

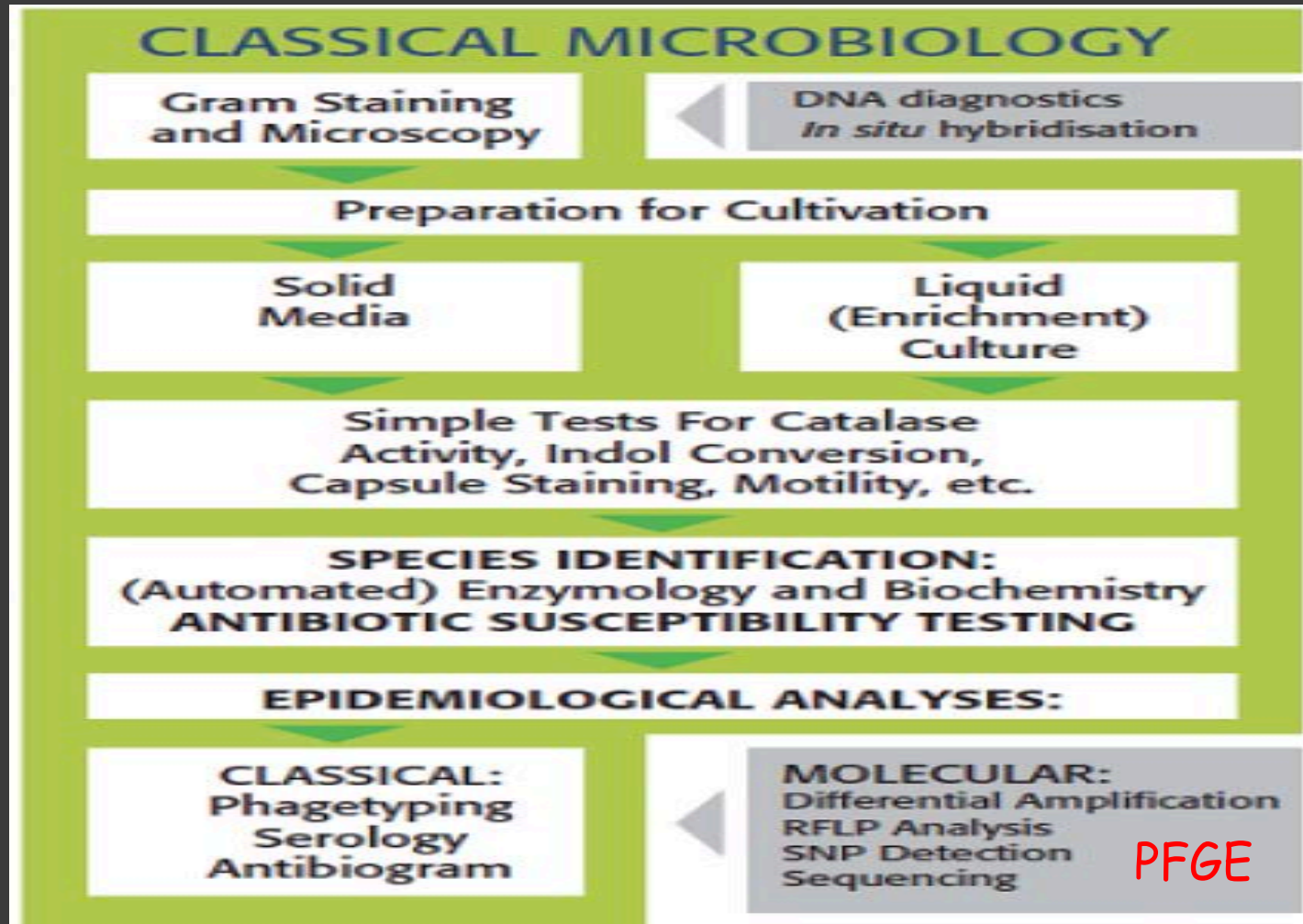
Réunion organisée par le Syndicat des Biologistes (SDB)
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Nouvelles méthodes d'identification bactérienne et d'étude de sensibilité aux antibiotiques

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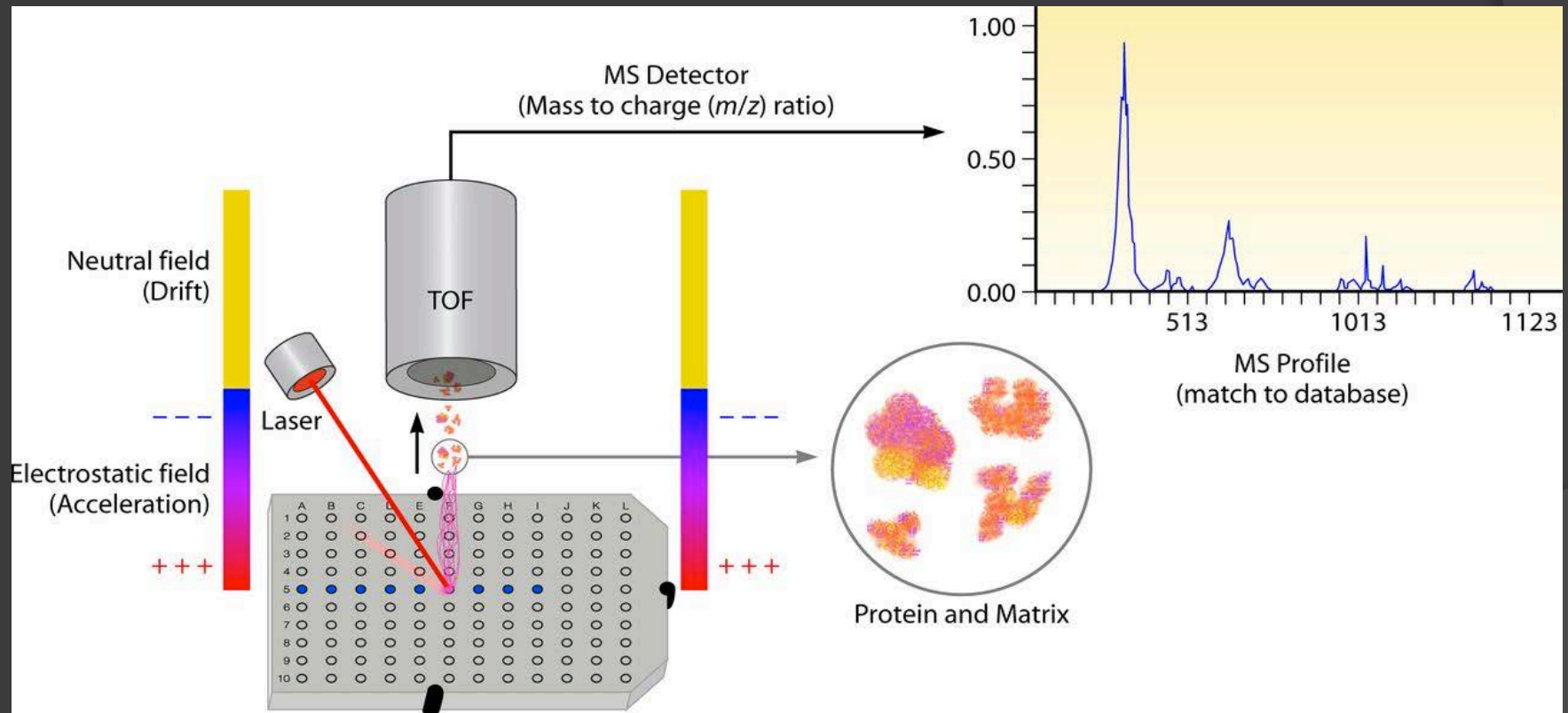
Classical bacteriology



Modern phenotypic approaches for bacteriological diagnosis

MALDI-TOF MS

Matrix-assisted laser desorption/ionization - Time-Of-Flight Mass Spectrometry



Commercial MALDI-TOF MS systems



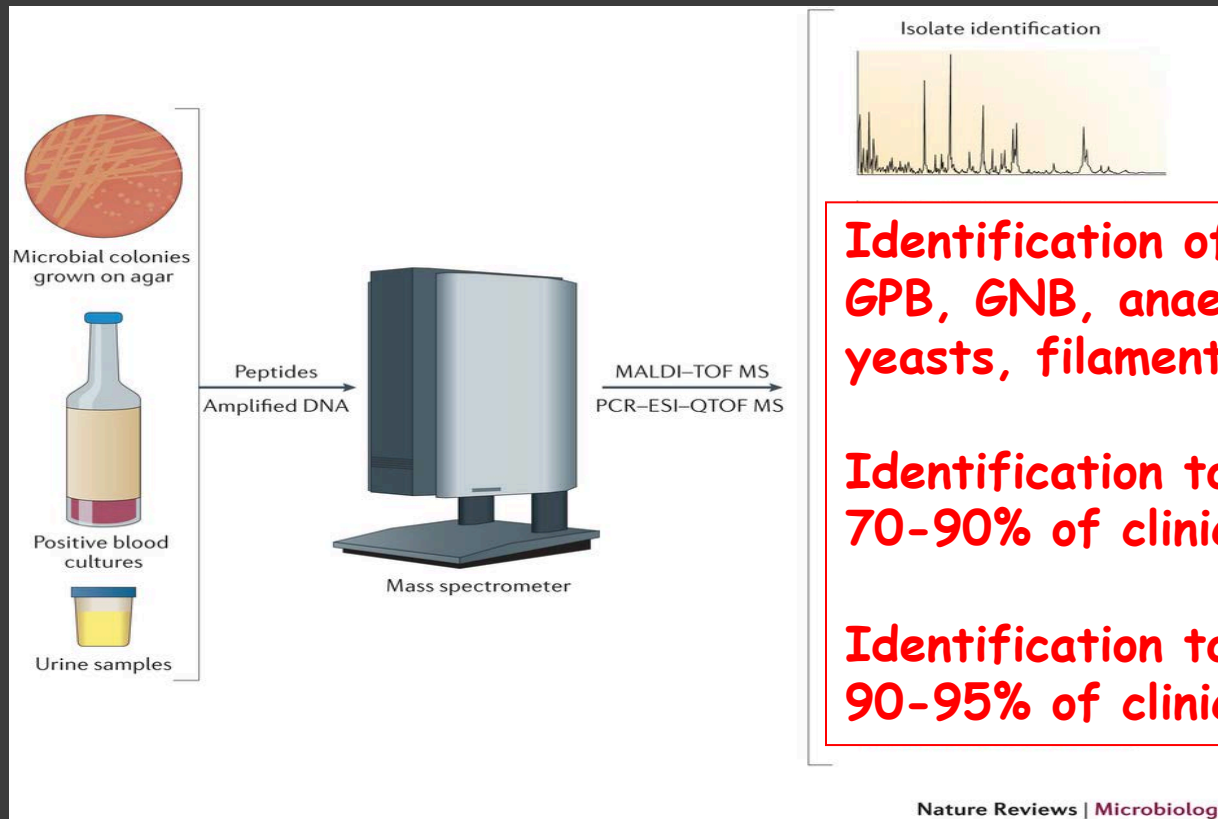
Vitek MS
(bioMérieux)



MALDI Biotyper CA system
(Bruker Daltonics)

Application: Identification

Fast and reliable identification of bacteria and fungi from pure cultures as well as from positive BCs and urines ($>10^5$ CFU/mL)



Identification of :
GPB, GNB, anaerobes, mycobacteria,
yeasts, filamentous fungi

Identification to the species level:
70-90% of clinical isolates

Identification to the genus level:
90-95% of clinical isolates

Nature Reviews | Microbiology

Advantages and limits

Detection method	Advantages	Disadvantages
Conventional; culture on microbiological media and identification by biochemical tests	• Sensitive • Inexpensive	• Lengthy and time consuming process • Might require 24–48 h
MALDI-TOF MS	• Fast (3-5') • Accurate • Less expensive than molecular and immunological-based detection methods (1-2 €) • Trained laboratory personnel not required	• High initial cost of the MALDI-TOF equipment (150 k€)

Some difficulties to identify some bacterial isolates:

- *E. coli* and *Shigella* spp.
- *S. pneumoniae* and *S. mitis/oralis*
- *Corynebacteria*
- *E. cloacae* complex
- Mucoid strains

Clinical impact

Prospective monocentric study (USA) comparing ID using MALDI-TOF MS vs. conventional method in patients with BCs positive for GNB (role of the antimicrobial stewardship+++)

