

BactoReal[®] Kit

Borrelia burgdorferi sensu lato

Manual



CE

IVD

For *in vitro* diagnostic use

REF

DHUB05053

Σ

50 reactions



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Index

1. Intended use.....	3
2. Product description.....	3
3. Pathogen information.....	3
4. Contents of the kit, stability and storage.....	4
5. Additionally required materials and devices.....	4
6. Precautions and safety information.....	4
7. Limitations.....	5
8. Preparation of samples.....	6
9. Preparation of real-time PCR.....	6
9.1. Pipetting scheme.....	6
9.2. Programming of temperature profile.....	7
10. Interpretation of PCR-data.....	7
11. Troubleshooting.....	8
11.1. No signal in FAM channel and Cy5 channel with controls and sample:.....	8
11.2 Valid results for controls, but no signal in FAM channel and Cy5 channel with sample:.....	8
11.3. Signal in FAM channel in negative control:.....	8
11.4. Signal in FAM channel in extraction negative control:.....	8
12. Specifications and performance evaluation.....	9
12.1. Kit performance.....	9
12.2. Limit of detection and linearity.....	10
12.3. Analytical specificity.....	10
12.4. Diagnostic evaluation.....	10
13. References.....	10

Explanation of symbols



Batch code



Catalogue number



Contains sufficient for <n> tests



This product fulfils the requirements of the European Directive 98/79 EC for *in vitro* diagnostic medical devices



Corrosion, GHS05



Use by



Manufactured by



Store at



For *in vitro* diagnostic use



Exclamation mark, GHS07

1. Intended use

BactoReal[®] Kit *Borrelia burgdorferi* sensu lato is an *in vitro* diagnostic test, based on real-time PCR, for the detection of DNA of *Borrelia* species belonging to the *Borrelia burgdorferi* sensu lato (s.l.) complex.

2. Product description

BactoReal[®] Kit *Borrelia burgdorferi* sensu lato detects the *ospA* gene of *Borrelia burgdorferi* s.l. species, namely *Borrelia burgdorferi* sensu stricto, *B. garinii*, *B. afzelii*, *B. valaisiana*, *B. lusitaniae*, *B. bavariensis*, *B. bissettii* and *B. spielmanii* (*B. spielmanii* is detected with lower sensitivity). This test allows the rapid and sensitive detection of DNA of *Borrelia burgdorferi* sensu lato from samples purified from tissue, blood, synovial or cerebrospinal fluid (e.g. with the QIAamp DNA Mini Kit, Qiagen).

A probe-specific amplification-curve in the FAM channel indicates amplification of *Borrelia burgdorferi* s.l. specific DNA. An internal DNA positive control (DNA IPC) is detected in Cy5 channel and is used as DNA extraction as well as real-time PCR inhibition control. The target for the DNA IPC is extracted with the sample.

This test has been validated with the Applied Biosystems[®] 7500 Fast Real-time PCR System (Thermo Fisher Scientific) and tested with a LightCycler[®] 480 Instrument II (Roche) and Mx3005P[®] QPCR System (Agilent), but is also compatible with other real-time PCR instruments which detect and differentiate fluorescence in FAM and Cy5 channel.

The test is based on real-time PCR. A specific DNA sequence of the pathogen genome is detected and amplified. The generated PCR-product is detected by an oligonucleotide-probe labelled with a fluorescent dye. This technology allows a sequence-specific detection of PCR amplicates.

Ingenetix ViroReal[®], BactoReal[®] and ParoReal Kits are optimized to run under the same thermal cycling conditions. RNA and DNA can be analysed in one run.

