Bedside diagnostics in dermatology

Viral, bacterial, and fungal infections

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Learning objectives
After completing this learning activity, participants should be able to describe and perform diagnostic tests that dermatologists can perform at the bedside; select the appropriate bedside technique for diagnosis of specific infectious dermatologic conditions; interpret micrographs to diagnose infectious dermatologic conditions using these bedside laboratory techniques; and judge appropriate situations for utilization of bedside laboratory techniques to save time or money in the timely diagnosis and treatment of patients with important infectious dermatologic diseases.

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Viral, bacterial, and fungal infections are frequently encountered in clinical practice, resulting in numerous cutaneous manifestations. Although diagnosis of these infections has changed over time because of technological advancements, such as polymerase chain reaction, bedside diagnostic techniques still play an important role in diagnosis and management, enabling rapid and low-cost diagnosis and implementation of appropriate therapies. This 2-part article will review both common and infrequent uses of bedside diagnostic techniques that dermatologists can incorporate into daily practice. This article examines the utility of bedside tests for the diagnosis of viral, bacterial, and fungal infections. The second article in this series reviews the use of bedside diagnostics for parasitic and noninfectious disorders. (J Am Acad Dermatol 2017;77:197-218.)

Key words: acid-fast; bedside diagnosis; cytology; Gram stain; slit-skin; Ziehl–Neelsen.

Bedside diagnostic tests and exfoliative cytology can yield rapid, reliable results that are especially helpful to confirm or exclude dermatologic diseases. Although other tests (including histopathology, polymerase chain reaction, and culture) play an undeniably important role, dermatologists should be aware of these tests and their potential to help expedite diagnosis in the clinic, on the inpatient wards with complex and critically ill patients, and in resource-limited settings

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Abbreviations used:

CBE:	chlorazol black E stain
H&E:	hematoxylin–eosin
HFMD:	hand-foot-mouth disease
HSV:	herpes simplex virus
KOH:	potassium hydroxide
MC:	molluscum contagiosum
PCR:	polymerase chain reaction
SJS:	Stevens–Johnson syndrome
SSSS:	staphylococcal scalded-skin syndrome
TEN:	toxic epidermal necrolysis
VZV:	varicella zoster virus

where other tests are unavailable. The focus of this 2-part continuing medical education series is the use of bedside diagnostic tests for rapid diagnosis of infectious and noninfectious disorders.

VIRAL INFECTIONS

Tzanck smear

Key points

- **Tzanck smear is an inexpensive, rapid, simple, noninvasive technique that is useful for diagnosing viral and bacterial infections and many inflammatory disorders**
- **A variety of stains are commercially available**

The Tzanck smear was initially introduced by Arnault Tzanck in 1947 for the cytologic examination of vesicular lesions to distinguish between blistering disorders.[1] Since that time, multiple uses have been described, including many in dermatology. As with other techniques, the utility of the tool is dependent on the user's experience.

The Tzanck smear is a simple, relatively noninvasive, rapid, inexpensive test that can be performed easily on multiple sites, including the mucosa.[2-5] For herpetic and other lesions, an early vesicular lesion is preferred for highest diagnostic yield.[6] The desired area is cleaned, the overlying crust or vesicle roof is incised and folded back, the base of the lesion is scraped with a no. 15 scalpel, and the contents are smeared thinly onto a glass slide (Supplemental Video 1, available at http://www.jaad.org). Specimens are air-dried and should be stained shortly after preparation to avoid cellular swelling and loss of nuclear detail. Depending on the stain used, fixation occurs either with alcohol (which is often incorporated into stains for self-fixing of the specimen) or heat. Stains used include May-Grunwald-Giemsa, Wright–Giemsa, and various modifications of these. These stains typically contain a combination of methylene blue, eosin, and Azure B.[2-5] Numerous stains are commercially available as kits (for example Quik-Dip [Mercedes Medical, Sarasota, FL], Wright-Giemsa [Sigma-Aldrich, St. Louis, MO], Hemacolor [Millipore Sigma, Billerica, MA], or Diff-Quik [Microptic, Barcelona, Spain]). Different stains result in variable coloring, but the nuclear features are the same. Evaluation of nuclear detail often requires ×20, ×40, or ×100 (oil-immersion) magnification.

Herpetic infections

Key points

- **Tzanck smear is most sensitive and specific when performed on early vesicular or pustular lesions**
- **Tzanck smear cannot differentiate between herpes simplex viruses 1 and 2 and varicella zoster virus**
- **Key cytologic features of herpetic infection include multinucleate keratinocytes, acantholysis, keratinocyte ballooning, and nuclear margination**

Herpes simplex viruses 1 and 2 (HSV1/2) and varicella zoster virus (VZV or HHV3) are exceedingly common viral infections worldwide. The characteristic clinical appearance of grouped vesicles on an erythematous base involving the orolabial or genital mucosa may not require additional confirmatory testing for HSV 1/2. However, atypical presentations can be a source of diagnostic confusion, especially in immunocompromised patients. Rapid confirmation of infection enables earlier treatment, institution of infection control measures, and avoidance of complications.

Several studies have shown that dermatologists can accurately and reliably diagnose herpetic infection using the Tzanck smear after proper training.[7-9] Diagnosis of herpetic infection depends upon visualization of the characteristic cytologic features. These features include "ballooning" of keratinocytes to sizes as great as 80 μm, multinucleation, and acantholysis. Nuclear changes include enlarged nuclei, peripheral margination of chromatin, nuclear molding, and blurry staining with "ground glass" cytoplasm. Cowdry type A bodies, which are intranuclear inclusion bodies surrounded by a subtle clear halo, are characteristic but may be difficult to find[6,10,11] (Fig 1). Tzanck smears cannot distinguish between HSV1/2 and VZV, and they are less sensitive for old or crusted lesions.[6,11,12]

Several published studies have compared Tzanck to other techniques, including viral culture, polymerase chain reaction (PCR), direct fluorescent antibody, biopsy with cytologic examination and immunohistochemical staining, and electron microscopy.[7,8,12-17] Reported sensitivity ranges from

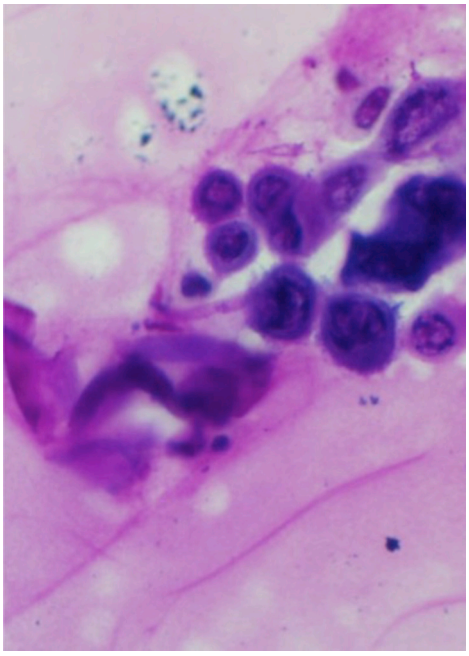

Fig 1. Tzanck smear of a herpetic infection shows multinucleation, margination of the chromatin, and nuclear molding.

40% to 76.9% and specificity up to 100% in comparative studies, depending on the age and stage of the infection and user experience. Tzanck smear of 1- to 3-day-old vesicles has a sensitivity of 78.7% to 100%, compared to 27% to 59.7% for older, eroded, or crusted lesions.[11,12] Tzanck smear performs better than direct fluorescent antibody testing (sensitivity, 50-85%; specificity, 99-100%) and viral culture (sensitivity, 52-93%; specificity, 100%) but is not as sensitive or specific as PCR.[7,11-13,15-17] The sensitivity of PCR is 92.4% to 100% for fresh lesions and 80.7% for older lesions (even up to 1 month), with a specificity of 83.7% to 98%.[8,12] The cost of PCR varies but averages approximately $80 per specimen, compared to reimbursement for provider interpreted Tzanck smear, for which Medicare pays <$10.

Other viral infections

Key points

- **The microscopic appearance of molluscum contagiosum is characterized by pathognomonic 30- to 35-μm virally transformed cells called Henderson–Patterson bodies**
- **Cryotherapy and dermoscopy also can help increase diagnostic yield**
- **Characteristic cytologic changes of Orf include Guarnieri bodies and acantholytic cells; in hand-foot-mouth disease, syncytial bodies are typical**

Molluscum contagiosum

Molluscum contagiosum (MC) is a common childhood viral infection that presents with firm, umbilicated papules. Single or few lesions can also be seen in adults. Widespread, extensive, or "giant" molluscum occurring in immunodeficient states, such as HIV infection, posttransplant, and iatrogenic immunosuppression can be more difficult to diagnose.[18] The differential diagnosis of diffuse umbilicated papules in an immunosuppressed patient also includes cryptococcosis, histoplasmosis, penicilliosis, paracoccidioidomycosis, coccidioidomycosis, and herpetic infection.[18] For less clinically obvious lesions, light cryotherapy to a lesion can accentuate the lesion's central umbilication, with clear change compared to background skin (Fig 2, *A*). Dermoscopy reveals white or yellow lobules surrounded by a crown of blood vessels that do not cross the centers of the lobules[19,20] (Fig 2, *B*).

Both Tzanck smear and potassium hydroxide (KOH) preparations also can be used to diagnose MC. To prepare the slide, the central part of the lesion is scraped using a no. 15 scalpel or 3-mm curette, then applied in a thin layer to a glass slide. Either KOH or Tzanck stain is applied. The virally transformed keratinocytes have large inclusions (30-35 μm), which are ovoid with homogenous basophilic or slightly eosinophilic staining on Tzanck smear (Fig 2, *C* and *D*). These Henderson–Patterson, or molluscum bodies, are pathognomonic for MC.

Other viral infections

Orf (ecthyma contagiosum) and milker's nodules are parapox viral infections that arise mainly in farmers or meat industry workers and result in localized skin infections that resolve spontaneously. Tzanck smear of a lesion reveals eosinophilic inclusions, Guarnieri bodies, acantholysis, necrotic cells, and leukocytes.[11]

Hand-foot-mouth disease (HFMD) is a common, highly infectious, self-limited disease caused by enteroviruses, most commonly coxsackie A16 and enterovirus 71. Atypical, severe presentations have been reported secondary to coxsackie A6.[21] The diagnosis can be made clinically, histologically, or by PCR. Tzanck smear can help differentiate HFMD from herpetic infection. In HFMD, affected nuclei are similarly sized, only slightly enlarged, and are grouped together in a large mass, whereas in herpetic infection, nuclei are much more enlarged, with characteristic changes in nuclear features.[11,22] Key microscopic features of viral infections are listed in Table I.