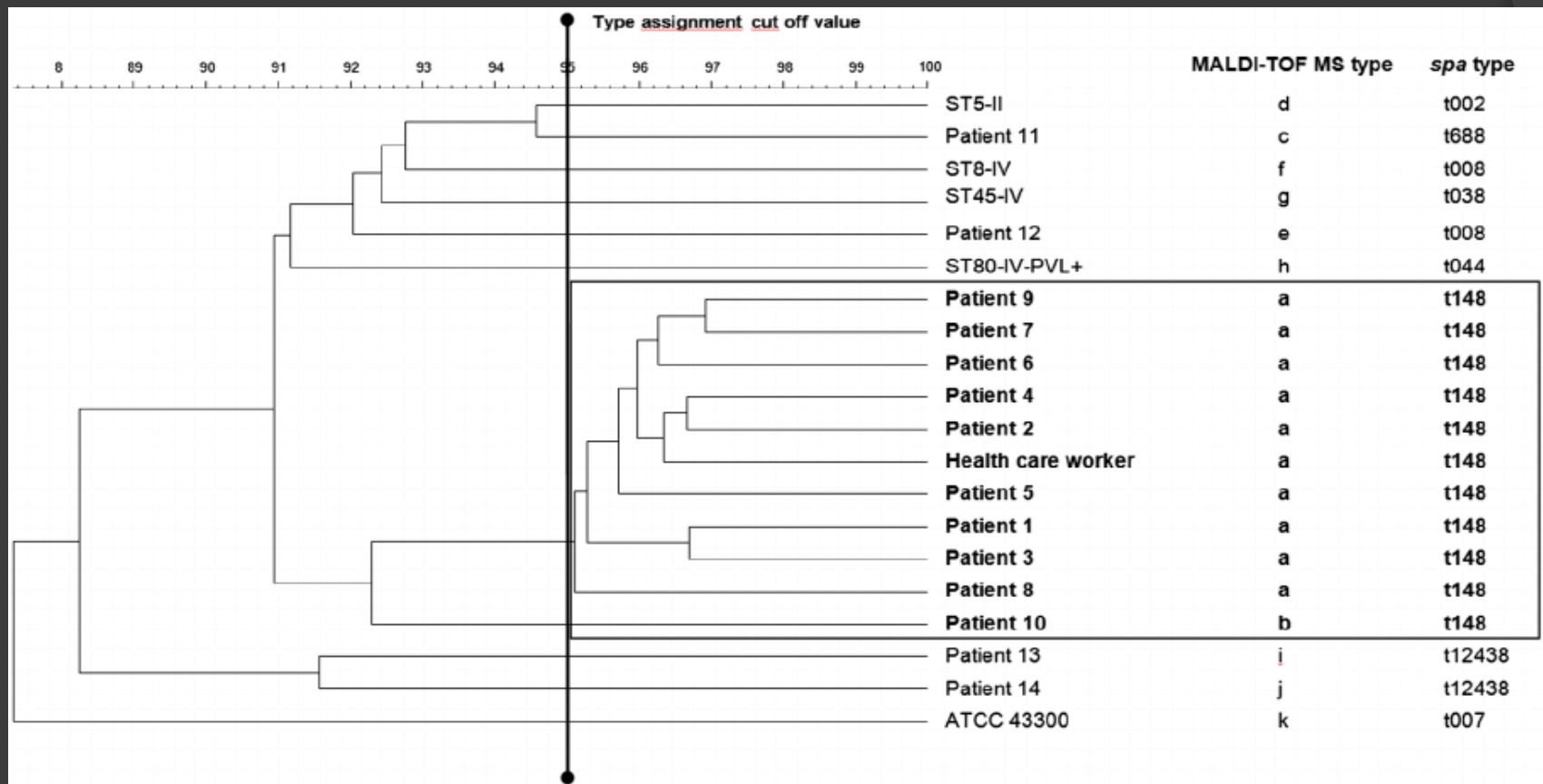
Outcome	Pre-intervention cohort (<i>n</i> = 157)	Intervention cohort (<i>n</i> = 112)	<i>P</i> value
30-Day all-cause mortality	33 (21)	10 (8.9)	0.01
60-Day all-cause mortality	48 (30.6)	14 (12.5)	0.001
Inpatient mortality	29 (18.5)	9 (8)	0.02

Table 4 Univariate and multivariate logistic regression— independent predictors of 30-day mortality.

Variable	Univariate analysis			Multivariate analysis ^a		
	OR	95% CI	<i>P</i> value	OR	95% CI	<i>P</i> value
Nosocomial acquisition	2.35	1.21–4.55	0.01	1.03	0.38–2.84	0.95
Pre-infection LOS	1.02	1.0–1.05	0.04	1.01	0.98–1.04	0.49
APACHE II	1.15	1.08–1.22	<0.001	1.18	1.10–1.27	<0.001
Respiratory source	2.13	1.08–4.23	0.03	0.82	0.35–1.96	0.66
Genitourinary source	0.31	0.1–0.7	0.003	0.37	0.17–1.12	0.07
MALDI-TOF MS + antimicrobial stewardship	0.37	0.17–0.78	0.009	0.28	0.12–0.71	0.008
Time to active therapy	1.00	1.00–1.01	0.02	1.00	1.00–1.01	0.007

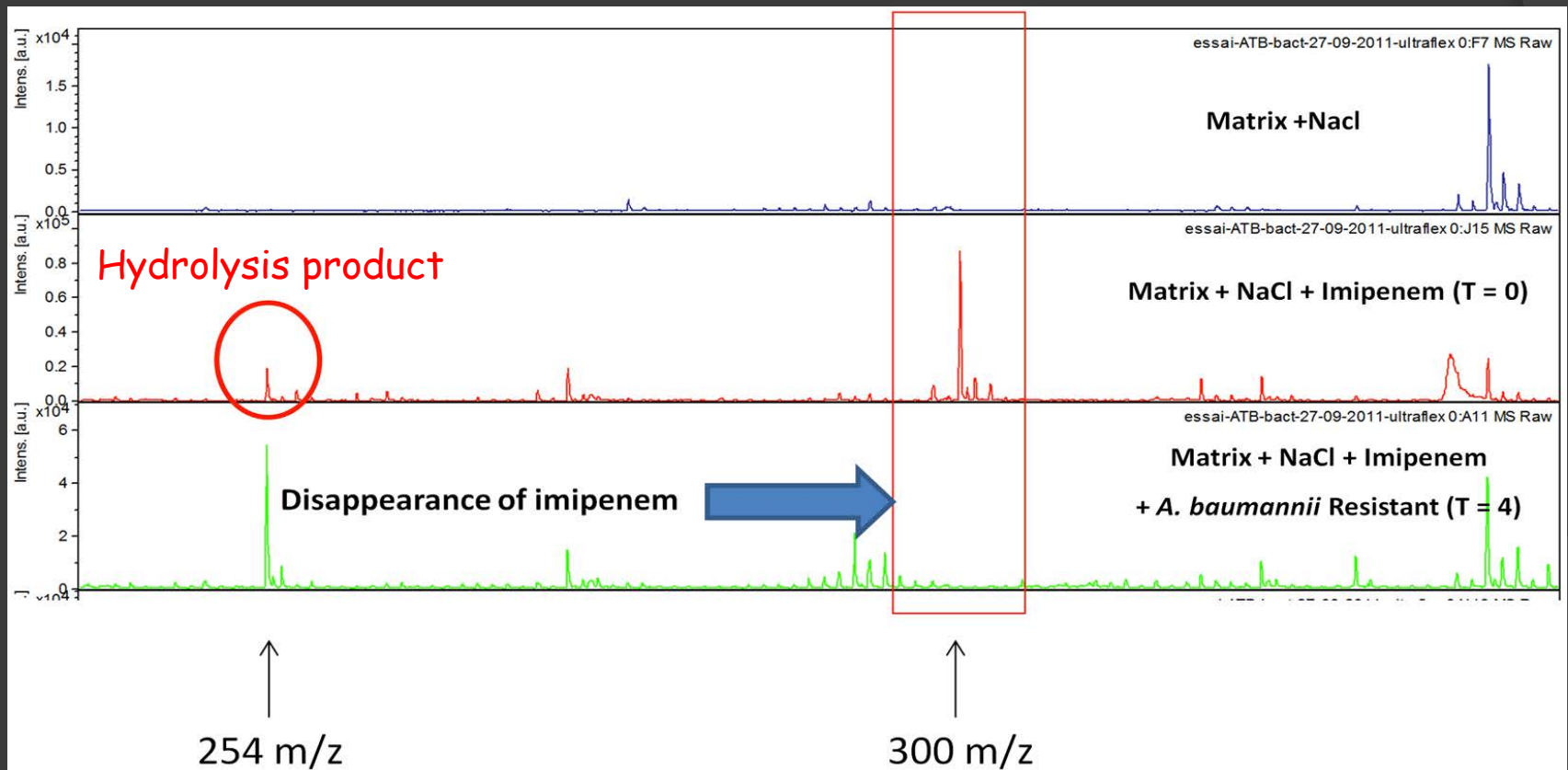
Application: Typing

Comparison of MRSA clinical isolates during an outbreak in a neonatal ICU



Application: Detection of resistance

Detection of an enzymatic activity → resistance to carbapenems by production of carbapenemases in GNB (incubation: 0.5-4 hours)

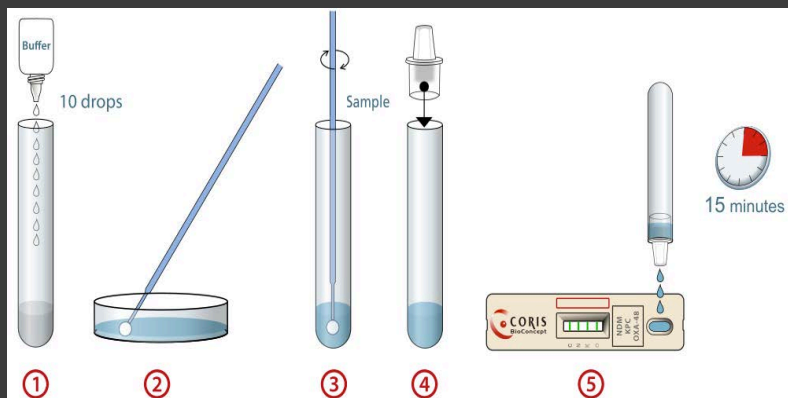


Chromogenic tests (ESBL-PE, CPE)

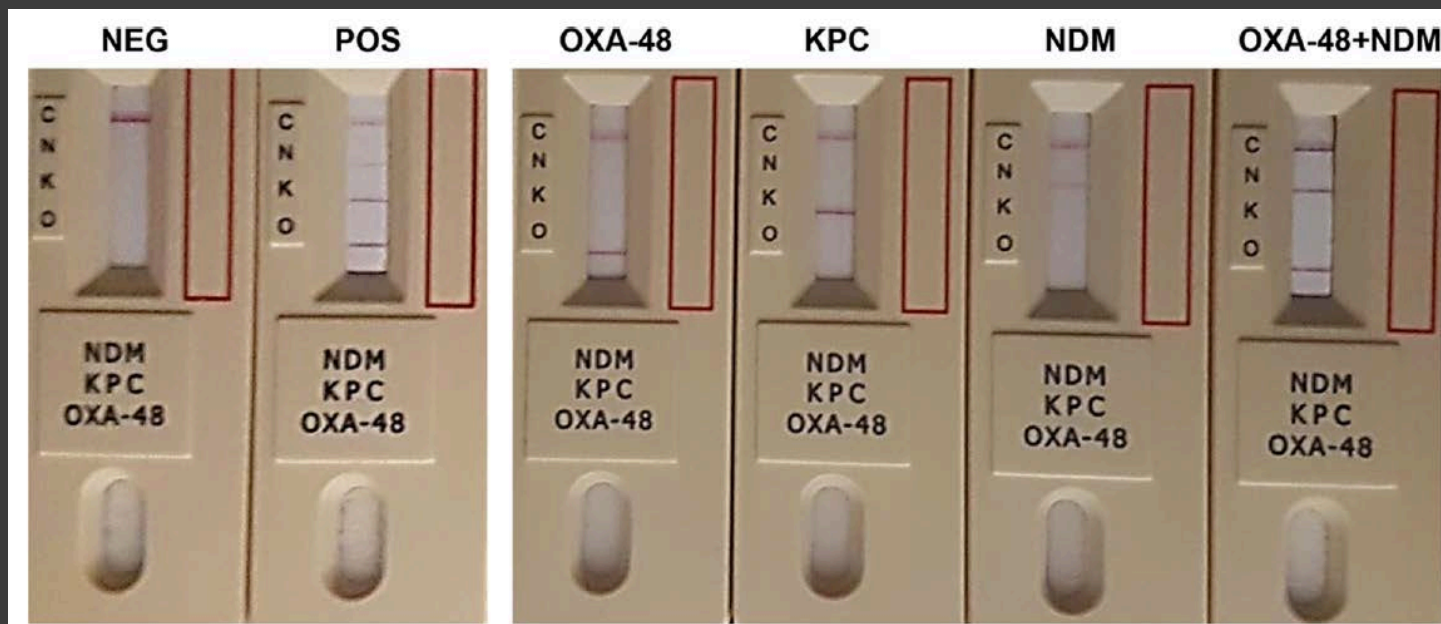
Principle/name of the test	Targeted enzymes	Required additional supplies	Delay for first/definitive results	Performances on cultured bacteria	Performances on clinical specimen
Colorimetric-chromogenic substrate					
β -Lacta test®	ESBL ^b	None	15 min	Sensitivity: 88% Specificity: 71%	Urines: sensitivity: 94%, specificity: 100% (positive blood culture: sensitivity: 95.7%, specificity: 100%)
β -CARBA test®	Carbapenemase	None	30 min	No direct comparison: sensitivity 87%, specificity 100%	
Colorimetric-non-chromogenic substrate					
Rapid ESBL NP test®	ESBL ^b	None	20 min	Sensitivity: 95% Specificity: 100%	Urines: sensitivity 98%, specificity 99.8%, positive blood culture: sensitivity 100%, specificity 100%
Rapid ESBL Screen kit®	ESBL ^b	None	30 min/2 h	Sensitivity: 92% Specificity: 83%	
Rapidec® Carba NP test	Carbapenemase	None	30 min/2 h	Sensitivity: 99% Specificity: 100%	Positive blood culture: preliminary experimental data
Rapid CARB Screen®	Carbapenemase	None	5 min/2 h	Sensitivity: 89.5% Specificity: 70.9%	
Rapid Carb Blue kit®	Carbapenemase	None	15 min/1 h	No direct comparison: sensitivity 100%, specificity 100%	

