

## IDEXX Summary

## 14C

**Topic:** Beta Trial Study report comparing Pseudalert\* versus the Millipore™ and Cetrимide membrane filtration methods in bottled waters for detection and enumeration of *Pseudomonas aeruginosa*

**Title:** “Comparison of the performance of the IDEXX Pseudalert\* test against the Millipore™ and Cetrимide membrane filtration methods at recovering confirmed *Pseudomonas aeruginosa* from bottled water samples”

**Author:** IDEXX Laboratories

**Date:** November 2010

### Report Highlights:

- Pseudalert was compared to the Millipore and Cetrимide membrane filtration methods at an independent laboratory that regularly tests bottled water samples.
- Fifty four water samples collected at a bottled water plant were included in this study.
- Data from the completed study showed:
  - Pseudalert had comparable detection of *P. aeruginosa* versus the Millipore and Cetrимide membrane filtration methods ( $p = 1.0$ )\*\* from naturally contaminated bottled water samples. Two methods are comparable if  $p > 0.05$ .
  - Pseudalert had comparable recovery ( $p = 0.174$ )\*\*\* to the Millipore method from bottled water samples spiked with *P. aeruginosa* strain ATCC 27853. Two methods are comparable if  $p > 0.05$
  - Pseudalert had superior recovery ( $p = 0.001$ )\*\*\* to the Cetrимide method from bottled water samples spiked with *P. aeruginosa* strain ATCC 27853. Two methods are comparable if  $p > 0.05$ †
- Pseudalert performed as well or better than the Millipore and Cetrимide membrane filtration methods for detection and quantification of *P. aeruginosa* in bottled water samples

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\*\*Based on the McNemar test for paired samples

\*\*\*Based on the Student's t-test (one tail; paired samples)

† Pseudalert method had significantly higher *confirmed* sensitivity versus the Cetrимide method. See page 5 of this Technical Note.

## Technical Note

Comparison of the performance of the IDEXX Pseudalert\* test against the Millipore™ Pseudomonas Selective Broth and Cetrимide Membrane Filtration methods at recovering confirmed *Pseudomonas aeruginosa* from bottled 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 laboratory located in Venezuela that evaluated the performance of the Pseudalert test prior to its launch in September 2010. Samples of water used in the production of bottled water products served as the test matrix for this study and were collected at a bottling plant at different stages of the manufacturing process. The microorganisms present in these water samples were from wild populations that occurred naturally in the environment and did not result from supplemental spiking activities. Additional water samples were collected that were spiked with the *P. aeruginosa* strain ATCC 27853 to simulate a contamination event. Testing occurred over the course of two months. This study compared the relative recovery of confirmed *P. aeruginosa* by Pseudalert after 24 hours of incubation against the Millipore™ Pseudomonas Selective Broth and Cetrимide Membrane Filtration methods.

### Procedure

1. Water samples (>300 mL) were collected at a bottling plant at different stages of the manufacturing process.
2. A 100 mL aliquot of each sample was processed and analyzed following the procedures outlined for the Millipore™ Pseudomonas Selective Broth method (Appendix A). Additional confirmation procedures were added (see description below).
3. A 100 mL aliquot of each sample was processed and analyzed following the procedures outlined for the Cetrимide Membrane Filtration method (Appendix B). Additional confirmation procedures were added (see description below).

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  $38\pm 0.5^{\circ}\text{C}$ .
5. Presumptive *P. aeruginosa* positive samples from each method were subjected to the following confirmation procedures:
  - Growth on Cetrимide agar
  - Skim milk agar hydrolysis
  - Growth at  $42^{\circ}\text{C}$
  - Oxidase test
  - Asparagine Broth (production of green fluorescent pigment)
  - Acetamide Broth (production of purple color)

## Results

Fifty four water samples collected at a bottled water plant were included in this study. Of these samples, ten were found to be naturally contaminated with *P. aeruginosa*. The ability of the Pseudalert, Millipore™, and Cetrimide methods to detect these natural *P. aeruginosa* populations<sup>1</sup> is shown below:

