BTO *Executive Summary*

Use of disinfection methods can occasionally lead to differences between culture and RT-PCR results in the detection of indicator organisms

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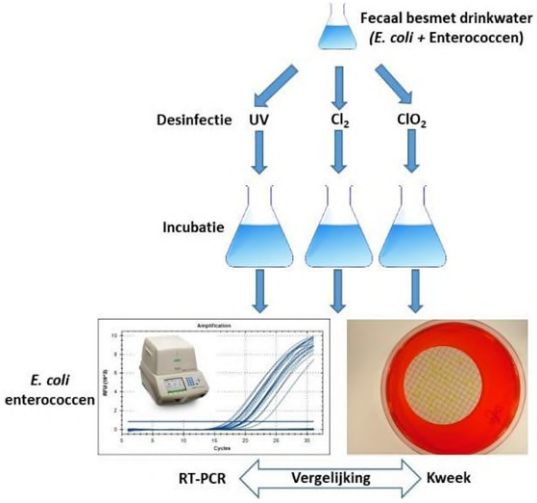
The recently approved RT-PCR method in certain situations detects faecal indicators that are not detected by the regular culture methods, particularly when disinfection steps are used in the treatment process. Measurements in practice and laboratory experiments demonstrate that this detection with RT-PCR of RNA of inactivated organisms can occur in cases where UV or low concentrations of Cl_2 or ClO_2 are used. It is not expected that detection of indicator organisms will also occur following the use of higher concentrations of Cl_2, for instance in the case that pipes are flushed after emergencies, and certainly not in the case of longer contact times, as laid down in the Drinking Water Hygiene Code. RT-PCR can be effectively used in all situations to demonstrate the *absence* of indicator organisms.



Schematic illustration of the research: impact of disinfection on the detection of E. coli *and enterococci with RT-PCR.*

Interest: insight into the occurrence of differences between culture and RT-PCR for correct assessment

As an indicator of the presence of a faecal contamination in drinking water, *E. coli* and enterococci are detected by means of a culture. Drinking water utilities and laboratories have invested a great deal in the development, implementation and validation of alternative methods to determine the presence of faecal contaminants more quickly. The use of RT-PCR makes it possible to demonstrate the presence of RNA of *E. coli* and enterococci selectively and quickly: with RT-PCR the analysis result is available within 4-6 hours. This makes rapid reaction possible following emergencies, or in the case of contaminations after work activities on the pipe network, and thus limits the health hazards for the customer. After the validation of the RT-PCR method, the Human Environment and Transport Inspectorate (ILT) granted temporary approval for the use of RT-PCR as an alternative method for the detection of *E. coli*.

During the application of the RT-PCR method in practice at the Evides drinking water utility, *E. coli* and enterococci were detected in a portion of the samples, while the regular culture methods in these samples did not detect any indicator organisms. It is possible that RNA of *E. coli* and enterococci is still detected after the indicator organisms have already been inactivated by treatment disinfection steps – such as UV and chlorine dioxide. To enable a correct interpretation of the measurement results, research was conducted into the effects that disinfection steps (UV, ClO_2 and Cl_2) have on the outcome of RT-PCR analyses.

Approach: measurements in practice and laboratory experiments

In order to understand the differences between culture and RT-PCR results:

- analyses were conducted on samples from the different treatment steps at Evides, in which UV and ClO_2 are applied as disinfection steps;
- laboratory experiments were conducted on artificially contaminated water samples, which were subjected to disinfection steps under controlled conditions.

Results: UV and low ClO_2 and Cl_2 concentrations have little effect on RT-PCR

Measurements at the Berenplaat treatment plant and laboratory experiments show that disinfection with UV or with low concentrations (0.4 mg/l) of ClO_2 result in no faecal indicators being detectable any longer by culture methods, while they are still detectable with RT-PCR. The application of Cl_2 in concentrations typically used after emergencies for the disinfection of contaminated pipes (5 mg/l) resulted, with a short contact time (30 minutes), in the disappearance of the culturable indicators and in the virtual disappearance of the signal with RT-PCR. With the use of a lower concentration of Cl_2 (0.5 mg/l) and of ClO_2 in a concentration of 0.4 mg/l, the microorganisms remain detectable with RT-PCR, but not by culture.

This means that nonculturable indicators can indeed be detected with RT-PCR in treatments where UV and/or ClO_2 are applied for disinfection, and in treatments using chlorination in low concentrations.

The degree to which detection occurs will depend on the physical removal of indicator organisms *before* the disinfection step, and on the resulting concentration of indicator organisms that then reaches the UV or ClO_2 disinfection step.

Application: RT-PCR suitable for detecting the absence of indicator organisms

The use of RT-PCR for the detection of indicator organisms can, in certain situations, lead to the detection in samples in which no culturable indicators are detected. This detection with RT-PCR can occur when UV or low concentrations of Cl_2 or ClO_2 are used for disinfection. It is not expected that detection of indicator organisms will also occur following the use of higher concentrations of Cl_2, for instance in the case that pipes are flushed after emergencies, and certainly not in the case of longer contact times, as laid down in the Drinking Water Hygiene Code. This means that RT-PCR can be used to demonstrate the *absence* of indicator organisms. In the detection of indicator organisms with RT-PCR, it is important that knowledge about the disinfection methods applied to the water be taken into account during the interpretation of the analysis results. In cases where UV or low ClO_2 concentrations are used as a disinfection step in the treatment, it is advisable to carry out a supplementary culture analysis to gain insight into the culturability of the detected indicator organisms.

Further research should show the frequency with which, and under which practice situations, RT-PCR methods can actually detect the nonculturable indicator organisms. As a basis for this research, the measurements carried out at every production site within the framework of the Analysis of Microbial Safety of Drinking Water (AMVD) can be used, supplemented with measurements on the water produced.

Report

This research is described in the report *Effect van desinfectie op detectie van indicatororganismen met RT-PCR* (BTO 2019.005).

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