Beyond the Microscope: Modern Diagnostics and AI for Identifying Onychomycosis

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ToeFX
show off your toenails

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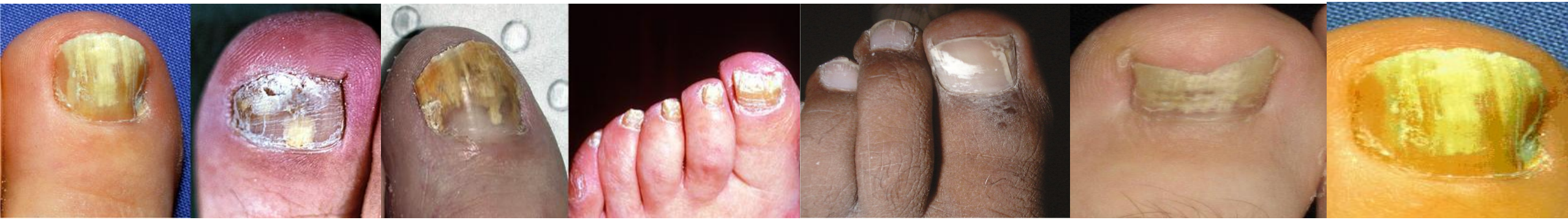
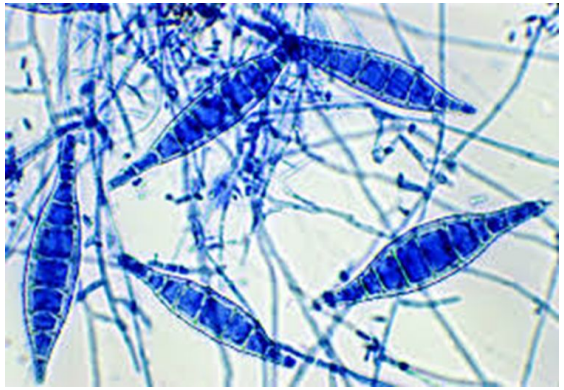
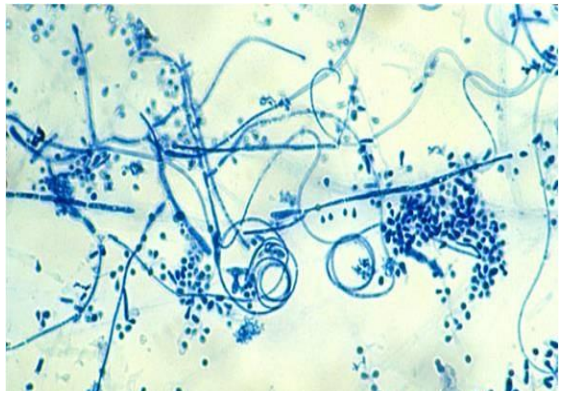
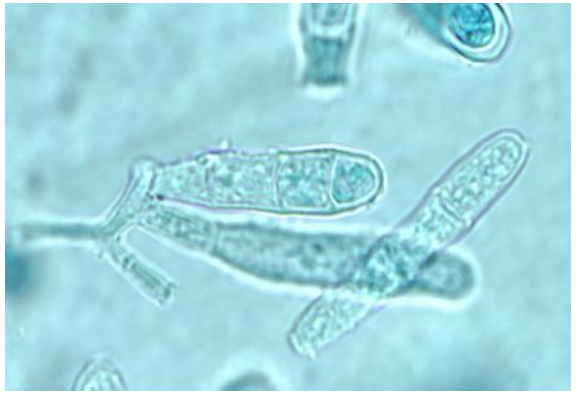
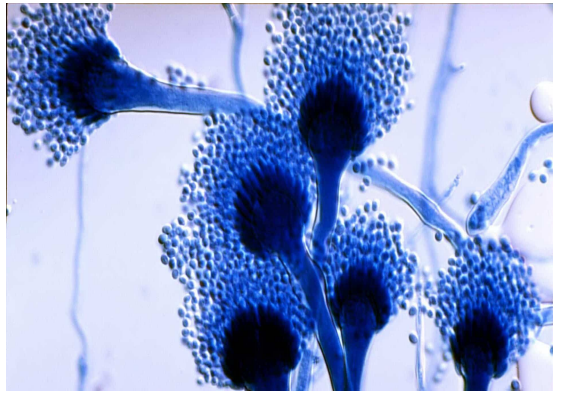
Onychomycosis



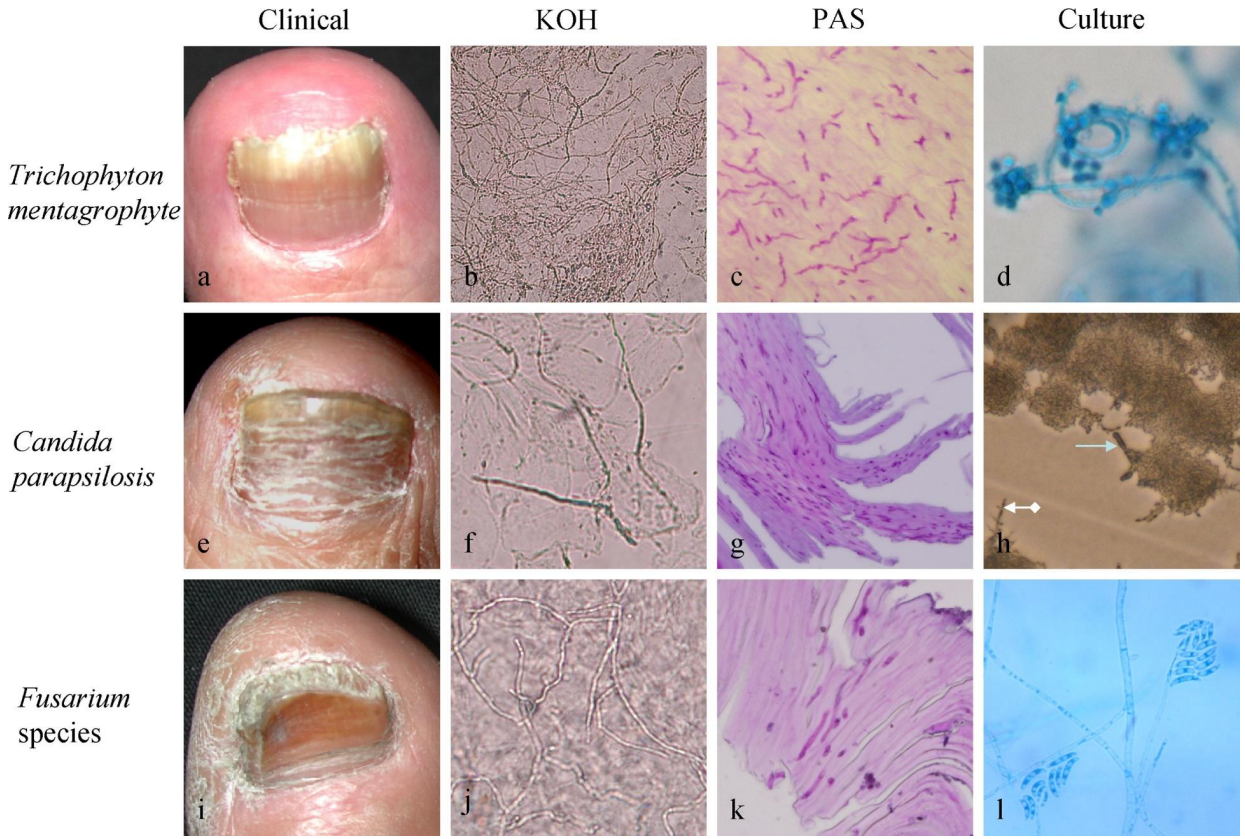
- Onychomycosis (OM) is a fungal infection of the fingernails or toenails that causes discoloration, thickening, and separation from the nail bed.
- Psychosocial and emotional effects resulting from onychomycosis are widespread and may have a significant impact on quality of life.
- OM represents a difficult-to-treat form of superficial fungal infections
- OM prevalence increases with age and is estimated at 10–15% in the world population

Agents: dermatophytes, yeasts, and saprophytic mold

- The dermatophytes are identified in 90% of the toenail and 50% of fingernail onychomycosis.
- Yeasts, particularly Candida Spp. cases account for 2% of onychomycosis, especially in fingernails.
- Nondermatophytic mold onychomycosis is cultured primarily from toenails including Fusarium, Aspergillus, Acremonium, Scytalidium, and Scopulariopsis
- Approximately 10% of cases of onychomycosis worldwide are thought to be caused by NDM.

Onychomycosis Diagnosis



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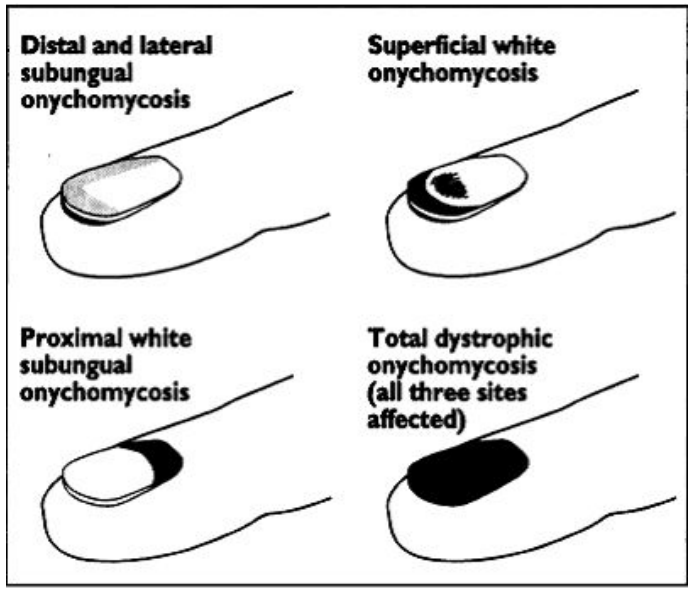
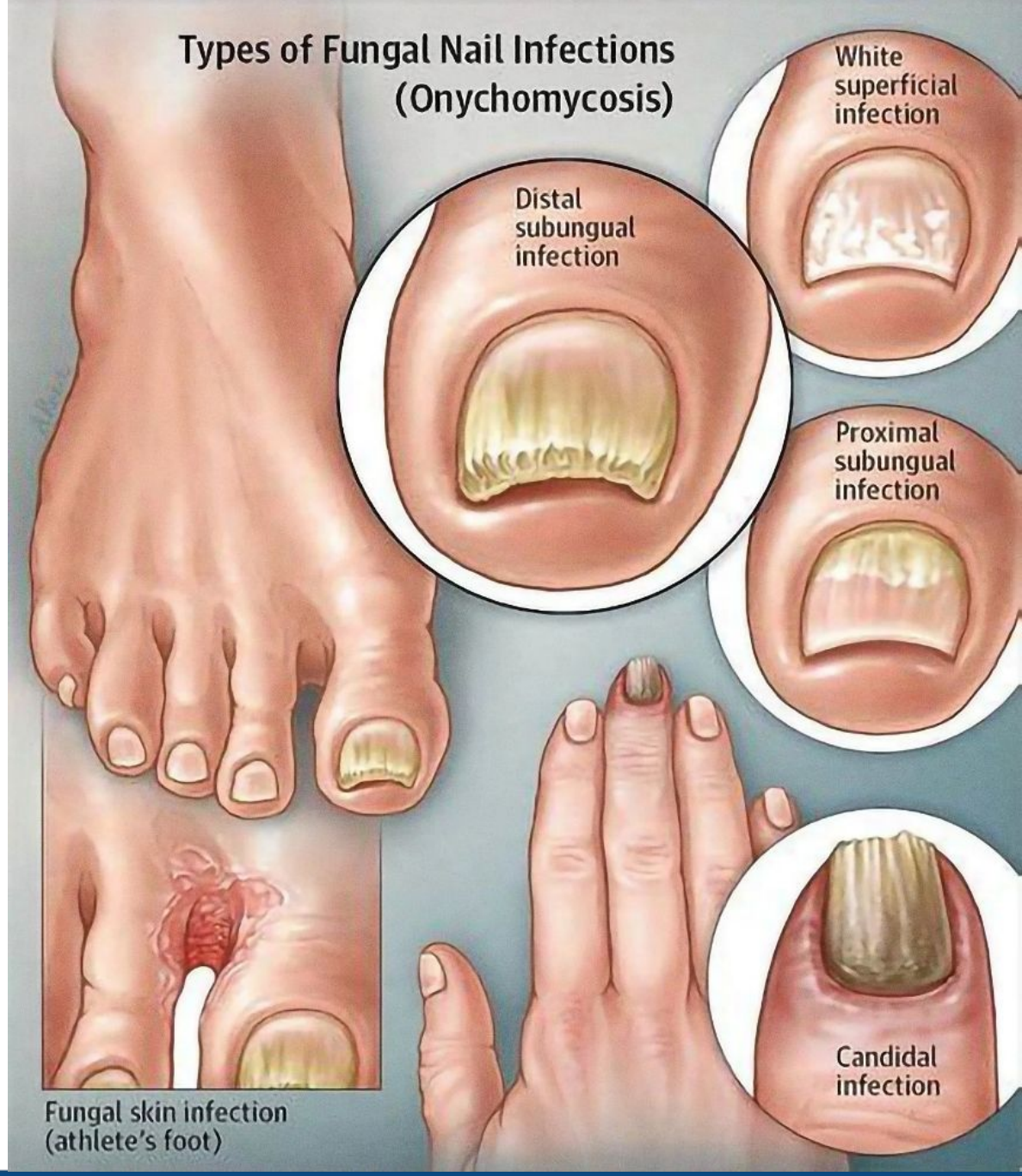
While culture is considered a standard diagnostic tool, it has several limitations, including its sensitivity, specificity, and length.

