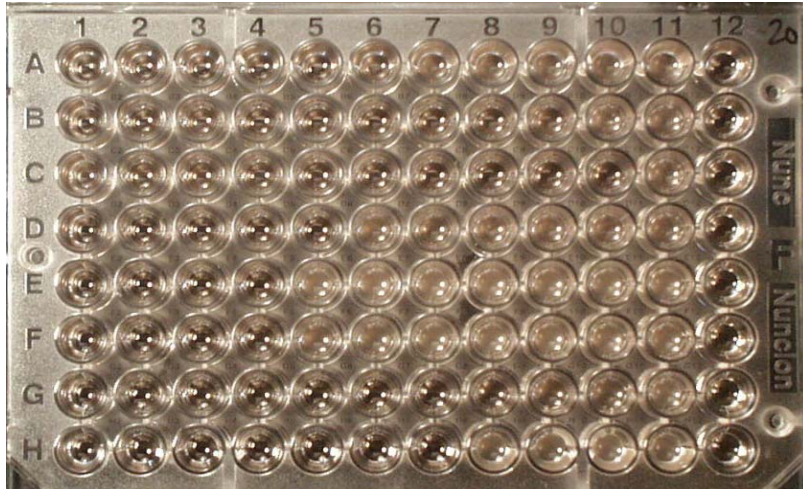


# Antifungal susceptibility testing: Which method and when?



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ISCIII

# Agenda

- Summary of current standards and selected commercial methods
- BPs and BP setting process
  - CLSI
  - EUCAST
- Indications for AFST
- Conclusion

# In vitro susceptibility testing

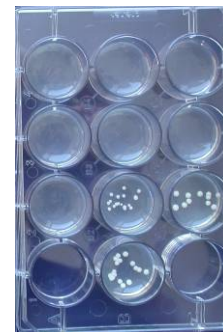
## ■ Broth-dilution

- Macro-format
- Micro-format
  - CLSI Reference method
  - EUCAST Reference method
  - Odds method
  - SensiTitre

## ■ Disk / Etest diffusion

## ■ Vitek

## ■ Agar dilution



# Yeast standards

## **EUCAST Definitive Document EDef 7.1: method for the determination of broth dilution MICs of antifungal agents for fermentative yeasts**

Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST)\*

**Keywords** Antifungal agents, EUCAST, fermentative yeasts, MICs, susceptibility testing method

*Clin Microbiol Infect* 2008; 14: 398–405

**Free at EUCAST web page !!!**

M27-A3

Vol. 28 No. 14

Replaces M27-A2

Vol. 22 No. 15

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**Reference Method for Broth Dilution  
Antifungal Susceptibility Testing of Yeasts;  
Approved Standard—Third Edition**

**155 \$ (non-member price including supplement)**

# CLSI M27-A <A2> <A3 & EUCAST

	M27-A	M27-A2	M27-A3	EUCAST
Glucose /Inoculum size		0.2%, 0.5-2.5 × 10 <sup>3</sup>		2%, 0.5-2.5 × 10 <sup>5</sup>
Plates & Reading		Round bottom & Visual		Flat & Spec.
Incubation time	48 h	24-48 h	24 h	24 h
End point	80% inhib.	50% inhib.	50% inhib.	50% inhib
<i>C. parapsilosis</i> ATCC 22019	2-8 µg/ml	0.5-4 / 1-4 µg/ml	0.5-4 µg/ml	0.5-2 µg/ml
<i>C. krusei</i> ATCC 6258	16-64 µg/ml	8-64 / 16-128 µg/ml	8-64 µg/ml	16-64 µg/ml

# Antifungal "S, R" breakpoints for *Candida*

Breakpoints given as: S:  $\leq X$ ; R:  $> Y$

Proposed Breakpoints- not yet approved!!

	CLSI	EUCAST (specific for)
Amphotericin B	$\leq 1$	$\leq 1$ ; $> 1$
Anidulafungin	$\leq 2$	$\leq 0.032$ ; $> 0.032$ ( <i>C. alb</i> ) $\leq 0.064$ ; $> 0.064$ ( <i>C. g,k,t</i> )
Caspofungin	$\leq 2$	-
Micafungin	$\leq 2$	-
Fluconazole	$\leq 8$ ; $> 32$	$\leq 2$ ; $> 4$ ( <i>C. a,t,p</i> )
Itraconazole	$\leq 0.125$ ; $> 0.5$	-
Posaconazole	-	$\leq 0.064$ ; $> 0.064$ ( <i>C. a,t,p</i> )
Voriconazole	$\leq 1$ ; $> 2$	$\leq 0.125$ ; $> 0.125$ ( <i>C. a,t,p</i> )

# Vitek 2, Sensititre, Etest & Ref methods

**Table 1.** Essential agreement (EA) rates between results given by VITEK 2 System, Sensititre Yeast-One and Etest and the reference procedures by antifungal agent

Antifungal agent	EA between AST procedures								
	VITEK/ EUCAST	VITEK/ CLSI24H	VITEK/ CLSI48H	ETEST/ EUCAST	ETEST/ CLSI24H	ETEST/ CLSI48H	SYOne/ EUCAST	SYOne/ CLSI24H	SYOne/ CLSI48H
AMB	98.7%	99.3%	100%	98.4%	97.4%	96.4%	97.9%	97.4%	96.0%
5FC	98.0%	98.6%	96.0%	96.4%	95.2%	95.2%	96.0%	95.2%	95.2%
FLC	97.5%	96.6%	96.2%	97.2%	96.4%	95.2%	97.2%	96.0%	95.6%
VRC	97.5%	96.8%	96.6%	95.2%	95.2%	95.2%	95.5%	95.6%	95.2%
<b>TOTAL</b>	<b>97.9%</b>	<b>97.8%</b>	<b>97.3%</b>	<b>96.8%</b>	<b>96.1%</b>	<b>95.5%</b>	<b>96.6%</b>	<b>96.1%</b>	<b>95.5%</b>

# Aspergillus Standards

**EUCAST Technical Note on the method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for conidia-forming moulds**

*Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST)\**

**Keywords** antifungal drugs, EUCAST Technical Note, filamentous fungi, resistance

*Clin Microbiol Infect* 2008; **14**: 982–984

**Free at EUCAST web page !!!**

M38-A2

Vol. 28 No. 16

Replaces M38-A

Vol. 22 No. 16

**Reference Method for Broth Dilution  
Antifungal Susceptibility Testing of  
Filamentous Fungi; Approved Standard—  
Second Edition**

**120 \$ (Non-member price)**

# Differences between CLSI M38-A2 & E. Dis 9.1

	CLSI M-38-A2	EUCAST-AFST E. DEF 9.1
Glucose	0.2 %	2%
Shape of well	Round bottom	Flat bottom
Inoculum (cfu/mL)	$0.5 - 2.5 \times 10^4$	$1-5 \times 10^5$
Incubation (h)	48	48
Reading	Visual	Visual
Endpoint	optically clear & MEC for echinocandins	optically clear & MEC for echinocandins

## A. fumigatus ECOFFs & BP suggestions

	ECOFF / Susceptible	Intermediate	Resistant
Amphotericin B	$\leq 1 \mu\text{g/ml}$	-	-
Itraconazole	$\leq 1 \mu\text{g/ml}$	2	>2
Voriconazole	$\leq 1 \mu\text{g/ml}$	2	>2
Posaconazole	$\leq 0.25 \mu\text{g/ml}$	0.5	>0.5

E-Test Method for Testing Susceptibilities of *Aspergillus* spp. to  
the New Triazoles Voriconazole and Posaconazole and  
to Established Antifungal Agents: Comparison  
with NCCLS Broth Microdilution Method

Ana Espinel-Ingroff<sup>1,2\*</sup> and A. Rezusta<sup>1,2</sup>

- **Voriconazole: 93.3 to 100% agreement (within 3 dilutions)**
  - independent of the E-test incubation time (for four of five species at both incubation times).
- **Amphotericin B, itraconazole, and posaconazole,**
  - E-test results were more dependent on the incubation time for certain species.
- **Posaconazole and *A. fumigatus*,**
  - highest agreement at 48 h (95.2%) (versus 90% at 24 h)

# Agenda

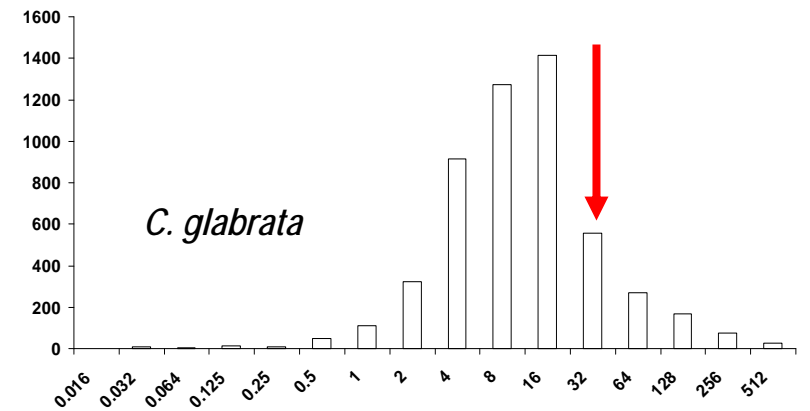
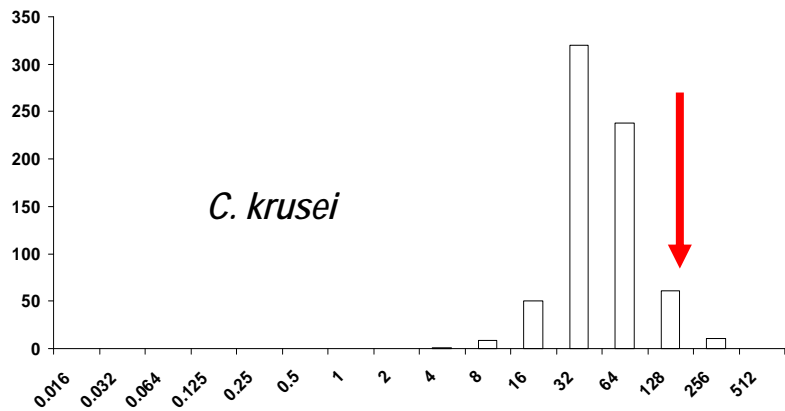
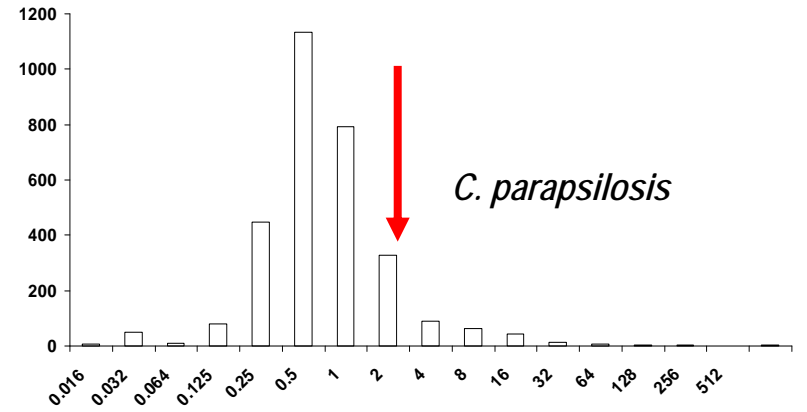
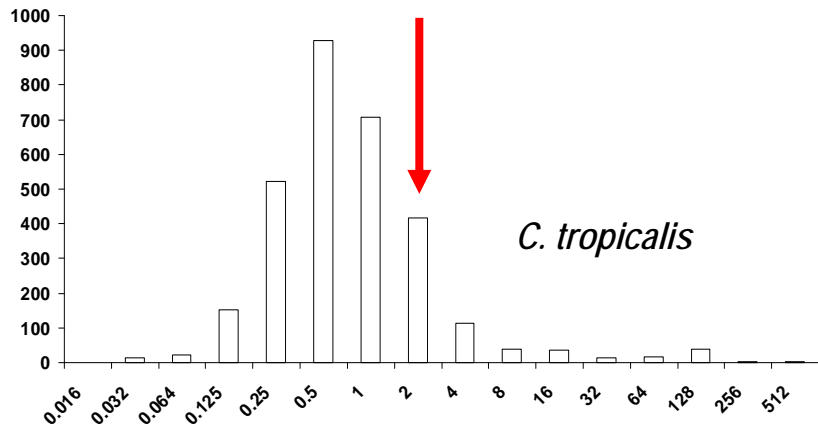
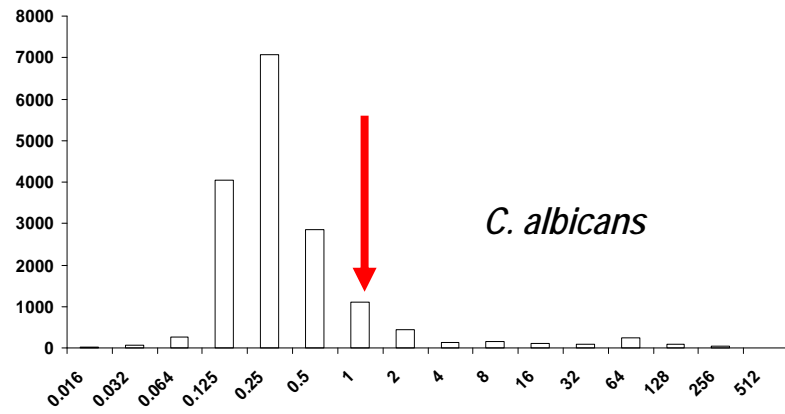
- Summary of current standards and selected commercial methods
- **BPs and BP setting process**
  - CLSI
  - EUCAST
- Indications for AFST
- Conclusion

