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Topic Overview

Skin biopsies are among the most commonly performed biopsies due to the frequency of skin conditions and easy access to lesions. However, the accuracy of skin biopsy interpretation and the resulting pathology report rely heavily on the proper execution of the biopsy procedure. Optimal biopsy procedure involves selecting the best locations to sample, using the most appropriate biopsy technique, ensuring correct storage and shipment of samples, and including comprehensive clinical information that helps the pathologist interpret histologic results.

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DERMATOLOGY

How to Take and Submit Skin Biopsies: Getting the Most Out of Your Pathology Report

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Skin biopsies are commonly performed because skin issues are common, the skin is readily accessible, and changes are often quickly noticed by the client or the veterinarian. Taking skin biopsies is a prevalent way to obtain detailed information about a skin lesion and guide the subsequent management of the case.

Skin biopsies are performed for various reasons, including achieving or confirming a diagnosis; guiding further testing; or, as a last resort, failure of lesion response to treatment. Some conditions, like demodicosis or other mite infestations, can be diagnosed without biopsies, and deep skin scrapes may suffice. However, a negative result from a skin scrape does not exclude the possibility of infection, which makes biopsies a potential subsequent step.

Biopsies may not always reveal pathogens, especially if pathogens are present in low numbers. Even when special stains are used, rare pathologic agents may remain undetected due to the low sensitivity of special stains on formalin-fixed, paraffin-embedded tissues. Cultures or polymerase chain reaction tests may be more sensitive for detecting a low number of pathogens. Nevertheless, a biopsy may identify specific agents even when scrapes and cultures return negative results, thus enabling a definitive diagnosis.

WHAT TO CONSIDER BEFORE PERFORMING BIOPSIES

Secondary Conditions

Before performing biopsies, first check for

Learning Objectives

- Numerous actions taken before a biopsy can improve the interpretation of biopsy results (e.g., managing treatment and biopsy site preparations before the biopsy procedure, appropriately preparing the biopsy site).
- Choosing the right locations and appropriate technique for a biopsy and submitting samples of the correct quantity and size are essential.
- The correct storage and shipping of the samples greatly influence the outcome of the biopsy interpretation.
- Incorrect biopsy technique and sample storage may result in tissue artifacts.
- Submitting additional clinical information along with the biopsy sample is crucial.
- Some cases may require additional biopsies if the lesions are not yet fully developed.
- Good communication between clinician and pathologist ensures the most thorough interpretation of the lesions.

secondary pyoderma, which can obscure the primary lesions. For cases with secondary pyoderma, treating the infection before performing the biopsy should be considered.

Corticosteroid Use

If the patient was treated with a corticosteroid before biopsy, the inflammatory cell profile may be altered, thereby complicating interpretation of histologic lesions. For instance, eosinophil counts may be significantly decreased after steroid therapy, making the diagnosis of allergic dermatitis challenging. Before performing the biopsy, it is advisable to discontinue oral corticosteroids for at least 2 weeks and injectable steroids for 4 to 8 weeks, depending on the half-life of the drug. However, when lesions are severe and stopping the corticosteroid medication would result in patient suffering, it is acceptable to proceed with biopsies without discontinuing the drugs.

Hair Removal

Hair should be trimmed using scissors; use of electric clippers should be avoided as they can cause artifacts and damage delicate features.

Cleansing

For superficial lesions, avoid scrubbing the lesion before performing the biopsy. Scrubbing may eliminate key diagnostic features (e.g., crusts containing acantholytic cells or infectious agents). The lesions should therefore either remain uncleansed or be gently cleansed with water and patted dry, ensuring that any crusts or scales remain undisturbed.

For deep dermal or nodular lesions, cleansing the skin by scrubbing the surface is acceptable because the diagnostic features in the deeper layers of the skin tissue remain visible.

Local Anesthesia

Injecting local anesthetics (e.g., lidocaine) into the subcutaneous tissue instead of the dermis helps avoid artifacts that can mimic the appearance of dermal edema.

