Clinical impact of MALDI-TOF on blood cultures

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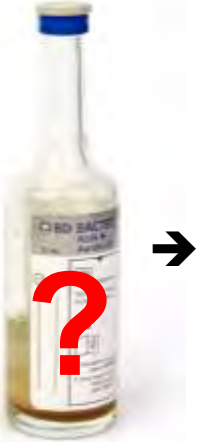
OUTLINE OF THE PRESENTATION

- Burden of sepsis in the clinical field
- Routine processing of blood cultures
- MALDI-TOF on positive blood cultures: the iris-Lab experience
- Discussion and perspectives

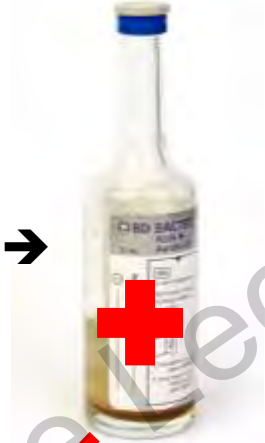
BURDEN OF SEPSIS IN THE CLINICAL FIELD

- Sepsis is a major cause of morbidity and mortality in hospitalized patients
- More than 35% of patients in ICU experience sepsis
  ➔ > 50% of patients who experience septic shock do not survive
- Timely initiation of antimicrobial therapy **is vital** to the treatment of bloodstream infections and sepsis.
- Significantly reducing the time to microbial identification and antimicrobial susceptibility testing (AST) **could decrease** the time to targeted antimicrobial therapy… **leading to a decrease in mortality, shortened hospital stay, and lower hospitalization costs**.

ROUTINE BLOOD CULTURES PROCESSING



1 – 5 days incubation



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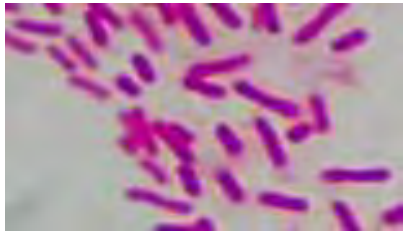


24 h

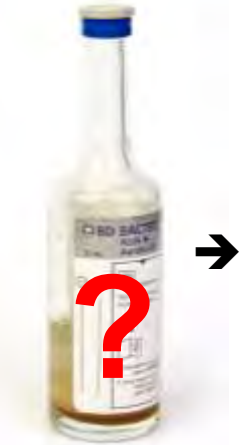
5 min

24 h

ID and AST results

Molecular tools

ROUTINE BLOOD CULTURES PROCESSING



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1 – 5 days incubation

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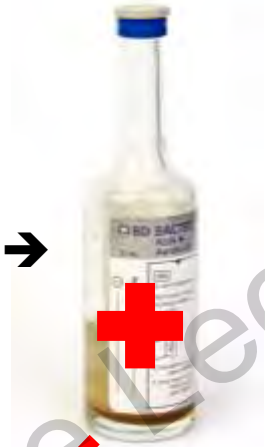

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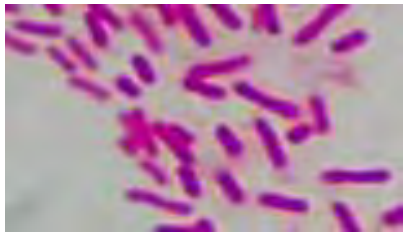


↓ 24 h

↓ 5 min

↓ 24 h

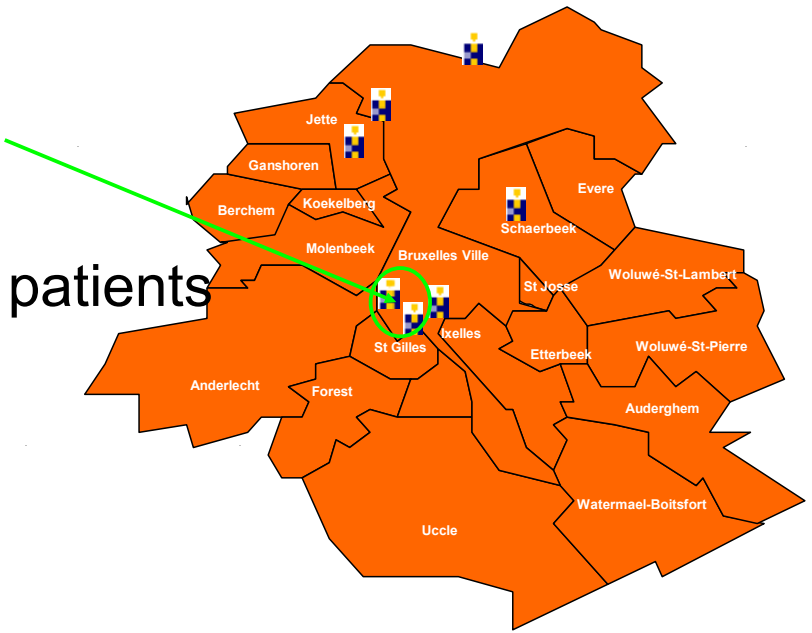
ID and AST results

MALDI-TOF MS

MALDI-TOF ID ON POSITIVE BLOOD CULTURES: THE IRIS-LAB EXPERIENCE

- **Iris-Lab**
  - **Saint-Pierre university hospital:** a 450-bed, regional centre for infectious diseases
  - **Jules Bordet Institute:** a 167-bed, totally dedicated to cancer
  - **Brugmann university hospital:** a 854-bed, organised in three distinct sites
  - **Queen Fabiola Children's University Hospital:** a 167-bed, entirely reserved for pediatrics medicine

- 45,000 blood cultures per year.
- Positivity rate of ~ 4-10% ➔ ± 1,250 patients



