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Posted Date: 3 September 2025

doi: 10.20944/preprints202509.0379.v1

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Article

Detection of Dermatophytes Using PCR Followed by Array Technology; A Validation Study

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Abstract

Infections of the skin, hair, and nails are frequently caused by dermatophytes, fungi that metabolize keratin, and present as tinea corporis, tinea capitis, tinea pedis, tinea unguium, and related conditions. In immunocompetent patients these infections are typically superficial, limited to the epidermis, but may still trigger immune reactions of variable severity. Diagnosis currently relies on culture, which is time-consuming, requiring up to four weeks and skilled microscopy. The Euroarray Dermatomycosis, a PCR-based biochip assay targeting 56 fungal species, offers a markedly shorter turnaround time of 48 hours. We conducted an analytical validation study assessing sensitivity, specificity, accuracy, reproducibility, limit of detection, and linearity. Hair, nail, and skin specimens from healthy donors were spiked with genomic DNA from dermatophytes and yeasts including *Trichophyton*, *Microsporum*, *Epidermophyton*, *Fusarium*, *Candida*, *Scopulariopsis*, and *Nannizzia*; plasmids were used for linearity and detection limit testing. The assay demonstrated high sensitivity (91.7–100%) and specificity (100%) across species, reproducibility ranging from 88.9–100%, and limits of detection as low as 10–100 copies/ml depending on specimen type and organism, with excellent linearity (R^2 0.9677–0.9987). In conclusion, Euroarray Dermatomycosis shows strong analytical performance and has the potential to significantly improve accuracy, reliability, and speed of fungal diagnosis. Furthermore, adopting molecular techniques for dermatophyte detection may facilitate future exploration of resistance mechanisms in fungi, providing earlier insights into emerging antifungal resistance patterns and supporting the development of targeted therapies.

Keywords: dermatophytes; array technology; diagnosis; dermatophytosis; tinea corporis; tinea capitis; tinea cruris; tinea pedis

1. Introduction

Dermatophytes are a group of fungi that hydrolyze keratin, and therefore can infect keratinized structures such as skin, hair, and nails, causing dermatophytoses. In immunocompetent hosts, these infections are generally restricted to the epidermis, yet they may still elicit immune responses of varying severity [1]. Major forms include tinea corporis (body, excluding feet, groin, face, scalp, or beard hair), tinea pedis (feet), tinea cruris (groin and upper thighs), tinea faciei (face), and tinea manuum (hands) [2]. Tinea capitis and tinea barbae affect scalp and beard hair, respectively, while tinea unguium (onychomycosis) is the most common nail infection [2]. Although superficial, dermatophytoses require careful clinical and laboratory evaluation. Diagnosis depends on clinical presentation, which varies by infection type. For instance, tinea pedis often presents as pruritic erosions between toes or a “moccasin” distribution on feet, while tinea cruris typically appears as erythematous lesions on the inner thigh. Because patients may harbor multiple infections simultaneously, thorough examination of skin, hair, and nails is essential [2].

Dermatophytes are classified into three main genera: Epidermophyton, Microsporum, and Trichophyton [3]. Their prevalence varies worldwide [4], with higher burdens in warm climates. For example, >70% of children in northern Brazil with dermatophytosis present with tinea capitis [5], while tinea pedis affects over 50% of adults in parts of Europe. Tinea cruris is more common in adults, whereas other types often predominate in children [6]. Globally, 10–15% of the population is expected to experience a dermatophyte infection. Females are more commonly affected, with peak incidence between ages 51–60 [4]. Onychomycosis shows a prevalence of 14% in the United States [7] and 8% in Canada [8].

Traditional diagnosis relies on culture and phenotypic identification. Methods include assessing colony morphology, pigmentation, texture, growth rate, microscopic features, temperature tolerance, and biochemical reactions [9,10]. Culture requires 3–4 weeks of incubation and is prone to contamination and external variability [9–11]. This process is time-consuming and demands specialized expertise, potentially delaying reliable identification and treatment [12]. Diagnosis is further complicated in immunocompromised patients, whose presentations may deviate from typical patterns [15]. Recent molecular advances have introduced new approaches, with PCR demonstrating high sensitivity and reproducibility compared to morphology-based methods [11,13].

PCR can generate characteristic DNA polymorphisms using repetitive sequences and gel banding patterns [10,14]. The GACA sequence has proven useful for dermatophyte identification [14], while the chitin synthase 1 [CHS1] gene of *Microsporum canis* has also shown strong diagnostic potential [13,16]. Although PCR improves turnaround time, sensitivity, and specificity, designing primers for each species remains challenging. Multiplex PCR, which amplifies multiple DNA targets simultaneously, enables detection of several organisms from one sample [11]. When combined with microarray technology, this allows identification of numerous dermatophytes within hours. After PCR amplification, microarray hybridization provides a comprehensive profile of detected species [12]. This study will evaluate the clinical application of combined PCR and microarray techniques for the rapid identification of dermatophytoses.

This work highlights how molecular platforms like PCR and microarray, beyond enabling rapid dermatophyte identification, can also be adapted for the detection of antifungal resistance genes. Incorporating resistance gene markers into diagnostic panels would allow earlier recognition of mutations that compromise therapy, ensuring more accurate treatment decisions. Such integration positions dermatophyte testing as a model for broader fungal diagnostics, where simultaneous detection of pathogens and resistance determinants is essential for addressing emerging antifungal resistance.

