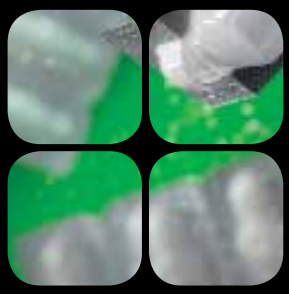

An ISO/TR 13843 Method Performance Validation for the Pseudalert*/Quanti-Tray* System for the Quantitative Detection of *Pseudomonas aeruginosa* in Water

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One IDEXX Drive • Westbrook, Maine 04092 USA

idexx.com

Executive Summary

The Pseudalert*/Quanti-Tray* method is designed for the most-probable-number (MPN) enumeration of *Pseudomonas aeruginosa* from swimming pool and spa water and from bottled water. The Pseudalert test is based on a bacterial enzyme technology that signals the presence of *P. aeruginosa* through the hydrolysis of a fluorogenic substrate diagnostic for the bacterium. The Quanti-Tray is designed to produce MPN quantitative bacterial counts from 100-mL samples using target-organism specific media. The Pseudalert/Quanti-Tray method was validated according to ISO/TR 13843:2000.

The method was challenged with pure cultures of 23 strains of *P. aeruginosa*, 9 strains of other species of *Pseudomonas*, and 15 strains of other common water bacteria. All 23 strains of *P. aeruginosa* produced strong blue fluorescence under 365-nm ultraviolet (UV) light, while none of the other bacteria produced fluorescence. The results of evaluations for sensitivity, specificity, selectivity, and robustness of counting are summarized in the table below.

Sensitivity	94%
Specificity	100%
Selectivity	- 0.30
False positive rate	<1%
False negative rate	6.5%
Efficiency	97%
Repeatability	0.012
Reproducibility	0.017

The data for counts after the recommended incubation period of 24–28 hours showed that for *P. aeruginosa* the method is robust, but that for some slower growing strains or weak enzyme producers there can be an increase in counts at 28 hours of incubation, compared to those at 24 hours of incubation. The Pseudalert results after 24 hours of incubation were found to be at least as sensitive as the ISO 16266 method (after 44 hours of incubation and confirmation). Incubation of Pseudalert beyond 24 hours slightly increased the number of counts. This is a well recognised phenomenon, particularly with environmental bacteria. However, provided that increases in counts after extended incubation are not excessive, for practical and operational considerations, counts after 24 hours of incubation are considered acceptable.

The Pseudalert medium has a stated shelf life of 12 months stored at 4–30°C. The data for three reference strains of *P. aeruginosa*, tested with the same batch of medium stored at 4°C and 30°C, showed no significant difference in the performance of the medium when stored up to 298 days. The two temperatures represent the extremes of the temperature range recommended for storage. Subsequent testing has established that Pseudalert is stable for at least one year when stored at 4–30°C.

The Pseudalert/Quanti-Tray method was compared with the ISO 16266 method using Pseudomonas CN medium, following the requirements of ISO 17994:2004. One hundred and thirty samples were prepared by inoculating dechlorinated swimming pool water with 13 strains of *P. aeruginosa* (made up of a selection of ATCC reference strains, environmental strains obtained from the U.K. Health Protection Agency, and environmental strains collected

by IDEXX Laboratories, Inc.), so as to generate 10 samples for each strain. The outcomes of the ISO 17994 one-tailed analyses are summarized below.

Study	MRD%	S.D.	x_L	x_H	Conclusion
Pseudalert at 24 hours vs. ISO 16266	6.36	29.99	1.10	11.19	Pseudalert significantly higher
Pseudalert at 28 hours vs. ISO 16266	12.00	30.35	6.68	16.76	Pseudalert significantly higher

References

ISO/TR 13843:2000(E) Water Quality – Guidance on validation of microbiological methods. Geneva: International Organisation for Standardization.

ISO 16266:2006 Water Quality – Detection and enumeration of *Pseudomonas aeruginosa* – Method by membrane filtration. Geneva: International Organisation for Standardization.

ISO 17994:2004 Water Quality – Criteria for establishing equivalence between microbiological methods. Geneva: International Organisation for Standardization.

1 Introduction

The Pseudalert*/Quanti-Tray* method is designed for the most-probable-number (MPN) enumeration of *Pseudomonas aeruginosa* from swimming pool and spa water and from bottled water.

Pseudalert is based on a bacterial enzyme technology that signals the presence of *P. aeruginosa* through the hydrolysis of a fluorogenic substrate diagnostic for the bacterium. The Pseudalert reagent is mixed with 100 mL of sample and incubated either as a presence/absence (PA) test or as an MPN test. *P. aeruginosa* is able to grow rapidly in the medium, which is rich in selected amino acids, vitamins, and other nutrients that the bacterium can utilize. When *P. aeruginosa* metabolizes a diagnostic enzyme substrate, the sample will produce blue fluorescence under 365-nm ultraviolet (UV) light. Pseudalert allows detection of *P. aeruginosa* at 1 cfu per 100 mL within 24 hours (26 hours for carbonated bottled water) with the suppression of selected, multiple, non-target bacteria in numbers up to 5×10^3 per 100-mL sample per non-target bacterium. In the presence/absence format, 100-mL or 250-mL samples can be analysed.

The Quanti-Tray is designed to produce quantitative bacterial counts from 100-mL samples using target-organism specific media. The medium/sample mixture is added to a Quanti-Tray pouch, which is then sealed in a Quanti-Tray* Sealer prior to incubation. The pouch is designed so that after sealing there are 51 wells containing reagent/sample mixture. The Sealer is a motor-driven, heated-roller instrument designed to seal a Quanti-Tray. The positive wells are counted, and the MPN of *P. aeruginosa* is determined from an appropriate table.

2 Scope of application of Pseudalert/Quanti-Tray method

The Pseudalert/Quanti-Tray method is primarily designed for the analysis of swimming pool and similar water and for the analysis of carbonated and non-carbonated bottled water.

3 Target organism identification (ISO/TR 13843 sections 10.2.1 and 9.2)

In the Pseudalert/Quanti-Tray method, *P. aeruginosa* are those bacteria that produce any degree of blue fluorescence under 365-nm UV illumination through the metabolism of a diagnostic fluorogenic substrate.

3.1 Pure culture challenge (ISO/TR 13843 section 10.2.1)

Reactions by target and non-target bacteria were confirmed by challenging Pseudalert with pure cultures of reference strains sourced from the American Type Culture Collection (ATCC), environmental strains of *P. aeruginosa*, other species of Pseudomonas and related pseudomonads, and selected Gram-negative and Gram-positive bacteria. Typical reactions of these reference and environmental strains are listed in Table 3.1-1.

Table 3.1-1 Reference strains of *P. aeruginosa*, other species of *Pseudomonas* and related pseudomonads, and selected Gram-negative and Gram-positive bacteria used for confirming typical positive and negative reactions with Pseudalert*

Note: Target bacteria (*P. aeruginosa*) were spiked at <50 cfu per 100 mL. Non-target bacteria were spiked at approximately 500–5,000 cfu per 100 mL.

Bacterium	Source	Reaction in Pseudalert
P. aeruginosa	ATCC[1] 10145[2]	Strong blue fluorescence under UV[3]
	ATCC 9027	Strong blue fluorescence under UV
	ATCC 25628	Strong blue fluorescence under UV
	ATCC 35422	Strong blue fluorescence under UV
	ATCC 35554	Strong blue fluorescence under UV
	Effluent isolate Pa 23	Strong blue fluorescence under UV
	Water isolate Pa 28	Strong blue fluorescence under UV
	Water isolate Pa 31	Strong blue fluorescence under UV
	Water isolate Pa 34	Strong blue fluorescence under UV
	Water isolate Pa 37	Strong blue fluorescence under UV
	Bottled water isolate 1	Strong blue fluorescence under UV
	Bottled water isolate 2	Strong blue fluorescence under UV
	Proficiency test isolate 1	Strong blue fluorescence under UV
	Proficiency test isolate 2	Strong blue fluorescence under UV
	Proficiency test isolate 3	Strong blue fluorescence under UV
	Spa water isolate Pa 42	Strong blue fluorescence under UV
	Spa water isolate Pa 43	Strong blue fluorescence under UV
	Spa water isolate Pa 44	Strong blue fluorescence under UV
	Spa water isolate Pa 45	Strong blue fluorescence under UV
	Clinical isolate Pa 46	Strong blue fluorescence under UV
	Clinical isolate Pa 47	Strong blue fluorescence under UV
	Clinical isolate Pa 48	Strong blue fluorescence under UV
Pseudomonas alcaligenes	Water isolate	No fluorescence under UV
Pseudomonas fluorescens	ATCC 13525[2]	No fluorescence under UV
	Bottled water isolate Pf 3	No fluorescence under UV
Pseudomonas huttiensis	ATTC 14670	No fluorescence under UV
Pseudomonas luteola	Water isolate	No fluorescence under UV
Pseudomonas mendocina	Water isolate Pm 1	No fluorescence under UV
Pseudomonas putida	ATCC 12633	No fluorescence under UV
Pseudomonas stutzeri	Cooling tower isolate	No fluorescence under UV
Pseudomonas sp.	Water isolate	No fluorescence under UV
Burkholderia cepacia	ATCC 17765	No fluorescence under UV
B. cepacia group	Bottled water isolate	No fluorescence under UV
Sphingomonas paucimobilis	ATTC 10829	No fluorescence under UV
Stenotrophomonas maltophilia	Water isolate	No fluorescence under UV
Enterobacter cloacae	Water isolate	No fluorescence under UV
Escherichia coli	ATCC 8739	No fluorescence under UV
	ATCC 11725	No fluorescence under UV
	ATCC 25922[2]	No fluorescence under UV
	Effluent isolate Esc 3	No fluorescence under UV
Serratia fonticola	ATCC 29845	No fluorescence under UV
	Cooling tower isolate	No fluorescence under UV
Serratia marcescens	ATCC 43862	No fluorescence under UV
Serratia sp.	Effluent isolate	No fluorescence under UV
Enterococcus casseliflavus	Broth contaminant	No fluorescence under UV
Enterococcus faecalis	ATCC 33186	No fluorescence under UV

1. American Type Culture Collection (ATCC)
2. ATCC 10145, ATCC 13525, and ATCC 25922 are used as quality control organisms for Pseudalert.
3. 365-nm UV light

3.2 Sensitivity, specificity, false positive/negative rates, efficiency, and selectivity studies (ISO/TR 13843 section 9.2)

In terms of microbiological methods, these characteristics are defined by ISO/TR 13843:

a) True positives: number of presumptive positives found positive

b) False negatives: number of presumptive negatives found positive

c) False positives: number of presumptive positives found negative

d) True negatives: number of presumptive negatives found negative

1) Sensitivity = $a/(a + b)$
   (the fraction of the total positives correctly assigned in the presumptive count)
2) Specificity = $d/(c + d)$
   (the fraction of the total negatives correctly assigned in the presumptive count)
3) False positive rate = $c/(a + c)$
   (the fraction of the observed positives wrongly assigned)
4) False negative rate = $b/(b + d)$
   (the fraction of the observed negatives wrongly assigned)
5) Efficiency (E) = $(a + d)/n$
6) Selectivity (F) = $\log_{10} [(a+c) / (a+b+c+d)]$

For Pseudalert*, these characteristics relate to the number of positive wells that actually contained the target bacterium (*P. aeruginosa*) and the number of negative wells that were truly negative for the target bacterium.

