



**Molecular detection of antimicrobial resistance**

Focus on carbapenemase-producing *Enterobacteriaceae* (CPE)



Pierre Bogaerts Ir PhD  
Belgian NRC for AR in *Enterobacteriaceae* and non-fermenters

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**Acknowledgment**

1. Pierre Bogaerts is co-inventors of
  - the BYG test
  - Immunochromatographic assay (OXA-48, KPC K-SeT)

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**Molecular Detection of Carbapenemases**

- Open discussion at anytime
- Why is it time for action?
- Nucleic acid amplification
- Only NA amplification?
- Future
- Conclusions



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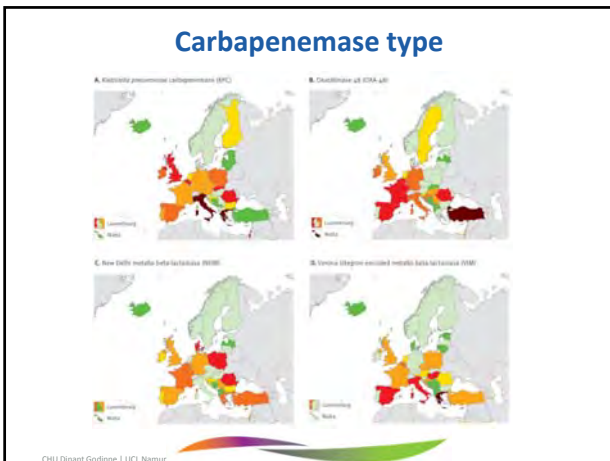
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### Carbapenemases

Classification	Enzyme (variant)	Most Common Bacteria
Class A (Active Serine70)	KPC (2-22)	<i>Enterobacteriaceae</i> (reports in <i>non fermenters</i> )
	GES (2,5,6,14)	<i>Pseudomonas</i> , <i>Acinetobacter</i>
Class B (metallo-β-lactamase)	Chromosomal	<i>Bacteroides</i> , <i>Aeromonas</i> , <i>Steno</i> , ...
	IMP (1-49),	<i>P. aeruginosa</i>
	VIM (1-46),	<i>Enterobacteriaceae</i>
	GIM, SPM, SIM, ...	<i>Enterobacteriaceae</i>
Class D (Active Serine70)	NDM (1-11)	<i>Acinetobacter</i> spp.
	OXA (-23,40,58, 72, 143...)	<i>Acinetobacter</i> spp.
	OXA-48 (162, (163, 247, 405), 181, 204, 232, 244, 245, 370, ...)	<i>Enterobacteriaceae</i>

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### Beta-lactamases

<http://www.lahey.org/studies>

Classification	Enzyme (variant)	Most Common Bacteria
Class A (Active Serine 70)	TEM (1-222) (ESBL or not)	<i>Enterobacteriaceae</i>
	SHV (1-190) (ESBL or not)	
	CTX-M (1-165) (ESBL)	<i>Acinetobacter</i> spp., <i>Pseudomonas</i>
	VEB (1-12), BEL (1-3), PER (1-8)	
	GES (1-24) (ESBL or carba)	
Class C (Cephalosporinases Active Serine 70)	CMY (1-131),	<i>Enterobacteriaceae</i>
	ACC (1-5),	
	ACT (1- 37)	
	DHA (1-23)	
	FOX (1-12)	
Class D (Active Serine 70)	MOX (1-11)	
	OXA (1-442): non ESBL, ESBL, Carbapenemases	<i>Acinetobacter</i> spp., <i>Pseudomonas</i> <i>Enterobacteriaceae</i>

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**Other Class of antibiotics**

Plasmidic Resistance to	Genes/variants	Most Common Bacteria
Fluoroquinolones	<i>qnrA, B, C, S</i> <i>qepA</i> <i>aac(6')-Ib-cr</i>	<i>Enterobacteriaceae</i>
Aminoglycosides	<i>armA</i> <i>rmtA to H</i> <i>nmpA</i> AAC ANT APH	<i>Enterobacteriaceae</i> <i>Acinetobacter</i> spp.
Colistin	<i>mcr-1</i> (Liu et al. Lancet, 2015)	<i>Enterobacteriaceae</i>

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**Resistance genes detection**

Nucleic acid amplification-based methods

On colonies

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**Detection**

- End-point:
  - ✓ Electron microscopy,
  - ✓ Reverse hybridisation,
  - ✓ Closed tube: colour, turbidity, fluorescence, melting, particules, biosensors, ...
- Real-time : closed tube/« quantitative »
  - ✓ Fluorescence, luminescence, turbidity, ...

