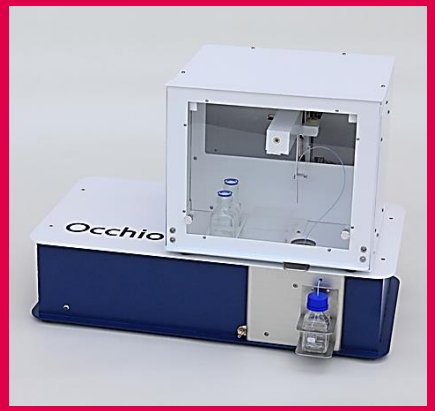


UCLL HOGESCHOOL

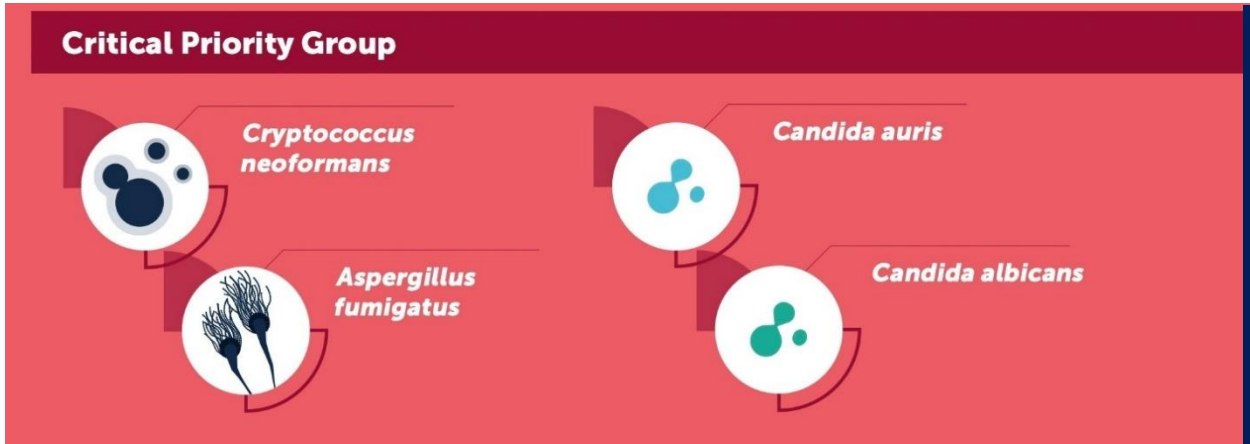
RESEARCH & EXPERTISE

Development of a new fast antifungal susceptibility test for *Candidozyma auris*

UCLL Sustainable resources
Ellen Van Hileghem
Maarten Hendrickx

Introduction

- *Candidozyma auris* (formerly *Candida auris*)
  - a yeast causing invasive candidiasis
  - first reported in 2009 in Japan
  - nosocomial infections
  - serious threat to global human health (WHO)
  - mortality between 30-60%



World Health Organization

Introduction

European Centre for Disease prevention and Control
11/9/2025

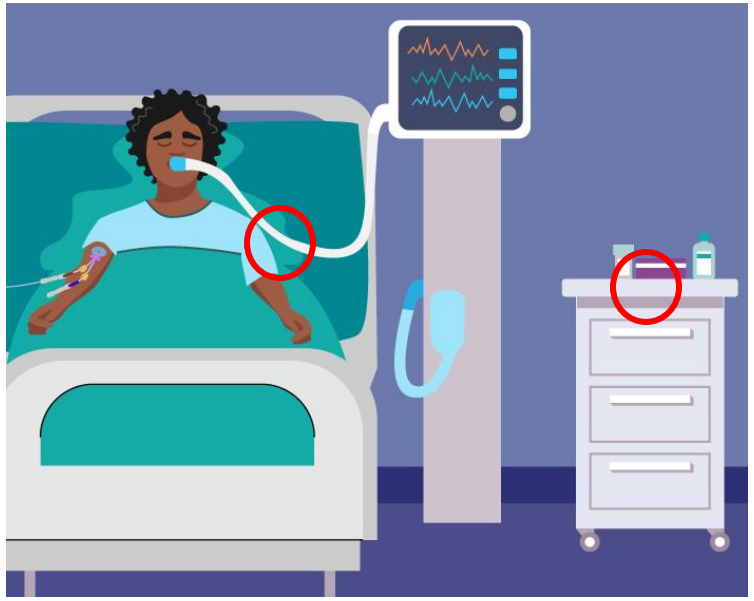
Drug-resistant fungus Candidozyma auris confirmed to spread rapidly in European hospitals: ECDC calls for urgent action

Press release
11 Sep 2025

The latest survey from the European Centre for Disease Prevention and Control (ECDC), the fourth of its kind, confirms that Candidozyma auris (formerly Candida auris) continues to spread quickly across European hospitals, posing a serious threat to patients and healthcare systems. Case numbers are rising, outbreaks are growing in scale, and several countries report ongoing local transmission. The findings highlight the importance of early detection and control of transmission to avoid widespread rapid dissemination.

Candidozyma auris (*C. auris*) is a fungus that usually spreads within healthcare facilities, is often resistant to antifungal drugs, and can cause severe infections in seriously ill patients. Its ability to persist on different surfaces and medical equipment and to spread between patients makes it particularly challenging to control. Between 2013 and 2023, EU/EEA countries reported over 4 000 cases, with a significant jump to 1 346 cases reported by 18 countries in 2023 alone. Five countries – Spain, Greece, Italy, Romania, and Germany – have accounted for most of the cases over the decade. Dr Diamantis Plachouras, Head of ECDC's Antimicrobial Resistance and Healthcare-Associated Infections Section, said:

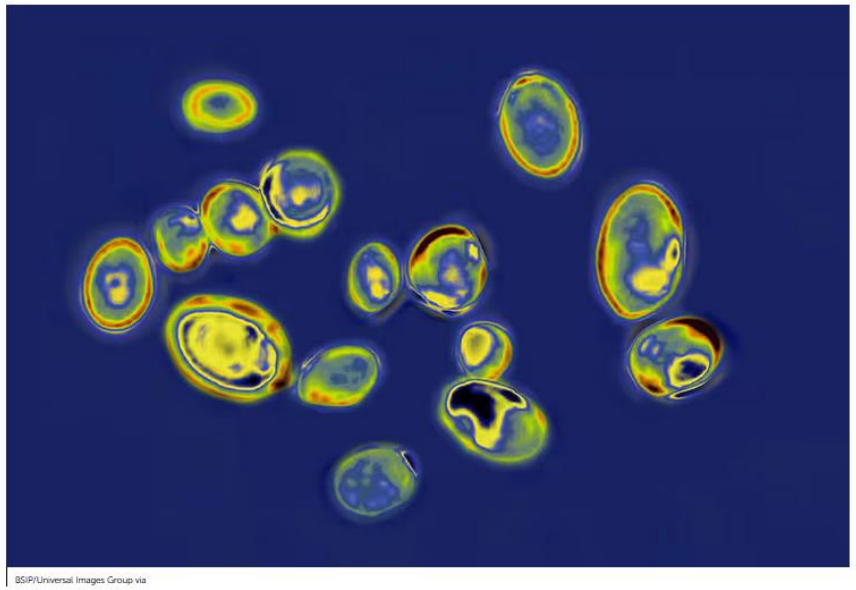
- *Candidozyma auris* (*C. auris*)
  - potential to cause outbreaks in health care settings
    - colonization of skin and surfaces
    - environmental persistence



Introduction

De Morgen
11/9/2025

Gevaarlijke schimmel candida auris rukt op in Europa, ook in België dook de soort al op



BSIP/Universal Images Group via

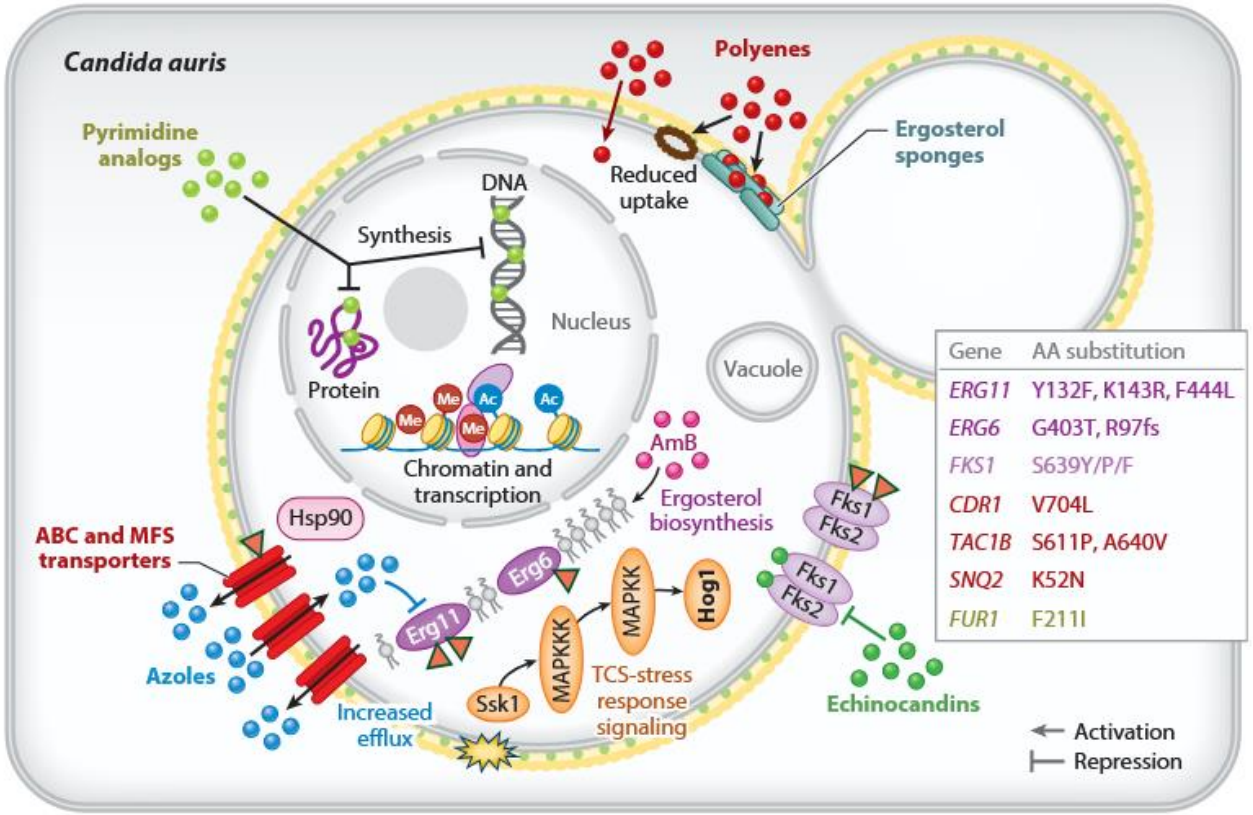
De gevaarlijke schimmel Candida auris is aan een Europese opmars bezig, dat blijkt uit een studie van het Europees Centrum voor ziektepreventie en -bestrijding. 'Op meerdere plekken is de soort plusminus gelijktijdig problemen beginnen te veroorzaken. Dat is intrigerend.'

