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Legionnaires' Disease Pseudoepidemic Due to Falsely Positive Urine Antigen Test Results

Maria Cristina Rota,[a] Stefano Fontana,[b] Carmen Montaño-Remacha,[a] Maria Scaturro,[b] Maria Grazia Caporali,[a] Vincenzo Vullo,[c] Laura Scorzolini,[c] Andreina Ercole,[d] Maria Luisa Ricci[b]

National Centre for Epidemiology, Surveillance and Health Promotion, National Institute of Health, Rome, Italy[a]; Infectious, Parasitic and Immune-Mediated Diseases Department, National Institute of Health, Rome, Italy[b]; Public Health and Infectious Diseases Department, Sapienza University of Rome, Rome, Italy[c]; Public Health Prevention Department, Local Health Unit Rome A, Rome, Italy[d]

Legionella pneumophila is an opportunistic human pathogen that can cause a severe respiratory syndrome known as Legionnaires' disease (LD). *L. pneumophila* accounts for 98% of all LD cases reported in Europe (1). Although culture of respiratory secretions and serology are also methods used for diagnosing LD, the urinary antigen test (UAT) is easy and fast to perform; consequently, ca. 77% of cases are diagnosed using this method (1). Several UATs with high specificities but varied sensitivities have been developed; most of them are able to reliably detect only Lp serogroup 1 antigen (2–4). In this brief report, we describe a pseudo-LD outbreak due to false-positive UAT results. Between December 2012 and January 2013, 18 community-acquired LD cases were reported by a university hospital in Rome, Italy. All cases were living in Rome and were diagnosed using one particular UAT (Xpect; Thermo Fisher Oxoid, Basingstoke, United Kingdom) in two independent laboratories of the same university hospital (the emergency department laboratory [herein lab 1] and the central hospital laboratory [herein lab 2]). Alerted by an increased number of reported cases, the local health authority started an epidemiological investigation in December 2012. A questionnaire was used to interview each patient, with collection of clinical data, individual risk factors, and possible exposures. Interviews showed that the patients' houses were located all over the city, and no common exposures were identified. By the end of January 2013, the outbreak was considered improbable since no common source or epidemiological link was found among the 18 LD cases and no further cases were reported in any other hospital in Rome. An incorrect laboratory diagnosis was hypothesized. The Hospital Infection Control Team was requested to verify which assay was used for the laboratory diagnosis of each case. Furthermore, a careful revision of clinical records of all LD cases was recommended. The in-depth analysis highlighted that only 9 out of the 18 reported cases fulfilled the current case definition (clinical and laboratory criteria). Despite the presence of clinical criteria, nine patients did not fulfill the case definition, because the same UAT (Xpect; Thermo Fisher Oxoid, Basingstoke, United Kingdom) performed by both lab 1 and lab 2 gave discordant results (the lab 1 result was positive, and the lab 2 result was negative). For some patients, both labs used the same UAT lots.

Respiratory secretions, sera, and urine samples were available from 4 of the 9 patients with discordant test results. In order to confirm the diagnosis, they were sent to the National Reference Laboratory for *Legionella*. Urine samples were boiled, centrifuged, and then tested by both Xpect and BinaxNOW (Alere, USA) UATs. All samples gave negative results. Therefore, only 9 cases were classified as confirmed.

As a precautionary measure, the Hospital Infection Control Team decided to withdraw the Xpect UAT from the laboratories and to replace it with two other commercially available kits. Following this, the incidence of LD later decreased to the expected hospital values. In mid-February 2013, an urgent security warning was issued by the Thermo Fisher Oxoid company about the use of 3 Xpect lots associated with possible false-positive results, inviting the users to reevaluate the results obtained and to consider running the tests again. One of the 3 lots was found to be among those used by the two laboratories of the university hospital. The specificities of UATs in detecting Lp serogroup 1 are known to be very high (97 to 100%), with a low probability of false-positive results (2–4). Enhanced surveillance allowed the prompt identification of this false cluster and the implementation of corrective measures earlier than the official communication from the UAT producer.

This experience highlighted two important issues in the diagnostics of LD that should be considered when an outbreak is suspected. First, the reliability of a test is never absolute. Results obtained can be impaired depending on the quality of the test but also on the procedures applied by different operators, while the application of standard operating procedures might help to achieve uniformity of performance of the test. Therefore, during the outbreak investigation, the National Reference Laboratory for *Legionella*, as per the recommendations of the European Centre for Disease Prevention and Control (ECDC) *Legionella* Reference Laboratory at Public Health England, London, provided laboratories with a modified protocol to result in a better performance of immune-chromatographic tests (unpublished data). All positive urine samples should be retested after boiling for 5 min and centrifugation (5 min at 12,000 × *g*), and only the supernatants remaining positive should be considered valid. This retesting is advisable because laboratories would normally follow the manufacturer's instructions indicating that unboiled samples should be tested (5). Second, the diagnosis of LD should always be based on complementary assays, such as culture, serology, and (in the future) PCR, once this method has been validated in interlaboratory studies (6, 7).

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Address correspondence to Maria Luisa Ricci, marialuisa.ricci@iss.it.

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