Overview of Hematoxylin Eosin and Periodic Acid Schiff McManus Staining to Identify *Subcutaneous mycoses*: A Histochemical and Morphologic Study

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Abstract— Some normal flora fungi can cause infection if there is a disruption in the protective barrier of the skin and mucous membranes or a defect in the immune system that allows them to enter, colonize and multiply in the body. Many fungal pathogens can produce subcutaneous lesions, given the complexity of patients at risk of infection and the variety of fungal pathogens. Direct microscopy is essential to detect fungi for appropriate treatment and patient recovery. This study aims to see the picture of staining between Hematoxylin Eosin (HE) with Periodic Acid Schiff (PAS) McManus to identify *Subcutaneous mycoses* fungi on histopathology tissue pieces. This study is a descriptive observational study with a cross-sectional design. The number of samples was total sampling. Hematoxylin Eosin (HE) and Periodic Acid Schiff (PAS) McManus staining were performed to detect *Subcutaneous mycoses* fungi and then read by 3 academicians with clear, less clear, and unclear categories. Based on the results of this study showed that there were 6 samples of fungal infection *Subcutaneous mycoses* obtained through Hematoxylin Eosin (HE) staining. Only 2 fungi were visible, and 4 fungi were less clear. In comparison, Periodic Acid Schiff (PAS) McManus staining of the 6 fungal samples was visible. In conclusion, the picture of PAS McManus staining is better than HE staining for detecting *Subcutaneous mycoses* fungi in histopathology tissue.

Keywords— Hematoxylin Eosin (HE), Periodic Acid Schiff (PAS) McManus, *Subcutaneous mycoses*.

DOI: 10.48047/ecb/2023.12.8.529

1. INTRODUCTION

Many fungal pathogens can produce subcutaneous lesions during the fungal infection disease process (Murray et al., 2021). Certain fungi usually enter traumatically through the skin, involving the integumentary system, including nails and hair, and generally cause skin inflammation (Hsu et al., 2012). *Subcutaneous mycoses* are commonly found in subtropical and tropical climates (Sukmawati, Ervianty, 2015), mainly due to moisture and temperature conditions that favor the growth and spread of causative agents. With an increase in the frequency of migration and tourism travel, these infections can be detected anywhere in the world (Sánchez et al., 2020).

Clinically seen as lesions on the skin surface, the pathogen rarely spreads to other body organs. The clinical course is generally chronic and insidious once established; most fungal infections are resistant to antifungal therapy (Murray et al., 2021). For example, superficial cuts or abrasions may expose environmental fungi that can infect the exposed dermis (Jawetz et al., 2016).

Some normal flora fungi can cause infection if there is a breakdown in the protective barrier of the skin and mucous membranes or if there is a defect in the host's immune system that allows them to enter, colonize and multiply within the host's body (Murray et al., 2021). In general, lesions become granulomatous and expand slowly from the area of implantation, and their expansion through the lymphatics draining the lesion is slow. These mycoses are usually confined to the subcutaneous tissues, but in rare cases, they become systemic and give rise to life-threatening diseases (Jawetz et al., 2016).

Fungal infections are caused by pathogenic fungi, which can adapt to the human body, usually a small number of pathogenic fungi. Most fungi live as normal flora and do not cause human infections because they fail to grow at 37°C (Chakrabarti, 2020).

Given the complexity of patients at risk of infection and the variety of fungal pathogens, fungal subcutaneous lesions pose considerable diagnostic and therapeutic challenges. Diagnosis depends on high clinical suspicion and obtaining appropriate material for culture and histopathology. Isolation and identifying the infecting organism are critical in properly managing the infection (Murray et al., 2021).

Some types of fungi cannot be diagnosed by culture, so it can only be done by histopathological examination using microscopy, namely by staining the tissue compared to the fungal culture, which takes a long time. Diagnostic accuracy is necessary to provide appropriate treatment for the patient's recovery (Kawilarang, 2022). Seeing fungal morphology and structure cannot be seen by the direct eye. One way to see the morphology and structure of fungi is by looking under a direct light microscope using Hematoxylin Eosin (HE) staining (Nafiu et al., 2023) and Periodic Acid Schiff (PAS) McManus on *Subcutaneous mycoses* fungi (Irawan et al., 2019).

Hematoxylin Eosin (HE) staining is the most widely used histological stain. The hematoxylin component stains the cell nucleus blue-black, showing good intranuclear detail. In contrast, eosin stains the cell cytoplasm and most connective tissue fibers in shades and intensities of pink, orange, and red (Bancroft et al., 2013). Hematoxylin Eosin (HE) staining has stood the test of time as the standard stain for histological examination of human tissue. This simple combination of dyes can reveal delicate cell and tissue structures (Chan, 2014).

Periodic Acid Schiff (PAS) McManus stain is usually added with a counterstain, which is used to show other tissue elements. To examine fungi, using a light green counterstain is more advisable because fungi are often weakly stained with hematoxylin staining. The cell nucleus will be stained blue using light green, and the background or surrounding tissue will be green (Yona et al., 2022). Direct microscopy is essential for infections caused by fungal infections; many other pathogenic fungi can be identified by direct microscopy examination (Chakrabarti, 2020).

