



# Uued meetodid karbapenemaaside produktsiooni avastamiseks

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# Introduction

- Chromogenic media
- Carba NP test I and II
- MALDI-TOF MS based functional  $\beta$ -lactamase assay
- RealTime PCR
- Conventional PCR

\* Production of any carbapenemase

\* Production of Class .. carbapenemase

# chromID CARBA



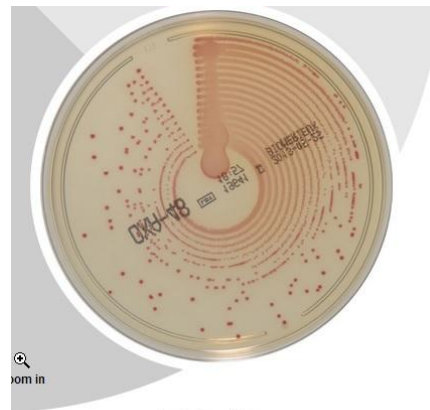
- Fast screening of carbapenemase-producing Enterobacteriaceae, especially **KPC, VIM and NDM-1 in 18-24 hours**
  - high sensitivity 97.4% [93.4-99.3]
  - high specificity 99.7% [98.9-100.0]
  - defined for use clinical specimens (stools or rectal swabs)
- chromID CARBA agar consists of a nutritive base combining different peptones. It contains:
  - A mixture of antibiotics which enable the selective growth of CPE
  - Three chromogenic substrates which enable the ID of the most frequently isolated CPE

# chromID CARBA related articles

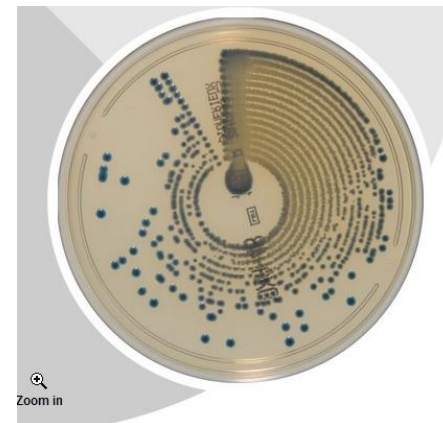
- Conclusions: chromID CARBA medium demonstrated good performance with the collection of CPE used also for CPE difficult to detect due to the low carbapenems MIC, such as OXA-48 and VIM-type producing strains. This method showed a high correlation with the routine procedure and in 2 cases a higher sensibility in the detection of carriers.
- **Evaluation of chromID CARBA agar medium (bioMérieux) performance for the detection of carbapenemase-producing Enterobacteriaceae**
- A. Piazza, A. Galano, S. Bracco, S. Asticcioli, T. Giani, B. Pini, E. Nucleo, F. Arena, M. Caltagirone, F. Luzzaro
- Published 28. may 2013

# chromID OXA-48

- Active surveillance of CPE
- chromID OXA-48 agar consists of a nutritive base combining different peptones. It contains:
  - A mixture of antibiotics which enable the selective growth of OXA-48 CPE
  - Three chromogenic substrates which enable the ID of the most frequently isolated CPE



Incubation : 18 hours  
*E. coli* OXA-48



Incubation : 18 hours  
*K. pneumoniae* OXA-48

# chromID OXA-48 related article

- Comparative evaluation of the recently developed chromogenic culture medium chromID OXA-48 (bioMérieux) with chromID CARBA (bioMérieux) and SUPERCARBA showed that chromID OXA-48 and SUPERCARBA media have the highest sensitivity for detection of OXA-48 producing Enterobacteriaceae (91% and 93%) comparatively to chromID CARBA (21 %). The chromID OXA-48 has the highest specificity, with 100%, as compared to 53% and 68% for the SUPERCARBA and chromID CARBA media for detecting those OXA-48 producers.
- **Comparative evaluation of a novel chromogenic medium (chromID OXA-48) for detection of OXA-48 producing Enterobacteriaceae.**
- [Girlich D](#), [Anglade C](#), [Zambardi G](#), [Nordmann P](#).
- Published 30.sep 2013

