



QUANTIDISC AND YEA METHOD COMPARISON

RECOVERY AND ENUMERATION OF
MESOPHILIC HETEROTROPHIC BACTERIA IN POTABLE WATER

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INTRODUCTION



- A comparative study was made of the heterotrophic plate count pour plate method versus the IDEXX Quantidisc® - YEA method to evaluate equivalence of the new method which uses chromogenic substrates
- 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.



INTRODUCTION



- The alert value is 5000 cfu / mL
- Drinking water is chlorinated, therefore the majority of plate count readings in the Johannesburg area are below 100 cfu/mL.
- The pour plate method relies on the target bacteria being able to metabolize the ingredients in the media



INTRODUCTION



- The IDEXX Quantidisc® -YEA method is a 50-well disc impregnated with substrate that targets 3 primary bacterial enzymes in a wide variety of species of bacteria typically found in drinking water.
- The samples used were routine samples from the greater Johannesburg area
- Sample bottles contain 0.5 mL of a 3% sodium thiosulphate solution to neutralize the chlorine



METHOD



- The water sample is shaken thoroughly
- Adjustable autopipettes were used
- A sterile blank was done for both methods with every batch analysed
- Pour plate method – two 1 mL aliquots pipetted into Petri dishes and poured with yeast extract agar



METHOD



- IDEXX Quantidisc® -YEA method – one 4 mL aliquot pipetted directly onto centre of Quanti Disc plate (only 0.5 mL absorbed)
- Both sets of plates incubated for 48 hours at 35°C
- The yeast extract agar plates were read manually on a colony counter



METHOD



- The IDEXX Quantidisc® -YEA discs were read on an IDEXX Quanti Disc reader using ultraviolet light at a wavelength of 365 nm, which reads fluorescing wells



RESULTS



- Values < 1.8 or > 391.2 cfu on the MPN table or values that were zero for both sets of results were excluded
- Results from 132 samples were used out of an initial 200 samples tested
- After the incubation period, the IDEXX Quantidisc[®] -YEA method yielded significantly higher results than that of the pour plate method



RESULTS



- Results evaluated using 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



RESULTS



- The relative mean difference is 66.57 and the upper and lower limits are positive, being 88.91 and 44.23 respectively
- In considering the one-sided evaluation (point 7.3 ISO 17994:2004), only the lower value of the maximum acceptable deviation (-D) is of concern



RESULTS



- 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



DISCUSSION



- The IDEXX Quantidisc ® -YEA method is more sensitive
- The multiple enzyme technology comprises 3 substrates:
 - 4 methyl umbelliferyl phosphate
 - 4 methyl umbelliferyl β -D glucoside
 - L-alanine -7 - amido – 4- methyl – coumarin



DISCUSSION



- These substrates are metabolized by the enzymes:
 - Alkaline phosphatase
 - β – D glucosidase
 - L – alanine – aminopeptidase
- These enzymes are common to a wide spectrum of mesophilic, heterotrophic bacteria found in water



DISCUSSION



- When the substrates are metabolized, fluorescence is produced which is read under UV light at 365 nm
- The possible reasons for a higher count are the following:
 - Dispersion of a sample in the pour plate method is manual or mechanical agitation after the plate is poured with agar, which is viscous, and possibly results in clumping. Perhaps the dispersion in the discs is more efficient



DISCUSSION



- The higher sensitivity could be due to the use of multiple substrates in the IDEXX Quantidisc® -YEA
- There is no loss of titre due to temperature variances such as that in the agar
- There is no compromise of medium due to re-heating



DISCUSSION



- There is no depletion of aerobic conditions for those organisms at the bottom of the Petri dish or possible drying out of those on the surface of the plate
- There is no variance in the counting because it is not subjective



CONCLUSIONS



- The IDEXX Quantidisc ® -YEA is user-friendly: quick and easy to use
- It eliminates the preparation, maintenance and QA of the agar
- There is less equipment to maintain such as steamers, plate pourers and water baths
- It is more sensitive when compared to the pour plate method



CONCLUSIONS



- 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 interfacing can eliminate transcription errors



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



REFERENCES



- . <http://quantidisc>
- 4. SANS 214: 2006 THE SOUTH AFRICAN NATIONAL STANDARD - Drinking Water