228 Midway Ln, Suite B
Oak Ridge, TN 37830
865-813-1700

Molecular Detection of *Legionella*

Quantitative Polymerase Chain Reaction Assays
for L. species and L. pneumophila

Assured Bio ID: CIH010210-1

Analyst: M. McGraw

Inspector:	Certified Industrial Hygienist	**Date Collected:**	1/1/2010
Job Number:	CI012369	**Date Received:**	1/2/2010
Project Name:	Legionella	**Date Reported:**	1/3/2010

Assured Bio ID: CIH010210-1-1
Sample ID: 1
Description: Cooling Tower 1

L. species: 74 cells/ mL
L. pneumophila: Below Detectable Limits

Assured Bio ID: CIH010210-1-2
Sample ID: 2
Description: Cooling Tower 2

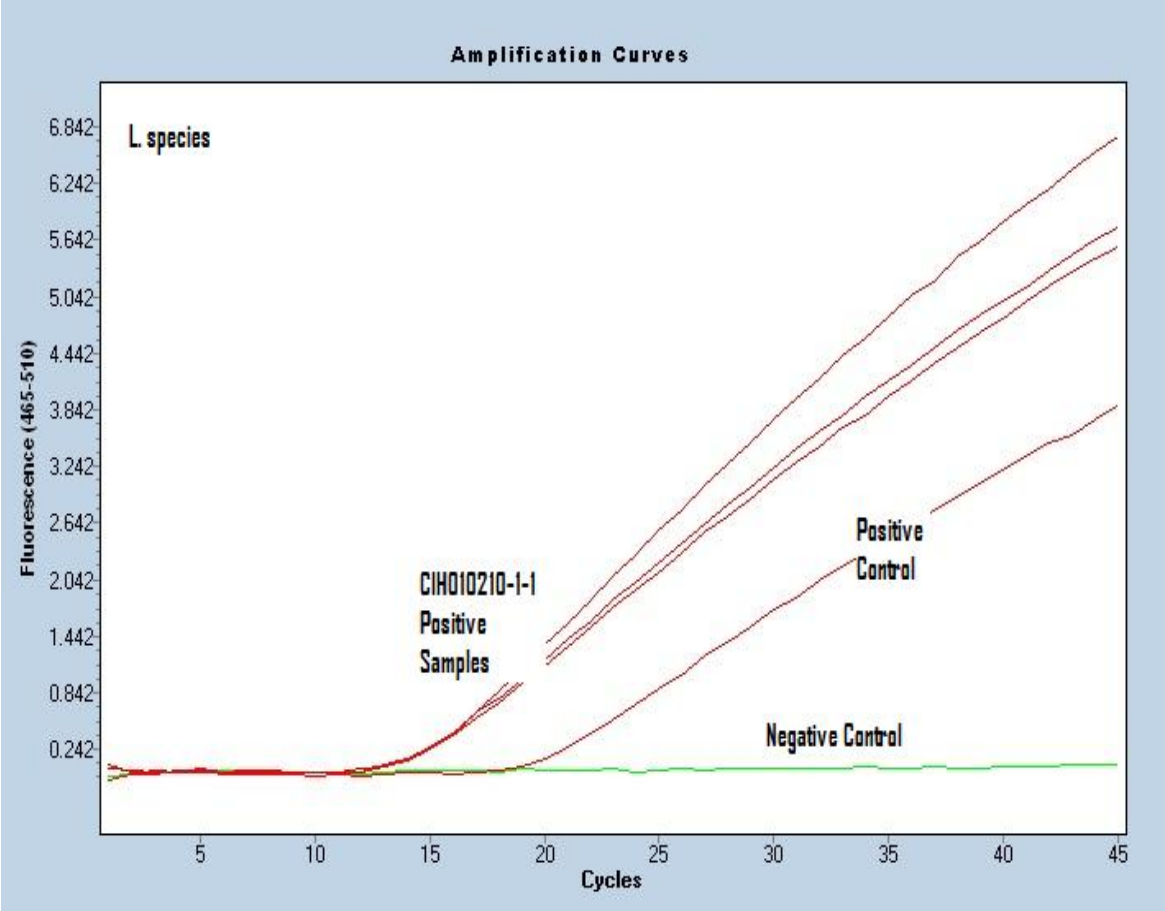
L. species: Below Detectable Limits
L. pneumophila: Below Detectable Limits

