ELISABETH PHARMACON Ltd.
Rokycanova 4437/5
615 00 Brno-Zidenice, Czech Republic
Phone: +420 542 213 851
E-mail: info@elisabeth.cz
Web: www.elisabeth.cz
VAT: CZ26258412

EliGene® Mycoplasma pneumoniae UNI

REF **90055-UNI (for 50 samples)**

Kit components:

5 x 150 µl **M. pneumoniae UNI Mix**
2 x 50 µl **PC DNA M. pneumoniae**
5 x 200 µl **IC DNA 01**
1 x 20 µl **Eli Blocker**
1 x 20 µl **ROX dye**
1 x **Instruction for Use**

Storage and shelf life after first opening:

All components of the kit must be transported and stored at -20 °C. Kit and remaining MasterMixes must be stored at -20 °C in a dark.

Intended use

EliGene® Mycoplasma pneumoniae UNI is intended for DNA diagnostics of pathogenic species *Mycoplasma pneumoniae* from isolated DNA sample.

Principle of the method

This diagnostic kit is based on RealTime PCR method. In this kit primers and fluorescent probes (FAM and HEX) for the detection of *Mycoplasma pneumoniae* DNA and internal control are used.

Introduction

Mycoplasma pneumoniae belonging to class *Mollicutes* has one of the smallest known genomes. It lacks a peptidoglycan cell wall and thus it is resistant to the effects of penicillins and other beta-lactam antibiotics. In humans it causes atypical pneumonia. *M. pneumoniae* infects the upper and lower respiratory tracts, leading to upper respiratory tract infection, bronchiolitis, tracheobronchitis, bronchitis and community-acquired pneumonia. Infections of the lower respiratory tract generally manifest with a cough, sometimes with dyspnoea, adenopathy, wheezing and, rarely, with respiratory failure. Although *M. pneumoniae* infections are usually mild, and many are asymptomatic, they are not always self-limiting. *M. pneumoniae* causes up to 40% of cases of community-acquired pneumonia, but *M. pneumoniae* respiratory tract infections are also associated with a wide range of extra-pulmonary manifestations, including neurological, cardiac, dermatological, musculoskeletal, haematological and gastrointestinal symptoms.

Primary sample collection, handling and storage

Clinical material:	Recommended DNA isolation procedure:
Throat swabs, Nasopharyngeal aspirates,	Manual: EliGene® Urine Isolation Kit
Sputum	Automatic: **ZEPHYRUS Magneto** (ELISABETH PHARMACON)

WARNING: To keep the sensitivity of the test we recommended strictly follows the pre-analytical procedures mentioned in this instruction. Specially do not change the recommended procedures of isolation as for stated amount of specimen, centrifugation force, etc.

Throat swabs:

These specimens should be collected before the treatment by antibiotics according to standard protocol. Specimens in cultivation medium can be stored at 4°C up to seven days.

Recommended procedure for swab specimens processing

1. Just before the DNA isolation immerse throat swabs in 1 ml of PBS. After agitating the swabs for 3 min, open the tube and by pressing the swab along the tubes wall press out the all solution from the swab. Discard the swab.
2. Centrifuge the tube for 15 minutes at 6000 x g or 10 minutes at 10 000 x g.

Manual isolation:

1. Aspirate supernatant. Resuspend pellet in 180 µl of molecular grade water with 200 µl of MI3 solution from EliGene® Urine Isolation Kit, and add 10 µl of Proteinase K and 20 µl of Internal Control DNA 01 (IC DNA 01). Vortex for 15 seconds and continues according to instructions in standard protocol in EliGene® Urine Isolation Kit.
2. Elution is done to 50 µl of elution buffer. Use DNA immediately after the isolation for the analysis or store DNA hours to one week at 4°C or freeze DNA at -20°C for longer period than one week.