Fast (<2 hours), reliable, cheap
Usable on colonies, positive BCs and urines
Clinical impact poorly investigated

Immunochromatographic tests (CPE)



112 CPE clinical isolates
100% sensitivity and specificity
<15 €



O.K.N. kit
(Coris)

Modern genotypic approaches for bacteriological diagnosis

Rapid molecular tests

- ✓ **Specific detection of bacterial pathogens:**
 - Screening for carriage: MRSA, VRE, CPE, GBS
 - Diagnosis of infection:
 - . *S. aureus*
 - . *C. difficile*
 - . *M. tuberculosis*

- ✓ **Detection of a panel of pathogens :**
 - Meningitis/Encephalitis
 - Bacteremia (positive BCs)
 - Gastroenteritis
 - Respiratory infections

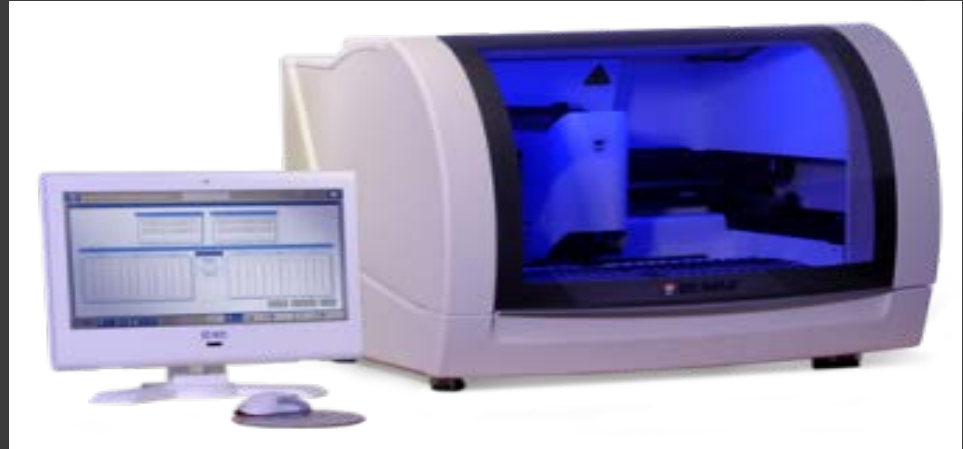
Commercial tests for MRSA

Organism detected	Assay	Company	Analysis platform	Probes	DNA target sequence	Specimen type	Time to result (h)
MRSA	BD GeneOhm MRSA ACP	Becton Dickinson	SmartCycler System	Molecular beacons	SCC <i>mec</i> at <i>orfX</i> junction.	Nasal swab	2.5
	BD MAX MRSA	Becton Dickinson	BD MAX System	Taqman ^R probes	SCC <i>mec</i> at <i>orfX</i> junction.	Nasal swab	2
	Xpert MRSA	Cepheid	GeneXpert Dx System	Taqman ^R probes	Insertion site (<i>attBsc</i>) of SCC <i>mec</i>	Nasal swab	1
	MRSA Advanced Test	Roche	LightCycler	FRET probes	Insertion site SCC <i>mec</i> at <i>orfX</i> junction	Nasal swab	2
	NucliSENS EasyQ MRSA	bioMérieux	EasyQ System (NASBA)	Molecular beacons	SCC <i>mec</i> at <i>orfX</i> junction and <i>mecA</i> gene for oxacillin resistance	Nasal swab	3

Sensitivity et specificity >90 %
NPV > 95%

Automated systems

BD MAX system
(Becton Dickinson)



~2 min HOT per sample

GeneXpert
(Cepheid)



Clinical impact

Cluster randomized crossover trial (2008-2010) in Belgium comparing the Xpert MRSA kit (Cepheid) to the enriched chromogenic culture

Outcome	Intervention	Control	P value
Median reporting time for MRSA admission screening (h)	11	88	<0.001
Median time from admission to isolation of newly detected MRSA carriers (h)	25	96	<0.001
Proportion of isolation days/total MRSA positive patient days (%)			
For newly detected MRSA carriers	82 (943/1147)	73 (528/724)	
For all MRSA carriers	79(1955/2461)	82(1949/2385)	
MRSA acquisition during hospital stay			
No cases/No patients at risk (%)	3.2	3.2	0.986
No cases/1 000 patient-days	2.6	2.8	0.692

Commercial tests for VRE

2 commercial kits for direct detection of VRE from stools/rectal swabs:

	Sens.	Spe.	PPV	NPV
Xpert <i>vanA/vanB</i>				
<i>vanA</i>	74-100	93-99	67-89	81-100
<i>vanB</i>	87-100	15-86	3-33	71-100
BD GeneOhm VanR				
<i>vanA</i>	43-88	96-100	82-100	67-97
<i>vanB</i>	75-100	21-85	7-37	100

Many false-positive results, especially for *vanB* → *van* genes identified in Gram-positive anaerobes:

- *vanB* : *Clostridium* spp., *Eggerthella lenta*, *Ruminococcus* spp.
- *vanD*, *vanG* : *Ruminococcus* spp.

Bourdon et al., Diagn Microbiol Infect Dis 2010 ;
Usacheva et al., Am J Clin Pathol 2010 ; Gazin et al., Eur J Clin Microbiol Infect Dis 2012

Clinical impact

Prospective study (2012) in France comparing Xpert *vanA/vanB* vs. enriched chromogenic culture

	Investigation of the first case in the diabetology unit (n=31 patients)	Investigation of a secondary case in the nephrology unit (n=22 patients)
From sampling, to sample reception	2.6 (1.7-2.6)	2.8 (1.1 – 3.8)
From sampling, to results	70.5 (69.4 – 70.5)	4.6 (4.0 – 18.9)
Total cost of microbiological testing	333.50	870.40
Overall loss of income (€)	13,968.70 to 85,175.00	0
Overall cost of the strategy (€)	14,302.20 to 86,175.50 ⁹	870.40 to 2,611.20 ⁹

→ Rapid decision about the best infection control strategy without loss of income due to discontinuation of patient transfers and admissions

Commercial tests for CPE

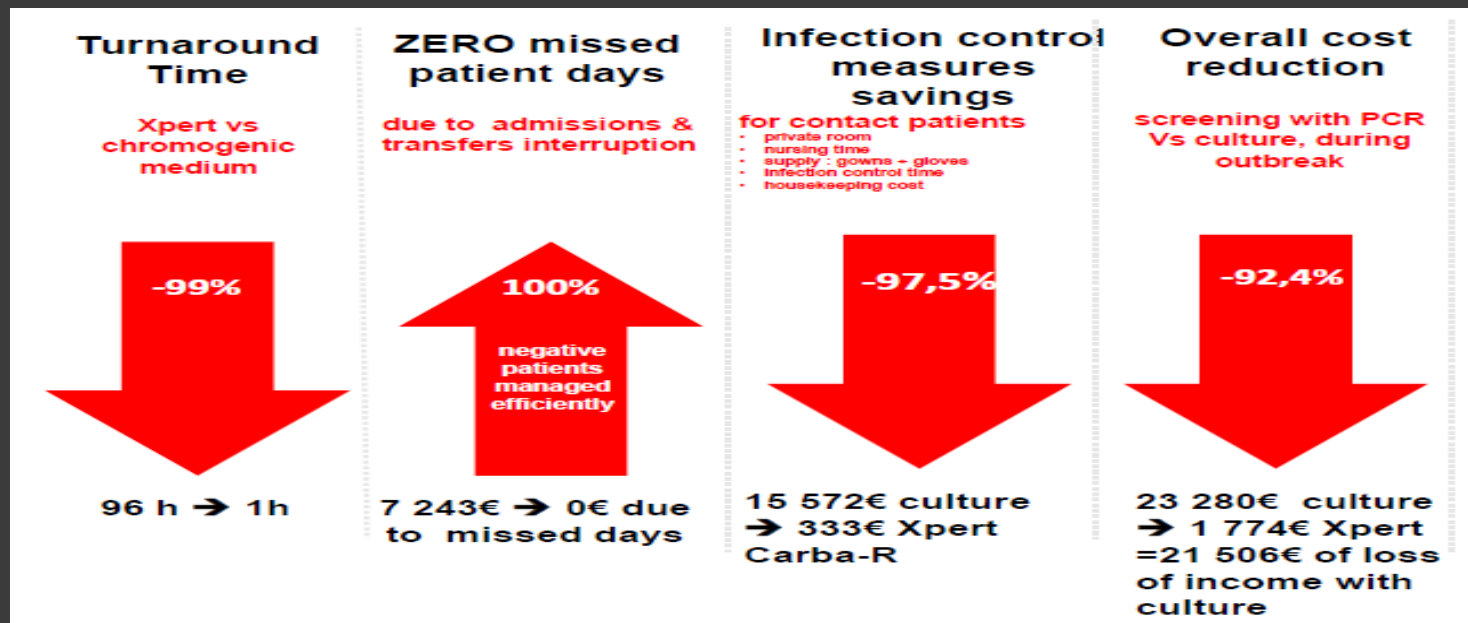
Numerous kits for detection directly from stools/rectal swabs

Test/Method	Turnaround time	Target species (n*)	Target carbapenemases	Sensitivity (%)	Specificity (%)	PPV† (%)	NPV‡ (%)
Check-Direct CPE	<180 min	Gram –negative bacilli (83–450)	KPC, VIM, NDM, OXA-48	97.1–100.0	94.0–100.0	100.0	0.0–70.0
LAMP/eazyplex® superBug complete A	25–60 min	Gram –negative bacilli (14–450)	KPC, VIM, NDM, OXA-48	100.0	100.0 (83.0 for OXA-48 like genes)	ND	ND
TaqMan PCR	<120 min	Enterobacteriaceae (59, 1308)	Classes A, B & D	100.0	100.0	ND	ND
NucliSENSEasyQKPC	<120 min	Enterobacteriaceae (300)	KPC only	100.0	100.0	ND	ND
Xpert® Carba-R kit	52 min	Gram-negative bacilli (450)	KPC, VIM, NDM, OXA-48	100.00	100.0 (83.0 for OXA-48 like genes)	ND	ND
Microarray (Alere technologies)	2–8 h	Gram-negative bacilli (117)	Classes A, B, D	98.2	97.4	ND	ND
Microarray (Verigene BC-GN)	2 h	Gram-negative bacilli (104)	Classes A, B, D	96.8	100.0	ND	ND
Microarray (Check-MDR CT101-103)	≤6 h	Gram-negative bacilli (57–187)	Classes A, B, D	90.5–100.0 (KPC=85.0)**	95.7–100.0	97.6–100.0	99.0–100.0
Xpert MDRO assay	<1 h	Gram-negative bacilli (328)	KPC, NDM, VIM	100.0	99.0–99.4	81.8–93.0	100.0

>90%

Clinical impact

Monocentric study (France, 2014) comparing the impact of the implementation of the Xpert Carba-R on infection control management and cost effectiveness respect to standard culture (1 OXA-48 case and 14 contact patients)



- No interruption of admissions and transfers
- No secondary case

Commercial tests for GBS

TABLE 1 PCR or nucleic acid amplification test (NAAT) validation for GBS screening[†]

PCR Studies	n, sample size	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Time to run test (mins)	Test – Site
Bergeron et al 2000 ²⁷	112	97.0 (82.5-99.8)	100 (86.9-100)	30-100	Conventional PCR vs. new fluorogenic PCR – Single center
Davies et al* 2004 ²⁸	803	94.0 (90.1-97.8)	95.9 (94.3-97.4)	40	IDI Strep – Multicenter
Gavino et al* 2007 ²⁹	55	95.8 (76.9-99.8)	64.5 (45.4-80.2)	<75	Xpert GBS Assay – Single center
Edwards et al 2008 ³⁰	784	91.1 (86.1-94.7) 79.3 (72.8-84.8)	96.0 (94.0-97.4) 95.4 (93.4-96.9)	75	Xpert GBS Assay vs. IDI Strep – Multicenter
Money et al* 2008 ³¹	190	90.7 (79.7-96.9)	97.6 (93.1-99.5)	99	IDI Strep – Canadian single center
El Helali et al 2009 ³²	968	98.5 (94.8-99.6)	99.6 (98.8-99.9)	<75	Xpert GBS Assay – French single center
Young et al* 2011 ³³	559	90.8 (84.6-95.2)	97.6 (95.6-98.8)	41	Xpert GBS Assay – Single center

[†] Vaginal/rectal samples and intrapartum standard culture as gold standard.

