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Original article

Optimising the diagnostic strategy for onychomycosis from sample collection to FUNGAL identification evaluation of a diagnostic kit for real-time PCR

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Summary

Onychomycosis is a common nail disorder mainly due to dermatophytes for which the conventional diagnosis requires direct microscopic observation and culture of a biological sample. Nevertheless, antifungal treatments are commonly prescribed without a mycological examination having been performed, partly because of the slow growth of dermatophytes. Therefore, molecular biology has been applied to this pathology, to support a quick and accurate distinction between onychomycosis and other nail damage. Commercial kits are now available from several companies for improving traditional microbiological diagnosis. In this paper, we present the first evaluation of the real-time PCR kit marketed by Bio Evolution for the diagnosis of dermatophytosis. Secondly, we compare the efficacy of the kit on optimal and non-optimal samples. This study was conducted on 180 nails samples, processed by conventional methods and retrospectively analysed using this kit. According to our results, this molecular kit has shown high specificity and sensitivity in detecting dermatophytes, regardless of sample quality. On the other hand, and as expected, optimal samples allowed the identification of a higher number of dermatophytes by conventional mycological diagnosis, compared to non-optimal samples. Finally, we have suggested several strategies for the practical use of such a kit in a medical laboratory for quick pathogen detection.

Key words: Onychomycosis, diagnosis, PCR, kit, direct examination, culture.

Introduction

Onychomycosis is a common nail pathology, most frequently caused by dermatophytes, a group of keratinophilic fungi made up of three genera: *Trichophyton*, *Microsporum* and *Epidermophyton*. To a lesser extent, moulds and yeast may also be responsible for nail disorders or isolated as contaminants or colonisers. The rate of onychomycosis is rising around the world, even if data in the literature is highly variable depending on the study, with prevalence in Europe of up to 22%.[1,2] Onychomycosis represents 50% of nail diseases, mostly with clinical aspects that may be confused with other onychopathies, such as psoriasis, lichen planus and trauma.[2] Therefore, a confirmation of the aetiology and a precise mycological identification is necessary in order to initiate the appropriate treatment, which may differ according to

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the fungus.[3] In 2007, the French Society of Dermatology recommended that samples from patients with suspected onychomycosis be referred to expert laboratories for analysis, before starting an antifungal treatment. Nevertheless, only a small percentage of practitioners (3.4% of generalists and 39.6% of dermatologists) use mycological diagnosis.[4,5] This data may be explained by several factors, such as the amount of time needed to obtain definitive mycological results and lack of culture sensitivity. This has contributed to clinicians losing confidence in mycological diagnosis.

In fact, the current gold standard for diagnosing onychomycosis is direct microscopic examination of biological samples and the results of their culture on fungal media. Direct examination should allow fungal elements to be observed quickly, whereas *in vitro* culture completes this information by providing the morphological identification of the fungus, at a genus and even species level. However, there are drawbacks to this traditional mycological diagnosis: whereas direct examination lacks specificity, culture is frequently associated with weak sensitivity and false negative results.[6] Moreover, growth of dermatophytes is often time consuming and requires up to 3–4 weeks and sometimes supplementary subcultures, in order to precisely identify the pathogen.[7]

In this context, molecular biology methods based on polymerase chain reaction (PCR) and targeting different genes have been gradually developed to complement microbiological diagnosis. Many molecular methods of PCR-RFLP,[8,9] PCR-Elisa,[10,11] nested PCR,[12] multiplex PCR[13] and real-time PCR[14] have been described as helping to diagnose dermatophytosis.[15] Recently, quick and reliable commercial kits have also been proposed for detecting onychomycosis. From DNA extraction to final diagnosis, these tools generally allow the enhancement of sensitivity and specificity and reduce diagnosis delay to a one day workflow. A technique developed by Brillowska-Dabrowska *et al.* includes a rapid two-step DNA extraction protocol and a duplex PCR, and has been adapted in a kit marketed by the Statens Serum Institute. This method combines a pan-dermatophyte PCR with a specific PCR for *T. rubrum*, the most commonly isolated dermatophyte in onychomycosis. This tool was evaluated by several authors. According to Kondori *et al.* the sensitivity, specificity, positive and negative predictive values are, respectively, 93%, 87%, 94% and 85%. Others such as Chandran *et al.* have reported results in the same range.[16–20] Recently, Mehlig *et al.* have reported the evaluation of a new multiplex-based PCR marketed under the name MycoDerm by Biotype Diagnostic. With this kit, 21 fungi belonging to the most relevant human pathogens causing dermatomycoses in Europe could be differentiated. Sensitivity, specificity, positive and negative predictive values of 87.3%, 94.3%, 87.3% and 94.3%, respectively, were calculated for dermatophytes.[21] Among the other kits available for detecting dermatophytes directly from biological samples, the company Fast Track Diagnostics has recently proposed a multiplex real-time PCR kit: FTD dermatophytes. Unfortunately, no study has been yet published about this kit. The PCR-Elisa marketed under the name 'Onychodiag' was further adapted to real-time PCR, by the same company, Bio Evolution (France), for the overall detection of dermatophytes, directly from samples.[10] This kit represents the only simplex real-time PCR for the general diagnosis of dermatophytosis.