Assured Bio ID: CIH010210-1-3
Sample ID: 3
Description: Cooling Tower 3

L. species: 16 cells/milliliter
L. pneumophila: 5 cells/milliliter

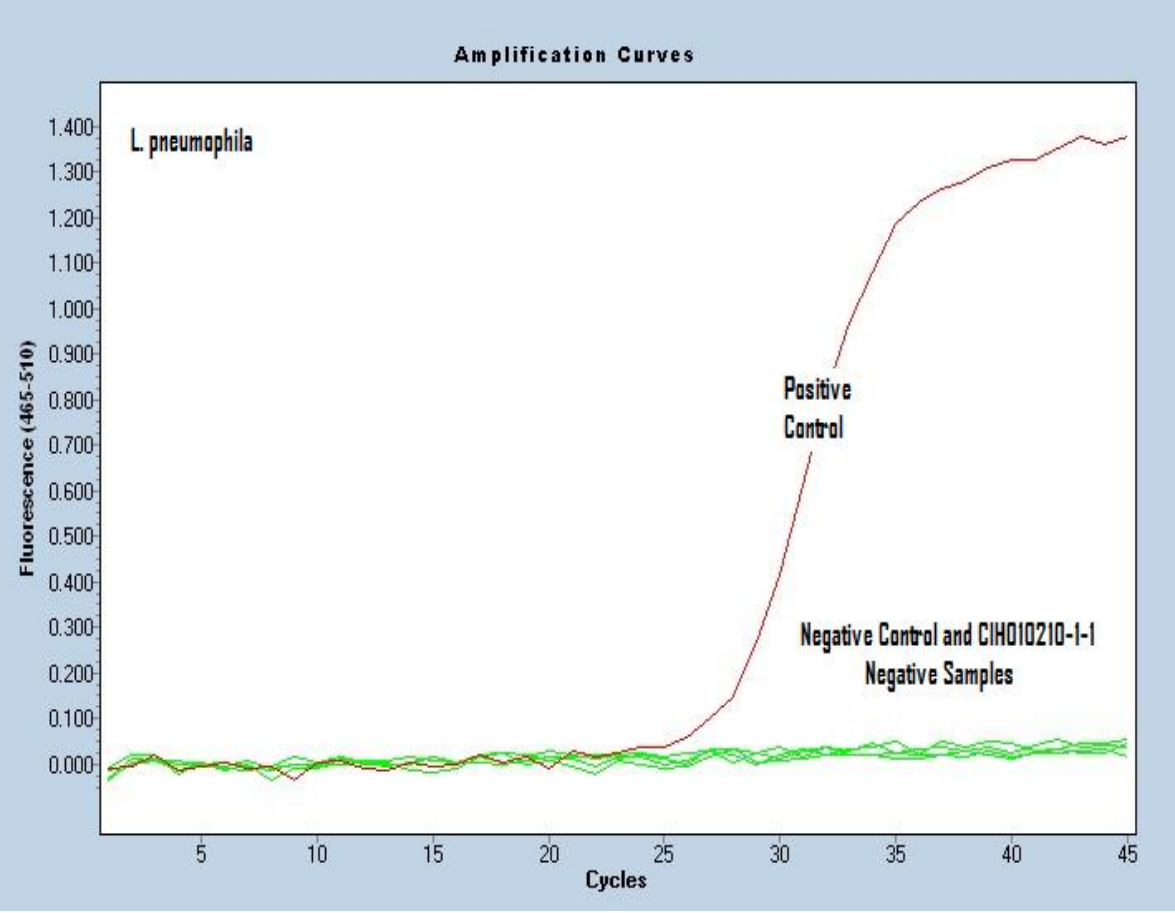
*Assured Bio Labs, LLC uses Legionella-specific PCR to detect the presence or absence of Legionella species listed above. Detection of Legionella species is facilitated via selective amplification of the 16S sector of rRNA. This section of the genome is highly selective for each individual species and inhibits amplification of non-target bacteria. To ensure no false positives or negatives have occurred each assay is spiked with both a positive and negative control.

Below is the raw data used to determine the results of the DNA analysis. The software uses an calculus algorithm to determine if the increase in florescence is statistically high enough to warrant a positive result. Each assay for each sample is run in triplicate to ensure reproducibility of result.



