Skin specimens for parasitic infection

Dr. Muntaha M. AL-Alouci

• Skin specimen

- The skin is the largest organ in the body. There are regional variations in the structure and function of skin between different sites in the body, and this is reflected in the microscopic appearance of the skin.
- The skin at all sites consists of three layers:
- (a) epidermis, which provides a protective waterproof covering; (b) dermis, which gives structural support and contains skin appendages and (c) subcutaneous fat.

- **Epidermis:** the epidermis is a keratinising stratified squamous epithelial layer. The cells arise from the basal layer and divide to form the spinous cell layer. At the granular layer cell death occurs and the dead cells form the keratin (horny) layer which is shed from the body.
- The epidermis also contains two other cell types: (a) melanocytes, which produce melanin pigment. These cells are scattered individually along the basal layer of the epidermis and (b) Langerhans cells which have a role in the immune-response of the body.

- **Dermis:** the dermis is the layer of connective tissue and elastic tissue containing blood and lymphatic vessels, nerves and nerve endings with skin appendage structures. The dermis is divided into the papillary dermis, which is the superficial structure that folds between the rete pegs of the epidermis and the reticular dermis (deeper dermis).
- Skin appendages: the skin appendage structure is derived from the epidermal cells which flow down into the dermis. These may form hair follicles and sebaceous glands which are closely associated with each other, forming a pilosebaceous unit. There are also eccrine and apocrine sweat glands. The skin appendage structures often extend into the subcutaneous fat.

- Subcutaneous fat: beneath the dermis is a layer of adipose tissue with an associated fibrovascular stroma. Hair follicles and sweat gland structures extend into it.
- Hair and nails: the hair and nails are specialized structures formed from keratin. They are located at specific specialized sites in the body

Types of skin specimens

• 1-Biopsy-specimens

A biopsy is a procedure to remove a piece of tissue or a sample of cells from body so that it can be analyzed in a laboratory.

- A skin (cutaneous) biopsy removes cells from the surface of body. A skin biopsy is used most often to diagnose skin conditions, including cancers or some infections. Skin biopsy procedures include:
- Shave biopsy. During a shave biopsy, the doctor uses a tool similar to a razor to scrape the surface of your skin.
- **Punch biopsy.** During a punch biopsy, the doctor uses a circular tool to remove a small section of your skin's deeper layers.
- Incisional biopsy. During an incisional biopsy, the doctor uses a scalpel to remove a small area of skin. Whether you receive stitches to close the biopsy site depends on the amount of skin removed.
- Excisional biopsy. During an excisional biopsy, the doctor removes an entire lump or an entire area of abnormal skin. You'll likely receive stitches to close the biopsy site.
- You receive a local anesthetic to numb the biopsy site before the procedure.







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Biopsy and Histopathological Examination



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Diagrammatic representation of excisional biopsy technique. a Incision around lesion. b Blunt under mining of mucosa of wound margins after removal of lesion. c Operation site after suturing.



art of it is removed, is eliber an existent forper misional beings. Suthermore, aspiration or met orier near a media to withingst a sample from i stor for examination.





a₁, **b**₁, **c**₁ Steps correspond to **a**, **b**, **c**, in a vertical cross-sectional view Obtain sterile, full-thickness, punch-biopsy specimens at the active border of the lesion. Some practitioners recommend having the specimen include both "affected" and "unaffected" (e.g., non ulcerated) tissue. Divide the specimen into 3 portions (or obtain multiple biopsy specimens):

• Use 1 sterile portion for leishmanial and other cultures (bacterial, mycobacterial, and fungal); the portion placed in leishmanial culture medium also can be used by CDC for PCR.

• Use 1 portion for impression smears

• Use 1 portion for histologic examination of tissue sections (fixed in 10% formalin; embedded in paraffin)—stained with H&E, Giemsa, and other special stains—to help exclude mycobacterial, fungal, and other infectious etiologies.

