Laboratory Diagnostics of Nail Fungal Disease: Old versus New (PCR) Techniques

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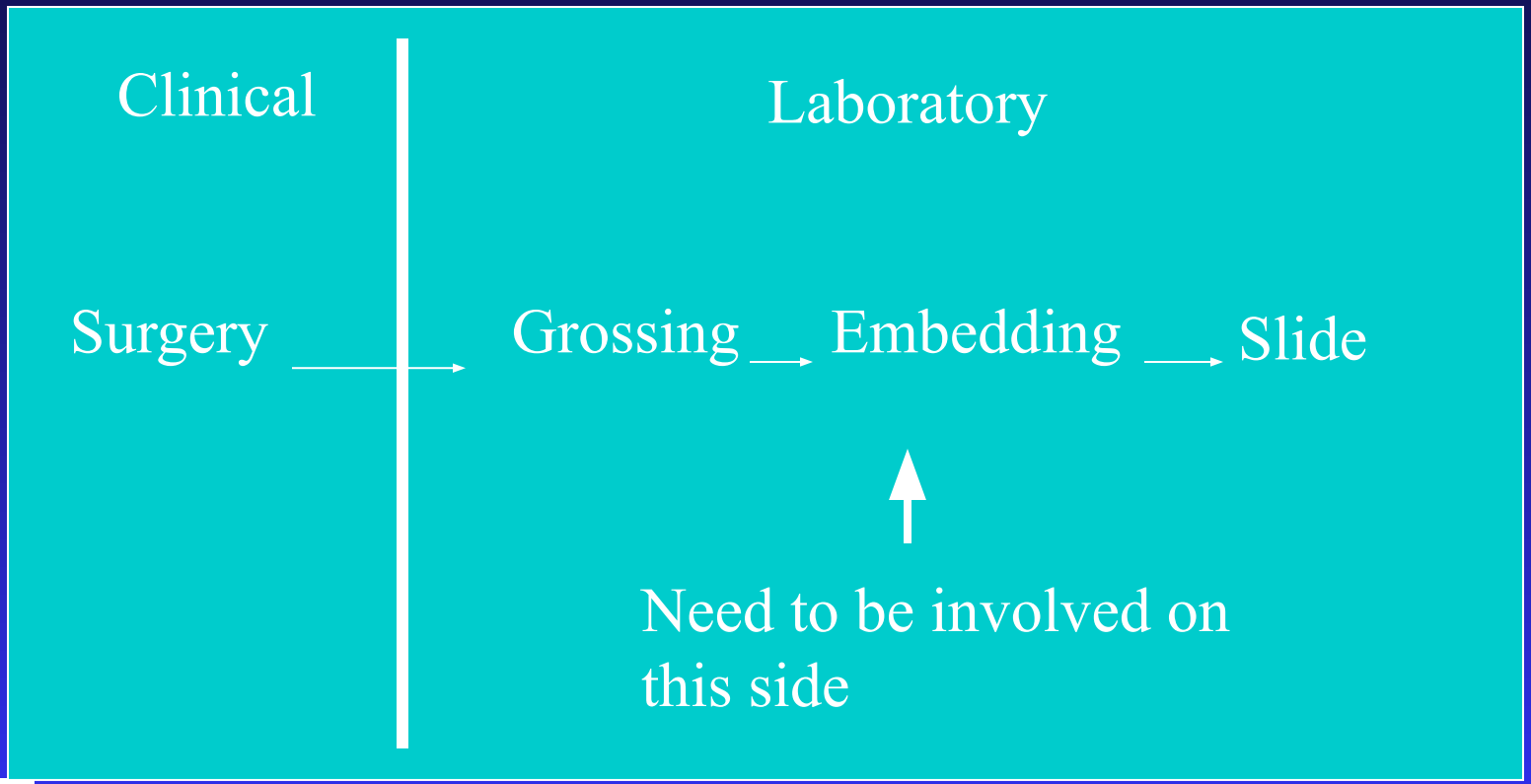
Objectives

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

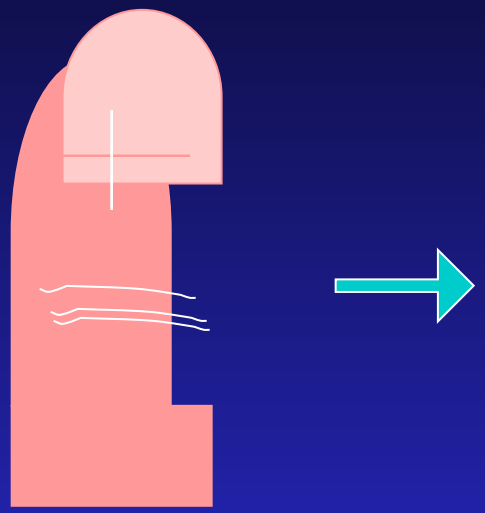
What can the nail surgeon do to submit a bed/matrix specimen for appropriate interpretation?

