

# How to Biopsy the Nail Unit

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# Objectives

- How/where to biopsy
- How to submit to laboratory
- Laboratory processing
  - Special stain utility
- Fungal diagnostics



# What can the nail surgeon do to submit a bed/matrix specimen for appropriate interpretation?



# Need to be involved in lab prep





# Need concise and clear guidelines for specimen submission:

# Orientation of tissue

- Clear information to histotechnicians
- Reproducible among different laboratories









#### Courtes of Dara Phose Ser Rich





#### Print template at www.ctapathology.com



# Histology Materials

















# Each specimen is different





# Pathologist review before grossing

- Number tissue blocks
- Unstained slides or levels at the start
- Special stains
- Importance of nail
- Reserve nail for fungal testing



# Finding the pigment

- H&E with initial levels
- MelanA IHC
- Fontana-Masson
- PAS fungus
  - Unstained slides



# Finding the pigment H&E levels





#### Finding the pigment Melanin stain (Fontana-Masson)





#### Finding the pigment Melanocyte market (melanA/Mart1





# Digital Myxoid/Mucous Cyst





# Digital Myxoid/Mucous CystMucin may be anywhere





# Digital Myxoid Cyst

# • Often don't see mucin

Scar

Reactive changes





# Mucin stain often required







Courtesy of Dr. Phoebe Rich





Print template at www.ctapathology.com



### Histology Materials

















#### 10% formalin





#### Nail Fungus Diagnostics





#### Submit specimen dry in an envelope





### Mold







## Mold vs Dermatophyte





# PCR replacing culture





## PCR Sample Collection



- Before collection, remove any traces of nail varnish.
- Take samples from nail areas with visible damage or staining (see Fig. 1a). Include any crumbly or soft material under the nail plate. If several nails are affected, take a pooled sample from all of them.
- Plane off sample material from the nail surface down to the deeper layers using a sterile, blunt (if required) scalpel, a sharp spoon, a ring curette or a milling tool. Retrieve any soft/crumbly material from under the nail using a small hook.
- Preferably, collect nail shavings or small pieces of the nail, not entire nails (see Fig. 1b).



# PCR Sample Collection

















FFPE tissue



#### PCR replacing culture

Protocol :

1/6/21 AL

Patient ID -

CT20-27456

Partial result	Result	Slide 1 Field B Chip 1
Cross contamination control	valid	
nternal Control	valid	
DNA positive control	not detected	have been been been been been been been
hybridisation specificity control	valid	- Don't form? come Dones Dones Done? Done Tel:
Dermatophyte (universal)	DETECTED	10 - 11 12 13 14 15 15 16 13 10
Trichophyton equinum	not detected	Ro - 11 10 10 10 10 10 10
Trichophyton tonsurans	not detected	Qu _ 1 0 0 H 900
Frichophyton interdigitale	DETECTED	Ben in the second secon
Frichophyton mentagrophytes	not detected	
r. interdigitale/mentagrophytes	not detected	Slide 1 Field B Chip 2
Frichophyton quinckeanum	not detected	
Frichophyton schoenleinii	not detected	
Frichophyton simii	not detected	. N. 11 10 10 10 10 10 10
F. quinckeanum/schoenleinii/simii	DETECTED	_ 14 10 14 10 10 10 <sup>-0</sup> 00
Frichophyton benhamiae(white/afr.)	not detected	a
Frichophyton benhamiae (yellow)	not detected	
F. bullosum/benhamiae (afr.)	not detected	10 TH 16 TH 10 TH -
F. concentricum/erinacei	not detected	Q <sub>11</sub> _ 11 12 13 13 13 14 Res
Frichophyton erinacei	not detected	
F. verrucosum/eriotrephon	not detected	Contract of the American State
Frichophyton 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	
Scopularionele bravicaulie	not detected	