*These studies also had comparisons with antepartum culture results.

Commercial tests for *S. aureus*/MRSA

Organism detected	Assay	Company	Analysis platform	Probes	DNA target sequence	Specimen type	Time to result (h)
<i>S. aureus</i> and MRSA	Xpert MRSA/SA SSTI	Cepheid	GeneXpert System	Taqman ^R probes	<i>spa</i> for <i>S. aureus</i> , <i>SSCmec</i> and <i>mecA</i> gene for methicillin resistance	Wound swab	<1
	Xpert SA Nasal Complete	Cepheid	GeneXpert System	Taqman ^R probes	<i>spa</i> for <i>S. aureus</i> , <i>SSCmec</i> and <i>mecA</i> gene for methicillin resistance	Nasal swab	<1
	Xpert MRSA/SA Blood Culture ^a	Cepheid	GeneXpert System	Taqman ^R probes	<i>spa</i> for <i>S. aureus</i> , <i>SSCmec</i> and <i>mecA</i> gene for methicillin resistance	Blood Culture	<1
	StaphSR	BD GeneOhm	Smart Cycler	Molecular beacons	<i>nuc</i> gene for <i>S. aureus</i> , Insertion site (<i>attBsc</i>) of <i>SSCmec</i> for methicillin resistance	Blood Culture	1–1.5
	BC-GP ^b	Nanosphere	Verigene	Gold nanoparticles	<i>gyrB</i> for <i>S. aureus</i> and <i>mecA</i> gene for methicillin resistance	Blood culture	2.5

Sensitivity et specificity >90 %
NPV > 95%

Performances in BSI (positive BCs)

Detection of *S. aureus*

Kit	No. HC	Sensitivity (%)	Specificity (%)
Xpert MRSA/SA BC	792	99.6	99.5
GeneOhm StaphSR	782	99.2	96.5

Detection of MRSA

Kit	No. HC	Sensitivity (%)	Specificity (%)
Xpert MRSA/SA BC	792	98.1	99.6
GeneOhm StaphSR	782	94.3	97.8

Possibility to use the Xpert MRSA/SA SSTI kit in PJIs and VAPs

Clinical impact

Monocentric study (USA) comparing the Xpert MRSA/SA BC kit (group 1) vs. conventional method (group 2) in patients whose BCs revealed Gram-positive cocci in clusters (GPCC)

TABLE 2. Data on Drug Therapy for Patients with Bacteremia due to Methicillin-Susceptible *Staphylococcus aureus* at the Michael E. DeBakey Veterans Affairs Medical Center in Houston, Texas (2008–2009)

Variable	Group 1 ^a (<i>n</i> = 12)	Group 2 ^b (<i>n</i> = 48)	<i>P</i> ^c
Mean time to initiate MSS drug therapy, hours	5.2	49.8	.007
Median time to initiate MSS drug therapy, hours	0	48.5	.004
Mean duration of MRS drug therapy, hours	19.7	80.7	.003
No. (%) of patients not initially treated with MRS drug	3 (25.0)	5 (10.4)	
No. (%) of patients treated with MRS drug for unrelated condition	3 (25.0)	4 (8.3)	
No. (%) of patients treated with MRS drug for staphylococcal bacteremia	6 (50.0)	39 (81.3)	.025

But no difference in time to initiation of therapy for MRSA between groups (*P* = 0.33)

Commercial tests for *C. difficile*

Assay	Method	Target	DNA extraction	HOT (min) [†]	TAT (min) [†]	Cost per test	Sens. (%)	Spe. (%)
BD GeneOhm Cdiff	RT-PCR	<i>tcdB</i>	Manual	45	120	\$47 [‡]	82-98	91-100
Illumigene <i>C. difficile</i>	LAMP	<i>tcdA</i>	Manual	10	60	\$33 [‡]	73-98	91-100
Xpert <i>C. difficile</i>	Multiplex RT-PCR	<i>tcdB</i> , <i>cdt</i> , <i>tcdCA117</i>	Automated	10	60	\$52 [‡]	93-100	91-99
BD MAX Cdiff	RT-PCR	<i>tcdB</i>	Automated	10	100	\$43 [‡]	90-98	98-100
Portrait <i>C. difficile</i>	HDA	<i>tcdB</i>	Automated	10	100	\$25	98	93
ProGastro Cd	RT-PCR	<i>tcdB</i>	Automated (easyMAG)	45	180	\$25	77-100	93-99
Seeplex Diarrhea ACE	Multiplex PCR	<i>tcdB</i>	Manual	N/A	240	\$41 [‡]	90	97

[†]HOT and TAT for a batch of 5 samples.

Limits :

Risk of false-negative results (mutations, deletions)

Do not detect free toxins in stools

Clinical benefit++

Detection of MTB

The Xpert MTB/RIF Molecular Beacon Assay

rpoB GENE 81 bp RIF RESISTANCE DETERMINING REGION



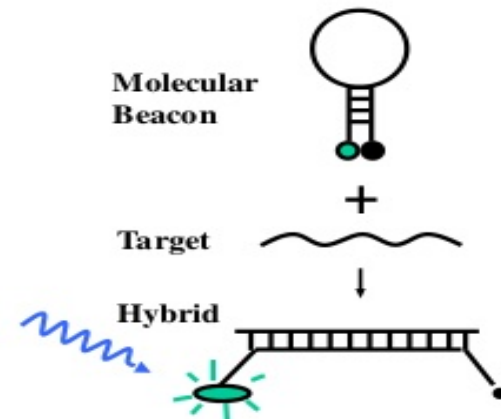
5-Probes bind to wild type (do not bind to mutant sequence)

1-Probe for SPC (*B. globigii*)

6-fluorescent dyes detected simultaneously

Delta Ct Max 5

<2 hours



Resp. and non-resp. samples:
Sensitivity of 100 % and 86 %
specificity of 100 % and 97 %

Broad molecular panels

→ Syndromic approach

POTENTIAL ADVANTAGES

- Increase diagnostic yield as multiple targets are tested in one sample
- Conserve and optimize analysis of samples difficult to obtain (spinal fluid, vitreous fluids, synovial fluids)
- Simplify ordering algorithm as only one test needs to be requested
- Streamline workflow in the laboratory and reduce hands-on time
- Potential saving in reagents by testing multiple organisms at once compared to testing each pathogen separately
- Standardize testing

POTENTIAL DISADVANTAGES

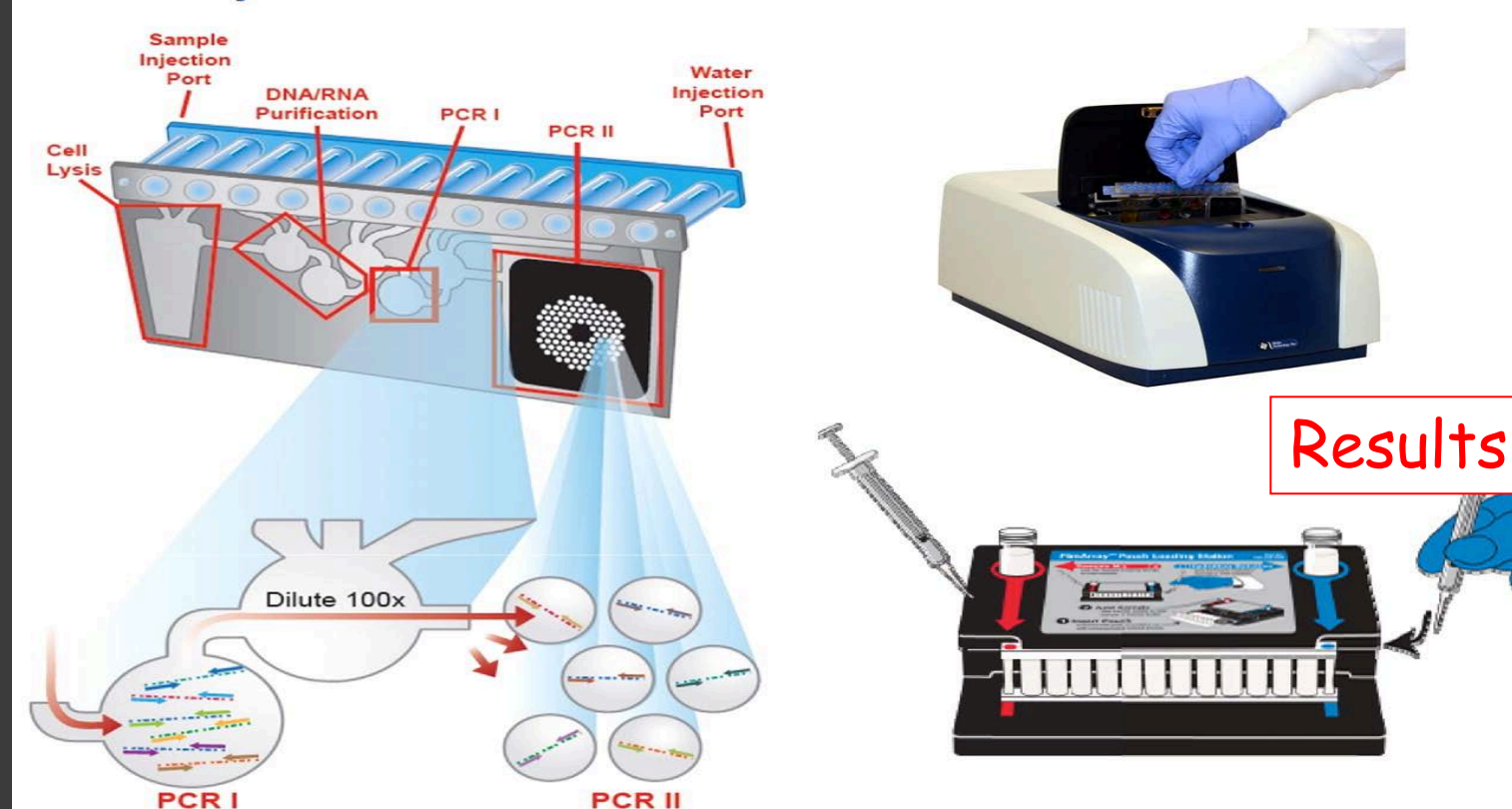
- False positive results due to cross reactivity or unspecific amplification caused by multiple primers/targets present in the reaction
- False-negative results due to use of preferential amplification of one target over another
- Negative internal control due to exhaustion of reagents in samples with a high amount of one particular target
- Added cost of testing targets that may not be necessary in some patient populations
- High cost of commercial kits and instruments

4 FDA-cleared systems (mostly automated)

FilmArray (Biofire, bioMérieux)

Principle: Two-stage nested PCR, the second stage involving parallel singleplex reactions followed by melt analysis

