IN FOCUS
https://doi.org/10.1071/MA22005

Diagnosis of dermatophytes: from microscopy to direct PCR

Sarah E. Kidd[A] and Gerhard F. Weldhagen[A]

For full list of author affiliations and declarations see end of paper

*Correspondence to:
Sarah E. Kidd
National Mycology Reference Centre, Microbiology and Infectious Diseases, SA Pathology, Frome Road, Adelaide, SA 5000, Australia.
Email: sarah.kidd@sa.gov.au

ABSTRACT

Dermatophyte fungi are a common cause of skin, nail and hair infections globally, ranging from mild to cosmetically disfiguring, or even invasive infections in rare cases. Specimens requiring fungal microscopy and culture for suspected dermatophyte infection make up a significant portion of the workload in diagnostic microbiology laboratories. Whilst still considered the gold standard, a dermatophyte culture-based method is labour intensive, has poor sensitivity, slow result turnaround time and requires significant expertise for identification of the fungi. Molecular diagnostics, especially real-time PCR, have the potential to improve diagnostic sensitivity, reduce labour requirements and decrease result turnaround times. Despite these advantages, a PCR-based approach may present some difficulties and disadvantages, most notably its diagnostic range and incompatibility with oral therapy prescribing requirements under the Pharmaceutical Benefits Scheme. Here we review current best practices and future prospects for laboratory diagnosis of dermatophyte infections, including the role of microscopy, culture and direct PCR.

Keywords: dermatophyte, *Epidermophyton*, fungal culture, medical mycology, microscopy, *Microsporum*, onychomycosis, real-time PCR, tinea pedis, *Trichophyton*.

Introduction

Dermatophyte fungi are a common cause of skin, nail and hair infections globally. The most common agents are *Trichophyton rubrum* and *T. interdigitale*,[1–3] but other dermatophytes belonging to the genera *Arthroderma*, *Trichophyton*, *Epidermophyton*, *Microsporum*, *Nannizzia*, *Paraphyton*, and *Lophophyton* (the latter three genera formerly classified within *Microsporum*) also cause infection. Infections commonly present as tinea pedis and onychomycosis, but can affect any keratinised area of the body, having low clinical acuity. However, some infections may be debilitating or invasive in immunocompromised or elderly patients.[4,5] The prevalence of onychomycosis is approximately 10% in the general population, but may increase to 50% in those aged >70 years.[6] Infections are usually transmitted by direct or indirect human contact, but may also be acquired from animal sources or soil, depending on the species. The prevalence of these infections and the species that cause them appears to vary significantly by geographic region, and is well reviewed by Nowicka and Nawrot.[3]

Occasionally, yeasts such as *Candida* spp. and non-dermatophyte moulds such as *Scopulariopsis* spp., *Aspergillus* spp., *Fusarium* spp., and *Acremonium* spp. may cause onychomycosis, but the diagnosis is complicated by these fungi also being common environmental contaminants.[7,8] Diagnostic guidelines require at least two subsequent isolations of these fungi in the absence of a dermatophyte and in the setting of direct microscopy exhibiting fungal hyphae not resembling dermatophytes.[9]

Effective antifungal agents are available for treatment of dermatophyte infections in topical or oral formulations, with the latter being necessary to treat refractory nail infections. However, for oral formulations to be prescribed through the Pharmaceutical Benefits Scheme (PBS), patients need to fulfil clinical requirements and laboratory diagnostic criteria including microscopy and dermatophyte culture.[10] Therefore, these infections form a significant laboratory workload and reliable, sensitive, and specific diagnostic methods are needed for effective treatment.

While much of mycology continues to utilise conventional methods such as microscopy and culture, the diagnosis of dermatophyte infections is increasingly becoming modernised through the use of real-time PCR assays. Here we review current best practices and future prospects for laboratory diagnosis of dermatophyte infections, including the role of

Received: 31 December 2021
Accepted: 5 February 2022
Published: 8 April 2022

Cite this:
Kidd SE and Weldhagen GF (2022) *Microbiology Australia* **43**(1), 9–13. doi:10.1071/MA22005

Table 1. Summary of available diagnostic methods for dermatophyte infections.

