

# Diagnóstico rápido de la candidemia



Barcelona, 17 de marzo de 2017



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Infección grave  
INSTITUTO DE INVESTIGACIÓN SANITARIA LA FE



# Diagnóstico micológico

EL tiempo es ORO



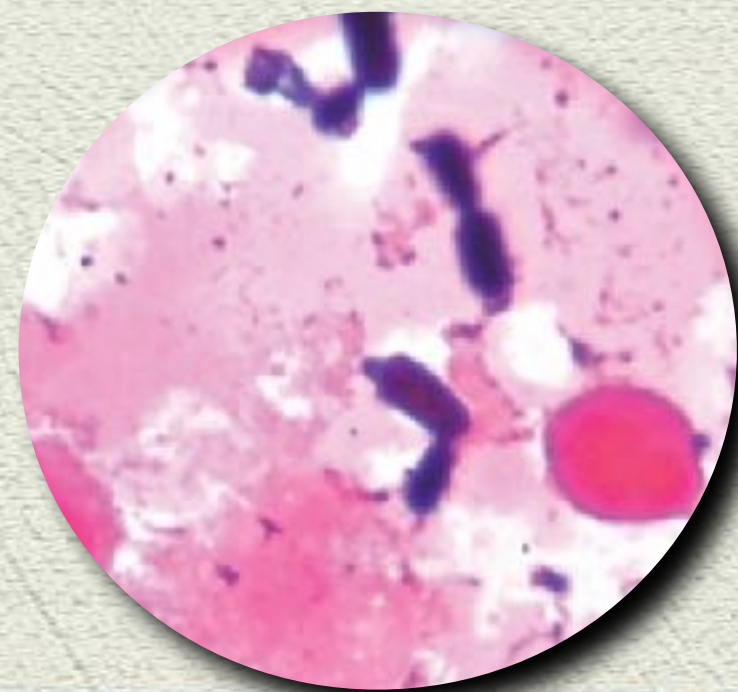
EL tiempo es VIDA



# Hemocultivo positivo...; identificar!



< 15'



★ Subcultivo en SDAC y CHROMagar

48 h

★ PNA-FISH

90'

★ Maldi-Tof directo

30'



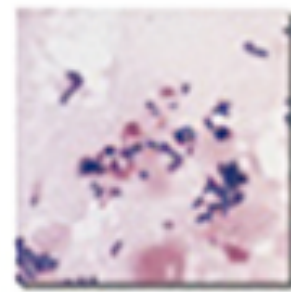
# Peptide Nucleic Acid Fluorescent In-situ Hybrdization

### Positive Blood Culture



15 Min.

## Gram Stain



GPCC



Yeast



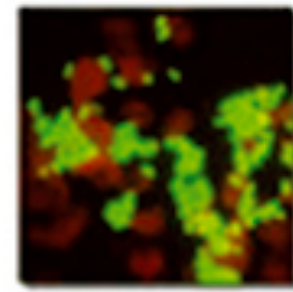
GPCPC

*S. aureus*  
➔  
PNA FISH™

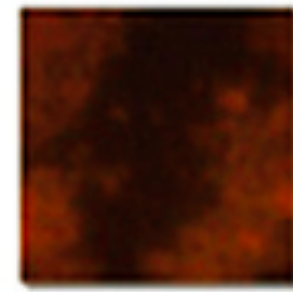
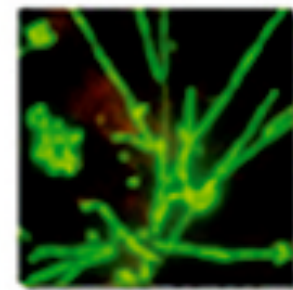
*C. albicans*  
➔  
PNA FISH™

*E. faecalis*  
➔  
PNA FISH™

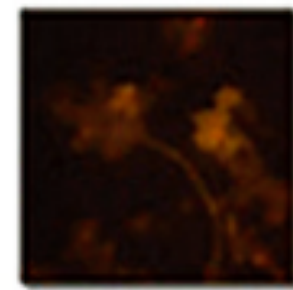
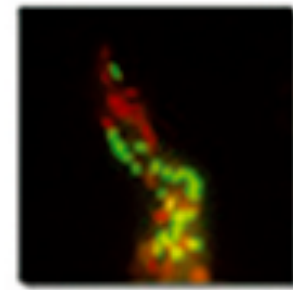
### Results (2.5 Hrs.)



*S. aureus*

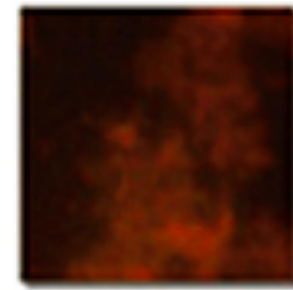
non-*S. aureus* GPCC

*C. albicans*

non-*C. albicans* Yeast

*E. faecalis* - Green

*E. faecalis* - Green non-enterococci GPCPC  
other enterococci - Red



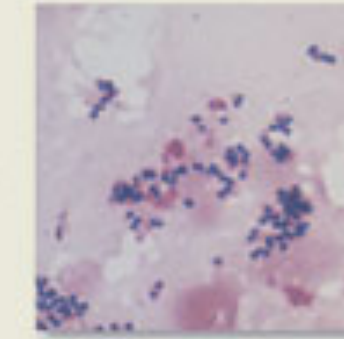
non-enterococci GPCPC

### Positive Blood Culture



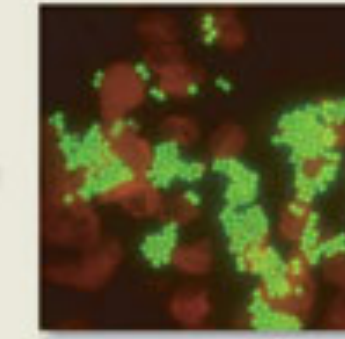
15 Min.

Gram stain



GPCC

2.5 Hrs.



*S. aureus*

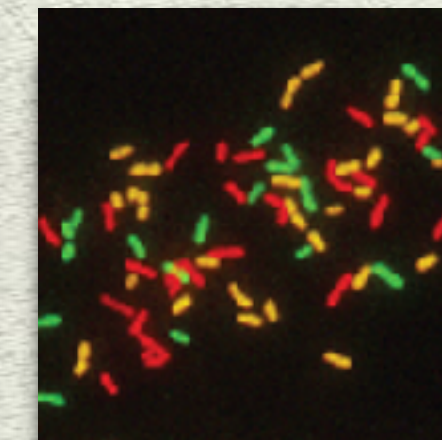
or



non-*S. aureus*



### Call to Physician



# GNR Traffic Light™ PNA FISH®

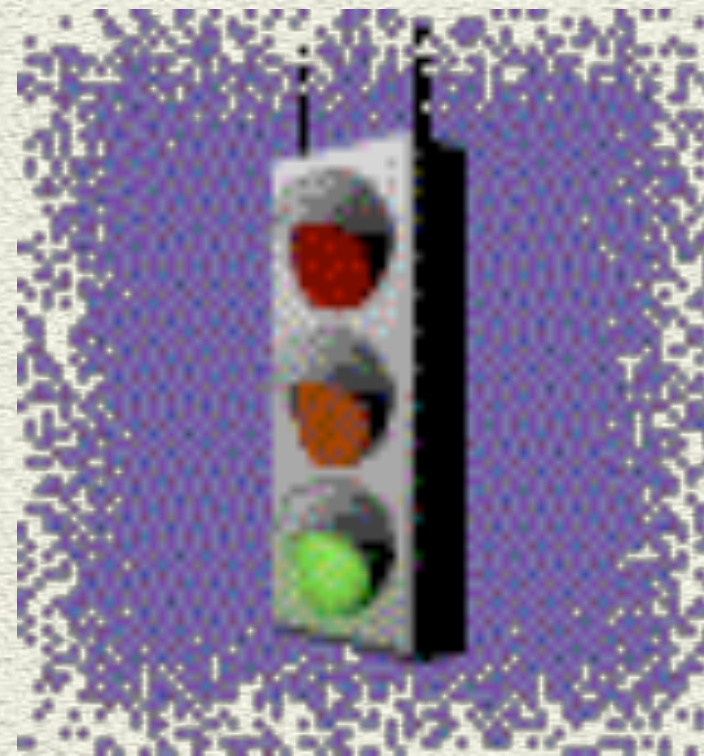
# Rapid identification of *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* from positive blood cultures



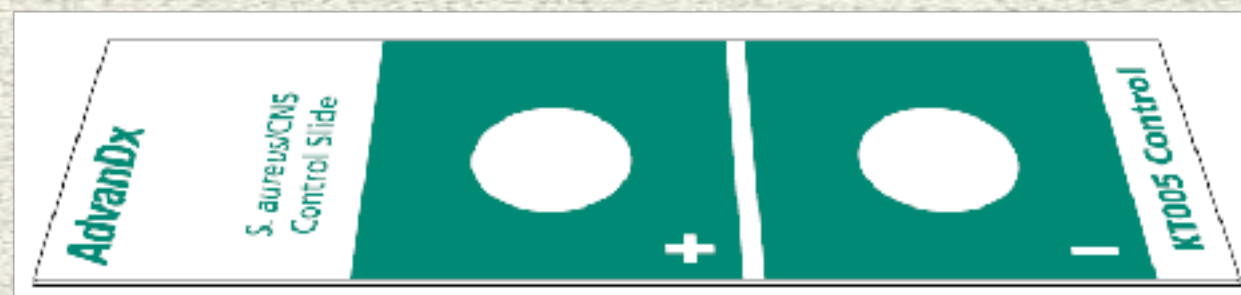
# PNA FISH

## Peptide Nucleic Acid Fluorescent In-situ Hybridization

- *C. glabrata* / *C. krusei*
- *C. tropicalis*
- *C. albicans* / *C. parapsilosis*



90'



*C. glabrata* o *C. krusei*

*C. albicans* o *C. parapsilosis*

*C. tropicalis*

Forrest GN, Curr Fun Infect Rep 2008; 2:221-6

**Yeast Traffic Light PNA FISH™**

AdvanDX; Woburn, MA



# PNA FISH

## Evaluation of the Yeast Traffic Light PNA FISH Probes for Identification of *Candida* Species from Positive Blood Cultures

Leslie Hall, Kara M. Le Febre, Sharon M. Deml, Sherri L. Wohlfiel, and Nancy L. Wengenack  
Division of Clinical Microbiology, Mayo Clinic, Rochester, Minnesota, USA

Hall L. J Clin Microbiol 2012; 50:1446

The Yeast Traffic Light PNA FISH kit (YTL) correctly identified *Candida* spp. in 207/216 (96%) positive blood cultures. Identical results were seen with known cross-reacting species and cultures containing *Candida lambica* and *Rhodotorula mucilaginosa*. The YTL provides rapid, reliable identification of the five common *Candida* species found in blood cultures.

## Evaluation of PNA-FISH Yeast Traffic Light for Rapid Identification of Yeast Directly from Positive Blood Cultures and Assessment of Clinical Impact

N. R. H. Stone, R. L. Gorton, K. Barker, P. Ramnarain, C. C. Kibbler  
Department of Microbiology, Royal Free Hospital, London, United Kingdom

Stone R. J Clin Microbiol 2013; 51:1301

The PNA-FISH Yeast Traffic Light assay was performed on 54 clinical isolates of yeasts inoculated into blood culture bottles. The assay showed high sensitivity (*Candida albicans*/*C. parapsilosis*, 100%; *C. glabrata*/*C. krusei*, 92.3%; *C. tropicalis*, 100%) and specificity (*C. albicans*/*C. parapsilosis*, 100%; *C. glabrata*/*C. krusei*, 94.8%; *C. tropicalis*, 100%). Case note review estimated a change in therapy in 29% of cases had the PNA-FISH result been available to the clinician.



# Evaluación de PNA FISH en 154

## hemocultivos con levaduras en HU La Fe

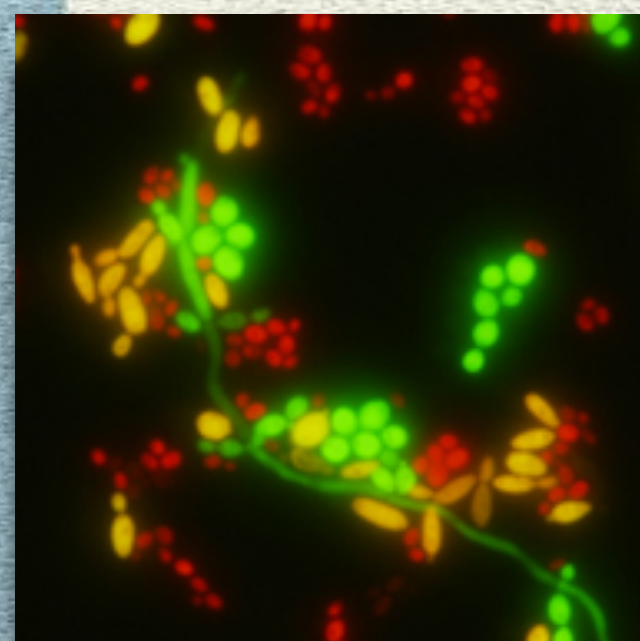


2015-2017

| Especie                      | PNA FISH (color) |           |          |           | Error identificación |
|------------------------------|------------------|-----------|----------|-----------|----------------------|
| <i>C. parapsilosis</i> (47)  | 47               |           |          |           |                      |
| <i>C. auris</i> (47)         |                  |           |          | 47        |                      |
| <i>C. albicans</i> (31)      | 30               |           |          | 1*        | 1                    |
| <i>C. glabrata</i> (12)      |                  |           | 6        | 6*        | 6                    |
| <i>C. tropicalis</i> (10)    |                  | 10        |          |           |                      |
| <i>C. guilliermondii</i> (3) |                  |           |          | 3         |                      |
| <i>C. krusei</i> (1)         |                  |           | 1        |           |                      |
| <i>C. lusitaniae</i> (2)     |                  |           |          | 2         |                      |
| <i>C. neoformans</i> (1)     |                  |           |          | 1         |                      |
| <b>TOTAL (154)</b>           | <b>77</b>        | <b>10</b> | <b>7</b> | <b>60</b> | <b>7</b>             |

Mala identificación  
 $7/93 = 7,5\%$

Utilidad práctica  
en nuestro hospital  
 $94/154 = 61\%$





# Accelerate Pheno™ system and PhenoTest™ BC kit



2017 FDA approved

Reduce (40 h) sensiblemente el tiempo de identificación y sensibilidad *in vitro* (CMI) de los hemocultivos (+)

