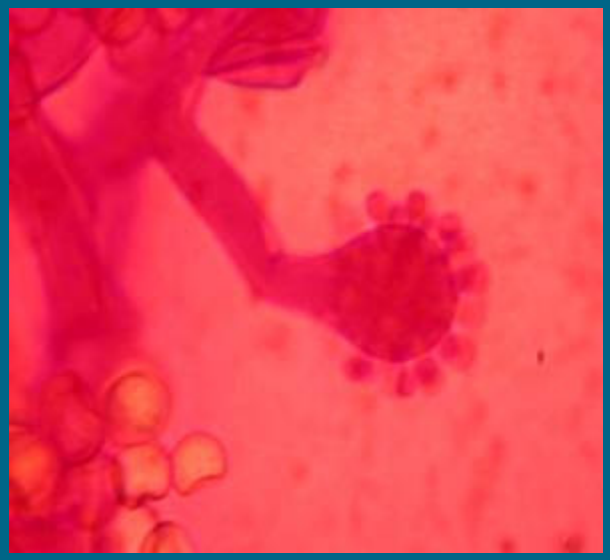
Instituto de Salud Carlos III

Emerging fungal pathogens: clinical usefulness of new diagnostic tools and identification methods



EDUCATIONAL WORKSHOP: Invasive infections due to rare or emerging yeasts and moulds: clinical aspects, diagnosis and treatment

15th ECCMID, Copenhagen

April 2nd 2005

MANUEL CUENCA-ESTRELLA

The Fungal Pathogens

- 120,000 validly described species of fungi
- >1,000,000 suspected species
- 200 species are recognized as primary pathogens of man and animals (around 100 as IFI)
- 5-10 new pathogens per year in last decade

Should Fungal Pathogens causing IFI be Identified at Species Level ?

NO

Fungal infections are considered the same

No therapeutic alternatives

Should Fungal Pathogens causing IFI be Identified at Species Level ?

YES, BUT
Surveillance Programs
Epidemiological reasons

Should Fungal Pathogens causing IFI be Identified at Species Level ?

YES

There is sufficient evidence to recommend that

Candida, azole agents and Blood cultures

	% resistant strains			
	n	Fluco	Itra	Vori
C. albicans	536	0,74	0,37	0,22
C. parap.	473	0,42	0,42	0
C. tropicalis	175	3,4	4	3,73
C. glabrata	151	29,1	29,1	10,5
C. krusei	76	100	6,5	9,3

Datos del Servicio de Micología. CNM. ISCIII

Aspergillus y Anfotericina B

There is some clinical correlation

	% strains with MICs ≥ 2 mg/l	
	n	Anfotericina B
A. fumigatus	268	1,80
A. flavus	65	23,1
A. terreus	65	53,8

Datos del Servicio de Micología. CNM. ISCIII

Scedosporium y Paecilomyces

There is some clinical correlation

		% de cepas resistentes a		
	n	Anfo B	Itra	Vori
S. apiospermum	46	91,3	95,6	13,3
S. prolificans	44	100	100	100
P. variotii	11	0	0	36,3
P. lilacinus	10	100	90	22,2

Datos del Servicio de Micología. CNM. ISCIII

Then...

It would be right to do that. If not, surveillance programs should be implemented at least

But, how can I do that taking into account the resources of my hospital, center, university... ?

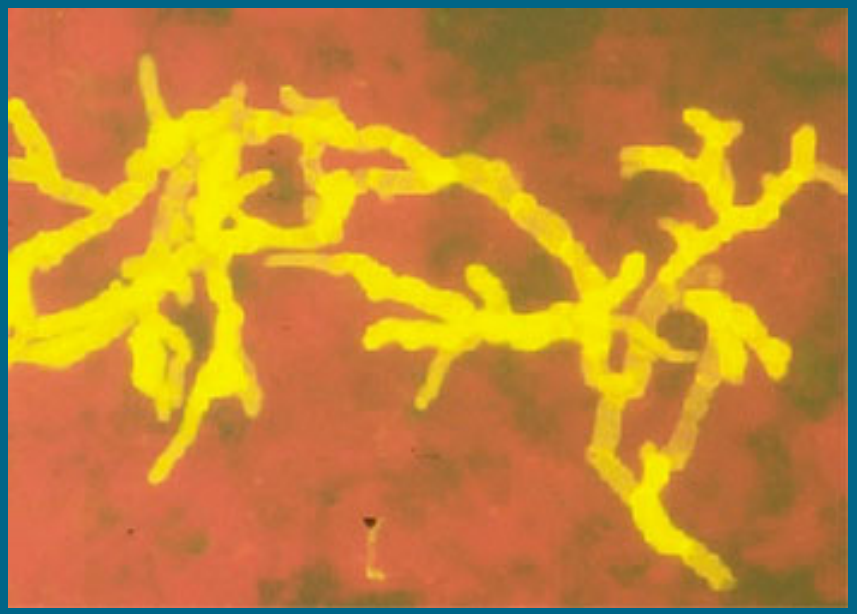
Are there new diagnostic tools with clinical usefulness ?

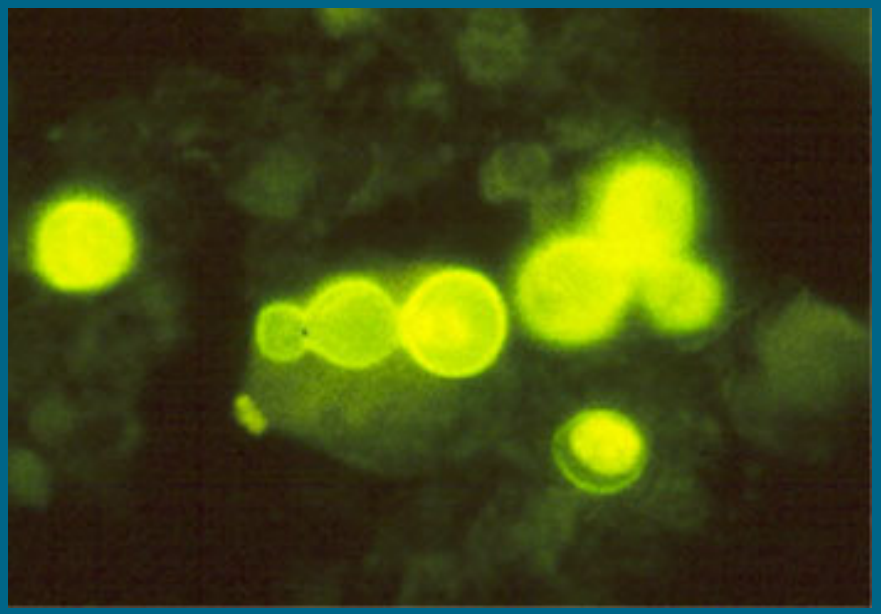
Laboratory procedures for diagnosis of IFI

- Direct Microscopic Examination
- Histopathologic studies
- Culture
  - Conventional identification
  - Molecular methods
- Serologic Testing
- Molecular Diagnostics