Marking

For areas affected by alopecia, marking the direction of hair growth by drawing a line with a Sharpie can greatly assist the pathologist during the trimming process (cutting the sample in smaller sections to fit the tissue cassettes). Marking allows for a cut that follows the hair follicles, which enables assessment of the entire length of the hair follicle (**FIGURE 1**). Note that a dot does not effectively indicate the direction of the cut.

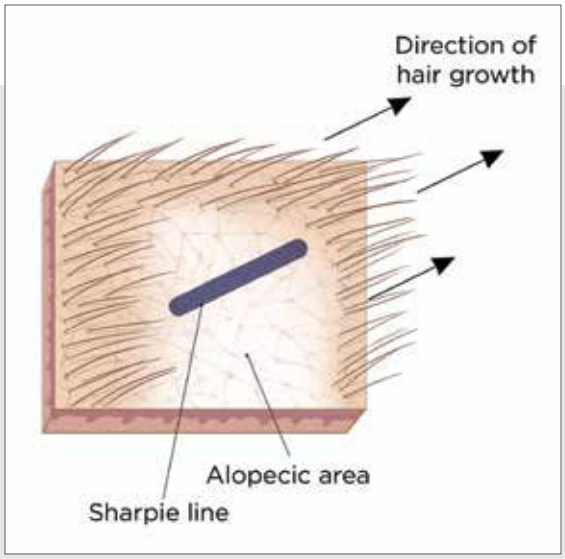

FIGURE 1. Recommended step before performing a biopsy of an alopecic area. A line is drawn in the direction of hair follicles to facilitate trimming along the entire length of the hair follicles.

Figure 1: Kip Carter

WHICH LESIONS TO BIOPSY

It is crucial to biopsy fully developed primary lesions, if clinically available, because later stages of lesions may reflect only chronicity of the changes (e.g., fibrosis) and the original cause for the lesions may no longer be visible. For instance, a pustule containing acantholytic cells is typically indicative of pemphigus foliaceus, but older crusts might reveal only secondary bacterial contamination and degenerated cells, making a definitive diagnosis of pemphigus foliaceus impossible. Early lesions may or may not be diagnostic. Before a diagnosis can be reached, repeated biopsy samples may need to be submitted. In some cases, collecting lesions from numerous stages of maturation may be helpful.

Skin lesions can be primary or secondary (**TABLE 1**). However, the same type of lesion may be primary in one disease and secondary in another. For instance, alopecia is a primary lesion in endocrinopathies resulting from hair cycle arrest; however, it can also occur as a secondary lesion (e.g., in allergic dermatitis)

TABLE 1 Primary and Secondary Skin Lesions

LESION	DESCRIPTION
PRIMARY	
Bulla	Circumscribed, fluid-filled lesion; >1 cm
Cyst	Closed cavity that contains fluid or semisolid material (e.g., keratin)
Macule	Flat lesion, same level as the skin; <1 cm
Nodule	Solid, elevated lesion, usually consisting of inflammatory cells or tumor cells
Papule	Solid, raised lesion; <1 cm
Patch	Flat lesion, same level as the skin; >1 cm
Plaque	Solid, raised lesion; >1 cm
Pustule	Circumscribed, elevated lesion filled with inflammatory cells (usually neutrophils or eosinophils)
Vesicle	Circumscribed, fluid-filled lesion; <1 cm
Wheal	Circumscribed, raised, usually erythemic lesion composed of edema
SECONDARY	
Callus	Thickened, often pigmented, and hyperkeratotic plaque in areas of chronic pressure trauma or friction (e.g., elbow)
Crust	Dried exudate on the surface
Epidermal collarette	Loose scales arranged in circular pattern around a central area of erythema (usually represents a ruptured pustule from bacterial folliculitis)
Erosion	Loss of superficial layers of the epidermis; the basement membrane is still intact
Eschar	Thick crust in association with an ulcer with incorporated collagen
Fissure	Linear, narrow tear or crack
Lichenification	Increased thickening and hyperpigmentation of the skin resulting from long-standing surface trauma or friction
Scale	Increased shedding of the stratum corneum
Ulcer	Transmural loss of the epidermis including the basement membranes

as a result of mast cell degranulation or trauma to the hair follicles (e.g., scratching or overgrooming due to pruritus).

