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Chla/Myco pneumo r-gene®

Respiratory Multi Well System r-gene®
Real Time PCR kit

Product code : 71-044

CE 0459
IVD

1. Product Description

Intended use:

Chla/Myco pneumo r-gene® allows for the rapid detection of *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* via the 5' nuclease technique, a real time analysis technology.

Chlamydia pneumoniae and Mycoplasma pneumoniae are bacteria responsible primarily for atypical pneumopathology. Their rapid detection is difficult to achieve with immunological or culture tests. Chla/Myco pneumo r-gene® however permits a simplification of etiological searches for atypical pneumopathogens. The rapidity and sensitivity of the results thus obtained contribute to tailoring an effective antibiotic treatment.

Principle:

The amplification premix is optimized for the amplification and the detection of *Chlamydia pneumoniae* (reading at 530 nm) and *Mycoplasma pneumoniae* (reading at 560 nm).
This real time duplex PCR is carried out on respiratory samples after DNA or RNA extraction (nasopharyngeal aspiration, nasal swabbing, nasal washing, bronchoalveolar liquid).

Amplified sequence :

- *Chlamydia pneumoniae* :
  OMP2 gene coding for a surface membrane protein.
  Fragment size amplified : 138 bp.

- *Mycoplasma pneumoniae* :
  P1 gene coding for a cytadhesine.
  Fragment size amplified : 105 bp.

Additional reagents/instruments required:

Other products from the Multi Well System r-gene® range listed in Chapter « Related Products » can be used in combination with this product.

- Validated extraction platforms:
  - **NucliSENS® easyMAG®**
  - MagNAPure Compact
  - QIAsymphony SP

- Validated Real-Time PCR amplification platforms:
  - LightCycler® 480
  - Applied Biosystems 7500 Fast, StepOne®
  - Stratagene® / Agilent / Versant® kPCR Molecular Systems AD
  - Rotor-Gene®
  - Dx Real-Time System (Bio-Rad)

Number of reactions:

60 reactions assuming a pipetting volume of 15 µL.
Final reaction volume: 25 µL.

Content:

R44	Amplification premix *Contains dNTPs, $MgCl_2$, amplification buffer, specific primers and probes, mix for targeted pathogens, Taq Polymerase, passive reference ROX®.*	2 x 450 µL
W0	Negative extraction and amplification control	2 x 1.8 mL
PC44	Positive control	1 x 300 µL

Package Insert : 1 Package Insert provided in the kit or downloadable from www.biomerieux.com/techlib

Storage:

Store at -18°C/-22°C before and after first opening, protected from light until the expiration date printed on the label, in the room reserved for preparation of the premixes.

⚠ Store the positive control before and after first opening at -18°C/-22°C in the same place as the extracted samples.

Each premix cannot undergo more than 11 freeze/thaw cycles.

Controls:

- The negative extraction and amplification control (**W0**)

This control (**W0**) must be extracted and amplified at the same time and using the same protocol as the patient samples. It verifies the absence of contamination during extraction and amplification.
Its signal is detected at 530 nm for the *Chlamydia pneumoniae* parameter and at 560 nm for *Mycoplasma pneumonia* parameter.

- The positive control (PC44)

This control (PC44) contains two plasmids which are specifically recognized by the primers and probes for *Chlamydia pneumoniae* and by the primers and probes for the *Mycoplasma pneumoniae* contained in the reagent mixture R44.
Systematically tested, it verifies the proper outcome of the amplification step.
Its signal is detected at 530 nm for the *Chlamydia pneumoniae* parameter and at 560 nm for the *Mycoplasma pneumoniae* parameter.

- The cellular control :

The cellular control checks the presence of cells in the samples. If Rhino&EV/Cc r-gene® (product code : 71-042) of the Respiratory Multi Well System r-gene® range is tested, cellular control is included.

If not, the CELL Control r-gene® (product code: 71-106) kit must be used.

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2. Warnings and Precautions

- This kit is intented for *in vitro* use only. The kit must be handled by a qualified staff member, in accordance with Good Laboratory Practice and handling instructions for molecular biology.
- Read all the instructions before starting the manipulation.

General warnings and precautions:

- Wear protective clothing, i.e: Disposable gloves, lab coat, safety goggles, mask.
- Avoid contact between the reagents and the skin. Wash immediately with copious amounts of water if contact occurs.
- Samples must be prepared under a biological safety hood.
- Never pipet by mouth.
- Do not smoke, eat or drink in dedicated work areas.
- Handle and dispose all specimens and materials as potentially infectious. After use: material, reagents and waste must be handled as potentially infectious.

