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Improvement of direct microscopy method for differential diagnosis of skin mycoses

Gulshat E. Bailina[1], Aziza E. Nesipbaeva[2], Elena V. Kukhar[3]

[1]Department of Veterinary medicine, Faculty of Veterinary and Livestock Technology, S.Seifullin Kazakh Agrotechnical Research University, Astana, Kazakhstan
[2]Department of Microbiology and Biotechnology, Faculty of Veterinary and Livestock Technology, S.Seifullin Kazakh Agrotechnical Research University, Astana, Kazakhstan
[3]Research platform of agricultural biotechnology, S. Seifullin Kazakh Agrotechnical Research University, Astana, Kazakhstan

Corresponding author: Gulshat E. Bailina: gulshat_010@mail.ru
Co-authors: (1: AN) nessipbayeva2510@mail.ru; (2: EK) kucharev@mail.ru
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Abstract

Background and Aim.In the practice of veterinary doctors, a number of methods for diagnosing dermatomycoses are used, methods of direct and luminescent microscopy are undeservedly ignored. Comparative analysis of staining methods for improving the method of direct microscopy, rapid and reliable detection of spores and mycelium in biomaterial, identification of pathogens and differential diagnosis of skin mycoses is of crucial importance for the effective treatment of animals

Materials and Methods. 47 clinical samples of wool and skin taken from farm animals were examined for dermatomycetes. Diagnosis was performed using direct microscopy, fluorescence microscopy with calcofluor, and culture medium culture Saburo.

Results. During the study of 47 samples of biological material, it was found that during microscopy the pathogen was detected in 10 cases (21.3%), during KOH microscopy - in 15 cases (29.9%), during KOH microscopy with other dyes in 11 cases (23.4%), during KOH microscopy with color calcofluor in 25 cases (53.2%). The efficiency of the KOH microscopy method with white calcofluor in comparison with direct microscopy was 60% higher, in comparison with KOH microscopy with other dyes - by 56%, classical KOH microscopy by 43.8%.

By the culture method, pathogens were detected in 28 cases (59.6%), of which 7 *Trichophyton spp.,* 1 *Microsporum spp.,* 14 *Aspergillius spp.,* 6 yeast. Growth was absent in 19 samples (40.4%), bacteria were detected in 3 samples (6.3%). In comparison with KOH microscopy with calcofluor white, positive results were confirmed in 89.3%.

Conclusion. The use of coloring with various dyes of morphological elements of micromycetes in pure cultures allows you to identify mycelial hyphae of various thicknesses, spores, conidia, other morphological structures and carry out a quick preliminary identification of dermatomycetes and opportunistic mold fungi in smears.

Keywords: dermatomycete; dye; macroconidia; microscopy; mycelium; spore.

Introduction

Early diagnosis of infections caused by classic dermatophytes or opportunistic micromycetes is critical for prompt, effective treatment. Diagnostic problems have been evident since these infections were first identified. Routine clinical assays often yield nothing. Developing specific serological and biochemical tests has been attempted for years, but progress has been slow. The lack of early diagnosis is associated with problems in treatment, since the inability to make an accurate diagnosis does not allow forchoosing the right strategy and tactics fortreatment measures. In addition, the clinical manifestations of skin mycoses are diverse (inflammation, allergic reaction) and depend on the animal'sbody, which makes it very difficult to diagnose diseases [1, 2, 3].

Laboratory results that allow the pathogen to be detecteddirectly in the diagnostic material are decisive in diagnosing mycoses. Early laboratory diagnostics involves using methods that include tests with a Wood lamp, direct microscopy with alkali, inoculation of clinical material on nutrient media of various compositions, and microscopic examination [4].

Fungal microscopy is a mandatory procedure for making a preliminary diagnosis, while obtaining positive microscopy results that confirmthe presence of infection regardless of the results of other diagnostic methods [5]. Usually, in mycological diagnostics, primary microscopy is carried out, which is an examination option when the material is obtained directly from the patient, or secondary microscopy is an examination of the culture of the pathogen obtained from a pre-taken sample [6].

Primary microscopy involves the use of colored and unpainted drugs. Direct microscopy is a valuable skill in treating skin infections and infestations; however, it is underusedin dermatology clinics. The clinical sample collection process is simple and not invasive. The samples can then be processed quickly and examined in the clinic; if properly configured, the result is available in minutes. In the context of superficial mycoses, direct microscopy provides important information about infection that other methods cannot provide. The presence of *Candida* and *Malassezia* hyphal forms, for example, can confirm active infection rather than colonization, information that cannot be obtained by culture or molecular methods [7].

Light and fluorescence microscopy are most often used to diagnose fungal infections. Fluorescence was first discovered by Frederick W. Herschel in 1845, who noted that the excitation of a quinine solution with UV rays resulted in blue emission. Later, George H. Stokes, who coined the term "fluorescence,"noted that the wavelength of the original UV radiation is shorter than that of fluorescence. The first attempts to use fluorophores to stain tissue, bacteria, and other pathogens in research were made in the early 1900s [8].

Potassium hydroxide solution, fluorescent dyes (calcofluor white, acridine orange), lactophenol cotton blue, Gram stain, etc. are used to conduct microscopy of test samples for the presence of fungi [9]. Direct microscopy with potassium hydroxide solution allows the detection and identification of fungal elements in the sample. Thus, according to the study by N. Subathra, N. Bharathi Santhose (2017), who used microscopy with KOH to diagnose onychomycosis, the method's sensitivitywas approximately 51%, which was not inferior to the culture method. To assess the diagnostic efficiency of two different microscopic methods in detecting fungal growth in affected nails with onychomycosis, *Subathra N.* et al. (2017) used the classical microscopy method with a 40% KOH solution and a modified microscopy method with 36% DMSO. The sensitivity of both methods was 50.6% and 48.2%, respectively [10]. Aroop Mohanty et al. (2021) reported that in diagnosingmucormycosis, the sensitivity and specificity of KOH microscopy are 64% and 91%, respectively [11].

