

**Topic:** IDEXX Trial Study report comparing Legiolert\* with Quanti-Tray\*/Legiolert versus the Centers for Disease Control (CDC) standard method: “Procedures for the Recovery of *Legionella* from the Environment”<sup>1</sup>

**Title:** Comparison of the performance of the IDEXX Legiolert test versus Centers for Disease Control (CDC) method: “Procedures for the Recovery of *Legionella* from the Environment” at recovering confirmed *Legionella pneumophila* from potable water samples.

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**Date:** April 2016

## Report Highlights:

- Legiolert with Quanti-Tray/Legiolert was compared to the CDC standard method at IDEXX Laboratories in Westbrook, ME.
- Over 900 potable water samples were screened for the presence of *Legionella pneumophila* by using membrane filtration combined with GVPC medium resulting in 170 positive samples. All 170 samples were examined via a split sample analysis, resulting in 114 data pairs with at least one non-zero value.
- Data from the completed study showed:
  - Legiolert has superior detection and quantification of *Legionella pneumophila* in potable water samples.
  - Legiolert accurately recovered very low concentrations of *Legionella pneumophila* (as low as 1 organism/10 mL of sample).
  - The false positivity rate of Legiolert was 1.5% (132 confirmed *Legionella pneumophila* positives out of 134 individual positive wells analyzed).

<sup>1</sup>*Procedures for Recovery of Legionella from the Environment*. U.S. Department of Health and Human Services Public Health Service, Centers for Disease Control and Prevention (CDC) Atlanta, GA 30333

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# Technical Note

Comparison of the performance of the IDEXX Legiolert\* with Quanti-Tray\*/Legiolert test versus Centers for Disease Control (CDC) method: "Procedures for the Recovery of *Legionella* from the Environment" at recovering confirmed *Legionella pneumophila* from potable water samples.

## Product Description

Legiolert is a test that detects *Legionella pneumophila* in potable water samples. This test is based on a bacterial enzyme detection technology that signals the presence of *Legionella pneumophila* through utilization of a substrate present in the Legiolert reagent. *Legionella pneumophila* cells grow rapidly and reproduce using the rich supply of amino acids, vitamins and other nutrients present in the Legiolert reagent. Actively growing strains of *Legionella pneumophila* use the added substrate to produce a brown color indicator when used with Quanti-Tray/Legiolert. Legiolert detects *Legionella pneumophila* at 1 organism in a 10 mL sample within 7 days via dilution of the 10 mL with 90 mL sterile, deionized water.

## Scope

This technical note contains data collected at IDEXX Laboratories in Westbrook, ME. The test matrix for this study was potable water. IDEXX Laboratories partnered with several commercial laboratories to collect water samples from locations across the United States. Represented samples from Alaska, Arizona, California, Florida, Georgia, Pennsylvania, and Texas were collected. The microorganisms present in these potable water samples were from wild populations that occurred naturally in the environment and did not result from supplemental spiking activities. Samples were screened for presumptive positives, resulting in 119 positive samples, of which 114 resulted in usable values for comparative analysis. This study compared the relative recovery of confirmed *L. pneumophila* by Legiolert after 7 days of incubation against CDC Procedures for the Recovery of *Legionella* from the Environment.

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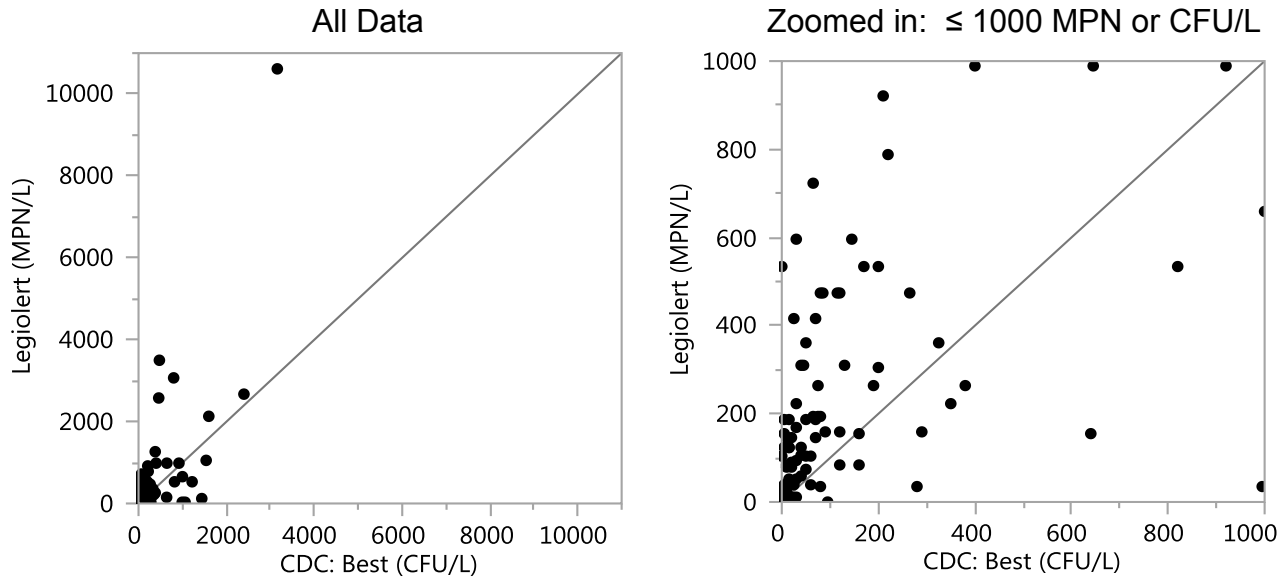
## Procedure

