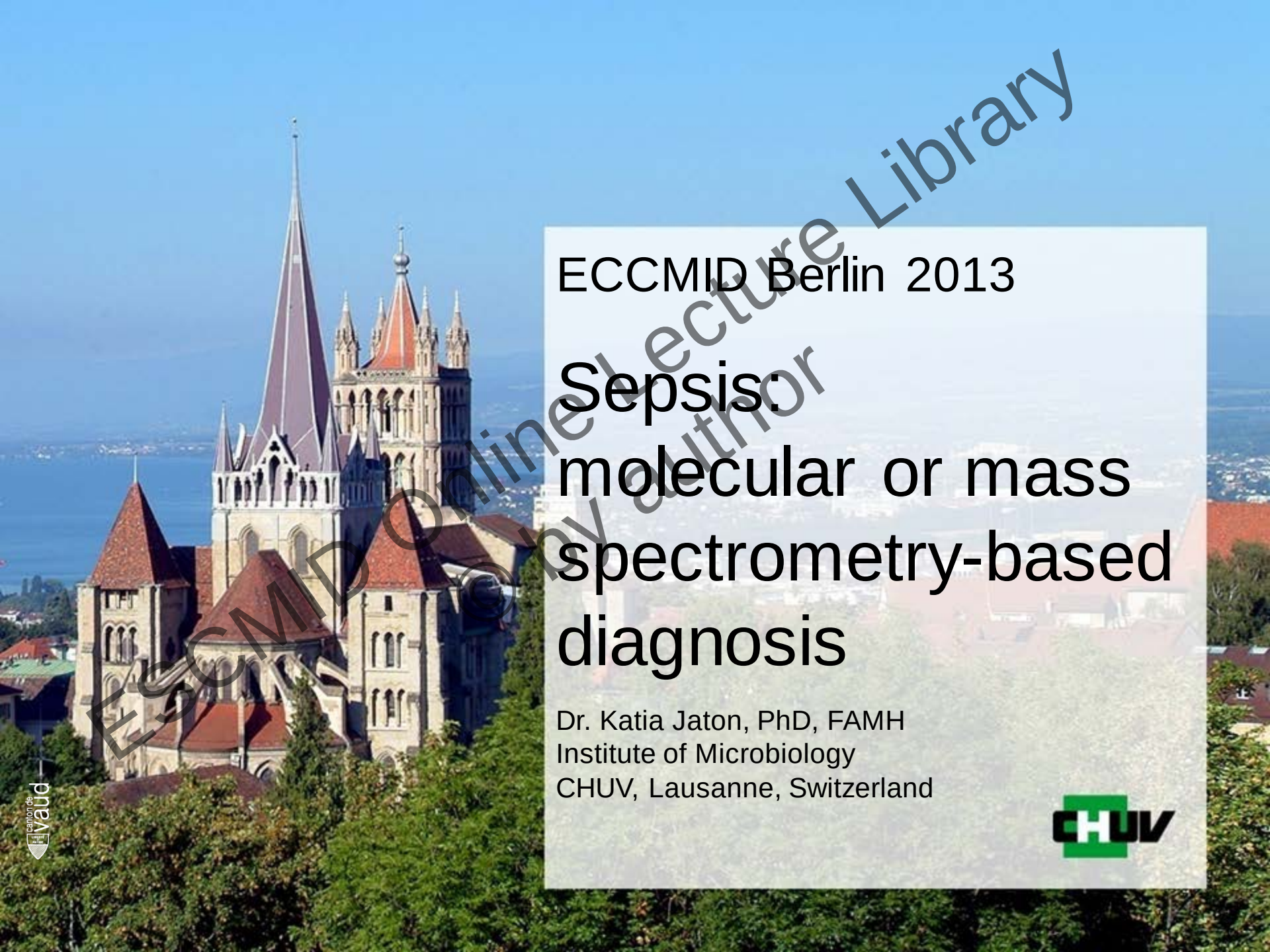


ECCMID Berlin 2013

Sepsis: molecular or mass spectrometry-based diagnosis

Dr. Katia Jaton, PhD, FAMH
Institute of Microbiology
CHUV, Lausanne, Switzerland

Objectives

To understand the importance of rapid diagnosis of sepsis

To appreciate the added value of MALDI-TOF-based identification of bacterial agents

To know the limits and advantages of molecular tests used on blood

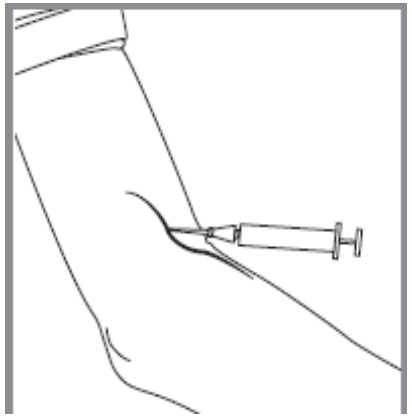
Sepsis: crucial to treat rapidly

Early empirical antibiotic therapy is crucial

Rapid detection of bacteria in blood

- To establish the presence of infection
- To reassure the clinician about the chosen empirical therapy
- To streamline AB treatment after assessment of the AB susceptibility of the isolate

Blood



Blood is easy to obtain and a commonly used specimen

Only 5 to 10% of all blood cultures are positive

A significant proportion of sepsis episode are microbiologically non-documented

Blood cultures

Positive cultures are detected:

- 90% first 24 hours
- 95% after 48 hours
- 99% after 72 hours

No manipulation when negative

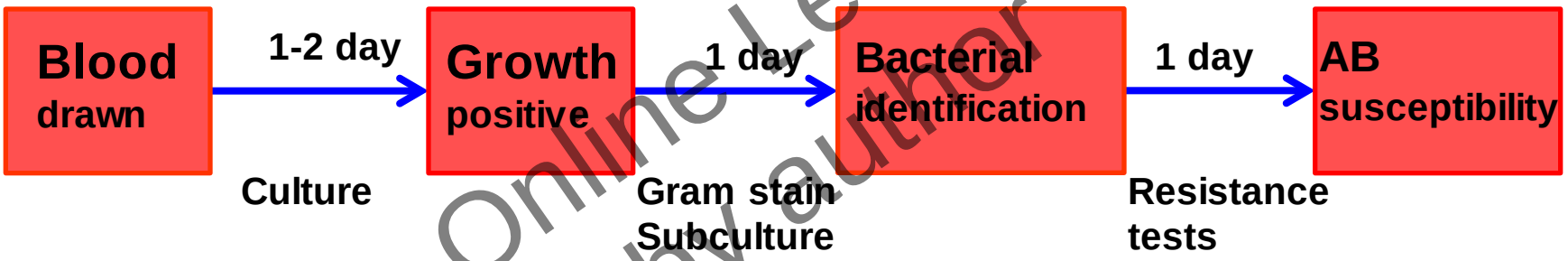
Specific broth for mycobacteria

10 ml of blood, less for children

Antibiotics removal (resins)

