Use of Innovative Enzymatic Method for the Determination of Pseudomonas aeruginosa in Spas, Pools and Hospital Waters

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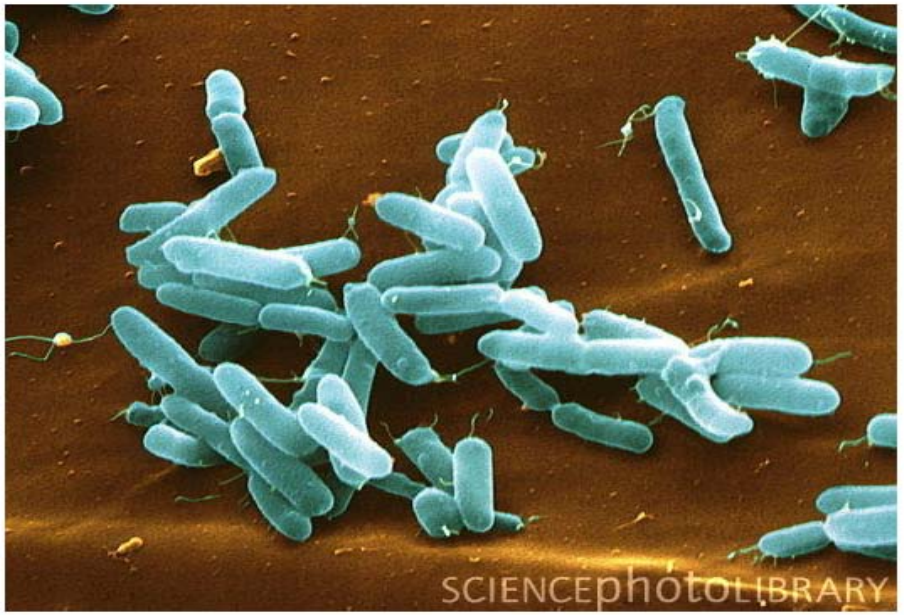
Objectives

- Introduction to microbiology of Pseudomonas aeruginosa
- Why test for Pseudomonas aeruginosa
- Methods
  - 15 Tube Most Probable Number -MPN
  - Membrane Filtration Methods- USA & Europe
  - Pseudalert
- Studies
- Q & A

Properties of Pseudomonas aeruginosa

- Family- Pseudomonadaceae
- Ubiquitous in soil & water & most surfaces
- Gram negative rod; 0.5 to 0.8 µm by 1.5-3.0 µm
- Catalase and Ctyochrome oxidase positive
- Single polar flagella
- Requires minimal nutrition needs
- Can resist high levels of chlorine
- Can form biofilms

Electron Microscope Picture of Pseudomonas aeruginosa



Why Test for Pseudomonas aeruginosa

- An opportunistic pathogen
  - Can infiltrate wounds
  - Weak immune systems
  - Elderly
  - Patients with severe burns
- Thrives at elevated temperatures; grows at 42°C
- Can result in
  - Swimmer's ear
  - Skin rash
  - Urinary Tract Infection
  - Gastrointestinal infection
  - Rare instances-pneumonia

