Information sessions UZ Leuven: national reference center activities

8-6-2023

Lize Cuypers PharmD PhD

Coordinator of national reference centers for human pathogens and rare diseases
Laboratory Medicine, UZ Leuven

Laboratory of Clinical Microbiology, KU Leuven

Recap important information

- **Accreditation** requested: please enter your name (and RIZIV/INAMI if applicable) in the chat box
- **Interactive sessions**: you can speak up by unmuting your microphone to ask questions or raise comments in the chat box

- No recording of the session but slides will be shared https://www.uzleuven.be/nl/laboratoriumgeneeskunde/nationale-referentiecentra-en-referentielaboratoria

National Reference Center for Mycosis (UZ Leuven and CHU Liège)

8-6-2023

Katrien Lagrou & Lize Cuypers

Marie-Pierre Hayette & Rosalie Sacheli

UZ LEUVEN

National Reference Center Mycosis

NRC for Mycosis

CHU de Liège

sciensano.be/nl/nrc-nrl/nationaal-referentiecentrum-nrc-voor-mycosis

sciensano

Home • Nationaal Referentiecentrum (NRC) voor Mycosis

Nationaal Referentiecentrum (NRC) voor Mycosis

Belangrijke berichten

Candida auris (Maart 2023)

Tot nu toe werd in België bij **8 patiënten** *Candida auris* geïsoleerd (tussen 2016 en 2022), echter bij twee
huisverblijf gevonden worden, dit voor een casus eind 2021
t einde van 2021 betrof het steeds een geïmporteerde
van een toenemend aantal *Candida auris* gevallen en
tra waakzaamheid voor de detectie en opvolging van *C.*

ntie in de omgeving en in asymptomatische patiënten wat
huizen. Daarenboven is deze gist potentieel multiresistent.
vestigde klinische gevallen (zie definitief globaal rapport

roup en Nationaal Referentie Centrum
len steeds op species niveau. Bij identificatieproblemen
t Nationaal Referentiecentrum voor Mycosen (UZ Leuven

Tests disponibles

1. In vitro gevoeligheid testen (gisten: Leuven en Luik / filamenteuze schimmels: Leuven)
2. Bevestiging van de aanwezigheid van Pneumocystis jirovecii door PCR (Leuven)
3. PCR voor de identificatie van dermatofyten in microscopische/kweeknegatieve en moeilijk te identificeren en/of verontreinigde dermatofytculturen (Luik)
4. Typering van stammen: alleen bij uitbraken (stam van invasieve infectie: Leuven/dermatofyten: Luik)
5. PCR voor de detectie van Aspergillus speciës (Leuven)
6. Bepaling van (1→3)-β-D-glucan in serum (Wako methode)
7. Panfungale PCR (Leuven)
8. Identificatie van een schimmel isolaat (Leuven en Luik)

Jaar	
2020	Rapport d'activité 2020-2021
2019	Rapport Dermatophytes 2018-2019 - CHU Liège (FR)

Verantwoordelijke laboratoria

Coördinator

- UZ Leuven/KU Leuven

Geassocieerd

- Centre Hospitalier Universitaire de Liège

Erkend door

- National Institute for Health and Disability Insurance (INAMI-RIZIV)

Formulaires de demande

- Aanvraagformulier Mycosis

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NRC request form

CHU de Liège

- Information on the laboratory and patient
- Information on the sample: necessary to know the **sample type** from which the fungus was isolated (same fungus can be a contaminant or the cause of a life threatening disease depending on the sample type)
- **Type of mycosis and host factors** are useful for interpretation

***GEGEVENS OVER HET STAAL**

*Identificatienummer: ……

*Afnamedatum: ……..

Resultaat rechtstreeks onderzoek……

……..

*Staaltype:

Isolaat: ❑ gist ❑ filamenteuze fungus

Geïsoleerd uit ……..

❑ weefsel ❑ BAL-vocht ❑ ander :……

KLINISCHE GEGEVENS

*Preciseer naargelang type mycose:

❑ Diepe mycose : ……..

……

❑ Subcutane mycose:……

……

❑ (muco)cutane/oppervlakkige mycose:……..

……

*Gastheerfactoren:

❑ Neutropenie ❑ HIV/AIDS ❑ Corticosteroïden

❑ Transplantatie : type……...

❑ Diabetes ❑ Behandeling met immuunsuppressiva

❑ Andere:……...

