Symposium 16: Antifungal susceptibility testing

Commercial tests

Joseph Meletiadis Ph. D.
Assistant Prof. in Mycology

Clinical Microbiology Laboratory, “Attikon” University Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece

TIMM 2015

Increased Interest in Antifungal Susceptibility Testing

- Different susceptibility profiles among isolates and species (susceptible-resistant)
- Changing epidemiology of isolated organisms (new species-acquired drug resistance)
- Newer drugs - more choices
- Drug discovery
- Optimizing therapy
- Predict patient outcome

ITMM 2013

Turner et al. *Expert Opin Emerg Drugs*. 2006;11(2):231–250.
Maertens et al. *Curr Med Chem-Anti-infective Agents.* 2002;1:65–81.

Reference methods

CLSI

- M27-A3 Yeast broth dilution
- M38-A2 Mould broth dilution
- M44-A2 Yeast disk diffusion
- M51-A Mould disk diffusion

EUCAST

- E.def. 7.2 Yeast broth dilution
- E.def. 9.1 Aspergillus broth dilution

IUMS 2015

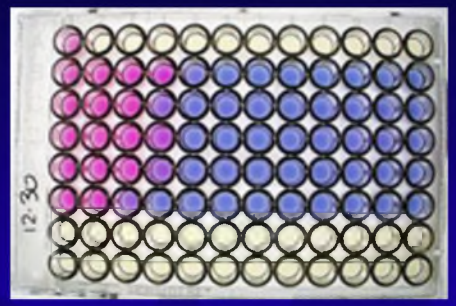
Challenges for reference methods

- ✓In-house preparation
- ✓Cumbersome for routine setting
- ✓Requires expertise particularly for visual reading of trailing growth
- ✓RPMI w/o glucose is a poor medium
- ✓Variation with caspofungin
- ✓Amphotericin B resistance
- ✓Echinocandins testing of *Aspergillus* spp.

ECMM 2015

Commercial tests

- ✓ **Broth microdilution (colorimetric) tests**
  - Sensititre YeastOne (FDA approved)
  - ATB fungus,
  - SensiQuatro,
  - Fungitest,
  - Candifast,
  - Micronaut,



- ✓ **Agar diffusion tests on strip, disc or tabled format**
  - Etest (FDA approved),
  - MIC test strips
  - NeoSensitab

- ✓ **Automated systems**
  - Vitek2 (FDA approved)

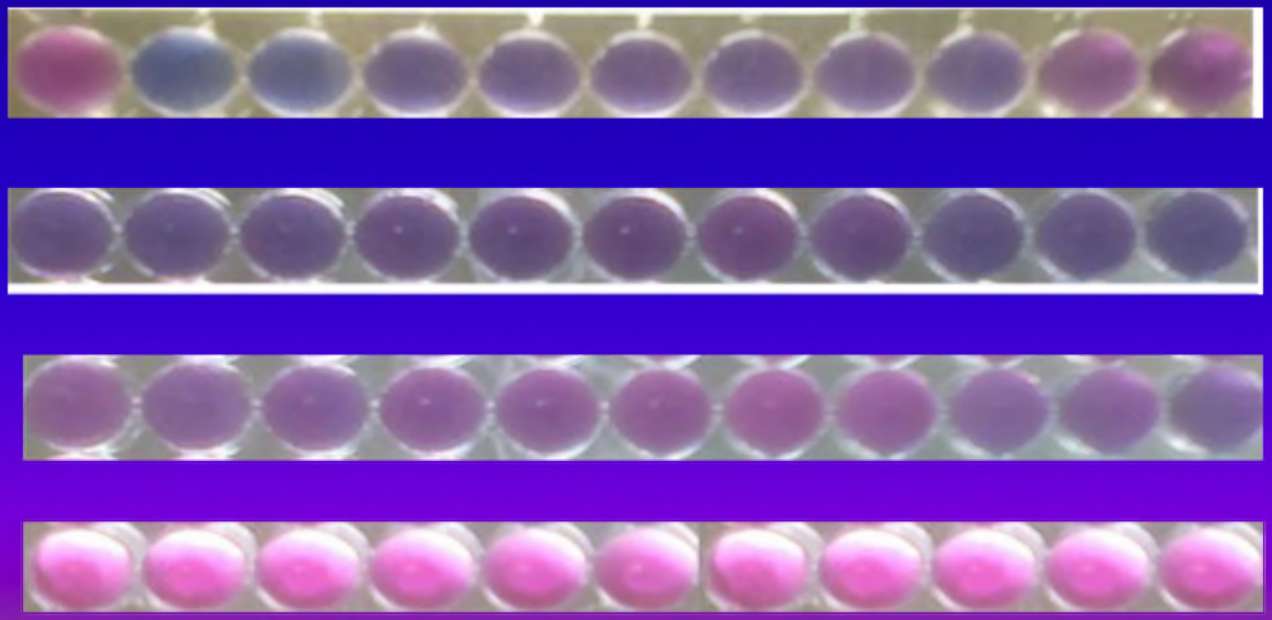
TMM 2017

Sensititre YeastOne

Drug concentrations

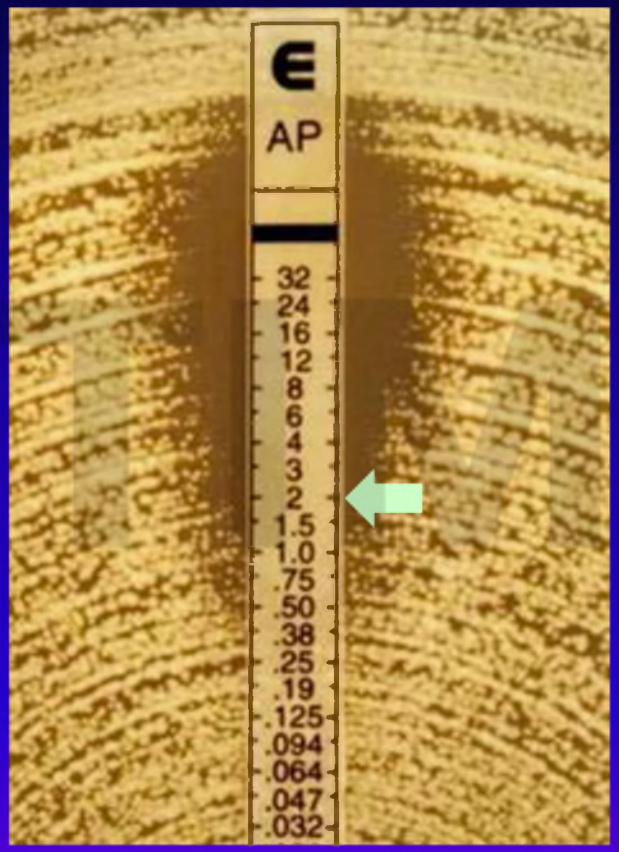
GC Low High

Red =growth, Purple=growth inhibition Blue = no growth.



