ESGMYC
ESCMID STUDY GROUP FOR MYCOBACTERIAL INFECTIONS
European Society of Clinical Microbiology and Infectious Diseases

Groupe Hospitalier Universitaire
SAINT-LOUIS
LARIBOISIÈRE
FERNAND-WIDAL

université PARIS DIDEROT

A year in Clinical Microbiology

Emmanuelle CAMBAU
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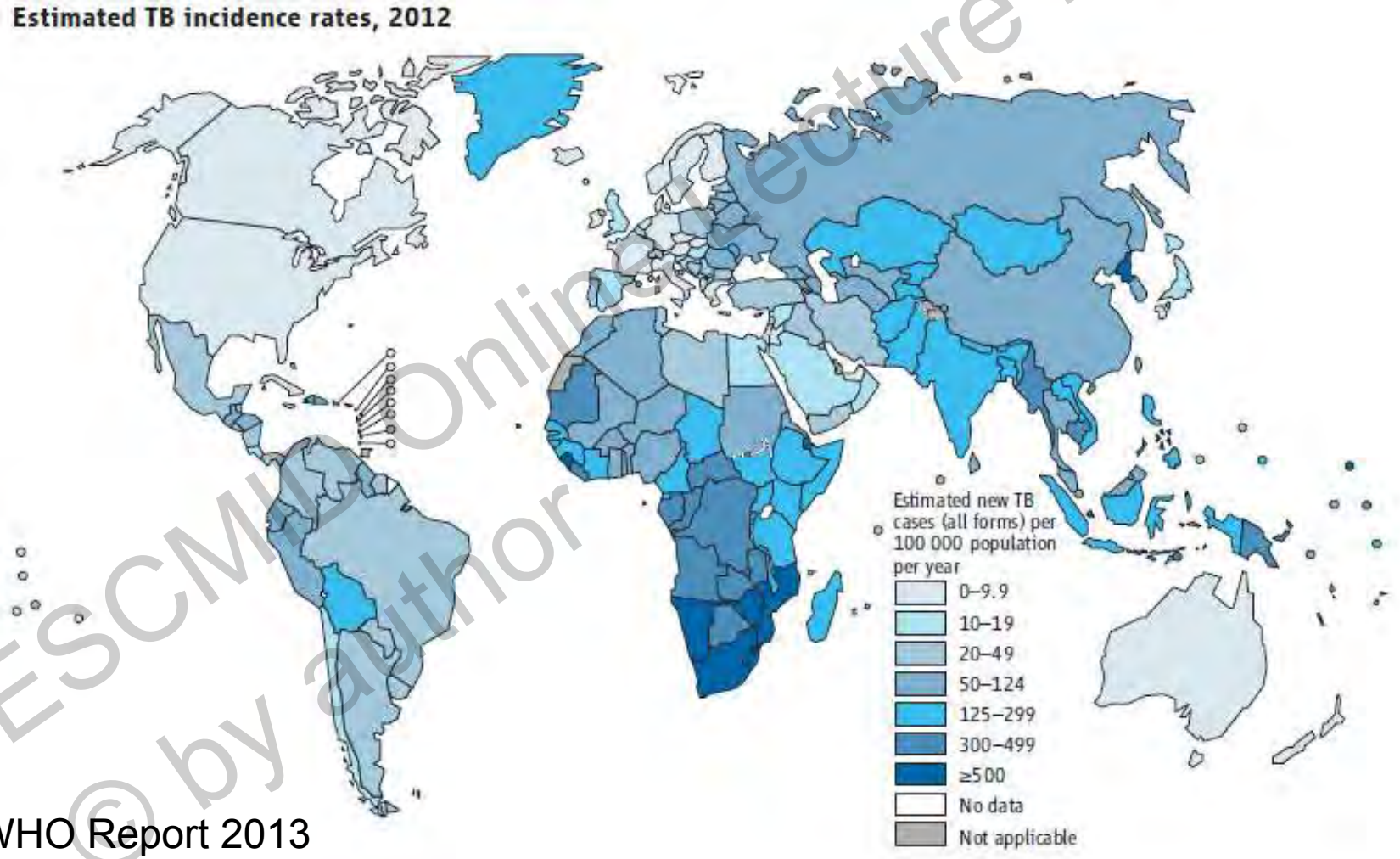
COI disclosure: consultancy for bioMerieux, Becton-Dickinson, Hain lifesciences

New anti-tuberculous drugs approved by EMA

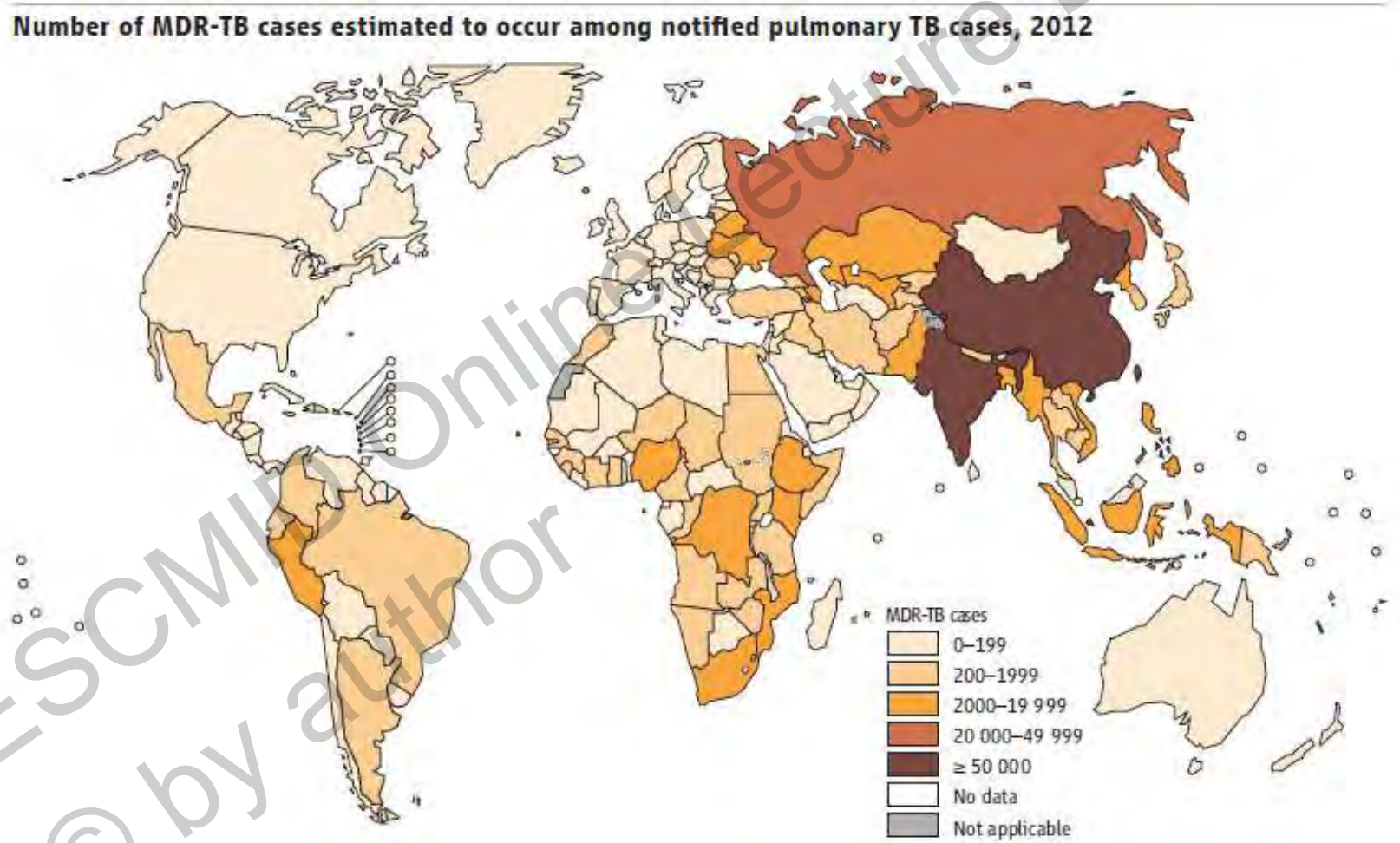
- Bedaquiline = TMC-207= R201970 (Janssen, Johnson and Johnson)
- Delamanid = OPC-67683 (Otsuka Pharmaceutical, Tokushima)

8.6 millions new cases TB in 2012
1.3 millions of deaths (15%)



