

Lower Extremity Specimen Collection Techniques





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Biopsy Overview

WHAT IS A BIOPSY?

- A biopsy is a small sample of lesional tissue for histopathologic evaluation
- Used to determine the presence and/or extent of a disease
- Guides both pharmacologic therapy (dermatitis) as well as surgical planning (neoplastic processes)
- Narrows the differential diagnoses and prevents delays in treatment

BIOPSY TIPS

- Biopsy is recommended prior to definitive excision
- Ablative procedures are contraindicated without a biopsy confirmation of the diagnosis
- When dealing with pigmented lesions, biopsy the darkest region and any area of elevation
- Be gentle when handling the biopsy to avoid crushing the sample
- Place the sample in fixative immediately after removal to avoid desiccation
- Samples taken for gout crystal analysis must be submitted in 100% alcohol (ETOH)
- Photographs of the pathology to be biopsied are recommended and may have utility in diagnosis

MEDICAL DOCUMENTATION

- Consider obtaining an informed consent
- Document the medical necessity for the procedure including pain and/or suspicion of malignancy
- Create a procedure note including the description of the pathology being sampled, i.e location, size, shape, color, etc.
- Document the specific laboratory test ordered
- Confirm review of pathology report and diagnosis consultation with the patient



Tips on submitting specimens to Bako Diagnostics

- Include a clinical description with all specimens
- Send clinical photos to laboratory by visiting bakodx.com and clicking the “Upload Image” button at the top right of the home page
- Multiple biopsies from the same lesion or within the same geographic region of a process may be submitted in the same vial (resulted as one specimen)
- Multiple biopsies from different locations should be submitted in different specimen vials (resulted as multiple specimens)
- Multiple biopsies should be labeled anatomic site specific



BakoDx headquarters in Alpharetta, GA



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Exam Room Supplies

RECOMMENDED LIST

Stock each exam room with the following to reduce prep time and improve efficiency when performing procedures:

- **70% isopropyl alcohol wipe**
- **Local anesthesia**
 - › Lidocaine with (or without) epinephrine, pre-filled 1cc or 3cc syringes, 27g or 30g needle
- **Sterile biopsy instruments**
 - › 2mm and 3mm punch biopsy, #10 blade scalpel, Miltex® BiopBlade™, curette, 10cc syringe with 18 gauge needle for needle aspiration biopsy, toenail nipper, forceps, needle holder, scissors*
- **Suture**
 - › Optional for punch biopsy >3mm or packing material such as Gelfoam®
 - › Hemostatic agent (35% aluminum chloride or Monsel's solution) to control bleeding
- **Bandage and gauze dressing**
- **Formalin fixative vials**
 - › For skin, soft tissue mass, and needle aspiration specimens
- **Reagent alcohol (ETOH) vials**
 - › For gout specimens and needle aspiration specimens
- **Onychodystrophy Collection Bags**
 - › For toenail specimens (onychomycosis) and dry skin specimens (keratin only)
- **Dermapak®**
 - › For skin PCR testing
- **ENFD kit**
 - › For Epidermal Nerve Fiber Density testing

*Most instruments are available for purchase through Bako Diagnostics.



Complimentary Supplies for Specimen Submission Available from Bako Diagnostics

- Onychodystrophy Collection Bags
- Specimen Vials
- Epidermal Nerve Fiber Density (ENFD) Biopsy Kit
- Dermapak®
- Specimen Submission Guide
- Requisition Forms
- Large Biohazard Bags
- Transport Box
- Clinical Pak and Airbill/Label for Mail
- Patient Education Brochures
- Biopsy Consent Forms
- Biopsy Refusal Form
- Post-Biopsy Instructions for Patients

