Strategy on Antibiotic Resistance
StAR

Bern – November 22nd, 2019
(11:00-11:25)

ifik
institut für infektionskrankheiten
Ein Institut der Universität Bern

UNIVERSITÄT BERN

Symposium Antibiotic Resistance StAR

MDROs:
molecular diagnostic tools

Prof. Andrea Endimiani, MD, PhD

Swiss Society for Microbiology, SSM

Institute for Infectious Diseases (IFIK) - University of Bern, Switzerland

In this short lecture …..

MDROs:

focus on Gram-negatives, especially CPE

Molecular:

detection of antimicrobial resistance genes/proteins

No colorimetric/ biochemical tests (e.g., NP tests)

No rapid ASTs (e.g., Accelerate Pheno System)

Diagnostic tools:

those "rapid", commercially available, and used in the clinical context for

Colonization (gut)

Confirmation (colonies)

Bacteremia

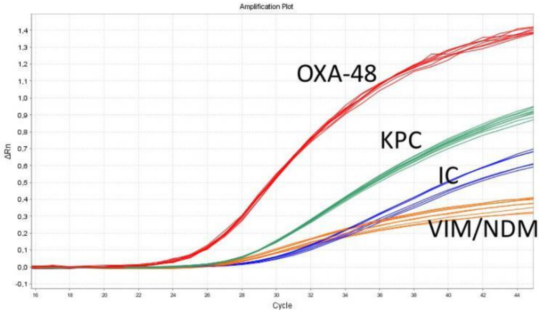
No metagenomics (NGS)

Check-Direct Screening (Check-Points)

Rectal swab

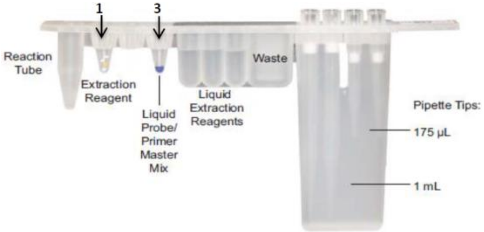
- **Real-time multiplex PCR**
- Rapid preparation
- Time to results (**<3 hrs**)

~30-50 Euro



BD MAX

Reagent strip

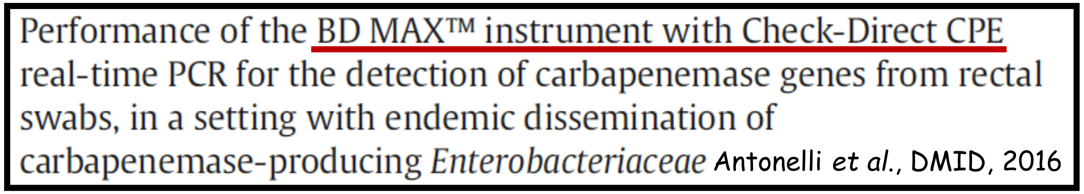


Check-Direct ESBL
Screen for BD MAX™

- CTX-M-1 group
- CTX-M-2 group
- CTX-M-9 group
- SHV-ESBLs

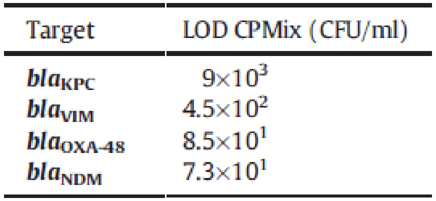
Check-Direct CPE /CPO
Screen for BD MAX™

- KPC
- OXA-48-like incl. OXA-181, OXA-232, OXA-244
- VIM
- NDM


Performance of the BD MAX™ instrument with Check-Direct CPE real-time PCR for the detection of carbapenemase genes from rectal swabs, in a setting with endemic dissemination of carbapenemase-producing *Enterobacteriaceae* Antonelli *et al.*, DMID, 2016

Florence, Italy
557 rectal swabs

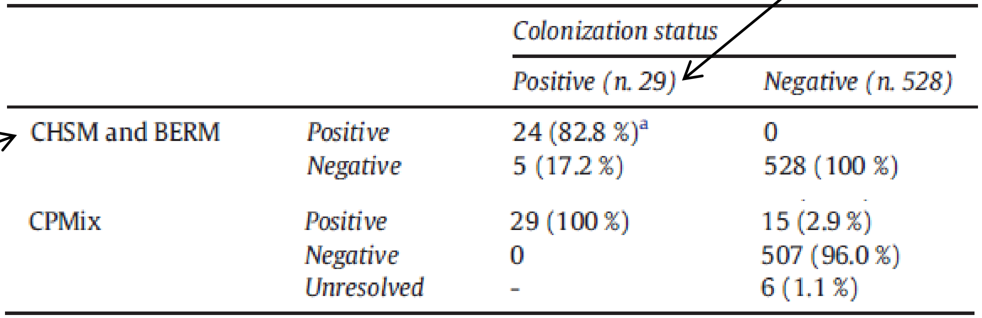
Limit of detection (LOD)



Target	LOD CPMix (CFU/ml)
bla$_{KPC}$	9×10^3
bla$_{VIM}$	4.5×10^2
bla$_{OXA-48}$	8.5×10^1
bla$_{NDM}$	7.3×10^1

23 KPC
5 VIM
1 OXA-48

Culture vs. Check-Direct CPE



		Colonization status	
		Positive (n. 29)	*Negative (n. 528)*
CHSM and BERM	*Positive*	24 (82.8 %)[a]	0
	Negative	5 (17.2 %)	528 (100 %)
CPMix	*Positive*	29 (100 %)	15 (2.9 %)
	Negative	0	507 (96.0 %)
	Unresolved	-	6 (1.1 %)

ChromID CARBA SMART
(w/wo broth enrichment)
[5 samples not detected]

TAT from 18-24 hrs (direct culture) or 48 hrs (broth enrichment) to 3 hrs

	KPC	VIM	OXA-48	NDM
Sensitivity	100%	100%	100%	-
Specificity	99.6%	97.6%	99.6%	100%

GeneXpert (Cepheid)

- Add aliquot to elution, vortex, transfer to port S
- Insert cartridge to station (overall, 1 min)
- Run time (<1 h)

~50 Euro

Real-time multiplex PCR

- Smart fluidic system
- Filtering and Sonication (DNA)
- Fluorescent-labeled hybr. probes (6 colors)

Xpert® MRSA/SA BC

Xpert® vanA/vanB

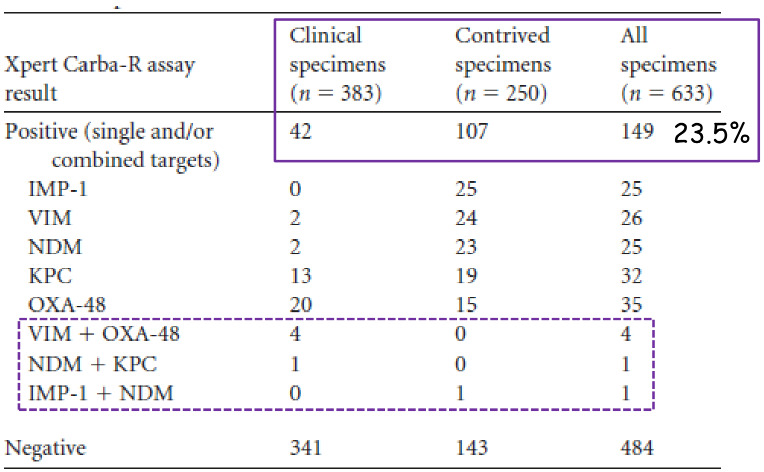
Xpert® Carba-R

KPC
NDM
OXA-48-like incl. OXA-181, OXA-232, OXA-244
VIM
IMP-1

Multisite Evaluation of Cepheid Xpert Carba-R Assay for Detection of Carbapenemase-Producing Organisms in Rectal Swabs JCM, 54:7; 2016

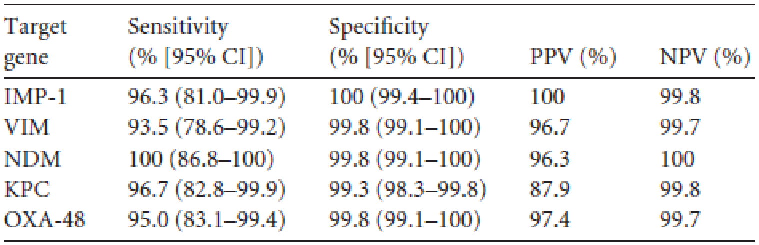
M. Tato,[a] P. Ruiz-Garbajosa,[a] M. Traczewski,[b] A. Dodgson,[c] A. McEwan,[c] R. Humphries,[d] J. Hindler,[d] J. Veltman,[e] H. Wang,[f] R. Cantón[a]

4 centers
(2 USA, 1 UK, 1 Spain)



Xpert Carba-R assay result	Clinical specimens ($n = 383$)	Contrived specimens ($n = 250$)	All specimens ($n = 633$)
Positive (single and/or combined targets)	42	107	149 23.5%
IMP-1	0	25	25
VIM	2	24	26
NDM	2	23	25
KPC	13	19	32
OXA-48	20	15	35
VIM + OXA-48	4	0	4
NDM + KPC	1	0	1
IMP-1 + NDM	0	1	1
Negative	341	143	484

