American Journal of Clinical Dermatology (2020) 21:697–709
https://doi.org/10.1007/s40257-020-00526-y

Bedside Diagnostics for Infections: A Guide for Dermatologists

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Published online: 19 June 2020
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Abstract

In dermatology, there are many bedside diagnostic tests that may aid in more rapid diagnosis and early initiation of appropriate therapy. When performed correctly, these bedside diagnostic tests can provide both sensitive and specific results. We discuss bedside diagnostic tests, such as the Tzanck smear, potassium hydroxide (KOH) preparation, and mineral oil preparation, with a specific focus on their use in diagnosing infectious dermatoses.

Key Points

Bedside diagnostic techniques for cutaneous infections may lead to early diagnosis and prompt initiation of the appropriate therapy.

With training, many bedside diagnostic tests are easy to perform and may have sensitivities comparable to those of more advanced methods.

In the USA, Clinical Laboratory Improvement Act (CLIA) laboratory regulations must be considered and may limit the use of bedside diagnostic tests in clinical practice.

1 Introduction

Infectious dermatoses constitute a major part of clinical practice in dermatology, both in the clinic and on inpatient wards. Rapid and accurate diagnosis is key to promptly initiating the appropriate therapy. Bedside diagnostic tests, when performed correctly, can provide both sensitive and specific results. When caring for critically ill patients on inpatient wards, prompt diagnoses are crucial and potentially lifesaving. Furthermore, bedside diagnostic tests may allow physicians in resource-poor areas to provide high-quality care. While many of these tests require a certain amount of training and skill to produce reliable results, in the right hands they may be of great value. We discuss bedside diagnostic tests for the diagnosis of infectious dermatoses.

2 Tzanck Smear Overview

The Tzanck smear, first described in 1947 by Dr. Tzanck, is a cytologic test best known for aiding in the diagnosis of *Herpesviridae* infections [1]. However, the Tzanck smear is a rapid bedside test that can be used to aid in the diagnosis of many infectious and noninfectious dermatologic diseases [2–4]. While the sensitivity and specificity of Tzanck smears is user dependent, competency in performing and interpreting Tzanck smears can be achieved with training and practice [5].

A Tzanck smear should be prepared from a recent lesion or intact vesicles for greatest diagnostic sensitivity [6]. For vesiculobullous diseases, such as herpes infection, it begins with unroofing intact vesicles or removing overlying crust from the desired lesion [6]. The base of the lesion is then

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scraped firmly, typically with a number 15 scalpel blade, to obtain an adequate sample (Fig. 1). The tissue sample should be smeared thinly across a clean microscopic slide and allowed to air dry, with or without the use of gentle heat [2]. Fixatives, such as methanol, may be used to preserve the sample and minimize artifacts caused by the drying process [3]. Such fixatives are often added to commercially available stains. Next, the slide is stained with Wright–Giemsa stain or methylene blue [6]. Modified Wright–Giemsa stains such as Diff-Quik or Hemacolor are widely available and commonly used and only require 1–2 min for adequate staining [3, 7]. Finally, the specimen is gently washed with water to remove excess stain and dried before applying a cover slip.

In the USA, laboratory requirements greatly limit the use of Tzanck smears. The Tzanck smear is considered a moderate-complexity test, which under the Clinical Laboratory Improvement Act (CLIA) of 1988 limits its use to certified laboratories. Many dermatology offices outside of academic centers do not pursue CLIA certification for this test and are therefore unable to perform Tzanck smears. Dermatology offices that offer Mohs surgery are also required to meet CLIA standards within their laboratories to process tissue. These laboratories may be used to perform Tzanck smears. It is also worth mentioning that a certain amount of training and skill is required to accurately perform and interpret Tzanck smears. Further, if clinicians do not regularly utilize

Fig. 1 Preparing a Tzanck smear. **a** The lesion is unroofed and **b** the base of the lesion is scraped and **c** smeared thinly onto a glass slide. **d** The slide is flooded with Wright-Giemsa for 1–2 min before, **e** rinsing with water. **f** The slide is rinsed until the runoff is clear

this test, their ability to reliably interpret the stain diminishes [5].

2.1 Herpesviridae

Infection with or reactivation of herpes simplex virus (HSV) types 1 or 2 and varicella zoster virus is frequently encountered in clinical practice. In such cases, prompt diagnosis and early initiation of treatment are essential to lessen disease severity and decrease complications such as postherpetic neuralgia. The Tzanck smear is not as sensitive as polymerase chain reaction (PCR) and is unable to differentiate between different types of herpetic infections, but it is more rapid to perform [2, 3]. The ideal lesion for preparing a Tzanck smear is an early intact vesicle. The vesicle is unroofed, and the base of the lesion or the underside of the blister roof is scraped to prepare a specimen using the procedure described in Sect. 2. A diagnosis of herpes virus infection or reactivation is confirmed by the presence of acantholytic keratinocytes that exhibit characteristic viral changes, including multinucleation, molding of nuclei, and margination of chromatin (Fig. 2) [8]. These multinucleated giant cells are sometimes referred to as Tzanck cells [7]. Immunofluorescent staining using herpes virus antibodies may add additional value to bedside smears in diagnosing herpetic infections; however, this requires the use of a fluorescent microscope and fluorochrome-labeled antibodies, neither of which are typically available in a clinic setting [9].

The sensitivity of the Tzanck smear for herpetic skin lesions differs widely between studies, likely because of differences in technique, physician training and experience, and duration of the lesion. In general, most studies have found that the Tzanck smear has a sensitivity around 80% [8, 10–12]. Furthermore, the sensitivity is greatest when performed on a vesicle or pustule compared with an eroded or crusted lesion [12]. However, PCR has a sensitivity > 95% when performed on early lesions and maintains a relatively high sensitivity even when performed on later lesions that are eroded or crusted [12]. Furthermore, PCR can also differentiate between types of herpes viruses. PCR is the most sensitive test for herpetic infections, whereas a viral culture, despite having notoriously low sensitivity, remains the most specific [13]. The Tzanck smear does not replace these tests but in skilled hands may lead to earlier initiation of the appropriate therapy and may be nearly as sensitive as PCR when performed on early lesions.

