

References

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IDEXX Water

One IDEXX Drive
Westbrook, ME 04092

Contact IDEXX today at: **(1) 800-321-0207**
or visit **idexx.com/water/Legiolert**

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Comparison of the
Legiolert[®]/Quanti-Tray[®]
method for the enumeration
of *Legionella pneumophila*
in potable and similar water
samples with the ISO reference
method (ISO 11731-2:2004)

Comparison of the Legiolert®/Quanti-Tray® method for the enumeration of *Legionella pneumophila* in drinking and similar water samples with the ISO reference method (ISO 11731-2:2004).

This is a short summary of a European ring trial which compared Legiolert®/Quanti-Tray® to the international standard method for the enumeration of *Legionella pneumophila* in drinking and similar water samples. The complete study, using results from naturally contaminated samples, has been published in *Letters in Applied Microbiology* (2017, Vol. 54, issue 4, pages 271-275, DOI: 10.1111/lam.12719) - Evaluation of most probable number method for the enumeration of *Legionella pneumophila* from potable and related water samples. D. P. Sartory, K. Spies, B. Lange, S. Schneider and B. Langer.

Legiolert® is a selective reagent that shows the presence of *L. pneumophila* through the production of a brown colour and/or turbidity after incubation at 39 ± 0.5°C in a humidified environment. When used with the Quanti-Tray® system, Legiolert® provides a confirmed MPN count of *L. pneumophila* after 7 days incubation.

The objective of the study was to compare Legiolert®/Quanti-Tray® with the ISO reference membrane filtration method (ISO 11731-2:2004), which uses Glycine-Vancomycin-Polymyxin-Cycloheximide medium (GVPC agar) for the enumeration of *L. pneumophila* from various drinking and similar waters. This ISO method is also the regulatory method in Germany as cited under the German drinking water ordinance which includes a technical action standard for *Legionella* of 100 cfu per 100 ml.

Participants in the study analysed 100 ml samples of naturally contaminated cold drinking water and related waters (principally hot tap water, showers and boiler outlets) which were taken from both domestic, public (e.g. hospitals, retirement homes and sports facilities) and industrial systems.

Four geographically widespread laboratories in Germany that routinely analyse water samples for *Legionella* (including water company and commercial laboratories) participated in this study. Experienced

analysts undertook the analyses and were trained in the Legiolert®/Quanti-Tray® procedure. The data generated were analysed by an independent consultant using the statistical procedures outlined in **The Microbiology of Drinking Water: Part 3** and according to ISO 17994:2014.

After excluding unusable data (i.e. where both counts are zero or one of the pair was above the counting range) there were 290 pairs of counts suitable for data analysis.

The paired differences between the counts were derived (Legiolert®/Quanti-Tray® count minus the ISO 11731-2 MF count) for the data. The number of pairs in which the Legiolert MPN count was higher (169) was greater than the number of pairs in which the ISO 11731-2 MF count was higher (111) (see Chart). Ten samples had equal counts. The median difference (Legiolert® minus ISO 11731-2) was + 1 with a range of - 117 to + 813.

