PATHOLOGY CORNER

The Morphologic Identification of Common Organisms That May Look Alike in the General Pathology Practice: A Brief Review

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Published: 24 October 2014
Ibnosina J Med BS 2014;6(5):
Received: 09 April 2014
Accepted: 08 July 2014
This article is available from: http://www.ijmbs.org

Abstract
Surgical Pathologists often rely on morphologic features in identifying organisms in their general practice. The aim of this paper is to provide a brief practical and illustrated reference, comparing the morphologic features of organisms commonly encountered in the general practice of pathology. This comparison will focus on pairs of organisms that may look alike, resulting in diagnostic difficulties. These paired look–alike organisms include: Histoplasma Capsulatum versus Pneumocystis, Falciparum Malaria versus Babesia Microti, Pseudohyphae (of Candida) versus True Fungal Hyphae (of Aspergillus), Septate Hyphae (as in Aspergillus) versus Aseptate Hyphae (as in Mucor), Fungal Hyphae versus Artifact, and Antibiotic-Altered Bacteria versus Other organisms. Key distinguishing morphologic features are compared to help avoid diagnostic pitfalls.

Key Words
Organisms, Morphologic Diagnosis

Introduction

Most infectious organisms can be accurately identified with certainty by microbiologic cultures or molecular based techniques, like polymerase chain reaction (PCR) based tests. However, for various reasons (e.g. unsuspected clinical diagnosis, suboptimal or inadequate specimen handling) pathologists often find themselves relying on morphologic findings alone for the diagnosis and identification of bacteria, fungi, parasites, and other organisms.

The ability to identify common organisms is essential in the practice of pathology. In fact, a microscopic morphologic identification may provide the advantages of rapid diagnosis and add specific details on the extent of tissue invasion by the suspected organism and the inflammatory response.

The limitations of morphology, however, include subjectivity and the need for high level of diagnostic skill drawn from extensive experience. For example, a recent

study from a major university-associated medical center, found that only 79% of fungi were correctly identified based on morphologic features alone (1). The challenge is naturally greater in separating organisms that show similar morphologic features and/or have similar size.

This paper describes key morphologic features of some clinically relevant organisms that appear similar morphologically, and thus can be easy to confuse. Cases are presented in pairs, linking one organism with another that represents the most likely differential diagnostic challenge.

Key Morphologic Features

Histoplasma Capsulatum versus Pneumocystis

These two organisms may present a pathologist with a diagnostic challenge on cytologic or histologic specimens obtained from lungs, especially on slides stained with Gomori Methenamine Silver (GMS). *Histoplasma capsulatum* can also be found in lymph nodes and bone marrow, among other sites. Pneumocystis is usually crescent shaped, circular, or oval shaped. Similarly, *Histoplasma capsulatum* can be a round or pear-shaped organism of slightly larger size. Distinguishing between the two organisms relies on size, and some subtle unique differences in their appearance (Table 1). *Histoplasma capsulatum* (Figure 1A &B) is approximately two to five micrometers, while Pneumocystis (Figure 1C&D) may be slightly larger, ranging from two to seven micrometers (2). Histoplasma is commonly intracellular, although extracellular clusters may be seen rarely with individual

Table 1. Comparison of differentiating features of *Histoplasma capsulatum* versus *Pneumocystis*.

	Histoplasma capsulatum	*Pneumocystis*
Site & cytologic features	Lung tissue, lymph nodes, bone marrow, and others	Limited to lung alveoli
	Predominantly intracellular (inside macrophages)	Extracellular
Shape & Size	Round, oval organisms (from 2-4 microns)	Round, oval to crescent-like (4-7 microns)
Form	Budding and pseudocapsule (in histology sections)	No budding or capsule

Table 2. Comparison between *Babesia microti* and *Plasmodium falciparum*.