This study is the first retrospective evaluation of Bio Evolution's real-time PCR kit, carried out on 180 nails, divided into optimal and non-optimal samples. After reporting our results, we will discuss the routine use of such a kit in the laboratory in order to optimise conventional diagnostic methods for onychomycosis.

Material and methodology

Clinical nail samples

The study was conducted in the Parasitology–Mycology laboratory at Nancy Hospital, in France. From November 2012 to May 2013, we collected 180 nail samples from 90 patients with suspected onychomycosis. The patients included in the study were referred to the laboratory by the Dermatology, Diabetology, Gastroenterology and Geriatrics Services at Nancy hospital or by private practitioners. All samples were collected by the same experienced mycologist. Each patient gave two samples: the first one was considered as non-optimal for conventional mycological diagnosis and consisted of clipping the free edge of the nail in the distal zone. The second sample was obtained from the infected nail bed as close as possible to the junction between diseased and healthy nail. Nail powder was also scraped from the underside of the nail bed.

Mycological diagnosis

Each sample was divided in three parts: (i) one part was examined microscopically in order to look for the presence of fungal elements. For this direct examination, observation using a chlorazole black stain was compared to Blankophor in order to determine which of these two techniques is the most sensitive for detecting hyphae, spores and yeast in the sample. When

using Blankophor, preparations were examined using a fluorescence microscope (Leica Type LB30T 020-519-500, Leica, Saint-Jorioz, France) with an excitation filter at 350–460 nm and a barrier filter at a wavelength of 420 nm. The whole slides were read at ×20 and ×40 objectives before being classified as negative or positive cases, regardless of the quantity of fungal elements; (ii) a second part of each sample was cultured on both a Sabouraud chloramphenicol agar slant and a Sabouraud chloramphenicol plus cycloheximide agar slant (Bio-Rad, Marnes-la-Coquette, France) for fungal identification. Culture tubes were incubated for up to 3–4 weeks at 27°C. Each culture was checked two times per week until there was optimal growth for fungal identification. The genus and species of fungi were identified by their macroscopic and microscopic appearance after lactophenol cotton blue staining; (iii) a third part of the sample was kept for molecular diagnosis.

Extraction of fungal DNA

Nail powder or nail pieces cut into small fragments with a surgical blade were used for DNA extraction. DNA was extracted and purified using the QIamp DNA mini kit® (Qiagen, Courtaboeuf, France) as recommended by the manufacturer with a slight modification: the incubation time of samples with proteinase K was extended from 45 to 90 min.

Polymerase chain reaction

PCR was performed using the real-time PCR kit marketed by Bio Evolution for dermatophytes diagnosis (reference: BE-A995). Briefly, the kit comprises all reagents necessary to perform 25 amplification tests, including a cellular control. For the PCR reaction, 5 µl of each DNA sample was mixed with 20 µl of the PCR ready mix provided in the kit and containing the primers and the cellular control. The reactions were performed using a StepOne Plus System (Applied Biosystem, Illkirch, France). The amplification programme consisted of an initial denaturation at 95°C for 30 s followed by 40 cycles of 5 s at 95°C and 40 s at 60°C and a final cooling step for 1 s at 37°C. The fluorescence was acquired during the hybridisation phase at 60°C on the two different channels.