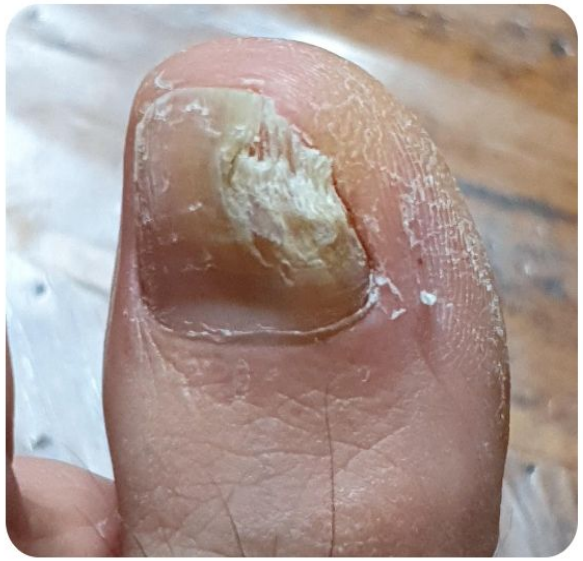
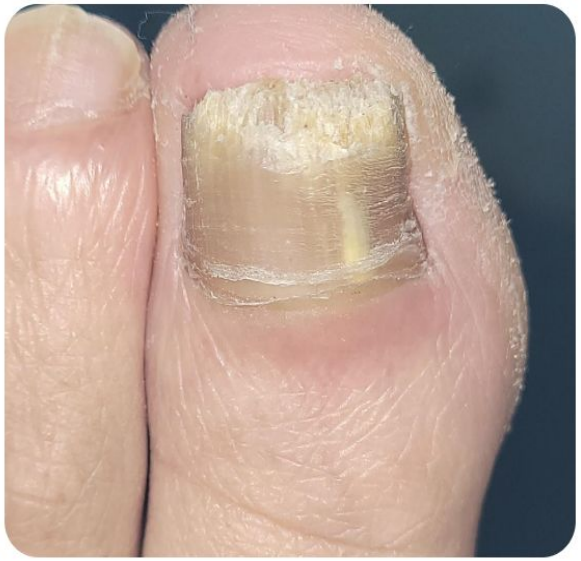
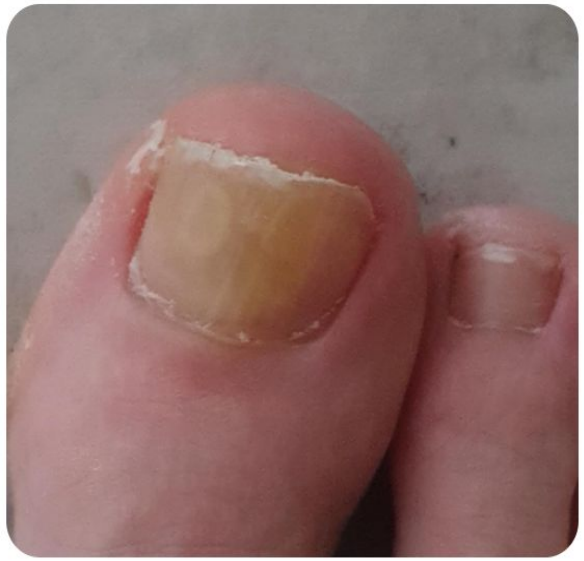
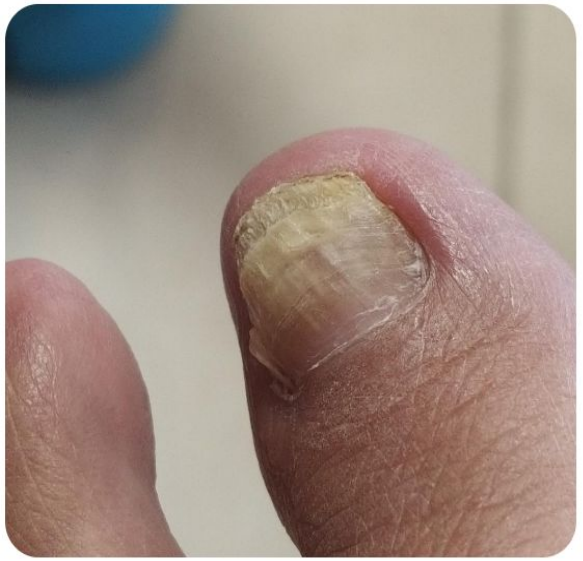
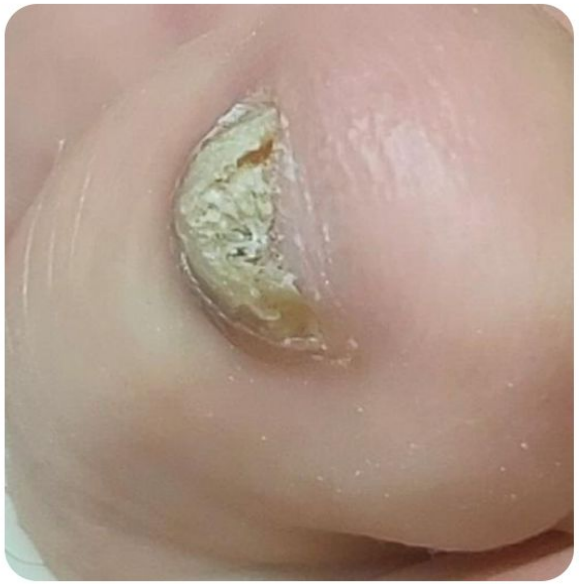
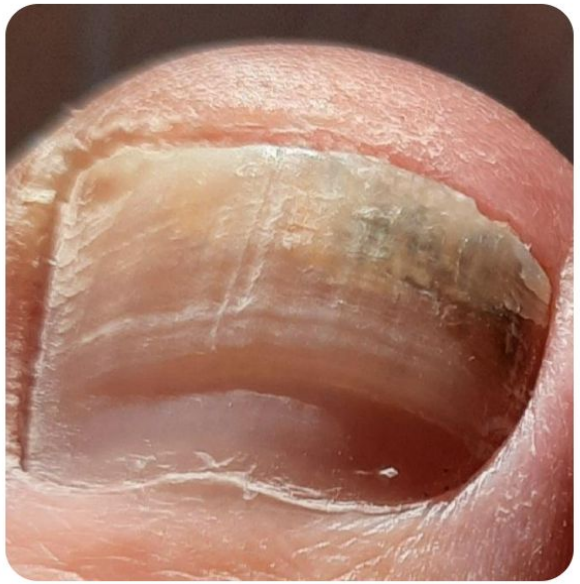
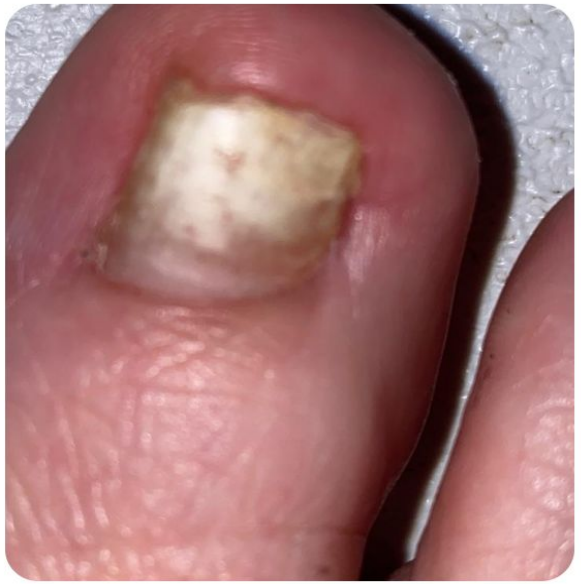
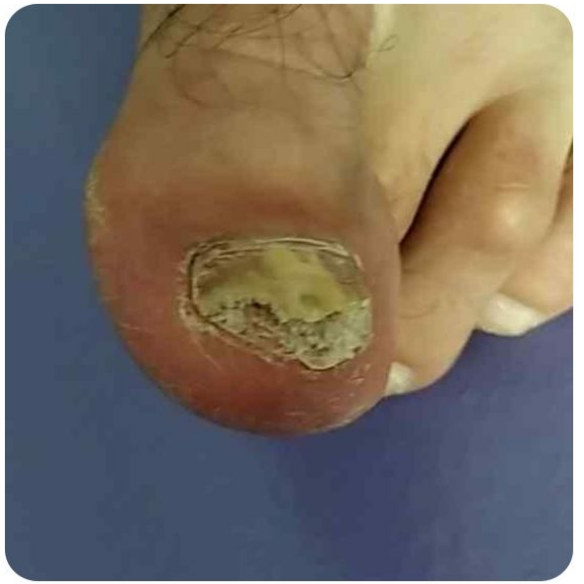
OM has four main clinical types

- Distal subungual onychomycosis (DSO)
- Proximal subungual onychomycosis (PSO)
- White superficial onychomycosis (WSO)
- Total dystrophic onychomycosis (TDO)



Patients may have a combination of these subtypes.



Differentiate onychomycosis by other similar nail pathologies, including:

Although these images might easily be mistaken for toenail fungus, each one illustrates a different, non-fungal condition — highlighting the need for quick, precise diagnosis.

Accurate diagnosis is crucial for successful treatment and requires identification of physical changes and positive laboratory analysis. Only 50% of nail problems are caused by onychomycosis.

