



Novel tests to detect coliphages in drinking water

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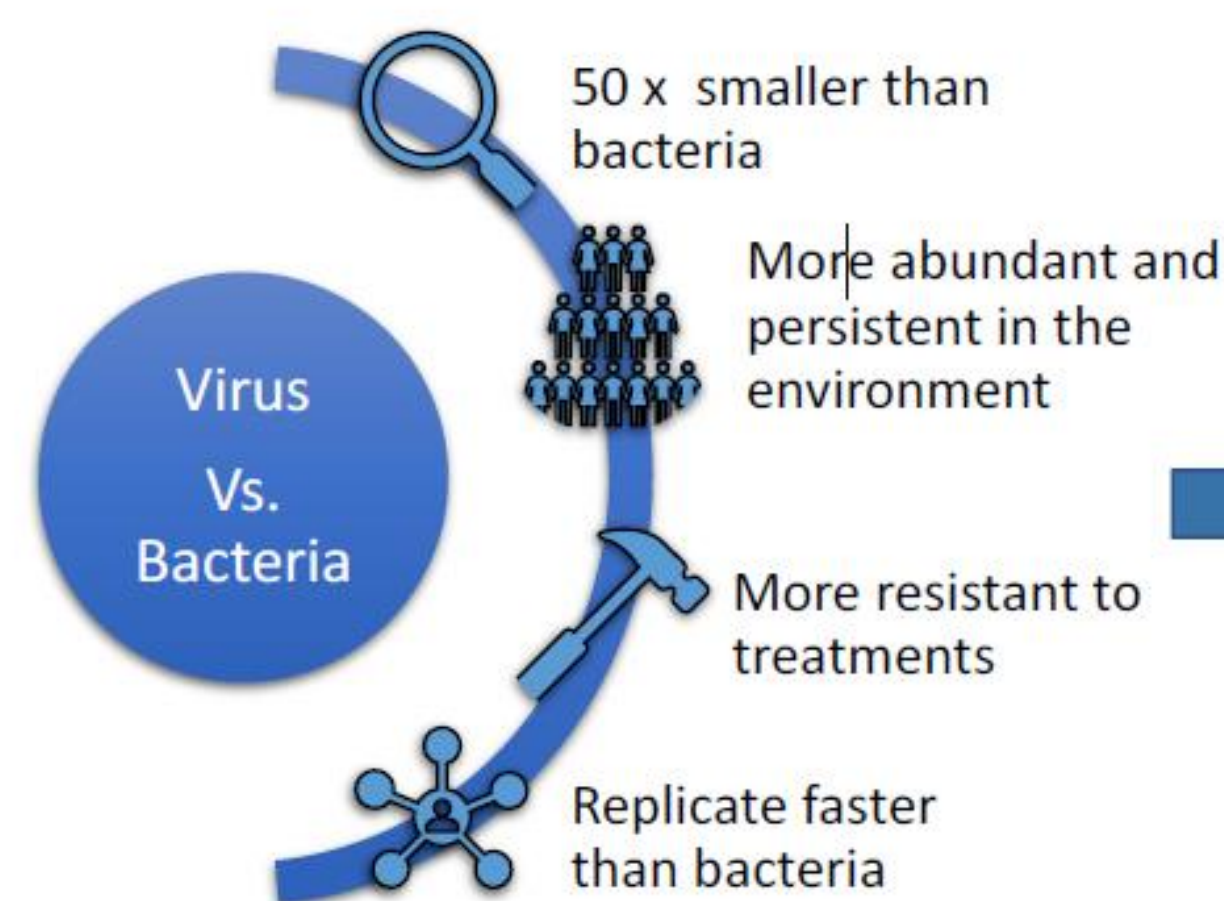


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Background

Bacteriophages (phages) are viruses that infect bacteria and are the most widely distributed and abundant biological form on Earth, estimated at $\sim 10^{31}$ particles in the biosphere. Bacteriophages that infect coliform bacteria are known as coliphages, and their survival and incidence in water environments resemble those of human viruses more closely than most commonly used bacterial indicator. Therefore, they have been proposed as good water quality indicators. On January 2021, the recast Drinking Water Directive 98/83/EC established somatic coliphages as a new indicator required for operational monitoring of the drinking water treatment process. Culture-based detection and enumeration of coliphages takes an average of 18 to 24 hours to yield results.

