Stains and Smears: Resident Guide to Bedside Diagnostic Testing

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Dermatology residents often are on the front line when it comes to treating patients with complicated skin disorders, frequently seeing these cases first before discussing their findings and plan with an attending physician. Bedside testing modalities can help facilitate arriving at a diagnosis quickly, allowing for rapid initiation of treatment. The potassium hydroxide (KOH) preparation, Tzanck smear, mineral oil preparation, and Gram stain are easy to perform quickly and provide valuable diagnostic information. The purpose of this article is to consolidate these frequently used tests into a useful reference for the training and practicing dermatologist.

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Dermatologists are fortunate to specialize in the organ that is most accessible to evaluation. Although we use the physical examination to formulate the initial differential diagnosis, at times we must rely on ancillary tests to narrow down the diagnosis. Various bedside testing modalities—potassium hydroxide (KOH) preparation, Tzanck smear, mineral oil preparation, and Gram stain—are most useful in diagnosing infectious causes of cutaneous disease.

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This guide serves as a useful reference for residents on how to perform these tests and which conditions they can help diagnose. Several of these procedures have no standard protocol for performing them; the literature is littered with various methodologies, fixatives, and stains, and as such, this article will attempt to describe a technique that is convenient and quick to perform with readily available materials while still offering high diagnostic utility.

KOH Preparation

A standard in the armamentarium of a dermatologist, the KOH preparation is invaluable to diagnose fungal and yeast infections. Although there are many available preparations including varying concentrations of KOH, dimethyl sulfoxide, and various inks, the procedure is similar for all of them.[1] The first step involves collecting the specimen, which can be scale from an active border of suspected cutaneous dermatophyte or *Malassezia* infection, debris from suspected candidiasis, or hair shafts plucked from an area of alopecia of presumed tinea capitis. A no. 15 blade can be used to scrape the specimen onto a microscope slide, though a second microscope slide can be used in lieu of a blade in patients who will not remain still, and then a coverslip is placed. Two drops of the KOH solution of your choice are then placed on opposite ends of the coverslip, allowing capillary action to spread the stain evenly. A paper towel can be folded in half and pushed down on the surface of the coverslip to spread the stain and soak up any excess, and this pressure also can help the KOH solution digest the keratin in the specimen. Briefly heating the underside of the slide (below boiling point) will help digest the keratin; this step is not necessary when you are using a KOH preparation with dimethyl sulfoxide. Although many

dermatologists view the slide almost immediately, ideally at least 5 minutes should pass before it is read. Particularly thick specimens may require additional digestion time, so setting them aside for later review may help visualize infectious agents. In a busy clinic where an immediate diagnosis may not be requisite and a prescription can be called in pending the result, waiting to review the slide may be feasible.

Tzanck Smear

The Tzanck smear is a useful cytopathologic test in the rapid diagnosis of herpetic lesions, though it cannot differentiate between herpes simplex virus type 1, herpes simplex virus type 2, and varicella-zoster virus. It also has shown utility for rapid diagnosis of protean other dermatologic conditions including autoimmune blistering disorders, cutaneous malignancies, and other infectious processes, though it has been superseded by histopathology in most cases.[2] An ideal sample is collected by scraping the base of a fresh blister with a no. 15 blade or a second microscope slide. The scrapings then are smeared onto another microscope slide and allowed to air-dry briefly. Then, Wright-Giemsa stain is dispensed to cover the sample and allowed to sit for 15 minutes before being washed off with sterile water. After air-drying, the sample is examined for the presence of clumped multinucleated giant cells, a feature that confirms herpetic infection and allows rapid initiation of antiviral medication.[3]

Mineral Oil Preparation

A mineral oil preparation has utility in diagnosing ectoparasitic infestation. In the case of scabies, a positive microscopic examination is diagnostic and requires no further testing, allowing for rapid initiation of therapy. This technique also is useful in diagnosing rosacea related to *Demodex*, which requires a treatment algorithm that differs from the classic papulopustular rosacea which it mimics.[4]

Mineral oil preparations can be rapidly performed and interpreted. Several drops of mineral oil are placed onto a microscope slide and a no. 15 blade is dipped into this oil prior to scraping the sample lesion. For scabies, a burrow is scraped repeatedly with the blade, and the debris is collected in the mineral oil. Occasionally, the mite can be dermoscopically visualized as a jet plane or arrowhead at the leading edge of a burrow; scraping should be focused in the vicinity of the mite.[5] A coverslip is applied to the microscope slide and examination for the mite, egg casings, and scybala can be performed with microscopy.[6] For *Demodex* infestation, a facial pustule can be expressed or several eyelash hairs can be plucked and suspended in mineral oil. Examination of this specimen is identical to scabies.

Gram Stain

The Gram stain is invaluable in classifying bacteria, and a properly performed test can narrow the identification of a causative organism based on cellular morphology. Although it is more technically complex than other bedside diagnostic maneuvers, it can be rapidly performed once the sequence of stains is mastered. The collected sample is smeared onto a glass slide and then briefly passed over a flame several times to heat-fix the specimen. Caution should be taken to avoid direct or prolonged flame contact with the underside of the slide. After fixation, the staining can be performed. First, crystal violet is instilled onto the slide and remains on for 30 seconds before being rinsed off with sink water. Then, Gram iodine is used for 30 seconds, followed by another rinse in water. Next, pour the decolorizer solution over the slide until the runoff is clear, and then rinse in water. Finally, flood with safranin counterstain for 30 seconds and give the slide a final rinse. After air-drying, it is ready to be interpreted.[7]

Final Thoughts

Although the modern dermatologist has access to biopsies, cultures, and sophisticated diagnostic techniques, it is important to remember these useful bedside tests. The ability to rapidly pin a diagnosis is particularly useful on the consultative service where critically ill patients can benefit from identification of a causative pathogen sooner rather than later. Residents should master these stains in their training, as this knowledge may prove to be invaluable in their careers.

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