Warnings and precautions for molecular biology:

- Amplification procedures require highly skilled techniques to avoid risk of sample contamination:
  - Use separate working places for sample preparation and amplification reactions. Movement in the laboratory must be in one direction only from the reagent preparation area to the amplification area. Allocate a set of lab coats and pipettes to each area. Never introduce an amplified product in reagent and/or sample preparation areas.
  - Pipettes used to handle samples are reserved for this purpose only. These pipettes must be positive displacement pipettes or pipettes equipped with filter tips. All tips must be sterile.
  - The pipettes used to aliquot reagents must be reserved only for this purpose. The necessary reagents for amplification are aliquoted in order to be used during one single experiment.
  - Tubes from different specimens and amplification premix must never be opened at the same time.
  - Used samples must be exclusively reserved for this analysis.

- Do not use reagents after expiration date printed on the labels.
- Do not substitute reagents from kits with different batch numbers or from other manufacturers.
- The reagents must be fully defrosted to room temperature before testing.
- The use of a metal cold block (+2/+8°C) is recommended for the manipulation of the reagents and the samples.
- Always perform preventive maintenance for workstations, for automated extraction, amplification, and centrifuge systems, according to the manufacturer's recommendations.

For more detailed information, see the product safety data sheet which can be downloaded from www.biomerieux.com/techlib

3. Product Handling

3.1 Extraction

Instruments	Kit	Sample volume	Sample Type	Protocol	Elution volume
NucliSENS® easyMAG® * (bioMérieux)	NucliSENS® easyMAG® easyMAG® Reagents	200 µL	Respiratory samples : nasopharyngeal aspiration, nasal swabbing, nasal washing, bronchoalveolar liquid	Specific B + 50 µL silica	50 µL
		400 µL			100 µL
MagNAPure Compact (Roche Diagnostics)	MagNAPure Compact Nucleic acid Isolation Kit I	200 µL		Total_NA_plasma_100_400	50 µL
		400 µL			100 µL
QIAsymphony SP (Qiagen)	QIAsymphony Virus/Bacteria Mini Kit	300 µL		Pathogen Complex 200	85 µL

* A proteinase K pre-treatment prior to extraction on the NucliSENS® easyMAG® automat may be required, in the event in which a sample is considered to be too slimy. In this case, add 10 µl of Proteinase K to 20 mg/ml of the sample and leave to incubate for 15 minutes at 56°C.

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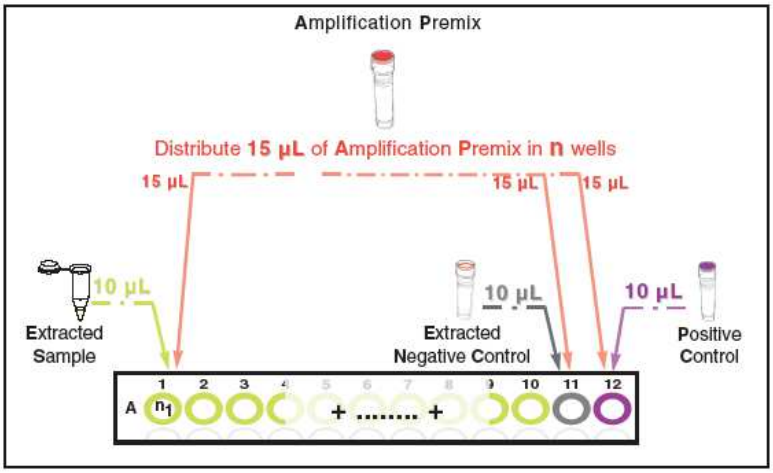
3.2 Amplification

Note: *To simplify this protocol, the device containing the amplification reaction mix will be referred to as a "well".*

3.2.1 Amplification preparation

- Plan **n** wells (***n****= number of samples + Positive Control + Negative Control)*

Note: *- Use the transparent plates (ref.: HSP9601) with the optical stoppers (ref.: TCS0803) for the Dx Real-Time System amplification device.*
- The use of a metal cold block (+2/+8°C) is recommended for the manipulation of the reagents and the samples.



- Centrifuge for 15 sec. (depending on the instrument).
- Place the microplate in the real time PCR instrument and run the program as described in the table below.

3.2.2 Amplification program

Steps		Time	Temperature	Cycles	Fluorescence Acquisition
Reverse Transcription		5 min.	50°C	1	-
Taq Polymerase Activation		15 min.	95°C	1	-
Amplification	Denaturation	10 sec.	95°C	45	-
	Annealing	40 sec.	60°C		530 + 560 nm
	Elongation	25 sec.	72°C		-

Note 1: *The temperature increases and decreases are set by default, which means at 100% or at their maxima.*

Note 2: *For LightCycler® 480, two optical systems exist: only "System II" is compatible with the use of the kit. The "System II" features, in its software a colour compensation to be activated.*

Note 3: ***On Applied Biosystems 7500 Fast only, select «ROX» as «PASSIVE REFERENCE» when programming.***

Note 4: ***On Applied Biosystems StepOne® only, select «NONE» in «PASSIVE REFERENCE» when programming.***

Note 5: *On Rotor-Gene®, calibrate the signal by clicking on «GAIN OPTIMISATION».*

