

A novel flow cytometry assay based on bacteriophage-derived proteins for *Staphylococcus* detection in blood

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Supplementary Material S1 - Assessment of the binding affinity of GFP-AMI_SH3 protein after decoration of *S. pneumoniae* R6st and *E. faecium* LMV-0-42 cells

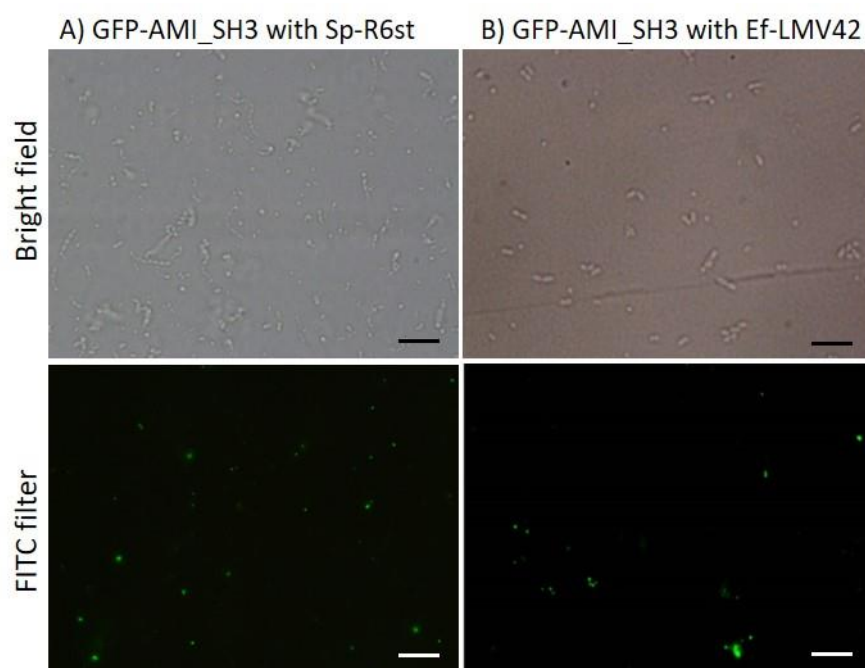


Figure S1 - Fluorescence microscopy images of *S. pneumoniae* R6st (A) and *E. faecium* LMV-0-42 (B) after incubation with GFP-AMI_SH3 protein. Observations were made in bright field and under FITC filter with the same exposure time to detect the presence/absence of fluorescing cells. Scale bar represents 10 μ m

Supplementary material S2 - Graphical representation of blood assays performed for *Enterococcus* species.

