LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN
MEDIZINISCHE FAKULTÄT
MAX VON PETTENKOFER-INSTITUT

Borrelia burgdorferi sensu lato complex Assay

For the detection of *Borrelia burgdorferi sensu lato* complex *(ospA- and p41(Flagellin)* genes) using the BD MAX™ system.

Instructions for use

(Version 2.0 – November 2018)

Attention

BD changes the format of the BD MAX™ - ExK™ DNA-Extraktion-Kits

Please take notice of changes in the protocol-settings (marked in red)

Contents

Introduction

Borrelia burgdorferi sensu lato is the causative agents of Lyme borreliosis. Currently 18 different *Borrelia* species are grouped in this *B. burgdorferi* sensu lato complex. Besides dermatitis being the most common manifestations of Lyme disease, frequent pathologies include arthritis, carditis, and neurological symptoms related to the inflammatory response of the host to *B burgdorferi*. The "Bb" panel consists of a real-time PCR system for the detection of the *ospA* gene and *p41* flagellin gene, both highly specific for *B. burgdorferi* sensu lato. The "Bb" panel has successfully been applied for detection of 16 different *Borrelia* species, such as *B. burgdorferi* sensu stricto, *B. garinii*, *B. afzelii*, *B. bavariensis*, *B. spielmanii* and *B. valaisiana*.

This protocol describes the system settings and setup protocols for running the "Bb" panel to detect *Borrelia burgdorferi* complex using the BD MAX™ system (assay target genes):

1. *ospA*
2. *p41 (Flagellin)*
3. *β-Actin*

The qPCR has been validated for cerebrospinal fluid, tissue sample and joint aspiration.

Contact information

For information regarding ordering dried snap-in tubes for the "Bb" assay:

info@biolegio.com

For information regarding to the protocol:

schubert@med.uni-muenchen.de

1. Protocol

This protocol describes the assay settings required for running the "Bb" - assay on the BD MAX™ system. The "Bb" snap-in assay contains primers and probes for the detection of *Borrelia burgdorferi* sensu lato complex – *ospA and p41*. Additionally, the assay provides primers and probe for β-actin of eukaryotic cells used as internal control for the DNA extraction and PCR performance.

Materials needed

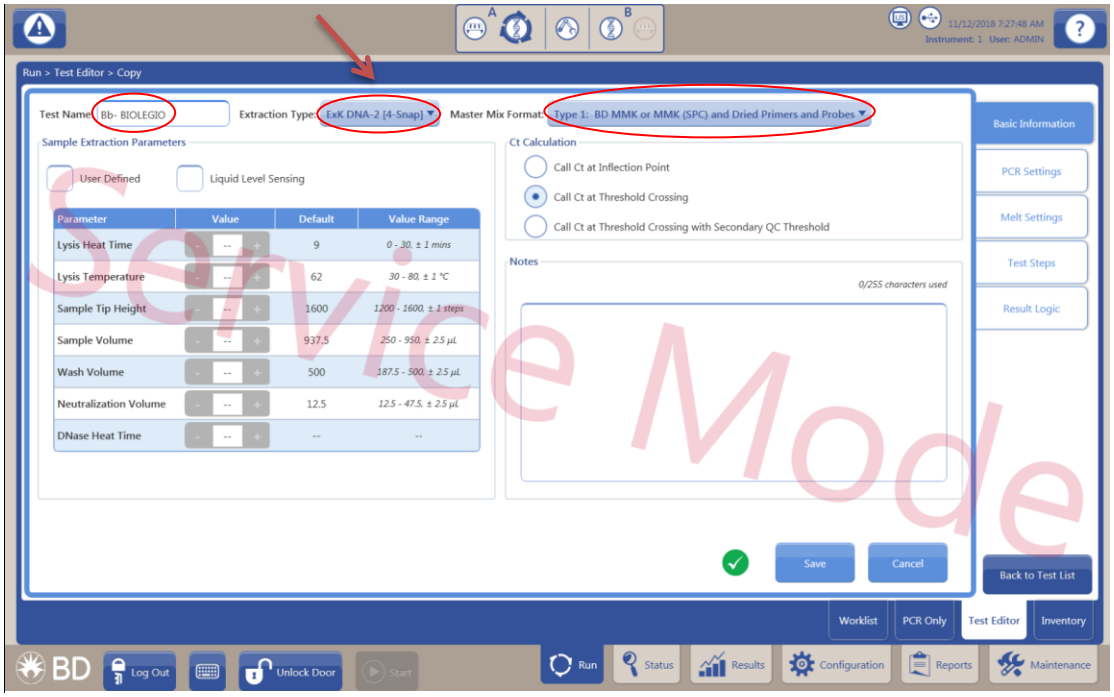
- BD MAX™ system
- BD MAX™ ExK DNA-2 Extraction Kit (BD cat.no: 442820)
  New product – 4-snap format
- BD MMK Mastermix **with** SPC (BD cat.no: 442829)
- BD MAX™ PCR Cartridges (BD cat.no: 437519)
- Dried snap-ins "Bb" (Biolegio cat no: BDT-14015)
- Eppendorftubes 1,5 ml
- Vortex Mixer
- Micropipettes
- Safeseal Filtertips
- Disposable gloves