Limitations of blood cultures

Long TAT



Antibiotic treatments prior sampling

Low sensitivity especially for fastidious bacteria

Intermittent bacteremia

New techniques

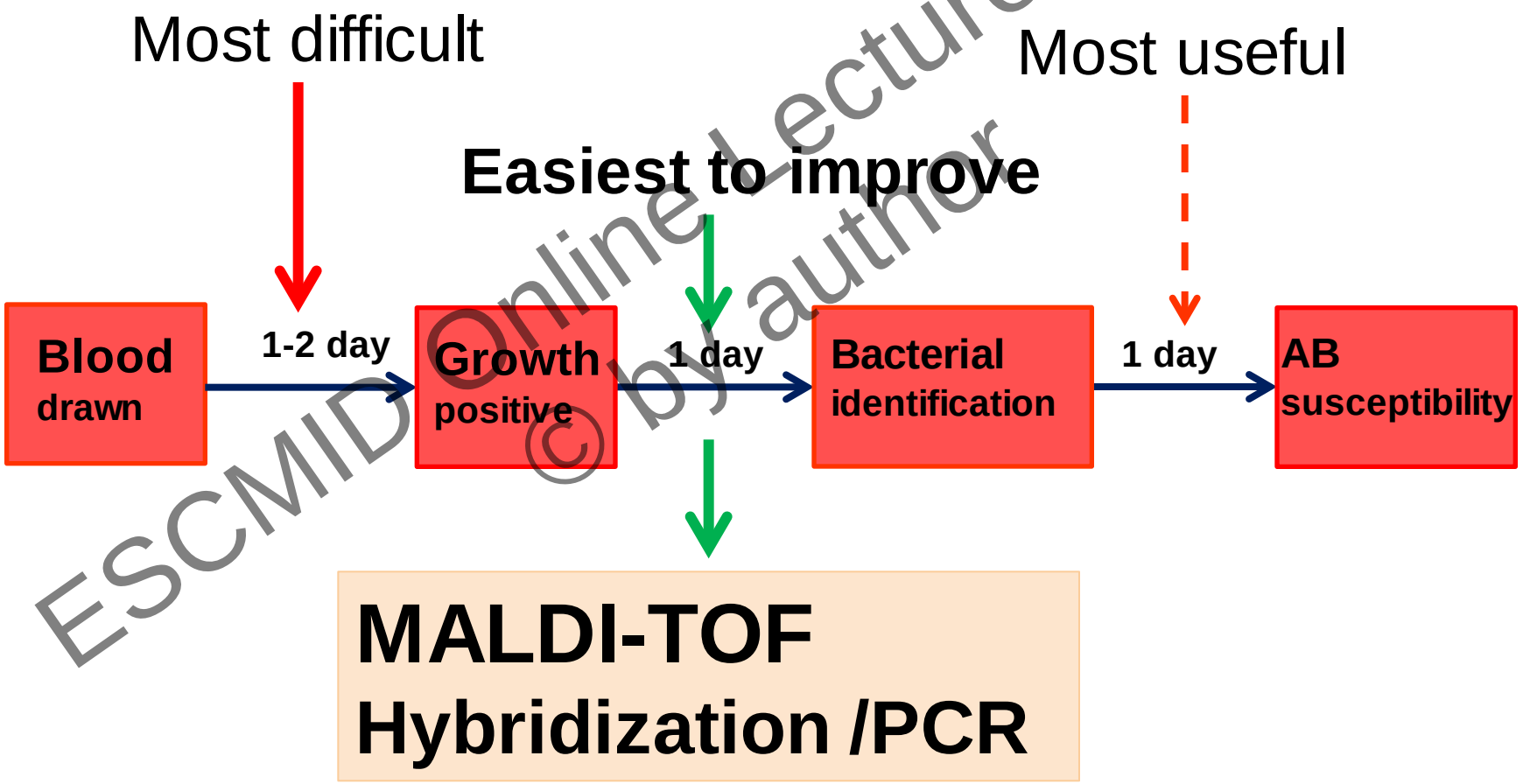
Increase SPEED (TAT < 6 hours)