2. Results

A PCR and microarray-based diagnostic test was evaluated for dermatophyte detection in nail, skin, and hair samples. Each specimen type was tested with dermatophytes typically pathogenic to that source. For nail samples (Table 1), eight dermatophyte species were assessed, and all showed 100% correct detection except for *Fusarium oxysporum*, which demonstrated only 33.3% accuracy. The overall sensitivity across all species was 96.0%, and 100% when the outlier species was excluded. Skin samples were tested for ten different species; six were detected with 100% accuracy, *Trichophyton mentagrophytes quinckii* at 96.0%, *T. mentagrophytes interdigitale* and *T. equinum* at 91.3%, and *T. concentricum* at only 30.8%. Overall sensitivity for skin was 94.3%, increasing to 97.9% when *T. concentricum* was excluded. Hair samples were tested for five species; three had 100% detection, *T. violaceum* had 96.0%, and *M. audouinii* had 85.7%, giving an overall sensitivity of 96.0%.

Certain species tested should be categorized as yeasts or molds rather than dermatophytes. To test specificity, each sample type was analyzed for classification as either dermatophyte or yeast/mold (Table 2). In nail samples (Table 2A), 15 species were identified as dermatophytes and five as yeasts/molds (*Candida* spp., *F. oxysporum*, and *S. brevicaulis*), with 100% correct detection in both categories. Hair samples (Table 2B) and skin samples (Table 2C) showed similar results, with all dermatophytes and all yeasts/molds detected with 100% specificity.

Reproducibility was assessed for each species (Table 3). Nail organisms showed 100% reproducibility except *F. oxysporum*. In skin, six species were 100% reproducible, *T. mentagrophytes quinkeanum* was 88.9%, *T. equinum* and *T. mentagrophytes interdigitale* were 77.8%, and *T. concentricum* showed 0%. In hair, three species were 100% reproducible, while *T. violaceum* and *M. audouinii* were 88.9% and 66.7%, respectively.

For limit of detection testing, the lowest gDNA concentration detected was recorded. In nail samples (Table 4), all species had 100% detection at all concentrations except *C. albicans* and *C. parapsilosis*. *C. albicans* showed 33.3% detection at 1.00E+02 copies/μl and no detection at 1.00E+01, while *C. parapsilosis* showed 33.3% detection at 1.00E+01. In skin samples (Table 5), *N. persicolor* and *T. interdigitale* had 100% detection at all concentrations, whereas others showed partial or no detection at lower concentrations, with *T. concentricum* showing 0% across all. In hair (Table 6), all species except *M. canis* had 100% detection. *M. canis* was detected at 75.0% in the highest concentration, 50% in the next, and 0% at lower levels. *T. violaceum* and *M. audouinii* were detectable across all concentrations but demonstrated 0 average intensity.

Linearity was assessed by comparing average signal intensities across concentration levels (Table 7). Graphs and R² values were generated for each species, with R² serving as the measure of linearity. For example, *T. interdigitale* in nail samples (Figure 1) demonstrated 97.9% linearity.

2.2. Figures, Tables and Schemes

All figures and tables should be cited in the main text as Figure 1, Table 1, etc.

Table 1. Accuracy of detection of tested dermatophytes in nail, skin, and hair samples.

Spike	Accuracy Dermatophyte detection in Nail samples		Accuracy Dermatophyte detection in Skin samples		Accuracy Dermatophyte detection in Hair samples	
	Count of Correct		Count of Correct		Count of Correct	
	Percent of Correct		Percent of Correct		Percent of Correct	
<i>T. interdigitale</i>	27	100.0%	27	100%	-	-
<i>T. rubrum</i>	27	100.0%	-	-	-	-
<i>C.albicans</i>	27	100.0%	-	-	-	-
<i>C.parapsilosis</i>	27	100.0%	-	-	-	-
<i>C.guillermundii</i>	27	100.0%	-	-	-	-
<i>E.floccosum</i>	27	100.0%	27	100%	-	-
<i>S.brevicaulis</i>	27	100.0%	-	-	-	-
<i>T.menta inter</i>	-	-	21	91.3%	-	-
<i>T.equinum</i>	-	-	21	91.3%	-	-
<i>T.violaceum</i>	-	-	27	100.0%	24	96.0%
<i>T. menta quinkeanum</i>	-	-	24	96.0%	-	-
<i>M.canis</i>	-	-	27	100.0%	18	100.0%
<i>N.persicolor</i>	-	-	27	100.0%	9	100.0%
<i>T.schoenleinii</i>	-	-	27	100.0%	-	-
<i>T.tonsurans</i>	-	-	-	-	27	100.0%
<i>M.audouinii</i>	-	-	-	-	18	85.7%
Sensitivity	189	100%	228	97.9%	96	96.3%

Table 2. Detection of organisms as either dermatophytes or yeast/mold in nail (A), hair (B.), and skin (C) samples. Y/M indicates yeast and mold, other than those that are also considered dermatophytes.

