

## qLAMP of specific sequences of phage genomes isolated from bacterial ghost cells for testing the virucidal and limited virucidal effect of washing procedures (01IF22787N)

Pathogens such as bacteria, fungi, or viruses can persist on contaminated textiles (e.g., work wear and ward laundry in hospitals, retirement and nursing homes) for several weeks, so that disinfecting reprocessing is necessary. Disinfecting textiles during reprocessing is a sensitive step that requires validated methods. In addition to adhering to the process parameters, it is recommended to regularly check the disinfection performance using bioindicators. Bioindicators have been established to verify the virucidal effect of washing processes. These are contaminated with Enterobacteriophage MS2, which belongs to the group of non-enveloped bacteriophages. A disadvantage is the long duration of the culture-based detection process (approx. 2-3 days).

One aim of the research project was to develop a method that would enable quantitative evaluation of the bioindicators without multiplying the bacteriophages by cultivating them with their host cells. In addition, a bioindicator contaminated with the enveloped phage phi6 was developed, as this phage resembled representative human pathogenic viruses in its thermo- and chemoresistance under the test conditions selected in laboratory studies.

Bacterial ghost cells are cell envelopes, i.e., “empty” bacterial cells that have been created by cell lysis and do not contain any cell components such as nucleic acids, etc. Bacterial ghost cells were generated by chemical lysis of host cells and then incubated with the bacteriophages. Lysis procedures were established to isolate the phage genome from the ghost cells without breaking down the phages. To amplify phage-specific genome segments, a method was used that is performed at a constant temperature (isothermal) and is known as loop-mediated isothermal amplification, or LAMP for short. In order to detect the amplification of the genome segment in real time, detection molecules, known as probes, were designed and suitable reaction conditions were determined. The probes consist of double-stranded DNA, with one strand functionalized with a fluorophore and the other with a quencher. In the initial state, the quencher suppresses the fluorescence of the fluorophore. During amplification of the genome segment, the fluorophore and quencher are separated (DARQ: “Detection of Amplification by Release of Quenching”) and the fluorescence quenching is reversed. By selecting fluorophores with different colors of fluorescence in the probe design, the fluorescence signal generated by DARQ-LAMP can be attributed to the respective phage (MS2 or phi6) even when the genome segments are amplified simultaneously. To quantify the number of phages, calibration was performed by measuring standard series with a known number of phage genomes or genome segments. Based on the data obtained, mathematical evaluation models were developed that enable the number of phages to be determined.

The research report is available on request from the wfk - Cleaning Technology Institute.



Schematic representation of the preparation and infection of bacterial ghost cells

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