Taylor & Francis
Taylor & Francis Group

REVIEW ARTICLE

🔓 OPEN ACCESS ⟳ Check for updates

Recent trends in rapid diagnostic techniques for dermatophytosis

Hassan Aboul-Ella (ID)[a], Rafik Hamed[b] and Heidy Abo-Elyazeed (ID)[a]

[a]Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Giza Egypt; [b]Bacteriology Biotechnology Diagnostics Department, Institute for Evaluation of Veterinary Biologics (CLEVB), Agricultural Research Center (ARC), Cairo, Egypt

ABSTRACT

Dermatophytosis is a common contagious disease of both humans and animals. It is caused by a group of filamentous fungi known as dermatophytes, including several genera and various species. An accurate diagnosis of dermatophytes as a causative agent of a skin lesion requires up to one month of conventional laboratory diagnostics. The conventional gold standard diagnostic method is a direct microscopic examination followed by 3 to 4 weeks of Sabouraud's dextrose agar (SDA) culturing, and it may require further post-culturing identification through biochemical tests or microculture technique application. The laborious, exhaustive, and time-consuming gold standard method was a real challenge facing all dermatologists to achieve a rapid, accurate dermatophytosis diagnosis. Various studies developed more rapid, accurate, reliable, sensitive, and specific diagnostic tools. All developed techniques showed more rapidity than the classical method but variable specificities and sensitivities. An extensive bibliography is included and discussed through this review, showing recent variable dermatophytes diagnostic categories with an illustration of weaknesses, strengths, and prospects.

1. Introduction

Dermatophytosis is a disease caused by a group of closely related fungi called dermatophytes. It includes three main genera and more than fifty species. Altogether, there are seven genera *Trichophyton, Microsporum, Epidermophyton, Nannizzia, Paraphaton, Lophophyton, Arthroderma*. All species of the first three genera and some of the *Nannizzia* species are obligate human pathogens. *Microsporum, Trichophyton*, and *Epidermphyton* are these main genera [1]. Of these, the genera *Trichophyton* and *Microsporum* contain zoophilic species. *Nannizzia* is geophilic but can infect animals and humans. The incidence of tinea in humans or ringworm in animals is relatively high, with reinfection and reoccurrence possibilities [2]. It is a highly contagious disease by which 20–25% of all worldwide population infected with it at any time around the year [3] with the highest prevalence of (19.7%) in developing countries [4,5]. Dermatophytosis is conventionally diagnosed using direct microscopic examination, gold standard culturing, and other identification techniques as microcultures and biochemical tests. This is a long, time-consuming process, requiring experts and specific diagnostic protocol [6]. The results were significantly affected by both objective and subjective factors: the amount of the sample, the pre-sampling applied treatments, and the examiner's experience. Developing a more rapid method or protocol to facilitate the diagnosis of dermatophytosis was the aim of many studies in the past few decades. All developed techniques shared rapidity and showed different levels of specificity and sensitivity. Through the following statistical representation Figure (1), the recent advances in dermatophytosis rapid diagnosis are categorized into five major sections, microscopic examination dependent techniques, modified rapid culture techniques, matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) based technique, molecular diagnostic techniques, and lateral flow-based techniques. The data is obtained from Scopus databases and analysed using a free author's analytical services available through Scopus official website "Scival analytics" combined with "Microsoft Excel" for data presentation. The represented data shows the high interest of scientific communities in the recent techniques developed to achieve a rapid diagnosis of dermatophytosis.

2. Microscopic examination dependent techniques

2.1. Bright-field light microscope

Microscopic examination in dermatophytes infection diagnosis has been used through different diagnostic techniques. Either direct microscopic examination of skin scrapings, hair, scales, and nails using a clarifying agent examined under 40x power of bright field microscope, or histological [7] histopathological [8] and even immunohistochemical examination [9] of biopsied tissue of a lesion from infected cases. Such techniques aim to demonstrate the different hair parasitism arrangements of arthrospores [10]. Several
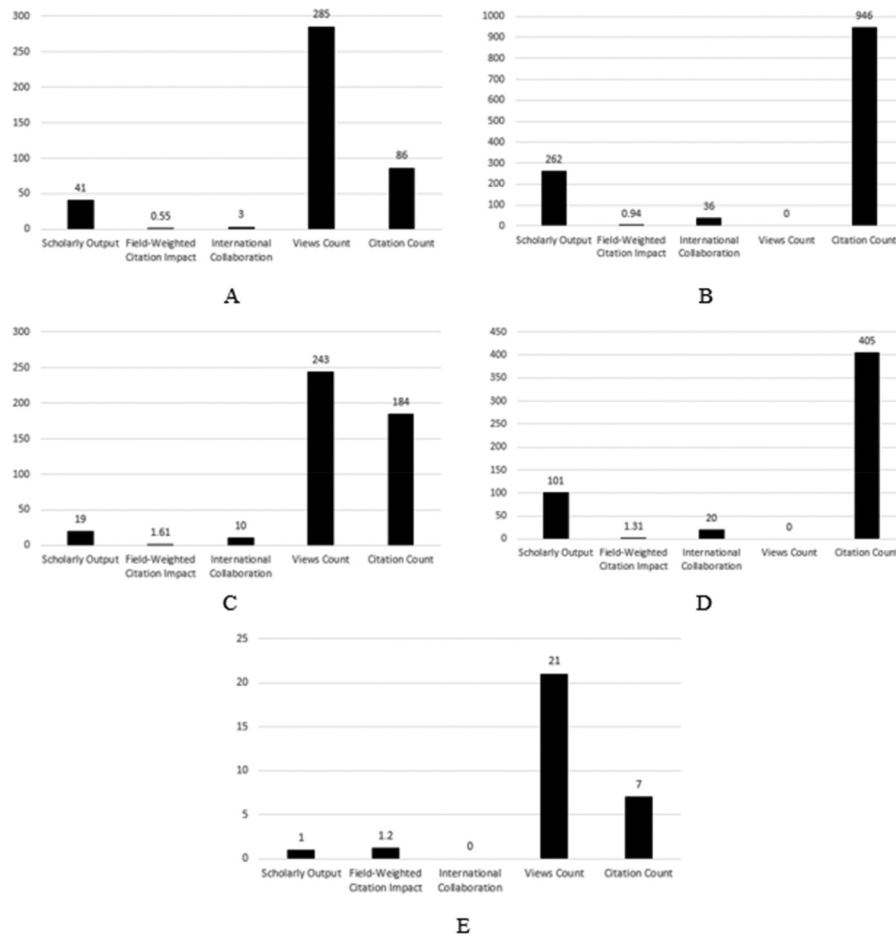
Figure 1. Statistical diagrams represent the scholarly output, field weight citation impact, international collaboration, views count, and citation count of studies on rapid diagnosis of dermatophytosis on the Y-axis and the number of each variant on the X-axis. A. microscopic examination dependent techniques, B. modified rapid culture techniques, C. MALDI-TOF based techniques, D. molecular diagnostic technique, E. lateral-flow based techniques.