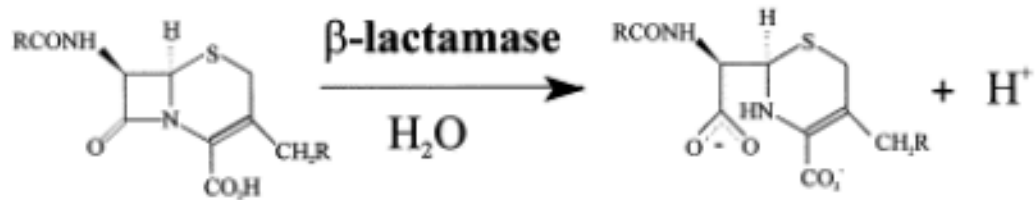
# Carba NP test

- Nordmann-Poirel biochemical test for rapid identification of Carbapenemase Types in Enterobacteriaceae and Pseudomonas spp.
- Carba NP test I - detection of carbapenamase presence
- Carba NP test II – detection of carbapenemase classes

# Carba NP test I

## The Carba NP (Normann-Poirel) test I

Hydrolysis of  $\beta$ -lactam bond releases protons decreasing pH and turning the color of phenol from red to orange/yellow





# Carba NP test I

## The Carba NP (Nordmann-Poirel) test I

### Material

- a. O/N culture of bacteria in MH, blood, or trypticase soya plates
- b. **Tube A:** Solution A: phenol red 0.5% w/v, zinc 0.1mM **[(-) control]**
- c. **Tube B:** Solution A plus imipenem 6mg/ml

### Method

1. Resuspend a 1/3 to 1/2 of a single calibrated loop of bacterial colonies in the four tubes
2. Incubate at 37°C for maximum 2h.
3. Optical reading of the color of each tube

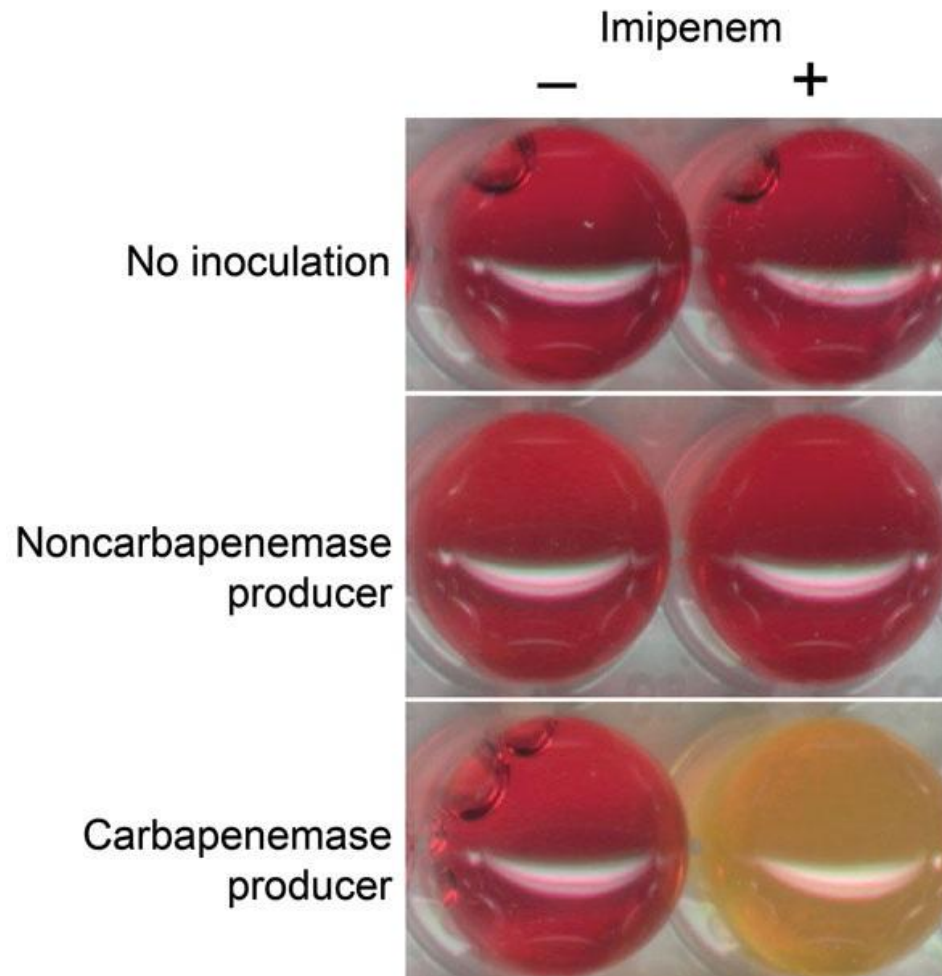
### Interpretation

	Tube A	Tube B
No carbapenemase	Red	Red
Carbapenemase Producer	Red	Orange/Yellow
Not interpretable	Yellow	Yellow

