Pr Boualem Sendid

Inserm U1285, CNRS UMR 8576
Institut de Microbiologie- CHU de Lille

DU d'Antibiothérapie et Chimiothérapie Anti-Infectieuse, 2023-2024

Epidemiology of Invasive Fungal Infections

Open Forum Infectious Diseases

MAJOR ARTICLE

Epidemiology and Clinical Features of Invasive Fungal Infection in a US Health Care Network

Brandon J. Webb,[1,2] Jeffrey P. Ferraro,[3,4] Susan Rea,[3] Stephanie Kaufusi,[3,5] Bruce E. Goodman,[3,5] and James Spalding[6]

Total of 3374 IFI episodes occurred in 3154 patients. The mean incidence of IFI was 27.2 cases/100 000 patients per year.

Table 1. Incidence (per 100 000 Patients) of IFI by Category and Year

	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2006–2015
All IFIs	30.2	27.2	23.2	25.4	24.3	26.7	28.7	28.1	26.1	31.8	27.2
Candida	18.3	15.1	14.9	16.3	17.2	17.4	16.4	17.2	14.4	19.5	15.0
Other yeast/yeast like	0.4	0.3	0.4	0.3	0.1	0.3	0.2	0.3	0.3	0.3	0.4
Cryptococcus	0.4	0.1	0.3	0.2	0.2	0.3	0.4	0.5	0.7	0.1	0.3
Aspergillus	2.4	2.8	1.4	1.8	1.9	2.4	3.3	2.7	2.9	2.5	2.4
Other hyaline mold	0.4	0.2	0.3	0.0	0.2	0.3	0.5	0.3	0.2	0.1	0.2
Mucorales	0.5	0.2	0.2	0.2	0.1	0.1	0.5	0.9	0.2	0.1	0.3
Dimorphic fungi	8.1	7.8	6.1	7.0	4.6	6.3	7.4	6.6	5.9	8.7	6.9
Dematiaceous mold	0.3	0.4	0.2	0.3	0.5	0	0.3	0.1	0.2	0.1	0.2

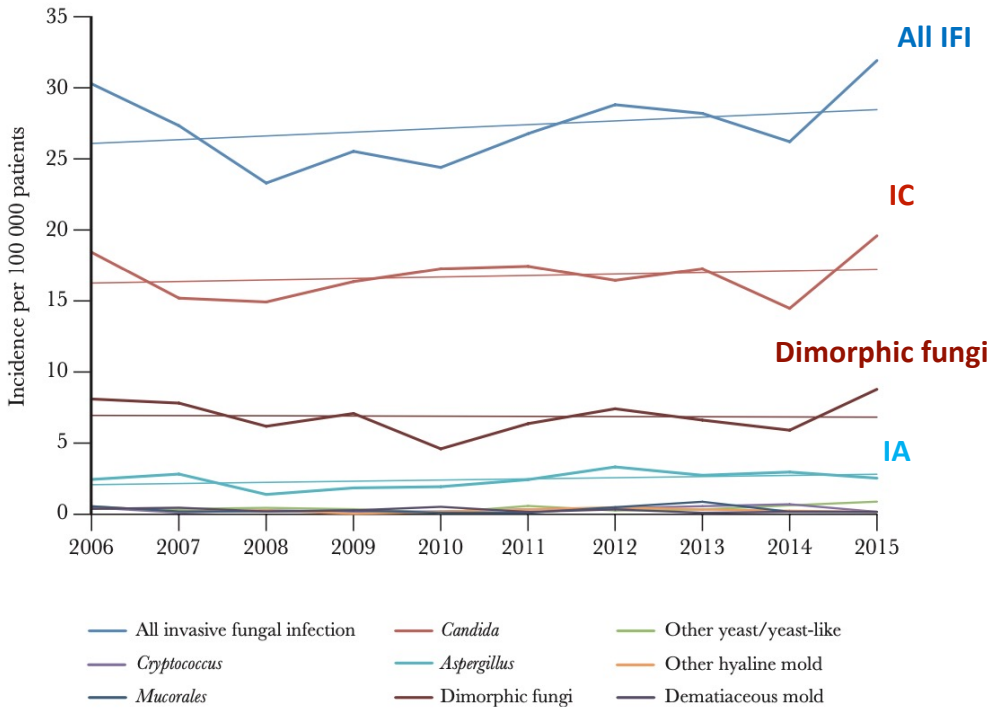


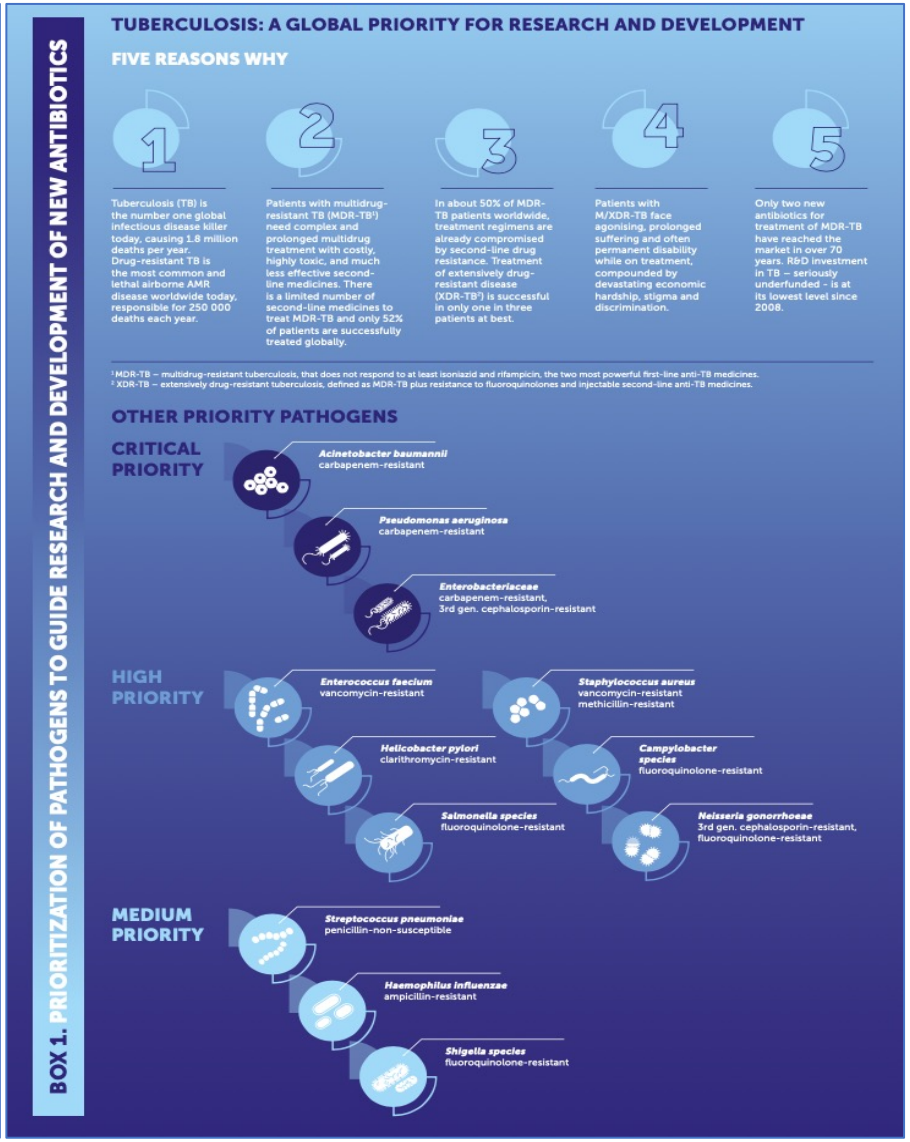
Figure 1. Incidence of invasive fungal infection (IFI) by category (per 100 000 patients).

Webb BJ *et al. Open Forum Infect Dis*. 2018

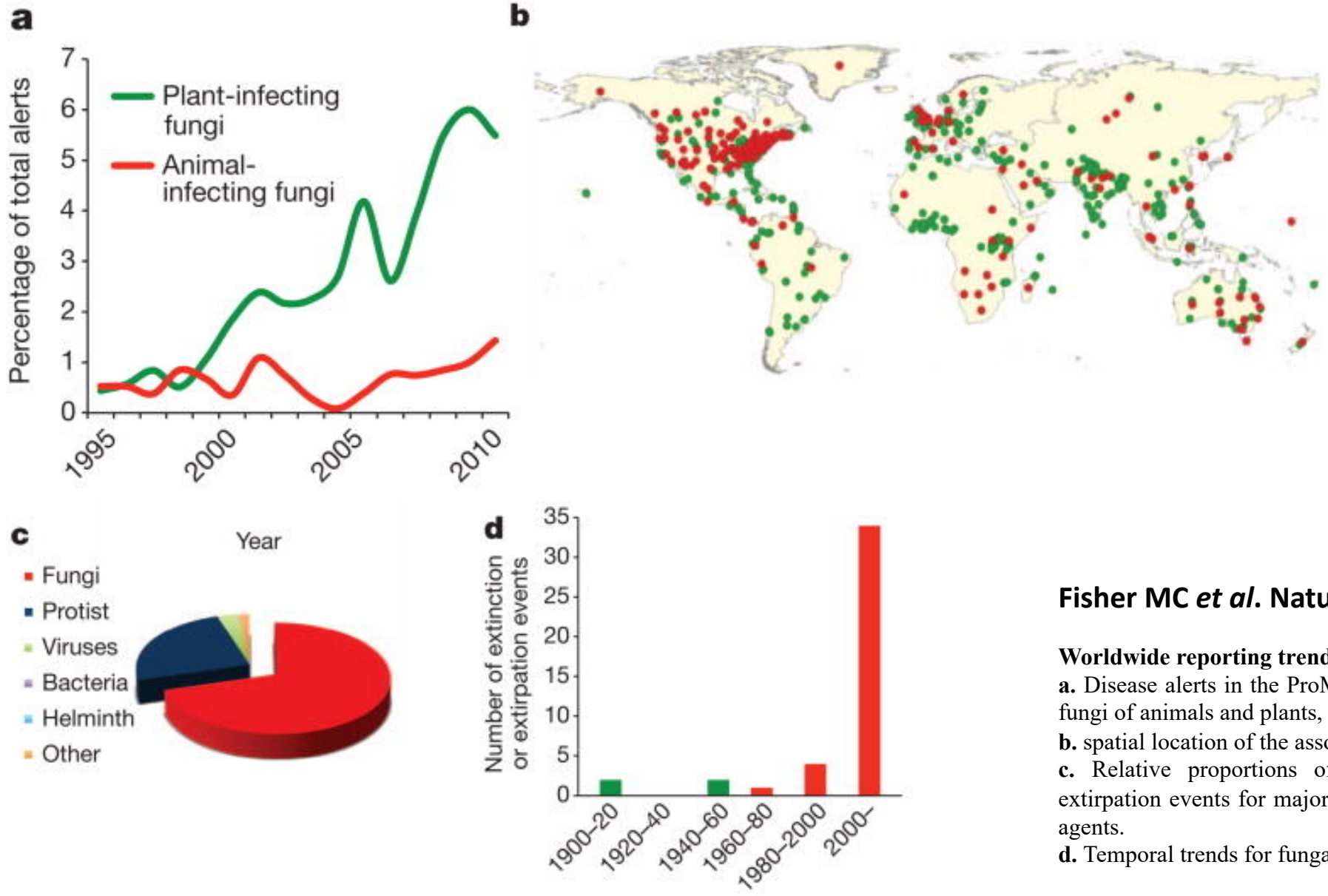
World Health Organization

WHO fungal priority pathogens list to guide research, development and public health action

Critical group	High group	Medium group
Cryptococcus neoformans	*Nakaseomyces glabrata (Candida glabrata)*	*Scedosporium* spp.
Candida auris	*Histoplasma* spp.	*Lomentospora prolificans*
Aspergillus fumigatus	Eumycetoma causative agents	*Coccidioides* spp.
Candida albicans	Mucorales	*Pichia kudriavzeveii (Candida krusei)*
	Fusarium spp.	*Cryptococcus gattii*
	Candida tropicalis	*Talaromyces marneffei*
	Candida parapsilosis	*Pneumocystis jirovecii*
		Paracoccidioides spp.



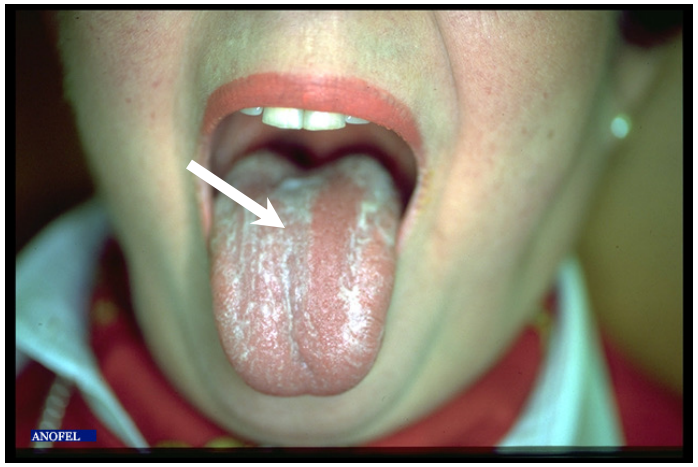
Emerging Fungal Threats to Animal, Plant and Ecosystem Health



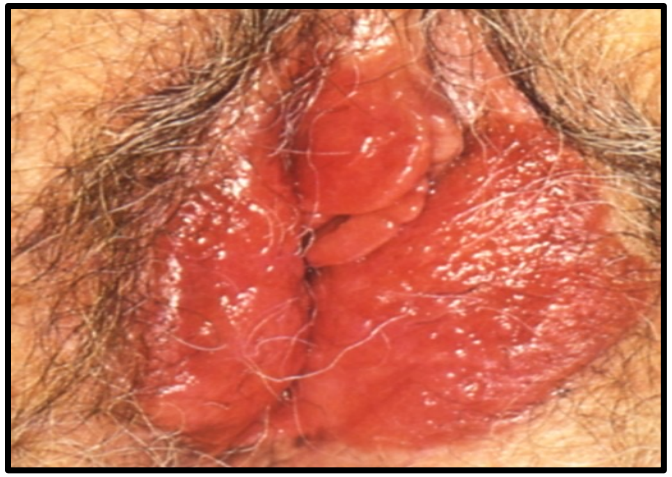
Fisher MC *et al*. Nature. 2012

Worldwide reporting trends in fungal EIDs.
a. Disease alerts in the ProMED database for pathogenic fungi of animals and plants, and the
b. spatial location of the associated reports.
c. Relative proportions of species extinction and/or extirpation events for major classes of infectious disease agents.
d. Temporal trends for fungal pathogens

