IDEXX Summary 14G

Topic: Beta Trial Study report comparing Pseudalert* versus ISO 16266:2006[1] in bottled mineral (still and sparkling) and thermal spa water samples for detection and enumeration of *Pseudomonas aeruginosa*

Title: **"**Comparing the performance of the IDEXX Pseudalert test against a modified version of the ISO 16266:2006 method used at a major bottled water company for the recovery and specific detection of confirmed *Pseudomonas aeruginosa* from water samples"

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Date: February 2011

Report Highlights:

- Pseudalert was compared to ISO 16266:2006 at a globally recognized, independent bottled water laboratory that regularly tests natural mineral water (still and sparkling) as well as water collected from thermal spas.

- Data from the completed study showed:
    - Pseudalert was able to accurately recover very low concentrations of *P. aeruginosa* (as low as 1 cfu/250 mL of sample) without interference from biological or chemical agents (i.e. minerals or added chlorine)
    - Thermal spa water samples naturally contaminated with *P. aeruginosa* at concentrations ranging from 1 to >150 bacteria per 250 mL the Pseudalert method, after 24 hours of incubation, was able to perfectly match the recovery of the modified ISO 16266:2006 method after 48 hours of incubation
    - Still water samples analyzed statistically using the McNemar's test calculated p-value was found to be 0.3711, which indicates that the two methods are statistically equivalent in their specificity and ability to recover the *P. aeruginosa* populations. Two methods are comparable if $p > 0.05$.
- Pseudalert performed as well or better than ISO 16266:2006 for detection and quantification of *P. aeruginosa* in mineral water and thermal spa water samples

1. ISO 16266:2006 Water quality — Detection and enumeration of *Pseudomonas aeruginosa* — Method by membrane Filtration, Geneva. International Standards Organization

Technical Note

Comparing the performance of the IDEXX Pseudalert test against a modified version of the ISO 16266:2006 method used at a major bottled water company for the recovery and specific detection of confirmed *Pseudomonas aeruginosa* from 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 a European bottled water testing facility during an evaluation of the Pseudalert test that started prior to its launch in September 2010.
The matrices tested in this study included several natural mineral waters (still and sparkling) as well as water collected from thermal spas. The natural mineral water and thermal water samples used in this study contained endogenous bacterial populations. Natural mineral water samples were also spiked with pure bacterial strains to test the specificity of both test methods.

Testing occurred over the course of several months and compared the relative recovery of confirmed *P. aeruginosa* by Pseudalert after 24 hours of incubation for still waters and after 26 hours of incubation for sparkling waters against the modified ISO 16266:2006 method after at least 48 hours (incubation + confirmation steps).

Procedure

1. Test matrices included:

    - Still natural mineral waters
    - Sparkling natural mineral waters containing different concentrations of CO_2
    - Thermal samples (before & after disinfection)
    - Rinse water

2. Two hundred and fifty milliliter aliquots of each sample were processed and analyzed following the modified ISO 16266:2006 method protocol.

    - Filtration 250ml on 0,45µm filters - incubation on CN agar at 42°C for 48 hours
    - Colonies that produce blue-green (pyocyanin) colour counted as confirmed *Pseudomonas aeruginosa* /250ml
    - All the other colonies are counted as presumptive *Pseudomonas aeruginosa* colonies/250ml and are confirmed by following tests :
        - Gram stain
        - Oxydase test
        - Pigment production on Kings A & B agars and/or API 20NE

3. Two hundred and fifty milliliter aliquots of each sample were processed and analyzed following the procedures outlined in the Pseudalert package insert for 250 mL detection using the IDEXX presence/absence device. Pseudalert was incubated for at least 24 hours at 38±0.5ºC in a dry incubator or a water bath.

4. Bacterial strains used for artificial contamination were previously subjected to the following tests for further characterization:

   - Pyocyanin production on Kings A agar
   - Pyoverdin production on Kings B agar
   - Growth on CN agar (36°C for 24 hours)
   - Growth on CN agar (42°C for 48 hours)
   - Growth on Nutrient Agar (4°C for 48 hours)
   - Growth on Nutrient Agar (42°C for 48 hours)
   - API 20NE identification (growth at 30°C)
   - 16S rRNA sequencing

5. Statistical analysis: The McNemar's test was used to compare the recovery of *Pseudomonas aeruginosa* by the Pseudalert and modified ISO 16266:2006 methods using the calculator found at the following website:

http://graphpad.com/quickcalcs/McNemar1.cfm

The McNemar's test is a non-parametric method used on nominal data. It is applied to 2 × 2 contingency tables (see below) with a dichotomous trait, with matched pairs of subjects, to determine whether the row and column marginal frequencies are equal.