Note 6: *On Stratagene® or Agilent or Versant® kPCR Molecular System AD select «NONE» in «REFERENCE DYE», when programming.*

Programming and analysis assistance sheets, per device type, downloadable at www.biomerieux.com/techlib

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4. Results

4.1 Validation of results

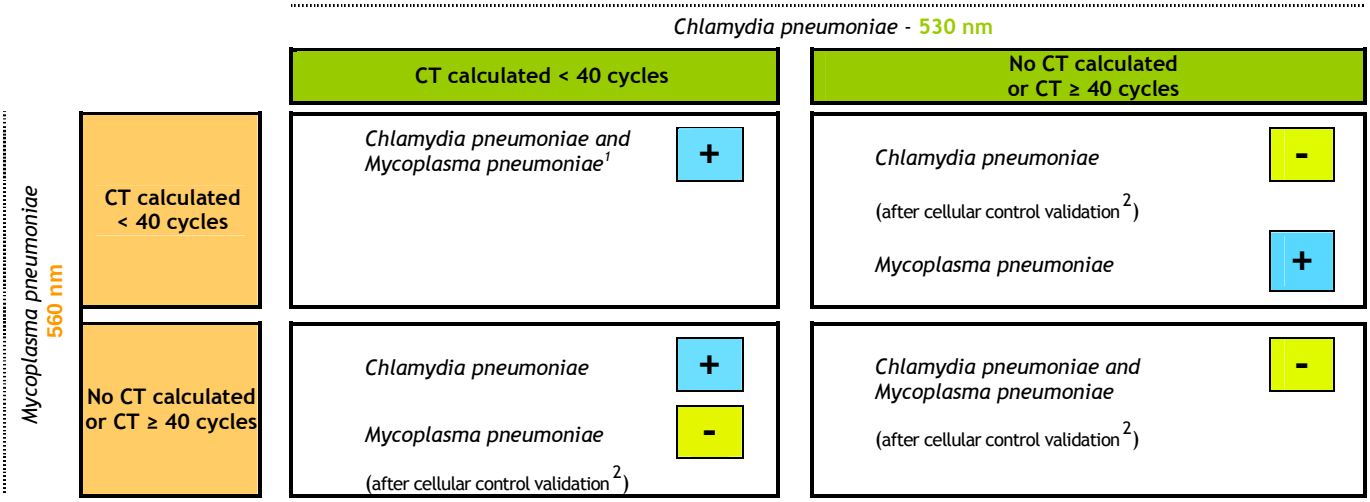
⚠ The test is only valid if all the following conditions are fulfilled. If this is not the case, all samples and controls must be tested again.

1st condition: The negative control should not give a detectable signal at **530 nm** neither at **560 nm**.

2nd condition: The positive control should give a detectable signal at both **530 nm** and **560 nm** below **32 cycles**.

4.2 Interpretation of results

- A positive sample displays a CT value.
- If a CT value can not be calculated, the sample is considered as negative or inhibited or contains a limited number of cells.
- A CT greater than or equal to 40 cycles corresponds to a sample that is below the detection.



1 - For Rotor-Gene® and Applied Biosystems StepOne® apparatus, a crosstalk 530 nm in 560 nm may be observed.

⚠ 2 – The quality of the sample taken and /or the inhibition for the pathogen search can be verified using the cellular control (CELL Control r-gene® - ref.: 71-106, also contained in the reagents: Rhino&EV/CC r-gene® - ref.: 71-042).

Note: The evaluation of the efficacy of the extraction and the detection of possible inhibitors is possible using the DICO Extra r-gene® - ref.: 71-101 / RICO Extra r-gene® - ref.: 71-105 reagent(s).

Note: Certain batches of transport media used for routing and storage of respiratory samples may contain traces of genomic DNA likely to give a false positive result beyond 35 cycles. For the same reasons, the use of inhibition controls IC1 (RICO Extra r-gene®) or IC2 (DICO Extra r-gene®) in combination with this cellular control may also engender weakly false positive signals beyond 35 cycles. In both cases, any sample with a CT for the cell control target greater than 35 cycles is considered to be a sample with not enough cells to be interpreted as valid. This sample must be retested or re-sampled.

IMPORTANT NOTE:
It is absolutely necessary to compare results obtained with this kit with other diagnostic investigation methods in order to define patient infectious status.

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5. Performances of the assay

5.1 Intra-Experiment and Inter-Experiment Reproducibility

5.1.1 Intra-Experiment Reproducibility

- **On *Chlamydia pneumoniae*:**

- The intra-experiment reproducibility studies for the Chla/Myco pneumo r-gene® kit were carried out on a *Chlamydia pneumoniae* sample diluted in an *Chlamydia pneumoniae*-negative nasopharyngeal sample.
- The samples were analysed 10 times on the LightCycler® 480 device (Roche) after NucliSENS® easyMAG® extraction (bioMérieux) (protocol: Specific B, sample volume: 400 µl, elution volume: 100 µl).
- The following table shows the average CTs obtained for each of the dilutions. The standard deviation and the variation coefficient were determined.
- The variation coefficient is between 0.83% and 1.77%. These values demonstrate the good intra-experiment reproducibility of the Chla/Myco pneumo r-gene® kit on a nasopharyngeal sample.

