Grossing and Preparation of Nail Unit Specimens: From Nail Clippings to Amputations and Everything in Between

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Only disclosure

CTA Pathology

Objectives

- Challenges in nail
- Specimen submission
- H&E and special stain
- Processing specific specimens
- Clippings for fungus

Why is the nail unit so daunting to dermatologist and dermatopathologists?

https://www.katiemoorevisualartist.com/the-louisiana-museum-of-modern-art-copenhagen/

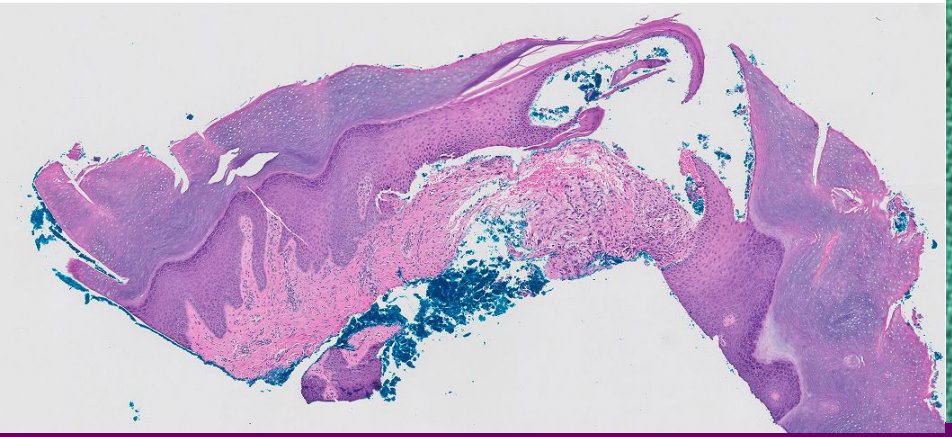
Why are nails so difficult?

- Poor sample
- Insufficient biopsies

https://collection.waikatomuseum.org.nz/objects/4306/bomb-fragments

Why are nails so difficult?

- Never see normal



https://cdn.ymaws.com/www.aocd.org/resource/resmgr/ddb_high/lichen_planus_3_high.jpg

Why are nails so difficult?

- Lack of orientation

Why are nails so difficult?

- Rare sample—little experience

Why are nails so difficult?

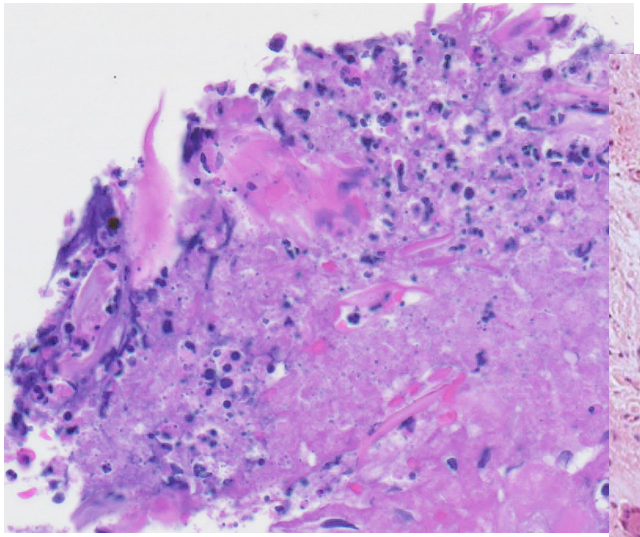
- Rare sample—little experience

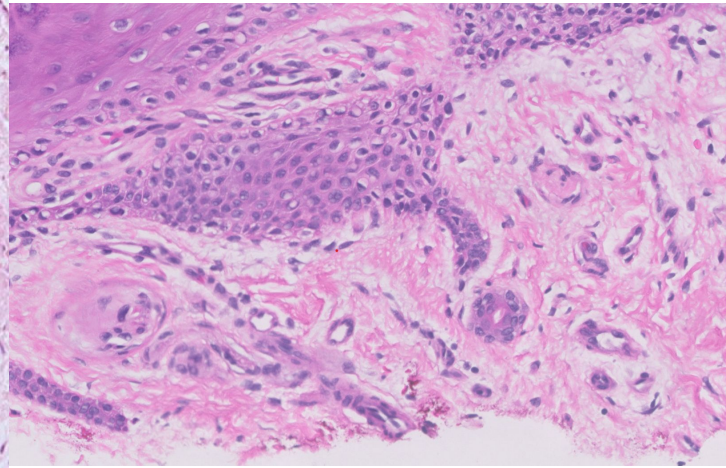
ʻiʻiwi

Main pollinator of lobelia

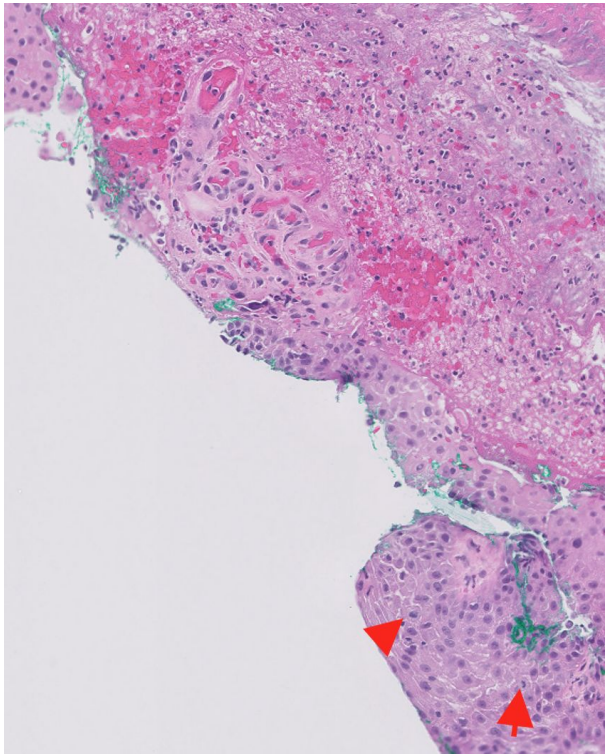
https://blog.nwf.org/2018/05/will-this-rare-hawaiian-forest-bird-go-extinct/

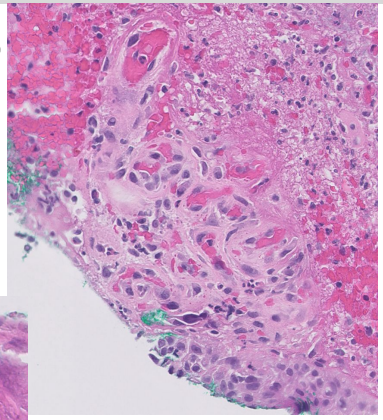
Why are nail so difficult?

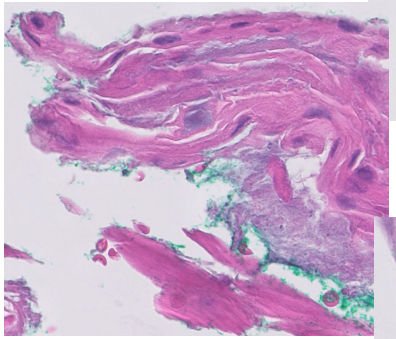


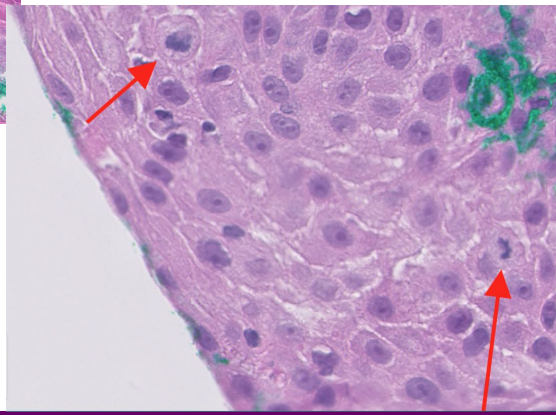


Reactive or Malignant?

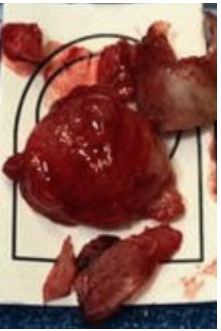


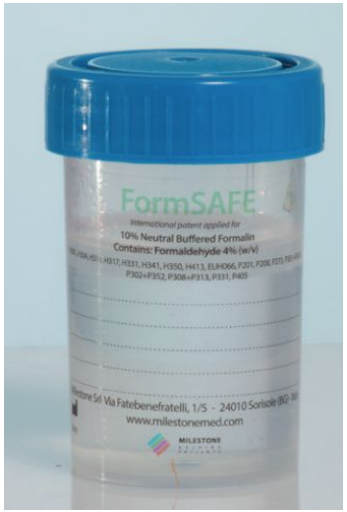


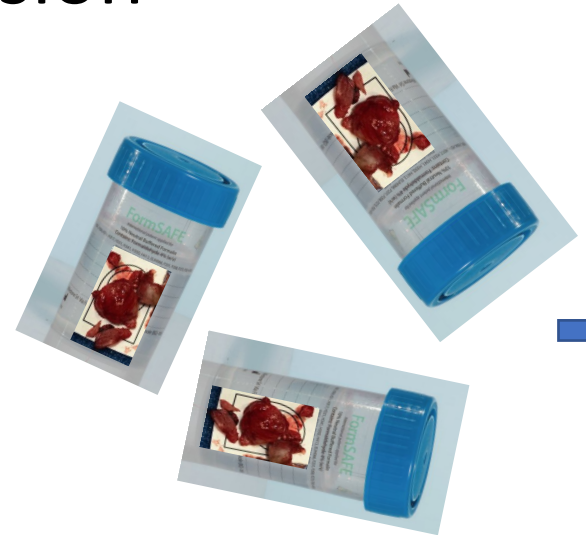




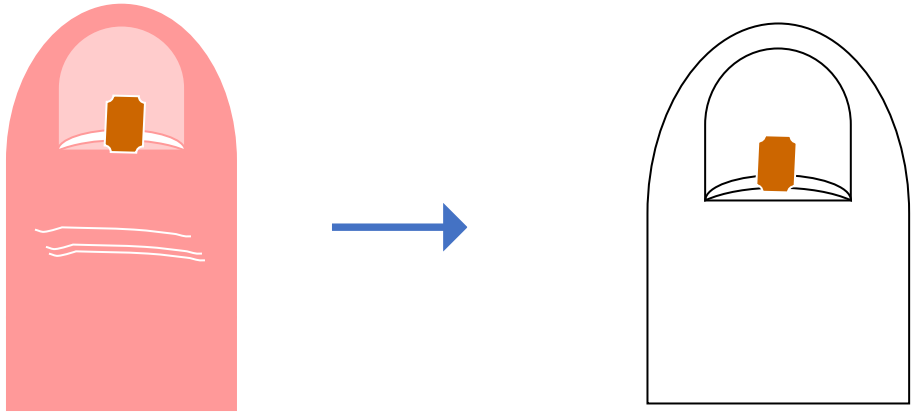
Specimen submission







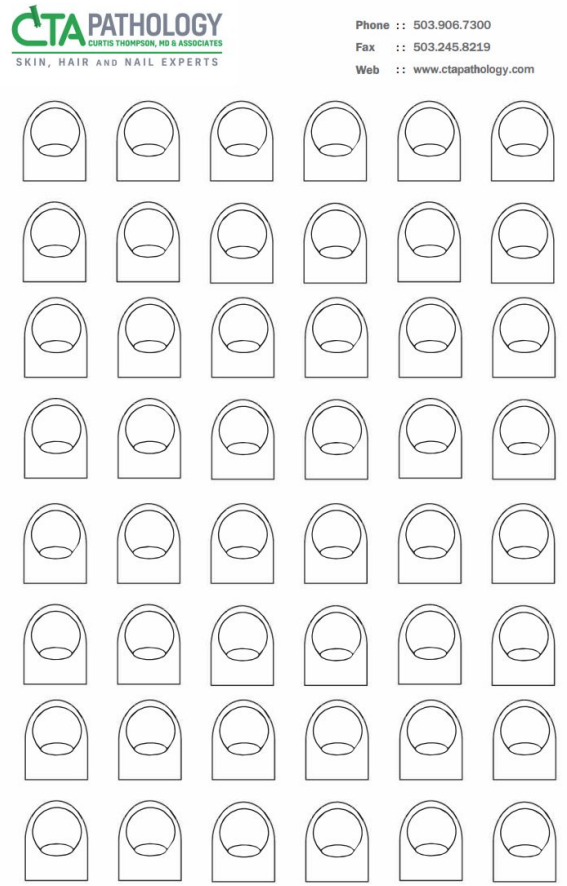
Order ensues . . .




