



**JNI** 15<sup>es</sup> Journées  
Nationales  
d'Infectiologie

Bordeaux  
et l'interrégion Aquitaine § Limousin



du mercredi 11 au vendredi 13 juin 2014  
Palais des Congrès de Bordeaux

# NOUVELLES TECHNIQUES : POUR QUELLES AVANCÉES EN MICROBIOLOGIE MÉDICALE

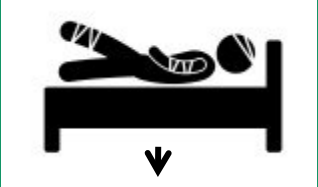
Dr Frédérique GOURIET

Fédération de Bactériologie Virologie et Hygiène hospitalière

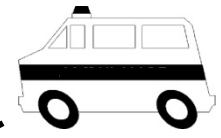
URMITE CNRS-IRD UMR 6236



15<sup>es</sup> JNI, Bordeaux  
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Syndrome and disease-based sampling kits



Transport

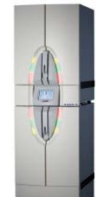
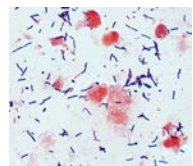
Immuno-chromatographic or agglutination assay



Real-time PCR

Point of care (POC) laboratory assays < 2 hours

### Direct examination and culture



Automated blood culture monitoring

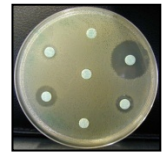


Diversified culture conditions

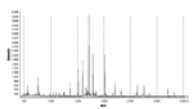
### Phenotypic identification and antibiotic susceptibility testing



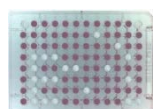
Manual biochemotype and antibiogram



Automated biochemical phenotype and antibiogram



MALDI-TOF MS



Phenotypic microarray

### Core laboratory

### Molecular detection and identification



PCR



Sequencing



Metagenomics

Unidentified or unusual bacterium

Local, national and /or international alert

Real-time genome sequencing



SMS message, Laboratory website

Strain collection<sup>2</sup>

New or atypical bacterium

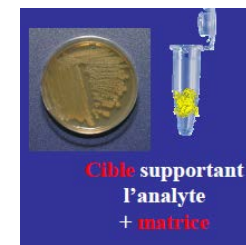
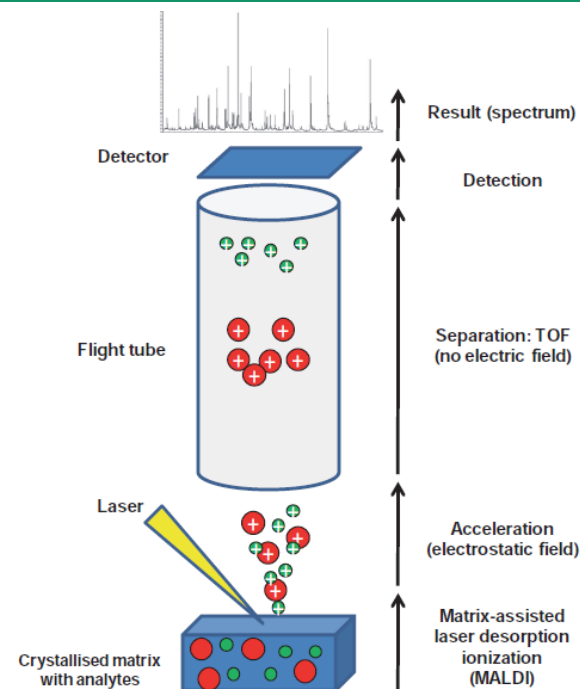
# Nouvelles applications de la spectrométrie de masse au laboratoire de Microbiologie



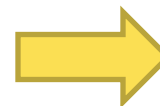
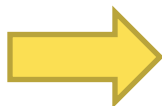
15<sup>es</sup> JNMI, Bordeaux  
du 11 au 13 juin 2014

# Nouvelles applications de la spectrométrie de masse au laboratoire de Microbiologie

- Identification de souche bactérienne
- identification directe sur flacon d'hémocultures
- Identification directe sur les urines
- Biotypage des bactéries
  - ➔ détection d'une épidémie
  - ➔ Surveillance d'une épidémie
  - ➔ Identification de clones
- Détection rapide de la résistance aux antibiotiques par MALDI-TOF
  - ➔ Carbapénémases chez les bactéries à gram négatif



# Apport de la Spectrométrie de masse pour le diagnostic microbiologique



Analyse MALDI-TOF



Micro-organisme inconnu

Dépôt sur une cible

Interprétation des données  
Banque de données

Overview Details **Images** Products Learn more Contact

MALDI Biotyper Realtime Classification Project: 20110228

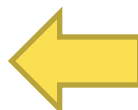
File View Tools Help

1 2 3 4 5 6 7 8 9 10 11 12

A B C D E F G H

- Not occupied
- Prepared
- Aborted
- Measured
- Zeroline spectrum
- Measured, classified green
- Measured, classified yellow
- Measured, classified red
- Zeroline spectrum, not classified

Prod. Ion	Name	Detected Species	Score	Comment	Validation
A2	A2	Acidiphilum acidophilum	2.712		species <input type="radio"/> genus-only <input type="radio"/> unknown <input type="radio"/>
A3	A3	Capnocytophaga necatrix	2.215		species <input type="radio"/> genus-only <input type="radio"/> unknown <input type="radio"/>
A4	A4	Azithromycin sulfonamide	2.556		species <input type="radio"/> genus-only <input type="radio"/> unknown <input type="radio"/>
A5	A5	Microbacterium maritimum	2.342		species <input type="radio"/> genus-only <input type="radio"/> unknown <input type="radio"/>
A6	A6	Proteus mirabilis	2.415		species <input type="radio"/> genus-only <input type="radio"/> unknown <input type="radio"/>
A7	A7	no reliable identification	1.251		species <input type="radio"/> genus-only <input type="radio"/> unknown <input type="radio"/>
A8	A8	Halomonas halodurificans	1.861		species <input type="radio"/> genus-only <input type="radio"/> unknown <input type="radio"/>
A9	A9	Leptobacterium roseum	2.392		species <input type="radio"/> genus-only <input type="radio"/> unknown <input type="radio"/>
A10	A10	Methylobacterium rhodesianum	1.794		species <input type="radio"/> genus-only <input type="radio"/> unknown <input type="radio"/>
A11	A11	Methylobacterium pili	2.417		species <input type="radio"/> genus-only <input type="radio"/> unknown <input type="radio"/>
A12	A12	Clostridium perfringens	2.105		species <input type="radio"/> genus-only <input type="radio"/> unknown <input type="radio"/>
R1	R1	no reliable identification	0.004		species <input type="radio"/> genus-only <input type="radio"/> unknown <input type="radio"/>



File Edit View Tools Help

1

2

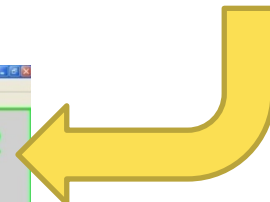
3

4

Method: mass-proteinase

Run: C:\Program Files\Applied Biosystems\MALDI\bin\maldi.exe

Prepared  For Calibration  For Measurement  For MS/MS Measurement  Aborted  Zeroline Spectrum



# Interprétation des résultats

- Comparaison par souches
- 3 niveaux d'identification
  - Log supérieur ou égal à 2: identification d'espèce
  - Log entre 1,7 et 2,0: identification de genre
  - Log < à 1,7: absence d'identification



Biologie moléculaire



Incrémentation de la Base de données Bruker Biotyper

Analyte Name	Organism (best match)	Score Value	Organism (second best)	Score Value
<a href="#">Enterococcus faecalis XY_121.BBB</a> (+++)(A)	Enterococcus faecalis	2.318	Enterococcus faecalis	2.318
<a href="#">Enterococcus faecalis XY_121.BBB</a> (+++)(A)	Enterococcus faecalis	2.311	Enterococcus faecalis	2.309
<a href="#">Proteus mirabilis XY_719.BBB</a> (+++)(A)	Proteus mirabilis	2.572	Proteus mirabilis	2.573
<a href="#">Proteus mirabilis XY_719.BBB</a> (+++)(A)	Proteus mirabilis	2.521	Proteus mirabilis	2.521
<a href="#">Pseudomonas aeruginosa MZ006.BBB</a> (+++)(A)	Pseudomonas aeruginosa	2.487	Pseudomonas aeruginosa	2.511

Analyte Name	Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
<a href="#">Pseudomonas aereali</a> (+++)	1 (+++)	Pseudomonas aeruginosa ATCC 27853 THL	2.407	<a href="#">287</a>
<a href="#">Staphylococcus aureu</a> (++)	2 (+++)	Pseudomonas aeruginosa 8147_2 CHB	2.31	<a href="#">287</a>
<a href="#">Staphylococcus aureu</a> (++)	3 (++)	Pseudomonas aeruginosa DSM 50071T HAM	2.27	<a href="#">287</a>
	4 (++)	Pseudomonas aeruginosa ATCC 27853 CHB	2.219	<a href="#">287</a>
	5 (++)	Pseudomonas aeruginosa 19955_1 CHB	2.183	<a href="#">287</a>
	6 (++)	Pseudomonas aeruginosa A07_08_Padu FLR	2.017	<a href="#">287</a>
	7 (+)	Pseudomonas jipjensis LMG 21316T HAM	1.751	<a href="#">198616</a>
	8 (-)	Pseudomonas citreoflois DSM 50332T HAM	1.627	<a href="#">53408</a>
	9 (-)	Pseudomonas indica DSM 14015T HAM	1.583	<a href="#">137658</a>
	10 (-)	Pseudomonas fluorescens LMG 2274T HAM	1.573	<a href="#">53412</a>

# Performances des différents Spectromètre disponible pour la microbiologie



## Matrix-assisted laser desorption ionization time-of-flight mass spectrometry, a revolution in clinical microbial identification

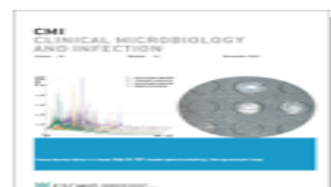
A. Bizzini<sup>1</sup>, G. Greub<sup>1,2</sup>

Article first published online: 15 JUL 2010

DOI: 10.1111/j.1469-0691.2010.03311.x

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Issue



Clinical Microbiology and Infection

Volume 16, Issue 11, pages 1614–1619, November 2010

**TABLE I.** Performance of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) identification with routine samples in clinical microbiology laboratories

Number of isolates tested	Manufacturer of the MALDI-TOF MS system	Overall correct identification at the species level (%)	Overall correct identification at the genus level (%)	Correct identification of Gram-negative bacteria at the species level (%)	Correct identification of Gram-positive bacteria at the species level (%)	References
1660 <sup>a</sup>	Bruker Daltonics GmbH	84.1	11.3	NA	NA	[11]
1371 <sup>a</sup>	Bruker Daltonics GmbH	91.7	2.8	88.8	88.0	[14]
720 <sup>a</sup>	Bruker Daltonics GmbH	93.6	NA	98.2	83.9	[13]
720 <sup>a</sup>	Shimadzu Corporation	88.3	NA	94.8	75.6	[13]
1116 <sup>b</sup>	Bruker Daltonics GmbH	95.2	4.8	93.8	97.7	[10]

NA, not available.  
<sup>a</sup>Prospective study.  
<sup>b</sup>Retrospective study.