		Mean of CTs	Standard Deviation	Variation Coefficient(%)
Chlamydia pneumoniae	$1{,}6.10^{-1}$ IFU/100µL	33,98	0,28	0,83%
	8.10^{-2} IFU/100µL	35,23	0,35	1,00%
	$3{,}2.10^{-2}$ IFU/100µL	37,23	0,68	1,77%

Intra-Experiment Reproducibility of the Detection of *Chlamydia pneumoniae* using the Chla/Myco pneumo r-gene® kit ref. : 71-044

- **On *Mycoplasma pneumoniae*:**

- The intra-experiment reproducibility studies for the Chla/Myco pneumo r-gene® kit were carried out on a *Mycoplasma pneumoniae* sample diluted in an *Mycoplasma pneumoniae*-negative nasopharyngeal sample.
- The samples were analysed 10 times on the LightCycler® 480 device (Roche) after NucliSENS® easyMAG® extraction (bioMérieux) (protocol: Specific B, sample volume: 400 µl, elution volume: 100 µl).
- The following table shows the average CTs obtained for each of the dilutions. The standard deviation and the variation coefficient were determined.
- The variation coefficient is between 1.51% and 3.63%. These values demonstrate the good intra-experiment reproducibility of the Chla/Myco pneumo r-gene® kit on a nasopharyngeal sample.

		Mean of CTs	Standard Deviation	Variation Coefficient(%)
Mycoplasma pneumoniae	180 CCU/100µL	34,79	0,53	1,51%
	90 CCU/100µL	36,26	0,92	2,53%
	36 CCU/100µL	38,48	1,40	3,63%

Intra-Experiment Reproducibility of the Detection of *Mycoplasma pneumoniae* using the Chla/Myco pneumo r-gene® kit ref. : 71-044

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5.1.1 Inter-Experiment Reproducibility

- **On *Chlamydia pneumoniae*:**

- The inter-experiment reproducibility studies for the Chla/Myco pneumo r-gene® kit were carried out on a *Chlamydia pneumoniae* sample diluted in an *Chlamydia pneumoniae*-negative nasopharyngeal sample.
- The samples were analysed 10 times on the Dx Real-Time System (Bio-Rad) after NucliSENS® easyMAG® extraction (bioMérieux) (protocol: Specific B, sample volume: 400 µl, elution volume: 100 µl) during 10 separate experiments.
- The following table shows the average CTs obtained for each of the dilutions. The standard deviation and the variation coefficient were determined.
- The variation coefficient is between 1.07 % and 1.66%. These values demonstrate the good inter-experiment reproducibility of the Chla/Myco pneumo r-gene® kit on a nasopharyngeal sample.

		Mean of CTs	Standard Deviation	Variation Coefficient(%)
Chlamydia pneumoniae	16 IFU/100µL	27,55	0,46	1,66%
	1,6 IFU/100µL	31,24	0,35	1,11%
	0,16 IFU/100µL	34,15	0,37	1,07%

Inter-Experiment Reproducibility of the Detection of *Chlamydia pneumoniae* using the Chla/Myco pneumo r-gene® kit ref. : 71-044

- **On *Mycoplasma pneumoniae*:**

- The inter-experiment reproducibility studies for the Chla/Myco pneumo r-gene® kit were carried out on a *Mycoplasma pneumoniae* sample diluted in an *Mycoplasma pneumoniae*-negative nasopharyngeal sample.
- The samples were analysed 10 times on the Dx Real-Time System (Bio-Rad) after NucliSENS® easyMAG® extraction (bioMérieux) (protocol: Specific B, sample volume: 400 µl, elution volume: 100 µl) during 10 separate experiments.
- The following table shows the average CTs obtained for each of the dilutions. The standard deviation and the variation coefficient were determined.
- The variation coefficient is between 1.52 % and 3.16 %. These values demonstrate the good inter-experiment reproducibility of the Chla/Myco pneumo r-gene® kit on a nasopharyngeal sample.