Courtesy of Dr. Phoebe Rich

Print template at www.ctapathology.com

Template



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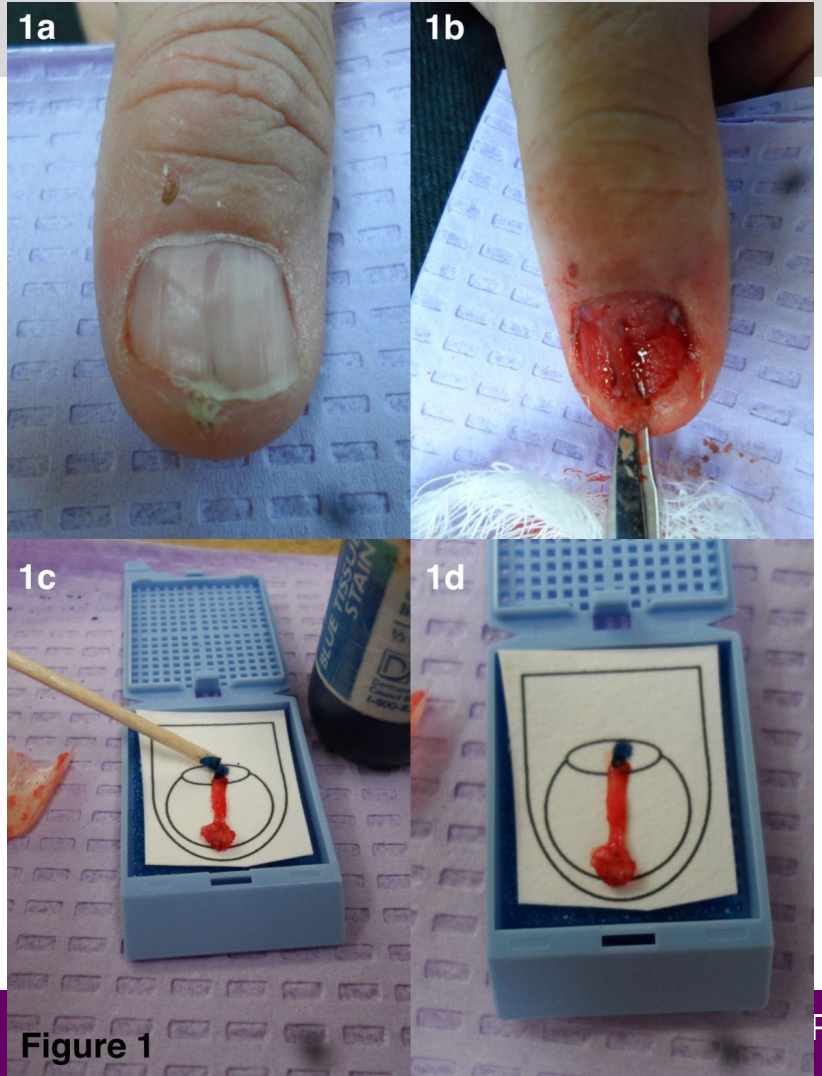
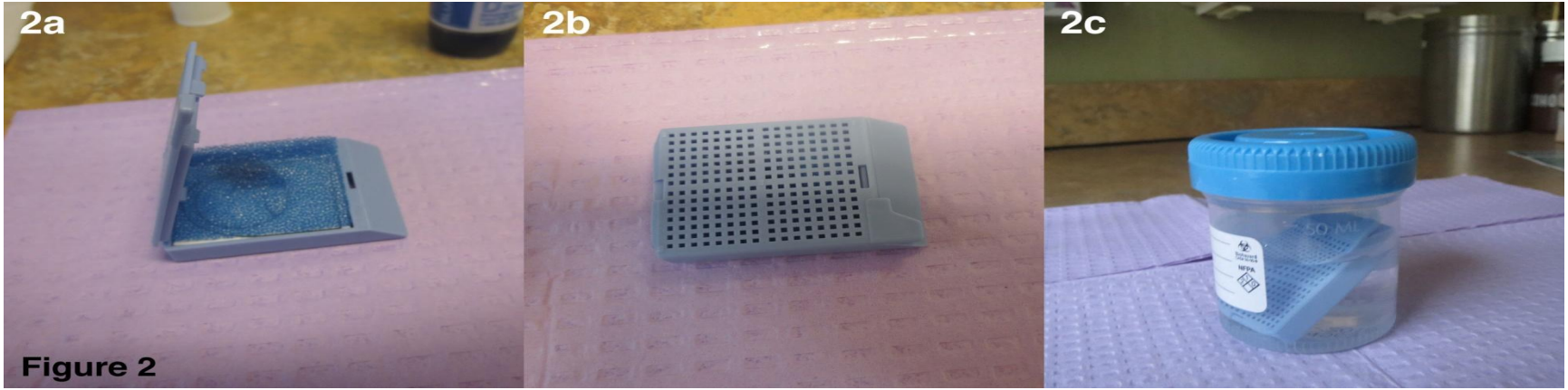


Figure 1

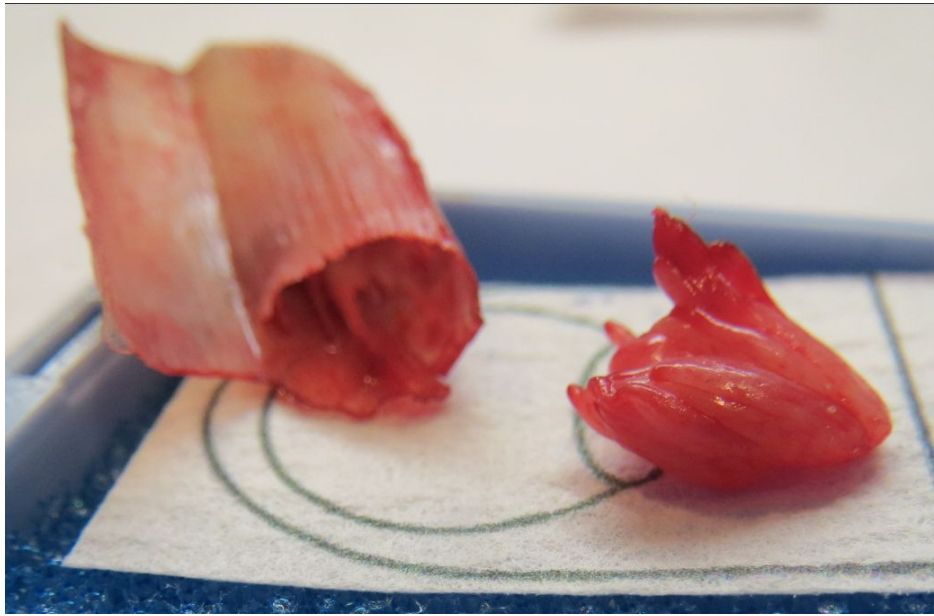

2a
2b
2c
Figure 2

Initial sections

- Number tissue blocks
- Unstained slides and levels
- Special stains
- Importance of nail

Brian Schapiro, MD

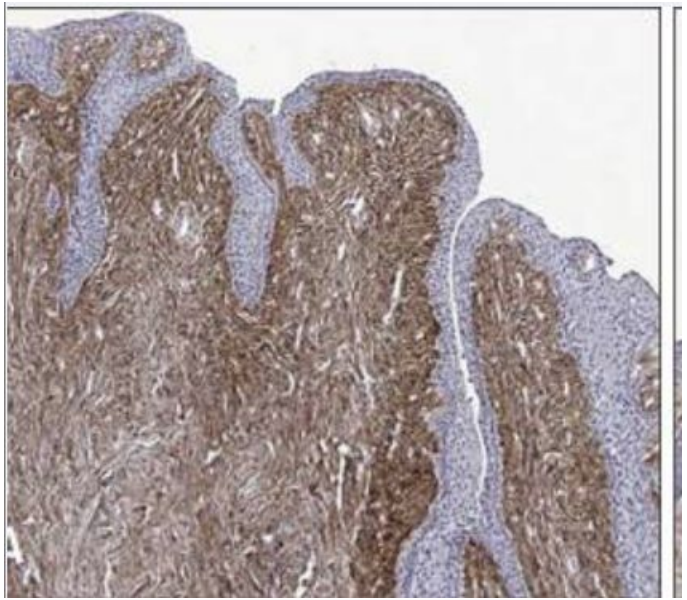
Clinical and gross examination



Thanks to Phoebe Rich, MD

Onychomatricoma

Onychodermis
CD13



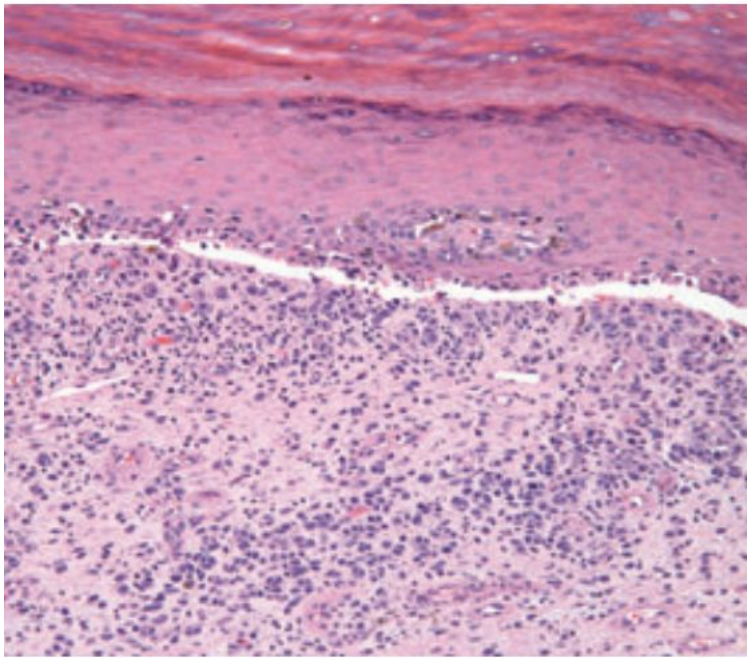
Onychomatricoma

Park CS et al. Ann Dermatol 30:27-8, 2018.

Nail plate submission?

