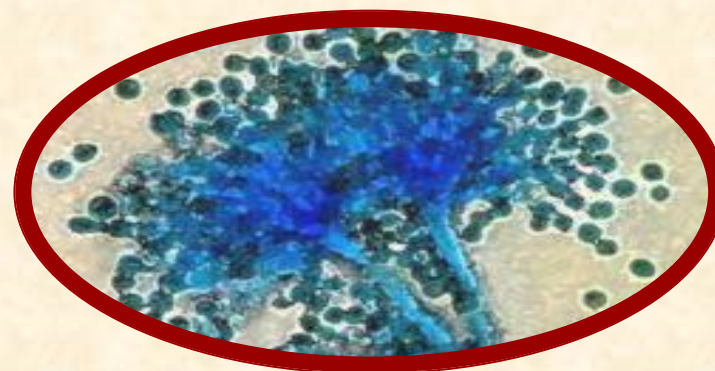




# XLVII CONGRESSO NAZIONALE AMCLI

10-13 Novembre 2018  
Palacongressi Rimini

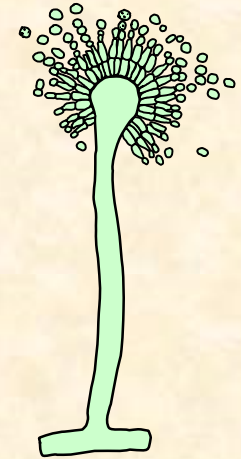


**Sessione Congiunta  
AMCLI - FADOI**

**INFEZIONI GRAVI IN  
MEDICINA INTERNA:  
GESTIONE E NUOVI FARMACI**

**Stefano Andreoni**  
**SCO Microbiologia e Virologia**  
**Azienda O/U Maggiore della Carità Novara**

# Classificazione delle patologie da *Aspergillus*



## Patologie in soggetti immunocompetenti

**Tossicosi o micotossicosi (da aflatossine)**  
Ingestione o inalazione di micotossine  
Ingestione di altri metaboliti

### Manifestazioni allergiche

Asma allergico  
Rinite allergica  
Sinusite allergica  
Alveolite allergica estrinseca  
Aspergillosi broncopolmonare allergica

### Infezioni superficiali o non invasive

Infezioni cutanee  
Otomicosi  
Sinusiti

## Infezioni associate a danni tissutali o a corpi estranei

Cheratiti e endoftalmiti  
Infezioni da ferite infette  
Osteomieliti  
Endocarditi da valvole infette  
Infezioni da protesi vascolari infette  
Aspergilloma (fungus ball)  
Peritoniti

## Infezioni nell'ospite immunocompromesso

Aspergillosi cutanea primaria  
Infezione seno-orbitaria  
Aspergillosi polmonare  
Tracheobronchite invasiva  
Aspergillosi cronica necrotizzante  
Aspergillosi acuta invasiva  
Aspergillosi del SNC  
Aspergillosi disseminata

Inalazione  
di conidi di  
*Aspergillus*

eliminazione

colonizzazione

infezione

ipersensibilizzazione

Nessuna  
sequela

aspergilloma seni  
sinusite cronica  
invasiva

Aspergilloma  
polmonare

Malattia polmonare  
cavitaria

Aspergillosi  
cronica  
necrotizzante

Paziente  
immunodepresso

Malattia  
polmonare cronica

Aspergillosi  
invasiva

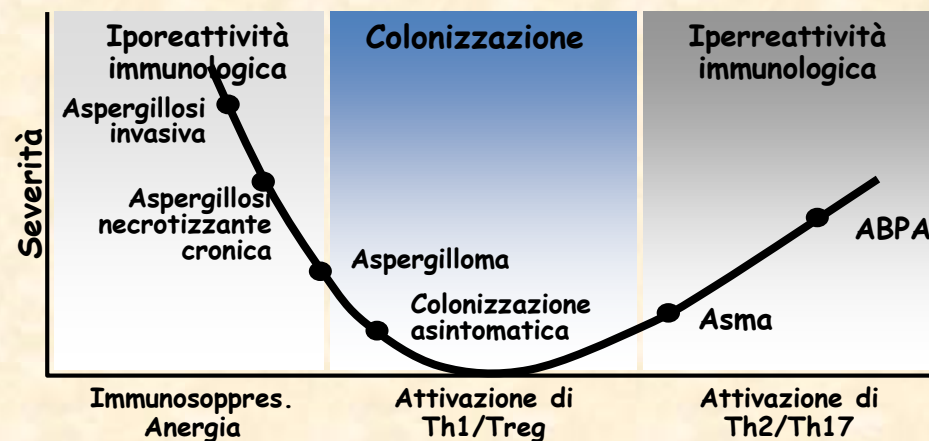
Asma

sinusite allergica

(ABPA)  
broncopneumopatia allergica

(EAA)  
alveolite allergica estrinseca

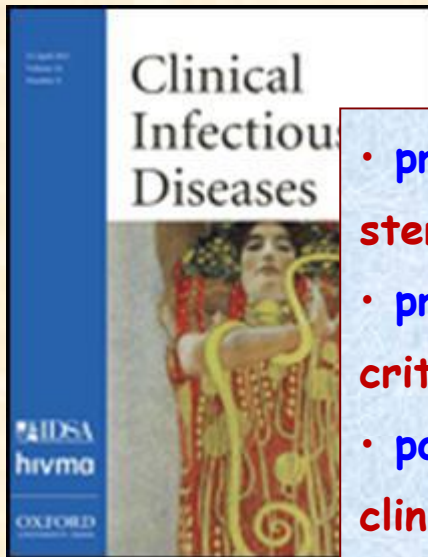
(da Clin Microbiol Rev 2009)



Revised **definitions of invasive fungal disease** from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (**EORTC/MSG**) Consensus Group (De Pauw B et al., CID, 2008)

«Invasive fungal diseases are important causes of morbidity and mortality. Clarity and uniformity in defining these infections are important factors in improving the quality of clinical studies»

The **revised definitions** retain the original classifications of “**proven**” “**probable**” and “**possible**” invasive fungal disease.

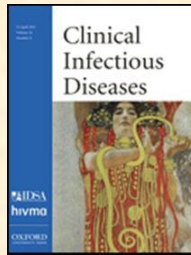


**Definizioni basate su tre livelli di certezza diagnostica\***

- **provata**, in presenza di evidenza istologica, citologica o colturale (da sito sterile) di invasione;
- **probabile**, in presenza di almeno un criterio relativo al tipo di paziente, un criterio clinico e un dato microbiologico;
- **possibile**, in presenza di un criterio relativo al tipo di paziente e un criterio clinico.

\* definizioni impiegate nei pazienti affetti da malattie ematologiche maligne, sottoposti a trapianto d'organo o di cellule staminali emopoietiche, nei pazienti affetti da malattie auto-infiammatorie trattati con terapia immunosoppressiva.

# Criteria for **probable** invasive fungal disease except for endemic mycoses (De Pauw B et al. Clin Infect Dis, 2008)



## Host factors<sup>a</sup>

- Recent history of neutropenia ( $<0.5 \times 10^9$  neutrophils/L [ $<500$  neutrophils/mm<sup>3</sup>] for  $>10$  days) temporally related to the onset of fungal disease
- Receipt of an allogeneic stem cell transplant
- Prolonged use of corticosteroids (excluding among patients with allergic bronchopulmonary aspergillosis) at a mean minimum dose of 0.3 mg/kg/day of prednisone equivalent for  $>3$  weeks
- Treatment with other recognized T cell immunosuppressants, such as cyclosporine, TNF- $\alpha$  blockers, specific monoclonal antibodies (such as alemtuzumab), or nucleoside analogues during the past 90 days
- Inherited severe immunodeficiency (such as chronic granulomatous disease or severe combined immunodeficiency)

## Clinical criteria<sup>b</sup>

### Lower respiratory tract fungal disease<sup>c</sup>

The presence of 1 of the following 3 signs on CT:

- Dense, well-circumscribed lesions(s) with or without a halo sign
- Air-crescent sign
- Cavity

### Tracheobronchitis

Tracheobronchial ulceration, nodule, pseudomembrane, plaque, or eschar seen on bronchoscopic analysis

### Sinonasal infection

Imaging showing sinusitis plus at least 1 of the following 3 signs:

- Acute localized pain (including pain radiating to the eye)
- Nasal ulcer with black eschar
- Extension from the paranasal sinus across bony barriers, including into the orbit

### CNS infection

## Mycological criteria

### Direct test (cytology, direct microscopy, or culture)

Mold in sputum, bronchoalveolar lavage fluid, bronchial brush, or sinus aspirate samples, indicated by 1 of the following:

Presence of fungal elements indicating a mold

Recovery by culture of a mold (e.g., *Aspergillus*, *Fusarium*, Zygomycetes, or *Scedosporium* species)

### Indirect tests (detection of antigen or cell-wall constituents)<sup>e</sup>

#### Aspergillosis

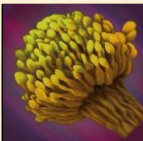
Galactomannan antigen detected in plasma, serum, bronchoalveolar lavage fluid, or CSF

Invasive fungal disease other than cryptococcosis and zygomycoses

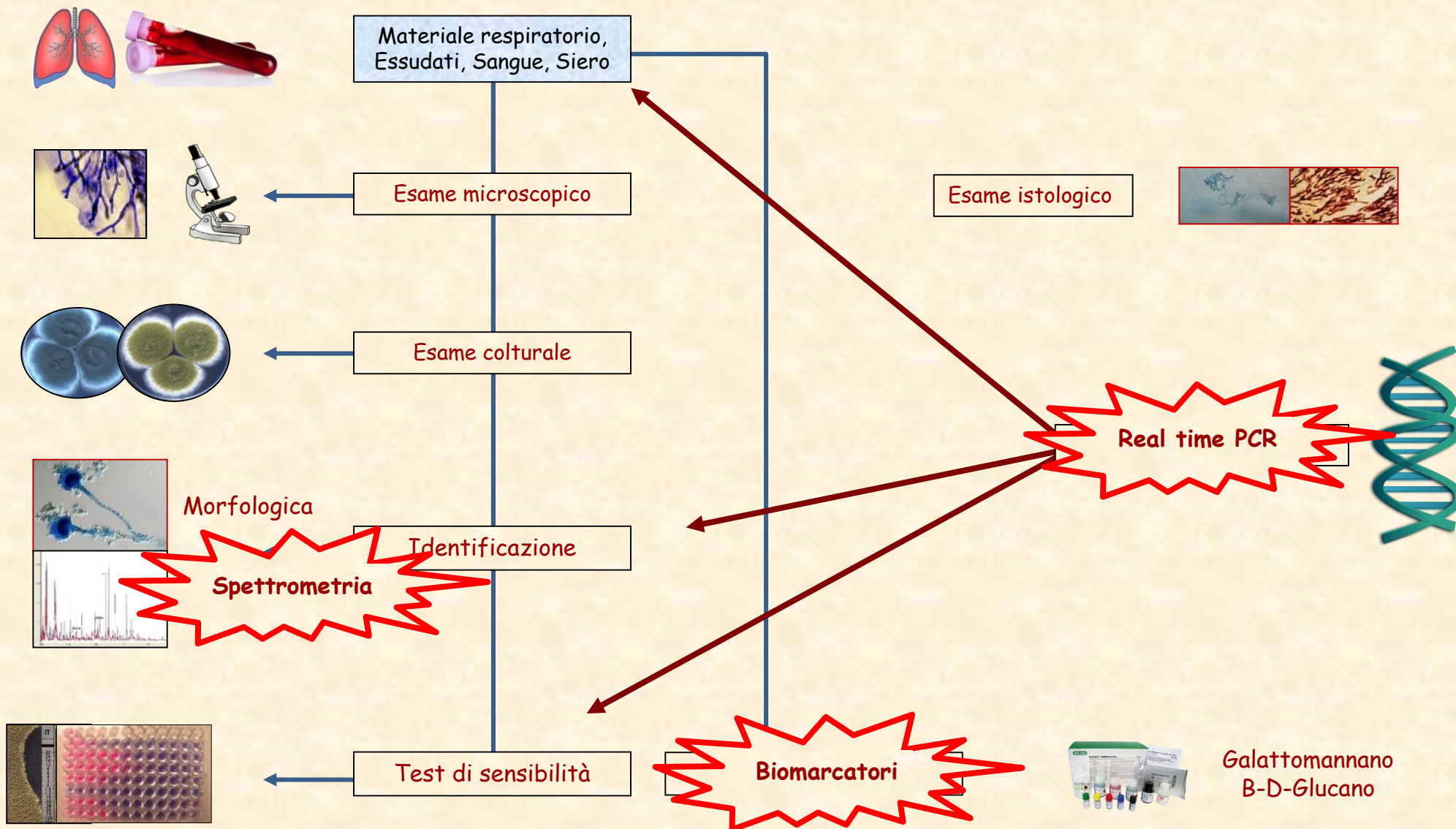
$\beta$ -D-glucan detected in serum

We had hoped that nucleic acid-detection tests, such as **PCR**, would have improved enough to incorporate the results of these tests into definitions.

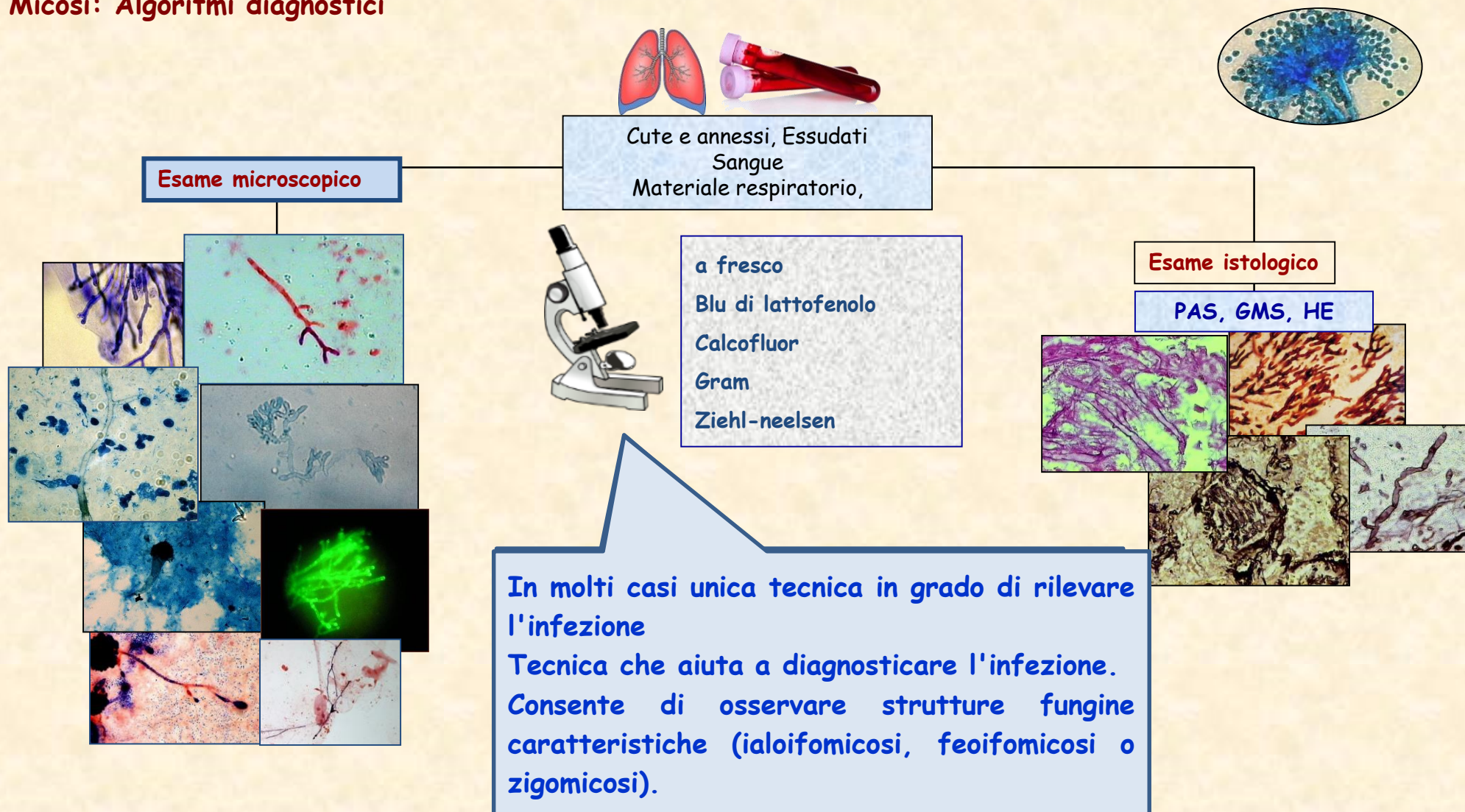
However, **standardization and validation have not yet been attained for these platforms**



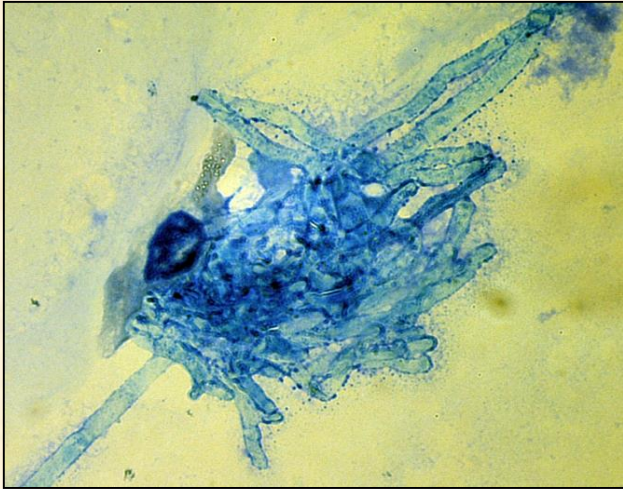
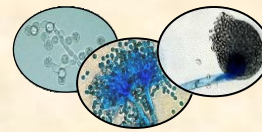
# DIAGNOSTICA DI LABORATORIO DELLE ASPERGILLOSI



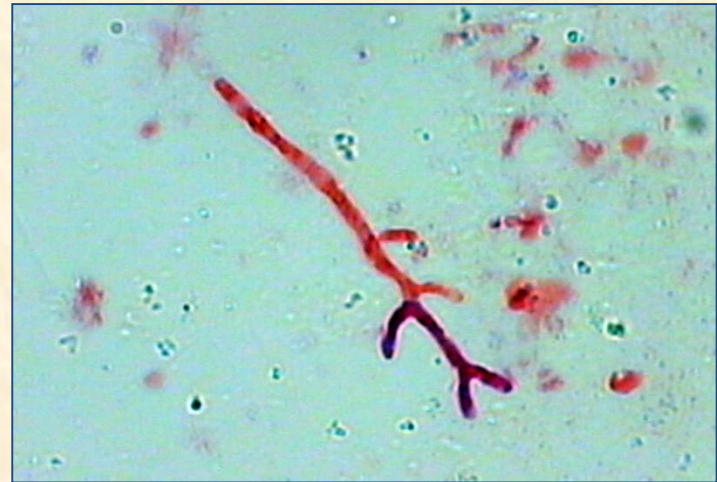
## Micosi: Algoritmi diagnostici



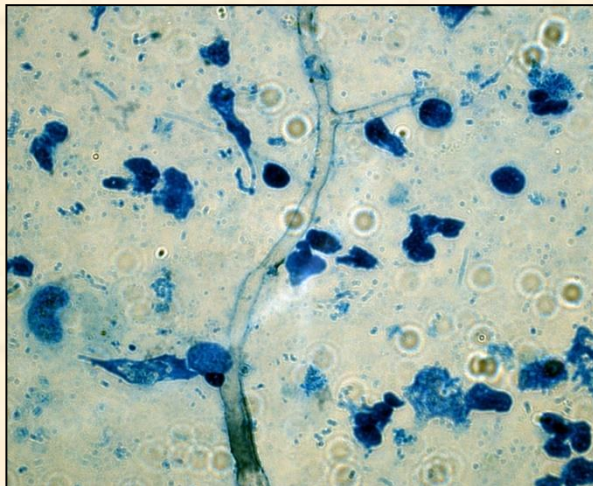
**Caratteri morfologici  
rilevabili all'esame microscopico diretto**



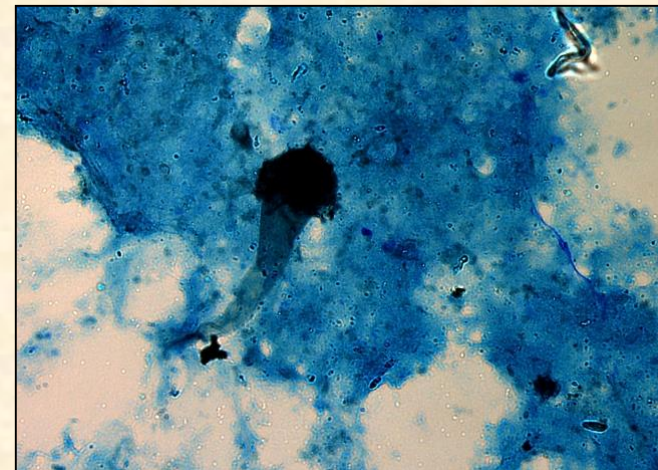
**Lavaggio broncoalveolare (Kinyoun)**



**Aspirato seno mascellare (Gram)**

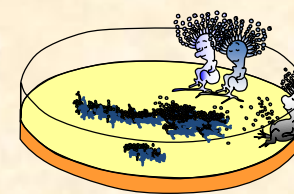


**Aspirato bronchiale (Kinyoun)**

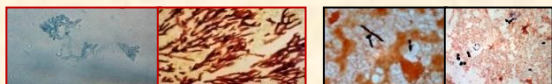


**Biopsia polmonare(Kinyoun)**

# Algoritmi diagnostici



## Esame microscopico



Sabouraud dextrose agar  
Malt extract agar,  
Potato dex agar, Rice agar  
Czapek agar

## Esame colturale

18-24 h 25 - 35°C.

