WLN, Rijksstraatweg 85, 9756 AD Glimmen. Tel.: 050 402 21 21, www.wln.nl
G. Wubbels, M. van der Wiel

water | onderzoek | advies

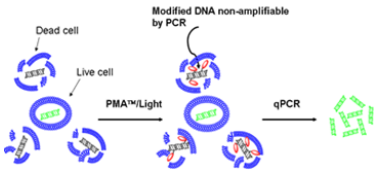
Quantification of infectious pathogenic Campylobacter species in water using QPCR and PMA

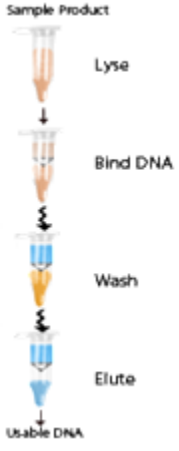
Introduction

Ingestion of Campylobacter via food or water is one of the main causes of acute diarrheal disease. Campylobacteriosis is normally a self-limiting disease, but complications like reactive arthritis or Guillain-Barre syndrome can occur. Especially birds and rodents are hosts of Campylobacter which are introduced in the drinking water pathway. Contamination of surface water is caused by excretion of campylobacter in the feces of these animals. To prevent infection with campylobacter via drinking water these bacteria have to be removed during purification of surface water meant for drinking water. A good and reliable method for detecting infectious Campylobacter species is very valuable in the Netherlands for the reason that there are now residual disinfectants used in drinking water to kill pathogenic bacteria. And therefore it is essential to know that during purification of surface water Campylobacter is diminished to acceptable levels to fulfill the legislation and to protect the consumer for disease. In this study we developed a quantitative real-time PCR for infectious pathogenic Campylobacter. To achieve this we combined the PCR with a Propidiummonoazide treatment (PMA) to detect just intact cells which can be infective.

Method

PMA treatment of a water sample before DNA isolation. Incubate sample and PMA for 5 min. In the dark in a final concentration of 50µM. Then expose the sample+PMA 5min. to a light source of 600W.



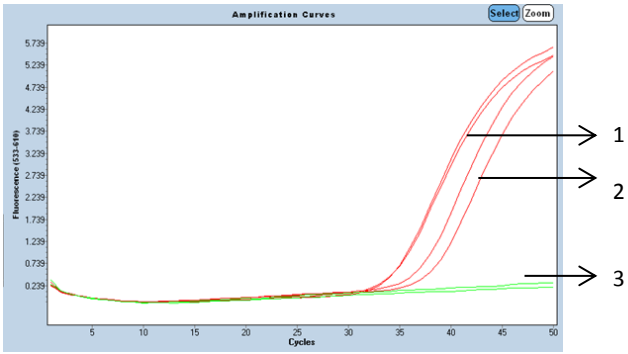


Primer/probe set for Campylobacter species:
16S gene:
C. species Forward: tga-ggg-aga-ggc-aga-tgg
C. species Reverse:: ggc-ggt-aca-ctt-aat-gcg-tta-g
C. species Probe: tca-cca-aga-ata-ccc-att-gcg-aag-g

Primer/probe set for internal control:
Plasmid with insert of dengue virus:
I.C. Forward: atg-aca-gcc-act-cct-ccg
I.C. Reverse: gga-acg-aac-caa-aca-gtc-ttc
I.C. Probe: agc-aga-gac-cca-ttc-cct-cag-agc

PCR program was as followed: 10min. Roomtemp. (uracil glycosylase), 5 min. 95°C (Denaturation), 50x 30sec. 95°C; 1min. 60°C; 30sec. 72°C.

Results



1) suspension A; 2) suspension A + PMA; 3) suspension A 30min 85˚C + PMA

This first result was a trial that showed us the working of PMA to a lab organism. In the table below you will see the results of another experiment. The result shows us a delta cp 1.28 between PMA treatment and a normal sample. That means that a factor 1.8 of the total cells is alive. (with a PCR efficiency of 67%)

SampleName	cDNA/reaction	cDNA / 500µl	Average Cp	delta Cp
Sample 1 +PMA	581.48	9691.25	32.76	1.28
Sample 1	1102.85	18380.76	31.48	

Conclusion

Our conclusion thus far is that the QPCR for Campylobacter in water can be used in routine; more experience has to build up with more real samples to understand the effect and usefulness of PMA treatment in comparison to infectious behavior of campylobacter and the effect of water treatment processes as UV-disinfection

Acknowledgment

Appl Environ Microbiol. 2010 Aug;76(15):5097-104. doi: 10.1128/AEM.00411-10. Epub 2010 Jun 18. Rapid quantification of viable Campylobacter bacteria on chicken carcasses, using real-time PCR and propidium monoazide treatment, as a tool for quantitative risk assessment.
Josefsen MH, Löfström C, Hansen TB, Christensen LS, Olsen JE, Hoorfar J. DK