

Supplementary Materials

Table S1: *In silico* analysis for the specificity of the BSG method using SCREENED. (A) From each animal, archaea, bacteria, fungi, plant protest and virus group from the NCBI Nucleotide RefSeq database, the number of sequences presenting a positive signal for the BSG method is indicated. (B) Description of the positive signals.

(A)		NCBI Nucleotide RefSeq database sequences groups						
		Animal	Archaea	Bacteria	Fungi	Plant	Protist	Virus
Sequence number with a positive signal for the BSG method		0	0	167	0	0	0	0
Total sequence number		75,548	54,464	11,507,253	86,250	46,015	89,957	10,108
(B)		Species	Frequency	Accession numbers				
<i>B. subtilis</i> group*	<i>B. altitudinis</i> (<i>B. pumilus</i> group)	9	NZ_CP012482.1; NZ_CP018574.1; NZ_CP068389.1; NZ_LDJZ01000007.1; NZ_QCWN01000053.1; NZ_QENO01000024.1; NZ_VTEM01000053.1; NZ_CP012329.1; NZ_CP012330.1					
	<i>B. amyloliquefaciens</i>	14	NC_014551.1; NC_020272.1; NZ_AWQY01000019.1; NZ_CP007242.1; NZ_CP014700.1; NZ_CP014783.1; NZ_CP018152.1; NZ_CP018200.1; NZ_CP038028.1; NZ_CP054479.1; NZ_CP061852.1; NZ_LDHQ01000012.1; NZ_QVEJ01000071.1; NZ_AJST01000001.1					
	<i>B. licheniformis</i>	11	NZ_AVEZ01000017.1; NZ_CP014842.1; NZ_CP022477.1; NZ_CP025226.1; NZ_CP065647.1; NZ_JFYM01000035.1; NZ_LDHH01000013.1; NZ_MIPQ01000020.1; NZ_MIZD01000018.1; NZ_NQLG01000457.1; NZ_VEGU01000065.1					
	<i>B. pumilus</i>	8	NZ_CP016784.1; NZ_CP058951.1; NZ_JACNYY010000068.1; NZ_JACNZG010000034.1; NZ_JACNZV010000074.1; NZ_UWJF01000028.1; NZ_VTEO01000003.1; NZ_VTEP01000003.1					
	<i>B. halotolerans</i> (<i>B. mojavensis</i> group)	4	NZ_CP029364.1; NZ_MBQV01000017.1; NZ_PVXA01000035.1; NZ_PVXC01000085.1					
	<i>B. safensis</i> (<i>B. pumilus</i> group)	8	NZ_CP015607.1; NZ_CP015611.1; NZ_CP018100.1; NZ_CP043404.1; NZ_JAEACH010000001.1; NZ_LDFH01000001.1; NZ_LDGD01000006.1; NZ_LZRH01000005.1					
	<i>B. stratosphericus</i> (<i>B. pumilus</i> group)	2	NZ_MWKO01000142.1; NZ_SPPG01000370.1					
	<i>B. subtilis</i>	42	NC_019896.1; NZ_BDCV01000028.1; NZ_CP009684.1; NZ_CP009796.1; NZ_CP013654.1; NZ_CP015375.1; NZ_CP021123.1; NZ_CP028202.1; NZ_CP028209.1; NZ_CP028213.1; NZ_CP028217.1; NZ_CP028218.1; NZ_CP028812.1; NZ_CP032315.1; NZ_CP046448.1; NZ_CP064818.1; NZ_JAAEBI010000014.1; NZ_JAAEBK010000011.1; NZ_JABFHE010000130.1; NZ_JA-BUOT010000018.1; NZ_KZ848487.1; NZ_LDFK01000020.1; NZ_LDGB01000018.1; NZ_LJSM01000017.1; NZ_LZOV01000074.1; NZ_MAFZ01000007.1; NZ_MAFZ01000010.1; NZ_ML241291.1; NZ_NQYD01000001.1; NZ_PYBK01000049.1; NZ_QFZO01000054.1; NZ_QSNC01000001.1; NZ_QVMX01000069.1; NZ_QZDE02000003.1; NZ_RFFO01000030.1; NZ_SIWZ01000001.1; NZ_VEGG01000015.1; NZ_VTEK01000005.1; NZ_WOVJ01000047.1; NZ_UAQD01000006.1; NZ_KN049967.1; NZ_CLNG01000048.1					
	<i>B. vallismortis</i>	1	NZ_CP026362.1					
	<i>B. velezensis</i>	51	NC_009725.2; NC_020410.1; NC_022075.1; NC_022081.1; NZ_CP009679.1; NZ_CP010556.1; NZ_CP011278.1; NZ_CP011347.1; NZ_CP011937.1; NZ_CP018007.1; NZ_CP020874.1; NZ_CP022556.1; NZ_CP022654.2; NZ_CP023341.1; NZ_CP023414.1; NZ_CP027429.1; NZ_CP028207.1; NZ_CP028211.1; NZ_CP028439.1; NZ_CP028440.1; NZ_CP029034.1; NZ_CP029296.1;					