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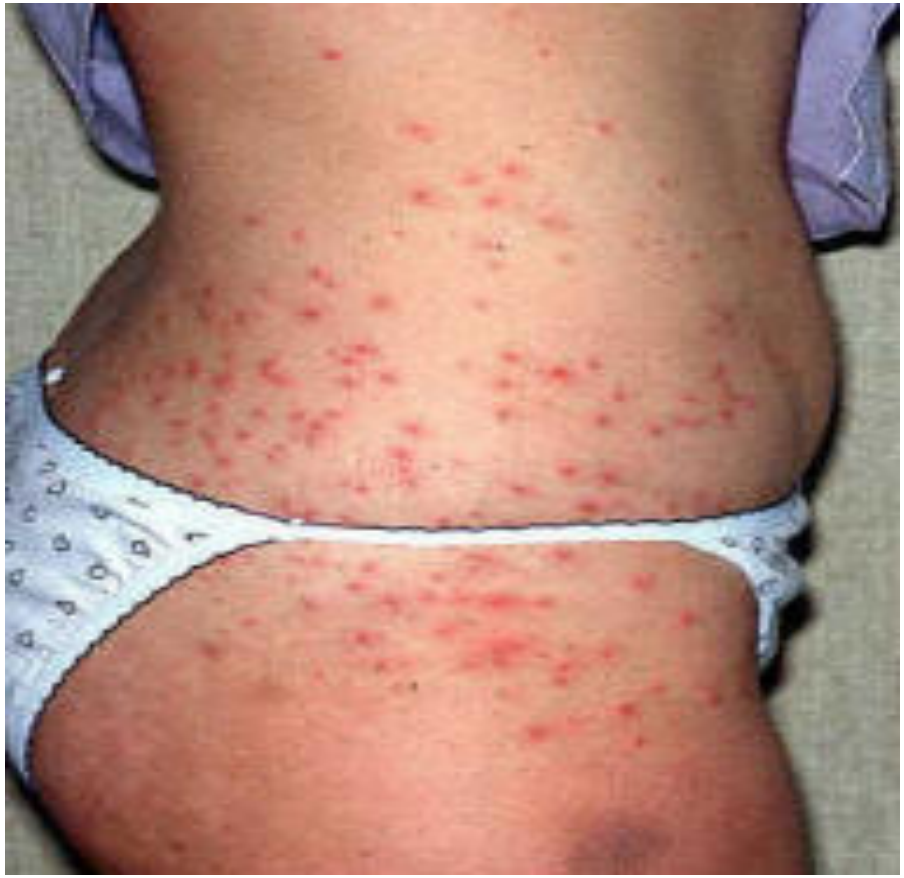
Pseudomonas aeruginosa skin rash



Pool – Spa

- Pools = a structure that is filled with water and used for swimming
- Spas = a structure that is filled with water and used for relaxation or invigoration (high temperature)

Regulatory – Pools/Spas

- Regulations for testing vary by country
- Most testing is performed due to a suspected cause of illness
- US Pool/Spa regulations are sporadic and may be at the state, county or regional/city level
- Most US labs use *Standard Methods* 9213

Methods

Methods for Pseudomonas aeruginosa

ISO 16266
(2006)
(2 + 6 days)

Membrane Filtration:
bottled water 250mL,
other water 100mL

↓

PACN agar
36 ± 2°C for 44 ± 4 h
(1st read at 22 ± 2 h)

↓

confirmation
(non-green only):
nutrient agar
36 ± 2°C for 22 ± 2 h

↓

oxidase test (red-brown)

↓

King's B medium
(oxidase +)
36 ± 2°C for up to **5 days**

↓

Acetamide broth
(red-brown, fluorescent)
36 ± 2°C for 22 ± 2 h
Add Nessler reagent (contains Hg)
Look for gas production (NH_4^+)

Positive control:
P. aeruginosa NCTC 10332
Negative control:
Escherichia coli NCTC 9001

EN 12780
(2007)
(2 + 1 days)

MF:
mineral & Bwater 250mL,
potable / pool water 100mL

↓

PACN agar
37 ± 1°C for 44 ± 4 h
(1st read at 22 ± 2 h)

↓

confirmation
(non-green only):
milk agar w/Centrimide
37 ± 1°C for 22 ± 2 h

↓

oxidase test

Positive control:
P. aeruginosa NCTC 10662
Negative control:
Escherichia coli NCTC 9001

MoDW
(2002)
(2 + 1 days)

MF:
treated water 100mL,
other water less
(aim for 20-80 cfu)

↓

PACN agar
37°C for 48 h

↓

confirmation (all):
milk agar
37°C for 24 h

***Standard Methods*/AWWA**
(2005) 9213E
(3 + 1 days)

MF:
natural water 200mL,
pool water ≥500mL
(aim for 20-80 cfu)