 Bako Diagnostics 4340 SHILOH ROAD ALPHARETTA, GA 30005 PH: 877-376-7284 • FAX: 770-475-0528		PHYSICIAN/CLINIC INFORMATION			
DATE COLLECTED: / / TIME COLLECTED: :		LAB USE ONLY SPCR1500000			
PATIENT INFORMATION					
LAST NAME: _____ FIRST NAME: _____ (ALL)					
STREET ADDRESS: _____ APT. # _____					
CITY: _____ STATE: _____ ZIP CODE: _____					
PHONE NUMBER: _____					
DATE OF BIRTH: / / AGE: _____ SEX: _____ RACE: _____					
BILL: <input type="checkbox"/> INSURANCE <input type="checkbox"/> PATIENT <input type="checkbox"/> Biomechanical Correlation (Plantar Skin)					
BILLING/INSURANCE INFORMATION (Attach a copy of primary / secondary insurance cards — both sides)					
SUBSCRIBER NAME/RELATIONSHIP TO SUBSCRIBER: <input type="checkbox"/> Self <input type="checkbox"/> Spouse <input type="checkbox"/> Dependent					
MEMBER ID: _____					
ADDRESS: _____					
CITY: _____ STATE: _____ ZIP: _____					
ADDITIONAL CLINICAL INFORMATION: (If clinical image is available, please print and attach or submit digitally at HTTPS://IMAGES.BAKODX.COM)					
ICD CODES (See back)					
SPECIMEN #1		SPECIMEN #2			
<input type="checkbox"/> Right <input type="checkbox"/> Left <input type="checkbox"/> Shave <input type="checkbox"/> Punch <input type="checkbox"/> Biopsy <input type="checkbox"/> Excision		<input type="checkbox"/> Right <input type="checkbox"/> Left <input type="checkbox"/> Shave <input type="checkbox"/> Punch <input type="checkbox"/> Biopsy <input type="checkbox"/> Excision			
NAIL UNIT DYSTROPHY (Fungal, Inflammatory, Neoplastic)		NAIL		NAIL UNIT DYSTROPHY (Fungal, Inflammatory, Neoplastic)	
<input type="checkbox"/> Higher Sensitivity and melanin screen (PAS/MS/FH) Dermatococcus / Melanoma		PLEASE INDICATE PRECISE SITE OF ORIGIN (1,2)		<input type="checkbox"/> Higher Sensitivity and melanin screen (PAS/MS/FH) Dermatococcus / Melanoma	

Example of BakoDx Requisition Form

Toenail Specimen Collection for Onychodystrophy

INDICATION

Toenail specimen collection for onychodystrophy/onychomycosis augments the physician's physical examination and is used to diagnose the presence of nail disease. Additionally, laboratory testing provides independent objective data that can assist physicians to determine a precise course of targeted patient treatment.

DIFFERENTIAL DIAGNOSIS

Lichen Planus	Pigmented Mold	Onychomycosis
Melanocytic Neoplasm	Onychogryphosis	Pseudomonas
Microtrauma	Onycholysis	Psoriasis

MATERIALS NEEDED

- 70% isopropyl alcohol wipe
- Nail Nippers
- Curette

PROCEDURE

1. Wipe toenail collection site with 70% isopropyl alcohol.
2. Local anesthesia may or may not be required.
3. Debride and discard distal nail clippings.
4. Obtain specimen from most proximal area of nail and hyponychium.
5. Use curette to obtain additional subungual debris.
6. Place nail and subungual debris into Onychodystrophy Collection Bag.

FIXATIVE

No fixative required, use Onychodystrophy Collection Bag.

Onychodystrophy Laboratory Tests

Anatomic pathology tests (PAS, GMS, FM) are sensitive tests designed to detect fungi and melanin, and allow for a diagnosis of microtrauma in cases of non-infectious nail dystrophy. PCR analysis is a specific test designed to identify the genus and species of causative organisms in cases of infectious nail dystrophy. Combination testing (PAS, GMS, FM, PCR) provides the most comprehensive evaluation of nail unit dystrophy, incorporates both the highest sensitivity and highest specificity, and enables precise targeted therapy for the underlying etiology.

PERIODIC ACID–SCHIFF (PAS)

Sensitive test for staining cell walls of fungi magenta to identify living fungi

GOMORI METHENAMINE SILVER (GMS)

Sensitive test for staining degenerated fungal organisms

FONTANA-MASSON (FM)

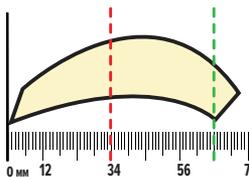
Sensitive test for melanin pigment (melanoma) and pigmented saprophytes

ONYCHODYSTROPHY PCR TEST (DNA)

Specific test that identifies genus and species with molecular genetic analysis and rapid detection of genetic material from dermatophytes, saprophytes, yeasts, and pseudomonas

IMPORTANT SPECIMEN INFORMATION

Minimum 3mm Best 6mm+



(Enlarged for detail)

Minimum specimen size is 3mm of nail and subungual debris for ordering a single test such as PAS or PCR. However, when ordering multiple tests such as PAS, GMS, FM, and PCR at the same time, 6mm or more of specimen is preferred.

PATIENT NAIL COLLECTION TECHNIQUE

1

Debride and *discard* distal nail clippings.

2

Obtain specimen from the most proximal area of nail and hyponychium.

3

Use curette to obtain *additional subungual debris*, as this will increase the potential yield.