studies and experiments have been tried on a different clarifying agent, during wet slide preparation, to achieve a better cleared microscopic view and less time during the slide preparation and examination. Potassium hydroxide (KOH), sodium hydroxide (NaOH), calcofluor white [11], Chicago sky blue [12], lactophenol cotton blue (LPCB), and a mixture of dimethyl sulphoxide (DMSO) and glycerine with NaOH or KOH.

2.2. Phase-contrast light microscope

A better bright-field microscopic view is achieved by contrast and clearance adjustment. That is obtained by lowering the light intensity to achieve maximum contrast and maximum cleared view. So, the phase-contrast microscope will be better because structures are more clearly delineated without loss of light. A 40x phase contrast microscope objective lens has been used to demonstrate the characteristic dermatophytes fungal elements in skin scrapings and nail samples [13].

2.3. Fluorescence microscope

Incorporation of fluorescence related techniques in the rapid diagnosis of dermatophytosis has been based on the fact that dermatophytes infected tissue with certain species produce fluorescence when either stained with hematoxylin/eosin (H and E) stain [8,14] or exposed to UV light. On the same bases, Wood's lamp usage during clinical examination of the cutaneous lesion was developed [15]. The main obstacle facing the natural fluorescence-based dermatophytes technique is this nature's limitation to a few species leads to overall independence on this technique in dermatophytes diagnosis. On the other hand, using a fluorescent dye as calcofluor white showed significant advances in conventional KOH wet mount [12].

Microscopic examination as a classical step in diagnosing dermatophytosis showed various microscope types and different examination techniques. The most uncomplicated and most rapid technique was using a bright-field microscope in association with one of the clarifying agents. By the time, it showed the lowest

specificity, sensitivity, and positive predictive value in comparison to other methods [10].

3. Modified rapid culturing techniques

3.1. Dermatophytes test medium (DTM)

Other than the conventional isolation of dermatophytes on mycological specific media, modified culturing media can provide a relatively rapid (2 weeks) presumptive identification of dermatophytes if compared with the conventional culturing [16]. Dermatophytes test medium (DTM) is one of those firstly developed media for rapid presumptive identification of dermatophytes [17]. Unfortunately, some non-dermatophytes mould (NDM), either pathogenic or saprophytic, can survive the type and concentration of the used antifungal agents. NDM can also produce alkaline products that change the colour of the media from straw yellow to red. That formed red colour equals or even more intense than the dermatophytes group itself. Moreover, some of NDM may share the dermatophytes group the same shape, site of the lesion, and sample type.

3.2. Dermatophytes identification medium (DIM)

A relatively rapid medium for dermatophytes presumptive identification has been developed as DTM modification avoiding the DTM drawback as the non-specific and false-positive NDM reactions [17]. Modifications to DTM were 37°C incubation, and the increased concentration of cycloheximide [18]. This modified DTM is dermatophytes identification medium (DIM) [18]. DIM has been used through certainly developed identification protocol, which showed a great advantage on the DTM in the meaning of sensitivity and specificity. Unfortunately, DIM has high false negatives and false positives, limiting its usefulness and usage [19].

3.3. Multi-chromogenic media-dependent protocol

A 2 days protocol is designed for rapid, simple differentiation of two closely similar dermatophytes species [20]. The developed protocol was designed as follows; a 2–20 days old primary isolates were sub-cultured on four different commercially available chromogenic media at different temperatures 4, 20, 25, 30°C with maximum temperature not reach 37°C as temperatures above 37°C would be unsuitable for the growth of most of the dermatophytes group. The reading strategy for those inoculated media was regular inspection from 2 hours to 7 days post incubation. This study [20] showed that Candiselect™ is a promising candidate for achieving rapid and accurate differentiation between

the two dermatophyte species studied (within a few hours). Despite the promising results, this technique is only able to distinguish between the two dermatophytes mentioned above. If a different dermatophyte is present, it needs to be further diagnosed, which may take even more time than the traditional culture.

3.4. Screening culture-slide method

A thin layer of DTM coats a transparent plastic slide. It principally resembles a rapid microculture technique in which samples are collected and applied on the thin layered media using transparent adhesive tape. The dermatophytes identification depends on micromorphological changes during daily culture-slide microscopic examination in association with some macromorphological colour characteristics [21].

All culturing-based techniques for rapid identification of dermatophytes, either developed for a wide range of dermatophytes identification or only a limited number of species, require a long time that acts as an actual obstacle against a real application of those mentioned studies in the routine diagnosis of dermatophytosis.