- *Candidozyma auris* (*C. auris*)
  - pronounced drug resistant strain
    - 90% for at least one antifungal
    - 30% for at least two antifungals
    - some strains resistant to all classes of antifungals

      → no treatment options

Introduction

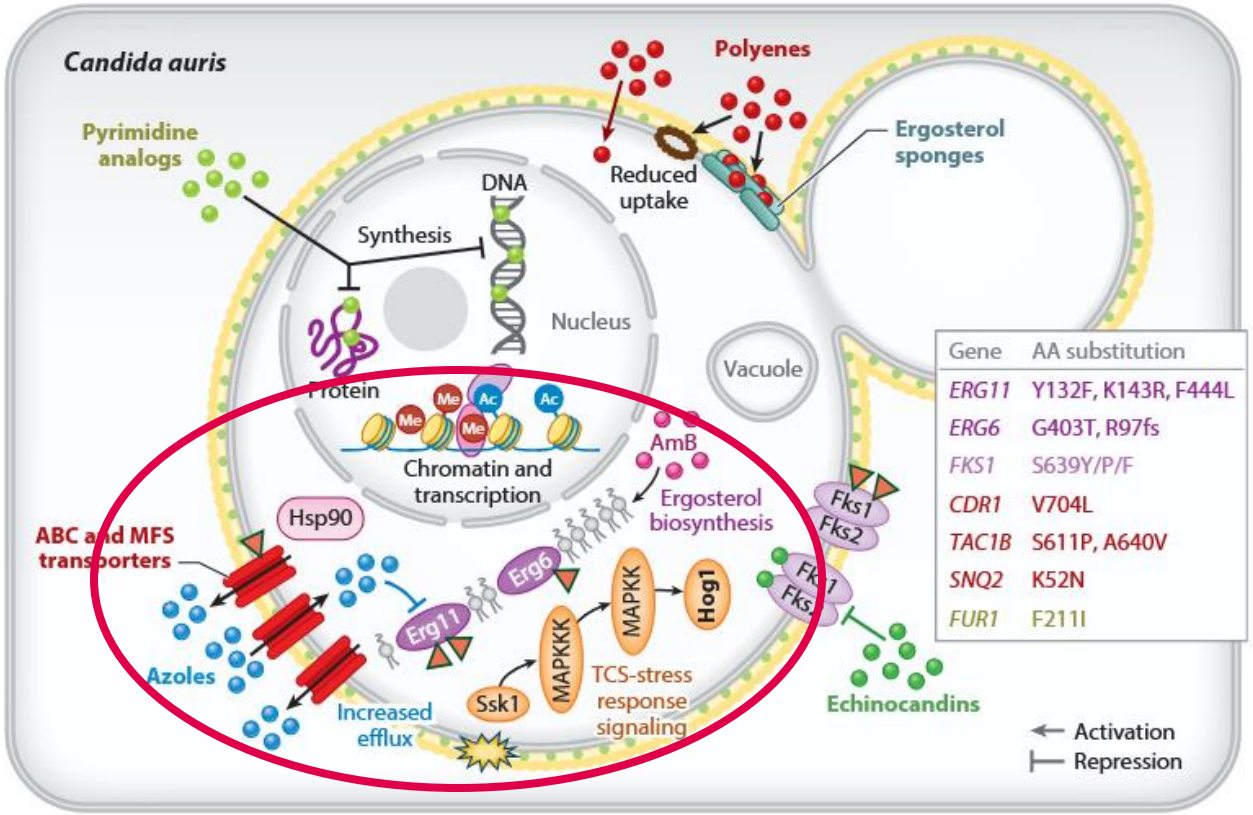
Antifungals



- Triazole class drugs
- Polyene class drugs
- Echinocandin class drugs

Introduction

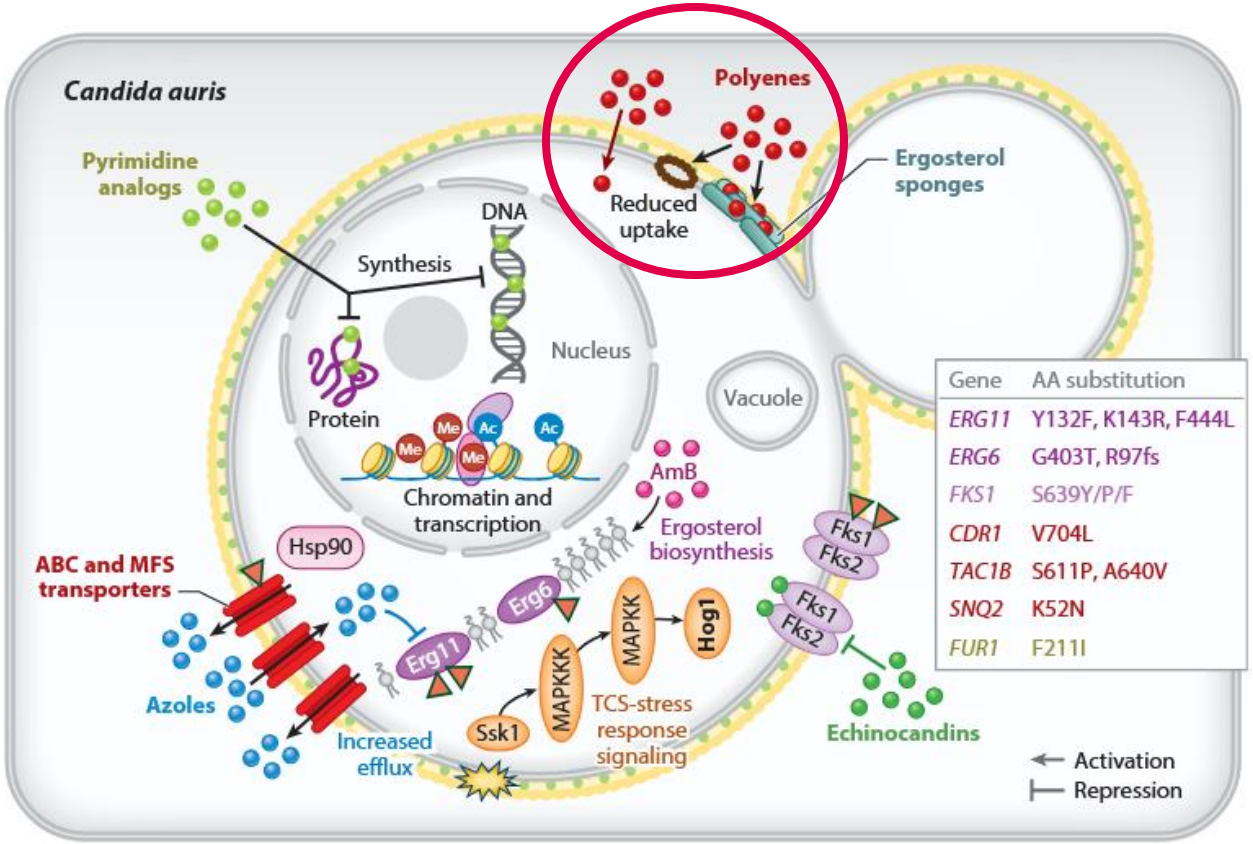
Antifungals



- Triazole class drugs
  - Inhibition of ergosterol synthesis
  - **Fluconazole**
- Polyene class drugs
- Echinocandin class drugs

Introduction

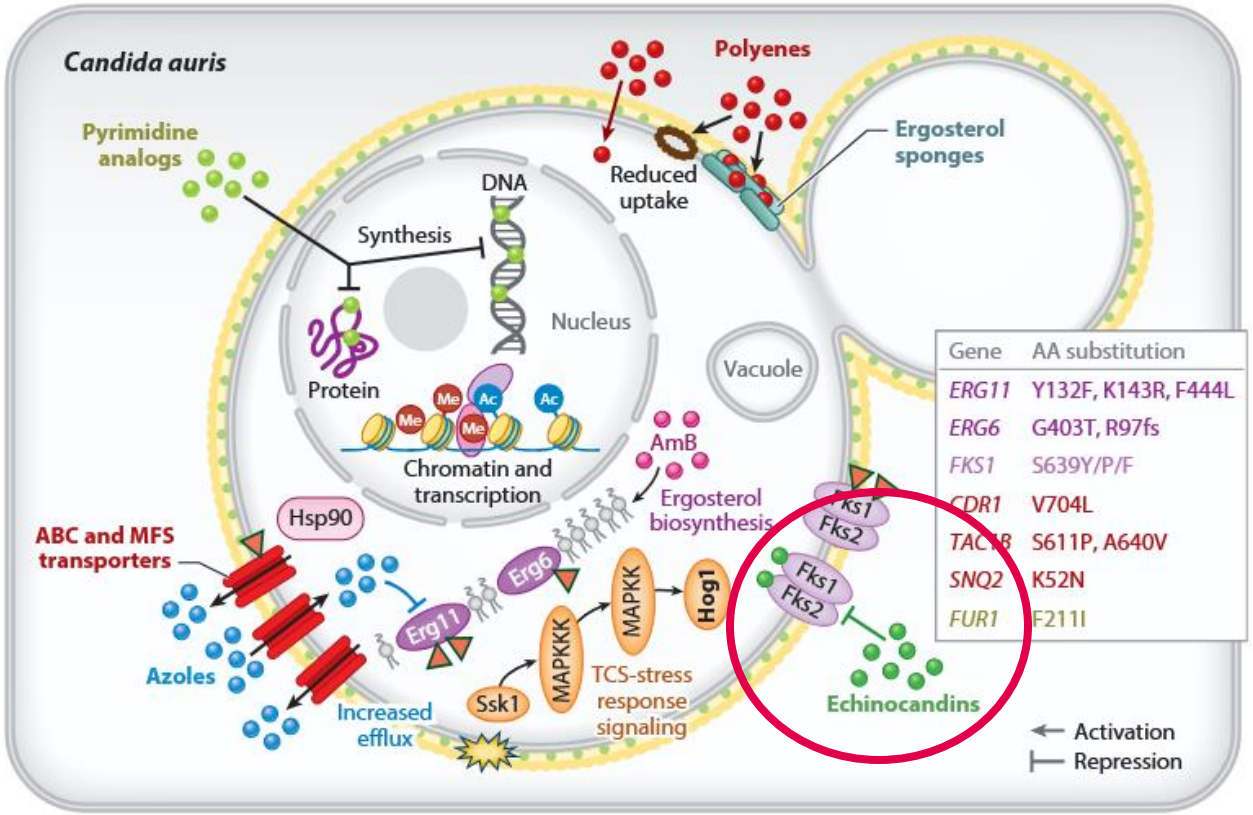
Antifungals



- Triazole class drugs
- Polyene class drugs
  - Complex with ergosterol
  - Cellmembrane distortion
  - **Amphotericin B**
- Echinocandin class drugs