Improve detection after AB therapy

Improve the SENSITIVITY

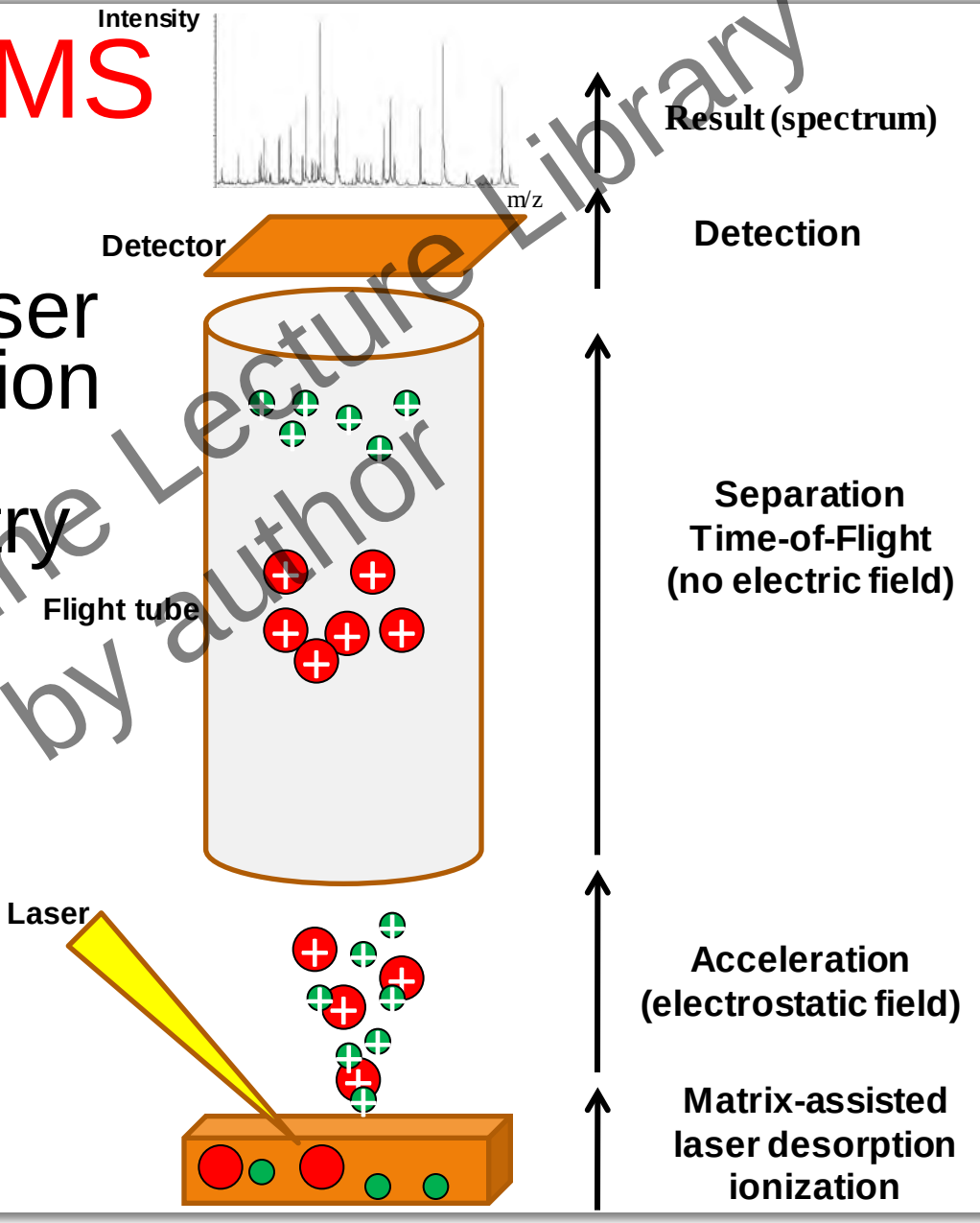
Improve detection of fastidious bacteria

3 steps to improve



MALDI-TOF MS

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry



Adapted from: Croxatto, Prod'hom & Greub, FEMS Microbiol Reviews 2011

Different spectra are obtained for different microbial species

E. faecium

S. aureus

E. coli

with spikes ranging from 1'000 to 20'000 m/z

MALDI-TOF MS: a revolution

Easy to use and rapid (< 5 min)

High throughput

Low cost

Accurate identification

Replace other conventional identification

Direct identification
in positive blood culture

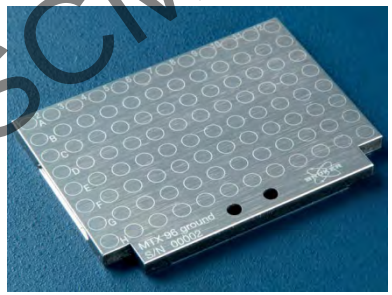
MALDI-TOF MS

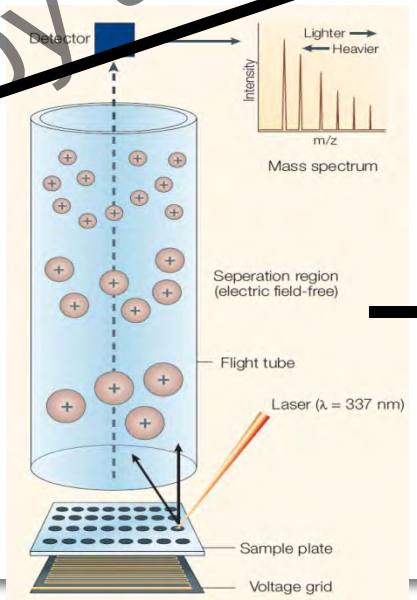
POSITIVE blood culture

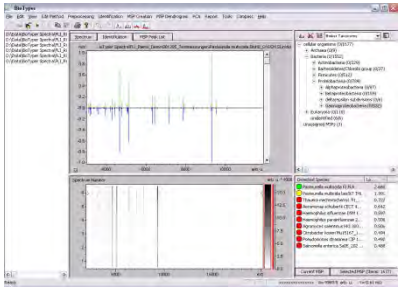
Preparation of a bacterial pellet 5 ml of the positive BC

Centrifugation

erythrocyte lysis with ammonium chloride







Prod'hom et al. J Clin Microbiol (2010)

126 positive blood cultures

Congruent identification at species level:

- 99% for gram positive *
  - * *S. caprae* instead of *S. capitis*
- 100% for gram negative

No identification for 22% of the cases

Prod'hom et al. J Clin Microbiol (2010)

Factors affecting MALDI-TOF identification

Presence of a capsule:

K. pneumoniae, H. influenzae, S. pneumoniae (n=13)

Close relatedness of bacteria within streptococci

S. pneumoniae, S. oralis, … (n=13)

Cell wall composition of Gram positive bacteria conferring high resistance to lysis (n=21)

Presence of residual blood proteins

Prod'hom et al. J Clin Microbiol (2010)

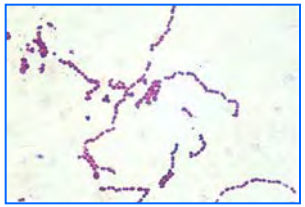
Literature: MALDI-TOF

Authors (year)	n	Concordant ID at species level
Prod'hom (2010)	126	78% (GN: 89%, GP: 72%)
Prod'hom (unpublished)	314	85% (GN: 89%, GP: 83%)
La Scola (2009)	599	66% (GN: 91%, GP: 49%*)
Ferreira 2010	300	43% (GN: 83%, GP: 32%)
Stevenson (2010)	212	80%
Ferroni (2010)	685	89% (312 spiked bottles)
Christner (2010)	277	94%
Ferreira (2010)	68	76%

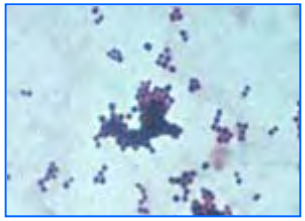
*Improved extraction for *Staphyloccocus* spp.: 38 to 75%

