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Comparative Evaluation of the Seegene Seeplex RV15 and Real-Time PCR for Respiratory Virus Detection

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Manuscripts

Reaction #	FAM channel	JOE channel	Cy5 channel
1	FLA	FLB	
2	RSV		
3	PF2	PF1	PF3
4	ADV	Enterovirus	
5			HRV
6	MPNV		
7	PDV	K-Ras	

Table I

Distribution of primer and probe combinations and probe dye-labels within each of the seven real-time RT-PCR reactions, i.e. reaction 4 had adenovirus and enterovirus primers and probes.

Virus		Sequence	Final Conc. (nM)
Influenza A (FLA)	Forward	CAAGACCAATCCTGTACCTCTG	900
	Reverse	TGCATTTGGACAAAGCGTCTAC	900
	Probe	FAM-AGTCCTCGCTCACTGGGCACGGT-BHQ1	200
Influenza B (FLB)	Forward	GGCAACACCTGTTCCATATTAAG	900
	Reverse	GCCTGTTAATGCAGACAAAACCTC	900
	Probe	JOE-TCATAGGCAGCTCCGAAGCAAGACATGA-BHQ1	200
Respiratory Syncytial Virus (RSV)	Forward	GAAGGGTCAAACATCTGTTTAAACAAG	900
	Reverse 1	GTTTCAGCTWGTGGGAAGAAAGATA	900
	Reverse 2	GTGTCAGCCTGTGGAAAGAAGGATA	900
	Probe	FAM-TGATCCTGCATTRTCACARTACCATCCTCT-BHQ1	200
Parainfluenza 1 (PF1)	Forward	ATTCAGACAGGATGGAACCGTTAA	900
	Reverse	GATACTAAGCTTTGTTGTGACCTCAT	900
	Probe	JOE-ACCAATGCCTTCAACTGTGTCTCCCGTG-BHQ1	200
Parainfluenza 2 (PF2)	Forward	AGAGATAACAGGGTTTGAGAATAATTCAT	900
	Reverse	CAAATGGAGTTTGGTGATTAAGGGTA	900
	Probe	FAM-TCCAGATGCTCGATCAACTATGTCCAGAGG-BHQ1	200
Parainfluenza 3 (PF3)	Forward	CGATTAGAGGCTTTCAGACAAGATG	900
	Reverse	CTGTTGAGACCGCATGATTGAC	900
	Probe	Cy5-CCACTGTGTCCACCGCTCAATACCAGCC-BHQ3	200
Enterovirus	Forward	CCCCTGAATGCGGCTAATCC	900
	Reverse	GTCACCATAAGCAGCCAATATAAGAA	900
	Probe	AACACGGACACCCAAGTAGTCGGTCC	200
Adenovirus (ADV)	Forward	GCCCCAGTGGTCTTACATGCACATC	900
	Reverse	GCCACGGTGGGGTTTCTAAACTT	900
	Probe	FAM-TGCACCAGACCCGGGCTCAGGTACTCCGA-BHQ1	200
Rhinovirus (HRV)	Forward 1	TGTGCTCRCTTTGAGTCCTC	900
	Forward 2	TGTGCTCAGTGTGCTTCCTC	900
	Reverse	TGAAACACGGACACCCAAAGTA	900
	Probe	Cy5-CCCTGAATGYGGCTAACCTTAAMCCTGC-BHQ3	200
Metapneumovirus (MPNV)	Forward	CATATAAGCATGCTATATTAAGAGTCTC	500
	Reverse	CCTATTCTGCAGCATATTTGTAATCAG	250
	Probe	FAM-TGYAATGATGAGGGTGTCACTGCGGTTG-BHQ1	500
K-Ras	Forward	GCCTGCTGAAAATGACTGAATATAAAC	600
	Reverse	TGATTCTGAATTAGCTGTATCGTCAAG	600
	Probe	JOE-TGCCTACGCCACAAGCTCCAACCTACCA-BHQ1	100
PDV	Forward	GCGGGTGCCTTTTACAAGAAC	600
	Reverse	CAGAATAAGCAAAATTGATAGGAACCAT	600
	Probe	FAM-TCTTTCTCAACCTCGTCCGTCACAAGT-BHQ1	100

Table II

Primer and probe sequences for each of the ten targets detected by the real-time respiratory RT-PCR system. The ADV primer-probe set is derived from those described in [Heim et al., 2003], and the MPNV set from [Maertzdorf et al., 2004]. The remaining primers and probes were developed in-house.