Introduction

Antifungals



- Triazole class drugs
- Polyene class drugs
- Echinocandin class drugs
  - 1,3-Beta-glucan synthase inhibition
  - Cellwall distortion
  - **Caspofungin**

Introduction

Antifungal susceptibility testing

EUCAST
EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

For *Candidozyma auris:*
EUCAST/CLSI guidelines not available

Introduction

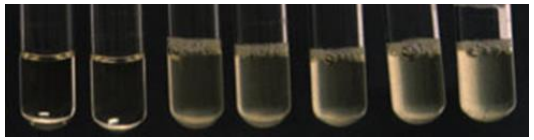
Antifungal susceptibility testing

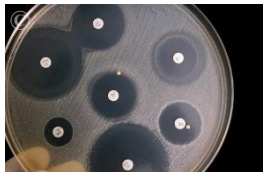
- Use CDC tentative breakpoints for MIC's

CDC	R ≥ (mg/l)
Caspofungin	2
Fluconazole	32
Amphotericin B	2

https://www.cdc.gov/candida-auris/hcp/laboratories/antifungal-susceptibility-testing.html

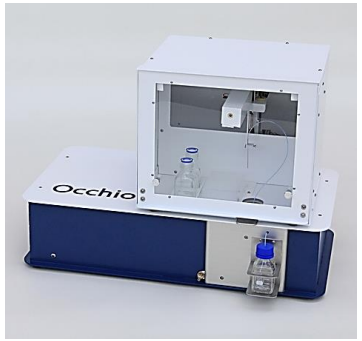
AIM





Conventional techniques

Slow: 24 – 48 hours



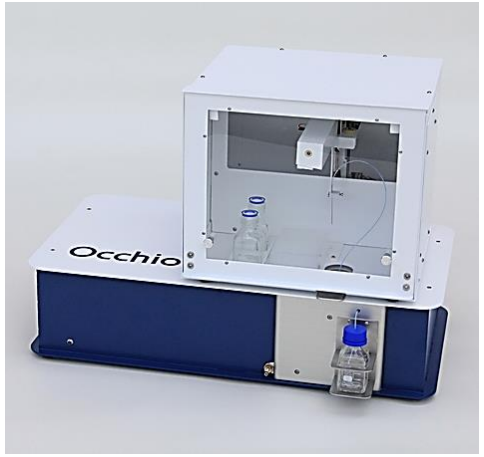
IPAC2 AR

Flow cytometer

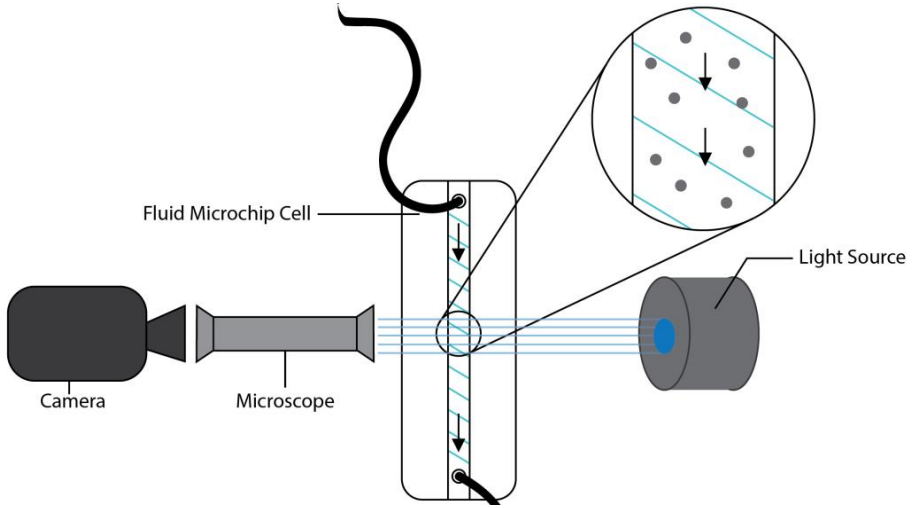
?? hours

IPAC2 AR

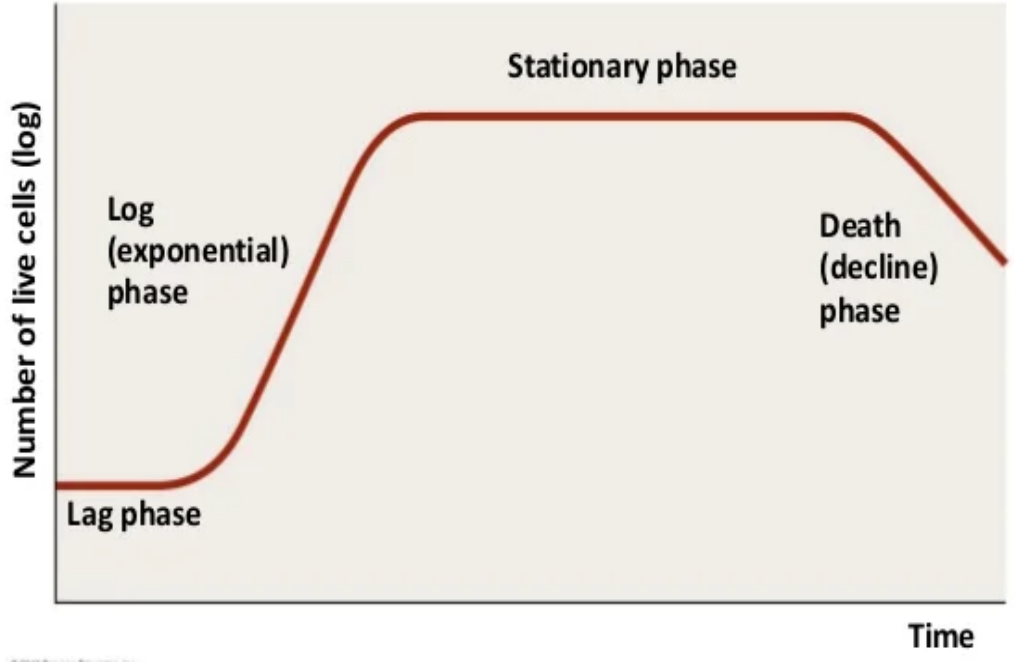
Occhio



- Incubation chamber (35 °C)
- 96-Deepwellplate
- Automated dispensing robot
- Cleaning solutions
- Sensitive camera
- Adjustable software (AI)

