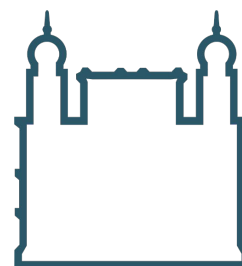


A multi-peptide mass spectrometry diagnostic approach applied to SEPSIS pathogen identification

- Michel Batista
- MS Facility
- Fiocruz Paraná
- Skyline User Group Meeting at ASMS
- 2024



Ministério da Saúde

FIOCRUZ - PARANÁ
Instituto Carlos Chagas

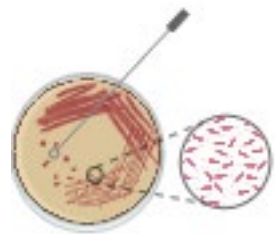
Background

- Sepsis is a bloodstream infection
- Needs fast and accurate diagnosis

47 - 50 million
cases
per year¹

At least
11 million
deaths per year²

1 in 5 deaths
worldwide is
associated
with **sepsis**³



Culture



MALDI-TOF



PCR



PCR/ESI-MS



LC-MS/MS

Cost

Sensitivity

Time to get the results

Complementing sepsis
diagnosis

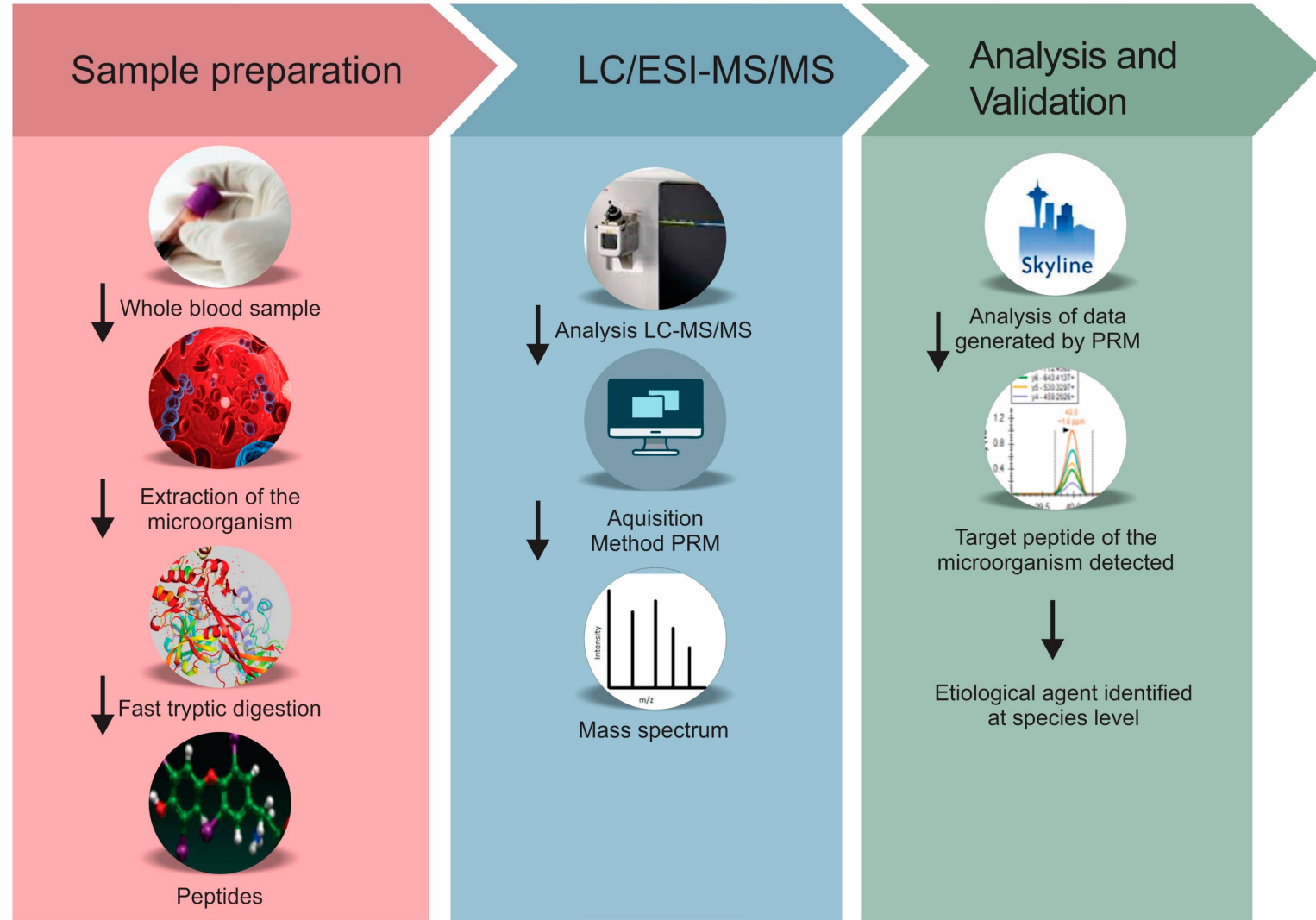
Identification

Resistance profiling


Main goal

- Establishing a protocol for identification of sepsis pathogens directly from blood through target proteomics