		Mean of CTs	Standard Deviation	Variation Coefficient(%)
Mycoplasma pneumoniae	18 000 CCU/100µL	29,54	0,45	1,52%
	1 800 CCU/100µL	33,49	1,06	3,16%
	180 CCU/100µL	36,37	0,86	2,37%

Inter-Experiment Reproducibility of the Detection of *Mycoplasma pneumoniae* using the Chla/Myco pneumo r-gene® kit ref. : 71-044

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5.2 Analytical sensitivity

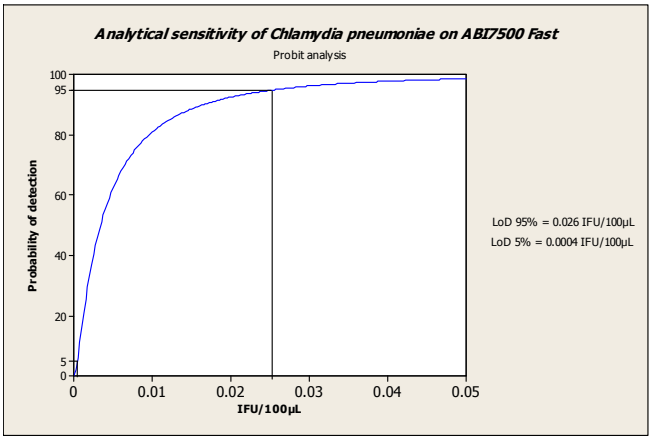
The analytical sensitivity (or detection limit) for the Chla/Myco pneumo r-gene® kit was determined using a range of dilutions of a sample of *Chlamydia pneumoniae* and a sample of *Mycoplasma pneumoniae* in a nasopharyngeal sample that has previously returned negative results for these viruses.

These *Chlamydia pneumoniae* samples contained 4.9.10-2 à 4.9.10-5 IFU/100 µl. These *Mycoplasma pneumoniae* samples contained 1 to 50 CCU/100 µl.
Each dilution was extracted 15 times using the NucliSENS® easyMAG® extraction automat (bioMérieux) on its Specific B program, and then amplified on the ABI 7500 Fast (Applied Biosystems) using the Chla/Myco pneumo r-gene® kit.

The graph to the right shows the probability analysis for the detection of *Chlamydia pneumoniae*.

It shows a 95% probability of detection of *Chlamydia pneumoniae* in a nasopharyngeal sample at 0.026 IFU/100 µl or 0.26 IFU/ml.

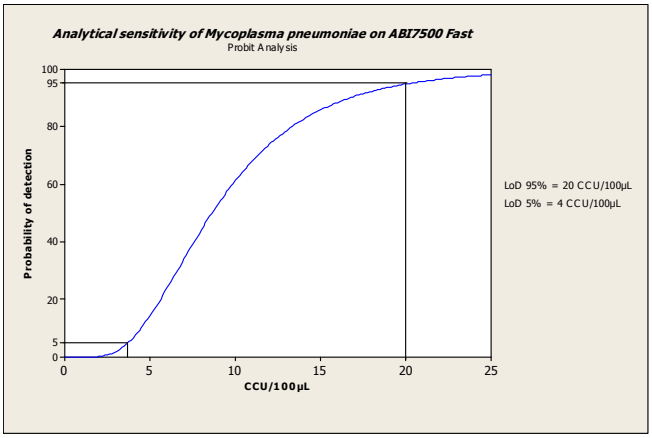
It shows a 5% probability of detection of *Chlamydia pneumoniae* in a nasopharyngeal sample at 0.0004 IFU/100 µl or 0.004 IFU/ml.



The graph to the right shows the probability analysis for the detection of *Mycoplasma pneumoniae*.

It shows a 95% probability of detection of Mycoplasma pneumoniae in a nasopharyngeal sample at 20 IFU/100 µl or 200 IFU/ml.

It shows a 5% probability of detection ofMycoplasma pneumoniae in a nasopharyngeal sample at 4 IFU/100 µl or 40 IFU/ml.



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5.3 Analytical specificity

The recognition specificity of the triggers and probes selected for the detection of the *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* viruses with the Chla/Myco pneumo r-gene® kit (ref. 71-044) was determined based on the analysis of the sequences present in the databases (viral, bacterial and human).
It was tested by experiment on the following pathogens in real time PCR:

- **Bacteria:**

  Acinetobacter baumanii,
  Bordetella bronchiseptica, Bordetella parapertussis, Bordetella pertussis,
  Branhamella catarrhalis,
  Candida albicans, Candida non albicans (tropicalis), Candida non albicans (utilis), Candida glabrata,
  Chlamydia pneumoniae, Chlamydia trachomatis serovar D, Chlamydia psittaci,
  Citrobacter freundii,
  Enterobacter cloacae, Enterobacter kobei,
  Escherichia coli,
  Haemophilus influenzae, Haemophilus parainfluenzae,
  Klebsiella pneumoniae, Klebsiella oxytoca,
  Legionella micdadei, Legionella pneumophila,
  Morganella morganii,
  Mycobacterium avium, Mycobacterium chelonae, Mycobacterium fortuitum, Mycobacterium gordonae, Mycobacterium intracellulare, Mycobacterium kansasii, Mycobacterium lentiflavum, Mycobacterium tuberculosis, Mycobacterium xenopi,
  Mycoplasma pneumoniae, Mycoplasma genitalium, Mycoplasma hominis, Mycoplasma orale, Mycoplasma salivarium, Mycoplasma fermentans, Mycoplasma penetrans,
  Nocardia asteroide,
  Proteus mirabilis,
  Pseudomonas aeruginosa,
  Raoultella ornithinolytica,
  Serratia marcescens,
  Staphylococcus aureus, Staphylococcus epidermidis,
  Stenotrophomonas maltophilia,
  Streptococcus agalactiae, Streptococcus constellatus.

