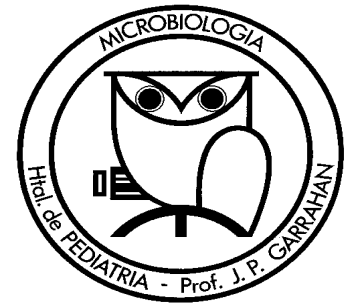
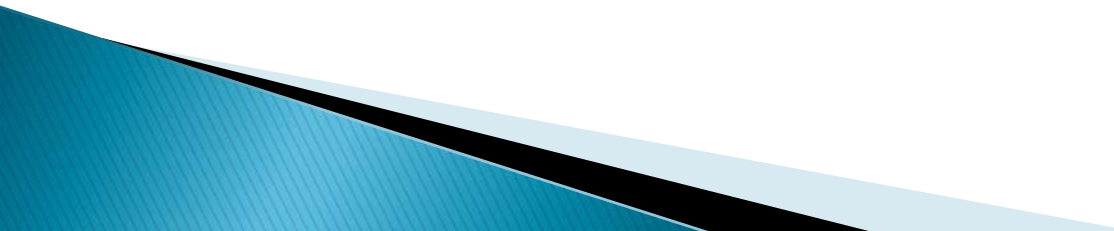


Hospital de Pediatría S.A.M.I.C.  
"Prof. Dr. Juan P. Garrahan"

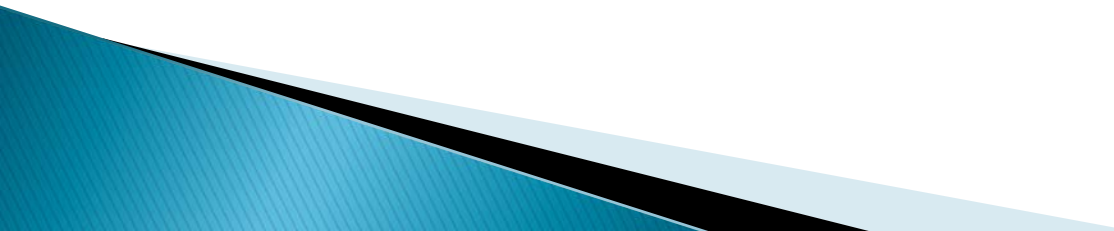


# Servicio de Microbiología

# Misión

- ▶ Equipo interdisciplinario
  - ▶ Realización de análisis microbiológicos destinados a la prevención, el diagnóstico y el seguimiento de las enfermedades infecciosas
- 

# Laboratorio de Microbiología en un hospital de alta complejidad

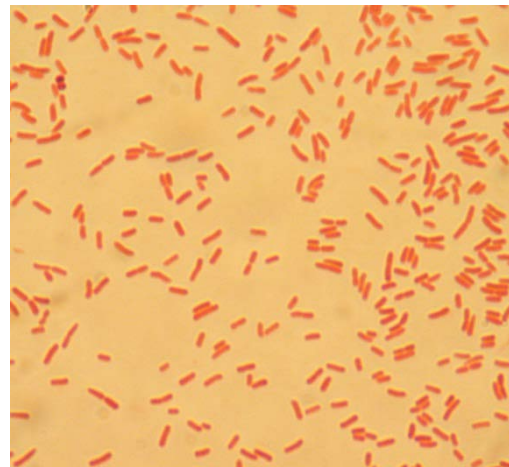
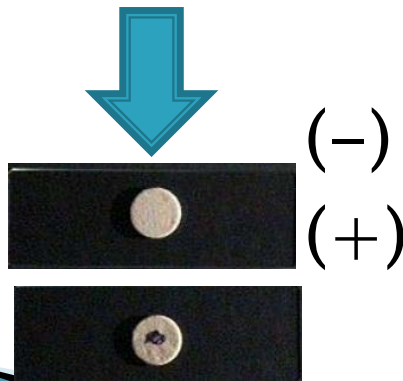
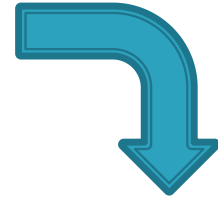
- ▶ Actividad asistencial
  - ▶ Actividad docente
  - ▶ Actividad de investigación aplicada a la clínica
- 

# Actividad asistencial

- ▶ MICOLOGIA
  - ▶ BACTERIOLOGIA
  - ▶ VIROLOGIA
- 

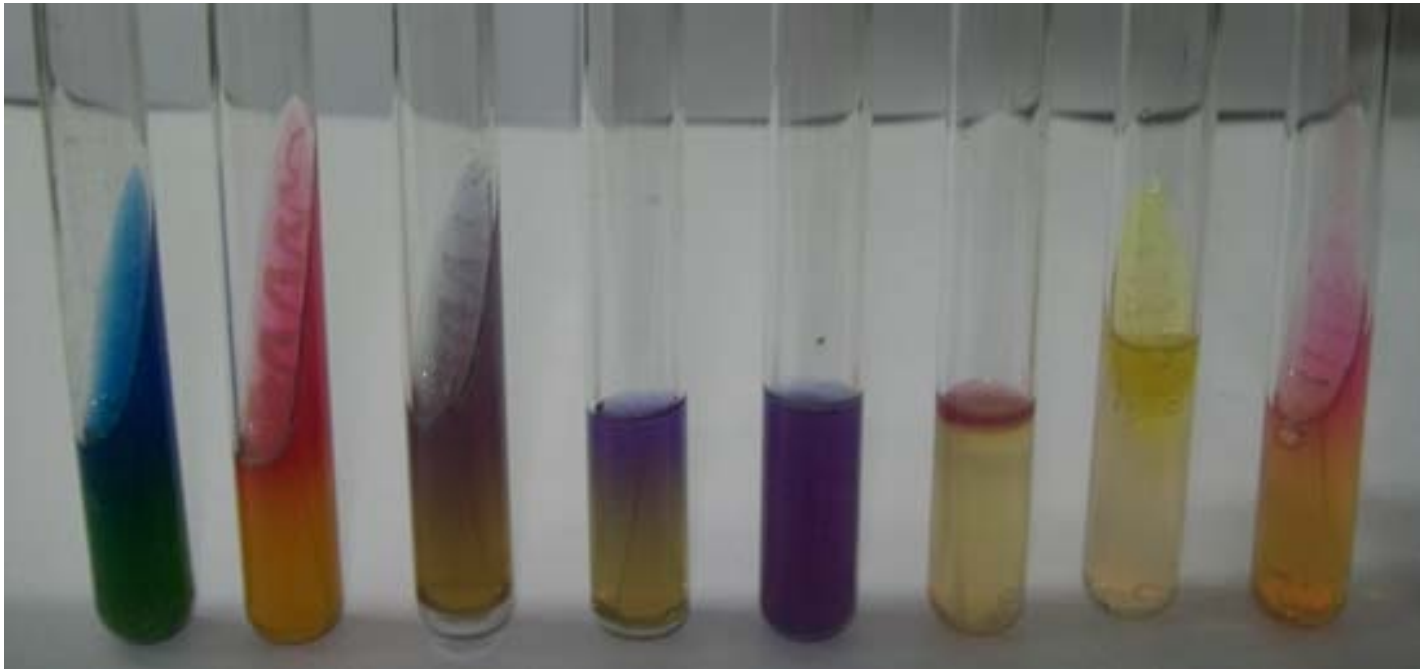
# TIPIFICACIÓN MANUAL

- ▶ MORFOLOGÍA DE LA COLONIA
- ▶ COLORACIÓN DE GRAM
- ▶ OXIDASA



# TIPIFICACIÓN MANUAL

- ▶ Marcha en tubos (enterobacterias)



# TIPIFICACIÓN MÉTODOS COMERCIALES

- ▶ SISTEMAS DE TIPIFICACIÓN CON GALERIAS DE SUBSTRATOS DESHIDRATADOS ESTANDARIZADOS (ej.: API, ROSCO) CON LECTURA DE LOS RESULTADOS SOBRE SOFTWARE DE IDENTIFICACIÓN.





# MÉTODOS COMERCIALES

- ▶ TABLETAS ROSCO (ROSCOZYM)



# TIPIFICACIÓN Y SENSIBILIDAD

- ▶ Métodos automatizados (Sist. Vitek 2C)



# Mesa de trabajo

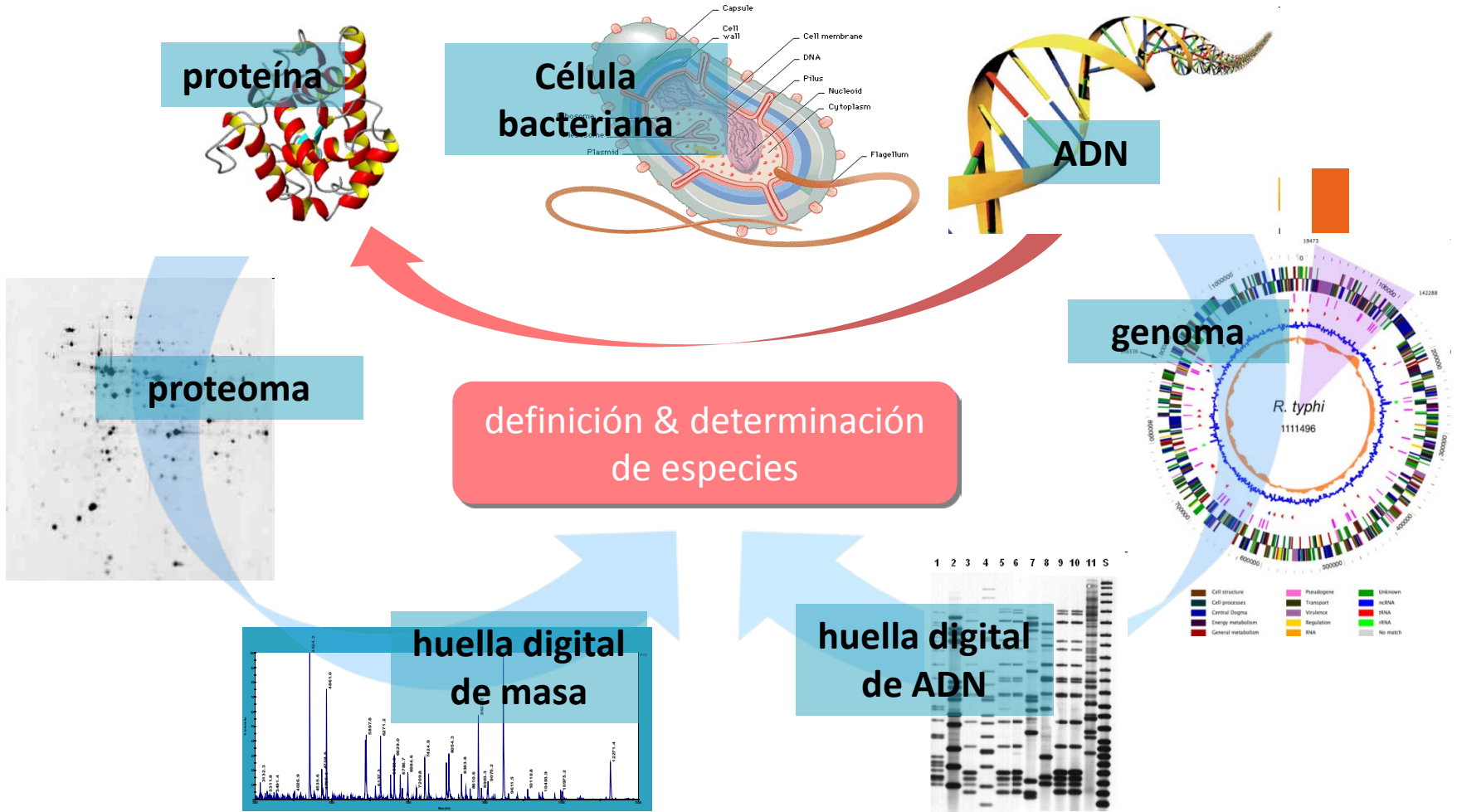


# Tarjeta sistema automatizado

- ▶ Tarjeta Vitek: identificación (60 determinaciones) y sensibilidad (CIM por microdilución)

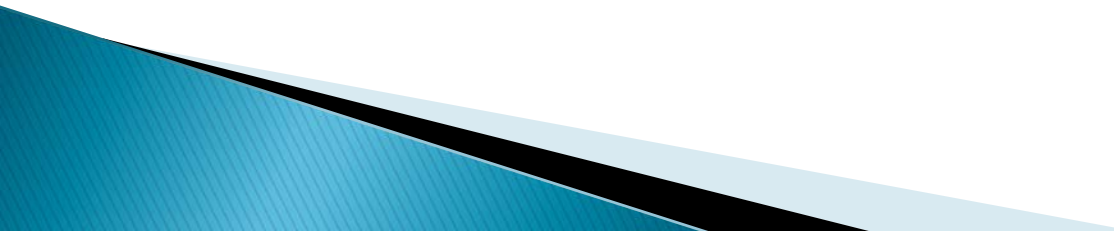


# Taxonomía moderna: Proteómica & Genómica



La huella digital de Masa está directamente relacionada con la secuencia genómica

# MALDI TOF : tecnología

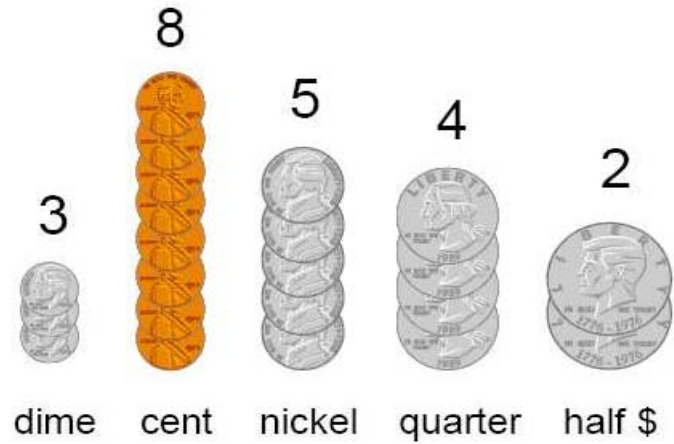
- ▶ La espectrometría de masa es una técnica analítica para determinar la composición elemental de una muestra
  - ▶ El principio del MS consiste en la ionización de compuestos químicos para generar moléculas cargadas y medir su relación masa-carga ( $m/r$ )
- 

- ▶ MALDI–TOF (Matriz Asistida por Desorción / Ionización con Laser a Tiempo de Vuelo), un tipo de masa específica, permite una detección de proteínas de bacterias intactas.

