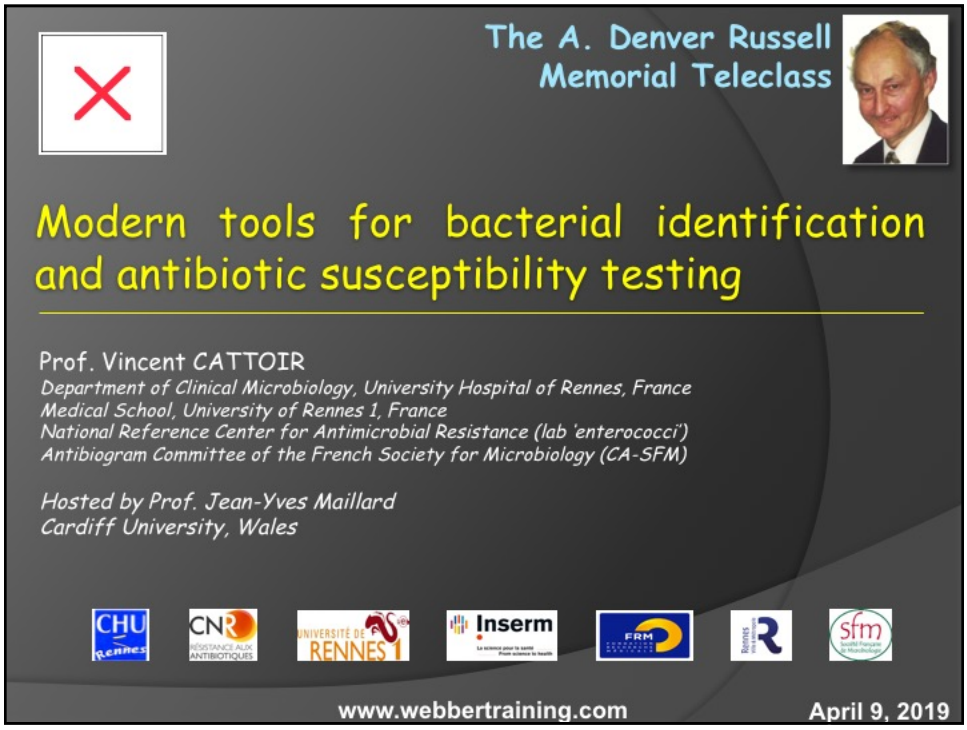
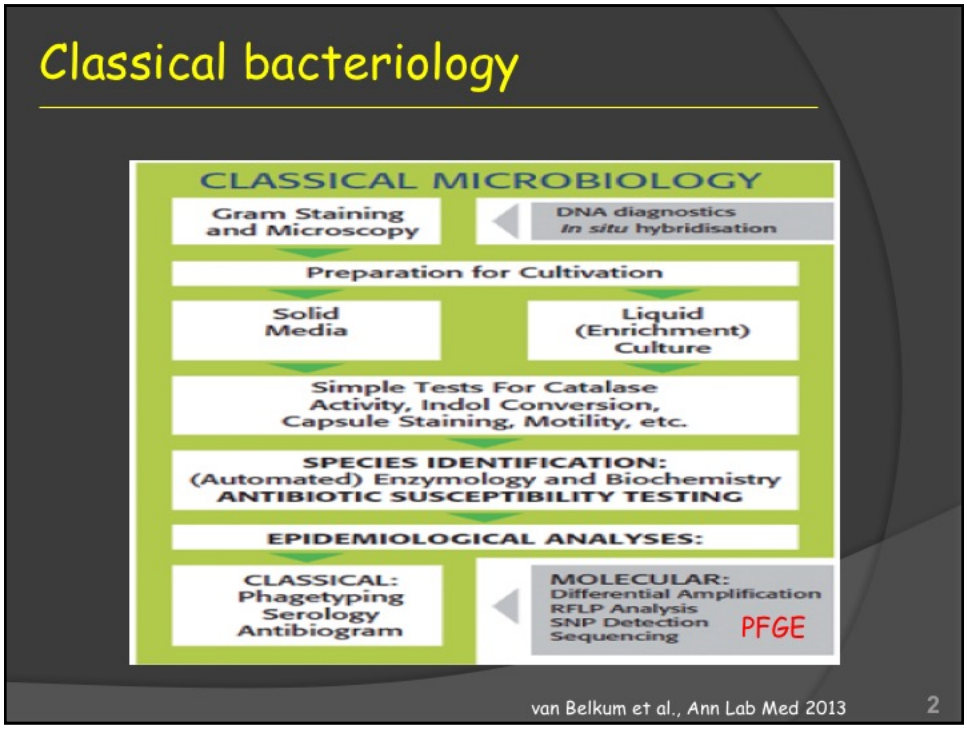

The A. Denver Russell Memorial Teleclass
Modern tools for bacterial identification and antibiotic susceptibility testing
Prof. Vincent CATTOIR
Department of Clinical Microbiology, University Hospital of Rennes, France
Medical School, University of Rennes 1, France
National Reference Center for Antimicrobial Resistance (lab 'enterococci')
Antibiogram Committee of the French Society for Microbiology (CA-SFM)
Hosted by Prof. Jean-Yves Maillard
Cardiff University, Wales
www.webbertraining.com
April 9, 2019

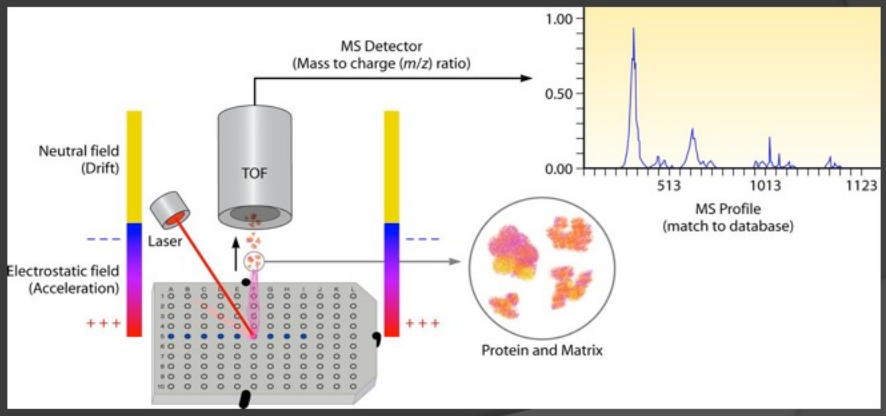

Classical bacteriology
CLASSICAL MICROBIOLOGY
Gram Staining and Microscopy
DNA diagnostics In situ hybridisation
Preparation for Cultivation
Solid Media
Liquid (Enrichment) Culture
Simple Tests For Catalase Activity, Indol Conversion, Capsule Staining, Motility, etc.
SPECIES IDENTIFICATION:
(Automated) Enzymology and Biochemistry
ANTIBIOTIC SUSCEPTIBILITY TESTING
EPIDEMIOLOGICAL ANALYSES:
CLASSICAL:
Phagetyping
Serology
Antibiogram
MOLECULAR:
Differential Amplification
RFLP Analysis
SNP Detection
Sequencing
PFGE
van Belkum et al., Ann Lab Med 2013
2