Automatic isolation:

1. Open the tubes and carefully aspirate all supernatant. Re-suspend pellets in 180 µl of PBS by vortexing and add 20 µl of Internal control IC DNA 01.
2. Isolate DNA from the sample by using MAGNETO BodyFluid DNA/RNA isolation kit according to protocol for Plasma samples with Elution to 50 µl of Elution buffer.

Nasopharyngeal aspirates (NPAs), sputum:

Homogenize NPAs and sputum specimens by adding an equal volume of a mucolytic agent (dithiotreitol, final concentration 4–6 mM). Centrifuge the specimen at 6000 x g for 15 minutes before DNA isolation.

Manual isolation:

1. Aspirate supernatant. Resuspend pellet in 180 µl of molecular grade water with 200 µl of MI3 solution from EliGene® Urine Isolation Kit, and add 10 µl of Proteinase K and 20 µl of Internal Control DNA 01 (IC DNA 01). Vortex for 15 seconds. Continue according to instructions in standard protocol in EliGene® Urine Isolation Kit.
2. Elution is done to 50 µl of elution buffer. Isolated DNA use immediately for the detection or store it hours to one week at 4 °C or freeze DNA at -20 °C for longer period than one week.

Automatic isolation:

1. Open the tubes and carefully aspirate all supernatant re-suspend pellets in 200 µl of PBS by vortexing and add 20 µl of Internal control IC DNA 01.
2. Isolate DNA from the sample by using MAGNETO BodyFluid DNA/RNA isolation kit according to protocol for Plasma samples with Elution to 50 µl of Elution buffer.

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Additional required equipment

- Automatic pipette 5-20 µl and sterile tips with filter DNA RNA free, DNase-, RNase- free (we recommended plastic with CE certificate for diagnostic purposes).
- Sterile stand DNA-, RNA- free, DNase-, RNase- free.
- Equipment for RealTime PCR – the kit is designed for RealTime Systems LightCycler 2.0, LightCycler 480 a LightCycler Nano (Roche), Quant Studio 3 a 5 (Thermofisher Scientific), RealTime System ABI 7500FAST (Applied Biosystems), Rotor-Gene 6000 nebo Rotor-Gene Q (Qiagen), CFX96 Touch Real-Time PCR Detection System (Bio-Rad), MIC qPCR Cycler (Bio Molecular Systems).
- Sterile plastic (strips, plates, tubes) DNase-, RNase- free compatible with given RealTime PCR system.
- Lab safety gloves

Configuration of Real Time instrument

- For *Mycoplasma pneumoniae* detection the probe labeled with FAM is used (exc. 494 nm – em. 518 nm)
- For Internal control the probe labeled with HEX is used (exc. 520 nm – em. 548 nm)

LightCycler® 2.0 (Roche):

For reaction use glass capillaries only.

Set up the following temperature profile:

Step 1 - Analysis mode "None", 1 Cycle

95°C	3 min	Ramp rate (20°C/s)	Acquisition mode "None"

Step 2 - Analysis mode "Quantification", 50 Cycles

95°C	5 s	Ramp rate (20°C/s)	Acquisition mode "None"
55°C	20 s	Ramp rate (5°C/s)	Acquisition mode "Single"
72°C	10 s	Ramp rate (20°C/s)	Acquisition mode "None"

The complete temperature profile can be up-loaded from Run Template "EliGene_UNI_LC2_v00.ixo". The Run Template can be imported to the software in menu "Navigator" by clicking to icon "Import" from the CD included in the kit.

LightCycler® 480 (Roche):

For reaction use white plates only. The usage of natural plates can lead to decreased sensitivity of the kit. Do not reuse plates; the contamination of your laboratory could occur during the manipulation with plates.

In option Detection format choose "Dual Color Hydrolysis probe".