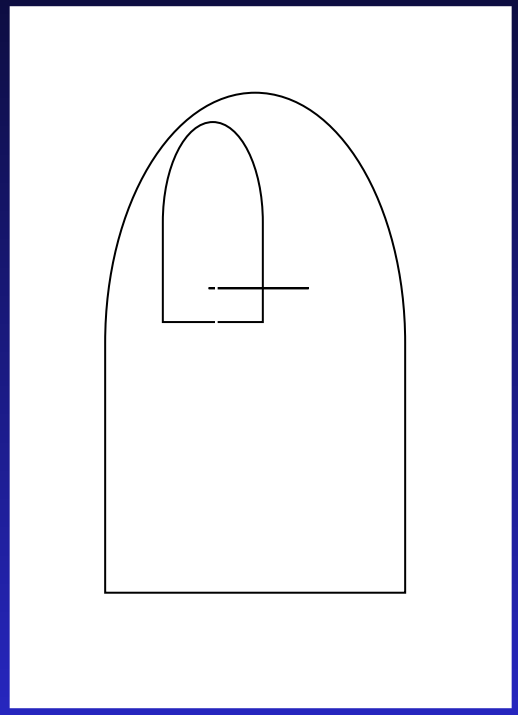
Need to be involved in lab prep

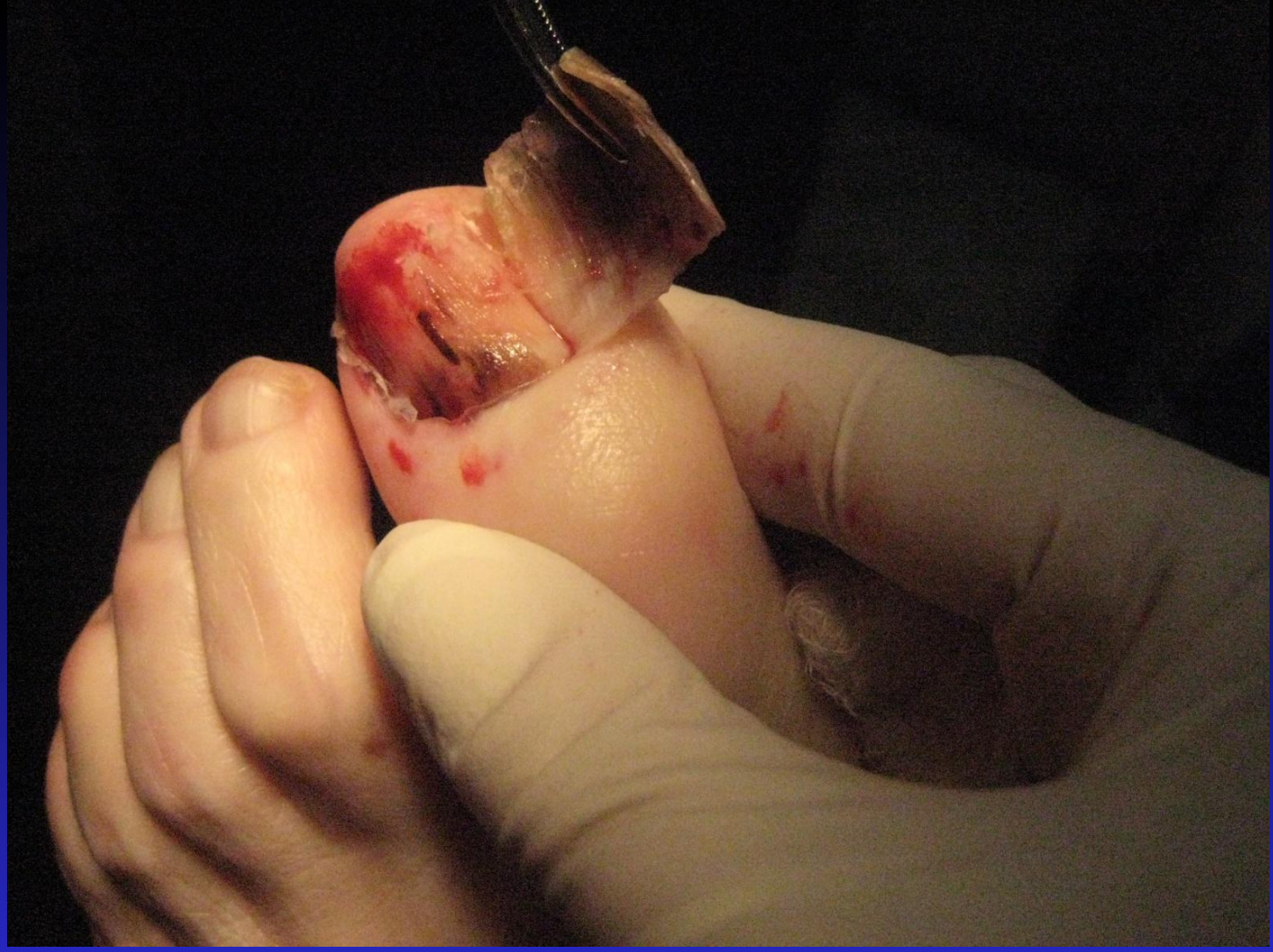


Need concise and clear guidelines for specimen submission:

- Orientation of tissue
- Clear information to histotechnicians
- Reproducible among different laboratories







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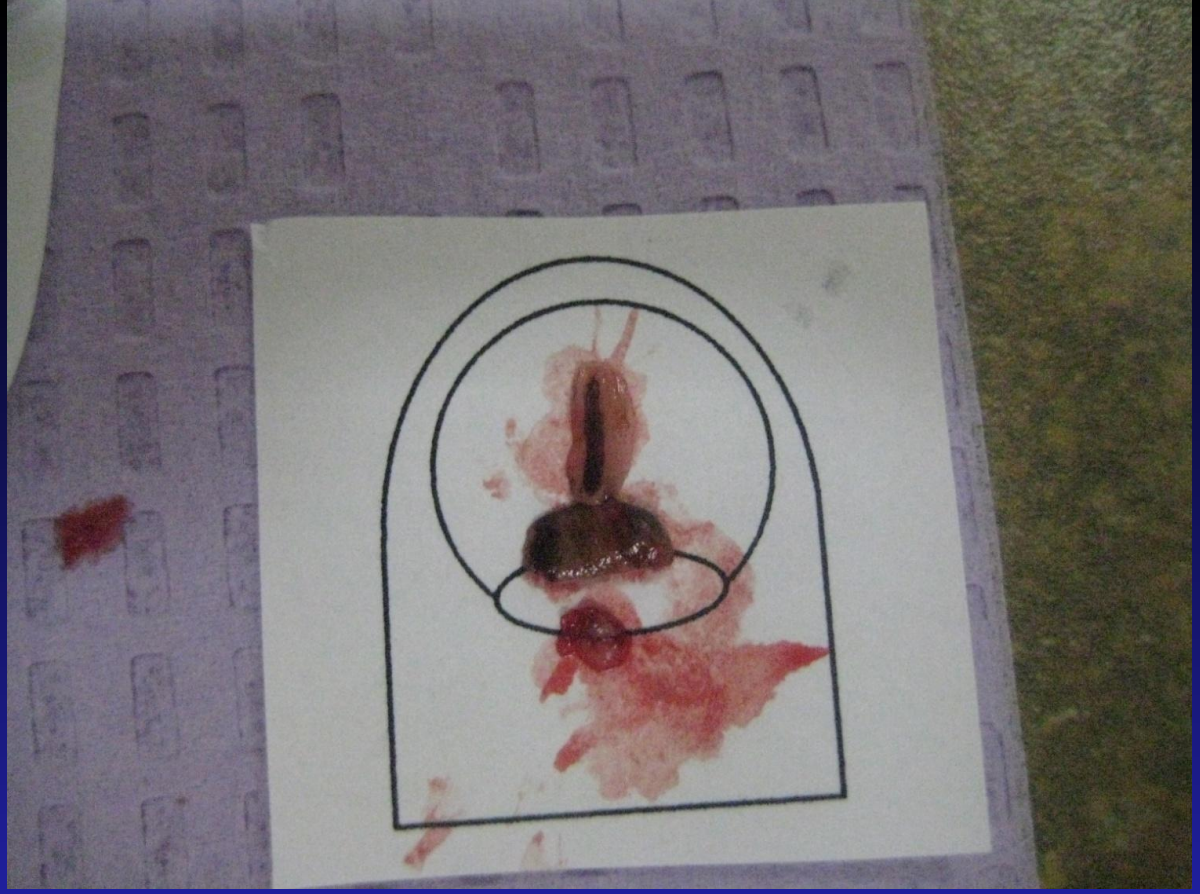
Courtesy of Dr. Phoebe Rich



Print template at www.ctapathology.com

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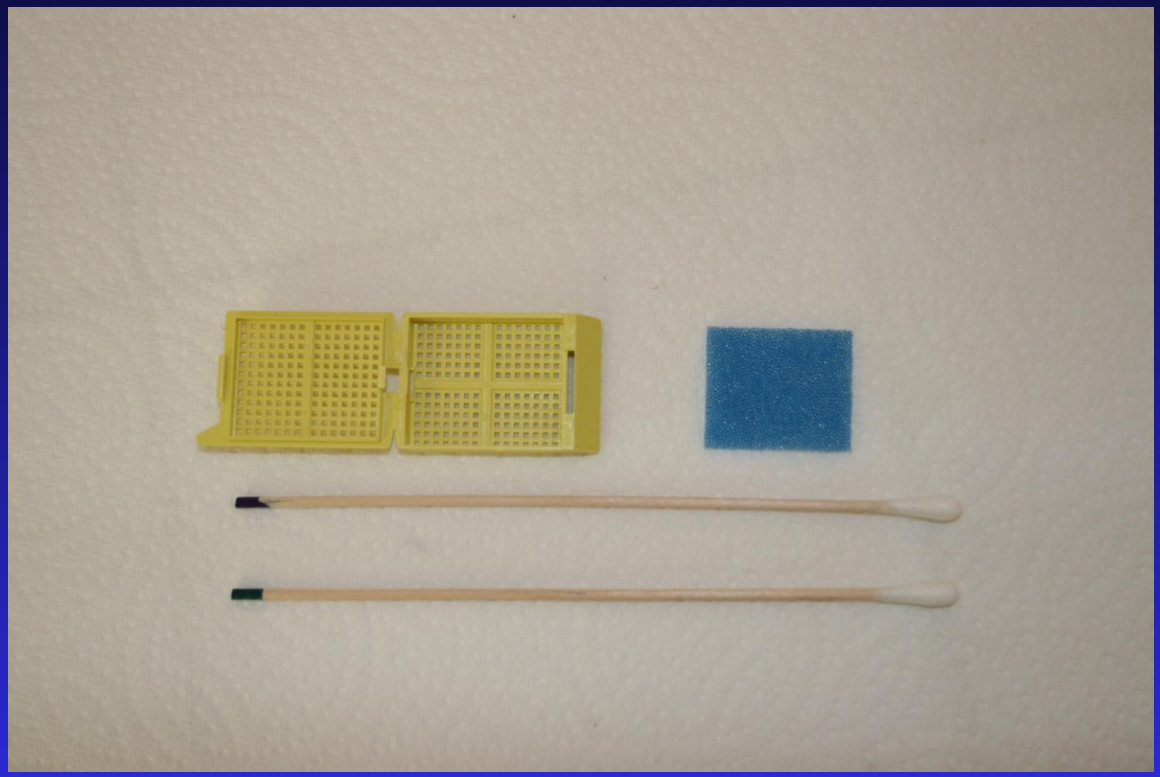
Histology Materials



