External Quality Assessment in Mycology

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National Collection of Pathogenic Fungi (NCPF)

2,500 strains:

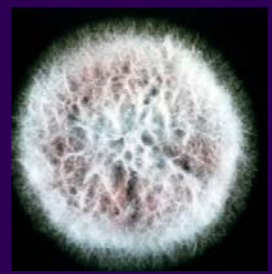
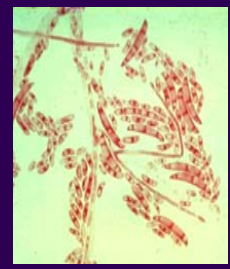
500 strains of dermatophytes and related organisms

900 mould strains from subcutaneous and deep-seated infections

900 pathogenic yeast strains

140 strains of dimorphic fungi



Currently all data being entered onto biolomics programme: historical data, DNA, photographs

www.hpaculturecollections.org.uk

Storage of isolates - lyophilisation

Storage of isolates
- cryopreservation in liquid N_2

Aims of NEQAS in mycology

Ensure that laboratories offering a mycology service are performing at a satisfactory level

Educational specimens

Provision of web-site information

Limitations:

Unable to send simulated specimens

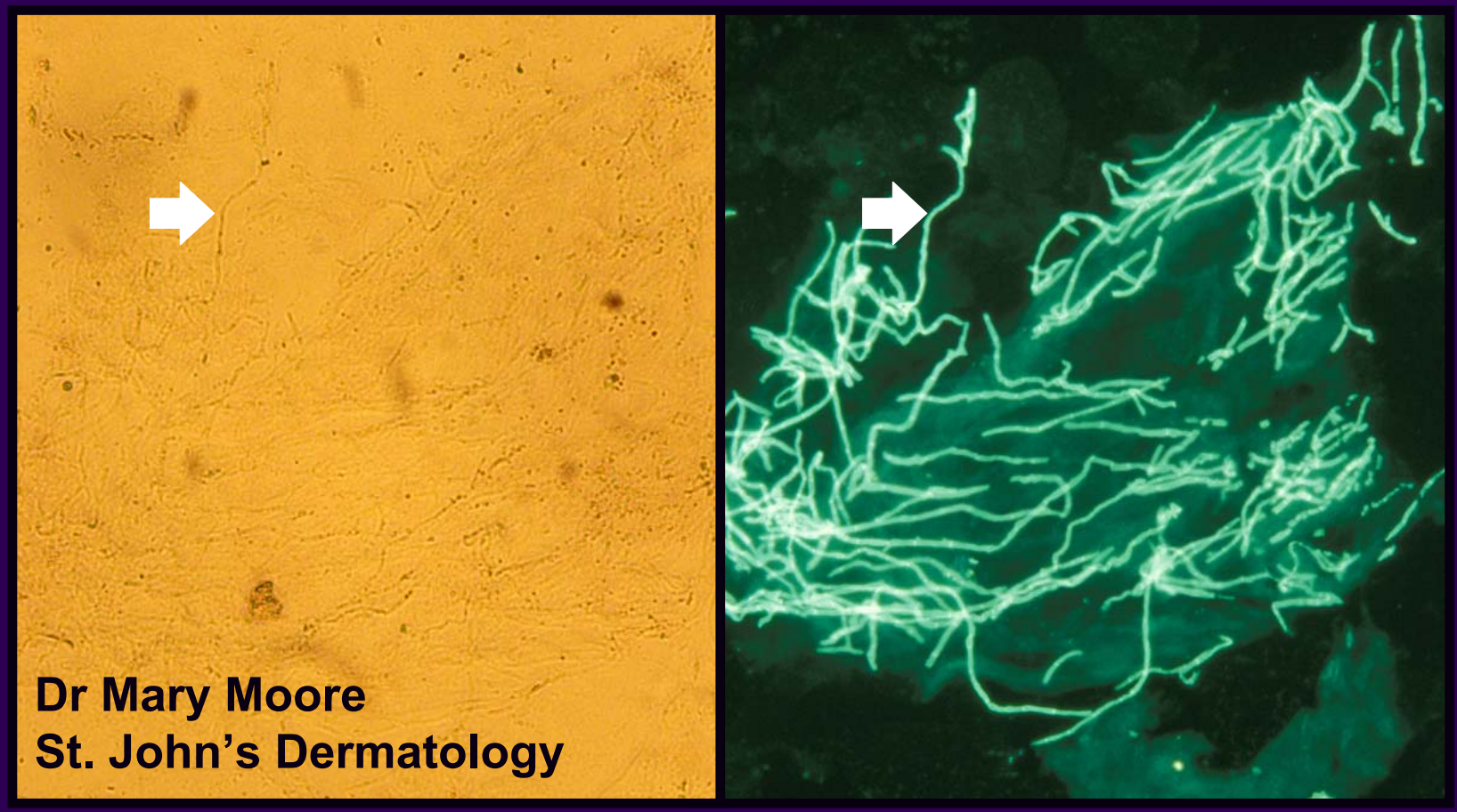
Limited to identification and susceptibility testing only

Difficulty is sending dermatophytes

The ideal QA scheme

- Identify yeasts & moulds
- Assess resistance patterns
- Microscopy of fluids, solid tissues
- Evaluate risk of an isolate to the patient
- Comment on possible therapy
- Scoring at different levels of expertise

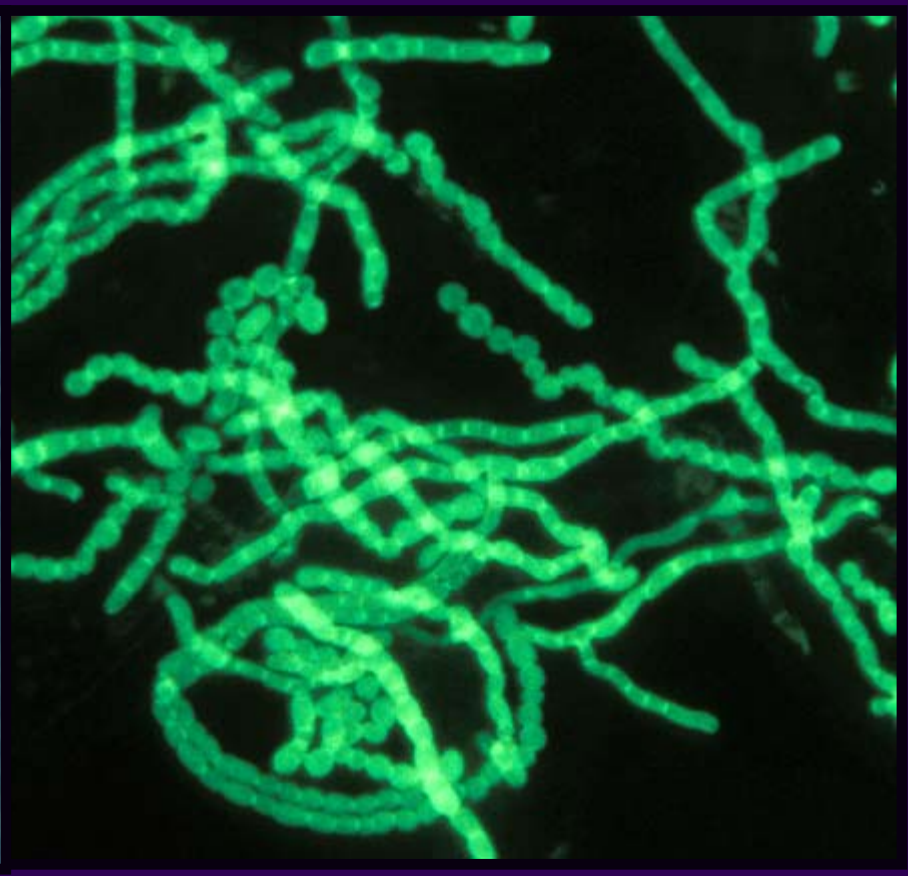
KOH squash - bright field fluorescence of nail tissue



Bright field

Fluorescence

Dermatophyte in skin - arthroconidia

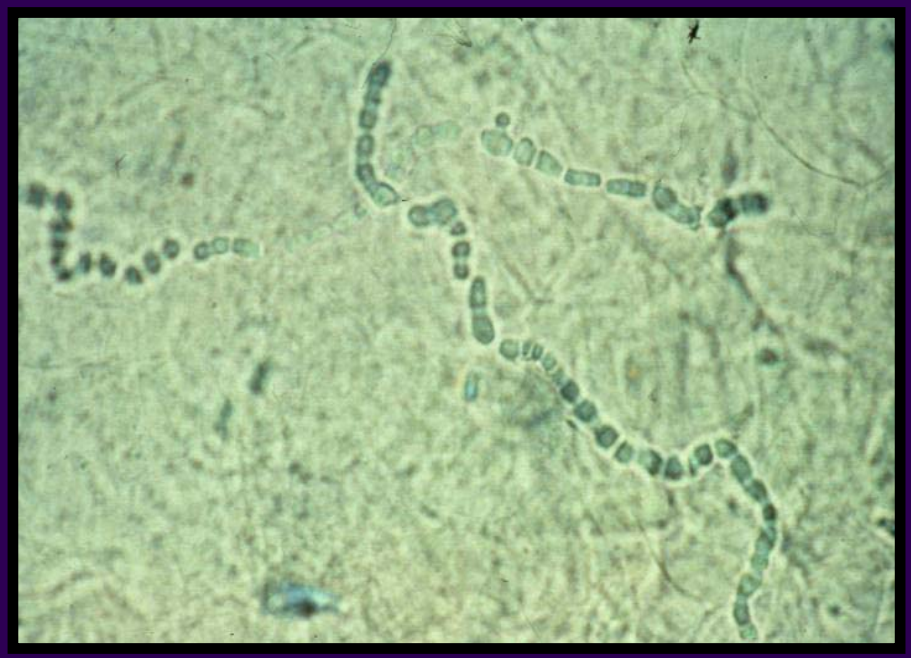


Arthroconidia are a very good indicator of dermatophytosis

KOH skin squash

Health Protection Agency

Arthroconidia



'Fungal mosaic'