	Plasmodium falciparum	*Babesia microti*
Location in RBC	Intracellular (in fresh smears)	Intracellular or extracellular
Shape	Usually round rings	Round or pear-shaped rings with a central clear zone
Size	1.2-2 micrometers	around 2 micrometers
Ring forms	1- 3 rings in a red blood cell	up to 12 rings, in a red blood cell (usually in pairs)
Chromatin dots	1-2 chromatin dots	1-3 chromatin dots
Other forms	Schizonts & gametocytes rarely seen (banana-shaped gametocytes are diagnostic for *P. falciparum*)	No other forms other than rings seen in peripheral blood

Table 3. Comparison of morphologic features between true hyphae versus pseudohyphae.		
	True Hyphae (e.g. *Aspergillus*)	Pseudohyphae (e.g. *Candida*)
Size & Shape	Regular width with parallel walls regular septation	Variable width, irregular constrictions (sausage link appearance)
Form	Branching at an acute angles	Irregular budding

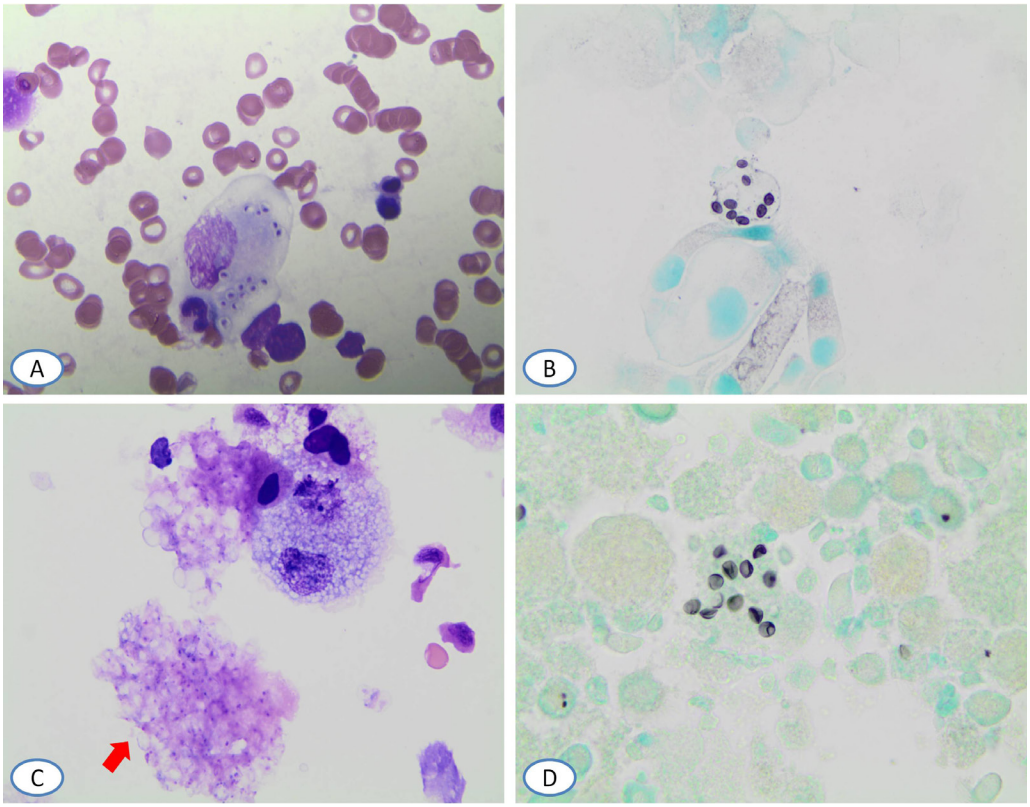

Figure 1. *Histoplasma capsulatum* with Wright Giemsa stain (A) and GMS stain (B). *Pneumocystis* with Wright Giemsa stain (C) (note: Extracellular location) and GMS stain (D).

yeasts showing narrow-based budding. Pneumocystis, on the other hand, is typically extracellular and does not demonstrate budding. On Wright-Giemsa stained slides, Pneumocystis organisms do not stain well and may not be easy to recognize. Budding yeasts of candida species (Figure 2A&B) may resemble Histoplasma capsulatum (Figure 2C), but their larger size and the presence of pseudohyphae (Figure 2D) can be helpful in differentiating between the two.