WHO Report 2013

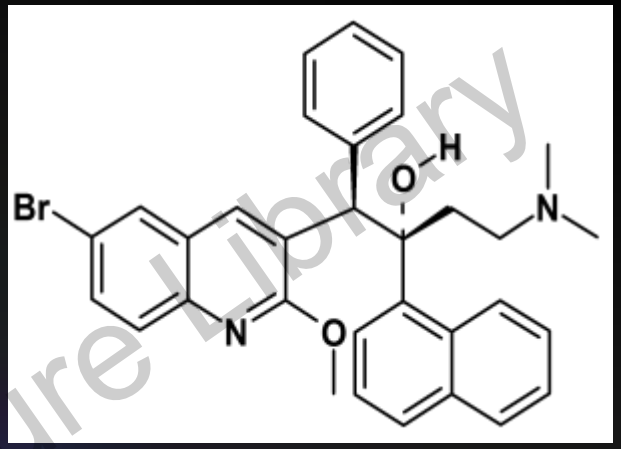
450 000 MDR-TB cases (3.6% new cases and 20.2% previously treated cases)
170 000 deaths (38%)



WHO Report 2013

Bedaquiline

diarylquinoline



H+ H+ H+ H+

Andries K et al.
Science 2005

ADP + Pi > ATP

Inhibition of subunit C of ATP synthase

In vitro activity of bedaquiline on *Mycobacterium tuberculosis*

Strains	N	MIC (mg/L)
fully susceptible	6	0.03-0.12
R rifampicin	1	0.03
R ethambutol	1	0.01
R pyrazinamide	1	0.03
R fluoroquinolones	3	0.06-0.12
R isoniazid	7	0.03-0.06
R rifampicin + isoniazid (MDR)	2	0.03

Andries Science 2005

No cross resistance with other antituberculous antibiotics

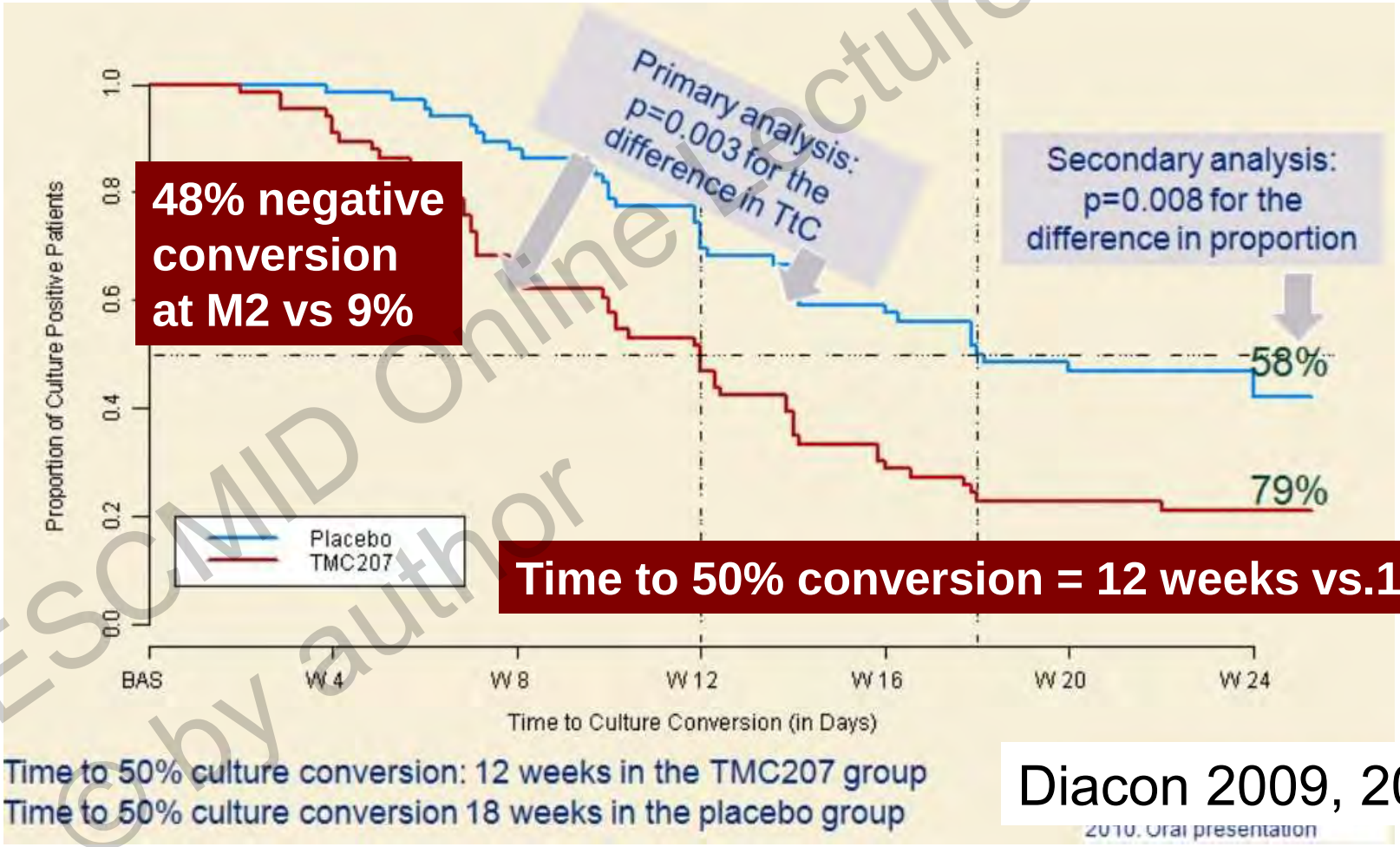
	MIC (mg/L) on 7H11	
	Wild type	Mutant BK12*
Bedaquiline	**0.03**	**4**
Isoniazid	0.12	0.12
Rifampicin	0.5	0.12
Ethambutol	2	4
Streptomycin	1	1
Amikacin	1	2
Moxifloxacin	0.25	0.25

*** : mutation A63P in *atpE***

Andries Science 2005

Efficacy of bedaquiline in MDR-TB

- South Africa, 47 MDR patients
- 5 drugs -combination + bedaquiline or placebo



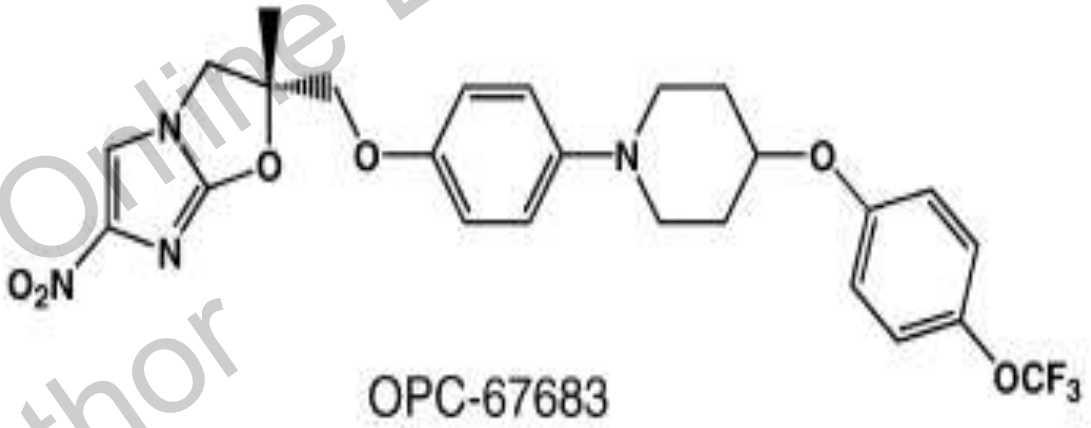
Diacon 2009, 2012

Delamanid (OPC-67683)