Toenails

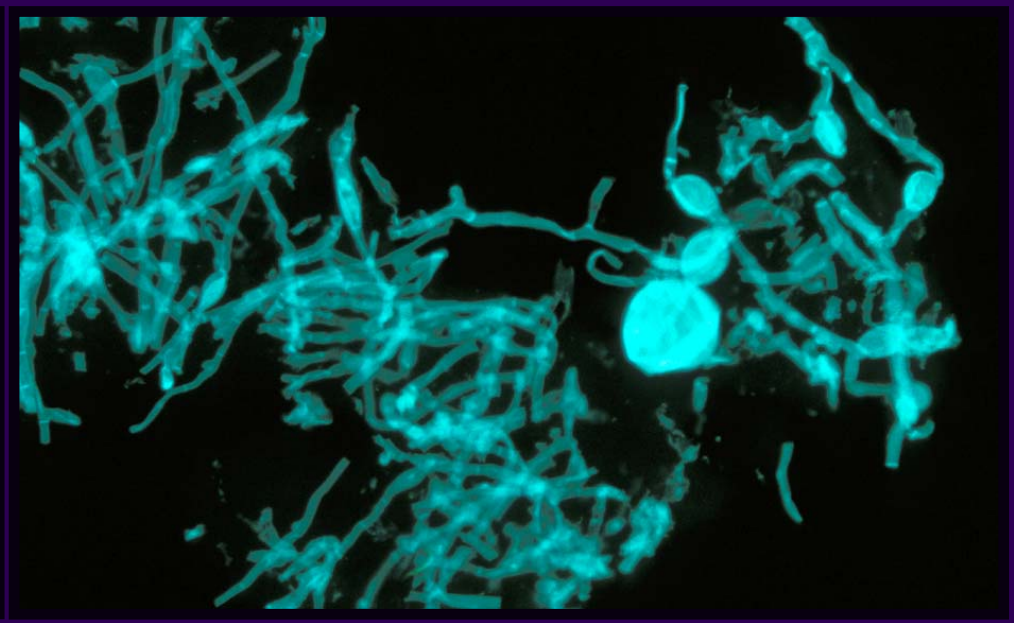
Health Protection Agency

Psoriasis

Fungal nail infection

Non-dermatophyte mould infection of nail tissue

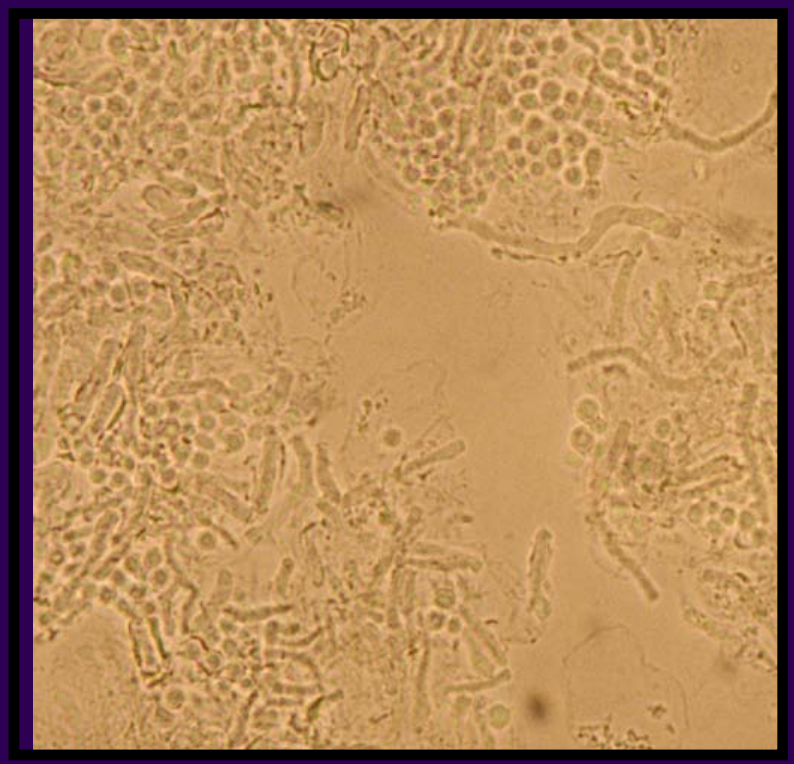
Pronounced fronds



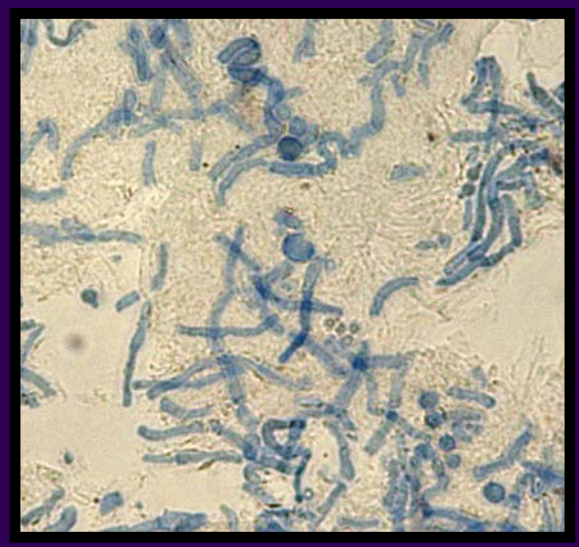
Large hyphal swelling

Pityriasis versicolor

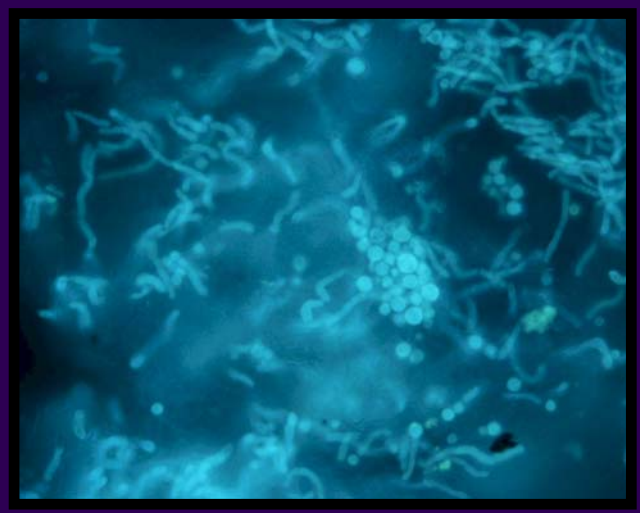
Malassezia furfur



30% KOH



Parker's stain



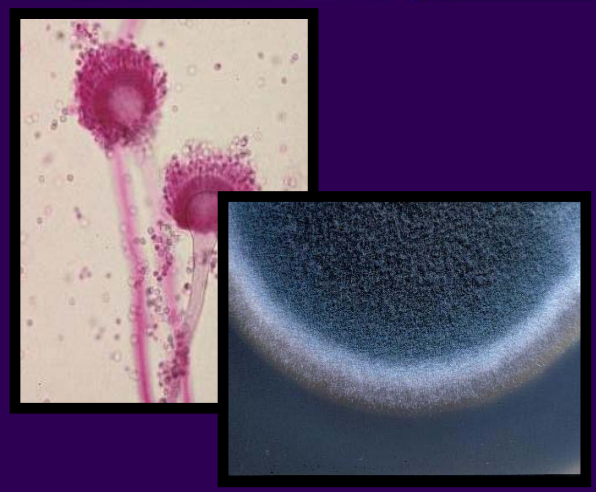
Fluorescence

Minimum species list

Yeast

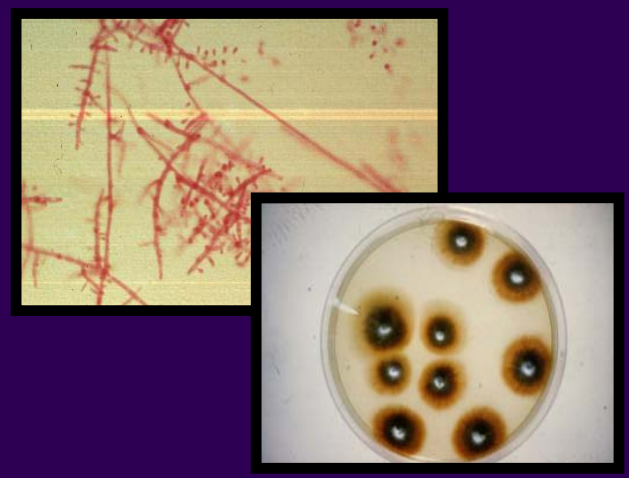
Candida albicans
C. glabrata
C.parapsilosis
C. tropicalis
C. krusei
Cr. neoformans

Mould



Aspergillus fumigatus
A. flavus
A. niger
A. terreus
Scopulariopsis brevicaulis
Fusarium solani
Scedosporium apiospermum
Rhizopus sp.

Dermatophytes



Trichophyton rubrum
T. interdigitale
T. tonsurans
Microsporum canis
Epidermophyton floccosum

Examples of scoring groups

Simple:	*Microsporum canis* *Trichophyton rubrum* *Aspergillus niger*
Advanced:	*Microsporum persicolor* *Aspergillus versicolor*
Genus only:	*Acremonium* *Alternaria*
Educational:	*Onycochola canadensis* *Cunninghamella bertholletiae*

Procedure

- 3 distributions per year
- 4 strains per distribution - dermatophyte
  other moulds
  yeast (now also susceptibility)
- Clinical information composed
- Several strains of each examined
- Yeast: subjected to commercial identification methods
  - AUXACOLOR, API and cornmeal agar plate
  - genomic sequences D1-D2 and ITS1 and 2
  - susceptibility - 2 ref labs , NCCLS (CLSI) strains
- Moulds: gross colonial and microscopic examination
  - genomic sequences
- Strains freeze-dried then re-examined before distribution and photographed for website

‘Referee’ laboratories

The 50 laboratories achieving the highest cumulative scores in the EQA specimens distributed during the previous year selected by computer