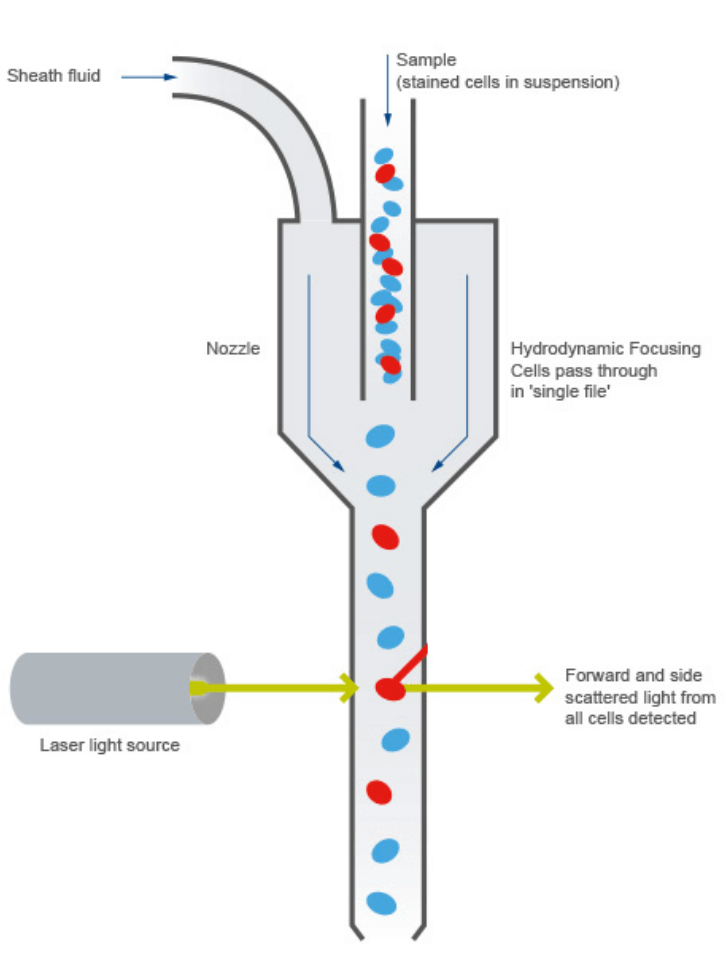


Quantification of microorganisms in suspension

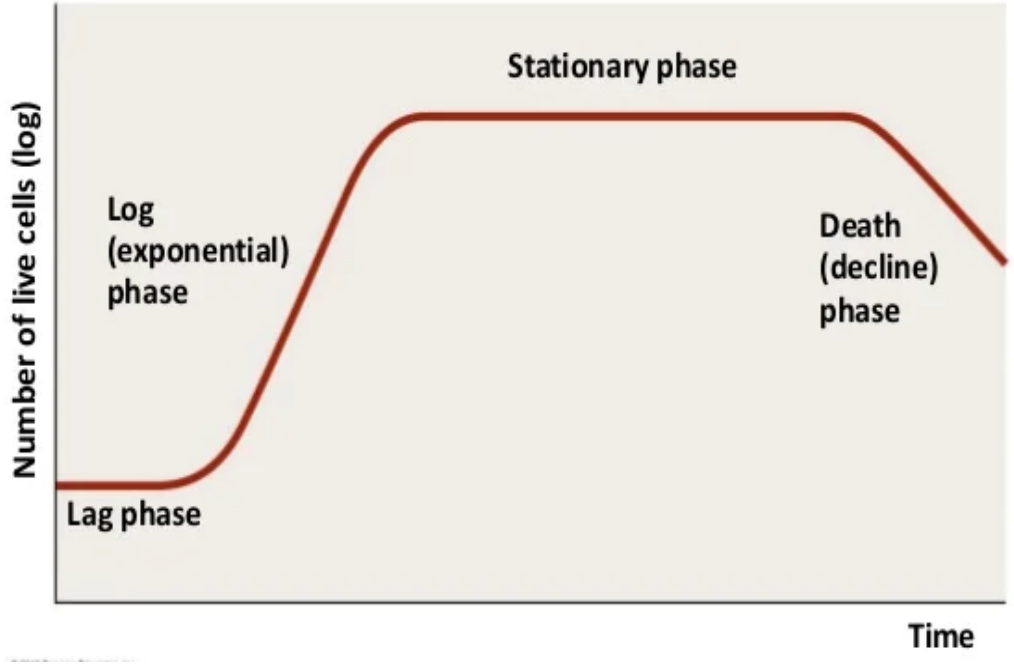


Flow cytometer

- Sample = cells in suspension
- Results = detection of forward and side scattered light
  ! <u>No</u> fluorescence



Quantification of microorganisms in suspension



Experimental work

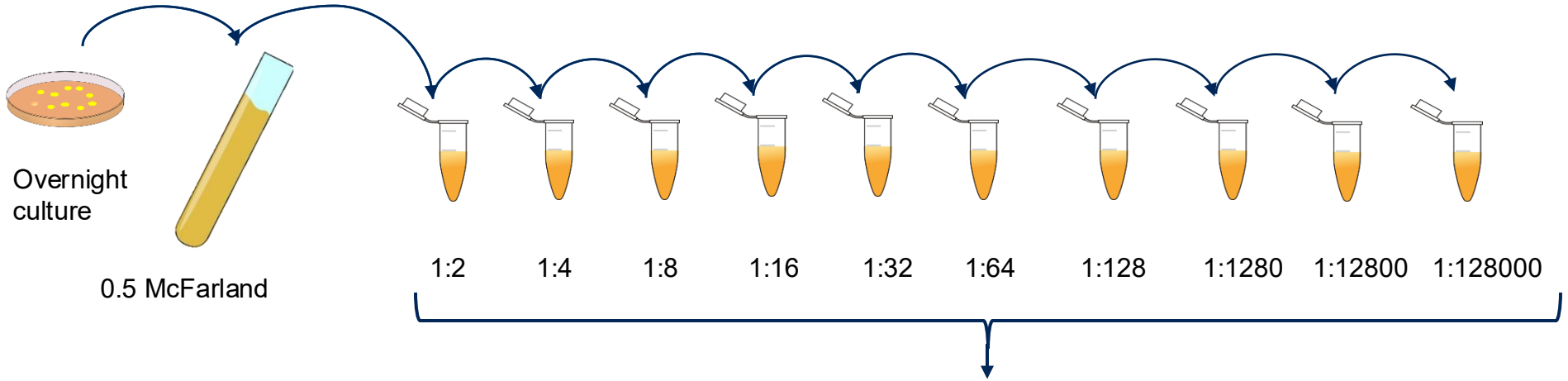
- Validation: Linear response
- Growth experiments
- MIC-tests
  - Conventional techniques: broth dilution, disk diffusion, E-test, Sensititre Vizion, Vitek 2 Compact

- Strains:
  - *C. auris* CBS 12373
  - *C. auris* B11220
  - *C. auris* B11222
  - *C. auris* B11224
  - *C. auris* B11230

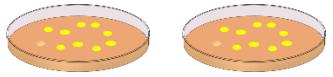
- Antimycotics:
  - Amphotericin B
  - Caspofungin
  - Fluconazole

Validation

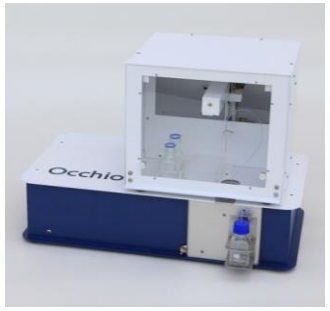
- Aim = finding a linear response between the number of yeasts and the response of IPAC2 AR/flow cytometer



