Sensitivity of *Legionella pneumophila* DNA detection in serum samples in relation to disease severity

In a recently published report, we assessed the performance of PCR as a diagnostic method and compared the performance of different PCR assays of serum samples from patients with Legionnaires’ disease (LD) (Diederen et al., 2007). Among the patients with proven LD, 54.4% tested positive in 5S rRNA PCR, 52.9% in *mip* gene PCR and 30.9% in 16S rRNA PCR in the first available serum sample. We were not able to investigate the relationship between test sensitivity and severity of disease. However, the association between Ct value in 5S PCR positive serum samples ($n=49$) and C-reactive protein value was determined, and showed a strong negative correlation (Pearson correlation coefficient $r=-0.63; P<0.0001$). As the urinary antigen test is less reliable in milder cases of LD, it would be plausible that the same holds true for the detection of *Legionella* DNA in serum (Yzerman et al., 2002). The aim of the described study was to assess the sensitivity of PCR as a diagnostic method and to investigate a possible relationship between test sensitivity and the severity of disease.

In this study, 68 outbreak-related patients (Bovenkarspel, The Netherlands) with confirmed LD according to the European Working Group for Legionella Infections (EWGLI) criteria were investigated (Den Boer et al., 2002; Yzerman et al., 2002). To investigate the relationship between test sensitivity and severity of disease, the patients were divided into two clinical categories for pneumonia. Patients were classified as category 1 (mild pneumonia and moderately severe) and category 2 (severe pneumonia). LD was defined as severe when two or more of the following conditions were present: (1) respiratory rate $>30$ min$^{-1}$; (2) chest radiograph showing bilateral involvement or involvement of multiple lobes; (3) shock; (4) PaO$_2$ $<60$ mmHg or arterial oxygen saturation $<92\%$. A real-time assay targeted at specific regions within the 5S rRNA gene was used (Diederen et al., 2007).

Samples included 136 serum samples obtained from 68 patients with LD. In one sample, inhibition of PCR occurred; this patient was excluded from the analysis. Among the patients with LD, 39% [26/67; 95% confidence interval (CI) 28–51%] tested positive in PCR in the first available serum sample, and this number increased to 54% [36/67; 95% CI 42–65%] if all serum samples were included in the calculations. The detection rate for all non-inhibited samples was 35% [47/135; 95% CI 27–43%]. For 58 patients, data on disease severity were available. In patients with severe pneumonia, 49% (19/39; 95% CI 34–64%) tested positive in the first available serum sample, increasing to 67% (26/39; 95% CI 51–79%) if all serum samples were included in the calculations. In patients with mild and moderately severe pneumonia, 37% (7/19; 95% CI 19–59%) tested positive in the first available serum sample, increasing to 53% (10/19; 95% CI 32–73%) if all serum samples were included.

The sensitivity of PCR on serum samples found in our study was relatively low compared to previous studies (Diederen et al., 2007; Lindsay et al., 2004). An important limitation of this study is the fact that the serum samples were stored at $-20$ °C and thawed repeatedly before the PCR assays were applied. The storage and thawing probably influenced the stability of *Legionella* spp. DNA present in the samples. In addition, we only tested a relatively small number of patients in both groups; the calculated sensitivities are therefore more uncertain as indicated by the confidence intervals that are provided here. Although we did observe a higher sensitivity in patients with more severe disease (49% vs 37%, $P=0.4$), these differences did not reach statistical significance. There is a need for larger, prospective studies to determine the role and added value of PCR on serum samples, and to further investigate the relationship between test sensitivity and the severity of disease.

**Correspondence:** Bram M. W. Diederen (bramdiederen@gmail.com)

- **1**Regional Laboratory of Public Health Haarlem, Boerhaavelaan 26, 2035 RC Haarlem, The Netherlands
- **2**Municipal Health Service Kennemerland, PO Box 5514, 2000 GM Haarlem, The Netherlands
- **3**Laboratory of Medical Microbiology and Immunology, St Elisabeth Hospital, PO Box 747, 5000 AS Tilburg, The Netherlands