# CLSI vs EUCAST breakpoint establishing procedure

	CLSI	EUCAST
MIC distributions	Cumulative % per species	Several data sets Epidemiological cutoffs per species
PK/PD	From animal models or humans	Target validated by means of Montecarlo simulations
MIC/Clinical outcome relationships	Rule 90-60	Data mining BP never higher than ECOFF unless supported by clinical data

# Fluconazole

**The proof of the concept  
or  
the perfect example**



## Summary of epidemiological cut-offs values for this bug-drug combination

	EUCAST methodology
<i>C. albicans</i>	$\leq 1$ mg/L
<i>C. parapsilosis</i>	$\leq 2$ mg/L
<i>C. tropicalis</i>	$\leq 2$ mg/L
<i>C. glabrata</i>	$\leq 32$ mg/L
<i>C. krusei</i>	$\leq 128$ mg/L

## Correlation of the MIC and Dose/MIC Ratio of Fluconazole to the Therapeutic Response of Patients with Mucosal Candidiasis and Candidemia<sup>▽</sup>

Juan L. Rodríguez-Tudela,<sup>1\*</sup> Benito Almirante,<sup>2</sup> Dolors Rodríguez-Pardo,<sup>2</sup> Fernando Laguna,<sup>3</sup>  
J. Peter Donnelly,<sup>4</sup> Johan W. Mouton,<sup>5</sup> Albert Pahissa,<sup>2</sup> and Manuel Cuenca-Estrella<sup>1</sup>

### ■ Candidaemia.

- 126 patients & 126 episodes

### ■ Oropharyngeal candidiasis.

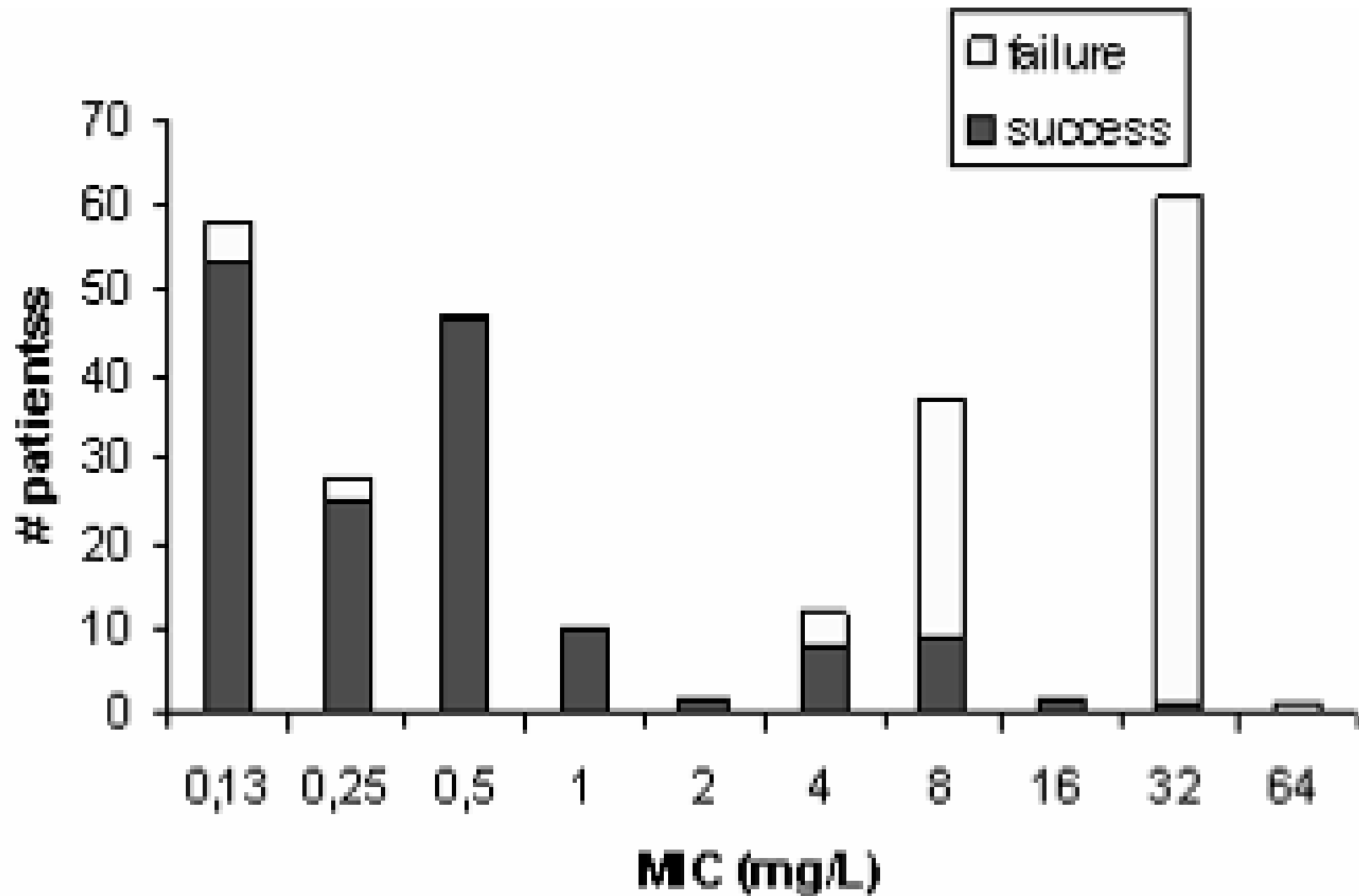
- 110 HIV+ patients & 132 episodes

■ A total of 258 episodes of *Candida* infection were available for analysis

## Data Mining Validation of Fluconazole Breakpoints Established by the European Committee on Antimicrobial Susceptibility Testing<sup>V</sup>

Isabel Cuesta,<sup>1</sup> Concha Bielza,<sup>2</sup> Pedro Larrañaga,<sup>2</sup> Manuel Cuenca-Estrella,<sup>1</sup> Fernando Laguna,<sup>3</sup>  
Dolors Rodríguez-Pardo,<sup>4</sup> Benito Almirante,<sup>4</sup> Albert Pahissa,<sup>4</sup> and Juan L. Rodríguez-Tudela<sup>1\*</sup>

# Distribution between successes and failures of 258 episodes



# EUCAST Clinical Data

MIC in mg/L	$\geq 100$ mg/d	
	No cure/Total	% response
< 0.5	124/133	93.2
1	10/10	100
2	2/2	100
4	8/12	66.7
8	9/37	24.3
>16	3/64	4.7

# Strategy to analyze results by data mining

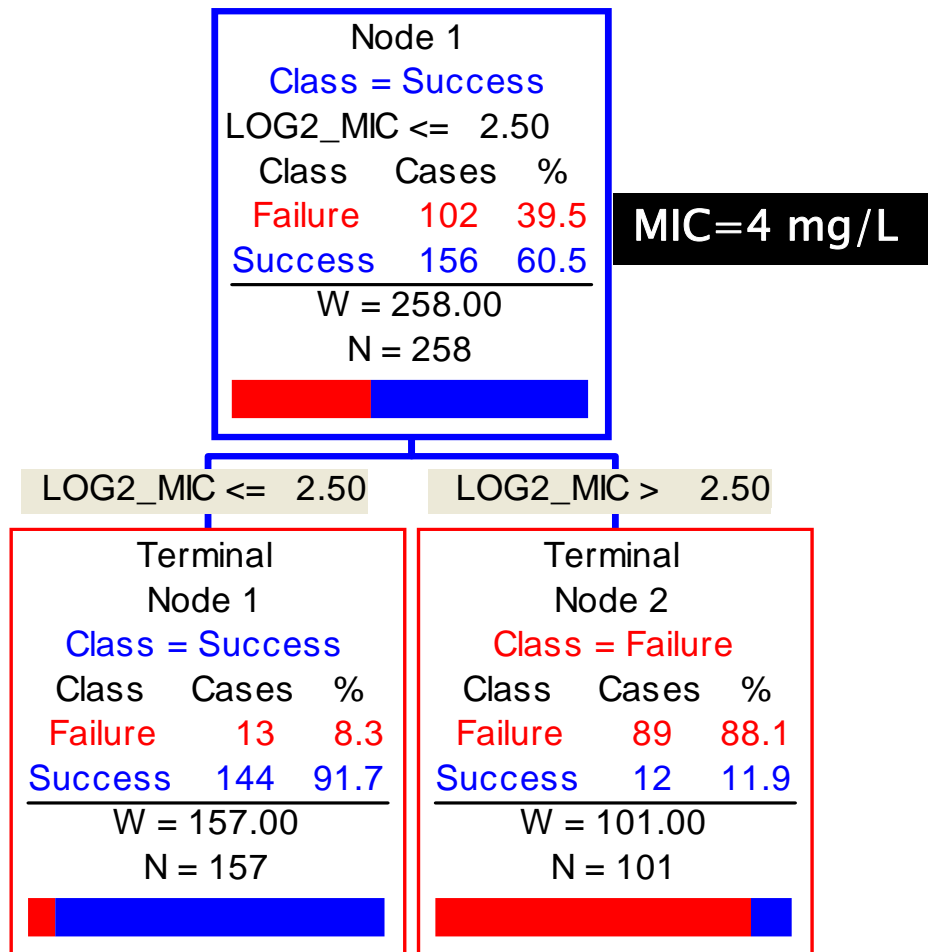
- Different models have been used:
  - Two decision trees: J48 and CART
  - A classification rule: One R
  - A probabilistic classifier: Naive Bayes
  - A logistic regression model: Simple logistic
- Performance of the tests were assessed by means of:
  - Sensitivity and specificity
  - False positive rate
  - Area under ROC curve
  - Matthews Correlation Coefficient (MCC)
- To check if different algorithms produce similar answers

