

Bedside Microscopy for the Beginner

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Novice microscopists may struggle with identifying both the appropriate lesion for bedside testing as well as preparation and interpretation of the specimen. This article will serve as a guide to identify what type of primary lesion should prompt consideration of microscopic evaluation in the outpatient setting, provide specific details about how to properly obtain and analyze such a specimen, and elaborate basic information about the interpretation thereof.

Cutis. 2018;102:E27-E30.

Dermatologists are uniquely equipped amongst clinicians to make bedside diagnoses because of the focus on histopathology and microscopy inherent in our training. This skill is highly valuable in both an inpatient and outpatient setting because it may lead to a rapid diagnosis or be a useful adjunct in the initial clinical decision-making process. Although expert microscopists may be able to garner relevant information from scraping almost any type of lesion, bedside microscopy primarily is used by dermatologists in the United States for consideration of infectious etiologies of a variety of cutaneous manifestations.^{1,2}

Basic Principles

Lesions that should be considered for bedside microscopic analysis in outpatient settings are scaly lesions, vesiculobullous lesions, inflammatory papules, and pustules¹; microscopic evaluation also can be useful for myriad trichoscopic considerations.^{3,4} In some instances, direct visualization of the pathogen is possible (eg, cutaneous fungal infections, demodicidosis, scabetic infections), and in other circumstances reactive changes of keratinocytes or the presence of specific cell types can aid in diagnosis (eg, ballooning degeneration and multinucleation of keratinocytes in herpetic lesions, an abundance of eosinophils in erythema toxicum neonatorum). Different types of media are used to best prepare tissue based on the suspected etiology of the condition.

One major stumbling block for residents when beginning to perform bedside testing is the lack of dimensional understanding of the structures they are searching for; for example, medical students and residents often may mistake fibers for dermatophytes, which typically are much larger than fungal hyphae. Familiarizing oneself with the basic dimensions of different cell types or pathogens in relation to each other (Table) will help further refine the beginner's ability to effectively search for and identify

Average Size of Cell Types and Pathogens Commonly Seen in Bedside Microscopy

Cell Type	Average Size (Diameter)
Dermatophyte	1–2 μm
<i>Pityrosporum</i> yeast	2–4 μm
RBC	7 μm
Herpetic nuclear inclusion bodies	3–8 μm
Resting lymphocyte	8–10 μm
Neutrophil	10–15 μm
Eosinophil	12–17 μm
Keratinocyte	30–40 μm
HSV-infected keratinocyte	Up to 80 μm
Scabies eggs	100–150 μm
<i>Demodex brevis</i>	150 to 200 μm
Scabies mite (adult male)	250 μm in length; 200 μm in width
<i>Demodex folliculorum</i>	300–400 μm
Scabies mite (adult female)	300–500 μm in length; ~300 μm in width

Abbreviations: HSV, herpes simplex virus; RBC, red blood cell.

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The author reports no conflict of interest.

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pathogenic features. This concept is further schematized in Figure 1 to help visualize scale differences.

Examination of the Specimen

Slide preparation depends on the primary lesion in consideration and will be discussed in greater detail in the following sections. Once the slide is prepared, place it on the microscope stage and adjust the condenser and light source for optimal visualization. Scan the specimen in a gridlike fashion on low power (usually $\times 10$) and then inspect suspicious findings on higher power ($\times 40$ or higher).

Dermatophytes

Fungal infections of the skin can present as annular papulosquamous lesions, follicular pustules or papules, bullous lesions, hypopigmented patches, and mucosal exudate or erosions, among other manifestations.⁵ Potassium hydroxide (KOH) is the classic medium used in preparation of lesions being assessed for evidence of fungus because it leads to lysis of keratinocytes for better visualization of fungal hyphae and spores. Other media that contain KOH and additional substrates such as dimethyl sulfoxide or chlorazol black E can be used to better highlight fungal elements.⁶