Single virus samples			Dual virus samples	
	<i>n</i>	<i>C_t</i> range [†]		<i>n</i>
FLA	9	18.5 - 31.4	ADV & HRV	2
FLB	9	22.7 - 37.6	PF1 & ADV	1
HRV	9	21.2 - 38.3	RSV & ADV	2
ADV	9	23.4 - 38.1	RSV & HRV	1
MPNV	8	22.4 - 35.0	HRV & MPNV	4
RSV	8	25.3 - 38.2	FLA & MPNV	2
PF1	13	23.8 - 37.9	FLA & RSV	4
PF2	6	25.3 - 35.3	PF3 & MPNV	1
PF3	11	25.3 - 37.5	PF2 & ADV	1

Table III

Characteristics of the 100 respiratory extracts tested by Seplex RV12. Either single targets (left-hand two columns) or two targets (right-hand columns) were detected in each sample by in-house multiplex RT-PCR. The target range of RV12 comprises these 9 viruses, together with two human coronavirus groups (229E/NL63 and OC43/HKU1). Also, RSV-A and RSV-B are detected separately.

† *C_t* ranges are derived from both single and dual virus samples.

Single virus samples			Dual virus samples	
	<i>n</i>	<i>C_t</i> range [†]		<i>n</i>
FLA	10	23.6 – 34.6	ADV & PF3	1
FLB	17*	18.8 – 32.5	FLB & HRV	1
HRV	4	23.5 – 36.0	FLB & PF3	1
ADV	10	20.4 – 32.4	FLA & PF2	1
MPNV	7	20.6 – 31.5	RSV & ADV	2*
RSV	9	23.4 – 36.4		
PF1	9	22.7 – 30.6		
PF2	5*	20.7 – 32.7		
PF3	7	23.3 – 32.6		

Table IV

Characteristics of the 84 respiratory extracts tested by Seplex RV15. Either single targets (left-hand two columns) or two targets (right-hand columns) were detected in each sample by in-house multiplex RT-PCR. Four FLB, two PF2, and both RSV-ADV dually-infected samples had been tested previously in the RV12 evaluation. All viruses in these repeated samples had been previously detected by the RV12 system. In addition to the 12 targets detected by RV12, RV15 also detects human bocavirus, human parainfluenza 4, and enterovirus.

† *C_t* ranges are derived from both single and dual virus samples.

(a)

Sample #	PCR result (C _t)	RV12
7	FLB (35.77)	None
29	FLB (35.70)	None
83	FLB (32.01)	None
94	FLB (37.56)	None
60	HRV (33.54)	None
43	MPNV (35.32)	None
32	PF1 (35.21)	RSVb
27	HRV (29.34), MPNV (35.62)	HRV, ADV
82	FLA (30.81), MPNV (35.94)	FLA
87	MPNV (26.57), PF3 (33.92)	MPNV
100	PF2 (28.5), ADV (38.11)	PF2
17	ADV (31.65)	ADV, 229E
18	ADV (26.85), RSV (32.64)	ADV, RSVa, HRV
39	RSV (30.24)	RSVa, OC43, ADV
47	ADV (33.44)	ADV, OC43
50	FLA (28.95)	FLA, HRV
55	HRV (32.69)	HRV, OC43
66	HRV (21.16)	HRV, RSVa
70	FLA (27.98)	FLA, RSVa
71	HRV (31.81)	HRV, RSVa
90	PF3 (31.81)	PF3, RSVb
102	PF2 (29.14)	PF2, HRV

(b)

Sample #	PCR result (C _t)	RV15
156	FLB (26.50)	None
158	FLB (30.30)	None
159	FLB (30.41)	None
160	FLB (29.46)	None
170	FLB (31.64)	None
142	ADV (30.02)	None
143	ADV (29.76)	None
150	ADV (23.38)	None
108	PIV-2 (26.98), FLA (28.70)	None
182	ADV (27.20)	HRV, RSV
107	MPNV (26.92)	MPNV, HRV
134	RSV (29.86)	RSVb, HEV
165	FLA (27.03)	FLA, OC43
176	FLB (25.13)	FLB, OC43
183	RSV (31.96)	RSVb, BocV

Table V

Summary of the discrepant results (highlighted in bold type) between the in-house real-time PCR and Seeplex® RV12 (a) and RV15 (b). Samples where Seeplex® missed targets detected by PCR are given first.

