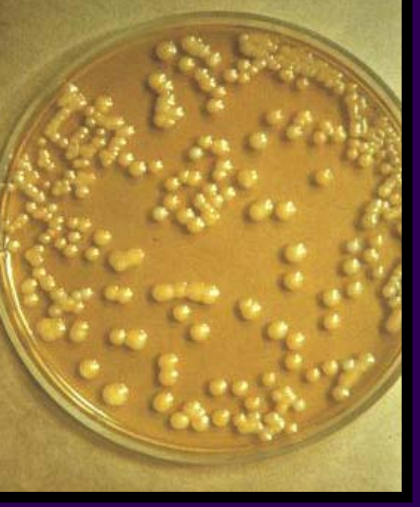
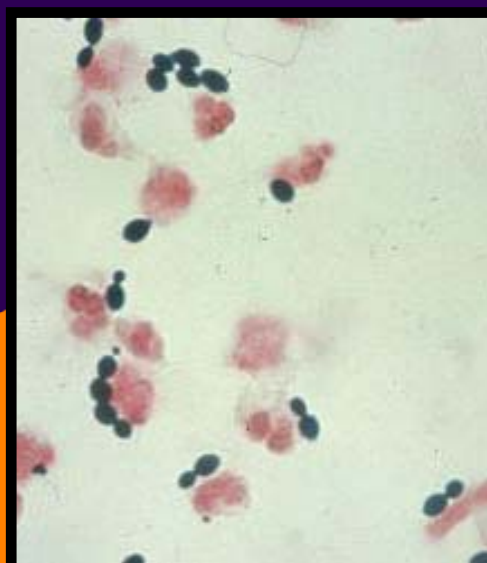


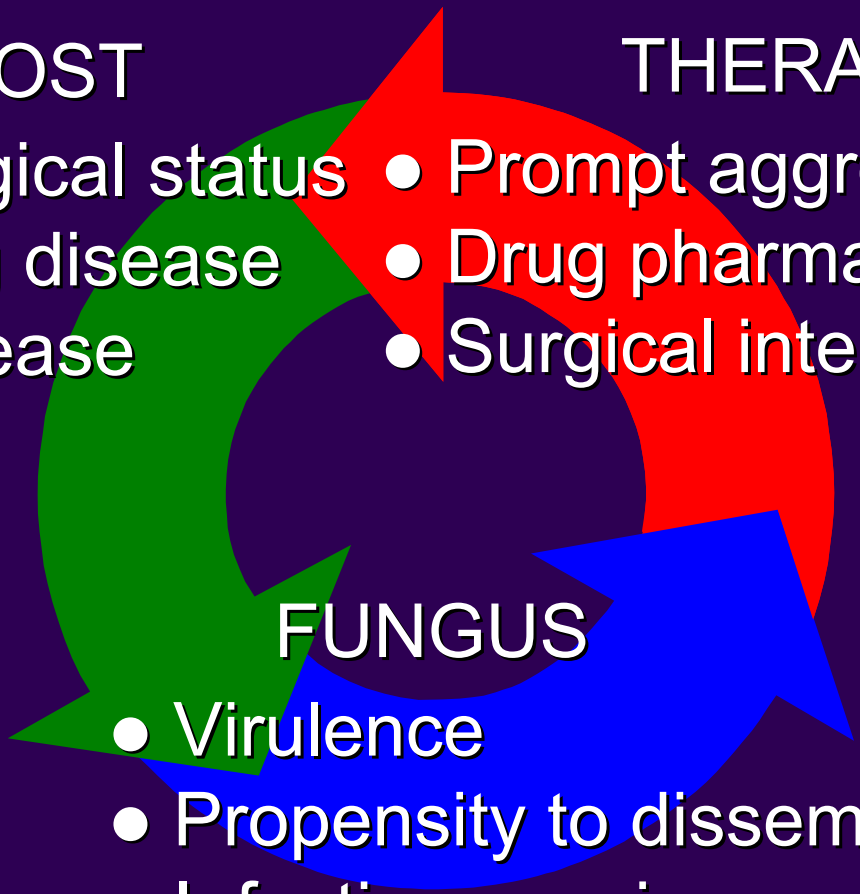
Fungal Update
- antifungal susceptibility testing

Dr Elizabeth M. Johnson
Director, HPA Mycology Reference Laboratory, Bristol, UK

London November 2008



Factors affecting outcome of IFI



HOST

- Immunological status
- Underlying disease
- Site of disease

THERAPY

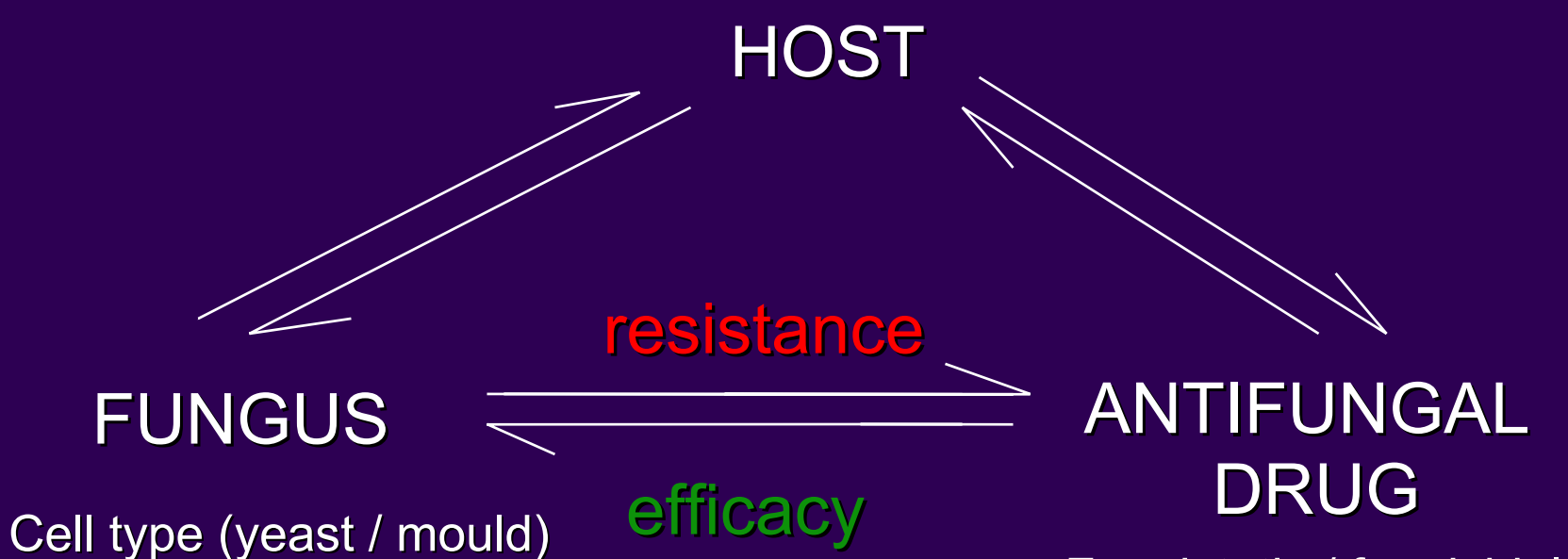
- Prompt aggressive therapy
- Drug pharmacokinetics
- Surgical intervention

FUNGUS

- Virulence
- Propensity to disseminate
- Infecting species
- Drug resistance

Factors influencing the outcome of antifungal therapy

Van t' Wout J. 1996



Cell type (yeast / mould)
Genomic stability
Size of population
Biofilms

Fungistatic / fungicidal
Dosing
Pharmacokinetics
Drug interactions

Minimum Inhibitory Concentration (MIC)

‘The MIC predicts how best to treat an infected test tube’