Nitro-imidazole compound

Needs to be activated (reduction) in the bacterial cell

Inhibits mycolic acid synthesis, effect on DNA



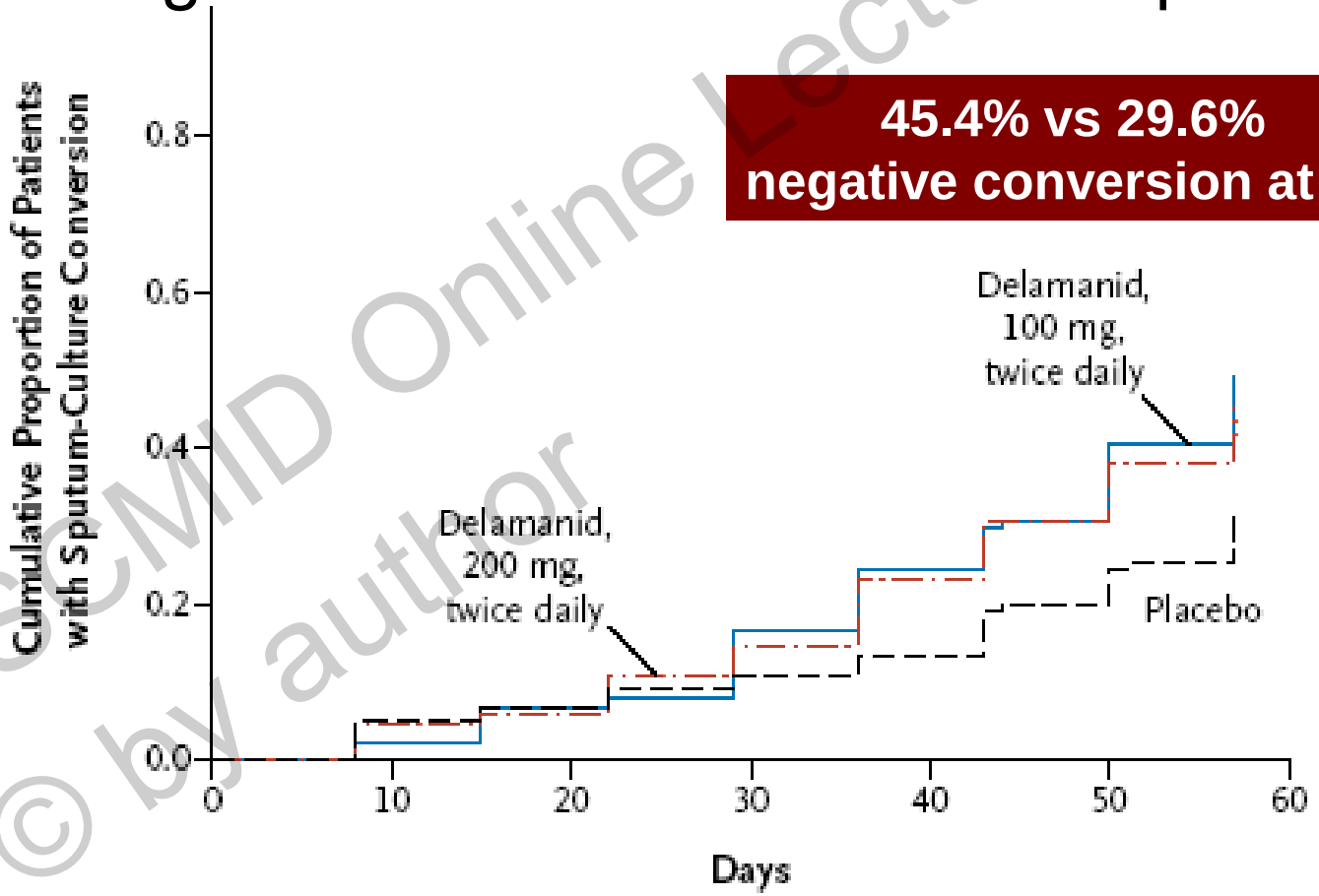
OPC-67683

MIC on *M. tuberculosis* : 0.012 mg/L

Matsumoto PLOS Medicine 2006, Rakech PLOS one 2014

Efficacy of delamanid in MDR TB

- 481 patients HIV- smear+ (Gler NEJM 2011)
- 5 drug-combination + delamanid or placebo



New antituberculosis drugs, regimens, and adjunct therapies: needs, advances, and future prospects

Alimuddin I Zumla, Stephen H Gillespie, Michael Hoelscher, Patrick P J Philips, Stewart T Cole, Ibrahim Abubakar, Timothy D McHugh, Marco Schito, Markus Maeurer, Andrew J Nunn

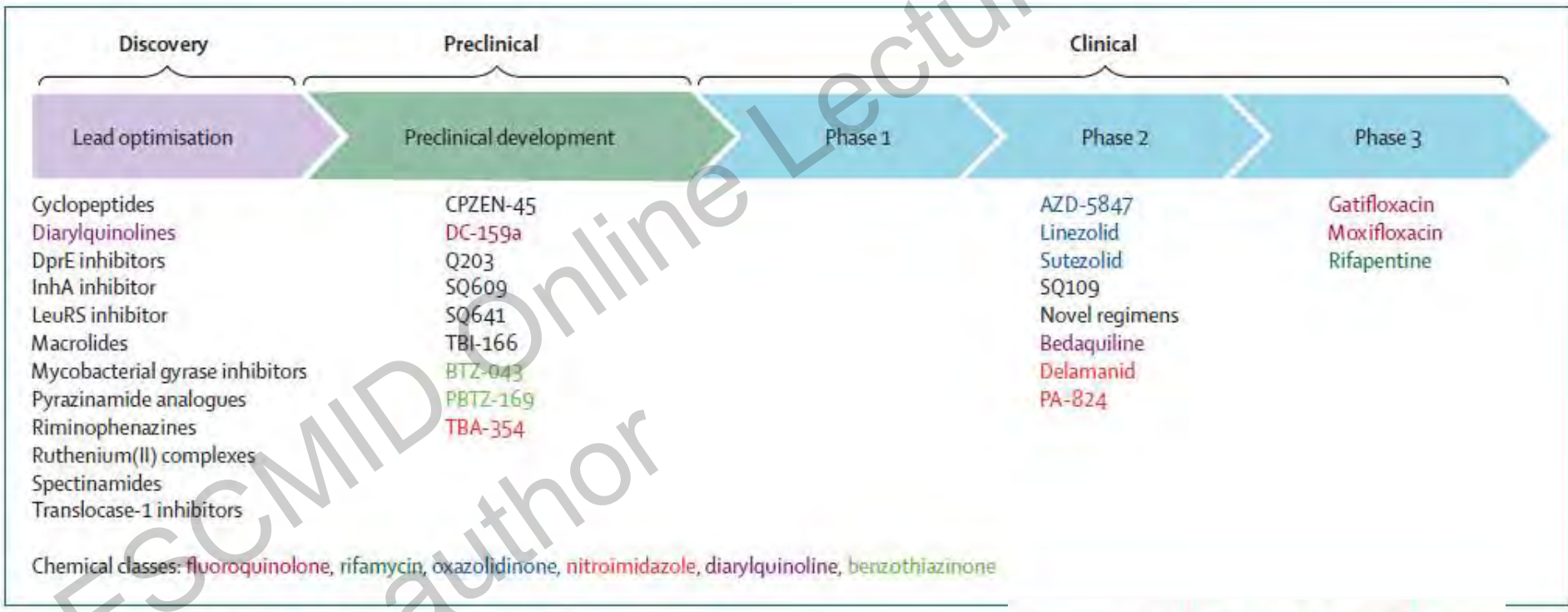


Figure 1: Global pipeline of new tuberculosis drugs[2,22-24,31-48]
Used with permission of the Stop TB Partnership Working Group on New Drugs.

Lancet Infect Dis 2014; 14: 327–40

Rapid molecular tests for diagnosis of MDR-TB

Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults (Review)

Steingart KR, Sohn H, Schiller I, Kloda LA, Boehme CC, Pai M, Dendukuri N

Cochrane Database of Systematic Reviews 2013;

http://www.thecochranelibrary.com

Advances in tuberculosis diagnostics: the Xpert MTB/RIF assay and future prospects for a point-of-care test

Stephen D Lawn, Peter Mwaba, Matthew Bates, Amy Piatek, Heather Alexander, Ben J Marais, Luis E Cuevas, Timothy D McHugh, Lynn Zijenah, Nathan Kapata, Ibrahim Abubakar, Ruth McNerney, Michael Hoelscher, Ziad A Memish, Giovanni Battista Migliori, Peter Kim, Markus Maeurer, Marco Schito, Alimuddin Zumla

Lancet Infect Dis 2013; 13: 349–61

www.thelancet.com/infection Vol 13 April 2013

WHO publications 2014

Nucleic acid amplification direct testing (NAAT) for tuberculosis

- PCR started in 1990 using
  - gene (Hermans JCM 1990)
  - RNA (Boddinghaus JCM 1990)
  - IS6110 (Thierry JCM 1990)
- 1996 : recommendations CDC / ATS
- 2008 : recommendations CDC / JAMA
- Pubmed
  - 5000 papers, 350 review, 8 meta-analyses
  - Sarmiento 2003: meta-analysis on smear negative specimens
  - Greco 2009: meta-analysis on smear positive specimens
  - Lawn 2013: review on Xpert®MTB/RIF®

ATS 1997, leven and Goosens 1997, Sarmiento 2003, Dinnes 2007, MMWR 2009;58-7-10, Lawn S LID 2013

Pr Emmanuelle CAMBAU

NAAT sensitivity for TB diagnosis

= We would like that all TB patients with Smear-positive specimens are NAAT+

- Cochrane review
  - Pooled sensitivity = **98%** (95% CrI 97- 99%)

