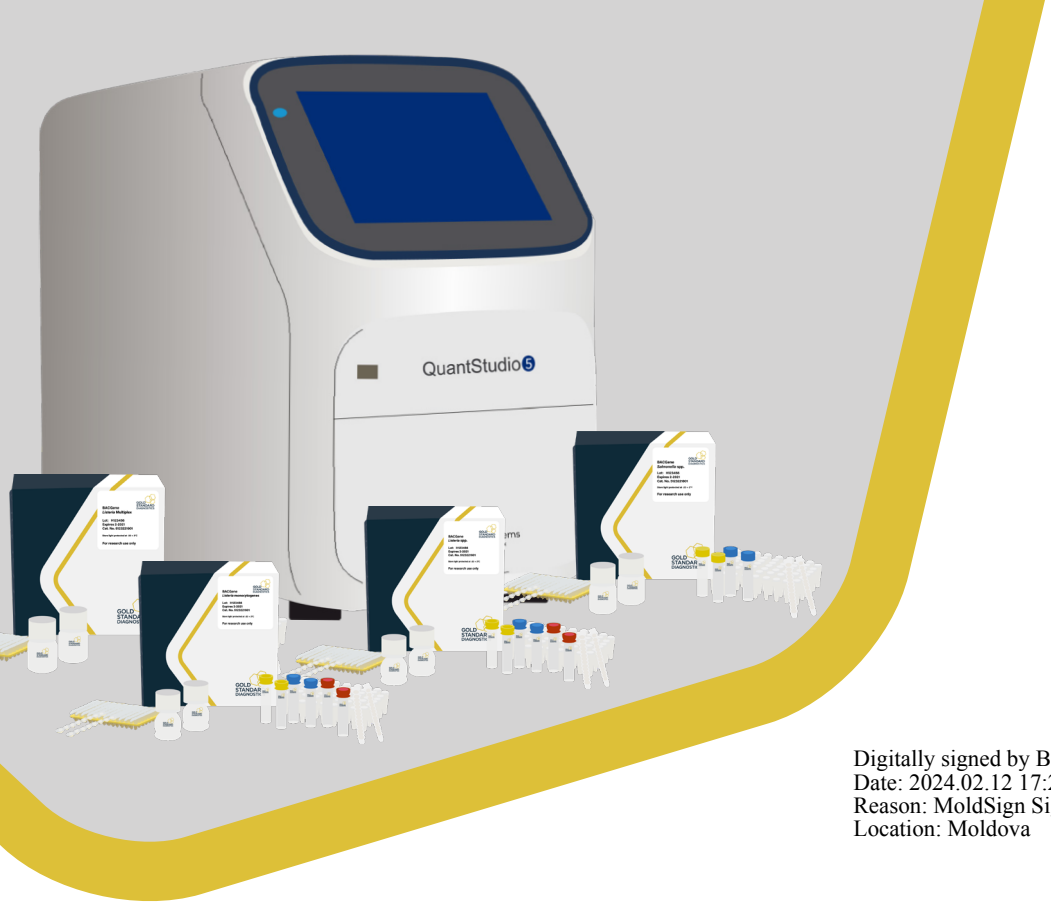

GOLD STANDARD DIAGNOSTICS

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BAC*Gene* Feasibility Study on QuantStudio™ 5

In order to give the outmost flexibility for food pathogen testing, the BAC*Gene* real-time PCR kits have been certified using a number of different instruments in accordance to EN ISO 16140 part 2 by NF Validation (Afnor Certification) and also by AOAC PTM certification.

To even further expand the cycler scope - although not being fully validated, nor certified - a feasibility study was conducted using BAC*Gene* *Salmonella* spp. (Cat. No. 5123221801) BAC*Gene* *Listeria* spp. (Cat. No. 5123222101), BAC*Gene* *Listeria monocytogenes* (Cat. No. 5123222001) and BAC*Gene* *Listeria* Multiplex (Cat. No. 5123221901) on the QuantStudio™ 5 real-time PCR system from Thermo Fisher Scientific™.

