Diagnostics Arsenal for Emerging Infectious Diseases

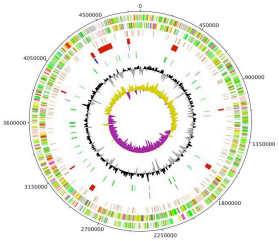
Niaz Banaei, MD
Stanford University

Overview

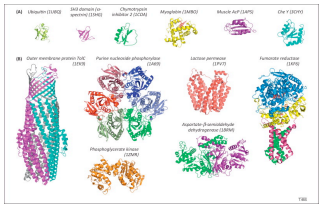
- Accurate diagnostics in 21st century

- Diagnostics for emerging pathogens
  - Ebola and Zika
  - Carbapenem-Resistant Enterobacteriaceae (CRE)
  - Malaria

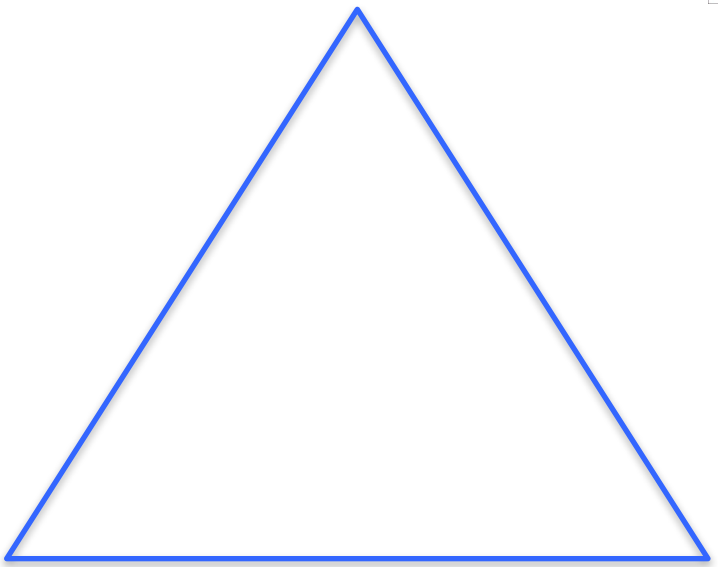
Recipe for POC Diagnostic Development

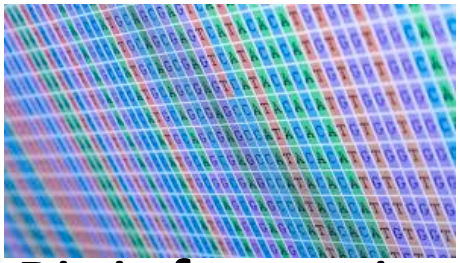


Genomics/
Proteomics Databases



Molecular
Diagnostic
Platforms





Bioinformatics
Tools

Diagnostics No Longer a Limiting Factor

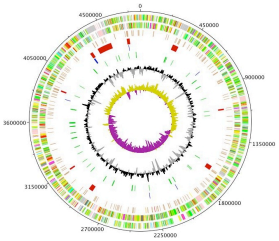
GeneXpert Omni, Cepheid

IMMY

Biofire, bioMérieu

Cobas Liat System, Roche

Recipe for POC Diagnostic Development



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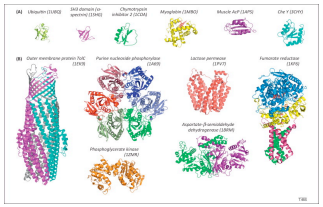

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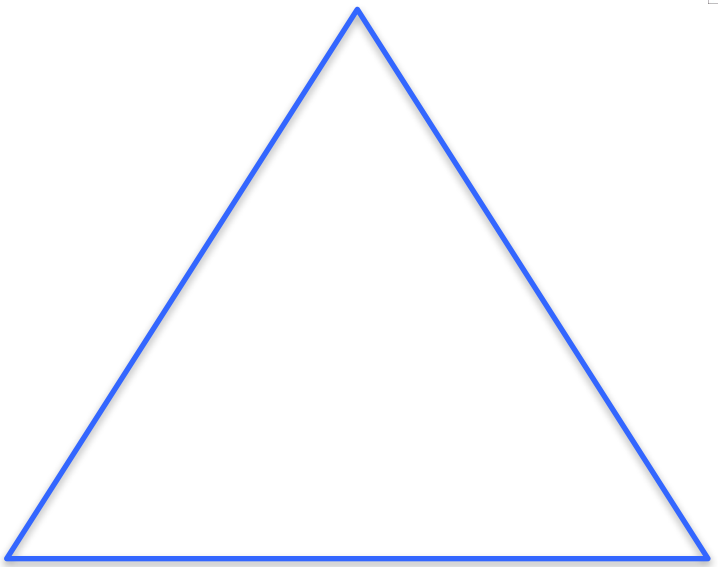

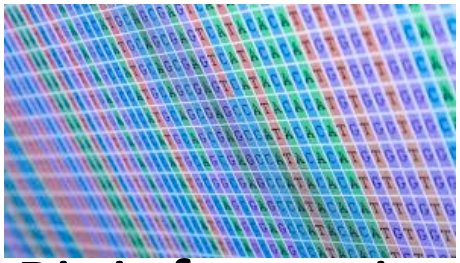

Bioinformatics
Tools

Health

Ebola global response was 'too slow', say health experts

23 November 2015 | Health

Healthcare workers were among those most at risk of catching Ebola

http://www.bbc.com/news/health-34877787

THE LANCET

Will Ebola change the game? Ten essential reforms before the next pandemic. The report of the Harvard-LSHTM Independent Panel on the Global Response to Ebola

Suerie Moon, Devi Sridhar, Muhammad A Pate, Ashish K Jha, Chelsea Clinton, Sophie Delaunay, Valnora Edwin, Mosoka Fallah, David P Fidler, Laurie Garrett, Eric Goosby, Lawrence O Gostin, David L Heymann, Kelley Lee, Gabriel M Leung, J Stephen Morrison, Jorge Saavedra, Marcel Tanner, Jennifer A Leigh, Benjamin Hawkins, Liana R Woskie, Peter Piot

"Although scientists had identified the Ebola nearly four decades earlier and basic research had advanced understanding of the disease, Ebola was not an attractive target for industry investment in research and development, nor was it high on the public health research agenda."

THE LANCET

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Recommendation 7: Establish a global facility to finance, accelerate, and prioritise research and development.

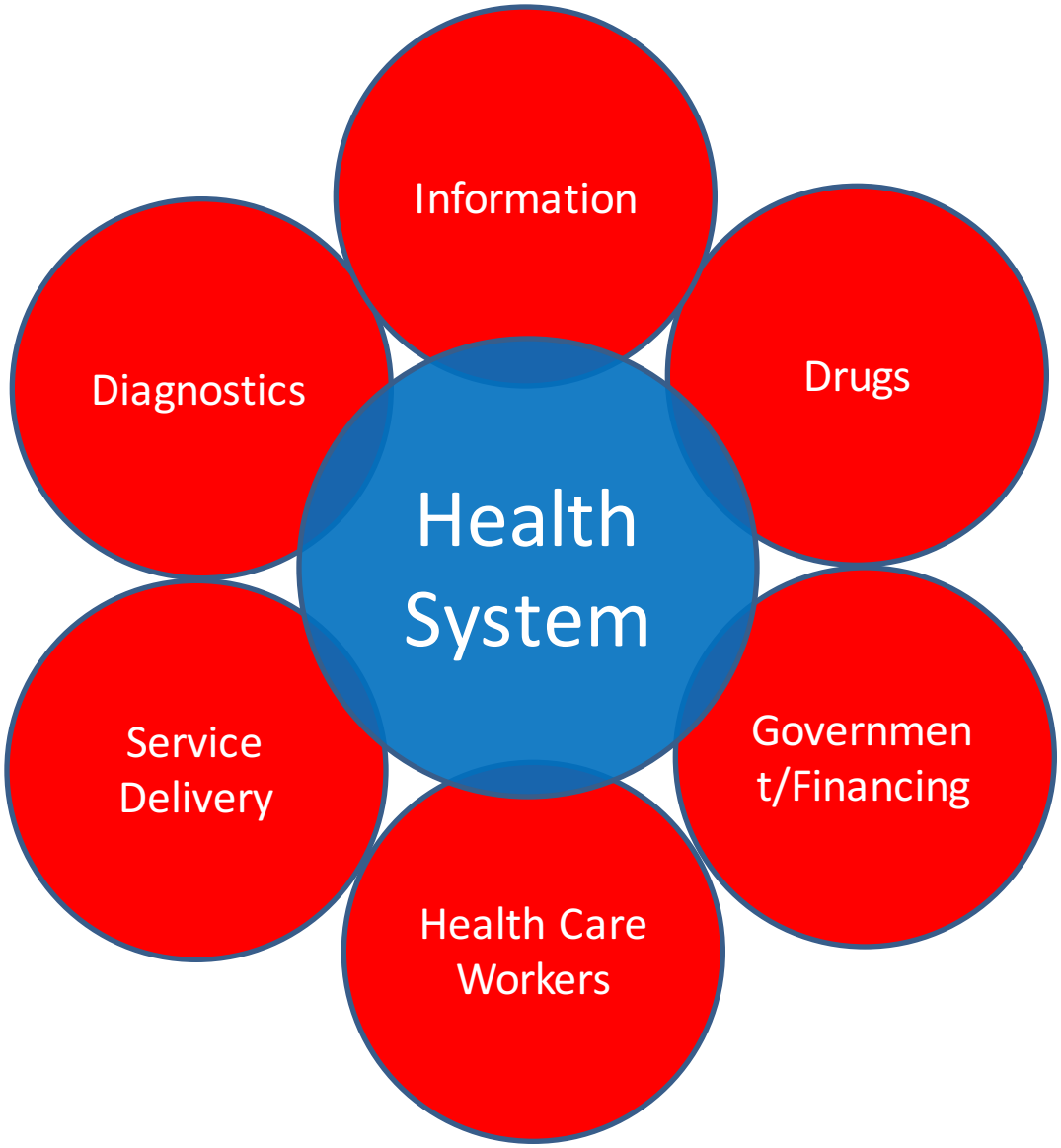
The UN Secretary General and the WHO Director-General should convene in 2016 a high-level summit of public, private, and not-for-profit research funders to establish a global financing facility for research and development for health technology relevant for major disease outbreaks. The facility would support manufacturing, research, and development for drugs, vaccines, diagnostics, and other non-pharmaceutical supplies (such as personal protective equipment) where the commercial market does not offer appropriate incentives. For known pathogens, the facility could invest in bringing candidate drugs, vaccines, technology platforms, and other relevant products through proof of concept, phase 1, and phase 2 testing in humans, so that they are ready for wider testing, manufacturing, and distribution when an outbreak strikes. During an outbreak the facility would rapidly mobilise finance for priority research and development projects, such as diagnostics for novel pathogens.

May 13, 2015

WHO Lists Cepheid's Xpert Ebola as Eligible for Procurement to Ebola Affected Countries

Xpert Ebola listed in World Health Organization Emergency Use Assessment and Listing program for diagnostic products in the context of global health emergency

Diagnostics are One Component of Health System

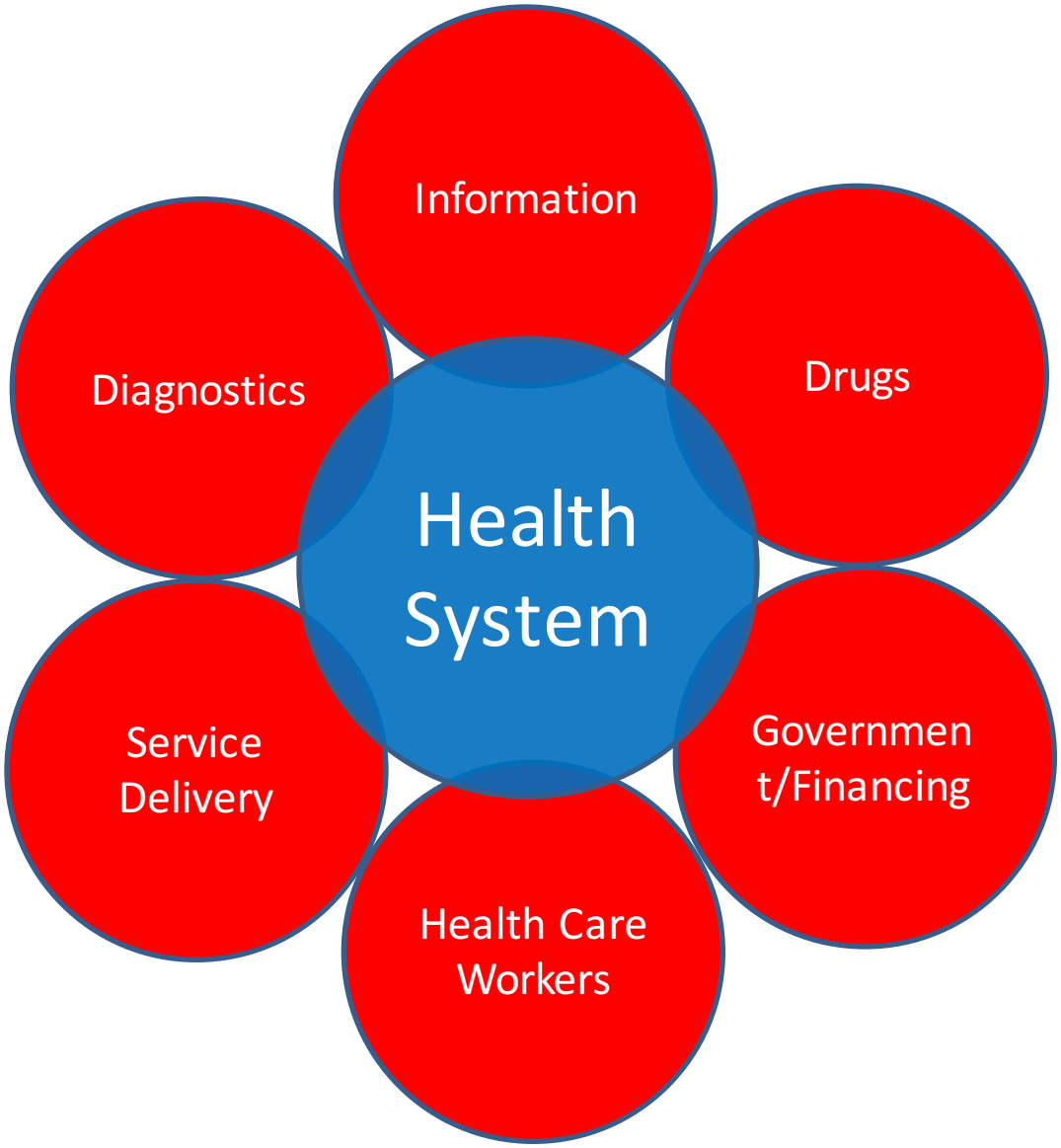


Laboratory Capacity is Lacking in Endemic Countries

Outbreak Response Requires a Health System

Laboratory Training is Needed in Endemic Countries

Diagnostics are One Component of Health System



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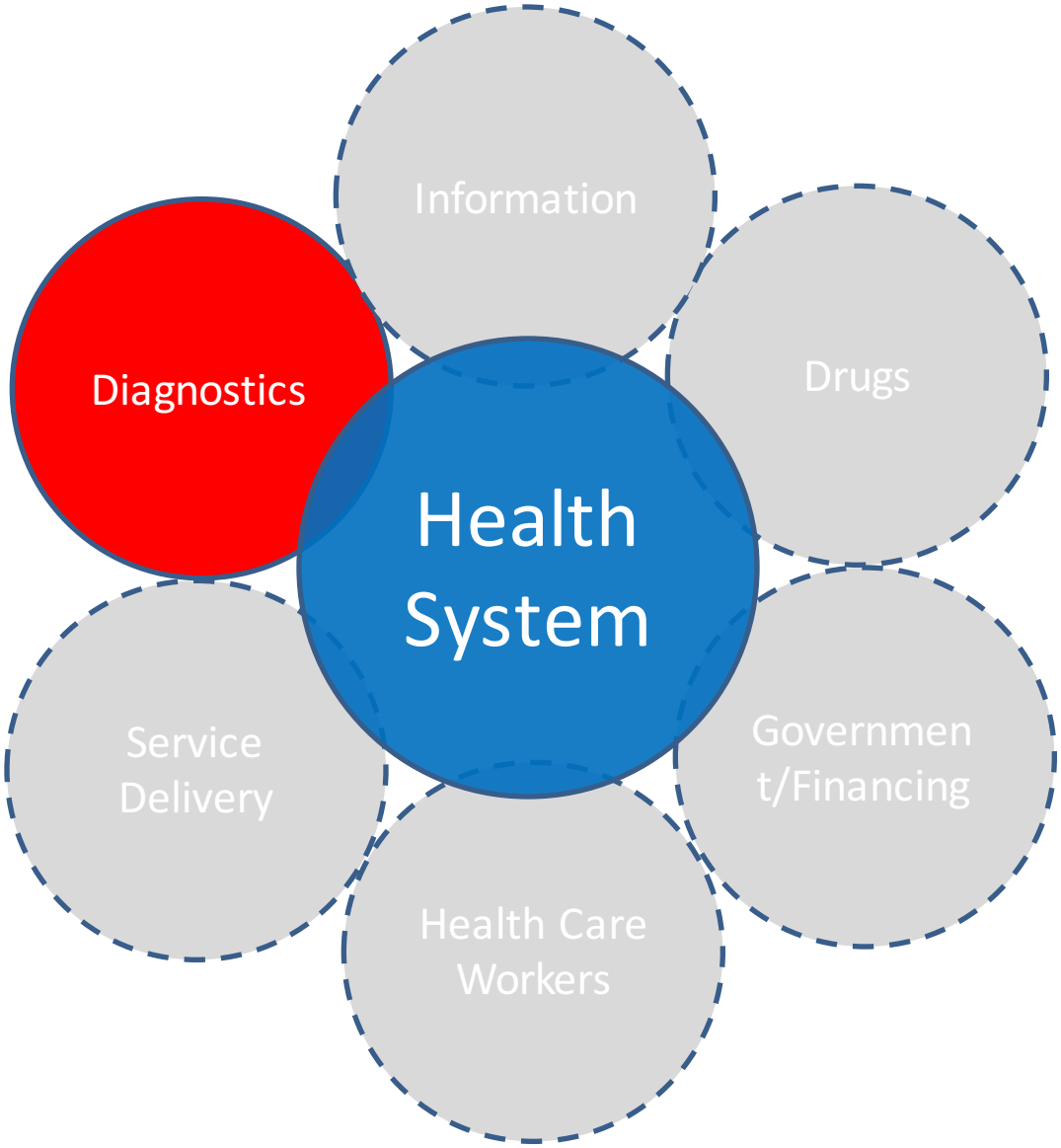

Public Health England laboratory in Port Loko, Sierra Leone

Credit: Jana Broadhurst

Burial team collecting dead bodies from a treatment center at Koidu Government Hospital where many healthcare workers had fallen victim due to inadequate infection control measures.

