Comparison of two different real-time PCR assays and chemistry for detection of norovirus genogroup II. Method of choice?

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Introduction

Human noroviruses, members of *Caliciviridae* family are one of the main cause of acute non-bacterial gastroenteritis in humans of all ages. They are highly contagious and can be spread between people by direct contact or through contaminated food and water, causing explosive outbreaks in environments where people are in close contact such as hospitals, homes, schools and other resorts. Sporadic cases are frequent, too. Noroviruses can not be propagated in cell cultures. Thus molecular assays (reverse transciptase-polymerase chain reaction in real- time (RT-PCR) for caliciviral detection have been developed. Recently real-time RT-PCR has been introduced (Dingle et al., 1995; Hoehne and Schreier, 2006; Kageyama et al., 2003).

Aim of the study

The aim of the study was to design and compare two different real-time RT-PCR assays for detection of norovirus genotypes belonging to genogroup II.

1. For targeting a 95 base-pair fragment in ORF1–ORF2 (open reading frame) junction of the genome, genogroup II specific primers (COG2F/COG2R) were used (Kageyama et al., 2003). For the purpose of our study the detection probe RING2-TP from the same author was slightly modified: in one assay a 20 base-long dual labelled fluorogenic probe (FAM-BBQ) (BBQ-probe) (Metabion) and in the other a 15 base-long TaqMan MGB probe (MGB-probe) (Applied Biosystems) was designed.
2. The linear range of detection and efficiency of amplification for both real-time RT-PCR assays combined with three different reagents were established. First one-step real- time RT-PCR was performed using AgPath-ID One-Step RT-PCR kit (Ambion) or OneStep RT-PCR Kit (Qiagen) and second two-step real- time RT-PCR reagent kit (Applied Biosystems) was tested.
3. In addition the comparison in the view of sensitivity of methods performed on two different real-time PCR instruments (StepOne and ABI PRISM 7900HT (Applied Biosystems)) was made.

Methods

Strain selection

To test efficiency of real-time RT-PCRs on a wider range of noroviruses, a group of different norovirus genotypes belonging to genogroup II, have been selected (II.1, II.2, II.3, II.4, II.6, II.7, II.10, II.b, II.c). In addition a strain from genogroup IV was also included. The starting RNA transcripts were kindly provided by RIVM, The Netherlands.

Real-time RT-PCR

Two assays using BBQ- or MGB-probe were combined with three different reagents and performed on two instruments:

1. Two-step RT and real-time PCR (Applied Biosystems)

RT was performed using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) followed manufacture's instructions. 5 µl of RNA transcript was added to a final volume of 20 µl RT reaction. Real-time PCR was performed using 900 nM forward primer, 900 nM reverse primer, 200 nmol BBQ-probe or MGB-probe and TaqMan Universal Master Mix (Applied Biosystems). A final reaction volume of 20 µl real-time PCR reaction contained 2 µl of corresponding cDNA. The designed real-time PCR protocol was performed on both instruments (2' at 50º C, 10' at 95º C, followed 45 cycles: 15" at 95º C and 1' at 56º C).

2. AgPath-ID One-Step RT-PCR Kit (Ambion) and 3. OneStep RT-PCR Kit (Qiagen)

The real-time RT-PCR was carried out in 25 µl reaction mixture containing 2 µl of RNA transcript, 900 nM forward primer, 900 nM reverse primer, 200 nmol BBQ-probe or MGB-probe, and AgPath-ID One-Step RT-PCR Kit or OneStep RT-PCR Kit. PCR amplification was performed on ABI PRISM 7900HT or StepOne instrument under following conditions: AgPath-ID One-Step RT-PCR kit (10' at 45º C, 10' at 95º C followed 45 cycles: 15" at 95º C, 45" at 56º C); OneStep RT-PCR kit (30' at 50º C, 15' at 95º C followed 45 cycles: 15" at 95º C, 1' at 56º C). To determine the dynamic range of detection and efficiency of amplification for both real-time RT-PCR assays using two different one-step RT-PCR reagents standard curves were generated using 10-fold serial dilutions of genotype II.2.

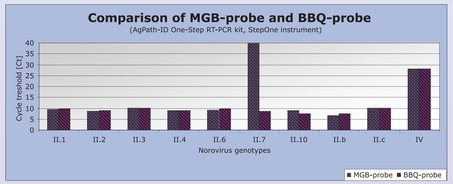
Results

Comparison of amplification efficiency and sensitivity between MGB-probe and BBQ-probe

Table 1: Average of Cycle thresholds (Ct) (ave Ct) and Standard deviation (SD) calculated from Cts differences between Ct values generated with MGB-probe and BBQ-probe assay ($Ct_{BBQ\text{-}probe}$ - $Ct_{MGB\text{-}probe}$)

	AgPath-ID One-Step RT-PCR Kit		High Capacity cDNA Reverse Transcription Kit		OneStep RT-PCR kit	
	ave ΔCt	SD	ave ΔCt	SD	ave ΔCt	SD
StepOne	-0.13	±0.51	-1.03	±1,03	-0.51	±0.60
ABI PRISM 7900HT	0.19	±0.33	-1,15	±0.80	0.54	±0.89

Figure 1: Comparison between $Ct_{MGB\text{-}probe}$ and $Ct_{BBQ\text{-}probe}$



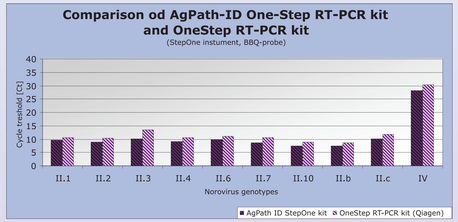
The sample II.7 was not detected using MGB-probe with any used RT-PCR reagents on any used instrument.