Assessment of the sensitivity, specificity, and selectivity of Pseudalert was achieved through the identification of positive isolates from positive fluorescing wells, generated using samples derived by spiking dechlorinated swimming pool water with 13 strains of *P. aeruginosa* (made up of a selection of ATCC reference strains, environmental strains obtained from the U.K. Health Protection Agency, and environmental strains collected by IDEXX Laboratories, Inc.). Information on the selected strains is presented in Appendix A, Table A-1.

Sufficient swimming pool water was spiked so as to provide ten, 100-mL samples for analysis for each strain of *P. aeruginosa*. After incubation, 25 fluorescing wells were selected from each set of the samples (2–3 wells from each sample) for each strain of *P. aeruginosa* used. These were selected to ensure that the whole range of fluorescence intensities encountered were included. Similarly, 25 negative wells for each strain were selected for isolation of any potential bacteria present. As a result, 325 positive wells and 325 negative wells were subcultured for isolation of any bacteria present. The isolates were identified using the BBL* Crystal* Enteric/NonFermenter (E/NF) Identification Kit and software (BD Diagnostic Systems, Sparks, MD, U.S.A.), a miniaturization identification method.

Isolates were obtained from 324 of the 325 fluorescing subcultured wells. The failure to obtain growth from one positive well was due to a laboratory error. The oxidase and BBL Crystal E/NF profiles and identifications of these isolates are presented in Appendix A, Table A-2. All were identified as *P. aeruginosa*.

Three hundred and twenty-five negative wells were subcultured, of which 21 yielded colonies that were oxidase positive. All were identified as *P. aeruginosa* by the BBL Crystal E/NF system. See Appendix A, Table A-3.

Table 3.2-1 Pseudalert*/Quanti-Tray* system sensitivity, specificity, false-positive and false-negative rates, efficiency, and selectivity

Characteristic	Results
Sensitivity	94%
Specificity	100%
False-positive rate	<1%
False-negative rate	6.5%
Efficiency	97%
Selectivity	-0.30

The outcomes of the data analyses show that, for *P. aeruginosa*, the Pseudalert/Quanti-Tray method is very sensitive and specific for the target bacterium, with a false-positive rate of less than 0.01 (i.e., <1 %).

A false-negative rate of 0.065 was also recorded. This may have been due to stressing of the bacteria when they were inoculated into swimming pool water, resulting in a slower response in the medium.

The method is very selective with a value of -0.30, which is better than the guidance value of -1 suggested by ISO/TR 13843 for colony count methods. The method can also be said to be very efficient for *P. aeruginosa*, with an efficiency value of 0.97.

4 Counting uncertainty (ISO 13843 section 10.2.1 and Annex B)

Repeatability and reproducibility are two estimates of the reliability that can be achieved with an analytical method. These can be assessments of the whole method, using appropriate natural samples, or of the counting uncertainty associated with reading the results of a method. The result produced by any method will depend on the ease with which analysts can count colonies or positive MPN reactions. This will be affected by the distinctiveness of colonial morphology of target and non-target organisms on an enumeration agar or the clarity of positive and negative reactions in broth-based MPN tests. Assessments of counting uncertainty can, therefore, provide an indication of any potential problems that could occur with wide adoption of a method.

Repeatability (r) and reproducibility (R) are defined as follows:

repeatability	Closeness of the agreement between the results of successive measurements of the same measurand, carried out under the same conditions of measurement
Reproducibility	Closeness of the agreement between the results of measurements of the same measurand, carried out under changed conditions of measurement

When applied to counting of microbiological colonies on an agar plate or of positive reactions in an MPN test, the same or changed ‘condition’ is the counting analyst. Thus, in this context, repeatability is the agreement in counts obtained by repeated counting by one analyst, and reproducibility is the agreement in counts obtained by repeated counting by two or more analysts. The assessment of reproducibility is generally more informative than the assessment of repeatability.

Annex B of ISO/TR 13843 provides guidance on the assessment of counting repeatability and reproducibility using relative standard deviations (RSDs) of repeated counts. It also recommends that, when using pure cultures, RSDs should ideally be less than 0.02 (i.e., not more than 2% deviation).

Counting uncertainty studies were conducted by IDEXX analysts. The data are presented in Appendix B. The counting was done so that each analyst was not aware of other analysts' counts. The number of positive wells was recorded separately for each analyst. The counting was undertaken after 24 hours of sample incubation and again after 28 hours of sample incubation. The calculated RSDs for positive wells from Pseudalert*/Quanti-Trays*, inoculated with a reference strain of *P. aeruginosa* (ATCC 27853) are shown below.

Table 4-1 Pseudalert repeatability and Reproducibility

	24 h incubation	28 h incubation
repeatability	0.012	0.016
Reproducibility	0.017	0.013

The values for the counting of positive fluorescing wells of *P. aeruginosa* are below the guidance value of <0.02 (ISO/TR 13843 section B1), indicating that reliable counting of positive wells can be achieved with the Pseudalert/Quanti-Tray method.

5 Robustness—time sensitivity (ISO/TR 13843 sections 10.2.2 and B.4)

Two aspects of robustness are relevant to the Pseudalert/Quanti-Tray test method:

1) The recommended incubation period of 24–28 hours
2) The shelf life of up to 12 months, with storage of the Pseudalert medium at 4–30°C away from light

5.1 Robustness of incubation period

Paired counts of analyses (conducted in duplicate) of suspensions of low numbers of 22 reference and environmental cultures of *P. aeruginosa* were counted after 24 hours and 28 hours of incubation. All samples were tested using the same batch of the Pseudalert test. Incubation was at 38°C ±0.5°C. The 44 paired results are presented in Appendix C, Table C-1.

Results were the same for 36 of the paired counts after the two incubation times. Of the remaining 8 paired counts, 7 had an increase of only 1 positive well at 28 hours. The remaining paired count had a significant increase for 1 of the duplicate samples of the *P. aeruginosa* strain (proficiency test isolate 1), with no increase in the other paired analysis. All the reference isolates and the great majority of the isolates from various water types showed no increase in positive wells at 28 hours of incubation.

The data show that, for *P. aeruginosa*, the recommended incubation period of 24–28 hours is robust, but that for some slower growing strains or weak enzyme producers there can be an increase in counts at 28 hours of incubation, compared to those at 24 hours of incubation.

Increases in counts over a prescribed incubation period (e.g., 20–24 hours or 40–48 hours for the membrane filtration method, such as ISO 16266) is a well recognised phenomenon, particularly with environmental bacteria. However, provided that increases in counts after extended incubation are not excessive, for practical and operational considerations, counts after 24 hours of incubation are considered acceptable.

5.2 Robustness of Pseudalert shelf life

Suspensions of low numbers of three ATCC *P. aeruginosa* reference strains were analysed using a single batch of Pseudalert tests that had been stored for up to 298 days at both 4°C and at 30°C. Replicate samples were analysed after 24 hours and after 28 hours of incubation.

Results are presented in Appendix C, in Tables C-2 and C-3, respectively.

The Pseudalert medium has a stated shelf life of 12 months, when stored at 4–30°C. The data for all three bacteria, tested with the same batch of medium and stored at the two temperatures, show no significant difference in medium performance with storage up to 298 days. The two temperatures represent the extremes of the recommended storage temperature range for the Pseudalert medium. Subsequent testing has established that Pseudalert is stable for at least 1 year when stored at temperatures ranging from 4–30°C.

6 Upper working limit (ISO/TR 13843 sections 10.2.4 and 6.3.3)

The Pseudalert/Quanti-Tray test is an MPN method with an arbitrary counting range of 0 to 201, set by the number of wells in the Quanti-Tray pouch. According to ISO/TR 13843 section 6.3.3, no upper limit can be set for MPN methods for practical reasons and for statistical reasons, "*because precision does not depend in a simple way on the number of particles introduced in the detection set.*"

7 Precision (ISO/TR 13843 sections 10.2.5 and 9.5.5)

The precision of an MPN method is described by the 95% confidence intervals calculated for each MPN value (ISO/TR 13843 section 9.5.5). The confidence intervals for counts from Pseudalert/Quanti-Tray, calculated using the program available from the U.S. Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM)[†] are given in Appendix D.

A theoretical repeatability for each MPN can be derived from the MPN and its confidence intervals. Relative uncertainty values for each MPN ($\log_{10}$SD, calculated from the MPN 95% confidence interval values) and repeatability (relative standard uncertainty) as coefficients of variation (CV%) are also presented in Appendix D. These Poisson values can be used as minimum precision values with which any appropriate data a laboratory may have (e.g., on variation in decanting the test volume or counting uncertainty) can be combined to derive an overall estimate of repeatability for each MPN.

8 Relative recovery (ISO/TR 13843 section 10.2.6 and ISO 17994)

The Pseudalert/Quanti-Tray method was compared with the ISO 16266 method using Pseudomonas CN medium, following the requirements of ISO 17994. One hundred and thirty samples were prepared by inoculating dechlorinated swimming pool water with 13 strains of *P. aeruginosa* (made up of a selection of ATCC reference strains, environmental strains obtained from the U.K. Health Protection Agency, and environmental strains collected by IDEXX Laboratories, Inc.), so as to generate 10 samples for each strain.