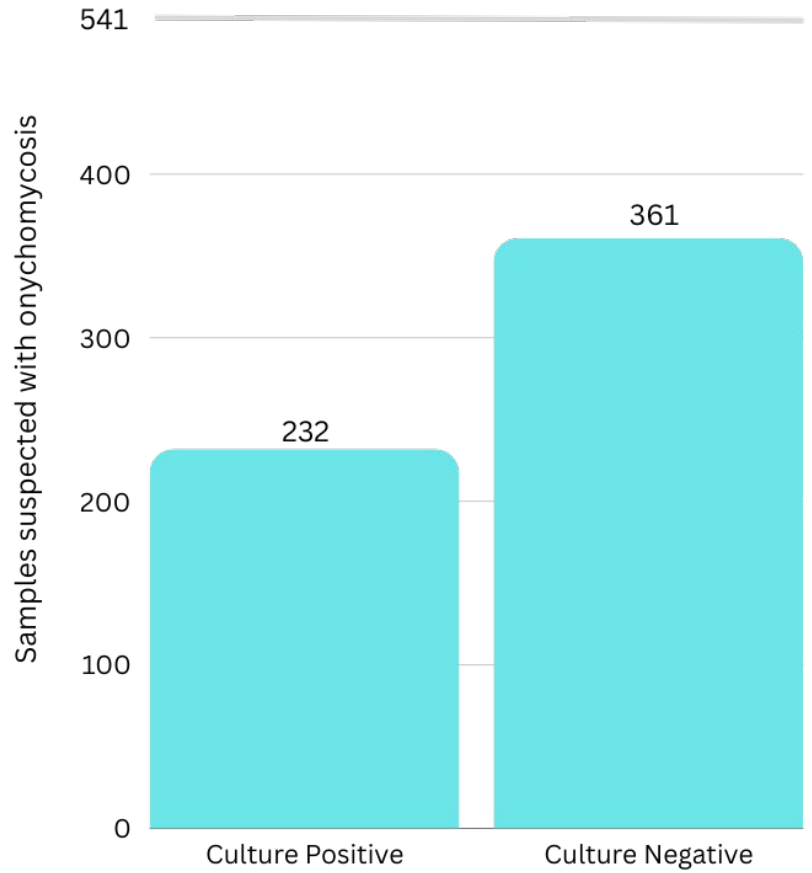
It is essential to know that almost half of the abnormal-appearing toenails are not mycotic, so mycological testing is vital to establish an accurate diagnosis and is especially important if systemic therapy is planned.

Why did we start doing this?

- ✔ 48/50 samples with a clinical assessment of onychomycosis by Dr. Rabinovich, foot and ankle specialist who sees fungus because he does nail avulsions, came back negative.
- ✔ Patient recruitment was very difficult.
- ✔ We worked with the LifeLabs team.

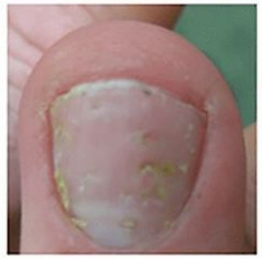
And the winner is…

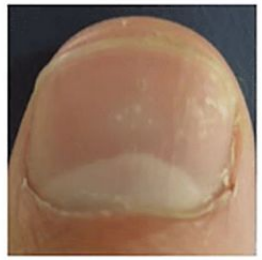
- ✔ All samples were collected in Hamilton, Ontario
- ✔ 593 samples with ‘suspected onychomycosis’ tested
- ✔ 361 were found culture negative
- ✔ 232 were culture positive



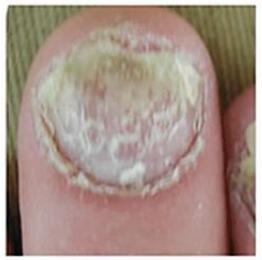
The main alternative to culture: NOTHING

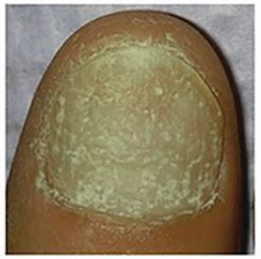
- ✔ Very much depends on the clinician
- ✔ All of these have some resemblance to fungus. None of them are fungus.

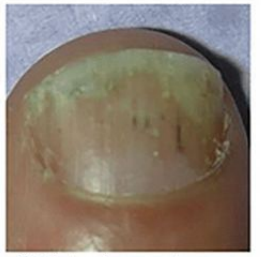

Pitting, leukonychia

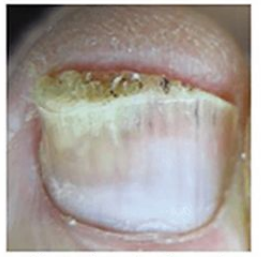

Leukonychia

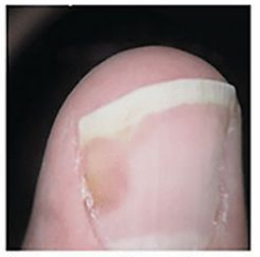

Red macules in lunula

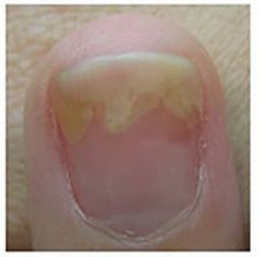

Crumbling


Trachyonychia


Splinter hemorrhages and onycholysis

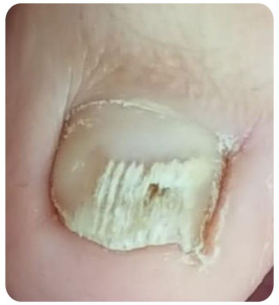
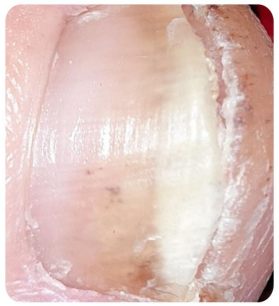
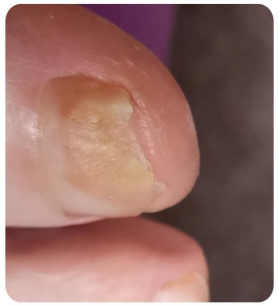
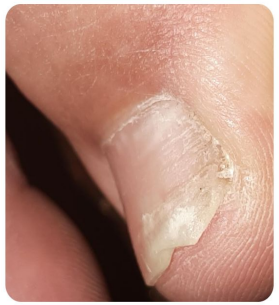

Hyperkeratosis and splinter hemorrhages


Salmon patch or oil spot dyschromia


Onycholysis and salmon patch dyschromia

Summary of diagnostic techniques

	Sensitivity	Specificity	Sensitivity	Disadvantages
KOH testing	61% (44–100%)	95% (75–100%)		It's an art! Cannot identify pathogen subtype
	Inexpensive, simple Quick results			
Fungal culture	56% (29–82%)	99% (83–100%)		
	Can identify pathogen subtype Delay in results (up to 1 month)			Low sensitivity
Histopathology	84% (61–93%)	89% (44–100%)		Expensive
	Most sensitive conventional test			Cannot specify pathogen subtypes
Nail dermoscopy			Bedside tool, non-invasive	Cannot demonstrate presence of
			fungi	

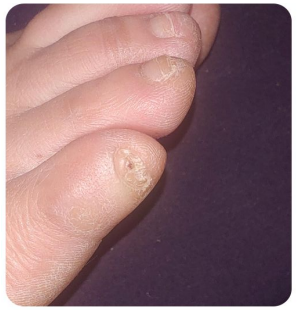
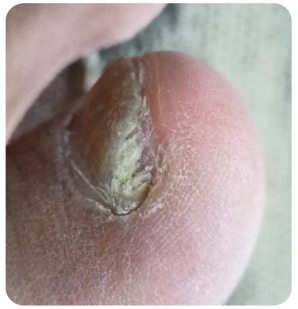
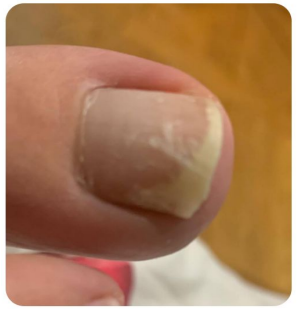
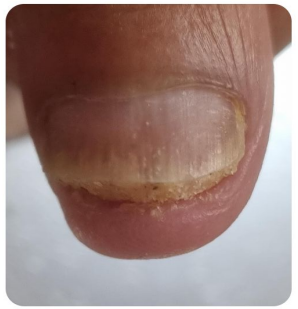
Benefits of Early Diagnosis of OM

Antifungal selection and effectiveness

Make informed decisions and plans

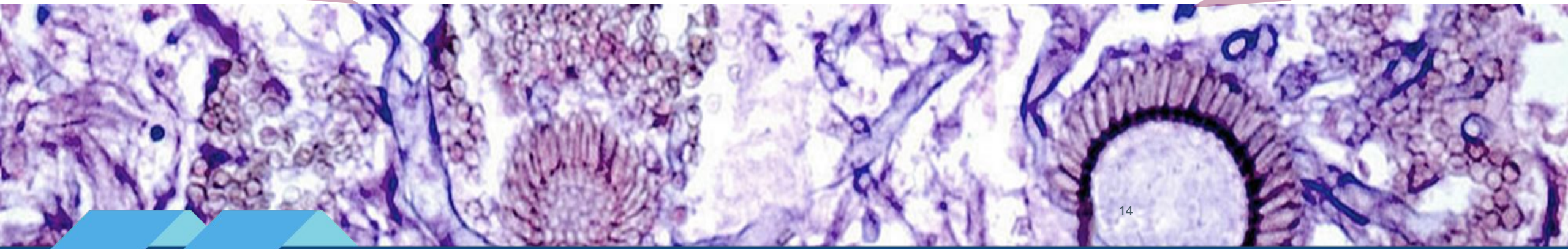
Improved Infection Control

Reducing healthcare costs

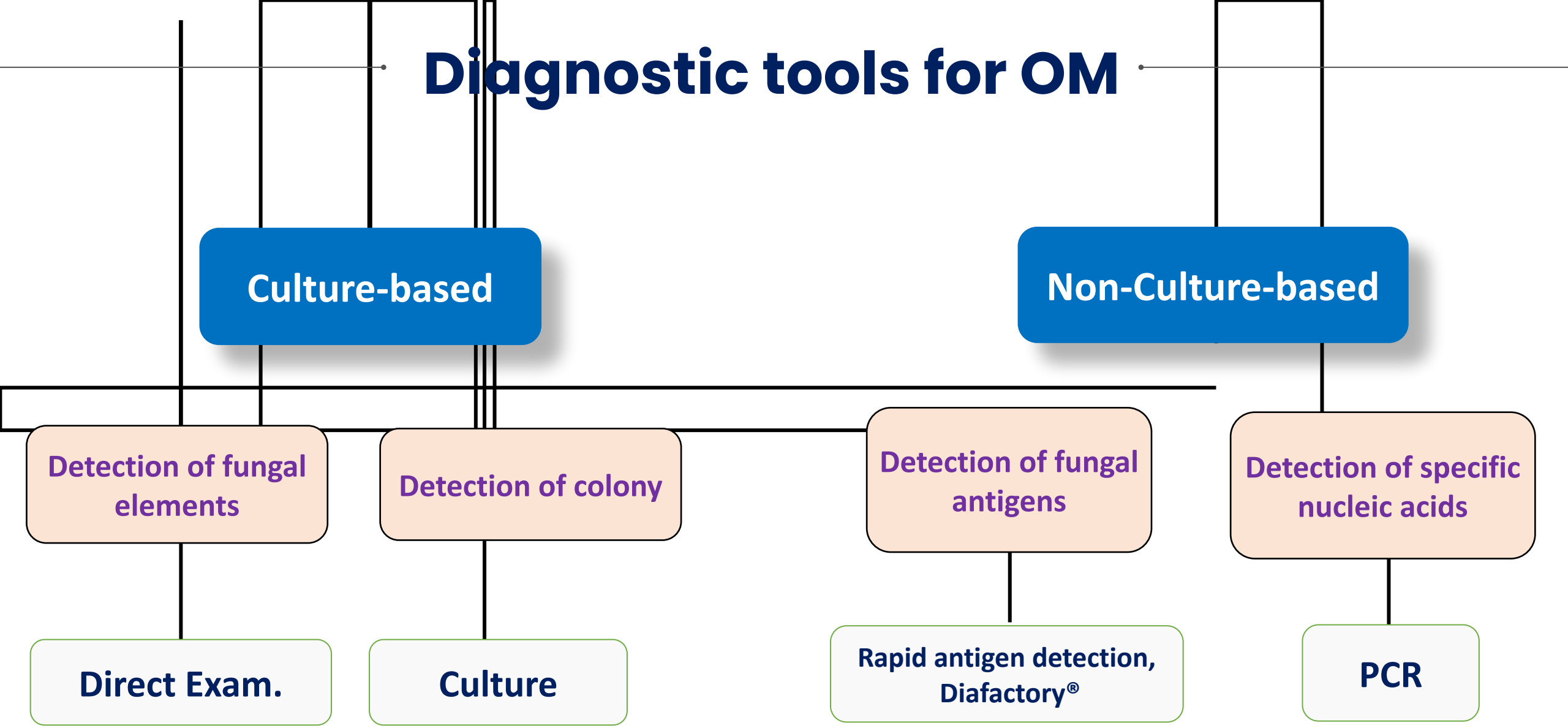





OM management complicating in most of countries

- Low prioritization of fungal infection by healthcare organizations and health ministries
- Some tests are expensive, most are not
- Lack of standardization and validation of tests in different countries
- A lack of trained personnel to undertake the tests
- Lack of target antifungal medications
- Lack of clinical and laboratory relations