Modern phenotypic approaches for bacteriological diagnosis

3

MALDI-TOF MS

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



Clark et al., Clin Microbiol Rev 2013

4

Commercial MALDI-TOF MS systems

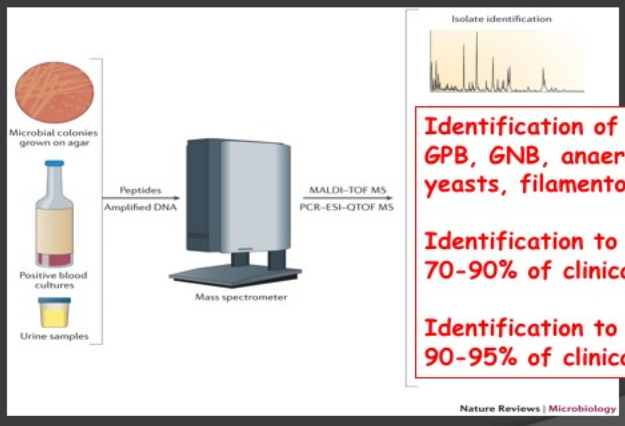
Vitek MS
(bioMérieux)

MALDI Biotyper CA system
(Bruker Daltonics)

5

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

Fournier et al., Nat Rev Microbiol 2013; Chen et al., J Clin Microbiol 2013; Feirrera et al., J Clin Microbiol 2013

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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

Singhal et al., Front Microbiol 2015 7

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 (n = 157)	Intervention cohort (n = 112)	P 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

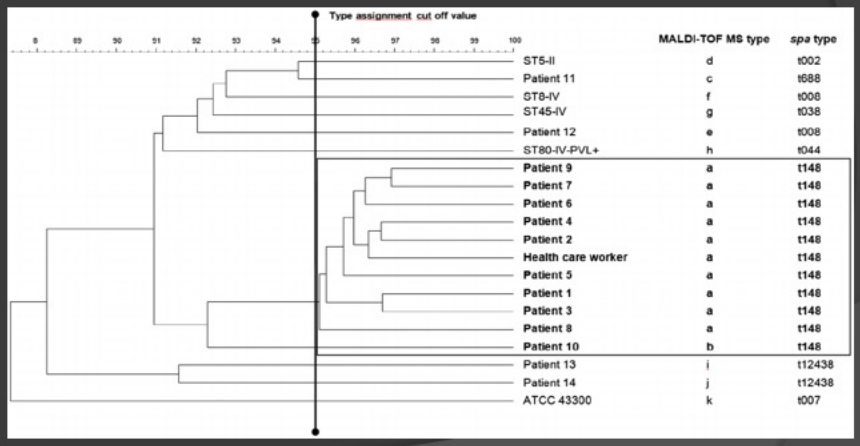
Table 4 Univariate and multivariate logistic regression— independent predictors of 30-day mortality.

Variable	Univariate analysis			Multivariate analysis[a]		
	OR	95% CI	P value	OR	95% CI	P 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

Perez et al., J Infect 2014 8

Application: Typing

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

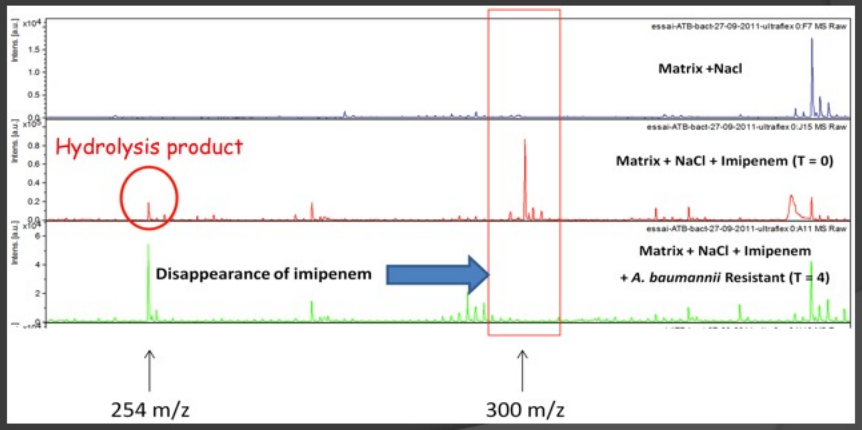


Steensels et al., Acta Clin Belg 2016

9

Application: Detection of resistance

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



Kempf et al., PLoS One 2016

10

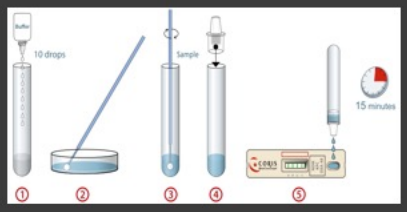
Chromogenic tests (ESBL-PE, CPE)

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					
ß-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: sensibility 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%	

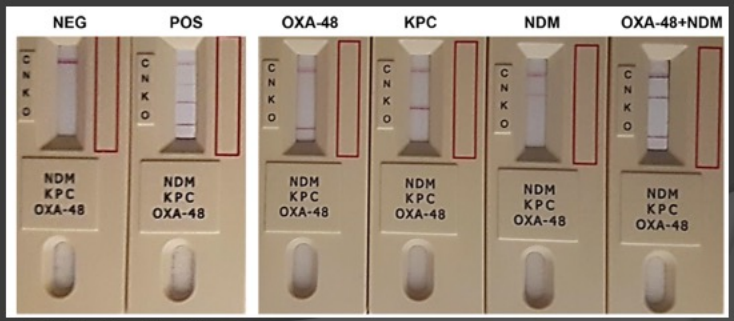
Fast (<2 hours), reliable, cheap
Usable on colonies, positive BCs and urines
Clinical impact poorly investigated

Decousser et al., Expert Rev Mol Diagn 2017 11

Immunochromatographic tests (CPE)



