

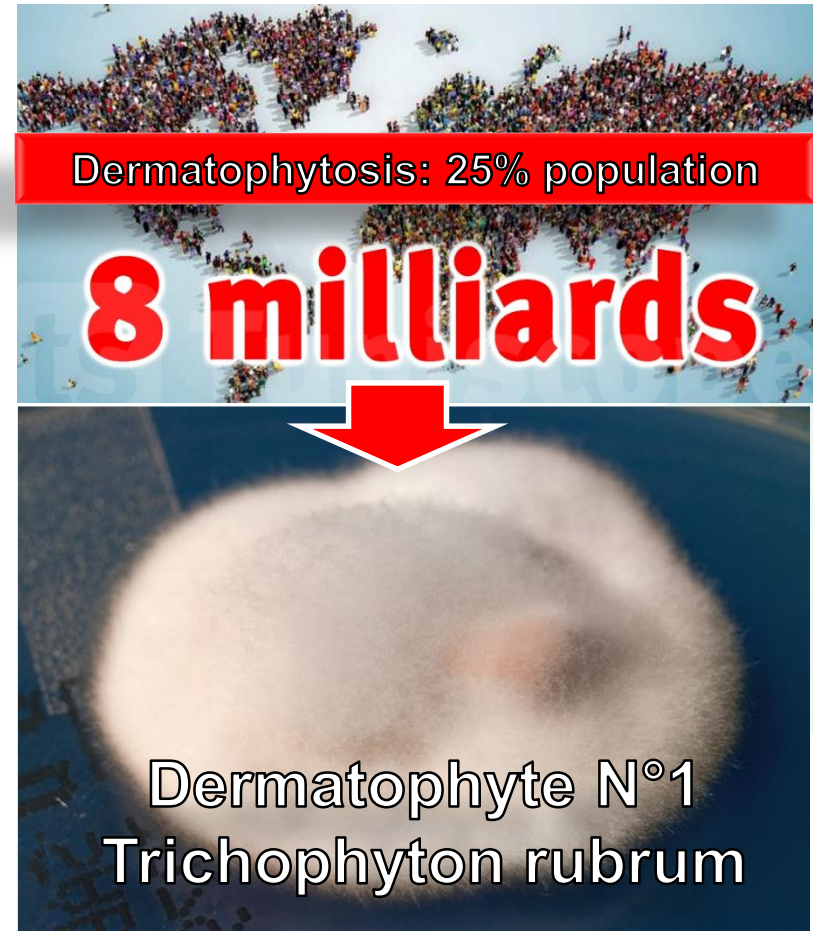
# Dermatophytes: diagnostic methods in the NRC Liège

Marie-Pierre Hayette, Rosalie Sacheli  
Service de Microbiologie clinique &  
National Reference Center for Mycosis, CHU Liège



## Dermatophytes: main facts

- Infect the skin, hair and nails  
→ tinea (...corporis, cruris, ...)
- Main causes of skin diseases  
(20-25% population)
- **Anthropophilic, zoophilic** or  
geophilic origin
- **>2018. Emergence of TER-  
resistant Trichophyton  
(indotineae) in Belgium**



## CENTRE DE RÉFÉRENCE POUR LES MYCOSES

## FORMULAIRE A ENVOYER AVEC L'ÉCHANTILLON AU CENTRE DE RÉFÉRENCE

Professeur Marie-Pierre HAYETTE

 Service de Microbiologie Clinique-CHU de Liège- Sart-Tilman B23- 4000 Liège  
 Tél. CNR 04/323.22.98 – Secrétariat 04.323.22.90 Fax 04/366.24.40- Email: [mphayette@chuliege.be](mailto:mphayette@chuliege.be)  
 Rosalie Sacheli, Responsable scientifique, [R.Sacheli@chuliege.be](mailto:R.Sacheli@chuliege.be)

## \*IDENTIFICATION DU LABORATOIRE QUI ENVOIE L'ÉCHANTILLON

 \*Nom du responsable : .....  
 \*Nom du laboratoire : .....  
 \*Adresse (ou cachet) : .....  
 Code postal + localité : .....  
 N° d'agrégation : .....  
 Tél. : ..... Fax : .....  
 Adresse Email contact : .....  
 \*Nom du médecin demandeur : .....  
 et N° INAMI : .....

## RENSEIGNEMENTS CONCERNANT LE PATIENT

 \*Nom (initiales/autre code) : .....  
 \*Sexe :  H  F  
 \*Date de naissance (ou âge) : .....  
 \*Code postal/Localité : .....  
 Nationalité : ..... Origine : .....  
 Séjour récent à l'étranger :  oui/durée : .....  non  
 Si oui, pays ou région : .....

## RENSEIGNEMENTS CONCERNANT L'ÉCHANTILLON

 \*Numéro d'identification : .....  
 Ex. microscopique direct : .....  
 Nature :  Levure  Filamenteux  Inconnu  
 \*Souche isolée de :  
 Prélèvement respiratoire : .....  
 Sang : .....  
 Phanères :  ongle pied  ongle main  cheveu  cuir chevelu  peau \*Si peau, préciser le site de prélèvement : .....  
 Autre : .....  
 \*Date de prélèvement : .....  
 \*Identification présumée : .....