3. Pathogen information

Borrelia are Gram-negative bacteria belonging to the *Spirochaetaceae*. The genus *Borrelia* consists of numerous species. Besides the species belonging to the *B. burgdorferi* sensu lato-complex there are also human pathogenic *Borrelia* species causing relapsing fever (*B. recurrentis*, *B. duttonii* and *B. hermsii*). Species belonging to the *B. burgdorferi* sensu lato-complex and causing Lyme disease (Lyme borreliosis) are *Borrelia burgdorferi* sensu stricto, *B. garinii*, *B. afzelii*, *B. valaisiana*, *B. lusitaniae*, *B. bavariensis*, *B. bissettii* and *B. spielmanii*. These are associated with different genospecies-specific symptoms. They are transmitted by ticks, mainly by *Ixodes ricinus*. Lyme disease is a multisystemic illness, which can affect various organs, especially the nervous system, skin, heart and joints.

4. Contents of the kit, stability and storage

Labelling	Content	Amount	Storage
Borrelia burgdorferi s.l. Assay Mix (green cap)	Primer and probe (FAM) for <i>Borrelia burgdorferi</i> s.l. detection	1 x 50 µl	-15 °C to -25 °C
DNA IPC-3 Assay Mix (yellow cap)	Primer and probe (Cy5) for DNA IPC detection	1 x 50 µl	-15 °C to -25 °C
DNA IPC Target (orange cap)	Target for DNA IPC (internal DNA positive control system)	1 x 100 µl	-15 °C to -25 °C
Borrelia burgdorferi s.l. Positive Control (red cap)	DNA positive control (approx. 10,000 target copies/µl)	1 x 25 µl	-15 °C to -25 °C
DNA Reaction Mix (white cap)	DNA amplification mix	1 x 500 µl	-15 °C to -25 °C until first use, then at +4°C
Nuclease-free water (blue cap)	Nuclease-free water	1 x 1000 µl	-15 °C to -25 °C

The components of BactoReal® Kit *Borrelia burgdorferi* sensu lato are stable until the expiry date stated on the label.

5. Additionally required materials and devices

- Reagents and devices for DNA-extraction
- Nuclease-free water for dilution of DNA IPC Target
- Disposable powder-free gloves
- Pipettes (adjustable)
- Sterile pipette tips with filters
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- Real-time PCR instrument which is able to detect and differentiate fluorescence in FAM and Cy5 channel
- Optical 96 well reaction plates or optical reaction tubes

6. Precautions and safety information

- For *in vitro* diagnostic use. The use of this kit is limited to persons instructed in the procedures of real-time PCR and *in vitro* diagnostics.
 - Clean benches and devices periodically.
 - Use sterile filter pipette tips.
 - Specimens should be handled as if infectious in accordance with safe laboratory procedures. Wear protective disposable powder-free gloves when handling kit reagents and specimens.
 - Use separated working areas for specimen preparation, reagent preparation and amplification. Supplies and equipment must be dedicated to each of these separate working areas and ensure workflow in the laboratory from pre- to post-PCR.
 - Be careful when handling samples and positive control to avoid cross contamination. Change gloves after handling of samples or positive control. Store positive or potentially positive material separately from reagents
 - Prevent contamination of work equipment and reagents with DNA/RNA, nuclease or amplification products by good laboratory practice.
 - Quality of DNA has a profound impact on the test performance. Ensure that the used DNA extraction system is compatible with real-time PCR technology.
 - Always include a negative control per PCR-run (nuclease-free water instead of sample).
 - For a valid interpretation of results, a negative control should be included during DNA-extraction (e.g. extraction of water instead of sample material), in order to exclude false-positive results due to contamination with DNA of pathogen during extraction.
 - Please note the expiry date of the kit.
 - Do not interchange or mix reagents from kits with different lot numbers.
 - Repeated thawing and freezing of kit components should be avoided. Protect kit components from light.
- Caution:** DNA IPC Target is stored in RNA/DNA stabilizer which contains Guanidinium thiocyanate/Triton X-100 (see MSDS, www.ingenetix.com).

- Use established laboratory practices according to your local safety regulations for discarding specimens, reagents and waste.

