Evaluation of a PCR-based microarray for rapid and sensitive dermatophyte diagnostics.

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Abstract

Dermatomycosis remains a global concern. While conventional culture is considered the gold standard for its diagnosis, it is laborious, lengthy and requires a high level of expertise. In contrast, novel molecular assays promise rapid yet sensitive and specific detection of dermatophytes. Here, we evaluate the performance of a novel PCR-based procedure in comparison to culture as reference.

Methods

A total of 223 isolates were collected from 05/2022 to 08/2022. These clinical samples were prospectively analysed in parallel by culture and the EUROArray Dermatomycosis.

For cultural analysis, sample material was plated and incubated on Sabouraud, Dermatophyte and Candida Agar. Primary material was digested by Proteinase K followed by automated extraction via easyMAG and subsequently used for the identification of up to 23 dermatophytes, 3 yeast and 3 mould species by the EUROArray.

The fungi identified were classified according to their pathogenicity:

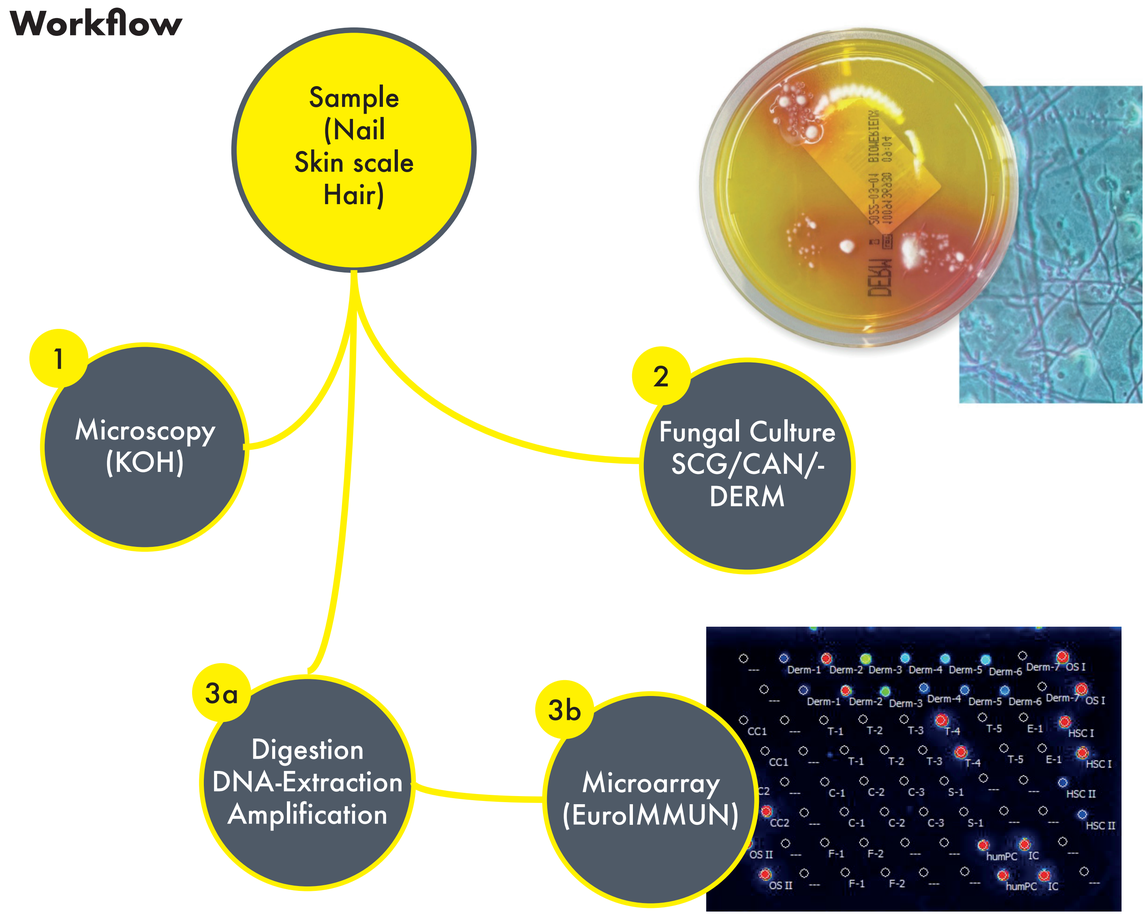
obligate pathogenic

Trichophyton spp.
Epidermophyton spp.
Microsporum spp.
Nannizzia spp.