HOW MANY BIOPSY SAMPLES TO SUBMIT

When lesions are multifocal, performing multiple skin biopsies enhances the likelihood of identifying a diagnostic feature in the samples. Typically, 3 to 4 biopsies are suggested, except when the lesion is focal and small enough to be entirely submitted through a single biopsy. If biopsy samples are collected from different locations on the body and lesions have a different appearance, submitting the samples in separate containers with proper labels will aid in the accurate interpretation of the lesions.

IDENTIFYING THE BEST LOCATION TO BIOPSY

Biopsy samples should be collected from the center of the lesion. Additional samples may be obtained from the lesion's edge and unaltered skin (**FIGURE 2A**). Collecting biopsies solely from the margins of lesions should be avoided because margins may only contain fibrosis and granulation tissue and lack diagnostic features (**FIGURE 2B**). However, for ulcers, vesicles, and bullae, biopsies should be taken at the lesion's edge, including the healthy epidermis (**FIGURE 2C**), because for these lesions, it is crucial to examine the transition from the vesicle or ulcer to healthy skin. Doing so is key to identifying the precise location of the separation between the epidermis and the dermis (e.g., a suprabasilar split in pemphigus vulgaris, a subepithelial split in subepithelial bullous diseases) or detecting any signs of interface dermatitis that may predispose to a vesicular lesion or ulcer. If a biopsy sample is obtained from the center of a lesion, the overlying epidermis may not be included in the sample, making a definitive diagnosis unlikely.

BIOPSY METHODS

Punch Biopsies

Among the several methods for performing a biopsy, the most commonly performed is the punch biopsy. A punch biopsy sample should ideally be at least 6 mm in diameter. Although 8-mm samples are preferred for their larger size, they also carry a marginally increased risk for contamination. Punch biopsy samples can be 4 mm if the lesions are very small or in sensitive areas (e.g., nasal planum, foot pads). Punch biopsies smaller than 4 mm are generally not recommended because they are usually nondiagnostic.

When performing punch biopsies, twist the punch in a single direction to prevent friction artifacts that can occur from moving it back and forth.

Avoid using punch biopsies for lesions deep within the tissue as a punch biopsy of deep lesions may yield only the superficial portion of the lesion and leave the main deeper lesion unexamined. For such cases, an excisional biopsy is recommended or a double punch biopsy could be considered (**FIGURE 3**).

Excisional/Elliptical and Wedge Biopsies

Excisional/elliptical biopsies are advised for larger lesions, such as big pustules; nodules; or vesicular, ulcerative lesions (**FIGURE 2C**). Wedge biopsies, although collected similarly to excisional biopsies, are triangular and primarily collected from areas like pinnal margins, footpads, or nodular lesions.

GENERAL BIOPSY TECHNIQUE

For all biopsy techniques, gentleness is key. To prevent tissue damage and artifact introduction, use only forceps with rounded edges and avoid those with teeth.