7. Limitations

- Optimal performance of this test requires appropriate specimen collection, transport and storage, as well as an appropriate DNA extraction procedure.
- DNA extraction and *Borrelia burgdorferi* s.l. detection have been validated for tissue, blood, synovial or cerebrospinal fluid with this kit. Test performance with other specimen types has not yet been assessed.
- A negative test result does not exclude the possibility of *Borrelia burgdorferi* s.l. infection, because test results may be affected by improper specimen collection, technical error, specimen mix-up or pathogen quantities below the assay sensitivity. The presence of PCR inhibitors may lead to invalid results.
- For this kit highly specific primers and probes have been selected. However, false-negative or less sensitive results might be obtained due to sequence heterogeneity within the target region of not yet described clinical subtypes. *Borrelia spielmanii* is detected with lower relative fluorescence intensities.
- Results should be interpreted in consideration of clinical and laboratory findings.

8. Preparation of samples

Extract samples with a DNA extraction system compatible with real-time PCR technology. An extraction negative control should be included during DNA-extraction (e.g. extraction of water instead of sample material).

The **DNA IPC Target** has to be added during extraction. The DNA IPC is used as a control of DNA extraction, identifies possible PCR inhibition and confirms the integrity of kit reagents.

Caution: The DNA IPC Target must not be added directly to the sample material but has to be pipetted to the lysis buffer.

→ For an elution volume of 50-100 µl: Per sample, spike 1 µl DNA IPC Target into lysis buffer.

→ For an elution volume of >100 µl or when using an automated extraction system: Per sample, spike 2 µl DNA IPC Target into lysis buffer.

9. Preparation of real-time PCR

- Include one negative control (water), one positive control and one extraction negative control per PCR run.
- It is recommended to analyse samples in duplicates, which increases the probability of pathogen detection and facilitates interpretation of results.
- Thaw DNA samples on ice.
- Thaw kit components at room temperature. When thawed, mix components, centrifuge briefly and keep on ice.
- Mix the DNA Reaction Mix to ensure homogeneity of solution.
- **Positive Control**
 - Use 1 µl of *Borrelia burgdorferi* s.l. Positive Control + 4 µl nuclease-free water. Pipette positive control at last.

9.1. Pipetting scheme

		Per sample
Preparation of Master Mix (mix well)	Nuclease-free Water*	3.0 µl
	DNA Reaction Mix	10.0 µl
	<i>Borrelia burgdorferi</i> s.l. Assay Mix	1.0 µl
	DNA IPC-3 Assay Mix	1.0 µl
	Total volume Master Mix	15.0 µl
Preparation of PCR	Master Mix	15.0 µl
	DNA-Sample*	5.0 µl
	Total volume	20.0 µl

*1-8 µl of the sample can be used. When using ≠ 5 µl sample, the volume of water has to be adjusted accordingly.

→ **If DNA IPC Target was not added during extraction:** Freshly dilute the DNA IPC Target 1:100 with nuclease-free water and add 1 µl per sample directly to the master mix.

Caution: The use of more than 1 µl diluted (1:100) DNA IPC Target per reaction causes inhibition of the real-time PCR reaction.

9.2. Programming of temperature profile

Please find further information on programming the real-time PCR instrument in the respective operator's manual. Take into consideration that some PCR-platforms have to be calibrated with the corresponding dye before performing a multiplex-PCR.

Select detection channel: FAM-NONE, 530 nm (for *Borrelia*)
Cy5-NONE, 667 nm (for DNA IPC-3)

Select reference dye (passive reference): ROX

Sample Volume: 20 µl

Temperature Profile:

Program 1	Program 2	Program 3
Cycles: 1 Analysis: None	Cycles: 1 Analysis: None	Cycles: 45 Analysis: Quantification Acquisition at 60°
50°C *2 min	95°C 20 sec	95°C 5 sec 60°C 1 min

For ABI PRISM® 7500:
Ramp speed: "Standard"

For LightCycler® 480 instrument:
Detection format: 2 Color Hydrolysis Probe

***Note:** If viral RNA should be also detected in the same PCR run, program 1 has to be prolonged to 15 min at 50°C. This temperature profile can be used for all ingenetix ViroReal®, BactoReal®, MycoReal and ParoReal kits for the detection of DNA or RNA.

