IDEXX Summary 14A

Topic: Beta Trial Study report comparing Pseudalert* versus *Standard Methods for the Examination of Water and Wastewater*[1] (SM 9213E) in pool/spa waters for detection and enumeration of *Pseudomonas aeruginosa*

Title: "Comparison of the performance of the IDEXX Pseudalert test against SM 9213E at recovering confirmed *Pseudomonas aeruginosa* from pool/spa water samples"

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Report Highlights:

- Pseudalert was compared to SM 9213E at an independent laboratory that regularly tests pool/spa waters
- Over 1,000 pool/spa samples were tested for *P. aeruginosa* using Pseudalert and SM 9213E
- Data from the completed study showed:
  - Pseudalert has comparable detection ($p = 1.0$) and quantification of *P. aeruginosa* versus SM 9213E. Two methods are comparable if $p > 0.05$.
  - Pseudalert accurately recovered very low concentrations of *P. aeruginosa* (as low as 1 cfu/100ml of sample)
  - Very high background flora (> 57,000 cfu/mL) did not interfere with the ability of Pseudalert to accurately detect and enumerate *P. aeruginosa*
- Pseudalert performed as well as SM 9213E for detection and quantification of *P. aeruginosa* in pool/spa water samples

1. APHA, AWWA, and WEF (American Public Health Association, American Water Works Association, and Water Environment Federation). 2005. *Standard Methods for the Examination of Water and Wastewater*. 21st ed. New York: APHA.

Technical Note

Comparison of the performance of the IDEXX Pseudalert* test versus *Standard Methods for the Examination of Water and Wastewater*[1] (SM 9213E) at recovering confirmed *Pseudomonas aeruginosa* from pool/spa water samples

Product Description

The Pseudalert test detects the presence of *Pseudomonas aeruginosa* in bottled, pool, and spa water samples. The test is based on a bacterial enzyme detection technology that signals the presence of *Pseudomonas aeruginosa* through the hydrolysis of a substrate present in the Pseudalert reagent. *Pseudomonas 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 *Pseudomonas aeruginosa* have an enzyme that cleaves the substrate to produce a blue fluorescence under UV light. Pseudalert detects *Pseudomonas aeruginosa* at 1 cfu in either 100 mL or 250 mL samples within 24 hours for non-carbonated water samples and within 26 hours for carbonated samples.

Scope

This technical note contains data collected at an independent certified laboratory that evaluated the performance of the Pseudalert test prior to its launch in September 2010. The test matrix for this study was pool/spa water, samples of which were collected at numerous locations as part of the laboratory's normal testing operation. The microorganisms present in these pool/spa water samples were from wild populations that occurred naturally in the environment and did not result from supplemental spiking activities. Testing occurred over the course of seven months and involved 1,008 separate pool/spa water samples. This study compared the relative recovery of confirmed *P. aeruginosa* by Pseudalert after 24 hours of incubation against the SM 9213E.

Procedure

1. Samples were collected at numerous locations as 2 x 200 mL aliquots from pool/spa sites maintained at > 30ºC.
2. When the samples arrived at the laboratory, composites were prepared by combining and thoroughly mixing the two samples from each collection site.
3. A 100 mL aliquot of each sample was processed and analyzed following the procedures outlined in the SM 9213E.
4. A 100 mL aliquot of each sample was processed and analyzed following the procedures outlined in the Pseudalert package insert for 100 mL quantification using the Quanti-Tray* device. Pseudalert was incubated for 24 hours at 38±0.5ºC.
5. The Heterotrophic Plate Count of each sample was determined following the procedure outlined in the *Standard Methods for the Examination of Water and Wastewater 20th Edition*[1] (SM 9215C)
6. Presumptive *P. aeruginosa* positive samples from both methods were confirmed by assaying the following reactions (which are enhancements from SM 9213E):
    - Oxidase
    - Arginine decarboxylase[2]
    - Growth on Centrimide agar[3]
    - Growth in Tryptic Soy Broth incubated at 41.5ºC
7. Random negative samples were also subjected to confirmation procedures.