2-4 gg

5-8 gg

10-12 gg

Bassa sensibilità subordinata alla qualità dei campioni, alle manipolazioni dei campioni, alle condizioni di crescita

Limitata capacità di riprodurre le condizioni ambientali dei tessuti infetti (ipossia).

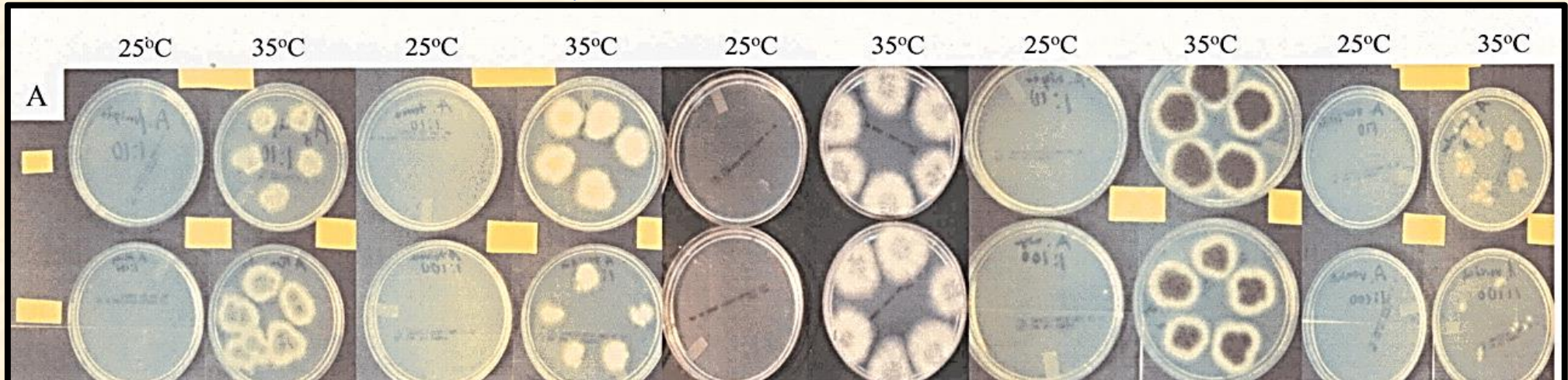


Emocoltura

*Aspergillus*

??

## Aspergillus hyphae in infected tissue: evidence of physiologic adaptation and effect on culture recovery (Tarrand J.J. et al., J Clin Microbiol. 2005)



L'incubazione a 35°C aumenta complessivamente l'isolamento di *Aspergillus* spp. da campioni clinici (31%). La coltura in ambiente microaerofilo a 35°C aumenta significativamente l'isolamento di *Aspergillus* spp. da varie fonti.

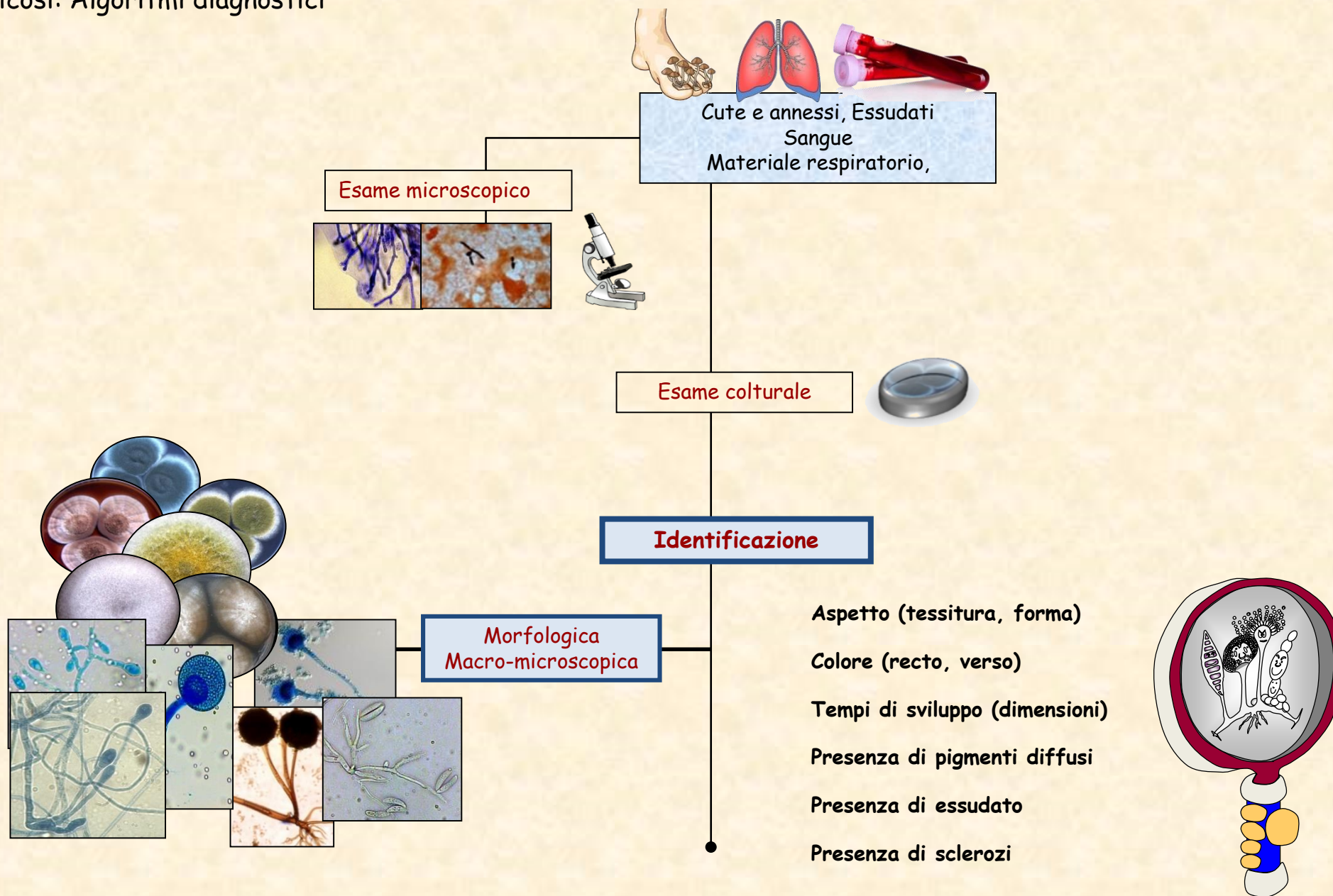
L'isolamento di *A. fumigatus* era significativamente più alto utilizzando plug di espettorato lavato rispetto agli standard per le indagini micologiche (Pashley C.H. et al., Medical Mycology, 2012)

La crescita di *A. fumigatus* non è favorita su campioni di espettorato diluiti-omogeneizzati.

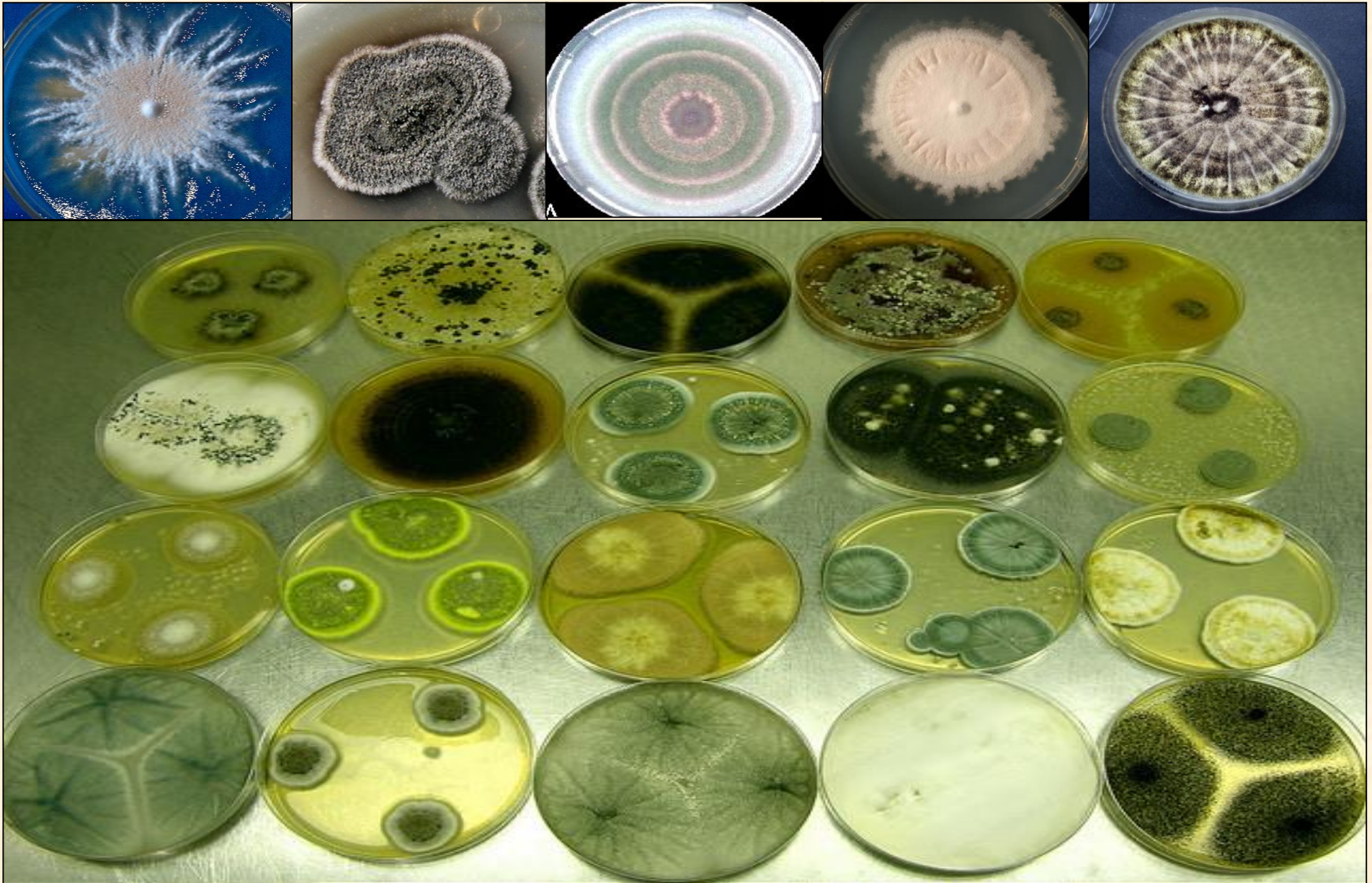
Culture of experimentally stressed *Aspergillus* hyphae at 35°C in 6% O<sub>2</sub>-10% CO<sub>2</sub> versus 25°C in room air for 3 days (A) and up to 14 days (B). Paired inocula for each dilution are shown side by side

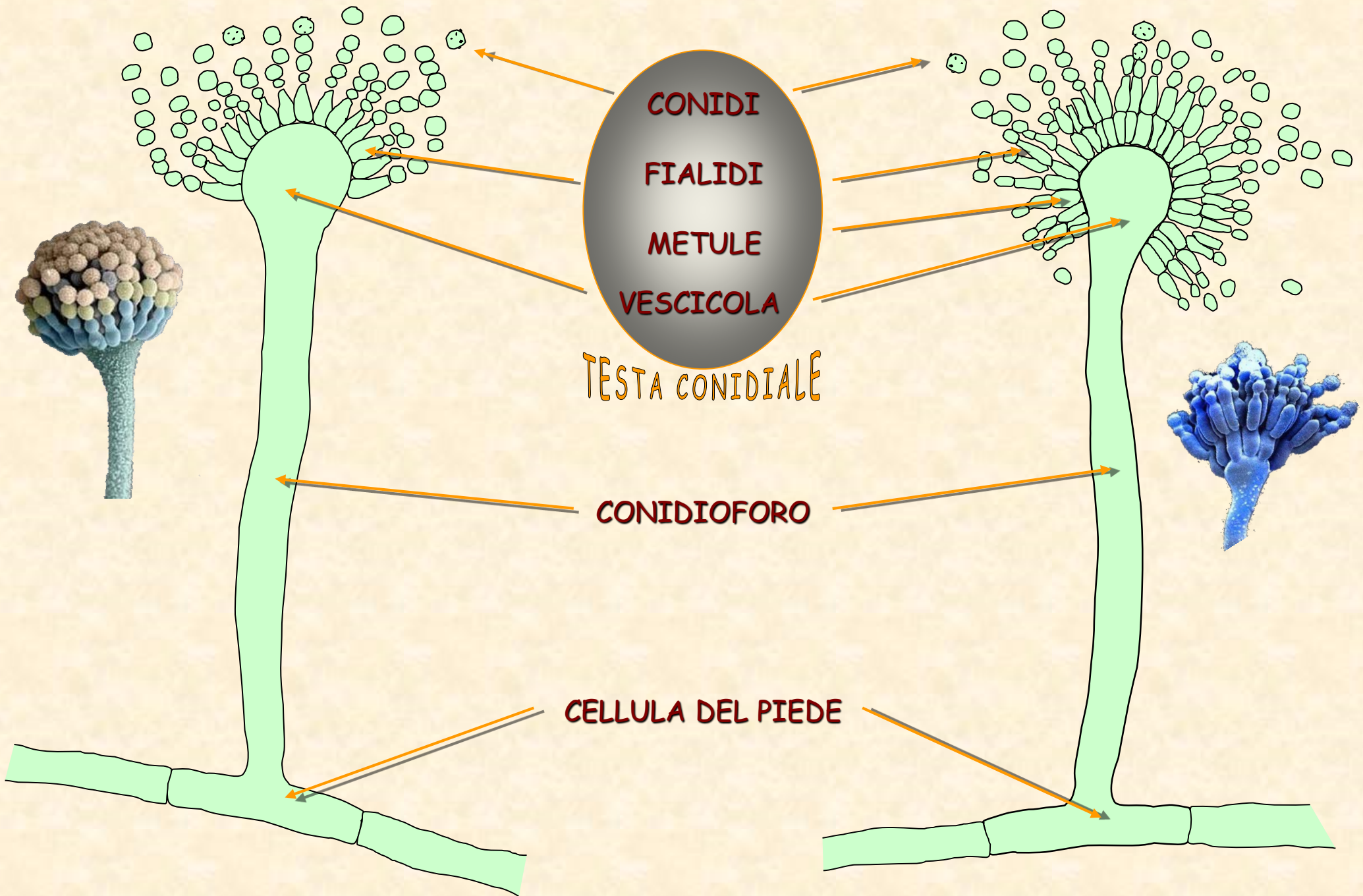
Significant increase of *Aspergillus* spp. from autopsy tissue and various clinical samples (+31%)

# Micosi: Algoritmi diagnostici

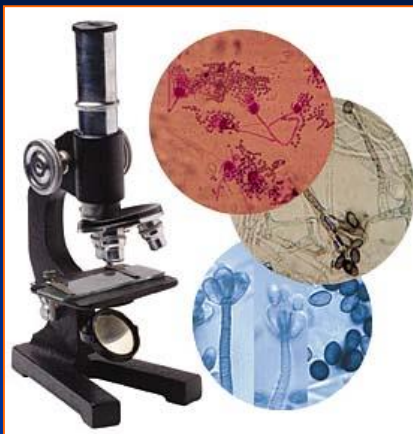


## CARATTERI TINTORIALI DI COLONIE DI *ASPERGILLUS*

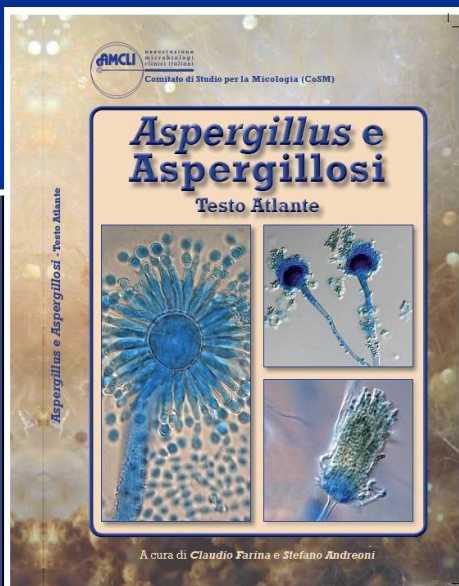
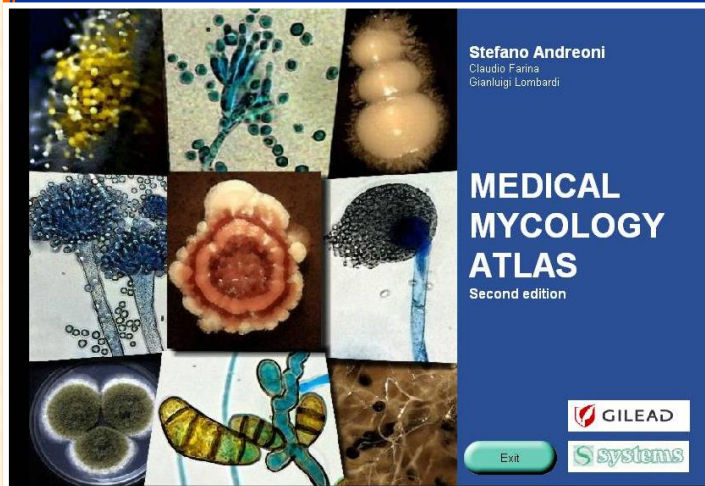