Pseudalert/Quanti-Tray pouches were counted after 24 hours and 28 hours of incubation. MPN values were rounded to the nearest whole integer. ISO 16266 plates were counted after 22 hours and 45 hours of incubation and confirmed according to ISO 16266.

Paired confirmed count data for the two methods were natural logarithm (ln) transformed, and the relative difference in counts for each pair (x_i) was calculated as $[\ln(a_i) - \ln(b_i)] \times 100$ % (a_i and b_i being the paired confirmed counts for the trial and reference methods respectively). From the data, a mean relative difference (MRD) and the standard deviation (SD) were calculated. Evaluation of equivalence is based on the MRD and the expanded uncertainty (U), based on the SD of the mean ($U = 2s/\sqrt{n}$), from which the lower (x_L) and higher (x_H) limits of the confidence interval are calculated. Based on this statistical approach and a one-sided evaluation, the outcomes of the comparative study were determined. Outcomes are presented in Table 8-1 (raw data available upon request).

[†] http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm109656.htm

The comparison of Pseudalert/Quanti-Tray MPNs counted after 24 and 28 hours of incubation with confirmed ISO 16266 after 44 hours of incubation resulted in the Pseudalert/Quanti-Tray method producing significantly higher counts at both times on average than did the ISO 16266 method. A total of 324 blue fluorescing wells from Pseudalert*/Quanti-Tray* were subcultured, and all were identified as *P. aeruginosa*. Thus, there were no false-positive reactions.

Table 8-1 Comparison of performance for Pseudalert/Quanti-Tray after incubation for 24 and 28 hours and ISO 16266 for the enumeration of *P. aeruginosa* from water

Study	MRD%	SD	x_L	x_H	Conclusion
Pseudalert at 24 hours vs. ISO 16266	6.36	29.99	1.10	11.19	Pseudalert significantly higher
Pseudalert at 28 hours vs. ISO 16266	12.00	30.35	6.68	16.76	Pseudalert significantly higher

MRD% = Mean relative difference expressed as a percentage
SD = Standard deviation

9 References

ISO/TR 13843:2000(E) Water Quality – Guidance on validation of microbiological methods. Geneva: International Organisation for Standardization.

ISO 16266:2006 Water Quality – Detection and enumeration of *Pseudomonas aeruginosa* – Method by membrane filtration. Geneva: International Organisation for Standardization.

ISO 17994:2004 Water Quality – Criteria for establishing equivalence between microbiological methods. Geneva: International Organisation for Standardization.

Appendix A Data from sensitivity, specificity, and selectivity studies (see section 3.2)

Table A-1 Source data of isolates of *P. aeruginosa* used to challenge Pseudalert*/Quanti-Tray*

Strain No.	Source
#1	HPA[1] PS-3, water isolate
#2	HPA PS-10, spa water isolate
#3	ATCC[2] 10145, type strain
#4	ATCC 27853, isolated from blood culture
#5	ATCC 3554, quality control strain for API products
#6	IDEXX collection, water isolate
#7	ATCC 9027, isolated from otitis externa infection
#8	ATCC 15442, isolate from animal room bottle water
#9	ATCC 25668, clinical isolate
#10	HPA PS-1 (= NCTC[3] 12951), source unknown, probably clinical
#11	HPA PS-7, ship's drinking water isolate
#12	HPA PS-9, same as NCTC 10332, type strain
#13	HPA PS-6, spa water isolate

1. HPA = UK Health Protection Agency collection
2. ATCC = American Type Culture Collection
3. NCTC = U.K. National Collection of Type Cultures

Table A-2 Identification of isolates from fluorescing wells from Pseudalert*/Quanti-Tray*

Strain No.	Isolate No.	Oxidase	Crystal E/NF profile	Identification	Confidence
#1	1.1	+	3413111351	*P. aeruginosa*	0.9997
#1	1.2	+	3413111351	*P. aeruginosa*	0.9997
#1	1.3	+	3413111351	*P. aeruginosa*	0.9997
#1	1.4	+	3413111351	*P. aeruginosa*	0.9997
#1	1.5	+	3413111351	*P. aeruginosa*	0.9997
#1	1.6	+	3413111351	*P. aeruginosa*	0.9997
#1	1.7	+	3413111351	*P. aeruginosa*	0.9997
#1	1.8	+	3413111351	*P. aeruginosa*	0.9997
#1	1.9			No growth (lab error)	
#1	1.10	+	3413111351	*P. aeruginosa*	0.9997
#1	1.11	+	3413111351	*P. aeruginosa*	0.9997
#1	1.12	+	3413111351	*P. aeruginosa*	0.9997
#1	1.13	+	3413111351	*P. aeruginosa*	0.9997
#1	1.14	+	3413111351	*P. aeruginosa*	0.9997
#1	1.15	+	3413111351	*P. aeruginosa*	0.9997
#1	1.16	+	3413111351	*P. aeruginosa*	0.9997
#1	1.17	+	3413111351	*P. aeruginosa*	0.9997
#1	1.18	+	3413111351	*P. aeruginosa*	0.9997
#1	1.19	+	3413111351	*P. aeruginosa*	0.9997
#1	1.20	+	3413111351	*P. aeruginosa*	0.9997
#1	1.21	+	3413111351	*P. aeruginosa*	0.9997
#1	1.22	+	3413111351	*P. aeruginosa*	0.9997
#1	1.23	+	3413111351	*P. aeruginosa*	0.9997
#1	1.24	+	3413111351	*P. aeruginosa*	0.9997
#1	1.25	+	3413111351	*P. aeruginosa*	0.9997
#2	2.1	+	3403111351	*P. aeruginosa*	0.9998
#2	2.2	+	3403111351	*P. aeruginosa*	0.9998
#2	2.3	+	3403111351	*P. aeruginosa*	0.9998
#2	2.4	+	3403111351	*P. aeruginosa*	0.9998
#2	2.5	+	3403111351	*P. aeruginosa*	0.9998
#2	2.6	+	3403111351	*P. aeruginosa*	0.9998
#2	2.7	+	3403111351	*P. aeruginosa*	0.9998
#2	2.8	+	3403111351	*P. aeruginosa*	0.9998
#2	2.9	+	3403111351	*P. aeruginosa*	0.9998
#2	2.10	+	3403111351	*P. aeruginosa*	0.9998
#2	2.11	+	3403111351	*P. aeruginosa*	0.9998
#2	1.12	+	3403111351	*P. aeruginosa*	0.9998
#2	2.13	+	3403111351	*P. aeruginosa*	0.9998
#2	2.14	+	3403111351	*P. aeruginosa*	0.9998
#2	2.15	+	3403111351	*P. aeruginosa*	0.9998
#2	2.16	+	3403111351	*P. aeruginosa*	0.9998
#2	2.17	+	3403111351	*P. aeruginosa*	0.9998
#2	2.18	+	3403111351	*P. aeruginosa*	0.9998
#2	2.19	+	3403111351	*P. aeruginosa*	0.9998
#2	2.20	+	3403111351	*P. aeruginosa*	0.9998
#2	2.21	+	3403111351	*P. aeruginosa*	0.9998
#2	2.22	+	3403111351	*P. aeruginosa*	0.9998
#2	2.23	+	3403111351	*P. aeruginosa*	0.9998

Strain No.	Isolate No.	Oxidase	Crystal E/NF profile	Identification	Confidence
#2	2.24	+	3403111351	*P. aeruginosa*	0.9998
#2	2.25	+	3403111351	*P. aeruginosa*	0.9998
#3	3.1	+	3003111351	*P. aeruginosa*	0.9998
#3	3.2	+	3003111351	*P. aeruginosa*	0.9998
#3	3.3	+	3003111351	*P. aeruginosa*	0.9998
#3	3.4	+	3003111351	*P. aeruginosa*	0.9998
#3	3.5	+	3003111351	*P. aeruginosa*	0.9998
#3	3.6	+	3003111351	*P. aeruginosa*	0.9998
#3	3.7	+	3003111351	*P. aeruginosa*	0.9998
#3	3.8	+	3003111351	*P. aeruginosa*	0.9998
#3	3.9	+	3003111351	*P. aeruginosa*	0.9998
#3	3.10	+	3003111351	*P. aeruginosa*	0.9998
#3	3.11	+	3003111351	*P. aeruginosa*	0.9998
#3	3.12	+	3003111351	*P. aeruginosa*	0.9998
#3	3.13	+	3003111351	*P. aeruginosa*	0.9998
#3	3.14	+	3003111351	*P. aeruginosa*	0.9998
#3	3.15	+	3003111351	*P. aeruginosa*	0.9998
#3	3.16	+	3003111351	*P. aeruginosa*	0.9998
#3	3.17	+	3003111351	*P. aeruginosa*	0.9998
#3	3.18	+	3003111351	*P. aeruginosa*	0.9998
#3	3.19	+	3003111351	*P. aeruginosa*	0.9998
#3	3.20	+	3003111351	*P. aeruginosa*	0.9998
#3	3.21	+	3003111351	*P. aeruginosa*	0.9998
#3	3.22	+	3003111351	*P. aeruginosa*	0.9998
#3	3.23	+	3003111351	*P. aeruginosa*	0.9998
#3	3.24	+	3003111351	*P. aeruginosa*	0.9998
#3	3.25	+	3003111351	*P. aeruginosa*	0.9998
#4	4.1	+	3403111251	*P. aeruginosa*	0.9998
#4	4.2	+	3403111251	*P. aeruginosa*	0.9998
#4	4.3	+	3403111251	*P. aeruginosa*	0.9998
#4	4.4	+	3403111251	*P. aeruginosa*	0.9998
#4	4.5	+	3403111251	*P. aeruginosa*	0.9998
#4	4.6	+	3403111251	*P. aeruginosa*	0.9998
#4	4.7	+	3403111251	*P. aeruginosa*	0.9998
#4	4.8	+	3403111251	*P. aeruginosa*	0.9998
#4	4.9	+	3403111251	*P. aeruginosa*	0.9998
#4	4.10	+	3403111251	*P. aeruginosa*	0.9998
#4	4.11	+	3403111251	*P. aeruginosa*	0.9998
#4	4.12	+	3403111251	*P. aeruginosa*	0.9998
#4	4.13	+	3403111251	*P. aeruginosa*	0.9998
#4	4.14	+	3403111251	*P. aeruginosa*	0.9998
#4	4.15	+	3403111251	*P. aeruginosa*	0.9998
#4	4.16	+	3403111251	*P. aeruginosa*	0.9998
#4	4.17	+	3403111251	*P. aeruginosa*	0.9998
#4	4.18	+	3403111251	*P. aeruginosa*	0.9998
#4	4.19	+	3403111251	*P. aeruginosa*	0.9998
#4	4.20	+	3403111251	*P. aeruginosa*	0.9998
#4	4.21	+	3403111251	*P. aeruginosa*	0.9998
#4	4.22	+	3403111251	*P. aeruginosa*	0.9998
#4	4.23	+	3403111251	*P. aeruginosa*	0.9998