## CADRE RÉSERVÉ AUX CAS DE SUSPICION DE RÉSISTANCE À LA TERBINAFINE

 Localisation de la lésion : .....  
 Lésion étendue :  Oui  Non  
 Patient sous traitement :  Oui  Non  
 Si oui, nom du traitement : .....  
 Séjour récent en Inde ou environs (préciser le pays) : .....

## INFORMATIONS CLINIQUES

 \*Selon le cas préciser les symptômes observés :  
 Mycose profonde : .....  
 Mycose sous-cutanée : .....  
 Mycose superficielle : .....  
 Dermatophytose étendue  Oui  Non  
 \*Facteurs associés :  
 Traitement par immunosuppresseurs  VIH  Corticoïdes  
 Hémopathie  
 Transplantation  d'organe  de M. osseuse : date : .....  
 Diabète  autre : .....  
 Commentaires : .....

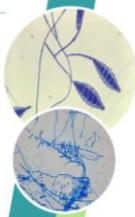
## INFORMATIONS ÉPIDÉMIOLOGIQUES

 Mode de transmission probable  
 Dermatophytes  
 Contact avec animal (préciser lequel) : .....  
 Epidémie en milieu scolaire  transmission intrafamiliale  
 Autres : .....

## ANALYSES DEMANDÉES

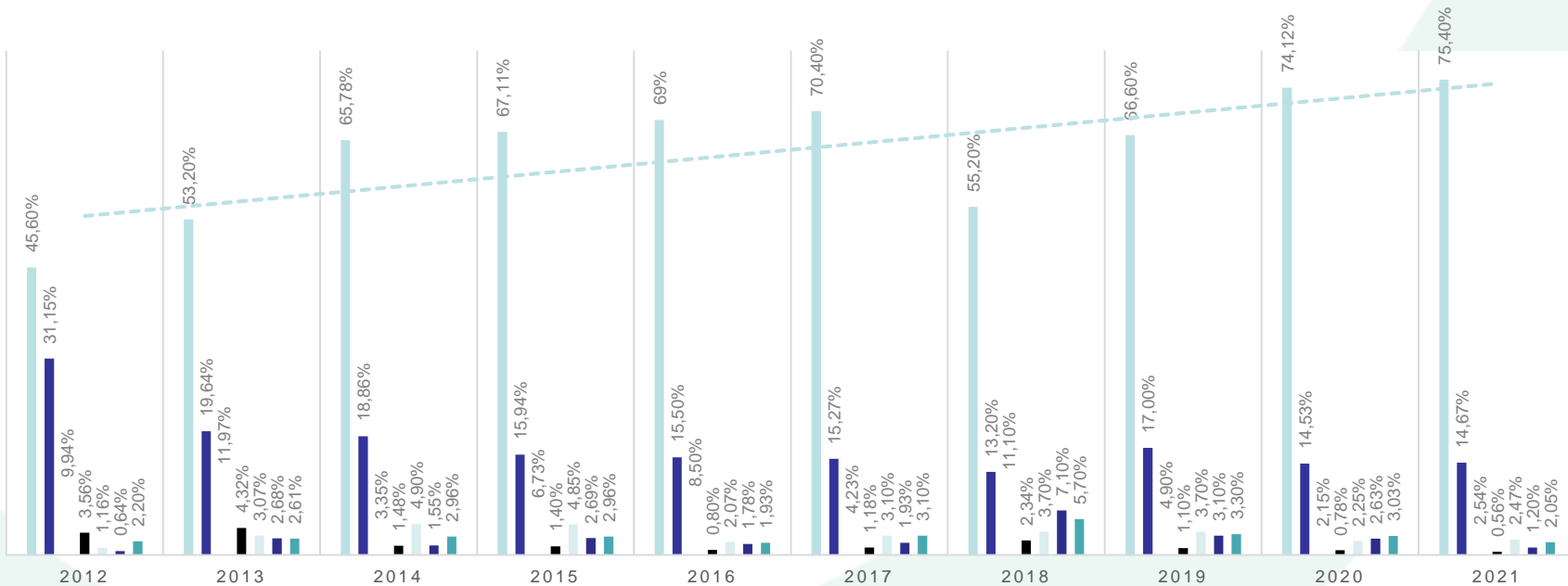
 Identification  Antifongogramme  
 PCR résistance à la terbinafine (si résistance clinique)  
 PCR pan-fongique (sur souche isolée en culture)  
 PCR dermatophytes (examen direct positif et culture négative)  
 Génotypage dermatophytes (WGS, en cas d'épidémie)