4. Matrix-assisted laser desorption/ ionization-time of flight mass spectrometry (MALDI-TOF) based techniques

This spectrum generation and assessment technique is considered an evolutionary step in the laboratory diagnosis as a highly reliable, accurate, easy to handle, and easy to incorporate in the regular laboratory workflow. At the same time, it overcomes the drawbacks of molecular techniques as high cost, experience needed during the application, and analysis of outcome results or drawbacks of conventional technique as long-time consumption and low specificity [22,23]. MALDI-TOF MS was firstly applied on a whole-cell scale, a fresh culture of suspected microbial colonies. By the time, it became able to deal directly with another type of culture, than solid agar plates as blood cultures and other applications have been incorporated in its system to improve its ability to perform a wide antimicrobial panel [24].

All systems installed for MALDI-TOF identify a wide scale of different bacteria and yeast species level [25,26]. Several trials have been done to apply such a technique in different identification protocols of moulds including dermatophytes [27]. All showed impactive results only when it was associating a laboratory-made database, which is laborious and time-consuming in most cases [28], with a trial to extend the existing MALDI-TOF fungal knowledge database to allow the better robust identification of clinically relevant dermatophytes [29], different studies showed an extensive range of accuracy 13.5% to

100% due to inconsistencies concerning critical steps of the routine pre-analysis preparation laboratory process [29–38]. According to the obtained results, some studies showed that MALDI-TOF is an excellent complementary for conventional culturing in routine dermatophytes diagnosis [29,33,36,39–41].

5. Molecular diagnostic techniques

5.1. Conventional PCR

Conventional PCR is the most straightforward format of a polymerase chain reaction (PCR) for a highly specific, sensitive, and accurate diagnosis. PCR in dermatophytosis diagnosis able to achieve the diagnostic level of dermatophytes detection or dermatophytes species identification through pan fungal, dermatophytes primer, or species-specific primers respectively [42,43]. Internal transcribed spacer (ITS) and 28s ribosomal DNA are the most used pan dermatophytes primers. They are followed by further outcome amplicon size determination using gel electrophoresis. Despite the sample size, condition, and preparation before applying the PCR reaction, even in mixed cultures, it showed a higher rate of identification than other conventional methods [44–48]. PCR results were affected by the animal species from which the sample was collected as animal behaviour differences affect dermatophytes nucleic acid load on the collected sample, which, by its role, reflected on the results [48]. Although the relatively low cost of conventional PCR compared to other molecular techniques, it requires post-amplification steps and cannot illustrate the situation of infection quantitively [49].

5.2. Quantitative/real-time PCR (qPCR)

Quantification of the fungal load of the infected sample can be achieved through the highly accurate, sensitive, and specific quantitative PCR. Real-time PCR depends on a pair of primers and labelled probes targeting pan or species-specific genes. Also, it showed less vulnerable steps to contamination due to less post-amplification handling [42,43]. Although its high cost, it showed a relatively affordable cost in case of high working routine laboratories that examine several samples per cycle [50]. qPCR is a useful diagnostic method in case of treatment effect follow up, differentiation between clinical infection and contamination by establishing a dermatophytes infection threshold. It showed higher sensitivity and specificity characteristics when compared with conventional methods [51–56]. Overall, qPCR showed a significantly higher sensitivity in dermatophytes diagnosis, species identification [53], and differentiation even in the presence of other keratinophilic NDM species in the same site of infection. Generally from previous studies, we cannot depend on

qPCR as a complete replacement to the gold-standard method as it showed both false positive and false negative results as it is affected by several factors as sample quality, differences in molecular targets, the used amplification methodology, and the DNA extraction protocols [57–59].

5.3. Nested PCR

Aiming to increase PCR specificity in dermatophytosis diagnosis, several targets nested PCR has been set up, especially in pan primers usage [60,61]. A primary amplicon resulting from the first pair of primers amplification cycle will be subjected to another cycle with another primer pair. In the second cycle, the resulted primary amplicon will act as a template for the second pair of primers. The overall sensitivity of nested PCR set was very high in several previous studies [49,62,63] incorporate a pan PCR protocol for dermatophytes diagnosis, also in comparison to the results obtained from KOH mount slide [64,65] and conventional culturing especially in case of that cultures which fail to achieve macroscopic growth [66]. However, the incidence of contamination and time consumption in comparison to the one set up PCR act as the main disadvantage in its application [2,61,67].

5.4. Multiplex PCR

One of those PCR techniques developed to be used in case of sample limitation with the need to detect multiple causative agents in the same sample using the same PCR reaction using two or more sets of primers. The nucleotide sequences of each forward and reverse primer in each chosen set should be checked for dimerization, which may lead to unspecific amplification. Several multiplex/duplex PCR have been designed [53] and tried multiple times [44,45] and showed a competitive sensitivity and specificity than culturing and KOH wet mounts with the highest accuracy in cases of onychomycoses and *T. rubrum* [51,67].

5.5. PCR-ELISA

A hybrid technique based on both PCR and Enzyme-linked immunosorbent assay (ELISA) in which a labelled nucleic acid amplicon is used instead of target analyte protein in microculture ELISA plates [68]. To increase its sensitivity, a specific probes hybridization was performed before ELISA application. Two hybridization formats have been developed. Either amplification of target genes in the presence of digoxigenin and biotin-labelled nucleotide probe to form a biotinylated PCR amplicon [69]. The fixed amplicon on the microtiter plate was detected using anti-digoxigenin peroxidase [70]. The other format is

based on amplification in the presence of a fluorescently labelled nucleotide probe, which is detected using horseradish peroxidase-conjugated anti fluorescein antibodies [71]. Although time consumption and exhaustive application, it was of low cost per sample compared to qPCR [50], and several designs have been developed through various studies [72–74]. However, it is not considered the most applicable technique in dermatophytes routine diagnosis due to its laborious effort and time consumption compared to other established methods.

