Respiratory Multi Well System (MWS) r-gene™ : simultaneous detection of infectious agents involved in respiratory diseases

Magro S., Resa C., Vignoles M., Bertrand M., Bes J., Berriot A., Dube M., Roques C., Pourque L., Anton C., Barranger C. and Joannes M.
ARGENE, Parc Technologique Delta Sud, 09340 VERNIOLLE, France
e-mail: stephane.magro@argene.com

Introduction

Respiratory infections are among the most common infections of humans worldwide and are caused by a large number of viral and bacterial agents. The most common Upper and Lower Respiratory Infections (URTI and LRTI) such as rhinitis, pharyngitis, laryngitis, bronchitis, bronchiolitis, and pneumonia can lead to Acute Respiratory Infections (ARI) which account for an estimated 75% of all acute morbidities in industrialized countries and continue to be the leading cause of acute illness worldwide. Populations at increased risk for developing a fatal respiratory distress are infants and young children, immunocompromised persons and the elderly.

Respiratory Multi Well System (MWS) r-gene™ is a brand new range of real-time PCR complete kits for the simultaneous detection of infectious agents involved in respiratory diseases by multiple detection strategies.

Materials and Methods

Extractions : NucliSENS® easyMAG™ extraction (bioMérieux) is validated for a volume of 400µL of sample eluted in 100µL, or 200µL of sample eluted in 50µL. For both volumes, 50µL of magnetic silica is used. For nasopharyngeal samples, Proteinase K (Novagen) pre-treatment is performed.

Amplifications : 10µL of extracted sample are added to 15µL of ready-to-use 71-04x r-gene™ amplification premix. For RNA targets, reverse transcriptase is added to perform one-step real time PCRs. The same protocol and the same ampiification program are used for the 6 kits :

71-040	Influenza A/B r-gene™	*Influenza* A
		Influenza B
71-041	RSV/hMPV r-gene™	Respiratory Syncytial Virus A,B
		human Metapneumovirus
71-043	AdV/hBoV r-gene™	52 Adenovirus serotypes
		human Bocavirus 1,2,3,4
71-044	Chla/Myco pneumo r-gene™	*Chlamydophila pneumoniae*
		Mycoplasma pneumoniae
71-045	hCoV/hPIV r-gene™	human Coronavirus 229E, NL63, OC43, HKU1
		human Parainfluenza virus 1,2,3,4
71-046	CELL Control r-gene™	Validation of the presence/abscence of cells

QCMD 2010 panels, *Influenza* A and B virus, Adenovirus and *Chlamydophila* and *Mycoplasma pneumoniae* were amplified on ABI 7500 Fast (Applied Biosystems) and/or Versant kPCR AD system (Siemens) and/or Dx Real-Time System (Bio-Rad) and/or LC480 (Roche), after NucliSENS® easyMAG™ extraction of 200µL of sample.

Study on 100 clinical samples : Residual clinical specimens were collected from subjects of all ages with respiratory symptoms presenting to the pediatric ward, the intensive care unit or emergency department of Dijon Hospital (France). Nasal wash/aspirates, broncho-alveolar lavages and tracheal aspirates from January to March 2010 and 2011 winter seasons were tested. 400µL of samples were extracted with NucliSENS® easyMAG™ and amplified on ABI 7500 Fast. Direct immunofluorescence assay was performed with monoclonal antibody anti RSV FITC (17-042-Argene).

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Results

QCMD 2010 Panel

				ABI7500Fast (Applied Biosystems)		Versant kPCR AD (Siemens)	
QCMD 2010 *Influenza* A and B virus	Sample contents	Matrix	CT Value (cycles)	*Influenza* A	*Influenza* B	*Influenza* A	*Influenza* B
	Influenza virus H3N2	VTM	30	30.1	neg	31.2	neg
	Influenza virus H1N1v	VTM	35	32.0	neg	32.7	neg
	Influenza virus H1N1	VTM	33	32.8	neg	33.8	neg
	Influenza A &B Neg	VTM	-	neg	neg	neg	neg
	Influenza virus H1N1v	VTM	35	32.0	neg	32.8	neg
	Influenza virus B	VTM	32	neg	31.9	neg	32.7
	Influenza virus B	VTM	39	neg	29.4	neg	29.8
	Influenza virus H1N1v	VTM	30	28.5	neg	29.5	neg
	Influenza virus H1N1	VTM	29	29.3	neg	29.8	neg
	Influenza A &B Neg	VTM	-	neg	neg	neg	neg
	Influenza virus H1N1v	VTM	38	neg	neg	37.6	neg
	Influenza virus H3N2	VTM	37	32.6	neg	33.7	neg

				ABI7500Fast (Applied Biosystems)		Versant kPCR AD (Siemens)	
QCMD 2010 Adenovirus	Sample contents	Matrix	Sample conc (Copies/mL)	AdV	hBoV	AdV	hBoV
	ADV type 1	VTM	447	34.3	neg	33.7	neg
	ADV type 41	VTM	113	neg	neg	neg	neg
	ADV type 4	VTM	64.121	28.6	neg	28.5	neg
	ADV type 1	VTM	767	33.6	neg	34.4	neg
	ADV type 1	VTM	4.613	26.6	neg	26.5	neg
	ADV type 1	VTM	4.055	32.5	neg	32.4	neg
	ADV Negative	VTM	-	neg	neg	neg	neg
	ADV type 34	VTM	1.225	33.5	neg	33.3	neg

