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Screening for tinea unguium by Dermatophyte Test Strip*

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Summary

Background The direct microscopy, fungal culture and histopathology that are necessary for the definitive diagnosis of tinea unguium are disadvantageous in that detection sensitivity is affected by the level of skill of the person who performs the testing, and the procedures take a long time.
Objectives The Dermatophyte Test Strip, which was developed recently, can simply and easily detect filamentous fungi in samples in a short time, and there are expectations for its use as a method for tinea unguium screening. With this in mind, we examined the detection capacity of the Dermatophyte Test Strip for tinea unguium.
Methods The presence or absence of fungal elements was judged by direct microscopy and Dermatophyte Test Strip in 165 nail samples obtained from residents in nursing homes for the elderly. Moreover, the minimum sample amount required for positive determination was estimated using 32 samples that showed positive results by Dermatophyte Test Strip.
Results The Dermatophyte Test Strip showed 98% sensitivity, 78% specificity, 84·8% positive predictive value, 97% negative predictive value and a positive and negative concordance rate of 89·1%. The minimum sample amount required for positive determination was 0·002–0·722 mg.
Conclusions The Dermatophyte Test Strip showed very high sensitivity and negative predictive value, and was considered a potentially useful method for tinea unguium screening. Positive determination was considered to be possible with a sample amount of about 1 mg.

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Conflicts of interest
The Dermatophyte Test Strip used for screening in this survey was provided for free by JNC Corporation.

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What's already known about this topic?

- Tinea should be diagnosed based on mycological examinations.
- Direct microscopy, culture and histopathology are employed to detect fungal elements.
- These methods require technical skill and/or consume time.

What does this study add?

- The Dermatophyte Test Strip visualizes mycotic antigens by immunochromatography.
- It allows easier and faster detection of fungi in samples, with very high sensitivity.
- It is a useful method for screening of tinea unguium.

Tinea unguium is estimated to occur in at least 10% of the population in advanced countries,[1,2] and is increasing along with ageing in all countries. There are other diseases similar to tinea unguium,[3] but discrimination is not easy. Thus, definitive diagnosis by mycological examinations (direct microscopy, fungal culture and histopathology) is necessary in order to start the treatment of tinea unguium. However, there are disadvantages in direct microscopy, such as detection sensitivity being affected by the level of skill of the person who performs the test. Fungal culture takes 2–3 weeks

before pathogenic fungi can be identified, in addition to having a low identification rate. Moreover, histopathology is also inferior in terms of rapidity. The recently developed Dermatophyte Test Strip (Fig. 1) visualizes mycotic antigens extracted from samples such as nails by immunochromatography, enabling rapid and easy discrimination, and thus may be effective in the screening of tinea unguium. There have been few preliminary reports of detecting dermatomycoses with the Dermatophyte Test Strip.[4–6] In this study, we investigated the detection capacity of the Dermatophyte Test Strip for tinea unguium using more nail samples, and we estimated the minimum amount of nail sample required for positive determination.

Materials and methods

Dermatophyte screening using nail samples

Screening subjects were nail samples collected from cases among residents in three nursing homes for the elderly in Tokyo, Japan. The Dermatophyte Test Strip was provided by JNC Corporation (Tokyo, Japan). This test strip is prepared as follows and as shown in Figure 1. BALB/c mice were immunized with the fungus components of *Trichophyton rubrum* to prepare a monoclonal antibody.[7] This antibody was found to react specifically to seven dermatophytes (*T. rubrum*, *T. mentagrophytes*, *T. violaceum*, *T. tonsurans*, *Microsporum gypseum*, *M. canis* and *Epidermophyton floccosum*).[7,8] A Dermatophyte Test Strip visualizes the complex of colloidal gold-labelled antibody and antigen by immunochromatography.

The presence or absence of dermatophyte in each nail sample was examined by direct microscopy and by a Dermatophyte Test Strip. A nail sample was cut into pieces, added to extraction solution[9] in a test tube, mixed by grinding with a plastic stick, and left to rest for 15 min. The test strip was then added, and a positive judgement was made after 5 min if a brown line was visible on the test strip.

Estimation of the minimum sample amount required for positive determination with a Dermatophyte Test Strip

The nail samples were homogenized in extraction solution and the supernatant was diluted twofold repeatedly. The test strip was put into the diluted solutions, and absorbance at the determination line was measured with a test strip reader (Nippon TechnoCluster Inc., Tokyo, Japan). A linear regression equation was calculated from the absorbance and dilution rates, and the maximum dilution rate was obtained at the limit of the absorbance value for visually recognizing the brown colour at the determination line. The minimum sample amount required for positive determination with a Dermatophyte Test Strip was calculated from the maximum dilution rate and the initial amount of the nail plate homogenized.

Data aggregation and analysis

The results were analysed by calculating the sensitivity, specificity, positive predictive value, negative predictive value and the positive and negative concordance rate of the Dermatophyte Test Strip, using microscopy as the standard.