↓

m-PA agar
41.5 ± 0.5°C for **72 h**

↓

confirmation: # of typical and
atypical, up to lab:
milk agar 35 ± 1°C for 24 h

DIN 38 411
(1983)

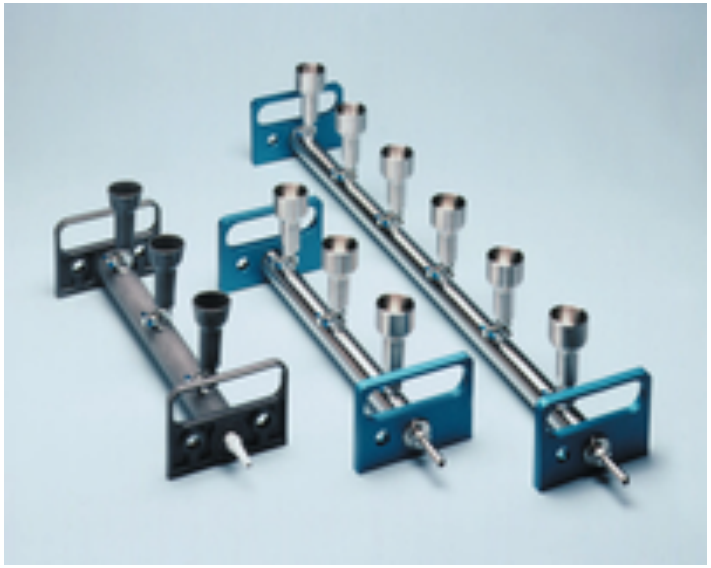
Multiple Tube Fermentation (MTF) Most Probable Number (MPN)

Standard Methods- 9213F MPN Test

- Presumptive test is either 5 tube X 10 mL or 15 tube (10 ml, 1 mL and 0.1 mL - 5 tubes for each dilution)
- Asparagine broth is the presumptive test and Acetamide broth is for confirmation
- 24 - 48 hours at 35-37°C - **presumptive test**
  - Presumptive is green fluorescence under a 365 nm UV light
  - **Confirmation** is a purple color within 24-36 hours at 35-37°C

Membrane Filtration

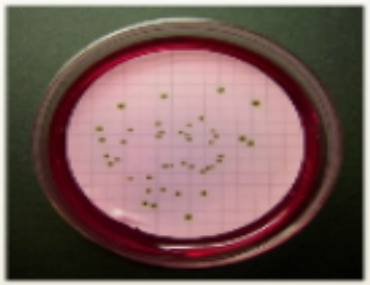
Membrane Filter Apparatus



PRIMARY MEDIUM USED

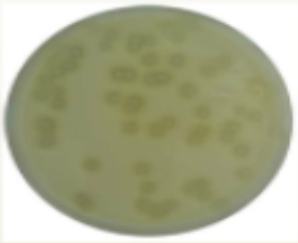
M-PA agar at 41.5 ± 0.5°C for 72h.

