



JNI
Tours
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Quoi de neuf en Biologie Moléculaire?

Chloé PLOUZEAU

Laboratoire de Bactériologie CHU de POITIERS



Quelles attentes?

Fiabilité

Facilité technique et Rapidité

Spectre large

Fiabilité et facilité technique

Rapidité

Automatisation

Automates « tout en un »

GeneExpert°
Cepheid



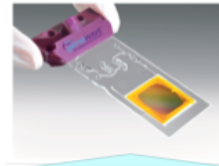
Très rapide
Simplicité+++
Pas de personnel spécialisé
Nombre de cibles limité

Techniques rapides et techniques automatisées

Gene Wave



GeneSpress®
Cartridge



- Simple cartridge design
- On-chip valves
- Direct sample loading
- Robust Assay Design methodology



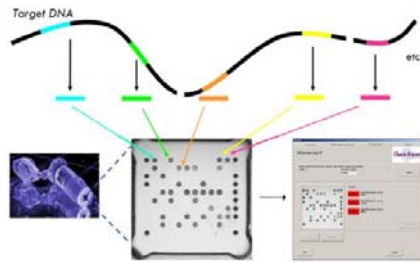
Extraction, amplification et détection/puce
Multiplex jusqu'à 50 cibles
<3 heures

Spectre large

PCR multiplexes T réel
Ou Puces/ Hybridation/ Migration

Données de
résistance et virulence

« Panels moléculaires »
Approche syndromique



bio-centric



Carbapenemases

	Check-MDR CT101 Catalog no. 10-0020	Check-MDR CT102 Catalog no. 10-0019	Check-MDR CT103 Catalog no. 10-0021
NDM	X	X	X
KPC	X	X	X
OXA-48	X	X	X
VIM	X	X	X
IMP		X	X

AmpCs

CMY 140X	X		X
ACC	X		X
DHA	X		X
ACTRMB	X		X
CMY II	X		X
FOX	X		X

ESBLs

CTX-M-1 group	X	X	X
CTX-M-1 like			X
CTX-M-15 like			X
CTX-M-3 like			X
CTX-M-32 like			X
CTX-M-2 group	X	X	X
CTX-M-8 & -25 group	X	X	X
CTX-M-9 group	X	X	X
TEM vt	X	X	X
TEM 104K	X	X	X
TEM 164S	X	X	X
TEM 164C		X	X
TEM 164H	X	X	X
TEM 238S	X	X	X
SHV vt	X	X	X
SHV 238S	X	X	X
SHV 238A	X	X	X
SHV 240K	X	X	X