Comparison between sensitivity achieved by performing on StepOne and ABI PRISM 7900HT instrument

Table 2: Average of Cycle thresholds (Ct) (ave Ct) and Standard deviation (SD) calculated from Cts differences between Ct values generated with MGB-probe or BBQ-probe assay with AgPath-ID One- Step RT-PCR kit or OneStep RT-PCR kit ($Ct_{AgPath\text{-}ID\ One\text{-}Step\ RT\text{-}PCR\ Kit}$ -$Ct_{OneStep\ RT\text{-}PCR\ kit}$).

	MGB-probe		BBQ-probe	
	ave ΔCt	SD	ave ΔCt	SD
StepOne	-2.25	±2.50	-1.46	±0.60
ABI PRISM 7900HT	-2.98	±0.7	-3.43	±1.00

Figure 2: Comparison between $Ct_{AgPath\text{-}ID\ One\text{-}Step\ RT\text{-}PCR\ Kit}$ and $Ct_{OneStep\ RT\text{-}PCR\ kit}$



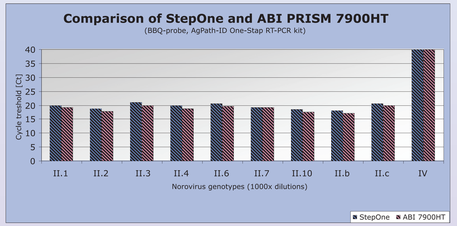
The Ct values obtained with AgPath-ID One-Step RT-PCR kit (Ambion) were always lower than using OneStep RT-PCR kit (Qiagen).

Determination of the linear range of detection and efficiency of amplification

Table 3: Average of Cycle thresholds (Ct) (ave Ct) and Standard deviation (SD) calculated from Cts differences between Ct values generated with MGB-probe or BBQ-probe assay with both RT-PCR reagents ($Ct_{ABI\ PRISM\ 7900HT}$ - $Ct_{StepOne}$).

	AgPath-ID OneStep RT-PCR Kit		OneStep RT-PCR kit	
	ave ΔCt	SD	ave ΔCt	SD
MGB-probe	-1.26	±0.44	-0.05	±0.23
BBQ-probe	-0.85	±0.32	1.09	±0.93

Figure 3: Comparison between $Ct_{StepOne}$ and $Ct_{ABI\ PRISM\ 7900HT}$



The sensitivity of method performed on ABI PRISM 7900HT is at average 0.6 times higher in comparison with the method performed on StepOne instrument.

To define the linear range of detection and efficiency of amplification for both assays, using two different one-step RT-PCR reagents, standard curves were generated (Figure 4 and Figure 5) and efficiency of amplification was calculated (Table 4).

Figure 4: Standard curves generated with both assays and AgPath-ID One-Step RT-PCR kit and OneStep RT-PCR kit performed on ABI PRISM 7900HT instrument.

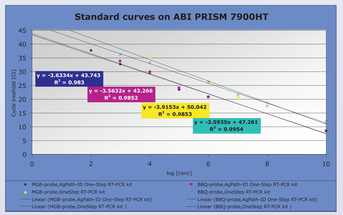


Figure 5: Standard curves generated with both assays and AgPath-ID One-Step RT-PCR kit and OneStep RT-PCR kit performed on StepOne instrument.

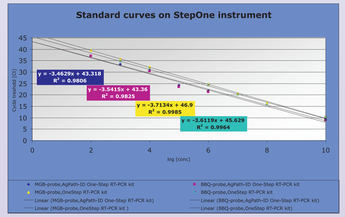


Table 4: Efficiency of amplification

Efficiency	AgPath-ID One-Step RT-PCR kit		OneStep RT-PCR kit	
	MGB-probe	BBQ-probe	MGB-probe	BBQ-probe
StepOne	94.4	91.6.	85.9	89.2
ABI PRISM 7900HT	88.5	90.8	85.9	89.8

In average the best amplification on both instruments was achieved combining BBQ-probe with AgPath-ID One-Step RT-PCR kit.

Conclusions

- BBQ-probe combined with AgPath-ID™ One-Step RT-PCR kit is considered the best solution. This combination of assay and RT-PCR chemistry offers more sensitive and reliable detection of norovirus genogroup II strains performed on both real-time PCR instruments.
- The sensitivity of methods performed on ABI PRISM 7900HT is higher in comparison with the same methods performed on StepOne instrument.

Reference

1] Dingle, K.E., Lambden, P.R., Caul, E.O. and Clarke, I.N. 1995. Human enteric Caliciviridae: the complete genome sequence and expression of virus-like particles from a genetic group II small round structured virus. J Gen Virol 76: 2349-2355.

2] Hoehne, M., Schreier, E. 2006. Detection of Norovirus genogroup I and II by multiplex real-time RT- PCR using a 3'-minor groove binder-DNA probe. BMC Infectious Diseases 2006, http://www.biomedcentral.com/1471-2334/6/69

3] Kageyama, T., Kojima, S., Shinohara, M., Uchida, K., Fukushi, S., Hoshino, F.B., Takeda, N., Katayama, K. 2003. Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. J Clin Microbiol, 41 (4): 1548-1557.