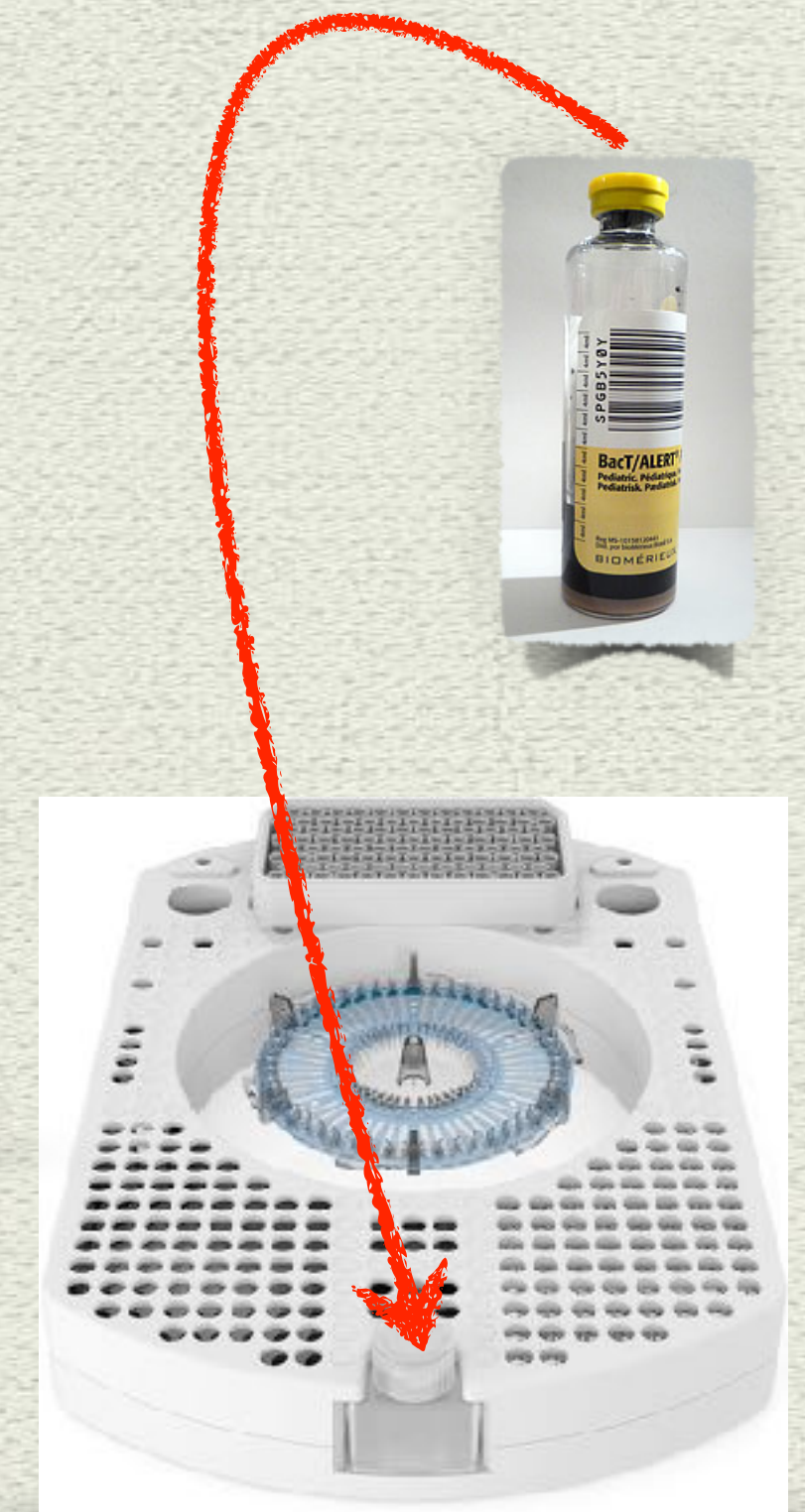
- ★ Identificación: FISH automatizado
- ★ Sensibilidad *in vitro*: análisis celular morfocinético (MCA)



- Precio elevado (400 €/muestra)
- Solo incluye 2 especies *Candida*
- No datos en candidemia

Tiempo medio resultados:

- Identificación: 90 min
- Sensibilidad antibiótica: 7 h





# Accelerate Pheno™ system and PhenoTest™ BC kit



## Microorganismos

### **Klebsiella spp.**

*K. oxytoca*  
*K. pneumoniae*

### **Enterobacter spp.**

*E. cloacae*  
*E. aerogenes*

### **Proteus spp.**

*P. mirabilis*  
*P. vulgaris*

### **Citrobacter spp.**

*C. freundii*  
*C. koseri*

### **Serratia marcescens**

### **Acinetobacter baumannii**

### **Pseudomonas aeruginosa**

11

### **Candida spp.**

*C. albicans*  
*C. glabrata*

2

### **Staphylococcus spp.**

*S. aureus*  
*S. capitis*  
*S. epidermidis*  
*S. haemolyticus*  
*S. hominis*  
*S. lugdunensis*  
*S. warneri*

### **Streptococcus spp.**

*S. agalactiae*  
*S. gallolyticus*  
*S. mitis*  
*S. oralis*  
*S. pneumoniae*

### **Enterococcus spp.**

*E. faecium*  
*E. faecalis*

14

## Antimicrobianos

Ampicilin

Ampicillin-Sulbactam

Ceftaroline

Piperacillin-Tazobactam

Erythromycin

Cefepime

Daptomycin

Ceftazidime

Linezolid

Vancomycin

Ceftriaxone

MRSA (Cefoxitin)

Ertapenem

MLSb (Erythro-clinda)

Meropenem

18

Amikacin

Gentamicin

Tobramycin

Ciprofloxacin

Aztreonam



# MALDI-TOF "directo" de hemocultivo

Direct MALDI-TOF Mass Spectrometry Assay of Blood Culture Broths for Rapid Identification of *Candida* Species Causing Bloodstream Infections: an Observational Study in Two Large Microbiology Laboratories



30'

We evaluated the reliability of the Bruker Daltonik's MALDI Biotyper system in species-level identification of yeasts directly from blood culture bottles. Identification results were concordant with those of the conventional culture-based method for 95.9% of *Candida albicans* (187/195) and 86.5% of non-*albicans Candida* species (128/148). Results were available in 30 min (median), suggesting that this approach is a reliable, time-saving tool for routine identification of *Candida* species causing bloodstream infection.



In conclusion, our study confirms that the Bruker Biotyper system is one of the more promising alternatives proposed to accelerate species-level ID of yeast isolates. Furthermore, almost 80% of our positive BCs were reported positive  $\leq 24$  h after sample entry (median time, 16 h). Consequently, with direct Bruker Biotyper assay of BC broth, physicians can realistically expect (in many cases) to receive species-level ID data for *Candida* isolates causing BSI within 24 h after the BC is drawn, a prospect that is unthinkable with conventional culture-based phenotypic testing.



# MALDI-TOF "directo" de hemocultivo

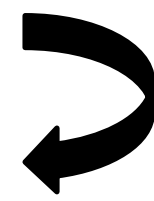
30'

Extraer 5 ml del hemocultivo y  
Centrifugar a 2000 rpm 2min



Descartar el sobrenadante  
lavar dos veces el sedimento con 1,5  
ml de agua destilada estéril

Centrifugar  
(12500 rpm, 2')



Incubar 10 min a 37 °C



Lavar con 1ml de  
agua destilada estéril  
X 2



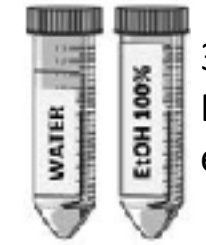
Re-suspender 1 ml de  
(SDS 0,1%, NaHCO 3  
0,015 M)



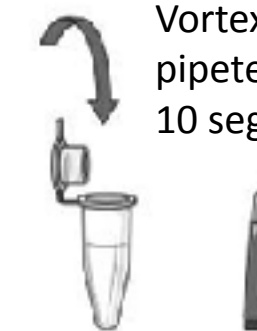
Centrifugar  
(12500 rpm, 2')



Centrifugar  
(12500 rpm, 2')



300µl de agua destilada estéril  
Re-suspender en 900µl de  
etanol absoluto



Vortex o  
pipeteo  
10 seg



Centrifugar  
(12500 rpm, 2')



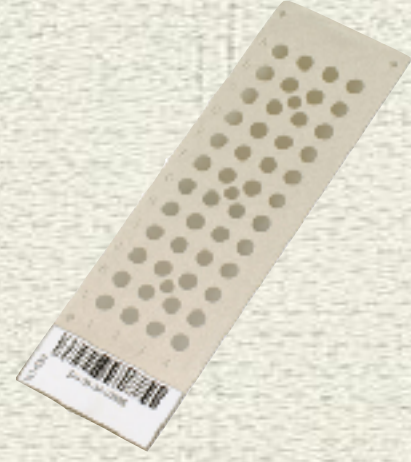
Descartar el  
sobrenadante  
dejar secar al  
aire.

Añadir 50µl de AF  
+  
50µl de  
Acetonitrilo

Centrifugar  
(12500 rpm, 2')



Añadir 1 µl de matriz (CHCA)  
Dejar secar





Identificación de 110 hemocultivos monomicrobianos después de extracción directa por MALDI TOF - MS



| Target Species                       | Nº. of different strains | Vitek MS identification results | Concordant identification [Nº (%)] | NO identification [Nº (%)] | Discordance [Nº (%)] |
|--------------------------------------|--------------------------|---------------------------------|------------------------------------|----------------------------|----------------------|
| <i>Escherichia coli</i>              | 22                       | 21                              | 21                                 | 1                          | 0                    |
| <i>Klebsiella spp.</i>               | 6                        | 6                               | 6                                  | 0                          | 0                    |
| <i>Enterobacter cloacae</i>          | 6                        | 5                               | 5                                  | 0                          | 1                    |
| <i>Serratia marcescens</i>           | 3                        | 3                               | 3                                  | 0                          | 0                    |
| <i>Pseudomonas aeruginosa</i>        | 3                        | 3                               | 3                                  | 0                          | 0                    |
| Other BGNNF                          | 3                        | 1                               | 1                                  | 2                          | 0                    |
| Rod Gram-negative                    | 43 (39,10%)              | 41                              | 39 (90,69)                         | 3                          | 1                    |
| STCN                                 | 31                       | 26                              | 26                                 | 4                          | 1                    |
| <i>Staphylococcus aureus</i>         | 5                        | 5                               | 5                                  | 0                          | 0                    |
| <i>Enterococcus spp.</i>             | 5                        | 5                               | 5                                  | 0                          | 0                    |
| <i>Streptococcus pneumoniae</i>      | 2                        | 2                               | 1                                  | 0                          | 1                    |
| <i>Streptococcus alpha hemolytic</i> | 3                        | 1                               | 1                                  | 2                          | 0                    |
| <i>Listeria monocytogenes</i>        | 2                        | 2                               | 2                                  | 0                          | 0                    |
| Otros BGP                            | 3                        | 2                               | 2                                  | 1                          | 0                    |
| Gram-positive                        | 51 (46,36)               | 43                              | 42 (82,35)                         | 7                          | 2                    |
| <i>Candida parapsilosis</i>          | 10                       | 10                              | 10                                 | 0                          | 0                    |
| <i>Candida albicans</i>              | 2                        | 2                               | 2                                  | 0                          | 0                    |
| <i>Candida glabrata</i>              | 2                        | 1                               | 1                                  | 1                          | 0                    |
| Other fungus                         | 2                        | 0                               | 0                                  | 1                          | 1                    |
| Fungus                               | 16 (14,54)               | 13                              | 13 (81,25)                         | 2                          | 1                    |
| TOTAL                                | 110 (100%)               |                                 | 94 (86,36)                         | 11 (10)                    | 4 (3,64)             |



# Ácidos nucleicos

FilmArray™ (BioFire DX)

Screening de 24 patógenos causales de Sepsis en 1 hora

Sangre total  
(>4ml)

Líquidos  
estériles (máx.  
disponible hasta  
10 ml)



Incubación (12h)  
\*según volumen muestra

Extracción+Amplificación+Detección HRM (1h)

(180 €/muestra)

El 90% de las infecciones bacterianas y candidiásicas graves son debidas a:

## Gram + Bacteria

*Staphylococcus*  
*Staphylococcus aureus*  
*Streptococcus*  
*Streptococcus agalactiae*  
*Streptococcus pyogenes*  
*Streptococcus pneumoniae*  
*Enterococcus*  
*Listeria monocytogenes*

## Gram - Bacteria

*Enterobacteriaceae*  
*Enterobacter cloacae complex*  
*Escherichia coli*  
*Klebsiella oxytoca*  
*Klebsiella pneumoniae*  
*Serratia*  
*Proteus*  
*Acinetobacter baumannii*  
*Haemophilus influenzae*  
*Neisseria meningitidis*

## Fungi

*Candida albicans*  
*Candida glabrata*  
*Candida krusei*  
*Candida parapsilosis*  
*Candida tropicalis*

## Antibiotic

### Resistance\*

*mecA*  
*vanA / vanB*  
*KPC*

Altun O. J Clin Microbiol 2013; 51:4130

Altun O. J Clin Microbiol 2015; 53:710



# PCR + nanopartículas + RM

RESEARCH ARTICLE

DIAGNOSTICS

## T2 Magnetic Resonance Enables Nanoparticle-Mediated Rapid Detection of Candidemia in Whole Blood

!!! Detecta 1 ufc/mL de *Candida* spp en  
< 3 h !!!

*Candida* spp. cause both local and disseminated infections in immunocompromised patients. Bloodstream infections of *Candida* spp., known as "candidemia," are associated with a high mortality rate (40%), which is mainly attributed to the long diagnostic time required by blood culture. We introduce a diagnostic platform based on T2 magnetic resonance (T2MR), which is capable of sensitive and rapid detection of fungal targets in whole blood. In our approach, blood-compatible polymerase chain reaction is followed by hybridization of the amplified pathogen DNA to capture probe-decorated nanoparticles. Hybridization yields nanoparticle microclusters that cause large changes in the sample's T2MR signal. With this T2MR-based method, *Candida* spp. can be detected directly in whole blood, thus eliminating the need for analyte purification. Using a small, portable T2MR detection device, we were able to rapidly, accurately, and reproducibly detect five *Candida* species within human whole blood with a limit of detection of 1 colony-forming unit/ml and a time to result of <3 hours. Spiked blood samples showed 98% positive agreement and 100% negative agreement between T2MR and blood culture. Additionally, performance of the assay was evaluated on 21 blinded clinical specimens collected serially. This study shows that the nanoparticle- and T2MR-based detection method is rapid and amenable to automation and offers clinicians the opportunity to detect and identify multiple human pathogens within hours of sample collection.