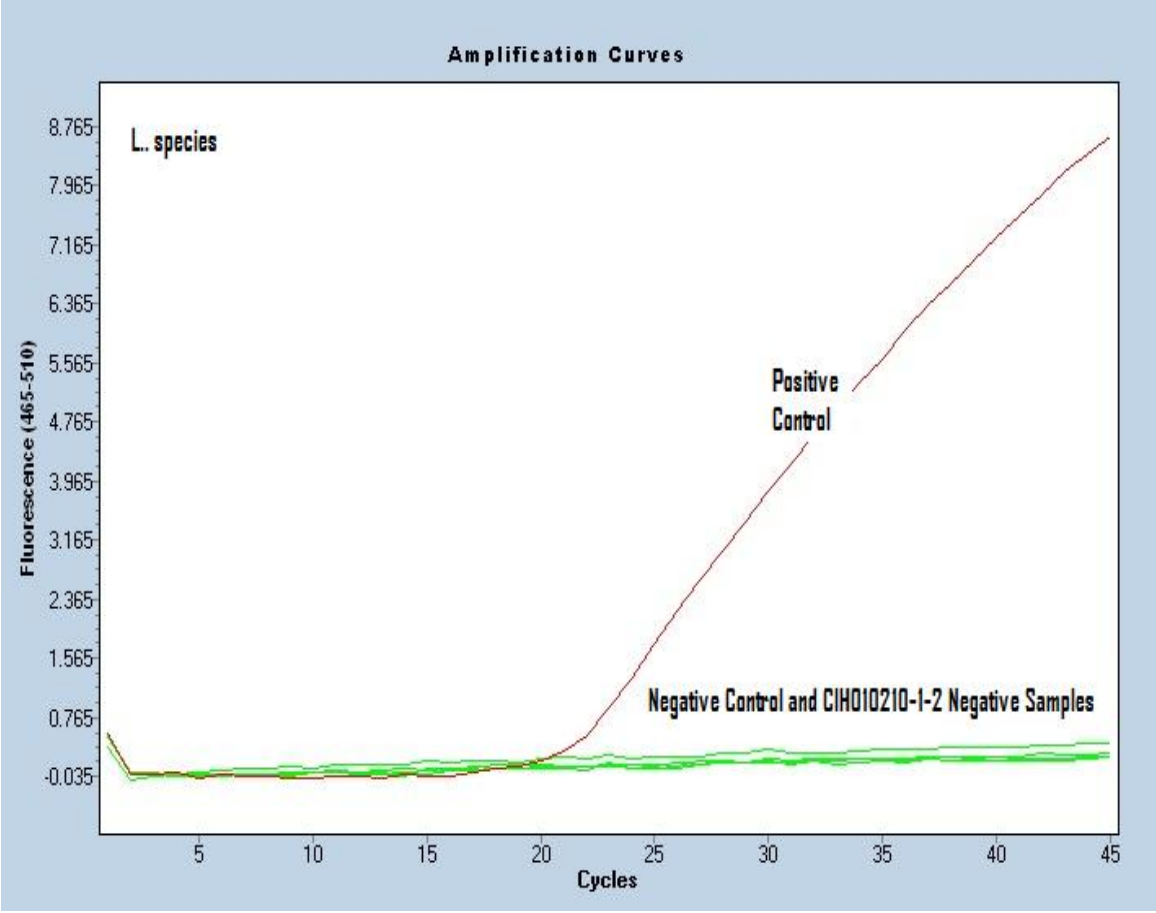
The y-axis depicts the amount of fluorescence detected within the 465-510 nm spectrum. The x-axis represents the cycle number within the PCR analysis. There are 45 cycles within this real-time-PCR process. If a product has not been detected after the 45th cycle insufficient DNA is present at the time of sampling and results are "Below Detectable Limits."

Below is the raw data used to determine the results of the DNA analysis. The software uses an calculus algorithm to determine if the increase in florescence is statistically high enough to warrant a positive result. Each assay for each sample is run in triplicate to ensure reproducibility of result.