- **Viruses:**

  Herpesvirus: HSV-1, HSV-2, VZV, CMV, EBV, HHV-6, HHV-7, HHV-8
  Polyomavirus : JCV, BKV
  Adenovirus 3, 4, 5, 8, 11, 12, 40
  Bocavirus 1
  Parvovirus B19
  Enterovirus : Coxsackievirus B2, A9 ; Echovirus 9, 25, 30 ; Poliovirus S3
  Parechovirus 1, 2 ;
  Rhinovirus 14, 87, 1B
  Influenza A, Influenza B
  Paramyxovirus : Parainfluenzavirus 1, 2, 3, 4 ; VRS A, VRS B ; hMPV A, hMPV B
  Coronavirus NL63.

⇒ No cross reaction *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* and no amplification was observed with any of the other pathogens tested.

NB: *Tests were also carried out on human DNA extracts that returned negative results for Chlamydia pneumoniae and Mycoplasma pneumoniae. These tests showed the lack of amplification of the sequence of human origin.*

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5.4 Test report CP/MP QCMD 2012 panel

11 samples were blind-tested with the Chla/Myco pneumo r-gene® kit during the European CP/MP checks proposed by the QCMD in 2012. 200 µl of each sample, extracted with the NucliSENS® easyMAG® (bioMérieux) were amplified on the ABI 7500 Fast (Applied Biosystems) with the R44 specific amplification premix provided in the Chla/Myco pneumo r-gene® kit.

⇒ 100% (11/11) of the samples tested in the CP/MP panel QCMD agreed with the QCMD results.

4 out of 11 samples in the CP/MP panel were *Chlamydia pneumoniae* positive.
All these samples were detected with the Chla/Myco pneumo r-gene® kit.
5 out of 11 samples in the CP/MP panel were *Mycoplasma pneumoniae* positive.
All these samples were detected with the Chla/Myco pneumo r-gene® kit.

The detection of low-concentration samples for *Chlamydia pneumoniae* (0.049 IFU/100 µl) confirms the good level of sensitivity of the Chla/Myco pneumo r-gene® kit.
The detection of low-concentration samples for *Mycoplasma pneumoniae* (5 CCU/100 µl) confirms the good level of sensitivity of the Chla/Myco pneumo r-gene® kit.
The lack of cross-reaction between *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* demonstrates the specificity of the *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* triggers and probes selected for the Chla/Myco pneumo r-gene® kit.

QCMD Results				**Chla/Myco pneumo r-gene® Results**	
				ABI Prism® 7500 Fast	
Panel Code	Sample Content and Matrix	Sample Concentration per 100µL	Sample Status	CT *Chlamydia pneumoniae*	CT *Mycoplasma pneumoniae*
CP.MP12-01	*C. pneumoniae* (STM)	0.49 IFU	Frequently detected **(Core)**	**32.46**	NEG
CP.MP12-02	*C. pneumoniae* (STM)	4.9 IFU	Frequently detected **(Core)**	**29.47**	NEG
CP.MP12-03	*M. pneumoniae* (STM)	500 CCU	Frequently detected **(Core)**	NEG	**35.27**
CP.MP12-04	CP/MP Negative (STM)	-	Negative	NEG	NEG
CP.MP12-05	*M. pneumoniae* (BAL)	250 CCU	Detected	NEG	**35.50**
CP.MP12-06	*M. pneumoniae* (STM)	250 CCU	Frequently detected **(Core)**	NEG	**36.22**
CP.MP11-07	*M. pneumoniae* (STM)	50 CCU	Detected	NEG	**37.85**
CP.MP12-08	*M. pneumoniae* (STM)	5 CCU	Infrequently detected	NEG	**39.13**
CP.MP12-09	*C. pneumoniae* (STM)	0.049 IFU	Detected	**35.97**	NEG
CP.MP12-10	CP/MP Negative (BAL)	-	Negative	NEG	NEG
CP.MP12-11	*C. pneumoniae* (BAL)	4.9 IFU	Frequently detected **(Core)**	**29.21**	NEG

STM: Sample Transport Medium.
BAL: Human Bronchoalveolar Lavage previously screened negative for *C. pneumoniae* and *M. pneumoniae*
CCU: Colour-Changing Units
IFU: Inclusion Forming Units

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5.5 Internal study of 100 clinical samples