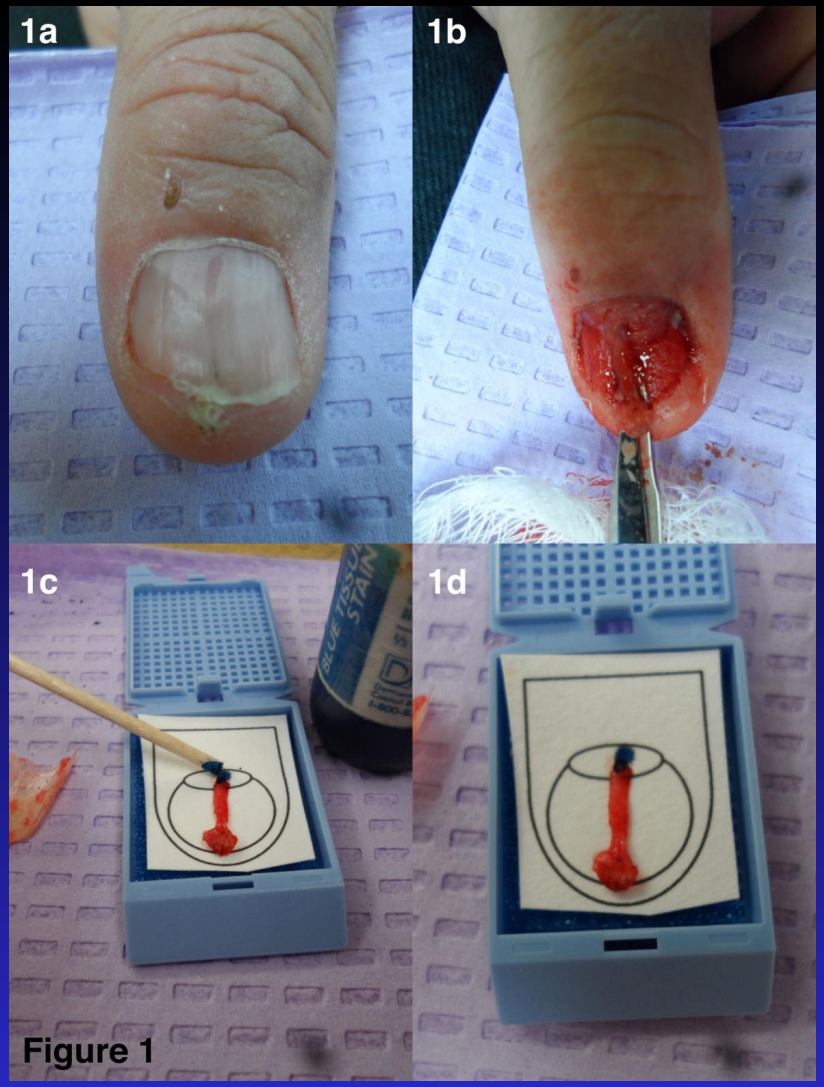


Figure 1

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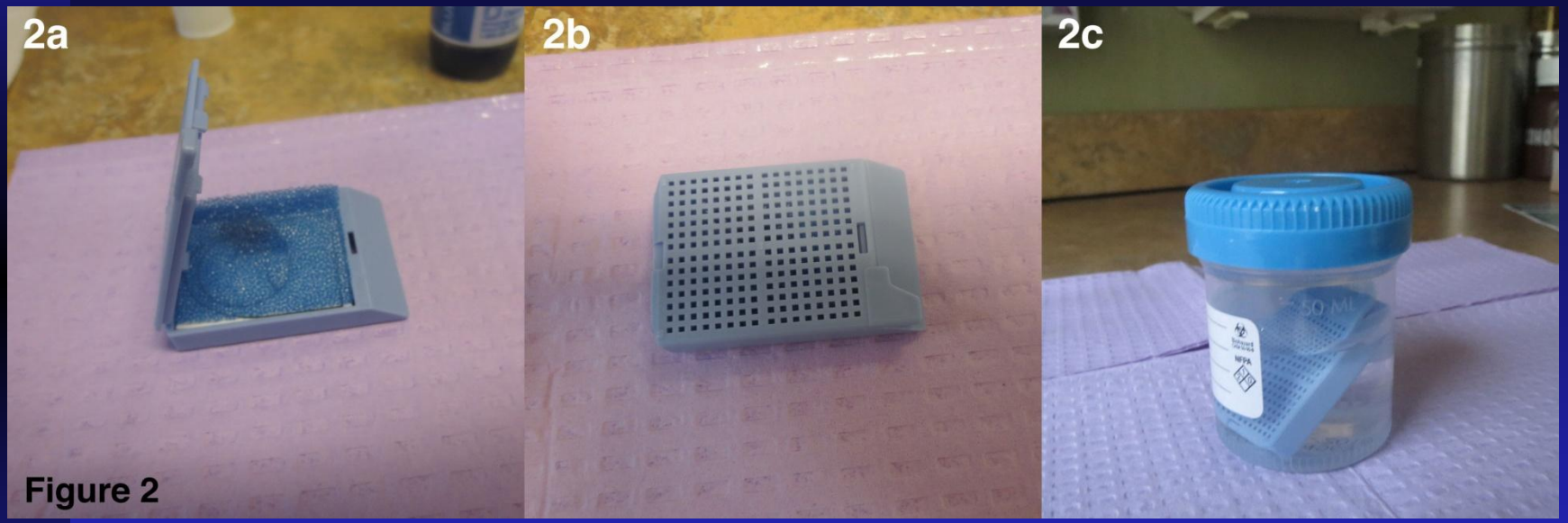