Based on the description above, this study aims to see the description of staining between Hematoxylin Eosin (HE) with Periodic Acid Schiff (PAS) McManus to identify *Subcutaneous mycoses* fungi.

2. MATERIALS AND METHODS

2.1 Ethical Approval

This study has been declared ethically sound by the Health Research Ethics Committee of the Faculty of Medicine, Airlangga University with a letter numbered **68/EC/KEPK/FKUA/2023** on March 13, 2023.

2.2 Research Methods

The type of research used is descriptive observational research. The research design used was a cross-sectional study. The sampling technique in this study was total sampling. Research Place at

Sudarma Laboratory, Surabaya. Research time was conducted in March-April 2023. Population: Paraffin blocks of stored fungal tissues that have been diagnosed with fungal infections at Sudarma Laboratory, Surabaya. Duration of storage of fungal tissue at Sudarma Laboratory, Surabaya: for 1 year. Research Sample: Paraffin blocks of fungal tissue stored and diagnosed with *Subcutaneous mycoses* fungal infection at Sudarma Laboratory, Surabaya.

2.3 Research Procedure

2.3.1 Cutting Paraffin Blocks with a Microtometer

Tissues that have been embedded in paraffin blocks are adjusted on a microtome and then cut with a thickness of 3-5 μm with a representative paraffin band cut.

2.3.2 Hematoxylin Eosin (HE) staining (Bancroft, et al., 2013) :

Perform the deparaffinization process to remove paraffin, Put the tissue slices into the water, Drop Gill's hematoxylin then let stand for 5 minutes, Rinse with distilled water to remove excess paint, "Blue" with ammonia water, Rinse with distilled water, Rinse with 70% alcohol, Counterstain with 1% alcoholic Eosin for 4 minutes, Dehydrate quickly with 95% alcohol (I) 30 seconds 95% alcohol (II) 30 seconds Blot with filter paper and dry the slide, Clean with Xylene (I) 5 min, Xylene (II) 5 min, Cover with entellan slide cover.

2.3.3 Periodic Acid Schiff (PAS) McManus staining (Bancroft, et al., 2013) :

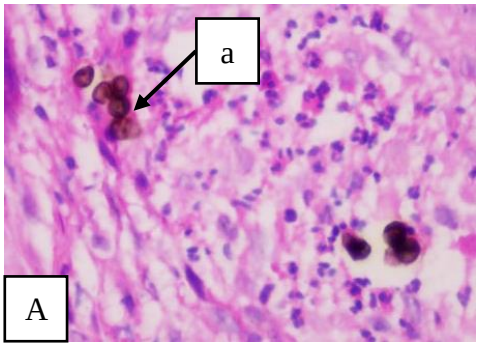
Deparaffinize the slide then put it in distilled water, Oxidize with 0.5% Periodic acid solution for 5 minutes, Rinse with distilled water, Then color with Schiff's reagent for 15 minutes, Rinse under running water for 10 minutes, Give counterstain with light green (working) for a few seconds, Blot until the tissue is dry, Dehydrate with Xylene twice for 5 minutes each, then cover with a slide cover that has been given entellan.

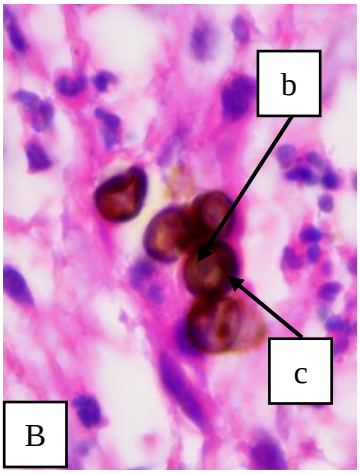
2.3.4 In this study, 1 sample of histopathology tissue pieces has been made and staining has been carried out with 2 different stains and then read by 3 academicians, namely researchers, clinical microbiology specialists, and Sudarma Laboratory analysis staff, Surabaya. The parameters used to measure the intensity of fungal staining in this study are clear, less clear, and unclear (Noronha, 2010).

3. RESULTS

Based on the observation of microscopic images of paraffin block preparations using histology slides, the quality of staining can be seen in the microscopic images below.