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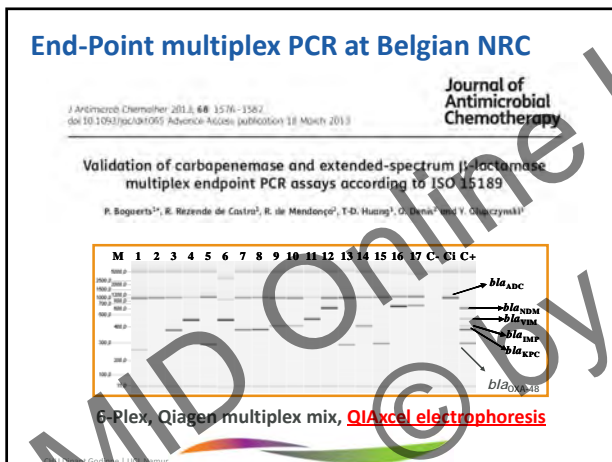
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### PCR based method as confirmatory test from isolated bacterial colonies

Gold standard: PCR-sequencing (detects all variants, costly, labor-intensive)

Author (yr)	N° of isolates (CPE)	Targeted <i>bla</i> genes	Method	Sens.	Spec.	Time to result (h)
Swayne (2011 and 2013)	59 (41) (+965 +343)	KPC, GES, IMI, SME, OXA-48, GES, IMP, VIM, NDM, SPM, SIM, GIM	RT TaqMan PCR	100	100	2
Van der zee (2014)	226	OXA-48, VIM, IMP, NDM, KPC	RT TaqMan PCR	100	100	4
Monteiro (2013)	58 (30)	KPC, GES, VIM, NDM, IMP, OXA-48	RT PCR Melting curves	100	100	3
Cuzon (2012)	187 (89)	ESBL, AmpC, KPC, VIM, NDM, IMP, OXA-48	Multiplex Ligation-PCR micro-array	98	100	6
Kaase (2012)	132 (94)	KPC, VIM, NDM, IMP, OXA-48	PCR reverse hybridization ELISA	97	99	3-4
ETC ....						



### Check-Points: Ligation-PCR-Array

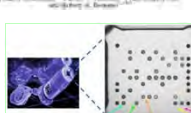


For epidemiology survey




Multicenter Evaluation of a New DNA Microarray for Rapid Detection of Clinically Relevant *bla* Genes from  $\beta$ -Lactam-Resistant Gram-Negative Bacteria<sup>1</sup>

Evaluation of a DNA Microarray (Check-MDR CT102) for Rapid Detection of TEM, SHV, and CTXM Extended-Spectrum  $\beta$ -Lactamases and of KPC, OXA-48, VIM, IMP, and NDM-1 Carbapenemases<sup>2</sup>

\*Thierry Bogaerts<sup>1</sup>, Annelies M. Hoogkamporen<sup>1</sup>, Erik-Jan Bogaerts<sup>1</sup>, Barbara Bogaerts<sup>1</sup>, Joël Vanhille<sup>1</sup>, Annelies Verbeke<sup>1</sup>, Tjallingii van der Wal<sup>1</sup>, Yvonne Chazarynki<sup>1</sup>, and Patrick Tulkens<sup>1</sup>

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**About 7 hours TAT  
Working by batch (24-48)**

NDM-1, KPC, AmpC and ESBL	Check-MDR CT101*	3 strains/array
NDM-1, KPC, OXA-48, VIM, IMP and ESBL	Check-MDR CT102*	3 strains/array
ESBL, AmpC, Carbapenemases + minor ESBL	Check-MDR CT103 + XL	1 strain/array