Sample Name	A. Hair
Expected	Detected with Dermatophytes
	Not detetced with Y/M

Spike	Accuracy UDA/YM detection in Nail, Hair, Skin samples			
	Detected Derm, Not detected YM		Not detected Derm, Detected YM	
	Count of detetced	% of Detected	Count of ND	% of Non detected
T. interdigitale	54	100.0%	0	0.0%
T. rubrum	54	100.0%	0	0.0%
C.albicans	0	0.0%	27	100.0%
C.parapsilosis	0	0.0%	27	100.0%
C.guillermundii	0	0.0%	27	100.0%
E.floccosum	27	100.0%	0	0.0%
F.oxisporum	0	0.0%	27	100.0%
S.brevicaulis	0	0.0%	27	100.0%
T.menta inter	27	100.0%	0	0.0%
T.concentricum	27	100.0%	0	0.0%
T.equinum	27	100.0%	0	0.0%
T.violaceum	54	100.0%	0	0.0%
E.floccosum	27	100.0%	0	0.0%
T. menta				
quinkeanum	27	100.0%	0	0.0%
M.canis	54	100.0%	0	0.0%
N.persicolor	54	100.0%	0	0.0%
T.schoenleinii	27	100.0%	0	0.0%
T.tonsurans	27	100.0%	0	0.0%
M.audouinii	27	100.0%	0	0.0%
N.persicolor	27	100.0%	0	0.0%
	540	100.0%	135	100.0%

Sample Name	Skin			
Expected	Detected with Dermatophytes			
	Not detected with Y/M			
	Accuracy UDA/YM detection in Nail, Hair, Skin samples			
	Detected Derm, Not detected YM		Not detected Derm, Detected YM	
Spike	Count of detected	% of Detected	Count of ND	% of Non detected
T. interdigitale	54	100.0%	0	0.0%
T. rubrum	27	100.0%	0	0.0%
C.albicans	0	0.0%	27	100.0%
C.parapsilosis	0	0.0%	27	100.0%
C.guillermundii	0	0.0%	27	100.0%
E.floccosum	27	100.0%	0	0.0%
F.oxisporum	0	0.0%	27	100.0%
S.brevicaulis	0	0.0%	27	100.0%
T.menta inter	9	100.0%	0	0.0%
T.concentricum	27	100.0%	0	0.0%
T.equinum	27	100.0%	0	0.0%
T.violaceum	54	100.0%	0	0.0%
E.floccosum	27	100.0%	0	0.0%
T. menta quinkeanum	27	100.0%	0	0.0%
M.canis	54	100.0%	0	0.0%
N.persicolor	54	100.0%	0	0.0%
T.schoenleinii	27	100.0%	0	0.0%
T.tonsurans	27	100.0%	0	0.0%
M.audouinii	27	100.0%	0	0.0%
N.persicolor	27	100.0%	0	0.0%
	495	100.0%	135	100.0%

Table 3. Reproducibility rates for the detection of different organisms in nail, skin, and hair samples. Total concordant refers to instances of no change and when the organisms were constantly detected. .

	Target Organism	Total Concordant	Total Discordant	Reproducibility
Nail	T. interdigitale	207	0	100.0%
	T. rubrum	207	0	100.0%
	C.albicans	207	0	100.0%
	C.parapsilosis	207	0	100.0%
	C.guillermundii	207	0	100.0%
	E.floccosum	207	0	100.0%
	F.oxisporum	23	184	11.1%
	S.brevicaulis	207	0	100.0%
Skin	T.interdigitale	207	0	100.0%
	T.menta inter	161	46	77.8%
	T.concentricum	0	207	0.0%
	T.equinum	161	46	77.8%
	T.violaceum	207	0	100.0%
	E.floccosum	207	0	100.0%
	T. menta quinkeanum	184	23	88.9%
	M.canis	207	0	100.0%
	N.persicolor	207	0	100.0%
	T.schoenleinii	207	0	100.0%
Hair	T.tonsurans	207	0	100.0%
	T.violaceum	184	23	88.9%

	M.audouinii	138	69	66.7%
	M.canis	207	0	100.0%
	N.persicolor	207	0	100.0%

Table 4. Detection levels at different concentrations of organisms in nail samples. Units are copy per micrometer (cp/mcl). When there is a detection, the light given off is quantified as average intensity. Higher means good detection, and is directly related to higher amount of copies .

Spike	LOD Dermatophyte detection in Nail samples									
	1,000	750	100	10	1	Average Intensity	1,000	750	100	10
	1.00E+03	7.50E+02	1.00E+02	1.00E+01	1.00E+00		1.00E+03	7.50E+02	1.00E+02	1.00E+01
	3	2	2	1	0		3	2	2	1
	Count of Detected						Percent of Detected			
T.interdigitale	3	3	3	3	3	9,482	100.0%	100.0%	100.0%	100.0%
T.rubrum	3	3	3	3	3	57,330	100.0%	100.0%	100.0%	100.0%
C.albicans	3	3	1	0	0	3,690	100.0%	100.0%	33.3%	0.0%
C.parapsilosis	3	3	3	1	1	48,884	100.0%	100.0%	100.0%	33.3%
C.guillermondii	3	3	3	3	3	14,915	100.0%	100.0%	100.0%	100.0%
E.floccosum	3	3	3	3	3	29,880	100.0%	100.0%	100.0%	100.0%
F.oxisporum	3	3	3	3	3	6,471	100.0%	100.0%	100.0%	100.0%
S.brevicaulis	3	3	3	3	3	28,481	100.0%	100.0%	100.0%	100.0%
Universal Dermatophyte	3	3	3	3	3		100.0%	100.0%	100.0%	100.0%

Table 5. Detection levels at different concentrations of organisms in skin samples. Units are copy per micrometer. .