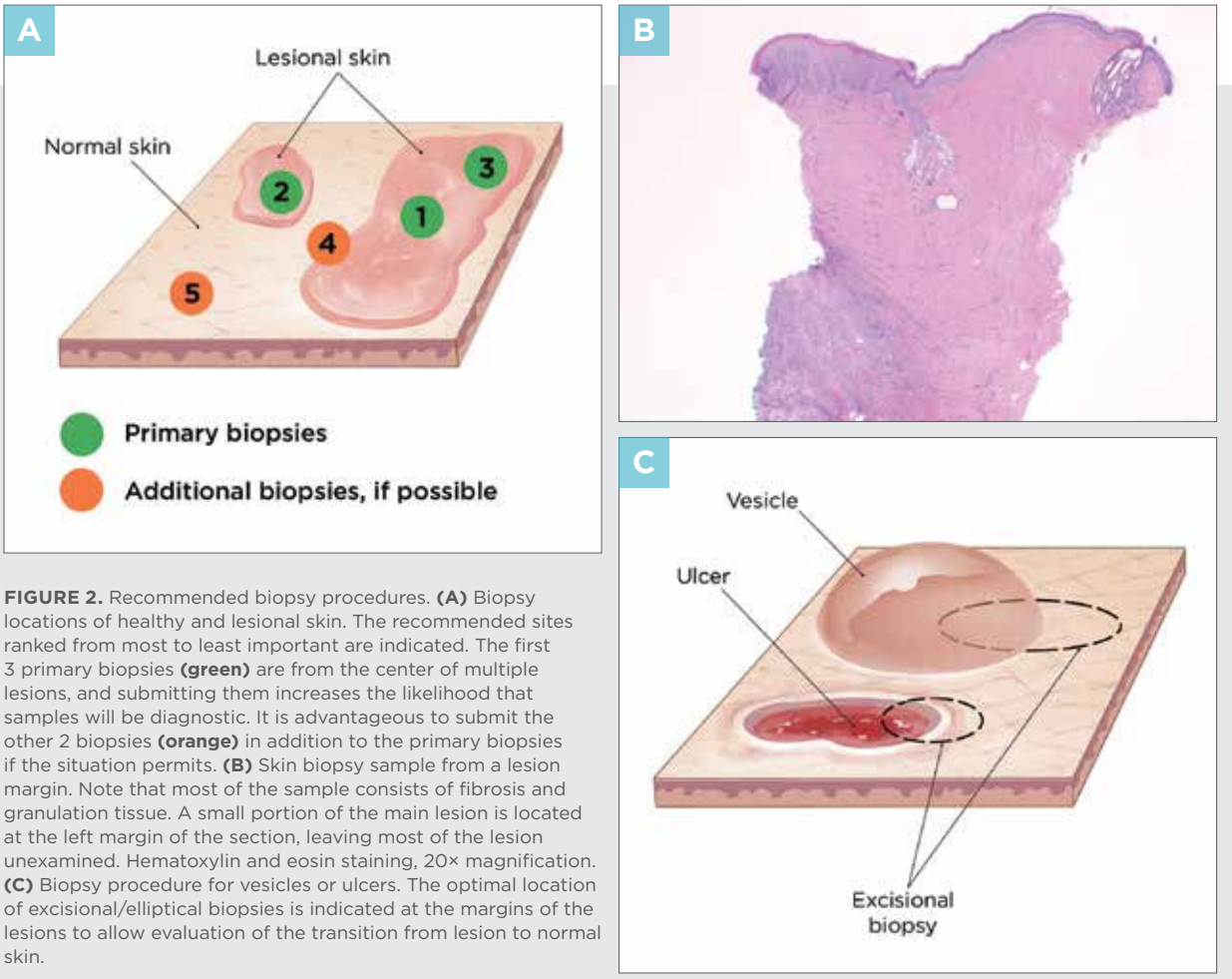

FIGURE 2. Recommended biopsy procedures. **(A)** Biopsy locations of healthy and lesional skin. The recommended sites ranked from most to least important are indicated. The first 3 primary biopsies **(green)** are from the center of multiple lesions, and submitting them increases the likelihood that samples will be diagnostic. It is advantageous to submit the other 2 biopsies **(orange)** in addition to the primary biopsies if the situation permits. **(B)** Skin biopsy sample from a lesion margin. Note that most of the sample consists of fibrosis and granulation tissue. A small portion of the main lesion is located at the left margin of the section, leaving most of the lesion unexamined. Hematoxylin and eosin staining, 20× magnification. **(C)** Biopsy procedure for vesicles or ulcers. The optimal location of excisional/elliptical biopsies is indicated at the margins of the lesions to allow evaluation of the transition from lesion to normal skin.