## AUTRES INFORMATIONS IMPORTANTES

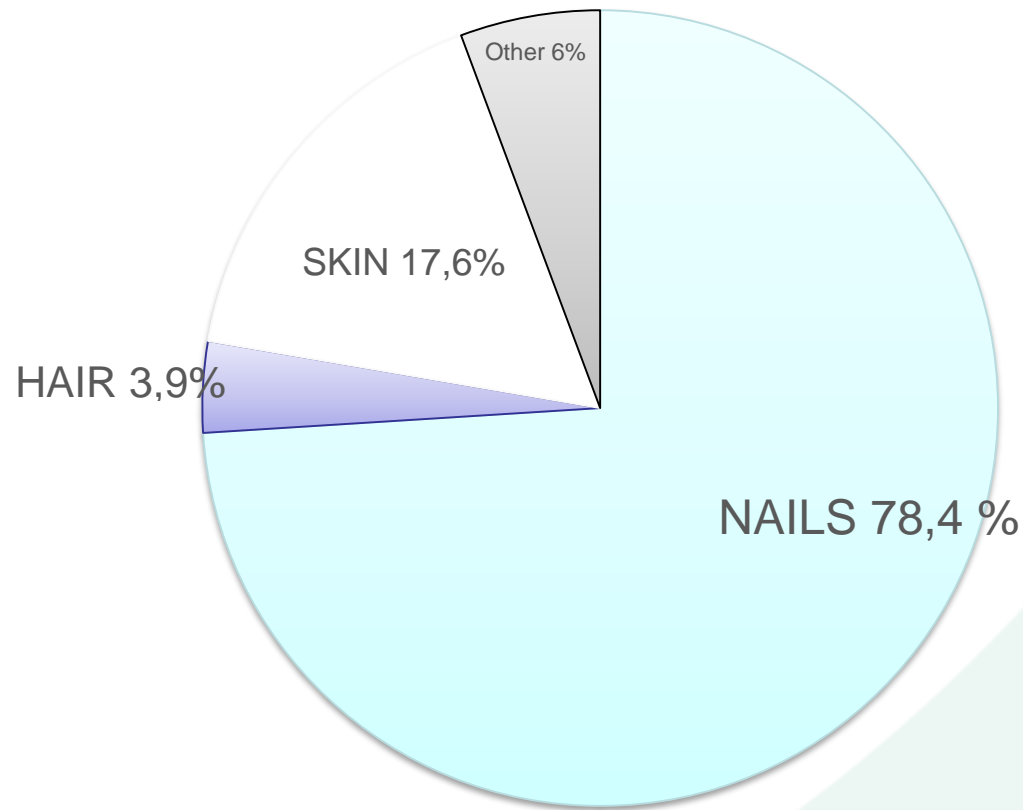


Mars 2023

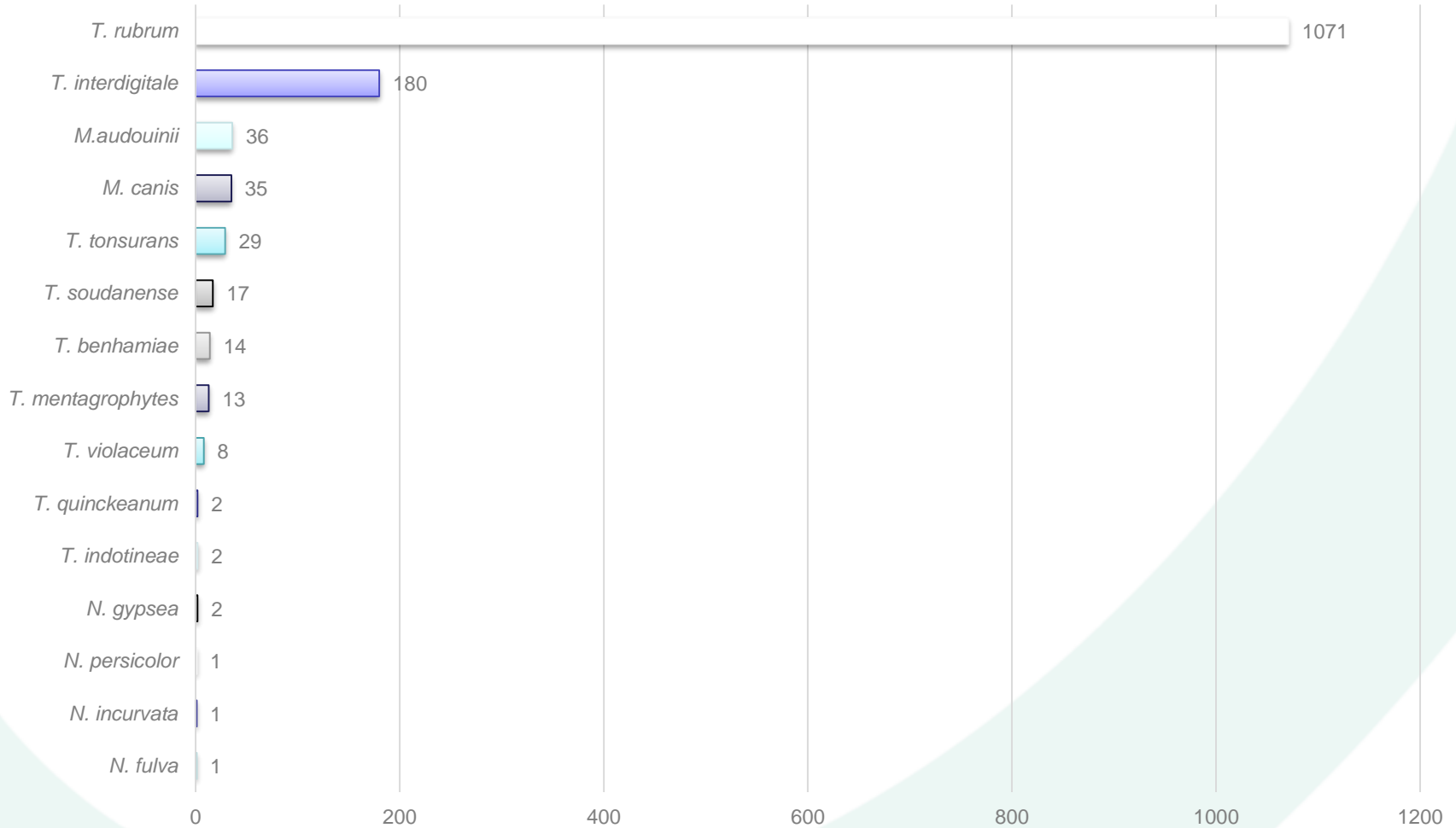
# Dermatophytes strains received by the two NRCs from 2012 to 2021