Spike	LOD Dermatophyte detection in Skin samples									
	1,000	750	100	10	1		1,000	750	100	10
	1.00E+0	7.50E+0	1.00E+0	1.00E+0	1.00E+0	Averag	1.00E+0	7.50E+0	1.00E+0	1.00E+0
	3	2	2	1	0	e	3	2	2	1
	Count of Detected					Intensit	Percent of Detected			
T.interdigitale	4	4	4	4	2	5,010	100.0%	100.0%	100.0%	100.0%
T.menta										
inter	1	1	1	1	1	0	25.0%	25.0%	25.0%	25.0%
T.concentricu										
m	0	0	0	0	0	0	0.0%	0.0%	0.0%	0.0%
T.equinum	2	1	0	0	0	1,378	50.0%	25.0%	0.0%	0.0%
T.violaceum	4	4	3	0	0	1,794	100.0%	100.0%	75.0%	0.0%
E.floccosum	4	4	4	3	3	7,544	100.0%	100.0%	100.0%	75.0%
T. menta										
quinkeanum	4	4	4	1	0	10,373	100.0%	100.0%	100.0%	25.0%
M.canis	3	2	2	2	0	2,714	75.0%	50.0%	50.0%	50.0%
N.persicolor	4	4	4	4	4	33,662	100.0%	100.0%	100.0%	100.0%
T.schoenleinii										
	4	4	4	3	0	18,366	100.0%	100.0%	100.0%	75.0%

Universal Dermatophyt e	4	4	4	4	4		100.0%	100.0%	100.0%	100.0%
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Table 6. Detection levels at different concentrations of organisms in skin samples. Units are copy per micrometer. .

Spike	LOD Dermatophyte detection in Hair samples										
	1,000750100101						1,00075010010				
	1.00E+03	7.50E+02	1.00E+02	1.00E+01	1.00E+00	Average	1.00E+03	7.50E+02	1.00E+02	1.00E+01	
	3	2	2	1	0		3	2	2	1	
	Count of Detected					Intensity	Percent of Detected				
T. tonsurans	4	4	4	4	0		5,010	100.0%	100.0%	100.0%	100.0%
T. violaceum	4	4	4	4	2	0	100.0%	100.0%	100.0%	100.0%	
M. audouinii	4	4	4	4	0	0	100.0%	100.0%	100.0%	100.0%	
M. canis	3	2	0	0	0	1,378	75.0%	50.0%	0.0%	0.0%	
N. persicolor	4	4	4	4	4	1,794	100.0%	100.0%	100.0%	100.0%	
Universal Dermatophyte	4	4	4	4	4		100.0%	100.0%	100.0%	100.0%	

Table 7. Linearity measurements for organism detected at different concentrations in nail, skin, and hair samples .

		Linearity						
Log10 Concentration		1	10	100	750	1,000	10,000	
		0.00	1.00	2.00	3.00	4.00	5.00	Sum
Nail	T. interdigitale (nail)	-	3988	7383.75	9200	12804	14032	47,407.8
	T. rubrum (nail)	-	42030	54028	59848	64319	66423	286,648.0
	C.albicans (nail)	-	2096	3184	3876	4628	4668	18,452.0
	C.parapsilosis (nail)	-	19520	38668	55579.75	64255	66399	244,421.8
	C.guillermondii (nail)	-	3308	7600	17052	22216	24400	74,576.0
	E.floccosum (nail)	-	9272	18776	29388	35512	56450	149,398.0
	F.oxisporum (nail)	-	2136	2508	6084	9716	11912	32,356.0
	S.brevicaulis (nail)	-	6048	16340	26732	44676	48608	142,404.0
Mean of Intensity	T.interdigitale (skin)	-	4512	5160	4648	4808	5920	25,048.0
	T.menta inter (skin)	-	0	0	0	0	0	0.0
	T.concentricum (skin)	-	0	0	0	0	0	0.0
	T.equinum (skin)	-	104	248	1360	1384	3792	6,888.0
	T.violaceum (skin)	-	16	1216	1464	1888	4384	8,968.0
	E.floccosum (skin)	-	3944	3536	6672	7512	16056	37,720.0
	T. menta quinkeanum (skin)	-	1080	1776	8848	15848	24312	51,864.0
	M.canis (skin)	-	8	0	1976	3120	8464	13,568.0
	N.persicolor (skin)	-	8344	11768	35520	52248	60431.5	168,311.5
	T.schoenleinii (skin)	-	5104	8304	21824	24312	32288	91,832.0
Hair	T.tonsurans (hair)	-	56	3280	6160	8104	9312	26,912.0
	T.violaceum (hair)	-	2544	3184	4000	6576	8456	24,760.0
	M.audouinii (hair)	-	72	784	672	816	1352	3,696.0
	M.canis (hair)	-	200	960	2240	2944	2848	9,192.0