3.1 *Chromoblastomycosis*





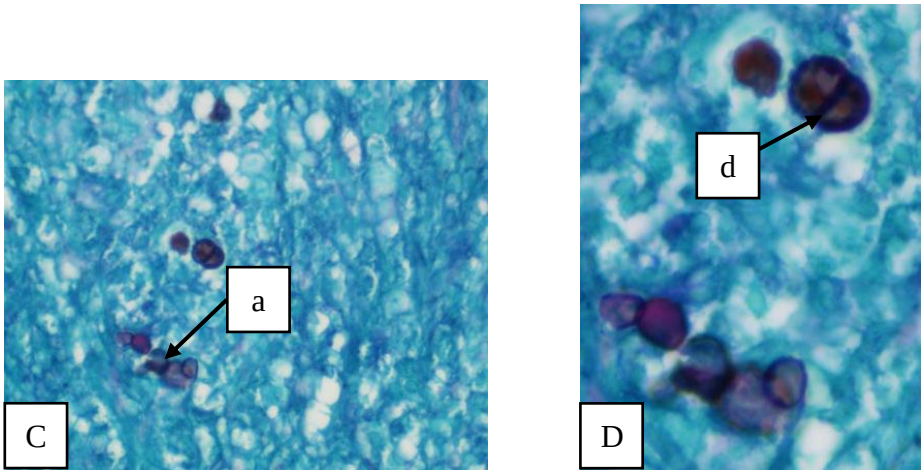


Figure 1. Microscopic slices of histology slides on *Chromoblastomycosis* fungal tissue. (A) HE magnification 450 times, (B) HE 1000 times, (C) PAS McManus magnification 450 times, (D) PAS McManus magnification 1000 times, (a) *Sclerotic bodies*, (b) brown pigmented, (c) thick-walled, (d) skeletal hyphae.

Interpretation of microscopic slices of histology slides on *Chromoblastomycosis* fungal tissue using Hematoxylin Eosin (HE) staining (Figure 1A & 1B) and Periodic Acid Schiff (PAS) McManus staining (Figure 1C & 1D) of visible fungi; *Sclerotic bodies* (5–12 μm in diameter), brown pigmented, thick-walled, occasionally brown, septate hyphae (3–8 μm in diameter).

3.2 *Mycetoma*

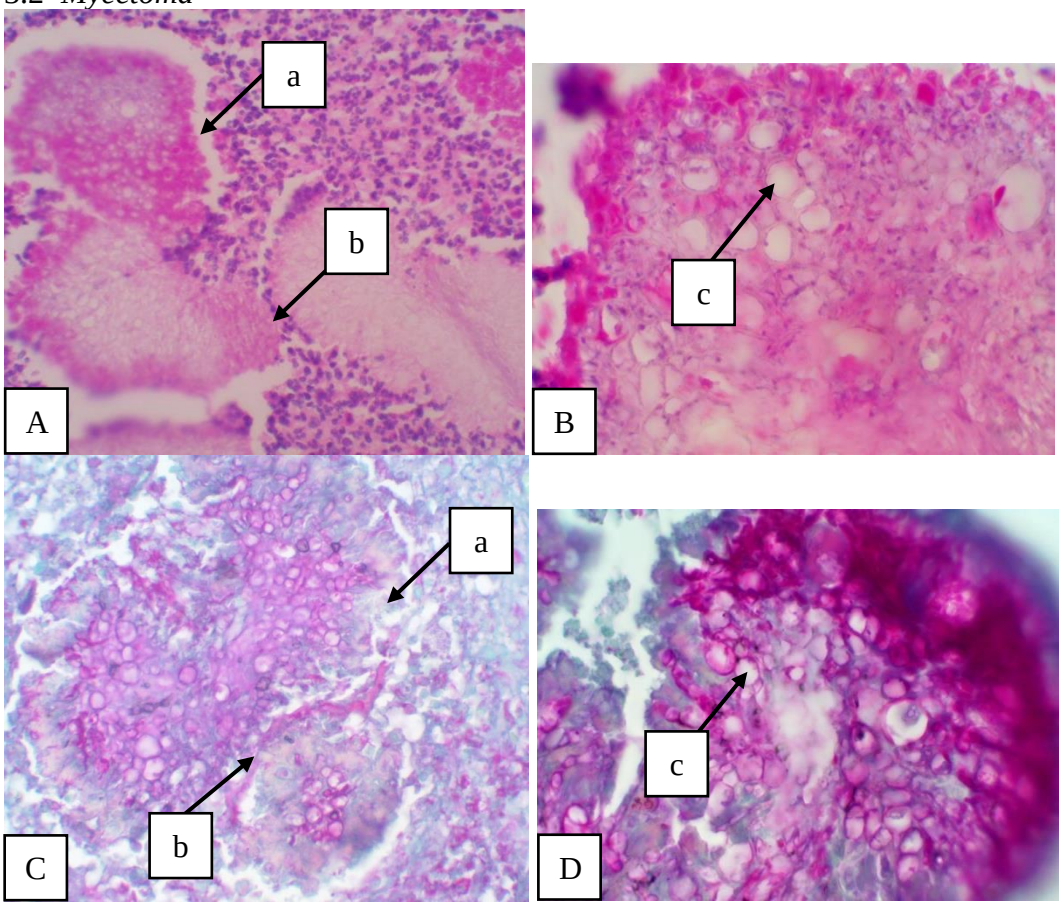


Figure 2. Microscopic slices of histology slides on *Mycetoma* fungal tissue. (A) HE magnification 450 times, (B) HE 1000 times, (C) PAS McManus magnification 450 times, (D) PAS McManus magnification 1000 times, (a) granule, (b) thin-walled and intertwined filaments, (c) chlamydospore.

Interpretation of microscopic slices of histology slides on *Mycetoma* fungal tissue using Hematoxylin Eosin (HE) staining (Figure 2A & 2B) and Periodic Acid Schiff (PAS) McManus staining (Figure 2C & 2D) of visible fungi; Granule (white, yellow, or red), narrow (0.5–1 μm in diameter), intertwined filaments, often numerous chlamydospores, especially at the periphery of the granule.