# 1941 samples received in 2021 for dermatophytes identification



# 1412/1941 dermatophytes identified in 2021 by both NRCs



# Laboratory diagnosis of dermatophytes at the NRC Mycosis Liège



- Fungal strains isolated from skin/hair/nails
- Identification:
  - Direct microscopy
  - Pan-dermatophytes PCR
  - PCR ITS (+EF1-alpha)+ sequencing
- Susceptibility testing for dermatophytes: EUCAST method
- Detection of Terbinafine resistance
  - SQLE PCR + sequencing
  - DermaGenius<sup>®</sup> resistance kit

# Algorithm

Dermatophyte and non-dermatophyte strains + NRC form



Sub-culture  
(7-14 days)

Sabouraud+antibiotics  
& Takashio medium

Incubation  
28°C

Identification  
(7-14 days)



Pan-dermatophytes PCR  
or  
ITS PCR+ sequencing  
(EF1-alpha)

- Antifungal susceptibility testing
- Detection of TER resistance

EUCAST method  
(Ref method)

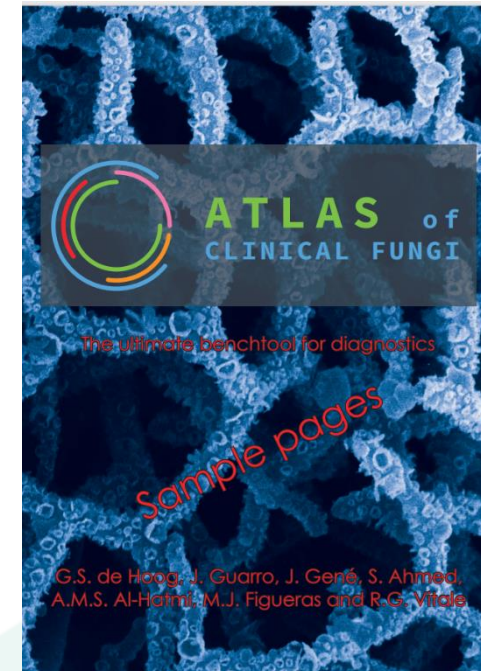
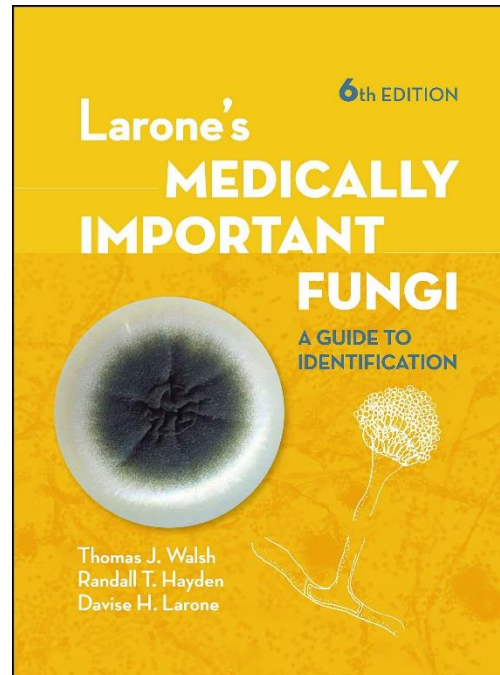
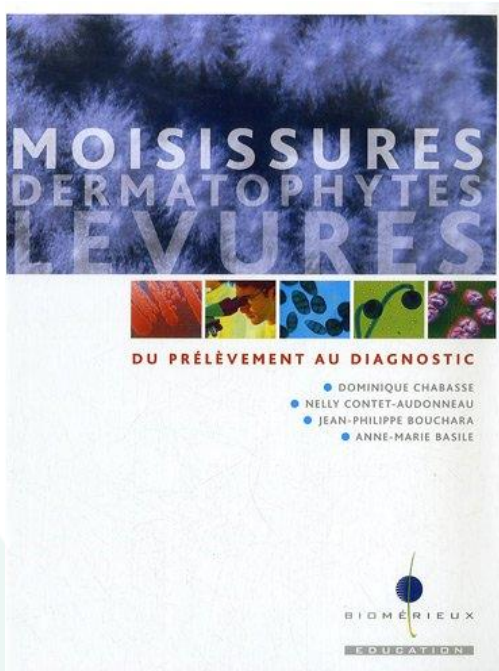
- SQLE PCR + Sequencing
- Commercial method (DermaGenius® Resistance)



Identification  
(7-14 days)



