RESPIRATORY MULTI WELL SYSTEM (MWS) R-GENE™: SIMULTANEOUS DETECTION OF INFECTIOUS AGENTS INVOLVED IN RESPIRATORY DISEASES

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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 Tract 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

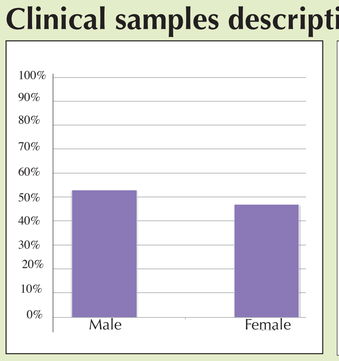
Extractions: NucliSENS® easyMAG® extraction (bioMérieux) is validated for a volume of 400µL of sample eluted in 100µL, or 200µL of sample eluted in 50µL. For both volumes, 50µL of magnetic silica is used. For nasopharyngeal samples, Proteinase K (Novagen) (20 mg/mL) pre-treatment is performed.
Amplifications: 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 amplification program are used for the following 6 kits :

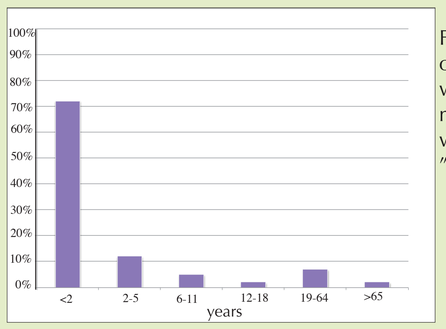
Reference	Designation	Targets
71-040	Influenza A/B r-gene™	Influenza A - Influenza B
71-041	RSV/hMPV r-gene™	RSV A,B - hMPV A,B
71-042	Rhino&EV/Cc r-gene™	Rhinovirus and Enterovirus - Validation of presence/absence of cells
71-043	AdV/hBoV r-gene™	52 Adenovirus serotypes - hBoV 1, 2, 3, 4
71-044	Chla/Myco pneumo r-gene™	*Chlamydophila pneumoniae* and *Mycoplasma pneumoniae*
71-045	hCoV/hPIV r-gene™	hCoV 229E, NL63, OC43, HKU1 - hPIV 1, 2, 3, 4

QCMD 2010 panels: Influenza A and B virus, Adenovirus , Rhinovirus/Enterovirus and Coronavirus QCMD 2010 panels were amplified on ABI 7500 Fast (Applied Biosystems) and/or Versant kPCR AD system (Siemens) and/or Dx real-time System (Bio-Rad), 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 (Applied Biosystems). Direct immunofluorecence assay was performed with monoclonal antibody anti-RSV FITC (17-042 Argene).

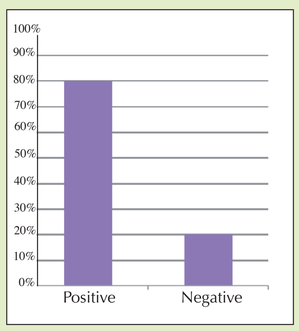
Results

Clinical samples description



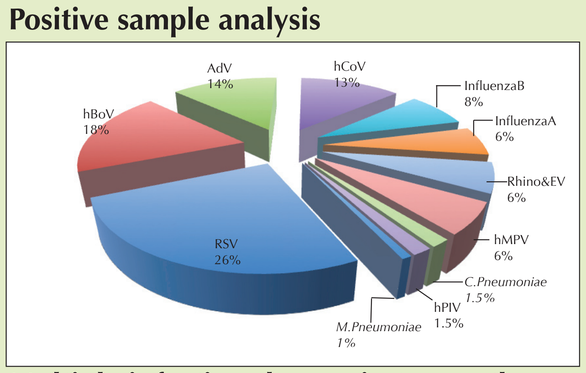


Fifty-three percent of the study subjects were male and the main age group was "< 2 years old " (72%).



The results show 80 positive samples for at least one pathogen and 20 negative.

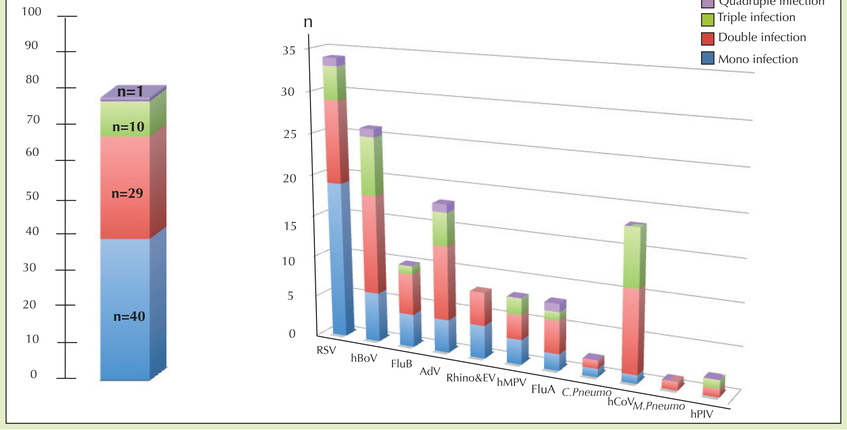
Positive sample analysis



On the 80 positive samples, 132 PCR were postive.

Among the 80 positive samples, Respiratory Syncytial Virus was the main pathogen detected (26%), followed by Bocavirus (18%), Adenovirus (14%) and Coronavirus (13%).
Among less frequent pathogens, we found the following viruses/bacteria : Influenza B (8%) ; Influenza A, Rhino/Enterovirus and human Metapneumovirus (hMPV) (6%) ; *C. pneumoniae* and Parainfluenzaviruses (1.5%) and *M.pneumoniae* (1%).
Rhino&EV/Cc r-gene™ kit allows to check the quality of the prelevment with the Cell control PCR.
All the samples have been found positive for cells, allowing their validation for diagnostic.