Strain No.	Isolate No.	Oxidase	Crystal E/NF profile	Identification	Confidence
#4	4.24	+	3403111251	*P. aeruginosa*	0.9998
#4	4.25	+	3403111251	*P. aeruginosa*	0.9998
#5	5.1	+	3403111211	*P. aeruginosa*	0.9996
#5	5.2	+	3403111211	*P. aeruginosa*	0.9996
#5	5.3	+	3403111211	*P. aeruginosa*	0.9996
#5	5.4	+	3403111211	*P. aeruginosa*	0.9996
#5	5.5	+	3403111211	*P. aeruginosa*	0.9996
#5	5.6	+	3403111211	*P. aeruginosa*	0.9996
#5	5.7	+	3403111211	*P. aeruginosa*	0.9996
#5	5.8	+	3403111211	*P. aeruginosa*	0.9996
#5	5.9	+	3403111211	*P. aeruginosa*	0.9996
#5	5.10	+	3403111211	*P. aeruginosa*	0.9996
#5	5.11	+	3403111211	*P. aeruginosa*	0.9996
#5	5.12	+	3403111211	*P. aeruginosa*	0.9996
#5	5.13	+	3403111211	*P. aeruginosa*	0.9996
#5	5.14	+	3403111211	*P. aeruginosa*	0.9996
#5	5.15	+	3403111211	*P. aeruginosa*	0.9996
#5	5.16	+	3403111211	*P. aeruginosa*	0.9996
#5	5.17	+	3403111211	*P. aeruginosa*	0.9996
#5	5.18	+	3403111211	*P. aeruginosa*	0.9996
#5	5.19	+	3403111211	*P. aeruginosa*	0.9996
#5	5.20	+	3403111211	*P. aeruginosa*	0.9996
#5	5.21	+	3403111211	*P. aeruginosa*	0.9996
#5	5.22	+	3403111211	*P. aeruginosa*	0.9996
#5	5.23	+	3403111211	*P. aeruginosa*	0.9996
#5	5.24	+	3403111211	*P. aeruginosa*	0.9996
#5	5.25	+	3403111211	*P. aeruginosa*	0.9996
#6	6.1	+	3413111351	*P. aeruginosa*	0.9997
#6	6.2	+	3413111351	*P. aeruginosa*	0.9997
#6	6.3	+	3413111351	*P. aeruginosa*	0.9997
#6	6.4	+	3413111351	*P. aeruginosa*	0.9997
#6	6.5	+	3413111351	*P. aeruginosa*	0.9997
#6	6.6	+	3413111351	*P. aeruginosa*	0.9997
#6	6.7	+	3413111351	*P. aeruginosa*	0.9997
#6	6.8	+	3413111351	*P. aeruginosa*	0.9997
#6	6.9	+	3413111351	*P. aeruginosa*	0.9997
#6	6.10	+	3413111351	*P. aeruginosa*	0.9997
#6	6.11	+	3413111351	*P. aeruginosa*	0.9997
#6	6.12	+	3413111351	*P. aeruginosa*	0.9997
#6	6.13	+	3413111351	*P. aeruginosa*	0.9997
#6	6.14	+	3413111351	*P. aeruginosa*	0.9997
#6	6.15	+	3413111351	*P. aeruginosa*	0.9997
#6	6.16	+	3413111351	*P. aeruginosa*	0.9997
#6	6.17	+	3413111351	*P. aeruginosa*	0.9997
#6	6.18	+	3413111351	*P. aeruginosa*	0.9997
#6	6.19	+	3413111351	*P. aeruginosa*	0.9997
#6	6.20	+	3413111351	*P. aeruginosa*	0.9997
#6	6.21	+	3413111351	*P. aeruginosa*	0.9997
#6	6.22	+	3413111351	*P. aeruginosa*	0.9997
#6	6.23	+	3413111351	*P. aeruginosa*	0.9997

Strain No.	Isolate No.	Oxidase	Crystal E/NF profile	Identification	Confidence
#6	6.24	+	3413111351	*P. aeruginosa*	0.9997
#6	6.25	+	3413111351	*P. aeruginosa*	0.9997
#7	7.1	+	3013111311	*P. aeruginosa*	0.9999
#7	7.2	+	3013111311	*P. aeruginosa*	0.9999
#7	7.3	+	3013111311	*P. aeruginosa*	0.9999
#7	7.4	+	3013111311	*P. aeruginosa*	0.9999
#7	7.5	+	3013111311	*P. aeruginosa*	0.9999
#7	7.6	+	3013111311	*P. aeruginosa*	0.9999
#7	7.7	+	3013111311	*P. aeruginosa*	0.9999
#7	7.8	+	3013111311	*P. aeruginosa*	0.9999
#7	7.9	+	3013111311	*P. aeruginosa*	0.9999
#7	7.10	+	3013111311	*P. aeruginosa*	0.9999
#7	7.11	+	3013111311	*P. aeruginosa*	0.9999
#7	7.12	+	3013111311	*P. aeruginosa*	0.9999
#7	7.13	+	3013111311	*P. aeruginosa*	0.9999
#7	7.14	+	3013111311	*P. aeruginosa*	0.9999
#7	7.15	+	3013111311	*P. aeruginosa*	0.9999
#7	7.16	+	3013111311	*P. aeruginosa*	0.9999
#7	7.17	+	3013111311	*P. aeruginosa*	0.9999
#7	7.18	+	3013111311	*P. aeruginosa*	0.9999
#7	7.19	+	3013111311	*P. aeruginosa*	0.9999
#7	7.20	+	3013111311	*P. aeruginosa*	0.9999
#7	7.21	+	3013111311	*P. aeruginosa*	0.9999
#7	7.22	+	3013111311	*P. aeruginosa*	0.9999
#7	7.23	+	3013111311	*P. aeruginosa*	0.9999
#7	7.24	+	3013111311	*P. aeruginosa*	0.9999
#7	7.25	+	3013111311	*P. aeruginosa*	0.9999
#8	8.1	+	3013111311	*P. aeruginosa*	0.9999
#8	8.2	+	3013111311	*P. aeruginosa*	0.9999
#8	8.3	+	3013111311	*P. aeruginosa*	0.9999
#8	8.4	+	3013111311	*P. aeruginosa*	0.9999
#8	8.5	+	3013111311	*P. aeruginosa*	0.9999
#8	8.6	+	3013111311	*P. aeruginosa*	0.9999
#8	8.7	+	3013111311	*P. aeruginosa*	0.9999
#8	8.8	+	3013111311	*P. aeruginosa*	0.9999
#8	8.9	+	3013111311	*P. aeruginosa*	0.9999
#8	8.10	+	3013111311	*P. aeruginosa*	0.9999
#8	8.11	+	3013111311	*P. aeruginosa*	0.9999
#8	8.12	+	3013111311	*P. aeruginosa*	0.9999
#8	8.13	+	3013111311	*P. aeruginosa*	0.9999
#8	8.14	+	3013111311	*P. aeruginosa*	0.9999
#8	8.15	+	3013111311	*P. aeruginosa*	0.9999
#8	8.16	+	3013111311	*P. aeruginosa*	0.9999
#8	8.17	+	3013111311	*P. aeruginosa*	0.9999
#8	8.18	+	3013111311	*P. aeruginosa*	0.9999
#8	8.19	+	3013111311	*P. aeruginosa*	0.9999
#8	8.20	+	3013111311	*P. aeruginosa*	0.9999
#8	8.21	+	3013111311	*P. aeruginosa*	0.9999
#8	8.22	+	3013111311	*P. aeruginosa*	0.9999
#8	8.23	+	3013111311	*P. aeruginosa*	0.9999

Strain No.	Isolate No.	Oxidase	Crystal E/NF profile	Identification	Confidence
#8	8.24	+	3013111311	*P. aeruginosa*	0.9999
#8	8.25	+	3013111311	*P. aeruginosa*	0.9999
#9	9.1	+	301311131	*P. aeruginosa*	0.9999
#9	9.2	+	301311131	*P. aeruginosa*	0.9999
#9	9.3	+	301311131	*P. aeruginosa*	0.9999
#9	9.4	+	301311131	*P. aeruginosa*	0.9999
#9	9.5	+	301311131	*P. aeruginosa*	0.9999
#9	9.6	+	301311131	*P. aeruginosa*	0.9999
#9	9.7	+	301311131	*P. aeruginosa*	0.9999
#9	9.8	+	301311131	*P. aeruginosa*	0.9999
#9	9.9	+	301311131	*P. aeruginosa*	0.9999
#9	9.10	+	301311131	*P. aeruginosa*	0.9999
#9	9.11	+	301311131	*P. aeruginosa*	0.9999
#9	9.12	+	301311131	*P. aeruginosa*	0.9999
#9	9.13	+	301311131	*P. aeruginosa*	0.9999
#9	9.14	+	301311131	*P. aeruginosa*	0.9999
#9	9.15	+	301311131	*P. aeruginosa*	0.9999
#9	9.16	+	301311131	*P. aeruginosa*	0.9999
#9	9.17	+	301311131	*P. aeruginosa*	0.9999
#9	9.18	+	301311131	*P. aeruginosa*	0.9999
#9	9.19	+	301311131	*P. aeruginosa*	0.9999
#9	9.20	+	301311131	*P. aeruginosa*	0.9999
#9	9.21	+	301311131	*P. aeruginosa*	0.9999
#9	9.22	+	301311131	*P. aeruginosa*	0.9999
#9	9.23	+	301311131	*P. aeruginosa*	0.9999
#9	9.24	+	301311131	*P. aeruginosa*	0.9999
#9	9.25	+	301311131	*P. aeruginosa*	0.9999
#10	10.1	+	3013111211	*P. aeruginosa*	0.9998
#10	10.2	+	3013111211	*P. aeruginosa*	0.9998
#10	10.3	+	3013111211	*P. aeruginosa*	0.9998
#10	10.4	+	3013111211	*P. aeruginosa*	0.9998
#10	10.5	+	3013111211	*P. aeruginosa*	0.9998
#10	10.6	+	3013111211	*P. aeruginosa*	0.9998
#10	10.7	+	3013111211	*P. aeruginosa*	0.9998
#10	10.8	+	3013111211	*P. aeruginosa*	0.9998
#10	10.9	+	3013111211	*P. aeruginosa*	0.9998
#10	10.10	+	3013111211	*P. aeruginosa*	0.9998
#10	10.11	+	3013111211	*P. aeruginosa*	0.9998
#10	10.12	+	3013111211	*P. aeruginosa*	0.9998
#10	10.13	+	3013111211	*P. aeruginosa*	0.9998
#10	10.14	+	3013111211	*P. aeruginosa*	0.9998
#10	10.15	+	3013111211	*P. aeruginosa*	0.9998
#10	10.16	+	3013111211	*P. aeruginosa*	0.9998
#10	10.17	+	3013111211	*P. aeruginosa*	0.9998
#10	10.18	+	3013111211	*P. aeruginosa*	0.9998
#10	10.19	+	3013111211	*P. aeruginosa*	0.9998
#10	10.20	+	3013111211	*P. aeruginosa*	0.9998
#10	10.21	+	3013111211	*P. aeruginosa*	0.9998
#10	10.22	+	3013111211	*P. aeruginosa*	0.9998
#10	10.23	+	3013111211	*P. aeruginosa*	0.9998