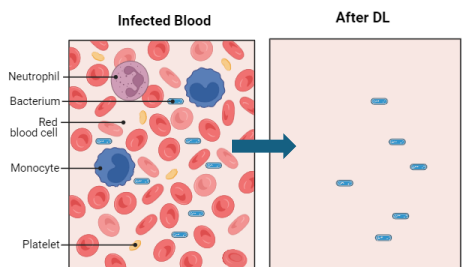
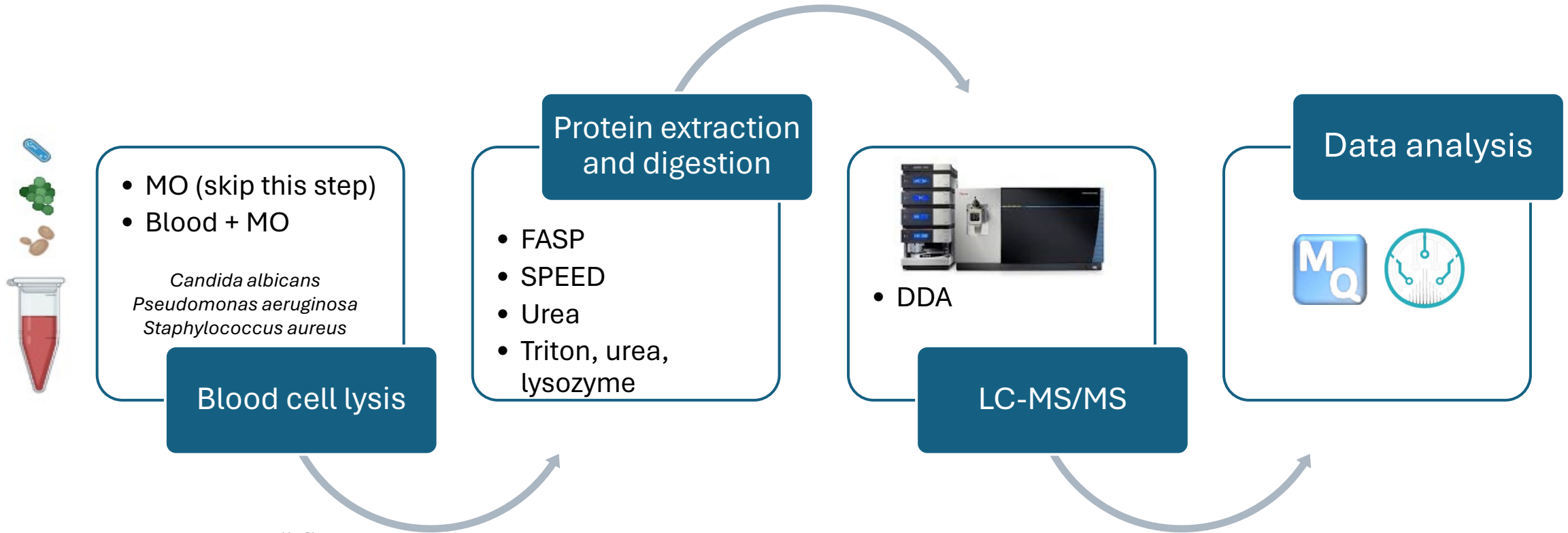
Candida albicans
Pseudomonas aeruginosa
Staphylococcus aureus



Specific goals

- 1) Defining protein extraction and digestion protocol 
- 2) Selecting microbial peptides for target analysis
- 3) Quantifying microorganism load in blood through target LC-MS/MS