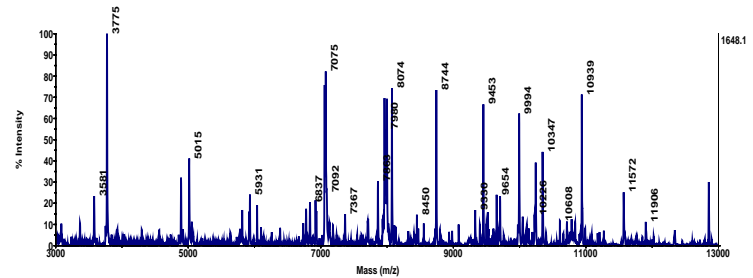
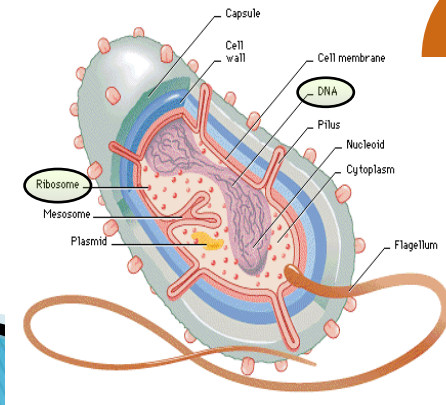
# MALDI - TOF: Tecnología



quantity

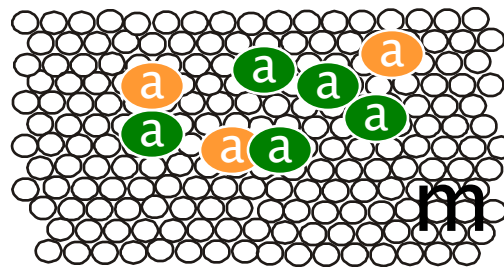
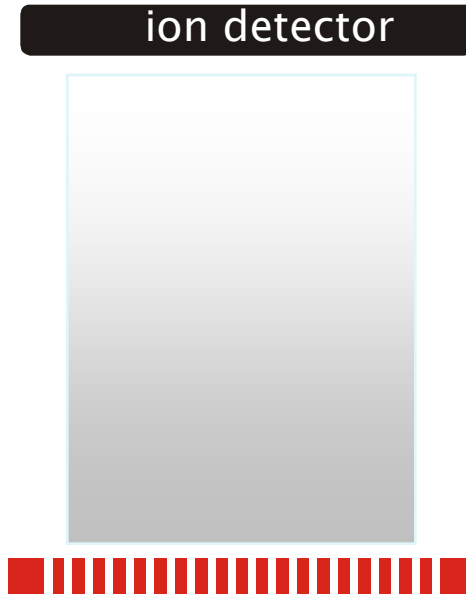


value



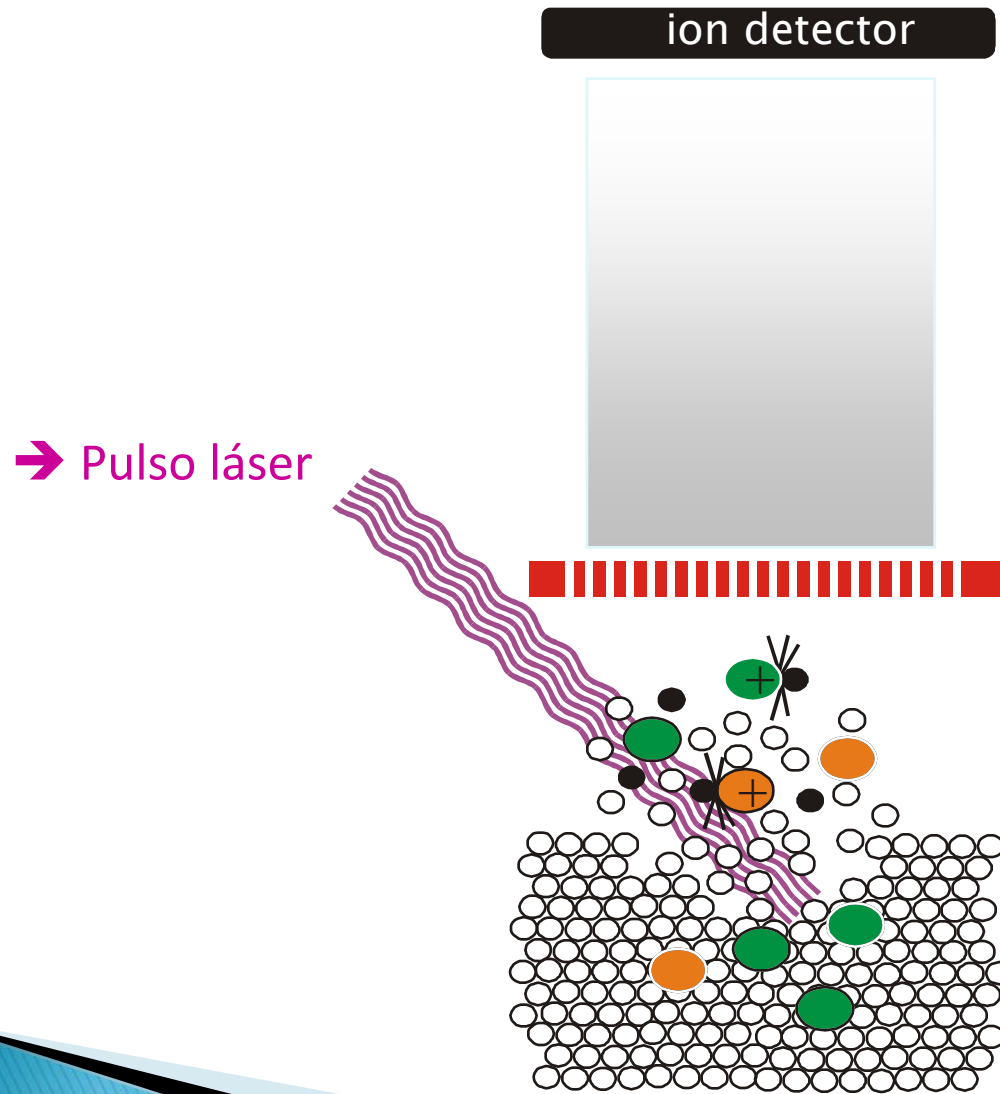


# MALDI-TOF: principio



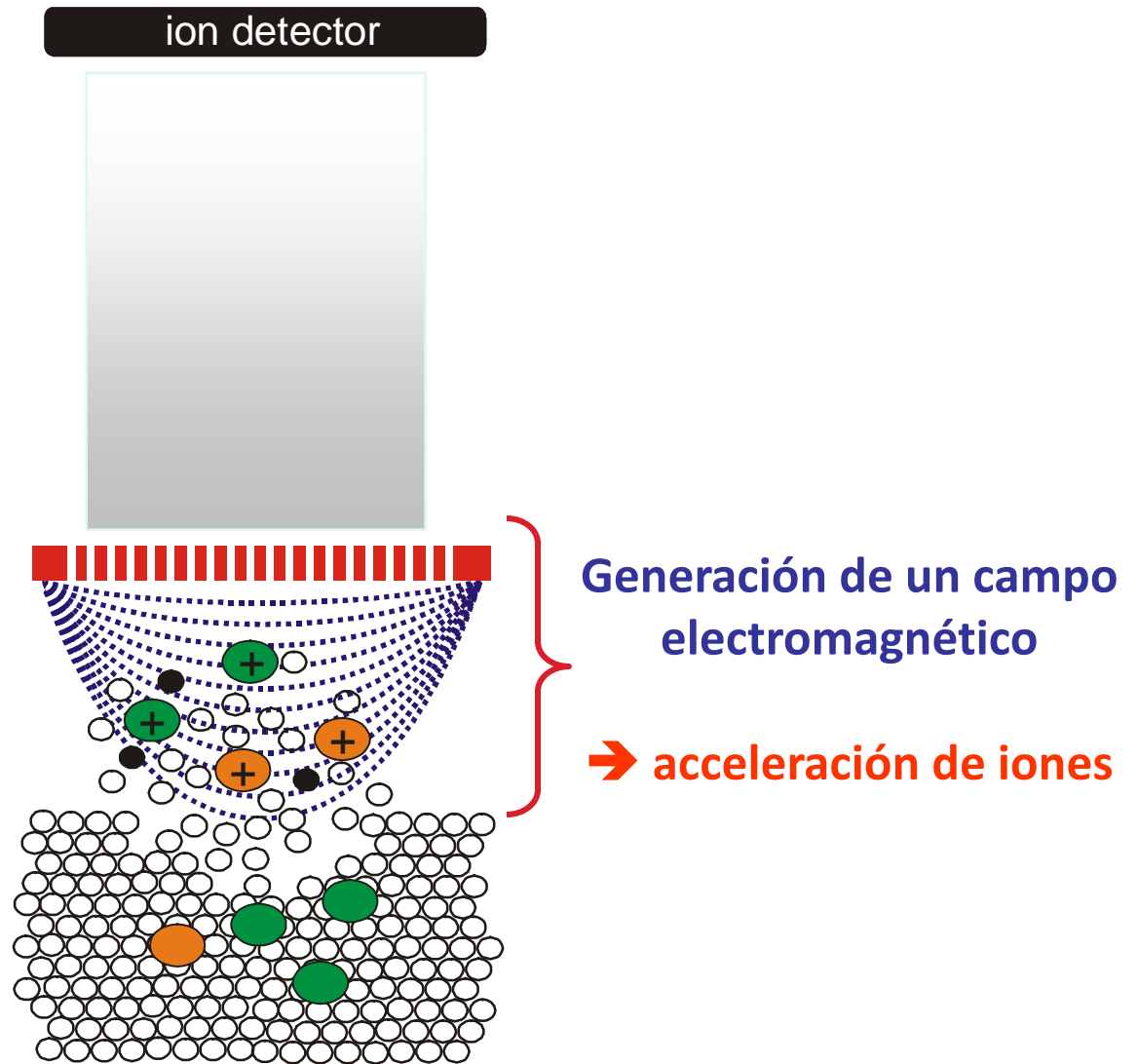
**Las moléculas del  
analito se  
incorporados a los  
cristales de la matriz**

# MALDI-TOF: principio



- desorción de la matriz
- ionización por transferencia de carga desde la matriz al analito

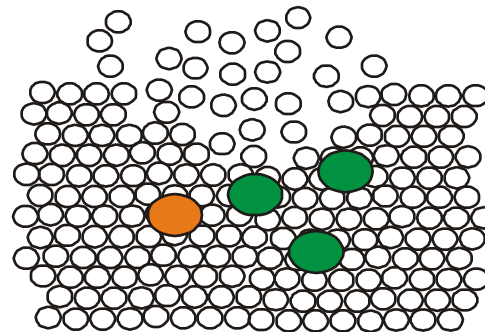
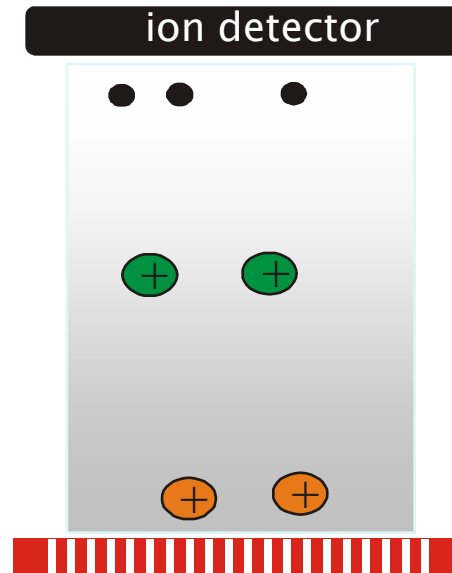
# MALDI-TOF: principio