	N.persicolor (hair)	-	17128	16448	26168	31760	30720	122,224.0
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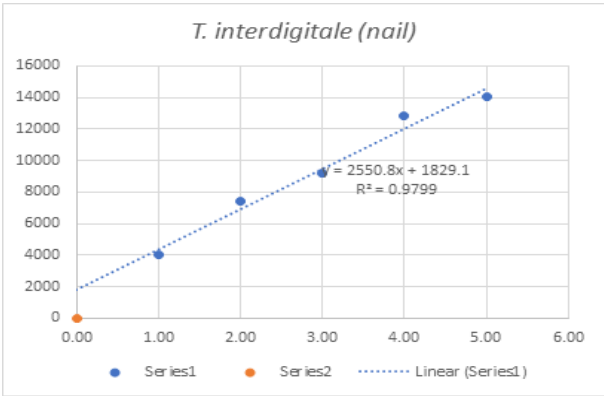
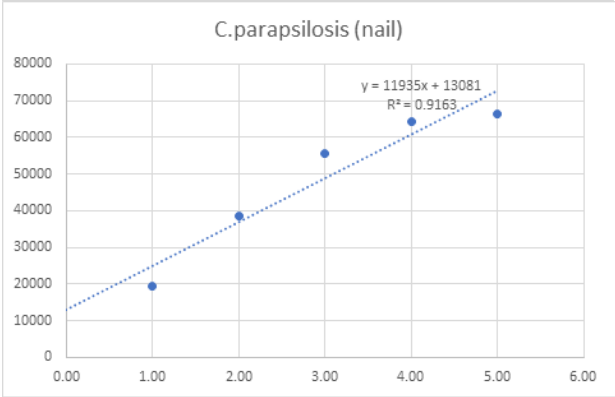
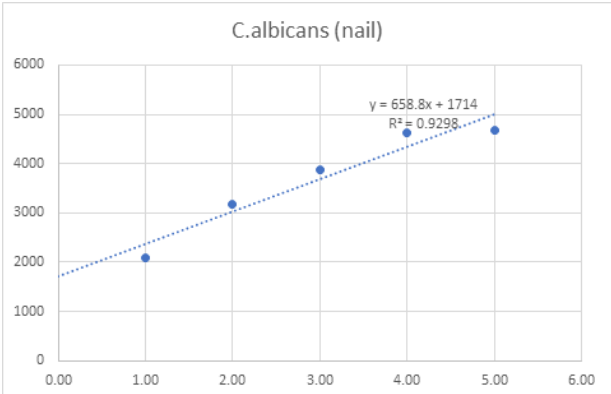
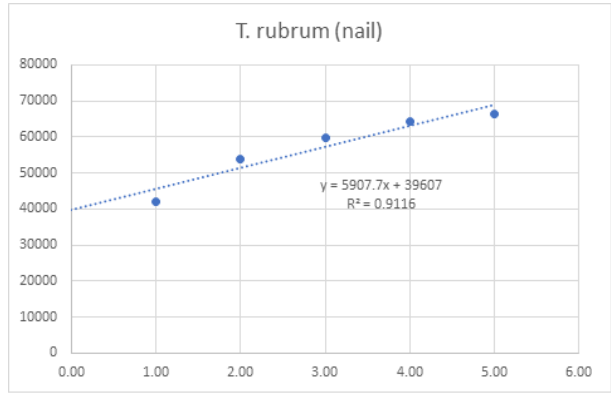


Figure 1. Graphs of linearity for organisms detected in nail samples .



