

# *Borrelia burgdorferi*

## 2013 EQA Programme Final Report

QAB114147 (BbDNA13)

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A UKAS accredited proficiency testing provider (no. 4385)

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## 1. Programme aims

To assess the qualitative detection of *Borrelia burgdorferi* sensu stricto (*B. burgdorferi* s.s) at different concentrations.

To assess the qualitative detection of *B. burgdorferi* genospecies complex at different concentrations.

Quantitative data presented in this report is for information only and does not form part of the formal EQA assessment

Participants are encouraged to read the QCMD Participants' Manual, which can be downloaded from the QCMD website. Any queries about this report should be addressed to the QCMD Neutral Office (neutraloffice@qcmd.org).

## 2. Programme details

BbDNA13			
Date of panel distribution	30/09/2013	Number of respondents	57
Number of participants	58	Number of datasets submitted	60
Number of countries	19	Number of qualitative datasets submitted	57
Number of qualitative and quantitative datasets submitted			3

One participant did not return results.

## 3. Panel composition

Sample code	Sample content	Sample * matrix	Sample conc. cells/ml	Sample status	Sample type
BbDNA13-03	<i>Borrelia garinii</i>	Culture medium	1.0x10E5	Frequently detected	Core
BbDNA13-05	<i>Borrelia garinii</i>	Culture medium	1.0x10E4	Frequently detected	Core
BbDNA13-07	<i>Borrelia garinii</i>	Culture medium	1.0x10E3	Frequently detected	Educational
BbDNA13-08	<i>Borrelia burgdorferi</i> s.s.	Culture medium	1.0x10E5	Frequently detected	Core
BbDNA13-10	<i>Borrelia burgdorferi</i> s.s.	Culture medium	1.0x10E4	Frequently detected	Core
BbDNA13-02	<i>Borrelia burgdorferi</i> s.s.	Culture medium	1.0x10E3	Detected	Educational
BbDNA13-04	<i>Borrelia afzelii</i>	Culture medium	1.0x10E5	Frequently detected	Core
BbDNA13-06	<i>Borrelia afzelii</i>	Culture medium	1.0x10E4	Frequently detected	Core
BbDNA13-01	<i>Treponema phagedenis</i>	Culture medium	1.0x10E5	Negative	Core
BbDNA13-09	<i>Borrelia</i> spp. negative	Culture medium		Negative	Core

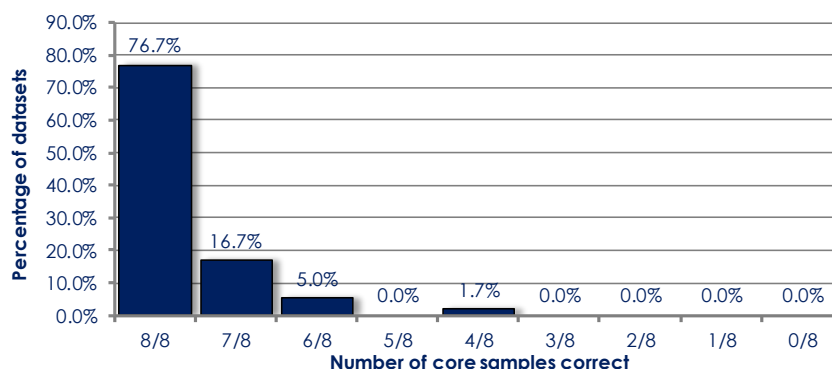
**Sample code:** QCMD panel sample codes for the samples distributed to participants.  
**Sample content:** content of the panel samples and, where applicable, the subtype or strain of the pathogen.  
**Sample matrix:** material used as a matrix in preparation of the panel samples.  
**Sample conc.:** predefined values for QCMD internal purposes only. Values should not be used by participants for method comparison or as a target for individual laboratory performance assessment.  
**Sample status:** the sample status assigned to each panel sample. Please see Appendix A for more information.  
**Sample type:** panel samples classified as core proficiency samples.

Homogeneity & stability assessment criteria can be found in the current version of the QCMD Participant Manual.

\*Culture medium: Borrelia MKP medium.

## 4. Programme results

### 4.1. Qualitative performance on the core proficiency samples



The QCMD EQA panels contain a range of samples, designed to look at different aspects of assay performance. Panel members are designated 'core proficiency samples' on the basis of scientific information, clinical relevance and clinical experience (published literature and professional clinical guidelines) and, where available and appropriate, established target performance limits taken from previous QCMD EQA distributions. Laboratories are expected to correctly analyse and report the core proficiency samples in order to show acceptable proficiency.