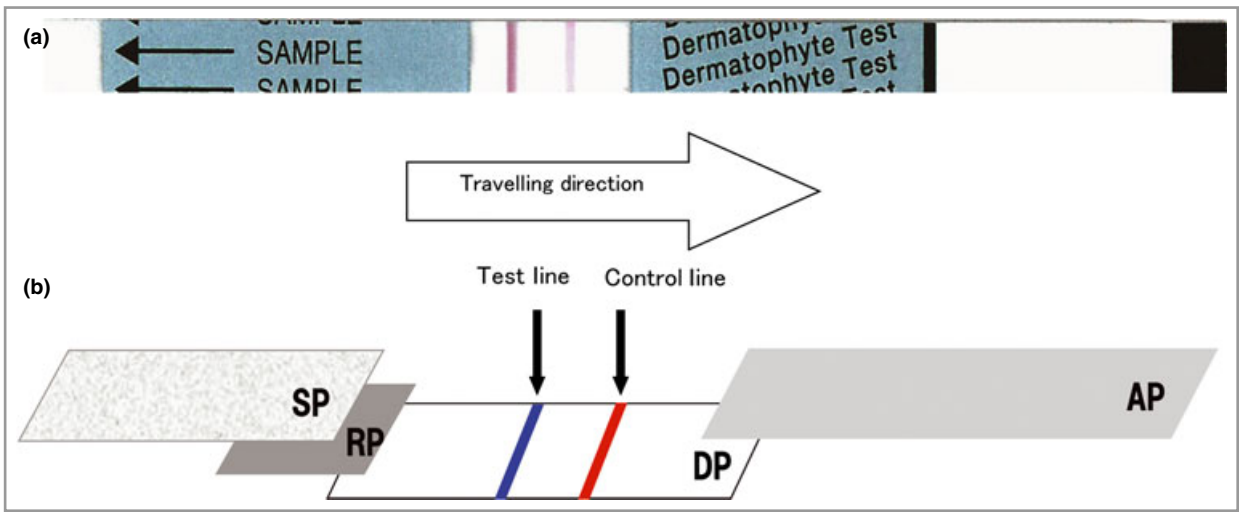

Fig 1. Dermatophyte Test Strip. (a) A representative strip with a positive test line and control line. (b) Schematic diagram of the strip. A monoclonal antibody (mAb) that specifically reacts with the polysaccharide that is present in the cell wall of dermatophytes is immobilized linearly on the test line portion of the test strip. The reagent, which turns red with humidity, is immobilized linearly on the control line portion. The polysaccharide is extracted with the extraction solvent and migrates with the colloidal gold-labelled mAb in the test strip. It is then absorbed by the immobilized mAb and produces a brown line that can be observed visually. This method is generally called immunochromatography, and the presence or absence of dermatophyte can be judged qualitatively. SP, sample pad (extracted samples are absorbed on this pad); DP, detection pad (test line and control line are located on this nitrocellulose membrane); RP, reagent pad (this pad includes colloidal gold-labelled mAb); AP, absorption pad (excess liquid is absorbed on this pad).

Ethics

All residents gave consent to participate in this study. This study was carried out in compliance with the Helsinki Declaration and the Ethical Guidelines for Epidemiological Research (the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labour and Welfare, Japan) and was approved by the institutional review board of the participating institutions.

Results

The detection rate of tinea unguium was examined in 165 nail samples (Table 1). Of 91 samples that were positive using direct microscopy, 89 were also positive using the Dermatophyte Test Strip, giving a sensitivity of 98%. Additionally, of 74 samples that were negative using direct microscopy, 58 were negative using the Dermatophyte Test Strip, giving a specificity of 78%. The positive predictive value was 84·8% (89/105) and the negative predictive value was 97% (58/60). The positive and negative concordance rate was 89·1% (147/165).

The calculated minimum sample amount required for positive determination with the Dermatophyte Test Strip was 0·002–0·722 mg, differing widely among samples.

Discussion

In an evaluation of onychomycosis, sensitivity was reportedly 80%, 52% and 92% in direct microscopy, fungal culture and histopathology with periodic acid–Schiff (PAS) staining, respectively.[10] The Dermatophyte Test Strip is expected to be used as a method to supplement direct microscopy. In this survey, it showed sensitivity as high as 98%, which is comparable with the positive rate for histopathology.[10,11] Moreover, the Dermatophyte Test Strip will likely become a screening method for tinea unguium because it has a high negative predictive value and can also significantly reduce the time needed for testing. When the Dermatophyte Test Strip result is negative, the case can be considered not to be tinea unguium. On the other hand, when the Dermatophyte Test Strip result is positive, definitive diagnosis should be performed by direct microscopy because the positive predictive value is not sufficiently high. In this way, laboratory labour can be saved and diagnostic efficiency can be enhanced compared with performing direct microscopy in all cases.

Because the Dermatophyte Test Strip is provided as a ready-to-use kit, fast and easy testing is possible without the need for dedicated instruments. It can be used in the private practice of dermatologists, as well as by family doctors deciding whether to refer patients to a dermatologist. It can also be used in hospitals for testing in laboratories. Outsourcing of PAS staining in Japan costs about 12 Euros, which is quite expensive and, moreover, it is not possible to diagnose disease rapidly in front of a patient. On the other hand, the Dermatophyte Test Strip currently costs 7·8 Euros and is considered reasonable in terms of cost.

The calculated minimum amount of nail sample required for positive determination was 0·722 mg at the highest; therefore, judgement by Dermatophyte Test Strip is considered possible if a sample of about 1 mg (approximately corresponding to a cube of 1 mm in edge length) can be collected.

In order to reduce the number of cases receiving unnecessary treatment and those not receiving necessary treatment, it is hoped that a Dermatophyte Test Strip method that enables sensitive, simple and easy tinea unguium screening in a short time will be studied further and will be utilized in actual clinical situations.

Table 1 Comparison of mycological evaluation between the Dermatophyte Test Strip and direct microscopy

Dermatophyte Test Strip	Direct microscopy		
	+	−	Total
+	89	16	105
−	2	58	60
Total	91	74	165

Sensitivity 98% (89/91), specificity 78% (58/74), positive predictive value 84·8% (89/105), negative predictive value 97% (58/60), concordance rate 89·1% (147/165).

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