Strain No.	Isolate No.	Oxidase	Crystal E/NF profile	Identification	Confidence
#10	10.24	+	3013111211	*P. aeruginosa*	0.9998
#10	10.25	+	3013111211	*P. aeruginosa*	0.9998
#11	11.1	+	3013111311	*P. aeruginosa*	0.9999
#11	11.2	+	3013111311	*P. aeruginosa*	0.9999
#11	11.3	+	3013111311	*P. aeruginosa*	0.9999
#11	11.4	+	3013111311	*P. aeruginosa*	0.9999
#11	11.5	+	3013111311	*P. aeruginosa*	0.9999
#11	11.6	+	3013111311	*P. aeruginosa*	0.9999
#11	11.7	+	3013111311	*P. aeruginosa*	0.9999
#11	11.8	+	3013111311	*P. aeruginosa*	0.9999
#11	11.9	+	3013111311	*P. aeruginosa*	0.9999
#11	11.10	+	3013111311	*P. aeruginosa*	0.9999
#11	11.11	+	3013111311	*P. aeruginosa*	0.9999
#11	11.12	+	3013111311	*P. aeruginosa*	0.9999
#11	11.13	+	3013111311	*P. aeruginosa*	0.9999
#11	11.14	+	3013111311	*P. aeruginosa*	0.9999
#11	11.15	+	3013111311	*P. aeruginosa*	0.9999
#11	11.16	+	3013111311	*P. aeruginosa*	0.9999
#11	11.17	+	3013111311	*P. aeruginosa*	0.9999
#11	11.18	+	3013111311	*P. aeruginosa*	0.9999
#11	11.19	+	3013111311	*P. aeruginosa*	0.9999
#11	11.20	+	3013111311	*P. aeruginosa*	0.9999
#11	11.21	+	3013111311	*P. aeruginosa*	0.9999
#11	11.22	+	3013111311	*P. aeruginosa*	0.9999
#11	11.23	+	3013111311	*P. aeruginosa*	0.9999
#11	11.24	+	3013111311	*P. aeruginosa*	0.9999
#11	11.25	+	3013111311	*P. aeruginosa*	0.9999
#12	12.1	+	3013111311	*P. aeruginosa*	0.9999
#12	12.2	+	3013111311	*P. aeruginosa*	0.9999
#12	12.3	+	3013111311	*P. aeruginosa*	0.9999
#12	12.4	+	3013111311	*P. aeruginosa*	0.9999
#12	12.5	+	3013111311	*P. aeruginosa*	0.9999
#12	12.6	+	3013111311	*P. aeruginosa*	0.9999
#12	12.7	+	3013111311	*P. aeruginosa*	0.9999
#12	12.8	+	3013111311	*P. aeruginosa*	0.9999
#12	12.9	+	3013111311	*P. aeruginosa*	0.9999
#12	12.10	+	3013111311	*P. aeruginosa*	0.9999
#12	12.11	+	3013111311	*P. aeruginosa*	0.9999
#12	12.12	+	3013111311	*P. aeruginosa*	0.9999
#12	12.13	+	3013111311	*P. aeruginosa*	0.9999
#12	12.14	+	3013111311	*P. aeruginosa*	0.9999
#12	12.15	+	3013111311	*P. aeruginosa*	0.9999
#12	12.16	+	3013111311	*P. aeruginosa*	0.9999
#12	12.17	+	3013111311	*P. aeruginosa*	0.9999
#12	12.18	+	3013111311	*P. aeruginosa*	0.9999
#12	12.19	+	3013111311	*P. aeruginosa*	0.9999
#12	12.20	+	3013111311	*P. aeruginosa*	0.9999
#12	12.21	+	3013111311	*P. aeruginosa*	0.9999
#12	12.22	+	3013111311	*P. aeruginosa*	0.9999
#12	12.23	+	3013111311	*P. aeruginosa*	0.9999

Strain No.	Isolate No.	Oxidase	Crystal E/NF profile	Identification	Confidence
#12	12.24	+	3013111311	*P. aeruginosa*	0.9999
#12	12.25	+	3013111311	*P. aeruginosa*	0.9999
#13	13.1	+	3013111351	*P. aeruginosa*	0.9999
#13	13.2	+	3013111351	*P. aeruginosa*	0.9999
#13	13.3	+	3013111351	*P. aeruginosa*	0.9999
#13	13.4	+	3013111351	*P. aeruginosa*	0.9999
#13	13.5	+	3013111351	*P. aeruginosa*	0.9999
#13	13.6	+	3013111351	*P. aeruginosa*	0.9999
#13	13.7	+	3013111351	*P. aeruginosa*	0.9999
#13	13.8	+	3013111351	*P. aeruginosa*	0.9999
#13	13.9	+	3013111351	*P. aeruginosa*	0.9999
#13	13.10	+	3013111351	*P. aeruginosa*	0.9999
#13	13.11	+	3013111351	*P. aeruginosa*	0.9999
#13	13.12	+	3013111351	*P. aeruginosa*	0.9999
#13	13.13	+	3013111351	*P. aeruginosa*	0.9999
#13	13.14	+	3013111351	*P. aeruginosa*	0.9999
#13	13.15	+	3013111351	*P. aeruginosa*	0.9999
#13	13.16	+	3013111351	*P. aeruginosa*	0.9999
#13	13.17	+	3013111351	*P. aeruginosa*	0.9999
#13	13.18	+	3013111351	*P. aeruginosa*	0.9999
#13	13.19	+	3013111351	*P. aeruginosa*	0.9999
#13	13.20	+	3013111351	*P. aeruginosa*	0.9999
#13	13.21	+	3013111351	*P. aeruginosa*	0.9999
#13	13.22	+	3013111351	*P. aeruginosa*	0.9999
#13	13.23	+	3013111351	*P. aeruginosa*	0.9999
#13	13.24	+	3013111351	*P. aeruginosa*	0.9999
#13	13.25	+	3013111351	*P. aeruginosa*	0.9999

Table A-3 Identification of isolates from negative wells from Pseudalert*/ Quanti-Tray*

Strain No	Isolate No	Oxidase	Crystal E/NF profile	Identification	Confidence
#1	N1.1			No growth	
#1	N1.2			No growth	
#1	N1.3			No growth	
#1	N1.4			No growth	
#1	N1.5	+	3413111351	*P. aeruginosa*	0.9997
#1	N1.6			No growth	
#1	N1.7			No growth	
#1	N1.8			No growth	
#1	N1.9			No growth	
#1	N1.10			No growth	
#1	N1.11			No growth	
#1	N1.12			No growth	
#1	N1.13			No growth	
#1	N1.14			No growth	
#1	N1.15			No growth	
#1	N1.16			No growth	
#1	N1.17	+	3413111351	*P. aeruginosa*	0.9997
#1	N1.18			No growth	
#1	N1.19			No growth	
#1	N1.20			No growth	
#1	N1.21			No growth	
#1	N1.22			No growth	
#1	N1.23			No growth	
#1	N1.24			No growth	
#1	N1.25			No growth	
#2	N2.1			No growth	
#2	N2.2			No growth	
#2	N2.3	+	3403111351	*P. aeruginosa*	0.9998
#2	N2.4			No growth	
#2	N2.5			No growth	
#2	N2.6			No growth	
#2	N2.7			No growth	
#2	N2.8			No growth	
#2	N2.9			No growth	
#2	N2.10			No growth	
#2	N2.11			No growth	
#2	N2.12			No growth	
#2	N2.13			No growth	
#2	N2.14			No growth	
#2	N2.15			No growth	
#2	N2.16			No growth	
#2	N2.17			No growth	
#2	N2.18			No growth	
#2	N2.19			No growth	
#2	N2.20			No growth	
#2	N2.21			No growth	
#2	N2.22			No growth	
#2	N2.23			No growth	
#2	N2.24			No growth	
#2	N2.25			No growth	