COLONY MORPHOLOGY



brownish-green/black colonies = presumptive *P. aeruginosa*

CONFIRMATION



Culture on milk agar at 37 ± 1°C for 22 ± 2h

Pseudalert 24 Hour Test

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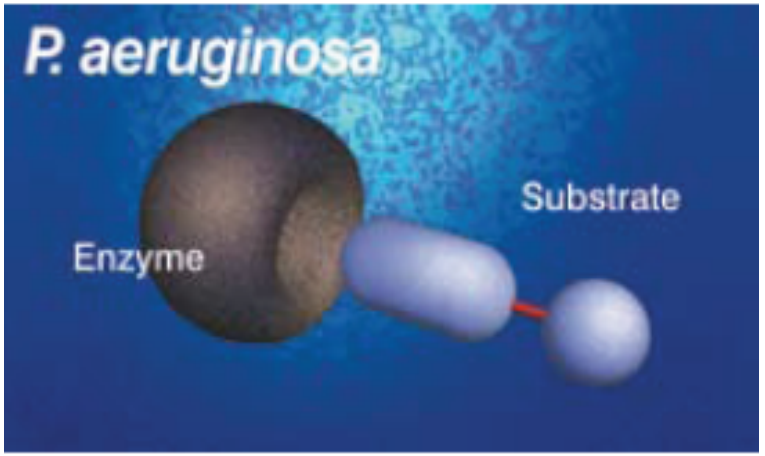
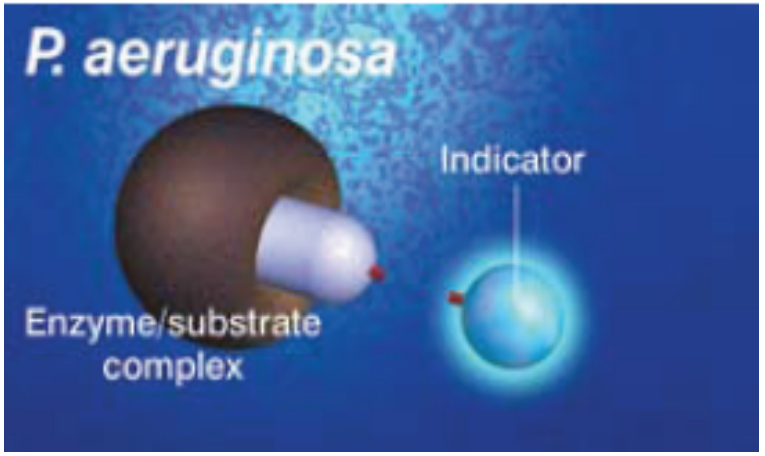
Pseudalert

- Pseudalert is based on Bacterial Enzyme Technology





Presence- Absence or Quantification Testing

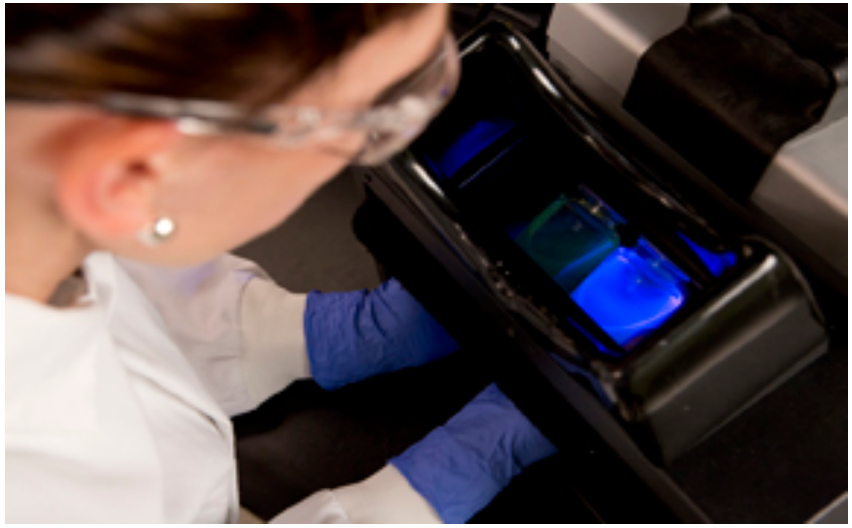
Procedure for either P/A or Quantification