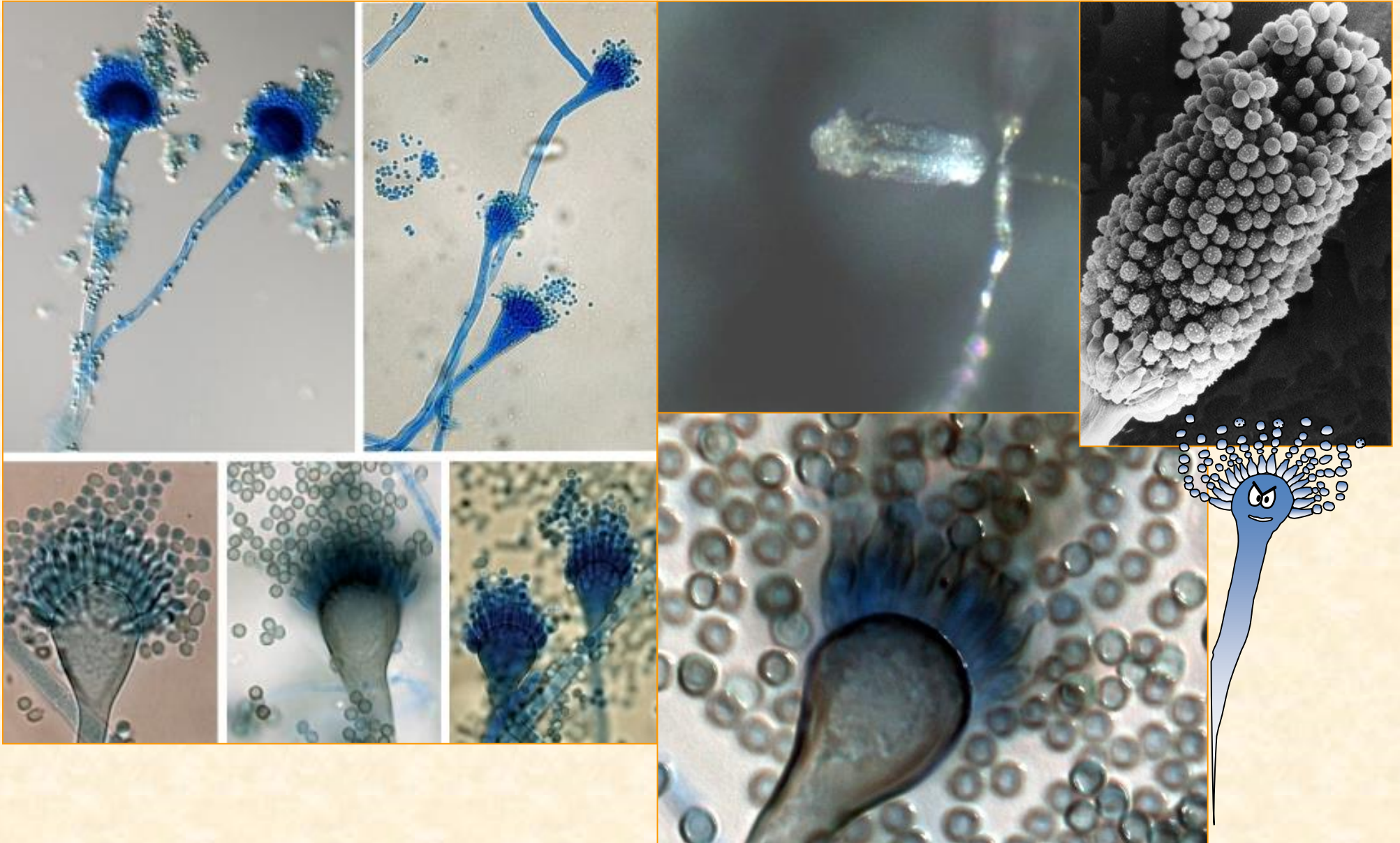
# Identificazione funghi filamentosi:



Testi di micologia  
Atlanti di micologia  
Chiavi dicotomiche  
Centri di riferimento  
Reti informatiche  
Controlli di qualità



## *A. fumigatus*: caratteri macro-microscopici



# Classificazione corrente di *Aspergillus*

(comprendente solo isolati implicati in malattie umane)

Subgenere	Sezione	Isolati implicati in infezioni umane
<i>Aspergillus</i>	<i>Aspergillus</i>	<i>A. chevalieri</i>
	<i>Restricti</i>	<i>A.chevalieri, A.restrictus, A.caesiellus, A.concius</i>
<i>Fumigati</i>	<i>Fumigati</i>	<i>A.fumigatus, A.lentulus, A.viridinutans, N.fischeri, N.pseudofischeri, N.udagawae, N.hiratsukae</i>
<i>Clavati</i>	<i>Clavati</i>	<i>A.clavatus, A.clavatoanicus</i>
<i>Nidulantes</i>	<i>Usti</i>	<i>A.ustus, A.deflectus</i>
	<i>Versicolores</i>	<i>A.versicolor, A.janus, A.sydowii, A.granulosus</i>
	<i>Terrei</i>	<i>A.terreus</i>
	<i>Flavipedes</i>	<i>A.carneus, A.flavipes, A.niveus</i>
	<i>Nidulantes</i>	<i>A.nidulans, A.quadrilineata, A.unguius</i>
<i>Circumdati</i>	<i>Circumdati</i>	<i>A.ochraceus</i>
	<i>Flavi</i>	<i>A.flavus, A.oryzae, A.alliaceus, A.tamarisii</i>
	<i>Nigri</i>	<i>A.niger, A.awamori</i>
	<i>Candidi</i>	<i>A.candidus</i>

# *Aspergillus* sezione *Fumigati* : specie accettate (23 *Neosartoria* species e 10 *Aspergillus* species)

Specie anamorfe ( <i>Aspergillus</i> )	Specie teleomorfe ( <i>Neosartoria</i> )	
<i>A. brevipes</i> <i>A. duricaulis</i> <i>A. fumigatiaffinis</i> <i>A. fumigatus</i> <i>A. anomalus</i> <i>A. fumigatus</i> var. <i>acolumnaris</i> <i>A. fumigatus</i> var. <i>ellipticus</i> <i>A. fumigatus</i> mut. <i>helvola</i> <i>A. phialiseptus</i> <i>A. neoellipticus</i> <i>A. arvii</i>  <i>A. fumisynnematus</i> <i>A. lentulus</i> <i>A. novofumigatus</i> <i>A. turcosus</i> <i>A. unilateralis</i> <i>A. brevipes</i> var. <i>unilateralis</i>  <i>A. viridinutans</i> <i>A. fumigatus</i> var. <i>sclerotiorum</i>	<i>N. assulata</i> <i>N. aurata</i> <i>N. aureola</i> <i>N. australensis</i> sp. nov. <i>N. coreana</i> <i>N. denticulata</i> <i>N. fennelliae</i> <i>N. otani</i> <i>N. ferenczii</i> sp. nov. <i>N. fischeri</i> <i>N. galapagensis</i> <i>N. glabra</i> <i>N. hiratsukae</i> <i>N. laciniosa</i> <i>N. multiplicata</i> <i>N. papuensis</i> sp. nov.	<i>N. pseudofischeri</i> <i>N. quadricincta</i> <i>N. primulina</i> <i>N. spinosa</i> <i>N. fischeri</i> var <i>spinosus</i> <i>S. fumigata</i> var. <i>verrucosa</i> <i>N. botucatensis</i> <i>N. paulistensis</i> <i>N. takakii</i> ? <i>N. spathulata</i> <i>N. stramenia</i> <i>N. tatenoi</i> <i>N. delicata</i> <i>N. udagawae</i> <i>N. warcupii</i> sp. nov.
	(da Samson R.A., et al. Studies in Micology, 2007)	

Origin, molecular identification MIC and MEC of different antifungal against clinical isolates of *Aspergillus* section *Fumigati* (Alcazar-Fuoli L et al. , AAC, 2008)(seml.)

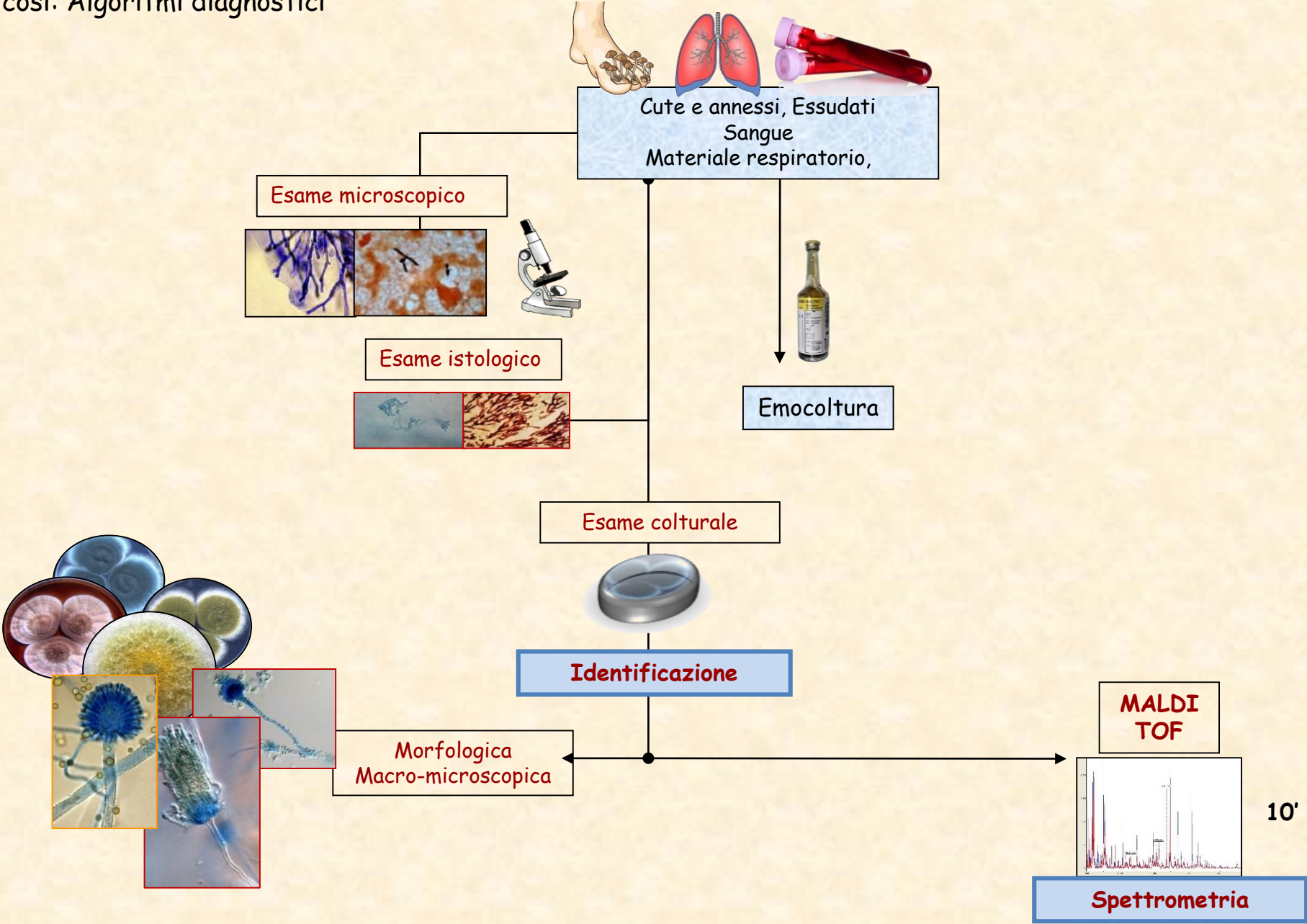
Isolate	Origin	Molecular identification	MIC µg/ml				MEC µg/ml		
			AMB	ITZ	VRZ	RVC	POS	CAS	MICA
ATCC 2004305	Reference strain	<i>A. fumigatus</i>	0.25-1.0	0.125-1.0	0.25-1.0	0.25-1.0	0.03-0.25	0.12-0.5	0.03
CNM-CM-1290	Sputum	<i>A. lentulus</i>	4.5	9.25	4.5	3.5	0.4	0.28	0.05
CNM-CM-3134	Sputum	<i>A. lentulus</i>	10	10.25	6	6	0.56	0.38	0.07
CNM-CM-3364	BAL	<i>A. lentulus</i>	8.4	8	5.6	5.6	2	0.18	0.03
CNM-CM-3537	Sputum	<i>A. lentulus</i>	7	8.25	3.5	3	0.28	0.42	0.04
CNM-CM-3599	Sputum	<i>A. lentulus</i>	2.66	0.5	4	2	0.5	0.26	0.03
CNM-CM-4420	Nail	<i>A. lentulus</i>	10.66	8	5.33	4	0.33	0.33	0.03
CNM-CM-4428	Skin	<i>A. lentulus</i>	12	7	5	5	0.62	0.29	0.03
CNM-CM-4063	BAS	<i>A. fumisynnematus</i>	1	0.25	0.83	0.83	0.12	0.5	0.03
CNM-CM-2280	Sputum	<i>A. fumigatiaffinis</i>	16	6.66	6.66	6	1.16	1.3	0.04
CNM-CM-3227	BAL	<i>A. fumigatiaffinis</i>	8	8	5.6	5	0.62	0.16	0.08
CNM-CM-3303	Skin	<i>N. hiratsukae</i>	0.5	0.12	0.5	0.37	0.06	0.34	0.03
CNM-CM-3305	Skin	<i>N. hiratsukae</i>	0.31	0.185	0.375	0.25	0.045	0.03	0.14
CNM-CM-3764	OPE	<i>N. hiratsukae</i>	1	0.33	1.66	1.33	0.16	0.26	0.04
CNM-CM-3769	Sputum	<i>N. pseudofischeri</i>	1	8	3.33	2	0.25	0.12	0.06
CNM-CM-2270	Sputum	<i>N. pseudofischeri</i>	0.17	8	3	3.5	0.31	0.51	0.26
CNM-CM-3914	Nail	<i>N. pseudofischeri</i>	0.25	0.125	0.125	0.125	0.125	0.125	0.125
CNM-CM-3147	OPE	<i>A. viridinutans</i>	0.58	14.4	4	5.66	0.41	0.67	0.2
CNM-CM-4518	Nail	<i>A. viridinutans</i>	0.37	16	4	4	0.25	1	0.03

a The MICs of amphotericin B (AMB), itraconazole (ITC), voriconazole (VCZ), ravuconazole (RVC), posaconazole (POS) are expressed as geometric means (GMs)  
b The MECs of caspofungin (CAS) and micafungin (MICA) are GMs; the MICs and MECs for the reference strain are ranges.  
BAL bronchoalveolar lavage; BAS bronchoalveolar aspirate; OPE oropharyngeal exudate.

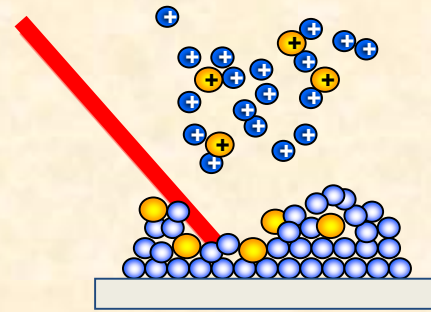
Nel genere *Aspergillus* si riconoscono due livelli di identificazione:

- identificazione a livello di *specie complex*. Ne sono un esempio *A. fumigatus complex* (sottogenere *Fumigati*, sezione *Fumigati*) e *A. flavus complex* (sottogenere *Circumdati*, sezione *Flavi*);
- identificazione delle specie all'interno di una sezione: ciò consente, ad esempio, la discriminazione di *A. fumigatus* da *A. lentulus* (entrambi membri della sezione *Fumigati*, sottogenere *Fumigati*).

Micosi: Algoritmi diagnostici



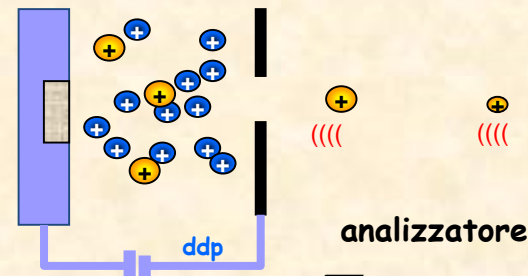
Lo spettrometro di massa è in grado di determinare la massa molecolare delle molecole presenti in una miscela.