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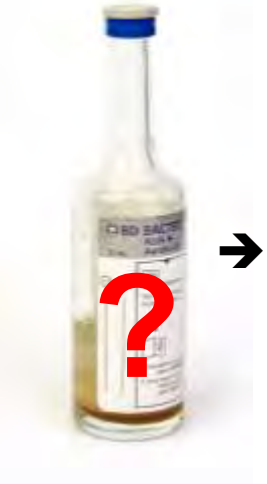

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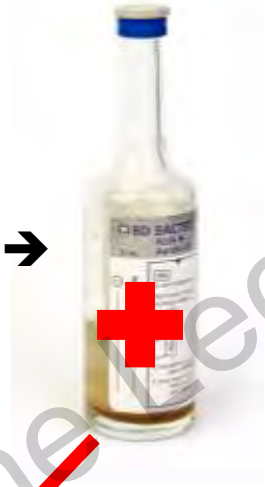

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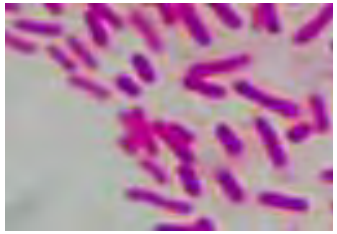


24 h

MALDI-TOF MS

MALDI-TOF MS identification from positive blood cultures: which method to use?

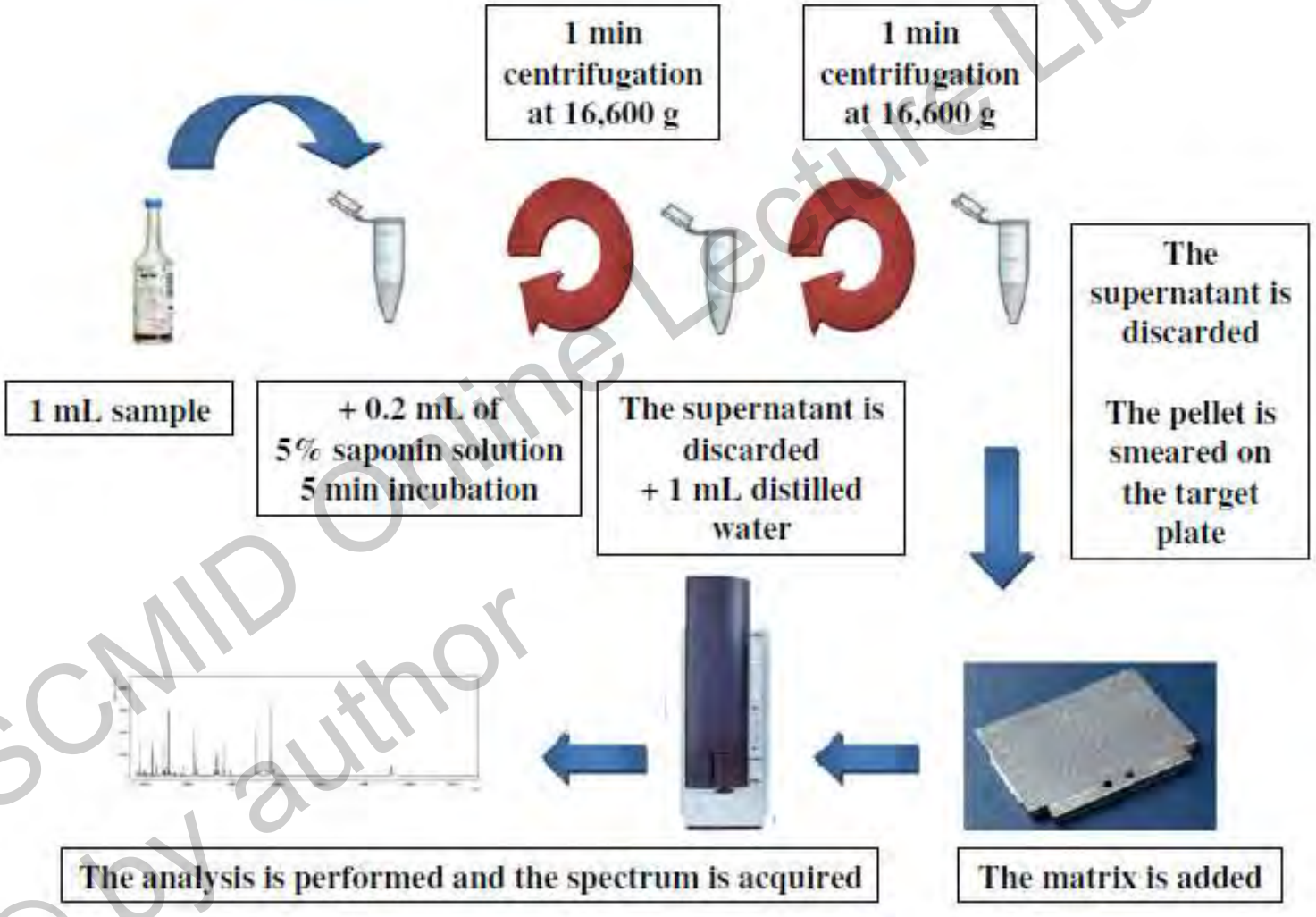
MALDI-TOF MS IDENTIFICATION FROM POSITIVE BLOOD CULTURES: WHICH METHOD TO USE?