Croxatto, Prod'hom & Greub. FEMS Microbiol Rev 2011

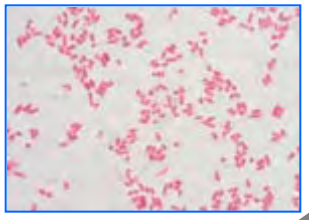
Pre-MALDI-TOF era



Gram + cocci
arranged in chains

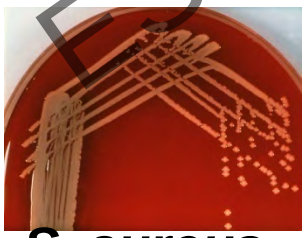


Gram + cocci
arranged in cluster



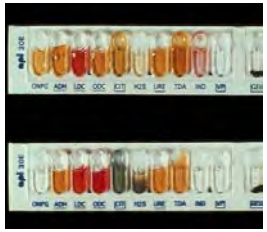
Gram - bacilli

Overnight incubation



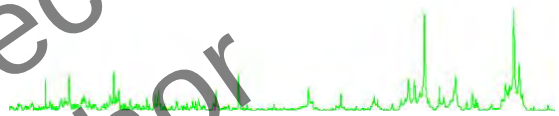
S. aureus

E. coli

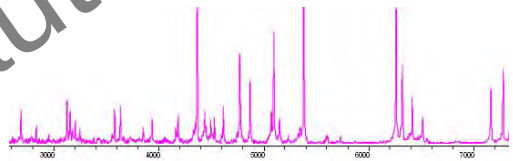


Post-MALDI-TOF era

E. faecium



S. aureus



E. coli

Clinical impact of the MALDI-TOF :

202 episodes gram negative bacteremia

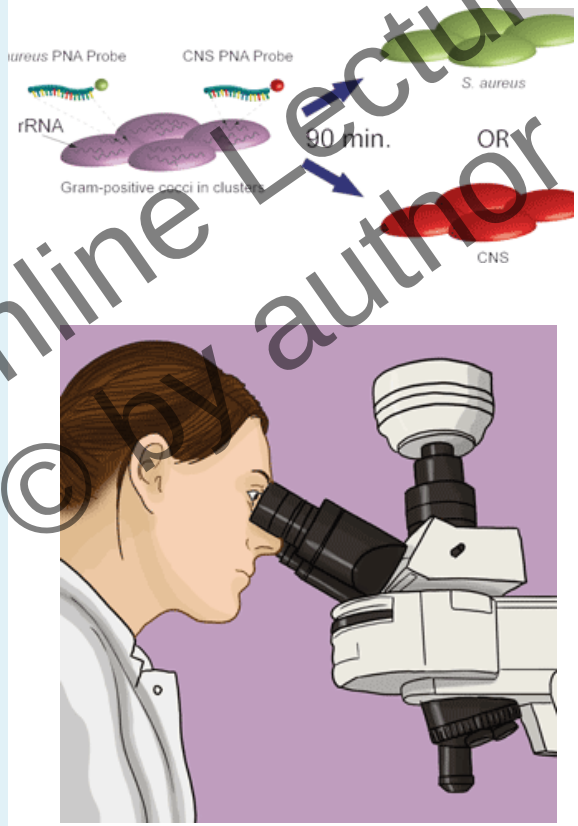
↓

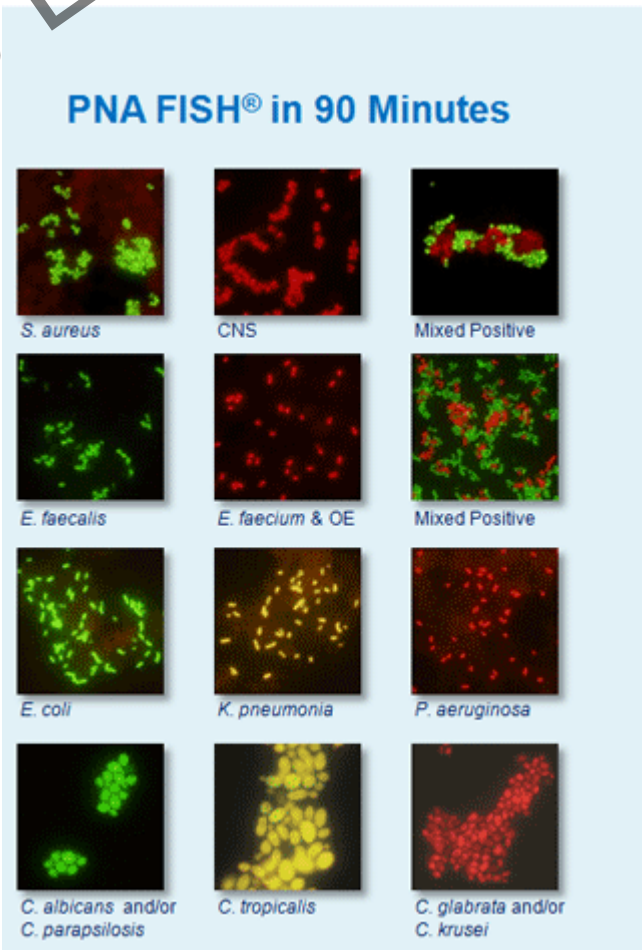
Impact on treatment :

Gram stain :	20.8%
MALDI-TOF identification:	35.1%
Broadening of the AB spectrum :	43.7%
Focused empirical AB therapy	25.0%

O. Clerc et al, Clin. Infect. Dis. 2013

Other approaches:
Probe Hybridization Assays (FISH)





http://www.advandx.com/Solutions/BSI-PNA-FISH.aspx

Gherna et al, 2009, JCM, 47: 247

Probe hybridization assays

Advantages

rRNA (viability)

