Respiratory picornavirus genotyping conventional nested RT-PCR ("Wisdom VP42 assay")

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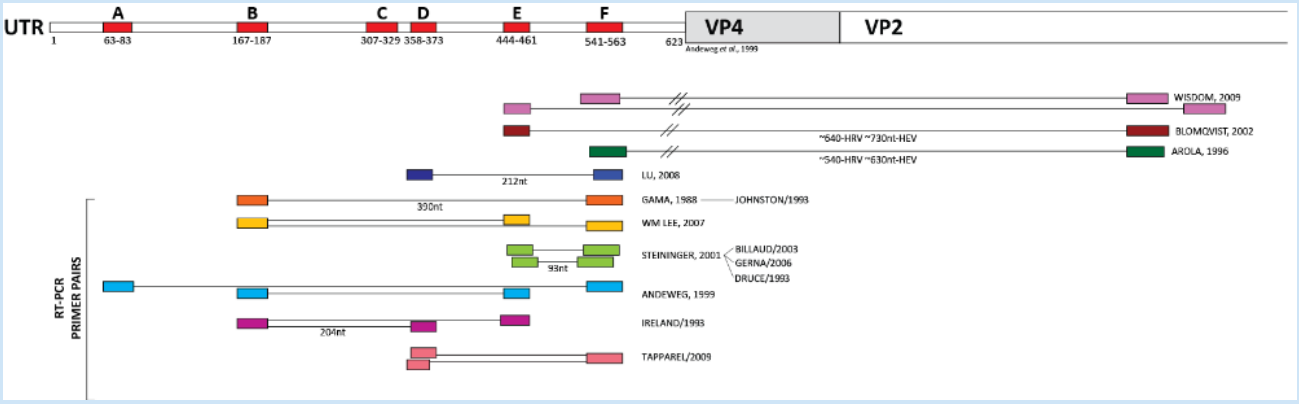
Abstract

This is my preferred, previously published [Ref 1], rhinovirus (RV) and enterovirus (EV) genotyping assay when seeking to characterize the genotypes of respiratory picornavirus identified after use of a screening real-time RT-PCR to test nucleic acid extracts from clinical samples.

I have not confirmed that it can detect every single RV genotype but I do know that it detects *many* from each of the three RV species (*Human rhinovirus A*, *Human rhinovirus B* and *Human rhinovirus C*) as well as at least some *Human enterovirus* (EV) genotypes.

The assay picks up EVs due to the shared genetic similarities in the 5'UTR target region. EVs can be discriminated using subgenomic sequencing (see VP42 typing assay protocol), or simply described as 'respiratory EVs' since there is no specific-specific vaccine or treatment available anyway.

This is a robust primary subgenomic sequencing assay. It is more sensitive than any VP1 protocols because it targets more conserved primer target sites. It produces a more reliable typing result than does the 5'UTR region alone.



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Protocol

Oligonucleotide sequences...

Step 1.

	Name	Sequence (5'-3')
RT-PCR (Round 1)	HRV_HEV VP42 OS	CCGGCCCCTGAATGYGGCTAA
	HRV_HEV VP42 OAS	ACATRTTYTSNCCAAANAYDCCCAT
PCR (Round 1)	HRV_HEV VP42 IS	ACCRACTACTTTGGGTGTCCGTG
	HRV_HEV VP42 IAS	TCWGGHARYTTCCAMCACCANCC

1. Expected amplicon sizes: Round 1: 380 base pairs; Round 2:
2. The naming used here is my in-house adaptation (FYI: 01 - forward / sense; 02 - reverse / antisense; .x - version of the design of this particular named oligonucleotide). If you prefer to be true to the original publication, please see Ref 1

Reagents

Step 2.

REAGENTS

SensiFAST Probe no ROX one-step kit BIO-76005 by Bioline

MyTaq HS DNA Polymerase BIO-21113 by Bioline

Reaction set-up

Step 3.

RT-PCR (Round 1)

Reagent	Vol (µl) 1x	Final reaction concentration
Nuclease-free water	1.4	N/A
SensiFAST no ROX One-Step Mix (2X)	10	1X
Primers (µM)[1]	6	600nM
$MgCl_2$	0	3mM
RNase inhibitor	0.4	Unknown
RT/Taq (?U/µl)	0.2	Unknown
Template	2	N/A

1. Both mixed to this final concentration
2. Dispense 18µL to each reaction tube.
3. Add 2µL of template (extracted RNA, controls or NTC [nuclease-free water])
4. Total reaction volume is 20µL

PCR (Round 2)

Reagent	Vol (µl) 1x	Final reaction concentration
Nuclease-free water	8.7	N/A
MyTaq Reaction Buffer (5X)	4	1X
Primers (µM)[1]	3.8	380nM
$MgCl_2$	1.4	4.75mM
MyTaq HS DNA Polymerase (5U/uL)	0.2	Unknown
1st round amplicon	2	N/A

1. Both mixed to this final concentration
2. Dispense 18µL into each reaction tube
3. NB: a 1:100 pre-dilution can be made first
4. Total reaction volume is 20µL

Amplification

Step 4.

RT-PCR (Round 1)

45°C	20 min	1X
94°C	2 min	1X
94°C	18 sec	|
50°C	21 sec	| 35X
72°C	90 sec	|
72°C	7 min	1X
4°C	∞	

95°C	1 min	1X
94°C	18 sec	|
50°C	21 sec	| 35X
72°C	90 sec	|
4°C	∞	

Amplification

Step 5.