NZ_CP029473.1; NZ_CP031694.1; NZ_CP033967.1;
 NZ_CP035533.1; NZ_CP036518.1; NZ_CP036527.1;
 NZ_CP039297.1; NZ_CP041145.1; NZ_CP042271.1;
 NZ_CP053717.1; NZ_CP059344.1; NZ_CP059855.1;
 NZ_CP060085.1; NZ_CP063157.1; NZ_CP063687.1;
 NZ_CP063768.1; NZ_JAAHBR010000031.1;
 NZ_JAAKXX010000009.1; NZ_KZ155841.1;
 NZ_LSZL01000040.1; NZ_MBDV01000014.1;
 NZ_MPHE01000018.1; NZ_MSXY01000073.1;
 NZ_PEMY01000036.1; NZ_PVHM01000026.1;
 NZ_RPOD01000030.1; NZ_VANQ01000027.1;
 NZ_VEWW01000017.1; NZ_VEWZ01000020.1

	<i>B. xiamenensis</i> (<i>B. pumilus</i> group)	2	NZ_CP017786.1; NZ_LDHZ01000020.1
Unclassified <i>Bacillus</i>	<i>Bacillus</i> sp.	15	NZ_BDJJ01000001.1; NZ_CP017072.1; NZ_CP019626.1; NZ_CP025258.1; NZ_CP027061.1; NZ_CP046653.1; NZ_CP060193.1; NZ_JAESPO010000027.1; NZ_JAESPP010000073.1; NZ_LKCP01000046.1; NZ_LKCP01000056.1; NZ_LMRK01000001.1; NZ_PJCT01000020.1; NZ_RPSG01000024.1; NZ_RPSI01000032.1

* <https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=653685>.

Table S2: Misclassification of NZ_CLNG01000048.1. From analysis performed in Table S1, a sequence (Accession number: NZ_CLNG01000048.1) classified as belonging to *Streptococcus pneumoniae* presented a PCR amplification for the BSG method. However, based on further investigations (see below), this sequence was shown as belonging to *Bacillus subtilis*.