# EUCAST clinical data

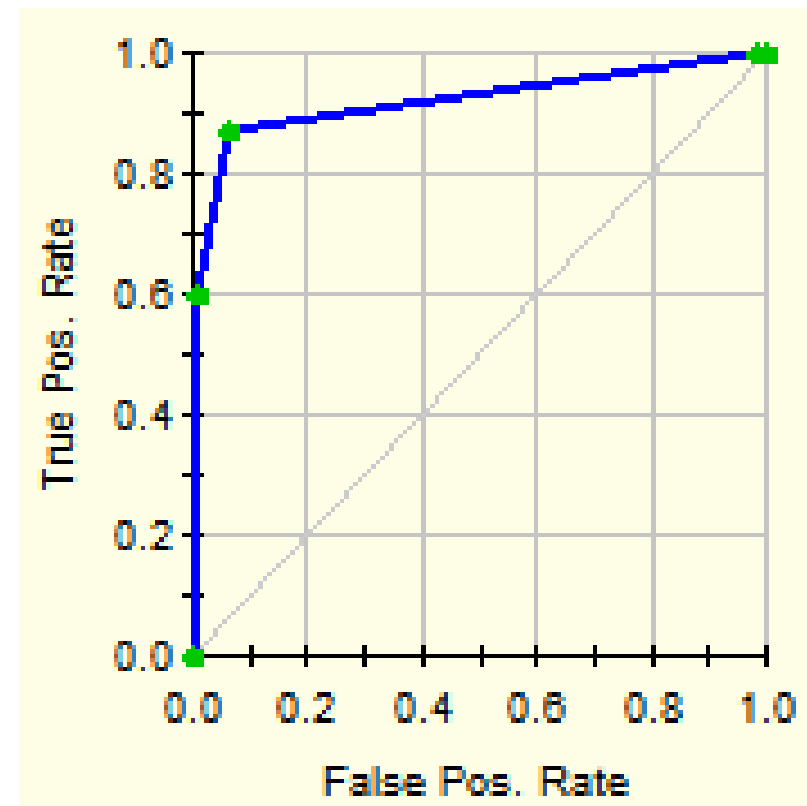
<b>Classifiers</b>	<b>MIC value for failures in mg/L</b>	<b>Sensitivity in %</b>	<b>False positive rate in %</b>	<b>Area under ROC curve</b>
<b>J48</b>	<b>&gt;4</b>	<b>87</b>	<b>8</b>	<b>0.86</b>
<b>CART</b>	<b>&gt;4</b>	<b>87</b>	<b>8</b>	<b>0.89</b>
<b>one R</b>	<b>&gt;4</b>	<b>87</b>	<b>8</b>	<b>0.89</b>
<b>Naïve Bayes</b>	<b>&gt;2</b>	<b>91</b>	<b>13</b>	<b>0.91</b>
<b>Simple Logistic</b>	<b>&gt;2</b>	<b>91</b>	<b>13</b>	<b>0.91</b>

# CART for all set: 258 cases

## Predicting failures



Relative error: 0.204  
ROC area: 0.89  
Sensitivity: 89%  
False positive rate: 8%



# Clinical BPs for fluconazole have been validated by data mining

Susceptible	Intermediate	Resistant
EUCAST		
$\leq 2$ mg/L	4 mg/L	$> 4$ mg/L

- Apply to *C. albicans*, *C. parapsilosis* and *C. tropicalis*
- But not to
  - *C. krusei* which is inherently resistant
  - *C. glabrata* because BPs divide wild type populations & only 9 cases for analysis

## Anidulafungin versus Fluconazole for Invasive Candidiasis

N Engl J Med 2007;356:2472-82.

Micafungin versus Caspofungin for Treatment  
of Candidemia and Other Forms of Invasive  
Candidiasis *Clinical Infectious Diseases* 2007;45:883-93

	<b>Fluconazole</b>	<b>Anidulafungin</b>	<b>Caspofungin</b>	<b>Micafungin</b>	
	800 mg/400 mg	200 mg/100 mg	70 mg/50 mg	100 mg	150 mg
<i>C. glabrata</i>	50	56	67	86	88

## % of species causing fungal infections

Specie	Candidemia N (%)	OPC N (%)	All N (%)
<i>Candida albicans</i>	73	132	205 (79.4)
<i>Candida parapsilosis</i>	27	---	27 (10.4)
<i>Candida tropicalis</i>	12	---	12 (4.6)
<i>Candida glabrata</i>	9	---	9 (3.4)
Other yeast*	5	---	5 (1.9)
All	126 (48.8)	132 (51.2)	258

Other yeast comprise an isolate of the following species: *C. krusei*, *C. guilliermondii*, *C. kefyr*, *C. dubliniensis* and *Geotrichum capitatum*

## Breakpoints for Susceptibility Testing Should Not Divide Wild-Type Distributions of Important Target Species<sup>▽</sup>

Maiken Cavling Arendrup,<sup>1\*</sup> Gunnar Kahlmeter,<sup>2</sup>  
Juan Luis Rodriguez-Tudela,<sup>3</sup> and J. Peter Donnelly<sup>4</sup>

- One *C. glabrata* susceptible (MIC = 2 mg/L) to fluconazole according EUCAST BPs

S	I	R
≤ 2	4	>4

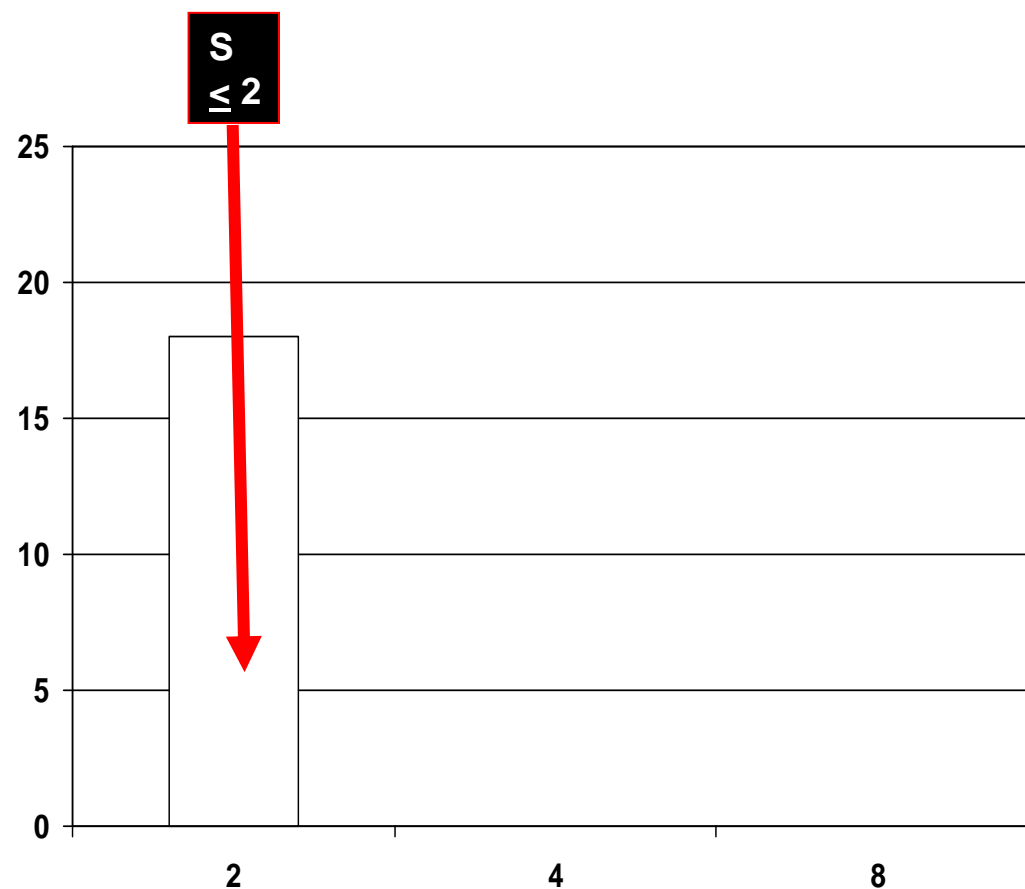
- 51 repetitions in different days

## Breakpoints for Susceptibility Testing Should Not Divide Wild-Type Distributions of Important Target Species<sup>▽</sup>