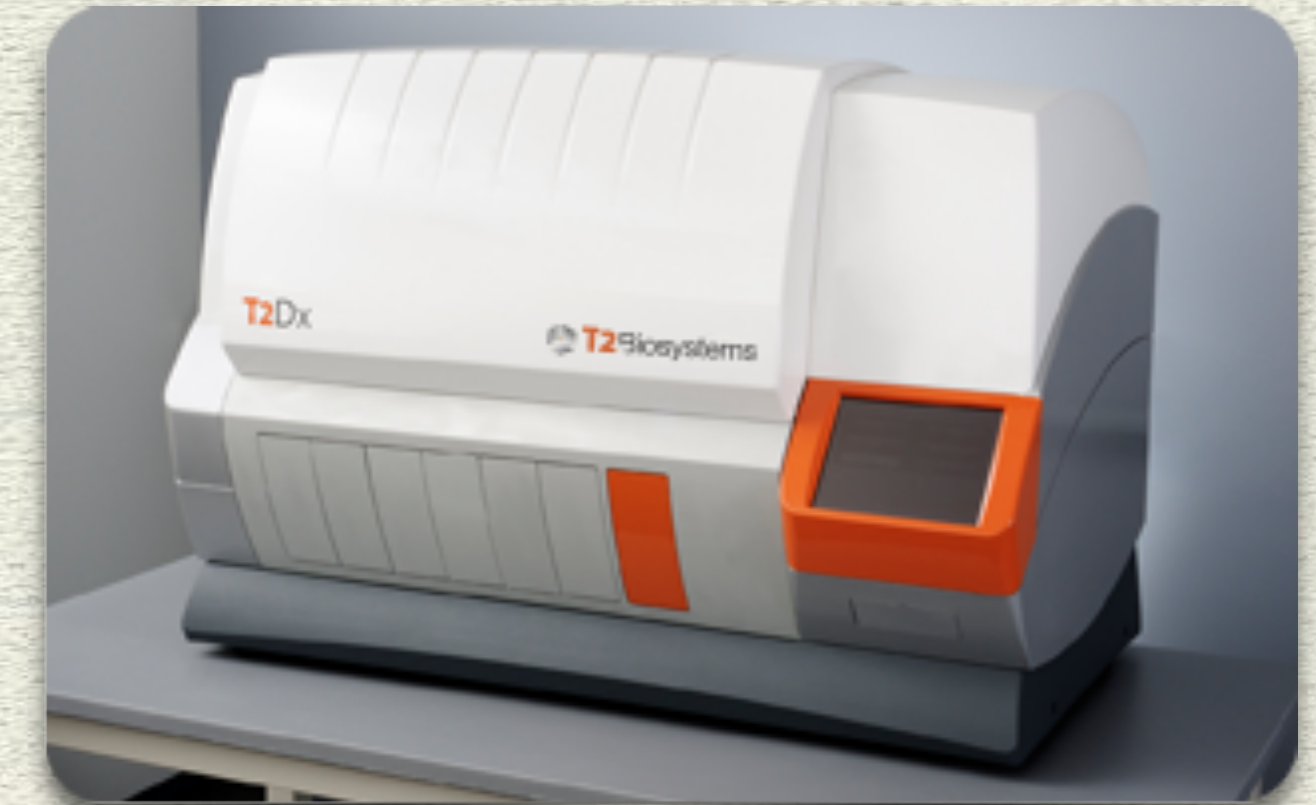
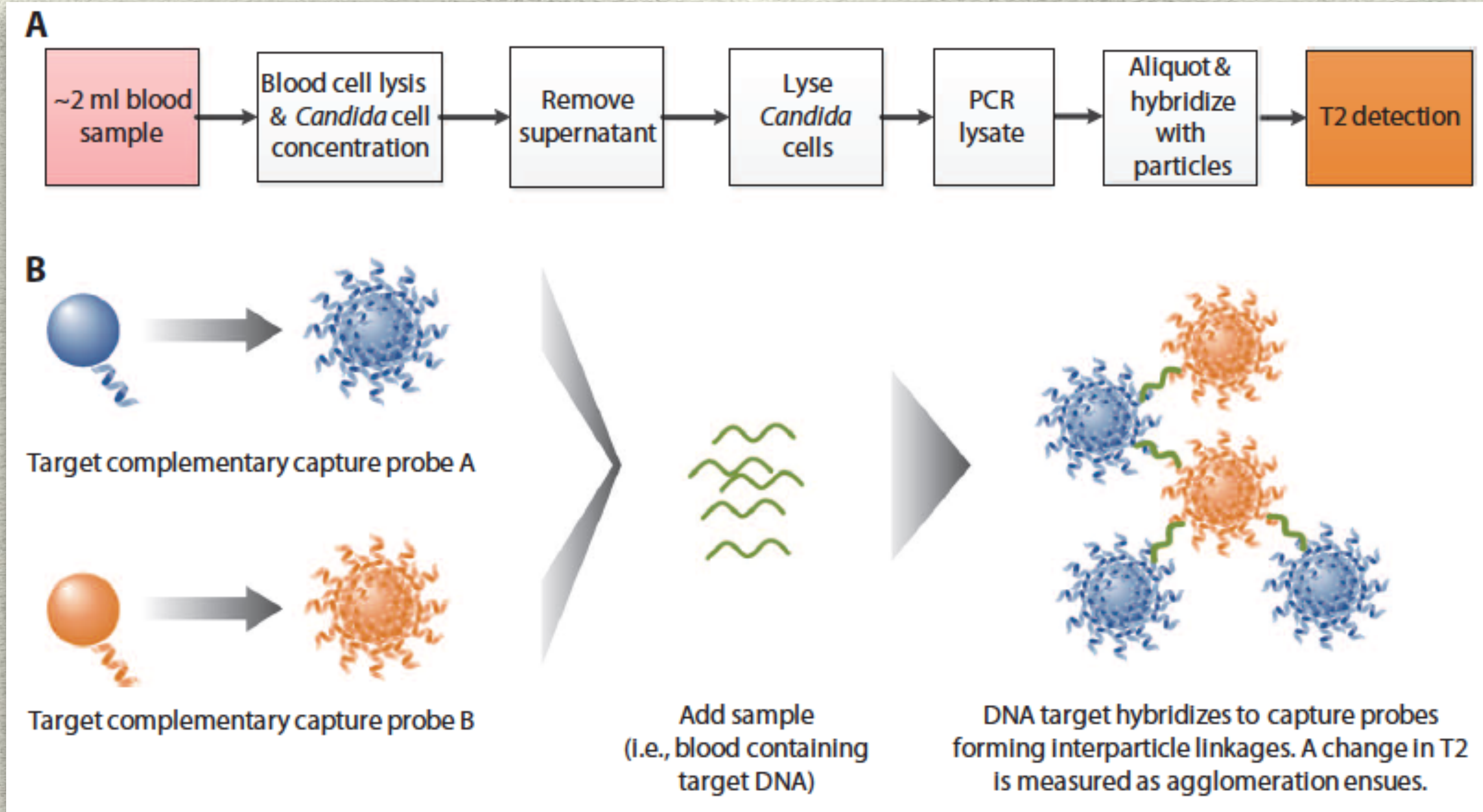


3 h





# PCR + nanopartículas + RM

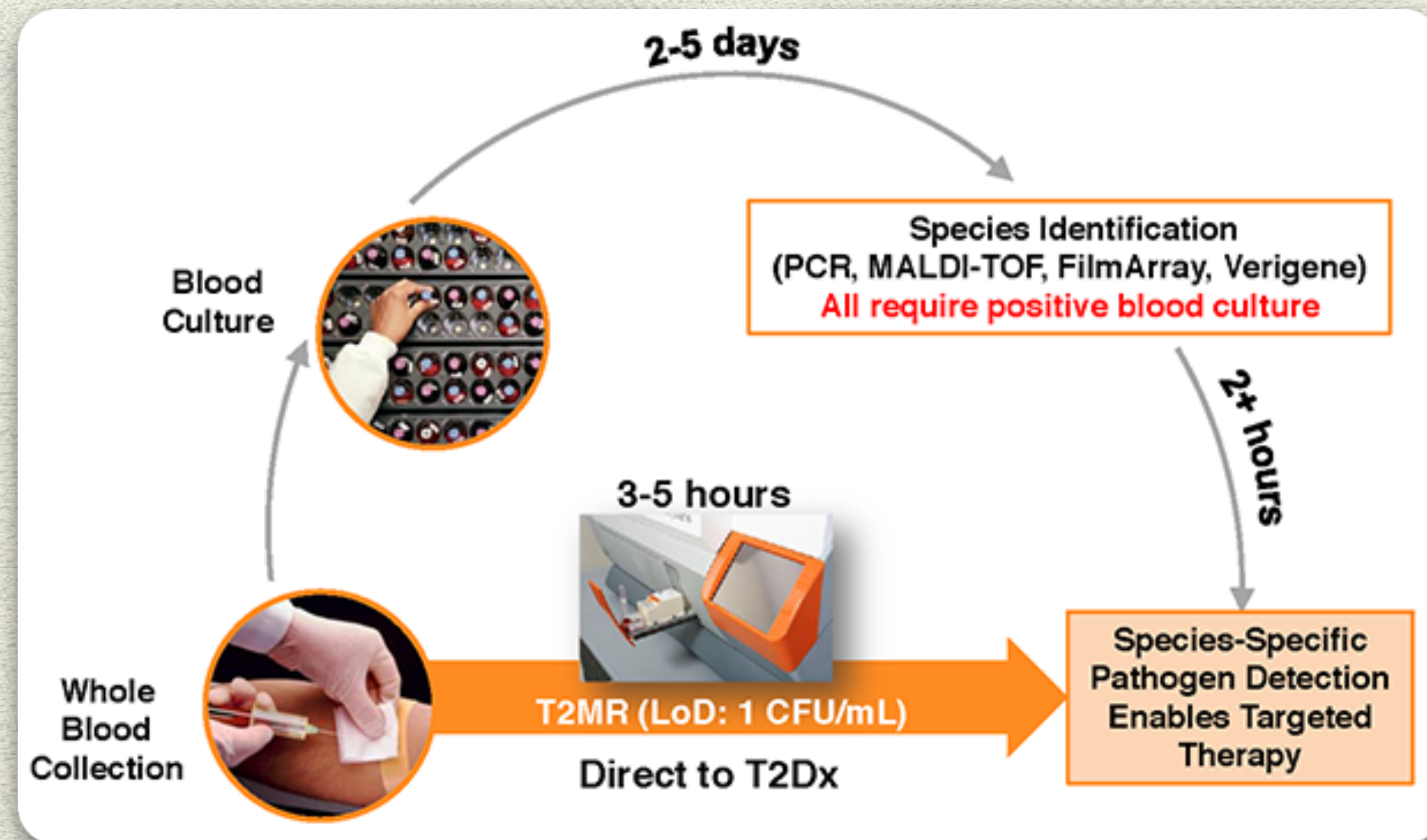


# T2Candida® (2014 FDA approved)

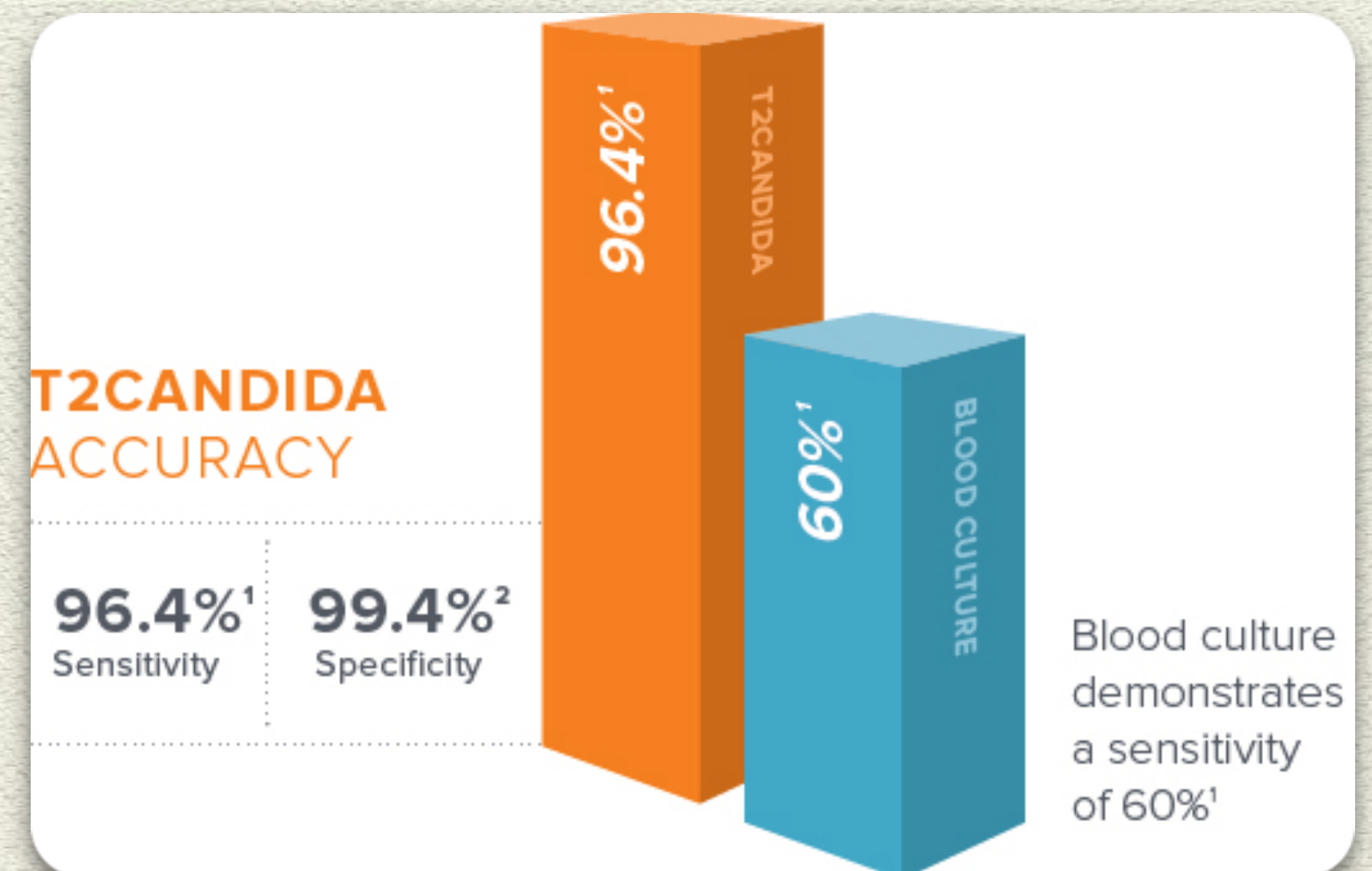
## T2Candida® (2015 CE Mark)



# PCR + nanopartículas + RM



T2Candida® (2014 FDA approved)



T2Candida® (2015 CE Mark)



# T2 Magnetic Resonance Assay for the Rapid Diagnosis of Candidemia in Whole Blood: A Clinical Trial

Eleftherios Mylonakis,<sup>1</sup> Cornelius J. Clancy,<sup>2</sup> Luis Ostrosky-Zeichner,<sup>3</sup> Kevin W. Garey,<sup>4</sup> George J. Alangaden,<sup>5</sup> Jose A. Vazquez,<sup>6</sup> Jeffrey S. Groeger,<sup>7</sup> Marc A. Judson,<sup>8</sup> Yuka-Marie Vinagre,<sup>9</sup> Stephen O. Heard,<sup>10</sup> Fainareti N. Zervou,<sup>1</sup> Ioannis M. Zacharioudakis,<sup>1</sup> Dimitrios P. Kontoyiannis,<sup>11</sup> and Peter G. Pappas<sup>12</sup>

**Background.** Microbiologic cultures, the current gold standard diagnostic method for invasive *Candida* infections, have low specificity and take up to 2–5 days to grow. We present the results of the first extensive multicenter clinical trial of a new nanodiagnostic approach, T2 magnetic resonance (T2MR), for diagnosis of candidemia.

**Methods.** Blood specimens were collected from 1801 hospitalized patients who had a blood culture ordered for routine standard of care; 250 of them were manually supplemented with concentrations from <1 to 100 colony-forming units (CFUs)/mL for 5 different *Candida* species.