2.2 Molluscum contagiosum

Molluscum contagiosum is often a simple clinical diagnosis based on the presence of characteristic grouped pearly skin-colored to erythematous umbilicated papules. However, atypical lesions may lack these characteristic findings, especially in immunosuppressed patients, and may pose more of a diagnostic challenge. In such cases, a Tzanck smear may be performed on contents expressed or scraped from the center of a lesion. The presence of large (30–35 μm) oval basophilic intracytoplasmic inclusion bodies known as molluscum bodies or Henderson–Patterson bodies (Fig. 3) are diagnostic of molluscum infection [14].

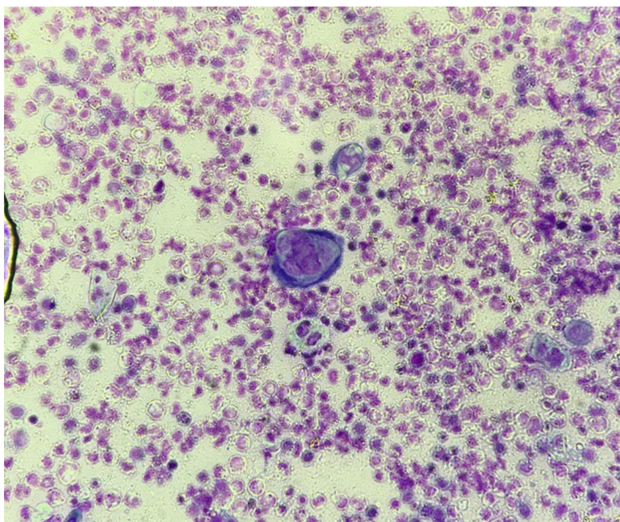

Fig. 2 Positive Tzanck smear from a patient with herpes simplex virus type 2, confirmed by polymerase chain reaction, showing multinucleated giant cells (× 10 objective)

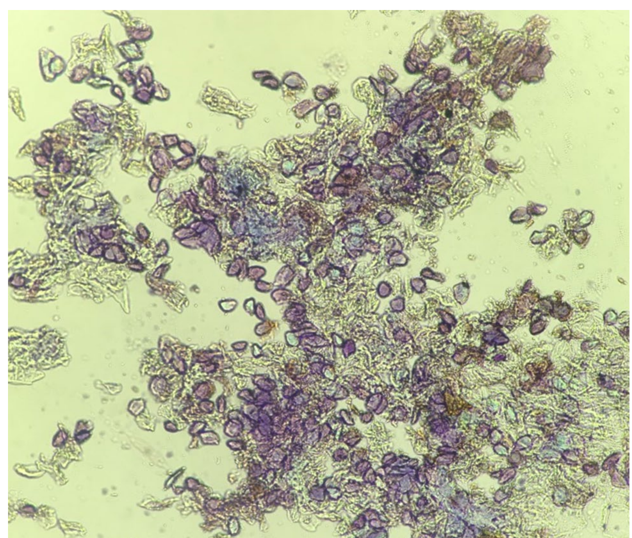

Fig. 3 Tzanck smear of a molluscum lesion showing numerous large oval Henderson–Patterson bodies (× 2 objective)

2.3 Bullous Impetigo

Bullous impetigo is a bacterial skin infection caused by *Staphylococcus aureus* that is common among children. It is characterized by the formation of flaccid vesicles, pustules, and bullae that are easily ruptured, leaving weeping eroded plaques, often with overlying yellow or honey-colored crust [15]. In approximately 30% of cases of impetigo, *S. aureus* produces exfoliatoxins A and B (ETA and ETB) from phage group II, types 55 and 71 [16]. ETA and ETB cleave desmoglein 1, leading to subcorneal acantholysis and the formation of flaccid bullae, similar to those seen in pemphigus foliaceus. While a Tzanck smear is not commonly used in the diagnosis of bacterial infections, in the correct clinical setting it may be used to aid in the diagnosis of bullous impetigo. When performed on lesions of bullous impetigo, a Tzanck smear may show dyskeratotic acantholytic keratinocytes with many cocci and neutrophils, thus supporting the diagnosis [17]. While this does not replace bacterial culture, it may facilitate earlier initiation of appropriate therapy. Occasionally in young children and adults with renal insufficiency, ETA and ETB can disseminate, leading to widespread bullae distant from the site of infection, called staph scalded skin syndrome (SSSS). Unfortunately, because bacteria and inflammatory cells are lacking at the sites of blistering, a Tzanck smear is unable to reliably differentiate between SSSS and immunobullous disease.

2.4 Blistering distal dactylitis

Blistering distal dactylitis is a superficial bacterial skin infection, most commonly caused by *Streptococcus pyogenes,* that manifests as tense bullae on an erythematous base of the volar fat pads of the distal fingers or toes [18]. It typically occurs in children and can be rapidly diagnosed by Gram stain or Tzanck smear prepared by scraping the base or roof of a bullous lesion. A Tzanck smear from an active lesion may reveal numerous cocci in chains (Fig. 4). While less common than *S. pyogenes*, blistering distal dactylitis may also be caused by *S. aureus*, in which case a Tzanck smear would reveal numerous cocci in clusters rather than chains.

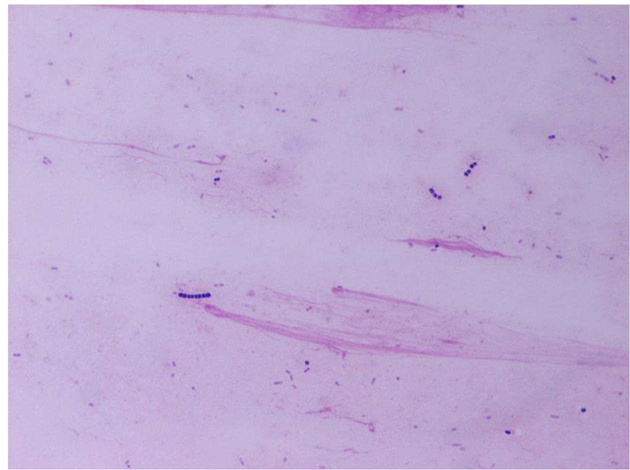

Fig. 4 Tzanck smear from a patient with blistering distal dactylitis showing numerous cocci in chains (×20 objective)

