# Usefulness of PCR-Elisa assay for detection of *Trichophyton rubrum, Trichophyton interdigitale, Epidermophyton floccosum* and *Microsporum canis* in skin scrapings and nails in routine laboratory diagnostics

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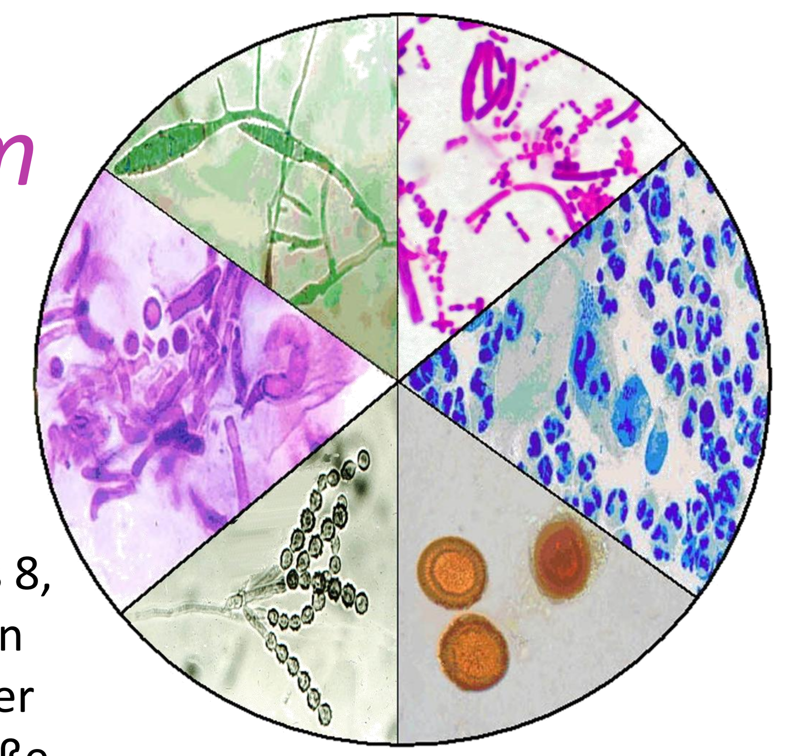
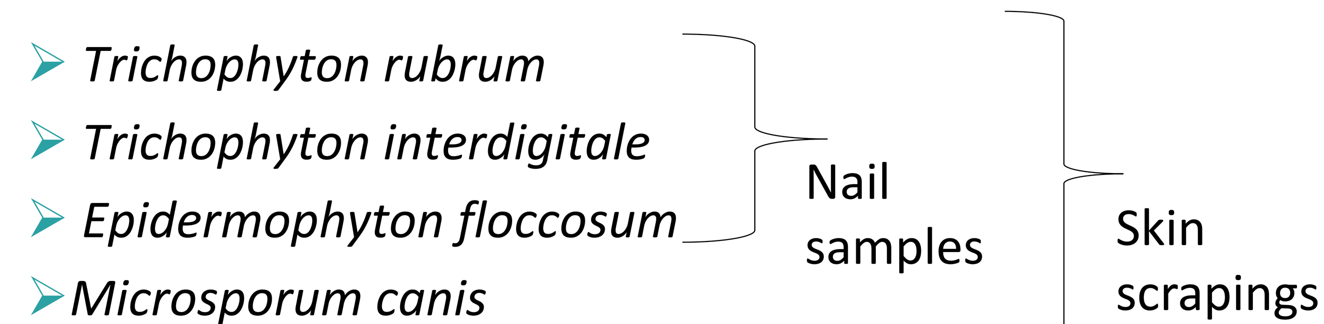


## Introduction

A uniplex PCR-Elisa assay has been used for direct assessment of dermatophytes in clinical samples.