#### 4.2. Qualitative analysis of the EQA data for all panel samples

The number (percentage) of correct qualitative results is presented below. Qualitative data were returned by participants as 'positive', 'negative' or 'not determined'. Not determined results were counted as incorrect for all panel samples (positive or negative).

QCMD organises datasets according to commercial and in-house technology groups, which are Conventional PCR, Real time PCR, NASBA, SDA, TMA and bDNA. Where datasets were reported as 'other' for a technology or kit method this was reviewed by the QCMD Neutral Office and assigned to an appropriate group where possible.

Table i: Number of correct qualitative results per panel member and technology type

Sample code	Sample content	Sample conc. cells/ml	Total datasets n=60		PCR							
					Conventional				Real time			
					Commercial <sup>a</sup> n=1		In-house <sup>b</sup> n=8		Commercial <sup>c</sup> n=17		In-house <sup>d</sup> n=34	
n	%	n	%	n	%	n	%					
<b>BbDNA13-03</b>	<i>Borrelia garinii</i>	1.0x10E5	59	98.3	1	100.0	8	100.0	17	100.0	33	97.1
<b>BbDNA13-05</b>	<i>Borrelia garinii</i>	1.0x10E4	59	98.3	1	100.0	8	100.0	17	100.0	33	97.1
<b>BbDNA13-07</b>	<i>Borrelia garinii</i>	1.0x10E3	57	95.0	1	100.0	7	87.5	17	100.0	32	94.1
<b>BbDNA13-08</b>	<i>Borrelia burgdorferi</i> s.s.	1.0x10E5	59	98.3	1	100.0	8	100.0	16	94.1	34	100.0
<b>BbDNA13-10</b>	<i>Borrelia burgdorferi</i> s.s.	1.0x10E4	58	96.7	1	100.0	8	100.0	15	88.2	34	100.0
<b>BbDNA13-02</b>	<i>Borrelia burgdorferi</i> s.s.	1.0x10E3	55	91.7	0	0.0	8	100.0	17	100.0	30	88.2
<b>BbDNA13-04</b>	<i>Borrelia afzelii</i>	1.0x10E5	59	98.3	1	100.0	8	100.0	17	100.0	33	97.1
<b>BbDNA13-06</b>	<i>Borrelia afzelii</i>	1.0x10E4	59	98.3	1	100.0	8	100.0	17	100.0	33	97.1
<b>BbDNA13-01</b>	<i>Treponema phagedenis</i>	1.0x10E5	52	86.7	1	100.0	8	100.0	14	82.4	29	85.3
<b>BbDNA13-09</b>	<i>Borrelia</i> spp. negative	-	55	91.7	1	100.0	7	87.5	15	88.2	32	94.1

Table ii: Qualitative performance scores per technology type

Sample code	Sample status	Total All technologies n=60		PCR																	
				Conventional								Real time									
				Commercial <sup>a</sup> n=1				In-house <sup>b</sup> n=8				Commercial <sup>c</sup> n=17				In-house <sup>d</sup> n=34					
0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3		
<b>BbDNA13-03</b>	Frequently detected	59	0	0	1	1	0	0	0	8	0	0	0	17	0	0	0	33	0	0	1
<b>BbDNA13-05</b>	Frequently detected	59	0	0	1	1	0	0	0	8	0	0	0	17	0	0	0	33	0	0	1
<b>BbDNA13-07</b>	Frequently detected	57	0	0	3	1	0	0	0	7	0	0	1	17	0	0	0	32	0	0	2
<b>BbDNA13-08</b>	Frequently detected	59	0	0	1	1	0	0	0	8	0	0	0	16	0	0	1	34	0	0	0
<b>BbDNA13-10</b>	Frequently detected	58	0	0	2	1	0	0	0	8	0	0	0	15	0	0	2	34	0	0	0
<b>BbDNA13-02</b>	Detected	55	0	5	0	0	0	1	0	8	0	0	0	17	0	0	0	30	0	4	0
<b>BbDNA13-04</b>	Frequently detected	59	0	0	1	1	0	0	0	8	0	0	0	17	0	0	0	33	0	0	1
<b>BbDNA13-06</b>	Frequently detected	59	0	0	1	1	0	0	0	8	0	0	0	17	0	0	0	33	0	0	1
<b>BbDNA13-01</b>	Negative	52	0	0	8	1	0	0	0	8	0	0	0	14	0	0	3	29	0	0	5
<b>BbDNA13-09</b>	Negative	55	0	0	5	1	0	0	0	7	0	0	1	15	0	0	2	32	0	0	2