sorgente

un composto viene ionizzato

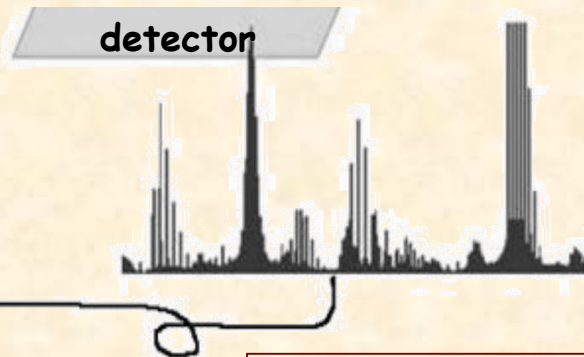
**Ionizzazione**



analizzatore

gli ioni vengono separati in base al proprio rapporto massa/carica

**Separazione Ioni**



**Elaborazione dati**

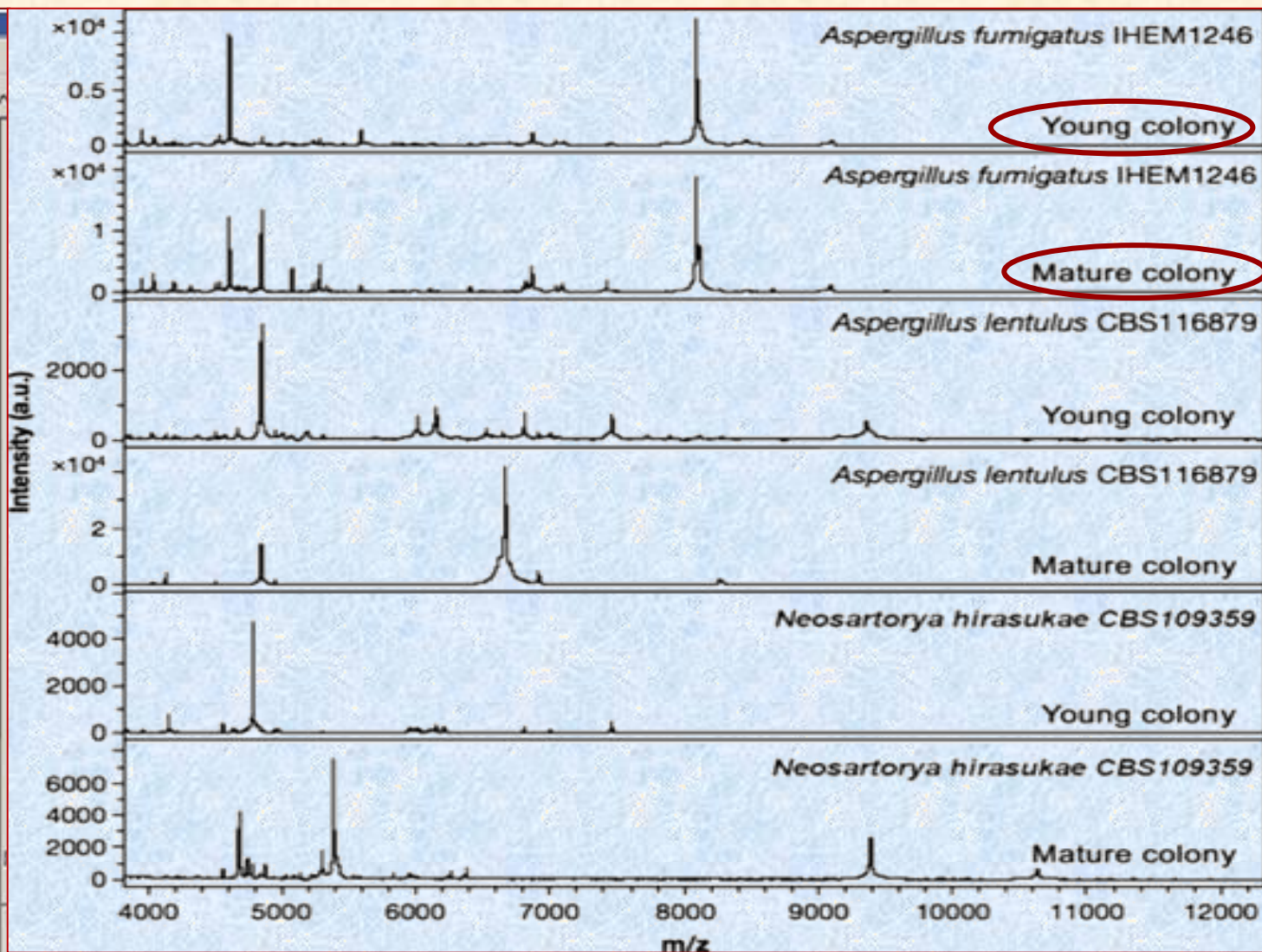
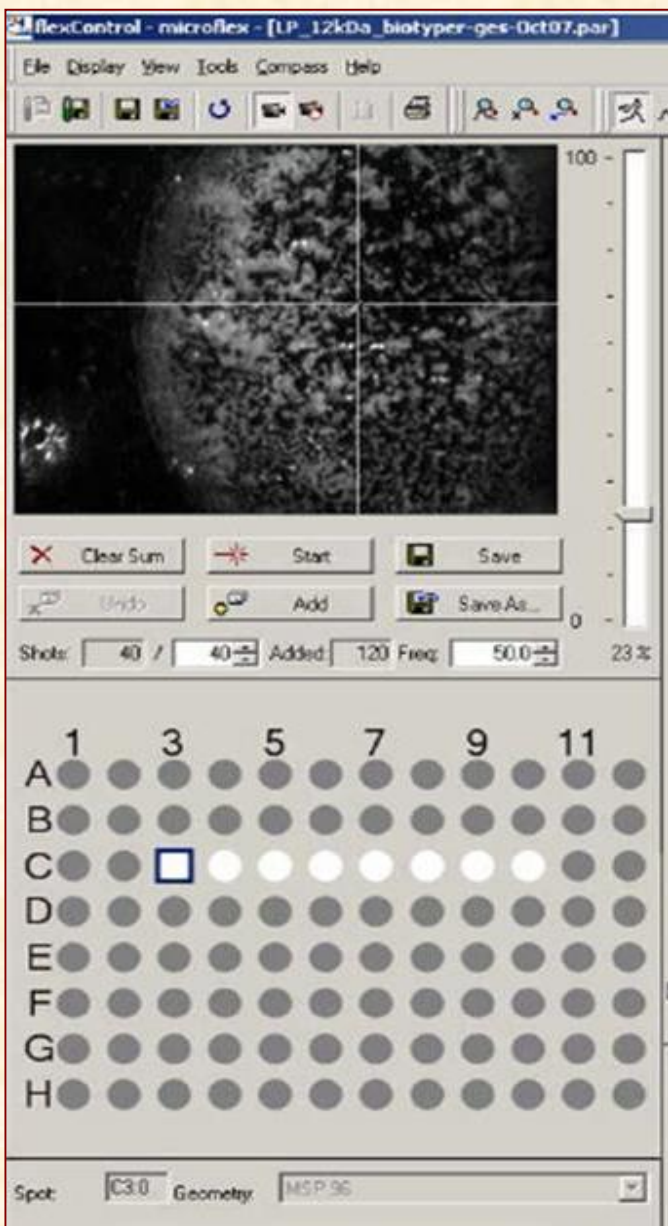
**Rivelazione Ioni**

gli ioni raggiungono un rilevatore dove l'energia ricevuta è convertita in un segnale elettrico



Il segnale elaborato (intensità, frequenza) è convertito in uno spettro di massa

# Representation of the specific spectral fingerprints of young and mature colonies for three selected species (Alanio A et al., CMI, 2010)





# Identification results for the 124 clinical and 16 environmental isolates of *Aspergillus* obtained by MALDI TOF MS\* (Alanio A et al., CMI, 2010)

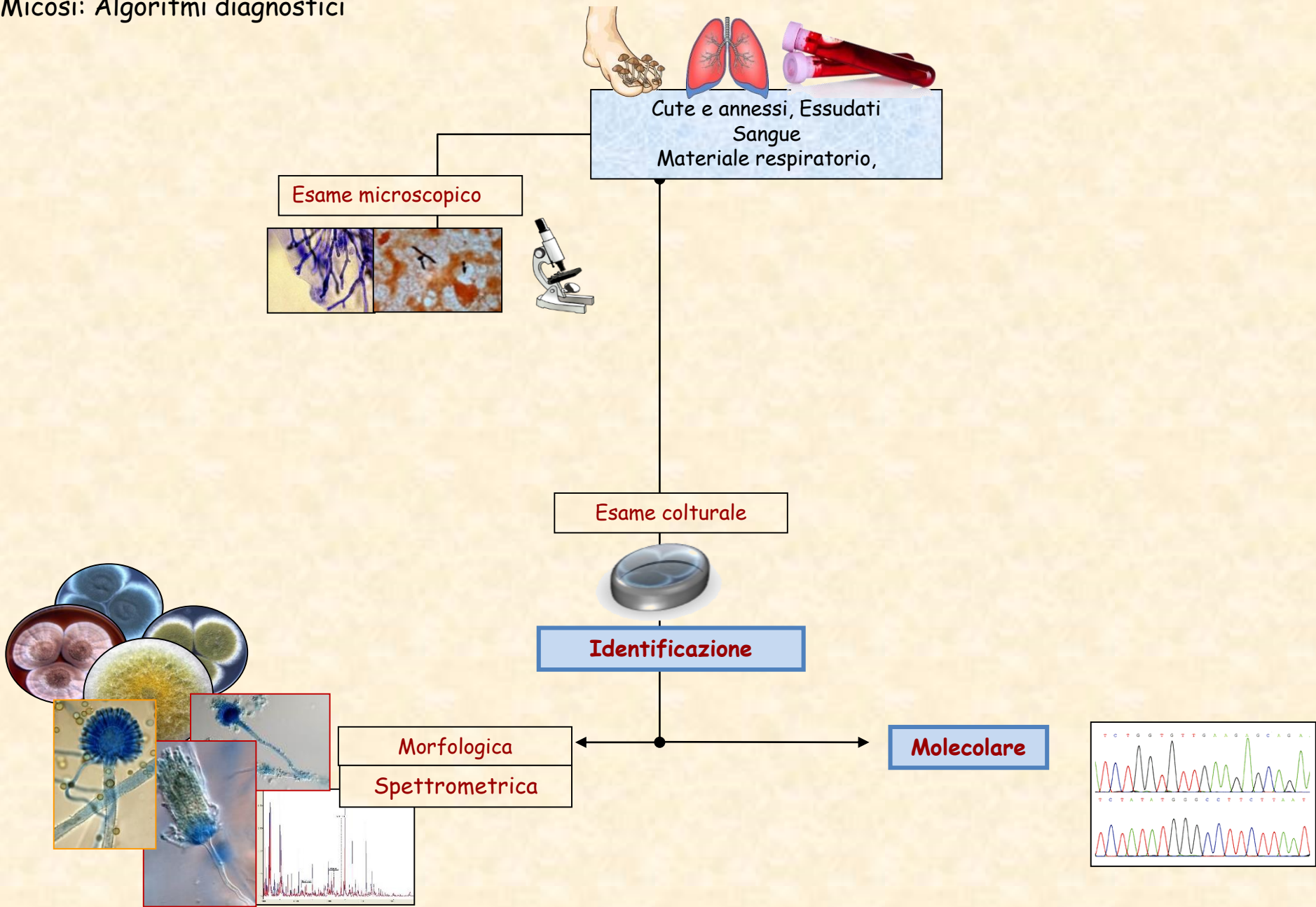
Section	Species	No. of isolates tested	No. of isolates correctly identified <sup>a</sup>	% of isolates correctly identified <sup>b</sup>
Fumigati	<i>Aspergillus fumigatus</i>	50	48	96
	<i>Aspergillus lentulus</i>	7	7	100
	<i>Neosartorya pseudofischeri</i>	5	5	100
	<i>Neosartorya fischeri</i>	1	1	-
	<i>Aspergillus fumigatiaffinis</i>	2	2	-
	<i>Aspergillus fumisynnematus</i>	1	1	-
	<i>Aspergillus viridinutans</i>	2	2	-
	<i>Neosartorya udagawae</i>	1	1	-
	<i>Neosartorya hiratsukae</i>	2	2	-
	<i>Aspergillus flavus</i>	13	13	100
Flavi	<i>Aspergillus tamaraii</i>	2	2	-
	<i>Aspergillus parvisclerotigenus</i>	1	1	-
Terrei	<i>Aspergillus terreus</i>	8	8	100
Nigri	<i>Aspergillus niger</i>	9	9	100
	<i>Aspergillus tubengensis</i>	1	1	-
Nidulantes	<i>Aspergillus foetidus</i>	2	2	-
	<i>Emericella nidulans</i>	4	4	-
	<i>Emericella quadrilineata</i>	1	1	-
	<i>Aspergillus sydowii</i>	7	7	100
	<i>Aspergillus versicolor</i>	3	3	-
Usti	<i>Aspergillus calidoustus</i>	14	14	100
	<i>Aspergillus pseudodeflectus</i>	1	1	-
Circumdati	<i>Aspergillus insuetus</i>	1	1	-
	<i>Aspergillus ochraceus</i>	2	2	-

a: In comparison with identification obtained by partial gene sequence-based method

b: Calculated only when the number of isolates was at least five

\*: matrix-assisted laser desorption ionization time-of-flight mass spectrometry

Micosi: Algoritmi diagnostici



# Identificazione molecolare da coltura (sequenziamento)



Per *Aspergillus* vi sono **due livelli di identificazione**:

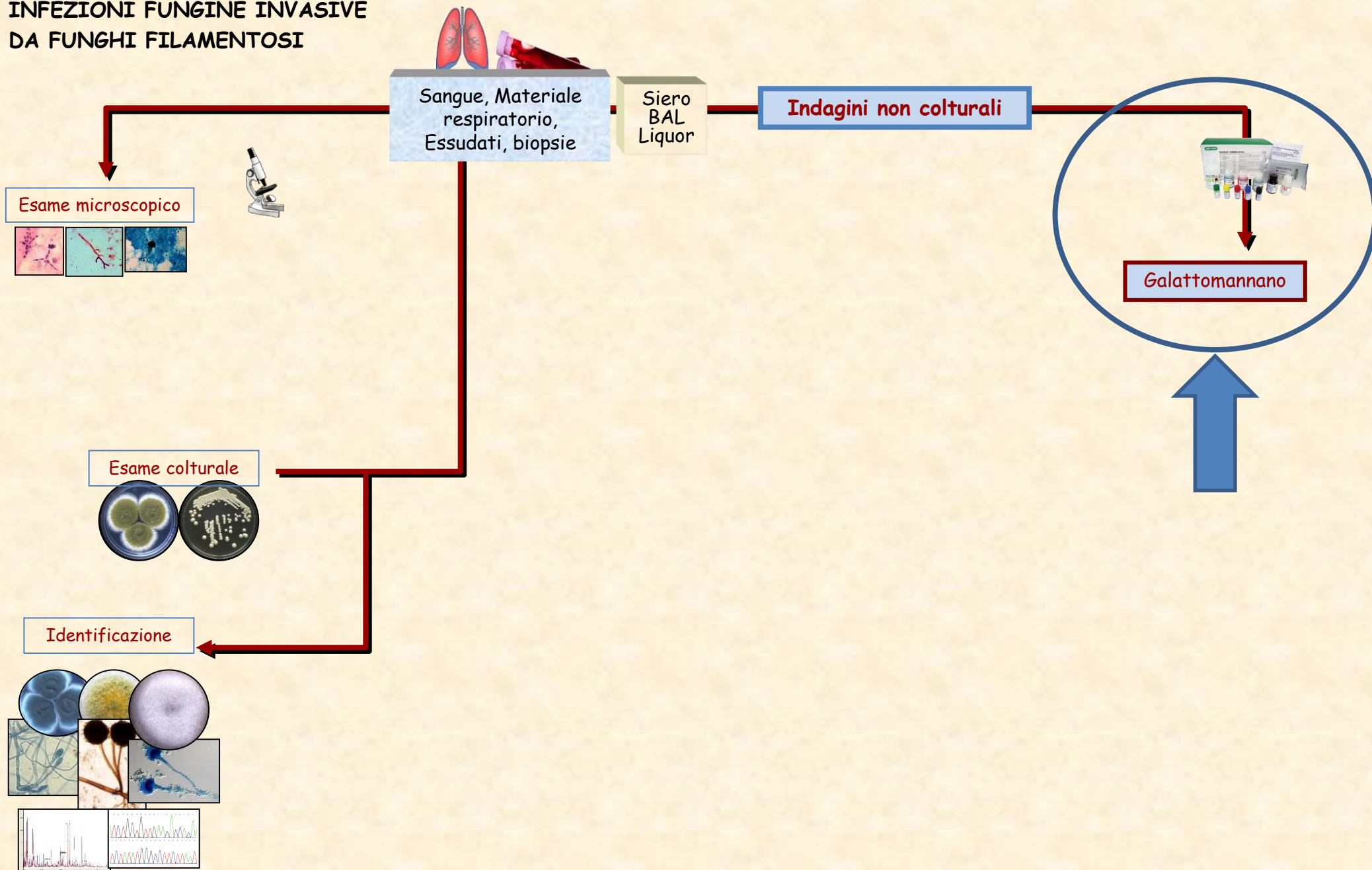
- a livello di **specie complex**, es. discriminazione di *A. fumigatus* complex da *A. flavus* complex
- a livello di **specie all'interno della sezione**; es. discriminazione di *A. fumigatus* da *A. lentulus* (entrambi membri della sezione *Fumigati*).

**Genere**  
**Specie**  
**Subspecie**

L'*International Subcommission on Fungal Barcoding* ha proposto il **segmento nucleare ribosomiale della regione ITS** come **principale barcode fungino** o come regione di *default* per l'identificazione a livello di specie complex

L'analisi di sequenze di **regioni codificanti proteine ( $\beta$ -tubulina, calmodulina)**, può consentire l'identificazione all'interno delle sezioni *Fumigati*, *Usti*, *Nigri*, e *Terrei*.

# INFEZIONI FUNGINE INVASIVE DA FUNGHI FILAMENTOSI



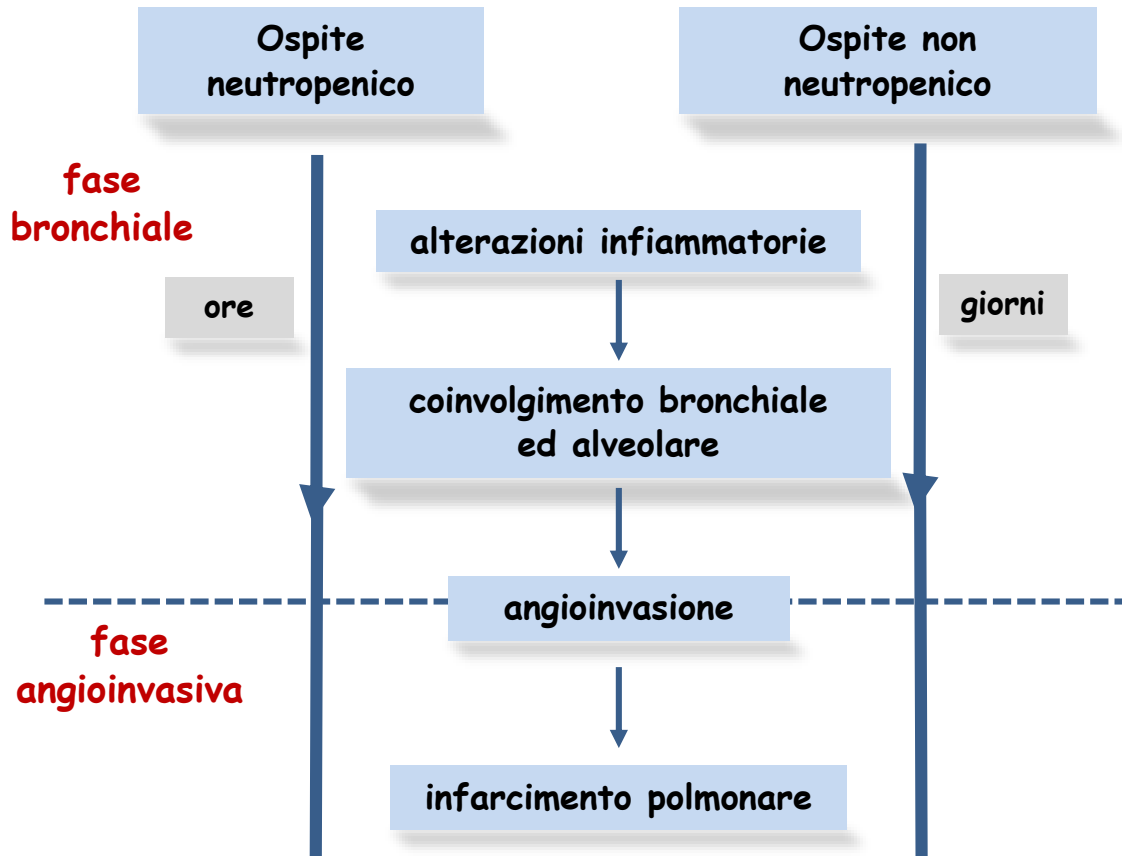
## INFEZIONI FUNGINE INVASIVE DA FUNGHI FILAMENTOSI



### ① Indicazioni: diagnosi di aspergillosi

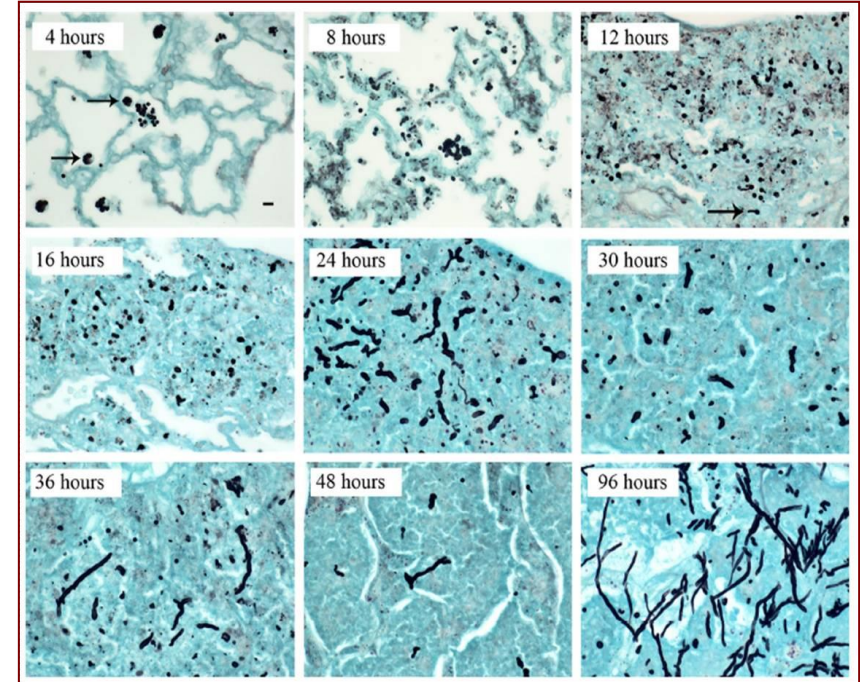
- **Pazienti neutropenici** (leucemia, trapianto allogenico di midollo): **test seriali** (ogni 3-4 gg).  
Cutoff: un campione singolo  $> 0.7$  o due campioni consecutivi  $> 0.5$ .
- I test seriali sono utili anche in **bambini neutropenici**.
- In **pazienti non neutropenici** la quantificazione seriale del GM non ha lo stesso valore diagnostico e pronostico
- La quantificazione nei campioni di **BAL (cutoff  $> 1$ )** o **nel liquor (cutoff  $> 0.5$ )** utile sia nei pazienti neutropenici che non neutropenici
- Valori nel siero  $> 1$  sono considerati segno di fallimento terapeutico sia negli adulti che nei bambini.
- Il valore prognostico di GM è riferibile a campioni di siero ma non di BAL

Evoluzione di aspergillosi polmonare invasiva nelle **fasi broncoalveolare ed angioinvasiva** in pazienti neutropenici e non neutropenici  
( da Nucci M., et al., 2013, Haematologica)



**I biomarker di Aspergillus nel BAL e nel siero forniscono informazioni complementari.**

- L'infezione in alcuni pazienti è diagnosticata mediante GM ritrovato nel siero e non nel BAL, e viceversa; talvolta GM si riscontra sia nel siero che nel BAL.