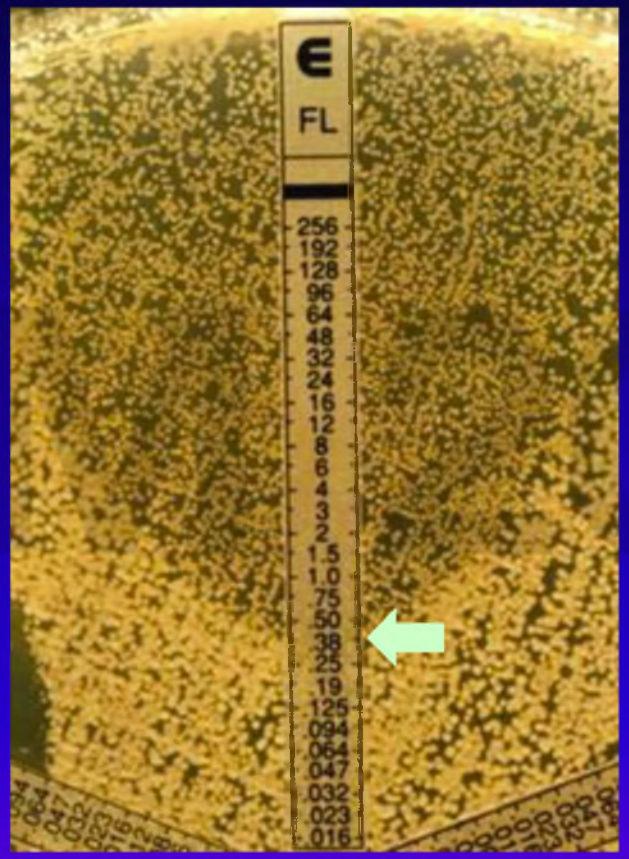
Etest

Amphotericin B



Read at 100% inhibition-clear zone

Fluconazole



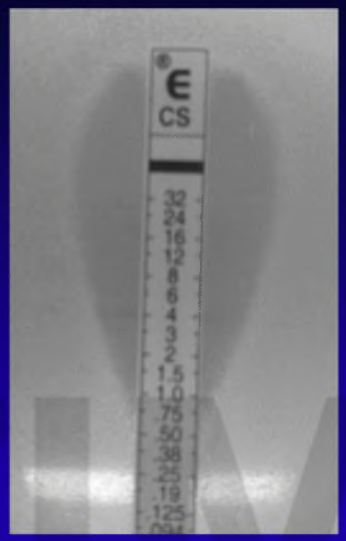
Ignore light growth and microcolonies

Caspofungin

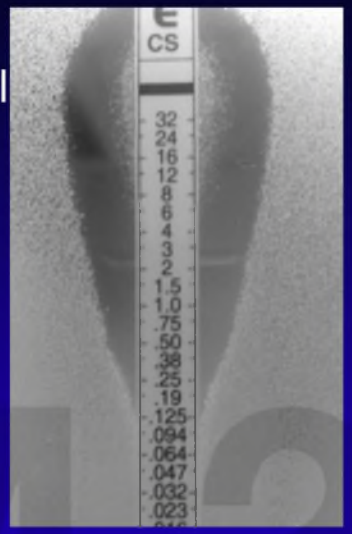
Bottom of the dip
Ignore microcolonies and regrowth

