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Evaluation of three Immunochromatographic Assays for Detection of *Legionella pneumophila* serogroup 1 Antigen in Urine Samples

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ABSTRACT

The Uni-Gold, the SAS and the Binax NOW immunochromatographic test (ICT) urinary antigen assays for the qualitative detection of *Legionella pneumophila* serogroup 1 were compared using 39 unfrozen and nonconcentrated urine samples from patients with Legionnaires´ disease (LD). The Uni-Gold antigen test detected the urinary antigen in 41% (16/39), the SAS antigen test in 61.5% (24/39), and the Binax NOW antigen test in 74.3% (29/39). The Binax NOW ICT assay showed the best results when detecting *L. pneumophila* urinary antigen.

Key words: *Legionella pneumophila*, antigen, urine sample, detection and immunochromatographic test.

Evaluación de tres métodos inmunocromatográficos para la detección del antígeno de *Legionella pneumophila* serogrupo 1 en muestras de orina

RESUMEN

Los métodos inmunocromatográficos Uni-Gold, SAS y Binax NOW para la detección cualitativa del antígeno de *Legionella pneumophila* serogrupo 1 en orina fueron comparados empleando 39 muestras de orina, sin congelar y sin concentrar, de pacientes con Enfermedad del Legionario. La prueba Uni-Gold detectó el antigen en el 41% de los casos (16/39), SAS en el 61,5% (24/39) y Binax NOW en el 74,3% (29/39). La prueba Binax NOW mostró los mejores resultados en la detección del antígeno de *L. pneumophila* serogrupo 1 en muestras de orina.

Palabras claves: *Legionella pneumophila*, antígeno, muestra urinaria, métodos inmunocromatográficos

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INTRODUCTION

Legionella is an important cause of both community-acquired and nosocomial pneumonia, and ***Legionella pneumophila*** serogroup 1 has been reported to be responsible for as many as 90% of ***Legionella*** infections. ***Legionellae*** are ubiquitous water-borne gram negative bacilli. Methods for diagnosing legionellosis include: culturing the organism from body fluids and tissues, visualizing the bacterium with direct fluorescent antibody staining, detecting serum antibody through indirect fluorescent antibody (IFA) and enzime-linked immunosorbent assays (ELISA), ***Legionella*** urinary antigen detection (UAD) and polimerase chain reaction (PCR). Experience has shown that approximately 92% of patients with cultural-proven serogroup 1 ***L. pneumophila*** pneumonia excrete detectable levels of urinary antigen[1]. The UAD is possible thanks to enzyme immunoassays (EIAs) or immunochromatographic (ICT) tests. Although the sensitivity of EIAs has been reported higher[2], ICT assays show important advantages over conventional EIAs: tests are very easy to perform (do not need special laboratory equipment) and results can be obtained much quicker, within 15 min.

The aim of our study was the evaluation of three ICT assays: the Uni-Gold ***Legionella*** antigen test, the SAS ***Legionella*** antigen test and the Binax NOW ***Legionella*** antigen test for the detection of ***L. pneumophila*** serogroup 1 in unfrozen and nonconcentrated urine samples.

MATERIAL AND METHODS

Patients: From 26 June to 19 July 2001, the city of Murcia, in the south-east of Spain, underwent through the world´s largest outbreak of LD. In July 2007, 39 frozen urine samples from patients who suffered from LD during the 2001 massive outbreak[3] were included in this study. A confirmed case of LD was defined as a patient who fulfilled the epidemiological criteria, who showed radiological signs of infiltration and for whom LD was confirmed by serology, considered the " Gold standard " for the diagnosis of LD and defined as a fourfold rise of antibody titers to ***L. pneu-***

mophila serogroup 1 in paired sera to ≥ 128.

Collection of urine samples and storage for 6 years: The 39 urine samples included in this study were collected between June and July 2001 among those patients who came along the hospital with LD. After receipt, samples were tested simultaneously using the Biotest *Legionella* urine antigen EIA (Biotest AG, Dreieich, Germany), Binax NOW ICT *Legionella* urinary antigen test (Binax, Portland, Maine) and Bartels ELISA *Legionella* urinary antigen (Intracel, Frederick, Md) ([2]). After the samples were stored at -80ºC for 6 years.