10. Interpretation of PCR-data

For analysis of PCR results select fluorescence display options 530 nm (FAM channel) for the *Borrelia* target and 667 nm (Cy5 channel) for the DNA IPC target. Samples with positive Ct or Cp-values are considered positive.

IMPORTANT: Please additionally check the amplification-curves manually. Samples should be inspected both in logarithmic and linear scale view and compared with the negative control.

	FAM channel <i>Borrelia</i> target	Cy5 channel DNA IPC target	Interpretation
Negative control	Negative	Negative / Positive ¹	Valid
Positive control	Positive	Negative / Positive ¹	Valid
Extraction negative control	Negative	Positive	Valid
Sample	Positive	Positive / Negative ²	Positive
Sample	Negative	Positive	Negative ³
Sample	Negative	Negative	Invalid

¹Only positive if the DNA IPC Target was added at a 1:100 dilution directly to the master mix

²High pathogen load in the sample can lead to reduced or absent signal of DNA IPC

³The positive signal of the DNA IPC excludes a possible PCR inhibition. However, IPC Ct-values should show comparable results. A shift of Ct-values can indicate a partial inhibition of PCR.

In case of invalid data, analysis has to be repeated with the remaining or newly extracted DNA sample (see 11. Troubleshooting).

11. Troubleshooting

11.1. No signal in FAM channel and Cy5 channel with controls and sample:

- Incorrect programming of the temperature profile or detection channels of the real-time PCR instrument.
→ Compare temperature profile and programming of detection channels with the protocol.
- Incorrect configuration of PCR reaction.
→ Check your work steps (see pipetting scheme) and repeat PCR, if necessary.

11.2 Valid results for controls, but no signal in FAM channel and Cy5 channel with sample:

- Incorrect programming of detection channels with the sample
→ Compare programming of detection channels with protocol.
- If the DNA IPC Target was added during extraction:
 - PCR reaction has been inhibited.
 - DNA extraction has failed.
 - The undiluted DNA IPC Target has not been added to lysis buffer of sample.
 - The extracted sample has not been added to PCR reaction.
→ No interpretation can be made. Make sure you use a recommended method for DNA isolation and stick closely to the manufacturer's instructions. Check your work steps.

11.3. Signal in FAM channel in negative control:

- A contamination occurred during preparation of PCR.
→ Repeat PCR with new reagents in replicates.
→ Strictly pipette positive control at last.
→ Make sure that work space and instruments are cleaned at regular intervals.

11.4. Signal in FAM channel in extraction negative control:

- A contamination occurred during extraction.
→ Repeat extraction and PCR using new reagents.
→ Make sure that work space and instruments are cleaned at regular intervals.

12. Specifications and performance evaluation

12.1. Kit performance

Performance of BactoReal® Kit *Borrelia burgdorferi* sensu lato with an Applied Biosystems® 7500 Fast Real-time PCR System (Thermo Fisher Scientific) is shown in Figure 1.