**Results.** T2MR demonstrated an overall specificity per assay of 99.4% (95% confidence interval [CI], 99.1%–99.6%) with a mean time to negative result of 4.2 ± 0.9 hours. Subanalysis yielded a specificity of 98.9% (95% CI, 98.3%–99.4%) for *Candida albicans*/*Candida tropicalis*, 99.3% (95% CI, 98.7%–99.6%) for *Candida parapsilosis*, and 99.9% (95% CI, 99.7%–100.0%) for *Candida krusei*/*Candida glabrata*. The overall sensitivity was found to be 91.1% (95% CI, 86.9%–94.2%) with a mean time of 4.4 ± 1.0 hours for detection and species identification. The group analysis showed a sensitivity of 92.3% (95% CI, 85.4%–96.6%) for *C. albicans*/*C. tropicalis*, 94.2% (95% CI, 84.1%–98.8%) for *C. parapsilosis*, and 88.1% (95% CI, 80.2%–93.7%) for *C. krusei*/*C. glabrata*. The limit of detection was 1 CFU/mL for *C. tropicalis* and *C. krusei*, 2 CFU/mL for *C. albicans* and *C. glabrata*, and 3 CFU/mL for *C. parapsilosis*. The negative predictive value was estimated to range from 99.5% to 99.0% in a study population with 5% to 10% prevalence of candidemia, respectively.

**Conclusions.** T2MR is the first fully automated technology that directly analyzes whole blood specimens to identify species without the need for prior isolation of *Candida* species, and represents a breakthrough shift into a new era of molecular diagnostics.

Table 3. Positive and Negative Predictive Values of T2 Magnetic Resonance Method for a Range of Prevalence of Candidemia

| Prevalence of Disease | 91.0% Sensitivity/98.1% Specificity |       |
|-----------------------|-------------------------------------|-------|
|                       | PPV                                 | NPV   |
| 1%                    | 32.6%                               | 99.9% |
| 2%                    | 49.4%                               | 99.8% |
| 5%                    | 71.6%                               | 99.5% |
| 10%                   | 84.2%                               | 99.0% |
| 20%                   | 92.3%                               | 97.8% |
| 35%                   | 96.3%                               | 95.3% |
| 50%                   | 98.0%                               | 91.6% |

VENTAJAS

- ★ Rapidez (<4 h)
- ★ VPN >99%
- ★ Límite detección: 1-3 UFC/mL



# PCR + nanopartículas + RM



T2Candida® (2014 FDA approved)

T2Candida® (2015 CE Mark)



# PCR + RM + nanopartículas

2-4 mL sangre !!!



Utilidad en niños 😊😊😊

- Detección directa de *Candida* en sangre completa
- 5 especies de *Candida* (*C. albicans*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, *C. glabrata*)
- Límite de detección 1 ufc/ml
- Evaluación en muestras de sangre inoculadas con *Candida*
- Concordancia: 98% (cultivos positivos); 100% (cultivos negativos)
- Sistema comercializado, detector portátil de sobremesa
- Tiempo: 3 h
- Manejo: 2 minutos
- Rápido, automatizado, fácil

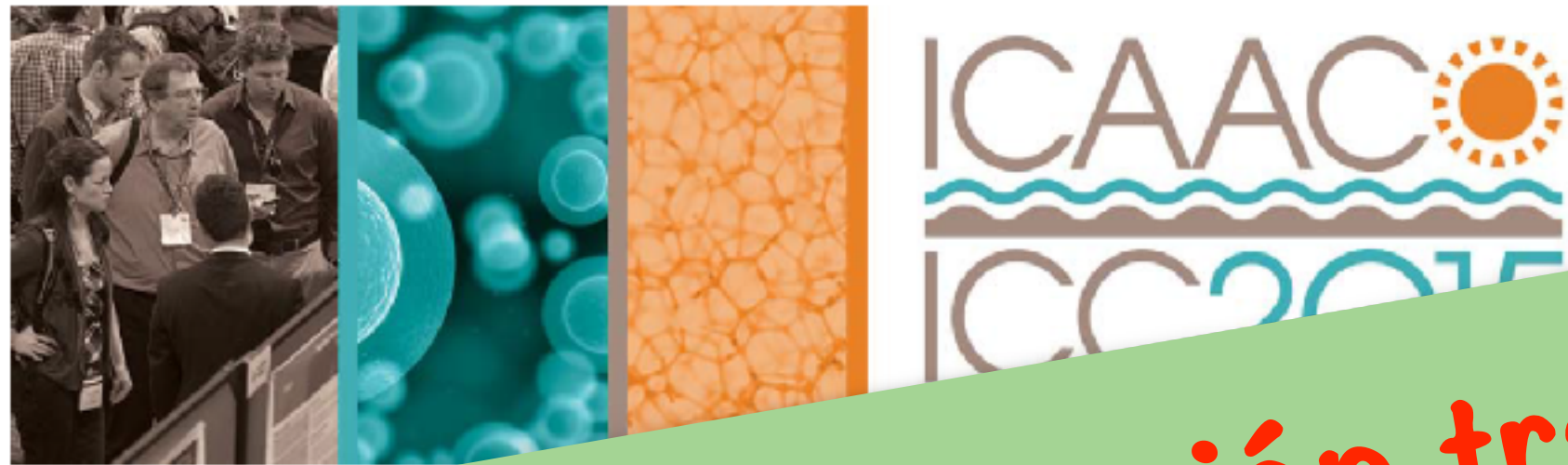
## RESULTADOS:

- ★ *C. albicans* / *C. tropicalis*
- ★ *C. krusei* / *C. glabrata*
- ★ *C. parapsilosis*





# PCR + RM + nanopartículas



Session: Methods of Care. Impact on Clinical

**T2candida is More Sensitive and Rapid Than Blood Culture for Detecting and Monitoring Invasive Candidiasis in Proven Cases of Infection**

P. Pappas<sup>1</sup>, M. Nguyen<sup>2</sup>, E. Mylonakis<sup>3</sup>; <sup>1</sup>Univ. of Alabama, Birmingham, Birmingham, AL, <sup>2</sup>Univ. of Pittsburgh Med. Ctr., Pittsburgh, PA, <sup>3</sup>Alpert Med. Sch. and Brown Univ., Providence, RI

T2MR and T2Candida: novel technology for the rapid diagnosis of candidemia and invasive candidiasis

Table 7. Summary of T2Candida detection of invasive candidiasis and candidemia.

| Disease detected                           | T2Candida     | Blood culture | Total <i>Candida</i> infections |
|--------------------------------------------|---------------|---------------|---------------------------------|
| Candidemia                                 | 31            | 33            | 33                              |
| Invasive candidiasis                       | 12            | 0             | 12                              |
| Probable or suspected invasive candidiasis | 10            | 0             | 10                              |
| Total cases                                | 53            | 33            | 55                              |
| Sensitivity                                | 96.4% (53/55) | 60% (33/55)   |                                 |

Pfaller M. Future Microbiol 2015; 11:103–17

**Conclusions:** T2C remained positive for proven candidemia and candidiasis infections in more patients and with greater frequency than BC, suggesting that T2C is more sensitive than BC for identifying invasive candidiasis. In one case, T2C identified a case of intra-abdominal candidiasis caused by *C. albicans* that was not detected by BC. T2C may be a useful tool for species identification, and patient monitoring for cases of invasive candidiasis to guide cessation of therapy, as well as improved source control.

Candidiasis invasiva con HC(-)



# The economic impact of rapid *Candida* species identification by T2Candida among high-risk patients

Sara Pinar Bilir<sup>\*1</sup>, Cheryl P Ferrufino<sup>1</sup>, Michael A Pfaller<sup>2,3</sup> & Julie Munakata<sup>1</sup>

## Impacto económico T2Candida

### BC vs T2

- Hospital 5100 ptes alto riesgo/año
- Prevalencia de candidemia 3%
- Ahorro anual/centro: \$5,858,448 (47.6%)
- Ahorro episodio candidemia: \$26,887 (48.8%)

The base case results were calculated using the assumption that in a hospital admitting 5100 high-risk patients per year [50], approximately 153 patients will be diagnosed with candidemia, assuming a 3% prevalence rate [71]. Based on

Using these assumptions, the annual cost for testing and treating the high-risk patients with the baseline BC-based strategy was estimated at US\$12,298,598 compared with US\$6,440,150 in the projected T2Candida-based diagnostic strategy for a potential annual savings of US\$5,858,448 (47.6%). The cost per tested

The estimated potential savings per patient with candidemia were US\$26,887, a 48.8% reduction in hospital costs. Rapid species

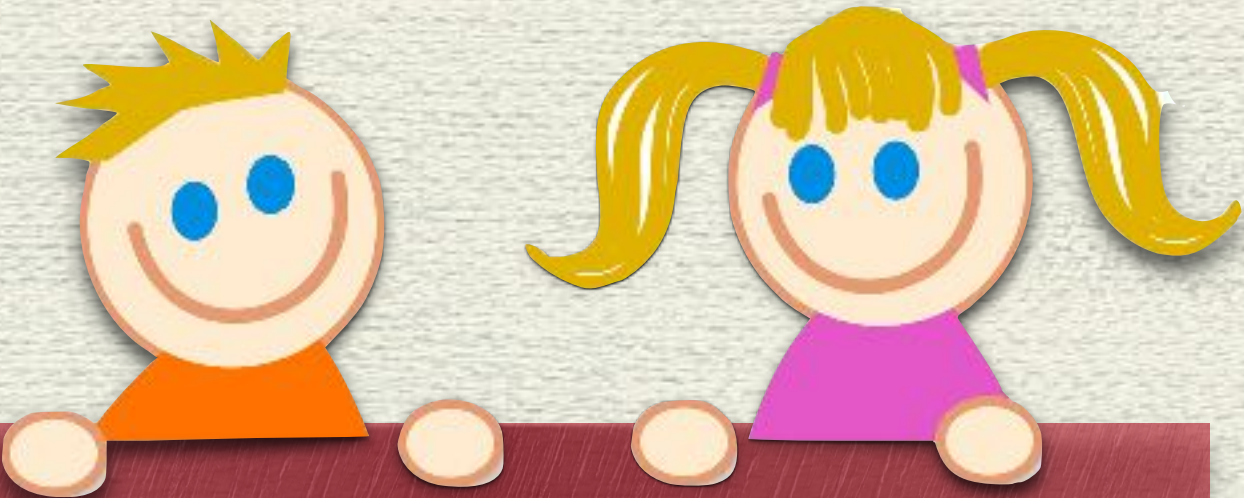


# T2Candida Provides Rapid and Accurate Species Identification in Pediatric Cases of Candidemia

During the study period, 15 whole-blood specimens were collected from pediatric patients, aged 5 to 12 years, with candidemia. Duplicate blood specimens were collected

ment; one specimen was subjected to blood culture and the other to the T2Candida panel. The following *Candida* species were identified by blood culture from these 15 patients: *Candida albicans* (4), *Candida parapsilosis* (7), *Candida glabrata* (3), and *Candida tropicalis* (1). The panel was also applied to nine whole blood specimens from pediatric patients who had negative blood cultures.

2 mL



100% concordancia con HC:  
● 15 niños con candidemia  
● 9 sin candidemia

Agreement Between T2Candida and Blood Culture Results for 24 Pediatric Samples Pipetted Directly Into a T2Candida Cartridge

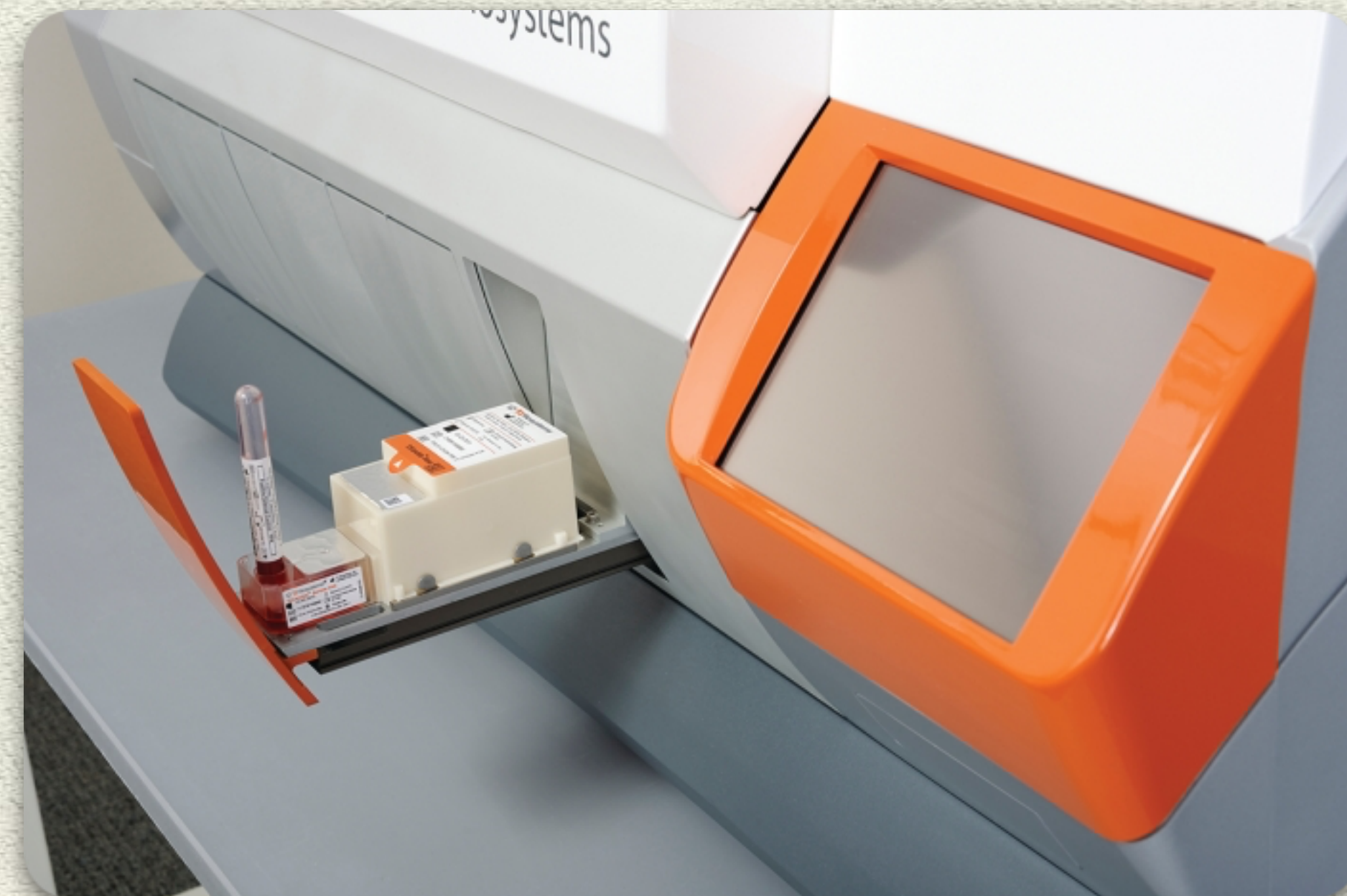
| Blood Culture Result  | T2Candida Result, No.                                  |                             |                                                    |          |
|-----------------------|--------------------------------------------------------|-----------------------------|----------------------------------------------------|----------|
|                       | <i>Candida albicans</i> /<br><i>Candida tropicalis</i> | <i>Candida parapsilosis</i> | <i>Candida krusei</i> /<br><i>Candida glabrata</i> | Negative |
| <i>C parapsilosis</i> | 0                                                      | 7                           | 0                                                  | 0        |
| <i>C albicans</i>     | 4                                                      | 0                           | 0                                                  | 0        |
| <i>C glabrata</i>     | 0                                                      | 0                           | 3                                                  | 0        |
| <i>C tropicalis</i>   | 1                                                      | 0                           | 0                                                  | 0        |
| Negative              | 0                                                      | 0                           | 0                                                  | 9        |

costs associated with a delayed candidiasis diagnosis.<sup>8,12</sup> A detailed economic model recently published estimated annual savings of \$5,858,448 for a hospital with just over 5,000 high-risk patients.<sup>12</sup> However, a detailed economic model specific to pediatric populations is needed for the T2Candida assay.