Pseudalert

Add Reagent to Sample

Mix well to Dissolve

Incubate and read results with a UV lamp at 365 nm

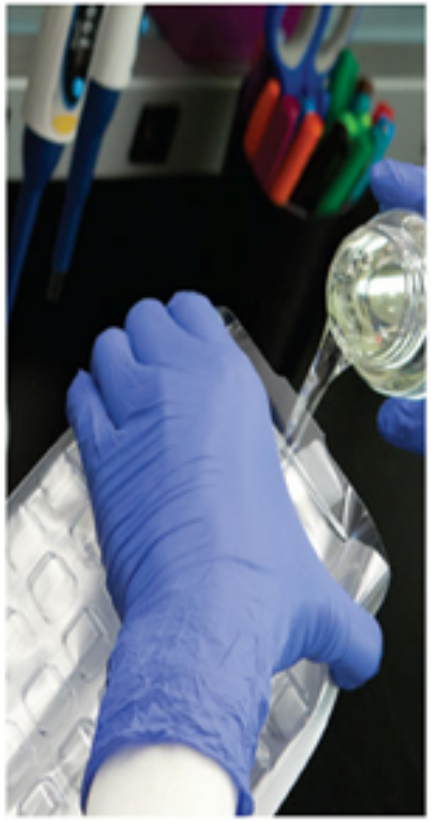


24-28 hours at 38± 0.5°C

Record results as P/A

Quantification

Add powder and mix.

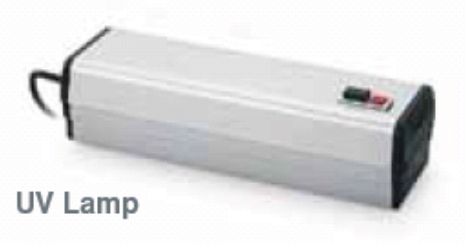


Pour into Quanti-Tray.

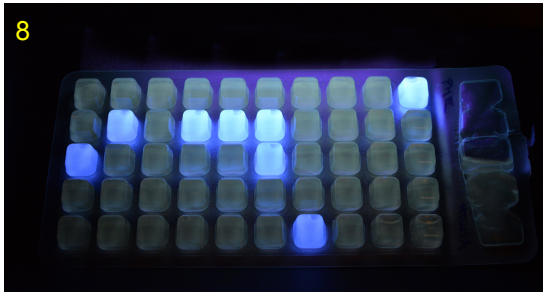
Seal Quanti-Tray.

Incubate and read results with a UV lamp at 365 nm

- 24-28 hours at 38± 0.5°C



- Record positive wells



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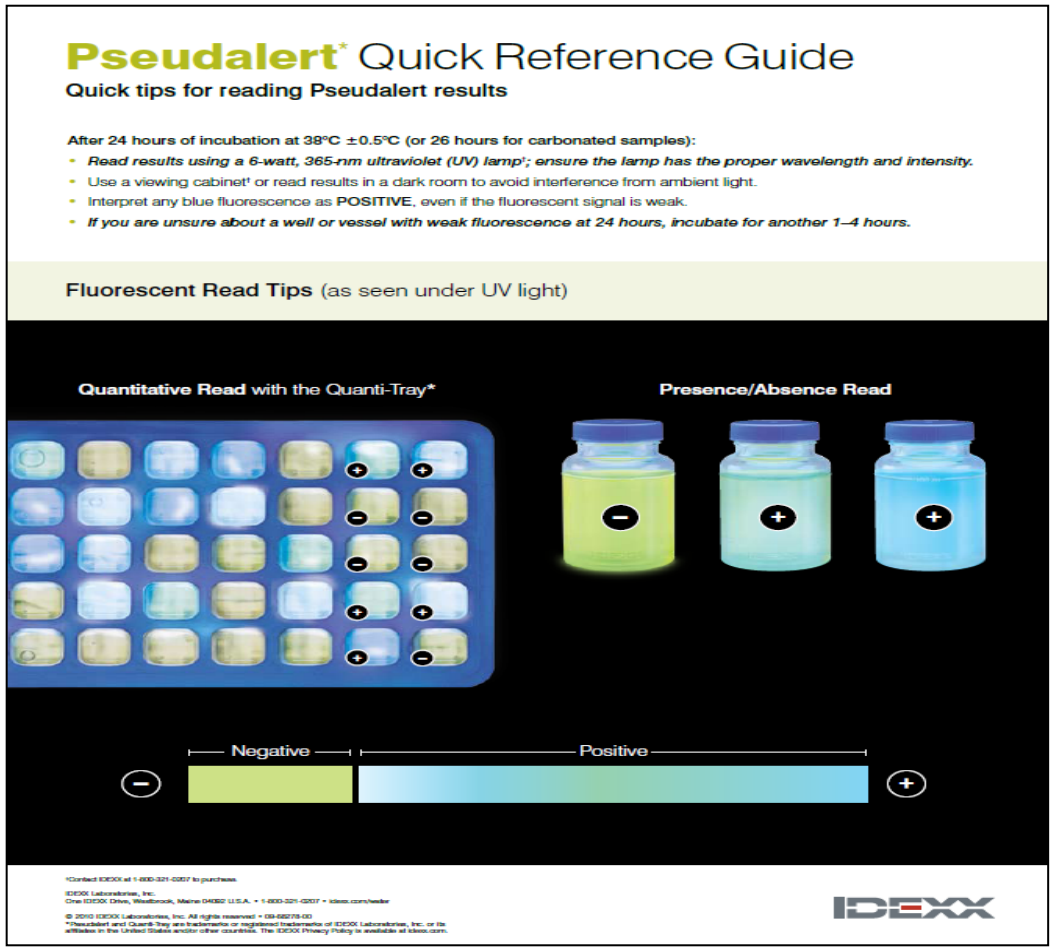
51-Well Quanti-Tray®
MPN Table