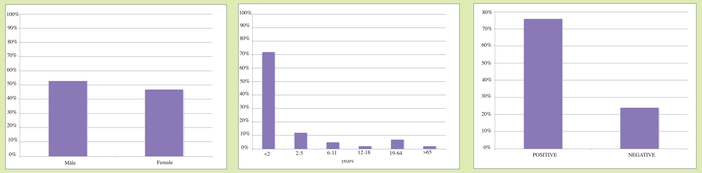
				Dx Real-Time System (Bio-Rad)		LC480 (Roche)	
QCMD 2010 *Chlamydophia & Mycoplasma pneumoniae*	Sample contents	Matrix	Sample conc	*CP*	*MP*	*CP*	*MP*
	C.pneumoniae	BAL	0.49 IFU/100µL	37.2	neg	36.0	neg
	C.pneumoniae	STM	4.9 IFU/100µL	29.4	neg	29.8	neg
	C.pneumoniae	STM	0.049 IFU/100µL	36.1	neg	36.1	neg
	CP/MP Negative	STM	neg	neg	neg	neg	neg
	M.pneumoniae	STM	50 CCU/100µL	neg	neg	neg	37.5
	C.pneumoniae	STM	4.9 IFU/100µL	29.2	neg	29.5	neg
	C.pneumoniae	BAL	4.9 IFU/100µL	33.3	neg	33.0	neg
	M.pneumoniae	BAL	50 CCU/100µL	neg	37.4	neg	36.5
	M.pneumoniae	STM	500 CCU/100µL	neg	34.6	neg	34.7
	M.pneumoniae	STM	5,000 CCU/100µL	neg	31.5	neg	32.0
	M.pneumoniae	BAL	500 CCU/100µL	neg	34.2	neg	34.5
	C.pneumoniae	STM	0.49 IFU/100µL	32.9	neg	32.8	neg

VTM : Virus Transport Medium
STM : Semple Transport medium
BAL : Human Braoenchoalveolar Lavage negative for CP and MP

H1N1v : New variant pandemic H1N1 strain
CCU: Color Changing Units
IFU: Inclusion Forming Units

29 samples out of 32 (91%) of the three panels were in agreement with QCMD expected results whatever the real time PCR platforms used, including low viral and bacterial loads. Only three low positive samples, *Influenza* A at 38 cycles, AdV41 at 113 copies/mL and *M. pneumoniae* at 50 CCU/100µL, were not detected or only with one platform. No cross reaction was observed.

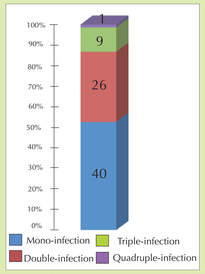
Study on 100 respiratory samples

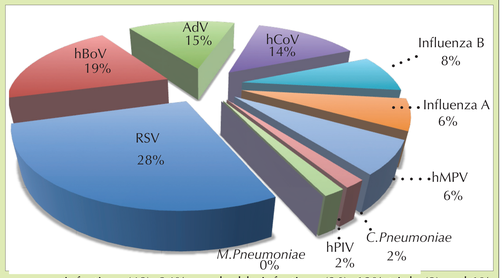


Fifty-three percent of the study subjects were male and the main age group was "< 2 years" (72%).
The results show 76 positive samples for at least one of the tested pathogens and 24 negative.

Positive sample analysis

On the 76 positive samples, 123 PCR were positive.





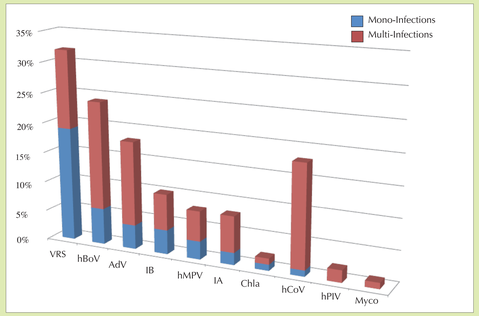
53% of the 76 positive samples were mono infections (40), 34% are double infections (26), 12% triple (9) and 1% quadruple (1).
Respiratory Syncytial Virus was the main pathogen detected (28%), followed by human Bocavirus (19%), Adenovirus (15%) and Coronavirus (14%).

Real Time PCR versus Immunofluorescence (on RSV)

		RSV DFA		
		+	-	
MWS 71-041 RSV/hMPV r-gene™	+	27	7	34
	-	1	65	66
		28	72	100

92% of results obtained with 71-041RSV real time PCR and Immunofluorescence assays were in agreement.
The 8 discrepant samples are 7 RSV positive PCR confirmed as positive in the second intention test, and one negative by PCR, which was detected as *Influenza* B positive (27.3 cycles).

Multiple infections by pathogen :



hCoV (22%), hBoV (20%) and AdV (17%) were the 3 viruses detected the most in the multiple infections, followed by RSV (16%).

Discussion

Multiparametric diagnosis demonstrates a high rate of respiratory pathogens detection using sensitive molecular-based assays among a large sample of subjects evaluated for respiratory syndromes in a hospital setting.

24 negative samples still remain without causal agent. These samples were not tested for Rhinovirus, Enterovirus or for bacteria such as *Bordetella*, *Legionella pneumophila* or *Streptococcus pneumoniae*, pathogens also involved in respiratory infections and which might be detected by the other Respiratory MWS r-gene™ real time PCR assays.

Conclusions

Respiratory Multi Well System (MWS) r-gene™ represents an innovative solution in response to the challenges in respiratory infections. Results presented in this study show the sensitivity, robustness and reliability of MWS r-gene™ kits.

Their practicability and compatibility with the major extraction and real time PCR platforms allow an immediate integration in most routine diagnostic laboratories.

These PCR assays can assist clinical laboratories in identifying respiratory pathogens infections in hospitalized patients and aid in patient management.