# MALDI-TOF: principio

Tubo TOF  
(vacío)  
<  $10^{-7}$  Torr

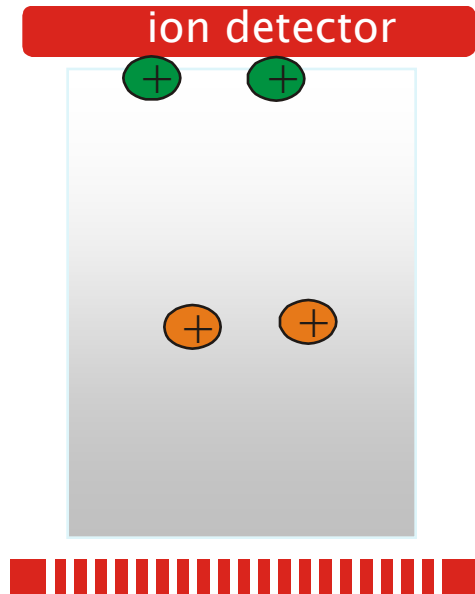
Longitud del tubo » 1.2 m



## Separación de iones:

- Migran a diferente velocidad en un campo libre (vacío)  
--- **Time Of Flight** ---
- Velocidad  
→ determinada por la relación masa/carga
- Aquí no hay más aceleración

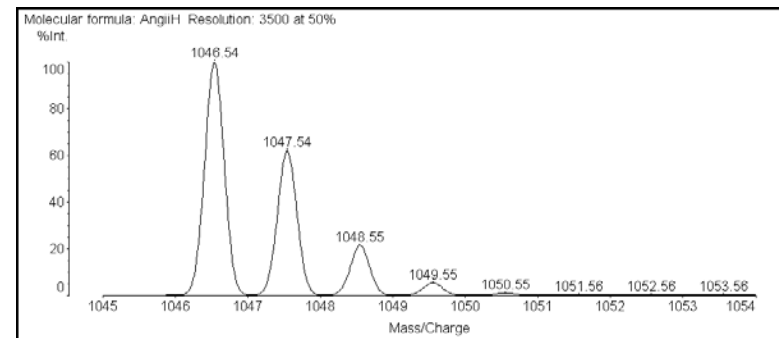
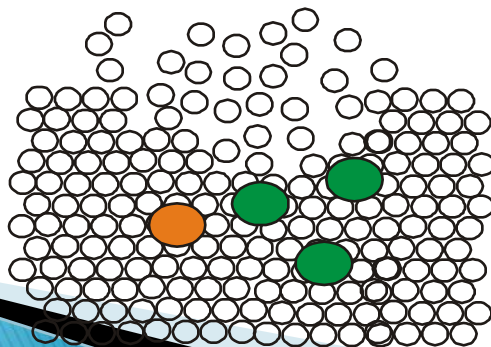
# MALDI-TOF: principio



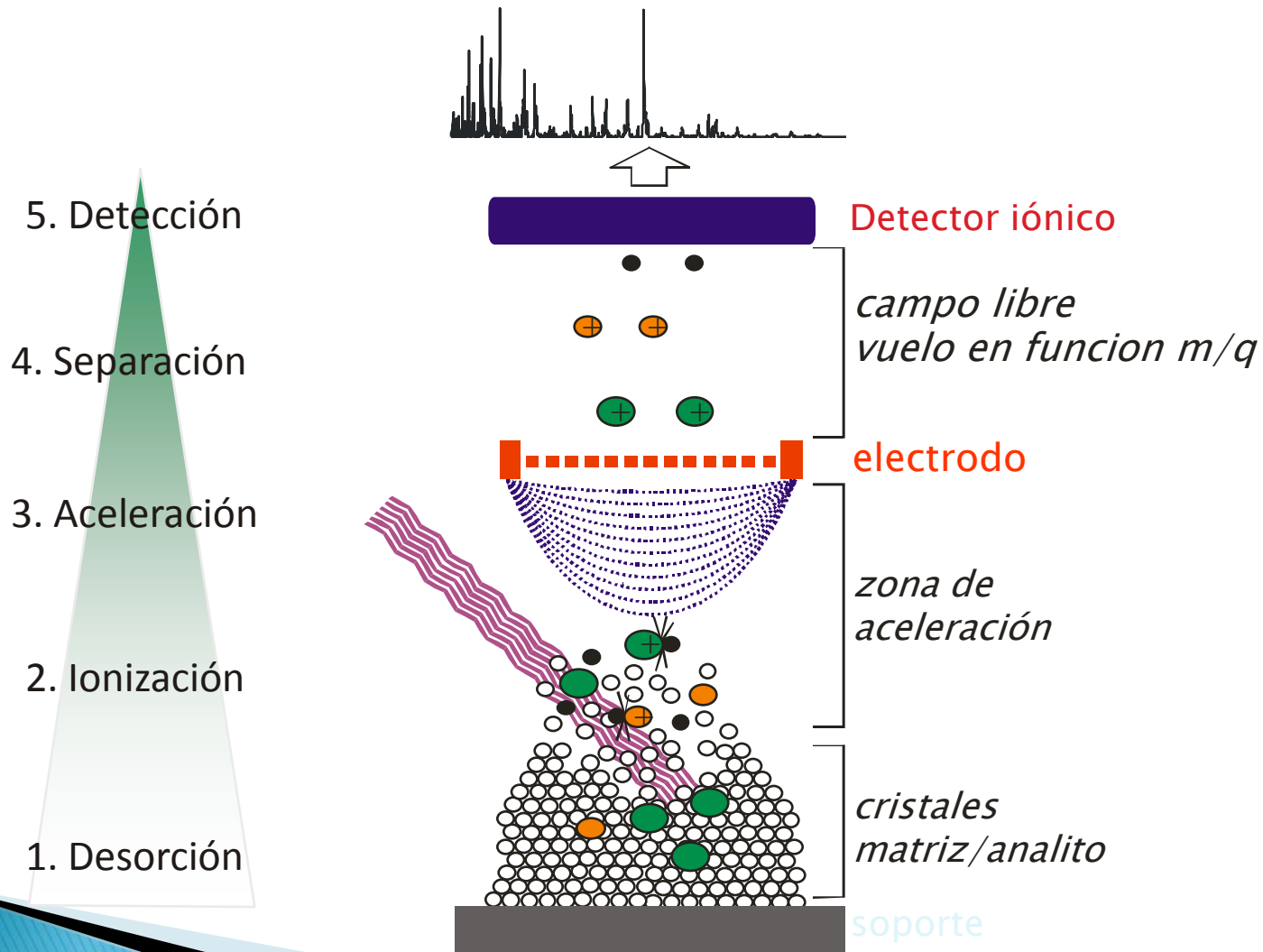
## Detector único

Se detectan los iones que alcanzan el detector según su TOF

Los TOFs son enviados al computador para su análisis y emisión del espectro



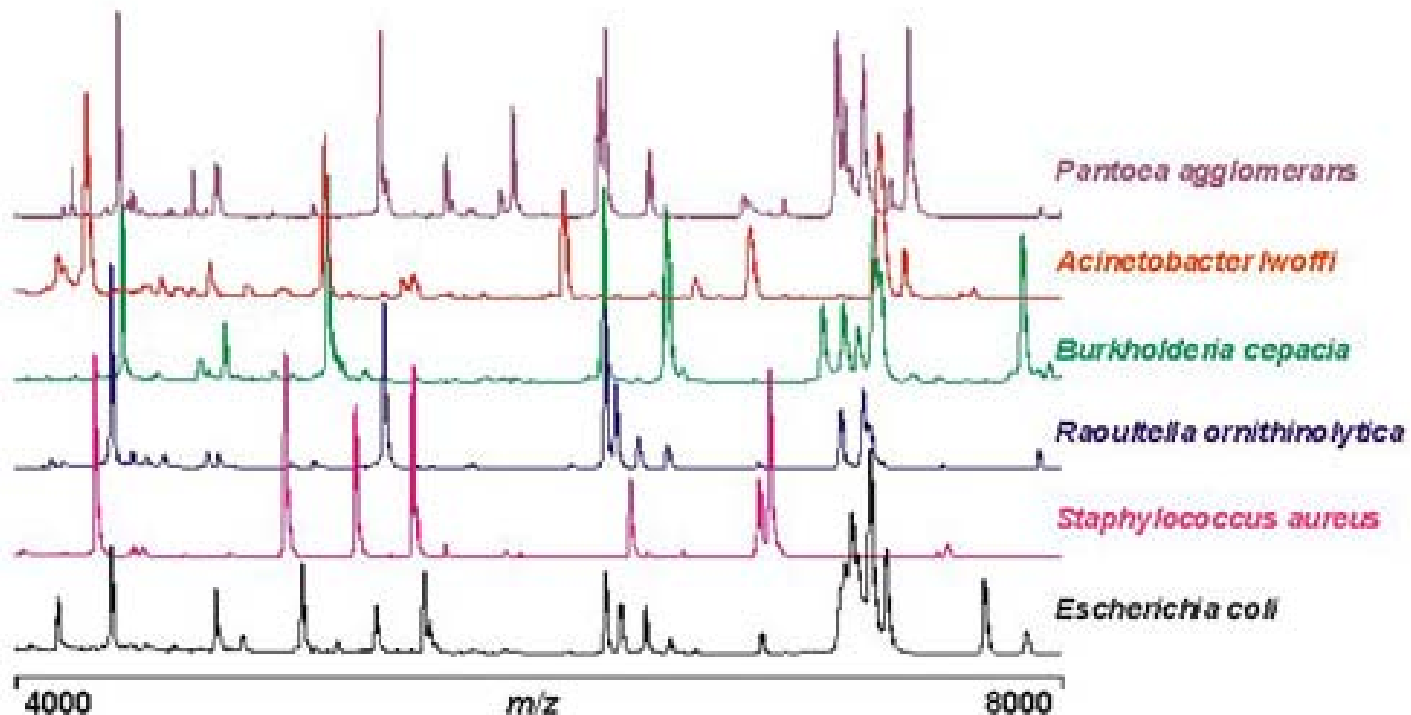
# En resumen:



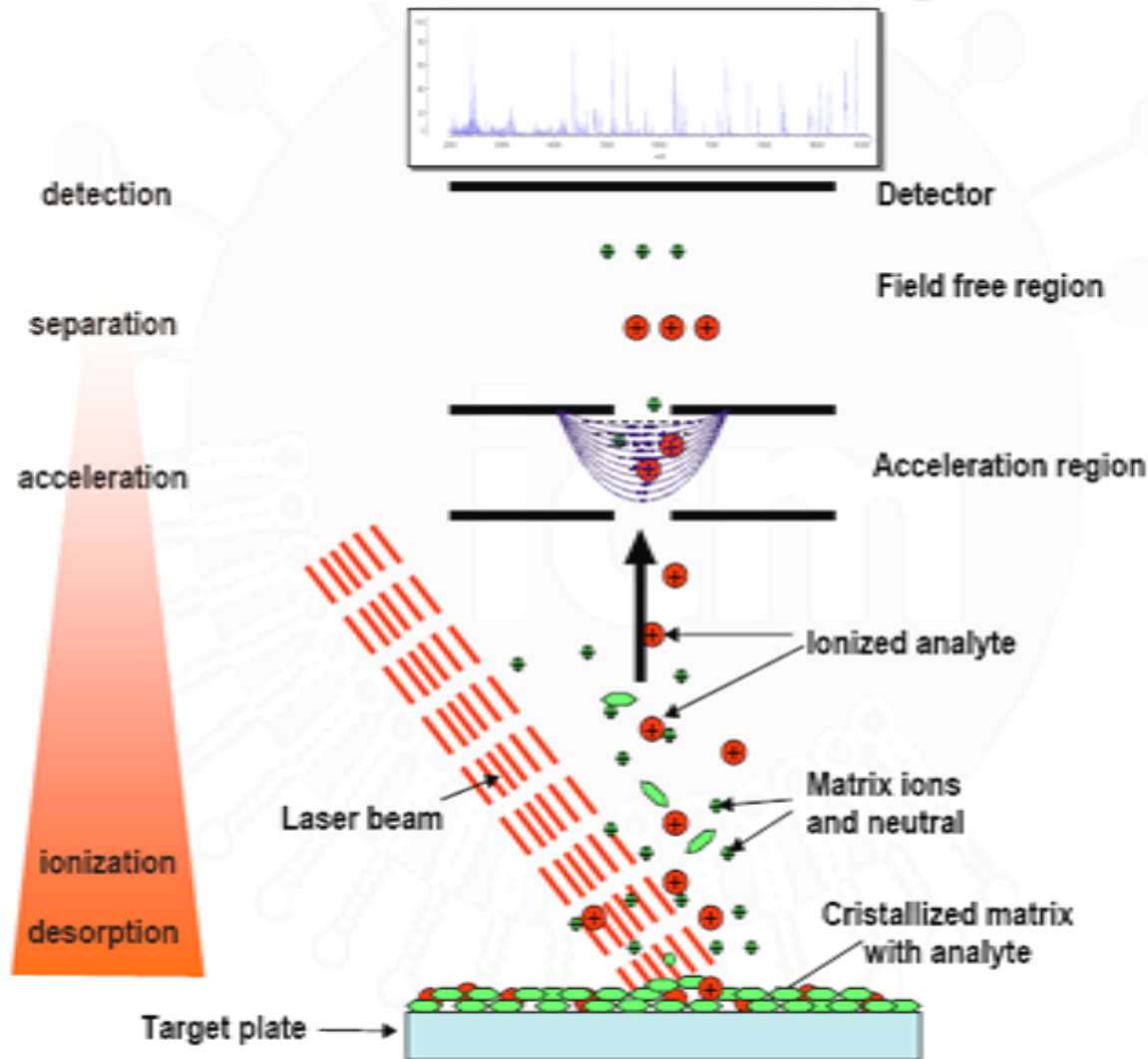
# Identificación de organismos con MALDI-TOF