Defining protein extraction and digestion protocol



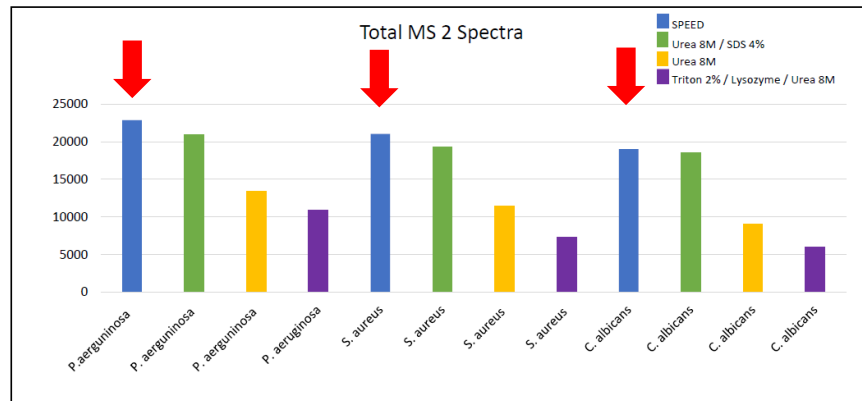
- 1) 500 mM sodium carbonate, 1% triton X-100 pH 10.5
- 2) 1 M Tris-HCl

Differential blood cell lysis - DBCL

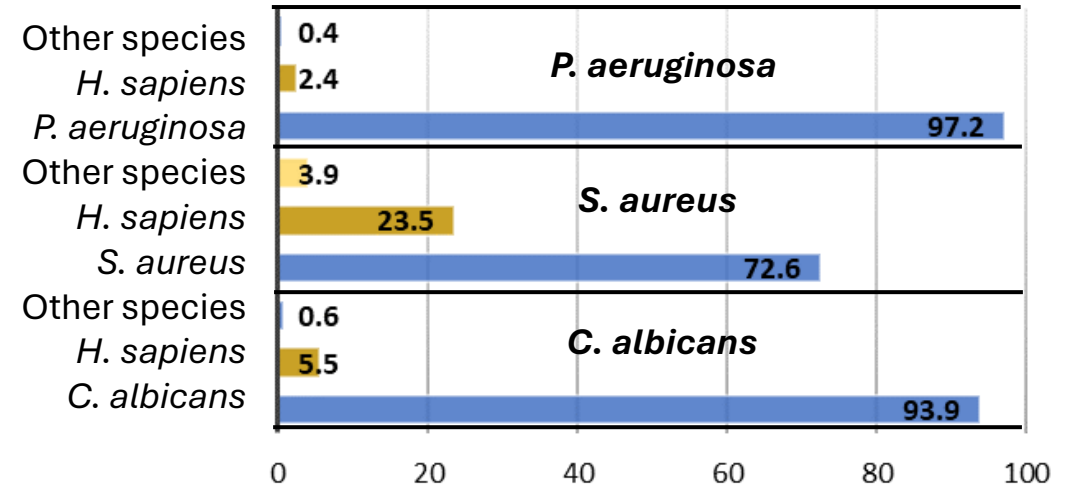
DDA against a database with 43 species

Defining protein extraction and digestion protocol

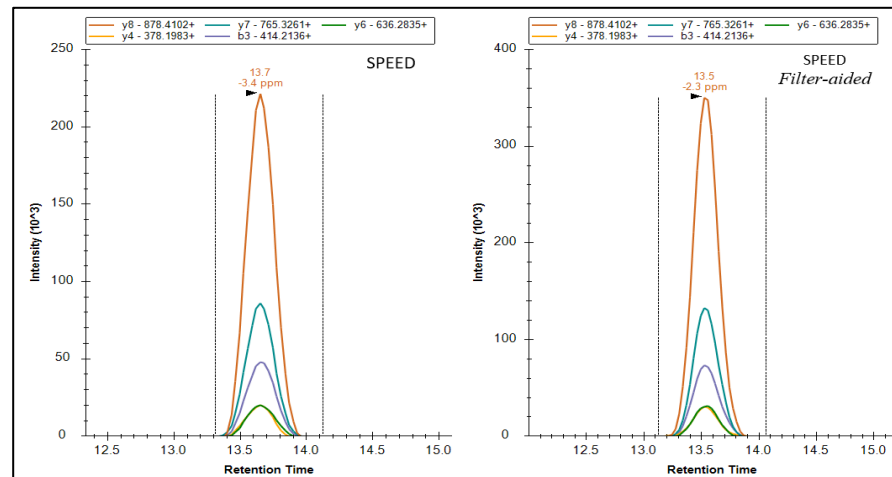
A Blood-free microorganism MSMS number



B Peptides identified in MO+blood after DBCL (%)