Dermatophytosis—Dermatophytes lead to superficial infection of the epidermis and epidermal appendages and present in a variety of ways, including site-specific infections manifesting typically as erythematous, annular or arcuate scaling (eg, tinea faciei, tinea corporis, tinea cruris, tinea manus, tinea pedis), alopecia with broken hair shafts, black dots, boggy nodules and/or scaling of the scalp (eg, tinea capitis, favus, kerion), and dystrophic nails (eg, onychomycosis).^{5,7} For examination of lesional skin scrapings, one can either use clear cellophane tape against the skin to remove scale, which is especially useful in the case of pediatric patients, and then press the tape against a slide prepared with several drops of a KOH-based medium to directly visualize without a coverslip, or scrape the lesion with a No. 15 blade and place the scales onto the glass slide, with further preparation as described below.⁸ For assessment of alopecia or dystrophic nails, scrape lesional skin with a No. 15 blade to obtain affected hair follicles and proximal subungual debris, respectively.^{6,9}

Once the cellular debris has been obtained and placed on the slide, a coverslip can be overlaid and KOH applied laterally to be taken up across the slide by capillary action. Allow the slide to sit for at least 5 minutes before analyzing to better visualize fungal elements. Both tinea and onychomycosis will show branching septate hyphae extending across keratinocytes; a common false-positive is identifying overlapping keratinocyte edges, which are a similar size, but they can be distinguished from fungi because they do not cross multiple keratinocytes.^{1,8} Tinea capitis may demonstrate similar findings or may reveal hair shafts with spores contained within or surrounding it, corresponding to endothrix or ectothrix infection, respectively.⁵

Pityriasis Versicolor and Malassezia Folliculitis—Pityriasis versicolor presents with hypopigmented to pink, finely scaling ovoid papules, usually on the upper back, shoulders, and neck, and is caused by *Malassezia furfur* and other *Malassezia* species.⁵ *Malassezia* folliculitis also is caused by this fungus and presents with monomorphic follicular papules and pustules. Scrapings from the scaly papules will demonstrate keratinocytes with the classic “spaghetti and meatballs” fungal elements, whereas *Malassezia* folliculitis demonstrates only spores.^{5,7}

Candidiasis—One possible outpatient presentation of candidiasis is oral thrush, which can exhibit white mucosal exudate or erythematous patches. A tongue blade can be used to scrape the tongue or cheek wall, with subsequent preparatory steps with application of KOH as described for dermatophytes. Cutaneous candidiasis most often develops in intertriginous regions and will exhibit erosive painful lesions with satellite pustules. In both cases, analysis of the specimen will show shorter fatter hyphal elements than seen in dermatophytosis, with pseudohyphae, blunted ends, and potentially yeast forms.⁵

Vesiculobullous Lesions

The Tzanck smear has been used since the 1940s to differentiate between etiologies of blistering disorders and is now most commonly used for the quick identification of herpetic lesions.¹ The test is performed by scraping the base of a deroofed vesicle, pustule, or bulla, and smearing the cellular materials onto a glass slide. The most commonly utilized media for staining in the outpatient setting at my institution (University of Texas Dell Medical School, Austin) is Giemsa, which is composed of azure II–eosin, glycerin, and methanol. It stains nuclei a reddish blue to pink and the cytoplasm blue.¹⁰ After being applied to the

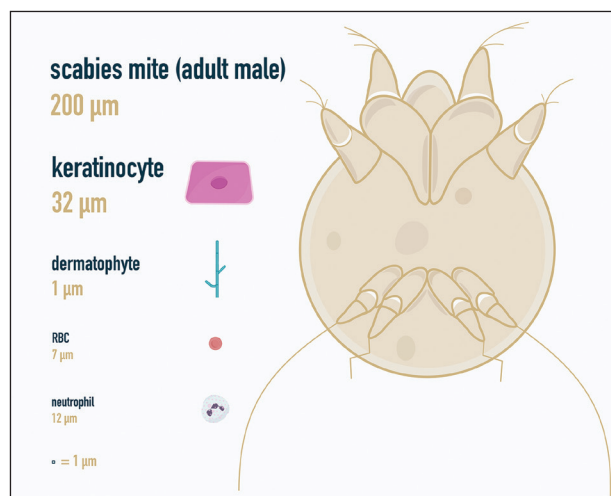


FIGURE 1. Schematic to help demonstrate relational size and scale of cell types and pathogens commonly seen in bedside microscopy. Dimensions listed pertain to diameter of micro-organisms. Image courtesy of Bogdan Mohora, MS (Austin, Texas).

slide, the cells are allowed to air-dry for 5 to 10 minutes, and Giemsa stain is subsequently applied and allowed to incubate for 15 minutes, then rinsed carefully with water and directly examined.