| No. | Sample Tested              | Pseudalert<br>(24hr)                      | Millipore<br>(48hr) | Cetrimide<br>(48hr) |
|-----|----------------------------|---|---------------------|---------------------|
|     |                            | <i>P. aeruginosa</i> Detected<br>(+ or -) |                     |                     |
| 1   | Processed water            | +   | +                   | +                   |
| 2   | Well water #2              | -   | -                   | -                   |
| 3   | Well water #4              | +   | +                   | -                   |
| 4   | Well water #9              | -   | -                   | -                   |
| 5   | Spring water #1            | -   | -                   | -                   |
| 6   | Spring water #2            | -   | -                   | -                   |
| 7   | Spring water #4            | -   | -                   | -                   |
| 8   | Spring water #4.1          | -   | -                   | -                   |
| 9   | Spring water #5            | -   | -                   | -                   |
| 10  | Spring water #6            | -   | -                   | -                   |
| 11  | Spring water #6,1          | -   | -                   | -                   |
| 12  | Filtered water CEP #1      | -   | -                   | -                   |
| 13  | Filtered Water CEP #2      | -   | -                   | -                   |
| 14  | Filtered Water CEP #3      | -   | +                   | +                   |
| 15  | Filtered water CEP #4      | +   | +                   | +                   |
| 16  | Filtered water CEP #PB     | -   | -                   | -                   |
| 17  | Filtered water Comedor #1  | -   | +                   | +                   |
| 18  | Filtered water Comedor #2  | -   | -                   | -                   |
| 19  | Filtered water CTP #1      | -   | -                   | -                   |
| 20  | Filtered water CTP #2      | -   | -                   | -                   |
| 21  | Tap water #1               | -   | -                   | -                   |
| 22  | Tap water #2               | -   | -                   | -                   |
| 23  | Tap water #3               | -   | -                   | -                   |
| 24  | Tap water #4               | -   | -                   | -                   |
| 25  | Industrial water carbon #3 | -   | -                   | -                   |
| 26  | Well water #2              | -   | -                   | +                   |
| 27  | Well water #3              | +   | +                   | +                   |

| No. | Sample Tested                   | Pseudalert<br>(24hr)                      | Millipore<br>(48hr) | Cetrimide<br>(48hr) |
|-----|---------------------------------|---|---------------------|---------------------|
|     |                                 | <i>P. aeruginosa</i> Detected<br>(+ or -) |                     |                     |
| 28  | Well water #4                   | +   | -                   | -                   |
| 29  | Filtered water EFE              | -   | -                   | -                   |
| 30  | Tap water EFE                   | -   | -                   | -                   |
| 31  | Drinking water # 1              | -   | -                   | -                   |
| 32  | Drinking water #2               | -   | -                   | -                   |
| 33  | Drinking water # 3              | -   | -                   | -                   |
| 34  | Drinking water #4               | -   | -                   | -                   |
| 35  | Drinking water # 5              | -   | -                   | -                   |
| 36  | Drinking water # 6              | -   | -                   | -                   |
| 37  | Drinking water # 7              | +   | +                   | +                   |
| 38  | Tap water # 1                   | +   | +                   | +                   |
| 39  | Tap water # 2                   | -   | -                   | -                   |
| 40  | Tap water # 3                   | -   | -                   | -                   |
| 41  | Tap water # 4                   | -   | -                   | -                   |
| 42  | Tap water # 5                   | -   | -                   | -                   |
| 43  | Tap water # 6                   | -   | -                   | -                   |
| 44  | Tap water # 7                   | -   | -                   | -                   |
| 45  | Drinking water # 7              | -   | -                   | -                   |
| 46  | Drinking water # 036            | -   | -                   | -                   |
| 47  | Processed water Tank A          | -   | -                   | -                   |
| 48  | Processed water Tank C          | -   | -                   | -                   |
| 49  | Processed water carbon filter 1 | -   | -                   | -                   |
| 50  | Processed water carbon filter 2 | -   | -                   | -                   |
| 51  | Processed water carbon filter 3 | -   | -                   | -                   |
| 52  | Processed water carbon filter 4 | -   | -                   | -                   |
| 53  | Processed water entry filter    | -   | -                   | -                   |
| 54  | Processed water cold water      | -   | -                   | -                   |

<sup>1</sup>The median count of *P. aeruginosa* in these positive samples was 7 cells/100 mL



Recovery of natural *P. aeruginosa* populations by these methods was analyzed statistically using the McNemar test for matched paired samples. The results of this analysis are shown below and showed comparable ( $p = 1.0$ ) recovery by Pseudalert against the two standard methods.

|            |   |           |    |
|------------|---|-----------|----|
|            |   | Cetrimide |    |
|            |   | +         | -  |
| Pseudalert | + | 5         | 2  |
|            | - | 3         | 44 |

