IN VITRO METHOD FOR DIAGNOSIS OF *Pneumocystis Jirovecii* INFECTION

DESCRIPTION OF THE TECHNOLOGY

Pneumocystis pneumonia (PcP) is a major clinical problem with high morbidity and mortality. It mainly affects immunosuppressed patients, for different reasons (HIV infection, transplants, chemotherapy, autoimmune diseases, etc.). In addition, it also plays an important role in the etiology of the chronic obstructive pulmonary disease (COPD), which is the fourth leading cause of mortality in the world; and also in neonatal respiratory distress syndrome, the main cause of morbidity and mortality in premature infants.

To date, the nested-PCR targeting the *Pneumocystis* mitochondrial large subunit rRNA (mtLSUrRNA) remains the gold standard technique for detecting *P. jirovecii* colonization. Nested-PCR consists of two sequential rounds of conventional PCR that make this technique highly sensitive, enabling the detection of low fungus burdens. This nested-PCR is the method of choice for detecting low *Pneumocystis jirovecii* loads in non-invasive samples such as oral washes, nasopharyngeal aspirates or in lung samples of colonized infants. However, this method is laborious, time consuming (8-10 hours), and entails considerable risk of contamination, increasing the risk of detecting false positives.

Research staff from the Fundació per al Foment de la Investigació Sanitària i Biomèdica de la Comunitat Valencia (FISABIO), the University of Valencia, the Andalusian Health Service, the University of Chile and the Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV, Mexico) have developed a new *in vitro* method for the diagnosis of *Pneumocystis jirovecii* infection. Their work aims to improve detection by developing a single-round PCR quantification assay based on the amplification of the Msg-A multicopy gene family, that is more cost efficient, faster (1 hour) and decreases the chances of contamination. On the other hand, this method also allows the quantification of the pathogen and has greater sensitivity.

MARKET APPLICATION SECTORS

Companies in the pharmaceutical sector and the clinical diagnosis sector.

TECHNICAL ADVANTAGES AND BUSINESS BENEFITS

Speed of detection: you can count on results in 1 or 2 hours.

Greater reliability since the technique reduces the possibility of contamination.

Greater sensitivity to detect *Pneumocystis jirovecii* than other protocols based on PCR or real-time PCR to improve the detection of *Pneumocystis*, so that non-invasive samples can be used.

It allows the quantification of the pathogen in the samples, another advantage that does not allow a conventional PCR or nested PCR.

It would allow its application in Point-of-Care diagnostic devices

CURRENT STATE OF DEVELOPMENT

The kit has been developed using 54 lung tissues obtained from autopsies of infants dead by sudden death.

INTELLECTUAL PROPERTY RIGHTS

Patent number P202030563, filed at the Spanish Patent and Trademark Office on June 10, 2020. PCT international extension is expected.

COLLABORATION SOUGHT

Companies interested in a license agreement to commercialize the technology or a technical cooperation agreement to continue with its development.

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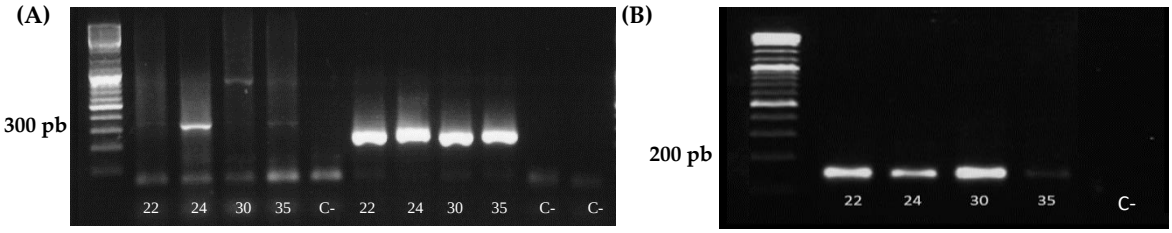

Figure. Electrophoretic migration in a 2% agarose gel of the amplification products obtained by two rounds of nested-PCR (A) and Real-time PCR Msg-A (B) from DNA extracts of lung samples 22, 24, 30 and 35. Lane 1: 1 kb plus ladder DNA marker.

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