A Comparison of the Efficiency Of Commercially Available Automated Nucleic Acid Extraction Platforms to Detect a Wide Range of Respiratory Viruses

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Objective: Molecular diagnostic (MDx) techniques are becoming increasing prevalent in pathology and microbiology laboratories and are especially useful for the detection of viral pathogens, which have traditionally been diagnosed by enzyme immunosorbent assays (EIA) or tissue culture. MDx are particularly useful due to the rapid turn around time and increased specificity achieved compared to conventional technologies. Automation of viral nucleic acid extraction prior to amplification and detection techniques, usually PCR based, markedly reduces hands-on time for laboratory workers and improves workflow. We sought to compare a wide range of commercially available systems in their ability to detect a bank of respiratory samples.

Validation of the GS Respiratory Viral kit: The ***EasyScreen*™ Respiratory Viral Detection Kit** detects 15 common respiratory tract viruses including; Influenza A, Influenza B, Rhinovirus, Enterovirus, RSV, human metapneumovirus, Parainfluenza 1-3, Coronaviruses (NL63, HKU1, 229E and OC43), Adenovirus and Bocavirus.

In order to test the ability of the kit to detect all viral types the assay was validated using the 2014 QCMD respiratory panel (QCMD, Glasgow, Scotland) and the RCPAQAP (Sydney, Australia) Influenza proficiency programs MAV-1, MAV-2 and MAV-3.

Sample extraction: Nucleic acids were extracted and purified according the the manufacturer's recommendations using the Nimbus automated nucleic acid purification and PCR set up platform (Genetic Signatures, Sydney, Australia)

Table 1. Results of the 2014 QCMD panels

	Performance of the GS Respiratory Viral Panels on 2014 QCMD Panels			
QCMD Panel	Core Samples	GS RVP Result	Educational Samples	GS RVP Result
Influenza A	5	5	2	1
Influenza B	5	5	1	1
RSV	6	6	2	2
Rhinovirus	7	7	3	3
Parainfluenza*	6	6	3	3
Coronavirus	6	6	4	4
Metapneumovirus	8	8	0	0
Adenovirus	9	9	1	1

* Parainfluenza 4 is not detected by the GS RVP Panels

Table 2. Results of RCPAQAP Influenza panels

Sample	Contents	Flu A copies/µl	GS RVP Result
MAV-1A	A/Brisbane/7/2010 A(H1N1) pdm	18830	Positive
MAV-1B	A/Canberra/35/2012 H3N2	1834.5	Positive
MAV-1C	B/Victoria/25/2012	N/A	Positive
MAV-1D	H5N1 Clade 2.3.2.1 (2010)	1167.05	Positive
MAV-1E	MDCK	N/A	Negative
MAV-1F	A/Perth/140/2012 A(H1N1) pdm	1695	Positive
MAV-2A	A/Brisbane/7/2010 A(H1N1) pdm	25045	Positive
MAV-2B	A/Turkey/NSW/10/2012 H9N2	164.4	Positive
MAV-2C	MDCK	N/A	Negative
MAV-2D	A/Victoria/361/2011 H3N2	388.9	Positive
MAV-2E	H5N1 Clade 2.3.2.1 (2010)	3520.5	Positive
MAV-2F	B/Darwin/40/2012	N/A	Positive
MAV-3A	A/Brisbane/7/2010 A(H1N1) pdm	173600	Positive
MAV-3B	A/Canberra/35/2012 H3N2	2439.5	Positive
MAV-3C	A/Brisbane/19/2012 A(H1N1) pdm	802	Positive
MAV-3D	MDCK	N/A	Negative
MAV-3E	A/Duck/Can Tho/NZ-S2-208/2008 H7N7	1444	Positive
MAV-3F	H5N1 Clade 2.3.4 (2006)	245.4	Positive
Neg Ctrl		N/A	Negative

Results: The 2014 QCMD panel results demonstrate the ability of the ***EasyScreen*™ Respiratory Viral Detection Kit** to correctly identify all core samples (Table 1). In addition, the assay correctly identified 15 of 16 educational samples. Further testing of the RCPAQAP Influenza panels resulted in positive signals from all Influenza A and Influenza B types (Table 2).