Credit: Jana Broadhurst

Delivering blood samples by motorbike to the Public Health England field diagnostic laboratory in Port Loko, Sierra Leone

Credit: Jana Broadhurst

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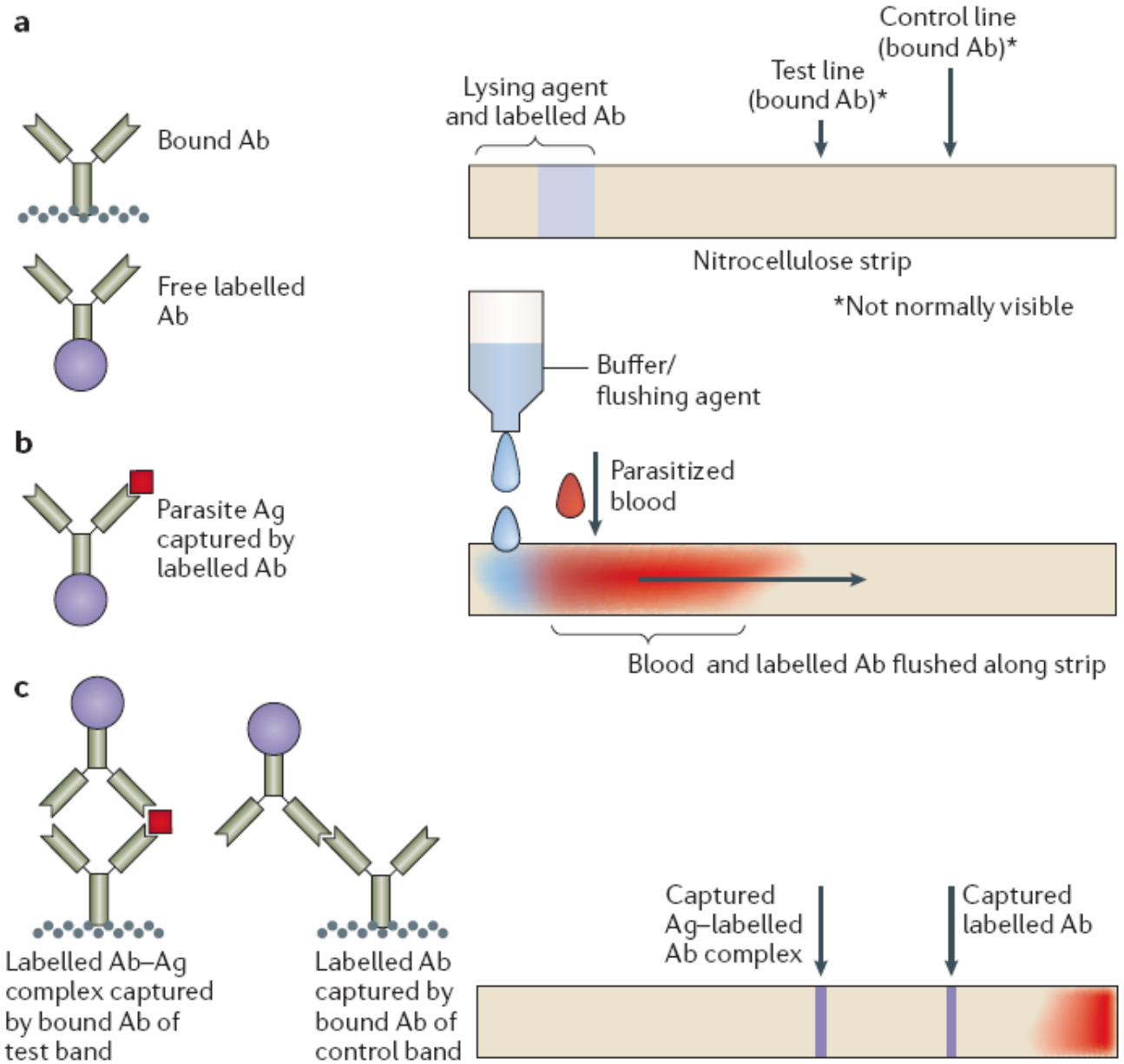
Point Of Care Ebola Diagnostics

Corgenix ReEBOV Antigen Rapid Test

Corgenix ReEBOV Antigen Rapid Test

Method: Dipstick immunoassay
Target: Detecting VP40 matrix protein
TAT: 15 to 25 minutes

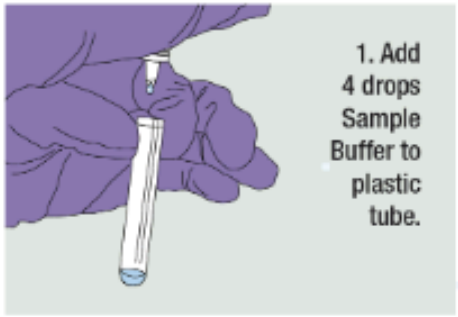
Lateral flow immunochromatographic assay



Bell et al Nature Reviews Microbiology 2006

ReEBOV™ Antigen Rapid Test Instructions

For Detection of Ebolavirus VP40 Antigen

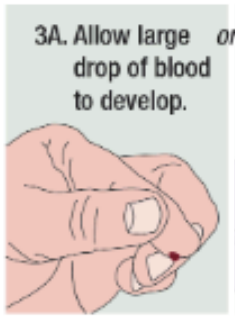


1. Add 4 drops Sample Buffer to plastic tube.

2A. Use safety lancet to perform finger stick.

or

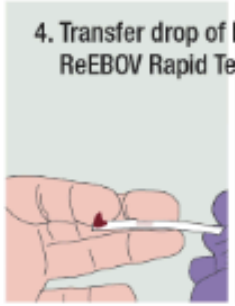
2B. Use a venipuncture.



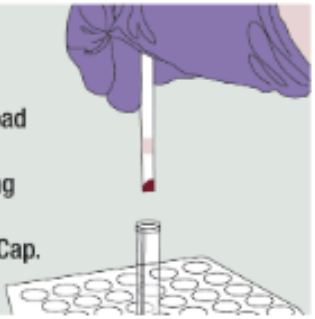
3A. Allow large drop of blood to develop.

or

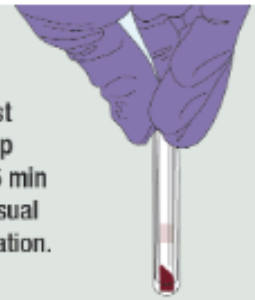
3B. Remove blood with pipette



4. Transfer drop of blood on to ReEBOV Rapid Test sample pad.



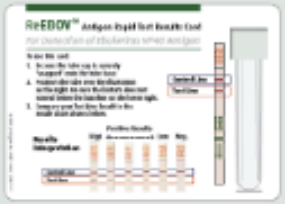
5. Place ReEBOV sample pad into tube containing sample. Replace Cap.

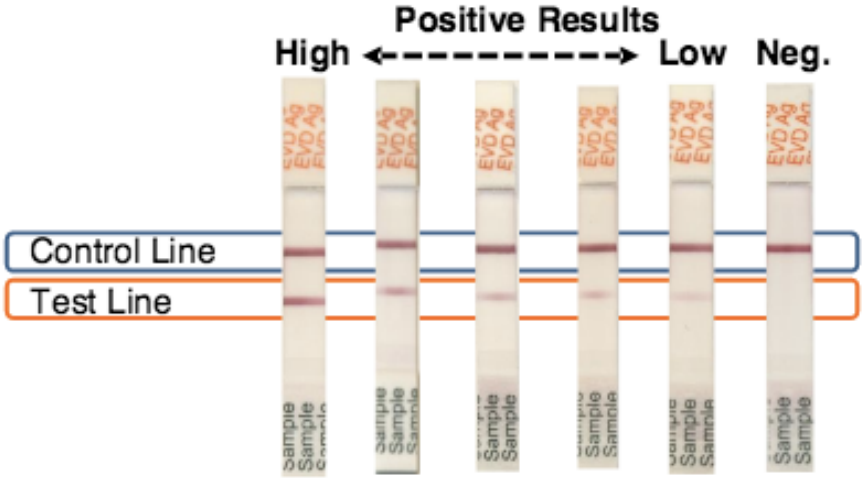


6. Allow ReEBOV Rapid Test to develop for 15-25 min before visual interpretation.

7. Visual Interpretation
   - Top line is control stripe
   - Bottom line is a positive test line

8. Use the Visual Aid card provided to assist in result interpretation.





ReEBOV Antigen Rapid Test kit for point-of-care and laboratory-based testing for Ebola virus disease: a field validation study

Mara Jana Broadhurst, John Daniel Kelly, Ann Miller, Amanda Semper, Daniel Bailey, Elisabetta Groppelli, Andrew Simpson, Tim Brooks, Susan Hula, Wilfred Nyoni, Alhaji B Sankoh, Santigi Kanu, Alhaji Jalloh, Quy Ton, Nicholas Sarchet, Peter George, Mark D Perkins, Betsy Wonderly, Megan Murray, Nira R Pollock

POC Evaluation

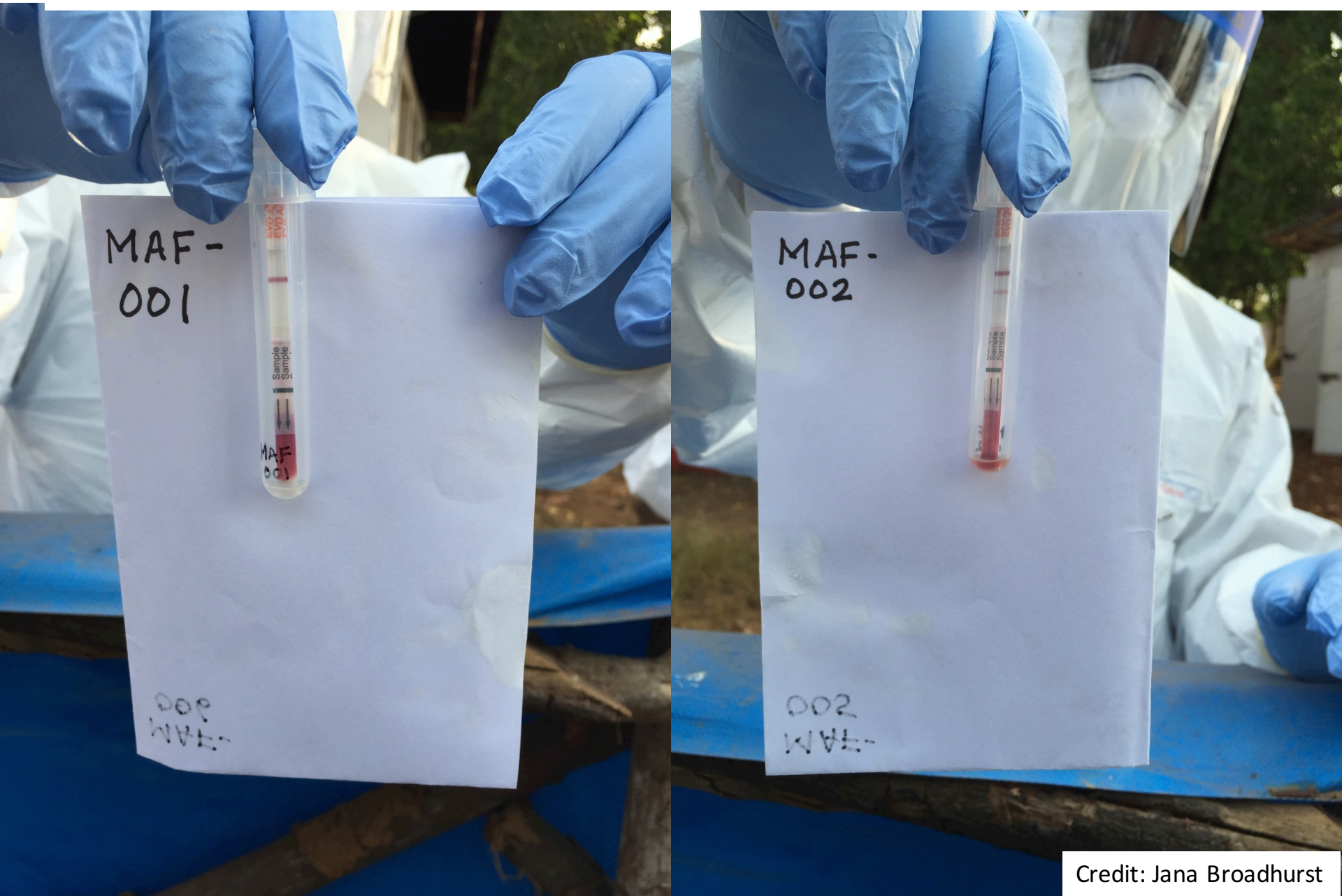
- n=106 individuals with suspected EVD in the field (Sierra Leone)
- Test: Corgenix ReEBOV Antigen Rapid Test on finger stick samples vs. realtime RT-PCR results (RealStar Filovirus Screen RT-PCR kit 1·0; altona Diagnostics GmbH) on venepuncture plasma samples

Lab Evaluation

- n=284 whole blood from individuals with suspected EVD
- Test: Corgenix ReEBOV Antigen Rapid Test vs realtime RT-PCR (Port Loko, Sierra Leone).

Broadhurst et al LANCET 2015 386:867

Alhaji Sankoh with a positive rapid diagnostic test performed on fingerstick blood at the point of care in an Ebola care center



Credit: Jana Broadhurst

RT-PCR testing inside the Public Health England laboratory in Port Loko, Sierra Leone

Credit: Jana Broadhurst

	ReEBOV rapid diagnostic test on fingerstick samples (point of care; n=105)	ReEBOV rapid diagnostic test on venepuncture whole blood samples (reference laboratory; n=277)
Prevalence (% of patients/specimens that tested positive by RT-PCR)	28/105 (26·7%)	45/277 (16·2%)
Sensitivity	28/28 (100%; 87·7–100)	45/45 (100%; 92·1–100)
Specificity	71/77 (92·2%; 83·8–97·1)	214/232 (92·2%; 88·0–95·3)
Negative predictive value	71/71 (100%; 94·9–100)	214/214 (100%; 98·3–100)
Positive predictive value	28/34 (82·4%; 65·5–93·2)	45/63 (71·4%; 58·7–82·1)

Data are n/N (%) or n/N (%; 95% CI). RT-PCR=real-time reverse transcription PCR. *The altona real-time RT-PCR assay was done on fresh venepuncture plasma samples.

Table 1*: Performance of the ReEBOV rapid diagnostic test versus real-time RT-PCR (altona)

- Rapid+/PCR-

6/16 (38%) positive by a referee real-time RT-PCR assay (Trombley assay)

- Rapid-/PCR-

3/18 (17%) positive by Trombley

Broadhurst et al LANCET 2015 386:867

GeneXpert Ebola PCR Assay

Feasibility of Xpert Ebola Assay in Médecins Sans Frontières Ebola Program, Guinea

Rafael Van den Bergh, Pascale Chaillet, Mamadou Saliou Sow, Mathieu Amand, Charlotte van Vyve, Sylvie Jonckheere, Rosa Crestani, Armand Sprecher, Michel Van Herp, Arlene Chua, Erwan Piriou, Lamine Koivogui, Annick Antierens

Médecins Sans Frontières

- Lab Evaluation

Tested 218 whole blood from patients with suspected EVD, on treatment, and convalescence

Compared Xpert to lab-developed RT-PCR

Sample type	No. positive by Xpert Ebola Assay	No. (%) positive by routine PCR
Total	26	18 (69)
Diagnosis 1	8	8 (100)
Diagnosis 2	0	0
After transfusion	12	9 (75)
Convalescent phase	6	1 (17)

Emerging Infectious Diseases 2016 22:210

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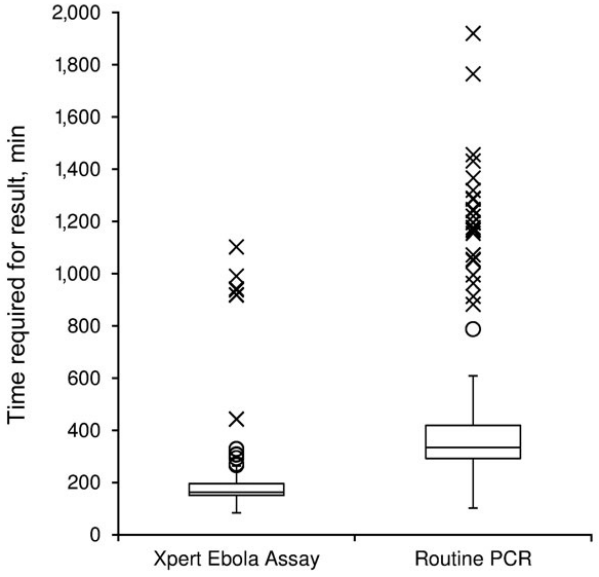
TAT receiving sample to resulting

Median 165 min 334 min



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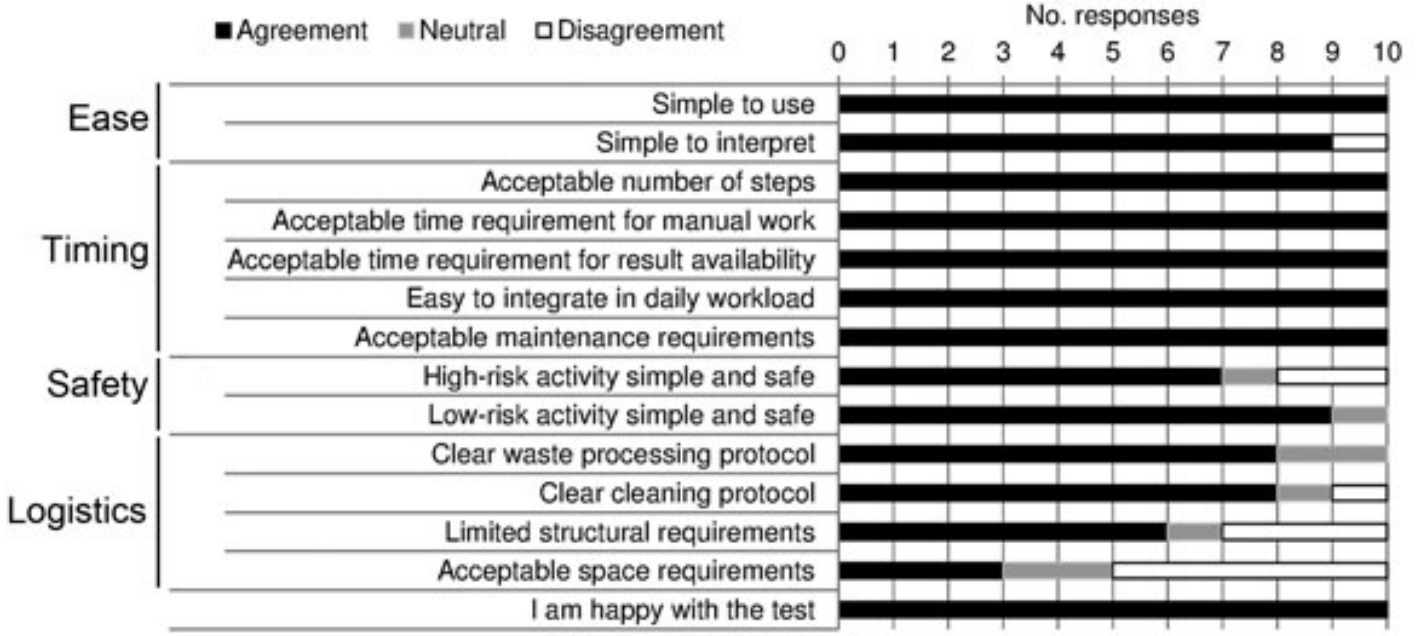


Emerging Infectious Diseases 2016 22:210

October 7-11 • San Diego, CA • www.idweek.org
IDWeek 2015
Advancing Science, Improving Care

LB-1. Field Laboratory Evaluation of the GeneXpert Ebola Assay for Diagnosis of Ebola Virus Disease in Sierra Leone

Amanda Semper, Jana Broadhurst, Jade Richards, Geraldine M. Foster, Andrew Simpson, Elisabetta Groppelli, Julie Johnson, J. Dan Kelly, Tim Brooks, Christopher H. Logue, Ann Miller, Megan B. Murray, and Nira R. Pollock, MD, PhD

Evaluation at a field biocontainment laboratory in Sierra Leone

- Individuals with suspected EVD
- Specimens: Whole blood (n = 218) and buccal swab (n = 71) specimens
- Tests: Xpert vs routine EVD testing by RT-PCR ("Trombley assay")
  - Compared whole blood sampling methods (pipette vs swab) on 84/218

Semper et al PLoS Medicine 2016

Loading up the Cepheid GeneXpert for delivery to the Public Health England field laboratory in Port Loko in preparation for the field validation study

Credit: Jana Broadhurst

Bringing the GeneXpert into the PHE field lab in Port Loko

Credit: Jana Broadhurst

October 7-11 • San Diego, CA • www.idweek.org
IDWeek 2015
Advancing Science, Improving Care

LB-1. Field Laboratory Evaluation of the GeneXpert Ebola Assay for Diagnosis of Ebola Virus Disease in Sierra Leone

- Invalid Xpert results

7/218 (3.2%) whole blood and 7/71 (9.9%) buccal swab

- Tromboley PCR+

On whole blood: 22/22 Xpert+ (Sensitivity 100%)
On buccal swab: 20/20 Xpert+ (Sensitivity 100%)

- Tromboley PCR-

On whole blood: 181/189 Xpert- (Specificity 95.8%) → 7/8 from previously PCR+ → Xpert Specificity 99.5%
On buccal swab 44/44 Xpert- (Specificity 100%)

- Whole blood sampled with pipette vs Swab concordant for 78/79 (98.7%)
  Sample reagent reduced EBOV titer by ≥6 logs (Pinsky et al PLoS One 2015)

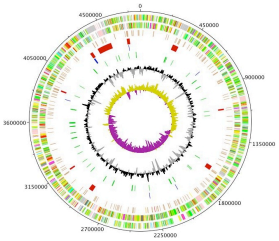
Semper et al PLoS Medicine 2016

Brazil state announces Zika fever emergency

1 December 2015 | Latin America & Caribbean

Aedes aegypti mosquito is found throughout tropical Africa and in parts of South America

Recipe for POC Diagnostic Development



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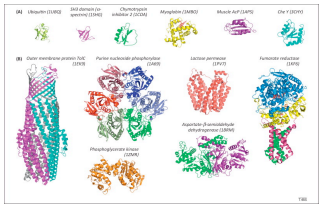