For Peer Review

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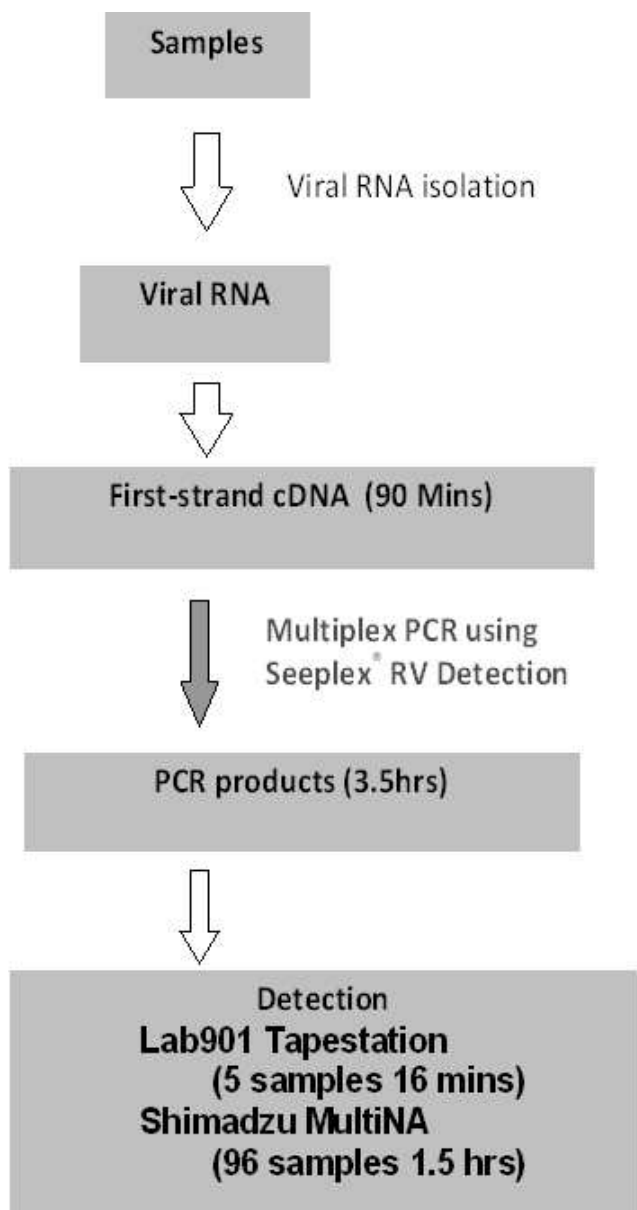


Figure 1: Workflow diagram of the Seeplex® diagnostic system.
30x56mm (300 x 300 DPI)