4

Place *dry* nail sample and subungual debris into Onychodystrophy Collection Bag.

IMPORTANT

Minimum 3mm Best 6mm+

Enlarged for detail

- Single test = 3mm (min)
- Multiple tests = 6mm (best)
- PAS, GMS, FM, PCR

5

Complete information on bag. Complete Requisition Form and prepare for shipping.

PATIENT EXAMPLE

Pre-Nail Debridement



Patient nail as they present in the office.

Post-Nail Debridement



Total area of nail and tissue removal during collection process, showing optimal specimen size of 6mm+.

TIPS FOR BEST SUBMISSIONS

- Do not submit specimen if your patient is undergoing antifungal therapy. Wait 7 days after topical treatment. Wait 60 days after systemic treatment.
- Do not use nail or skin softener.
- Do not place a patient in a betadine whirlpool.
- Do not submit initial distal nail clippings.
- Do not submit specimen in formalin.



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Punch Biopsy

INDICATION

Punch biopsies are ideal when a small part of a much larger lesion is submitted for histopathology such as sampling tumors that are too large to be shaved or inflammatory conditions that have a deep dermal component.

DIFFERENTIAL DIAGNOSIS

Carcinomas	Melanoma	Psoriasis
Dermatitis	Neoplasm	Tinea Pedis
Diabetic Ulcers	Neoplastic Ulcers	Tumors
Eczema	Pigmented Lesions	Verruca

MATERIALS NEEDED

- 2mm or 3mm punch biopsy with or without plunger
- 70% isopropyl alcohol wipe
- 1cc to 3cc lidocaine with epinephrine in a 1cc to 3cc syringe, 27g or 30g needle
- Hemostatic agent (35% aluminum chloride or Monsel's solution)
- Topical antibiotic
- Gauze pad
- Bandage

INSTRUMENT



PROCEDURE

A multiple 2mm punch approach is warranted for large lesions and non-specific dermatitis as two small punches allow for more rapid wound-healing, better tissue sampling, and usually do not need suture closure. A single 3mm punch can be used on a smaller pigmented lesion.

1. Prepare biopsy site with 70% isopropyl alcohol wipe for 10 seconds.
2. Administer local anesthesia as a raised wheal at the biopsy site.
1cc lidocaine with epinephrine in a 3cc syringe, 27g or 30g needle.
3. Gently press the punch instrument onto the skin to ensure sampling dermal and subcutaneous tissue.

Do not include normal tissue with biopsy, except bullous lesions where you should include the area of attachment.

4. Rotate punch clockwise/counterclockwise and cut to the level of the subcutaneous fat.
5. Gently elevate the biopsy core and cut the connective tissue at the base, if needed.
6. Place the biopsy into formalin fixative.
7. Apply hemostatic agent (35% aluminum chloride) to biopsy site or suture defect closure (if needed).
8. Apply topical antibiotic and bandage.

FIXATIVE

Formalin vial



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Ulcer Biopsy

INDICATION

Although most lower extremity ulcers may be pressure induced, secondary to venous or arterial complications, or neuropathic in origin, some may not be. Patients presenting with chronic non-healing wounds (1 month or longer duration) or wounds not responding to treatment, should be considered for biopsy (punch or curettage) to determine the presence or absence of an alternate pathology.

DIFFERENTIAL DIAGNOSIS

Basal Cell Carcinoma	Pyoderma Gangrenosum
Dermatitis	Squamous Cell Carcinoma
Melanoma	Vasculitis