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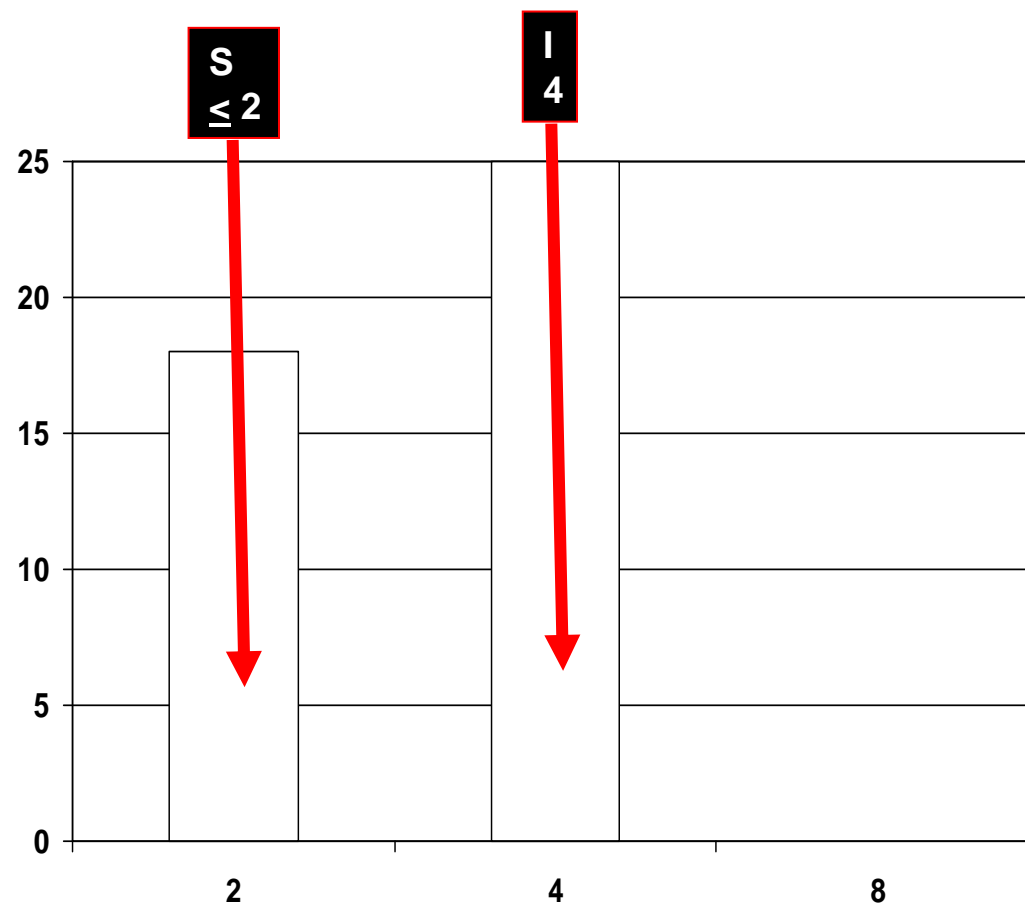
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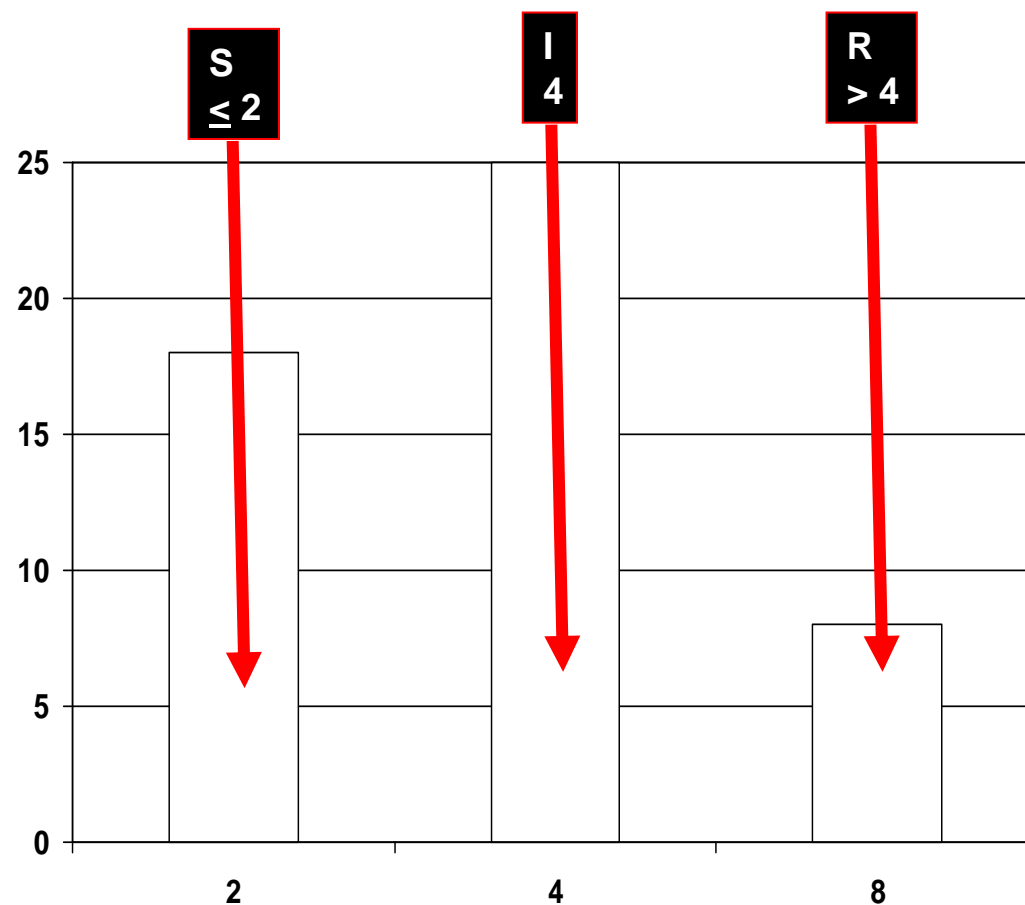
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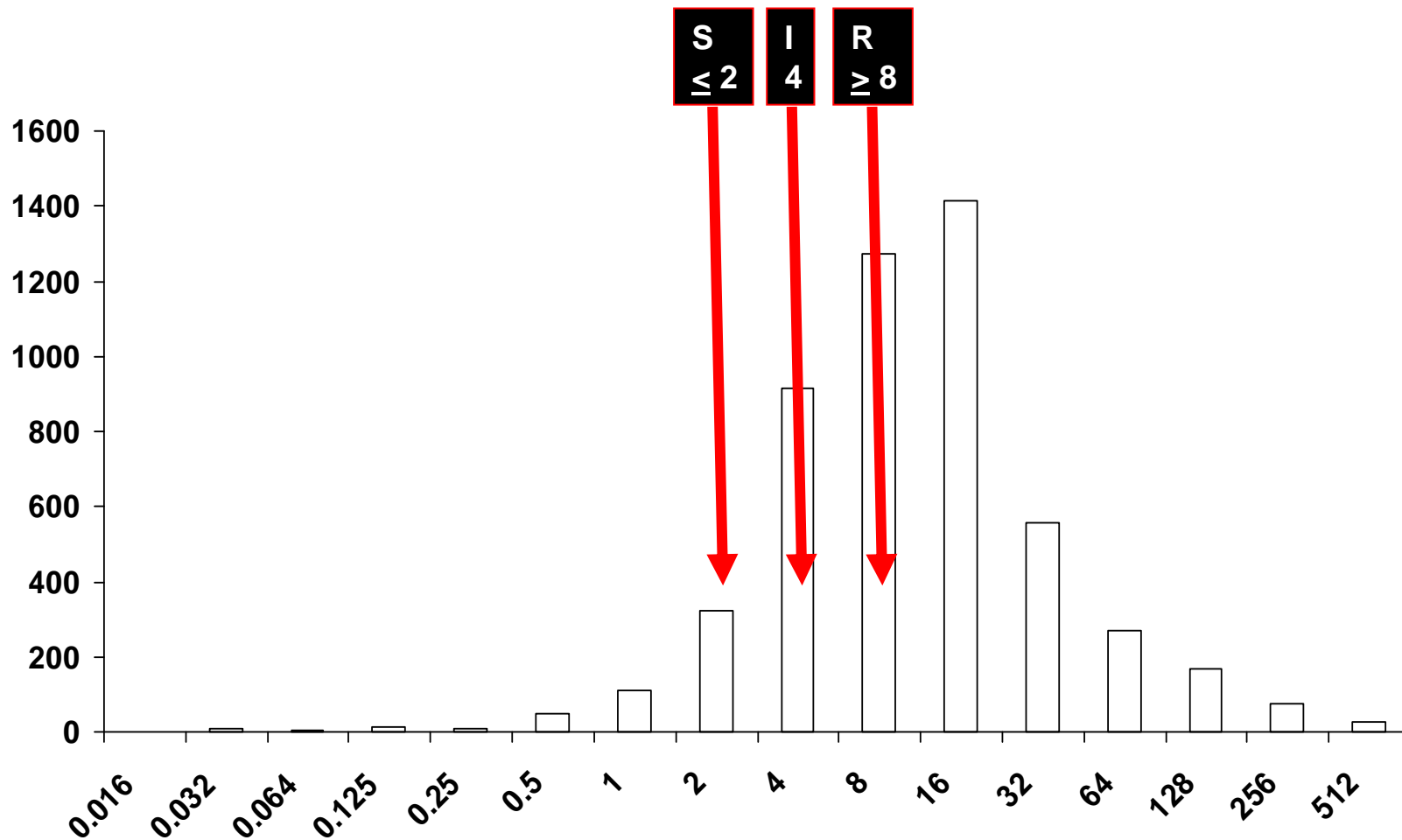
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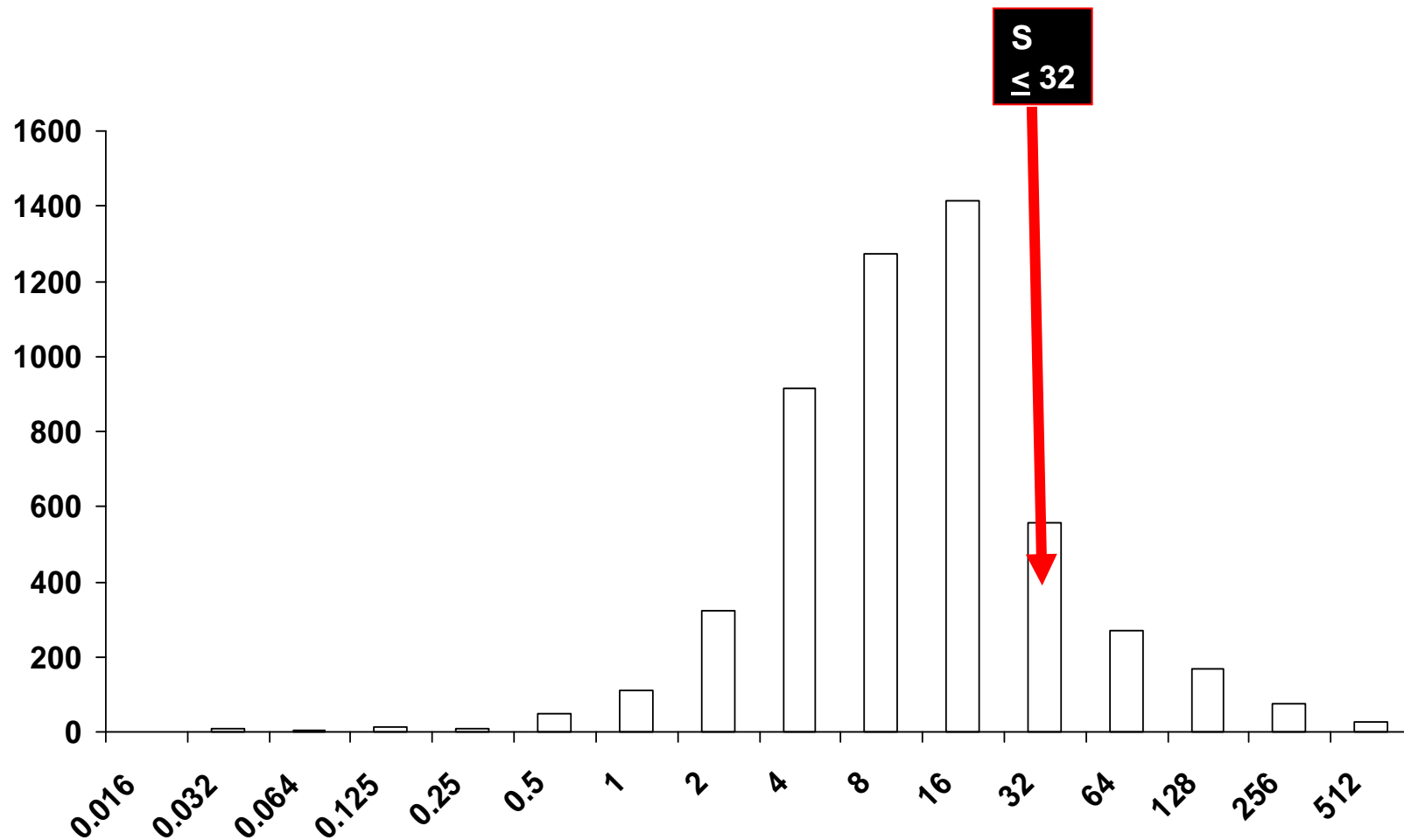