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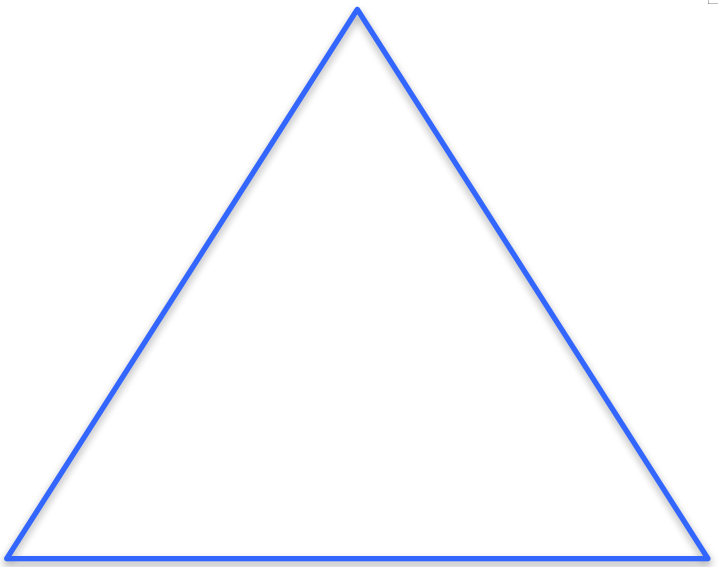
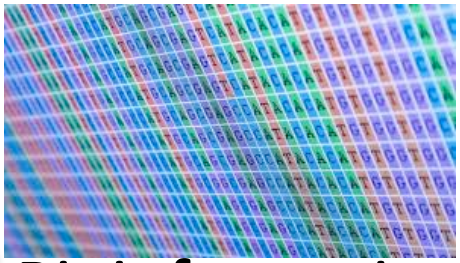
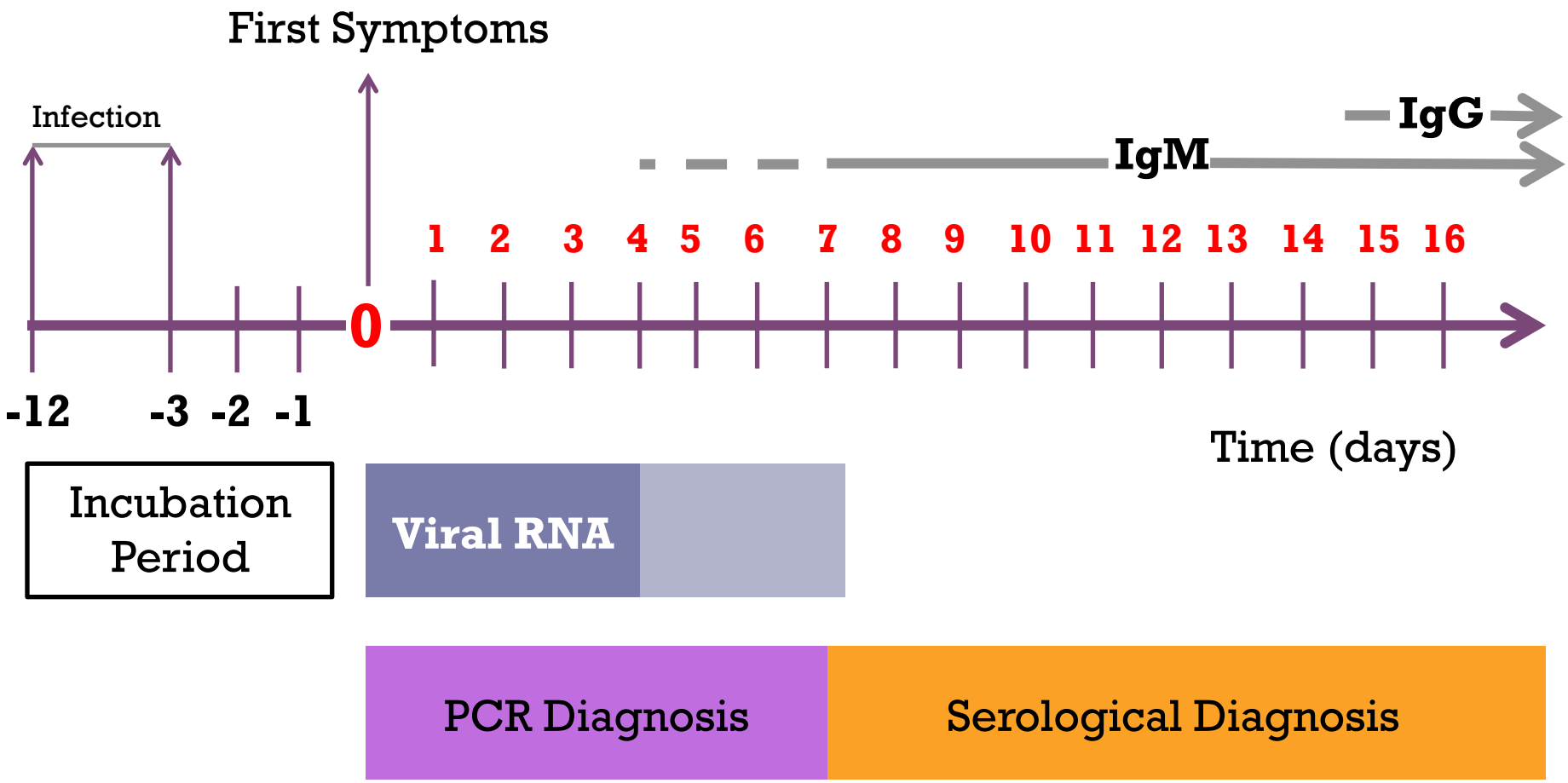




Bioinformatics
Tools

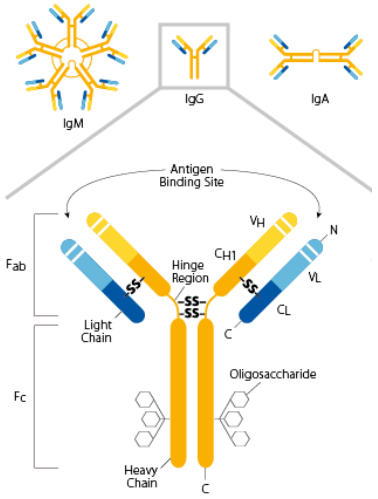
Zika Virus Diagnostics



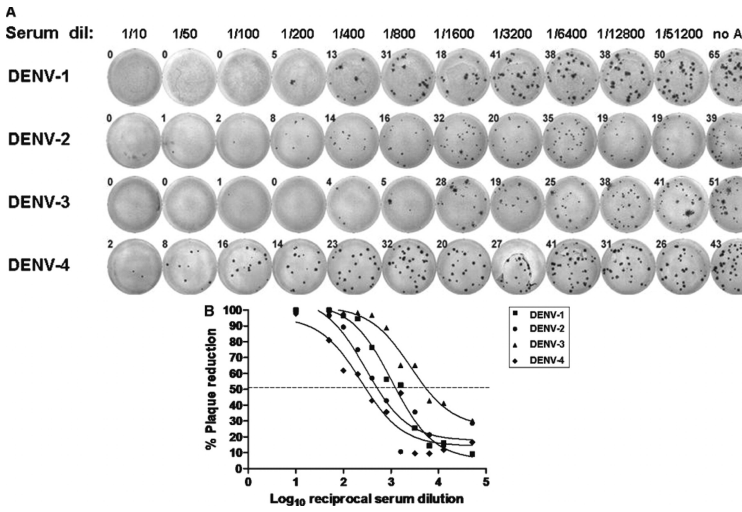
Slide courtesy of Desiree Lebeaud and Michele Barry

Zika Virus Diagnostics: Serology

- ZIKV IgM Antibodies
  - CDC IgM ELISA: extensive flavivirus cross reaction.
  - Previous Infection or vaccination with other flaviviruses (*e.g.*, DENV, JEV, YFV) may result in false positive results.
  - Note: returning travelers frequently have an initial diagnosis of dengue, due to false-positive DENV IgM tests.
  - CDC IgM ELISA received FDA EUA
  - Euroimmun offers CE marked Zika virus ELISA assays



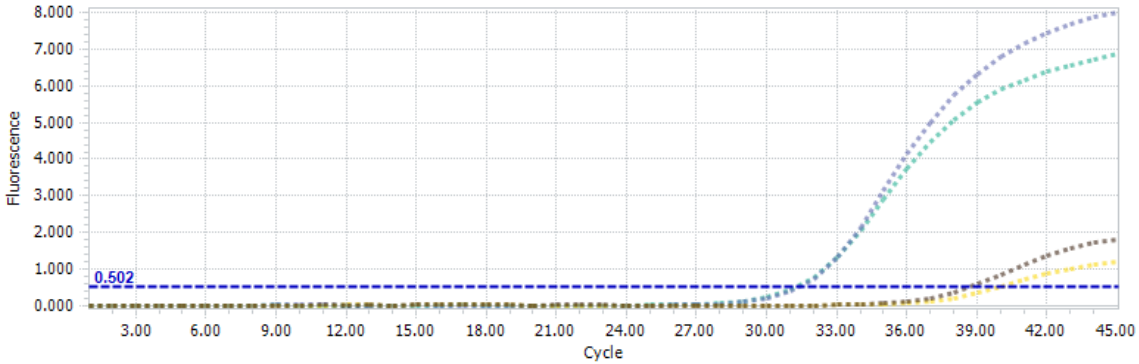
- Plaque Reduction Neutralization Testing (PRNT)
  - PRNT90 may help distinguish ZIKV infection from infection with DENV or other flaviviruses.
  - ≥4-fold rise in titer in acute and convalescent sera or ≥4-fold greater ZIKV titer compared to other flaviviruses in a single specimen.



- NS1 Antigen Testing
  - Not currently available

Slide courtesy of Ben PInsky

Zika Virus Diagnostics: Nucleic Acid Amplification Testing



CENTERS FOR DISEASE CONTROL AND PREVENTION

- RNA Detection
  - Serum: short duration of RNAemia, lower viral loads compared to DENV or CHIKV.
  - Saliva: higher rate of detection, duration similar to serum.
  - Urine: may be detectable 7 or more days after undetectable in serum.

- ZIKV CDC real-time, reverse transcriptase-PCR (rRT-PCR)
  - Most commonly performed globally.
  - Designed based on sequences from the 2007 Yap Islands outbreak.
    - Lanciotti *et al.,* Emerging Infectious Diseases 2008

- Other rRT-PCR Options
  - Altona Diagnostics offers a CE marked rRT-PCR assay.
  - Several laboratory developed ZIKV rRT-PCR assays have been published.

Slide courtesy of Ben PInsky

Stanford Zika Virus Testing

		CDC ZIKV rRT-PCR		
		Positive	Negative	Total
Stanford Multiplex ZIKV	Positive	25	31*	56
	Negative	1	76	77
	Total	26	107	133

- Stanford Multiplex ZIKV/CHIKV/DENV rRT-PCR
  - Detects and distinguishes between these three co-circulating arboviruses.
  - ZIKV was detected in significantly more Nicaraguan samples using the Stanford assay than the CDC ZIKV rRT-PCR ($p<0.001$).
  - In a larger set of samples from patients with suspected arboviral infection
    - 73.7% (255/346) tested positive.
    - Co-infections accounted for 20.1% (53/255) of the positives: DENV-CHIKV (n=36), CHIKV-ZIKV (n=10), DENV-ZIKV (n=3), and DENV-CHIKV-ZIKV (n=4).

Slide courtesy of Ben PInsky

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  - Ebola and Zika
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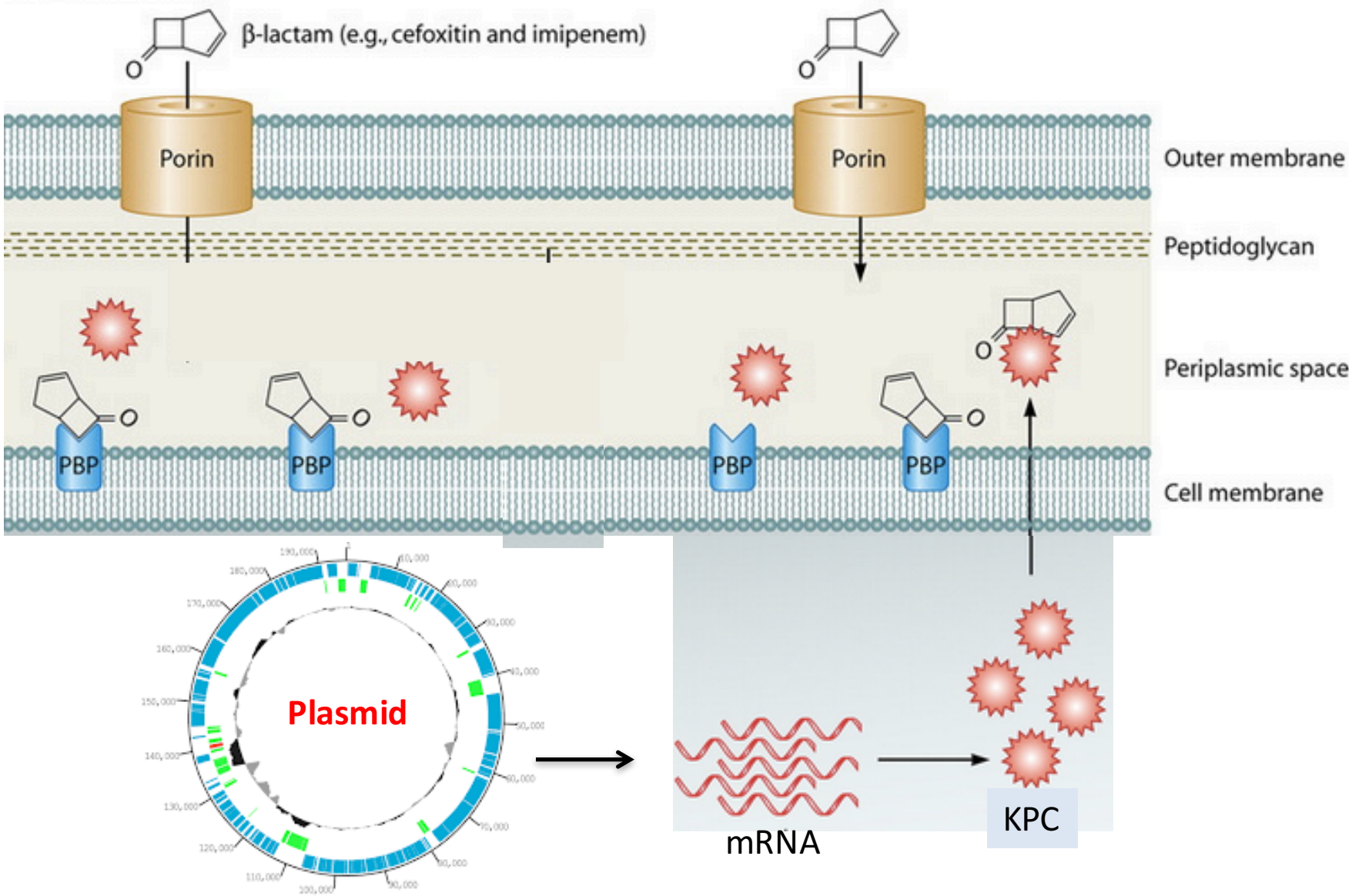
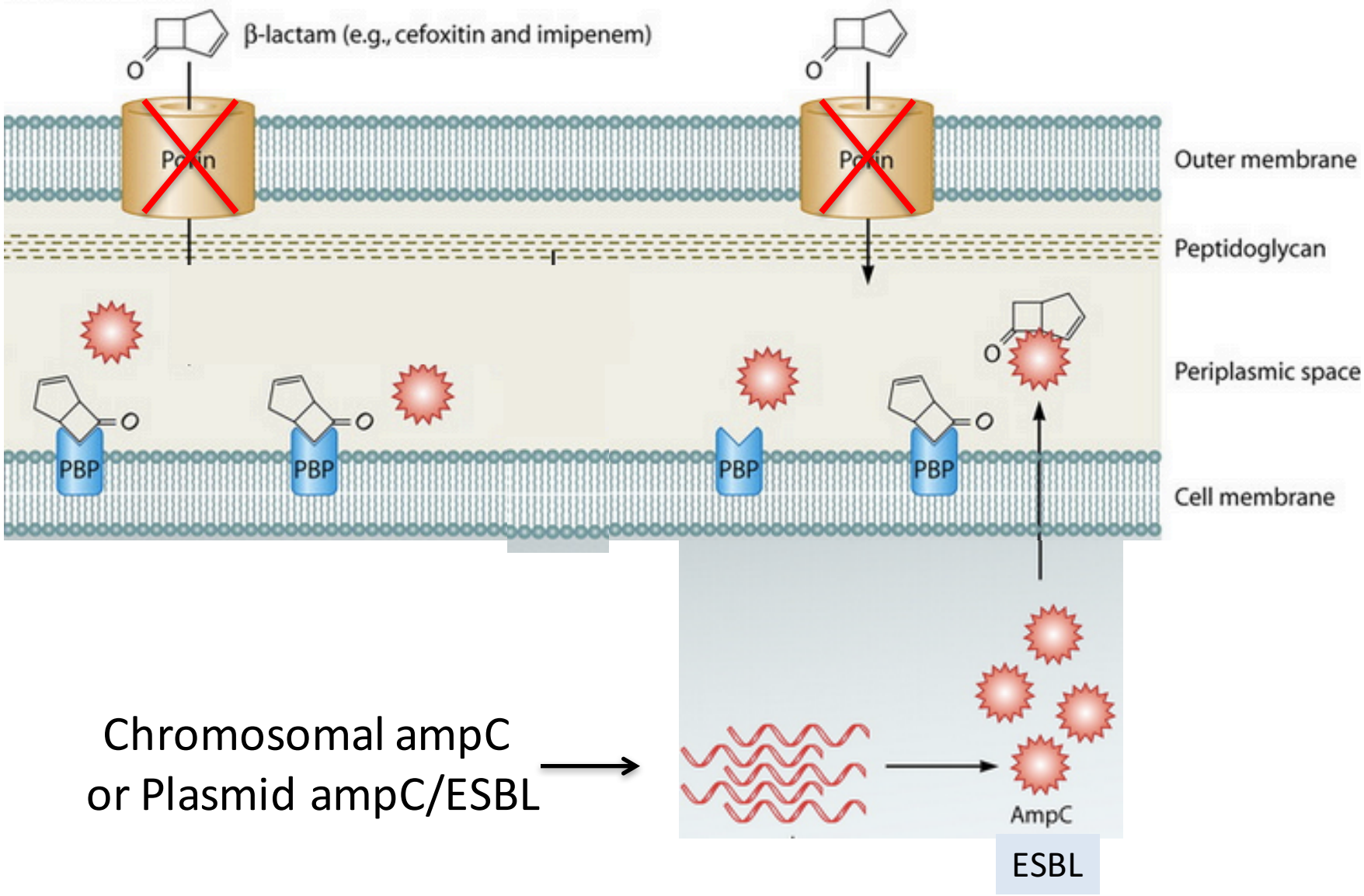
Carbapenem-Resistant Enterobacteriaceae (CRE)

2015 CDC definition: Resistant to imipenem, meropenem, doripenem or ertapenem

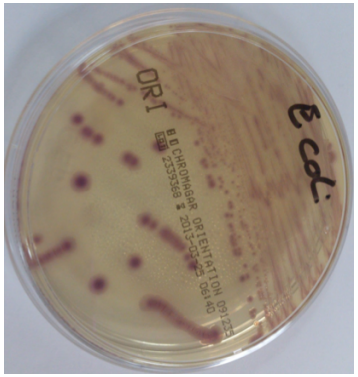
Mechanism of Carbapenem Resistance



Mechanism of Carbapenem Resistance



Indian man with pyelonephritis.
Visiting daughter in Silicon Valley.
Ureteral stent placed in India for kidney stones.
Urine culture: *E. coli* >100,000/mL

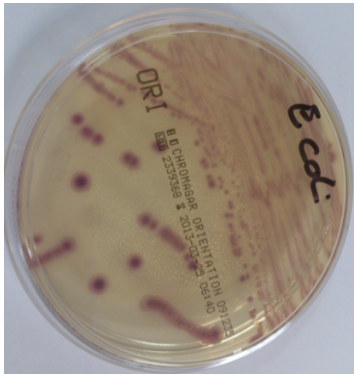


Susceptibility

	MIC by Vitek 2	
Amikacin	>=64 ug/mL ...	RESISTANT
Amoxicillin/Clavulanic Acid	>=32 ug/mL ...	RESISTANT
Ampicillin	>=32 ug/mL ...	RESISTANT
Cefazolin	>=64 ug/mL ...	RESISTANT [1]
Cefepime	>=64 ug/mL ...	RESISTANT
Cefoxitin	>=64 ug/mL ...	RESISTANT
Ceftazidime	>=64 ug/mL ...	RESISTANT
Ceftriaxone	>=64 ug/mL ...	RESISTANT
Ciprofloxacin	>=4 ug/mL (...	RESISTANT
Doxycycline		
Ertapenem	>=8 ug/mL (...	RESISTANT
Gentamicin	>=16 ug/mL ...	RESISTANT
Imipenem		
Levofloxacin	>=8 ug/mL (...	RESISTANT
Meropenem	8 ug/mL (MIC)	RESISTANT
Nitrofurantoin	64 ug/mL (MIC)	INTERMEDIATE
Piperacillin/Tazobactam	>=128 ug/mL...	RESISTANT
Tetracycline	>=16 ug/mL ...	RESISTANT
Tobramycin	>=16 ug/mL ...	RESISTANT
Trimethoprim/Sulfamethoxazole.	>=320 ug/mL...	RESISTANT

Disk Diffusion and E-test results

Indian man with pyelonephritis.
Visiting daughter in Silicon Valley.
Ureteral stent placed in India for kidney stones.
Urine culture: *E. coli* >100,000/mL