"Dip effect" phenomenon

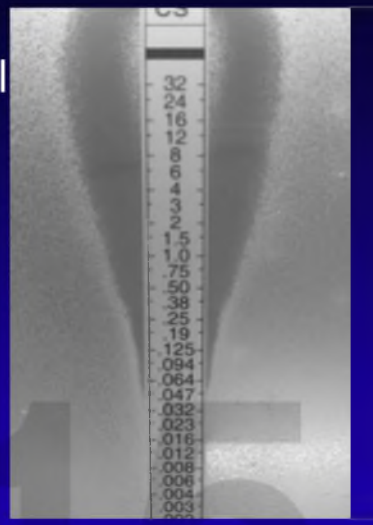
MIC=2 mg/l

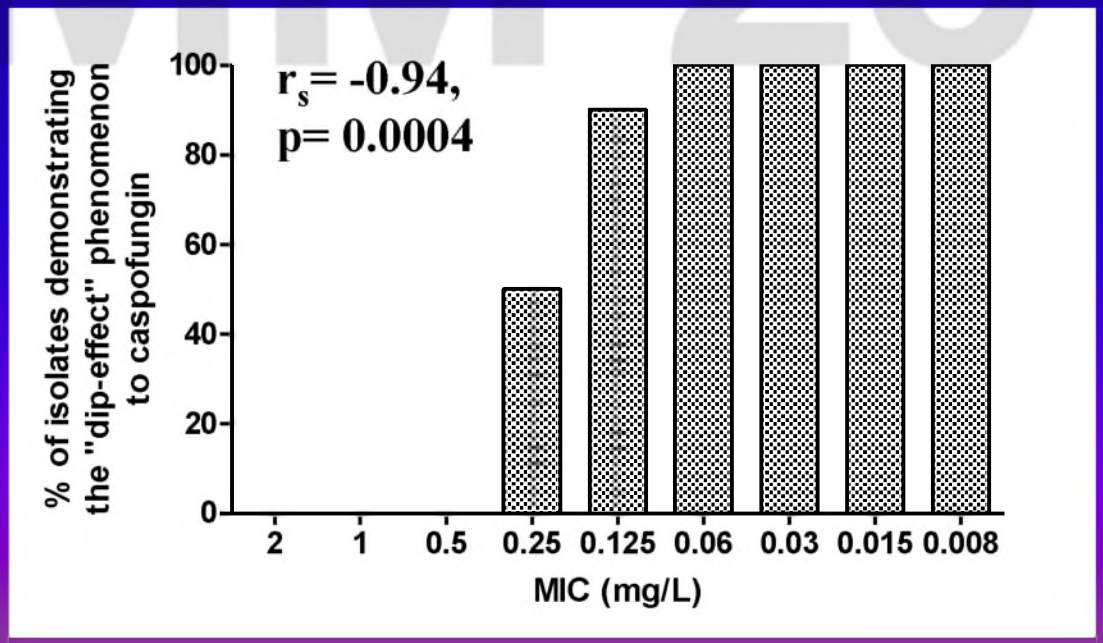


MIC=0.12 mg/l



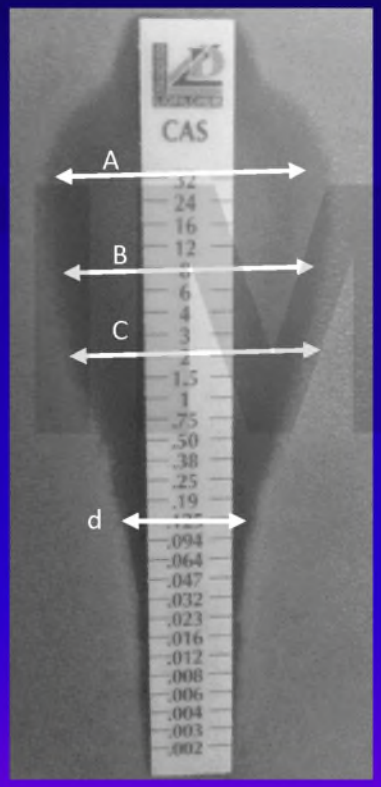
MIC=0.06 mg/l

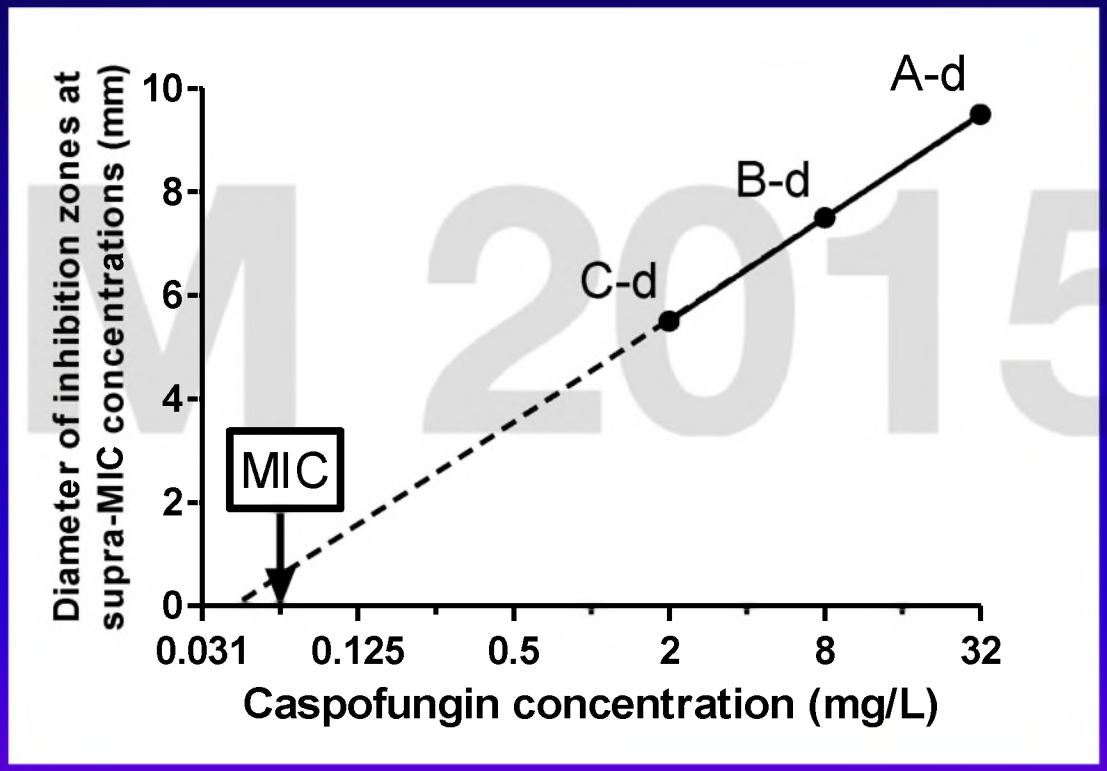




Siopi et al JCM 2015

New approach of caspofungin MIC determination for isolates with "dip effect"





Increased agreement with CLSI from 63% to >86%

Siopi et al JCM 2015

Vitek

AST-YS06 (412 610)			S Breakpoint	
Antifungal compound	Card conc.	MIC range reported	EUCAST	CLSI
Amphotericin B	1, 4, 16, 32	≤0.25 - ≥16	1	1
Fluconazole	1, 4, 8, 16	≤1 - ≥64	2	2
Flucytosine	4, 8, 16, 64	≤1 - ≥64	-	4
Voriconazole	0.5, 1, 4, 8	≤0.12 - ≥8	0.12	0.12
NEW Caspofungin	1, 4, 8	≤0.25 - ≥4	-	0.25/0.125

C. glabrata

TIMM 2015

Astvad AAC 2013

Test-specific ECV

TABLE 3 Comparison of ECVs obtained

Species	No. of isolates tested	Agent	Sensititre YeastOne	CLSI[b]
C. albicans	944	FZ	2 (94.4)	0.5 (98.1)
	942	ITR	0.5 (96.7)	0.12 (95.0)
	673	POS	0.25 (97.6)	0.06 (98.4)
	849	VOR	0.06 (96.7)	0.03 (99.0)
C. parapsilosis	642	FZ	4 (86.1)	2 (93.2)[c]
	642	ITR	0.5 (99.4)	0.5 (99.7)
	392	POS	0.12 (93.9)	0.25 (99.3)[c]
	559	VOR	0.06 (86.9)	0.12 (97.8)[c]
C. tropicalis	175	FZ	8 (94.9)	2 (98.4)
	175	ITR	1 (93.7)	0.5 (97.8)
	125	POS	2 (93.6)	0.12 (97.8)
	167	VOR	0.25 (90.4)	0.06 (97.3)
C. glabrata	212	FZ	128 (98.6)	32 (91.5)
	212	ITR	4 (95.8)	2 (95.2)
	173	POS	4 (96.0)	2 (96.1)
	205	VOR	2 (98.5)	0.5 (90.4)
C. krusei	53	FZ	256 (100)	64 (99.8)
	53	ITR	1 (98.1)	1 (99.0)
	33	POS	1 (100)	0.5 (99)
	48	VOR	1 (97.9)	0.5 (99.4)