Method	Direct microscopy[3,11–14]	Culture[3,14,15]	Culture identification by MALDI-ToF[16–18]	Culture identification by ITS sequencing[19]	Direct detection by RT-PCR[20–26]
Depth of identification	• Differentiates dermatophytes from other moulds and yeast	• Dependent on staff expertise and available culture media	• 37 species level identifications • 4 genus level identifications (Bruker filamentous fungi database v. 4.0)	• Both genus and species level identification of dermatophytes represented in validated/curated genetic data repositories	• Most commonly to genus level • Species and species complex level identification dependent on assay design
Advantages	• Fast result turnaround time • Good sensitivity when an optical brightener is used	• Grow the aetiological agent for identification and subsequent studies • Identify non-dermatophyte causes of onychomycosis • Data may be used for epidemiological purposes	• Direct identification from semisolid media possible • Cost effective • Reduced need for morphological identification • Species library regularly updated • Fungal taxonomy regularly updated • Creation of in-house species library possible	• Gold standard – results are unequivocal • Sequence data can be shared and compared between laboratories • Online databases are publicly available	• Reduced sample preparation • Increased sensitivity • Reduced turnaround time • Reduced requirement for morphological expertise • TGA approved commercial kits available • In-house assay detection chemistry can be suited to a specific demographic • Use some existing laboratory equipment
Disadvantages	• Low specificity • Differentiating dermatophyte hyphae/elements from those of other fungi requires significant expertise	• Low sensitivity • Takes 2–4 weeks to grow, and additional time for identification • Morphological identification requires significant expertise	• May need subculture to improve spectral analysis – increases workload and turnaround time • Species library is restricted to research use only (RUO) for many laboratories	• Requirement for additional equipment and dedicated laboratory space • Needs highly trained and skilled staff • Databases may not be adequately curated (e.g. GenBank) • Costs frequently exceed Medical Benefit Scheme remuneration	• Detection chemistry may not be compatible with high throughput laboratories • Requirement for additional equipment and dedicated laboratory space • May detect DNA from non-viable dermatophytes post-treatment • May not detect species causing non-dermatophyte onychomycosis • Methods not included in current PBS prescribing criteria

microscopy, culture and direct PCR, and Table 1 summarises the advantages and disadvantages of each.

Microscopy

Direct microscopy of specimens is an essential component of the diagnostic pathway for cutaneous fungal infections. It provides a relatively fast result demonstrating the presence or absence of fungal elements indicative of infection. More importantly, a skilled microscopist can differentiate dermatophyte hyphae from that of non-dermatophyte moulds and yeast pseudohyphae, and the budding yeast cells of *Candida* spp. from those of *Malassezia* spp. This alone may be sufficient for a diagnosis of dermatophyte and dermatophyte-like infections. However, observation of fungal elements does not necessarily indicate causation of infection or demonstrate viability of the fungal elements and it is not possible to determine the genus or species. Therefore, microscopy is a low-specificity diagnostic technique.

Traditionally, skin and nail specimens are digested in 10–20% potassium hydroxide (KOH) as a clearing agent for 3–16 h prior to microscopy, having a false-negative rate of 5–15%.[11] Staining with chlorazol black E can provide additional contrast for visualising the fungal elements (Fig. 1*a*). However, optical brightener stains such as Calcofluor White or Blankophor, which bind to cellulose and chitin in the fungal cell wall and fluoresce under UV light (Fig. 1*b*), significantly improve the sensitivity of microscopy (82–91% vs 74–85% KOH preparation alone)[12,13] and allows more rapid scanning of slides. Optical brightener stains are recommended in laboratory guidelines for dermatophyte studies.[14]

Culture

Culture remains the gold standard for diagnosis of dermatophyte and other fungal infections, yielding a specific aetiological agent that can be identified to species level. However, culture has low sensitivity,[15] requires 2–4 weeks

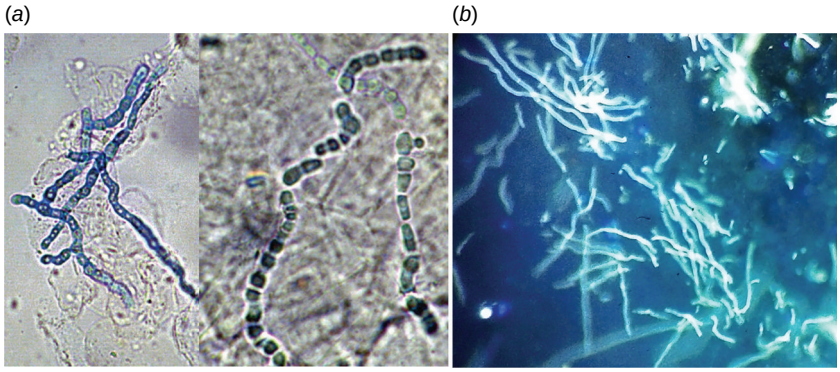

Fig. 1. KOH wet mounts of nail tissue containing dermatophyte hyphae with arthroconidia, stained with (*a*) chlorazol black, viewed under a light microscope, and (*b*) calcofluor white, viewed under a UV microscope. Image credit: (*a*) courtesy of David Ellis, Mycology Online (www.mycology.adelaide.edu.au).