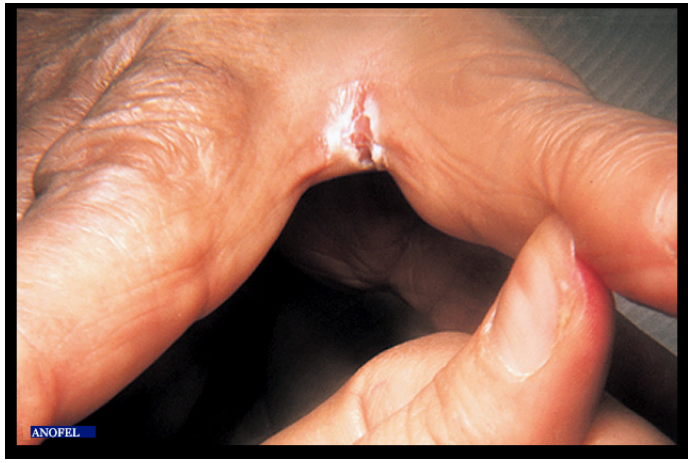
Mycoses superficielles



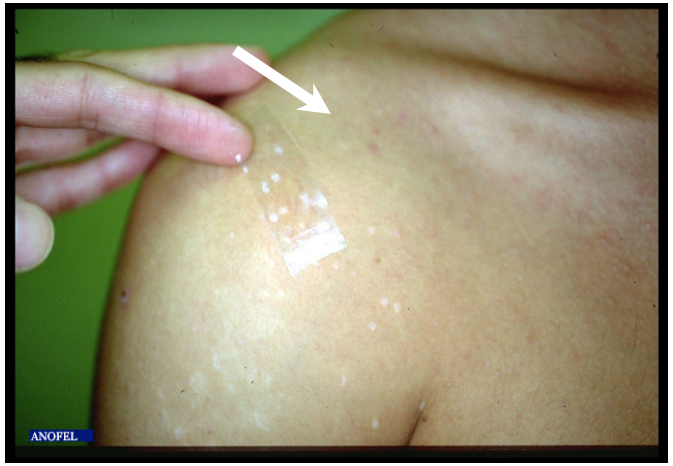
Candidose buccale



Candidose génitale

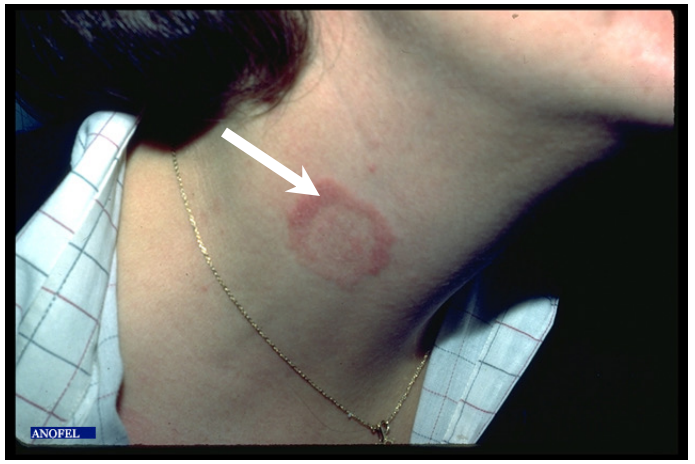


Candidose interdigito-palmaire



Pityriasis versicolor

Pityriasis versicolor

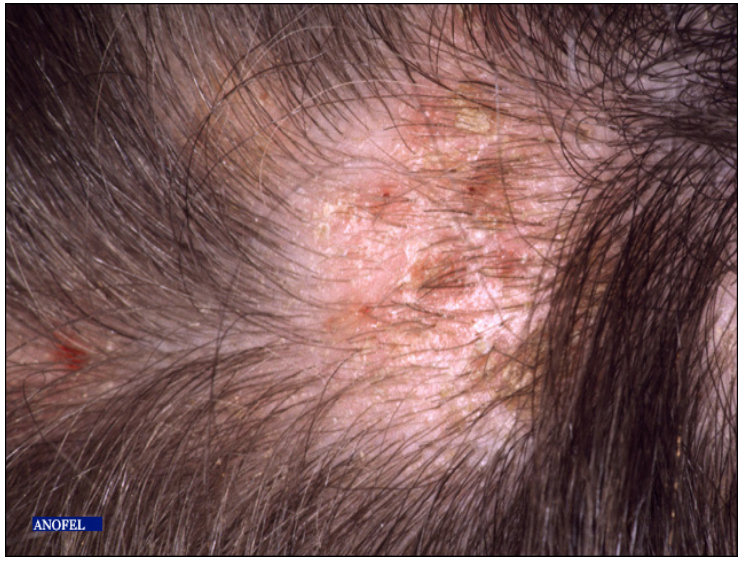


Herpes circiné

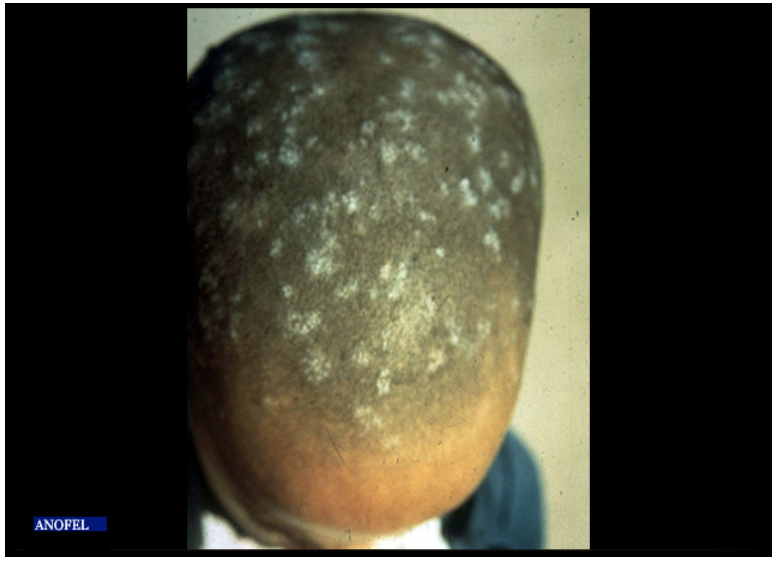
Mycoses superficielles

Atteintes à dermatophytes

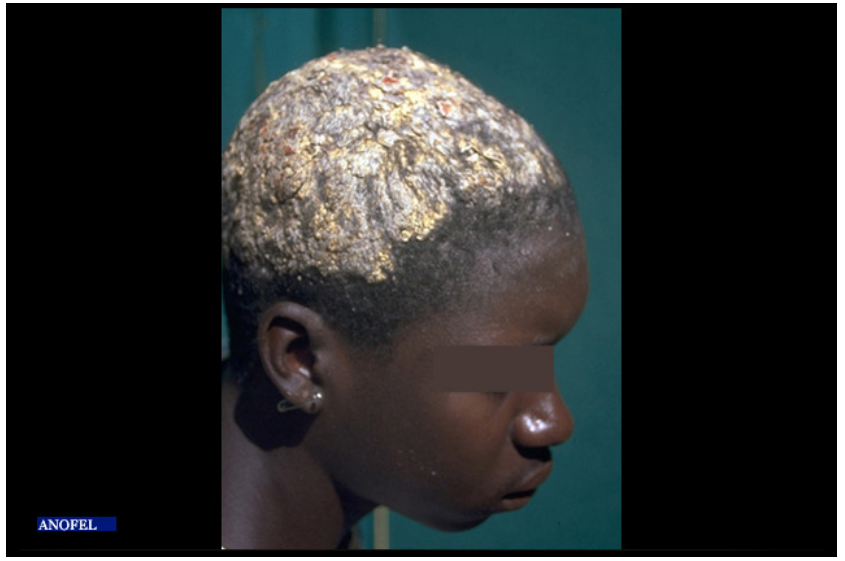
Teignes microsporiques



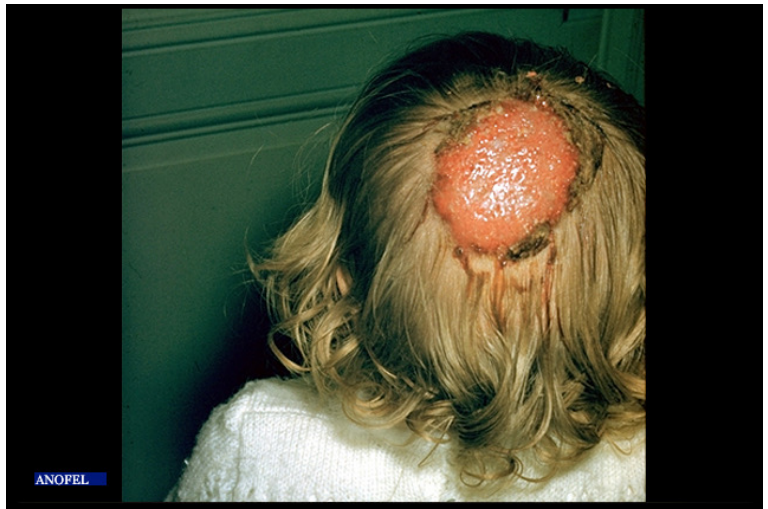
Teignes trichophytiques



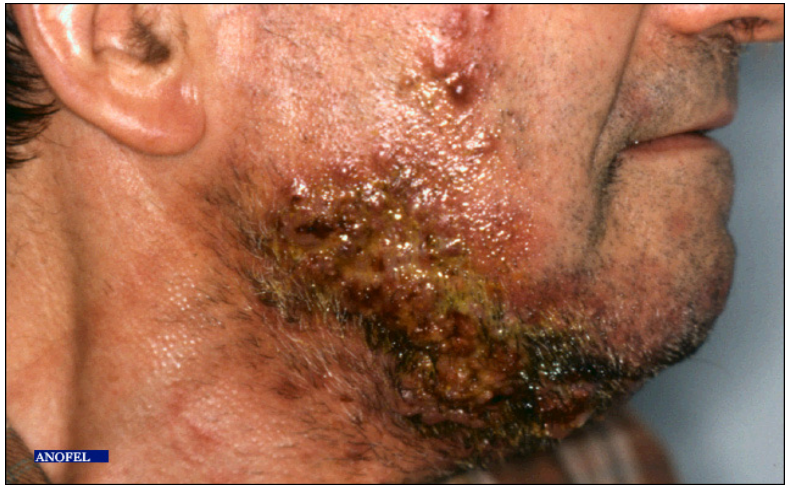
Favus



Teignes inflammatoires: kérion



Teignes inflammatoires: sycosis

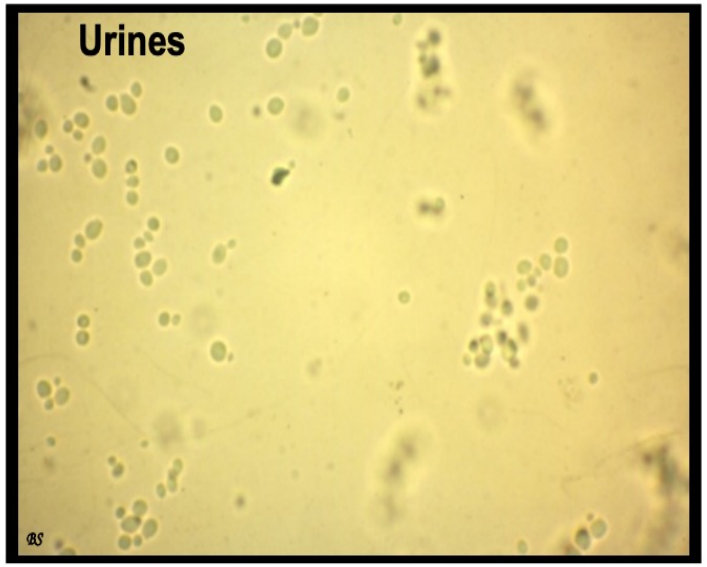


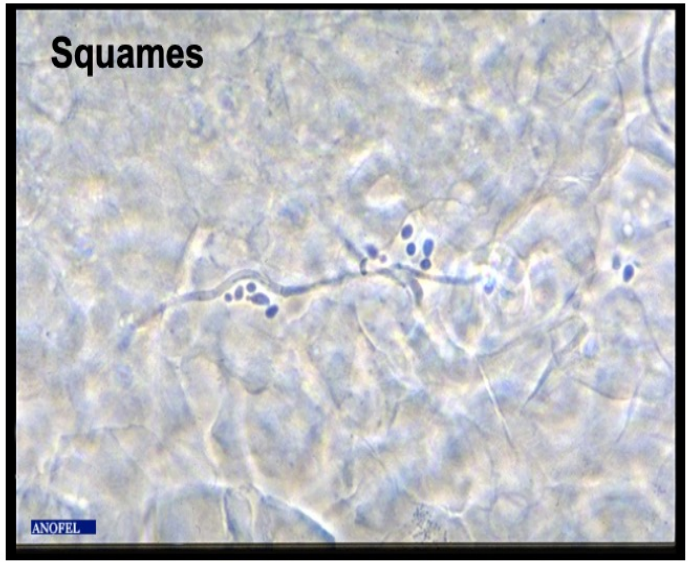
Diagnostic des mycoses superficielles

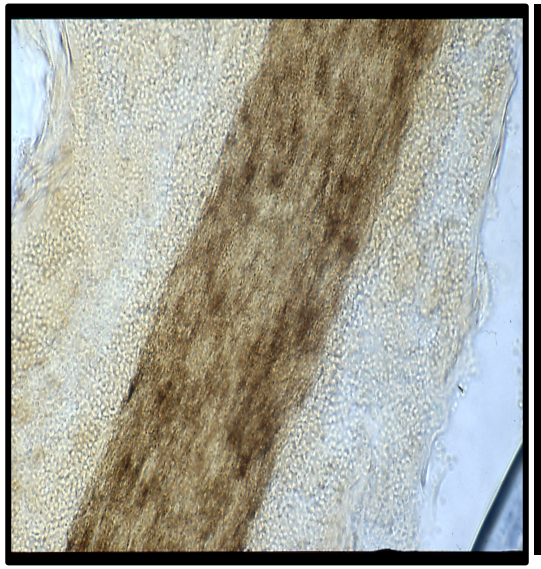
Diagnostic aisé fondé sur les méthodes conventionnelles :

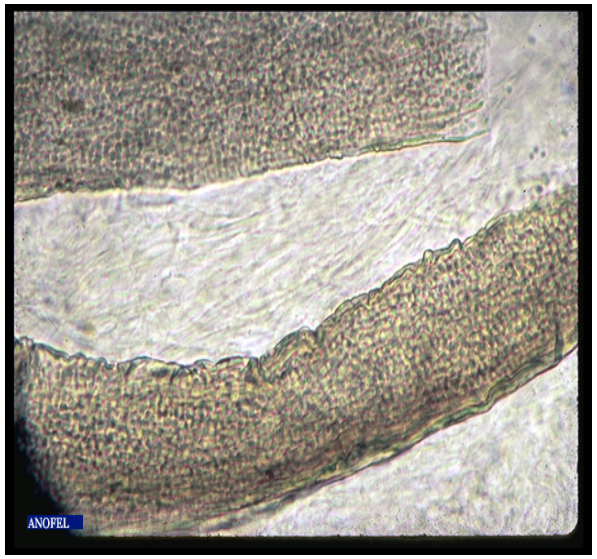
- Prélèvements (écouvillons, squames, cheveux, ongles…)
- Examen direct (éléments morphologiques d'orientation)
- Mise en culture (isolement de l'agent pathogène)
- Identification (Méthodes phénotypiques, MALDI-TOF)

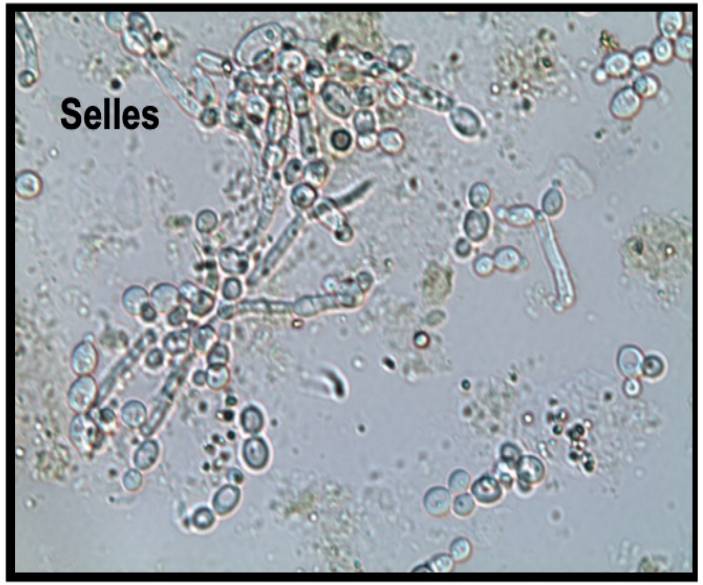
Examen direct à l'état frais ou après coloration

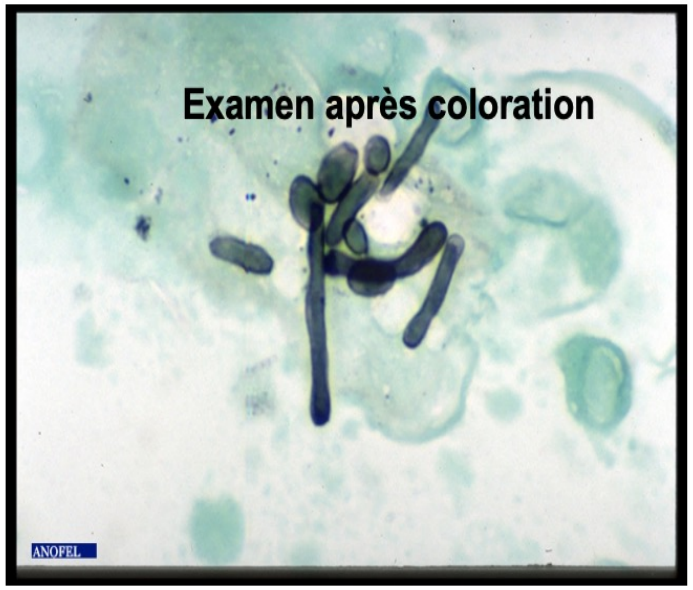


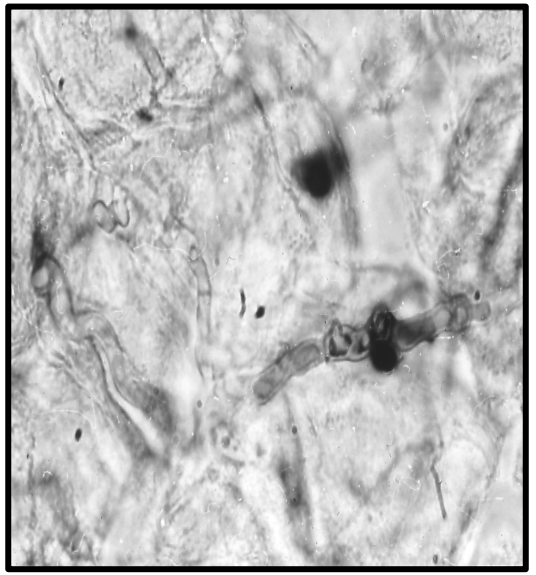


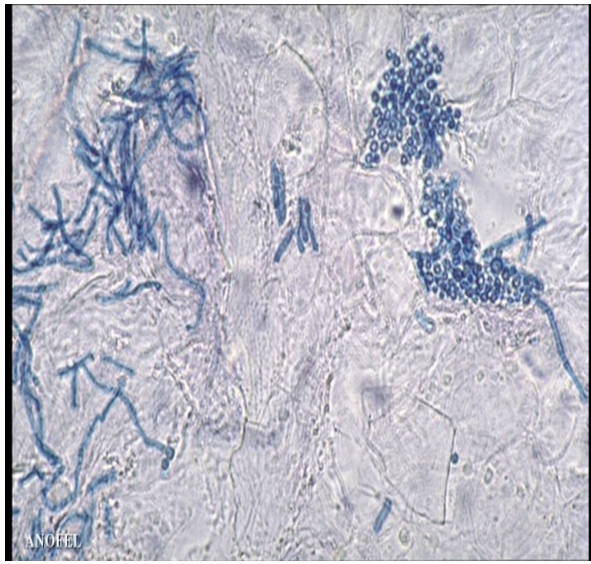






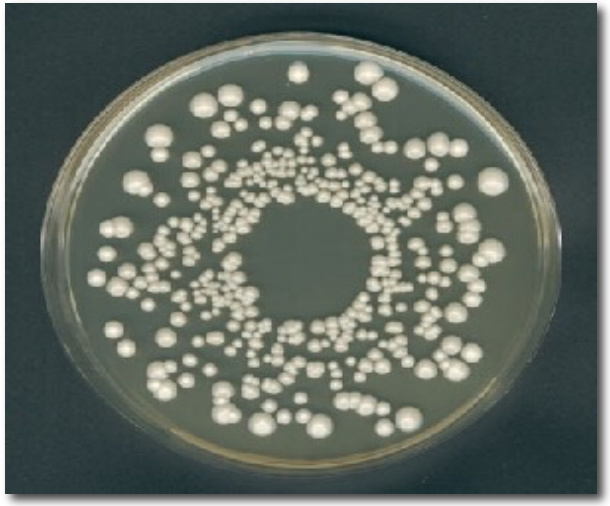


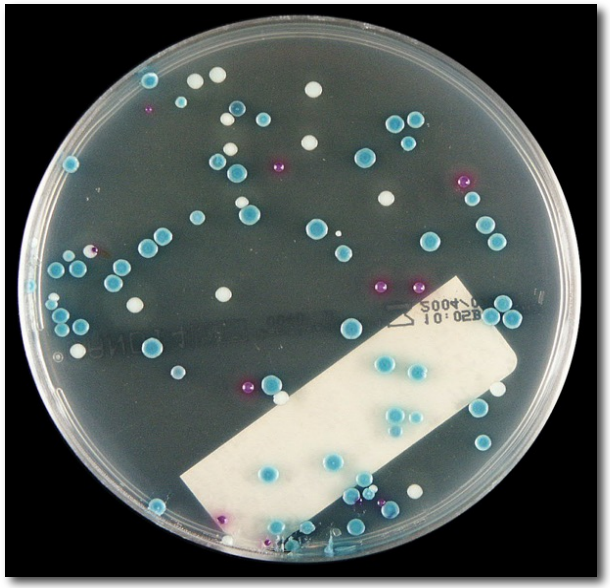




Identification présomptive : culture sur milieu chromogéniques

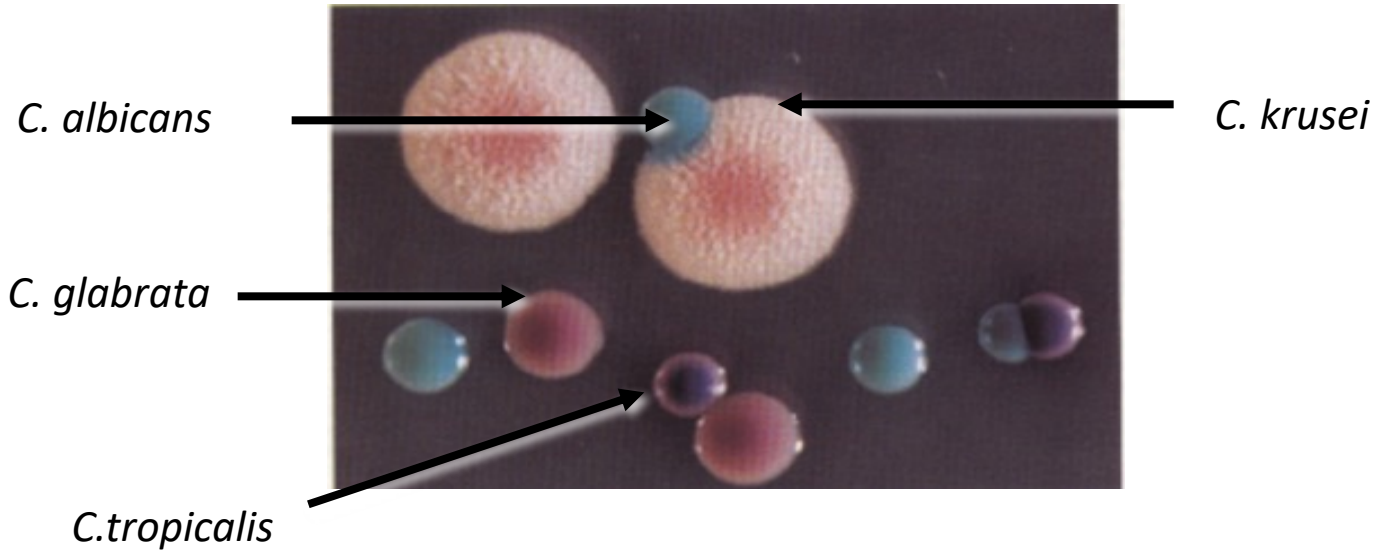
Milieu Sabouraud





Milieu Candi*Select*4 ®

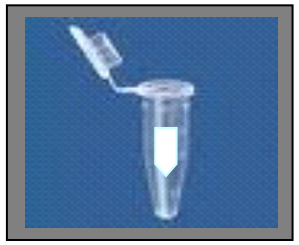
Avantages
- Isolement et identification des 4 principales espèces
- Détection des mélanges d'espèces
- Différents milieux : CHROMagar, CandiSelect4 Candichrom, albicans ID, fluoroplate…