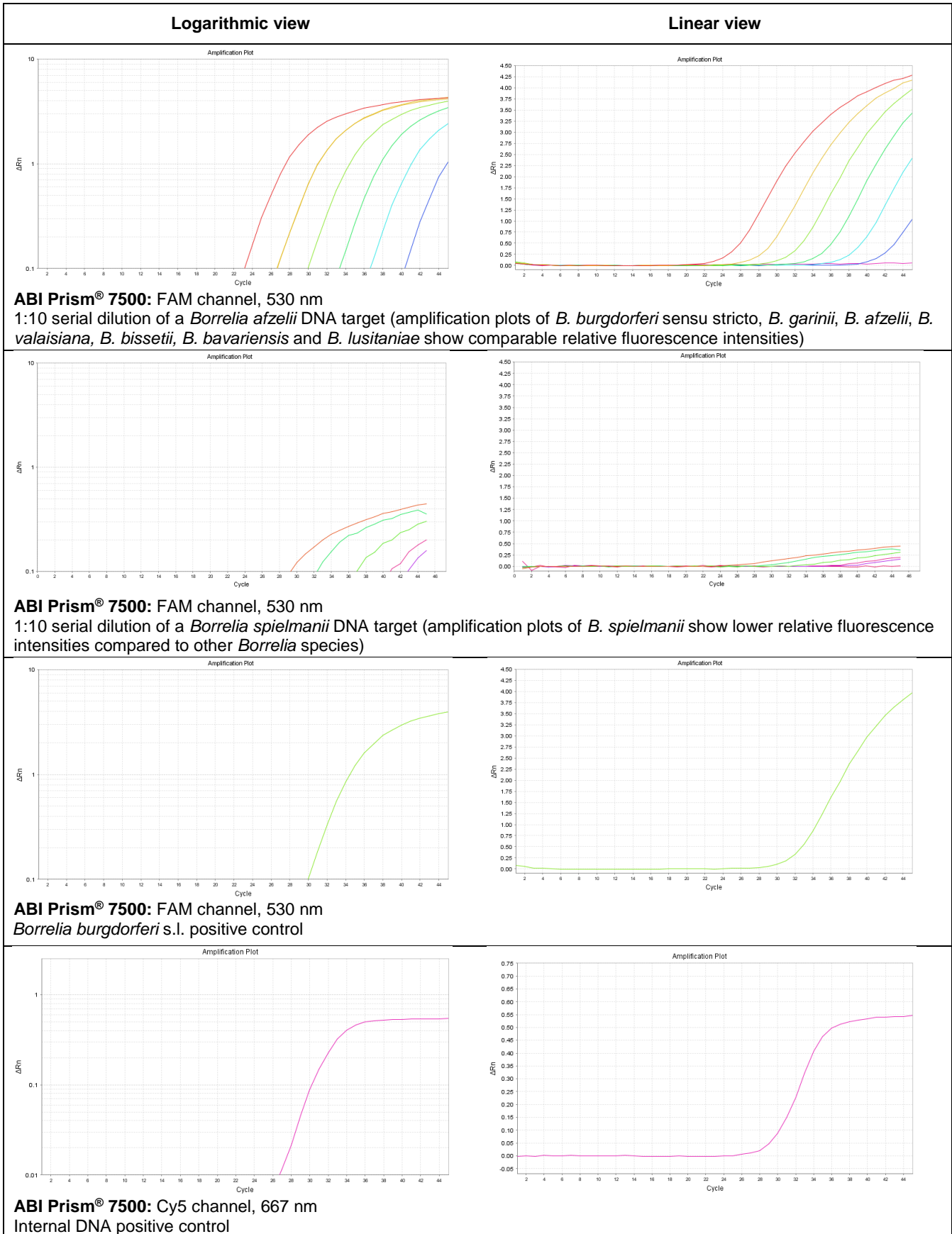


Figure 1 Performance of BactoReal® Kit *Borrelia burgdorferi* sensu lato

12.2. Limit of detection and linearity

BactoReal® Kit *Borrelia burgdorferi* sensu lato was tested with a 10-fold dilution series of a plasmid containing a fragment of *Borrelia afzelii* DNA. At least 10 target copies/reaction could be detected.

The **limit of detection** (LoD95 = smallest number of copies of target DNA which can be detected in 95% of cases) is 42 target copies/reaction.

