FAST AND SENSITIVE DETECTION OF *PSEUDOMONAS AERUGINOSA* IN CLINICAL SETTINGS USING ENGINEERED REPORTER PHAGES

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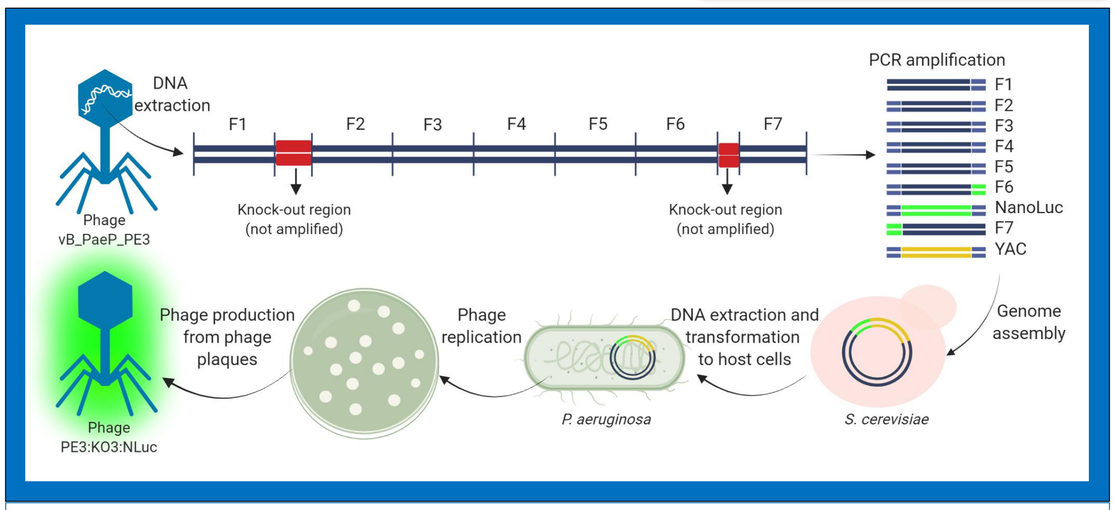
INTRODUCTION

Pseudomonas aeruginosa **is a bacterial pathogen responsible for a wide range of healthcare-associated infections, such as surgical site infections, bacteraemia, urinary tract infections, and mostly, pneumonia. As a result, the World Health Organization (WHO) identified it as one of the top priority pathogens that urgently calls for the development of novel treatments. Bacteriophages (phages) have emerged as a promising therapeutic approach and their properties can be further improved through engineering. This opens an extensive variety of possibilities, allowing to assemble chimeric phages with new functions. Considering the slow turnover of conventional diagnostic methods and the problems associated with the current molecular and immunogenic methods, this study aimed at assembling a bioluminescence-based reporter phage for the fast and sensitive detection of *P. aeruginosa* in clinical care.**

METHODS

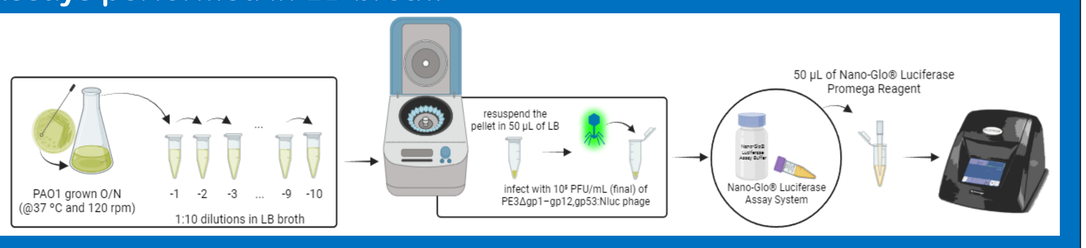
Phage engineering



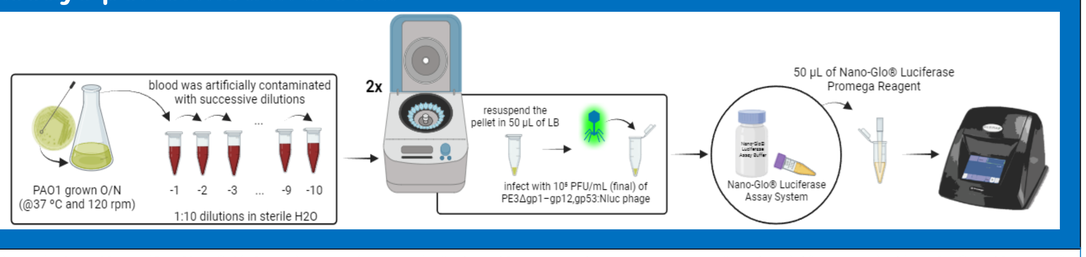
Pseudomonas **phage vB_PaeP_PE3 was genetically engineered using the yeast-based phage engineering platform. The genome of this phage has been previously reduced by deleting genes with unknown function, and in this study, this phage genome was used as a scaffold for the insertion of the NanoLuc® luciferase gene which encodes the NanoLuc® luciferase enzyme able to produce a bright and improved luminescence. This gene was swapped with gene gp53 that encodes a hypothetical protein with unknown function.**

