Mycobacteriophage: A demonstration of the reduction of *M. smegmatis* in various matrices.

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Background: Mycobacterial disease is a major cause of fatality worldwide, with approximately 1.5 million deaths for every 10 million infections. These infections are difficult to treat due to the intrinsic resistance of its mycolic acid rich cell wall to many antibiotics. There is potential for mycobacteriophage (MP) to be used therapeutically for multidrug- and extensively-drug resistant infections. *Mycobacterium smegmatis* mc^2 155 is a useful substitute for slow-growing pathogenic mycobacteria, as it propagates quickly under lab conditions. This feature of *M. smegmatis* increases the pace of analysis, by ensuring the quick isolation and characterisation of MP and acting as a model organism for proof-of-concept studies.

Methods: For instance, *M. smegmatis* was used to spike various matrices before applying phage to provide insights into which matrices permit phage infection. Here we demonstrate the application of a recently characterised MP, LOCARD, to various mediums spiked with *M. smegmatis*, including broth, milk and simulated intestinal fluid. MP survival in simulated gastric fluid was also examined to determine whether encapsulation could enhance delivery to the intestinal environment.

Conclusion: Through reduction in bacterial numbers or phage propagation, we infer the environments which permit phage-host interaction sufficiently to reduce the mycobacterial burden of each matrix. From these rapidly generated results, we can hypothesise and

extrapolate how slow-growing mycobacterium species and their phages may interact in these conditions, helping guide the design of future experiments and therapies.