Cada especie posee un patrón de picos diferente y característico

→ Lo que permite la Identificación de cada organismo



# MALDI – TOF: Tecnología



**Mass Spectrometry:** the velocity of the ion depends on the mass-to-charge ( $m/z$ ) ratio



**Time-Of-Flight** to the detector is recorded



Ions are accelerated by an electric field of known strength



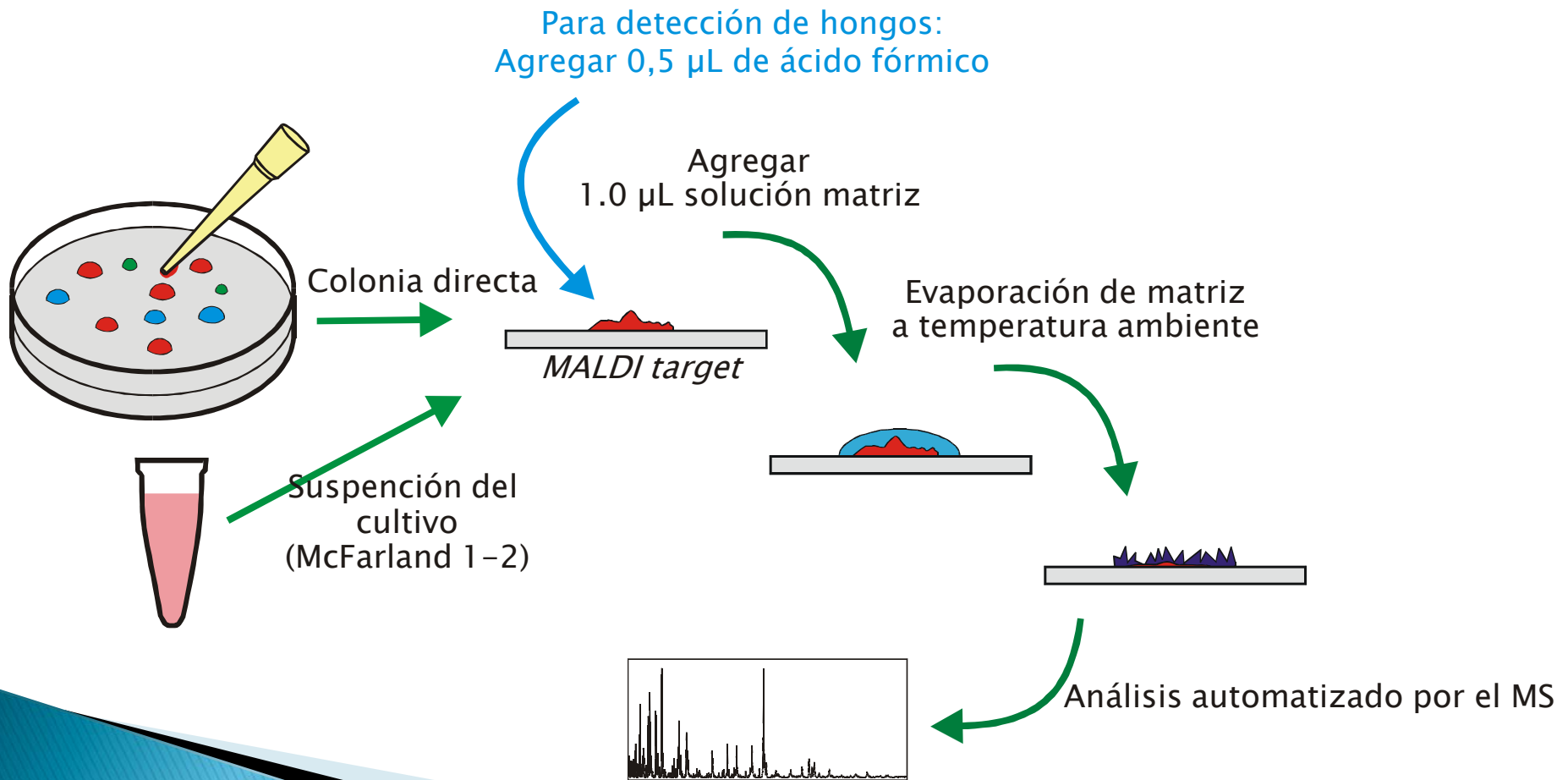
**Laser Desorption/Ionization:** matrix is ionized by the laser pulse and transfers part of its charge (+) to the molecules (proteins) to be analyzed



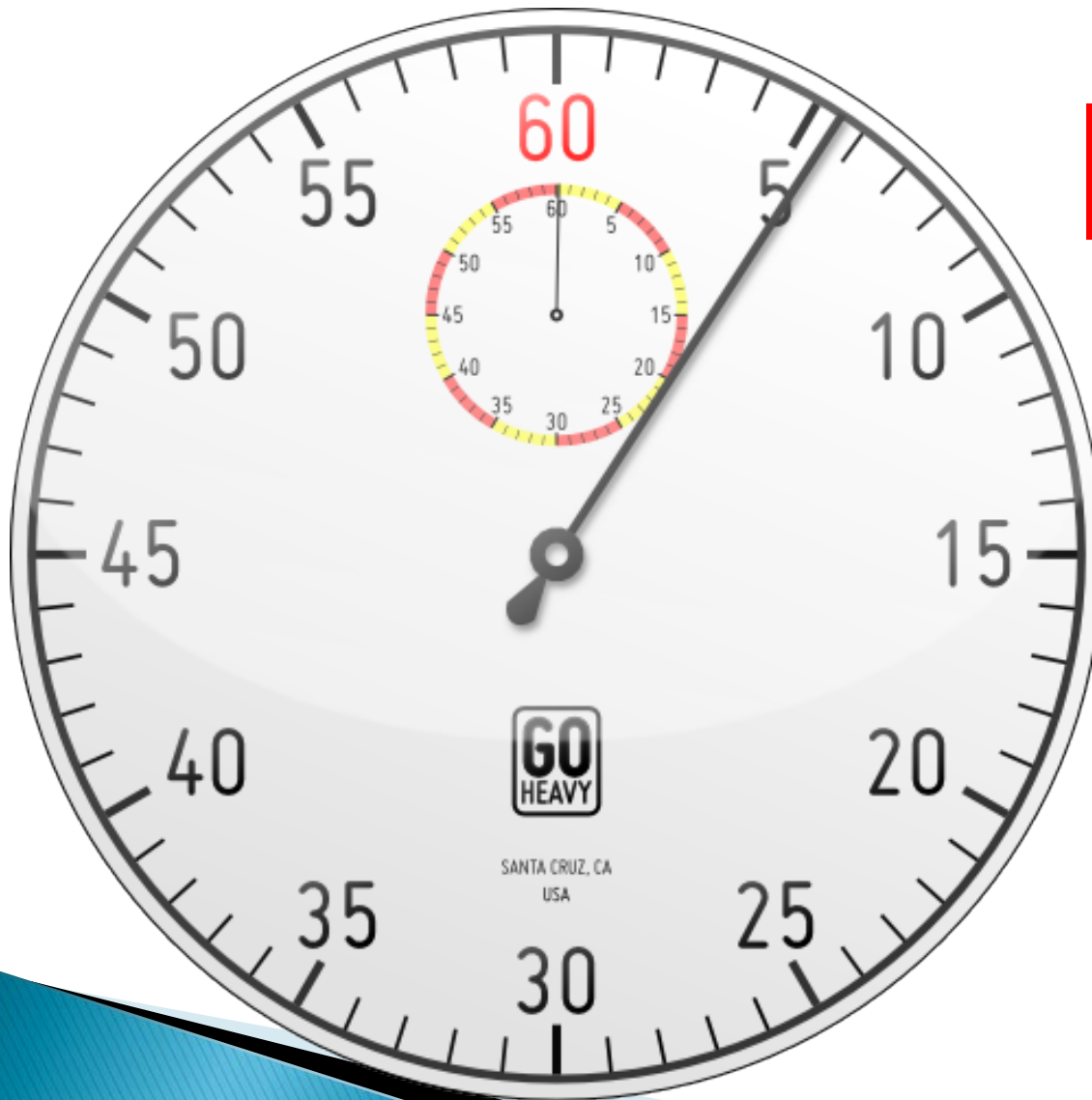
**Matrix-Assisted:** sample is embedded in a matrix\* to protect the biomolecule from being destroyed by direct laser beam and to facilitate vaporization and ionization



# Preparación de la muestra



Valor para el usuario...

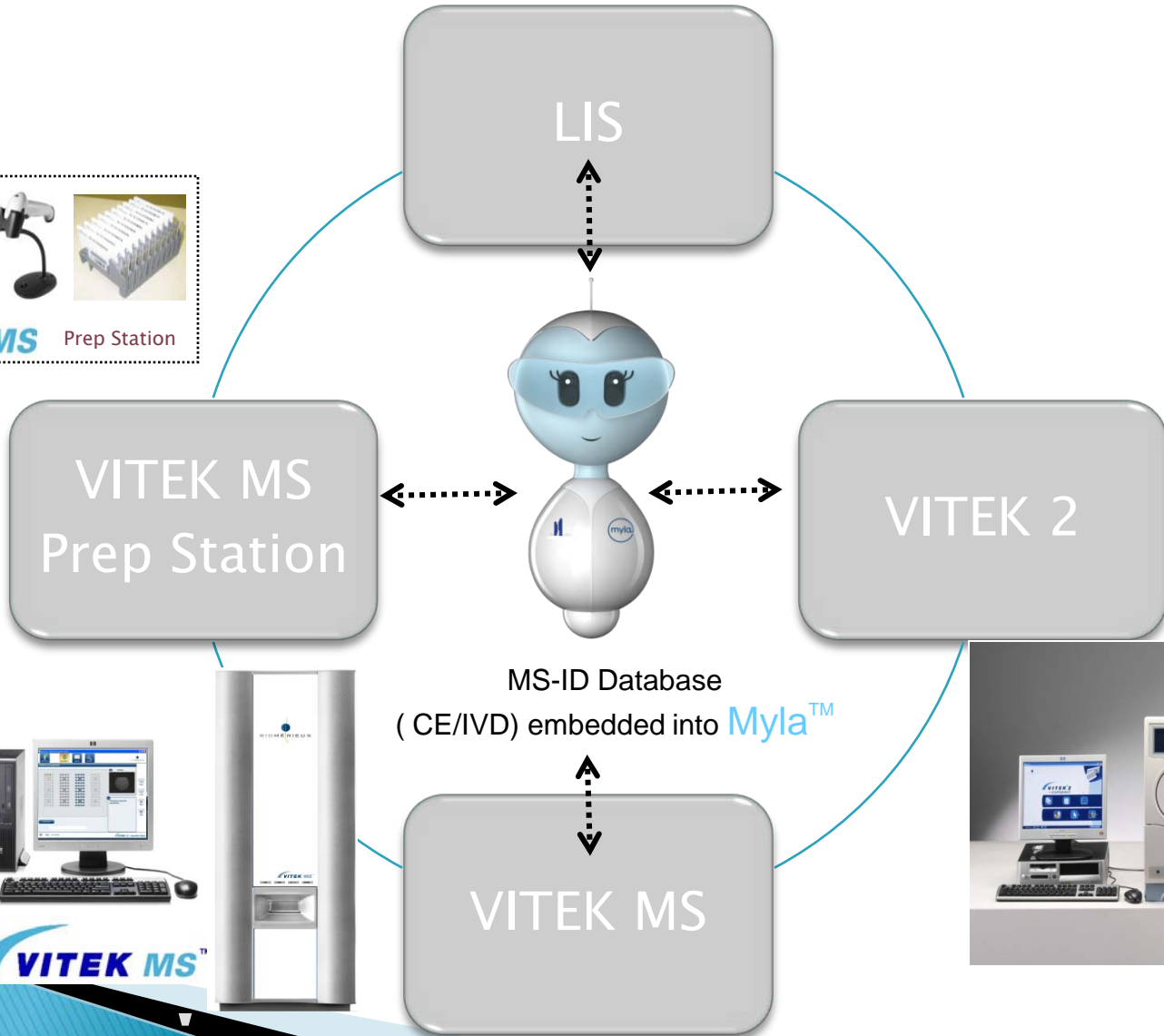


**¡¡¡Rápidez!!!!**

**Entre 45" y 1'30" por  
aislamiento**



**30 muestras en aprox. media  
hora**



# Valor para el usuario...

**Acertado**

THEME

## MAUDI-TOF MS: a new laborato for the diagnosis of clinical infec

In a recent publication Bruudage and Shanks highlighted the importance of secondary infections in viral diseases, using the example of the 1918 influenza pandemic [1]. A large number of deaths, perhaps as many as 30% of the total fatalities, were probably due to bacterial superinfections. This contextually explains the need for quick and reliable identification of bacterial organisms that are the primary or secondary aetiological agents of infectious episodes.

Standard methods for the identification of microorganisms

Until standardised test systems such as API<sup>®</sup> and VITEK<sup>®</sup> 2 (bioMérieux), or PHOENIX<sup>®</sup> (BD Diagnostics) were developed, identification of clinical microorganisms was based on traditional

need for cultivation. DNA sequencing after amplification by PCR is particularly useful for the identification of microorganisms at the specific level, be they bacteria, moulds or viruses. After amplification with non-specific primers, DNA sequences can be determined and compared with known sequences from databases (e.g. BLAST) and DNA sequencing is also necessary for phylogenetic analysis because with genetic similarities with other microorganisms can be detected and thus robust phylogenetic relationships established [2].