Taking the measures and criteria as defined by Gold Standard Diagnostics internal quality standards - the compatibility of the BAC*Gene* kits in scope can be given.

Application Note –

QuantStudio™ 5 Real-Time PCR System

Can BAC*Gene* kits be used on the QuantStudio™ 5 real-time PCR system from Thermo Fisher Scientific™?

Yes, compatibility was confirmed - Furthermore, BAC*Gene* kits in scope have not been fully validated nor certified by Afnor or AOAC PTM for the use on the QuantStudio™ 5 system.

2 Scope of the Study

This feasibility study was run by performing standard MasterMix Quality Control testing complying with Gold Standard Diagnostics internal quality standards confirming the expected parameters within the given specification range. Data evaluation was performed according to defined guidelines. Furthermore, in order to ease the use of BAC*Gene* on QuantStudio™ 5 (QS5), a MS Excel evaluation sheet (version 2.08) was setup and is available upon request and compatible to be used with QS5 software version up to 2.6.

3 Materials & Methods

3.1 Materials

3.1.1 Test Kit and Cycler

***Salmonella* spp.**	***Listeria* spp.**	***Listeria monocytogenes***	***Listeria monocytogenes* and *Listeria* spp.**	**Cycler Specifications**
BAC*Gene Salmonella* spp. Cat. No. 5123221901 Lot.No. H039023, Expiry Date 27.11.2025	BAC*Gene Listeria* spp. Cat. No. 5123222101 Lot No. H022033, Expiry Date 05.03.2025	BAC*Gene Listeria* monocytogenes Cat. No. 5123222001 Lot.No. H063122, Expiry Date 12.12.2024	BAC*Gene Listeria* Multiplex Cat. No. 5123221901 Lot No. H117023, Expiry Date 28.12.2024	QuantStudio™ 5 ID-Number: 272512192 Up to Software V2.6

3.1.2 Cycler Settings

- ***Salmonella* spp.:**

Plate Setup	Instrument Settings	Analysis Settings
Reporter 1: FAM / Quencher: None (*Salmonella* spp.)	Run mode: Standard	Auto baseline
Reporter 2: CY5 / Quencher: None (IPC)	Sample volume: 25 µL	Auto threshold
Passive reference: (none)		

- ***Listeria* spp.:**

Plate Setup	Instrument Settings	Analysis Settings
Reporter 1: VIC / Quencher: None (*Listeria* spp.)	Run mode: Standard	Auto baseline
Reporter 2: CY5 / Quencher: None (IPC)	Sample volume: 25 µL	Auto threshold
Passive reference: (none)		

- ***Listeria monocytogenes*:**

Plate Setup	Instrument Settings	Analysis Settings
Reporter 1: VIC / Quencher: None (*Listeria monocytogenes*)	Run mode: Standard	Auto baseline
Reporter 2: CY5 / Quencher: None (IPC)	Sample volume: 25 µL	Auto threshold
Passive reference: (none)		

- ***Listeria* spp. and *Listeria monocytogenes*:**

Plate Setup	Instrument Settings	Analysis Settings
Reporter 1: VIC / Quencher: None (*Listeria* spp.)	Run mode: Standard	Auto baseline
Reporter 2: ROX / Quencher: None (*Listeria monocytogenes*)	Sample volume: 25 µL	Auto threshold
Reporter 3: CY5 / Quencher: None (IPC)		
Passive reference: (none)		

3.2 Method Design

Using positive control DNA, a dilution was performed obtaining a concentration of 10 copies/reaction. Later on, diluted positive control DNA, positive controls (C+) and negative controls (C-) were tested via QuantStudio™ 5 real-time PCR system.
The feasibility experiments using all 4 BAC*Gene* kits were designed the same with identical number of replicates. Replicates representation is shown in the table below:

	Positive control (C+)	Negative control (C-)	DNA: 10 cp/rxn (LOD)
Replicates	5	33	10

4 Results and Discussion

Table 1: Data representation using BAC*Gene Salmonella* spp. acc. to test design. The Cq-values represent mean values of respective repetitions.