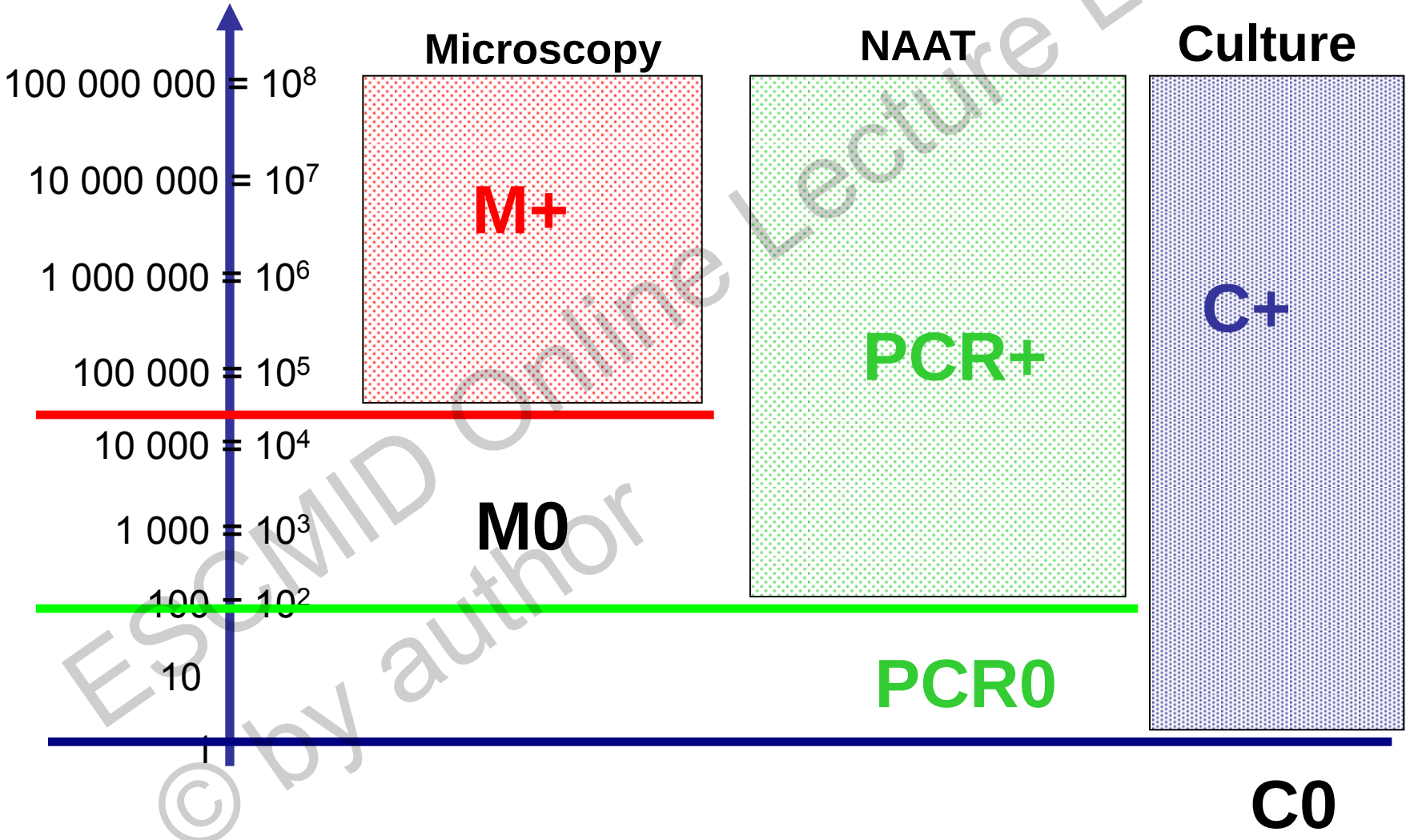
- WHO study group review
  - Pooled sensitivity = **98%** (95%CrI 97-99%)

NAAT sensitivity for TB diagnosis

= We would like that even TB patients with Smear-negative specimens are NAAT+

- Cochrane review
  - Sensitivity : **67%** (95% CrI 58% -74%)
- LID Review (S. Lawn et al. 2013)
  - Sensitivity : **75%** (range: 47% - 83%)
  - Extrapulmonary TB : **77%** (range 25%-97%)
- WHO expert group review
  - Sensitivity : **68%** (95% CrI 61%-74%)

Sensitivity of NAAT for detecting tuberculosis bacilli



NAAT Performances for TB: **specificity**

= We would like that patients <u>without</u> TB are NAAT negative

- Cochrane review
  - Specificity : **98%** (95% CrI 97% to 99%)
- LID Review
  - Specificity : **98.6%** (range 88.9% - 100%)
- WHO study group review
  - Specificity : **99%** (95% CrI 98% to 99%)

Pr Emmanuelle CAMBAU

Molecular detection of rifampicin resistance

Sensitivity = all Resistant strains are detected
Specificity = no Susceptible strains are detected

- Cochrane review
  - sensitivity : **94%** (95% CrI 87% to 97%)
  - specificity : **98%** (95% CrI 97% to 99%)
- WHO study group review
  - sensitivity : **95%** (95%CrI 90-97%)
  - specificity : **98%** (95%CrI 97-99%)

Positive predictive value for detection of rifampicin resistance

For 1000 patients and a specificity of 98%

Prevalence of resistance	30%	2%
Nb of resistant isolates	300	20
Nb of false resistant test	14	20
PPV of detection of rifampicin resistance	96%	50%

Karen R Steingart1, et al. Cochrane Database Syst Rev. 2013

Positive predictive value for detection of rifampicin resistance

For 1000 patients and a specificity of 98%

Prevalence of resistance	30%	2%
Nb of resistant isolates	300	20
Nb		
PPV of detection of rifampicin resistance	96%	50%

Confirmation with another molecular test and with phenotypic determination is mandatory

Karen R Steingart1, et al. Cochrane Database Syst Rev. 2013

The new clinical bacteriology laboratory

Automated susceptibility testing

Identification

Inoculation
Culture
incubation

Blood culture automate

Molecular detection

Whole genome sequencing

Point-Counterpoint: The Automated Clinical Microbiology Laboratory: Fact or Fantasy?

N.A. Ledeboer, S. Dallas, P.H. Gilligan

JCM Accepts, published online ahead of print on 19 March 2014
J. Clin. Microbiol. doi:10.1128/JCM.00686-14

Pros

- Cost saving for hospital directors (less technicians)
- Less skilled workers
- Higher throughput
- Image collection
- Can work 24/24

Cons

- High cost for machines
- Large footprints
- Less skilled workers
- Not adapted to the emergency needed for infections (batch working)
- Not include molecular methods or new techniques

Point-Counterpoint: The Automated Clinical Microbiology Laboratory: Fact or Fantasy?

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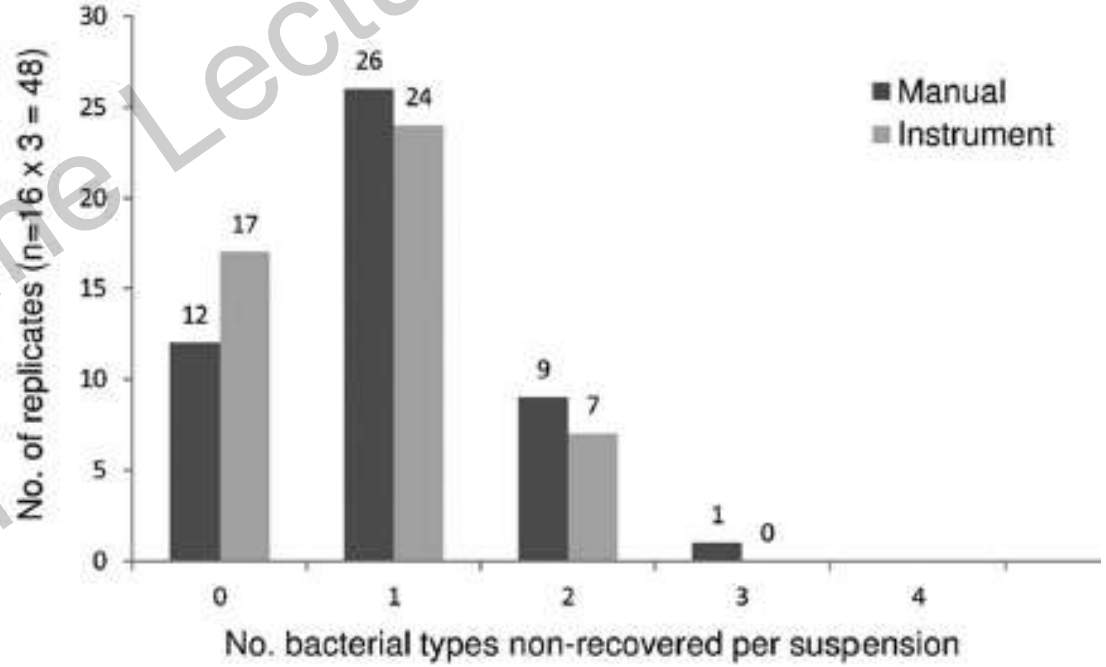
Pros	Cons
• **Cost saving for hospital**	• **High cost for machines**
• **Image collection**	**infections (batch working)**
• **Can work 24/24**	• **Not include molecular methods or new techniques**

There are no data thus far that shows that automation of microbiologic culture processes improves patient outcomes

Automated versus Manual Sample Inoculations in Routine Clinical Microbiology: a Performance Evaluation of the Fully Automated InoqulA Instrument

P. Froment, H. Marchandin, P. Vande Perre and B. Lamy
J. Clin. Microbiol. 2014, 52(3):796. DOI: 10.1128/JCM.02341-13.

16 Polymicobic suspensions
48 Replicates
2 to 4 different bacteria



The automate improved the quality and standardization of bacterial isolation from monomicrobial and polymicrobial clinical specimens

The World Sepsis Declaration

Sepsis is one of the most common, least-recognized illnesses in both the developed and developing world. Globally, 20 to 30 million patients are estimated to be afflicted every year, with over 6 million cases of neonatal and early childhood sepsis and over 100,000 cases of maternal sepsis.