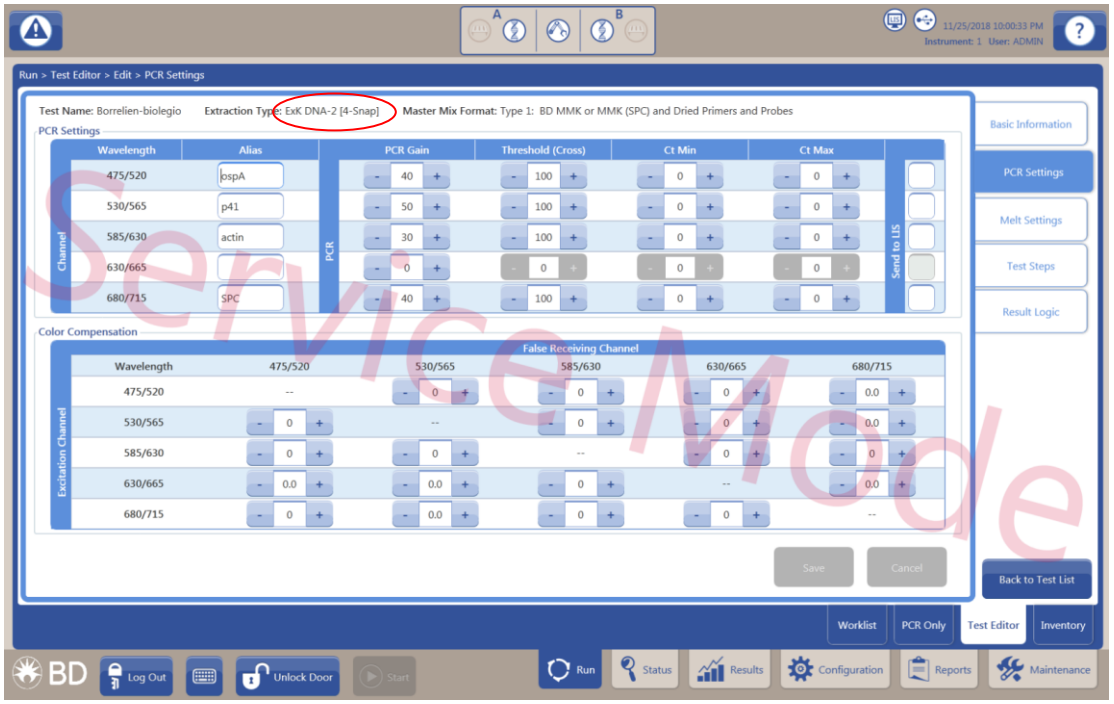
1.1 Run settings

The assay is performed on the BD MAX™ system with use of the BD MMK with SPC in combination with the ExK DNA-2 Kit for the extraction.

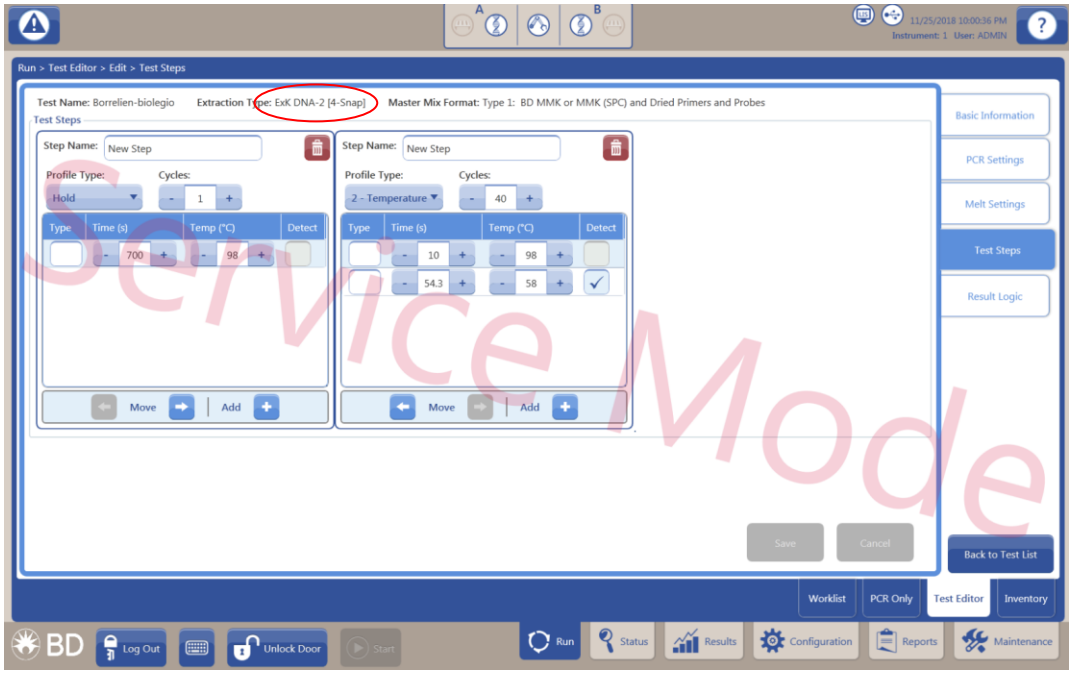
The BD MAX software version 4.70A or 4.72A is essential for the test procedure with the "4-snap extraction Kit".

