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Are Oropharyngeal Swabs Suitable as Samples for *Legionella*-Specific PCR Testing?

In a recent study by McDonough et al., a *Legionella* cluster was identified through retrospective PCR analysis of 240 throat swab samples from cases of pneumonia among young and otherwise healthy U.S. military recruits (4). Results were confirmed by sequence analysis. No diagnostic evidence other than PCR results supported their findings. Although we appreciate the systematic description of their cluster, we question the validity of the use of PCR with oropharyngeal swabs for the diagnosis of Legionnaires' disease (LD). The interpretation of these results is problematic and must be applied with caution.

Culture diagnosis remains the gold standard for diagnosis of LD and is the most specific diagnostic procedure. Currently, a positive culture, a positive urinary antigen test, or a fourfold or greater rise in antibody titer against *Legionella pneumophila* is definitive of a confirmed case, and PCR-positive samples are classified only as presumptive by the European Working Group on Legionella Infections (http://www.ewgli.org/) (2). During an epidemic or in a setting with an unusual high prevalence, a specificity of 100% is not an essential prerequisite for a diagnostic test. However, when the prevalence of infection is low, even a modest loss of specificity will result in false-positive findings. This holds true especially for new (commercial) diagnostic methods for which clinical specificity is not yet well defined, such as PCR. False-positive PCR results have been reported previously (1). The quality performance of 46 participating laboratories for the detection of *Legionella* spp. by two quality control exercises was investigated in 2004 and 2005 (5). The rate of false positivity ranged from 4.0% in 2004 to 8.2% in 2005.

Oropharyngeal swabs may be a suitable sample for PCR testing, but this application has been evaluated only in a small study, in which five of six samples from patients with LD tested positive (6). We conducted a study using oropharyngeal swabs obtained from a group of hospitalized patients with pneumonia. Specimens from 242 adults admitted to hospital with community-acquired pneumonia were tested (7). For the detection of *Legionella*, two assays targeted at specific regions within the 5S rRNA gene (detects all *Legionella* species) and the *mip* gene (detects only *L. pneumophila*) were used (3). *L. pneumophila* PCR was positive in only 3 out of 11 confirmed cases (27%) of LD. These findings indicate that oropharyngeal swabs are not a reliable sample for *Legionella* PCR.

McDonough et al. (4) performed confirmatory nucleic acid amplification testing and sequence analysis. The logic behind confirmatory testing is based upon two assumptions. The assumptions are that failure to confirm a positive result means that the initial positive result was likely a false positive and that confirming the initial positive result increases confidence that it was a correct result. A problem is that by applying discrepant analysis, the test under evaluation is used to define a true-positive result, and new tests under evaluation should be compared to an independent gold standard (e.g., other diagnostic tests). In addition, discrepant analysis involves post hoc testing of specimens that were positive in the initial evaluation, and such selective testing of specimens is biased in favor of the new test.

The predictive values of PCR testing of oropharyngeal swabs for *Legionella* spp. are not yet sufficiently characterized. Of all the common pneumonia pathogens, *Legionella* species probably present the greatest risk for PCR contamination, given the organism's environmental habitat (8). Even if there truly was a cluster of infections with *L. pneumophila*, McDonough et al. should have given more consideration to gaining additional laboratory evidence for the occurrence of LD.

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Author's Reply

I agree in principle with most of the statements and note that the paper already discusses many of these issues. The strongest support for the association between *Legionella pneumophila* and pneumonia in this study does not come from the demonstrated validity of the methods but from the coclustering in time and space of the *L. pneumophila*-positive oropharyngeal swabs and the pneumonia patients from which they were collected. Sequencing provides more than just confirmation of the original PCR results, as sequencing of even short amplicons provides much

more specificity than PCR (by demonstrating that the amplicons did not arise from nonspecific cross-reactions but rather from the target species). I am the first to admit that causality was not demonstrated and that the methods used were suboptimal by necessity (the article says these things explicitly), and I hope that our paper will serve as a catalyst to inspire the use of more traditional methods of *L. pneumophila* surveillance in crowded and susceptible communities of young adults.

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