Statistical analysis

Agreement between the three diagnostic methods was assessed using Cohen's Kappa coefficient. Homogeneity between the three diagnoses was tested using a marginal homogeneity test, which is a generalisation of the McNemar's test (Table 1).[22]

As mycological examination was an imperfect gold standard, a Latent Class Analysis (LCA) was used to estimate the sensitivity and specificity of each test and the prevalence of dermatophytes in the population under study.[23] LCA was performed using PROC LCA (https://methodology.psu.edu/downloads/proclcalta). In this study, a unique population of 90 samples and a unique latent class (the prevalence of dermatophytes) was considered. The LCA model took into account the results of the three tests: direct examination, culture and PCR. All statistical analyses were made using SAS 9.2 (SAS Institute, Cary Inc., North Carolina, USA). All hypotheses were tested using a two-tailed type I error rate of 5%.

Results

Clinical nail samples and participants

Of the 180 nail samples collected from 90 patients with suspected onychomycosis, 47.8% were from females (43) and 52.2% (47) from males. The mean age of the 90 patients in the study was 70.3 with a median of 69.5 and standard deviation of 16.6 years. The most common lesions observed were total damage of the nail in 42.2% (38) of cases, followed by distal lesions with onycholysis in 31.1% (28) of cases and distal lesions with hyperkeratosis in 24.4% (22) of the samples. No proximal nail affection was found. In 48.9% (44) of the samples, only one location was observed, whereas the most frequent associations concerned inter-toe and foot plant in 29% (26) of cases.

Direct examination results

The impact of sample quality on the direct examination method was compared for each patient by considering two factors: the sample type (optimal or non-optimal) and direct examination method (use

Table 1 Relation between the Kappa Index and the concordance level between diagnosis methods.

Concordance	Kappa Index
Excellent	≥0.80
Good	$0.60 \leq \kappa < 0.80$
Medium	$0.40 \leq \kappa < 0.60$
Mediocre	$0.20 \leq \kappa < 0.40$
Bad	$0 \leq \kappa < 0.20$
Execrable	<0

of chlorazole black or fluorescence). Overall, by using chlorazole black, 43 positive results were found on optimal samples, whereas 37 were detected on the non-optimal samples. With the use of fluorescence, six and four additional positive results were detected in optimal and non-optimal samples respectively (Table 2). For optimal samples, the Kappa index value is 0.8672, showing an excellent concordance between both methods. For non-optimal samples, the Kappa index value is 0.774 and reflects a good concordance between both direct examination methods.

According to these results, better quality samples along with the use of fluorescence tends to improve the number of positive direct examinations compared to chlorazole black and non-optimal samples. Nevertheless, due to the small difference between the numbers of positive samples in each condition, this conclusion needs to be treated with caution.

Culture results

Of the 180 overall samples, 55.6% (50) and 58.9% (53) of the cultures came from non-optimal and optimal samples respectively, and were positive, regardless of the fungal identification. From these cultures, six additional dermatophyte strains were isolated in the optimal samples compared to the non-optimal samples. In the non-optimal samples, seven additional non-dermatophytes fungi were found (five yeast and two moulds). Three samples remain negative in culture for non-optimal samples, whereas the corresponding cultures were positive in the optimal samples (data not shown). According to these results, better sample quality statistically correlates to obtaining dermatophytes in culture (marginal homogeneity test, $P = 0.0277$, risk $\alpha = 0.05$).

Table 2 Results of the direct examination on the 90 non-optimal or optimal samples by using either chlorazole black or fluorescence.

	Fluorescence		
Chlorazole black	–	+	Total
Optimal samples			
–	41	6	47
+	0	43	43
Total	41	49	90
Non-optimal samples			
–	46	7	53
+	3	34	37
Total	49	41	90

On optimal and non-optimal samples, all fungi included, 39.6% (21/53) and 30% (15/50) of dermatophytes were obtained by culture, respectively, corresponding to 23.3% (21/90) and 16.6% (15/90) of the dermatophytes isolated for each sample category (Table 3). Among dermatophytes, *Trichophyton rubrum* was found in 76% (16/21 cultures) or 66.6% (10/15 of these cultures) and *T. mentagrophytes* in 14% (3/21) or 20% (3/15), of optimal and non-optimal samples respectively. One *Epidermophyton floccosum* and one *Trichophyton terrestre*, both for non-optimal and optimal samples were also obtained (data not shown). The six additional dermatophytes detected in the optimal samples correspond to six *T. rubrum* strains.

PCR results

When comparing the number of dermatophytes found by culture and the molecular method, a larger number of dermatophytes was detected with this molecular kit: only 23.3% (21/90) and 16.7% (15/90) respectively of the optimal and non-optimal samples, obtained from the same patients, were found positive in culture, whereas the PCR resulted in 34.4% (31/90) of positive cases whatever the sample quality (Table 3). In fact, according to these results, PCR overcomes sample quality and this is currently one of the key elements in proper mycological diagnosis.