Plasmodium falciparum versus *Babesia microti*

Both of these parasites are transmitted to humans by the bite of an arthropod vector. Babesiosis is contracted from the Ixodes tick, while malaria is usually acquired following a bite by an infected female Anopheles mosquito. Ring forms of *Plasmodium falciparum* and Babesia share common features with subtle morphologic differences observed. Babesia rings are usually smaller and tend to be located centrally in red blood cells (Figure 3A). They can be round or pear shaped, as opposed to Plasmodium, which are typically round. Furthermore, the interior of the Babesia rings may show a clear zone (Figure 3B), while

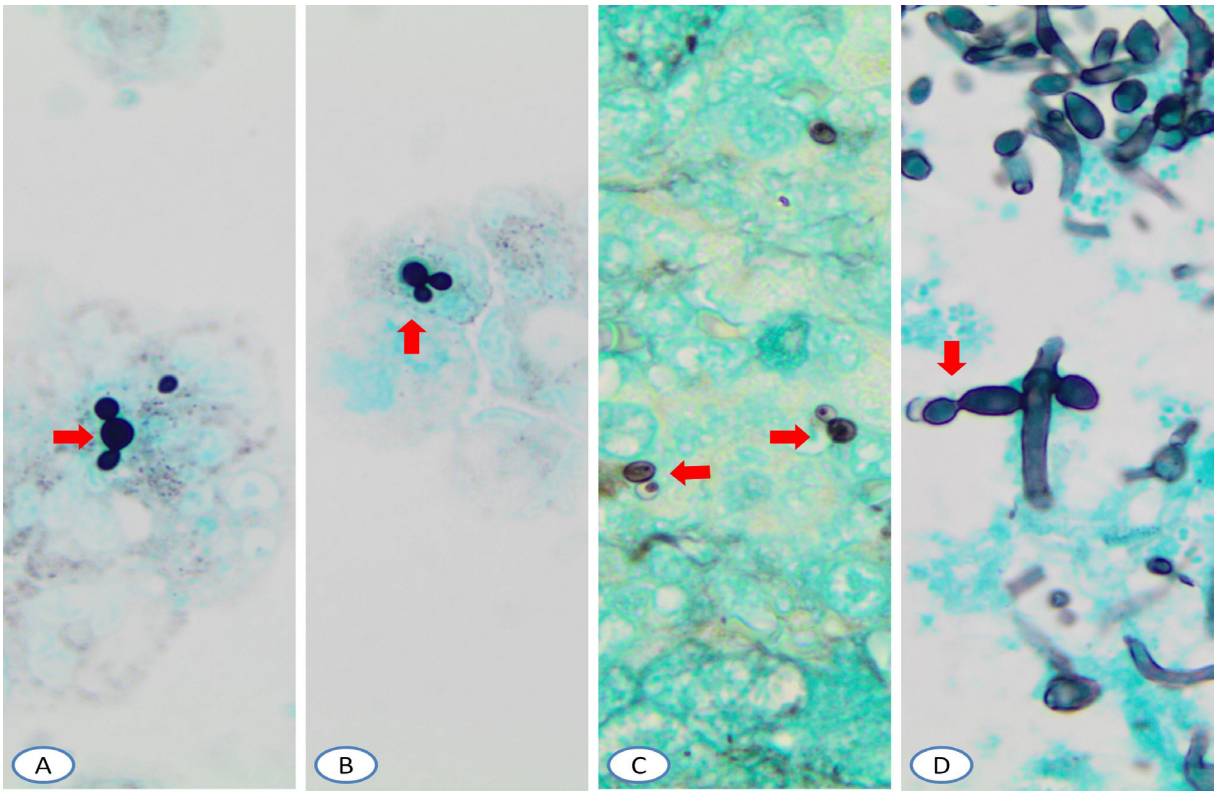

Figure 2. *Candida albicans* with GMS stain (A&B), *Histoplasma capsulatum* with GMS stain (C), and Pseudohyphae of *Candida albicans* with GMA stain (D).