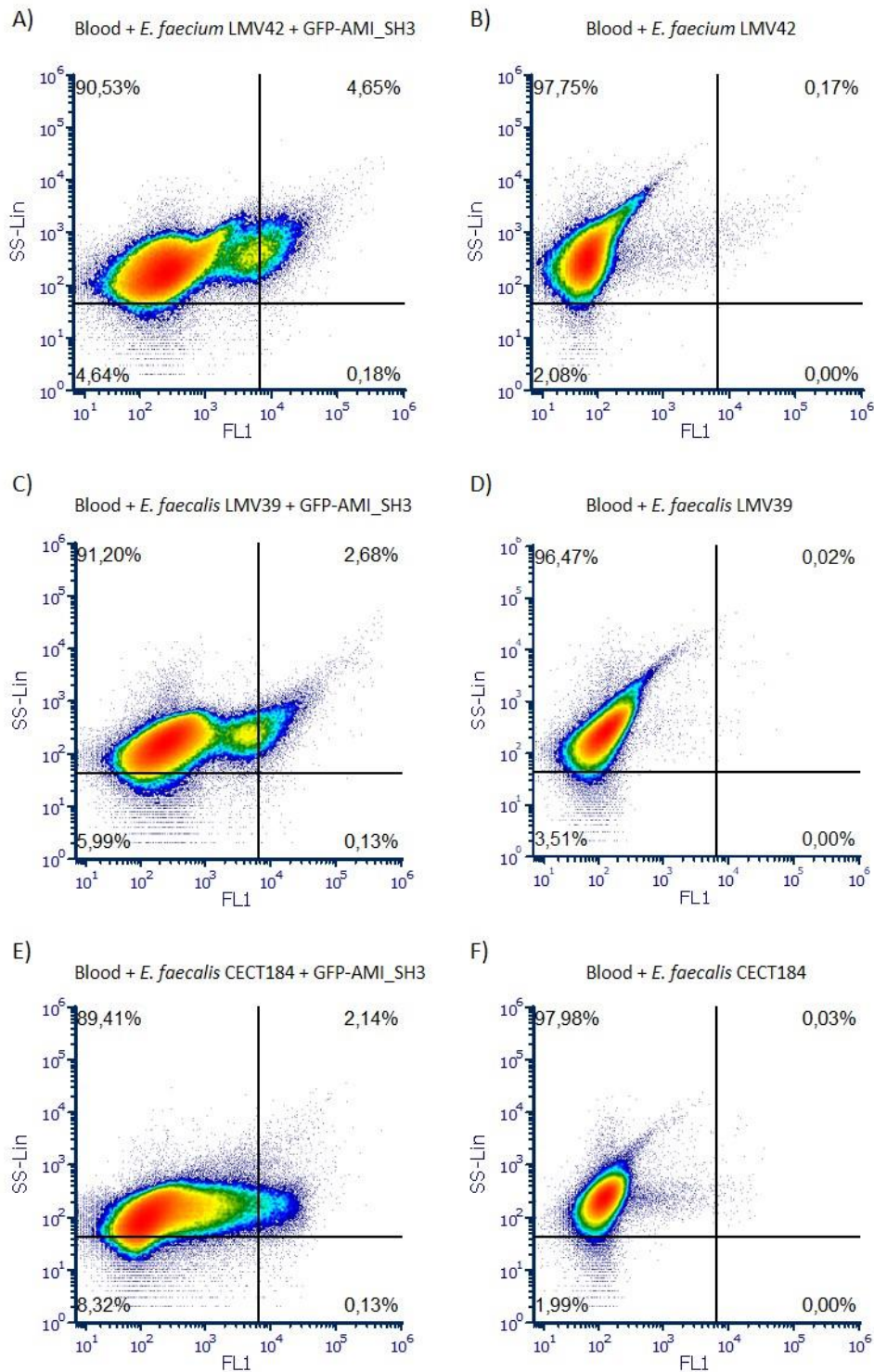


Figure S2 - Graphical representation of blood samples analysis by flow cytometry. Representative dot plots showing side scattering (SS) and green fluorescence intensity (FL1) of blood culture with *E. faecium* LMV-0-42 (A), *E. faecalis* LMV-0-39 (C) and *E. faecalis* CECT184 (E) incubated with GFP-AMI_SH3 protein; and the respective blood cultures with unlabelled *E. faecium* LMV-0-42 (B), *E. faecalis* LMV-0-39 (D) and *E. faecalis* CECT184 (F).

Supplementary Material S3 – Gene sequences

Ami (Amidase domain, 633 bp)

AAAAAAGAAACAGCTAAGAAAAGTGCAAGTAAAACCTCCTGCACCTAAAAA
GAAAGCAACACTAAAAGTTTCTAAGAACCATATTA ACTATAACAATGGATAA
ACGTGGTAAGAAACCTGAAGGAATGGTAATACACAACGATGCGGGTCGTTT
TTCAGGACAACAATATGAAAACCTTTAGCTAAAGCCGGTTATGCTAGATA
CGCTAATGGTATTGCTCACTACTATGGTTTCAGAAGGTTATGTATGGGAAGCA
ATAGATGCTAAGAATCAAATTGCTTGGCATAACAGGTGATGGAACAGGAGCT
AACTCAGGTAACCTTTAGATTTGCAGGTATTGAAGTCTGTCAATCAATGAGTG
CTAGTGATGCTCAATTCCTTAAAAACGAACAAGCAGTATTCCAATTCACAGC
AGAGAAATTCAAAGAATGGGGTCTTACTCCTAACCGTAAGACTGTAAGATT
GCACATGGAGTTCGTTCTACAGCTTGTCCTCACCGTTCAATGGTACTTCAT
ACAGGATTCAATCCAGTAACTCAAGGAAGACCATCACAAGCAATAATGAAT
AAATTA AAAAGATTATTTTCATTAAACAAATTA AAAACTACATGAGTAATGGA
ACTTCAAGTTCTACTGTA

SH3 (SH3 domain, 444 bp)

AGACCATCACAAGCAATAATGAATAAATTA AAAAGATTATTTTCATTAAACAA
ATTAAAAACTACATGAGTAATGGAACCTTCAAGTTCTACTGTAGTTAAAAAA
GGTAAAACAAGTAGTGCAAGTACACCGGCAACTAGACCTGTAACAGGTTCT
TGGA AAAAAGAACCAGTTCGGAACCTTGGTACAAACCGGAATCGGCAACGTTT
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CTCCAGTAGGAGGTAACCTCCCTGCAGGTGCTACAATTGTATATGACGAGG
TAGCAATCCAAGCAGGTCATATTTGGATTGGTTATAACGCTTATAATGGTAA
CAGAGTATATTGCCCTGTTAGAACATGTCAAGGTGTTCCACCAAACCATATA
CCTGGTGTTGCCTGGGGTGTATTCAAAGGTTAA

AMISH3 (Amidase and SH3 domains, 984 bp)

AAAAAAGAAACAGCTAAGAAAAGTGCAAGTAAAACCTCCTGCACCTAAAAA
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TGGTACAAACCGGAATCGGCAACGTTTGTTAATGGTAACCAACCTATAGTA
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CAGGTGCTACAATTGTATATGACGAGGTAGCAATCCAAGCAGGTCATATTT
GGATTGGTTATAACGCTTATAATGGTAACAGAGTATATTGCCCTGTTAGAAC
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AAAGGTAA