3.3 *Lobomycosis*

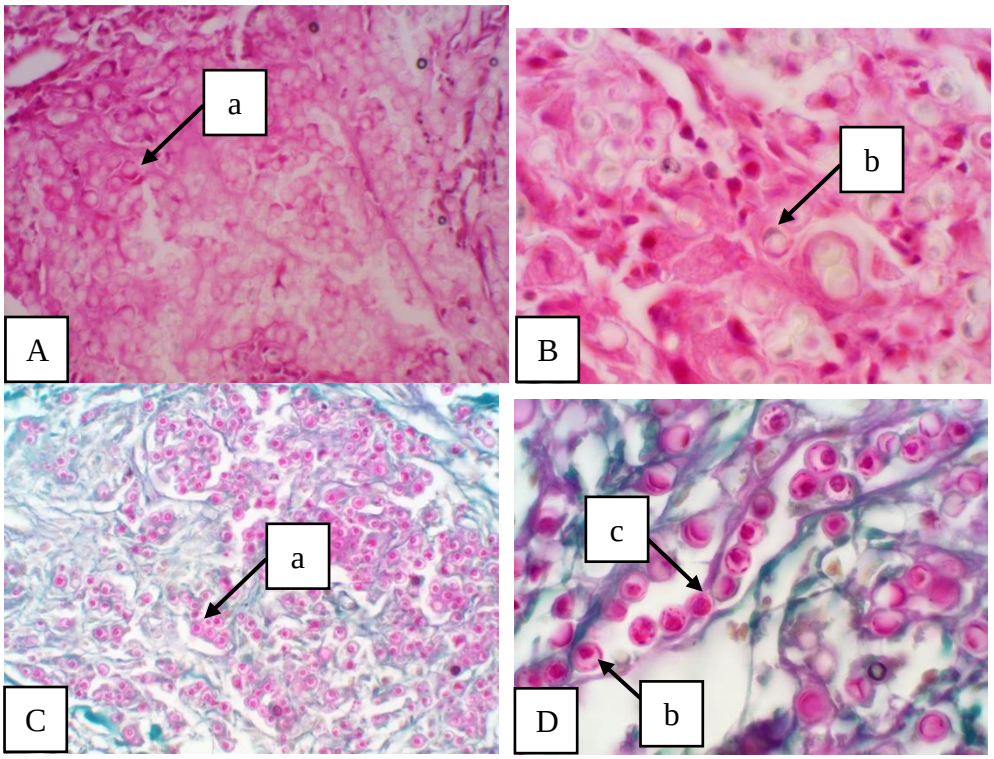
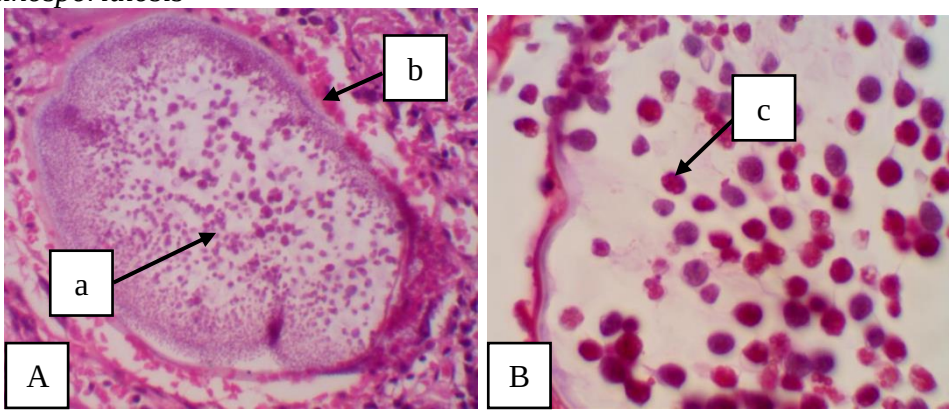


Figure 3. Microscopic slices of histology slides on *Lobomycosis* fungal tissue. (A) HE magnification 450 times, (B) HE 1000 times, (C) PAS McManus magnification 450 times, (D) PAS McManus magnification 1000 times, (a) round shape, (b) thick cell wall, (c) sequential budding and forming cell chains.

Interpretation of microscopic slices of histology slides on *Lobomycosis* fungal tissue using Hematoxylin Eosin (HE) staining (Figure 3A & 3B) and Periodic Acid Schiff (PAS) McManus staining (Figure 3C & 3D) of visible fungi; The appearance was spheric to oval and yeastlike. The fungi are 6 to 12 μm in diameter and have a thick, double-refractile cell wall. Reproduces by sequential budding and usually forms chains of cells connected by narrow.