Rapid

Polymicrobial

Specific

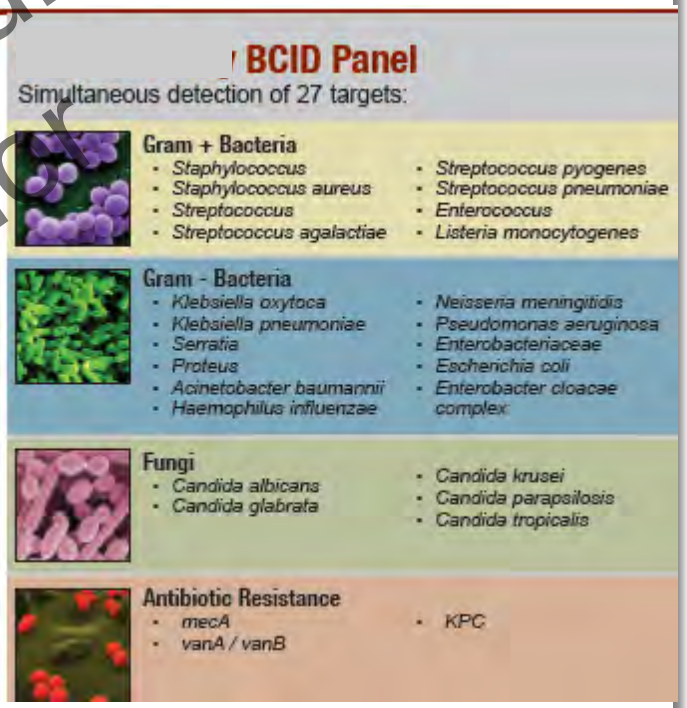
Limitations

Probes available only for classical pathogens

Closely related species

High cost

Other approaches:
PCR on positive blood cultures

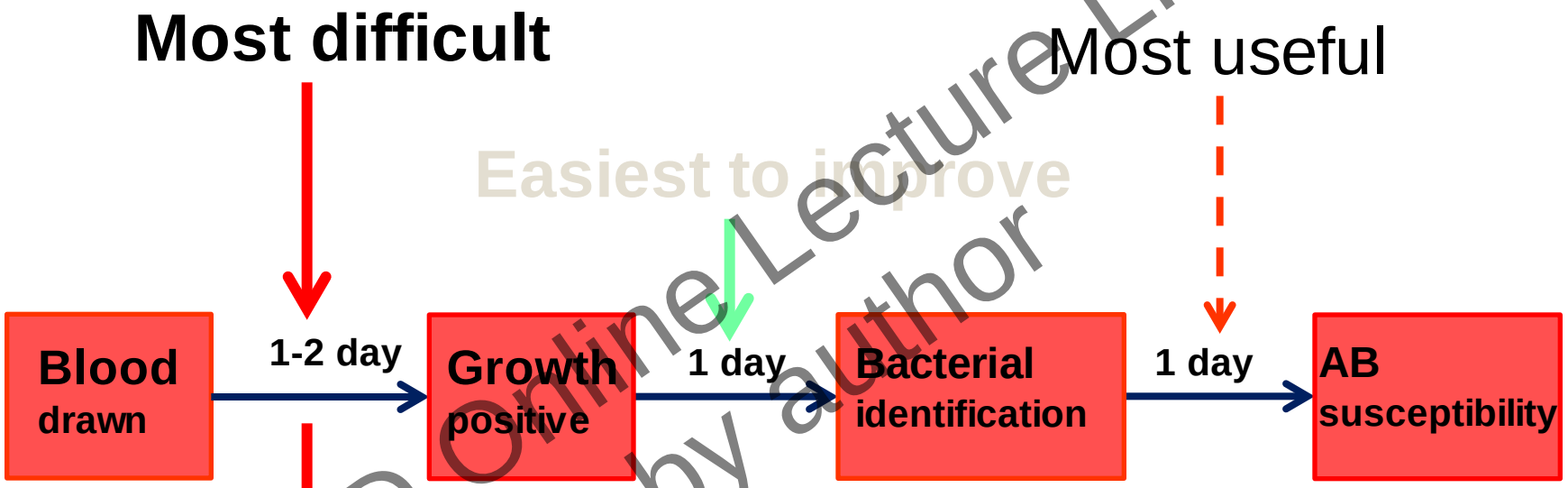


Identification available only for classical pathogens
High cost

Literature: Hybridization and PCR

Authors (Year)	Assay	Principle	Pathogen detected	TAT (h)	Detection limit
Ly (2008)	PNA-FISCH	Fluorescence based hybridization probes	>10	1.5 -3	NA
Lindholm (2004)	AccuProbe	Cheminuminescent DNA probes	5	2.5	NA
Eigner (2005)	GenoType assay	Broad-range amplification + hybridizatuion	Gram pos/neg pannel	5	10^4/ml
Tissari (2010)	Prove-it Sepsis	Multiplex PCR with microarray	> 30	2.5	NA

