IDEXX Summary 14B

Topic: Beta Trial Study report comparing Pseudalert* versus EN ISO 16266:2008[1] in pool/spa waters for detection and enumeration of *Pseudomonas aeruginosa*

Title: **"**Comparison of the performance of the IDEXX Pseudalert* test against the EN ISO 16266:2008 method at recovering confirmed *Pseudomonas aeruginosa* from pool/spa water samples"

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Report Highlights:

- Pseudalert was compared to EN ISO 16266:2008 at an independent laboratory that regularly tests pool/spa waters.

- Data from the completed study showed:
  - Pseudalert was able to accurately detect and quantify the presence of *P. aeruginosa*, even in the presence of very high bacterial populations (>1,120 cfu/mL).
  - Pseudalert had comparable detection and quantification of *P. aeruginosa* versus EN ISO 16266:2008 ($p = 0.082$)** from naturally contaminated pool/spa water samples. Two methods are comparable if $p > 0.05$.
  - Pseudalert has better recovery ($p = 0.009$)** of *P. aeruginosa* versus EN ISO 16266:2008 from pool/spa water samples spiked with different strains of the bacterium. 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)

- Pseudalert performed as well or better than EN ISO 16266:2008 for detection and quantification of *P. aeruginosa* in pool/spa water samples

1. ISO 16266: Water quality — Detection and enumeration of *Pseudomonas aeruginosa* — Method by membrane Filtration, Geneva. International Standards Organization; The text of ISO 16266:2006 has been approved by CEN as **EN ISO 16266:2008** without any modification; COMITÉ EUROPÉEN DE NORMALISATION, Brussels, Belgium

** Based on student's t-test (one tail; paired samples)

Technical Note

Comparison of the performance of the IDEXX Pseudalert* test against the EN ISO 16266:2008[1] method 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* rapidly grows and reproduces 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 located in Germany 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 prone to natural *P. aeruginosa* contamination. 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. Additional pool/spa water samples were collected that were unlikely to be contaminated with naturally occurring *P. aeruginosa*. These samples were spiked with seven *P. aeruginosa* isolates that were collected from environmental sources and displayed different morphological characteristics on the EN ISO 16266:2008 media. Testing occurred over the course of several months. This study compared the relative recovery of confirmed *P. aeruginosa* by Pseudalert after 24 hours of incubation against the EN ISO 16266:2008 method.

Procedure

1. Samples (>200 mL) were collected at numerous locations (maximum of five collections per site) in accordance with ISO 19458[2] specifications. Storage time was recorded and care was taken to test within 12 to 18 hours of collection, however, some samples were tested within 48 hours of collection.
2. A 100 mL aliquot of each sample was processed and analyzed following the procedures outlined in EN ISO 16266:2008. Additional confirmation procedures were added (see description below) based on the morphological characteristics of the presumptive positive samples.
3. 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 - 28 hours at 38±0.5ºC.
4. The Heterotrophic Plate Count of each sample was determined following the HPC method[3] incubated at both 20ºC and 36ºC.
5. Presumptive *P. aeruginosa* positive samples from the EN ISO 16266:2008 method were subjected to confirmation procedures based on the following scenarios:
    - Blue/Green colonies = oxidase & API 20NE
    - Fluorescent colonies = oxidase, acetamidase, growth at 42ºC, and API 20NE
    - Red/Brown colonies = oxidase, acetamidase, Kings B (fluorescence), growth at 42ºC, and API 20NE
6. Pseudalert positive wells were confirmed in the following way:
    - Blue fluorescent wells = oxidase, acetamidase, growth at 42°C, and API 20NE

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Results

Eighty six pool water samples were included in this study. Of these samples, sixteen were found to be naturally contaminated with *P. aeruginosa*. The ability of Pseudalert and EN ISO 16266:2008 (shown below as ISO 16266) to detect and quantify these natural *P. aeruginosa* populations is shown below:

Sample name	Pseudalert (MPN/100mL)	ISO 16266 (cfu /100mL)	HPC 20˚C (cfu / mL)	HPC 36˚C (cfu / mL)
indoor whirlpool (fra), filtrate	>201	>500 (pres.)	0	39
indoor pool (fra2b), filtrate	74	48	1	440
indoor whirlpool (fra), filtrate	19	28	0	3
natural pool, MU1, swimmer	0	2	250	1120
natural pool, MU1, wading pool	9	4	570	700
natural pool, MU2, nonswimmer	1	0	100	120
natural pool, MU3, swimmer	2	0	220	488
natural pool, MU3, wading pool	34	not analyzable	>300	>300
open air pool (oes), pool	29	32	0	45
open air pool (sto), swimmer	38	42	>300	>300
natural pool, MU4, swimmer	2	2	450	580
natural pool, MU4, nonswimmer	2	0	490	800
natural pool, MU4, wading pool	6	0	760	1080
indoor whirlpool (fra), filtrate	3	2	0	0
indoor pool (fra5), filtrate	6	3	0	0
indoor pool (fra6), filtrate	201	186	0	3

(pres.) = *P. aeruginosa* present in sample but exact concentration could not be determined.