# Problèmes d'identification :Discordance MALDI\_Tof et identification classique

## Phénotypique en spectrométrie de masse



### Matrix-assisted laser desorption ionization time-of-flight mass spectrometry, a revolution in clinical microbial identification

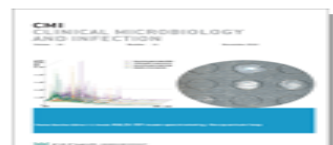
A. Bizzini<sup>1</sup>, G. Greub<sup>1,2</sup>

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Issue



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**TABLE 2.** Examples of discordant results between conventional and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) identification

Conventional ID	MALDI-TOF MS ID	16S rDNA sequencing ID	Explanation	References
<i>Enterobacter cloacae</i>	<i>Enterobacter hormaechei</i>	<i>E. hormaechei</i>	Conventional methods such as Vitek 2 system (BioMérieux) do not identify some species of the <i>E. cloacae</i> complex group	[14]
<i>Stenotrophomonas maltophilia</i> <i>S. maltophilia</i>	<i>Pseudomonas hibiscicola</i> <i>Pseudomonas beteli</i>	<i>S. maltophilia</i> <i>S. maltophilia</i>	Invalid taxonomic name (heterotypic synonyms of <i>S. maltophilia</i> that should not be used according to the recent taxonomy)	[10,11,14]
<i>Shigella sonnei</i> <i>Shigella flexneri</i>	<i>Escherichia coli</i> <i>Escherichia coli</i>	<i>Shigella sonnei</i> <i>Shigella flexneri</i>	Absence of enough reference spectra in the MALDI-TOF MS database, leading to insufficient discriminative power for closely related species	[10,11,14]
<i>Streptococcus pneumoniae</i> <i>Streptococcus mitis</i> <i>Propionibacterium acnes</i>	<i>Streptococcus parasanguinis</i> <i>Streptococcus pneumoniae</i> <i>Eubacterium bradyi</i>	<i>Streptococcus pneumoniae</i> <i>Streptococcus mitis</i> <i>P. acnes</i>	Incorrect reference spectra in the MALDI-TOF MS database	[14]
<i>Streptococcus dysgalactiae</i>	<i>Streptococcus pyogenes</i> or <i>Streptococcus dysgalactiae</i> on repeated independent deposits	<i>Streptococcus dysgalactiae</i>	Inconsistent results, owing either to difficult-to-differentiate strains or a lack of sufficient reference spectra in the MALDI-TOF MS database	[14]

ID, identification.



# MALDI-TOF mass spectrometry tools for bacterial identification in clinical microbiology laboratory

Etienne Carbonnelle<sup>a,c,\*</sup>, Cécile Mesquita<sup>a</sup>, Emmanuelle Bille<sup>b,c</sup>, Nesrine Day<sup>a</sup>, Brunhilde Dauphin<sup>d</sup>, Jean-Luc Beretti<sup>b</sup>, Agnès Ferroni<sup>b</sup>, Laurent Gutmann<sup>a,c</sup>, Xavier Nassif<sup>b,c</sup>

**Table 1**

Summary of major studies using MALDI-TOF for bacterial identification. GN: Gram negative, GP: Gram positive.

Authors	Sample		Id species level	Id genus level	Main identification difficulty	Comments
Serg et al. [32]	Routine (n=1660)	all routine samples	83.8%	95%	<i>Propionobacterium acnes</i> , <i>Streptococcus pneumoniae</i> , <i>Stenotrophomonas maltophilia</i> , <i>Shigella</i> sp.	First line method of identification
van Veen et al. [36]	Routine (n=980)	all routine samples	92%	98.8%	<i>Streptococcus pneumoniae</i> , anaerobic bacteria	
Blondiaux et al. [37]	Routine (n=362)	all routine samples	72.9%	87%	viridans streptococci group. <i>Shigella</i> sp.	
Prod'homme et al. [42]	Blood (n=126)	positive blood culture	77.8%, GN: 89.1%, GP: 71.6%	78.7%, GN: 89.1%, GP: 72.9%	<i>Streptococcus mitis</i> group, <i>Staphylococcus</i> sp.	The presence of a capsule explain partially the low identification rate of <i>S. pneumoniae</i> , <i>H. influenzae</i> , <i>K. pneumoniae</i>
La Scola et al. [43]	Blood (n=599)	positive blood culture	76%	76%	<i>Streptococcus</i> sp., polymicrobial samples	
Stevenson et al. [44]	Blood (n=212)	positive blood culture (179), spiked bottles (33)	80.2%	80.2%	<i>Streptococcus mitis</i> group <i>Propionobacterium acnes</i>	
Ferroni et al. [45]	Blood (n=685)	positive blood culture (388), spiked bottles (312)	89%	98%	<i>Streptococcus pneumoniae</i> , <i>Streptococcus mitis</i> group	For mixed culture, most abundant germ was in most cases identified. Fast method
Christner et al. [46]	Blood (n=277)	positive blood culture	94.2%	95%	Cocci Gram +	Mismatching mostly resulted from insufficient bacterial count and occurred preferentially with Gram +
Ferreira et al. [47]	Blood (n=300)	positive blood culture	42.6%, GN: 83.3%, GP: 31.8%	71.6%, GN: 96.6%, GP: 65.7%	<i>Streptococcus mutans</i> , <i>Staphylococcus</i> sp., <i>Staphylococcus aureus</i>	No mixed culture
Ferreira et al. [48]	Urine (n=220)	positive urine samples	91.8%, GN: 93.6%, GP: 56.6%	92.7%, GN: 94.6%, GP: 66.6%	<i>Streptococcus</i> sp., <i>Enterococcus</i> sp., <i>Raoultella</i> sp.	Best results with high bacterial account >10 <sup>5</sup> CFU/mL, <i>E. coli</i> >10 <sup>5</sup> CFU/mL: 97.6% correct id rate, 5 mixed cultures: 3 identifications

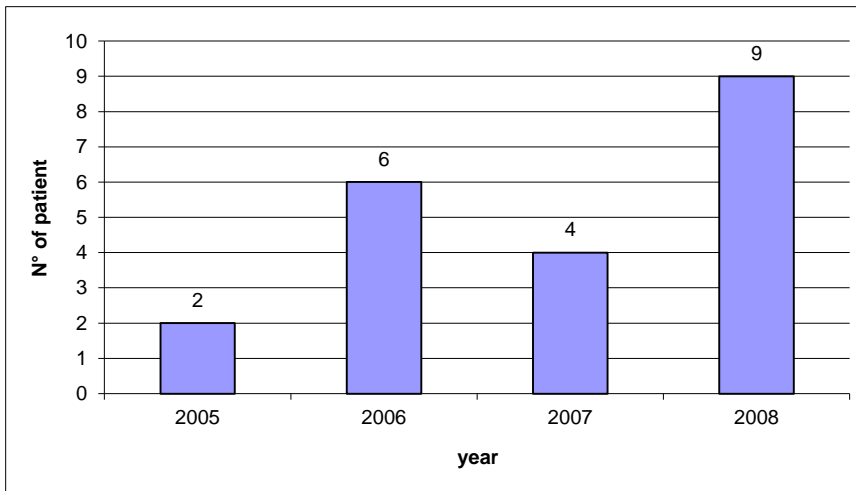
# Identification phénotypique classique / spectrométrie de masse

- **Problèmes du choix du système d'identification:**
  - Gram
  - Tests d'orientation (catalase, oxydase...)
- **Diversité phénotypique:**
  - Base de données: nombre importants de souches par espèce
  - Chevauchement des phénotypes
    - Choix multiples
- **Souches déficientes**
  - Variant
  - Inoculum insuffisant
- Choix préalable inutile
- Colonie ou échantillon hémoculture/urines
- Une seule base de donnée pouvant être constituée par une seule souche par espèce modulable
- Profils protéiques provenant en grande partie par des protéines ribosomales
- Résultat rapide
- Coût concurrentiel

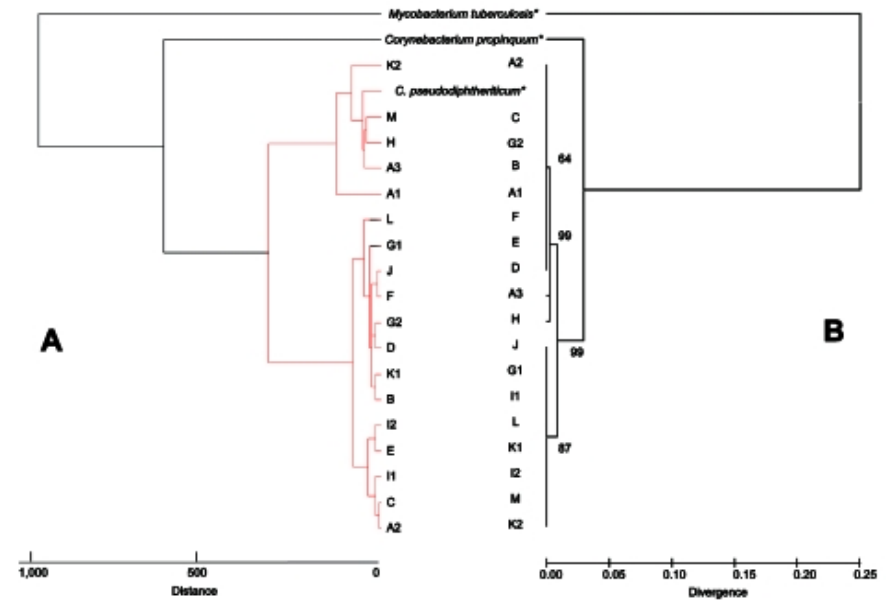
# Détection d'une épidémie

## Outbreak of *Corynebacterium pseudodiphtheriticum* Infection in Cystic Fibrosis Patients, France

Fadi Bittar,<sup>1</sup> Carole Cassagne,<sup>1</sup> Emmanuelle Bosdure, Nathalie Stremier, Jean-Christophe Dubus, Jacques Sarles, Martine Reynaud-Gaubert, Didier Raoult, and Jean-Marc Rolain



18 souches  
Issues de 13 patients



Arbre phylogénétique position des *Corynebacterium* spp. comparaisons MALDI-TOF (A) et séquences partielle ARN -sous unité beta de polymerase gene *rpoB* (B).