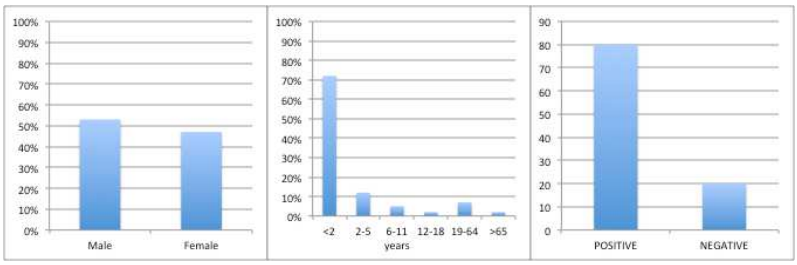
5.5.1 Description of the study

A study was carried out on 100 clinical samples collected from subjects of all ages who had respiratory symptoms and were seen at the Pediatrics Department, Intensive Care or the Emergency Department of the Dijon University Hospital (France). The samples were nasal secretions, broncho-alveolar washes or tracheal aspirations collected during the winters of 2010 and 2011, from January to March.
The samples were extracted on the NucliSENS® easyMAG® (bioMérieux). 400 µl of samples were extracted in accordance with the Specific B protocol, after the proteinase K pre-treatment (10 µl of Proteinase K added to 20 mg/ml of the sample and leave to incubate for 15 minutes at 56°C, then eluted in 100 µl.
The extracts were amplified with the following kits on the ABI 7500 Fast (Applied Biosystems), in accordance with the protocol described in the technical sheets:

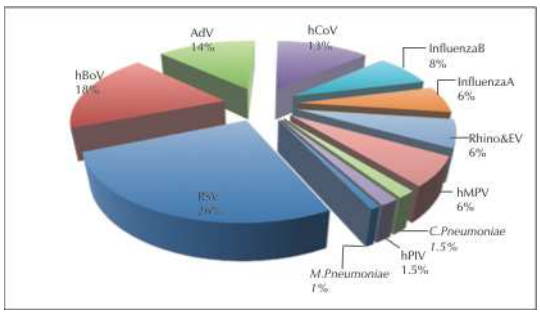
Reference	Designation	Targets
71-040	Influenza A/B r-gene®	Influenza A - Influenza B
71-041	RSV/hMPV r-gene®	RSV A,B - hMPV A,B
71-042	Rhino&EV/Cc r-gene®	Rhinovirus and Enterovirus -Validation of presence/absence of cells
71-043	AdV/hBoV r-gene®	52 Adenovirus serotypes - hBoV 1, 2, 3, 4
71-044	Chla/Myco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

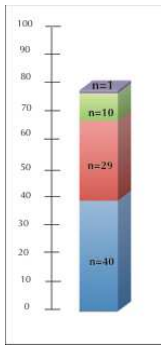
5.5.2 Results

The male/female distribution is balanced (53% / 47%), with the under 2s age group being the largest (72% of samples). 80 samples were detected as positive for one of the pathogens tested. 20 samples were negative. All the samples were detected as positive in the cells using the cell indicator from the 71-042 Rhino&EV/Cc r-gene® kit, enabling a diagnostics result to be returned.



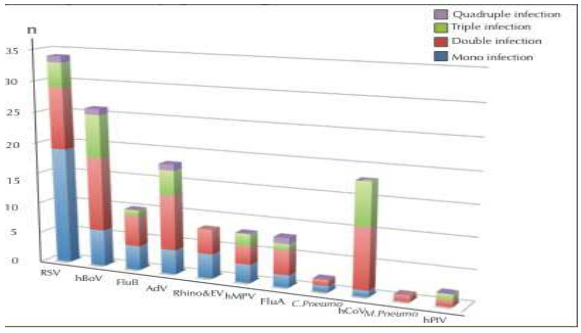
The analysis of the 80 positive samples allowed 132 positive PCR to be determined, along with a detection distribution for the different pathogens shown in the diagrams to the right.
The virus detected the most was the Respiratory Syncytial Virus (RSV - 26%), followed by Bocavirus (hBoV - 18%), the Adenovirus (AdV – 14%) and the Coronavirus (hCoV – 13%).
As regards co-infections (cf. bar chart to the right), 50% of the 80 positive results were mono-infections (40), 36% were double infections (29), 13% were triple infections (10) and 1% were quadruple infections (1).





The analysis of the co-infections shows that it is the Bocavirus that are the most often involved (hBoV – 22%), followed by the Coronavirus (hCoV – 19%) and then the Respiratory Syncytial Virus (RSV – 16%).

20 samples of the 100 samples tested remained negative.



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5.6 Clinical Studies

5.6.1 Clinical Study on Respiratory Samples – Clemenceau UH Virology Laboratory (Caen – France)

A retrospective clinical study was carried out at the virology laboratory of UH Clemenceau, Caen on 208 respiratory samples (nasal aspirations and nasal swabbing with virological transport media) which had been characterised by the routine techniques of the laboratory.
The extraction of the samples was carried out using the QIAsymphony extraction automat(QIAGEN) with the QIAsymphony Virus/Bacteria Mini kit and following the Pathogen Complex 200 protocol as of 300 µl of sample (85 µl elution).
The amplification was carried out using the Dx Real-Time System instrument (Bio-Rad).

a/ Analysis of the results the *Chlamydia pneumoniae* parameter:

Sensitivity = 100%
Specificity = 100%

		Reference		
		+	-	
Chla / Myco pneumo r-gene® 71-044	+	4	0	*4*
	-	0	204	*204*
		4	*204*	***208***

The two techniques correlate at 100%. 4 samples were positive and 204 were negative in both techniques.

b/ Analysis of the results the *Mycoplasma pneumoniae* parameter:

Sensitivity = 100%
Specificity = 100%

		Reference		
		+	-	
Chla / Myco pneumo r-gene® 71-044	+	6	0	*6*
	-	0	202	*202*
		6	*202*	***208***

The two techniques correlate at 100%. 6 samples were positive and 202 were negative in both techniques.