Set up the following temperature profile:

Step 1 - Analysis mode "None", 1 Cycle

95°C	3 min	Ramp rate (4.4°C/s)	Acquisition mode "None"

Step 2 - Analysis mode "Quantification", 50 Cycles

95°C	5 s	Ramp rate (4.4°C/s)	Acquisition mode "None"
55°C	20 s	Ramp rate (2.2°C/s)	Acquisition mode "Single"
72°C	10 s	Ramp rate (4.4°C/s)	Acquisition mode "None"

Step 3 - Analysis mode "None", 1 Cycle

40°C	1 min	Ramp rate (2.2°C/s)	Acquisition mode "None"

The complete temperature profile can be up-loaded from Run Template "EliGene_UNI_LC480_v00.ixo". The Run Template can be imported to the software in menu "Navigator" by clicking to icon "Import" from the CD included in the kit.

QuantStudio 3 and 5 (ThermoFisher Scientific):

Use the Experiment type," Presence/Absence", Chemistry "TaqMan Probes", and Run Mode "Standard". As reporter dyes use FAM (*Mycoplasma pneumoniae*), VIC/HEX (IC DNA 01) and ROX as a passive reference dye.

Set up the following temperature profile:

Holding stage

95°C 3 min Ramp rate (1.6°C/s)

Holding stage

Cycling stage – 50 cycles

95°C 5 s Ramp rate (1.6°C/s)

55°C 20 s Ramp rate (1.6°C/s) Data collection ON

72°C 10 s Ramp rate (1.6°C/s)

Post-Read Stage

40°C 1 min Ramp rate (1.6°C/s)

Collect emission signal at the second step at 55 °C

The complete temperature profile can be up-loaded from Run Template "EliGene_UNI_QS3_v00.edt" or "EliGene_UNI_QS5_v00.edt". The Run Template can be copied from the CD included in the kit.

LightCycler® Nano (Roche):

For reaction use clear strips only.

In "Run Settings" menu choose "Hydrolysis Probes" option and "High Quality" option.

In "Profile menu" set up the following temperature profile:

Step 1 - Hold

95°C 3 min Ramp rate (5°C/s)

Step 2 – 3-Step Amplification, 50 cycles

95°C 5 s Ramp rate (5°C/s)

55°C 20 s Ramp rate (4°C/s) "Acquire" signal

72°C 10 s Ramp rate (5°C/s)

Step 3 - Hold

40°C 1 min Ramp rate (4°C/s)

In "Samples" menu click in window "Targets" (upper right window) on icon "+" and choose FAM dye as "Target 1". Then click once again on icon "+" and choose HEX dye as "Target 2". In window "Samples" (upper left window) click on icon "+" and add your samples. Then assign the samples with positions and Targets FAM and HEX as an Unknown sample (Samples) or Standard.

The complete temperature profile can be up-loaded from Run Template "EliGene_UNI_LC_NANO_v00.ppf". The Run Template can be copied from the CD included in the kit.

RealTime System ABI7500 (Applied Biosystems):

Use the program module for absolute quantification (Plate Type ”Quantitation-Standard Curve” experiment for ABI 7500FAST). In case of ABI7500FAST use “7500 (96wels)” instrument type.

Set up the following temperature profile:

Holding stage

95°C	3 min	

Cycling stage – 50 cycles

95°C	5 s	
55°C	20 s	Data collection ON
72°C	10 s	

Collect emission signal at the second step – 55°C.

The complete temperature profile can be up-loaded from Run Template “EliGene_UNI_ABI_v00.edt”. The Run Template can be copied from the CD included in the kit.

CFX96 Touch Real-Time PCR Detection System (Bio-Rad):

In Startup Wizard Create a new Experiment for CFX96 instrument and Create New Protocol.

Set up the following temperature profile:

Step 1	95°C	3 min
Step 2	95°C	5 s
Step 3	55°C	20 s + Plate Read
Step 4	72°C	10 s
Step 5	*GOTO Step 2*	*50x*
Step 6	40°C	20 s

Enter the Sample Volume 20 µl

Collect emission signal at the Step 3 at 55° C.