### Molecular methods for rapid screening of CPE from rectal swab/stools

Author (yr)	Targets	Method	Sens. %	Spec. %	Detection limit (CFU/PCR)	Process Time
NAAS (2013)	OXA-48 and variant	RT PCR (LNA)	100	100	20-50 CFU/mL of stool (100mg)	4h
Giani (2012)	KPC	In house end-point PCR	100	86	1	3-4h
Pournaras (2012)	KPC, VIM	In house end-point PCR	94.4	86	NR	4h
Singh (2012)	KPC	RT PCR (TaqMan) FAM labeled reporter probes	97	96.6	1-10	24 h* (culture: 64-72h)
Richter (2012)	KPC-2/-12	Fast RT PCR (TaqMan) MGB probe	100	98	1	≤ 2h
Vasoo (2013)	KPC, NDM	RT PCR (Light Cycler) Simple lysis extraction (soiled spec.)	89.1 100	-	2-10	1.5-4 h

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### Cepheid GeneXpert® System

**Cepheid**

- FDA cleared, all in one
- Random access
- Hands-on time < 5 min
- So easy Real-Time PCR (50 min)

**Xpert Carba-R**  
Differential diagnosis and detection of KPC, KPC-2, KPC-8, and OXA-48 from screening OXA-48 & KPC-232

WHO: AST PCR-based at POC April 2014

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### amplex eazyplex® SuperBug CRE

**amplex**

- The swab is taken using E-swab
- Transfer 25 µl from the E-Swab into 500 µl RALF – buffer
- The **LAMP** assays are run on the Genie®II at 66°C for **30 minutes**
- Carbapenemases/ESBL genes (2 panels)
  - ✓ KPC
  - ✓ NDM
  - ✓ VIM
  - ✓ OXA-48/181
  - ✓ CTX-M-G1 or OXA-58
  - ✓ CTX-M-G9 or OXA-23

freeze dried

OXA-181

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**Surveillance – Targeted screening with laboratory extractor and RT thermocycler**

1 Perianal swab → 2 DNA Extraction → 3 Check-Direct CPE

40 min      2 h

OXA-48 (including 181)  
KPC  
NDM/VIM (one colour)

ABI7500    CFX96    LC480

**Check-Direct CPE for BD MAX™**

1 Or → 2 Check-Direct CPE

50 min Extraction      1.5 h PCR

OXA-48  
KPC  
NDM  
VIM

KPC, OXA-48, NDM

**BD MAX™ CRE**

**BD MAX™ CRE Assay**  
*For Research Use Only (RUO)*

➤ BD MAX™ CRE Assay KPC, OXA-48 and NDM rectal swab RT-PCR (2 h)

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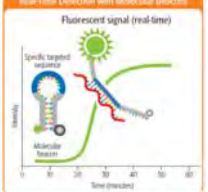


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### bioMérieux easyQ KPC



- KPC only. In stool
- NASBA (RNA amplification)
- 2 hour to result (48 samples)

25 CHU Dijon - Centre de Diagnostic - I.U.C.L. - Nancy

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### FOCUS Diagnostics Simplexa™ Molecular Assays



- KPC in stool (BDO commercialized ?)
- 5 min hands-on time
- 1 h test
- No random access

WHO: AST PCR-based at POC April 2014

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### curetis Unyvero®



- Unyvero™ P50 Pneumoniae cartridge (RUO)
- Low hands-on time
- 5 hours from respiratory sample