2.5 Fungal Infections

Sporotrichosis or Rose gardener's disease is caused by the dimorphic fungus *Sporothrix schenckii*, which is commonly found in soil [19]. It typically affects gardeners, farmers, and veterinarians and poses a diagnostic challenge because it can have a variable and nonspecific presentation. Following inoculation, lesions may appear as erythematous papules, pustules, nodules, or ulcers with proximal sporotrichoid spread along lymphatics [20]. However, solitary lesions may also occur. The gold standard for diagnosis is fungal culture with Sabouraud agar, which typically takes at least 4 days to yield fungal growth [21]. In 2004, Civila et al. [22] demonstrated that characteristic asteroid bodies composed of a central round cell surrounded by club-shaped hyaline elements could be found in approximately 85% of cases of cutaneous sporotrichosis using direct smear. These smears were prepared using expressed purulence and scrapings from the base of eroded lesions. While asteroid bodies are fairly nonspecific, in the right clinical setting when fungal infection is suspected they can provide useful information, thus offering a more rapid and inexpensive diagnostic tool. It is worth mentioning that often in invasive fungal infections, such as in sporotrichosis, organisms may be very sparse and difficult to find on smears or even tissue sections. As such, Tzanck smear should not be used to rule out infection but, in the right setting, may lead to earlier initiation of the appropriate therapy. When erosions or ulcers are present, a smear may be prepared by scraping the base of these lesions. Further, when lesions are composed primarily of nodules and plaques, scrapings may be prepared from biopsy specimens.

Tzanck smears may also aid in the diagnosis of cryptococcosis, an opportunistic infection caused by *Cryptococcus neoformans* or *Cryptococcus gattii* [23]. Cryptococcosis is typically transmitted via inhalation of spores from bird droppings and, when disseminated to the skin, manifests as umbilicated papules or nodules with a predilection for the head and neck or meningoencephalitis in immunosuppressed patients (particularly patients with HIV/AIDS and solid organ transplant recipients) [24]. Cutaneous cryptococcosis is typically a manifestation of disseminated infection and can be severe and life-threatening, necessitating prompt diagnosis and early initiation of therapy [25–29]. The Tzanck smear, when performed on an infectious lesion,

may demonstrate spherical spores (4–12 µm) with large capsules and yeast forms with narrow-based budding [3, 28, 29]. The diagnosis may be further confirmed by histology, culture, or cryptococcal antigen detection in urine or cerebrospinal fluid.

Similarly, *Histoplasma capsulatum* is a dimorphic fungus endemic to the Ohio and Mississippi river valley that is found in soil contaminated with bird and bat feces [30]. Following inhalation of spores, disseminated disease may develop in immunocompromised patients, especially those with HIV, and may present as mucocutaneous umbilicated papules, nodules, or ulcers [31, 32]. Negatively staining budding yeast on a Tzanck smear from an infectious lesion may suggest a diagnosis of histoplasmosis while awaiting confirmatory tests such as fungal culture or serologies [33, 34].

Blastomyces dermatitidis is also found in the soil of the Ohio and Mississippi river valley and is transmitted through the inhalation of spores [35]. Blastomycosis most often causes pulmonary disease but when disseminated may also have cutaneous manifestations such as erythematous nodules that progresses to verrucous or ulcerated plaques that are often studded with pustules [36]. A Tzanck smear may be prepared by scraping the base of a lesion and smearing it thinly on a glass slide to visualize large spherical broadly based budding yeast characteristic of *Blastomyces spp.* [37]. The organisms in a cutaneous lesion are typically not abundant. Since they are difficult to find, it is important to remember that culture remains the gold standard for diagnosis but can take weeks and lead to delays in therapy. More recently, a urine antigen test has been developed that further aids in early diagnosis [38].

2.6 Leishmaniasis

Transmitted by sandflies, leishmaniasis is a mucocutaneous infection caused by the protozoa *Leishmania* spp. During a blood meal, the sand fly transmits extracellular motile promastigotes that are phagocytized and transform into amastigotes and then divide within macrophages [39]. Leishmaniasis typically presents with solitary or multiple mucocutaneous ulcers that heal slowly over a few months. Sporotrichoid spread may also be observed in cutaneous leishmaniasis. The most sensitive diagnostic test for leishmaniasis, with a sensitivity of approximately 97%, is the detection of parasitic DNA by PCR [40]. However, PCR is not always available in undeveloped and developing countries where leishmaniasis is common. When performed by an experienced physician, a Tzanck smear may allow direct visualization of multiple small (2–4 µm) round amastigotes, also called Leishman–Donovan bodies, within the cytoplasm of large macrophages, with a sensitivity up to 90% (Fig. 5) [41–43]. Micheletti et al. [44] described a modified technique, known as the thick drop method, where the border of an active lesion is gently cut with a no. 15 scalpel blade to obtain a few drops of blood. The expressed drops of blood are placed onto a glass slide without smearing, then dried before staining with Wright–Giemsa. Again, visualization of intracytoplasmic amastigotes is diagnostic. This method yields a sensitivity ranging from 64 to 77% [44]. Another alternate technique, presented by Sousa et al. [45] is the Press-Imprint-Smear, which is prepared using a punch biopsy specimen from the border of a suspected lesion, which is then compressed between two glass slides. The expressed contents are examined after fixation and staining with Wright–Giemsa. Sousa et al. [45] reported identifying amastigotes on 85.3% of Press-Imprint-Smears when prepared from highly suspicious lesions.

3 Mineral Oil Preparation

Scabies, sometimes referred to as the "seven-year itch," is caused by the mite *Sarcoptes scabiei var. hominis* that is estimated to infect 300 million patients per year [46, 47].