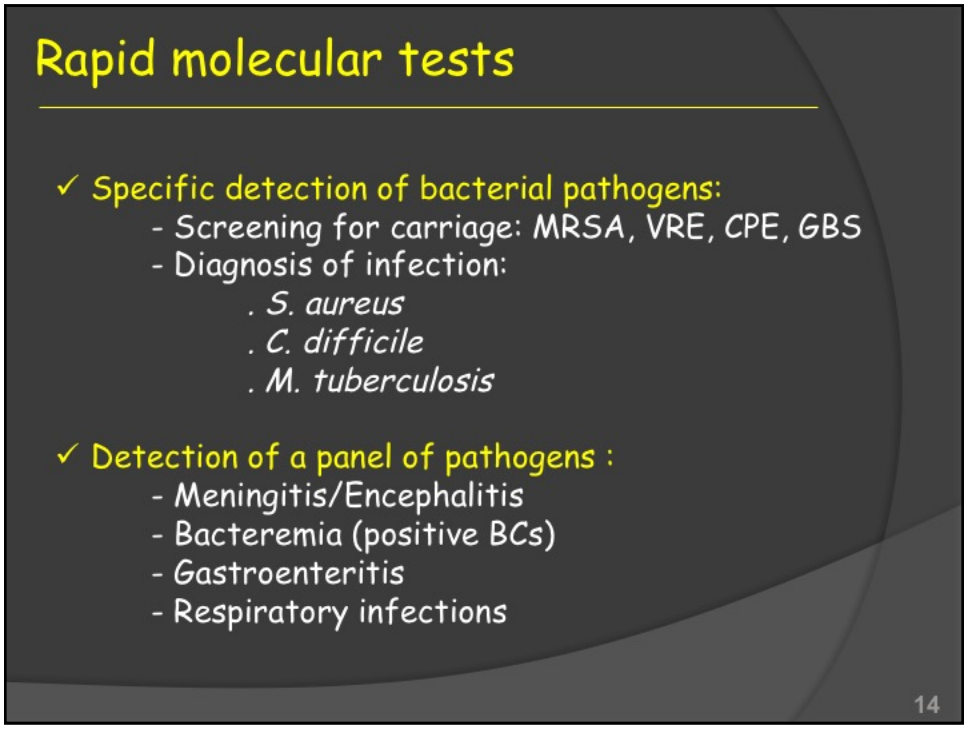
112 CPE clinical isolates
100% sensitivity and specificity
<15 €



O.K.N. kit (Coris)

Wareham et al., J Clin Microbiol 2017 12


Modern genotypic approaches for bacteriological diagnosis
13


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
14

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*mec* at *orfX* junction.	Nasal swab	2.5
	BD MAX MRSA	Becton Dickinson	BD MAX System	Taqman® probes	SCC*mec* at *orfX* junction.	Nasal swab	2
	Xpert MRSA	Cepheid	GeneXpert Dx System	Taqman® probes	Insertion site (*attBsc*) of SCC*mec*	Nasal swab	1
	MRSA Advanced Test	Roche	LightCycler	FRET probes	Insertion site SSC*mec* at *orfX* junction	Nasal swab	2
	NucliSENS EasyQ MRSA	bioMerieux	EasyQ System (NASBA)	Molecular beacons	SSC*mec* at *orfX* junction and *mecA* gene for oxacillin resistance	Nasal swab	3

Sensitivity et specificity >90 %
NPV > 95%

Palavecino, Meth Mol Biol 2014

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Automated systems

BD MAX system
(Becton Dickinson)

~2 min HOT per sample

GeneXpert
(Cepheid)

Widen et al., J Clin Microbiol 2014

16

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

Roisin et al., PLoS One 2014

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Commercial tests for VRE

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

	Sens.	Spe.	PPV	NPV
Xpert *vanA/vanB*				
vanA	74-100	93-99	67-89	81-100
vanB	87-100	15-86	**3-33**	71-100
BD GeneOhm VanR				
vanA	43-88	96-100	82-100	67-97
vanB	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

18

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[a]	870.40 to 2,611.20[a]

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

Birgand et al., Antimicrob Resist Infect Control 2013 19

Commercial tests for CPE

Numerous kits for detection directly from stools/rectal swabs

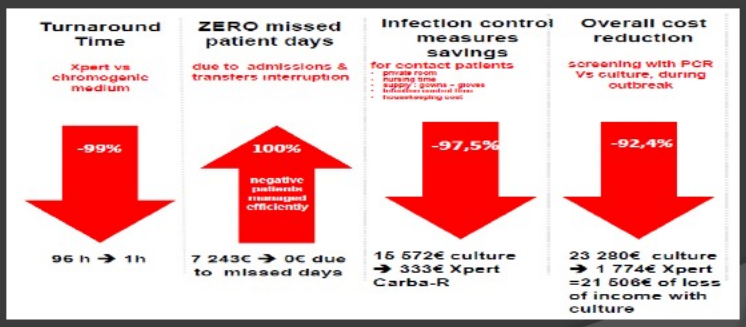
Test/Method	Turnaround time	Target species (n*)	Target carbapenemases	Sensitivity (%)	Specificity (%)	PPV† (%)	NPV‡ (%)
Check-Direct CPE	<180 min	Gram –negative bacili (83–450)	KPC, VIM, NDM, OXA-48	97·1–100·0	94·0–100·0	100·0	0·0–70·0
LAMP/eazyplex® superBug complete A	25–60 min	Gram –negative bacili (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 bacili (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 bacili (117)	Classes A, B, D	98·2	97·4	ND	ND
Microarray (Verigene BC-GN)	2 h	Gram-negative bacili (104)	Classes A, B, D	96·8	100·0	ND	ND
Microarray (Check-MDR CT101-103)	≤6 h	Gram-negative bacili (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 bacili (328)	KPC, NDM, VIM	100·0	99·0–99·4	81·8–93·0	100·0

>90%

Sekyere et al., J Appl Microbiol 2015 20

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

Dubouix-Bourandy et al., ECCMID 2015 21

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.

http://contemporaryobgyn.modernmedicine.com/ 22

Commercial tests for *S. aureus*/MRSA

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

Sensitivity et specificity >90 %
NPV > 95%

Palavecino, Meth Mol Biol 2014

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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

Buchan et al., J Clin Microbiol 2015

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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] (n = 12)	Group 2[b] (n = 48)	P[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)

Parta et al., Infect Control Hosp Infect 2010 25

Commercial tests for *C. difficile*

Assay	Method	Target	DNA extraction	HOT (min)†	TAT (min)†	Cost per test	Sens. (%)	Spe. (%)
BD GeneOhm Cdiff	RT-PCR	*tcdB*	Manual	45	120	$47‡	82-98	91-100
Illumigene *C. difficile*	LAMP	*tcdA*	Manual	10	60	$33‡	73-98	91-100
Xpert *C. difficile*	Multiplex RT-PCR	*tcdB, cdt, tcdC*Δ*117*	Automated	10	60	$52‡	93-100	91-99
BD MAX Cdiff	RT-PCR	*tcdB*	Automated	10	100	$43‡	90-98	98-100
Portrait *C. difficile*	HDA	*tcdB*	Automated	10	100	$25	98	93
ProGastro Cd	RT-PCR	*tcdB*	Automated (easyMAG)	45	180	$25	77-100	93-99
Seeplex Diarrhea ACE	Multiplex PCR	*tcdB*	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++