3.4 *Rhinosporidiosis*



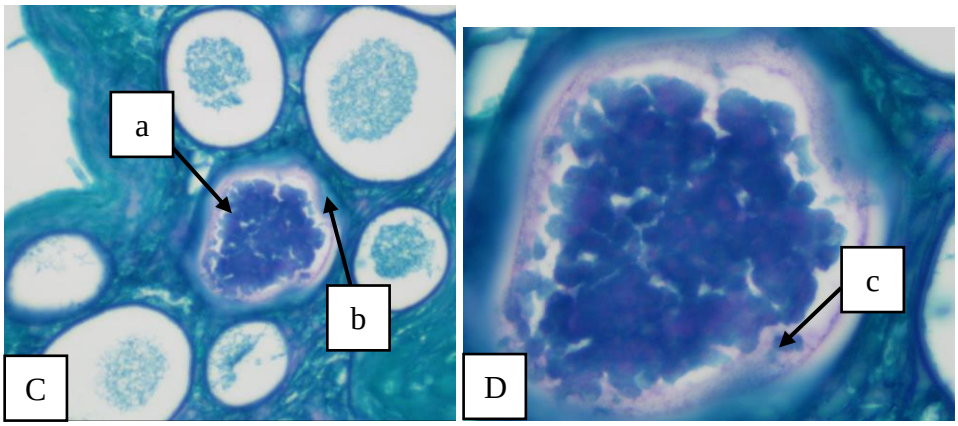


Figure 4. Microscopic slices of histology slides on *Rhinosporidiosis* fungal tissue. (A) HE magnification 450 times, (B) HE 1000 times, (C) PAS McManus magnification 450 times, (D) PAS McManus magnification 1000 times, (a) large and round sporangia, (b) thick-walled, (c) endospores.

Interpretation of microscopic slices of histology slides on *Rhinosporidiosis* fungal tissue using Hematoxylin Eosin (HE) staining (Figure 4A & 4B) and Periodic Acid Schiff (PAS) McManus staining (Figure 4C & 4D) of visible fungi; Large, round sporangia (100–350 μm in diameter when mature), sporangia thick-walled (≤5 mm), endospores vary in size (1–10 μm in diameter), endospores are arranged in zonal pattern in mature sporangia.

3.5 *Actinomycosis*

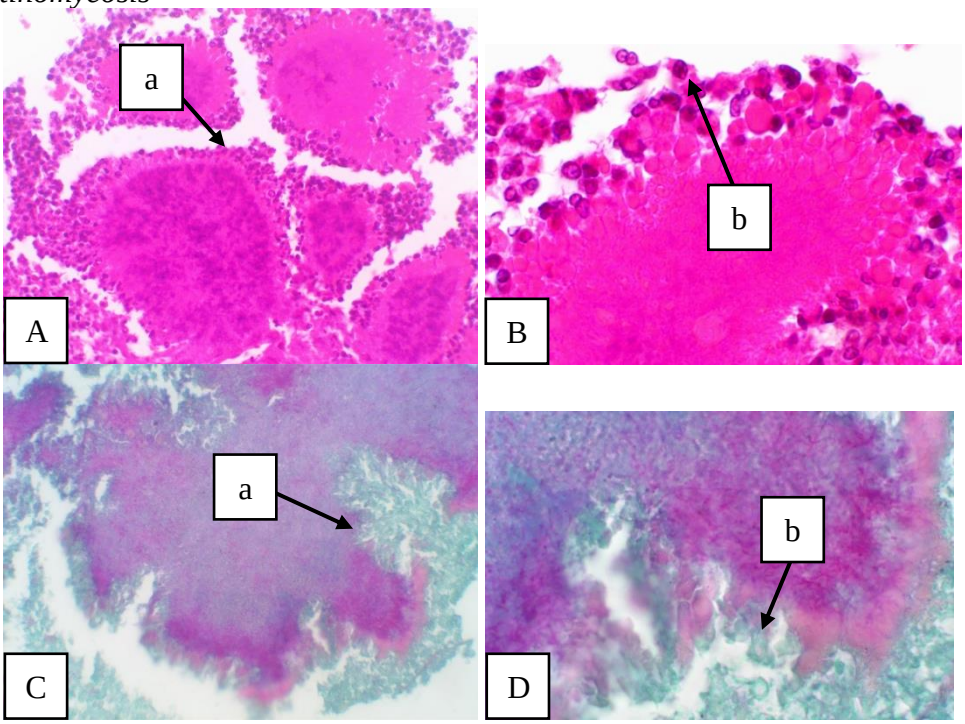


Figure 5. Microscopic slices of histology slides on *Actinomycosis* fungal tissue. (A) HE 450 times magnification, (B) HE 1000 times, (C) PAS McManus 450 times magnification, (D) PAS McManus 1000 times magnification, (a) granule, (b) branched filament.

Interpretation of microscopic slices of histology slides on *Actinomycosis* fungal tissue using Hematoxylin Eosin (HE) staining (Figure 5A & 5B) and Periodic Acid Schiff (PAS) McManus staining (Figure 5C & 5D) of visible fungi; "Sulfur granules" (30-3,000 μm or more in diameter), delicate (<1 μm in diameter) and branched filaments.