Methods: Nucleic acids were extracted from 96 random primary clinical samples received at the microbiology department of St. Vincent's Hospital, Darlinghurst, Sydney. The extaction systems utilised comprised the Qiasymphony (Qiagen, Hilden, Germany), Kingfisher-Flex (Thermo, Waltham, USA), EasyMAG (Biomerieux, Marcy l'Etoile, France), MagNA Pure 96 (MP96) (Roche, Pleasanton, USA) and Nimbus (Hamilton, Reno, USA). To ensure all samples contained the same amount of starting nucleic acids 100µl of primary sample was added to each platform. If a larger volume was recommended by the supplier the volume was adjusted using sterile molecular biology grade water. Samples were extracted according to the manufacturers recommendations and PCR carried out using the Genetic Signatures' ***EasyScreen*™ Respiratory Viral Detection Kit**. The real-time PCR reactions were carried out on a Bio-Rad CFX384 machine (Hercules, USA). Each set of samples from each system were then ran simultaneously on the same PCR plate to ensure consistency between platforms and thus amplification efficiency could be determined precisely for each extraction instrument.

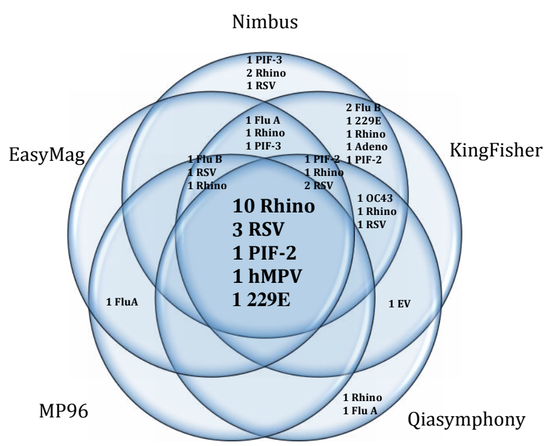

Figure 1. Platform comparison schematic

Table 3. Results of the 2014 QCMD panels

Sample #	Nimbus	Kingfisher	Qiasymphony	EasyMag	MP96
1	PIF-2	PIF-2	PIF-2	PIF-2	PIF-2
2	PIF-2	PIF-2			
3	hMPV	hMPV	hMPV	hMPV	hMPV
4	OC43	OC43	OC43		
5	Flu A	Flu A		Flu A	
6	RSV	RSV	RSV	RSV	
7	hRV	hRV	hRV	hRV	hRV
8	RSV	RSV	RSV	RSV	
9	hRV	hRV	hRV	hRV	
10	hRV	hRV	hRV	hRV	hRV
11	RSV				
12	RSV		RSV		
13	PIF-3				
14	229E	229E	229E	229E	229E
15	hRV	hRV	hRV	hRV	hRV
16	hRV	hRV	hRV	hRV	hRV
17	hRV	hRV	hRV	hRV	hRV
18	RSV	RSV	RSV		
19	hRV, PIF-2, RSV	hRV, PIF-2, RSV	hRV, PIF-2, RSV	hRV, PIF-2, RSV	hRV, RSV
20	hRV	hRV		hRV	
21			hRV		
22	Influenza A		Influenza A	Influenza A	
23	hRV	hRV	hRV	hRV	hRV
24	hRV				
25	229E	229E			
26	hRV	hRV, EV	hRV, EV	hRV	hRV
27	hRV	hRV	hRV	hRV	hRV
28	hRV	hRV		hRV	hRV
29	PIF-3	PIF-3	PIF-3	PIF-3	
30	hRV	hRV			
31	hRV				
32	RSV	RSV	RSV	RSV	RSV
33	Flu B	Flu B		Flu A, Flu B	Flu A, Flu B
34			Flu A		
35	hRV	hRV	hRV	hRV	hRV
36	RSV	RSV	RSV	RSV	
37	Flu B, Adeno	Flu B, Adeno			
38	RSV	RSV		RSV	RSV
39	hRV	hRV	hRV	hRV	hRV
40	hRV	hRV	hRV		
EC Fail	0	0	3	4	17
Positive	38	32	27	26	19

Results: The 96 freshly collected clinical samples represented the type of infections that would typically be received by a testing laboratory. The different extraction platforms were found to vary considerably in the levels of positivity achieved (Figure 1 and Table 3). The Nimbus was found to have the highest positivity rate (39.6%), compared to the MP96 with the lowest positivity rate (19.8%). The positivity rate correlated with the results obtained for the internal extraction control, where 0% of samples were shown to be insufficient nucleic acids extracted on the Nimbus, compared to 17.7% of specimens purified on the MP96 indicating either inhibition or inefficient purification. The clinical specimens used for the MP96 were exactly the same as for the EasyMag which had 4.2% of specimens with insufficient sample, indicating the difference was unlikely to be due to sample degradation.

Conclusions: The choice of automation depends on throughput and ease of use considerations. Open platforms (Nimbus and KingFisher) allow end-users more freedom to tailor assays to achieve improved sensitivity and specificity. Closed systems on the other generally can offer ease of use, but have limited options for optimisations. Laboratories should however be aware that different platforms will not always generate consistent data and that purification is as important as PCR performance.

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