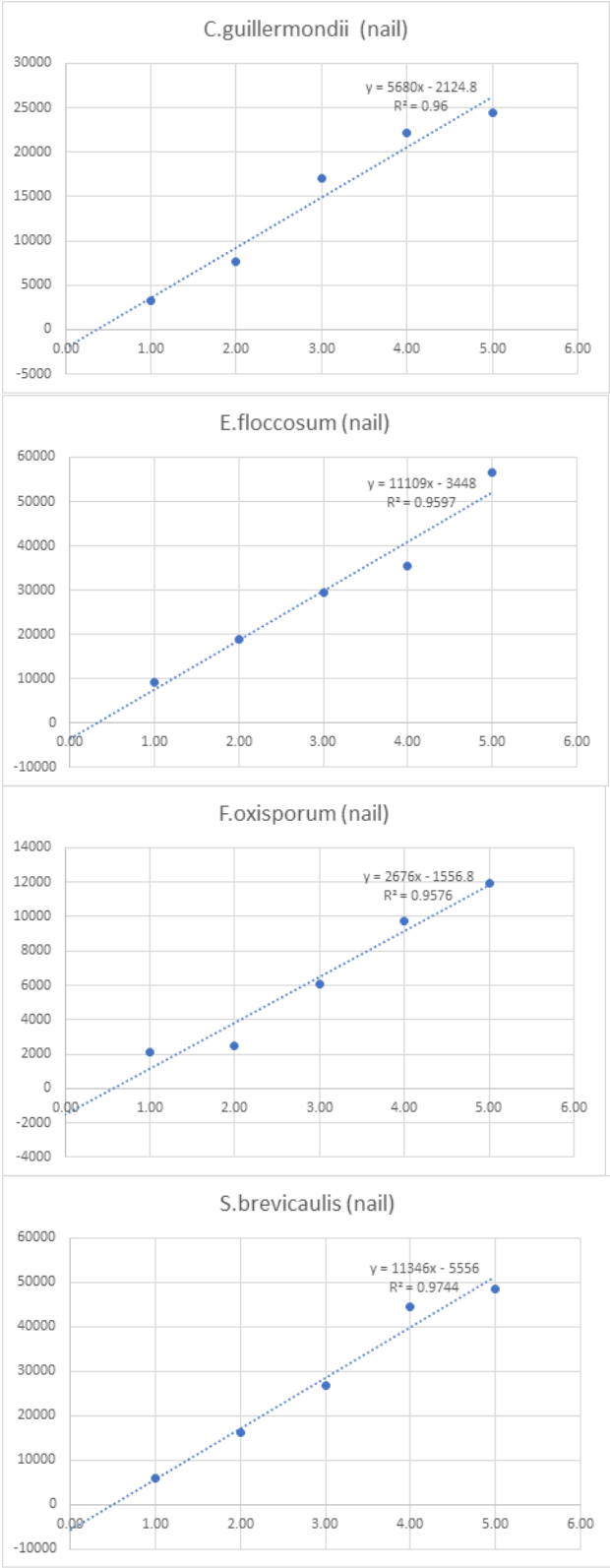
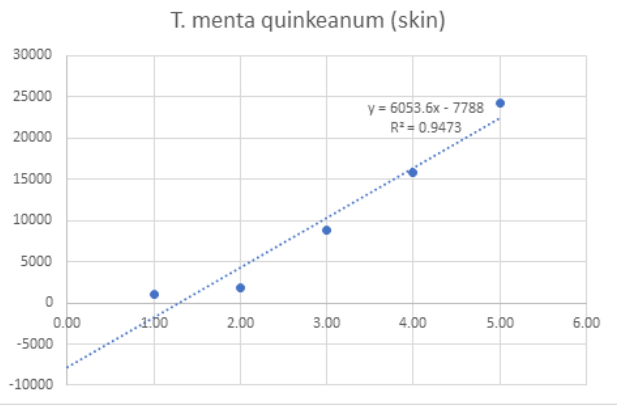
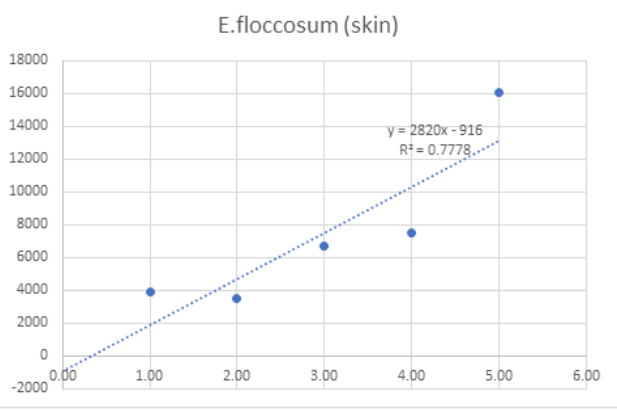
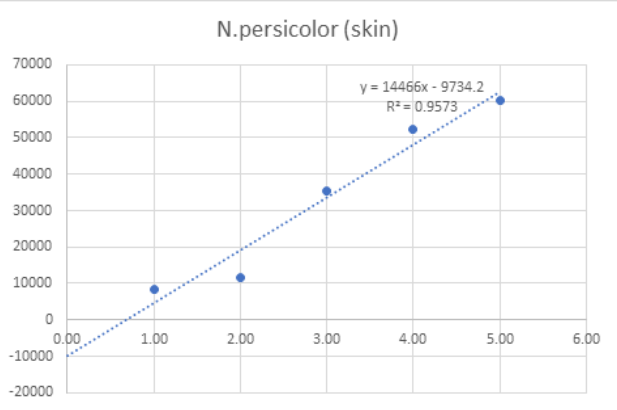
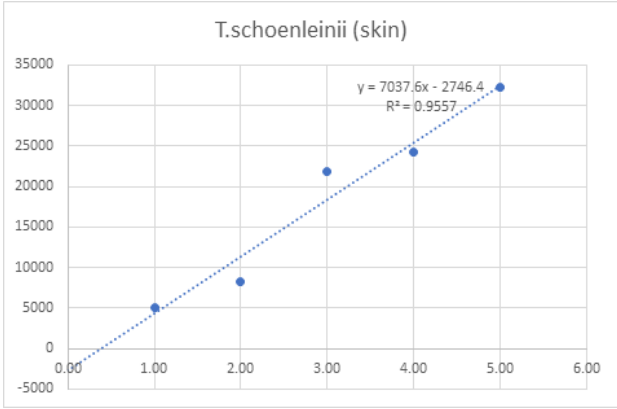


Figure 2. Graphs of linearity for organisms detected in skin samples .