Both biochemical and genetic analyses are reliable, but their use may be time-consuming and their cost comparatively high. There is thus a need for progress towards more rapid and cheap but accurate methods of identi-

**Sept 2010**

■ 1021 clinical isolates tested

■ 98% correct ID<sup>1</sup>



Figure 1. Number of papers referring to the use of MALDI-TOF MS in investigations with bacteria, 2000-2005. Source: PubMed, <http://www.ncbi.nlm.nih.gov/pubmed/>.

OPEN ACCESS Freely available online

## Matrix-Assisted Laser Desorption Ionization Flight Mass Spectrometry for the Identification of Clinically Relevant Bacteria

Cinzia Benagli<sup>1</sup>, Viviana Rossi<sup>1</sup>, Marisa Dolina<sup>1</sup>, Mauro Tonolla<sup>1,2</sup>, Orlan

<sup>1</sup> Cantonal Institute of Microbiology, Bellinzona, Switzerland, <sup>2</sup> Microbiology Unit, Plant Biology Department, Universit

**Abstract**

**Background:** Matrix-assisted laser desorption ionization-time of flight mass spectrometry reliable identification of microorganisms, particularly clinically important pathogens.

**M**atrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) is an emerging technology recently applied to the problem of bacterial species identification. We evaluated two MALDI-TOF MS systems with 720 nonselectively inoculated bacterial colonies under routine clinical laboratory conditions. Isolates were analysed in parallel on both devices, using the manufacturers' default recommendations. We compared MS with conventional biochemical test system identifications. Discordant results were resolved with "gold standard" 16S rDNA gene sequencing. The first MS system (Bruker) gave high-confidence identifications for 680 isolates, of which 674 (99.1%) were correct; the second MS system (Shimadzu) gave high-confidence identifications for 630 isolates, of which 625 (99.4%) were correct.

**Jan 2011**

■ 1019 clinical isolates tested

■ 98% correct ID<sup>1</sup>

**Cite this article as:** Benagli C, Rossi V, Dolina M, Tonolla M, Orlan A (2010) Matrix-assisted laser desorption ionization-time of flight mass spectrometry for the identification of clinically relevant bacteria. *Journal of Clinical Microbiology* 48:1169-1175. doi:10.1128/JCM.01891-09

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**Competing Interests:** The authors have declared that no competing interests exist.

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Journal of Clinical Microbiology, Apr. 2010, p. 1169-1175  
 0095-1177/10/\$12.00 doi:10.1128/JCM.01891-09  
 Copyright © 2010, American Society for Microbiology. All Rights Reserved.

## Comparison of Two Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry Methods with Conventional Phenotypic Identification for Routine Identification of Bacteria to the Species Level<sup>1</sup>

Abdelsalam Cherkaoui,<sup>1,\*</sup> Jonathan Hibbs,<sup>2</sup> Stéphane Emonet,<sup>2</sup> Manuela Tangomo,<sup>2</sup> Myriam Girard,<sup>2</sup> Patrice Francois,<sup>2</sup> and Jacques Schrenzel<sup>1,2</sup>

<sup>1</sup> Clinical Microbiology Laboratory and <sup>2</sup> Genetic Research Laboratory, <sup>3</sup> Service of Infectious Diseases, University of Geneva Hospitals (HUG), CH-1211 Geneva 14, Switzerland

Received 23 September 2009; returned for modification 23 November 2009; accepted 8 February 2010

**Bacterial identification relies primarily on culture-based methodologies requiring 24 h for isolation and an additional 24 to 48 h for species identification. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) is an emerging technology recently applied to the problem of bacterial species identification. We evaluated two MALDI-TOF MS systems with 720 nonselectively inoculated bacterial colonies under routine clinical laboratory conditions. Isolates were analysed in parallel on both devices, using the manufacturers' default recommendations. We compared MS with conventional biochemical test system identifications. Discordant results were resolved with "gold standard" 16S rDNA gene sequencing. The first MS system (Bruker) gave high-confidence identifications for 680 isolates, of which 674 (99.1%) were correct; the second MS system (Shimadzu) gave high-confidence identifications for 630 isolates, of which 625 (99.4%) were**

**Apr 2010**

■ 680 clinical isolates tested

■ 99% correct ID's

Pathogens are developing for fast typical outbreak. Rapid molecular diagnosis on any elongated fibrous cleave specimen can be use of such a detect

be considered a separate reagent, the quality control issues of such testing become more formidable with each additional gene target. Cost and workload requirements for microscopy or

logy (15, 21). Compared with conventional phenotype- or PCR-based identification, MALDI-TOF MS shows rapid turnaround time, low sample volume requirements, and modest reagent costs. Peptide or protein mass-to-charge (m/z) values form mass spectral peaks, indicating the molecular masses and charge densities of components present in a biological sample. These spectra can generate pathogenomic patterns that provide enhanced identifications of particular species and even genotypes within species. Due to their unannotated lines and readily interpretable data, MALDI-TOF MS has long been

\* Corresponding author. Mailing address: Clinical Microbiology Laboratory, Service of Infectious Diseases, University of Geneva Hospitals (HUG), CH-1211 Geneva 14, Switzerland. Phone: 41 22 7327521; Fax: 41 22 7327312; E-mail: [abdelsalam.cherkaoui@hug.ch](mailto:abdelsalam.cherkaoui@hug.ch)  
<sup>1</sup> Published ahead of print on 17 February 2010.

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Con relación al gold standard: secuenciación

# Principales beneficios:

- ▶ Rapidez:  
Con un resultado rápido de ID, conociendo la prevalencia de la Institucion se dirige mejor el tratamiento empírico.
- ▶ Costo - Eficiencia.
- ▶ Alta Performance.  
98% de concordância com Gold Standard secuenciacion.
- ▶ Facilidad de Uso / Mejora en el flujo de trabajo.  
Integración informática,
- ▶ Sistema aprobado por ANMAT para uso clínico.

**CONCLUSION:**

**Laboratorio**

**Estandarización y Calidad**

**Disminución del flujo de trabajo**

**Paciente**

**Impacto Clínico Positivo**

**Disminución de Tiempo de Internación**

**Disminución de Morbi/Mortalidad**

**Institución**

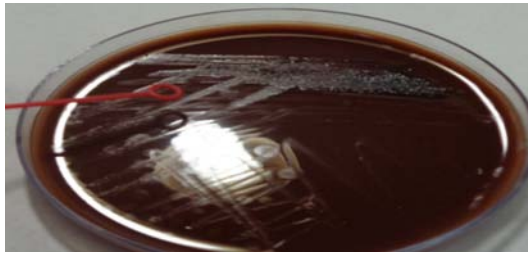
**Ahorro de ATB**

**Ahorro de Costos Global**

**Menor Toxicidad**

**Menor Impacto Ecológico**

# Identificación bacteriana directa de hemocultivos positivos utilizando tecnología MALDI TOF (Matrix Assisted Laser Desorption Ionization Time Of Flight Mass spectrometry) en pacientes pediátricos.



- 1ml sangre( AE filtración)
- Anadir :
  - Lisante 1: 100ul agua destilada
  - TLisante2: 100ul Sodium Dodecyl sulfate SDS 5%
- Vortexear
- Incubar durante 5-10minutos
- Centrifugar 1min 13000g
- Descartar sobrenadante
- Resuspender el sedimento con 1ml de agua destilada
- Centrifugar 5min a 13000g
- Retirar sobrenadante



- Aplicar de tubo con L1 o L2 2 el sedimento en tarjeta
- Anadir :
  - ✓1ul matriz (HCCA) y dejar secar
  - ✓0,5ul Ácido fórmico (dejar secar)+0,5ul matriz (HCCA) y dejar secar



En el análisis de AE y AN, los mejores protocolos de ID fueron

- 1 ) **para bacilos Gram negativos (BGN)**: CC con 59,5% y **87,1%** de ID correcta respectivamente (p: 0,010),
- 2 ) para cocos **Gram positivos (CGP) racimos**: L1 con AF con 62,5% y **87,5 %** de ID correcta respectivamente (p:0,004)
- 3 ) para **CGP cadenas**: CC con **70,6%** y 69,2% de ID correcta respectivamente (p:0,925)



Impacto de programa de uso ATB asociado a nuevas tecnologías para la detección precoz y tratamiento de las bacteriemias por BGN en niños hospitalizados

*Unidad principal de analisis* : demora en horas del tratamiento antibiotico adecuado .

Estudio prospectivo . Pre intervención : 6 meses  
Post intervención : 6 meses

La información microbiológica se difundió en tiempo real mediante grupo de whats up entre microbiología , infectología y área de farmacia .

Episodios : 120

Modificación del tratamiento ATB inicial : 55 % vs 62 %

La mediana de demora en la adecuación ATB fue 48 hs vs 24 hs

Requirieron UCI : 22 pac vs 12 pac

Mediana días en UCI : 25 vs 20

Mediana días de internación : 29 vs 3

La mediana días de negativización hemocultivos : 3 vs 3

Fallecieron 12 % vs 8.3 %

La mortalidad a 30 días fue de 17 % vs 10 %

**La identificación rápida asociado a un programa para el uso adecuado de ATB permitió optimizar los tratamientos definitivos en forma precoz .**

# FilmArray

*Diagnostico Sindrómico Rápido y  
Preciso*

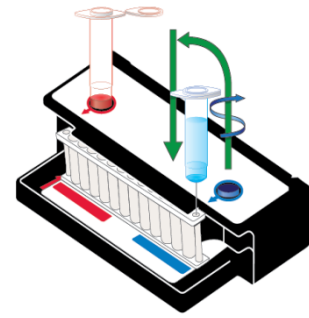
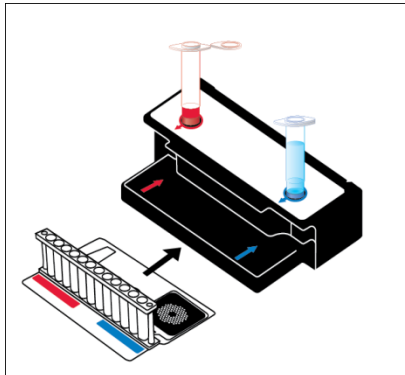
FilmArray®

# FilmArray

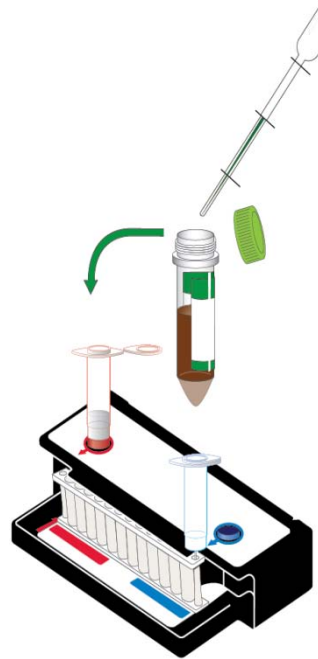
## La herramienta más rápida para obtener los mejores resultados

- **Fácil** – Dos minutos de preparación
- **Rápido** – Resultados en una hora
- **Completo** –
  - RP- 20 targets (17 virus & 3 bacterias)
  - BCID- 27 targets (19 bacterias, 4 levaduras & 3 resistencias a antibióticos)
  - GI- 22 targets (13 bacterias, 4 parásitos & 5 virus)
  - ME -15 targets (6 bacterias, 7 virus & 1 hongo)
- **Sistema Cerrado** – bajo riesgo de contaminación
- **Diagnóstico molecular** – Alta sensibilidad y especificidad.