Le Guern et al., Expert Rev Mol Diagn 2013 26

Detection of MTB

The Xpert MTB/RIF Molecular Beacon Assay

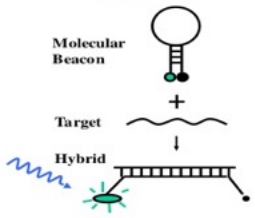
rpoB GENE 81 bp RIF RESISTANCE DETERMINING REGION

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



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

Malbruny et al., Int J Tuberc Lung Dis 2011 27

Broad molecular panels

➔ Syndromic approach

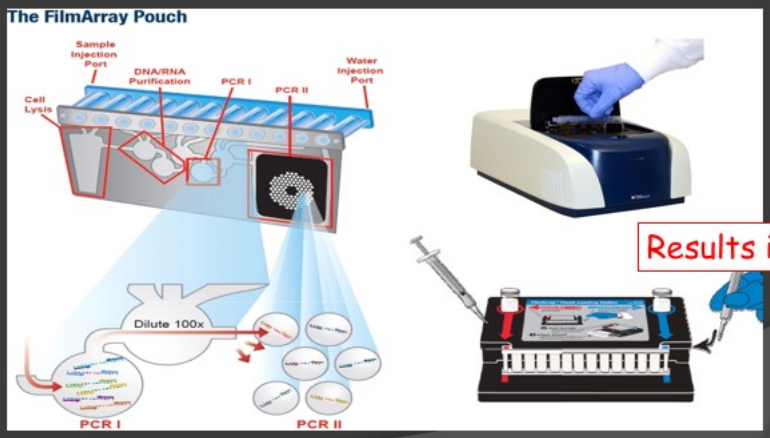
POTENTIAL ADVANTAGES	Increase diagnostic yield as multiple targets are tested in one sample
	Conserve and optimize analysis of samples difficult to obtain (spinal fluid, vitreous fluids, synovial fluids)
	Simplify ordering algorithm as only one test needs to be requested
	Streamline workflow in the laboratory 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)

https://www.aacc.org/publications/cln/articles/2015/april/one-sample-multiple-results 28

FilmArray (Biofire, bioMérieux)

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

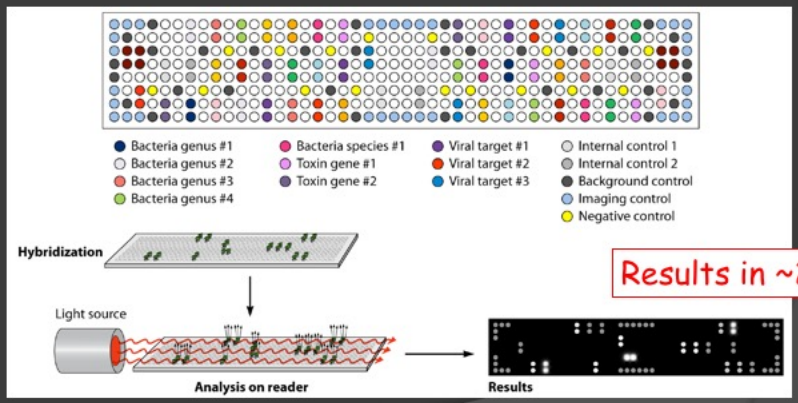


Results in ~1 h

29

Verigene (Nanosphere)

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



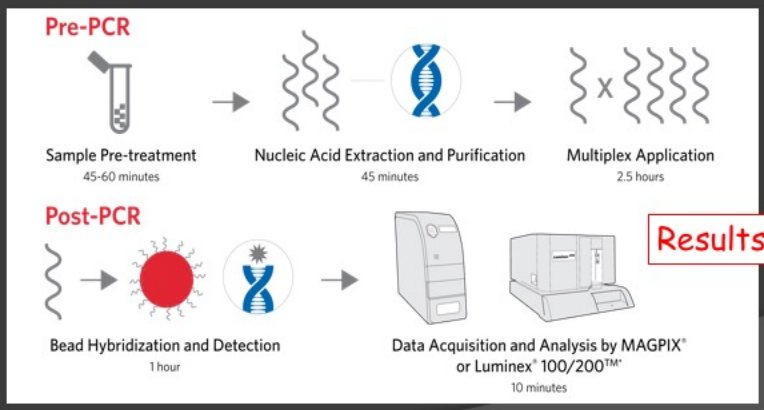
Results in ~2 h

Buchan & Ledeboer, Clin Microbiol Rev 2015

30

xTAG (Luminex)

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



Results in ~5 h

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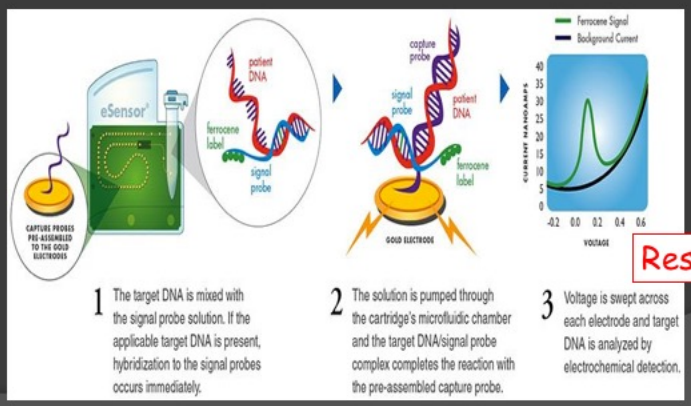
ePlex (GenMark Dx)

Principle: Competitive DNA hybridization and electrochemical detection (eSensor)



Results in ~1.5 h

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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	*E. coli* K1	2/2	100	34.2–100	1,557/1,558	99.9	99.6–100
	H. influenzae	1/1	100		1,558/1,559	99.9	99.6–100
	L. monocytogenes	0/0			1,560/1,560	100	99.8–100
	N. meningitidis	0/0			1,560/1,560	100	99.8–100
	S. agalactiae	0/1	0.0		1,558/1,559	99.9	99.6–100
	S. pneumoniae	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	*C. neoformans*/*C. gattii*	1/1	100		1,555/1,559	99.7	99.3–99.9

Leber et al., J Clin Microbiol 2015 33

Bacteremia (positive BCs)

2 automated systems

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

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

Patel, Mayo Clin Proc 2016 34

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]