Flow cytometer



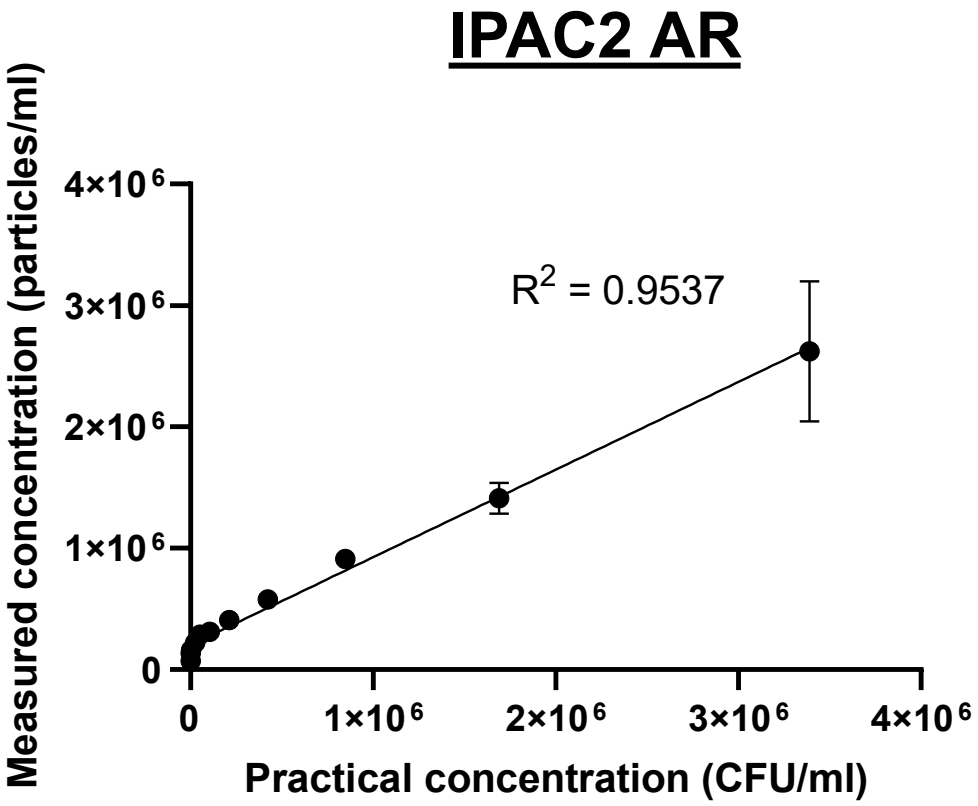
Practical concentration

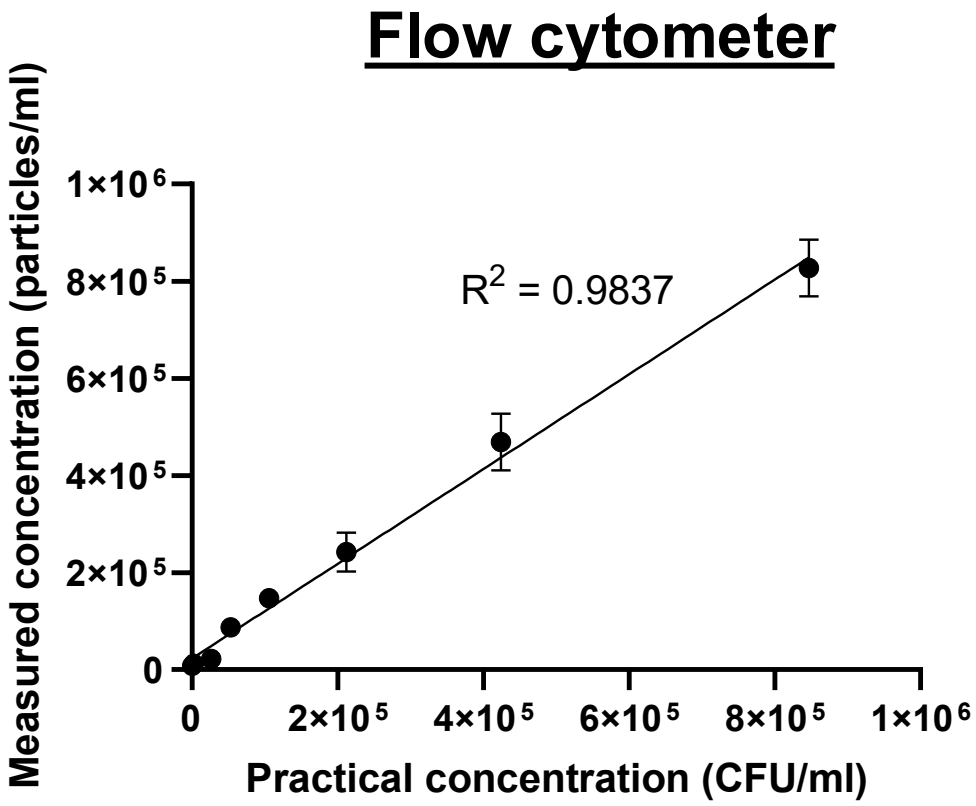


IPAC2 AR

Validation

***C. auris* CBS 12373**

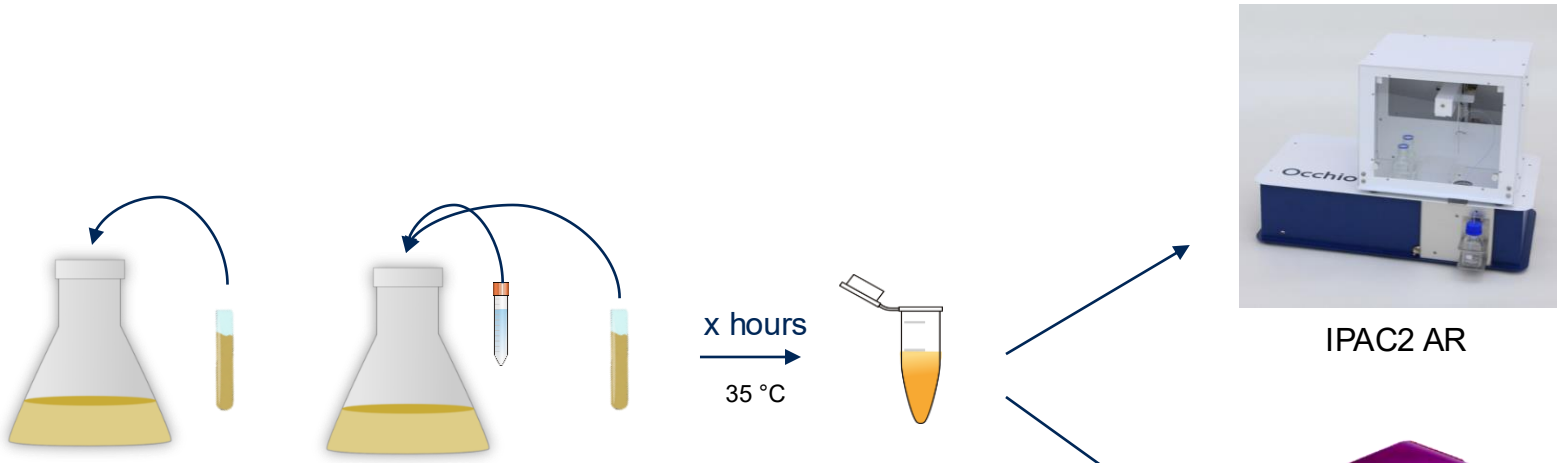




Growth experiments

CDC	R ≥ (mg/l)
C = Caspofungin	2
F = Fluconazole	32
A = Amphotericin B	2

- Aim = to detect growth of *C. auris* in the presence or absence of an antimycotic



C. auris

C. auris + 4 mg/l Caspofungin
C. auris + 64 mg/l Fluconazole
C. auris + 4 mg/l Amphotericin B

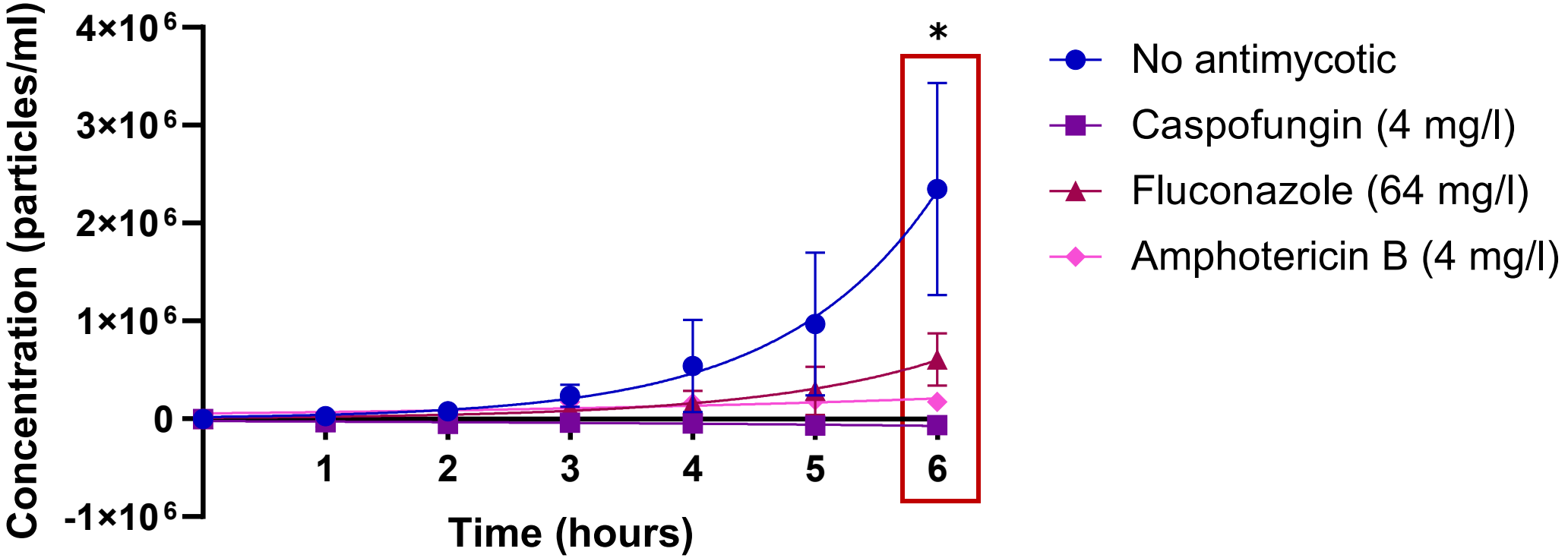
IPAC2 AR

Flow cytometer

Growth experiments

CDC	R ≥ (mg/l)
C = Caspofungin	2
F = Fluconazole	32
A = Amphotericin B	2

C. auris CBS 12373: IPAC2 AR

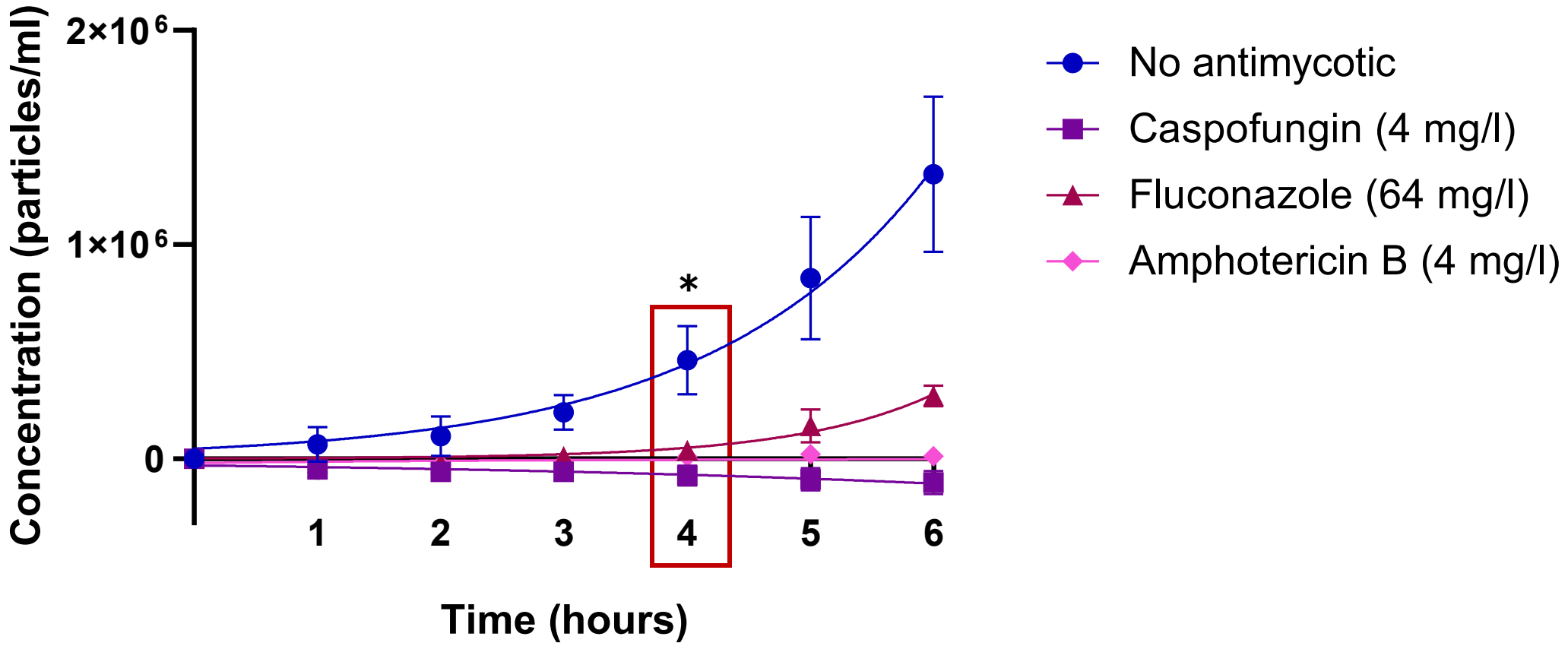


Two-way ANOVA (n=3)
* $p < 0.05$

Growth experiments

CDC	R ≥ (mg/l)
C = Caspofungin	2
F = Fluconazole	32
A = Amphotericin B	2

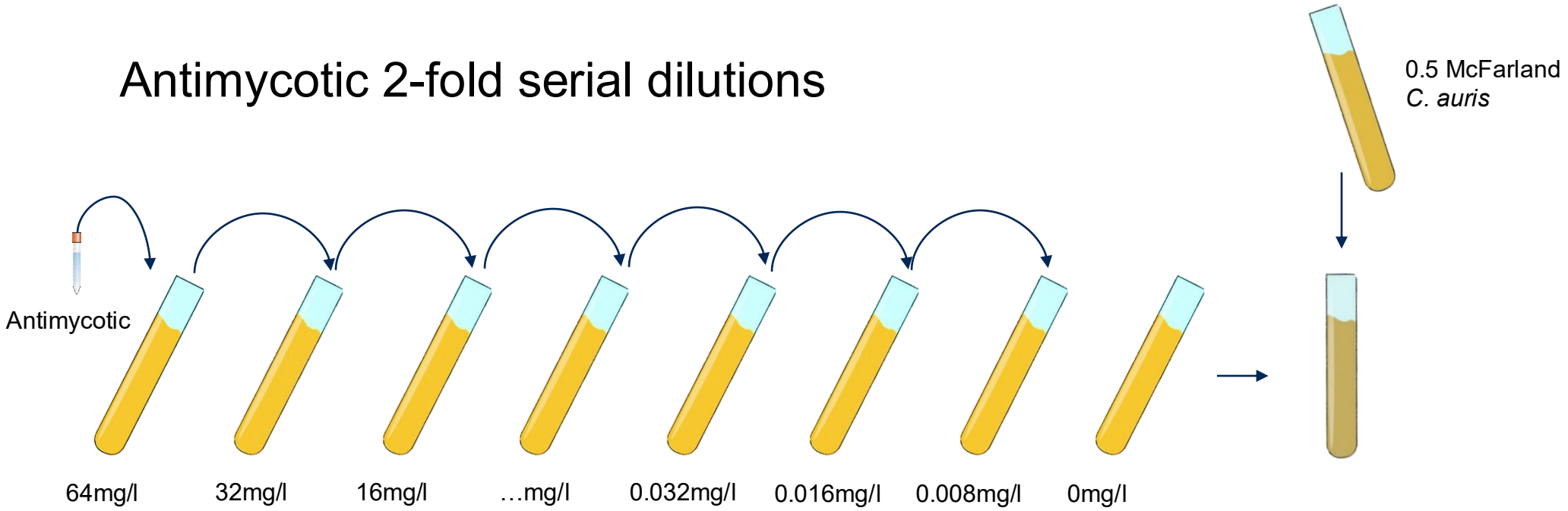
C. auris CBS 12373: Flow cytometer



Two-way ANOVA (n=3)
* $p < 0.05$

MIC (Minimum inhibitory concentration)