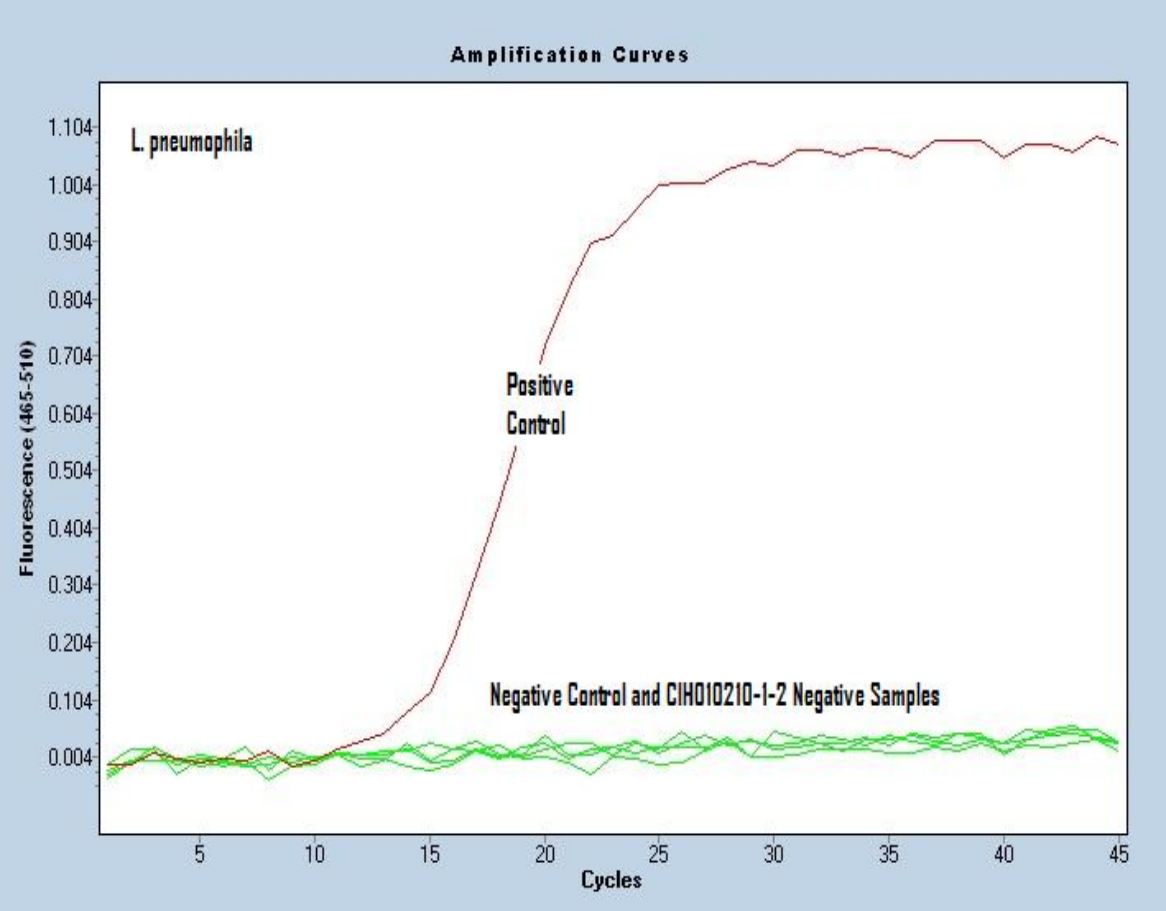
The y-axis depicts the amount of fluorescence detected within the 465-510 nm spectrum. The x-axis represents the cycle number within the PCR analysis. There are 45 cycles within this real-time-PCR process. If a product has not been detected after the 45th cycle insufficient DNA is present at the time of sampling and results are "Below Detectable Limits."

Below is the raw data used to determine the results of the DNA analysis. The software uses an calculus algorithm to determine if the increase in florescence is statistically high enough to warrant a positive result. Each assay for each sample is run in triplicate to ensure reproducibility of result.



