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EliGene® Borrelia UNI (flagellin)

CE

REF 90024-UNI (for 50 samples)

Kit components:

5 x 150 µl **Borrelia UNI Mix**
2 x 50 µl **PC DNA Borrelia**
5 x 200 µl **IC DNA 01**
1 x 20 µl **Eli Blocker**
1 x 20 µl **ROX dye**
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® Borrelia UNI is intended for DNA diagnostics of pathogenic species *Borrelia garinii, Borrelia afzelii* and *Borrelia burgdorferi sensu stricto* from isolated DNA.

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 *Borrelia* DNA and internal control are used.

Introduction

Lyme borreliosis is a disease caused by spirochete *Borrelia burgdorferi*. The detection of this bacterium could be made from any clinical material using the DNA diagnostics. There can be found three pathogenic species of *Borrelia* in European Union: *B. b. sensu stricto, B. afzelii* and *B. garinii*. EliGene® Borrelia UNI Kit contains mix of primers and probes that are specific to all three mentioned *Borrelia* species. EliGene® Borrelia UNI Kit has a high sensitivity that allows detecting 1–10 Borrelia genomes in 5 µl of DNA sample. DNA diagnostics is useful in cases of *Borrelia* suspicion (anamnesis of tick or insect that suck the blood). For diagnostic purposes following clinical specimen are recommended: a) whole EDTA blood (when the patient has a temperature and in acute period of the disease); b) urine; c) cerebrospinal fluid (in cases of neuroborreliosis); d) synovial fluid; e) tissue (skin biopsy).

Primary sample collection, handling and storage

Clinical material:

Blood, urine, CSF, synovial fluid, tissue

Recommended DNA isolation procedure:

Manual: EliGene® Urine Isolation Kit (ELISABETH PHARMACON)

Automatic: **ZEPHYRUS Magneto** (ELISABETH PHARMACON)

Blood:

It is recommended to take blood in patients with suspicion of *Borrelia* whose have the fever. Non-clotting blood takes into fluid EDTA. For the diagnostic purposes of *Borrelia*, it is necessary to isolate DNA from the sample during the day of blood taking. The blood sample is necessary to be transported stored at 4 °C in any event do not freeze the blood sample!

Manual isolation:

Add 10 µl of Proteinase K and 20 µl of Internal Control DNA 01 (IC DNA 01) to the sample and then continue according to the standard protocol of EliGene® Urine Isolation Kit (ELISABETH PHARMACON) for DNA isolation from blood. Isolated DNA use immediately for the detection or store it hours to one day at 4 °C. The concentration of isolated DNA is getting lower in every freezing process and there is a threat of false negative results. The alternative way to the immediate DNA isolation in case of manual isolation is the possibility to carry out a partial isolation of DNA. The sample is heated with Proteinase K and Lysis buffer in the first step. Next steps of DNA isolations are carried out later. Lysed sample is stored at 4 °C and in a few days the isolation is finished.

Automatic isolation:

Add 20 µl of Internal Control DNA 01 (IC DNA 01) and isolate DNA from the sample by using MAGNETO BodyFluid DNA/RNA isolation kit according to protocol for blood samples with elution to 50 µl of Elution buffer.

CSF:

According to standard protocol take the sample of the CSF into sterile tubes. Samples must be stored and transported at 4 °C. For the diagnostic purposes, it is recommended to isolate DNA from the sample during the day of taking sample. For DNA isolation at least 200 µl of sample must be used.

Manual isolation:

Add 20 µl of Proteinase K to the sample and 20 µl of Internal Control DNA 01 (IC DNA 01) and then continue according to the standard protocol of EliGene® Urine Isolation Kit (ELISABETH PHARMACON) for DNA isolation from blood. 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:

Add 20 µl of Internal Control DNA 01 (IC DNA 01) and 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.