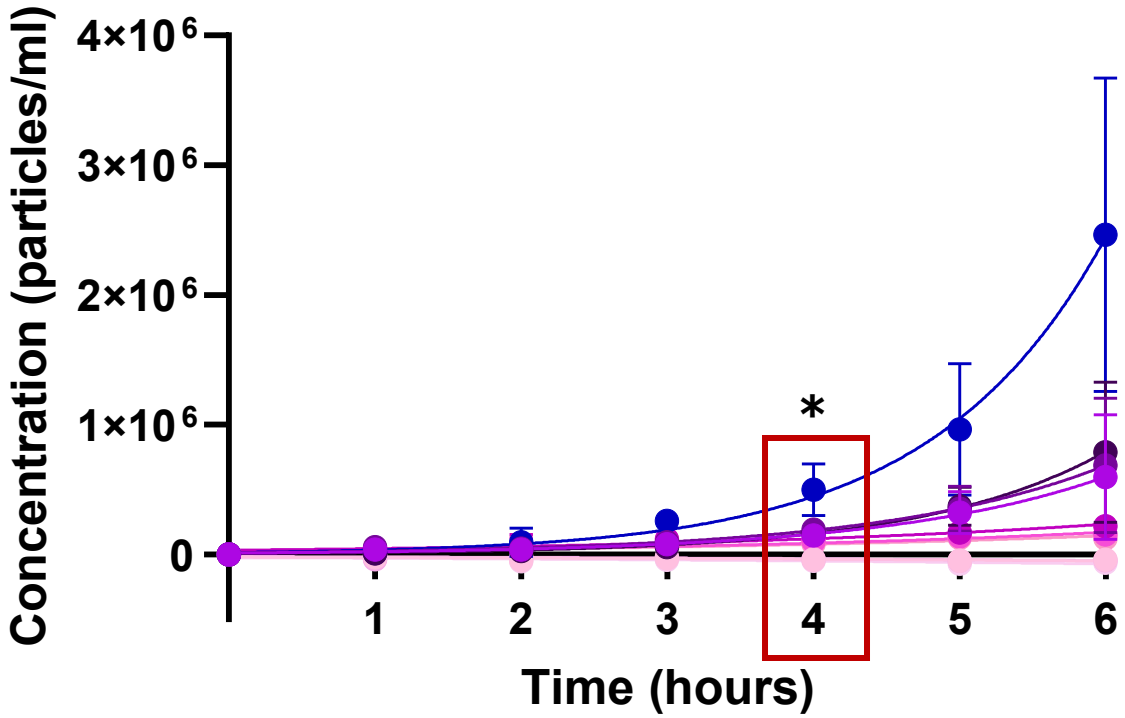
- Aim = finding the lowest concentration of an antimycotic that inhibits the growth of *C. auris*



CDC	R ≥ (mg/l)
C = Caspofungin	2

MIC Minimum inhibitory concentration

C. auris CBS 12373: IPAC2 AR



- No antimycotic
- Caspofungin (0.016 mg/l)
- Caspofungin (0.03 mg/l)
- Caspofungin (0.06 mg/l)
- Caspofungin (0.125 mg/l)
- Caspofungin (0.25 mg/l)
- Caspofungin (0.5 mg/l)
- Caspofungin (1 mg/l)
- Caspofungin (2 mg/l)
- Caspofungin (4 mg/l)

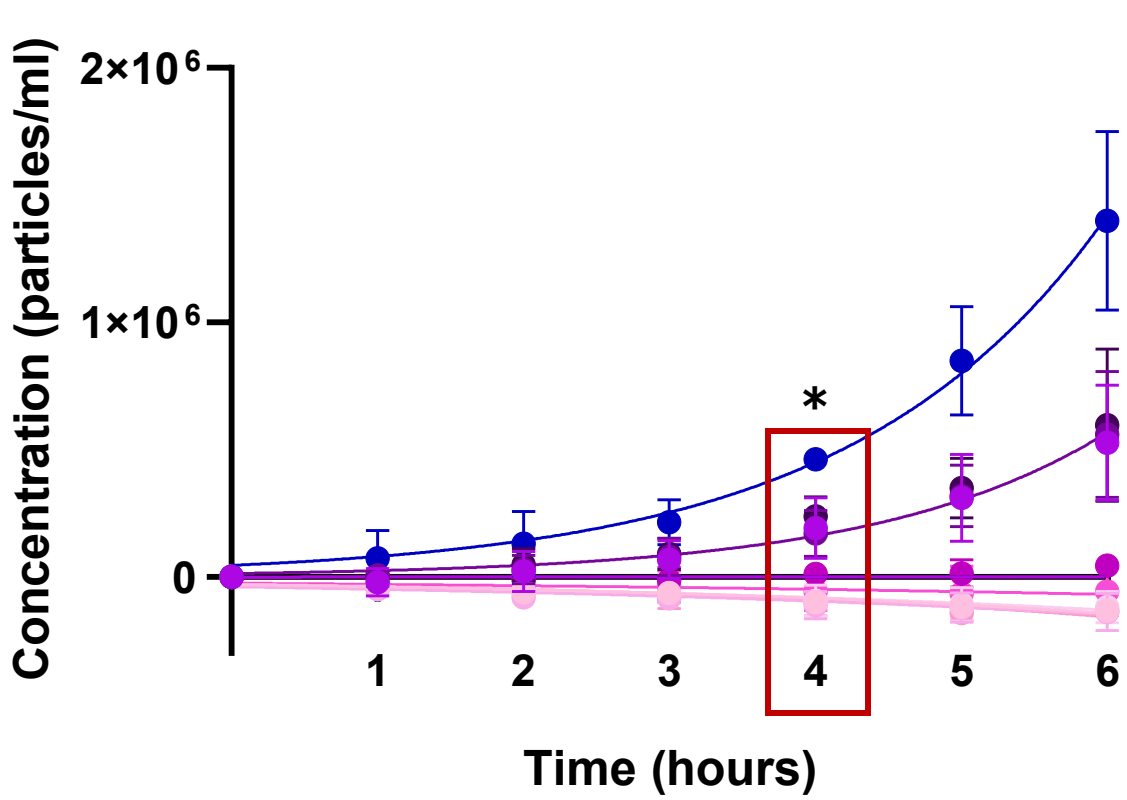
Susceptible

Two-way ANOVA (n=3)
* $p < 0.05$

CDC	R ≥ (mg/l)
C = Caspofungin	2

MIC Minimum inhibitory concentration

C. auris CBS 12373: Flow cytometer



- No antimycotic
- Caspofungin (0.016 mg/l)
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- Caspofungin (0.125 mg/l)
- Caspofungin (0.25 mg/l)
- Caspofungin (0.5 mg/l)
- Caspofungin (1 mg/l)
- Caspofungin (2 mg/l)
- Caspofungin (4 mg/l)