Walker et al., J Clin Microbiol 2016

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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			
Campylobacter species	✓	✓	✓
Clostridium difficile	✓	✓	
Plesiomonas shigelloides	✓		
Salmonella species	✓	✓	✓
Yersinia enterocolitica	✓		✓
Vibrio cholerae	✓	✓	
Vibrio species	✓		✓
Enteroaggregative *Escherichia coli*	✓		
Enteropathogenic *Escherichia coli*	✓		
Enterotoxigenic *Escherichia coli*	✓	✓	
Shiga toxin–producing *Escherichia coli*	✓	✓	✓
Escherichia coli O157	✓	✓	
Shigella species (enteroinvasive *Escherichia coli*)	✓	✓	✓
Parasites			
Cryptosporidium species	✓	✓	
Cyclospora cayetanensis	✓		
Entamoeba histolytica	✓	✓	
Giardia lamblia	✓	✓	
Viruses			
Adenovirus serotypes 40/41	✓	✓	
Astrovirus	✓		
Norovirus	✓	✓	✓
Rotavirus	✓	✓	✓
Sapovirus	✓		

Patel, Mayo Clin Proc 2016

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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 (229[E], 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*

Popowitch et al., J Clin Microbiol 2013

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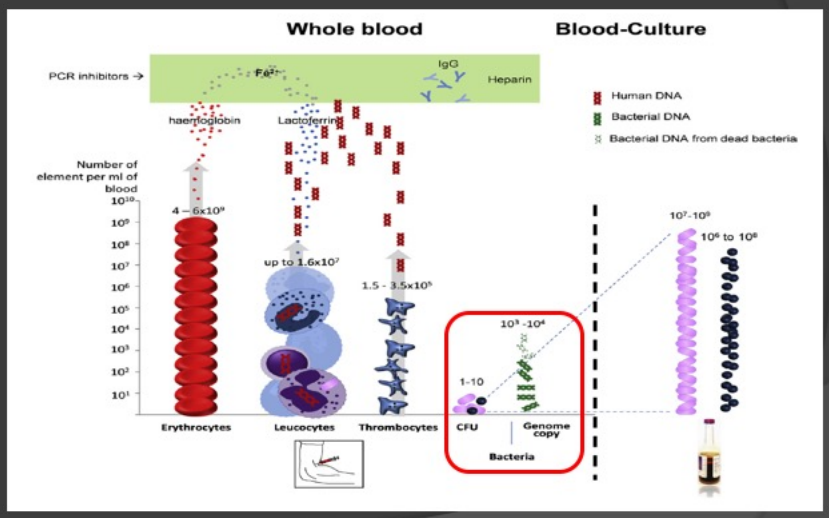
Bloodstream infections (BSI)



Opota et al., Clin Microbiol Infect 2015

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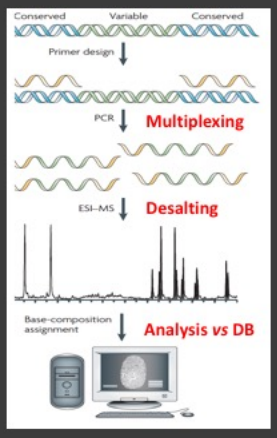
Diagnosis of BSI directly from blood

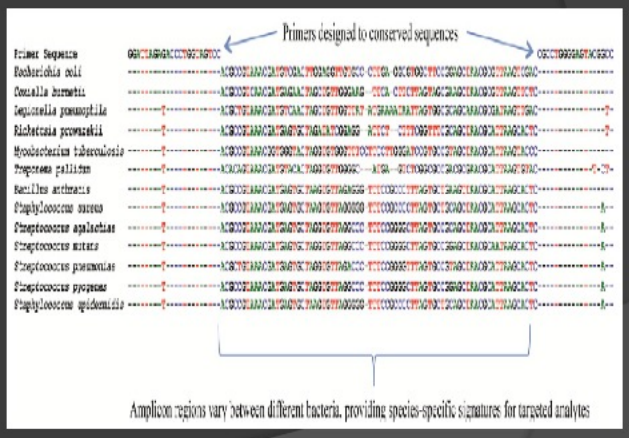
System	Method	Time to result (hours)	Blood volume (mL)	Microorganism coverage	Resistance and virulence markers	Sensitivity, specificity, and correlation with conventional methods (%)	Comments
SepsiTest Molzym, Bremen, Germany	Broad-range PCR + sequencing	6	1–10[a]	>345 bacteria (Gram positive and Gram 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	*mecA*[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	*mecA*, *vanA/B*	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)	*mecA*, bla_{KPC}, *vanA/B*	50–91[d], 98–99, 79–97	Pros: universal, detection of mixed bacterial populations, semiquantitative; Cons: no interventional studies

Opota et al., Clin Microbiol Infect 2015 39

PCR-ESI-MS

PCR-ElectroSpray Ionization-Mass Spectrometry





Ecker et al., Nat Rev Microbiol 2008 ; Lavigne et al., Clin Chem Lab Med 2012 ; Wolk et al., J Mol Diagn 2012 40

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: mecA, vanA, vanB and kpc	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})

Ecker et al., Nat Rev Microbiol 2008 ; Lavigne et al., Clin Chem Lab Med 2012 ; Wolk et al., J Mol Diagn 2012

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NGS in bacteriology

✓ Genomics (Whole Genome Sequencing – WGS)
- List of virulence genes
- In silico antibiogram
- Molecular typing

✓ Metagenomics
- Microbiota diversity
- Personalized medicine

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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

MacCannell, Clin Microbiol Newsl 2016

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In silico antibiogram of *S. aureus*

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

12 ATB

Antimicrobial agent	No. of isolates resistant by phenotype: Susceptible by genotype[b]	No. of isolates resistant by phenotype: Resistant by genotype	No. of isolates susceptible by phenotype: Susceptible by genotype	No. of isolates susceptible by phenotype: Resistant by genotype[b]	Total no. of isolates	Very major error rate (%) (95% CI)	Major error rate (%) (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
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 %

Gordon et al., J Clin Microbiol 2014

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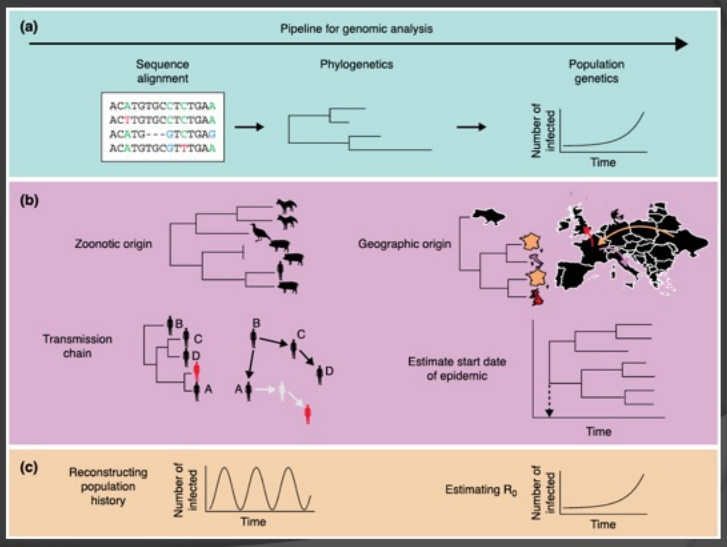
Molecular epidemiology by WGS



