

# Procedures in Family Practice

## Diagnostic Procedures of the Skin

### Part One: Wood's Light, KOH Slide, Gram's Stain, and Cultures

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The diagnosis of skin lesions involves the same principles and methodology required in other medical problems. Visual recognition alone and "shotgun" therapy is not a satisfactory clinical approach. A disciplined and careful examination of lesions, establishment of a differential diagnosis, and selection of appropriate procedures are frequently necessary for cutaneous diseases.

The indications, limitations, interpretation, and techniques of diagnostic procedures must be well understood to obtain reliable information. Not all tinea capitis will reveal fluorescence with Wood's light examination, but the Wood's light may be particularly helpful in the diagnosis of tinea versicolor, erythrasma, porphyria, and tuberous sclerosis. Bacterial growth on cultures taken from the skin does not necessarily mean infection. Because the eczematous skin teems with bacteria, there must be a careful interpretation of the culture results within the context of the clinical situation. This paper is the first in a two-part series dealing with selected cutaneous procedures which are useful to the family physician in everyday practice.

The diagnosis of cutaneous diseases may seem deceptively simple to non-dermatologists because of the visual and physical accessibility of the skin lesions. Too often, the diagnosis is attempted on visual recognition alone. Failing this, a multidirectional, "shotgun" therapy may be tried, complicating the disease. Unfortunately, the logical reasoning and careful selection of procedures that are second nature in most diagnostic problems are frequently neglected in cutaneous medicine.

However, skin lesions present significant diagnostic clues which require a careful and disciplined examination. These include descriptive morphology (the specific and detailed appearance of lesions), the configuration of the lesions (the interrelationship of lesions to each other, including grouping, annularity, and linearity), and the body distribution.<sup>1</sup> Historical information should include the course of the disease, symptomatology, previous treatment which may complicate the eruption, drug history, review of systems, family history, and allergic background.

Physical examination of the patient as well as of the entire skin and mucous membrane is often necessary. These historical and examination requirements for skin diseases are no different from those called for in

many medical problems, and frequently are equally important.

Often the eruption will suggest a primary diagnosis which must be substantiated, and other possibilities excluded. Cutaneous procedures may be helpful and necessary in diagnosis.

#### Selection of Procedures

The physician should establish some diagnostic considerations and acquire knowledge of the biologic aspects and their potential etiology in order to choose an appropriate test. In addition, the limitations of and information from the tests must be understood. It is of little value to do a fungal culture for tinea versicolor because it will not grow, potassium hydroxide examination (KOH) for erythrasma which is caused by a bacterium, or culture for *C. albicans* from the mouth which is often found normally in this site. It is the understanding of these interrelationships of diagnosis and selection of cutaneous procedures that is critical to intelligent interpretation of the results. If a specimen is obtained with a procedure, the nature of the lesion and the method of obtaining the specimen are equally important in the interpretation of the results.

#### Wood's Light Examination

Hairs infected with some dermatophytes were found to fluoresce when exposed to ultraviolet light passed through a Wood's glass filter. This filter allows light greater than 365 nm to pass through. (Ten angstroms are equivalent to 1 nanometer; nm is the preferred current designation.)

Unfortunately, only a few dermatophytes invading hair cause fluorescence (Table 1). Of these, the more common in the United States are *Microsporum audouini* and *Microsporum canis*. Both of these fungi fluoresce a fairly brilliant blue-green. However, *Trichophyton tonsurans*, which is the most frequent cause of tinea capitis in many areas of the United States, does not fluoresce. *Trichophyton schoenleinii*, the cause of favus more commonly seen in Europe, may fluoresce a faint green color. Wood's light examination of the glabrous skin, groin, nails, palms, and soles is not helpful in the diagnosis of dermatophyte infections because of the absence of fluorescence, but may

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be valuable in tinea versicolor and erythrasma.

The light should warm up for about one minute, and the patient should be examined in a very dark room. Although fluorescence can be appreciated in a room with ordinary shades, the lighting may still be too intense. It is better to use a room with occlusive shades (black shades) or a windowless room. The characteristic fluorescence should be seen in broken-off hair. Since the invasion of the hair begins within the follicle, the plucked hair

should have fluorescence in the intra-follicular (subepidermal) portion of the hair. For the same reasons, in the periphery of an active lesion representing early infection, fluorescence may be observed only in the intrafollicular part of the hair shaft.