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The two associated labs have a different focus

CHU de Liège

UZ Leuven: invasive mycoses

- Mucorales PCR
- β-D-glucan
- Susceptibility testing of all *Aspergillus* isolates

CHU Liège: dermatophytoses

- PCR dermatophytes
- Susceptibility testing of dermatophytes

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Available tests NRC for mycosis at UZ Leuven

ISOLATES

- Identification: microscopy, MALDI, ITS sequencing
- Susceptibility testing: Sensititre or EUCAST

- Beta-D-glucantest (serum): Wako

SAMPLES

- Molecular tests
  - *Pneumocystis*: confirmation context
  - *Aspergillus* (species + resistance): galactomannan + and culture -
  - Mucorales
  - Panfungal (18S)
- Typing in the context of outbreaks

***AANGEVRAAGDE TESTEN**

1056 ❑ Identificatie
Vermoedelijke identificatie :……..
1055 ❑ Gevoeligheidsbepaling
1760 ❑ Pneumocystis PCR (BAL) *(enkel ter confirmatie van dubieuze immuunfluorescentie of PCR)*
1520 ❑ Pneumocystis PCR (ASPIRAAT) *(enkel ter confirmatie van dubieuze immuunfluorescentie of PCR)*
5734 ❑ PCR Aspergillus (BIOPTEN) (species- en resistentie-detectie) *(indien rechtstreeks onderzoek positief en cultuur negatief)*
5733 ❑ PCR Aspergillus (BAL) (species- en resistentie-detectie) *(indien galactomannaan positief en cultuur negatief)*
5732 ❑ PCR Aspergillus (DIVERSE VOCHTEN) (species- en resistentie-detectie) *(indien rechtstreeks onderzoek positief en cultuur negatief)*
5805 ❑ PCR Mucorales (BAL) **
5807 ❑ PCR Mucorales (BIOPTEN) **
5806 ❑ PCR Mucorales (BLOED) **
5808 ❑ PCR Mucorales (DIVERSE VOCHTEN) **
6005 ❑ Panfungale PCR (BIOPTEN) **
5799 ❑ Panfungale PCR (DIVERSE VOCHTEN) **
5999 ❑ β-D-glucantest (SERUM)
❑ Genotypering *(enkel in geval van uitbraak)*

**** Voorafgaand contact met NRC noodzakelijk**

What’s new?

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National Reference Center Mycosis

Panfungal PCR: not for BAL!

Aanvraag panfungale PCR in het kader van referentiefunctie mycose

In het kader van onze referentiefunctie als NRC voor mycose, voeren we een panfungale PCR uit ter detectie en identificatie van fungi in normaal steriele stalen indien dit niet lukt via cultuur. Parallel met de panfungale PCR wordt indien voldoende staalvolume aanwezig is, een Aspergillus species PCR uitgevoerd gezien dit een frequent pathogeen is en deze specifieke PCR een hogere gevoeligheid heeft dan een panfungale PCR. Bij onvoldoende staalvolume, wordt enkel de panfungale PCR uitgevoerd. In het kader van onze referentieactiviteit willen we echter vragen enkel biopten en normaal steriele lichaamsvochten (dus geen BAL vochten) op te sturen voor deze analyse, enkel indien microscopisch de aanwezigheid van fungi aangetoond werd. Uit eigen ervaring en literatuur weten we dat de kans om een fungaal pathogeen te detecteren en te identificeren buiten deze context heel klein is. We hopen met deze richtlijnen duidelijkheid te brengen over de context waarin deze analyse aangevraagd kan worden. Meer informatie kan je vinden op de website van Sciensano of in onze labogids.

prof. apr. klin. biol. Katrien Lagrou

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Mucorales PCR – MucorGenius (Pathonostics)

- Multiplex real-time PCR for the **detection of Mucorales species DNA** (*Rhizopus* species, *Mucor* species, *Lichtheimia* species, *Cunninghamella* species and *Rhizomucor* species = 90% of the Mucorales species that cause invasive mucormycosis infections)

- For specimen types: BAL, biopsies, blood and normal sterile fluids

- Diagnosis of **mucormycosis**
- **High mortality rate** in immunocompromised patients
- **Difficult diagnosis**
- **Frequent co-infection with *Aspergillus* species**: same extract for *Aspergillus* and *Mucorales* PCR

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Mucorales PCR on serum: good performance for diagnosis of mucormycosis

Several commercial assays available

Multicenter prospective evaluation in patients with suspicion of invasive mould disease (host factor, suggestive imaging and clinical symptoms) in 9 university hospitals in France (n= 232)

- Screening twice weekly with **mucorales PCR on serum samples**

Good performance of qPCR detection of circulating DNA in serum:

85% sensitivity; 90% specificity

Early marker: positive 4 days before mycological or histopathological examination

12% mixed infection

13/40 patients with mucormycosis had a mixed Aspergillus/Mucorales infection

100% mortality rate if qPCR remains positive despite appropriate antifungal treatment