Susceptible

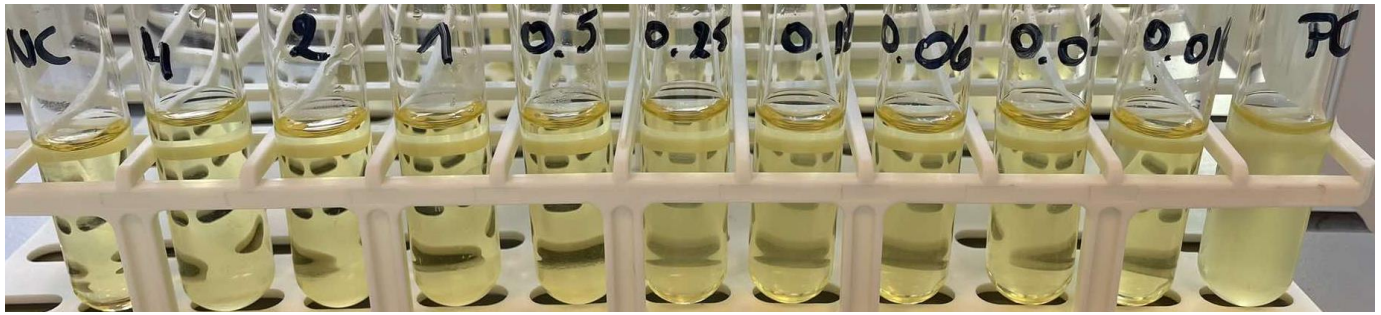
Two-way ANOVA (n=3)
* $p < 0.05$

CDC	R ≥ (mg/l)
C = Caspofungin	2

MIC Minimum inhibitory concentration

C. auris CBS 12373: Conventional techniques

Broth dilution test



MIC = 0.5 mg/l

Susceptible

E-test

MIC = 0.125 mg/l

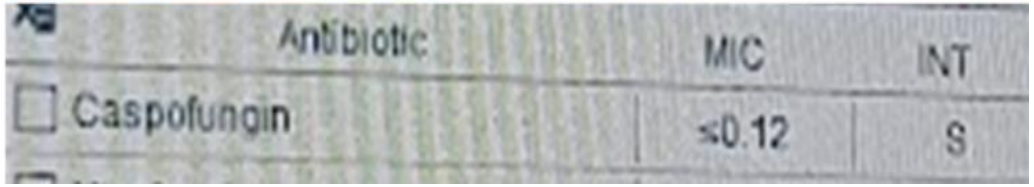
Susceptible

Sensititre Vizion

MIC = 0.25 mg/l

Susceptible

Vitek 2 Compact



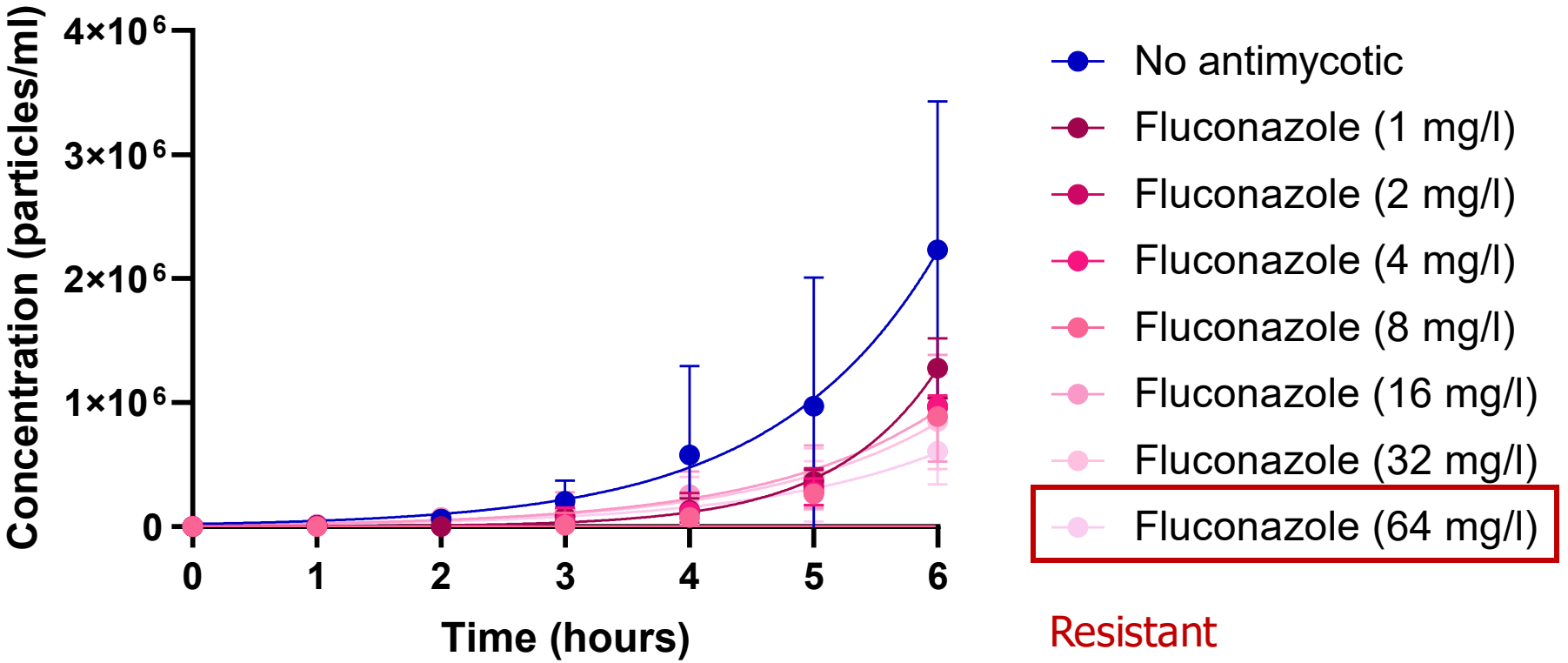
MIC ≤ 0.12 mg/l

Susceptible

CDC	R ≥ (mg/l)
F = Fluconazole	32

MIC Minimum inhibitory concentration

C. auris CBS 12373: IPAC2 AR



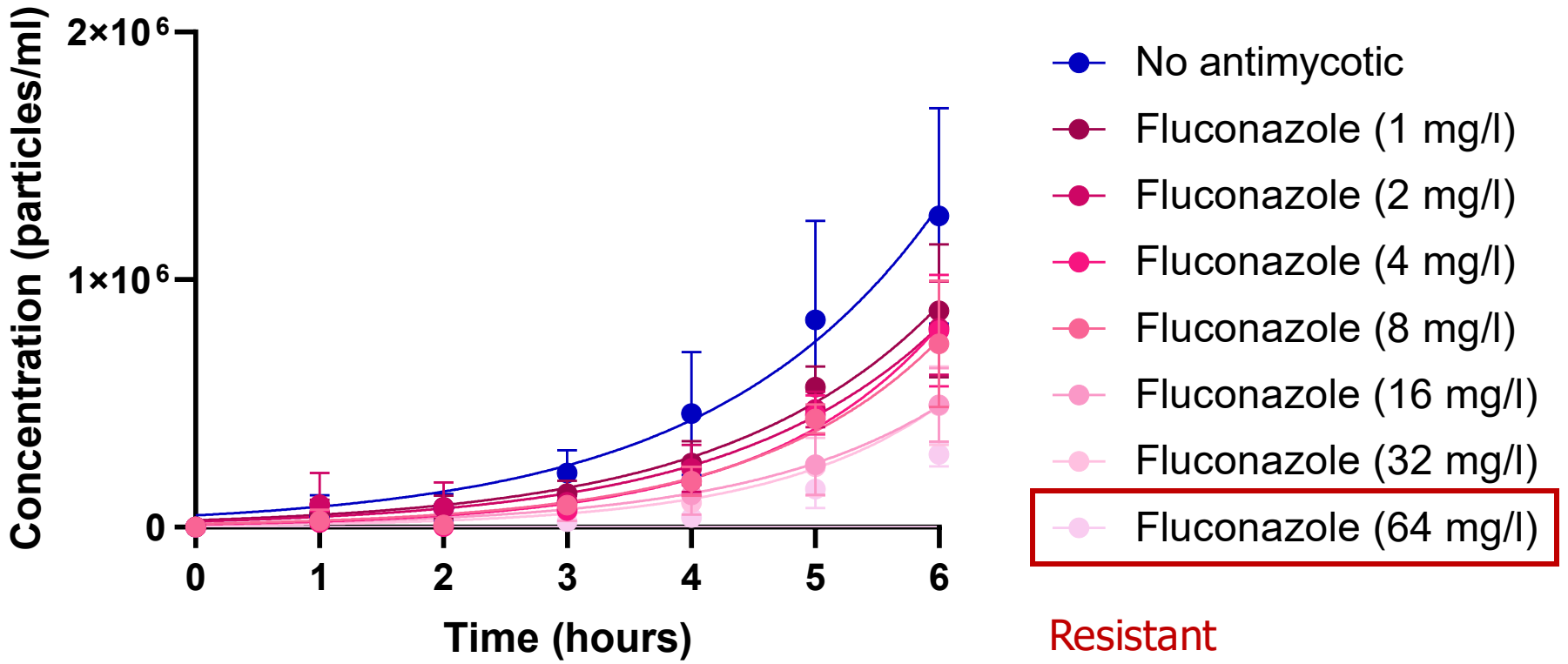
Resistant

Two-way ANOVA (n=3)
* $p < 0.05$

CDC	R ≥ (mg/l)
F = Fluconazole	32

MIC Minimum inhibitory concentration

C. auris CBS 12373: Flow cytometer



Resistant

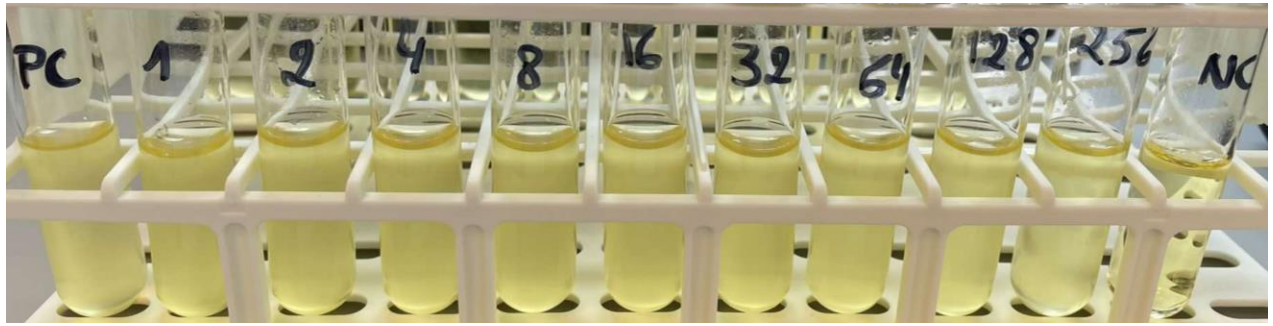
Two-way ANOVA (n=3)
* $p < 0.05$

CDC	R ≥ (mg/l)
F = Fluconazole	32

MIC Minimum inhibitory concentration

C. auris CBS 12373: Conventional techniques

Broth dilution test



MIC > 256 mg/l

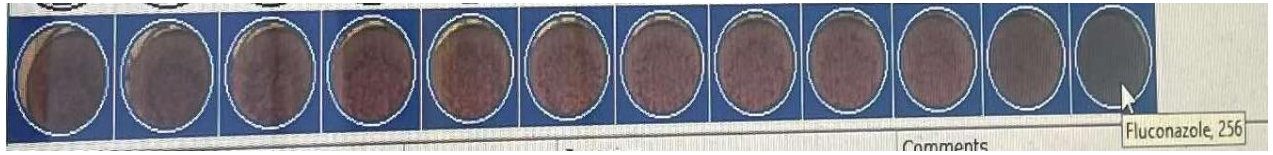
Resistant

E-test

MIC > 256 mg/l

Resistant

Sensititre Vizion



MIC > 256 mg/l

Resistant

Vitek 2 Compact

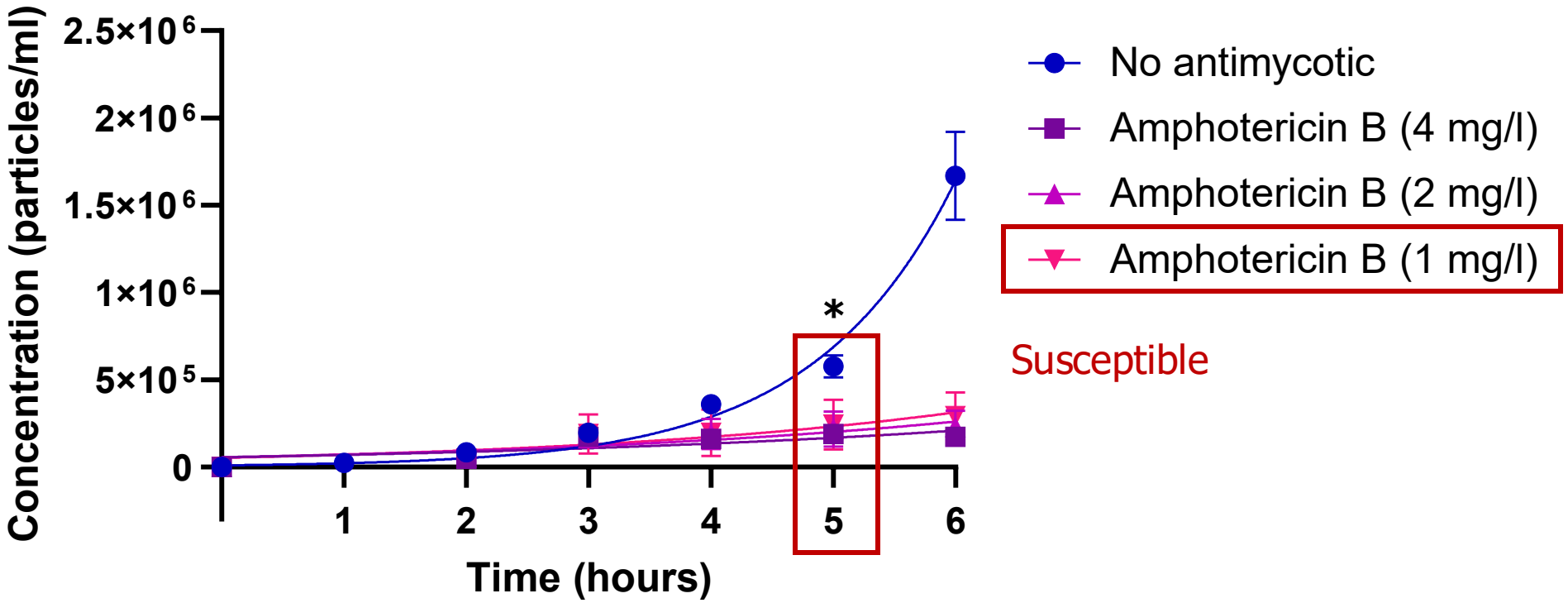
MIC = 32 mg/l

Resistant

CDC	R ≥ (mg/l)
A = Amphotericin B	2

MIC Minimum inhibitory concentration

C. auris CBS 12373: IPAC2 AR

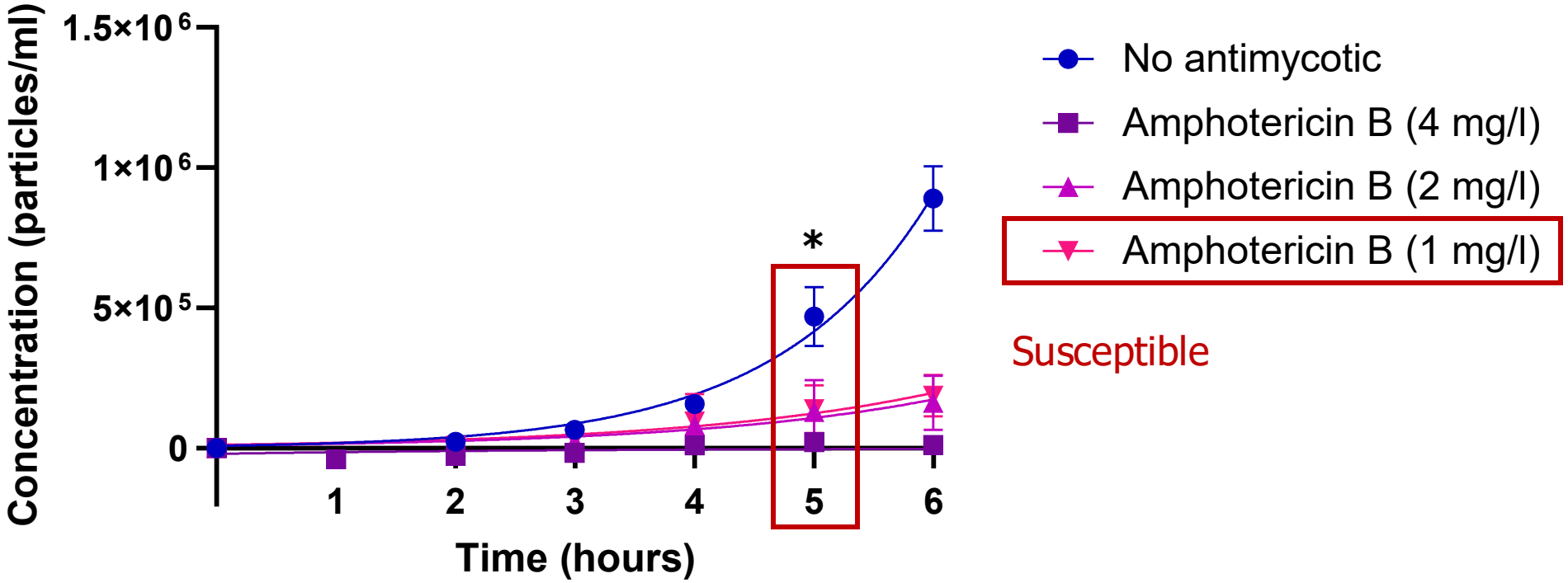


Two-way ANOVA (n=3)
* $p < 0.05$

CDC	R ≥ (mg/l)
A = Amphotericin B	2

MIC Minimum inhibitory concentration