(Hope W.W., et al., 2010, AAC)

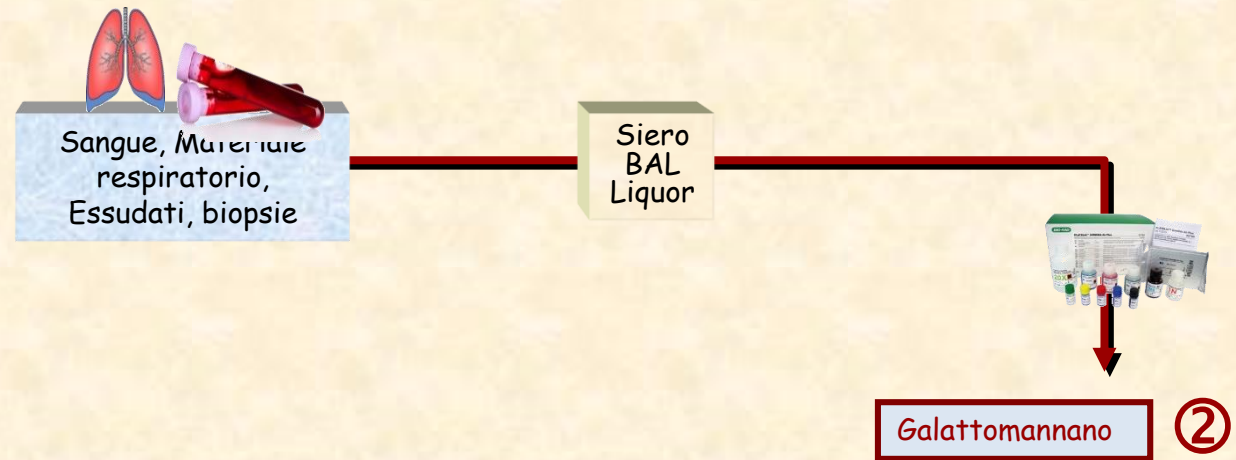
Istopatologia del polmone in modello animale (conigli neutropenici); prime 96 h di IPA

Cambiamenti parenchima polmonare: fagocitosi di conidi (macrofagi alveolari) (<4 ore dall'inoculazione), infiltrato infiammatorio, germinazione di conidi e formazione ife dopo 8 ore.

Alterazioni infiammatorie antecedenti l'invasione ifale dei vasi sanguigni (**fase "broncoalveolare"**)

L'infarcimento polmonare si verifica dopo 24 ore (**fase angioinvasiva**).

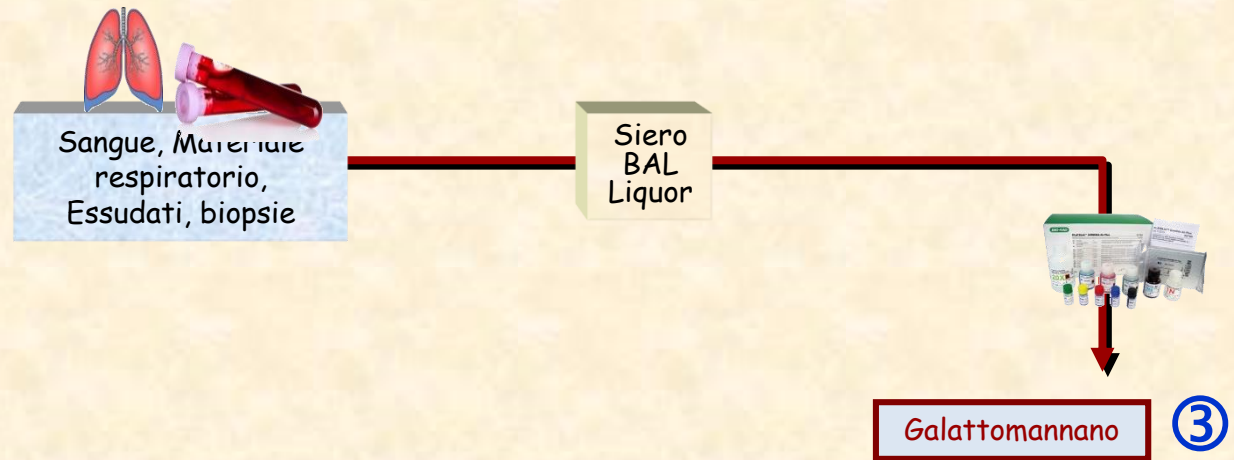
## INFEZIONI FUNGINE INVASIVE DA FUNGHI FILAMENTOSI



### ② Falsi positivi

- Reattività crociata con esoantigeni (batteri-funghi)
- Induzione da chemioterapia citotossica (ciclofosfamide)
- Bambini prematuri (83%) e neonati
- Mucosa intestinale danneggiata (assorbimento)
- Batteri colonizzanti l'intestino del neonato (*Bifidobacterium sp.*)
- Dieta con cereali e latte
- Tamponi di cotone
- Antibiotici con GM
- Soluzioni che contengono GM: emoderivati, immunoglobuline

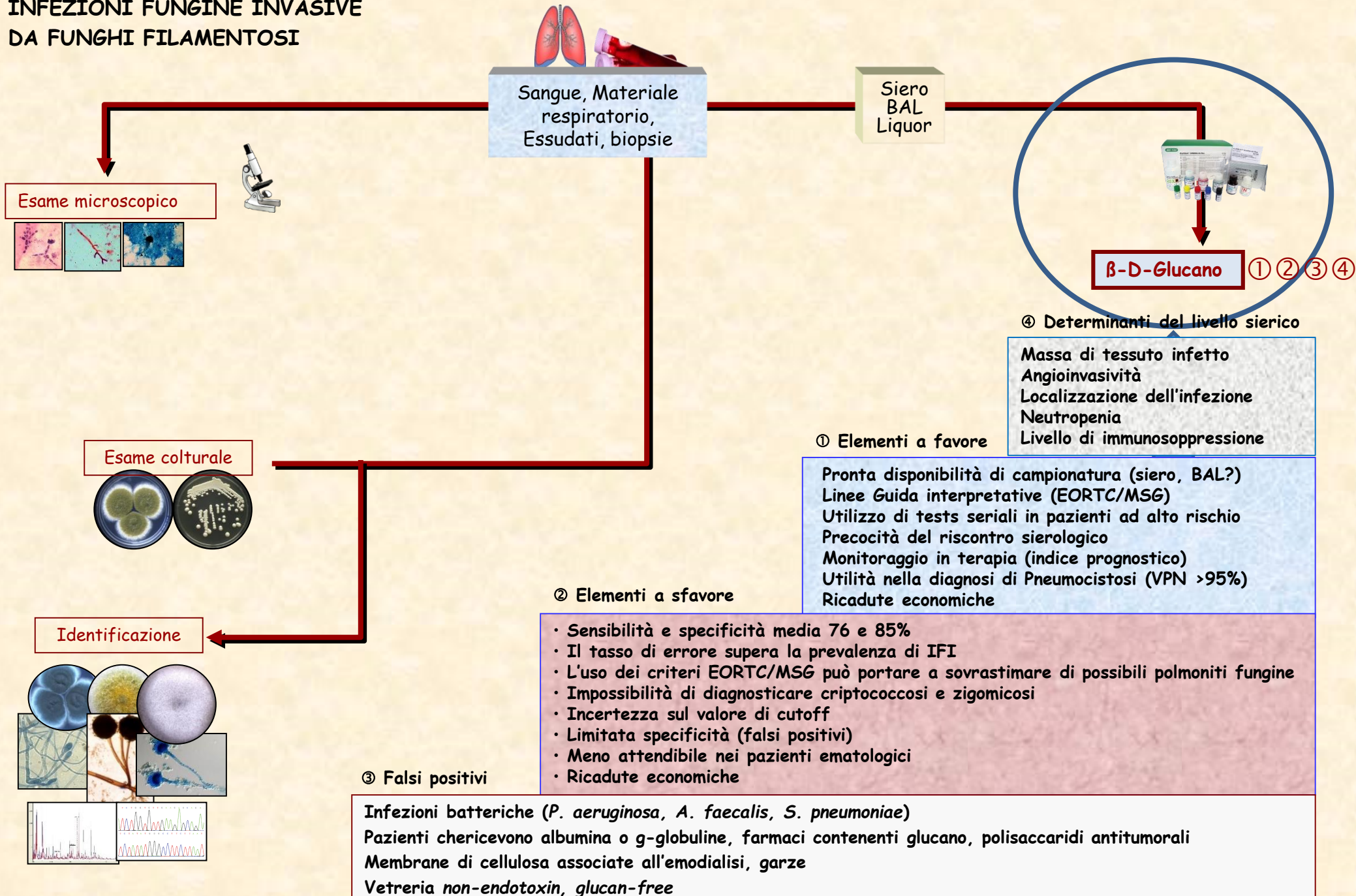
## INFEZIONI FUNGINE INVASIVE DA FUNGHI FILAMENTOSI



### ③ Falsi negativi

- Uso concomitante di antifungini
- Scarsa angioinvasione (HSCT)
- Presenza di anticorpi anti-aspergillus
- Carica fungina bassa
- Uso inappropriato (frequenza, conservazione)

# INFEZIONI FUNGINE INVASIVE DA FUNGHI FILAMENTOSI



**Diagnostic performance of single tests and different test combinations** in patients with proven/probable IFD from GM, BDG, culture, microscopy, or histopathology, and in patients with no IFD (BochD, CMI, 2016)

Test (combination) and condition	Sensitivity, % (n=37)	Specificity, % (n=29)	PPV, %	NPV, %
PCR (BAL)	32 (12/37)	100 (29/29)	100	54
PCR (blood)	5 (2/37)	97 (28/29)	67	44
GM (BAL)	43 (16/37)	97 (28/29)	94	57
GM (blood)	19 (7/37)	100 (29/29)	100	49
BDG (BAL)	73 (27/37)	48 (14/29)	64	58
BDG (blood)	65 (24/37)	97 (28/29)	96	68
Chip (BAL)	16 (6/37)	86 (25/29)	60	45
Chip (blood)	11 (4/37)	86 (25/29)	50	43
PCR (BAL) OR GM (BAL)	<b>La combinazione di biomarcatori è superiore al singolo uso nella diagnosi di IFD. Integrare campioni di sangue e BAL in un algoritmo diagnostico è un approccio vantaggioso.</b>			
PCR (blood) OR GM (blood)				
PCR (BAL) OR BDG (blood)				
GM (BAL) OR BDG (blood)	92 (34/37)	93 (27/29)	94	90
PCR (BAL) OR GM (BAL) OR BDG (BAL)	78 (29/37)	48 (14/29)	66	64
PCR (BAL) OR GM (BAL) OR BDG (blood)	92 (34/37)	93 (27/29)	94	90
PCR (BAL) OR GM (BAL) OR BDG (blood) OR Chip (BAL)	97 (36/37)	79 (23/29)	86	96
PCR (BAL) OR GM (BAL) OR BDG (blood) OR Chip (BAL, non-IA)	97 (36/37)	86 (25/29)	90	96
Positivity was defined by a positive result of either test and negativity by negative results for all tests ("OR"). IFD, invasive fungal disease; IA, invasive aspergillosis; non-IA, non- <i>Aspergillus</i> fungi; PCR, <i>Aspergillus</i> -specific polymerase chain reaction; Chip, multifungal DNA-microarray; GM, galactomannan; BDG, 1,3- $\beta$ -D-Glucan; BAL, bronchoalveolar lavage fluid; PPV, positive predictive value; NPV, negative predictive value; DOR, diagnostic odds ratio.				

## Performance characteristics of each test and combinations of tests with BALF and serum for Chronic Pulmonary Aspergillosis (CPA)<sup>a</sup>

Assay(s)	Sensitivity (% [no./total])	Specificity (% [no./total])	PPV	NPV	PLR	NLR	DOR (95% CI)
<b>With BALF</b>							
GM test (ODI $\geq$ 0.5)	77.8 (21/27)	90.0 (108/120)	63.6	94.7	7.78	0.25	31.5 (10.6–93.3)
$\beta$ DG test (ODI $\geq$ 100)	77.8 (21/27)	72.5 (87/120)	38.9	93.5	2.83	0.31	9.2 (3.4–24.9)
PCR 1	86.7 (26/30)	84.2 (101/120)	57.8	96.2	5.47	0.16	34.6 (10.8–110.4)
PCR 2	66.7 (20/30)	94.2 (113/120)	74.1	91.9	11.4	0.35	32.3 (11.0–94.7)
GM test and PCR 1	66.7 (18/27)	97.5 (117/120)	85.7	92.9	26.7	0.34	78.0 (19.3–315.6)
GM test and PCR 2	55.6 (15/27)	98.3 (118/120)	88.2	90.8	33.3	0.45	73.8 (15.0–361.8)
$\beta$ DG test and PCR 1	66.7 (18/27)	96.7 (116/120)	81.8	92.8	20.0	0.35	58.0 (16.2–208.2)
$\beta$ DG test and PCR 2	48.2 (13/27)	99.2 (119/120)	92.9	89.5	57.8	0.52	110.5 (13.4–909.6)
GM test and $\beta$ DG test	66.7 (18/27)	98.3 (118/120)	90.0	92.9	40.0	0.34	118.0 (23.6–590.6)
GM test or PCR 1	96.3 (26/27)	76.7 (92/120)	48.1	98.9	4.13	0.05	85.4 (11.1–658.1)
GM test or PCR 2	85.2 (23/27)	85.8 (103/120)	57.5	96.3	6.01	0.17	34.8 (10.7–113.3)
$\beta$ DG test or PCR 1	96.3 (26/27)	60.0 (72/120)	35.1	98.6	2.41	0.06	39.0 (5.1–297.1)
$\beta$ DG test or PCR 2	92.6 (25/27)	67.5 (81/120)	39.1	97.6	2.85	0.11	26.0 (5.9–115.2)
GM test or $\beta$ DG test	88.9 (24/27)	65.0 (78/120)	36.4	96.3	2.54	0.17	14.9 (4.2–52.2)

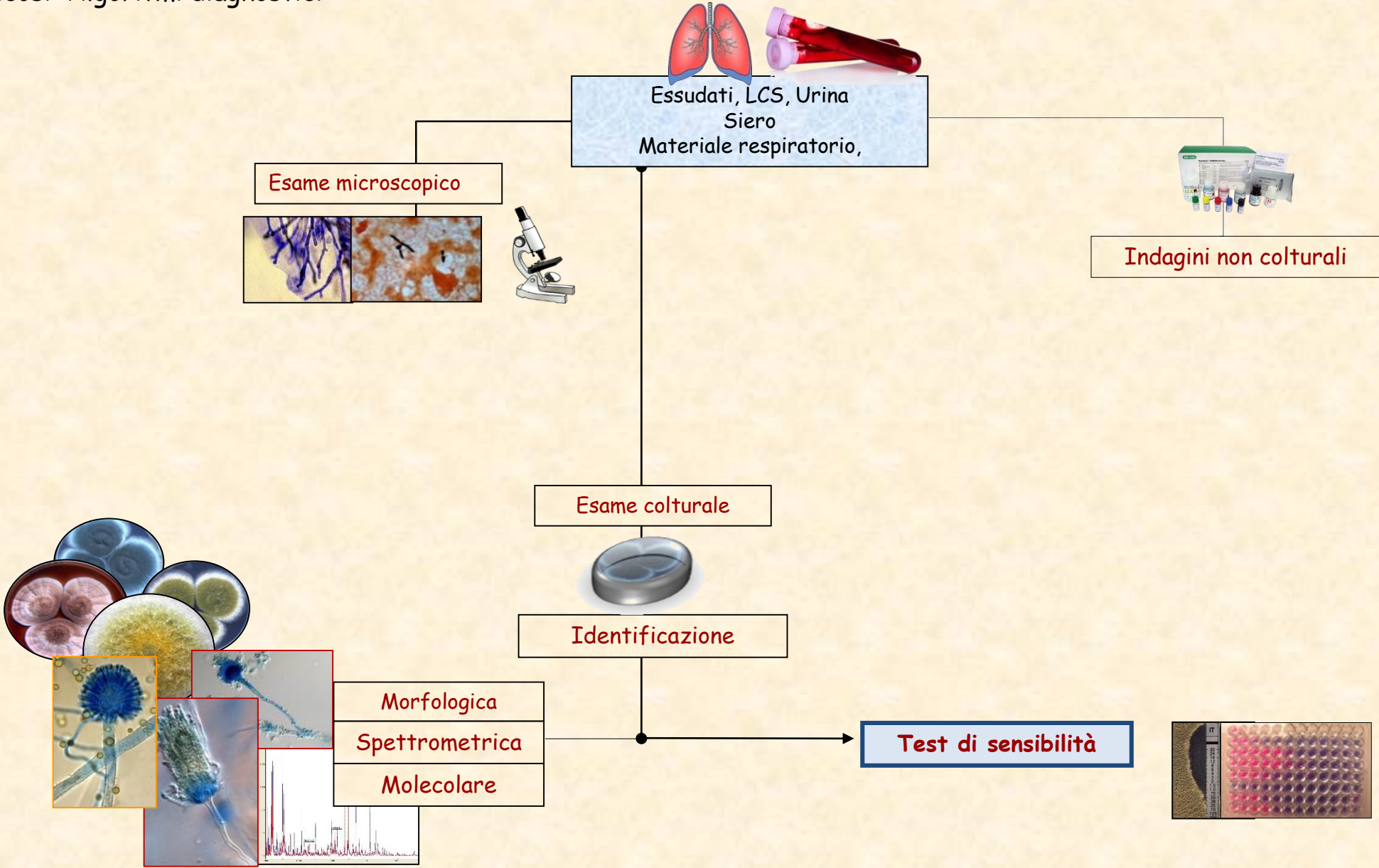
La combinazione dei test GM e  $\beta$ DG ha avuto il più alto rapporto di probabilità diagnostica: la combinazione dei test GM e  $\beta$ DG su BALF era più utile di qualsiasi singolo test per la diagnosi di CPA

<sup>a</sup> $\beta$ DG, beta-D-glucan; CI, confidence interval; DOR, diagnostic odds ratio; GM, galactomannan; NLR, negative likelihood ratio; NPV, negative predictive value; ODI, optical density index; PLR, positive likelihood ratio; PPV, positive predictive value.