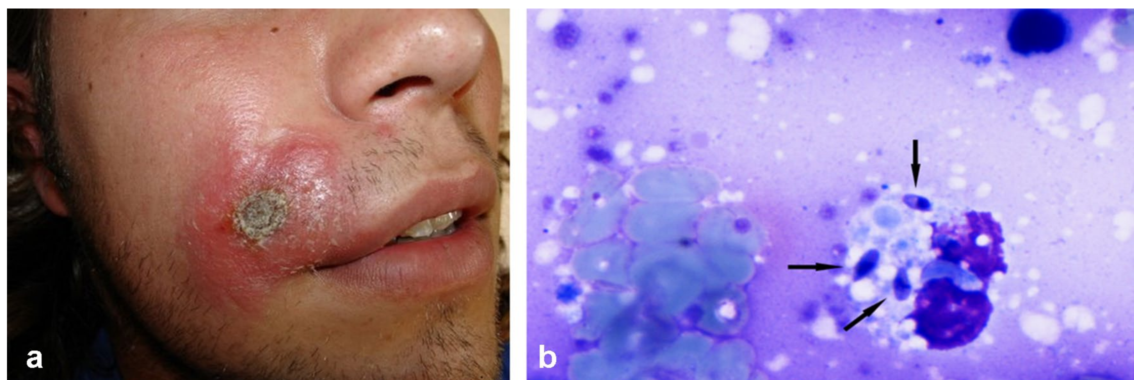

Fig. 5 Clinical image of patient with **a** leishmaniasis and **b** a Tzanck smear of the lesion showing multiple intracytoplasmic amastigotes (Leishman–Donavon bodies), as indicated by the *black arrows* (×40 objective under oil immersion)

It is passed through direct contact with an infected person, with the most important risk factor being crowded living conditions, such as those seen in prisons, military barracks, and schools or daycares. Following infection, the female mite burrows into the stratum corneum where it lives for 3–4 weeks, depositing eggs. Symptoms typically take 4–6 weeks to develop and are the result of an allergic response to mites and their detritus, particularly their feces, known as scybala. Scabies manifests clinically as erythematous papules and vesicles that often coalesce into linear and serpiginous burrows with associated intense pruritus. Burrows are most often observed in the finger web spaces, around the wrists, elbows, axillae, feet, waist band, and genitalia [47]. However, generalized and atypical presentations are common [48–50]. Clinical suspicion of scabies is best confirmed with a mineral oil preparation. First, a drop of mineral oil is deposited on a glass slide. Then, a no. 15 blade is dipped in the mineral oil and used to scrape a suspected burrow. Finally, the scraped contents are smeared on the glass slide and microscopically examined [51]. If the scraping has been performed successfully, direct visualization of adult mites, nymphs, eggs, and scybala is possible (Fig. 6b). When used in conjunction with dermoscopy to identify and scrape suspected burrows, mineral oil preparation has a sensitivity of approximately 75% [52]. In children and less cooperative patients, a sharp curette may be used rather than a scalpel blade [53]. Of note, mineral oil preserves scybala, whereas potassium hydroxide does not [54].

In an otherwise healthy patient with non-crusted scabies, typically only 10–15 mites live on the entire body [55]. Therefore, it is critical that the location of scraping be carefully selected to increase diagnostic yield. Ideally, an intact burrow should be chosen. Dermoscopy can be used to aid in proper location selection. When examining a burrow dermoscopically, a small dark triangular structure in front of a white featureless area can often be seen at the end of a burrow. This is known as the delta-wing sign and represents the dark-appearing head and front legs of a mite followed by the white or translucent body (Fig. 6a) [56, 57]. Scraping these areas is the most likely way to yield a mite on microscopic exam. Furthermore, if the delta-wing sign has disappeared upon reexamination of the location under dermoscopy after scraping, one can be sure a mite has been removed. In fact, it has been suggested that the presence of a delta-wing sign on dermoscopy is enough to confirm the diagnosis, meaning scraping and therefore microscopic exam may not be necessary [58, 59].

It is hypothesized that scratching in scabies has an evolutionary advantage by helping to decrease mite burden and control mite population numbers. Therefore, in immunosuppressed patients and those with an impaired ability to scratch, crusted scabies may develop where the number of mites is much more numerous [47]. This manifests as scaly crusted plaques and is much more contagious and difficult to treat.

Another genus of mites known to infest humans are the demodex mites, specifically *Demodex folliculorum* and *Demodex brevis* [60]. Both species are most prevalent on the face as they prefer areas of high sebum production. *D. folliculorum* is found in the superficial portion of hair follicles, whereas *D. brevis* resides in sebaceous glands. Demodex mites are extremely common and present on nearly all adults as commensal organisms [61]. However, when overgrown, demodex mites are thought to contribute to rosacea and blepharitis [62, 63]. This has led to the recent development of therapies targeted at decreasing demodex loads in the treatment of rosacea [64, 65]. A mineral oil preparation can be performed using much the same technique as just described for scabies. Hair follicles in the affected area are scraped to prepare a microscopic slide. *D. folliculorum* will appear as a slender mite, approximately 0.3–0.4 mm long with four pairs of short legs (Fig. 7), whereas *D. brevis* will appear much shorter [60]. *D. brevis* is rarely seen on superficial scrapings as it lives deeper in the pilosebaceous unit.

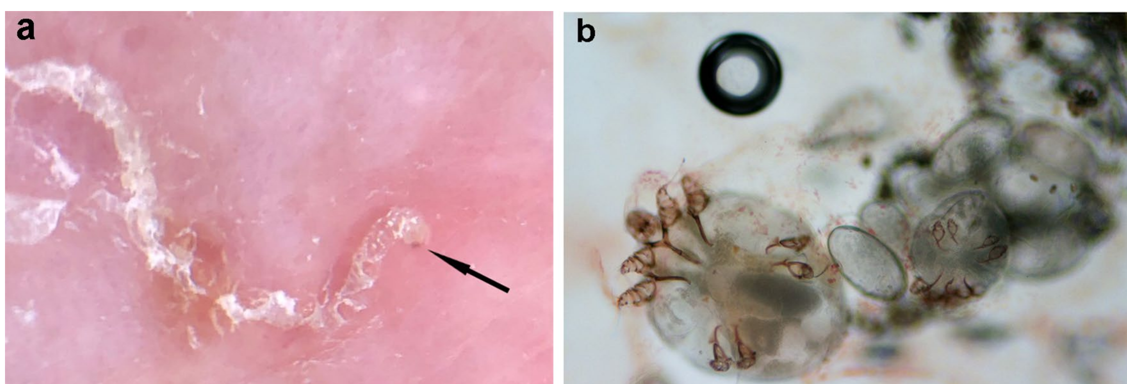

Fig. 6 **a** Clinical image of a scabies burrow with delta-wing sign at leading edge indicated by black arrow (image courtesy of Anthony Linfante, MD). **b** Mineral oil preparation showing adult scabies mite with adjacent egg and nymph (×40 objective)