1. Samples were collected from various locations as 200-1000 mL aliquots by commercial laboratories and shipped cold to IDEXX Laboratories.
2. CDC method: A 200 mL aliquot of each sample was processed and analyzed following the procedures outlined in the CDC method. Briefly, 200 mL was concentrated by membrane filtration and resuspended in 5 mL of sterile, deionized water. Concentrates were analyzed by direct plating of 0.5 mL of both untreated and acid treated aliquots. Acid treated aliquots were plated in duplicate. Selective plating was done on BCYE (buffered charcoal yeast extract) and BCYE containing GVPC (glycine, vancomycin, polymyxin B, cycloheximide).
3. Legiolert: A 10 mL aliquot of each sample was processed and analyzed following the procedures outlined in the Legiolert protocol for 10 mL quantification using the Quanti-Tray/Legiolert device. Briefly, 10 mL of test sample was diluted 10-fold by addition to 90 mL of sterile, deionized water and examined using Legiolert reagent and Quanti-Tray/Legiolert. Legiolert was incubated for 7 days at  $39 \pm 0.5^\circ\text{C}$  with  $\geq 85\%$  relative humidity.
4. Presumptive *Legionella pneumophila* positive samples from both methods were confirmed by assaying the following reactions:
  - Growth on BCYE
  - No growth on TSA with 5% sheep's blood
5. In order to properly compare Legiolert to the CDC method for the specific detection of *Legionella pneumophila*, presumptive positive samples from the traditional culture method were screened for the presence of *Legionella* other than *L. pneumophila* (e.g. *L. bozeman*, *L. anisa*, *L. micdadei*) by examining isolates for fluorescence during exposure to 365-nm (or comparable) UV light. If further analysis was required to confirm the identity of potential non-*pneumophila* isolates, then latex agglutination (Oxoid) was performed. Data resulting from the isolation of non-*pneumophila* species of *Legionella* by the CDC method was filtered from the analysis.

## Results

Of the original screened potable water samples 119 contained confirmed *L. pneumophila* by at least one of the test methods. Data from 5 of these samples was not usable for statistical comparison and were TNTC (too numerous to count) on all CDC standard method plates.

The Youden plots below visually compare MPN vs. CFU for Legiolert vs. the CDC standard method.



### Legiolert vs. CDC method “Best”

T-test results for Legiolert vs. CDC Best

Statistic	vs. CDC: Best
Legiolert mean	299.3
CDC mean	161.1
Mean Difference	138.2
Std Error	55.0
Upper 95%	246.8
Lower 95%	29.6
N	165
Correlation	0.750
t-Ratio	2.514
DF	164
Prob >  t	0.0129
Prob > t	0.0065
Prob < t	0.99

$p < 0.05$  indicates a significant difference

The false positivity rate of Legiolert was 1.5% (132 confirmed *Legionella pneumophila* positives out of 134 individual positive wells analyzed).

## Conclusions

The data presented above clearly demonstrates the favorable detection and quantification of *Legionella pneumophila* by the Legiolert method compared to the CDC method. Legiolert was able to accurately recover very low concentrations of *Legionella pneumophila* (as low as 1 organism/10 mL of sample) without interference from the heterotrophic bacterial populations or chemical residues present in the potable water samples.

Based on these data we conclude that, after 7 days of incubation, Legiolert performs as well as the CDC method for the specific detection and quantification of *Legionella pneumophila* from potable water matrices.