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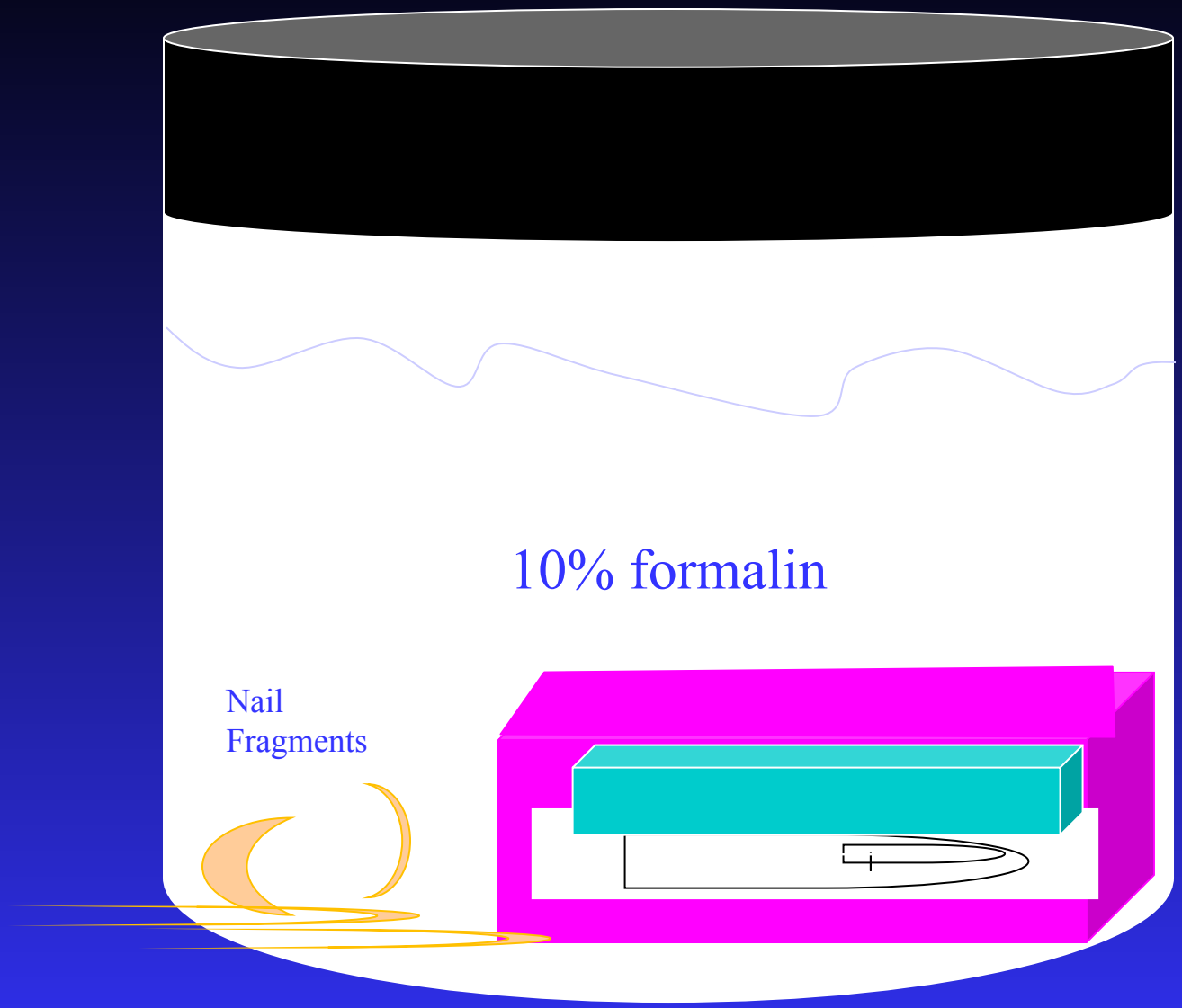
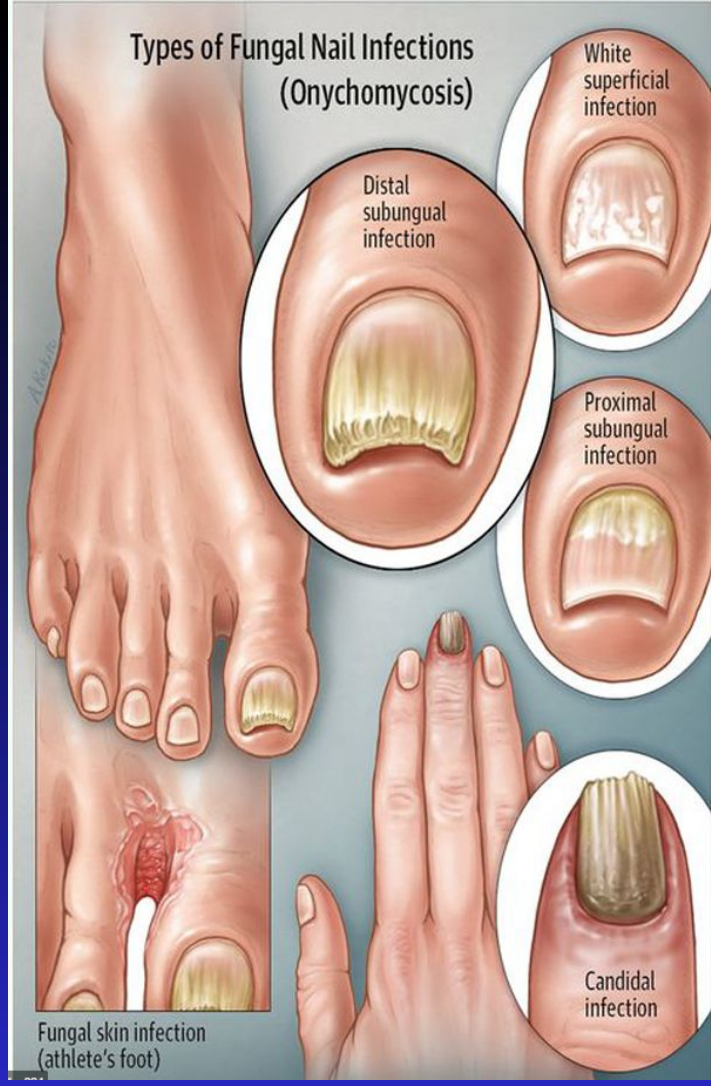