C 16 h digestion 15 min digestion



C. albicans – patient sample

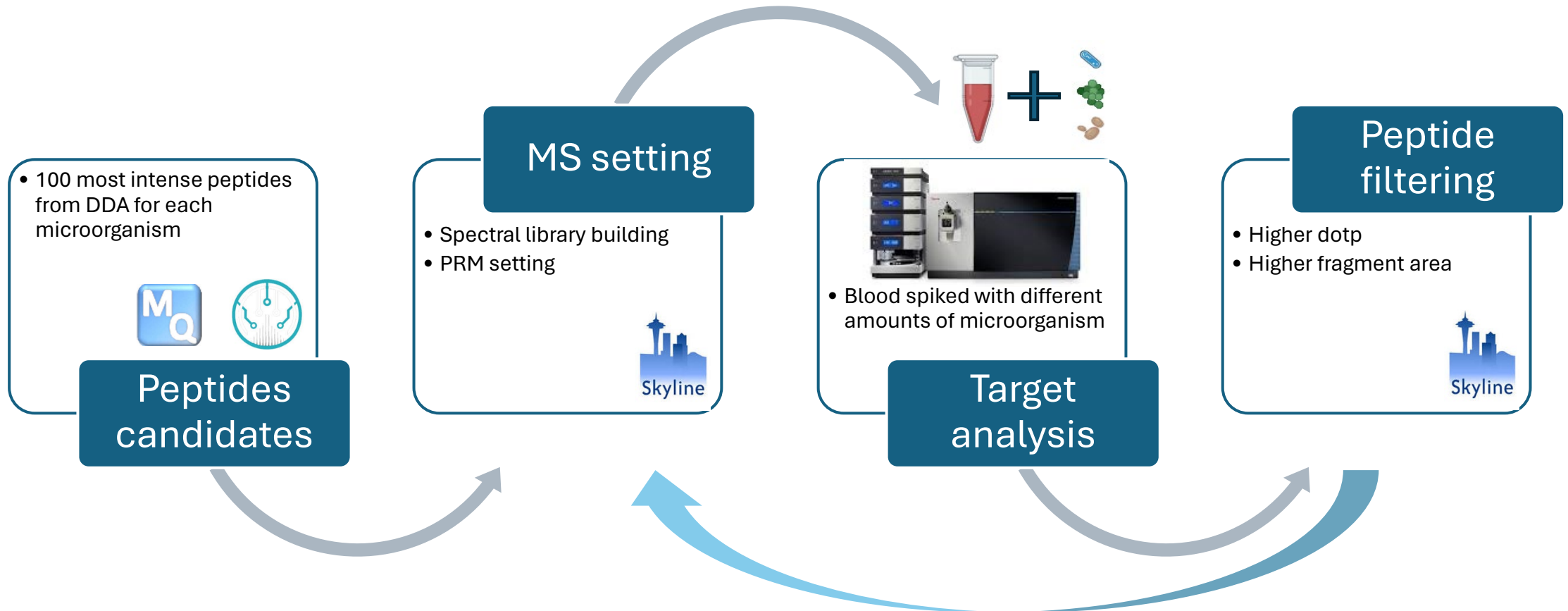
DBCL+SPEED *filter aided*

- ✓ Efficient
- ✓ Easy
- ✓ Low cost
- ✓ Deal with abundant blood proteins
- ✓ Fast

Specific goals

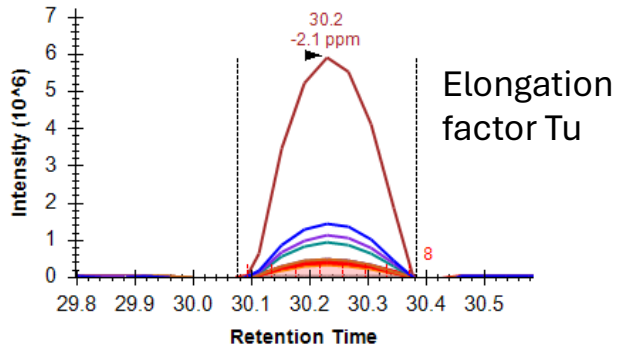
- 1) Defining protein extraction and digestion protocol ✓
- 2) Selecting microbial peptides for target analysis ←
- 3) Quantifying microorganism load in blood through target LC-MS/MS