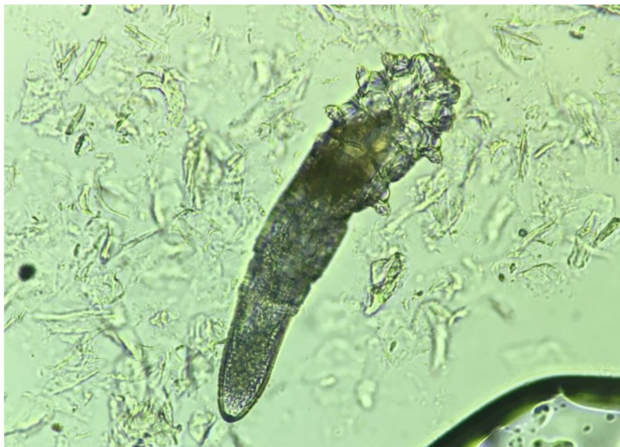

Fig. 7 Mineral oil preparation demonstrating Demodex folliculorum (×40 objective)

Repeat scrapings can also be performed to assess response to therapy.

4 Potassium Hydroxide Overview

Potassium hydroxide (KOH) is a strong base that is commonly used in the diagnosis of superficial fungal infections because of its ability to dissolve keratin, making fungi more visible [66, 67]. The KOH preparation is a simple and inexpensive test for diagnosing dermatophyte and yeast infections of the skin, hair, and nails. For skin infections, it starts with scraping the skin with a glass slide or no. 15 scalpel blade to obtain scale from the affected area. The area may be gently rubbed with an alcohol pad prior to scraping to help accentuate and release the scale. The scrapings are then placed on a glass slide and 1–2 drops of 10–20% of KOH are applied, followed by a cover slip. Dimethyl sulfoxide (DMSO) is often added to KOH to catalyze its penetration of keratinocytes and thick pieces of scale. Gentle heat can also be used to help speed the effects of KOH but is unnecessary when DMSO is used. The slide should be left for 10–15 min before being examined to allow the KOH to dissolve the surrounding keratin and make fungi more visible. Furthermore, counterstains such as Chlorazol black E, which stains chitin, Swartz–Lamkins, or Wright–Giemsa can be added to enhance the visibility of fungi (Fig. 8) [68]. Similarly, Calcofluor-white can be added to make fungal elements fluoresce [69].

4.1 Candida

Mucocutaneous candidiasis is a fungal infection caused by the *Candida* genus of yeast, most commonly *Candida albicans*, that often affects the oral and vaginal mucosa, skin, and nails. Cutaneous candidiasis manifests as erythematous macerated plaques in intertriginous areas with peripheral satellite pustules. It has a proclivity for warm, moist, and occluded areas. Additional risk factors include immunosuppression, hyperglycemia, and systemic and topical steroids. KOH preparation, when performed on candida lesions, may reveal branching pseudohyphae and oval budding yeast (Fig. 9) [70]. Furthermore, Wright–Giemsa staining may be used to better visualize candida elements and their morphology.

4.2 Tinea versicolor

Tinea versicolor, caused by *Malassezia* spp., is often diagnosed clinically based on the presence of round to oval erythematous, hypopigmented, or hyperpigmented patches with fine scale on the chest, back, or upper arms [71]. However, KOH preparation can still be a valuable, simple, and inexpensive diagnostic test. When performed on overlying scale, direct microscopic exam of a KOH preparation will reveal abundant yeast forms with short hyphae with the characteristic “spaghetti and meatballs” appearance (Fig. 10) [72]. It is worth mentioning that it is difficult to distinguish between

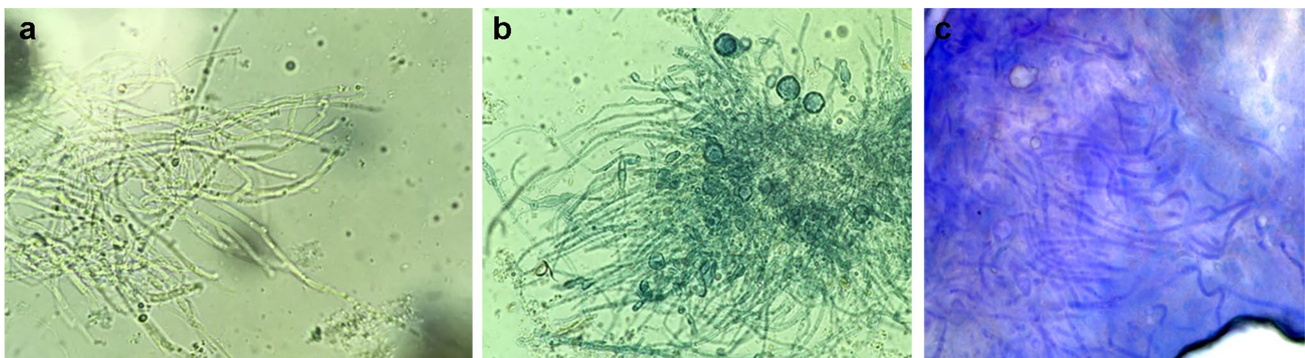

Fig. 8 Direct microscopy of hyalohyphomycotic fungal infection with **a** 20% potassium hydroxide (KOH), **b** Chlorazol black E, and **c** Wright–Giemsa staining; all prepared from the same lesion (×40 objective)