Other stains that can be used to perform the Tzanck smear include commercial preparations that may be more accessible in the inpatient settings such as the Wright-Giemsa, Quik-Dip, and Diff-Quick.^{1,10}

Examination of a Tzanck smear from a herpetic lesion will yield acantholytic, enlarged keratinocytes up to twice their usual size (referred to as ballooning degeneration), and multinucleation. In addition, molding of the nuclei to each other within the multinucleated cells and margination of the nuclear chromatin may be appreciated (Figure 2). Intranuclear inclusion bodies, also known as Cowdry type A bodies, can be seen that are nearly the size of red blood cells but are rare to find, with only 10% of specimens exhibiting this finding in a prospective review of 299 patients with herpetic vesiculobullous lesions.¹¹ Evaluation of the contents of blisters caused by bullous pemphigoid and erythema toxicum neonatorum may yield high densities of eosinophils with normal keratinocyte morphology (Figure 3). Other blistering eruptions such as pemphigus vulgaris and bullous drug eruptions also have characteristic findings.^{1,2}

Gout Preparation

Gout is a systemic disease caused by uric acid accumulation that can present with joint pain and white to red nodules on digits, joints, and ears (known as tophi). Material may be expressed from tophi and examined immediately by polarized light microscopy to confirm the diagnosis.⁵ Specimens will demonstrate needle-shaped, negatively birefringent monosodium urate crystals on

polarized light microscopy (Figure 4). An ordinary light microscope can be converted for such use with the lenses of inexpensive polarized sunglasses, placing one lens between the light source and specimen and the other lens between the examiner's eye and the specimen.¹²

Parasitic Infections

Two common parasitic infections identified in outpatient dermatology clinics are scabies mites and *Demodex* mites. Human scabies is extremely pruritic and caused by infestation with *Sarcoptes scabiei* var *hominis*; the typical presentation in an adult is erythematous and crusted papules, linear burrows, and vesiculopustules, especially of the interdigital spaces, wrists, axillae, umbilicus, and genital region.^{1,13} Demodicidosis presents with papules and pustules on the face, usually in a patient with background rosacea and diffuse erythema.^{1,5,14}

If either of these conditions are suspected, mineral oil should be used to prepare the slide because it will maintain viability of the organisms, which are visualized better in motion. Adult scabies mites are roughly 10 times larger than keratinocytes, measuring approximately 250 to 450 μm in length with 8 legs.¹³ Eggs also may be visualized within the cellular debris and typically are 100 to 150 μm in size and ovoid in shape. Of note, polaroscopic examination may be a useful adjunct for evaluation of scabies because scabetic spines and scybala (or fecal material) are polarizable.¹⁵

Two types of *Demodex* mites typically are found in the skin: *Demodex folliculorum*, which are similarly sized to scabies mites with a more oblong body and occur most commonly in mature hair follicles (eg, eyelashes), and *Demodex brevis*, which are about half the size (150–200 μm) and live in the sebaceous glands of vellus hairs (Figure 5).¹⁴ Both of these mites have 8 legs, similar to the scabies mite.