Diagnostic tools for OM



Accuracy of Diagnostic Testing for OM

Test	Pretest probability	Sensitivity*	Specificity	LR+	LR–	PV+	PV–
Potassium hydroxide preparation	62%	55.9% to 80% (80%)	95%	9.6	0.4	96.3%	55.9%
Fungal culture	56%	23% to 84.6% (56%)	99%	17.3	0.4	99.4%	52%
Biopsy plus periodic acid–Schiff stain	65%	81% to 91.6% (84%)	89%	7.2	0.2	93.4%	75.4%
Polymerase chain reaction	32%	83%	84%	5.2	0.2	71%	91%

LR– = negative likelihood ratio; LR+ = positive likelihood ratio; PV– = negative predictive value; PV+ = positive predictive value.
*—Values in parentheses were used to calculate likelihood ratios.

Although the combination of culture and direct examination has been the gold standard in recent years, its false-negative rate ranges from 15% up to 80%.

www.aafp.org/afp

100 years of culture

Culture Medium:
Sabouraud Dextrose Agar (SDA) or Dermatophyte Test Medium (DTM)

** SDA with chloramphenicol (an antibiotic) and cycloheximide (an antifungal selective for dermatophytes) can prevent contamination from bacteria and non-dermatophyte fungi.

Inoculation Technique:

- Place the nail clipping or scraped material onto the surface of the agar.
- Ensure that the sample is in close contact with the medium for optimal fungal growth.
- Gently press down without embedding the material deeply.

Incubation:

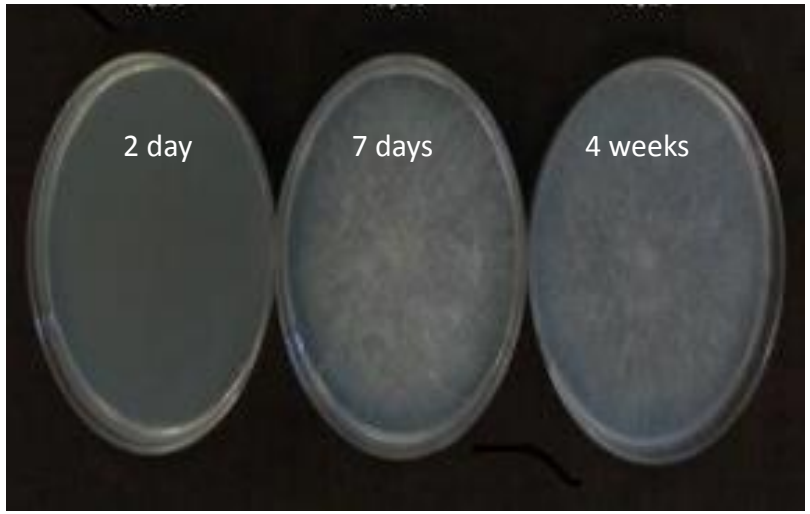
- **Temperature**: Incubate the culture at 25-30°C, which is optimal for *T. rubrum* growth.
- **Duration**: Allow 1–3 weeks for visible growth, as *T. rubrum* is slow-growing. Check the plate periodically to observe colony morphology.

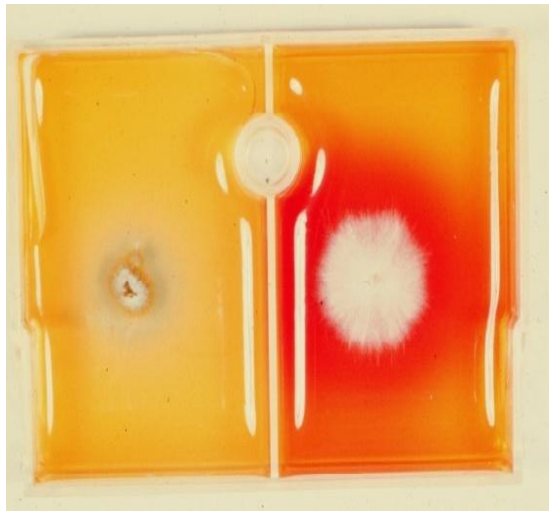
Observation and Identification:

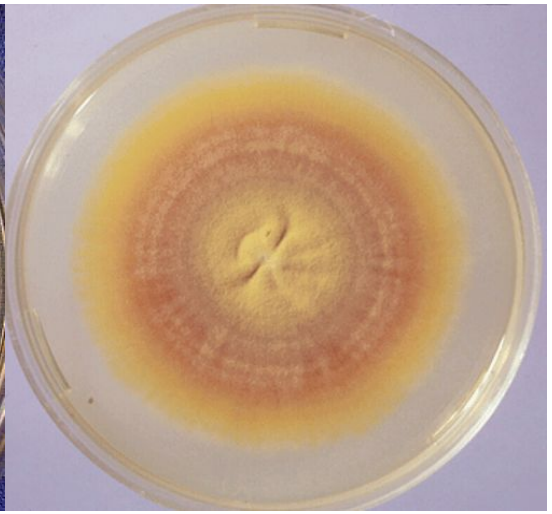
- After colonies appear, examine their morphology. Colonies of *T. rubrum* are usually white to cream on the surface, with a red or wine-colored reverse.

Culture

- Fungal species identification may take many days (results in 3 to 4 weeks) .
- Some species fail to sporulate on routine media.
- Requiring repeated testing to achieve acceptable sensitivity.
- Phenotypic characters are unstable and can change with environmental changes.





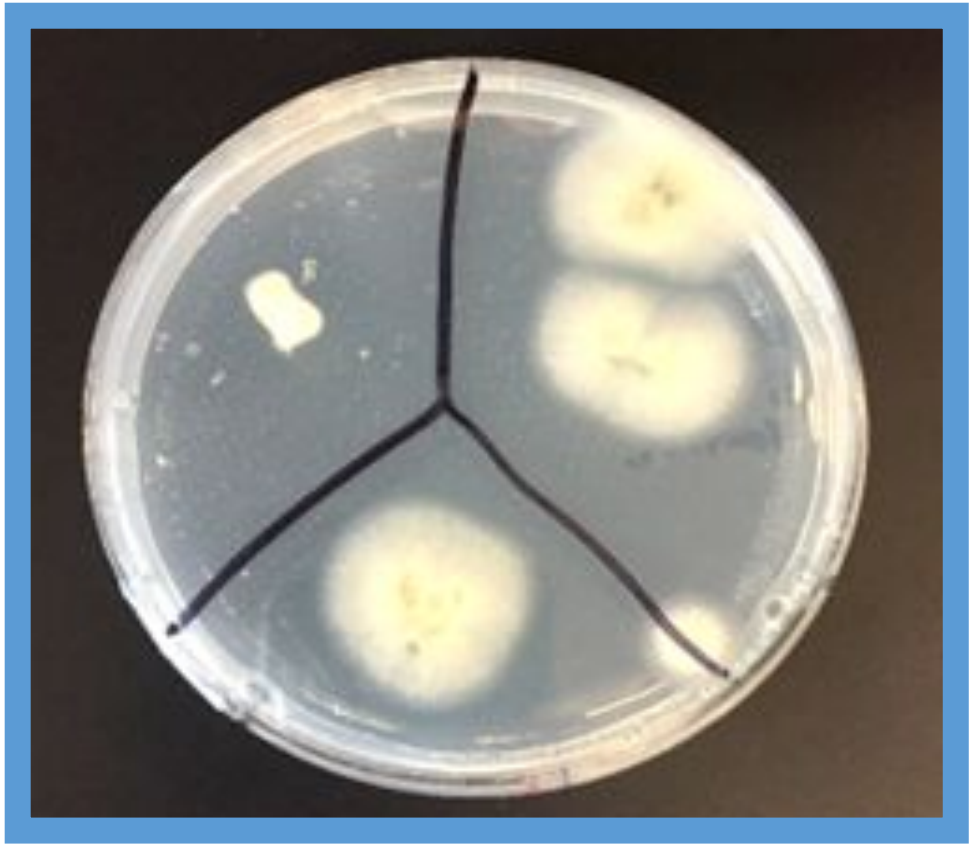


Commercial lab process

Step 1: Digest the nail in KOH, search under an optical microscope for mycelia.

Step 2: Culture a fungal colony.

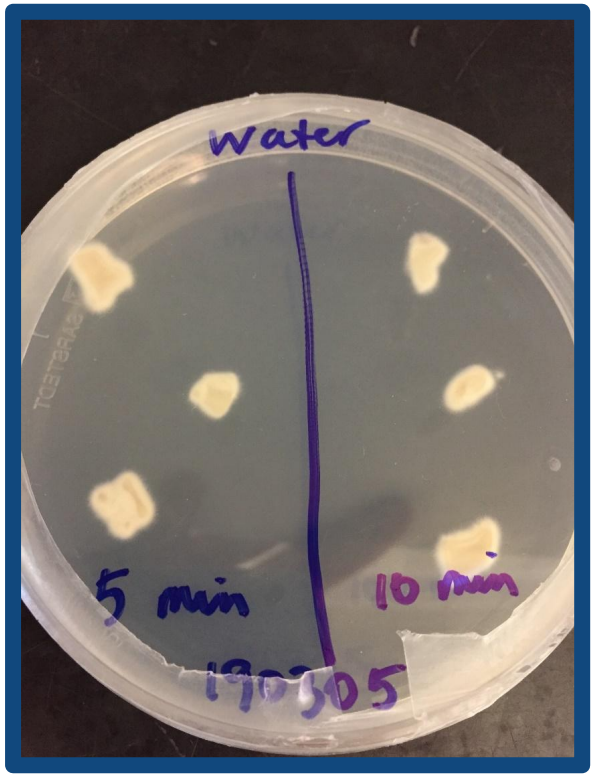
Step 3: Wait a minimum of 3 weeks.



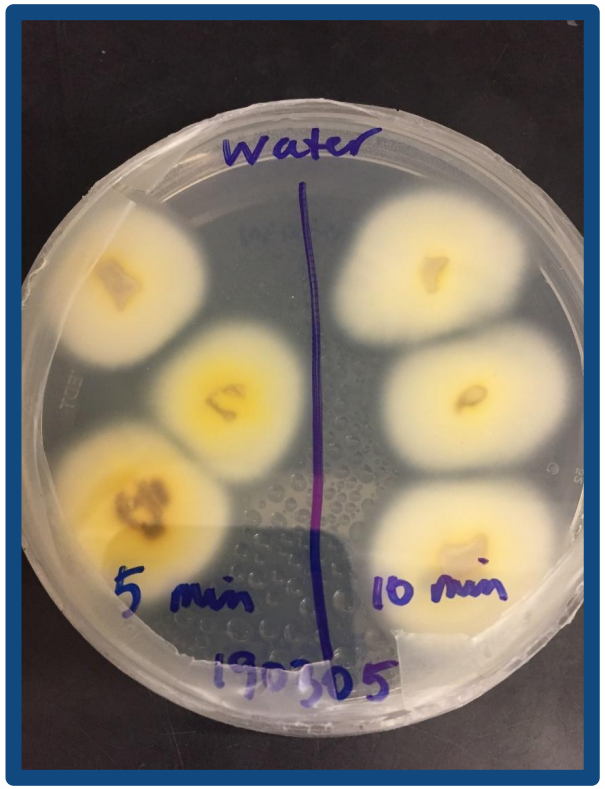
Trichophyton rubrum
(T. rubrum) grown by ToeFX

Slow

Day 2



Day 7-14



Low Sensitivity

The sensitivity of culture for diagnosing onychomycosis can be relatively low, with some studies reporting sensitivity rates between 30% and 50%. This low sensitivity is influenced by several factors:

Fungal Viability: The success of a culture depends on the viability of the fungal organisms present in the nail sample. If the sample is improperly collected or stored, the fungi may be non-viable, leading to a false-negative result.