Susceptibility

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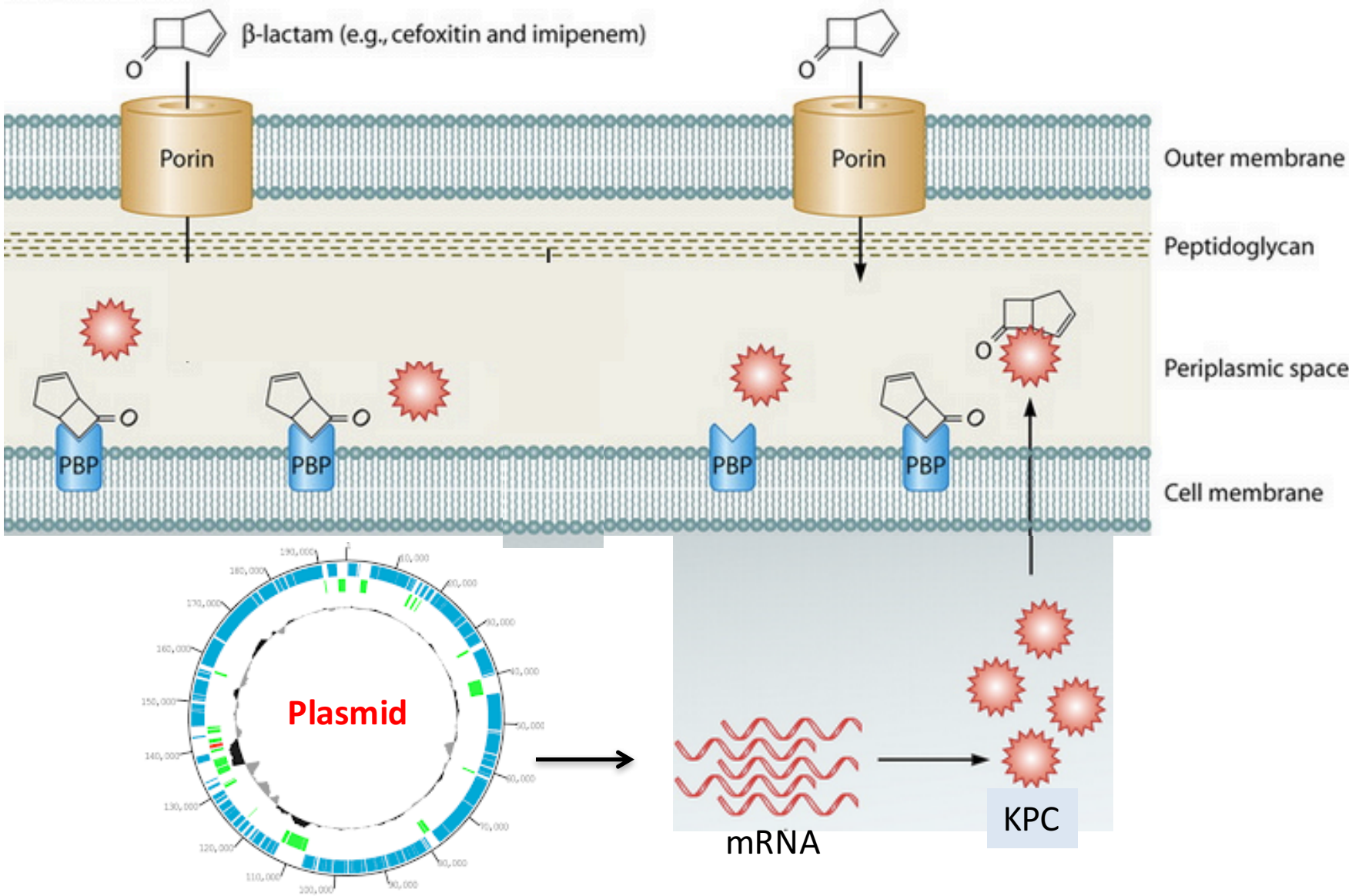
Cefazolin	>=64 ug/mL ...	RESISTANT [1]			
Cefepime	>=64 ug/mL ...	RESISTANT			
Cefoxitin	>=64 ug/mL ...	RESISTANT			
Ceftazidime	>=64 ug/mL ...	RESISTANT			
Ceftolozane/Tazobactam				>256 ug/mL ...	R
Ceftriaxone	>=64 ug/mL ...	RESISTANT			
Ciprofloxacin	>=4 ug/mL (...	RESISTANT			
Doxycycline				>256 ug/mL ...	R
Ertapenem	>=8 ug/mL (...	RESISTANT	RESISTANT		
FOSFOMYCIN			SUSCEPTIBLE		
Gentamicin	>=16 ug/mL ...	RESISTANT			
Imipenem			RESISTANT		
Levofloxacin	>=8 ug/mL (...	RESISTANT			
Meropenem	8 ug/mL (MIC)	RESISTANT	RESISTANT		
Nitrofurantoin	64 ug/mL (MIC)	INTERMEDIATE			
Piperacillin/Tazobactam	>=128 ug/mL...	RESISTANT			
Polymixin B				1 ug/mL (MIC)	No interp
Tetracycline	>=16 ug/mL ...	RESISTANT			
Tobramycin	>=16 ug/mL ...	RESISTANT			
Trimethoprim/Sulfamethoxazole.	>=320 ug/mL...	RESISTANT			

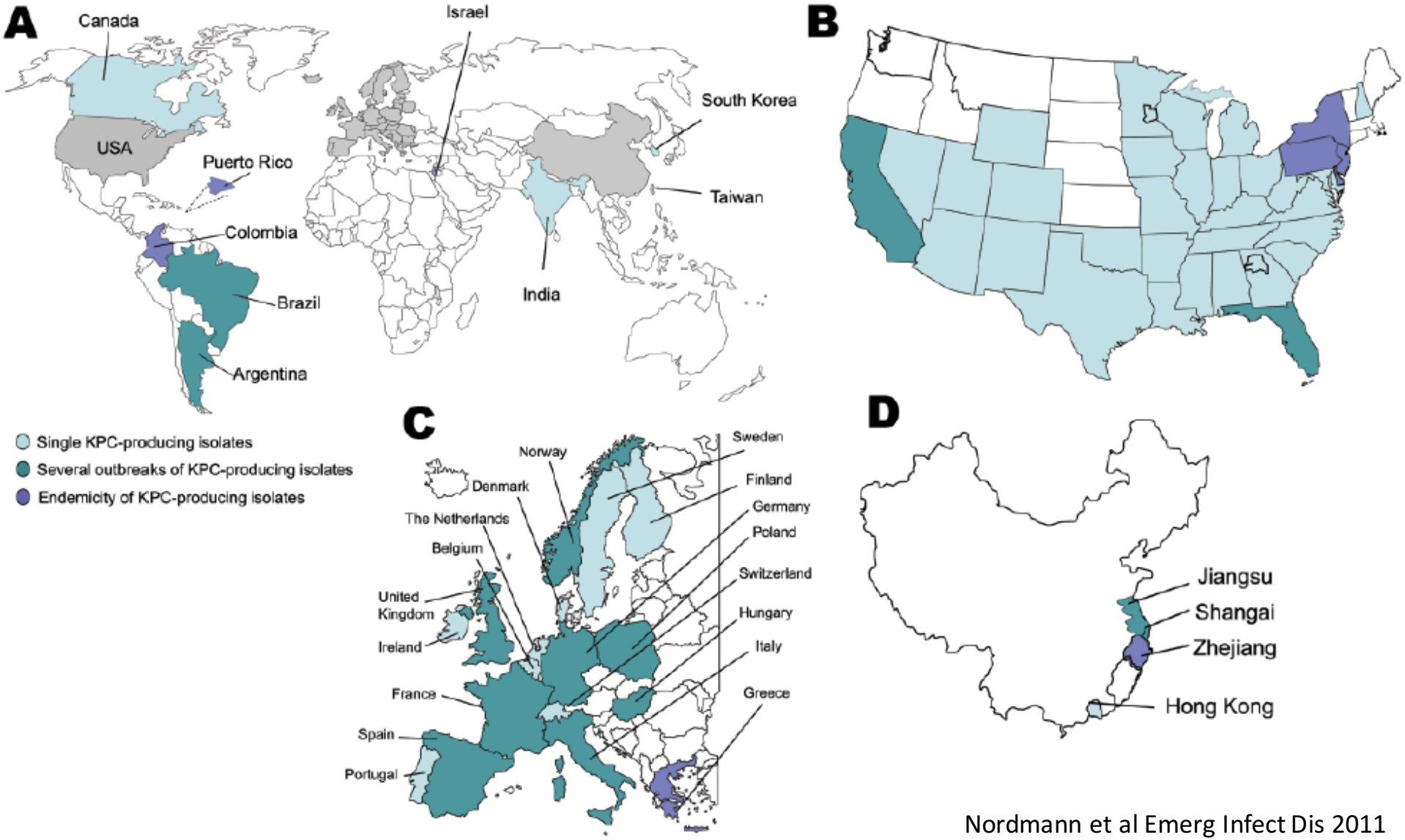
Mechanism of Carbapenem Resistance



Emergence of Carbapenemases

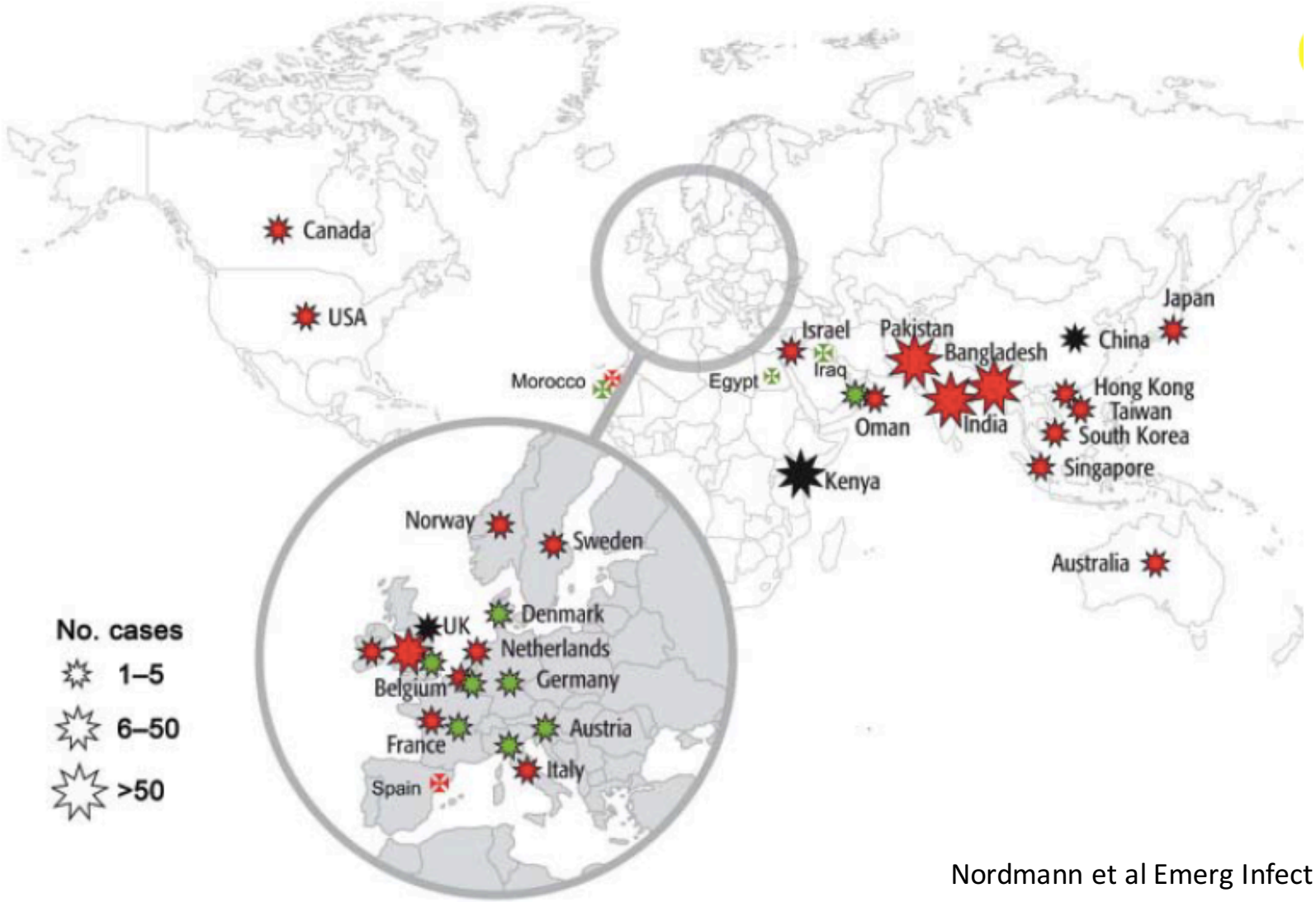
Enzyme	Molecular Class	Location	Year of Isolation	City,Country of origin
IMP	B	Plasmid	1991	Japan
KPC	A	Plasmid	1996	NC, USA
VIM	B	Plasmid	1996	Verona, Italy
OXA-48-like	D	Plasmid	2001	Turkey
NDM	B	Plasmid	2008	India

Geographic Distribution of *Klebsiella pneumoniae* Carbapenemase (KPC) producers



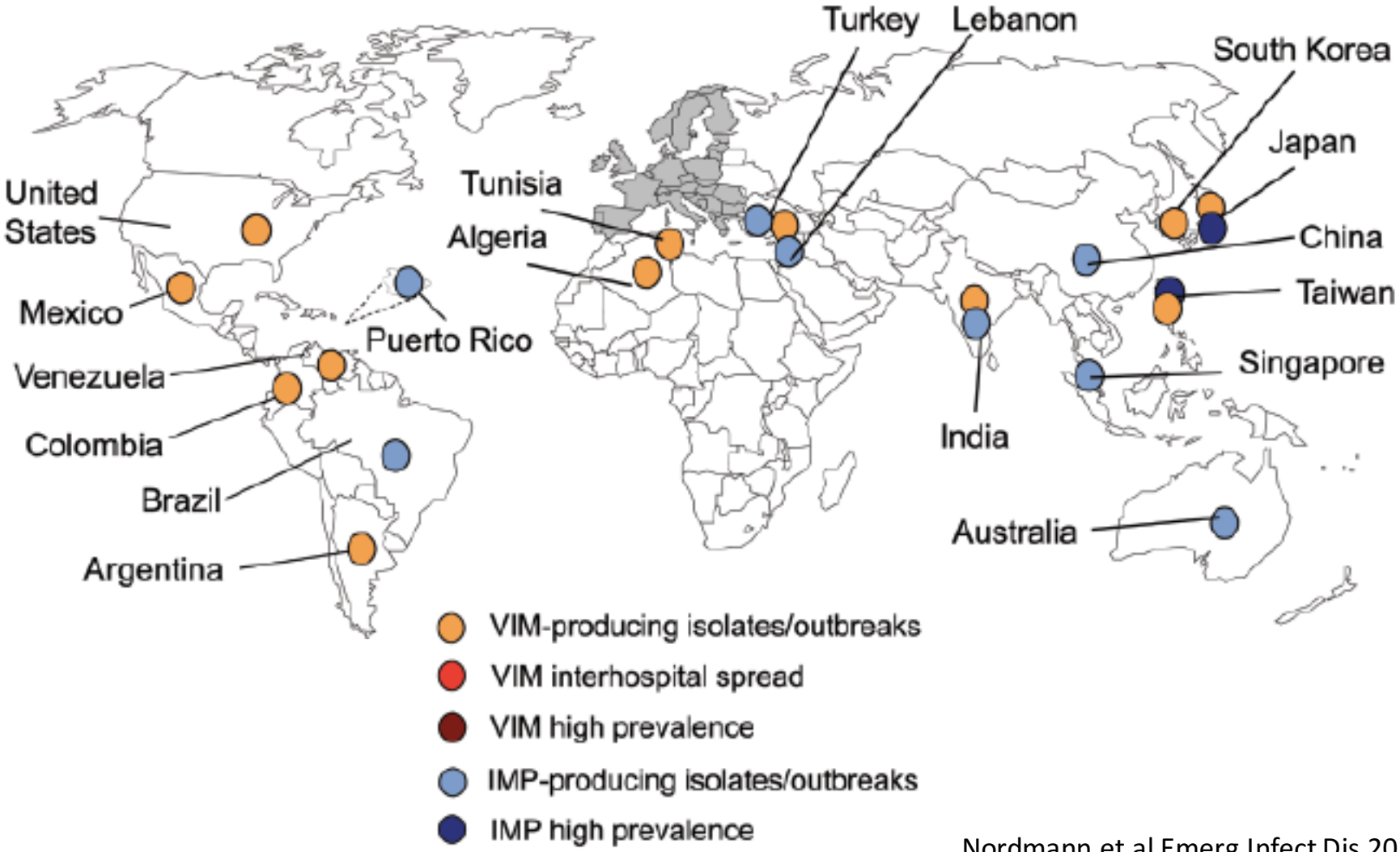
Nordmann et al Emerg Infect Dis 2011

Geographic Distribution of New Delhi Metallo-β-Lactamase Producers



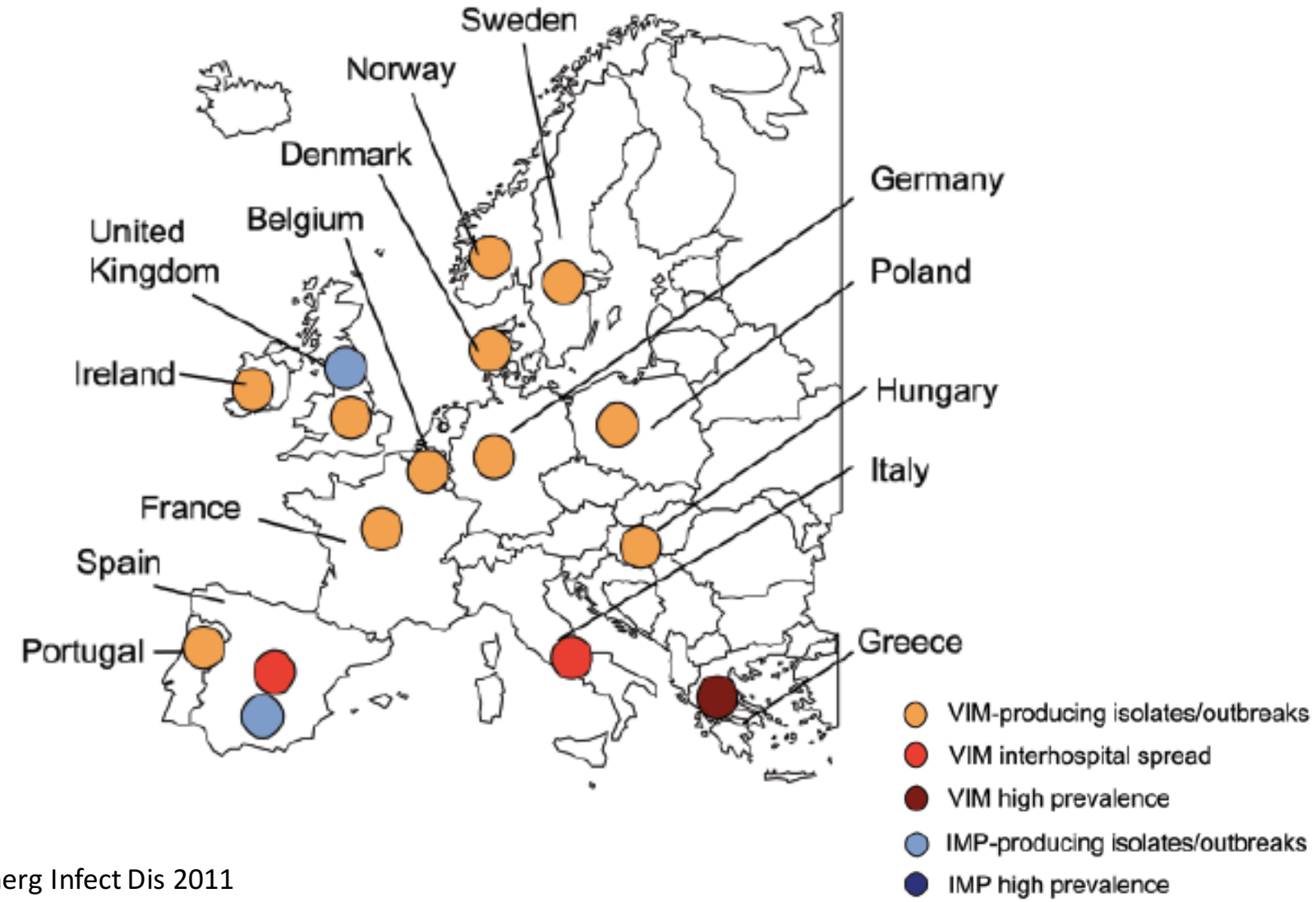
Nordmann et al Emerg Infect Dis 2011

Distribution of Verona Integron-Encoded Metallo-β-Lactamase (VIM) & IMP Producers



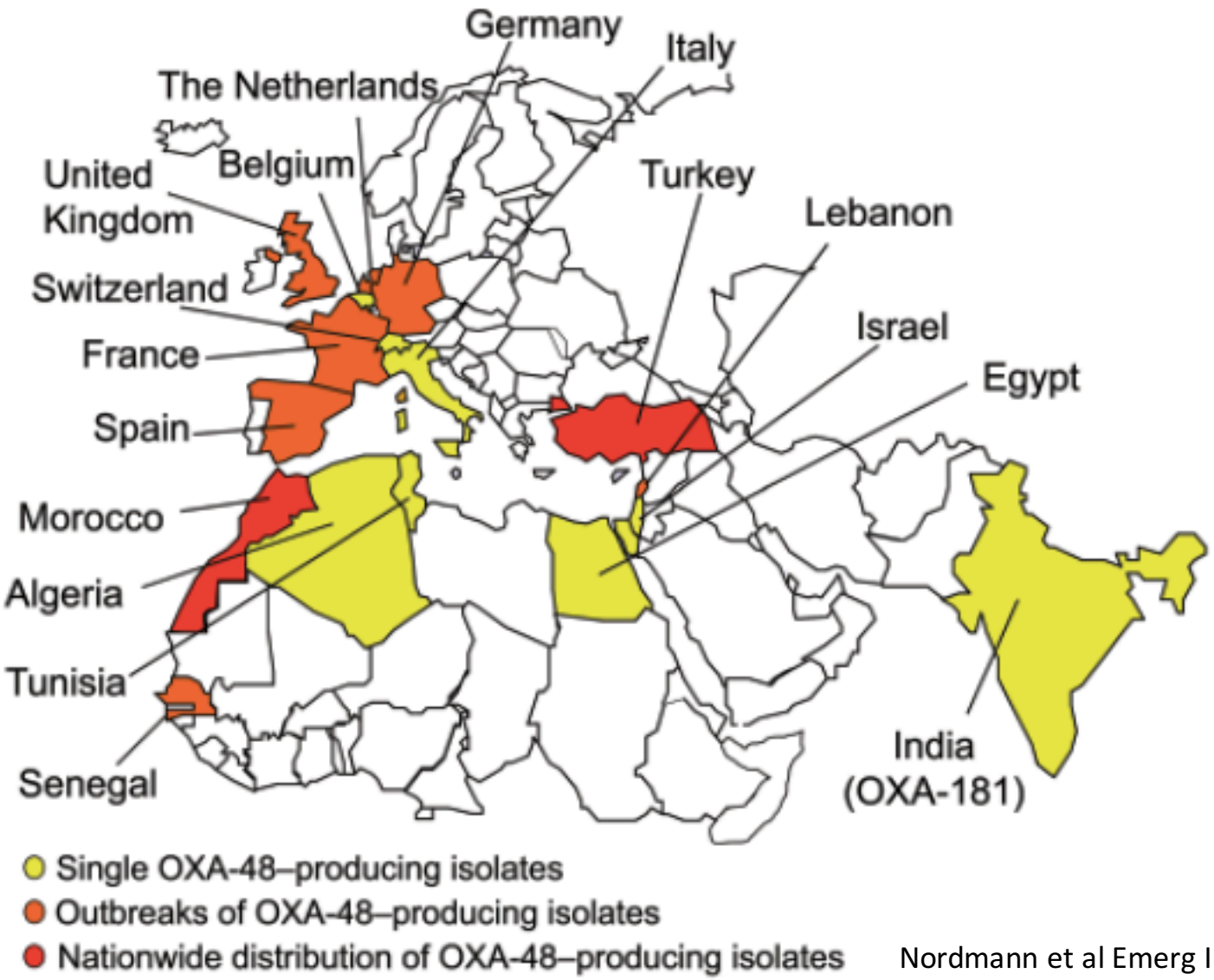
Nordmann et al Emerg Infect Dis 2011

Distribution of Verona Integron-Encoded Metallo-β-Lactamase (VIM) & IMP Producers