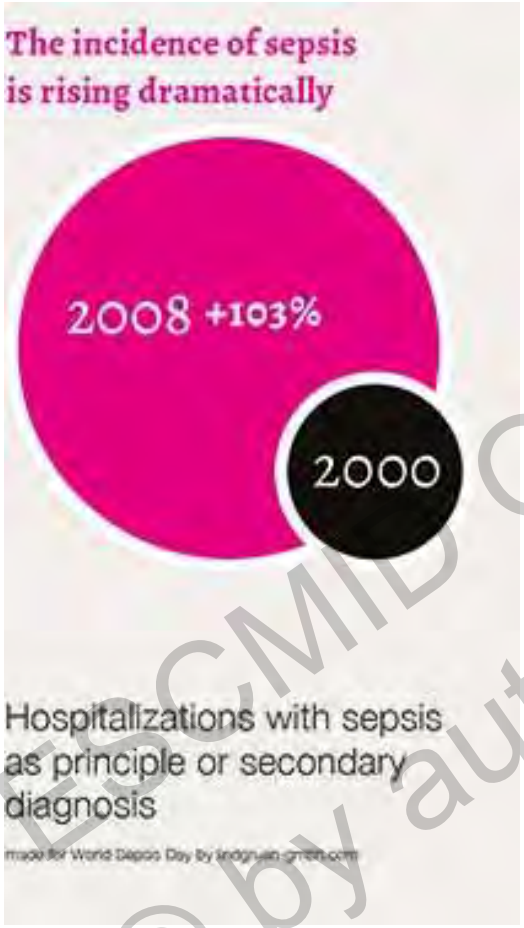
Worldwide, a person dies from sepsis every few seconds.

GSA
WFSICCM
WFPICCS
WFCCN
ISF
Sepsis Alliance
IFEM
AAP
GSS
CSCC

September 13 2013 | World Sepsis Day

www.**world-sepsis-day**.org

Do we have good diagnosis tests for sepsis in CM labs?



Sepsis is a medical emergency

Unfortunately, sepsis is still often overlooked and recognized too late.

Early sepsis treatment is cost effective.
It reduces the number of hospital and critical care bed days for patients.

Clinical Chemistry 59:11
1649–1656 (2013)

Work Flow Analysis of Around-the-clock Processing of Blood Culture Samples and Integrated MALDI-TOF Mass Spectrometry Analysis for the Diagnosis of Bloodstream Infections

Wilhelm Schneiderhan,[1] Alexander Grundt,[1] Stefan Wörner,[1,2] Peter Findeisen,[1] and Michael Neumaier[1*]

Comparison of 2 Blood Culture Media Shows Significant Differences in Bacterial Recovery for Patients on Antimicrobial Therapy

790 • CID 2013:56

Rebecca Zadroga,[1,2] David N. Williams,[2,3] Richard Gottschall,[4] Kevan Hanson,[4] Vickie Nordberg,[4] Marcia Deike,[4] Mike Kuskowski,[5] Lisa Carlson,[6] David P. Nicolau,[7] Christina Sutherland,[7] and Glen T. Hansen[2,4,8]

Emerging Technologies for Rapid Identification of Bloodstream Pathogens

Atul Kothari[1], Margie Morgan[2], David Haake[1,3]

Clinical Infectious Diseases Advance Access published April 24, 2014

Emerging technologies for rapid identification of bloodstream infections
Kothari A. et al, CID 2014

List of techniques that can be used

Table 1. Tests for Rapid Identification of Bloodstream Pathogens

Blood Culture Assays	Pathogens Detected	Resistance Markers	Turnaround Time (after blood cultures turn positive)
PNA-FISH	*Staphylococcus aureus*, CoNS, *Enterococcus fecalis*, Other Enterococci, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Candida albicans*, *Candida parapsilosis*, *Candida glabrata*, *Candida krusei*, *Candida tropicalis*	No	1.5-3 hours
QuickFISH	*Staphylococcus aureus*, CoNS, *Enterococcus fecalis*, Other Enterococci, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*	No	<30 minutes
MALDI-ToF*	Gram positive and negative bacteria, yeast, fungi, filamentous fungi, Mycobacteria	In Development	10-30 minutes
Gene Xpert MRSA/SA	*Staphylococcus aureus*	*mec A*	<1 hour
Verigene Gram Positive Blood Culture (BC-GP)	*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus lugdunensis*, *Streptococcus anginosus group*, *Streptococcus agalactiae*, *Streptococcus pneumonia*, *Streptococcus pyogenes*, *Enterococcus fecalis*, *Enterococcus fecium*, *Staphylococcus* spp., *Streptococcus* spp., *Listeria* spp.	*mec A*, *van A*, *van B*	2.5 hours
Verigene Gram Negative Blood Culture (BC-GN)**	*Escherica coli*, *Shigella* spp., *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Acenitobacter* spp., *Proteus* spp., *Citrobacter* spp., *Enterobacter* spp.	KPC, NDM, CTX-M, VIM, IMP, OXA	2 hours
Film Array Blood Culture Identification (BC ID)	*Staphylococcus aureus*, *Staphylococcus* spp, *Streptococcus agalactiae*, *Streptococcus pneumonia*, *Streptococcus pyogenes*, *Streptococcus* spp., *Enterococcus* spp., *Listeria monocytogenes*, *Hemophilus influenza*, *Neisseria meningitides*, *Enterobacter cloacae* complex, *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Acenitobacter baumanii*, *Proteus* , *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, *Candida tropicalis*	*mec A*, *van A*, *van B*	1 hour
Whole Blood Assays	**Pathogens Detected**	**Resistance Markers**	**Turnaround Time**
Light Cycler*** SeptiFast	*Staphylococcus aureus*, CoNS, *Streptococcus spp.*, *Streptococcus pneumonia*, *Enterococcus fecalis*, *Enterococcus fecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Enterobacter cloacae / aerogenes*, *Serratia marcescens*, *Acenitobacter baumanii*, *Proteus mirabilis* , *Stenotrophomonas maltophila*, *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, *Candida tropicalis*, *Aspergillus fumigatus*	No	6 hours
SeptiTest***	>300 different pathogens	No	8-12 hours
T2 Candida****	*Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, *Candida tropicalis*	No	3 hours

*Biomerieux instrument FDA approved, Bruker pending approval

**pending FDA approval

***not available in US

Emerging technologies for rapid identification of bloodstream infections
Kothari A. et al, CID 2014

List of studies examining impacts on outcomes

Table 2. Studies Examining Impacts on Clinical Outcomes and Healthcare Costs

Intervention	Year of Publication	Antimicrobial Stewardship Intervention	Mortality Benefit	Length of Stay	Cost Saving per patient	Reference
PNA-FISH	2006	Yes	Not studied	-2 days	$4005	Forrest, 2006 [25]
PNA-FISH	2011	No	Not studied	+2.2 days	Not studied	Holtzman 2011 [27]
PNA-FISH	2006	Yes	Not studies	Not studied	$1729*	Forrest 2006 [28]
PNA-FISH	2008	No	Yes (16.8% vs 7.9%)	-2 days*	$19441*	Ly 2008 [26]
Gene Xpert MRSA/SA	2010	Yes	Yes (18% vs 26%)*	-6.2 days	$21387	Bauer 2010 [30]
MALDI-ToF	2012	Yes	Yes (5.8% vs 10.7%)*	-1.8 days	$19547*	Perez 2012 [31]
MALDI-ToF	2013	Yes	Yes (12.7% vs 20.3%)	-2.8 days*	Not studied	Huang 2013 [33]
Verigene BC-GP	2013	Yes	No	-21.7 days	$60729	Sango 2013 [35]

*not statistically significant

Emerging technologies for rapid identification of bloodstream infections
Kothari A. et al, CID 2014

