



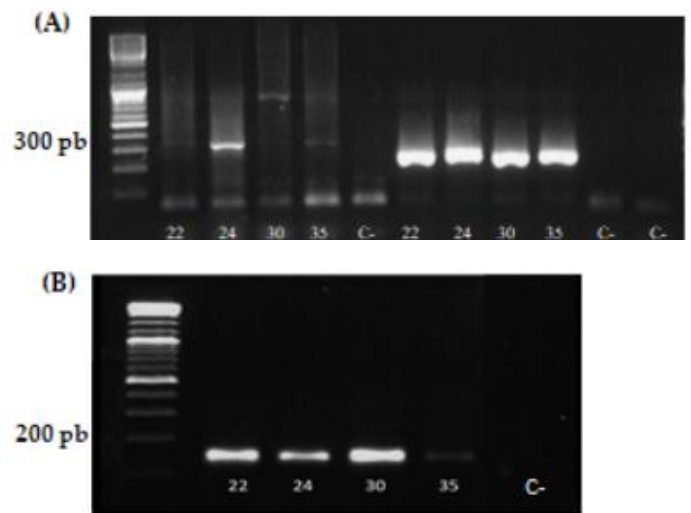
IN VITRO METHOD FOR DIAGNOSIS OF PNEUMOCYSTIS JIROVECI INFECTION

Description

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 València (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.



*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.*

Technical Advantages

- 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..

State of development and Intellectual Property Rights

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

Patent file at Spanish Patent and Trademark Office with registration number P202130735 and priority date July 28, 2021.