Courtesy of Dr. Phoebe Rich

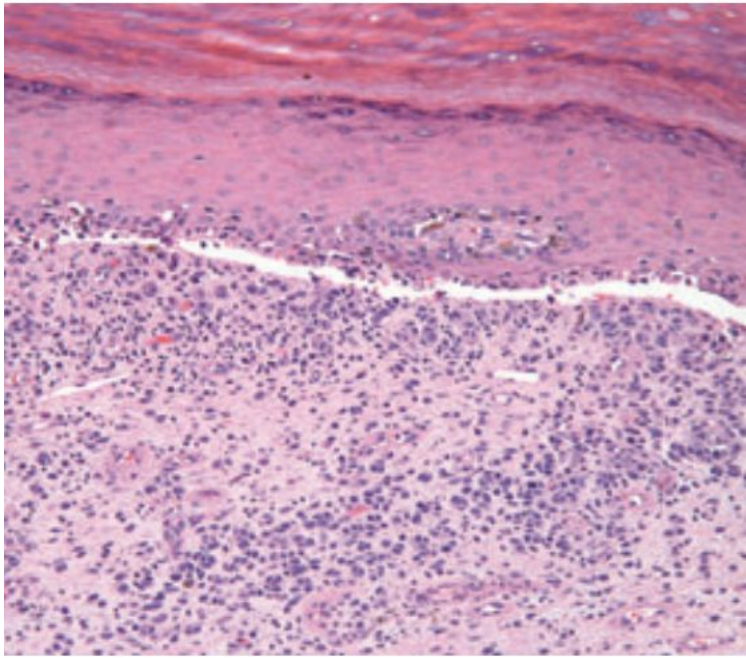
Nail plate submission?



Ruben BS and McCalmont TH. J Cutan Pathol 37:1028-9, 2010

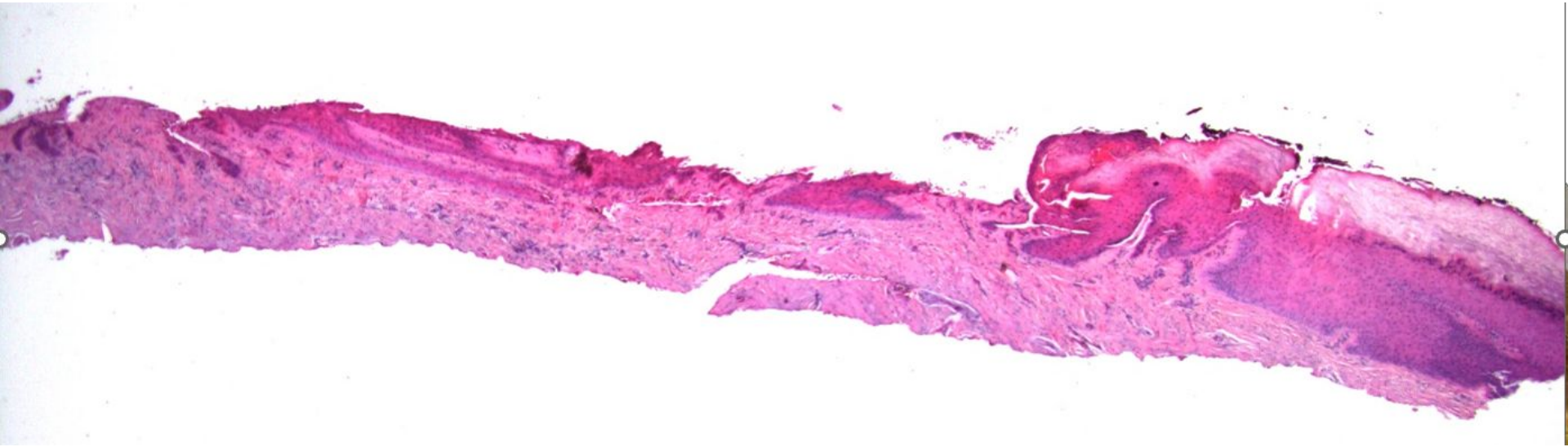
Nail plate submission?

Melanoma in-situ

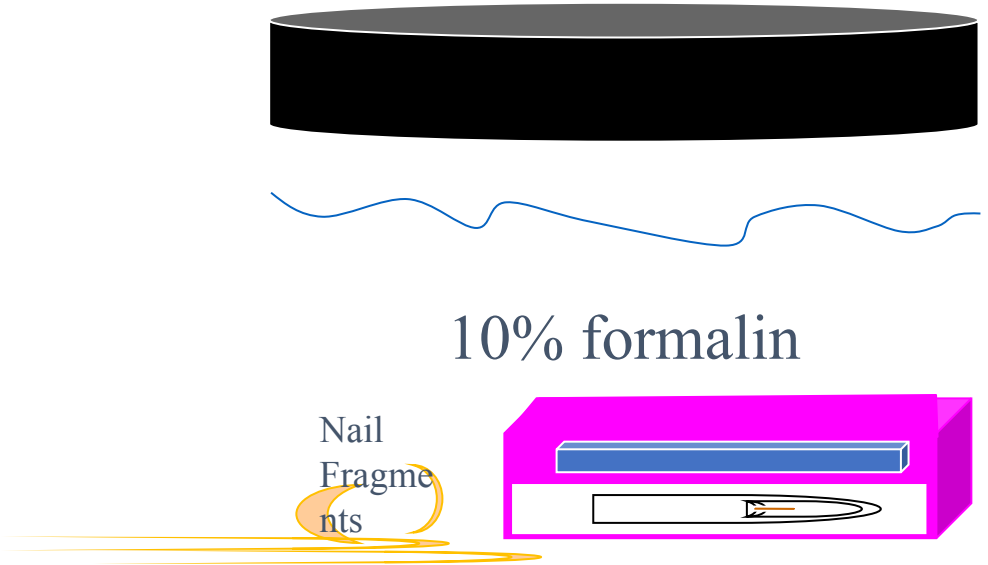


Ruben BS and McCalmont TH. J Cutan Pathol 37:1028-9, 2010

Nail plate submission?



One specimen charge

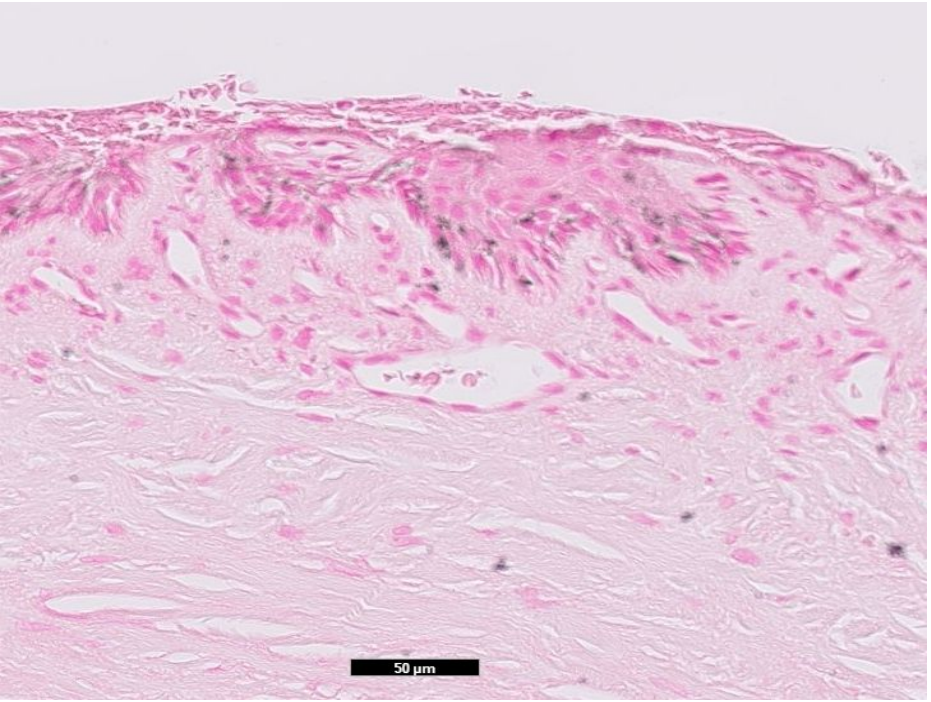


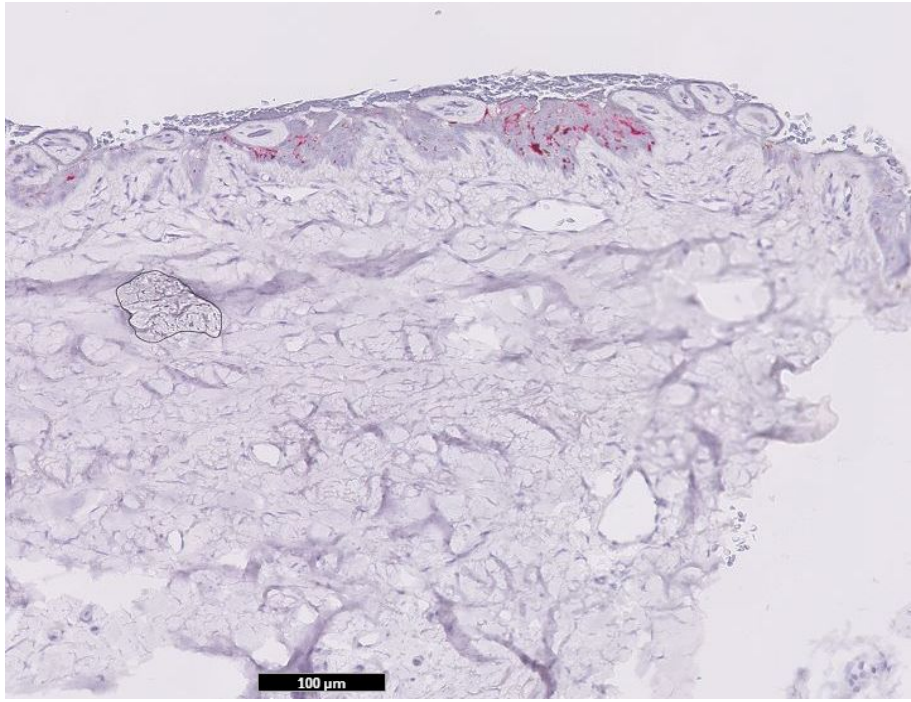
Embedding

Preordering for Longitudinal melanonychia

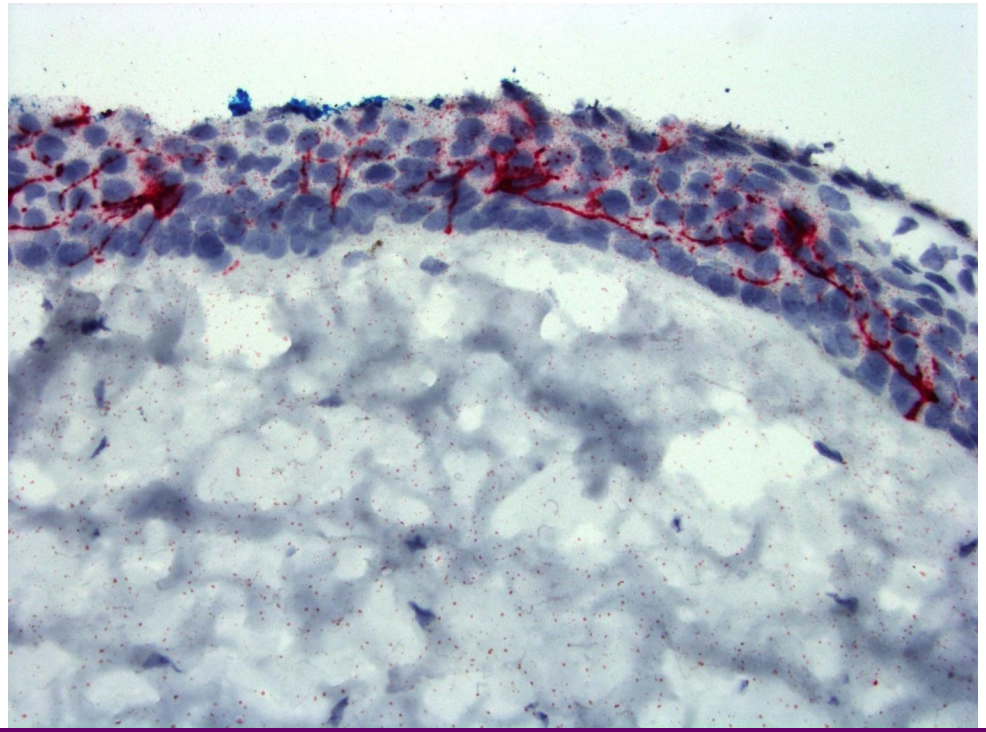
- H&E levels X3
- MelanA
- Fontana-Masson
- PAS fungus
- Unstained slides