#### PCR replacing culture

#### Patient ID : CT20-27456

Test result	Result	
Dermatophyte	Multiple infection	
Yeast/Mould	Fusarium solani	

Patient ID : CT20-27456				
Partial result	Result			
Cross contamination control	valid			
Internal Control	valid			
DNA positive control	not detected			
Hybridisation specificity control	valid			
Dermatophyte (universal)	DETECTED			
menophyton equinam	noi delected	_		
Trichophyton tonourane	not detected			
Trichophyton interdigitale	DETECTED			
Trichophyton mentagrophytes	not detected			
T. interdigitale/mentagrophytes	not detected			
Trichophyton quinckeanum	not detected			
Trichophyton schoenleinii	not detected			
Triehophyton cimii	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			
1. verrucosum/enotrepnon	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	-		

1/6/21 AL Protocol 1

age





# PCR Experience to date

- Works well after PAS (formalin fixed)
- Correlates with PAS but some PAS negative are positive with PCR
- Co-infection common
- Insurances cover test



#### 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.





#### Study at CTA Pathology—PAS versus PCR

#### Conclusion

- Sensitivity (true positive rate) for PCR molecular analysis that is ~15% superior to a PAS only stain.
- The results justify stopping the use of PAS based on this sensitivity difference alone.
- Since PCR identifies an array of different dermatophyte, yeast and mold species on the initial test, thereby ensuring that initial treatment is specific and appropriate.
- Traditional culture, which is insensitive and slow, is no longer needed.
- The cost and time savings of not performing a culture is significant.





#### **EUROArray Dermatomycosis**



#### Test principle:

Multiplex-PCR amplification of target sequences plus subsequent microarray hybridization for detection of PCR products

#### Detection range:

- 23 dermatophytes + 3 yeast + 3 molds
- Universal dermatophyte detection covering in total 50 species
- Integrated control reactions

Dermatophyte	species	
Anthropophilic	M audouinii	T. verrucosum
T. tonsurans	Zoophilic	T. eriotrephon
T. interdigitale	T. equinum	M. canis
T. schoenleinii	T. mentagrophytes* (T. interdigitale)	N. persicolor* (M. persicolor)
T. concentricum	T. simii	Geophilic
T. rubrum	T. quinckeanum* (T. mentagrophytes)	N. fulva* (M. fulvum)
T. violoaceum	T. erinacei	N. gypsea* (M. gypseum)
E. floccosum	T. bullosum	N. incurvata' (M. incurvatum)
M. ferrugineum	T. benhamiae* (A. benhamiae)	
New nomenclature (Ho	og et al, Mycopathologia: 2017 Feb; 182(1-2):5-31)	
C parapsilosis	C. quilliermondii	E. oxysporum
C albicans	E solani	Sc. brevicaulis



#### EUROArray Dermatomycosis -Workflow



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#### **EUROArray Dermatomycosis**

**BIOCHIP 1** 



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# EUROArray dermatomycosis results from software

Slide	Field	Chip	Patient ID	Parameter	Result type	Result
1	A	1	0	DNA positive control	Partial result	DETECTED
	A	1	0	Hybridisation specificity control	Partial result	valid
L	A	1	0	Dermatophyte (universal)	Partial result	DETECTED
L	A	1	0	Trichophyton equinum	Partial result	not detected
L	Α	1	0	Trichophyton tonsurans	Partial result	not detected
Ĺ	A	1	0	T. interdigitale/mentagrophytes	Partial result	DETECTED
1	A	1	0	Trichophyton interdigitiale	Partial result	DETECTED

7	Α	1	30	Negative control	Test result	valid
7	Α	1	30	Cross contamination control	Partial result	valid
7	Α	1	30	Internal Control	Partial result	valid
7	Α	1	30	Hybridisation specificity control	Partial result	valid

\*\*Without a negative control the assay is automatically invalid\*\*

Assay would need to be repeated if:

- **1.** End-user forgets to include negative control
- 2. Negative control result is "invalid"

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# Positive Result for *Trichophyton rubrum*



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