Panel très complet

Temps technique +++
Techniciens de BM
Pièce dédiée



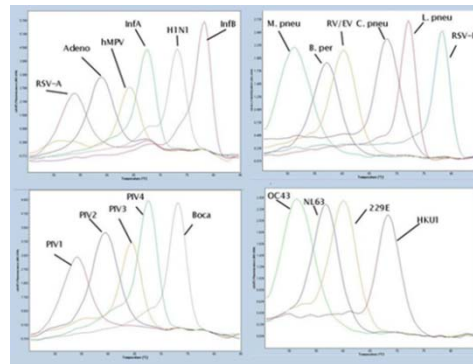
Check point

Spectre large

PCR multiplexes T réel
Ou Puces/ Hybridation/ Migration

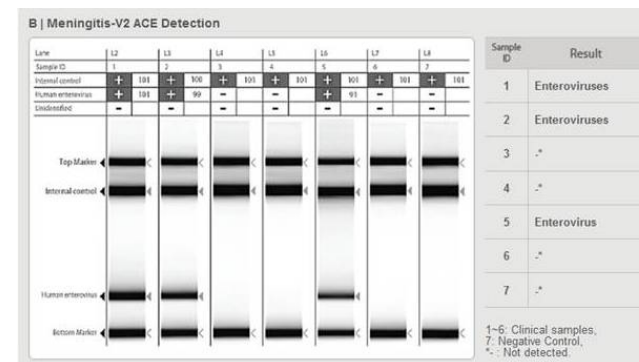
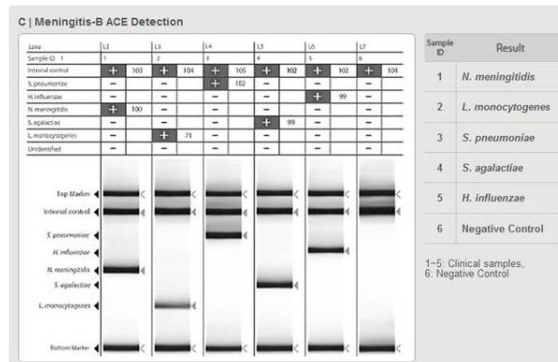
Données de
résistance et virulence

« Panels moléculaires »
Approche syndromique



Respi Finder

Seeplex



CE-IVD

Grand nombre de cibles
Infections mixtes
Temps technique++
personnel BM

Automatisation

Spectre large

Données de
résistance et virulence

Approche syndromique

PCR couplée à la spectrométrie de masse



PLEX-ID

Principe

Mesure de la **composition** en bases
des **Amplicons**
par **Spectrométrie de Masse**



PLEX-ID
Rapid Microbiology

1h30

Abbott
A Promise for Life

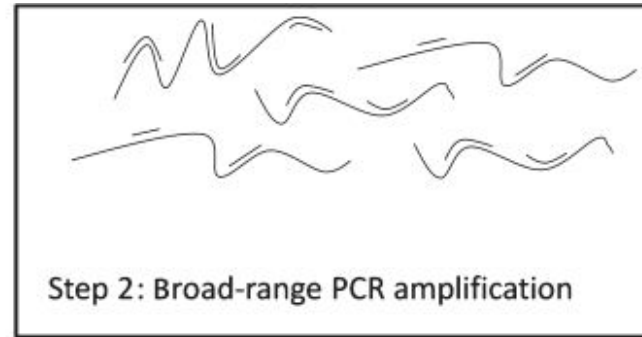
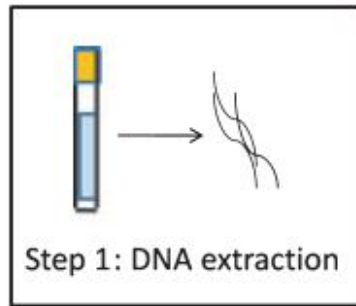
6h

La PCR –ESI-TOF

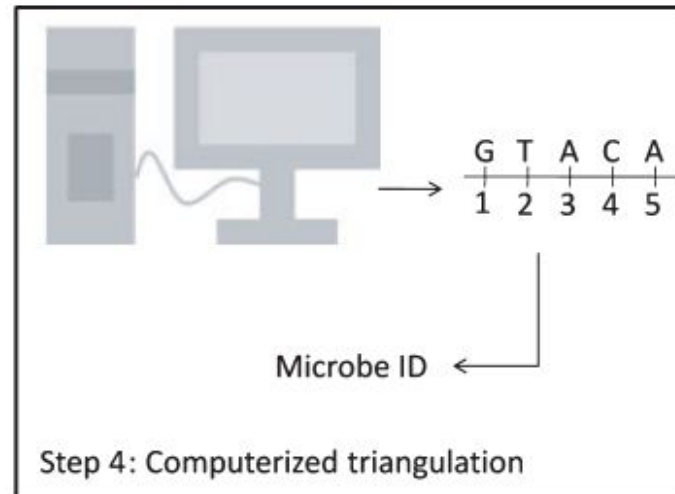
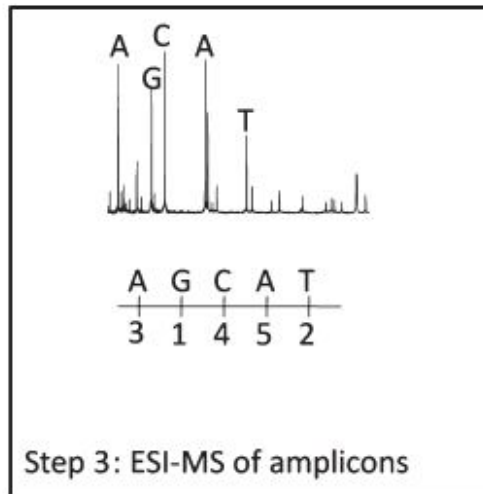
Electro Spray Ionisation