> 80% of these correct - included in EQA scoring

< 80% of these correct - excluded from EQA scoring

Susceptibility testing: not scored if not 80% consensus

Trichophyton tonsurans

Distribution	Correct ID (% labs)
1987	44
1989	62
1993	59
1997	78
2002	65
2008	33

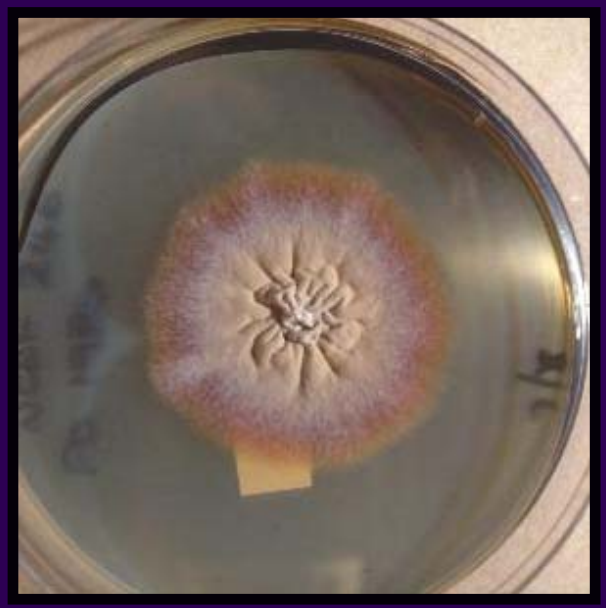
Incidence in UK survey

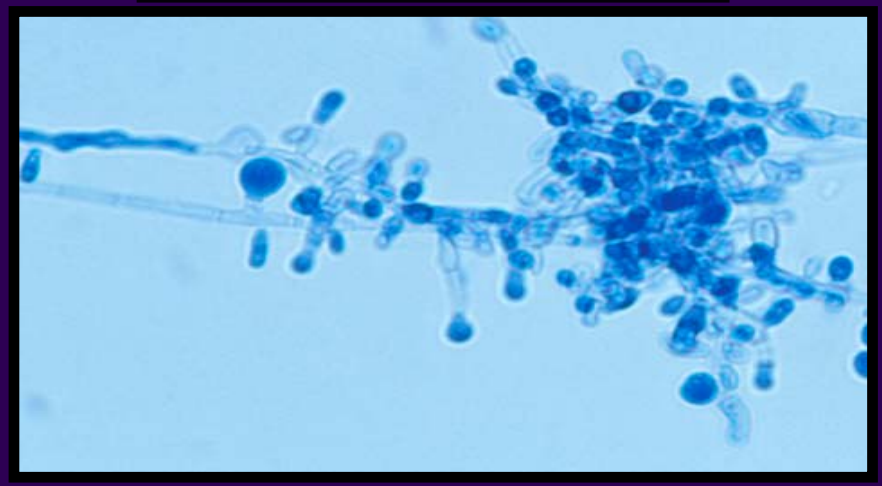
1980: 17 cases (0.3%)

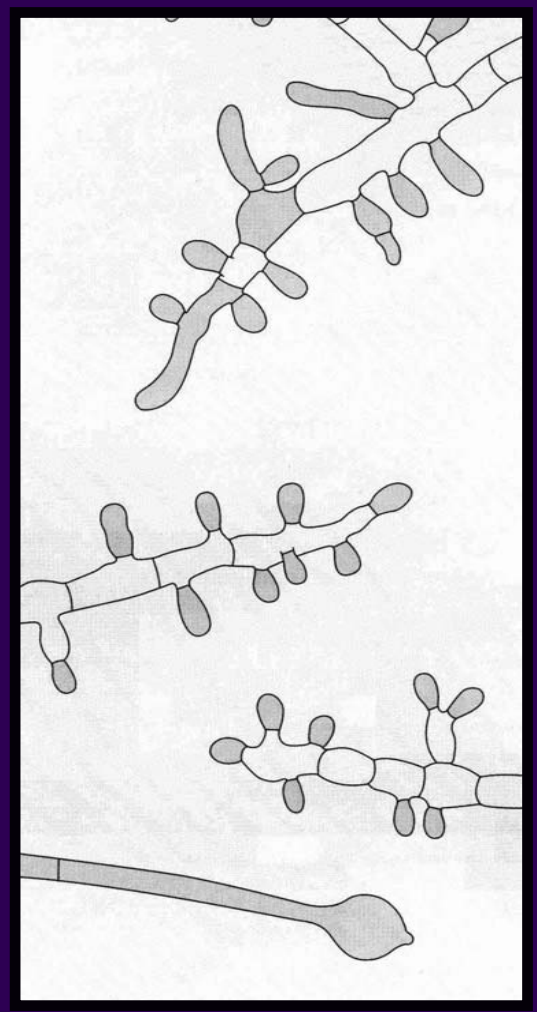
2000: 1227 cases (4.6%)

Poor general performance with a particular specimen leads to the inclusion of an educational resume on specific identification features for that species

Trichophyton tonsurans



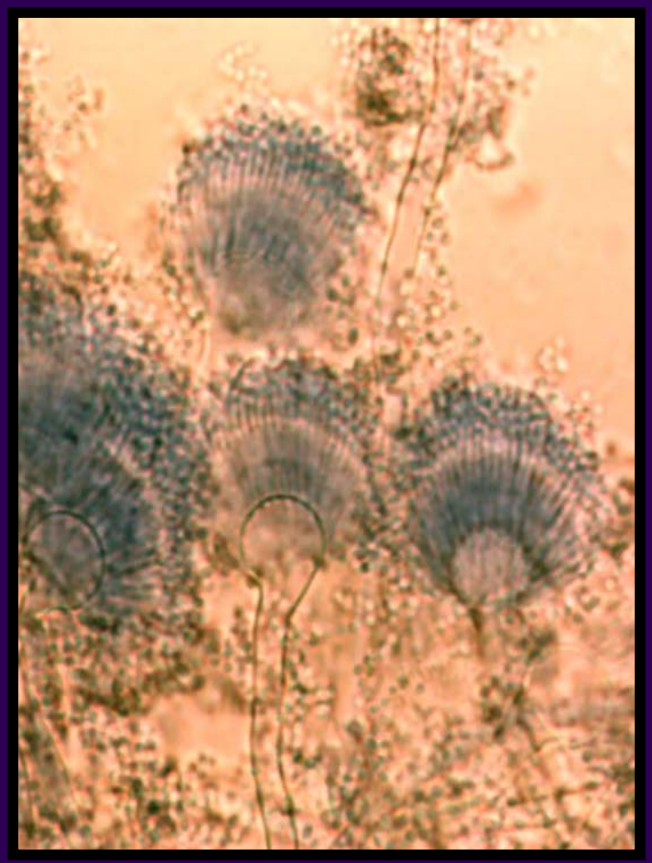


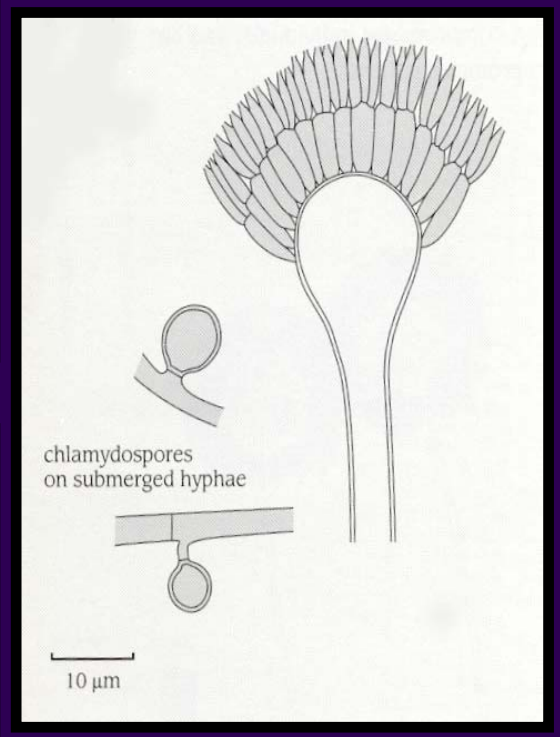


Aspergillus terreus

Distribution	Correct ID (% labs)
1989	63
1992	79
1994	91
1997	94
1999	97
2002	93

Aspergillus terreus culture and microscopy



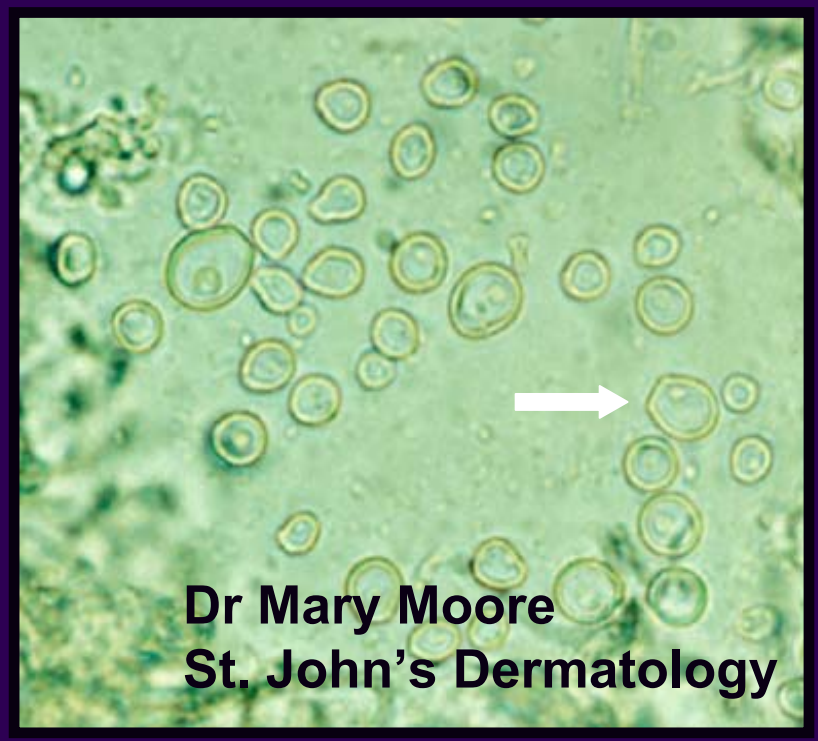


Scopulariopsis brevicaulis

Distribution	Correct ID (% labs)
1990	68
1992	82
1996	88
2000	88
2004	97

Scopulariopsis brevicaulis onychomycosis

Grossly hyperkeratotic nail with brown discoloration



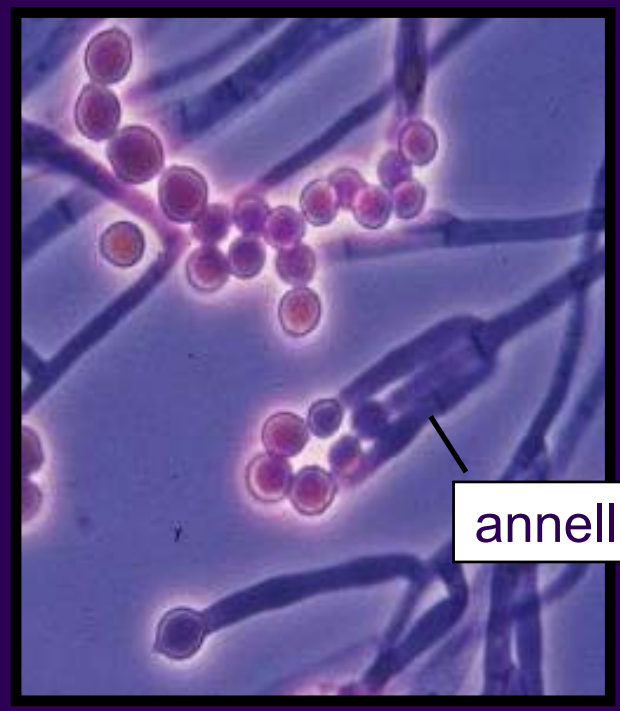
Lemon-shaped conidia in the nails have thick, bright, refractile walls and a truncate base