In Table 4, by only considering the presence of hyphae from direct examination, PCR enabled the detection of one additional positive case in the optimal samples and two positive cases in the non-optimal ones, whereas direct examinations and cultures were negative. In nine and 14 optimal and non-optimal samples, for which direct examination was positive

Table 3 Overall results of direct examination, cultures and PCR both on optimal and non-optimal samples (all fungal elements considered at direct examination).

Culture	PCR	DE	Number	Percentage (%)
Optimal samples				
–	–	–	32	35.6
–	–	+	27	30
–	+	+	10	11.1
+	+	+	21	23.3
Total			90	100
Non-optimal samples				
–	–	–	38	42.2
–	–	+	21	23.3
–	+	+	16	17.8
+	+	+	15	16.6
Total			90	100

Table 4 Results of direct examination, cultures and PCR both on optimal and non-optimal samples, by considering the only presence of hyphae at direct examination.

Culture	PCR	DE (hyphae)	Number	Frequency (%)
Optimal samples				
–	–	–	40	44.4
–	–	+	19	21.1
–	+	–	1	1.1
–	+	+	9	10
+	+	+	21	23.3
Non-optimal samples				
–	–	–	44	48.9
–	–	+	15	16.7
–	+	–	2	2.2
–	+	+	14	15.6
+	+	+	15	16.7

while the culture was found negative, additional dermatophytes were detected by PCR. Overall, when sample cultures were positive for dermatophytes, direct examinations and PCR correlate and were also positive. Although culture results tend to affected by the sample quality, PCR was not affected by this factor: 31 samples were found positive by PCR with or without positive direct examinations and cultures, regardless of the quality of the samples. This result supports the kit's good efficacy and sensitivity.

Regarding the 19 and 15 optimal and non-optimal samples that were positive for direct examinations and negative with PCR, the cultures were either negative or resulted in moulds. No dermatophyte was identified within these samples by panfungal PCR and sequencing. On the contrary, in four and five optimal and non-optimal samples, for which moulds were identified by cultures, PCR detected dermatophytes, meaning that even when moulds are present in samples, the kit is able to specifically detect dermatophytes. This data support PCR's good specificity.

Sensitivity and specificity of the methodology

In this study, by choosing to observe every fungal element as a criterion of positivity for direct examination, a sensitivity of 100% is obtained for direct examination and a specificity of 100% is found for culture. PCR also reaches 100% sensitivity and specificity. In this model, the specificity of the culture is enhanced by the sample quality. However, by selecting the only observation of filamentous hyphae as a criterion of positivity for direct examination, even if 100% sensitivity and specificity rates are still obtained for direct examination and culture, the specificity of the PCR, although remaining high, is slightly reduced at 95.6%. Note that the sensitivity of the culture is enhanced in both cases, in higher quality samples (Table 5).

Discussion

This study was conducted on 180 samples divided into optimal and non-optimal nail fragments and aimed at evaluating the real-time PCR kit marketed by Bio Evolution for onychomycosis diagnosis. The quality of samples, and direct examination method were also tested, although it is already well known that better quality sampling influences the mycological results and that fluorescence may favour the observation of fungal elements.[24]

Regarding this first evaluation of the Bio Evolution real-time PCR kit, an accurate and specific diagnosis of onychomycosis due to dermatophytes can be quickly reached. This molecular tool demonstrated a good specificity and can also be used even if other non-dermatophytic fungi such as moulds are present in the samples.

The gold standard for onychomycosis diagnosis still relies on direct observation of optimal samples followed by a culture that may need up to 3–4 weeks before it can be interpreted. This biological diagnosis of onychomycosis is based on good mycological knowledge and requires experienced investigators.[15]

Direct observation of biological specimens is often sufficient to quickly determine the presence of fungal elements, but it does not enable the distinction between dermatophytes and moulds.[7] Authors have already reported that 15%–50% of the positive samples in microscopy remain negative in culture, showing higher sensitivity of direct observation compared to culture.[15] Moreover, although it is quick and economic, it gives false-negative results in 5%–15% of

Table 5 Sensitivity and specificity of the tests by considering either only hyphae at the direct examination or all fungal elements, on non-optimal and optimal samples.