- 51 repetitions in different days

# BPs divide wild-type population of *C. glabrata*

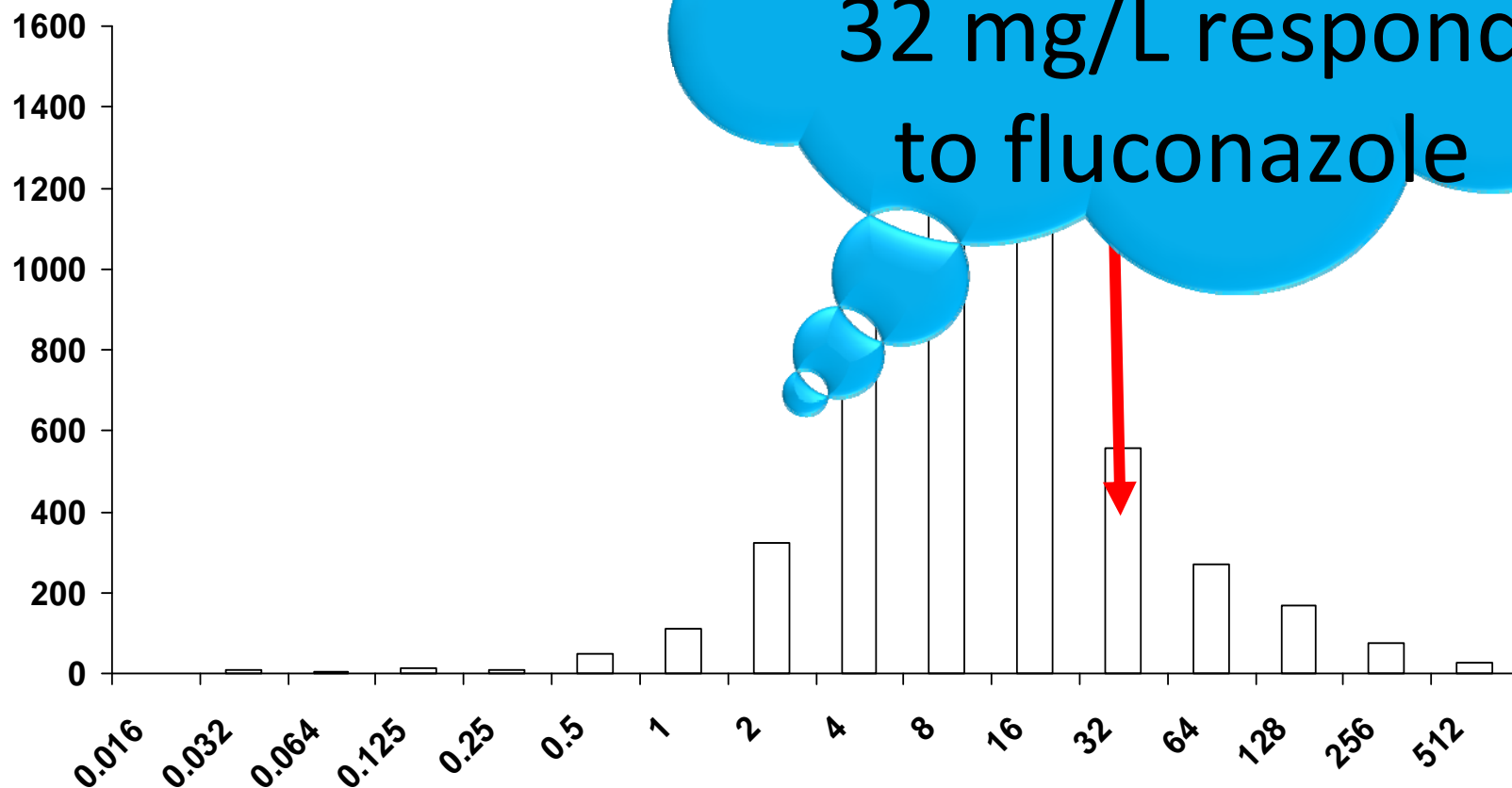


# BPs divide wild-type population of *C. glabrata*

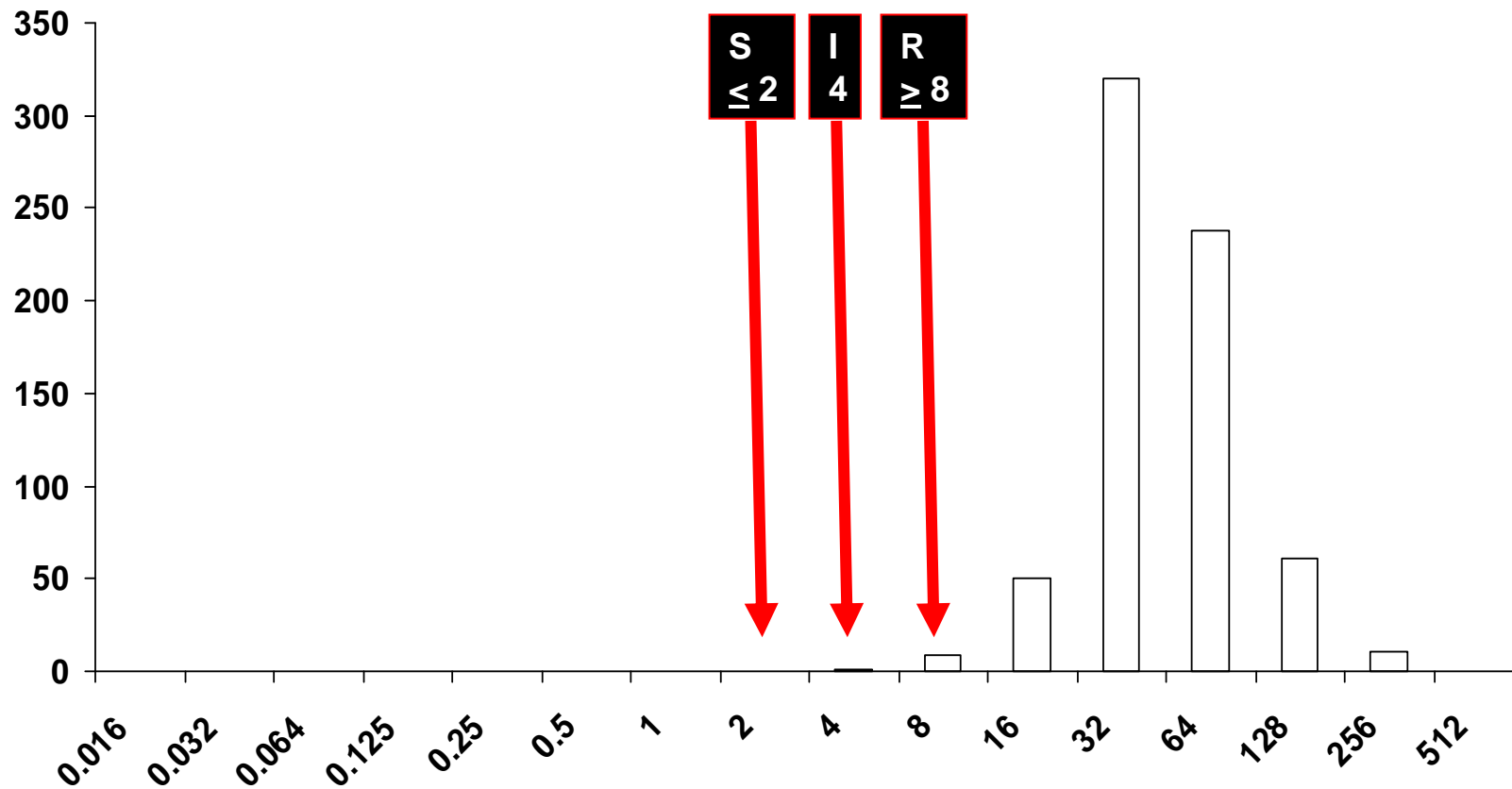


BPs divide

We do not have clinical data showing MICs  $\leq$  32 mg/L respond to fluconazole



# BPs identify *C. krusei* as intrinsically resistant to FZ



Evaluation by Data Mining Techniques of Fluconazole Breakpoints  
Established by the Clinical and Laboratory Standards Institute  
(CLSI) and Comparison with Those of the European  
Committee on Antimicrobial Susceptibility  
Testing (EUCAST)<sup>∇</sup>

Isabel Cuesta,<sup>1</sup> Concha Bielza,<sup>2</sup> Manuel Cuenca-Estrella,<sup>1</sup>  
Pedro Larrañaga,<sup>2</sup> and Juan L. Rodríguez-Tudela<sup>1\*</sup>

# Voriconazole

■ CLSI vs EUCAST

# Step 1: Voriconazole MIC distribution





## ■ MIC distribution

TABLE 1. Susceptibility of *Candida* species to voriconazole by MIC: ARTEMIS Global Antifungal Surveillance Program, 2001 to 2004<sup>a</sup>

Species (no. of isolates tested)	Cumulative % of strains at MIC ( $\mu\text{g/ml}$ ):										
	0.007	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8
<i>C. albicans</i> (4,701)	80	94	98	99	99	99	99	99	99	100	
<i>C. glabrata</i> (1,183)	1	1	3	14	40	70	86	92	95	99	100
<i>C. parapsilosis</i> (1,253)	19	61	81	90	95	98	99	99	99	99	100
<i>C. tropicalis</i> (963)	9	27	64	93	99	99	99	99	99	99	100
<i>C. krusei</i> (243)				1	13	61	95	100			
<i>C. lusitaniae</i> (110)	70	92	94	97	98	98	99	100			
<i>C. guilliermondii</i> (128)		6	15	66	93	98	99	99	100		
<i>C. kefyr</i> (38)	47	87	97	100							
<i>C. pelliculosa</i> (28)			4	4	64	93	100				
<i>Candida</i> spp. (55) <sup>b</sup>	18	40	51	80	89	98	98	100			
All <i>Candida</i> isolates (8,702)	48	64	75	82	88	94	98	99	99	99	100

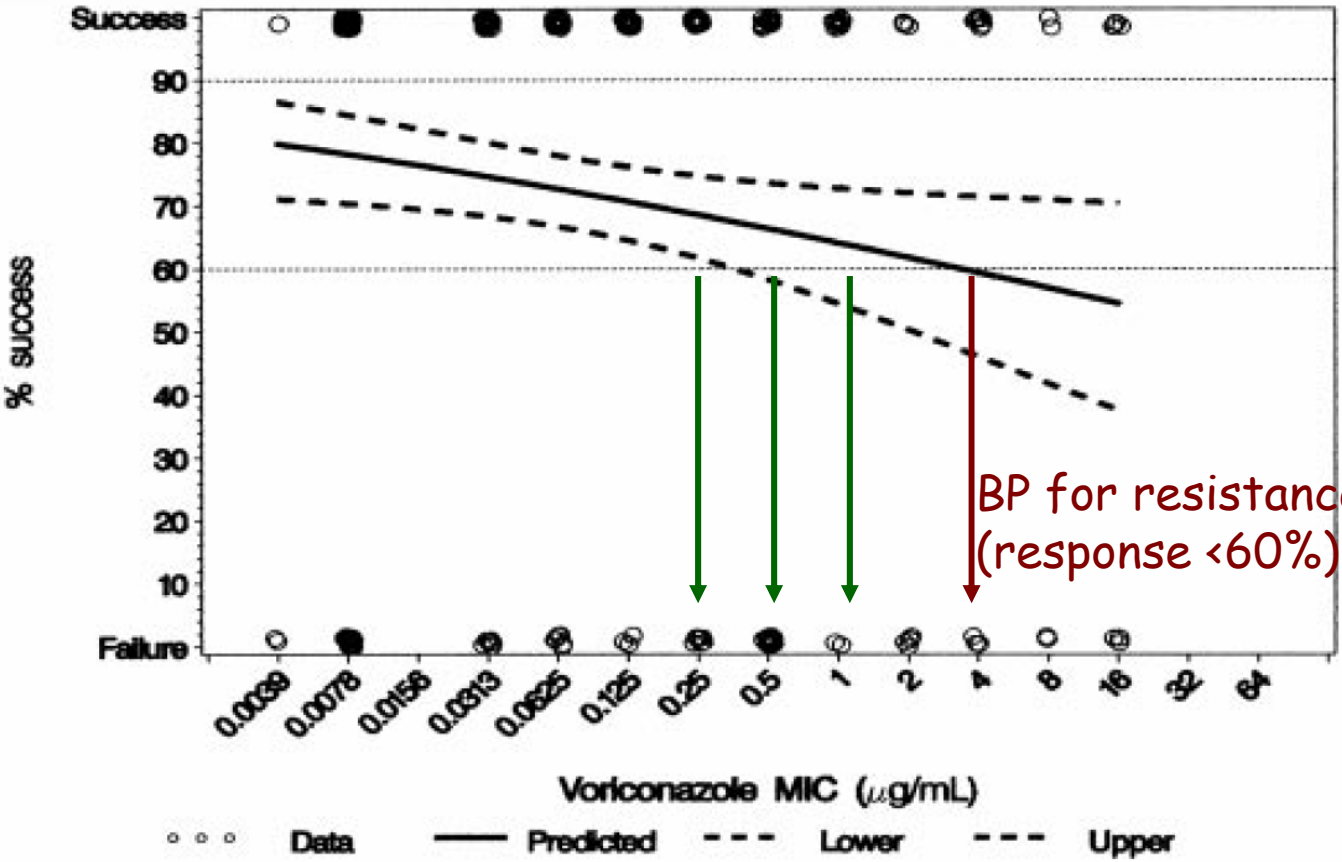
## Step 2: MIC - In vivo outcome

TABLE 3. *Candida* species, geometric mean MICs, and investigator-assessed response to voriconazole therapy<sup>a</sup>

Species	No. of isolates tested	Geometric mean MIC ( $\mu\text{g/ml}$ )	% Success
<i>C. albicans</i>	96	0.0164	72 
<i>C. parapsilosis</i>	34	0.0266	85 
<i>Candida</i> spp.	12	0.0712	92
<i>C. tropicalis</i>	51	0.1283	73 
<i>C. krusei</i>	9	0.3650	78
<i>C. glabrata</i>	47	0.7937	55 

<sup>a</sup> Broth microdilution MICs were determined in accordance with CLSI M27-A2. Baseline isolates from studies 603, 608, 309/604, and 301/606 were used.

# Step 2 Logistic analysis MIC-outcome




249 cases  
 BP suggestion  
 R >4 µg/ml  
 S ≤0.25 / 0.5 / 1 µg/ml ??