For filter settings use the “Scan Mode” All Channels but in Plate Manager select for the samples only fluorophores FAM and HEX. Then assign the samples with positions and Targets FAM and HEX as an “Unknown” sample or “Standard”.

The complete temperature profile can be up-loaded from Run Template “EliGene_UNI_CFX96_v00.edt”. The Run Template can be copied from the CD included in the kit.

Rotor-Gene 6000 or Q (Qiagen):

In the “New Run” window choose “Three Step” run

Choose the appropriate “Rotor Type” and click “Next”.

Set up the following temperature profile:

Holding stage

95°C	3 min	

Cycling stage – 50 cycles

95°C	5 s	
55°C	20 s	Acquiring in channels “Green” and “Yellow”

72°C 10 s

The complete temperature profile can be up-loaded from Run Template "EliGene_UNI_RG6000_v00.ret". The Run Template can be copied from the CD included in the kit.

MIC qPCR (Bio Molecular Systems Pty Ltd):

In Run Setup Create a new Run Profile for the MIC instrument. Enter the Sample Volume of 20 µl and Temperature Control "Fast TAQ (v3)".

Set up the following temperature profile:

Hold

95°C 3 min

Cycling – 50 cycles

95°C 5 s

55°C 20 s Acquire on "Green" and "Yellow"

72°C 10 s

Hold after cycling

40°C 60 s

The complete temperature profile can be up-loaded from Run Template "EliGene_UNI_MIC_v00.mictemplate". The Run Template can be copied from the CD included in the kit.

Reagent preparation

- To avoid the contamination keep all tubes closed and follow the instructions.
- Before the usage, all reagents must be completely thawed, briefly mix on vortex and shortly spin.
- In the step of Proteinase K addition of Isolation protocol add 20 µl of Internal Control DNA 01 (IC DNA 01) to isolated sample. In no case add the internal control to isolated DNA just before the analysis.
- **If you do not use all the volume of MasterMix, store the tube at dark at temperature 4°C up to 14 days. For long-term storage use the freezer (-20 °C, dark). MasterMix should not go through more than five freeze- thaw cycles.**

WARNING: The contamination in laboratory space is also possible. Use separate pipette for MasterMixes, separate pipette for positive controls and separate pipette for samples. Follow all recommendations for laboratories of DNA analyses.

Preparation of Reaction Mix for the instruments LightCycler 480, Rotor-Gene, CFX96 and MIC qPCR

1. Detection: Take one microtube with M. pneumoniae UNI Mix. After the thawing pipette 15 µl of the Mix to amplification microtubes or plate and add 5 µl of isolated DNA. During the pipetting of samples be careful to avoid cross-contamination of samples.
2. Positive control: Take one microtube with M. pneumoniae UNI Mix. After the thawing pipette 15 µl of Mix to amplification microtube or plate and add 5 µl of PC DNA M. pneumoniae. During the pipetting of positive control be careful to avoid contamination of the other samples.

Insert the micro tubes or plate with samples to the RealTime PCR instrument and run the program according to chapter "Configuration of Real Time instrument" above.

Preparation of Reaction Mix for LightCycler® 2.0

1. Preparation of MasterMix: Prepare the MasterMixes by mixing EliBlocker solution with M. pneumoniae UNI Mix. Take one tube of M. pneumoniae UNI Mix and thaw the content at the room temperature. Immediately after the thawing spin shortly the microtubes and add 3.0 µl of EliBlocker solution to microtube with M. pneumoniae UNI Mix. Mix gently by pipetting up and down and shortly spin.
2. Detection: Take one microtube with M. pneumoniae MasterMix. Pipette 15 µl of the Mix to amplification microtube or plate and add 5 µl of isolated DNA. During the pipetting of samples be careful to avoid cross-contamination of samples.
3. Positive control: Take one microtube with M. pneumoniea MasterMix. Pipette 15 µl of the Mix to amplification microtube or plate and add 5 µl of PC DNA M. pneumoniae. During the pipetting of positive control be careful to avoid contamination of the other samples.