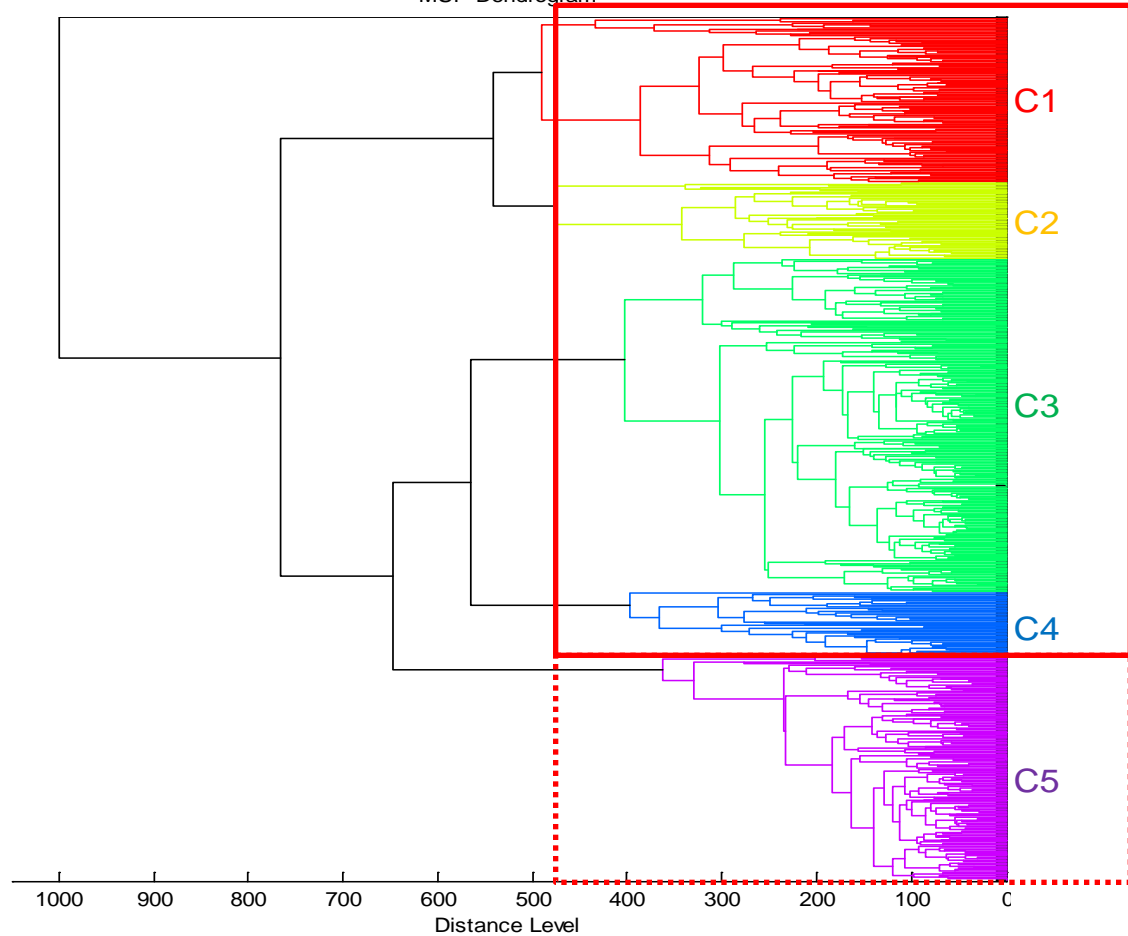
Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 16, No. 8, August 2010

# Surveillance d'une épidémie

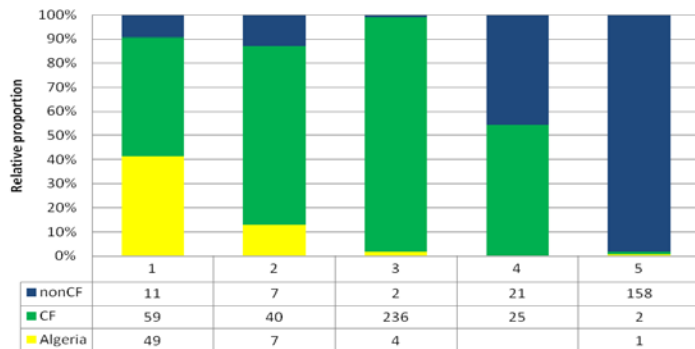
## Staphylococcus aureus et mucovicosose

- 622 isolats
- 5 clusters
- Cluster associations
  - Origine Geographical
    - Algérie, ST80 (C1)
    - Marseille (C2-C5)
  - Origine Clinique
    - Patients non-mucovicosique (C5)
    - Patients mucovicosique
      - C1 and C2 : children
      - C3 and C4 : adults