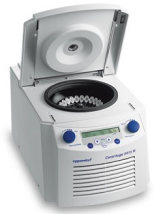


Milieu CHROMagar™ Candida

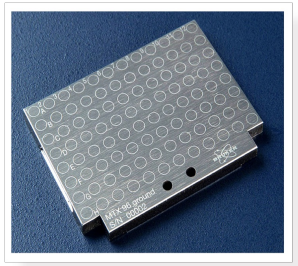
Identification par spectrométrie de masse

Extraction acide formique
Acétonitrile





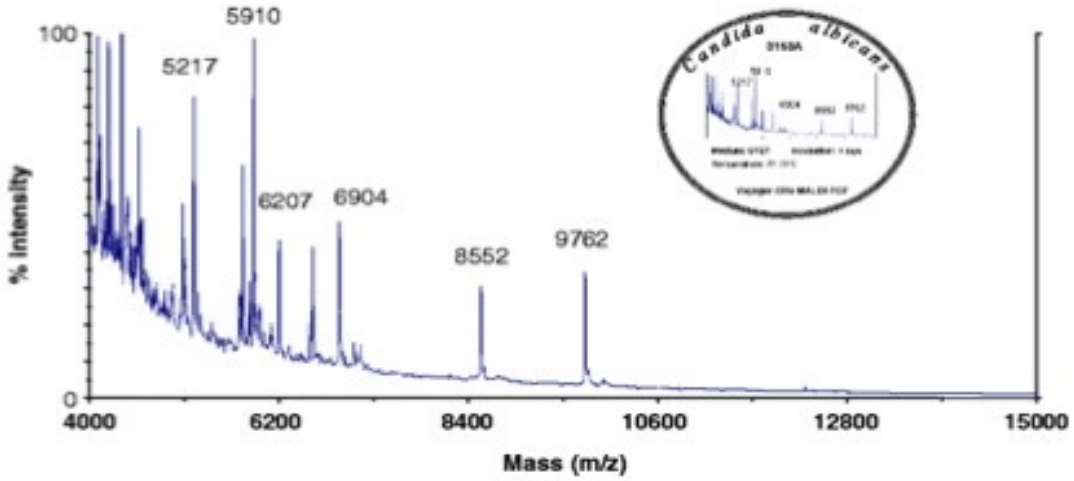
Extrait + matrice



Analyse

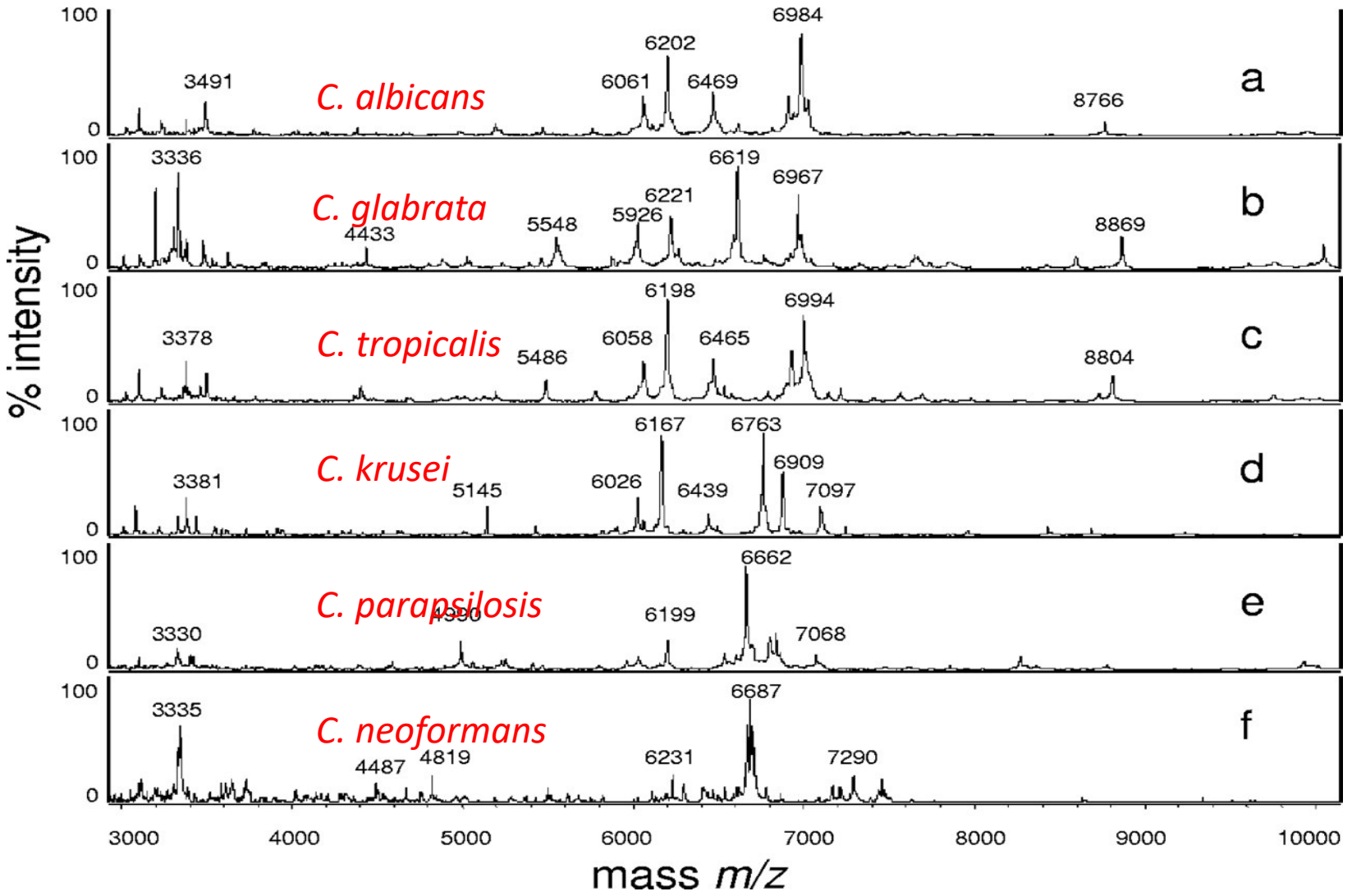
15 min

MALDI-TOF BiFlex III®
(Bruker Daltonics™)



Profil peptidique spécifique d'espèce

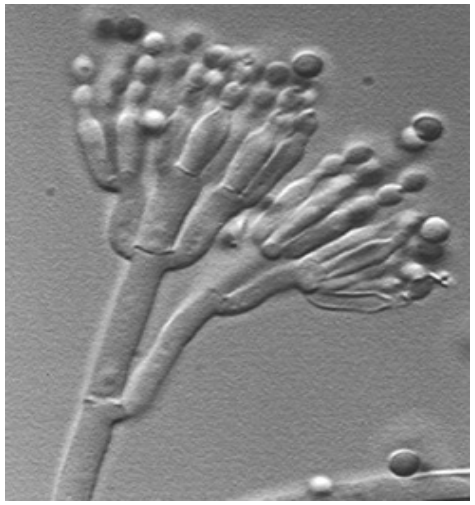
Identification par spectrométrie de masse (MALDI-TOF: Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry)



Examen microscopique des cultures

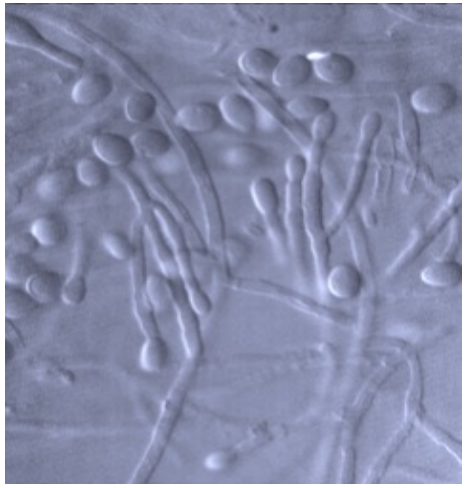
Alternaria

Penicillium



Fusarium

Scedosporium



Aspergillus

Trichophyton

Diagnostic biologique des infections fongiques Invasives

Statistics of the 10 most significant human invasive fungal infections

Disease (most common species)	Location	Estimated life-threatening infections/ year at that location*	Mortality rates (% in infected populations)*
Opportunistic invasive mycoses			
Aspergillosis (*A. fumigatus*)	worldwide	>200,000	30-95
Candidiasis (*C. albicans*)	worldwide	>400,000	46-75
Cryptococcosis (*Cryptococcus neoformans*)	worldwide	>1,400,000	20-70
Mucormycosis (Rhizopus oryzae)	worldwide	>10,000	30-90
Pneumocystis (*P. jirovecii*)	worldwide	>400,000	20-80

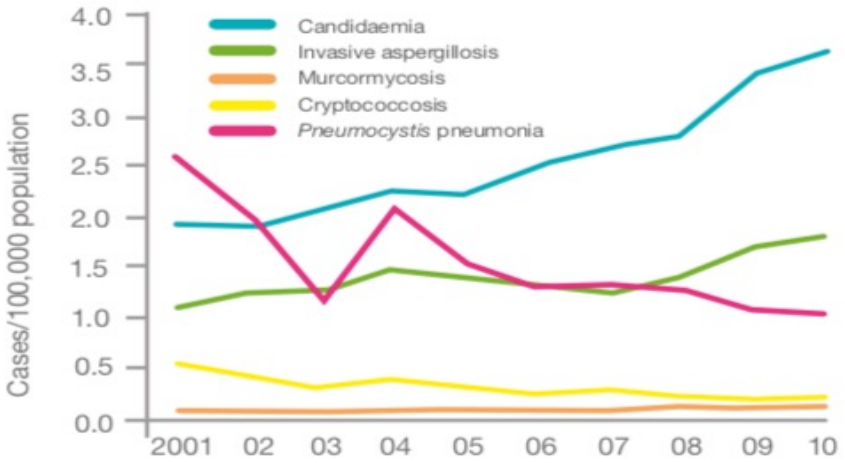
IFI kill 1,5 million /year
= Tuberculosis, more than malaria

Brown GD *et al.* 2012 Sci Transl Med.

Impact médico-économiques des Infections Fongiques Invasives

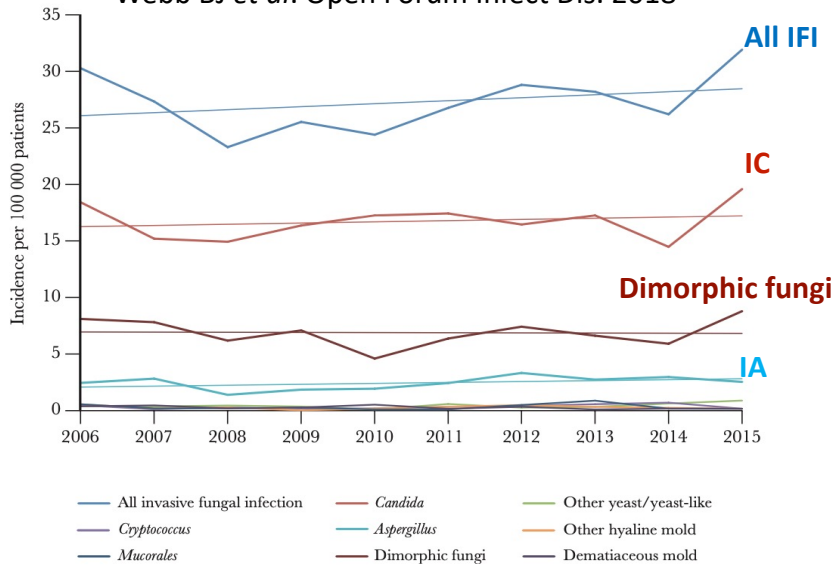
Rates of invasive fungal infections in France (2001-2010)

Bitar D *et al., Bull Epidemiol Hebdo,* 2013



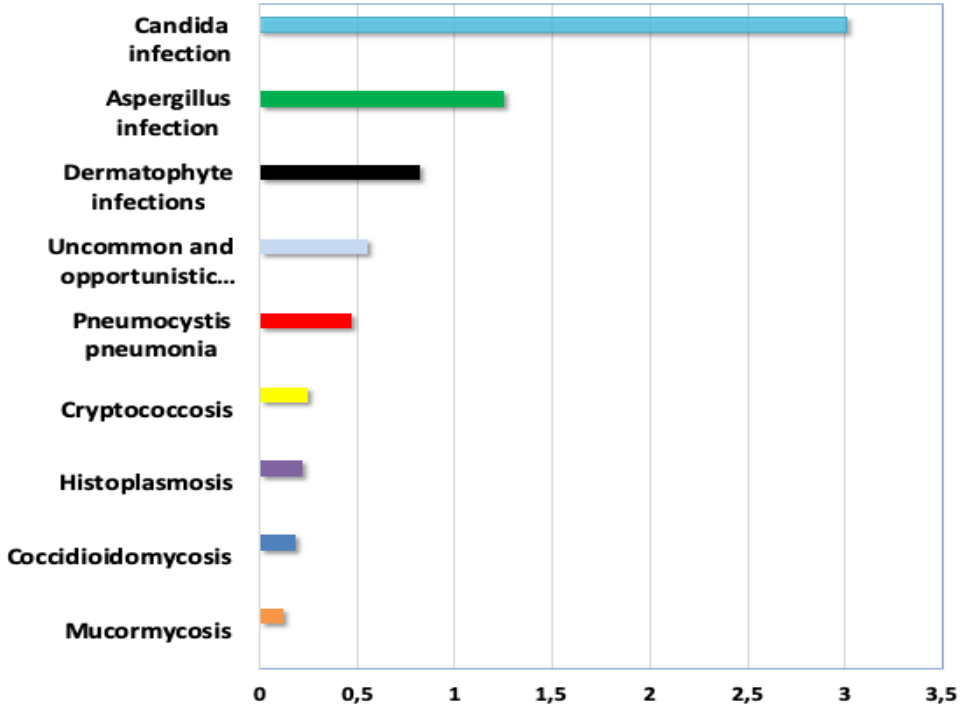
Rates of invasive fungal infections in USA (2006-2015)

Webb BJ *et al*. Open Forum Infect Dis. 2018



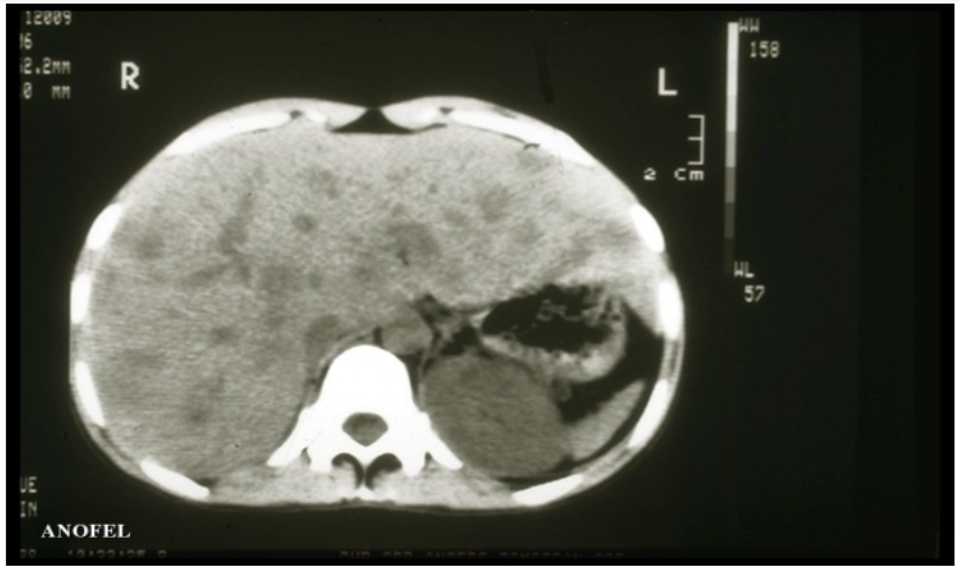
Annual Estimated Total Inpatient and Outpatient Costs for Fungal Diseases in United States*