<sup>b</sup>21/30 with the Fungitec G test (cutoff  $\geq$  20) and 9/30 with the Wako Turbidimetric assay (cutoff  $\geq$  11).

<sup>c</sup>All with Fungitec G test (cutoff  $\geq$  20).

Micosi: Algoritmi diagnostici



Characteristics and **intrinsic resistance profiles of *Aspergillus* species**  
(Van Der Linden JWM, Medical Mycology, 2011) (mod.)

Species	Characteristics	Amphotericin B	Azoles	Echinocandins
<i>A. lentulus</i>	Newly recognized to cause IA	R	R	V
<i>A. udagawae</i>	Uncommon cause of IA	R	R (vor)	
<i>N. pseudofisherii</i>	Uncommon cause of IA	V	R	
<i>A. fumigatiaffinis</i>	No cases of IA reported	R	R	
<i>A. viridinutans</i>	Newly recognized to cause IA in patients with primary immunodeficiencies	R	R	
<b><i>A. flavus</i></b>	Common in dry climates	R		
<b><i>A. nidulans</i></b>	Primarily causes IA in patients with CGD	R		
<i>A. tetrazonus</i>	Newly recognized to cause IA in CGD			R
<b><i>A. terreus</i></b>	Propensity to disseminate with positive blood cultures	R		
<i>A. alabamensis</i>	No cases of IA reported	R		
<b><i>A. niger</i></b>	Uncommon cause of IA; Common cause of otomycosis		V	
<i>A. tubingensis</i>	Newly recognized to cause keratitis and IA		V	
<i>A. calidoustus</i>	Uncommon cause of IA; Propensity to disseminate		R	V
<b><i>A. versicolor</i></b>	Uncommon cause of IA; Common cause of onychomycosis	R	V	
<i>A. sydowii</i>	Newly recognized to cause onychomycosis; Uncommon cause of IA.	R	V	
<i>A. persii</i>	Newly recognized to cause onychomycosis	R		R

R= Reduced susceptibility; V= Variable susceptibility; vor: voriconazole

2008

# Emergence of Azole Resistance in *Aspergillus fumigatus* and Spread of a Single Resistance Mechanism

Eveline Snelders<sup>1,2</sup>, Henrich A. L. van der Lee<sup>1,2</sup>, Judith Kuijpers<sup>1,2</sup>, Anthonius J. M. M. Rijs<sup>1,2</sup>, János Varga<sup>3,4</sup>, Robert A. Samson<sup>3</sup>, Emilia Mellado<sup>5</sup>, A. Rogier T. Donders<sup>6</sup>, Willem J. G. Melchers<sup>1,2</sup>, Paul E. Verweij<sup>1,2\*</sup>

## Background

Resistance to triazoles was recently reported in *Aspergillus fumigatus* isolates cultured from patients with invasive aspergillosis. The prevalence of azole resistance in *A. fumigatus* is unknown. We investigated the prevalence and spread of azole resistance using our culture collection that contained *A. fumigatus* isolates collected between 1994 and 2007.

## Conclusions

Azole resistance has emerged in *A. fumigatus* and might be more prevalent than currently acknowledged. The presence of a dominant resistance mechanism in clinical isolates suggests that isolates with this mechanism are spreading in our environment.

2015

# Emergence of Azole Resistance in *Aspergillus*

Nathan P. Wiederhold, PharmD<sup>1,2</sup> Thomas F. Patterson, MD<sup>2,3</sup>

Semin Respir Crit Care Med 2015;36:673–680.

## Abstract

Resistance to the azole antifungals itraconazole, voriconazole, and posaconazole in *Aspergillus* species is a growing concern. This is especially alarming for *A. fumigatus*, where acquired resistance has been documented in patients with invasive disease caused by this species that were exposed to these agents, as well as in azole-naïve individuals. The primary mechanisms of resistance that have been described in clinical strains include different point mutations in the *CYP51A* gene, which encodes the enzyme responsible for converting lanosterol to ergosterol via demethylation. Some

# Nuova fase nella gestione dell'aspergillosi

Emergenza di ceppi di *Aspergillus* spp. meno sensibili agli antifungini, principalmente *A. fumigatus* resistenti agli azoli.

## Motivi:

- Terapia a lungo termine con azoli in pazienti con aspergillosi cronica
- Uso notevole degli azolici in agricoltura

## Meccanismi di resistenza:

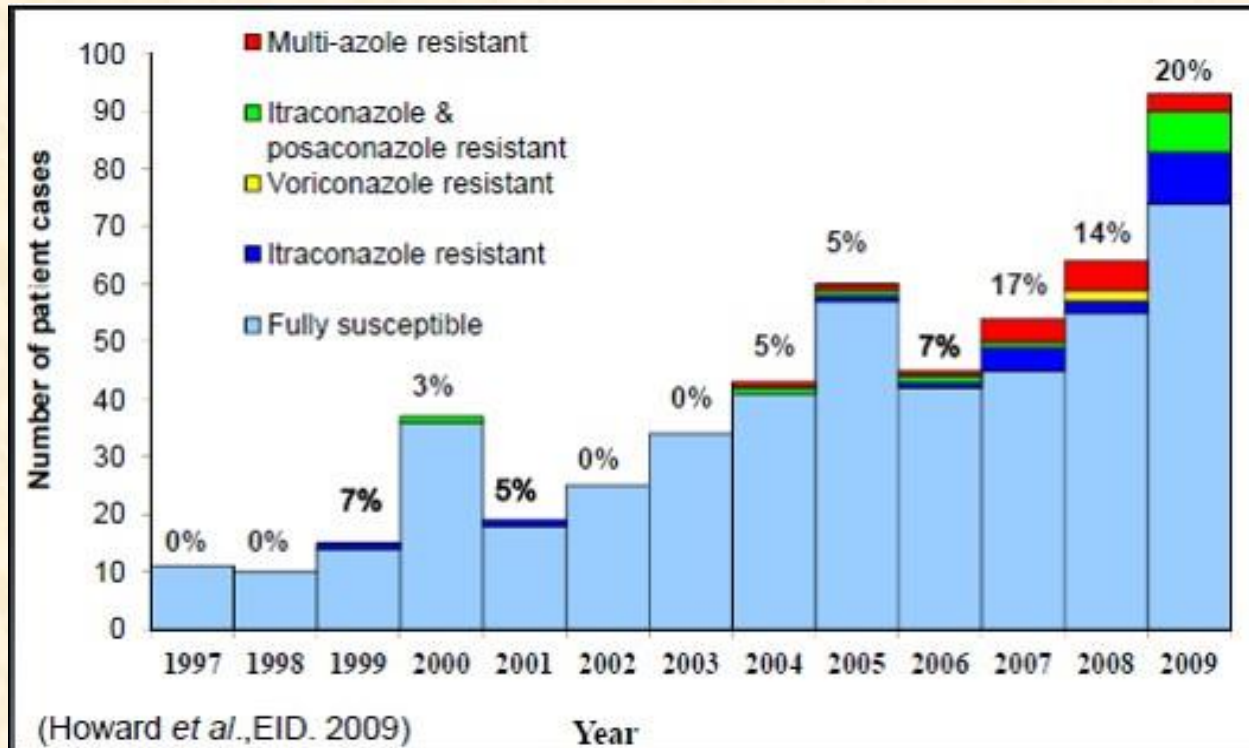
- ripetizione tandem 34-bp TR<sub>34</sub> nella regione promotrice del gene target azolico *CYP51A* e una mutazione puntiforme nel gene target stesso che comporta una sostituzione amminoacidica L98H (TR<sub>34</sub>/L98H).

Nella pratica clinica, il riconoscimento tempestivo di infezioni da miceti resistenti agli antifungini potrà consentire di ottimizzare la terapia e l'outcome del paziente.

# Emergenza di resistenza agli azoli in *Aspergillus* nell'Unione Europea

~ 6% di resistenza agli azoli nei centri di riferimento (DK e NL)

0.4-4.8 % resistenza agli azoli in 13 centri con più di 60 ceppi testati nello studio SCARE



## Meccanismi

### TR-L98H

- >90% di R in NL di origine ambientale

### Altre mutazioni *Cyp51*

- la maggior parte dopo terapia prolungata con azoli

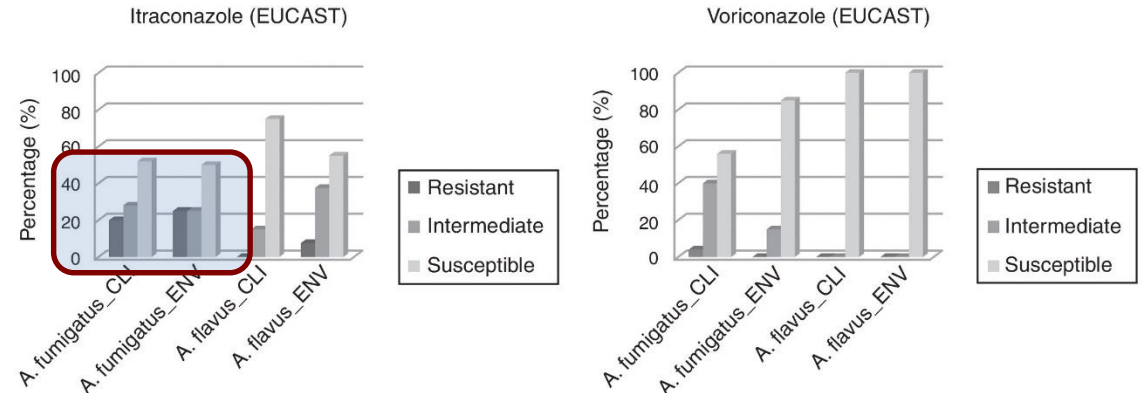
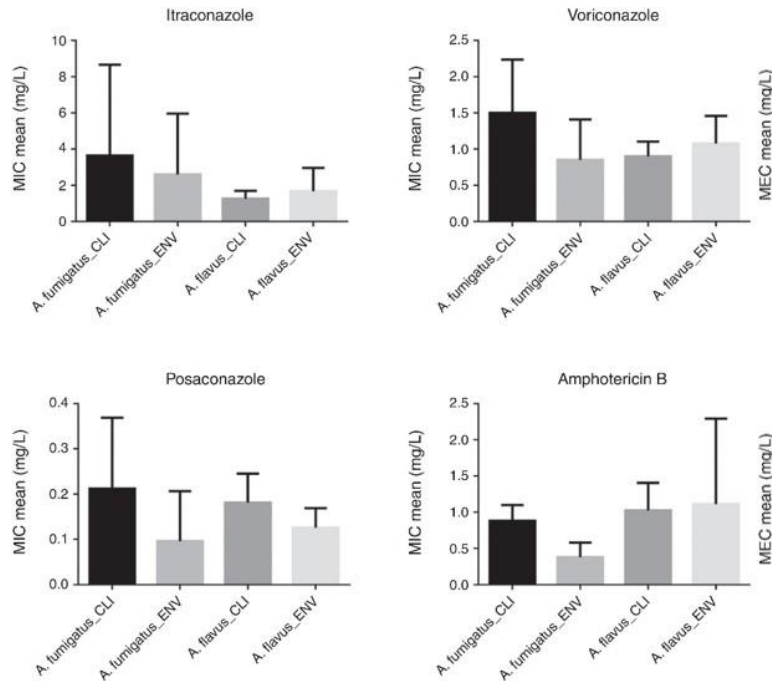
### Meccanismi non *Cyp51*

- 43% in Manchester

Bueid JAC 2010; Howard EID 2009; Verweij, von der Linden, Arendrup (SCAPE)

# In vitro antifungal susceptibility of clinical and environmental isolates of *Aspergillus fumigatus* and *Aspergillus flavus* in Brazil (Denardi B.L., Braz J Infect Dis, 2018)

Minimal inhibitory concentration (MIC) mean of itraconazole, voriconazole, posaconazole, amphotericin B, for *A. fumigatus* and *A. flavus* clinical (CLI) and environmental (ENV) isolates.



Susceptibility profile of *Aspergillus fumigatus* and *Aspergillus flavus* clinical (CLIN) and environmental (ENV) isolates for azoles, following the EUCAST breakpoints, and amphotericin B by EUCAST, CLSI breakpoints.

Con i breakpoint proposti da EUCAST, rispettivamente il 20% e il 25% degli isolati clinici e ambientali di *A. fumigatus*, sono risultati resistenti a itraconazolo (concentrazione minima inibitoria, MIC > 2,0 mg/L).



# Metodi di riferimento per lo studio delle sensibilità delle muffe agli antifungini



Microdiluizione in brodo CLSI M38-A2, 2008

Disco-diffusione CLSI M51-A, 2010

Cutoff Epidemiologici CLSI M59, 2016

Performance standards CLSI M61, 2017



Microdiluizione in brodo: 2011 (ver.3.0)



Microdiluizione in brodo: 2018 (ver.9.0)



*breakpoint clinici*



*cut-off epidemiologici (ECV)*

# European Committee on Antimicrobial Susceptibility Testing

## Antifungal Agents European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs Version 9.0, valid from 2018-02-12

- MIC method (EUCAST standardised broth microdilution method)
- Medium: RPMI1640-2% glucose, MOPS as buffer
- Inoculum: Final  $1 \times 10^5$  –  $2.5 \times 10^5$  cfu/mL
- Incubation: 48h
- Reading: Visual, complete inhibition for amphotericin B and azoles (MIC), aberrant growth endpoint for echinocandins (MEC).
- Quality control: *A. fumigatus* ATCC 204305, *A. flavus* ATCC 204304 (read after 18-24 h)

Antifungal agent	MIC breakpoint (mg/L)											
	<i>A. flavus</i>		<i>A. fumigatus</i>		<i>A. nidulans</i>		<i>A. niger</i>		<i>A. terreus</i>		Non-species related breakpoints <sup>1</sup>	
	S ≤	R >	S ≤	R >	S ≤	R >	S ≤	R >	S ≤	R >	S ≤	R >
Amphotericin B	IE <sup>2</sup>	IE <sup>2</sup>	1	2	Note <sup>3</sup>	Note <sup>3</sup>	1	2	-	-	IE	IE
Anidulafungin	IE	IE	IE	IE	IE	IE	IE	IE	IE	IE	IE	IE
Caspofungin	IE	IE	IE	IE	IE	IE	IE	IE	IE	IE	IE	IE
Fluconazole	-	-	-	-	-	-	-	-	-	-	-	-
Isavuconazole	IE <sup>2</sup>	IE <sup>2</sup>	1	1	0.25	0.25	IE <sup>2</sup>	IE <sup>2</sup>	1	1	IE	IE
Itraconazole <sup>4</sup>	1	2	1	2	1	2	IE <sup>2,5</sup>	IE <sup>2,5</sup>	1	2	IE <sup>5</sup>	IE <sup>5</sup>
Micafungin	IE	IE	IE	IE	IE	IE	IE	IE	IE	IE	IE	IE
Posaconazole <sup>4</sup>	IE <sup>2</sup>	IE <sup>2</sup>	0.125 <sup>6</sup>	0.25 <sup>6</sup>	IE <sup>2</sup>	IE <sup>2</sup>	IE <sup>2</sup>	IE <sup>2</sup>	0.125 <sup>6</sup>	0.25 <sup>6</sup>	IE	IE
Voriconazole <sup>4</sup>	IE <sup>2</sup>	IE <sup>2</sup>	1	2	IE	IE	IE <sup>2</sup>	IE <sup>2</sup>	IE <sup>2</sup>	IE <sup>2</sup>	IE	IE

"IE" indicates that there is insufficient evidence that the species in question is a good target for therapy with the drug. An MIC with a comment but without an accompanying S, I or R categorisation may be reported.

2. The ECOFFs for these species are in general one step higher than for *A. fumigatus*.

3. There are too few MIC data to establish ECOFFs and hence to suggest any breakpoints.

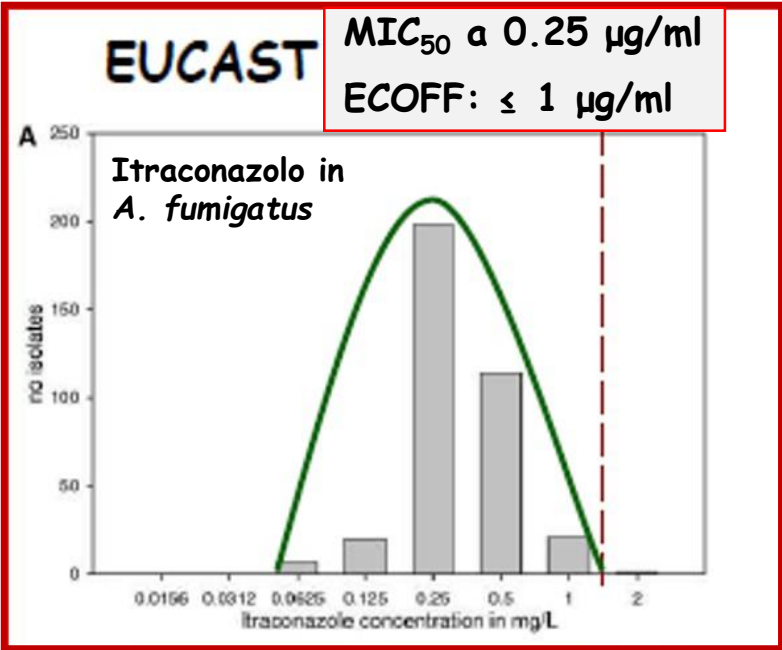
4. Monitoring of azole trough concentrations in patients treated for fungal infection is recommended.

6. Provided adequate drug exposure has been confirmed using therapeutic drug monitoring (TDM).

# MIC distributions and epidemiological cut-off (ECOFF) values (mg/L)

	0.002	0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512	ECOFF
<i>Aspergillus flavus</i>	0	0	2	2	0	11	59	162	116	25	11	0	0	4	0	0	0	0	0	1
<i>Aspergillus fumigatus</i>	0	0	1	2	8	16	155	895	832	202	54	30	14	58	121	1	0	0	0	1
<i>Aspergillus nidulans</i>	0	0	0	0	2	7	33	39	19	4	5	1	2	4	0	0	0	0	0	1
<i>Aspergillus niger</i>	0	0	0	0	0	1	6	18	104	117	21	7	5	25	3	0	0	0	0	4
<i>Aspergillus terreus</i>	0	0	0	1	5	24	200	341	54	5	3	0	2	1	0	0	0	0	0	0.5
<i>Aspergillus versicolor</i>	0	0	0	0	0	1	2	4	11	10	1	0	1	1	0	0	0	0	0	ND
<i>Aspergillus sydowii</i>	0	0	0	0	0	1	8	8	18	10	1	2	1	7	0	0	0	0	0	ND

The table includes MIC distributions available at the time breakpoints were set. They represent combined distributions from multiple sources and time periods. The distributions are used to define the epidemiological cut-offs (ECOFF) and give an indication of the MICs for organisms with acquired or mutational resistance mechanisms. They should not be used to infer resistance rates. When there is insufficient evidence no epidemiological cut-off has been determined (ND).