Li et al., Genome Biol 2014

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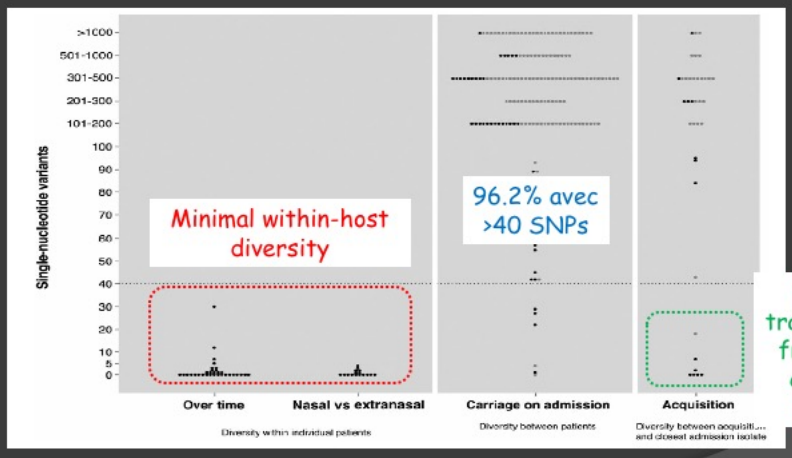
Transmission mapping



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

Price et al., CID 2014

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Current limits of WGS

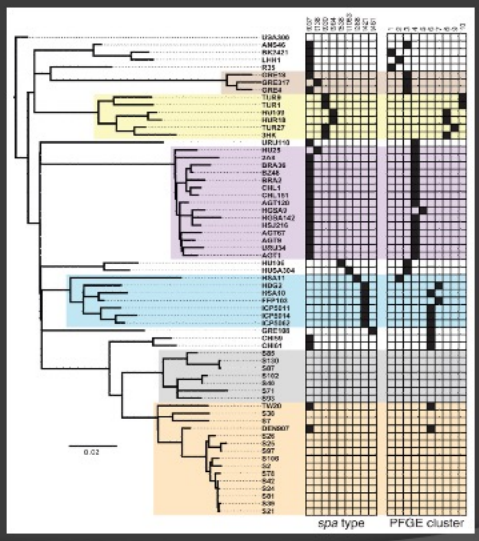
Current limitations

- 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?

Sherry et al., J Clin Microbiol 2013 ; Croucher & Didelot, Curr Opin Microbiol 2015 ; Gilchrist et al., Clin Microbiol Rev 2015 47

'Molecular clock'



Evolution and diversification within a bacterial population by accumulation of mutations over time

May depend of different settings, hosts or lineages

In *S. aureus* :
-Mutation rate from 2.0 to 3.4 × 10^{-6} mutations per site/year
-ie 5.6 to 9.5 mutations / year
-ie 1 SNV / 5-10 weeks

Definition of a clone ? Species specific?

Harris et al., Science 2010 ; Price et al., J Hosp Infect 2013 ; Price et al., Clin Infect Dis 2014 48

Third-generation NGS

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

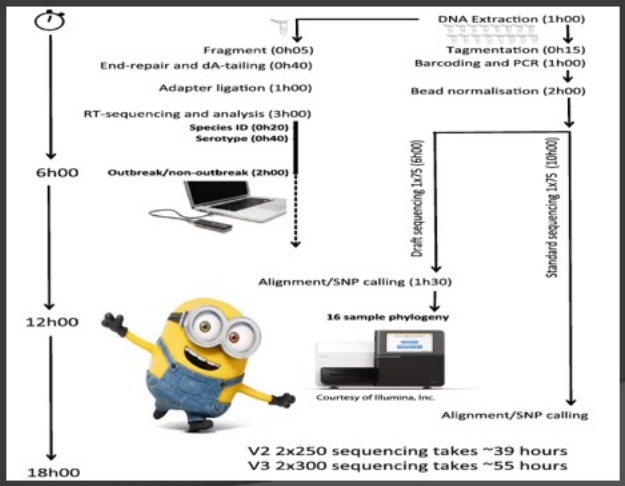
Platform	Instrument	Form factor	Sequencing technology[a]	Instrument cost	Read length (bp)	Run time (modes)	Single read output (modes)[c]	Sequence output (modes)	Approx cost per isolate (bacterial)
Pacific BioSciences	RSII	Capital	SMRT	$700,000	10,000-15,000	4 h	50,000	1 Gb	$500-600
	Sequel	Capital	SMRT	$350,000	10,000-20,000	6 h	350,000	7 Gb	TBD
Oxford Nanopore	MinION Mkl	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

[a]SBS, sequencing by synthesis; SMRT, single-molecule real-time sequencing.
[b]TBD, to be determined.