	Pseudalert Positive	Pseudalert Negative	Row total
ISO 16266 Positive	*a*	*b*	*a + b*
ISO 16266 Negative	*c*	*d*	*c + d*
Column total	*a + c*	*b + d*	*n*

Marginal homogeneity implies that row totals are equal to the corresponding column totals, or

$$(a + b) = (a + c)$$
$$(c + d) = (b + d)$$

Since the a and the d on both sides of the equations cancel, this implies b = c; this is the basis of the McNemar test, it uses only the number of discordant pairs.

McNemar calculates the difference between the proportions (expressed as a percentage) with 95% confidence interval

$$X^2 = [(b - c) -1]^2 / (b + c)$$

When the (two-sided) P-value is greater or equal to the conventional 0.05, the conclusion is that there is no significant difference between the two proportions, so in this case, both methods are

equivalent and recover *P. aeruginosa* effectively. A P-value of less than 0.05 indicates that both methods give significantly different results.

Results

A total of 187 individual water samples representing 59 independent water types were included in this study.

Thermal waters

Nine of the 18 thermal and rinse water samples from the thermal spa were found to be naturally contaminated with *P. aeruginosa*. The ability of Pseudalert (after 24 hours of incubation) and the modified ISO 16266:2006 method to detect these natural *P. aeruginosa* populations is shown below:

Table 1: Recovery of Natural *P. aeruginosa* Populations from Thermal Water Samples

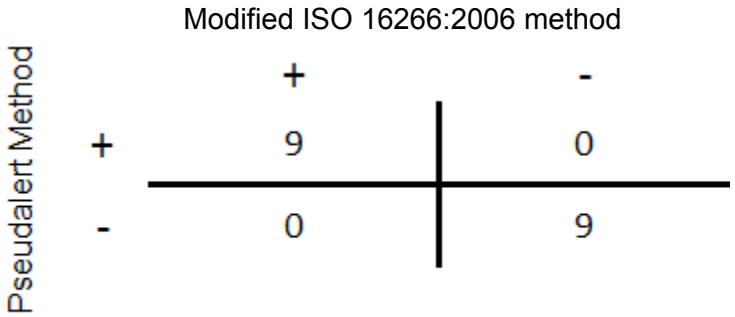
Date Tested	Sample Identification	Water Type	HPC (cfu/mL)	Standard Method		IDEXX Pseudalert	
				Presumptive (cfu/250mL)	Confirmed (cfu/250mL)	Vessel (+ or -)	Incubation (water/dry)
02-Nov-10 (before disinfection)	Sample #1	Thermal mineral	2.00E+00	0	0	-	Water
02-Nov-10 (before disinfection)	Sample #2	Thermal mineral	tntc	tntc	tntc	+++	Water
02-Nov-10	Sample #3	Chlorinated	0.00E+00	0	0	-	Water
02-Nov-10	Sample #4	Network drinking	4.30E+01	1	1	+++	Water
03-Nov-10 (1 day after disinfection)	Sample #5	Thermal mineral	6.00E+00	0	0	-	Water
03-Nov-10 (1 day after disinfection)	Sample #6	Thermal mineral	1.60E+01	>150	>150	+++	Water
03-Nov-10	Sample #7	Network drinking	2.00E+00	0	0	-	Water
05-Nov-10 (3 days after disinfection)	Sample #8	Thermal mineral	5.00E+00	0	0	-	Water
05-Nov-10 (3 days after disinfection)	Sample #9	Thermal mineral	tntc	tntc	tntc	+++	Water
05-Nov-10	Sample #10	Network drinking	5.80E+01	0	0	-	Water
16-Nov-10 (before disinfection)	Sample #11	Thermal mineral	8.00E+00	0	0	-	Water
16-Nov-10 (before disinfection)	Sample #12	Thermal mineral	2.00E+00	9	9	+++	Water
16-Nov-10 (before disinfection)	Sample #13	Thermal mineral	5.00E+00	>100	>100	+++	Water
16-Nov-10	Sample #14	Network drinking	2.00E+00	0	0	-	Water
18-Nov-10 (2 days after disinfection)	Sample #15	Thermal mineral	1.80E+01	0	0	-	Water
18-Nov-10 (2 days after disinfection)	Sample #16	Thermal mineral	6.00E+00	37	37	+++	Water
18-Nov-10 (2 days after disinfection)	Sample #17	Thermal mineral	tntc	tntc	tntc	+++	Water
18-Nov-10	Sample #18	Network drinking	1.00E+00	5	5	+++	Water

