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Multiplex RT-qPCR for RSV A and B Typing

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Albert Campoy¹, Maria Iglesias¹, Sonia Vázquez-Morón^{1,2}, Francisco Pozo^{1,2}, Inma Casas^{1,2}

¹Laboratory of Reference and Research in Respiratory Viruses, National Centre for Microbiology, Instituto de Salud Carlos III, 28220 Majadahonda, Spain;

²CIBER de Epidemiología y Salud Pública (CIBERESP), ISCIII, Madrid, Spain.



Albert Campoy

National Institute of Health Carlos III

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Protocol status: Working

We use this protocol and it's working

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Abstract

This SOP describes a multiplex RT-qPCR assay for the detection and typing of respiratory syncytial virus (RSV) into subgroups A and B, enabling simultaneous identification of both targets in a single reaction.

Troubleshooting

Materials

1 Primers:

Primer	Sequence (5'-3')
RSAB1F	TTCTTGTTAGGTGTWGGATCTGCAAT
RSAB1R	GTTACACCTGCATTRACACTRAATTC
RP-F	GACTTCAGCATGGCGGTGT
RP-R	GAGCGGCTGTCTCCACAAGT

2 Probes:

Probe	Sequence (5'-3')
RSVA-FAM	TCTACTATCCACAAACAAGGCTGTAGTC
RSVB-HEX	TTCCAACAATCTGCTGTTCTTCTGCTG G
RP-TxRd	TTCTGACCTGAAGGCTCTGCGCG

Master Mix Preparation

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Reagent	Use []	1x (ul)
H2O		5.25
2x PCR Master Mix		12.5
RSAB1F Primer	40 uM	0.5*
RSAB1A Primer		
RP-F Primer	20 uM	0.5*
RP-R Primer		
RSV A-FAM Probe	10 uM	0.25

Reagent	Use []	1x (ul)
RSV B-HEX Probe		0.25
RP-TxRd	2.5 uM	0.25
SuperScript III RT/Platinum		0.5

*This volume refers to a pull composed of the two primers

Cycling Conditions

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A	B	C
52°C	30 min	
95°C	2 min	
95°C	15 seg	50x
55°C	1 min 30 seg	