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Rapid detection of viable *Legionella pneumophila* in tap water

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Key words
Legionella pneumophila, viable, rapid detection, rRNA, reverse transcription-PCR

Aim of the study
This study was performed to:
(i) develop a rapid method to detect viable *L. pneumophila* of all serogroups in tap water samples as an alternative to the bacteriological reference method (ISO 11731);
(ii) compare the two methods by analyzing water samples from public sports facilities in the Canton of Basel-Landschaft, Switzerland.

Material and methods
To detect living cells of *L. pneumophila*, an assay was developed which was based on a nutritional stimulation of RNA synthesis and subsequent detection of an increase in precursor 16S rRNA (reverse transcription PCR; RT-PCR) as an indicator for viability. For quantification, a real-time quantitative PCR (qPCR) was performed. As assay performance parameters, the analytical specificity (inclusivity and exclusivity), the limit of detection, and the repeatability were evaluated. In a field study, the developed assay was compared to the standard method of ISO 11731 using 102 tap water samples from public sports facilities in the Canton of Basel-Landschaft, Switzerland.

Results and significance
The developed assay is able to detect all strains of *L. pneumophila* belonging to serogroups 1-14. All other tested *Legionella* spp. and all bacterial strains of other genera tested negative for this target. The limit of detection of the assay was 1'000 cells/L regarding viability (RT-PCR) and 100 cells/L regarding quantification (qPCR).

Applied to the field samples, the new molecular assay as well as the bacteriological analysis (ISO method) revealed each a positive result in 21 (21%) samples for *L. pneumophila*. Diagnostic specificity and sensitivity of the assay were 97% and 91%, respectively, with respect to the official microbiological criterion for *L. pneumophila* for water from showers (1000 CFU/L) and taking the ISO method as the reference. Statistically, the two methods did not differ significantly in the frequency of not matching results and the agreement of both methods was substantial.

The new method is sensitive and specific for *L. pneumophila* and allows results to be obtained within 8 h upon arrival, compared to one week or more by the ISO method. It represents a useful tool for a rapid detection of viable *L. pneumophila* of all serogroups in water by molecular biology. It could be used as an alternative to the ISO method for official water analysis for legionellae and particularly when a short test time is required.

Publications, posters and presentations
Boss, R.; Baumgartner, A.; Kroos, S.; Blattner, M.; Fretz, R.; Moor, D. (2018) Rapid detection of viable *Legionella pneumophila* in tap water by a qPCR and RT-PCR based method. J. Appl. Microbiol. https://doi.org/10.1111/jam.13932

Boss, R. (2017) Molekularbiologischer Nachweis lebender *L. pneumophila* in Wasser. Oral presentation at the meeting of "ERFA molekulare Diagnostik Mikroorganismen", arranged for members of cantonal laboratories in Switzerland and other interested parties. June 2017.

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