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## About IDEXX Laboratories

IDEXX Laboratories, Inc. is the global market leader in diagnostics and information technology solutions for animal health and water and milk quality. Headquartered in Maine, IDEXX employs over 6,000 people in more than 175 locations around the world.

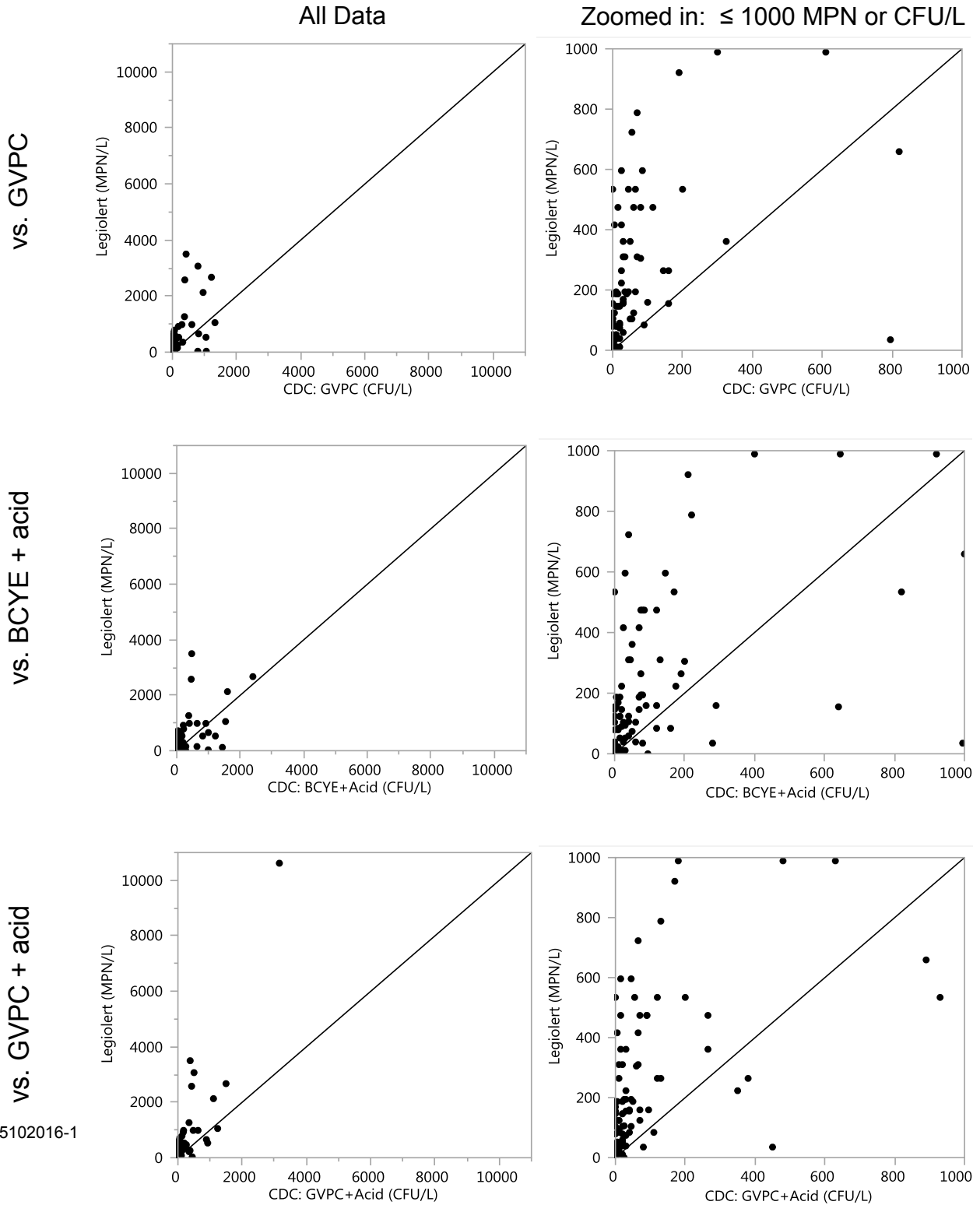
IDEXX is the world leader in microbiology testing technologies that ensure safe water. As the world's preferred provider of innovative drinking-water microbiology test kits, IDEXX is known for its breakthrough products. We provide easy, rapid, accurate and cost-effective water-testing solutions. Our sales, customer service and technical support teams serve customers in over 100 countries and our products have governmental approval in 40+ countries world-wide.