L. Millon et al. CID 2022

The rise of *Candida auris* infections…

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National Reference Center Mycosis

Rise of *Candida auris* infections

Source: Meijer et al. 2022

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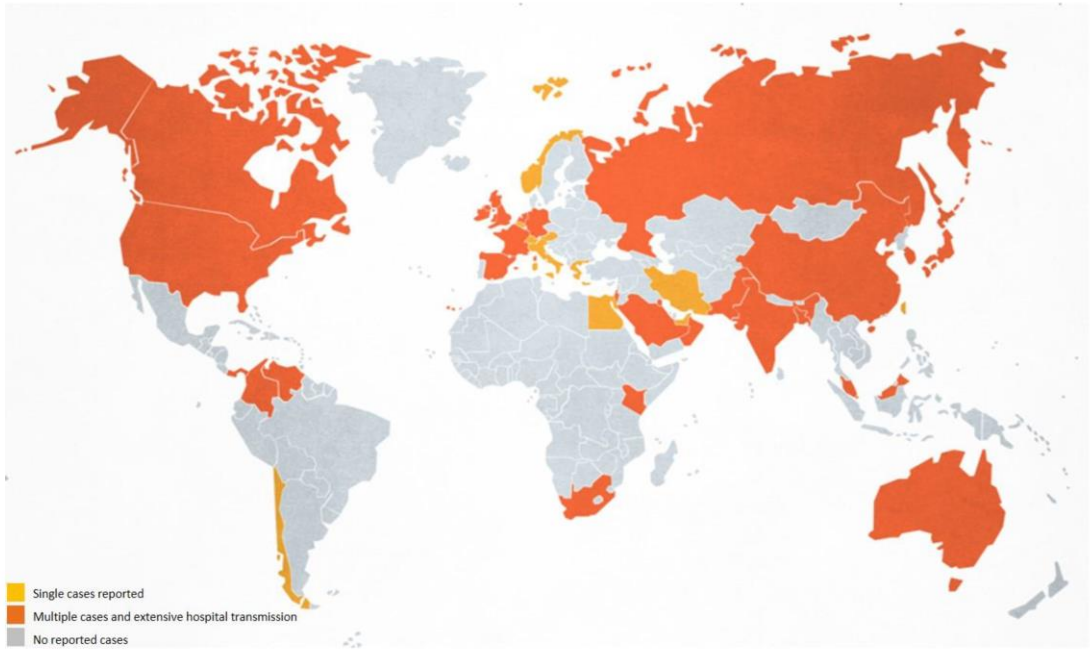
National Reference Center Mycosis

Candida auris outbreaks and endemicity in Europe

Rapid risk assessment: Candida auris outbreak in healthcare facilities in northern Italy, 2019 2021

Risk assessment

21 Feb 2022



RAPID COMMUNICATION

Increasing number of cases and outbreaks caused by *Candida auris* in the EU/EEA, 2020 to 2021

Anke Kohlenberg[1] , Dominique L Monnet[1] , Diamantis Plachouras[1] , *Candida auris* survey collaborative group[2]
1. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
2. The members of the *Candida auris* survey collaborative group are listed under Collaborators and at the end of the article

Source: Website ECDC, Sabino et al. 2020 and Geremia et al. 2023

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Candida auris also in Belgium

Voor u uitgelegd Candida auris

DeMorgen.

Schimmel 'Candida auris' aan wereldwijde opmars bezig: 'Ook bij ons is er verhoogde waakzaamheid'

'Ziekenhuisschimmel' *Candida auris* is aan een wereldwijde opmars bezig. Het aantal gevallen blijft bij ons tot dusver beperkt, maar waakzaamheid is volgens experts ook in België geboden. Candida auris heeft twee eigenschappen die de schimmel extra problematisch maken.

DIETER DE CLEENE 27 maart 2023, 03:00

Een rusthuis in New York. Volgens de gezondheidsinstanties verspreidt Candida auris zich in de VS aan 'alarmerend' tempo. Beeld NYT

Newsflash Belgium – recommendations RAG and NRC

Candida auris (March 2023)

Now in Belgium, **8 patients** were isolated from Candida auris (between 2016 and 2022), but in two patients, no link with a foreign hospital residence could be found, for a case at the end of 2021 and one during the first half of 2022. Before the end of 2021, it was always an imported infection. This finding, together with the reporting of an increasing number of Candida auris cases and outbreaks in other countries in Europe, requires additional vigilance for the detection and follow-up of *C. auris* fallen.

C. auris has an unusual ability to persist in the surrounding area and in asymptomatic patients which has contributed to outbreaks in different hospitals. In addition, this yeast is potentially multiresistant. Adequate isolation measures are important for confirmed clinical cases (see final global report Quality Control Sciensano enquête 2018/2 (external link)).