5.6. PCR-Restriction fragment length polymorphism (PCR-RFLP)

PCR-RFLP is another hybrid technique based on PCR and further restricted fragment length polymorphism step using restriction enzymes [75,76]. To a certain extent, it resembles a low-cost replacement of nested PCR as it was able to detect different dermatophytes species and even different NDM species from a multi-causes' onychomycoses cases [61,77–81] but could not detect variation within the same species [82,83]. Principally, this assay depends on the amplification of target sequences, which shows species-specific variation; the amplicon was then treated with specific restriction enzymes; finally, amplicon sequencing or gel electrophoresis step was performed to compare the molecular weight of the bands to standard ladder [61,79]. However, it seems a relatively low cost, easy to be performed design and higher sensitivity than conventional culture methods [84]. Due to this technique's laborious, exhaustive, and time-consuming nature and restriction enzyme requirement, it is excluded from the routine dermatophytes' diagnosis scheme [85].

Other molecular assays have been developed or under development in the field of rapid, molecular dermatophytosis diagnosis. Mainly based on or hybrid with a PCR step as PCR – high resolution melt assay (PCR-HRM) [86], PCR-pyrosequencing [87], single primer-PCR [88], arbitrary primed-PCR [89], PCR-reverse line blot [90], fingerprinting [91], genomic or oligonucleotide array [92] and proteomic analysis techniques [92,93], isothermal amplification techniques [94] and random primer amplification polymorphic DNA (RAPD) [95]. All showed promising results on the research levels, not for routine clinical use.

6. Lateral flow-based techniques

Lateral flow-based techniques have been developed for rapid detection of various infectious and analytical agents during the past few years [96] that meets the affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end-users

(ASSURED) criteria of the world health organization (WHO) [97]. A lot of lateral flow assays with different accuracy, specificity, and sensitivity were developed. Immunochromatography lateral flow assay (ILFA), nucleic acid lateral flow immunochromatographic assay (NALFIA) [98] and nucleic acid lateral flow assay (NALFA). NALFA can be based either on hetero-thermal amplification (PCR-NALFA) [99,100] or isothermal amplification (RPA-NALFA) [101]. Prospectively, Clustered Regularly Interspaced Short Palindromic Repeats/*cas*12 lateral flow assay (CRISPR/*cas*12-LFA) [102,103] could be developed for diagnosis of dermatophytes. CRISPR/*cas*12-LFA developed either using the DETECTR system [104,105] or SHERLOCK system [106–110]. Each lateral flow assay can be developed in different formats, typical sandwich, competitive, and multiplex [111]. Few studies that have been done as a preliminary step in applying a lateral flow-based technique considered this assay as a rapid method of dermatophytes identification.

In a single-arm comparative study through which a dermatophyte has been detected within 222 samples, a comparison between the conventional direct microscopic examination, highly specific PCR, and a recently developed monoclonal antibody-based lateral flow was performed to diagnose onychomycosis. The accuracy obtained from the three compared methods was as follows sequentially; 90.5%, 76.6%, and 92.5%, while 45 samples showed the difference in the obtained results accuracy between the three compared methods, were relied on PCR result as a conclusive judgement [112].

Further investigations [113] showed that a dermatophyte lateral flow kit is the prober candidate as a rapid diagnostic technique, replacing the highly advanced complex to deal with molecular techniques and the conventional long-time consuming techniques.

Till the current date, there are no polyclonal antibodies, and all trials were for monoclonal antibodies-based lateral flow kit for rapid diagnosis of dermatophytosis [60,112–116]. Such a polyclonal antibodies – based lateral flow kit will achieve the complicated formula characterized by rapidity, field applicability, and low cost of production as the only problem facing the monoclonal based lateral flow is the high cost of development and production.

7. Conclusion

Several successfully developed diagnostic techniques for dermatophytosis are now available to substitute the conventional methods. A lot of those techniques are more reliable and more impactive on the diagnosis of dermatophytosis. Some showed high-cost problems or requirements of experts; others showed quite sensitivity, specificity, or rapidity, others need a lengthy pre-examination preparation. Nowadays, the lateral flow-based techniques are considered a promising

competitor to both conventional laboratory-based diagnostic methods and advanced molecular-based diagnostic techniques. Enhancing the lateral flows' diagnostic accuracy, sensitivity, and specificity is the recent challenge and prospect for dermatophytosis diagnosis.

Disclosure statement

All authors state a declaration that no conflict of interest.

ORCID

Hassan Aboul-Ella http://orcid.org/0000-0002-6367-5334
Heidy Abo-Elyazeed http://orcid.org/0000-0003-0640-2644

References

[1] de Hoog HS, Dukik K, Monod M, et al. Toward a novel multilocus phylogenetic taxonomy for the dermatophytes. Mycopathologia. 2017;182(1–2):5–31.

[2] Yang G, Zhang M, Li W. Direct species identification of common pathogenic dermatophyte fungi in clinical specimens by semi-nested PCR and restriction fragment length polymorphism. Mycopathologia. 2008;166(4):203–208.

[3] Teklebirhan G, Bitew A. Prevalence of dermatophytic infection and the spectrum of dermatophytes in patients attending a tertiary hospital in Addis Ababa, Ethiopia. Int J Microbiol. 2015;2015:653419.

[4] Dogra S, Shaw D, Rudramurthy SM. Antifungal drug susceptibility testing of dermatophytes: laboratory findings to clinical implications. Indian Dermatol Online J. 2019;10(3):225–233.

[5] Organization WH. Epidemiology and management of common skin diseases in children in developing countries. Geneva World Heal Organ. 2005;54.

[6] Frosch M, Harmsen D, Schwinn A, et al. Molecular differentiation of dermatophyte fungi. Mycoses. 1999;42(1–2):67–70.

[7] Gianni C, Cerri A. Usefulness of histological examination for the diagnosis of onychomycosis. Dermatology. 2001;202(4):283–288.