Microscopic examination

Technique	Useful to...	It takes...
Calcofluor	Fungi	1 min

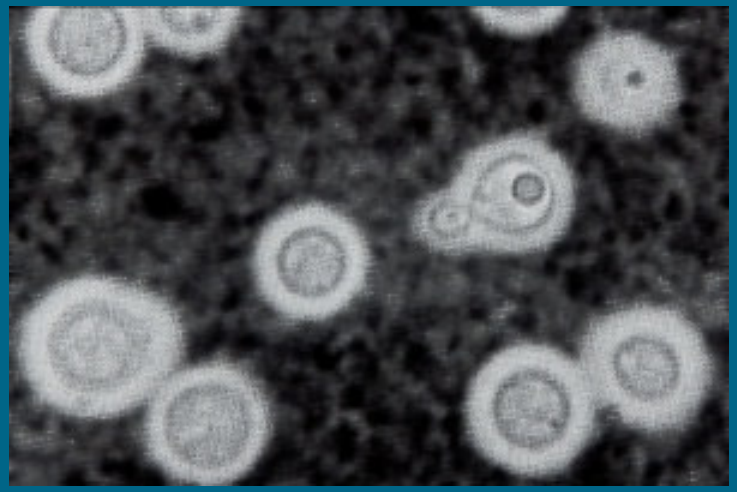




BAL samples

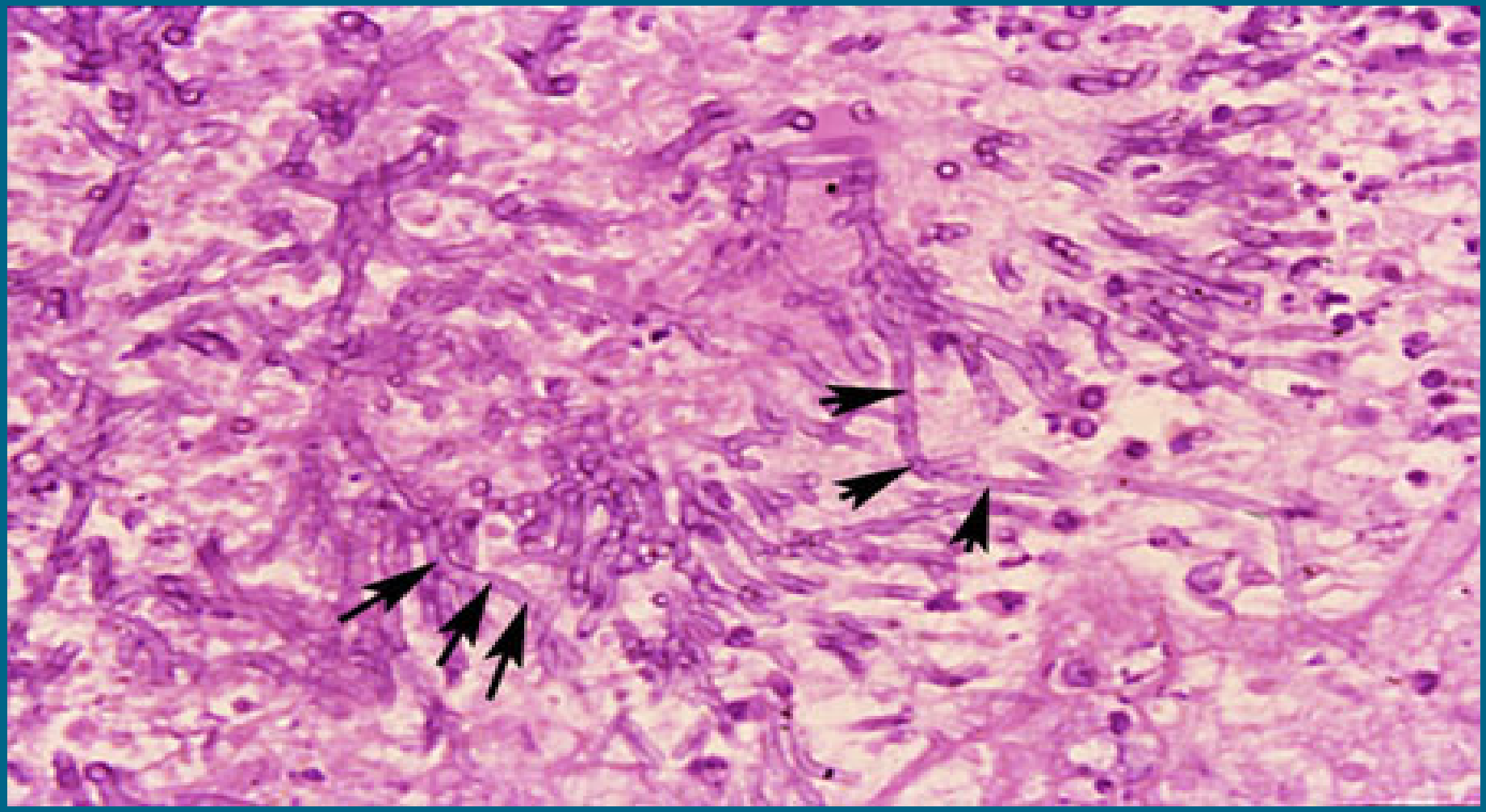
Microscopic examination

Method	Useful to...	It takes...
Indian Ink	*C. neoformans* in CSF	1 min

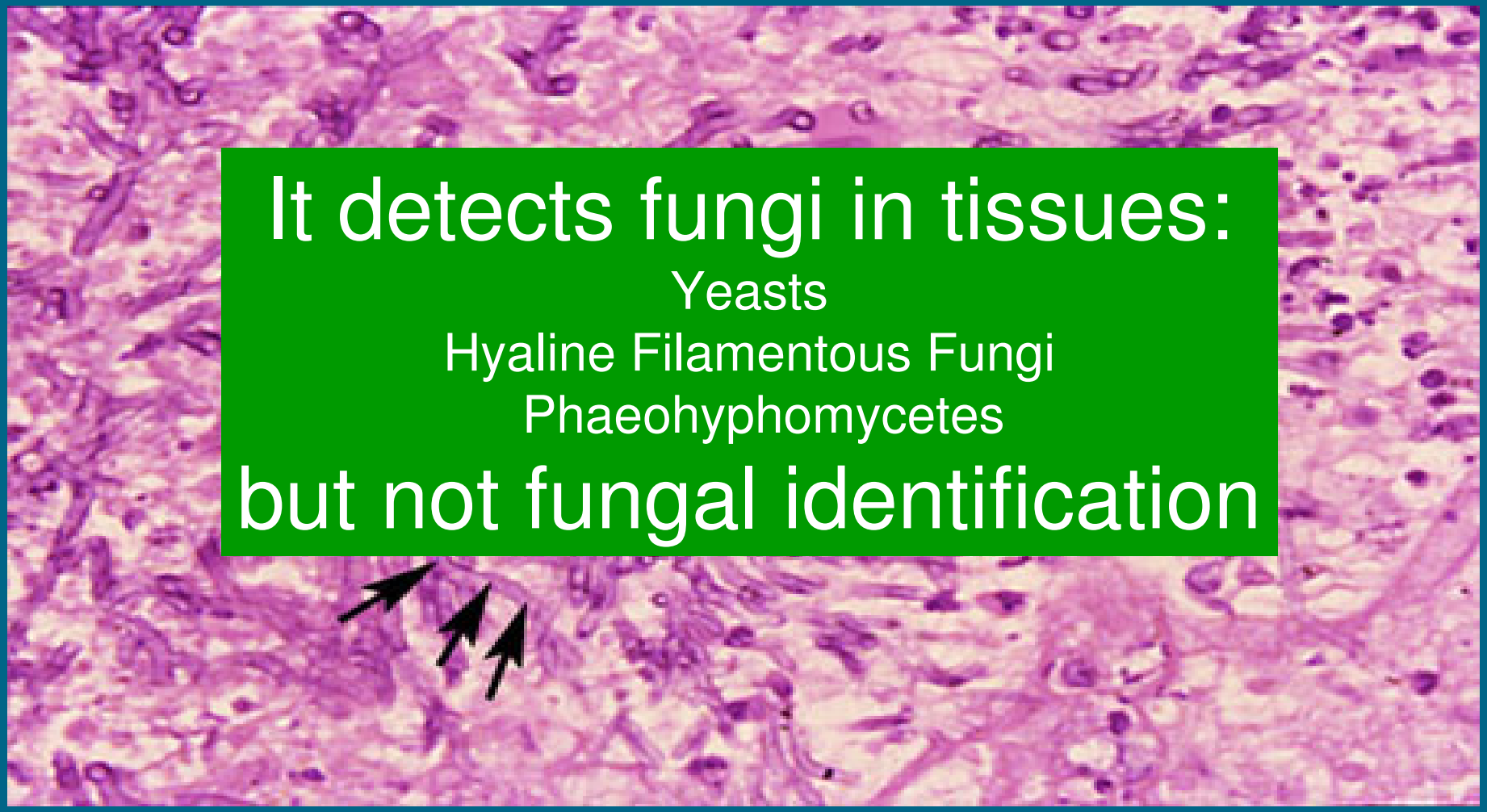


CSF

Histopathologic studies



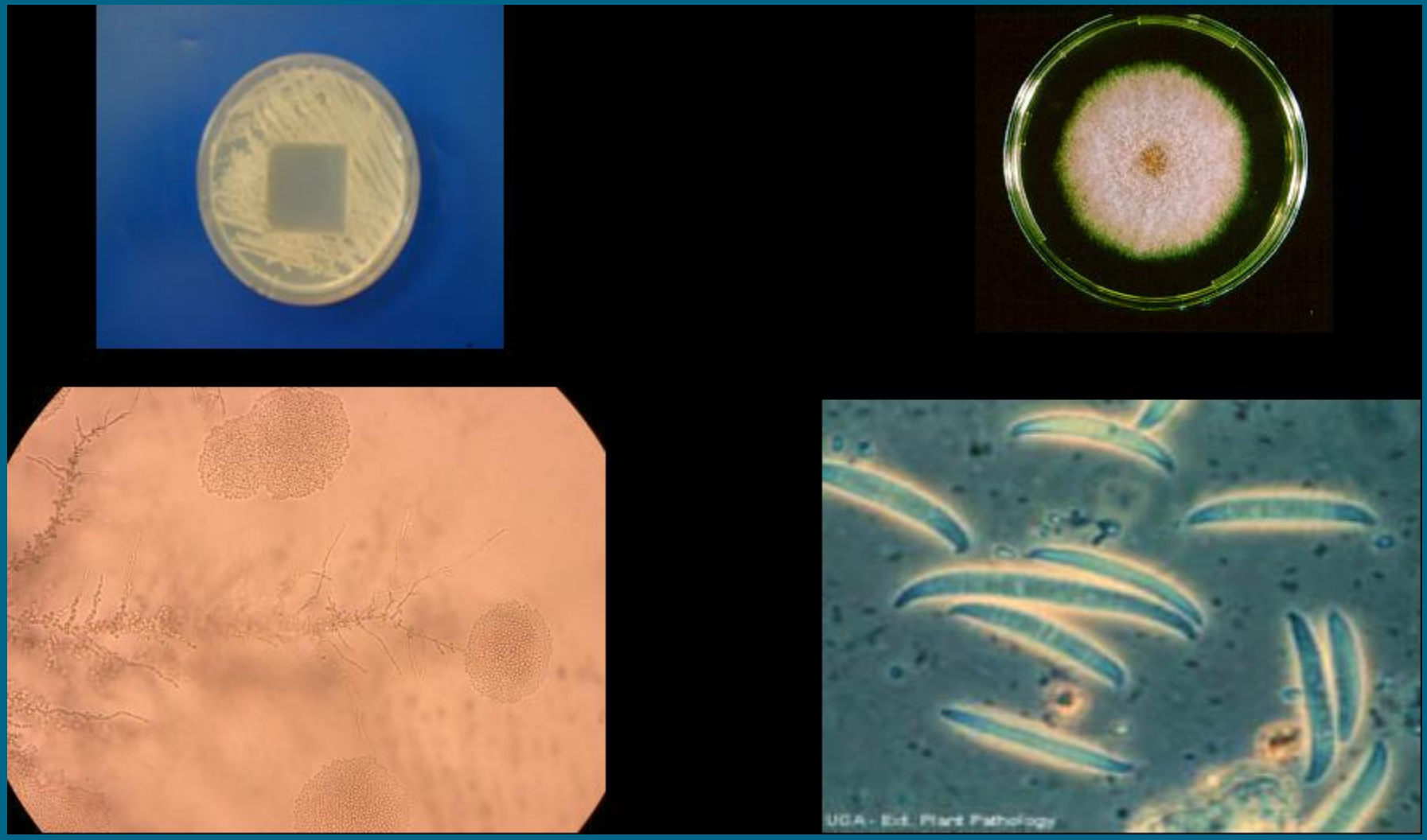
Histopathologic studies



Identification with Histopathologic studies

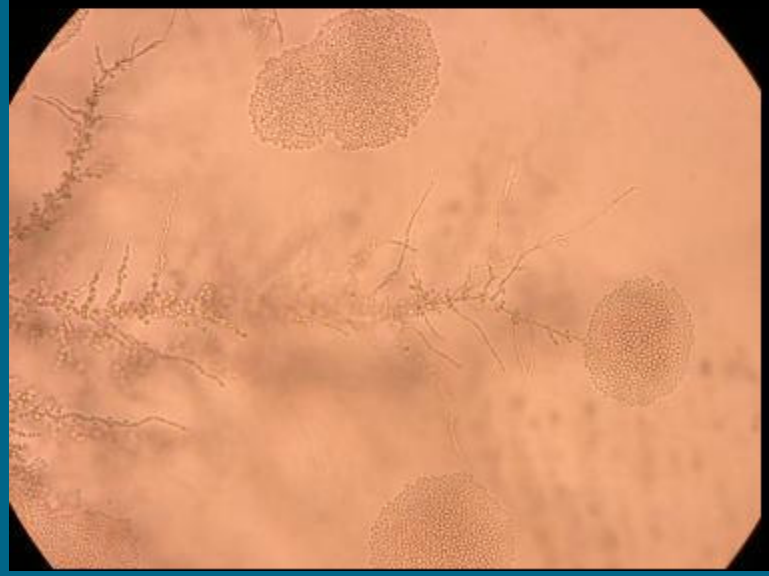
COCCIDIOIDOMYCOSIS

Cultures
Conventional Identification

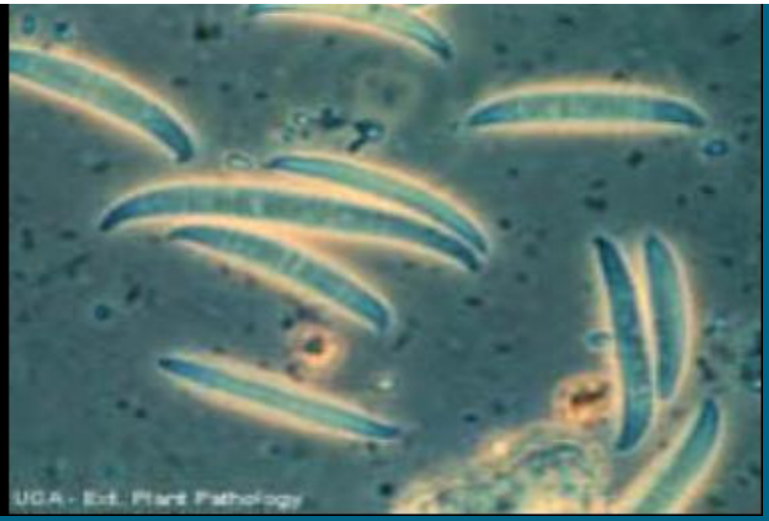


Cultures
Conventional Identification

Candida parapsilosis on CMA

