



UK Clinical Virology Network



Performance of in-house real-time PCR assays for the detection of respiratory syncytial virus (RSV) types A and B

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Summary

Laboratories across the UK are using a range of different in-house assays for the detection of viral targets. A small-scale assessment of ten such assays in use for the detection for RSV A and B infections indicated that the assays performed at a similar level of sensitivity and with the majority showing 100% specificity.

Introduction

Although UK laboratories are using different in-house assays for the detection of viral targets, their performance has not been compared. To address this, the Clinical Virology Network (CVN) and the HPA Microbiological Diagnostics Assessment Service set up an assessment of in-house real-time PCR assays to gain some insight into the performance of assays being used across UK diagnostic laboratories and to inform on the adoption of best practice. The CfI Respiratory Virus Unit provided specimens and initial testing for a panel of specimens which was then distributed to members of the CVN and HPA Molecular Diagnostics Forum (MDF).

Materials and Methods

The CVN and MDF invited laboratories to submit Standard Operating Procedures of in-house real-time assays that are in regular diagnostic use to the study. A summary of these protocols is given in Appendix 2.

Titred laboratory strains of RSV A and RSV B viruses were provided by the CfI Respiratory Virus Unit to generate a specimen panel for distribution across participating labs in MDF and CVN. There were insufficient volumes of original patient specimens to include in the panel, plus relatively low viral titres meant only limited dilutions could be generated. The specimen panel consisted of 500µl aliquots of tissue culture grown virus diluted in either RSV-negative nose and throat swab material (NTS) or virus transport medium (VTM). Appropriate dilutions were chosen based on the routine diagnostic assay of the CfI Respiratory Virus Unit. The panel composition is given in Table 1.

Table 1: Specimen panel composition

Sample type	No. of tests	Details
RSV A (Long)	7	Virus positive tissue-culture material diluted in VTM. A dilution series of five samples, plus two additional low and high samples diluted in NTS
RSV A (A2)	2	Virus positive tissue-culture samples at low and high concentrations diluted in NTS
RSV B	9	Virus positive tissue-culture material diluted in VTM. A dilution series of five samples, plus two additional low and high samples diluted in NTS or VTM material tested in duplicate
Mixed viruses	3	RSV A (Long) + RSV B at three different dilutions; two diluted in VTM and one in mixed VTM and NTS.
Negative	4	One NTS and three VTM samples
TOTAL	25	

The panel numbers were randomised and the panel distributed on dry ice to the participant laboratories, to be stored at -70°C and tested within 10 working days of receipt.

Results

Ten laboratories registered for inclusion in the study (including one NASBA assay), all of which returned results. Respondents provided information on Ct (cycle at which the signal crosses the threshold signifying positivity), type (where available), run validation, and interpretation of results.

Inter-laboratory Ct values are given in Appendix 3 (together with details of the panel composition). Table 2 summarises the number of positives obtained by each laboratory for the RSV A and RSV B dilutions: these ranged between 6 and 7 for RSV A (Long), and 6 and 9 for RSV B. Both dilutions of the RSV A A2 strain were found to be positive by all the laboratories, as were the mixed viral samples. Three laboratories (A, D and F) detected all of the samples expected to contain RSV. There was a suggestion that one laboratory (E) might be detecting RSV B at a lower sensitivity (6/9) compared to RSV A (7/7). There were no false positive results.

Table 2: Number of positive reactions from each laboratory

Lab Identification	RSV A (Long) dilutions (n=7)	RSV B dilutions (n=9)	Total
A	7	9	16
B	6	7	13
C	7	8	15
D	7	9	16
E	7	6	13
F	7	9	16
G	6	9	15
H	6	7	13
I	6	9	15
J	7	9	16
Average (mean)	6.6	8.2	14.8

The majority of laboratories reported the presence or absence of RSV, with only four distinguishing between RSV A and B (Appendix 4). Two of these four laboratories (Labs C and H) correctly identified all of the single type specimens where a positive result was obtained (with a single weakly amplified specimen being untypable by one laboratory). Two laboratories (Labs E and I) incorrectly identified RSV A specimens as RSV B (one RSV Long and one RSV A2 strain). Two laboratories also identified mixed RSV A and B specimens: two of three by Lab E and a single example by Lab I.

Conclusion

There are a range of in-house real-time PCR assays in use across the UK, which use different chemistries and platforms (Appendix 2). This study provides an initial indication of their performance although it should be noted that both the small number of specimens and variations in methodologies (e.g. initial sample volume extracted, volume of nucleic acid in final reaction etc) limit the conclusions that can be drawn.