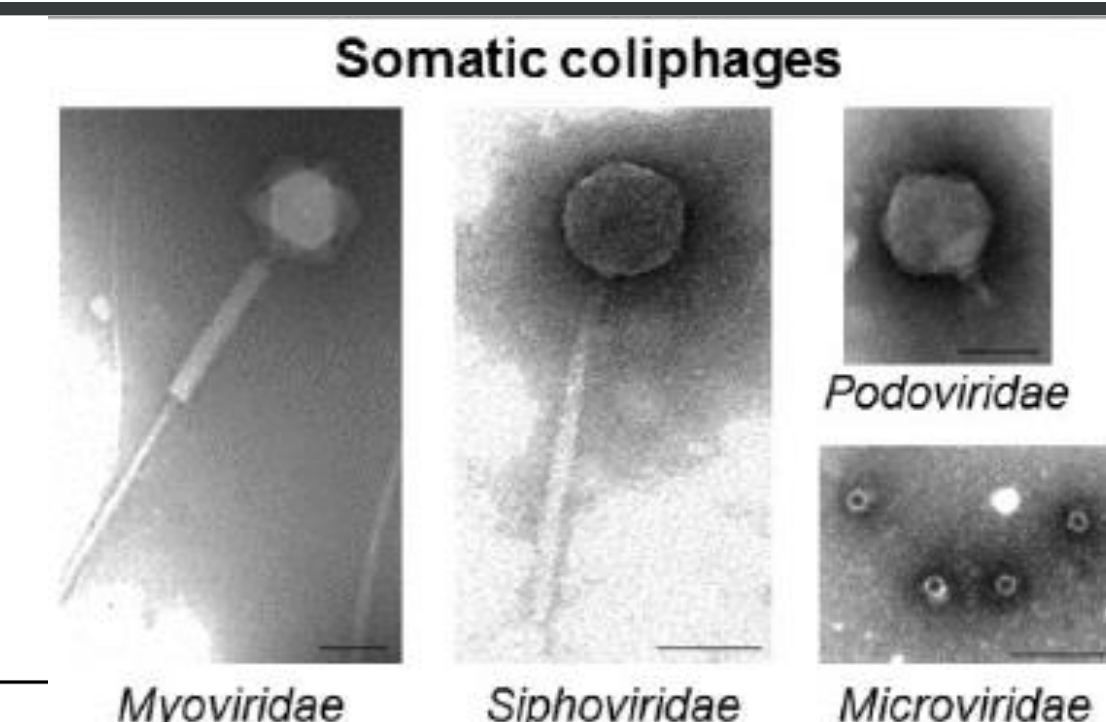
Why viral indicators?



23.12.2020 [EN] Official Journal of the European Union L 431/1

DIRECTIVE (EU) 2020/2184 OF THE EUROPEAN PARLIAM AND OF THE COUNCIL of 16 December 2020 on the quality of water intended for human consumption (recast)

Operational parameter	Reference value	Unit	Notes
Somatic coliphages	50 (for raw water)	Plaque Forming Units (PFU)/100 ml	This parameter shall be measured if the risk assessment indicates that it is appropriate to do so. If it is found in raw water at concentrations > 50 PFU/100 ml, it should be analysed after steps of the treatment train in order to determine log removal by the barriers in place and to assess whether the risk of a breakthrough of pathogenic viruses is sufficiently under control.



Current methods for detection and enumeration of somatic coliphages

International Standardization Office. ISO

10705-1: 2002. Water quality. Detection and enumeration of bacteriophages.

Part 1: Enumeration of F-specific RNA bacteriophages

10705-2: 2002. Water quality. Detection and enumeration of bacteriophages.