[8] Cubells JR, Martínez AM, Leboráns LM. Fluorescence microscopy as a diagnostic tool for dermatophytosis. Am J Dermatopathol. 2016;38(3):208–210.

[9] Bonenberger TE, Ihrke PJ, Naydan DK. Rapid identification of tissue micro-organisms in skin biopsy specimens from domestic animals using polyclonal BCG antibody. Vet Dermatol. 2001;12(1):41–47.

[10] Quinn PJ, Carter ME, Markey BK, et al. Clinical veterinary microbiology, section 3: mycology, The dermatophytes. Wolfe publishing. 1994;67:381–390.

[11] Haldane DJ, Robart E. A comparison of calcofluor white, potassium hydroxide, and culture for the laboratory diagnosis of superficial fungal infection. Diagn Microbiol Infect Dis. 1990;13(4):337–339.

[12] Mourad B, Ismail M, Hawwam S. Evaluation of the efficacy of fluorescent staining and chicago sky blue staining as methods for diagnosis of dermatophytosis in hair and nails. Clin Cosmet Investig Dermatol. 2019;12:751–758.

[13] Roberts GD. Detection of fungi in clinical specimens by phase-contrast microscopy. J Clin Microbiol. 1975;2(3):261–265.

[14] Idriss MH, Elston D. The diagnostic value of fungal fluorescence in onychomycosis. J Cutan Pathol. 2013;40(4):385–390.

[15] Gupta LK, Singhi MK. Wood's lamp. Indian J Dermatol Venereol Leprol. 2004;70:131–135.

[16] Li X-F, Shen Y-N, Chen W, et al. A new medium for diagnosis of dermatophyte infection. Eur J Dermatol. 2009;19(1):34–37.

[17] Salkin IF, Padhye AA. A new medium for the presumptive identification of dermatophytes. J Clin Microbiol. 1997;35(10):2660–2662.

[18] Gromadzki S, Ramani R, Chaturvedi V. Evaluation of new medium for identification of dermatophytes and primary dimorphic pathogens. J Clin Microbiol. 2003;41(1):467–468.

[19] Banerjee U, Talwar V. Evaluation of modified dermatophyte test medium for isolation of dermatophytes: A preliminary report: bewertung eines modifizierten Dermatophyten-Testmediums zur Isolierung von Dermatophyten Eine vorläufige Mitteilung. Mycoses. 1984;27(9):465–469.

[20] Mayser P. A simple and rapid method to differentiate Arthroderma benhamiae from Microsporum canis. J Dtsch Dermatol Ges. 2013;11(4):322–327.

[21] Gip L. A rapid screening method for the detection of pathogenic fungi on the skin. Mycoses. 1981;24(1):17–26.

[22] Wjst M, Van Den BD. Time-of-flight mass spectrometry. Methods Mol Biol. 2013;1015:71–85.

[23] Croxato A, Prod G, Greub G. Applications of MALDI - TOF mass spectrometry in clinical diagnostic microbiology. FEMS Microbiol Rev. 2012;36(2):380–407.

[24] Mimica MJ, Martino MDV, Pasternak J, et al. MALDI-TOF MS in the clinical microbiology laboratory. J Bras Patol Med Lab. 2013;49(4):256–259.

[25] Tartor Y, Taha M, Mahboub H, et al. Yeast species associated with diseased fish: occurrence, identification, experimental challenges, and antifungal susceptibility testing. Aquaculture. 2018;488:134–144.

[26] Bille E, Dauphin B, Leto J, et al. MALDI-TOF MS Andromas strategy for the routine identification of bacteria, mycobacteria, yeasts, Aspergillus spp. and positive blood cultures. Clin Microbiol Infect. 2011;18(11):1117–1125.

[27] Intra J, Sarto C, Tiberti N. Genus-level identification of dermatophytes by MALDI-TOF MS after 2 days of colony growth. Lett Appl Microbiol. 2018;67(2):136–143.

[28] Cassagne C, Normand A-C, L'Ollivier C. Performance of MALDI-TOF MS platforms for fungal identification. Mycoses. 2016;59(11):678–690.

[29] De Respinis S, Monnin V, Girard V, et al. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry using the vitek ms system for rapid and accurate identification of dermatophytes on solid cultures. J Clin Microbiol. 2014;52(12):4286–4292.

[30] Erhard M, Hipler U, Burmester A, et al. Identification of dermatophyte species causing onychomycosis and tinea pedis by MALDI-TOF mass spectrometry. Exp Dermatol. 2007;17(4):356–361.

[31] Nenoff P, Erhard M, Simon JC, et al. MALDI-TOF mass spectrometry – a rapid method for the identification of dermatophyte species. Med Mycol. 2012;51(1):17–24.

[32] Alshawa K, Beretti J-L, Lacroix C, et al. Successful identification of clinical dermatophyte and Neoscytalidium species by matrix-assisted laser desorption ionization – time of flight. J Clin Microbiol. 2012;50(7):2277–2281.

[33] Calderaro A, Motta F, Montecchini S, et al. Identification of dermatophyte species after implementation of the in-house MALDI-TOF MS database. Int J Mol Sci. 2014;15(9):16012–16024.

[34] Karabıçak N, Karatuna O, İlkit M. Evaluation of the bruker matrix-assisted laser desorption – ionization time-of-flight mass spectrometry (MALDI-TOF MS) system for the identification of clinically important dermatophyte species. Mycopathologia. 2015;180 (3–4):165–171.

[35] Theel ES, Hall L, Mandrekar J, et al. Dermatophyte identification using matrix-assisted laser desorption ionization – time of flight mass spectrometry. J Clin Microbiol. 2011;49(12):4067–4071.

[36] Ste CL. MALDI-TOF-based dermatophyte identification. Mycopathologia. 2016;182(1–2):183–192.

