

PCR-based diagnosis of Dermatophytic infections: on the way to a consensus

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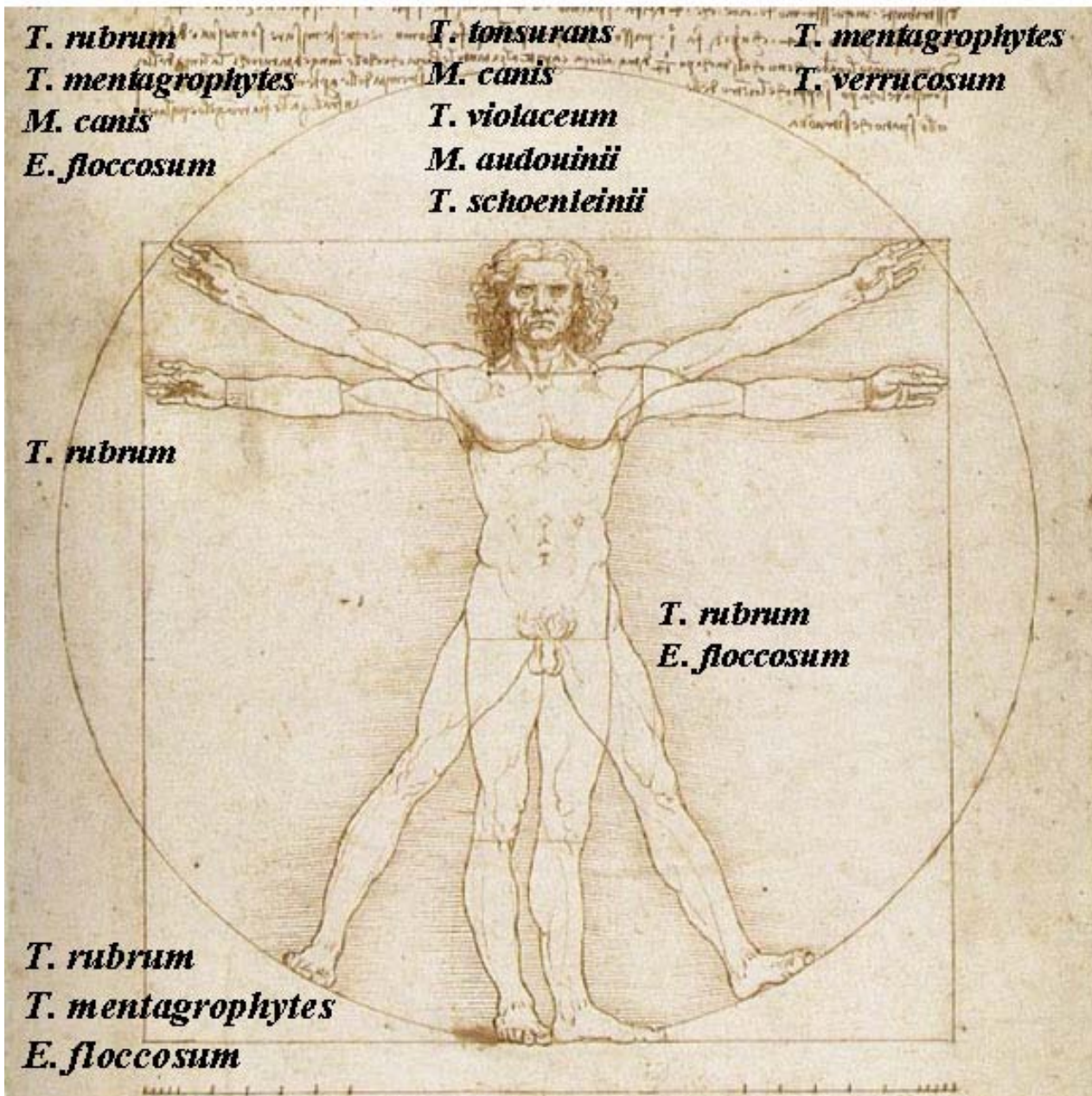
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Recently, new PCR-based assays have been developed for the detection and identification of dermatophytes, directly from clinical specimens. These assays can distinguish one or a few major dermatophyte species, sometimes in combination with a general, genus or dermatophyte, detecting assay. This emergence of PCR-based methods is interesting, as until recently, direct microscopy and culture were the only methods in common use in the diagnosis of dermatophytic infections. The PCR-based assays are of course very sensitive, rapid and easy to perform without requiring much expertise when standardized, but they can't always convey the information that conventional mycology conveyed (for example, they don't always distinguish zoophilic from anthropophilic strains). At the moment, it is not clear if these methods can replace the conventional mycology. Even their usefulness in complementing the standard diagnostic methodology (and how this should be practically achieved) is unclear.