Key to Table i and ii

- Sample code:** QCMD panel sample codes for the samples distributed to participants.
- Sample content:** content of the panel samples and, where applicable, the subtype or strain of the pathogen.
- Sample conc.:** predefined values for QCMD internal purposes only. Values should not be used by participants for method comparison or as a target for individual laboratory performance assessment.
- Total datasets:** number and percentage of datasets reporting the correct qualitative result for each panel sample
- Sample status:** the sample status assigned to each panel sample. Please see Appendix A for more information.
- Total. All technologies:** number of datasets awarded each score (0 to 3). A breakdown of the results for all datasets is also provided based on technology

- a: Clonit *Borrelia burgdorferi* (n=1)
- b: Details not presented.
- c: Artus *Borrelia* LC PCR kit (n=4), Diagenode Dia-BorBurg-050 (n=1), GeneProof *Borrelia burgdorferi* PCR kit (n=4), gerbion diarella *Borrelia* real time PCR kit (n=1), gerbion ixSave*Borrelia* (n=1), Sacace *Borrelia burgdorferi* sensu lato (n=1), Sacace Tick Borne Encephalitis Real-TM (n=1), Tib Molbiol Light Mix *Borrelia* spp. (n=4)
- d: Details not presented.

### 4.3. Qualitative analysis of the EQA data by assay target gene

Participants were asked to specify the target gene of their assay when submitting their results. Out of the 60 qualitative datasets received by QCMD 88% (n=53) contained information on the target gene.

Sample code	Sample content	Sample conc. cells/ml	Total datasets n=60		OspA <sup>a</sup> n=18		Fla <sup>b</sup> n=17		16s <sup>c</sup> n=8		Other <sup>d</sup> n=10		Not <sup>e</sup> reported n=7	
			n	%	n	%	n	%	n	%	n	%	n	%
<b>BbDNA13-03</b>	<i>Borrelia garinii</i>	1.0x10E5	59	98.3	18	100.0	17	100.0	8	100.0	9	90.0	7	100.0
<b>BbDNA13-05</b>	<i>Borrelia garinii</i>	1.0x10E4	59	98.3	18	100.0	17	100.0	8	100.0	9	90.0	7	100.0
<b>BbDNA13-07</b>	<i>Borrelia garinii</i>	1.0x10E3	57	95.0	18	100.0	16	94.1	8	100.0	8	80.0	7	100.0
<b>BbDNA13-08</b>	<i>Borrelia burgdorferi</i> s.s.	1.0x10E5	59	98.3	18	100.0	17	100.0	8	100.0	10	100.0	6	85.7
<b>BbDNA13-10</b>	<i>Borrelia burgdorferi</i> s.s.	1.0x10E4	58	96.7	18	100.0	17	100.0	8	100.0	10	100.0	5	71.4
<b>BbDNA13-02</b>	<i>Borrelia burgdorferi</i> s.s.	1.0x10E3	55	91.7	17	94.4	14	82.4	8	100.0	10	100.0	6	85.7
<b>BbDNA13-04</b>	<i>Borrelia afzelii</i>	1.0x10E5	59	98.3	18	100.0	17	100.0	8	100.0	9	90.0	7	100.0
<b>BbDNA13-06</b>	<i>Borrelia afzelii</i>	1.0x10E4	59	98.3	18	100.0	17	100.0	8	100.0	9	90.0	7	100.0
<b>BbDNA13-01</b>	<i>Treponema phagedenis</i>	1.0x10E5	52	86.7	16	88.9	14	82.4	6	75.0	10	100.0	6	85.7
<b>BbDNA13-09</b>	<i>Borrelia</i> spp. negative	-	55	91.7	17	94.4	13	76.5	8	100.0	10	100.0	7	100.0

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**Sample content:** content of the panel samples and, where applicable, the subtype or strain of the pathogen.  
**Sample conc:** predefined values for QCMD internal purposes only. Values should not be used by participants for method comparison or as a target for individual laboratory performance assessment.  
**Total datasets:** number and percentage of datasets reporting the correct qualitative result for each panel sample. A breakdown of the results for all datasets is also provided based on target gene.

- a: outer surface protein A gene.
- b: flagellin gene.
- c: 16s gene;
- d: 23S (n=2), hbb gene (n=1), ospA + Fla (n=2), ospA + Fla + recA (n=1), p66 (n=1), RecA (n=2), rrf-rrl region (n=1).
- e: no target gene information returned by participant.

### 4.4 Quantitative results per laboratory

Three participants returned a total of three datasets containing quantitative results; these are shown below.