**Kaitlin B et al. Clin Infect Dis 2020*

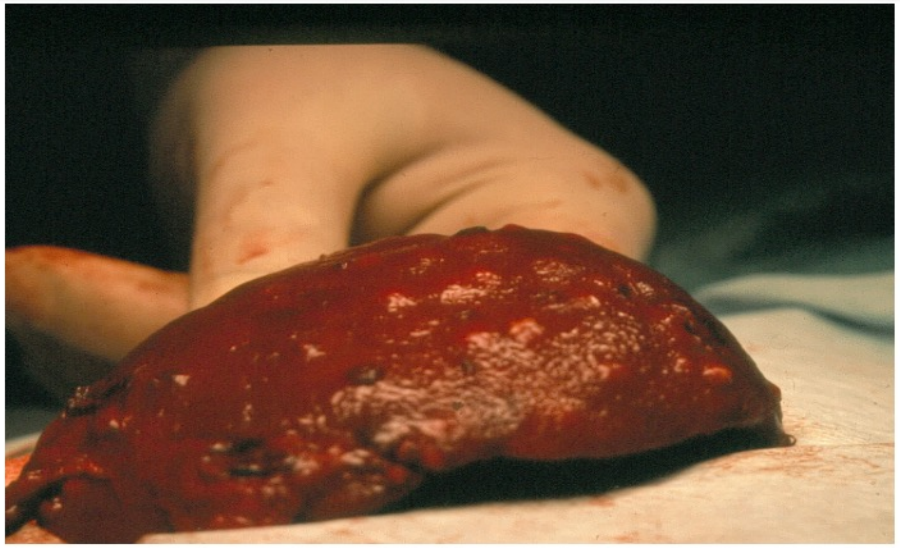


Annual Fungal Disese Costs (billion US$)

Candidoses invasives

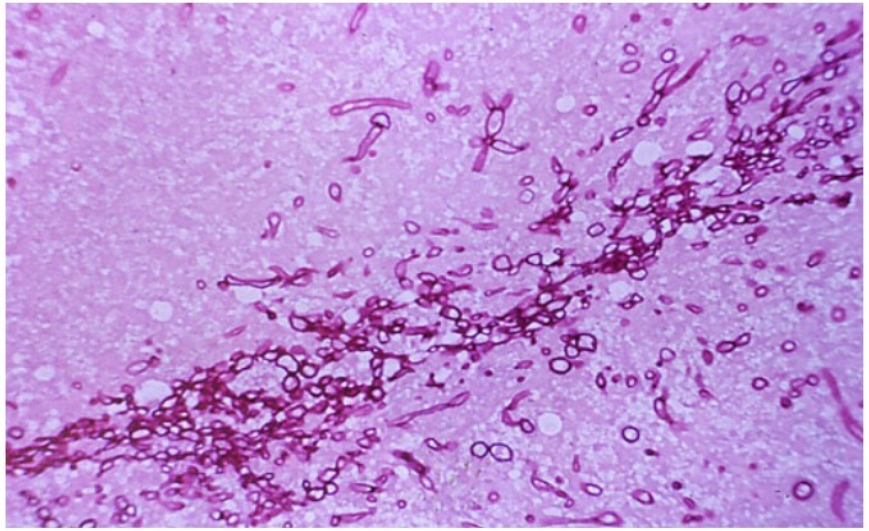


Candidose hépatique : Importance de l'imagerie



Examen macroscopique

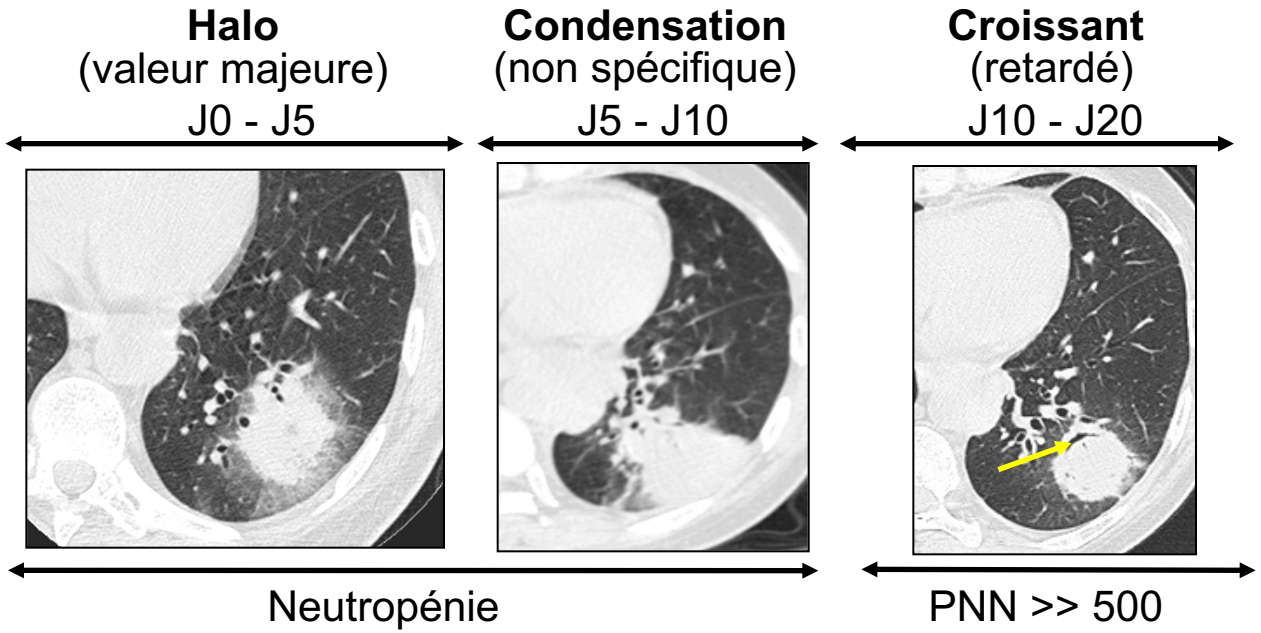
Micro-abcès

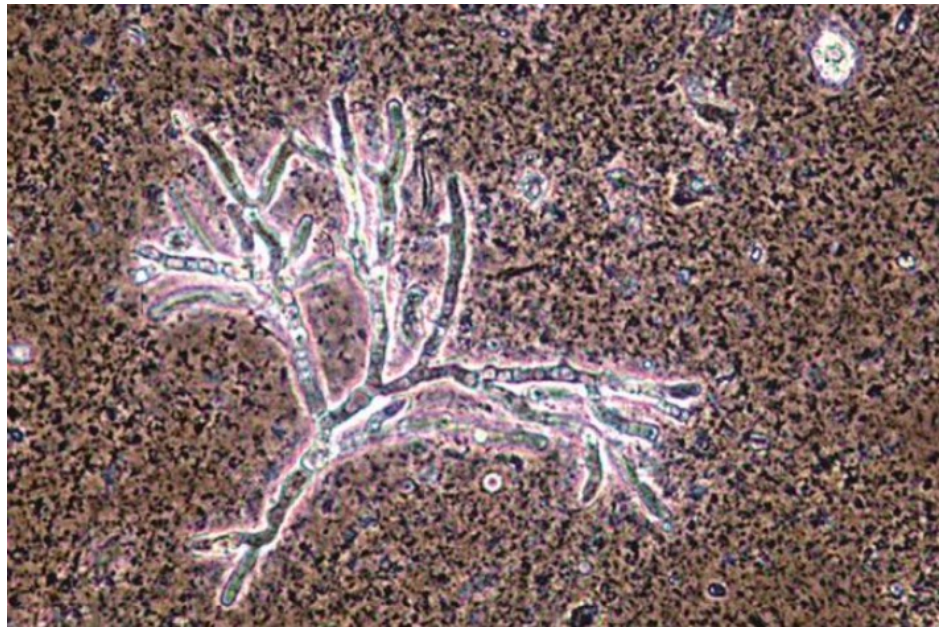


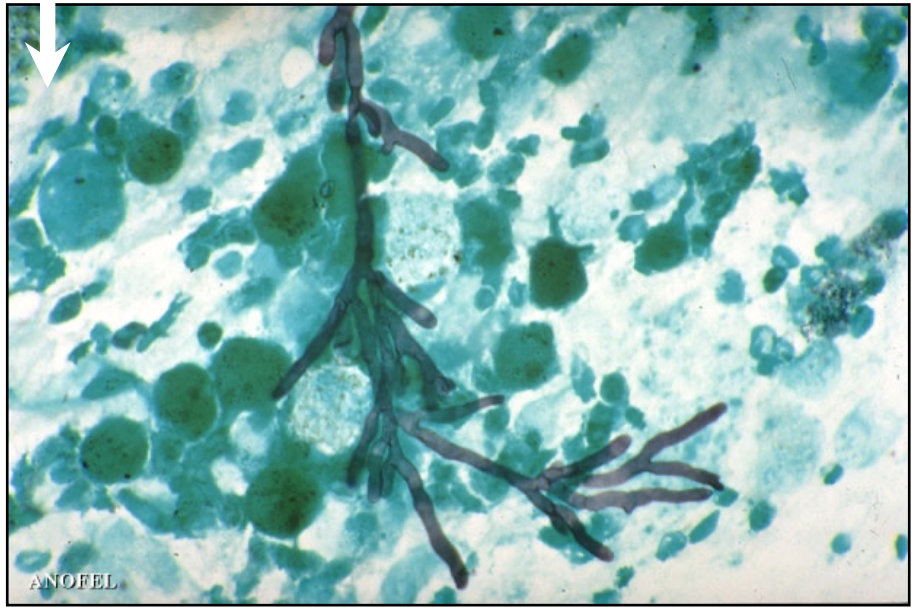
Examen histologique : Filaments + levures

