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A new dermatophytosis lab on chip immunochromatography-based diagnostic era

Hassan Aboul-Ella[1*], Rafik Soliman[1], Rafik Hamed[2], Heidy Abo-Elyazeed[1]

[1]Department of Microbiology, Faculty of Veterinary Medicine, Cairo University
Hasanabo@cu.edu.eg, Rafiksoliman108@hotmail.com, Heidyshawky86@hotmail.com
[2] Department of Microbiology, Institute for evaluation of veterinary biologics (CLEVB), Agricultural Research Center (ARC), Abbasia, Cairo, Egypt.
Rafikhamed1010@hotmail.com

[1]Department of Microbiology, Faculty of Veterinary Medicine, Cairo University
Hasanabo@cu.edu.eg, Rafiksoliman108@hotmail.com,
Heidyshawky86@hotmail.com
[2] Department of Microbiology, Institute for evaluation of veterinary biologics (CLEVB), Agricultural Research Center (ARC), Abbasia, Cairo, Egypt.
Rafikhamed1010@hotmail.com

KEYWORDS

Dermatophytosis, lateral flow, lateral immunochromatographic assay, rapid diagnostics, lab on a chip, field test, assured test, bedside test.

SHORT SUMMARY

The development, standardization, and evaluation of a new point-of-care (POC) test for the rapid identification of dermatophytosis cases were covered in the current study. This test was based on the specific interactions between dermatophyte antigens and their particular antibodies. Finding the dermatophyte antigens in scrap samples of hair, nails, and skin from animals with dermatological problems might help make a diagnosis more accurate than using the time-consuming, expensive, and widely used direct wet-prepared slides. With various testing techniques and clinical findings, the newly developed kit may be able to offer clinicians a preliminary and competitive test result that will help them make the right diagnosis and start treating impacted people. In addition, the application of the new lateral flow immunochromatographic assays (LFIAs) is quicker and simpler than that of existing techniques, such as direct wet mounts, isolation techniques, and molecular techniques, particularly in the majority of medical facilities, remote rural areas, and low-income nations or regions. The need for an accurate, quick, specific, and sensitive diagnosis of dermatophytosis grows as a result of the pervasive nature of dermatophyte diseases. The current serological LFIAs are intended to be a more effective and affordable method of treating dermatophytes' affections in this case by requiring only three easy procedures and five to seven minutes from the time the sample is collected to the reading of the results. It should be clearly explained that after evaluating 100 clinical samples, the newly developed kit did not exhibit any real limits of detection (LoD) relating to the type and nature of the sample that was collected, the clinical stage of the diseases, or other factors. To fully cover the LoD-related topic, however, more in-depth sample testing will be needed in the future.

EXTENDED ABSTRACT

Motivation: Dermatophytosis is a widely spread contagious zoonotic disease, affecting both man (tinea) and animals (ringworm). This disease is caused by a group of closely related keratinophilic fungi known collectively as the dermatophytes group. Although the wide distribution of dermatophytosis cases throughout the whole world and its adverse clinical effect on human health, economical effect on productive animals, and pet animal welfare, there is no rapid accurate diagnostic tool for such disease. Up to date, dermatophytosis diagnosis is limited to direct, and rapid with low sensitivity and specificity techniques. Several sophisticated wet mount techniques, using highly skilled personnel, require molecular assays and gold standards with long-time consuming conventional culturing and biochemical testing. All of the previously

mentioned existing diagnostic methods are lab-confined techniques with no availability of field, handheld, or bedside tests. The different aspects of difficulties facing dermatophytosis diagnosis lead to mistaken diagnosis and faulty prescription of antifungals with their expected long list of side effects on man and animals. The current conducted study tries to accomplish the difficult equation by achieving an accurate, sensitive, specific, user-friendly, rapid, robust, device-less, deliverable to end-users, and economic cost for the development and production of diagnostic kits.

Methodology: through the current study the methodological procedures have been organized into three different stages, as follows:

Pre-kit development stage (preliminary stage): Designing a suitable immunization protocol for the White New-Zealand rabbits used. It was accomplished based on prior literature, the nature of the used antigens, the formulated immunization emulsions, and the regular antibodies title. These procedures included the isolation, selection, and preparation of species-specific dermatophytes antigens [1-3], the preparation of the two different immunization emulsions for each dermatophytes species-specific antigen. Finally, an effective species-specific polyclonal antibody separation and purification method based on caprylic acid was employed [7] and assessed using an agar gel precipitation test [8].

Kit development stage (developmental and standardization stage): The previously synthesized polyclonal antibodies and the colloid gold nanoparticles were accomplished [9–11]. Additionally, using the nitrocellulose membrane, the sample application pad, the conjugate pad, the adsorbent pad, and the carrier polyvinyl card (PVC) in a proper manner of layering and orienting those layers, the loading of various bio-reactants as well as the overall layering and fabrication of the lateral flow solid phase (multilayered sheet) were carried out [15, 16]. Furthermore, a formula for an extraction solution that was successful in experiments was created and put to the test for applicability and repeatability [17–21].

Post-kit development stage (evaluation stage): At this step, the new kit was really tested against two major, well-established diagnostic procedures, one of which is mycological culturing, the gold standard procedure in the field of diagnostic mycology. With actual hands-on experience and the establishment of the kit application processes, the true competitive and replacement potential of the new kit was considerably clarified and shown [22, 23].

Results:

After using this kit to examine 100 animal samples, the results were shown in Tables 1, 2, and 3 as coefficients of the kit and the culture and the kit and the direct microscopy, respectively, were 0.44 and 0.76. Because of this, the newly designated and developed kit, which is based on lateral flow immune-chromatographic assay, demonstrated a very promising competitive diagnostic result within 5-7 minutes through three simple to follow steps and through an easily interpreted result that is described as one ruby red spot means a negative result, while two ruby red spot means a positive result. Figure 1.

Formulae

The specificity and sensitivity of the newly developed kit were calculated as follows;

Specificity= T-/ ((T-) + (F+)) x 100%

Sensitivity= T+/ ((T+) + (F-)) x 100%

The LFIAs gave a specificity of 98% and a sensitivity of 76.9%. The Cohen's kappa statistic was calculated to measure the level of agreement between the newly developed kit and the standard culture as follows;

Cohen's kappa coefficient (k) = (po – pe) / (1 – pe), where (po) is the relative observed agreement among raters and (pe) is the hypothetical probability of chance agreement

Tables and Figures

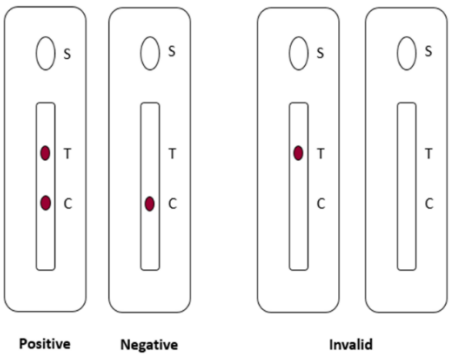

Figure 1: Illustration of the valid (positive/negative) and the invalid visible outcome results

Table (1): comparing LFIA and microscopic slide results

		Direct microscopic examination (DME)	
	Results	Positive	Negative
LFIA	Positive	**6**	**9**
	Negative	**3**	**82**

Table (2): comparing culture and LFIA results

		LFIA	
	Results	Positive	Negative
Culture	Positive	**14**	**6**

Table (3): comparing microscopic slide and culture results

		Culture	
	Results	Positive	Negative
DME	Positive	**5**	**4**
	Negative	**15**	**76**

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