CHU Dijon - Centre de Diagnostic - I.U.C.L. - Nancy

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### Unyvero™ P50 Panel

#### COVERAGE – CLINICALLY RELEVANT PATHOGENS AND ANTIBIOTIC RESISTANCES

Detected Microorganisms		Antibiotic Resistance Marker	Resistance against
Gram-positive bacteria	Staphylococcus aureus	meC4	Oxacillin
	Staphylococcus epidermidis	meA	Mazoxilol
Non-fermenting bacteria	Pseudomonas aeruginosa	meA/E	Macrolide
	Acinetobacter baumannii	ermA	Macrolide / Lincosamide
	Legionella pneumophila	ermB	Macrolide / Lincosamide
	Moraxella catarrhalis	ermC	Macrolide / Lincosamide
Enterobacteriaceae	Enterobacter spp.	bla <sub>TEM-1</sub>	1 <sup>st</sup> Gen. Cephalosporin
	Escherichia coli	bla <sub>TEM-1</sub>	1 <sup>st</sup> Gen. Cephalosporin
	Klebsiella pneumoniae	bla <sub>TEM-1</sub>	1 <sup>st</sup> Gen. Cephalosporin
	Shigella sonnei	bla <sub>TEM-1</sub>	1 <sup>st</sup> Gen. Cephalosporin
Other	Haemophilus influenzae	bla <sub>TEM-1</sub>	1 <sup>st</sup> Gen. Cephalosporin
	Chlamydia pneumoniae	bla <sub>TEM-1</sub>	1 <sup>st</sup> Gen. Cephalosporin
Fungal	Candida albicans	ERG11	Multidrug resistance
	Aspergillus fumigatus	orfT	Multidrug resistance, Sulfoxonazole

ESBL VS non-ESBL  
MIR-ACT  
ISA001-DNA-S1

CHU Dinant Godinne, LUCL Namur WHO: AST PCR based at POC, April 2014

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### PCR/ESI-MS, Abbott

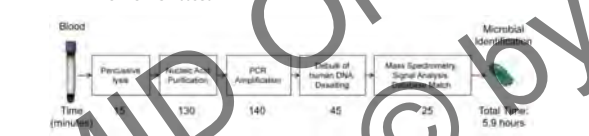
**COVERAGE**

750+ Bacteria, Candida and 4 Antibiotic Resistance Markers: meC4, vanB, vanA and kpc

**SAMPLE TYPE**

Anti EDTA whole blood, Serum fluids and tissues

PCR ESI-TOF based



Total Time: 5.9 hours

Bacconi, JCM, 2014

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### Resistance genes detection

Nucleic acid amplification-based methods

On Blood culture

CHU Dinant Godinne, LUCL Namur

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### Nanosphere Verigene®



- US/FDA-Cleared all inclusive
- Positive Blood Culture Bottle†
- PCR if necessary + functionalized with gold nanoparticles for detection
- Random Access
- Hands-On Time <5 minutes
- Run Time <2.5 hours

©2011 Datascope Genzyme, LLC, Boston

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### Panels Nanosphere Verigene

**Panel GRAM-**

**Species**

- Escherichia
- Klebsiella
- Pseudomonas
- Serratia
- Genus
- Acinetobacter
- Proteus
- Citrobacter
- Enterobacter spp.

**Resistance**

- KPC
- NDM
- CTX-M
- VIM
- IMP
- OXA

**Panel GRAM+**

**Species**

- Staphylococcus spp.
- Streptococcus spp.
- Micrococcus spp.
- Listeria spp.

**Resistance**

- mecA
- vanA
- vanB

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### FilmArray® Blood Culture panel

**1 Test. 27 Targets. All in about an hour.**

**Gram + Bacteria**

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

**Gram - Bacteria**


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

**Yeast**

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

**Antibiotic Resistance**

- vanA - vancomycin resistance
- vanB - vancomycin resistance
- KPC - carbapenem resistance



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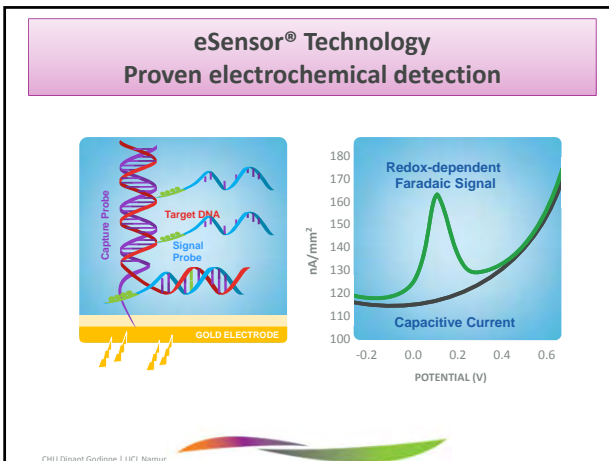
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### The ePlex™ Blood Culture Identification Gram-Positive Panel