Nail Fungus Diagnostics

- Sampling an issue



Submit specimen dry in an envelope

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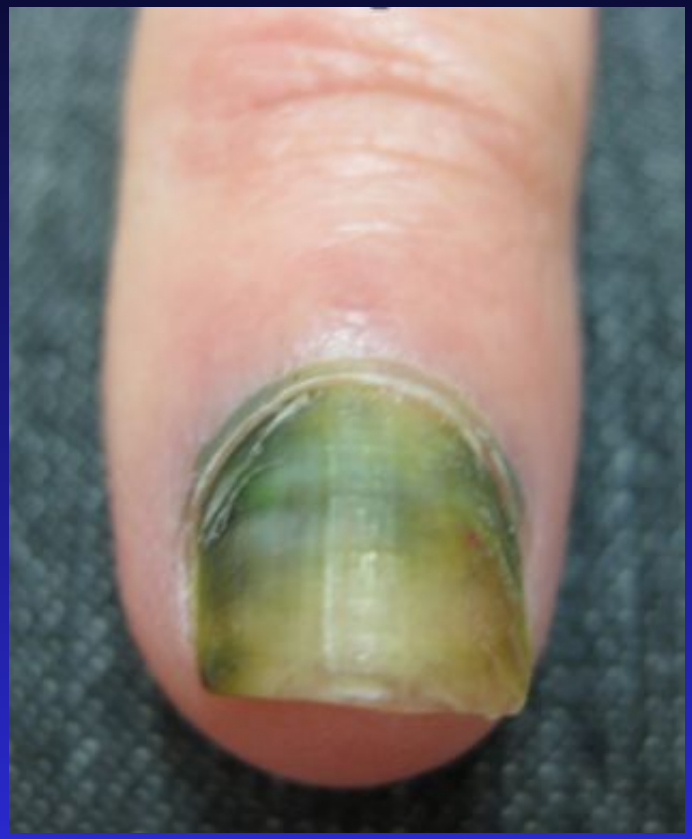
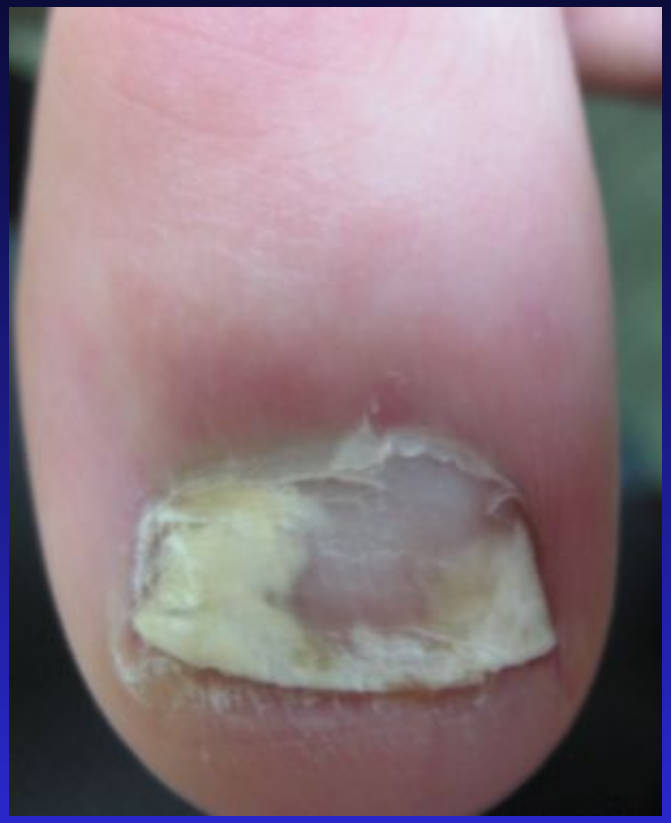
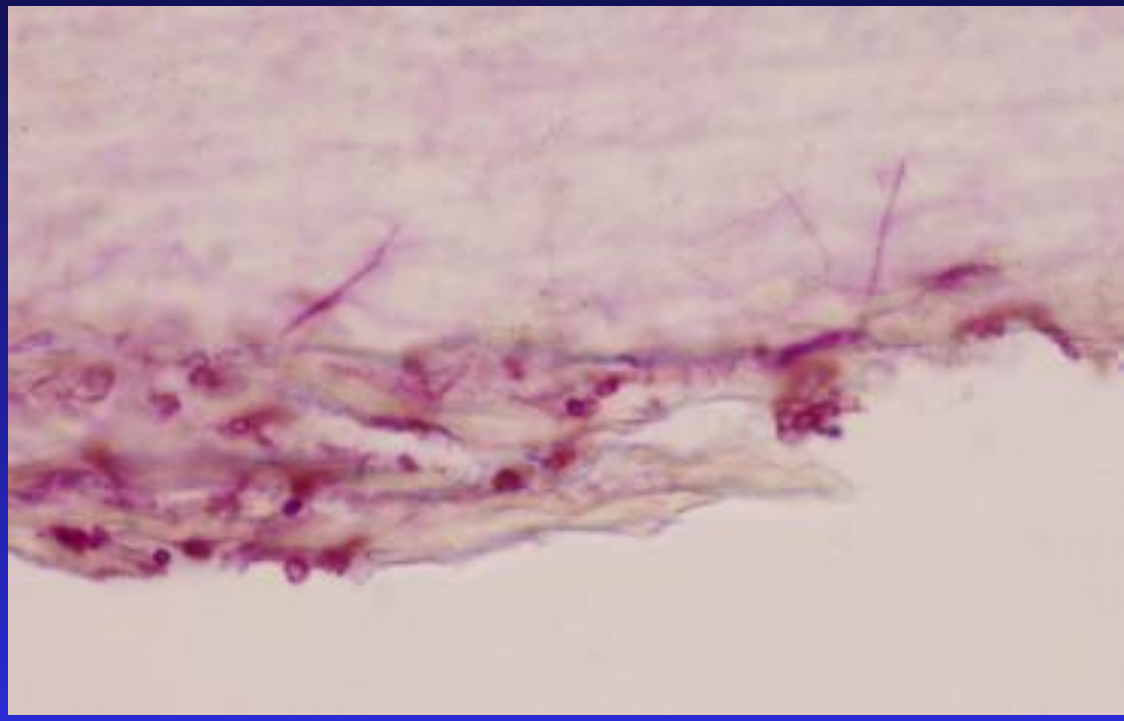
Mold



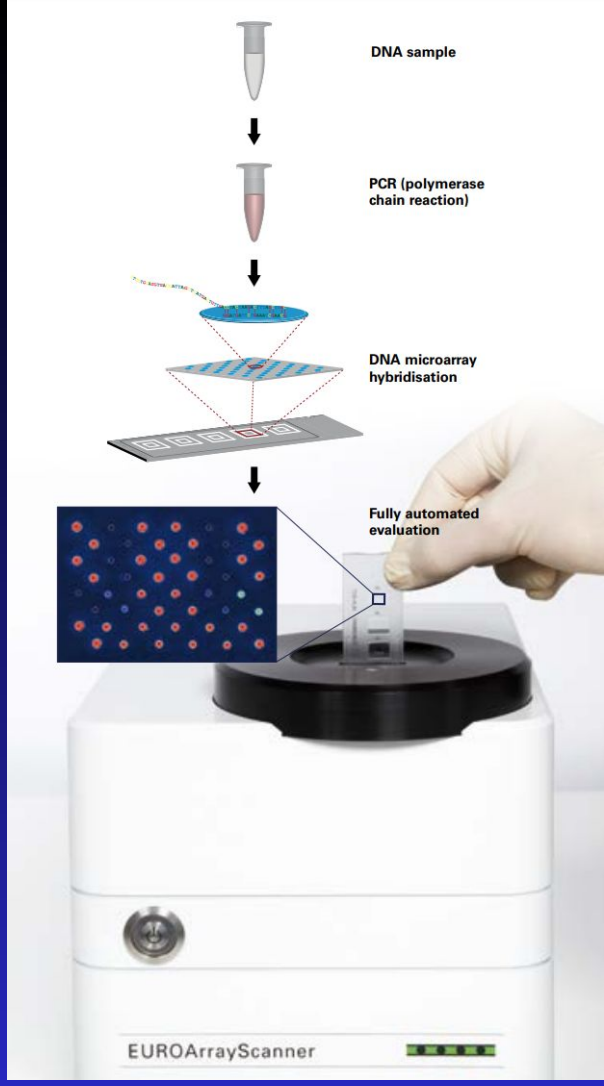


Mold vs Dermatophyte

- Invades vertical to nail plate.