Urine, Synovial fluid:

According to standard protocol, take the sample of the urine or synovial fluid into sterile tubes. Samples must be stored and transported at 4 °C. For the diagnostic purposes, it is necessary to isolate DNA from the sample during the day of taking. The sample of urine and synovial fluid must be centrifuged at 6000 x g for 20 minutes before the DNA isolation. **In no case lower the centrifugation force or time of centrifugation!** Use 8–15 ml of urine specimen. In the case that laboratory do not have centrifuge for large volumes, aliquot urine specimen to lower volumes but overall volume must be at least 8 ml.

Manual isolation:

1. Aspirate supernatant. Take care; all urine must be aspirated before the re-suspending! 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 100 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 180 µl of PBS by vortexing and add 20 µl of Internal control DNA 01 (IC DNA 01).

   WARNING: The rest of urine in the tube can influence the purity of isolated DNA.

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.

Tissue:

Store the tissue immediately after the excise in the refrigerator at 4 °C and transport it to the laboratory during the same day.

Manual isolation:

For the diagnostic purposes, it is necessary to isolate DNA using UltraClean Tissue DNA Kit (MoBio) from the sample during the day of taking.

Add 20 µl of Internal Control DNA 01 (IC DNA 01) to the sample in the beating microtube. 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:

Cut up to 10 mg tissue sample into small pieces, add 200 µl Lysis Buffer, 6 µl Proteinase K and 20 µl of Internal Control DNA 01 (IC DNA 01) and isolate DNA from the sample by using Prepito DNA Tissue10 Kit according to protocol with elution to 50 µl of Elution buffer.

Additional required equipment

- Automatic pipette 5–20 µl and sterile tips with filter DNA-, RNA- free, DNase-, RNase- free (we recommend 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 *Borrelia* DNA 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 (Borrelia), 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_LCNANO_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_ABI7500_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 BORRELIA UNI Mix and after the thawing 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.
2. Positive control: Take one microtube with BORRELIA UNI Mix and after the thawing pipette 15 µl of mix to amplification microtube or plate and add 5 µl of PC DNA Borrelia. During the pipetting of positive control be careful to avoid contamination of other samples. Use separate pipette for positive controls!

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 MasterMix by mixing Eli Blocker solution with BORRELIA UNI Mix. Take one tube of BORRELIA UNI Mix and thaw the content at the room temperature. Immediately after the thawing spin shortly the microtube and add 3 µl of Eli Blocker solution to one microtube with BORRELIA UNI mix. Mix gently by pipetting up and down and shortly spin.
2. Detection: Take one microtube with MasterMix and pipette 15 µl of mix to glass capillary 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 MasterMix and pipette 15 µl of MasterMix to glass capillary and add 5 µl of PC DNA Borrelia. During the pipetting of positive control be careful to avoid contamination of other samples. Use separate pipette for positive controls!

Insert the glass capillaries 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 MasterMix by mixing ROX dye solution with BORRELIA UNI Mix. Take one tube of BORRELIA UNI Mix and thaw the content at the room temperature. Immediately after the

thawing spin shortly the microtube and add 3 µl of ROX dye solution to one microtube with BORRELIA UNI mix. Mix gently by pipetting up and down and shortly spin.

2. Detection: Take one microtube with MasterMix and pipette 15 µl of 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 MasterMix and pipette 15 µl of MasterMix to amplification microtube or plate and add 5 µl of PC DNA Borrelia. During the pipetting of positive control be careful to avoid contamination of other samples. Use separate pipette for positive controls!

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 *Borrelia*: The positive result is characterized by amplification and growth of signal in FAM channel (530). 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).

Cp value corresponds to the quantity of positive result. The value of “Score” must be higher than 3.5.

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 *Borrelia*: The positive result is characterized by amplification and growth of signal in FAM channel (465-510). 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).

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 *Borrelia*: The positive results are characterized by amplification and growth of signal in FAM channel (510-528). 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).

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 *Borrelia*: The positive results are characterized by amplification and growth of signal in FAM channel (em. 518 nm). 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).

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 *Borrelia*: The positive result is characterized by amplification and growth of signal in FAM channel (Green). 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).