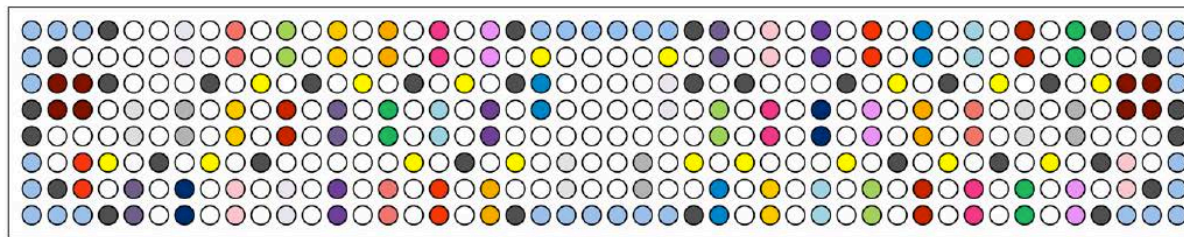
The FilmArray Pouch



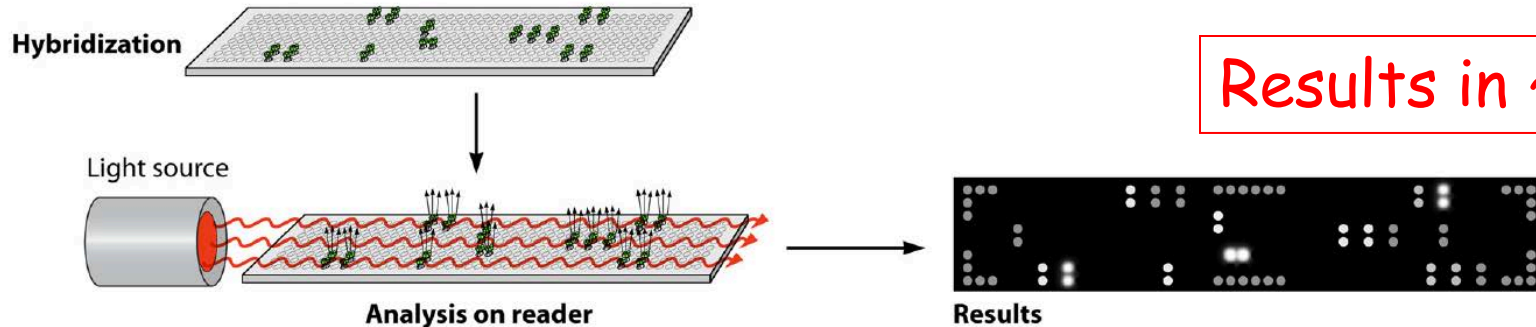
Verigene (Nanosphere)



Principle: Multiplex PCR followed by solid-microarray detection using nanoparticle-conjugated probes



- | | | | |
|---------------------|-----------------------|-------------------|----------------------|
| ● Bacteria genus #1 | ● Bacteria species #1 | ● Viral target #1 | ○ Internal control 1 |
| ○ Bacteria genus #2 | ● Toxin gene #1 | ● Viral target #2 | ● Internal control 2 |
| ● Bacteria genus #3 | ● Toxin gene #2 | ● Viral target #3 | ● Background control |
| ● Bacteria genus #4 | | | ● Imaging control |
| | | | ● Negative control |



Results in ~2 h

xTAG (Luminex)

Principle: Initial multiplex PCR by liquid-microarray detection using microbead-conjugated probes

Pre-PCR



Sample Pre-treatment
45-60 minutes

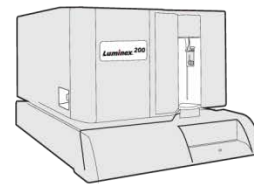
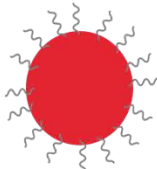


Nucleic Acid Extraction and Purification
45 minutes



Multiplex Application
2.5 hours

Post-PCR



Bead Hybridization and Detection
1 hour

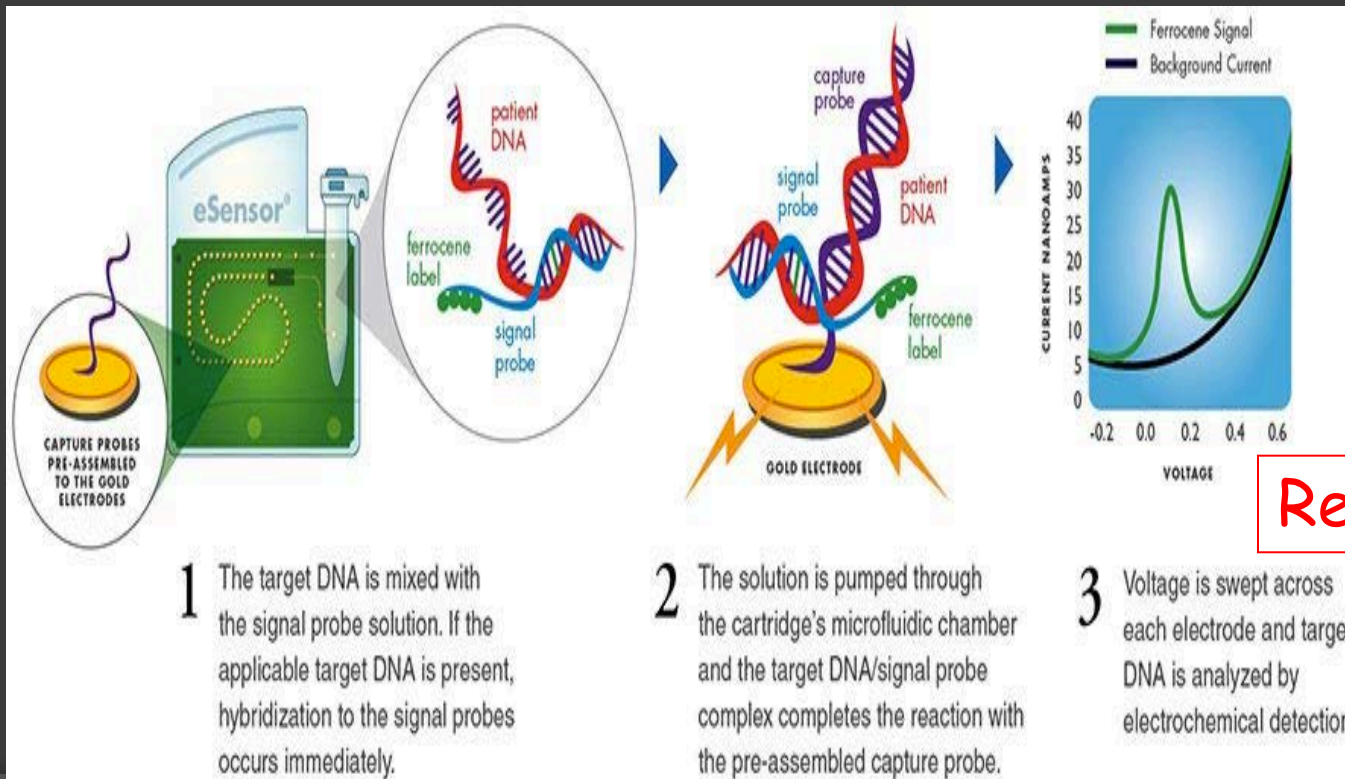
Data Acquisition and Analysis by MAGPIX®
or Luminex® 100/200™*
10 minutes

Results in ~5 h

ePlex (GenMark Dx)



Principle: Competitive DNA hybridization and electrochemical detection (eSensor)



Meningitis/Encephalitis

n = 1,643 CSF		Sensitivity/PPA ^b			Specificity/NPA ^b		
Analyte		TP/(TP + FN) ^c	%	95% CI	TN/(TN + FP) ^c	%	95% CI
Bacteria							
6 {	<i>E. coli</i> K1	2/2	100	34.2–100	1,557/1,558	99.9	99.6–100
	<i>H. influenzae</i>	1/1	100		1,558/1,559	99.9	99.6–100
	<i>L. monocytogenes</i>	0/0			1,560/1,560	100	99.8–100
	<i>N. meningitidis</i>	0/0			1,560/1,560	100	99.8–100
	<i>S. agalactiae</i>	0/1	0.0		1,558/1,559	99.9	99.6–100
	<i>S. pneumoniae</i>	4/4	100	51.0–100	1,544/1,556	99.2	98.7–99.6
Viruses							
7 {	CMV	3/3	100	43.9–100	1,554/1,557	99.8	99.4–99.9
	EV	44/46	95.7	85.5–98.8	1,507/1,514	99.5	99.0–99.8
	HSV-1	2/2	100	34.2–100	1,556/1,558	99.9	99.5–100
	HSV-2	10/10	100	72.2–100	1,548/1,550	99.9	99.5–100
	HHV-6	18/21	85.7	65.4–95.0	1,532/1,536	99.7	99.3–99.9
	HPeV	9/9	100	70.1–100	1,548/1,551	99.8	99.4–99.9
	VZV	4/4	100	51.0–100	1,553/1,556	99.8	99.4–99.9
Yeast							
1 {	<i>C. neoformans</i> / <i>C. gattii</i>	1/1	100		1,555/1,559	99.7	99.3–99.9

Bacteremia (positive BCs)

2 automated systems

Gram-positive bacteria	Gram-negative bacteria	Candida species
FilmArray Blood Culture Identification Panel (BioFire Diagnostics, LLC)		
<i>Staphylococcus</i> species	<i>Klebsiella oxytoca</i>	<i>Candida albicans</i>
<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>	<i>Candida glabrata</i>
<i>Streptococcus</i> species	<i>Serratia</i> species	<i>Candida krusei</i>
<i>Streptococcus agalactiae</i>	<i>Proteus</i> species	<i>Candida parapsilosis</i>
<i>Streptococcus pyogenes</i>	<i>Acinetobacter baumannii</i>	<i>Candida tropicalis</i>
<i>Streptococcus pneumoniae</i>	<i>Haemophilus influenzae</i>	
<i>Enterococcus</i> species	<i>Neisseria meningitidis</i>	
<i>Listeria monocytogenes</i>	<i>Pseudomonas aeruginosa</i>	19+5
	Enterobacteriaceae	
	<i>Escherichia coli</i>	
	<i>Enterobacter cloacae</i> complex	
Resistance genes	Resistance genes	3
<i>mecA</i>	<i>bla_{KPC}</i>	
<i>vanA/vanB</i>		

Verigene (Nanosphere, Inc.)		
Gram-Positive Blood Culture Test		Gram-Negative Blood Culture Test
<i>Staphylococcus aureus</i>		<i>Escherichia coli</i>
<i>Staphylococcus epidermidis</i>		<i>Klebsiella pneumoniae</i>
<i>Staphylococcus lugdunensis</i>		<i>Klebsiella oxytoca</i>
<i>Streptococcus anginosus</i> group		<i>Pseudomonas aeruginosa</i>
<i>Streptococcus agalactiae</i>		<i>Acinetobacter</i> species
<i>Streptococcus pneumoniae</i>		<i>Citrobacter</i> species
<i>Streptococcus pyogenes</i>		<i>Enterobacter</i> species
<i>Enterococcus faecalis</i>		<i>Proteus</i> species
<i>Staphylococcus</i> species	11	8
<i>Streptococcus</i> species		
<i>Listeria</i> species		
Resistance genes		Resistance genes
<i>mecA</i>		<i>bla</i> _{NDM}
<i>vanA</i>	3	<i>bla</i> _{KPC}
<i>vanB</i>		<i>bla</i> _{OXZ}
		<i>bla</i> _{VIM}
		<i>bla</i> _{CTX-M}
		5

Clinical impact

Retrospective monocentric (USA) evaluating the clinical impact of rapid PCR (Verigene BC-GN) on positive BCs (n = 195 patients)

Clinical outcome	Pre-BC-GN	Post-BC-GN	P value
Mean time from initial Gram stain to BC-GN identification, h	NA ^a	3.5	NA
Mean time from initial Gram stain to organism identification, h	37.9	10.9	<0.001 ^b
Mean time from initial Gram stain to effective therapy, h			
All cases	10.2	6.5	0.12 ^b
Cases on suboptimal empirical therapy	30.3	19.1	0.12 ^b
No. of cases in which therapy was de-escalated	33	37	0.66 ^c
Mean time from initial Gram stain to de-escalation, h	40.9	34.1	0.14 ^b
Recurrence of bacteremia, no. (%)	8 (8.2)	3 (3.1)	0.21 ^c
Mean total length of stay in hospital, days	15.2	18.0	0.52 ^b
Mean length of hospital stay after positive culture, days	9.7	9.4	0.87 ^b
Mean length of stay in ICU, days	16.2	12.0	0.03 ^b
30-day mortality, no. (%)	19 (19.2)	8 (8.1)	0.04 ^c
ESBL cases, no.	15	11	0.53 ^c
Length of stay in hospital, days	12.0	13.5	0.59 ^b
Mean time to effective therapy, h (no.) ^d	41.4 (9)	7.3 (9)	0.04 ^b
30-day mortality, no. (%)	4 (26.7)	0 (0)	0.11 ^c