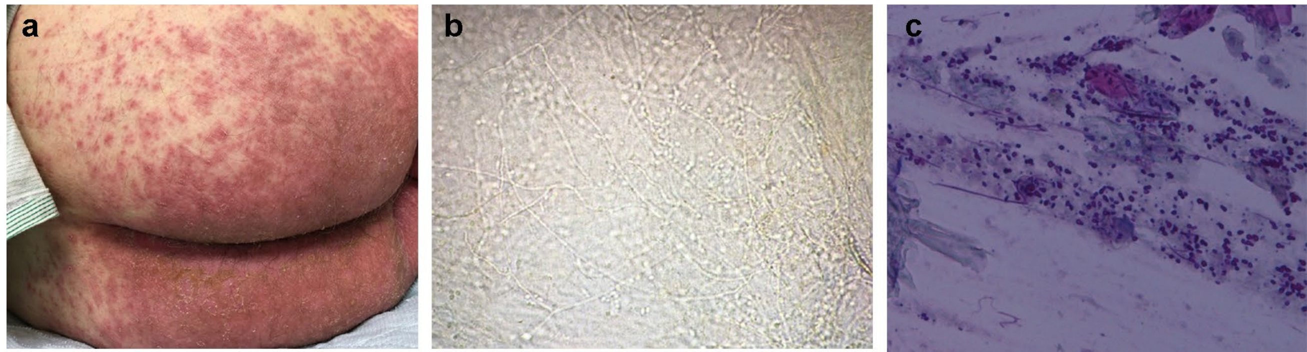

Fig. 9 **a** Candida intertrigo with scraping showing numerous budding yeast with **b** potassium hydroxide (KOH) (×40 objective) and **c** Wright–Giemsa (×40 objective)

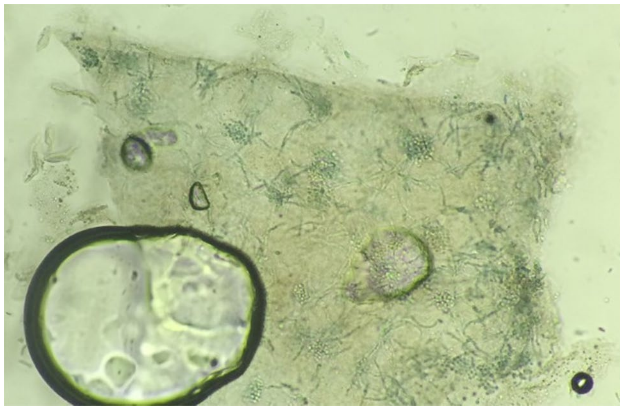

Fig. 10 Potassium hydroxide (KOH) preparation with Chlorazol black E of tinea versicolor showing yeast and short hyphal elements with "spaghetti and meatballs" appearance (×20 objective)

Candida and *Malassezia* on KOH preparation, so the clinical presentation must be considered when interpreting KOH preparations.

4.3 Dermatophytes

Dermatophytoses are a heterogenous group of superficial fungal infections of the skin, hair, or nails caused by the fungal genera *Trichophyton*, *Microsporum*, and *Epidermophyton*. Dermatophytes utilize keratin as a nutrient source and colonize keratinized tissue, leading to a heterogenous group of disorders that includes tinea corporis, tinea pedis, tinea capitis, tinea cruris, tinea faciei, tinea manuum, and onychomycosis. When used in dermatophyte infections, a KOH preparation of peripheral scale may reveal branching septate hyphae that cross keratinocyte cell walls (Fig. 11) [66, 73]. KOH preparation cannot differentiate between genera of dermatophytes, but the distinction is rarely clinically relevant. Of note, partial treatment with antifungals may lead to a falsely negative KOH preparation. Further, in the case of Majocchi's granuloma, a variant of tinea corporis in which the dermatophyte has invaded the hair follicle, a KOH preparation may also be negative.

In the case of tinea capitis, dermatophytes infect the hair shafts, so a KOH preparation must contain hair shafts to be diagnostic. Involved hair is typically more brittle, and scraping a scaly alopecic plaque will usually yield hair fragments. In the USA, the endothrix *Trichophyton tonsurans* has become the most common cause of tinea capitis and can be seen on KOH preparation as abundant spores within hair shafts [74, 75]. The second most common cause of tinea capitis is the small-spore ectothrix *Microsporum canis*, which appears as numerous spores restricted to the exterior of hair shafts, often encasing them.

5 Gram Stain

Gram staining, introduced in 1884 by Hans Christian Gram, is used to categorize and identify bacterial species based on morphology and the properties of their cell walls [76]. It is seldom used in the clinic setting but remains an important diagnostic tool and critical part of inpatient care when bacterial infection is suspected. The rapid results obtained from Gram staining enable more accurate and precise antibiotic therapy while awaiting bacterial culture results. First, a specimen is obtained from a tissue sample or scraping and spread thinly onto a glass slide. It is then fixed with gentle heat or allowed to air dry. Next, the slide is stained with crystal violet for 30–60 s before being rinsed with water. Then, an iodine-based mordant is added for an additional 30–60 s to fix the crystal violet [77]. After rinsing again with water, the slide is bathed with a decolorizing solvent such as ethanol or acetone until the runoff is clear and rinsed yet again with water. Finally, the slide is counterstained with safranin dye for 30–60 s before a final rinse and drying [14]. Gram staining divides bacteria into two large groups, Gram-positive bacteria that retain crystal violet within a