Selection of peptides for target analysis

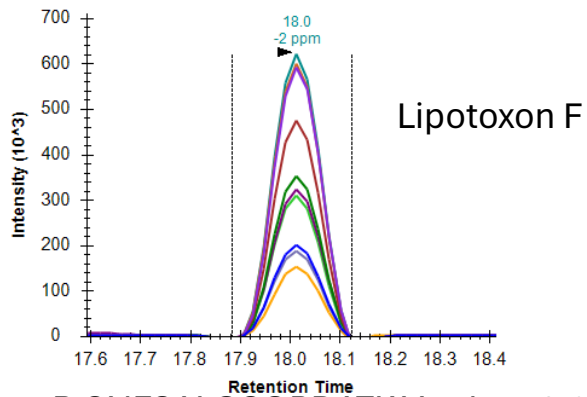


P. aeruginosa

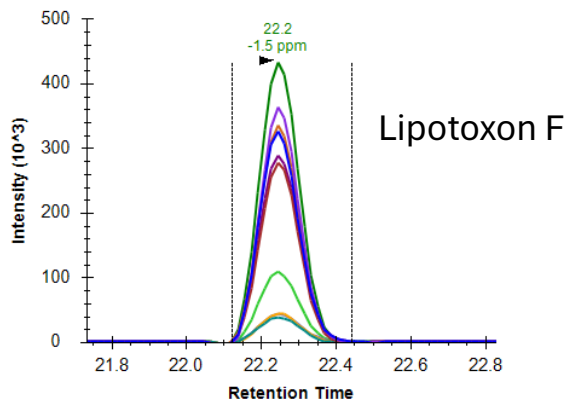
R.AGENVGILLR.G - dotp 0.98



R.NAEAQLQNASAQR.A - dotp 0.97

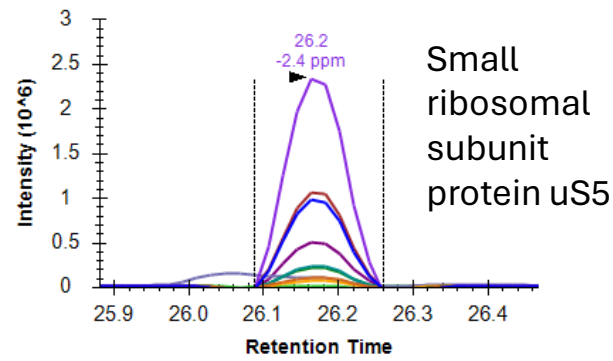


R.SNFSALQSQPDATK.V - dotp 0.85

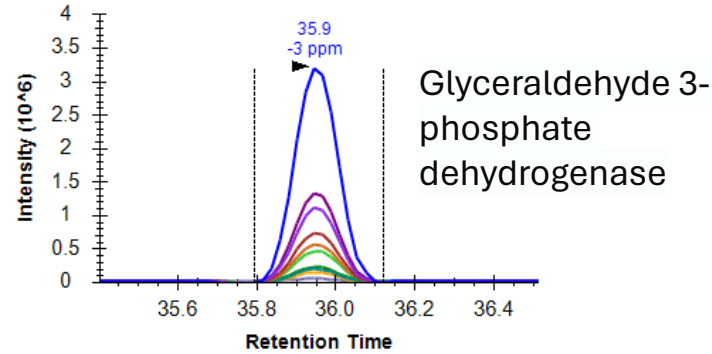


S. aureus

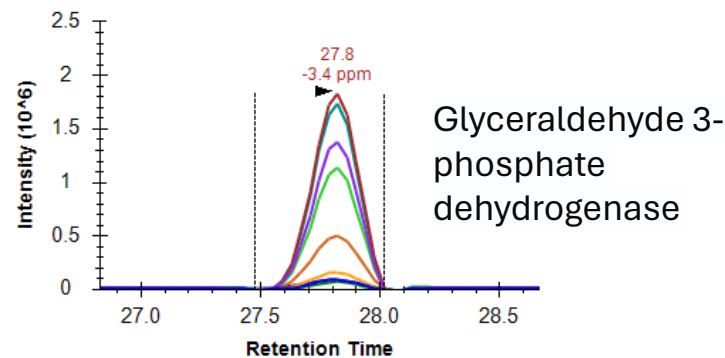