20 Bacterial Targets	3 Resistance Genes
<i>Bacillus cereus</i> group	<i>mecA</i>
<i>Bacillus subtilis</i> group	<i>mecC</i>
<i>Corynebacterium</i>	<i>vanA/vanB</i>
<i>Enterococcus</i>	
<i>Enterococcus faecalis</i>	
<i>Enterococcus faecium</i>	
<i>Lactobacillus</i>	
<i>Listeria</i>	
<i>Listeria monocytogenes</i>	
<i>Micrococcus</i>	
<i>Propionibacterium</i>	
<i>Staphylococcus</i>	
<i>Staphylococcus aureus</i>	
<i>Staphylococcus epidermidis</i>	
<i>Staphylococcus saprophyticus</i>	
<i>Streptococcus</i>	
<i>Streptococcus agalactiae</i>	
<i>Streptococcus anginosus</i>	
<i>Streptococcus pneumoniae</i>	
<i>Streptococcus pyogenes</i>	

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### The ePlex™ Blood Culture Identification Gram-Negative Panel

25 Bacterial Targets	6 Resistance Genes family
<i>Acinetobacter baumannii</i>	<i>CTX-M</i>
<i>Bacteroides fragilis</i>	<i>IMP</i>
<i>Citrobacter</i>	<i>KPC</i>
<i>Citrobacter freundii</i>	<i>NDM</i>
<i>Cronobacter sakazakii</i>	<i>OXA-carba (OXA-48, other OXAs...)</i>
<i>Enterobacter</i>	<i>VIM</i>
<i>Enterobacter cloacae</i> complex	
<i>Escherichia coli</i>	
<i>Fusobacterium</i>	
<i>Fusobacterium necrophorum</i>	
<i>Haemophilus influenzae</i>	
<i>Klebsiella oxytoca</i>	
<i>Klebsiella pneumoniae</i>	
<i>Morganella morganii</i>	
<i>Neisseria meningitidis</i>	
<i>Pantoea agglomerans</i>	
<i>Prevotella</i>	
<i>Proteus</i>	
<i>Proteus mirabilis</i>	
<i>Pseudomonas</i>	
<i>Pseudomonas aeruginosa</i>	
<i>Salmonella</i>	
<i>Serratia</i>	
<i>Serratia marcescens</i>	
<i>Stenotrophomonas maltophilia</i>	

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**CORIS BioConcept**

## TRAPIST In development

The Rapid Advanced PCR and Immunoassay SysTem

MICROFLUIDIC CHIP

ANALYSIS PLATFORM

60 min

Extraction not included

CHU Dijon-Gadonne, UCL Namur

**CORIS BioConcept**

## Multiplex PCR amplification Multiplex oligochromatography detection

Into the loading tank:  
Extracted DNA sample and  
MULTIPLY PCR reagents

95°C 72°C 55°C

Liquid flow

Fluorescent PCR products to the OC

CHU Dijon-Gadonne, UCL Namur

**CORIS BioConcept**

## Targets

Gram-negative		Gram-positive	
Espèce/Genre	Résistance	Espèce/Genre	Résistance
<i>Acinetobacter baumannii</i>	KPC	<i>Enterococcus faecalis</i>	
<i>Pseudomonas aeruginosa</i>	NDM	<i>Enterococcus faecium</i>	
<i>Enterobacter</i> sp.	CTX-M	<i>Listeria monocytogenes</i>	
<i>Escherichia coli</i>	VIM	<i>Staphylococcus</i> sp.	mecA
<i>Serratia marcescens</i>	IMP	<i>Staphylococcus aureus</i>	vanA
<i>Klebsiella oxytoca</i>	OXA-48	<i>Staphylococcus epidermidis</i>	vanB
<i>Klebsiella pneumoniae</i>	OXA-23	<i>Streptococcus</i> sp.	
<i>Neisseria meningitidis</i>	OXA-24	<i>Streptococcus agalactiae</i>	
<i>Proteus mirabilis</i>	OXA-58	<i>Streptococcus pyogenes</i>	
<i>Citrobacter freundii</i>		<i>Streptococcus pneumoniae</i>	