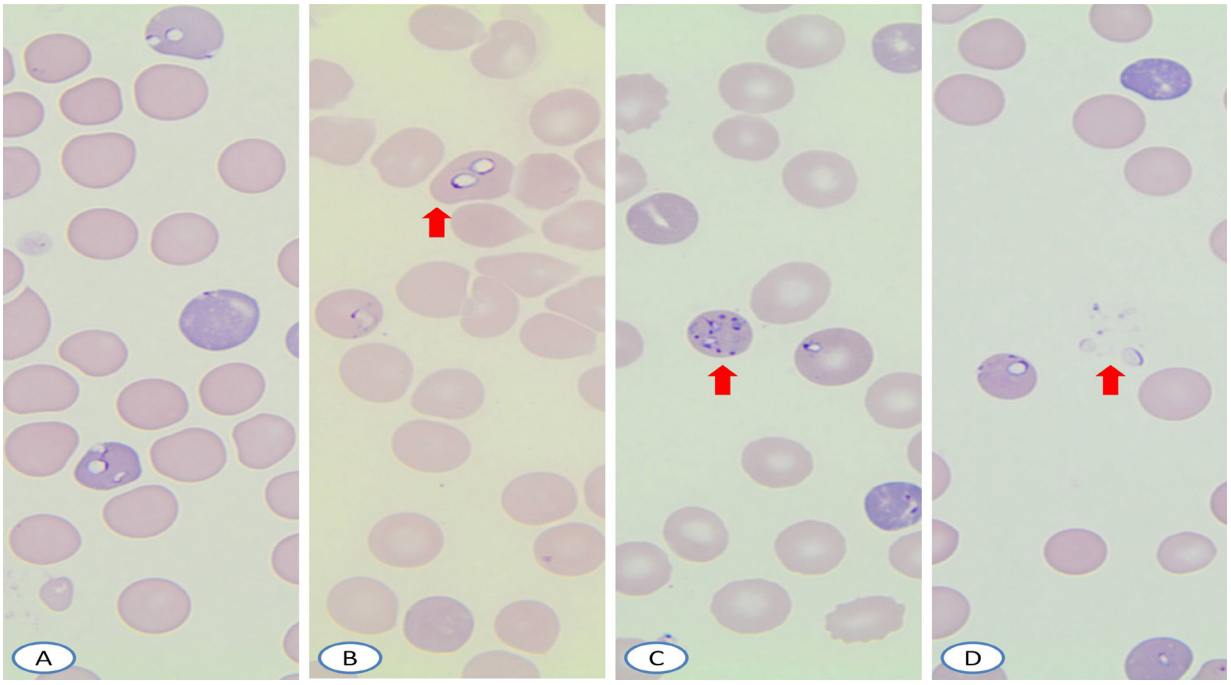

Figure 3. Babesia ring forms: (A) smaller and centrally located in red blood cells, (B) intereroir of ring with clear zone, (C) with many ring forms in a single red blood cell, and (D) Extracellular ring forms of Babesia (Arrow).