- Centrifugations
- Lysis buffers
- Sepsityper kit

- Cut-offs
- Methodology
- Mass spectrometer
- BC bottles used
- Inclusion criteria

La Scola, Plos One 2009
Christner, ECCMID 2010
Prod'hom JCM 2010
Stevenson, JCM 2010
Ferroni, JCM 2010
Ferreira, CMI 2010
Moussaoui, CMI 2010
Schmidt, EJCMID 2011
Kok, PlosOne 2011
Fothergill, JCM 2012
Lagacé-Wiens, JCM 2012
Meex, JMM 2012
Martiny, EJCMID 2012
Wüppenhorst, EJCMID 2012
Yaman, DMID 2012
Saffert, DMID 2012
Wimmer, JCM 2012
López Roa, CMI 2012
Vlek, PlosOne 2012
Buchan, JCM 2012
Spanu , JCM 2012
Loonen, EJCMID 2012
Klein, JMM 2012
Juiz, EJCMID 2012
March-Rosselló, EJCMID 2013
…

IN HOUSE METHOD PROTOCOL



Martiny, Eur J Clin Microbiol Infect Dis 2012

Comparison of an in-house method and the commercial Sepsityper™ kit for bacterial identification directly from positive blood culture broths by matrix-assisted laser desorption-ionisation time-of-flight mass spectrometry

D. Martiny · A. Dediste · O. Vandenberg

- Comparison with Sepsityper method
  - Analytical performance
- Optimisation of cutoff values
  - 1,7 and 2 (recommended)
  - 1,4 and 1,6 (adapted)
- 2 x 5-weeks period

Martiny, Eur J Clin Microbiol Infect Dis 2012

Rate of correct identification N=59		In-House method		Sepsityper method	
		Genus	Species	Genus	Species
Recommended cut-off values	GN (n=22)	90.1%	72.7%	68.2%	54.5%
	GP (n=37)	62.2%	31.4%	73.0%	45.7%
	All	72.9%	47.4%	71.2%	49.1%
Adapted cut-off values	GN (n=22)	90.1%	81.8%	72.7%	59.1%
	GP (n=37)	83.8%	68.6%	81.1%	74.3%
	All	86.4%	73.7%	78.0%	68.4%

- MALDI-TOF MS identification from positive BC is feasible
- It provides faster identification results

Gold standard: routine identification

Study	BC	db	GN (%)	N	GP (%)	N	Yeasts (%)	N	Total (%)	N	PM	N	CO	Procedure
La Scola et al., 2009	B	Biotyper 2881	94	125	37	197	0		59	322	1/2 (18x)	22	SA	~~DC + TFA~~
			87	100	67	140			76	240				DC + FA
Prod'Hom et al., 2010	B	Biotyper 2	79	48	42	74	0		57	122	1/2	4	2	NH_4Cl lysis
Ferroni et al., 2010	BA	Andromas	92.6	189	81.5	103	100	20	86.9	312	0		SA	SL Spiked samples
			92.8	139	89.2	223	100	11	90.9	373	1/2 ID	15		SL + slidex CI
Stevenson et al., 2010	B	Biotyper 2	79,5	78	56.7	134	0		65.1	212	1/2 ID (9x)	10	SA	SS + lysis
Christner et al., 2010	B	Biotyper 2	NR						95-75	277	1/2 (13x)	16	1.3 -2	DC
Moussaoui et al., 2010	B	Biotyper 3290	98.9	187	92.9	295	excluded		95.2	482	36%	21	SA	SS
Schmidt et al., 2011	B	Saramis V3.3.2	97.7	43	61.7	60	0		76.7	103	0		SA	0.5 µl +DHB+FA
	BA		81.4		21.7				46.6					0.5 µl +DHB+FA
	BA		67.4		13.3				35.9					0.5 µl +DHB+FA
Ferreira et al., 2010	B	Biotyper 3290	83.3	61	31.8	239	5.6 (genus)	18	39.6	318	0		2	DC
Kok et al., 2011	B	Biotyper 2	79.7 87.2	187	46.3 68.4	285	NR		59.4 74.8	507	1/2 (20x)	31	2 1.7	ST
Fuglsang-Damgaard et al., 2011	BA	EB	79.3 89.9	169	6.7 42.4	316	0 0 5.3	19	28.6 55.4	583*	21.6% 67.6%	37	2 none	SS + lysis
Yan et al., 2011	B	Biotyper 3476	NA	NA	NA	NA	100	42	100	42	NA	NA	2 1.7	Adapted ST
Spanu et al., 2012	B	Biotyper 2	NA	NA	NA	NA	91.3	346	91.3	346	NA	NA	SA	DC
Juiz et al., 2012	B	Biotyper 3	87.5	24	44.3	61	0		56.5	85	0		2	In-house
			95.8		83.6				87.1					ST
Loonen et al., 2012	BA	Biotyper 3740	78.7	47	21.2	52	0		48.5	99	1/2 species	2	2	DC
			25.5		5.8				15.2		0			MolYsis
			91.5		42.3				65.7		1/2 genus			ST
Wuppenhorst et al., 2012	BA	Biotyper 3	93.8	64	72.3	148	0		77.8	212	excluded		1.4	SL
Lagacé-Wiens et al., 2012	BA	Biotyper 3	95.0	20	56.4	39	50	2	68.9	61	1/2 ID	2	2	ST
Chen et al., 2013	B	Biotyper 4500	88.7 99.1	106	72 96	75	0		81.8 97.8	181	2/2 (n=5) 1/2 (n=16)	21	2 1.7	ST (+in house)
		VITEK MS >20000	89.6 98.1		68 85.3				80.7 92.9		the majority sp.		98% 90%	
Fothergill et al., 2013	BA	Saramis	82.5 86.0	57	76.0 76.7	150	83.3 88.9	18	78.2 79.9	225	46.4% 1 to species	28	≥ 75%	Lysis-filtration
Foster, 2013	B	VITEK MS 2	83.7 84.7	92	90.1 95.7	161	0		88.1 92.1	253	0		SA	DC + lysis

ULB

BC, blood culture; B, Bactec; BA, BacTalert; db, database; EB, enriched Biotyper, GP, Gram-positive bacteria; GN, Gram-negative bacteria; PM, polymicrobial cultures; CO, cutoff; SA, specific algorithm; FA, formic acid; TFA, trifluoroacetic acid; SL, saponin-lysis; SS, serum separator tubes; DC, differential centrifugations; ST, Sepsityper