Performance for different targets



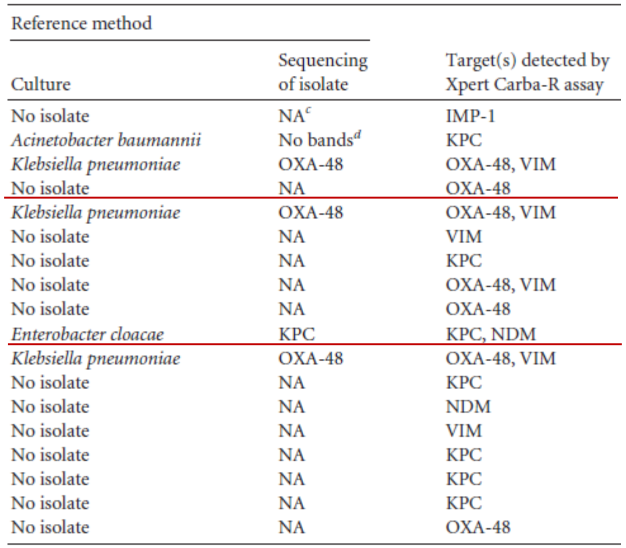
Target gene	Sensitivity (% [95% CI])	Specificity (% [95% CI])	PPV (%)	NPV (%)
IMP-1	96.3 (81.0–99.9)	100 (99.4–100)	100	99.8
VIM	93.5 (78.6–99.2)	99.8 (99.1–100)	96.7	99.7
NDM	100 (86.8–100)	99.8 (99.1–100)	96.3	100
KPC	96.7 (82.8–99.9)	99.3 (98.3–99.8)	87.9	99.8
OXA-48	95.0 (83.1–99.4)	99.8 (99.1–100)	97.4	99.7

Sensitivity: 96.6%
Specificity: 98.6%

PPV: 95.3%
NPV: 99.0%

Results in 32-48 min

Discrepant results

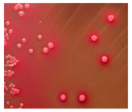


Reference method: Culture	Sequencing of isolate	Target(s) detected by Xpert Carba-R assay
No isolate	NA[c]	IMP-1
Acinetobacter baumannii	No bands[d]	KPC
Klebsiella pneumoniae	OXA-48	OXA-48, VIM
No isolate	NA	OXA-48
Klebsiella pneumoniae	OXA-48	OXA-48, VIM
No isolate	NA	VIM
No isolate	NA	KPC
No isolate	NA	OXA-48, VIM
No isolate	NA	OXA-48
Enterobacter cloacae	KPC	KPC, NDM
Klebsiella pneumoniae	OXA-48	OXA-48, VIM
No isolate	NA	KPC
No isolate	NA	NDM
No isolate	NA	VIM
No isolate	NA	KPC
No isolate	NA	KPC
No isolate	NA	KPC
No isolate	NA	OXA-48

Evaluation of a New Commercial Microarray Platform for the Simultaneous Detection of β-Lactamase and *mcr-1* and *mcr-2* Genes in *Enterobacteriaceae*

Odette J. Bernasconi,[a,b] Luigi Principe,[c] Regula Tinguely,[a] Aneta Karczmarek,[d] Vincent Perreten,[e] Francesco Luzzaro,[c] Andrea Endimiani[a]

Journal of Clinical Microbiology
October 2017 Volume 55 Issue 10



Confirmatory test after ASTs

≥ 8 hrs

85 Euro

New CT103XL (Check-Points)

Broad-spectrum: TEM and SHV

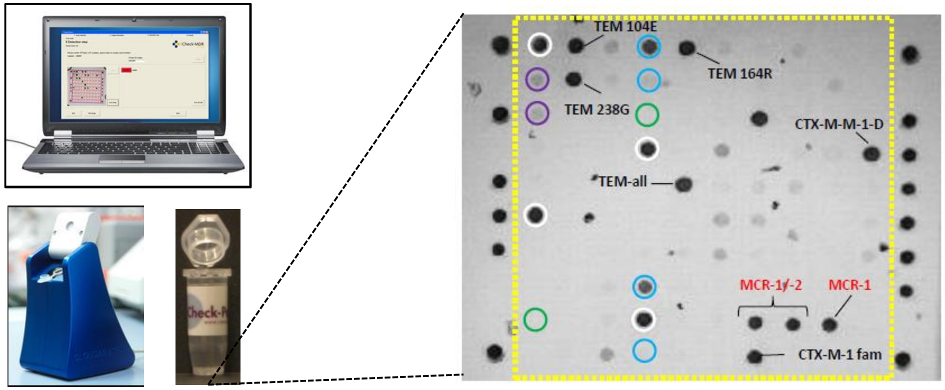
ESBLs: TEM, SHV, CTX-M, BEL, PER, GES, VEB

pAmpCs: CMY, DHA, FOX, ACC-1, ACT/MIR

Carba: KPC, NDM, VIM, IMP, GIM, SPM, OXA-48-like $^{\text{incl. 181/-232/-244}}$, -23, -24, -58

Mcr-1 and *mcr-2*

E. coli strain



Sensitivity and Specificity for all target genes: both ~100%

Oxford NANOPORE Technologies

Rapid WGS

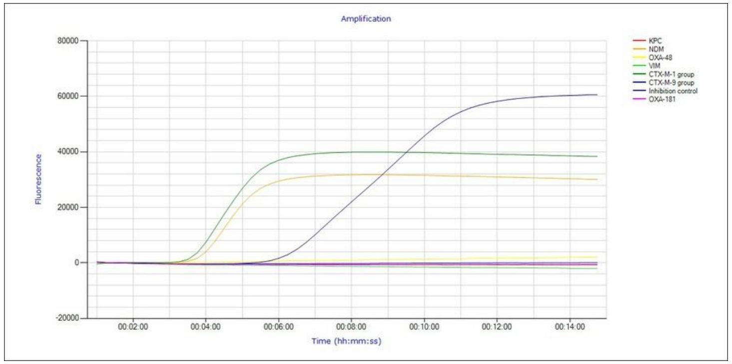
In few hrs
enough reads/coverage
to reports all ARGs

6 strains in 1 cell
~80 CHF each

Eazyplex (Amplex Diagnostics)

Genie II platform

- Preparation (**5 min**)
- No DNA extraction/purification
- Run time (**<30 min**)



LAMP
Loop-mediated isothermal Amplification

Real-time fluorescent measurement

50 CHF

eazyplex® MRSA

S. aureus
mecA
mecC
S. epidermidis

eazyplex® VRE

eazyplex® SuperBug mcr-1

eazyplex ® SuperBug Acineto

eazyplex® SuperBug complete

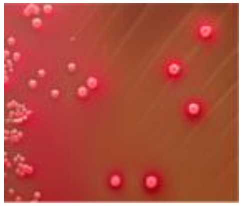
	SuperBug complete A	SuperBug complete B	SuperBug complete C
NDM	X	X	X
VIM	X	X	X
KPC	X	X	X
OXA-48	X	X	X
OXA-23	X	X	
OXA-40	X	X	
OXA-58	x		
OXA-181		x	X
IMP			x

eazyplex® SuperBug CRE

KPC,
NDM,
OXA-48 incl. *OXA-244*
and OXA 181,
VIM, as well as
CTX-M-1 and
CTX-M-9 group

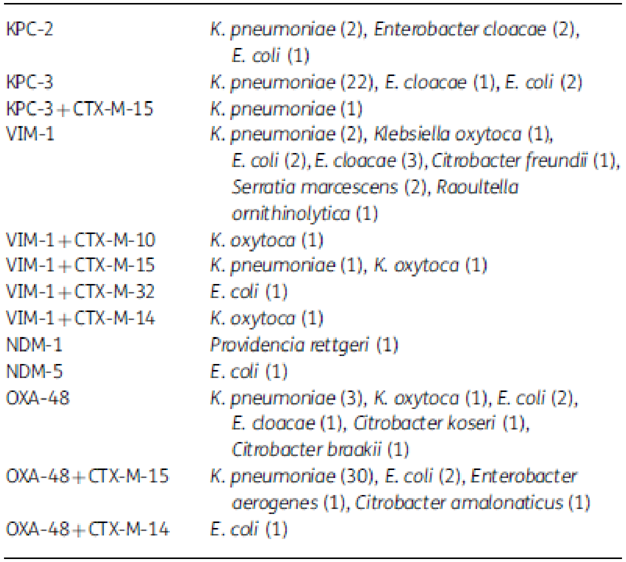
Evaluation of the eazyplex® SuperBug CRE system for rapid detection of carbapenemases and ESBLs in clinical Enterobacteriaceae isolates recovered at two Spanish hospitals

J Antimicrob Chemother 2015; **70**: 1047–1050



94 CPE

100% agreement
with PCR/Sequencing of *bla* genes



KPC-2	*K. pneumoniae* (2), *Enterobacter cloacae* (2), *E. coli* (1)
KPC-3	*K. pneumoniae* (22), *E. cloacae* (1), *E. coli* (2)
KPC-3+CTX-M-15	*K. pneumoniae* (1)
VIM-1	*K. pneumoniae* (2), *Klebsiella oxytoca* (1), *E. coli* (2), *E. cloacae* (3), *Citrobacter freundii* (1), *Serratia marcescens* (2), *Raoultella ornithinolytica* (1)
VIM-1+CTX-M-10	*K. oxytoca* (1)
VIM-1+CTX-M-15	*K. pneumoniae* (1), *K. oxytoca* (1)
VIM-1+CTX-M-32	*E. coli* (1)
VIM-1+CTX-M-14	*K. oxytoca* (1)
NDM-1	*Providencia rettgeri* (1)
NDM-5	*E. coli* (1)
OXA-48	*K. pneumoniae* (3), *K. oxytoca* (1), *E. coli* (2), *E. cloacae* (1), *Citrobacter koseri* (1), *Citrobacter braakii* (1)
OXA-48+CTX-M-15	*K. pneumoniae* (30), *E. coli* (2), *Enterobacter aerogenes* (1), *Citrobacter amalonaticus* (1)
OXA-48+CTX-M-14	*E. coli* (1)

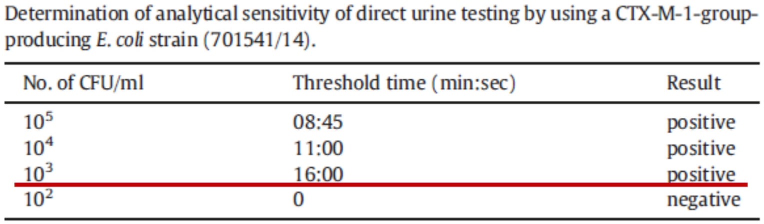
Extended-spectrum β-lactamase (ESBL) detection directly from urine samples with the rapid isothermal amplification-based eazyplex® SuperBug CRE assay: Proof of concept☆