Mycoses profondes

Aspergillose invasive

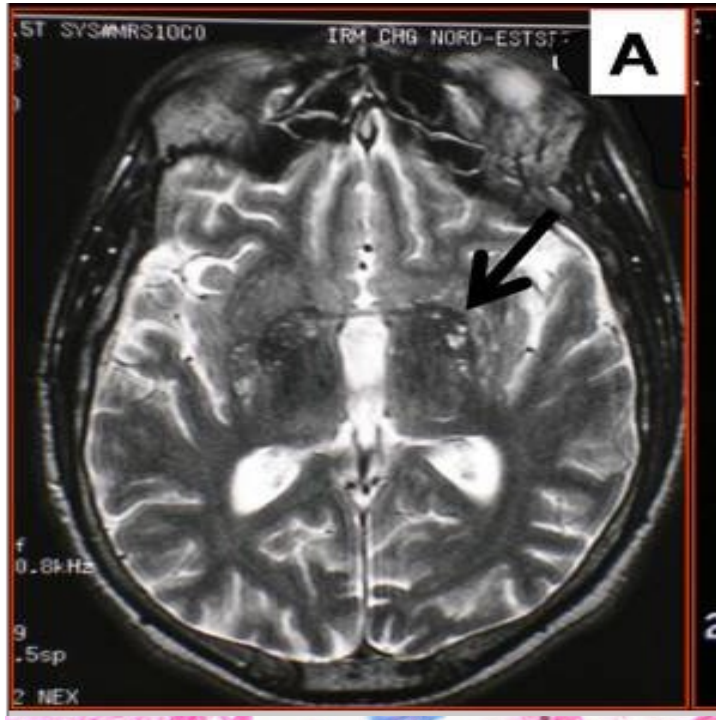


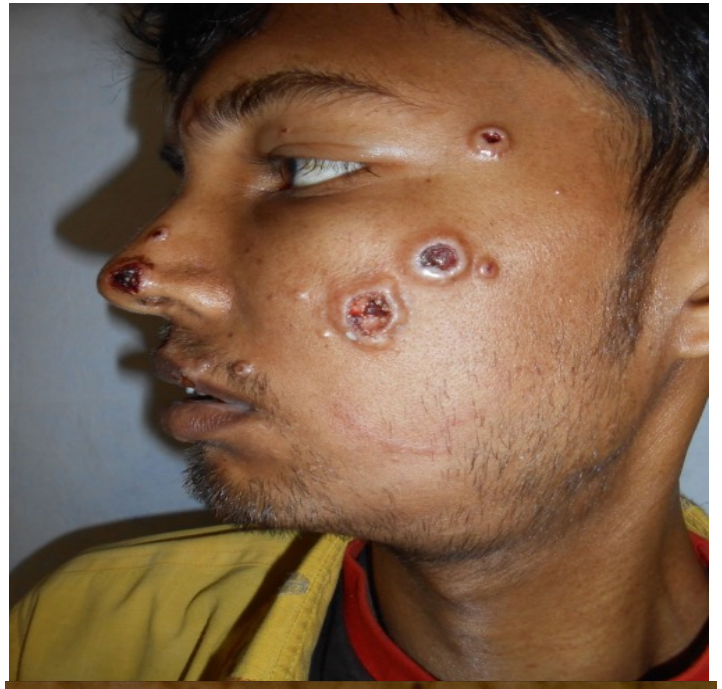


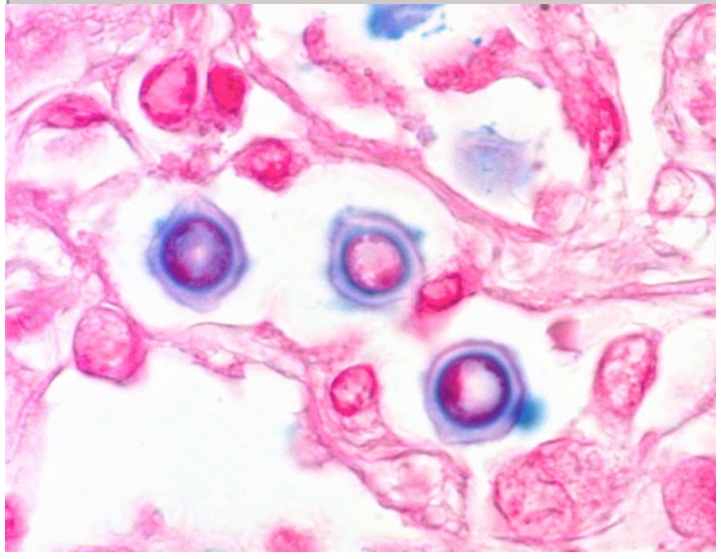


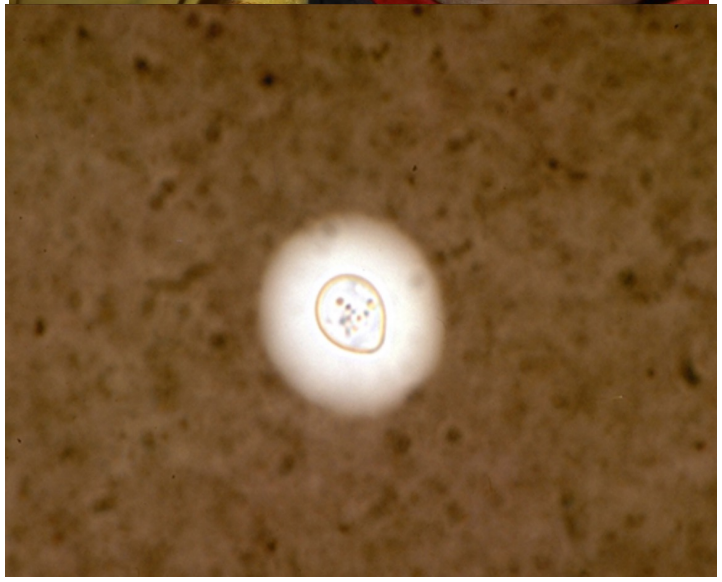
Examen direct du LBA

Cryptococcose neuroméningée

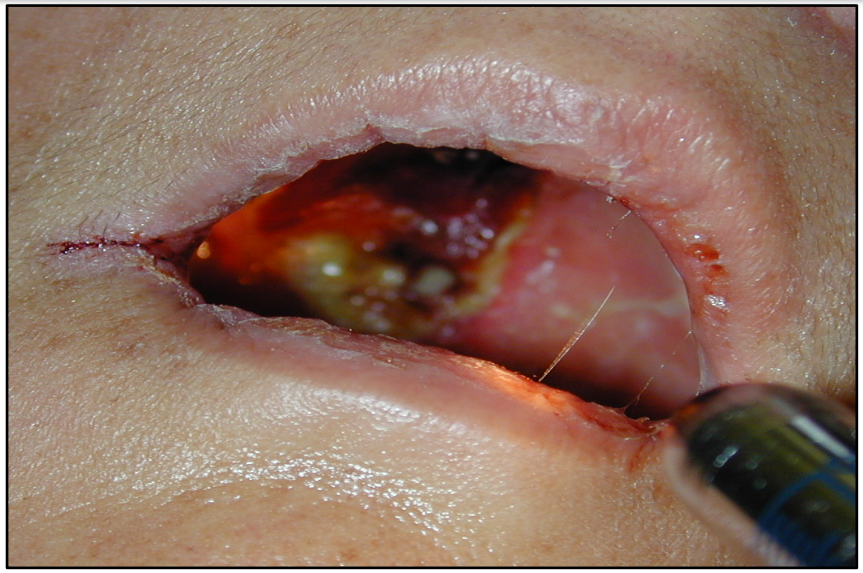




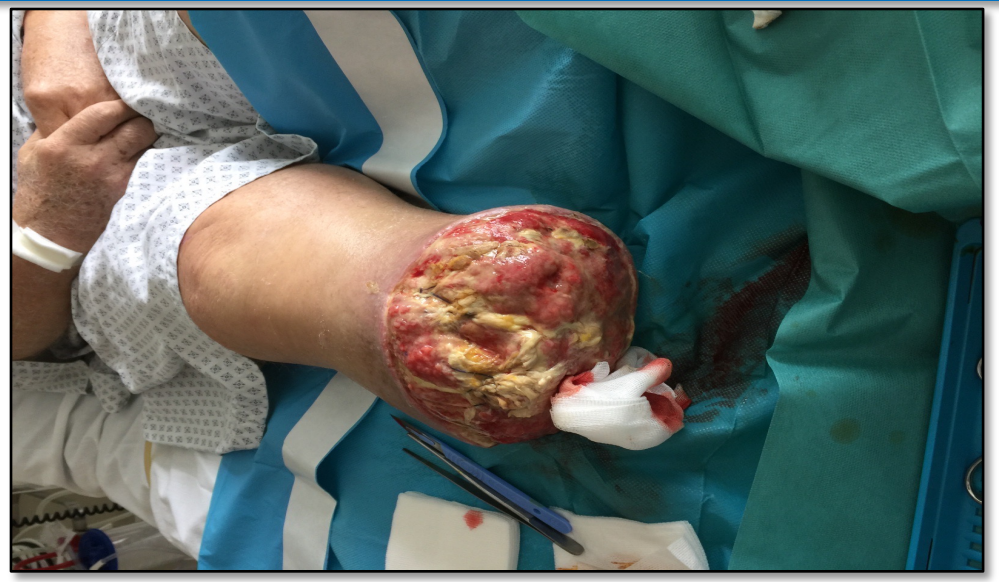




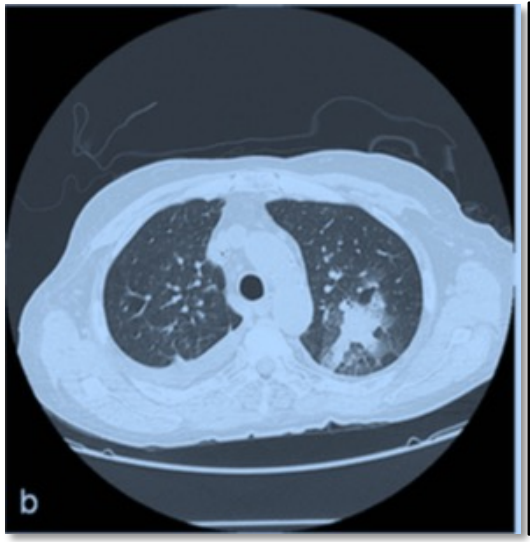
Mucormycoses invasives

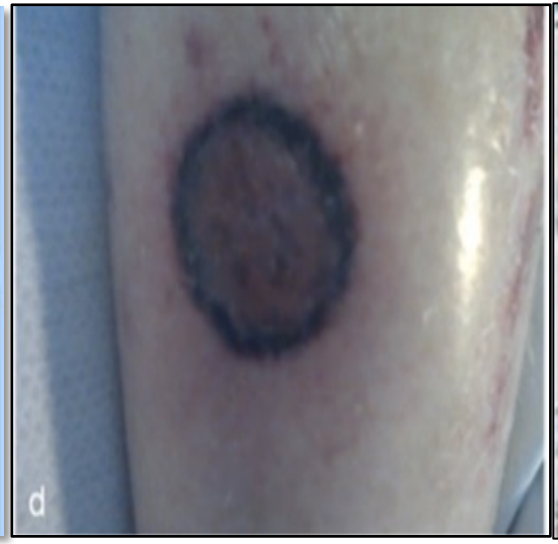


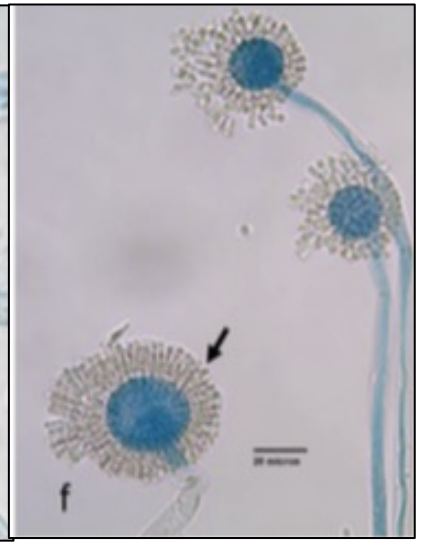
Mucormycose rhino-orbito-cérébrales



Mucormycose post-traumatique

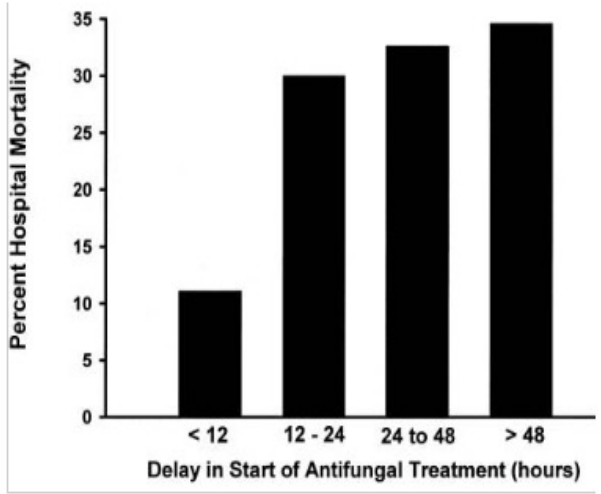


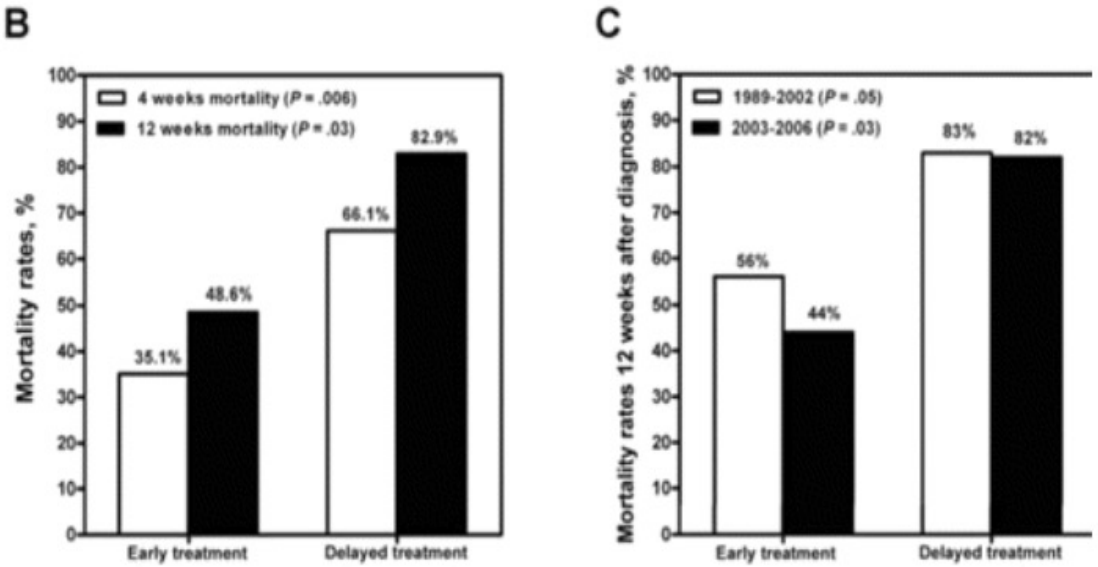


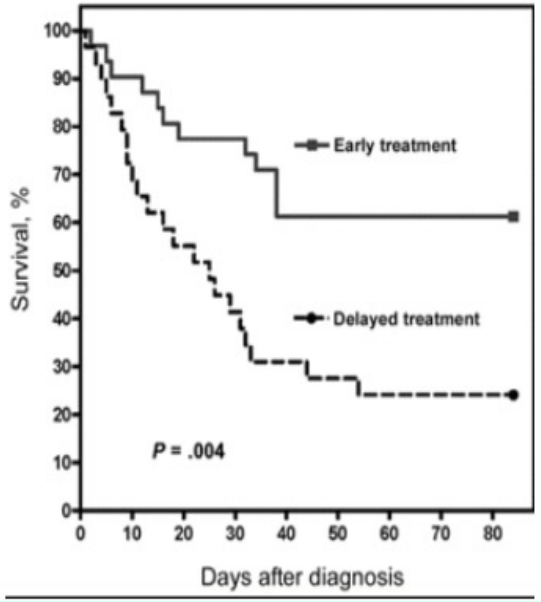


Mucormycose disséminée

Importance du diagnostic précoce des infections fongiques invasives







Morrell *et al*. 2005. AAC; Greene *et al*. CID. 2008; Chamilos *et al*. CID. 2008

Approches et outils disponibles

Mycologie conventionnelle

Histologie

Alternatives à la culture (biologie moléculaire, Marqueurs sériques)

Examen direct

Culture

Séquençage panfongique

Détection d'ADN circulant ou *in situ* (RT-PCR)

Détection des antigène circulant/ Anticorps spécifiques

- Orientation diagnistique
- Peu sensible

- Valeur diagnostique : sites stériles
- Tests de sensibilité aux ATF

- Spécifique (Ac monoclonaux),
- Invasif
- Diagnostic de certitude

- Sensibilité faible

Standardisation ? Résultat dépendant de matrice, gène cible, type de PCR

Performances variables selon les population de patients

Diagnostic biologique des candidoses invasives

CONVENTIONAL METHODS

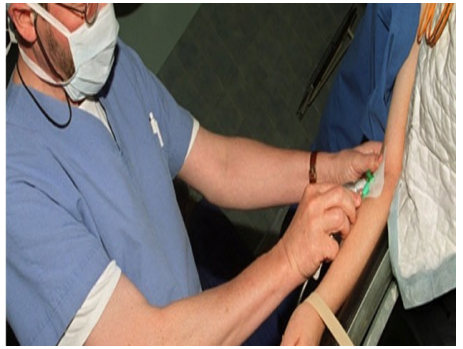
Culture

- Blood
- other sterile body sites
- urines
- other sites (Diff. diagnosis colonisation- infection)

Insensitive (less than 50% for BC), time consuming for antifungal susceptibility testing

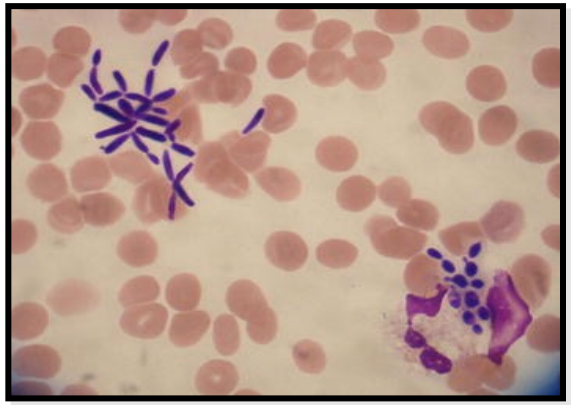
Time for species identification reduced by MALDI-TOF

Sampling (10-20 mL of Blood)



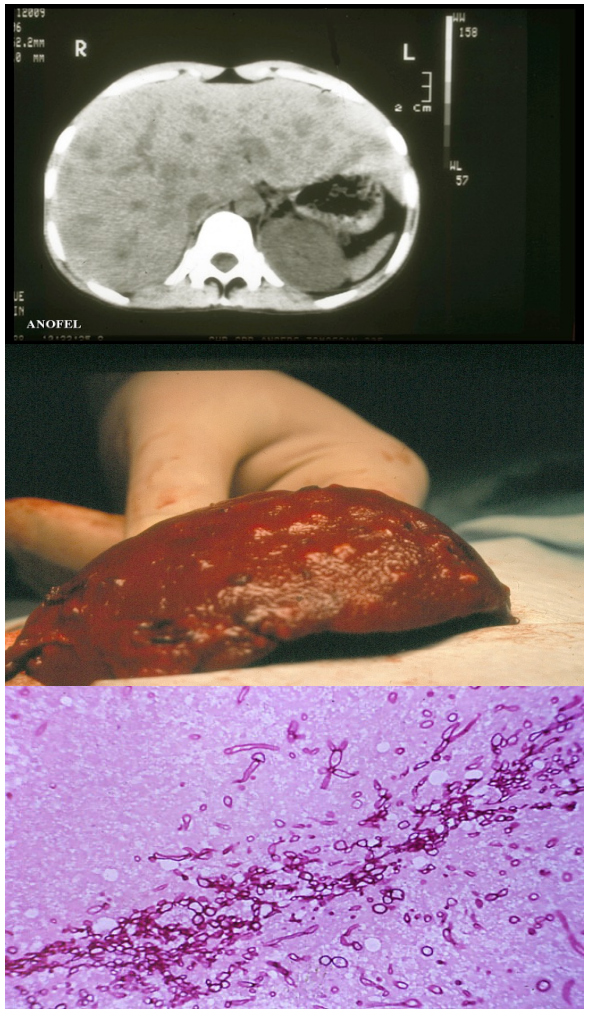
Incubator (35°C, > 5 days)

Microscopic examination



HISTOLOGY

- biopsies (liver, skin)
- low yield (patients under AFT)
- rarely performed in patients with aplasia



NON-CULTURE BASED METHODS

Candida **– indirect tests**

- **antigen**
- **antibodies**
- **fungal DNA detection**

Mostly evaluated in onco-hematology and ICU patients
Antigen and PCR tests evaluated in neonates
Very few commercially available tests

Comparison of (1→3)-β-D-Glucan, Mannan/Anti-Mannan Antibodies, and Cand-Tec *Candida* Antigen as Serum Biomarkers for Candidemia

56 candidemia, 200 controls (100 bacteremia, 100 sterile blood cultures)

	Sensitivity, %	Specificity, %
BDG	87.5	85.5
Mn	58.9	97.5
Anti-Mn	62.5	65
Mn-Anti-Mn	89.3	63
BDG+Mn	89.3	85
Cand Tec Candida Ag	13	93.9

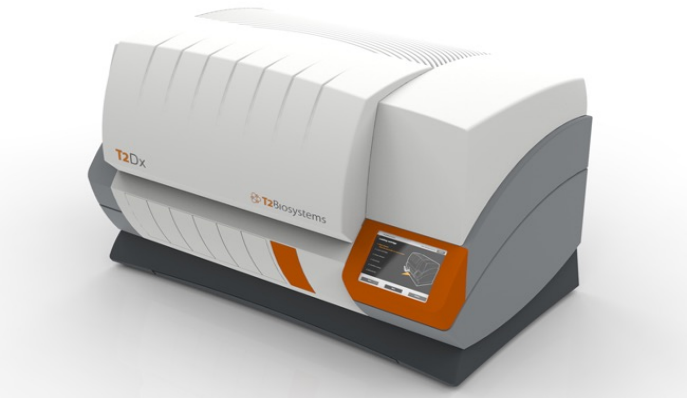
Held J, J Clin Microbiol 2013

(1,3)-βD- glucans in IC diagnosis

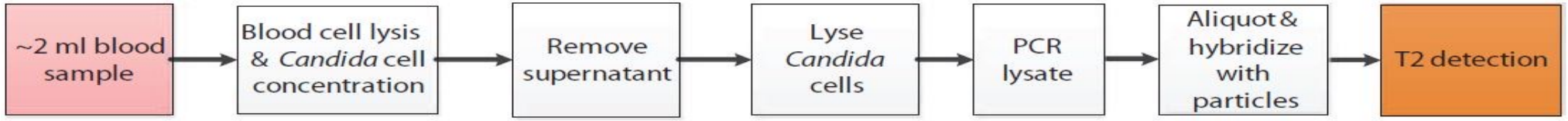
References	Patient type	Number patients/ samples (mean)	IC type (cases)	Cutoff[b]	Sensitivity (%) (95 % CI)	Specificity (%) (95 % CI)	PPV (%) (95 % CI)	NPV (%) (95 % CI)	Proven IC BG[b] (median)
Tissot et al. [38]	Surgical (abdominal) pancreatitis	89/921 [9]	IAC (29)	≥80	65 (46–82)[a]	78 (63–90)[a]	68 (48–84)[a]	77 (61–88)[a]	223
León et al. [65]	SAC	176/766 (4.3)	C, IAC (31)	≥80	51.6 (34–69)	86.9 (78–92)	59.3 (40–75)	83.0 (73–89)	259
Del Bono et al. [64]	Surgical	152/152 (1)	C (53)	≥80	62	98	98.4	57.3	324
Posteraro et al. [63]	Medical/ surgical	95/130 (1.3)	C (13 + 1 M)	≥80	92.9 (66–99)	93.7 (85–90)	72.2 (46–90)	98.7 (92–99)	500
Mohr et al. [62]	Surgical	57/239 (4)	C [3]	≥80	100[a]	59[a]	NDA	NDA	171

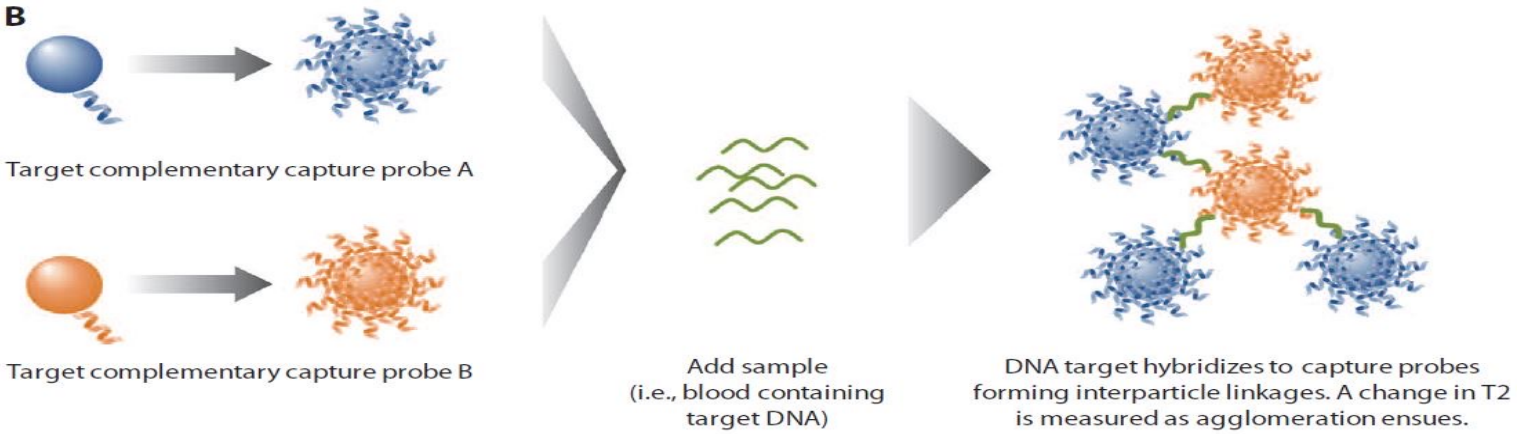
Leon C, Intensive Care Med 2014

T2 Candida for the Diagnosis of Candidemia and Invasive *Candida* Infections



T2 Candida : workflow





Beyda et al. Diagn Microbiol Infect Dis. 2013; Neely et al. 2013. Sci Transl Med; Mylonakis et al. 2015. CID; Bilir et al. 2015. Future Microbiol

T2 Candida for the Diagnosis of Candidemia and Invasive *Candida* Infections

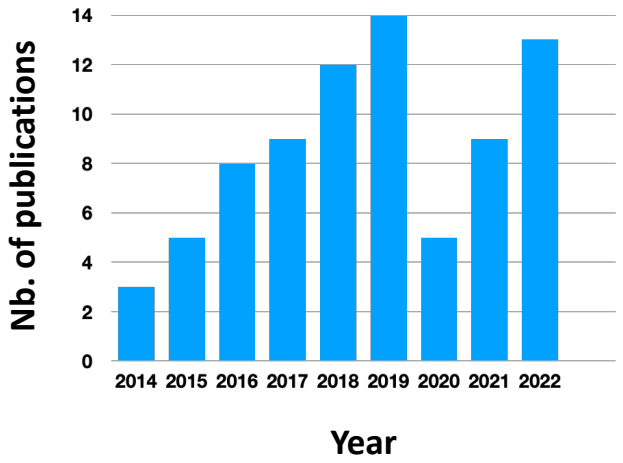
T2Candida® Panel: US FDA approved and CE marked Nucleic Acid Amplification (NAA) platform for the qualitative detection by magnetic resonance of 5 common *Candida* species :
C. albicans/*C. tropicalis*, *C. parapsilosis*, *C. krusei*/*C. glabrata* (95% of *Candida* isolates)

- Fully automated (T2Dx Instrument; T2 Biosystems®), 5 mL Whole blood (EDTA).
- Turn around time for analysis: < 5h
- Limit of detection: 1 - 3 CFU/mL

Neely et al. 2013. Sci Transl Med; Beyda et al. Diagn Microbiol Infect Dis. 2013; Mylonakis et al. 2015. CID; Bilir et al. 2015. Future Microbiol

T2 Candida for the Diagnosis of Candidemia

77 publications since 2014



Clinical Infectious Diseases

MAJOR ARTICLE

IDSA Infectious Diseases Society of America · hivma hiv medicine association · OXFORD

Detecting Infections Rapidly and Easily for Candidemia Trial, Part 2 (DIRECT2): A Prospective, Multicenter Study of the T2Candida Panel

Cornelius J. Clancy,[1] Peter G. Pappas,[2] Jose Vazquez,[3] Marc A. Judson,[4] Dimitrios P. Kontoyiannis,[5] George R. Thompson III,[6] Kevin W. Garey,[7] Annette Reboli,[8] Richard N. Greenberg,[9] Senu Apewokin,[10] G. Marshall Lyon III,[11] Luis Ostrosky-Zeichner,[12] Alan H. B. Wu,[13] Ellis Tobin,[14] M. Hong Nguyen,[1] and Angela M. Caliendo[15]

T2C was sensitive for diagnosing candidemia at the time of positive BC. In patients receiving antifungal therapy, T2C identified bloodstream infections that were missed by companion BCs.

