Strategy on Antibiotic Resistance StAR

Bern – November 22nd, 2019

(11:00-11:25)

institut für infektionskrankheiten
Ein Institut der Universität Bern

UNIVERSITÄT BERN

Symposium Antibiotic Resistance StAR

MDROs: molecular diagnostic tools

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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

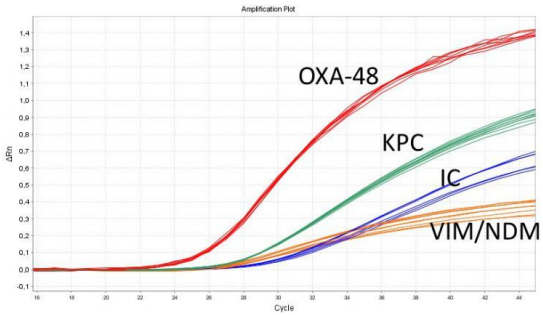
No metagenomics (NGS)

Check-Direct Screening (Check-Points)

Rectal swab

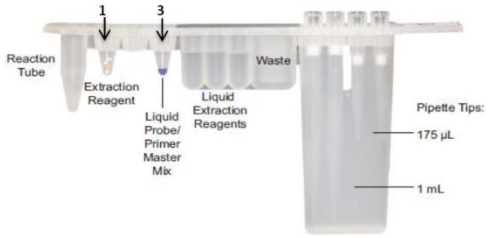
- **Real-time multiplex PCR**
- Rapid preparation
- Time to results (**<3 hrs**)

~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

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

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

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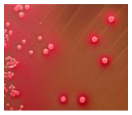
Journal of Clinical Microbiology
October 2017 Volume 55 Issue 10



Confirmatory test after ASTs

≥ 8 hrs

85 Euro

New CT103XL (Check-Points)

Broad-spectrum: TEM and SHV

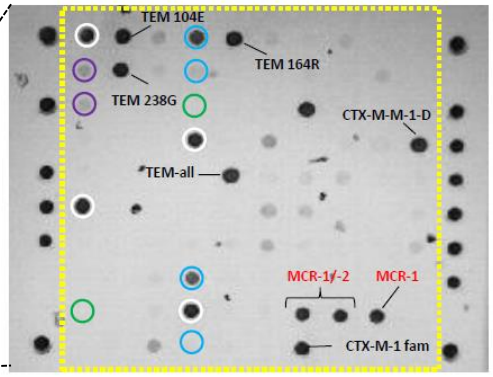
ESBLs: TEM, SHV, CTX-M, BEL, PER, GES, VEB

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

Carba: KPC, NDM, VIM, IMP, GIM, SPM, OXA-48-like [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

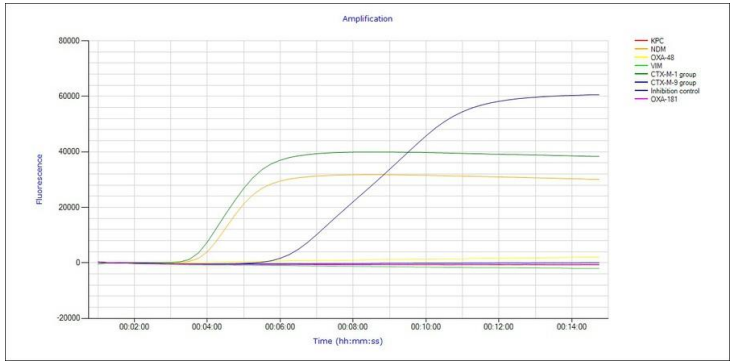
In few hrs
enough reads/coverage
to reports all ARGs

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

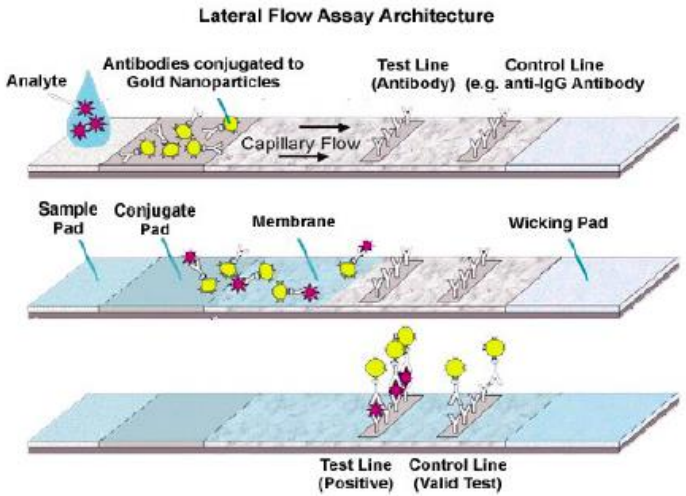
eazyplex® SuperBug complete

	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

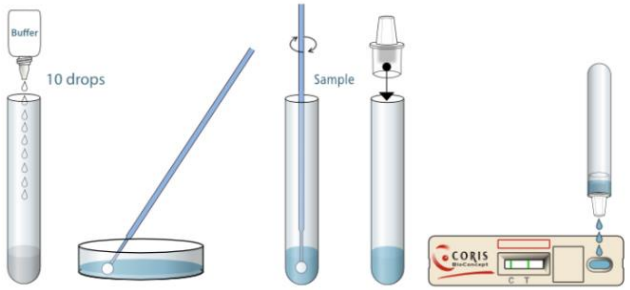
KPC,
NDM,
OXA-48 incl. OXA-244
and OXA 181,
VIM, as well as
CTX-M-1 and
CTX-M-9 group