Hinic V. *et al.*, J Microb Meth; 119, 2015

50 urine

30 were CTX-M +

LOD in Urine



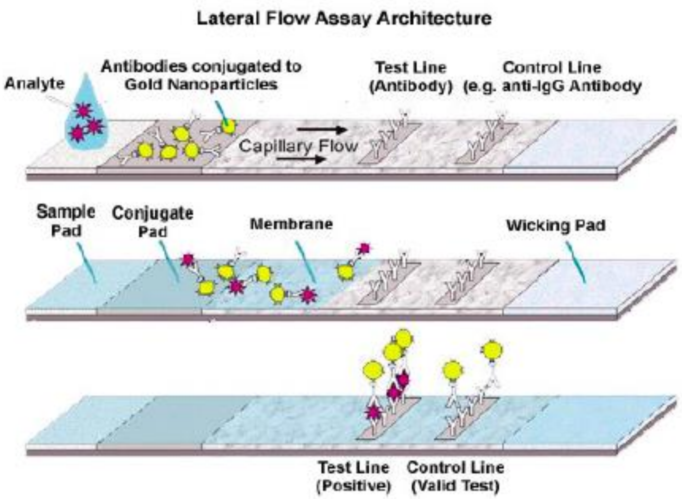
Determination of analytical sensitivity of direct urine testing by using a CTX-M-1-group-producing *E. coli* strain (701541/14).

No. of CFU/ml	Threshold time (min:sec)	Result
10^5	08:45	positive
10^4	11:00	positive
10^3	16:00	positive
10^2	0	negative

Sensitivity: 100%
Specificity: 97.9%

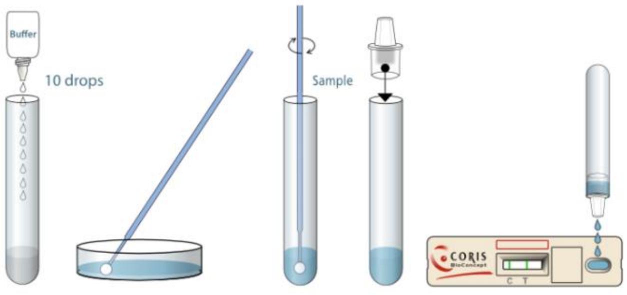
Avg. 8 min

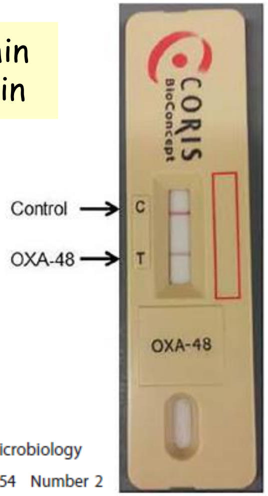
Lateral Flow Assays / Immunochromatographic Tests



2x10^6 CFU/mL
(colonies or blood culture)

Within
15 min



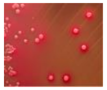


Journal of Clinical Microbiology
February 2016 Volume 54 Number 2

TARGET	PRODUCT NAME
OXA-48	OXA-48 *K*-SeT
KPC	KPC *K*-SeT
OXA-48 & OXA-163 & KPC	RESIST-3 O.O.K. *K*-SeT
OXA-48 & KPC & NDM	RESIST-3 O.K.N. *K*-SeT
OXA-23	OXA-23 *K*-SeT
OXA-48 & KPC NDM & VIM	RESIST-4 O.K.N.V.
OXA-48 & OXA-163 & KPC NDM & VIM	RESIST-5 O.O.K.N.V.
IMP	IMP *K*-SeT

15 Euro

Youri Glupczynski*, Stéphanie Evrard, Te-Din Huang and Pierre Bogaerts
J Antimicrob Chemother 2019; **74**: 1284–1287



Target	*n*	sensitivity (95% CI)	specificity (95% CI)
Global performance of the RESIST-4 *K*-SeT assay for the collection of retrospective and prospective clinical isolates (*n* = 479)			
OXA-48-like incl. *OXA-181, OXA-232, OXA-244*	112	100 (95.9–100)	100 (98.8–100)
VIM	104	99 (94.0–99.9)	100 (98.7–100)
NDM	61	100 (92.6–100)	100 (98.9–100)
KPC	31	100 (86.3–100)	100 (99.0–100)
Other carbapenemases/non-carbapenemases	184		
Target	*n*	positive predictive value (95% CI)	negative predictive value (95% CI)
Positive and negative predictive values of the RESIST-4 *K*-SeT assay for the collection of prospective clinical isolates (*n* = 345)			
OXA-48-like incl. *OXA-181*	90	100 (94.9–100)	100 (98.2–100)
VIM	65	100 (93.4–100)	100 (98.3–100)
NDM	22	100 (81.5–100)	100 (98.5–100)
KPC	19	100 (79.1–100)	100 (98.5–100)
Other carbapenemases/non-carbapenemases	151		

Type NDM : NDM-1 -4 -5 -6 -7 -9
Type KPC : KPC-2 -3
Type IMP : IMP-1 -8 -11
Type VIM : VIM-1 -2 -4 -19
OXA-48-like : OXA-48 -162 -181 -204 -232 -244 -517 -519 -535
Non-carbapenemases (cross-reactivity) : OXA-163 and OXA-405 (OXA-48-like extended spectrum oxacillinases with very weak carbapenemase activity).

+

Simplified Testing Method for Direct Detection of Carbapenemase-Producing Organisms from Positive Blood Cultures Using the NG-Test Carba 5 Assay

Giordano L *et al.*

Only *K. pneumoniae*

Antimicrobial Agents and Chemotherapy July 2019 Volume 63 Issue 7 e00550-19

Easy/rapid extraction from +BCs:
40 μL + 5 drops of extraction buffer

KPC, n=48
OXA-48-like, n=21
VIM, n=19
NDM, n=15
Combinations, n=8

Spiked BCs:
109/111 (98.2%) CPE detected

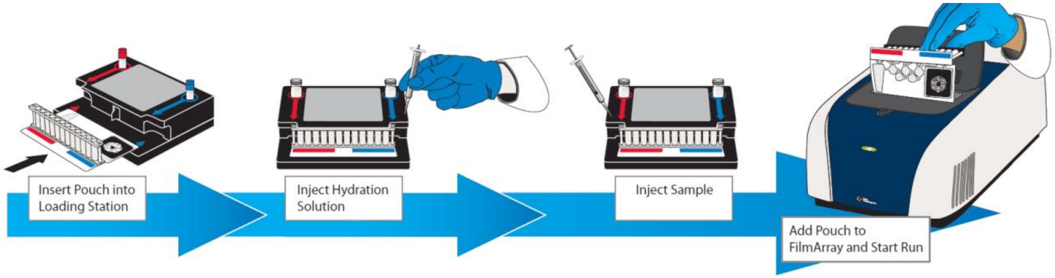
KPC, n=123
OXA-48-like, n=1
VIM, n=2

Clinical BCs:
124/126 (98.4%) CPE detected

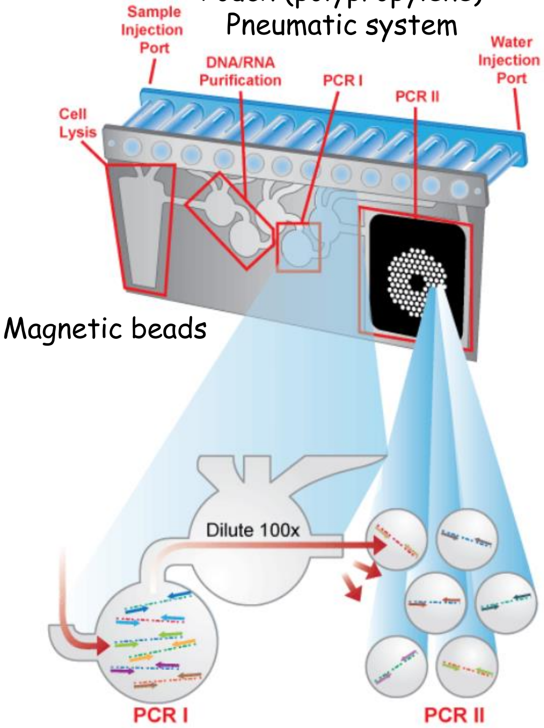
Overall:
Sensitivity: 98.3%
Specificity: 100%

BioFire FilmArray (bioMérieux)

+



Pouch (polypropylene)
Pneumatic system

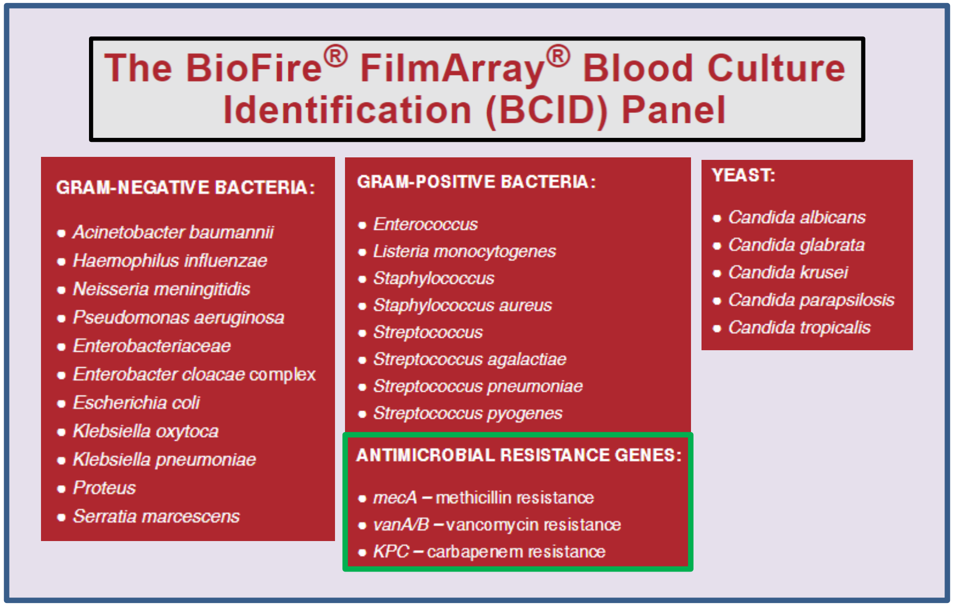


Multiplex PCR
(RT-PCR for RNA target)