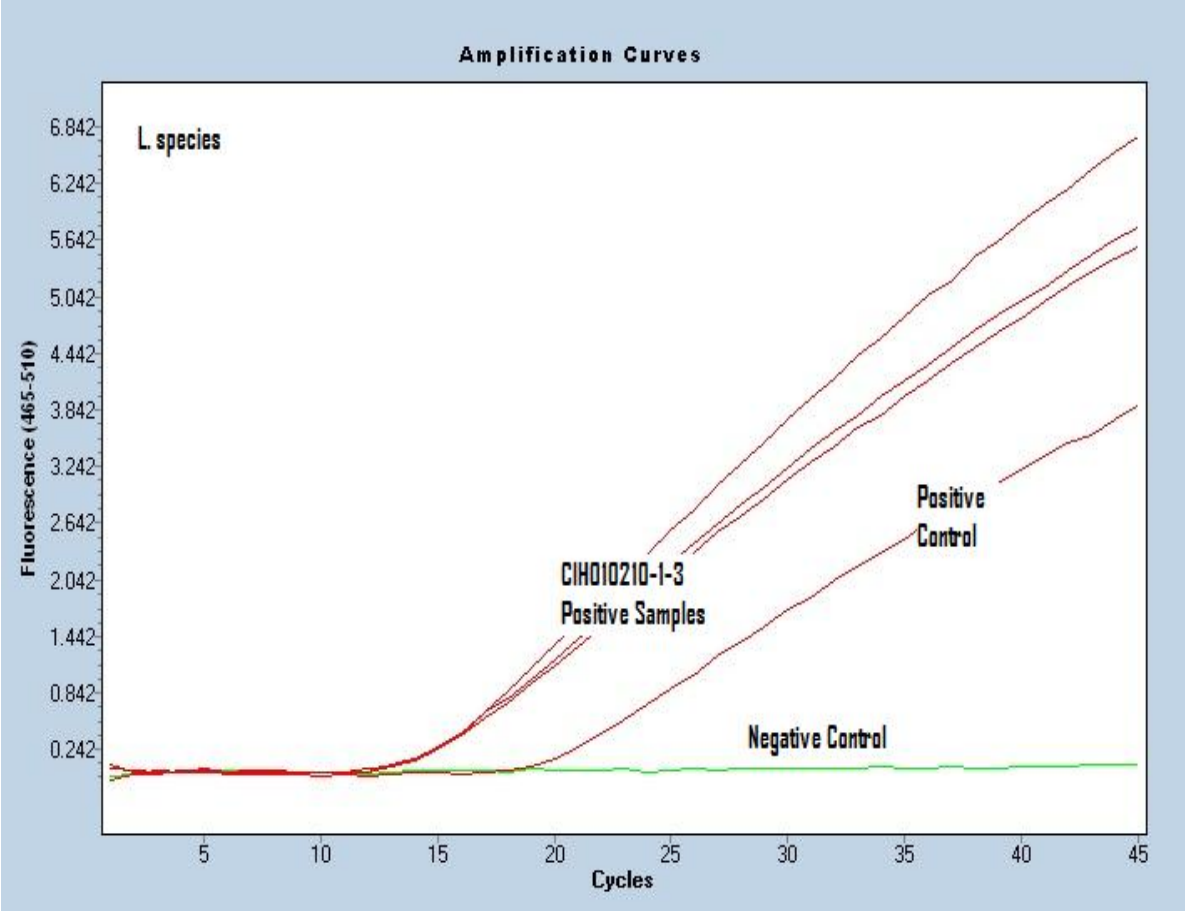
The y-axis depicts the amount of fluorescence detected within the 465-510 nm spectrum. The x-axis represents the cycle number within the PCR analysis. There are 45 cycles within this real-time-PCR process. If a product has not been detected after the 45th cycle insufficient DNA is present at the time of sampling and results are "Below Detectable Limits."

Below is the raw data used to determine the results of the DNA analysis. The software uses an calculus algorithm to determine if the increase in florescence is statistically high enough to warrant a positive result. Each assay for each sample is run in triplicate to ensure reproducibility of result.