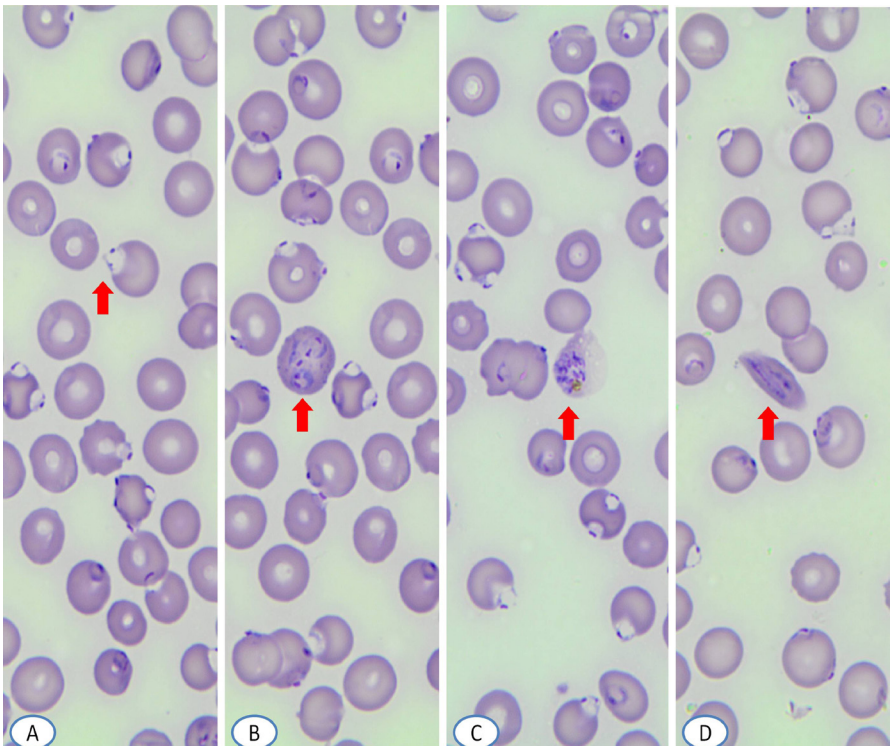

Figure 4. *Plasmodium falciparum* ring forms: (A) in the margins of infected cells and (B) showing multiple dots per ring (Wright-Giemsa stain). Note: Rings located in center of red blood cells do not show central clear zone, unlike Babesia. (C) *Plasmodium falciparum* schizont and (D) banana-shaped gametocyte (Wright-Giemsa stain).