Sample code	Sample content	Sample conc. cells/ml	Dataset a copies/μl	Dataset b Geq/ml	Dataset c cells/ml
<b>BbDNA13-03</b>	<i>Borrelia garinii</i>	1.0x10E5	19900	8900000	33575100
<b>BbDNA13-05</b>	<i>Borrelia garinii</i>	1.0x10E4	2400	900000	4912840
<b>BbDNA13-07</b>	<i>Borrelia garinii</i>	1.0x10E3	135	75300	452129
<b>BbDNA13-08</b>	<i>Borrelia burgdorferi</i> s.s.	1.0x10E5	377	132000	645232
<b>BbDNA13-10</b>	<i>Borrelia burgdorferi</i> s.s.	1.0x10E4	37	12100	70118
<b>BbDNA13-02</b>	<i>Borrelia burgdorferi</i> s.s.	1.0x10E3	3	1757	11397
<b>BbDNA13-04</b>	<i>Borrelia afzelii</i>	1.0x10E5	71	44000	5020280
<b>BbDNA13-06</b>	<i>Borrelia afzelii</i>	1.0x10E4	11	1960	587867

**Sample code:** QCMD panel sample codes for the samples distributed to participants.  
**Sample content:** content of the panel samples and, where applicable, the subtype or strain of the pathogen.  
**Sample conc:** predefined values for QCMD internal purposes only. Values should not be used by participants for method comparison or as a target for individual laboratory performance assessment.

- a: extraction method; MagNA Pure Compact Nucleic Acid Isolation Kit I. Platform; Lightcycler 2.0
  - b: extraction method; QIAamp DNA Mini Kit (Qiagen). Platform; Lightcycler 2.0
  - c: extraction method; Easymag Biomerieux. Platform; ABI 7500 Real-Time PCR System
- Assay; Artus Borrelia LC PCR kit  
 Assay; Artus Borrelia LC PCR kit  
 Assay; Realtime

## 5. Comments

### General Comments

The number of participants in the 2013 *Borrelia burgdorferi* EQA programme was 58. This represents an increase of 11.5% on 2012 (n=52).

All datasets returned used PCR based assays, the majority of PCR datasets were generated using real time PCR assays (85%; n=51). Of the real time PCR datasets 34 (66%) were generated using in-house developed assays.

Quantitative data was returned to QCMD in 3 (5%) datasets, this is a similar proportion to 2012(7.5%). Of those who returned quantitative data all included qualitative data in their dataset.

### Qualitative analysis

Forty six (76.7%) datasets returned by participants reported all core samples correctly. This was slightly less than the 81.1% of datasets that correctly reported all core samples in the 2012 EQA programme and was mainly due to the inclusion of a *Treponema phagedenis* specificity negative core samples in this year's EQA panel.

Almost all datasets were able to correctly detect *Borrelia burgdorferi* s.s panel samples down to a concentration of approximately  $1.0 \times 10^4$  cells/ml. This performance is similar to that of the 2012 EQA programme.

Eight (13.3%) false positive results were reported for the specificity negative sample (BbDNA13-01) containing *Treponema phagedenis* indicating that some assays have difficulty specifically detecting *Borrelia* spp. Five (8.3%) false positive results were reported on the true negative panel sample (BbDNA13-09) which compares to the 3.8% false positivity in 2012.

A total of 53 datasets contained information on the target genes used. The most commonly targeted gene was the outer surface protein A gene which was targeted either individually or in combination with other genes in 37.7% of datasets.

### Quantitative analysis

Participants returned three quantitative datasets which did not allow full quantitative analysis.

Although the numbers of quantitative results remains low this area may develop in the future. The variability in the quantitative results returned indicates that the availability of EQA and reference materials will be of value in the establishment of quantitative assays.

## Acknowledgements

Thank you to Prof. Dr. Andreas Sing for his contribution to this report.  
Data analysis and report generation were performed by the QCMD Neutral Office.

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## Appendix A

### Assigning the sample status

Sample status is assigned by peer-group consensus, based on the qualitative results returned by all participants. It is not a measure of the 'strength' of a positive sample nor is it technology-dependent, and is used solely for the scoring of the EQA data. The rationale for the sample status is:

**Frequently detected:** More than 95% of datasets recorded the correct positive result.

**Detected:** Between 65% and 95% of datasets recorded the correct positive result.

**Infrequently detected:** Less than 65% of datasets recorded the correct positive result.

**Negative:** A panel sample that does not contain the target and produces an unequivocal negative result.

### Scoring system for qualitative EQA data

The scores awarded for qualitative EQA data were based on the sample status. The scoring system is represented in the following table, where 0 is 'highly satisfactory' and 3 is 'highly unsatisfactory'. Colour has been included as an extra visual aid.

### Scoring system based on the assigned sample status

Sample status	Participant's result		
	Negative	Not determined	Positive
Frequently detected	3	3	0
Detected	2	2	0
Infrequently detected	1	1	0
Negative	0	3	3