Clancy et al. Clin Infect Dis 2018

MAJOR ARTICLE

T2 Magnetic Resonance Assay for the Rapid Diagnosis of Candidemia in Whole Blood: A Clinical Trial

Eleftherios Mylonakis,[1] Cornelius J. Clancy,[2] Luis Ostrosky-Zeichner,[3] Kevin W. Garey,[4] George J. Alangaden,[5] Jose A. Vazquez,[6] Jeffrey S. Groeger,[7] Marc A. Judson,[8] Yuka-Marie Vinagre,[9] Stephen O. Heard,[10] Fainareti N. Zervou,[1] Ioannis M. Zacharioudakis,[1] Dimitrios P. Kontoyiannis,[11] and Peter G. Pappas[12]

First prospective multicenter study (Direct 1) : 1801 hospitalized patients who had a BC ordered for routine standard of care; 250 of them were manually supplemented with concentrations from <1 to 100 colony CFUs/mL for 5 different Candida species :

Sensitivity/Specificity : 91.1%/ 99,4%,
Sensitivity : 88.17% for subgroup K/G
92.3% for subgroup A/T
94,27% for P

Mylonakis et al.

Clin Infect Dis 2015

J Antimicrob Chemother 2018; **73** Suppl 4: iv27–iv30
doi:10.1093/jac/dky046

Journal of Antimicrobial Chemotherapy

Impact of rapid, culture-independent diagnosis of candidaemia and invasive candidiasis in a community health system

M. E. Patch[1]*, E. Weisz[1], A. Cubillos[1], S. J. Estrada[1,2] and M. A. Pfaller[3]

- The implementation of T2Candida improves time to appropriate antifungal therapy for candidaemic patients (34 h for BC *versus* 6 h for T2Candida, P = 0.0147)

Patch et al. JAC 2018

T2 Candida for the Diagnosis of Invasive *Candida* Infections

Performances of T2C in the detection of invasive candidiasis in combination with β-glucans and/or mannan

Open Forum Infectious Diseases

BRIEF REPORT

Performance of the T2Candida Panel for the Diagnosis of Intra-abdominal Candidiasis

Frederic Lamoth[1,2,a] Cornelius J. Clancy,[3,a] Frederic Tissot,[1] Kevin Squires,[3] Philippe Eggimann,[4] Ursula Flückiger,[5] Martin Siegemund,[6] Christina Orasch,[7] Stefan Zimmerli,[8] Thierry Calandra,[1] Oscar Marchetti,[1,9] Minh H. Nguyen,[3,b] and Pierre-Yves Bochud[1,b]; on behalf of the Fungal Infection Network of Switzerland (FUNGINOS)

Table 1. Performance of T2Candida and 1,3-Beta-D-Glucan for Detection of Intra-abdominal Candidiasis

	T2Candida	1,3-Beta-D-Glucan
Sensitivity,	33.3 (13.3–59.0)	83.3 (58.6–96.4)
Specificity, %	93.3 (77.9–99.2)	66.7 (47.2–82.7)
Positive predictive value,[a] %	71.1 (35.7–91.6)	55.2 (41.6–68.0)
Negative predictive value,[a] %	74.0 (66.9–80.0)	89.0 (73.7–95.9)
Positive likelihood ratio	5.00 (1.13–22.18)	2.50 (1.45–4.32)
Negative likelihood ratio	0.71 (0.51–1.00)	0.25 (0.09–0.72)

Values in brackets are 95% confidence intervals.

Cohort of 48 IAC patients : IAC was **present** in **100%** of cases with **concordant positive** T2Candida/1,3-beta-D-glucan and **absent** in 90% of **concordant negative** results.

Clinical Infectious Diseases

MAJOR ARTICLE

418 specimen pairs (406 enrolled, 12 spiked) included in analysis

Multicenter Prospective Study of Biomarkers for Diagnosis of Invasive Candidiasis in Children and Adolescents

Brian T. Fisher,[1,2] Craig L. K. Boge,[1] Rui Xiao,[2] Sydney Shuster,[1] Dawn Chin-Quee,[3] John Allen IV,[3] Shareef Shaheen,[3] Randall Hayden,[4] Sri Suganda,[4] Theoklis E. Zaoutis,[1,2] Yeh-Chung Chang,[3] Dwight E. Yin,[5] Anna R. Huppler,[6] Lara Danziger-Isakov,[7] William J. Muller,[8] Emmanuel Roilides,[9] José Romero,[10] Paul K. Sue,[11] David Berman,[12] Rachel L. Wattier,[13] Natasha Halasa,[14] Alice Pong,[15] Gabriela Maron,[16] Pere Soler-Palacin,[17] Susan C. Hutto,[18] Blanca E. Gonzalez,[19] Christine M. Salvatore,[20] Sujatha Rajan,[21] Michael Green,[22] Elizabeth Doby Knackstedt,[23] Sarmistha B. Hauger,[24] and William J. Steinbach[3]

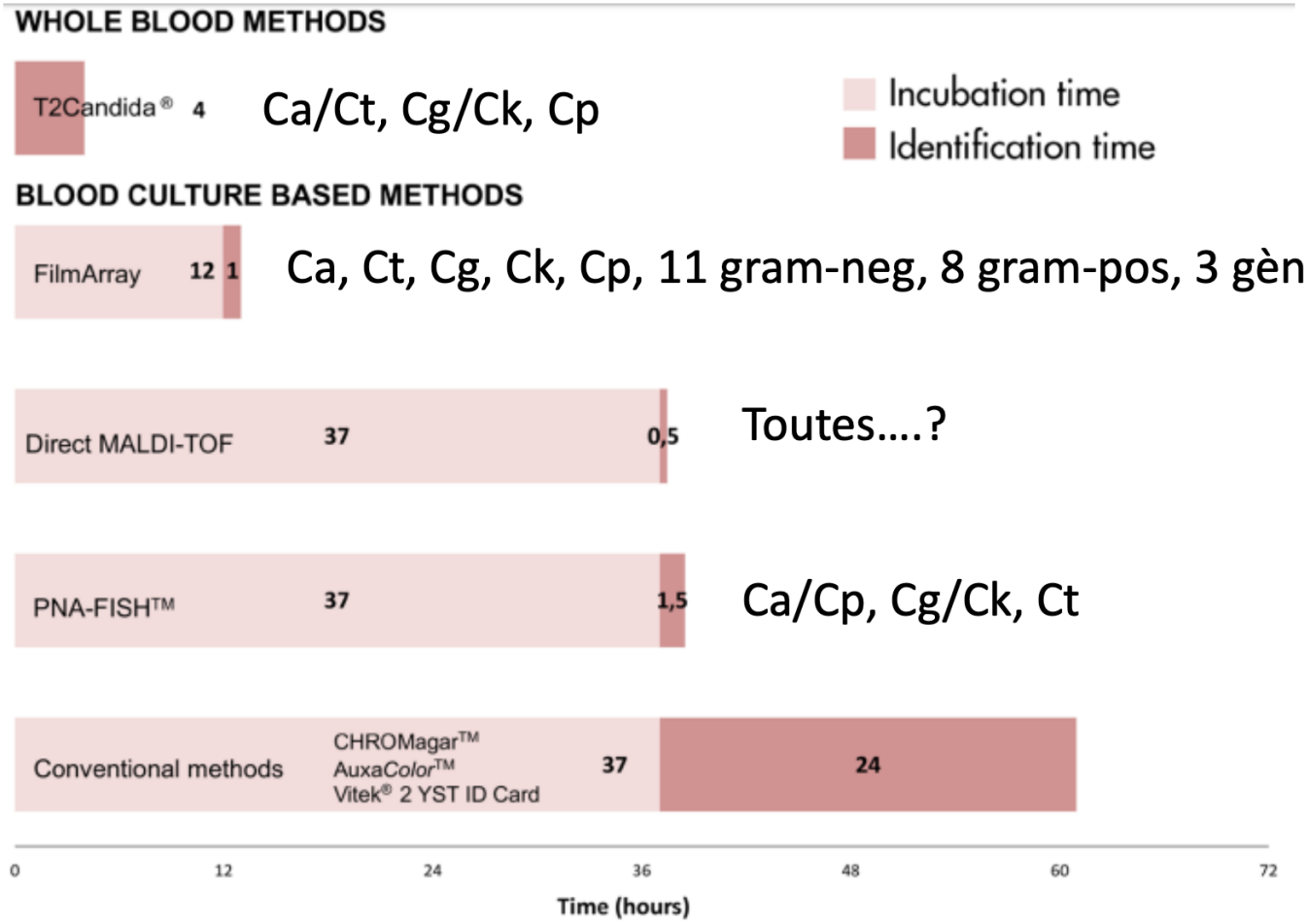
Table 7. Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value for the T2Candida and Platelia *Candida* Antigen Plus Results Used in Combination

Test	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive Predictive Value % (95% CI)	Negative Predictive Value % (95% CI)	Specimens Exceeding Cutoff n
T2Candida only	77.3 (54.6, 92.2)	97.2 (95.1, 98.6)	60.7 (40.6, 78.5)	98.7 (97.0, 99.6)	28
Platelia *Candida* Antigen Plus only	40.9 (20.7, 63.7)	97.2 (95.1, 98.6)	45.0 (23.1, 68.5)	96.7 (94.5, 98.3)	20
At least one test positive	86.4 (65.1, 97.1)	94.7 (92.0, 96.7)	47.5 (31.5, 63.9)	99.2 (97.7, 99.8)	40
Both tests positive	31.8 (13.9, 54.9)	99.8 (98.6, 100.0)	87.5 (47.4, 99.7)	96.3 (94.0, 97.9)	8

A total of 418 specimen pairs (406 enrolled, 12 spiked) were included in analysis : **T2Candida alone** or **in combination with mannan antigen** may be beneficial for rapid detection of *Candida* species in children with concern for IC.

Time to yeast identification

Ibáñez-Martínez et al. Rev Esp Quimioter 2017



Ca/Ct, Cg/Ck, Cp

Se 91.1%, Sp 99.4%

Ca, Ct, Cg, Ck, Cp, 11 gram-neg, 8 gram-pos, 3 gènes R ATB

Toutes….?

Peptide nucleic acid-fluorescence in situ hybridization (PNA-FISH)

Ca/Cp, Cg/Ck, Ct

Se 99.2%, Sp 99.9%

Conventional methods + MALDI-TOF = 24-30 depending on Candida species

Figure 2 | Graphic representation of time (hours) to yeast identification depending on employed technique.

Diagnostic biologique des aspergilloses invasives

Diagnostic biologique des aspergilloses invasives

- Mycologie conventionnelle
- Antigène galactomannane dans le sérum et le LBA (+LCR)
- Lateral Flow Device? (situation d'urgence)
- β-(1,3)-D-glucanes

Patterson et al. 2016. CID (IDSA); Marchetti et al. 2012. BMT (ECIL); Avni et al. 2012. JCM; Arvanitis et al. 2014. JCM; Prattes et al. 2016. Curr Fungal Infect Rep

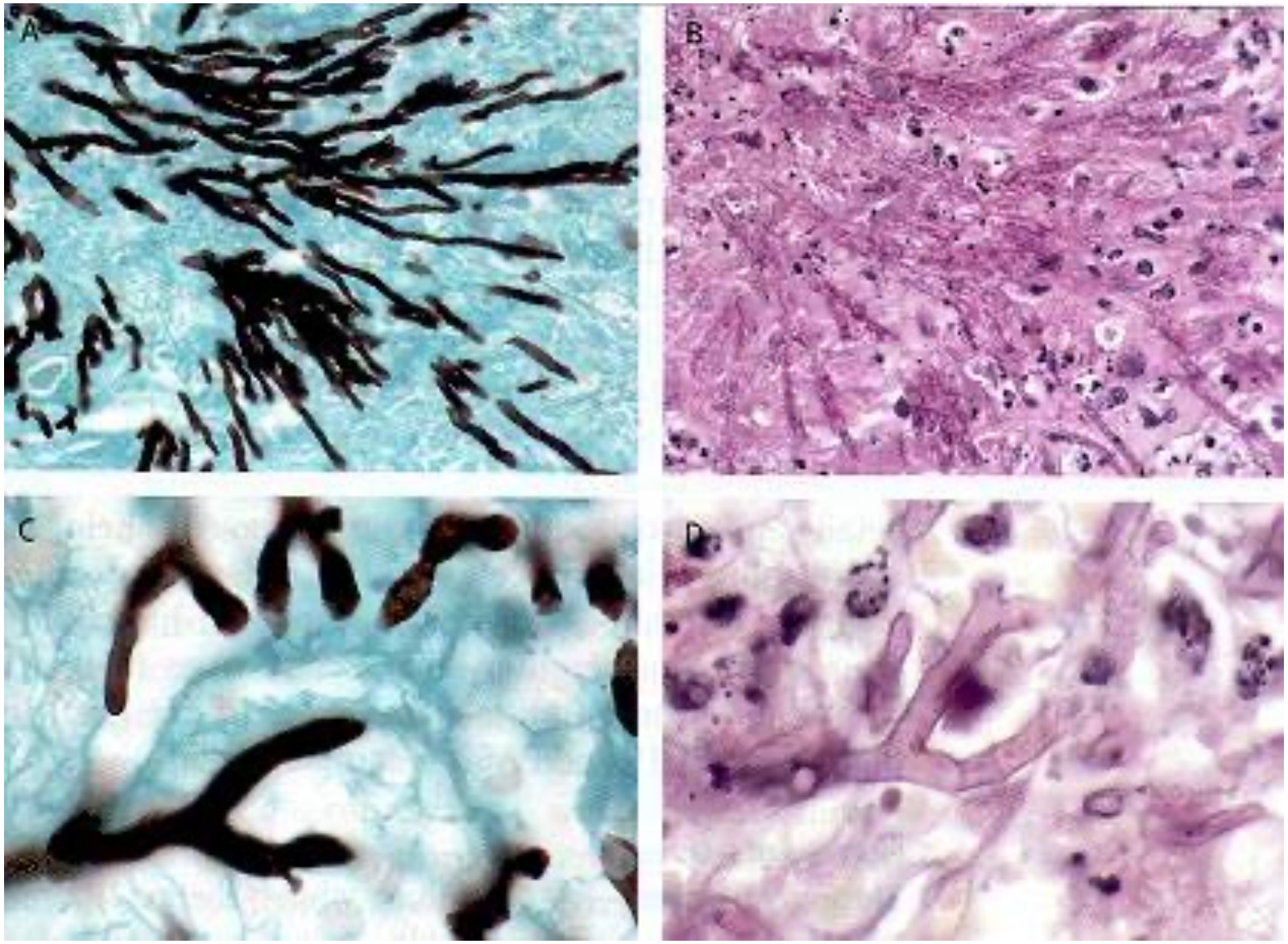
Aspergillose invasive- Mycologie conventionnelle

- **Diagnostic conventionnel**

Examen direct des
tissus
prélèvements endobronchiques (crachat, aspirations trachéales, LBA)

	Crachat/LBA	tissus
Etat frais	préparation ± KOH	
Colorations de routine	Gram	HE
Colorations spécifiques	GMS, PAS, BT	GMS, PAS
Agents fluorescents	Calcofluor white	
Colorant fluorescent	Uvitex 2B	
	Blankophor	

Aspergillose invasive- Diagnostic Conventionnel



In: Hope et al., *Lancet Infectious Diseases* 5: 609, 2005

Aspergillose invasive- Diagnostic Conventionnel

DIAGNOSTIC HISTOLOGIQUE

Améliorer la détection des filaments par immunohistochimie

Anticorps monoclonaux

- **WF-AF-1 (Dako) [1)]**
- **EB-A1 [2)]**

Sensibilité pour AI prouvée par la culture : 89-94%
Résultats spécifiques de genre ou d'espèce

[1)] Choi JK et al., *Am J Clin Pathol* 121: 18, 2004
[2)] Pierard GE et al., *Am J Clin Pathol* 96: 373, 1991
Verweij PE et al., *Am J Clin Pathol* 49: 798, 1996