Time of Flight

Tout type de
prélèvement

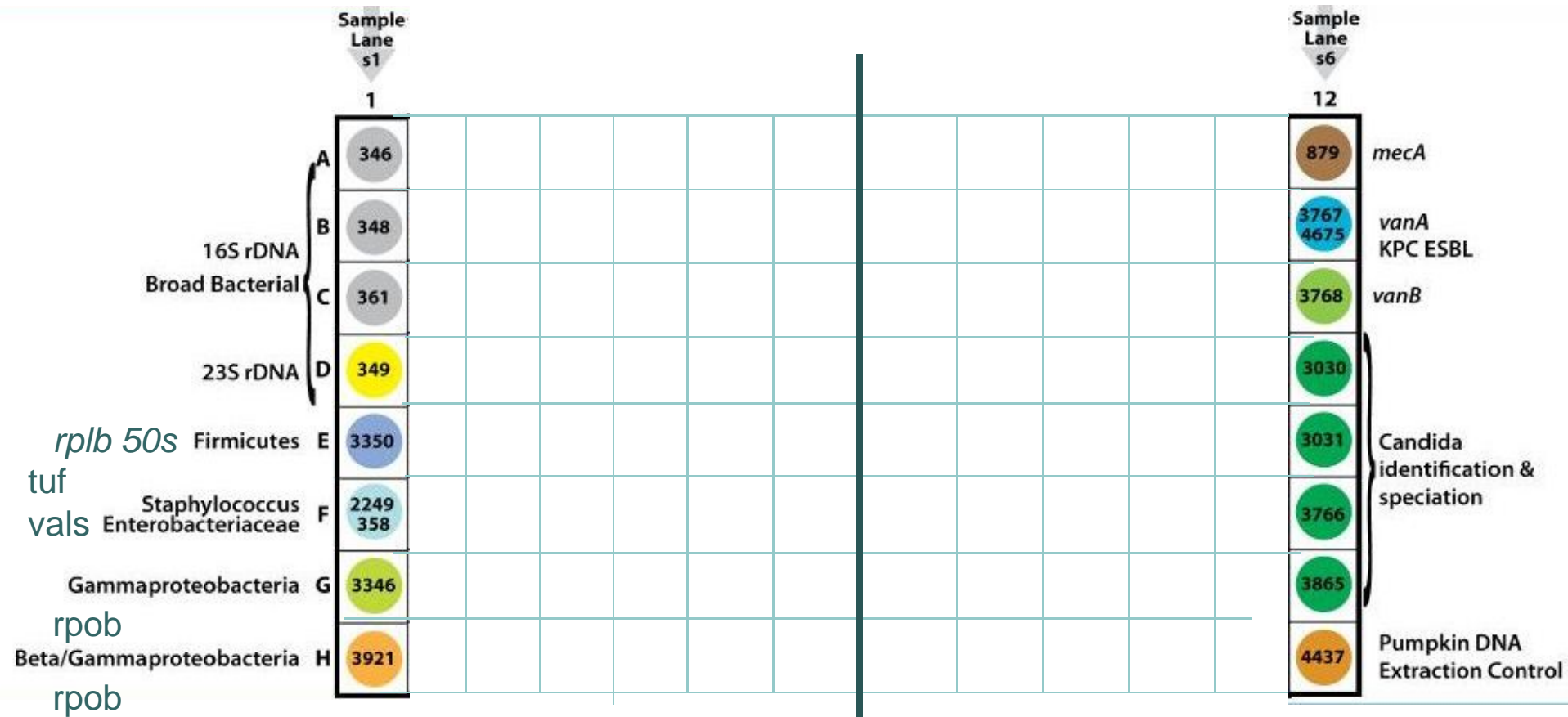


Grand nombre de
cibles



Microbe ID

Kit BAC SPECTRUM



Réactif dans les plaques (sauf RT) : polymerase, primers et calibrant interne (POS et calibrant pour le SM)

16 puits/ échantillons et 6 échantillons/ plaque

PLEX-ID Broad Fungal Assay

- 16 well assay with 16 broad-range primer pairs
- Detects Yeast & Molds
- More than 3500 species in the database
- Unknown detections will be reported and linked to the closest match

<i>LSU rRNA</i> (broad)	A	3030	5181	<i>mtDNA SSU</i> (Ascomycetes)
<i>LSU rRNA</i> (broad)	B	5185	4837	<i>B-tubulin</i> (Fusarium)
<i>LSU rRNA</i> (broad)	C	3766	5178	<i>SSU rRNA</i> (broad)
<i>LSU rRNA</i> (broad)	D	5186	5172	<i>mtDNA cytb</i> (Mucorales)
<i>mtDNA SSU</i> (Candida)	E	3865	5174	<i>SSU rRNA</i> (Mucorales)
<i>mtDNA SSU</i> (Candida)	F	3867	4836	<i>SSU rRNA</i> (misc)
<i>mtDNA SSU</i> (Aspergillus)	G	3862	5187	<i>LSU rRNA</i> (broad)
<i>mtDNA SSU</i> (Cryptococcus)	H	4145	4437	extraction control

PLEX-ID Viral IC / Broad Viral I

Alphaherpesvirus Polyomavirus	A	3398 4849
Betaherpesvirus Adenovirus	B	4100 943
Beta/Gammaherpesvirus Parvovirus	C	3408 3118
Gammaherpesvirus Polyomavirus Adenovirus	D	3407 4843 5155
Gammaherpesvirus Parvovirus	E	3405 3110
Enterovirus	F	3758
	G	3760
Pumpkin EC	H	4437