C. auris CBS 12373: Flow cytometer



Susceptible

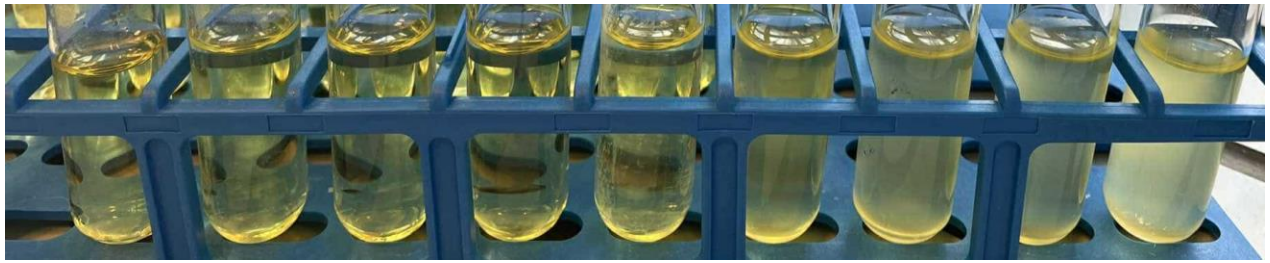
Two-way ANOVA (n=3)
* $p < 0.05$

CDC	R ≥ (mg/l)
A = Amphotericin B	2

MIC (Minimum inhibitory concentration)

C. auris CBS 12373: Conventional techniques

Broth dilution test



MIC = 0.5 mg/l

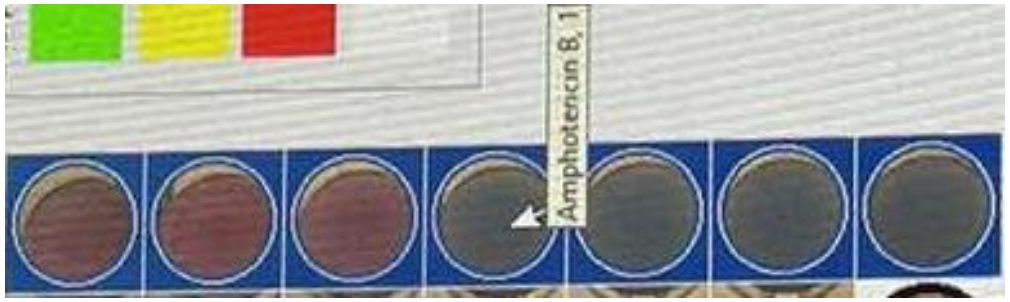
Susceptible

E-test

MIC = 0.32 mg/l

Susceptible

Sensititre Vizion



MIC = 1 mg/l

Susceptible

Vitek 2 Compact

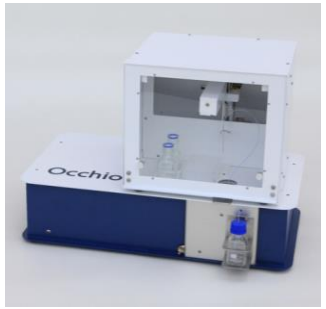
MIC ≤ 0.25 mg/l

Susceptible

Conclusion

CDC	R ≥ (mg/l)
C = Caspofungin	2
F = Fluconazol	32
A = Amphotericin B	2

	C. auris B11220			*C. auris* B11224			*C. auris* B11230			*C. auris* CBS12373			*C. auris* B11222			Time (hours)
	C	F	A	C	F	A	C	F	A	C	F	A	C	F	A	
IPAC2 AR (Occhio)	S	S	S	R	R	S	S	R	S	S	R	S	S	R	S	**4-7**
Flow cytometer	S	S	S	R	R	S	S	R	S	S	R	S	S	R	S	**4-7**
Broth dilution test	S	S	S	R	R	S	S	R	S	S	R	S	S	R	S	**24**
E-test (Biomerieux)	S	S	S	R	R	S	S	R	S	S	R	S	S	R	S	**24**
Disk diffusion (Rosco)	/	S	S	/	R	S	/	R	S	/	R	S	/	R	S	**24**
Sensititre Vizion	S	S	S	R	R	S	S	S[1]	S	S	R	S	S	R	S	**24**
Vitek 2 Compact[2]	S	S	S	S	R	S	S	S	S	S	R	S	S	R	S	**24**

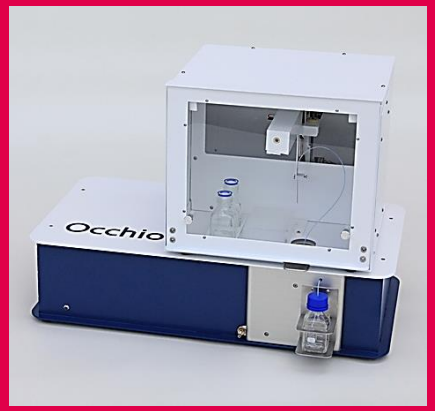


IPAC2 AR

Flow cytometer

C. auris:
4-7 hours

[1] After 48 hours S becomes R.
[2] Wrong identification of *C. auris.*
/ Not available.



Questions?

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RESEARCH & EXPERTISE

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