There are potential errors in interpretation of Wood's light examination, and it is important to understand its limitations (Table 2). The specific fluorescent color should be sought and not just any fluorescence. Lint may appear bright white. Faint fluores-

cence may be emitted by scales, ointments, and dried soap. However, if the characteristic fluorescent color is carefully identified as emanating from hair, many errors of interpretation may be avoided.

Another use of the Wood's light is for the diagnosis and treatment of tinea versicolor caused by *Malassezia furfur*. The active lesions show a variable fluorescence which may suggest the diagnosis. Confirmation calls for potassium hydroxide (KOH) examination of skin scrapings, which reveal short hyphae and budding yeasts ("spaghetti and meatballs"). More important, the Wood's light examination may help to establish the extent of the eruption because some of the lesions may not be easily seen with ordinary light. These unrecognized lesions, if untreated, serve as a source for perpetuation of the eruption. Wood's light and KOH examination are very useful in follow-up evaluation to determine whether or not the eruption is clear.

Erythrasma, caused by a bacterium *Corynebacterium minutissimum*, is a reddish-brown, crinkly-scaled eruption. The lesions do not have the active border which is evident in tinea infections and may appear on various parts of the body. Between the toes and occasionally in the crural crease it will cause maceration. The Wood's light should induce a coral-red fluorescence. In the intertriginous areas *C. minutissimum*, if present, may not be the only cause of the cutaneous changes. Intertrigo, seborrheic dermatitis, psoriasis, and other bacterial and fungal infections are additional diagnoses that might be considered, depending on the part of the body involved.

Wood's light examination is useful in the diagnosis of porphyria. In symptomatic porphyria (acquired porphyria cutanea tarda) the urine will fluoresce a bright, pink-orange color. The fluorescence may be accentuated by acidifying the urine. This is accomplished by adding an equal volume of 1.5 N HCl to the test tube.<sup>2</sup> Normal urine should be used as control. In porphyria variegata (mixed porphyria) the urine will fluoresce in the acute stage and the stool will fluoresce in the stage of remission. A specimen of stool on the rectal examining glove or stool mixed with equal parts of amyl alcohol, glacial

Table 1. Wood's Light Fluorescence of Hair

Dermatophyte	Wood's Light
* <i>Microsporum audouini</i>	+ blue-green
* <i>Microsporum canis</i>	+
<i>Microsporum distortum</i>	+
<i>Microsporum ferrugineum</i>	+
* <i>Trichophyton tonsurans</i>	- (may have dull white color in blond and grey-haired patients)
<i>Trichophyton schoenleini</i>	± Usually negative. May fluoresce greyish white, faint green.
<i>Trichophyton verrucosum</i>	-
<i>Trichophyton equinum</i>	-

\*Most important in the United States

Table 2. Errors in Wood's Light Examination

Not all dermatophytes causing tinea capitis produce fluorescence with Wood's light examination.
Other causes of fluorescence may be lint, soap, scale, ointments.
It is not helpful for infections of the skin except for tinea versicolor and erythrasma (bacterium).
Recommendations:
Room should be dark.
Characteristic fluorescence should be observed.
Identify fluorescence as emanating from the hair; plucked hair may have fluorescence only in the intrafollicular part.

acetic acid and ether may show fluorescence.<sup>3</sup> In congenital porphyria the teeth and urine fluoresce, as do the red cells with fluorescent microscopy. In erythropoietic protoporphyria the red cells also fluoresce, but the urine does not. There is no fluorescence in acute intermittent porphyria.

The Wood's light may also help to identify the hypomelanotic lesions of tuberous sclerosis.<sup>4,5</sup> These are shaped like the mountain ash leaflet and are less bright (hypomelanotic) than are those of vitiligo (amelanotic). The macules are present at birth and appear before other lesions of tuberous sclerosis. Therefore, ash-leaf-shaped, hypopigmented macules, usually over the trunk and extremities and more visible under Wood's light, are characteristic of tuberous sclerosis.

Teeth discolored by tetracycline, nails yellow from mepacrine, and some skin tumors in patients taking tetracycline<sup>6</sup> may also demonstrate variable fluorescence.

#### Potassium Hydroxide (KOH) Slide Examination

The potassium hydroxide (KOH) slide preparation can be one of the most revealing yet quick and simple diagnostic tests available to the busy practitioner. In essence, a skin or hair specimen is taken from the patient and placed on a slide. Although the standard procedure adds 10 to 15 percent potassium hydroxide solution, which is then covered by a cover slip and heated gently with thicker specimens, we prefer 20 percent potassium hydroxide with dimethyl sulfoxide\* and no heating. The KOH swells and separates the epithelial cells or keratin of hair so that they may easily be examined microscopically. The specimen can then be scanned for fungi, parasites, viral invasion, foreign bodies, etc. It is of primary importance that a proper specimen be obtained and this technique changes depending on the area one is examining, or for what one is searching.