MacCannell, Clin Microbiol Newsl 2016 49

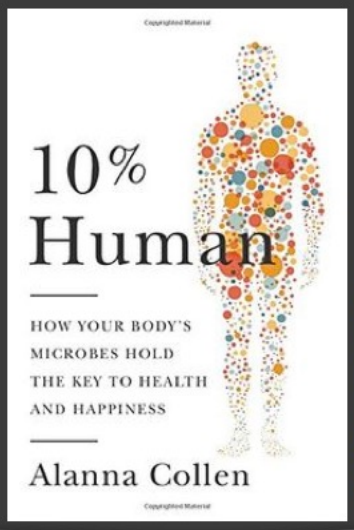
Applications - MinION

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

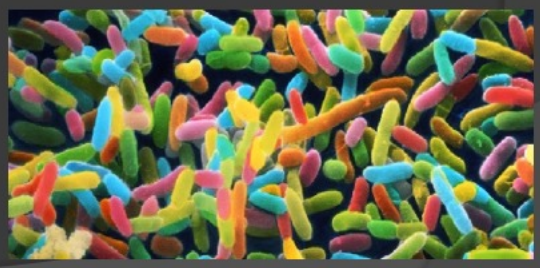


Quick et al., Genom Biol 2015 50

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



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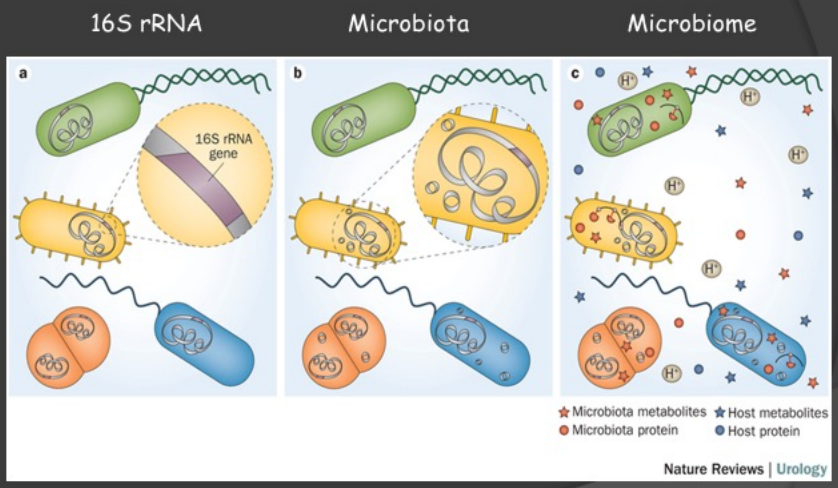
Microbiota & Microbiome



Whiteside et al., Nat Rev Urol 2015

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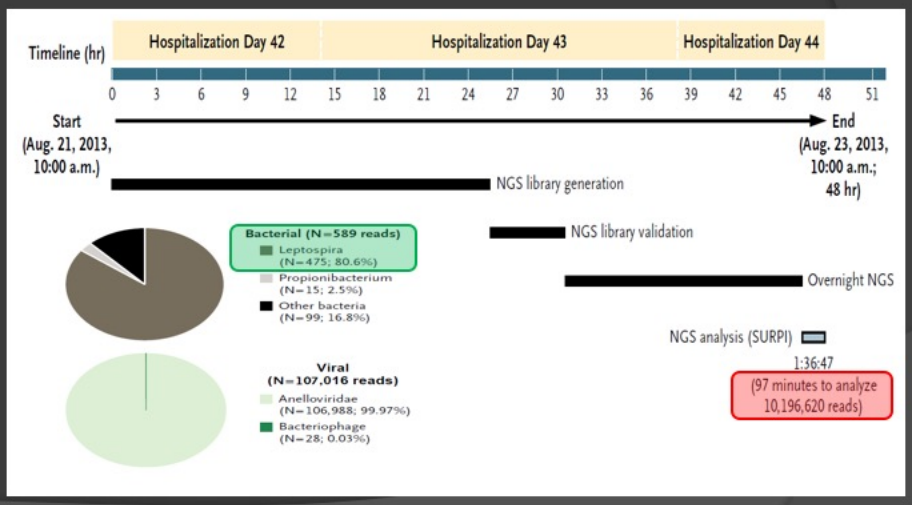
Diagnosis by metagenomics

Diagnosis of a case of neuroleptospirosis (MiSeq)



Wilson et al., New Engl J Med 2014

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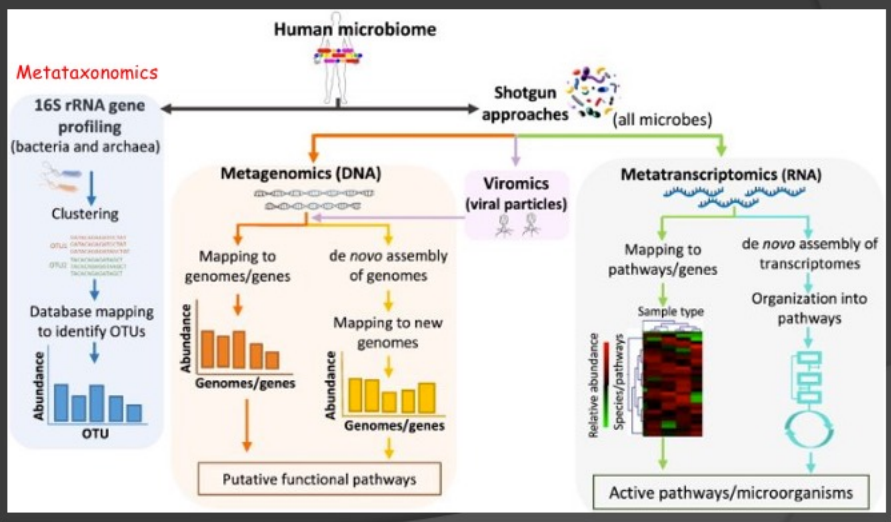
Metagenomics



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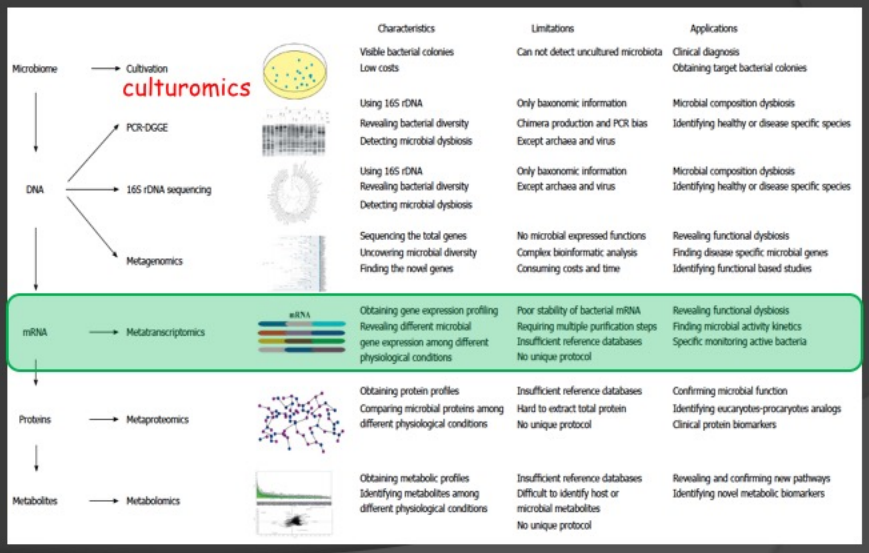
Study of the gut microbiota