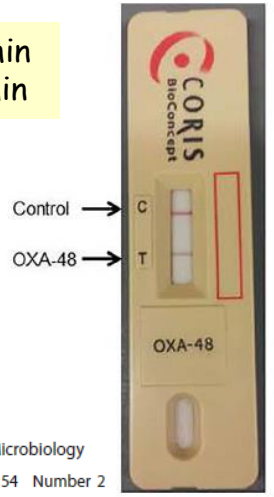
Lateral Flow Assays / Immunochromatographic Tests



2x10⁶ CFU/mL
(colonies or blood culture)

Within 15 min





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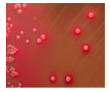
February 2016 Volume 54 Number 2

15 Euro

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

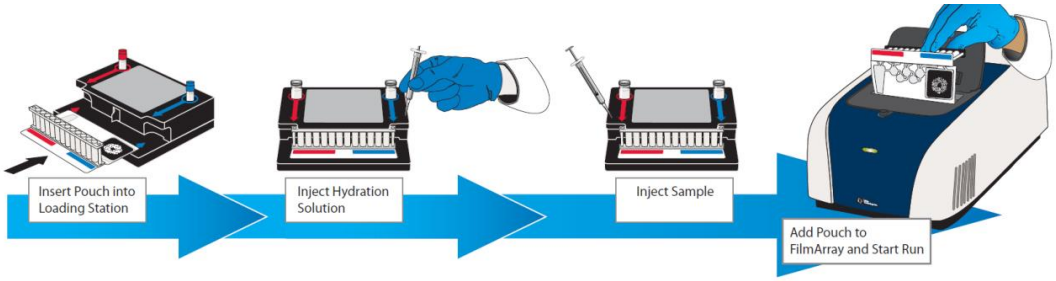
Youri Glupczynski*, Stéphanie Evrard, Te-Din Huang and Pierre Bogaerts
J Antimicrob Chemother 2019; **74**: 1284–1287



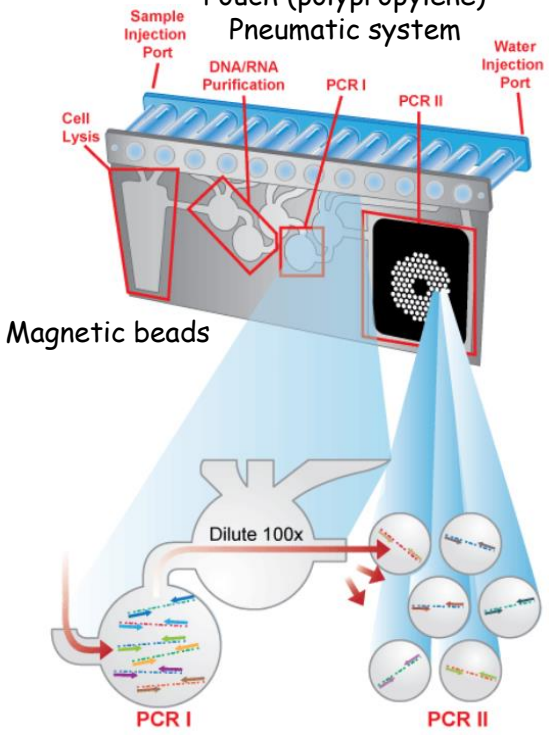
Target	*n*	sensitivity (95% CI)	specificity (95% CI)
Global performance of the RESIST-4 *K*-SeT assay for the collection of retrospective and prospective clinical isolates (*n* = 479)			
OXA-48-like incl. *OXA-181*, *OXA-232*, *OXA-244*	112	100 (95.9–100)	100 (98.8–100)
VIM	104	99 (94.0–99.9)	100 (98.7–100)
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		

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:

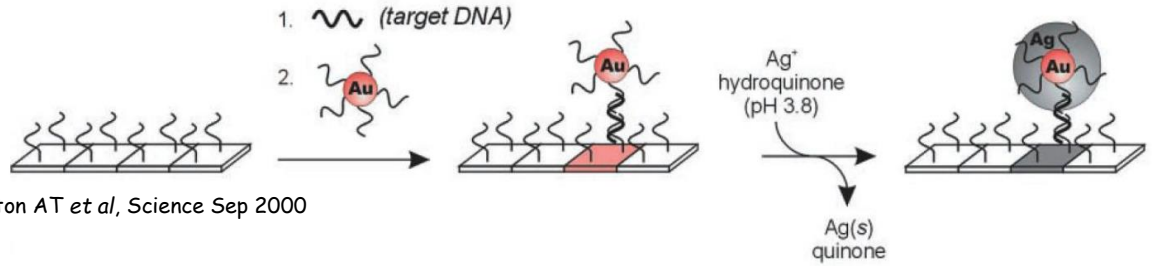
- *mecA* – methicillin resistance
- *vanA/B* – vancomycin resistance
- *KPC* – carbapenem resistance

YEAST:

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

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

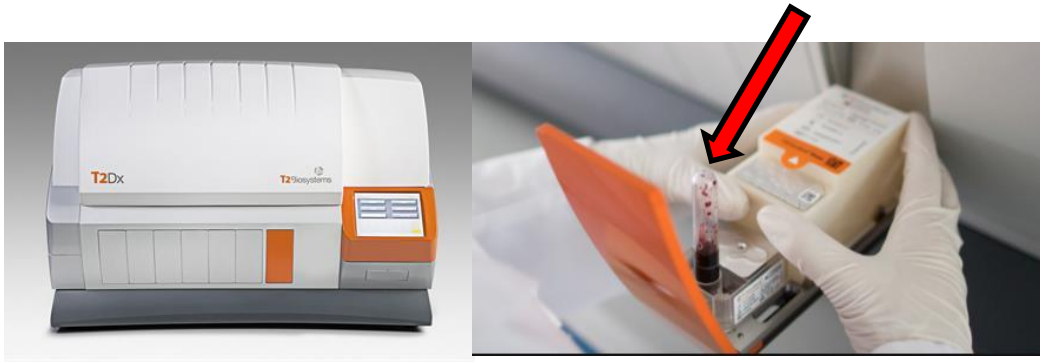
Species	Genus	Resistance
Escherichia coli	*Acinetobacter* spp.	CTX-M (ESBL)
Klebsiella pneumoniae	*Citrobacter* spp.	IMP (carbapenemase)
Klebsiella oxytoca	*Enterobacter* spp.	KPC (carbapenemase)
Pseudomonas aeruginosa	*Proteus* spp.	NDM (carbapenemase)
Serratia marcescens		incl. *OXA-48* OXA (carbapenemase)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

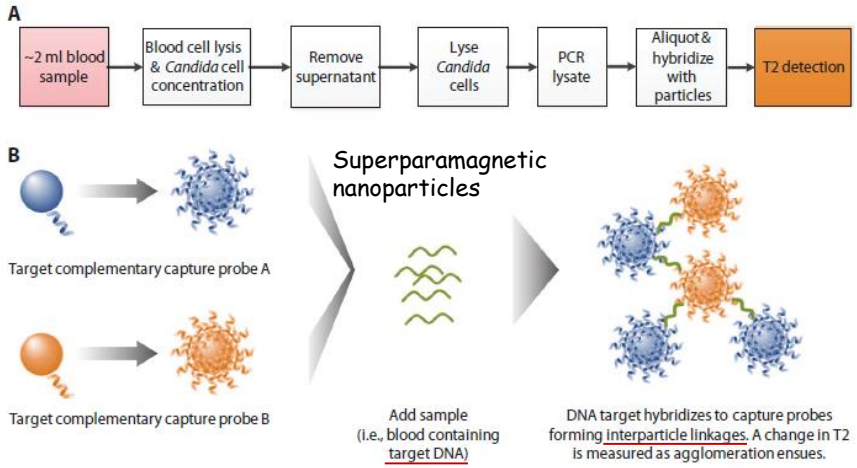
~50 Euro

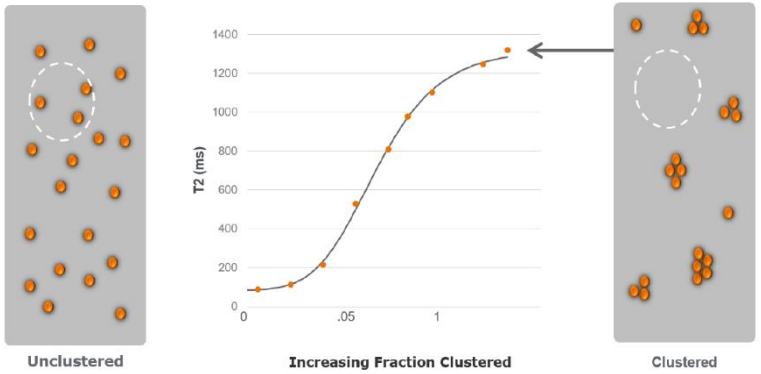
T2 Magnetic Resonance, T2MR (T2 Biosystems)



- 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

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Ein Institut der Universität Bern

UNIVERSITÄT BERN

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