MSP Dendrogram



Relative proportion of groups in different clusters

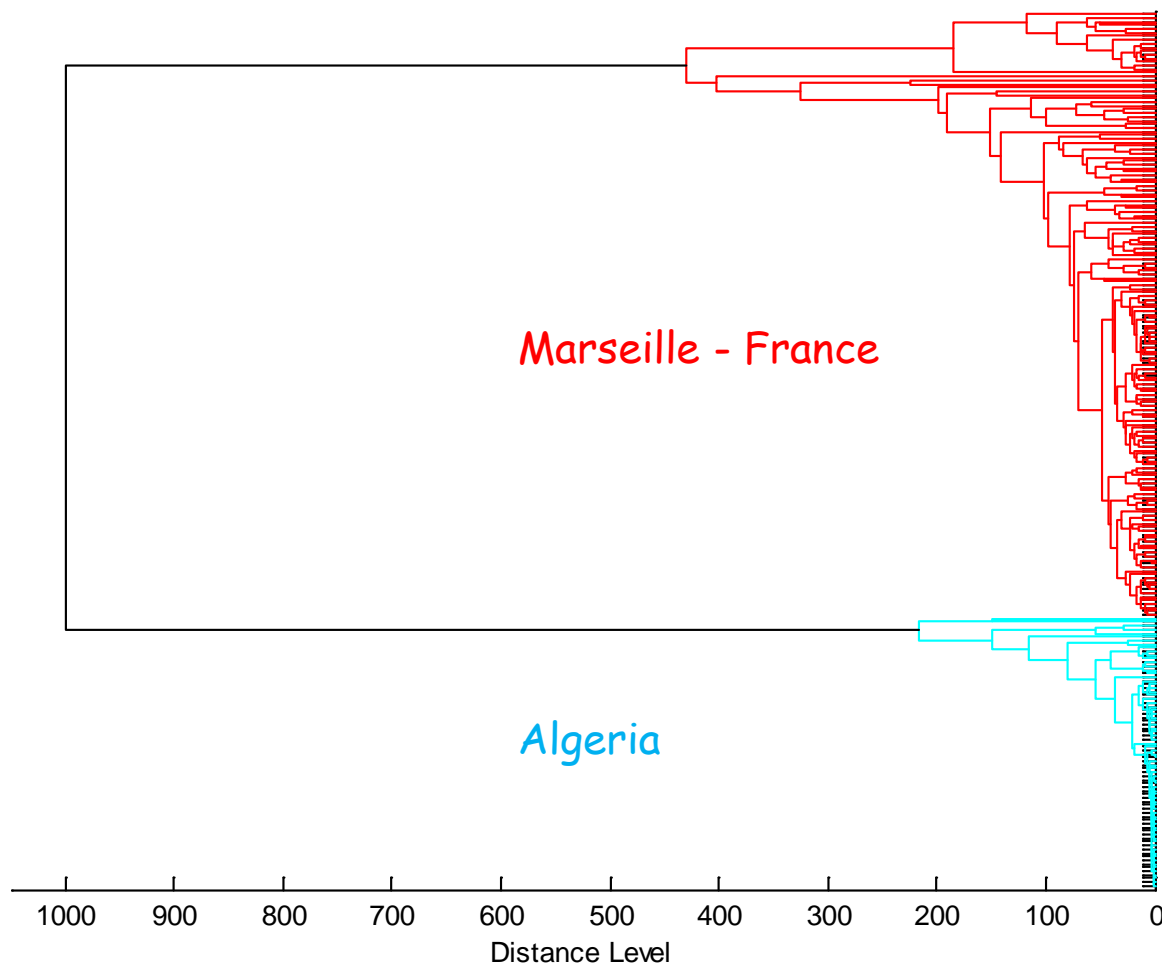


➔ Clusters identification de phénotypes

# Epidémie et origine géographique

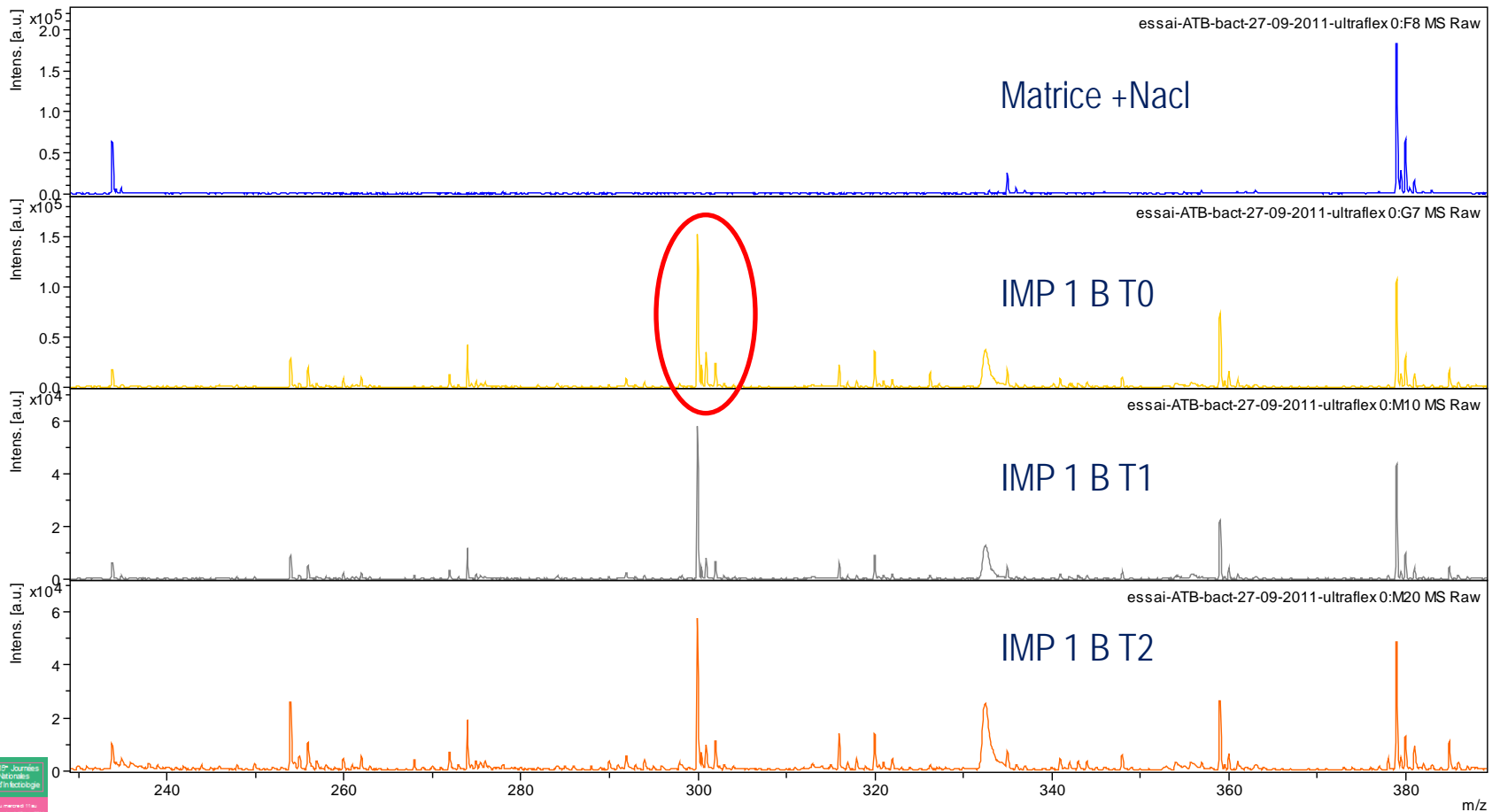
## *Klebsiella pneumoniae*

MSP Dendrogram



# Détection des carbapénémases par MALDI-TOF

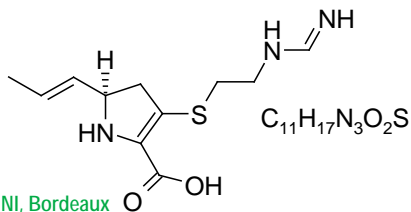
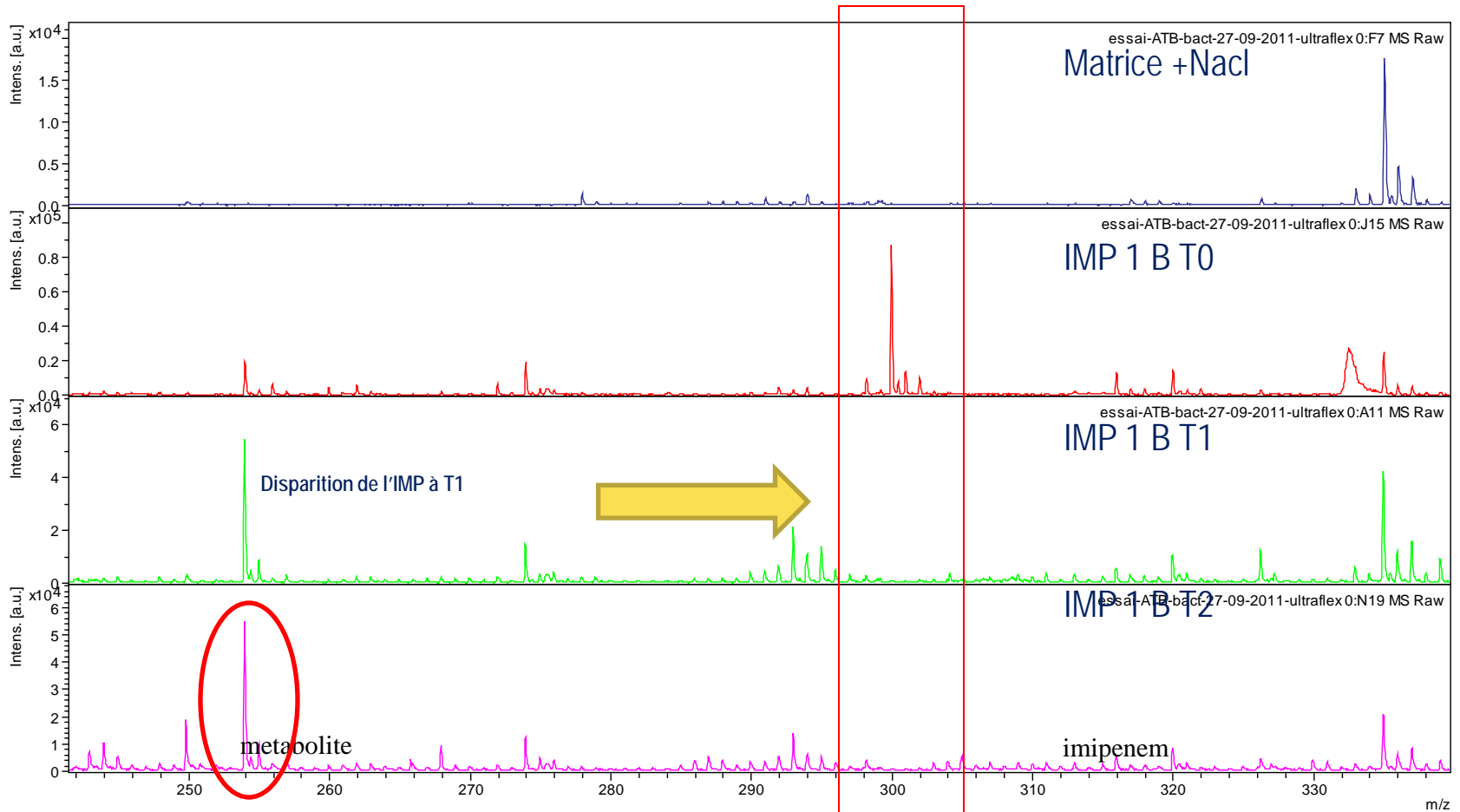
*Klebsiella pneumoniae* Sensible Imipénème – Test Imipénème  
IMP : pic spécifique à 300 m/z



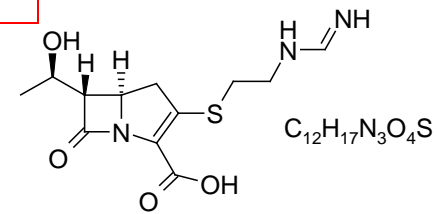
# Détection des carbapénémases par MALDI-TOF

*Klebsiella pneumoniae* NDM-1 Positive – Test Impénème

IMP : pic spécifique à 300 m/z

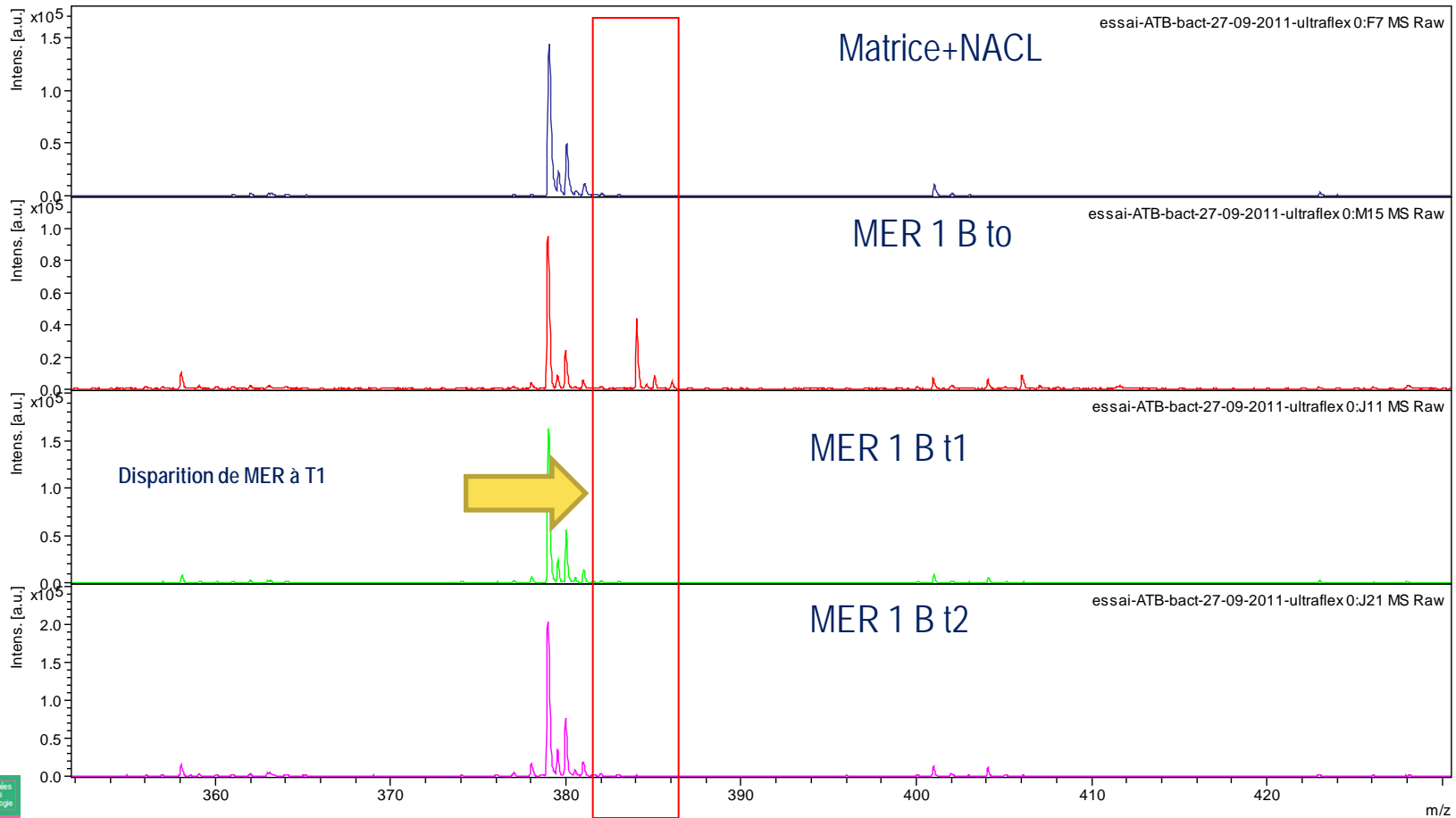


carbapénémase



# Détection des carbapénémases par MALDI-TOF

*Klebsiella pneumoniae* NDM-1 Positive – Test Méropénème  
MER: pic spécifique à 384 m/z