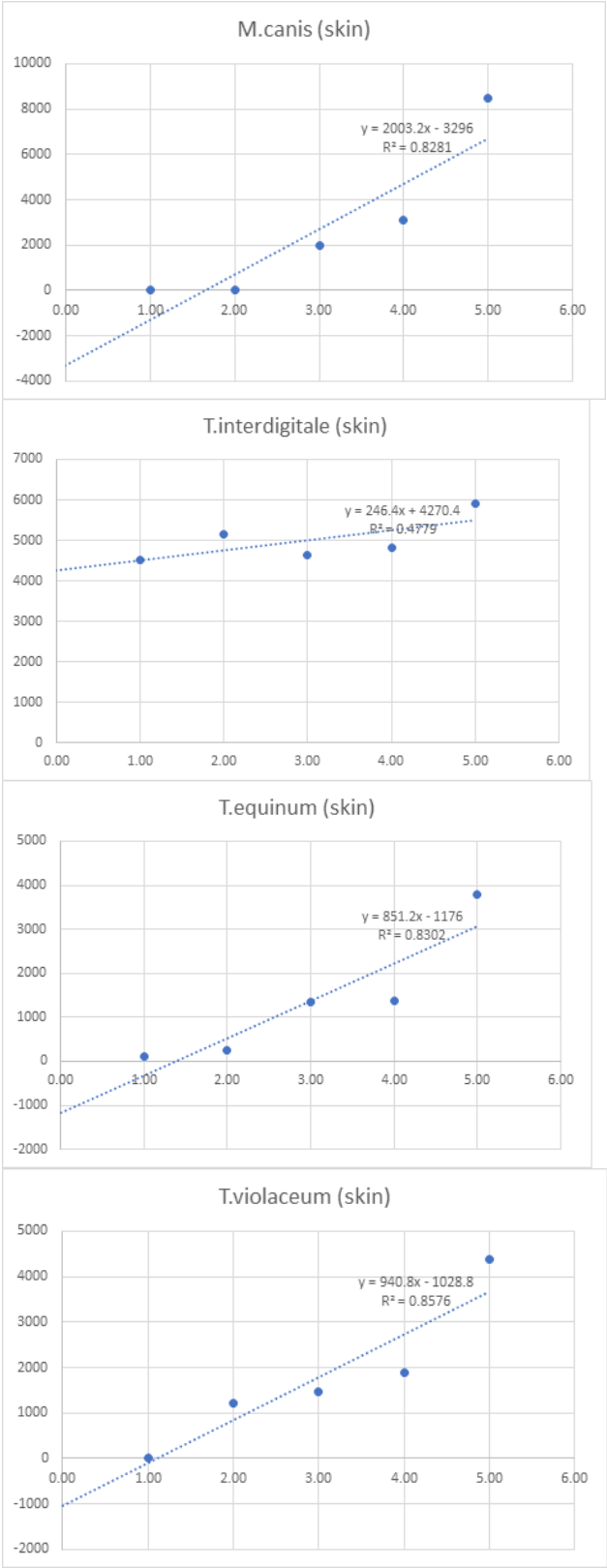
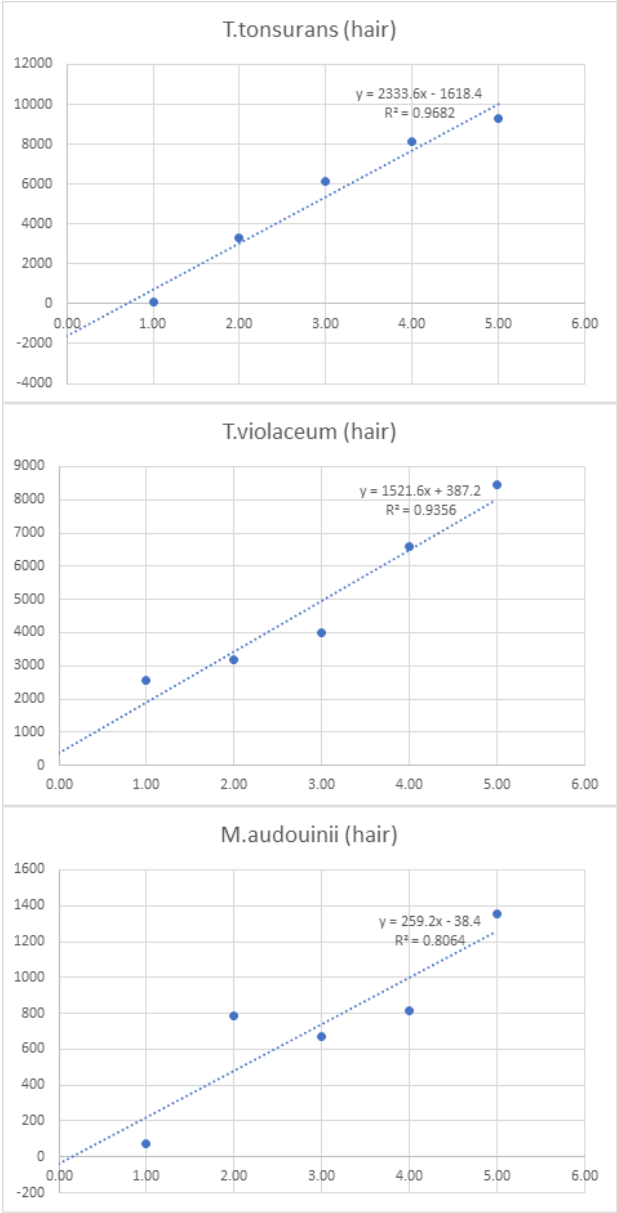
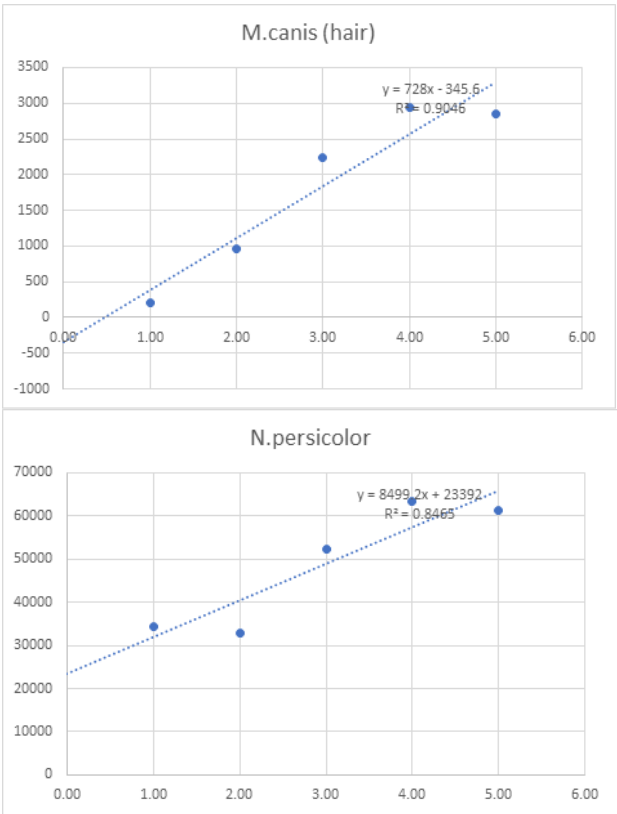


Figure 3. Graphs of linearity for organisms detected in hair samples .