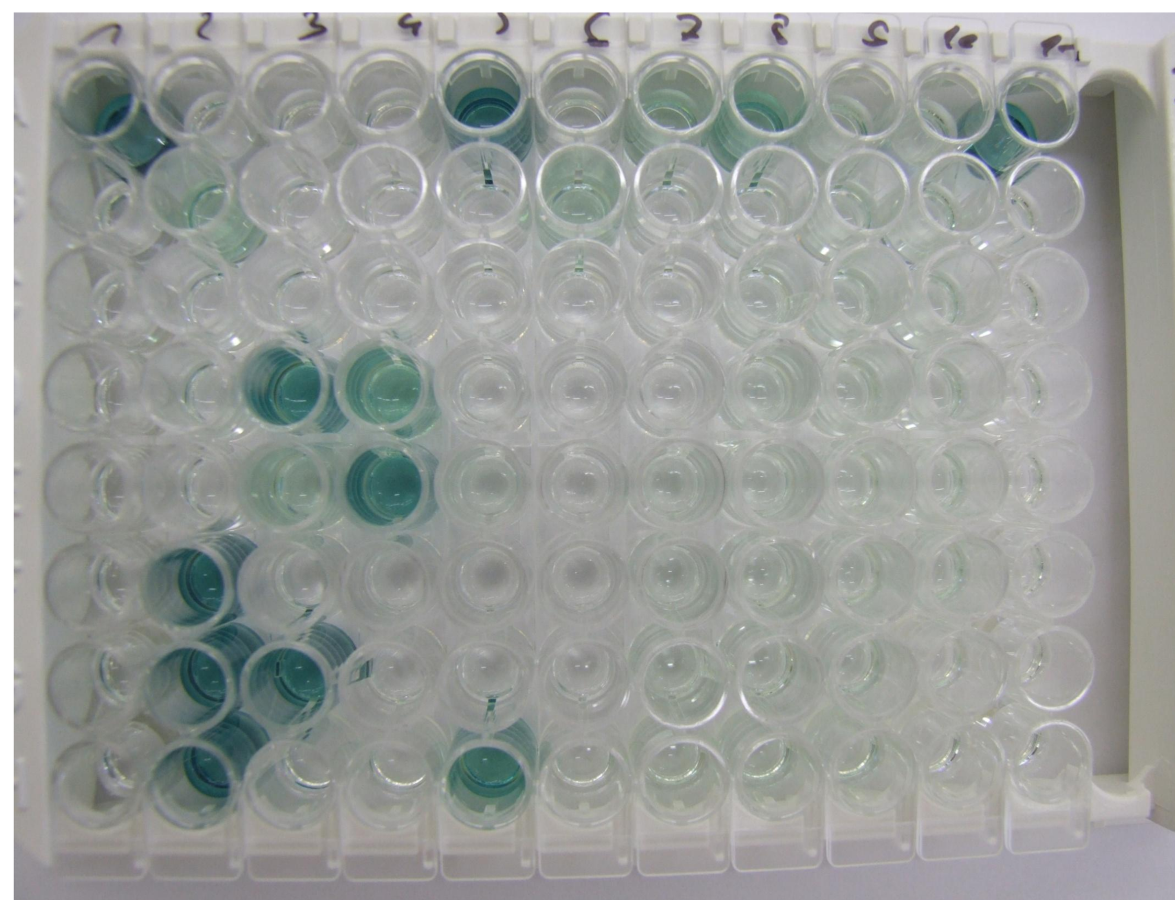

## Methods

The specific target sequence of the primers (one of them labeled by digoxigenin ) was the topoisomerase II gene. DNA isolation was carried out by Qiagen QIAamp DNA Mini Kit. The PCR products were hybridized by biotinylated probes. The biotinylated hybrids were immobilized on streptavidin-coated wells, and detected by using peroxidase-labeled anti-digoxigenin antibody in a colorimetric reaction. Nail specimens were investigated for *Trichophyton* (*T.*) *rubrum*, *T. interdigitale*, and *Epidermophyton floccosum*. Skin scrapings were tested for *Microsporum canis*, additionally. The sensitivity of the PCR was compared to those of the fluorescence preparation (Calcofluor®) and cultural isolation.

## Diagnostic sensitivity and specificity Cultivation vs. native preparation (Calcofluor)

| | Native preparation negative | Native preparation positive | All together |
|---|---|---|---|
| Cultivation positive | 223 | 938 | 1161 |
| Cultivation negative | 2017 | 486 | 2503 |
| All together | 2240 | 1424 | 3664 |

**Native preparation (Calcofluor)**
Sensitivity 80.1 % Specificity 80.6 %



Visualization of the PCR product by Elisa-Technique

- The PCR products were hybridized by biotinylated probes. The biotinylated hybrids were immobilized on streptavidin-coated wells, and detected by using peroxidase-labeled anti-digoxigenin antibody in a colorimetric reaction.