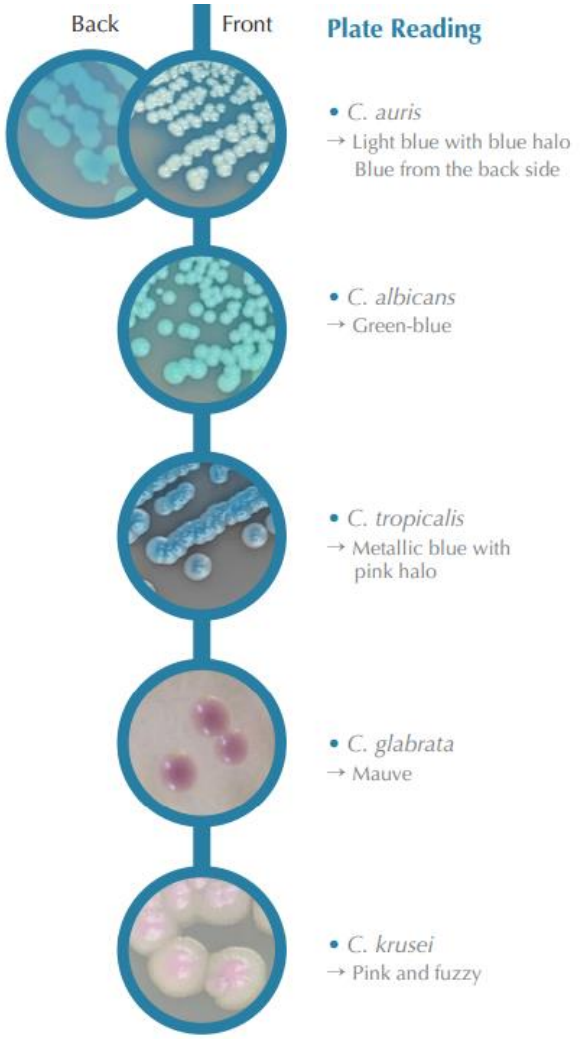
Recommendations Belgian Risk Assessment Group and National Reference Center

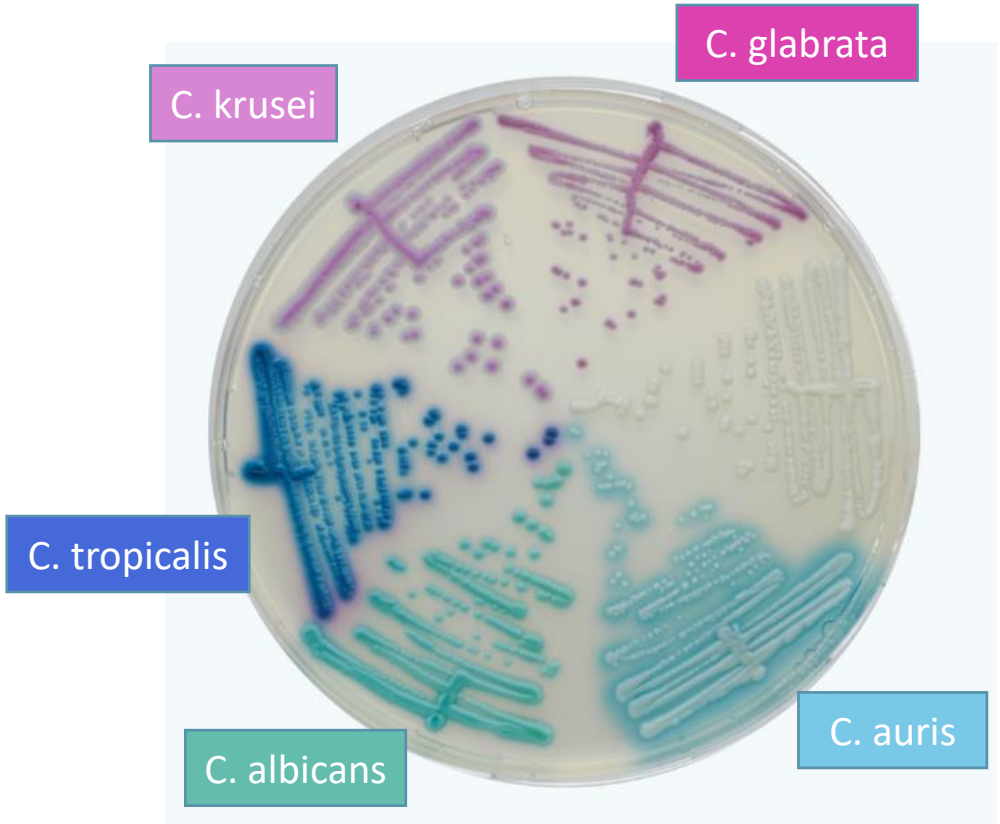
- **Always identify yeasts from normally sterile samples at species level.** In case of identification problems, isolates can be sent to the National Reference Center for Mycosis (UZ Leuven ou CHULiège).
- **Send all yeasts identified as Candida auris to the NRC for confirmation of identification and sensitivity determination.**
- **Send all yeasts identified as Candida haemulonii or Candida pseudohaemuloniii to the NRC.**
- Send all yeasts that are resistant to fluconazole (except for Candida glabrata and Candida stool) to the NRC.