No. of wells giving positive reaction	MPN per 100 ml sample	95% Confidence Limits Lower	Upper
0	<1.0	0.0	3.7
1	1.0	0.3	5.6
2	2.0	0.6	7.3
3	3.1	1.1	9.0
4	4.2	1.7	10.7
5	5.3	2.3	12.3
6	6.4	3.0	13.9
7	7.5	3.7	15.5
8	8.7	4.5	17.1
9	9.9	5.3	18.8
10	11.1	6.1	20.5
11	12.4	7.0	22.1
12	13.7	7.9	23.9
13	15.0	8.8	25.7
14	16.4	9.8	27.5
15	17.8	10.8	29.4
16	19.2	11.9	31.3
17	20.7	13.0	33.3
18	22.2	14.1	35.2
19	23.8	15.3	37.3
20	25.4	16.5	39.4
21	27.1	17.7	41.6
22	28.8	19.0	43.9
23	30.6	20.4	46.3
24	32.4	21.8	48.7
25	34.4	23.3	51.2
26	36.4	24.7	53.9
27	38.4	25.4	56.6
28	40.6	28.0	59.5
29	42.9	29.7	62.5
30	45.3	31.5	65.6
31	47.8	33.4	69.0
32	50.4	35.4	72.5
33	53.1	37.5	76.2
34	56.0	39.7	80.1
35	59.1	42.0	84.4
36	62.4	44.6	88.8
37	65.9	47.2	93.7
38	69.7	50.0	99.0
39	73.8	53.1	104.8
40	78.2	56.4	111.2
41	83.1	59.9	118.3
42	88.5	63.9	126.2
43	94.5	68.2	135.4
44	101.3	73.1	146.0
45	109.1	78.6	158.7
46	118.4	85.0	174.5
47	129.8	92.7	195.0
48	144.5	102.3	224.1
49	165.2	115.2	272.2
50	200.5	135.8	387.6
51	> 200.5	146.1	infinite

IDEXX Sales and Technical Support
1-800-321-0207 or 1-207-856-0496
www.idexx.com/water

Quick Reference Guide



Pseudalert* Quick Reference Guide

Quick tips for reading Pseudalert results

After 24 hours of incubation at 38°C ±0.5°C (or 26 hours for carbonated samples):

- ***Read results using a 6-watt, 365-nm ultraviolet (UV) lamp[2]; ensure the lamp has the proper wavelength and intensity.***
- Use a viewing cabinet[1] or read results in a dark room to avoid interference from ambient light.
- Interpret any blue fluorescence as **POSITIVE**, even if the fluorescent signal is weak.
- ***If you are unsure about a well or vessel with weak fluorescence at 24 hours, incubate for another 1–4 hours.***

Fluorescent Read Tips (as seen under UV light)

[1]Contact IDEXX at 1-800-321-0207 to purchase.