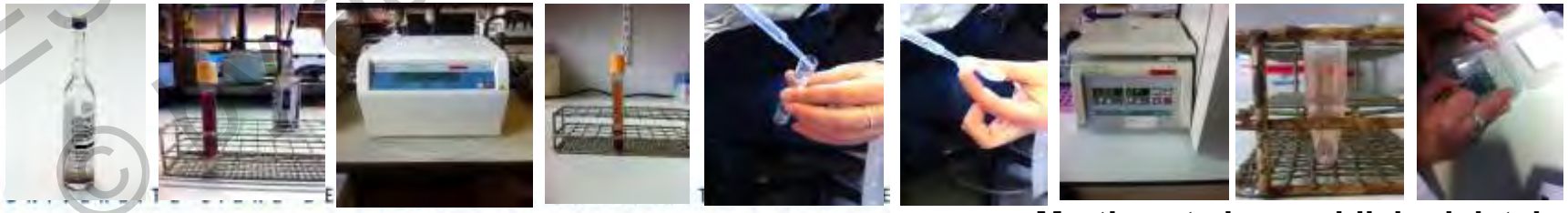
MALDI-TOF MS IDENTIFICATION FROM POSITIVE BLOOD CULTURES: WHICH METHOD TO USE?

PRACTICAL PARAMETERS	Sepsityper method	In-house method	
Cost (€)/analysis	7.45	0.72	
Time to perform analysis (min)	20 – 40	20	
Ease of use	++	+++	

Martiny, Eur J Clin Microbiol Infect Dis 2012

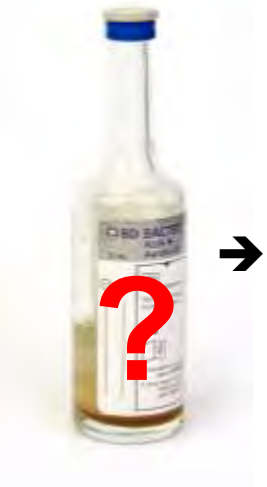
MALDI-TOF MS IDENTIFICATION FROM POSITIVE BLOOD CULTURES: WHICH METHOD TO USE?

PRACTICAL PARAMETERS	Sepsityper method	In-house method	SST
Cost (€)/analysis	7.45	0.72	0,27
Time to perform analysis (min)	20 – 40	20	20
Ease of use	++	+++	++++

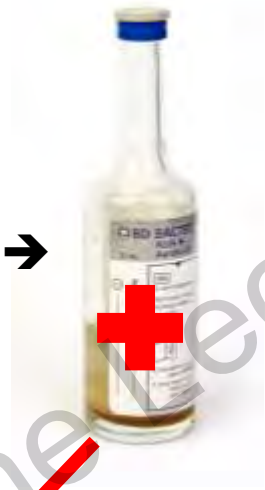


Martiny et al. unpublished data's

ROUTINE BLOOD CULTURES PROCESSING



1 – 5 days incubation



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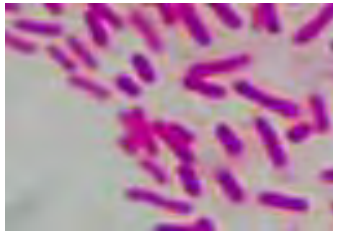


24 h

MALDI-TOF MS

What is the clinical impact of a RMI directly from positive blood cultures by MALDI-TOF MS on patient management

WHAT CLINICAL IMPACT OF MTMS IDENTIFICATION FROM POSITIVE BLOOD CULTURES?

Still debated

- **For:**
  - Rapid identification of more virulent organisms with predictable resistance to antibiotics ➔ faster adoption of the appropriate antibiotic.
  - Help to identify the origin of the sepsis if it remains unknown.

- **Against:**
  - No information concerning the AB susceptibility.
  - Improving TTI has no clinical impact if the use of RMI is delayed by the clinician!!!!

Emonet, Expert Rev Anti Infect Ther, 2011
Croxatto, FEMS Microbiol Rev. 2012
Nori, J. Clin Microbiol. 2013

ORIGINAL ARTICLE 10.1111/1469-0691.12282

Impact of rapid microbial identification directly from positive blood cultures using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry on patient management

D. Martiny[1], F. Debaugnies[1], D. Gateff[2], M. Gérard[3], M. Aoun[4], C. Martin[3], D. Konopnicki[3], A. Loizidou[4], A. Georgala[4], M. Hainaut[5], M. Chantrenne[1], A. Dediste[1], O. Vandenberg[1,6] and S. Van Praet[2]

1) *Department of Microbiology, Saint-Pierre University Hospital and Jules Bordet Institute,* 2) *Hospital Pharmacy, Saint-Pierre University Hospital,* 3) *Division of Infectious Diseases, Saint-Pierre University Hospital,* 4) *Division of Infectious Diseases, Jules Bordet Institute,* 5) *Paediatric Department, Saint-Pierre University Hospital and* 6) *Infectious Diseases Epidemiological Unit, Public Health School, Université Libre de Bruxelles, Brussels, Belgium*

- Multidisciplinary team
- 2 phases
  - Prospective analysis
  - Retrospective analysis

Martiny et al., Clin Microbiol Infect 2013

MALDI-TOF MS IDENTIFICATION FROM POSITIVE BLOOD CULTURES: WHAT CLINICAL IMPACT?