Cut-off epidemiologici (ECOFF) in *Aspergillus* (EUCAST)

	AmB	Itra	Vor	Posa	Isa
<i>A. flavus</i>	4	1	2	0,5	2
<i>A. fumigatus</i>	1	1	1	0,25	2
<i>A. nidulans</i>	ND	1	1	0,25	0,25
<i>A. niger</i>	1	4	2	0,5	4
<i>A. terreus</i>	4	0,5	2	0,25	1
<i>A. versicolor</i>	ND	ND	ND	ND	-
<i>A. sydowii</i>	ND	ND	ND	ND	-

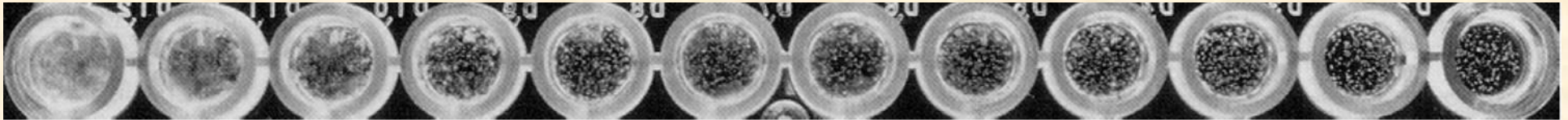
ND: insufficienti evidenze per determinare cut off epidemiologici

# Echinocandine: attività in vitro vs *Aspergillus*

(Arikan S et al., AAC, 46:3084, 2002)

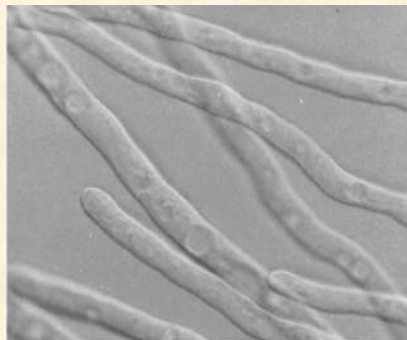
Controllo di crescita

Concentrazione crescente di Echinocandine



Sana

Punto di transizione:  
**MEC**  
(Minimum Effective  
Concentration)



Aberrante



# Sensititre YeastOne (Y10)

Metodo (brododiluizione) colorimetrico in micropiastra che impiega come indicatore l'Alamar blue.

A1: non agente antimicotico: controllo di crescita

A12-H11: Amfotericina B (0.12-18 µg/ml)

A2-A11: Anidulafungina (0.015-8 µg/ml)

B1-11: Micafungina (0.008-8 mg/ml)

C1-11: Caspofungina (0.008-8 µg/ml)

D1-11: 5-Fluorocitosina (0.06-64 µg/ml)

E1-11: Posaconazolo (0.008-8 µg/ml)

G1-11: Voriconazolo (0.008-8 µg/ml)

D1-11: Itraconazolo (0.015-16 µg/ml)

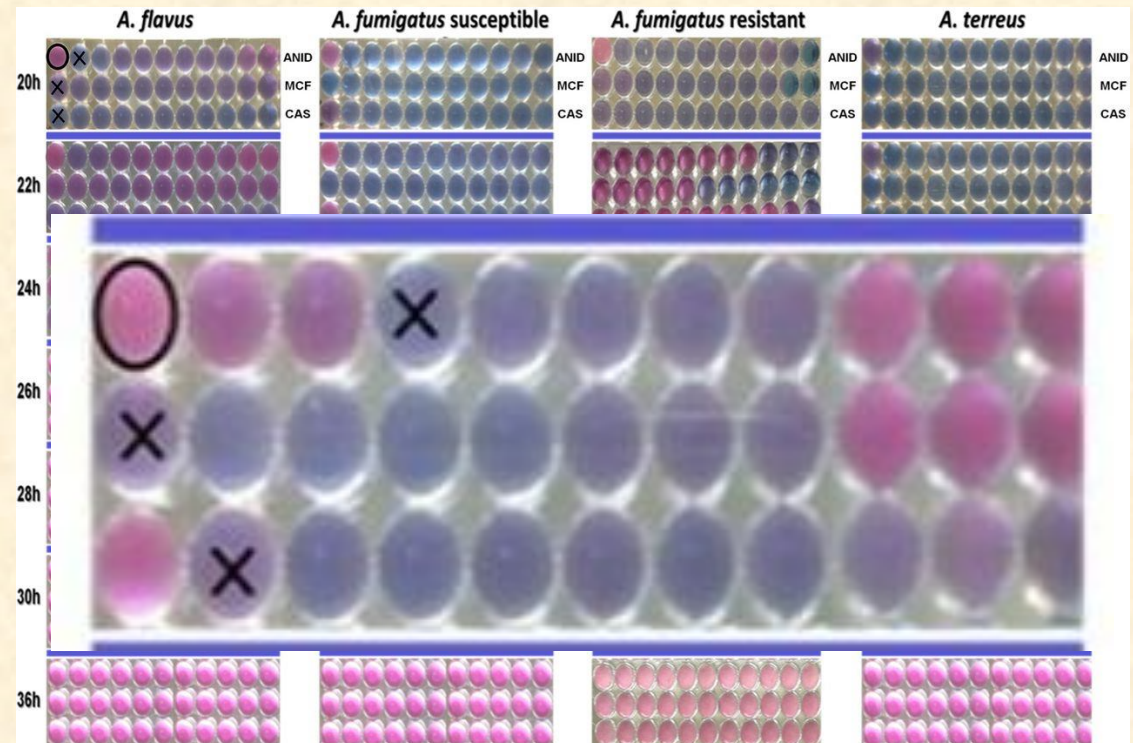
H1-12: Fluconazolo (0.125-256 µg/ml)

BLU: mancata crescita

PORPORA: inibizione parziale

ROSSO: crescita

Comparative Evaluation of Sensititre YeastOne and CLSI M38-A2 Reference Method for Antifungal Susceptibility Testing of *Aspergillus* spp. against Echinocandins (Siopi M, JCM, 2017)



Gli endpoint colorimetrici YO erano da 2 a 3 diluizioni inferiori alle concentrazioni effettive minime (MEC) CLSI di caspofungina e da 1 a 2 diluizioni rispetto ai MEC CLSI di micafungina.

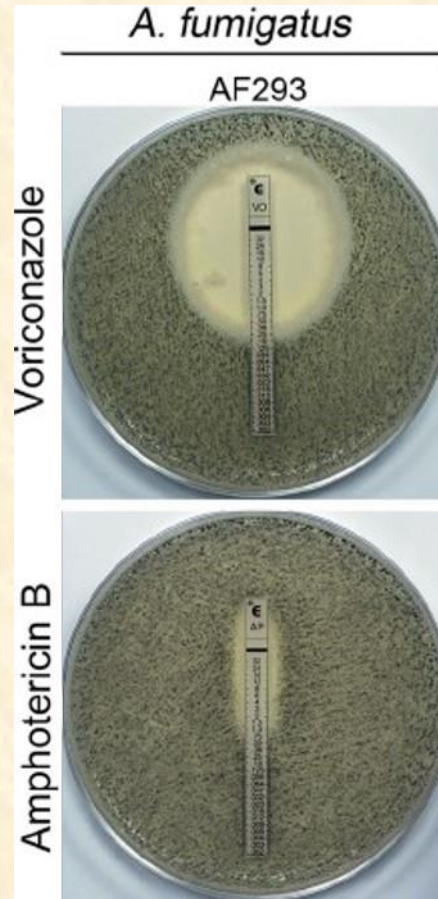
## Metodo in agar (gradiente diffusione)

Terreno più indicato RPMI 1640

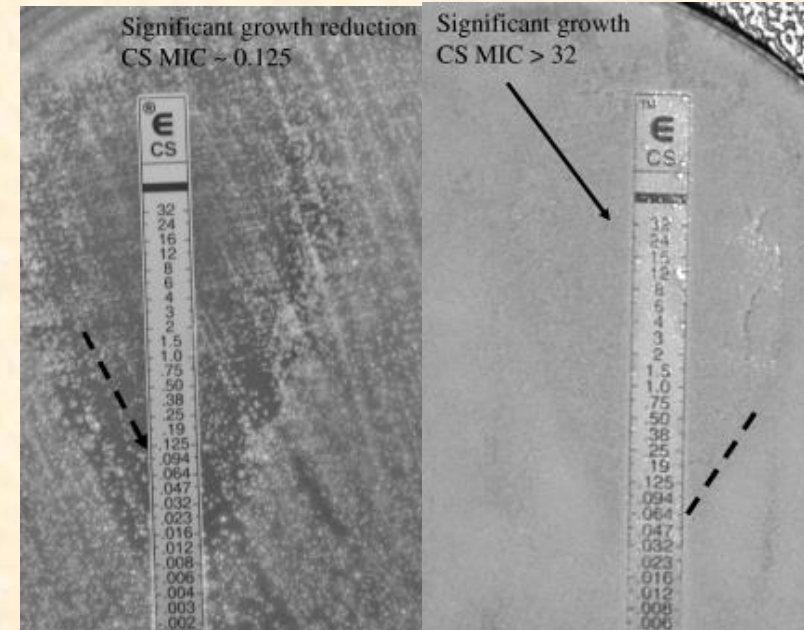


Inoculo  $\sim 10^6$  UFC/ml

McF	$10^6$ UFC/ml	%T (530 nm)
0.5	0.4-4.6	78-82%



(Sugui, JCM, 2012)



(Arendrup, AAC, 2008)

# Comparing Etest and Broth Microdilution for Antifungal Susceptibility Testing of the Most-Relevant Pathogenic Molds (Lamoth F., JCM, 2015)

Essential agreement between CLSI broth microdilution and Etest results

Species	Essential agreement (%) <sup>a</sup>				
	AMB	VCZ	POS	CSP	MCF
<i>A. fumigatus</i> (n = 74)	99	95	89 <sup>b</sup>	96	100
<i>A. flavus</i> (n = 29)	97	100	83 <sup>b</sup>	100	100
<i>A. terreus</i> (n = 25)	16 <sup>b</sup>	100	64 <sup>b</sup>	96	100
<i>A. niger</i> (n = 12)	100	100	83 <sup>b</sup>	100	100
<i>A. ustus</i> (n = 8)	100	100	100	14 <sup>b</sup>	57 <sup>b</sup>
<i>A. versicolor</i> (n = 6)	100	100	100	80 <sup>b</sup>	100
<i>Mucoromycotina</i> (n = 35)	91	100	94	100	100
<i>Fusarium</i> spp. (n = 34)	94	100	100	100	100
<i>S. apiospermum</i> (n = 20)	80 <sup>b</sup>	90	90	63 <sup>b</sup>	37 <sup>b</sup>
<i>S. prolificans</i> (n = 5)	100	100	100	100	100
<i>P. lilacinum</i> (n = 30)	100	100	97	100	100
<i>P. variotii</i> (n = 4)	100	100	100	50 <sup>b</sup>	100
<i>Scopulariopsis</i> spp. (n = 8)	75 <sup>b</sup>	100	100	43 <sup>b</sup>	29 <sup>b</sup>

<sup>a</sup> Essential agreement was within  $\pm 2$  dilutions. AMB, amphotericin B; VCZ, voriconazole; POS, posaconazole; CSP, caspofungin; MCF, micafungin.

<sup>b</sup> Essential agreement of <90% for MIC/MEC values obtained with the Etest and BMD methods.

## Comparing Etest and Broth Microdilution for Antifungal Susceptibility Testing of the Most-Relevant Pathogenic Molds (Lamoth F., JCM, 2015)

Proposed recommendations for use of the Etest method for antifungal susceptibility testing of molds

Drug	Recommendation	
	<i>Aspergillus</i> spp.	Non- <i>Aspergillus</i> spp. <sup>a</sup>
Amphotericin B	Yes; exception: <i>A. terreus</i>	Yes; exceptions: <i>S. apiospermum</i> and <i>Scopulariopsis</i> spp.
Voriconazole	Yes; caveat: ability to detect azole-resistant isolates not yet proven	Yes
Posaconazole	No (or use with extreme caution); lower MICs with Etest vs BMD	Yes
Caspofungin/ micafungin	Yes; exception: <i>A. ustus</i> (poor agreement with BMD for this species, which demonstrated MICs across the testing range)	Yes; exceptions: <i>S. apiospermum</i> , <i>P. variotii</i> , and <i>Scopulariopsis</i> spp. (poor agreement with BMD for these species, which demonstrated MICs across the testing range)

<sup>a</sup>*Mucoromycotina*, *Fusarium* spp., *S. apiospermum*, *S. prolificans*, *P. lilacinum*, *P. variotii*, and *Scopulariopsis* spp.

# Identificazione molecolare



**Genere**

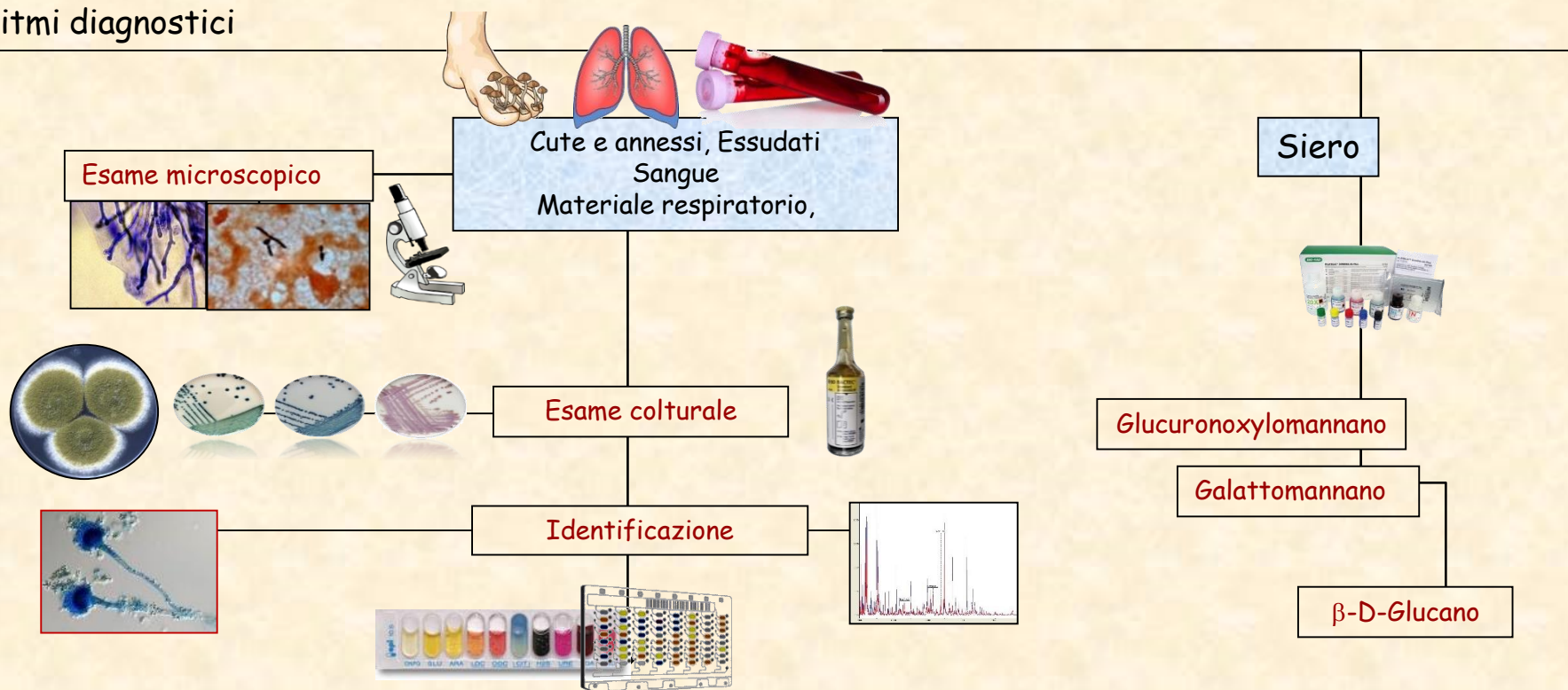
**Specie**

**Subspecie**

**da coltura**

**da campione**

# Micosi: Algoritmi diagnostici



PCR

① ②

① Nessun saggio molecolare è incluso tra i criteri stabiliti dal Gruppo di studio di cooperazione dell'Organizzazione Europea per la Ricerca e Cura del Cancro/Istituto Nazionale di allergie e malattie infettive (EORTC/MSG) (European Organisation for Research and Treatment of Cancer/Mycoses Study Group per definire malattie fungine invasive.

② variabili

- Tipo di campione biologico: sangue, siero o BAL
- Volume del campione
- Sistemi di estrazione del DNA
- Sistema di amplificazione (PCR Real-time, nested)
- Gene target (18S, 28S ITS ITS1, ITS2)
- Sistemi di identificazione di sequenze amplificate
- Controlli di processo
- Tipo e fase di malattia
- Grandezza della popolazione studiata
- Precocità del rilevamento
- Tipo di ac.nucleico rilevabile (vitale, non vitale)



PCR

Aspergillus Polymerase Chain Reaction: Systematic Review of Evidence for Clinical Use in Comparison With Antigen Testing (White P.L., et al., CID, 2015)

Performance of Fungal Biomarker Assays for the Detection of Invasive Aspergillosis as Determined by the Relevant Published Meta-analyses

Assay													
	GM-EIA				β-D-Glucan				PCR				
	Leeflang [7]	Pfeiffer [8]	Zou [5]	Guo [6]	Lamoth [9]	Karageorgopoulos [10]	He [11]	Onishi [12]	Tuon [15]	Sun [16]	Avni [18]	Mengoli [17]	Arvanitis [19]
Parameter	Serum		BAL		Plasma/Serum				BAL			Blood	
Sensitivity, %	79.3	79.3	83.6	85.7	56.8	77.1	77.0	77.3	78.4	79.6	76.8	88.0	84.0
Specificity, %	80.5	86.3	89.4	89.0	97.0	85.3	81.3	83.4	93.7	94.1	94.5	75.0	76.0
PPV, %	30.3	46.9	64.4	66.7	55.6	30.2	28.7	33.4	74.1	79.2	79.9	36.7	41.3
NPV, %	97.3	96.5	95.9	96.0	97.1	97.8	97.3	97.2	94.9	94.2	93.4	97.4	95.9
Positive LR	4.06	5.81	7.89	7.81	18.90	5.24	4.12	4.65	12.43	13.38	13.87	3.52	3.50
Negative LR	0.26	0.24	0.18	0.16	0.44	0.27	0.28	0.27	0.23	0.22	0.25	0.16	0.21
DOR	15.8	24.2	43.0	48.6	43.0	19.4	14.7	17.2	53.8	61.7	56.52	22.1	16.6

Gli autori sostengono che vi siano prove sufficienti, almeno equivalenti a quelle utilizzate per includere i tests GM-EIA e β-D-glucano e che la PCR sia ormai abbastanza matura per l'inclusione nelle definizioni EORTC/MSG.

# Molecular Tools for the Detection and Deduction of Azole Antifungal Drug Resistance Phenotypes in *Aspergillus* Species (Dudakova A., CMR, 2017)

PCR-based assays to characterize molecular resistance azole mechanisms in *Aspergillus* spp.