Gastroenteritis

Target	Targets on the panel		
	FilmArray Gastrointestinal Panel (BioFire Diagnostics, LLC)	xTAG Gastrointestinal Pathogen Panel (Luminex Corporation)	Verigene Enteric Pathogens Test (Nanosphere, Inc.)
Bacteria			
<i>Campylobacter</i> species	✓	✓	✓
<i>Clostridium difficile</i>	✓	✓	
<i>Plesiomonas shigelloides</i>	✓		
<i>Salmonella</i> species	✓	✓	✓
<i>Yersinia enterocolitica</i>	✓		✓
<i>Vibrio cholerae</i>	✓	✓	
<i>Vibrio</i> species	✓		✓
Enteraggregative <i>Escherichia coli</i>	✓		
Enteropathogenic <i>Escherichia coli</i>	✓		
Enterotoxigenic <i>Escherichia coli</i>	✓	✓	
Shiga toxin—producing <i>Escherichia coli</i>	✓	✓	✓
<i>Escherichia coli</i> O157	✓	✓	
<i>Shigella</i> species (enteroinvasive <i>Escherichia coli</i>)	✓	✓	✓
Parasites			
<i>Cryptosporidium</i> species	✓	✓	
<i>Cyclospora cayetanensis</i>	✓		
<i>Entamoeba histolytica</i>	✓	✓	
<i>Giardia lamblia</i>	✓	✓	
Viruses			
Adenovirus serotypes 40/41	✓	✓	
Astrovirus	✓		
Norovirus	✓	✓	✓
Rotavirus	✓	✓	✓
Sapovirus	✓		

Respiratory infections

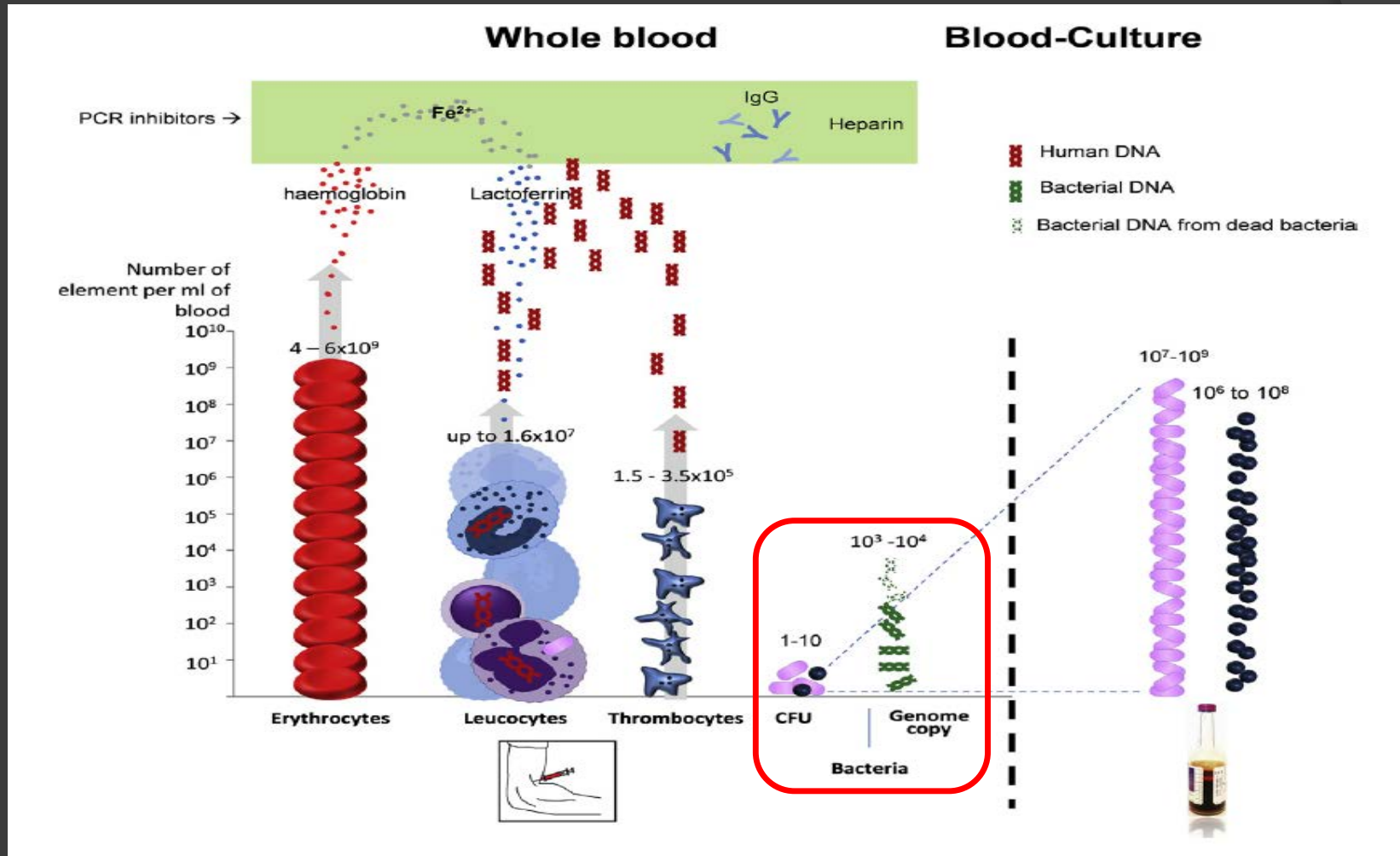
From nasopharyngeal swab specimens

Assay ^a	Manufacturer	Methodology	Preextraction required	Viruses reported ^b
FilmArray RP ^c	BioFire Diagnostics	Endpoint melt curve analysis	No	AdV; CoV HKU1, NL63; influenza virus A (H1/2009, H1, H3); influenza virus B; MPV; PIV1, -2, -3, -4; RSV; RhV/EV
eSensor RVP	GenMark Dx	Voltammetry	Yes	AdV (C, B/E); influenza virus A (H1/2009, H1, H3); influenza virus B; MPV; PIV1, -2, -3; RSV (A/B); RhV
xTAG RVPv1	Luminex Molecular Diagnostics	Fluorescence-labeled bead array	Yes	AdV; influenza virus A (H1, H3); influenza virus B; MPV; PIV1, -2, -3; RSV (A/B); RhV/EV
xTAG RVP fast	Luminex Molecular Diagnostics	Fluorescence-labeled bead array	Yes	AdV; influenza virus A (H1, H3); influenza virus B; MPV; RSV; RhV/EV
ePlex RPP	GenMark Dx	Voltammetry	No	AdV; CoV (229E, HKU1, NL63, OC43); influenza virus A (H1/2009, H1, H3); influenza virus B; MPV; PIV1, -2, -3, -4; RSV (A/B); RhV/EV

*Bacteria detected: *B. pertussis*, *M. pneumoniae*, *C. pneumoniae*

**Bacteria detected: *M. pneumoniae*, *C. pneumoniae*

Bloodstream infections (BSI)

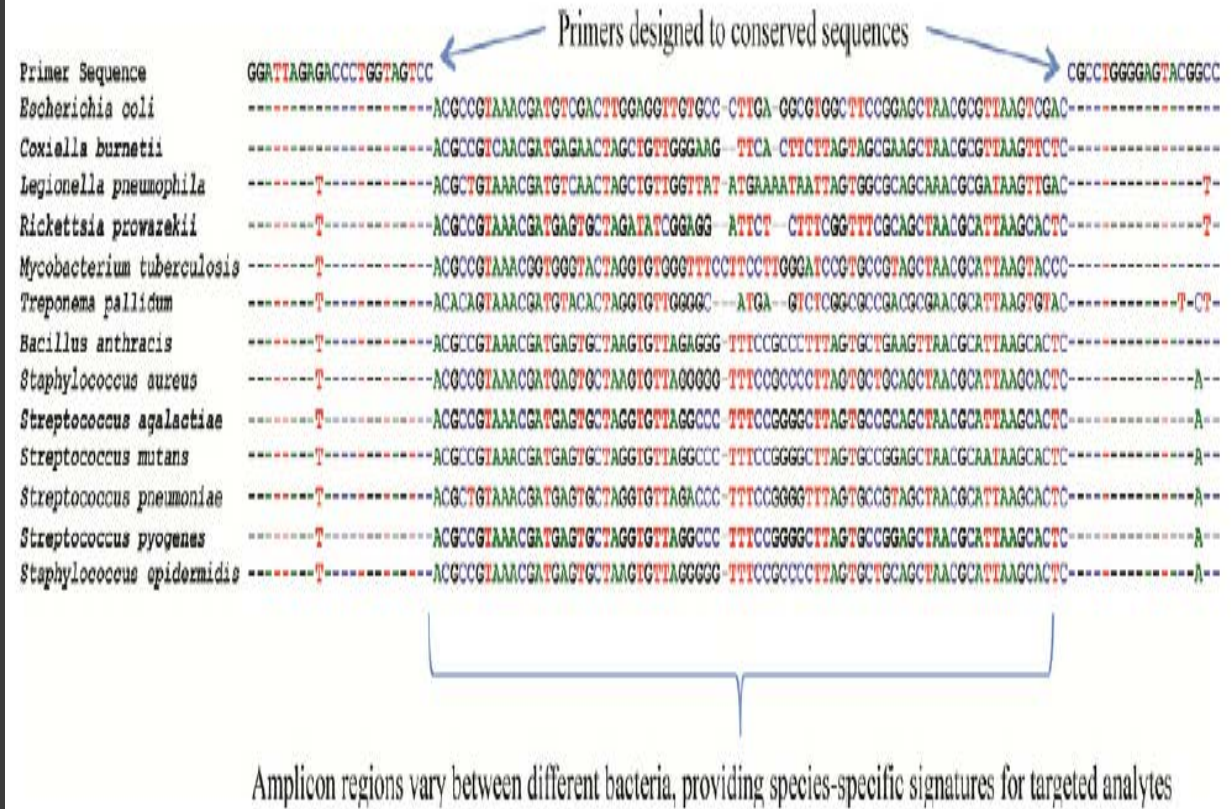
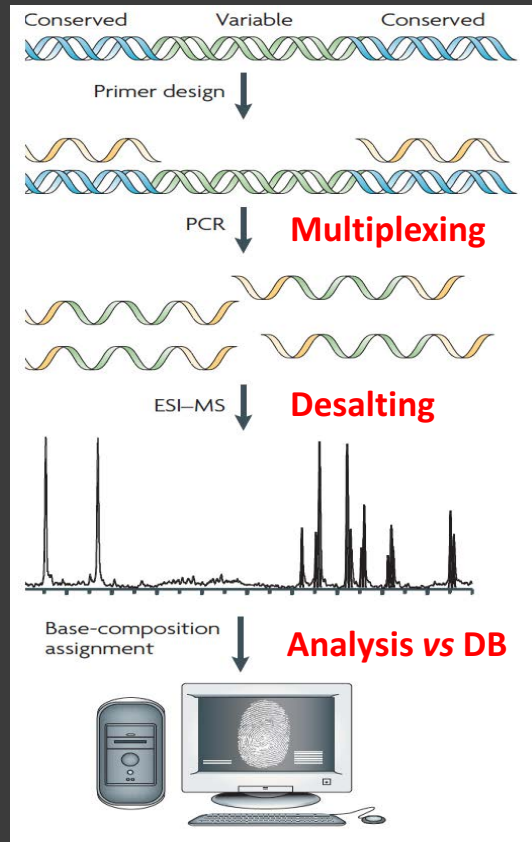


Diagnosis of BSI directly from blood

System	Method	Time to result (hours)	Blood volume (mL)	Microorganism coverage	Resistance and virulence markers	Sensitivity, specificity, and correlation with conventional methods (%)	Comments
SepsiTest Molzym, Bremen, Germany	Broad-range PCR + sequencing	6	1–10 ^a	>345 bacteria (Gram positive and Gram negative) and fungi	0	21–87, 85–96, NR	Pros: can be used in other sterile samples; Cons: variable sensitivity and specificity
SeptiFast Roche Molecular System, Basel, Switzerland	Multiple broad-range real-time PCR	3.5–5	1.5	6 Gram positive, 8 Gram negative, 5 fungi	<i>mecA</i> ^b	43–95, 60–100, 43–83	Pros: time to result; Cons: variable sensitivity and specificity, no quantification
MagicPlex Seegene, Seoul, Korea	Multiple PCR + multiplex real-time PCR	3–5	1	21 bacteria (Gram positive and Gram negative) at species level (90 at genus level), 6 fungi	<i>mecA</i> , <i>vanA/B</i>	37–65, 77–92, 73	Pros: fast; Cons: limited number of studies, succession of reaction and device, no quantification
VYOO SIRS-Lab, Jena, Germany	Multiplex PCR + electrophoresis	8	5	14 Gram positive, 18 Gram negative, 7 fungi	0	NR, NR, 70	Pros: highly sensitive; Cons: limited number of studies, several manual steps
PLEX-ID, Abbott Molecular, Carlsbad, CA, USA	Multiplex broad-range PCR/ESI-MS	6	1.25–5 ^c	Up to 800 (Gram positive, Gram negative, fungi)	<i>mecA</i> , <i>bla</i> _{KPC} , <i>vanA/B</i>	50–91 ^d , 98–99, 79–97	Pros: universal, detection of mixed bacterial populations, semiquantitative; Cons: no interventional studies