+++ = high fluorescence
++ = moderate fluorescence
tntc = too numerous to count

Unpsiked sample
Target spike
Non-target spike

Both the Pseudalert and the modified ISO 16266:2006 methods were able to recover the environmental populations of *P. aeruginosa* that contaminated these water samples. The two methods performed equally (no statistical analysis required) and were able to recover *P. aeruginosa* when present at concentrations as low as 1 cfu/250 mL.

Figure 1: 2 x 2 Table Comparing the Ability of each Method to Detect Natural *P. aeruginosa* Populations from Thermal Water Samples



The intensity of the fluorescent signal produced by Pseudalert after 24 hours of incubation was consistently high (+++) even when low concentrations of bacteria were present. Pseudalert was able to recover *P. aeruginosa* populations that persisted in the water samples as early as one day after disinfection. This suggests that Pseudalert may be able to recover injured *P. aeruginosa* populations at least as well as the modified ISO 16266:2006 method.

Spiked natural mineral waters

The ability of Pseudalert (after 24 hours of incubation) and the modified ISO 16266:2006 method to recover spiked *P. aeruginosa* strains and suppress the detection of spiked non-target bacterial populations in still water samples is shown below. For this part of the study the Pseudalert method was incubated in either a dry incubator or a water bath.

Table 2: Recovery of Spiked Bacterial Populations from Still Water Samples Incubated in a Dry Incubator

Date Tested	Sample Identification	Water Type	Spiked Strain	Spike (cfu/250mL)	Rep	HPC (cfu/mL)	Standard Method		IDEXX Pseudalert	
							Presumptive (cfu/250mL)	Confirmed (cfu/250mL)	Vessel (+ or -)	Incubation (water/dry)
15-Sep-10	Sample #19	Still	*P. migulae*	2.50E+06	1	/	0	0	-	Dry
					2	/	0	0	-	
					3	/	0	0	-	
					4	/	0	0	-	
					5	/	0	0	-	
15-Sep-10	Sample #20	Still	*P. migulae*	2.50E+06	1	7.80E+04	0	0	-	Dry
					2	7.20E+04	0	0	-	
					3	8.20E+04	0	0	-	
27-Sep-10	Sample #21	Still	*P. alcaligenes*	2.50E+06	1	/	tntc	0	-	Dry
					2	/	tntc	0	-	
					3	/	tntc	0	-	
					4	/	tntc	0	-	
					5	/	tntc	0	-	
27-Sep-10	Sample #22	Still	*P. alcaligenes*	2.50E+06	1	4.60E+04	tntc	0	-	Dry
					2	1.80E+04	tntc	0	-	
					3	2.20E+04	tntc	0	-	
22-Sep-10	Sample #23	Still	*P. jessenii*	2.50E+06	1	/	0	0	-	Dry
					2	/	0	0	-	
					3	/	0	0	-	
					4	/	0	0	-	
					5	/	0	0	-	
22-Sep-10	Sample #24	Still	*P. jessenii*	2.50E+06	1	1.90E+04	0	0	-	Dry
					2	2.70E+04	0	0	-	
					3	5.90E+04	0	0	-	
20-Oct-10	Sample #25	Still	*P. fluorescens*	2.50E+06	1	/	0	0	-	Dry
					2	/	0	0	-	
					3	/	0	0	-	
					4	/	0	0	-	
					5	/	0	0	-	
20-Oct-10	Sample #26	Still	*P. fluorescens*	2.50E+06	1		0	0	-	Dry
					2	/	0	0	-	
					3	/	0	0	-	
20-Oct-10	Sample #27	Still	*P. aeruginosa* strain NE 55	100	1	/	45	45	+++	Dry
					2	/	38	38	+++	
					3	2.50E+05	34	34	+++	
					4	/	51	51	+++	
					5	/	41	41	+++	
20-Oct-10	Sample #28	Still	*P. aeruginosa* strain NE 55	8	1	/	6	6	+++	Dry
					2	/	7	7	+++	
					3	5.60E+05	5	5	+++	
					4	/	7	7	+++	
					5	/	8	8	+++	