Strain No	Isolate No	Oxidase	Crystal E/NF profile	Identification	Confidence
#3	N3.1			No growth	
#3	N3.2			No growth	
#3	N3.3			No growth	
#3	N3.4	+	3003111351	*P. aeruginosa*	0.9998
#3	N3.5			No growth	
#3	N3.6			No growth	
#3	N3.7			No growth	
#3	N3.8			No growth	
#3	N3.9			No growth	
#3	N3.10	+	3003111351	*P. aeruginosa*	0.9998
#3	N3.11			No growth	
#3	N3.12			No growth	
#3	N3.13	+	3003111351	*P. aeruginosa*	0.9998
#3	N3.14			No growth	
#3	N3.15			No growth	
#3	N3.16			No growth	
#3	N3.17			No growth	
#3	N3.18			No growth	
#3	N3.19	+	3003111351	*P. aeruginosa*	0.9998
#3	N3.20			No growth	
#3	N3.21			No growth	
#3	N3.22	+	3003111351	*P. aeruginosa*	0.9998
#3	N3.23			No growth	
#3	N3.24			No growth	
#3	N3.25	+	3003111351	*P. aeruginosa*	0.9998
#4	N4.1			No growth	
#4	N4.2			No growth	
#4	N4.3			No growth	
#4	N4.4	+	3403111251	*P. aeruginosa*	0.9998
#4	N4.5			No growth	
#4	N4.6			No growth	
#4	N4.7			No growth	
#4	N4.8			No growth	
#4	N4.9			No growth	
#4	N4.10	+	3403111251	*P. aeruginosa*	0.9998
#4	N4.11			No growth	
#4	N4.12			No growth	
#4	N4.13	+	3403111251	*P. aeruginosa*	0.9998
#4	N4.14			No growth	
#4	N4.15			No growth	
#4	N4.16			No growth	
#4	N4.17			No growth	
#4	N4.18	+	3403111251	*P. aeruginosa*	0.9998
#4	N4.19			No growth	
#4	N4.20			No growth	
#4	N4.21			No growth	
#4	N4.22			No growth	
#4	N4.23			No growth	
#4	N4.24			No growth	
#4	N4.25	+	3403111251	*P. aeruginosa*	0.9998

Strain No	Isolate No	Oxidase	Crystal E/NF profile	Identification	Confidence
#5	N5.1			No growth	
#5	N5.2			No growth	
#5	N5.3			No growth	
#5	N5.4			No growth	
#5	N5.5			No growth	
#5	N5.6			No growth	
#5	N5.7			No growth	
#5	N5.8			No growth	
#5	N5.9			No growth	
#5	N5.10			No growth	
#5	N5.11			No growth	
#5	N5.12			No growth	
#5	N5.13			No growth	
#5	N5.14			No growth	
#5	N5.15			No growth	
#5	N5.16			No growth	
#5	N5.17			No growth	
#5	N5.18			No growth	
#5	N5.19			No growth	
#5	N5.20			No growth	
#5	N5.21			No growth	
#5	N5.22			No growth	
#5	N5.23			No growth	
#5	N5.24			No growth	
#5	N5.25			No growth	
#6	N6.1	+	3413111311	*P. aeruginosa*	0.9999
#6	N6.2			No growth	
#6	N6.3			No growth	
#6	N6.4			No growth	
#6	N6.5			No growth	
#6	N6.6			No growth	
#6	N6.7			No growth	
#6	N6.8			No growth	
#6	N6.9			No growth	
#6	N6.10			No growth	
#6	N6.11			No growth	
#6	N6.12			No growth	
#6	N6.13			No growth	
#6	N6.14			No growth	
#6	N6.15			No growth	
#6	N6.16			No growth	
#6	N6.17			No growth	
#6	N6.18			No growth	
#6	N6.19			No growth	
#6	N6.20			No growth	
#6	N6.21			No growth	
#6	N6.22			No growth	
#6	N6.23			No growth	
#6	N6.24			No growth	
#6	N6.25			No growth	

Strain No	Isolate No	Oxidase	Crystal E/NF profile	Identification	Confidence
#7	N7.1			No growth	
#7	N7.2			No growth	
#7	N7.3			No growth	
#7	N7.4			No growth	
#7	N7.5			No growth	
#7	N7.6			No growth	
#7	N7.7			No growth	
#7	N7.8			No growth	
#7	N7.9			No growth	
#7	N7.10			No growth	
#7	N7.11			No growth	
#7	N7.12			No growth	
#7	N7.13			No growth	
#7	N7.14			No growth	
#7	N7.15			No growth	
#7	N7.16			No growth	
#7	N7.17			No growth	
#7	N7.18			No growth	
#7	N7.19			No growth	
#7	N7.20			No growth	
#7	N7.21			No growth	
#7	N7.22			No growth	
#7	N7.23			No growth	
#7	N7.24			No growth	
#7	N7.25			No growth	
#8	N8.1			No growth	
#8	N8.2			No growth	
#8	N8.3			No growth	
#8	N8.4			No growth	
#8	N8.5			No growth	
#8	N8.6			No growth	
#8	N8.7			No growth	
#8	N8.8			No growth	
#8	N8.9			No growth	
#8	N8.10			No growth	
#8	N8.11			No growth	
#8	N8.12			No growth	
#8	N8.13			No growth	
#8	N8.14	+	3013111211	*P. aeruginosa*	0.9998
#8	N8.15			No growth	
#8	N8.16			No growth	
#8	N8.17			No growth	
#8	N8.18			No growth	
#8	N8.19			No growth	
#8	N8.20			No growth	
#8	N8.21			No growth	
#8	N8.22			No growth	
#8	N8.23			No growth	
#8	N8.24			No growth	
#8	N8.25			No growth	

Strain No	Isolate No	Oxidase	Crystal E/NF profile	Identification	Confidence
#9	N9.1			No growth	
#9	N9.2			No growth	
#9	N9.3			No growth	
#9	N9.4			No growth	
#9	N9.5			No growth	
#9	N9.6			No growth	
#9	N9.7			No growth	
#9	N9.8			No growth	
#9	N9.9			No growth	
#9	N9.10			No growth	
#9	N9.11			No growth	
#9	N9.12	+	3013111211	*P. aeruginosa*	0.9998
#9	N9.13			No growth	
#9	N9.14	+	3413111311	*P. aeruginosa*	0.9999
#9	N9.15			No growth	
#9	N9.16			No growth	
#9	N9.17			No growth	
#9	N9.18			No growth	
#9	N9.19			No growth	
#9	N9.20			No growth	
#9	N9.21	+	3413111211	*P. aeruginosa*	0.9999
#9	N9.22			No growth	
#9	N9.23	+	3413111211	*P. aeruginosa*	0.9999
#9	N9.24			No growth	
#9	N9.25			No growth	
#10	N10.1			No growth	
#10	N10.2			No growth	
#10	N10.3			No growth	
#10	N10.4			No growth	
#10	N10.5			No growth	
#10	N10.6			No growth	
#10	N10.7			No growth	
#10	N10.8			No growth	
#10	N10.9			No growth	
#10	N10.10			No growth	
#10	N10.11			No growth	
#10	N10.12			No growth	
#10	N10.13			No growth	
#10	N10.14			No growth	
#10	N10.15			No growth	
#10	N10.16			No growth	
#10	N10.17			No growth	
#10	N10.18			No growth	
#10	N10.19			No growth	
#10	N10.20			No growth	
#10	N10.21			No growth	
#10	N10.22			No growth	
#10	N10.23			No growth	
#10	N10.24			No growth	
#10	N10.25			No growth	

Strain No	Isolate No	Oxidase	Crystal E/NF profile	Identification	Confidence
#11	N11.1			No growth	
#11	N11.2			No growth	
#11	N11.3			No growth	
#11	N11.4			No growth	
#11	N11.5			No growth	
#11	N11.6			No growth	
#11	N11.7			No growth	
#11	N11.8			No growth	
#11	N11.9			No growth	
#11	N11.10			No growth	
#11	N11.11			No growth	
#11	N11.12			No growth	
#11	N11.13			No growth	
#11	N11.14			No growth	
#11	N11.15			No growth	
#11	N11.16			No growth	
#11	N11.17			No growth	
#11	N11.18			No growth	
#11	N11.19			No growth	
#11	N11.20			No growth	
#11	N11.21			No growth	
#11	N11.22			No growth	
#11	N11.23			No growth	
#11	N11.24			No growth	
#11	N11.25			No growth	
#12	N12.1			No growth	
#12	N12.2			No growth	
#12	N12.3			No growth	
#12	N12.4			No growth	
#12	N12.5			No growth	
#12	N12.6	+	3013111311	*P. aeruginosa*	0.9999
#12	N12.7			No growth	
#12	N12.8			No growth	
#12	N12.9			No growth	
#12	N12.10			No growth	
#12	N12.11			No growth	
#12	N12.12			No growth	
#12	N12.13			No growth	
#12	N12.14			No growth	
#12	N12.15			No growth	
#12	N12.16			No growth	
#12	N12.17			No growth	
#12	N12.18			No growth	
#12	N12.19			No growth	
#12	N12.20			No growth	
#12	N12.21			No growth	
#12	N12.22			No growth	
#12	N12.23			No growth	
#12	N12.24			No growth	
#12	N12.25			No growth	

Strain No	Isolate No	Oxidase	Crystal E/NF profile	Identification	Confidence
#13	N13.1			No growth	
#13	N13.2			No growth	
#13	N13.3			No growth	
#13	N13.4			No growth	
#13	N13.5			No growth	
#13	N13.6			No growth	
#13	N13.7			No growth	
#13	N13.8			No growth	
#13	N13.9			No growth	
#13	N13.10			No growth	
#13	N13.11			No growth	
#13	N13.12			No growth	
#13	N13.13			No growth	
#13	N13.14			No growth	
#13	N13.15			No growth	
#13	N13.16			No growth	
#13	N13.17			No growth	
#13	N13.18			No growth	
#13	N13.19			No growth	
#13	N13.20			No growth	
#13	N13.21			No growth	
#13	N13.22			No growth	
#13	N13.23			No growth	
#13	N13.24			No growth	
#13	N13.25			No growth	

Appendix B Counting uncertainty for fluorescing wells from Pseudalert*/Quanti-Tray* inoculated with *P. aeruginosa* ATCC 27853 (see section 4)

Table B-1 Repeatability data for reading of positive wells after 24-hour Pseudalert* incubation