- Microscopy
- Reference books

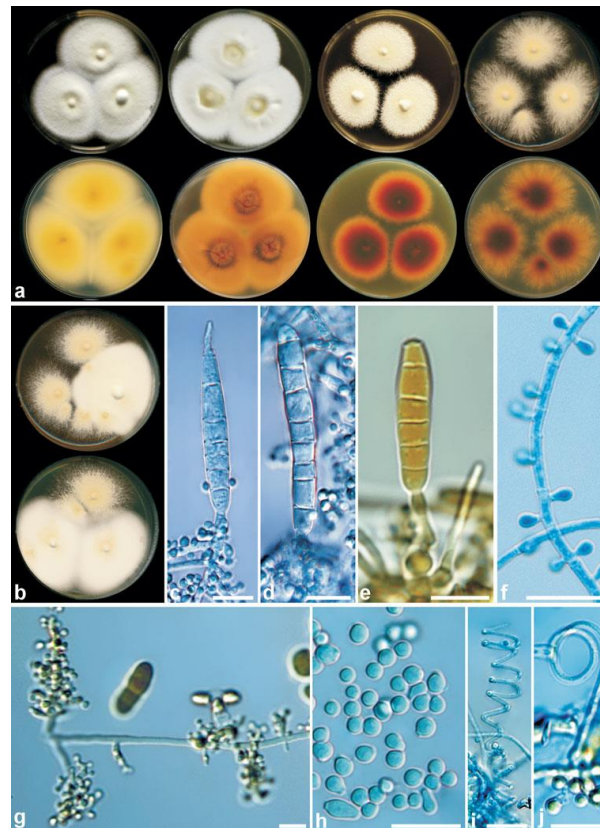


# Microscopy can be tricky

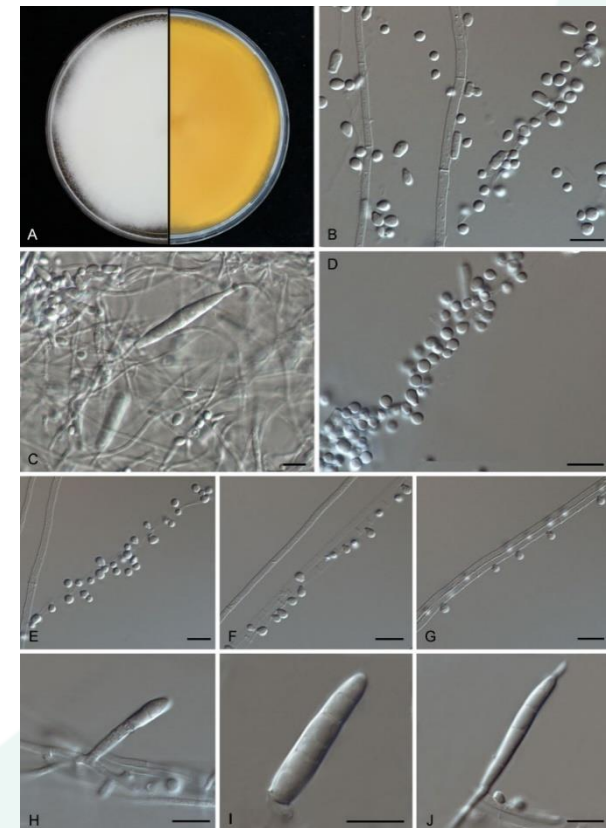
*T. indotineae*



*T. interdigitale*



*T. mentagrophytes*



# PAN-DERMATOPHYTE PCR if suspicion of dermatophyte

## DermaGenius 2.0 . (validated)

### Multiplex Total

- *Candida albicans*
- *Trichophyton mentagrophytes*
- *Trichophyton interdigitale*
- *Trichophyton rubrum*
- *Trichophyton tonsurans*
- *Trichophyton soudanense*
- *Trichophyton benhamiae*
- *Trichophyton verrucosum*
- *Trichophyton violaceum*
- *Microsporum canis*
- *Microsporum audouinii*
- *Epidermophyton floccosum*

### Multiplex Ongles

- *Candida albicans*
- *Trichophyton interdigitale*
- *Trichophyton rubrum*

- *Candida albicans*
- 11 dermatophytes species
- Direct detection of dermatophytes in nail, skin and hair samples or on strains

Once per week,  
result on the  
same day

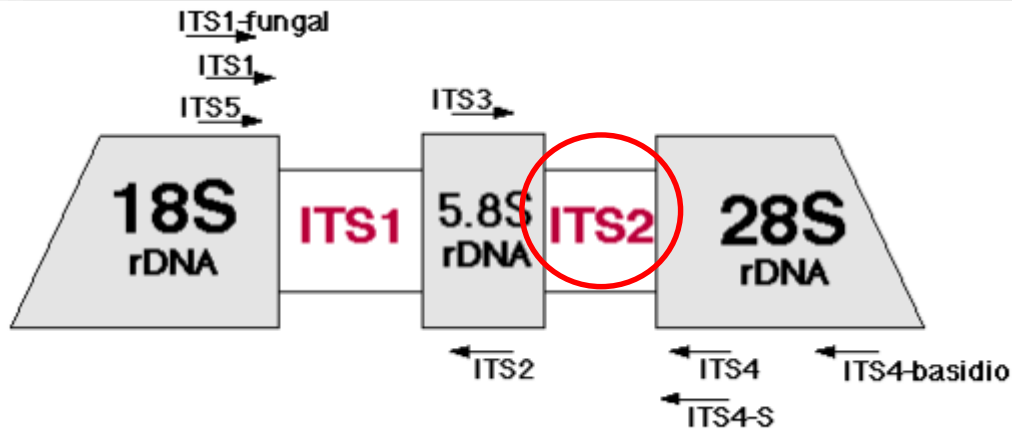
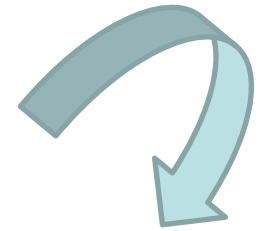
## DermaGenius 3.0 (being validated)

