per swab (10 x 10cm), or  $\sim 1$  CFU/cm<sup>2</sup>. In a small proportion of cases (7%) the results did not match. Samples were positive when tested with MicroSnap and negative when tested with traditional methods. In contrast, 4% of samples were detected positive with traditional methods and negative with MicroSnap. This suggests that MicroSnap was better at recovering samples than the traditional method.

### Summary

In summary, the results of microbiological methods are naturally very variable and must be interpreted with care and recognition of their limitations.

Pragmatism and practical solutions are required to establish 'reasonable expectations' for the results from microbiological methods.

Results from environmental samples are subject to even greater variation. Therefore, qualitative measurements and trend analysis provide the most meaningful information.

MicroSnap can offer a simple, rapid and cost effective alternative to traditional plate counts. ■