Fungal Burden: In early or mild cases of onychomycosis, the fungal burden may be low, meaning that the number of fungal cells present in the sample may be insufficient to grow in culture. This can make detection difficult in less severe infections.

Sampling Errors

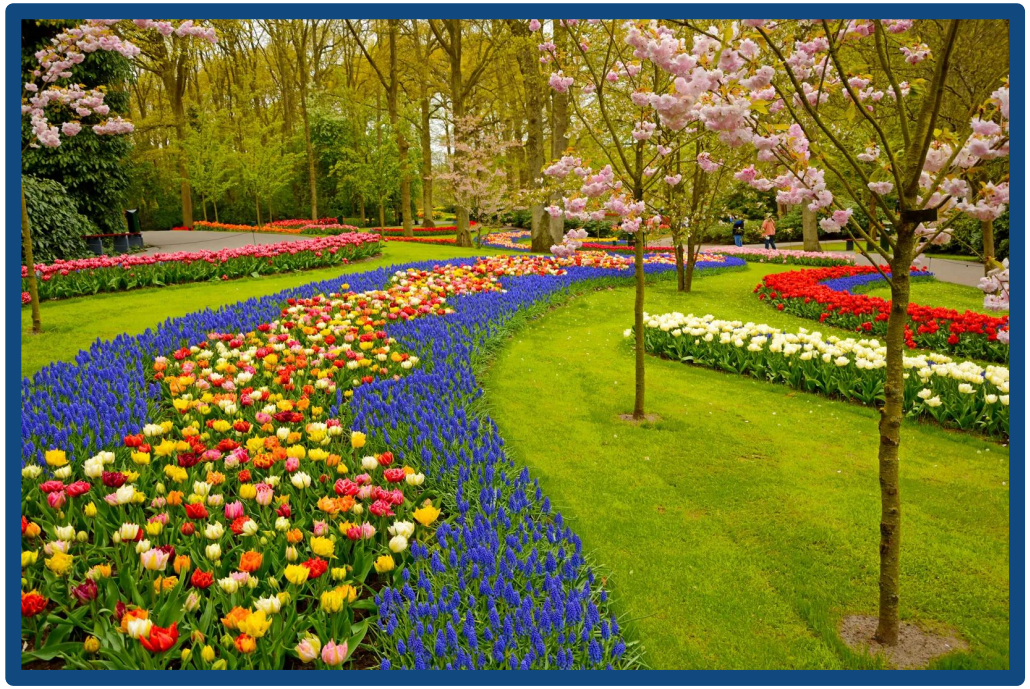
Onychomycosis affects the deeper layers of the nail, but superficial nail clippings or samples that do not contain infected tissue from the nail bed may result in negative cultures, even when the patient has an active fungal infection.

Antifungal Treatment:
Patients who have used topical or systemic antifungal agents prior to sample collection may have a reduced fungal load in the nails, leading to false-negative culture results. Even residual antifungal agents in the sample can inhibit fungal growth in the culture medium.

Anecdote: in 2022

We started seeing an overwhelming number of

- Rhizoporus
- **Overgrowth of Contaminants:** Cultures are susceptible to contamination by environmental fungi and bacteria. Overgrowth of these contaminants can obscure the growth of the pathogenic fungi, leading to inaccurate results.
- We called up Jianping Xu, a world-renowned fungus expert at McMaster University and he laughed. He said these are garden-variety fungi, ie literally these are common fungi found in a garden
- Either the technician had been gardening, or someone had left a window open in the laboratory, and these species were dominating
- It's POSSIBLE that these were real results, but I should say that the results merited further investigation, very very atypical species



Direct Microscopy

Fast Technique

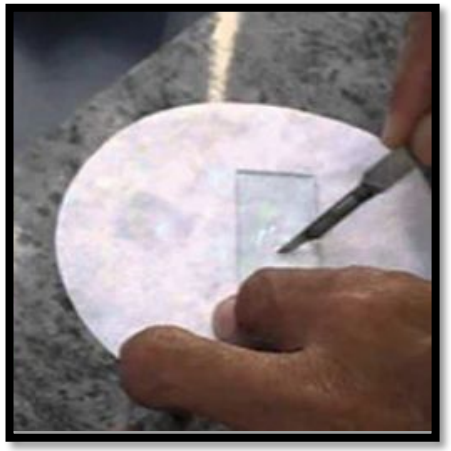
Inexpensive

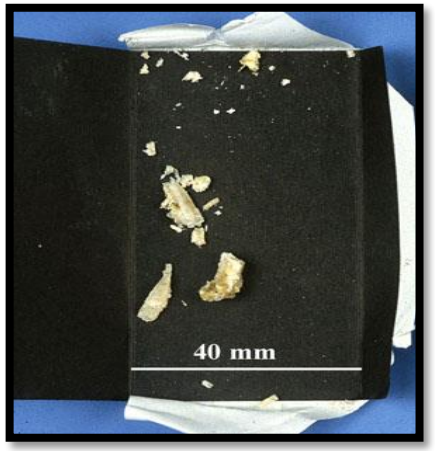
Highly variable sensitivity, depending on the observer's experience

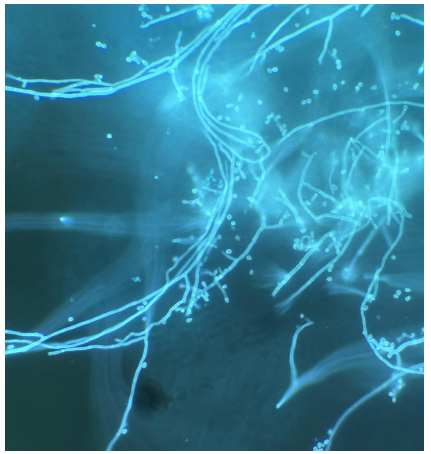
It can furthermore reveal co-infections dermatophytes or yeasts with other molds.

It may be confusing in case of atypical hyphae or co-infection.

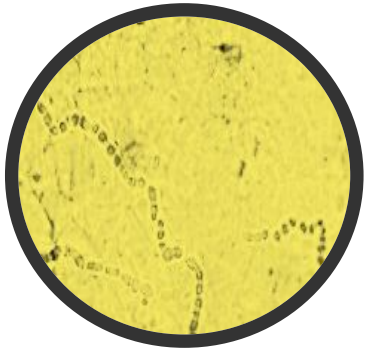
These methods, however, are not able to identify a fungus to the genus or species level.



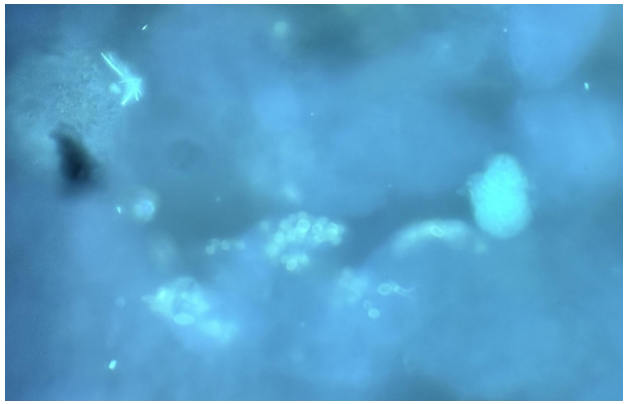




T. rubrum hyphae and Spores



KOH mount



Yeast and budding cells

PCR Testing for OM

Providing targeted treatment for onychomycosis starts with identifying the genus and species.

Exact

Determine the genus and species of the infectious organism to provide the safest, most effective oral and topical treatments

Accurate

Assess patient results with high confidence
99.9% analytical specificity, 86% clinical sensitivity[1]
Correlates highly with histopathology

Cost-effective

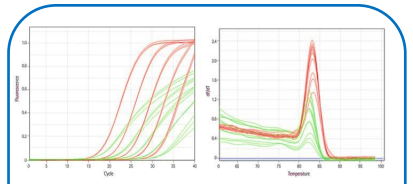
Eliminate unnecessary additional testing, focus on the relevant agents of disease, and prevent treatment course changes

Determining the exact infectious agents to treat leads to improved patient outcomes.

Benefits of Nail PCR Testing

1. High Sensitivity and Specificity
2. Rapid Turnaround Time
3. Broad Pathogen Detection
4. Identification of Difficult-to-Culture Pathogens
5. Differentiation Between Pathogens with Similar Symptoms
6. Quantification of Pathogen Load
7. Improved Infection Control

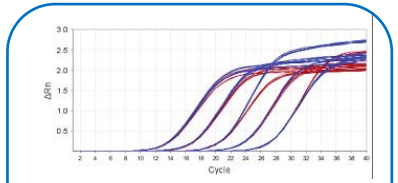
Innovation in the PCR Testing Workflow



Pan-fungal Real time PCR Testing

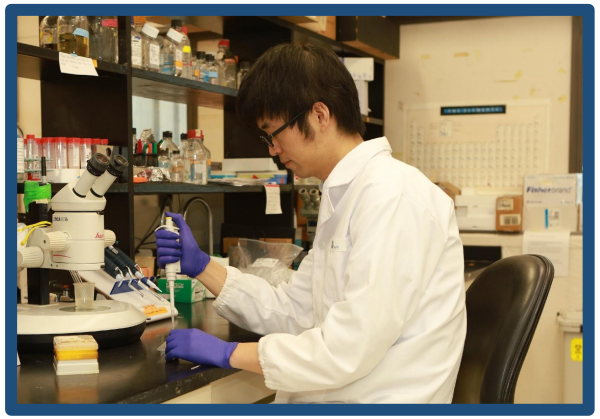
\+
- **Dermatophytes**
  - *T. rubrum*
  - *T. mentagrophytes*
  - *Microsporum spp*
  - *Epidermophyton spp*
- **Candida**
  - *Candida albicans*
  - *Candida parapsilosis*
  - *Candida guilliermondii*
  - *Candida tropicalis*
- **Aspergillus and Other Molds**
  - *Aspergillus spp*
  - *Acremonium spp*
  - *Fusarium spp*
  - *Scytalidium spp*
  - *Scopulariopsis spp*