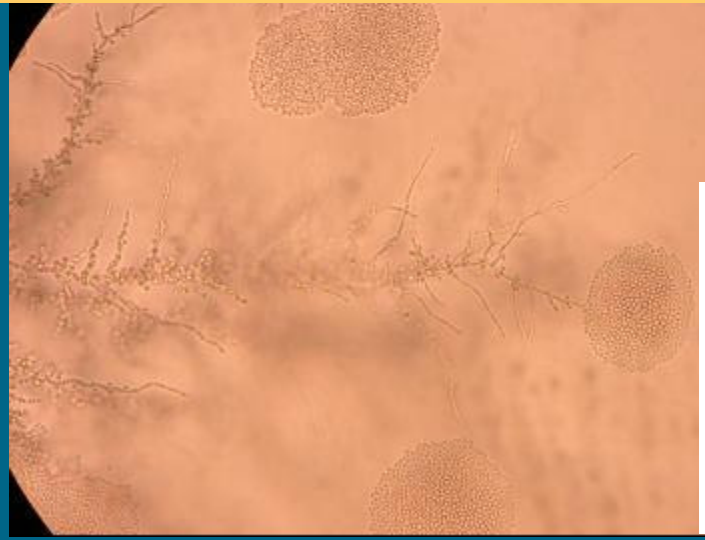


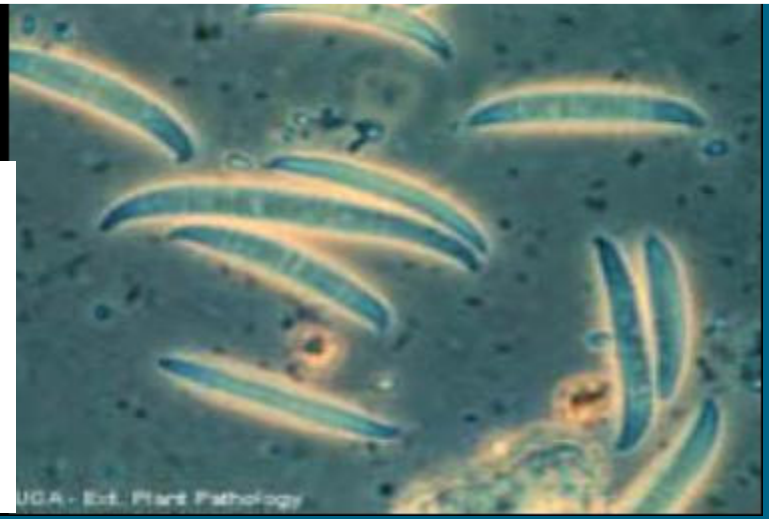
Fusarium solani on MEA



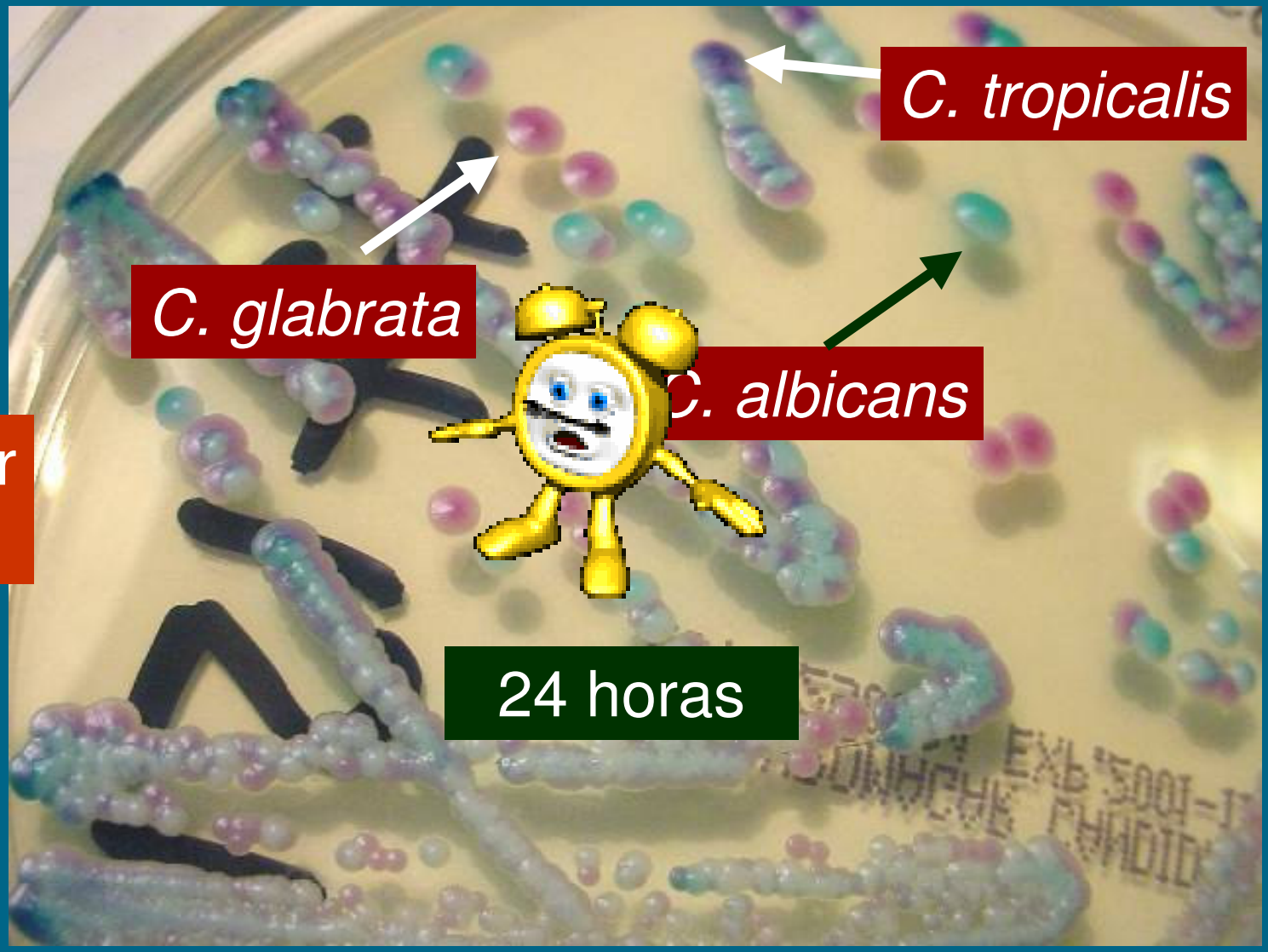
Cultures
Conventional Identification

But for most of fungi it takes 1 week to months





Something new in conventional identification



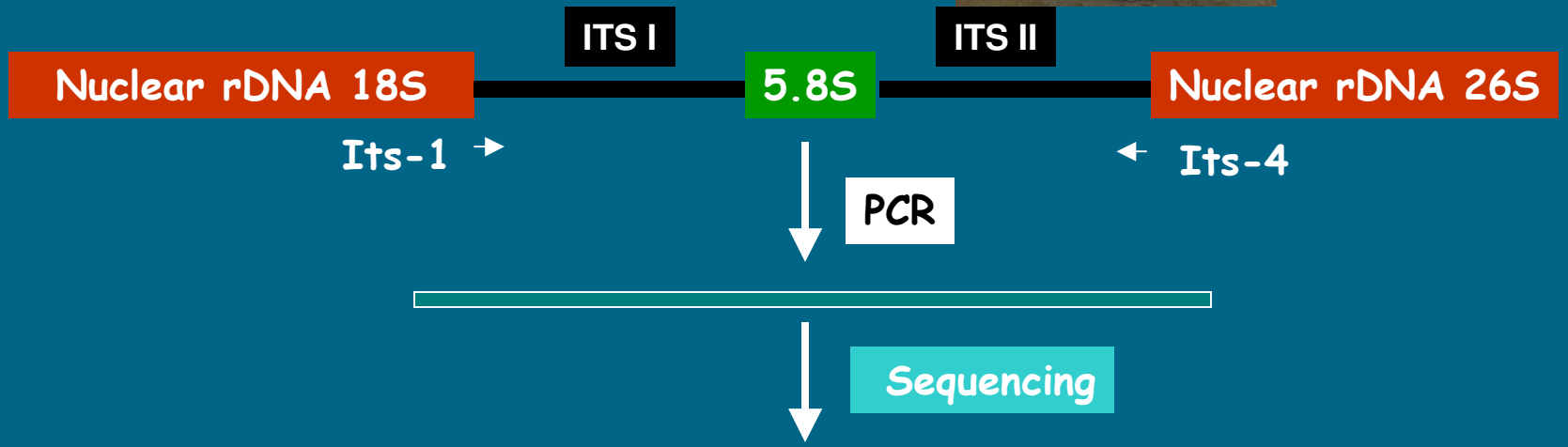
Chromagar *Candida*

Cultures
Molecular Methods

Yeast or filamentous fungi

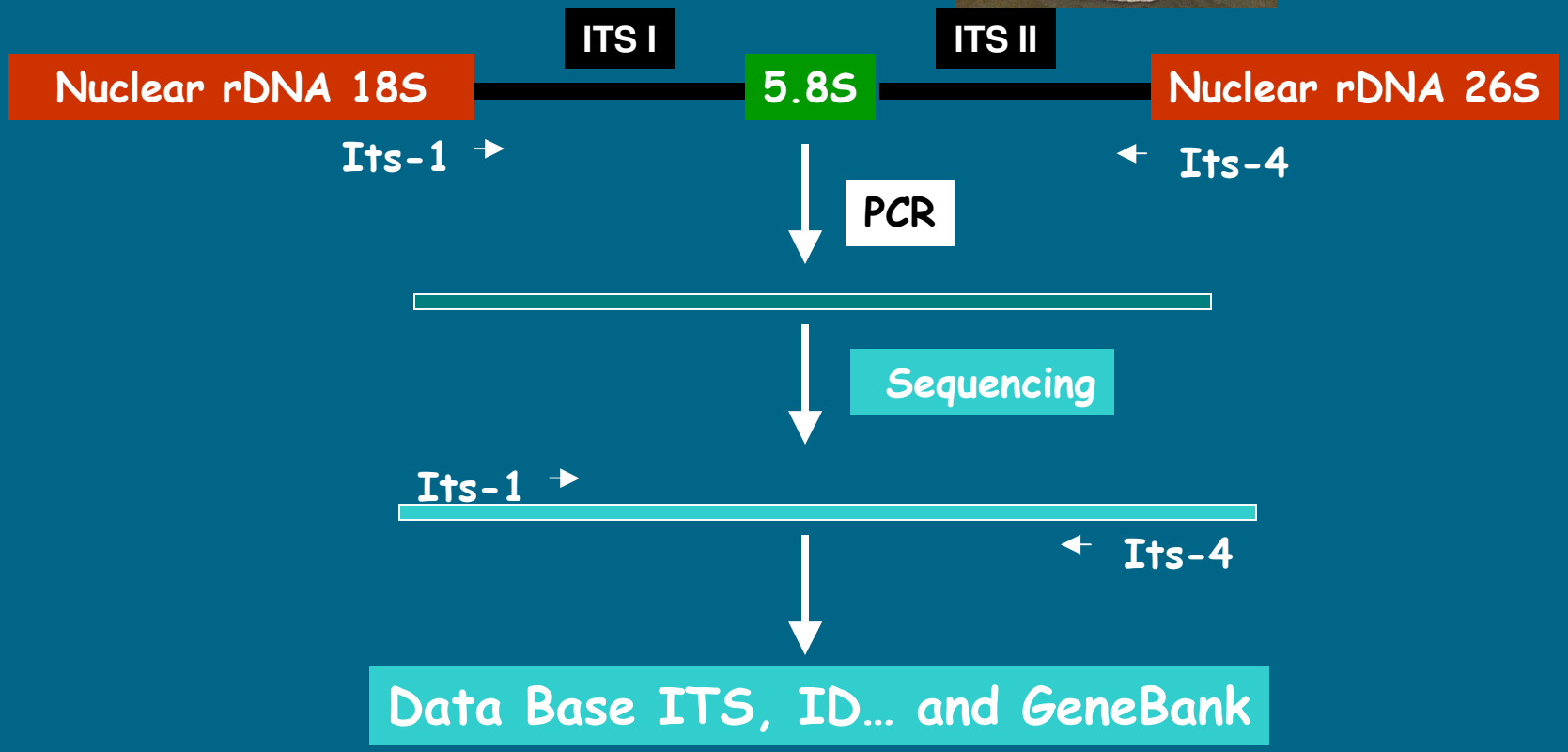
Cultures
Molecular Methods

Yeast or filamentous fungi



Cultures
Molecular Methods

Yeast or filamentous fungi



Cultures
Molecular Methods

Ye[illegible]st or filamentou

[illegible]26S

It could take 48 h to identification

Its-4

[illegible]ata Base ITS, ID… and GeneBank

But Pitfalls

- Non standardized methods
- Expensive equipments
- Lack of experience
- Non reliable data base for emerging-rare fungi

But Pitfalls

- Non
- Expe
- Lack
- Non ng-rare fung

They are available only at some reference centers that are normally away from clinical practice

Serologic Testing

- Conventional techniques:
  - They seldom exhibit clinical utility
  - Serology for dimorphic filamentous primary pathogenic fungi
  - Detection of capsular antigen of *C. neoformans*
- New techniques:
  - Galactomannan for *Aspergillus*
  - Mannan for *Candida*
  - Other antigen-antibody tests
  - Detection of beta-glucan