Multiplex real-time PCR
and HRM analysis

- Preparation of the pouch
- Add pouch to FilmArray station (overall, **2 min**)
- Run time of about **1 h**

~100 Euro



The BioFire® FilmArray® Blood Culture Identification (BCID) Panel

GRAM-NEGATIVE BACTERIA:

- *Acinetobacter baumannii*
- *Haemophilus influenzae*
- *Neisseria meningitidis*
- *Pseudomonas aeruginosa*
- *Enterobacteriaceae*
- *Enterobacter cloacae* complex
- *Escherichia coli*
- *Klebsiella oxytoca*
- *Klebsiella pneumoniae*
- *Proteus*
- *Serratia marcescens*

GRAM-POSITIVE BACTERIA:

- *Enterococcus*
- *Listeria monocytogenes*
- *Staphylococcus*
- *Staphylococcus aureus*
- *Streptococcus*
- *Streptococcus agalactiae*
- *Streptococcus pneumoniae*
- *Streptococcus pyogenes*

ANTIMICROBIAL RESISTANCE GENES:

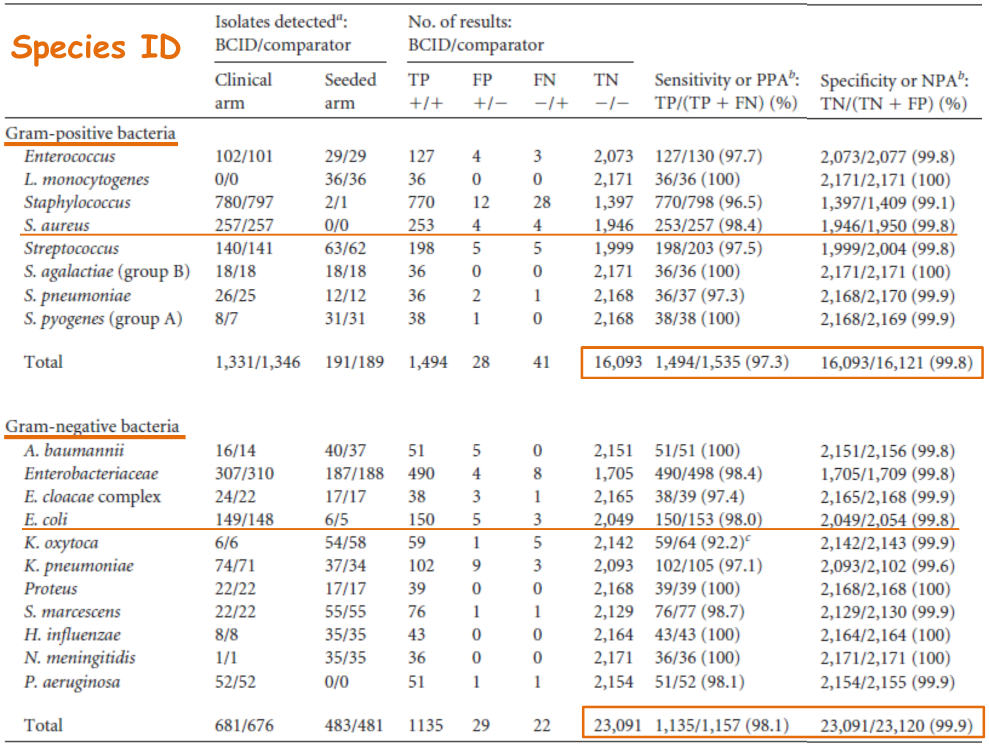
- *mecA* – methicillin resistance
- *vanA/B* – vancomycin resistance
- *KPC* – carbapenem resistance

YEAST:

- *Candida albicans*
- *Candida glabrata*
- *Candida krusei*
- *Candida parapsilosis*
- *Candida tropicalis*

Evaluation of the FilmArray Blood Culture Identification Panel: Results of a Multicenter Controlled Trial

Salimnia H.. *et al.*, J Clin Microbiol; 54:3, 2016



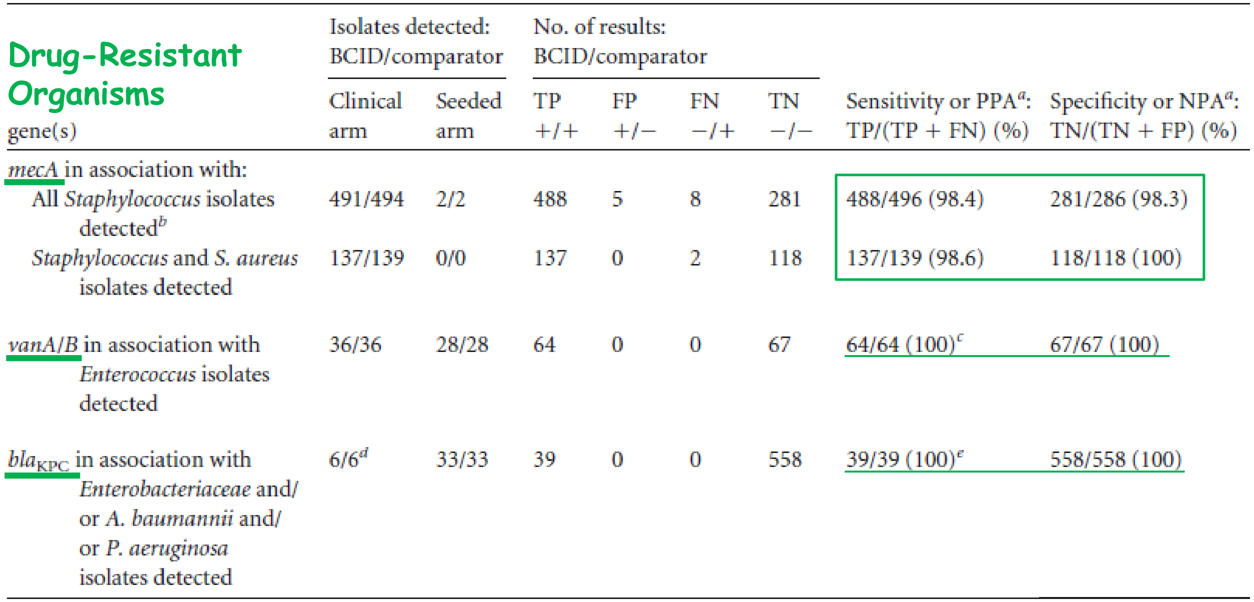
Species ID	Isolates detected[a]: BCID/comparator		No. of results: BCID/comparator					
	Clinical arm	Seeded arm	TP +/+	FP +/−	FN −/+	TN −/−	Sensitivity or PPA[b]: TP/(TP + FN) (%)	Specificity or NPA[b]: TN/(TN + FP) (%)
Gram-positive bacteria								
Enterococcus	102/101	29/29	127	4	3	2,073	127/130 (97.7)	2,073/2,077 (99.8)
L. monocytogenes	0/0	36/36	36	0	0	2,171	36/36 (100)	2,171/2,171 (100)
Staphylococcus	780/797	2/1	770	12	28	1,397	770/798 (96.5)	1,397/1,409 (99.1)
S. aureus	257/257	0/0	253	4	4	1,946	253/257 (98.4)	1,946/1,950 (99.8)
Streptococcus	140/141	63/62	198	5	5	1,999	198/203 (97.5)	1,999/2,004 (99.8)
S. agalactiae (group B)	18/18	18/18	36	0	0	2,171	36/36 (100)	2,171/2,171 (100)
S. pneumoniae	26/25	12/12	36	2	1	2,168	36/37 (97.3)	2,168/2,170 (99.9)
S. pyogenes (group A)	8/7	31/31	38	1	0	2,168	38/38 (100)	2,168/2,169 (99.9)
Total	1,331/1,346	191/189	1,494	28	41	16,093	1,494/1,535 (97.3)	16,093/16,121 (99.8)
Gram-negative bacteria								
A. baumannii	16/14	40/37	51	5	0	2,151	51/51 (100)	2,151/2,156 (99.8)
Enterobacteriaceae	307/310	187/188	490	4	8	1,705	490/498 (98.4)	1,705/1,709 (99.8)
E. cloacae complex	24/22	17/17	38	3	1	2,165	38/39 (97.4)	2,165/2,168 (99.9)
E. coli	149/148	6/5	150	5	3	2,049	150/153 (98.0)	2,049/2,054 (99.8)
K. oxytoca	6/6	54/58	59	1	5	2,142	59/64 (92.2)[c]	2,142/2,143 (99.9)
K. pneumoniae	74/71	37/34	102	9	3	2,093	102/105 (97.1)	2,093/2,102 (99.6)
Proteus	22/22	17/17	39	0	0	2,168	39/39 (100)	2,168/2,168 (100)
S. marcescens	22/22	55/55	76	1	1	2,129	76/77 (98.7)	2,129/2,130 (99.9)
H. influenzae	8/8	35/35	43	0	0	2,164	43/43 (100)	2,164/2,164 (100)
N. meningitidis	1/1	35/35	36	0	0	2,171	36/36 (100)	2,171/2,171 (100)
P. aeruginosa	52/52	0/0	51	1	1	2,154	51/52 (98.1)	2,154/2,155 (99.9)
Total	681/676	483/481	1135	29	22	23,091	1,135/1,157 (98.1)	23,091/23,120 (99.9)