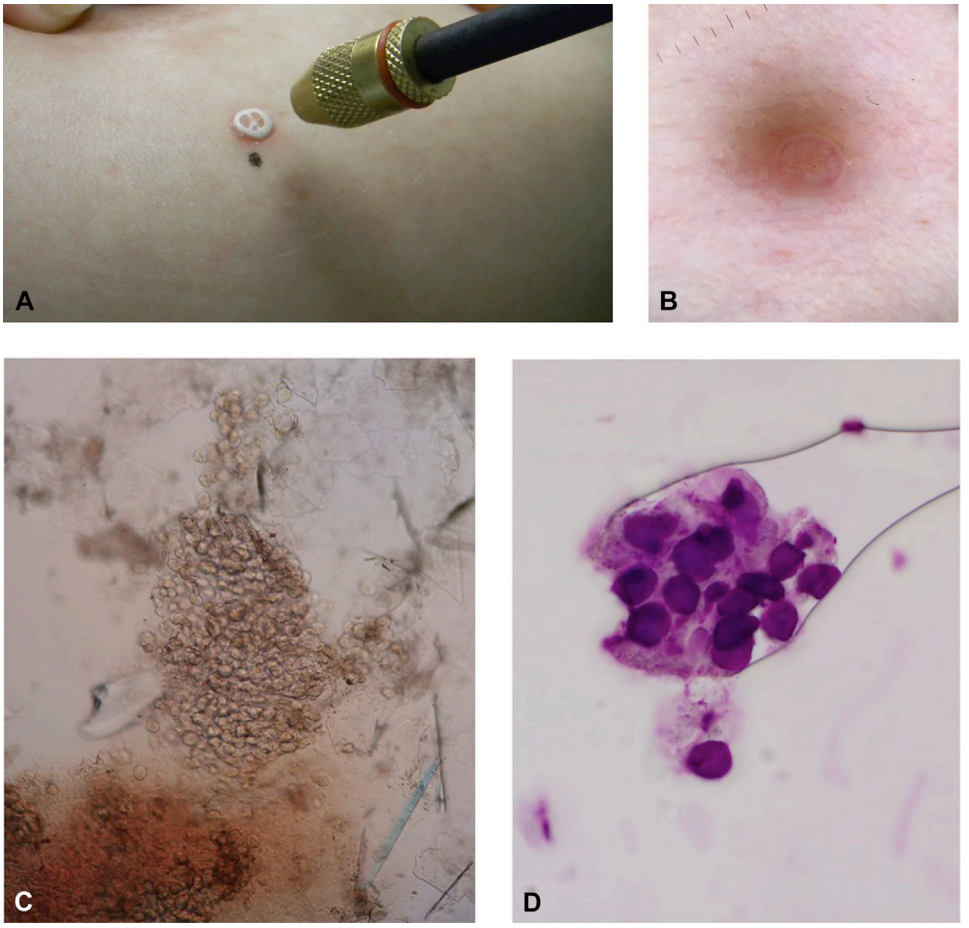

Fig 2. Molluscum contagiosum bedside diagnostics. **A**, Cryotherapy shows central umbilication. **B**, Dermoscopy demonstrates white or yellow lobules surrounded by a crown of blood vessels. **C**, Potassium hydroxide preparation demonstrates monomorphic ovoid cells that have been virally transformed. **D**, Tzanck preparation highlights the monotonous, homogenous pigmentation with pink or basophilic cytoplasm. (Photograph in **A** courtesy of Brian Swick, MD.)

Table I. Key features of viral infections using bedside diagnostics

Disease	Technique and stain	Microscopic appearance
Herpetic infections (herpes simplex virus or varicella zoster virus)	Tzanck smear performed on base of lesion	Multinucleate keratinocytes, acantholysis, keratinocyte ballooning, and nuclear margination
Molluscum contagiosum	Tzanck or potassium hydroxide	Monomorphic, cuboidal, pathognomonic 30-35 μm virally transformed cells
Hand-foot-mouth disease	Tzanck on base of the lesion	Syncytial bodies: nuclei are similarly sized, only slightly enlarged, and can be grouped together in a larger mass
Orf and milker's nodules	Tzanck on base of lesion	Eosinophilic inclusions, Guarnieri bodies, acantholysis, necrotic cells, and leukocytes

BACTERIAL INFECTIONS

Gram stain

Key point

- **Gram stain enables rapid confirmation of the presence of bacteria and helps guide early empiric therapy for Gram-positive or -negative organisms**

In 1884, Hans Christian Gram first described the technique for bacterial staining that later would bear his name.[23] This method has since been slightly modified, but the basic principles remain the same. Ionic interactions are responsible for the coloration of bacteria.[24-26] Gram-positive organisms are stained purple by crystal violet, whereas Gram-negative

organisms are stained pink by the safranin counterstain. Atypical mycobacterial organisms can sometimes stain slightly positive or equivocally.

The procedure involves spreading a thin layer of the substance to be identified onto a slide and fixing it either by air-drying or heat. Alcohol can be used to aid fixation. Crystal violet is applied and then rinsed with water or dilute iodine after 30 to 60 seconds. Next, Gram iodine is applied, and the specimen is rinsed again with water after 30 to 60 seconds. Decolorizer, such as acetone or alcohol, is applied until the runoff is clear. Finally, safranin is applied as a counterstain for an additional 30 to 60 seconds. The slide is rinsed and left to dry.[27]

GRAM-POSITIVE AND -NEGATIVE BACTERIAL INFECTIONS

Folliculitis

Key points

- **Folliculitis is a common inflammation or infection of the hair follicle that can be caused by several bacterial and nonbacterial organisms**
- **Gram stain can provide initial diagnostic clues to the causative organism**

Although folliculitis can be caused by physical or chemical injury, most cases are caused by bacterial, fungal, viral, and parasitic infections. Factors that increase the risk of folliculitis include immunosuppression, chronic kidney disease, diabetes mellitus, poor hygiene, and chronic wounds.[28] *Staphylococcus aureus*, a Gram-positive organism, is the most common cause of folliculitis.[29] Gram-negative folliculitis can occur because of *Pseudomonas*, *Proteus*, *Klebsiella*, *Escherichia*, and *Enterobacter* and can be a complication of chronic antibiotic treatment in acne.[30-32] Gram-negative folliculitis can also occur after exposure to contaminated warm water, most commonly from a spa pool, Jacuzzi, or swimming pool.

In addition to bacterial causes of folliculitis, fungal and noninfectious etiologies exist. These are discussed below and in the second article in this series.

Gram stains are the bedside diagnostic test of choice for folliculitis, although KOH preps of pustular lesions can help rule out fungal etiologies. Initial identification of the causative microbe (usually Gram-positive or -negative cocci or rods) involves Gram stain followed by culture. Gram stain results enable antibiotic therapy to be tailored appropriately pending the final culture results. Tzanck smear also can be useful to help identify noninfectious causes of folliculitis (discussed in more detail in the second article in this series).[28,33,34]

GRAM-POSITIVE INFECTIONS

Key points

- **Staphylococcal infections can be diagnosed using Gram stain**
- **Frozen section examination can help differentiate staphylococcal scalded-skin syndrome from toxic epidermal necrolysis**

Impetigo and staphylococcal scalded-skin syndrome

Impetigo is a common superficial skin infection characterized by pustules and honey-colored crusted erosions.[35] Causative organisms of nonbullous impetigo include *Streptococcus* and *Staphylococcus* spp. Bullous impetigo, which presents as small vesicles that evolve into flaccid bullae with clear then purulent fluid, is primarily caused by exfoliative toxin A- and B-producing *S aureus*.[36,37]

A diagnosis of impetigo is based on the clinical presentation, Gram stain, and culture. The Infectious Diseases Society of America guidelines advocate using Gram stain for identification of the causal organism, especially for atypical cases.[38] In addition, Tzanck smear of an erosion or base of a bullous lesion may be used to distinguish bullous impetigo from other clinical mimics, including pemphigus and herpes simplex infection. Typical findings of bullous impetigo include dyskeratotic acantholytic cells with occasional cocci in clusters. Typical findings of pemphigus are discussed in the next article in this series. The sensitivity and specificity of Tzanck preparations for diagnosing impetigo have been reported to be as high as 92% and 100%, respectively.[11]

Staphylococcal scalded-skin syndrome (SSSS), the systemic form of bullous impetigo, is caused by exfoliative toxins A and B, which cleave desmoglein-1 at the epidermal granular layer. SSSS occurs mostly in neonates and children <5 years of age and presents with flexural then generalized erythema and desquamation.[39] Tzanck preparation can help rapidly differentiate SSSS from Stevens–Johnson syndrome/toxic epidermal necrolysis (SJS/TEN).[39] Tzanck smear reveals broad, superficial, acantholytic keratinocytes, as in bullous impetigo, but typically no bacteria and minimal inflammation are seen.[11,40] This appearance differentiates SSSS from SJS/TEN, in which necrotic, cuboidal basal keratinocytes are diagnostic.[41] Another rapid diagnostic test or alternative biopsy technique that can be performed in lieu of obtaining a biopsy specimen is the "jelly

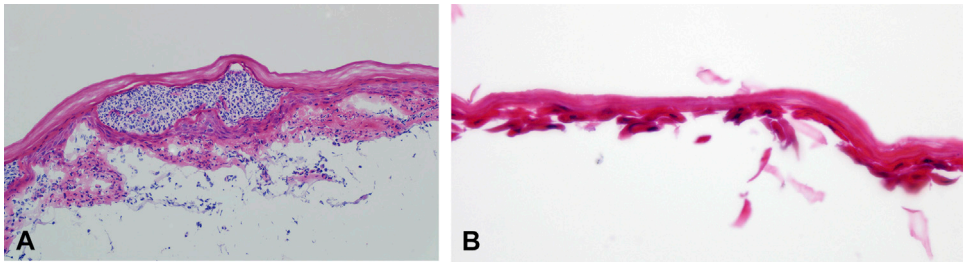

Fig 3. Jelly roll preparation can help differentiate infections. **A**, Bullous impetigo has an intraepidermal split with acantholysis, neutrophils, and cocci forms. **B**, Staphylococcal scalded-skin syndrome demonstrates acantholysis of viable cells with a split in the granular layer. These are differentiated from toxic epidermal necrolysis, which has full-thickness necrosis. (Photograph in **B** courtesy of Brian Swick, MD.)

roll'' technique, which involves peeling off a blister roof or scraping it with a blade, wrapping the sample around a cotton swab, placing the sample in sterile saline, and then processing the section in a cryostat, followed by hematoxylin–eosin (H&E) staining. Histopathology is similar to that seen using Tzanck smear (Fig 3) but also shows the level of the split: intraepidermal at the granular layer for SSSS and subepidermal caused by full epidermal necrosis in SJS/TEN.

