



Abstract

Legionella and other waterborne pathogens cause approximately 25% of healthcare-associated infections. Infection prevention depends on determining if the hospital water system is a reservoir for these pathogens. We evaluated the GeneSystems real-time quantitative PCR (qPCR) technology for the rapid detection of *Legionella* species and *Pseudomonas aeruginosa* in water samples collected from 18 healthcare facilities and 1 long-term care facility. 100 samples were tested and qPCR results were compared to standard culture. qPCR detected *L. pneumophila* in 29% of samples and *Legionella* spp. in 67% of samples compared to 16% and 17% by standard culture. Sensitivity/specificity of the *L. pneumophila* and *Legionella* species qPCR (with culture as the standard) was 100%/85% and 100%/40%, respectively. 22% of samples were culture positive for *P. aeruginosa*. Sensitivity/specificity for culture positive samples was 100%/100% for enriched samples. The *Legionella pneumophila* and *P. aeruginosa* qPCR are recommended for evaluating the risk of exposure from water within healthcare facilities.

Background

Legionella environmental surveillance of hospital water systems is important in determining the risk for hospital acquired Legionnaires' disease. *Legionella* culture is the gold standard, however, rapid methods like real-time quantitative PCR (qPCR) could be used to obtain a rapid evaluation of *Legionella* contamination. The GeneSystems qPCR can identify *Legionella pneumophila* and *Legionella* bacteria in water samples in 3 hours versus 7-10 days for culture method. While testing has been done using cooling tower samples, evaluation with hospital potable water samples is needed to demonstrate the utility of this technology for routine *Legionella* analysis. *Stenotrophomonas maltophilia*, *Acinetobacter* species, and *Pseudomonas aeruginosa* are waterborne pathogens which cause significant morbidity and mortality and infection has been linked to hospital water. We also evaluated real-time, qualitative PCR for detection of *P. aeruginosa* in water samples

Objectives

1. Evaluate the sensitivity and specificity of PCR vs. culture in detecting the presence of *Legionella pneumophila* and *Legionella* species.
2. Evaluate the utility of GeneSystems' qPCR method for *Legionella* risk assessment of drinking water by comparison to standard culture method.
3. Evaluate the performance of GeneSystems' qualitative PCR for detection of *P. aeruginosa* in drinking water samples.

Materials and Methods

100 potable warm water samples (1 L) were collected from 18 healthcare institutions and one building housing senior citizens and split for testing.

Legionella Analysis

qPCR - Total DNA was extracted from 500 ml using the GeneExtract instrument (in approximately 2 h). (GeneSystems, Bruz, France)

Approximately 200 μ L of purified extract is recovered. qPCR were performed with the GeneDisc Cycler with GeneDisc *L. pneumophila* (sg 1 to 15) and a separate GeneDisc *Legionella* spp. (approximately 1 h per disc). The genome units of DNA per sample were expressed as GU/L. For a 500 mL sample: Level of Detection ($_{LOD}$ PCR) equals: 330 GU/L; Level of Quantification ($_{LOQ}$ PCR) equals: 1666 GU/L. If the GU was less than the $_{LOD}$ PCR, the sample was reported as "Absence".

Culture - Analysis for all *Legionella* species, including *pneumophila*, was performed directly and after concentration. Buffered Charcoal Yeast

Extract (BCYE) medium and BCYE with dyes, glycine, vancomycin, and polymyxin (DGVP) were used for culture isolation. Concentration was achieved by filtering 100 mL of the sample through a 47 mm diameter, 0.2- μ m pore size polycarbonate filter membrane. The membrane was transferred to 10 ml of original sample and vortexed to suspend the concentrate. Results were obtained in 7 days after humidified incubation at 36°C (1).

Pseudomonas aeruginosa analysis with enrichment

Qualitative PCR - 100 mL of the sample was filtered through a membrane filter (mixed cellulose ester, 0.45- μ m, 47 mm diameter) which was transferred to a 50 mL sterile tube containing 10 mL *Brucella* broth. After a 15 h incubation, the extraction was carried out with 2 mL of the broth culture. Bacterial cells were isolated after a series of centrifugations and incubations, then lysed. The bacterial DNA was recovered by purification with dedicated resin supplied in the lysis buffer. The final DNA solution is analyzed using the *Pseudomonas aeruginosa* GeneDisc Pack.

Culture - 100 mL aliquot of each sample was tested following the ASTM International Standard Test Method [D 5246 – 92 (Reapproved 2004)].

Briefly, 100 mL sample was concentrated on a filter membrane (as used for *Legionella*) which was placed on the surface of M-PA-C agar [Modified *Pseudomonas* Agar, C (3rd formula)]. Plates were incubated at 42°C for 48-72 h. If isolated, *P. aeruginosa* will demonstrate characteristic colonies with dark centers.