I. General Objectives

- A. To work towards forming a consensus on standard PCR-based diagnostic methodology, able to complement or even replace the current standard in dermatophyte diagnosis, direct microscopy and culture.
- B. To bring together clinicians, scientists, veterinarians, laboratorians and others united by their common interests on dermatophyte diagnosis and other diverse aspects of dermatophyte science.
- C. To motivate young scientists (i.e. molecular biologists, taxonomists, etc) working in mycology to join dermatophyte research and motivate clinicians to incorporate scientific advances in their work.
- D. To build a database with dermatophyte sequences, representing the global dermatophyte variability. This database could work complementary to other similar databases (e.g. the CBS dermatophyte database).
- E. As a secondary aim, to start registering the contemporary geographic distribution of the various dermatophyte species and strains, thus paving the way for a future systematic work on global epidemiology.
- F. To preserve dermatophyte strains (in international Culture Collections, eg. CBS etc.), make them available to the international scientific community, thus encouraging further research.
- G. To form a platform for the free exchange of ideas on the topic.

II. Specific Objectives

- A. To determine the dermatophyte species needed to be included in a PCR-based diagnostic assay, working simultaneously towards an agreement on an operational dermatophyte species concept. A few species are causing the majority of dermatophyte infections but they tend to differ among countries and areas. For example, the major species isolated from scalp dermatophytosis (a children's disease) in USA is *Trichophyton tonsurans*, but in Southern Europe *Microsporum canis* and in sub-Saharan Africa a mixture of species such as African *Trichophyton rubrum* (which is a mixture of *T. soudanense*, *T. gourvilli*, *T. raubitschekii*, *Trichophyton violaceum* and *Microsporum audouinii*)
- B. To determine the ideal genomic target for such a PCR. Such a potential target should combine good intraspecies stability with good interspecies discriminatory power. So far the internal transcribed spacer (ITS) has been the main focus of interest but similar areas like the intergenic spacer (IGS) or structural genes like chitin synthase 1 have been also investigated. In a multiplex PCR, the possibility of multiple targets may have to be investigated. The current release of dermatophyte whole genomes greatly facilitates target selection.
- C. To determine the required sensitivity and specificity of the chosen assays in a clinical setting. PCR-based assays are generally very sensitive with a specificity that is primarily determined during the design phase.
- D. To work towards a synthesis of the currently available methods. The already developed assays would have to be assessed so as to decide which one performs best.
- E. To appraise the PCR-based assays in comparison with the standard methodologies (direct microscopy and culture). Laboratory assays have to be checked in the clinical setting and their true and false predictive values to be accurately determined. For this, "gold standard" methodology and criteria of positivity would have to be discussed and agreed.

F. To investigate the possibility for new promising assays and even methodologies (i.e. probe based). Species and genus specific DNA probes have been shown to be able to discriminate dermatophytes in diverse PCR-based diagnostic assays but the final format of such an assay has to be evaluated and decided.

G. To recommend a standard DNA extraction procedure. This is an important point that should be settled but has not been systemically studied.

Expected outcomes

A consensus standardized extraction and PCR protocol distributed to the working group participating laboratories will be assessed.

A database of target's sequences from dermatophytes originating in diverse countries and areas will be formed and made publicly available.

Collection of representative strains from different geographic areas. A consensus dermatophyte panel (positive controls) will be made available for research or diagnosis.

Encourage collaborative publication of results (whitepapers, working group sessions in ISHAM Congresses and other international Mycological and Medical events) during the working group's operating period.

Expected duration of the working group's operations

The first two years will be devoted to a. the buildup of the group, b. collecting data, c. create and activate a platform for the exchange of ideas, d. find sponsors and e. starting protocol trials. Conclusion of the laboratory activities is anticipated at the end of the third year and publication and general dissemination of generated results will be done in the fourth year of the WG operating period.



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