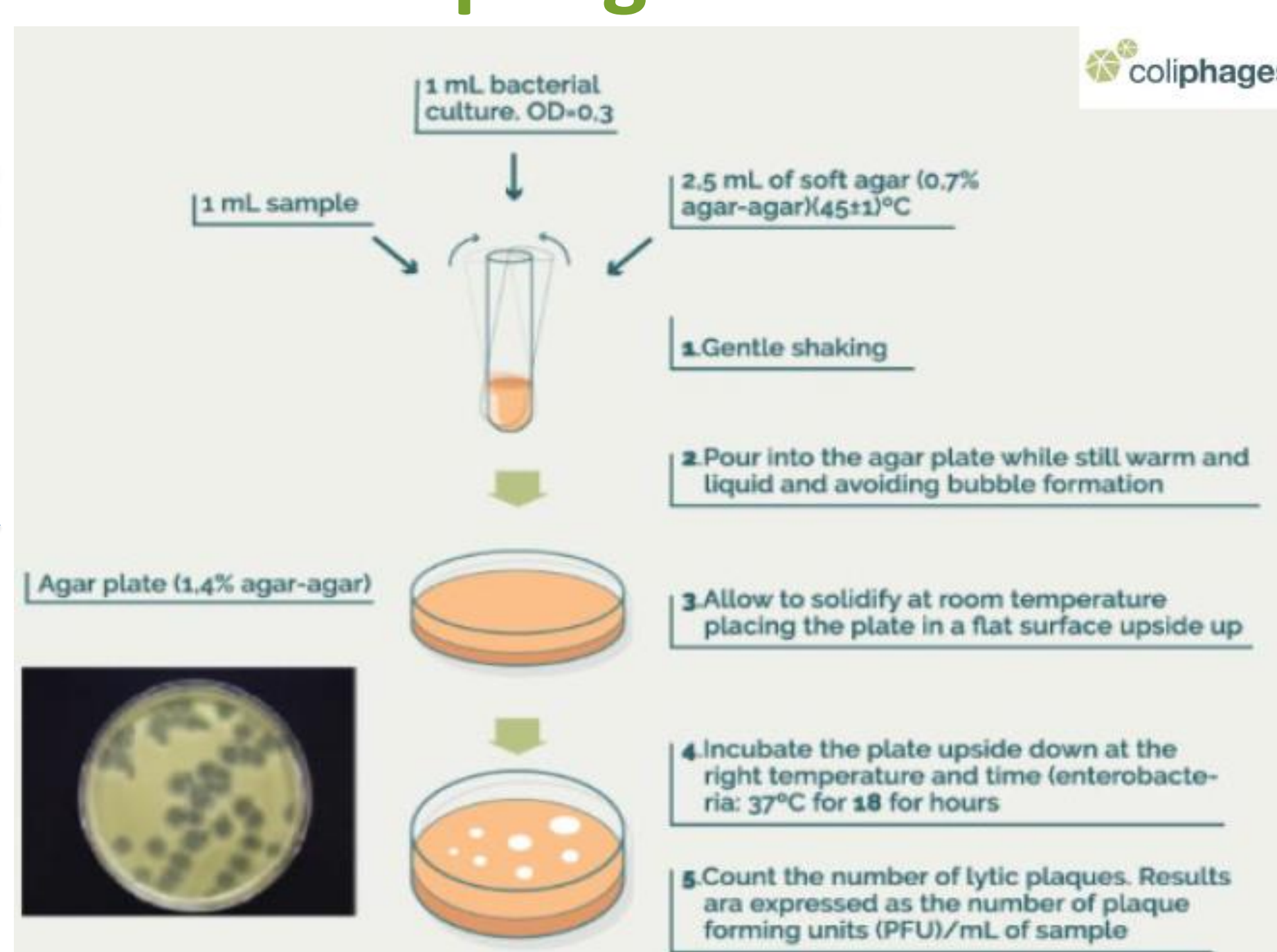
Part 2: Enumeration of somatic coliphages

10705-3: 2003. Water quality. Detection and enumeration of bacteriophages.

Part 3: Validation of methods for concentration of bacteriophages from water

10705-4: 2001. Water quality. Detection and enumeration of bacteriophages.