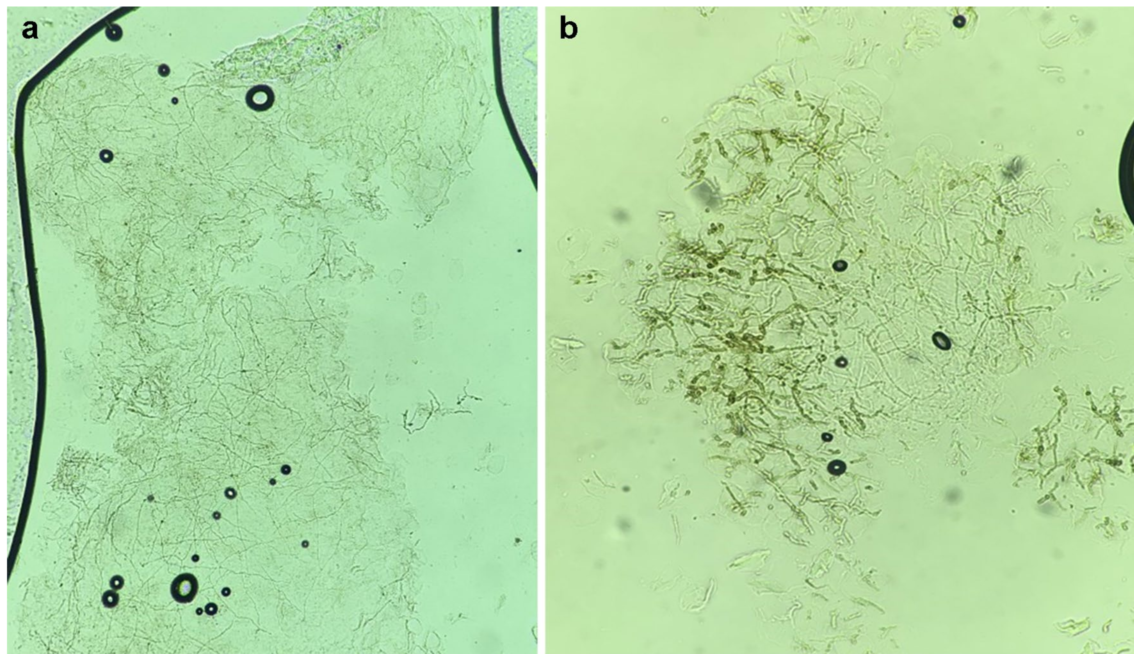

Fig. 11 Potassium hydroxide (KOH) preparations of **a** tinea corporis showing long branching hyphae (×20 objective) and **b** tinea nigra showing pigmented hyphae (×20 objective)

thick peptidoglycan cell wall, and Gram-negative bacteria that have only a thin layer of peptidoglycan that does not retain crystal violet and is counterstained with safranin. Crystal violet causes Gram-positive bacteria to appear purple, whereas counterstained Gram-negative bacteria appear pink because of the safranin. Using Gram staining, bacteria can be further subdivided based on their morphology. It is important to note that Gram staining cannot directly identify bacterial species. However, it is much quicker than bacterial culture, and bacterial species can often be inferred based on morphology when the clinical setting is considered.

It is important to note that, under CLIA, non-endocervical or urethral Gram stains are considered high complexity [78]. Therefore, in the USA, laboratory requirements significantly limit the use of Gram staining in clinic settings. As bedside Gram stains are not routinely performed by dermatologists in the USA, many dermatologists likely do not feel comfortable performing or interpreting them.

6 Acid-Fast Stain

Acid-fast stain is a bacterial stain used to identify acid-fast organisms, such as *Mycobacterium* spp., with cell walls made up primarily of mycolic acid. Mycolic acid is a thick waxy lipid that stains poorly with traditional preparations such as Gram staining [79]. First, a sample is smeared thinly on a glass slide and fixed by allowing it to air dry or applying heat. Then, absorbent paper is placed over the slide and flooded with carbol fuchsin stain to ensure the sample remains saturated with stain throughout the heating process. The sample is gently heated until steam is produced and is maintained at this heat for 5 min. After the heating process, the paper may be removed, and the slide is rinsed with water before being de-stained with acid-alcohol (typically hydrochloric acid in isopropyl alcohol or methanol) until the runoff is clear. Finally, the slide is counterstained with methylene blue or malachite green, rinsed, and allowed to dry. During this process, the thick waxy cell walls of acid-fast bacteria protect against de-staining and retain the carbol fuchsin stain, whereas non-acid-fast bacteria do not. If the process is performed correctly, acid-fast bacteria will appear red, whereas non-acid-fast organisms will appear blue because of the counterstaining [79]. While not widely used by dermatologists in the USA, acid-fast staining can be immensely helpful in developing countries where infections with acid-fast organisms, particularly mycobacterial species, are common.

The primary utility of acid-fast staining is in diagnosing mycobacterial infections, most notably tuberculosis [80]. Cutaneous tuberculosis, caused by the acid-fast bacillus *Mycobacterium tuberculosis*, can have a variable clinical presentation, whether due to direct inoculation as in tuberculous chancre and tuberculosis verrucosa cutis or to spread of endogenous infection as in scrofuloderma and lupus vulgaris. On acid-fast stain, *M. tuberculosis* appears as bright red thin rods that often clump together because of their waxy cell walls [79–81]. Similarly, *Mycobacterium*

leprae, the causative organism of Hansen's disease, can be visualized using acid-fast staining and closely resemble *M. tuberculosis*. The slit-skin smear is a rapid and inexpensive diagnostic test that utilizes acid-fast staining for the diagnosis of Hansen's disease and is often used in resource-poor areas where leprosy is endemic [82]. Samples are obtained from active lesions and commonly affected sites, including the earlobes, elbows, and knees, by making small incisions of 3–5 mm ("slits") with a scalpel blade and then using the blade to scrape the wound edges [83]. Local anesthetic is not routinely used because active plaques are often anesthetic. The obtained dermal tissue and fluid is then transferred to a glass slide. After fixing the samples with formalin, the procedure is very similar to that of a traditional acid-fast stain. Slit-skin smears can be used to diagnose leprosy and to monitor treatment response. While slit-skin smear has little utility in paucibacillary disease, its sensitivity approaches 80 and 100% in multibacillary and lepromatous leprosy, respectively, when performed by an experienced physician [82]. PCR is more sensitive, especially in pauci-bacillary disease, but it is also much more expensive and not widely available.

7 Wood's Lamp

Wood's lamp, developed in 1903 by Robert William Wood, emits ultraviolet light with a wavelength of approximately 365 nm [84, 85]. Aside from delineating dyspigmentation in vitiligo and melanoma, it can also be used in the diagnosis of infectious dermatoses by inducing fluorescence of certain infectious organisms. Fluorescence occurs when porphyrin-producing organisms absorb ultraviolet light and emit visible light. For example, *Corynebacterium minutissimum*, the organism responsible for erythrasma, produces coproporphyrin III, which causes it to fluoresce coral red (Fig. 12) [86]. Therefore, erythrasma can be differentiated from tinea and candidal intertrigo, which share many similar clinical features, by examination under Wood's lamp. *Cutibacterium acnes*, previously *Propionibacterium acnes*, also fluoresces red under Wood's lamp because of the production of porphyrins. It has been suggested that quantitative measurements of fluorescence may serve as a surrogate for *C. acnes* burden and response to antimicrobial therapy in the treatment of acne [87]. Finally, infections due to *Pseudomonas aeruginosa* can be identified by their blue-green fluorescence under Wood's lamp due to the production of the siderophore, pyoverdine [88].