Usually, time required for obtaining positive results is as follows:

- KPC producers: 2 to 30 min
- OXA-48 like producers: 30 min to 1h
- Metallo- $\beta$ -lactamases (NDM, VIM, IMP): 15 min to 1h

# Carba NP test I results



## CARBAPENEMASES

### KPC

Molecular Class A

- hydrolyze penicillins, 3<sup>rd</sup> generation cephalosporins, aztreonam and carbapenems
- plasmid accommodated
- partially inhibited by clavulanate, tazobactam & sulbactam
- inhibited by boronic acid and its derivatives
- not inhibited by chelating agents
- not inhibited by cloxacillin

### MβLs

Molecular Class B1

- hydrolyze penicillins, 3<sup>rd</sup> generation cephalosporins and carbapenems
- plasmid accommodated
- not inhibited by clavulanate, tazobactam & sulbactam
- not inhibited by boronic acid and its derivatives
- inhibited by chelating agents
- not inhibited by cloxacillin

### OXA-48

Molecular class D

- hydrolyze penicillins, older cephalosporins, carbapenems & temocillin
- plasmid accommodated
- not inhibited by clavulanate, tazobactam & sulbactam
- not inhibited by boronic acid and its derivatives
- not inhibited by chelating agents
- not inhibited by cloxacillin

# Carba NP test II

## The Carba NP (Nordmann-Poirel) test II

### Material

- a. O/N culture of bacteria in MH, blood, or trypticase soya plates
- b. **Tube A:** Solution A: phenol red 0.5% w/v, zinc 0.1mM **[(-) control]**
- c. **Tube B:** Solution A plus imipenem 6mg/ml **[(+ control)]**
- d. **Tube C:** Solution A plus imipenem 6mg/ml and tazobactam 40mg/ml **[KPC inhibition]**
- e. **Tube D:** Solution A without zinc plus imipenem 6mg/ml and EDTA 0.03M **[MβL inhibition]**

### Method

1. Resuspend a 1/3 to 1/2 of a single calibrated loop of bacterial colonies in the four tubes
2. Incubate at 37°C for maximum 2h.
3. Optical reading of the color of each tube

# Carba NP test II

## The Carba NP (Nordmann-Poirel) test II

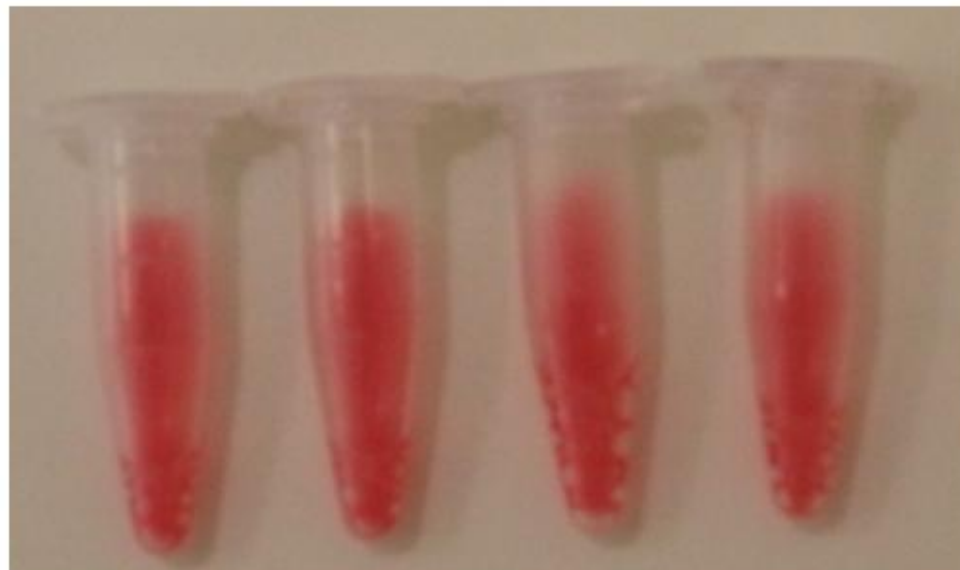
### Interpretation

	No antibiotic (tube A)	Imipenem (tube B)	Imipenem+ tazobactam (tube C)	Imipenem+EDTA (tube D)
No carbapenemase	Red	Red	Red	Red
Ambler class A carbapenemase	Red	Orange/Yellow	Red	Orange/Yellow
Ambler class B carbapenemase	Red	Orange/Yellow	Orange/Yellow	Red
Ambler class D carbapenemase	Red	Orange/Yellow	Orange/Yellow	Orange/Yellow
No interpretable	Yellow	Yellow	Yellow	Yellow