Insert the micro tubes or plate with samples to the RealTime PCR instrument and run the program according to chapter “Configuration of Real Time instrument” above.

Preparation of Reaction Mix for instruments QuantStudio 3 and 5 and ABI7500

1. Preparation of MasterMix: Prepare the MasterMixes by mixing ROX dye solution with M. pneumoniae UNI Mix. Take one tube of M. pneumoniae UNI Mix and thaw the content at the room temperature. Immediately after the thawing spin shortly the microtube and add 3.0 µl of ROX dye solution to microtube with M. pneumoniae UNI Mix. Mix gently by pipetting up and down and shortly spin.
2. Detection: Take one microtube with M. pneumoniae MasterMix. Pipette 15 µl of the Mix to amplification microtubes or plate and add 5 µl of isolated DNA. During the pipetting of samples be careful to avoid cross-contamination of samples.
3. Positive control: Take one microtube with M. pneumoniae MasterMix. Pipette 15 µl of each Mix to individual amplification microtubes or plate and add 5 µl of PC DNA M. pneumoniae. During the pipetting of positive control be careful to avoid contamination of the other samples.

Insert the micro tubes or plate with samples to the RealTime PCR instrument and run the program according to chapter “Configuration of Real Time instrument” above.

Result reading

LightCycler® 2.0 (Roche):

Analyze real-time PCR results in channels 530 and 560 (LightCycler® Software 4.X) respectively, using the Qualitative detection module of the LightCycler® Analysis Software.

Positive result for *Mycoplasma pneumoniae*: The positive result is characterized by amplification and growth of signal in FAM channel (530) of M. pneumoniae UNI Mix reaction. In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (560) in M. pneumoniae UNI Mix.

Cp value corresponds to the quantity of positive result. The value of “Score” must be higher than 4.5.

LightCycler® 480 (Roche):

In “Sample Editor” menu choose “Abs Quant” workflow.

In menu “Analysis” choose “Abs Quant/2nd Derivative Max” option.

In Analysis window click to “Color Comp” icon and choose Universal CC FAM (510)-VIC (580) calibration. Analyze results by clicking to icon “Calculate”.

Positive result for *Mycoplasma pneumoniae*: The positive result is characterized by amplification and growth of signal in FAM channel (465-510) of M. pneumoniae UNI Mix reaction. In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (533-580) in M. pneumoniae UNI Mix.

The values of Concentration correspond to the quantity of positive result; "Negative" means negative result. Positive result is characterized by increasing of fluorescence signal in selected channel.

LightCycler® Nano (Roche):

In "Analysis" menu click in window "Select Analysis" on icon "+" and choose "Automatic Quantification".

Positive result for *Mycoplasma pneumoniae*: The positive result is characterized by amplification and growth of signal in FAM channel (510-528) of M. pneumoniae UNI Mix reaction. In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (530-548) in M. pneumoniae UNI Mix.

The values of Concentration correspond to the quantity of positive result; "Negative" means negative result. Positive result is characterized by increasing of fluorescence signal in selected channel.

RealTime System ABI7500 (Applied Biosystems):

In "Analysis Settings" choose "Automatic Treshold" and "Automatic Baseline" option and analyze results.

Positive result for *Mycoplasma pneumoniae*: The positive result is characterized by amplification and growth of signal in FAM channel (em. 518 nm) of M. pneumoniae UNI Mix reaction. In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (em. 548 nm) in M. pneumoniae UNI Mix.

The values of Qty correspond to the quantity of positive result, "result undet." means negative result. Positive result is characterized by increasing of fluorescence signal in given channel.