Multiple infections by respiratory pathogens



40/80 positive samples were single infected (50%), 29 were double infected (36%), 10 triple infected (13%) and 1 quadruple infected (1%).

Bocavirus (n=20), Coronavirus (n=17), and Respiratory Syncytial Virus (n=15) were the 3 viruses mostly detected in the multiple infections, followed by Adenovirus (n=14).

Real time PCR versus Immunofluerescence (on RSV)

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

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

2010 QCMD Panels

				ref. 71-040 Influenza A/B r-gene™			
				ABI 7500 Fast (Applied Biosystems)		Versant kPCR AD (Siemens)	
	Sample contents	Matrix	Expected CT Value*	Flu A CT Value*	Flu B CT Value*	Flu A CT Value*	Flu B CT Value*
QCMD 2010 Influenza A and B virus	Influenza virus H3N2 (Core)	VTM	30	30.1	neg	31.2	neg
	Influenza virus H1N1v (Core)	VTM	35	32.0	neg	32.7	neg
	Influenza virus H1N1	VTM	33	32.8	neg	33.8	neg
	Influenza A & B Neg (Core)	VTM	-	neg	neg	neg	neg
	Influenza virus H1N1v (Core)	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 (Core)	VTM	30	28.5	neg	29.5	neg
	Influenza virus H1N1 (Core)	VTM	29	29.3	neg	29.8	neg
	Influenza A & B Neg (Core)	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

H1N1v : new variant pandemic H1N1 strain

				ref. 71-043 AdV/hBoV r-gene™			
				ABI 7500 Fast (Applied Biosystems)		Versant kPCR AD (Siemens)	
	Sample contents	Matrix	Expected Sample concentration (Copies/mL)	AdV CT Value*	hBoV CT Value*	AdV CT Value*	hBoV CT Value*
QCMD 2010 Adenovirus	ADV type 1	VTM	447	34.3	neg	33.7	neg
	ADV type 41	VTM	113	neg	neg	neg	neg
	ADV type 1 (Core)	VTM	64,121	28.6	neg	28.5	neg
	ADV type 4 (Core)	VTM	767	33.6	neg	34.4	neg
	ADV type 1 (Core)	VTM	4,613	26.6	neg	26.5	neg
	ADV type 1 (Core)	VTM	4,055	32.5	neg	32.4	neg
	Negative (Core)	VTM	-	neg	neg	neg	neg
	ADV type 34	VTM	1,225	33.5	neg	33.3	neg

				ref. 71-042 Rhino&EV/Cc r-gene™			
				ABI 7500 Fast (Applied Biosystems)		Dx real-time sytem (bio-Rad)	
	Sample contents	Matrix	Expected Results	Rhino&EV CT Value*	Cc CT Value*	Rhino&EV CT Value*	Cc CT Value*
QCMD 2010 Rhinovirus/ Enterovirus&Coronavirus	Rhinovirus 42	VTM/1x10^{-2}	Positive	29.7	31.9	29.8	32.1
	Rhinovirus 8	VTM/1x10$^{-2.5}$	Positive (Core RV)	27.4	32.6	28.7	32.8
	Rhinovirus 72	VTM/1x10^{-2}	Positive (Core RV)	22.4	32.3	21.6	32.7
	Coronavirus 229E	VTM/1x10^{-4}	Negative (Core CV)	neg	neg	neg	33.6
	Rhinovirus 90	VTM/1x10^{-4}	Positive (Core RV)	30.0	36.5	30.7	39.4
	Coronavirus OC43	VTM/1x10^{-5}	Negative (Core CV)	neg	36.6	neg	40.3
	Rhinovirus 16	VTM/1x10^{-4}	Positive (Core RV)	33.6	36.6	33.8	38.5
	Coronavirus 229E	VTM/1x10^{-6}	Negative	neg	neg	neg	neg
	Rhinovirus 16	VTM/1x10^{-5}	Positive	neg	neg	neg	neg
	Coronavirus NL63	VTM/1x10^{-3}	Negative	neg	neg	neg	neg
	Coxsackievirus A21	VTM/1x10^{-3}	Positive	27.8	36.1	33.3	neg
	Coronavirus NL63	VTM/1x10^{-5}	Negative	neg	36.8	neg	neg

VTM : Virus Transport Medium * Cycles

100% of Core proficiency samples (18/18) of the three tested panels were in agreement with QCMD expected results on each real time PCR platforms used, including low viral loads. Among the "challenging" samples 11 samples on 14 (80%) of the three panels were in agreement with QCMD expected results. Only three low positive samples, Influenza A at 38 cycles, Adenovirus 41 at 113 copies/mL and Rhinovirus 16 diluted at 1.10^{-5}, were not detected or only with one platform.
No cross reaction was observed.

Discussion

Multiparametric diagnosis demonstrates a high rate of respiratory virus detection using sensitive molecular-based assays among a large sample of subjects evaluated for respiratory syndromes in a hospital setting.
20 negative samples still remain without causal agent. These samples were not tested for bacteria such as *Bordetella* or *Legionella pneumophila*, pathogens also involved in respiratory infections and which might be detected by the other Respiratory MWS r-gene™ real time PCR.

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 allows an immediate integration in most routine diagnostic laboratories.
These PCR assays should assist clinical laboratories in identifying respiratory pathogens infections in hospitalized patients and aid in patient management.