Usually, time required for obtaining positive results is as follows:

- KPC producers: 2 to 30 min
- OXA-48 like producers: 30 min to 1h
- Metallo- $\beta$ -lactamases (NDM, VIM, IMP): 15 min to 1h

	No antibiotic (tube A)	Imipenem (tube B)	Imipenem+ tazobactam (tube C)	Imipenem+EDTA (tube D)
No carbapenemase	Red	Red	Red	Red



Internal control  
(Lysis Buffer+inoculum)  
+  
Solution A without IMP

**negative**

Lysis Buffer+inoculum  
+  
Solution A with IMP

**negative**

Lysis Buffer+inoculum  
+  
TZB  
+  
Solution A with IMP

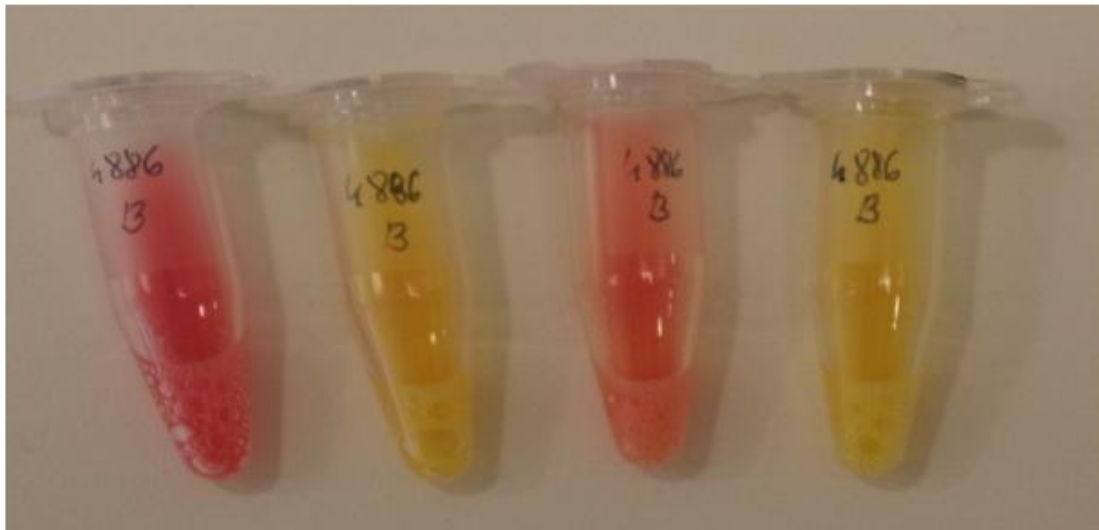
**negative**

Lysis Buffer+inoculum  
+  
EDTA  
+  
Solution A with IMP

**negative**

**Interpretation → negative for carbapenemase**

	No antibiotic (tube A)	Imipenem (tube B)	Imipenem+ tazobactam (tube C)	Imipenem+EDTA (tube D)
Ambler class A carbapenemase	Red	Orange/Yellow	Red	Orange/Yellow



Internal control  
(Lysis Buffer+inoculum)  
+  
Solution A without IMP

**negative**

Lysis Buffer+inoculum  
+  
Solution A with IMP

**positive**

Lysis Buffer+inoculum  
+  
TZB  
+  
Solution A with IMP

**negative**

Lysis Buffer+inoculum  
+  
EDTA  
+  
Solution A with IMP

**positive**

**Interpretation → class A carbapenemase (e.g. KPC)**

	No antibiotic (tube A)	Imipenem (tube B)	Imipenem+ tazobactam (tube C)	Imipenem+EDTA (tube D)
Ambler class B carbapenemase	Red	Orange/Yellow	Orange/Yellow	Red



