How to Submit a Nail Specimen

Erica Reinig, MD[a], Phoebe Rich, MD[b], Curtis T. Thompson, MD[a,b,c,*]

KEYWORDS

• Biopsy • Microscopy • Histology • Tissue • Sectioning • Orientation • Cassette • Formalin

KEY POINTS

- Proper specimen orientation is crucial for accurate grossing as well as proper tissue embedding and sectioning, significantly improving pathologic diagnostic ability.
- Close communication with the laboratory, including a thorough clinical history and differential and instructions to the laboratory (ie, initial level hematoxylin and eosin stain [H&E] sections), is important.
- Submitting nail biopsy specimens with a specific protocol that includes placing the tissue on a drawing of a nail allows for preservation of orientation and prevents loss of tissue.
- A simple protocol on nail plate specimens greatly improves adherence of the plate to the glass slide for H&E and periodic acid–Schiff (PAS) sections.

INTRODUCTION

Laboratory technicians and pathologists often fear receiving a nail unit specimen because there are significant challenges in both getting the nail plate to adhere to a glass slide and because the soft tissue specimens of the nail unit matrix and bed are often small and fragmented. Interpretation by the pathologist is challenging, not only because of the often difficult nature of the specimen but also because orientation at the microscopic level is tricky, especially when examining a diseased nail unit.

When routine skin specimens are obtained in a clinic, the specimens are usually placed free floating in a container with an appropriate amount of formalin (10% formaldehyde) before the specimen is sent to the laboratory. With nail unit specimens, however, placing these specimens free in formaldehyde results in loss of orientation and frequent loss of critical tissue needed to make a diagnosis. Maintenance of tissue integrity and orientation streamlines specimen processing—from grossing to embedding to sectioning—and significantly improves pathologic diagnostic ability. Thus, in the clinic, nail unit specimens require additional work to preserve tissue integrity and orientation.

It is helpful to be able to send nail unit specimens to a laboratory with expertise in nail specimen processing. However, the clinic is often required to send to a variety of laboratories. By preparing a nail unit specimen in the clinic in a way that preserves orientation and prevents loss of tissue, the specimen may thus be processed and interpreted in a variety of laboratories with better success. Thus, the onus is on the clinician and the clinic to submit nail specimens in a manner as discussed later.

Clear communication with the laboratory is important in nail unit specimen submission. Of primary importance is instruction to the laboratory to pay close attention to small fragments of tissue.

Disclosures: None.

[a] Department of Pathology, Oregon Health Sciences University, Portland, OR, USA; [b] Department of Dermatology, Oregon Health Sciences University, Portland, OR, USA; [c] Department of Biomedical Engineering, Oregon Health Sciences University, Portland, OR, USA
* Corresponding author. PO Box 230577, Portland, OR 97281.
E-mail address: cthompson@cta-lab.com

Dermatol Clin 33 (2015) 303–307
http://dx.doi.org/10.1016/j.det.2014.12.012
0733-8635/15/$ – see front matter Published by Elsevier Inc.

Instructions to perform initial level sections and unstained sections on positively charged slides are important because the small nail matrix/bed specimens may not survive refacing the paraffin block for additional sections. Also important is a clear clinical history and differential, especially because the histologic features of some nail tumors such as onychopapilloma are not distinct. A pathologist not given a clear differential will diagnose an onychopapilloma as a verruca, as both have hyperkeratosis and hypergranulosis. Similarly, a diagnosis of a mold infection requires direction by the clinician to the laboratory to consider a mold; otherwise, the microbiology laboratory will consider the mold a contaminant and not characterize the mold.

For submission in a way that preserves specimen orientation, a couple of methods have previously been proposed. George and colleagues[1] describe a technique of marking the epithelial surface with colored ink, dipping the specimen in glacial acetic acid to fix the ink, and then placing it in formalin for transport. Although this technique may certainly improve orientation by maintaining the ability to identify the epithelial surface, placing the specimen floating free in formalin will lead to a loss of proximal-distal and medial-lateral orientation, and small fragments of potentially diagnostic tissue may be lost. Richert and colleagues[2] describe a different technique for submission in which the specimen is placed on cardboard with a nail diagram and covered with a sheet of filter paper. The cardboard and filter paper are then stapled together so that the specimen remains flat and oriented, preventing tissue loss.

A SIMPLE TECHNIQUE FOR SPECIMEN SUBMISSION

The authors have developed a technique that incorporates aspects of both of these methods, but which may be more practical, particularly in clinics where nail unit sampling is not routine. Borrowing from ophthalmologic tissue processing, the specimen is oriented by placing it on a cartoon printout of a nail (**Fig. 1**) in the same location as its in vivo location. Multiple tissue fragments may be placed on a single nail cartoon in the area from which they came. Any type of paper may be used for the cartoon, and the cartoon may be drawn by hand with a pen or pencil or printed and cut out (a cartoon printout may currently be found at www.cta-lab.com/nail_resources.html). The cartoon should be small enough to fit flat within a tissue cassette. Wetting the paper slightly with formalin before placing the tissue on it prevents histologic drying artifacts in the tissue.

After placing the specimen on the nail cartoon, the orientation may be further improved by carefully inking one or more edges of the specimen and the corresponding cartoon paper (see **Fig. 1**). Very precise inking is best done using the wooden end of a cotton swab rather than the cotton end (see **Fig. 1**C). Because many lesions are pigmented, avoiding black ink is important to prevent confusion of ink with melanin. Thus, green or blue ink is best.

The specimen on the cartoon printout is then placed in a tissue cassette. Tissue cassettes and the sponges that go inside them to secure tissue must be purchased in bulk, so requesting a handful of cassettes and sponges from your local histopathology laboratory is best. After placing 1 or 2 tissue sponges over the cartoon holding the tissue, the cassette is closed securely. The cassette can then be placed in an appropriately sized container with formalin for fixation and transportation to the laboratory (**Fig. 2**).

On receipt in the laboratory, the cassette holding the nail unit specimen may be sent directly through overnight processing without opening the cassette. The tissue may also undergo gross sectioning before overnight processing. Small fragments may move around a little on the cartoon during the overnight processing, but the overnight processing adds significant strength to the soft tissue and often leads to the ability to perform more precise gross sectioning. The histotechnologist should be encouraged to consult the pathologist for advice on orientation, inking, and gross sectioning. For instance, features of a presumed onychopapilloma are best seen with longitudinal proximal-to-distal sections, whereas features of an onychomatricoma are best seen with transverse sections. In addition, a fragmented specimen may be separated by the histotechnologist into multiple separate cassettes or blocks. Finally, the laboratory should be instructed to cut 5 to 10 unstained sections on positively charged slides with the initial hematoxylin and eosin stain (H&E) sections for use with additional H&E or special stains; this prevents loss of potentially critical tissue needed to make an accurate diagnosis.

NAIL PLATE SUBMISSION

Nail plate associated with a matrix/bed specimen should be submitted separately from the soft tissue if possible. This separate submission is because the histotechnologist is often most concerned about sectioning the very hard nail plate, and the diagnostic tissue is usually the matrix/bed specimen. Failure to separate the soft tissue from the plate may result in loss of diagnostic