Galactommanane sérique

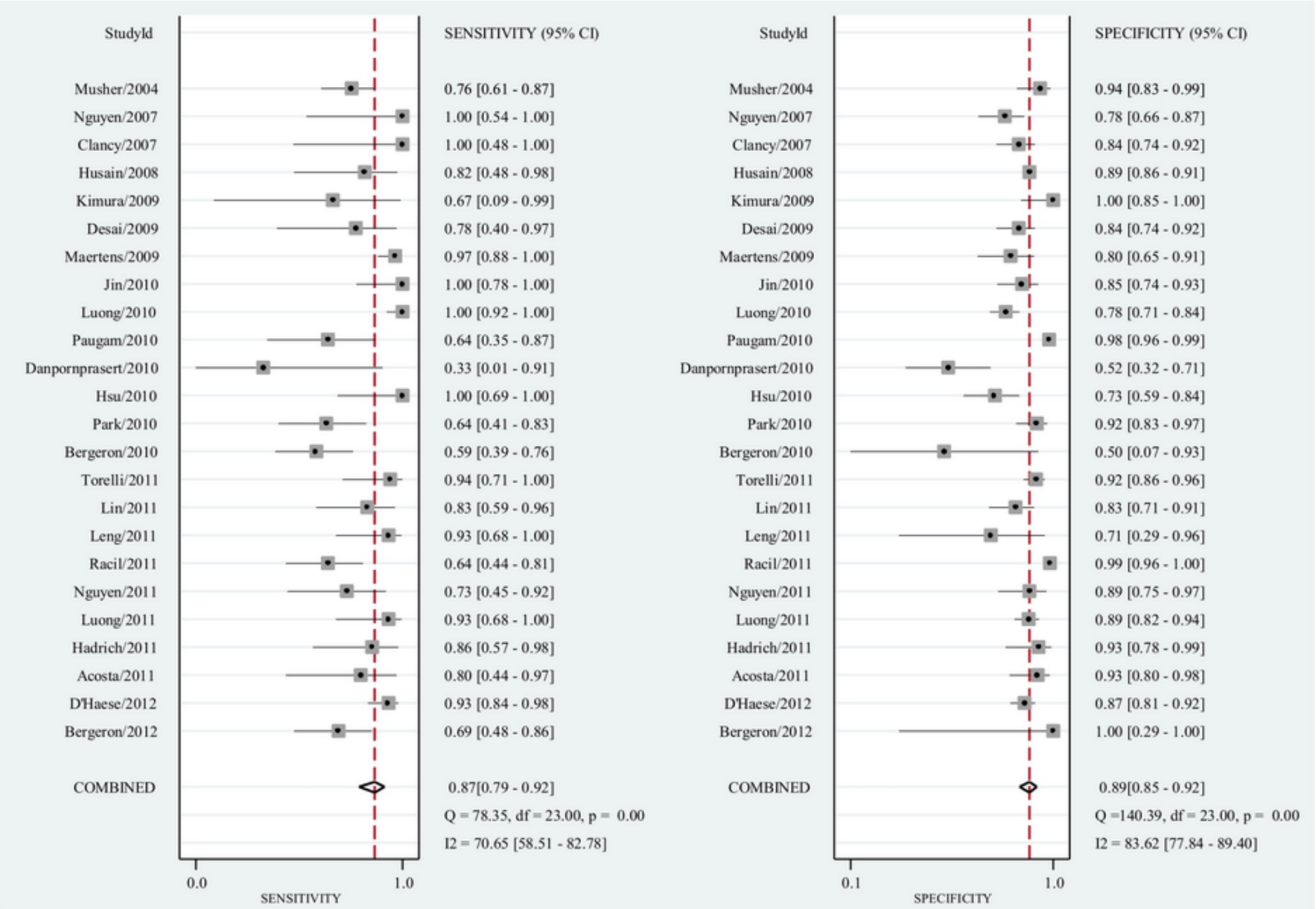
Polysaccharide libéré par les champignons du genre *Aspergillus* au cours de leur croissance
Méthode de détection standardisée : kit ELISA Platelia Aspergillus Ag (Bio-Rad®)

54 études, 5 660 patients, 586 avec une forme prouvée ou probable (EORTC/MSG)
Patients neutropéniques
→ DOI à 0.5 **(Se / Sp) : 78% / 85%**
(Leeflang *et al.*, Cochrane Database Syst Rev, 2015)

1 109 patients admis en réanimation
26 formes prouvées
Remarque : 10 patients neutropéniques
→ DOI à 0.5 : **Se de 42%**
(Meersseman *et al.,* Am J Respir Crit Care Med, 2008)

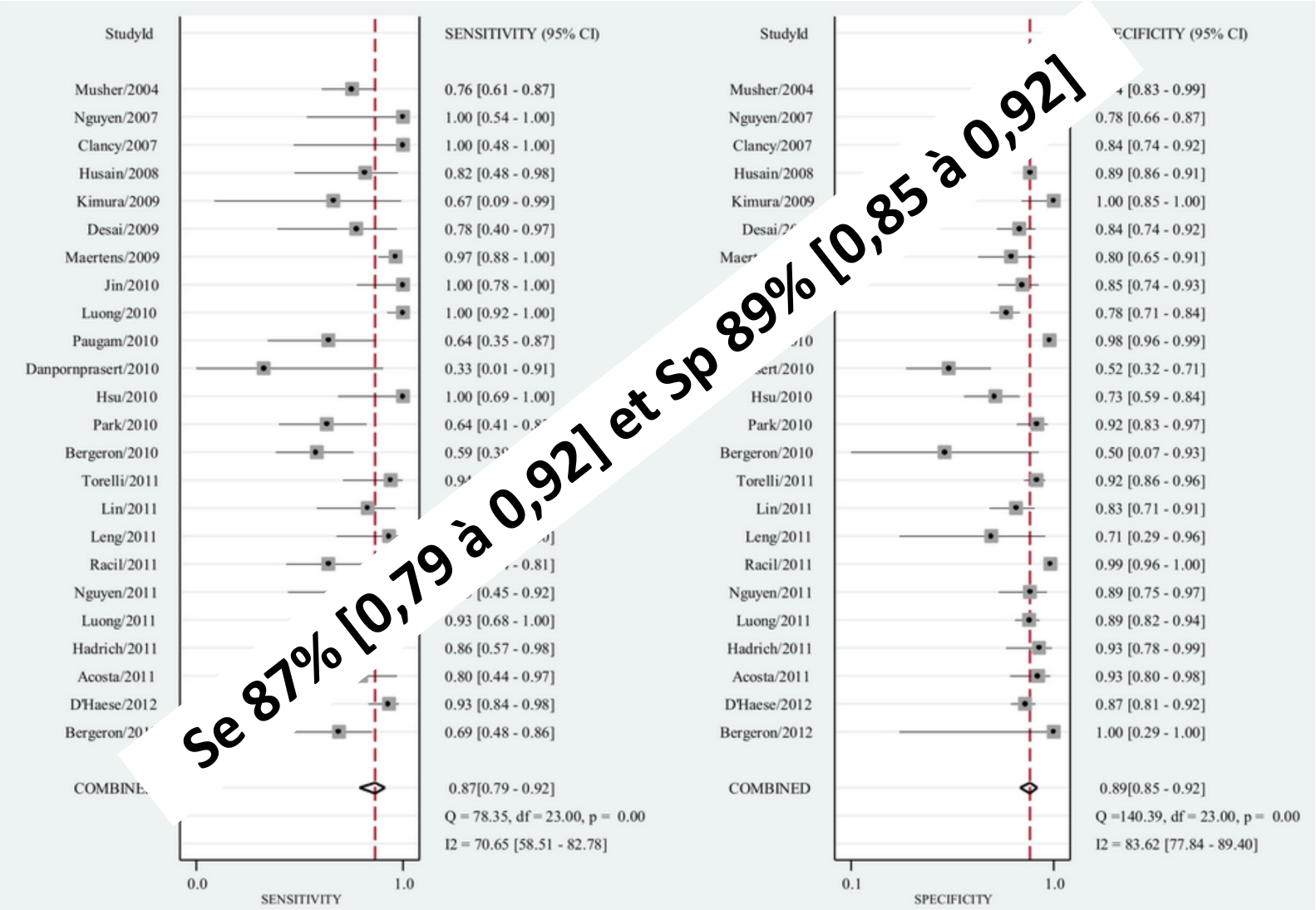
Analyse de 126 cas d'AI chez des transplantés d'organes solides (poumon, cœur, foie, rein)
Incidence de 6,5%, 2,9%, 1,8 et 0,6% respectivement
→ DOI à 0.5 : **Se de 50,6%**
(Gioia *et al.*, Mycoses, 2021)

Détection du galactommanane dans le LBA



(Zou *et al.*, Plos One, 2012)

Détection du galactommanane dans le LBA



(Zou *et al.*, Plos One, 2012)

Apport de la PCR au diagnostic des API

→ Entrée de la PCR Aspergillus dans les critères EORTC/MSG du diagnostic des API (Donnelly *et al.*, Clin Infect Dis., 2020)

PCR positive à deux reprises dans le plasma, le sérum ou le sang total
PCR positive dans le LBA à deux reprises ou en dupliqua
Association d'une PCR sérique/plasma/sang total positive et d'une PCR dans le LBA

Apport de la PCR au diagnostic des API

- PCR: manque de standardisation
  - ADN circulant: Se 84% Sp 76%
  - LBA: Se 77% Sp 94%

Méta-analyse Cochrane : 18 études - 2000-2013.

– 19 cohortes
  - Patients onco-hémato (adultes-enfants)
  - Haut risque IA → Prévalence moy de IA (prouvée ou probable) : 12% (2.5 à 30.8 %).

– Différentes techniques de qPCR (PCR maison).
  - Cible : *A. fumigatus*

– Valeurs intrinsèques

Critère diagnostique	Sensibilité moy (IC 95%)	Spécificité moy (IC 95%)
1 PCR pos	80,5 (73 - 86.3)	78.5 (67.8 - 86.4)
2 PCR pos	58 (36.5 -76.8)	96 (89.6 - 98.6)

Cruciani M et coll., *Cochrane Database of Systematic Reviews* 2015

Apport de la PCR au diagnostic des API
Performances variables selon les populations étudiées

- Patients d'hématologie à haut risque

25 études, 2 595 patients inclus
Pour un répliquat **Se / Sp : 84% / 76%**
Pour deux PCR consécutives positives **Se / Sp : 64% / 95%**
(Arvanitis *et al.*, JCM, 2014)

- HM, HSCT, SOT

34 études
Pour un répliquat **Se / Sp : 79,2% / 79,6%**
Pour deux PCR consécutives positives **Se / Sp : 59,6% / 95,1%**
Très bonne VPN (95%)
(Cruciani *et al.*, Cochrane Library, 2019)

- Analyse en sous-groupe réalisée sur la cohorte précédente pour déterminer l'impact de la chimioprophylaxie sur les performances de la PCR.

→Pas de perte de Se, par contre perte de Sp (perte de 26% si l'on considère une seule PCR positive, perte de 12% si l'on considère deux ou plus résultats de PCR)
(Cruciani *et al.*, J Antimicrob Chemother, 2021)

Apport de la PCR au diagnostic des API

Tests commerciaux

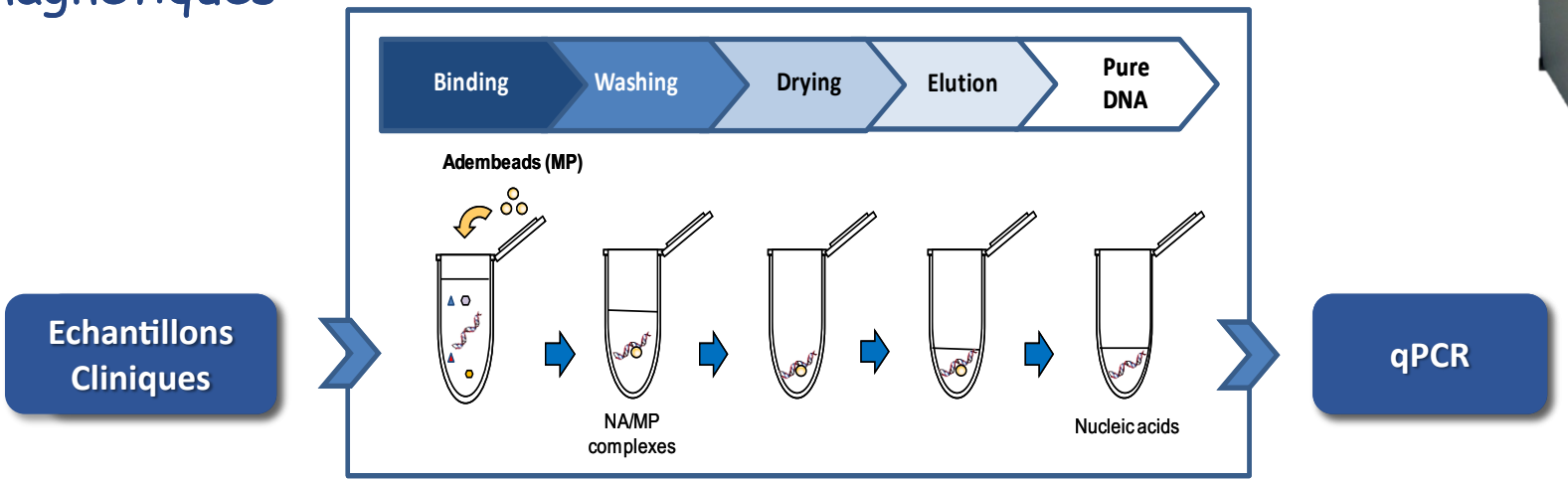
- MycoGENIE (Ademtech), AsperGenius (PathoNostics), Fungiplex (Renishaw).
- Sang, LBA, +/-biopsies
- AsperGenius (PathoNostics):
  - 2 multiplex
  - 5 espèces aspergillaires + mutation TR34, L98H, T289A, et Y121F (CYP51A)
  - Se 84.2%, Sp 91.4%, VPP 76.2%, and VPN 94.6%

Nécessité d'évaluation prospective multicentrique pour préciser les performances diagnostiques dans différentes populations de patients

MycoGENIE *Aspergillus :* Détection d'ADN *A. fumigatus* dans le sérum

Détection d'ADN *d'Aspergillus fumigatus* et de la mutation TR34/L98H

❑ Purification d'Acides Nucléiques à l'aide de billes magnétiques



- ✓ Automatisé
- ✓ Pas de centrifugation
- ✓ ADN de grande qualité
  - Pas de dégradation
  - Pas d'inhibiteurs PCR
  - Grande concentration

- Plaques pré-remplies
- Prêtes à l'emploi
- 12 extractions / run
- Durée 35min
- Volume d'élution 50µl

Performances du test MycoGENIE pour le diagnostic des aspergilloses invasives

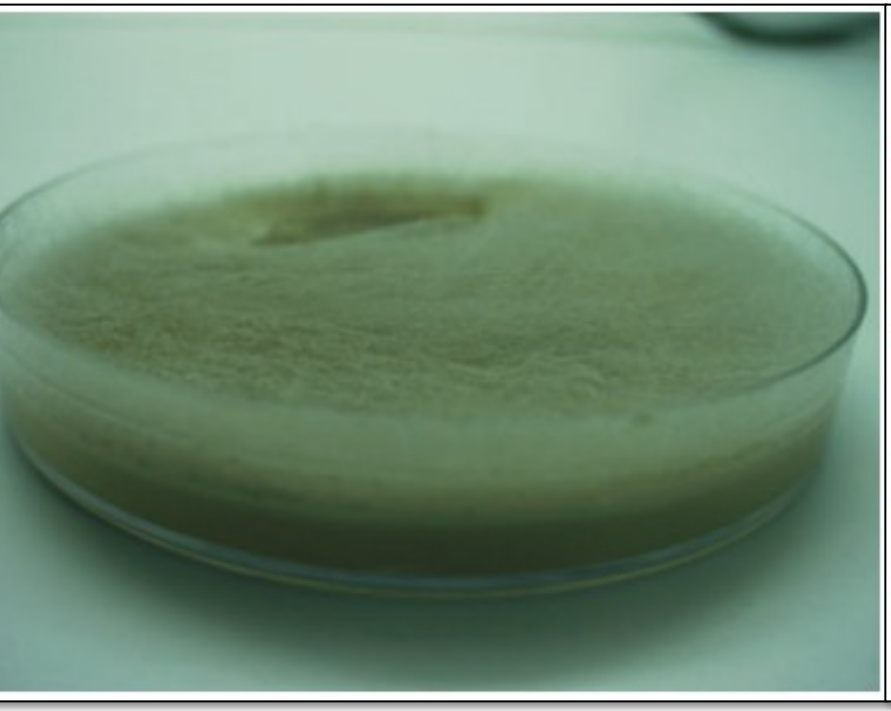
- Dannaoui *et al.*, JCM, 2017 :
  - Respiratoires n=88 / 62 patients : Se 92,9%; Sp : 90,1%
  - Sérums n= 69 / 16 patients AI prouvée-probable et 13 patients non AI
    - Se 100%; Sp 84,6
  - LOD : Aspergillus : 1 copie TR34/L98H : 6 copies
- Denis *et al.*, J.Mol. Diagn., 2018
  - LBA n=73 / 31 AI probables avec Se 100%; Sp : 71%

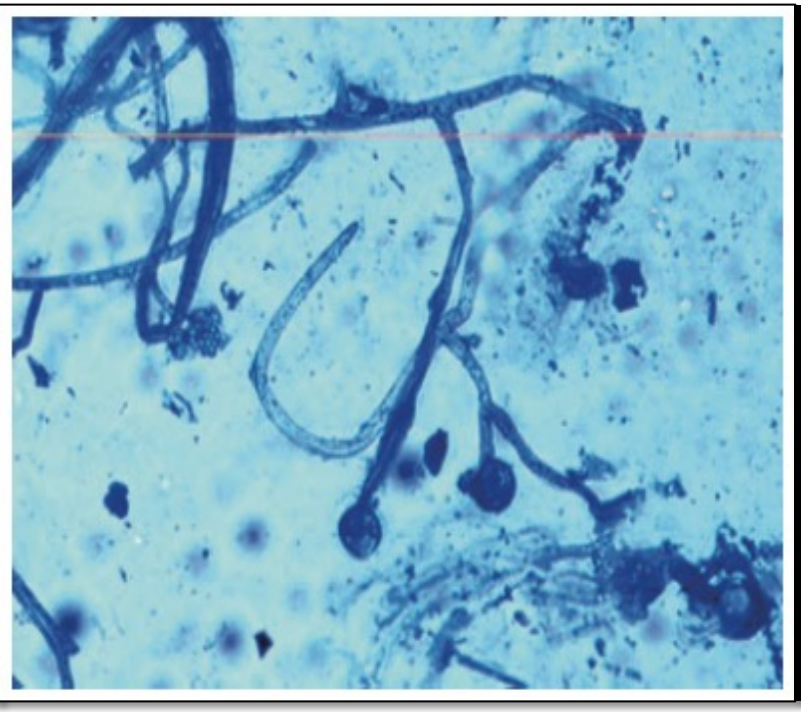
EORTC/MSGERC IPA case definitions

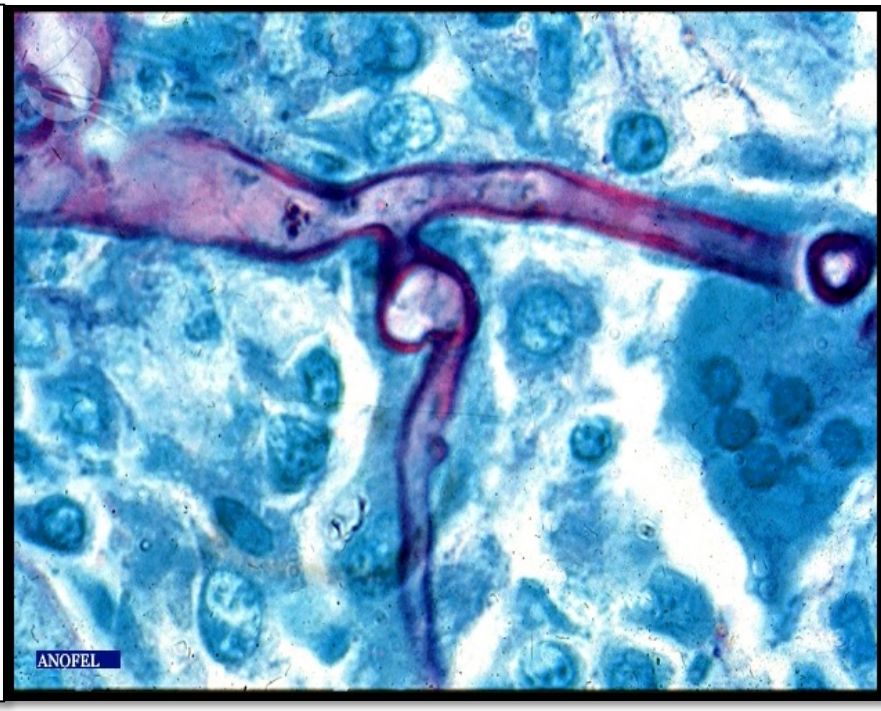
Criteria		Clinical	Radiological	Mycological
EORTC/MSGERC Donnelly JP, CID 2020	**Proven IPA**	-	-	**Lung biopsy**, at least 1: • Histo/cytopathologic or direct microscopic examination (hyphae + tissue damage) • Positive culture from tissue
	Probable IPA	**Host factors:** • Neutropenia • Malignant hemopathy • Transplant • Prolonged corticosteroids >0.3mg/kg >3weeks/2months • Immunosuppressive drugs…	At least 1 **CT pattern**: • Dense, well-circumscribed lesion (±halo) • Air crescent sign • Cavity • Consolidation	At least 1: • Positive direct microscopy or culture of a respiratory sample (sputum, tracheal aspirate, BAL) • BAL GM ≥1 • serum GM ≥1 • BAL GM ≥0.8 and serum GM ≥0.7 • *2x* positive *Aspergillus* PCR (serum or BAL)
	Possible IPA	*idem*	*idem*	-