Variation was observed in the detection of RSV between laboratories but all laboratories were able to identify RSV. Of those laboratories offering subtyping of RSV A and RSV B, the majority of samples were correctly differentiated.

Acknowledgements

We would like to thank VRD for providing the titred viruses, VTM and NTS material and baseline test data used for this assessment.

Appendices

Appendix 1: Panel details

CVN no.	Virus	Dilution	Titre (pfu/ml)	Diluted in
RSV 13	RSV A (Long)	10^{-2}	6×10^5	VTM
RSV 05	RSV A (Long)	10^{-3}	6×10^4	VTM
RSV 01	RSV A (Long)	10^{-4}	6×10^3	VTM
RSV 15	RSV A (Long)	10^{-5}	6×10^2	VTM
RSV 03	RSV A (Long)	10^{-6}	6×10^1	VTM
RSV 18	RSV A (Long)	10^{-3}	6×10^4	NTS
RSV 25	RSV A (A2)	10^{-3}	6×10^4	NTS
RSV 22	RSV A (Long)	10^{-5}	6×10^2	NTS
RSV 04	RSV A (A2)	10^{-5}	6×10^2	NTS
RSV 09	RSV B	10^{-1}	4×10^4	VTM
RSV 21	RSV B	10^{-2}	4×10^3	VTM
RSV 02	RSV B	10^{-3}	4×10^2	VTM
RSV 06	RSV B	10^{-4}	4×10^1	VTM
RSV 16	RSV B	10^{-5}	4	VTM
RSV 12	RSV B	10^{-2}	4×10^3	NTS
RSV 20	RSV B	10^{-2}	4×10^3	VTM
RSV 11	RSV B	10^{-4}	4×10^1	VTM
RSV 23	RSV B	10^{-4}	4×10^1	VTM
RSV 14	RSV A (Long)+RSV B	$10^{-3}+10^{-4}$		VTM
RSV 19	RSV A (Long)+RSV B	$10^{-5}+10^{-2}$		VTM+NTS
RSV 17	RSV A (Long)+RSV B	$10^{-5}+10^{-4}$		VTM
RSV 07	Negative			NTS
RSV 08	Negative			VTM
RSV 10	Negative			VTM
RSV 24	Negative			VTM

VTM: viral transport medium; NTS: nose and throat swab

Appendix 2: Assay summary from SOP's provided

Lab ID	PCR platform	Extraction method (vol in/elution vol)	Reverse transcription (vol extract added/total RT vol)	Volume of RT reaction added (total rxn vol)	Chemistry/detection method ¹	RSV Gene target(s)	Comments	Reference(s)
A	ABi Prism 7500 fast	Roche MagNAPure Compact (200µl/100µl)	One-step RT-PCR using Invitrogen Superscript III/Platinum taq (10µl/30µl)	N/A	DLP	N gene	Co-extracted BMV RNA internal control tested in a parallel multiplex.	Gunson <i>et al.</i> 2005: J Clin Virol. 33 :341-4.
B	ABi Prism 7500 fast	Thermo Fisher Scientific Kingfisher 96 (100µl/75µl)	Random hexamers (36µl/50µl)	5µl (20µl)	DLP	N gene	Internal control; two duplex reactions run: FluA/FluB; hMPV/RSV	-
C ²	ABi 7500 fast	Roche MagNAPure (Total NA) (200µl/50µl)	One step RT-PCR Qiagen Multiplex RT-PCR (5µl/20µl)	N/A	DLP	L gene	Shared primers and separate A and B probes. Can be run with MS2 in multiplex	-
D	Corbett RotorGene 3000	Roche MagNAPure Compact (200µl/60µl)	One-step RT-PCR using Invitrogen Superscript III/Platinum taq (5µl/25µl)	N/A	DLP	NP-Gene	Co-extracted MS2 RNA internal control reactions run: as a Triplex with PIV and Adenovirus	-
E	Corbett RotorGene 3000	Corbett X-tractor Gene machine (700µl/100µl)	SuperScript III Platinum One-Step Quantitative RT-PCR system (7.5µl/25µl)	N/A	DLP	N gene	Pre-lysed samples (X-tractor Gene liquid reagent pack) extracted. Duplex RSV A & B; internal control added after lysis and prior to extraction. Internal control used MS2 phage.	-
F	ABi 7500	Qiagen MDx (300µl/100µl)	Random hexamers (20µl/40µl)	5µl (30µl)	DLP (MGB)	N gene		-
G	Roche 480	Roche MagNAPure (200µl/50µl)	SuperScript III Platinum One-Step Quantitative RT-PCR system used (5µl/25µl)	N/A	DLP	N gene		Hu <i>et al.</i> 2003: J Clin Micro. 41 :149-54.
H	Corbett RotorGene	Qiagen EZ1 (400µl/120µl)	One-step RT-PCR using Qiagen QuantiTect Multiplex RT-PCR NR kit (10µl/30µl)	N/A	DLP	N gene	SOP under review. 3 multiplex reactions: FluA/FluB/hMPV; PIV1/2/3; RSV A/RSV B/HRV	FluA/B/PIV3 : Templeton <i>et al.</i> 2004: J Clin Micro. 42 (4):1564-9. RSV A/B/HRV : Gunson <i>et al.</i> 2005: J Clin Virol. 33 :341-4. PIV : Kuypers <i>et al.</i> 2006: J Clin Micro. 44 (7) :2382-8.
I	ABi Prism 7500	Qiagen BioRobot 9604 or QIAamp MiniElute Virus Kit (200µl/200µl)	One-step RT-PCR (specific primer) (10µl/25µl)	N/A	DLP	N gene	Triplex: rhinovirus/RSV A/RSV B	RSV : van Elden <i>et al.</i> 2003: J Clin Microbiol. 41 (9):4378-81. Rhinovirus : Bredius <i>et al.</i> 2004: Pediatr Infect Dis J. 23 (6):518-22 RSV : Moore <i>et al.</i> 2006: Eur J Clin Microbiol infect Dis. 25: 167-74
J	NucliSens EasyQ	bioMérieux EasyMag (200µl/60µl)	NASBA	5ul (20µl)	Real-time NASBA	Fusion	No Ct values obtained; internal control coextracted	

