FLUORESCENT *IN SITU* HYBRIDIZATION APPLIED TO THE RAPID DETECTION OF FUNGI IN UNGUEAL HISTOLOGICAL SECTIONS

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Background: Fluorescent *In situ* Hybridization (FISH) is a powerful technique with increasingly applications in microbiology. Fluorescently-labeled oligonucleotide probes specifically target and hybridize to rRNA so that the whole cell can be visualized directly by fluorescence microscopy [1]. In clinical research it allows to detect directly the presence of the etiologic agent of disease on small samples of patient´s tissue [2].

Objective: To report a FISH method optimization for detection of fungi in ungueal histological sections that can be used for diagnosis of onychomycosis.

Methods: Clinical samples were constituted by ungueal sections of clinically suspected onychomycosis nails collected by podologists in the North of Portugal and embedded in parafin. We modified and adapted the FISH technique used to filamentous fungi in biofilms by [3]. The method comprises of four basic steps: Deparafination of the paraffin embedded sections; Enzymatic digestion of the tissue sample with proteinase k; Hybridization with the fluorescent labeled eukaryotic probe EUK516, 5'-ACCAGACTTGCCCTCC-3', to homologous fragments of rRNA; Mounting and observation by fluorescent microscopy.

Results: FISH with the EUK516 showed distinctive positive reactions in all samples from infected nails. No fluorescence was observed in the sections of the negative control samples (healthy nails). A weak autofluorescence of the sections was detected, but did not interfere with the detection of fungi, that were distinctly observed by a strong, red positive signal. We found enzymatic digestion with proteinase K a critical step for the background reduction of the tissue section.

Conclusions: FISH has been proven to be a rapid, precise and cost effective technique for the detection of fungi within the nail clinical samples.

References:

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