List of studies examining impacts on outcomes

Table 2. Studies Examining Impacts on Clinical Outcomes and Healthcare Costs

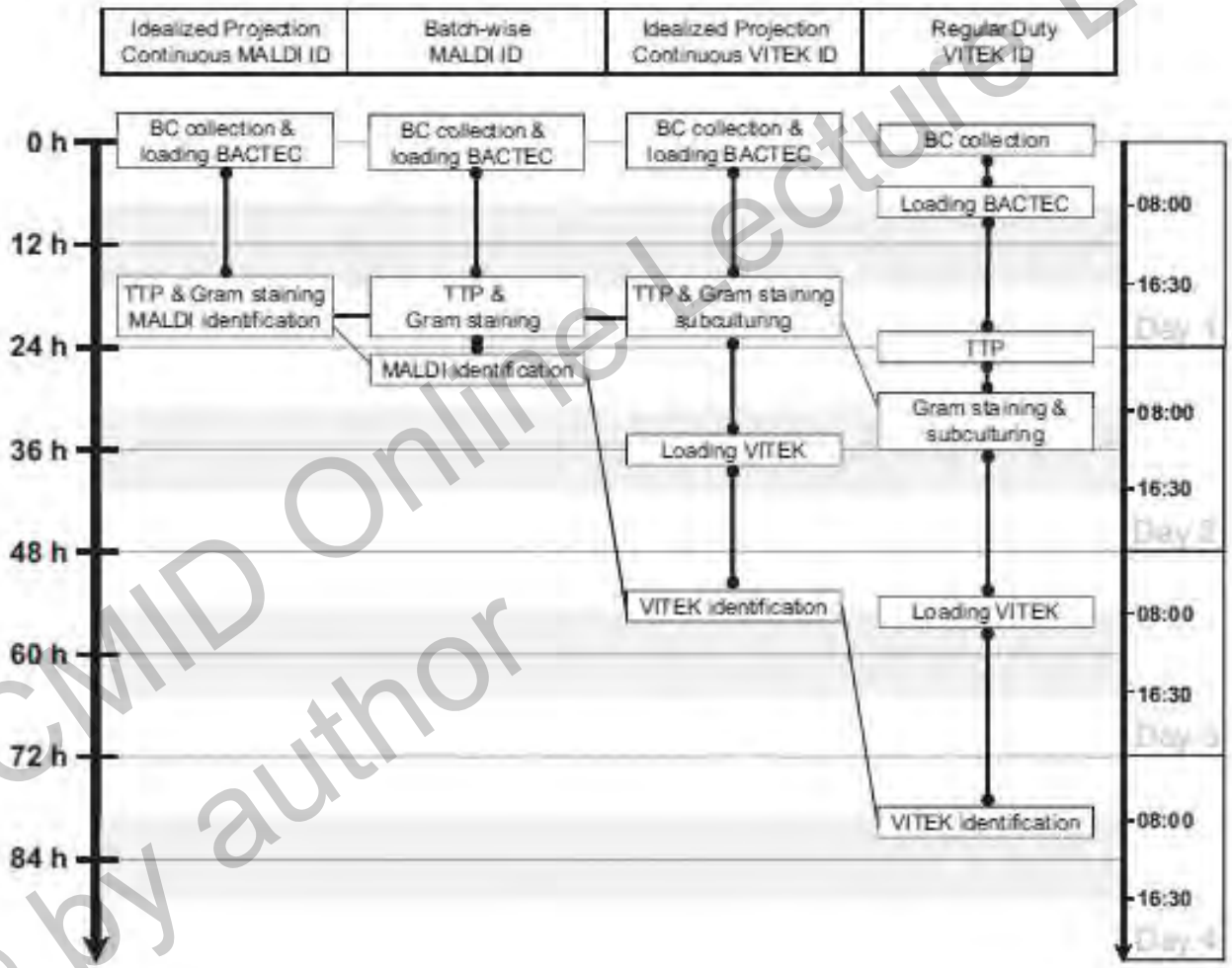
Intervention	Year of Publication	Antimicrobial	Mortality Benefit	Length of Stay	Cost Saving per	Reference