Internal control  
(Lysis Buffer+inoculum)  
+  
Solution A without IMP

**negative**

Lysis Buffer+inoculum  
+  
Solution A with IMP

**positive**

Lysis Buffer+inoculum  
+  
TZB  
+  
Solution A with IMP

**positive**

Lysis Buffer+inoculum  
+  
EDTA  
+  
Solution A with IMP

**negative**

**Interpretation → class B carbapenemase (metallo-beta-lactamase VIM, NDM)**



	No antibiotic (tube A)	Imipenem (tube B)	Imipenem+ tazobactam (tube C)	Imipenem+EDTA (tube D)
Ambler class D carbapenemase	Red	Orange/Yellow	Orange/Yellow	Orange/Yellow



Internal control  
(Lysis Buffer+inoculum)  
+  
Solution A without IMP

**negative**

Lysis Buffer+inoculum  
+  
Solution A with IMP

**positive**

Lysis Buffer+inoculum  
+  
TZB  
+  
Solution A with IMP

**positive**

Lysis Buffer+inoculum  
+  
EDTA  
+  
Solution A with IMP

**positive**

**Interpretation → class D carbapenemase (e.g. OXA-48)**

	No antibiotic (tube A)	Imipenem (tube B)	Imipenem+ tazobactam (tube C)	Imipenem+EDTA (tube D)
Ambler class A carbapenemase	Red	Orange/Yellow	Red	Orange/Yellow

	No antibiotic (tube A)	Imipenem (tube B)	Imipenem+ tazobactam (tube C)	Imipenem+EDTA (tube D)
Ambler class B carbapenemase	Red	Orange/Yellow	Orange/Yellow	Red



Internal control  
(Lysis Buffer+inoculum)  
+  
Solution A without IMP

**negative**

Lysis Buffer+inoculum  
+  
Solution A with IMP

**positive**

Lysis Buffer+inoculum  
+  
TZB  
+  
Solution A with IMP

**positive**

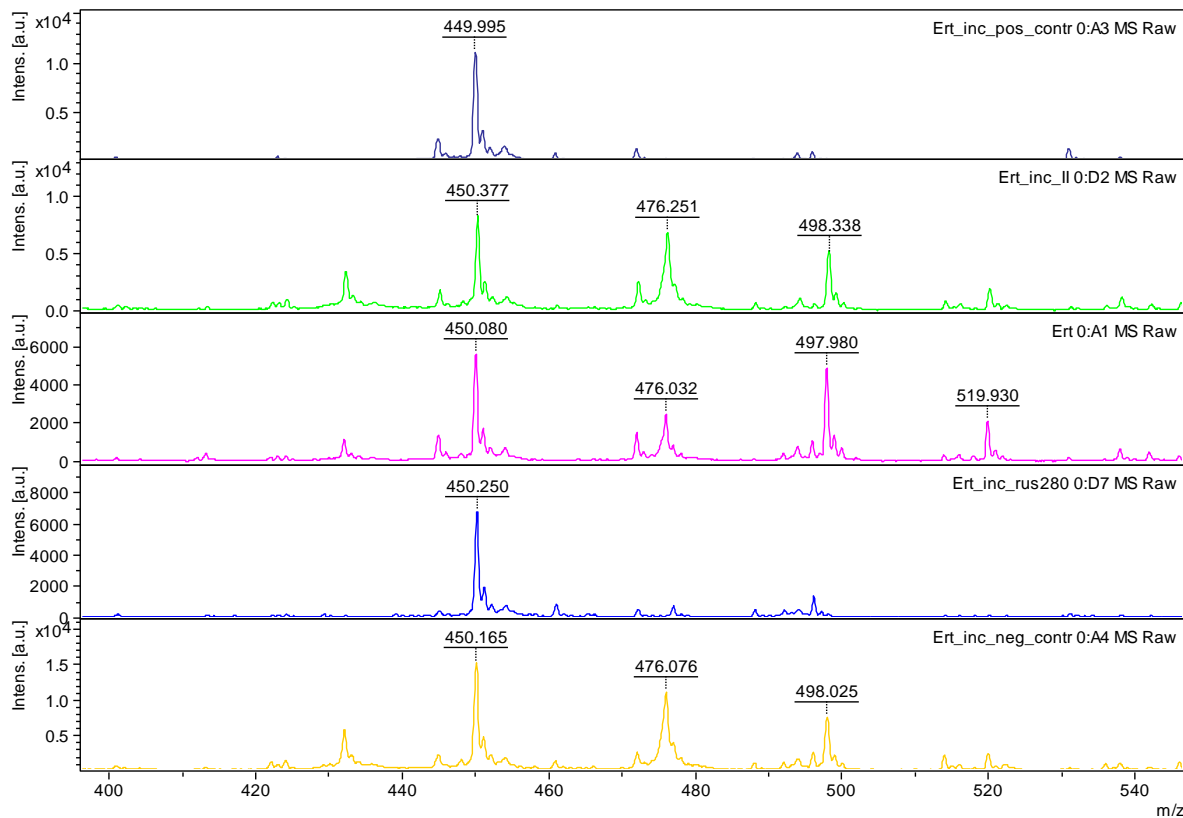
Lysis Buffer+inoculum  
+  
EDTA  
+  
Solution A with IMP