8 centers in USA

July 2012 - Feb 2014

2,207 BC samples

- 1,568 clinical
- 639 seeded



Drug-Resistant Organisms gene(s)	Isolates detected: BCID/comparator		No. of results: BCID/comparator					
	Clinical arm	Seeded arm	TP +/+	FP +/−	FN −/+	TN −/−	Sensitivity or PPA[a]: TP/(TP + FN) (%)	Specificity or NPA[a]: TN/(TN + FP) (%)
mecA in association with:								
All *Staphylococcus* isolates detected[b]	491/494	2/2	488	5	8	281	488/496 (98.4)	281/286 (98.3)
Staphylococcus and *S. aureus* isolates detected	137/139	0/0	137	0	2	118	137/139 (98.6)	118/118 (100)
vanA/B in association with *Enterococcus* isolates detected	36/36	28/28	64	0	0	67	64/64 (100)[c]	67/67 (100)
bla_{KPC} in association with *Enterobacteriaceae* and/or *A. baumannii* and/or *P. aeruginosa* isolates detected	6/6[d]	33/33	39	0	0	558	39/39 (100)[e]	558/558 (100)

COMING SOON: THE BIOFIRE® FILMARRAY® BLOOD CULTURE IDENTIFICATION 2 (BCID2) PANEL*

More species

More ARGs

GRAM-POSITIVE BACTERIA:

- *Enterococcus faecalis**
- *Enterococcus faecium**
- *Listeria monocytogenes*
- *Staphylococcus*
    - *Staphylococcus aureus*
    - *Staphylococcus epidermidis**
    - *Staphylococcus lugdunensis**
- *Streptococcus*
    - *Streptococcus agalactiae*
    - *Streptococcus pneumoniae*
    - *Streptococcus pyogenes*

GRAM-NEGATIVE BACTERIA:

- *Acinetobacter calcoaceticus-baumannii* complex
- *Bacteroides fragilis**
- Enteric Bacteria
    - *Enterobacter cloacae* complex
    - *Escherichia coli*
    - *Klebsiella aerogenes**
    - *Klebsiella oxytoca*
    - *Klebsiella pneumoniae* group
    - *Proteus*
    - *Salmonella**
    - *Serratia marcescens*
- *Haemophilus influenzae*
- *Neisseria meningitidis*
- *Pseudomonas aeruginosa*
- *Stenotrophomonas maltophilia**

YEAST:

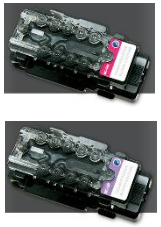
- *Candida albicans*
- *Candida auris**
- *Candida glabrata*
- *Candida krusei*
- *Candida parapsilosis*
- *Candida tropicalis*
- *Cryptococcus neoformans/gattii**

ANTIMICROBIAL RESISTANCE GENES:

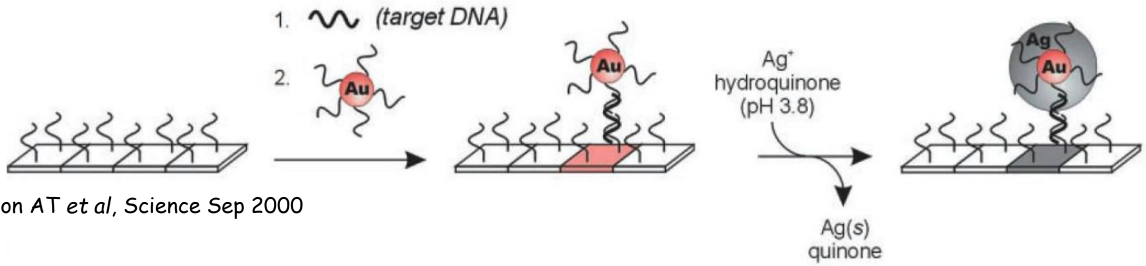
- **Carbapenemases**
    - IMP*
    - KPC
    - OXA-48-like* (?)
    - NDM*
    - VIM*
- **Colistin Resistance**
    - mcr-1*
- **ESBL**
    - CTX-M*
- **Methicillin Resistance**
    - *mec*A/C
    - *mec*A/C and MREJ (MRSA)*
- **Vancomycin Resistance**
    - *van*A/B

Preliminary data presented at ECCMID 2019

Verigene System (Luminex)



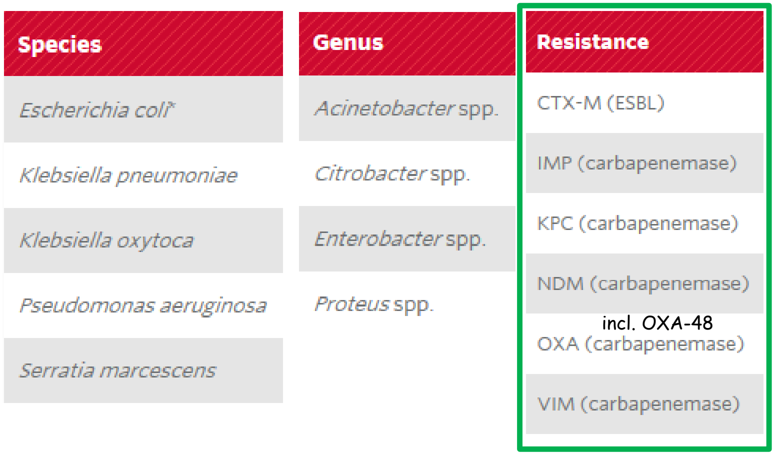
- Load cartridge, consumables, and sample (**5 min**)
- Automated sample preparation and processing
- Place slide from cartridge in reader (**2,5 hrs**)



Taton AT *et al*, Science Sep 2000

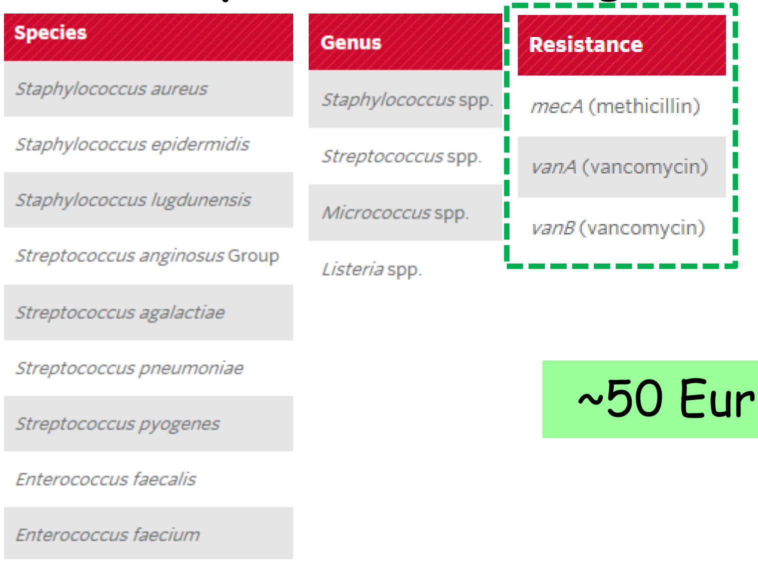
Microarray approach by using Au-nanoprobe as reporter and silver reduction to enhance signal

Gram-negatives cartridge



Species	Genus	Resistance
*Escherichia coli**	*Acinetobacter* spp.	CTX-M (ESBL)
Klebsiella pneumoniae	*Citrobacter* spp.	IMP (carbapenemase)
Klebsiella oxytoca	*Enterobacter* spp.	KPC (carbapenemase)
Pseudomonas aeruginosa	*Proteus* spp.	NDM (carbapenemase)
Serratia marcescens		incl. OXA-48 OXA (carbapenemase)
		VIM (carbapenemase)

Gram-positives cartridge

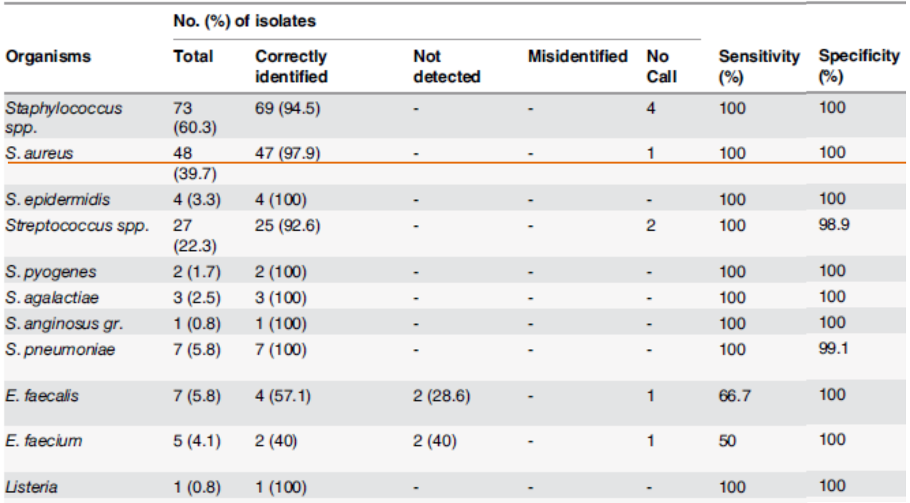


Species	Genus	Resistance
Staphylococcus aureus	*Staphylococcus* spp.	*mecA* (methicillin)
Staphylococcus epidermidis	*Streptococcus* spp.	*vanA* (vancomycin)
Staphylococcus lugdunensis	*Micrococcus* spp.	*vanB* (vancomycin)
Streptococcus anginosus Group	*Listeria* spp.	
Streptococcus agalactiae		
Streptococcus pneumoniae		
Streptococcus pyogenes		
Enterococcus faecalis		
Enterococcus faecium		