[37] Respinis SDE, Tonolla M, Pranghofer S, et al. Identification of dermatophytes by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Med Mycol. 2013;51(5):514–521.

[38] Ollivier CL, Cassagne C, Normand A-C, et al. A MALDI-TOF MS procedure for clinical dermatophyte species identification in the routine laboratory. Med Mycol. 2013;51(7):713–720.

[39] Packeu A, De Bel A, l'Ollivier C, et al. Fast and accurate identification of dermatophytes by matrix-assisted laser desorption ionization – time of flight mass spectrometry : validation in the clinical laboratory. J Clin Microbiol. 2014;52(9):3440–3443.

[40] Mohammed YF, Salem EZ, Shahin IMI, et al. Applicability of Fourier transform infrared (FTIR) spectroscopy in rapid identification of some Candida and dermatophyte species infections in humans. Int J Dermatol. 2016;55(10):1164–1171.

[41] Suh S, Grosso KM, Carrion ME. Multilocus phylogeny of the Trichophyton mentagrophytes species complex and the application of matrix-assisted laser desorption/spectrometry for the rapid identification of dermatophytes. Mycologia. 2018;110(1):118–130.

[42] Jensen RH, Arendrup MC. Molecular diagnosis of dermatophyte infections. Curr Opin Infect Dis. 2012;25(2):126–134.

[43] Verrier J, Monod M. Diagnosis of dermatophytosis using molecular biology. Mycopathologia. 2016;182 (1–2):193–202.

[44] Brillowska-dabrowska A, Saunte DM, Arendrup MC, et al. Five-hour diagnosis of dermatophyte nail infections with specific detection of Trichophyton rubrum. J Clin Microbiol. 2007;45(4):1200–1204.

[45] Brillowska-Dabrowska A, Nielsen SS, Nielsen HV. Optimized 5-hour multiplex PCR test for the detection of tinea unguium : performance in a routine PCR laboratory. Med Mycol J. 2010;48(6):828–831.

[46] Kondori N, Afshari P, Stro L. Comparison of dermatophyte PCR kit with conventional methods for detection of dermatophytes in skin specimens. Mycopathologia. 2013;176(3–4):237–241.

[47] Brasch J, Beck-Jendroschek V, Gläser R. Fast and sensitive detection of Trichophyton rubrum in superficial tinea and onychomycosis by use of a direct polymerase chain reaction assay. Mycoses. 2010;54(5):313–317.

[48] Spiliopoulou A, Bartzavali C, Jelastopulu E, et al. Evaluation of a commercial PCR test for the diagnosis of dermatophyte nail infections. J Med Microbiol. 2015;64(Pt 1):25–31.

[49] Cafarchia C, Gasser RB, Figueredo LA, et al. An improved molecular diagnostic assay for canine and feline dermatophytosis. Med Mycol J. 2013;51 (2):136–143.

[50] Kupsch C, Ohst T, Pankewitz F, et al. The agony of choice in dermatophyte diagnostics – performance of different molecular tests and culture in the detection of T. rubrum and T. interdigitale. Clin Microbiol Infect. 2016;22(8):735.e11–7.

[51] Bergmans AM, Van der Ent M, Bohm N, et al. Evaluation of a single-tube real-time PCR for detection and identification of 11 dermatophyte species in clinical material. Clin Microbiol Infect. 2009;16 (6):704–710.

[52] Wisselink GJ, Van Zanten E, Kooistra-Smid AM. Trapped in keratin ; a comparison of dermatophyte detection in nail, skin and hair samples directly from clinical samples using culture and real-time PCR. J Microbiol Methods. 2011;85(1):62–66.

[53] Arabatzis M, Bruijnesteijn van Coppenraet LES, Kuijper EJ, et al. Diagnosis of common dermatophyte infections by a novel multiplex real-time polymerase chain reaction detection/identification scheme. Br J Dermatol. 2007;157(4):681–689.

[54] Alexander CL, Shankland GS, Carman W, et al. Introduction of a dermatophyte polymerase chain reaction assay to the diagnostic mycology service in Scotland. Br J Dermatol. 2011;164(5):966–972.

[55] Sharma R, Rajak RC, Pandey AK, et al. Internal transcribed spacer (ITS) of rDNA of appendaged and non-appendaged strains of Microsporum gypseum reveals Microsporum appendiculatum as its synonym. Antonie Van Leeuwenhoek. 2006;89(1):197–202.

[56] Miyajima Y, Satoh K, Uchida T, et al. Rapid real-time diagnostic PCR for Trichophyton rubrum and Trichophyton mentagrophytes in patients with tinea unguium and tinea pedis using specific fluorescent probes. J Dermatol Sci. 2013;69(3):229–235.

[57] Ohst T, Kupsch C, Gräser Y. Detection of common dermatophytes in clinical specimens using a simple quantitative real-time TaqMan polymerase chain reaction assay. Br J Dermatol. 2016;174(3):602–609.

[58] Bergman A, Heimer D, Kondori N, et al. Fast and specific dermatophyte detection by automated DNA extraction and real-time PCR. Clin Microbiol Infect. 2013;19(4):E205–E211.

[59] Motamedi M, Mirhendi H, Zomorodian K, et al. Clinical evaluation of β – tubulin real-time PCR for rapid diagnosis of dermatophytosis, a comparison with mycological methods. Mycoses. 2017;60 (10):692–696.

[60] Verrier J, Monod M. Diagnosis of dermatophytosis using molecular biology. Mycopathologia. 2017;182 (1):193–202.

[61] Verrier J, Baudraz-rosselet F, Monod M. Oral terbinafine and itraconazole treatments against dermatophytes appear not to favor the establishment of Fusarium spp . in nail. Dermatology. 2014;228(3):225–232.

[62] Uchida T, Makimura K, Ishihara K, et al. Comparative study of direct polymerase chain reaction, microscopic examination and culture-based morphological methods for detection and

identification of dermatophytes in nail and skin samples. J Dermatol. 2009;36(4):202–208.