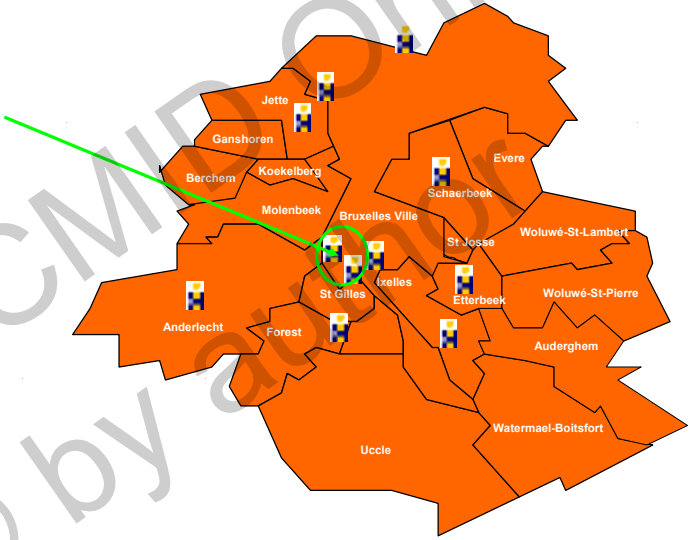
- **AIMS OF THE STUDY**
  - ***Prospective analysis:*** to evaluate the **theoretical impact** of RMI from positive blood cultures on the clinical management of bacteraemic patients in our hospitals.
  - ***Retrospective analysis:*** to evaluate the **real impact** of the rapid identification.

Martiny et al., Clin Microbiol Infect 2013

MATERIALS AND METHODS

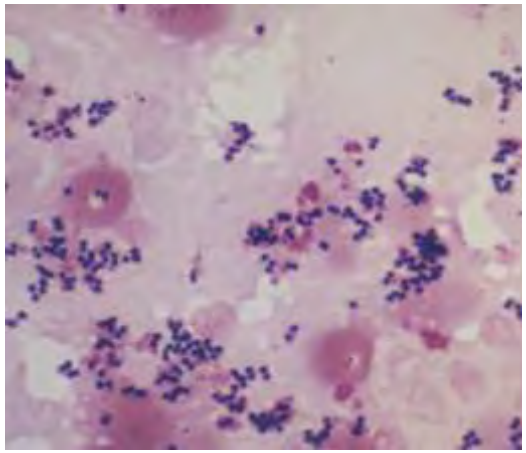
Location

- Brussels
  - Porte de Hal laboratory
    - St-Pierre: public hospital experienced in ID
      dedicated to cancer



Samples and inclusion criteria

- 6 months period (Sep 11- March 12)
- 1st positive BC for each new episode
- Staphylococcal morphology at the Gram
  - Contamination
  - *S. aureus* infection
  - Catheter-related infection



MALDI-TOF MS rapid identification

- Microflex LT
- Bactec
- Db 3995
- Twice daily
- In house protocol

Results classification

- Reliable or unreliable?
  - Lowered cut-off criteria
    - 1.4 genus ; 1.6 species
    - 0.3 log
  - Discrepancies Gram/RMI

- To be communicated to the IDS?
  - Medical Microbiologist experience

PROSPECTIVE ANALYSIS

FIRST STEP

New Positive Blood Culture

New episode or staphylococcal morphology at the Gram staining ?

YES → Inclusion in the study

SECOND STEP

Communication of the Gram staining result to the IDS and data collection:

* Patient data (sexe, age, medical unit, clinical presentation)
* Current antibiotic treatment
* IDS therapeutic decision based on the Gram staining result

LAST STEP

Communication of the RMI result to the IDS and data collection:

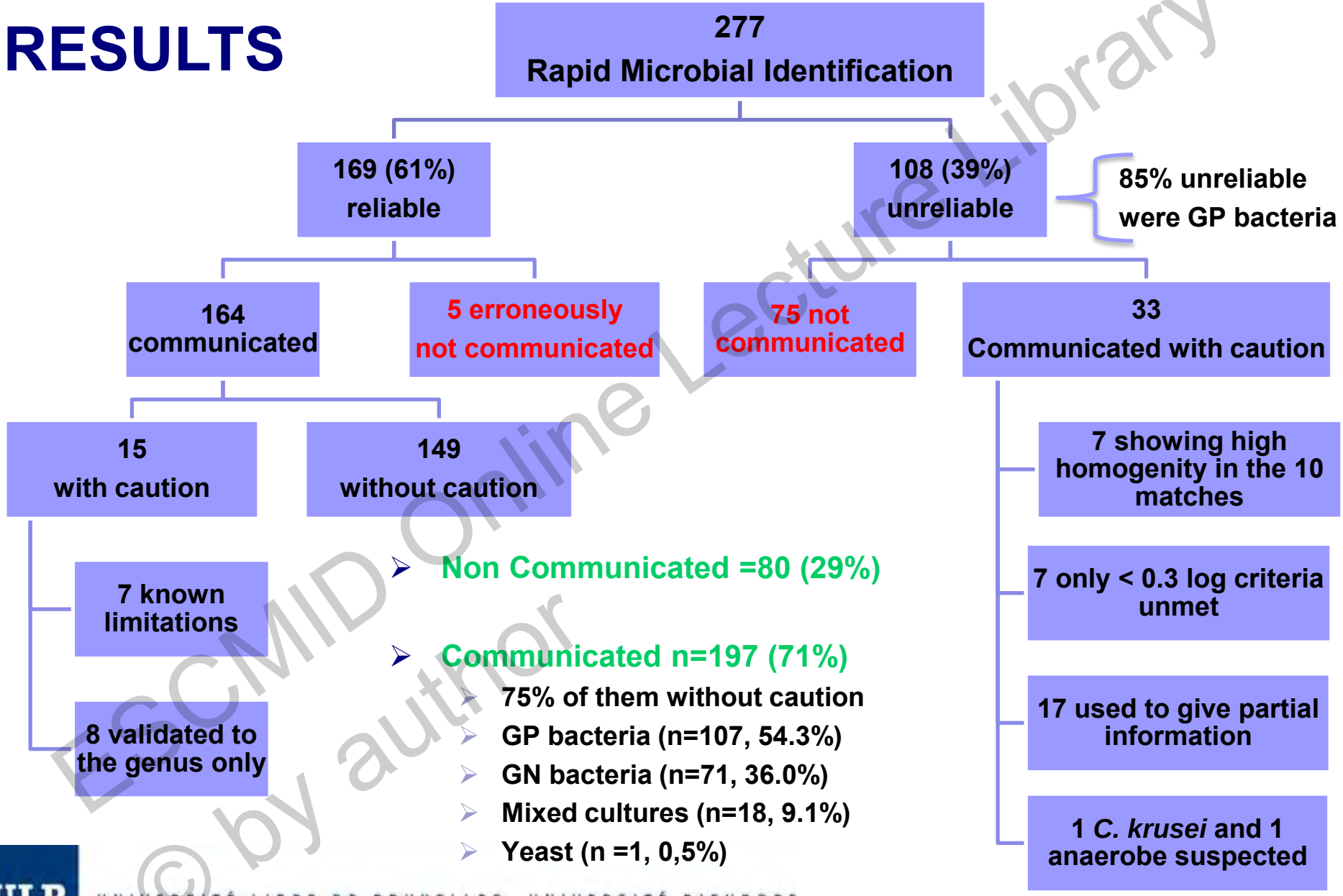
* Does the RMI result modify the IDS's first therapeutic decision ?
* Is the RMI result helpful for the general management of the patient ?