Sensitivity and Specificity tests

Assays performed in LB broth:



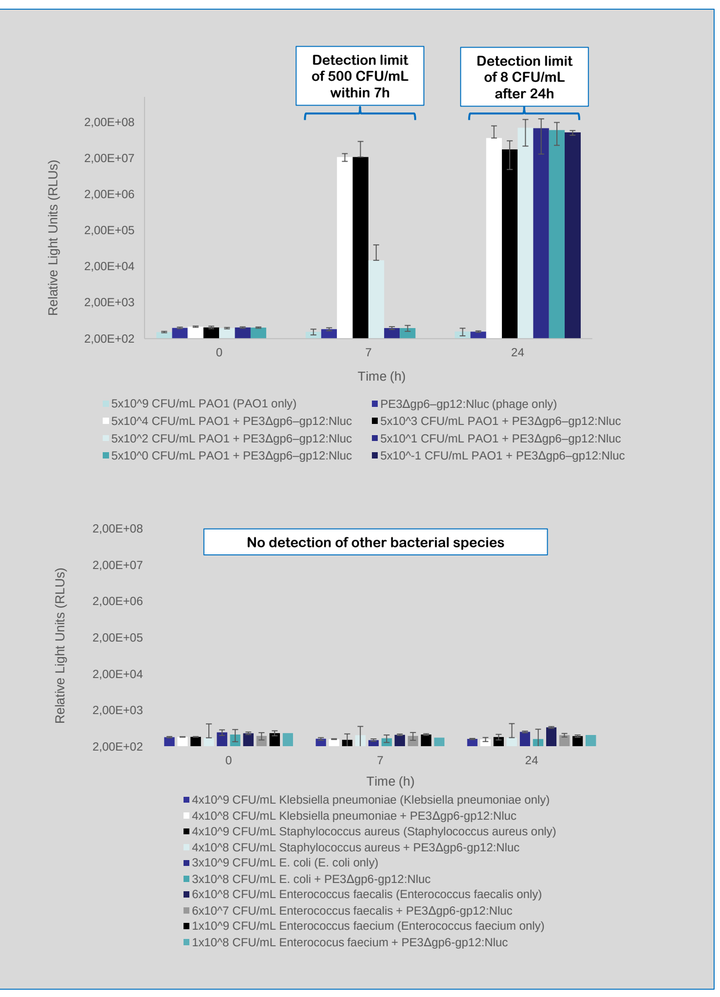
Assays performed in blood:



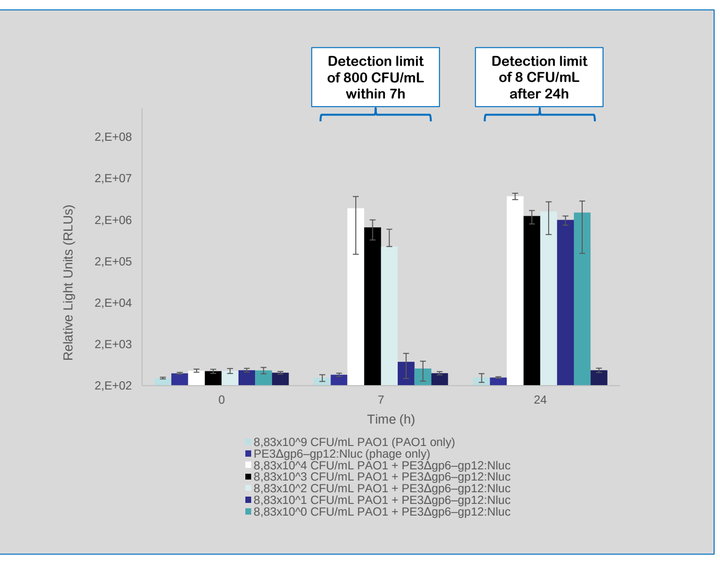
The detection limit of this phage-based detection system was evaluated through the infection of serial dilutions of *P. aeruginosa* suspensions with the reporter phage (vB_PaeP_PE3Δgp1-gp12,gp53:Nluc), and subsequent quantification of luminescence (in relative light units, RLUs).

RESULTS

Assays performed in LB broth



Assays performed on blood



CONCLUSIONS

In this study, we were able to successfully engineer a reporter phage that is capable of reliably detect 500 CFU in 1 mL of sample contaminated with *P. aeruginosa* PAO1 within 7 h or an average of 1 CFU/mL after 24 h, and no false positives were observed. Similar results were also obtained when the reporter phage was tested in blood, being capable of detecting an average of 8 CFU/mL within 24 hours. Overall, compared to culture-dependent methods, the NanoLuc-based reporter phage allows a fast and sensitive detection of *P. aeruginosa* cells using a simple protocol, 41 h faster than culture-dependent approaches. Therefore, this phage-based detection system is a promising alternative to the common methods for the accurate detection of *P. aeruginosa* in clinical settings.

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