Vol = volume; eln = elution; rxn = reaction. 1. DLP: Dual-labelled probes (also known as TaqMan or hydrolysis probes); MGB: minor groove binder; NASBA: nucleic acid sequence-based amplification. 2. Subsequent to the completion of this study, this laboratory changed their reaction mix to Invitrogen SSIII Platinum RTPCR and retested the samples, whereby all of the RSV-positive samples were detected.

Appendix 3: Individual laboratories Ct results

CVN no.	Virus	Dilution	Laboratory ID - Ct values													
			A(1)	A(2)	B(1)	B(2)	B(3)	C	D	E(1)	E(2)	F	G	H(1)	H(2)	I
RSV 13	RSV A (Long)	10-2	21.90	22.17	27.30	27.70	27.15	27.8	20.1	24.42	24.79	25	31	24.46	22.52	22.07
RSV 05	RSV A (Long)	10-3	25.67	25.94	32.05	31.01	31.73	31.5	22.9	28.18	27.98	29	35	25.34	25.30	25.48
RSV 01	RSV A (Long)	10-4	30.19	30.63	35.67	35.00	35.61	34.0	26.6	32.32	31.98	31	39	28.29	28.46	27.95
RSV 15	RSV A (Long)	10-5	34.73	35.27	38.62	38.14	38.16		30.7	36.11	36.40	35	42			31.72
RSV 03	RSV A (Long)	10-6	37.05	37.15		41.82			32.8	40.05	40.33	38		38.60	39.22	
RSV 18	RSV A (Long)	10-3	28.30	28.25	31.83	32.32	31.71	31.1	22.9	29.02	28.96	30	37	27.88	25.54	25.09
RSV 25	RSV A (A2)	10-3	26.66	26.15	25.90	26.73	25.83	27.2	19.3	25.32	25.08	27	31	26.77	26.19	21.46
RSV 22	RSV A (Long)	10-5	36.02	36.08	38.90	38.71	37.23		30.4	35.66	36.76	36	42	38.13	34.85	33.77
RSV 04	RSV A (A2)	10-5	29.22	29.06	33.56	35.54	33.71	34.3	27.3	32.49	32.68	34	39	33.80	35.11	30.50
RSV 09	RSV B	10-1	19.01	18.85	32.18	31.64	31.56	27.1	17.1	30.31	29.96	22	25	19.74	19.28	17.92
RSV 21	RSV B	10-2	25.41	25.59	36.18	35.66	35.80	30.5	20.0	31.96	32.02	25	31	23.63	24.04	20.91
RSV 02	RSV B	10-3	25.88	26.35	40.09	39.01	39.39	33.2	24.4	36.92	37.49	28	35	24.65	24.67	23.99
RSV 06	RSV B	10-4	29.90	29.52		41.61	43.11	35.0	29.7			32	37	34.08	33.96	27.30
RSV 16	RSV B	10-5	34.60	35.04					30.9			34	44			30.03
RSV 12	RSV B	10-2	22.69	22.44	35.65		36.50	30.5	20.5	37.52	36.74	26	31	27.43	23.02	20.28
RSV 20	RSV B	10-2	23.74	23.67	35.90	35.71	36.04	30.4	18.5	31.91	31.96	25	31	23.41	23.32	20.62
RSV 11	RSV B	10-4	29.35	29.54			44.35	36.0	29.0			32	36			27.19
RSV 23	RSV B	10-4	32.10	31.93	41.08	40.93	44.00	37.6	28.5	37.45	40.55	31	42	38.00	37.47	27.25
RSV 14	RSV A (Long)+RSV B	10-3(A)+10-4(B)	25.67	25.55	31.85	32.19	31.58	30.9	23.1	28.72	28.82	31	36	25.37	25.36	25.17(A);26.99(B)
RSV 19	RSV A (Long)+RSV B	10-5(A)+10-2(B)	25.08	25.18	35.89	38.39	35.43	30.2	19.8	35.64(A);31.66(B)	35.32(A);32.13(B)	26	31	29.86	24.46	21.27
RSV 17	RSV A (Long)+RSV B	10-5(A)+10-4(B)	24.89	25.21	35.14	36.21	35.43	30.4	20.6	37.50(A);39.98(B)	37.13(A);37.57(B)	26	32	27.34	23.68	21.26