Analyst A : Pseudalert reads @ 24 hrs +ve wells					
Sample	**Read 1**	**Read 2**	**Mean**	**SD**	**RSD sq**
10-1	18	18	18	0.000	0.000
10-2	21	21	21	0.000	0.000
10-3	12	12	12	0.000	0.000
10-4	19	19	19	0.000	0.000
10-5	19	19	19	0.000	0.000
10-6	13	13	13	0.000	0.000
10-7	23	23	23	0.000	0.000
10-8	15	16	15.5	0.707	0.002
10-9	20	20	20	0.000	0.000
10-10	15	15	15	0.000	0.000
20-1	33	33	33	0.000	0.000
20-2	29	29	29	0.000	0.000
20-3	32	33	32.5	0.707	0.000
20-4	26	26	26	0.000	0.000
20-5	32	33	32.5	0.707	0.000
20-6	28	28	28	0.000	0.000
20-7	26	25	25.5	0.707	0.001
20-8	35	35	35	0.000	0.000
20-9	30	30	30	0.000	0.000
20-10	35	34	34.5	0.707	0.000
40-1	44	43	43.5	0.707	0.000
40-2	43	43	43	0.000	0.000
40-3	43	43	43	0.000	0.000
40-4	42	42	42	0.000	0.000
40-5	42	42	42	0.000	0.000
40-6	40	41	40.5	0.707	0.000
40-7	44	44	44	0.000	0.000
40-8	35	36	35.5	0.707	0.000
40-9	43	43	43	0.000	0.000
40-10	36	36	36	0.000	0.000
60-1	46	45	45.5	0.707	0.000
60-2	47	47	47	0.000	0.000
60-3	47	47	47	0.000	0.000
60-4	46	46	46	0.000	0.000
60-5	48	48	48	0.000	0.000
60-6	48	48	48	0.000	0.000
60-7	46	46	46	0.000	0.000
60-8	45	45	45	0.000	0.000
60-9	49	49	49	0.000	0.000
60-10	48	46	47	1.414	0.001
80-1	50	49	49.5	0.707	0.000
80-2	49	50	49.5	0.707	0.000
80-3	50	50	50	0.000	0.000

Analyst A : Pseudalert reads @ 24 hrs +ve wells					
Sample	**Read 1**	**Read 2**	**Mean**	**SD**	**RSD sq**
80-4	51	51	51	0.000	0.000
80-5	50	50	50	0.000	0.000
80-6	50	50	50	0.000	0.000
80-7	50	50	50	0.000	0.000
80-8	51	50	50.5	0.707	0.000
80-9	51	51	51	0.000	0.000
80-10	50	49	49.5	0.707	0.000
				Sum RSD	0.007
				RSDc	0.012

Table B-2 Repeatability data for reading of positive wells after 28-hour Pseudalert* incubation

Analyst A : Pseudalert reads @ 28 hrs +ve wells					
Sample	**Read 1**	**Read 2**	**Mean**	**SD**	**RSD sq**
10-1	20	20	20	0.000	0.000
10-2	21	21	21	0.000	0.000
10-3	12	12	12	0.000	0.000
10-4	22	20	21	1.414	0.005
10-5	19	19	19	0.000	0.000
10-6	14	14	14	0.000	0.000
10-7	26	26	26	0.000	0.000
10-8	17	16	16.5	0.707	0.002
10-9	21	21	21	0.000	0.000
10-10	15	15	15	0.000	0.000
20-1	33	33	33	0.000	0.000
20-2	31	31	31	0.000	0.000
20-3	33	33	33	0.000	0.000
20-4	27	27	27	0.000	0.000
20-5	33	33	33	0.000	0.000
20-6	28	28	28	0.000	0.000
20-7	26	26	26	0.000	0.000
20-8	35	33	34	1.414	0.002
20-9	30	30	30	0.000	0.000
20-10	35	35	35	0.000	0.000
40-1	43	43	43	0.000	0.000
40-2	43	43	43	0.000	0.000
40-3	43	43	43	0.000	0.000
40-4	42	42	42	0.000	0.000
40-5	42	42	42	0.000	0.000
40-6	41	41	41	0.000	0.000
40-7	44	44	44	0.000	0.000
40-8	36	36	36	0.000	0.000
40-9	45	45	45	0.000	0.000
40-10	36	36	36	0.000	0.000
60-1	45	45	45	0.000	0.000
60-2	49	48	48.5	0.707	0.000
60-3	48	45	46.5	2.121	0.002
60-4	46	46	46	0.000	0.000
60-5	48	48	48	0.000	0.000
60-6	49	48	48.5	0.707	0.000
60-7	46	46	46	0.000	0.000
60-8	45	45	45	0.000	0.000
60-9	49	49	49	0.000	0.000
60-10	46	46	46	0.000	0.000
80-1	49	49	49	0.000	0.000
80-2	49	50	49.5	0.707	0.000
80-3	50	50	50	0.000	0.000
80-4	51	51	51	0.000	0.000
80-5	50	50	50	0.000	0.000
80-6	50	50	50	0.000	0.000
80-7	50	50	50	0.000	0.000
80-8	50	50	50	0.000	0.000
80-9	51	49	50	1.414	0.001

Analyst A : Pseudalert reads @ 28 hrs +ve wells					
Sample	**Read 1**	**Read 2**	**Mean**	**SD**	**RSD sq**
80-10	49	51	50	1.414	0.001
				Sum RSD	0.012
				RSDc	0.016

Table B-3 Reproducibility data for reading of positive wells after 24-hour Pseudalert* incubation

Three analysts – Pseudalert reads @ 24 hrs +ve wells						
Sample	**A**	**B**	**C**	**Mean**	**SD**	**RSD sq**
10-1	18	18	19	18.3	0.577	0.001
10-2	21	21	21	21.0	0.000	0.000
10-3	12	12	12	12.0	0.000	0.000
10-4	19	19	20	19.3	0.577	0.001
10-5	19	19	19	19.0	0.000	0.000
10-6	13	13	14	13.3	0.577	0.002
10-7	23	23	24	23.3	0.577	0.001
10-8	15	16	17	16.0	1.000	0.004
10-9	20	20	20	20.0	0.000	0.000
10-10	15	15	15	15.0	0.000	0.000
20-1	33	33	33	33.0	0.000	0.000
20-2	29	29	31	29.7	1.155	0.002
20-3	32	33	34	33.0	1.000	0.001
20-4	26	26	26	26.0	0.000	0.000
20-5	32	33	33	32.7	0.577	0.000
20-6	28	28	28	28.0	0.000	0.000
20-7	26	25	26	25.7	0.577	0.001
20-8	35	35	36	35.3	0.577	0.000
20-9	30	30	30	30.0	0.000	0.000
20-10	35	35	35	35.0	0.000	0.000
40-1	44	43	43	43.3	0.577	0.000
40-2	43	43	43	43.0	0.000	0.000
40-3	43	43	43	43.0	0.000	0.000
40-4	42	42	42	42.0	0.000	0.000
40-5	42	42	42	42.0	0.000	0.000
40-6	40	40	40	40.0	0.000	0.000
40-7	44	43	43	43.3	0.577	0.000
40-8	35	35	36	35.3	0.577	0.000
40-9	43	43	45	43.7	1.155	0.001
40-10	36	36	36	36.0	0.000	0.000
60-1	46	45	46	45.7	0.577	0.000
60-2	47	47	47	47.0	0.000	0.000
60-3	47	46	46	46.3	0.577	0.000
60-4	46	46	46	46.0	0.000	0.000
60-5	48	48	48	48.0	0.000	0.000
60-6	48	48	48	48.0	0.000	0.000
60-7	46	46	46	46.0	0.000	0.000
60-8	45	44	45	44.7	0.577	0.000
60-9	49	49	49	49.0	0.000	0.000
60-10	48	47	47	47.3	0.577	0.000
80-1	50	49	49	49.3	0.577	0.000
80-2	49	49	49	49.0	0.000	0.000
80-3	50	50	50	50.0	0.000	0.000
80-4	51	51	51	51.0	0.000	0.000
80-5	50	50	50	50.0	0.000	0.000
80-6	50	50	50	50.0	0.000	0.000
80-7	50	49	50	49.7	0.577	0.000
80-8	51	50	50	50.3	0.577	0.000
80-9	51	49	50	50.0	1.000	0.000

Three analysts – Pseudalert reads @ 24 hrs +ve wells						
Sample	**A**	**B**	**C**	**Mean**	**SD**	**RSD sq**
80-10	50	49	49	49.3	0.577	0.000
					Sum RSD	0.015
					RSDc	0.017

Table B-4 Reproducibility data for reading of positive wells after 28-hour Pseudalert* incubation

Three analysts – Pseudalert reads @ 28 hrs +ve wells						
Sample	**A**	**B**	**C**	**Mean**	**SD**	**RSD sq**
10-1	20	20	20	20.0	0.000	0.000
10-2	21	21	21	21.0	0.000	0.000
10-3	12	13	13	12.7	0.577	0.002
10-4	22	22	22	22.0	0.000	0.000
10-5	19	19	19	19.0	0.000	0.000
10-6	14	14	14	14.0	0.000	0.000
10-7	26	27	27	26.7	0.577	0.000
10-8	17	18	18	17.7	0.577	0.001
10-9	21	22	21	21.3	0.577	0.001
10-10	15	15	15	15.0	0.000	0.000
20-1	33	33	33	33.0	0.000	0.000
20-2	31	31	31	31.0	0.000	0.000
20-3	33	34	34	33.7	0.577	0.000
20-4	27	27	27	27.0	0.000	0.000
20-5	33	33	33	33.0	0.000	0.000
20-6	28	28	28	28.0	0.000	0.000
20-7	26	26	26	26.0	0.000	0.000
20-8	35	36	36	35.7	0.577	0.000
20-9	30	30	31	30.3	0.577	0.000
20-10	35	35	35	35.0	0.000	0.000
40-1	43	43	45	43.7	1.155	0.001
40-2	43	43	43	43.0	0.000	0.000
40-3	43	43	44	43.3	0.577	0.000
40-4	42	42	42	42.0	0.000	0.000
40-5	42	42	42	42.0	0.000	0.000
40-6	41	40	41	40.7	0.577	0.000
40-7	44	44	44	44.0	0.000	0.000
40-8	36	36	36	36.0	0.000	0.000
40-9	45	44	45	44.7	0.577	0.000
40-10	36	36	37	36.3	0.577	0.000
60-1	45	45	46	45.3	0.577	0.000
60-2	49	48	48	48.3	0.577	0.000
60-3	48	46	46	46.7	1.155	0.001
60-4	46	46	46	46.0	0.000	0.000
60-5	48	48	48	48.0	0.000	0.000
60-6	49	48	48	48.3	0.577	0.000
60-7	46	46	46	46.0	0.000	0.000
60-8	45	45	45	45.0	0.000	0.000
60-9	49	49	49	49.0	0.000	0.000
60-10	46	47	47	46.7	0.577	0.000
80-1	49	49	49	49.0	0.000	0.000
80-2	49	49	49	49.0	0.000	0.000
80-3	50	50	50	50.0	0.000	0.000
80-4	51	51	51	51.0	0.000	0.000
80-5	50	50	49	49.7	0.577	0.000
80-6	50	50	50	50.0	0.000	0.000
80-7	50	50	50	50.0	0.000	0.000
80-8	50	50	50	50.0	0.000	0.000
80-9	51	50	51	50.7	0.577	0.000

Three analysts – Pseudalert reads @ 28 hrs +ve wells						
Sample	**A**	**B**	**C**	**Mean**	**SD**	**RSD sq**
80-10	49	49	49	49.0	0.000	0.000
					Sum RSD	0.008
					RSDc	0.013

Appendix C Robustness data for Pseudalert*/Quanti-Tray* (see section 5)

Table C-1 Replicate counts from suspensions of 22 reference and environmental strains of *P. aeruginosa* from Pseudalert*/Quanti-Tray* after 24 hours and 28 hours of incubation.