for growth, and potentially a further 2 weeks for identification. Species identification needs detailed examination of colony and microscopic morphology, requiring specialised media and significant expertise.

Culture media for dermatophyte studies should include a specialised isolation medium such as Lactritmel or Dermatophyte Test Medium, containing antibiotics and cycloheximide; this minimises growth of fast growing bacterial and fungal contaminants, allowing the slower growing dermatophyte to grow. Sabouraud's dextrose agar containing antibiotics but not cycloheximide is also recommended for nail specimens to allow growth of potential causes of non-dermatophyte onychomycosis.

The identification of dermatophytes and other fungi requires specialised training and expertise, something of a dying art in the era of laboratory automation. Commercial matrix assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-ToF) databases have the potential to identify common dermatophyte species; however, many species, for example, *Trichophyton benhamiae*, *T. equinum*, *T. erinacei*, *T. eriotrephon*, *T. interdigitale*, *T. mentagrophytes* and *T. tonsurans*, cannot be reliably differentiated.[16] Optimised protein extraction methodologies combined with supplemented databases may be far more accurate,[17,18] although significant initial setup is required. DNA sequencing for species identification using the Internal Transcribed Spacer (ITS) rDNA region may be useful in some cases, but sequence databases contain numerous entries for dermatophytes with incorrect identifications that complicates making a reliable identification, and particularly between *T. interdigitale* and *T. mentagrophytes*.[19]

Real-time PCR assays

Given the slow and labour-intensive nature of conventional methods, many laboratories are looking towards real-time PCR for detection of dermatophytes in clinical samples.[20–26] The primary advantage of this approach is the decreased turnaround time over culture, with results typically available within 1–3 days of receipt in the laboratory. Additionally, the sensitivity of PCR is around 20–30% higher than culture, in part because dermatophyte DNA can be detected even in the presence of fast-growing fungal contaminants that might overgrow the slower-growing dermatophytes in cultures.[15,20,21,25] PCR-based identification reduces the need for morphologists, who could be utilised more effectively diagnosing fungi from life-threatening infections.

PCR assays may be commercial or in-house designed. In-house assays have the benefit of being customisable to existing laboratory platforms, as well as to include species that best represent the local epidemiology,[23] and fungi involved in non-dermatophyte infections of the skin and nails. Commercial assays typically have the benefit of regulatory approval, lessening the validation requirements on the laboratory. The sensitivity of dermatophyte PCR assays has invariably been shown to exceed that of culture.[20–26]

Some commercial assays include pan-dermatophyte detection and/or specific primers for detection of a limited number of species. For example, the Dermatophyte PCR Test (SSI Diagnostica, Denmark), utilises pan-dermatophyte primers targeting the CHS1 gene as well as *T. rubrum*-specific ITS2 primers, the DermaGenius Nail multiplex assay (PathoNostics, The Netherlands) contains species-specific primers for *T. rubrum*, *T. interdigitale* and *C. albicans* only, and the Dermatophytes Real-Time PCR (EurobioPlex, France) is designed to detect six dermatophyte species. The 'Dermatophytes and other Fungi' multiplex tandem PCR (MT-PCR) (AusDiagnostics, Sydney) detects at least 14 dermatophyte species, as well as four *Candida* species and two non-dermatophyte moulds, with species identification made possible by specific primers and melt curve analysis (Fig. 2). This assay does not currently differentiate all of the species that it detects,[21,22] but differences in melt curve may be suggestive of certain species, requiring further validation. Given the limitations of species differentiation with PCR assays, it may only be possible to report the species complex, genus, or simply 'dermatophyte detected'. This loss of species resolution limits insight into the potential origins of infection (i.e. anthropophilic vs zoophilic), but for most clinicians the faster turnaround time coupled with increased sensitivity, appears to be an acceptable trade-off.