Finding the Pigment
Fontana-Masson and MelanA

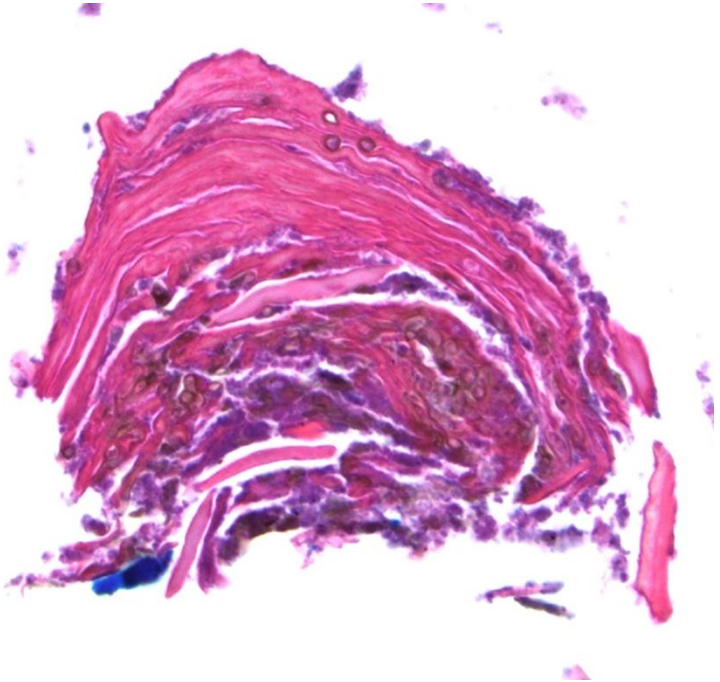




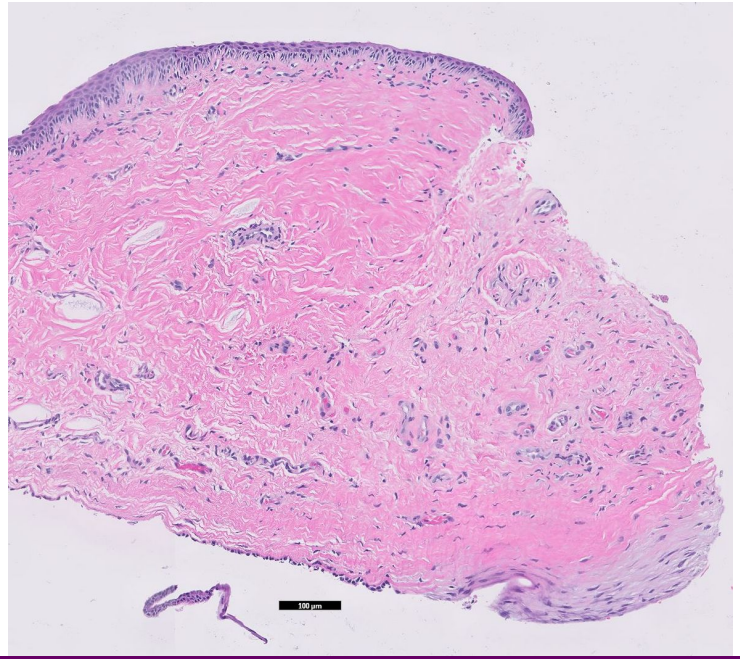
MelanA

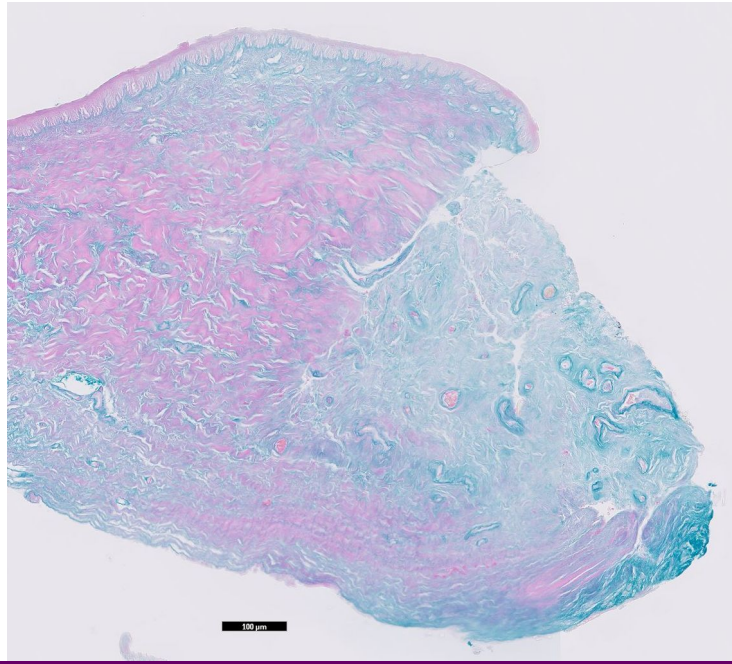


Pigmented fungus



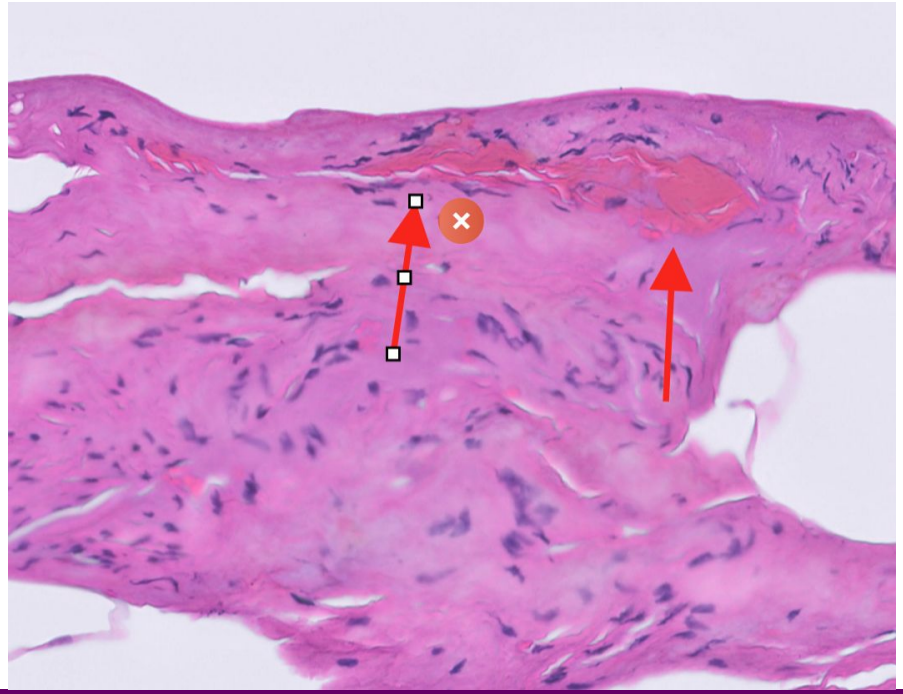
Mucin stain for myxoid cyst



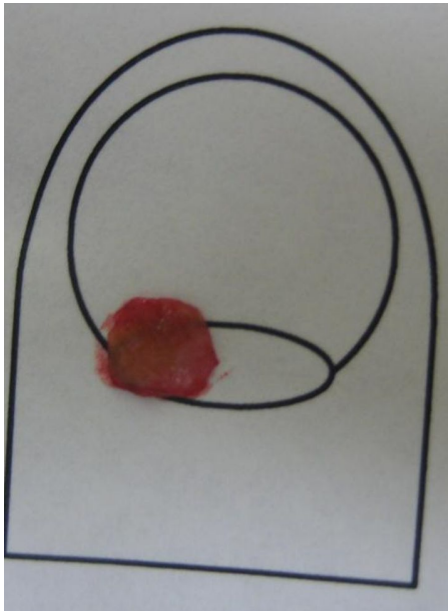


Dilute Fontana-Masson

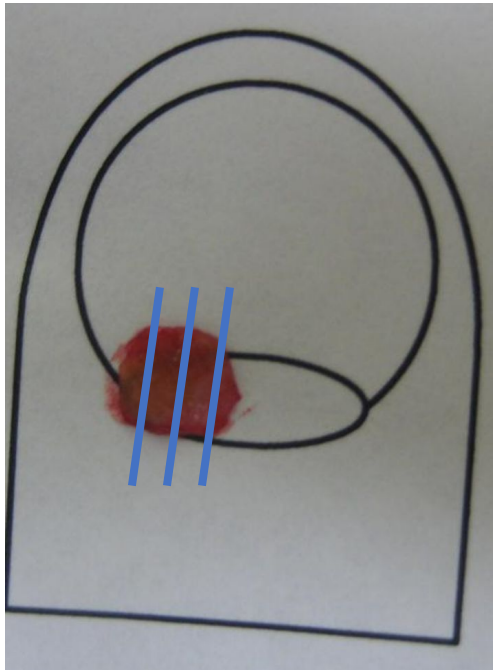
Perl’s iron does not work



Shave biopsy

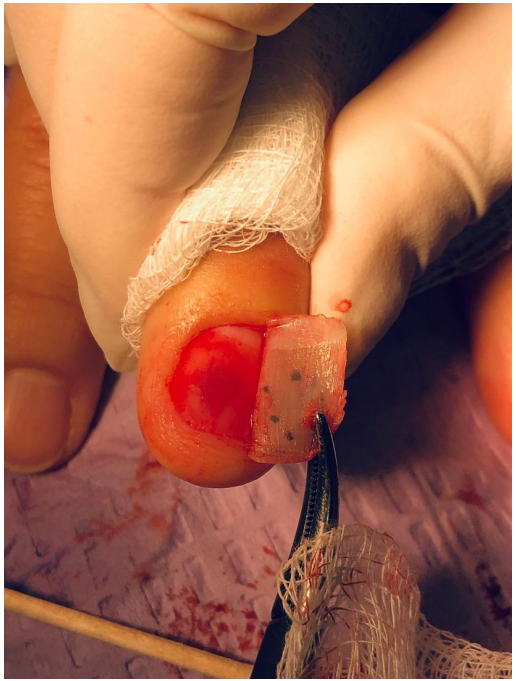


Shave biopsy



- Section after overnight fixation
- Preorders
  - Levels and unstained
  - MelanA, Fontana-Masson
  - PAS