Hospitals facing an outbreak of *C. auris* (i.e. two or more cases with a potential link in time, place, or person) are asked to involve the Outbreak Support Team (Ost). Contact can be made through the regional infection control teams.

Source: Website Sciensano NRC page mycosis

Implementation CHROMagar™ Candida plus





Source: Website Chromagar

Candida auris cases in Belgium

- Communication by Agentschap Zorg & Gezondheid Vlaanderen (24/4/2023): awareness and mandatory notification outbreak setting
- Total of 9 *Candida auris* cases evaluated at the NRC mycosis, UZ Leuven

Year of isolation	Age category	Gender	Infection status	Sample type	Import
2016	50-60	F	Infection	Blood	Yes
2019	60-65	M	Colonization	Urine	Yes
2019	50-60	M	Colonization	Wound	Yes
2019	50-60	F	Colonization	Wound	Yes
2021	50-60	M	Infection	Blood	No
2022	>65	F	Colonization	Urine	No
2022	50-60	M	Infection	Blood	Yes
2022	>65	F	Infection	Blood	Yes
2023	20-30	F	Colonization	Wound	Yes

Not al Belgian cases were imported…

1 Kuwait

infection

1 Kuwait

colonisation

1 S Africa

colonisation

1 India

colonisation

1 Belgium

infection

1 Kuwait

infection

1 Belgium

colonisation

1 Albania

infection

1 Rhodes

colonisation

2016	2017	2018	2019	2020	2021	2022	2023

Susceptibility testing of *Candida auris*

Year	Fluconazole	Amphotericine B	Voriconazole	Anidulafungine	Posaconazole	Itraconazole	Flucytosine
2016	>64	0.50	>4	0.125	>4	>4	0.25
2019	128	1	1	0.50	0.03	0.13	0.13
2019	>128	0.50	>8	8	>8	>8	1
2019	>128	1	2	0.50	0.03	>8	0.13
2021	32	0.25	0.13	0.25	0.06	8	0.06
2022	>128	1	2	1	0.13	0.25	>64
2022	>128	0.50	4	1	0.13	0.25	>64
2022	128	0.50	0.50	0.50	0.03	0.13	0.13
2023	>128	1	0.50	4	0.016	0.064	0.25

Better to be prepared

- Newsflash NRC mycosis
- Mandatory notification of *Candida auris* cases
- Implementation CHROMagar™ Candida plus
- Set-up whole genome sequencing approach (long-read sequencing ONT) and pipeline for potential future outbreaks
- Screening of wastewater (region Leuven) for *Candida auris*

National surveillance study azole resistance in invasive aspergillosis (2022 – 2023)

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National Reference Center Mycosis

National surveillance study 2011-2012

- **One-year** prospective surveillance study
- Participation of **18 hospitals**
- **220 isolates from 182 patients**

Journals.ASM.org

Nationwide Surveillance of Azole Resistance in *Aspergillus* Diseases

Edith Vermeulen,[a] Johan Maertens,[a,b] Annelies De Bel,[c] Eric Nulens,[d] Jerina Boelens,[e] Ignace Surmont,[f] Anna Mertens,[g] An Boel,[h] Katrien Lagrou[a,i]

Department of Microbiology and Immunology, Catholic University Leuven, Leuven, Belgium[a]; Department of Haematology, University Hospitals Leuven, Leuven, Belgium[b]; Department of Microbiology and Infection Control, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel (VUB), Brussels, Belgium[c]; Department of Microbiology, AZ Sint-Jan Brugge-Oostende AV, Bruges, Belgium[d]; Department of Microbiology, Ghent University Hospital, Ghent, Belgium[e]; Department of Microbiology, Heilig Hart Ziekenhuis Roeselare-Menen, Roeselare, Belgium[f]; Department of Microbiology, ZNA Hospitals site Middelheim, Antwerp, Belgium[g]; Department of Clinical Microbiology, Onze-Lieve-Vrouwziekenhuis, Aalst, Belgium[h]; National Reference Center for Mycosis, Department of Laboratory Medicine, University Hospitals Leuven, Leuven, Belgium[i]