3. Discussion

Conventional dermatophyte detection methods include direct microscopic examination and culture. Direct microscopy with potassium hydroxide (KOH) is rapid but has limited sensitivity (17). The current gold standard remains culture on species-specific media, which can take up to 4 weeks (18). Periodic acid-Schiff (PAS) staining provides high sensitivity for histological detection but cannot precisely identify the pathogen [19]

The validation results presented here confirm the high sensitivity and specificity of the Euroarray Dermatomycosis PCR-based assay. Compared to culture, it provides accurate species-level identification of dermatophytes and other fungi within 48 hours. Faster turnaround facilitates timelier initiation of targeted therapy, which improves outcomes and helps prevent further spread of infection. The assay consistently achieved sensitivities above 94% across sample types, and 100% when outliers (*Fusarium oxysporum* in nails, *T. concentricum* in skin) were excluded. These outliers likely reflect limitations in probe hybridization or strain-specific genomic variability.

Specificity was strong, with reliable discrimination between dermatophytes and non-dermatophyte yeasts or molds, a critical advantage in mixed infections or atypical presentations. This ability reduces the risk of inappropriate antifungal use, contributing to antimicrobial stewardship. Reproducibility was high overall, though *F. oxysporum* in nails and *T. concentricum* in skin highlighted areas requiring probe refinement. Limit of detection testing confirmed assay reliability for low-burden infections, particularly in nail and hair samples, though probe optimization is needed for some skin species. Linearity was excellent for nails and hair ($R^2 > 0.90$), with greater variability in skin specimens, suggesting further optimization.

The clinical importance of this work extends to antifungal resistance. Rising terbinafine resistance in *T. rubrum*, *T. mentagrophytes*, and *T. indotineae* is driven by mutations in the squalene epoxidase (SQLE) gene [20], while efflux pump overexpression and ergosterol alterations confer resistance to azoles [21]. Although the Euroarray does not yet target resistance markers, its molecular design and demonstrated performance provide a foundation for integrating resistance genotyping. Expanding probe sets to include known resistance mutations would enable early recognition of resistant strains, real-time monitoring of therapeutic effectiveness, and more precise tailoring of antifungal regimens.

In summary, this study demonstrates that the Euroarray Dermatophylosis assay is a highly sensitive, specific, and time-efficient diagnostic platform. With further integration of resistance detection, this technology has the potential to become a comprehensive clinical tool for both accurate dermatophyte identification and surveillance of antifungal resistance mechanisms.

4. Materials and Methods

4.1. Sample Collection and DNA Extraction

Clinical materials consisted of hair stubs, nail clippings, and skin scales collected under sterile conditions to minimize contamination. Non-viable cells such as crusts were also acceptable for testing. Samples were disinfected prior to collection. DNA extraction was performed using the EUROArray SwiftX-traction kit following the manufacturer's instructions. In some runs, automated extraction was performed on the EUROArray Workstation to ensure consistency and reproducibility.

4.2. PCR Amplification

Extracted DNA was subjected to multiplex PCR with pre-formulated, ready-to-use reagents supplied by EUROIMMUN. This step amplified pathogen-specific gene segments while simultaneously incorporating fluorescent labels. Internal amplification controls were included in each reaction to verify the success of PCR and to rule out inhibition.

4.3. Microarray Hybridization and Detection

Amplified products were hybridized to the EUROArray Dermatophylosis BIOCHIP, a glass microarray slide with immobilized oligonucleotide probes specific to dermatophytes and common non-dermatophyte fungi. Hybridization was performed at 55 °C under controlled conditions. Slides were then washed to remove unbound DNA and scanned using the EUROArrayScanner.

4.4. Data Analysis

Fluorescent signals from hybridized probes were automatically analyzed using EUROArrayScan software. Results were interpreted against internal positive and negative controls, with each probe corresponding to a specific fungal species. Reports were automatically generated and archived for traceability.

4.5. gDNA

gDNA for all the tested fungi were provided by Euroimmune- Germany

5. Conclusions

In conclusion, the Euroarray Dermatophylosis PCR-based assay showed high sensitivity, specificity, and reproducibility for the detection of dermatophytes and related fungi in nail, skin, and hair samples, while reducing turnaround time significantly compared to conventional culture. A few species such as *Fusarium oxysporum* and *T. concentricum* demonstrated lower performance, but overall the validation confirmed the strength and reliability of this molecular approach. By allowing rapid and accurate species identification, this technique provides clear advantages in supporting timely clinical management, reducing diagnostic uncertainty, and preventing inappropriate antifungal use. Furthermore, the molecular design of the assay creates an opportunity to integrate resistance genotyping in the future, enabling early recognition of antifungal resistance mechanisms and supporting more precise treatment strategies against emerging resistant strains.

Author Contributions: Conceptualization, Z.D.; methodology, Z.D. and S.C.; validation, Z.D. and S.C.; resources, A.D. and S.C.; data curation, Z.D. and S.C.; writing—original draft preparation, S.C. and A.D.; writing—review and editing, Z.D., S.C., and A.D.; visualization, S.C. and A.D.; supervision, Z.D.; project administration, Z.D.; funding acquisition, Z.D. All authors have read and agreed to the published version of the manuscript.”.

Funding: “This research received no external funding”.

Conflicts of Interest: “The authors declare no conflicts of interest.”.

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