PCR-ESI-MS

PCR-ElectroSpray Ionization-Mass Spectrometry



Iridica platform (Abbott)

Technology PCR-ESI-MS
(new version of the Plex-ID)

Expensive equipment



ASSAY	COVERAGE	SAMPLE TYPE
BAC BSI BAC SFT	750+ Bacteria, Candida and 4 Antibiotic Resistance Markers: <i>mecA</i> , <i>vanA</i> , <i>vanB</i> and <i>kpc</i>	5ml EDTA whole blood Sterile fluids and tissues
BAC LRT	Identical coverage as BAC BSI and BAC SFT with additional semi-quantitative threshold	BAL and ETA
Fungal	200+ fungi	BAL and Isolates
Viral IC	130+ viruses in 13 reporting groups	Plasma

Direct detection
from blood ~6-8 h
Detection of R genes
(*mecA*, *vanA*, *vanB*,
bla_{KPC})

NGS in bacteriology

✓ Genomics (Whole Genome Sequencing - WGS)

- List of virulence genes
- In silico antibiogram
- Molecular typing

✓ Metagenomics

- Microbiota diversity
- Personalized medicine

Current NGS platforms

Platform	Instrument	Form factor	Sequencing technology ^a	Instrument cost	Read length (bp)	Run time (modes)	Single read output (modes) ^c	Sequence output (modes)	Approx cost per isolate (bacterial)
Illumina	MiniSeq	Benchtop	SBS	\$50,000	2 x 150	24 h/17 h	25 M/8 M	7.5 Gb/ 2.4 Gb	TBD
	MiSeq(Dx)	Benchtop	SBS	\$99,000	2 x 300	56 h	25 M	15 Gb	\$60-70
	NextSeq(Dx)	Benchtop	SBS	\$250,000	2 x 150	29 h/26 h	400 M/130 M	120 Gb/ 39 Gb	\$50-60
	HiSeq (various models)	Capital	SBS	\$750,000	2 x 125	6 days/40 h		1 Tb/ 180 Gb	\$50-60
ThermoFisher IonTorrent	PGM	Benchtop	Semiconductor	\$50,000	400	7 h	5.5 M	2 Gb	\$60-70
	Proton	Benchtop	Semiconductor	\$150,000	200	4 h	83 M	10 Gb	\$60-70
	S5	Benchtop	Semiconductor	\$65,000	400	2.5 h /4 h	5 M/80 M	15 Gb	\$50-60
	S5XL	Benchtop	Semiconductor	\$150,000	400	2.5 h/4 h	5 M/80 M	15 Gb	\$50-60

In silico antibiogram of *S. aureus*

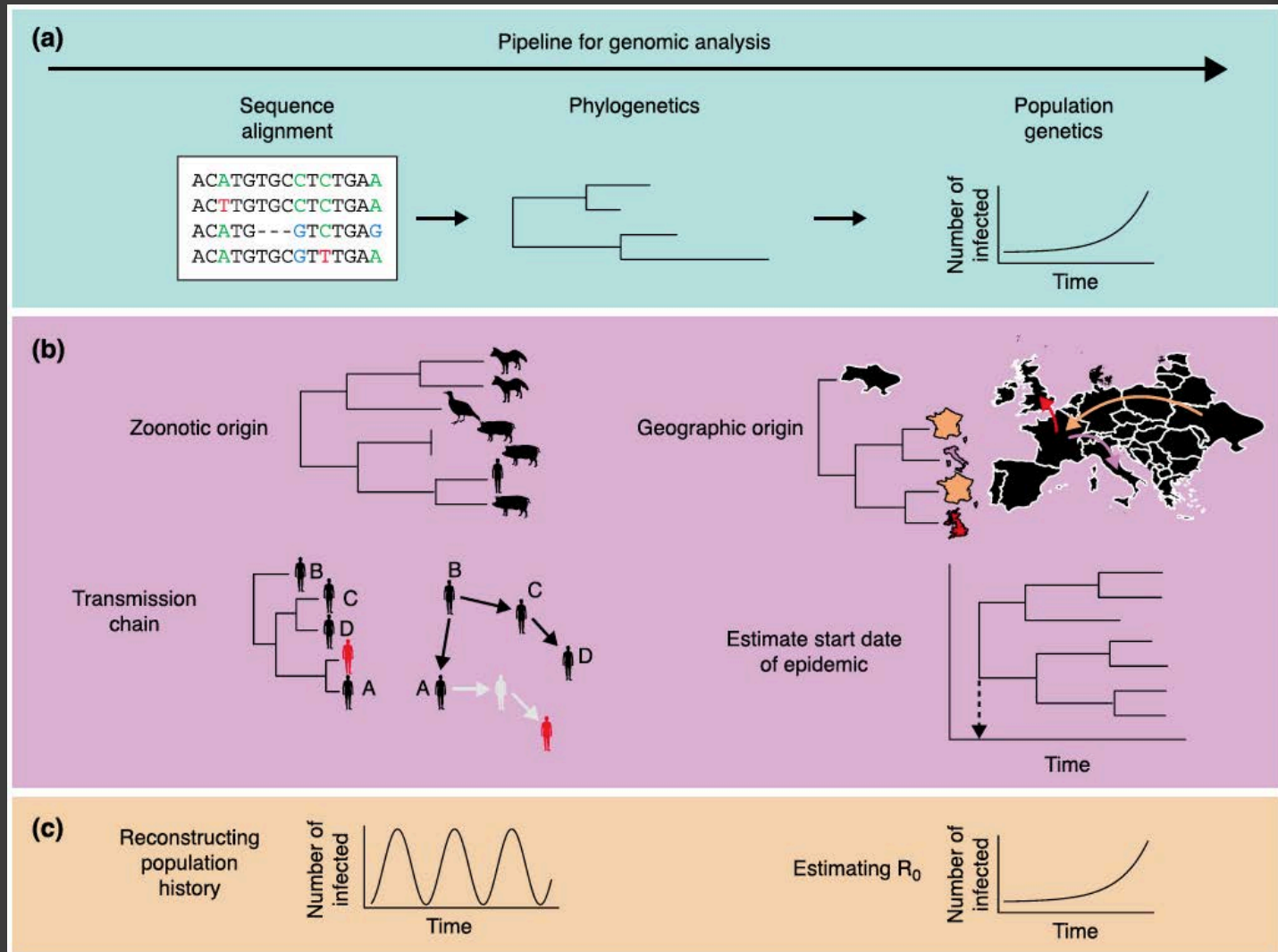
Creation of a panel of resistance mechanisms (501 reference strains) tested against 401 clinical isolates:

12 ATB

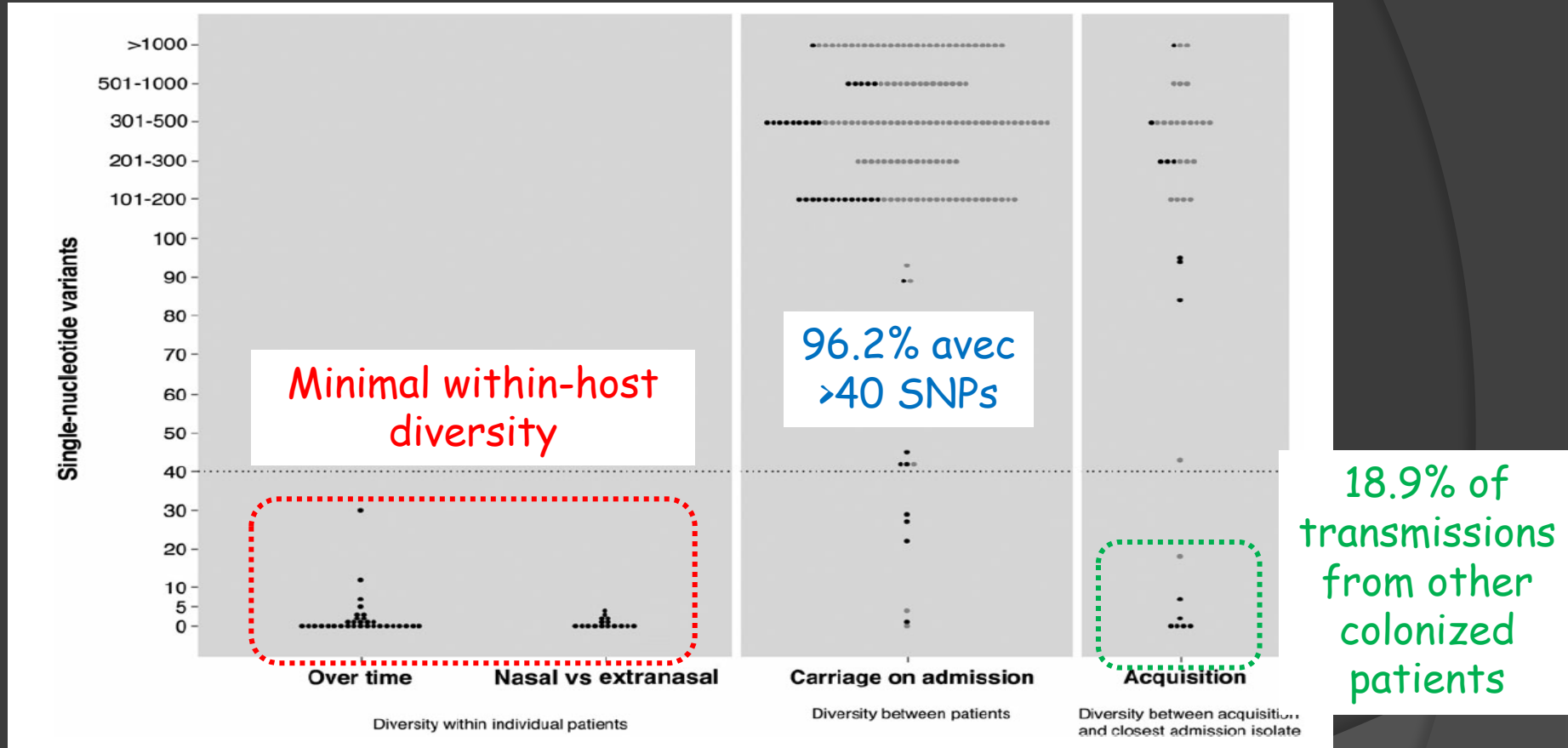
Antimicrobial agent	No. of isolates resistant by phenotype		No. of isolates susceptible by phenotype		Total no. of isolates	Very major error rate (%) (95% CI)	Major error rate (%) (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
	Susceptible by genotype ^b	Resistant by genotype	Susceptible by genotype	Resistant by genotype ^b					
Penicillin	3 (2)	379	84	25 (9)	491	0.6 (0.1–1.8)	5.1 (3.3–7.4)	0.99 (0.98–1.00)	0.77 (0.68–0.84)
Methicillin	2 (1)	55	432	2 (1)	491	0.4 (0.05–1.5)	0.4 (0.05–1.5)	0.96 (0.87–0.99)	1.00 (0.98–1.00)
Ciprofloxacin	6 (4)	64	420	1 (0)	491	1.2 (0.4–2.6)	0.2 (0.05–1.1)	0.91 (0.82–0.96)	1.00 (0.98–1.00)
Erythromycin	4 (2)	79	405	3 (3)	491	0.8 (0.2–2)	0.6 (0.1–1.8)	0.95 (0.87–0.98)	0.99 (0.98–1.00)
Clindamycin	2 (2)	77	2	0	81	2.5 (0.3–8.6)	0.0 (0–4.4)	0.97 (0.90–1.00)	1 (0.20–1.00)
Tetracycline	0	18	471	2 (2)	491	0.0 (0–0.7)	0.4 (0.05–1.5)	1.00 (0.78–1.00)	1.00 (0.98–1.00)
Vancomycin	0	0	491	0	491	0.0 (0–0.7)	0.0 (0–0.7)	N/A ^c	1.00 (0.99–1.00)
Fusidic acid	4 (4)	39	448	0	491	0.8 (0.2–2)	0.0 (0–0.7)	0.91 (0.77–0.97)	1.00 (0.99–1.00)
Trimethoprim	2 (2)	1	197	2 (1)	202	1.0 (0.1–3.5)	1.0 (0.1–3.5)	0.33 (0.02–0.87)	0.99 (0.96–1.00)
Gentamicin	2 (2)	2	487	0	491	0.4 (0.05–1.5)	0.0 (0–0.7)	0.50 (0.09–0.91)	1.00 (0.99–1.00)
Mupirocin	0	2	489	0	491	0.0 (0–0.7)	0.0 (0–0.7)	1.00 (0.20–1.00)	1.00 (0.99–1.00)
Rifampin	0	5	486	0	491	0.0 (0–0.7)	0.0 (0–0.7)	1.00 (0.46–1.00)	1.00 (0.99–1.00)
Overall	25 (19)	644	4,410	35 (16)	5,112	0.5 (0.3–0.7)	0.7 (0.5–0.9)	0.97 (0.95–0.98)	0.99 (0.99–1.00)

FDA cutoffs : <1,5 % <3 %

Molecular epidemiology by WGS



Transmission mapping



Conventional methods falsely identified 3 patient-to-patient transmissions (all MRSA) and failed to detect 2 acquisitions and 4 transmissions (2 MRSA)

Current limits of WGS

Current limitations

- Cost of personal genome sequencing platforms
- Speed of data analysis
- Limited user-friendly bioinformatics platforms available for sequence assembly and data analysis
- Education because of poor knowledge of genomics and bioinformatics among diagnostic microbiologists
- Limited reference sequence data for many species
- Difficulties with DNA extraction directly from clinical samples

+ Interpretative criteria for typing?