# T2 Candida en España

- ★ Estudio prospectivo
- ★ 2 hospitales (HUP La Fe, HU La Paz)
- ★ Pacientes críticos adultos
- ★ Pacientes críticos pediátricos





October 26-30 • New Orleans, LA • [www.idweek.org](http://www.idweek.org)



# IDWeek<sup>2016</sup><sup>TM</sup>

Advancing Science, Improving Care



## T2 Magnetic Resonance Poster Sessions

Thursday, October 27, 2016: 12:30-2:00pm

[205. Detection of \*Borrelia\* in Early-Stage Lyme Disease Using T2 Magnetic Resonance](#)

[T2 Biosystems](#)

*T2 Biosystems*

Friday, October 28, 2016: 12:30-2:00pm

[1560. Antifungal Prescribing During Initial Implementation of Candidemia Early Detection and Species Identification Testing with T2Candida Panel](#)

*Riverside Community Hospital*

[1567. Utilization of T2Candida<sup>®</sup> Panel for the Rapid Detection of \*Candida\* Species in a Large Community Hospital](#)

*Huntsville Hospital*

[1569. T2 Magnetic Resonance Improves Timely Management of Candidemia](#)

*Henry Ford Hospital*

[1571. Novel T2 Magnetic Resonance Assay Compared to Standard Blood Cultures for Detection of Candidemia](#)

*Henry Ford Hospital*





# Novel T2 Magnetic Resonance Assay Compared to Standard Blood Cultures for Detection of Candidemia.

Shamik Dwivedi D.O., Eloy Ordaya M.D., Brie Kezlarian M.D., Rachel Kenny PharmD, Robert Tibbetts PhD, Linoj Samuel PhD, George Alangaden M.D.

Henry Ford Hospital, Detroit



## Methods

Observational, retrospective, cross-sectional evaluation of patients with suspected candidemia who had T2 performed from 11/2015 - 6/2016 at Henry Ford Health System, a large healthcare system in Detroit, MI. TAT, discordant results of T2 and BC, and PPV/NPV were assessed.

## Results

- Of the first 200 patients, 50 (25%) had positive T2 and/or BC:
  - 11/50 (22%) had dual positive results and 39/50 (78%) had discordant results
- Overall, possible reasons for discordance were:
  - Different dates of blood collection (69%), antifungal therapy before blood collection (57%), and *Candida* sp. not detected by T2 (4%)
- The average TAT:
  - T2 → 11 hours (range 3-41 hours) vs. BC → 51 hours (range 3-120 hours) to preliminary result ( $p < 0.0001$ ) and 6 days (range 3-13 days) to final results.
- The positive and negative predictive values for T2 were 22% and 96% for specimens collected within 24 hours vs. 33% and 90% overall.

## 200 pacientes

- T2+/BC+: 50
- T2+/BC-: 22
- T2-/BC+: 17
- VPP: 22%
- VPN: 96%
- 11 h (3-41h)

## Conclusion

- Performance characteristics of T2 in routine clinical practice differ from those reported in clinical trials.
- Targeted use of T2 in only high-risk pts and uniformity in sample collection will be important for accurate interpretation of this test.
- The low PPV differs from that noted in early clinical trials and requires further investigation.
- The rapid TAT and good NPV of T2 will support future antimicrobial stewardship efforts.

Table 2. Characteristics of patients with discordant T2 and blood culture results.

| Characteristics                            | T2 + / BC -<br>( 22 patients ) | T2 - / BC +<br>( 17 patients ) | p value |
|--------------------------------------------|--------------------------------|--------------------------------|---------|
| <b>Demographic</b>                         |                                |                                | NS      |
| - Male sex                                 | 11 (50%)                       | 6 (35%)                        |         |
| - Age, mean (range)                        | 58.5 (19 - 87)                 | 58.7 (22 - 80)                 |         |
| <b>Medical history</b>                     |                                |                                | NS      |
| - Immuno-compromised                       | 11 (50%)                       | 9 (53%)                        |         |
| - Diabetes mellitus                        | 5 (23%)                        | 7 (41%)                        |         |
| - Renal failure (requiring dialysis)       | 4 (18%)                        | 4 (24%)                        |         |
| - TPN                                      | 8 (36%)                        | 5 (29%)                        |         |
| - Central Venous Catheter                  | 19 (86%)                       | 13 (77%)                       |         |
| - Prolonged ICU stay (>10 days)            | 15 (68%)                       | 11 (65%)                       |         |
| - Abdominal surgery                        | 5 (23%)                        | 3 (18%)                        |         |
| <b>Clinical presentation</b>               |                                |                                | NS      |
| - Fever                                    | 15 (68%)                       | 13 (77%)                       |         |
| - Hypotension                              | 12 (55%)                       | 9 (53%)                        |         |
| - Chorioretinitis                          | 0                              | 1 (6%)                         |         |
| - Deep seated infection                    | 3 (14%)                        | 1 (6%)                         |         |
| - Colonization                             | 3 (14%)                        | 7 (41%)                        |         |
| - Prior positive blood cultures            | 1                              | --                             |         |
| <b>Laboratory</b>                          |                                |                                | NS      |
| - Leukocytosis                             | 14 (64%)                       | 6 (35%)                        |         |
| - Neutropenia                              | 4 (18%)                        | 1 (6%)                         |         |
| <b>Blood culture positive</b>              |                                |                                |         |
| - <i>C. albicans</i>                       | --                             | 5 (29%)                        | --      |
| - <i>C. glabrata</i>                       | --                             | 7 (41%)                        | --      |
| - <i>C. lusitaniae</i>                     | --                             | 2 (12%)                        | --      |
| - <i>C. parapsilosis</i>                   | --                             | 3 (18%)                        | --      |
| <b>T2MR positive*</b>                      |                                |                                |         |
| - <i>C. albicans/C. tropicalis</i>         | 10 (46%)                       | --                             | --      |
| - <i>C. parapsilosis</i>                   | 4 (18%)                        | --                             | --      |
| - <i>C. glabrata/C. krusei</i>             | 8 (36%)                        | --                             | --      |
| <b>Candida score (range)</b>               | 2.1 (0 - 4)                    | 2.2 (0 - 4)                    | NS      |
| <b>Candidemia presentation</b>             | 14 (64%)                       | 15 (88%)                       | NS      |
| <b>Treatment</b>                           | 21 (96%)                       | 16 (94%)                       | NS      |
| <b>Average length of treatment (range)</b> | 10.8 (2 - 20)                  | 11 (1 - 25)                    | NS      |
| <b>Death</b>                               | 8 (36%)                        | 10 (59%)                       | NS      |





# Antifungal Prescribing During Initial Implementation of Candidemia

## Early Detection and Species Identification Testing with T2Candida Panel



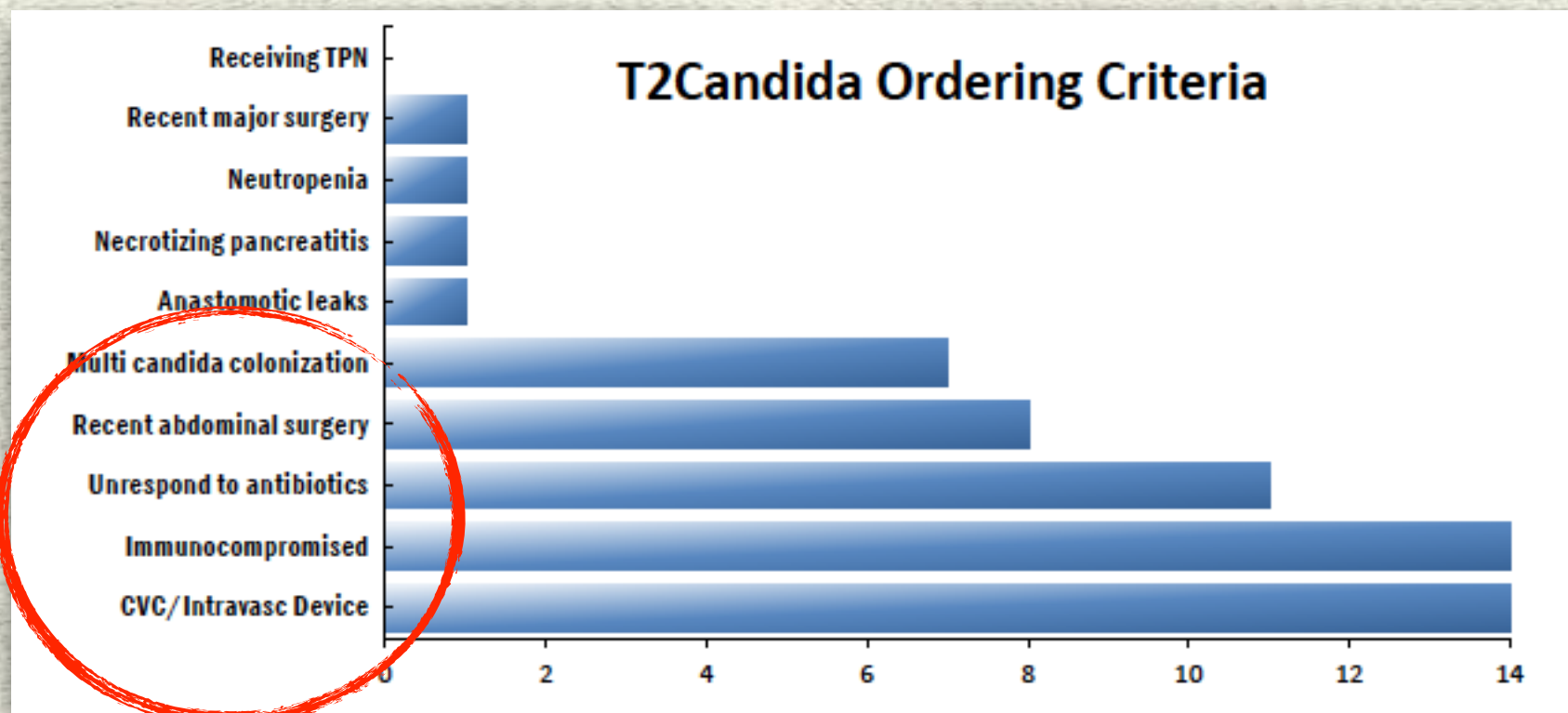
Falguni Patel, PharmD, BCPS, Elizabeth Young, PharmD,  
Department of Pharmaceutical Services, Riverside Community Hospital, Riverside, CA

- Valid T2 results were reported in 59 patients.
- 98% (58/59) of patients met hospital criteria for T2 in at-risk patients. Concomitant blood cultures were ordered on 38/59 (64%) patients.
- Antifungals had been prescribed for 11/59 patients (18.6%) prior to T2 ordering for empiric treatment of invasive fungal disease.
- T2 resulted in 3 to 5 hours, and was available in the hospital reporting system in an average of 6.3 hours.

### 59 pacientes

- T2+/BC+: 3
- T2+/BC-: 3
- T2-/BC+: 0
- ⌚ 3-5 h
- Tto dirigido < 6h

| Patient # | T2Candida species              | Regular blood culture species | Antifungal started < 6 hrs | Antifungal started < 9 hrs | Antifungal start based on T2Candida results |
|-----------|--------------------------------|-------------------------------|----------------------------|----------------------------|---------------------------------------------|
| 1         | <i>C.albicans/C.tropicalis</i> | <i>C.albicans</i>             | ✓                          |                            | micafungin                                  |
| 2         | <i>C.parapsilosis</i>          | Negative                      | ✓                          |                            | fluconazole                                 |
| 3         | <i>C.albicans/C.tropicalis</i> | Negative                      | ✓                          |                            | fluconazole                                 |
| 4         | <i>C.albicans/C.tropicalis</i> | Negative                      | ✓                          |                            | fluconazole                                 |
| 5         | <i>C.albicans/C.tropicalis</i> | <i>C.albicans</i>             |                            | ✓                          | fluconazole                                 |
| 6         | <i>C.albicans/C.tropicalis</i> | <i>C.albicans</i>             | ✓                          |                            | fluconazole                                 |



- Of the 59 T2 results, 6/59 (10%) were positive, 5/6 (83%) *C.albicans/C.tropicalis*, 1/6 (17%) *C.parapsilosis*. Of the patients with positive T2 results none had been prescribed antifungal therapy prior to the test.
- Appropriate antifungal therapy with either micafungin or fluconazole was ordered in positive T2 patients within 6 hours of hospital system reporting for 5/6 (83%) and within 9 hours in 1/6 (17%).
- All positive T2 patients had had concomitant blood cultures drawn with 3/6 (50%) reported as positive and those cultures reported *C.albicans* in agreement with their T2 results.
- Of the 53 patients with negative T2 results, none had subsequent positive fungal blood culture results and 8 had been on antifungals. Of those 8 patients, antifungal therapy was discontinued in accordance to clinical status.