Disease (number of patients)

- Invasive aspergillosis (122)

- ABPA (39)
- Chronic pulmonary aspergillosis (10)
- Aspergillus bronchitis (7)
- Aspergilloma (5)

triazole resistance %

4.6%

7.0%

5.5%

- **6 TR34/L98H**
- **1 TR46/Y121F/T289A**
- **2 non-Cyp51A**

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National surveillance study 2022-2023

- **One-year** prospective surveillance study: April 2022 – March 2023
- Focus solely on **invasive aspergillosis**

- All Belgian laboratories invited to send *Aspergillus species* complex isolates cultured from clinical samples, **when judged clinically relevant according to consensus definitions**
  - EORTC/MSGERC
  - (modified) *Asp*ICU
  - Expert case definitions for IAPA
  - COVID ECMM/ISHAM criteria
- Request to send **all clinically relevant isolates, to prevent selection bias**

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Consensus definitions on invasive aspergillosis

Defining pulmonary aspergillosis (in addition to Table 2 of Koehler *et al.)*

	Host factors	Clinical factors	Mycological factors
Proven	-		Direct histopathology or microscopy of fungal elements Positive culture (on sterile site) Positive PCR (on sterile site)
EORTC/MSGERC Probable	Neutropenia Haematological malignancy AlloHSCT Solid organ transplant Prolonged corticosteroids Immunosuppressants Inherited severe immunodeficiency GVHD	CT findings or tracheobronchitis	Microscopy Culture Galactomannan* or LFA (blood, BAL) PCR (blood, BAL)
***Asp*ICU**	ICU patient (not necessary if BAL microscopy and culture are positive)	Compatible signs and symptoms and abnormal medical imaging chest X-ray or CT scan	Positive culture Modified AspICU: positive galactomannan*
IAPA	Patient with influenza requiring ICU admission	Tracheobronchitis, pulmonary or cavitating infiltrate	Microscopy Culture Galactomannan* (blood, BAL)
CAPA	Patient with COVID requiring ICU admission	Tracheobronchitis, pulmonary or cavitating infiltrate	Microscopy Culture Galactomannan* or LFA (blood, BAL) PCR (blood, BAL)

* Galactomannan optimal cut-off values dependent on the specimen type: ≥0.8-1 for BAL, while >0.5 for serum

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Study application form

UZ LEUVEN | LABORATORIUMGENEESKUNDE

ASPERGILLUS SURVEILLANCE 2022 (LWS study 865)

Paper form needs to accompany the isolate to the 8th floor!

Information necessary for internal registration:

UZL lab: Maak een nieuw pseudo-ID aan en geef ook geboortedatum en geslacht in

Sample ID (*LWS UZ Leuven veld "naam"*): .....

Date of birth (*dd/mm/yyyy*): ..... Gender: ☐ M or ☐ F

Sample isolate date (*dd/mm/yyyy*): .....

Name of clinical biologist (*LWS UZ Leuven veld "voorschrijver"*): .....

Name of the laboratory: .....

Contact details: .....

Requested tests: ☒1056 (identificatie) ☒ 1055 (gevoeligheidsbepaling EM gekweekte fungus)

Results will be send to you according to your standard delivery preferences in our LWS

Isolate information:

Morphologic species complex identification: ☐ *A. fumigatus complex* ☐ *A. flavus complex*

☐ *A. niger complex* ☐ *A. terreus complex* ☐ *unknown* ☐ *other:* .....

Origin of the sample (BAL, sputum, etc): .....

In case susceptibility test performed: Method: ..... Results: .....

Patient information:

Underlying disease: .....

Clinical relevance of *Aspergillus spp.* (cultured):

- ☐ Invasive disease: proven
- ☐ Invasive disease: probable disease (EORTC/MSGERC criteria)
- ☐ Influenza-associated pulmonary aspergillosis (IAPA)
- ☐ COVID-19-associated pulmonary aspergillosis (CAPA)
- ☐ Invasive aspergillosis in critically ill patients (Modified *Asp*ICU criteria)

Antifungal therapy initiated:

Date of initiation: ..... Antifungal drug(s) initiated: .....

Antifungal drugs given during the last year (specify antifungal drug and treatment period):

☐ Antifungal prophylaxis: ..... → period: .....

☐ Antifungal treatment: ..... → period: .....

Sample ID (*LWS UZ Leuven veld "naam"*): .....

Date of birth (*dd/mm/yyyy*): ..... Gender: ☐ M or ☐ F

Sample isolate date (*dd/mm/yyyy*): .....