Scopulariopsis brevicaulis

Culture

On cycloheximide free medium, colonies have a powdery brown surface

Microscopy



Chains of rough-walled annelloconidia are formed in basipetal succession

Candida parapsilosis

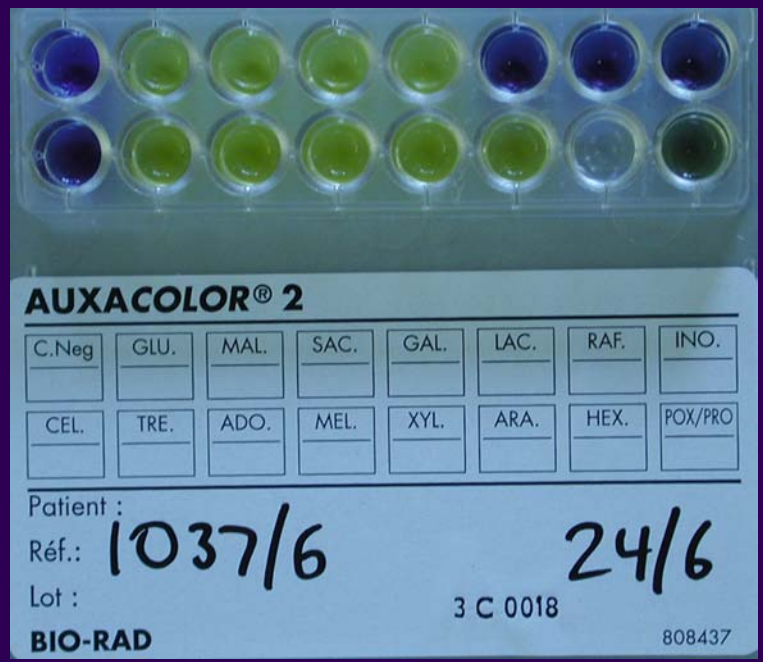
Distribution	Correct ID (% labs)
1987	66
1992	82
1995	92
1997	91
2001	75
2004	93
2008	92

Common mis-identifications:

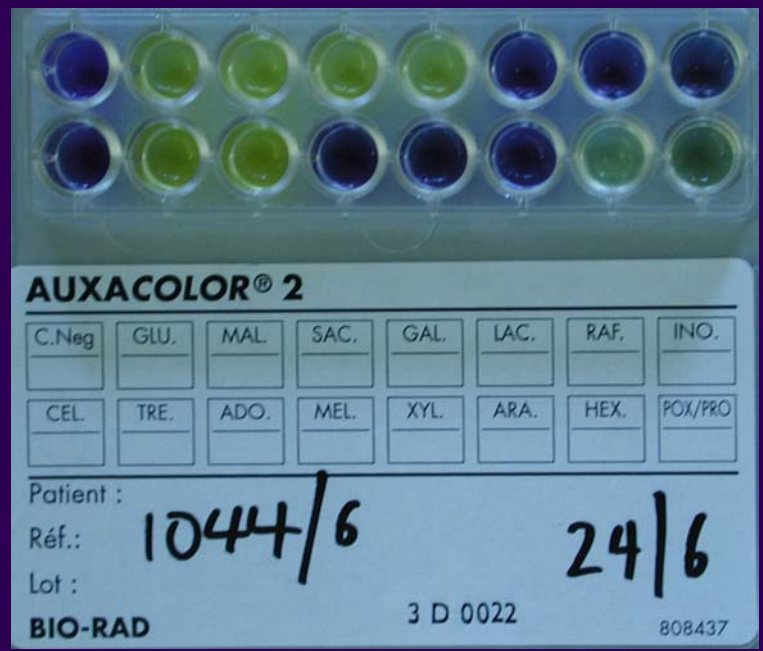
Candida albicans 6; *C. famata* 2; *C. krusei* 5; *C. sake* 3

Commercial kit for yeast identification
Auxacolor 2

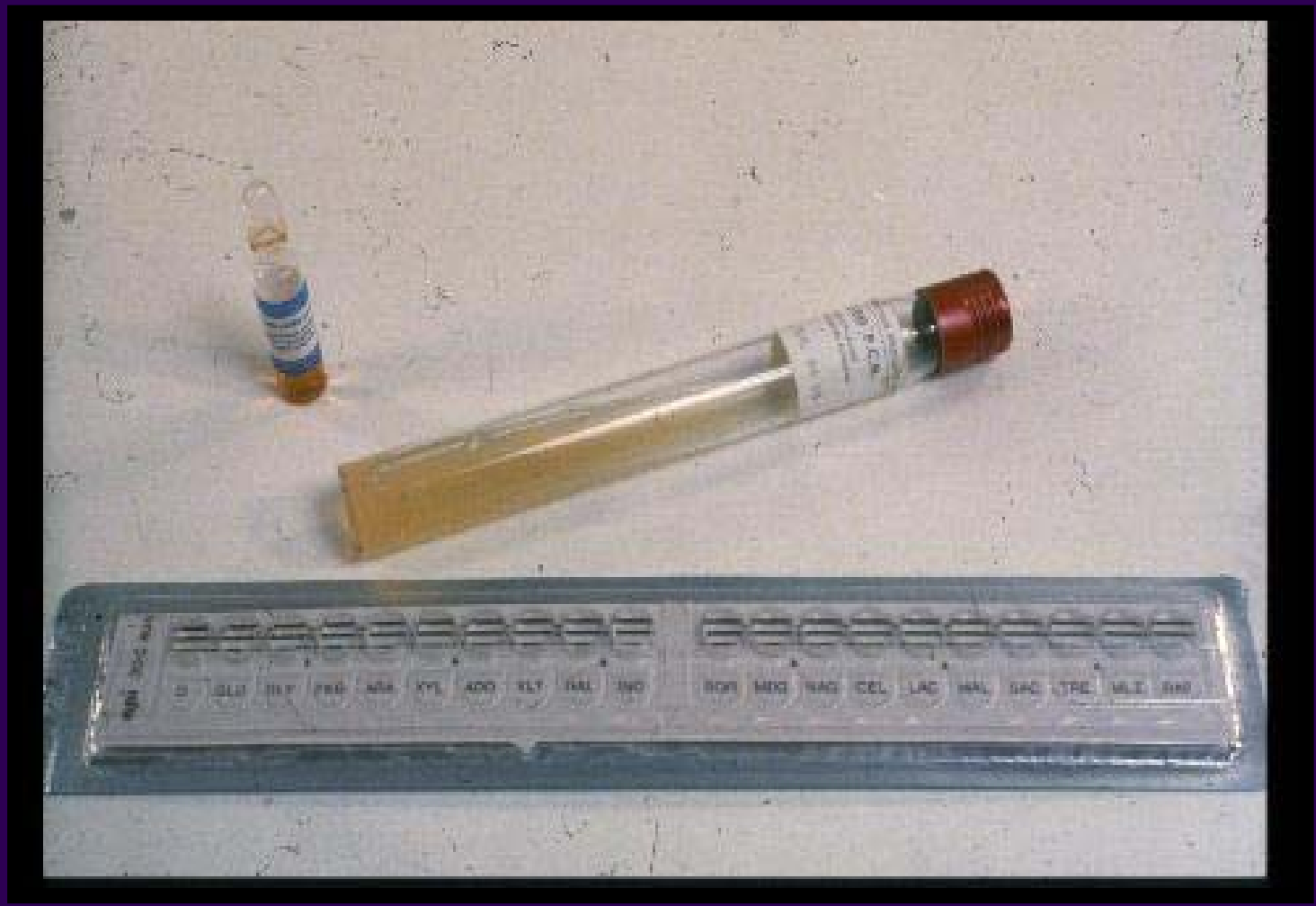
C. parapsilosis



C. dubliniensis

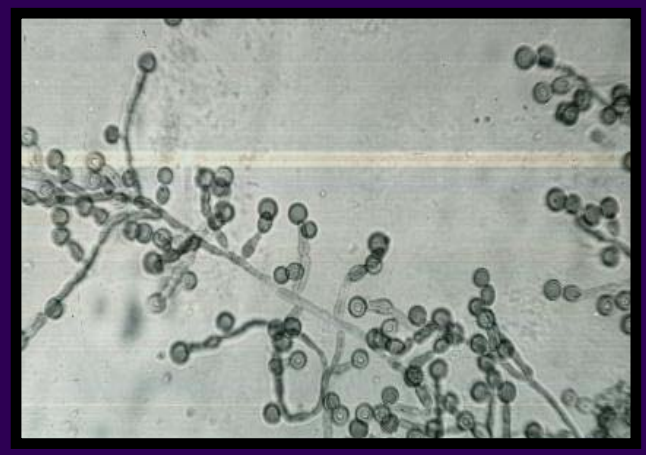


Commercial kit for yeast identification
API (20C 32 !D)



The cardinal rule for yeast identification

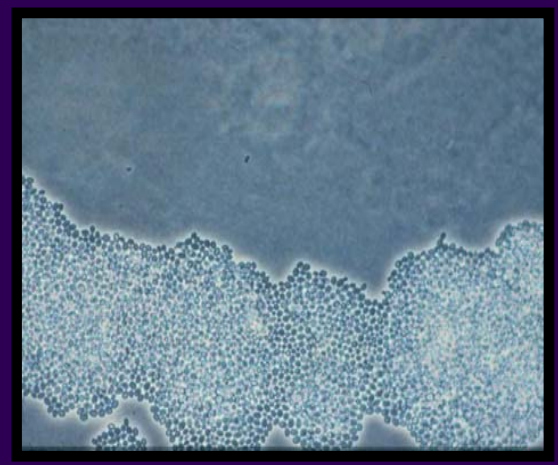
Look at it under the microscope



Candida albicans

Candida parapsilosis

Candida glabrata



The importance of clinical details /travel history/past infections

CASE 1

June 2005: 'Yeast from blood culture for ID and susceptibilities'

Patient: anonymised, no DOB, no clinical details, travel etc.