Recovery of natural *P. aeruginosa* populations by both methods was analyzed statistically using the paired t-test and showed comparable (p=0.082) recovery. One sample (natural pool, MU3, wading pool) was excluded from this analysis because the EN ISO 16266:2008 method was unreadable due to non-target bacterial interference on the membrane filter. Another sample (indoor whirlpool (fra), filtrate) was also excluded from the analysis because the recovered *P. aeruginosa* populations exceeded the counting range of both methods.

The majority of the pool/spa water samples had high heterotrophic bacterial contamination that did not interfere with the performance of the Pseudalert test.

Fourteen pool/spa water samples, which did not contain natural *P. aeruginosa* contamination, were spiked with environmental isolates described in the following table:

Strain	isolated from (origin)	Bacterial ID	colony color on CN agar	fluorescence	Api 20 NE profile	Api 20 NE confirmation
101	river water (Rhine)	*P. aeruginosa*	brownish-green	+	1154475	99.9%
102	open air pool	*P. aeruginosa*	toxic-green	++	1154575	99.5%
103	river water (Main river)	*P. aeruginosa*	green	(+)	1354575	99.9%
104	carbonized water dispenser	*P. aeruginosa*	brownish-green	+	1054475	99.4%
105	cooling tower	*P. aeruginosa*	brownish	+	1154575	99.5%
106	carbonized water dispenser	*P. aeruginosa*	petrol	(+)	1054555	98.4%
107	underground hydrant (pipe construction)	*P. aeruginosa*	reddish-brown	+	1154575	99.5%

++ = strong fluorescence
+ = moderate fluorescence
(+) = weak fluorescence

The ability of Pseudalert and EN ISO 16266:2008 (shown below as ISO 16266) to detect and quantify these spiked *P. aeruginosa* populations is shown below:

Sample name	Pseudalert (MPN/100mL)	ISO 16266 (cfu /100mL)	HPC 20°C (cfu / mL)	HPC 36°C (cfu / mL)	Spike Strain
open air pool (lan1), pool	109	49	1	0	101
indoor pool (ben1), pool	130	49	0	0	102
open air pool (ben2), pool	130	16	0	1	103
artificial lake (ben3), lake	38	0	n.a.	n.a.	104
open air pool (hep1), pool	70	1	n.a.	n.a.	105
lake (arguk1), lake	130	33	58	124	106
indoor pool (dar1), pool	10	6	n.a.	n.a.	101
indoor pool (fra11) whirlpool	31	19	n.a.	n.a.	102
indoor pool (fra12), whirlpool	10	8	n.a.	n.a.	104
natural pool (MU5+), wading pool	118	100	172	504	103
natural pool (MU5+), nonswimmer	50	75	376	416	104
open air pool (Obe1), wading pool	78	80	0	2	105
open air pool (Oes), swimmer	66	50	0	0	106
open air pool (Rei), filtrate	38	65	0	0	107

n.a. = not applicable (HPC count not determined)

Recovery of spiked *P. aeruginosa* populations by both methods was analyzed statistically using the paired t-test and showed a non-comparable (p = 0.009)) recovery. Pseudalert was able to consistently recover more *P. aeruginosa* from these spiked samples than the EN ISO 16266:2008 method.

Conclusions

The data presented above clearly demonstrates the favorable detection and quantification of *P. aeruginosa* by Pseudalert when compared against the EN ISO 16266:2008 method with pool/spa water samples. 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 1,120 cfu/mL) did not interfere with the ability of Pseudalert to accurately detect and quantify the presence of *P. aeruginosa*. The EN ISO 16266:2008 method experienced significant non-target bacterial interference with one of the pool/spa water samples that did not pose a problem for Pseudalert. The Pseudalert method was also shown to recover spiked *P. aeruginosa* isolates better than the EN ISO 16266:2008 method. The reason for this discrepancy is unclear and unexpected since these isolates were originally recovered from pool/spa water sources using the EN ISO 16266:2008 method.

Based on these data we conclude that, after 24 hours of incubation, Pseudalert performs at least as well as the EN ISO 16266:2008 method at the specific detection and quantification of *P. aeruginosa* from pool/spa water matrices.

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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 or acceptance in 36 countries world-wide.

References

1. ISO 16266: Water quality — Detection and enumeration of *Pseudomonas aeruginosa* — Method by membrane Filtration, Geneva. International Standards Organization; The text of ISO 16266:2006 has been approved by CEN as a **EN ISO 16266:2008** without any modification; COMITÉ EUROPÉEN DE NORMALISATION, Brussels, Belgium
2. ISO 19458:2006 Water Quality – Sampling for microbiological analysis, Geneva. International Standards Organization
3. German Drinking Water Directive (Trinkwasserverordnung - TrinkwV) by May 21st 2001 (obligatory since January 1st 2003) in annex 5 number 1