\-

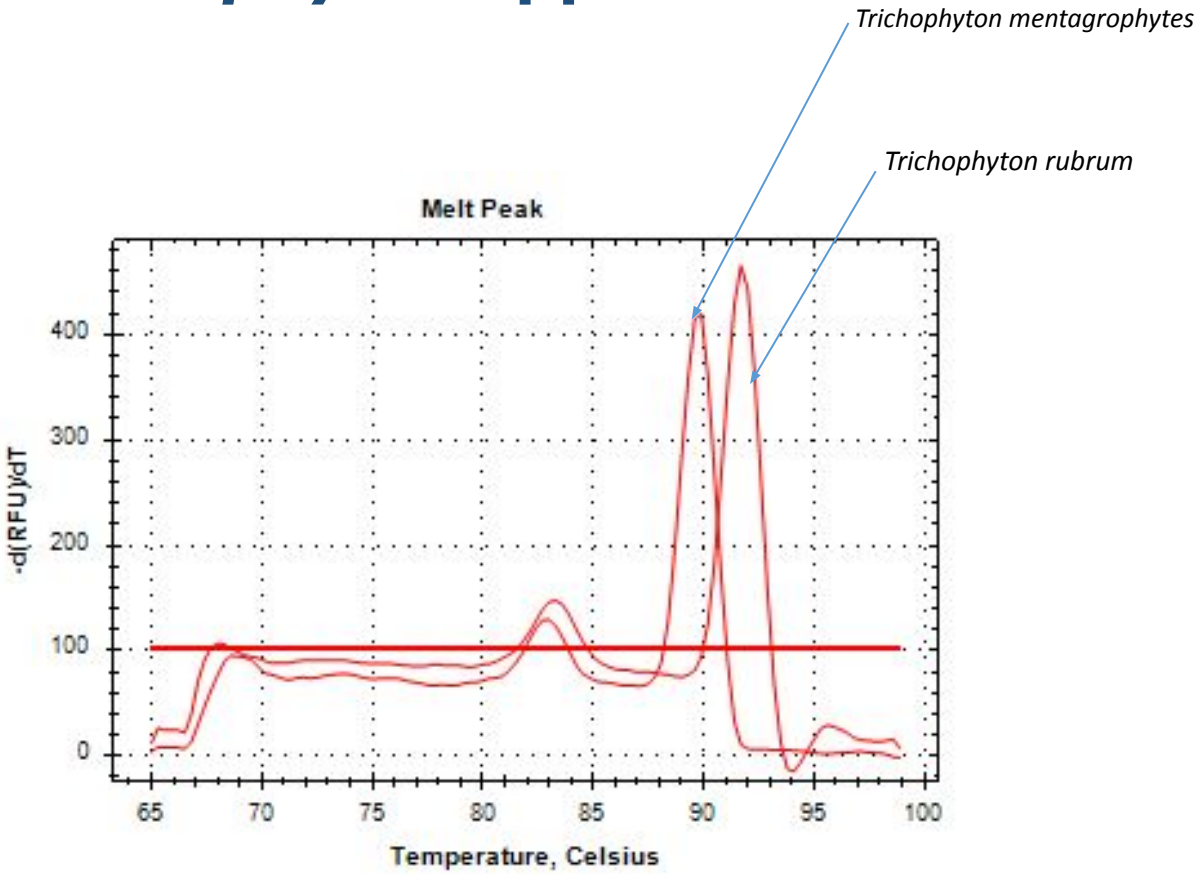


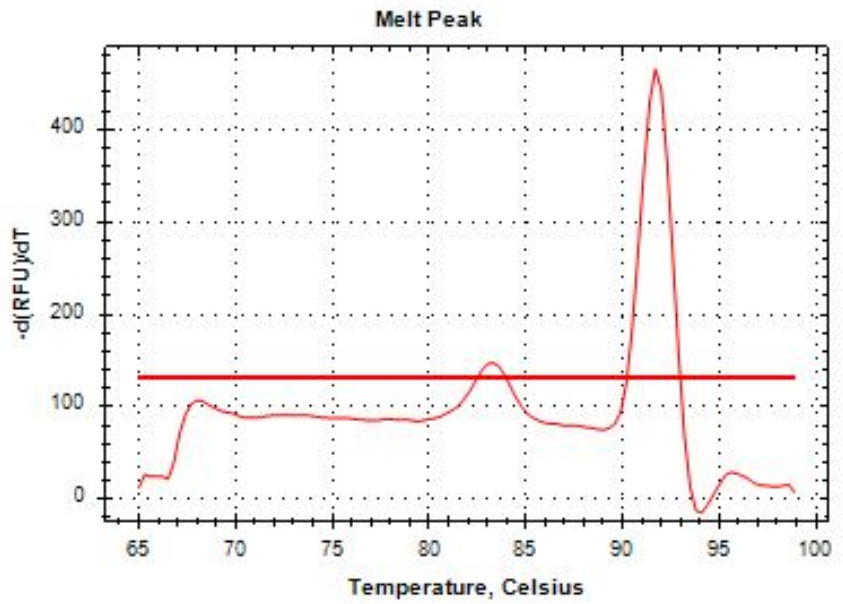
Taqman Real time PCR Testing

- **Dermatophytes** → \+ / \-
- **Candida** → \+ / \-
- **Aspergillus** → \+ / \-
- ***Pseudomonas aeruginosa*** → \+ / \-
- \- → **No further Testing**



Trichophyton Spp.

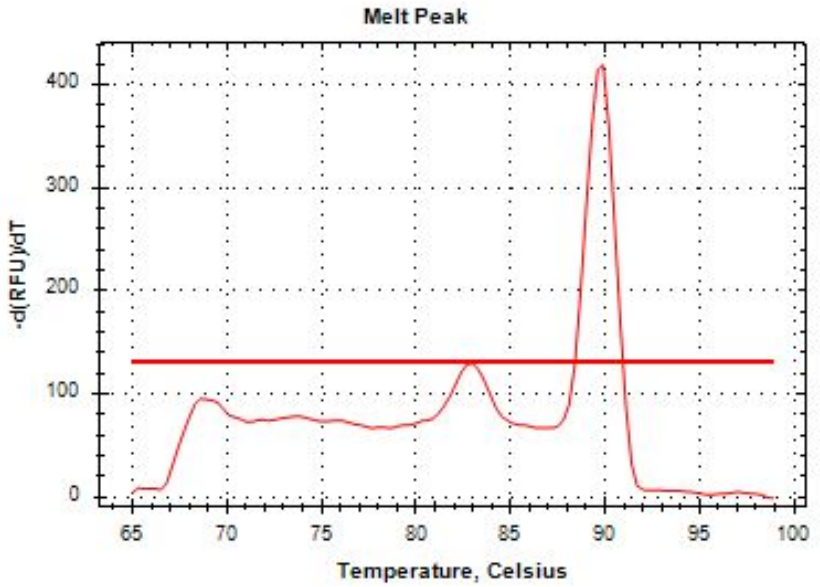




Trichophyton rubrum

91.80

83.10

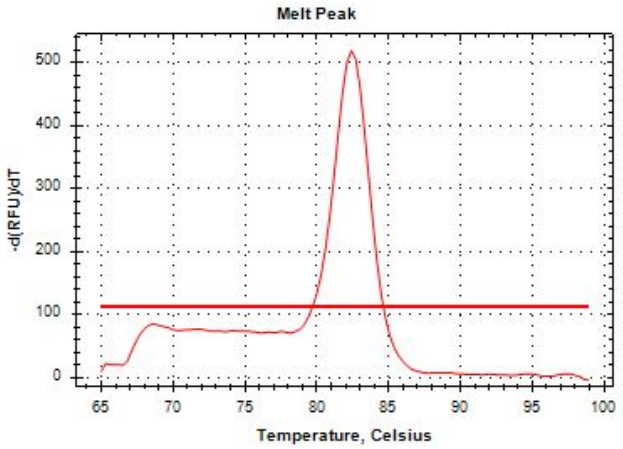


Trichophyton mentagrophytes

90.30

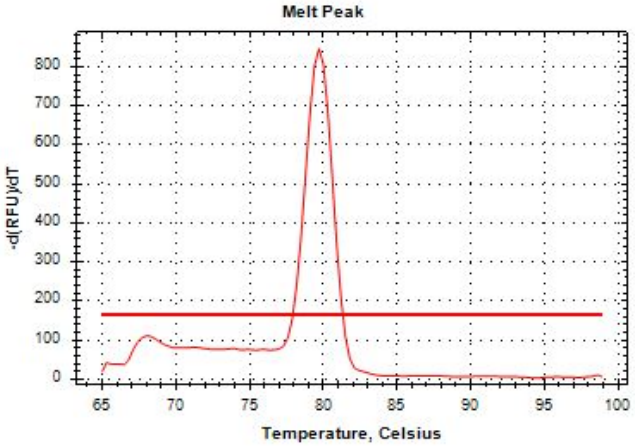
83.10

Candida Spp.