H.B. Levine

Susceptibility testing - issues

Format - disc test / agar incorporation / broth dilution

Inoculum standardization

Medium / drug solvents

Temperature

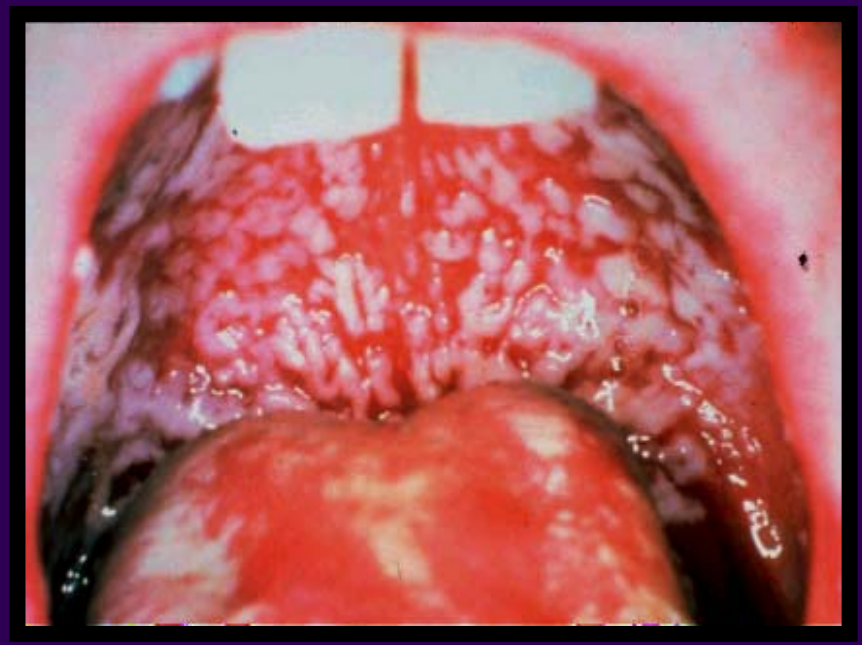
Duration

Static or shaken

Reading - endpoint interpretation

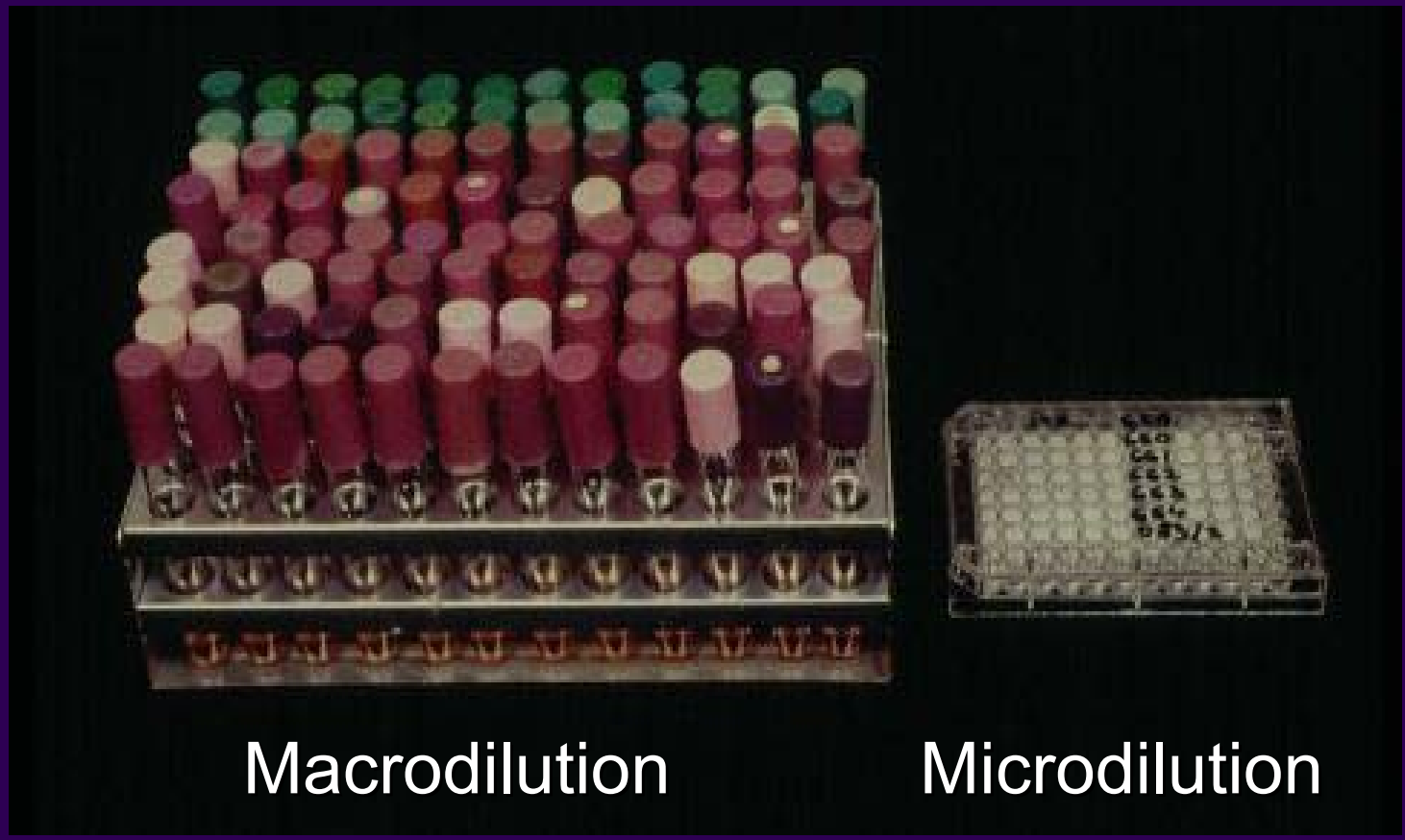
Breakpoint interpretation - *in vivo* - *in vitro* correlation
- normal ranges
- attainable blood concentrations

Oropharyngeal candidosis in AIDS



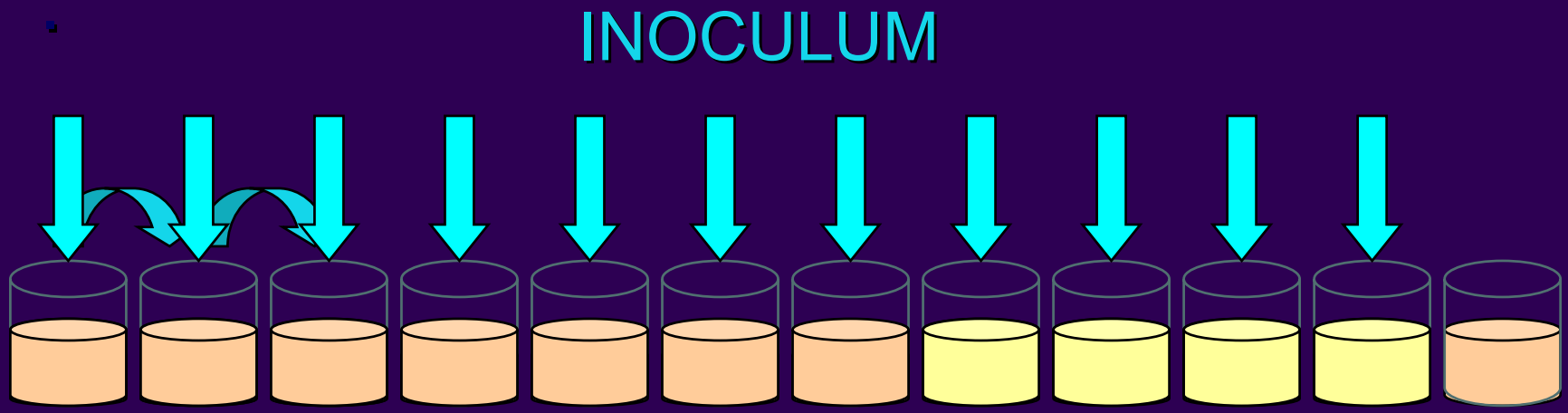
Homogeneity of underlying disease
Emerging resistance encountered
Visible condition
Treated with oral antifungals
Differing regimens
Response easily evaluable

CLSI (NCCLS) broth dilution (M27-A2)



EUCAST modification - extra glucose
- flat-bottom plates
- read at 24 hours

MIC testing

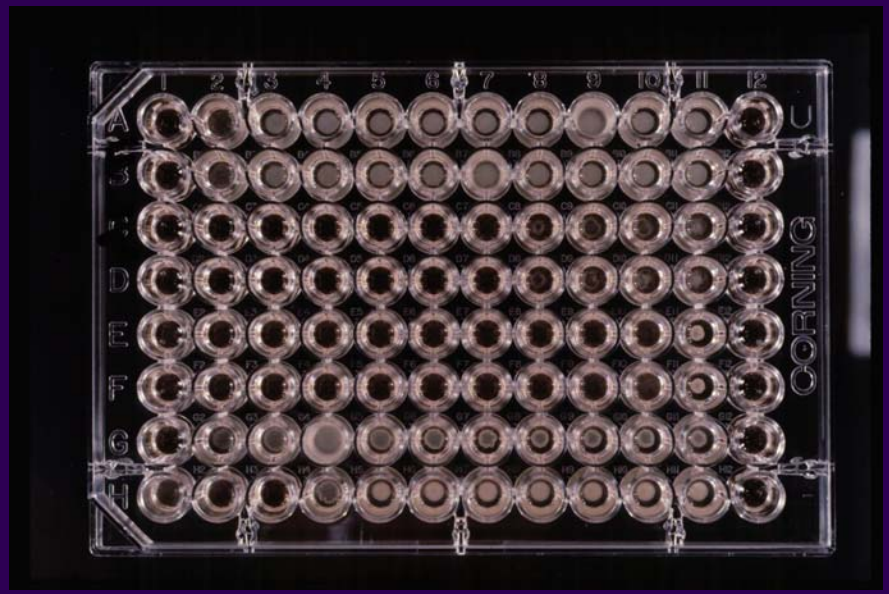


mg/L	64	32	16	8	4	2	1	0.5	0.25	0.125	GROWTH CONTROL
mg/L	16	8	4	2	1	0.5	0.25	0.125	0.06	0.03	NEGATIVE CONTROL

MIC 1.0 mg/L or 0.25 mg/L
depending on concentration range

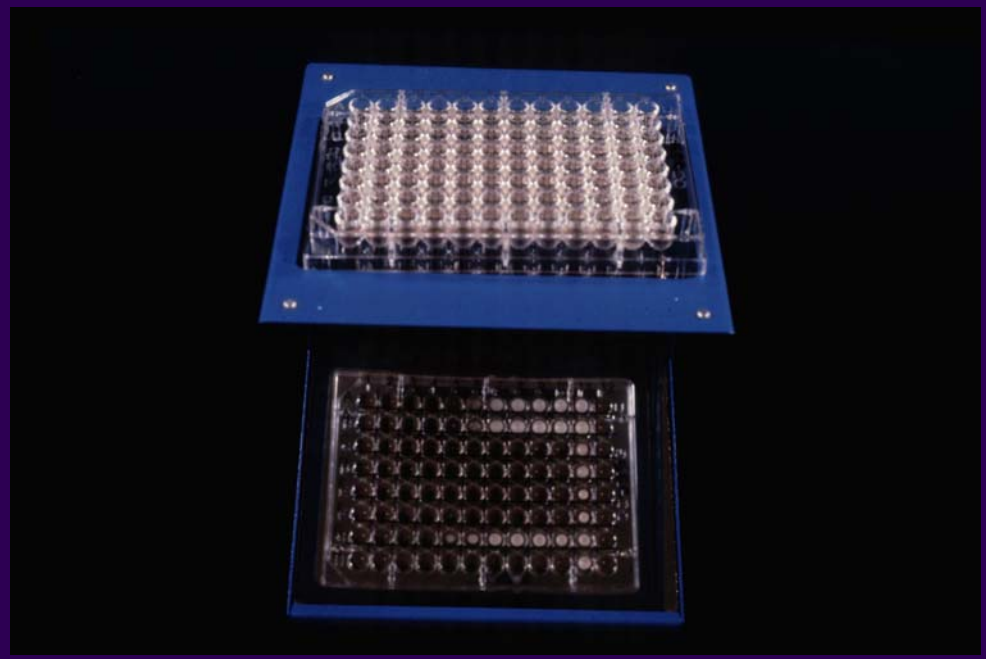
Reading of microtitre plates

Microtitre plate

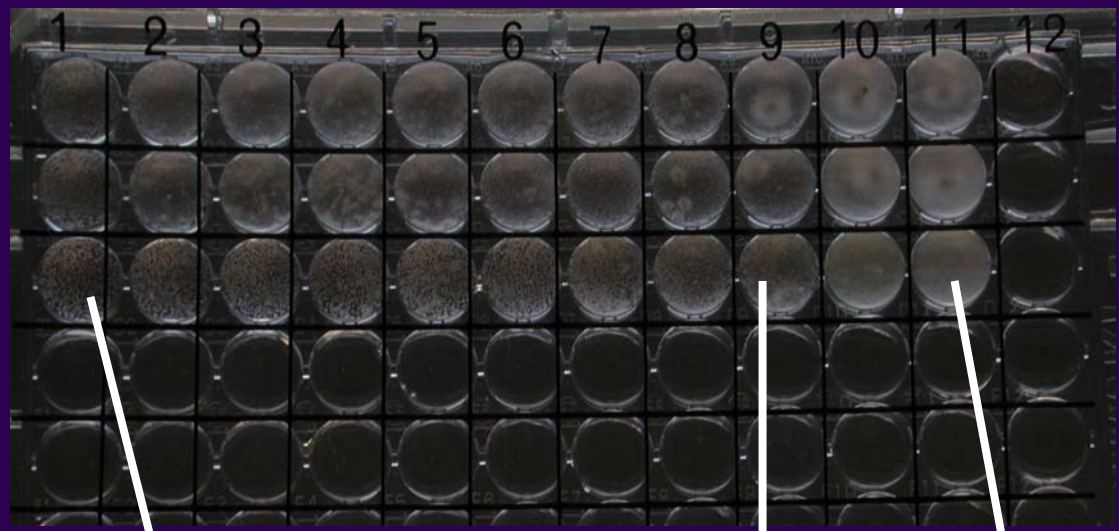


Reading on a spectrophotometer is possible if flat bottom plates are used - this produces a non-subjective reading