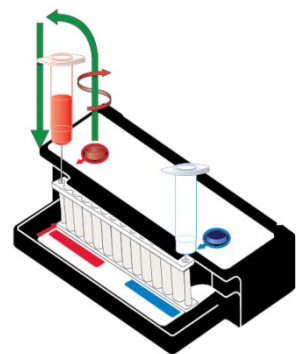
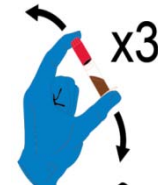




Hidratar el pouch

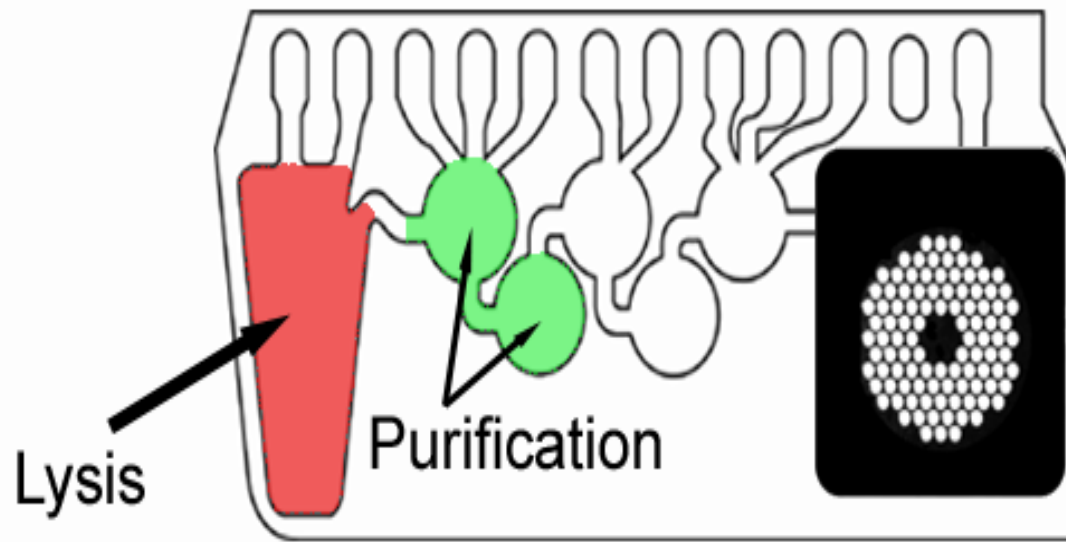


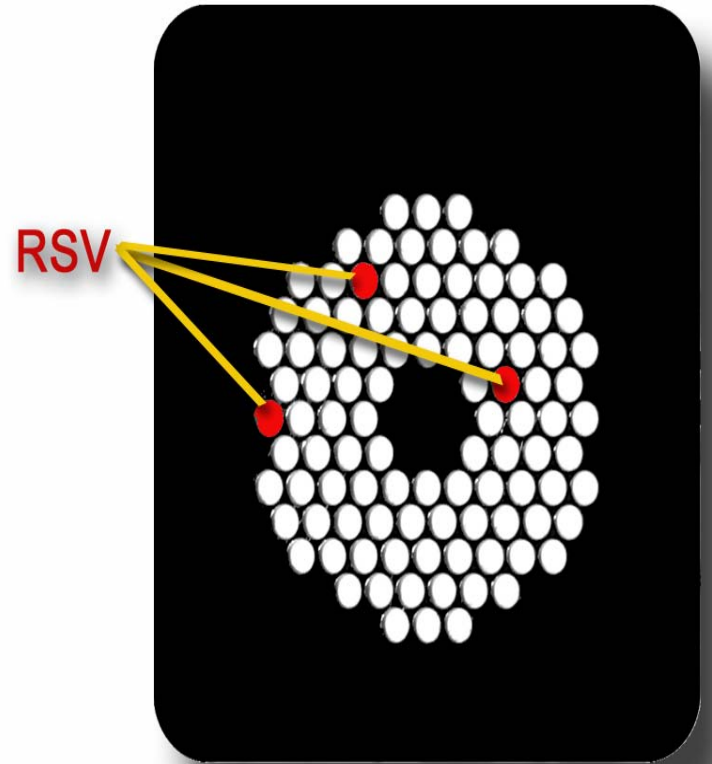
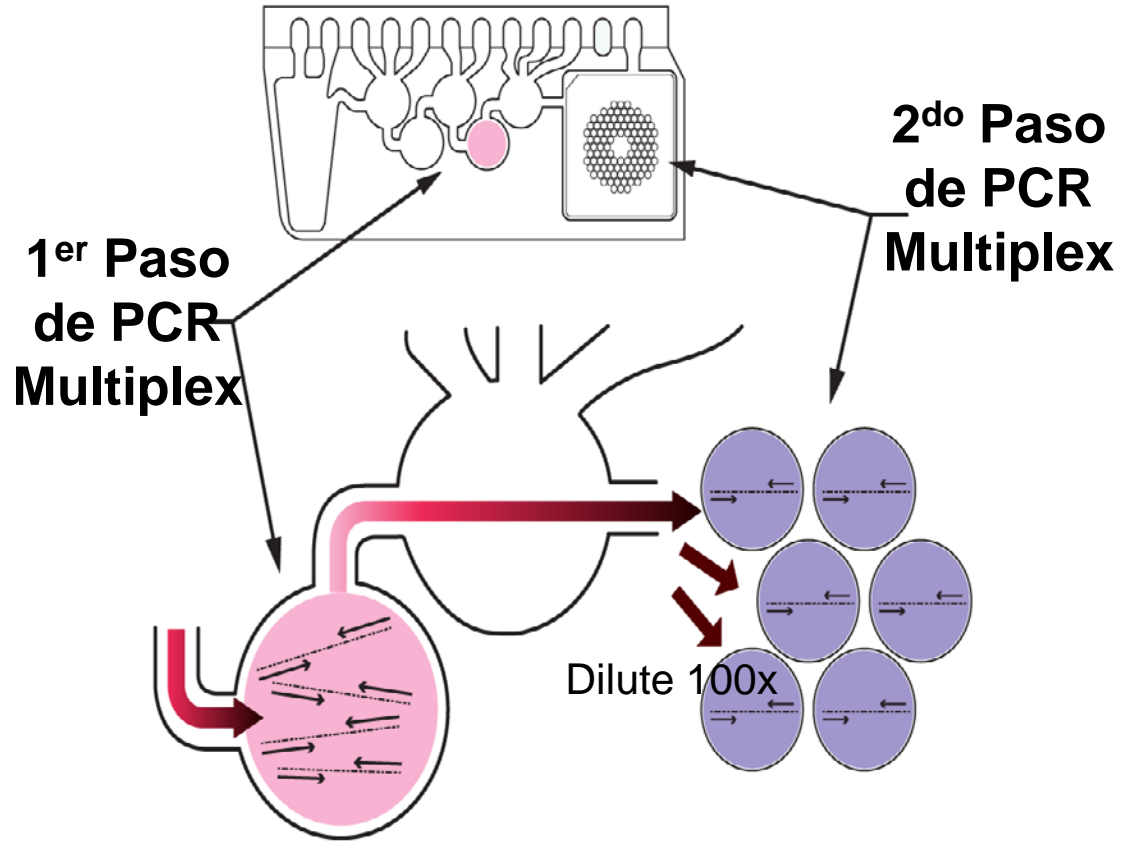
Preparar la muestra



Cargar la muestra







# Panel Respiratorio

## Muestra Directa: Hisopado Nasofaringeo

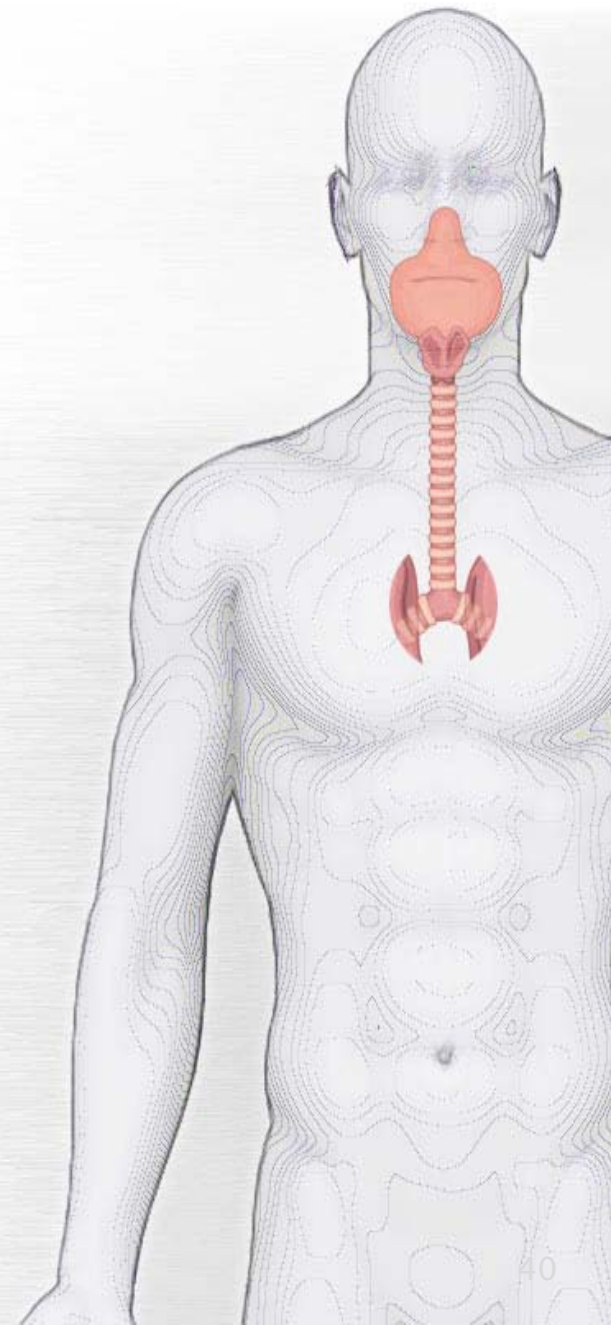
### Virus

Adenovirus  
Coronavirus 229E  
Coronavirus HKU1  
Coronavirus OC43  
Coronavirus NL63  
Human Metapneumovirus  
Human Rhinovirus/  
Enterovirus  
Influenza A  
Influenza A/H1  
Influenza A/H1-2009  
Influenza A/H3  
Influenza B

Parainfluenza 1  
Parainfluenza 2  
Parainfluenza 3  
Parainfluenza 4  
RSV

### Bacterias

Bordetella pertussis  
Chlamydophila pneumoniae  
Mycoplasma pneumoniae





# Panel SEPSIS

## Muestra: Hemocultivo Positivo

### Gram + Bacterias:

*Enterococcus*

*L. monocytogenes*

### **Staphylococcus**

*S. aureus*

### **Streptococcus**

*S. agalactiae*

*S. pyogenes*

*S. pneumoniae*

### Resistencia Antibiótica:

*mecA*

Van A/B

KPC

### Gram - Bacterias:

*A. baumannii*

*H. influenzae*

*N. meningitidis*

*P. aeruginosa*

### **Enterobacteriaceae**

*Enterobacter*

*cloacae complex*

*E. coli*

*K. oxytoca*

*K. pneumoniae*

*Proteus*

*S. marcescens*

### Levaduras:

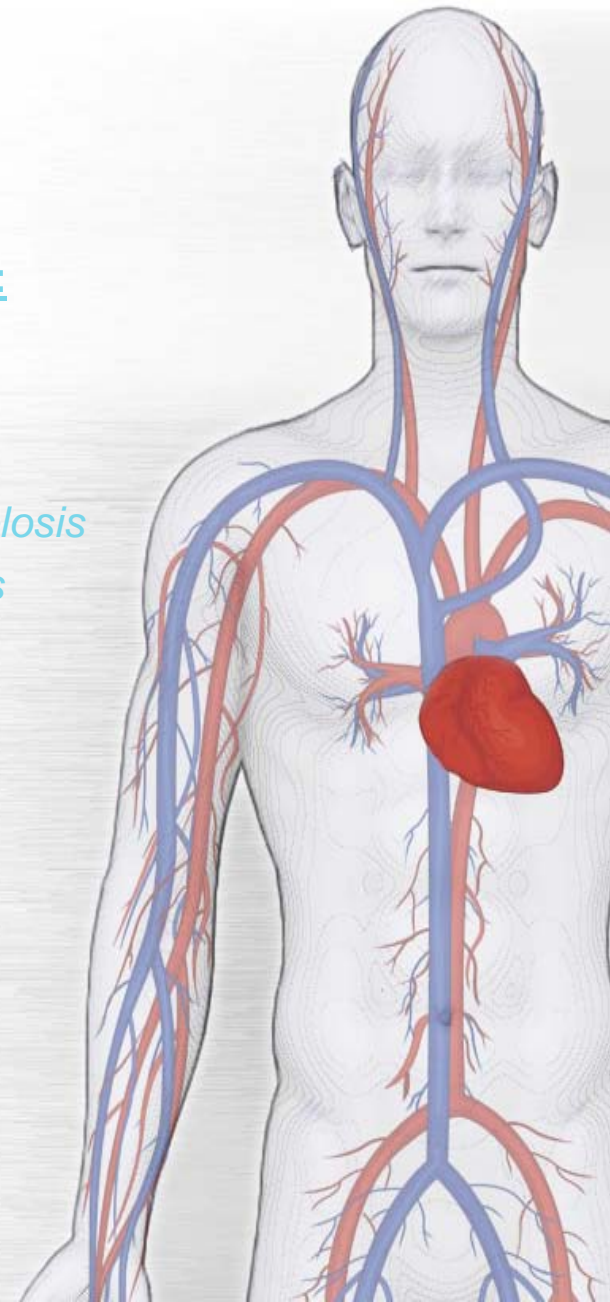
*C. albicans*

*C. glabrata*

*C. krusei*

*C. parapsilosis*

*C. tropicalis*



# Panel GASTROINTESTINAL

## Muestra: Materia Fecal

### Bacterias:

*Campylobacter*

*Clostridium difficile* (Toxin A/B)

*Plesiomonas shigelloides*

*Salmonella*

*Vibrio*

*Vibrio cholerae*

*Yersinia enterocolitica*

### *E. coli* / *Shigella* diarreogénicas

*E. coli* O157

Enteraggregative *E. coli* (EAEC)

Enteropathogenic *E. coli* (EPEC)

Enterotoxigenic *E. coli* (ETEC)

Shiga-like toxin-producing *E. coli* (STEC)

*Shigella*/Enteroinvasive *E. coli* (EIEC)