Despite the venerable age of the direct microscopy method and its well-deserved advantages (simplicity, ease of implementation, etc.), it is constantly being improved. Additional use of various dyes is proposed to increase the reliability and sensitivity of this method. Schiff'sreagent is widely used as a broad-spectrum dye for detecting fungi [12]. Broadwater Devin R. et al. (2022), using a modified method of staining with the Schiff reagent, recognized this method as the most optimal for identifying fungi and not taking much time during analysis. The Schiff reagent stains the cell walls of Candida spp., Malassezia fungi, namely, polysaccharide complexes [13].

Using lactophenol blue,Vacharavel Shamly et al. (2014) noted its ability to stain thin hyphae.This finding allowed the authors to identify pathogensmore easilyduring microscopic examination [14].

Megha Tandon (2023) recognized PAS staining as a simple and rapid alternative to cultivation. As a result of the studies, the method's sensitivitywas 76.2%, and the specificity was 42.9% [15].

When comparing the staining results with rose Bengal and lactophenol blue during rapid identification of pathogens from chili leaves, I. Barman et al. (2018) found spores of "C. assamicum," "C. annuum,"and "C. Frutescen." The authors concluded that lactophenol-trypan blue is more suitable for staining spores of irregular rod shape and spores of spherical shape, pink Bengal dye [16].

Recently, the introduction of direct wool microscopy with the addition of white calcofluor luminescent substance has intensified in veterinary mycological laboratories [4].

Calcofluor is a fluorochrome dye withanaffinity for chitin and cellulose. Its peculiarity is that when a drop of dye is added to a preparation with KOH, it is absorbed by parts of the fungus [17]. The combined use of white calcofluor with potassium hydroxide (KOH) solution makes detecting both young and mature fungus hyphae possible, which increases fungal infection's detectabilityby 10% compared to the standard KOH method [18].

The use of white calcofluor dye to identify fungal pathogens showed a high sensitivity of the method. For example, according to Vishal Punjabic et al. (2020), 85% and 60% of cases, were positive when comparing the staining performance of the sensitivity of calcofluor white with acridine orange. Calcofluor white also showed a high predictive value of a positive result (63%), in contrast to acridine orange (57.1%) [19].

In the diagnosis of fungal keratitis, Dalia Moemen et al. (2019) calcofluor white showed high sensitivity and specificity (99.44% and 90.91%, respectively), not inferior to the dye methylene blue in these indicators (92.31% and 80%, respectively) [20].

Schottelius J. (2000) developed combined staining of fungal spores with calcofluor white with trichrome blue and subsequent incubation with methylene blue. Staining made it possible to distinguish microsporidium spores, spotted, bright white under the influence of calcofluor, while Candida spores had a reddish-violet color [21].

Our research aims to compare staining methods to improve the direct microscopy method, rapid and reliable detection of spores and mycelium in biomaterial, identification of pathogens, and differential diagnosis of skin mycoses.

Materials and Methods

The object of the study is a biomaterial taken from farm animals with suspected dermatomycoses in the amount of 47 samples. Biological material (hair andskin scales) was taken from the periphery of the lesions [22] andanalyzed according to the requirements for microscopic studies on mushrooms [7].

Dyes used: lactophenol cotton blue, Romanovsky dye, Giemsa reagent, Schiff reagent, methylene blue, Bengal pink, white calcofluor, andfuchsine.

In order toobtain a 10% potassium hydroxide solution, 10 g of caustic potassium wasweighed, and 50 ml of distilled water was added. The mixture was stirred until the substance was completely dissolved and the solution volume was100 ml.

Bengal pink dye was prepared by diluting 0.25 g of rose Bengal powder per 100 ml of water.

To obtain 0.1% white calcofluor dye, 0.1 g of powder was dissolved in 100 ml of distilled water under low heat [16].

KOH microscopy was performed as follows: skin scrapings and hair were fixed on a slide, 10% KOH alkali solution was added. The glass was heated over the burner flame without boiling to accelerate material clarification; then, it was covered with a slide and viewed under a microscope [7].

Direct microscopy was used to detect fungal elements in biological material. Microscopy of biomaterial samples for the detection of mycelium or spores was carried out under an increase of ×10 on an Olympus BX43 (2020) fluorescent microscope [15].

The test material was clarified with 10% KOH to detect fungal elementsand then stained with calcofluor white [23].

A cultural mycological studyinvolving isolating a pure culture of fungi from biological material was conducted to establish the species affiliation [24]. The biomaterial was cultured on Sabouraud agar to detect zoophilic fungi and Chapek agar to detect micromycetes in 28ºS. Before sowing, the material was kept in 70% alcohol for 5 minutes.

The cultures that were obtained were identified based on cultural and morphological characteristics using identifiers [25, 26, 27] and taking into account the growth characteristics of the colonies.

Staining of smears of pure cultures of micromycetes was carried out by a simple method: 20 μL of any dye was applied to the slide, fixed for 5 minutes, and microscopy was performed.

Scotch preparations were prepared from young budding culturesby placing 10 cm long self-adhesive tape (Scotch 3M®) on a glass stick;the sticky side touchedthe surface of the formed colony of the test culture. The tape was fixed on glassandexamined under a microscope under various magnifications.

Results

Morphological identification of fungi remains the foundation of any clinical laboratory. Microscopic analysis of characteristic fungal structures is one of the most important parts of fungal identification [7]. Therefore, we diagnosed dermatomycoses by the gold standard method with additional coloring with calcofluor:

- classical KOH microscopy without coloring,
- coloring of biomaterial and pure culture of pathogens with various dyes (lactophenol cotton blue, Romanovsky dye, Giemsa reagent, Schiff reagent, methylene blue, Bengal pink),
- white staining of biomaterial with calcofluor,
- cultural and morphological identification of pure culture of pathogens with staining of smears with various dyes.

A total of 47 samples from farm animals were studied, of which 28 samples revealed the presence of pathogenic micromycetes: 7 *Trichophyton* spp., 1 *Microsporum* spp., 14 *Aspergillius* spp., and 6 yeast of different species.