NZ_CLNG01000048.1 blasted against nr/nt NCBI database

Sequences producing significant alignments									
Download ▼ New Select columns ▼ Show 100 ▼ ?									
<input checked="" type="checkbox"/> select all 100 sequences selected									
GenBank Graphics Distance tree of results New MSA Viewer									
	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
<input checked="" type="checkbox"/>	Bacillus subtilis strain SRCM102751 chromosome, complete genome	Bacillus subtilis	8547	1.353e+05	99%	0.0	99.87%	4047680	CP028217.1
<input checked="" type="checkbox"/>	Bacillus subtilis strain SEM-9 chromosome, complete genome	Bacillus subtilis	8543	1.354e+05	99%	0.0	99.85%	4121982	CP021123.1
<input checked="" type="checkbox"/>	Bacillus subtilis strain SRCM103629 chromosome, complete genome	Bacillus subtilis	8543	1.354e+05	99%	0.0	99.85%	4129860	CP035413.1
<input checked="" type="checkbox"/>	Bacillus subtilis subsp. subtilis strain SRCM101392, complete genome	Bacillus subtilis ...	8540	1.353e+05	99%	0.0	99.85%	4147173	CP021921.1
<input checked="" type="checkbox"/>	Bacillus subtilis strain 75 chromosome, complete genome	Bacillus subtilis	8534	1.353e+05	99%	0.0	99.83%	4156459	CP045825.1
<input checked="" type="checkbox"/>	Bacillus subtilis strain PR10 chromosome, complete genome	Bacillus subtilis	8534	1.354e+05	99%	0.0	99.83%	4073059	CP040528.1
<input checked="" type="checkbox"/>	Bacillus subtilis strain H19 chromosome, complete genome	Bacillus subtilis	8534	1.353e+05	99%	0.0	99.83%	4063004	CP039935.1
<input checked="" type="checkbox"/>	Bacillus subtilis strain SRCM103637 chromosome, complete genome	Bacillus subtilis	8534	1.352e+05	99%	0.0	99.83%	4027238	CP035414.1
<input checked="" type="checkbox"/>	Bacillus subtilis strain SRCM103641 chromosome, complete genome	Bacillus subtilis	8534	1.352e+05	99%	0.0	99.83%	4110403	CP035390.1
<input checked="" type="checkbox"/>	Bacillus subtilis subsp. subtilis strain SRCM100761, complete genome	Bacillus subtilis ...	8534	1.352e+05	99%	0.0	99.83%	3988630	CP021889.1
<input checked="" type="checkbox"/>	Bacillus subtilis subsp. subtilis strain SRCM100757, complete genome	Bacillus subtilis ...	8534	1.352e+05	99%	0.0	99.83%	3988649	CP021499.1
<input checked="" type="checkbox"/>	Bacillus subtilis subsp. subtilis strain SRCM101444, complete genome	Bacillus subtilis ...	8534	1.352e+05	99%	0.0	99.83%	3988602	CP021498.1
<input checked="" type="checkbox"/>	Bacillus subtilis strain GQJK2, complete genome	Bacillus subtilis	8534	1.354e+05	99%	0.0	99.83%	4072961	CP020367.1
<input checked="" type="checkbox"/>	Bacillus subtilis strain SRCM102749 chromosome, complete genome	Bacillus subtilis	8528	1.353e+05	99%	0.0	99.81%	4192708	CP028213.1
<input checked="" type="checkbox"/>	Bacillus subtilis subsp. subtilis strain MH-1 chromosome, complete genome	Bacillus subtilis ...	8528	1.351e+05	99%	0.0	99.81%	4184648	CP032853.1
<input checked="" type="checkbox"/>	Bacillus subtilis strain SRCM103697 chromosome, complete genome	Bacillus subtilis	8528	1.352e+05	99%	0.0	99.81%	4072580	CP035395.1
<input checked="" type="checkbox"/>	Bacillus subtilis strain SRCM103773 chromosome, complete genome	Bacillus subtilis	8528	1.353e+05	99%	0.0	99.81%	4052954	CP035397.1
<input checked="" type="checkbox"/>	Bacillus subtilis strain SRCM103576 chromosome, complete genome	Bacillus subtilis	8528	1.352e+05	99%	0.0	99.81%	4012178	CP035402.1

Presence of *Bacillus* and *Streptococcus* population in the sample based on Kraken analysis on the assembly.

Streptococcus pneumoniae strain SMRU2731, whole genome shotgun sequence

NCBI Reference Sequence: NZ_CLNG01000048.1

[FASTA](#) [Graphics](#)

Go to: ☐

LOCUS NZ_CLNG01000048 7703 bp DNA linear CON 23-OCT-2020
 DEFINITION Streptococcus pneumoniae strain SMRU2731, whole genome shotgun sequence.
 ACCESSION NZ_CLNG01000048 [NZ_CLNG01000000](#)
 VERSION NZ_CLNG01000048.1
 DBLINK BioProject: [PRJNA224116](#)
 BioSample: [SAMEA1028711](#)
 Assembly: [GCF_001140205.1](#)