3.6 *Basidiobolomycosis*

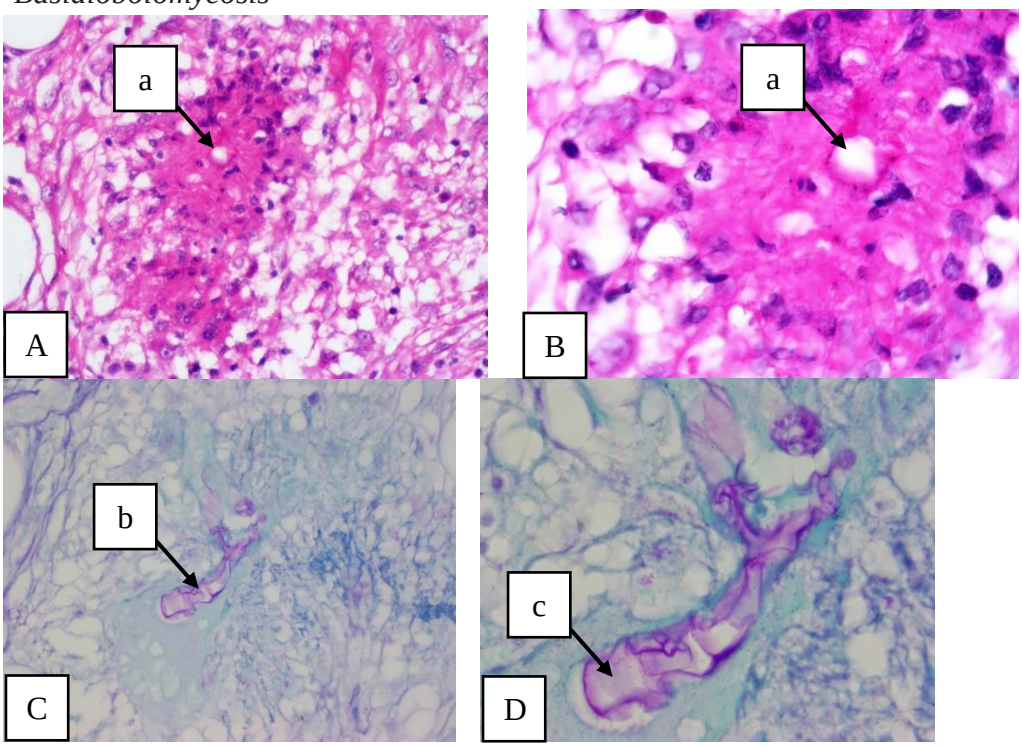


Figure 6. Microscopic slices of histology slides on *Basidiobolomycosis* fungal tissue. (A) HE magnification 450 times, (B) HE 1000 times, (C) PAS McManus magnification 450 times, (D) PAS McManus magnification 1000 times, (a) *Splendore-Hoeppli* phenomenon, (b) hyphae, (c) thin-walled

Interpretation of microscopic slices of histology slides on *Basidiobolomycosis* fungal tissue using Hematoxylin Eosin (HE) staining (Figure 6A & 6B) and Periodic Acid Schiff (PAS) McManus staining (Figure 6C & 6D) of visible fungi; The hyphal elements are sparse and often appear as hyphal fragments surrounded by *Splendore-Hoeppli* phenomenon. The hyphal fragments are thin-walled.

Table 1. Microscopic observation results based on the category of *Subcutaneous mycoses* fungal infection in paraffin block preparations.

Mycosis	Staining	Reader 1	Reader 2	Reader 3	Conclusions
Chromoblastomycosis	Hematoxylin Eosin (HE)	Clear	Clear	Clear	Clear
	Periodic Acid Schiff (PAS) McManus	Clear	Clear	Clear	Clear

Mycetoma	Hematoxylin Eosin (HE)	Less Clear	Less Clear	Less Clear	Less Clear
	Periodic Acid Schiff (PAS) McManus	Clear	Clear	Clear	Clear
Lobomycosis	Hematoxylin Eosin (HE)	Less Clear	Less Clear	Less Clear	Less Clear
	Periodic Acid Schiff (PAS) McManus	Clear	Clear	Clear	Clear
Rhinosporidiosis	Hematoxylin Eosin (HE)	Clear	Clear	Clear	Clear
	Periodic Acid Schiff (PAS) McManus	Clear	Clear	Clear	Clear
Actinomycosis	Hematoxylin Eosin (HE)	Less Clear	Less Clear	Less Clear	Less Clear
	Periodic Acid Schiff (PAS) McManus	Clear	Clear	Clear	Clear
Basidiobolomycosis	Hematoxylin Eosin (HE)	Less Clear	Less Clear	Less Clear	Less Clear
	Periodic Acid Schiff (PAS) McManus	Clear	Clear	Clear	Clear

Based on the recapitulation of Table 1, it shows that of the 6 research samples from the *Subcutaneous mycoses* fungal infection category obtained through Hematoxylin Eosin (HE) staining, only 2 fungi are clearly visible and 4 fungi look less clear than 6 research samples while McManus' Periodic Acid Schiff (PAS) staining of the 6 fungal samples is clearly visible.

4. DISCUSSION

Histopathologic examination of biopsies, surgical resection, and autopsy specimens should always begin with hematoxylin and eosin staining. However, many fungi stain poorly; more than this method is needed to show fungal elements in the tissue. There are several specialized stains to detect and view fungi; the operator should request these if a fungal infection is suspected. Periodic Acid Schiff (PAS) McManus staining is among the procedures commonly used for specific staining of fungal cells (Richardson, Warnock, 2012).