R.ATIDGLQNLK.N - dotp 0.98



R.TLAYLAELSK.- dotp 0.94

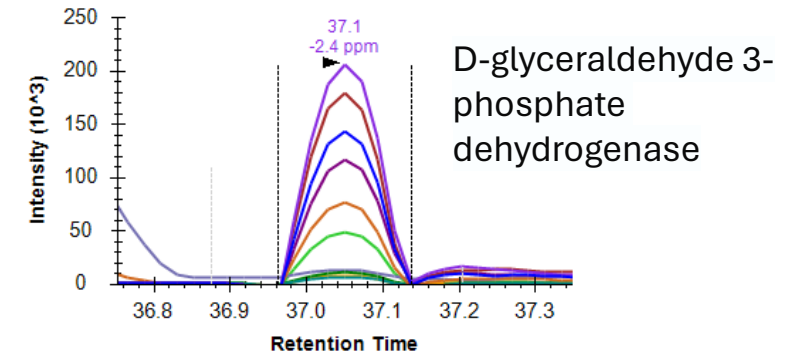


K.VLISAPATGDLK.T dotp 0.96

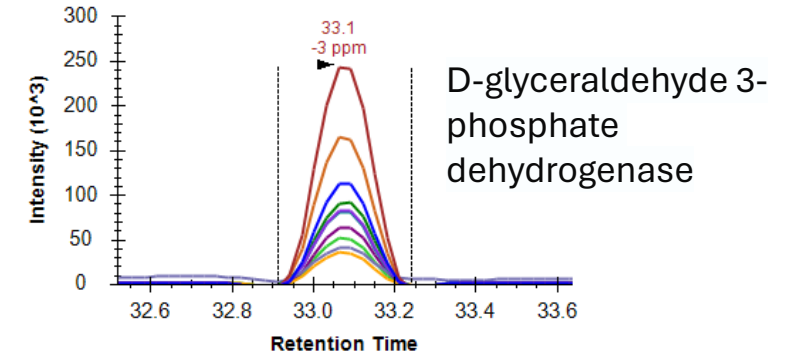


C. albicans

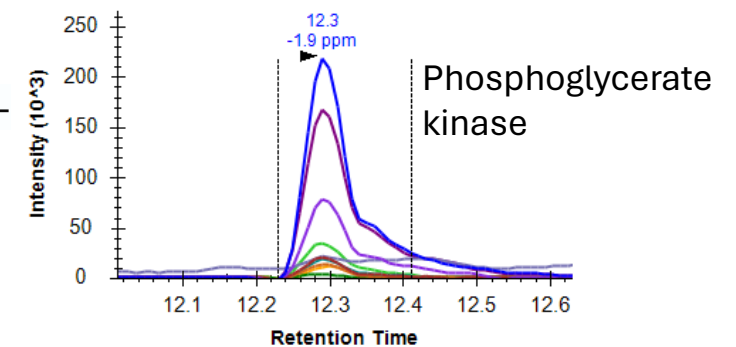
K.AGILLSPTFVK.L - dotp 0.95



K.AASYEEIAQAIK.K - dotp 0.96



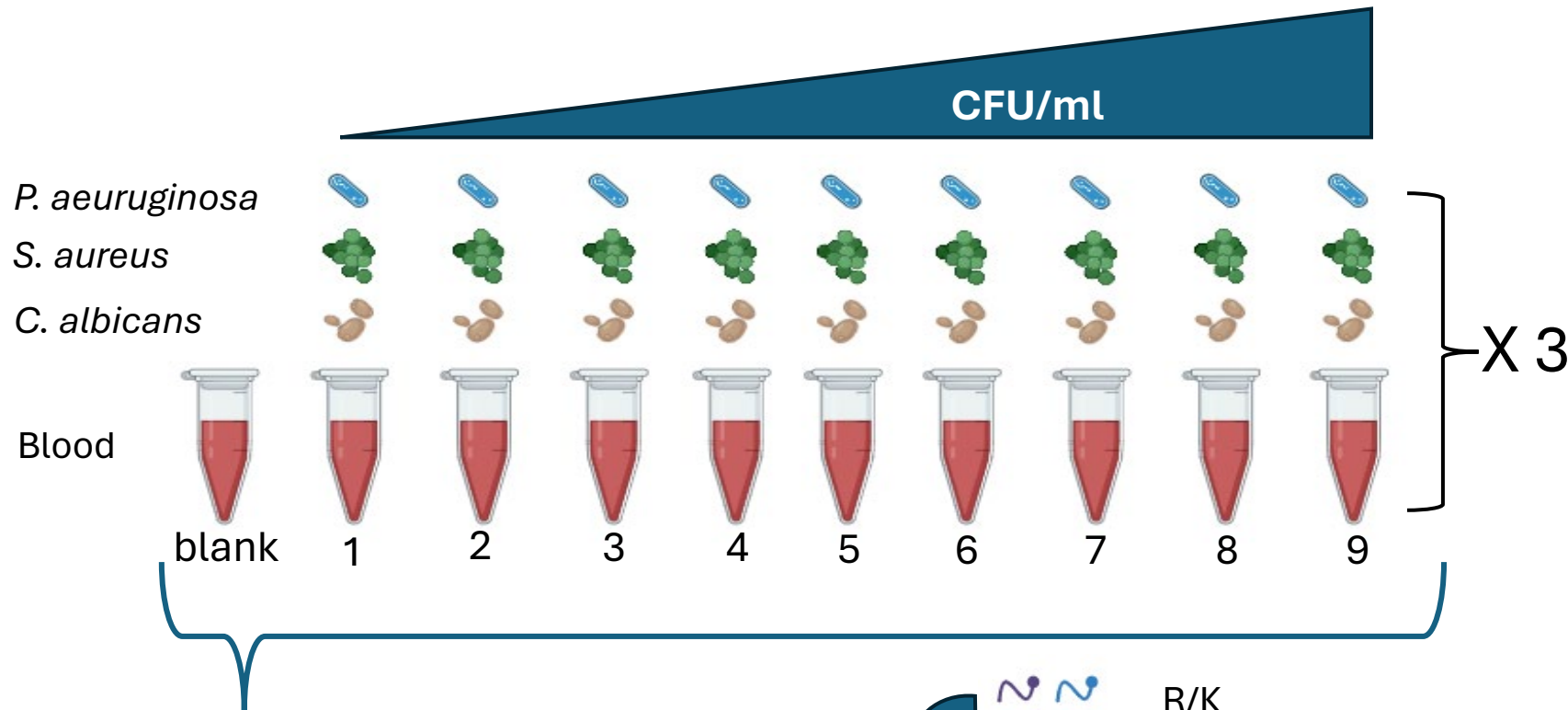
R.YHIEEGSSK.D dotp - 0.98



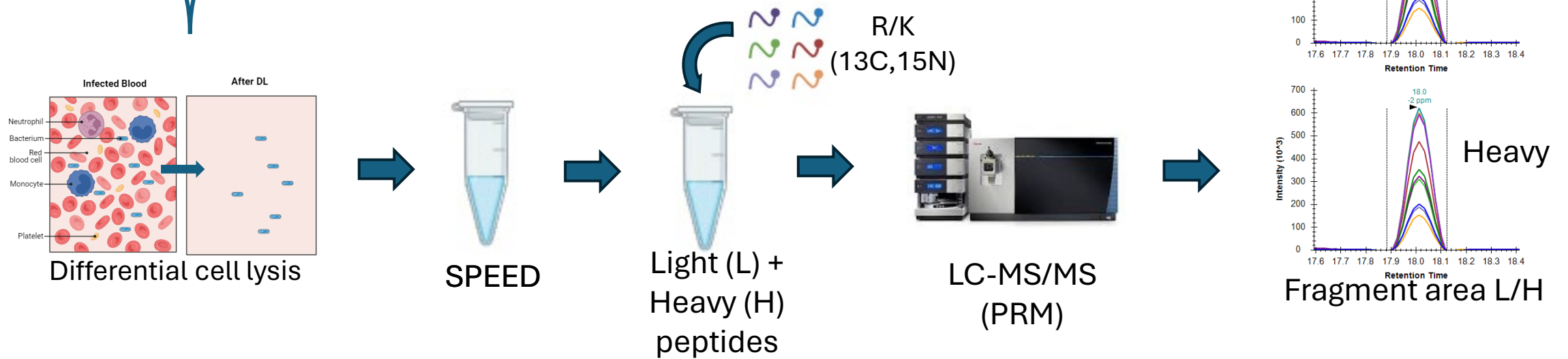