Reading mirror



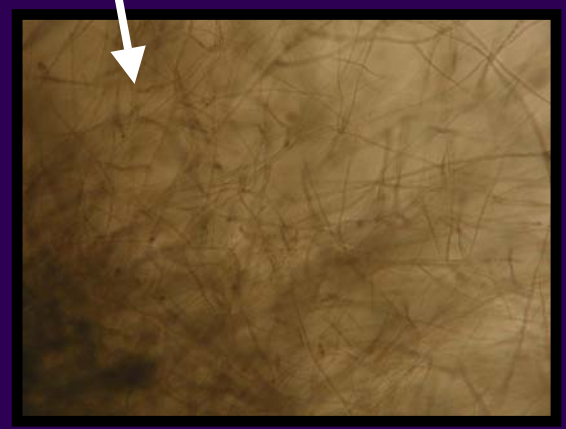
NCCLS method for caspofungin against mould (MEC)



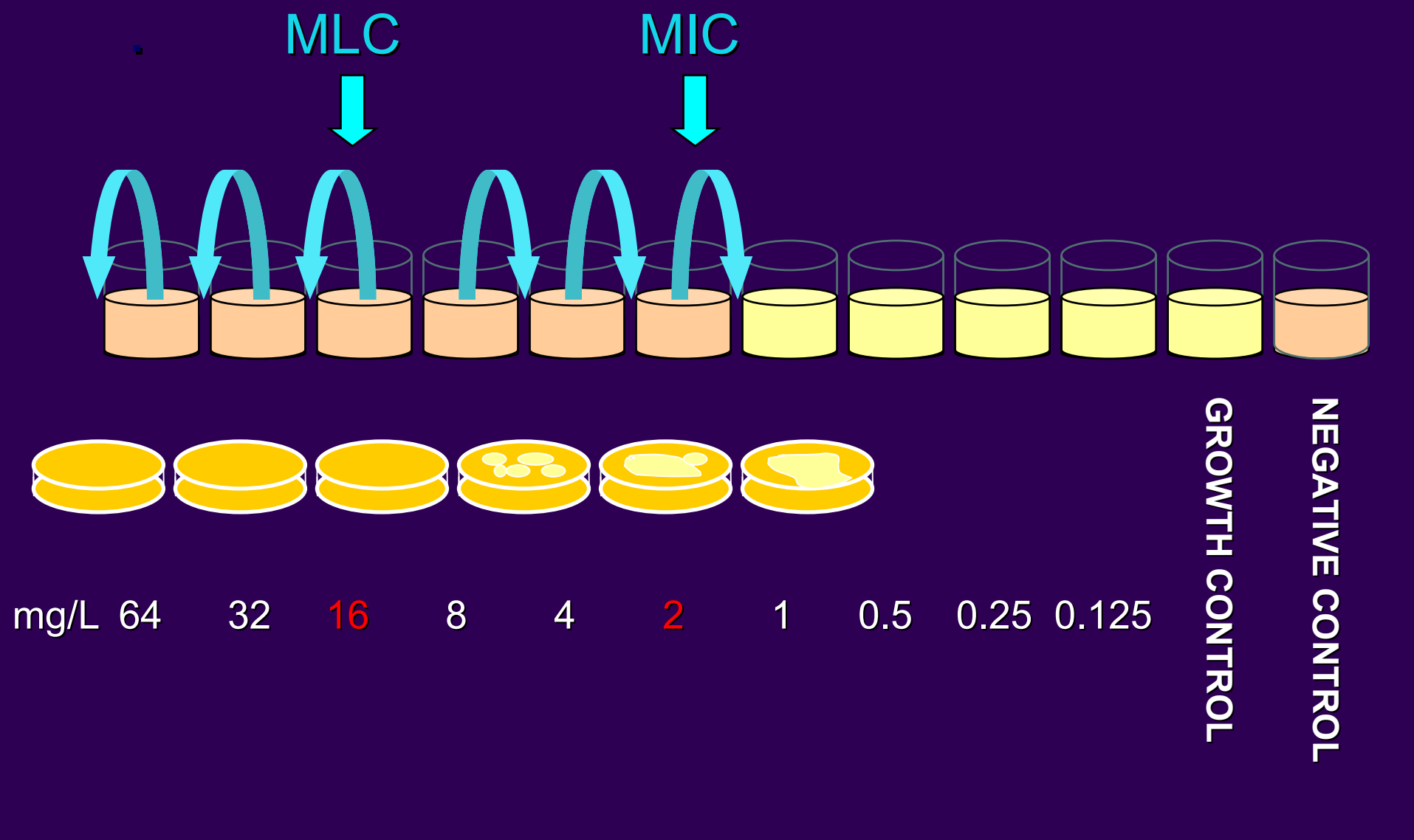
64 mg/L

0.25 mg/L

Control



Minimum Lethal (Fungicidal) Concentration (MLC/MFC)



Yeast species implicated in infection

(number referred to the Mycology Reference Laboratory for susceptibility testing 2004 and 2005)

Health Protection Agency

***C. albicans* (3420)**

***C. glabrata* (1460)**

***C. parapsilosis* (734)**

***C. tropicalis* (368)**

***C. krusei* (144)**

***C. lusitaniae* (123)**

***C. guilliermondii* (73)**

***C. dubliniensis* (40)**

***C. inconspicua* (29)**

***C. famata* (23)**

***C. kefyr* (21)**

C. chiropterum

C. ciferrii

C. haemulonii

C. humicola

C. lipolytica

C. norvegensis

C. pelliculosa

C. pintolopesii

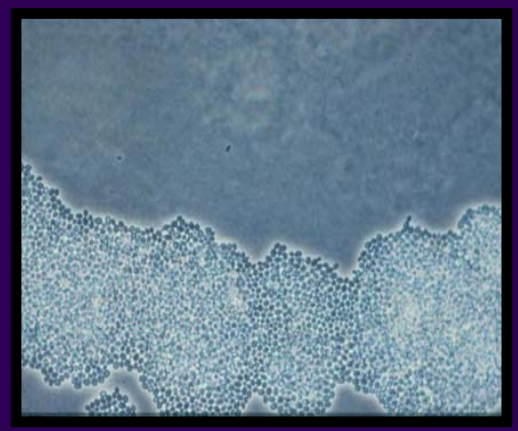
C. pulcherrima

C. rugosa

C. utilis

C. blankii

C. zeylanoides



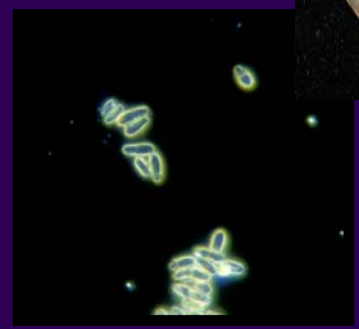
Pichia anomola (= *Candida pelliculosa*) (8)

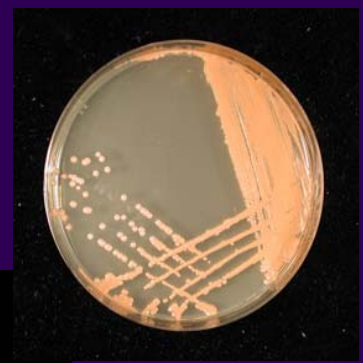
Saccharomyces cerevisiae (80)

Trichosporon spp. (25)

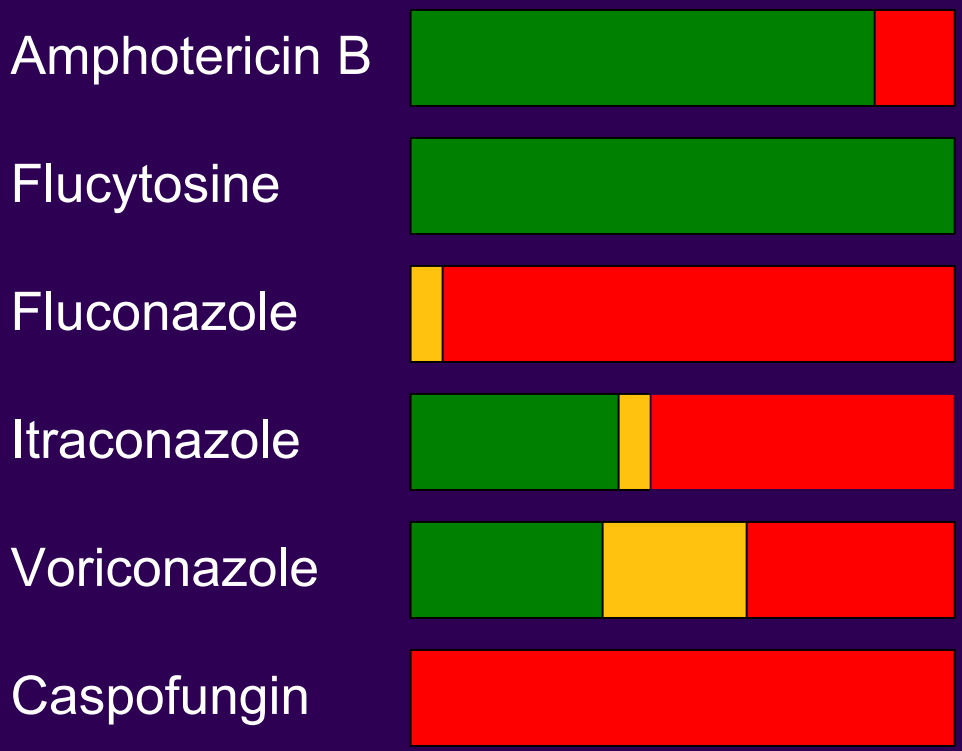
Rhodotorula spp. (52)

Malassezia spp. (36)





Rhodotorula mucilaginosa (99 strains)