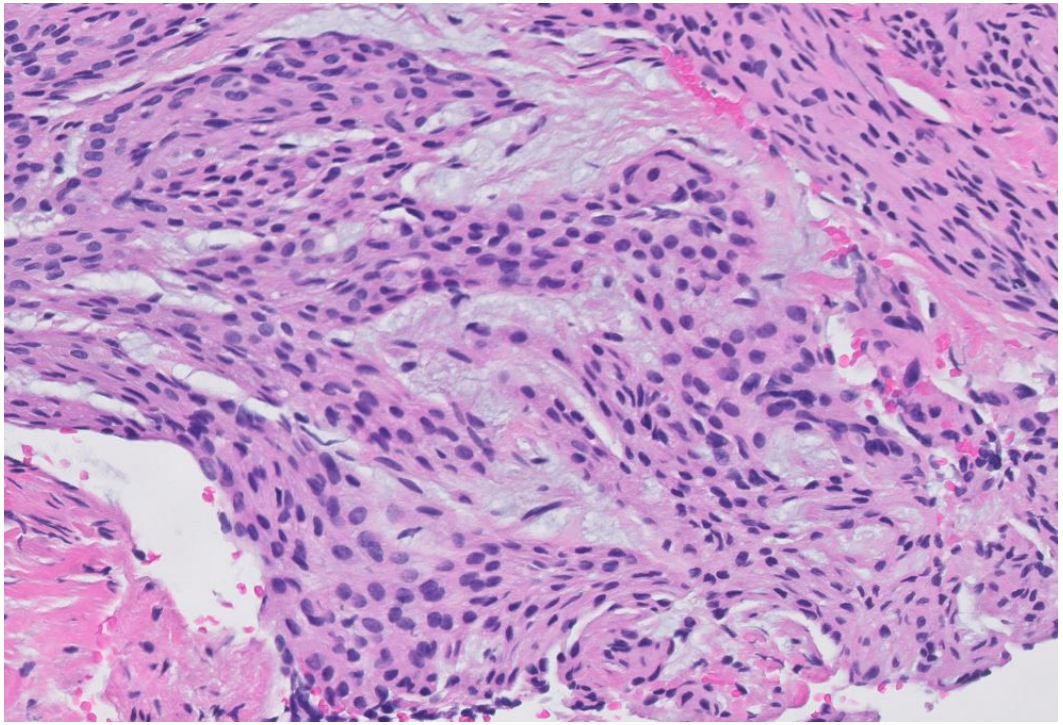
Excision nail unit epithelium



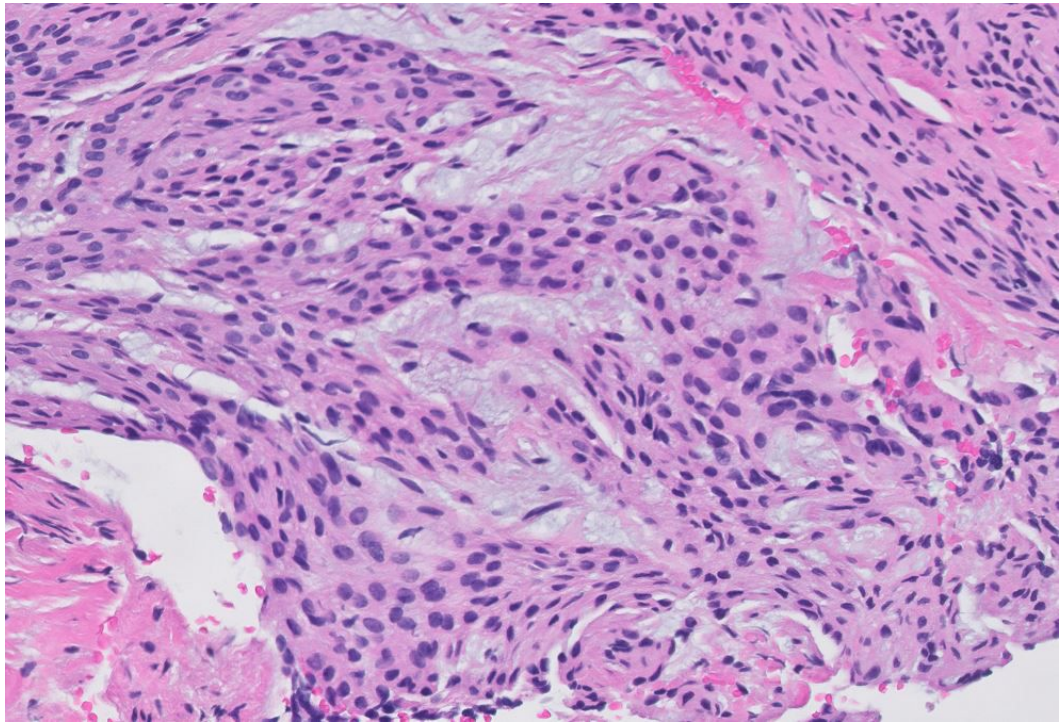
Excision nail unit epithelium

- Section after overnight fixation
- Two cassettes
- Preorders
  - Levels and unstained

Glomus tumor



Glomus tumor



Margins not necessary to report

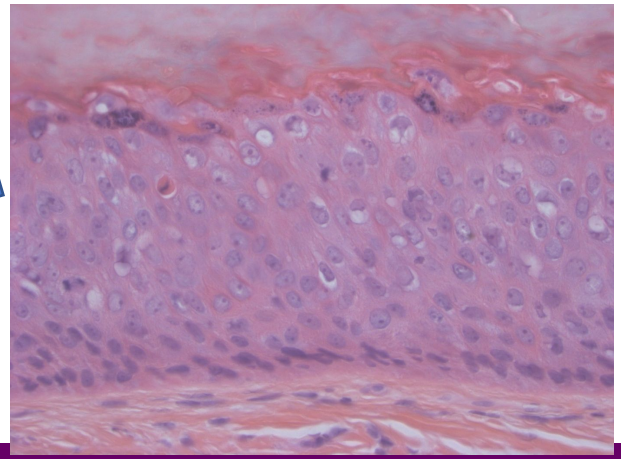
Excisional (wedge) biopsy

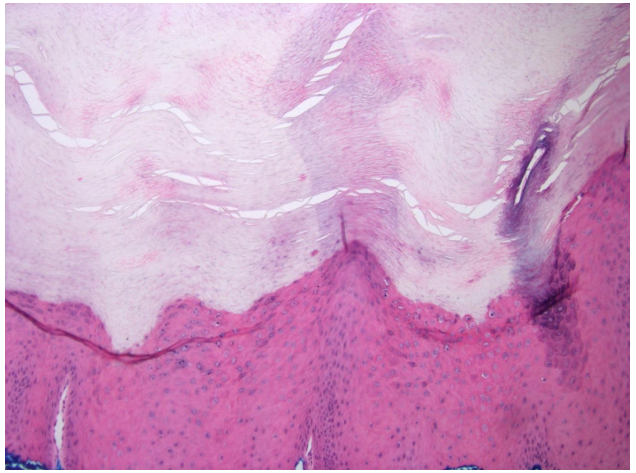
Excisional (wedge) biopsy

Excisional (wedge) biopsy

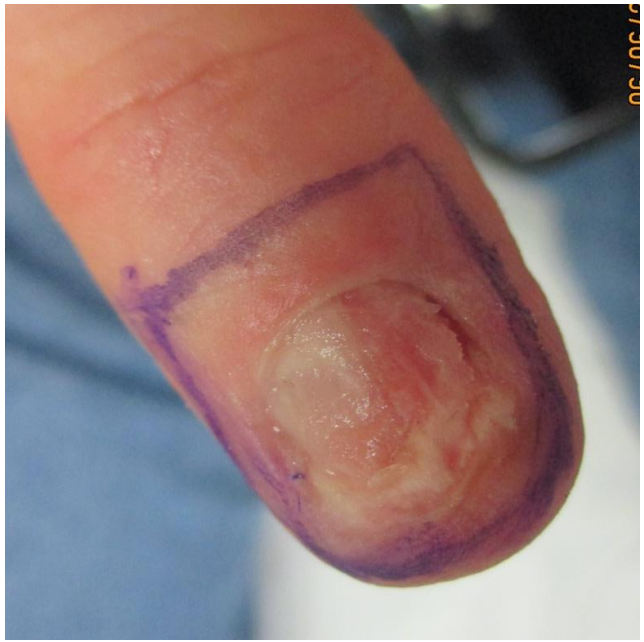
- Section after overnight fixation
- Levels and unstained
- PAS

