Research and Reviews: Journal of Molecular Genetics and Medicine

Extended Abstract
Vol. 4, Iss. 3
2020

MICROBIOLOGY 2020: The use of RT-PCR techniques of *E.coli* and *Enterococci* for fast detection of Fecal pollution in drinking water

G. H. Wubbels
Delft University, Netherlands

Abstract:

Recently new methods are developed to detect within four hours fecal pollution in drinking water . These methods are RNA-based and specially designed for the fecal target-organisms. For both E.coli and the Intestinal Enterococci a detection limit is achieved of approximately 1 living cell/100 ml. This low level detection in real water samples is very different from other publications and makes it unique in the field of Polymerase Chain Reaction techniques . Experiments wherein a comparison is made with standardized ISO culture methods showed that the Rerversed Transcriptase RNA methods for E.coli and Enterococci are more sensitive and accurate than the culture techniques. By choosing RNA instead of DNA sequences for targeting both bacteria the focus is laid on potentially surviving organisms instead of dead or not-culturable. This makes it possible to use it in both situations with or without using chlorine in distributing drinking water.

Experiences in practical situations in real fecal polluted drinking water situations confirmed the former findings with laboratory experiments.

Nowadays the methods are being used in the north of the Netherlands to detect a fecal pollution in early stage and is used to build up a database with information about the effect of working on hygienic base in difficult situations.

In the Netherlands tap water is distributed without disinfection. Therefore the produced distributed water is real drinking water. To be sure that the drinking water in the Netherlands is safe to drink by the consumers, a lot of effort is put into the production of drinking water and an exhausted monitoring program is fulfilled. In normal situations the water is safe to drink in respect to microbiological hazards.

However the highest risk of contamination of drinking water is not at the production plant but is in the distribution network when accidently the infrastructure is damaged. In the situations when there is a leakage or even a breakage of the system and the water pressure falls down intrusion with dirt and possible fecal waste can occur. Consumers in the surrounding of the breakage are closed down as soon as possible and the situation is restored. But before the water is free to transport again always a microbiological sample is taken and analysed for fecal pollution measured by E.coli and Enterococci. These analyses have to be done according to the standard ISO methods ISO 9308-1and ISO 7899-2 , which takes 44 hours at least before it is known whether the water is safe to drink or not. Sometimes however there ae situations when it is not possible to wait that long before the water can be distributed again and the system has to be connected as soon as possible. This is one of the main reasons why hygienic working is integrated in the Dutch Law.

New Molecular techniques makes it possible to fasten up the analyses of fecal contamination compared to culturing. Several Polymerase Chain Reaction (PCR) methods and PCR-like methods are already common in other branches and are also used in some water analyses. The problem with the PCR methods is to get enough signal for

This work is partly presented at the Event on 12th Edition of International Conference on Microbiology, Antibiotics and Public Health Conference scheduled during **August 19-20, 2020** held at Berlin**,** Germany

the detection of low concentrations of bacteria and to deal with dead or alive bacteria. The detection levels of normal PCR is too high and detection of dead bacteria is not interesting. That is why we developed a method based on detection of Ribosomal RNA (RT-PCR). Living bacteria always contains a lot of ribosomes and ribosomal RNA to stay alive by building enzymes for assimilation. The more the bacteria reaches the dead phase the less Ribosomes and Ribosomal RNA is present in the cell. So by looking at Ribosomal RNA, the detection level can be lowered to one cell per volume sample and the detection of dead cells is negligible.

For the comparison of the methods with the reference methods validation according ISO 16140 is chosen. For the RT-PCR methods both the primer and probes for E.coli and Enterococci were in house developed. Target genes were 16S Ribosomal RNA gene for E.coli and the 23S Ribosomal RNA gene for enterococci. Extraction was done with the NucliSENS Magnetic Extraction kit of Biomerieux and the RT-PCR was done in a Roche Light cycler 480 II PCR machine with the HawkZO5 Fast One-step RT-PCR Master Mix from Roche. Special care was taken by choosing the extraction kit and PCR kit with very low contaminating E.coli DNA/RNA concentrations. For the validation of the categorical parameters (specificity, sensitivity and accuracy) samples spiked with reference strains from environmental or animal source and real water samples were used.

Ac brief description of the RT-PCR method:

100 ml water sample is filtrated over 0.22 µm polycarbonate filter. The filter is folded and transported into an Eppendorf tube, followed by lysis and extraction with NucliSENS Extraction Kit. The resulting elution is divided into 4 wells from a 96 wells PCR-plate. Two duplicates with and without addition of positive control. The RT-PCR scheme is 5 minutes at 55°C, 5 minutes 60°C, 5 minutes 65°C followed by 50 cycles of 5 seconds at 92°C, 40 seconds at 60°C and 1 second at 72°C.Detection is done with HEX-label (533nm-610nm).

Biography :

Gerhard Wubbels has a long working history in the field of Water Biology. He has a lot experiences in microbiology and molecular biology and combines this knowledge for optimizing quality control of water. Besides Legionella, pathogenic bacteria and biofouling in drinking water systems his field of interest is risk-analysis and water quality (water Safety Plans and Hazard Analyses of Critical Control Points). In 2010 starting at WLN with introducing and developing molecular methods for drinking water control. In 2018 first these methods for fecal pollution are accepted as national methods for drinking water control in the Netherlands.

This work is partly presented at the Event on 12th Edition of International Conference on Microbiology, Antibiotics and Public Health Conference scheduled during **August 19-20, 2020** held at Berlin**,** Germany