GUM clinic number - referring lab ?*Cryptococcus* so HIV +ve??

Initial observations at MRL in Cat III - Yeast compatible with *Cryptococcus*, plus ? second smaller yeast

Attempts to purify 2nd yeast failed, *Cryptococcus* processed for ID (Auxacolour plus Dalmau plate) and susceptibilities, plus molecular confirmation of ID

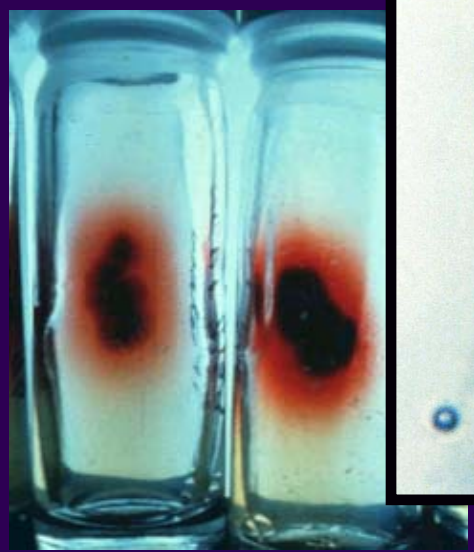
Confirmed as *C. neoformans*, full susceptibilities established

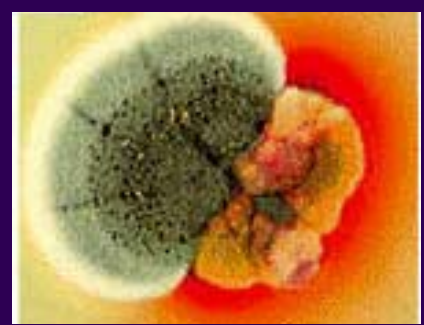
Case 1 continued…….

However, noticed t t on microscopy of Dalmau plate few *F* 3-4 weeks) post receipt, the original plate of angely dark pink in areas.

Needle mounts of p udimentary phialides

2nd "isolate" confirm





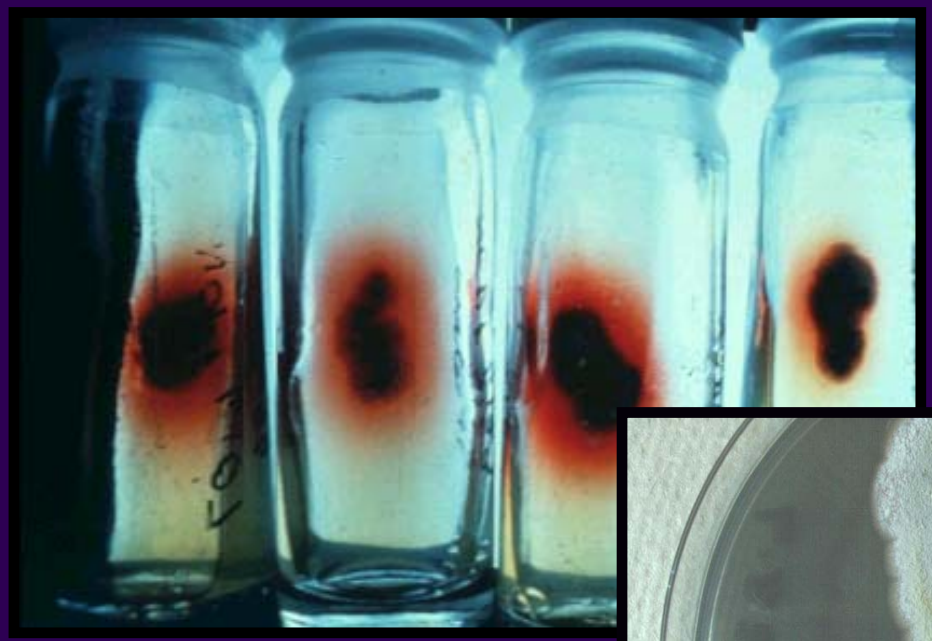
Case 1 continued…….

After contacting the referring lab, who elicited further clinical details confirmed that patient was young, HIV+ and had recently returned from extensive travelling in Thailand

Could this have been avoided ? Probably
Any yeast from B/C from an HIV + patient with links to Thailand would automatically be treated as a potential dimorphic pathogen (*P. marneffei*)

Picture confused by presence of a second pathogen

Penicillium marneffei



mould form at
30ºC or less

yeast form at 37ºC

Spring 2008 Distribution

Penicillium aculeatum

No growth (or conversion) at 37°C

Phialides too long, delicate (*P. marneffei* 6-8μm long, NEQAS isolate 10-14μm)

P. marneffei is predominantly biverticillate

Case 2

12th March 2008: 'Candida sp.' on slope from blood culture
'?septicaemia'

49 year old male

Processed for ID and susceptibilities

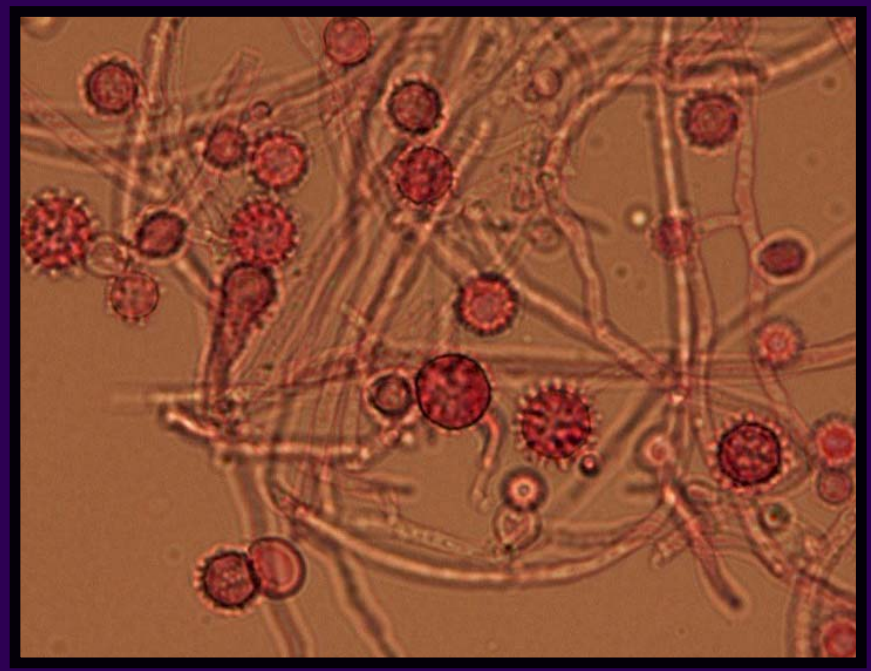
17th March 2008: V. poor growth in susceptibility tests (35°C)
Standard procedure to incubate such yeasts at room temp. in addition to 35ºC
- mould colony observed after 4 days