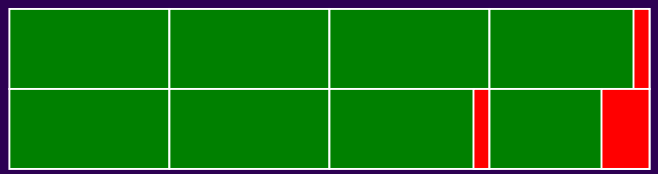


Spectrum of activity against yeast species

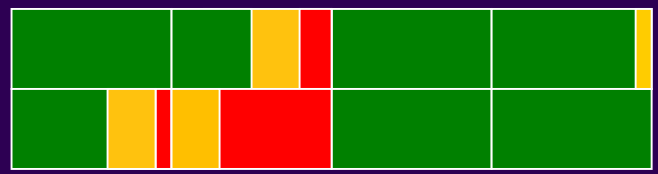
Candida albicans	***Candida glabrata***	***Candida parapsilosis***	***Cryp. neoformans***
Candida tropicalis	***Candida krusei***	***Candida lusitaniae***	***Trichosporon* spp.**

susceptible intermediate/SDD resistant

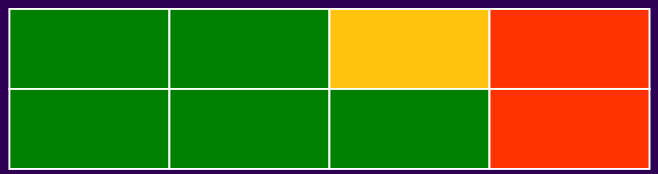
Amphotericin B



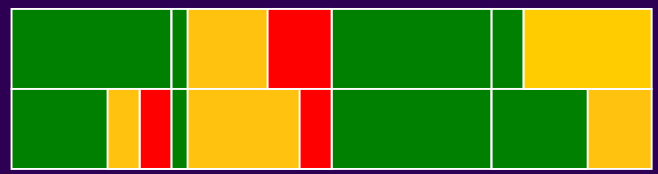
Fluconazole



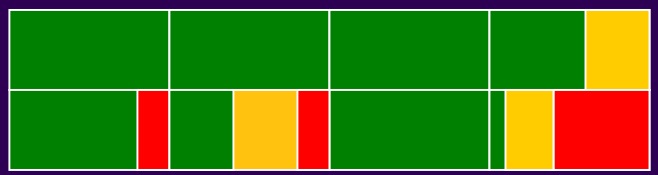
Caspofungin



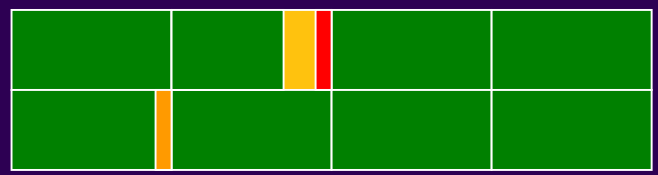
Itraconazole



Flucytosine



Voriconazole



Antifungal drug resistance

Intrinsic (primary) resistance:

The strain was resistant to the drug before treatment started

Acquired (emergent) resistance:

The strain became resistant during treatment

CLSI (NCCLS) reference method: breakpoints

	Fluconazole	Itraconazole	Flucytosine
Susceptible	≤ 8	≤ 0.125	≤ 4.0
Intermediate*	16 - 32	0.25 - 0.5	8.0 - 16
Resistant	≥ 64	≥ 1	≥ 32

* susceptible - dose dependent

1,295 patient-episode-isolate events: 692 mucosal, 603 invasive candidosis
12 published clinical studies - 13.3% resistance

	≤ 8.0 mg/L	16 - 32 mg/L	≥ 64 mg/L
Success	85% (841/993)	67% (87/130)	42% (72/172)

Strong relationship between MIC, fluconazole dose and outcome

Pfaller *et al.* 2006 CMR 17:2:435-447

The 90 - 60 Rule

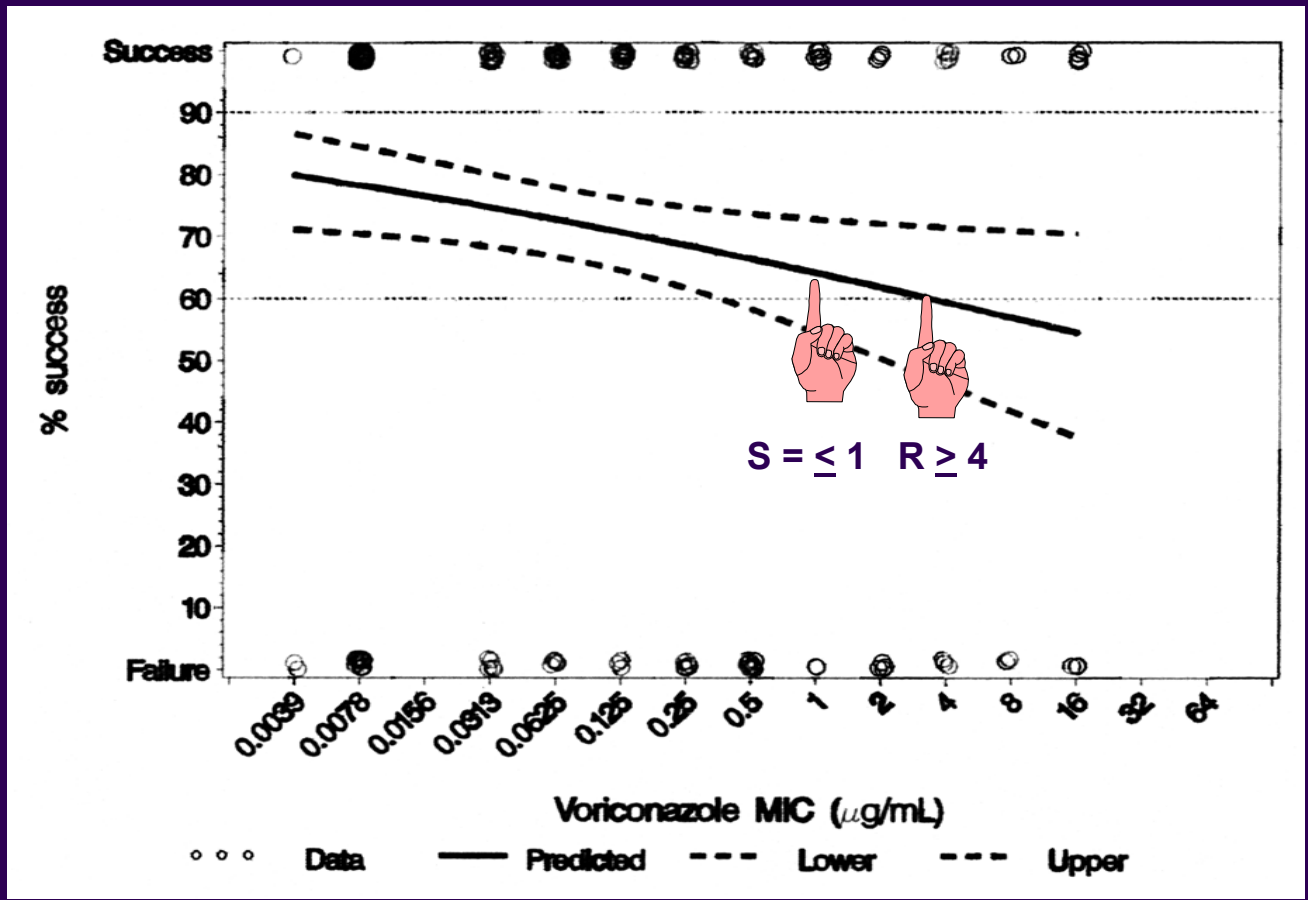
Applies with azole drugs against yeasts

S isolates respond ~90% of the time
R isolates respond ~60% of the time

Rex *et al.* Clin Infect Dis 35:982-989, 2002

Correlation of MIC with outcome for *Candida* species tested against voriconazole: analysis and proposal for interpretive breakpoints

Pfaller *et al*. 2006 JCM 44:3 819-826



S = ≤ 1
R = ≥ 4.0

8,702 clinical isolates
MIC_{90} 0.25 mg/L
MIC_{99} ≤ 1.0 mg/L

249 patients from 6 phase III clinical trials statistically significant (P = 0.021) correlation between MIC and investigator end-of-treatment assessment of outcome

Activity of voriconazole, itraconazole,fluconazole and amphotericin B in vitro against 1763 yeast from 472 patients in the voriconazole phase III clinical studies

Johnson *et al*. 2008 IJAA 32: 511-514

109 isolates had voriconazole MIC ≥ 4.0 mg/L

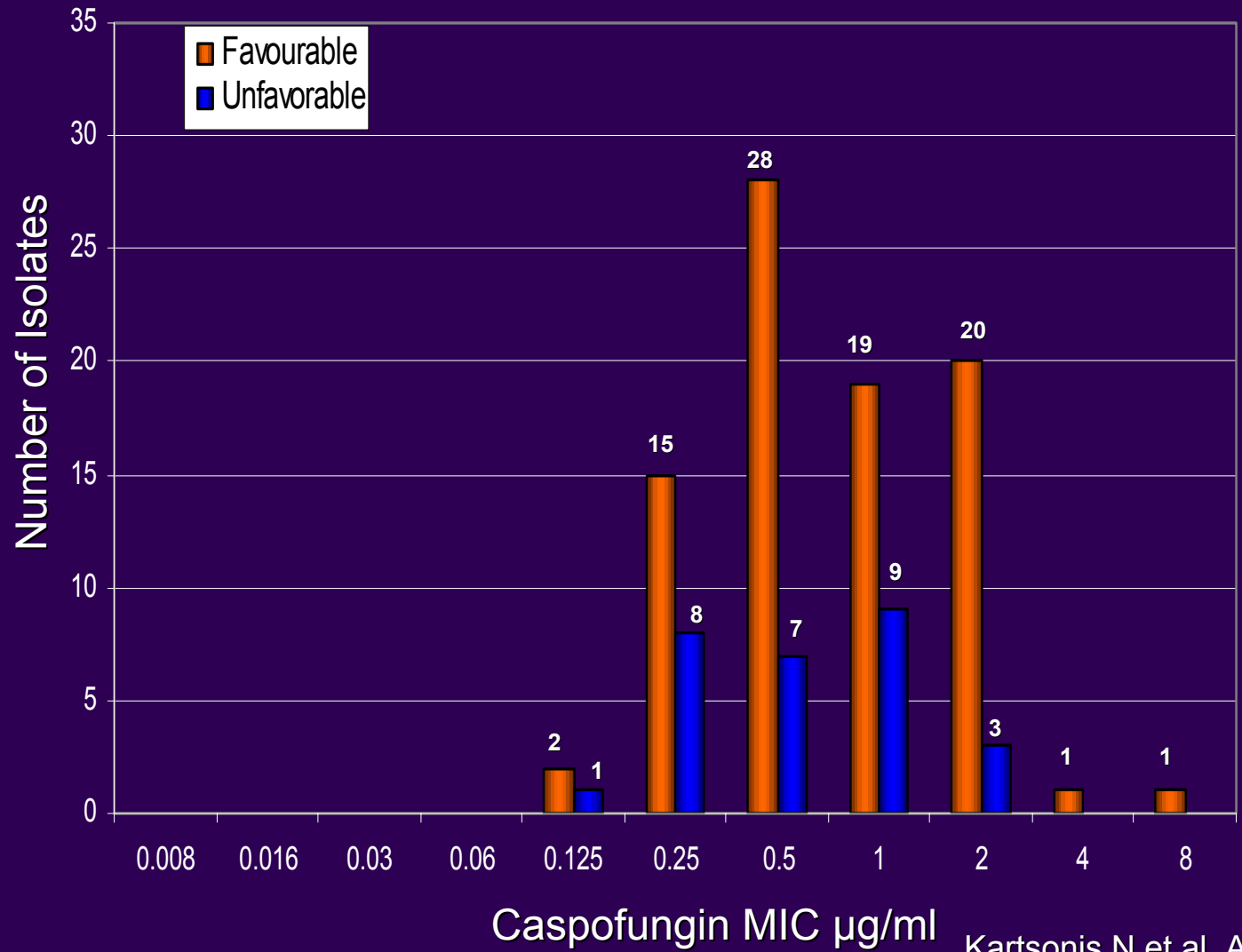
Candida albicans
Candida glabrata
Candida tropicalis