Part 4: Enumeration of bacteriophages infecting *Bacteroides fragilis*



Applying Standard methods still > 16 h

Purpose: Develop a novel molecular method to detect somatic coliphages in drinking water faster than standard methods

Material and Methods

Phase 1: Bioinformatics

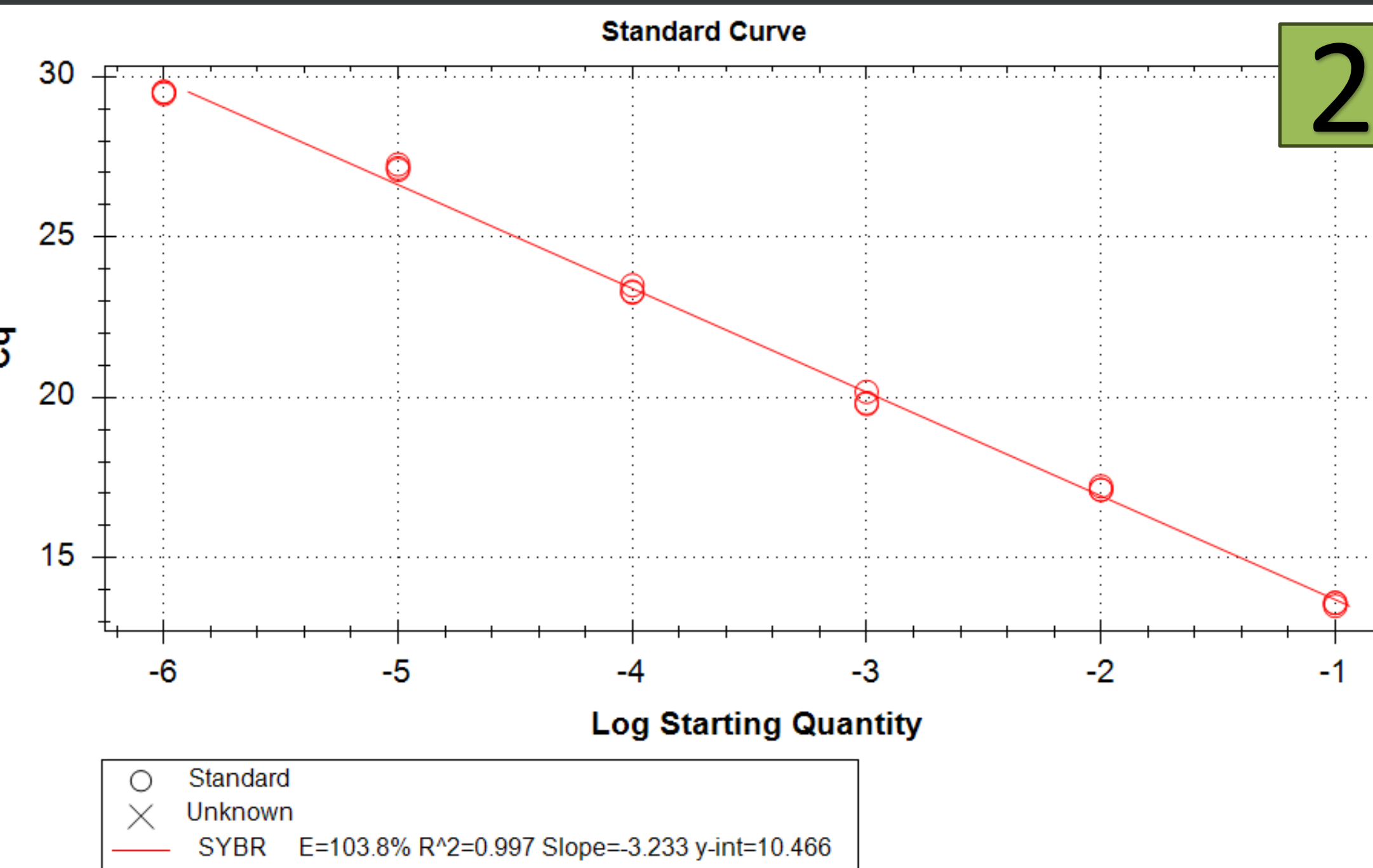
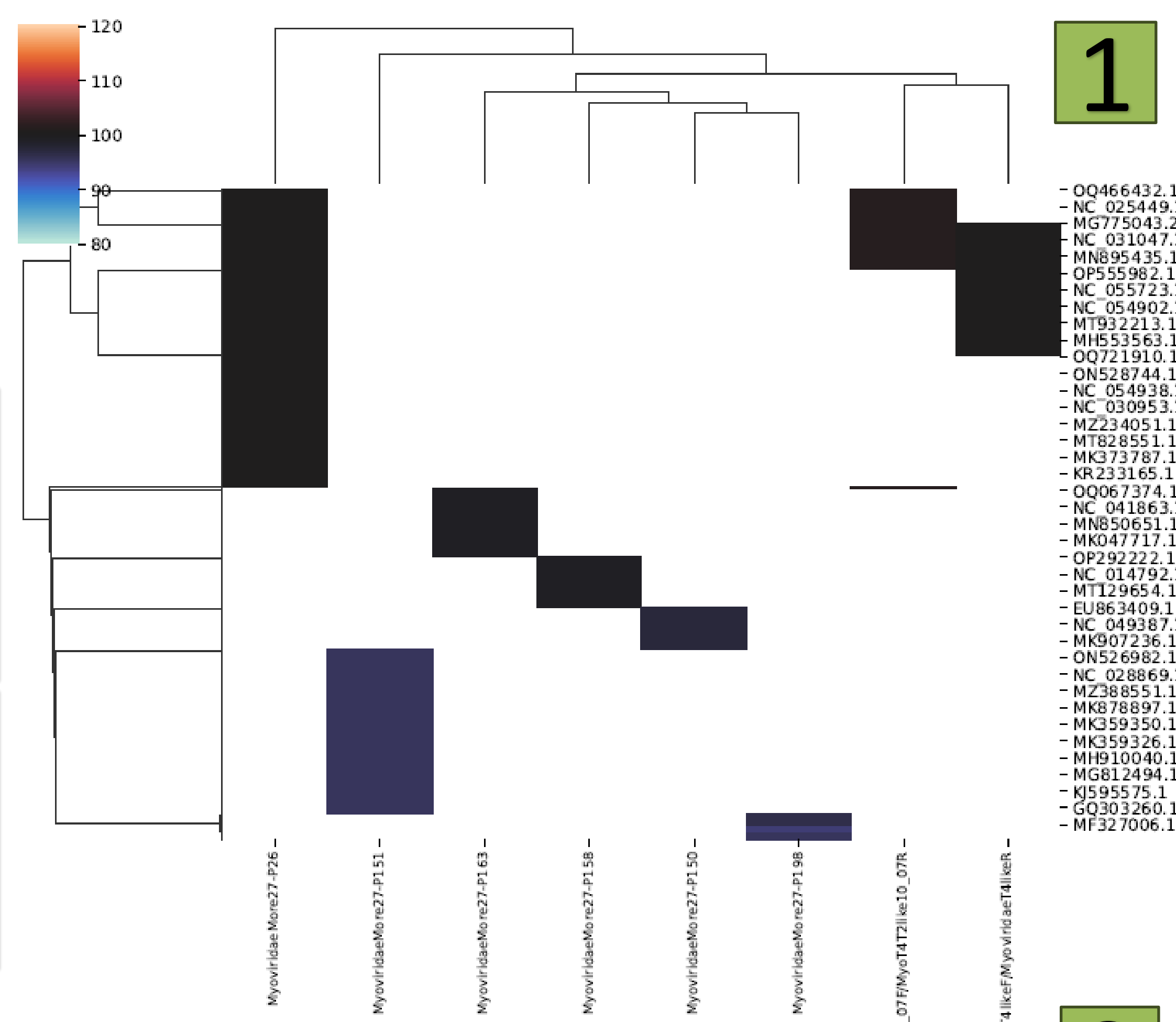
Full genome sequence alignment
Family specific primer approach
Primer design
In silico PCR

