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 increasingly prevalent in pathology and microbiology laboratories, and are especially useful for the detection of viral pathogens, which have traditionally been detected by enzyme immunosorbent assays (EIA) or tissue culture. MDx techniques are particularly useful due to the rapid turnaround time and increased specificity achieved compared to conventional technologies. Automation of viral nucleic acid extraction prior to amplification and detection, usually PCR based, markedly reduces hands-on time for laboratory workers and improves workflow. A bank of respiratory samples was used to compare the performance of a wide range of commercially available systems.

STUDY 1: Quality Assurance Programs

The ***EasyScreen*™ Respiratory Virus Detection Kit (#RV001)** 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.

The Detection Kit was validated using the 2014 QCMD Respiratory Panel (QCMD, Glasgow, Scotland). The kit was also tested against the RCPAQAP (Sydney, Australia) Influenza proficiency programs MAV-1, MAV-2 and MAV-3 after release.

Nucleic acids from both programs were extracted and purified according the the manufacturer's recommendations using the Hamilton Nimbus (GS1 branded) nucleic acid purification and PCR set up platform (Genetic Signatures, Sydney, Australia)

Table 1. Results of the 2014 QCMD panels

	2014 QCMD Results			
QCMD Panel	Core	RV001	Educational	RV001
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	RV001 Result
MAV-1A	A/Brisbane/7/2010 A(H1N1) pdm	Positive
MAV-1B	A/Canberra/35/2012 H3N2	Positive
MAV-1C	B/Victoria/25/2012	Positive
MAV-1D	H5N1 Clade 2.3.2.1 (2010)	Positive
MAV-1E	MDCK	Negative
MAV-1F	A/Perth/140/2012 A(H1N1) pdm	Positive
MAV-2A	A/Brisbane/7/2010 A(H1N1) pdm	Positive
MAV-2B	A/Turkey/NSW/10/2012 H9N2	Positive
MAV-2C	MDCK	Negative
MAV-2D	A/Victoria/361/2011 H3N2	Positive
MAV-2E	H5N1 Clade 2.3.2.1 (2010)	Positive
MAV-2F	B/Darwin/40/2012	Positive
MAV-3A	A/Brisbane/7/2010 A(H1N1) pdm	Positive
MAV-3B	A/Canberra/35/2012 H3N2	Positive
MAV-3C	A/Brisbane/19/2012 A(H1N1) pdm	Positive
MAV-3D	MDCK	Negative
MAV-3E	A/Duck/Can Tho/NZ-S2-208/2008 H7N7	Positive
MAV-3F	H5N1 Clade 2.3.4 (2006)	Positive
Neg Ctrl		Negative

Results: The ***EasyScreen*™ Respiratory Virus Detection Kit** correctly identified the all core samples, and 15 of 16 educational samples of the 2014 QCMD Respiratory Panel (Table 1).

Further testing of the RCPAQAP Influenza panels resulted in positive signals from all Influenza A and Influenza B types (Table 2).

STUDY 2: Clinical Samples

Nucleic acids were extracted from 96 fresh, random primary clinical samples received at the Microbiology department of St. Vincent's Hospital, Darlinghurst, Sydney. The extraction systems utilised comprised the Qiasymphony (Qiagen), Kingfisher-Flex (Thermo), easyMAG (Biomerieux), MagNA Pure 96 (MP96) (Roche) and Nimbus (Hamilton).
Samples were extracted according to the manufacturers recommendations using an 100μL aliquot. If a larger volume of nucleic acid was recommended by the supplier, the volume was adjusted using sterile molecular biology grade water. PCR was carried out using the Genetic Signatures' ***EasyScreen*™ Respiratory Virus Detection Kit**. The real-time PCR reactions were carried out on a CFX-384 (BIO-RAD). Each set of samples were then amplified simultaneously on the same PCR plate to ensure consistency and allow comparison between extraction platforms.

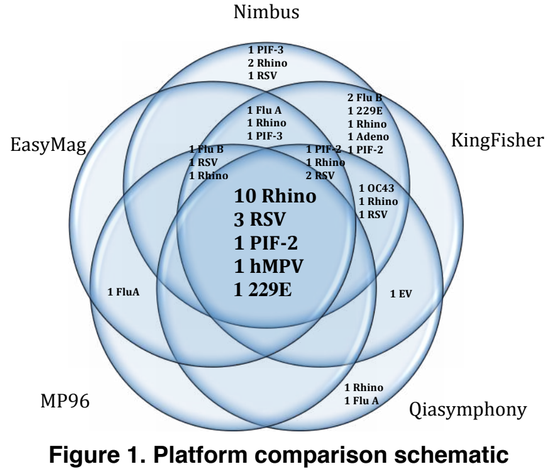

Figure 1. Platform comparison schematic

Table 3. Results of the clinical samples

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	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		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: Clinical samples represented typical infections encountered in a testing laboratory. The extraction platforms were found to vary considerably in the levels of positivity achieved (Figure 1, 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 internal extraction control, used to measure sufficient nucleic acid material post-extraction, showed 0% failure rate on the Nimbus, compared to 4.2% on the easyMAG and 17.7% on the MP96. Failure rate is indicative of either inhibition or inefficient purification. It is unlikely that sample degradation had an impact on the failure rate as aliquots were taken from the same freshly collected tube and extracted immediately.

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

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