facultative pathogenic

Cladophialiphora spp. *Cladosporium spp.* *Fusarium spp.*
Chaetomium spp. *Penicillium spp.* *Candida spp.*
Acremonium spp. *Scedosporium spp.* *Trichoderma spp.*
Aspergillus spp. *Aureobasidium spp.* *Scopulariopsis spp.*

Workflow



Results

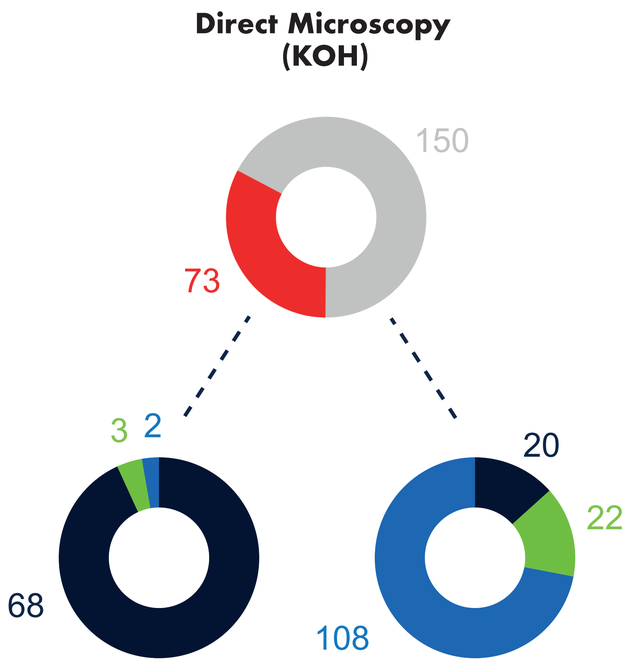


Fig. 1 | Human primary material was treated with KOH and used for microscopic examination to directly detect fungal components. Shown (dashed line) are the corresponding results of the positive and negative microscopic examination in the microarray.