Botryomycosis

Botryomycosis is an uncommon chronic skin infection due to masses of bacteria causing subcutaneous nodules that can progress to ulcers or draining sinuses.[42,43] Risk factors include HIV, alcoholism, diabetes, and other immunocompromised states. Trauma, surgery, or foreign body–penetrating injuries may precede infection. Common organisms include *S aureus* and *Pseudomonas aeruginosa*. Other pathogens include other Gram-positive and -negative organisms and anaerobic bacteria; it is rare for multiple organisms to infect a single patient simultaneously.[44,45] The differential diagnosis includes actinomycosis and mycetoma (see other sections).[45]

Microscopic examination of "grains," clumps of bacteria eliminated through the skin, or purulent material can assist in diagnosis. Gram stain can be performed on either the crushed grains or purulent material and may demonstrate either Gram-positive or -negative bacteria. In addition, Tzanck smear and staining of the crushed grain material will demonstrate suppurative inflammation with neutrophils and histiocytes. Occasionally, islands of radiating amorphous, eosinophilic material will be visible around clumps of bacteria, demonstrating the Splendore–Hoeppli phenomenon.[46,47]

GRAM-NEGATIVE BACTERIAL INFECTIONS

Key points

- **Ecthyma gangrenosum and meningococcemia are life-threatening conditions caused by Gram-negative bacteria**
- **Rapid diagnosis is possible using bedside touch prep and Gram stain**

Ecthyma gangrenosum

Ecthyma gangrenosum, which presents as necrotic, purpuric, and occasionally bullous skin lesions in patients with neutropenia or other forms of immunosuppression, is a life-threatening condition classically associated with *P aeruginosa* bacteremia[48-50] (Fig 4, *A*). Ecthyma gangrenosum can also occur in the absence of bacteremia because of primary inoculation[51] and may secondarily disseminate. Besides *P aeruginosa*, localized or disseminated infection with other Gram-negative bacteria, Gram-positive bacteria, angioinvasive fungi, and viruses can produce similar skin lesions.[52-54]

A diagnosis typically requires obtaining a biopsy specimen and staining with H&E and bacterial, fungal, and acid-fast tissue cultures. Unfortunately, the results of histopathologic examination and culture may not be readily available and can cause a delay in diagnosis and treatment. Touch preparations have been shown to be useful in patients presenting with ecthyma gangrenosum–like lesions and can help guide potentially life-saving empiric treatment. To perform a touch preparation, the underside of a skin punch biopsy specimen is smeared onto 2 glass slides, the first for Gram stain, H&E, or modified Giemsa–Wright stain and the second for KOH preparation with chlorazol black as a counterstain, if available. To avoid contamination, do not use a specimen that will be submitted for tissue culture. Other techniques that

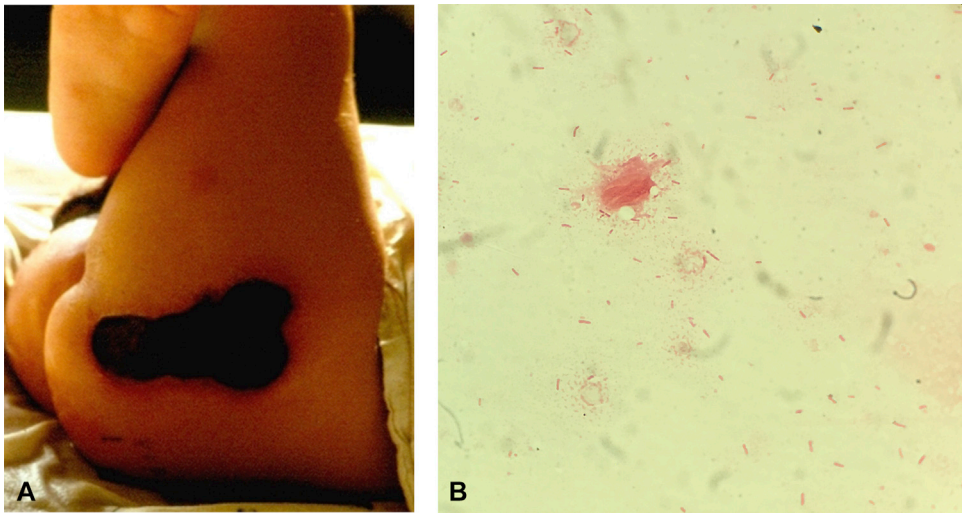

Fig 4. Ecthyma gangrenosum. **A**, Clinical appearance with necrotic epidermis and gray to violaceous border, often on the buttocks or upper thighs. **B**, Touch preparation with Gram stain demonstrates numerous Gram-negative rods. (Oil immersion; original magnification: **B**, ×100.)

may increase the yield of a touch preparation include smearing the fluid from a hemorrhagic or purulent blister, as well as scraping the edges of the skin defect made from the biopsy specimen with a no. 15 blade and smearing the residue on glass slides. Hemorrhagic necrosis can indicate *P aeruginosa* infection, while diffuse inflammatory cells are more often associated with Gram-positive infections.[55] Bacterial organisms may be visible, and Gram stain can help identify the causative agent (Fig 4, *B*). A KOH preparation may also reveal fungal organisms, enabling rapid adjustment of therapy (see the section on angioinvasive fungal section below).

Meningococcemia

Neisseria meningitidis is a Gram-negative diplococcus that can cause life-threatening meningitis and meningococcemia. Meningococcal infection can present as purpura fulminans, characterized by noninflammatory retiform purpura, palpable purpuric papules and nodules, or petechial rash, coagulopathy, and altered mental status.[56,57] Early recognition is crucial to reduce mortality and control the disease.

While cerebrospinal fluid (CSF) culture is the criterion standard for diagnosis, bedside Gram staining of representative skin lesions is minimally invasive and can more rapidly confirm the diagnosis.[58,59] One study found that Gram staining of CSF provided diagnostic data in only 4 of 18 patients, whereas Gram stains of skin lesions of those same patients were diagnostic in 12 (86%) of the 14 cases not identified by CSF examination.[59] The results of a skin biopsy procedure may also be positive even after the CSF has cleared. In meningococcemia, rapid diagnosis can be achieved through either Gram stain of a hemorrhagic vesicle, papule, or macule aspirate or by performing a touch preparation of a skin biopsy specimen and smearing the collected material onto a glass slide. Alternatively, a no. 15 scalpel can be used to scrape the base of a purpuric or hemorrhagic lesion. Gram staining is most likely to be positive when the patient is bacteremic. While a negative result does not rule out meningococcal infection, a positive test revealing Gram-negative diplococcic can lead to immediate life-saving intervention.

ACID-FAST BACTERIAL INFECTIONS

Acid-fast stains

Key point

- **Ziehl–Nielsen and intensified Kinyoun stains are useful for staining acid-fast bacteria**

Before the development of Ziehl–Nielsen stain, visualization of acid-fast bacteria (AFB) was less than ideal because of the presence of mycolic acid inhibiting entry of colorant into the cells. Ziehl–Nielsen stain uses heat to incorporate the stain into cell walls. More recently, other stains have been developed that allow for detection of these bacteria, some of which have advantages over traditional stains.[60]

To prepare Ziehl–Nielsen or Kinyoun stain, a small amount of material approximately 20 mm × 10 mm is placed in the center of a slide, then dried and fixed, either with or without heat. A small sample works best,

because clumps of organisms make it difficult to observe the characteristics of individual cells. The sample should be placed in the central area of the slide to ensure an even stain. For Ziehl–Nielsen, first a piece of absorbent paper to fit the slide is placed over the sample and saturated with the carbolfuchsin stain. Next, the underside of the slide is heated until steam rises but does not boil. The preparation must be kept moist with stain and steaming for 5 minutes, repeating the heating as needed. The slide is rinsed and then decolorized using 3% hydrochloric acid alcohol (3% hydrochloric acid in 95% alcohol) for 2 to 5 minutes, or until the runoff is clear. A methylene blue or malachite green counterstain is applied for 5 minutes and then rinsed.

Kinyoun stain uses the Kinyoun carbolfuchsin stain initially and does not require additional heat. It is decolorized after 3 minutes with 3% hydrochloric acid alcohol, then rinsed with water and decolorized again. A methylene blue counterstain is applied as with the Ziehl–Nielsen stain.[27] AFB appear red with both stains, and other bacteria stain blue or green depending on the counter stain.

MYCOBACTERIAL INFECTIONS

Cutaneous tuberculosis

Key points

- ***Mycobacterium tuberculosis* is the most common cause of cutaneous tuberculosis presenting in the skin**
- **Cytology can assist in the identification of acid-fast bacteria**

Cutaneous tuberculosis (TB) is most commonly caused by *Mycobacterium tuberculosis*. Additional etiologic agents include *Mycobacterium bovis* and *Mycobacterium canetti*.[61,62] Cutaneous TB is uncommon but can be seen most frequently in developing countries where TB infection is prevalent.[63,64] Although there are several clinical manifestations of cutaneous TB, scrofuloderma and lupus vulgaris are 2 of the more important forms of cutaneous tuberculosis that lend themselves to bedside diagnosis. Both forms represent active mycobacterial infection in the skin rather than a hypersensitivity reaction to indolent infection. In scrofuloderma, skin lesions are caused by direct extension of underlying TB from lymph nodes, bone, or joints, while lupus vulgaris is a chronic, progressive form of cutaneous TB occurring in patients with a moderate or high degree of immunity.