Specific goals

- 1) Defining protein extraction and digestion protocol ✓
- 2) Selecting microbial peptides for target analysis ✓
- 3) Quantifying microorganism load in blood through target LC-MS/MS ←

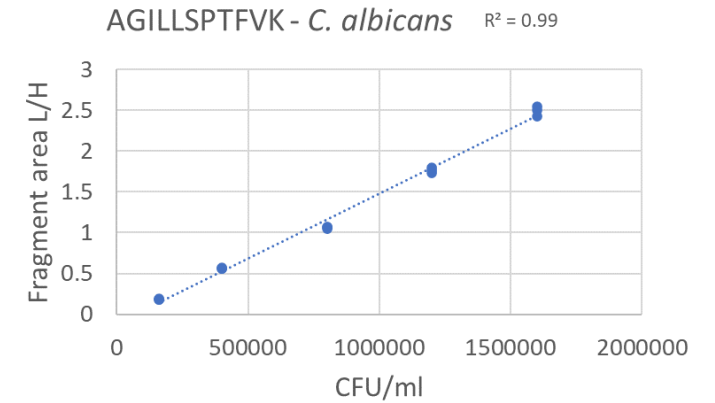
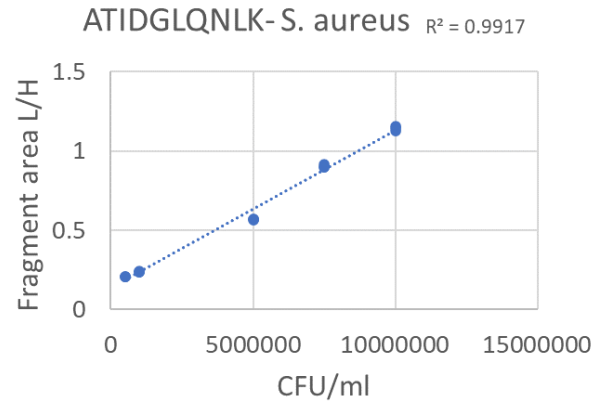
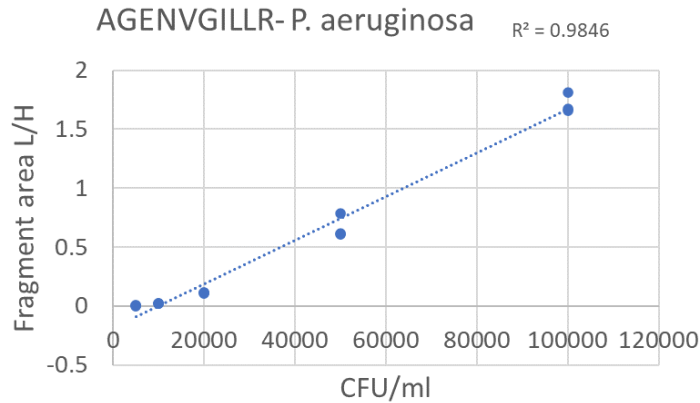
Quantifying microorganisms in blood