The KOH slide examination can be effectively applied to the following

\*20 percent KOH with dimethyl sulfoxide  
distilled water 60 cc  
potassium hydroxide 20 gm  
dimethyl sulfoxide 40 cc  
Dimethyl sulfoxide is available from Fisher Scientific.

kinds of problems:

1. *Fungal infections* can be demonstrated in skin, hair, and nails in the form of hyphae, hyphal fragments (arthrospores), and yeast cells.

a. *Scalp Infections* — In ringworm of the scalp (tinea capitis) areas showing hair loss are scraped with a #15 scalpel blade taking care to remove any black dots. (Stubs of hairs that break off a few millimeters above the scalp should be plucked, especially if they show fluorescence under Wood's light.) The material obtained should be placed in KOH on a slide and examined microscopically. The presence of fungi will be demonstrated as masses of small round bodies (arthrospores) seen packed within the infected hair (endothrix) or found both inside and outside the hair (ectothrix).

b. *Glabrous Skin Infections* — The raised, advancing border of these skin lesions should be scraped since this is the location of the active fungi. It may be necessary to wipe the area with alcohol beforehand to remove debris, body oil, or ointments, which could interfere with the microscopic examination. In vesicular tinea infections, the top of the blister should be cut off and examined because this should contain a mesh of hyphae. This material should be placed on a slide with KOH and examined for hyphal filaments growing through the cells.

c. *Nail Infections* — In nail infections the nail should be pared with a scalpel and the scrapings discarded until an area of crumbly material is reached. This material along with subungual debris is prepared in the same manner as skin specimens and examined for hyphae.

d. *Yeast Infections* — In yeast infections, such as that found in the diaper area, it is important to get a specimen during the early stages. The small pustules near the advancing edge of the lesion should be scraped. Throat specimens for thrush should be obtained by scraping the area with a wooden tongue depressor. Vaginal specimens from a candidiasis can be taken with a swab. This material is processed in the same way as other skin specimens and microscopic examination should reveal budding yeast cells and pseudo-hyphae (elongated budding yeast cells resembling hyphae). Because simple budding yeast can be found as normal flora of the

body, it is imperative to find the pseudo-hyphae in order to consider the yeast pathogenic.

2. *Parasite infestation*, such as that due to *Sarcoptes scabiei*, the scabies mite, can be identified by shaving the entire burrow from the body with a scalpel blade. This specimen is placed in KOH on a slide and examined for the mite itself or the large brown eggs that the mite leaves in the skin.

3. *Molluscum contagiosum*, a viral disease of the epithelium, can be identified with a KOH preparation by expressing the lesion and examining microscopically for large masses of smooth, oval, molluscum bodies, which will be tightly packed.

#### Gram's Stain

The Gram's stain provides a rapid method to determine the presence and certain characteristics of bacteria. It is probably not used often enough in evaluating skin diseases.

There are a number of pustular skin diseases that are not bacterial in origin. Examples of these are some viral infections, subcorneal, pustular dermatosis, and pustular psoriasis. In these eruptions, Gram's stain of purulent material from intact lesions would show that no bacteria were present. In a few instances the Gram's stain of cutaneous lesions of gonococcemia may reveal the characteristic gram negative diplococci of *N. gonorrhoeae*.

The presence of bacteria on Gram's stain examination does not necessarily mean infection. The normal skin, and especially eczematous skin, harbors significant bacteria. Phagocytosis of bacteria has been considered evidence of infection by some.<sup>7</sup> As will be discussed in the section on bacterial cultures, infection must be based on clinical-bacteriological correlation that requires careful interpretation and judgment.

The principle of Gram's stain depends on the ability of some bacteria to retain the violet-iodine stain in the presence of alcohol. Those which keep the violet-iodine color are called gram positive (streptococci, staphylococci, pneumococci, and diphtheroids). Bacteria losing the violet-iodine color in the presence of alcohol will fix the red counterstain safranin and are referred to as gram negative (*Neisseria*, enteric bacilli, *Proteus vulgaris*, *Pseudomonas aeruginosa* and the genus *Hemophilus*).