While obtaining a biopsy specimen and assessing tissue culture are typically performed for cutaneous TB, fine-needle aspiration (FNA) can be used to obtain samples for cytology and can be more sensitive than histology.[65] Scrofuloderma is more likely than other forms of cutaneous TB to have diagnostic findings on cytology after Ziehl–Neelsen or other AFB staining. Typically, scrofuloderma shows caseating necrosis with or without granulomas.[65] AFB are more likely to be seen when caseating necrosis is present.[65]

As with scrofuloderma, lupus vulgaris is typically diagnosed using culture and histopathology despite the lack of tubercle bacilli in histologic samples.[61] Cytology of FNA of lupus vulgaris skin lesions only rarely has visible AFB organisms and more commonly reveals epithelioid granulomas without caseous necrosis. Nonetheless, cytology samples are more likely to be AFB-positive than traditional histology.[65]

Leprosy

Key points

- **Slit-skin smear is useful for diagnosing leprosy**
- **Response to treatment can be monitored with this technique**

Slit-skin smear. Leprosy is caused by *Mycobacterium leprae*, an obligate intracytoplasmic parasite of macrophages and Schwann cells that cannot be cultured. The clinical presentation is variable, which can make diagnosis and response to treatment difficult. While PCR is sensitive, it remains expensive and is unavailable in many parts of the world. Slit-skin smear is effective as a bedside diagnostic tool and is used extensively in leprosy-endemic areas.[66]

In the slit-skin smear procedure, a sample is taken from a small incision in the skin. To ensure that an adequate sample is taken, incisions are made in multiple locations, typically the earlobes, elbows, and knees. Samples can also be taken from active lesions.[67] Target sites are first cleaned with alcohol or acetone. The skin is pinched, and a small linear incision is made in the skin. No local anesthesia is typically necessary. Before withdrawing the blade, the wound is scraped to obtain a 5- to 7-mm long sample of dermis and fluid, which is smeared on a slide.[68] The smear is made in a linear or circular manner on the slide, no larger than a pencil eraser (5-7 mm), beginning peripherally and ending in the center, leaving a central "button" (2-4 mm) that can be easily focused upon with the microscope (Supplemental Video 2, available at http://www.jaad.org). After drying, slides are placed on a staining rack and flooded with 10% formalin for 15 minutes for fixation. Ziehl–Neelsen or Kinyoun stains are performed as above. Slides should be air-dried rather

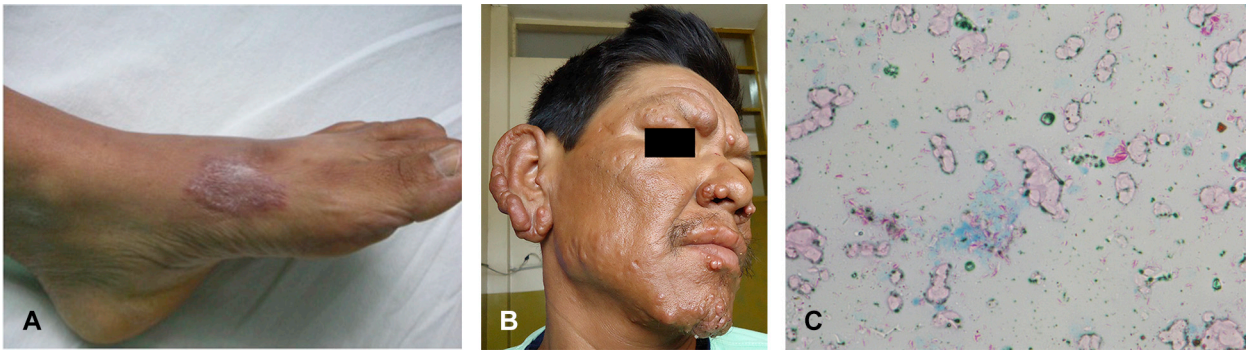

Fig 5. **A**, Patients on the tuberculoid pole of leprosy have few organisms and a high immune response with few hypopigmented anesthetic lesions compared to those on the lepromatous pole (**B**), who have a low immune response and numerous organisms with indurated plaques. **C**, A slit-skin smear performed on a patient with lepromatous leprosy demonstrates numerous acid-fast bacilli after staining. (Oil immersion; original magnification: **C**, ×100.)

than heat-fixed. Repeat smears should be obtained from multiple areas to increase the diagnostic yield. Positive smears are defined by the presence of acid-fast (red) bacterial rods (Fig 5).

Slit-skin smear allows for an estimation of the bacterial index, measuring the number of AFB present. This value is an estimate of the bacterial load and allows for monitoring of disease progression and response to treatment.[68] In multibacillary leprosy (>5 lesions), the sensitivity of slit-skin smear is 59.8%, compared to 85.9% for PCR. In paucibacillary cases, the difference is more substantial (1.8% vs 75.4%, respectively). While cases of lepromatous or histoid leprosy can be diagnosed with 100% sensitivity by either slit-skin or PCR, there is definite increased diagnostic value to PCR in diagnostically challenging cases ($P < .0001$).[66] Still, in cases with high pretest probability and in places where the cost and availability of PCR are an issue, slit-skin smear is a rapid and useful bedside diagnostic test.

Buruli ulcer

Key points

- **Buruli ulcer is caused by *Mycobacterium ulcerans***
- **Samples from edematous areas can help improve the diagnostic yield**

Buruli ulcer, caused by *M ulcerans*, is the third most common mycobacterial disease after tuberculosis and leprosy and is frequently observed in Africa and other humid, tropical regions.[69] This condition typically begins with a painless nodule that slowly breaks down to form an ulcer with extensively undermined edges, the result of digestion of the skin and soft tissue by a macrolide toxin (mycolactone). Cases are diagnosed according to clinical appearance. Microscopic examination of swabs from ulcers or smears from biopsy specimens using Ziehl–Neelsen stain is a first-line test in many endemic countries because of its low cost and rapid results. Sensitivity varies with the clinical lesion examined, ranging from about 40% in the ulcerative form to 60% in the nodular and 80% in the edematous form.[70] Two samples are recommended to confirm the diagnosis.[71] The swabs are more likely to be positive if taken from beneath the undermined edges of ulcers compared to the ulcer center.[71] The sensitivity of PCR and histopathology does not vary with the clinical form of the disease and is as high as 98% and 90%, respectively.[70-72] In many endemic areas, however, there is no access to either confirmatory test.

Table II provides a summary of key diagnostic features for these infections.

FUNGAL INFECTIONS

Key points

- **Direct microscopy using KOH is a simple, inexpensive, and fast way to diagnose fungal infections**
- **The addition of other stains, including chlorazol black E and calcofluor white, may improve the diagnostic yield**

Direct microscopy of fungal elements commonly uses KOH to help dissolve the background keratinocytes for enhanced visualization of hyphae or yeast forms. To achieve the highest yield from skin scrapings, identify high-yield sites, such as the leading edge of an annular lesion or pustules, if present. Before scraping, wipe the target area with alcohol to enhance control of the scale and prevent loss to the surrounding environment. A no. 15 scalpel is used to scrape the scale or pustule at a

Table II. Key clinical and histologic features of bacterial infections using bedside diagnostics

Disease	Technique and stain	Microscopic appearance
Folliculitis	Tzanck smear followed by Gram stain of pustule	GPC or GNR (oil immersion); eosinophils, pityrsporum
Ecthyma gangrenosum	Touch preparation or slit-skin with both rapid H&E and KOH with chlorazol stain	Hemorrhagic necrosis, GNR, histiocytes, lymphocytes, hyphae if fungal
Botryomycosis	Tzanck smear or Gram stain of lesion edge, ulcer base, or drainage	Balls of GPC or GNR
Meningococcemia	Gram stain of petechial lesions	Gram-negative diplococci
Impetigo/staphylococcal scalded-skin syndrome	Tzanck smear of bullous lesion, "jelly roll" and frozen section for H&E of blister roof; alternatively, Gram stain of purulent material	GPC in clusters or chains, neutrophils, dyskeratotic acantholytic cells; intraepidermal split
Scrofuloderma	FNA with acid-fast stain of lesion	Caseous necrosis with or without granulomas; AFB possible
Lupus vulgaris	FNA with acid-fast stain of lesion	Epithelioid granulomas with occasional necrosis; AFB possible
Leprosy	Slit-skin of earlobes, elbows, knees, and active lesions	Pink to red rods positive with AFB
Buruli ulcer	FNA or touch preparation with acid-fast stain from undermined edges of ulcers	Pink to red rods positive with AFB

AFB, Acid-fast bacilli; *FNA*, fine-needle aspiration; *GNR*, Gram-negative rod; *GPC*, Gram-positive cocci; *H&E*, hematoxylin–eosin; *KOH*, potassium hydroxide.

perpendicular angle, and the scale is applied to a focal area of a glass slide. One drop of KOH (10-20%) is applied, and a coverslip is added. If only KOH is used without other dissolving agents, gentle heating of the sample will accelerate the degradation of keratinocytes. One should avoid overheating the sample, because crystals will develop, precluding accurate evaluation. If the KOH contains dimethyl sulfoxide 40%, then heating is unnecessary. To enhance contrast under the microscope and improve visualization, one should reduce microscope illumination by lowering the condenser until epithelial cells are clearly visible (Fig 6). The specimen should be scanned at 4× to find cells and evaluated at 10×, 20×, or 40× magnification to identify fungal elements. If the scale is thick, it may be necessary to break up clumps of cells by gently pushing on the coverslip with the blunt end of a pen.