Hematoxylin Eosin (HE) stain is a routine stain used in everyday pathological diagnostics and, as a universal stain, is the primary contrast method in medical diagnosis for biopsy specimens (Ankle, Joshi, 2011) and has stood the test of time as a standard stain for histological examination of human tissues. This simple combination of dyes can reveal delicate cell and tissue structures (Chan, 2014). Periodic Acid Schiff (PAS) McManus stain is usually added with a counterstain, which is used to show other tissue elements. Fungi in the cell nucleus are stained blue, and with light green, all the background will become green (Yona et al., 2022).

The histopathologic examination can also provide insight into the diagnostic significance of some culture isolates. Histopathologic evaluation of granulomatous inflammation, giant cell reaction, necrotizing inflammation, and others should include a careful search for fungal morphologic elements. Seeing fungal morphology in tissue structures provides irrefutable evidence of invasive infection (Subhashini et al., 2019).

In Hematoxylin Eosin (HE) staining to see the morphology and shape of the color of the fungal tissue *Chromoblastomycosis* looks contrast so that it is visible *Sclerotic bodies* (diameter 5-12 μm), brown pigmented fungi, thick-walled and sometimes brown, skeletal hyphae (diameter 3-8 μm). Then in Periodic Acid Schiff (PAS) McManus staining to see the morphology and shape of the color of the fungal tissue, *Chromoblastomycosis* looks more contrast so that it looks more clearly *Sclerotic bodies* (5-12 μm), brown pigmented fungi, thick-walled and sometimes brown, skeletal hyphae (diameter 3-8 μm).

Clinically, the infection presents as verrucous nodules or plaques, usually on the extremities. Histopathology shows dark brown, thick-walled round fungi (5-12 μm) primarily clustered in the dermis. The epidermis showed hyperkeratosis and pseudoepithelomatous hyperplasia, and the dermis showed granulomatous and suppurative inflammation with abscess formation. (Ghosh et al., 2019). A biopsy is performed to confirm the diagnosis.

In most cases, a tuberculoid-type granuloma is reported, consisting of lymphohistiocytic infiltration, epithelial cells, and multinucleated Langhans giant cells. Langhans giant cells are large cells formed by the fusion of epithelioid cells in which macrophage cells are activated due to continuous cytokine activity in delayed hypersensitivity, so they are a hallmark of many granulomatous conditions. *Sclerotic bodies* are found in the stratum corneum and subsequent layers of the epidermis and skin. *Sclerotic bodies* can also be found in granulomatous and giant cell processes. Special staining is not required to observe the lesions as they are easily observed in tissue stained with Hematoxylin Eosin (Krzyściak et al., 2014).

The picture of filaments and chlamydospores on tiny slices of *Mycetoma* fungal tissue histology slides in Hematoxylin Eosin (HE) staining is almost the same color as the surrounding tissue structure so that it looks less contrast and looks less transparent. In contrast, in Periodic Acid Schiff (PAS) McManus staining, the picture of filaments and chlamydospores can be seen more clearly on tiny slices of *Mycetoma* fungal tissue histology slides.

The disease is characterized by three clinical signs and symptoms: subcutaneous swelling, multiple sinuses and discharge, and a painless mass. Histopathologic identification of the causative agent of *Mycetoma* was established in the 1950s. The fungus can be identified using Hematoxylin Eosin (HE) staining (Siddig, Fahal, 2017). Histologically, HE staining shows suppurative granulomas (composed of neutrophils) surrounding typical granules in the subcutaneous tissue. The neutrophil infiltrate is seen surrounded by palisading histiocytes (palisading granuloma) on its outer region, where the inflammatory infiltrate is composed of multiple cellular arrays consisting of lymphocytes, plasma cells, eosinophils, macrophages, and visible fibrosis. Larger, multinucleated cells are often also seen. HE is staining, and Gram staining sections show thin filamentous bacteria in cases of *actinomycetoma* and thick stick-shaped structures in *Eumycetoma* (Alam et al., 2009).

The description of cell chains and yeast cell connectors for *Lobomycosis* fungal tissue using Hematoxylin Eosin (HE) staining looks less contrast. It looks less transparent, while for McManus PAS staining to see the morphology of cell chain images and yeast cell connectors in *Lobomycosis* fungal tissue is different in color from the surrounding tissue structure so that it looks more contrast and clearer.

The most common manifestation of *Lobomycosis* is a nodular keloid appearance with a slow and insidious onset (Arenas et al., 2019). Initial routine histopathologic microscopic examination and Periodic Acid Schiff (PAS) staining histochemistry of ulcerated skin, tissue sections were covered by skin. In the epidermal layer, the epithelial surface and acanthosis discontinuities were seen. In the dermis, epithelioid histiocytes appear to form granuloma structures with a multinucleated giant cell distribution, mixed inflammatory cell distribution (PMN neutrophils and lymphoplasmatic cells), and fibrosis and necrotic tissue remnants. There are focal chains, tetrads, and yeast-like cells with 6-12 micrometer thick-walled spores that are refractive, intracellular, and extracellular (Saputra et al., 2022).