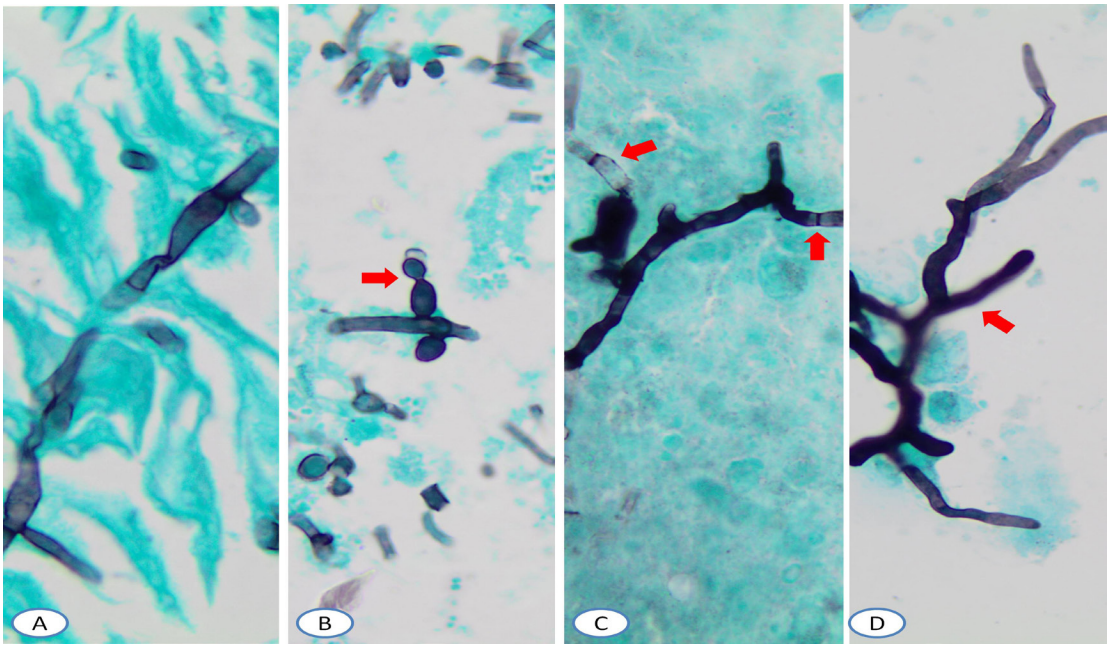

Figure 5. *Candida albicans* pseudohyphae (A&B, Wright GMS stain). Hyphae of *Aspergillus fumigatus* (C&D, GMS stain).

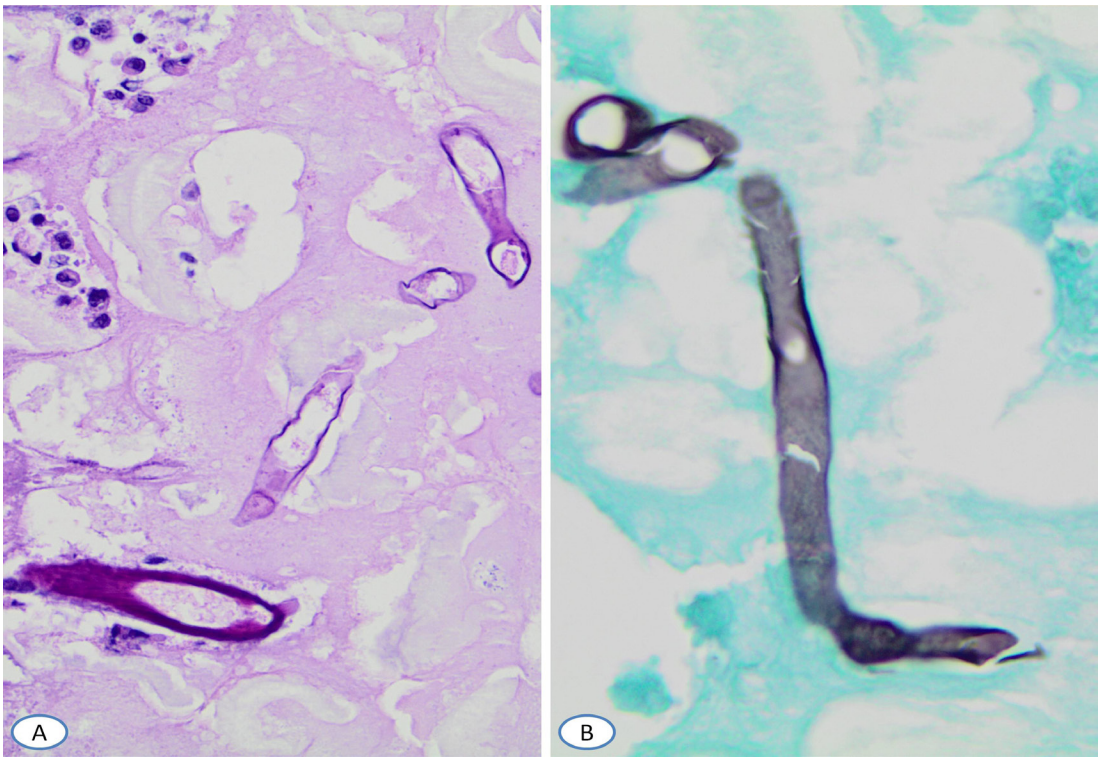

Figure 6. Aseptate and wide Hyphae of Mucor (A) with H&E stain and (B) with GMS stain. Note the ninety degree angle branching in (B).