For onychomycosis, obtaining subungual debris, overlying superficial scale (superficial onychomycosis), and the most proximal portion of the nail plate affected will increase the diagnostic yield.[73] When evaluating hair specimens, a Wood's lamp can help identify ectothrix organisms, in which case fluorescing hair should be epilated for evaluation. Scale should be obtained using a no. 15 scalpel. Alternate methods for obtaining scale include using a disposable toothbrush, culturette, or cotton tip. These methods may be safer, easier, and met with less resistance than using a scalpel blade in pediatric populations.[74,75]

Additional staining techniques exist that may be helpful for learners or inexperienced microscopists. Counterstain solutions containing KOH can help provide contrast between the hyphae and spores and background material.[73] Chlorazol black E (CBE) is a chitin-specific stain that turns fungal elements green to turquoise in color and is commercially available with KOH and dimethyl sulfoxide.[76,77] Swartz–Lamkins fungal stain combines KOH and Parker blue ink and stains hyphae blue against the background.[77] Calcofluor white binds to cellulose and chitin and fluoresces when exposed to ultraviolet light; this requires a fluorescent microscope, making it difficult to use in the clinical setting (Fig 7). Alternatively, a twin Wood's lamp light (365 nm) can be placed behind the microscope and shined onto the slide surface. When preparing a sample for evaluation, 1 drop of KOH and 1 drop of calcofluor white are added to the specimen.[78]

Superficial mycoses

Key points

- **Dermatophyte infection can be readily diagnosed using direct microscopy with KOH or CBE**
- **Candidiasis, pityrosporum versicolor, and pityrosporum folliculitis can be distinguished based upon morphologic appearance**

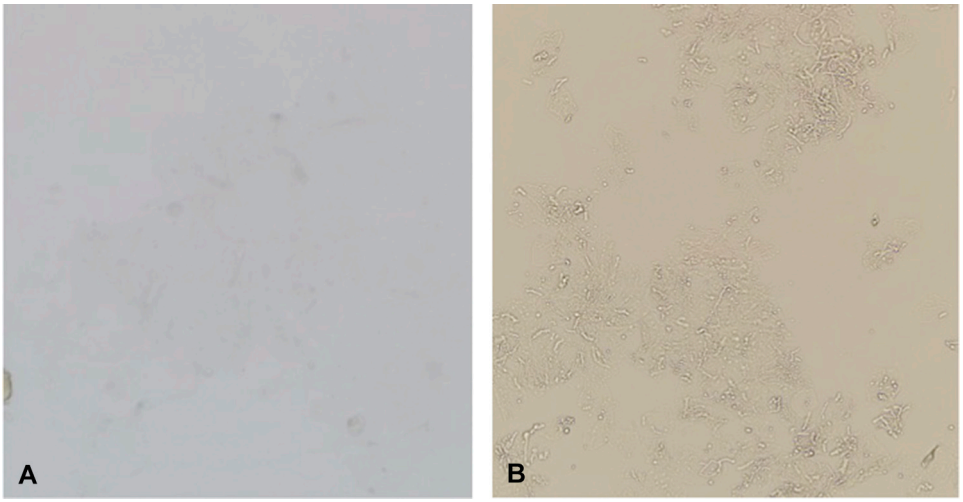

Fig 6. Condenser location matters for evaluation. For increased contrast for dermatophytes in potassium hydroxide preparations, the microscope condenser should lowered. When the condenser is up, epithelial cells and fungus will not be visualized (**A**); the same slide can demonstrate organisms with proper condenser position (**B**).

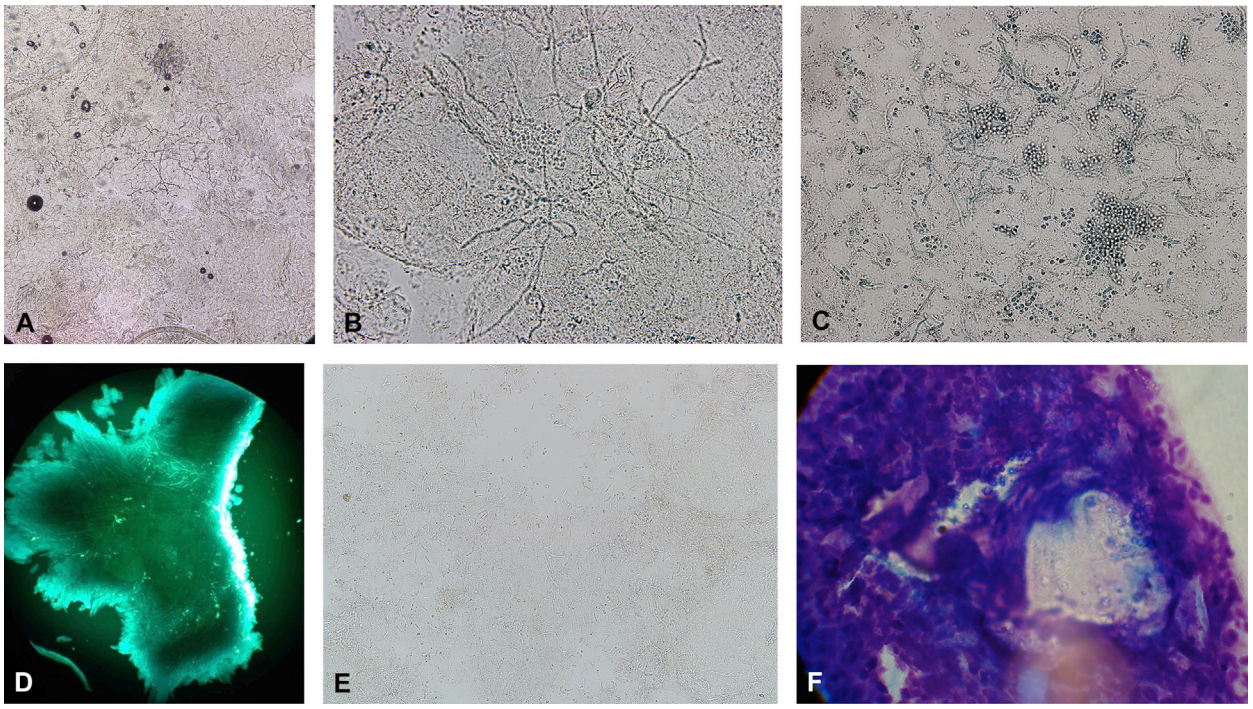

Fig 7. Superficial fungal infections can be easily detected using bedside diagnostics. **A**, Dermatophytes are identified by hyphal forms extending past cell walls on potassium hydroxide examination, **B**, Candidal infection can demonstrate spores or pseudohyphae. **C**, Chlorazol black E will cause the fungal forms to be turquoise in color compared to the background. In this case of tinea versicolor, there are short hyphal elements and spores. **D**, Calcofluor white will fluoresce the fungal elements in onychomycosis, making it easier to identify organisms. **E**, Potassium hydroxide preparation of tinea versicolor demonstrates short hyphal elements and spores. **F**, Pityrosporum folliculitis can demonstrate budding yeasts within a pustule or hair shaft. (Photograph in **B** courtesy of Brian Swick, MD. **F**, Potassium hydroxide examination; original magnifications: **A**, ×10; **B**, ×20; **D**, ×4; **E**, ×10; **F**, ×20.)

Superficial mycotic infections are caused by dermatophytes, Candida, and Pityrosporum (*Malassezia* spp.). These are common infections that result in clinically diverse presentations, including tinea capitis, tinea barbae, tinea corporis, tinea pedis, tinea cruris, tinea unguium, tinea versicolor, pityrosporum folliculitis, cutaneous candidiasis, intertrigo, nail unit infection (paronychia), vulvovaginitis, balanitis, thrush, and angular cheilitis. Risk factors for developing superficial mycoses include a history of diabetes mellitus, immunosuppression, antecedent antibiotics, and corticosteroid use.[75] History and clinical appearance may be highly suggestive, but confirmation of diagnosis is important because the differential diagnosis for these infections is broad and includes noninfectious etiologies. The presence of branching, septate hyphae extending across keratinocyte cell walls, within the nail plate, or within the hair shaft is diagnostic of dermatophyte infection[74-76,78] (Fig 7, *A-C*). Common mimickers include cell borders or membranes that have not dissolved, as well as air bubbles that are small, variably sized, round, and lack budding. Pseudohyphae and yeast forms are seen in candidal infection (Fig 7, *B*).

Tinea versicolor often can be diagnosed clinically based upon white, pink, salmon, red, tan, or brown patches with slight scale that can be accentuated with a slide, fingernail, or lateral pressure adjacent to the patches.[79,80] Direct microscopy using KOH is the simplest and least expensive way to diagnose dermatophyte infections either with a blade, microscope slide, or with the application of a piece of tape to remove superficial scale. Direct microscopy of tinea versicolor often demonstrates florid yeast forms and short hyphae ("spaghetti and meatballs" or "penne and peas"; Fig 7, *E*).

Published studies have evaluated the sensitivity and specificity of KOH compared to CBE, calcofluor white, fungal culture, PCR, and periodic acid–Schiff on formalin-fixed tissue with varying results depending on the specimen (eg, onychomycosis compared to skin scrapings) and study.[73,75,76,78,81-87] For superficial skin infections, such as tinea corporis, tinea pedis, and tinea versicolor, KOH, CBE, and calcofluor white have similar results.[78,86] Direct comparisons of KOH and calcofluor white demonstrate similar sensitivities (88% and 92%), specificities (both 95%), positive predictive values (73% and 74%), and negative predictive values (98% and 99%), respectively.[78] For onychomycosis, direct identification with KOH has published sensitivities ranging from 80% to 91%, with false negative reports ranging from 5% to 15%.[75,82,85] Periodic acid–Schiff of the nail plate is reported to be the most sensitive (92-98.8%), with CBE also highly sensitive (94.3%). Fungal culture has an overall lower sensitivity (23.8-79.3%).[82,85,87]

SUBCUTANEOUS MYCOSES

Key points

- **Chromoblastomycosis is an infection that presents with verrucous nodules and plaques that is most common in tropical and subtropical climates and is characterized by pigmented bodies ("copper pennies") seen on direct microscopy**
- **Lobomycosis is endemic in rural areas of Central and South America and is characterized by keloidal plaques, which can be diagnosed by direct visualization of single or coupled budding, thick-walled spherules with a thin connection ("pop beads"); the organism cannot be cultured**
- **Application of vinyl adhesive tape is an alternative method of obtaining a sample suitable for direct visualization**

Subcutaneous mycotic infections result from infection with slow-growing fungi affecting the dermis and subcutaneous tissue, resulting in verrucous and keloidal plaques and subcutaneous nodules (Table III). Direct visualization is essential for diagnosis of these entities, which are difficult to culture and most common in resource-limited settings.

