THE WATER MANAGEMENT SOCIETY

Rapid Microbiology Industry Liaison Group

MPN (Pseudalert™) Factsheet

These guides are intended to give unbiased views regarding a number of technologies and test kits and their potential capabilities.

The opinions expressed are the views of individual members of the Rapid Microbiology Industry Liaison Group and are supplied in good faith. Additional third party opinions are provided in the attached literature references and on-line links. The WMSoc cannot be held responsible for any misunderstanding or subsequent misapplication of this information.

The manufacturer should be contacted regarding details about availability, pricing, repairs, calibration, QA/QC recommended protocols, validation data, and performance data, etc.

The factsheets cannot give advice regarding specific water testing alert levels – these need to be advised by the regulators.

PSEUDALERT SUMMARY TABLE

- This test detects *Pseudomonas aeruginosa*. It does not detect other *Pseudomonas* species
- Limit of detection 1 CFU/100 mL (250 mL for presence/absence) in 24-28 hr
- No sample concentration step required
- Very simple and robust method that can be performed in a basic test area with minimum facilities
- ISO 16266-2:2018 Water Quality - Detection and enumeration of *Pseudomonas aeruginosa* - Part 2: Most probable number method

Method	Use of Most Probable Number technique to quantify *Pseudomonas aeruginosa*
Bacteria detected	*P. aeruginosa*
Pre-concentration as per ISO 11731	N/A
Algorithm to convert results to CFU	None required. Calculated / determined MPN is scientifically equivalent to CFU.
Can differentiate between live and dead cells	LIVE only
Will detect Viable But Non-Culturable (VBNC) bacteria	May encourage growth due to liquid growth medium
Interference from biocides and other water treatment additives	Not suitable for use with sparkling or coloured water
Use with complex water samples e.g. from cooling towers	YES
Laboratory or field	Laboratory or clean area with required equipment
Are results comparable to current plate counts?	Yes - validated against ISO 16266:2006 - Comparison done according to ISO 17994 – refer to ISO 16266-2:2018
Would current plate technique still be required?	No
False positive False negative	< 1% 6.5%
Could rapid test give a positive result whilst culture test gives negative result?	Possibly as liquid culture growth medium may enhance/encourage growth of VBNC

Suitable verification data should be supplied by any laboratories undertaking the testing (or UKAS accreditation).
All tests should have positive and negative control data available - irrespective of whether laboratory or field-based.
N.B. Not all methods are suitable for field-based testing.

1.	General	
i.	Name of Test:	**Pseudalert**
ii.	Scientific principles / basis for test:	**This test is based on a bacterial enzyme detection technology that signals the presence of *P. aeruginosa* through the hydrolysis of a substrate in the Pseudalert reagent. *P. aeruginosa* cells rapidly grow and reproduce using the rich supply of amino acids, vitamins and other nutrients present in the Pseudalert reagent. Actively growing strains of *P. aeruginosa* have an enzyme that cleaves the substrate in the reagent to produce blue fluorescence under ultraviolet light**
iii.	Sensitivity: Specificity: Limit of detection:	**94%** **100%** **1 CFU/100 mL (or 250 mL for presence/absence) within 24-28 hr**
iv.	Scientific publication references:	**Various - contact manufacturer for complete list**
v.	Patents:	**Yes**
vi.	Countries sold into:	**All major countries**
vii.	Manufacturer: Supplier:	**IDEXX**
viii.	Commercially available:	**Yes**
ix.	Micro-organism species detected:	***Pseudomonas aeruginosa***
x.	Lab based: Field based:	**Yes** **Basic test area and equipment needed**
xi.	Can the test be used to determine operational control? Trend analysis?	**Yes** **Yes**
xii.	Independent end-user data:	**Yes**
xiii.	Method validated by third party:	**Various – contact manufacturer for full list**

2.	Application details	
i.	Sample quality required:	**Can be used for drinking waters, bottled waters, swimming pool waters, spa waters and environmental surface water samples**
ii.	What sample preparation on-site is required:	**Minimal, very simple sample preparation procedure**
iii.	Does the sample need to be tested within a prescribed time scale (courier)?	**As per ISO 16266-2:2018**
iv.	Sample bottle type: Sample volume required:	**120-500 mL** **100 mL for MPN; 100 or 250 mL for presence absence**

3.	Analytical procedures	
i.	Does procedure require initial isolation of test organism by culture?	**No**
ii.	Which other substances and/or microorganisms are potential interferences or inhibitors?	**Appears to be unaffected by typical levels of other water bacteria Contact supplier for latest information on potential chemical interferences Unsuitable for sparkling or coloured waters**
iii.	What additional equipment will be required?	**Incubator:- 38 ± 0.5 °C; UV lamp. For quantification: Quanti-Tray sealer & trays**
iv.	Is equipment specialised?	**Yes, but inherently very simple to use**
v.	Is the process automated? Could it be automated?	**Sample preparation is manual and very simple Not currently required**
vi.	Does sample need pre-treatment prior to analysis?	**No**
vii.	Is training provided?	**Video instruction provided online**
viii.	How long will test take before results are available?	**24-28 hr**
ix.	How many samples can be analysed?	**Will be dependent on resources but could be up to 100-200 per technician. Typically up to 1- 2 min "hands on" time per sample**
x.	What units are results expressed in?	**MPN per unit volume tested, scientifically equivalent to CFU per unit volume tested**
xi.	Does the result correlate with standard analytical procedures such as plating?	**Yes**
xii.	Is specialised training required to conduct test and interpret results?	**No but advised, to demonstrate evidence of competency. WMSoc training course available: HTM04-01- Monitoring the Risk of Waterborne Pathogens - Best Practices - W038**
xiii.	Are results reproducible:	**Yes**
xiv.	What errors (if any) could occur with analysis (weak link)?	**Method appears to be simple and robust**
xv.	Has test been validated for environmental samples?	**Yes**
xvi.	Does the final result include VBNC?	**Possibly - it will encourage growth of VBNC**
xvii.	Does it detect live or dead cells, or both?	**Live only - does not detect dead cells**
xviii.	Has test been used in an EQA process or could it be?	**Yes**
xix.	Will it be possible for a user organisation to gain UKAS ISO 17025 accreditation?	**Yes**

Glossary:

Algorithms - can enable calculation between different measures (e.g. MPN to CFU)

Colony forming units - used to estimate the number of viable bacteria or fungal cells in a sample

Genus - a way of classifying bacteria. Genus comes above species & below family

Sensitivity - (also called the true positive rate or probability of detection) measures proportion of positives that are correctly identified as such

Species - a group of living things that all share common characteristics and that are all classified as alike in some manner

Specificity - (also called the true negative rate) measures proportion of negatives that are correctly identified as such

Strain - a particular variety of bacteria

Viable - the ability (of bacteria) to multiply

List of abbreviations:

ATP – Adenosine tri-phosphate

CFU – Colony Forming Units

EQA – External quality assurance

GU – Genomic unit

IMS – immunomagnetic separation

LOD - Limit of detection - the lowest quantity of bacteria that can be distinguished from the absence of that bacteria (a blank value) with a stated confidence level (generally 99%).

MPN - Most Probable Number

MALDI ToF – Matrix Assisted Laser Desorption/Ionization Time of Flight

NF Validation - Third party certification

PCR – Polymerase Chain Reaction

qPCR – Quantitive Polymerase Chain Reaction

RTPCR - Real Time Polymerase Chain Reaction

VBNC – Viable but Non-Culturable