All cross-resistant to itraconazole most to fluconazole

Resistant isolates (34): 56% response to voriconazole
Susceptible isolates (261): 71% response to voriconazole

23 base-line resistant, 8 developed on treatment, 3 different species

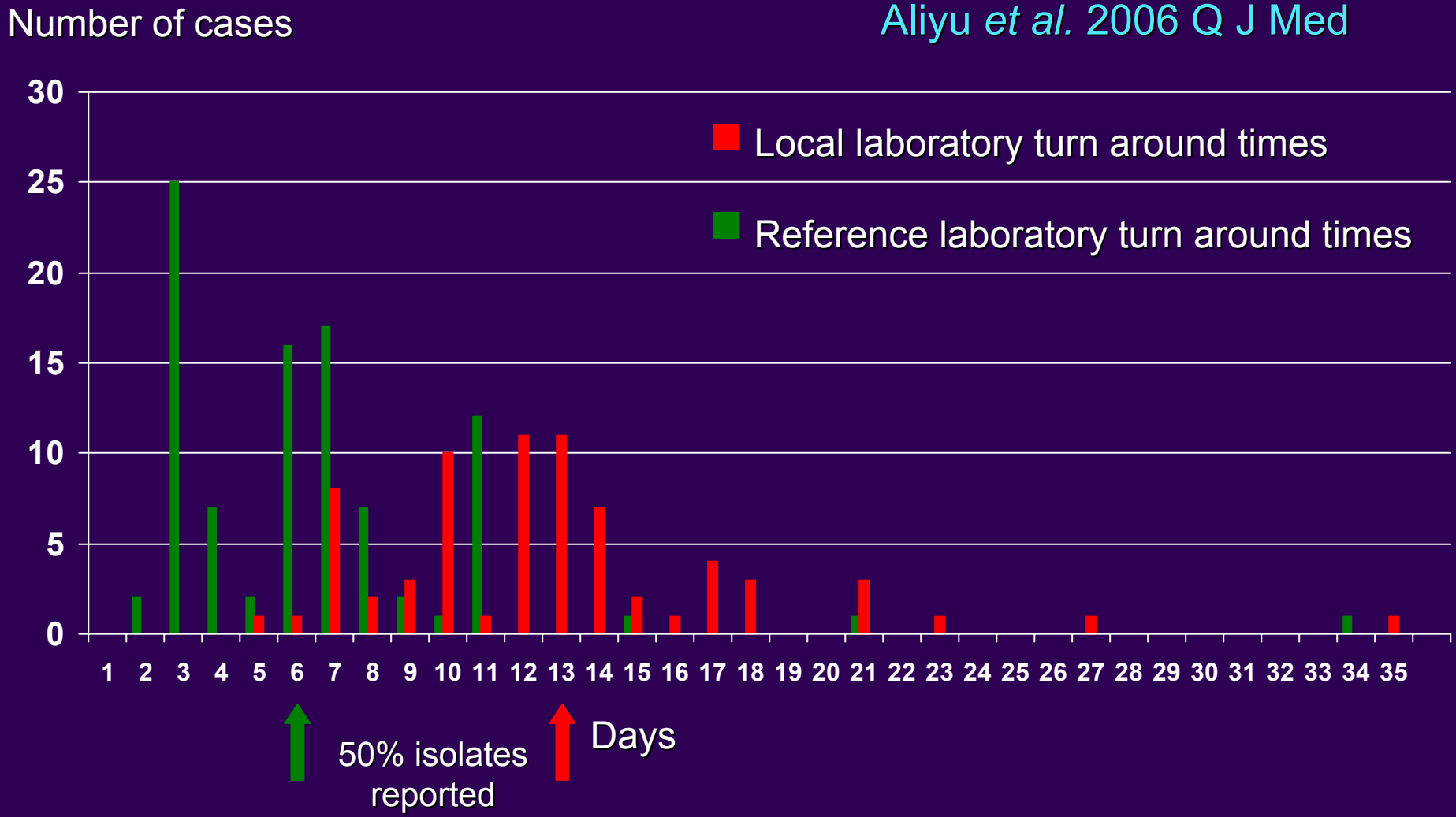
Correlation of caspofungin MICs to overall outcomes for patients with invasive candidiasis (MITT population)



MIC	Unfavourable Outcome
0.008	-
0.016	-
0.03	-
0.06	-
0.125	33%
0.25	35%
0.5	20%
1	32%
2	13%
4	0%
8	0%

Kartsonis N et al, AAC 2005 49:3616-3623

Turn-around times for local laboratory (final verification of results) compared to reference laboratory turn-around



Susceptibility testing methods

Yeast

CLSI (NCCLS) M27-A2

CLSI (NCCLS) M44-P disc diffusion

EUCAST

Disc diffusion / neosensitabs

Yeast one (sensititre)