Numbers in parentheses represent multiple tests on the same sample. Boxes shaded in dark grey represent negative results. Negative samples RSV 07, RSV 08, RSV 10 and RSV 24 were all found to be negative and are not shown (Lab D reported a failed internal control reaction for RSV 07). Lab H reported that they would normally test in triplicate but a technical error led to insufficient specimen being available.

Appendix 4: Typing and qualitative test results

CVN no.	Virus	Dilution	Laboratory ID - RSV type				
			C	E	I	H	J (qualitative)
RSV 13	RSV A (Long)	10-2	RSV A	RSV A	RSV A	RSV A	POS
RSV 05	RSV A (Long)	10-3	RSV A	RSV A	RSV A	RSV A	POS
RSV 01	RSV A (Long)	10-4	RSV A	RSV A	RSV A	RSV A	POS
RSV 15	RSV A (Long)	10-5		RSV A	RSV A		POS
RSV 03	RSV A (Long)	10-6		RSV A		RSV A	POS
RSV 18	RSV A (Long)	10-3	RSV A	RSV A	RSV B	RSV A	POS
RSV 25	RSV A (A2)	10-3	RSV A	RSV B	RSV A	RSV A	POS
RSV 22	RSV A (Long)	10-5		RSV A	RSV A	RSV A	POS
RSV 04	RSV A (A2)	10-5	RSV A	RSV A	RSV A	RSV A	POS
RSV 09	RSV B	10-1	RSV B	RSV B	RSV B	RSV B	POS
RSV 21	RSV B	10-2	RSV B	RSV B	RSV B	RSV B	POS
RSV 02	RSV B	10-3	RSV B	RSV B	RSV B	RSV B	POS
RSV 06	RSV B	10-4	RSV B		RSV B	RSV B	POS
RSV 16	RSV B	10-5			RSV B		POS
RSV 12	RSV B	10-2	RSV B	RSV B	RSV B	RSV B	POS
RSV 20	RSV B	10-2	RSV B	RSV B	RSV B	RSV B	POS
RSV 11	RSV B	10-4	RSV B		RSV B		POS
RSV 23	RSV B	10-4	untypeable	RSV B	RSV B	RSV B	POS
RSV 14	RSV A (Long)+RSV B	10-3(A)+10-4(B)	RSV A	RSV A	RSV A and B	RSV A	POS
RSV 19	RSV A (Long)+RSV B	10-5(A)+10-2(B)	RSV B	RSV A and B	RSV B	RSV B	POS
RSV 17	RSV A (Long)+RSV B	10-5(A)+10-4(B)	RSV B	RSV A and B	RSV B	RSV B	POS

Numbers in parentheses represent multiple tests on the same sample. Boxes shaded in dark grey represent negative results. Lab C performs typing reactions independently of detection testing. Negative samples RSV 07, RSV 08, RSV 10 and RSV 24 were all found to be negative.