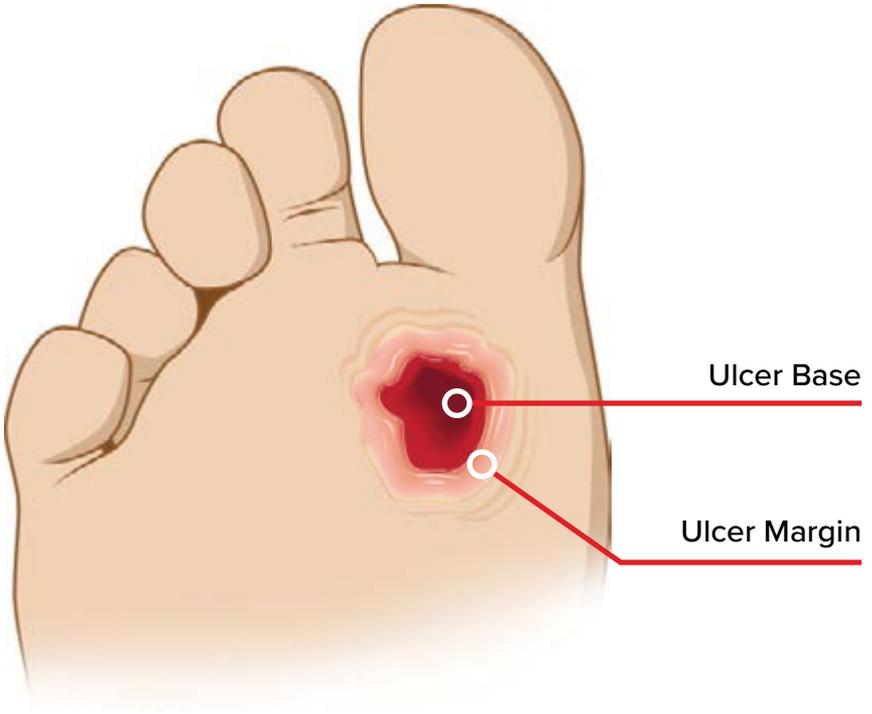
MATERIALS NEEDED

- 2mm or 3mm punch biopsy (see pages 11-12)
- Curette (see pages 23-24)
- eSwab™ for wound culture, if microbiological culture is also needed

PROCEDURE

Ideally, two biopsies should be performed to include both the base of the ulcer (for malignancy) and the wound margin (for inflammatory causes).

- See procedure for punch biopsy (see pages 11-12)
- See procedure for Curette (see pages 23-24)



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Nail Unit Biopsy

INDICATION

The nail unit is composed of the nail plate, bed, matrix, hyponychium, and proximal and lateral nail folds. Biopsies taken for histopathologic examination are intended to rule out pathologies such as inflammatory or neoplastic processes. This biopsy connotes more than a nail sampling for onychomycosis.

Linear melanonychia will require matrical sampling. A nail unit biopsy involving matrical tissue may result in a defect to the nail plate, which may resolve over time.

DIFFERENTIAL DIAGNOSIS

Acral Lentiginous Melanoma	Nail Bed Tumor	Pyogenic Granuloma
Longitudinal Melanonychia	Nail Dystrophy	Squamous Cell Carcinoma
Melanoma	Nail Psoriasis	Subungual Verruca Vulgaris

MATERIALS NEEDED

- 2mm or 3mm punch biopsy (see Punch Biopsy, pages 11-12)

INSTRUMENT

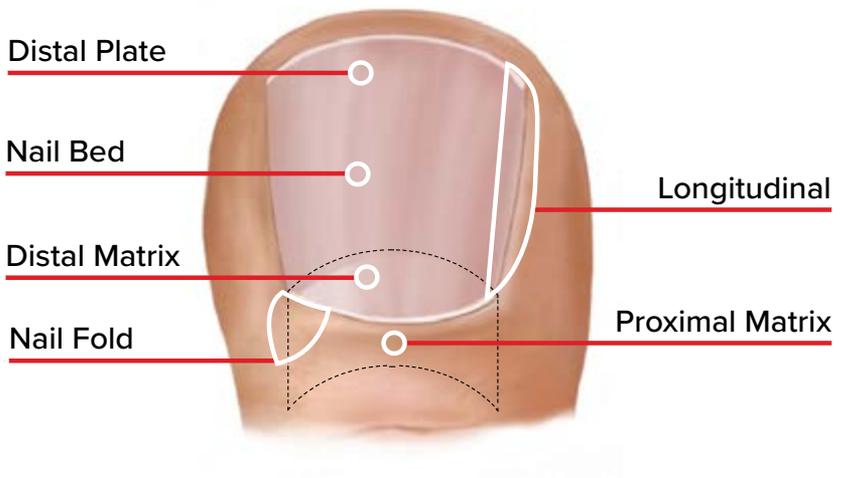


PROCEDURE

Biopsy procedures of the nail unit can vary based on location and differential diagnosis. Longitudinal biopsies are primarily utilized for sampling the lateral or medial nail unit including nail fold.

Whereas, punch biopsies may be utilized for lesions of the matrix or subungual regions (see Punch Biopsy, pages 11-12) .

