qPCR based detection of different rotavirus genotypes from stool samples in a single experiment

Ion Gutiérrez-Aguirre[1], Andrej Steyer[2], Jana Boben[1], Mateja Poljšak-Prijatelj[2], Kristina Gruden[1] and Maja Ravnikar[1]

[1] Department of Plant Physiology and Biotechnology, National Institute of Biology (NIB), Ljubljana, Slovenia

[2] Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

INTRODUCTION:

Rotaviruses are the major cause of acute diarrhea in animals, infants and children under 5 years old. Viral genome consists of 11 dsRNA segments. Six structural proteins (VP1-VP4, VP6 and VP7) and six non-structural proteins (NSP1-NSP6). The most common rotaviral group is group A, infecting both humans and animals. According to the VP4 and VP7 antigenic and molecular characterization, group A rotaviruses are further classified into different p and g types. The most frequent genotype combinations in humans are G1 P[8], G2 P[4], G3 P[8], G4 P[8] and G9 P[8]. These are also the most predominant global genotypes.

In case of natural disasters, i.e., earthquakes, floods, etc., fecal waters can mix with potable waters. Rotaviruses can then, along with other pathogenic agents, be present in water supplies, thus constituting a possible risk for the population. The development of a fast and sensitive qPCR detection method which could detect very low rotavirus concentrations and cover as many rotavirus genotypes as possible would help to prevent such risks.

OBJECTIVES:

Design a sensitive TaqMan® qPCR approach for the detection all rotaviral genotypes found till the date in Slovenia, G1P[8], G2P[4], G3P[8], G4P[8], G8P[8], G9P[8] and G12P[8].

METHODS:

-Stool samples were characterized and genotypized by RT-nested PCR followed by sequencing of VP4 and VP7 genes and comparison with database sequences.

-Initial rotavirus concentration (part/ml) in the stool samples was estimated by Electron Microscope (EM) counting using, as standard, known concentration lateks beads of similar size as rotaviral particles **(Figure 2)**.

-The qPCR approach was targeted to the VP2 gene and based on an alignment of 7 different rotavirus strains. Primer Express program (ABI) was used to design reverse and forward primers and a MGB-probe. A mixture of forward and reverse primers and a degenerated probe were used in order to overcome the high sequence variability among rotavirus strains and, therefore, detect as many rotavirus genotypes as possible.

-Total RNA was extracted using TRIzol (Invitrogen) and RT-PCR was performed using High Capacity cDNA archive kit (ABI). 10µl qPCR reactions were monitored in a ABI PRISM 7900 HT (ABI).

RESULTS:

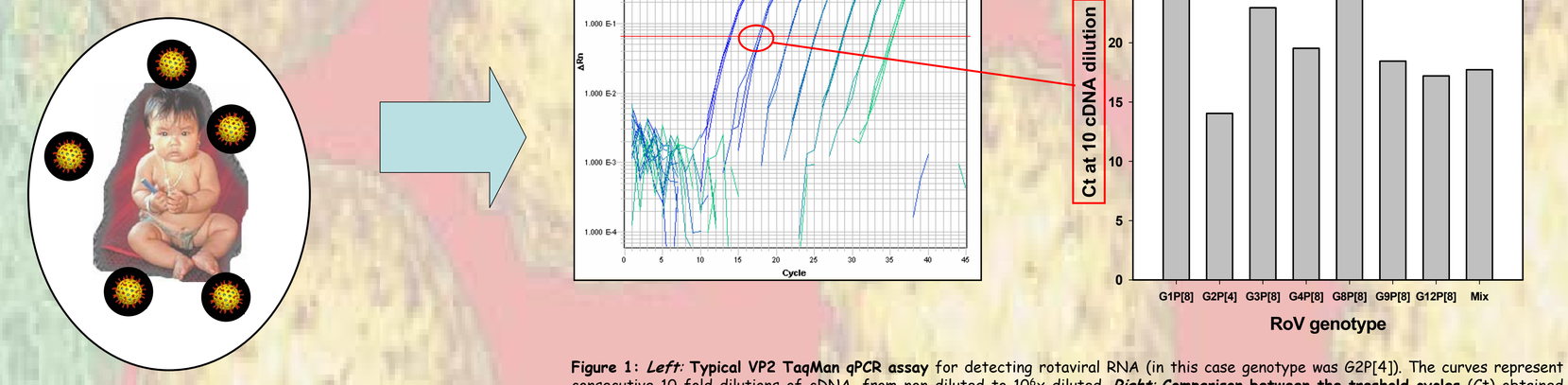

Figure 1: ***Left:*** **Typical VP2 TaqMan qPCR assay** for detecting rotaviral RNA (in this case genotype was G2P[4]). The curves represent consecutive 10 fold dilutions of cDNA, from non diluted to 10^6x diluted. ***Right:*** **Comparison between the treshold cycles** (Ct) obtained with the 10X diluted cDNA for the different tested rotaviral genotypes.