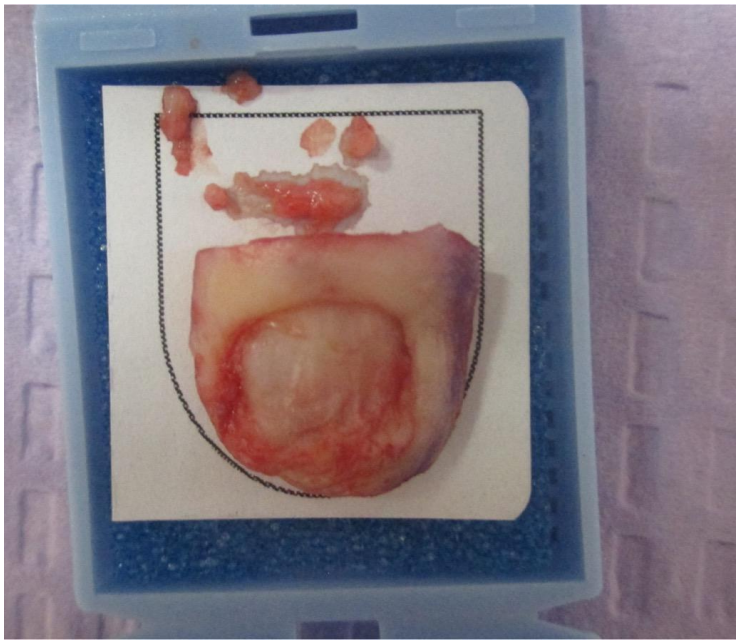
Squamous cell carcinoma—HPV-induced



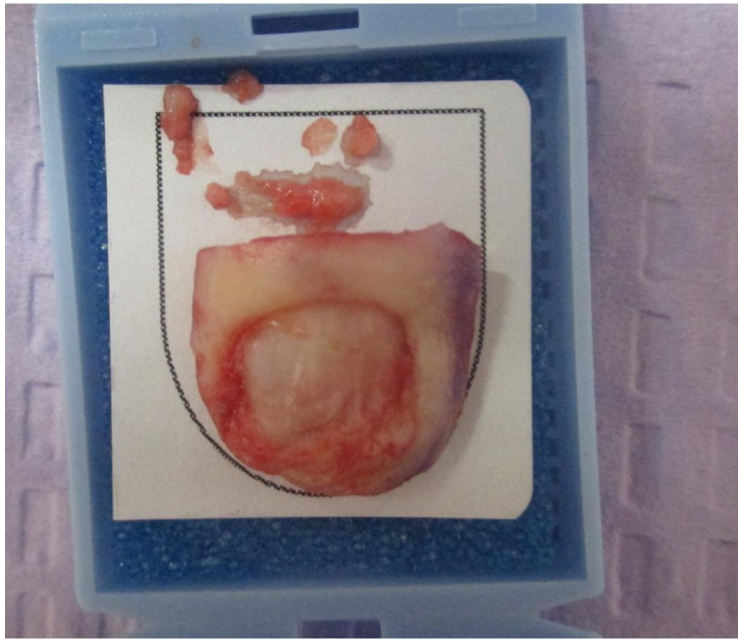


En bloc re-excision of SCC



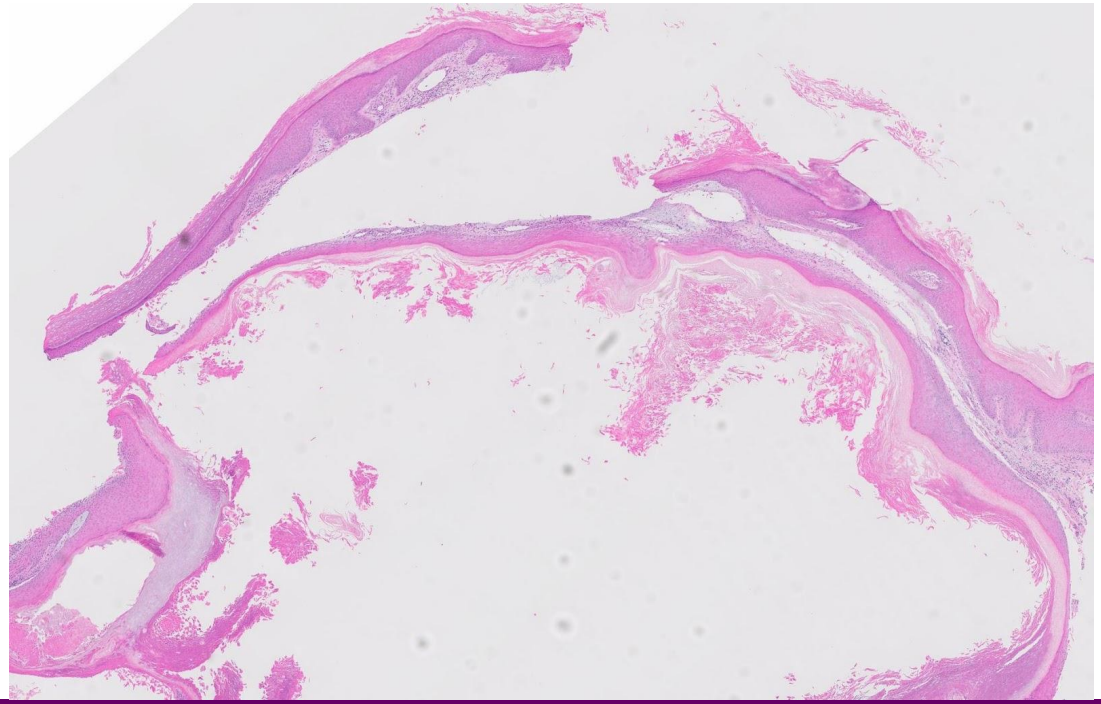


En bloc re-excision of SCC

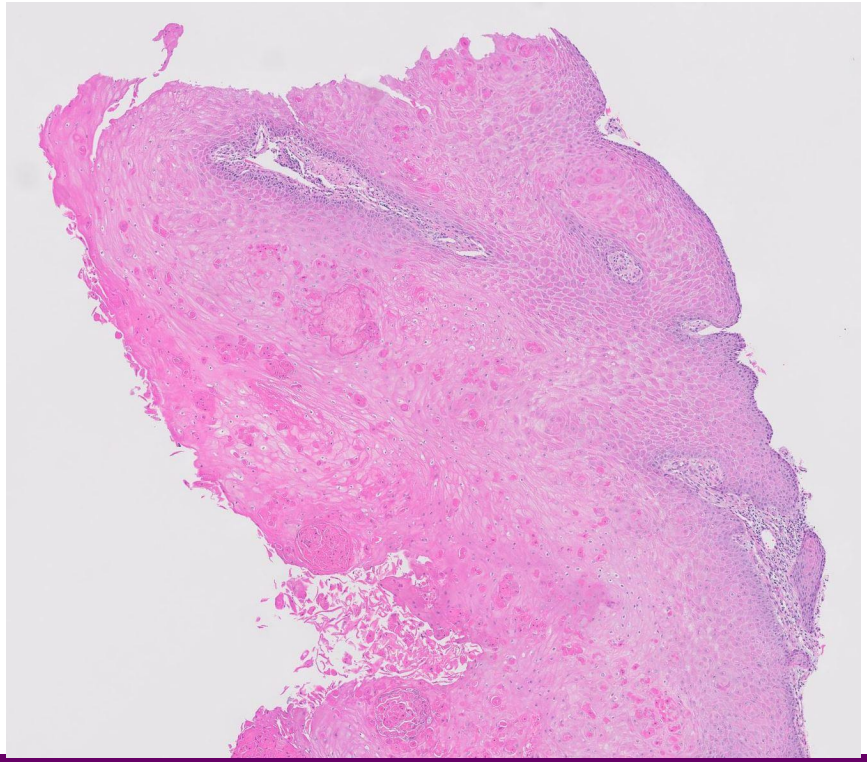


- Submit fragments in separately
- Margins and orientation important
- Levels and unstained