### Protozoos:

*Cryptosporidium*

*Cyclospora cayetanensis*

*Entamoeba histolytica*

*Giardia lamblia*

### Virus:

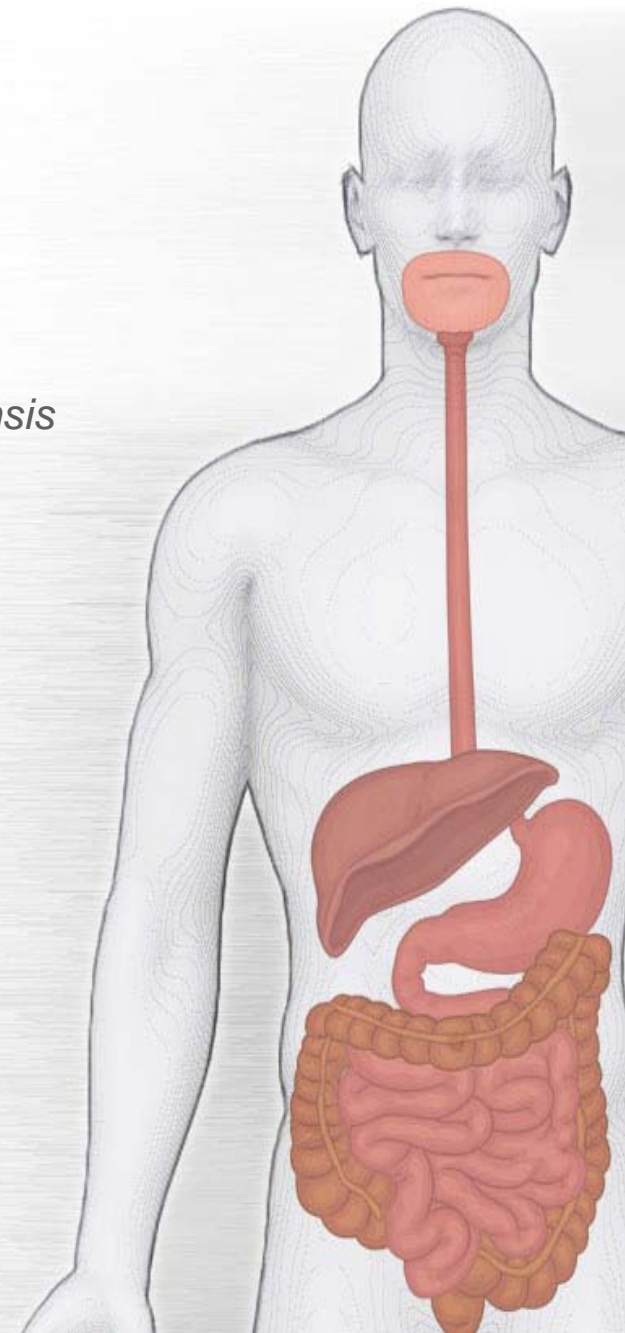
Adenovirus F 40/41

Astrovirus

Norovirus GI/GII

Rotavirus A

Sapovirus



# Panel Meningitis/Encefalitis (ME)

Registrado en ANMAT julio 2016

TIPO DE MUESTRA: 200µl Líquido Cefaloraquideo (LCR) – puncion lumbar

## **Bacteria:**

*E. coli* K1

*H. influenzae*

*L. monocytogenes*

*N. meningitidis* (encapsulated)

*S. agalactiae*

*S. pneumoniae*

## **Hongos:**

*Cryptococcus neoformans/gattii*

## **Virus:**

Cytomegalovirus (CMV)

Enterovirus (EV)

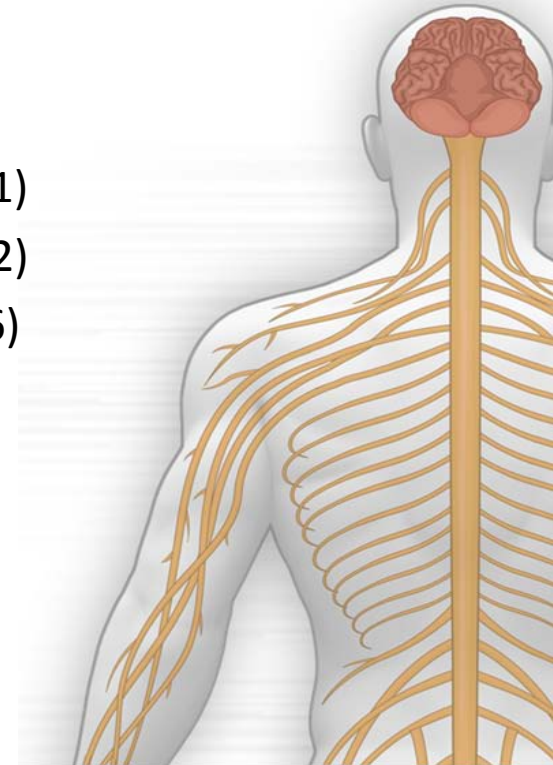
Herpes simplex type 1 (HSV-1)

Herpes simplex type 2 (HSV-2)

Human herpesvirus 6 (HHV-6)

Human Parechovirus (HPeV)

Varicella zoster virus (VZV)



# Panel ME Valor Clínico

## Impact of in-hospital enteroviral polymerase chain reaction testing on the clinical management of children with meningitis

Lyons, T. W., McAdam, A. J., Cohn, K. A., Monuteaux, M. C., Nigrovic, L. E.

### Abstract

**BACKGROUND:** Enteroviral meningitis is a common cause of meningitis in children which requires only supportive care. **OBJECTIVE:** To evaluate the impact of implementing an in-hospital enteroviral polymerase chain reaction (EVPCR) testing protocol on the clinical management of children with meningitis. **DESIGN:** Retrospective cohort study. **POPULATION:** Children <19 years old with meningitis. **INTERVENTION:** EVPCR testing differed by time period: send-out testing protocol from July 1, 2006-June 23, 2008 (pre-period) versus in-house testing protocol from June 24, 2008-June 30, 2010 (post-period). **MEASUREMENTS:** Test turnaround time, test utilization, length of stay, and duration of parenteral antibiotics. **RESULTS:** Of the 441 study patients, 216 (49%) presented during the post-period. Median age was 2.9 months (interquartile range, 1.5-96 months). Test turnaround time decreased with the in-house test (53 hours pre vs 13 hours post,  $P < 0.001$ ), and test utilization increased (28% pre vs 62% post,  $P < 0.001$ ). Among children with a positive EVPCR test, both length of stay (44 hours pre vs 28 hours post,  $P = 0.005$ ) and duration of parenteral antibiotics (48 hours pre vs 36 hours post,  $P = 0.04$ ) decreased in the post-period. No change in either of these outcomes was observed in children with meningitis and a negative EVPCR test. **CONCLUSION:** In-house EVPCR testing reduced test turnaround time, increased test utilization, and reduced both length of stay and duration of parenteral antibiotics for children with a positive result. Clinicians caring for children with meningitis should have access to in-hospital EVPCR testing.

Source: <http://www.ncbi.nlm.nih.gov/pubmed/>

[Find this article and related articles at Pub Med](#)

J Hosp Med. 2012 Sep;7(7):517-20. doi: 10.1002/jhm.1947. Epub 2012 May 16.

## Con PCR EV (derivada vs in house)

- Retrospectivo: 225 pre, 216 post.
- TAT (53 hs vs 13 hs)
- Utilización del test (28% vs 62%)
- LOS (44hs vs 28 hs)
- Duración de antibiotico parental (48 hs vs 36 hs)

# Panel ME Valor Clínico y Económico

**Rapid detection of enterovirus in cerebrospinal fluid by a fully-automated PCR assay is associated with improved management of aseptic meningitis in adult patients**

Giulieri SG, Chapuis-Taillard C, Manuel O, Hugli O, Pinget C, Wasserfallen JB, Sahli R, Jatou K, Marchetti C, Meylan P

## Abstract

### BACKGROUND:

Enterovirus (EV) is the most frequent cause of aseptic meningitis (AM). Lack of microbiological documentation results in unnecessary antimicrobial therapy and hospitalization.

### OBJECTIVES:

To assess the impact of rapid EV detection in cerebrospinal fluid (CSF) by a fully-automated PCR (GeneXpert EV assay, GXEA) on the management of AM.

### STUDY DESIGN:

Observational study in adult patients with AM. Three groups were analyzed according to EV documentation in CSF: group A = no PCR or negative PCR (n=17), group B = positive real-time PCR (n = 20), and group C = positive GXEA (n = 22). Clinical, laboratory and health-care costs data were compared.

### RESULTS:

Clinical characteristics were similar in the 3 groups. Median turn-around time of EV PCR decreased from 60 h (IQR (interquartile range) 44-87) in group B to 5h (IQR 4-11) in group C (p<0.0001). Median duration of antibiotics was 1 (IQR 0-6), 1 (0-1.9), and 0.5 days (single dose) in groups A, B, and C, respectively (p < 0.001). Median length of hospitalization was 4 days (2.5-7.5), 2 (1-3.7), and 0.5 (0.3-0.7), respectively (p < 0.001). Median hospitalization costs were \$5458 (2676-6274) in group A, \$2796 (2062-5726) in group B, and \$921 (765-1230) in group C (p < 0.0001).

### CONCLUSIONS:

Rapid EV detection in CSF by a fully-automated PCR improves management of AM by significantly reducing antibiotic use, hospitalization length and costs.

Source: <http://www.ncbi.nlm.nih.gov/pubmed/>

[Find this article and related articles at Pub Med](#)

[J Clin Virol](#). 2015 Jan;62:58-62. doi: 10.1016/j.jcv.2014.11.001. Epub 2014 Nov 10.

- ▶ **PCR totalmente automatizada EV**
- ▶ **TAT** baja 55 hrs (60 hrs pre vs 5 hrs post)
- ▶ **LOS** disminuye 3.5 dias (4 dias pre vs. 0.5 hrs post)
- ▶ **Duración de antibióticos parenteral** baja 12 hrs (1 dia pre vs 0.5 dia post)
- ▶ **Ahorro \$4,537 en costos hospitalario** (\$5,458 pre vs. \$921 post)

# **SEPSIS: CADA MINUTO CUENTA**

## **Terapia Intensiva - Emergencias**

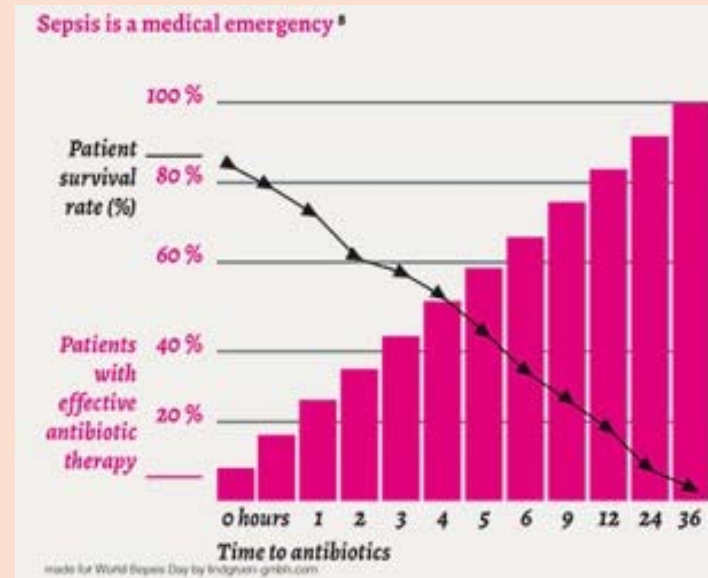
# SEPSIS: CADA MINUTO CUENTA



**EN ARGENTINA MUEREN 10.736 PACIENTES POR SEPTICEMIA AL AÑO**  
(Estadísticas Vitales, MinSal 2013)

**29,4 MUERTES/DIA**

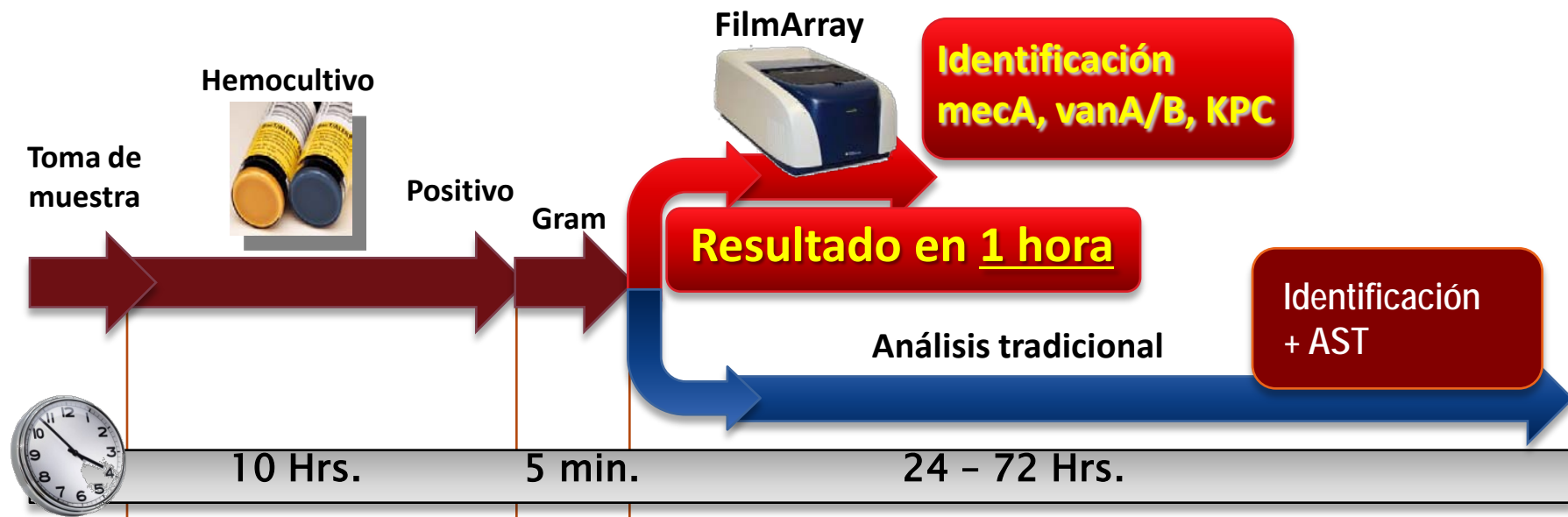
- Tasa de mortalidad entre **10-38%**.
- Cada hora decrece un **7.6%** la sobrevivida en pacientes sépticos.
- **1/3** de pacientes sépticos graves, reciben una terapia antibiótica inapropiada.
- El tratamiento empírico inadecuado lleva a mayor morbi-mortalidad e incrementa la resistencia a los antibióticos.