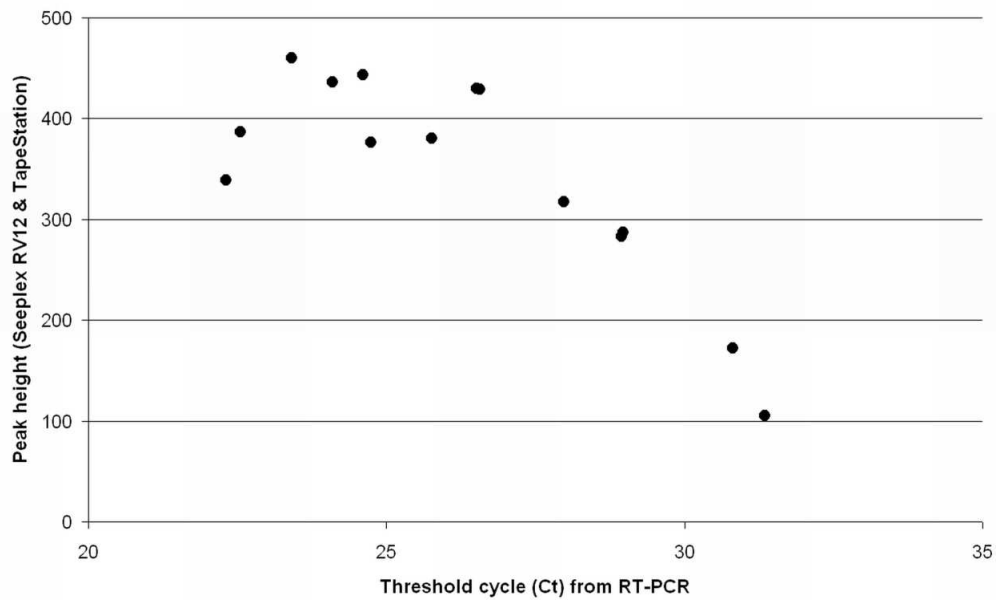


Figure 2: Plot showing how peak heights obtained using Seeplex® RV12 and the TapeStation microelectrophoresis platform display negative correlation with the threshold cycles obtained from the RT-PCR method. Data shown is from Influenza A-positive samples.
105x64mm (300 x 300 DPI)

Title Page

Title: Comparative Evaluation of the Seegene Seeplex RV15 and Real-Time PCR for Respiratory Virus Detection

Running Title: Real-Time PCR and Seeplex Systems for Respiratory Virus Detection

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1 Abstract

2 Respiratory virus infections contribute substantially both to hospitalisations of young
3 children, and to morbidity in immunocompromised patients such as those with
4 haematological malignancies. Their rapid and accurate diagnosis is essential to patient
5 management.

6 To evaluate the prospective utility of Seeplex® DPO technology in respiratory virus
7 diagnosis, a panel of 99 respiratory samples positive by real-time RT-PCR for one or
8 more viruses was assayed by the Seegene Seeplex® RV12 system. As well as being
9 able to detect all ten viruses in the real-time RT-PCR system with the exception of
10 enteroviruses, RV12 can also distinguish between the two subgroups of RSV and
11 detect two subgroups of coronaviruses.

12 Seven of the 9 viruses in common with the RT-PCR were detected reliably by RV12.

13 Eleven samples RT-PCR-positive for Metapneumovirus and 5 samples positive for

14 Influenza B were not detected by RV12. Seegene developed a second-generation

15 system, RV15, which not only allowed detection of 3 additional viruses, but also

16 addressed the potential problems with RV12 specificity. To address these concerns,

17 84 respiratory samples positive for a range of viruses by real-time PCR were assayed

18 with RV15. The results of this evaluation improved significantly upon those seen with

19 RV12.

20 The high throughput capabilities and potential lower technical requirements afforded

21 by the Seeplex® system may offer an alternative to real-time RT-PCR systems.

22 Text

23 Introduction

24 Timely and accurate diagnosis of respiratory virus infection is essential to patient care
25 in settings as diverse as haematopoietic stem cell transplant recipients [Jalal et al.,
26 2007; Piralla et al., 2009], childhood bronchitis and pneumonia [Shay et al., 2001;
27 Michelow et al., 2004] and the seasonal influenza epidemics implicated in substantial
28 annual mortality [Mook et al., 2008]. As such infections often present with similar
29 symptoms, laboratory analysis is essential.

30 Many laboratories rely still on non-molecular approaches such as direct
31 immunofluorescence and viral culture. However, in the UK, various real-time PCR-
32 based systems have been employed for a number of years, their utility having been
33 demonstrated largely by comparison with these more traditional methods [Templeton
34 et al., 2004; Bustin and Mueller, 2005; Freymuth et al., 2006; van de Pol et al., 2007;
35 Mahony et al., 2007; Kuypers et al., 2009] (reviewed in Mahony, 2008). Emerging
36 technologies thus have to demonstrate significant benefit against these existing tools
37 to achieve widespread adoption [Pabbaraju et al., 2008; Arens et al., 2010].

38 The novel Seeplex® system exploits Dual Priming Oligonucleotides (DPOs) as PCR
39 primers, each comprising a standard primer-length 5' portion which initiates priming,
40 separated by multiple inosine residues from a shorter, 6-12 nucleotide 3' component
41 which determines duplex extension. As well as facilitating melting temperature
42 regulation during primer design, mispriming and PCR artefacts such as primer
43 dimerisation are almost eliminated, allowing simultaneous and efficient amplification
44 of multiple complex targets in single reactions [Chun et al., 2007].

45 The system summary in Figure 1 shows a standard nucleic acid extraction, a cDNA
46 step, and then a DPO PCR, in which the products of each target virus have different

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3 47 nucleotide lengths. Post-PCR product analysis employs an automated bench-top
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5 48 system with which target detection interpretations are inferred from PCR product
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8 49 sizes.