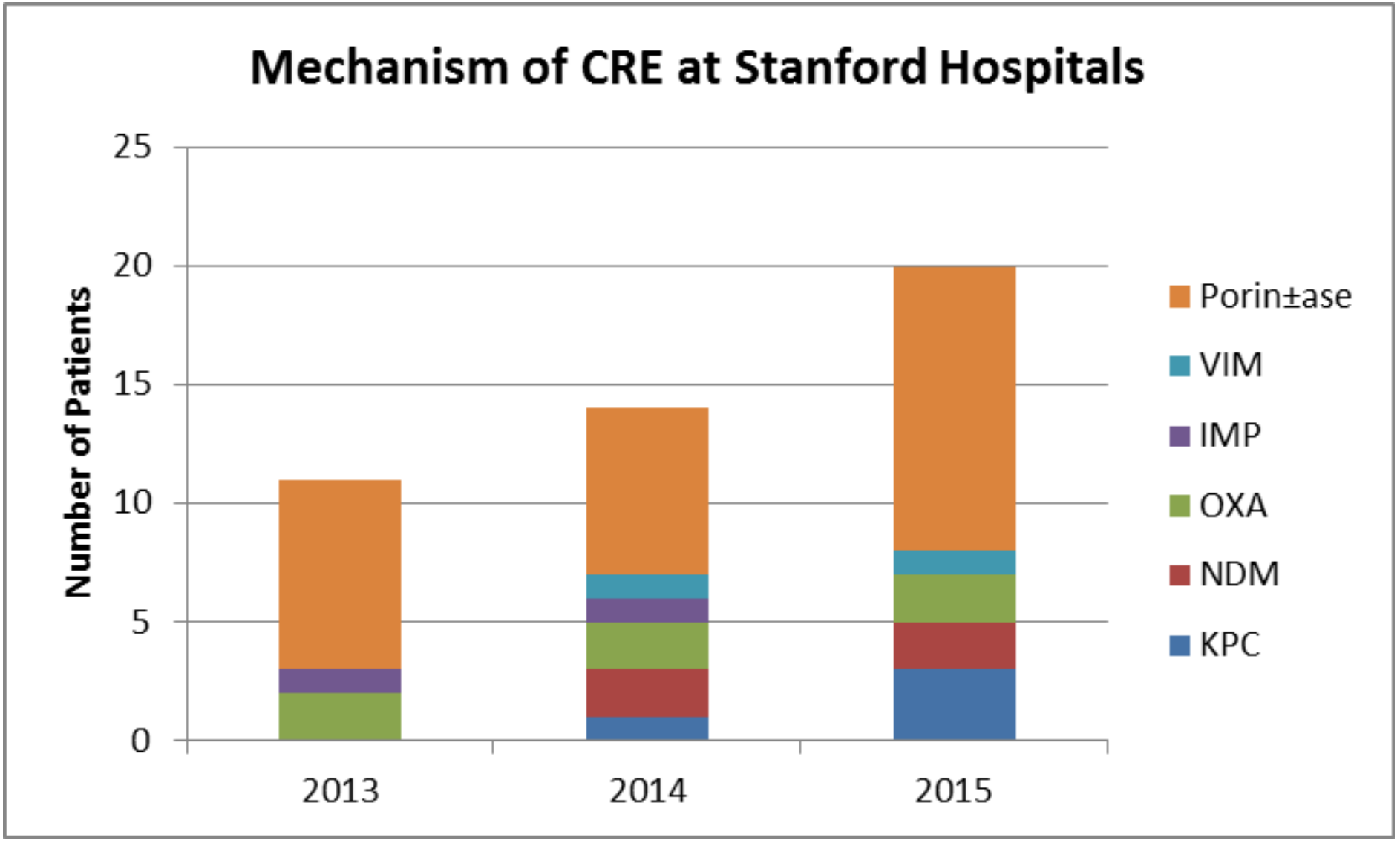


Nordmann et al Emerg Infect Dis 2011

Geographic Distribution of Oxacillinase-48-like (OXA-48-like) Type Producers



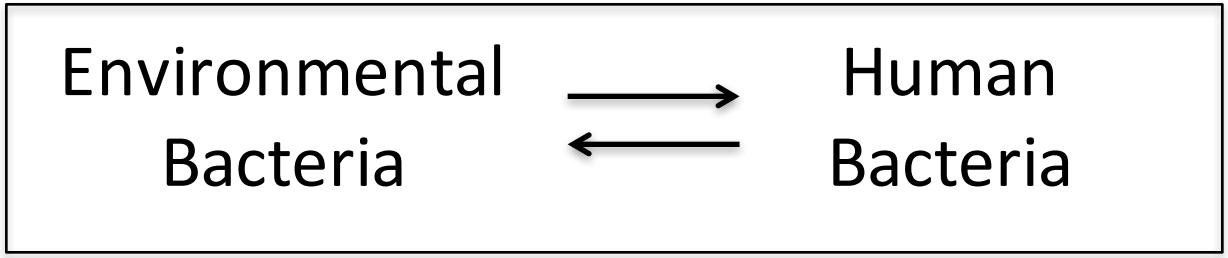
Nordmann et al Emerg Infect Dis 2011


Mechanism of CRE at Stanford Hospitals
Number of Patients
25
20
15
10
5
0
2013
2014
2015
Porin±ase
VIM
IMP
OXA
NDM
KPC

Origins of Carbapenemases

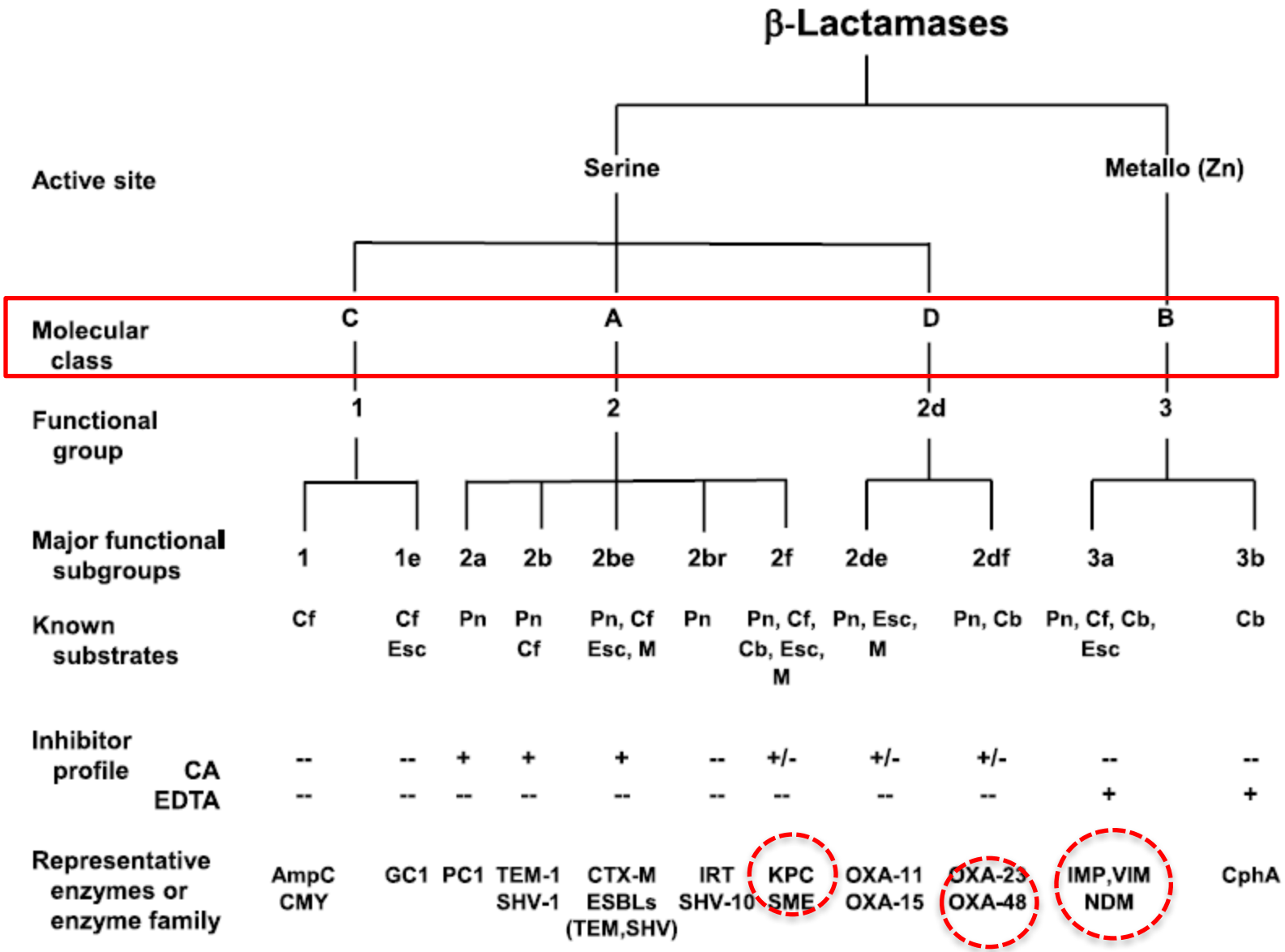
- Carbapenem design inspired by thienamycin, produced by the soil organism *Streptomyces cattleya*

- Carbapenemases originate from environmental organisms: OXA-48-like from *Shewanella* spp.; NDM from Pseudoxanthomonas

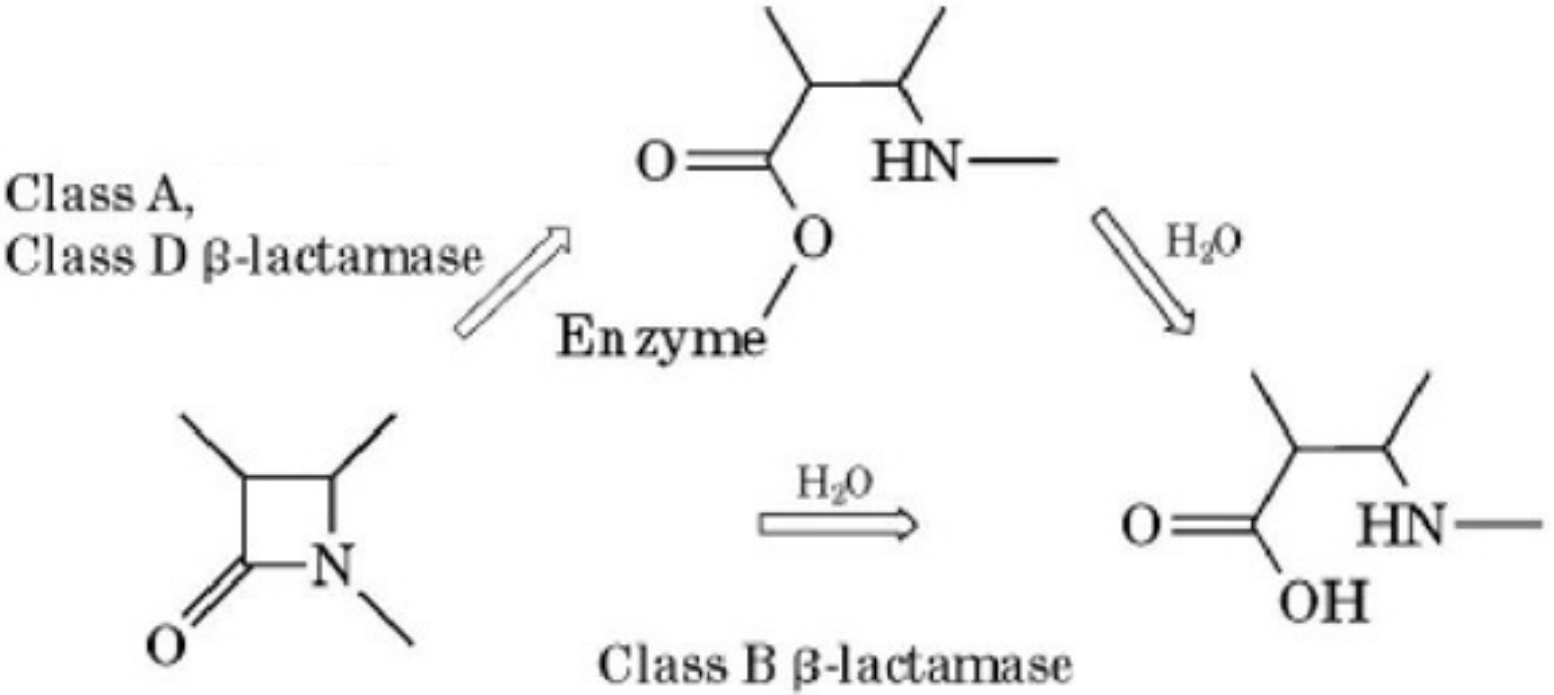


Queenan & Bush Clin Micro Rev 2007

Carbapenemase Classification

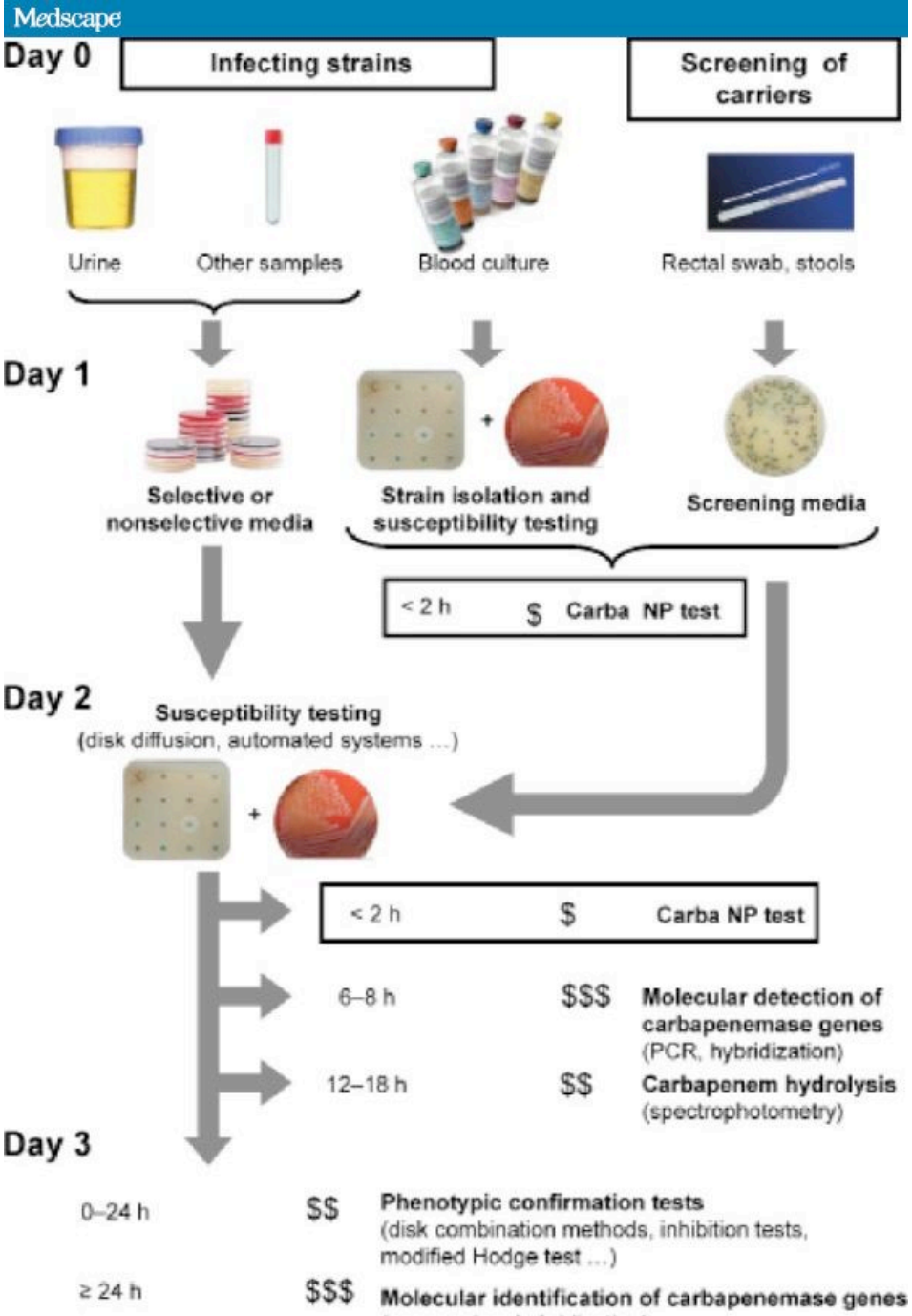


Carbapenemase Mechanisms



Laboratory Diagnostics for CRE

	Method	Need Culture	Duration (hrs)	Cost	Reveals Mechanism
Phenotypic	ChromAgar	Yes	24	$	No
	Carba NP	Yes	0.5-2	$	No
	MALDI	Yes	0.5	$	No
Genotypic	Xpert PCR	Optional	1	$$	Yes
	LDT PCR	Optional	2	$	Yes



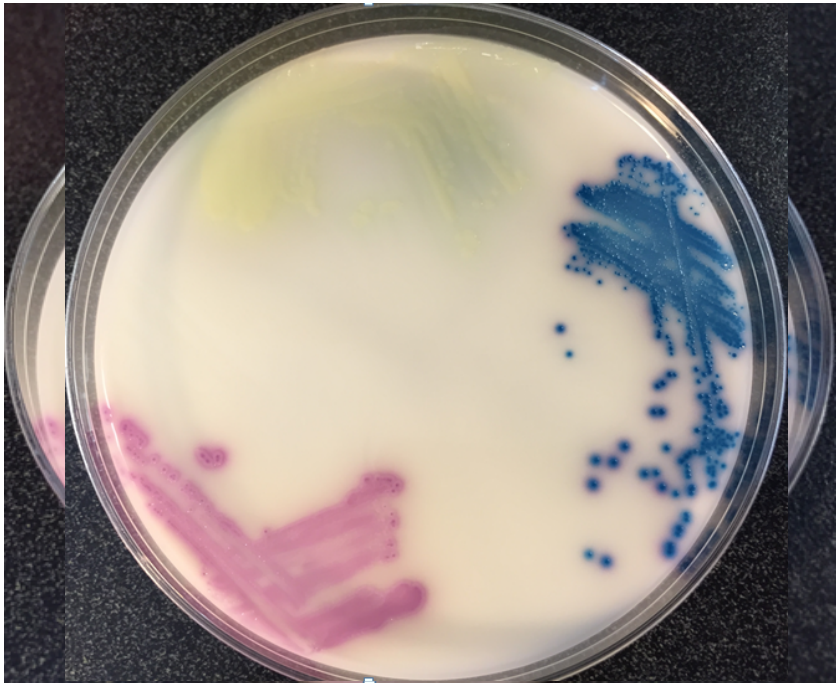
Nordmann et al Emerg Infect Dis 2012

Culture Based Detection of CREs

HardyCHROM™ CRE Agar

Organism	Description	Photo	Color
Klebsiella, *Enterobacter*, and *Serratia* spp.	Dark blue colonies		
Escherichia coli	Pink colonies with dark pink centers		

The main problem remains detection of OXA-48 producers that are susceptible to cephalosporins and have low-level resistance to carbapenems when not co-producing an ESBL



Significant Variations in the Level of Resistance to Carbapenems for Each Enzyme

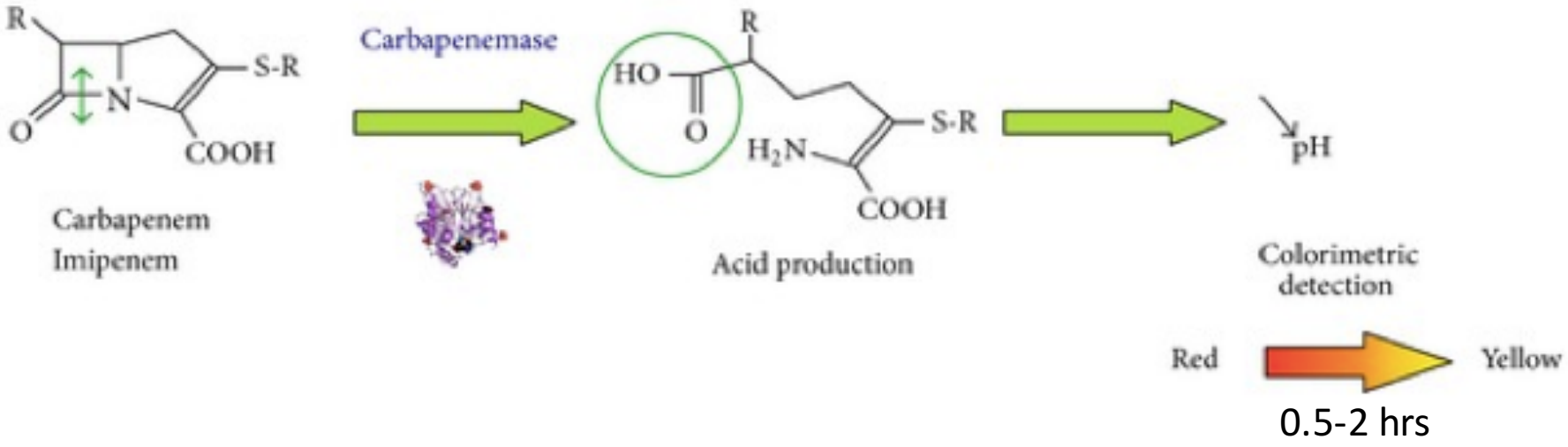
	MIC, mg/L		
Carbapenemase	Imipenem	Meropenem	Ertapenem
KPC	0.5–>64	1–>64	0.5–>64
Metallo β-lactamases†	0.5–>64	0.25–>64	0.5–>64
OXA-48 type	1–>64	0.5–>64	0.25–>64

*KPC, *Klebsiella pneumoniae* carbapenemase; OXA-48, oxacillinase-48.
†Including New Delhi metallo-β-lactamase-1.