Rotor-Gene 6000 or Q (Qiagen)- version 1.7 and higher:

Click to "Analysis" icon in the menu and choose Analysis option "Quantitation". In "Quantitation Analysis" window choose "Dynamic Tube" and "Slope Correct" option.

Positive result for *Mycoplasma pneumoniae*: The positive result is characterized by amplification and growth of signal in FAM channel (Green) of M. pneumoniae UNI Mix reaction. In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (Yellow) in M. pneumoniae UNI Mix.

The values of "Calc. conc." correspond to the quantity of positive result; "Negative" means negative result. Positive result is characterized by increasing of fluorescence signal in given channel.

QuantStudio 3 and 5 (ThermoFisher Scientific):

In "Analyse Settings" choose "Automatic Threshold" and "Automatic Baseline" option and analyze results.

Positive result for *Mycoplasma pneumoniae*: The positive result is characterized by the growth of fluorescence signal in FAM channel (em. 518 nm). In a case of negative results, the amplification will not occur.

The Internal Control must be amplified in each sample. The Internal Control amplification is characterized by the

growth of fluorescence signal in HEX/VIC channel (em. 548 nm).

CFX96 Touch Real-Time PCR Detection System (Bio-Rad):

In Data Analysis window choose "Quantification". In "Settings" menu choose option "Baseline Threshold" and select "Baseline Cycles" option as "Auto Calculated" and Single "Threshold" option as "Auto Calculated".

In Data Analysis window select a single fluorophore (FAM or HEX) by the clicking the box next to the fluorophore name located under the amplification chart and read the results for individual samples.

Positive result for *Mycoplasma pneumoniae*: The positive result is characterized by the growth of fluorescence signal in FAM channel (em. 518 nm). In a case of negative results, the amplification will not occur.

The Internal Control must be amplified in each sample. The Internal Control amplification is characterized by the growth of fluorescence signal in HEX channel (em. 548 nm).

MIC qPCR (Bio Molecular Systems):

To view and analyze acquired data, click the small cross next to Cycling under Analysis module and then select the target – Non-Assay Green (FAM) and Non-Assay Yellow (HEX). In the down right corner **increase** "**Fluorescence Cut-off Level**" **to 20 %**. A quantification cycle (Cq) value is displayed for each positive well. This is the cycle number at which the sample fluorescence rises above the threshold (the red horizontal line near the bottom of the amplification curve chart). In the Samples panel on the right, click sample names to hide or display the associated amplification curves and result data.

Positive result for *Mycoplasma pneumoniae*: The positive result is characterized by the growth of fluorescence signal in Green channel. In a case of negative results, the amplification will not occur.

The Internal Control must be amplified in each sample. The Internal Control amplification is characterized by the growth of fluorescence signal in Yellow channel.

Interpretation of results

Negative result:

If the increasing of amplification signal in FAM channel of M. pneumoniae UNI Mix does not appear before cycle number 45, the result of test should be interpreted as probably *Mycoplasma pneumoniae* DNA negative or with concentration of DNA below the detection limit of this kit (10 genomic DNA/reaction). The signal for Internal Control must be positive – see article Quality control. This result does not exclude the occurrence of *Mycoplasma pneumoniae* infection because results of this test are dependent on proper sample collection and elaboration. Results are also dependent on enough quantity of analyzed *Mycoplasma pneumoniae* DNA.

Positive result:

Amplification signal in FAM channel (465-510) of M. pneumoniae UNI Mix appears before cycle number 45. *Mycoplasma pneumoniae* DNA was detected in the sample. The sample is *Mycoplasma pneumoniae* DNA positive.

WARNING: The contamination in laboratory space is also possible. Use separate pipette for Master mixes, separate pipette for positive controls and separate pipette for samples. Follow all recommendations for laboratories of DNA analyses.

Inhibited sample:

In the case that increasing of amplification signal specific for *Mycoplasma pneumoniae* in FAM channel and also increasing of amplification signal specific for internal control in HEX channel is not observed, it is necessary to repeat the analysis. The best, there is to use DNA samples prepared by new DNA isolation.