Polyomavirus BK-Virus*
JC-Virus*

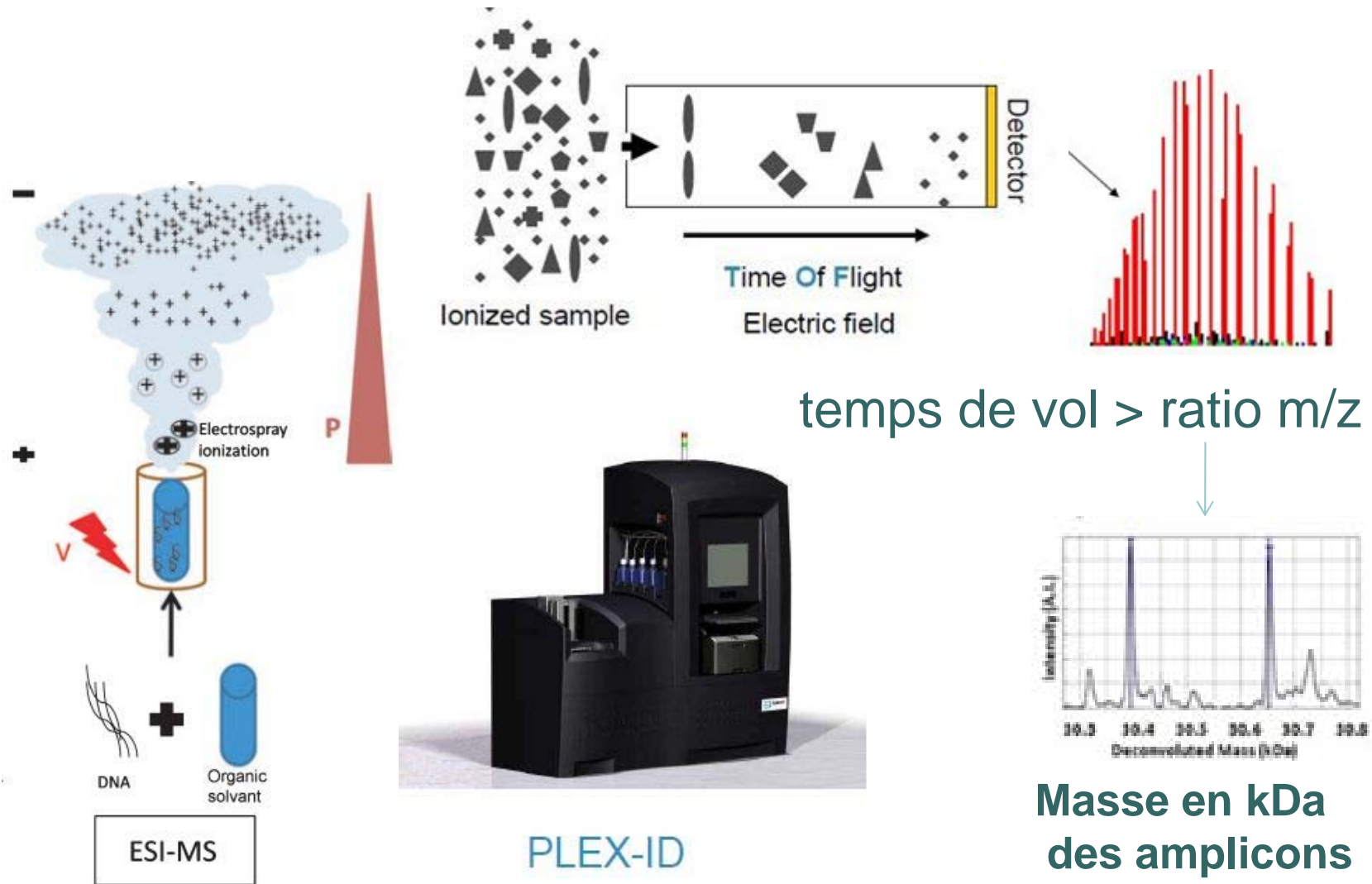
Adenovirus Human Adenovirus*
(2-54, A-F)

Herpesvirus Cytomegalovirus (CMV, HHV-5*)
Epstein-Barr-Virus (EBV, HHV-4*)
Herpes-Simplex-Virus-1 (HSV-1, HHV-1*)
Herpes-Simplex-Virus-2 (HSV-2, HHV-2*)
Kaposi-Sarcoma-Associated-Herpesvirus (KSHV, HHV-8*)
Varicella-Zoster-Virus (VZV, HHV-3*)