CHU Dijon-Gadonne, UCL Namur

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### Resistance markers « molecular » detection

Is molecular always nucleic acid  
amplification ?  
On colonies not clinical samples




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### Detection of CPE on bacterial culture Current imipenem hydrolysis colorimetric tests



**Carba NP**  
Nordmann et al., EID 2012

**RAPIDEC CARBA NP**,  
bioMérieux,  
Dortet et al., 2015

**Blue Carba.**  
Pires et al., JCM 2013  
Pasteran, JCM 2015

**Rapid CARB Screen**, ROSCO Diagnostica  
Huang et al., JCM, 2014

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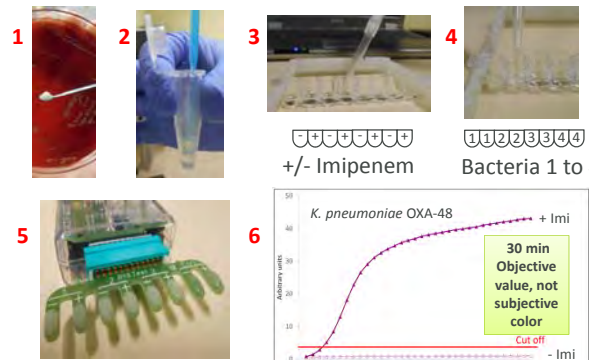
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### BYG Test: Electrochemical detection of CPE



1 2 3 4

5 6

$\ominus\ominus\ominus\ominus\ominus\ominus\oplus$   $\oplus\oplus\oplus\oplus\oplus\oplus$   
+/- Imipenem Bacteria 1 to 4

*K. pneumoniae* OXA-48

30 min  
Objective  
value, not  
subjective  
color

Cut off

Bogaerts P., Yunus S. et al. JCM 2015

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**OXA-48 or KPC immunochromatographic K-Set**

The Kit      10 drops lysis buffer      Just touch one colony

Scratch the colony on the bottom (3-5 sec)      Load 3 drops      Wait for max 15 min

© 2011 Diagnostics Gateway, LLC, Nazareth

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**OXA-48 K-Set**

From bacterial culture: results within 10-15 min max.

IC →      ← IC

**OXA-48 +**      **OXA-48 Neg**

MAX 15 min

Also KPC K-Set and combo duplex test (OXA-48 & KPC)

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**Resistance genes detection**

The Future  
Next Generation Sequencing

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### WGS-NGS

Fragmentation and adapter ligation  
Genomic DNA

Array-based sequence capture

Removal of unbound fragments

Elution of enriched library

Next-Generation Sequencing

**Found to most genomic sequencing data HELP!**

- 80 % of the genome
- are better reading of certain Zone
- Sensitivity
- Quantitation

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JCM Accepts, published online ahead of print on 30 October 2013  
J. Clin. Microbiol. doi:10.1128/JCM.02452-13  
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**Rapid whole genome sequencing for the detection and characterization of microorganisms directly from clinical samples**

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- 35 urines UTI
- Concordance with culture and WGS
- Data on resistance and typing
- Bioinformatics !
- To be continued !

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### Genome analysis softwares for Resistance genes

- The Comprehensive Antibiotic Resistance Database,  
✓ CARD: <http://arpcard.mcmaster.ca>
- ARDB-Antibiotic Resistance Genes Database  
✓ ARDB: <http://ardb.cbcb.umd.edu>
- ResFinder  
✓ <http://genomicepidemiology.org>

- ✓ Many genes but relevant role in resistance ?
- ✓ Not the same genes detected by different softwares

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### Conclusions

- Molecular test is to be adapted to the purpose
  - ✓ Routine
  - ✓ Screening
  - ✓ Outbreak
  - ✓ Epidemiology/surveillance
- Complexity of the interpretation: what means detected?
- Not only PCR
- Not only nucleic acid amplification
- Patient's benefit

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Carbapenemases detection April 2014

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