**positive**

**Interpretation → class B carbapenemase (e.g. VIM) + class A (e.g. KPC)**

# MALDI-TOF MS based functional $\beta$ -lactamase assay

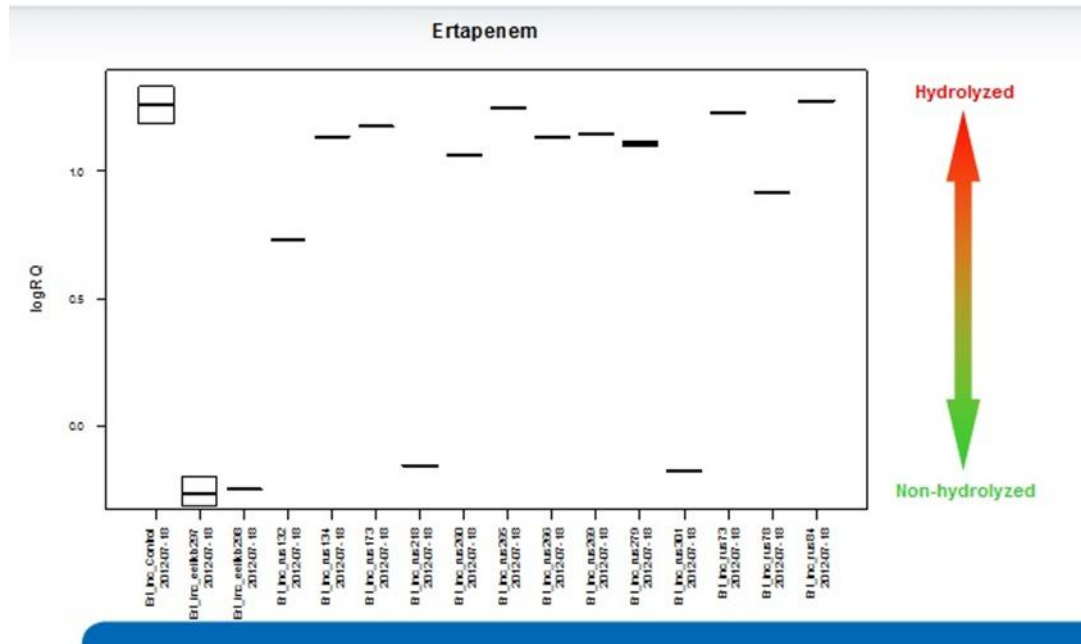
- First protocols published in 2011
- The main point: detection of antibiotic degradation products using MALDI-TOF spectrometer



# Automated evaluation – new software tool by Bruker

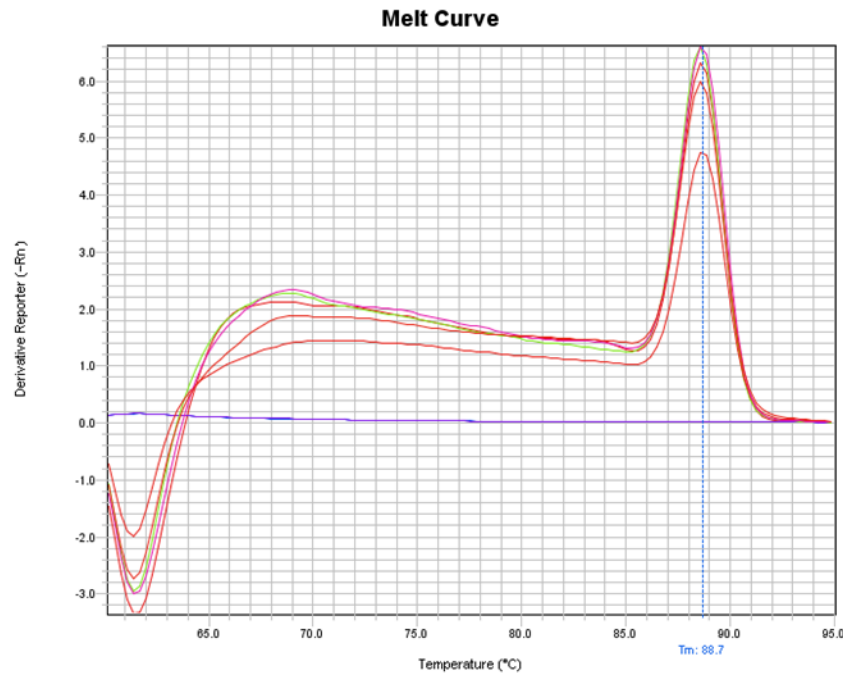
- Software is still not commercially available
- Demonstrates degradation stage of antibiotic