TIMM 2015

Canton AAC 2013

Sensititre YeastOne and 24h CLSI

Species (no. of isolates tested)	Antifungal agent	% of MICs by category[a,b]			CA%	% of errors[c]		
		S	I	R		VME	ME	Minor
C. albicans (174)	Anidulafungin	100.0	0.0	0.0	100.0	0.0	0.0	0.0
	Caspofungin	100.0	0.0	0.0	98.3	0.0	0.0	1.7
	Micafungin	100.0	0.0	0.0	100.0	0.0	0.0	0.0
C. glabrata (87)	Anidulafungin	95.4	3.4	1.2	93.1	1.1	0.0	5.8
	Caspofungin	83.9	11.5	4.6	89.7	1.1	1.1	8.1
	Micafungin	97.6	1.2	1.2	98.9	0.0	0.0	1.1
C. parapsilosis (90)	Anidulafungin	98.9	1.1	0.0	96.7	0.0	0.0	3.3
	Caspofungin	100.0	0.0	0.0	100.0	0.0	0.0	0.0
	Micafungin	100.0	0.0	0.0	100.0	0.0	0.0	0.0
C. tropicalis (73)	Anidulafungin	98.6	1.4	0.0	100.0	0.0	0.0	0.0
	Caspofungin	100.0	0.0	0.0	100.0	0.0	0.0	0.0
	Micafungin	98.6	0.0	1.4	100.0	0.0	0.0	0.0
C. krusei (81)	Anidulafungin	100.0	0.0	0.0	98.8	0.0	0.0	1.2
	Caspofungin	76.5	23.5	0.0	69.1	1.2	0.0	29.7
	Micafungin	100.0	0.0	0.0	100.0	0.0	0.0	0.0
C. lusitaniae (75)	Anidulafungin	100.0		0.0	100.0	0.0	0.0	0.0
	Caspofungin	100.0		0.0	100.0	0.0	0.0	0.0
	Micafungin	98.7		1.3	98.7	1.3	0.0	0.0
All *Candida* spp. (580)	Anidulafungin	99.1	0.7	0.2	98.3	0.2	0.0	1.5
	Caspofungin	94.1	5.0	0.9	93.6	0.3	0.2	5.9
	Micafungin	99.3	0.2	0.5	99.6	0.2	0.0	0.2

Pfaller AAC 2002

TIMY 2016

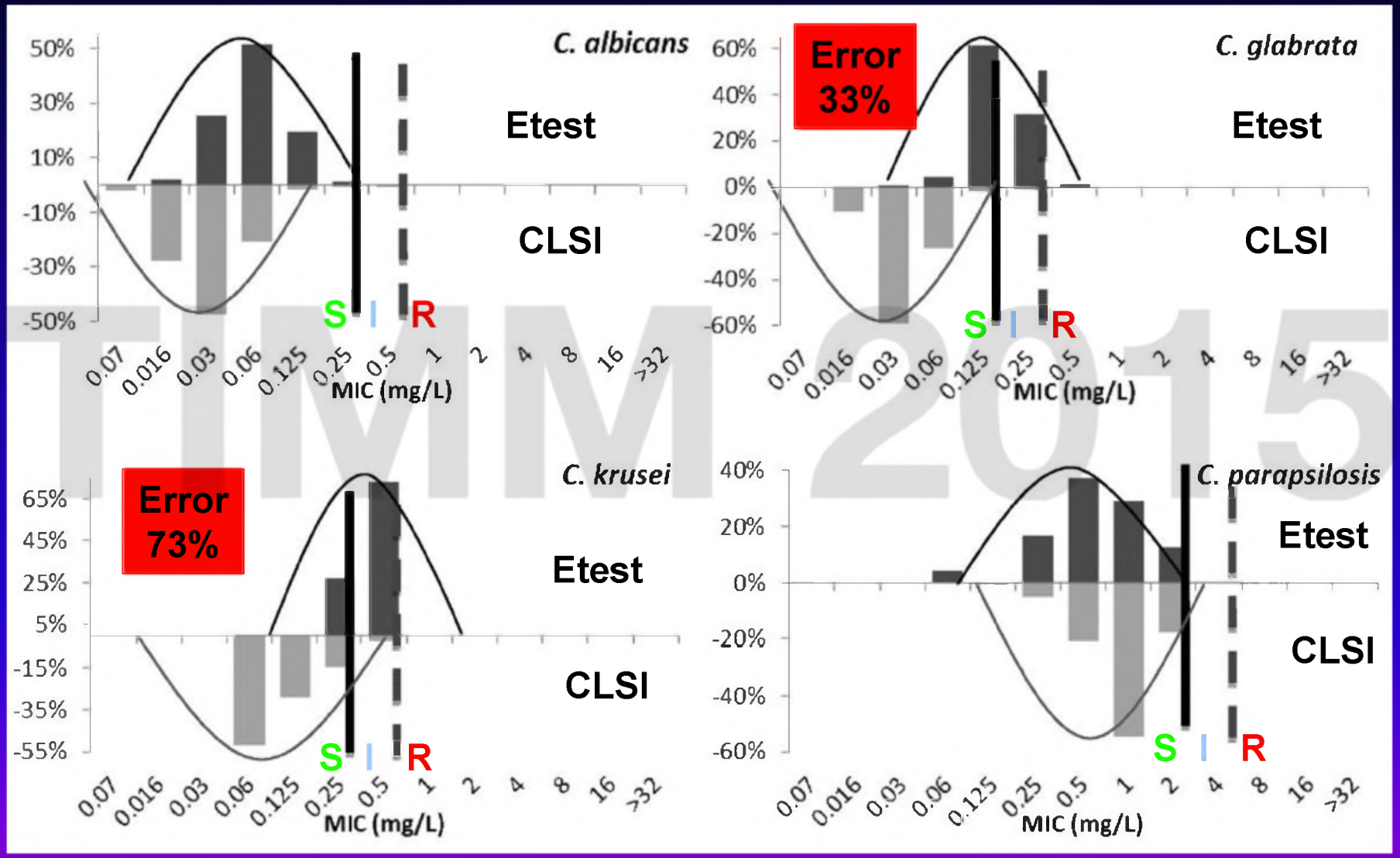
Sensititre YeastOne ECV of echinocandins and detection of fks mutatns

Drug	Species N	CLSI-ECV	SYO-ECV	Mutants identified (%)
Anidulafungin	C. albicans N=3118	0.12	0.12	10/11(91)
	C. glabrata N=2520	0.12	0.12	28/30(93)
	C. krusei N=408	0.25	0.25	3/3(ND)
	C. tropicalis N=652	0.12	0.5	4/6(ND)
	C. dubliniensis N=131	0.12	0.25	1/1(ND)
	Total			**46/51(90)**
Caspofungin	C. albicans N=6007	NA	0.25	11/11(100)
	C. glabrata N=3188	NA	0.25	26/30(87)
	C. krusei N=493	NA	1	2/3(ND)
	C. tropicalis N=1016	NA	0.25	5/6(ND)
	C. dubliniensis N=186	NA	0.25	1/1(ND)
	Total			**45/51(88)**
Micafungin	C. albicans N=4862	0.03	0.06	11/11(100)
	C. glabrata N=2763	0.03	0.03	30/30(100)
	C. krusei N=444	0.25	0.25	3/3(ND)
	C. tropicalis N=983	0.06	0.06	6/6(ND)
	C. dubliniensis N=123	0.12	0.12	1/1(ND)
	Total			**51/51(100)**

