# Human rhinovirus screening real-time RT-PCR ("modified Lu assay")

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## Abstract

This is an excellent rhinovirus screening assay which I have used on thousands of sample extracts mostly originating from acutely ill paediatric patients, spanning well over a decade's worth of collection dates.

I have not confirmed that it can detect every single 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* genotypes.

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

The assay was created and first described in 2008 by Xiaoyan Lu and colleagues at the US CDC [Ref 1]. In collaboration with Lu et al., we published a small update that included Lu's modified forward primer design. We routinely use it with this primer (hence the 'mod' text added to the primer name) but without the expensive special base, as outlined in Step 1.

## Before start

- If using a different brand or model of real-time thermocycler, check the concentration of ROX is adequate.
- Method assumes the user is familiar with the thermocycler and software used to run the protocol.

## Materials

SensiFAST Probe no ROX one-step kit BIO-76005 by [Bioline](#)

## Protocol

**Step 1.**

| Name | Sequence (5'-3') |
|---|---|
| panHRV_LU_01_ mod2 | CY+AGCC+TGCGTGGY |
| panHRV_02.2 | GAAACACGGACACCCAAAGTA |
| panHRV_TM | TCCTCCGGCCCCTGAATGYGGC |

1. The original Lu *et al*. forward primer [Ref 1] was modified [Ref 2].
2. We do not use the '**P**' originally designated at the '**Y**' position [see Ref 1 for details]. This is an expensive pyrimidine derivative degeneracy that mimics a **C/T** mix
3. The '+' represents an LNA '**A**' or '**T**' base
4. 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 and Ref 2.

Reagents
**Step 2.**

REAGENTS

SensiFAST Probe no ROX one-step kit BIO-76005 by [Bioline](#)

Reaction set-up
**Step 3.**

The assay has been used on both a Rotor-Gene 6000 and a Rotor-Gene Q real-time thermocycler

Prepare sufficient mix for the number of reactions.

Include a suitable 'dead volume' as necessary if using a robotic dispenser.

| Reagent | Vol. (ul) x1 | Final reaction concentration |
|---|---|---|
| Nuclease-free water | 0.8 | N/A |
| SensiFAST no ROX One-Step Mix(2X) | 10 | 1X |
| Primers (2μM)[1] | 4 | 400nM |
| Probe (2μM) | 1 | 100nM |
| $MgCl_2$ (25mM) | 1.6 | 5mM |
| RNase inhibitor | 0.4 | Unknown |

| | | |
|---|---|---|
| RT (?U/µl) | 0.2 | Unknown |
| Template | 2 | N/A |

1.  Both mixed tot his final concentration

2.  Dispense 15µL to each reaction well.

3.  Add 5µL of template (extracted RNA, controls or NTC [nuclease-free water] )

4.  Total reaction volume is 20µL

## Amplification
**Step 4.**

| | | |
|---|---|---|
| 45°C | 20min | 1X |
| 94°C | 2min | 1X |
| | | |
| 94°C | 5sec | \| 55X |
| 60°C | 60sec* | \| |

*Fluorescence acquisition step

## Result analysis
**Step 5.**

The definition used for a satisfactory positive result from a real-time fluorogenic PCR should include each of the following:

1. A **sigmoidal curve** – the trace travels horizontally, curves upward, continues in an exponential rise and followed by a curve towards a horizontal plateau phase

2. A **suitable level of fluorescence** intensity as measured in comparison to a positive control (y-axis)

3. A **defined threshold ($C_T$) value** which the fluorescent curve has clearly exceeded (Fig.1 arrow), which sits early in the log-linear phase and is <40 cycles

A flat or non-sigmoidal curve or a curve that crosses the threshold with a $C_T$ >40 cycles is considered a negative result. NTCs should not produce a curve.
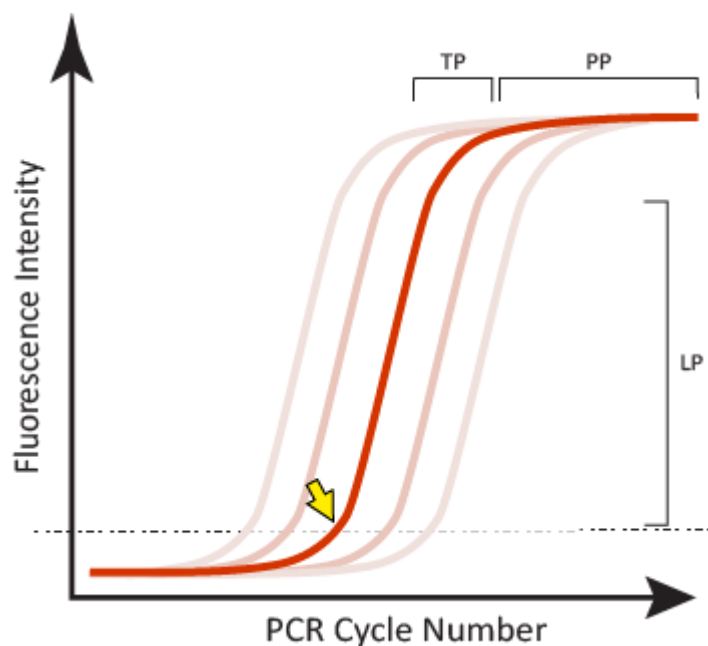
**Figure 1**. Examples of satisfactory sigmoidal amplification curve shape when considering an assay's fluorescent signal output. The crossing point or threshold cycle ($C_T$) is indicated (yellow arrow); it is the value at which fluorescence levels surpass a predefined (usually set during validation, or arbitrary) threshold level as shown in this normalized linear scale depiction. LP-log-linear phase of signal generated during the exponential part of the PCR amplification; TP-a slowing of the amplification and accompanying fluorescence signal marks the transition phase; PP-the plateau phase is reached when there is little or no increase in fluorescent signal despite continued cycling.