Source	Replicate	Number of positive wells		
		24 h	28 h	% change
ATCC[1] 10145	1	9	9	0
	2	11	11	0
ATCC 9027	1	16	16	0
	2	12	12	0
ATCC 25628	1	21	21	0
	2	22	22	0
ATCC 35422	1	11	11	0
	2	15	15	0
ATCC 35554	1	6	6	0
	2	7	7	0
Effluent isolate Pa 23	1	2	2	0
	2	3	3	0
Water isolate Pa 28	1	10	10	0
	2	6	6	0
Water isolate Pa 31	1	23	23	0
	2	18	18	0
Water isolate Pa 34	1	3	3	0
	2	6	6	0
Water isolate Pa 37	1	19	19	0
	2	22	22	0
Bottled water isolate 1	1	16	16	0
	2	11	11	0
Bottled water isolate 2	1	18	18	0
	2	18	19	+ 6 %
Proficiency test isolate 1	1	24	24	0
	2	24	29	+ 21 %
Proficiency test isolate 2	1	16	17	+ 6 %
	2	14	15	+ 7 %
Proficiency test isolate 3	1	16	17	+ 6 %
	2	12	12	0
Spa water isolate Pa 42	1	10	10	0
	2	8	8	0
Spa water isolate Pa 43	1	14	15	+ 7 %
	2	20	20	0
Spa water isolate Pa 44	1	25	25	0
	2	22	22	0
Spa water isolate Pa 45	1	29	29	0
	2	25	25	0
Clinical isolate Pa 46	1	11	12	+ 9 %
	2	9	9	0
Clinical isolate Pa 47	1	32	32	0
	2	23	23	0
Clinical isolate Pa 48	1	15	15	0
	2	13	14	+ 8 %

Table C-2 Replicate counts from suspensions of three strains of *P. aeruginosa* from Pseudalert*/Quanti-Tray* after 24 hours and 28 hours of incubation using Pseudalert stored at 4°C for up to 298 days

Day post manufacture	Number of positive wells								
	P. aeruginosa ATCC 10145			*P. aeruginosa* ATCC 27853			*P. aeruginosa* ATCC 35554		
	24 h	28 h	% change	24 h	28 h	% change	24 h	28 h	% change
0	37 36	38 37	+ 3 % + 3 %	15 22	26 36	+ 73 % + 64 %	28 28	28 33	0 + 18 %
27	37 37	41 37	+ 11 % 0	25 23	29 30	+ 16 % + 29 %	20 31	23 31	+ 15 % 0
40	31 26	31 26	0 0	22 27	23 27	+ 5 % 0	29 33	29 33	0 0
54	32 25	32 25	0 0	27 31	27 32	0 + 3 %	42 29	42 30	0 + 3 %
89	33 36	33 37	0 + 3 %	26 29	27 29	+ 4 % 0	29 30	29 30	0 0
117	26 32	26 32	0 0	30 32	32 32	+ 7 % 0	28 32	29 32	+ 4 % 0
152	34 27	35 28	+ 3 % + 4 %	22 30	22 31	0 + 3 %	36 31	36 32	0 + 3 %
180	19 17	19 19	0 + 12 %	23 17	24 17	+ 5 % 0	24 29	24 29	0 0
208	18 13	18 13	0 0	18 17	18 17	0 0	26 31	26 31	0 0
243	29 27	29 27	0 0	16 25	17 26	+ 6 % + 4 %	23 21	23 21	0 0
277	25 22	26 22	+ 4 % 0	17 19	17 21	0 + 11 %	18 25	18 26	0 + 4 %
298	34 18	34 18	0 0	11 17	11 17	0 0	23 24	23 24	0 0

Table C-3 Replicate counts from suspensions of three strains of *P. aeruginosa* from Pseudalert*/Quanti-Tray* after 24 hours and 28 hours of incubation using Pseudalert stored at 30°C for up to 298 days

Day post manufacture	Number of positive wells								
	P. aeruginosa ATCC 10145			*P. aeruginosa* ATCC 27853			*P. aeruginosa* ATCC 35554		
	24 h	28 h	% change	24 h	28 h	% change	24 h	28 h	% change
0	37 36	38 37	+ 3 % + 3 %	15 22	26 36	+ 73 % + 64 %	28 28	28 33	0 + 18 %
27	35 31	35 31	0 0	20 20	36 32	+ 80 % + 60 %	23 38	25 40	+ 9 % + 5 %
40	34 26	34 27	0 + 4 %	26 21	27 21	+ 4 % 0	30 29	30 29	0 0
54	24 28	24 29	0 + 4 %	30 25	30 25	0 0	29 34	30 35	+ 4 % + 3 %
89	34 36	34 36	0 0	34 24	35 26	+ 3 % + 8 %	21 25	22 25	+ 5 % 0
117	37 33	37 33	0 0	21 28	21 28	0 0	29 23	29 23	0 0
152	26 27	27 30	+ 4 % + 11 %	24 32	25 34	+ 4 % + 6 %	26 34	26 34	0 0
180	20 20	20 21	0 + 5 %	25 26	26 27	+ 4 % + 4 %	27 24	27 24	0 0
208	28 15	28 15	0 0	25 18	27 18	+ 8 % 0	34 27	34 27	0 0
243	28 23	28 24	0 + 4 %	25 20	25 21	0 + 5 %	21 30	21 30	0 0
277	20 22	20 23	0 + 5 %	14 16	14 16	0 0	23 23	23 23	0 0
298	20 16	20 16	0 0	16 17	17 17	+ 6 % 0	19 30	19 30	0 0

Appendix D Ninety-five percent confidence intervals and theoretical repeatabilities for each MPN value (see Section 7)

Table D-1 Ninety-five percent confidence intervals and theoretical repeatabilities for each MPN value for the 51-well Pseudalert*/Quanti-Tray*

Number of wells with positive reaction	MPN per 100-mL sample	95% Confidence Intervals		Theoretical repeatability	
		Lower	Upper	Repeatability standard deviation ($log_{10}SD$)	Relative standard uncertainty (%CV)
0	<1.0	0.0	3.7	-	-
1	1.0	0.3	5.6	0.3243	74.7
2	2.0	0.6	7.3	0.2768	63.7
3	3.1	1.1	9.0	0.2329	53.6
4	4.2	1.7	10.7	0.2038	46.9
5	5.3	2.3	12.3	0.1858	42.8
6	6.4	3.0	13.9	0.1699	39.1
7	7.5	3.7	15.5	0.1587	36.5
8	8.7	4.5	17.1	0.1479	34.1
9	9.9	5.3	18.8	0.1403	32.3
10	11.1	6.1	20.5	0.1343	30.9
11	12.4	7.0	22.1	0.1274	29.3
12	13.7	7.9	23.9	0.1226	28.2
13	15.0	8.8	25.7	0.1187	27.3
14	16.4	9.8	27.5	0.1143	26.3
15	17.8	10.8	29.4	0.1109	25.5
16	19.2	11.9	31.3	0.1071	24.7
17	20.7	13.0	33.3	0.1042	24.0
18	22.2	14.1	35.2	0.1014	23.3
19	23.8	15.3	37.3	0.0987	22.7
20	25.4	16.5	39.4	0.0964	22.2
21	27.1	17.7	41.6	0.0947	21.8
22	28.8	19.0	43.9	0.0928	21.4
23	30.6	20.4	46.3	0.0908	20.9
24	32.4	21.8	48.7	0.0890	20.5
25	34.4	23.3	51.2	0.0872	20.1
26	36.4	24.7	53.9	0.0865	19.9
27	38.4	26.4	56.6	0.0845	19.5
28	40.6	28.0	59.5	0.0835	19.2
29	42.9	29.7	62.5	0.0824	19.0

Number of wells with positive reaction	MPN per 100-mL sample	95% Confidence Intervals		Theoretical repeatability	
		Lower	Upper	Repeatability standard deviation (log_{10}SD)	Relative standard uncertainty (%CV)
30	45.3	31.5	65.6	0.0813	18.7
31	47.8	33.4	69.0	0.0804	18.5
32	50.4	35.4	72.5	0.0794	18.3
33	53.1	37.5	76.2	0.0786	18.1
34	56.0	39.7	80.1	0.0778	17.9
35	59.1	42.0	84.4	0.0773	17.8
36	62.4	44.6	88.8	0.0763	17.6
37	65.9	47.2	93.7	0.0760	17.5
38	69.7	50.0	99.0	0.0757	17.4
39	73.8	53.1	104.8	0.0753	17.3
40	78.2	56.4	111.2	0.0752	17.3
41	83.1	59.9	118.3	0.0754	17.4
42	88.5	63.9	126.2	0.0754	17.4
43	94.5	68.2	135.4	0.0760	17.5
44	101.3	73.1	146.0	0.0766	17.6
45	109.1	78.6	158.7	0.0778	17.9
46	118.4	85.0	174.5	0.0797	18.3
47	129.8	92.7	195.0	0.0824	19.0
48	144.5	102.3	224.1	0.0869	20.0
49	165.2	115.2	272.2	0.0953	21.9
50	200.5	135.8	387.6	0.1162	26.8
51	> 200.5	146.1	infinite	-	-