When studying unpainted primary biomaterial from animals by direct microscopy, structures resembling fungal mycelium were found on hair surfaces in 10 cases (21.2%). Mycelium growth inside the affected hair was also detected (Figure 1).

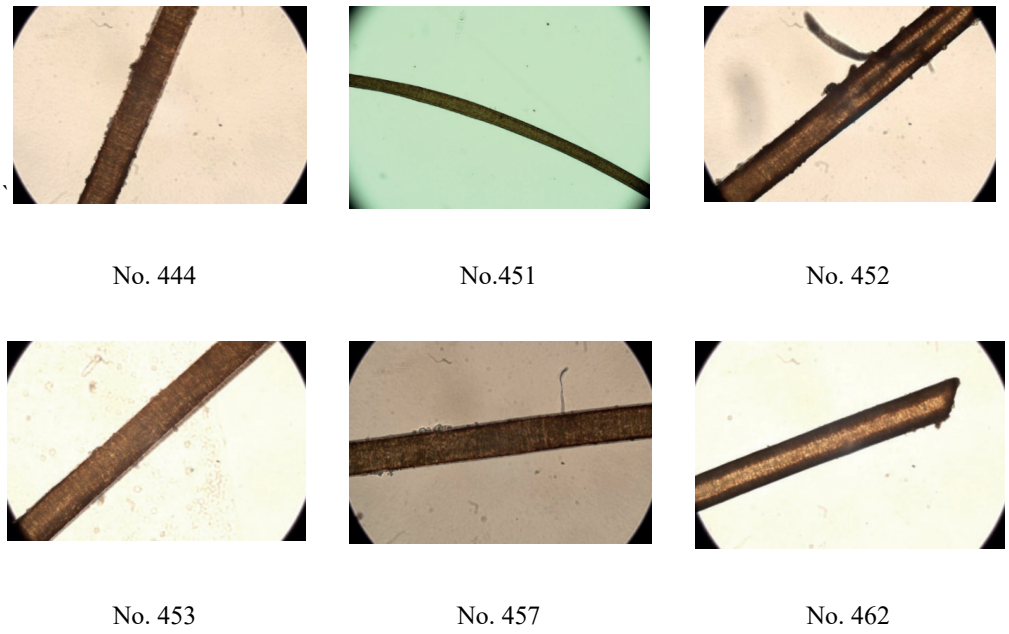


Figure 1 – Microscopy of affected animal hair

In Figure 1, external hair lesions are visible, manifested as a fluffy coating or threads in certain areas of the hair: samples No. 440, No. 452, No. 457. In some hair, the core is affected andfilled with mushroom spores. Especially strong lesions of the inner space of the hair were detected in samples No. 440, No. 444, and No. 457.

Detecting lesions of the hair core is difficult with microscopy of dark-colored hair. Identifying mycelium on the hair'ssurface is also difficult, and itcan be mistaken for glass defects and other artifacts.

Subsequently, we conducted a classic KOH test, in which the hair is enlightened in an alkali solution, which facilitates the detection of spores and mycelium [28]. Figure 2 shows the results of the KOH microscopy of animal hair.

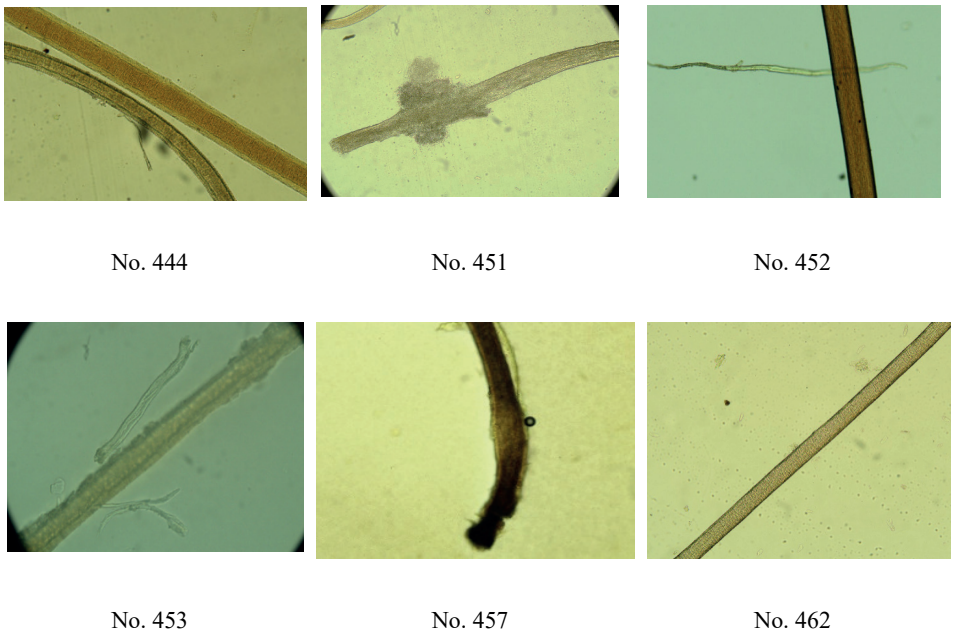


Figure 2 – KOH microscopy of affected hair, magnification ×10

Figure 2 shows various defects and lesions on the hair: in samples No. 440 and No. 457, focal thickenings and mycelium loosening were detectable. In samples No. 462 and No. 452, spore accumulation appeared inside the hair; numerous foci of hair loosening with stratification of the superficial hair membrane, and notches were also noted. Sample No. 453 revealed “pegs”characteristic wedge-shaped perforations and erosion on the hair surface, and hair thinning was observed [29]. KOH microscopy showed a positive result in 18 (38.2%) cases.