MALDI-TOF MS

RETROSPECTIVE ANALYSIS

- Using billing, medical and nursing files
- To check the compliance to IDS recommandations

RESULTS



- Non Communicated =80 (29%)
- Communicated n=197 (71%)
  - 75% of them without caution
  - GP bacteria (n=107, 54.3%)
  - GN bacteria (n=71, 36.0%)
  - Mixed cultures (n=18, 9.1%)
  - Yeast (n =1, 0,5%)

CLINICAL IMPACT OF MALDI-TOF MS RMI FROM POSITIVE BLOOD CULTURES

ADULT PATIENTS

- **Saint-Pierre (n=90)**
  - **11.1% ΔAB (n=10)**
  - 11.1% contamination (n=10)
  - 4.44% other benefits (n=4)
- **Jules Bordet (n=67)**
  - **14.9% ΔAB (n=10)**
  - 6.0% contamination (n=4)
  - 7.5% other benefits (n=5)

PAEDIATRIC PATIENTS

- **Saint-Pierre (n=40)**
  - 2.5% ΔAB (n=1)
  - **37.5% contamination (n=15)**

Gram stain result led to the modification of patient treatment in 17% of cases.

RMI hastened the use of appropriate antimicrobial treatment in more than 13% of cases in the adult population.

PROSPECTIVE ANALYSIS: AB modification

Saint-Pierre adults (n=10)

S. epidermidis	moxifloxacin	Respiratory distress syndrome	avoid vancomycin	Based on the Gram staining, IDS would have covered MRSA
	AMC	Respiratory distress syndrome cardiac failure	+ vancomycin	Same bacteria previously found in the patient's blood cultures
E. faecalis + *C. freundii*	PTZ	Shock of unknown origin	+ amikacin	Based on the Gram staining, IDS would have chosen vancomycin and metronidazole. According to the RMI amikacin is added to cover *C. freundii*
*K. sedentarius**	meropenem	Septic shock < peritonitis	+ ornidazol	Anaerobic bacteria suspected
S. aureus	moxifloxacin	COPD, ex drug user, fever and cough	+ vancomycin	Exclusion of a contamination -> early treatment administration
	PTZ	Multiple myeloma, raising CRP		
	PTZ + amikacin	Shock, urinary infection, suspected endocarditis		
E. faecium	SXT	Cirrhosis		Because of the identified microorganism
Enterococcus sp.	meropenem	Septic shock < abdominal fistula	potential + vancomycin	Because of the identified microorganism, to cover *E. faecium*
Salmonella sp.	PTZ	Osteonecrosis, fever	shift to levofloxacin	Because of the identified microorganism

The modification of the empirical treatment consisted of the addition of a new drug in 80% (8/10).

Jules Bordet adults (n=10): AB de-escalation

E. coli	levofloxacin metronidazole	Patient receiving high doses of corticosteroids, Chills	stop metronidazole	antibacterial spectrum reduction
S. pyogenes	vancomycin moxifloxacin	Earn, Nose and Throat neoplasy, pulmonary infection	stop vanco	Same bacteria previously found in a drainage fluid
S. agalactiae	PTZ	Systematic blood culture collection for patients receiving high doses of corticosteroids	aminoglycoside addition	Look for pelvic infection symptoms Finally considered as a post-chimiotherapy sepsis
A. genomospecies	ceftriaxon	Prostatic carcinoma, fever		Because of the identified microorganism
E. faecalis	temocillin	Endometrial cancer, pyelonephritis, fever	AMC instead of PTZ	Based on the Gram staining, IDS would have chosen PTZ
E. coli	moxifloxacin IV	Deterioration of the general state, suspicion of pulmonary infection	ciproxin PO shift	Same bacteria previously found in the urine sample
A. genomospecies	AMC	Hepatic disease, fever	meropenem instead of PTZ	Based on the Gram staining, IDS would have chosen PTZ
K. pneumoniae	0	Earn, Nose and Throat neoplasy	avoid amikacin addition	P. aeruginosa was suspected in an infected wound Based on the Gram staining, IDS would have started PTZ and amikacin-> only PTZ
E. faecalis	0	Abdominal surgery	avoid vancomycin addition	Based on the Gram staining, IDS would have chosen PTZ and vancomycin
S. epidermidis	0	Medullary graft, Systematic blood culture collection for patients receiving high doses of corticosteroids		Based on the Gram staining, IDS would have chosen vancomycin

The modification of the empirical treatment consisted of a de-escalation of the AB treatment in 80% of cases → cessation of treatment in 20% (2/10), changes in the treatment in 30% (3/10) and prevention of useless treatments in 30% of cases.