Parvovirus Human Erythrovirus V9
Human Erythrovirus VX
Human Parvovirus B19*

Enterovirus* Human Echovirus
Human Enterovirus
Human Rhinovirus

2) Electro-spray-ionisation MS



Dessalage

D'après Emonet et al. CMI 2010

3) Conversion de la masse en composition en acides nucléiques

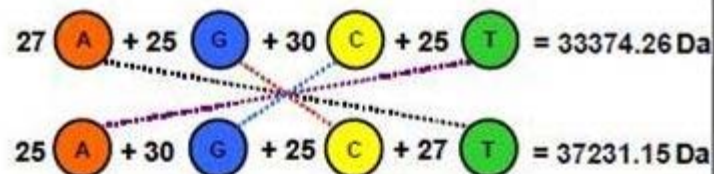
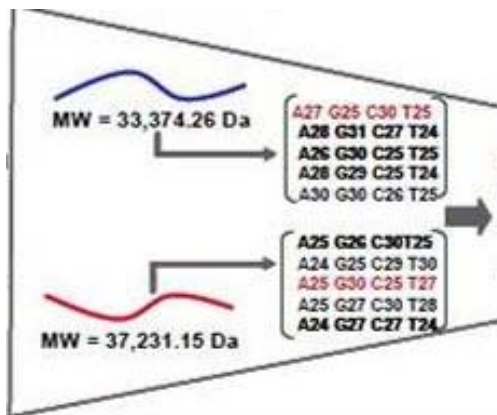
Chaque base= une masse connue



A = 313.0576 amu
G = 329.0526 amu
C = 289.0464 amu
T = 304.0461 amu

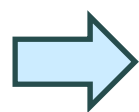
Relation entre masse globale d'un amplicon
Et sa composition en base

Spectro : Masse globale pour chaque brin (sens et antisens)



Plusieurs combinaisons possibles pour chaque brin

Une seule solution pour avoir les deux brins complémentaires



Aw Gx Cy Tz « signature moléculaire »

Comparée à la base de donnée pour chaque amorce

	Organisme	Masse	Base Composition
PCR/Target Region 1	Bacillus anthracis	35278.823	A26 C34 C27 T27
	Escherichia coli	35641.855	A22 G39 C29T25
	Staph aureus	35240.807	A24 G35 C30 T25
PCR/Target Region 2	Bacillus anthracis	35174.799	A25 C32 C30 T27
	Escherichia coli	35870.920	A27 G33 C27 T29
	Staph aureus	35744.918	A29 G29 C30 T28

« Triangulation » recoupement des résultats de toutes les amorces

Sample ID	Type	Code	Assay Name
	Direct Blood	BCARD1	BCA Resistance
	Assay Protocol		Assay Protocol Version
	BCA Assay Test		1

Error:

Flags: Unknown

Analysis Results

Reference Database: 01.3.0.0-A1.20.0.3

CONTROL

Detection	Q.Score	Level
Extraction Control	1	400

BACTERIA

Detected Organism	Q.Score	Level
Klebsiella pneumoniae	1	400
Streptococcus pyogenes	0.94	30

FUNGI

Detected Organism	Q.Score	Level
Not detected		

 GENETIC
RESISTANCE
MARKER

Detected Marker	Result	Q.Score	Level
macA	Not detected	n/a	n/a
vanA	Not detected	n/a	n/a
vanB	Not detected	n/a	n/a
kpc	Positive	1	400

Comment:

Rapport d'identification avec un score (qualité et nbre de primer positif)