Control procedure

EliGene® Mycoplasma pneumoniae UNI Kit involves internal control (IC DNA 01) and positive control (PC DNA M. pneumoniae). Internal isolation control follows the quality of DNA isolation and detects mistakes in the isolation process. It detects the occurrence of an inhibition of amplification process. In the case that the sample is *Mycoplasma pneumoniae* DNA negative, the Cp of internal control must be Cp < 35. In the case of strongly positive samples usually the internal control amplification is not detected.

Positive control follows the proper function of MasterMix. Minimal Cp of positive control must be 35 or less. The Cp higher than 35 for positive control can't be accepted and DNA detection must be repeated with new sample. In the case of repeatedly higher Cp contact manufacturer ELISABETH PHARMACON.

Use negative control for each run. As negative control use the water for molecular biology used in your laboratory. For negative control use the pipette for DNA samples.

Reference material:

To monitor the all examination process covering DNA isolation and RealTime PCR detection is possible to use specimen positive for *Mycoplasma pneumoniae*. The commercial positive material is not available.

Troubleshooting:

1. If there is no amplification of Internal Control, there is some problem in the isolation of DNA or the kit is after the expiration date or there is RealTime instrument breakdown.
2. If there is no amplification of Positive Control, the kit is after the expiration date or there is RealTime instrument breakdown.

Performance characteristics

Analytical performance characteristics:

Kit EliGene® Mycoplasma pneumoniae UNI has a very high sensitivity - detects 10 genomic or plasmid DNA added to the amplification mix. Analytical sensitivity of the test was verified as follows. The plasmid with *Mycoplasma* sequence was prepared and diluted to desired concentration of target sequence. The addition of human DNA to sample in common concentration has no effect to sensitivity of the method.

Analytical sensitivity is 10 copies of *Mycoplasma pneumoniae* in reaction mix.

Analytical specificity of method is 100%. Analytical specificity of method was verified by searching the DNA database NCBI (GenBank, http://www.ncbi.nlm.nih.gov/) for the sequences of primers and probes and by addition of human DNA to mastermix. 50 different samples did not give false positive result. False positive result was not observed after the addition of DNA from these organisms *B. burgdorferi sensu lato*, *M. tuberculosis*, *M. bovis*, *M. cansasii*, *M. xenopii*, *M. avium*, *M. marinum*, *Mycoplasma hominis and genitalium Lactobacillus*, *Enterococcus faecalis*, genus *Pseudomonas*, *E. coli*, *A. niger*, *C. albicans*, *S. aureus*, *S. agalactiae*, *Ch. trachomatis*, *U. urealyticum,* Adenovirus, HBV, EBV, CMV, HSV1, HSV2, VZV.

Clinical specificity was tested on human blood and serum samples. 50 samples of different human DNA isolated from the blood and serum did not give false positive result.

Diagnostic performance characteristics:

Within the frame of testing the functional characteristics of EliGene® Mycoplasma pneumoniae UNI kit overall 50 clinical specimens were analyzed. From these 50 clinical specimens were 9 positive. EliGene® Mycoplasma pneumoniae UNI kit analyzed all 9 samples as positive. Totally 41 clinical specimens were properly by EliGene® Mycoplasma pneumoniae UNI kit analyzed as negative.

Sensitivity: 100%

Specificity: 100%

Measuring interval

The kit enables the detection of 10^1 - 10^8 of *Mycoplasma pneumoniae* DNA molecules in reaction Mix.

Internal control of quality

As an internal control of quality the Internal Control for checking the process of DNA isolation together with Positive Control for functional control of MasterMix and as a reference sample is used.

Limitation of the examination procedure

The sensitivity of kit depends on handling with specimen (isolation of DNA). It is strictly recommended to use isolation kits and procedures mentioned above.

