

Figure 2. Analytical performance of IMMY anti-*Sporothrix* antibodies LFA to the clinical form and cross-reactivity analysis

<u>Clinical form</u>	<u>Sensitivity</u>	<u>95% CI</u>
Lymphocutaneous (n=59)	83.05%	71.03%-91.56%
Fixed cutaneous (n=27)	77.78%	57.74%-91.38%
Ocular (n=13)	92.31%	63.97%-99.81%
Mixed form (n=1)	100.00%	2.50%-100.00%

<u>Cross-reactivity analysis:</u>		
	<u>Number of samples</u>	<u>% positive</u>
Healthy volunteers	100	26,0% (26/100)
Disease		
Cryptococcosis	32	6,3% (2/32)
Candidemia	27	14,8% (4/27)
Paracoccidioidomycosis	14	14,3% (2/14)
Aspergillosis	10	0% (0/10)
Histoplasmosis	9	20% (2/10)
Fusariosis	4	0% (0/4)
Lobomycosis	1	0% (0/1)
Chromoblastomycosis	1	0% (0/1)
<i>Trichosporonosis</i>	1	100% (1/1)
Mucormycosis	1	0% (0/1)

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Research on molecular diagnostic methods and clinical application of common pathogenic dermatophytes in tinea capitis

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Objective: The main objective of this study was to design and develop a detection system based on qRT-PCR that can quickly and accurately identify the pathogenic fungi of tinea capitis, in order to improve the diagnostic ability of tinea capitis.

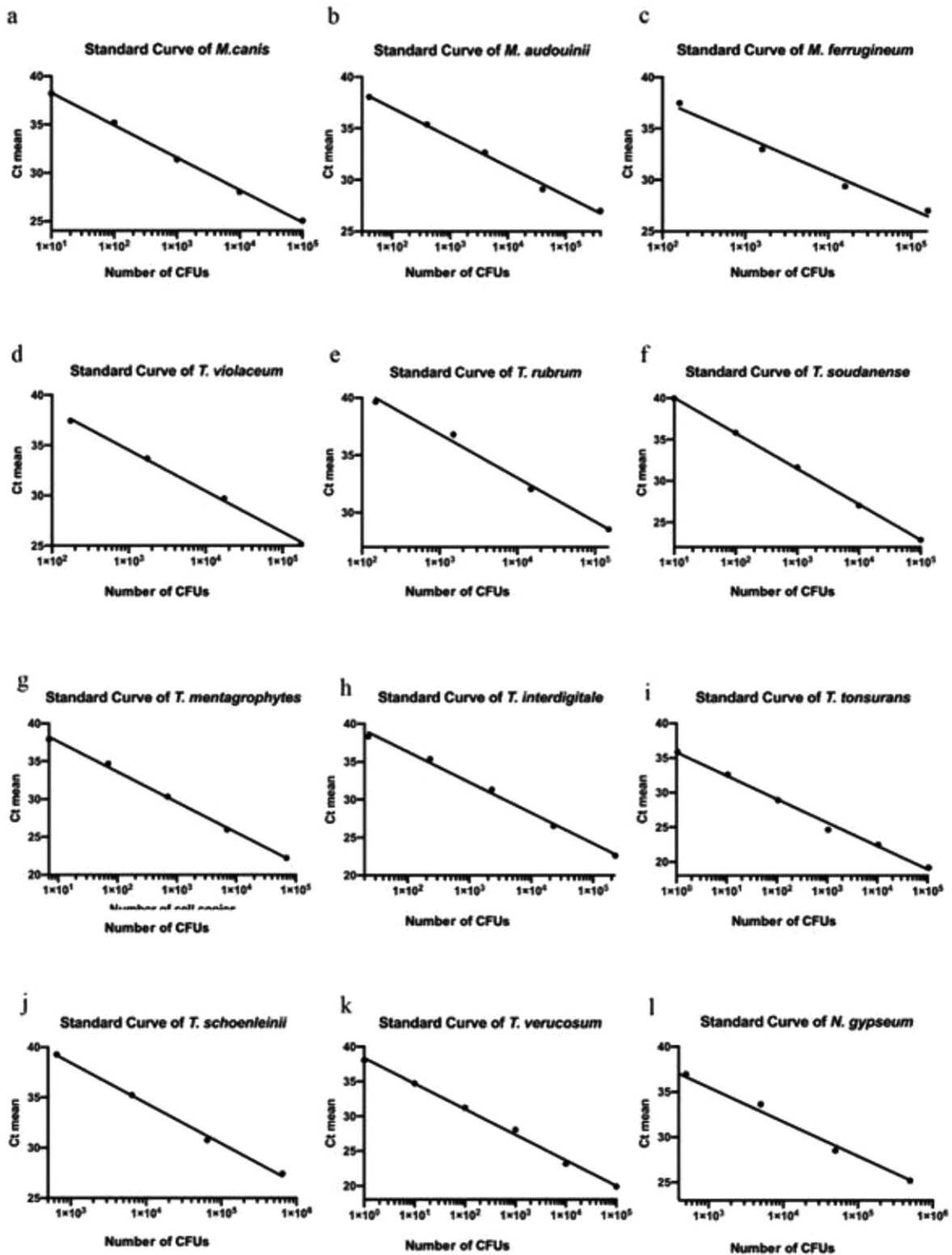
Methods:

1. A total of 8 isolates of *Microsporum* spp., 29 isolates of *Trichophyton* spp., 3 isolates of *Namitzia gypseae*, 6 isolates of non-dermatophytic filamentous fungi, *Malassezia* spp., and *Candida* spp. were included in this study.
2. Primer Express Software (V3.0) was used to design specific primers and TaqMan probe for qRT-PCR assay. The specificity of each system was validated using the above fungal isolates. The standard curve of each system was constructed by using the DNA of the standard substance of fungal isolates to find the minimum detection.
3. The clinical specimens from confirmed and suspected patients with tinea capitis were collected. Fungal DNA was extracted from clinical samples and detected by a two-step qRT-PCR system. The results of qRT-PCR were analyzed comprehensively and compared with a fungal microscope and fungal culture.
4. As a supplementary interpretation, the next generation sequencing targeted amplicon was conducted in 14 clinical samples that generated objectional results between fungal culture and qRT-PCR.

Results:

1. The molecular diagnostic system for tinea capitis herein consists of seven single-tube qRT-PCR assays designed on the complex and species level, which include the group of *M. canis* complex, *T. rubrum* complex, *T. mentagrophytes* complex, *T. tonsurans*, *T. schoenleinii*, *T. verrucosum*, and of *N. gypseae*. The analytical specificity of each group meets the design expectation.
2. The minimum detection limit of the *M. canis* complex group, *T. rubrum* complex group, *T. schoenleinii* group, and *N. gypseae* group was 100 Colony Forming Units (CFUs), the counterpart of which for *T. mentagrophytes* complex group, *T. tonsurans* and *T. verrucosum* were 10 CFUs.
3. A total of 351 clinical specimens were collected, including 231 cases confirmed of tinea capitis, 100 suspected of tinea capitis, and 20 with non-tinea capitis. Positive fungal microscopy and/or fungal culture were the gold criteria for diagnosis. Compared with the diagnostic gold standard, the sensitivity and efficacy of qRT-PCR, the combination of qRT-PCR and fungal microscopy, the combination of qRT-PCR and fungal culture were 93.1% and 93.6%, 100% and 100%, and 96.1% and 96.4%, respectively. The diagnostic specificity for cases of non-tinea capitis was 100%. The coincidence rate between qRT-PCR and fungal culture was 95.16%. The positive rate of qRT-PCR in suspected cases was 48%.
4. Amplification sequencing results confirmed that dermatophytes existed in 13 of 14 samples. Consistent with qRT-PCR, there were two species of dermatophytes mixed infection in 4 samples.

Conclusions: The seven single-tube qRT-PCR assays validated in this study can rapidly detect a variety of pathogenic fungi causing tinea capitis, with a high level of sensitivity and specificity. The combination of qRT-PCR and traditional mycological identification methods can further improve the diagnostic efficacy of tinea capitis.



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Comparative evaluation of Wako β -glucan test and Fungitell assay for the diagnosis of invasive fungal infections

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Objectives: Serum 1,3 β -D-glucan (BDG) has shown wide utility as a broad fungal biomarker for invasive fungal disease (IFD). Fungitell assay (USA) is the only FDA-approved kit available for the detection of BDG. We planned to compare the performance of the Wako assay (Japan) with that of the Fungitell assay in patients with Invasive aspergillosis (IA) and invasive

candidiasis (IC). We defined optimal cutoff values in the Wako assay to reliably exclude IFD (mainly due to *Aspergillus* and *Candida*).

Methods: A retrospective performance assessment study on archived patients' serum samples, collected as part of routine clinical care at PGIMER, Chandigarh, India was performed. Only adult patients qualifying as proven/probable IFD as defined by EORTC/MSG criteria were included. Serum samples from patients with risk factors for IFD and not meeting the criteria for proven/probable disease (who had no evidence of IFD) were included as controls. A positivity threshold of 80 pg/ml was used for Fungitell assay. For the Wako, the optimum positivity threshold or cut-off was determined on subsequent analysis. We also noted the time to positivity for BDG test in the Wako assay.

Result: A total of 157 individuals, including 97 patients with IFD (33 IA and 64 IC) and 60 non-IFD controls were included in the study. Mean age of the participants was 40.8 ± 16.4 years and 63% were males. The mean BDG levels of Wako assay in various patient groups are depicted in Figure 1. A significantly higher BDG value was noted in patients with IFD vs. controls