Samples	Non-optimal (%)		Optimal (%)	
Test	Sensitivity	Specificity	Sensitivity	Specificity
By considering hyphae and yeast at direct examination				
DE	100	64.4	100	54.2
Culture	48.4	100	67.7	100
PCR	100	100	100	100
By considering only hyphae at direct examination				
DE	100	74.6	100	67.8
Culture	53	100	71.1	100
PCR	100	95.6	100	97.8

cases.[25] In this study, we compared microscopic results by using two different methods on optimal and non-optimal samples. Optimal samples allow the detection of a higher number of positive direct examinations compared to non-optimal samples, due to the quality of sampling. Nevertheless, using either chlorazole black or fluorescence, results of direct examinations were unchanged regardless of sample quality. The only advantage of fluorescence, in our study, is that it is easier to read the microscopic examination than with chlorazole black. Even if our results are consistent with those of others, differences in performance are more apparent in other studies. By comparing different microscopic techniques; KOH, chlorazole black and acridine orange, Panasiti *et al.*[26] concluded that the latter two were better than KOH at detecting fungal elements. According to Abdelrahman *et al.*, use of fluorescence such as calcofluor enhances the sensitivity and specificity of direct examination by 22% and 6% respectively. With this marker, a higher quantity of fungal elements could be more easily observed than with KOH.[27] These results are along the same lines as those of Haldane *et al.*[28] and Hamer *et al.*[29]

While direct examination is the most sensitive step in mycological diagnosis, in terms of specificity, culture remains the reference to which the other techniques are generally compared, even if it may be linked to false-negative results. The main drawback of culture remains the slow growth of dermatophytes, delaying the results by up to 6 weeks in the event of supplementary tests to enhance frequent weak sporulation.[15] Mycological culture is highly specific, but negative results associated with a positive microscopic direct examination are found in up to 40% of cases, impeding its sensitivity.[20] In our study, the different LCA models resulted in a 100% specificity for culture.

Negative cultures may be the consequence of a previous antifungal treatment or may be due to already dead dermatophytes present in the distal part of the nail. False-positive results may be due to contaminant nail fungi that have no role in the pathology.

In our study, by comparing the results of PCR and culture, we have highlighted the concomitant presence of moulds and dermatophytes in several samples. Moulds may also represent aetiological agents for onychomycosis, even if their prevalence remains uncertain, due to the difficulty of proving their role in onychopathic pathogenesis. During the last decade, a growing prevalence of non-dermatophytes such as *Fusarium* spp. has been observed in onychomycosis.[30] Like other moulds such as *Scytalidium* sp., *Fusarium* spp. are relatively insensitive to treatments, justifying an accurate diagnosis of pathogens in order to find the best treatment. The Bio Evolution PCR showed a good specificity in these cases in our study and allowed the detection of dermatophytes within samples whose growth had been masked by the presence of a mould. Therefore, use of a specific PCR for dermatophytes, combined with conventional mycological diagnosis, could offer a quick method for specifying the aetiological agents of onychomycosis.

In this study, PCR and conventional mycological diagnosis were both performed on optimal and non-optimal samples. Only a few studies have evaluated the impact of sample quality on mycological results. Our results are along the same lines as those of Menotti *et al.* and Savin *et al.*: these results showed that PCR may offer a reliable diagnosis even if the quality of samples is non-optimal, for example, when it was not collected by an experienced mycologist or a dermatologist. The Onychodiag kit evaluated in the study by Savin *et al.* also enabled the sample quality to be overcome and enhanced sensitivity with regard to standard diagnosis.[10] It is now well known that a sample from the proximal zone of the nail reduces the failure rate of culture compared to a sample taken in a more distal zone of the nail. In this context, one of the advantages of PCR is the need for a small quantity of DNA enabling samples to be handled that could not be treated by conventional diagnosis, regardless of the sampling zone.

In contrast to our study, some authors have reported false-negative results with PCR methods.[31] This may be due to the DNA extraction step, that may be non-optimal, possibly carrying PCR inhibitors. Therefore, according to studies, it is increasingly common to use an internal control that highlights up to 5% of inhibitors.[32] The inhibitors are then removed by a 50% dilution of the DNA used for the PCR. Divergences between PCR and culture results may also be related to the aliquots, which may not contain any further viable fungal elements for the culture, whereas PCR may detect DNA contained in dead fungal elements.[33]

The important variability of dermatophytosis prevalence in studies is highly dependent on the tools and also on the included patients, that may be made up of a hospital population or generalist-based population or that may come from dermatologists. This makes comparison between studies difficult. In this study, most of the samples came from patients from geriatrics, which explains the high mean age (70.2 years) of the studied population; these patients have a risk factor related to their age for the development of mycoses.