« Quantification relative » grâce à un contrôle interne

Accès aux données spectrales et aux compositions moléculaires pour chaque amorce

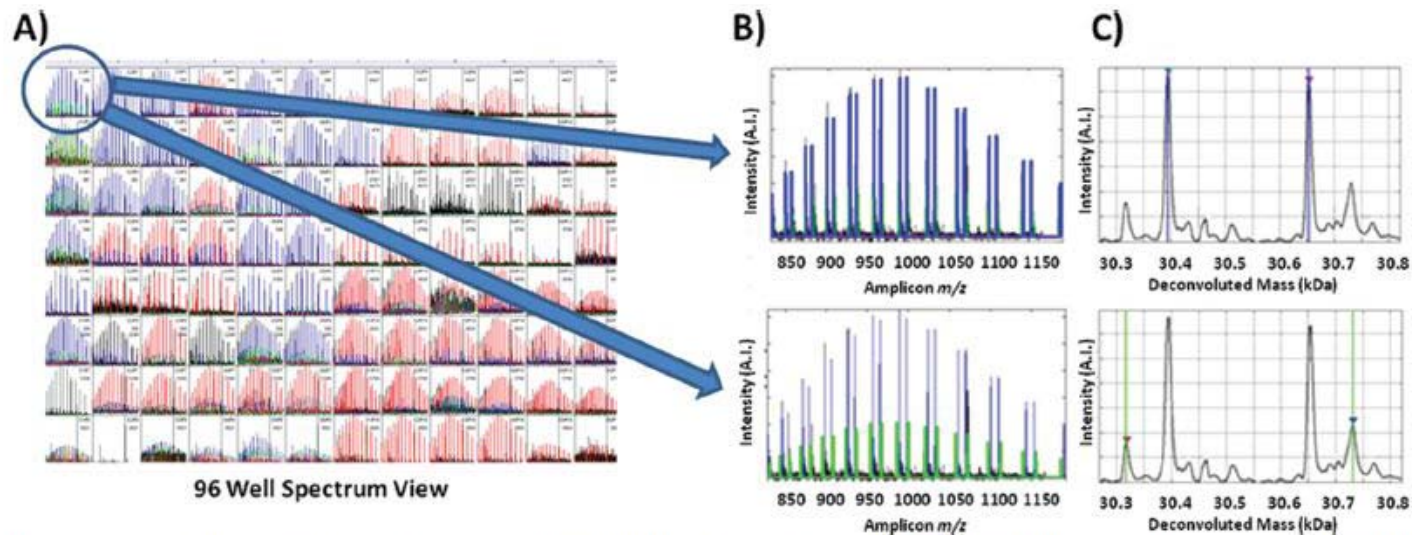


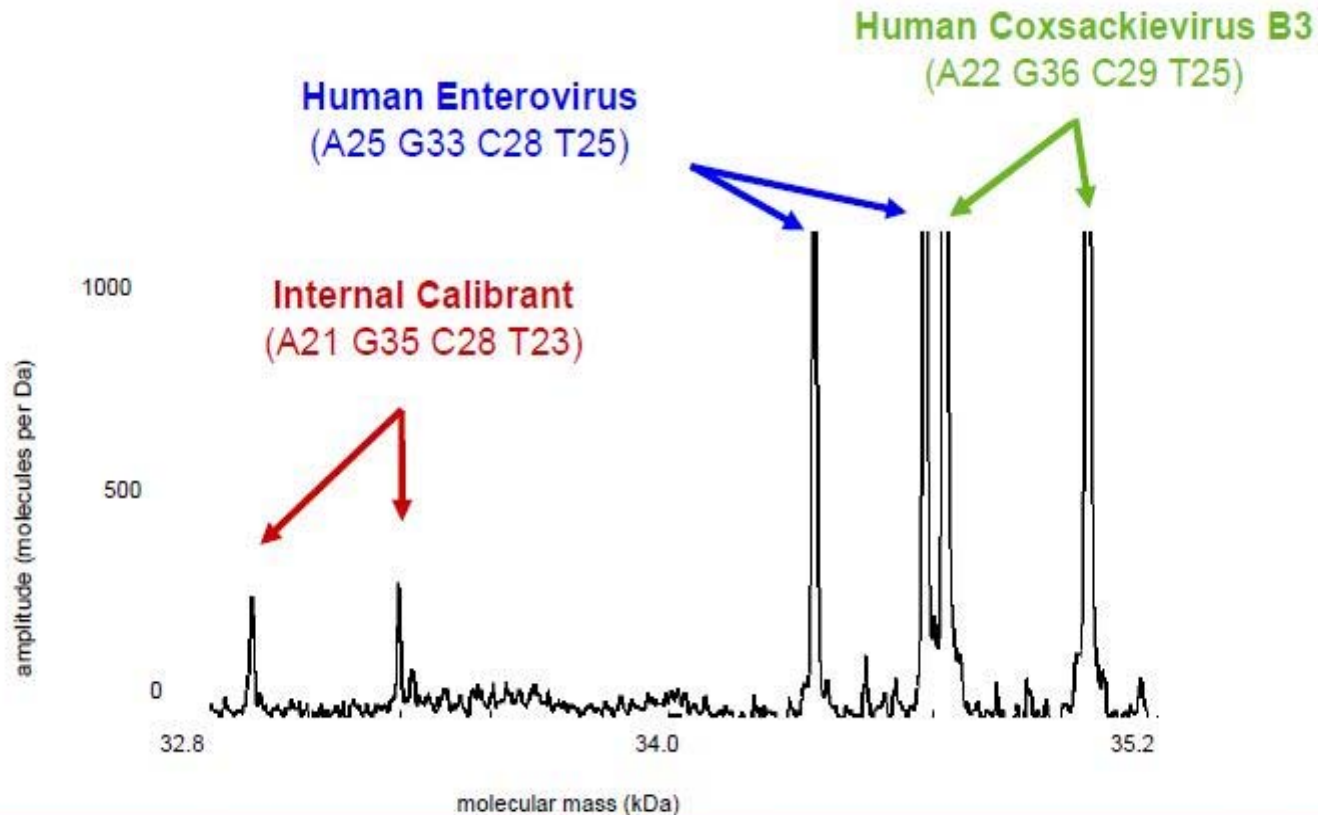
FIG. 1. Determining the base composition from mass spectral data: example using BCB115. (A) The spectra are collected and displayed as a 96-well plate format. Mass spectra can be viewed independently for each of the 96 wells. (B) Electro-spray-generated mass spectra. This sample contains the PCR amplicons from two different organisms: *Bacteroides thetaiotaomicron* (top) and *Staphylococcus aureus* (bottom). (C) Deconvoluted mass spectra showing molecular masses of the forward and reverse strands of each PCR amplicon. An algorithm is used to calculate the base composition from the molecular mass and was calculated to be 30A-27G-23C-19T (top) and 27A-30G-21C-21T (bottom).

