

QUANTIDISC AND YEA METHOD COMPARISON: RECOVERY AND ENUMERATION OF MESOPHILIC HETEROTROPHIC BACTERIA IN POTABLE WATER.

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Abstract

Historically, heterotrophic plate counts are enumerated using a pour plate method, whereby plates are read manually. This analysis is important to evaluate the maintenance of water disinfection in a reticulation system. Advances in media and technology have made it possible to do this evaluation using defined substrate media and enumeration is performed by means of instrumentation using software specifically developed for this purpose.

A comparative study was made of the recovery and enumeration of mesophilic, aerobic bacteria in potable water using the IDEXX Quanti Disc® - YEA method and pour plate method. The IDEXX Quanti Disc® - YEA method recovered significantly more bacteria than the pour plate method.

The IDEXX Quanti Disc® - YEA method eliminates media preparation, sterilization and dispensing, making it faster, more accurate and having less Quality Assurance steps. The instrumentation eliminates subjectivity in reading results and calculations and data transfer to the database can be interfaced. This method is only suitable for samples where the plate count is expected to be low, such as in drinking water. The heterotrophic plate count done on IDEXX Quanti Disc® - YEA does not need to be done in duplicate.

The recovery is possibly higher because the nutrient value of the media is not compromised by re-heating, so more stressed organisms are able to grow. Dispersion of the sample is possibly improved.

Key words: IDEXX Quanti Disc® - YEA, pour plate method, Quality Assurance, recovery, enumeration, aliquot, colony forming units. (cfu).

1. Introduction

A comparative study was made of the heterotrophic plate count pour plate method using yeast extract agar and the IDEXX Quanti Disc® - YEA method to evaluate the equivalence of the new method which uses chromogenic substrates. [1].

In SANS 241: 2006 Edition 6.1, [4] the pour plate method for plate count is used to ascertain the disinfection achieved and maintained in drinking water. The alert value is 5000cfu/mL. Drinking water is chlorinated, therefore the majority of plate count readings in the Johannesburg area are below 100cfu/mL.

The pour plate method relies on the target bacteria being able to metabolize the ingredients in the media whereas the IDEXX Quanti Disc® - YEA method is a 50-well disc impregnated with substrate that targets 3 primary bacterial enzymes in a wide variety of species of bacteria that are typically found in drinking water. The samples were from the routine daily rounds sampled by Johannesburg Water over the greater Johannesburg area. Sample bottles contain 0.5 mL of a 3% sodium thiosulphate solution which is sufficient to neutralize the chlorine present in the water samples.

2. Method

- The water sample is mixed thoroughly.
- Adjustable autopipettes were used.
- A blank of sterile distilled water was done for both methods with every batch of sample analysed.
- The pour plate method was tested by pipetting duplicate 1 mL aliquots of sample into Petri dishes which were then poured with yeast extract agar and mixed.
- The IDEXX Quanti Disc® - YEA was tested by pipetting a 4 mL aliquot of sample (only 0.5 mL is absorbed) [1] onto the Quanti Disc plate. The pipetting onto the Quanti Disc must be done directly onto the centre of the disc with the pipette held upright. Allow three seconds to elapse before replacing the lid to ensure the distribution of sample.
- Both sets of plates were incubated for 48 hours at $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$.
- The yeast extract agar plates were read manually on a colony counter, the average calculated and reported. The IDEXX Quanti Disc® - YEA plates were read on an IDEXX Quanti Disc reader using ultraviolet light at a wavelength of 365 nm which counts the fluorescing wells and uses an MPN table.

3. Results

MPN values that were either less <1.8 or >391.2 and samples yielding zero for both methods were not used for data analysis.

The results from 132 samples were finally used out of an initial 200 samples tested.

After 48 hours incubation at $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$., the IDEXX Quanti Disc® - YEA method gives significantly higher results than the pour plate method. The results were evaluated following the guidelines in ISO 17994:2004 section 7.3. This requires that the alternative method is equivalent according to the one-sided evaluation.

The lower D value is set at -10. The relative mean difference is 66.57 and the upper and lower limits are positive, being 88.91 and 44.23 respectively. Considering one-sided evaluation (point 7.3 ISO 17994:2004), only the lower value of the maximum acceptable deviation (-D) is of concern. When $x_L > 0$ the trial method has a significantly higher recovery than the pour plate method.

In a one-sided evaluation, the alternative method is considered acceptable even though the methods are not mathematically equivalent. [2]

An example of some results obtained is shown in the graph below: Log values of results were used to make the range of sampling points easier.