BP for resistance  
 (response <60%)

# CART analysis of studies # 603, 608, 309/604 & 301/606


Node 1			
Class = yes			
MIC ≤ 0.19			
Class Cases %			
no	70	28.1	
yes	179	71.9	
<hr/>			
W = 249.00			
N = 249			




MIC ≤ 0.19

MIC > 0.19

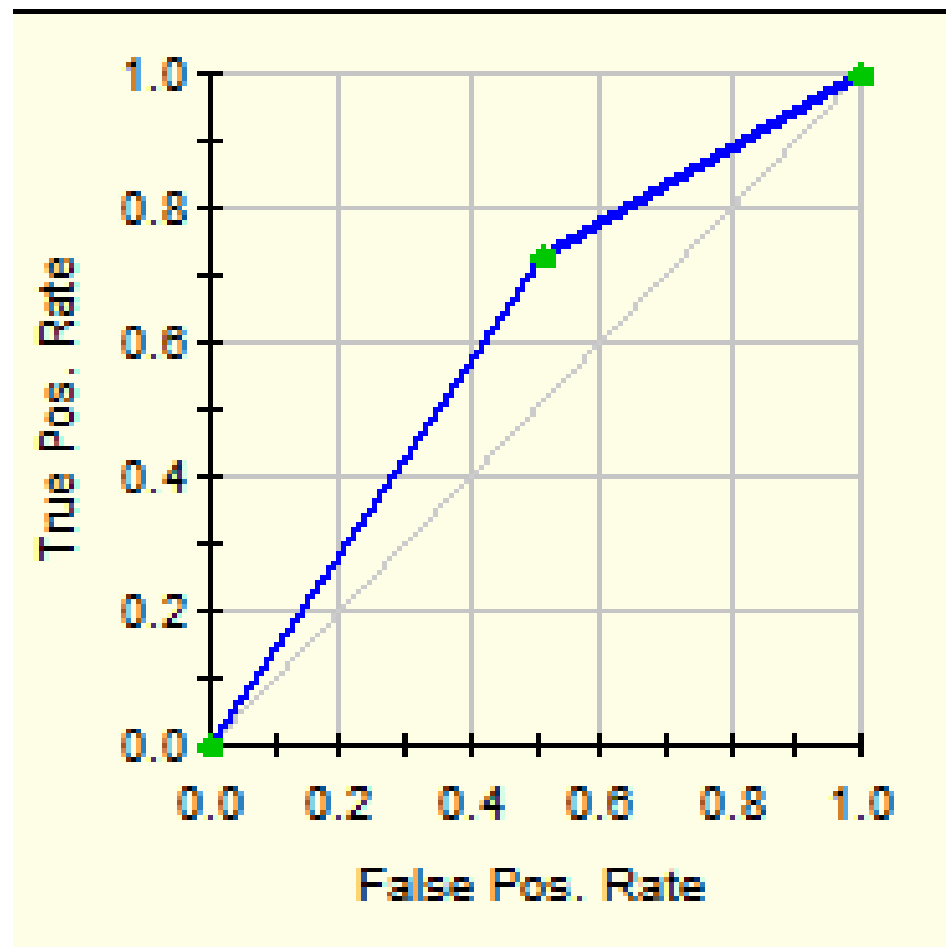
Terminal Node 1			
Class = yes			
Class Cases %			
no	36	21.6	
yes	131	78.4	
<hr/>			
W = 167.00			
N = 167			



Terminal Node 2			
Class = no			
Class Cases %			
no	34	41.5	
yes	48	58.5	
<hr/>			
W = 82.00			
N = 82			



ROC area for VZ: 0.608

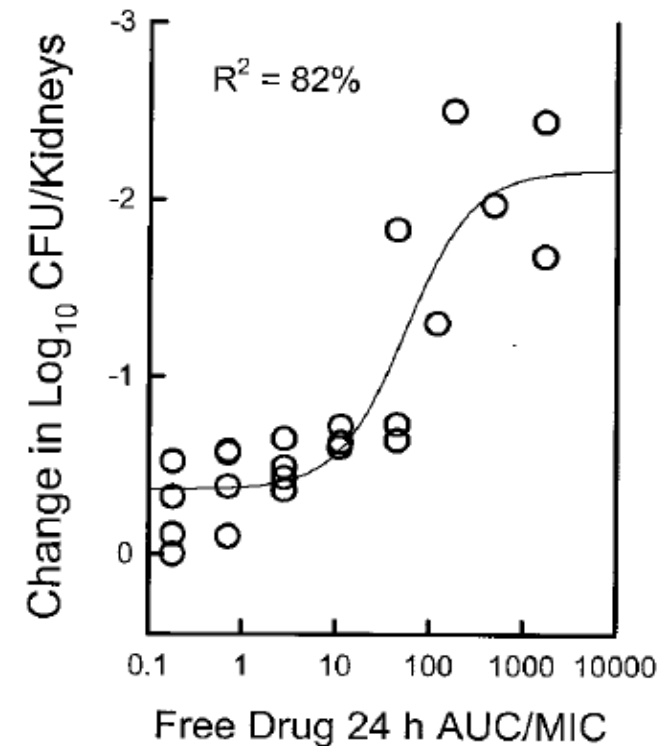


# Step 3: PK/PD data

Mouse model

Isolates: 10 *C. albicans* MIC 0.007 - 0.25 µg/ml

<i>C. albicans</i> strain	MIC (mg/liter)		ED <sub>50</sub> (mg/kg/24 h)	24-h AUC/MIC ratio	
	Voriconazole	Fluconazole		Total drug	Free drug
K1	0.007	0.25	7.93	81.4	17.9
W2	0.015	0.5	14.1	97	21.2
580	0.03	4.0	26.3	237	52
98-210	0.03	16	16.9	76	16.6
1490	0.03	0.5	19.7	60.8	13.3
5810	0.03	0.5	14.3	50.3	11.3
2-76	0.06	0.25	26	115	25.3
2183	0.06	>128	24.8	102	22.5
98-17	0.12	16	28.1	72	15.8
98-234	0.25	32	97.5	265	58
Mean ±SD					24 ± 17