Results

Legionella - Six of 19 institutions (32%) were positive for *Legionella* by culture; 17/100 samples yielded *L. pneumophila* and 1/100 yielded species other than *L. pneumophila*. *L. pneumophila* PCR (LP PCR) was positive for 29/100 samples and *Legionella* species PCR (LG PCR) was positive for 67/100 samples. Sensitivity and specificity data are presented in Table 1, both using culture as the "Gold Standard" for defining True Positive, and using PCR as the "Gold Standard" for defining True Positive. Figures 3 and 4 depict typical PCR curves showing PCR cycles versus fluorescence units as detected and calculated by the GeneDisc Cycler.



Figure 1. *L.pneumophila* on BCYE.



Figure 2. *P. aeruginosa* on M-PA-C.

P. aeruginosa - Twenty-two samples from 9 institutions were culture positive for *P. aeruginosa*. *P. aeruginosa* PCR (PA PCR) was positive for 25/98 samples.

Other Waterborne Pathogens - In addition to *Legionella* and *Pseudomonas*, *Stenotrophomonas maltophilia* and *Acinetobacter* species were also isolated from 7 and 1 sample, respectively.

Table 1. PCR was positive more often than culture for *L. pneumophila* (LP), *Legionella* species (LG) and *P. aeruginosa* (PA). Sensitivities and specificities are shown for culture versus PCR using culture as the "Gold Standard" and using PCR as the "Gold Standard".

| | <i>Legionella</i> Test Results | | | Sensitivity and Specificity | | | | |
|--------|-----------------------------------|-----|-------|-----------------------------|-------------|----------------------|-------------|--------|
| | Culture | | Total | Culture as Gold Standard | | PCR as Gold Standard | | |
| | Neg | Pos | | Sensitivity | Specificity | Sensitivity | Specificity | |
| LP PCR | Neg | 71 | 0 | 71 | 100.0% | 84.5% | 55.2% | 100.0% |
| | Pos | 13 | 16 | 29 | | | | |
| Total | | 84 | 16 | 100 | | | | |
| LG PCR | Neg | 33 | 0 | 33 | 100.0% | 39.8% | 25.4% | 100.0% |
| | Pos | 50 | 17 | 67 | | | | |
| Total | | 83 | 17 | 100 | | | | |
| | <i>P. aeruginosa</i> Test Results | | | Sensitivity and Specificity | | | | |
| | Culture | | Total | Culture as Gold Standard | | PCR as Gold Standard | | |
| | Neg | Pos | | Sensitivity | Specificity | Sensitivity | Specificity | |
| PA PCR | Neg | 72 | 1 | 73 | 95.7% | 94.7% | 86.2% | 98.6% |
| | Pos | 4 | 21 | 25 | | | | |
| Total | | 76 | 22 | 98 | | | | |

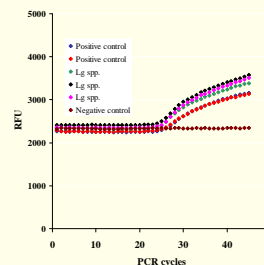


Figure 3. PCR curve of a water sample demonstrating presence of *Legionella* species.

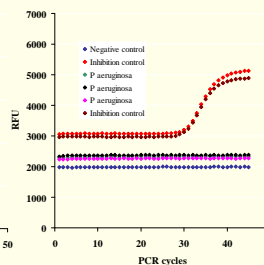


Figure 4. PCR curve of a water sample demonstrating absence of *P.aeruginosa*.

Conclusions

Legionella - *L. pneumophila* PCR can be used with confidence to evaluate the risk of exposure to *L. pneumophila* from hospital water distribution systems. We note, the differential pathogenicity among the 16 serogroups of *L. pneumophila* would necessitate serotyping of isolates from cultures to adequately assess the risk of Legionnaires' disease (2). The low specificity of the *Legionella* species PCR compared to culture may preclude the meaningful use of the species PCR in evaluating disease risk from *Legionella* species in potable water samples. This weak correlation was also seen with cooling tower samples (3). The frequency of positive PCR results in the absence of disease suggests that this result should be interpreted with caution. It may be that *Legionella* species (or genetically-related species) are easy to detect by PCR and more difficult to detect by culture.

P. aeruginosa - Given the excellent correlation between culture and PCR for the detection of *P. aeruginosa*, PCR could be used as an alternative to culture for environmental surveillance of hospital water systems.

Discussion and Implications

The advantages of PCR include the ability to detect microorganisms that cannot be cultivated on artificial media and more rapid detection for those that are slow-growers. The GeneSystems *Legionella* PCR would be useful in situations where rapid results are needed, such as outbreak investigations and following remediation efforts. Under these circumstances, the cost of rapid methods such as PCR is not a factor. Potable water systems of hospitals are often implicated as sources of outbreaks of hospital-acquired Legionnaires' disease. The accuracy of the *Legionella pneumophila* PCR test will be important tool in outbreak investigations.

References

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