# Détection des carbapénémases par MALDI-TOF

Table 1. Characterization of the 149 bacterial strains analyzed and data summary of imipenem hydrolysis assay utilizing MALDI-TOF MS

Strain type :No. of isolates (Location of isolates [ No.of isolates])	MALDI-TOF analysis			
	Range of the ratio of the area of imipenem/metabolite based on the time of incubation		No. of isolates detected as carbapenemase producers based on the time of incubation	
	2h	4h	2h	4h
<b>Carbapenem-resistant strains (70)</b> (imipenem MIC >8 mg/L)	<0.01-1.77	<0.01-0.26	67	70
<i>K. pneumoniae</i> KPC :1	<0.01	<0.01	1	1
<i>K. pneumoniae</i> NDM-1: 2	<0.01	<0.01	2	2
<i>P. aeruginosa</i> VIM : 2	<0.01	ND	2	2
<i>P. aeruginosa</i> IMP : 2	<0.01	ND	2	2
<i>A.baumannii</i> bla <sub>OXA23</sub> -like : 57 (Marseille [17], Algeria [40])	<0.01-1.77	<0.01-0.39	54	57
<i>A.baumannii</i> bla <sub>OXA24</sub> -like : 3 (Algeria)	<0.01	0.02-0.04	3	3
<i>A.baumannii</i> bla <sub>OXA23</sub> -like + bla <sub>OXA24</sub> -like : 3 (Algeria)	<0.01-0.48	<0.01-0.26	3	3
<b>Carbapenem-susceptible strains (79)</b> (imipenem MIC ≤2 mg/L)	<0.01-12.86	<0.01 -14.84	0	0
<i>K. pneumoniae</i> ESBL : 31 (Algeria)	ND	0.64-14.84	0	0
<i>K. pneumoniae</i> non ESBL : 4 (Algeria)	ND	1.24-5.82	0	0
<i>Escherichia coli</i> ATCC 25922 (1)	1.64	1.17	0	0
<i>A. baumannii</i> : 43 (Marseille [1], Algeria [42])	0.61-12.86	0.76 -3.26	0	0

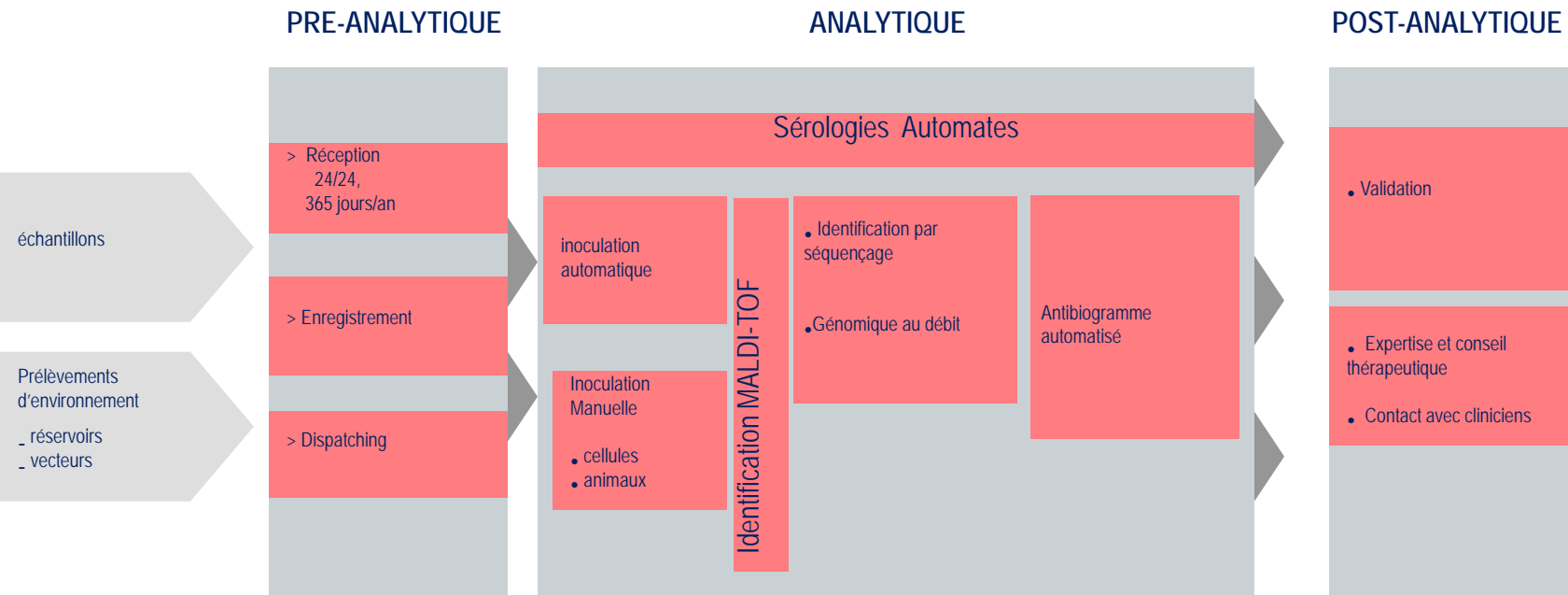
Sensitivity = 100% ; Specificity = 100% (t=4h)

# Diagnostic rapide en microbiologie médicale un véritable challenge

- Les tests microbiologiques améliorent la prise en charge des malades si les résultats sont disponibles dans le temps du soin.

# Organisation du laboratoire central en microbiologie

Regroupement géographique des laboratoires sur un seul site



Limites des laboratoires cœur: résultats retardés dans les tests de routine

**Création de laboratoires secondaire d'urgence**

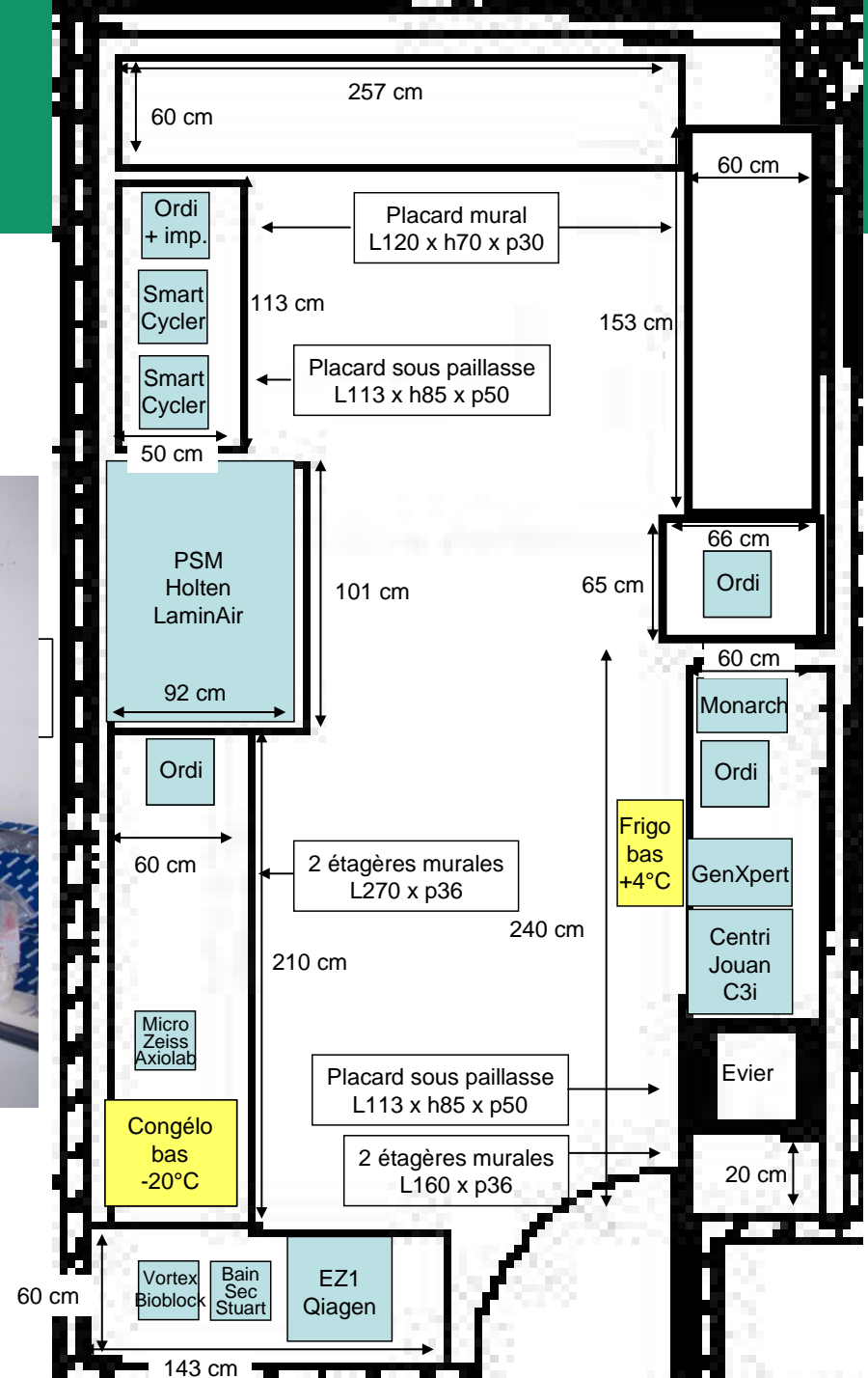


15<sup>es</sup> JN1, Bordeaux  
du 11 au 13 juin 2014

# Objectif: laboratoire d'urgence

- Prise en charge médicale rapide des maladies infectieuses en fournissant les résultats des analyses microbiologiques pendant que les malades sont encore en service d'urgence => Résultats  $\leq$  4 heures
- Afin de répondre à 4 questions cruciales
- 1 – Hospitalisation nécessaire ?
- 2 – isolement requis ?
- 3 –traitement anti-infectieux nécessaire ?
- 4 –analyses complémentaires nécessaires ?