Chromoblastomycosis is a subcutaneous fungal infection that most commonly occurs in tropical and subtropical climates. The causative organisms of chromoblastomycosis are extremely slow-growing and difficult to culture, so direct microscopic examination is an essential diagnostic tests. Black dots within the verrucous plaques of chromoblastomycosis represent transepidermal elimination of the organism (Fig 8, *A*). Scraping these pigmented areas with a scalpel blade results in the highest diagnostic yield for KOH preparations. Pigmented bodies, also called sclerotic bodies, muriform bodies, medlar bodies, or copper pennies, are pathognomonic; these are 4- to 12-μm globe-shaped, cigar-colored, thick-walled structures with occasional small septate hyphae[88,89] (Fig 8, *B*).

Lobomycosis is endemic in rural areas of Central and South America and is characterized by keloidal plaques. The organism, *Lacazia loboi,* cannot be cultured, so direct visualization is necessary for diagnosis (Fig 9, *A*). Scraping scale or the keloidal

Table III. Key fungal infections with clinical and microscopic features

Disease	Clinical features	Microscopic appearance
Superficial mycoses		
Dermatophytes	Erythematous patches with superficial, leading edge scale	Hyphae extending across keratinocyte cell walls, within the nail plate, or within the hair shaft
Candidiasis	Bright red patches with satellite papules and pustules	Pseudohyphae and yeast forms
Pityrosporum (tinea) versicolor	Pink, brown, or pale patches with fine overlying scale, accentuated with stretching of the skin	Florid yeast forms and short hyphae ("penne" and meatballs)
Pityrosporum folliculitis	Red papulopustules	Yeast forms
Subcutaneous mycoses		
Chromoblastomycosis	Verrucous and keloidal plaques and subcutaneous nodules with black dots	4-12 μm globe-shaped, cigar-colored, thick-walled structures with occasional small septate hyphae ("pigmented bodies," copper pennies, or Medlar bodies)
Lobomycosis	Keloidal plaques	9-10 μm single or coupled (2) thick-walled spherules with a thin connection ("brass knuckles or pop beads")
Eumycetoma	Tumefaction (swelling), sinus formation, and grains	Grains macroscopically; thick, broad hyphae, often with septations
Systemic mycoses		
Cryptococcus	Umbilicated papulopustules, cellulitic plaques, ulcers, and abscess	Tzanck: encapsulated 5-10 μm round, dark-walled, pleomorphic yeast with clear gelatinous capsults; India ink stains the background, revealing the extracellular capsule
Histoplasmosis	Oral and perianal ulcerations, umbilicated papules, nodules, and plaques	Small (2-4 μm), with a pseudocapsule
Blastomycosis	Annular verrucous raised plaques with irregular borders and atrophic central clearing; crusted papules and plaques and subcutaneous nodules	8-15 μm round to oval organisms with thick, double-refractile walls; broad-based, single budding
Paracoccidioidomycosis	Ulcerative and eroded plaques of the oral and perianal mucosa, perioral granulomatous plaques; cutaneous lesions: crusted papules, plaques, nodules, verrucous plaques, or ulcers	Variably sized, 5-50 μm, thick-walled, round cells with narrow (2-10 μm) budding ("mariner's wheels and Mickey Mouse")
Coccidioidomycosis	Granulomatous papules, plaques, and verrucous lesions; abscesses, chronic ulcers, subcutaneous nodules, and sinus tracts	10-80 μm, variably sized spherules filled with endospores
Angioinvasive mycoses		
Aspergillus	Violaceous, indurated plaques, necrotic eschars, annular "bull's eye" infarcts, and small erythematous macules and papules	Septate, thin hyphae; acute angle branching
Scedosporium spp. and *Fusarium* spp.		Septate, irregular hyphae with bubbly cytoplasm; branching is 45-90°
Mucomycosis		Ribbon-like, aseptate hyphae with wide angle branching

plaque with a scalpel blade will obtain a specimen; direct visualization with KOH demonstrates 9- to 10-μm single or coupled thick-walled spherules with a thin connection[90-92] (Fig 9, *B*). Morphologically, these structures have been compared to "pop beads" or "brass knuckles."

The vinyl adhesive tape test has been described as a useful test for chromoblastomycosis, lobomycosis, and paracoccidiodomycosis infections.[91] Instead of using a no. 15 scalpel to scrape the lesion, the sample is collected by applying clear vinyl tape to the lesion, being sure to rub the scaly, crusty areas to remove

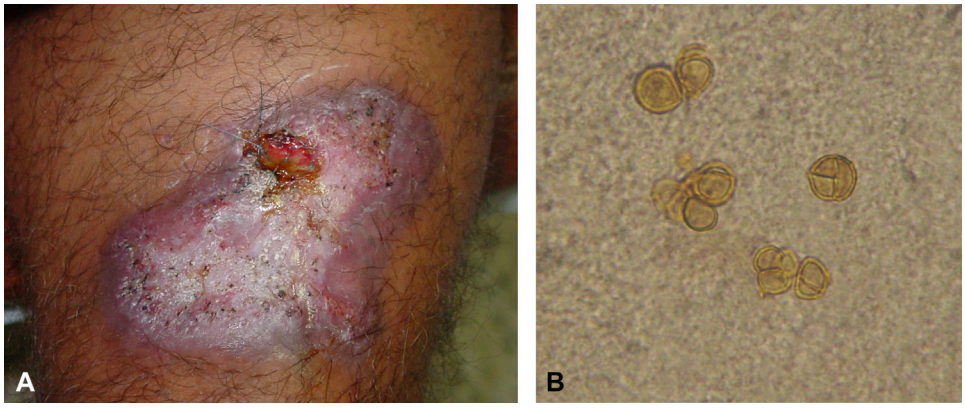

Fig 8. Chromoblastomycosis. **A**, Clinically, there are verrucous papules and plaques associated with central scarring and numerous dark dots. **B**, Scraping of the dark dots will demonstrate the pigmented spores (Medlar bodies, copper bodies) under potassium hydroxide examination. (Oil immersion; original magnification: **B**, ×100.)

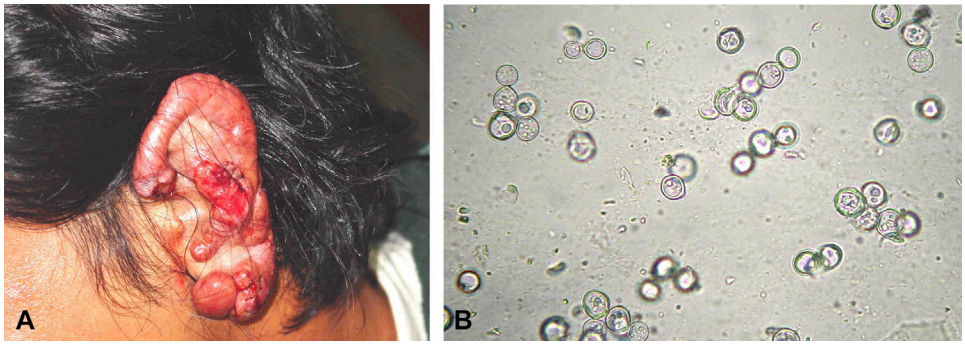

Fig 9. Lobomycosis. **A**, The clinical presentation are keloid plaques, often on ears or extremities. **B**, Using potassium hydroxide, 9- to 10-μm single or coupled thin-walled spherules with a thin connection will be observed. (Oil immersion; original magnification: **B**, ×100.)

the upper layers of the epidermis, then applying the tape to a slide with KOH. Attention should be focused on the black dots on the tissue surface, sites of transepidermal elimination of organisms, and >1 slide should be prepared to increase the diagnostic yield.[91]

Mycetomas are indolent infections characterized by tumefaction and draining sinuses and caused by either bacteria (actinomycetoma) or fungi (eumycetoma); they are most commonly found in tropical and subtropical locations (Fig 10, *A*). Botryomycosis has a similar clinical presentation (see bacteria section).[93] Grains eliminated through the sinus tracts can be seen with the naked eye. Direct examination may provide useful information, because certain organisms result in distinct colors. Black grains always signify a fungus is the causative organism[94] (Fig 10, *B* and *C*; Table IV). Grains can be obtained by squeezing sinus tracts to promote grain discharge, sometimes with the help of a no. 15 scalpel or forceps. Grains are then applied to a slide, KOH is added, and pressure with another slide or the blunt end of a pen over a coverslip is used to flatten the grains. Fungi in eumycetomas will have thick, broad hyphae, often with septations[95] (Fig 10, *D*). Actinomycetomas are characterized by bacteria with thin, fine filaments (Fig 10, *E*). Additional staining can be performed, including Gram stain or Grocott methenamine silver stain if further identification is needed.

SYSTEMIC FUNGAL INFECTIONS

Key points

- **Cutaneous lesions may be the presenting manifestation of disseminated fungal infections, including cryptococcosis, histoplasmosis, blastomycosis, paracoccidioidomycosis, and coccidioidomycosis**
- **Direct microscopy with KOH, CBE, and Tzanck smear can demonstrate differentiating morphologic features that allow for rapid diagnosis that can be confirmed with culture**