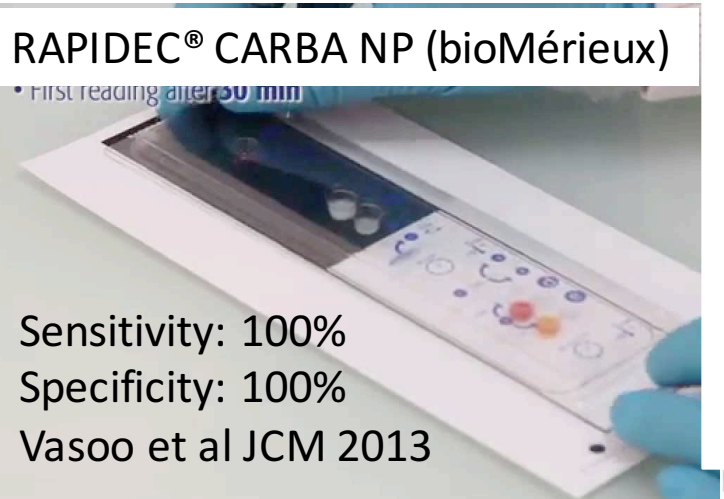
Carbapenem and oxyimino-β-lactam MICs are commonly higher for species with derepressed production of their chromosomally encoded AmpCs

Nordmann et al Emerg Infect Dis 2011
Tzouvelekis et al Clin Micro Rev 2012

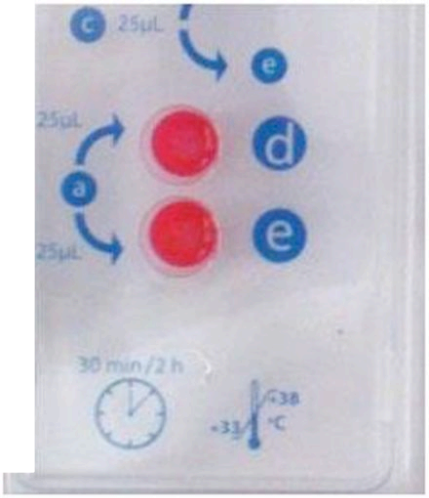
Phenotypic Detection of Carbapenemases: Carba NP (Nordmann et al Emerg Infect Dis 2012)



RAPIDEC® CARBA NP (bioMérieux)



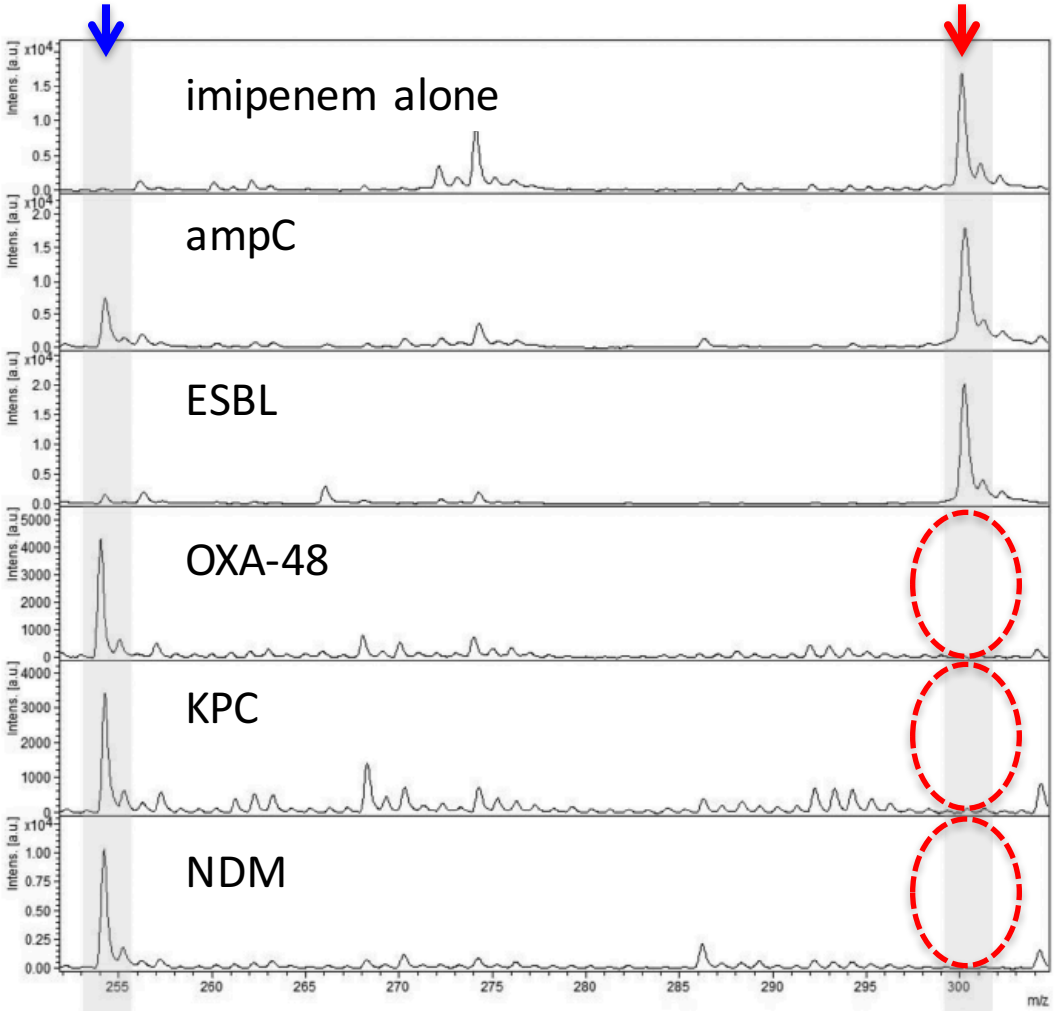
Sensitivity: 100%
Specificity: 100%
Vasoo et al JCM 2013



Phenotypic Detection of Carbapenemases: MALDI-TOF Mas Spectometry

metabolite (254 Da **m/z)**

imipenem: 300 Da **m/z**



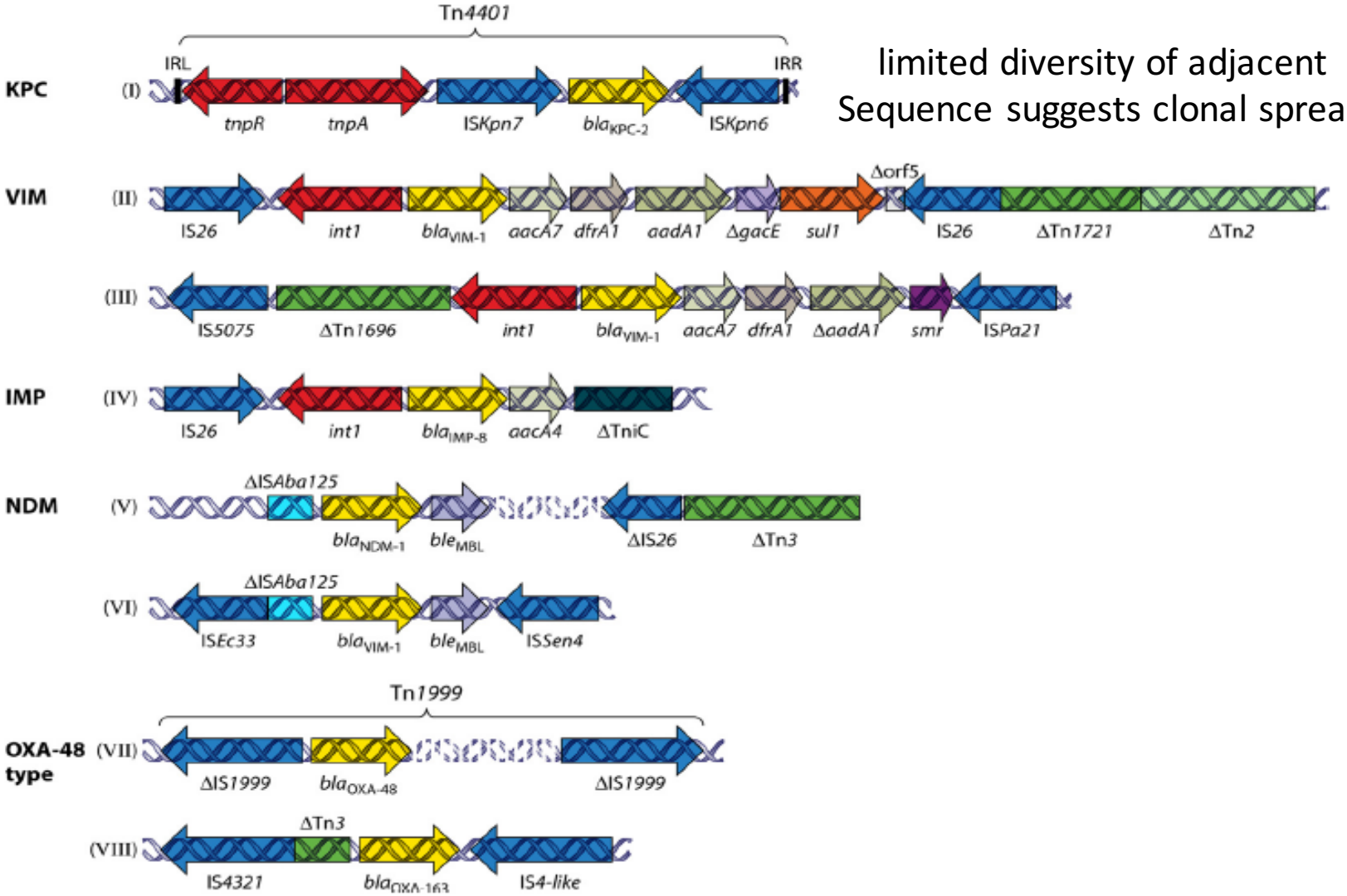
MALDI-TOF spectra of imipenem hydrolysis assays after a 20-min incubation at 37°C

$$\frac{\text{metabolite}}{(\text{metabolite} + \text{imipenem})} \geq 0.82$$

Training set: 77 CPE & 146 non-CPE
Sensitivity: 97.8%
Specificity: 97.8%

Lasserre et al JCM 2015

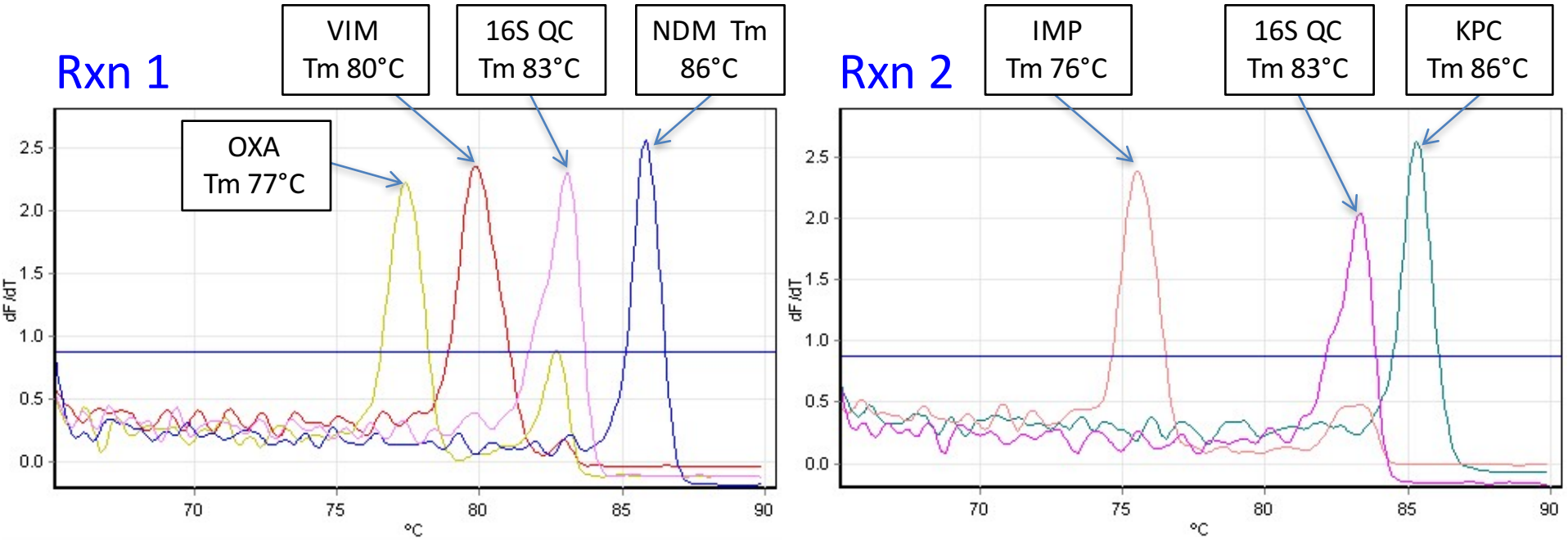
Nucleic Acid Testing



limited diversity of adjacent
Sequence suggests clonal spread

Tzouvelekis et al Clin Micro Rev 2012

Stanford Multiplex CRE PCR



Tan, Budvytiene et al Unpublished

Indian man with pyelonephritis.
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Ureteral stent placed in India for kidney stones.
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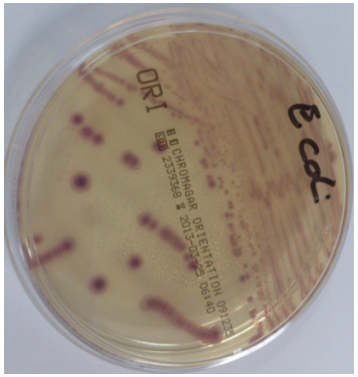

Susceptibility

	MIC by Vitek 2		Disk Diffusion	MIC by Etest	
Amikacin	>=64 ug/mL ...	RESISTANT			
Amoxicillin/Clavulanic Acid	>=32 ug/mL ...	RESISTANT			
Ampicillin	>=32 ug/mL ...	RESISTANT			
Cefazolin	>=64 ug/mL ...	RESISTANT [1]			
Cefepime	>=64 ug/mL ...	RESISTANT			
Cefoxitin	>=64 ug/mL ...	RESISTANT			
Ceftazidime	>=64 ug/mL ...	RESISTANT			
Ceftolozane/Tazobactam				>256 ug/mL ...	R
Ceftriaxone	>=64 ug/mL ...	RESISTANT			
Ciprofloxacin	>=4 ug/mL (...	RESISTANT			
Doxycycline				>256 ug/mL ...	R
Ertapenem	>=8 ug/mL (...	RESISTANT	RESISTANT		
FOSFOMYCIN			SUSCEPTIBLE		
Gentamicin	>=16 ug/mL ...	RESISTANT			
Imipenem			RESISTANT		
Levofloxacin	>=8 ug/mL (...	RESISTANT			
Meropenem	8 ug/mL (MIC)	RESISTANT	RESISTANT		
Nitrofurantoin	64 ug/mL (MIC)	INTERMEDIATE			
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Tetracycline	>=16 ug/mL ...	RESISTANT			
Tobramycin	>=16 ug/mL ...	RESISTANT			
Trimethoprim/Sulfamethoxazole.	>=320 ug/mL...	RESISTANT			

Mechanism Guides Therapy for CRE

Enzyme	Class	Drug
KPC	A	Ceftazidime/Avibactam 100% Aztreonam/Avibactam 100%
IMP	B	Aztreonam/Avibactam 100%
VIM	B	Aztreonam/Avibactam 100%
NDM	B	Aztreonam/Avibactam 94%
OXA-48-like	D	Ceftazidime/Avibactam 93% Aztreonam/Avibactam 100%
None (Porin change +ESBL & or ampC)	A/C	Aztreonam/Avibactam: 97% Ceftazidime/Avibactam 100% Ceftolozane/Tazobactam ≈50%

Vasoo et al AAC 2016

NOW AVAILABLE

Xpert® Carba-R:

On-demand molecular test detects and differentiates KPC, NDM, VIM, OXA-48 and IMP-1.

- *Rapid and accurate detection of carbapenemase-producing Gram-negative bacteria — with differentiation among the five major families of resistance genes*
- *Results in less than an hour to help guide implementation of epidemiological measures to control the spread of these microorganisms*
- *Fast, easy, and cost-effective, actionable information to help prevent widespread outbreaks*

CE IVD In Vitro Diagnostic Medical Device

FDA approved for culture

Disadvantage of PCR is that they can assess the presence of only already known resistance genes.