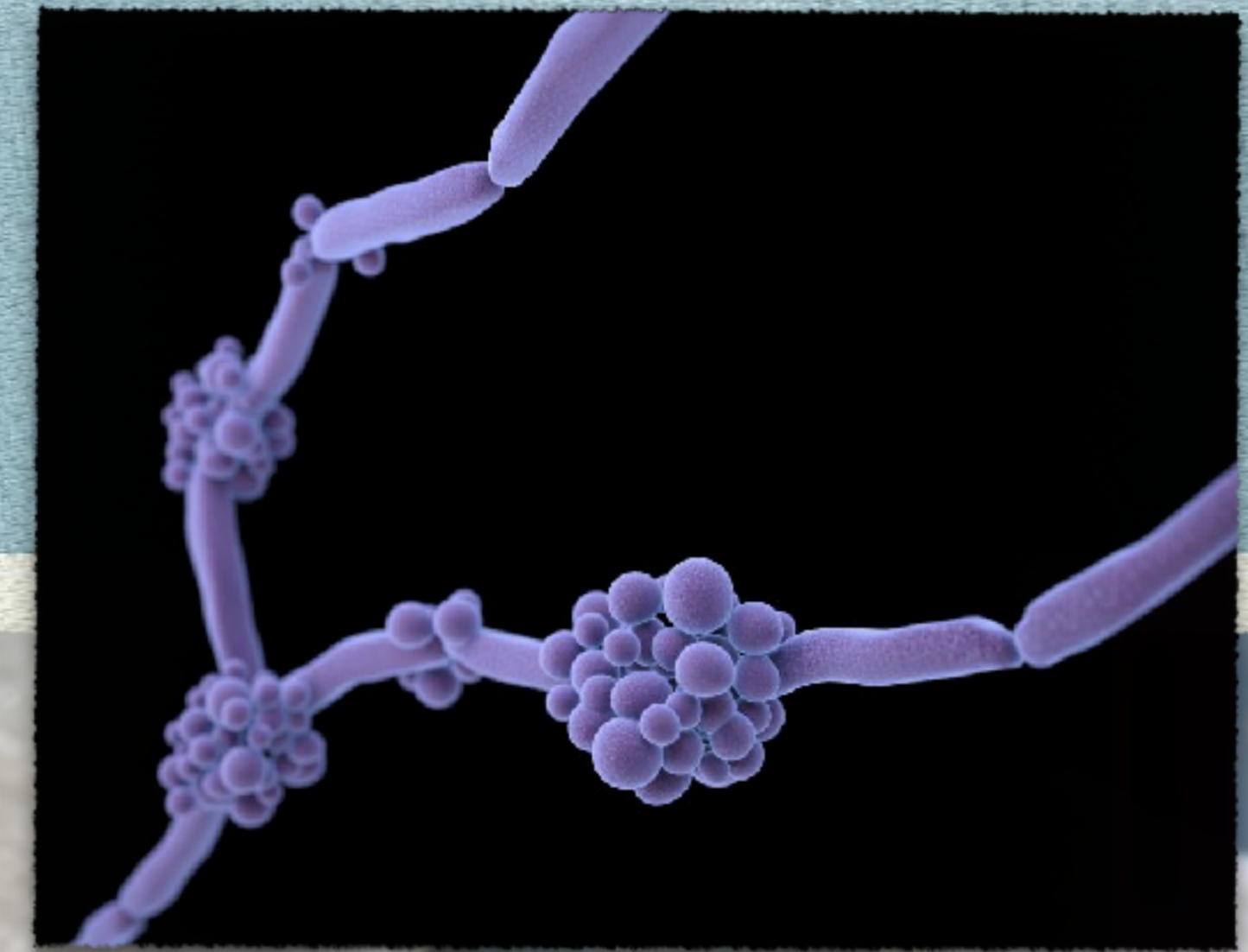






# Candida auris

- ★ Relacionada con Candida haemulonii
- ★ Trasmisibilidad muy elevada (<4 horas)
- ★ Agente causal de brotes hospitalarios
- ★ **No se identifica por las técnicas comerciales:** API, Auxacolor, Vitek ID, etc.
- ★ CHROMAGAR: colonias blancas-rosáceas
- ★ Mauditof: Bruker (ok), VitekMS (solo en RUO)
- ★ Identificación definitiva: Secuenciación ITS
- ★ **Resistente a fluconazol** (CMI > 256 mg/L) y voriconazol (CMI > 2 mg/L)
- ★ Ocasionalmente resistente a anfo B y candinas: multiresistente





# Identificación *C. auris*

## BIOQUÍMICA

Table 1. Biochemical identification

| Isolate no. | Species tested             | API <sup>a</sup>            | BD Phoenix <sup>b</sup> | Vitek-2 <sup>c</sup>                             | MicroScan <sup>d</sup>   |
|-------------|----------------------------|-----------------------------|-------------------------|--------------------------------------------------|--------------------------|
| 1           | <i>Candida auris</i>       | <i>Rhodotorula glutinis</i> | <i>C. catenulata</i>    | <i>C. haemulonii</i>                             | <i>C. famata</i>         |
| 2           | <i>C. auris</i>            | <i>R. glutinis</i>          | <i>C. haemulonii</i>    | <i>C. haemulonii</i>                             | <i>C. famata</i>         |
| 3           | <i>C. auris</i>            | <i>R. glutinis</i>          | <i>C. haemulonii</i>    | <i>C. haemulonii</i>                             | <i>C. famata</i>         |
| 4           | <i>C. auris</i>            | <i>R. glutinis</i>          | <i>C. haemulonii</i>    | <i>C. haemulonii</i>                             | <i>C. lusitanae</i>      |
| 5           | <i>C. auris</i>            | <i>R. glutinis</i>          | <i>C. haemulonii</i>    | <i>C. haemulonii</i>                             | <i>C. guilliermondii</i> |
| 6           | <i>C. auris</i>            | <i>R. glutinis</i>          | <i>C. haemulonii</i>    | <i>C. haemulonii</i>                             | <i>C. famata</i>         |
| 7           | <i>C. auris</i>            | <i>R. glutinis</i>          | <i>C. haemulonii</i>    | <i>C. haemulonii</i>                             | <i>C. guilliermondii</i> |
| 8           | <i>C. auris</i>            | <i>R. glutinis</i>          | <i>C. haemulonii</i>    | <i>C. haemulonii</i>                             | <i>C. parapsilosis</i>   |
| 9           | <i>C. auris</i>            | <i>R. glutinis</i>          | <i>C. haemulonii</i>    | <i>C. haemulonii</i>                             | <i>C. guilliermondii</i> |
| 10          | <i>C. auris</i>            | <i>R. glutinis</i>          | <i>C. haemulonii</i>    | <i>C. haemulonii</i>                             | <i>C. guilliermondii</i> |
| 11          | <i>C. duobushaemulonii</i> | <i>R. glutinis</i>          | <i>C. parapsilosis</i>  | <i>C. haemulonii</i>                             | <i>C. guilliermondii</i> |
| 12          | <i>C. duobushaemulonii</i> | <i>R. glutinis</i>          | <i>C. parapsilosis</i>  | <i>C. haemulonii</i>                             | <i>C. guilliermondii</i> |
| 13          | <i>C. haemulonii</i>       | <i>R. glutinis</i>          | <i>C. haemulonii</i>    | <i>C. haemulonii</i> /<br><i>Kodamaea ohmeri</i> | <i>C. catenulata</i>     |
| 14          | <i>C. duobushaemulonii</i> | <i>R. glutinis</i>          | <i>C. parapsilosis</i>  | <i>C. haemulonii</i>                             | <i>C. parapsilosis</i>   |
| 15          | <i>C. haemulonii</i>       | <i>R. glutinis</i>          | No identification       | <i>C. haemulonii</i> /<br><i>Kodamaea ohmeri</i> | <i>C. parapsilosis</i>   |

<sup>a</sup> Identification at 48 hours and 72 hours of incubation; API doesn't have *C. auris*, *C. haemulonii*, *C. duobushaemulonii* in its library.

## MALDI-TOF

Table 2. Identification by MALDI-TOF MS <sup>a</sup>

| Isolate no. | Species tested             | Bruker Biotyper                    |                                    |                              | Vitek-MS                       |                                |
|-------------|----------------------------|------------------------------------|------------------------------------|------------------------------|--------------------------------|--------------------------------|
|             |                            | FDA library <sup>b</sup>           | + RUO <sup>c</sup> library         | Full-tube extraction (score) | IVD <sup>d</sup> library       | + RUO library <sup>e</sup>     |
|             |                            | Direct on-plate extraction (score) | Direct on-plate extraction (score) |                              | Direct extraction (% identity) | Direct extraction (% identity) |
| 1           | <i>Candida auris</i>       | No ID <sup>f</sup>                 | <i>C. auris</i> (1.75)             | <i>C. auris</i> (2.19)       | No ID                          | <i>C. auris</i> (99)           |
| 2           | <i>C. auris</i>            | No ID                              | No ID                              | <i>C. auris</i> (2.02)       | No ID                          | <i>C. auris</i> (98)           |
| 3           | <i>C. auris</i>            | No ID                              | <i>C. auris</i> (1.80)             | <i>C. auris</i> (2.04)       | No ID                          | <i>C. auris</i> (99)           |
| 4           | <i>C. auris</i>            | No ID                              | <i>C. auris</i> (1.73)             | <i>C. auris</i> (2.10)       | No ID                          | <i>C. auris</i> (99)           |
| 5           | <i>C. auris</i>            | No ID                              | No ID                              | <i>C. auris</i> (1.95)       | No ID                          | <i>C. auris</i> (99)           |
| 6           | <i>C. auris</i>            | No ID                              | No ID                              | <i>C. auris</i> (1.97)       | No ID                          | <i>C. auris</i> (87)           |
| 7           | <i>C. auris</i>            | No ID                              | No ID                              | <i>C. auris</i> (2.05)       | No ID                          | <i>C. auris</i> (99)           |
| 8           | <i>C. auris</i>            | No ID                              | <i>C. auris</i> (1.73)             | <i>C. auris</i> (1.99)       | No ID                          | <i>C. auris</i> (99)           |
| 9           | <i>C. auris</i>            | No ID                              | No ID                              | <i>C. auris</i> (2.00)       | No ID                          | <i>C. auris</i> (92)           |
| 10          | <i>C. auris</i>            | No ID                              | <i>C. auris</i> (1.75)             | <i>C. auris</i> (1.92)       | No ID                          | <i>C. auris</i> (99)           |
| 11          | <i>C. duobushaemulonii</i> | <i>C. duobushaemulonii</i> (2.29)  | <i>C. duobushaemulonii</i> (2.29)  | Not tested                   | No ID                          | No ID                          |
| 12          | <i>C. duobushaemulonii</i> | <i>C. duobushaemulonii</i> (2.19)  | <i>C. duobushaemulonii</i> (2.19)  | Not tested                   | No ID                          | No ID                          |
| 13          | <i>C. haemulonii</i>       | <i>C. haemulonii</i> (2.29)        | <i>C. haemulonii</i> (2.29)        | Not tested                   | <i>C. haemulonii</i>           | <i>C. haemulonii</i>           |

Mizusawa M, J Clin Microbiol, 2017; 55:638



# Diagnóstico candidiasis invasora por *C. auris*

PCR tiempo real

RealCycler AURI-U/AURI-G®

Sensibilidad: 1 copia /  $\mu$ L

- ★ Sangre total (EDTA)
- ★ Líquidos orgánicos
- ★ Exudados, ...
- ★ HC +



- Listo para usar
- Alta sensibilidad y especificidad
- Resultados rápidos (2 horas aprox.)
- Marcado CE-IVD (diagnóstico in vitro)

**PROGENIE**  
MOLECULAR



# Identificación *C. auris*

CMI muy elevada a fluconazol  
(256 mg/L)