+++ = high fluorescence

++ = moderate fluorescence

tntc = too numerous to count

Non-target spike

Target spike

Table 3(a): Recovery of Spiked Bacterial Populations from Still Water Samples (1 thru 10) incubated in a Water Bath Incubator

Date Tested	Sample Identification	Water Type	Spiked Strain	Spike (cfu/250mL)	Rep	HPC (cfu/mL)	Standard Method		IDEXX Pseudalert	
							Presumptive (cfu/250mL)	Confirmed (cfu/250mL)	Vessel (+ or -)	Incubation (water/dry)
01-Sep-10	Sample #29	Still	/	/	1	1.20E+05	0	0	-	Water
					2	1.10E+05	0	0	-	
					3	1.10E+05	0	0	-	
01-Sep-10	Sample #30	Still	/	/	1	1.60E+05	0	0	-	Water
					2	1.40E+05	0	0	-	
					3	1.40E+05	0	0	-	
01-Sep-10	Sample #31	Still	/	/	1	2.40E+04	0	0	-	Water
					2	2.00E+04	0	0	-	
					3	1.30E+04	0	0	-	
01-Sep-10	Sample #32	Still	/	/	1	2.30E+04	0	0	-	Water
					2	1.10E+04	0	0	-	
					3	1.70E+04	0	0	-	
07-Sep-10	Sample #33	Still	*P. aeruginosa* strain NE 63	200	1	/	39	39	+++	Water
					2	/	46	46	+++	
					3	/	44	44	+++	
					4	/	41	41	+++	
					5	/	32	32	+++	
07-Sep-10	Sample #34	Still	*P. aeruginosa* strain NE 63	50	1	/	15	15	+++	Water
					2	/	9	9	+++	
					3	/	13	13	+++	
					4	/	11	11	+++	
					5	/	15	15	+++	
07-Sep-10	Sample #35	Still	*P. aeruginosa* strain NE 63	8	1	/	1	1	++	Water
					2	/	1	1	-	
					3	7.50E+04	2	2	++	
					4	/	1	1	++	
					5	/	1	1	++	
07-Sep-10	Sample #36	Still	*P. aeruginosa* strain NE 63	2	1	/	0	0	-	Water
					2	/	0	0	-	
					3	7.10E+04	0	0	-	
					4	/	1	1	-	
					5	/	0	0	-	
13-Oct-10	Sample #37	Still	*P. aeruginosa* strain NE 64	100	1	/	30	30	+++	Water
					2	/	30	30	+++	
					3	/	25	25	+++	
					4	/	32	32	+++	
					5	/	46	46	+++	
13-Oct-10	Sample #38	Still	*P. aeruginosa* strain NE 64	8	1	/	3	3	+++	Water
					2	/	2	2	+++	
					3	1.70E+04	2	2	+++	
					4	/	5	5	+++	
					5	/	4	4	+++	

+++ = high fluorescence

++ = moderate fluorescence

tntc = too numerous too

Unspiked sample

Target spike

Table 3(b): Recovery of Spiked Bacterial Populations from Still Water Samples (11 thru 21) incubated in a Water Bath Incubator