- Nail bed biopsies typically require nail avulsion prior to procedure
- Suspected melanocytic neoplasms, i.e. linear melanonychia require matrical biopsies (see video using QR code)



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Epidermal Nerve Fiber Density Punch Biopsy

INDICATION

Epidermal Nerve Fiber Density (ENFD) biopsy provides a definitive diagnosis of small fiber peripheral neuropathy and an assessment of its degree of severity (normal, borderline, mild, moderate, severe). Symptoms suggestive of small fiber neuropathy of the foot or leg include: pain, burning, tingling, prickling, paresthesia. This affects the feet first and progresses upward with a “stocking-and-glove” distribution. Symptoms may result from external triggers such as wearing socks or touching bedsheets. Muscle strength, reflexes, nerve conduction velocity test (NCVT), and electromyography (EMG) may be normal and unaffected.

DIFFERENTIAL DIAGNOSIS

- **Metabolic:** diabetes mellitus, metabolic syndrome, hyperlipidemia
- **Amyloidosis:** non-inherited forms, lymphoma, plasma cell dyscrasias
- **Autoimmune:** Sjogren’s syndrome, vasculitis/polyarteritis nodosa
- **Idiopathic:** there is no identifiable cause
- **Infectious:** HIV, hepatitis C, Lyme disease
- **Inherited:** Fabry’s or Tangier’s disease, familial amyloid polyneuropathy
- **Toxic:** chemotherapy, alcoholism, solvent exposure

MATERIALS NEEDED

- ENFD Kit from Bako Diagnostics
 - Kit includes 3mm punch, forceps, scissors, alcohol wipe, gauze, bandage, Zamboni’s fixative, buffer rinse, cryoprotectant, cold pack, shipping supplies
- 3cc to 5cc lidocaine with epinephrine in a 5cc syringe, 25g or 27g needle

INSTRUMENT



PROCEDURE

The most studied ENFD biopsy locations are 10cm distal to the greater trochanter of the femur and 10cm proximal to the lateral malleolus where normative values are well-established.

1. Mark biopsy site and prep with alcohol wipe. Infiltrate lidocaine with epinephrine proximal to, but not directly at, the biopsy site using an inverted “V” pattern. Finish with an iodine wipe.
2. Perform 3mm punch biopsy staying perpendicular to the skin’s surface to a depth of approximately 4mm, through the epidermis and into the dermis.
3. Gently remove the biopsy specimen with atraumatic forceps, elevating from the dermis and using caution not to crush the epidermal surface.
4. Apply hemostatic agent, topical antibiotic, gauze and bandage. In patients with high risk for infection, a single suture may be used.

PROCESSING SPECIMEN

When removing the sample, use atraumatic forceps, being careful to grasp the biopsy deep to the surface epithelium. Be GENTLE and avoid crushing the surface epithelium.

1. Gently place biopsy specimen in Zamboni’s fixative (vial #1) and immediately refrigerate for 8 hours minimum. Zamboni is a weak acid; sample may NOT remain for more than 24 hours. Do NOT allow the specimen to freeze.
2. After at least 8 hours in refrigerator, but not more than 24 hours, pour out Zamboni’s into blue tray while keeping specimen in vial.
3. Refill with buffer rinse (vial #2), pour out, repeat buffer rinse, pour out.
4. Refill with cryoprotectant (vial #3), screw on blue cap tightly.
5. Shipping option via FedEx® or UPS® (use cool-pack and Styrofoam® cooler).



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Shave Biopsy

INDICATION

Shave biopsies are effective for sampling superficially located lesions such as pigmented lesions and other neoplastic processes. Papular and macular lesions are ideally sampled via shave technique which provides the broadest lesional surface area.

DIFFERENTIAL DIAGNOSIS

Melanoma	Squamous Cell Carcinoma	Benign Nevi
Basal Cell Carcinoma	Kaposi's Sarcoma	Verucca

MATERIALS NEEDED

- #10 scalpel or #15 scalpel
- Forceps
- 70% isopropyl alcohol wipe
- 1cc to 3cc lidocaine with epinephrine in a 1cc to 3cc syringe, 27g or 30g needle
- Hemostatic agent (35% aluminum chloride or Monsel's solution)
- Topical antibiotic
- Gauze pad
- Bandage

INSTRUMENT



PROCEDURE

The chief advantage of a shave biopsy is the ability to better sample the dermal-epidermal junction. Where a punch biopsy typically samples 2-3mm length of epidermis, a shave biopsy most commonly samples 5mm to 2cm of junctional tissue.

1. Prepare biopsy site with 70% isopropyl alcohol wipe for 10 seconds.
2. Administer local anesthesia fanned out into the dermis beneath the lesion and biopsy site.
3. Enter skin at 10-15° angle and create a smooth divot, continuously use a sawing motion, moving blade underneath lesion.
 - For papules (elevated) - directly undermine the lesion for removal.
 - For macules (flat) - score preceding edge and utilize forceps to elevate sample throughout the procedure if visualization is needed.
4. Place the biopsy into formalin fixative.
5. Apply hemostatic agent (35% aluminum chloride) to biopsy site.
6. Apply topical antibiotic and bandage.