Moved all material to CAT III
– Confirmed as *Histoplasma capsulatum*

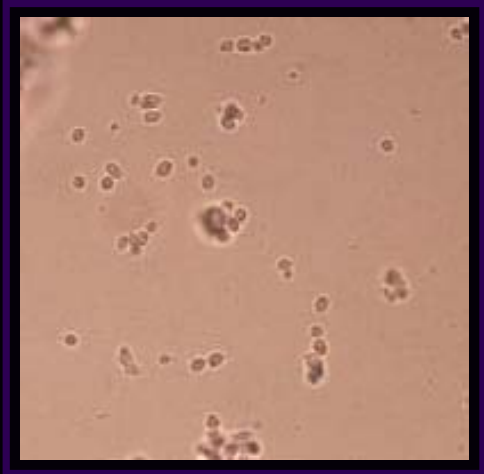
Histoplasma capsulatum

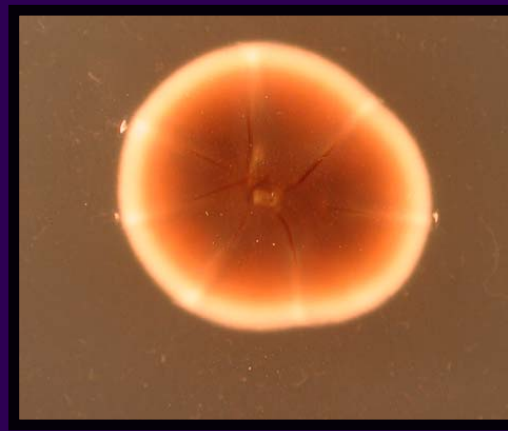
Health Protection Agency

Mycelial form

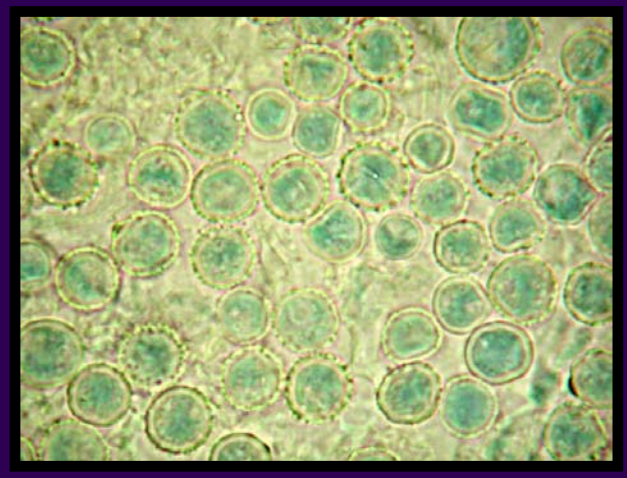


Yeast form

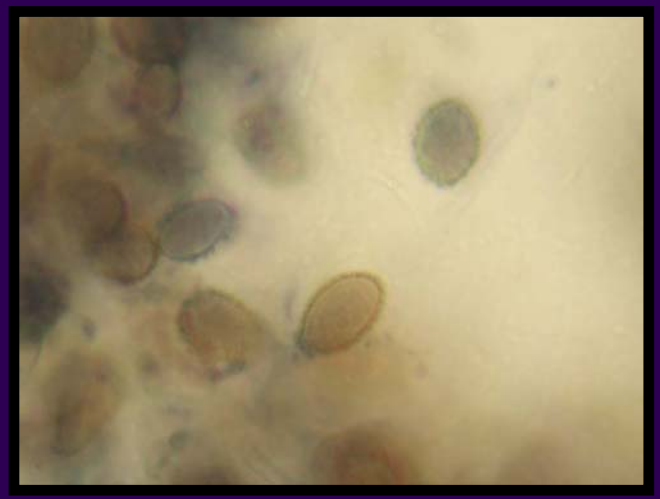




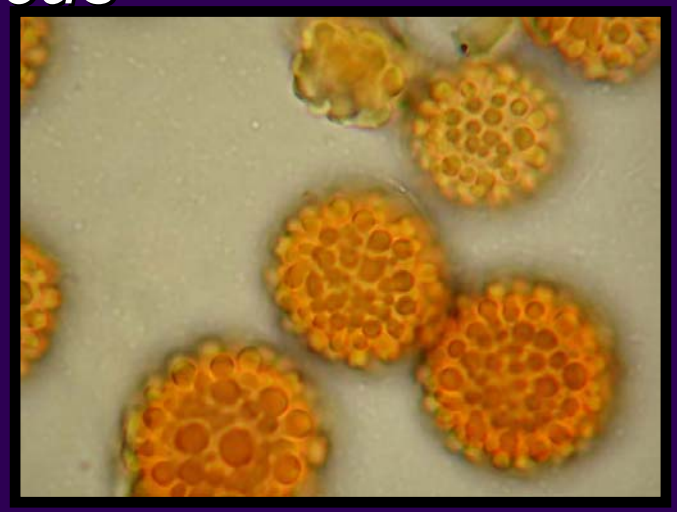
Histoplasma capsulatum macroconidia look-alikes



Corynascus



Ctenomyces



Sepedonium

Case 2 continued……

Contacted referring lab/clinicians:

Reminder – received at MRL 12/3/08 ?septicaemia

Patient admitted to ICU with pneumonia on 17/2/08; died on 26/2/08 !!!

HIV status remained unknown, no travel history had been taken

Referring lab had maintained organism at 35^{o}C or above – so unlikely to have been significant exposure to the infectious mould form. Verified correct disposal of materials/ surveillance of staff.

Subject of RIDDOR and HSE reports.

Could this have been prevented ???

Cases 3 and 4 (primary impact on referring labs, MRL involvement was for ID, advice on prohylaxis/infection control)

Case 3: Microbiology Lab received BAL from a 59 y/o female
OP specimen - 'Previous fungal infection'
no further details or travel history

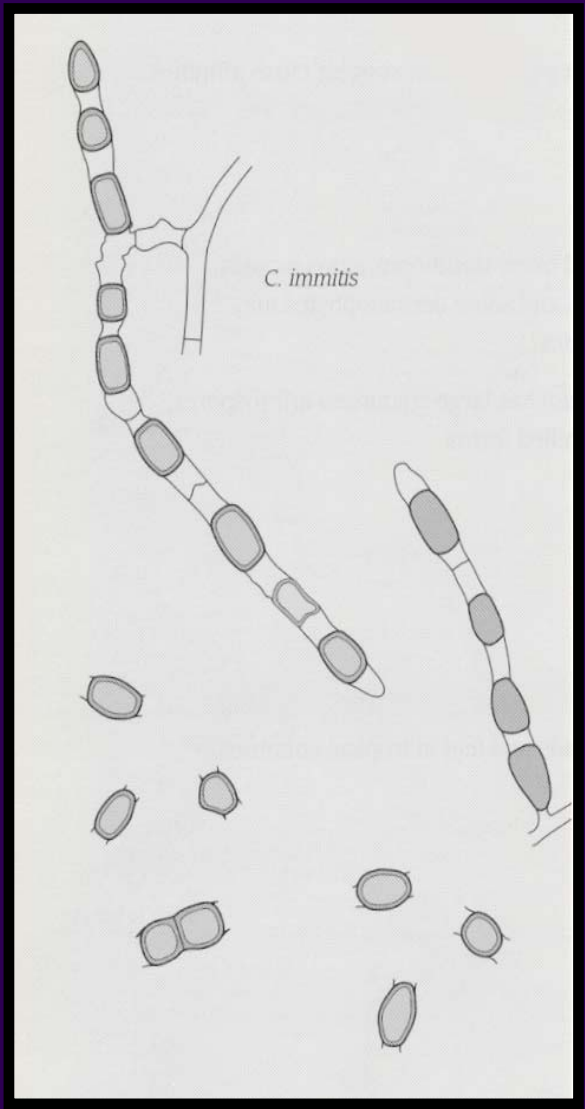
Please don't do this !!

Processed BAL in hood - when only a white mould grew this was brought out to be examined on the open bench!!!

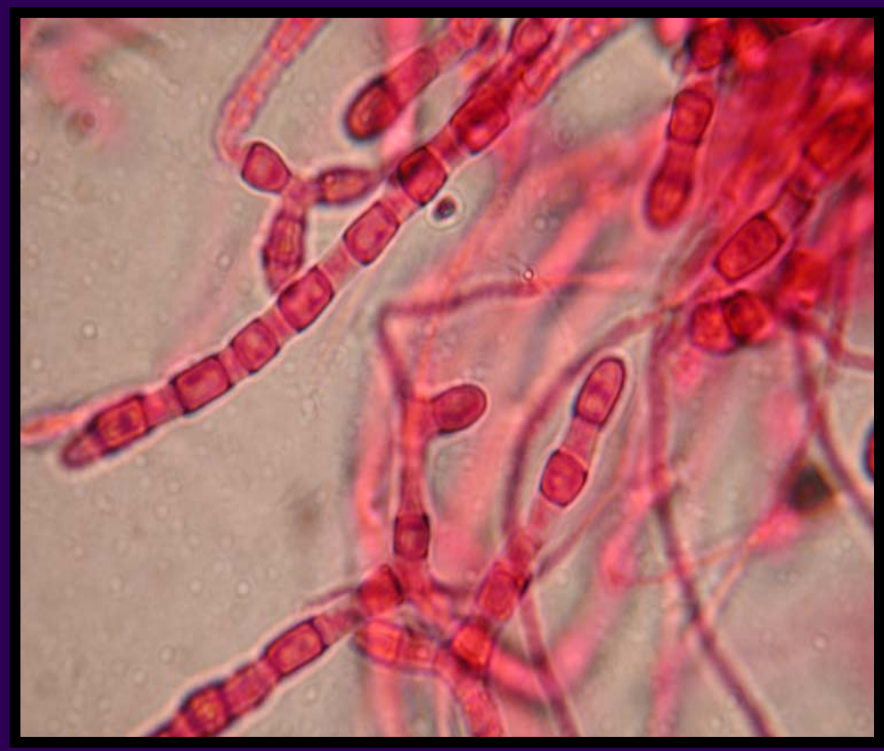
Arthrosporic mould
Microscopy consistent with *Coccidioides immitis*

Referred to MRL - Confirmed as *C. immitis* by microscopy and specific exo-antigen test

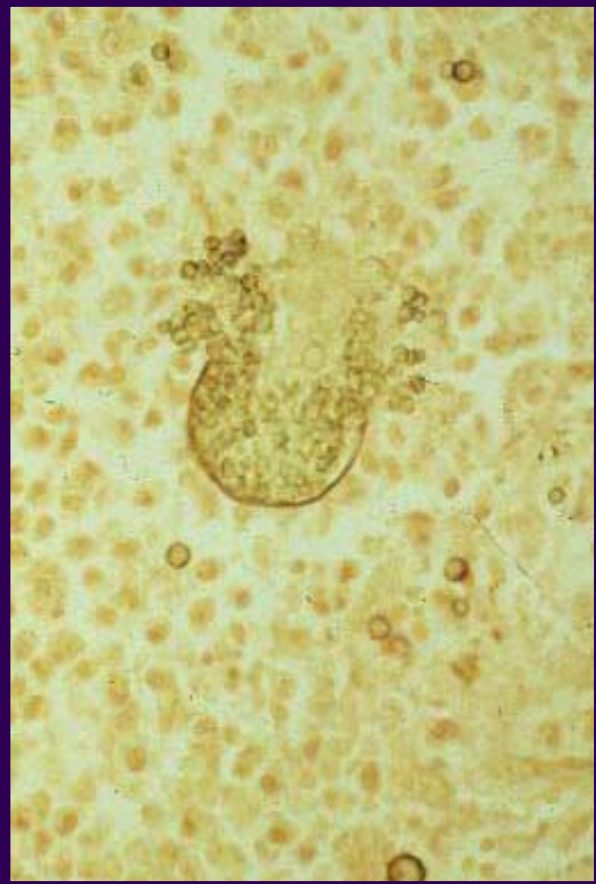
Coccidioides immitis



Arthroconida formation
Cells split by rhexolysis
Cell wall material left on spore



Coccidioides immitis life cycle



Case 3 continued……..

Retrospectively, emerged that the previous IFI had been confirmed as coccidioidomycosis in USA
Appropriate links to endemic area

Could this have been prevented??

Probably - if full clinical details had been supplied

Consequences:

HSE reports

Total deep clean - infectious dose is ONLY 10 arthroconidia!