Figure 2A, 2C and Figure 3: Kip Carter

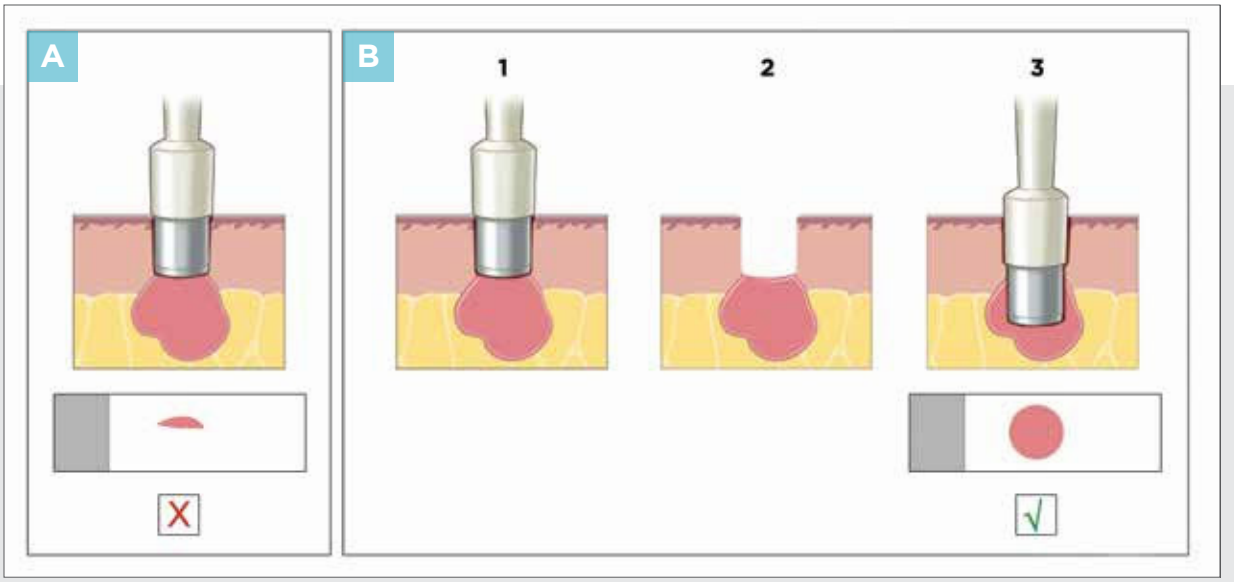

FIGURE 3. Pictogram of a double punch biopsy procedure. **(A)** If the lesion is deep within the tissue, a punch biopsy will only show the most superficial lesion. **(B)** It is recommended to take a second punch biopsy sample at the same location after the first, which will enable visualization of the deep lesion.

For example, for fragile tissue manipulation, Adson-Brown or Bishop-Harmon forceps can be used. Grasp the biopsy samples from the bottom, being careful not to squeeze as squeezing can cause artifacts that may render the sample nondiagnostic (**FIGURE 4A AND 4B**). Procedures such as sedation, suture techniques, and postoperative care are also relevant to the biopsy procedure but are beyond the scope of this article.

SAMPLE FIXATION

Biopsy samples should be immediately immersed in a fixative solution. If samples are left without a fixative for too long, autolysis greatly impedes histologic evaluation. Even a few minutes under intense light from surgical lamps while suturing the biopsy site can induce artifacts.

As a fixative, 10% neutral buffered formalin is strongly recommended. Formalin is a 37% to 40% water solution of formaldehyde, which is equivalent to a 4% paraformaldehyde solution. Formalin should be handled carefully because it causes skin irritation, can cause serious eye damage, and is considered carcinogenic.[1] Formalin is considered superior to formalin-free fixatives, such as 70% alcohol and acetone; however, because of the potential health hazards and carcinogenic nature of formalin, alternatives to replace formalin could be considered.[2]

Not immersing samples in an appropriate fixation solution can lead to fixation artifacts. Similarly, fixation artifact may arise if the samples are not immersed in a sufficient volume of formalin. A ratio of 1:10 (specimen:formalin) is recommended.

Consequently, selecting an appropriate container for the samples is crucial. Small tubes with narrow diameters do not provide adequate space for the proper specimen:formalin ratio. Furthermore, samples can be difficult to remove from the tubes as the tissue hardens with formalin fixation and may be stuck to the bottom of the tube. To ease the removal of samples from the container and ensure an accurate specimen:formalin ratio, use of round, straight-sided, wide-mouth jars with a broad opening is advised.

If a tissue cassette is used to additionally contain the sample in a jar (e.g., to separate samples from different locations), the sample should not be thicker than the height of the cassette as this leads to squeeze artifacts.