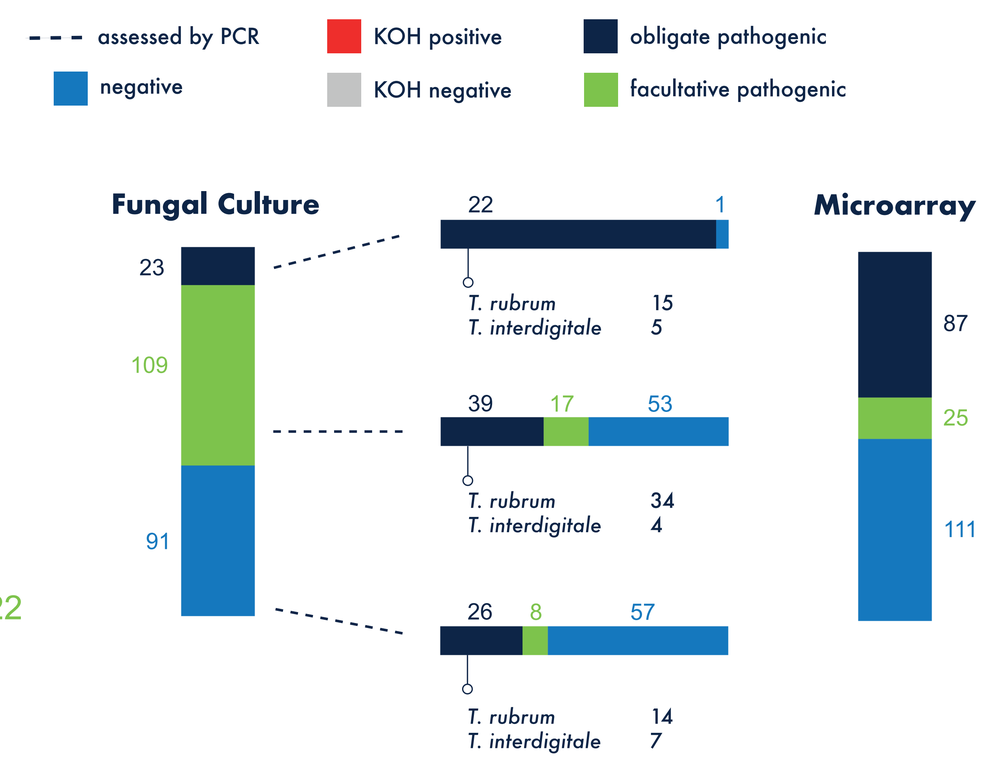


Fig. 2 | Patient primary material (nails, skin scales, swabs) was used in parallel for fungi cultivation as well as molecular biological examination by means of PCR-based microarray. The identified fungi were divided into obligate pathogens (dark blue) and facultative pathogens (green). The cultural determination was then compared with the microarray results (corresponding dashed line).

Using the **Microarray**, obligate pathogenic dermatophytes could be identified even in negative cultures and cultures with only facultative pathogens.
It represents a much more sensitive method for dermatophyte diagnostic.

In the **fungal culture**, obligate pathogenic dermatophytes could not always be detected, resulting in a sensitivity of 25% compared to the Microarray.

Slow-growing pathogens were overgrown by fast-growing molds, making correct analysis more difficult.

Direct **microscopy (KOH)** however is a sensitive method for the detection of fungal elements and on the other hand, is a sensitive method and complements the microarray as quality control.

Turnaround Time and Limitations

Turnaround Time

TAT mean is drastically reduced when speciment is tested with Microarray compared to fungal culture:

Fungal Culture:	25.5 d
Microarray:	3.4 d

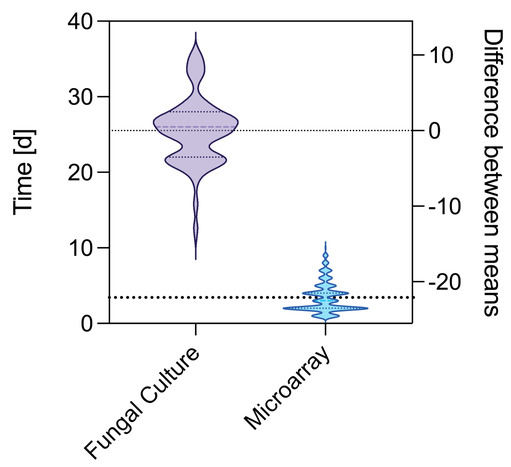


Fig. 3 | Turnaround Time of patient sample analyzed for funal colonization by culture compared to PCR-based microarray. Data (n = 235) displayed as mean ± SD.

Limitations

Insufficient genomic material of the pathogen due to sampling error

Extensive panel, but not every pathogen detected

Susceptibility testing still requires culture

Conclusion and Summary

- Performance and Sensitivity of Microarray shows superior properties in detection of dermatophytes.
- In 200 cultures, 65 pathogenic dermatophytes could still be detected thanks to microarray.
- Turnaround Time is reduced to max. 4 days compared to up to 26 days with analysis by culture.
- The Microarray is capable of detecting even culturally challenging pathogens.

Acknowledgement and Disclosure

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The authors have no conflicts of interest to declare that are relevant to the content of this article. The study was carried out with no financial contribution of the manufacturer.

Literatur
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