Isolate information:

Morphologic species complex identification: ☐ *A. fumigatus complex* ☐ *A. flavus complex*

☐ *A. niger complex* ☐ *A. terreus complex* ☐ *unknown* ☐ *other:* .....

Origin of the sample (BAL, sputum, etc): .....

Patient information:

Underlying disease: .....

Clinical relevance of *Aspergillus spp.* (cultured):

- ☐ Invasive disease: proven
- ☐ Invasive disease: probable disease (EORTC/MSGERC criteria)
- ☐ Influenza-associated pulmonary aspergillosis (IAPA)
- ☐ COVID-19-associated pulmonary aspergillosis (CAPA)
- ☐ Invasive aspergillosis in critically ill patients (Modified *Asp*ICU criteria)

Antifungal therapy initiated:

Date of initiation: ..... Antifungal drug(s) initiated: .....

Antifungal drugs given during the last year (specify antifungal drug and treatment period):

☐ Antifungal prophylaxis: ..... → period: .....

☐ Antifungal treatment: ..... → period: .....

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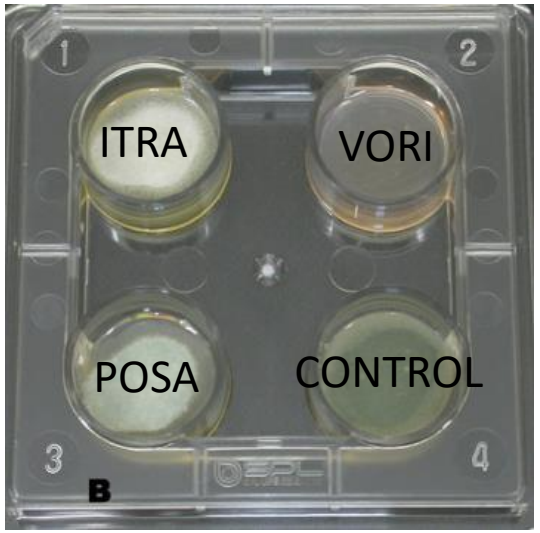
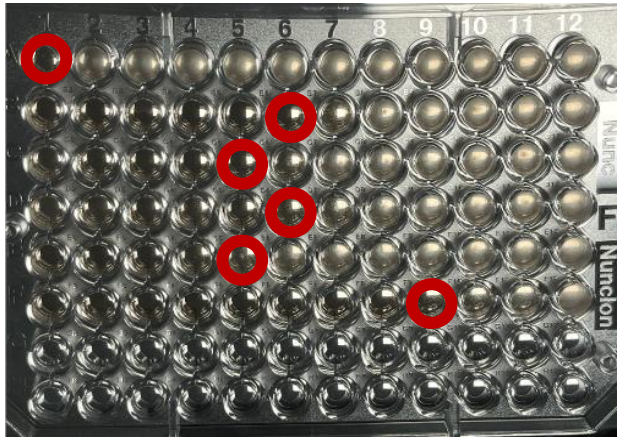
Triazole resistance detection in *Aspergillus* species



Triazole resistance screening agar for *Aspergillus fumigatus* (VIPcheck®)

AMPHO B
VORI
POSA
ITRA
ISA



Broth microdilution reference method (EUCAST)

CYP51A sequencing

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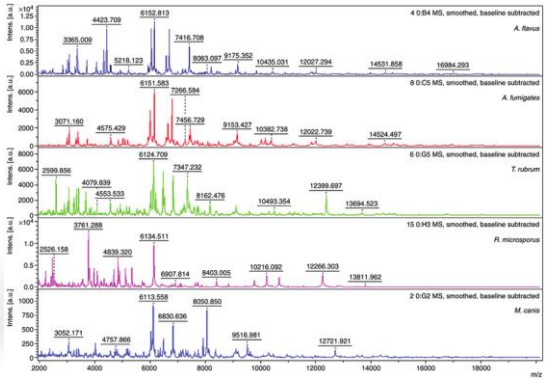
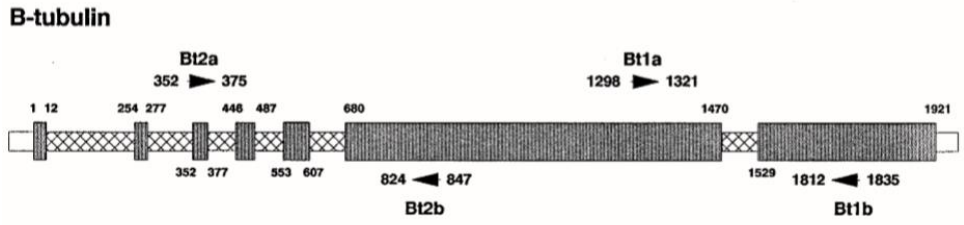
Mycosis

