Direct identification of bacteria from positive blood cultures by MALDI-TOF MS : MALDI Sepsityper kit (Bruker) VS home made saponin method for bacterial extraction

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OBJECTIVES

The aim of this study was to evaluate the direct identification of microorganisms from BacT/ALERT® (bioMérieux) positive blood cultures using the Microflex MALDI-TOF MS (Bruker). We compared two different bacterial extraction methods : the MALDI Sepsityper kit (Bruker) method and a home made saponin lysis method.

MATERIALS

Clinical samples

Blood cultures from inpatients (CHULg) in March/April 2011.
BacT/ALERT® 3D system (bioMérieux) with :

- FA bottles with charcoal
- SN bottles without charcoal

if + → - subculture and conventional ID (Maldi ± biochemichal test) = Reference method
- direct ID + subculture and conventional ID

⇨ **113 Positive BacT/ALERT SN bottles from 77 patients submitted to direct ID of bacteria by MALDI-TOF MS (Bruker).**

METHODS : Bacterial extraction systems

MALDI Sepsityper kit (Bruker)

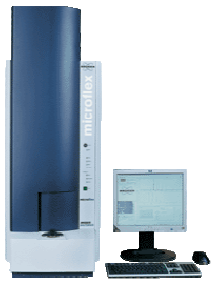
- Harvest 1 ml blood culture broth in a test tube
- Add 200 µl Lysis buffer and centrifuge
- Add 1 ml Washing buffer and centrifuge
- Suspend pellet in water

Saponin method (« home made »)

- Harvest 500 µl blood culture broth in a test tube
- Add 400 µl of 5% saponin and centrifuge
- Add 1 ml water and centrifuge
- Suspend pellet in water

Bacterial pellet after red blood cells lysis

- Standard Bruker extraction (Ethanol/formic acid) for MALDI bacterial profiling
- Spotting of 2 µl extract onto MALDI target, overlay with HCCA matrix
- MALDI-TOF measurement



Microflex MALDI-TOF MS (Bruker)
MALDI BioTyper DB Update V3.1.2.0

« CHU » species acceptation criteria: ID accepted if the first three results having the best matches are identical, whatever the scores were.

RESULTS

107 monomicrobial blood cultures:

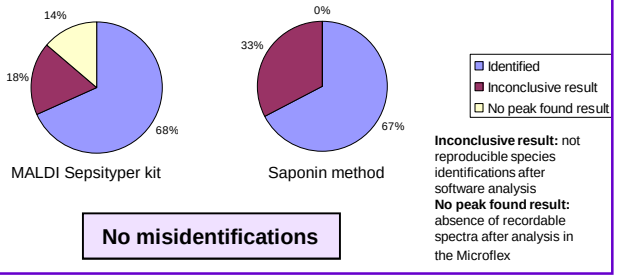
Identified to species by the reference method (number)	MALDI Sepsityper Kit Identified to species (%)	Saponin method Identified to species (%)	
Enterobacteriaceae (36)	32 (89%)	36 (100%)	
Non fermenting bacilli (1)	1 (100%)	0 (0%)	
Gram negative fastidious bacteria (2)	1 (50%)	0 (0%)	
Gram negative anaerobes (1)	0 (0%)	1 (100%)	
Gram negative bacteria (40)	**34 (85%)**	**37 (93%)**	**p ≥ 0.05**
Staphylococcus aureus (15)	12 (80%)	12 (80%)	
Coagulase negative Staphylococci (39)	23 (59%)	15 (38%)	
Beta-hemolytic streptococci (3)	1 (33%)	2 (61%)	
Streptococcus pneumoniae (5)	1 (20%)	4 (80%)	
Enterococci (2)	1 (50%)	2 (100%)	
Gram positive bacilli (2)	0 (0%)	0 (0%)	
Gram positive anaerobes (1)	1 (100%)	0 (0%)	
Gram positive bacteria (67)	**39 (58%)**	**35 (52%)**	**p ≥ 0.05**
TOTAL (107)	**73 (68%)**	**72 (67%)**	**p ≥ 0.05**

Matching scores: significatively higher with MALDI Sespityper kit for Gram negative bacteria but comparable with the 2 methods for Gram positive bacteria.

6 polymicrobial blood cultures:

Reference method Identified species from colonies	MALDI Sepsityper Kit/Saponin method Identified species from broth
Bacillus sp. / S. hominis	*S. hominis*
K. oxytoca / E. coli / Enterobacter asburiae	*E. coli*
K. oxytoca / E. coli / Enterobacter asburiae	*E. coli*
K. oxytoca / E. coli / Enterobacter asburiae	*E. coli*
K. pneumoniae / E. coli	*K. pneumoniae*
K. pneumoniae / E. coli	*K. pneumoniae*

Summary of the results for the 107 monomicrobial cultures



Inconclusive result: not reproducible species identifications after software analysis
No peak found result: absence of recordable spectra after analysis in the Microflex

No misidentifications

CONCLUSION

Direct identification with the home made saponin method based on our defined algorithm gave equivalent results than with the MALDI Sepsityper kit. Furthermore the saponin method is much less expensive. Globally, direct ID reduced drastically the turn around time for the identification of pathogens present in positive blood cultures.

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