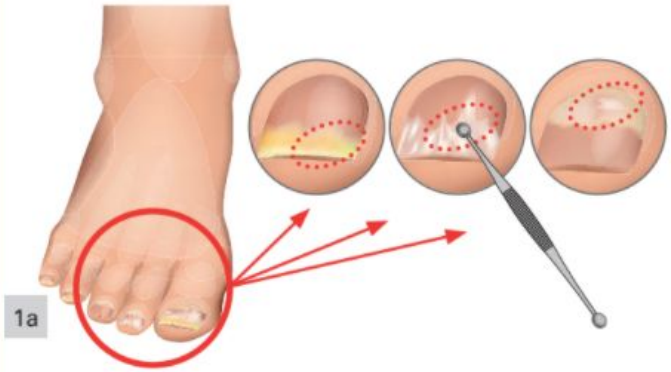
PCR replacing culture

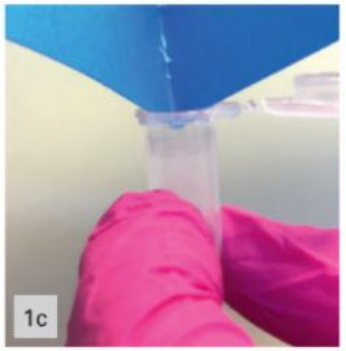


PCR Sample collection

- Remove any nail varnish
- Clean area 70% ethanol and let dry briefly

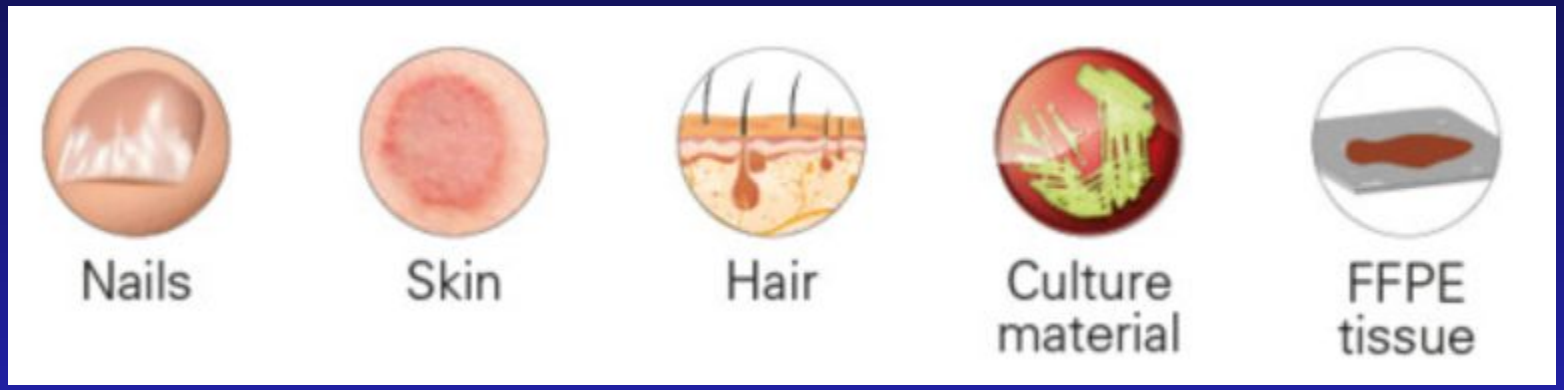
PCR Sample collection





- 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



PCR replacing culture

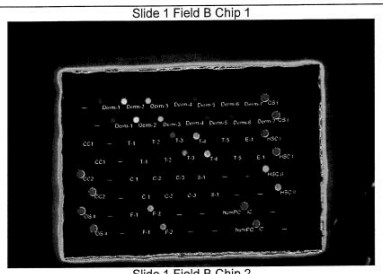
Patient ID : CT20-27456

Protocol : 1/6/21 AL

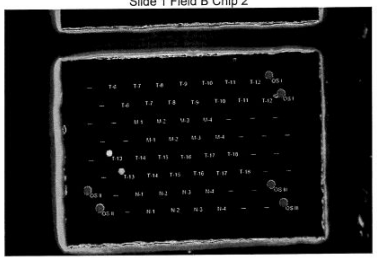
Page : 1

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/eriotrephon	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



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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

Page : 1

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/eriotrephon	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

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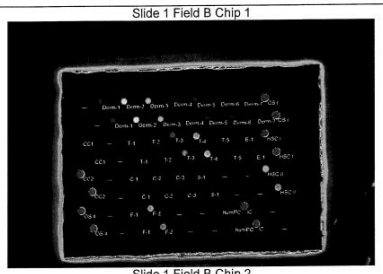

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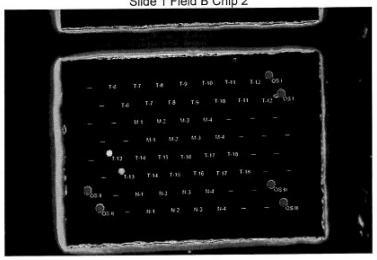

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Reimbursement for PCR testing

87481-1 87798-13

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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.

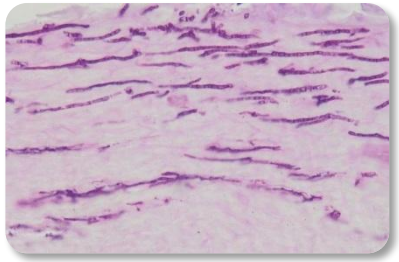
Classical Dermatophyte Diagnostics

Culture

- Species identification possible
- **Diagnosis within <u>2 - 6 weeks</u>**
- Sensitivity only 50 - 80 %
- Very experienced staff required / subjective analysis
- False-negative results possible, since some fungal species don‘t grow

Native microscopy

- Diagnosis <1h
- **<u>No</u> species identification possible**
- Subjective analysis
- False-positive results possible due to contamination/artifacts
- False-negative results possible, not each sample contains fungi particles



Histology

- Higher sensitivity than culture/native preparation
- Diagnosis within <u>some days</u>
- **<u>No</u> species identification possible**

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NEW: EUROArray Dermatomycosis

Test principle:

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

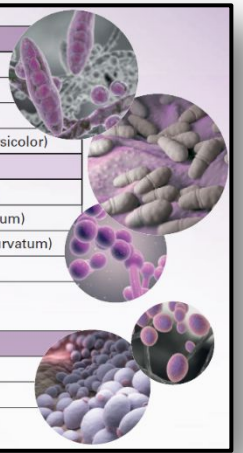
Detection range:

- **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 (Hoog et al, Mycopathologia: 2017 Feb; 182(1-2):5-31)

Yeast/Moulds		
C. parapsilosis	C. guilliermondii	F. oxysporum
C. albicans	F. solani	Sc. brevicaulis



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NEW: EUROArray Dermatomycosis

Advantages:

Quick results:

- < 24 h for results
- Early pathogen-specific treatment possible due to species identification
- Quick identification of human and animal carriers (avoiding epidemic spread)

Sensitivity: **> 98 %**
Time: **3 - 4 h**

Higher sensitivity:

- Species identification also possible after therapy start (no vital cells necessary)
- Less false-negative results at multiple infections
- Less false-negative results for nail material
- Identification of multiple infections possible

Standardized and easy workflow and evaluation:

- Fully automated data evaluation and archiving of the results
- No highly experienced staff necessary

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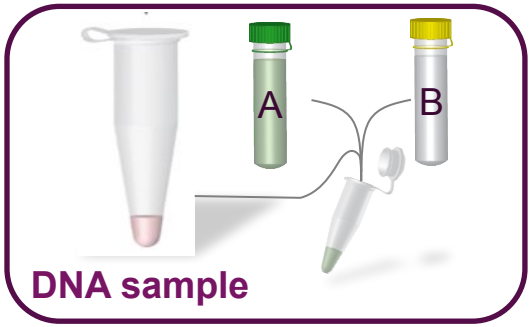
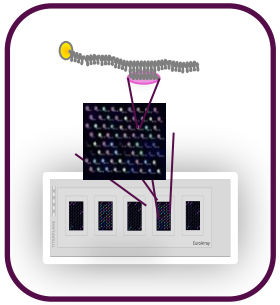
EUROArray Dermatomycosis - Workflow

