
Supplementary information

Assessing biofilm inhibition and immunomodulatory activity of small amounts of synthetic host defense peptides synthesized using SPOT-array technology

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Assessing biofilm inhibition and immunomodulatory activity of small amounts of synthetic host defense peptides synthesized using SPOT-array technology

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Supplementary Table 1 | List of control samples to be included in the biofilm and the immunomodulatory screening assays of the SPOT-peptides

Controls for Biofilm Inhibition Assay	
1	Positive control (+Ctr.) ¹ → Positive growth control containing only bacteria to define 100% biofilm growth
2	Negative control (-Ctr.) ¹ → Sterile medium without bacteria nor any treatment to define 0% biofilm growth
Controls for the Immunomodulatory Assay	
1	Media control (Media) ¹ → Evaluate the immune response of PBMCs/or any other immune cells in media alone
2	LPS ¹ → Lipopolysaccharide (LPS) treated cells; LPS is used as an immune cell stimulator and to investigate the innate immune response. ▲ NOTE. You will need to use this only if you are studying the inhibitory effect of peptides on immune mediators, e.g. cytokines induced by LPS.
3	Vehicle ² → Endotoxin-free water used to dissolve the peptides; used to determine the influence of peptide delivery vehicle (i.e. water) on immunomodulatory activity. Also, serve as a negative control for the cytotoxicity assay
4	Triton ³ → Cell lysis reagent used as a positive control in the cytotoxicity screening assay
Comparators (For both biofilm inhibition and immunomodulatory assays)	
1	PC ² → original (parent) peptide(s) made on the same cellulose membrane as the one used to synthesize all the SPOT-peptides
2	PP ² → Same parent peptide(s) prepared at 95% purity after synthesis by conventional Solid Phase Peptide Synthesis (SPPS)
3	Empty membrane Spot ² ; ensure that cellulose membrane or residual chemicals from synthesis do not interfere with biofilm and/or immunomodulatory assays.
4	Extra control peptide(s) ³ , which can be random or specifically chosen peptides; used for QC testing and for initial tests to standardize experimental conditions
5	Antibiotics/chemical compounds ³ against biofilm or immunomodulatory agents; used to compare the activity of the SPOT-peptides to well-known active compounds

¹Required on every microtiter plate; ² Not necessarily to be included in each array screen;

³optional controls to include that can enhance the screening diagnosis