Phase 2: Method Validation

In vivo PCR
Standards Development
Method Validation: Efficiency, specificity, LOD, LOQ

Phase 3: Environmental samples testing

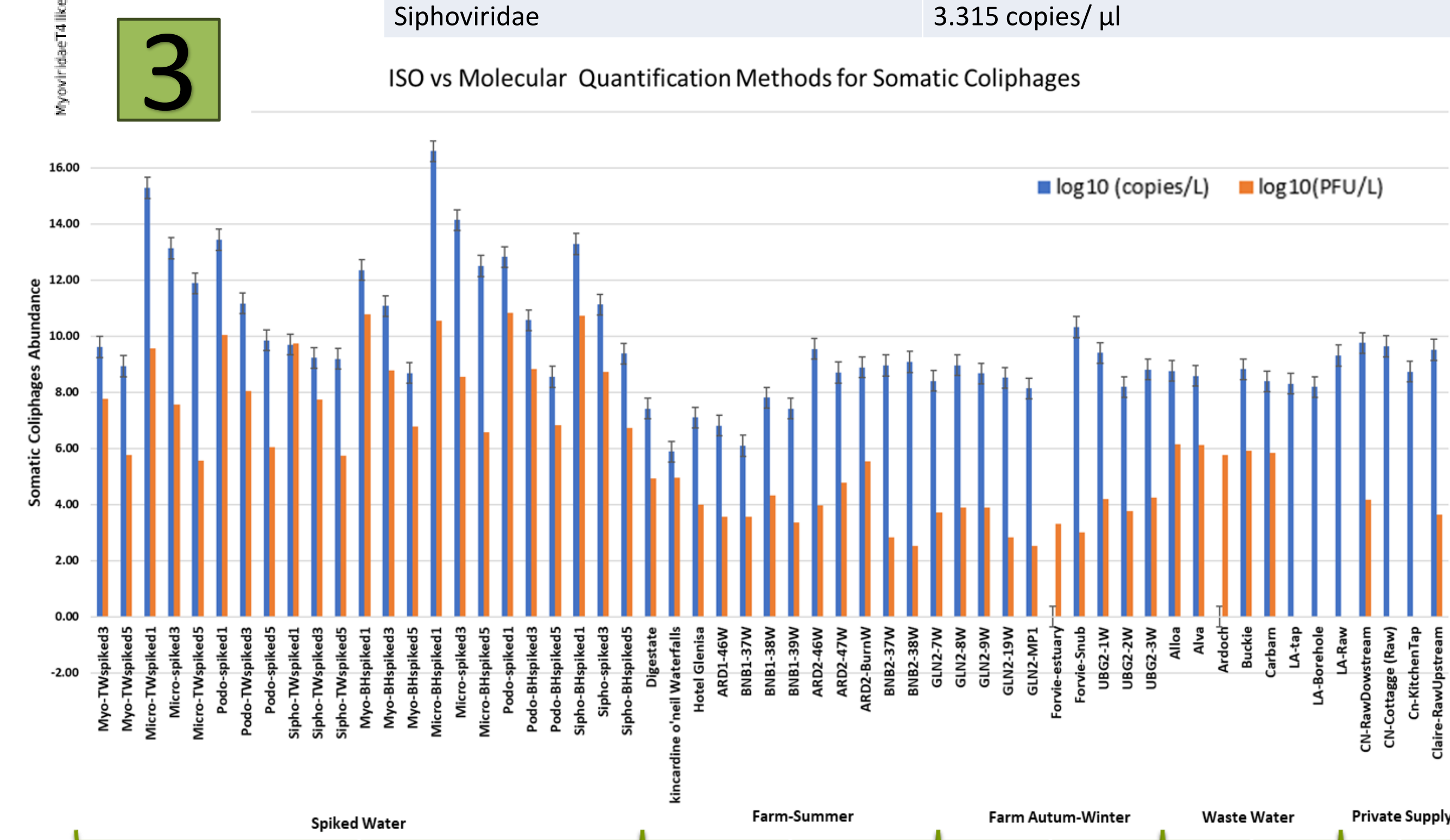
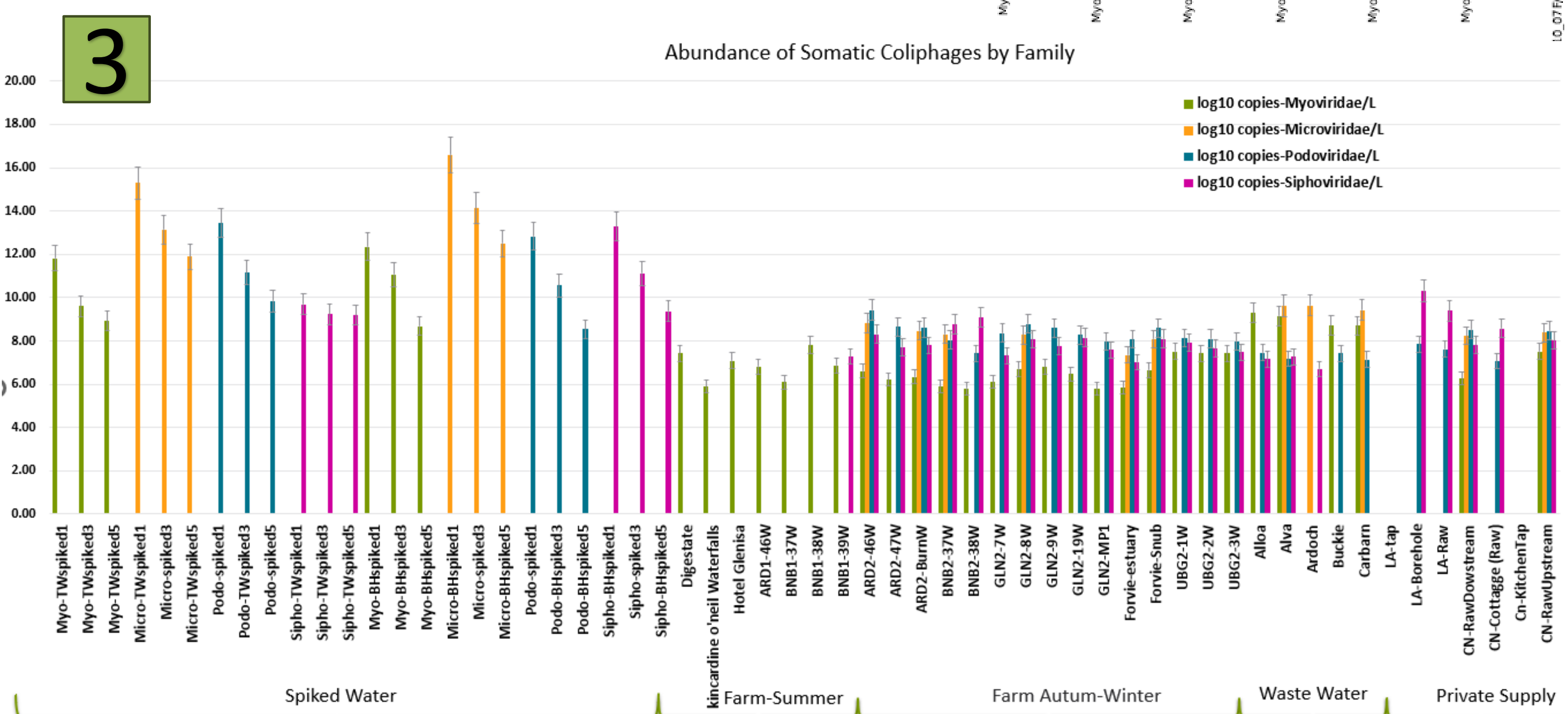
Application of molecular method on environmental samples
Comparison of results with ISO Method



METHOD VALIDATION PARAMETERS

Efficiency	90-110%
R2 (Coeff. Of determination)	0.993-0.997
Myoviridae	10.4 copies/ μ l
Microviridae	2.69 copies/ μ l
Podoviridae	26.5 copies/ μ l
Siphoviridae	3.315 copies/ μ l

ISO vs Molecular Quantification Methods for Somatic Coliphages



Results

Development of a molecular method to detect somatic coliphages in drinking water
8 hours protocol
Cost effective
Detects somatic coliphages by family

Future Work

Workshop to train stakeholders in molecular method application
Use of the method to map somatic coliphages in Scottish catchments, feed current quality indicators models, produce outbreak predictions, and develop management recommendations.



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