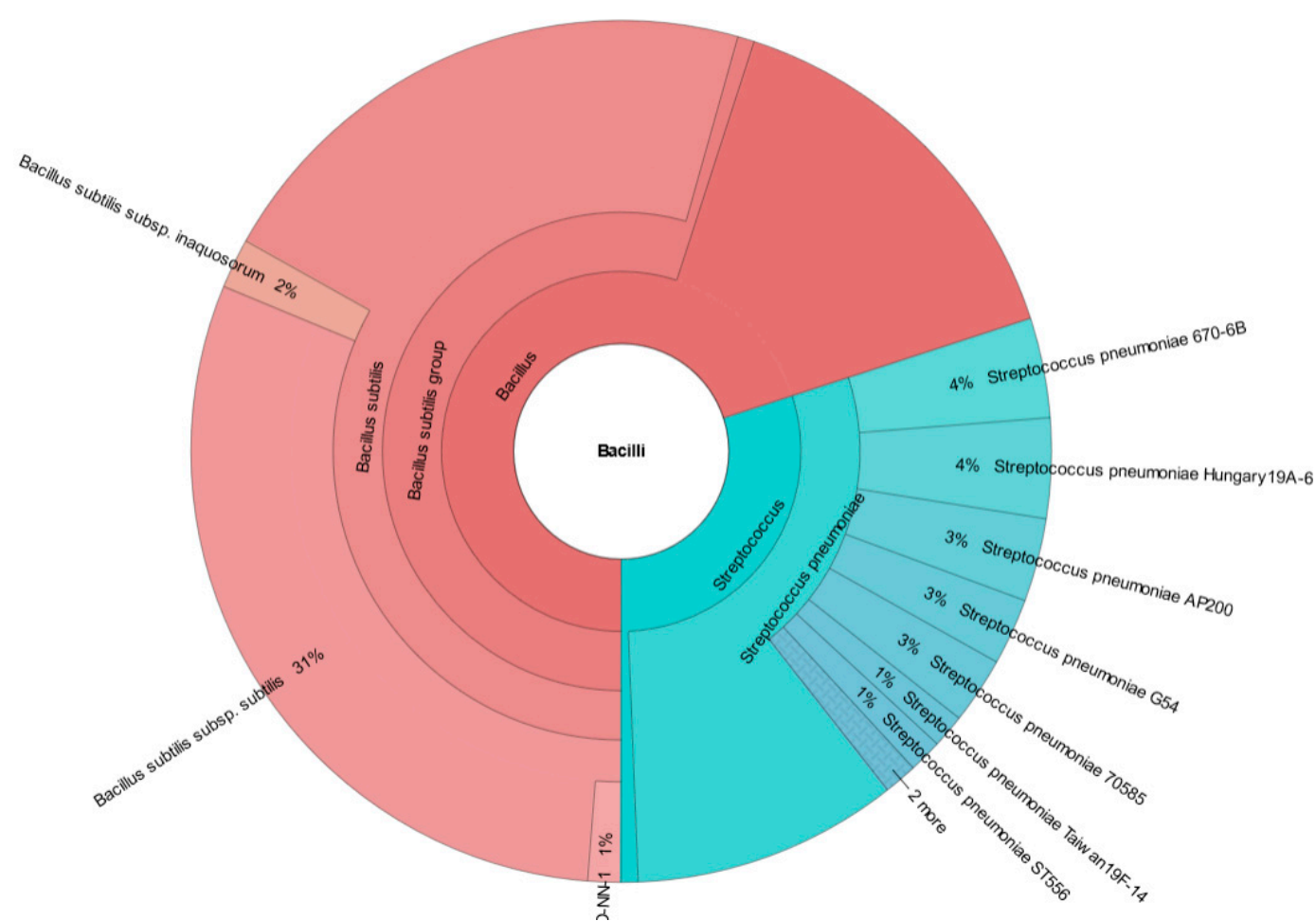


Table S3: Calculation of LOD_{95%} based on the POD curve for the developed real-time PCR BSG method. To this end, based on Table 3, the “Validation of qualitative PCR methods within a single laboratory” web-tool (<https://quodata.de/content/validation-qualitative-pcr-methods-single-laboratory>) was used as previously described [36–37].

Please enter your data

1st column: Number of copies of the target DNA sequence (= nominal copies)

2nd column: Number of positive test results

3rd column: Number of PCR replicates

[Click here to insert example data](#)

0.1	0	12
1	1	12
5	6	12
10	8	12
20	12	12
100	12	12

[Clear data](#)

Start the calculation

Results

Plausibility Check

The plausibility check indicates no irregularities.

POD curve and LOD_{95%}

The LOD_{95%} is 22.025 with a 95 % confidence interval of [14.426, 33.770].

The figure below summarises the results. The blue diamonds characterise the laboratory-specific RODs. The blue curve denotes the mean POD curve along with the corresponding 95 % confidence range highlighted as the grey band. The POD curve under ideal conditions is displayed as the black dashed curve.