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 Borrelia: 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 Borrelia: 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 Borrelia: 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 does not appear before cycle number 50, the result of test should be interpreted as probably *Borrelia* DNA negative or with concentration of *Borrelia* DNA below the detection limit of this kit (10 genomic DNA/reaction). The signal for Internal Control must be positive. This result does not exclude the occurrence of *Borrelia* infection because results of this test are dependent on proper sample collection and elaboration. Results are also dependent on enough quantity of analyzed *Borrelia* DNA.

Positive result:

Amplification signal in FAM channel will appear before cycle number 45. *Borrelia* DNA was detected in the sample. The sample is *Borrelia* DNA positive.

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.

Inhibited sample:

In the case that increasing of amplification signal in FAM channel (specific for *Borrelia*) as well as increasing of amplification signal in HEX channel (specific for Internal Control) is not observed, it is necessary to repeat the analysis. The best would be to use new DNA prepared by new extraction process.

Control procedure

EliGene® Borrelia UNI Kit involves Internal Control DNA 01 (IC DNA 01) and Positive Control (PC DNA Borrelia). 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 *Borrelia* 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 *Borrelia* DNA. The commercial positive material is not available.

Troubleshooting:

1. If there is no amplification of Internal control DNA 01 (IC DNA 01), 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® Borrelia UNI has a very high sensitivity - detects 2–20 genomic DNA added to the amplification mix.

Analytical sensitivity of the test was verified as follows. DNA from pure culture of *B. afzelii, B. garinii* and *B. b. s. stricto* was isolated and diluted. 2, 20 and 200 *Borrelia* genomes were added to MasterMix. Each concentration in each species was tested for three times. Detection of *Borrelia* was successfully for 100% in all samples containing 20 and more *Borrelia*. In samples containing 2 *Borrelia* per MasterMix was found the sensitivity 44,4 % (4 from 9 samples). The addition of human DNA in usual concentration has no effect to the sensitivity of the method.

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. Further, false positive result was not observed after the addition of gDNA from these organisms: *C. trachomatis*, *M. tuberculosis*, *M. bovis*, *M. cansasii*, *M. xenopii*, *M. avium*, *M. marinum*, *Lactobacillus*, *Enterococcus faecalis*, genus. *Pseudomonas*, *E. coli*, *A. niger*, *C. albicans*, *S. aureus*, *S. agalactiae*, *N. gonorrhoeae*, *U. urealyticum* Adenovirus, HBV, EBV, CMV, HSV1, HSV2, VZV.

Clinical specificity was tested on 486 clinical samples. In this clinical study the specificity 100% of probe and primers design was found.

Diagnostic performance characteristics:

The clinical sensitivity and specificity of EliGene® Borrelia UNI kit is 100%.

Measuring interval

The kit enables the detection of 10^1–10^8 of *Borrelia* DNA molecules in reaction mix.

Internal control of quality

As an internal control of quality the Internal control DNA 01 (IC DNA 01) 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.

The success of *Borrelia* detection is conditional on taking sample from the patient in the suitable time (having temperature). The sensitivity of *Borrelia* detection depends on taking the sample (whole no clotting blood, in chronic patients is better to use urine), the way of storage (store at 4 °C), the way of elaboration (DNA isolation, day of taking the sample, detection immediately after the isolation), method of the isolation of DNA (we strictly recommend to use above mentioned DNA isolation kits EliGene® Urine Isolation Kit or MAGNETO BodyFluid DNA/RNA isolation kit).

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

- 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 (Borrelia UNI Mix) are disposable and therefore must be used once only in the preparation of the reaction mixture.
- The tubes containing IC DNA 01 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

Barbara A. Bannister, Norman T. Begg and Stephen H. Gillespie: Infectious Disease. Blackwell Science, 2th Ed., 2000

Hytönen J, Hartiala P, Oksi J, Viljanen MK. 2008. Borreliosis: recent research, diagnosis, and management. Scand J Rheumatol. 37(3):161-172

Priem S, Rittig MG, Kamradt T, Burmester GR, Krause A. 1997. An optimized PCR leads to rapid and highly sensitive detection of Borrelia burgdorferi in patients with Lyme borreliosis. J Clin Microbiol. 35(3): 685–690

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.

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