Serologic Testing

- Conventional techni[illegible]:
  - They [illegible]om exh[illegible]ilit[illegible]
  - Serolo[illegible]
  - D[illegible]*oformans*
- N[illegible]
  - A[illegible]
  - Other [illegible]ribo[illegible] tests
  - Detec[illegible]n of beta-glucan

Galactomannan is a outstanding contribution to the diagnosis of aspergillosis in onco-haematological patients

Serologic Testing

- Other new techniques need validation
- Beta-D-glucan detection can be useful for some patients suffering from IFI
- Then, for emerging pathogens…

(1,3)-Beta-D-Glucan Detection (Glucatell® test)

New

FDA license for IFI, May 21st, 2004

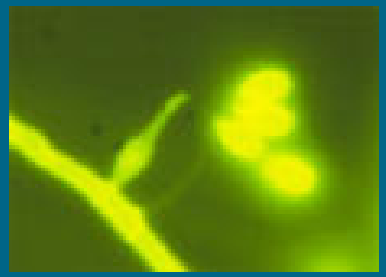
Detection :

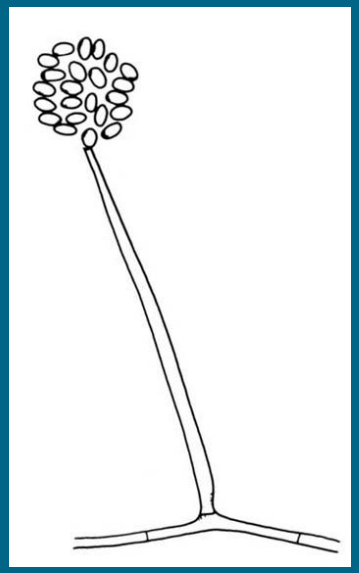
- Mucorales
- *Cryptococcus* spp

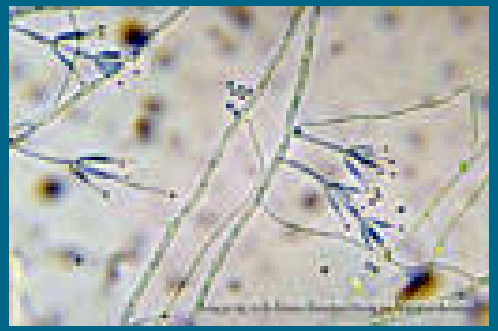
Clinical sample : serum

It takes 2 h, but
not iD at species level

Panfungal Detection







Glucatell® (1.3)-Beta-DG Establishing Cut-off

- 30 healthy adults
- 30 patients nonneutropenic with symptomatic Candidemia

Odabasi et al. CID'04

Glucatell® (1.3)-Beta-DG Establishing Cut-off

- 30 healthy adults
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Healthy			
Mean ± SD	Range		
17 ± 34 pg/ml	0-86 pg/ml		

Odabasi et al. CID'04

Glucatell® (1.3)-Beta-DG Establishing Cut-off

- 30 healthy adults
- **30 patients nonneutropenic with symptomatic Candidemia**

Healthy		**Candidemia**	
Mean ± SD	Range	**Mean ± SD**	**Range**
17 ± 34 pg/ml	0-86 pg/ml	**2,999 ± 5,190**	**36-22,263**

Odabasi et al. CID'04

Only 2 serum samples ≥ 60 pg/ml

Only 1 serum sample < 60 pg/ml

CUTOFF

≥ 60 pg/ml

- 30 pati… ptomatic Candiden…

He…		…idemia	
Mean ± SD			Range
17 ± 34 pg/ml	0-86 pg/ml	2,999 ± 5,190	36-22,263

Odabasi et al. CID'04

Glucatell® (1.3)-Beta-DG Diagnostic study

- 300 haematologic patients (283 evaluable)
  - prophylaxis with itra or caspo
  - serum samples twice a week
    - 2,070 specimens
    - 7.3 specimens/patient
- Definition of fungal infection by EORTC/MSG criteria
- Glucan assay was performed in a blinded fashion

Odabasi et al. CID'04

Glucatell® (1.3)-Beta-DG Diagnostic study

- 15 Proven fungemia
  - 6 *C. glabrata*
  - 3 *T. asahii*
  - 2 *C. krusei*
  - 1 *C. albicans, C. tropicalis, C. parapsilosis* & *Fusarium* sp
- 1 Proven fungal pneumonia by *A. terreus*
- 4 probable fungal pneumonia
  - 2 *A. fumigatus*
  - 1 *A. terreus* & *C. albicans*; 1 *Fusarium*
- 33 possible fungal pneumonia

Odabasi et al. CID'04

Glucatell® (1.3)-Beta-DG

15 cases of Proven fungemia

	Max. BG levels	No ⊕ results	Days before
Mean	927	3	9.71
SD	981	1.9	8.2
Range	61-3,422	1-8	-32 → +2

Odabasi et al. CID'04

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Odabasi et al. CID'04

Glucatell® (1.3)-Beta-DG

5 Proven & probable fungal pneumonia

	Max. BG levels	No ⊕ results	Days before
Mean	**530**	**1.6**	**11.4**
SD	**881**	**1.3**	**9.5**
Range	**81-2,105**	**1-4**	**-23 → +1**

Odabasi et al. CID'04

Glucatell® (1.3)-Beta-DG

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Range	81-2,105	1-4	**-23 → +1**

Odabasi et al. CID'04

Glucatell® (1.3)-Beta-DG

No. of BG ⊕ serum samples	Proven or probable IFI			
	% S	% Sp	% PPV	% NPV

Odabasi et al. CID'04

Glucatell® (1.3)-Beta-DG

No. of BG ⊕ serum samples	Proven or probable IFI			
	% S	% Sp	% PPV	% NPV
1 ⊕ result	100	90	43	100

Odabasi et al. CID'04

Glucatell® (1.3)-Beta-DG

No. of BG ⊕ serum samples	Proven or probable IFI			
	% S	% Sp	% PPV	% NPV
1 ⊕ result	100	90	43	100
≥ 2 ⊕ results	**65**	**96**	**57**	**97**

Odabasi et al. CID'04

Glucatell® (1.3)-Beta-DG

No. of BG ⊕ serum samples	Proven or probable IFI			
	% S	% Sp	% PPV	% NPV
1 ⊕ **result**	100	90	43	100
≥ 2 ⊕ **results**	65	96	57	97
≥ 3 ⊕ results	**60**	**99**	**80**	**96**

Odabasi et al. CID'04

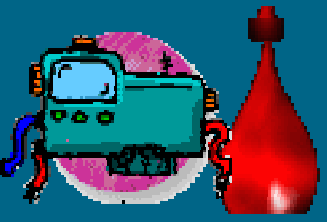
False positive results in this study

- 10 Non colonized patients
  - 10 (3.5%) patients w/o IFI
- 2 colonized (oral & urinary)
- No increase in BG serum levels

False positive results in literature

Surgical gauzes containing BG. Nakao et al' 97

Immunoglobulin products. Ikemura et al' 89



Hemodialysis with cellulose membranes. Kato et al' 01

Summary for Glucatell® (1.3)-Beta-DG

- A new tool able to detect many fungal pathogens, but not useful for identification
- Often precedes the microbiological or clinical diagnosis
- Few cases of filamentous fungal infections in this study
- We need studies with other patients

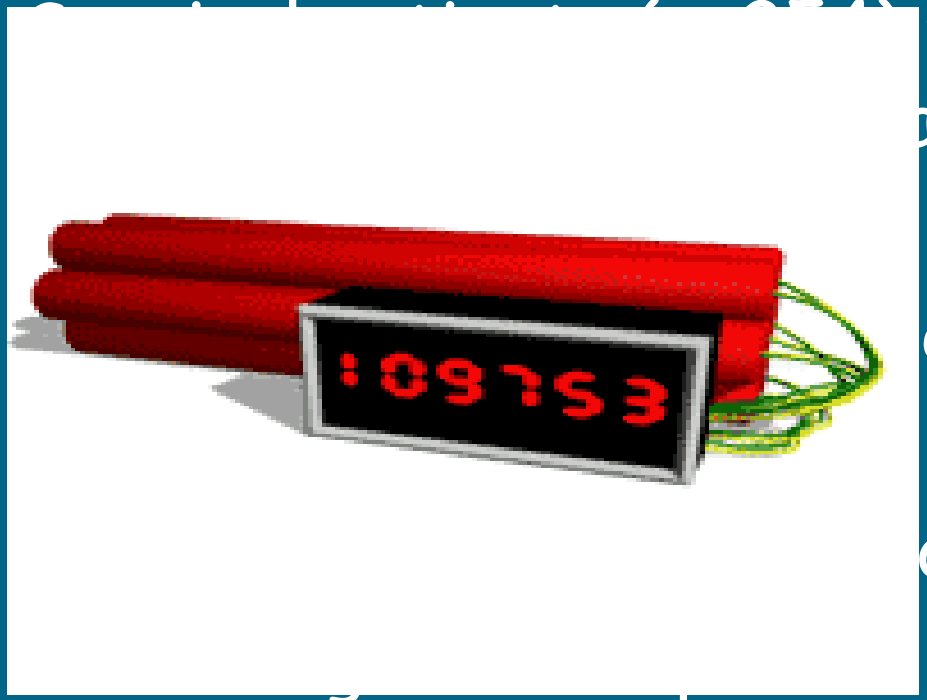
Glucatell® (1.3)-Beta-DG in surgical patients