TIMM 2015

Espinel-Ingroff AAC 2015

Caspofungin Etest MICs of wild-type isolates



Etest breakpoint of 0.5 mg/l for *C. glabrata* & *C. krusei*

Arendrup JCM 2012

Caspofungin Etest MICs and detection of *Candida* fks mutants

TABLE 6. MIC distributions of caspofungin *versus Candida* spp. with and without *fks1* and/or *fks2* mutations using the CLSI and EUCAST broth microdilution methods and the Etest method[a]

Species (no. of isolates tested)	Test	No. of isolates at indicated MIC (no. of isolates showing mutation)[b]								
		≤0.03	0.06	0.12	0.25	0.5	1	2	4	≥8
C. albicans (52)	CLSI	**15**	**23**	**3**		2 (2)	1 (1)	3 (3)	2 (2)	3 (3)
	Etest	**22**	**15**	**4**	2 (2)			3 (3)	2 (2)	4 (4)
C. glabrata (53)	CLSI	**1**	**20**	**7**	3 (1)	1	8 (3)	5 (4)	2	6 (6)
	Etest	**2**	**12**	**11**	7 (1)	4	5 (4)	5 (3)	4 (3)	3 (3)

[a] The data were compiled from Arendrup et al. (2) and Table 5 (this publication).
[b] MICs are in μg/ml. Bold values indicate WT MICs.

TIMM 2015

Pfaller JCM 2010

Agreement between Etest and EUCAST for *Candida* and *Cryptococcus* spp.

Yeast species	Total (n = 690)	AMB% (n = 655)	FCZ% (n = 669)	ITZ% (n = 198)	VRZ% (n = 588)
Candida albicans	362	80.9 (345)	66.9 (354)	72.5 (109)	88.3 (308)
Candida glabrata	113	69.2 (104)	72.7 (110)	67.7 (31)	81.6 (98)
Candida parapsilosis	69	64.7 (68)	78.3 (69)	92.3 (13)	86.4 (59)
Candida tropicalis	55	61.1 (54)	85.2 (54)	50.0 (14)	81.3 (48)
Cryptococcus neoformans	31	73.1 (26)	76.7 (30)	72.7 (11)	86.4 (22)
Other species	60	75.9 (58)	76.9 (52)	65.0 (20)	90.6 (53)
Very major errors		0.2%	2.7%	0%	5.9%
Major/Minor errors		1.2/21.2%	5.5/13.3%	9.1/33.1%	7.5/-%

Yeast species	Total (n = 690)	5FC% (n = 314)	CAS% (n = 244)
Candida albicans	362	95.8 (166)	80.0 (120)
Candida glabrata	113	100.0 (46)	66.7 (45)
Candida parapsilosis	69	92.3 (26)	89.7 (29)
Candida tropicalis	55	100.0 (25)	60.0 (20)
Cryptococcus neoformans	31	35.0 (20)	100.0 (4)
Other species	60	74.2 (31)	57.7 (26)
Very major errors		0%	0.4%
Major/Minor errors		4.1/5.4%	11.9/-%

Dannaoui CMI AAA 2009

ITMM 2015

Sensititre YeastOne and Etest for testing *Aspergillus* with amphotericin B and azoles