4 staff most at risk in regional lab – itraconazole prophylaxis for 6 weeks to cover potential incubation period

Case 4

May/June 2008: Regional Lab received a CSF from 66 y/o female
'meningeal enhancement'

White mould cultured 'tried to ID, but no spores'
Referred to MRL

Received in MRL 6th June
Mould from sterile deep site - ∴ handled in Cat III

16th June – initial cultures grown sufficiently – no obvious structures. Sub-cultured to additional media

24th June - Athrosporic mould, typical of *Coccidioides immitis*
Immediately alerted Regional Lab.

Confirmation by rRNA gene sequencing 3/7/08

Case 4 continued…….

Regional Lab unaware of any relevant travel history. However, patient had a previous diagnosis and chemotherapy for nasal carcinoma

MRL contacted by regional lab with additional information. Although the patient didn't have relevant 'travel history' involving an endemic area, she lived for 6 months each year in her second home in Arizona!!!!!!!!!!

Could it have been avoided?

Consequences: HSE - RIDDOR report for referring lab
Deep clean
Prophylaxis – 6 staff; fluconazole for 6 weeks

MRL procedures for organisms from deep sites

All moulds isolated or received from deep sites processed in class 1 hood in Cat III UNTIL they are proven NOT to be Hazard Group 3 pathogens - sputum, BAL, blood, tissue, skin biopsies, CSF, abscess fluid etc. + all isolates without site specified – many dermatophytes processed unnecessarily in Cat III

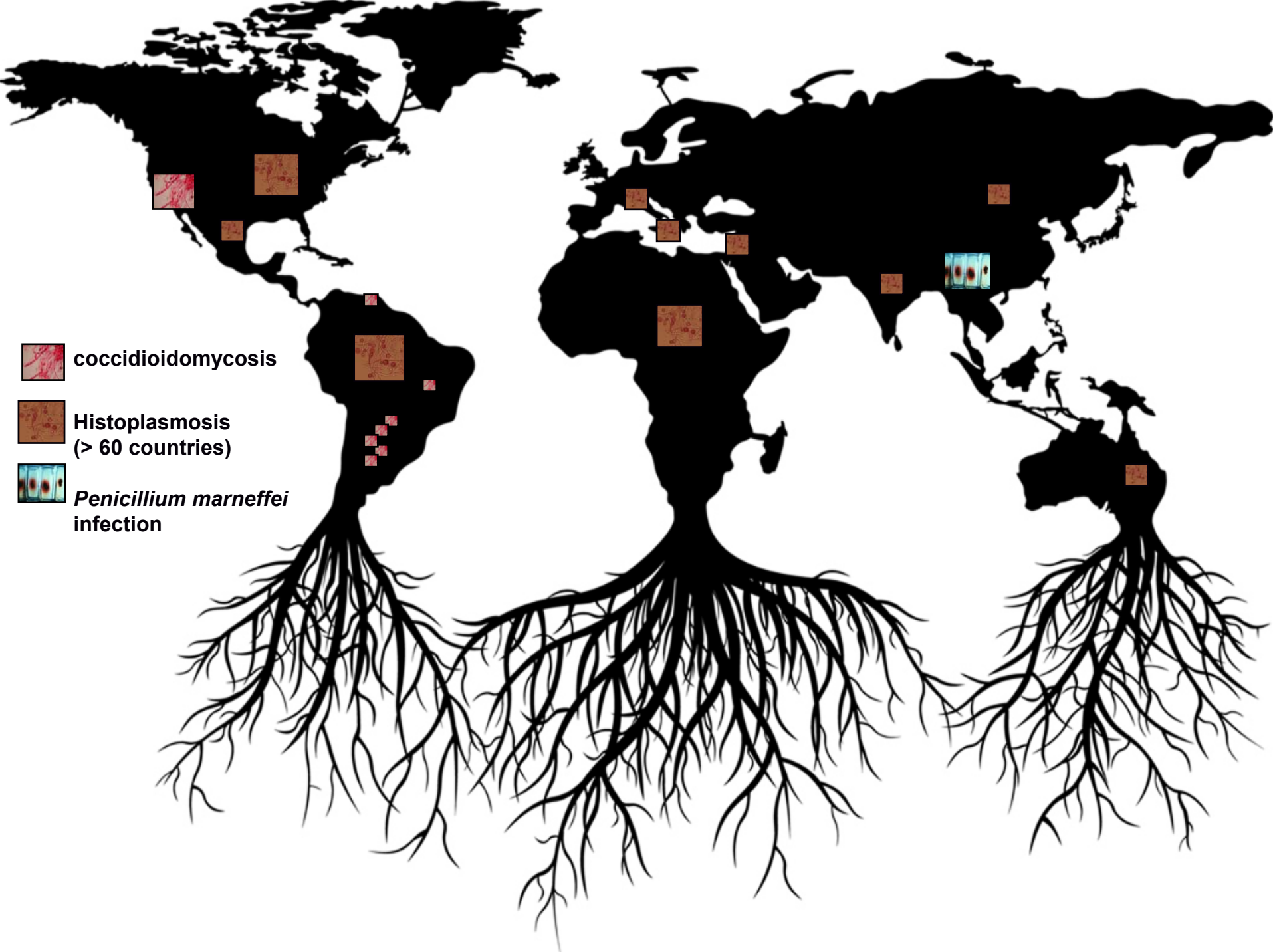
Yeasts from blood cultures if dimorphic pathogen is suspected

Warning signals:

Travel to: Arizona (*C. immitis*)
S. America (*H. capsulatum, B. dermatitidis, P. brasiliensis*)
Thailand (*P. marneffei*)
S. Asia (*C. bantiana*)
Middle East esp. Saudi Arabia (*R. mackenzei*)

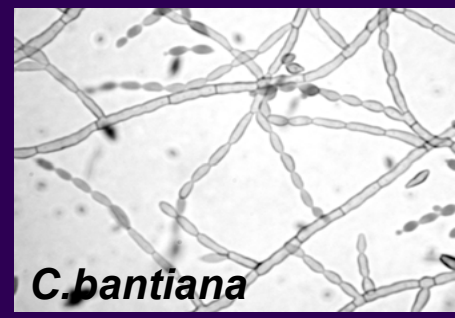
Caving (*H. capsulatum*)

HIV+ve (esp. *H. capsulatum; P. marneffei*)

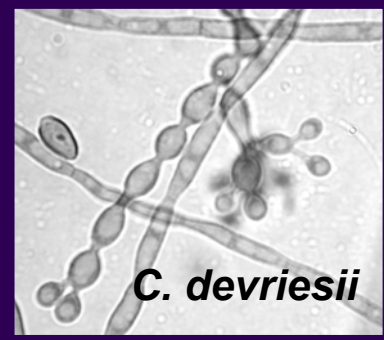

coccidioidomycosis
Histoplasmosis
(> 60 countries)
Penicillium marneffei
infection

Hazard Category 3 pathogens received / identified at MRL 2004 - 2008

2 x *Cladophialophora bantiana*
– (1 x renal infection cat, first isolation in UK)


C.bantiana

1 x *Cladophialophora devriesii*
– human chromoblastomycosis
First UK isolation. Only 2nd human case worldwide.


C. devriesii

4 x *Coccidioides immitis*

25 x *Histoplasma capsulatum*

2 x *Penicillium marneffei*

1 x *Ramichloridium mackenzei*
(now *Rhinocladiella mackenzei*).


R. mackenzei

Major changes in taxonomy

A higher-level phylogenetic classification of the *Fungi*

Mycological Research 111 Part 5 (2007) 509 - 547

Hibbett *et al.* (= 67 authors!!! - major taxonomic effort involving collaboration of the great and the good in fungal taxonomy)

A major and comprehensive phylogenetic classification of the kingdom *Fungi*

Supported by AFTOL - Assembling the Fungal Tree of Life

KEY changes

The revised classification accepts:

1	kingdom	*Fungi*
	(invalid name until 1980 as missing Latin diagnosis)	
1	sub-kingdom	*Dikarya* (new)
7	phyla	suffix: *-mycota*
10	sub-phyla	suffix: *-mycotina*
35	classes	suffix: *-mycetes*
12	sub-classes	suffix: *-mycetidiae*
129	orders	suffix: *-ales* (19 new)

All scientific names regardless of rank should be *italicised*

KEY changes

~~Deuteromycetes~~
~~Fungi Imperfecti~~

The most dramatic shifts in classification relative to previous works include the groups that have previously been classified in the basal fungal lineages *Chitridiomycota* and *Zygomycota* which have long been recognised as being polyphyletic

Zygomycota not accepted
- first published 1954 without a Latin diagnosis .:. invalid

Now distributed between:

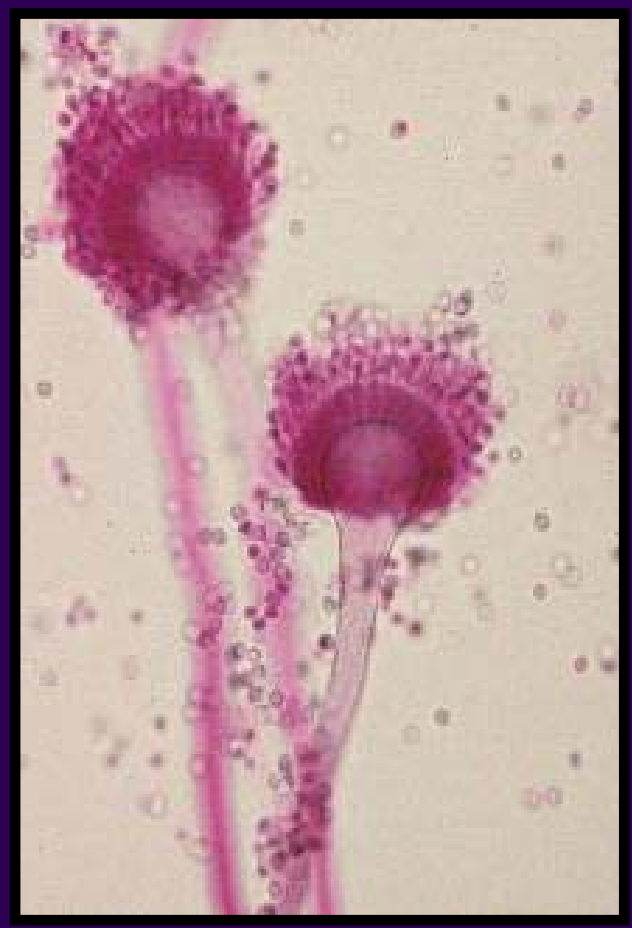
Glomeromycota
Mucoromycotina subphylum nov. (most pathogens)
Entomphthoromycotina
Kickxellomycotina
Zoopagomycotina