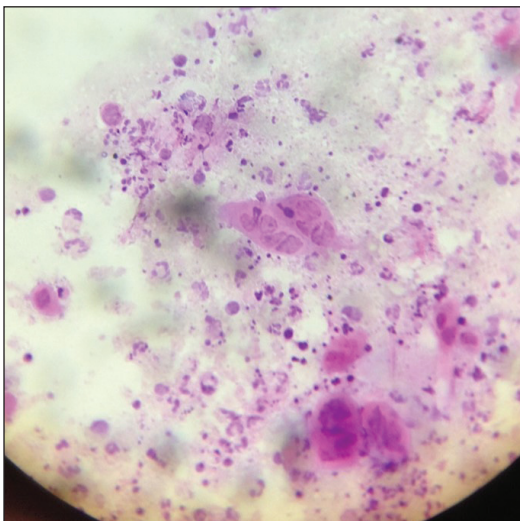


FIGURE 2. Ballooning degeneration and multinucleation of keratinocytes secondary to infection by herpes simplex (prepared with Giemsa stain). Image courtesy of Aron Gewirtzman, MD (Austin, Texas).

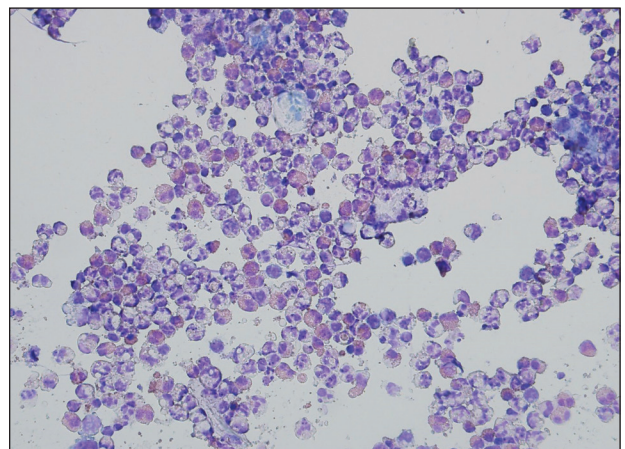


FIGURE 3. A predominance of eosinophils without keratinocyte abnormalities in erythema toxicum neonatorum (prepared with Diff-Quick). Image courtesy of Rachel McAndrew, MD (Austin, Texas).

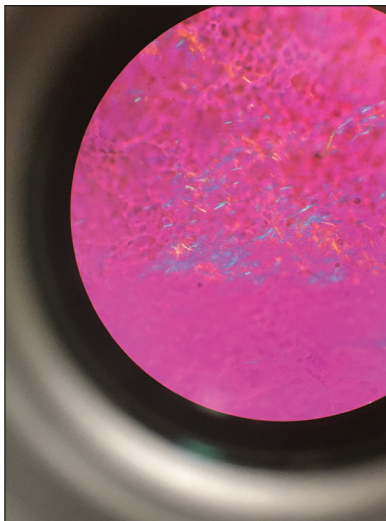


FIGURE 4. Needle-shaped, negatively birefringent crystals taken from a draining lower extremity tophus. Image courtesy of Paul Massey, MD (Boston, Massachusetts).



FIGURE 5. *Demodex* mite from a patient with rosacea and new-onset central facial pustules (prepared with mineral oil). Image courtesy of Candelario Antonio Rodriguez Vivian, MD (Monterrey, Mexico).

Hair Preparations

Hair preparations for bulbar examination (eg, trichogram) may prove useful in the evaluation of many types of alopecia, and elaboration on this topic is beyond the scope of this article. Microscopic evaluation of the hair shaft may be an underutilized technique in the outpatient setting and is capable of yielding a variety of diagnoses, including monilethrix, pili torti, and pili trianguli et canaliculi, among others.³ One particularly useful scenario for hair

shaft examination (usually of the eyebrow) is in the setting of a patient with severe atopic dermatitis or a baby with ichthyosiform erythroderma, as discovery of trichorrhexis invaginata is pathognomonic for the diagnosis of Netherton syndrome.¹⁶ Lastly, evaluation of the hair shaft in patients with patchy and diffuse hair loss whose clinical impression is reminiscent of alopecia areata, or those with concerns of inability to grow hair beyond a short length, may lead to diagnosis of loose anagen syndrome, especially if more than 70% of hair fibers examined exhibit the classic findings of a ruffled proximal cuticle and lack of root sheath.⁴

Final Thoughts

Beside microscopy is a rapid and cost-sensitive way to confirm diagnoses that are clinically suspected and remains a valuable tool to acquire during residency training.

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