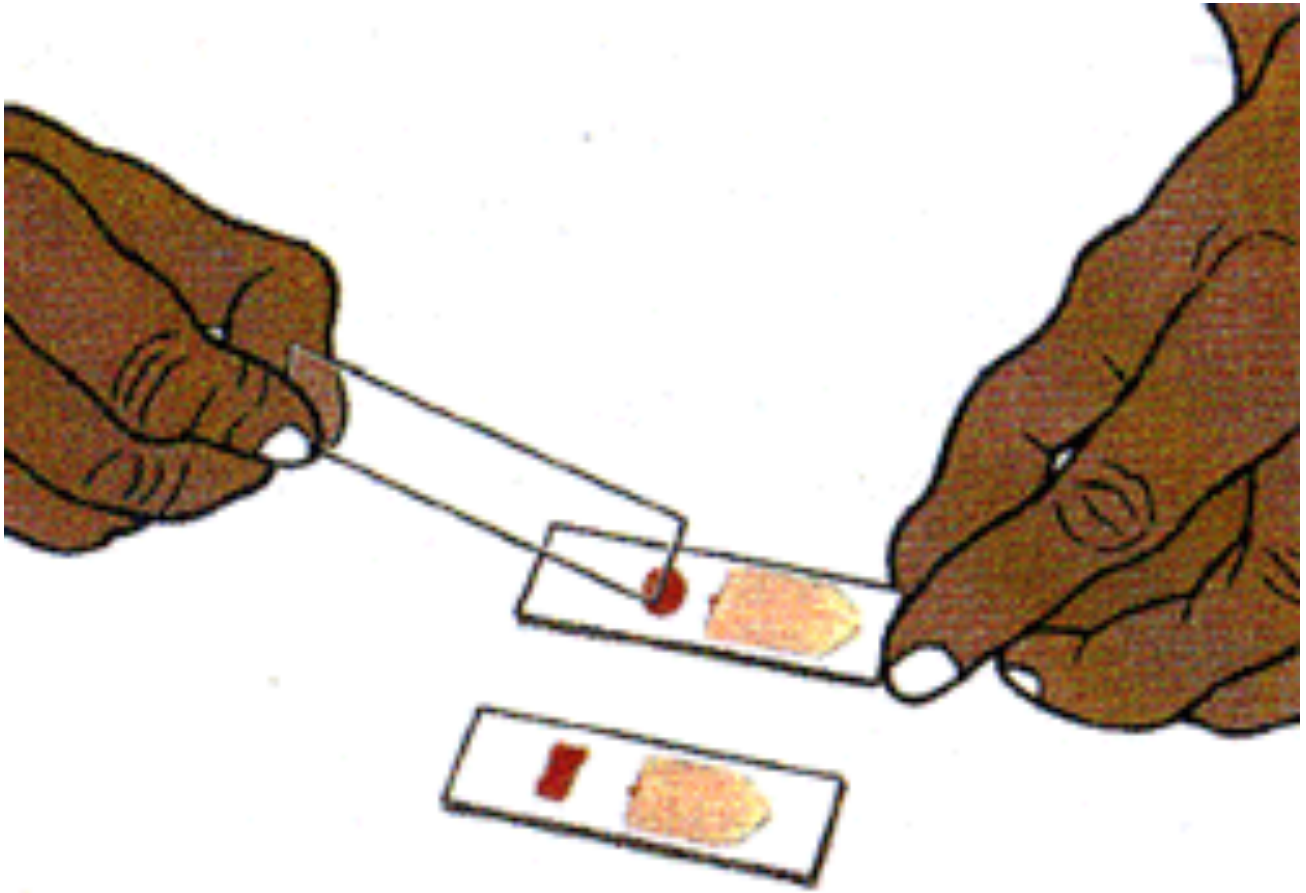
Cephei
A better w

Overview

- Accurate diagnostics in 21st century

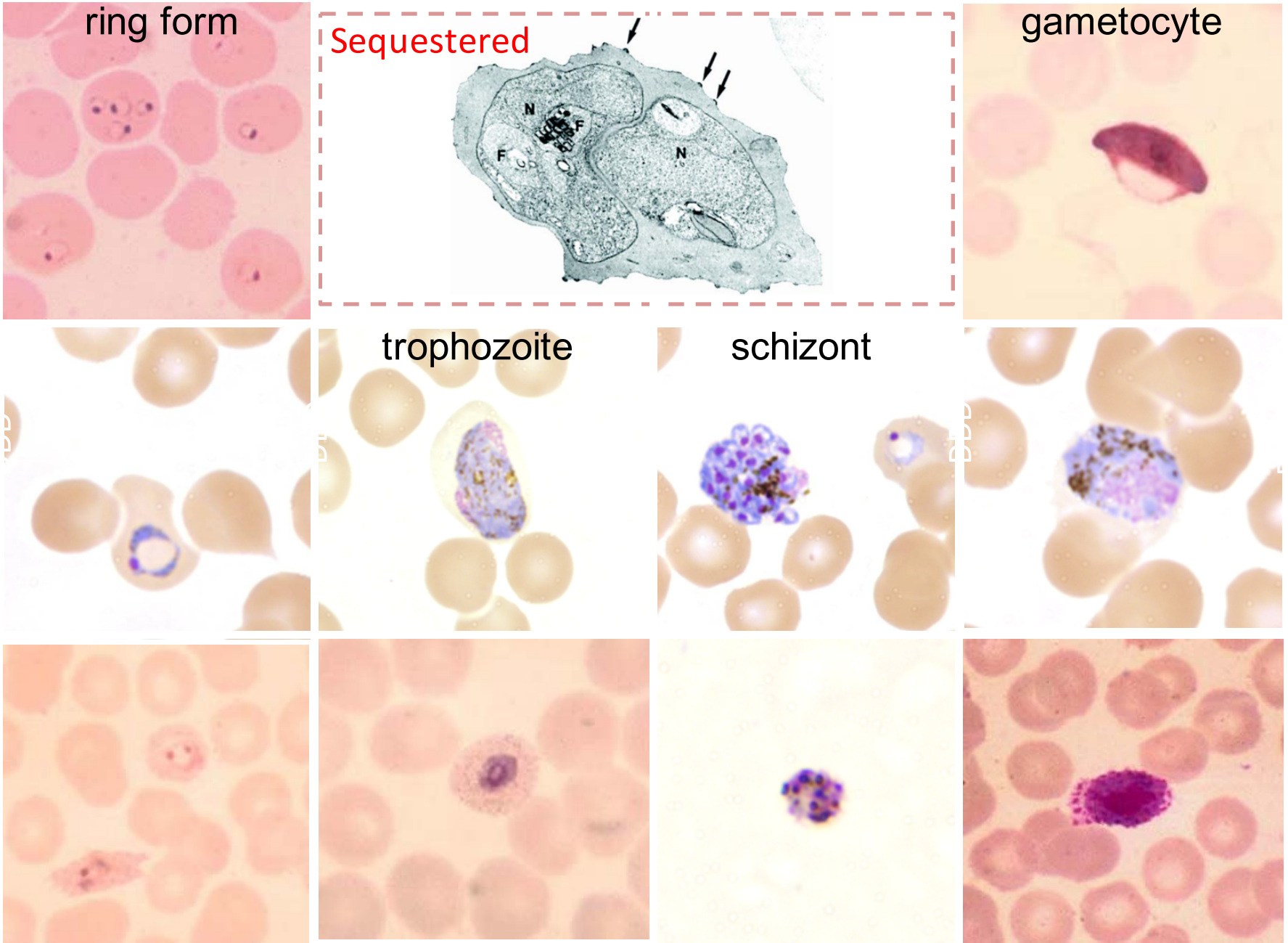
- Diagnostics for emerging pathogens
  - Ebola and Zika
  - Carbapenem-Resistant Enterobacteriaceae (CRE)
  - Malaria

Diagnosis of Malaria by Light Microscopy



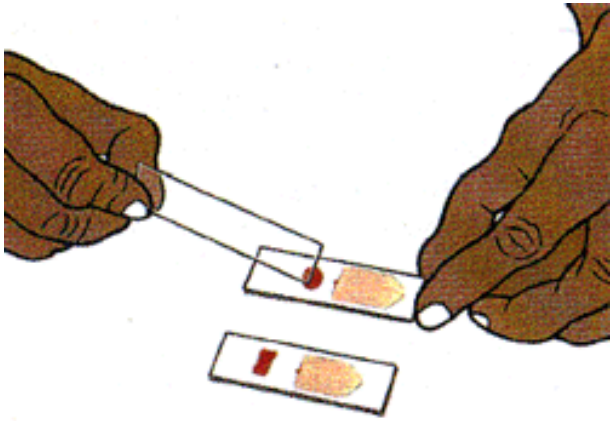
- Prepare slide immediately <1 hr after draw
- q8 h or q12 X 3 before malaria is r/o

Diagnosis of Malaria by Microscopy

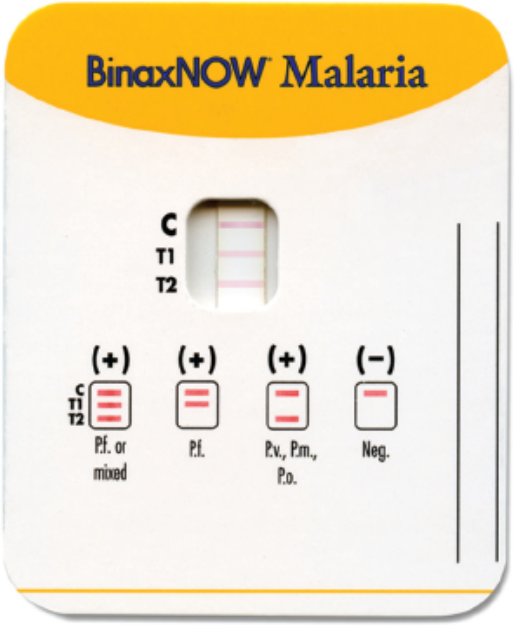


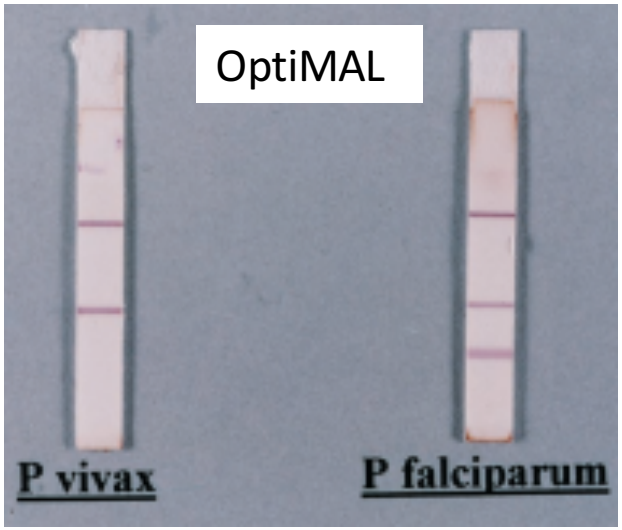
Light Microscopy ("Gold Standard")

- Wreight-Giemsa stain cheap and durable
- Glass slides reusable
- Thin smear
    - Helpful for species identification
    - Allows % parasitemia
- Thic smear
    - More sensitive in diagnosing malaria("Gold Standard")
- Monitor therapy
    - Cure = 75% reduction in first 48h



Nonmicroscopic Rapid Diagnostic Tests



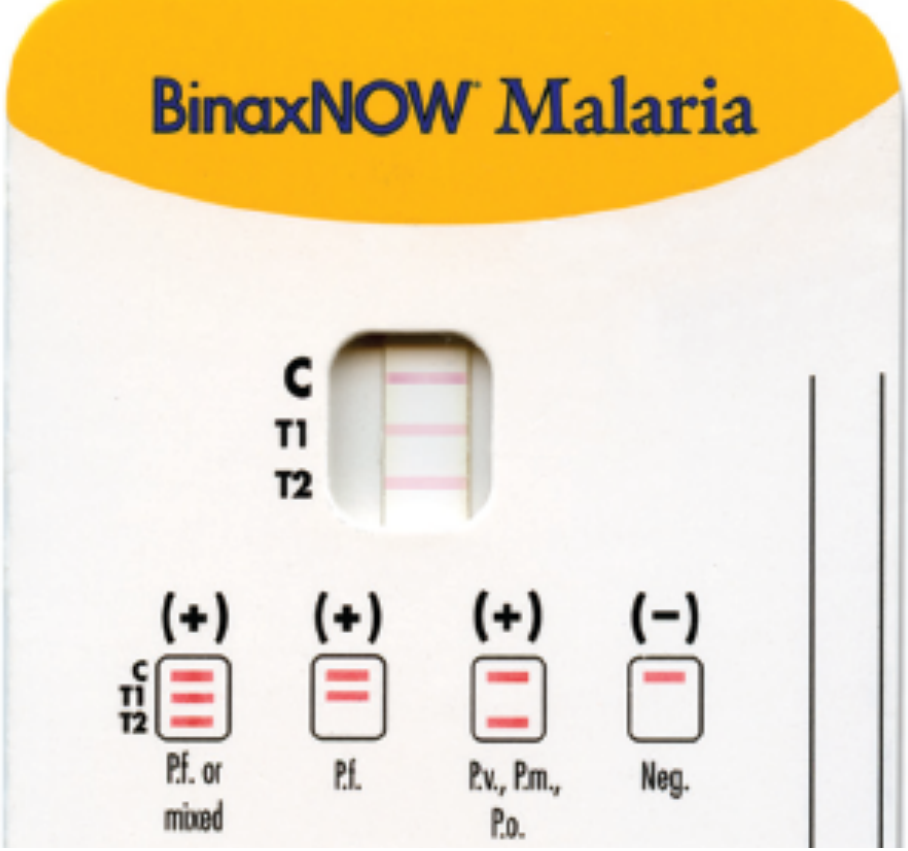


Performance of Malaria Rapid Diagnostics in Endemic and Non-Endemic Areas

Reference	MRDD*,†	Reference standard‡	Location	Blind study	Overall sensitivity (%)	Overall specificity (%)	*P. falciparum* sensitivity (%)	*P. falciparum* specificity (%)	*P. vivax* sensitivity (%)	*P. vivax* specificity (%)
Endemic										
Coleman *et al.* (2002b)	ICT Pf/Pv	GTTS	Thailand	Yes			35.4	99.7	2	99.9
Ferro *et al.* (2002)	OptiMAL	GTTS	Colombia	Unk§	99	94	90.6	98.6	96.5	97.6
Huong *et al.* (2002)	OptiMAL		Vietnam	Yes			50	100	73.7	100
	ICT Pf/Pv						83	100	20	100
Mason *et al.* (2002)	OptiMAL	GTTS	Myanmar	Yes	49		42	97	47¶	96.9¶
	ICT Pf/Pv				74		86	77	3¶	100¶
Non-endemic										
Gatti *et al.* (2002)	ICT Pf/Pv	GTTS	Italy	Unk	84	89	94	95	58¶	100¶
Iqbal *et al.* (2002)	OptiMAL	GTTS	Kuwait	Yes			87	99	79	97
	ICT Pf/Pv						81	99	58	98
Jelinek *et al.* (2001)	ICT Pf/Pv	GTTS	Europe	No			88	99	77	100
Playford & Walker (2002)	OptiMAL	GTTS and PCR	Australia	Yes	78	98	85	96	80	97
	ICT Pf/Pv				63	97	97	90	44	100
Rubio *et al.* (2001)	OptiMAL	GTTS and PCR	Europe	No	62	84				
	ICT Pf/Pv				68	97				

Limitations in sensitivity (100–500 parasites/μl), and robustness under field conditions.

Murray Trop Med & Intern Health 2003



BinaxNOW accuracy:

Plasmodium genus
Sensitivity is 92.9% (13/14)

Specificity is 99.8% (464/465)

Plasmodium species
***P. falciparum* 88.9% (8/9)**
non-*falciparum* 100% (7/7)

Correlation with parasitemia

High parasitemia (1.0%, or 50,000/l): 100% (2/2)
Medium parasitemia (0.1 to1.0%, or 5,000 to 50,000/l): 100% (12/12)
Low parasitemia (0.1%, or 5,000/l): 40% (2/5)

DiMaio et al JCM 2012

Limitations of Binax

Persistence of HRP-2 after parasite clearance

Humar et al. detected circulating HRP-2 antigen in 68% of treated patients on day 7, and in **27% it was still present on day 28** (Am J Trop Med Hyg 1997 **56:**44)

Gene deletion for the production of HRP-2

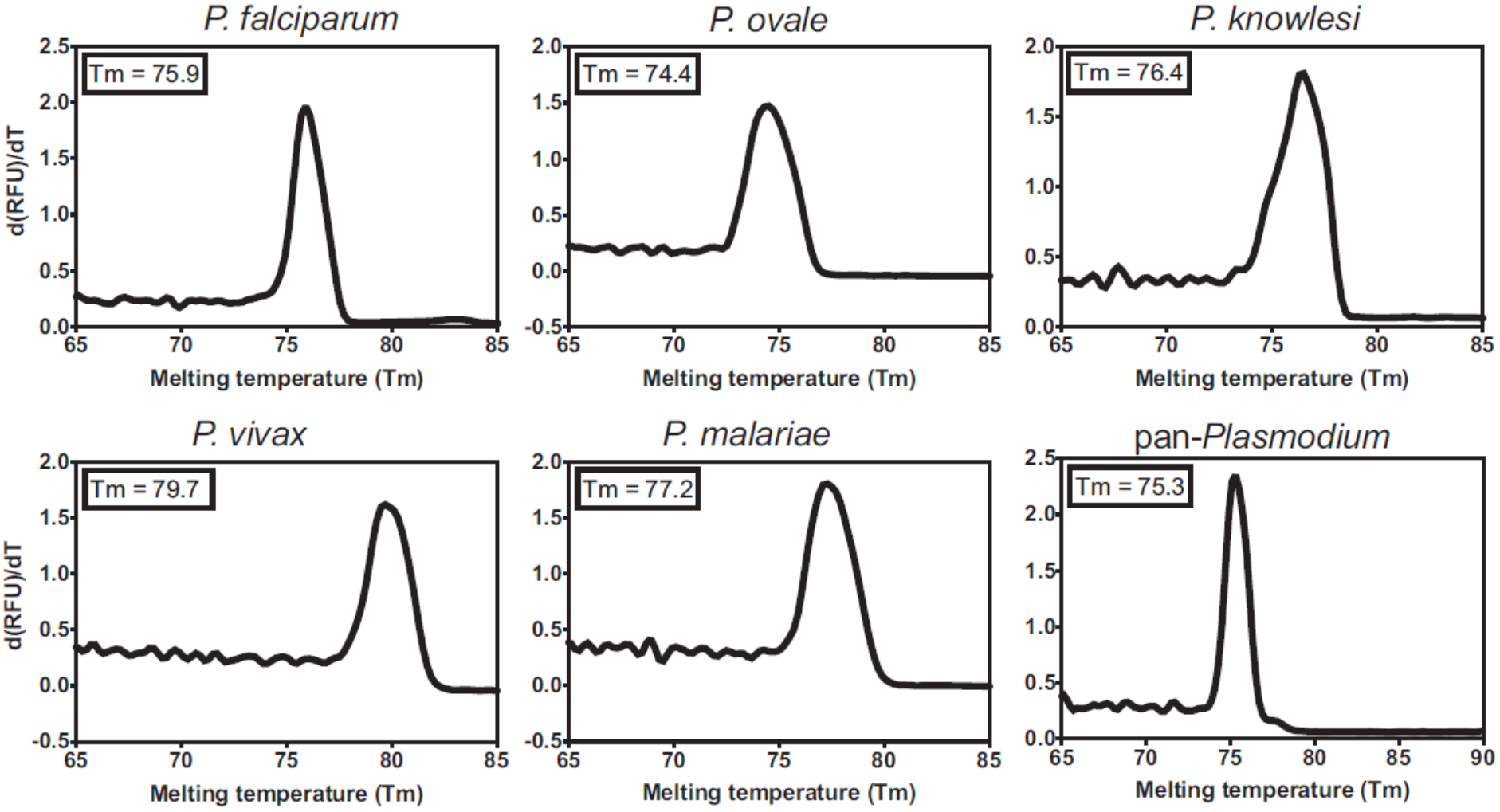
70% in Peru (per Joe Vinetz at UCSD)

False-positive results

Cross-reaction with serum rheumatoid factor

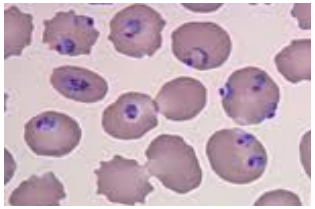
Moody Clin Microbiol Rev. 2002 15:66-78

Malaria Detection and Species Identification by Real-Time PCR



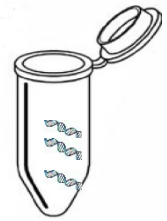
Lefterova et al JCM 2015 PMID:25972416

Malaria Detection and Species Identification by Real-Time PCR



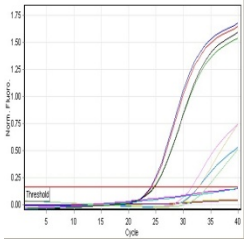
EDTA Blood

DNA extraction



Qiagen blood mini kit

Real-time PCR



- Qiagen RotorGene
- FastStart SYBR Green Master mix
- Singleplex reactions

Lefterova et al JCM 2015 PMID:25972416

Diagnostics No Longer a Limiting Factor

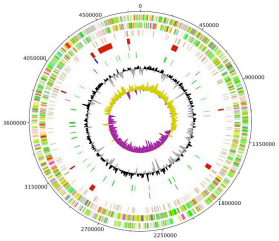
GeneXpert Omni, Cepheid

IMMY

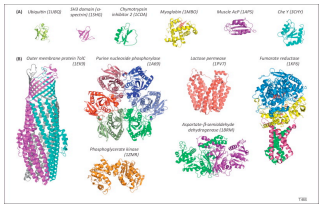
Biofire, bioMérieu

Cobas Liat System, Roche

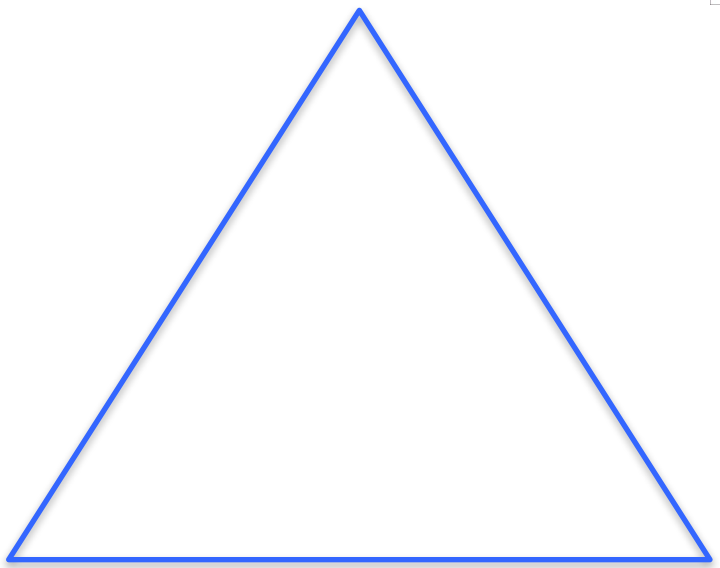
Summary I

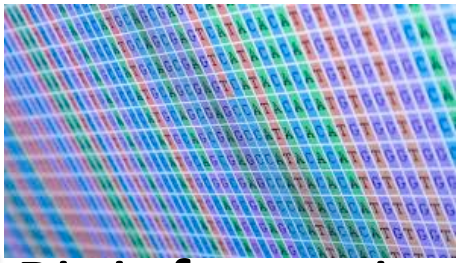


Genomics/
Proteomics Databases



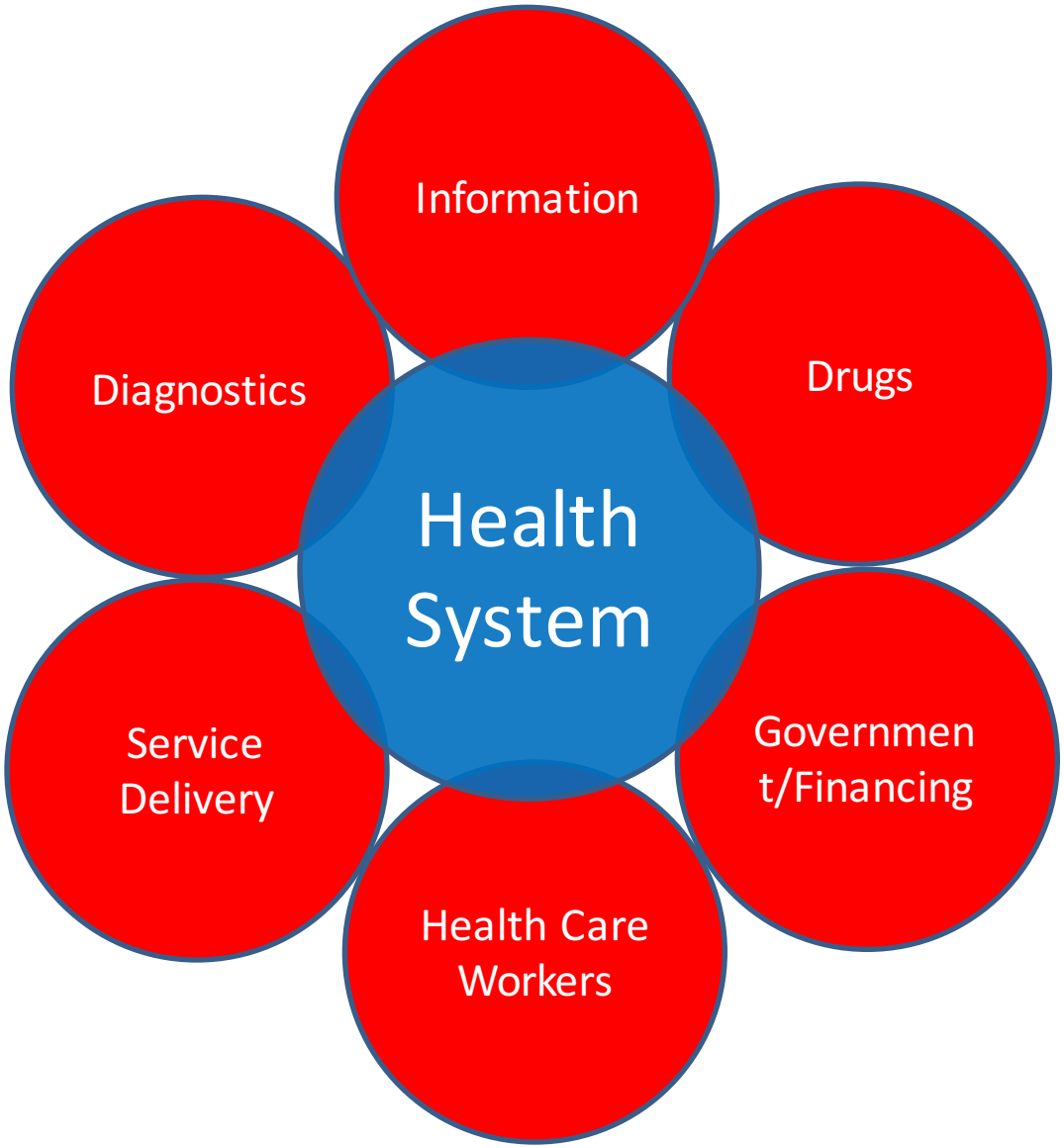
Molecular
Diagnostic
Platforms





Bioinformatics
Tools

Summary II


Information
Diagnostics
Drugs
Health System
Service Delivery
Governmen t/Financing
Health Care Workers