Problems in the KOH microscopic diagnosis of skin mycoses, expressed in the uniformity of mycelial lesions by pathogens of different types of dermatomycetesandthe absence of clearly visible spores and conidia, make it possible to make only an approximate preliminary diagnosis of skin mycosis or its appendages and do not allow identification ofmicromycetes to genus and species. There is also a possibility of a laboratory assistant’smistake in identifying the mycelium of dermatomycetes, which in KOH preparations looks imperceptible, has very thin, transparent, barely noticeable hyphae, and provided that there is a visible mycelium of opportunistic fungi, the novice researcher may make an erroneous diagnosis or not notice the presence of the pathogen at all.

To improve the quality of microscopy, we have developed a technology for coloring micromycete smears in biomaterial and in pure culture. The classic dyes lactophen blue, methylene blue, and Schiff’s reagent were selected for further studies to identify micromycetes in biological material from farm animals. Microscopy was performed at magnifications ×10, ×40, and ×100. When staining biomaterial samples with these dyes, the possibility of rapid detection of fungal mycelium in the biomaterial thickness (hair and scales) was detected in 11 (23.4%) cases (Figure 3).

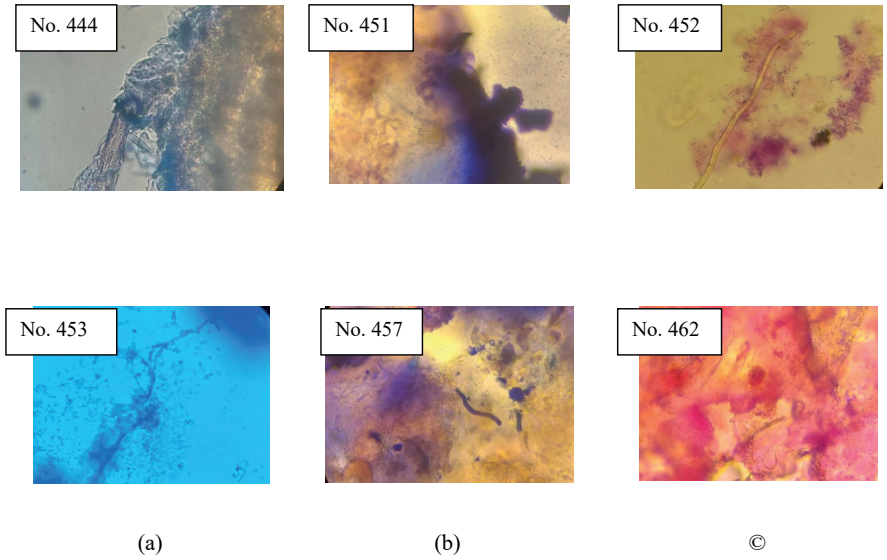


Figure 3 – Identification of fungal structures of micromycetes in the biomaterial when stained with classical dyes: a - lactophenol blue, b - methylene blue, c - Schiff'sreagent (the arrow indicates mycelium)

As can be seen from Figure 3, lactophenol blue stains mycelium well, but it also stains the general background. Methylene blue and Schiff'sreagent, along with the staining of mycelial hyphae, also stain skin scales, which complicates the diagnosis. Background staining of the tissue made it difficult to identify the morphological structures of micromycetes, which was doubtful due to the similarity in some cases with convoluted forms of bacteria, either with blood vessels or with a hair follicle. Also, in some cases, the general background of the smear was strongly colored, so the mycelium hyphae didn`t have clear boundaries.

Further searches for the optimal method of coloring served as the basis for using calcofluor white dye for coloring fungal components and conducting luminescent microscopy. 0.1% fluorescent calcofluor white dye and 10% potassium hydroxide solution were usedfor microscopy. Analysis was performed on an unfiltered fluorescent microscope (Figure 4) and with a blue filter (Figure 5).

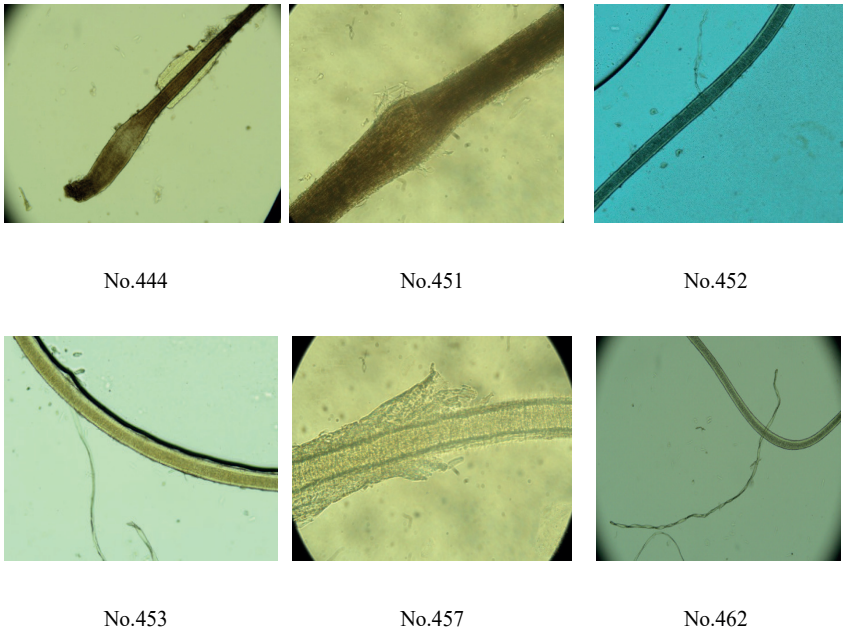
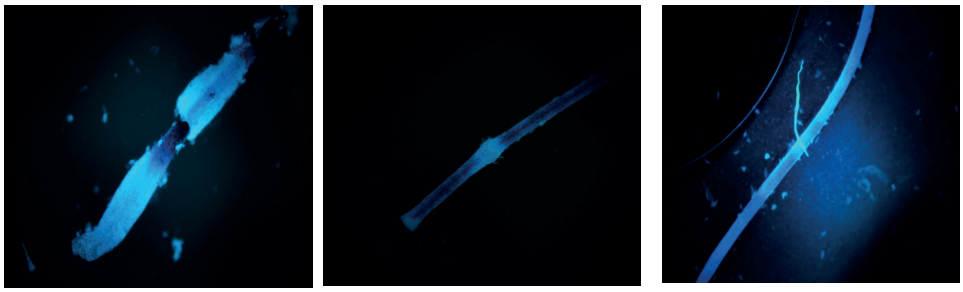
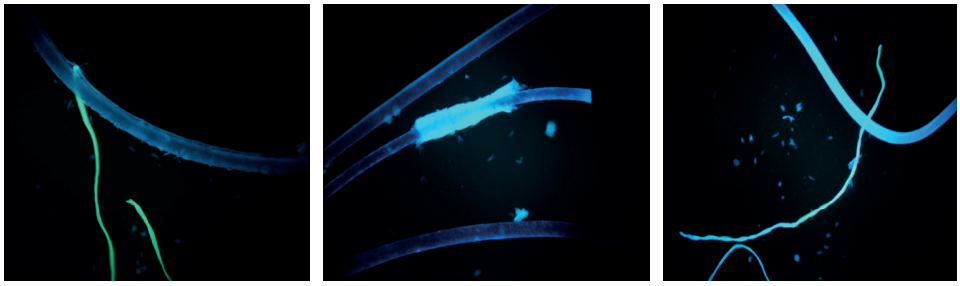