## References

- 1 *Procedures for Recovery of Legionella from the Environment*. U.S. Department of Health and Human Services Public Health Service, Centers for Disease Control and Prevention (CDC) Atlanta, GA 30333

# Appendix

Data comparisons Legiolert vs. individual CDC method conditions. No comparison to BCYE without pretreatment is shown as nearly all plates were overgrown.



T-test results for Legiolert vs. individual CDC method plates:

Statistic	vs. CDC: Best	vs. CDC: BCYE+Acid	vs. CDC: GVPC	vs. CDC: GVPC+Acid
Legiolert mean	299.3	256.3	250.3	301.0
CDC mean	161.1	146.9	90.4	99.5
Mean	138.2	109.3	159.8	201.5
Difference				
Std Error	55.0	35.0	35.8	54.6
Upper 95%	246.8	178.5	230.6	309.3
Lower 95%	29.6	40.2	89.0	93.7
N	165	134	147	164
Correlation	0.750	0.619	0.629	0.871
t-Ratio	2.514	3.127	4.461	3.691
DF	164	133	146	163
Prob >  t	0.0129	0.0022	<.0001	0.0003
Prob > t	0.0065	0.0011	<.0001	0.0002
Prob < t	0.99	1.00	1.00	1.00

p < 0.05 indicates a significant difference

Sample	Legiolert	CDC method			
	(MPN/L)	Best (CFU/L)	BCYE + acid (CFU/L)	GVPC (CFU/L)	GVPC + acid (CFU/L)
1	310	45	45	35	10
2	596	30	30	25	15
3	52	15	15	0	5
4	32	10	0	5	10
5	90	20	20	20	10
6	534	170	170	45	55
7	159	290	290	100	95
8	474	85	85	15	15
9	84	160	160	20	40
10	35	280	280	0	0
11	194	75	75	10	30
12	106	40	40	0	25
13	194	80	80	45	25
14	0	95	95	0	25
15	361	50	50	50	15
16	0	0	0	0	0
17	361	50	50	30	30
18	39	25	25	10	5
19	146	70	70	15	20
20	10616	3160	TNTC	TNTC	3160
	*TNTC – Too numerous to count				

	<b>Legiolert</b>	<b>CDC method</b>			
<b>Sample</b>	<b>(MPN/L)</b>	<b>Best (CFU/L)</b>	<b>BCYE + acid (CFU/L)</b>	<b>GVPC (CFU/L)</b>	<b>GVPC + acid (CFU/L)</b>
21	0	0	TNTC	0	0
22	0	0	0	0	0
23	104	60	60	55	20
24	3501	475	475	430	385
25	187	15	15	10	5
26	310	130	130	70	65
27	3071	800	TNTC	800	505
28	596	145	145	85	45
29	223	30	20	25	30
30	416	70	70	25	65
31	194	80	80	35	25
32	187	50	5	15	50
33	39	20	0	0	20
34	124	15	15	5	10
35	723	65	40	55	65
36	2580	460	460	390	430
37	124	5	0	5	0
38	35	5	0	0	5
39	104	50	25	50	45
40	35	995	995	795	450
41	0	0	0	0	0
42	0	0	0	0	0
43	123	15	15	0	0
44	104	0	0	0	0
45	155	160	0	160	30
46	474	115	75	115	90
47	534	0	0	0	0
48	534	200	0	65	200
49	0	0	0	0	0
50	0	0	0	0	0
51	39	5	0	0	5
52	84	10	10	0	10
53	0	15	15	0	0
54	187	5	5	0	0
55	264	75	75	25	10
	*TNTC – Too numerous to count				



	<b>Legiolert</b>	<b>CDC method</b>			
<b>Sample</b>	<b>(MPN/L)</b>	<b>Best (CFU/L)</b>	<b>BCYE + acid (CFU/L)</b>	<b>GVPC (CFU/L)</b>	<b>GVPC + acid (CFU/L)</b>
56	23	0	TNTC	TNTC	TNTC
57	416	25	25	5	5
58	194	65	TNTC	65	45
59	0	5	0	5	0
60	155	5	5	0	0
61	534	820	820	200	120
62	155	640	640	30	40
63	124	1440	1440	60	70
64	0	0	0	0	0
65	22	0	0	0	0
66	124	40	40	0	10
67	11	0	0	0	0
68	11	10	10	0	0
69	0	0	0	0	0
70	52	30	30	10	20
71	0	0	0	0	0
72	84	120	120	90	110
73	10	0	0	0	0
74	11	10	0	10	0
75	11	20	10	20	0
76	39	60	60	20	30
77	187	70	70	40	20
78	146	10	0	10	0
79	146	20	20	20	20
80	0	0	0	0	0
81	11	20	20	10	0
82	659	1000	1000	820	890
83	10	10	10	10	0
84	11	30	30	0	20
85	74	50	50	20	30
86	474	80	80	60	70
87	921	210	210	190	170
88	79	20	0	10	20
89	169	30	10	30	0
90	0	20	20	0	0
*TNTC – Too numerous to count					