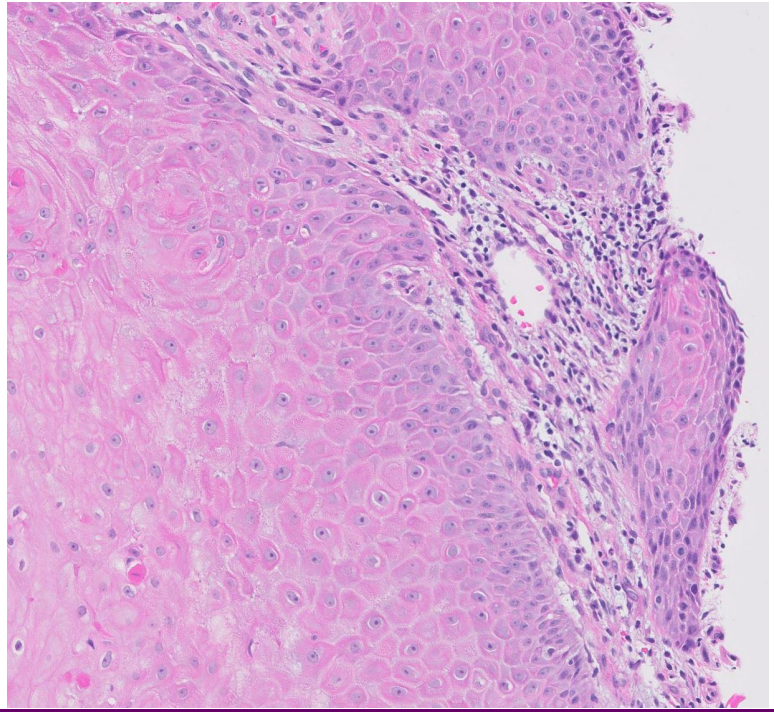
Amputation

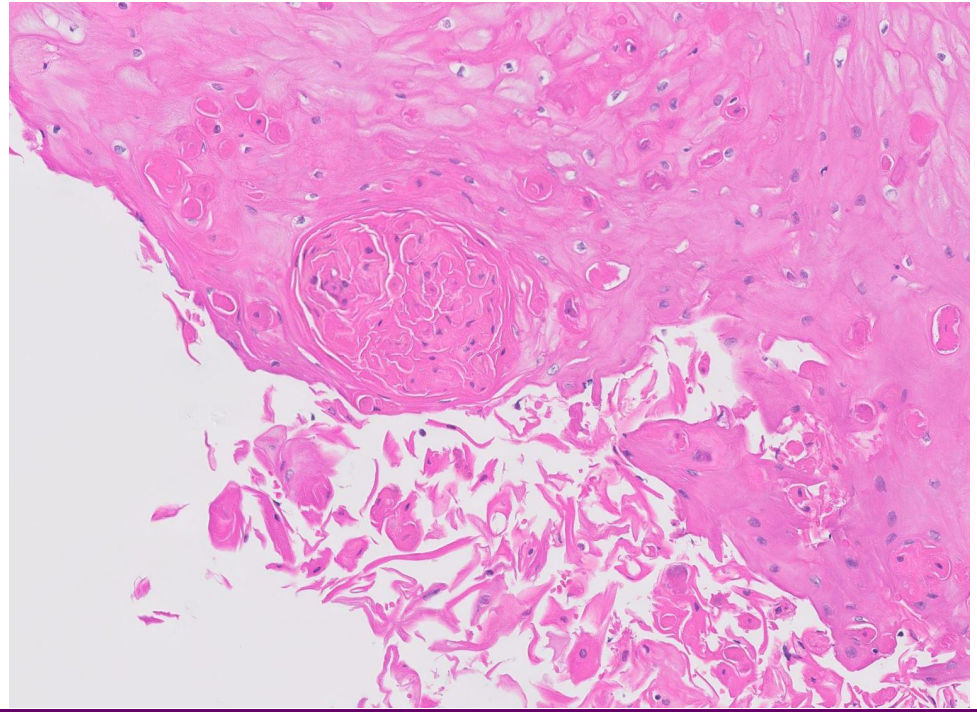


Amputation

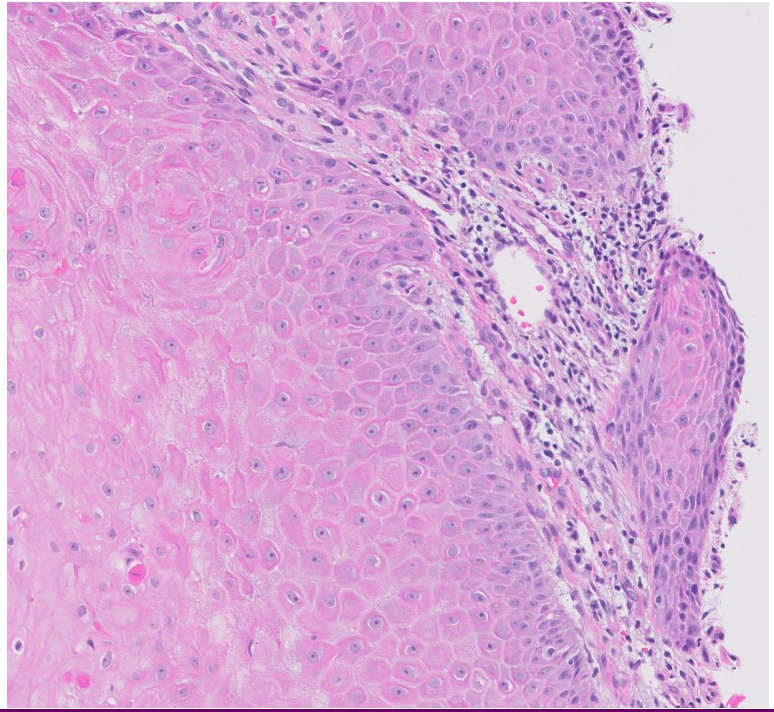


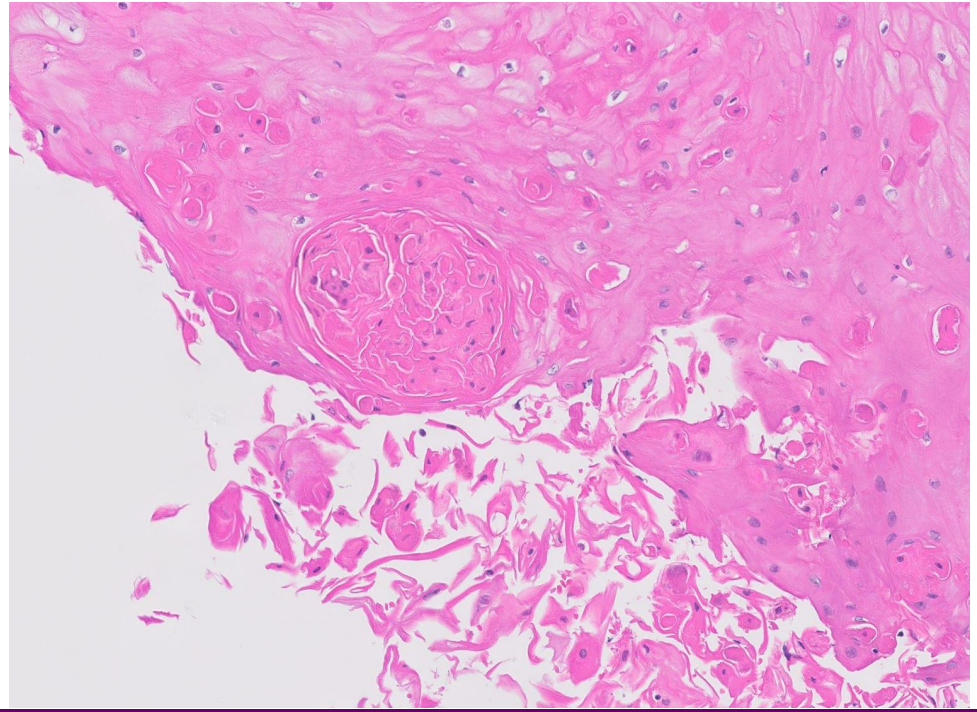
Amputation



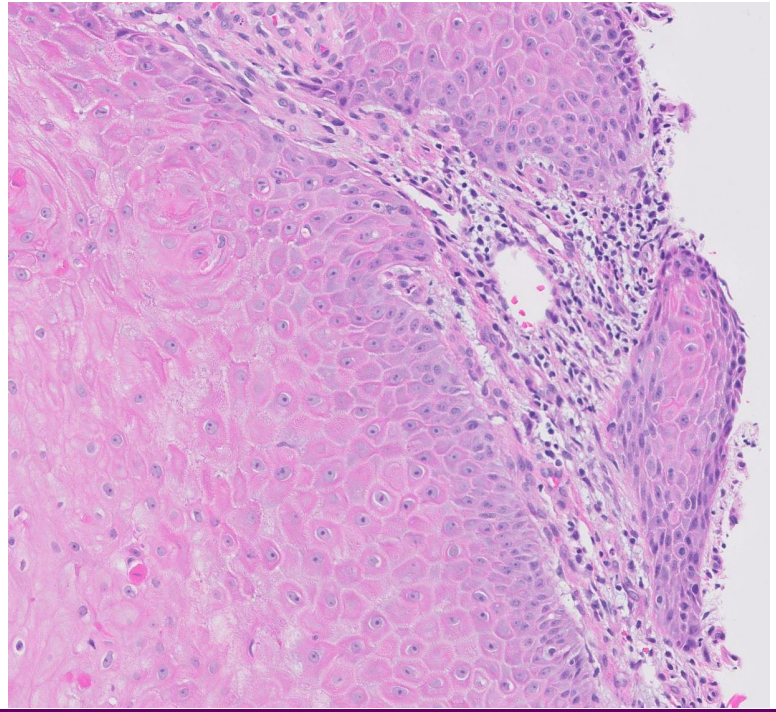


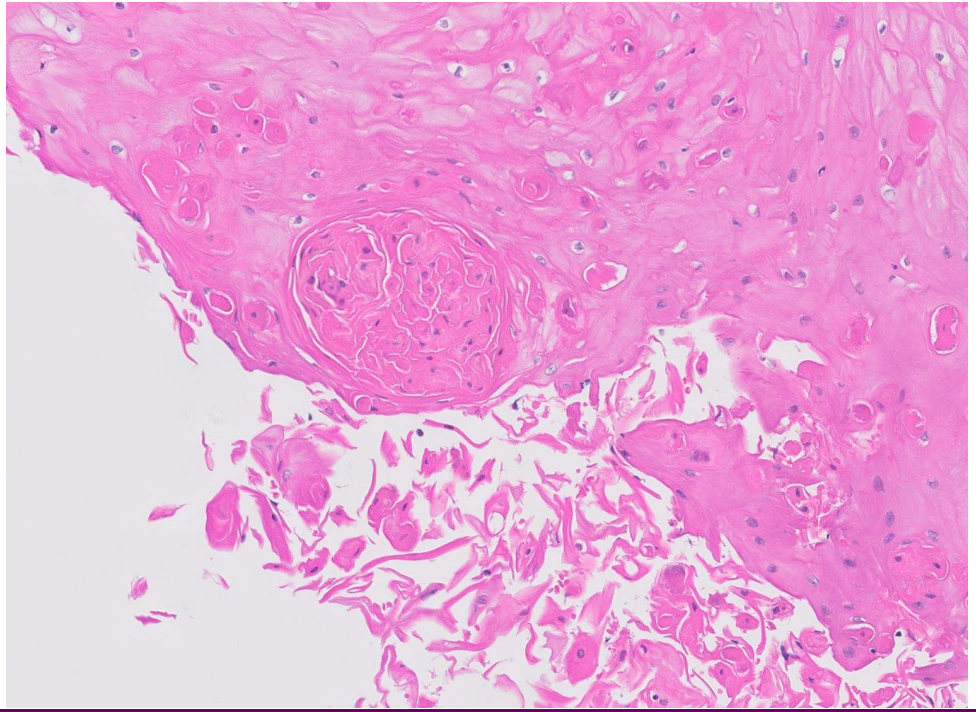
Amputation





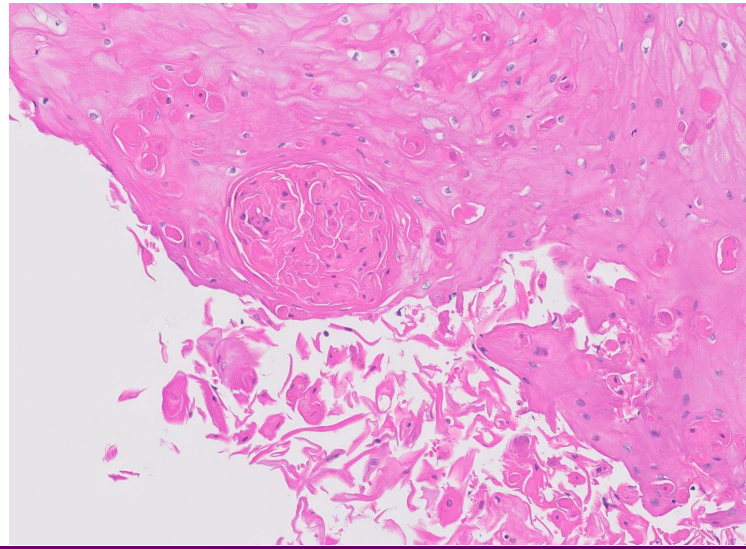
Subungual Keratoacanthoma



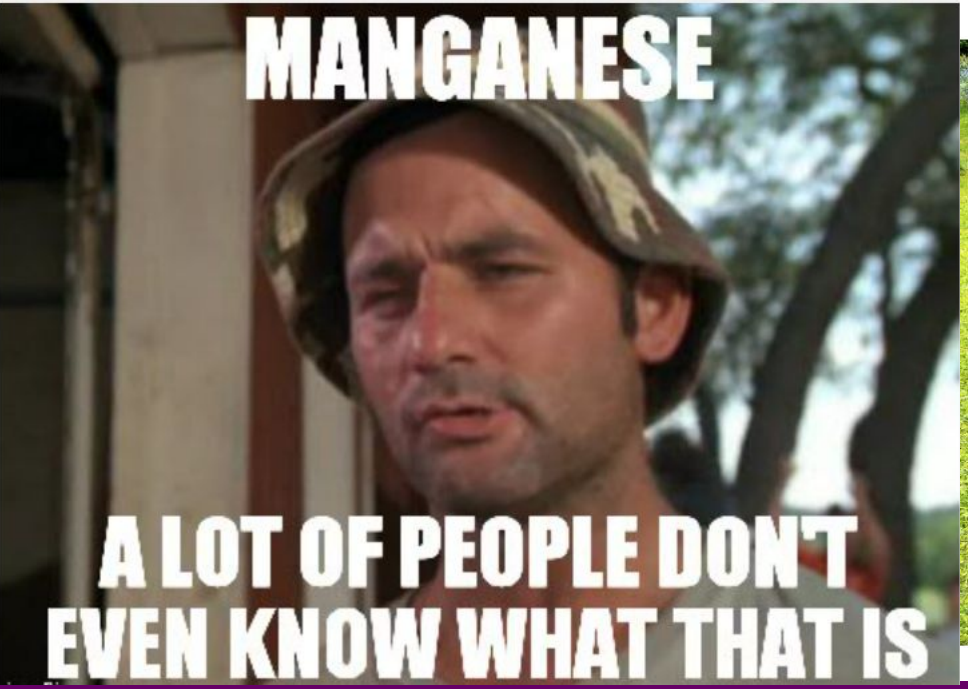


Subungual Keratoacanthoma

- Don’t amputate—need to call clinician
- Destroys bone and does not regress
- Biopsy is curative (shells out)



Clipping




Types of Fungal Nail Infections
(Onychomycosis)
White superficial infection
Distal subungual infection
Proximal subungual infection
Candidal infection
Fungal skin infection
(athlete's foot)

Submit specimen dry in an envelope

Nail adherence to glass slide

Periodic Acid-Schiff's Stain (P.A.S.) Stain for Nails

Procedure:

1. Place a small amount of Gelatin in water bath
2. Cut ribbon at 4um and float onto gelatin water bath
3. Using positively charged slide, pick up desired sections for PAS & H&E slides.
4. Place in 65 deg. C oven for 45 minutes. (More time for "difficult" specimens)
5. Deparaffinize slides using Xylene or Xylene substitutes and hydrate through alcohols (or place PAS slides on programmed de-paraffin run and H&E slides on H&E program on stainer).
6. Gently rinse slide in running tap water.
7. Rinse slide in distilled water.
8. **DO NOT "DIGEST" SLIDES!!!**
9. Place slide in 1% Periodic Acid for 10 minutes.
10. Gently rinse slide quickly in distilled water.
11. Place slide in Schiff's Solution for 10 minutes.
12. Gently rinse slides in warm- hot tap water for 5 minutes.
13. Place slide in Light Green Stain as needed to reach desired background intensity. (Approx. 30 seconds)
14. Dehydrate slide through 3 changes of Absolute Alcohol.
15. Clear slide through 3 changes of Xylene or Xylene substitute.
16. Coverslip using permanent mounting media.