Data analysis with the new software tool



# Multiplex RealTime PCR

- Used in BEEp project
- *bla*<sub>IMP</sub>, *bla*<sub>VIM</sub>, *bla*<sub>GIM</sub>, *bla*<sub>OXA-48</sub>, *bla*<sub>NDM</sub> and *bla*<sub>KPC</sub> genes' detection

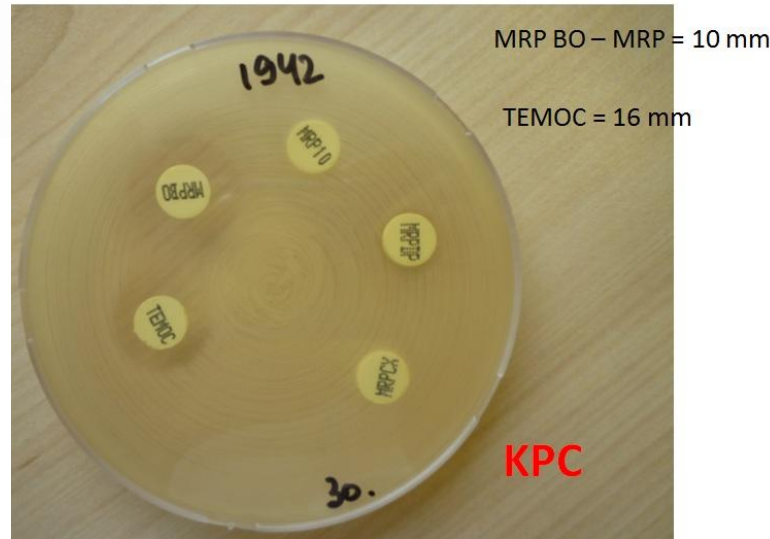
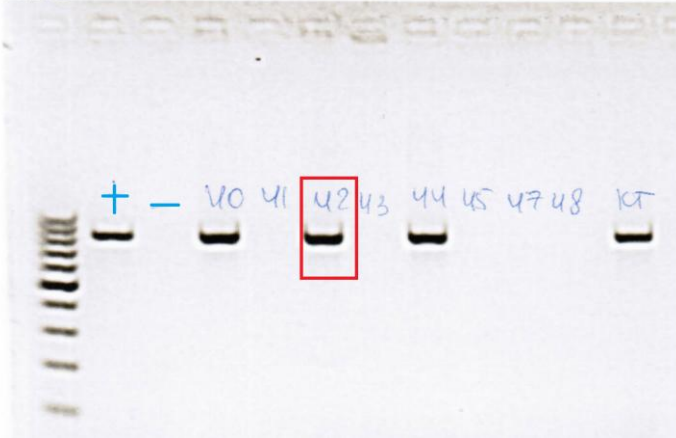


# PCR

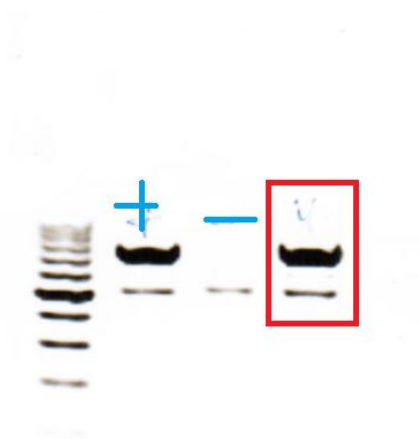
- Used in EuSCAPE EQA
- 100% correlation in EQA answers
- Protocol for *bla*<sub>VIM</sub>, *bla*<sub>OXA-48</sub>, *bla*<sub>NDM</sub> and *bla*<sub>KPC</sub> genes' detection
- Simple in reproduction in routine lab