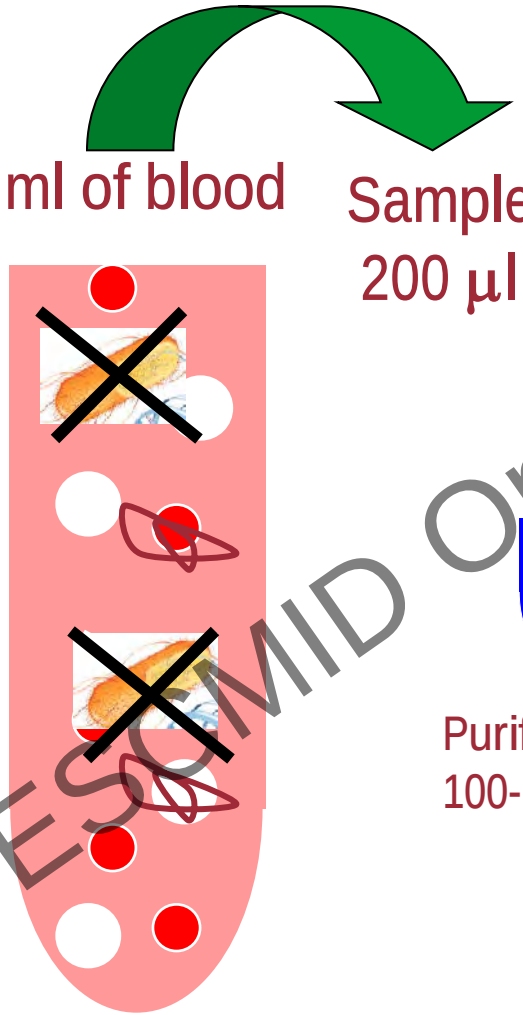
3 steps to improve



PCR DNA/RNA

Broad range
Multiplex PCR
Specific RT-PCR

Blood : not easy



Low amount of bacteria

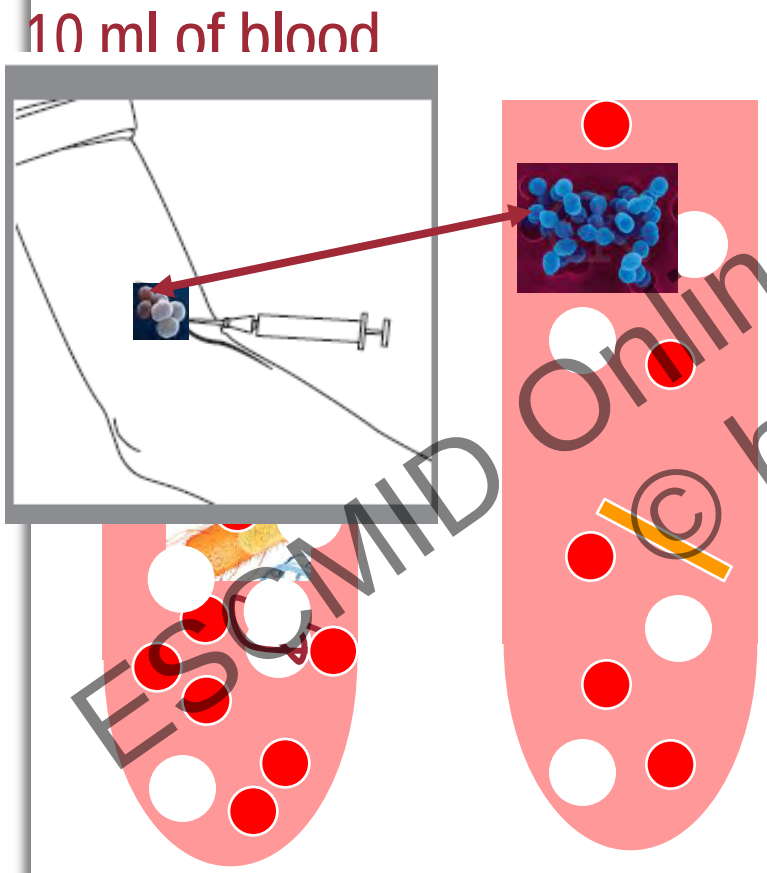
Often less than 1 to 10 CFU/ml

Presence of dead bacteria (DNAemia)

Presence of PCR inhibitors

Contamination with bacteria from the skin

Blood : not easy



Low amount of bacteria

Often less than 1 to 10 CFU/ml

Presence of dead bacteria (DNAemia)

Presence of PCR inhibitors

Contamination with bacteria from the skin

The questions:

Relation between amount of bacterial DNA in the blood with clinical signs of sepsis?

How fast is bacterial DNA cleared?

Origin of bacterial DNA: blood or skin?

Advantages of PCR

High sensitivity

Fastidious or slow growing microorganisms can be detected with sensible specific PCR

Bartonella, Coxiella, T. whipplei, …(endocarditis)

Not impact of AB

Quantitative results (monitoring)

Literature:

Specific PCR for given pathogens

Authors (Year)	Problem	Pathogen
Fenollar (2004)	Endocarditis	*Coxiella burnetii*
Zeaiter et al (2003)	Endocarditis	*Bartonella henselae*
Darton et al (2009)	Meningitis	*Neisseria meningitidis*
Bernal-Martinez (2011)	Aspergillosis	*Aspergillus fumigatus*
Edouard (2012)	Whipple	*Tropheryma whipplei*
Giulieri et al (2012)	Rickettsiosis	*Rickettsia* sp

Broad-range PCR

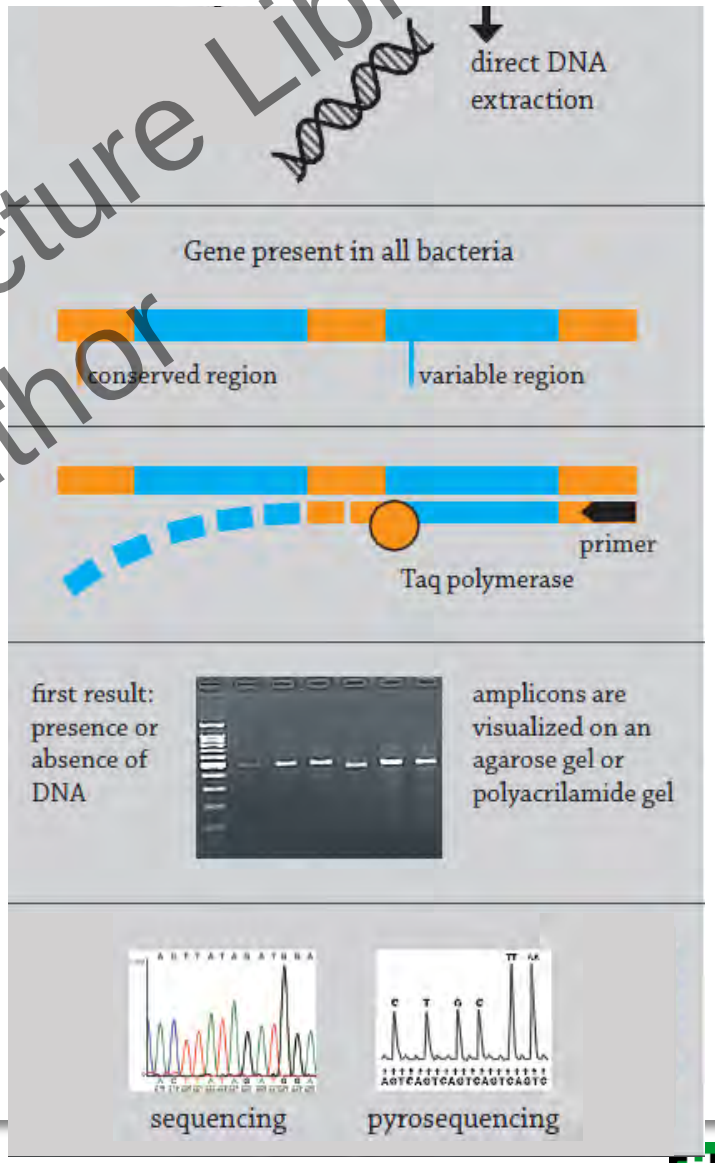
DNA extraction

Conserved target gene:
16S, 23S rRNA genes

Mostly home made PCR

Electrophoresis to see amplicons

Sequencing



Broad-range PCR studies

Many studies, still very challenging

Variable sensitivity (42%-100%)