Sample No.	Organisms ID	Base Composition									
		16S (346)	16S (348)	16S (361)	23S (349)	rplB (3350)	tufB (367)	valS (358)	rpoB(3346)		
BCB115	<i>Bacteroides thetaiotaomicron</i>	A30 G27 C23 T19	-	A30 G31 C23 T26	A30G29C23T19	-	-	-	A24 G29C27T32		
	<i>Staphylococcus aureus</i>	A27 G30 C21 T21	A30G29C29T30	A29 G30 C25 T24	-	A16 G23 C21 T19	A43 G28C19T35	-	-		
	Primers	rpo B(3921)	mec A	bla _{IPC}	van A / van B	Candida		25S (3030)	25S (3031)	25S (3766)	mito. rDNA (3865)
	<i>Bacteroides thetaiotaomicron</i>	-	-	-	-	-	-	-	-	-	-
	<i>Staphylococcus aureus</i>	-	A22 G11 C21 T21	-	-	-	-	-	-	-	-

FIG. 3. Base composition data obtained from sample BCB115, which contained a mixture of the Gram-negative anaerobe *Bacteroides thetaiotaomicron* and the Gram-positive methicillin-resistant *Staphylococcus aureus*. The composition of the PCR amplicon produced by each primer is shown.

Resolving Mixtures: Enterovirus double detection

Clinical Research Study results obtained at Athogen, Irvine, CA, tissue sample





Conclusions

Intérêt de la PCR-ESI-MS / Spectro masse?

Pas de culture / plus rapide

Virologie

Plus de donnée sur résistance et virulence

A améliorer

Automatisation de la chaîne extraction/PCR

Quantification

Nouveautés Oui Mais à quel prix???

Machine : 500 000 € / maintenance 46 000 €/An

Kits : 50 à 100 € par échantillon (*Viral IC = 75 euros/test*)

Besoin d'études médico-économiques