[63] Nagao K, Sugita T, Ouchi T, et al. Identification of Trichophyton rubrum by nested PCR analysis from paraffin-embedded specimen in Trichophytia profunda acuta of the glabrous skin. Japanese journal of medical mycology. 2005;46(2):129–132.

[64] Garg J, Tilak R, Singh S, et al. Evaluation of pan-dermatophyte nested PCR in diagnosis of onychomycosis. J Clin Microbiol. 2007;45 (10):3443–3445.

[65] Garg J, Tilak R, Garg A, et al. Rapid detection of dermatophytes from skin and hair. BMC Res Notes. 2009;2:60.

[66] Verrier J, Kra L, Bontems O. Dermatophyte identification in skin and hair samples using a simple and reliable nested polymerase chain reaction assay. Br J Dermatol. 2013;168(2):295–301.

[67] Petinataud D, Berger S, Contet-audonneau N. Molecular diagnosis of onychomycosis. J Mycol Med. 2014;24(4):287–295.

[68] Kondori N, Abrahamsson A-L, Ataollahy N, et al. Comparison of a new commercial test, Dermatophyte-PCR kit, with conventional methods for rapid detection and identification of Trichophyton rubrum in nail specimens. Med Mycol J. 2010;48 (7):1005–1008.

[69] Beifuss B, Bezold G, Gottlöber P, et al. Direct detection of five common dermatophyte species in clinical samples using a rapid and sensitive 24-h PCR–ELISA technique open to protocol transfer. Mycoses. 2009;54(2):137–145.

[70] Sue MJ, Yeap SK, Omar AR, et al. Application of PCR-ELISA in molecular diagnosis. Biomed Res Int. 2014;2014:653014.

[71] Santana AE, Taborda CP, Severo JS, et al. Development of enzyme immunoassays (ELISA and Western blot) for the serological diagnosis of dermatophytosis in symptomatic and asymptomatic cats. Med Mycol. 2017;56(1):95–102.

[72] Winter I, Uhrlaß S, Krüger C, et al. Molecular biological detection of dermatophytes in clinical samples when onychomycosis or tinea pedis is suspected. A prospective study comparing conventional dermatomycological diagnostics and polymerase chain reaction. Hautarzt. 2013;64(4):283–289.

[73] Savin C, Huck S, Rolland C, et al. Multicenter evaluation of a commercial PCR – enzyme-linked immunosorbent assay diagnostic kit (Onychodiag) for diagnosis of dermatophytic onychomycosis. J Clin Microbiol. 2007;45(4):1205–1210.

[74] Hayette M, Sacheli R. Dermatophytosis, trends in epidemiology and diagnostic approach. Curr Fungal Infect Rep. 2015;9(3):164–179.

[75] Mohammadi R, Abastabar M, Mirhendi H, et al. Use of restriction fragment length polymorphism to rapidly identify dermatophyte species related to dermatophytosis. Jundishapur J Microbiol. 2015;8(6): e17296.

[76] Begum J, Dev K, Lingaraju MC. Recent advances in the diagnosis of dermatophytosis. J Basic Microbiol. 2020;60(4):293–303.

[77] Machouart-dubach M, Lacroix C, de Chauvin MF, et al. Rapid discrimination among dermatophytes, Scytalidium spp ., and other fungi with a PCR-restriction fragment length polymorphism ribotyping method. J Clin Microbiol. 2001;39(2):685–690.

[78] Bontems O, Hauser PM, Monod M. Evaluation of a polymerase chain reaction-restriction fragment length polymorphism assay for dermatophyte and non-dermatophyte identification in onychomycosis. Br J Dermatol. 2009;161(4):791–796.

[79] Monod M, Bontems O, Zaugg C, et al. Fast and reliable PCR/sequencing/RFLP assay for identification of fungi in onychomycoses Printed in Great Britain. J Med Microbiol. 2006;55(Pt 9):1211–1216.

[80] Arabatzis M, Xylouri E, Frangiadaki I, et al. Rapid detection of Arthroderma vanbreuseghemii in rabbit skin specimens by PCR – RFLP. Vet Dermatol. 2006;17(5):322–326.

[81] Kamiya A, Kikuchi A, Tomita Y, et al. PCR and PCR — RFLP techniques targeting the DNA topoisomerase II gene for rapid clinical diagnosis of the etiologic agent of dermatophytosis. J Dermatol Sci. 2004;34(1):35–48.

[82] El Damaty H, Tartor Y, Mahmmod Y. Species identification, strain differentiation, and antifungal susceptibility of dermatophyte species isolated from clinically infected arabian horses. J Equine Vet Sci. 2017;59:26–33.

[83] Elavarashi E, Kindo AJ, Kalyani J. Optimization of PCR – RFLP directly from the skin and nails in cases of dermatophytosis, targeting the ITS and the 18S ribosomal DNA regions. J Clin Microbiol. 2013;7 (4):646–651.

[84] Verrier J, Pronina M, Peter C, et al. Identification of infectious agents in onychomycoses by PCR- terminal restriction fragment length polymorphism. J Clin Microbiol. 2012;50(3):553–561.

[85] Rasmussen HB. Restriction fragment length polymorphism analysis of PCR-amplified fragments (PCR-RFLP) and gel electrophoresis – valuable tool for genotyping and genetic fingerprinting. 2010

[86] Didehdar M, Khansarinejad B, Amirrajab N, et al. Development of a high-resolution melting analysis assay for rapid and high-throughput identification of clinically important dermatophyte species. Mycoses. 2016;59(7):442–449.

[87] Gharizadeh B, Norberg E, Loffler J, et al. Identification of medically important fungi by the PyrosequencingTM technology. Identifizierung medizinisch wichtiger Pilze mittels PyrosequencingTM-Technik. Mycoses. 2004;47 (1–2):29–33.