PCR use and format	Target
<b>Analyses of <i>A. fumigatus</i> cyp51A</b> Real-time PCR Mixed-format real-time PCR PCR-RFLP Nested PCR, two amplicons Conventional one-step and nested PCR <sup>a</sup>  PCR with TaqMan probes  Primer extension LightCycler endpoint genotyping AsperGenius (PathoNostics BV, Maastricht, The Netherlands) multiplex real-time PCR and melting curve analysis <sup>a</sup> Real-time RT-PCR	Single regions (~100 bp) TR- and amino acid substitution-specific regions Multiple single regions (180–270 bp) F1, TR region to L98; F2, G54 to N266 Single regions (100–173 bp) for TR <sup>34</sup> /L98H and M220 Single regions (103–143 bp) for TR <sup>46</sup> and the L98H substitution  TR, G54, L98, G138, M220 L98H and Y121F substitutions TR <sup>34</sup> and 15 polymorphic sites L98H, Y121F substitutions TR <sup>34</sup> , L98H, Y121F, T289A substitutions  <i>cyp51A</i> gene expression
<b>Analyses of <i>A. fumigatus</i> genes other than cyp51A</b> PCR plus sequencing Real-time RT-PCR  PCR with TaqMan probes PCR plus sequencing PCR plus sequencing, real-time RT-PCR	Full <i>cyp51B</i> gene in four amplicons <i>cyp51B</i> gene expression <i>mdr1</i> and <i>mdr4</i> gene expression <i>cyp51B</i> S35, P294 Full <i>hapE</i> gene <i>cyp51A</i> , <i>mdr1</i> -4, <i>atrF</i> , <i>cdr1B(abcG1)</i> , <i>mfs56</i>
<b>Analyses of <i>A. flavus</i> cyp51 genes</b> PCR plus sequencing PCR plus sequencing, TaqMan probes PCR plus sequencing	<i>cyp51A</i> and -B genes plus 5' and 3' regulatory sequences <i>cyp51A</i> , -B, and -C genes plus 5' and 3' regulatory sequences <i>cyp51A</i> , -B, and -C genes plus 5' and 3' regulatory sequences

<sup>a</sup>Non-culture-based, direct investigation of clinical (respiratory) samples was possible after confirmation of *Aspergillus* DNA in the sample by use of diagnostic *Aspergillus* PCR assays

# PCR-based detection of *Aspergillus fumigatus* Cyp51A mutations on bronchoalveolar lavage: a multicentre validation of the AsperGenius assay (R) in 201 patients with haematological disease suspected for invasive aspergillosis (Chong GM, J Antimicrob Chemother, 2016)

Studio retrospettivo in due parti.

- Prestazioni diagnostiche PCR species.
- Prestazioni PCR discriminante *A. fumigatus* WT da quelli con mutazioni in Cyp51A.

Campioni di BAL con GM positivo ( $\geq 1,0$ ) e/o coltura positiva considerati controlli positivi

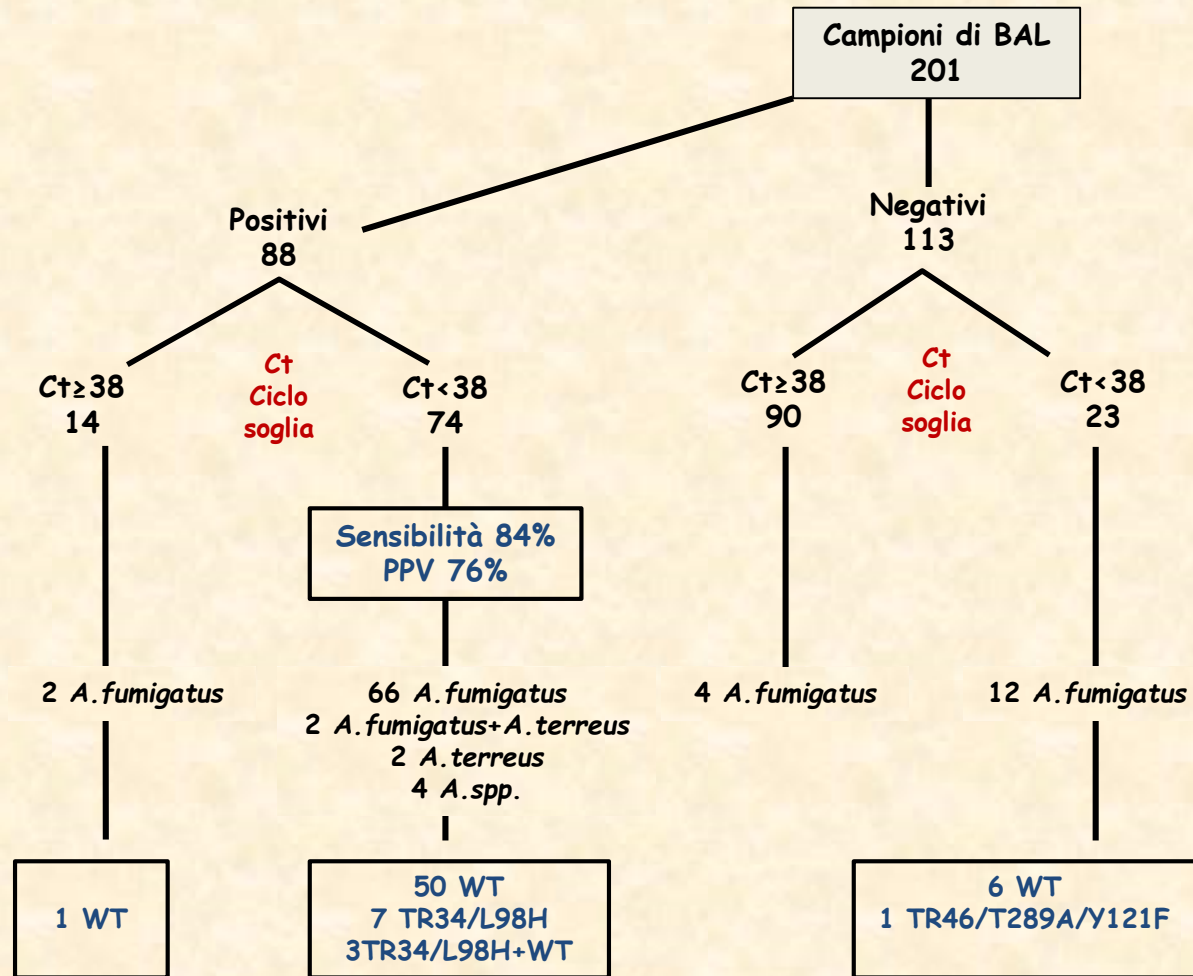
88 campioni risultati positivi: 74 (84,1%) erano positivi per PCR species

32 di 74 (43,2%) campioni di BAL erano negativi alla coltura e solo GM positivi

23 campioni da controlli negativi erano PCR specie positivi: 5 provenivano da pazienti con IA accertata, probabile o non class. (3 diagnosticati su GM sierico positivo, 1 su coltura sinusale e 1 su biopsia polmonare)

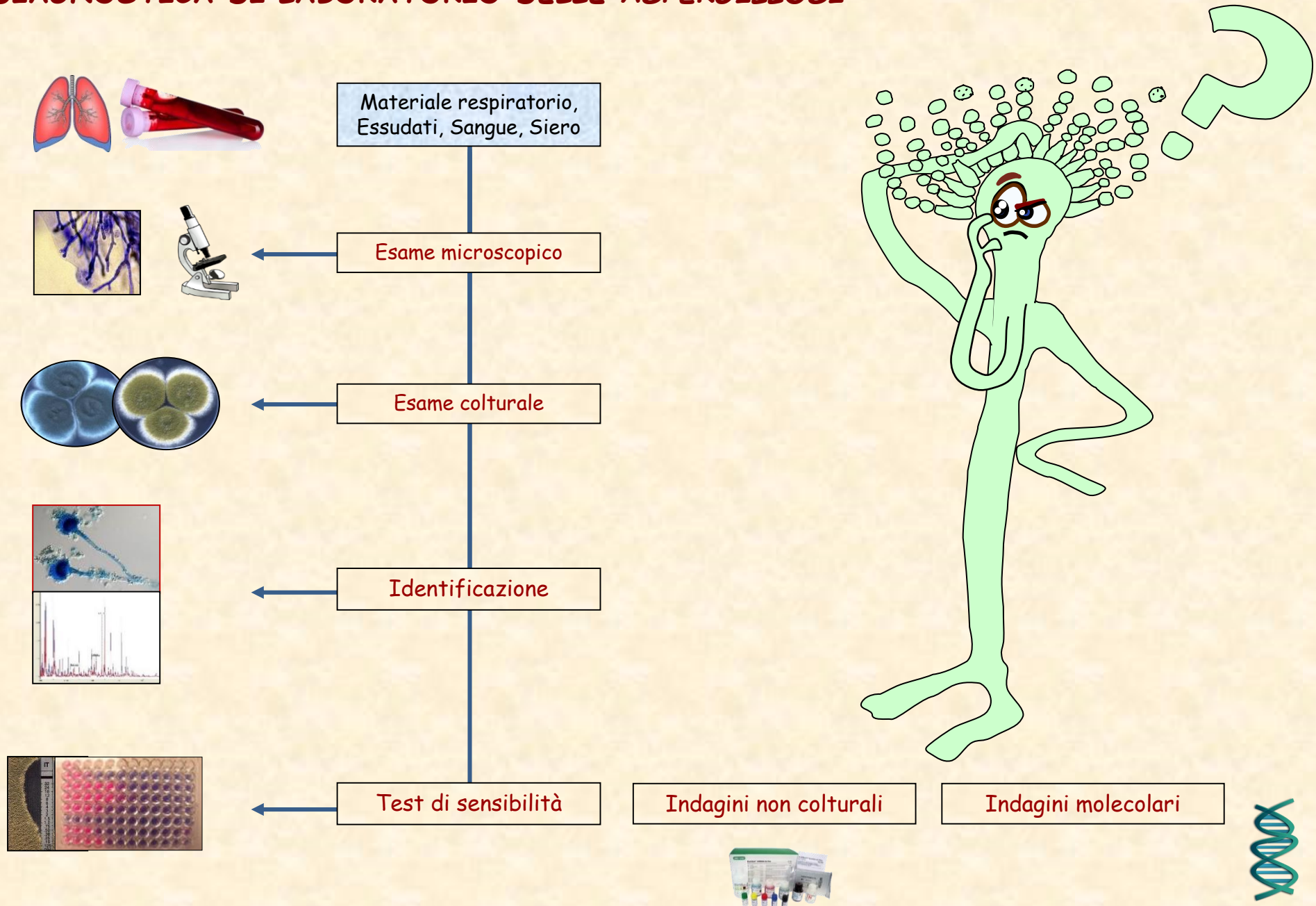
Alla PCR resistance, 57 pz avevano un *Aspergillus* WT e 11 una RAM Mutazione

Il fallimento del trattamento con azoli è stato osservato in 12/45 pazienti con WT *A. fumigatus* rispetto a 6/8 pazienti con *A. fumigatus* con RAM.



La mortalità a sei settimane era 2,6 volte più alta nei pazienti con RAM rilevata (17,8% senza versus 50,0%)

# DIAGNOSTICA DI LABORATORIO DELLE ASPERGILLOSI

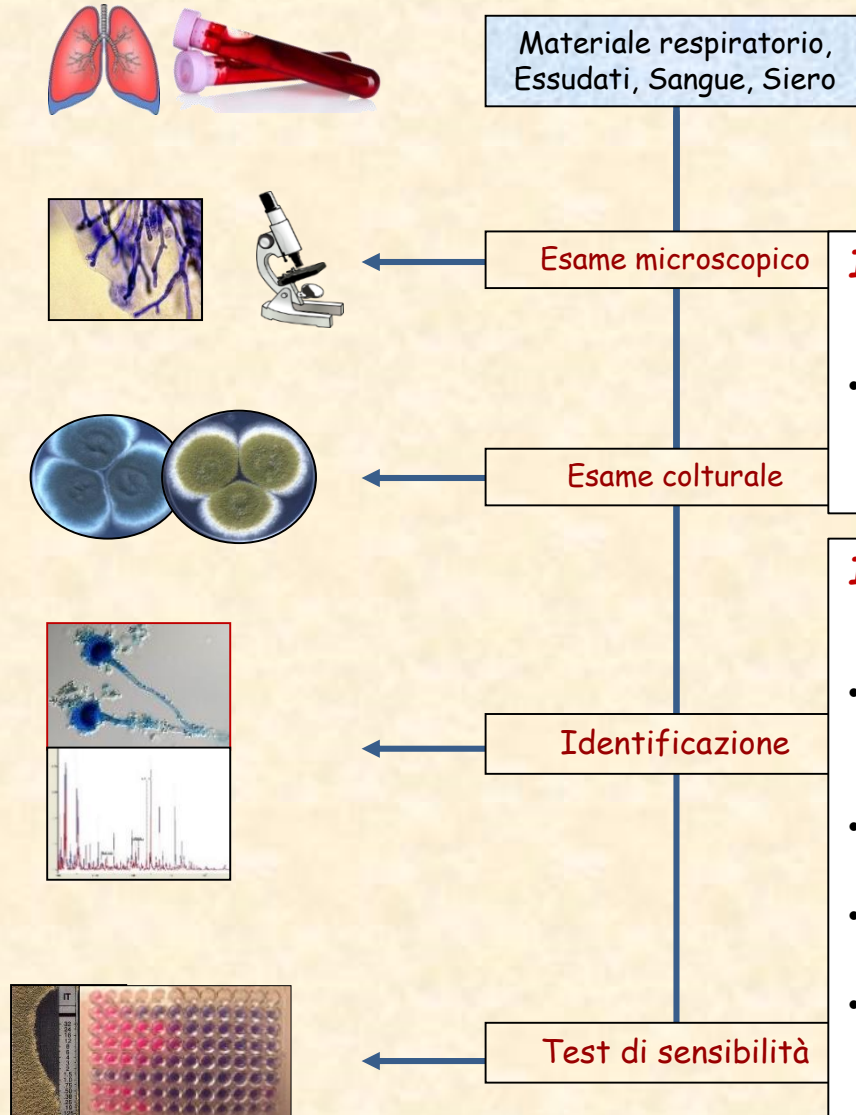


# DIAGNOSTICA DI LABORATORIO DELLE ASPERGILLOSI

Clinical Infectious Diseases

IDSA GUIDELINE

(Patterson T.F., Clin Inf Dis, 2016)



**I. Come può essere stabilita una diagnosi di aspergillosi invasiva? Come deve essere identificato *Aspergillus* ?**

- Fino a quando gli strumenti molecolari non saranno utilizzati più diffusamente, raccomandiamo di sottoporre i campioni di tessuti e liquidi per l'esame simultaneo istopatologico/citologico e colturale.

**II. Come possono essere utilizzati galattomannano e  $\beta$ -D-Glucano per la diagnosi di aspergillosi ?**

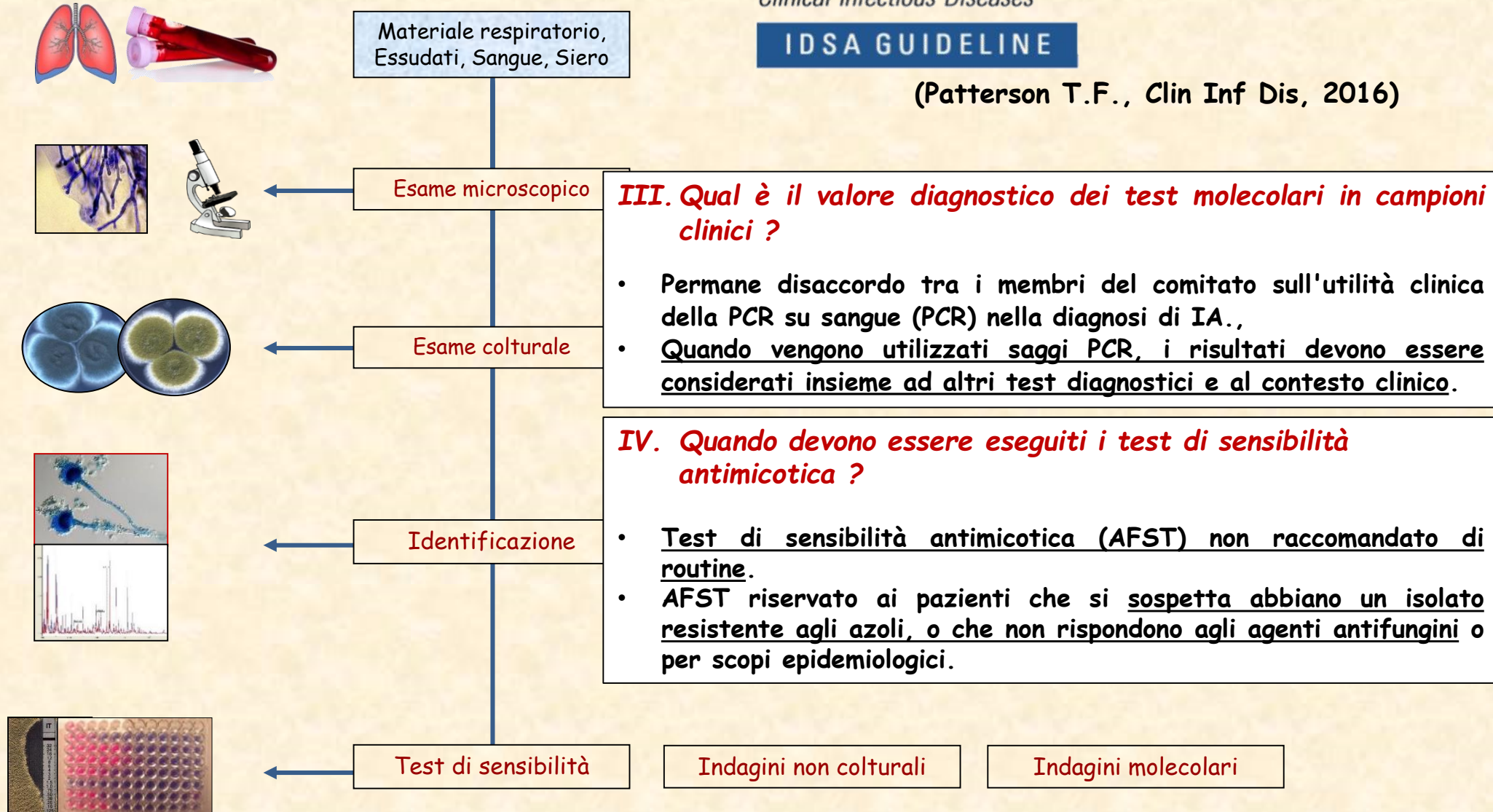
- Galattomannano (GM) su siero e BAL raccomandato per la diagnosi di IA in pazienti adulti e pediatrici in alcune sottopopolazioni di pazienti (neoplasie ematologiche, HSCT).
- GM non raccomandato per lo screening sierico di routine in pazienti che ricevono terapia o profilassi antifungina.
- GM non raccomandato per lo screening in pazienti SOT o pazienti con malattia granulomatosa cronica (CGD).
- Test sierici per  $\beta$ -D-glucano raccomandati per la diagnosi di IA in pazienti ad alto rischio (neoplasia ematologica, HSCT allogenico), ma non sono specifici per *Aspergillus*.

# DIAGNOSTICA DI LABORATORIO DELLE ASPERGILLOSI

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Grazie per l'attenzione