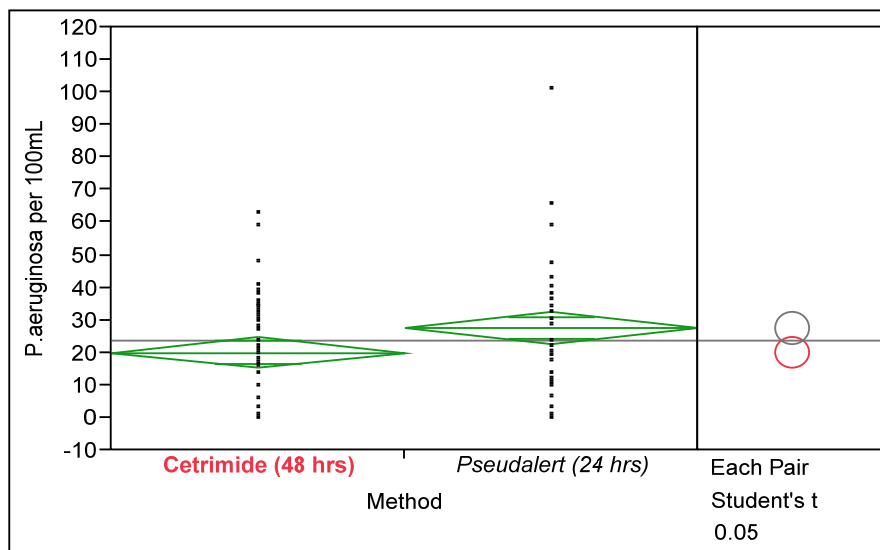
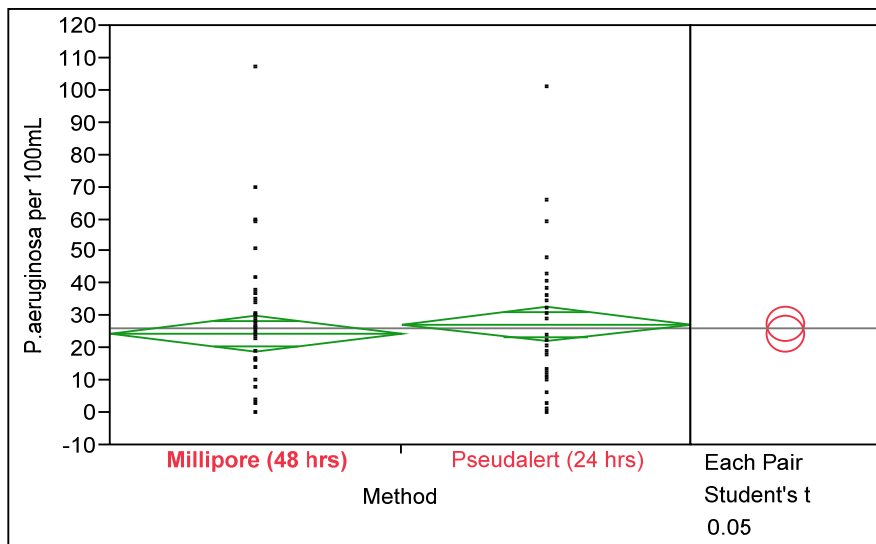
|            |   |           |    |
|------------|---|-----------|----|
|            |   | Millipore |    |
|            |   | +         | -  |
| Pseudalert | + | 6         | 1  |
|            | - | 2         | 45 |

The ability of the Pseudalert, Millipore™, and Cetrimide methods to detect a spiked *P. aeruginosa* strain (ATCC 27853) in these fifty four water samples is shown below:

| No. | Sample Tested              | Pseudalert                                | Millipore | Cetrimide |
|-----|----------------------------|---|-----------|-----------|
|     |                            | (24hr)                                    | (48hr)    | (48hr)    |
|     |                            | <i>P. aeruginosa</i> Detected (per 100mL) |           |           |
| 1   | Processed water            | n/a                                       | n/a       | n/a       |
| 2   | Well water #2              | 11.1                                      | 0         | 39        |
| 3   | Well water #4              | 6.4                                       | 3         | 48        |
| 4   | Well water #9              | 101.3                                     | 8         | 32        |
| 5   | Spring water #1            | 17.8                                      | 0         | 0         |
| 6   | Spring water #2            | 11.1                                      | 0         | 0         |
| 7   | Spring water #4            | 1   | 0         | 0         |
| 8   | Spring water #4.1          | 1   | 0         | 0         |
| 9   | Spring water #5            | 9.9                                       | 0         | 0         |
| 10  | Spring water #6            | 1   | 0         | 0         |
| 11  | Spring water #6.1          | 6.4                                       | 0         | 0         |
| 12  | Filtered water CEP #1      | 9.9                                       | 28        | 16        |
| 13  | Filtered Water CEP #2      | 13.7                                      | 19        | 31        |
| 14  | Filtered Water CEP #3      | 28.8                                      | 37        | 17        |
| 15  | Filtered water CEP #4      | 40.6                                      | 37        | 24        |
| 16  | Filtered water CEP #PB     | 20.7                                      | 17        | 17        |
| 17  | Filtered water Comedor #1  | 42.9                                      | 51        | 59        |
| 18  | Filtered water Comedor #2  | 28.8                                      | 27        | 30        |
| 19  | Filtered water CTP #1      | 23.8                                      | 27        | 30        |
| 20  | Filtered water CTP #2      | 23.8                                      | 35        | 17        |
| 21  | Tap water #1               | 30.6                                      | 28        | 22        |
| 22  | Tap water #2               | 30.6                                      | 28        | 21        |
| 23  | Tap water #3               | 30.6                                      | 25        | 28        |
| 24  | Tap water #4               | 42.9                                      | 27        | 22        |
| 25  | Industrial water carbon #3 | 3.1                                       | 14        | 6         |
| 26  | Well water #2              | 22.2                                      | 17        | 18        |
| 27  | Well water #3              | 23.8                                      | 4         | 22        |

| No. | Sample Tested                   | Pseudalert                                | Millipore | Cetrimide |
|-----|---------------------------------|---|-----------|-----------|
|     |                                 | (24hr)                                    | (48hr)    | (48hr)    |
|     |                                 | <i>P. aeruginosa</i> Detected (per 100mL) |           |           |
| 28  | Well water #4                   | 19.2                                      | 14        | 22        |
| 29  | Filtered water EFE              | 23.8                                      | 0         | 14        |
| 30  | Tap water EFE                   | 34.4                                      | 25        | 17        |
| 31  | Drinking water # 1              | 1   | 4         | 0         |
| 32  | Drinking water #2               | 0   | 10        | 1         |
| 33  | Drinking water # 3              | 12.4                                      | 59        | 0         |
| 34  | Drinking water #4               | 65.9                                      | 107       | 63        |
| 35  | Drinking water # 5              | 28.8                                      | 70        | 18        |
| 36  | Drinking water # 6              | 3.1                                       | 60        | 17        |
| 37  | Drinking water # 7              | >200                                      | 109       | 106       |
| 38  | Tap water # 1                   | 32.4                                      | 26        | 34        |
| 39  | Tap water # 2                   | 36.4                                      | 27        | 0         |
| 40  | Tap water # 3                   | 32.4                                      | 31        | 20        |
| 41  | Tap water # 4                   | 32.4                                      | 38        | 0         |
| 42  | Tap water # 5                   | 34.4                                      | 16        | 10        |
| 43  | Tap water # 6                   | 38.4                                      | 23        | 0         |
| 44  | Tap water # 7                   | 34.4                                      | 34        | 3         |
| 45  | Drinking water # 7              | 22.2                                      | 24        | 28        |
| 46  | Drinking water # 036            | 47.8                                      | 30        | 36        |
| 47  | Processed water Tank A          | 38.4                                      | 27        | 35        |
| 48  | Processed water Tank C          | 42.9                                      | 37        | 38        |
| 49  | Processed water carbon filter 1 | 42.9                                      | 29        | 33        |
| 50  | Processed water carbon filter 2 | 30.6                                      | 35        | 31        |
| 51  | Processed water carbon filter 3 | 59.1                                      | 24        | 41        |
| 52  | Processed water carbon filter 4 | 42.9                                      | 34        | 31        |
| 53  | Processed water entry filter    | 42.9                                      | 27        | 27        |
| 54  | Processed water cold water      | 47.8                                      | 42        | 28        |

The recovery of the spiked *P. aeruginosa* strain by the Pseudalert method was compared statistically against the Millipore and Cetrimide methods using the Student's t-test (one tail; paired samples). Values greater than or equal to 0.05 denote comparable (similar) recovery. This analysis (shown below) indicated that the Pseudalert method had comparable recovery ( $p = 0.174$ ) to the Millipore method. Recovery for Pseudalert ( $p = 0.001$ ) versus to the Cetrimide method indicates Pseudalert had a superior confirmed recovery versus Cetrimide.