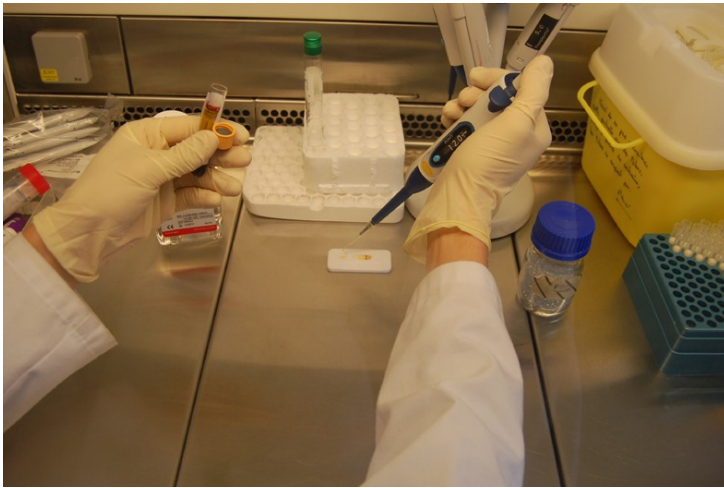
# Point of care à l'hôpital de la Timone/ et hôpital Nord



# Critères de sélection des analyses au POC

- Résultats en moins de 4 heures, transmission SMS
- bonne valeur prédictive
- dépistage par syndrome
- Résultats modifient la prise en charge

## Immuno-chromatographie



## PCR en temps réel





# Exploration par syndrome

PNEU

## Pneumonia

- Flu ICT
- VRS ICT
- **Mycoplasma pneumoniae PCR**
- **Whooping-cough (B. pertussis) PCR**
- **Coxiella burnetii PCR**
- **Staphylococcus aureus (ICU only) PCR**
- **Pneumocystis jiroveci PCR**
- Legionella urinary antigen
- Pneumococcal urinary antigen

MENIN

## Meningitis

- Cytology
- **Enterovirus PCR**
- **Herpes virus PCR**
- **Varicella zoster virus PCR**
- **Meningococcus PCR**
- **Pneumococcus PCR**
- Cryptococcus

GYNECO

## Gynecology

- HIV ICT
- **Group B streptococcus PCR**
- **Atopobium vaginae PCR**

GASTRO

## Gastro-enteritis, diarrhoea

- Rotavirus – adenovirus ICT
- Norovirus ICT
- Clostridium difficile - Helicobacter pylori ICT

ANG

## Pharyngitis

- Group A streptococcus ICT
- Infectious mononucleosis MNI test

Tropical fever

## Tropical fever

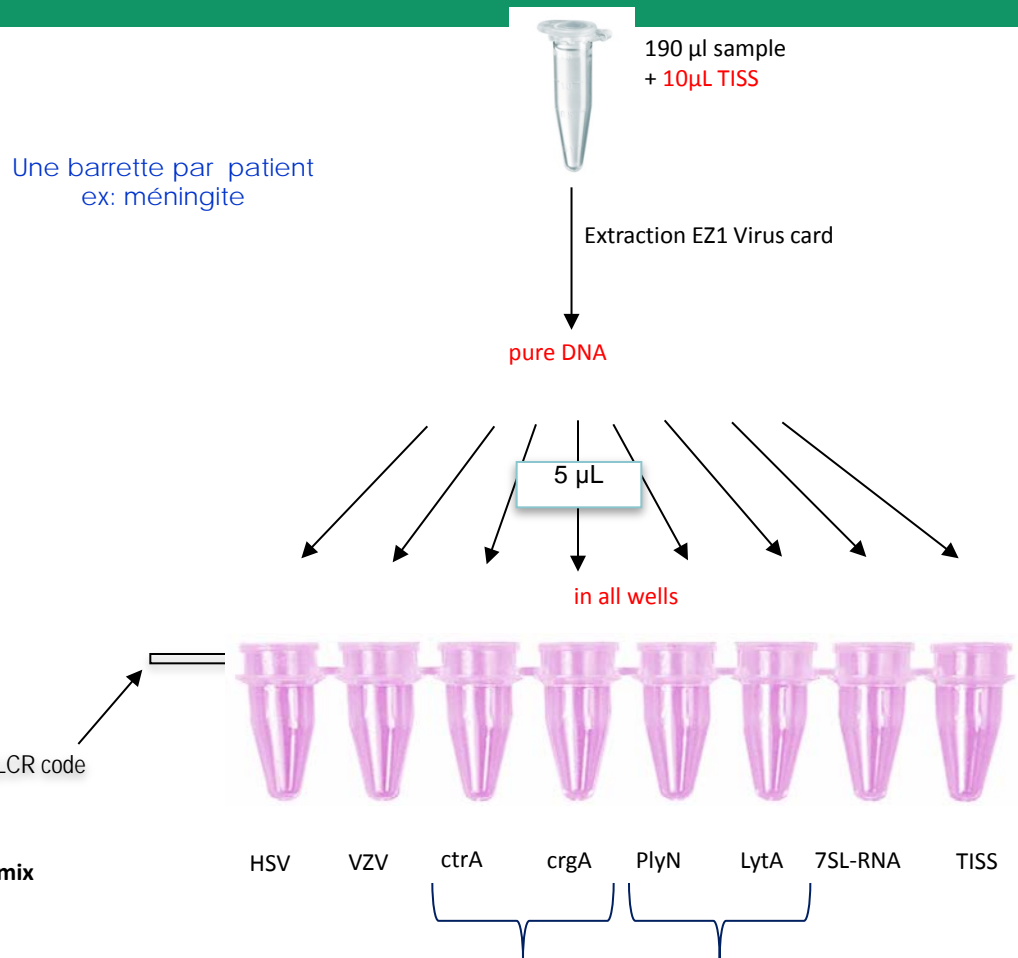
- Malaria PCR
- Malaria ICT
- Dengue ICT

Other

## Others

- Blood exposure accident = HIV
- Tetanus toxin ICT
- Procalcitonin

# Real-time PCR assays: syndrome-specific strips



Strip 2

CONTRÔLE NEGATIF - rien à rajouter – prêt à l'emploi

Strip 3

CONTRÔLE POSITIF- Rien à rajouter – prêt à l'emploi

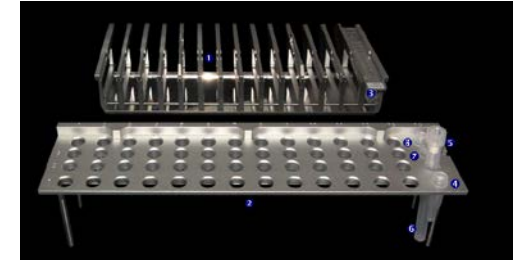
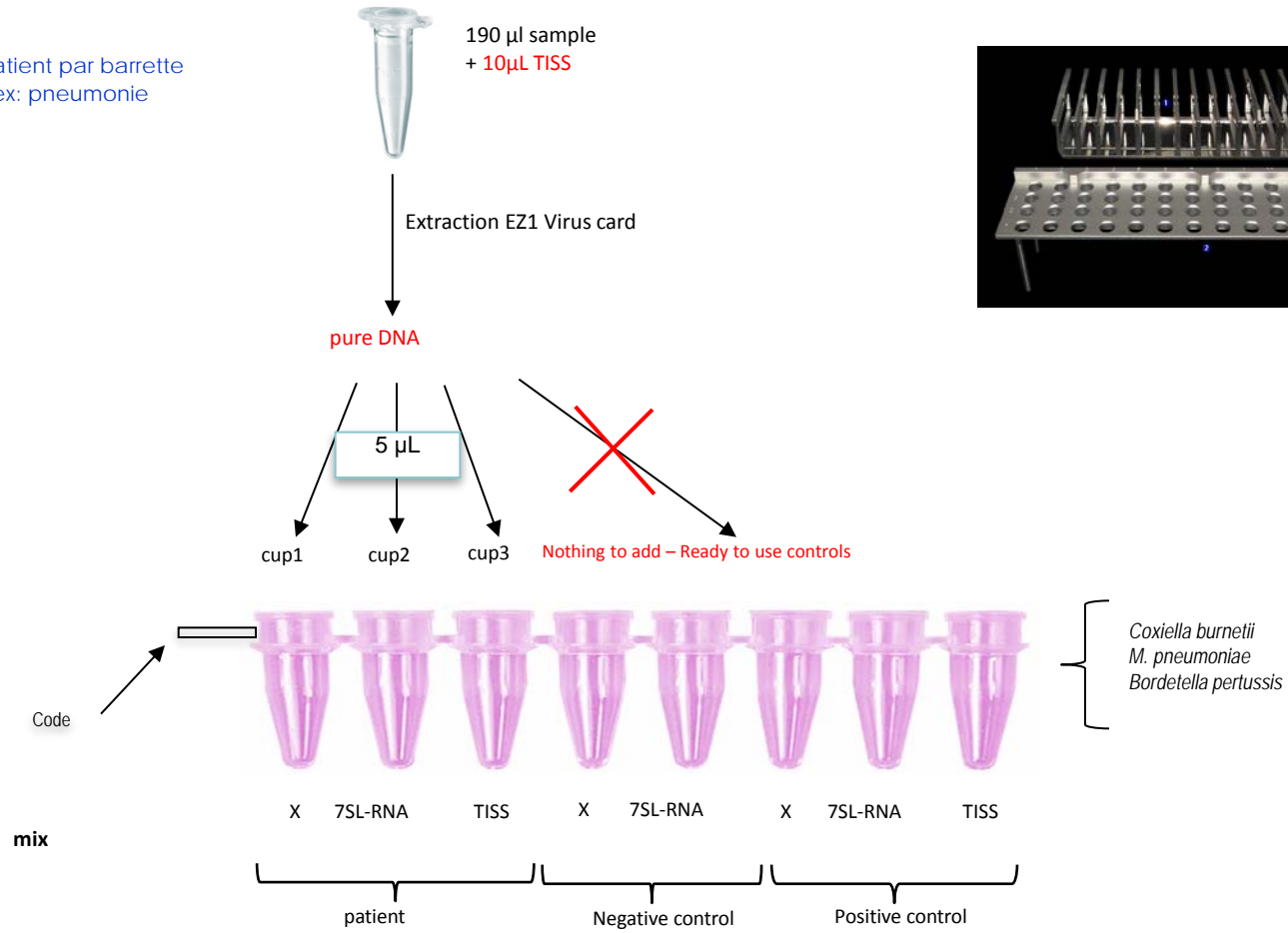