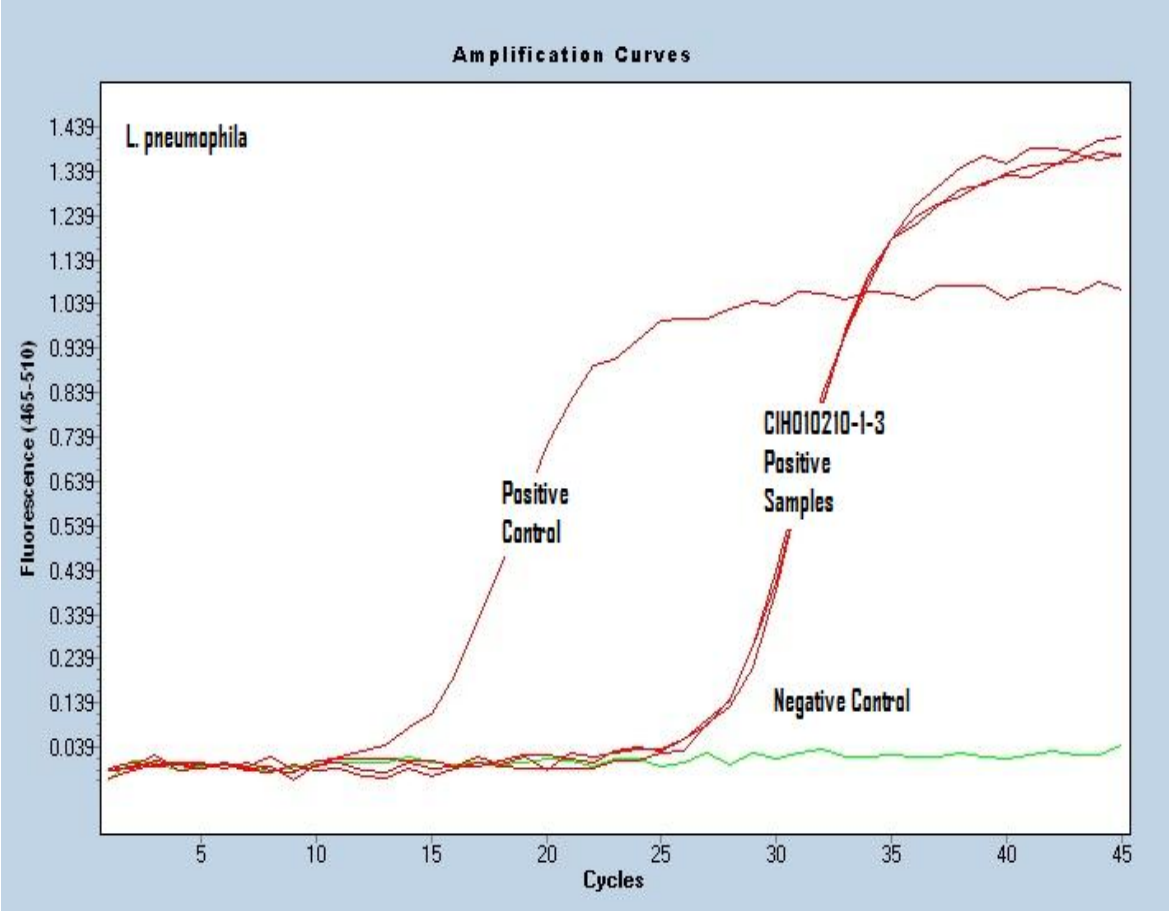
The y-axis depicts the amount of fluorescence detected within the 465-510 nm spectrum. The x-axis represents the cycle number within the PCR analysis. There are 45 cycles within this real-time-PCR process. If a product has not been detected after the 45th cycle insufficient DNA is present at the time of sampling and results are "Below Detectable Limits."

Below is the raw data used to determine the results of the DNA analysis. The software uses an calculus algorithm to determine if the increase in florescence is statistically high enough to warrant a positive result. Each assay for each sample is run in triplicate to ensure reproducibility of result.



The y-axis depicts the amount of fluorescence detected within the 465-510 nm spectrum. The x-axis represents the cycle number within the PCR analysis. There are 45 cycles within this real-time-PCR process. If a product has not been detected after the 45th cycle insufficient DNA is present at the time of sampling and results are "Below Detectable Limits."

Below is the raw data used to determine the results of the DNA analysis. The software uses an calculus algorithm to determine if the increase in florescence is statistically high enough to warrant a positive result. Each assay for each sample is run in triplicate to ensure reproducibility of result.



The y-axis depicts the amount of fluorescence detected within the 465-510 nm spectrum. The x-axis represents the cycle number within the PCR analysis. There are 45 cycles within this real-time-PCR process. If a product has not been detected after the 45th cycle insufficient DNA is present at the time of sampling and results are "Below Detectable Limits."

Notes:

The *Legionella* bacteria can be found naturally in the environment, primarily in water. The bacteria grow best in warm waters such as found in hot tubs, cooling towers, hot water tanks, large plumbing systems, or parts of the air-conditioning systems of large buildings.