IDEXX Laboratories, Inc.
One IDEXX Drive, Westbrook, Maine 04092 U.S.A. • 1-800-321-0207 • idexx.com/water

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Summary

MF ~ 22 steps

Eight Steps

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Pseudalert Studies

IDEXX Beta Trial report 14A. October 2010. Comparison of the performance of the Pseudalert test against SM 9213E (m-PA) from pool/spa water samples.

Beta Trial Report 14A con't

- Performed by an independent lab that regularly tests pools and spas.
- One thousand and eight pool & spa samples were evaluated against Standard Methods 9213E, m-PA.
- Pseudalert found to be equivalent to SM 9213E.
- Pseudalert able to recover very low concentrations (as low as 1/100 mL).
- High background flora (57000/mL) did not interfere
- Seventeen samples were found positive out of the total tested.

Beta Trial report 14A con't

		SM 9213E		
		+	-	
Pseudalert	+	17	2	19
	-	3	986	989
	Total	20	988	1008

Beta Trial 14B con't

- Performed by an independent lab that tests pools and spas in Europe.
- Pseudalert was found to be equivalent to the ISO 16266 MF method for pool and spa samples. A p value (=/> 0.05) calculated = 0.082.
- A total of 86 samples were tested -16 natural samples were positive. Seventy samples were spikes with strains of P. aeruginosa.
- Pseudalert detected and quantified P. aeruginosa even in the presence of very high bacteria population (>1120 CFU/mL).
- Pseudalert accurately detected very low levels of P. aeruginosa (as low as 1/100mL).

Evaluation of an MPN Method for the Rapid Enumeration of *Pseudomonas aeruginosa* from Swimming Pool and Spa Waters: David Sartory

- Compare the recovery of *Pseudomonas aeruginosa* by Pseudalert/Quanti-Tray to that by PACN agar (ISO 16266)
- Originally intended to use routine samples from swimming pools and spa pools
- Additional samples of swimming pool and spa pool waters inoculated with *Pseudomonas aeruginosa*
- Spiking trials undertaken by 6 UK laboratories and 1 German laboratory

Results

- 481 paired results: 23 were removed (>the upper limit for method)
- 458 paired counts for analysis
  - PACN mean CFU count = 39
  - Pseudalert mean MPN count = 45
  - Relative mean difference (ISO 17994) = - 0.4 (CI - 6.9 to + 6.1)
- ISO 17994 analysis revealed equivalent results between methods

Use of Pseudalert and Quanti-Tray for the Detection of *Pseudomonas aeruginosa* in 24 Hours for Spa Waters by a Private Lab

- Seventy spa samples were evaluated from public spas.
- Comparison of Pseudalert against Standard Methods 9213E, m-PA.
- One sample was positive and all the other samples were spiked with 3 different concentrations of an ATCC Pseudomonas strain (low, mid & high).
- Mean values: Pseudalert = 36.7/100mL and MF = 33.8/100 mL (n =39)
- Pseudalert is a suitable alternative for testing with results in 24 hours and no confirmation required.

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Hall, N. et al. (2011) Incidence of *Pseudomonas aeruginosa* in Private Spa Water. Presented at the World Aquatic Health Conference, Seattle, WA

- Spa samples consisted of 65 private spas and 12 public spas.
- Pseudalert test was compared to Standard Methods 9213F.
- Pseudalert test was found to be equivalent to Standard Methods
  - Sensitivity : 94.5%
  - Specificity: 100 %
- Sixty three % of private spas were positive and may pose a threat.

Conclusions

- Based on the studies presented, Pseudalert performs as well as the existing US and European methods in 24 hours and confirmation is not required.
- A number of labs both in the US and in Europe are now using Pseudalert for their everyday testing
- It can be performed either as a Present-Absence test or Quantification.

Thank you
Questions