15<sup>es</sup> JN, Bordeaux  
du 11 au 13 juin 2014



# Real-time PCR assays: syndrome-specific multiplexed strips

1 patient par barrette  
ex: pneumonie



# P.O.C: PCR en temps réel



## Xpert® Flu

Detection of Flu virus de type A and B, and H1N1 2009

Preparation : 2 min Result : 77 min

## Xpert® EV

Molecular detection of enterovirus meningitis

Preparation : 5 min Result : 150 min

## Xpert® *C. difficile*

Detection of toxigenic et epidemic strains of *Clostridium difficile*

Preparation : 2 min Result : 47 min

## Xpert® GBS

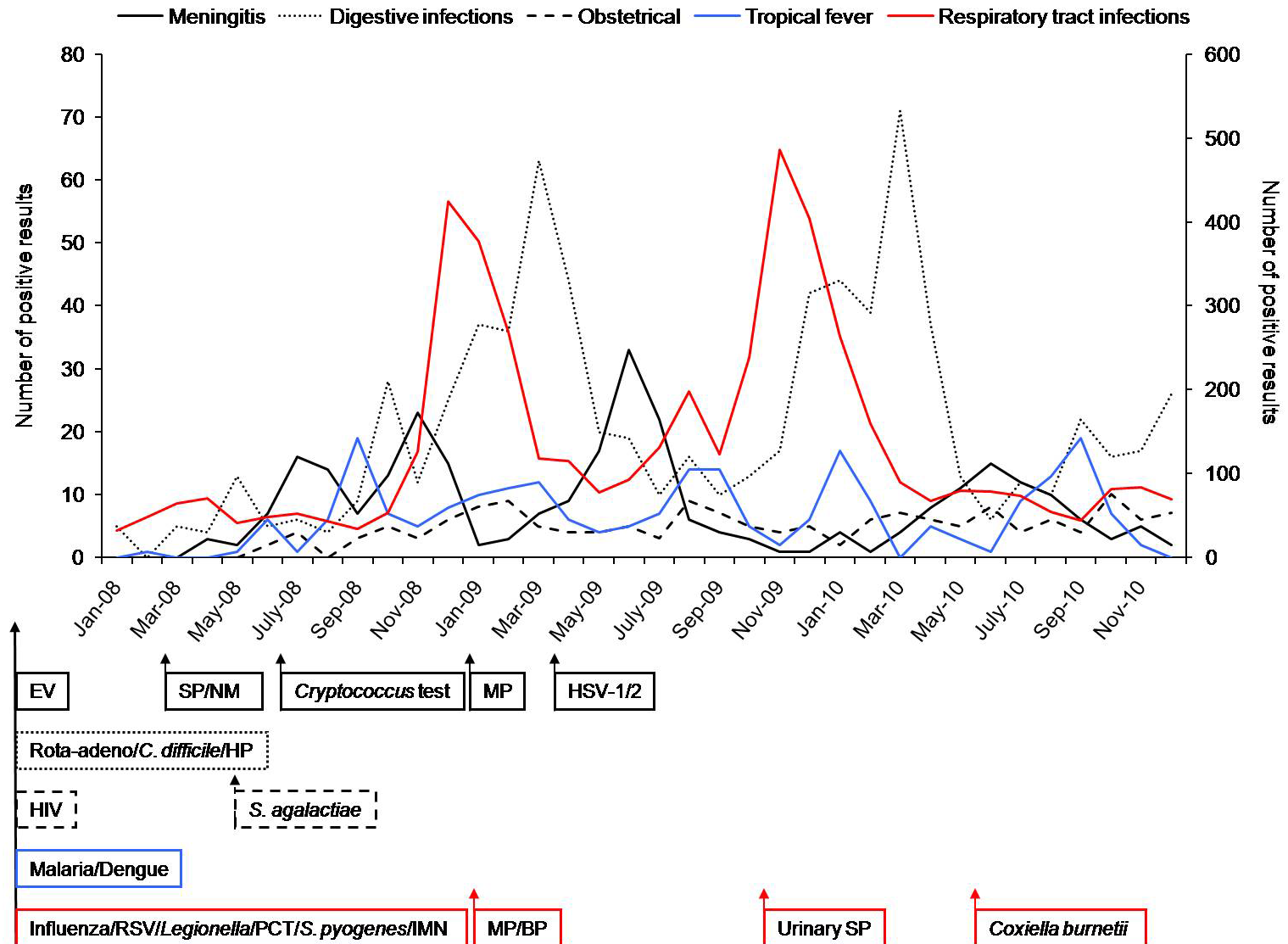
Intrapartum detection of *Streptococcus group B*

Preparation : 1 min Result : 52 min

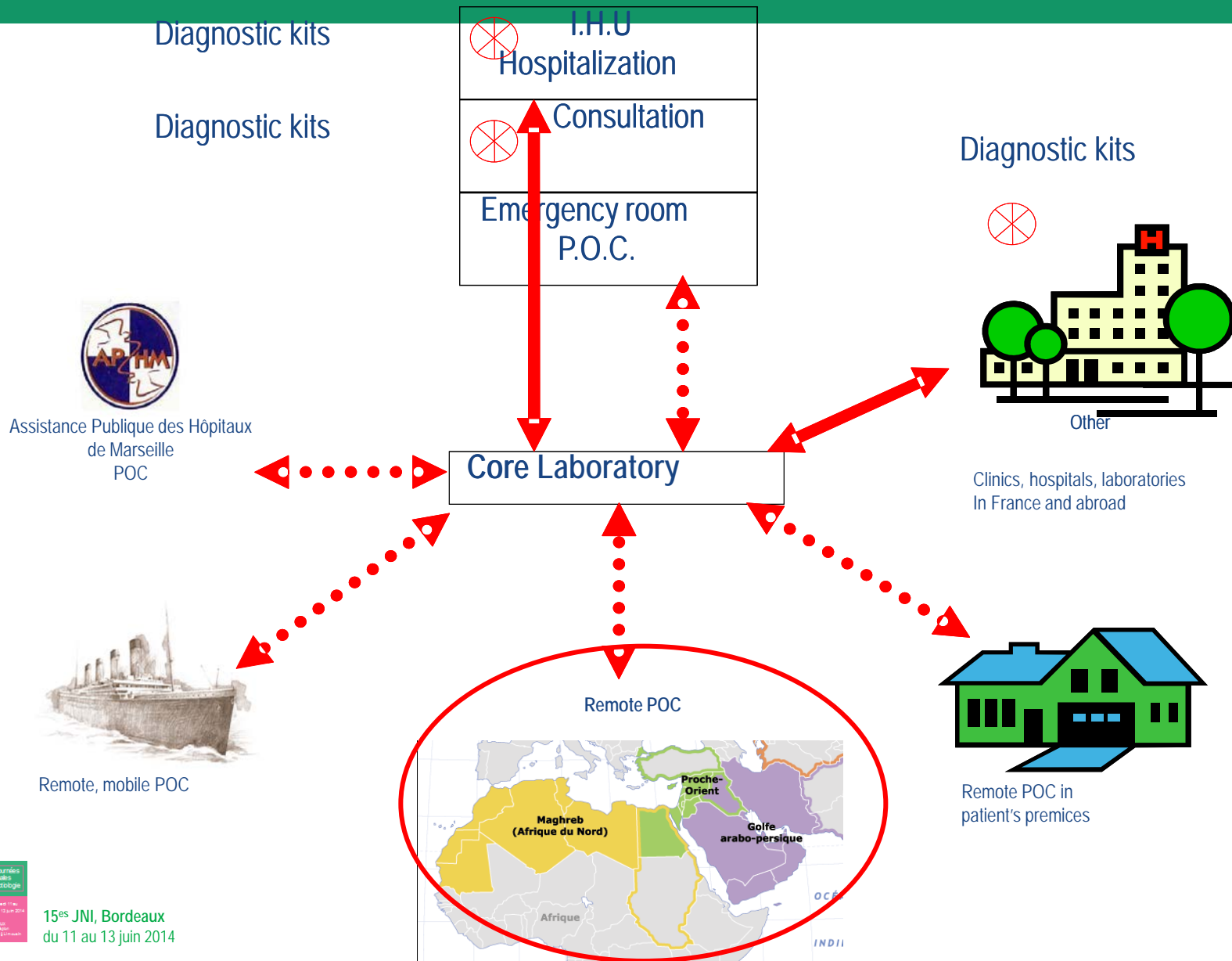
# Activité exponentielle

	2008			2009			2010		
	Test	Diagnostics	%	Test	Diagnostics	%	Test	Diagnostics	%
influenza virus	1390	102	7	12455	1489	12	2551	25	1
RSV	1537	378	25	3300	297	9	2171	451	21
<i>S. pneumoniae</i>				67	12	18	967	63	7
<i>L. pneumophila</i>	168	1	1	405	4	1	954	9	1
<i>M. pneumoniae</i>				668	25	4	1184	47	4
<i>C. burnetii</i>							244	0	0
<i>B. pertussis</i>				561	20	4	761	10	1
Procalcitonin	1169	206	18	1090	169	16	774	104	13
<i>S. pyogenes</i>	1817	339	19	2912	567	19	2210	420	19
EBV	82	9	11	143	13	9	146	16	11
EV	296	84	28	482	92	19	416	58	14
HSV-1/2	3	0	0	114	3	3	251	3	1
<i>S. pneumoniae</i>	267	10	4	479	10	2	410	14	3
<i>N. meningitidis</i>	272	6	2	480	3	1	403	6	1
<i>M. pneumoniae</i>				38	1	3	144	0	0
Rotavirus/adeno	537	105	20	1075	313	29	1221	295	24
<i>C. difficile</i>	352	7	2	518	10	2	535	17	3
<i>H. pylori</i>	33	4	12	49	4	8	60	1	2
<i>S. agalactiae</i>	149	21	14	352	64	18	417	66	16
HIV	199	2	1	401	4	1	269	5	2
Plasmodium spp	277	50	18	367	90	25	382	83	22
Dengue virus	45	4	9	66	6	9	15	2	13
<i>C. tetani</i>				16	8	50	21	18	86
<b>Total</b>	<b>8593</b>	<b>1328</b>	<b>15</b>	<b>26038</b>	<b>3204</b>	<b>12</b>	<b>16506</b>	<b>1713</b>	<b>10</b>

# Activité exponentielle



# Perspectives: POC délocalisé, Afrique



# Approche syndromique du diagnostic en microbiologie médicale

Place des nouveaux outils moléculaires

# Approche syndromique : kit péricardique

## Serological test

*Toxoplasma gondii*  
*Human immunodeficiency virus*  
*Cytomegalovirus*  
*Parvovirus B19*  
*Ebstein barr virus*  
*Hepatitis B, C virus*  
*Borrelia burgdorferi*  
*Mycoplasma pneumoniae*  
*Chlamydia spp.*  
*Brucella melitensis*  
*Legionella pneumophila*  
*Coxiella burnetii*  
*Bartonella quintana - B. henselae*  
*Rickettsia conorii - R. typhi*