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5.6.2 Clinical Study on Respiratory Samples – St Etienne UH Virology Laboratory (France)

A retrospective clinical study was carried out at the virology laboratory of UH St Etienne on 88 respiratory samples (nasal aspirations and nasal swabbing and bronchoalveolar fluids) which had been characterised by the routine techniques of the laboratory.
The extraction des samples was carried out using the NucliSENS® easyMAG® extraction automat (bioMérieux) in accordance with the Specific B protocol as of 400 µl of sample after the proteinase K pre-treatment (add 10 µl of Proteinase K to 20 mg/ml of the sample and leave to incubate for 15 minutes at 56°C)(100 µl elution).
The amplification was carried out using the ABI 7500 Fast (Applied Biosystems) instrument (Bio-Rad).
This study was carried out in parallel on 8 respiratory kits that are currently available on the market.
The results were analysed relative to an expected result, which was the same for the different kits tested, and is marked as reference in the following tables.

a/ Analysis of the results the *Chlamydia pneumoniae* parameter:

Sensitivity = 100%
Specificity = 97,67%

		Reference		
		+	-	
Chla / Myco pneumo r-gene® 71-044	+	2	2	*4*
	-	0	84	*84*
		2	*86*	***88***

The two techniques correlate at 97.73%. 2 samples were positive and 84 were negative for both techniques.
2 additional samples were detected with the Chla/Myco pneumo r-gene® kit (71-044).

b/ Analysis of the results the *Mycoplasma pneumoniae* parameter:

Sensitivity = 100%
Specificity = 98,73%

		Reference		
		+	-	
Chla / Myco pneumo r-gene® 71-044	+	9	1	*10*
	-	0	78	*78*
		9	*79*	***88***

The two techniques correlate at 98.86%. 9 samples were positive and 78 were negative for both techniques. 1 additional sample was detected with the Chla / Myco pneumo r-gene® kit (71-044).

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6. References

Programming and analysis assistance sheets, per device type, downloadable at www.biomerieux.com/techlib

1) M. Bertrand, M. Vignoles, J. Bes, S. Magro, C. Barranger, M. Joannes.
Development of a new diagnostic tool for the detection of Chlamydia pneumoniae and Mycoplasma pneumoniae in a duplex real-*time PCR*. Poster 21st ECCMID / 27th ICC 2011

2) S. Magro, M. Bertrand, C. Resa, M. Vignoles, J. Bes, M. Dube, A. Berriot, C. Roques, L. Pourque, C. Anton, C. Barranger, M. Joannes.
Respiratory Multi Well System (MWS) r-gene® : simultaneous detection of infectious agents involved in respiratory diseases. Poster CVS 2011

3) Resa C., Bertrand M., Bes J., Vignoles M., Magro S., Barranger C., Daval P., Manoha C., Auvray C., Pothier P., Joannes M.
Respiratory Multi Well System r-gene® : Simultaneous Detection of Infectious Agents Involved in Respiratory Diseases. Poster ESCV 2011

4) Bertrand M., Vignoles M., Bes J., Magro S., Barranger C., Joannes M.
Development of a new diagnostic tool for the detection of C. pneumoniae and M. pneumoniae in a duplex real-time PCR. Poster ESCV 2011

7. Related Products

Respiratory Multi Well System r-gene®

- *Influenza* A/B r-gene® ref.: 71-040
- RSV/hMPV r-gene® ref.: 71-041
- Rhino&EV/Cc r-gene® ref.: 71-042
- AdV/hBoV r-gene® ref.: 71-043
- Chla/Myco pneumo r-gene® ref.: 71-044
- HCoV/HPIV r-gene® ref.: 71-045

Meningo-Encephalitis Multi Well System r-gene®

- Parechovirus r-gene® ref.: 71-020

Controls Range

- DICO Ampli r-gene® ref.: 71-100
- DICO Extra r-gene® ref.: 71-101
- RICO Extra r-gene® ref.: 71-105
- CELL Control r-gene® ref.: 71-106

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8. Index of symbols

Symbol	Meaning
REF	Catalogue number
IVD	In Vitro Diagnostic Medical Device
	Manufacturer
	Temperature limit
	Use by
LOT	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Protect from light
	Keep dry
CE 0459	Identification of notified body

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