PROSPECTIVE ANALYSIS: other benefits

S. pettenkofferi	Pulmonary infection	investigation for CVC infection	Species related to catheter-related infections Finally considered as a contamination
S. gallolyticus	COPD, dyspnea and fever	blood cultures control + additionnal medical investigations	Suspicion of endocarditis, cardiac echography Patient died
S. pyogenes	Cardiac arrest	help in excluding patient error	Same Gram staining for blood cultures collected from another patient in the unit suggested ID error; RMI showed a different identification and misidentification was excluded. Patient died
S. aureus	Pulmonary infection	additionnal medical investigations	Same bacteria previously found in the patient's blood cultures Search for complicated *S. aureus* infection -> spondylodiscitis
S. agalactiae	Systematic blood culture collection for patients receiving high doses of corticosteroids	additionnal medical investigations	Look for pelvic infection symptoms Finally considered as a post-chemotherapy sepsis
K. pneumoniae	Ovarian carcinoma, chills	catheter removed	Same bacteria previously found in the patient's blood cultures
S. epidermidis	Ovarian carcinoma, fever	catheter removed	Same bacteria previously found in the patient's blood cultures
S. epidermidis	Multiple myeloma, no fever	blood culture control, early treatment instructions	To exclude catheter-related infection. *S. epidermidis* was previously found in blood cultures but no sign of catheter infection was observed. In case of fever, IDS advised to add vancomycin
S. epidermidis	Ovarian carcinoma, catheter placement	early CVC control	To check if there are infection signs at the catheter insertion site This patient had experienced *S. epidermidis* catheter-related infection in the preceeding weeks (see 123).

CLINICAL IMPACT OF MALDI-TOF MS RMI FROM POSITIVE BLOOD CULTURES

RETROSPECTIVE ANALYSIS

- RMI in 1h35 vs 25h43 with the conventional methods ($p<0.001$)
- Good compliance to IDS recommendations
- Modification of the AB treatment within 4h was met in only 50% of cases

CLINICAL IMPACT OF MALDI-TOF MS RMI FROM POSITIVE BLOOD CULTURES

- **Vlek et al.**
  - 4 months study
  - Intervention (n=89) vs control period (n=164)
  - ↓28.8h to get a species ID
  - ↑11.3% appropriate treatment within 24h

- **Clerc et al.**
  - 1 year single-arm observational study
  - Gram-negative bacteria (n=202)
  - Gram-stain → 20.8% modification vs 17% in our study
  - RMI → 35.1% modification, 43.7% broadening

Vlek, PlosOne, 2012
Clerc, CID, 2012

CLINICAL IMPACT OF MALDI-TOF MS RMI FROM POSITIVE BLOOD CULTURES

Some other studies on the clinical impact of RMI

- Reliable ID obtained in 66 -99%

- Time to effective treatment : 30h ➔ 20h
- Time to optimal treatment: 90h ➔ 47h

- 58h earlier ID if introduction of BC vials and MALDI 24/7

Lagacé-Wiens , JCM 2012, Klein J Med Microbiol. 2012

CLINICAL IMPACT OF MALDI-TOF MS RMI FROM POSITIVE BLOOD CULTURES

- RMI better guides antimicrobial management by leading to a modification of AB and/or an early confirmation of contamination.

- Implementation of antimicrobial stewardship programs and an adaptation of the workflow are needed.

- However, with the emergence of multiresistant bacteria, valid prediction of susceptibility based on the species alone is no longer possible ➔ early prediction of AB susceptibility is of great interest.

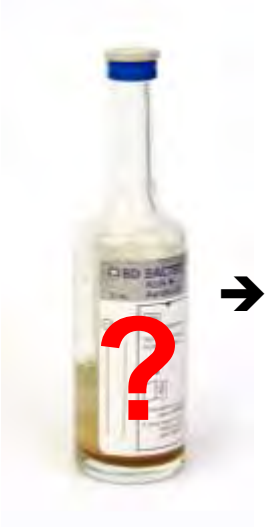
RAPID DETECTION OF AB RESISTANCE FROM POSITIVE BLOOD CULTURES BY MALDI-TOF MS

- MTMS allows the detection of a limited number of specific determinants in the resistance mechanisms of bacteria.
- But does not provide a complete picture of antibiotic resistance.

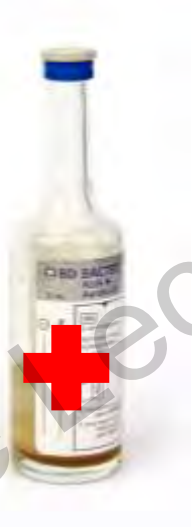
- Different approaches
  - detection of peaks of metabolites
  - mass spectral changes
  - drug containing isotope-labelled growth medium
- Gram + versus Gram –
- % of AB Resistance

Marinach C, Proteomics 2009
Burkhardt, JCM 2011
Ledeboer , JCM 2011
Nagy, J Med Microbiol. 2011
Sparbier, JCM 2011
Wybo, JCM 2011
Hrabak, JCM 2012
De Carolis, JCM 2012
Hrabák, CMR 2013
Sparbier K, JCM 2013
Alvarez-Buylla, JCM 2013
Vella A, JCM 2013
Demirev, J Am S Mass Spect 2013
Carvalhaes, JAC 2014
Jung, JCM 2014
L. Dortet, CMI 2014
Lasch, J Microbiol Methods 2014
…….