Create a full process assay in the test editor named "Bb - assay" and use the following parameters:





Edit the test steps using the following settings:



1.3. Sample preparation

➢ Cerebrospinal fluid and joint aspiration

- Cerebrospinal fluid and joint aspiration (1 ml) need to be centrifuged at a speed of 16000 g for 5 minutes, then remove 700 µl of the supernatant and resuspend the pellet in the remaining 300 µl .
- Extraction will be done with the "BD MAX™ ExK DNA-2 Extraction Kit"

➢ Tissue sample

- Transfer the sample to a sterile Petri dish (e.g.), than cut the sample into small pieces using a sterile scalpel.
- Transfer the cut specimen to a 2 ml Epi containing 200 µl lysis-buffer (e.g. High Pure PCR Template Preparation Kit, Fa. Roche). Then add 40 µl of Proteinase K Enzyme. Incubate at 56°C / 60 min/ 500 rpm in a thermomixer or until the sample becomes liquid.
- After this step the extraction will be done with the "BD MAX™ ExK DNA-2 Extraction Kit"

Transfer 200 µl of the liquid sample into a BD MAX™ DNA Sample Buffer Tube and close the tube with a blue septum cap. Ensure complete mixing by vortexing the sample.

1.4. Setting up the experiment

a. Create a worklist on the BD MAX instrument using the "Bb" assay (created in step 1.2) and label the lanes with the sample names.

b. Load the prepared Sample Buffer Tubes into their corresponding position in the BD MAX racks.

c. Load the BD MAX racks with the corresponding Unitized Reagent Strip.

d. Snap in the BD Extraction tubes (position 1), MMK tubes (position 2) and "Bb"- tubes (position 3) into the Reagent Strip, position 4 will be empty.

e. Load the racks and cartridges into the BD MAX and Start Run

2. Result interpretation

2.1 For a run to be valid

- No BD MAX System failures.
- Negative Control (optional) has a Cq value of -1 for all channels, accept channel 680/ 715 with the SPC-control from the BD-Kit
- Positive control (optional) has a Cq value for channel 475/520 , 530/565, 585/ 630 and 680/715

2.2 Interpretation if run is valid

- A Cq value of -1 indicates a negative result
- A Cq value for channel 475/ 520 (ospA) and/ or 530/ 565 (p41) indicates a positive result for *Borrelia burgdorferi* sensu lato complex.
- The β-actin extraction control (channel 585/630) should always give a Cq value, except for cerebrospinal fluid (CSF) with low cell counts. In this case the SPC-control has to be assessed.
- The SPC-control (channel 680/715) should always give a Cq value, if there is no other target positive. A negative value for the SPC together with negative Cq value

in every other channel indicates inhibition. Therefore this sample should be repeated.

- All curves need to be visually checked for right interpretation.

3. Validation

3.1 Targets

3.1.1. *Borrelia burgdorferi* : *ospA* (GenBank Acc. No.: X14407)

3.1.2. *Borrelia burgdorferi:* *p41* (GenBank Acc. No.: X15661)

3.2. Sensitivity / Specificity / Analytical Sensitivity

- The sensitivity and specificity was determined using 11 defined positive and 25 defined negative samples, which were unequivocally assessed positive and negative by 2 independent other PCRs, respectively.
- The analytical sensitivity was determined by log-step dilutions of several *Borrelia burgdorferi* strains (e.g. *B. garinii*, *B. afzelii* and *B. bavariensis*)
- All PCRs have been used in our clinical microbiological diagnostic laboratory under accreditation. They having passed constantly external inter-laboratory comparison programmes (twice a year).

Target	analytical sensitivity
Borrelia burgdorferi ospA	10^3 copies/ ml
Borrelia burgdorferi p41	10^3 copies/ ml

Disclaimer:

MvP-Institut is not responsible for the results on the "Bb" assay on the BD MAX system. Using the „open protocol", the respective laboratory itself is responsible for the validation of the assay.