Identification of all isolates to species level



MALDI-TOF mass spectrometry



β-tubulin **sequencing**

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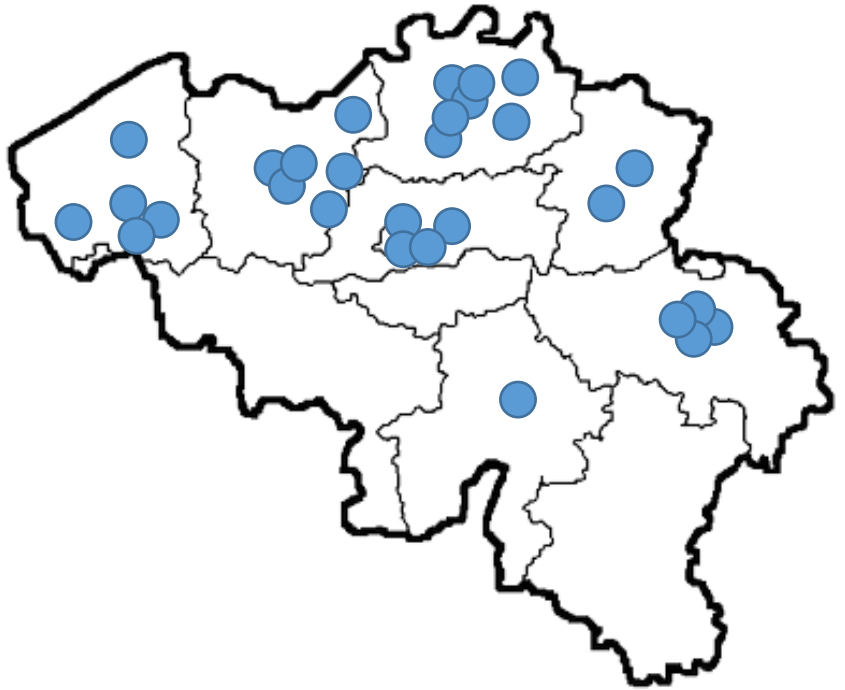
National Reference Center Mycosis

National surveillance study 2022-2023: dataset

- Participation of **29 hospitals**
- **325 isolates** (+ 2 still under evaluation for inclusion criteria)



- Median age: 66 years – 60.9% male
- Mainly respiratory samples: 43.7% BAL, 23.1% bronchial aspirate and 20.6% sputum
- **Classification of invasive disease**:
  - Proven 4%
  - Probable 55.1%
  - Putative 16.6%
  - CAPA 17.5%
  - IAPA 6.8%

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National Reference Center Mycosis

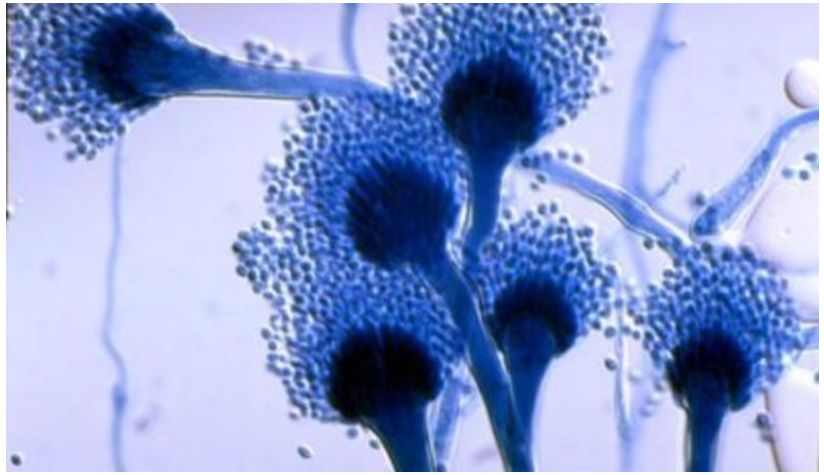
National surveillance 2022-2023: triazole resistance

- **Identification** to species complex level – MALDI and β-tubulin sequencing ongoing:
  - ***A. fumigatus* species complex 90.5%**
  - *A. niger* complex 4.3%
  - *A. flavus* complex 2.8%

Aspergillus flavus *Aspergillus fumigatus* *Aspergillus niger*

- **Triazole resistance** screening for 294 *A. fumigatus* species complex isolates: **9.9% !!!**
  - **69% TR34/L98H**
  - 20.7% TR46/Y121F/T289A

- Ongoing detailed data analysis



Source: Microbe Online and website Adama

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Thanks to…

National reference center for mycosis

Rita Merckx
Veerle Gerils
Robina Aerts
Otto Van de Gaer
Isabelle Devenyns
Joke Kayenbergh
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