AMB 48h→

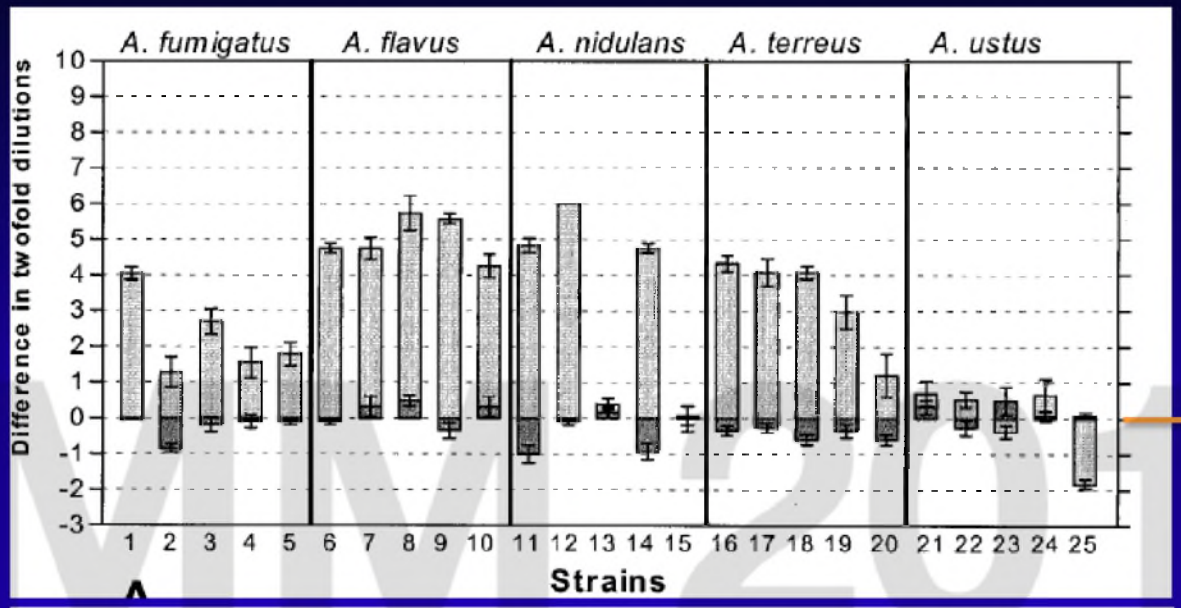
Etest
24h>48h
89% vs. 43%

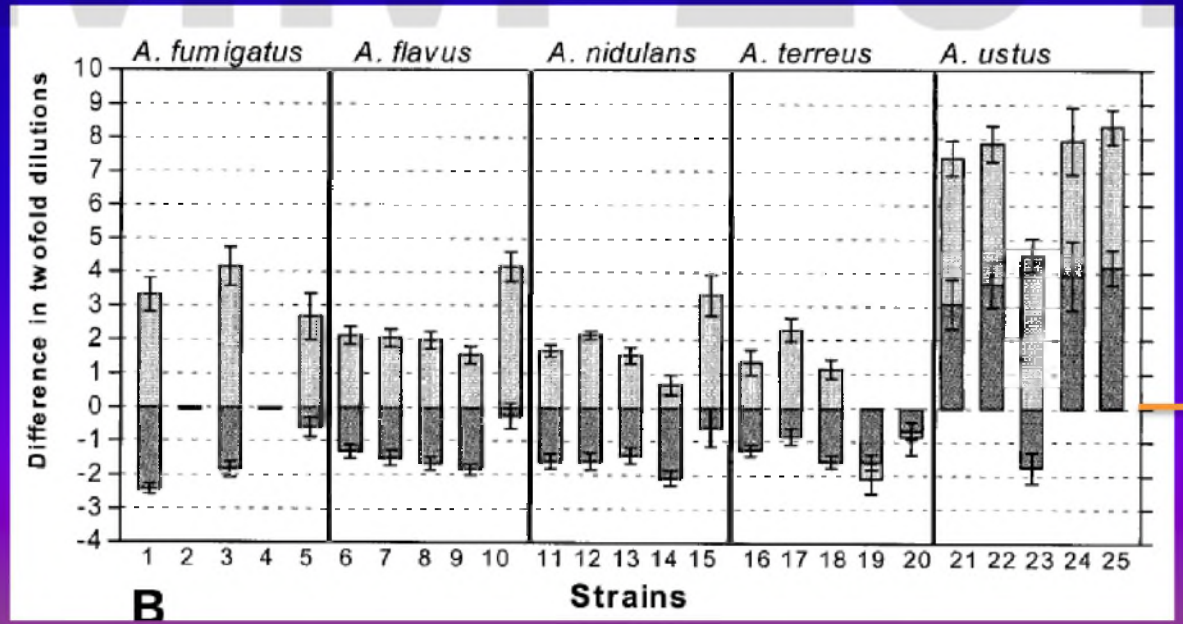
Sensititre
24h=48h
99% vs. 100%

ITRA 48h→

Etest
24h>48h
73% vs 56%

Sensititre
24h<48h
45% vs 85%





Etest

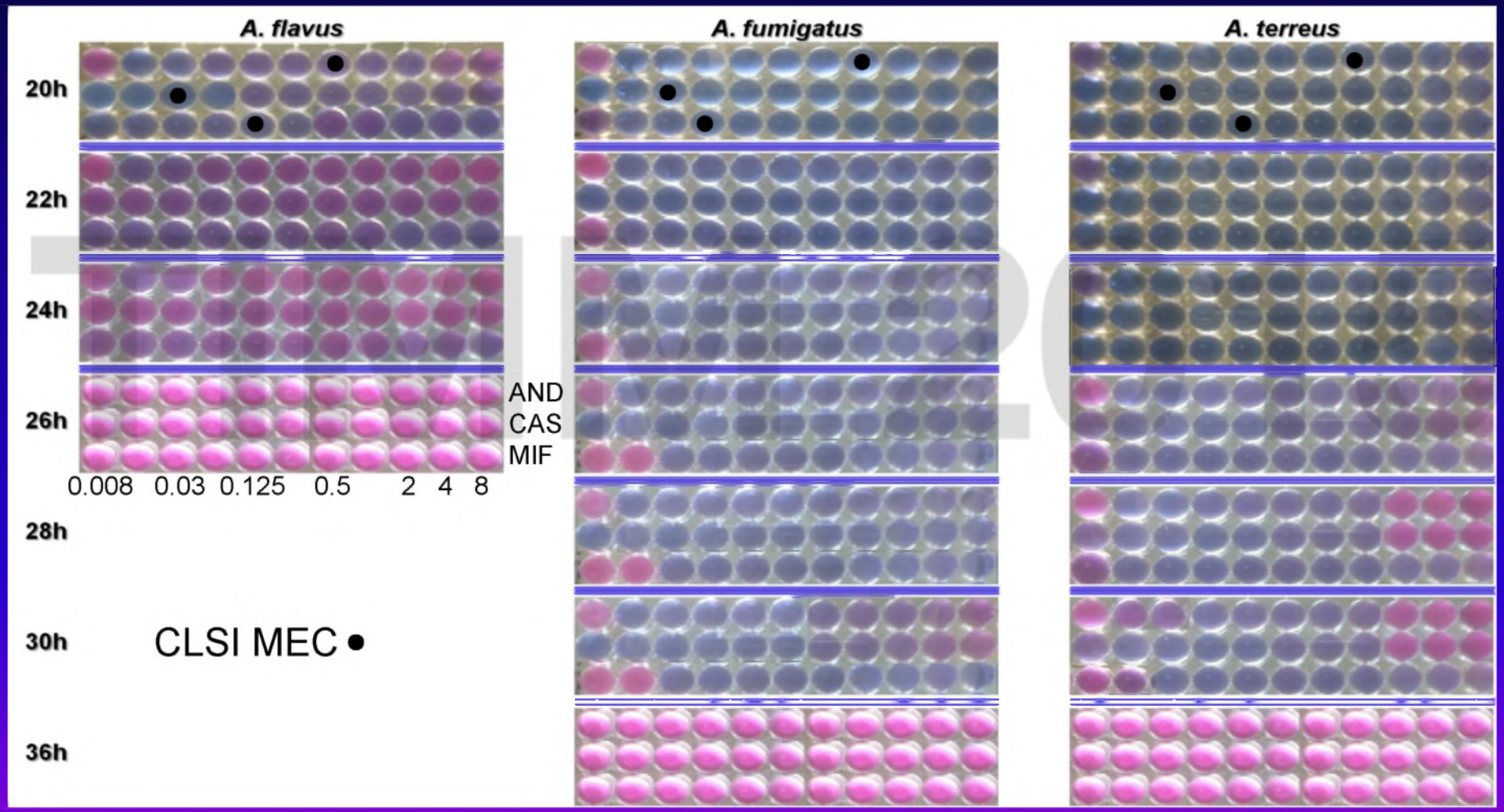
Sensittre

CLSI

CLSI

Meletiadis AAC 2002

Sensititre YeastOne for *Aspergillus* and echinocandins



Agreement=67-100% for AND, 0% for CAS and 0-33% for MIC

Siopi et al ECCMID 2013

Etest and echinocandin testing of *Aspergillus* spp.