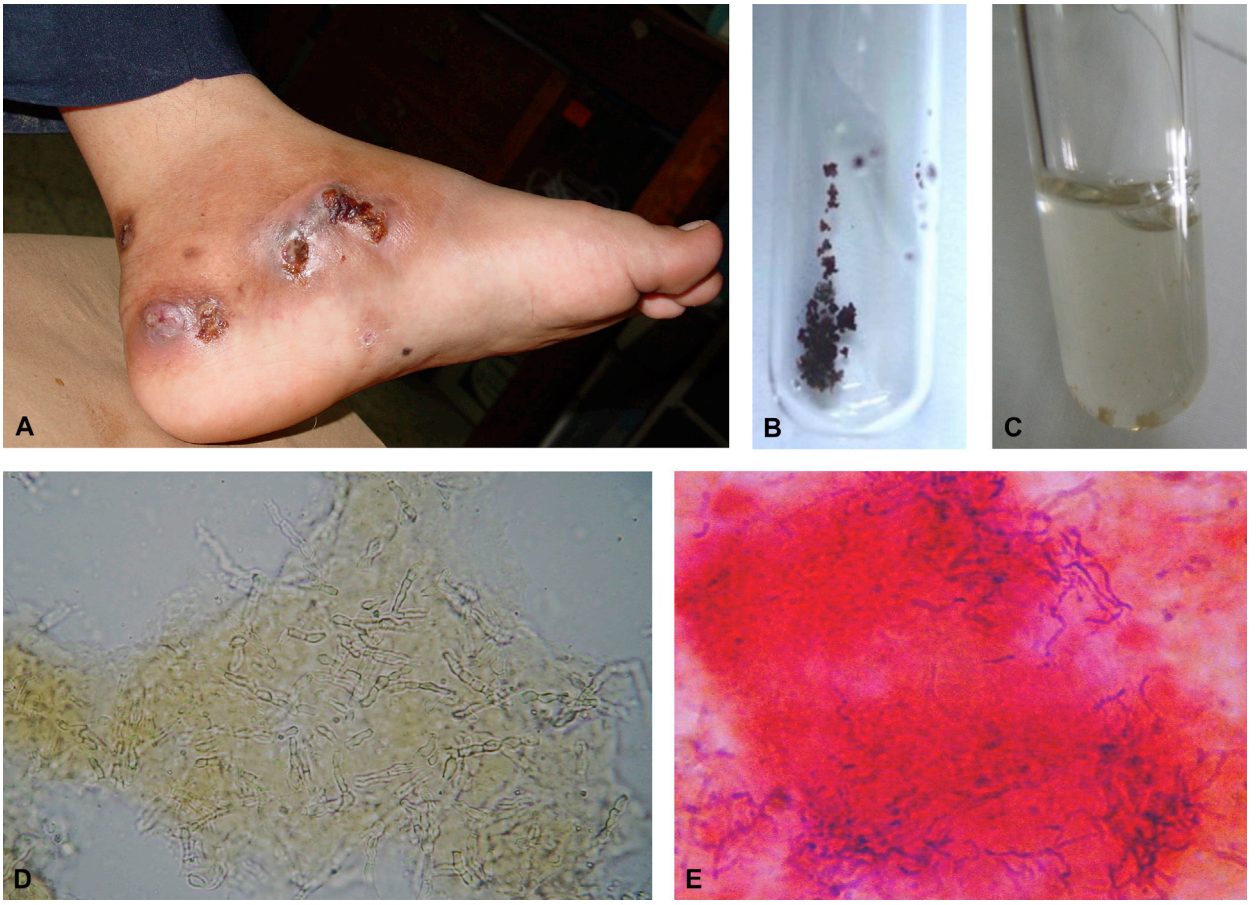

Fig 10. Mycetomas. **A**, The key clinical features are swelling (tumefaction), sinus tract formation, and grains. **B**, Black grains always signify a fungal organism. **C**, Yellow grains could represent bacterial or fungal organisms. **D**, Potassium hydroxide examination of hyphae confirms eumycetoma or a fungal origin. **E**, Gram stain with presence of Gram-positive filamentous bacteria confirms actinomycetoma as the diagnosis. (**D** and **E**, Oil immersion; original magnifications: **D** and **E**, ×100.)

Table IV. Mycetoma grain color and associated organisms*

Grain color	Organisms
Black	*Madurella* spp. (*M mycetomatis*, *M grisea*), *Leptospheria senegalensis*, *Exophiala jeanselmei*, *Pyrenochaeta romeroi*, *Curvularia lunata*, *Phialophora verrucosa*, and *Phytophthora parasitica*
Pale	*Pseudoallescheria boydii*, *Aspergillus* spp., *Fusarium* spp., *Acremonium* spp., *Phaeoacremonium* spp., *Neotestudina rosatii*, *Nocardia* spp. (*N brasiliensis*, *N cavae*, and *N asteroides*), and *Actinomadura madurae*
Red	*Actinomadura pelleitieri*
Yellow	*Streptomyces somaliensis*

*Data from Ramos-E-Silva et al.[92]

Systemic dimorphic fungal infections that can have cutaneous manifestations include cryptococcosis, histoplasmosis, blastomycosis, paracoccidioidomycosis, and coccidioidomycosis. Primary infection often occurs via inhalation, leading to mild or asymptomatic pulmonary disease. In susceptible hosts, hematogenous dissemination to multiple organs, including the skin, can occur. Importantly, cutaneous manifestations can be the presenting sign of systemic illness. However, direct inoculation of the skin can also occur, leading to primary cutaneous disease. Table III contains an overview of these infections.

Direct microscopic examination can yield rapid diagnosis and enable faster initiation of therapy in patients who are often systemically ill.

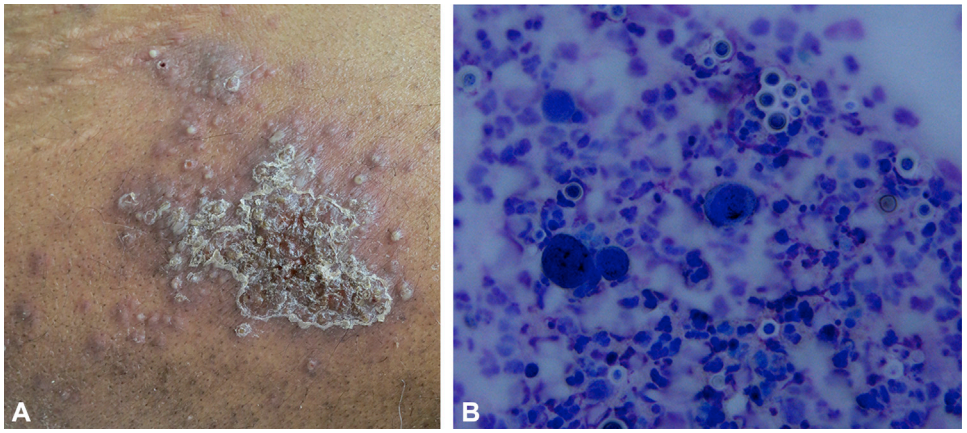

Fig 11. Cryptococcus. **A**, Numerous umbilicated papules, pustules, and plaques are one of the presentations of cutaneous Cryptococcus. **B**, Tzanck smear demonstrates yeast forms with a gelatinous capsule as demonstrated by a halo. (Original magnification: **B**, ×40.)

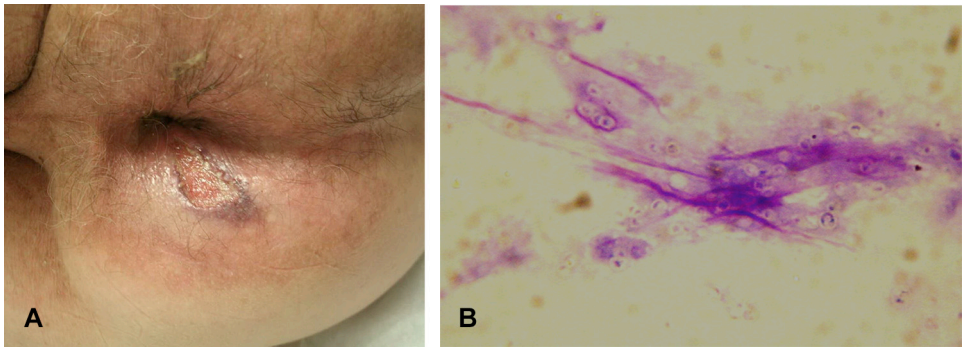

Fig 12. Histoplasmosis. **A**, Perianal ulcer, one of the manifestations of histoplasmosis. **B**, Giemsa stain can help highlight the very small organisms that have artifactual clearing around them. (Oil immersion; original magnification: **B**, ×100.)

Cryptococcosis is caused by *Cryptococcus* spp., most commonly *Cryptococcus neoformans*, which is found worldwide in bird droppings. It can have varied clinical presentations in immunocompromised patients, including umbilicated. resembling molluscum contagiosum. To obtain a specimen, one can use a no. 15 blade to scrape contents from the center of an umbilicated lesion, crust, papule, or pustule. Tzanck smear demonstrates 5- to 10-μm encapsulated, round, dark-walled, pleomorphic yeast with clear, unstained gelatinous capsules (Fig 11). India ink stains the background, revealing the extracellular capsule.[76,96,97] Capsules stain with methylene blue, Alcian blue, and mucicarmine.

Histoplasmosis (*Histoplasmosis capsulatum)* is found worldwide but is endemic to the Midwest, especially in states that border the Ohio River valley and Mississippi River. Cutaneous lesions include oral and perianal ulcerations, umbilicated papules, nodules, and plaques. Direct microscopic examination of lymph nodes or cutaneous lesions can be performed using a Tzanck smear (Giemsa stain), which has greater sensitivity than a KOH preparation. On examination, the organisms are small (2-4 μm), with a pseudocapsule.[76,98] If macrophages are present, engulfed intracellular organisms may be visualized (Fig 12).

Paracoccidioidomycosis is an infection endemic to South and Central America that can present with pulmonary symptoms, lymphadenopathy, and hepatosplenomegaly. It preferentially involves the mucous membranes with ulcerative and eroded plaques of the oral mucosa (moriform or Aguiar-Pupo stomatitis) and oropharynx, perioral granulomatous plaques, and perianal involvement. Cutaneous lesions may appear as scattered crusted papules, verrucous plaques, nodules, or ulcers and result from hematogenous spread in up to 25% of infections[99] (Fig 13, *A* and *B*). Direct visualization using a KOH preparation can be more sensitive than culture secondary to contaminating organisms, with a diagnostic yield of >90%. Organisms are variably