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10 50 The RV12 system enables simultaneous detection of 12 respiratory viruses in 2
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12 51 reactions per sample. Its performance when compared to traditional diagnostic
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14 52 methods has been demonstrated frequently [Yoo et al., 2007; Drews et al., 2008; Roh
15
16 53 et al., 2008; Kim et al., 2009], with potential time and resource savings when
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18 54 compared to more modern methods, particularly in high throughput settings where
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20 55 prompt diagnosis is important. More recently, the RV15 kit has been developed that
21
22 56 targets 15 viruses in 3 reactions.

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27 57 No studies evaluating either system against real-time PCR-based respiratory virus
28
29 58 detection methods have yet been published. The results of a comparative evaluation of
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31 59 serial iterations of the Seeplex® system with the multiplex PCR-based method that
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33 60 has been used for 5 years as the primary diagnostic assay for respiratory virus
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35 61 detection in a major London hospital are described.
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62 **Materials and Methods**

63 Samples were nasopharyngeal aspirates, bronchio-alveolar lavages, or respiratory
64 swabs (nose and/or throat) submitted to the laboratory for routine testing. Aspirates
65 and lavages were diluted in saline and centrifuged to separate cellular material from
66 mucoid and liquid components. Swabs were transported in viral transport medium and
67 extracted directly. Two automated nucleic acid extractors were employed at different
68 times during the course of this evaluation – the NucliSENS easyMAG platform
69 (Biomérieux, Marcy l’Etoile, France) and the QIAamp One-For-All Nucleic Acid Kits
70 on the Qiagen BioRobot MDx DSP (Qiagen, Crawley, UK). Immediately prior to
71 extraction, samples were supplemented with a fixed quantity of phocine distemper
72 virus (PDV), obtained from cell culture supernatant and stored in lots at -80°C.
73 Additionally, all samples that underwent easyMAG extraction were pre-incubated at
74 56°C for 10 minutes with Buffer AL and 30mAU/ml proteinase K (both Qiagen).
75 Nucleic acid extracts were stored at -80°C between extraction and analysis.

76 **Real-Time RT-PCR.**

77 Each sample extract was subjected to seven simultaneous real-time RT-PCRs, with
78 each reaction detecting between one and three targets (Table I). The seventh reaction
79 constituted a duplex control reaction, with primers and probes targeting both a human
80 gene from the *ras* family (K-Ras) [Hilbe et al., 2003] and Protein H (haemagglutinin)
81 of the PDV internal control RNA.

82 Mastermixes and samples were dispensed robotically into the Rotorgene Gene-Disc
83 reaction vessels by a CAS1200 liquid handler (both Qiagen).

84 Quantitect Multiplex RT-PCR Mix NoRox (Qiagen), specific primers and probe
85 combinations (Table II, all obtained from Sigma-Genosys, Gillingham, UK), and 10µl
86 of nucleic acid extract were mixed in 25µl reactions. Real time cycling, detection and

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2
3 87 post-run analysis were performed on the 72-well Rotorgene platform (Qiagen) with
4
5 88 fixed fluorescence thresholds, allowing direct comparison both between samples and
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7
8 89 between runs. Positive controls (a panel of plasmids containing the specific PCR
9
10 90 target as inserts) for each respiratory virus target were included in each run, and
11
12 91 compared to expected threshold cycle (C_t) values. For each sample, C_t values for the
13
14 92 PDV and K-Ras control reactions were compared to expected values before parallel
15
16 93 PCR results were considered valid.