AMB

ANI

MIC

CAS

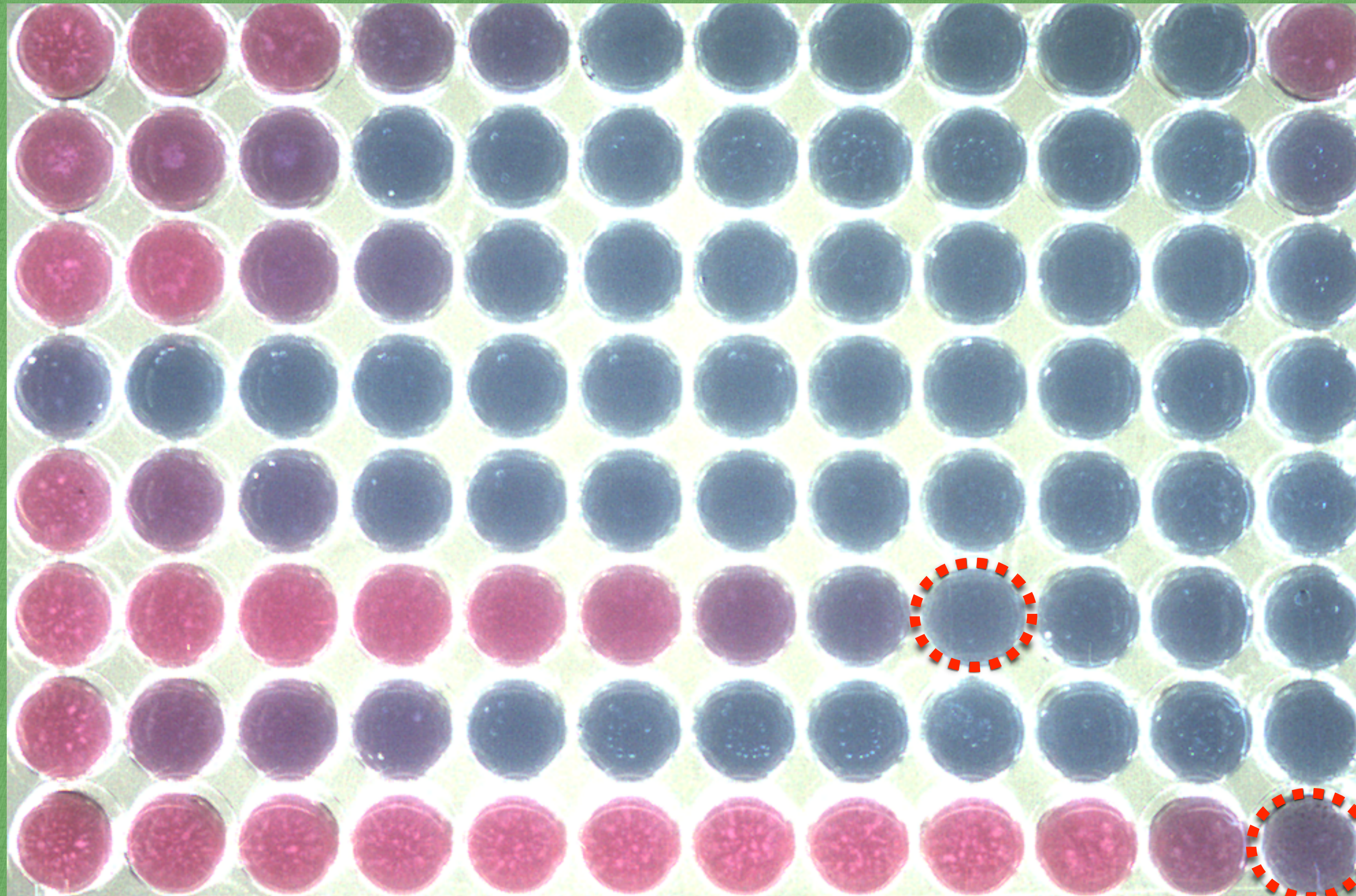
5FL

POS

VOR

ITR

FLU

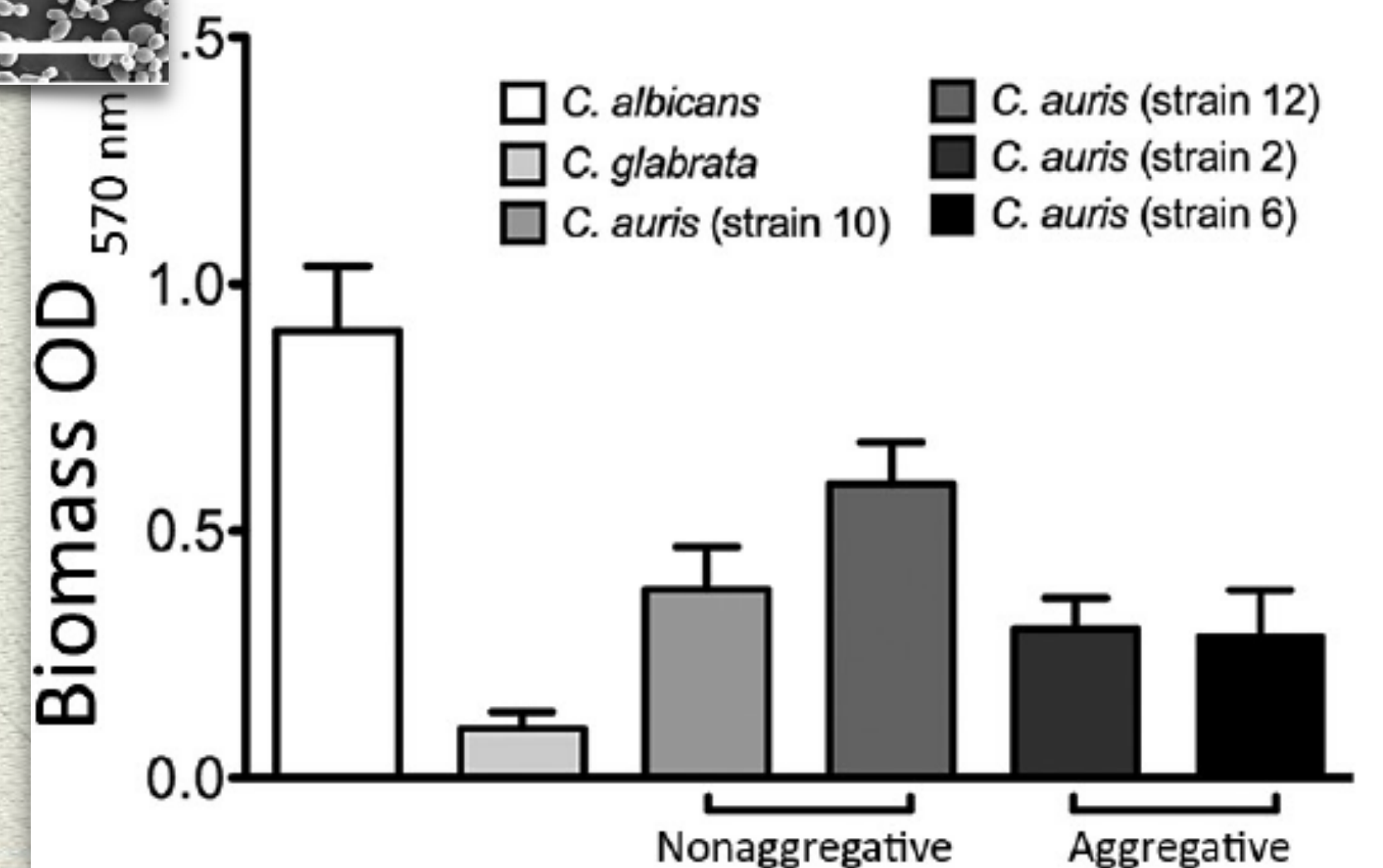
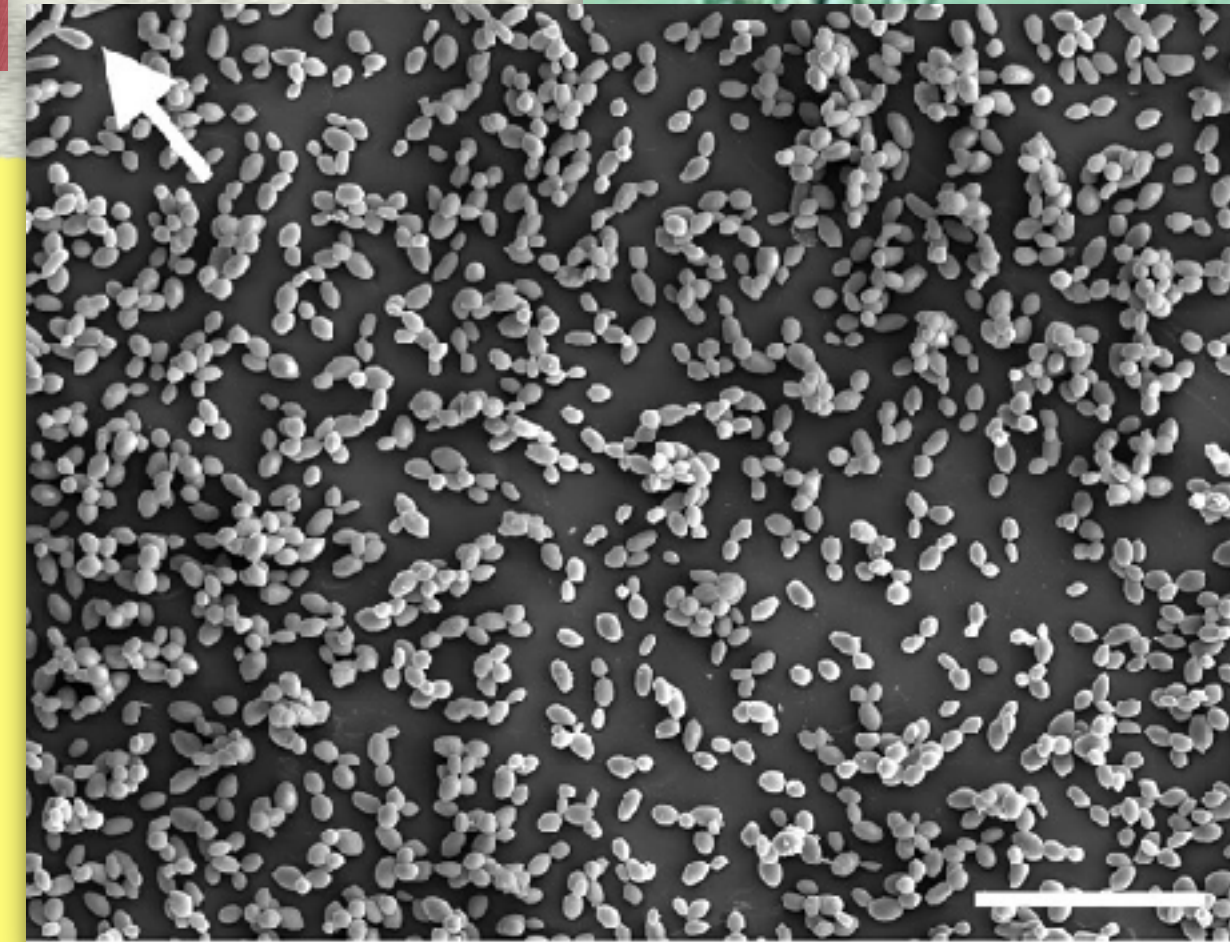
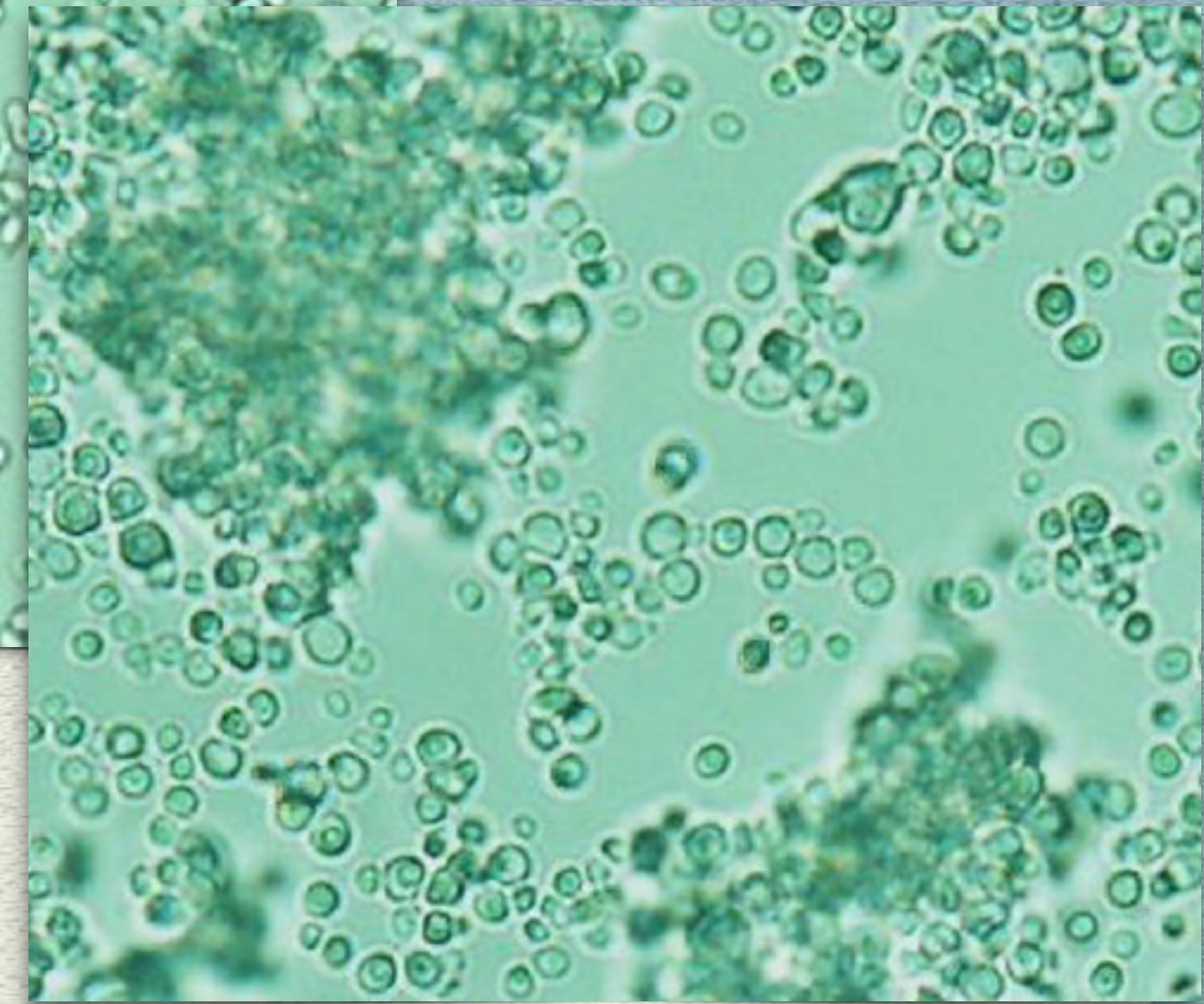
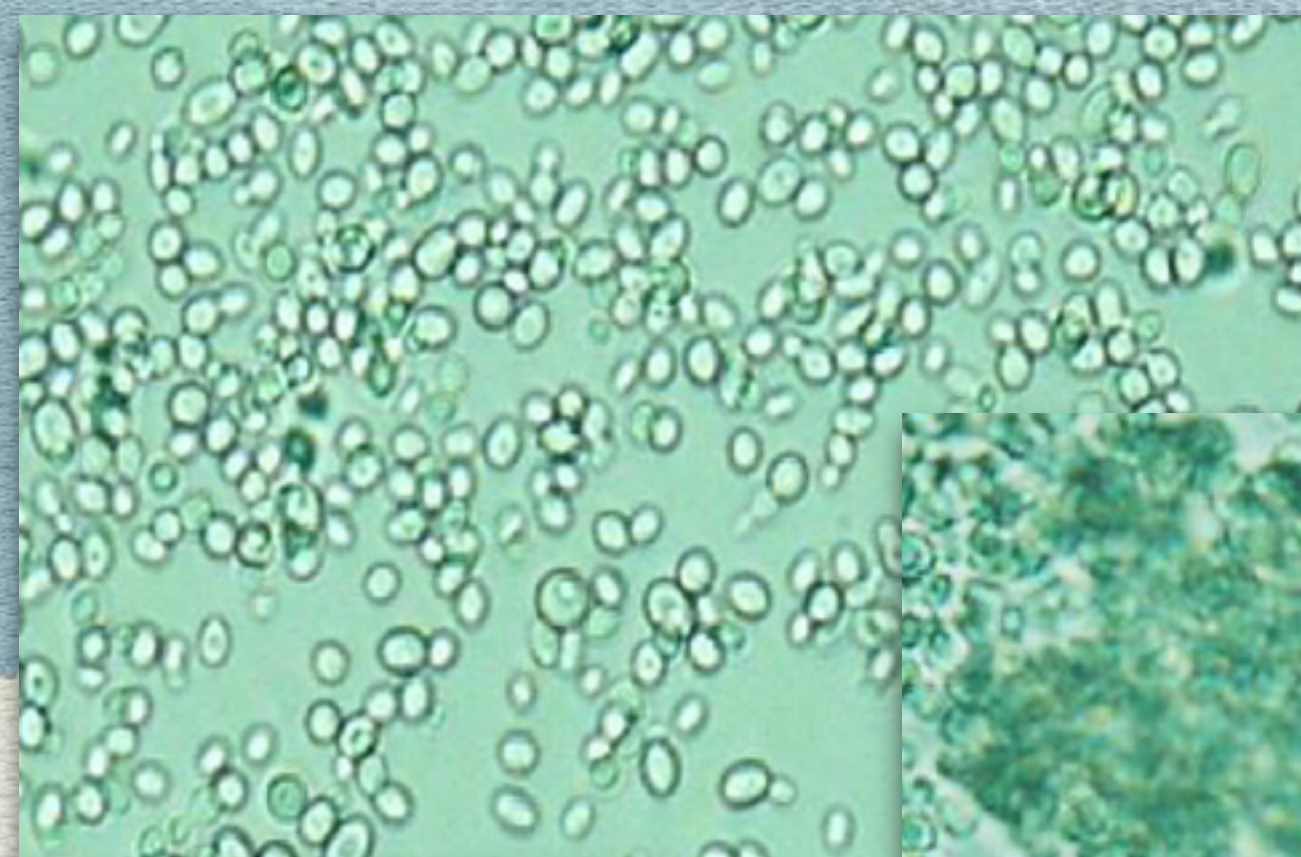




# C. auris: virulencia

Similar a *C. albicans*

- Actividad fosfolipasa
- Actividad proteinasa
- Fenotipo células no-agregadas:
  - ◆ Formación biopelículas (?)
  - ◆ Letalidad vs. *Galleria* > *C. albicans*