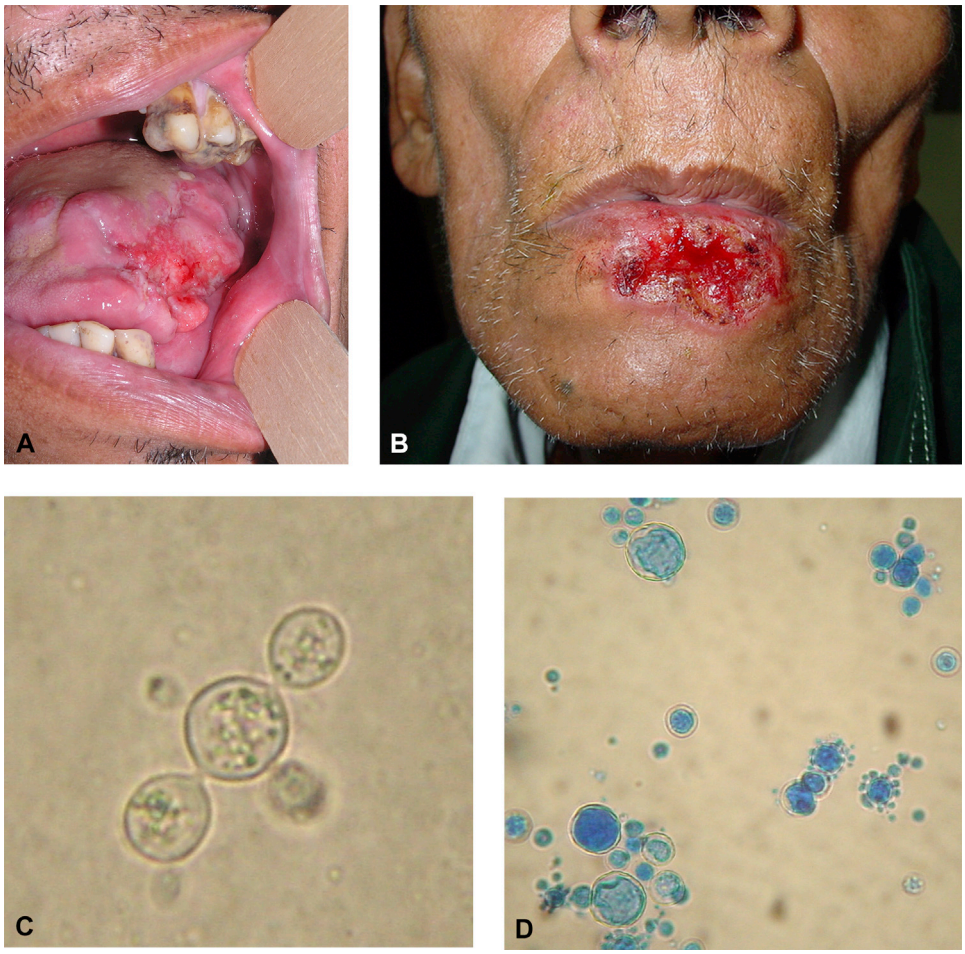

Fig 13. Paracoccidioidomycosis. **A**, Intraoral involvement is common with ulcerated plaques with small red dots. **B**, Perioral lesions are also common, with similar ulcerated plaques with hemorrhagic crust. **C**, Using potassium hydroxide, variably sized yeast forms with narrow budding can be seen, which may represent a mariner's wheel or Mickey Mouse. **D**, Lactophenol can provide additional contrast compared to background bacterial elements and will color the fungus blue. (Oil immersion; original magnification: **C** and **D**, ×100.)

sized, 5 to 50 μm, thick-walled, round cells with narrow (2-10 μm) budding[76,100] (Fig 13, *C*). Morphologically, they are said to have a "Mickey Mouse" or "Mariner's wheel" appearance. One can enhance visualization of the fungus with use of lactophenol cotton blue, especially if the specimen has significant contamination. This technique is performed by first adding alcohol to the sample to fix it, then adding the lactophenol/cotton blue stain to the specimen (Fig 13, *D*). The phenol and lactic acid kill live organisms, while preserving the fungal structures, and the cotton blue stains chitin in the cell walls blue. Another test reported to be successful in the diagnosis of paracoccidioidomycosis is the vinyl adhesive tape method, with technique the same as that used for chromoblastomycosis and lobomycosis.[91]

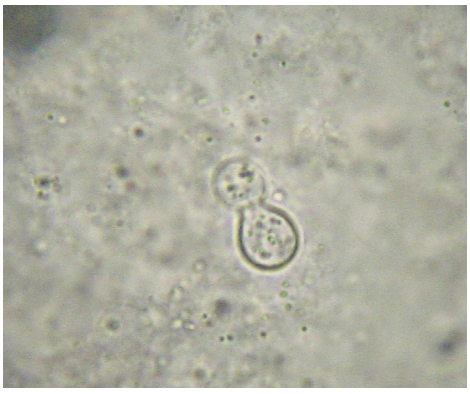

Fig 14. Blastomycosis. Potassium hydroxide examination will demonstrate broad-based budding organisms. (Oil immersion; original magnification: ×100.)

Blastomycosis is caused by *Blastomyces dermatitidis* and occurs predominantly in North America, with the highest number of reported cases in the Midwest and Canada. In the skin, it can present as annular, verrucous, raised plaques with irregular borders and atrophic central clearing (so-called "stadium seating").

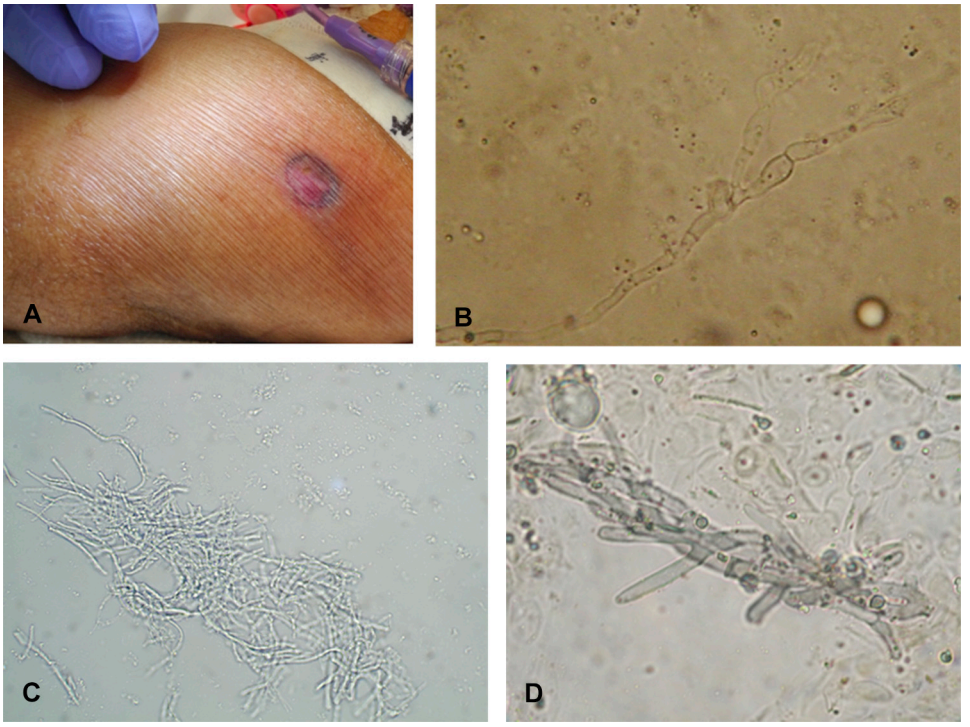

Fig 15. Angioinvasive fungal infection. **A**, Clinically, there are violaceous papules, plaques, or nodules as the fungus is targeting the blood vessels, resulting in significant hemorrhage. **B**, *Aspergillus* spp. will have septations and demonstrate acute angle branching. **C**, *Fusarium* spp. have smaller and more irregular hyphae with septae and bubbly cytoplasm. The branching will be more variable, from 45° to 90°. **D**, Mucormycosis are irregular, broader (10-25 μm), and aseptate hyphae with wide angle branching. (**B**, Oil immersion; original magnifications: **B**, ×100; **C** and **D**, ×40.)

Crusted papules and plaques and subcutaneous nodules also occur.[101] Direct microscopy with KOH reveals 8- to 15-μm round to oval organisms with thick, doubly refractile walls. Broad-based, single budding is also observed[76,102] (Fig 14).

Coccidioides immitis and *Coccidioides posadasii* are the causative organisms in coccidioidomycosis, which is endemic in the western/southwest United States and northern Mexico. Cutaneous manifestations include granulomatous papules, plaques, and verrucous lesions, abscesses, chronic ulcers, subcutaneous nodules, and sinus tracts, characteristically on the head and neck.[103-105] Direct microscopy after KOH or Tzanck smear enables visualization of the organisms, which are variably sized, 10- to 80-μm spherules filled with endospores.[76,104]

ANGIOINVASIVE FUNGAL INFECTIONS

Key points

- **Angioinvasive fungal infections (*Aspergillus, Fusarium, Scedosporium, Mucor, Rhizopus, Cunninghamella,* and *Lichtheimia* [*Absidia*]), are associated with high morbidity and mortality**
- **Early diagnosis of skin lesions using touch preparation can identify life-threatening cases of disseminated infection**

Aspergillus, *Fusarium*, *Scedosporium*, *Mucor*, *Rhizopus*, *Cunninghamella*, and *Lichtheimia (Absidia)* are ubiquitous saprophytic organisms that can cause devastating angioinvasive fungal infection with high morbidity and mortality.[106-109] Risk factors include neutropenia and other forms of immunosuppression. Skin involvement can occur from direct inoculation or, more frequently, from hematogenous spread. Cutaneous manifestations include violaceous, indurated plaques, necrotic eschars, annular "bull's eye" infarcts, and small erythematous macules and papules[110-114] (Fig 15, *A*).

Direct microscopic examination can help diagnose these infections. Touch preparation is performed by smearing the base of a punch biopsy specimen on a slide or by scraping a small amount of tissue from the wound base. KOH, CBE, calcofluor white, or other special stains can be used for immediate evaluation.[112,114] The microscopic appearance can predict the causative organism and enable rapid institution of appropriate therapy. *Aspergillus* spp.

have thin, septate hyphae with acute angle branching[95] (Fig 15, *B*). *Scedosporium* spp. and *Fusarium* spp. also have septae but demonstrate smaller and more irregular hyphae and bubbly cytoplasm; branching is 45° to 90° (Fig 15, *C*). Mucormycosis (zygomycetes), including *Mucor*, *Rhizopus*, *Cunninghamella,* and *Lichtheimia (Absidia)*, have irregular, broad (10-25 μm), ribbon-like, aseptate hyphae with wide angle branching[112,114] (Fig 15, *D*). Distinguishing mucormycosis from other angioinvasive fungal infections is crucially important because these organisms are resistant to voriconazole, the first-line antifungal therapy for *Aspergillus*.

In conclusion, direct, provider-performed microscopy enables rapid diagnosis and early initiation of treatment in numerous viral, bacterial, and fungal infections, providing a therapeutic advantage that is particularly significant in resource-limited and high acuity settings. Development of these bedside diagnostic skills through practice and frequent use is necessary to establish confidence. The second article in this continuing medical education series discusses the use of bedside diagnostic tests for parasitic disorders and noninfectious dermatologic conditions.

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