~50 Euro

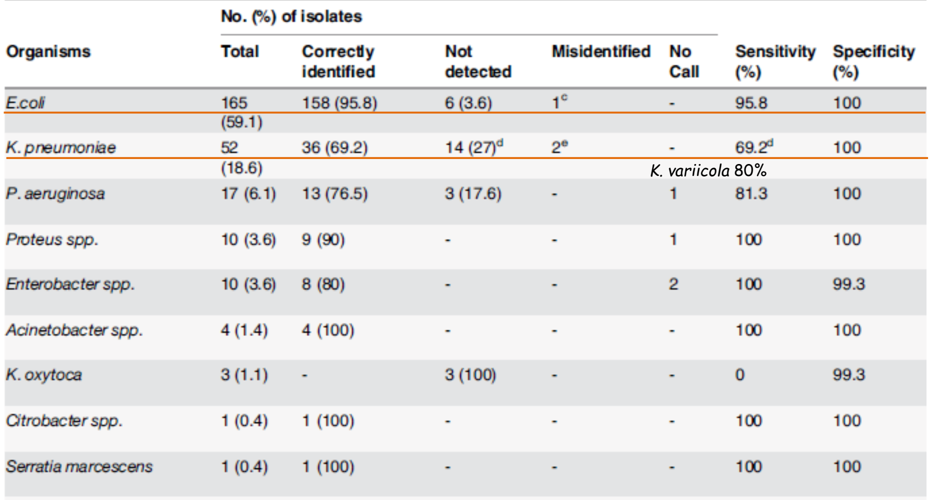
Hong Kong - Multicenter study (4 hospitals, 2014)
364 BCs (114 Gram-pos; 250 Gram-neg)

ID for Gram-positives (agreement 89.6%)



Organisms	No. (%) of isolates						
	Total	Correctly identified	Not detected	Misidentified	No Call	Sensitivity (%)	Specificity (%)
Staphylococcus spp.	73 (60.3)	69 (94.5)	-	-	4	100	100
S. aureus	48 (39.7)	47 (97.9)	-	-	1	100	100
S. epidermidis	4 (3.3)	4 (100)	-	-	-	100	100
Streptococcus spp.	27 (22.3)	25 (92.6)	-	-	2	100	98.9
S. pyogenes	2 (1.7)	2 (100)	-	-	-	100	100
S. agalactiae	3 (2.5)	3 (100)	-	-	-	100	100
S. anginosus gr.	1 (0.8)	1 (100)	-	-	-	100	100
S. pneumoniae	7 (5.8)	7 (100)	-	-	-	100	99.1
E. faecalis	7 (5.8)	4 (57.1)	2 (28.6)	-	1	66.7	100
E. faecium	5 (4.1)	2 (40)	2 (40)	-	1	50	100
Listeria	1 (0.8)	1 (100)	-	-	-	100	100

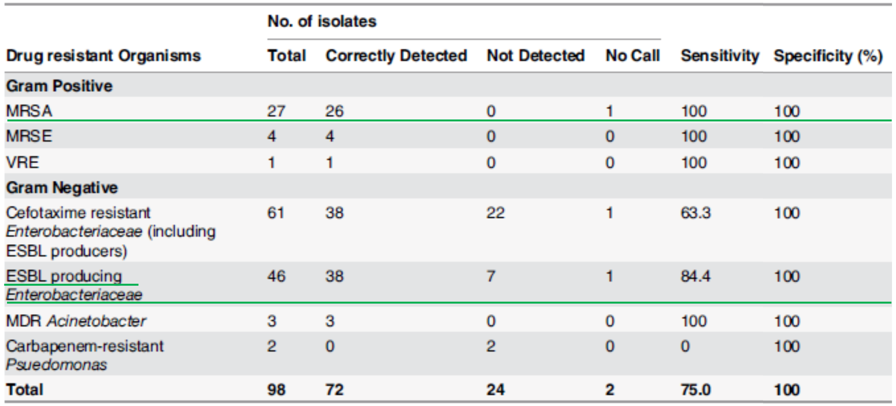
ID for Gram-negatives (agreement 90.5%)



Organisms	No. (%) of isolates						
	Total	Correctly identified	Not detected	Misidentified	No Call	Sensitivity (%)	Specificity (%)
E.coli	165 (59.1)	158 (95.8)	6 (3.6)	1^{c}	-	95.8	100
K. pneumoniae	52 (18.6)	36 (69.2)	14 (27)^{d}	2^{e}	-	69.2^{d}	100
P. aeruginosa	17 (6.1)	13 (76.5)	3 (17.6)	-	1	81.3	100
Proteus spp.	10 (3.6)	9 (90)	-	-	1	100	100
Enterobacter spp.	10 (3.6)	8 (80)	-	-	2	100	99.3
Acinetobacter spp.	4 (1.4)	4 (100)	-	-	-	100	100
K. oxytoca	3 (1.1)	-	3 (100)	-	-	0	99.3
Citrobacter spp.	1 (0.4)	1 (100)	-	-	-	100	100
Serratia marcescens	1 (0.4)	1 (100)	-	-	-	100	100

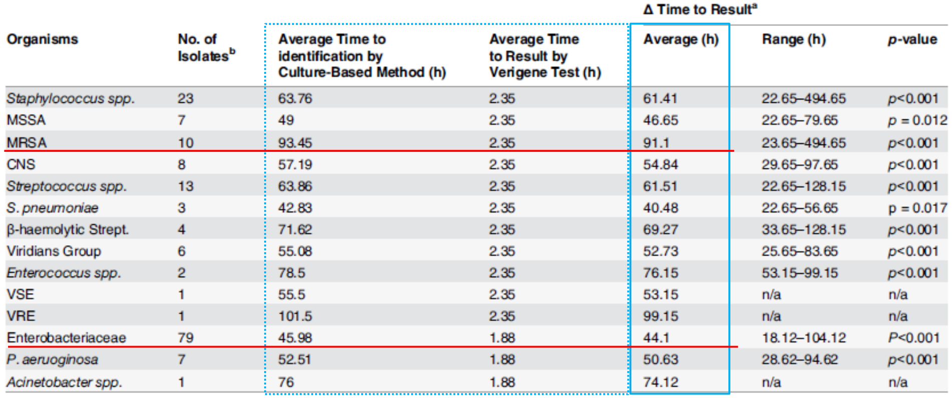
K. variicola 80%

Drug-Resistant Organisms



Drug resistant Organisms	No. of isolates					
	Total	Correctly Detected	Not Detected	No Call	Sensitivity	Specificity (%)
Gram Positive						
MRSA	27	26	0	1	100	100
MRSE	4	4	0	0	100	100
VRE	1	1	0	0	100	100
Gram Negative						
Cefotaxime resistant *Enterobacteriaceae* (including ESBL producers)	61	38	22	1	63.3	100
ESBL producing *Enterobacteriaceae*	46	38	7	1	84.4	100
MDR *Acinetobacter*	3	3	0	0	100	100
Carbapenem-resistant *Psuedomonas*	2	0	2	0	0	100
Total	**98**	**72**	**24**	**2**	**75.0**	**100**

Time to Results (40-99 hrs faster than routine)



Organisms	No. of Isolates^{b}	Average Time to identification by Culture-Based Method (h)	Average Time to Result by Verigene Test (h)	Δ Time to Result^{a}		
				Average (h)	Range (h)	*p*-value
Staphylococcus spp.	23	63.76	2.35	61.41	22.65–494.65	*p*<0.001
MSSA	7	49	2.35	46.65	22.65–79.65	*p* = 0.012
MRSA	10	93.45	2.35	91.1	23.65–494.65	*p*<0.001
CNS	8	57.19	2.35	54.84	29.65–97.65	*p*<0.001
Streptococcus spp.	13	63.86	2.35	61.51	22.65–128.15	*p*<0.001
S. pneumoniae	3	42.83	2.35	40.48	22.65–56.65	p = 0.017
β-haemolytic Strept.	4	71.62	2.35	69.27	33.65–128.15	*p*<0.001
Viridians Group	6	55.08	2.35	52.73	25.65–83.65	*p*<0.001
Enterococcus spp.	2	78.5	2.35	76.15	53.15–99.15	*p*<0.001
VSE	1	55.5	2.35	53.15	n/a	n/a
VRE	1	101.5	2.35	99.15	n/a	n/a
Enterobacteriaceae	79	45.98	1.88	44.1	18.12–104.12	*P*<0.001
P. aeruoginosa	7	52.51	1.88	50.63	28.62–94.62	*p*<0.001
Acinetobacter spp.	1	76	1.88	74.12	n/a	n/a