Nevertheless, by considering the positive direct examinations associated with positive culture for moulds, the rate of negative culture for dermatophytes corresponds to 52% (27/52) and 43% (25/58), respectively, in accordance with results from literature.[3,18,19]

Studies involving molecular tools have compared molecular results with culture and direct examination. Nevertheless, due to possible false-negative results in culture and in direct examination, their use as reference includes a bias, so the real nature of the infection is not accounted for. In the absence of a reference standard (the gold standard is being questioned because it is not reliable), the LCA statistical method was chosen here to assess the accuracy of the results. LCA calculates the results of the tests based on an informatics model by taking into account the PCR, culture, microscopy and calculating the prevalence of dermatophytes in the studied population. Excepting Bergman *et al.*, few authors have already used the LCA method.[34] Thus, it is difficult to compare the sensitivity and specificity of the PCR between studies because authors often calculate them according to direct examination or culture.

The higher cost of PCR compared to conventional mycological methods is currently a limiting factor that could in the future evolve in accordance with lower market prices of reagents. The higher cost of molecular analyses should also be reported along with a possible reduction of conventional diagnostics tests and, above all, the savings generated by using a better targeted therapeutic strategy, avoiding the high cost of antifungals.

This technique could also be used in patients who have already received an antifungal treatment that could inhibit the fungal culture. In addition, this most sensitive test could also be useful for an early and quick detection of relapse in patients where the nail appears clinically cured, although there is a residual presence of undetected dermatophytes by standard methods. In these cases, PCR could offer accurate monitoring of infection, leading to the most appropriate treatment.

Finally, an important question is to know how laboratories equipped with molecular tools should include them in their diagnostic strategy for onychomycosis: PCR completes the current diagnosis and helps to fill the gaps. It is necessary to define a balance between the cost of diagnosis, the speed of response, the certainty of results transmitted to clinicians, the importance of epidemiology, and laboratory work organisation. Molecular kits like the Bio Evolution one should thus be integrated into a diagnostic strategy combining the strengths of each diagnosis tool, i.e.: speed of direct examination, epidemiological specificity of culture and sensitivity of the molecular tool.

We propose three main strategies: (i) in the first category, PCR would be performed directly after collecting the samples, in parallel with conventional mycological diagnosis. PCR in first-line testing enables a quick and reliable diagnosis for clinicians, however, this scheme involves a high cost; (ii) in the second strategy, PCR could be performed only on samples that were positive in direct examination, even those showing hyphae in direct examination (PCR could thus be dedicated to rapidly distinguishing moulds and dermatophytes). This involves having the best direct examination possible to enable a more effective screening technique. Positive results could thus be known within 24 h and negative samples from direct examination could be then cultured in case of false-negative results at direct examination and to ensure the detection and identification of all pathogens, yeast included; (iii) in the third strategy: the PCR would be performed in second-line testing, i.e. after about a week of culture, only on samples resulting in negative cultures. However, one of the major advantages of PCR would be partly lost: its speed.

As a conclusion, from our point of view, obtaining a fungal culture remains essential in terms of epidemiology, for example when tracing the infection's origins. Therefore, molecular tools that clearly present advantages should not fully replace conventional diagnosis, but should be used alongside mycological tools. The kit proposed by Bio Evolution PCR does not offer the possibility of distinguishing the species within dermatophytes, but this limitation, if it is important for epidemiology, has no effect on the treatment decision because dermatophytes have almost similar sensitivities to antifungal drugs.[35] Despite its drawbacks, this PCR is a promising tool and may advantageously complete the mycological diagnosis in patients with suspected onychomycosis.

Some clinicians appear to lack confidence in mycological diagnosis and prescribe a treatment without a biological confirmation of the diagnosis.[36] Molecular methods such as this kit offer an opportunity to confront these biases: (i) reporting time of results is reduced compared to culture. In fact, PCR results can be generally obtained in a one day workflow; (ii) the high sensitivity of PCR could balance the disadvantages of mycological diagnosis such as false-negative cultures; (iii) suitably used, PCR could also minimise charges implemented by supplementary medical visits and additional analyses.

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Competing interests

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