# 1942 strain – KPC positive KP

Image1  
1381493776 - 004    *KPC*  
anastasia 11. oktober 2013. a. 15:16:42 B=0,00 C=1,00 G=1,00  
I=0,120s



# 1943 strain – OXA positive KP



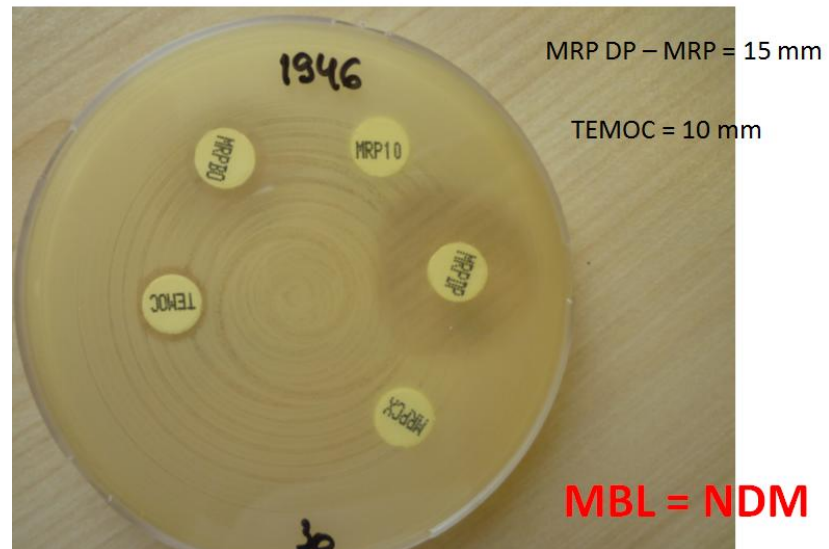
MR = MR DP = MR BO = MR CX

TEMOC = 9

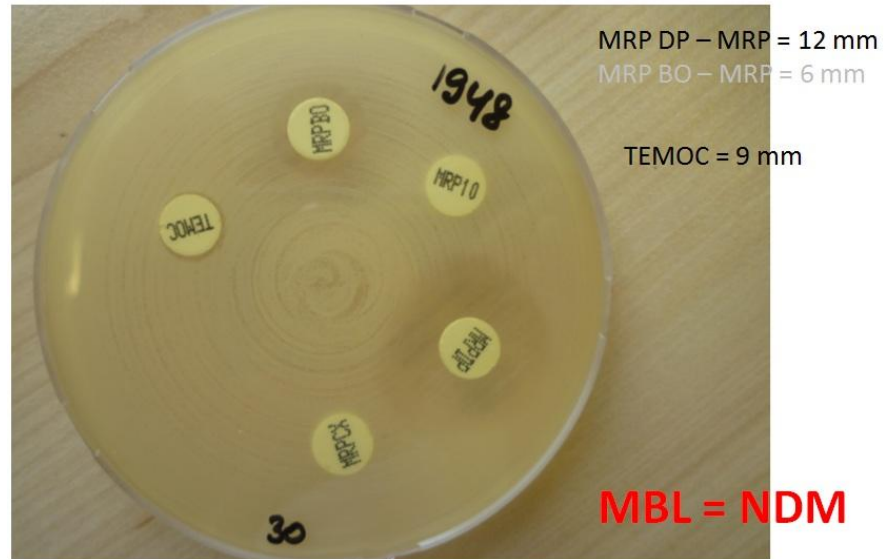
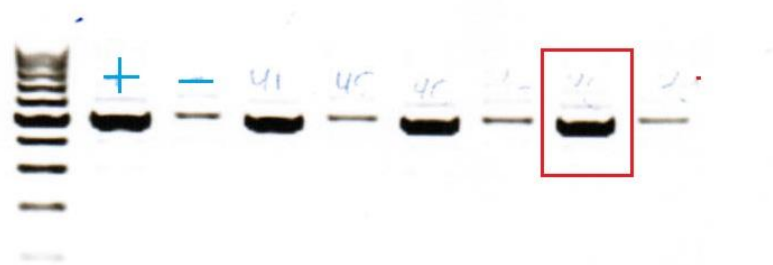
OXA – 48 group



# 1946 strain – NDM positive KP



# 1948 strain – NDM positive KP



# Täna tähelepanu eest!