	***Salmonella* spp. (FAM)**				**IPC (CY5)**			
Sample	Cq-values	EPF-values	Overall positives	Final results	Cq-values	EPF-values	Overall positives	Score
Positive control (C+)	> 30	Passed	5/5	Positive	> 25	Passed	5/5	Valid
Negative control (C-)	0	Passed	0/33	Negative	> 25	Passed	33/33	Valid
DNA: 10 cp/rxn (LOD)	> 30	Passed	10/10	Positive	> 25	Passed	10/10	Valid

Table 2: Data representation using BAC*Gene Listeria* spp. acc. to test design. The Cq-values represent mean values of respective repetitions.

	***Listeria* spp. (VIC)**				**IPC (CY5)**			
Sample	Cq-values	EPF-values	Overall positives	Final results	Cq-values	EPF-values	Overall positives	Score
Positive control (C+)	> 30	Passed	5/5	Positive	> 25	Passed	5/5	Valid
Negative control (C-)	0	Passed	0/33	Negative	> 25	Passed	33/33	Valid
DNA: 10 cp/rxn (LOD)	> 30	Passed	10/10	Positive	> 25	Passed	10/10	Valid

Table 3: Data representation using BAC*Gene Listeria monocytogenes*. acc. to test design. The Cq-values represent mean values of respective repetitions.

	***Listeria Monocytogenes* (VIC)**				**IPC (CY5)**			
Sample	Cq-values	EPF-values	Overall positives	Final results	Cq-values	EPF-values	Overall positives	Score
Positive control (C+)	> 25	Passed	5/5	Positive	> 25	Passed	5/5	Valid
Negative control (C-)	0	Passed	0/33	Negative	> 25	Passed	33/33	Valid
DNA: 10 cp/rxn (LOD)	> 35	Passed	10/10	Positive	> 25	Passed	10/10	Valid

Table 4: Data representation using BAC*Gene Listeria* Multiplex. acc. to test design. The Cq-values represent mean values of respective repetitions.

	***Listeria* spp. (VIC)**			***Listeria Monocytogenes* (ROX)**				**IPC (CY5)**			
Sample	Cq-values	EPF-values	Overall positives	Cq-values	EPF-values	Overall positives	Final results	Cq-values	EPF-values	Overall positives	Score
Positive control (C+)	> 25	Passed	5/5	> 25	Passed	5/5	Positive	> 25	Passed	5/5	Valid
Negative control (C-)	0	Passed	0/33	0	Passed	0/33	Negative	> 25	Passed	33/33	Valid
DNA: 10 cp/rxn (LOD)	> 35	Passed	10/10	> 35	Passed	10/10	Positive	> 25	Passed	10/10	Valid

Using BAC*Gene Salmonella* spp., BAC*Gene Listeria* spp., BAC*Gene Listeria monocytogenes* and BAC*Gene Listeria* Multiplex kits, the diluted positive control DNA was reliably detected in the respective reporter channels representing the amplification of corresponding pathogens' DNA. All replicates of prepared control DNA at concentration of 10 copies/reaction, which simulate a close value to the estimated kit limit of detection (LOD), were amplified with respected Cq-values. Results of the internal positive control (IPC) demonstrate the absence of PCR inhibition and therefore, the validity of the data. Moreover, the results of all replicates for positive control (C+) as well as negative control (C-) were as expected.

5 Conclusion

This conducted feasibility study on diluted positive control DNA to different concentrations shows the compatibility of BAC*Gene Salmonella* spp., BAC*Gene Listeria* spp., BAC*Gene Listeria monocytogenes* and BAC*Gene Listeria* Multiplex to be run on QuantStudio™ 5 using software up to V2.6.