Acknowledgements

Stanford University
- Indre Budvytiene
- Rajiv Gaur
- Cynthia Trung
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- Stanford Pathology
- Merck Inc

CLSI Interpretive Guidelines

Table 2. Breakpoint values (MIC, mg/L) for carbapenems according to guidelines in Europe (EUCAST) and the United States (CLSI), September 2010*

	EUCAST		CLSI	
Carbapenem	S	R	S	R
Ertapenem	≤0.5	>1	≤0.25	≥1
Imipenem	≤2	>8	≤1	≥4
Meropenem	≤2	>8	≤1	≥4

*EUCAST, European Committee on Antimicrobial Susceptibility Testing (www.eucast.org/clinical_breakpoints); CLSI, Clinical and Laboratory Standards Institute; S, sensitive; R, resistant.

Nordmann et al Emerg Infect Dis 2011

Urgent Need for Measures to Contain Carbapenemase-Producing Eneterics

- Complete and continuous epidemiological data
  - Global data: systematic reports from Africa, the Balkans, the Middle East, and vast areas in Asia
  - Multi-institution data from developed countries
- Implementation of effective yet affordable and sustainable measures against CPE spread
- Identifying the most effective drug combinations
  - Dosing regimen of colistin, carbapenems
- Resources from international public health organizations should be mobilized and allocated appropriately

Tzouvelekis et al Clin Micro Rev 2012

TABLE 4. Substrate and inhibition profiles of the carbapenemases

Molecular class	Functional group	Enzyme	Hydrolysis profile[a]					Inhibition profile[b]		Reference(s)
			Penicillins	Early cephalosporins	Extended-spectrum cephalosporins	Aztreonam	Carbapenems	EDTA	Clavulanic acid	
A	2f	NMC	+	+	+	+	+	−	+	124
		IMI	+	+	+	+	+	−	+	183
		SME	+	+	±	+	+	−	+	179
		KPC	+	+	+	+	+	−	+	4
		GES	+	+	+	−	±	−	+	174, 219
B1	3	IMP	+	+	+	−	+	+	−	224
		VIM	+	+	+	−	+	+	−	224
		GIM	+	+	+	−	+	+	−	224
		SPM	+	+	+	−	+	+	−	224
D	2d	OXA	+	+	±	−	±	−	±	225

Queenan & Bush Clin Micro Rev 2007

Biochemical Identification of Enzymes

- KPC
  - Boronic acid-based inhibition testing
  - Performed with imipenem or meropenem
- NDM, VIM, IMP
  - Etest MBL strip (bioMérieux)
  - EDTA inhibits MBL activity
- OXA-48-like
  - No inhibition test is available

Mechanism Guides Therapy for CRE

Enzyme	Class	Drug
KPC	A	Ceftazidime/Avibactam 100% Aztreonam/Avibactam 100%
IMP	B	Ceftazidime/Avibactam 9% Aztreonam/Avibactam 100%
VIM	B	Ceftazidime/Avibactam 0% Aztreonam/Avibactam 100%
NDM	B	Ceftazidime/Avibactam 0% Aztreonam/Avibactam 94%
OXA-48-like	D	Ceftazidime/Avibactam 93% Aztreonam/Avibactam 100%
None (Porin change +ESBL & or ampC)	A/C	Aztreonam/Avibactam: 97% Ceftazidime/Avibactam 100% Ceftolozane/Tazobactam ≈50%

Vasoo et al AAC 2016

Mechanism Guides Therapy for CRE

Enzyme	Class	Drug
KPC	A	Ceftazidime/Avibactam Aztreonam/Avibactam
IMP	B	Aztreonam/Avibactam
VIM	B	
NDM	B	
OXA-48-like	D	Ceftazidime/Avibactam Aztreonam/Avibactam
Porin +ESBL/ampC	A/C	Ceftazidime/Avibactam Aztreonam/Avibactam

Among 108 KPC, 32 NDM, 11 IMP, 14 OXA-48-like, 5 IMI, 4 VIM, and 3 SME producers:
aztreonam-avibactam active against all except two NDM with ↑MICs of 8/4 & 16/4 mg/L;
ceftazidime-avibactam active against all KPC, IMI, SME, and most OXA-48-like (93%) but not class B

Among 29 enterics: 18 ESBL (10 porin loss), 11 AmpC (3 porin loss), 1 ESBL+AmpC:
aztreonam-avibactam active against all except one (8/4 mg/L); ceftazidime-avibactam active against all

Older antibiotics: colistin 88%; tigecycline 79%; and fosfomycin 78%

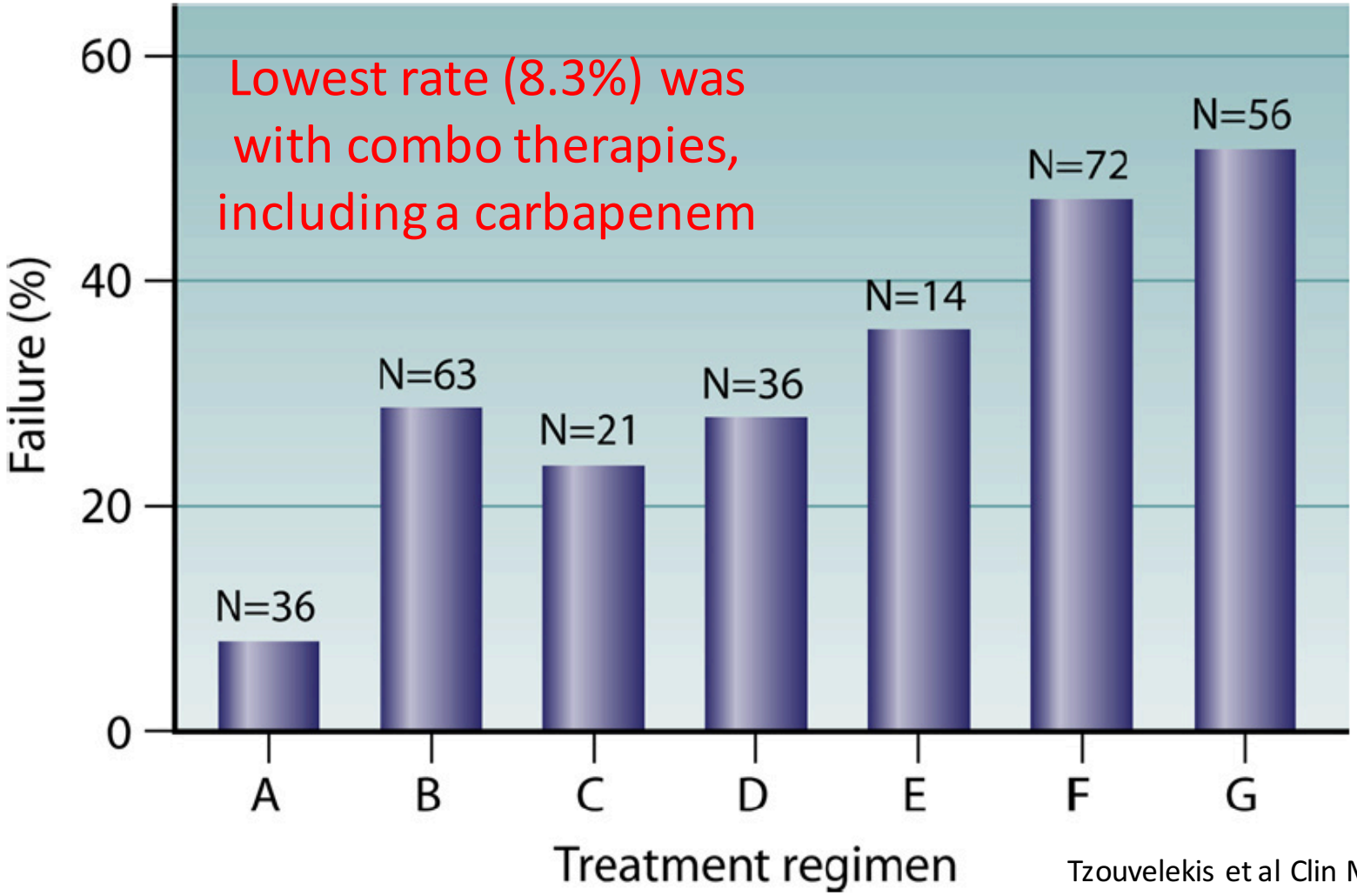
Vasoo et al AAC 2016

TABLE 3 Comparative susceptibilities of carbapenemase-producing Gram-negative bacilli to various antimicrobials

Antimicrobial[a]	% susceptibility with resistance mechanism shown (total no. of isolates)						
	KPC (108)	NDM (32)	OXA-48 group (14)	IMP (11)	IMI (5)	VIM (4)	SME (3)
β-Lactams and β-lactam–β-lactamase inhibitor combinations							
ATM	2	9	14	18	100	100	0
AZA	100	94	100	100	100	100	100
CAZ	2	0	14	0	80	0	67
CZA	100	0	93	9	100	0	100
CRO[b]	1	0	14	0	100		67
FEP[c]	2	0	14	0	80	0	33
ETP[b]	2	0	7	0	0		0
MEM	5	0	43	36	20	0	0
TZP	1	0	0	27	100	0	67
MEC[b]	0	16	43	18	100		33
Aminoglycosides							
GEN	63	16	36	0	80	0	100
TOB	9	9	36	9	80	0	100
AMK	51	41	43	82	100	0	100
Fluoroquinolones							
LVX	11	13	14	36	100	0	100
CIP	10	0	7	18	80	0	67
Other antimicrobial agents							
SXT[b]	21	9	14	45	80		100
NIT[b]	14	25	7	27	20		
FOF[b]	78	78	71	73	100		100
CST[d]	87	91	100	91	40	75	
TGC[b,d]	81	69	93	55	100		100

Vasoo et al AAC 2016

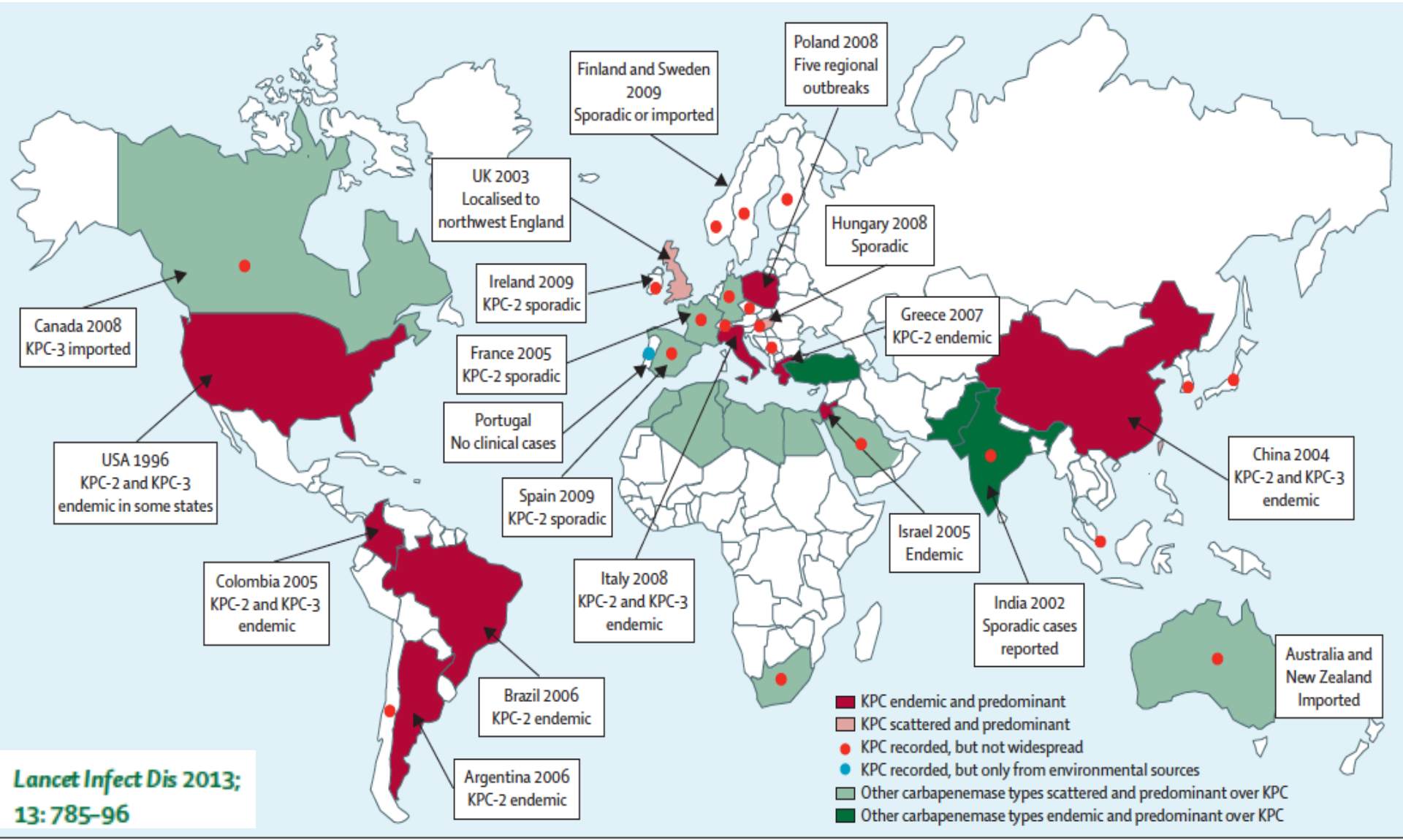
Clinical Outcomes of Carbapenemase-Producing *Klebsiella pneumoniae*



Tzouvelekis et al Clin Micro Rev 2012

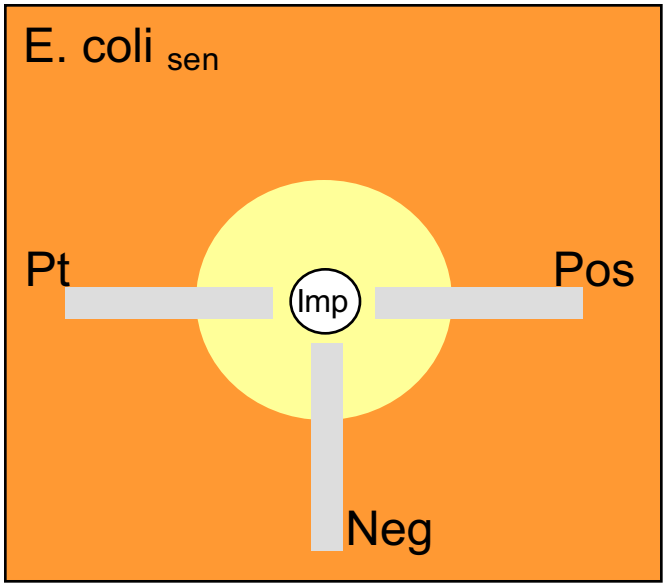
A, combination tx with 2 active drugs, one was a carbapenem; B, combination tx with 2 active drugs, not including a carbapenem; C, monotx with an aminoglycoside; D, monotx with a carbapenem; E, monotherapy with tigecycline; F, monotherapy with colistin; G, inappropriate tx.

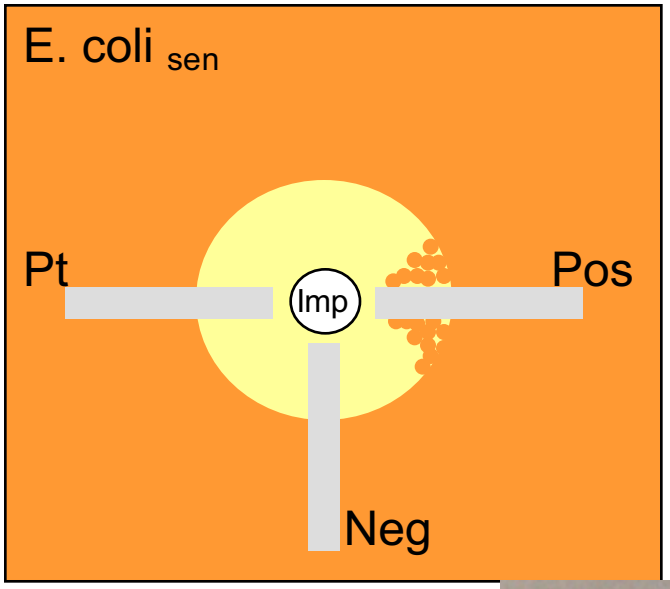
Geographic Distribution of *Klebsiella pneumoniae* Carbapenemase (KPC) producers

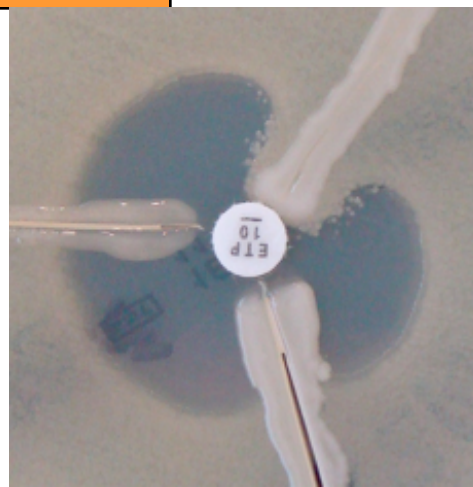


Lancet Infect Dis 2013; 13: 785–96

Phenotypic Detection of Carbapenemases: Modified Hodge Test







Sensitivity: 98% (weak detection of NDM producers)
Specificity: 80% (high level AmpC producers)

Vasoo et al JCM 2013
Nordmann et al Emerg Infect Dis 2011

THE LANCET

Will Ebola change the game? Ten essential reforms before the next pandemic. The report of the Harvard-LSHTM Independent Panel on the Global Response to Ebola

Suerie Moon, Devi Sridhar, Muhammad A Pate, Ashish K Jha, Chelsea Clinton, Sophie Delaunay, Valnora Edwin, Mosoka Fallah, David P Fidler, Laurie Garrett, Eric Goosby, Lawrence O Gostin, David L Heymann, Kelley Lee, Gabriel M Leung, J Stephen Morrison, Jorge Saavedra, Marcel Tanner, Jennifer A Leigh, Benjamin Hawkins, Liana R Woskie, Peter Piot

Panel 1: **Summary of system weaknesses exposed across four phases of the Ebola outbreak**

Pre-outbreak and Phase 1: December, 2013–March, 2014

- Inadequate national investment and donor support for building national health systems capable of detecting and responding to disease outbreaks
- Inadequate arrangements to monitor country commitments to do so

Phase 2: April, 2014–July, 2014

- Little incentive for countries to report outbreaks early
- Insufficient overall technical capacity among national and international teams
- WHO slow to mobilise global attention or assistance

Phase 3: August, 2014–October, 2014

- Government and private sector disregard for WHO recommendations regarding travel and trade restrictions
- Slow global operational response
- Unclear responsibility for international coordination
- Weak channels for lessons from previous Ebola outbreaks
- Little access to therapies in limited supply, or medical evacuation for national as opposed to international health workers
- Poor understanding of the importance of community engagement

Phase 4: October, 2014–September, 2015

- Weak coordination of global operational response
- Inadequate transparency on resource flows
- Weak accountability for use of funds at all levels