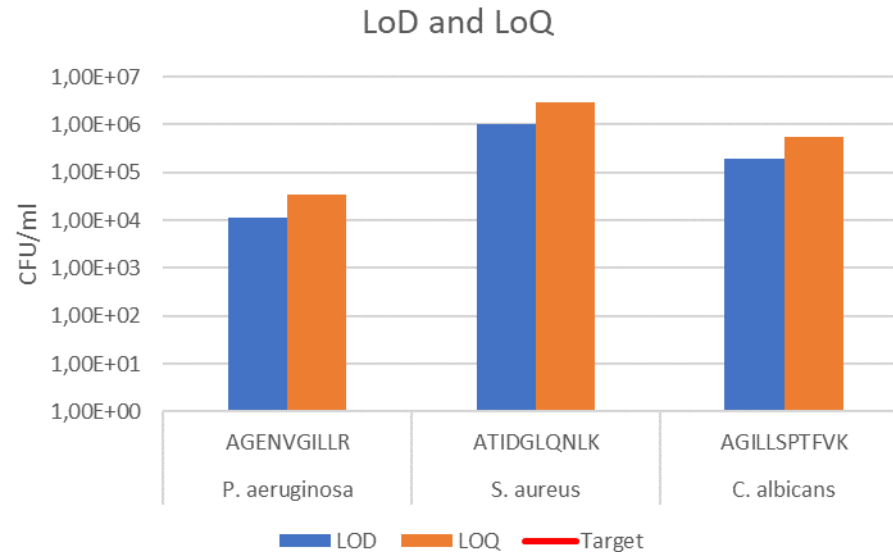
Curve points (CFU/mL)	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>C. albicans</i>
Blank	0,00E+00	0,00E+00	0,00E+00
1	5,00E+02	2,50E+04	4,00E+03
2	1,00E+03	5,00E+04	8,00E+03
3	5,00E+03	2,50E+05	4,00E+04
4	1,00E+04	5,00E+05	8,00E+04
5	2,00E+04	1,00E+06	1,60E+05
6	5,00E+04	2,50E+06	4,00E+05
7	1,00E+05	5,00E+06	8,00E+05
8	1,50E+05	7,50E+06	1,20E+06
9	2,00E+05	1,00E+07	1,60E+06



Quantifying microorganisms in blood



LoQ = 10 x (standard error / angular coefficient)
 LoD = 3.3 x (standard error / angular coefficient)



LoD (CFU/ml)
P. aeruginosa - 1.14x10⁴
S. aureus - 9.98x10⁵
C. albicans - 1.89x10⁵

Quantifying microorganisms in blood

- Patient samples analysis

Patient	Positive blood culture	LC/ESI-MS/MS	Number of identified target peptides
1	<i>S. aureus</i>	<i>Undetectable</i>	0
2	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	03
3	<i>S. aureus</i>	<i>S. aureus</i>	03
4	<i>S. epidermidis</i>	<i>undetectable</i>	0
5	<i>C. albicans</i>	<i>C. albicans</i>	03
6	<i>S. aureus</i>	<i>S. aureus</i>	03
7	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	03
8	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	01*

*Identified only the peptide AGENVGILLR

85.7%
sensitivity

Specific goals

- 1) Defining protein extraction and digestion protocol ✓
- 2) Selecting microbial peptides for target analysis ✓
- 3) Quantifying microorganism load in blood through target LC-MS/MS ✓

Concluding remarks

- ✓ Fast sample prep protocol for microbial identification directly from blood
- ✓ Workflow for microbial peptide selection for target LC-MS/MS
- ✓ Achievement of 85.7% sensitivity in patient samples
- ✓ Need for decreasing LoD and LoQ (sample prep, pre-cultivation, MRM)
- ✓ Detecting proteins related to microbial resistance to antibiotics
- ✓ More patient samples, and species...

Acknowledgment



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