Morphology and color form of *Rhinosporidiosis* fungal tissue using Hematoxylin Eosin (HE) staining looks contrast so that it is visible large and round sporangia and endospores vary in size (diameter 1-10 μm) and endospores arranged in a zonal pattern in mature sporangia. Then using Periodic Acid Schiff (PAS) McManus staining to see the morphology and shape of the color of the *Rhinosporidiosis* fungal tissue shows contrast so that it is visible large and round sporangia and endospores vary in size (diameter 1-10 μm) and endospores arranged in a zonal pattern in mature sporangia.

Rhinosporidiosis is characterized by the development of polyps on the mucosa, which may be soft and friable with distinct strawberry-like areas. Diagnosis is based on cytologic or histopathologic demonstration of thick-walled sporangia of various sizes with numerous endospores. Endospores will exit through gaps in the sporangia wall, forming an empty, collapsed shape (Philip et al., 2023). The morphological structure varies in diameter from 100 to 350 μm, enveloped in chitin walls visible as white dots on the mucosa containing sporangiospores (6-12 μm). Generally, endospores and sporangia are embedded amidst inflammatory cell infiltrates (Tong et al., 2023), visible as pinhead-sized yellowish spots within the polyp. Microscopically, these structures vary in size, corresponding to different stages in the organism's development, and have a dense eosinophilic wall enclosing smaller rounded structures containing amorphous eosinophilic material. The microscopic features of these organisms are enhanced using Grocott-Gomori's methenamine silver, Periodic Acid Schiff (PAS), and mucicarmine stains. Rhinosporidium seeberi has a very similar morphology to Coccidioides, but the sporangia and endospores are more significant than the spherules (Arias et al., 2021).

It was found that the morphology of *Actinomycosis* fungi using Hematoxylin Eosin (HE) staining for the morphology of granule and filament images on microscopic slices of histology slides looks less contrasted and looks less transparent, while in Periodic Acid Schiff (PAS) McManus staining the morphology and shape of the *Actinomycosis* fungal tissue is different in color from the surrounding tissue structure so that it looks more contrasted and looks clear for granule and filament images.

Actinomycosis is an invasive bacterial disease of filamentous Gram-positive bacilli, mainly belonging to the human commensal flora of the oropharynx, gastrointestinal tract, and urogenital tract. *Actinomycosis* commonly leads to malignancy, tuberculosis, or nocardiosis, spreading continuously and progressively and often forming abscesses (Valour et al., 2014). Biopsy samples showed extensive epithelioid granulomatous reaction with Langhans giant cells, fibrosis, and 4n destruction and focal remodeling. Fragments with squamous epithelium showed intraepithelial neutrophil infiltration and subepithelial aggregation of lymphocytes and numerous plasma cells. On the biopsy surface, filamentous bacterial colonies were identified. The colonies were positive for gram stain and PAS but not for Ziehl-Neelsen's stain, consistent with Actinomyces spp., the acid content of the bacteria was not detected (Kaldas et al., 2020).

There is a phenomenon of *Splendore-Hoeppli* in Hematoxylin Eosin (HE) staining from the observation of microscopic slices of histology slides, but not necessarily visible hyphal elements of *Basidiobolomycosis* fungi, while in Periodic Acid Schiff (PAS) McManus staining the morphology and shape of the fungal tissue is different in color from the surrounding tissue structure so that it looks more contrast and looks more straightforward for the description of the hyphal elements of *Basidiobolomycosis* fungi.

Tissue diagnosis by full-thickness slice biopsy often shows *the Splendore-Hoeppli* phenomenon characterized by dense eosinophilic infiltrates with granulomata showing extensive fungal hyphae and mixed inflammatory infiltrates on histopathology results after HE staining showing granulomata with giant cells containing lymphocytes, neutrophil histiocytes, plasma cells, and eosinophilic cells (Rajkiran et al., 2023). Amorphous eosinophilic cells, also known as *the Splendore-Hoeppli* phenomenon and hyphal fragments of septa 10 μm in diameter. These hyphal fragments sometimes appear in the center of *Splendore-Hoeppli* phenomenon material or are found in the cytoplasm of multinucleated giant cells (Brun et al., 2018).

5. CONCLUSIONS

After analyzing Hematoxylin Eosin (HE) staining and Periodic Acid Schiff (PAS) McManus staining to detect fungal infections of *Subcutaneous mycoses* in histopathological tissue pieces, the conclusions obtained in this study are Hematoxylin Eosin (HE) staining in fungal infections of *Subcutaneous mycoses* is visible in *Chromoblastomycosis* and *Rhinosporidiosis* fungi, while less clear in *Mycetoma*, *Lobomycosis*, *Actinomycosis*, and *Basidiobolomycosis* fungi. Then, in Periodic Acid Schiff (PAS) McManus staining in fungal infections, *Subcutaneous mycoses* are visible in *Chromoblastomycosis*, *Mycetoma*, *Lobomycosis*, *Rhinosporidiosis*, *Actinomycosis*, and *Basidiobolomycosis* fungi. Periodic Acid Schiff (PAS) McManus staining is better than Hematoxylin Eosin (HE) staining for detecting *Subcutaneous mycoses* fungal infection in histopathology tissue structure sections.

ACKNOWLEDGMENTS

We would like to thank Sudarma Laboratory, Surabaya, Indonesia, and the Faculty of Medicine, Airlangga University, Surabaya, Indonesia for their willingness to assist in the research process.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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