Samples that become frozen during transit may lead to freeze artifacts (**FIGURE 4C**). To avoid freezing of samples, alcohol (e.g., ethanol, methanol, isopropanol) should be added to the formalin (1 part alcohol:9 parts formalin).

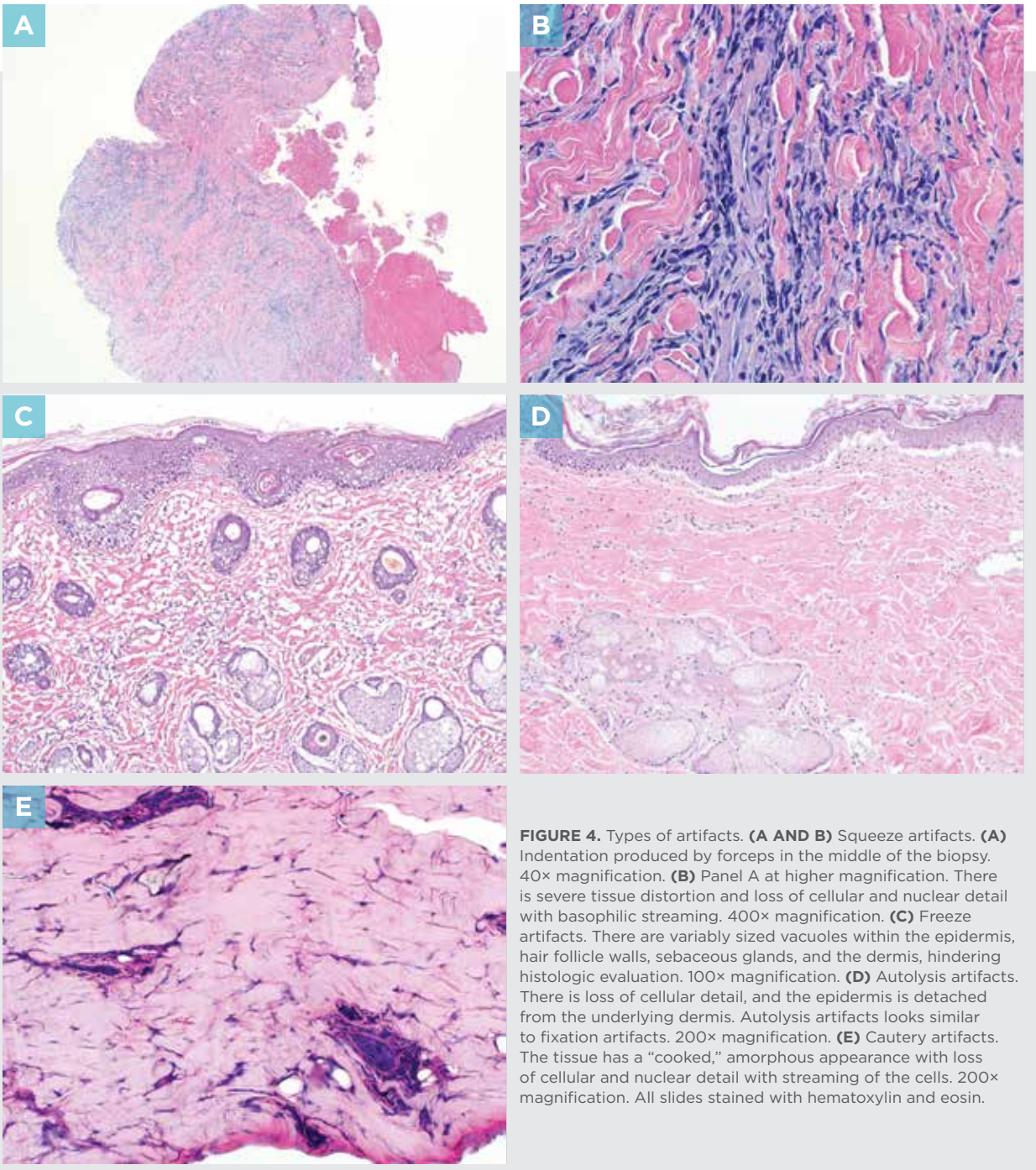

FIGURE 4. Types of artifacts. **(A AND B)** Squeeze artifacts. **(A)** Indentation produced by forceps in the middle of the biopsy. 40× magnification. **(B)** Panel A at higher magnification. There is severe tissue distortion and loss of cellular and nuclear detail with basophilic streaming. 400× magnification. **(C)** Freeze artifacts. There are variably sized vacuoles within the epidermis, hair follicle walls, sebaceous glands, and the dermis, hindering histologic evaluation. 100× magnification. **(D)** Autolysis artifacts. There is loss of cellular detail, and the epidermis is detached from the underlying dermis. Autolysis artifacts looks similar to fixation artifacts. 200× magnification. **(E)** Cautery artifacts. The tissue has a "cooked," amorphous appearance with loss of cellular and nuclear detail with streaming of the cells. 200× magnification. All slides stained with hematoxylin and eosin.

TYPES OF ARTIFACT

Squeeze Artifacts

These very common artifacts result from squeezing of biopsy specimens during sampling (**FIGURE 4A**). The tissue is usually severely distorted with streaming of cells and nuclei, which renders the affected areas nondiagnostic due to the loss of recognizable cellular and nuclear details (**FIGURE 4B**).

To Avoid: Be very gentle, avoid squeezing samples, and avoid use of cassettes when the sample is thicker than the cassette height.

Prefixation Artifacts

Anesthetics (e.g., lidocaine) injected into the dermis may mimic edema or even bullous disease.[3] Bleeding and separation of collagen bundles can result.

To Avoid: Inject anesthetics into the subcutaneous tissue rather than the dermis.

Fixation Artifacts

Improper fixation will cause tissue autolysis, resulting in loss of cellular detail, karyorrhexis, and karyolysis. The epidermis may separate from the underlying dermis and mimic a vesiculobullous lesion.[3] Fixation artifact looks like autolysis artifact.

To Avoid: Use 10% neutral buffered formalin and the appropriate amount of fixative (1 part fixative:10 parts specimen).

Freeze Artifacts

When tissue is frozen and subsequently thawed (as may occur during postal transit in cold weather), freeze artifact can result, leading to variably sized vacuoles in the epidermis, hair follicle walls, and dermis. Severe freeze artifact can render the sample nondiagnostic because the vacuoles may obscure any underlying lesions (**FIGURE 4C**).

To Avoid: Add alcohol to the fixative (1 part alcohol:9 parts formalin).

