qPCR as an alternative to the method of double agar overlay applied for phage enumeration

Hans Duyvejonck[1,2], Maya Merabishvili[1,3,4], Jean-Paul Pirnay[3] , Daniel De Vos[3], Gilbert Verbeken[3], Mario Vaneechoutte[1], Stefan Vermeulen[2], Els Van Mechelen[2]

1 Laboratory Bacteriology Research, Department of Clinical Chemistry, Microbiology and Immunology, Faculty of Medicine and Health Sciences, University of Ghent, Corneel Heymanslaan 10, 9000 Ghent, Belgium
2 Department of Biosciences, Faculty of Education, Health and Social Work, University College Ghent, Keramiekstraat 80, 9000 Ghent, Belgium
3 Laboratory for Molecular and Cellular Technology, Burn Wound Center, Queen Astrid Military Hospital, Bruynstraat 1, 1120 Brussels
4 The Eliava Institute of Bacteriophages, Microbiology and Virology, Gotua 3, 0160 Tbilisi, Georgia

Introduction

BFC-2 has been developed as a therapeutic phage cocktail active against clinical strains of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Acinetobacter baumannii* (Merabishvili et al. 2009, Merabishvili et al. 2014). The cocktail contains five different phages. To enumerate phage titers in a precise and rapid way during the production process of phage therapeutics and clinical trials, we developed a qPCR platform.

Enumeration of bacteriophages by the double agar overlay method (DAO) is still considered as the golden standard in phage quantification. However, some well-known limitations of the method (laboriousness, reproducibility) led us to develop the qPCR platform. We started with the in silico design and evaluation of primers, followed by the selection of at least one primer pair for each bacteriophage based on efficiency (Fig. 1) and specificity within the platform conditions (Ta 60 °C, [$MgCl_2$] 3.0 mM and [Primer] 2.0 µM) and evaluated the stability of the phages under different conditions (Fig. 2).

Study set up and results

Figure 1. qPCR platform for enumeration of the BFC-2 phages

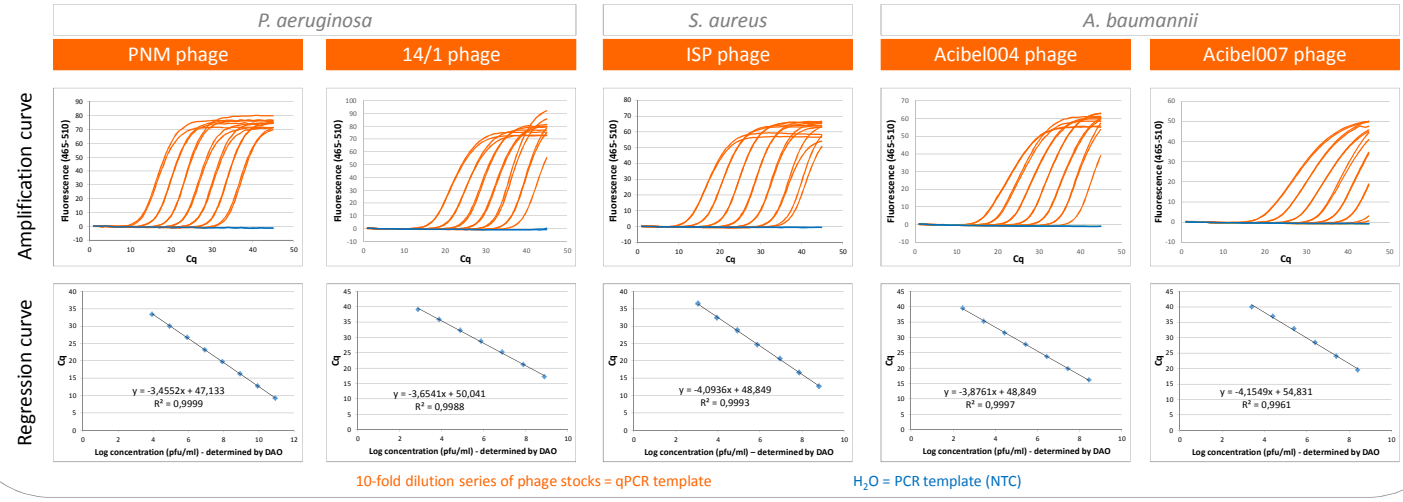
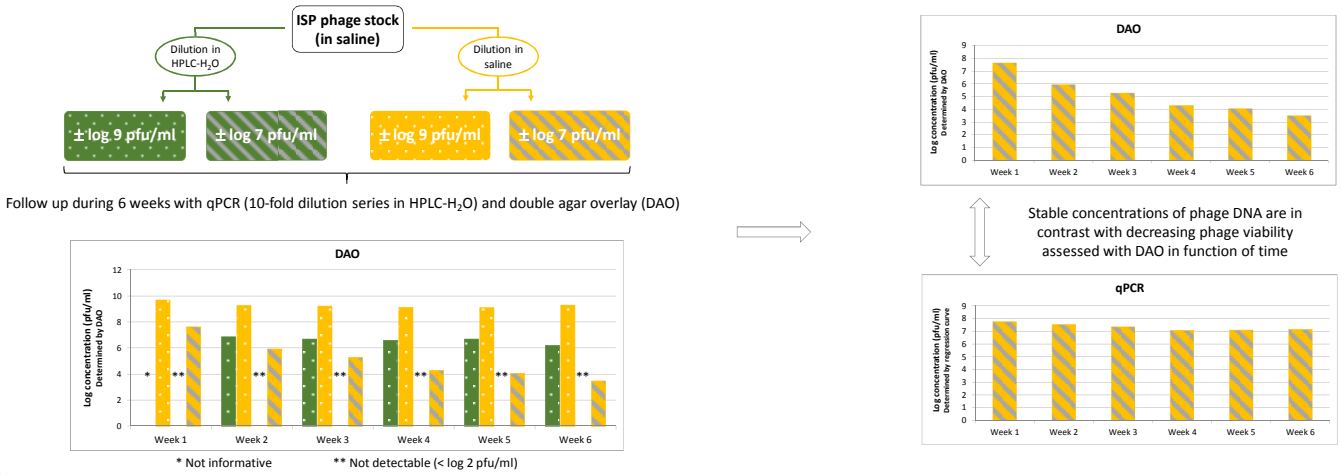


Figure 2. Influence in function of time of different storage conditions on the phage stability analyzed by means of qPCR and DAO



Discussion

The qPCR platform has been optimized for the enumeration of all 5 phages from the BFC-2 cocktail. Based on the concentration of phage in saline as determined with the plaque assay, the detection limit of the qPCR system was log 3 pfu/ml.

Concerning phage stock stability during long periods, we observed that storage conditions have a major influence on the infectivity of the phage determined by DAO. However, according to qPCR data the DNA titer remained stable. Our results showed that there was no correlation between numbers of active phage particles (plaque assay) and amplifiable phage DNA (qPCR) in phage suspensions after different storage periods. Further research is needed to elucidate the mechanisms behind decreasing infectivity of phage stock. Storage in saline at high titers was the best storage condition.

References

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Acknowledgments

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