	<b>Legiolert</b>	<b>CDC method</b>			
<b>Sample</b>	<b>(MPN/L)</b>	<b>Best (CFU/L)</b>	<b>BCYE + acid (CFU/L)</b>	<b>GVPC (CFU/L)</b>	<b>GVPC + acid (CFU/L)</b>
91	23	10	10	0	0
92	159	90	90	30	70
93	59	40	40	30	20
94	10	10	0	10	0
95	22	0	0	0	0
96	310	40	40	30	20
97	1269	380	380	380	350
98	989	920	920	610	630
99	264	190	190	160	120
100	1057	1540	1540	1330	1240
101	35	80	80	0	0
102	0	10	10	0	0
103	11	10	10	0	10
104	534	1220	1220	1050	930
105	94	30	30	0	10
106	79	10	10	0	0
107	788	220	220	70	130
108	0	0	0	0	0
109	159	120	120	30	40
110	0	0	0	0	0
111	0	10	0	10	0
112	23	10	10	0	10
113	305	200	200	80	60
114	0	0	0	0	0
115	989	400	400	300	180
116	2133	1600	1600	960	1110
117	2674	2400	2400	1220	1500
118	10	10	0	10	0
119	474	120	120	80	90
120	0	0	0	0	0
121	474	265	TNTC	TNTC	265
122	223	350	175	TNTC	350
123	264	190	190	145	130
124	0	0	0	TNTC	0
125	989	645	645	TNTC	480
	*TNTC – Too numerous to count				

	<b>Legiolert</b>	<b>CDC method</b>			
<b>Sample</b>	<b>(MPN/L)</b>	<b>Best (CFU/L)</b>	<b>BCYE + acid (CFU/L)</b>	<b>GVPC (CFU/L)</b>	<b>GVPC + acid (CFU/L)</b>
126	264	380	TNTC	TNTC	380
127	361	325	TNTC	325	265
128	10	5	TNTC	TNTC	5
129	35	1060	TNTC	1060	80
130	15725	TNTC	TNTC	TNTC	TNTC
131	0	0	0	0	0
132	0	0	TNTC	0	0
133	0	0	0	0	0
134	0	0	0	0	0
135	0	0	TNTC	0	0
136	0	0	0	TNTC	0
137	0	0	TNTC	0	0
138	0	0	TNTC	0	0
139	0	0	TNTC	0	0
140	0	TNTC	TNTC	TNTC	TNTC
141	0	0	0	TNTC	0
142	0	0	TNTC	0	0
143	0	0	0	TNTC	0
144	0	0	0	0	0
145	0	0	TNTC	TNTC	0
146	0	0	TNTC	0	0
147	0	0	0	0	0
148	0	0	TNTC	0	0
149	0	0	0	TNTC	0
150	0	0	TNTC	TNTC	0
151	0	0	TNTC	TNTC	0
152	0	0	0	0	0
153	0	TNTC	TNTC	TNTC	TNTC
154	0	0	TNTC	TNTC	0
155	0	0	TNTC	TNTC	0
156	0	0	TNTC	0	0
157	0	0	0	0	0
158	0	TNTC	TNTC	TNTC	TNTC
159	0	0	TNTC	TNTC	0
160	0	TNTC	TNTC	TNTC	TNTC
	*TNTC – Too numerous to count				

	<b>Legiolert</b>	<b>CDC method</b>			
<b>Sample</b>	<b>(MPN/L)</b>	<b>Best (CFU/L)</b>	<b>BCYE + acid (CFU/L)</b>	<b>GVPC (CFU/L)</b>	<b>GVPC + acid (CFU/L)</b>
161	0	0	TNTC	0	0
162	0	0	TNTC	0	0
163	0	0	0	0	0
164	0	0	0	0	0
165	0	0	0	0	0
166	0	0	TNTC	0	0
167	0	0	TNTC	0	0
168	0	10	10	0	0
169	0	0	TNTC	0	0
170	0	0	TNTC	0	0
*TNTC – Too numerous to count					