Variable specificity (70% -98%)

Many studies on neonates and children

Larger cohorts are needed

Results are difficult to interpret

Literature: Broad-range PCR

Authors	Patients	N	Sensitivity	Specificity
Ley (1998)	Neutropenic or Bone marrow transplants	90	82%	75%
Sleigh (2001)	ICU patients	126	54%	72%
Shang (2005)	Neonates	172	100%	98%
Amman (2007)	Pediatric cancer			
Ohlin (2008)	Newborn infants	288	42	95
Reier-Nilsen (2009)	Infants	48	67	88

Multiplex PCR

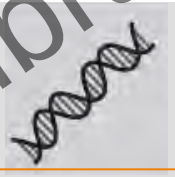
DNA extraction

Highly polymorph target gene: ITS

Commercial (SeptiFast®)
Light cycler amplification

Specific fluorogenic probes for different pathogens

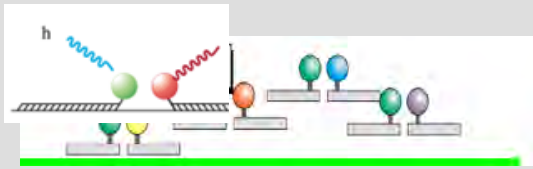
Melting temperature, array, …



Gene present in all bacteria

Internal transcript spacer

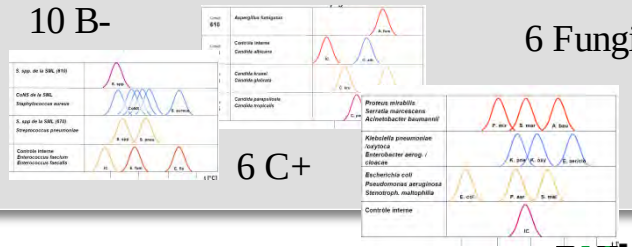
Gram +
Gram-
Fungi



10 B-

6 Fungi

6 C+



Septifast^{R} PCR studies

Neutropenic patients, intensive care units, endocarditis,...

Variable sensitivity (42%-100%)

Variable specificity (60 to100%)

Dependant on :

patient population

prior antibiotic treatment.

Limitations of Septifast ^{R}

Need for automation of DNA extraction

Extraction time consuming and cumbersome

Few samples per batch

Detection of commonly encountered bacteria and fungi

Difficult to interpret:

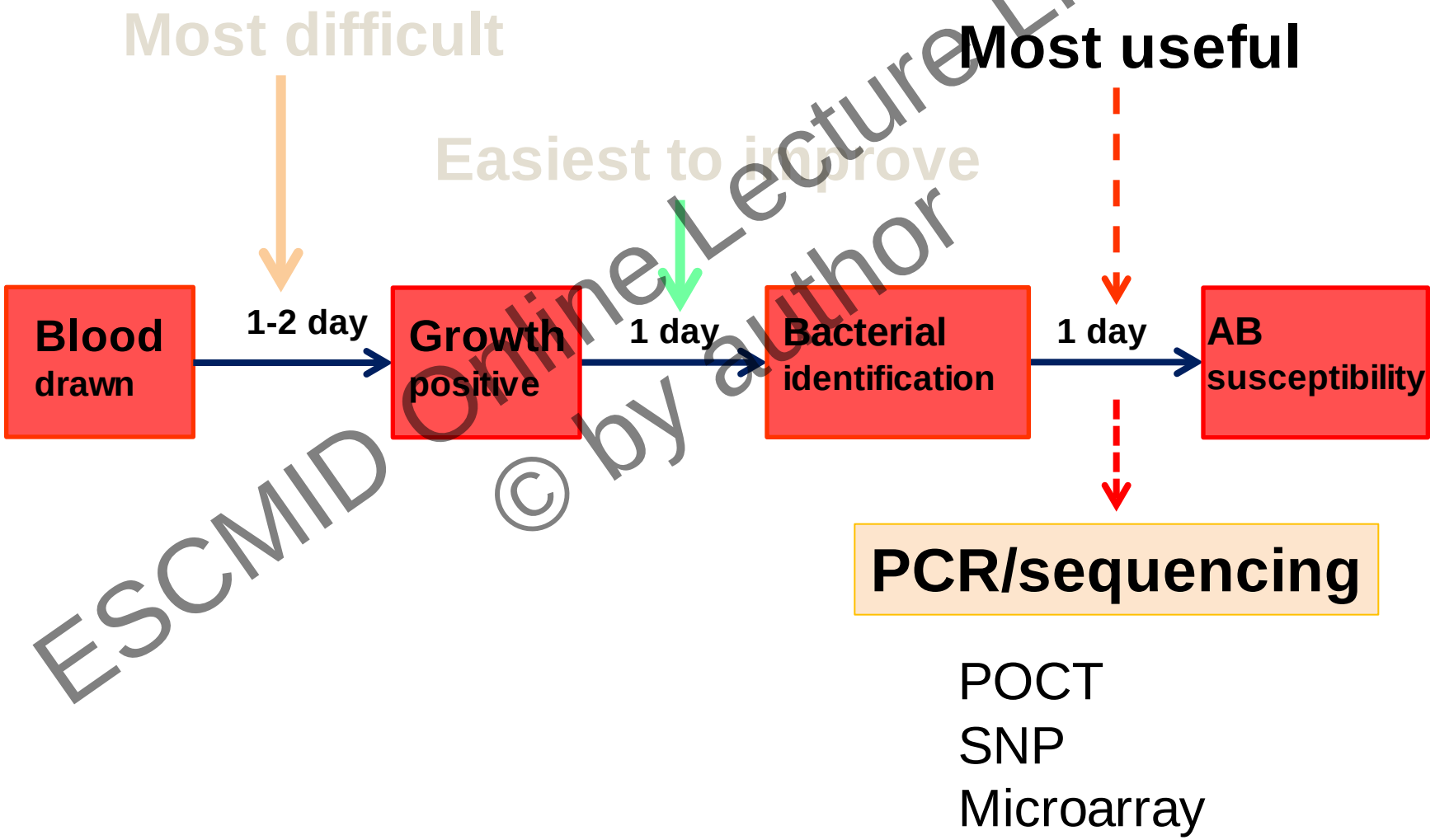
Septifast^{R} often positive when blood culture is negative i.e. prior AB, fungi, …