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20 94 **Seeplex® RV12.**

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22 95 Ninety-nine respiratory extracts stored at -80°C were selected that previously had
23
24 96 tested positive by RT-PCR for one or more respiratory virus targets. With the
25
26 97 exception of enterovirus and the internal control virus (PDV), which were not
27
28 98 detected by RV12, all viral targets in the RT-PCR were represented (Table III).
29
30 99 Random hexamer-primed cDNA synthesis products were generated using the
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32 100 Revertaid system (Fermentas, York, UK), according to the manufacturer's
33
34 101 instructions, and stored at -20°C until use.
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36 102 Each cDNA preparation was subjected to the RV12 PCR procedure according to the
37
38 103 manufacturer's instructions (Seegene, Seoul, South Korea). Briefly, parallel 20 μl
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40 104 reactions were set up, each containing RV12 mastermix, 8-MOPS contamination
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42 105 control reagent, and 3 μl of cDNA. One of each pair was supplemented with 4 μl
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44 106 primer mix A, the other with 4 μl primer mix B. Thermal cycling conditions were as
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46 107 follows: 15 minutes at 94°C , followed by 40 cycles of 94°C for 30s, 60°C for 90s, and
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48 108 72°C for 90s, followed by a single incubation of 10 minutes at 72°C . Completed
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50 109 reactions were analysed using the MultiNA platform (Shimadzu Corporation, Kyoto,
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52 110 Japan), coupled to a dedicated Seegene software template. Analysis output comprised
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3 111 both a qualitative interpretation of presence or absence of each virus target, and a
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5 112 determination of peak intensity.
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8 113 **Seeplex® RV15.**
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10 114 Eighty-four respiratory extracts stored at -80°C testing positive for one or more
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12 115 RT-PCR virus targets (Table IV) were subjected to cDNA synthesis using the Mu-LV
13
14 116 system (Invitrogen, Paisley, UK), according to the manufacturer's instructions.
15
16 117 Reaction products were stored at -20°C until use. Eight samples of the 84 had been
17
18 118 part of the previous RV12 analysis.
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20 119 Each cDNA preparation was subjected to the RV15 PCR procedure according to
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22 120 Seegene's instructions. Briefly, parallel 20µl reactions were set up for each sample, as
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24 121 per the RV12 kit, but with three reactions per sample (primer mixes A, B & C) rather
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26 122 than the two in the earlier kit. Thermal cycling conditions were as per the RV12 kit.
27
28 123 Completed reactions were analysed using the Tape Station platform (Lab901,
29
30 124 Edinburgh, UK) linked to a specific Seeplex® RV15 interpretation software module.
31
32 125 Again, results were presented both qualitatively and semi-quantitatively, according to
33
34 126 the migration rate and band intensity of each species present within completed
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36 127 reactions.
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38 128 **Redesigned Influenza B Primers.**
39
40 129 During the evaluation, a new panel of influenza B primers was developed by Seegene
41
42 130 to replace those in the RV15 kit. The cDNA preparations of the 19 FLB-positive (by
43
44 131 RT-PCR) samples analysed by RV15 were subjected to amplification using these
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46 132 primers under the same conditions as for the primer mixes of the RV12 and RV15
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48 133 kits, but in monoplex reactions. Analysis was performed as per RV15, above.
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134 **Results**

135 **Seeplex® RV12.**

136 All targets detected by the RT-PCR were also detected by Seeplex® RV12 in 88 of
137 the 99 nucleic acid extracts tested with the Seeplex® RV12 system. In 7 of the
138 remaining 11 samples, single infections were missed (4xFLB, one each of HRV, PF1,
139 and MPNV), and in four samples where two viruses had been detected by RT-PCR,
140 the virus target with the higher C_t was missed by RV12 (2xMPNV, 1xPF3 and
141 1xADV). Comparison of MultiNA peak heights and RT-PCR C_t values for each virus
142 target revealed a broad negative correlation between the two parameters, with the
143 exception of HRV, where no correlation was observed (Figure 2).

144 In all instances where PF2 (7 samples), FLA (15), and/or RSV (15) were detected by
145 RT-PCR, these virus targets were detected with RV12 also. For each instance where
146 PF1, PF3, HRV, and/or ADV were detected by RT-PCR, all except one of each virus
147 target were detected by Seeplex® RV12. Each instance of undetected virus
148 represented a sample whose C_t was found in the higher part of the range for that virus.
149 FLB and MPNV targets were detected less frequently. Four of the 5 FLB-positive
150 samples with the highest C_t values, and the 3 MPNV-positive samples with the
151 highest C_t values were not detected by RV12.

152 Conversely, ADV, RSV, HRV and MPNV were detected by RV12 in extracts where
153 the RT-PCR system had failed to do so (in 3, 4, 4, and 1 sample respectively), and
154 with the exception of the RSV detections, had high peak heights, consistent with high
155 target concentrations in the initial samples.

156 **Seeplex® RV15.**

157 Eighty four extracts from respiratory samples RT-PCR-positive for at least one
158 respiratory virus were tested by RV15. With the exception of ADV and FLB, all

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3 159 viruses detected by RT-PCR were also detected by RV15, including all instances of
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5 160 MPNV (7/7). Inverse correlations between C_t and RV15 band intensity were less
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8 161 pronounced than that seen with RV12.

