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# Letter to the Editor

## Caution on Interpretation of *Legionella* Results Obtained Using Real-Time PCR for Environmental Water Samples

In an article entitled "Quantitative Real-Time *Legionella* PCR for Environmental Water Samples: Data Interpretation" (2), the authors reported their results using quantitative real-time PCR to detect *Legionella* in environmental water samples. The authors found high false-positive rates for the PCR test if culture was used as the reference standard (Table 1), which is in agreement with the work of other investigators (3, 5) who found a higher positive rate of water samples with the PCR method than with culture, and the authors suggested that the high positive rate may be due to the presence of viable but nonculturable *Legionella* cells in water samples (6). However, no data were provided.

PCR detects both viable and nonviable cells by amplifying the target nucleic acids in the sample based on the assumption that the nucleic acids being copied are from live cells (4). We wish to postulate another hypothesis: the false-positive results are due to the presence of nonviable *Legionella* cells in water samples. Disinfection of cooling towers and potable water systems for *Legionella* have been applied widely. If disinfection is performed, it is likely that the water samples will contain nonviable *Legionella* cells which were killed by the disinfection measures. The remaining nucleic acids in the dead cells may still be recovered and amplified by the PCR. This can explain why no correlation was observed between culture and PCR results.

The detection of viable *Legionella* cells may be achieved by the detection of *mip* mRNA as the target, reverse transcription (to form cDNA), and then PCR amplification. The basis of detecting mRNA and not rRNA is that most of the bacterial mRNA have a short half-life (<2 min). Thus, detecting *mip* mRNA would indicate the presence of metabolically active (living) *Legionella* cells that must be present within a few minutes prior to the sample process. However, this method is technically more difficult and less sensitive (1).

False-positive readings of *Legionella* samples could lead to unnecessary and expensive emergency decontamination procedures. Using the PCR result for *Legionella* samples may overestimate the risk of infection. The PCR results must be applied with caution. Culture remains the reference standard for testing *Legionella* in environmental samples.

TABLE 1. Sample positivity comparison by culture and PCR methods[a]

| Water sample type | No. of culture-positive samples/no. studied (%) | No. of PCR-positive samples/no. studied (%) |
|---|---|---|
| Cooling tower (study 1) | 9/36 (25 ) | 36/36 (100) |
| Hot water (study 1) | 55/128 (43) | 117/128 (91) |
| Hot water (study 2) | 41/92 (45 ) | 76/92 (83 ) |

[a] As reported by Joly et al. (2).

### REFERENCES

1. **Eej, A. K., M. H. Mahbubani, and R. M. Atlas.** 1991. Detection of viable *Legionella pneumophila* in water by polymerase chain reaction and gene probe methods. Appl. Environ. Microbiol. **57:**597–600.
2. **Joly, P., P.-A. Falconnet, J. André, N. Weill, M. Reyrolle, F. Vandenesch, M. Maurin, J. Etienne, and S. Jarraud.** 2006. Quantitative real-time *Legionella* PCR for environmental water samples: data interpretation. Appl. Environ. Microbiol. **72:**2801–2808.
3. **Wellinghausen, N., C. Frost, and R. Marre.** 2001. Detection of legionellae in hospital water samples by quantitative real-time LightCycler PCR. Appl. Environ. Microbiol. **67:**3985–3993.
4. **Wilson, I. G.** 1997. Inhibition and facilitation of nucleic acid amplification. Appl. Environ. Microbiol. **63:**3741–3751.
5. **Yamamoto, H., Y. Hashimoto, and T. Ezaki.** 1993. Comparison of detection methods for *Legionella* species in environmental water by colony isolation, fluorescent antibody staining, and polymerase chain reaction. Microbiol. Immunol. **37:**617–622.
6. **Yamamoto, H., Y. Hashimoto, and T. Ezaki.** 1996. Study of nonculturable *Legionella pneumophila* cells during multiple-nutrient starvation. FEMS Microbiol. Ecol. **20:**149–154.

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