 Tissue impression smears (touch preparations) Grasp the biopsy specimen with forceps. To avoid making a bloody smear, some practitioners recommend briefly placing the cut surface on gauze or a paper towel to remove excess blood. However, blotting the specimen might remove amastigotes that are present on the surface. • Filet the specimen to increase surface area. Gently press the tissue—with a rolling or circular motion—onto a glass microscope slide. Repeat in a parallelowdowntheslide. • Air dry the slide, fix it in methanol, and stain with Giemsa. Alternatively, CDC can fix/stain the slide (as well as make the impression smears after receipt of the tissue). After making the smears, the tissue is not sterile but still is usable (e.g., for PCR).

- **2-Needleaspirates:** Fine needle aspiration is a type of <u>biopsy</u> procedure. a thin needle is inserted into an area of abnormal-appearing tissue or body fluid. As with other types of biopsies, the sample collected during fine needle aspiration can help make a diagnosis or rule out conditions such as <u>cancer</u>.
- it is generally considered a safe procedure. Complications are infrequent.

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"Draw up" ~0.1 mL of preservative-free sterile 0.9% saline into a 1.0–3.0 mL syringe (the better suction obtained with syringes at the larger end of the range may be advantageous). For ulcerative skin lesions, insert the needle, through intact sterile skin, into the dermis of the active border (see figure below). small-gauge needles are particularly useful for facial lesions



- Repeatedly move the needle back and forth under the skin, tangentially to the ulcer, simultaneously rotating the syringe and applying gentle suction, until pink-tinged tissue juice is noted in the hub of the needle. If necessary (if no aspirate is obtained), inject 0.05–0.1 mL saline under the skin and
 - resume suction. After the aspirate is obtained, withdraw the needle from the skin and discharge the aspirate into the leishmanial culture medium (each aspirate into a different tube).
 - Although thin smears of aspirates can be made, they typically are suboptimal, unless a cytospin preparation is used.

• 3-Dermal scrapings (for thin smears)

- Skin Scraping: is a diagnostic procedure that involved scrape of skin lesion with a scalpel blade or curette
- Skin scraping is a basis technique in dermatology that is applied in a high proportion of cases, its purpose is to detect the presence of microscopically ectoparasitesincluding mites and sarcoptes
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 - -It enables both the full thickness of the epidermis and the contents of the hair follicles to be sampled
 - -It is most commonly used in the diagnosis of parasitic infestations such as sarcoptic mange, cheyletiellosis and demodicosis
 - Generally several sites are sampled. Mites can be very difficult to find in some cases

- Procedure
- first thoroughly deride the relevant portions of ulcerative lesions; then apply pressure to ensure good hemostasis (to avoid making a bloody smear). A convenient location for obtaining specimens from ulcerative lesions is the area immediately adjacent to or beneath the active border (e.g., beneath the necrotic lip of the lesion).
 - The diagnosis of scabies can be confirmed by the demonstration of the mites or eggs. Because the mites are located under the surface of the skin, scrapings must be made from the infected area.
- Materials:

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- 1 x 3 glass slides
- sterile scalpel
- mineral oil
- sterile screwcap cup, 4 oz. (must be large enough to hold the glass slides)
- applicator sticks
- scotch tape

- Skin Scraping Technique:
- Place a drop of mineral oil on a sterile scalpel blade. Mites will adhere to the oil and skin scales will mix with the oil. The refractivity differences will be greater between the mite and the oil.
- Allow some of the oil to flow onto the papule.
- Scrape vigorously six or seven times to remove the top of the papule. (There should be tiny flecks of blood in the oil.)
- Transfer the oil and scraped material to a glass slide (an applicator stick can be used).
- Add 1 or 2 extra drops of mineral oil to the slide and stir the mixture. Any large clumps can be crushed to expose hidden mites.
- Place another slide on top of the slide with the material and tape the 2 slides together at each end to prevent them from coming apart.
- Place in a sterile cup large enough to hold the slides so that the lid can be screwed on firmly.
- Label the container with the appropriate patient information.
- Fill out a requisition and request: skin scraping to r/o scabies.
- Submit to laboratory for examination.



 Skin scraping for KOH examination & Involves microscopic examination of stratum corneum to visualize fungal elements & KOH solution causes separation and destruction of the stratum corneum cells