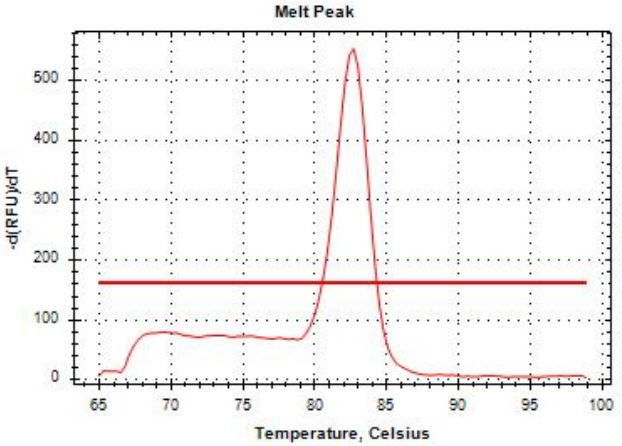
Candida albicans

82.50



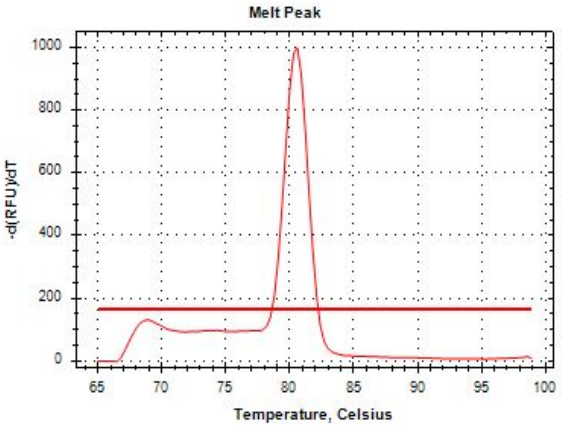
Candida parapsilosis

80



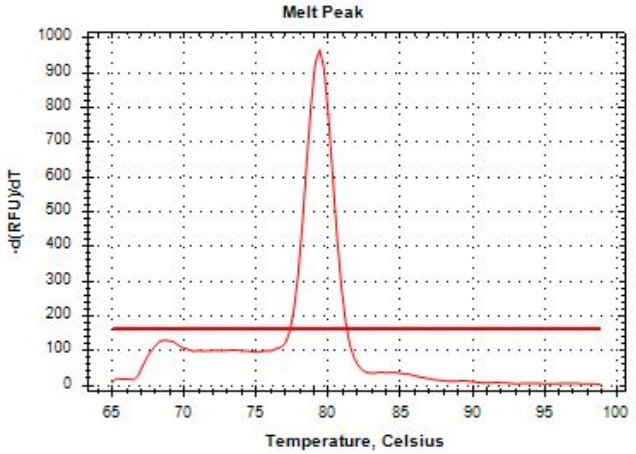
Candida tropicalis

82.50



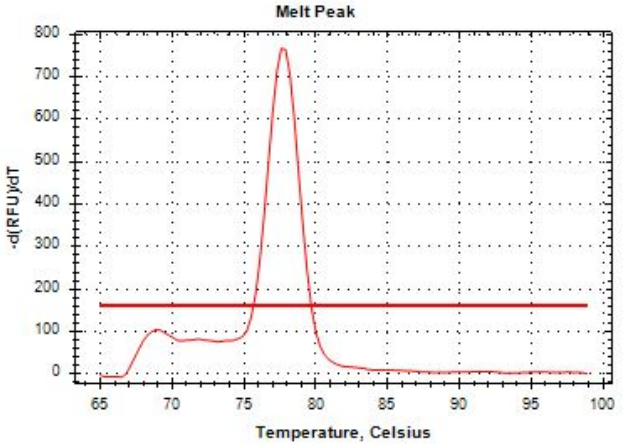
Candida krusei

80.40



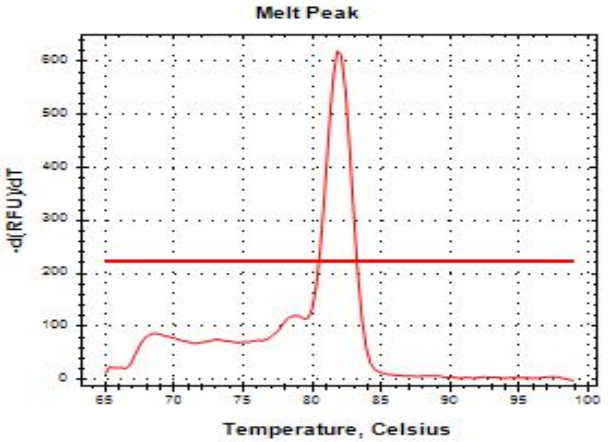
Candida auris

82.50



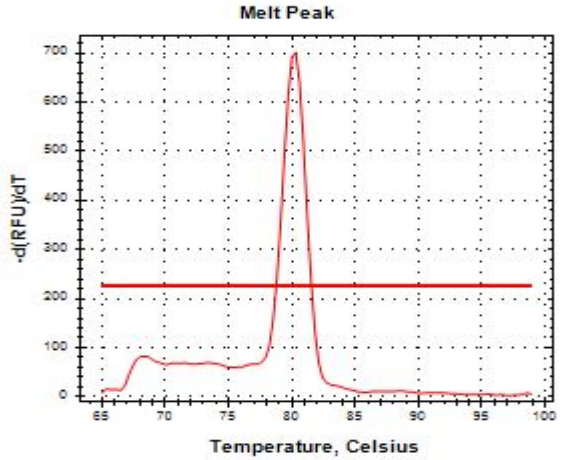
Candida lusitaniae

77.70



Candida guilliermondii

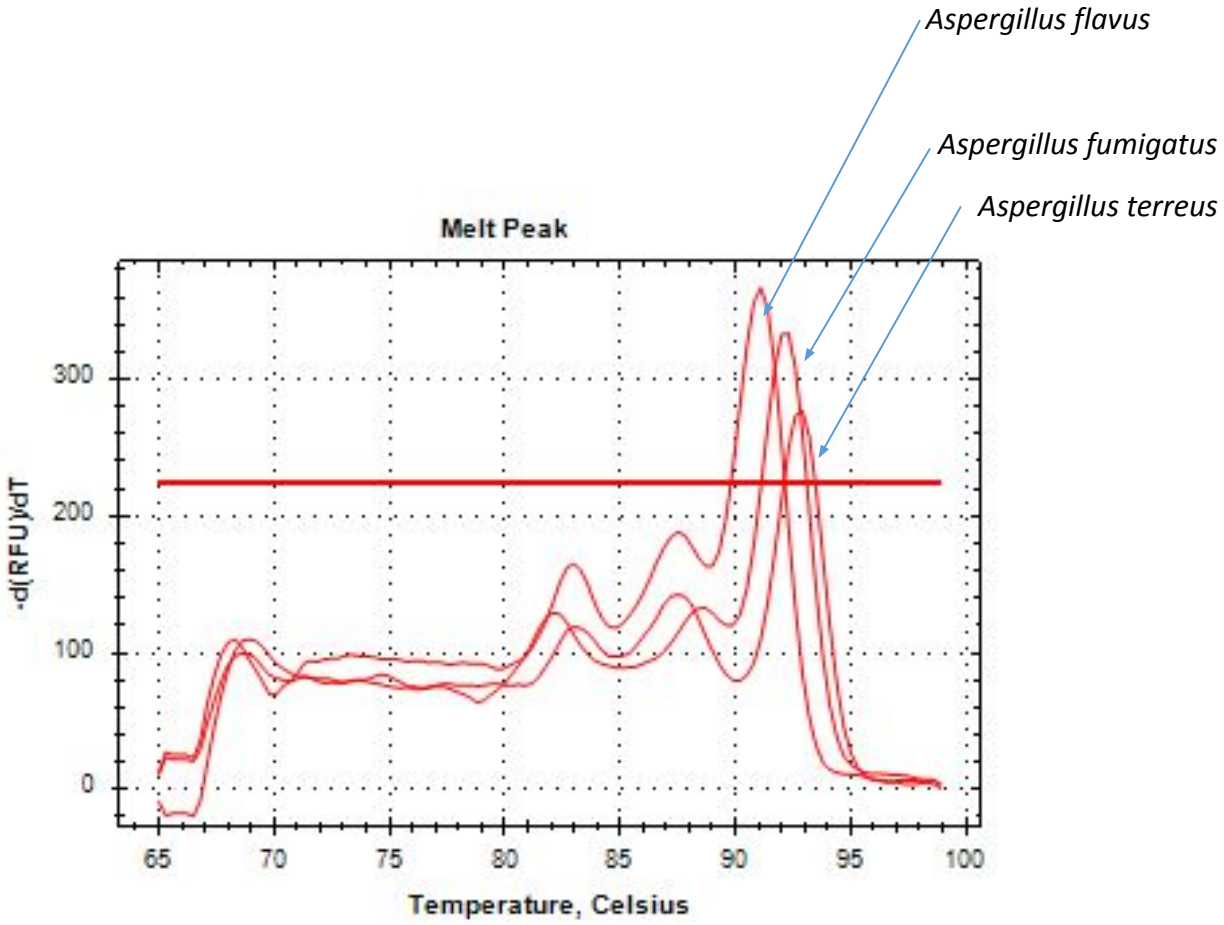
81.90

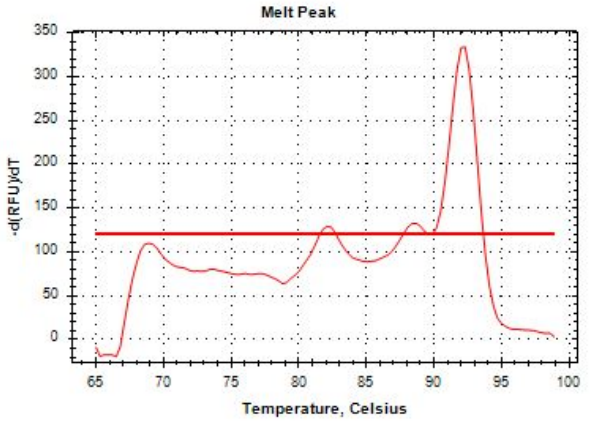


Candida orthopsilosis

80.10

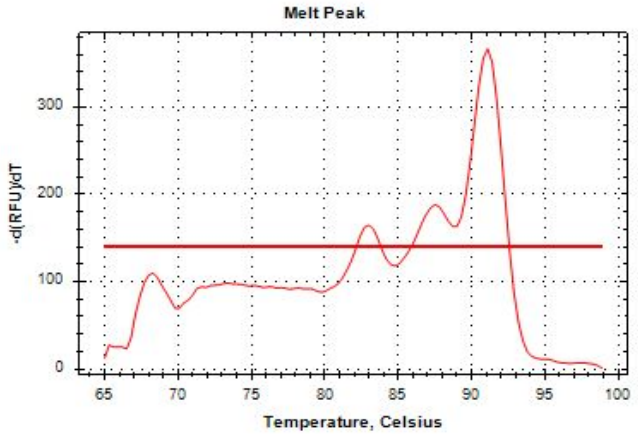
Aspergillus Spp.





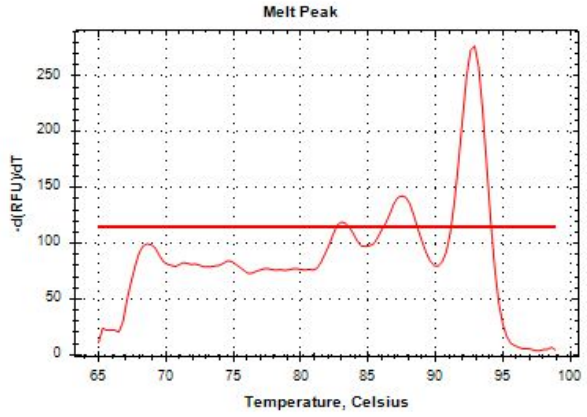
Aspergillus fumigatus

92.10
88.50
83.20



Aspergillus flavus

91.20
87.60
83.68

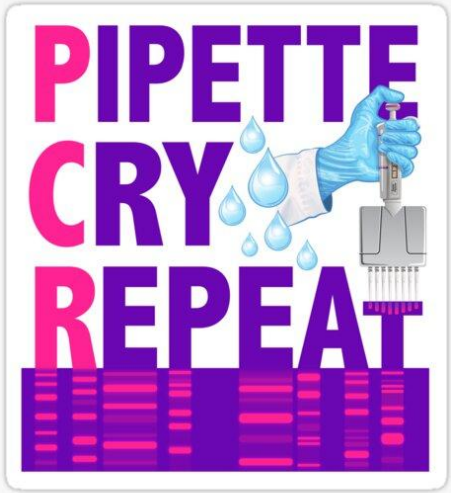


Aspergillus terreus

92.70
87.60
83.61

PCR: the DNA photocopier

It is difficult to detect tiny fragments of fungal DNA. **Making multiple copies of the fungal DNA makes detection much easier.**

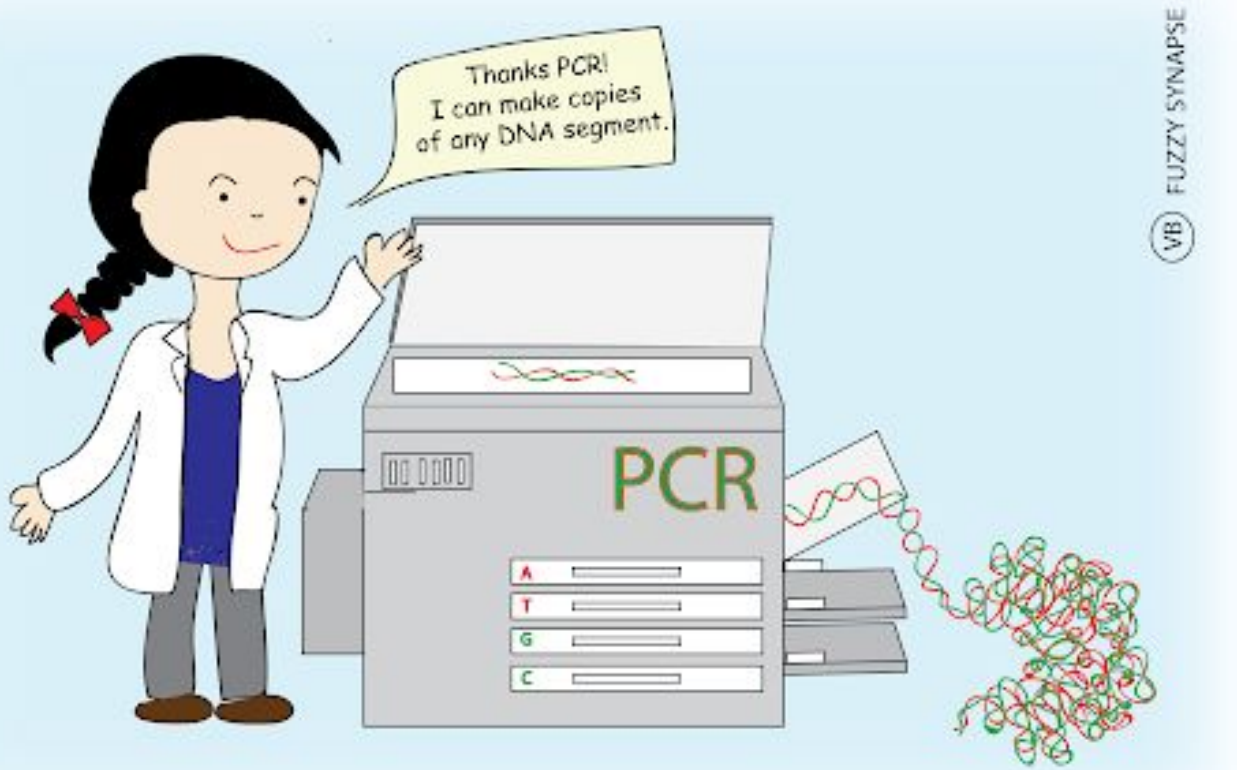


Polymerase Chain Reaction (PCR)

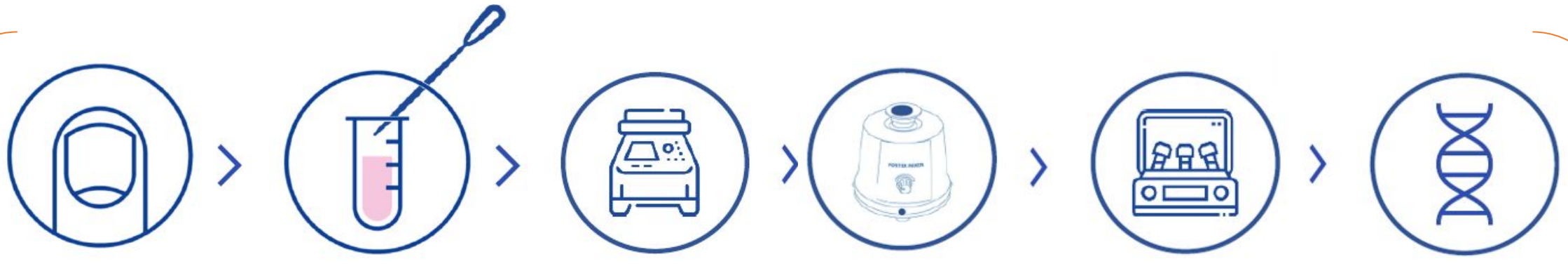
A technique used to make many copies of (“amplify”) a specific target region of DNA.