Figure 4 – Detection of spores and mycelium of micromycetes in affected hair, white color with calcofluor, magnification ×10, without filter



No.444 No.451 No.452



No.453 No.457 No.462

Figure 5 – Luminescence of micromycete mycelium and affected hair in biomaterial, white calcofluor staining, ×10, blue filter

A comparative analysis of images taken with a fluorescent microscope, with and without using a blue filter, found that the possibility of detecting the mycelium of fungi and affected hair is higher in the second case. Figure 5 clearlyshows luminous fungal mycelial filaments in the biological material under the blue filter of the luminescent microscope. The detection percentage was 21.3%.

Mycelium in KOH smears was clear and poorly visible in the field of view. The percentage of detection was 29.9%. Samples stained with calcofluor revealed hair affected by the overgrown mycelium of fungi. A luminous mycelium wasvisible, located directly on the affected hair. The mycelium of strain No. 453 had clearly defined boundaries, was detected as bright apple-green fluorescence, and was subsequently identified as *Microsporumcanis.*

Thus, the color of calcofluor white smears allows faster and clearer detection of affected hair. The mycelium on affected hair in such smears fluoresces blue, making the fungus hyphaevisible. Therefore, direct microscopy of biomaterial smears stained with calcofluor white is a rapid and sensitive method for laboratory diagnosis of mycoses.

Myceliumdetectionserved as the basis for the initial diagnosis of skin mycosis and the continuation of work on isolating primary cultures from biomaterial and culture and morphological analysis of pure cultures of mycromycetes, the formation of colonies of which was noted from the 4th to the 12th day of incubation (Figure 6).

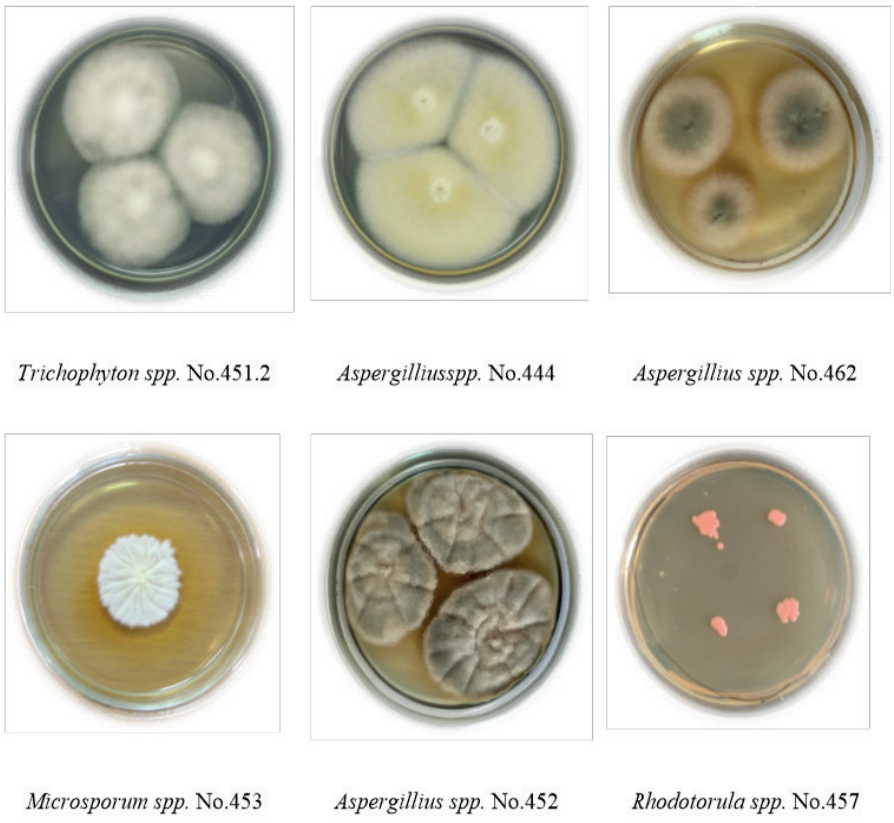
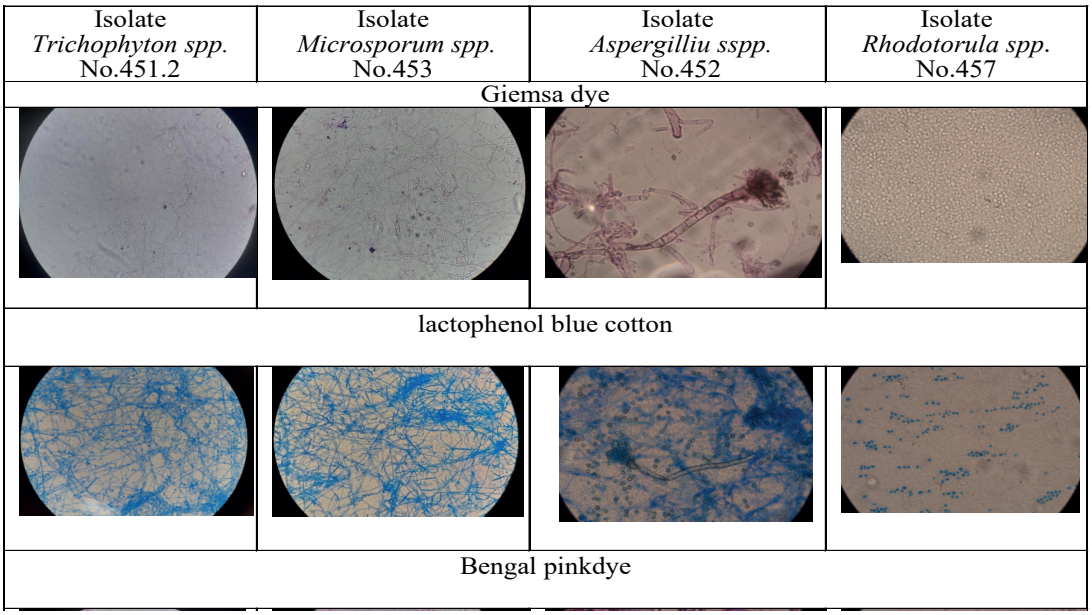