Date Tested	Sample Identification	Water Type	Spiked Strain	Spike (cfu/250mL)	Rep	HPC (cfu/mL)	Standard Method		IDEXX Pseudalert	
							Presumptive (cfu/250mL)	Confirmed (cfu/250mL)	Vessel (+ or -)	Incubation (water/dry)
13-Oct-10	Sample #39	Still	*P. aeruginosa* strain NE 64	2	1	/	0	0	-	Water
					2	/	0	0	-	
					3	4.50E+04	0	0	-	
					4	/	0	0	-	
					5	/	0	0	-	
21-Sep-10	Sample #40	Still	*P. aeruginosa* strain NE 55	100	1	/	56	56	+++	Water
					2	/	63	63	+++	
					3	/	56	56	+++	
					4	/	86	86	+++	
					5	/	71	71	+++	
21-Sep-10	Sample #41	Still	*P. aeruginosa* strain NE 55	8	1	/	5	5	+++	Water
					2	/	8	8	+++	
					3	1.80E+04	3	3	+++	
					4	/	5	5	+++	
					5	/	5	5	+++	
21-Sep-10	Sample #42	Still	*P. aeruginosa* strain NE 55	2	1	/	0	0	-	Water
					2	/	0	0	++	
					3	5.20E+04	2	2	-	
					4	/	2	2	++	
					5	/	1	1	-	
21-Sep-10	Sample #43	Still	*P. aeruginosa* strain NE 78	100	1	/	49	49	+++	Water
					2	/	53	53	+++	
					3	1.20E+04	52	52	+++	
					4	/	48	48	+++	
					5	/	48	48	+++	
21-Sep-10	Sample #44	Still	*P. aeruginosa* strain NE 78	8	1	/	7	7	+++	Water
					2	/	3	3	+++	
					3	4.50E+04	3	3	+++	
					4	/	3	3	+++	
					5	/	3	3	+++	
21-Sep-10	Sample #45	Still	*P. aeruginosa* strain NE 78	2	1	/	0	0	-	Water
					2	/	1	1	++	
					3	4.70E+04	0	0	-	
					4	/	0	0	-	
					5	/	0	0	-	
27-Sep-10	Sample #46	Still	*P. gessardii*	2.50E+06	1	/	0	0	-	Water
					2	/	0	0	-	
					3	/	0	0	-	
					4	/	0	0	-	
					5	/	0	0	-	
27-Sep-10	Sample #47	Still	*P. gessardii*	2.50E+06	1	1.30E+04	0	0	-	Water
					2	7.20E+04	0	0	-	
					3	5.10E+04	0	0	-	
22-Sep-10	Sample #48	Still	*P. stutzeri*	2.50E+06	1	/	tntc	0	-	Water
					2	/	tntc	0	-	
					3	/	tntc	0	-	
					4	/	tntc	0	-	
					5	/	tntc	0	-	
22-Sep-10	Sample #49	Still	*P. stutzeri*	2.50E+06	1	8.60E+04	tntc	0	-	Water
					2	7.10E+04	tntc	0	-	
					3	6.50E+04	tntc	0	-	

++ = moderate fluorescence
tntc = too numerous too

Target spike
Non-target spike

Both the Pseudalert and modified ISO 16266:2006 methods were able to accurately recover and detect the spiked *P. aeruginosa* strains from the still water samples using the spiked cultures (strains NE 55, 63, 64, and 78) even when the bacteria were present at a final concentration as low as 1 cfu/250mL.
The intensity of the fluorescent signal produced by Pseudalert (after 24 hours of incubation) was moderate (++) to high (+++) even when low concentrations of bacteria were present. An interpretation guide is available on the IDEXX website (www.idexx.com/water) which can assist in the interpretation of a positive and negative sample.
Non-spiked samples containing natural flora and samples artificially contaminated by non-target bacterial strains at concentrations exceeding $2.0x10^6$ bacteria per sample, did not show any false positive detection, demonstrating the good specificity of Pseudalert. At these concentrations the modified ISO 16266:2006 method recovered two of these strains (*P. stutzeri* and *P. alcaligenes*) at levels that were too numerous to count on the CN agar and required subsequent confirmation tests to exclude them as possible *P. aeruginosa* strains.

Figure 2: 2 x 2 Table Comparing the Ability of each Method to Detect Spiked *P. aeruginosa* Populations from Still Water Samples (Dry & Wet Incubation combined)

Pseudalert Method	Modified ISO 16266_2006 Method: +	Modified ISO 16266_2006 Method: -
+	56	1
-	4	74

When this data is analyzed statistically using the McNemar's test the calculated p-value was found to be 0.3711, which indicates that the two methods are statistically equivalent in their specificity and ability to recover the *P. aeruginosa* populations

The ability of Pseudalert (after 26 hours of incubation) and the modified ISO 16266:2006 method to recover spiked *P. aeruginosa* strains in Sparkling water samples is shown below. For this part of the study the Pseudalert method was incubated in a water bath in accordance with the product package insert.