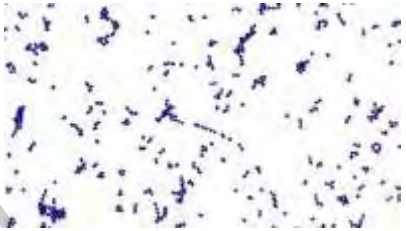
FUTURE PERSPECTIVES

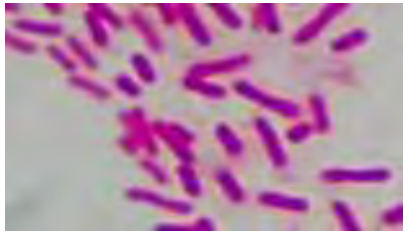


1 – 5 days incubation



15 min





24 h

5 min

24 h

ID and AST results

MALDI-TOF MS

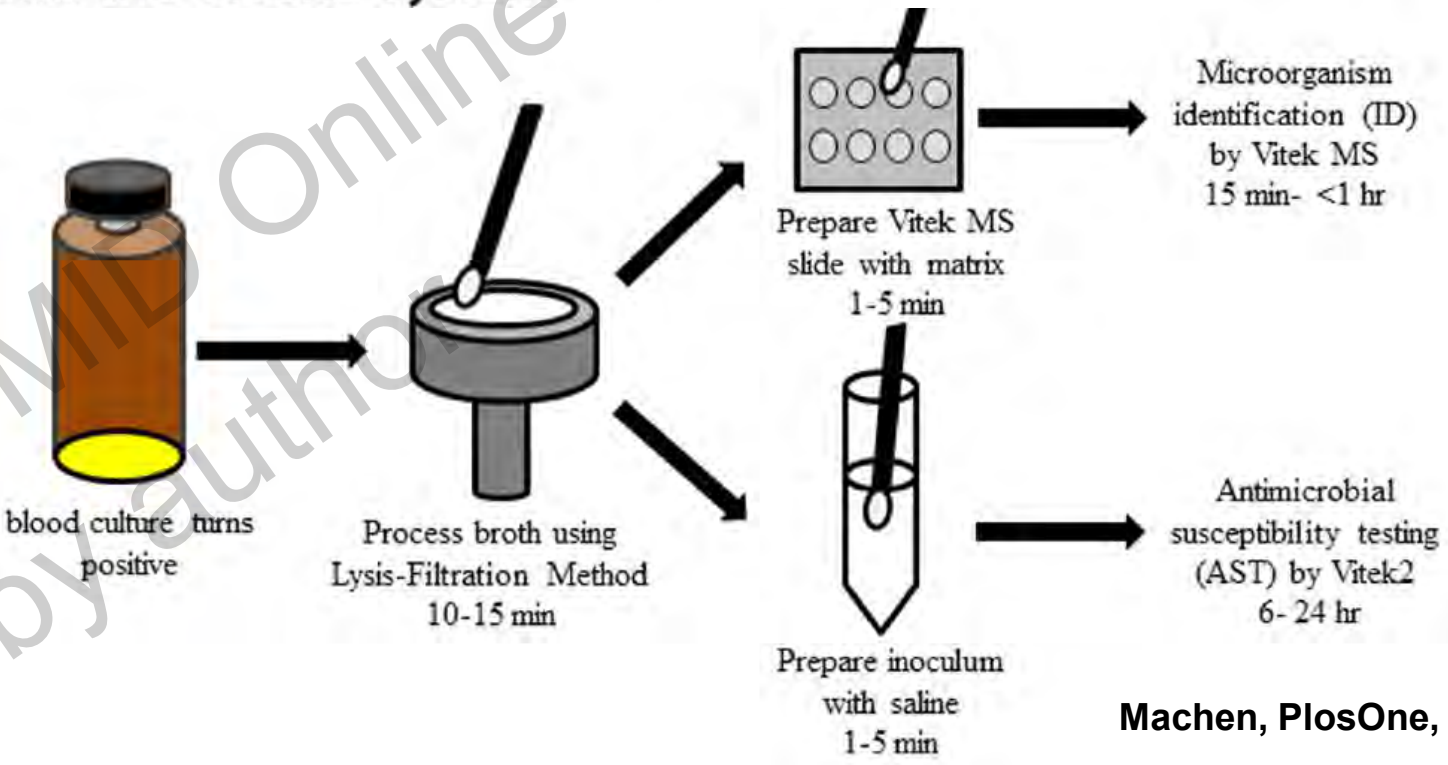
Gram positive	Gram negative
vanA, vanB ?	Non fermentative
mecA ?	ESBL
	Carbapenemase

FUTURE PERSPECTIVES

OPEN ACCESS Freely available online

PLOS | ONE

Same Day Identification and Full Panel Antimicrobial Susceptibility Testing of Bacteria from Positive Blood Culture Bottles Made Possible by a Combined Lysis-Filtration Method with MALDI-TOF VITEK Mass Spectrometry and the VITEK2 System



Machen, PlosOne, 2014

TAKE HOME MESSAGES

- Observations are only valid for similar organisations:
  - Different impact if the laboratory is open 24/7/365 and has a skilled staff 24/7/365.
  - Different impact if IDS is available for instant discussion regarding AB θ
  - Higher impact in medical institutions without IDS
  - Different impact according to the microbial ecology
- Difficulty in identifying accurate indicators
- As the time to ID or AST result is not the same as the time appropriate therapy ➔ further studies are needed to definitely assess the clinical impact of Maldi-tof on blood cultures.

TAKE HOME MESSAGES

PATIENT

ACKNOWLEDGEMENTS

- Iris-Lab and the Brussels Public Hospital Network
  - I would like to express my thanks to all the individuals and teams who collaborated on our different MALDI-TOF MS studies.
  - My thanks go especially to Delphine Martiny and her research team who implemented most of the techniques described in this talk.
- Additional request of information's may be sent to the following e-mail address: microbiologie@iris-lab.be