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Performance of dermatophyte PCR detection and identification at Statens Serum Institut, Denmark

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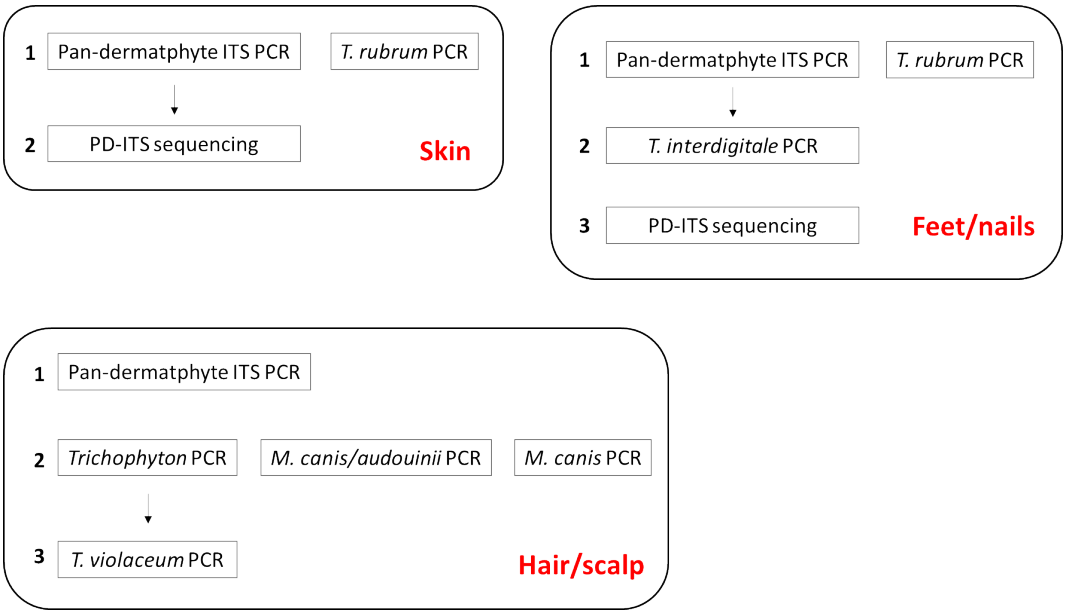
Background: Molecular techniques are increasingly used for diagnosing dermatophytosis improving speed and sensitivity when compared to classical culturing and microscopy. This study provides a 2.5 year performance overview of our improved conventional *in-house* dermatophyte PCR at Statens Serum Institut, Denmark.

Material/methods: DNA from patient samples (nails/feet, skin, scalp/hair) were extracted using a patented two-step method, developed in this laboratory (Brillowska-Dabrowska, J Clinical Microbiol, 2007, 45:1200-4). To accommodate variation in dermatophyte species distributions at different body sites, the DNA was subjected to PCR flows designed for each specimen-type (Figure). A novel ITS-targeting pan-dermatophyte PCR (PD-ITS) was designed for screening and in parallel, a *Trichophyton rubrum* specific PCR was applied for feet/nail and skin samples. Other species-specific PCR analyses or Sanger sequencing (Macrogen, Holland) using an internal forward primer to avoid interference with the internal control was ordered for PD-ITS positive samples.



Results: Among 4172 samples, 33.3% were positive, 65.8% negative and <1% inconclusive. The positivity rates ranged from ~25-40%, being highest in feet/nail samples. Genus identification sufficient for guiding treatment was obtained in ~98% of all dermatophyte positive samples ranging from ~90% in hair/scalp samples to >98% in feet/nail samples. Identification to species level was achieved for 96.3% overall of dermatophyte positive samples while the remaining 3.7% primarily consisted of *Trichophyton* species without identification to species level (23/52).

	Skin No. (%)	Hair/scalp No. (%)	Feet/nails No. (%)	Total No. (%)
Number of samples	1382 (100)	226 (100)	2564 (100)	4172
Negative	1032 (74.7)	162 (71.7)	1551 (60.5)	2745 (65.8)
Inconclusive	14 (1.0)	7 (3.1)	15 (0.6)	36 (0.9)
Dermatophyte positive	336 (24.3)	57 (25.2)	998 (38,9)	1391 (33.3)
Identification at least to genus level	329/336 (97.1)	51/57 (89.5)	982/998 (98.4)	1362/1391 (97.9)
Identification to species level	322/336 (95.8) *T. rubrum*: 248 (73.8) *T. interdigitale*: 45 (13.4) Other species: 29 (8.6)	42/57 (73.7) *M. canis*: 13 (22.8) *M. audouinii*: 14 (24.6) Other species: 15 (26.3)	975/998 (97.7) *T. rubrum*: 830 (83.2) *T. interdigitale*: 142 (14.2) Other species: 3 (0.4)	1339 (96.3)

Conclusions: Molecular diagnostics offer superiority in speed and sensitivity compared to conventional methods, yet, PCR methods are limited to only discovering targeted species. Pan-dermatophyte designs may overcome this fact and the current approach, coupling our pan-dermatophyte PCR with Sanger sequencing, offers a simple strategy to both detect and identify dermatophytes. Indeed, in combination with relevant species specific PCR assays a rate of >95% among +1300 dermatophyte positive samples were identified to species level signifying the value of the diagnostic strategy. Moreover, due to ongoing taxonomic revisions within dermatophytes, relying largely on DNA profiles and pathogenicity rather than conventional morphological features, molecular methods detecting DNA signatures may provide an even higher diagnostic advantage in the future.