PCR assays may not detect non-dermatophyte fungi that cause onychomycosis, which have an incidence of around 2–20%.[7,8,27,28] The AusDiagnostics panel includes targets for *Aspergillus* spp., *Scopulariopsis* spp., and four *Candida* species but not *Fusarium* spp., *Acremonium* spp., and *Neoscytalidium* spp. Since these may be environmental contaminants or opportunistic skin flora, they may be detected in the absence of infection and should be reported with caution, potentially utilising reflex cultures where non-dermatophyte onychomycosis is suspected.

No.	Sa	Gene	Call	Corrected melt	Take-off	Calculated ct	Concentration
N1	2...	*Trichophyton* spp.	Present	84.71	12.52	20.33	39 758
N2	2...	*T. rubrum* complex	Present	84.77	12.65	20.46	36 546
N3	2...	*Mentagrophytes*					
N4	2...	*Microsporum* spp.					
N5	2...	*Microsporum canis*					
N6	2...	*E. floccosum*					
N7	2...	*Nannizzia gypsea*					
N8	2...	*Scopulariopsis* spp.					
N9	2...	*Aspergillus* spp.					
N10	2...	*Candida* \ Meyeroz...					
N11	2...	Candida2	Present (C. parapsil...	79.72	24.55	32.36	18
N12	2...	SPIKE	Present	81.8	14.67	22.48	10 000

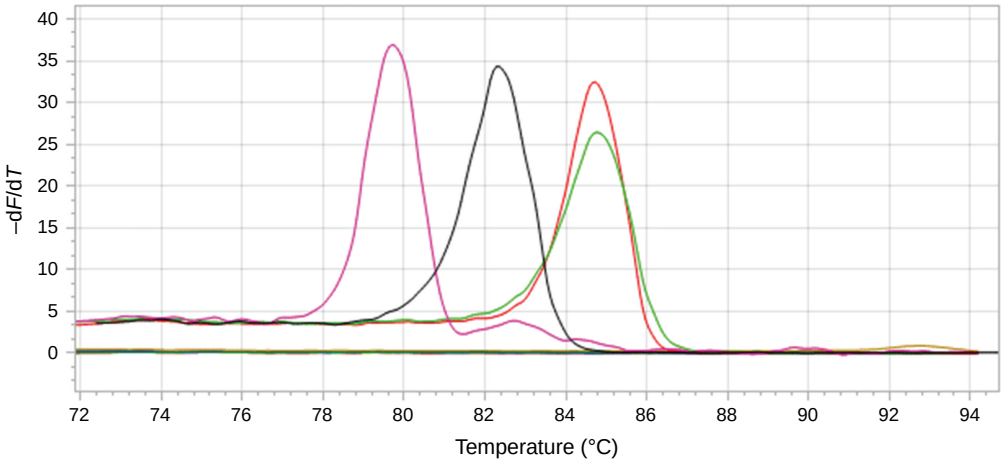

Fig. 2. Screenshot of Dermatophytes and other Fungi MT-PCR result display (AusDiagnostics Multiplex-Tandem PCR Results Software ver. 1.7.14) from a nail scraping, demonstrating melt curve detection of *Trichophyton* species (red), further identified as *T. rubrum* complex (green), trace quantities of a *Candida* species (pink) likely representing skin flora, and an internal PCR control (black).

Real-time PCR detection of squalene epoxidase mutations conferring terbinafine resistance has recently been described.[29] However, the significance and clinical utility of such assays are currently limited, as susceptibility breakpoints for dermatophytes remain tentative or unavailable.[30]

Despite the superior sensitivity of PCR compared to culture, dermatophyte detection in nail specimens requires confirmation by either microscopy or culture in order to satisfy Australian PBS requirements for prescription of oral terbinafine. This represents additional work, and does not always provide the necessary confirmation. Some of these cases may be attributed to the detection of DNA from non-viable dermatophytes (i.e. already inactivated by antifungal treatment).

Conclusions

PCR detection of dermatophyte infection brings many benefits to laboratories that are increasingly stretched for resources. However, currently, such advances come at the cost of accurate speciation, and the associated ecological and epidemiological information that accompanies it. Current PBS requirements and the increasingly apparent role of non-dermatophyte fungi in cutaneous infections means that there remains a role for microscopy and culture in routine diagnostic laboratories.