**La Identificación rápida de patógenos y mecanismos de resistencia mejora el tiempo en administrar la terapia correcta.**

Kumar A, Roberts D, Wood KE, et al.: Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med, 34: 1589-1596, 2006.

# SOLUCION DEL DIAGNOSTICO RAPIDO EN SEPSIS



## Resultado en 1 hora de ID patógeno y Mecanismos de Resistencia permite:

- **Tratamiento Dirigido:** Mejor elección de ATB, sin efectos secundarios no deseados ni incremento de COSTOS (Banerjee R et al. ICAAC Sept 2014)
- **Menor tiempo en UCI y Mortalidad:** En Infecciones causadas por *S. aureus* y *mecA* asociado, permitió: el **des-escalamiento** de terapia empírica en **44hs vs 67 hs**, **Mortalidad (10% vs 23%)** y **estancia en UCI (3 vs 7 días)** (Kim J et al. 115<sup>th</sup> Gral Meeting ASM. May2015)
- **Reducción de infección nosocomial:** Reconocimiento temprano de microorganismos Gram+/- , hongos específicos y resistencias. Optimización de la terapia basado en un programa de ATS (Timbrook, T et al. Journal of Clinical Microbiology. Apr 2015)



# URGENCIAS MICROBIOLÓGICAS

## GASTROINTESTINALES

-Síndrome Urémico Hemolítico (SUH): Responsable del 20% de trasplantes de riñón en niños y adolescentes

- 13,9 niños <5 años infectados cada 100.000

- ATB CONTRAINDICADO en caso de sospecha o confirmación



**ProNCEZ**  
Programa Nacional de Control  
de Enfermedades Zoonóticas



**Ministerio de  
Salud**  
Presidencia de la Nación

## Panel Gastrointestinal FilmArray

Muestra Directa: Materia Fecal

### Bacteria:

*Campylobacter*

(*C. jejuni*, *C. coli*, and *C. upsaliensis*)

*Clostridium difficile* (toxin A/B)

*Plesiomonas shigelloides*

*Salmonella*

*Vibrio*

(*V. parahemolyticus*, *V. vulnificus*,  
*V. cholerae*)

*Vibrio cholerae*

*Yersinia enterocolitica*

### Diarrheagenic *E. coli* / *Shigella*

Enteraggregative *E. coli* (EAEC)

Enteropathogenic *E. coli* (EPEC)

Enterotoxigenic *E. coli* (ETEC)

Shiga-like toxin-producing *E. coli* (STEC)

*E. coli* O157

*Shigella*/Enteroinvasive *E. coli* (EIEC)

### Parasites:

*Cryptosporidium*

*Cyclospora cayentanensis*

*Entamoeba histolytica*

*Giardia lamblia*

### Viruses:

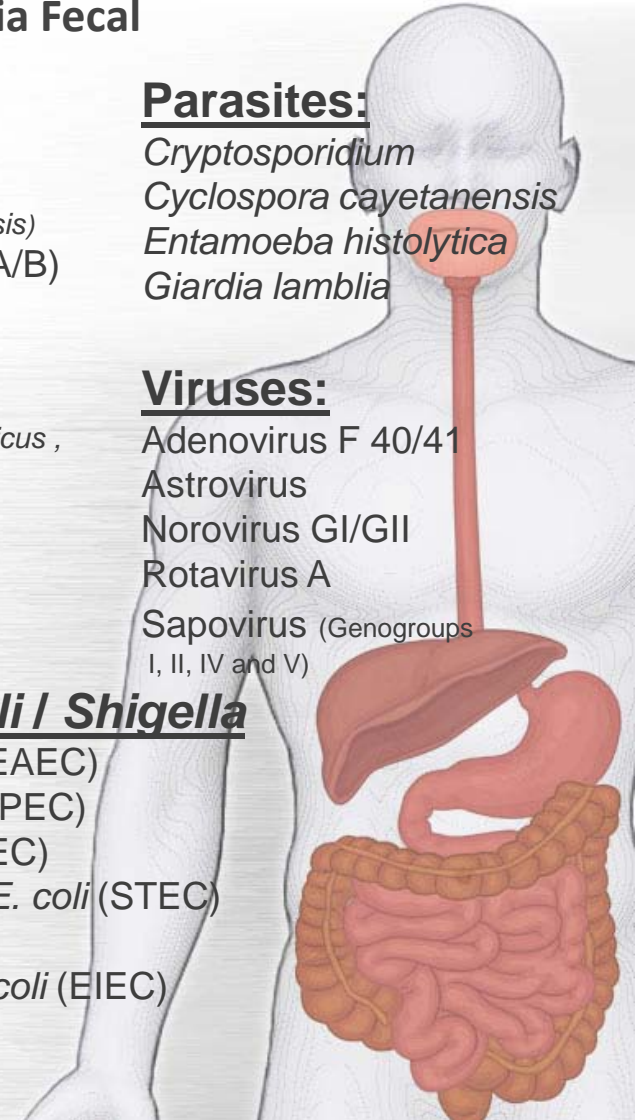
Adenovirus F 40/41

Astrovirus

Norovirus GI/GII

Rotavirus A

Sapovirus (Genogroups  
I, II, IV and V)



# A QUE PACIENTES ANALIZAR?

## INFECCIONES GASTROINTESTINALES

### ✓ **Húesped**

- ✓ **Inmunocompetente**
- ✓ **Inmunocomprometido**

### ✓ **Adquisición**

- ✓ **Comunidad**
- ✓ **Asociado al cuidado de la salud (nosocomial, geriátricos, centro de cuidados)**
- ✓ **Viajeros**
- ✓ **Comunidades cerradas (guarderías, hoteles, cruceros, restaurantes)**

### ✓ **Tipo**

- ✓ **Acuoso o secretora**
- ✓ **Inflamatorio (leucocitos y sangre)**

### ✓ **Origen**

- ✓ **No infeccioso**
- ✓ **Infeccioso**
  - **Bacteriano, Viral, Parasitario**
  - **>80 % resuelve espontáneamente con agua y electrolitos**
  - **<20% son prolongadas (>14 días)**

# **INFECCIONES RESPIRATORIAS Y GASTROINTESTINALES**

## **PATOLOGIAS ANUALES CON IMPACTO ESTACIONAL Y CICLICO**

- Colapso Hospitalario - Necesidad Edilicia para Aislamientos
- Incremento en la Morbi-Mortalidad
- Infecciones intrahospitalarias
- Decisión Clínica en tratamiento antiviral o antibiótico - Uso racional.
- Riesgo de incremento de Resistencias Antibióticas

# SOLUCION DEL DIAGNOSTICO RAPIDO CON FILMARRAY

**Resultado en 1 hora de Identificación de patógeno permite:**

**Aislamiento rápido y efectivo:** Debido al rápido resultado, el Panel Respiratorio de FilmArray sirvió para un cohorting efectivo de los pacientes (**1.4 hs vs 7 hs**) (Xu, et al. *Am J Clin Pathol.* 2013)

**Diagnóstico completo:** Debido a su alta sensibilidad, El panel GI de FilmArray detectó:

- Un patógeno en el **54,2%** de los casos vs métodos tradicionales que detectan un **18,1%**
- **Coinfecciones** en el **16,4%** de las muestras.

**Disminución significativa del tiempo de informe de resultado:**

- Rápida toma de muestra y envío al lab : 1 hora comparada con 19 horas (guardia médica)
- Prueba mas rápida : TAT de **2h vs 17h**, Mano de obra: **2 minutos vs 5hs** (Poelman R. Point of impact testing in emergency department: diagnostics of viral infections – Poster CVS 2015)

# Preparación, Amplificación y Detección Integrada de la Muestra



SIN INFRAESTRUCTURA



**SIMPLICIDAD**  
2 MINUTOS DE PROCESAMIENTO



**REACTIVOS LISTOS PARA USAR**  
CONSERVACION A TEMPERATURA AMBIENTE



BIOSEGURIDAD

FilmArray™ BCID Panel		BIOFIRE
Run Summary		
Sample ID: BCV_2012_1432_20_8	Run Date: 20 May 2013	Run Time: 1:41:54
Organisms Detected: Enterobacteriaceae Abiotrophia parvositonia	Controls: Passed	
Applicable Antibiogram: KPC - Detected	Reference Gene:	
Result Summary and Interpretation		
Antibiotic Resistance Genes		
<input checked="" type="checkbox"/> Detected	KPC (Klebsiella resistance gene)	
<input checked="" type="checkbox"/> Not Detected	bla <sub>TEM</sub> (beta-lactamase resistance gene)	
<input checked="" type="checkbox"/> Not Detected	bla <sub>SHV</sub> (beta-lactamase resistance gene)	
⚠️ NOTE: Interpretation requires an active high resolution. An elevated Ct for any organism associated organism gene across files for which antimicrobial resistance, bioactivity, or sensitivity assays are available and available within a file.		
Gene Positive Results		
Not Detected	Enterobacteriaceae	
Not Detected	Labetalactamase	
Not Detected	Shigella sonnei	
Not Detected	Shigella sonnei	
Not Detected	Shigella sonnei (Group B)	
Not Detected	Shigella sonnei (Group C)	
Not Detected	Shigella sonnei (Group A)	
Gene Negative Results		
Not Detected	Acinetobacter baumannii	
Not Detected	Enterobacteriaceae	
Not Detected	Enterobacteriaceae complex	
Not Detected	Enterobacteriaceae	
Not Detected	Abiotrophia parvositonia	
Not Detected	Prion	
Not Detected	Serratia marcescens	
Not Detected	Haemophilus influenzae	
Not Detected	Neisseria meningitidis	
Not Detected	Pseudomonas aeruginosa	
Yeast		
Not Detected	Candida albicans	
Not Detected	Candida glabrata	
Not Detected	Candida kefyr	
Not Detected	Candida parapsilosis	
Not Detected	Candida tropicalis	
Run Details		
Panel: BCID Panel	Protocol: BCID	
Run Status: Completed	Operator: KJW	
Serial No.: 0031274	Instrument: PA205	
Lot No.: 120113		

REPORTE EN UNA HORA



# Los Paneles

**Panel Respiratorio**  
FDA Mayo 2011  
ANMAT Sept 2015

**Panel BCID**  
FDA Junio 2013  
ANMAT Sept 2015

**Panel GI**  
FDA Mayo 2014  
ANMAT Sept 2015

**Panel Meningitis**  
ANMAT Julio 2016

Panel Respiratorias Bajas

Panel Infecciosas Tropicales

**FilmArray Platform**

## THE FILMARRAY LOWER RESPIRATORY TRACT INFECTION (LRTI) PANEL

Simultaneous detection of 30 Pathogens and 7 Antibiotic Resistance Markers:



### Bacteria

- *Acinetobacter calcoaceticus-baumannii* complex
- *Chlamydophila pneumoniae*
- *Enterobacter cloacae/aerogenes*
- *Escherichia coli*
- *Haemophilus influenzae*
- *Klebsiella oxytoca*
- *Klebsiella pneumoniae*
- *Legionella pneumophila*
- *Moraxella catarrhalis*
- *Mycoplasma pneumoniae*
- *Proteus* spp.
- *Pseudomonas aeruginosa*
- *Serratia marcescens*
- *Staphylococcus aureus*
- *Stenotrophomonas maltophilia*
- *Streptococcus agalactiae*
- *Streptococcus pneumoniae*
- *Streptococcus pyogenes*



### Antibiotic Resistance Markers

- CTX-M (ESBL)
- IMP (Carbapenem resistance)
- KPC (Carbapenem resistance)
- mecA/C - MREJ
- NDM (Carbapenem resistance)
- OXA-48-like (Carbapenem resistance)
- VIM (Carbapenem resistance)



### Viruses

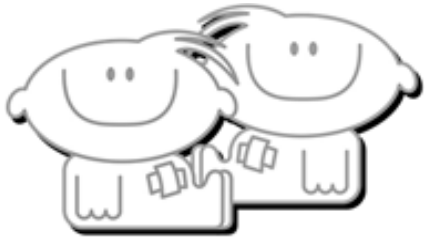
- Adenovirus
- Coronavirus
- Human Rhinovirus/Enterovirus
- Human Metapneumovirus
- Influenza A
- Influenza B
- Parainfluenza Virus
- Respiratory Syncytial Virus
- Coronavirus MERS



### Fungi

- *Aspergillus* spp.
- *Cryptococcus* spp.
- *Pneumocystis jirovecii*

Aún a la  
espera de  
aprobación  
de FDA!



Hospital de Pediatría S.A.M.I.C.  
"Prof. Dr. Juan P. Garrahan"



***Muchas gracias***