- |   |   |
|---|---|
| > <i>C. albicans</i>  | > <i>C. parapsilosis</i>                |
| > <i>S. brevicaulis</i>   |   |
| > <i>T. interdigitale/T. mentagrophytes (avec T. indotinea)</i> | > <i>T. quinckeanum/T. schoenleinii</i> |
| > <i>T. rubrum/T. soudanense</i>                                | > <i>T. mentagrophytes ITS type 4</i>   |
| > <i>T. tonsurans</i>   | > <i>T. violaceum</i>                   |
| > <i>T. benhamiae*</i>  | > <i>T. verrucosum</i>                  |
| > <i>M. canis</i>   | > <i>M. audouinii</i>                   |
| > <i>E. floccosum</i>   | > <i>N. gypsea</i>                      |

- *C. albicans/parapsilosis*
- *S. brevicaulis*
- 12 dermatophytes species/complexes

CHU de Liège

# ITS PCR + sequencing only on fungal strains



For identification of any fungi or non-dermatophytes or dermatophytes that are not recognised by DermaGenius

Very good identification of most dermatophytes except for the differentiation of ***T indotineae***  
→ PCR EF1-alpha  
→ →WGS (cf. *R Sacheli*)

TAT : 5-7 days



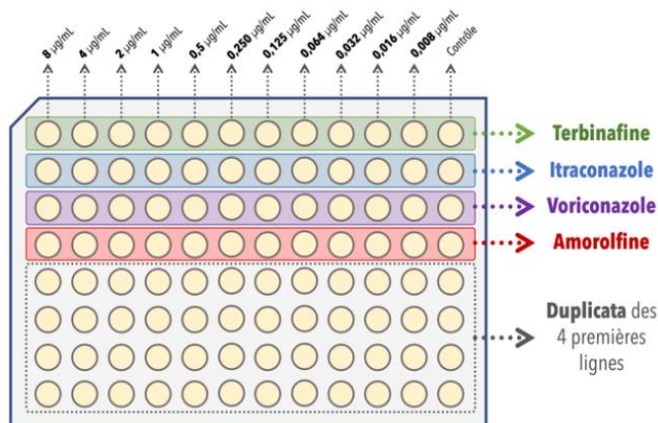
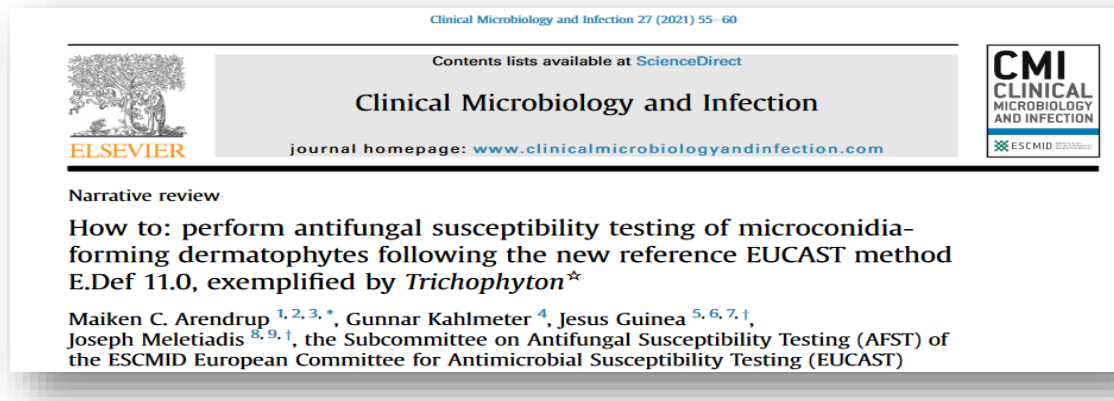
WESTERDIJK  
FUNGAL BIO  
DIVERSITY  
INSTITUTE

# TECHNIQUES

- Samples: dermatophytes strains
- Identification:
  - Direct microscopy/sub-culture
  - PCR pan-dermatophytes
  - PCR ITS + sequencing
- **Antifungal susceptibility testing for dermatophytes**
- **SQLE PCR + sequencing: determination of TER resistance**

# Susceptibility testing for dermatophytes

- EUCAST method E.Def.11.0 microdilution method (Arendrup et al. 2021 & 2020)



TAT: after reception of the strain:  
12-15 days

# EUCAST interpretation

Overview of antifungal ECOFFs and clinical breakpoints for yeasts, moulds and dermatophytes using the EUCAST E.Def 7.3, E.Def 9.4 and E.Def 11.0 procedures