Wood's lamp can also aid in the diagnosis of fungal infections, most notably, tinea capitis. As previously discussed, most cases of tinea capitis are due to *T. tonsurans* and *M. canis*. It may be prudent to differentiate between the two because terbinafine is known to be more effective in treating *T. tonsurans*, whereas griseofulvin is more effective against

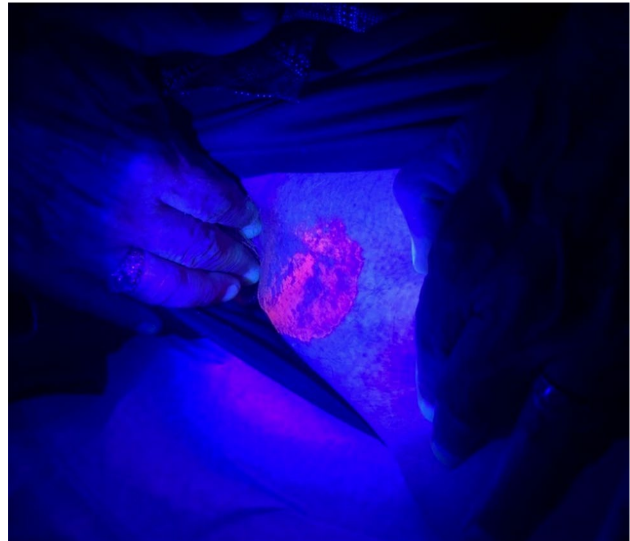

Fig. 12 Erythrasma showing coral-red fluorescence under Wood's lamp

M. canis [89]. They can be easily and quickly distinguished because *M. canis* fluoresces bright green because of the production of pteridine (Fig. 13), whereas *T. tonsurans* does not [90]. Lastly, *Malassezia furfur,* in tinea versicolor, may also emit orange-yellow fluorescence under Wood's lamp.

8 Conclusion

Cutaneous bacterial, fungal, viral, and even protozoal infections lead to significant morbidity and mortality and are commonly encountered in clinical practice. Prompt diagnosis is paramount in many infectious dermatoses. While bedside techniques cannot replace the more advanced and specific diagnostic methods such as PCR, culture, and immunofluorescence, they are generally much faster and, in skilled hands, may have comparable sensitivities. Such tests are especially valuable in resource-poor areas where infectious diseases are common. Further, when caring for critically ill patients, bedside tests can lead to early initiation of proper treatment or help to guide therapy while awaiting more specific testing.

Under CLIA, Tzanck smears and acid-fast stains are considered moderate-complexity tests, and most Gram stains are considered high-complexity tests, which, unfortunately, may discourage their use in an outpatient clinic setting [78]. CLIA has also created a subcategory of moderate-complexity testing called provider-performed microscopy (e.g., KOH and mineral oil preparations) [91], which allows providers to perform a limited number of tests without having to meet all of the regulatory requirements of moderate-/high-complexity testing. While bedside diagnostic tests such as the

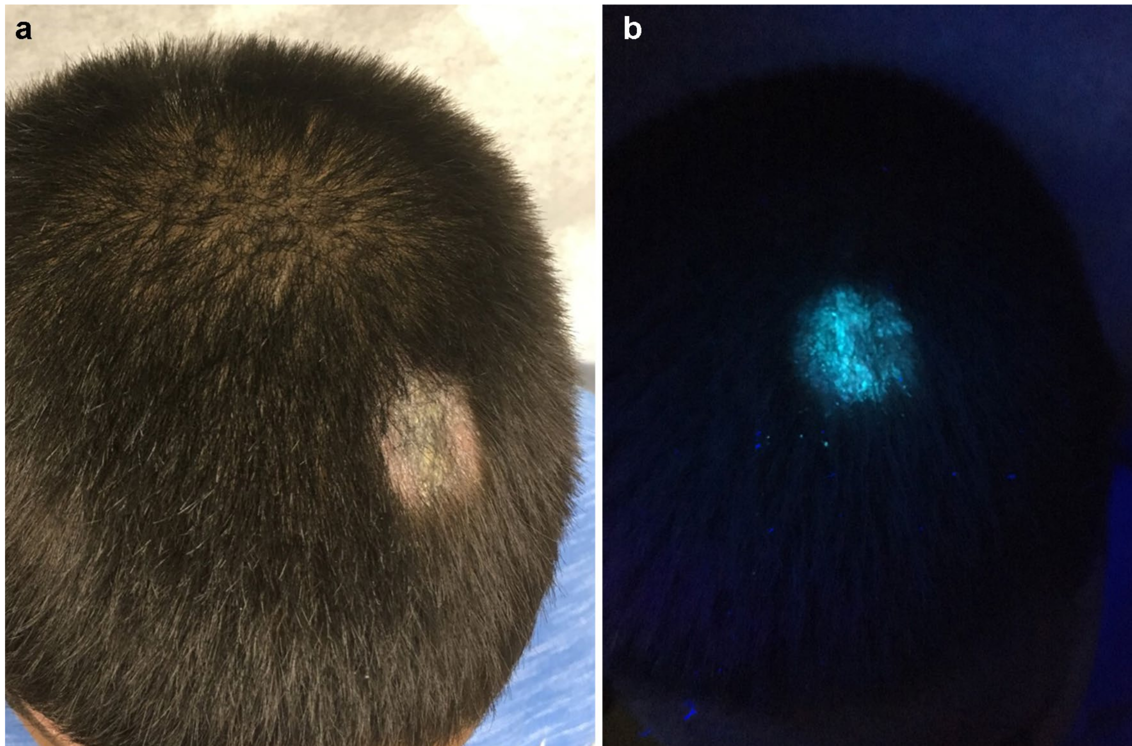

Fig. 13 **a** Tinea capitis secondary to *Microsporum canis*, **b** showing bright green fluorescence under Wood's lamp

Tzanck smear, KOH preparation, and mineral oil preparation are somewhat user dependent, with training they can be performed accurately and with significant reproducibility. These tests are valuable additions to the diagnostic repertoire in clinical and inpatient dermatology and—when applied correctly—can produce rapid and accurate results.

Compliance with Ethical Standards

Funding No sources of funding were used to conduct this study or prepare this manuscript.

Conflict of interest Brent Kelly, Frank Winsett, and Shaunak Patel have no conflicts of interest that are directly relevant to the content of this article.

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