PCR: paternity tests for fungus

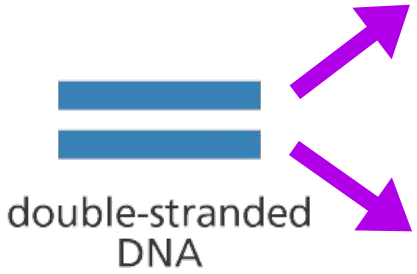
- ✔ Dermatophytes: T rubrum
- ✔ Candida
- ✔ Aspergillus
- ✔ Bacteria

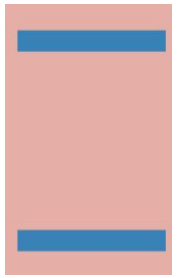


DNA Extraction Procedure



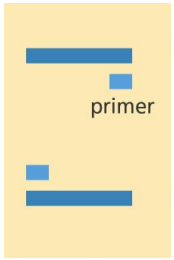
The Science of PCR





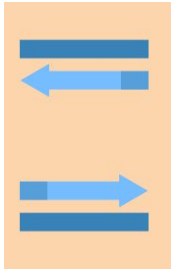
Denaturation (96 C)

Heat the reaction strongly to separate, or denature, the DNA strands. This provides single-stranded template for the next step.



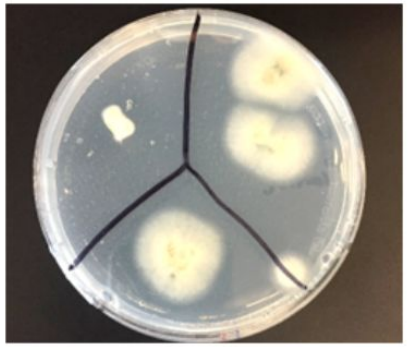
Annealing (55-65 C)

Cool the reaction so the primers can bind to their complementary sequences on the single-stranded template DNA.



Extension (72 C)

Raise the reaction temperatures so Taq polymerase extends the primers, synthesizing new strands of DNA.

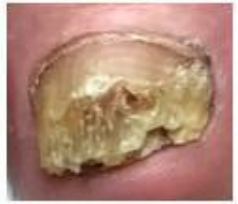
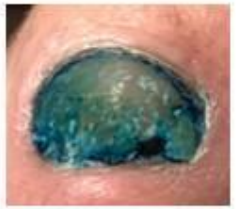
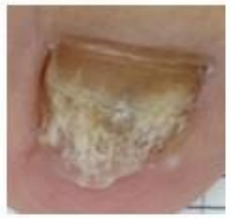
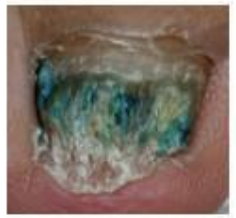
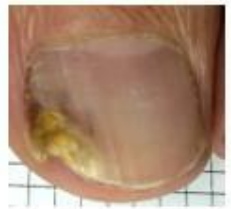
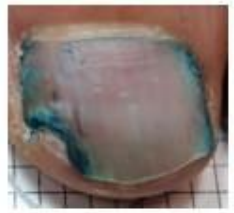
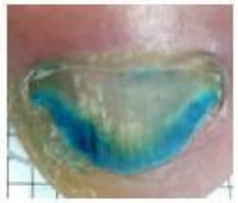
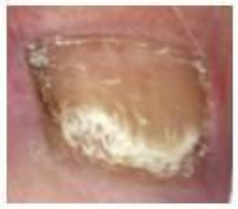
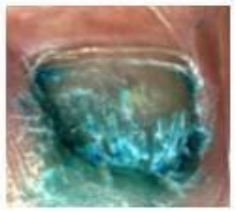


	Time	Specificity	Volume
Culture	3 weeks	55%	10 mg
qPCR	24 hours	99%	2 mg

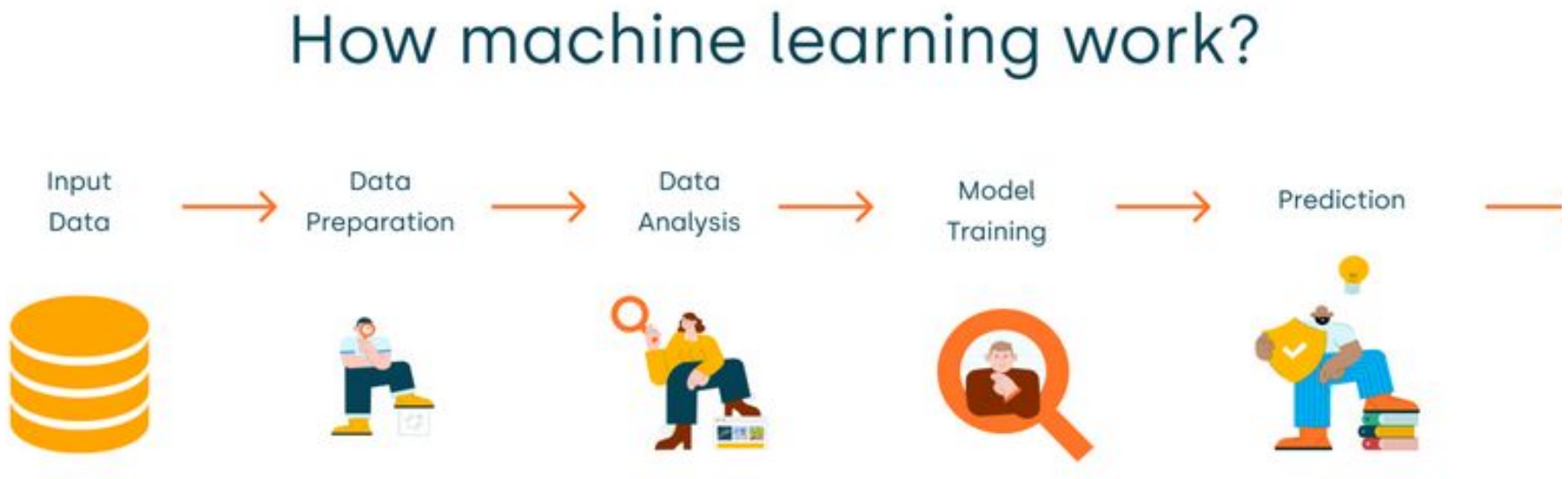
New Diagnostic Technique

- In-vivo staining

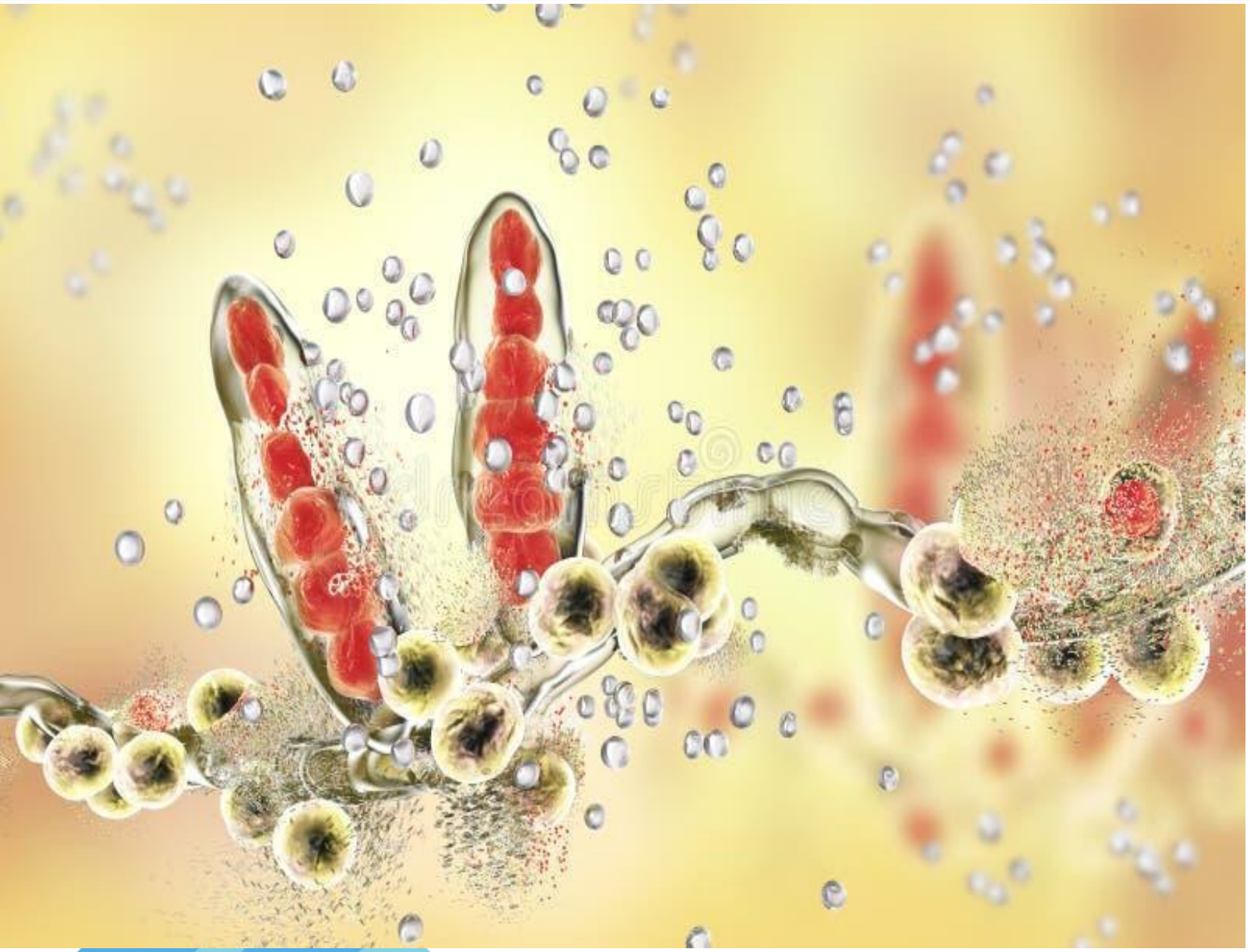
First in vivo stain for fungal infections

Baseline	Two weeks after ToeStain application	Baseline	Two weeks after ToeStain application
			
			
			

Machine Learning **AI as a screening tool for onychomycosis and Marketing Tool**



[Show AI Link]



Thank You

McMaster University

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