ATB Fungus

Fungitest

E-test

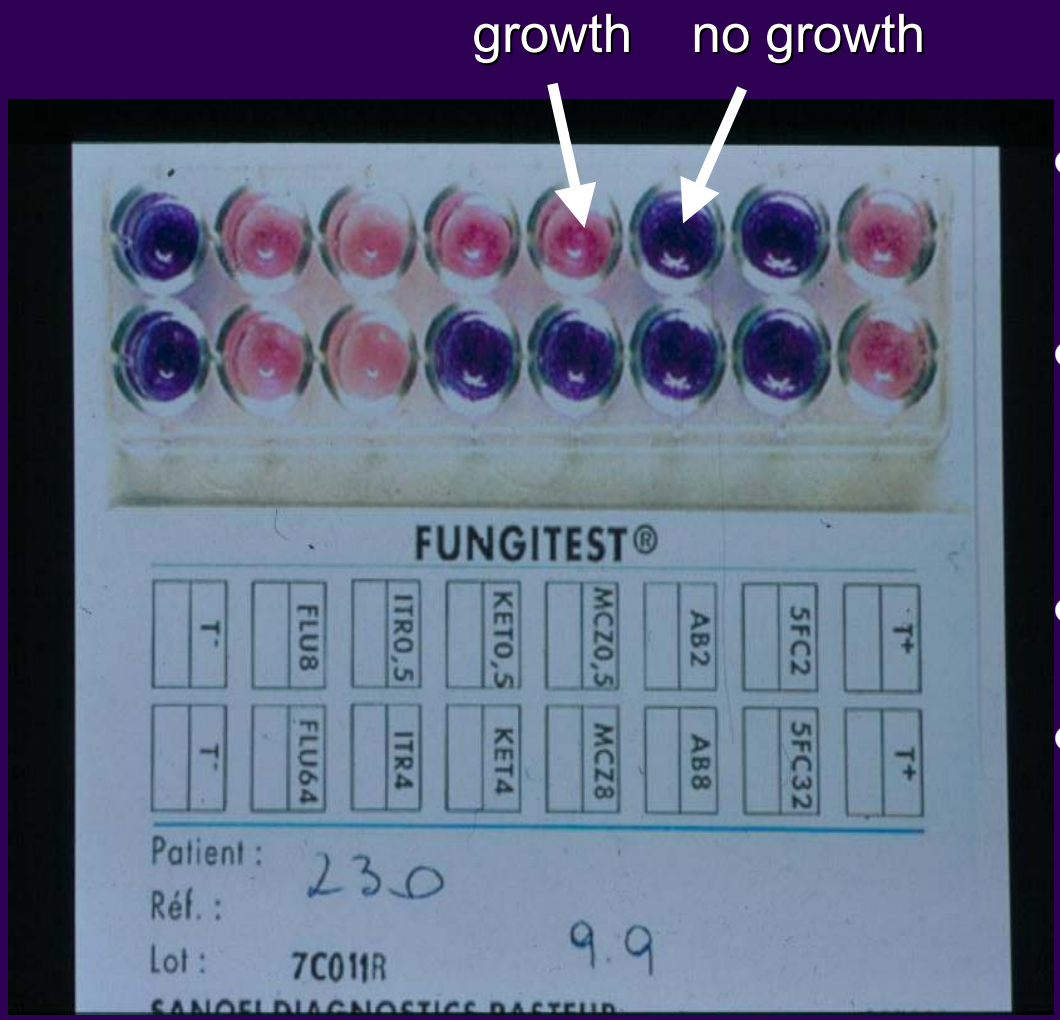
Mould

CLSI (NCCLS) M38-A

Agar dilution

E-test

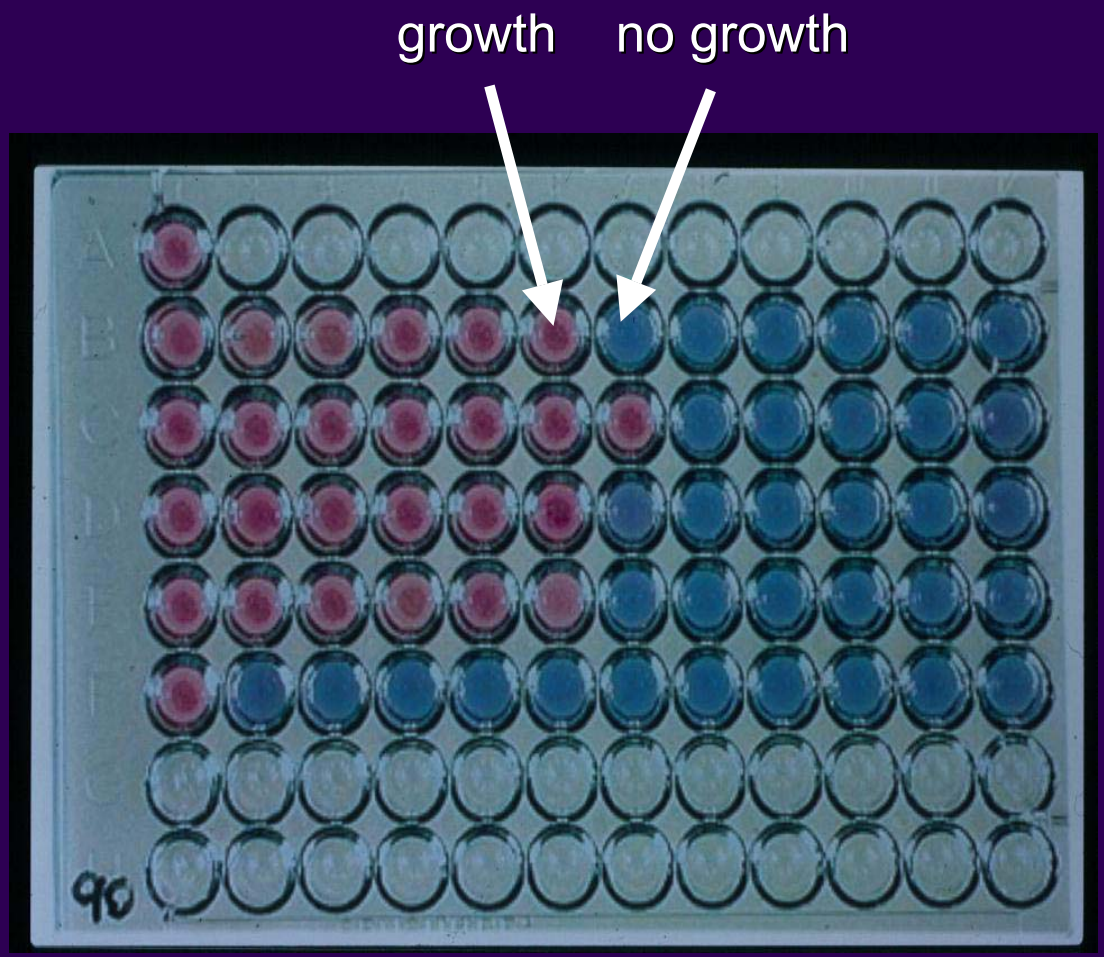
Fungitest - Diagnostics Pasteur



- Low and high concentration of each of 6 antifungal agents
- Concentrations do not correspond with all accepted breakpoints
- One test organism per strip
- Reasonable correlation with NCCLS method except with *Candida glabrata*

Davey *et al.* 1998 JCM 36:926-30

Yeast One (TREK)



- Same format as CLSI (NCCLS) microtitre method
- Chromogenic substrate for ease of reading
- Different drug in each row therefore one test organism per plate. Voriconazole and caspofungin now CE marked
- Good correlation with CLSI (NCCLS) method

Davey *et al.* 1998 JAC 42:439-444

Etest for yeasts

- Strips impregnated with a concentration gradient of antifungal agent
- MIC value rather than just susceptibility category
- Good correlation with CLSI (NCCLS) method
- Some false resistance due to interpretation of background growth

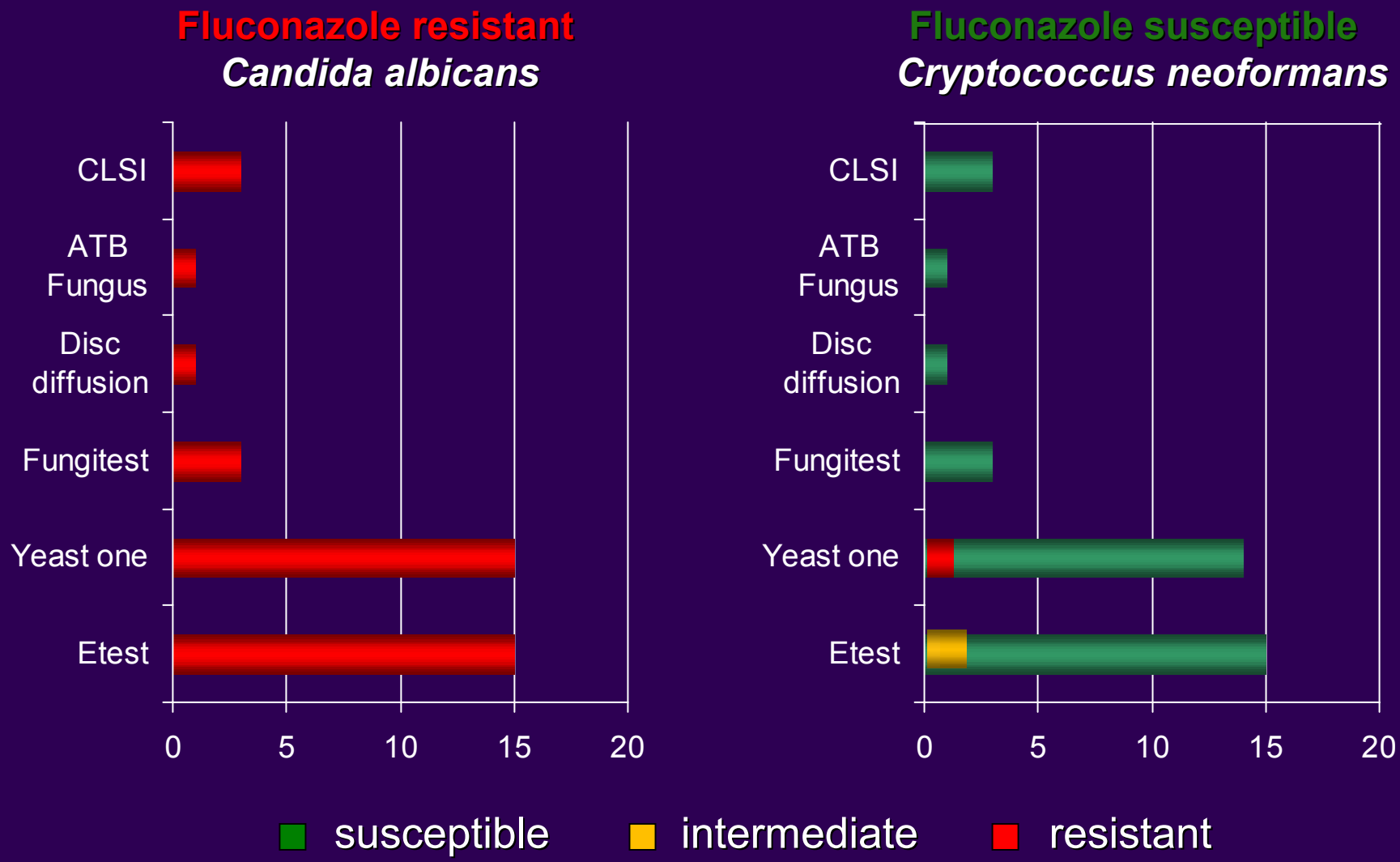
Warnock *et al.* 1998 JAC 42:321-331

NEQAS antifungal susceptibility testing

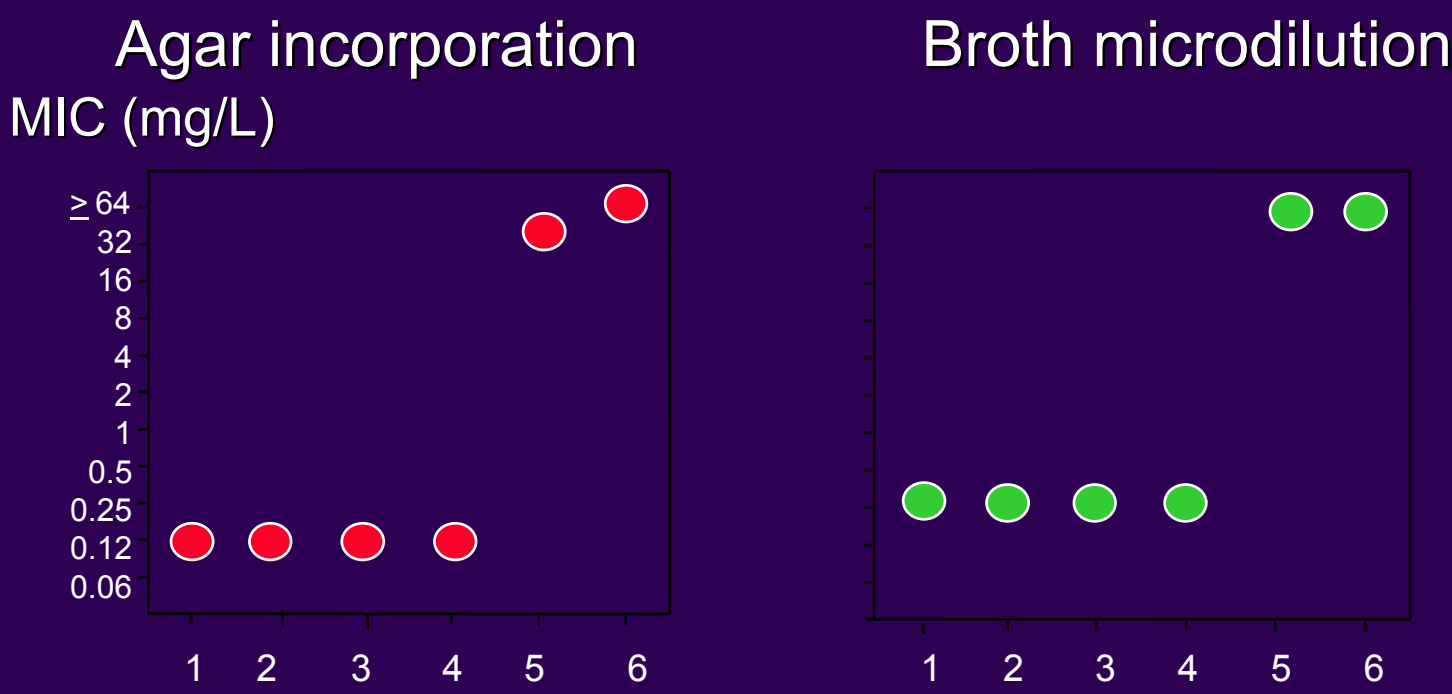
- Yeast isolates only
- Three distributions each year - two strains
- Strains selected and tested against antifungal panel
- Strains lyophilised and then 4 ampoules re-tested in Bristol Manchester (+ EUCAST) and at QAL (Etest)
- Susceptibility categories agreed
- Strains distributed
- Results analysed and reported