TABLE 2. Caspofungin *in vitro* susceptibility test results for clinical *Aspergillus* isolates[a]

Species	Isolates (no.)	BMD (μg/ml)				MGM Etest (μg/ml)				RPMI Etest (μg/ml)			
		Range	MEC_{90}	MEC_{50}	GM	Range	MIC_{90}	MIC_{50}	GM	Range	MIC_{90}	MIC_{50}	GM
A. flavus	51	0.064–0.25	0.125	0.125	0.136	0.002–1.0	0.25	0.032	0.034				
	48	0.032–0.50	0.25	0.125	0.128					0.002–0.125	0.064	0.016	0.023
A. fumigatus	72	0.016–2.0	0.25	0.25	0.137	0.008–0.50	0.064	0.032	0.033				
	100	0.016–4.0	0.50	0.25	0.129					0.002–4.0	0.064	0.032	0.039
A. nidulans	41	0.064–2.0	0.25	0.125	0.137	0.002–0.064	0.032	0.016	0.014				
	31	0.032–4.0	0.25	0.064	0.128					0.002–4.0	0.032	0.016	0.023
A. niger	90	0.064–0.25	0.25	0.125	0.136	0.008–0.50	0.064	0.032	0.026				
	75	0.032–0.50	0.125	0.064	0.127					0.002–0.125	0.032	0.016	0.020
A. terreus	19	0.016–0.25	0.25	0.25	0.137	0.008–0.064	0.032	0.016	0.019				
	18	0.032–1.0	0.50	0.125	0.128					0.008–0.50	0.125	0.064	0.073

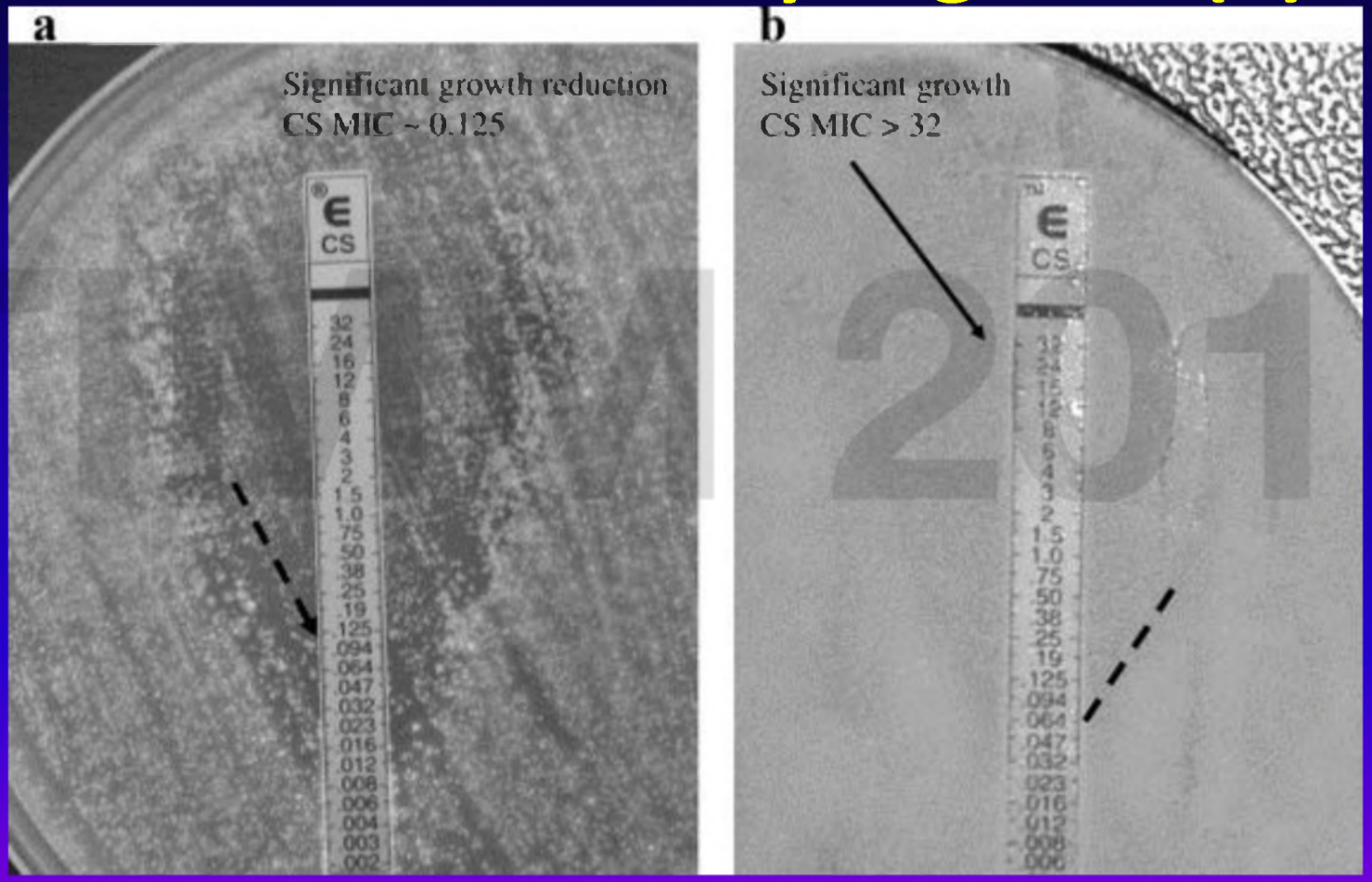
[a] MEC_{90} and MEC_{50}, 90% and 50% minimum effective concentration, respectively; MIC90 and MIC50, 90% and 50% minimum inhibitory concentration, respectively; GM, geometric mean.

TIMM 2015

Essential Agreement (mean, range) 16(2-33)% 31 (11-67)%

Fuller JCM 2010

Etest and detection of caspofungin resistance in *Aspergillus* (?)



CLSI MEC= 0.25 mg/l for both isolates

Arendrup AAC 2008

Vitek and *Candida*

154 yeast isolates
4 R to AMB
18 R to FC
85 R to FLC
57 R to VRC

14-18h for Candida
34 h for *C. neoformans*,
R. mucilaginosa,
D. capitatus.