The technique is as follows:\*

1. Heat fix the specimen by running the slide through a flame;
2. flood the slide with ammonium oxalate crystal violet for one minute and rinse with tap water;
3. flood the slide with Gram's iodine for one minute and rinse with tap water;
4. decolorize with ethyl alcohol for 15 seconds and rinse with tap water;
5. counterstain with safranin for 30 seconds and rinse in tap water;
6. dry and examine under oil immersion.

## Microbiological Procedures

### 1. Bacterial Cultures

Bacterial cultures should be obtained from a lesion that is likely to give the most specific and relevant information. For example, purulent material from an intact pustule is more representative than the contents from a ruptured, crusted lesion. Since eczematous skin, and to a lesser degree normal skin, teems with bacteria, it is difficult to distinguish pathogens from simple bacterial colonization. This is perhaps more true for *Staphylococcus aureus* than for other bacteria. This aspect of bacterial growth on skin is not adequately appreciated by physicians. A culture of *S. aureus* is not diagnostic of bacterial infection of the skin. The physician must interpret the bacterial culture in the context of the clinical situation. However, this is not as simple as it may seem. Nummular eczema may have the honey-colored crusts of impetigo, and bacterial cultures repeatedly produce *S. aureus*. But this dermatosis is not a simple pyoderma, and treatment with antibiotics is not adequate in the management of this skin disease.

If an intact pustule is available, material for culture can be obtained by aspirating it with a large-bore needle and syringe, or by opening the lesion with a needle, #11 blade, or hemolet, depending on the thickness of the wall of the pustule. Crusts should be removed from the heavily encrusted lesions and cultures obtained from the base. In a number of instances the base of the lesion will produce Group A streptococci (the more significant bacterial organism), and the crust *S. aureus*. For other

lesions, cultures of the exudate may be satisfactory.

If the material is not directly inoculated into culture media, the applicators must be kept from drying out. A transport material such as a *Culturette\*\** is satisfactory. Some consideration of the etiologic agent must be made on a clinical basis to select the appropriate media and atmospheric conditions. For example, anaerobic organisms require special containers and *Neisseria* needs chocolate agar with high CO<sub>2</sub>.

### 2. Fungal Cultures

Obtaining fungal culture material for dermatophytes requires special care, not only to establish whether infection has occurred, but also to make optimum the probability of a positive culture. Broken-off hairs, especially those that fluoresce, as in some instances of tinea capitis, are most likely to result in positive cultures. In other sites, scrapings from the active edges or the roof are most likely to result in positive cultures. Clippings of the discolored nails or scrapings from chronic scaling lesions (tinea pedis) may also be diagnostic. If *Candida albicans* infection is suspected, scrapings from satellite pustules or the scalloped edges are most likely to be productive. Cultures from the mouth for *Candida albicans* are not diagnostic because of the high recovery of this yeast in normal mouths. Instead, a KOH examination for pseudo-hyphae is more specific. Although the dermatophytes may withstand some drying, it is preferable to inoculate the specimen directly in the culture media. *Candida albicans* will not withstand drying, and specimens should be inoculated directly or be placed in an appropriate transport media. There are a number of sources for appropriate media in tubes and large-mouth perfume bottles that are suitable for office practice. Although Sabouraud's agar is a well-established culture media, we prefer Sabouraud's cyclohexamide, chloramphenicol agar (Mycosel).† This media inhibits fungal saprophytes and bacteria and probably adversely affects no more than two percent of *Candida albicans*.

\*\*Culturette tubes are available from Marion Scientific Corporation, Rockford, Illinois.

†Mycosel is available from Baltimore Biological Laboratories.

For those *not* trained in the identification of fungi the use of D.T.M. (Dermatophyte Test Media)‡ may be particularly helpful. This media incorporates a color indicator which turns red when acted on by the alkaline metabolite produced by a dermatophyte so that a pathogen can be recognized by a color change. In spite of some false positives, the media can be useful in proving mycotic infection.

Cultures for deep fungi and acid fast organisms can be obtained from lesion exudate, but it is usually more desirable to obtain tissue, especially from the edge of an ulcer. Half of the specimen can be submitted for histopathologic examination and the other half for culture. These specimens should not be allowed to dry out.

All of the cutaneous procedures which have been described are of diagnostic value when carefully selected and applied to appropriate clinical situations. The second and last part of this series will deal with various kinds of skin biopsy, touch imprints, the Tzanck smear and immunofluorescent studies.

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\*Gram's stain sets of chemicals are available from Difco Laboratories, Detroit, Michigan.

‡D.T.M. (Dermatophyte Test Media) is available from Schering Corporation.