Diagnosis of periodontal infections using quantitative polymerase chain reaction (qPCR) technique

Brief description

Molecular biology techniques for the detection and / or quantification of pathogenic bacteria associated with periodontal diseases. Molecular biology has become a very useful tool in the practice of the current dentistry allowing the study of microbial communities in the oral cavity, including species which are not likely to be grown in the laboratory. These techniques are based on the physical characterization of nucleic acid molecules. Among them, polymerase chain reaction (PCR) stands out, which aims to generate a large number of copies of a specific DNA fragment from a minimal amount, making it easier to identify with high probability the bacteria present in a sample.

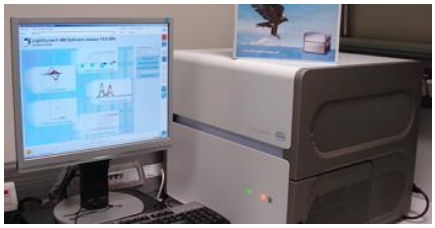

Figura 1: *Equipo LightCycler® 480 II (Roche) del que disponemos en el laboratorio de investigación de la Facultad de Odontología, UCM.*

How does it work?

For application, the first step is the collection and preparation of the samples, considering the conditions for sampling, preservation, and transport. In general, microbiological samples are taken from the areas to be evaluated, usually the gingival sulcus, using sterile paper points. These are placed in a sterile, empty vial and sent to the laboratory for analysis via PCR. To ensure proper preservation of the sample, it is recommended to freeze the vial if it is not sent to the laboratory immediately after collection, allowing for the accumulation of several frozen samples before shipment. It is important to note that if joint analysis of the sample by PCR and conventional anaerobic culture is requested, the paper points should be collected in a vial containing sterile reduced transport fluid (RTF), which helps maintain the anaerobic condition of the samples. In the laboratory, the bacteria present on the paper points are dispersed by agitation, followed by extraction and purification of bacterial deoxyribonucleic acid (DNA) for subsequent analysis using PCR techniques to detect their presence.

For DNA extraction, commercial kits are used with protocols that include chemical, enzymatic, and/or physical agents to release the genetic material, followed by purification of the extracted material. Amplification techniques are then carried out in machines known as thermal cyclers, either using qualitative PCR, with specific primers targeting selected bacteria, or quantitative PCR, which also incorporates fluorescent labeling for the detection of these bacteria in samples, enabling the simultaneous quantification of different species with the optimization of multiplex quantitative PCR.

What problem does it solve?

- qPCR provides accurate measurements by detecting specific sequences of selected organisms during the exponential amplification phase, making it more effective and precise for the diagnosis and treatment of periodontal diseases.
- The ability to perform enhanced multiplex assays allows for the simultaneous detection of multiple targets in a single sample, reducing both costs and time.

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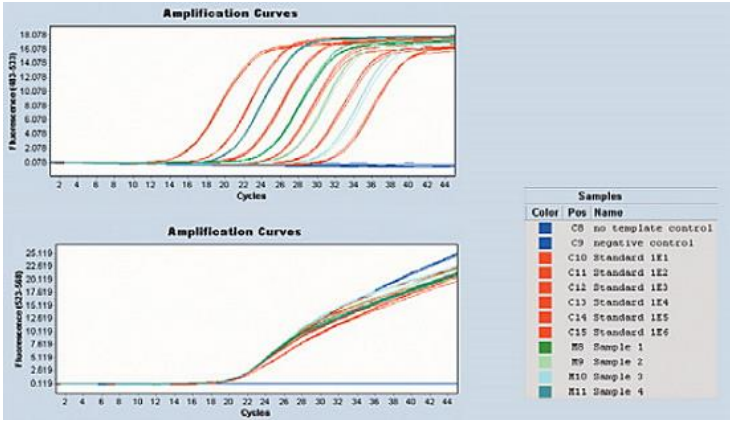

Figura 2: *Ejemplo de un ensayo de cuantificación absoluta utilizando el equipo LightCycler® 480 II (Roche) realizado en el laboratorio de investigación de la Facultad de Odontología, UCM.*

What future products will it develop?

Quantitative PCR provides clear assistance in the rapid diagnosis and treatment of periodontal diseases, particularly in cases where conventional diagnosis cannot be applied. Furthermore, these techniques have proven useful results in the field of Epidemiology, as they enable the identification of the likely source of infection, mode of transmission, and the gateway of the microorganisms involved in infections.

Competitive advantages compared to other research

Currently, quantitative PCR is considered a complementary or alternative procedure to conventional culture, as it allows the study of species that are not cultivable in the laboratory, as well as those with slow or difficult growth. Additionally, it reduces processing times and the time required to obtain results, providing significant advantages for rapid diagnosis in daily clinical practice. Moreover, PCR is more specific and sensitive than culture, as it relies on the detection of specific DNA sequences of bacterial genes, offering the possibility to distinguish closely related bacteria.

Where has it been developed?

This technique has been developed in the Laboratory of Periodontal Research, Department of Stomatology III at the Faculty of Dentistry. The research laboratory uses molecular biology techniques as a support for research and as an aid in diagnosis. The laboratory offers researchers various options, carried out by highly qualified personnel and using high-performance equipment. The laboratory provides technical and analytical advice on these samples, also participating in the development of studies and the optimization of new methodologies being developed in this field.

Y además...

The technology exposed provides assistance in research and to clinicians in two ways:

- Incorporating microbiological analysis into the protocol and variables of research within the field of dental research.
- Assist in the diagnosis and treatment of their patients to clinicians, by provifing advice based on microbiological analysis of samples.

Researcher in charge

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