References

1. Ghannoum MA *et al.* (2000) A large-scale North American study of fungal isolates from nails: the frequency of onychomycosis, fungal distribution, and antifungal susceptibility patterns. *J Am Acad Dermatol* **43**, 641–648. doi:10.1067/mjd.2000.107754
2. Ilkit M, Durdu M (2015) Tinea pedis: the etiology and global epidemiology of a common fungal infection. *Crit Rev Microbiol* **41**, 374–388. doi:10.3109/1040841X.2013.856853
3. Nowicka D, Nawrot U (2021) Tinea pedis-An embarrassing problem for health and beauty: a narrative review. *Mycoses* **64**, 1140–1150. doi:10.1111/myc.13340
4. Teo TSP *et al.* (2021) Mycetoma caused by *Microsporum canis* in a patient with renal transplant: a case report and review of the literature. *Transpl Infect Dis* **23**, e13516. doi:10.1111/tid.13516
5. Wang R *et al.* (2021) Invasive dermatophyte infection: a systematic review. *Mycoses* **64**, 340–348. doi:10.1111/myc.13212
6. Thomas J *et al.* (2010) Toenail onychomycosis: an important global disease burden. *J Clin Pharm Ther* **35**, 497–519. doi:10.1111/j.1365-2710.2009.01107.x

7. Bongomin F *et al.* (2018) A review of onychomycosis due to *Aspergillus* species. *Mycopathologia* **183**, 485–493. doi:10.1007/s11046-017-0222-9
8. Ellis DH *et al.* (1997) Non-dermatophytes in onychomycosis of the toenails. *Br J Dermatol* **136**, 490–493. doi:10.1111/j.1365-2133.1997.tb02128.x
9. Shemer A *et al.* (2009) New criteria for the laboratory diagnosis of nondermatophyte moulds in onychomycosis. *Br J Dermatol* **160**, 37–39. doi:10.1111/j.1365-2133.2008.08805.x
10. Pharmaceutical Benefits Scheme (PBS) (2021) General schedule, antifungals for systemic use, Terbinafine. Available at https://www.pbs.gov.au/medicine/item/2285G-2804N-4011D [Accessed 23 December 2021]
11. Weitzman I, Summerbell RC (1995) The dermatophytes. *Clin Microbiol Rev* **8**, 240–259. doi:10.1128/CMR.8.2.240
12. Weinberg JM *et al.* (2003) Comparison of diagnostic methods in the evaluation of onychomycosis. *J Am Acad Dermatol* **49**, 193–197. doi:10.1067/S0190-9622(03)01480-4
13. Haghani I *et al.* (2013) Comparison of diagnostic methods in the evaluation of onychomycosis. *Mycopathologia* **175**, 315–321. doi:10.1007/s11046-013-9620-9
14. Public Health England (2016) UK Standards for Microbiology Investigations. SMI B39. Investigation of Dermatological Specimens for Superficial Mycoses. Available at https://www.gov.uk/government/publications/smi-b-39-investigation-of-dermatological-specimens-for-superficial-mycoses [Accessed 13 December 2021]
15. Ghannoum M *et al.* (2018) Examining the importance of laboratory and diagnostic testing when treating and diagnosing onychomycosis. *Int J Dermatol* **57**, 131–138. doi:10.1111/ijd.13690
16. Bruker Daltonics GmbH & Co. KG. (2021) MBT Filamentous Fungi Library 4.0 Release Notes. Revision D Doc. no. 5027071.
17. L'Ollivier C *et al.* (2013) A MALDI-TOF MS procedure for clinical dermatophyte species identification in the routine laboratory. *Med Mycol* **51**, 713–720. doi:10.3109/13693786.2013.781691
18. Shaw D (2021) Matrix-assisted laser desorption/ionisation-time of flight mass spectrometry: protocol standardisation, comparison and database expansion for faster and reliable identification of dermatophytes. *Mycoses* **64**, 926–935. doi:10.1111/myc.13285
19. Normand AC *et al.* (2018) Nucleotide sequence database comparison for routine dermatophyte identification by internal transcribed spacer 2 genetic region DNA barcoding. *J Clin Microbiol* **56**, e00046–18. doi:10.1128/JCM.00046-18
20. Arabatzis M *et al.* (2007) Diagnosis of common dermatophyte infections by a novel multiplex real-time polymerase chain reaction detection/identification scheme. *Br J Dermatol* **157**, 681–689. doi:10.1111/j.1365-2133.2007.08100.x
21. Ross IL *et al.* (2020) Detection and identification of dermatophyte fungi in clinical samples using a commercial multiplex tandem PCR assay. *Pathology* **52**, 473–477. doi:10.1016/j.pathol.2020.03.002
22. Lin BB *et al.* (2021) Multiplex RT-PCR provides improved diagnosis of skin and nail dermatophyte infections compared to microscopy and culture: a laboratory study and review of the literature. *Diagn Microbiol Infect Dis* **101**, 115413. doi:10.1016/j.diagmicrobio.2021.115413
23. Kabtani J *et al.* (2021) Real-time PCR assay for the detection of dermatophytes: comparison between an in-house method and a commercial kit for the diagnosis of dermatophytoses in patients from Dakar, Senegal. *J Fungi (Basel)* **7**, 949. doi:10.3390/jof7110949
24. Cuchí-Burgos E *et al.* (2021) Commercial real time PCR implementation for rapid diagnosis of onychomycosis: a new workflow in a clinical laboratory. *Enferm Infecc Microbiol Clin (Engl Ed)* **39**, 326–329. doi:10.1016/j.eimc.2020.05.026
25. Spiliopoulou A *et al.* (2015) Evaluation of a commercial PCR test for the diagnosis of dermatophyte nail infections. *J Med Microbiol* **64**, 25–31. doi:10.1099/jmm.0.079962-0
26. Hayette MP *et al.* (2019) Clinical evaluation of the DermaGenius® Nail real-time PCR assay for the detection of dermatophytes and *Candida albicans* in nails. *Med Mycol* **57**, 277–283. doi:10.1093/mmy/myy020
27. Laroche L *et al.* (2021) Epidemiological analysis of nail aspergillosis and non-dermatophyte moulds. Criteria for the involvement of *Aspergillus* in 102 cases of onychomycosis at Montpellier University Hospital, France (1991–2019). *Mycoses* **64**, 1546–1553. doi:10.1111/myc.13366
28. Moreno G, Arenas R (2010) Other fungi causing onychomycosis. *Clin Dermatol* **28**, 160–163. doi:10.1016/j.clindermatol.2009.12.009
29. Singh A *et al.* (2021) Evaluation of DermaGenius® resistance real-time polymerase chain reaction for rapid detection of terbinafine-resistant *Trichophyton* species. *Mycoses* **64**, 721–726. doi:10.1111/myc.13271
30. The European Committee on Antimicrobial Susceptibility Testing (2020) Overview of antifungal ECOFFs and clinical breakpoints for yeasts, moulds and dermatophytes using the EUCAST E.Def 7.3, E.Def 9.3 and E.Def 11.0 procedures. Version 2. Available at http://www.eucast.org