Wang et al., World J Gasctroenterol 2015 55

Culturomics

18 culture conditions

Culture conditions for culturomics standardization
Preincubation in aerobic blood culture bottle with rumen fluid and then 5% sheep blood agar under aerobic conditions at 37°C
Preincubation in anaerobic blood culture bottle with rumen fluid and then 5% sheep blood agar under anaerobic conditions at 37°C
Preincubation in anaerobic blood culture bottle and then 5% sheep blood agar under anaerobic conditions at 37°C
Preincubation under aerobic conditions in Trypticase soy broth and then 5% sheep blood agar under aerobic conditions at 37°C
Preincubation under anaerobic conditions in 5% sheep blood broth and then 5% sheep blood agar under anaerobic conditions at 28°C
Preincubation under aerobic conditions in 5% sheep blood broth and then 5% sheep blood agar under aerobic conditions at 28°C
Preincubation under anaerobic conditions in 5% sheep blood broth and then 5% sheep blood agar under anaerobic conditions at 37°C
Preincubation under aerobic conditions in 5% sheep blood broth and then 5% sheep blood agar under aerobic conditions at 37°C
Preincubation in anaerobic blood culture bottle with stool filtered at 5 µm and then 5% sheep blood agar under anaerobic conditions at 37°C
Preincubation in aerobic blood culture bottle with stool filtered at 5 µm and then 5% sheep blood agar under aerobic conditions at 37°C
Preincubation in aerobic blood culture bottle with 5 ml sheep blood and then 5% sheep blood agar under aerobic conditions at 37°C
Preincubation in anaerobic blood culture bottle with 5 ml sheep blood and then 5% sheep blood agar under anaerobic conditions at 37°C
Preincubation in anaerobic blood culture bottle after thermic shock at 80°C during 20 min and then 5% sheep blood agar under anaerobic conditions at 37°C
Preincubation in anaerobic blood culture bottle with 5 ml rumen fluid and sheep blood and then 5% sheep blood agar under anaerobic conditions at 37°C
Preincubation in aerobic blood culture bottle with 5 ml rumen fluid and sheep blood and then 5% sheep blood agar under aerobic conditions at 37°C
Preincubation under aerobic conditions in brain heart infusion broth with 5% sheep blood and then 5% sheep blood agar under aerobic conditions at 37°C
Preincubation under anaerobic conditions in marine broth and then 5% sheep blood agar under anaerobic conditions at 37°C
Preincubation in aerobic marine broth and then 5% sheep blood agar under aerobic conditions at 37°C

Lagier et al., Clin Microbiol Rev 2015 56

Challenges in bioinformatics

Processing and long-term archiving of huge amounts of data (x 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

Caboche et al., Pathogens 2014

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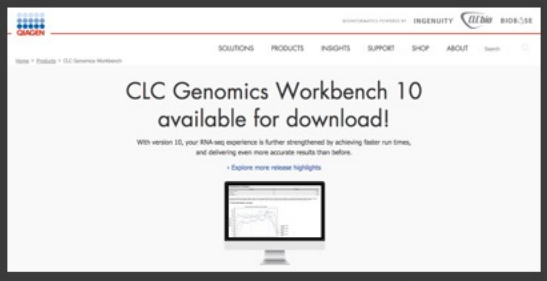
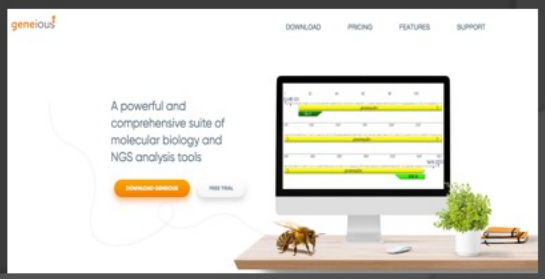
Softwares





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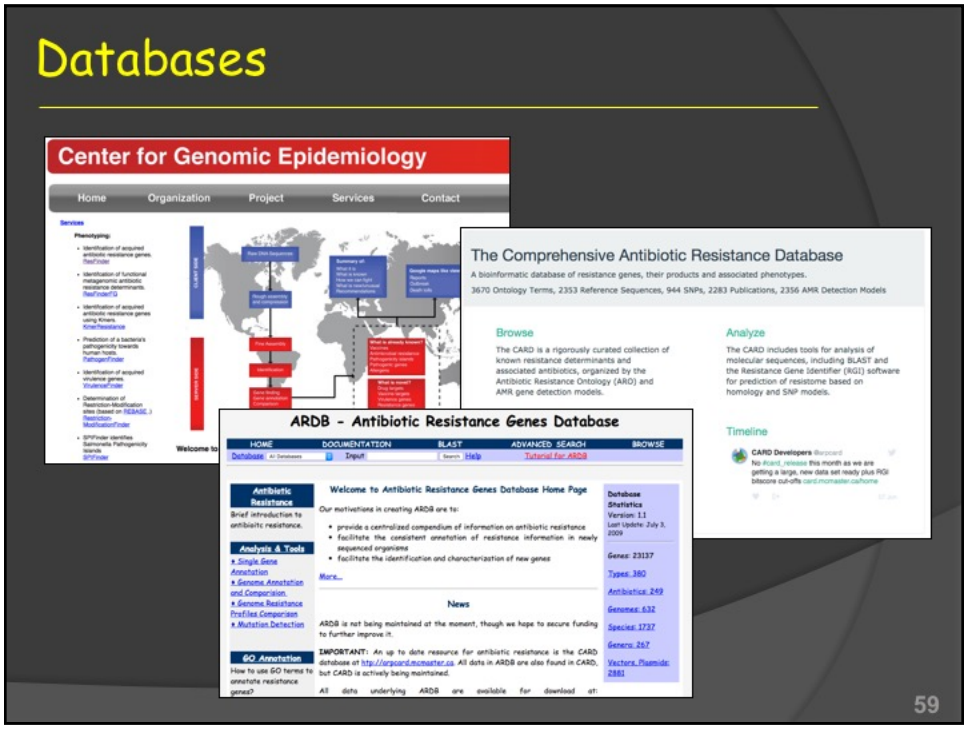

www.webbertraining.com/schedulep1.php	
April 18, 2019	INFECTION CONTROL ISSUES IN HEALTHCARE CONSTRUCTION, PART 1 - RENOVATION Speaker: **Andrew Streifel**, University of Minnesota
May 3, 2019	*(FREE … WHO Teleclass - Europe … Special Lecture for 5 May)* CLEAN CARE FOR ALL - IT'S IN YOUR HANDS Speaker: **Prof. Didier Pittet** and **Prof. Benedetta Allegranzi**, World Health Organization, Geneva **Sponsored by the World Health Organization Infection Control Global Unit**
May 16, 2019	IMMIGRANT AND REFUGEE POPULATIONS: A PUBLIC HEALTH AND POLICY PERSPECTIVE ON A CONTINUTING GLOBAL CRISIS Speaker: **Prof. Sotirios Tsiodras**, National and Kapodistrian University of Athens, Greece
May 27, 2019	*(FREE Teleclass – Broadcast live from the IPAC Canada conference)* To be announced Speaker: **To be confirmed**
May 29, 2019	*(FREE Teleclass – Broadcast live from the IPAC Canada conference)* ONE HEALTH: THE RISKS AND REWARDS OF LOVING ANIMALS Speaker: **Prof. Jason Stull**, Ohio State University


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