Figure 6 – Pure micromycete cultures isolated from biomaterial

Figure 6 shows colonies of primary fungal cultures of *Trichophyton spp., Microsporum spp., Aspergillius spp.* and yeast isolate *Rhodotorula spp.,* isolated from biological material of farm animals. Trichophyton spp (16.9%), less often Microsporumspp (2.1%), was detected in the culture method. Opportunistic fungi *Aspergilliusspp* were identified in 29.7% of cases, and yeast in 12.7% of cases.

The next step was comparative staining of yeast cells, spores and fungal mycelia from pure cultures of some *Rhodotorula spp.* yeast isolates. No. 457, mold fungi *Aspergillius spp.* No. 452 and *Trichophyton dermatomycetes spp.* №451.2, *Microsporum spp.* No. 453 with various dyes: methylene blue, Giemsa dye, Bengal pink, fuchsin, Schiff dye, Romanovsky dye, and lactophenol blue cotton (Figure 7).



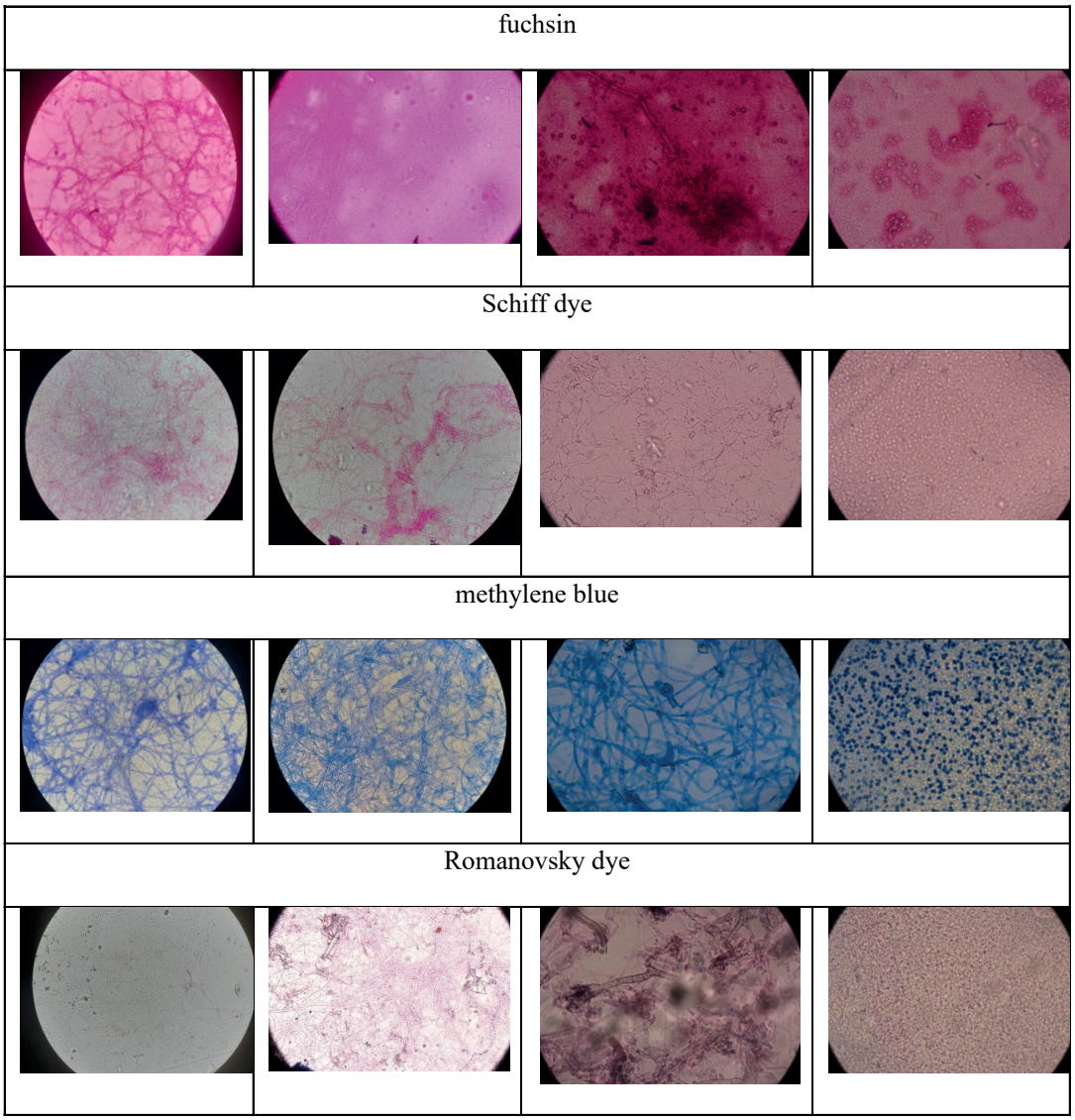


Figure 7 – Results of comparative coloration of yeast and mycelial fungi with various dyes

Figure 7 shows that when stained with methylene blue, all structural components of the mycelial fungi acquire a saturated blue tint. Romanovsky, Giemza, and Bengal pinkdyespartially stain hyphae and spores of mushrooms. Lactophenol blue cotton also stains all morphological structures of mycelial fungi of the genus Trichophyton (hyphae, mycelium, spores, conidia).