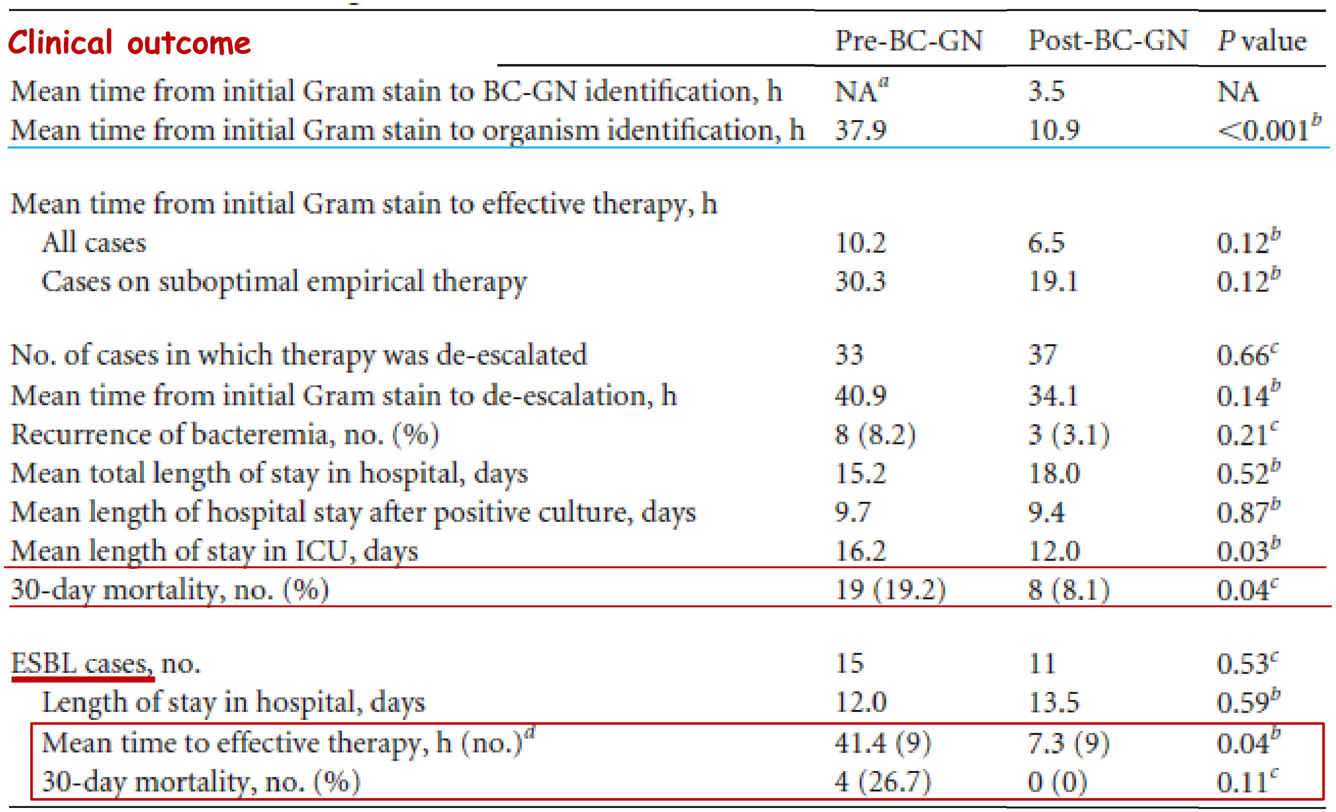
PLOS ONE | DOI:10.1371/journal.pone.0139728 October 2, 2015

Clinical Impact of Laboratory Implementation of Verigene BC-GN Microarray-Based Assay for Detection of Gram-Negative Bacteria in Positive Blood Cultures

Walker T. *et al.*, J Clin Microbiol; 54:7, 2016

Los Angeles
May 2013 - Nov 2013
Dec 2014 – May 2015

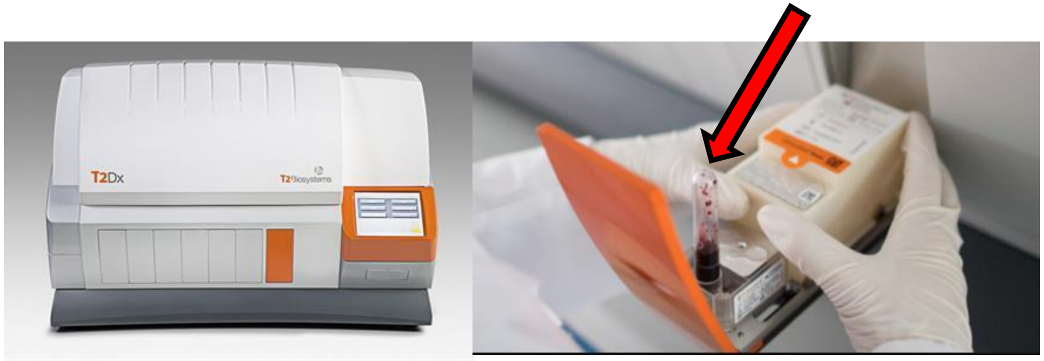
6-month period before Verigene (n=98) vs. 6-month period after Verigene (n=97)



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]

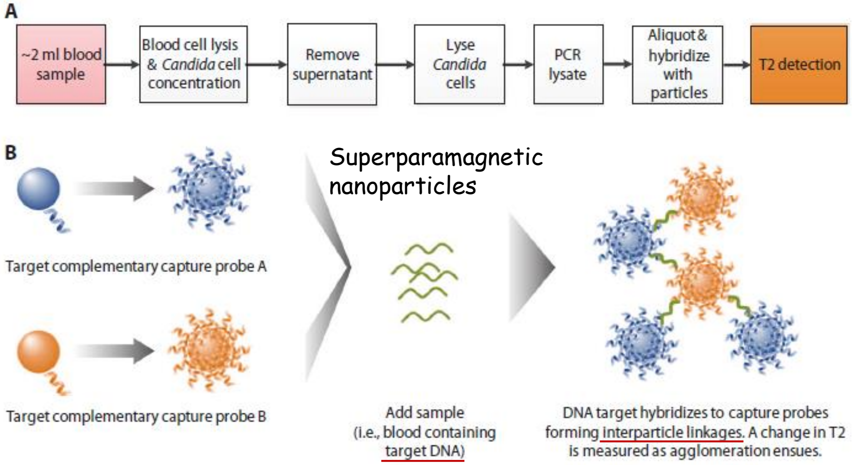
Saved
11,661 USD/case

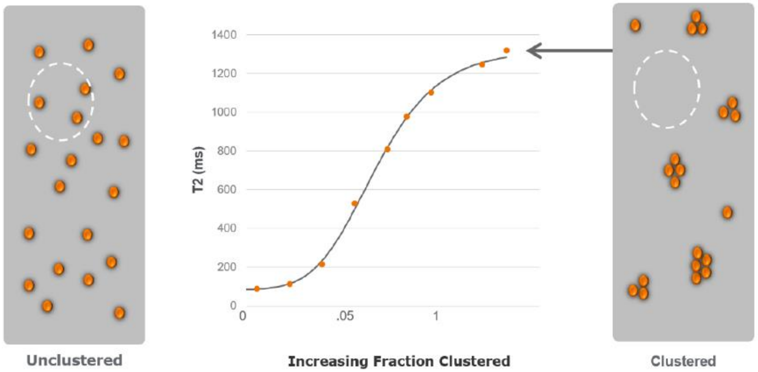
T2 Magnetic Resonance, T2MR (T2 Biosystems)



- Sample transferred to T2Dx (5 min)
- **No extraction/purification**
- Time to results (**~3-5 … max 9 hrs**)
- **Limit of detection: 1 CFU/mL**

267 CHF





Available T2Bacteria Kit

- Gram-negatives: *E. coli, K. pneumoniae, P. aeruginosa, A. baumannii*
- Gram-positives: *S. aureus, E. faecium*

} ESKAPEc

J Antimicrob Chemother 2018; **73** Suppl 4: iv20–iv26

Rome, 2017: Emergency and ICU

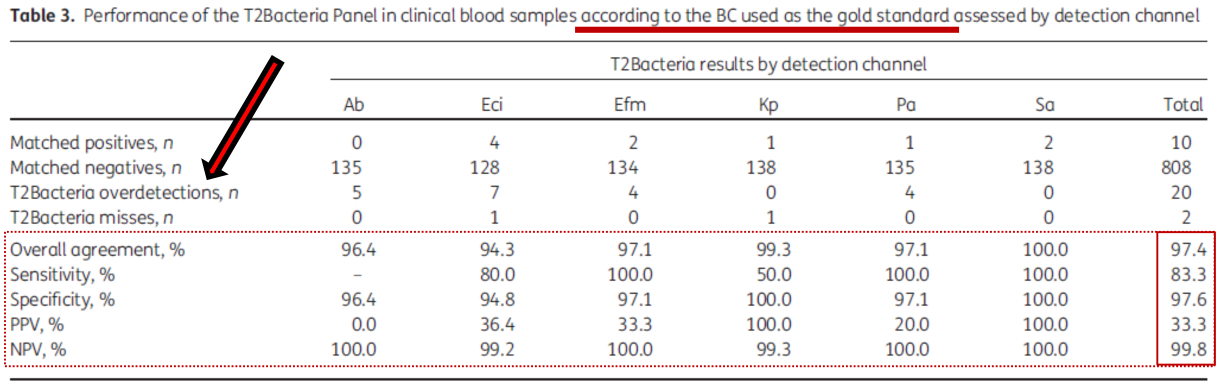
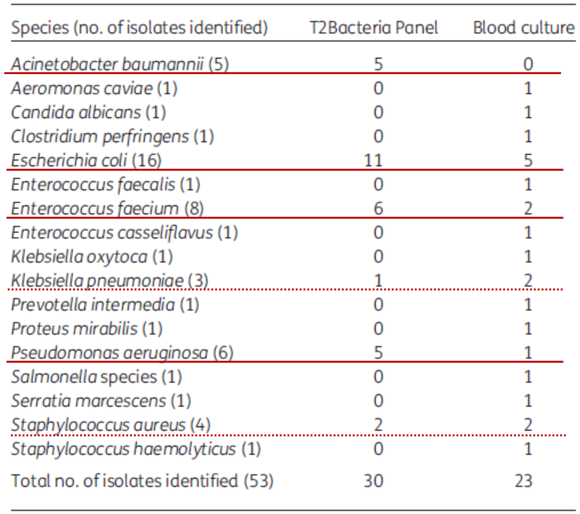


Table 3. Performance of the T2Bacteria Panel in clinical blood samples according to the BC used as the gold standard assessed by detection channel