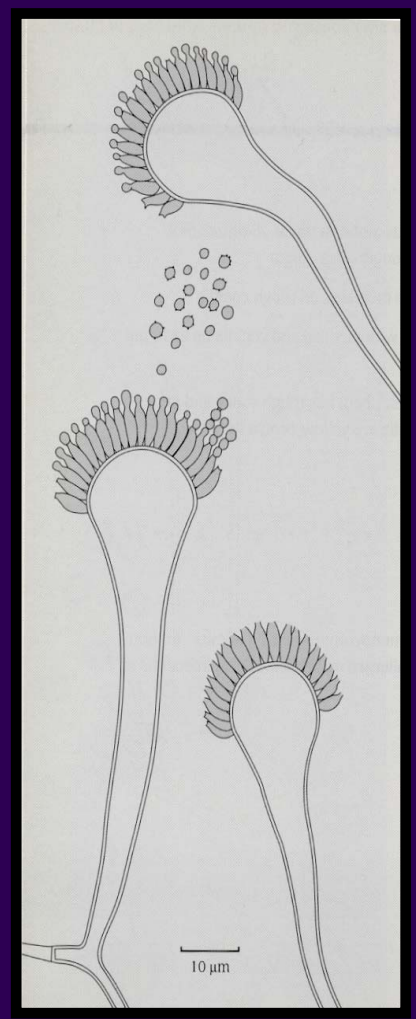
Mucoromycosis??

Zygomycota may be resurrected and validated when relationships are more clearly resolved

Aspergillus fumigatus complex

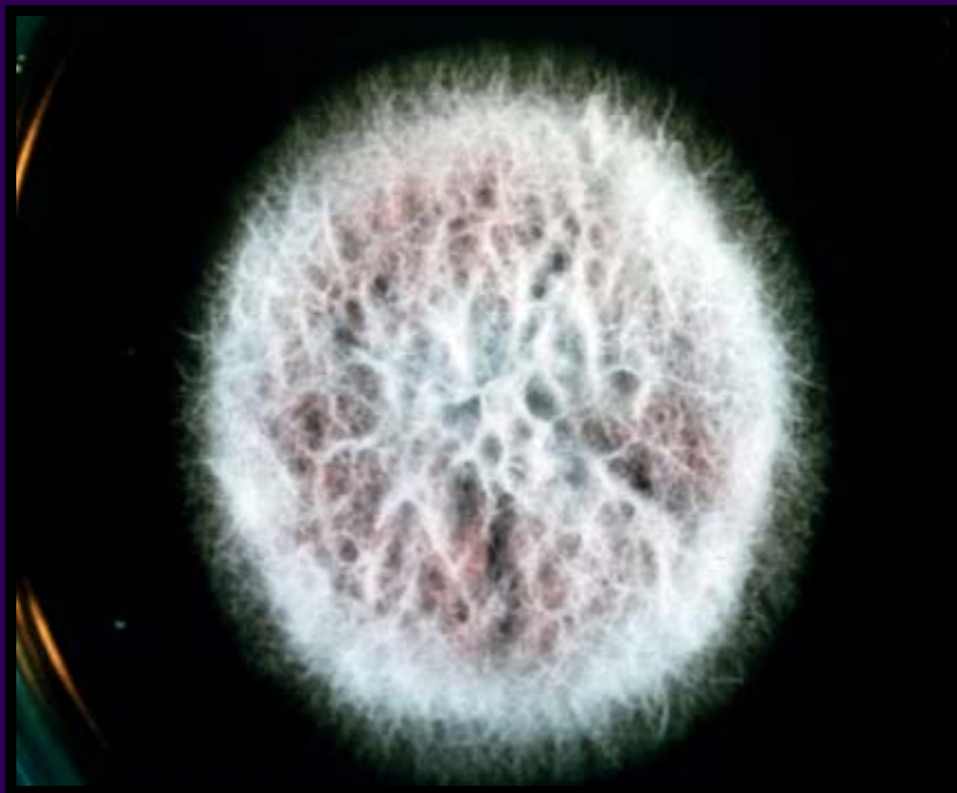
Review of the literature reveals that *Aspergillus* section *Fumigati* comprises in excess of 29 species of *Neosartorya* and 14 *Aspergillus*

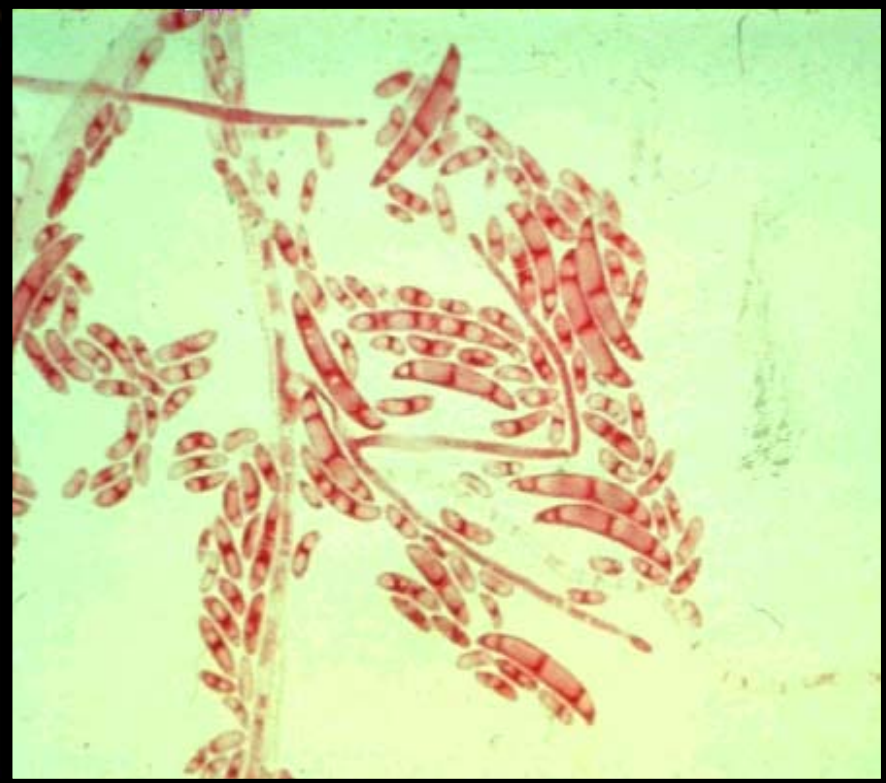




Fusarium solani ×

Fusarium solani species complex





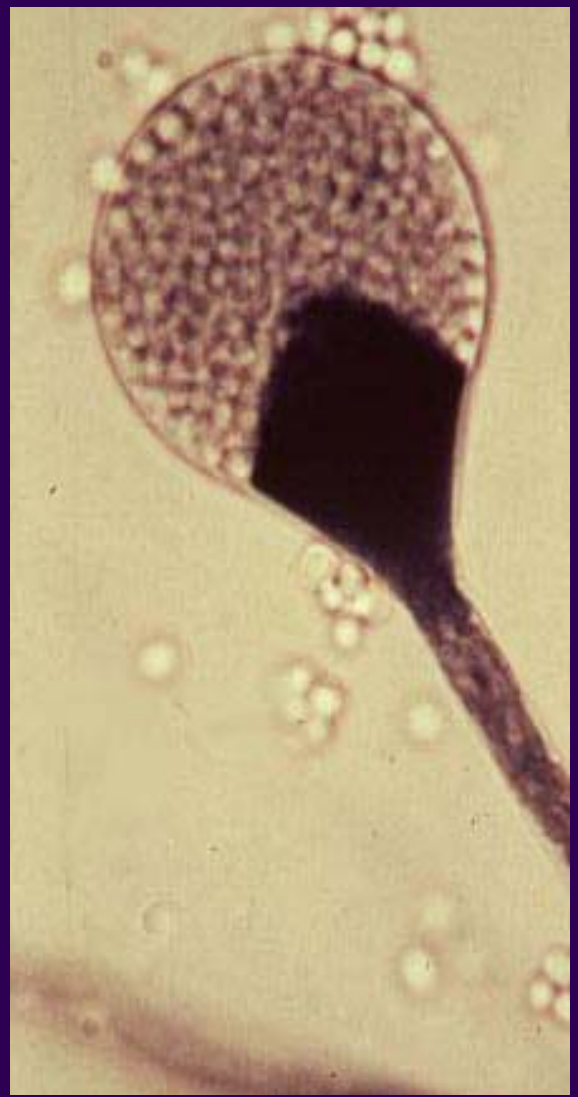
Species complex contains at least 45 different species
At least 20 implicated in infection

Absidia corymbifera

↓

Mycocladus corymbiferus

Health Protection Agency



NEQAS Mycology EQA

- ➔ Voluntary participation but CPA requirement
- ➔ Scoring system to help labs assess their own performance
- ➔ No penalties - educational not punitive
- ➔ Advice available by ‘phone, email or letter after each distribution
- ➔ Help offered to persistent low scorers

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Identification of
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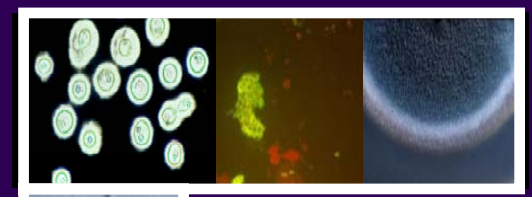

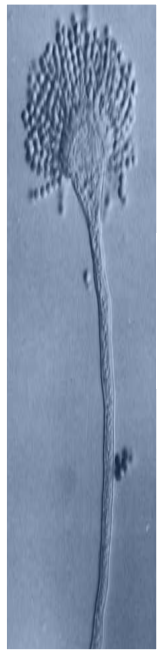

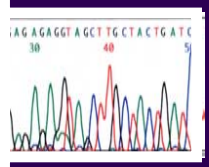

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