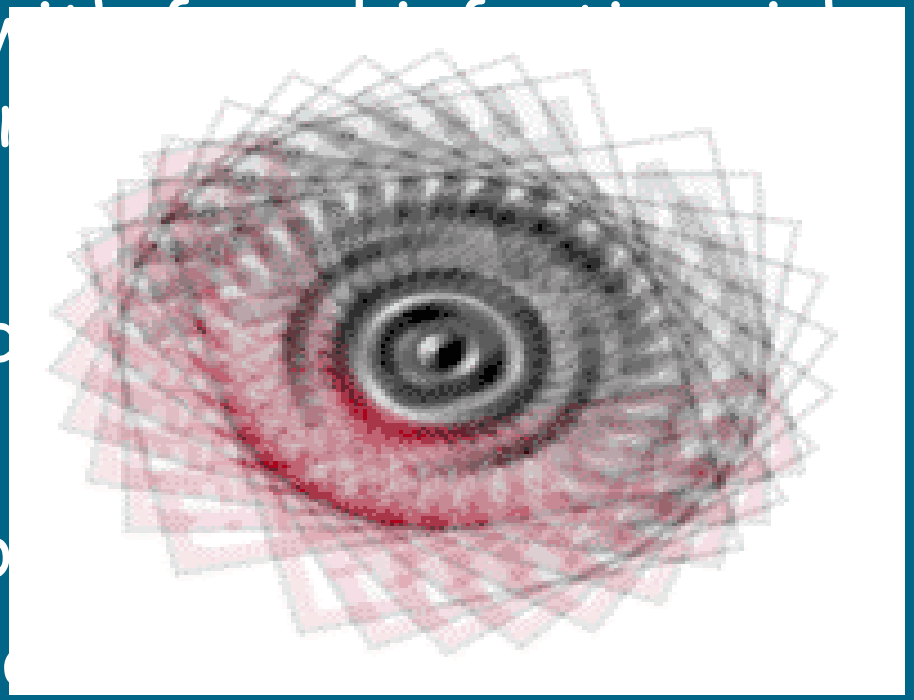
Takesue World J Surg 2004

- Surgical patients (n=254) with fungal infection risk, *Candida* spp. colonization and fever
- Fluconazole administered
- Beta-D-Glucan kinetically determined
- 32 positive patients
  - 15 (46.9%) responded to fluconazole
- 9% of negative responded as well
- OR of beta-D-glucan in colonized patients: 12.9 (2.07-80.73)

Glucatell® (1.3)-Beta-DG in surgical patients

Takesue World J Surg 2004

- 

- 
- OR of beta-D-glucan in colonized patients: 12.9 (2.07-80.73)

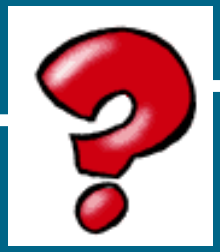
Molecular Diagnostics

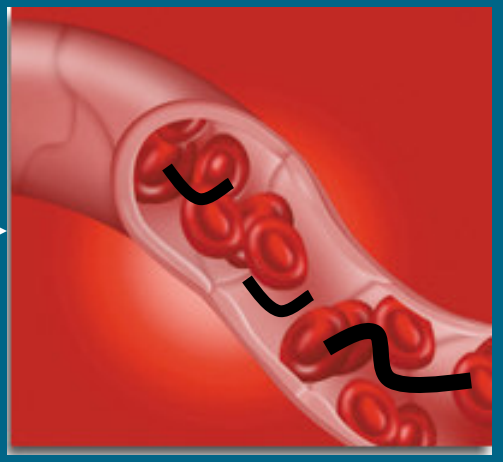
- Several approaches, many studies for distinct pathogens without clinical validation
- New breakthroughs preclude further analysis of many molecular diagnostics
- Lack of commercial methods:
  - No standardization
  - No quality control
- Lack of comparative multicenter studies

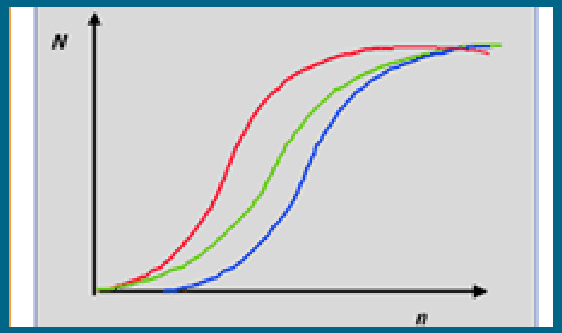
Molecular Diagnostics

- Seve… …for distinct pathoge…
- New brea… …nalysis of many mo…
- Lack of comm…
  - No stand…
  - No qua…
- Lack … …udies

But…



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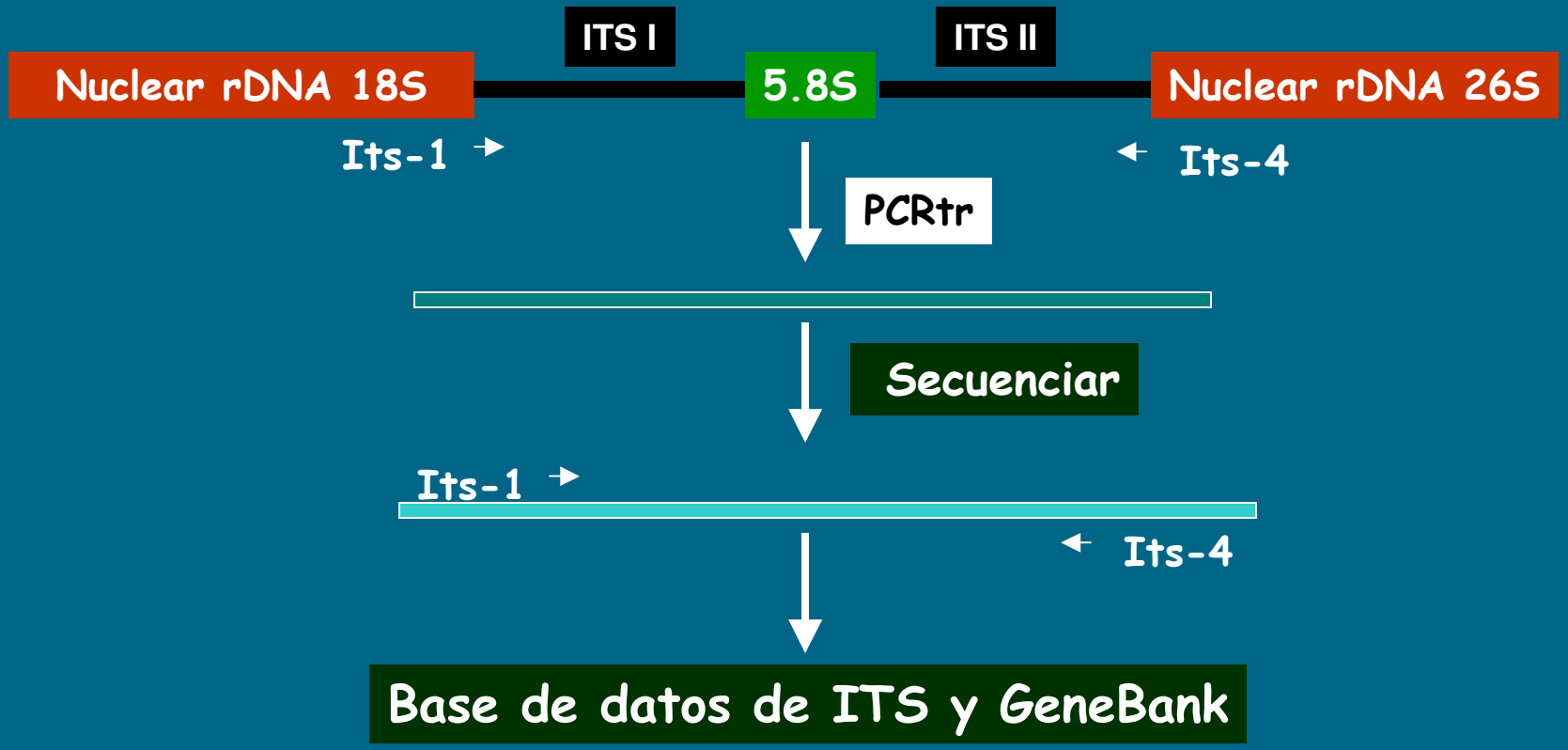
Spanish Net for study of transplant patients