falciparum rings do not show clear a clear zone unless they bulge out at the periphery of red cells. It is common for both parasites to show multiple ring forms in a single red blood cell: Babesia can exhibit up to twelve (Figure 3C), while *Plasmodium falciparum* does not usually display more than three. Tetrads of ring forms, when found, favor Babesia (3). Babesia ring forms may be seen outside RBC's even in a fresh sample (Figure 3D), a feature that is not seen with Plasmodium falciparum, unless the smear is made from an old sample. The rings of *Plasmodium falciparum* are most commonly found in the margins of infected cells (Figure 4A). Babesia rings show one to three chromatin dots, while Plasmodium rings most often have two, though single dots are frequently encountered (Figure 4B). Only ring forms of Babesia are encountered in the peripheral blood smear, unlike Plasmodium falciparum infections where other forms such as Schizonts (Figure 4C) and gametocytes are seen. Banana-shaped Gametocytes are diagnostic of Plasmodium Falciparum (Figure 4D). Red blood cells infected with Plasmodium falciparum and Babesia are not enlarged; a feature that also helps differentiates these two infections from Plasmodium vivax and ovale, where infected red blood cells are larger than their uninfected neighbors. Table 2 shows a comparative list of distinctive features.

Pseudohyphae versus True Fungal Hyphae

Depending on the surrounding environment, certain fungi can present as hyphae or yeast. Some yeast can form large buds that resemble hyphae (called pseudohyphae). Candida typically presents as small yeast (3-6 microns) with thin pseudohyphae that show constrictive narrowing (Figures 5A&B). Candida species may also show true hyphae (2). Aspergillus always appears as true hyphae, which branch dichotomously at forty-five degree angles, showing septation without narrowing (Figures 5C&D). Table 3 compares true hyphae versus pseudohyphae.

Septate Hyphae versus Aseptate Hyphae

Branching hyphae of Zygomycetes species also express some similarities to Aspergillus. The main differences between the two are shown in the type and width of branching. Aspergillus usually displays septations and has acute angle branching, frequently at acute angles. Mucor is typically aseptate and branches at ninety-degree angles. Mucor is approximately one and a half to two times broader with a width of up to twenty-five micrometers (2) (Figure