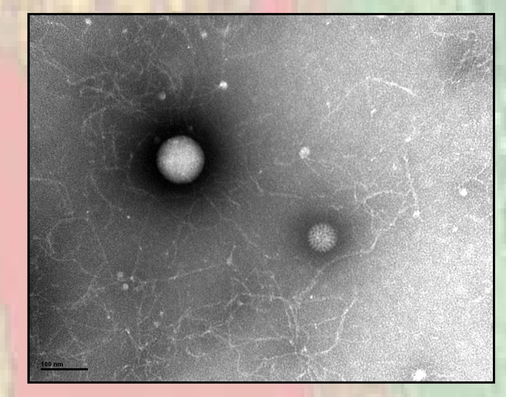

Figure 2: Estimation of the rotavirus (right down) concentration in initial stool samples by counting under EM together with latex beads (upper left) of similar size and known concentration

Table 1: Estimation of the L.O.D. Initial concentration (estimated by EM) of each Rotavirus sample used in the experiments shown in Fig3. From this values, taking into account the dilutions originated from the RNA isolation, RT mix, and qPCR mix, the number of target copies applied to the reaction were calculated (we assumed that the yields of RNA isolation and RT were of 100%). The minimum detectable number of target copies per reaction (L.O.D.) was approximately estimated for each genotype based on the data from Fig3.

Genotype	Initial Conc. (part/ml)	Target copies per reaction (non diluted cDNA)	L.O.D. (copies in reaction)
G1P[8]	$1{,}7x10^{11}$	$1{,}13x10^{8}$	113
G2P[8]	$6{,}1x10^{10}$	$4{,}06x10^{7}$	4,06
G3P[8]	$2{,}04x10^{10}$	$1{,}36x10^{7}$	13,6
G4P[8]	$1{,}29x10^{10}$	$8{,}6x10^{6}$	8,6
G8P[8]	$1{,}64x10^{10}$	$1{,}09x10^{7}$	1090
G9P[8]	$1{,}51x10^{10}$	$1{,}00x10^{7}$	10
G12P[8]	$4{,}59x10^{10}$	$3{,}06x10^{7}$	30,6
Mixture	$4{,}01x10^{10}$	$2{,}67x10^{7}$	26,7

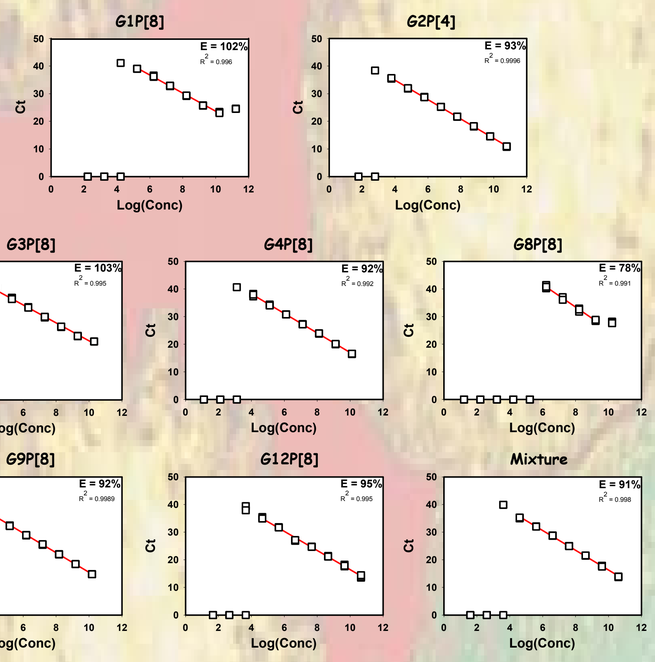

Figure 3: Characterization of the qPCR reaction for each of the rotavirus genotype tested. Dilutions of cDNA (from non diluted to 10^9x diluted) were applied by triplicate to 10 µl of final reaction volume. The Ct vs Log (conc) plot was built for each case. Indicated points were used in order to obtain the linear regression (red line). The obtained efficiency (E) and R^2 are indicated in each graphic. Concentration of initial samples was estimated as indicated in Fig2. The yield of RNA isolation and reverse transcription steps was assumed to be 100%.

CONCLUSIONS:

-We have developed a TaqMan qPCR method, which can detect most common rotavirus genotypes with a single approach, despite inherent sequence variability. The test is suitable for being used as screening test for the presence of Rotavirus in stool samples.

-Preliminary estimations, situate the LOD in the range of 10^1-10^3 target copies/reaction. As the assumed yield of 100% for the RNA isolation and RT steps is probably lower, it is likely that we are detecting even lower number of copies.

-This approach, in combination with an appropiate concentration method, will be used in order to monitor environmental water samples for the presence of Rotavirus.