	T2Bacteria results by detection channel						
	Ab	Eci	Efm	Kp	Pa	Sa	Total
Matched positives, *n*	0	4	2	1	1	2	10
Matched negatives, *n*	135	128	134	138	135	138	808
T2Bacteria overdetections, *n*	5	7	4	0	4	0	20
T2Bacteria misses, *n*	0	1	0	1	0	0	2
Overall agreement, %	96.4	94.3	97.1	99.3	97.1	100.0	97.4
Sensitivity, %	–	80.0	100.0	50.0	100.0	100.0	83.3
Specificity, %	96.4	94.8	97.1	100.0	97.1	100.0	97.6
PPV, %	0.0	36.4	33.3	100.0	20.0	100.0	33.3
NPV, %	100.0	99.2	100.0	99.3	100.0	100.0	99.8



Species (no. of isolates identified)	T2Bacteria Panel	Blood culture
Acinetobacter baumannii (5)	5	0
Aeromonas caviae (1)	0	1
Candida albicans (1)	0	1
Clostridium perfringens (1)	0	1
Escherichia coli (16)	11	5
Enterococcus faecalis (1)	0	1
Enterococcus faecium (8)	6	2
Enterococcus casseliflavus (1)	0	1
Klebsiella oxytoca (1)	0	1
Klebsiella pneumoniae (3)	1	2
Prevotella intermedia (1)	0	1
Proteus mirabilis (1)	0	1
Pseudomonas aeruginosa (6)	5	1
Salmonella species (1)	0	1
Serratia marcescens (1)	0	1
Staphylococcus aureus (4)	2	2
Staphylococcus haemolyticus (1)	0	1
Total no. of isolates identified (53)	30	23

Time to ID: 5.5 ± 1.4 hrs *vs.* 25.2 ± 15.2 hrs (P<0.001)

Time to negative: 6.1 ± 1.5 hrs *vs.* 120 hrs (P<0.001)

Exposed to AB treatment!

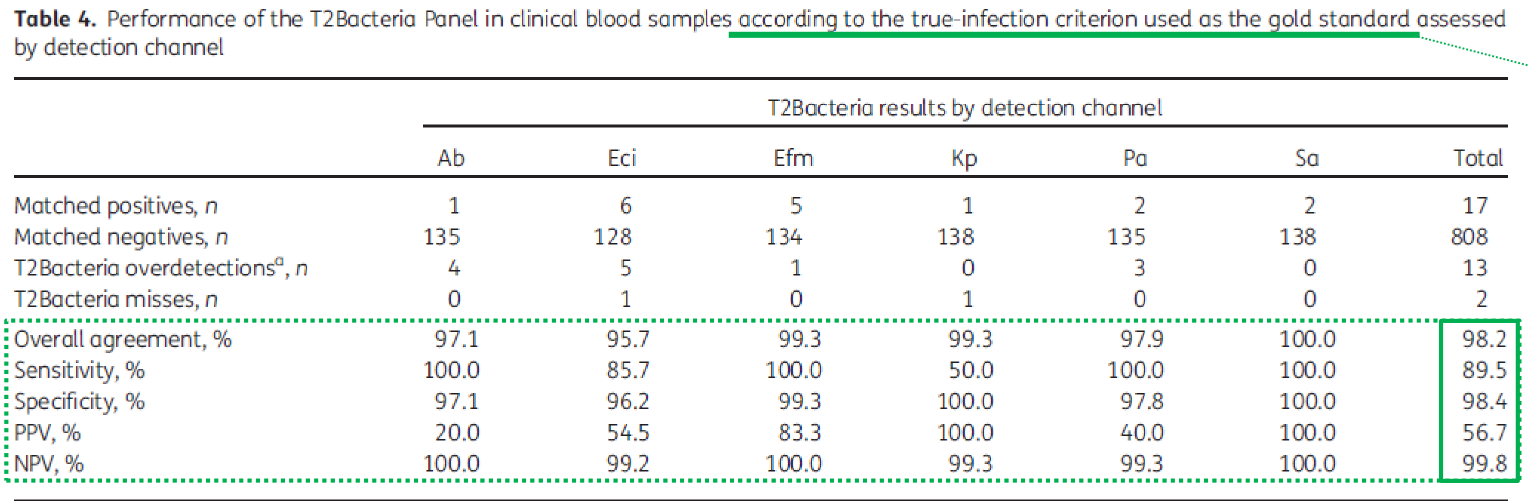


Table 4. Performance of the T2Bacteria Panel in clinical blood samples according to the true-infection criterion used as the gold standard assessed by detection channel

	T2Bacteria results by detection channel						
	Ab	Eci	Efm	Kp	Pa	Sa	Total
Matched positives, *n*	1	6	5	1	2	2	17
Matched negatives, *n*	135	128	134	138	135	138	808
T2Bacteria overdetections[a], *n*	4	5	1	0	3	0	13
T2Bacteria misses, *n*	0	1	0	1	0	0	2
Overall agreement, %	97.1	95.7	99.3	99.3	97.9	100.0	98.2
Sensitivity, %	100.0	85.7	100.0	50.0	100.0	100.0	89.5
Specificity, %	97.1	96.2	99.3	100.0	97.8	100.0	98.4
PPV, %	20.0	54.5	83.3	100.0	40.0	100.0	56.7
NPV, %	100.0	99.2	100.0	99.3	99.3	100.0	99.8

e.g., secondary infection with the same organism

See also: Nguyen MH *et al.* Ann Intern Med. 2019 Jun 18;170(12):845-852

IDWeek 2019: Poster - Snyder J *et al.*

T2Resistance Panel RUO

Gram-negative marker
KPC
OXA-48 (?)
NDM/VIM/IMP
CTX-M 14/15
AmpC(CMY/DHA)

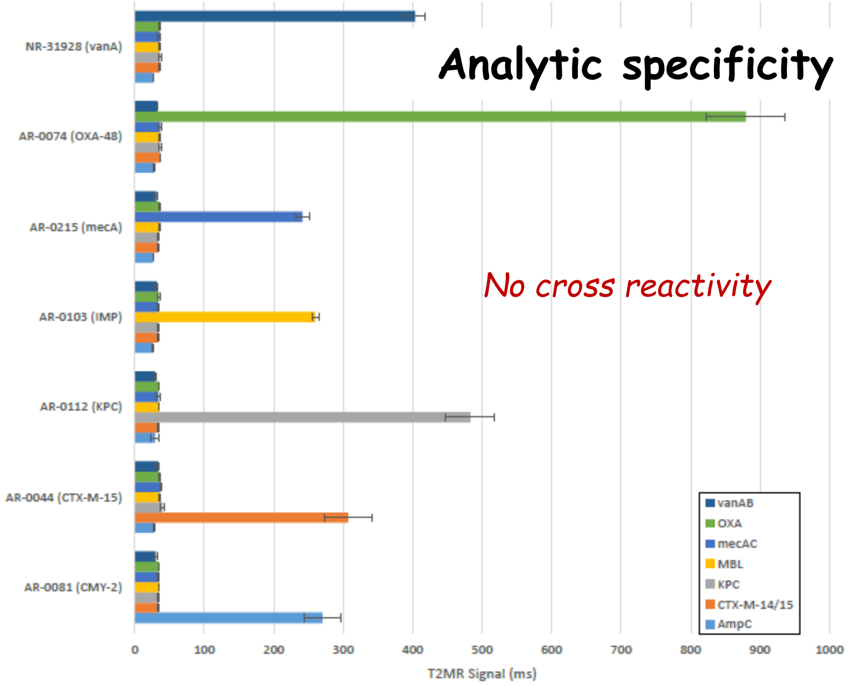
Gram-positive marker
vanA/B
mecA/C

Site	ID	BC Species	BC Gene	Time to mechanism result (hh:mm:ss)	T2MR Result	Time to T2MR result (h:mm:ss)	Agreement between BC and T2MR
Perugia	2	*Klebsiella pneumoniae*	KPC	48:36:00†	KPC	6:33:18	✓
Perugia	14	*Klebsiella pneumoniae*	KPC	94:45:00†	KPC	4:40:06	✓
Perugia	12	Negative	Negative	Negative	Negative	4:51:54	✓
Gemelli	23	Negative	Negative	Negative	NDM	4:52:35	T2MR detects NDM
Gemelli	30	*Klebsiella pneumoniae*	KPC	7:00:00	KPC	5:53:37	✓
Perugia	9	*Klebsiella pneumoniae*	KPC	19:09:00†	KPC	6:11:34	✓
Gemelli	33	*Klebsiella pneumoniae*	KPC	24:45:00	KPC	6:55:23	✓

Analytic sensitivity

Target	Channel	Species	Strain	Preliminary Limit of Detection (CFU/mL)
KPC-3	KPC	*Klebsiella pneumoniae*	CDC AR-0112	3
OXA-48	OXA	*Klebsiella pneumoniae*	CDC AR-0848	5
VIM-1	MBL	*Klebsiella pneumoniae*	CDC AR-0076	3
NDM-1		*Acinetobacter baumannii*	CDC AR-0037	7
IMP-1		*Pseudomonas aeruginosa*	CDC AR-0103	7
DHA-2	AmpC	*Klebsiella pneumoniae*	CDC AR-0079	5
CMY-2		*Escherichia coli*	CDC AR-0081	3
CTX-M-14	CTX-M	*Klebsiella pneumoniae*	CDC AR-0079	5
CTX-M-15		*Klebsiella pneumoniae*	CDC AR-0848	5
mecA	mecA/C	*Staphylococcus aureus*	CDC AR-0215	5
mecC		*Staphylococcus aureus*	ATCC BAA-2312	5
vanA	van	*Enterococcus faecium*	BEI NR-31928	3
vanB		*Enterococcus faecium*	JMI 1031982	5

Analytic specificity



No cross reactivity

ifik
institut für infektionskrankheiten
Ein Institut der Universität Bern

UNIVERSITÄT BERN

THANK YOU!

Dr. Odette J. Bernasconi, PhD

Edgar I. Campos-Madueno, MSc

Thomas Büdel, BSc

FNSNF Swiss National Science Foundation

Grant No. 170063

72 NRP Antimicrobial Resistance National Research Programme

NRP72 Grant No. 177378