Various species of *Legionella* cause infection in people exposed to common water related sources and have been implicated in community and nosocomial outbreaks. *Legionella* infections rarely affect organs outside of the lungs but soft tissue abscesses have also been attributed to Legionellosis, infection due to *Legionella* bacteria.

Legionella pneumophila accounts for about 90% of the cases of Legionellosis followed by ***Legionella micdadei*** responsible for about 8% of reported cases. Cases of Legionellosis caused by other species are rare but do occur. People most at risk of infection are people with suppressed immune systems such as HIV and Chemotherapy patients. People who are generally healthy show the least risk of infection and fatality due to Legionellosis. Legionnaires' disease is not known to be transmitted from person to person and there are no recorded infections of animals.

Methods of Analysis

Assured Bio Labs, LLC uses the following Standard Operating Procedures for the analysis of samples:

ViaScan/ Culturable Bacteria from Bulk Material: 125

ViaScan/ Culturable Bacteria from a Swab: 126

ViaScan/ Culturable Bacteria from an Air Sample: 138

Bacterial Species ID for Dominant Organisms: 117, 118, 119, 120

Bacteria Species Id of Enteric Gram Negative Bacteria: 142

Selected References

1. Barbaree, J.M, et al. “Protocol for Sampling Environmental Sites for *Legionellae.”* 1987. Applied and Environmental Microbiology. Vol 53, No 7. 1454-1458.
2. Campbell, J. Bibb, W.F., Lambert, M. A., Eng, S., Steigerwalt, A. G., Allard, J. Moss, C.W., and Brenner, D.J. 1984. “*Legionella sainthelensi*: A new Species of *Legionella* isolated From Water Near Mt. St. Helens.” Applied and Environmental Microbiology. Vol 47, No 2. 369-373.
3. Giglo, S., Monis, P.T., Saint, C.P. “*Legionella* Confirmation Using Real-Time PCR and SYTO9 is an Alternative to Current Methodology.” 2005. Applied and Environmental Microbiology. Vol 71, No 12. 8944-8948.
4. Gubler, J.G.H, Schorr, M., Gaia, V., Zbinden, R., and Altwegg, M. “Recurrent Soft Tissue Abscesses Caused by *Legionella cincinnatiensis*.” 2001. Journal of Clinical Microbiology. Vol. 39, No 12. 4568-4570.
5. Johnson, K.M. and Huseby, J.S.1997 “Lung Abscess Caused by *Legionella micdadei*.” Chest: Official publication of the American College of Chest Physicians.
6. Macher, J., Ed. 1999. *Bioaerosols: Assessment and Control*. ACGIH, Cincinnati, Ohio.
7. Ta, A.C., J.E. Stout, V.L. Yu, and M.M. Wagener. “Comparison of Culture Methods for Monitoring *Legionella* Species in Hospital Potable Water Systems and Recommendations for Standardization of Such Methods”. 1995. Journal of Clinical Microbiology.
8. Thomas, E., Gupta, N.K., Westhuizen, N.G., Chan, E., and Bernard, K. “Fatal Legionella maceachernii Pneumonia in Canada.” 1992. *Journal of Clinical Microbiology*. Vol 30, No 6. 1578-1579.
9. U.S. Department of Health and Human Services, Centers for Disease Control. “Procedures for the Recovery of *Legionella* from the Environment”. Jan. 2005.
10. Wadowsky, R.M., Yee, R. B. “Glycine-Containing Selective Medium for Isolation of *Legionellaceae* from Environmental Specimens.” 1981. Applied and Environmental Microbiology. Vol 42, No 5. 768-772.
11. Wistreich G.A. *Microbiology Laboratory: Fundamentals and Applications*. 1997. Prentice Hall, Upper Saddle River, New Jersey.
12. Yang, G., et al. “Dual detection of Legionella pneumophila and Legionella species by real-time PCR targeting the 23S-5S rRNA gene spacer region.” 2009. Clinical Microbiology and Infection.
13. Yaradou, D. F., et al. “Integrated Real-Time PCR for Detection and Monitoring of *Legionella pneumophila* in Water Systems.” 2007. “Applied and Environmental Microbiology. Vol 73, No 5. 1452-1456.

Limitations