FIXATIVE

Formalin vial



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Saucerization Biopsy

INDICATION

Saucerization is a form of shave biopsy which may extend to a deeper dermal depth than a routine scalpel shave biopsy. This procedure is ideally used for larger macules and infiltrative lesions.

DIFFERENTIAL DIAGNOSIS

Basal Cell Carcinoma	Dermal Sarcomas	Melanoma
Benign Nevi	Dermatofibroma	Squamous
Cell Carcinoma	Kaposi's Sarcoma	Verucca

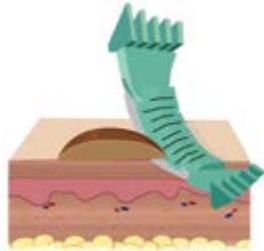
MATERIALS NEEDED

- Miltex® BiopBlade™ (bendable blade)
- Forceps
- 70% isopropyl alcohol wipe
- 1cc to 3cc lidocaine with epinephrine in a 1cc to 3cc syringe,
- 27g or 30g needle Hemostatic agent (35% aluminum chloride or Monsel's solution)
- Topical antibiotic
- Gauze pad
- Bandage

INSTRUMENT



PROCEDURE



This technique is a more aggressive method of sampling deeper dermal regions, therefore care must be taken as to not extend beyond the dermal layers.

1. Prepare biopsy site with 70% isopropyl alcohol wipe for 10 seconds.
2. Administer local anesthesia fanned out into the dermis beneath the lesion and biopsy site raising a wheel with lidocaine and epinephrine.
3. Bow the BiopBlade utilizing thumb and middle finger on the sides of the instrument, while using the index finger centrally for stabilization.
 - Bowing with light pressure = shallow biopsy
 - Bowing with heavier pressure = deeper biopsy
4. Enter the skin at 10-15 degree angle. Using a rocking motion, cut through the dermis undermining the lesion, leaving a smooth divot.
Warning: too much bowing or pressure on the BiopBlade may result in gouging
5. Place the biopsy into formalin fixative.
6. Apply hemostatic agent (35% aluminum chloride) to biopsy site.
7. Apply topical antibiotic and bandage.

FIXATIVE

Formalin vial



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Curettage

INDICATION

Curettage can be used for sampling superficial lesions which can be “scraped off” the skin surface, such as small, scaly lesions, interdigital tissue, ulcer bases.

DIFFERENTIAL DIAGNOSIS

Superficial Skin Infections	Ulcers
Skin Tumors	Verruca
Squamous Cell Carcinoma	Web Space Maceration

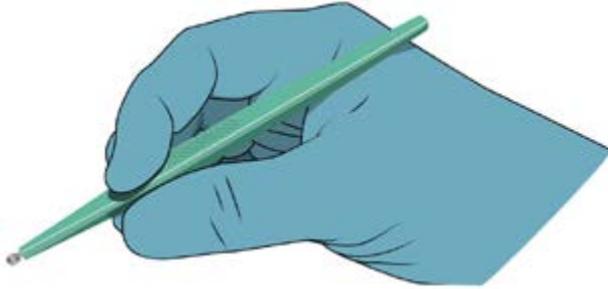
MATERIALS NEEDED

- Dermal curette (2mm, 3mm, 4mm)
- Optional Items
 - › 70% isopropyl alcohol wipe
 - › 1cc to 3cc lidocaine with epinephrine in a 1cc to 3cc syringe, 27g or 30g needle
 - › Hemostatic agent (35% aluminum chloride or Monsel’s solution)
 - › Topical antibiotic
 - › Gauze pad
 - › Bandage

INSTRUMENT



PROCEDURE



1. Anesthesia may or may not be necessary
2. Prepare biopsy site with 70% isopropyl alcohol wipe for 10 seconds
3. Hold instrument like a pencil with index finger on flat textured area
4. Gently scrape curette across biopsy site, collecting tissue within loop
Warning: too much pressure on curette may result in gouging
5. Several passes may be required for adequate tissue acquisition.
For web space, curettage is used to remove a portion of macerated tissue.
6. Deposit specimen into formalin fixative
7. Hemostatic agent, antibiotic, bandage may or may not be required

FIXATIVE

Formalin vial



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Web Space Tissue Collection and Testing

INDICATION

Web space tissue collection can involve a variety of sampling methods depending on the pathology. Surface keratin may be collected via a simple skin scraping and submitted for a PCR (DNA-based) test to rule out the possibility of a superficial web space infection, including fungal and bacterial etiologies. A punch biopsy, shave biopsy, or curettage may be performed for suspected conditions such as dermatitis, benign or malignant neoplasms, and non-healing wounds.