Data availability. Data sharing is not applicable as no new data were generated or analysed during this study.

Conflicts of interest. The authors declare no conflicts of interest.

Declaration of funding. This work did not receive any specific funding.

Author affiliation

[A]National Mycology Reference Centre, Microbiology and Infectious Diseases, SA Pathology, Frome Road, Adelaide, SA 5000, Australia.

Biographies

Sarah Kidd is the Head of the National Mycology Reference Centre at SA Pathology, as well as Adjunct Senior Lecturer at the University of Adelaide. She completed a Bachelor of Medical Science with Honours (1999) and a PhD in Medicine (2003), both at the University of Sydney, and has undertaken postdoctoral training and research at the University of British Columbia, Vancouver, Canada, and at Monash University–The Alfred Hospital, Melbourne. She is a Fellow of the Australian Society for Microbiology (FASM), Secretary of the Australia and New Zealand Mycoses Interest Group (ANZMIG), convenes the biennial Mycology Masterclass, and provides mycology expertise to the Royal College of Pathologists of Australasia Quality Assurance Programs.

Gerhard Weldhagen is the supervising pathologist for the National Mycology Reference Centre at SA Pathology. He completed his undergraduate studies in 1993, receiving a Bachelor's degree in Medicine and Surgery (MBChB) from the University of Pretoria, South Africa. This was followed by a Master's degree in Clinical Microbiology (MMed (Path) cum laude) and subsequently a PhD in Microbiology, conferred by the University of Pretoria in 2002 and 2005 respectively. After settling in Australia during 2009, a Fellowship of the Royal College of Pathologists of Australasia (FRCPA) was attained during the same year. Current interests include the role of molecular assays in diagnostic microbiology, including mycology.