*not statistically significant

Impact of rapid technologies on
- Mortality
- Cost
- length of stay in hospital

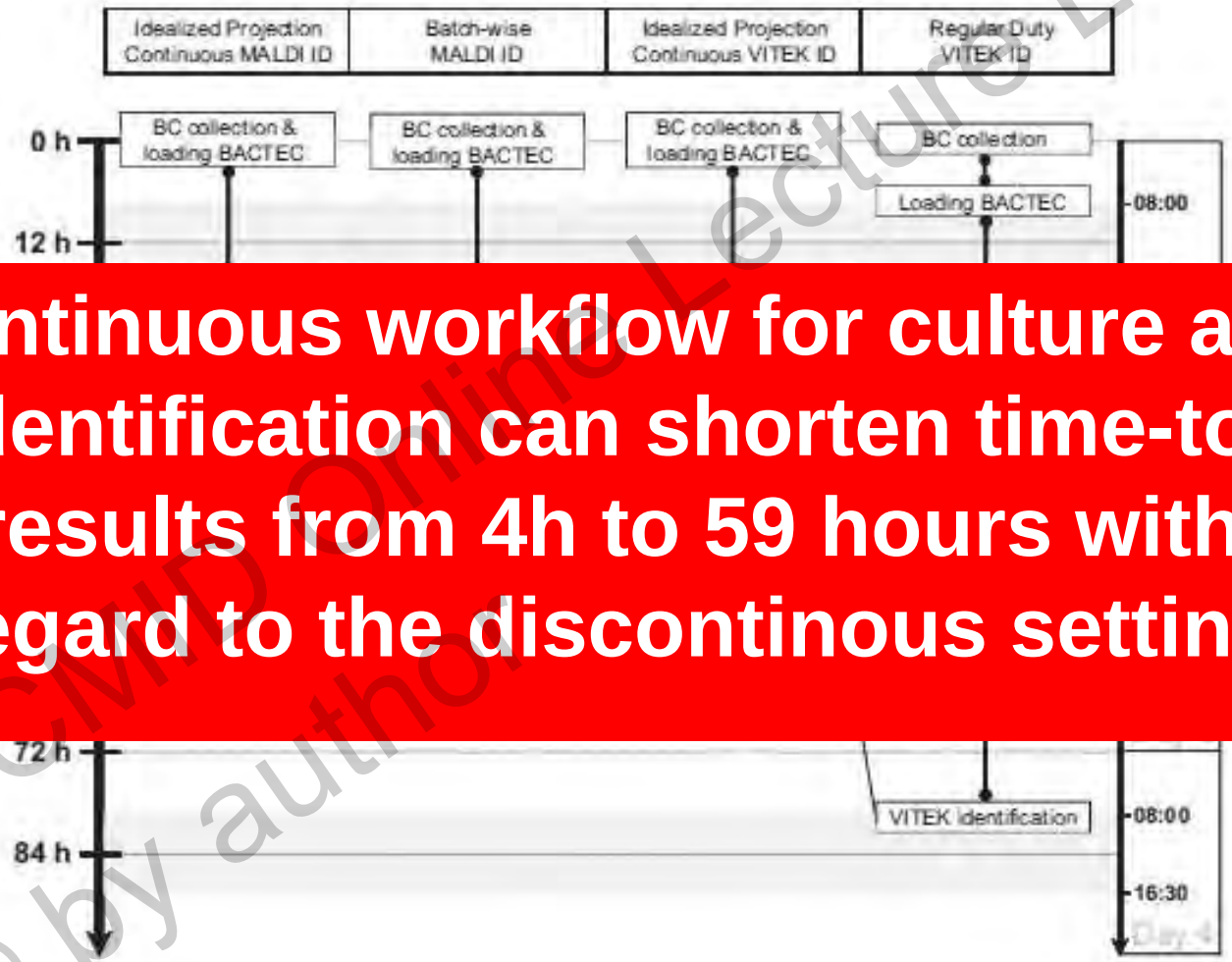
Needs to be more often examined

Workflow for detection and identification of pathogens in Blood cultures



Schneiderhan et al. 2013

Workflow for detection and identification of pathogens in Blood cultures



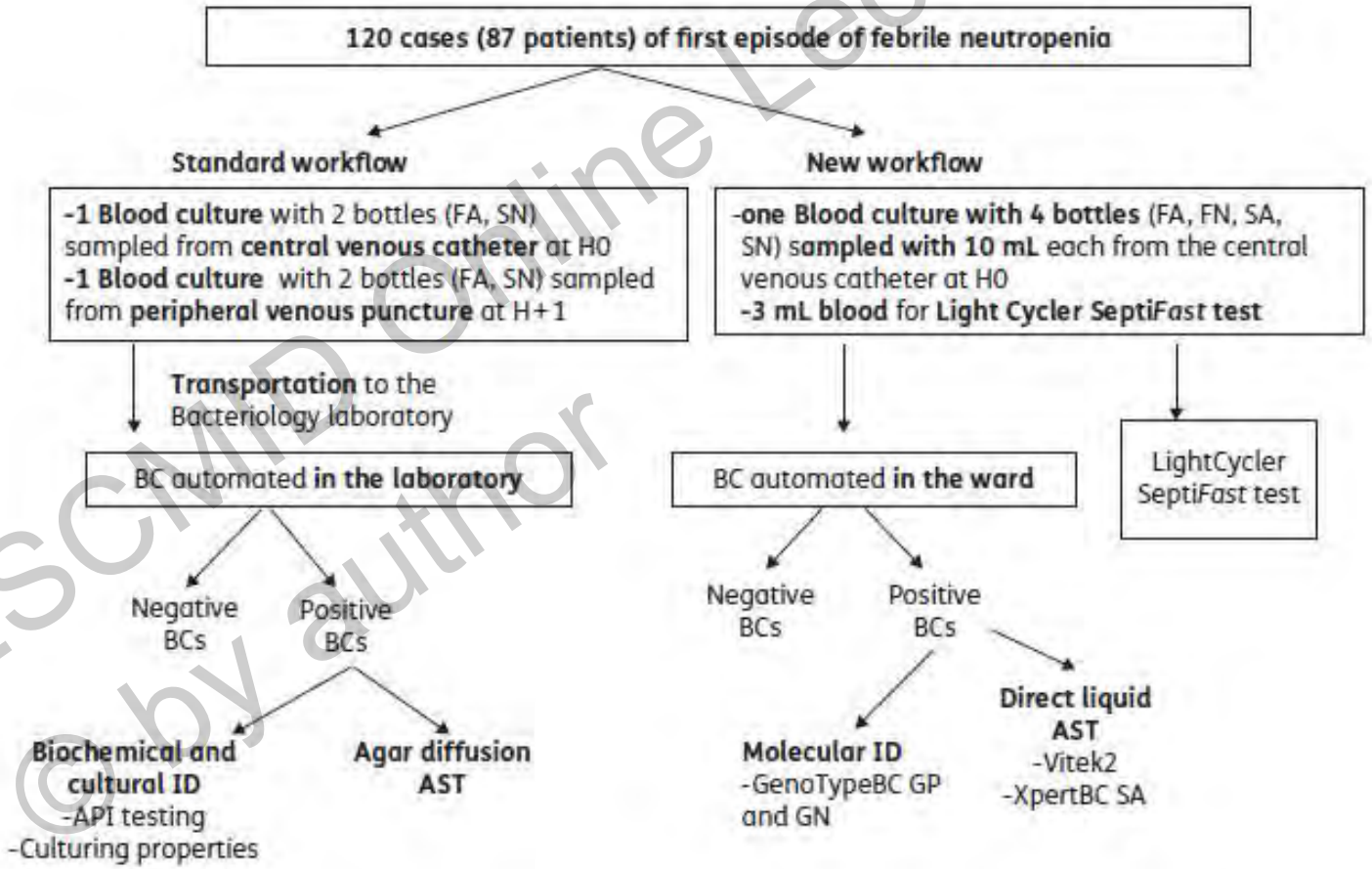
Continuous workflow for culture and identification can shorten time-to-results from 4h to 59 hours with regard to the discontinous setting

Schneiderhan et al. 2013

A new workflow for the microbiological diagnosis of febrile neutropenia in patients with a central venous catheter

Cecile Pautas[1], Emilie Sbidian[2]†, Yosr Hicheri[1,3]†, Sylvie Bastuji-Garin[2,4], Stéphane Bretagne[2,5], Celine Corbel[6], Laetitia Gregoire[7], Sébastien Maury[1,2], Lilia Merabet[6], Catherine Cordonnier[1,2] and Emmanuelle Cambau[6,8,9]*†

Journal of Antimicrobial Chemotherapy 2013



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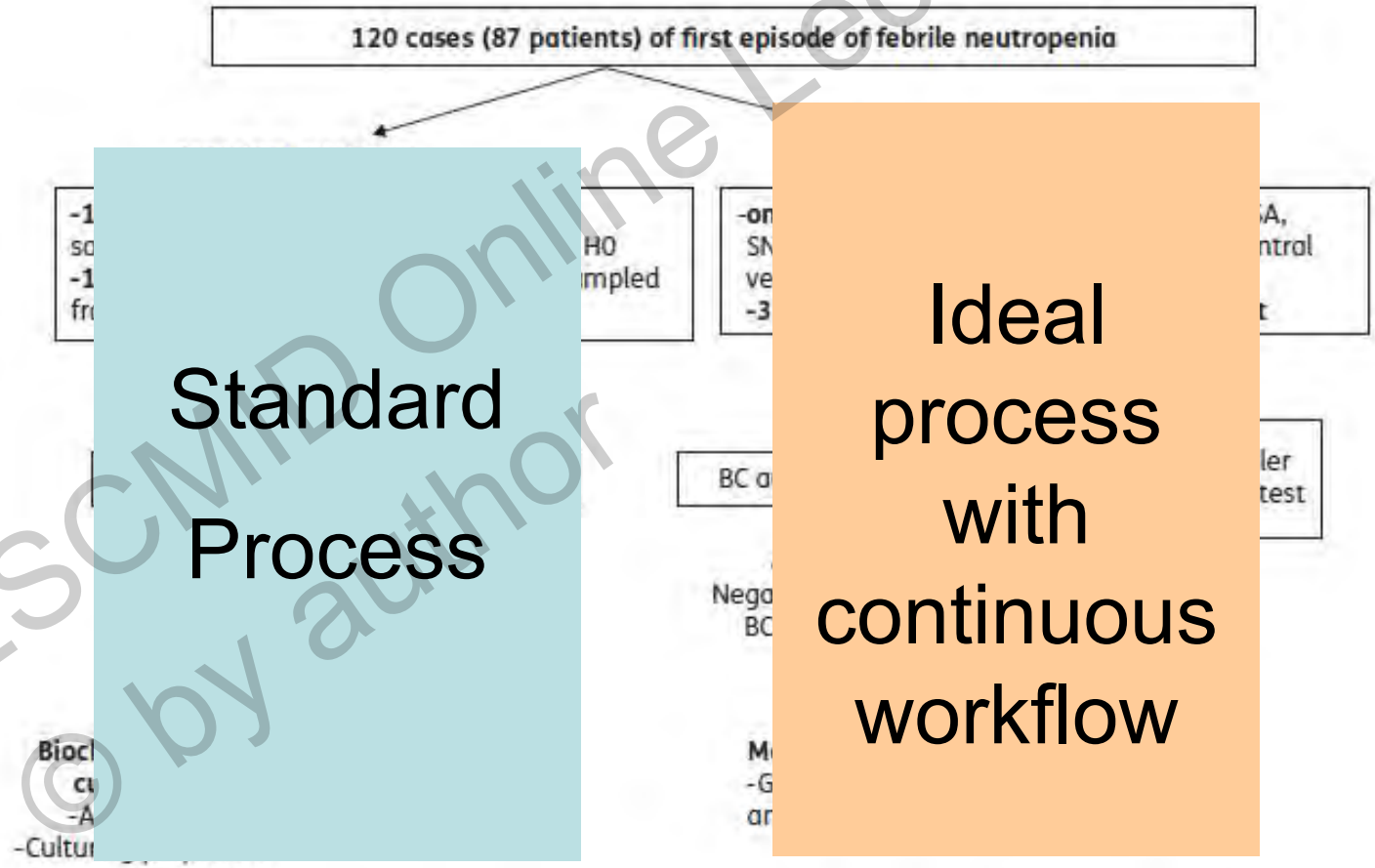
Journal of Antimicrobial Chemotherapy 2013



Continuous workflow decreased time-to-results to less than 24h

Table 1. Turnaround times for blood culture and identification and susceptibility testing results for 29 episodes positive with both wor

Time to microbiological results	Median time (range)	
	standard workflow	study workflow
Time to BC-positive signal[a]		
all episodes (n=29)	13 h 01 (9 h 31–43 h 33)	12 h 25 (7 h 55–25 h 37)
episodes with streptococci (n=13)	10 h 39 (9 h 31–13 h 37)	9 h 55 (7 h 55–17 h 55)
episodes with CoNS (n=7)	20 h 13 (17 h–43 h 27)	16 h 24 (14 h 34–25 h 18)
episodes with other bacteria (n=9)	14 h 10 (11 h 18–21 h 55)	16 h 54 (11 h 18–25 h 37)
Time to results of identification[b]		
all episodes (n=26)	69 h (22 h–405 h)	18 h 16 (11 h 74–55 h 37)
episodes with streptococci (n=11)	156 h (82 h 48–405 h)	15 h 55 (12 h 57–47 h 55)
episodes with CoNS (n=6)	66 h (50 h–75 h)	21 h 58 (18 h 53–31 h 18)
episodes with other bacteria (n=9)	36 h 12 (22 h–69 h)	21 h 55 (16 h 20–37 h 23)
Time to results of antibiotic susceptibility testing (AST)		
methicillin-resistance in staphylococci (n=9)[c]	70 h 36 (50 h–130 h)	17 h 30 (15 h 51–31 h 18)
all episodes with valid AST results (n=18)[d]	56 h (22 h–245 h)	22 h 10 (13 h 10–43 h 37)