Table 4: Recovery of Spiked Bacterial Populations from Sparkling Water Samples (11 thru 21) incubated in a Water Bath Incubator

Date Tested	Sample Identification	Water Type	Spiked Strain	Spike (cfu/250mL)	Rep	HPC (cfu/mL)	Standard Method		IDEXX Pseudalert	
							Presumptive (cfu/250mL)	Confirmed (cfu/250mL)	Vessel (+ or -)	Incubation (water/dry)
01-Sep-10	Sample #50	Sparkling	/	/	1	1.50E+00	0	0	-	Water
					2	1.90E+00	0	0	-	
					3	1.20E+00	0	0	-	
01-Sep-10	Sample #51	Sparkling	/	/	1	1.00E-01	0	0	-	Water
					2	3.00E-01	0	0	-	
					3	5.00E-01	0	0	-	
20-Oct-10	Sample #52	Sparkling 3,9g/l CO2 (4°C)	*P. aeruginosa* strain NE 55	100	1	/	39	39	+++ (48h)	Water
					2	/	39	39	+++ (48h)	
					3	1.00E+00	46	46	+++ (48h)	
					4	/	31	31	+++ (48h)	
					5	/	35	35	+++ (48h)	
20-Oct-10	Sample #53	Sparkling 3,9g/l CO2 (4°C)	*P. aeruginosa* strain NE 55	8	1	/	5	5	+++ (48h)	Water
					2	/	6	6	+++ (48h)	
					3	0.00E+00	7	7	+++ (48h)	
					4	/	7	7	+++ (48h)	
					5	/	8	8	+++ (48h)	
24-Nov-10	Sample #54	Sparkling 3,9g/l CO2 (4°C)	*P. aeruginosa* strain NE 63	100	1	1.00E+00	74	74	+++	Water
					2	1.00E+00	76	76	+++	
					3	/	84	84	+++	
24-Nov-10	Sample #55	Sparkling 6g/l CO2 (4°C)	*P. aeruginosa* strain NE 63	100	1	3.00E+00	55	55	+++	Water
					2	4.00E+00	52	52	+++	
					3	/	59	59	+++	
24-Nov-10	Sample #56	Sparkling 5,3g/l CO2 (4°C)	*P. aeruginosa* strain NE 63	100	1	0.00E+00	52	52	+++	Water
					2	0.00E+00	64	64	+++	
					3	/	77	77	+++	
02-Dec-10	Sample #57	Sparkling 3,9g/l CO2 (4°C)	*P. aeruginosa* strain NE 55	100	1	1.00E+00	31	31	+++	Water
					2	2.00E+00	46	46	+++	
					3	/	41	41	+++	
02-Dec-10	Sample #58	Sparkling 6g/l CO2 (4°C)	*P. aeruginosa* strain NE 55	100	1	3.00E+00	33	33	+++	Water
					2	2.00E+00	23	23	+++	
					3	/	36	36	+++	
02-Dec-10	Sample #59	Sparkling 5,3g/l CO2 (4°C)	*P. aeruginosa* strain NE 55	100	1	0.00E+00	29	29	+++	Water
					2	0.00E+00	36	36	+++	
					3	/	38	38	+++	

+++ = high fluorescence
++ = moderate fluorescence
tntc = too numerous too count

Unpsiked sample
Target spike
Non-target spike

Both the Pseudalert and modified ISO 16266:2006 methods were able to accurately recover and detect the spiked *P. aeruginosa* strains from the Sparkling water samples using the bacterial cultures (strains NE 55 and 63) even when the bacteria were present at a final concentration as low as **5** cfu/250 mL. The Pseudalert method experienced a delay in the detection of *P. aeruginosa* strain NE 55 for the first two sets of Sparkling water tested. A strong fluorescent signal (+++) was not detected

with these samples until after 48 hours of incubation which is beyond the recommended incubation period of the method. This is likely the result of incomplete de-gassing of the test sample which is a requirement of the Pseudalert method when testing Sparkling water samples. The presence of CO_2 in the water sample can slow the detection of *P. aeruginosa* so it's critical that this gas is removed prior to incubation. Subsequent testing with Sparkling water samples containing different concentrations of CO_2 yielded more encouraging results (see data collected in Nov and Dec). A statistical analysis of this data suggests a bias in favor of the modified ISO 16266:2006 method ($p<0.05$) which is primarily due to the delayed recovery of the first two samples tested. Repeated testing with more sparkling water samples will likely correct this discrepancy.