PANFUNGAL RT-PCR



Spanish Net for study of transplant patients

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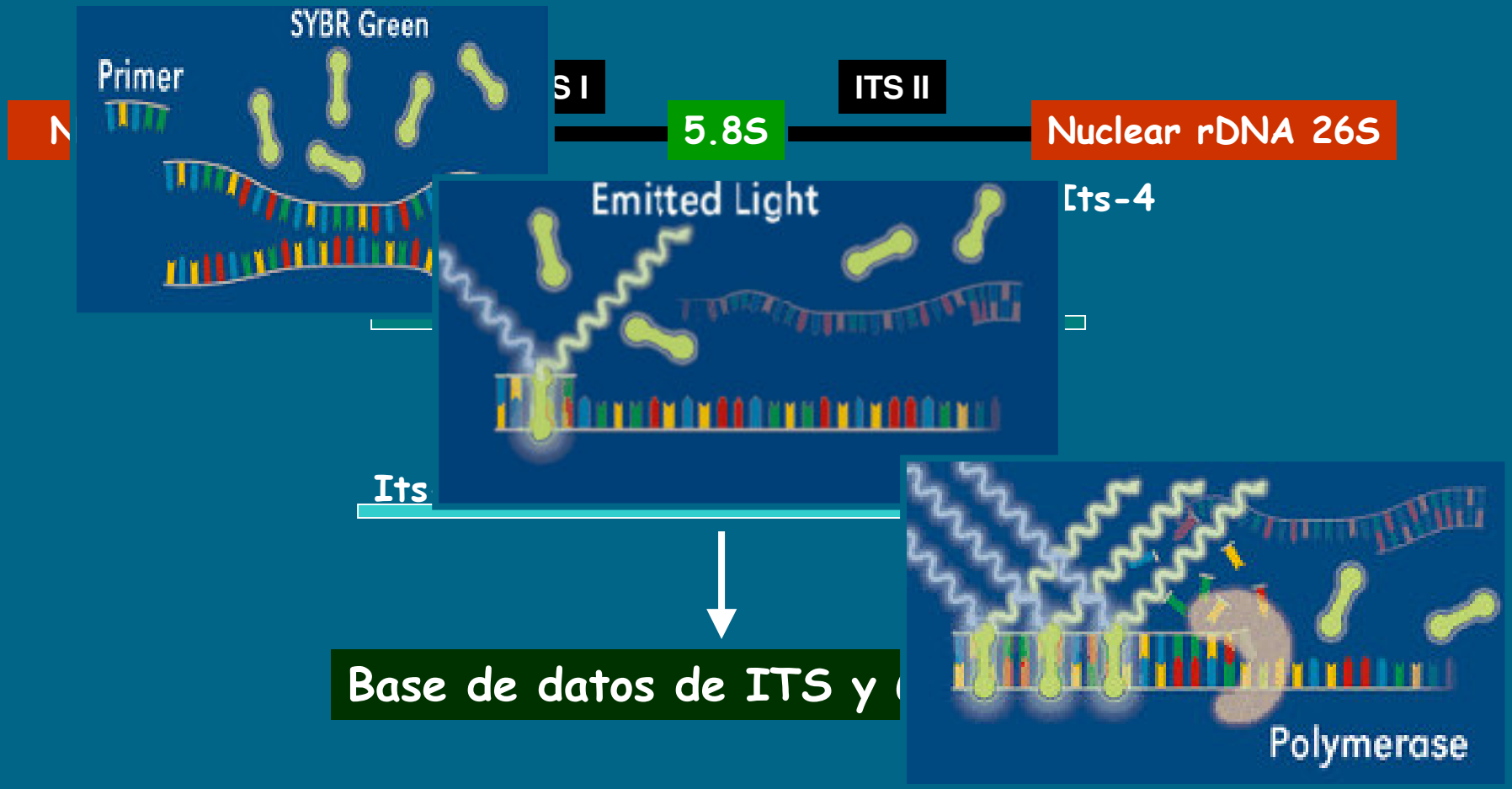
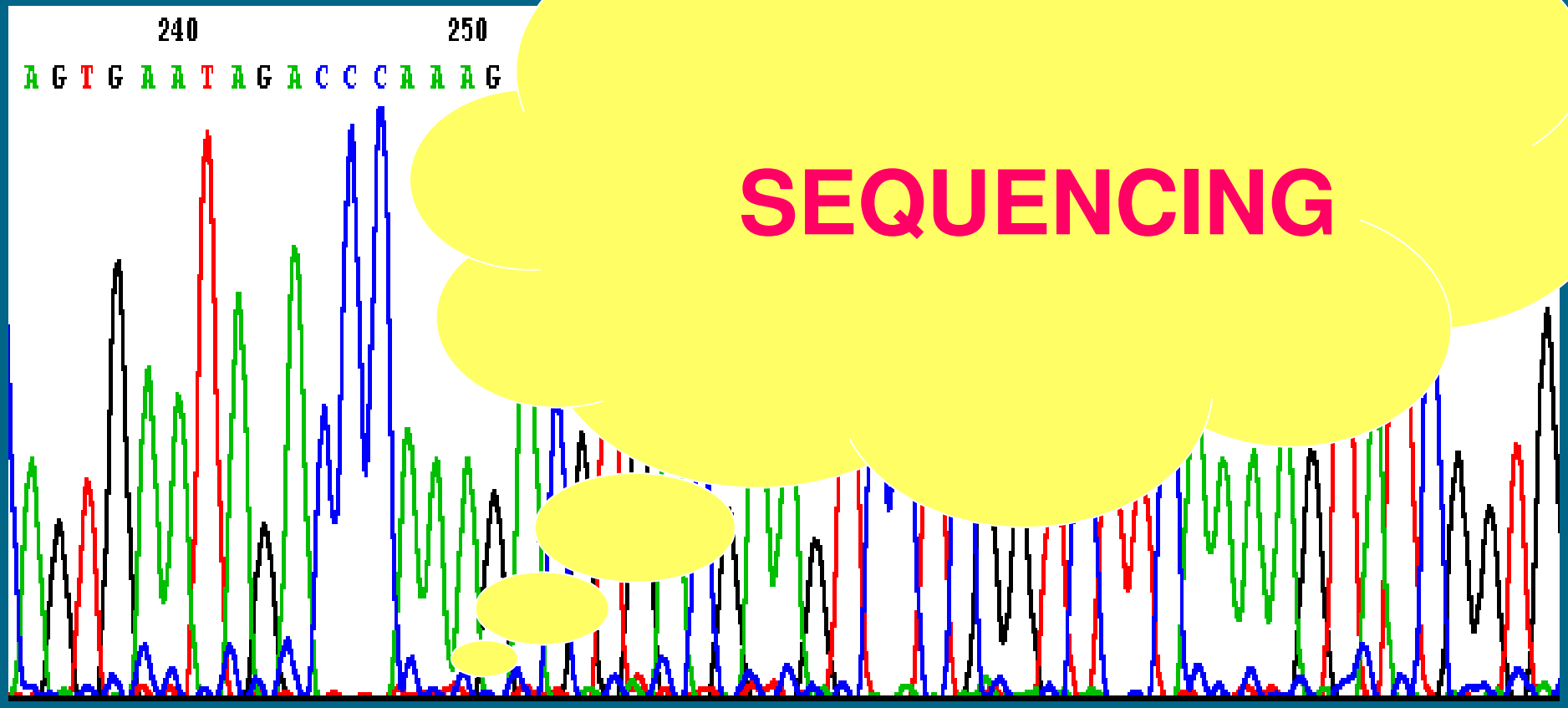
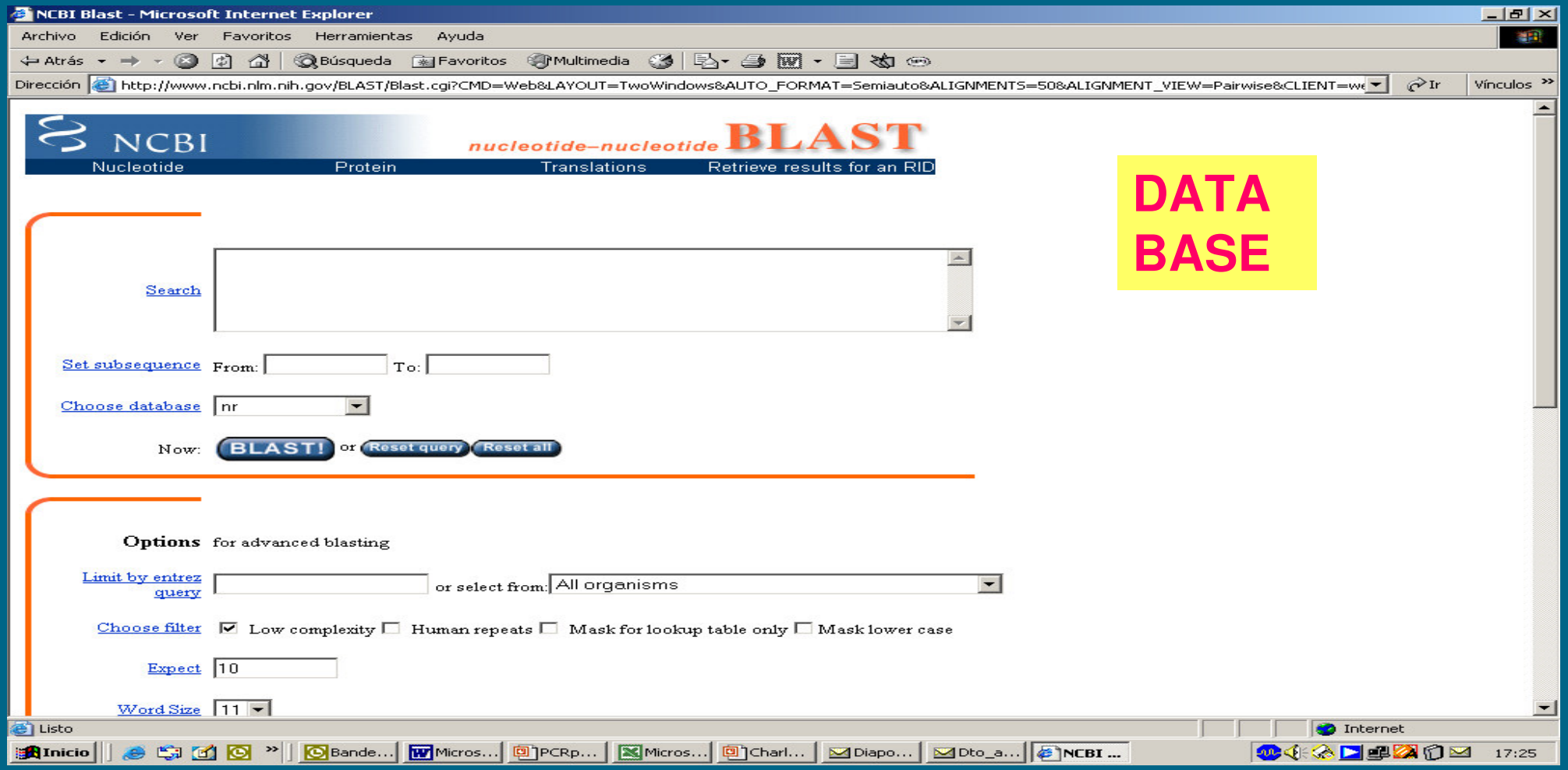