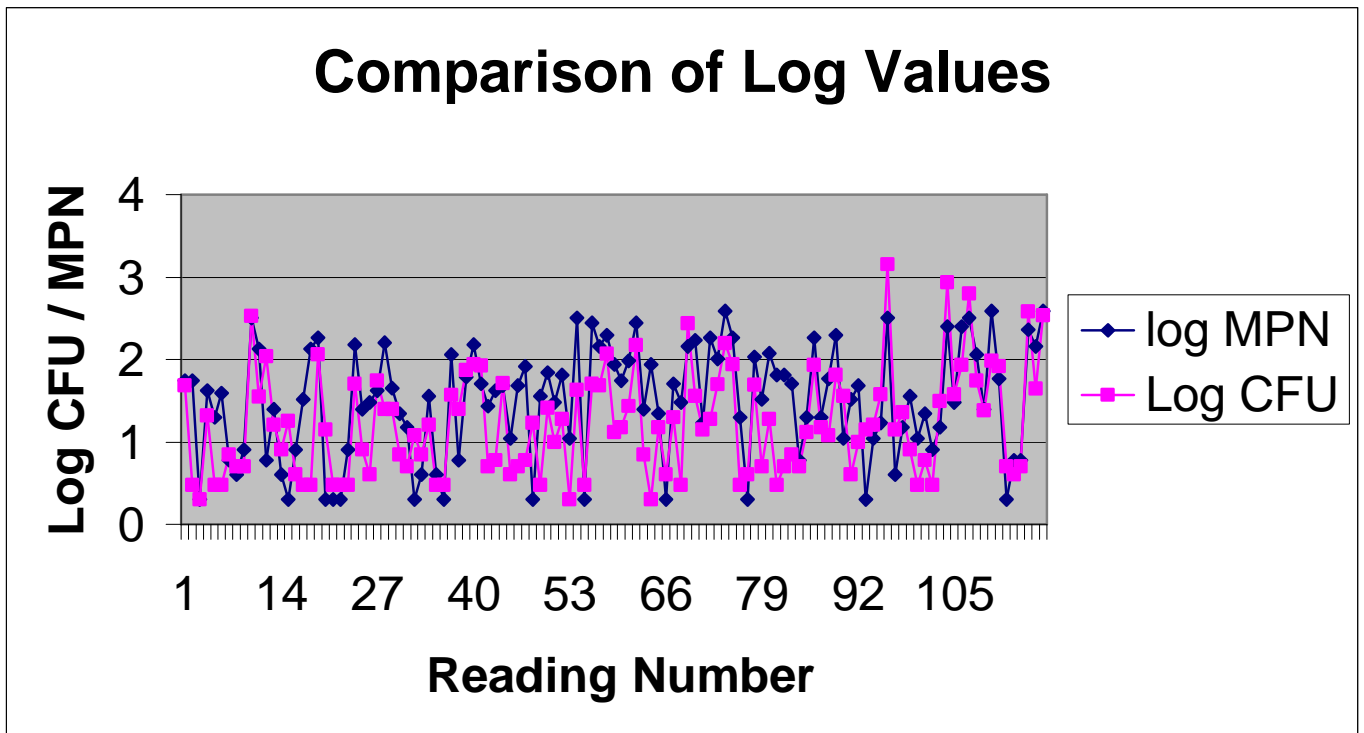


Figure 1. Log values of MPN and cfu values vs. frequency of occurrence.

4. Discussion

The IDEXX Quanti Disc® - YEA method is more sensitive. The multiple enzyme technology comprises three substrates viz. 4 methyl umbelliferyl phosphate
4 methyl umbelliferyl β -D glucoside and
L-alanine -7-amido-4-mthyl-coumarin.

These substrates are metabolized by the enzymes: alkaline phosphatase, β -D-glucosidase and L-alanine-aminopeptidase respectively. These enzymes are common to a wide spectrum of mesophilic, heterotrophic bacteria found in water. When the substrates are metabolized, fluorescence is produced which is read under ultra-violet light at 365 nm.

The possible reasons for a higher count are the following:

- Dispersion of a sample is effected in the pour plate method by mechanical or manual agitation of the plate after the plate is poured with agar, which is viscous and it possibly results in clumping. Perhaps the dispersion of the sample is better with the IDEXX Quanti Disc ® - YEA method because there is nothing to impede the sample.
- The higher sensitivity could be due to the use of multiple substrates.
- There is no loss of titre due to temperature variances in the agar when it is poured.
- There is no compromise of medium due to reheating.
- There is no depletion of aerobic conditions for those organisms at the bottom of the Petri dish or drying on the surface of the agar plate.
- There is no variance in counting because it is not subjective.

5. Conclusions

The IDEXX Quanti Disc ® - YEA is user-friendly: quick and easy to use.

It eliminates the preparation, maintenance and QA of the agar.

There is also less equipment like steamers, plate pourers and water baths to maintain.

It is a more sensitive test when compared to the pour plate method.

It is acceptable when evaluated according to ISO 17994:2004. The results and images can be stored and retrieved if necessary, it is compatible with LIMS software and transcription errors are eliminated by interfacing.

6. References

1. Sartory, D.P; Gu, H and Chen, C.-M (2008) Comparison of a novel MPN method against the Yeast Extract Agar (YEA) pour plate method for the enumeration of heterotrophic bacteria from drinking water. Water Research 42: 3489-3497.
2. ISO 17994: 2004 Water quality – Criteria for establishing equivalence between microbiological methods.
3. <http://quantidisc>
3. SANS 241: 2006 THE SOUTH AFRICAN NATIONAL STANDARD – Drinking water.