Literature:

Commercial assay SeptiFast ®

Authors (Year)	Patients	N	Sensitivity %	Specificity %
Lamoth (2010)	Neutropenic	86	50	64
Guido (2012)	Neutropenic	166	91.3	88.1
Pasqualini (2012)	Sepsis	391	56.1	91.7
Rath (2012)	Liver transplant	107	80.9	70
Rath 2012	Non Liver transplant	118	57.6	80
Kasper (2013)	Neonate	46	90.5	80
Leitner (2013)	ICU	57	42.9	88.2

3 steps to improve



Molecular antibiotic susceptibility

Different mechanisms of resistance

Not always at DNA level

Large number of resistance genes

Importance of interpretation

Changing knowledge of mechanisms

Commonly tested resistance genes

*mec*A for MRSA

*van*A, *van*B for VRE

*rpo*B for M. tbc resistance to rifampicine

TEM, SHV et CTX-M for BLSE

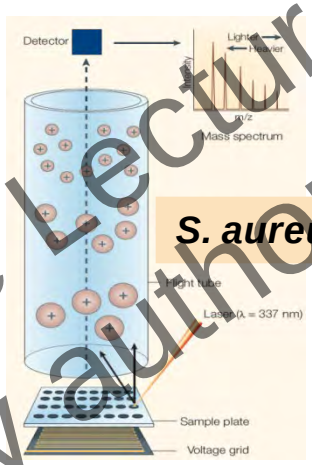
bla_{KPC}, bla_{VIM} *et* bla_{IMP} for *Enterobacteriaceae*

POCT- PCR:
Rapid diagnosis of MRSA bacteremia

POSITIVE blood culture

→ Cocci + →

→



S. aureus →

Molecular POCT MRSA

Specificity 100%

Sensitivity 99%

TAT : **MALDI-TOF → GenXpert**

Median time 97 minutes

Clerc O et al, Clin Infect Dis. 2013.

Added value

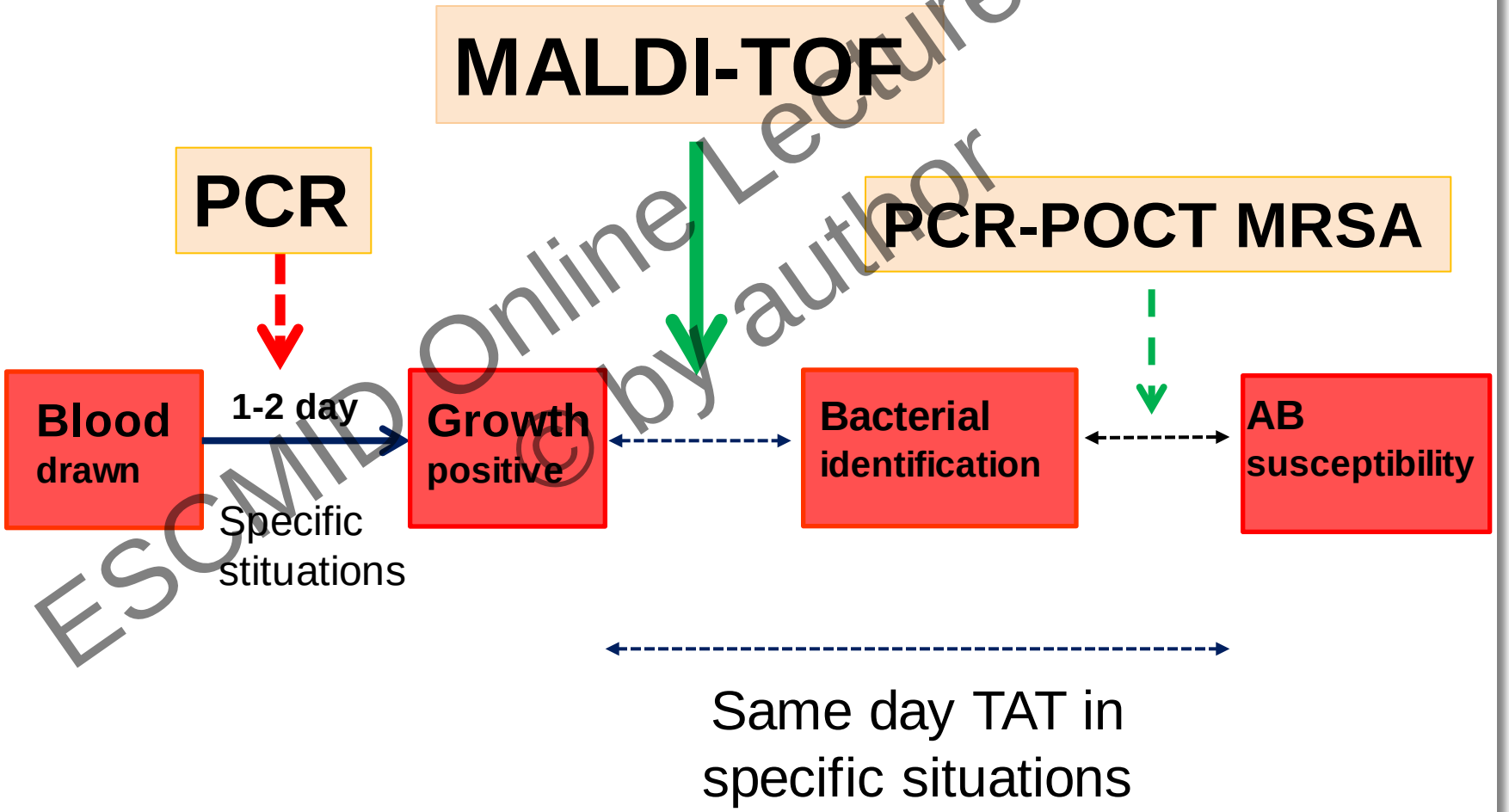
Clinical impact

Less unnecessary coverage of MRSA in cases of MSSA bacteremia

Early diagnosis of unsuspected MRSA bacteremia.

Clerc O et al, Clin Infect Dis. 2013.

Conclusions



Thank you for your attention