Third-generation NGS

- Long-read sequencing (3-20 kb)
- No amplification of DNA fragments
- High error rates but recent improvements

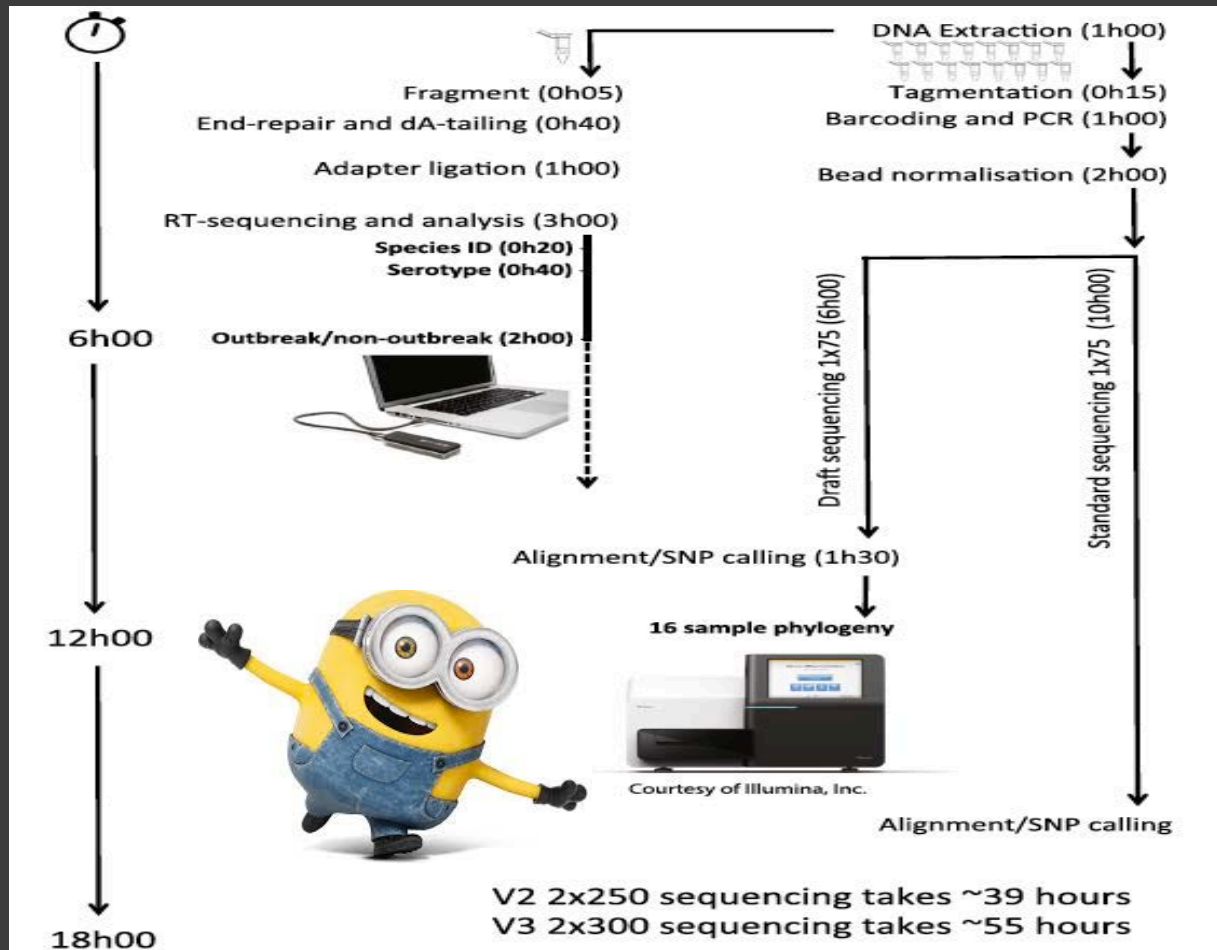
Platform	Instrument	Form factor	Sequencing technology ^a	Instrument cost	Read length (bp)	Run time (modes)	Single read output (modes) ^c	Sequence output (modes)	Approx cost per isolate (bacterial)
Pacific BioSciences	RSII	Capital	SMRT	\$700,000	10,000-15,000	4 h	50,000	1 Gb	\$500-600
	Sequel	Capital	SMRT	\$350,000	10,000-20,000	6 h	350,000	7 Gb	TBD
Oxford Nanopore	MinION MkI	Portable	Nanopore	\$1,000	>10,000	1 min to 48 h	2.2 M/ 4.4 M	Up to 42 Gb	TBD
	PromethION	Benchtop	Nanopore	TBD ^b	>10,000	1 min to 48 h	625 M/ 1.25 B	6 Tb/ 12 Tb	TBD

^aSBS, sequencing by synthesis; SMRT, single-molecule real-time sequencing.

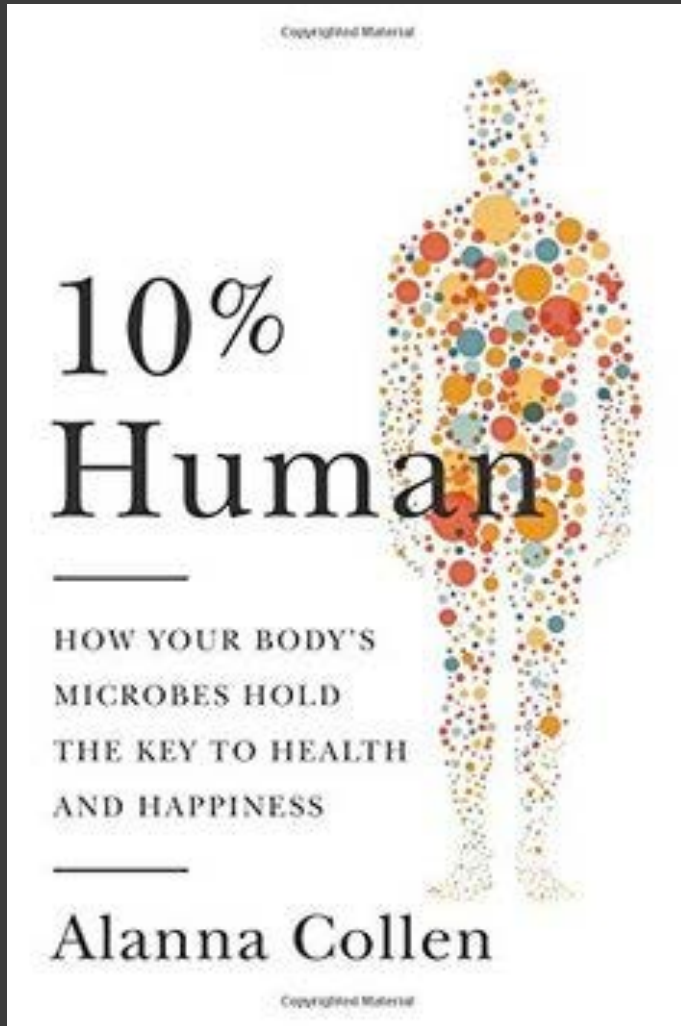
^bTBD, to be determined.

Applications - MinION

Hospital outbreak of *Salmonella* Enteritidis (>30 cases)



Human microbiota and microbiome

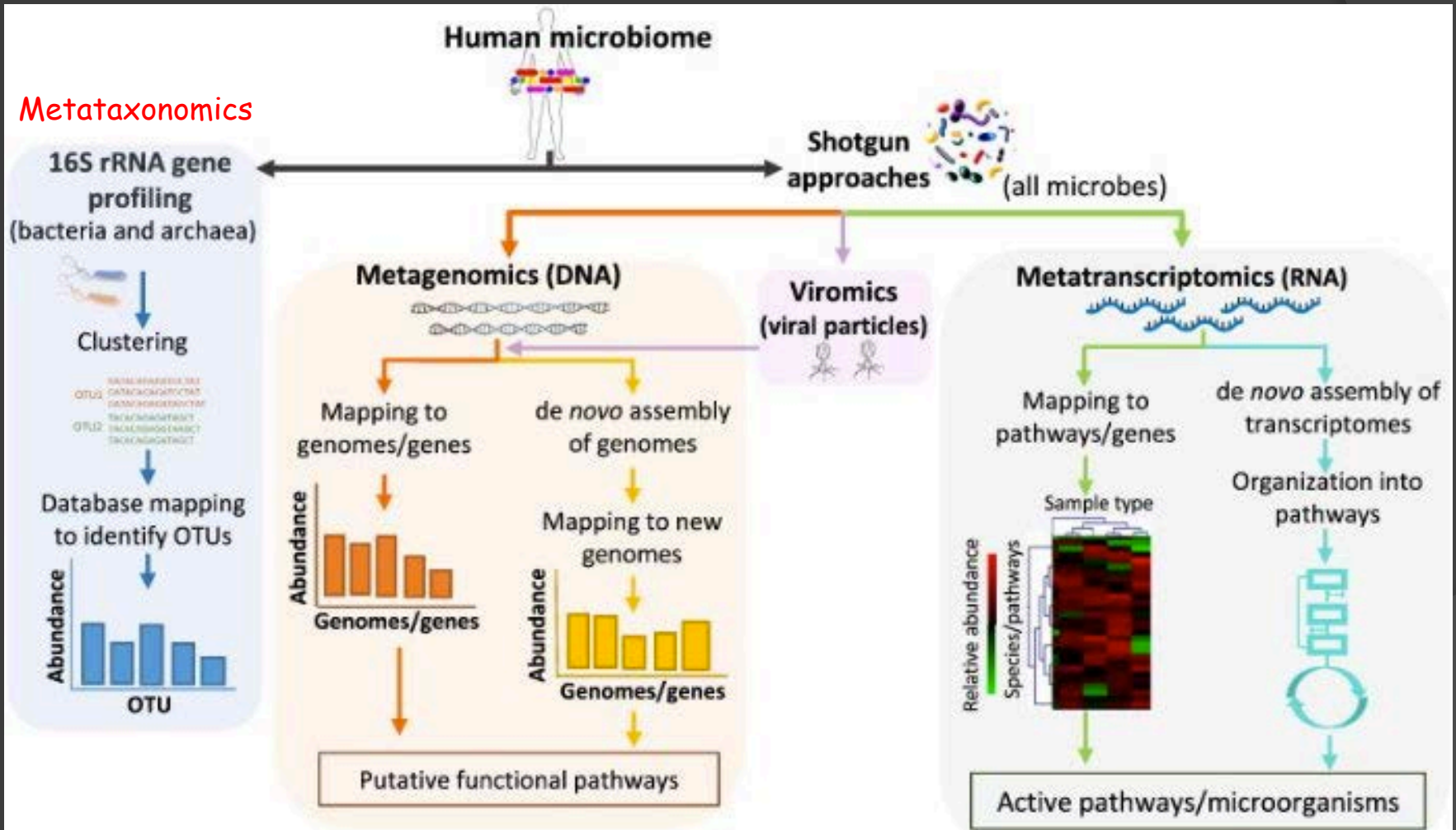


10^{14} bacteria, ie :

- 10 x more than human cells
- 1-3 % du poids (1-2 kg)
- >1000 different species
- 150 x more of bacterial genes
- No identical human microbiota



Metagenomics



Challenges in bioinformatics

Processing and long-term archiving of huge amounts of data ($\times 10,000$ compared to 1995)

Simplification of bioinformatic tools (user-friendly softwares)



Need of academic training for clinical microbiologists

Improvement of DB quality
Integration and standardization of meta-data