Autolysis Artifacts

After tissue death, the resultant enzyme loss, mitochondrial damage, and RNA and protein degradation can quickly lead to karyorrhexis, karyolysis, and loss of cellular detail. Similar to fixation artifacts, the epidermis may separate from the dermis beneath, causing cleft formation. The longer the tissue remains outside a living organism, the more severe the autolysis (**FIGURE 4D**).

To Avoid: Immediately immerse samples in an appropriate fixative using the correct ratio of samples:fixative.

Cautery/Fulguration Artifacts

When a sample is excised with electrosurgical or laser-cutting techniques, the tissue might exhibit a

BOX 1 Tips for Submitting Biopsy Samples

- Include patient signalment: breed, age, sex, skin/coat color.
- Indicate location of samples *and* lesion distribution on the animal.
- Provide a brief history, including clinical signs (e.g., pruritus, fever), previous therapy, and response to treatment.
    - Include relevant information only, not the patient's entire clinical history. Sorting through unnecessary information can be very time-consuming for the pathologist.
    - Write legibly or type out the clinical history. Deciphering illegible handwriting can be a time-consuming and frustrating task for all parties involved.
    - Do not use acronyms or abbreviations as they may not be used universally, leading to confusion for the pathologist.
    - Use appropriate terms to describe the lesions (e.g., pustules, macules, vesicles, erythema).
    - Indicate the duration of the skin issues and how they have developed over time.
    - Include results of other performed diagnostic tests (e.g., skin scrapes, cultures, previous histologic findings).
- Remember that pathologists are not clinicians and usually do not administer treatments. As such, they might not be acquainted with specific medication names, the interpretation of drug dosages, or clinical values.
- Pathologists do not judge if clinical suspicions are not confirmed; however, clinical differentials enable the pathologist to address the listed suspicions. Suspicions can be confirmed or reasons can be provided as to why the histologic lesions do not fit the clinical suspicion.
- Whenever available, include images in your email or submission, even if they are not of high quality. Clinical photographs are extremely helpful for interpreting histologic lesions.
- If the pathology report and interpretation do not match the clinical picture or behavior of the lesion, consider phoning or emailing the pathologist to discuss the case. Such discussion may help add missing information, provide an overall picture, and potentially plan how to proceed (e.g., repeating biopsies).