Conclusions

The data presented above clearly demonstrates the favorable recovery and specific detection of *P. aeruginosa* by Pseudalert when compared against the modified ISO 16266:2006 method with water samples collected and analyzed by the European bottled water testing laboratory. Based on this data we can make the following conclusions:

Pseudalert Sensitivity and Trueness –

Pseudalert was able to accurately recover very low concentrations of *P. aeruginosa* (as low as 1 cfu/250 mL of sample) without interference from biological or chemical agents (i.e. minerals or added chlorine) present in the water samples.

When testing thermal water samples naturally contaminated with *P. aeruginosa* at concentrations ranging from 1 to >150 bacteria per 250 mL the Pseudalert method, after 24 hours of incubation, was able to perfectly match the recovery of the modified ISO 16266:2006 method after 48 hours of incubation. Three of these samples were contaminated with *P. aeruginosa* at concentrations less than 10 cfu per 250 mL yet still produced a strong (+++) fluorescent signal with Pseudalert

When testing Still water samples spiked with various *P. aeruginosa* strains at concentrations ranging from 1 to 86 (average ~ 18) bacteria per 250mL the Pseudalert method, after 24 hours of incubation, was able to statistically match the recovery of the modified ISO 16266:2006 method ($p>0.05$) after 48 hours of incubation.

When testing Sparkling water samples spiked with various *P. aeruginosa* strains at concentrations ranging from 5 to 77 (average ~ 40) bacteria per 250 mL the Pseudalert method, after 26 hours of incubation, was able to match the recovery of the modified ISO 16266:2006 method with 18 of the 28 samples tested. 10 of the samples, spiked with between 5 and 46 (average ~ 22) bacteria per 250 mL, were only detected after 48 hours of incubation which suggests that residual gas may have remained in the samples which slowed the growth of the bacteria in the Pseudalert reagent.

Pseudalert Specificity –

Pseudalert was shown to be highly specific for the detection of *P. aeruginosa* in the water samples tested. There were no reported incidences of false positive responses with any of the samples tested.

Around 15 non-spiked samples, containing endogenous flora ranging from <1 to 1,6.10^6 bacteria/ml were analysed. None of these samples resulted in a false positive response with Pseudalert. Likewise, the presence of these bacteria did not interfere with the detection of natural or spiked *P. aeruginosa* populations in the other samples.

48 Still water samples were spiked with high concentrations (~10^4 cfu/mL) of non-aeruginosa Pseudomonas species from the bacterial culture collection and none of them were able to cause a false positive response with Pseudalert. Two of the strains (*P. stutzeri* and *P. alcaligenes*) were able to grow on CN agar from the modified ISO 16266:2006 method and required secondary confirmation testing to exclude them as true positive samples. The Pseudalert method was able to suppress the growth of both these false positive strains.

Pseudalert Robustness –

The Pseudalert method maintained its specificity and sensitivity whether the vessels were incubated in a dry incubator or a water bath for all the non-sparkling water samples tested. When non-aeruginosa Pseudomonas strains were spiked into Still water samples at a concentration of ~10^4 cfu/mL, the test was able to suppress their detection under both incubation conditions. When *P. aeruginosa* strains was spiked into Still water samples, Pseudalert was able to accurately detect it after 24 hours of incubation with both incubation conditions.

When testing Sparkling water samples with Pseudalert we found a delayed response in the detection of a spiked *P. aeruginosa isolate* with 10 of the 28 samples tested. This suggests that residual gas may have remained in the test sample which slowed the detection of the spiked bacteria. This occurrence points towards a possible lack of robustness with the recommended degassing procedure provided by the manufacturer.

Based on these data we conclude that Pseudalert performs at least as well the modified ISO 16266:2006 method at recovering and accurately detecting the presence of *P. aeruginosa* from bottled water matrices. More work should be undertaken to improve the performance with Sparkling water samples possibly by introducing a more robust degassing procedure.

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 Westbrook, Maine; IDEXX employs over 4,800 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 39 countries world-wide.

References

1. ISO 16266:2006 Water quality — Detection and enumeration of *Pseudomonas aeruginosa* — Method by membrane Filtration, Geneva. International Standards Organization