[88] Elavarashi E, Kalyani J, Sudha R, et al. Application of PCR fingerprinting using (GACA) 4 primer in the rapid discrimination of dermatophytes. Indian J Med Microbiol. 2014;32(3):236–239.

[89] Liu D, Coloe S, Baird R, et al. Application of PCR to the identification of dermatophyte fungi. J Med Microbiol. 2000;49(6):493–497.

[90] Bergmans AMC, Schouls LM, van der Ent M. Validation of PCR – reverse line blot, a method for rapid detection and identification of nine dermatophyte species in nail, skin and hair samples. Clin Microbiol Infect. 2008;14(8):778–788.

[91] Pounder JI, Williams S, Hansen D, et al. Repetitive-sequence-PCR-based DNA fingerprinting using the DiversiLab system for identification of commonly encountered dermatophytes. J Clin Microbiol. 2005;43 (5):2141–2147.

[92] Han HW, Hsu MM-L, Choi JS, *et al*. Rapid detection of dermatophytes and *Candida albicans* in onychomycosis specimens by an oligonucleotide array. BMC Infect Dis. 2014;14:581.

[93] Tartor YH, Abo ME, Shymaa H. Towards a rapid identification and a novel proteomic analysis for dermatophytes from human and animal dermatophytosis. Mycoses. 2019;62(12):1116–1126.

[94] Yo A, Yamamoto M, Nakayama T, *et al*. Detection and identification of *Trichophyton tonsurans* from clinical isolates and hairbrush samples by loop-mediated isothermal amplification system. J Dermatol. 2016;43(9):1037–1043.

[95] Shafiee S, Khosravi AR, Tamai IA. Comparative study of *Microsporum canis* isolates by DNA fingerprinting. Mycoses. 2014;57(8):507–512.

[96] Huang Y, Xu T, Wang W, *et al*. Lateral flow biosensors based on the use of micro- and nanomaterials: a review on recent developments. Microchim Acta. 2020;187 (1):70.

[97] Wu G, Zaman MH. Low-cost tools for diagnosing and monitoring HIV infection in low-resource settings. Bull World Health Organ. 2012;90(12):914–920.

[98] Koets M, Koets M, H Wichers J, *et al*. Nucleic acid lateral flow immunoassay for the detection of pathogenic bacteria from Food. Czech J Food Sci. 2009;27:350–353.

[99] Pecchia S, Da Lio D. Development of a rapid PCR-nucleic acid lateral flow immunoassay (PCR-NALFIA) based on rDNA IGS sequence analysis for the detection of *Macrophomina phaseolina* in soil. J Microbiol Methods. 2018;151:118–128.

[100] Babu BK, Saxena AK, Srivastava AK, *et al*. Identification and detection of Macrophomina phaseolina by using species specific oligonucleotide primers and probe. Mycologia. 2007;99(6):797–803.

[101] Ivanov AV, Safenkova IV, Zherdev AV, *et al*. Nucleic acid lateral flow assay with recombinase polymerase amplification: solutions for highly sensitive detection of RNA virus. Talanta. 2020;210:120616.

[102] Li Y, Li S, Wang J, *et al*. CRISPR/Cas Systems towards next-generation biosensing. Trends Biotechnol. 2019;37(7):730–743.

[103] Sashital DG. Pathogen detection in the CRISPR – cas era. Genome Med. 2018;10(1):32.

[104] Broughton JP, Deng X, Yu G, *et al*. CRISPR-Cas12-based detection of SARS-CoV-2. Nat Biotechnol. 2020;38(7):870–874.

[105] Brandsma E, Verhagen HJ, Van de Laar TJ, *et al*. Rapid, sensitive and specific SARS coronavirus-2 detection: a multi-center comparison between standard qRT-PCR and CRISPR based DETECTR. J Infect Dis. 2020;641.

[106] Kellner MJ, Koob JG, Gootenberg JS. SHERLOCK : nucleic acid detection with CRISPR nucleases. Nat Protoc. 2020;15(3):1311.

[107] Gronowski AM. Who or what is SHERLOCK? JIFFCC. 2018;29:201–204.

[108] Myhrvold C, Freije CA, Gootenberg JS, *et al*. Field-deployable viral diagnostics using CRISPR-Cas13. Science. 2018;360(6387):444–448.

[109] Gootenberg JS, Abudayyeh OO, Kellner MJ, *et al*. Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. Science. 2018;360(6387):439–444.

[110] Gootenberg JS, Abudayyeh OO, Lee JW, *et al*. Nucleic acid detection with CRISPR-Cas13a/C2c2. Science. 2017;356(6336):438–442.

[111] Sajid M, Kawde A-N, Daud M. Designs, formats and applications of lateral flow assay : A literature review. J Saudi Chem Soc. 2015;19(6):689–705.

[112] Tsunemi Y, Hiruma M. Clinical study of dermatophyte test strip, an immunochromatographic method, to detect tinea unguium dermatophytes. J Dermatol. 2016;43(12):1417–1423.

[113] Wakamoto H, Miyamoto M. Development of a new dermatophyte-detection device using immunochromatography. J Med Diagn Methods. 2016;5(2). DOI:10.4172/2168-9784.1000216.

[114] Higashi Y, Miyoshi H, Takeda K, *et al*. Evaluation of a newly-developed immunochromatography strip test for diagnosing dermatophytosis. Int J Dermatol. 2012;51(4):406–409.

[115] Burnham marusich A, Orne C, Kvam A, *et al*. Lateral flow immunoassay for rapid detection of dermatophytes in clinical specimens. ECCMID. 2018;2399.

[116] Tsunemi Y, Takehara K, Miura Y, *et al*. Screening for tinea unguium by dermatophyte test strip. Br J Dermatol. 2014;170(2):328–331.