Urinary antigen tests: The presence of *L. pneumophila* antigens serogroup 1 in urine samples after six years of freezing storage was investigated by using the Uni-Gold (Trinity Biotech PLC, Wicklow, Irland), the SAS (SA Scientific, San Antonio, TX, USA) and the Binax NOW (Binax, Portland, Maine) *Legionella* urinary antigen tests. The three of them are qualitative ICT assays. This three ICT tests consist of a test device in which rabbit anti-*L. pneumophila* serogroup 1 antibody is adsorbed onto nitrocellulose membrane (the patient line) and goat anti-rabbit IgG (the control line) is adsorbed onto the same membrane as a second stripe. Rabbit anti-*L. pneumophila* serogroup 1 antibodies are conjugated to colloidal gold particles dried onto an inert fibrous support. The tests were used as specified by the manufacturers. *L. pneumophila* serogroup 1 antigen captured by immobilized anti-*L. pneumophila* serogroup 1 antibodies reacts to bind conjugate antibody. Immobilized goat anti-rabbit IgG also captures conjugated antibody, forming the control line. The reactions were read within 15 min.

RESULTS AND DISCUSSION

When using unfrozen and nonconcentrated urine samples, the Uni-Gold antigen test detected the *L. pneumophila* serogroup 1 antigen in 16 of the 39 urine samples (41%), the SAS antigen test in 24 of the 39 urine samples (61.5%), and the Binax NOW antigen test in 29 of the 39 urine samples (74.3%).

Diagnosing infections by detecting soluble microbial molecules in urine is an antique resource. Since in 1918 it was demonstrated not only the presence in high concentrations of the polysaccharide molecules in urine but also their permanence for weeks, the development of urinary antigen detection systems started. In 1979, because of the difficulty of obtaining quality respiratory samples, the special laboratory media required for growing *L. pneumophila* and the not helpful in acute diagnosis serum antibody detection for which greater sensitivity is observed with samples obtained at least 6 weeks after symptom onset, made the detection of *Legionella* antigen in urine an alternative approach to diagnosing LD[4].

Since then, a variety of methods detecting Legionella urinary antigen have demonstrated sensitivities comparable to other established detection methods and specificities of 100%. In addition, urine is an easily obtainable specimen available in large volumes, that is why it can be concentrated and thus may test positive when other body fluids are negative.

Since antigen detection in urine has proved to be a sensitive and rapid method for the detection of *L. pneumophila* serogroup 1, this technique has become one of the most-used tools for the diagnosis of Legionnaires´ disease (LD)[5,6]. Urinary antigen detection (UAD) is a world-wide method used for the diagnosis of *L. pneumophila*, *Streptococcus pneumoniae* and other pathogens whose antigens are excreted through the patient´s urine in enough concentration for its detection for a long period of time. This method has many advantages: the specimen collection is easy and a large quantity of it is available for concentration. It is also possible to detect the antigen after starting antimicrobial therapy, when cultures are negative. These methods have demonstrated an adequate sensitivity and specificity and significantly increase the etiologic diagnosis of pneumonia. However, UAD also presents some disadvantages: 8% of patients with legionellosis do not excrete the *Legionella* antigen in their urine[4], it is only possible the detection of *L. pneumophila* serogroup 1 by the comercially available assays and no difference between relapse or reinfection may be found due to antigen persistence in urine. Thus, *Legionella* UAD cannot substitute culture and serologic testing but as the ideal single test to diagnose legionellosis has not yet been developed, laboratorians wishing to provide optimum LD diagnostic should offer the three of them.

The comparison between the three ICT assays showed the highest sensitivity for the Binax NOW when using nonconcentrated samples. This is in accordance with previous results[5,6,7,8]. In conclusion, the Binax NOW urinary antigen test is superior to the SAS and the Uni-Gold *Legionella* urinary antigen assays for the diagnosis of infections caused by *L. pneumophila* serogroup 1 when using nonconcentrated samples. However, the three tests considerably reduce the time needed to achieve detection, and at least two of them, Binax NOW and SAS *Legionella* urinary antigen assays, provide levels of sensitivity similar to those of the EIA [2,9] when using nonoconcentrated samples.

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