Results

Twenty samples of the original 1,008 pool/spa water samples tested contained confirmed *P. aeruginosa* by at least one of the test methods. The relative recovery of *P. aeruginosa* by each method is summarized in the chart below:

Month Tested	Sample No.	Pseudalert (MPN/100mL)	SM 9213 (cfu/100mL)	Confirmation	HPC (cfu/mL)
March	1	9	4	*P.aeruginosa*	40
	2	2	2	*P.aeruginosa*	<10
	3	>201	>100	*P.aeruginosa*	10260 (est)
	4	6	3	*P.aeruginosa*	10
April	5	201	>100	*P.aeruginosa*	80
	6	>201	>100	*P.aeruginosa*	>57000 (est)
	7	>201	>100	*P.aeruginosa*	>57000 est
May	8	6	>100	*P.aeruginosa*	60
	9	0	1	*P.aeruginosa*	<10
	10	5	1	*P.aeruginosa*	<10
	11	5	3	*P.aeruginosa*	40
	12	74	34	*P.aeruginosa*	80
	13	101	>100	*P.aeruginosa*	<10
June	14	>201	>100	*P.aeruginosa*	>57,000 est
July	15	4	12	*P.aeruginosa*	320
	16	1	0	*P.aeruginosa*	<10
	17	0	1	*P.aeruginosa*	10
August	18	6	2	*P.aeruginosa*	10
	19	34	27	*P.aeruginosa*	>57000
	20	22	27	*P.aeruginosa*	>57000

The presence or absence of confirmed *P. aeruginosa* by each method was compared statistically using the McNemar test for matched paired samples. The results of this analysis are shown below (Note – both methods had one sample that yielded a presumptive positive result for *P. aeruginosa* that could not be confirmed):

Only samples containing confirmed *P. aeruginosa*

Pseudalert \ SM 9213E	+	-
+	17	1
-	2	0

p = 1.0

All Samples

Pseudalert \ SM 9213E	+	-
+	17	2
-	3	986

p = 1.0

Conclusions

The data presented above clearly demonstrates the comparable detection (p>0.05) and quantification of *P. aeruginosa* by the Pseudalert method compared to the standard method. Pseudalert was able to accurately recover very low concentrations of *P. aeruginosa* (as low as 1 cfu/100mL of sample) without interference from the heterotrophic bacterial populations or chemical residues present in the pool/spa water samples. Even the presence of very high bacterial populations (in excess of 57,000 cfu/mL) did not interfere with the ability of Pseudalert to accurately detect and quantify the presence of *P. aeruginosa*. Both methods produced one test that was presumptively positive for the presence of *P. aeruginosa* but failed to be confirmed. The bacterial isolate that produced this result in Pseudalert was reintroduced into the test but failed to reproduce the positive response.

Based on these data we conclude that, after 24 hours of incubation, Pseudalert performs as well as the SM 9213E at the specific detection and quantification of *P. aeruginosa* from pool/spa water matrices.

For technical questions, please contact:

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Pseudalert Quick Reference Guide

About IDEXX Laboratories

IDEXX Laboratories, Inc. is the global market leader in diagnostics and information technology solutions for animal health and water and milk quality. Headquartered in Maine, IDEXX employs over 4,700 people in more than 60 locations around the world.
IDEXX is the world leader in microbiology testing technologies that ensure safe water. As the world's preferred provider of innovative drinking-water microbiology test kits, IDEXX is known for its breakthrough products. We provide easy, rapid, accurate and cost-effective water-testing solutions. Our sales, customer service and technical support teams serve customers in over 75 countries and our products have governmental approval in 36 countries world-wide.

References

1. APHA, AWWA, and WEF (American Public Health Association, American Water Works Association, and Water Environment Federation). 2005. *Standard Methods for the Examination of Water and Wastewater*. 21st ed. New York: APHA.

2. McFaddin, J.F. *Biochemical Tests for Identification of Medical Bacteria*. 3rd Edition. Philadelphia: Lippincott Williams & Wilkins, 2000. p.120.
3. Zimbro, Mary Jo and David A. Power, eds. *Difco and BBL Manual*. Sparks: Becton, Dickinson and Company, 2005. p.135.