The assay shows **linearity** over the range of 100 to 1,000,000 target copies/reaction with a slope of -3.35 ± 0.1 and a R_2 of 0.99 as shown in Figure 2.

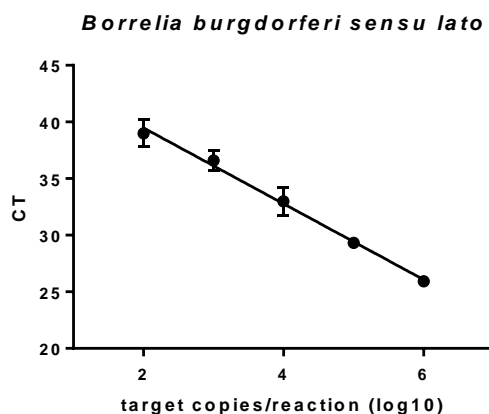


Figure 2 Ten-fold dilution series of a *Borrelia afzelii* DNA standard plotted against CT

12.3. Analytical specificity

The selection of highly specific primers and probes ensures analytical specificity. Primers and probes have been checked for possible homologies to currently published sequences by sequence comparison analysis. This validates the detection of so far known *Borrelia burgdorferi* s.l. strains.

Analytical specificity has been further evaluated by testing genomic DNA of adenovirus and of bacteria not belonging to the *Borrelia burgdorferi* sensu lato complex (*Borrelia hermsii*, *Borrelia miyamotoi*, *Bordetella pertussis*, *Bordetella parapertussis*, *Chlamydomphila pneumoniae*, *Haemophilus influenzae*, *Legionella pneumophila*, *Leptospira* sp., *Listeria monocytogenes*, *Neisseria meningitidis*, *Mycoplasma pneumoniae* and *Treponema phagedenis*). No cross-reactions have been observed.

Furthermore, genomic DNA of bacteria belonging to the *Borrelia burgdorferi* sensu lato complex was tested (*B. afzelii*, *B. burgdorferi* sensu stricto, *B. bavariensis*, *B. bissettii*, *B. coriaceae*, *B. garinii*, *B. lusitaniae*, *B. spielmanii*, *B. turdi*, *B. valaisiana* and *B. japonica*). *Borrelia spielmanii* was detected with lower relative fluorescence intensities compared to other *Borrelia* species. *Borrelia japonica*, a non-humanpathogenic *Borrelia* species was not detected. All other *Borrelia* isolates were detected and showed comparable relative fluorescence values.

12.4. Diagnostic evaluation

Diagnostic evaluation was performed with 80 DNA isolates from clinical specimens tested with accredited reference methods. Specimens included one tissue biopsy, one stool sample, 46 cerebrospinal fluids, 27 synovial fluids and 5 EDTA-blood samples. With the reference methods 42 samples were positive for *Borrelia burgdorferi* s.l. (*B. afzelii* n=6, *B. burgdorferi* sensu stricto n=6, *B. bavariensis* n=3, *B. bissettii* n=3, *B. garinii* n=16, *B. spielmanii* n=2, *B. valaisiana* n=4, *B. japonica* n=1) and 38 were negative (*B. hermsii* n=1, *B. miyamotoi* n=1, *Leptospira* sp. n=2, *Treponema* sp. n=1 and samples negative for *Borrelia burgdorferi* s.l. n=33).

With BactoReal® Kit *Borrelia burgdorferi* sensu lato 41 out of the 42 positive samples were also positive, albeit the two *B. spielmanii* positive samples showed a lower relative fluorescence intensity and were assessed questionable positive. The *Borrelia japonica* positive sample was tested negatively (non-humanpathogenic species).

The 38 samples negative with the reference methods were also negative with BactoReal® Kit *Borrelia burgdorferi* sensu lato.

13. References

O'Connell S. 2010. Lyme borreliosis: current issues in diagnosis and management. *Curr. Opin. Infect. Dis.* 23:231-5.