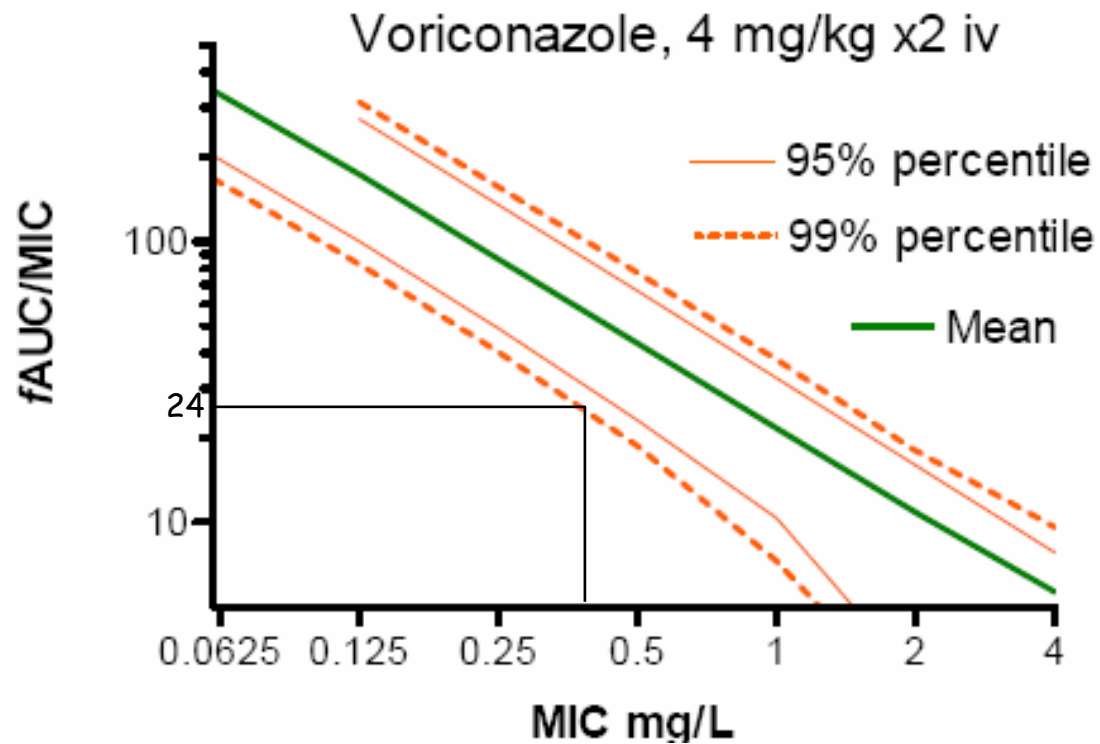


Human PK/PD

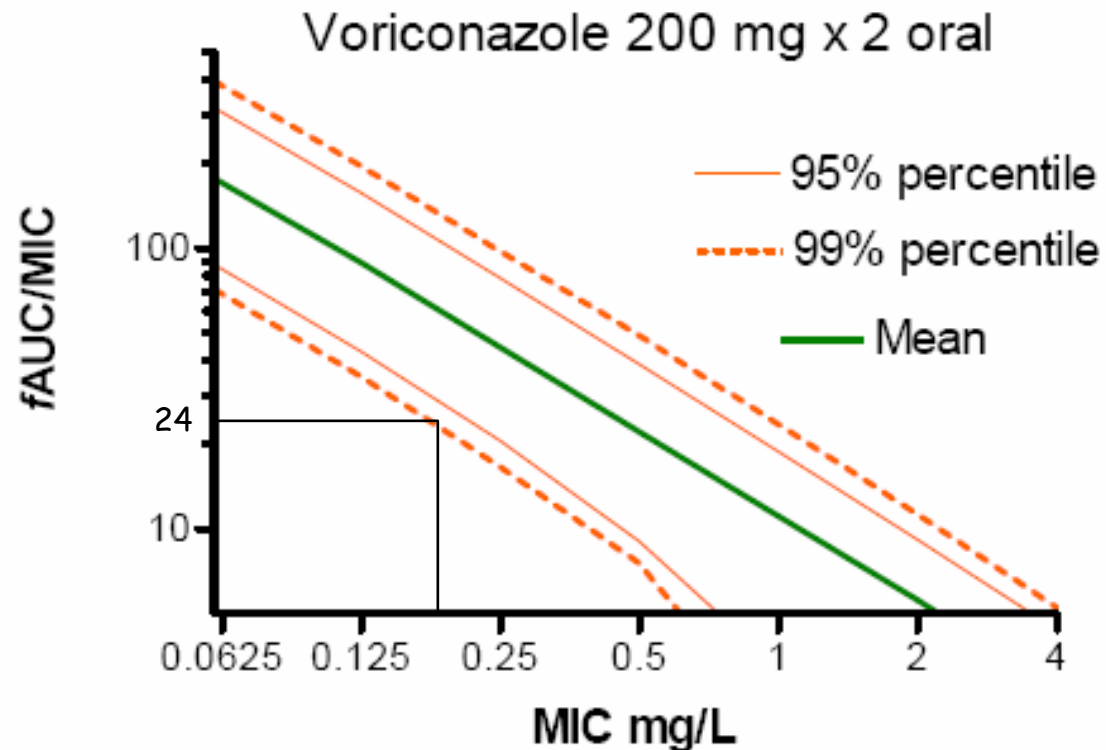
AUC ~ 20 µg\*h/ml

→ supports a susceptibility BP of 1 µg/ml

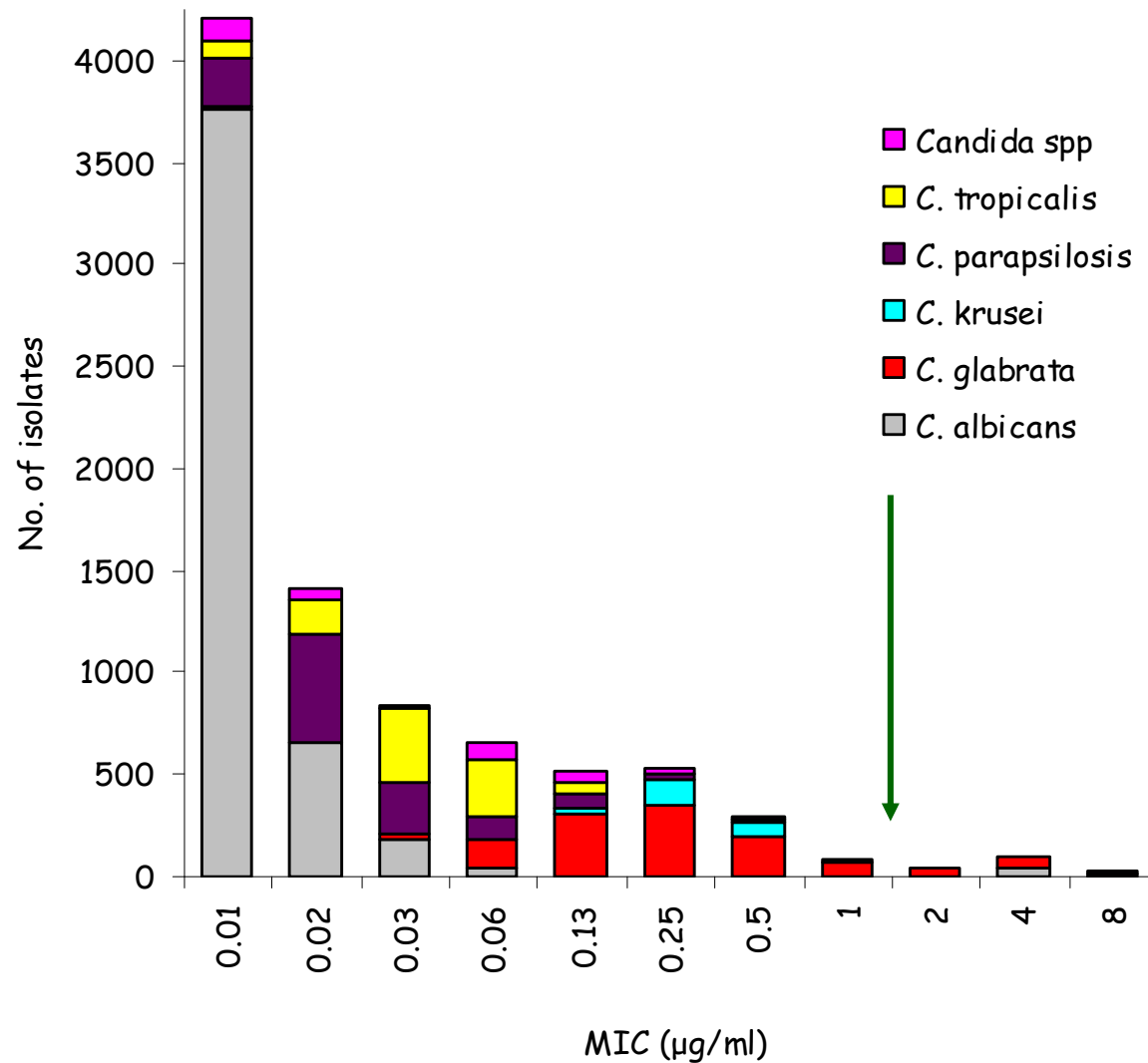
# Montecarlo simulation for a 24 PD target



# Montecarlo simulation for a 24 PD target



# Choice of susceptibility breakpoint



## Arguments for $S \leq 1 \mu\text{g/ml}$

### MIC distributions

Not bisect *C. glabrata* population  
 $\leq 1$  includes 99% of isolates

### Outcome

<60% response if MIC >4 µg/ml  
for average not for 95%  
population

### PK/PD

Serum levels frequently  $\geq 1 \mu\text{g/ml}$   
AUC/MIC will be  $\geq 24$ .

# European concerns

- Outcome ↓ *C. glabrata*
  - 55% versus 72-83% for *C. albicans*, *C. tropicalis* and *C. parapsilosis*
- Logistic regression for CLSI BPs
  - based on average population and not the lower 95% CI
- Montecarlo simulations:
  - Target AUC/MIC ~ 24 covers 99% population at a
    - MIC of 0.25 mg/L for iv
    - MIC of 0.125 for oral
- EUCAST data mining does not show any statistical support for in vitro in vivo outcome

# EUCAST approach for Voriconazole

## ■ ECOFFs as breakpoint

- *C. glabrata* and *C. krusei* No breakpoints, Insufficient data

Voriconazole	Rationale for the EUCAST clinical breakpoints, version 1																22 May 2008
2. MIC wild type distributions (based on MIC-values determined with EUCAST, CLSI and Etest methodology)																	
Species	0.0 02	0.0 04	0.0 08	0.0 16	0.0 32	0.0 64	0.1 25	0.2 5	0.5	1	2	4	8	16	32	64	ECOFF <sub>≤</sub> (mg/L)*
<i>Candida albicans</i>	50	996	513 9	523 5	11 07	53 0	20 7	10 6	63	42	18	13	18	22	78	6	<b>0.125</b>
<i>Candida glabrata</i>	0	5	26	63	19 2	53 4	12 18	12 44	78 1	34 1	19 6	13 9	69	20	2	6	<b>1</b>
<i>Candida krusei</i>	0	0	2	10	23	29	15 2	48 9	42 4	11 9	30	10	1	0	0	0	<b>1</b>
<i>Candida parapsilosis</i>	0	6	287	132 6	51 0	19 7	12 3	77	23	16	3	2	1	0	0	0	<b>0.125</b>
<i>Candida tropicalis</i>	0	28	133	602	93 4	73 2	27 4	11 3	51	32	8	2	10	20	16	3	<b>0.125</b>

# **Echinocandins BPs**

# Step 1 MIC distributions & ECOFFs

## ■ Anidulafungin

2. MIC wild type distributions (based on MIC-values determined with EUCAST and Etest methodology*)																			
Species	≤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	Tot	ECOFF <sub>≤</sub> (mg/L)**	
<i>Candida albicans</i> Full distributions	284	<u>360</u>	208	77	16	6	4	1	1							1	958	0.016-0.032	
					618	3	0	0	1	1	0	0	0	0	0	0	623		
				233	5	1	0	1	0	0	0	0	0	0	0	0	240		
<i>Candida glabrata</i> Full distributions	55	38	60	<u>149</u>	62	8	6	8	2	2	0	0	2	0	0	0	392	0.032-0.064	
					233	1	1	0	2	0	0	0	0	0	0	0	237		
				0	13	49	16	1	0	1	1	0	0	0	0	0	81		
<i>Candida krusei</i> Full distributions	2	1	12	<u>21</u>	7	12	2	3	0	0	0	0	0	0	0	0	60	0.064	
					58	7	0	0	0	0	0	0	0	0	0	0	65		
																	0		
<i>Candida parapsilosis</i>	0	3	1	0	4	6	2	36	78	<u>171</u>	96	13	7	0	0	2	419	4	
<i>Candida tropicalis</i> Full distributions	18	19	12	<u>30</u>	22	4	4	1	0	0	0	0	0	0	0	0	110	0.032-0.064	
					120	2	0	0	0	0	0	0	0	0	0	0	122		
				8	22	11	0	0	0	0	0	0	0	0	0	0	41		
<i>Candida guilliermondii</i>	0	0	0	0	0	1	0	2	5	<u>17</u>	6	1	0	0	1	0	33	4	

## Step 2: MIC - outcome data

■ No such data using EUCAST!

■ Anidulafungin superior to fluconazole

- *C. albicans* (135 patients) (81% × 62%) P<0.05
- *C. tropicalis* (22 patients) (93% × 50%) P<0.05
- *C. glabrata* (38 patients) (56% × 50%)
- *Candida* spp. (7 patients) (75% × 67% )

} Good targets  
for  
anidulafungin

■ Anidulafungin inferior to fluconazole

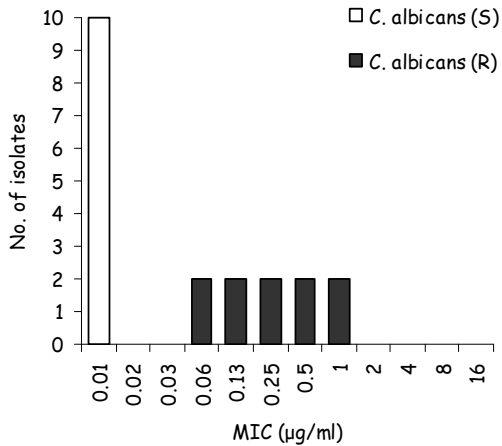
- *C. parapsilosis* (23 patients) (64% × 83%)

} Poor target  
for  
anidulafungin

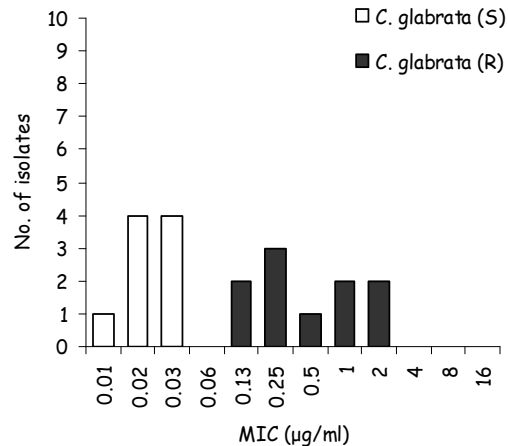
# Step 2: MIC distribution *fks* mutants

HZA

*C. albicans*



*C. glabrata*



MICs for *fks* mutants

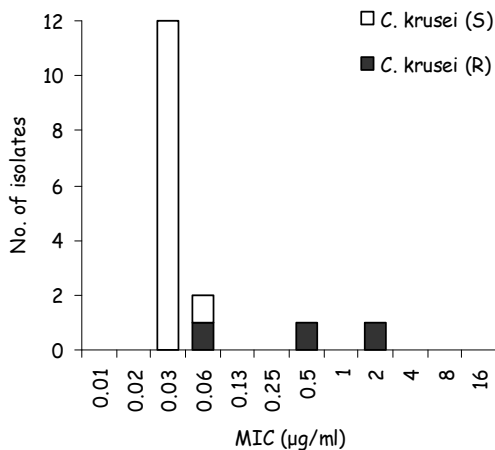
*C. albicans* >0.03

*C. glabrata* >0.06

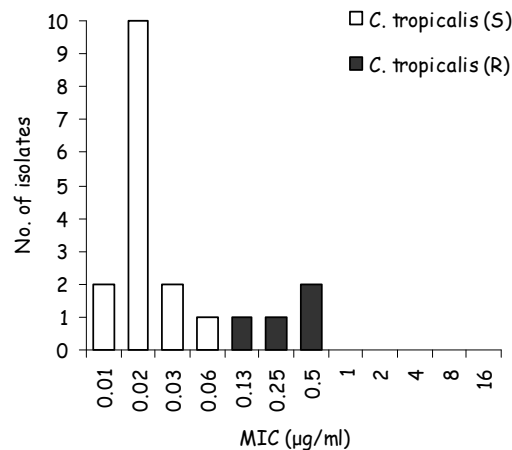
*C. krusei* >0.03/ >0.255

*C. tropicalis* >0.06

*C. krusei*



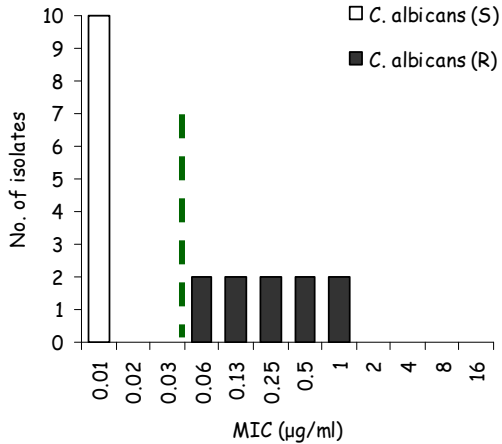
*C. tropicalis*



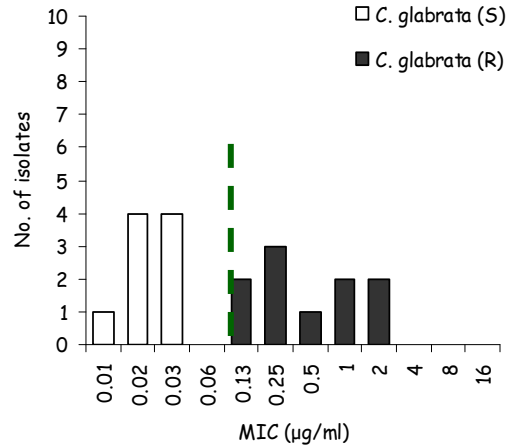
# Step 2: MIC distribution *fks* mutants