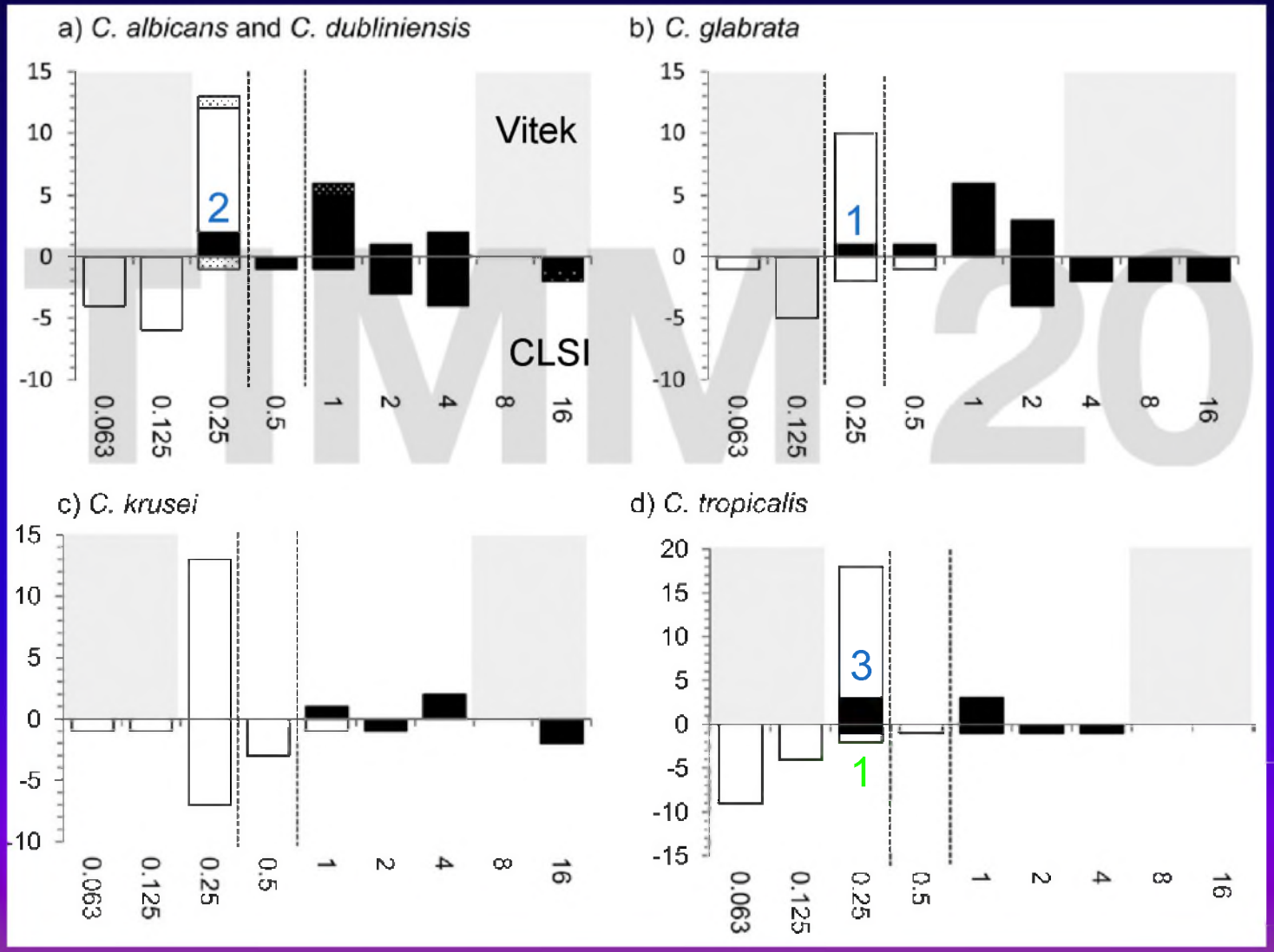
Antifungal agent[a]	Vitek and EUCAST	Vitek and CLSI24H	Vitek and CLSI48H
AMB	98.7	99.3	100
5FC	98.0	98.6	96.0
FLC	97.5	96.6	96.2
VRC	97.5	96.8	96.6
Total	97.9	97.8	97.3

Antifungal agent[a]	Vitek				
	VME	ME	MiE	>4 dil.	3-4 dil.
AMB				0	2
5FC				0	3
FLZ	8	0	23		
VOR	0	0	10		
Total	8	0	33	0	5

ILM 2015

Cuenca-Estrella AAC 2010

Vitek and detection of fks *Candida* mutants



Vitek
94% CA
19.4% VME

CLSI
88.4% CA
3.5% VME

EUCAST
99% CA
3.3% VME

WT isolates
fks mutant isolates
C. dubliniensis wt
C. dubliniensis fks mutant

Afstvad AAC 2013

Conclusions

- Commercial tests can provide rapid, less labor intensive and amenable to automation in vitro susceptibility data facilitating MIC determination in routine settings.
- MIC distributions should mirror the reference test, otherwise they may provide incorrect classifications using the reference breakpoints
- Commercial tests should separate wild type and mutant strains and detect resistance reproducibly across labs
- Differences (mainly minor errors) in performance of each test should be considered and results should be corroborated by reference methods in case of discrepancies.

ICMM 2015

HOW TO TRANSLATE EUCAST TO COMMERCIAL TESTS??

STATENS SERUM INSTITUT

- AFST tab
  - Rationale documents

Anidulafungin MIC Peak for *C. glabrata* 0.016 mg/L

2. 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*
Candida albicans **	284	360	208	77	16	6	4	1	1	0	0	0	0	0	0	1	0	0	0	0.03
*Candida glabrata***	55	38	60	149	62	8	6	8	2	2	0	0	2	0	0	0	0	0	0	0.06
Candida guilliermondii	0	0	0	0	0	1	0	2	5	17	6	1	0	0	0	0	0	0	0	ND
*Candida krusei***	2	1	12	21	7	12	2	3	0	0	0	0	0	0	0	0	0	0	0	0.06
Candida parapsilosis	0	3	1	0	4	6	2	36	78	171	96	13	7	0	0	2	0	0	0	4
Candida tropicalis **	18	19	12	30	22	4	4	1	0	0	0	0	0	0	0	0	0	0	0	0.06

8. Clinical breakpoints

Non-species-related breakpoints	There is insufficient evidence to set non-species-related breakpoints.
	Breakpoints were based on PK data, microbiological data and clinical experience. *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

Anidulafungin BP for *C. glabrata* 0.06 mg/L

Conclusions

- Commercial tests can provide rapid, less labor intensive and amenable to automation in vitro susceptibility data facilitating MIC determination in routine settings.
- MIC distributions should mirror the reference test, otherwise they may provide incorrect classifications using the reference breakpoints
- Commercial tests should separate wild type and mutant strains and detect resistance reproducibly across labs
- Differences (mainly minor errors) in performance of each test should be considered and results should be corroborated by reference methods in case of discrepancies.

ICMM 2015

Acknowledgement

(in chronological order)

University of Thessaloniki, Greece

Emmanuel Roilides

Radboud University and CWZ , Nijmegen, Netherlands

Paul Verweij

Johan Mouton

Jacque Meis

Peter Donnelly

Den de Paw

Cornel University, NY, USA

Tom Walsh

University of Athens, Greece

Loukia Zerva

Mariza Siopi

and all collaborators

EUCAST AFST subcommittee

Maiken Arendrup

Johan Mouton

Susan Howard

Jesus Guinea

Katrien Lagrou

Cornelia Lass-Florl (former)

Manuel Cuenca-Estrella (former)