"cooked" appearance due to thermal injury, resembling a thermal or chemical burn. This type of handling may give the tissue an amorphous appearance with diminished cellular detail, and the epidermis may separate from the underlying dermis (**FIGURE 4E**).

To Avoid: Avoid using electrosurgical or laser cutting to excise skin samples. Use a scalpel or punch whenever possible. When using thermal cutting, add an additional biopsy margin to account for the cautery artifact.

SAMPLE SUBMISSION

What Information to Submit

For neoplastic lesions, a diagnosis can frequently be made without a comprehensive clinical history or an elaborate description of the lesions. In contrast, for non-neoplastic samples, it is essential to submit additional information for the precise interpretation of histologic findings and to maximize the amount of information derived from the skin biopsy submission (**BOX 1**). Correlating histologic lesions with the clinical picture is of paramount importance, thereby making good communication between the clinician and the pathologist essential.

How to Send

After the sample is collected and immersed in 10% buffered formalin in a suitable, leak-proof, plastic container, the jar should be labeled and placed inside a plastic ziplock bag including absorbent material to soak up potential leaks. Submission forms should not be placed inside the ziplock bag as leakage can make paperwork unreadable. Instead, the submission form should be included separately in the shipping box along with the bagged container. An ice pack is not required for formalin-fixed specimens.

Where to Send

For straightforward cases, submitting samples to a general diagnostic laboratory may suffice. However, for clinically complex cases, it is highly recommended to submit the samples to a service specializing in dermatopathology.

SUMMARY

Maximizing the efficacy of a biopsy submission requires several considerations: selecting the optimal locations for biopsies, using the most appropriate biopsy technique, ensuring correct storage and shipment of samples, and including comprehensive clinical information. Even when all the required information is provided and the best possible biopsy specimens are submitted, some cases may still require additional biopsies because the diagnostic lesions might not be present during the initial biopsy or they may not have developed fully. Good communication between the clinician and pathologist will enable correlation of the histologic results with the clinical picture and will ensure the most thorough interpretation of skin lesions possible. TVP

References

1. Shetty JK, Babu HF, Laxminarayana KPH. Histomorphologic assessment of formalin versus nonformalin fixatives in diagnostic surgical pathology. *J Lab Physicians*. 2020;12(4):271-275. doi:10.1055/s-0040-1722546
2. Protano C, Buomprisco G, Cammalleri V, et al. The carcinogenic effects of formaldehyde occupational exposure: a systematic review. *Cancers (Basel)*. 2021;14(1):165. doi:10.3390/cancers14010165
3. Taqi SA, Sami SA, Sami LB, Zaki SA. A review of artifacts in histopathology. *J Oral Maxillofac Pathol*. 2018;22(2):279. doi:10.4103/jomfp.JOMFP_125_15

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CE QUIZ QUESTIONS

1. **How many biopsy samples are recommended when the patient has multiple lesions?**
   a. 1
   b. 2
   c. 3 to 4
   d. 6 to 7

2. **For a vesicular lesion, the best location to take a biopsy sample from is the center of the lesion.**
   a. True
   b. False

3. **Which stage of lesion is preferred for biopsy?**
   a. Early
   b. Fully developed
   c. Late

4. **What type of artifact produces large vacuoles within the tissue?**
   a. Squeeze artifact
   b. Cautery artifact
   c. Fixation artifact
   d. Freeze artifact

5. **Providing clinical information facilitates the interpretation of non-neoplastic lesions.**
   a. True
   b. False