* Pautas et al. JAC 2013

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Table 1. Turnaround times for blood culture and identification and susceptibility testing results for 29 episodes positive with both wor

Time to microbiological results	Median time (range)	
	standard workflow	study workflow
Time to BC-positive signal[a]		
all ep		
episo		
episo		
episo		
Time to		
all ep		
episo		
episodes with CoNS (n=6)	66 h (30 h–93 h)	21 h 58 (18 h 33–31 h 18)
episodes with other bacteria (n=9)	36 h 12 (22 h–69 h)	21 h 55 (16 h 20–37 h 23)
Time to results of antibiotic susceptibility testing (AST)		
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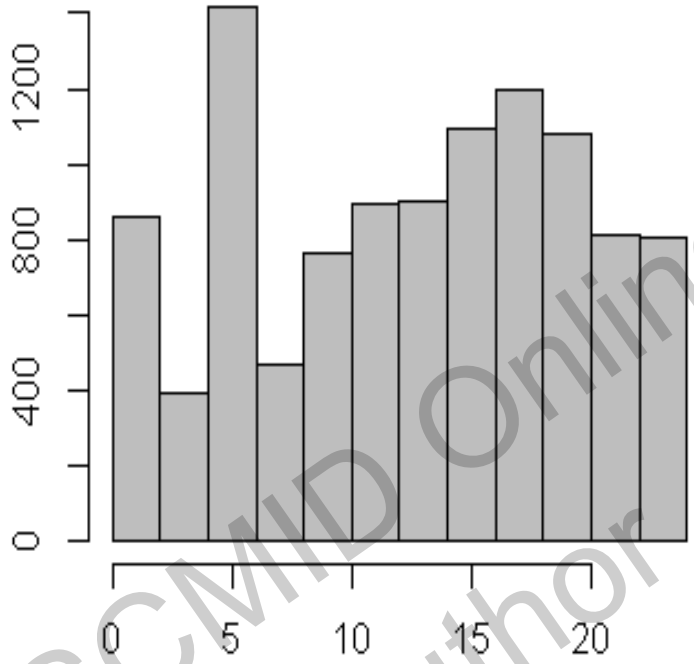
all episodes with valid AST results (n=18)[d]	56 h (22 h–245 h)	22 h 10 (13 h 10–43 h 37)

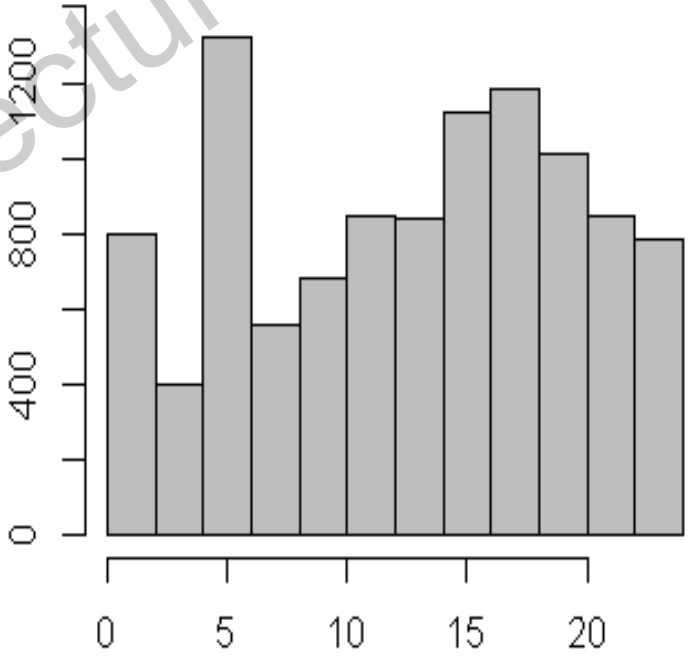
Median time to positivity of BC = 12h25
Median time to identification = 18h16
Median time to AST results = 22h10

* Pautas et al. JAC 2013

Have you looked at what time the blood cultures were collected?



2011

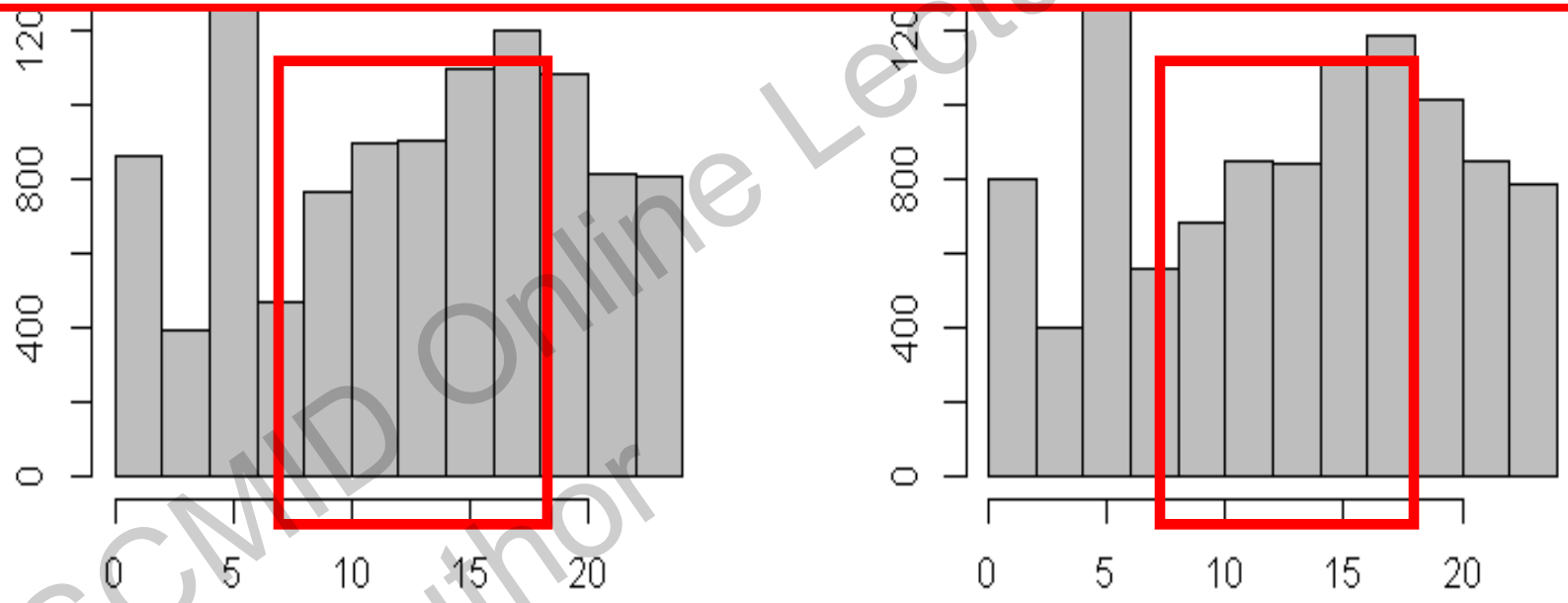


2012

Data for Lariboisière-Fernand Widal, 1000-bed University Hospital

Have a look at the time when the blood cultures are collected!

50% of BC are sampled during the day



50% of BC are sampled during the night

MDR bacteria are still evolving crazy

Proc Natl Acad Sci U S A. 2014 Apr 1;111(13):4988-93. doi: 10.1073/pnas.1321364111. Epub 2014 Mar 17.

Molecular dissection of the evolution of carbapenem-resistant multilocus sequence type 258 Klebsiella pneumoniae.

Deleo FR[1], Chen L, Porcella SF, Martens CA, Kobayashi SD, Porter AR, Chavda KD, Jacobs MR, Mathema B, Olsen RJ, Bonomo RA, Musser JM, Kreiswirth BN.
Author information

N Engl J Med. 2014 Apr 17;370(16):1524-31. doi: 10.1056/NEJMoa1303359.

Transferable vancomycin resistance in a community-associated MRSA lineage.

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World Alliance Against Antibiotic Resistance (WAAAR)

1. Awareness of antibiotic resistance (ATB-R)
2. Financed national plans for fighting against ATB-R
3. Antibiotics of assured quality in every part of the world
4. Integrated surveillance of ATB-R and ATB use
5. Development of new diagnostic tests
6. Revisit the use of ATB and its business
7. Educational efforts to change
8. Prevention of ATB-R bacteria transmission including environment
9. Increase research and drug developement
10. “lives saved by antibiotics” in the list of the world immaterial heritage (UNESCO)

Thank you for your attention