## Results

In 1414 out of 3664 samples (duration of the study 10 months) dermatophytes could be detected using cultivation and/or PCR. 960 (68 %) samples were positive both in culture and PCR, in 201 (14 %) samples a dermatophyte grew, but PCR was negative. In 253 samples (18 %) the culture was negative, however PCR was positive. The diagnostic sensitivity of the Calcofluor® preparation was 80.1 %, when compared to culture. The specificity was found to be 80.6 %. The diagnostic sensitivity of the dermatophyte culture was 82.1 %, the specificity 100 %. The sensitivity of the PCR was 85.8 %, higher than that of cultivation. Among 1414 detected dermatophytes 68.8 % were T. rubrum, 20.1 % *T. interdigitale*, 0.8 % *Epidermophyton floccosum*, and 0.3 % *Microsporum canis*.

## Diagnostic sensitivity and specificity Cultivation vs. PCR

| | PCR negative | PCR positive | All together |
|---|---|---|---|
| Cultivation positive | 201 | 960 | 1161 |
| Cultivation negative | 2250 | 253 | 2503 |
| All together | 2451 | 1213 | 3664 |

**Cultivation**
Sensitivity 82.1 %
Specificity 100.0 %

**PCR**
Sensitivity 85.8 %

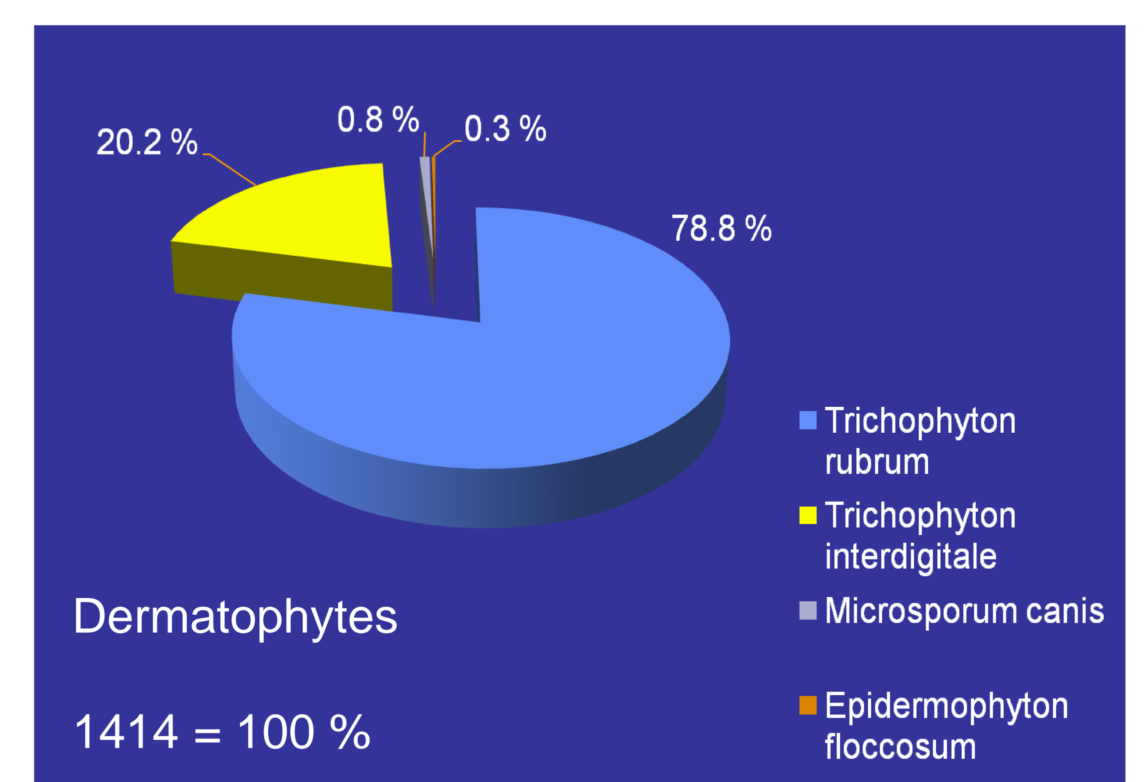
## Comparison cultivation *vs.* PCR

**Clinical samples tested n = 3664**

| Dermatophyte detected by cultivation and/or PCR | 1414 | 100 % |
|---|---|---|
| Out of these were: | | |
| Cultivation & PCR positive | 960 | 68 % |
| Cultivation positive/PCR negative | 201 | 14 % |
| Cultivation negative/PCR positive | 253 | 18 % |

## Discussion

In conclusion, the PCR-Elisa assay completes conventional laboratory diagnostics of dermatophyte infections. The assay appears to exhibit high sensitivity and specificity. This rapid method reduces duration of laboratory diagnostics and is a cost-effective procedure. The technique could prove suitable for use in the routine examination of clinical specimens in dermatology and is a promising option for rapid and direct identification of dermatophytosis.

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