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10 162 Three of 9 ADV-positive samples were not detected by RV15. All four FLB-positive
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12 163 samples that had also been tested with RV12 remained FLB-positive by RV15.

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15 164 However, a further 5 previously untested FLB-positive samples failed to signal with
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17 165 RV15. Again, viruses were detected by RV15 that were not detected by the RT-PCR:
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19 166 HRV, enterovirus, coronavirus, and bocavirus (1, 1, 2, and 1 sample respectively).
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21 167 The RT-PCR system did not target either coronavirus or bocavirus. Table V details
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23 168 the discrepant results between the RT-PCR and Seeplex® systems.

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27 169 **Redesigned Influenza B Primer Set.**

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29 170 Using the new primer set designed to overcome the FLB-detection limitations seen
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31 171 with RV12 and RV15, all FLB-positive samples in which FLB had been detected by
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33 172 RV15 were FLB-positive by the new primer set, as were four of the five samples
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35 173 FLB-negative by RV15. The single sample where FLB was not detected by Seeplex®
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37 174 had given a C_t of 30 by RT-PCR. The lack of amplification controls in this assay
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41 175 series precluded detection of PCR inhibition.
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176 Discussion

177 An in-house multiplex RT-PCR system and two iterations of a novel PCR-based
178 technology for the detection of respiratory viruses have been compared. In the first
179 instance, 99 samples were evaluated, and in the second, 84. A further panel of 19
180 FLB-positive samples was tested by a revised primer set within the novel Seplex®
181 system. Whilst direct comparison of the two systems is limited, due to the sample sets
182 having been pre-determined to be positive for one or more target viruses by one
183 system, it can be stated that the overall performance of the Seplex® RV12 and RV15
184 systems was very good, with several instances of viruses being detected where the
185 RT-PCR system had either failed or had been unable to do so.

186 Furthermore, with each virus, some negative correlation was observed between RT-
187 PCR threshold cycle and Seplex® peak intensity, suggesting the latter could also be
188 employed semi-quantitatively.

189 The frequency of instances where targets detected by RT-PCR were missed by
190 Seplex® was low, and may have been influenced by repeated freeze-thaw cycles of
191 affected samples, nearly all of which were at the upper end of the relevant C_t range
192 (i.e. with lower target concentrations). However, both MPNV and FLB targets were
193 detected poorly by RV12, with concomitant low band intensities from samples where
194 these targets were detected. Revision of the primer sets was a key component of the
195 development of RV15. RV15 gave a 100% detection rate of all viruses detected by
196 RT-PCR, including seven MPNV-positive samples, but no improvement in FLB
197 detection. The latter primer set was modified further, and retesting the panel of known
198 FLB-positive samples gave substantially greater success. Although these new primers
199 have since been included in the RV15, further evaluation within a standard Seplex®
200 multiplex PCR format is required.

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3 201 A limitation to the Seeplex® system is its internal control facility. The artificial
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5 202 targets included in each PCR mastermix allows validation only of the PCR step,
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8 203 whereas the addition of PDV to each extraction allows the RT-PCR system also to
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10 204 control for both the RNA extraction and the reverse transcription step. Inhibition of
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12 205 the RT-PCR system, detected by poorly-amplifying PDV target, occurs with a not
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14 206 insignificant frequency; it is essential to report that a lack of virus detection is not due
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16 207 to any technical deficiency within the system.

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19 208 In the laboratory setting, the Seeplex® system looks to compare favourably with
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21 209 existing technologies where high throughput, ease of use, low technical requirements,
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23 210 and a fully kit-based format are factors. Equally useful is the ability of the RV15
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25 211 system to detect bocavirus and coronaviruses, whose clinical impact is becoming
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27 212 increasingly clear [Esposito et al., 2008; Brodzinski and Ruddy, 2009; Garcia-Garcia
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29 213 et al., 2010].

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32 214 Since the completion of this work, the Seeplex® RV15 kit now includes the
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34 215 redesigned influenza B primer set, human RNase P whole process control and now
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36 216 comprises a one-step RT-PCR system rather than the two-step systems employed in
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38 217 these evaluations.

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41 218 In summary, the Seeplex® RV15 produces results comparable to the in house system.
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43 219 The high throughput capabilities and low technical requirements afforded by
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45 220 Seeplex® recommend it as a practical alternative to real-time RT-PCR.
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