Yeast is well stained with dyes: lactophenol blue cotton and methylene blue. Schiff dye stains yeast pink, which is explained by the high concentration of polysaccharides in the yeast cell wall.

Discussion and Conclusion

The composition of fungal cell walls is relatively simple and includes substances not typically found in animal and plant hosts (e.g., chitin). It is assumed thatidentification of pathogen-specific molecular targets of fungal wall components becomes possible [30]. As is known, the rigid cell wall of fungi is a multilayered structure consisting of chitin microfibrils embedded in a matrix of small polysaccharides, proteins, lipids, inorganic salts, and pigments, providing skeletal support and shape to the cells enclosed in them. Chitin is a (β 1–4)-bonded polymer of N-acetyl-D-glucosamine (GlcNAc). The major polysaccharides of the cell wall matrix are composed of non-cellulosic glucans such as glycogen-like compounds, mannans (mannose polymers), chitosan (glucosamine polymers), and galactans (galactose polymers). Small amounts of fucose, rhamnose, xylose, and uronic acids may be present. Glucan belongs

to a large group of D-glucose polymers having glycosidic bonds. The most common glucans that make up the cell wall have a β configuration. Polymers with (β 1-3)- and (β 1–6)-linked glucosyl units with different bond ratios of 1-3 and 1-6 are common [31].

As a rule, the transparent unpainted mycelium of dermatomycetes is clearly visible in smears of pure culture of pathogenic fungi and practically undetected in smears of pathological material. Various staining methods have been proposed for better detection of mycelial structures. However, not all mushrooms present in hair and scrapings stain equally well. Keratinized cells are resistant to the penetration of dyes and make it difficult to stain fungi inside the hair and scales. However, colored preparations have several important advantages over potassium hydroxidetreated samples. Stained preparations reveal small morphological details of fungi more clearly [32]. Our results confirm the authors'data, although some dyes showed a better result (Figure 7).

When using methods of staining with various dyes of morphological elements of micromycetes in pure cultures, we found that when staining with methylene blue, all structural components of mycelial fungi acquire a saturated blue tint, which makes it possible to identify mycelial hyphae of various thicknesses and conduct a quick preliminary identification of dermatomycetes and opportunistic mold fungi in smears.

Staining with methylene blue mycelium of dermatomycetes makes it easy to identify in smears a characteristic thin mycelium and other morphological elements: rocket-shaped mycelium of fungi of the genus Microsporum, microconidia, macroconidia, arthrospores and other structural elements of fungi of the genus *Trichophyton*. Lactophenol blue cotton also stains all morphological structures of *Trichophyton* mycelial fungi (hyphae, mycelium, spores, conidia). In contrast, on a light background, the mycelium manifests itself as saturated blue threads, and spores in the form of dark blue morphological structures.

Romanovsky, Giemza, and Bengal pinkdyespartially stain hyphae and spores of mushrooms, the pattern in the field of view is not as clear as when staining with lactophenol blue cotton dye. However, coloring with Romanovsky, Giemza, methylene blue, Schiff, fuchsin,andBengal pink dyes allows you to quickly and reliably identify the characteristic fusiform macroconidia of the fungi of the genus *Microsporum*.

Yeast is well stained with lactophenol blue cotton, methylene blue, and Schiff dye. Given that living cells are not stained with methylene blue, and stained blue cells are considered dead [3], this stainingmethod can provide data on the age of the culture.

Schiff dye stains yeast pink, which is explained by the high concentration of polysaccharides in the cell wall of yeast. Of the disadvantages of the dye, background staining of the smear can be noted, which makes it difficult to record the results.

KOH in various concentrations is used as a keratolytic agent to detect fungi in tissues [33]. However, it does not stain fungal elements. Therefore, delicate translucent fungal elements avoid detection. Several authors reported low sensitivity and specificity of KOH preparations [34]. Saxena et al. reported 68% sensitivity and 40% specificity of KOH [35].

The combined use of white calcofluor with potassium hydroxide (KOH) solution is believed to increase the detectability of fungal infection compared to the standard KOH method [18]. The affinity of the fluorochrome dye calcofluor for chitin allows the mycelium and spores of the fungus to absorb the drug when a drop of KOH with the dye is added. Itcauses the formation of a complex of β-1.3 and β-1.4 polysaccharides and a complex of white calcofluor with caustic potassium, which gives a characteristic glow under the blue filter of a luminescent microscope [17].

Using calcofluor allowed Rasconi S. et al. (2009) to perform preliminary identification of chitin of microscopic molds stained with calcofluor in pathological material [36]. In our studies, for direct microscopic detection of fungal structures, the biomaterial was stained with calcofluor white dye, which made it possible to quickly and efficiently identify hair lesions of early diagnosis of mycoses within 30-60 seconds (Figure 8).