DNA isolation & PCR setup

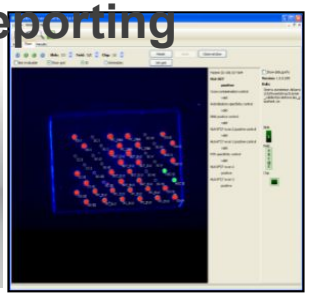


Ready to use PCR Master Mix solutions A & B



Hybridization

Result reporting



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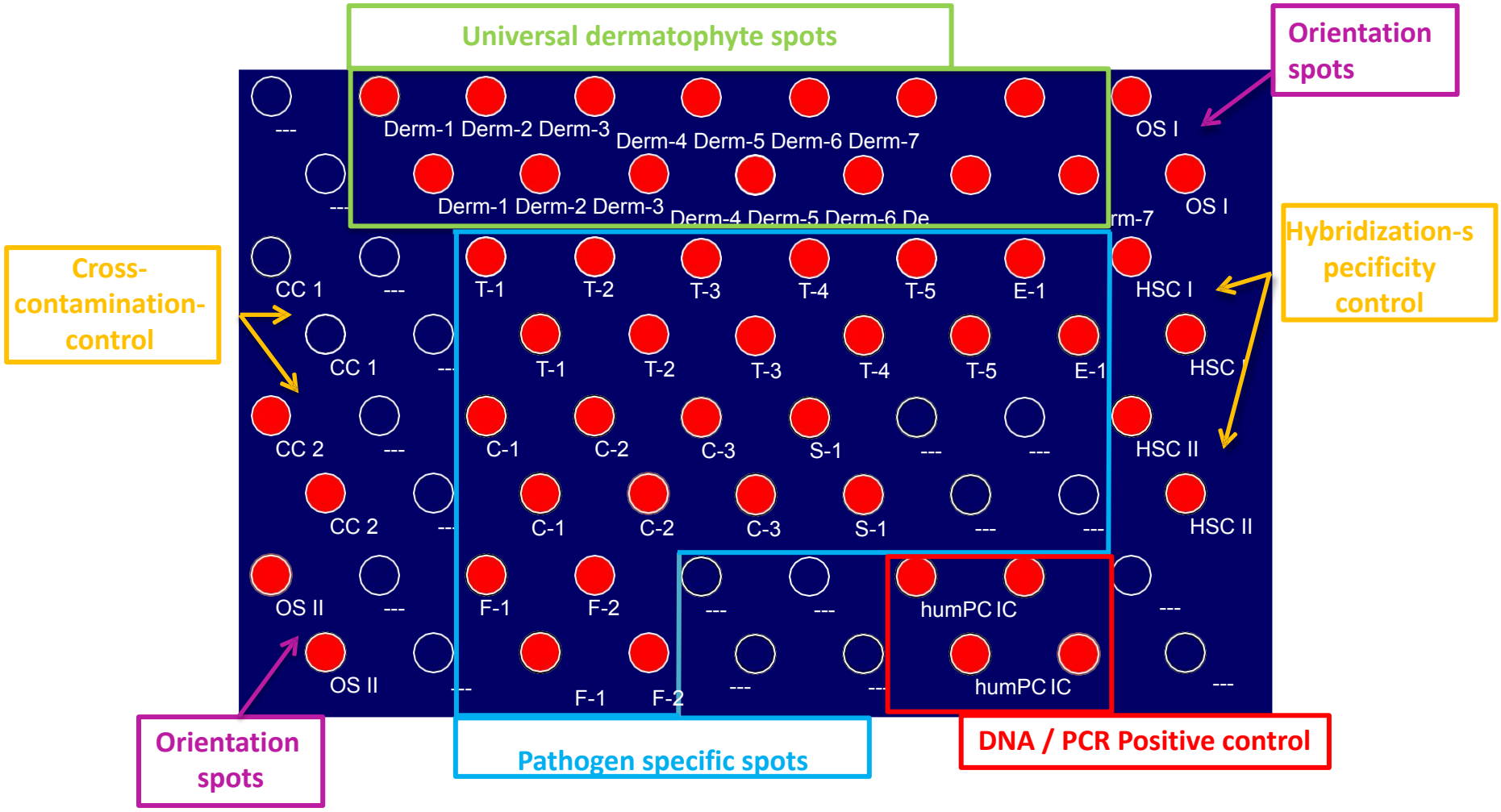
EUROArray Dermatomycosis

BIOCHIP 1



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EUROArray Dermatomycosis – Report

Patient ID :	1	**Test :**	Dermatomycosis
Result from :	31.08.2017	**Protocol :**	Demo EUROArray Dermatomycosis
Print date :	14.03.2018 07:42:46	**Scan date :**	29.08.2017 14:40:50
Patient name :	1	**Page :**	1

EUROIMMUN Medizinische Labordiagnostika AG

Automatic evaluation with the EUROArrayScan software

Partial result	Result
Cross contamination control	valid
Internal Control	valid
DNA positive control	DETECTED
Hybridisation specificity control	valid
Dermatophyt (universell)	DETECTED
Trichophyton equinum	not detected
Trichophyton tonsurans	not detected
T. interdigitale/mentagrophytes	not detected
Trichophyton interdigitiale	not detected
Trichophyton mentagrophytes	not detected
T. quinckeanum/schoenleinii/simii	not detected
Trichophyton quinckeanum	not detected
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Trichophyton benhamiae(yellow)	not detected
Trichophyton concentricum	not detected
T. bullosum/T. benhamiae(afr.)	not detected
Trichophyton erinacei	not detected
T. verrucosum/eriotrephon	not detected
Trichophyton rubrum	DETECTED
Trichophyton violaceum	not detected
Epidermophyton floccosum	not detected
Nannizzia fulva	not detected
Nannizzia gypsea	not detected
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Candida guilliermondii	not detected
Candida albicans	not detected
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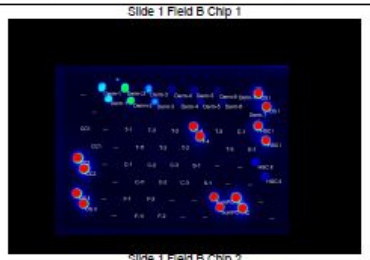
Fusarium oxysporum	not detected
Scopulariopsis brevicaulis	not detected

Slide 1 Field B Chip 1



Slide 1 Field B Chip 2

Patient ID : 1 **Protocol :** Demo EUROArray Dermatomycosis

Page : 2

Test result	Result
Dermatophyte	Trichophyton rubrum
Yeast/Mold	not detected

Signature : _____

The EUROArray system provides an automated evaluation report

Reports may be printed with or without EUROArray images

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EUROArray Dermatomycosis

Advantages:

Fast

Much faster identification of fungal species than in culture:
Less than 20 h from sample to result instead of 2-5 weeks

Simple

In contrast to classical methods no expert needed:
Easy to perform procedure and automated evaluation

Reliable

Higher sensitivity (>98 %) than with culture:
Reliable detection after therapy start & of multiple infections

Specific

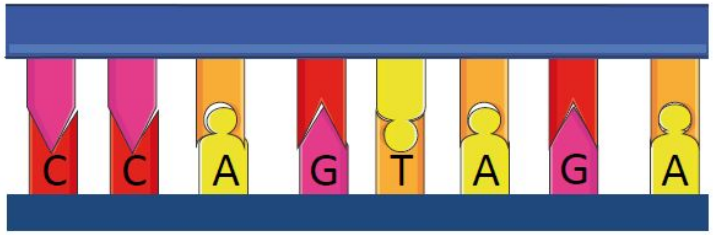
Precise identification of closely related species:
No reliable differentiation with culture or other MDx-tests

Extensive

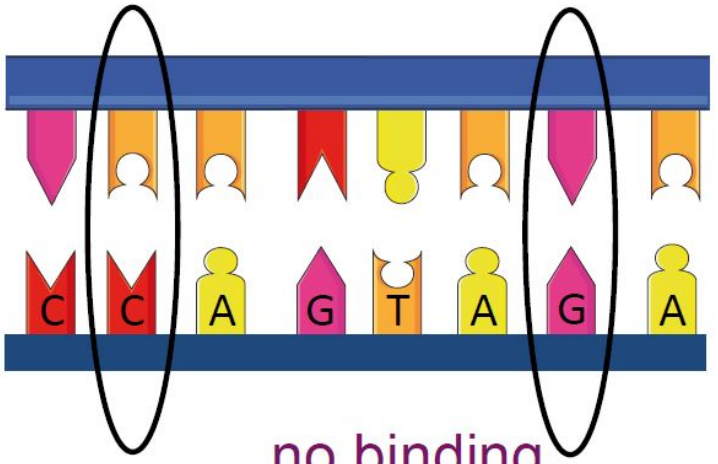
Worldwide broadest PCR-based test for dermatophytes:

Detection of PCR products by hybridization

- EUROArray spots: short single stranded DNA probes
- Bases pair highly specific by the formation of hydrogen bonds (AT&GC)
- In case of a perfect matching of probe and sample DNA, a strong binding is achieved
- Already one mismatch will either reduce the pairing strength of two DNA molecules or prevent it completely

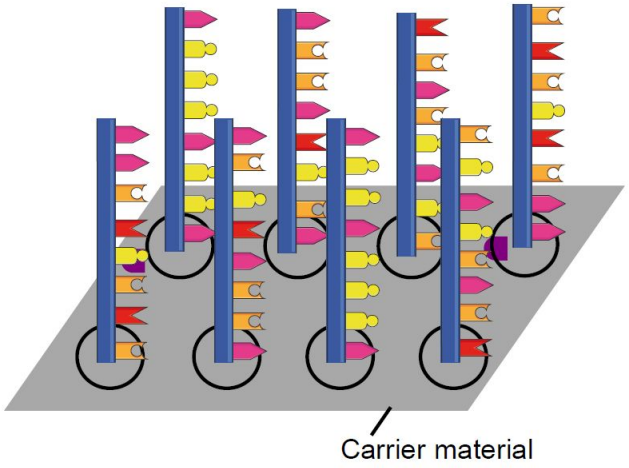


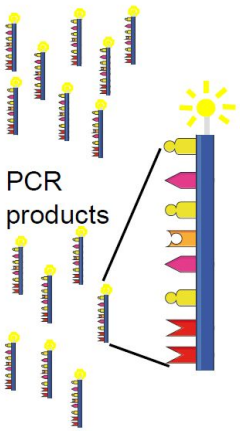
stable binding

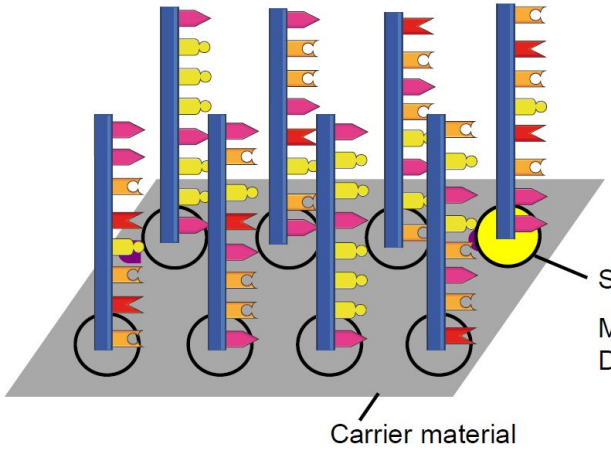


no binding

Detection of PCR products on an array







EUROArray dermatomycosis results from software

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

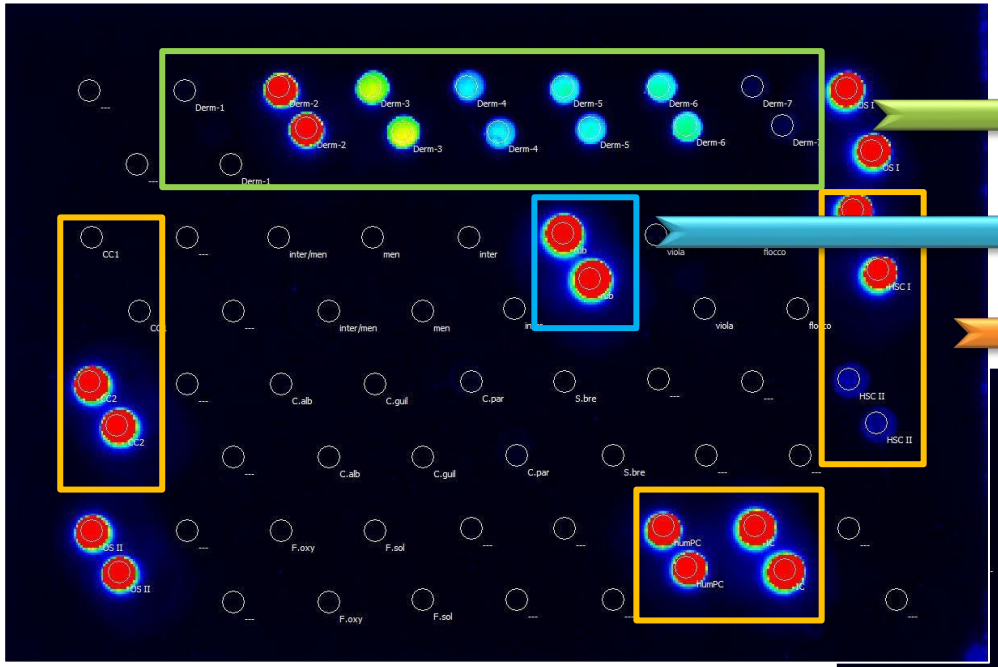
7	**A**	**1**	**30**	**Negative control**	**Test result**	**valid**
7	A	1	30	Cross contamination control	Partial result	valid
7	A	1	30	Internal Control	Partial result	valid
7	A	1	30	Hybridisation specificity control	Partial result	valid

****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”**

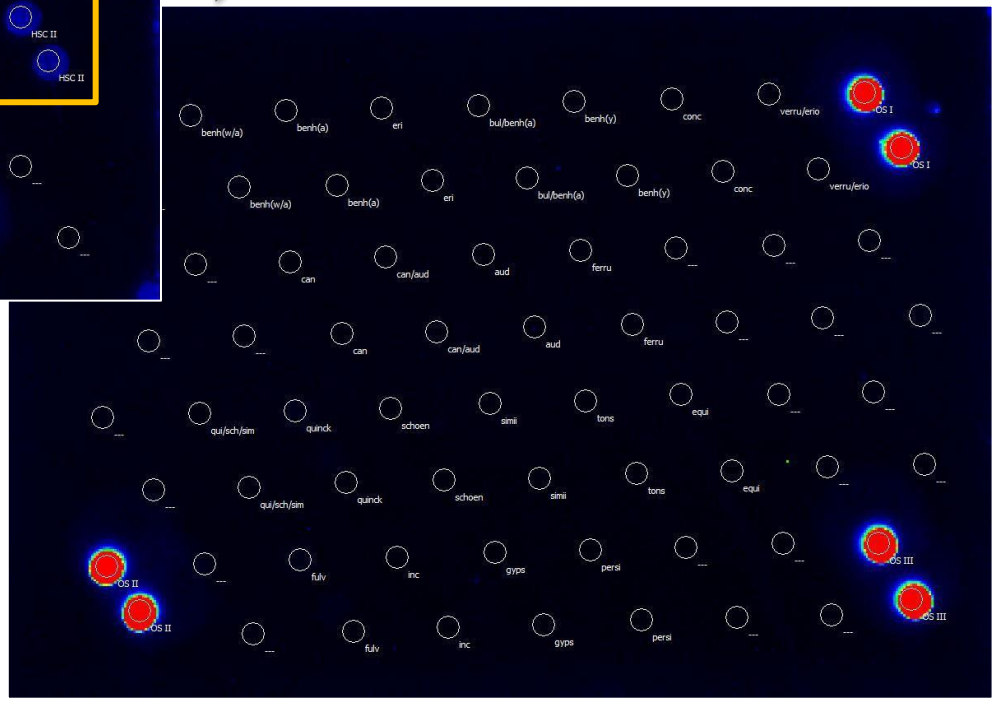
Positive Result for *Trichophyton rubrum*



Dermatophyte "detected"

***Trichophytum rubrum* "detected"**

Controls "valid/detected"



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Thanks!

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SKIN, HAIR AND NAIL EXPERTS