DIFFERENTIAL DIAGNOSIS

Bacterial infection/MRSA	Erythrasma
Benign/malignant Neoplasm	Fungal Infection
<i>Candida intertrigo</i>	Psoriasis
Cellulitis	Tinea Pedis
Eczema	Xerosis

LABORATORY TESTING

The BakoDx Web Space Infection PCR test is submitted with a simple skin scraping. The assay targets:

- *Candida* species
- *Corynebacterium minutissimum*
- Dermatophytes
- Gram-negative bacteria
- *Staphylococcus aureus*, and if positive, reflex to a genetic marker associated with methicillin resistance (*mecA*).

For routine histopathologic examination:

- Punch biopsy (see pages 11-12)
- Shave biopsy (see pages 19-20)
- Curettage (see pages 23-24)

MATERIALS NEEDED FOR PCR TEST

- #10 / #15 scalpel or dermal curette



- Dermapak®

The BakoDx Dermapak® is a collection pack envelope designed specifically for secure transportation of skin scrapings and its absorbent action reduces risk of bacterial overgrowth. It features built-in tape to form a seal and keep the skin specimen contained.



BIOPSY MATERIALS NEEDED FOR HISTOPATHOLOGY

- 2mm or 3mm punch biopsy (see pages 11-12)
- #10 or #15 scalpel (see pages 19-20)
- Dermal curette (see pages 23-24)

Web Space Tissue Collection and Testing (continued)

SKIN SCRAPING PROCEDURE

The skin scraping collection method for web space specimens (for Bako's Web Space Infection PCR) test can be performed using a scalpel or dermal curette. Skin preparation with alcohol, betadine, or other antiseptic is generally not required. Local anesthesia may, or may not, be needed if sampling the superficial epidermis.

1. Gently scrape macerated or exfoliated interdigital skin with a sterile scalpel or curette.
2. Place the collected debris directly in the center of the Dermapak® collection transport system. Clinically visible specimen is recommended.
3. If necessary, the surgical instrument can be wiped on the inside surface of the collection pack to ensure optimal specimen acquisition
4. Post-procedure wound dressings may, or may not, be required.
5. Close the Dermapak® by folding down flap A, followed by flap B, and then flap C.
6. Remove the adhesive backing strip, fold flap D down, and press down firmly.
7. Complete the patient identifying information on the rear panel and place the Dermapak® in a resealable plastic bag.

BIOPSY PROCEDURES

- Punch (see pages 11-12)
- Shave (see pages 19-20)
- Curettage (see pages 23-24)

FIXATIVE

- No fixative is required for PCR (DNA) analysis; submit dry or moist specimen in foldable Dermapak®
- Samples for routine histology and dermatopathologic review such as punch, shave, or curette biopsy must be submitted in formalin



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Incisional/Excisional Biopsy

INDICATION

An incisional biopsy removes a small piece of tissue from a larger lesion. An excisional biopsy is the removal of the entire lesion including full thickness skin to include a portion of the subcutis. An elliptical excision using a length to width ratio of 3:1 will allow for minimal skin tension at the surgical site.

Depending on the size of the incisional biopsy, the same 3:1 ratio may be of benefit.

DIFFERENTIAL DIAGNOSIS

Actinic Keratosis	Neoplasm	Squamous Cell Carcinoma
Basal Cell Carcinoma	Neoplastic Ulcers	Verruca
Kaposi's Sarcoma	Pigmented Lesions	
Melanoma	Skin Tumors	

MATERIALS NEEDED

- #10 or #15 scalpel
- Forceps
- 70% isopropyl alcohol wipe
- 1cc to 3cc lidocaine with epinephrine in a 3cc syringe, 27g or 30g needle
- Hemostatic agent (35% aluminum chloride or Monsel's solution) or Suture
- Topical antibiotic
- Gauze pad
- Bandage

INSTRUMENT



PROCEDURE

1. Prepare biopsy site with 70% isopropyl alcohol wipe for 10 seconds.
2. Administer local anesthesia fanned out into the dermis beneath the lesion and biopsy site with 1cc to 3cc lidocaine with epinephrine in a 3cc syringe, 27g or 30g needle.
3. Using a scalpel, cut down through the lesion into the dermis with a 3:1 ratio to include a section of the most representative area of the lesion.
Depending on the size of the incisional biopsy, the same 3:1 ratio may be of benefit.
4. Place the biopsy into formalin fixative.
5. Apply hemostatic agent (35% aluminum chloride) or wound closure, if necessary, to biopsy site.
6. Apply topical antibiotic and bandage.