Base de datos de ITS y

Spanish Net for study of transplant



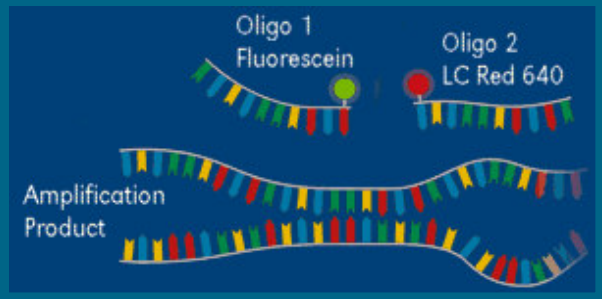
Spanish Net for study of transplant patients

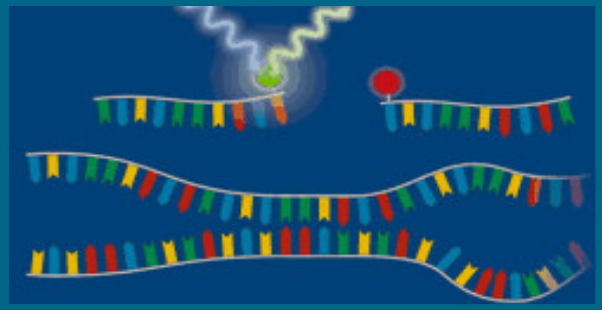


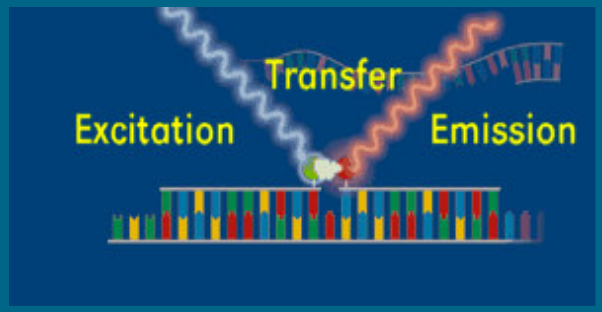
DATA BASE

Spanish Net for study of transplant patients

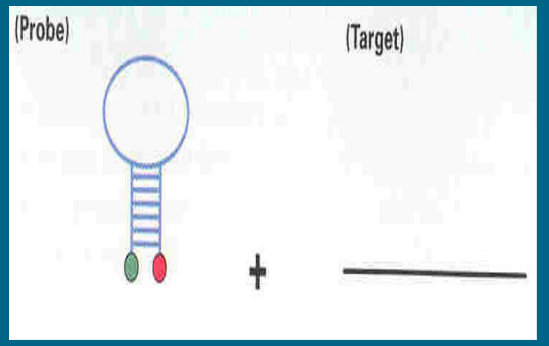
SPECIFIC RT-PCR



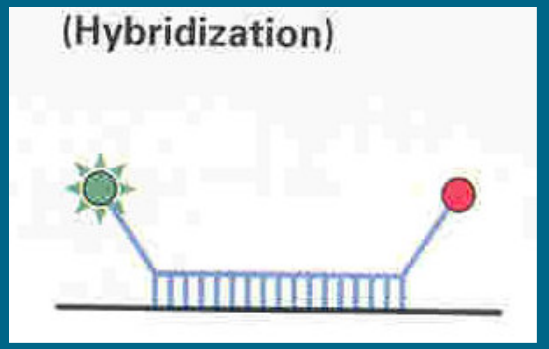




FRET PROBES



MOLECULAR BEACONS



Spanish Net for study of tr atients

Candida albicans

Candida tropicalis

Candida parapsilosis

Candida glabrata

Candida krusei

Aspergillus fumigatus

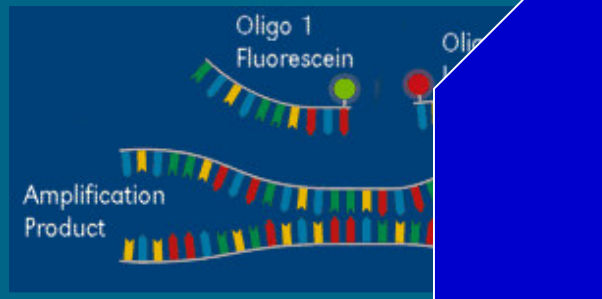
Aspergillus terreus

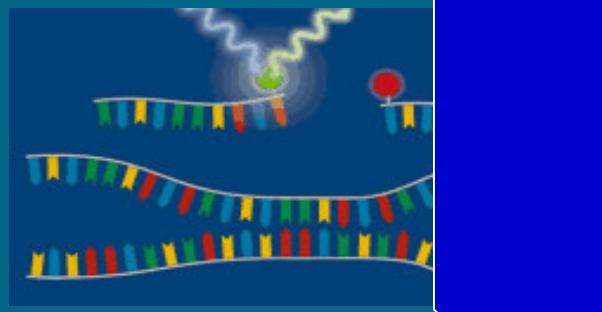
Aspergillus flavus

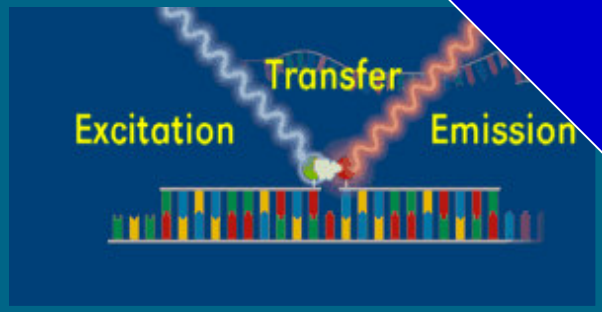
Scedosporium apiospermum

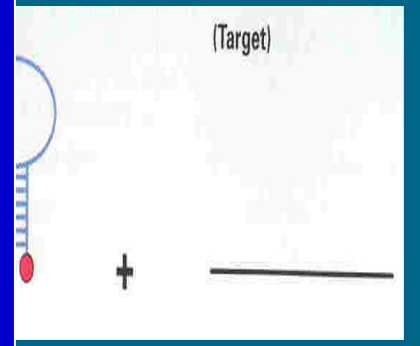
Scedosporium prolificans

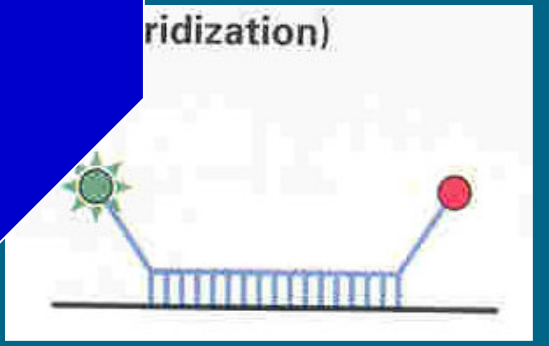
Other emerging fungal pathogens











Conclusions

- Identification at species level of emerging fungal pathogens is getting more useful for treating IFIs
- Several studies have showed differences in antifungal susceptibility profile with some clinical correlation
- In any case, epidemiological surveys are compulsory in order to know prevalence of emerging fungi in your geographical area

Conclusions II

- Identification of emerging fungal pathogens by conventional methods is difficult and time-consuming
- New diagnostic tools:
  - Beta-D-Glucano is useful for early diagnosis of IFI, even due to emerging pathogens, but identification at species level is not achieved
  - Molecular diagnostics could be useful, but we need validation