# *C. auris* en HUP La Fe

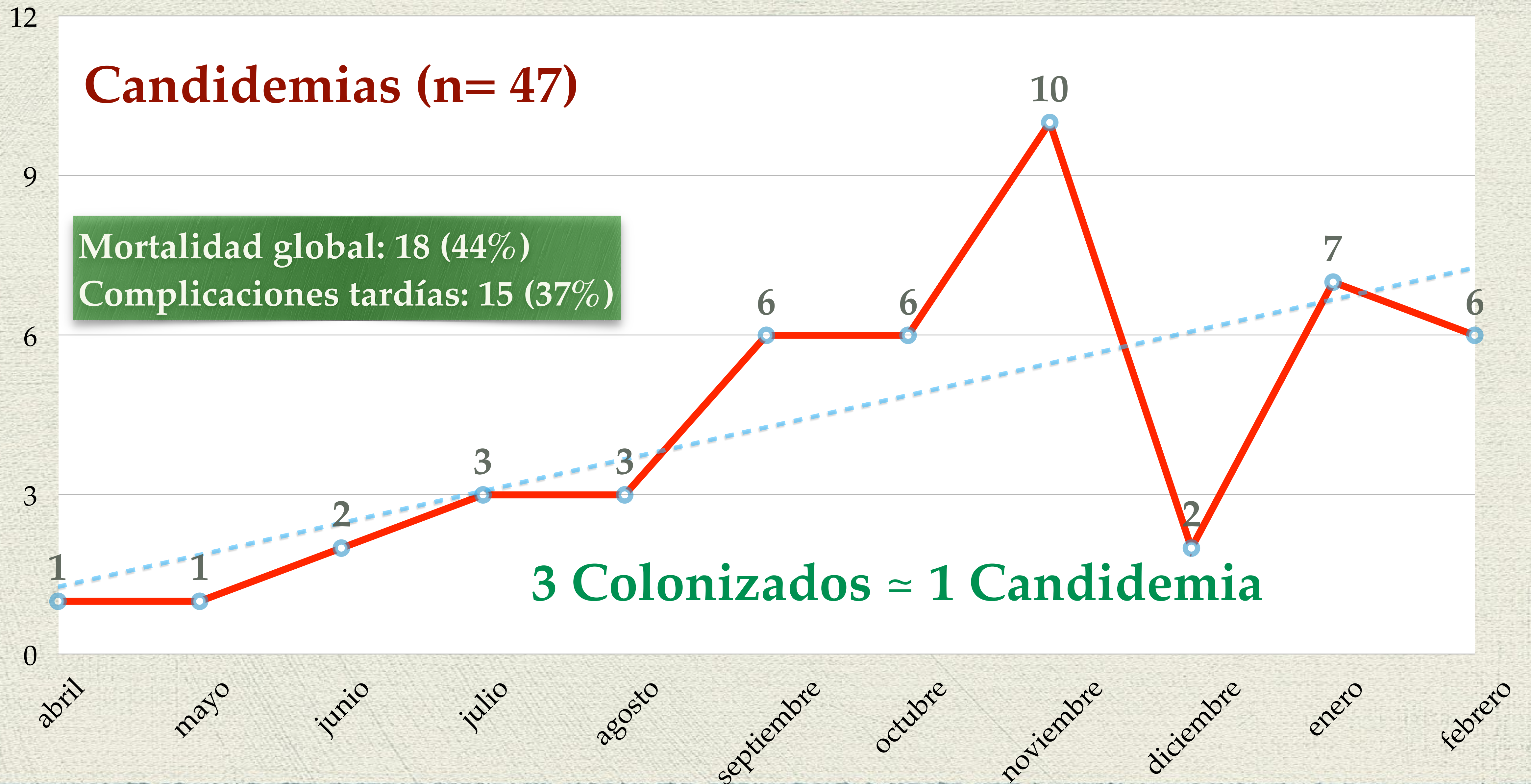
Pacientes colonizados (n= 154)

Casos nuevos/mes



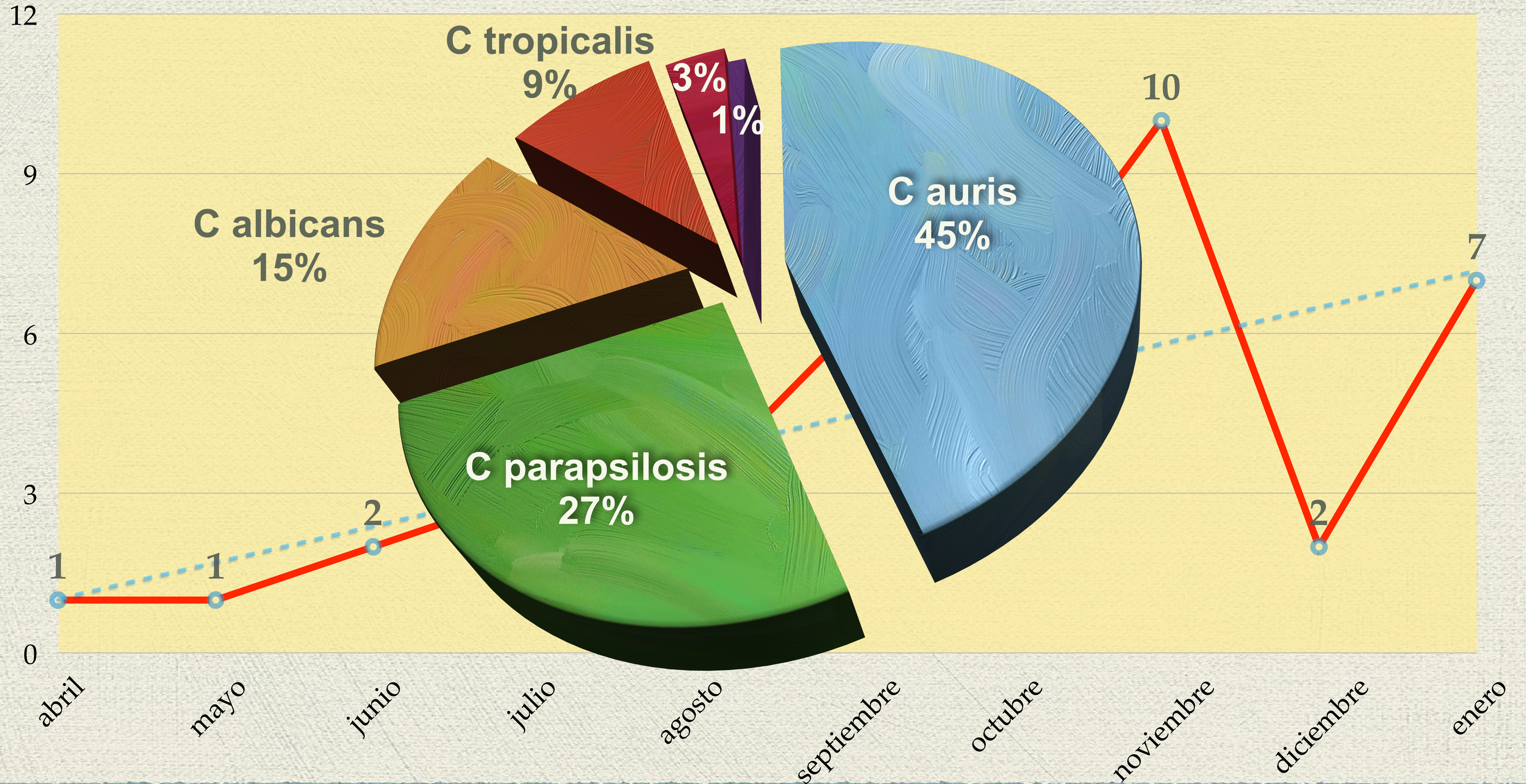


# *C. auris* en HUP La Fe





# 92 candidemias en HUP La Fe







## Revista Iberoamericana de Micología

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Original article

### Nosocomial fungemia by *Candida auris*: First four reported cases in continental Europe

Alba Cecilia Ruiz Gaitán<sup>a</sup>, Ana Moret<sup>b</sup>, José Luis López Hontangas<sup>c</sup>, José Miguel Molina<sup>c</sup>,  
Ana Isabel Aleixandre López<sup>b</sup>, Alicia Hernández Cabezas<sup>c</sup>, Juan Mollar Maseres<sup>d</sup>,  
Rabat Chouman Arcas<sup>c</sup>, María Dolores Gómez Ruiz<sup>c</sup>, Miguel Ángel Chiveli<sup>b</sup>, Emilia Cantón<sup>a</sup>,  
Javier Pemán<sup>a,c,\*</sup>

doi: 10.1016/j.riam.2016.11.002





## al outbreak of the globally *Candida auris* in a European

Open Access



RAPID RISK ASSESSMENT

## *Candida auris* in healthcare settings – Europe

19 Decer

## Guidance for the laboratory Investigation, management and prevention and control for



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E IGUALDAD

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SANIDAD Y CONSUMO

DIRECCIÓN GENERAL DE  
SALUD PÚBLICA, CALIDAD E  
INNOVACIÓN

Centro de Coordinación de  
Alertas y Emergencias Sanitarias

RESEARCH ARTICLE

## Epidemiolo

## *Candida c* in healt



## Informe sobre la emergencia de *Candida auris* en Europa

21.12.2016

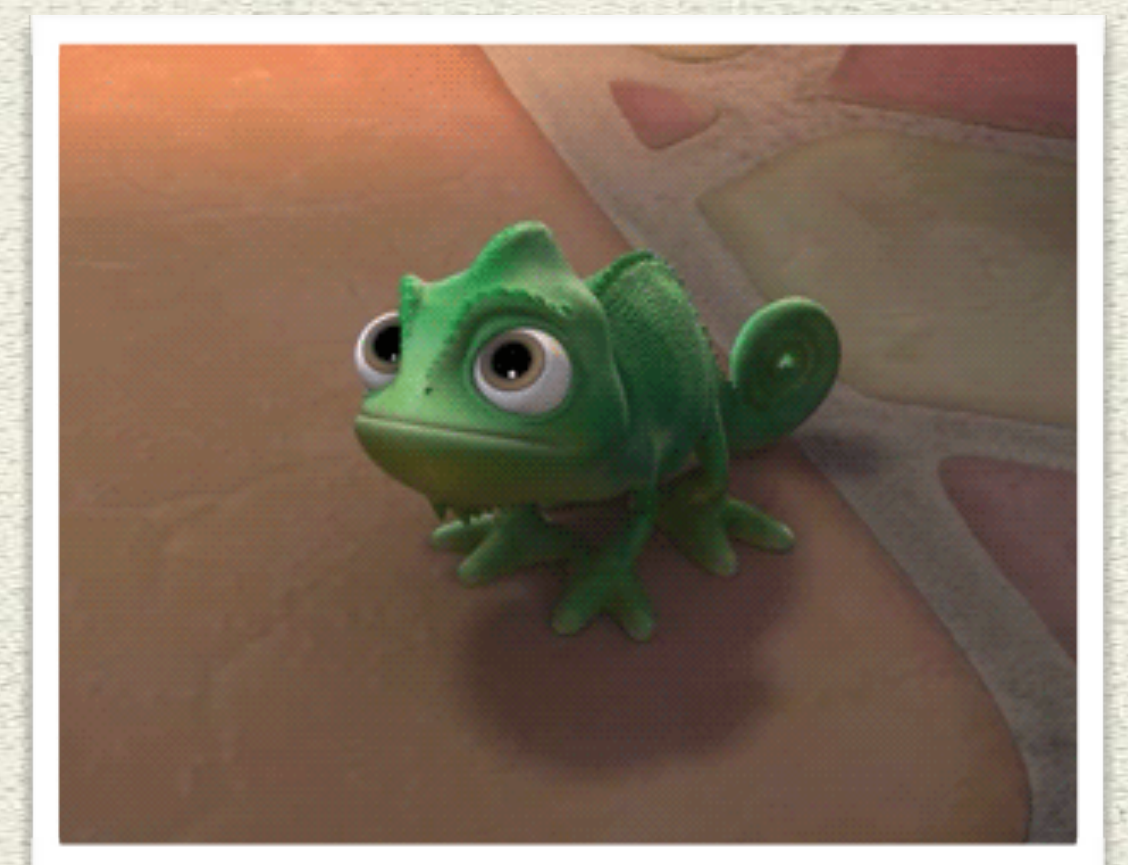
antidrug-resistant *Candida auris*: 'new kid on the  
block' in hospital-associated infections?



# T2 Candida

- Rapidez (<4 h)
- Manejo sencillo (< 10 min)
- Vol sangre mínimo (niños)
- VPN >99%
- Límite detección 1-3 ufc/mL
- CI sin candidemia
- Monitorización terapia
- Ahorro costes asociados

- Sólo 5 especies
- Agrupa 4 especies (A/T, G/K)
- Logística deficiente
- Caducidad corta reactivos
- Precio elevado (300 €)



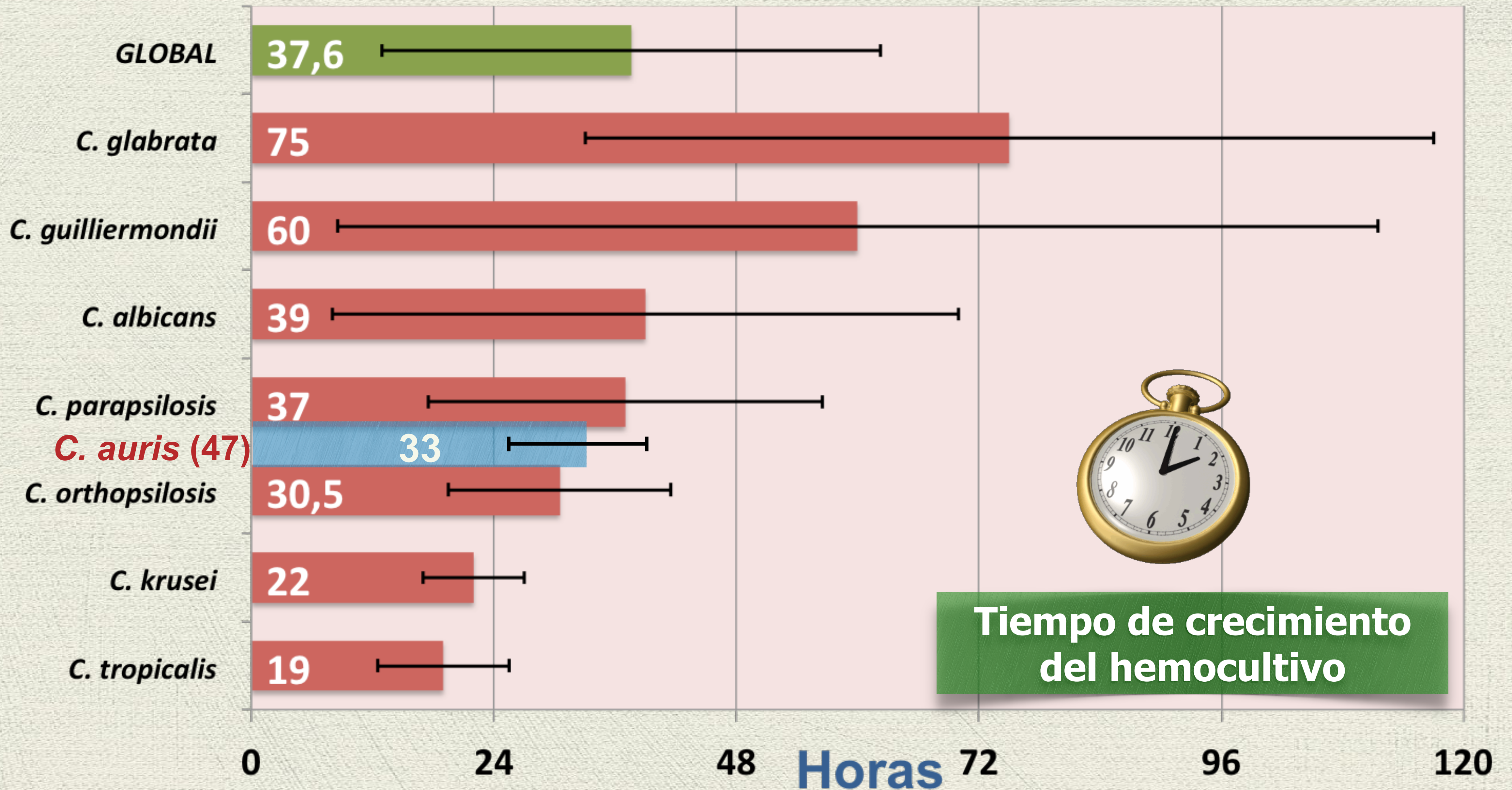


# ¿Podemos agilizar el diagnóstico de la **Candidiasis** Invasora?





## 234 candidemias Unidades Críticas HUyP La Fe (2005-2013)





# EL tiempo es... VIDA



## Hemocultivo (37 h)







**!! Muchas  
gracias !!**

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