FIXATIVE

Formalin vial



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Fine Needle Aspiration

INDICATION

Fine needle aspiration biopsy is intended for the sampling of subcutaneous mass lesions beyond the reach of routine skin biopsy techniques.

Fine Needle Aspiration vs Punch Biopsy

- If skin moves over soft tissue mass freely, then fine needle aspiration is likely indicated
- If skin is tethered to the underlying mass and they move in concert, then punch biopsy may be used

Do not surgically remove a soft tissue mass without establishing a definitive diagnosis as to its etiology, otherwise the potential for limb sparing surgery may be diminished.

DIFFERENTIAL DIAGNOSIS

- Benign Soft Tissue Masses (giant cell tumor, lipoma, plantar fibroma, etc.)
- Cysts (epidermal inclusion, ganglion, etc.)
- Kaposi's Sarcoma
- Malignant Soft Tissue Masses (sarcoma, lymphoma, etc.)

MATERIALS NEEDED

- 70% isopropyl alcohol wipe
- 3cc lidocaine in a 3cc syringe, 27g
- 10cc syringe
- 18g to 21g needle (21g preferred)
- Bandage

IMPORTANT SPECIMEN INFORMATION

A syringe body containing the sample with the needle REMOVED is acceptable, but formalin or cytology fixative is preferred.

CLIA regulations do not allow needles to be submitted to the laboratory. Be sure to remove the needle if submitting in syringe or flush specimen into appropriate fixative.

PROCEDURE



The purpose of needle aspiration biopsy is to harvest fluid, cells, and small pieces of tissue from the soft tissue lesion or mass.

1. Prepare biopsy site – alcohol wipe, raise local anesthetic wheal.
2. 10cc syringe with 21-gauge needle - inserted into soft tissue mass.
3. Draw syringe plunger back to create a vacuum.
4. Maintain vacuum and redirect needle into 4 quadrants of mass.
5. Let tension off the plunger and withdraw needle.
6. If fluid is obtained, flush fluid into fixative container.
7. If no fluid is obtained, draw fixative into syringe, then flush fixative back into fixative container.
8. Do NOT discard the needle prior to flushing and submitting to laboratory.
9. Dry tap can be an indication for open punch biopsy.

FIXATIVE

Formalin vial, reagent alcohol (ETOH) vials or empty vial (for larger fluid samples)

Reagent alcohol (ETOH) vials MUST be used when gout is suspected.



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Bone Biopsy

INDICATION

A bone biopsy removes a small representative sample of bone from a suspicious area for histopathologic evaluation to determine if cancer, infection, or other pathologies are present. Care should be taken to avoid contamination if an infectious etiology is suspected and culture is desired.

DIFFERENTIAL DIAGNOSIS

- Abnormal x-ray findings
- Benign Bone Tumors (Osteochondroma, Giant Cell Tumor, Osteoid Osteoma, Osteoblastoma, Enchondroma, etc.)
- Malignant Bone Tumors (Chondrosarcoma, Osteosarcoma, Chordoma, Ewing Tumor, Fibrosarcoma, etc.)
- Osteitis
- Osteomyelitis

MATERIALS NEEDED

- Bone trephine 2mm to 5mm
- Bone rongeur
- Bone curette
- Surgical saw

PROCEDURE

There are many different techniques available to obtain a bone biopsy and they all depend on the specific patient pathology present and the personal choice of the physician.

The two most common bone biopsy procedures include the following techniques:

Trephine technique: this can be performed percutaneous or open. If an ulcer or wound is present, avoid those areas and insert trephine through clean skin. Insert a 2mm to 5mm bone trephine to the bone and obtain a core sample.

Open surgical technique: this is a standard open surgical procedure performed down to bone. Utilizing a surgical bone instrument such as a rongeur, curette, surgical saw, or trephine, obtain at least 3mm of sample bone for histopathologic analysis.

Additional methods of obtaining a bone biopsy include: joint arthroplasty with bone biopsy, partial foot amputation with associated bone biopsy, bone marrow biopsy, and wound excision with corticotomy.

If infection is suspected, repeat the same procedure and send the second bone sample for microbiological culture.

FIXATIVE

- Empty sterile vial or ESwab vial, if microbiological culture is also needed
- Formalin vial, if microbiological culture is not needed



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