## Conclusions

The data presented above clearly demonstrates the favorable detection and quantification of *P. aeruginosa* by Pseudalert when compared against the Millipore and Cetrимide methods with water samples collected from a bottled water plant. Pseudalert was able to accurately recover very low concentrations of *P. aeruginosa* (as low as 1 cfu/100mL of sample). The Pseudalert method showed comparable recovery with a limited number of samples containing naturally occurring populations of *P. aeruginosa* when compared against the Millipore and Cetrимide methods. Pseudalert was also shown to detect and accurately quantify the spiked *P. aeruginosa* strain ATCC 27853 with comparable recovery against the Millipore method and superior recovery against the Cetrимide method. The Cetrимide method seemed prone to significantly underestimate the spiked *P. aeruginosa* population in a large number of the samples tested. In seven cases (see samples 5, 6, 9, 33, 39, 41, and 43) the Cetrимide method failed to detect the presence of the spiked *P. aeruginosa* strain despite being present in Pseudalert at a concentration of at least 10 cfu/100 mL. This represented nearly 13% of the total number of samples tested.

Based on these data we conclude that after 24 hours of incubation Pseudalert performs at least as well as the Cetrимide and Millipore methods at the specific detection and quantification of *P. aeruginosa* from bottled water matrices.

For technical questions, please contact:

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## About IDEXX Laboratories

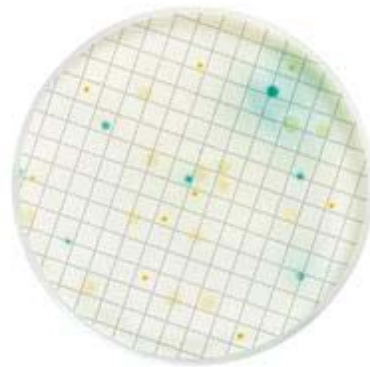
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# Appendix A

## Millipore™ Pseudomonas Selective Broth Method

1. Filter 100mL of water sample through a Millipore (catalog number GSWG047S1) 0.22µM nitrocellulose membrane filter.
2. Add 2mL of Millipore Pseudomonas Select Broth (catalog number MHA000P2P) to a sterile 47mm Millipore Petri-Pad device (catalog number PD20047SO) containing sterile 47mm absorbent pad (catalog number AP10045S1).
3. Place membrane filter onto pad of Petri-Pad device using sterile forceps being careful to avoid the creation of air bubbles underneath the filter.
4. Incubate device at 35 ± 2°C for 48 hours
5. Count colonies that are blue-green in color



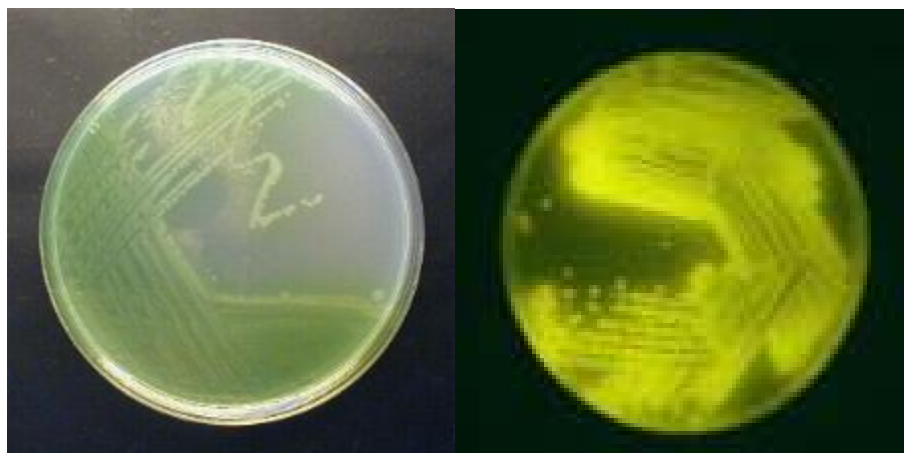
6. Proceed with confirmation of all blue-green colonies



## Appendix B

### Cetrimide Membrane Filtration Method

1. Filter 100mL of water sample through a Millipore™ (catalog number GSWG047S1) 0.22µM nitrocellulose membrane filter.
2. Place membrane filter onto a Cetrimide agar plate (Merck catalog number 1.05284 with 0.1% added glycerol) using sterile forceps being careful to avoid the creation of air bubbles underneath the filter.
3. Incubate device at  $37 \pm 1^\circ\text{C}$  for 48 hours
4. Count colonies that are blue-green in color and fluoresce under UV



5. Proceed with confirmation of all blue-green/fluorescent colonies.