Negative result does not exclude the occurrence of *Mycoplasma pneumoniae* infection because results of this test are dependent on proper sample collection and elaboration. Results are also dependent on enough quantity of analyzed *Mycoplasma pneumoniae* DNA.

Biological reference intervals

Not applicable information for this kit.

Warning

After mixing MasterMix is stable for 2 weeks at -20 °C. Do not freeze tubes with MasterMix repeatedly! Do not mix components of the kits of different lots.

Warnings and general precautions

This kit is intended for *in vitro* use only.

- Handle and dispose of all biological samples as if they were capable of transmitting infective agents. Avoid direct contact with the biological samples. Avoid splashing or spraying. The materials that come into contact with biological samples must be treated with 3% sodium hypochlorite for at least 30 minutes or autoclaved at 121 °C for one hour before disposal.
- Handle and dispose of all reagents and all assay materials as if they were capable of transmitting infective agents. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be treated and disposed of in compliance with the appropriate safety standards. Disposable combustible materials must be incinerated. Liquid waste containing acids or bases must be neutralized before disposal.
- Wear suitable protective clothing and gloves and protect eyes/face.
- Never pipette solutions by mouth.
- Do not eat, drink, smoke or apply cosmetic products in the work areas.
- Wash hands carefully after handling samples and reagents.
- Dispose of leftover reagents and waste in compliance with regulations in force.
- Read all the instructions provided with the kit before running the assay.
- Follow the instructions provided with the kit while running the assay.
- Do not use the kit after the expiry date.
- Only use the reagents provided in the kit and those recommended by the manufacturer.
- Do not mix reagents from different batches.
- Do not use reagents from other manufacturer´s kit.

Warnings and precautions for molecular biology

- Molecular biology procedures, such as extraction, reverse transcription, amplification and detection of nucleic acids, require qualified staff to prevent the risk of erroneous results, especially due to degradation of the nucleic acids contained in the samples or due to sample contamination by amplification products.
- It is necessary to have separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions.
- It is necessary to have lab coats, gloves and tools which are exclusively employed in the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never transfer lab coats, gloves or tools from the area designed for the amplification/detection of amplification products to the area designed for the extraction/preparation of the amplification reactions.
- The samples must be exclusively employed for this type of analysis. Samples must be handled under a laminar safety box. Tubes containing different samples must be never opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases, free from DNA and RNA.
- Reagents must be handled under PCR box or laminar flow box. The reagents required for amplification must be prepared in such a way that they can be used in a single session. The pipettes employed to handle the reagents must be used exclusively for this purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases, free from DNA and RNA.
- Amplification products must be handled in such way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be employed exclusively for this specific purpose.

Warnings and precautions specific to components of the kit

- The tubes containing mix (M. pneumoniae UNI Mix) are disposable and therefore must be used once only in the preparation of the reaction mixture.
- The tubes containing IC DNA 01 and PC DNA M. pneumoniae are disposable and therefore must be used once only in the preparation of the reaction mixture.
- These mixes carry the following safety warnings (P):

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P281 Use personal protective equipment as required.

Literature

Sánchez-Vargas FM, Gómez-Duarte OG. 2008. Mycoplasma pneumoniae-an emerging extra-pulmonary pathogen. Clin Microbiol Infect. 14(2):105-17.

Symbols

	Catalog number
	Upper limit of temperature
	Batch code
	Use by (last day of month)
	*in vitro* diagnostic medical device
	Fulfilling the requirements of European Directive 98\79\EC for *in vitro* diagnostic medical device.
	Contains sufficient for "N" tests
	Attention, consult instructions for use
	Manufacturer

Manufacturer

ELISABETH PHARMACON Ltd.
Rokycanova 4437/5, 615 00 Brno, Czech Republic Tel.: +420 542 213 851, +420 542 213 827
E-mail: info@elisabeth.cz