Version 3.0, valid from 2022-01-18

Species	Drug	ECOFF (mg/L)	Clinical Breakpoints (mg/L)				Recommendation for area of technical uncertainty (ATU) results
		WT ≤	S ≤	I	R >	ATU	
<i>T. indotinea</i> <sup>2</sup>	Amorolfin	(0.5) <sup>1</sup>	ND		ND		
	Amphotericin B	ND	ND		ND		
	Anidulafungin	ND	ND		ND		
	Micafungin	ND	ND		ND		
	Fluconazole	ND	ND		ND		
	Isavuconazole	ND	ND		ND		
	Itraconazole	(0.25) <sup>2</sup>	ND		ND		
	Posaconazole	ND	ND		ND		
	Terbinafine	(0.125)	ND		ND		
	Voriconazole	(1)	ND		ND		
<i>T. rubrum</i>	Amorolfin	(0.125)	ND		ND		
	Amphotericin B	ND	ND		ND		
	Anidulafungin	ND	ND		ND		
	Micafungin	ND	ND		ND		
	Fluconazole	ND	ND		ND		
	Isavuconazole	ND	ND		ND		
	Itraconazole	(0.25) <sup>2,3</sup>	ND		ND		
	Posaconazole	ND	ND		ND		
	Terbinafine	(0.03)	ND		ND		
	Voriconazole	(0.125)	ND		ND		

Reading with spectrophotometer  
 Interpretation: no BP  
 ECOFFs only for 2 species, 3 drugs  
*T. indotinea*=  
 TERB: 0,125µg/ml, ITRA 0.25µg/ml  
 VOR : 1µg/ml AMOR 0,5µg/ml

**Comments:**

<sup>1</sup> ECOFFs indicated in brackets ( ) are tentative.

<sup>2</sup> TECOFFs against *T. indotinea* (formerly the Indian variant of *T. interdigitale*) and *T. rubrum* were determined based on a shared isolate collection tested in 10 laboratories as part of a recently published study (Multicentre validation of a EUCAST method for the antifungal susceptibility testing of microconidia-forming dermatophytes; J Antimicrob Chemother, 2020) and a comment supporting the name change ( J Antimicrob Chemother, 2022).

<sup>3</sup> MIC distributions were wider than normally, the TECOFF is therefore associated with uncertainty. They apply to MICs determined using E.Def 11.0 and with 50% endpoint criteria.

# SQLE PCR + Sequencing

- If TER resistance is detected by EUCAST method
- In case of clinical resistance
- In case of extended tinea corporis/cruris (suspicion of *T. indotineae*)

## Terbinafine Resistance of *Trichophyton* Clinical Isolates Caused by Specific Point Mutations in the Squalene Epoxidase Gene

Tsuyoshi Yamada,<sup>a,b</sup> Mari Maeda,<sup>a</sup> Mohamed Mahdi Alshahni,<sup>b</sup> Reiko Tanaka,<sup>c</sup> Takashi Yaguchi,<sup>c</sup> Olympia Bontems,<sup>d</sup> Karine Salamin,<sup>d</sup> Marina Fratti,<sup>d</sup> Michel Monod<sup>d</sup>

Download Graphics

unnamed protein product  
Sequence ID: Query\_455905 Length: 93 Number of Matches: 1

Range 1: 1 to 93 Graphics Next Match Previous Match

Score	Expect	Method	Identities	Positives	Gaps
186 bits(473)	6e-64	Compositional matrix adjust.	92/93(99%)	92/93(98%)	0/93(0%)
Query 323	MFLGDSLNMRRHPLTGGGMTVAFNDVLLRNLLSPEAVPDLSDTKLVKQLSKFHWQRKSL	382			
Sbjct 1	MFLGDSLNMRRHPLTGGGMTVAFNDVLLRNLLSPEAVPDLSDTKLVKQLSKFHWQRKSL	60			
Query 383	ISVINILAQSLYLIFAA <sup>397</sup> SKHMFSLPLLVS <sup>415</sup> GY	415			
Sbjct 61	ISVINILAQSLYLIFAA <sup>397</sup> SKHMFSLPLLVS <sup>415</sup> GY	93			

Substitution in position 397 of F to L (F397L)

TAT: 5-7 days after culture



# DermaGenius<sup>®</sup> resistance kit

## DermaGenius<sup>®</sup> Resistance RT-PCR

Détection des mutations sur le gène squalène époxydase à l'origine de la résistance à la Terbinafine.

Détection additionnelle des souches de Trichophyton concernées.

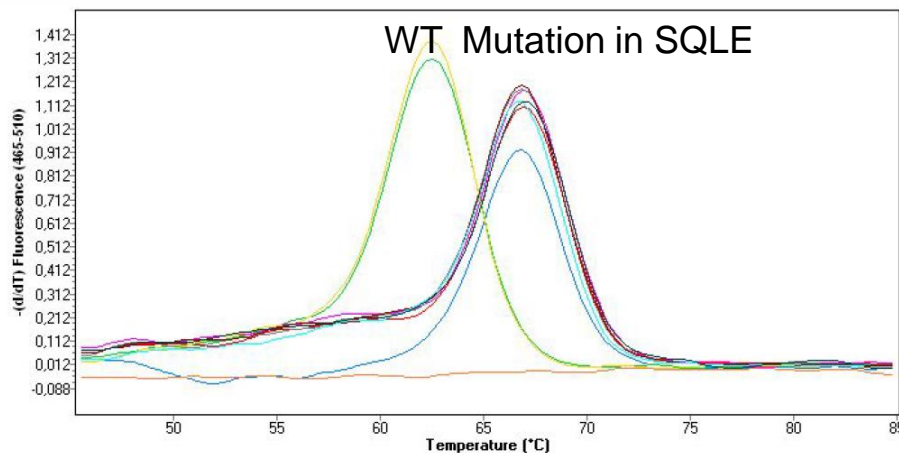
### Détection spécifique des mutations et des espèces Trichophyton:

- > Mutations du **SQLE** : Leu393Phe, Phe397Leu, Leu393Ser, Phe397Ile, Phe397Val
- > T. interdigitale/T. mentagrophytes (avec T. indotinea)      > T. quinckeanum/T. schoenleinii
- > T. rubrum/T. soudanense    > T. mentagrophytes ITS type 4
- > T. tonsurans    > T. violaceum

### Validé sur plusieurs thermocycleurs RT-PCR:

- > LightCycler 480 II (Roche)
- > CFX96 (Biorad)
- > Quantstudio 5 (Thermo Fisher Scientific)
- > Mic qPCR Cycler (Bio Molecular Systems)
- > CFX Opus 96 (Biorad)
- > Rotor-Gene Q (Qiagen)

- Validated, on 10R/20S T indotinea/interdigitale(ok)
- Also validated by the team of Anuradha Chowdhary (*Singh A., Mycoses, 2021*)
- Accreditation pending



Distributed by Teco Medical group

# Main mutations identified on the SQLE gene

+++

Leu393Phe  
Leu393Ser  
Phe397Leu

TER R

MIC  $\geq$  1 mg/L

Leu335Phe  
Ser395Pro  
Gln408Leu  
His440Tyr  
Ser443Pro

TER R but

▼ CMI  $\geq$  0,2 to  
1 mg/L

Ala448Thr

▼ sensitivity  
azoles

>2016

## Emergence of Difficult-to-Treat Tinea Corporis Caused by *Trichophyton mentagrophytes* Complex Isolates, Paris, France

Sarah Dellière,<sup>1</sup> Brune Joannard,<sup>1</sup> Mazouz Benderdouche, Anselme Mingui, Maud Gits-Muselli, Samia Hamane, Alexandre Alanio, Antoine Petit, Germaine Gabison, Martine Bagot, Stéphane Bretagne  
Dellière S, *EID*, 2022



## *Trichophyton indotinea*, from epidemiology to therapeutic

Arnaud Jabet<sup>a,b,\*</sup>, Anne-Cécile Normand<sup>b</sup>, Sophie Brun<sup>c</sup>, Eric Dannaoui<sup>d,e,f</sup>, Claude Bachmeyer<sup>g</sup>, Renaud Piarroux<sup>b,h</sup>, Christophe Hennequin<sup>a,i</sup>, Alicia Moreno-Sabater<sup>a,j</sup>

<sup>a</sup>Service de Parasitologie-Mycologie, Hôpital Saints-Antoine, AP-HP, 75012 Paris, France

<sup>b</sup>Service de Parasitologie-Mycologie, Hôpital La Pitié-Salpêtrière, AP-HP, 75013 Paris, France

<sup>c</sup>Service de Parasitologie-Mycologie, Hôpital Avicenne, AP-HP, 93009 Bobigny, France

<sup>d</sup>Unité de Parasitologie-Mycologie, Service de Microbiologie, Hôpital Necker, AP-HP, 75015 Paris, France

<sup>e</sup>UR Dynamic 7380, UPEC, EmA, USC ANSES, Faculté de Santé, 94000 Créteil, France

<sup>f</sup>Faculté de Médecine, Université Paris Cité, 75006 Paris, France

<sup>g</sup>Service de Médecine interne, Hôpital Tenon, AP-HP, 75020 Paris, France

<sup>h</sup>Institut Pierre Louis d'Epidémiologie et de Santé Publique, Inserm, Sorbonne Université, 75013 Paris, France

<sup>i</sup>Centre de Recherche Saint-Antoine, CRSA, Inserm, Sorbonne Université, 75012 Paris, France

<sup>j</sup>Centre d'Immunologie et des Maladies Infectieuses, (CIM-PARIS), Inserm U1135, Sorbonne, 75013 Paris, France



Article

## Belgian National Survey on Tinea Capitis: Epidemiological Considerations and Highlight of Terbinafine-Resistant *T. mentagrophytes* with a Mutation on SQLE Gene

Rosalie Sacheli<sup>1,\*</sup><sup>lb</sup>, Saadia Harag<sup>2</sup>, Florence Dehavay<sup>2</sup>, Séverine Evrard<sup>3</sup>, Danielle Rousseaux<sup>4</sup>, Akole Adjetey<sup>1</sup>, Laurence Seidel<sup>5</sup><sup>lb</sup>, Kim Laffineur<sup>6</sup><sup>lb</sup>, Katrien Lagr and Marie-Pierre Hayette<sup>1</sup>

# Conclusion

- We focus on dermatophytes
- !! Emergence of TER resistant *T. indotineae* = difficult to identify and to treat !
- Send your strains to us for characterisation AND CALL !!



04.323.22.98