## PCR on nasal swab

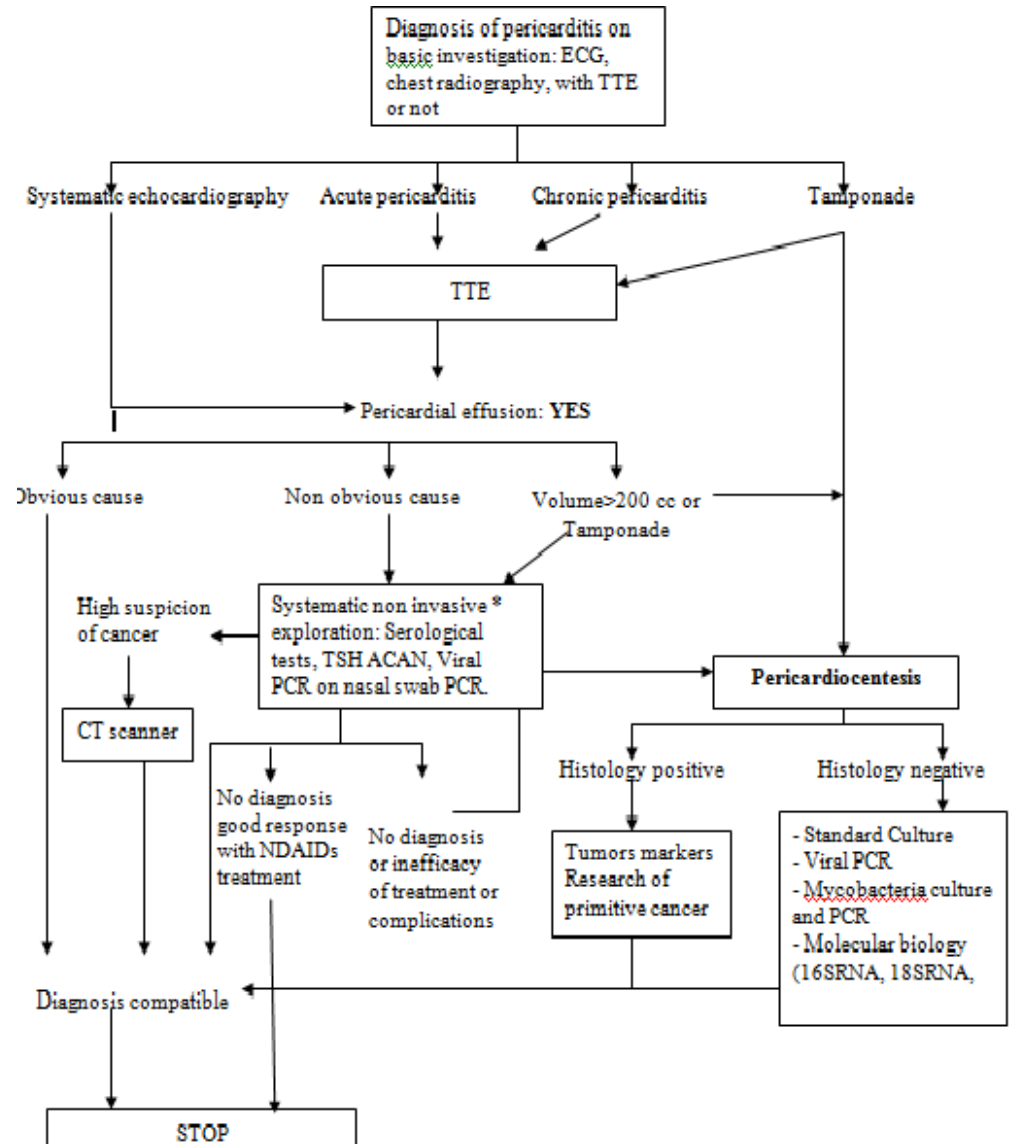
*Enterovirus*  
*Adenovirus*  
*Influenza virus*

## Non infectious tests

Thyroid Stimulating Hormone  
 Antinuclear antibodies

## Cultures

Cell cultures  
 Blood culture



# Application métagénomique

## Samples, diagnosis and metagenomic results.

Sample	Age <sup>a</sup>	Sex	Metavir identifier <sup>b</sup>	Diagnosis	Pathological agent (hospital diagnostic tests)	Most abundant viral type in viral metagenomes <sup>c</sup>
P1	34	M	LPC_P1	Idiopathic pericarditis	-	<i>Anelloviridae</i> (57.04%) <i>Retroviridae</i> (35.78%) Bacteriophages (4.74%)
P2	73	F	LPC_P2	Idiopathic pericarditis	-	<i>Anelloviridae</i> (68.76%) <i>Retroviridae</i> (29.68%)
P3	81	M	LPC_P3	Idiopathic pericarditis	-	Bacteriophages (33.69%) <i>Anelloviridae</i> (26.05%)
P4	6 months	M	LPC_P4	Sudden infant death	Polymicrobial infection	Bacteriophages (49.32%) <i>Retroviridae</i> (31.07%)
P5	66	M	LPC_P5	Idiopathic pericarditis	-	<i>Retroviridae</i> (90.51%)
P6	43	F	LPC_P6	Idiopathic pericarditis	-	<i>Anelloviridae</i> (60.23%) <i>Retroviridae</i> (28.58%)
P7	64	M	LPC_P7	Idiopathic pericarditis	-	<i>Anelloviridae</i> (98.59%)
P8	88	M	LPC_P8	Idiopathic pericarditis	-	<i>Retroviridae</i> (90.9%)

Detection : Torque teno virus et papillomavirus dans le liquide péricardique pour la 1ere fois chez l'homme

[PLoS One](#). 2014 Apr 1;9(4):e93367. 2014.

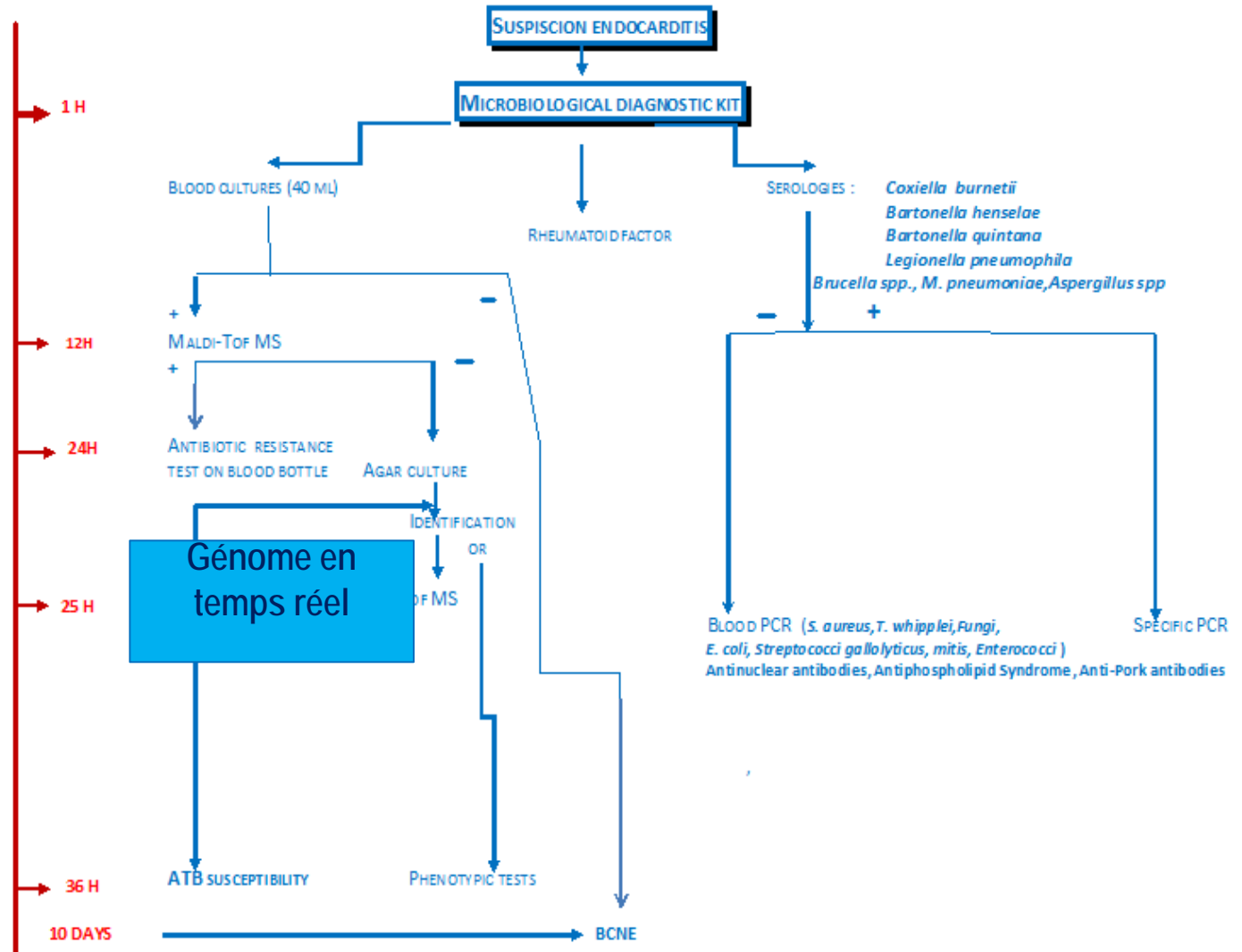
**Viral communities associated with human pericardial fluids in idiopathic pericarditis.**

[Fancello L](#), [Monteil S](#), [Popgeorgiev N](#), [Rivet R](#), [Gouriet F](#), [Fournier PE](#), [Raoult D](#), [Desnues C](#).





# Approche syndromique : ex Kit endocardite



# Application : génome en temps réel

- Génome en temps réel de *S.epidermidis*

*Staphylococcus epidermidis* souche isolée de 3 hémocultures sur 3 chez responsable d'une endocardite aortique chez un homme de 26 ans  
génomme de 2.5-Mb :sequence unique avec 32 genes de virulence.  
CSUR P278

[J Clin Microbiol.](#) 2013 May;51(5):1617-21.

Deciphering genomic virulence traits of a *Staphylococcus epidermidis* strain causing native-valve endocarditis.

[Fournier PE](#), [Gouriet F](#), [Gimenez G](#), [Robert C](#), [Raoult D](#).

# remerciements

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Merci pour votre attention