HZA

*C. albicans*



*C. glabrata*



MICs for *fks* mutants

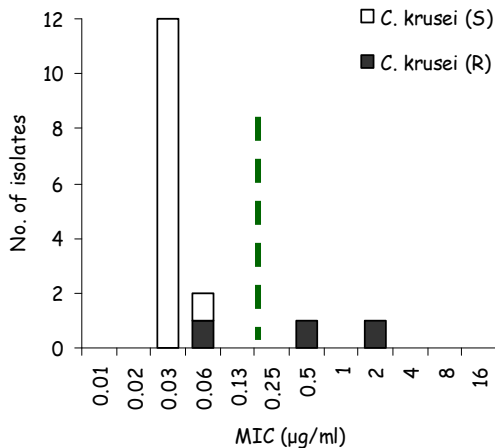
*C. albicans* >0.03

*C. glabrata* >0.06

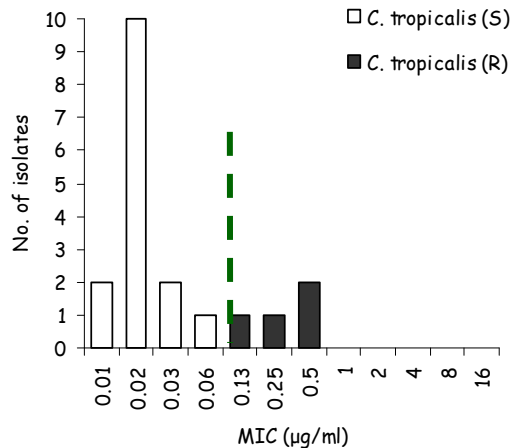
*C. krusei* >0.03/ >0.255

*C. tropicalis* >0.06

*C. krusei*



*C. tropicalis*



# Step 3: No PK/PD → ECOFFs as BP

## Clinical breakpoints

*C. albicans*: S ≤0.03, R >0.03 mg/L

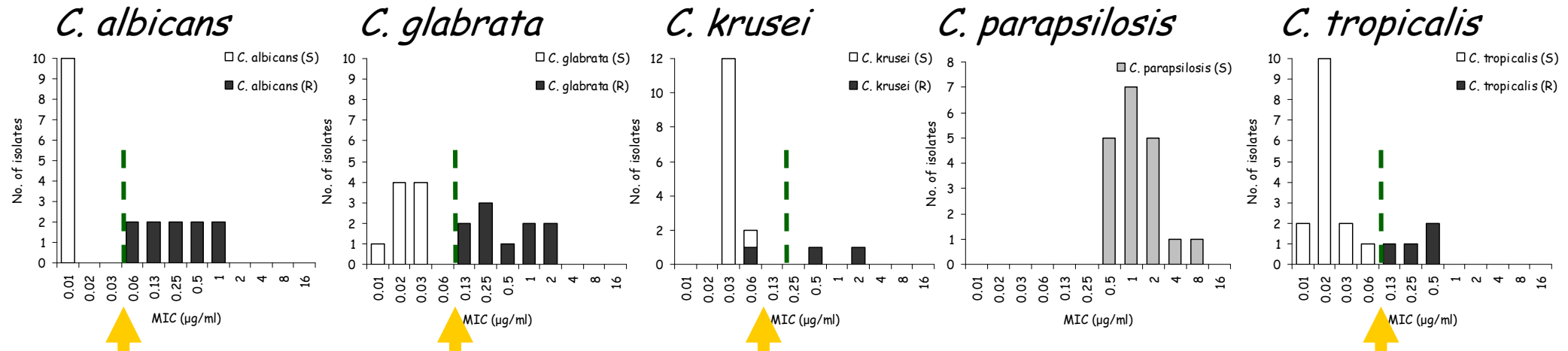
*C. glabrata*: S ≤0.06, R >0.06 mg/L

*C. tropicalis*: S ≤0.06, R >0.06 mg/L

*C. krusei*: S ≤0.06, R >0.06 mg/L

*C. parapsilosis* & *C. guilliermondii*: no Breakpoints (not good targets)

HZA



# Mis-classifications using CLSI BP

- CLSI BP: Susceptible  $\leq 2 \mu\text{g/ml}$

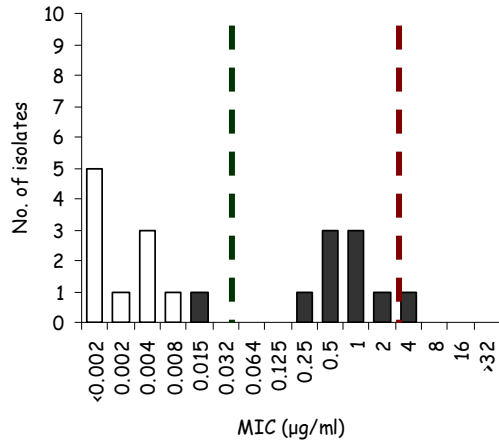
*fks* hot spot mutants classified as "S"

	Anidulafungin	Caspofungin	Micafungin
VMEs CLSI	89.2%	60.7%	92.9%

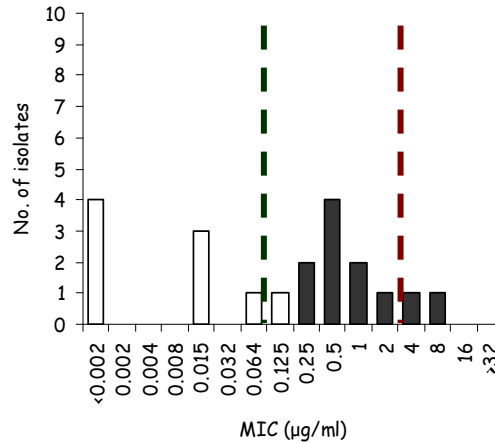
# Etest and Anidulafungin

HZA

*C. albicans*

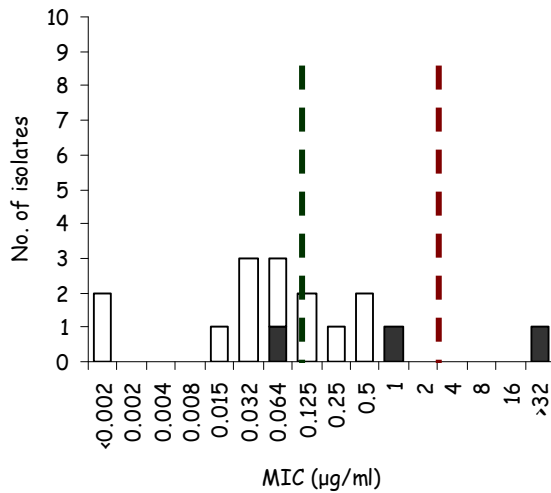


*C. glabrata*

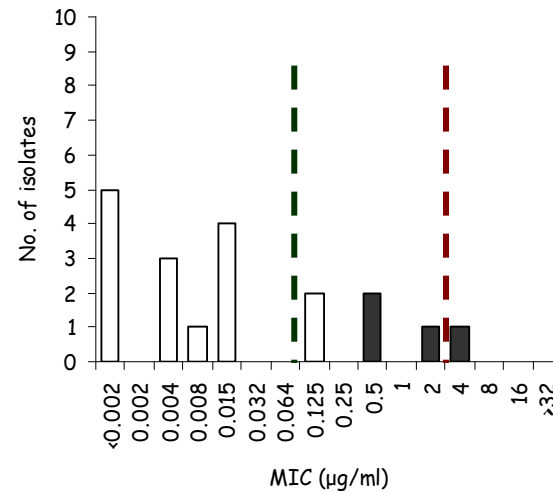


EUCAST draft BP  
CLSI BP

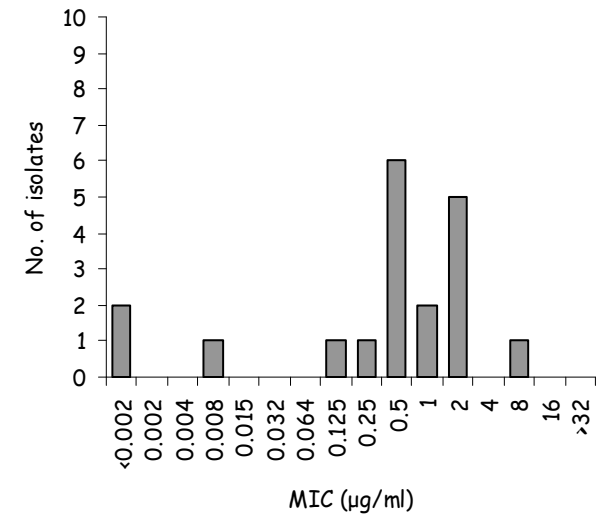
*C. krusei*



*C. tropicalis*



*C. parapsilosis*



# Agenda

- Summary of current standards and selected commercial methods
- BPs and BP setting process
  - CLSI
  - EUCAST
- Indications for AFST
- Conclusion

# When?

## ■ Guiding therapy

- Invasive mycoses
- Immunocompromised patients
- Failure cases
- Breakthrough isolates
- preceding AF therapy

## ■ Surveillance etc.

- Epidemiologic studies
- Uncommon species
- New compounds
- Cross resistance issues
- Breakpoint development process

# How?

- Standardized methodology
  - EUCAST
  - CLSI
- Commercial method validated against reference methodologies
  - ASTY, Etest, Sensititre, Vitek
- Careful selection of appropriate breakpoints

# Conclusion - Why do we differ??

## ■ CLSI BPs

- species with highest MIC drives the BP
- BPs have not been adjusted to change in methodology
- Risk of misclassifying R isolates as S. Very major errors !!!!

## ■ EUCAST BPs

- Conservative approach
- Clinical correlation has to have statistical support
- if data arise showing isolates with resistance mechanisms are good targets BPs have to be raised

## ■ My recommendation: Use EUCAST BPs

# Antifungal "S, R" breakpoints for *Candida*

Breakpoints given as: S:  $\leq X$ ; R:  $> Y$

Proposed Breakpoints- not yet approved!!

	CLSI	EUCAST (specific for)	NRMM (2009)
Ampho	$\leq 1$	$\leq 1$ ; $> 1$	$\leq 1$ ; $> 1$
Anidula	$\leq 2$	$\leq 0.032$ ; $> 0.032$ ( <i>C. alb</i> ) $\leq 0.064$ ; $> 0.064$ ( <i>C. g,k,t</i> )	$\leq 0.125$ DK $\leq 0.5$ SE
Caspo	$\leq 2$	-	$\leq 1$ DK, NO $\leq 2$ SE, FI
Mica	$\leq 2$	-	-
Fluco	$\leq 8$ ; $> 32$	$\leq 2$ ; $> 4$ ( <i>C. a,t,p</i> )	$\leq 2$ ; $> 4$
Itra	$\leq 0.125$ ; $> 0.5$	-	$\leq 0.125$ ; $> 0.5$
Posa	-	$\leq 0.064$ ; $> 0.064$ ( <i>C. a,t,p</i> )	$\leq 0.125$ DK, SE
Vori	$\leq 1$ ; $> 2$	$\leq 0.125$ ; $> 0.125$ ( <i>C. a,t,p</i> )	$\leq 0.125$



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Thank you for your attention