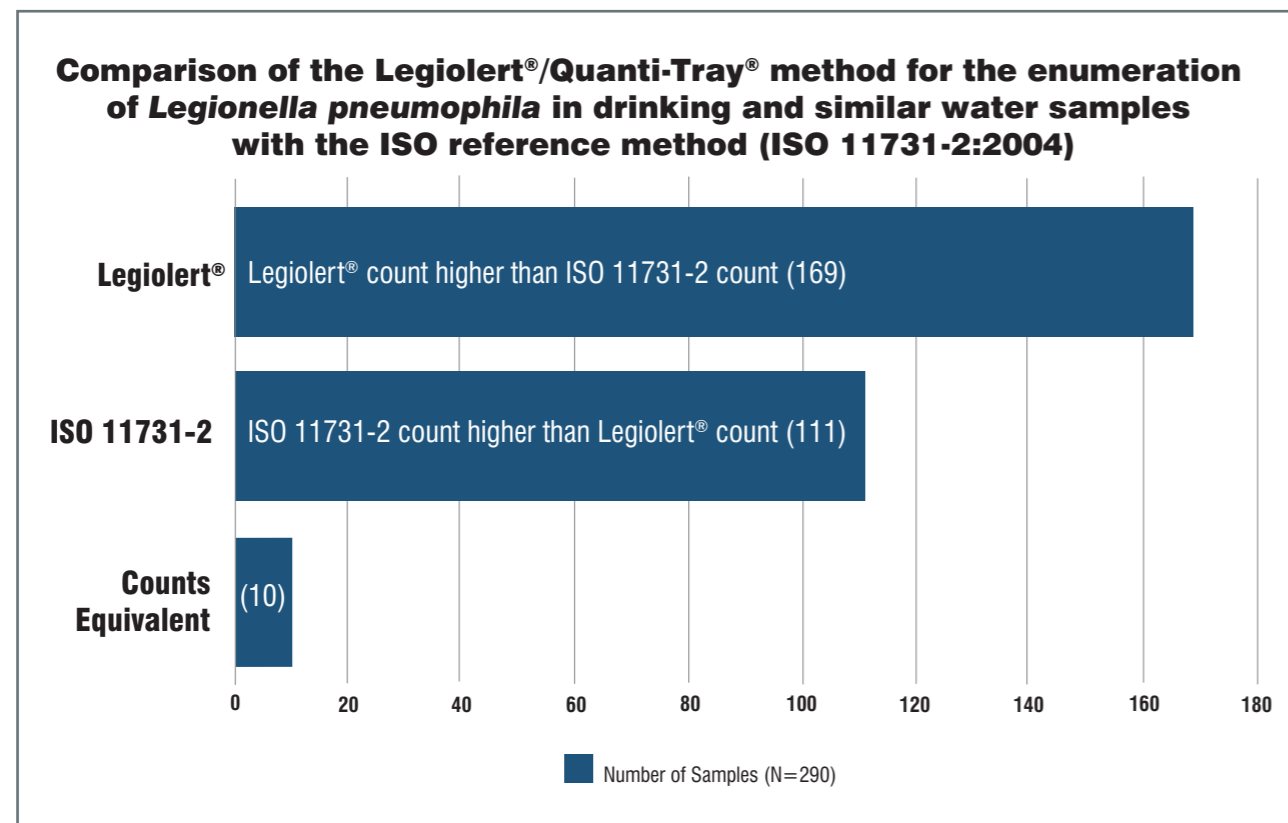
The paired results data for the *L. pneumophila* counts were compared using the mean relative difference procedure of ISO 17994:2014. This statistical procedure has been accepted by the European Commission for the comparison of two water microbiology methods. The relative difference (x) of each pair of count was calculated using the equation $x = 100 (\ln(a) - \ln(b))$, where $\ln(a)$ is the natural logarithm of the count by the Trial Method and $\ln(b)$ is the natural logarithm of the count by the Reference Method, for each sample for each analysis. The relative performance between the Trial Method and the Reference Method was computed. Data with a zero count by one method had one added to each count prior to log-transformation. Since the objective of the study was to compare a Trial Method with an

established Reference Method in terms of being not different, the 'two-sided' comparison according to ISO 17994:2014 is appropriate. The value of the upper and lower limits was set at +10 and -10 as suggested by ISO 17994:2014.

The results of the mean relative difference analyses are summarised in the Table below. The data from two laboratories (2 and 3) resulted in outcomes where the trial method (Legiolert®/Quanti-Tray®) yielded significantly higher counts of *L. pneumophila* compared to those for the ISO 11731-2 MF method. For the remaining two laboratories (1 and 4) the outcomes are 'inconclusive' meaning that more samples are needed to be analysed before a conclusive outcome could be determined. However, the spread of the confidence intervals (XL and XU) are indicative of probable comparability of the two methods. Combining the data from all four laboratories resulted in an outcome that showed Legiolert®/Quanti-Tray® produced significantly higher counts of *L. pneumophila*.

The Legiolert® test is read as confirmed *L. pneumophila*. To test whether there was any potential bias in favour of Legiolert®/Quanti-Tray® through false-positive reactions, a total of 1143 positive wells from 284 samples covering all sample types used in the study were subcultured for confirmation by serology. Of these, 1105 isolates (96.7%) were confirmed as *L. pneumophila*, indicating a high specificity for Legiolert.

These results demonstrate that Legiolert®/Quanti-Tray® is a suitable alternative, and potentially superior, replacement method for the ISO 11731-2 method for the analysis of *L. pneumophila* from potable water and similar water samples.



Laboratory	Number of Results	Mean Relative Difference	Standard Deviation	W*	X _L †	X _U ‡	Outcome
1	65	12.4	120.1	29.8	- 17.4	42.2	Inconclusive
2	65	35.5	117.3	29.1	6.4	64.6	Methods different
3	77	73.1	188.9	43.1	30.1	116.2	Methods different
4	85	17.8	201.1	44.1	- 26.3	62.0	Inconclusive
Combined	290	35.3	166.4	19.6	15.7	54.8	Methods different

* Half width of the "confidence interval" around the mean relative difference

† value of the relative difference at the lower "confidence limit"

‡ value of the relative difference at the upper "confidence limit"