UK NEQAS susceptibility testing methods

Health Protection Agency

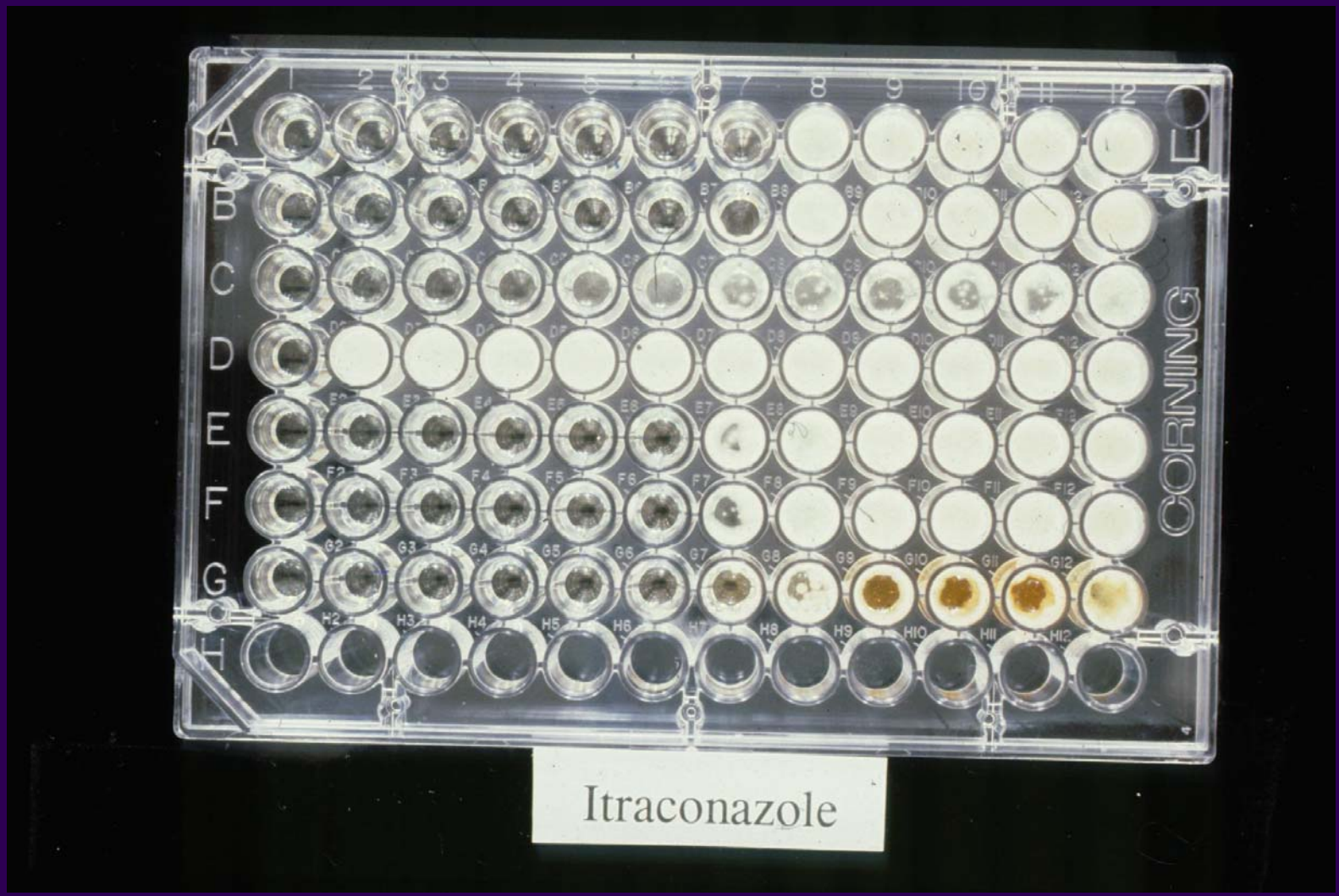


Itraconazole MIC testing
Aspergillus fumigatus

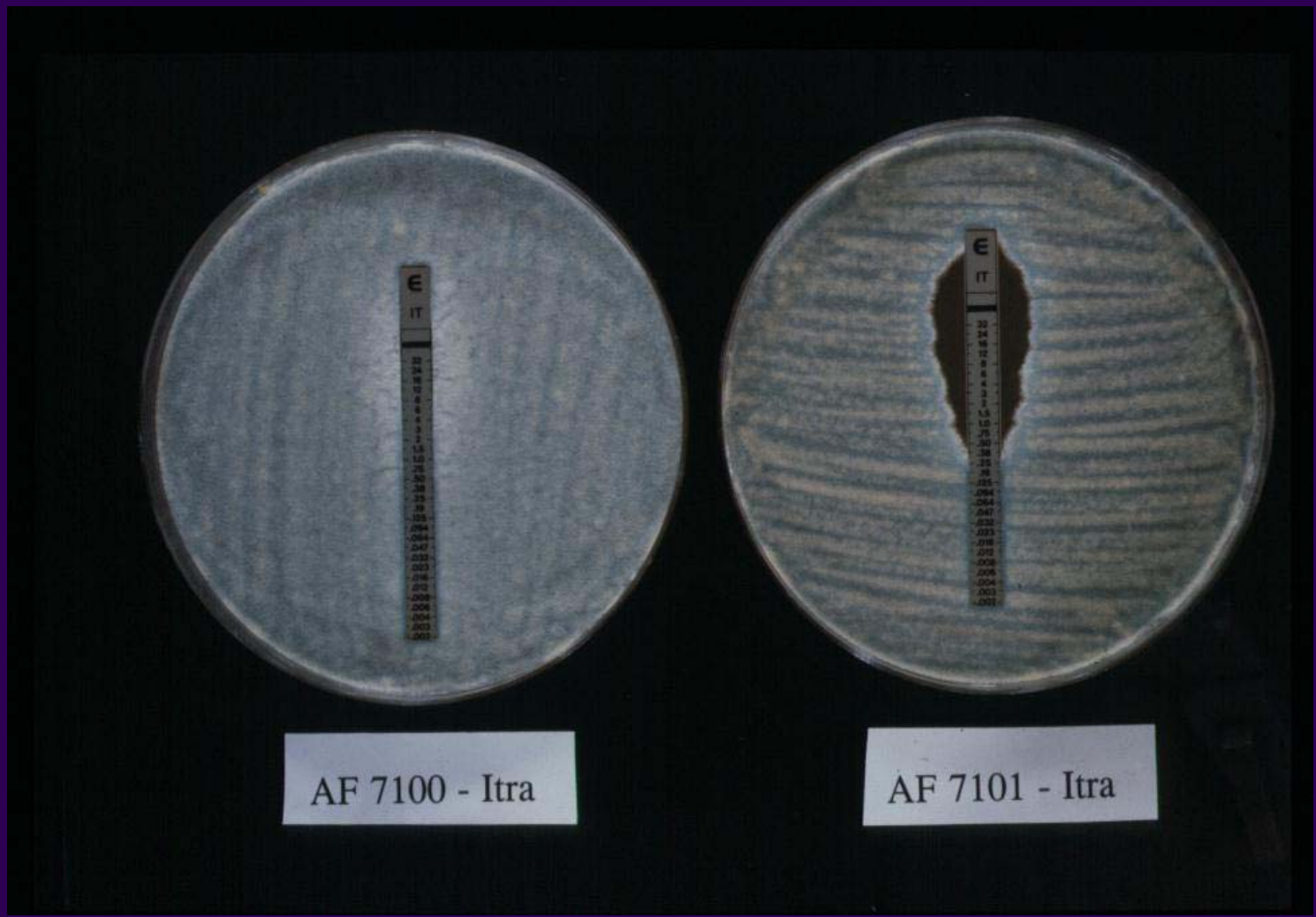


Denning *et al.* 1997 JAC

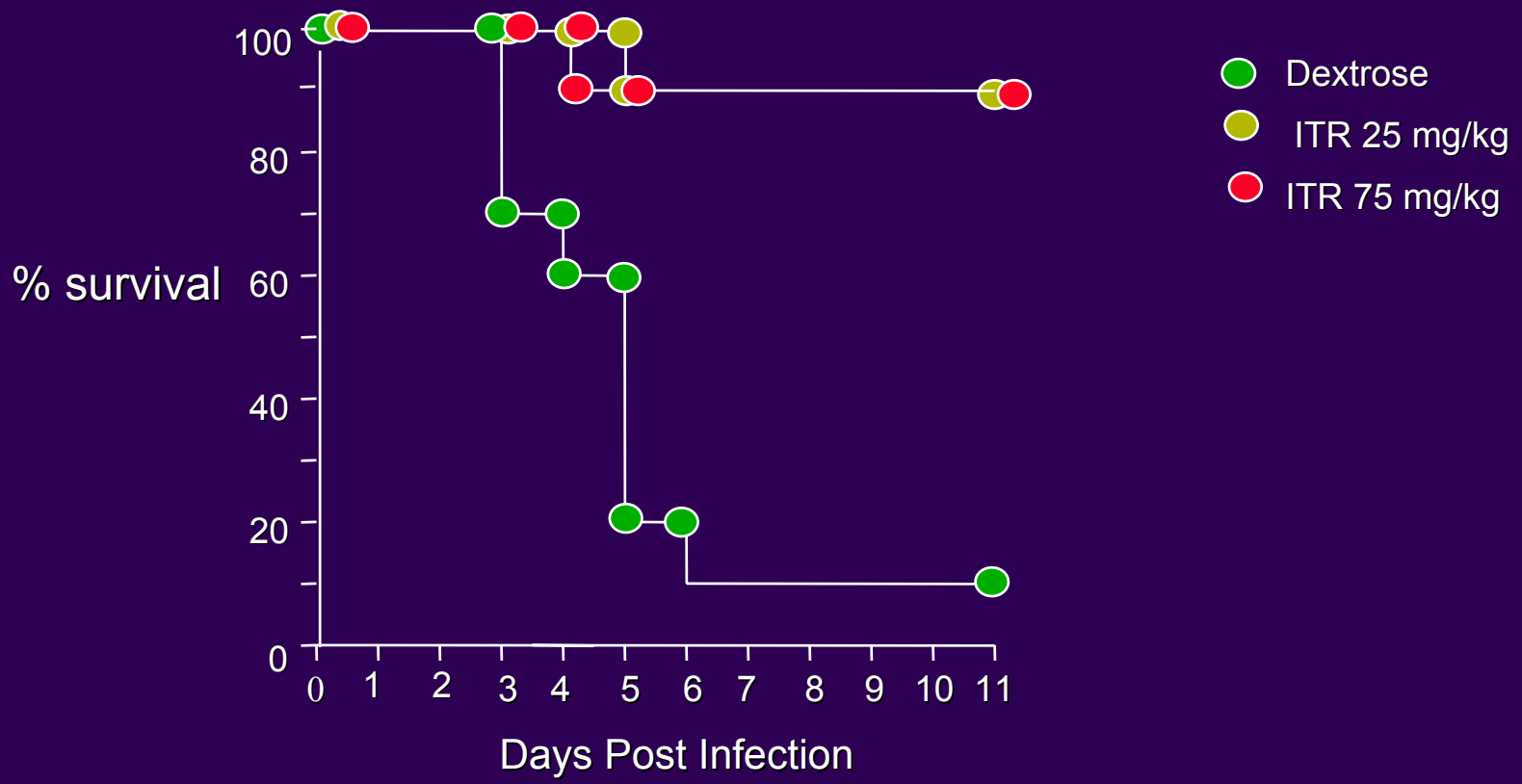
CLSI method for mould MIC



Etest for moulds

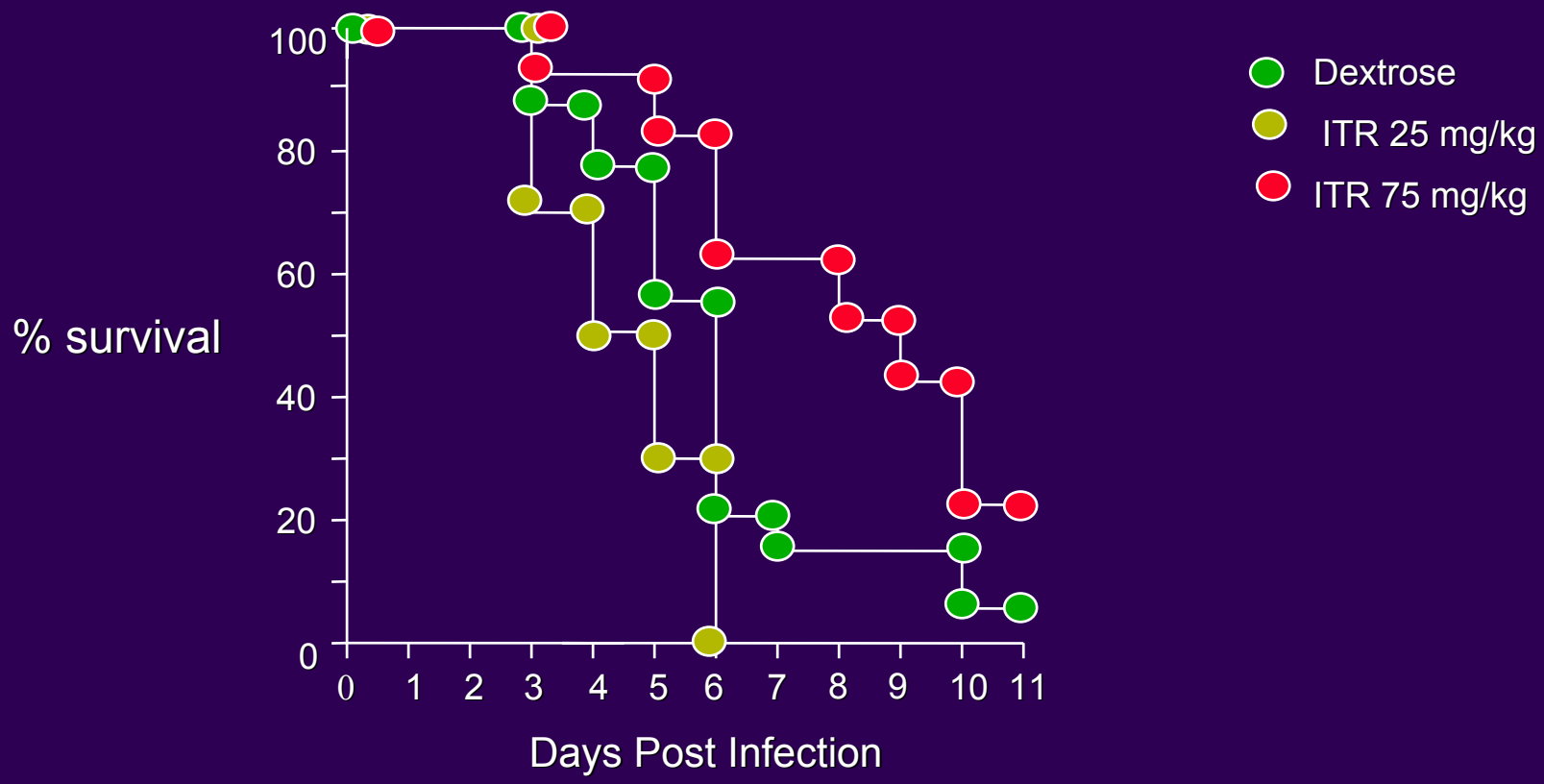


Survival curves in a neutropenic mouse model infected with an itraconazole susceptible *A. fumigatus* strain (MIC 0.25 mg/L)



Denning *et al.* 1997 JAC

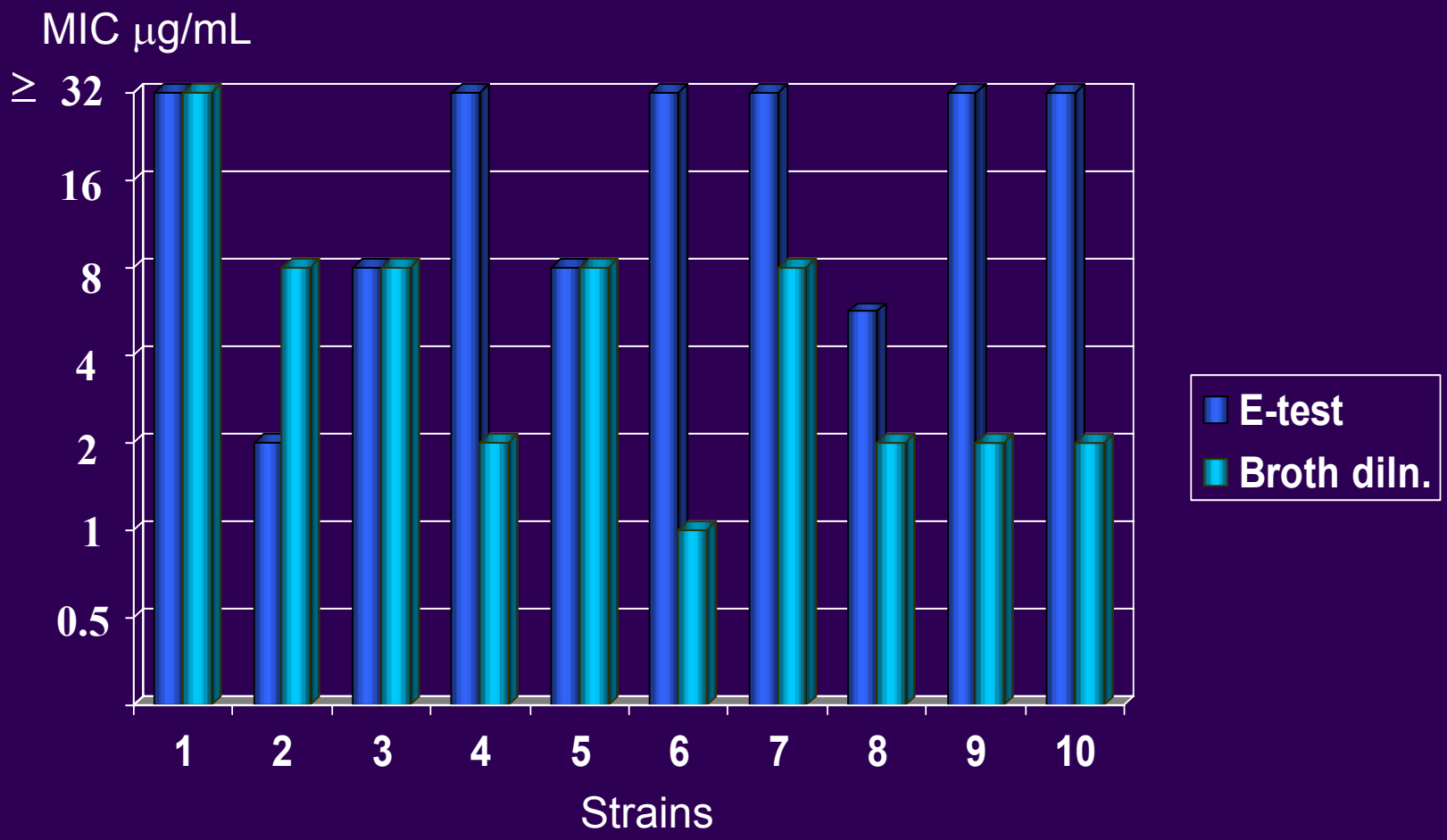
Survival curves in a neutropenic mouse model infected with an itraconazole resistant *A. fumigatus* strain (MIC >64 mg/L)



Denning *et al.* 1997 JAC

Etest result with *Scedosporium apiospermum*

Comparison of E-test result for amphotericin B with broth microdilution result for 10 strains of *Scedosporium apiospermum*



Antifungal drug resistance

Innate resistance

Most common
Predictable if organism speciated (sometimes only a percentage)
Defines an antifungal agents spectrum of activity

Emergent resistance

Currently rare
Rare in invasive disease
Very rare with moulds

Issues

Multi-resistant organisms (esp. *Scedosporium prolificans*)
Over the counter drugs (esp. azoles)

Susceptibility testing

HOW?

CLSI (NCCLS) method or CLSI validated method
Standardized methods for yeasts and moulds

WHEN?

Identification is crucial - predictable innate resistance
Candida krusei, C. glabrata and *C. tropicalis*
Yeasts from recalcitrant infections (mucosal and deep)
All moulds from deep infections
Should not be used as the sole criterion for selecting therapy

WHY?

Evidence for *in vitro-in vivo* correlation not perfect but at least as good as for bacteria
Emergent resistance in some yeasts
New agents - therapeutic choices
Emerging pathogens