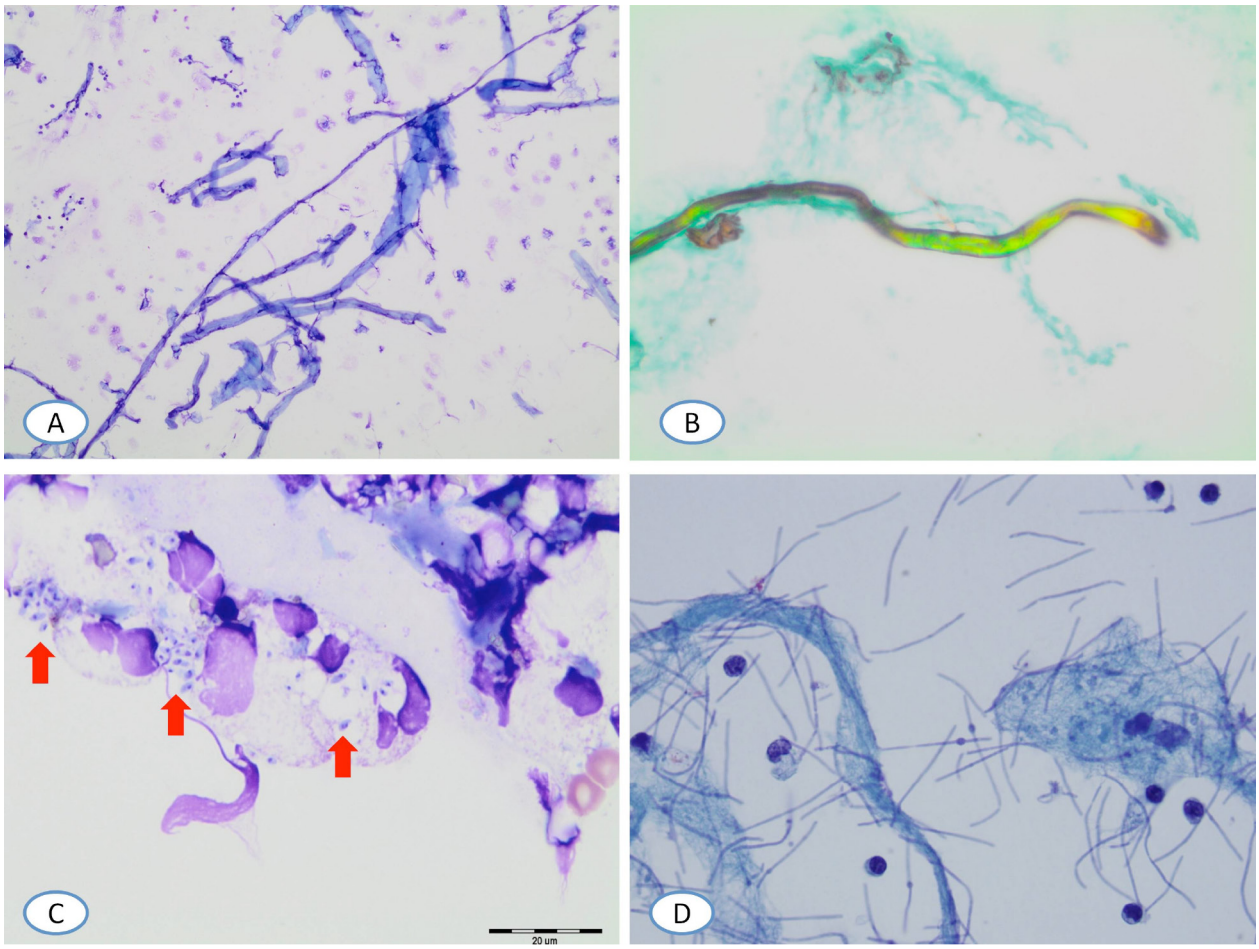

Figure 7. Synthetic Fibers on a Wright-Giemsa stained cytology specimen (A) and under polarizable light (B). Dead, swollen, *Streptococcus pneumoniae* (C, Wright Giemsa stain) and antibiotic-altered *Pseudomonas aeruginosa* (D, Diff-Quick stain).

6A&B).

Fungal Hyphae versus Artifact

Lint and other synthetic fibers may contaminate a specimen at the time of collection, or during processing and staining. They may look like fungal hyphae on Wright-Giemsa stained slides, and may take GMS staining appearing as long, thin, and, occasionally, branchlike structures. Hyphae are the basic structural units of molds and can show septation, whereas lint fibers are generally not septate. Using polarized light, lint fibers are typically refractile, unlike fungal hyphae (Figure 7A&B).

Antibiotic-Altered Bacteria versus Other Organisms

Artifactual findings in cytology or histology may mimic organisms and can cause unnecessary work up or diagnostic pitfalls (4). Artifactual changes may also make familiar and common organisms exhibit an unfamiliar morphology, causing diagnostic difficulties and uncertainties. Dead bacteria, especially those with capsules, may swell, mimicking the size and/or shape of fungal yeasts or some parasites (Figure 7C). Antibiotic treatment may cause bacteria to replicate without complete division, giving the appearance of different bacteria, or even fungi (Figure 7D) (5).

Conclusion

In an ideal situation, an infectious process is suspected clinically ahead of obtaining the specimen, and applicable ancillary studies (i.e. microbiologic cultures, molecular tests, or serologic tests) are utilized to confirm a presumptive morphologic diagnosis. For certain specimens, ancillary testing may not be possible, cost efficient, or even available. Under these conditions, the full burden of making a definitive diagnosis is placed, fairly or unfairly, on the shoulders of the pathologist's unaided morphologic recognition of organisms. In coping with such challenging situations, pathologists find themselves investing precious time and valuable resources searching the literature and trying to corroborate a morphologic impression. This paper

may serve as a quick reference in this regard, and can help make the search and accurate diagnosis easier.

Acknowledgements
The authors would like to thank Maria Nunez for her help with the manuscript.

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