Diagnostic des mucormycoses invasives

- Mycologie conventionnelle
- Histologie +++
- Pas de test de détection de polysaccharides circulants







Diagnostic des mucormycoses invasives

Détection d'ADN circulant par PCR pour le diagnostic des zygomycoses

JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2008, p. 3690–3702 Vol. 46, No. 11
0095-1137/08/$08.00+0 doi:10.1128/JCM.00917-08

Detection of a Molecular Biomarker for Zygomycetes by Quantitative PCR Assays of Plasma, Bronchoalveolar Lavage, and Lung Tissue in a Rabbit Model of Experimental Pulmonary Zygomycosis▽

Miki Kasai,[1]† Susan M. Harrington,[2]†‡ Andrea Francesconi,[1] Vidmantas Petraitis,[1,3] Ruta Petraitiene,[1,3] Mara G. Beveridge,[1] Tena Knudsen,[1] Jeffery Milanovich,[1] Margaret P. Cotton,[1] Johanna Hughes,[1] Robert L. Schaufele,[1] Tin Sein,[1] John Bacher,[4] Patrick R. Murray,[2] Dimitrios P. Kontoyiannis,[5] and Thomas J. Walsh[1]*

Quantitative Polymerase Chain Reaction Detection of Circulating DNA in Serum for Early Diagnosis of Mucormycosis in Immunocompromised Patients

Laurence Millon,[1,2] Fabrice Larosa,[3] Quentin Lepiller,[2,4] Faezeh Legrand,[3] Steffi Rocchi,[1] Etienne Daguindau,[3] Emeline Scherer,[1,2] Anne-Pauline Bellanger,[1,2] Joel Leroy,[5] and Frederic Grenouillet[1,2]

Clin Infect Dis 2013, 56(10): e95-101.

Diagnostic des mucormycoses invasives

La détection d'ADN peut précéder de plusieurs jours la positivité de l'histologie

Table 1 Clinical, Histological, and Mycological Findings, Antifungal Treatment, Results of Quantitative Polymerase Chain Reaction Assays, and Outcomes for 10 Patients With Proven Mucormycosis

Patient No.	Sex[a]/ Age	Underlying Disease	Localization of Infection, Date[b] of First Clinical Symptoms/First Radiological Signs	Date[b] of Fever Onset	Positive Histology (Day 0)/Molecular Diagnosis	Positive Mycological Cultures (Day)/ Identification	Antifungal Therapy (Day of Initiation of L-AmB, POS)	Outcome at Day 90	qPCR Result Cq (Day)		Days From 1st Positive PCR to 1st Clinical Signs[c]	Days From 1st Positive PCR to Positive Histology[d]
1	F/65	Undernutrition, alcoholism	Disseminated D −1/not done	D −5	Skin/not done	Skin, urine (D0)/*L. corymbifera*	FLU	Death (D1)	Acory	Neg (D −8, D −1)	/	/
2	M/37	HL	Disseminated D −16/not done	D −28	Lung, liver/not done	Lung, liver (D0)/*L. corymbifera*	L-AmB (D −1)	Death (D0)	Acory	28 (D −3), Neg (D −10)	+13	−3
3	M/51	ALL	Rhinocerebral D −1/D −1	D −9	Sinus/*Lichtheimia corymbifera*	Sinus (D0)/*L. corymbifera*	L-AmB (D0) POS (D0)	Death (D3)	Acory	36 (D −8), 32 (D −5), 30 (D −1), 25 (D0), 27 (D2), Neg (D −18, D −15, D −12)	−7	−8
4	F/48	Polytrauma	Cutaneous D −5/not done	D0	Skin/*Lichtheimia* sp	Skin (D0)/negative	FLU, CAS	Death (D2)	Acory	27 (D0), Neg (D −2, D −9)	+5	0
5	M/59	NHL, diabetes mellitus	Disseminated D −5/D −22	D −14	Kidney/*Lichtheimia* sp	Cerebrospinal fluid (D −10)/ *Lichtheimia ramosa*	FLU, CAS, L-AmB (D0)	Alive	Acory	32 (D −23) 38 (D −8), Neg (D −1, D5)	−18	−23
6	F/41	PNH, SAA	Disseminated D −30/D −4	D −17	Sinus/*Rhizopus* sp	Sinus (D0)/*R. oryzae*	CAS, POS (D −11) L-AmB (D0) deferasirox (D0)	Death (D15)	Muc1	36 (D −10), 35 (D −1), 38 (D2), 36 (D6), 38 (D9), 34 (D13)	+20	−10
7	F/13	AML	Pulmonary[e] D −62/D −61	D −65	Lung/*Rhizopus oryzae*	Not done	VOR, L-AmB (D −60)	Alive	Muc1	35 (D −68), 40 (D −62), 36 (D −55), 38 (D −21), 36 (D10), Neg (D −75, D29)	−6	−68
8	M/57	MDS, allo-HSCT	Rhino-cerebral D −18/D −11	D0	Sinus/not done	Sinus (D0)/*R. pusillus*	ITR (D −96), L-AmB (D0) POS (D0)	Alive	Rmuc	39 (D −18), 39 (D −1), Neg (D −11, D −8, D9, D13)	0	−18
9	F/55	Renal transplant	Disseminated D −24/D −11	D −30	Lung, heart/ *Rhizomucor pusillus*	Lung, heart (D0)/*R. pusillus*	CAS	Death (D0)	Rmuc	29 (D −4), 22 (D −3), Neg (D −24, D −19)	+20	−4
10	M/60	AML	Disseminated D −50/D −47	D −50	Liver, spleen/ *Rhizomucor* sp	Liver, spleen (D0)/ negative	VOR, L-AmB (D −44) POS (D −36)	Alive	Rmuc	41 (D −49), 28 (D −46), Neg (D −52, D −42, D −39, D −36, D −18, D −13, D −1)	+1	−49

Million L, Clin Infect Dis 2013; 56(10):e95-101.

Diagnostic des mucormycoses invasives

Early diagnosis and monitoring of mucormycosis by detection of circulating DNA in serum: retrospective analysis of 44 cases collected through the French Surveillance Network of Invasive Fungal Infections (RESSIF)

L. Millon [1, 2, *], R. Herbrecht [3], F. Grenouillet [1, 2], F. Morio [4, 5], A. Alanio [6, 7, 8, 9], V. Letscher-Bru [10, 11], S. Cassaing [12], T. Chouaki [13, 14], C. Kauffmann-Lacroix [15], P. Poirier [16], D. Toubas [17, 18], O. Augereau [19], S. Rocchi [2], D. Garcia-Hermoso [7, 8], S. Bretagne [6, 7, 8, 9], French Mycosis Study Group

Effectif : 19 mucormycoes probables, 25 confirmées (réseau RESSIF)
Sensibilité : 81% (au moins un sérum + par patient)

Précocité du diagnostic : PCR positive en moyenne 9 jours (0-28 jrs) avant la positivité des examens mycologiques; au moins 2 jrs (0-24 jrs) avant l'imagerie.

Valeur pronostique :
Le taux de survie à 84 jours était significativement plus élevé chez les patients ayant une PCR+ qui se négative sous traitement que ceux pour lesquels la PCR reste constamment positive (48% and 4%, respectivement; p <10 6).

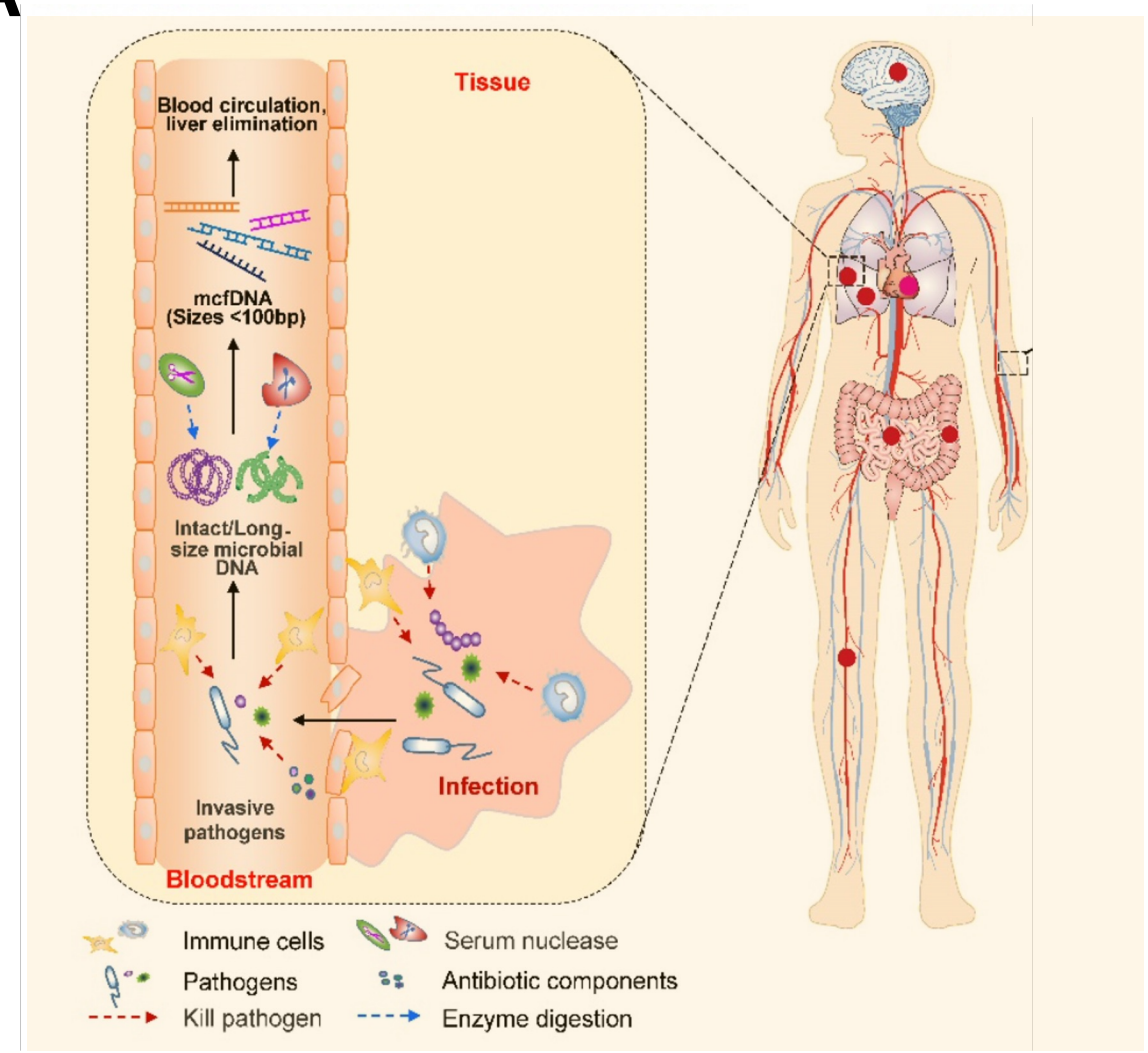
Million L, Clin Infect Dis 2013; 56(10):e95-101.

noscendo

Cell free DNA

In blood and other body fluids

- Urine
- CSF
- Ascites
- Sputum
- BAL
- Synovial fluid
- Other….

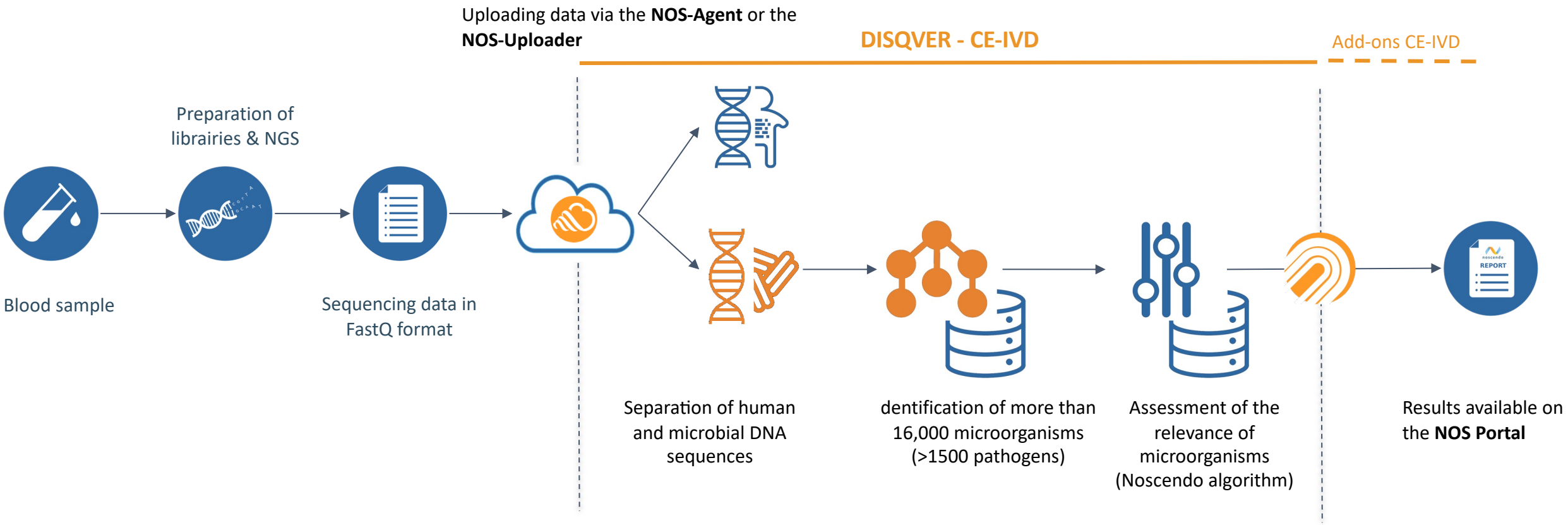


Pathogen genetic material is present in the cfDNA fractions of the different body fluids

- **Translocation from other compartments**

Han et al 2020 Theranostics

Methodology



Estimation of relevance

name	taxonomy_id	taxonomy_lvl	assigned_reads	relevance
Achromobacter sp. AONIH1	1758194	S	2	0.01
Achromobacter xylosoxidans	85698	S	6	0.02
Cloacibacterium normanense	237258	S	1	0.07
Cutibacterium acnes	1747	S	5	0.05
Delftia tsuruhatensis	180282	S	7	0.06
Escherichia coli	562	S	**11**	**16**
Homo sapiens	9606	S	35,362,170	**NA**
Human gammaherpesvirus 4	10376	S	**8**	**16**
Microbacterium sp. No. 7	1714373	S	2	0.97
Moraxella osloensis	34062	S	1	0.00
Plasmodium ovale	36330	S	**22**	0.03
Rhizopus delemar	936053	S	**47**	0.99
Stenotrophomonas maltophilia	40324	S	3	0.02
Streptococcus pneumoniae	1313	S	**1919**	**16**
Wuchereria bancrofti	6293	S	7	0.04

Available tools for the diagnosis of IFI

Dx Tools	Invasive Candidiasis	Cryptococcosis	Invasive Aspergillosis	Mucormycosis	Fusariosis	Histopalasmosis	Pneumocystosis
Direct examination/ Histology	Morphological aspects of IFI, Poor sensitivity						
Specificities		Capsule Indian Ink, other type of staining					Specific aspects IF
Culture Based Methods	Poor sensitivity						
Antigen	Mannan Spécific Transient Poor sensitivity Serum, (CSF) Diagnosis Deep-seated localisation	Glucuronoxylomannan Sensitivity +++ Spscificity +++ Serum, CSF (BAL, urine) Diagnosis Prognosis	Galactomannan Sensitivity host- and clinical entity dependant Specificity Cross reaction Other fungi Serum, BAL, CSF (bronchial aspiration) Screening, Diagnosis, Prognosis		Some species cross react with GM	Specific Ag Not available in Fr Cross react with GM	
β– D1,3 Glucans	Species dependant		Disappointing		Sensitivity 50%	Scare studies	NPV Long-time persistance
PCR	T2MR (5 species) Blood (CSF/AH) Necker/Marseille (few species)	In house Method	*A. fumigatus* /spp All matrices Resistance, included in EORTC/MSGERC IPA case definitions	(4 species in Lille)	In house Method	In house Method	Interpretation threshold, diffirenciate infection/colonization
Panfungal Sequencing	Moderate sensitivity if direct examination is positive !						
NGS/WGS	Sensistivity, quantification and interpretation ?						

Take home messages !

- Diagnostic des mycoses superficielles aisé, rapide
- Délais de rendu des résultats réduits de 24-48 h par rapport aux critères phénotypiques
- Mycologie conventionnelle utile pour l'isolement de l'agent pathogène, réalisation des tests de sensibilité aux antifongiques
- Diagnostic des mycoses invasive est difficile (Mycologie conventionnelle + histologie insuffisants) :
  - Candidoses invasives : Recours aux biomarqueurs (mannane, b-glucanes), T2MR pour améliorer la sensibilité des hémocultures
  - Aspergilloses invasives : Recours au galactomannane sérique, LBA, PCR in situ et PCR dans le sang
  - Mucormycoses : recours à la PCR (améliorer la sensibilité et la précocité du diagnostic)