Results:

Basement membrane, Fungi, Glycogen and Mucin: PINK TO RED
Other tissue: Green

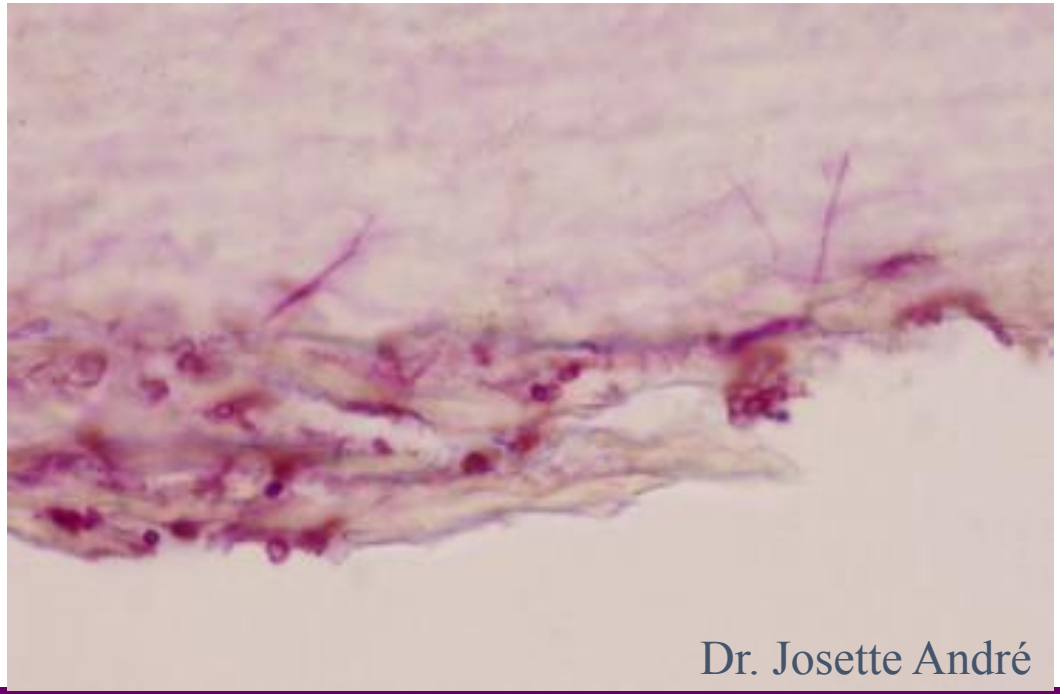
Reference: American Master Tech Scientific, Inc. "PAS Kit Procedure."

Fun(gul) Fact

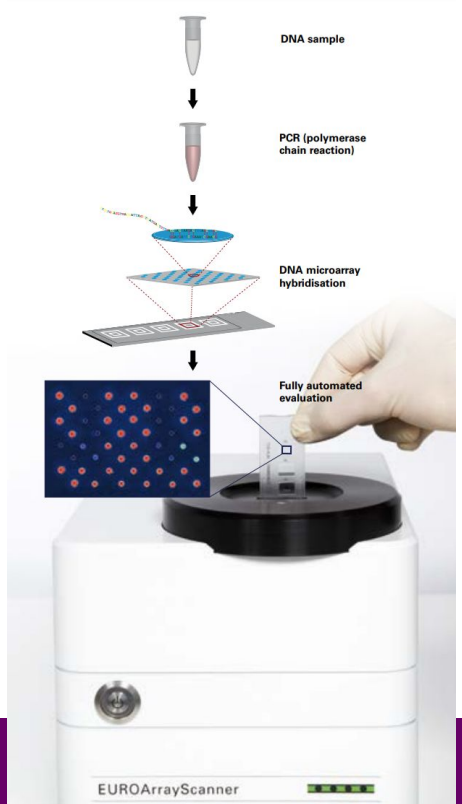
50% of patient presenting with nail changes suspicious for fungus are not fungus.

Mold

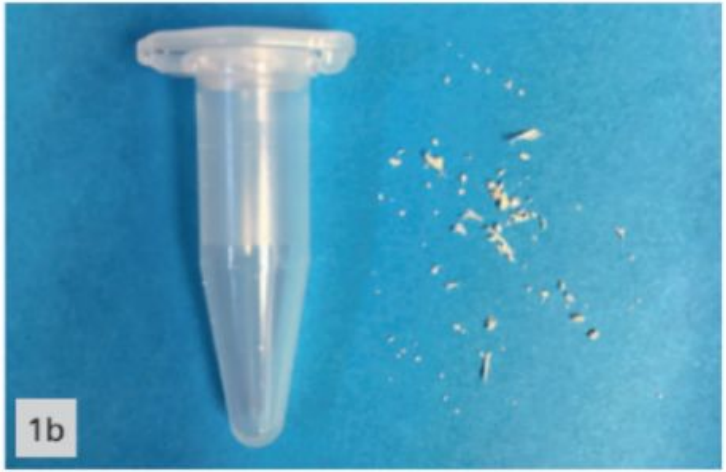
Mold vs Dermatophyte



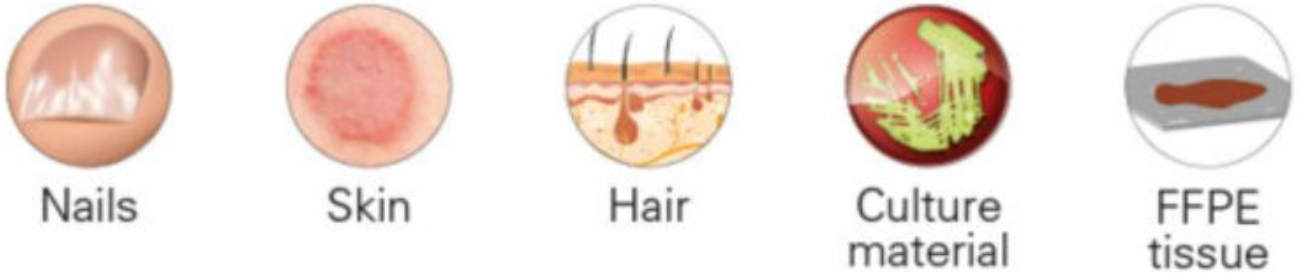
PCR replacing culture



PCR Sample Collection

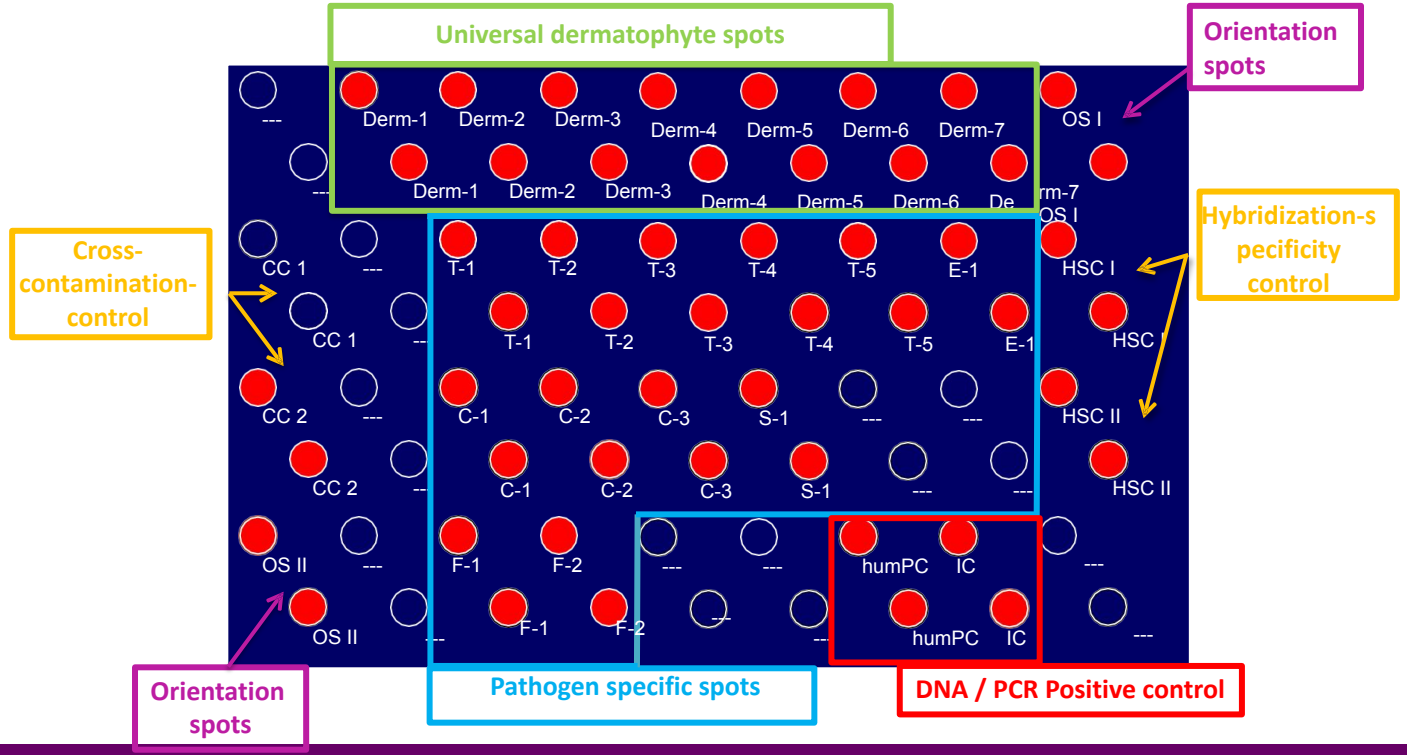


PCR Sample



EUROArray Dermatomycosis

BIOCHIP 1



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PCR replacing culture

Patient ID : CT20-27456

Test result	Result
Dermatophyte	**Multiple infection**
Yeast/Mould	**Fusarium solani**

Patient ID : CT20-27456 **Protocol :** 1/6/21 AL
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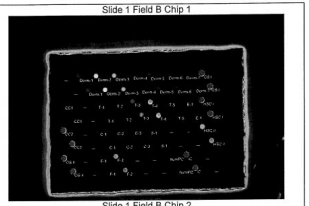
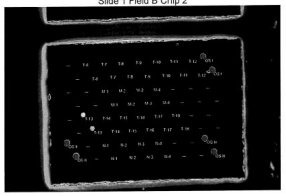
Partial result	Result
Cross contamination control	valid
Internal Control	valid
DNA positive control	**not detected**
Hybridisation specificity control	valid
Dermatophyte (universal)	**DETECTED**
Trichophyton equinum	not detected
Trichophyton tonsurans	not detected
Trichophyton interdigitale	**DETECTED**
Trichophyton mentagrophytes	not detected
T. interdigitale/mentagrophytes	not detected
Trichophyton quinckeanum	not detected
Trichophyton schoenleinii	not detected
Trichophyton simii	not detected
T. quinckeanum/schoenleinii/simii	**DETECTED**
Trichophyton benhamiae(white/afr.)	not detected
Trichophyton benhamiae (yellow)	not detected
T. bullosum/benhamiae (afr.)	not detected
T. concentricum/erinacei	not detected
Trichophyton erinacei	not detected
T. verrucosum/ehotrephon	not detected
Trichophyton rubrum	**DETECTED**
Trichophyton violaceum	not detected
Epidermophyton floccosum	not detected
Nannizzia fulva	not detected
Nannizzia gypsea	not detected
Nannizzia incurvata	not detected
Nannizzia persicolor	not detected
Microsporum canis	not detected
Microsporum ferrugineum	not detected
Microsporum audouinii	not detected
M. canis/audouinii	not detected
Candida parapsilosis	not detected
Candida guilliermondii	not detected
Candida albicans	not detected
Fusarium solani	**DETECTED**
Fusarium oxysporum	not detected
Scopulariopsis brevicaulis	not detected

Slide 1 Field B Chip 1



Slide 1 Field B Chip 2



Study at CTA Pathology—PAS versus PCR

- Eight-two (82) samples were tested with both PCR and PAS.
  - PCR molecular test identified 73% of samples as positive,
  - PAS only identified 59%.
- Two (12) samples were negative by traditional PAS but positive for PCR.
- One (1) sample was positive by PAS and negative with PCR.

PAS versus PCR

- **Conclusion**
  - Sensitivity (true positive rate) ~15% superior
  - Speciation stops need for culture

Take home

- Nail adherence protocol important
- Treat delicate bed/matrix specimens with care
- Preorder levels, special stains, unstained
- Dilute Fontana-Masson
- Clipping—nail adherence important
- PCR much better than PAS

Thanks!

Mahalo!

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Gracias!

Mālō 'aupito

César Baldaccini, “Le Pouce,” Qatar