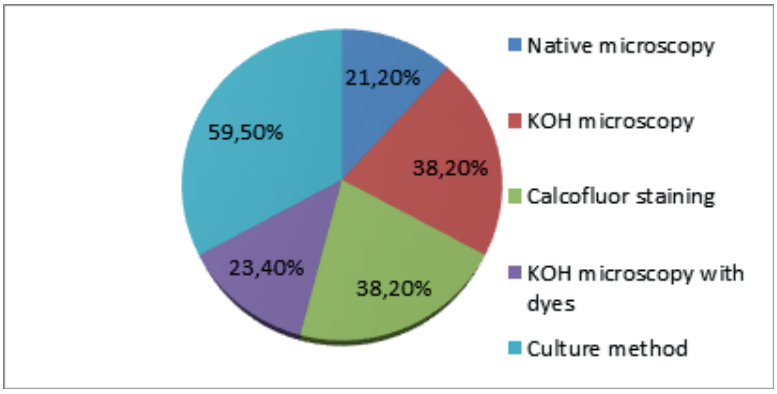


Figure 8 – Comparative results of biomaterial microscopy methods

As can be seen from Figure 8, the results of the mycological examination of 47 samples of biological material showed that during microscopy, the pathogen was detected in 10 cases (21.3%), during KOH microscopy in 15 cases (29.9%), during KOH microscopy with other dyes in 11 cases (23.4%), during KOH microscopy with calcofluor staining in 25 cases (53.2%).

The efficiency of the KOH microscopy method with white calcofluor compared with direct microscopy was 60% higher, compared with KOH microscopy with other dyes by 56%, classical KOH microscopy by 43.8%.

Identifying pathogens in samples of biological material using white calcofluor made it possible not only to quickly and reliably identify the pathogen and damage to hair or skin scales, but also to carry out differential diagnostics. This is because the mycelium of *Microsporum* spp. gives a bright apple-green fluorescence under a blue fluorescent microscope filter.

Our data are consistent with those of various authors Vishal Punjabi et al. (2020) [19], Dalia Moemen et al. (2019) [20], Schottelius J. [21], and several others. These results make it possible to recommend the use of direct microscopy with calcofluor staining for the rapid diagnosis of dermatomycoses in veterinary clinics of the Republic of Kazakhstan.

Pathogens were detected in 28 cases (59.6%) by the culture method. Compared with KOH microscopy with calcofluor white, positive results were confirmed in 89.3%. Practically similar results were obtained by Pihet M. et al. (2017) [37], who spoke of 93.8% detection of the pathogen in microscopy with calcofluor in comparison with the culture method. During the initial isolation of skin mycosis pathogens, fungal growth was obtained in 28 cases (59.6%), and growth was absent in 19 samples (40.4%).

According to the results, of the total number of pathogens, dermatomycetes were detected in 19% of cases: *Trichophyton spp.*16.9%, *Microsporum spp.* 2.1%. Opportunistic fungi *Aspergillius* spp. were detected in 29.7% of cases, yeast in 12.7% of cases. In 6.3% of cases, bacteria were detected, and in 40.4%, fungigrowthwas negative (Figure 9).

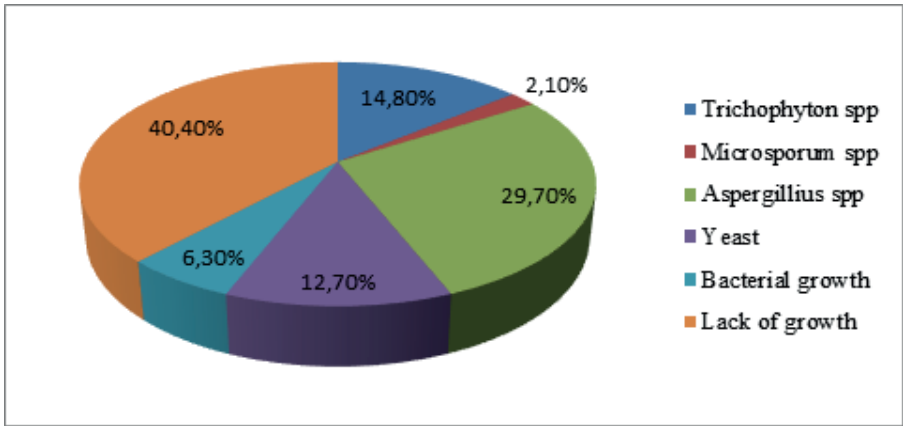


Figure 9 – Spectrum of pathogens isolated from farm animals by culture method

The staining of pure cultures of dermatomycetes, mycelial molds, and yeast also showed a higher diagnostic value when using the method of staining smears with various dyes in direct microscopy of scotch preparations.

Thus, using various dyes for coloring smears and biomaterials makes it possible to improve the direct microscopy method for the rapid and reliable detection of spores and mycelium, both in biomaterial and in pure culture. Thismethodwill make it possible to correctly identify pathogens quickly and carry out differential diagnostics of skin mycoses with high reliability.

As a result ofthemycological examination of 47 samples of biological material, it was revealed that during microscopy, the pathogen was detected in 10 cases (21.3%), during KOH microscopy in 15 cases (29.9%), during KOH microscopy with other dyes, in 11 cases (23.4%), during KOH microscopy with calcofluor staining, in 25 cases (53.2%).The efficiency of the KOH microscopy method with white calcofluor compared with direct microscopy was 60% higher, compared with KOH microscopy with other dyes, by 56%, classical KOH microscopyby 43.8%. The culture method, detected pathogensin 28a cases (59.6%), of which 7 *Trichophyton spp.*, 1 *Microsporum spp.*, 14 *Aspergillius spp*., 6 yeast. Growth was absent in 19 samples (40.4%), bacteria were detected in 3 samples (6.3%). Compared with KOH microscopy with calcofluor white, positive results were confirmed in 89.3%. Application of coloring with various dyes of morphological elements of micromycetes in pure cultures makes it possible to identify mycelial hyphae of various thicknesses, spores, conidia,andother morphological structures and to carry out rapid preliminary identification of dermatomycetes and opportunistic mold fungi in smears.

Authors' Contributions

EK and GB: Concept development, design and planning of the study, data collection and analysis, critical review of the article and final approval, research, statistical analysis. AN and GB: Conducted a comprehensive literature search and conducting research. All the authors have read, reviewed and approved the final version of the manuscript.

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