

Study results

Automation of liquid handling with the OT-2

for

***BACGene Salmonella* spp.**

***BACGene Listeria* Multiplex**

***BACGene Listeria* spp.**

BACGene Listeria monocytogenes

January 2024



Table of Content

1	General Information.....	3
1.1	Participants.....	3
2	Scope	3
3	Test plan.....	5
3.1	Accuracy of the liquid transfer	5
3.2	Accuracy of incubation temperatures	6
3.3	Cross-contamination.....	7
3.4	Robustness.....	8
3.4.1	Evaporation during lysis	8
3.5	Stability	9
3.5.1	Positive control stability	9
3.5.2	PCR mix stability	9
3.5.3	PCR-plate stability after addition of samples	9
3.6	Matrix testing	10
4	Test Results.....	11
4.1	Accuracy of the liquid transfer	11
4.2	Accuracy of incubation temperature.....	11
4.3	Cross contamination.....	11
4.4	Robustness.....	12
4.4.1	Evaporation during lysis	12
4.4.2	Positive control stability	13
4.4.3	PCR mix stability	14
4.4.4	PCR-plate stability after addition of samples	15
4.5	Matrix test.....	16
4.5.1	BACGene <i>Salmonella</i> spp.	16
4.5.2	BACGene <i>Listeria monocytogenes</i>	21
4.5.3	BACGene <i>Listeria</i> spp.....	25
4.5.4	BACGene <i>Listeria</i> Multiplex	32
5	Annex	41

1 General Information

1.1 Participants

Authors

Dr. Nadine Göhring, Laura Bleichner

Company

Gold Standard Diagnostics Freiburg GmbH

Engesserstraße 4

D-79108 Freiburg im Breisgau

GERMANY

Phone: +49 160 967 399 98

2 Scope

For increasing the ease-of-use of the Gold Standard Diagnostics real-time kits for pathogen detection, the BACGene kits, an automated workflow for all liquid handling-steps including the lysis buffer preparation, sample input, lysis and PCR setup on the OT-2 from Opentrons Labware Inc. was developed. The OT-2 liquid handler is an open platform system with a dedicated workflow for BACGene that has been thoroughly tested.

Currently in-scope of the protocol are the following kits:

1. BACGene *Salmonella* spp. (EGS 38/01 – 03/15)
2. BACGene *Listeria* Multiplex (EGS 38/05 – 03/17)
3. BACGene *Listeria monocytogenes* (EGS 38/03 – 01/17)
4. BACGene *Listeria* spp. (EGS 38/02 – 01/17)

All four kits have been validated for the detection of *Salmonella* and respectively *Listeria* for a broad range of food and environmental samples.

There are no changes in the kits, all components are exactly the same, but the automated workflow differs slightly from the manual workflow, see Table 1. Steps which are not included in the automated workflow are marked with a ✖ including a note why the respective step is not part of the automated workflow. For visualizing the whole workflow, also the sampling at the beginning and closing, mixing and transfer of the PCR plate are included, as they are general steps, they are marked in grey.

Table 1 BACGene workflow for comparison of manual and automated steps.

Step	#	Step	Manual workflow	OT-2 workflow	Note
Sampling	1	Sampling of enrichment samples into cluster tubes	✓	✓	-
Lysis preparation	1	Thawing of lysis buffer base, PK and Lysozyme	✓	✓	-
	2	Preparation of final lysis buffer by adding enzymes into the lysis buffer base	✓	✓	-
	3	Dispensing of final lysis buffer into lysis plate	✓	✓	-
Lysis	1	Addition of samples to lysis buffer	✓	✓	-
	2	Closing lysis plates with domed caps	✓	✗	Step redundant, lysis is performed with open tubes
	3	Incubation at 37 °C for 20 min	✓	✓	-
	4	Transfer to 2 nd heat block	✓	✗	Step redundant, 1 block on OT-2
	5	Incubation at 95 °C for 10 min	✓	✓	-
	6	Cooling at 4 °C for 5 min	✓	✗	Step redundant, cooling step only required to reduce pressure in closed lysis tube
PCR	1	Spinning down for liquid removal in caps	✓	✗	Step redundant, spinning needed for removing liquid from domed caps
	2	Opening of lysis plate	✓	✗	Step redundant, lysis is performed with open tubes
	3	Transfer into PCR plate	✓	✓	-
	4	Addition of C+	✓	✓	-
	5	Closing of PCR plate ¹ with optical caps	✓	✓	-
	6	Mixing and spinning down of plate	✓	✓	-
	7	Transfer into real-time cyclers and start PCR	✓	✓	-

¹ In the automated workflow the whole PCR plate is opened and placed to the deck in the beginning.

This proposal summarizes the plan for the internally generated tests for the automated workflow on the OT-2, in order to show its equivalence to the already validated and certified manual workflow.

3 Test plan

3.1 Accuracy of the liquid transfer

During the BACGene lysis and PCR preparation, various volumes (method-depending) are transferred in the respective steps. The aim of the accuracy of the liquid transfer study is to proof, that all volumes transferred by the OT-2 are within the previously validated acceptance criteria for the manual workflow, see Table 2.

Table 2 Steps and criteria for accuracy testing of the liquid transfer.

Method	Step	Dispensing mode	Volume transferred	Acceptance criterion
<i>BACGene Listeria</i>				
<ul style="list-style-type: none"> • <i>BACGene Listeria</i> Multiplex • <i>BACGene Listeria</i> spp. • <i>BACGene Listeria monocytogenes</i> 	Dispensing of lysis buffer to lysis plate	Single dispense	70 µL	± 15 %
<i>BACGene Salmonella</i> spp.	Dispensing of lysis buffer to lysis plate	Single dispense	90 µL	± 15 %
<i>BACGene Listeria</i>				
<ul style="list-style-type: none"> • <i>BACGene Listeria</i> Multiplex • <i>BACGene Listeria</i> spp. • <i>BACGene Listeria monocytogenes</i> 	Dispensing of enrichment samples to lysis buffer	Multichannel dispense (8-channel)	30 µL	± 20 %
<i>BACGene Salmonella</i> spp.	Dispensing of enrichment samples to lysis buffer	Multichannel dispense (8-channel)	10 µL	± 20 %
<i>BACGene Listeria</i>				
<ul style="list-style-type: none"> • <i>BACGene Listeria</i> Multiplex • <i>BACGene Listeria</i> spp. • <i>BACGene Listeria monocytogenes</i> 	Dispensing of lysates to PCR mix	Multichannel dispense (8-channel)	5 µL	± 20 %
<i>BACGene Salmonella</i> spp.				

Each condition was tested in 8 replicates. The complete accuracy test was performed three individual times. Evaluation was done based on mean values for every condition.

3.2 Accuracy of incubation temperatures

During the BACGene lysis, two incubation temperatures are part of the protocol: 20 min at 37 °C and 10 min at 95 °C. The aim of the accuracy study of the incubation temperatures is to proof, that both temperatures reached by the OT-2 heating module, are within the previously validated acceptance criteria for the manual workflow: 37 °C \pm 2 °C and 95 °C \pm 5 °C.

For measuring the temperature, several wells within a lysis plate were filled with 100 μ L water (corresponds to the lysis volume) and temperature is measured with an external thermometer.

The complete accuracy test was performed three individual times. Evaluation is done based on mean values measured temperatures. Temperature differences must be within the criteria listed in Table 3 below.

Table 3 Criteria for temperature accuracy

Step	Temperature	Acceptance criterion
1	37 °C	\pm 2 °C
2	95 °C	\pm 5 °C

3.3 Cross-contamination

As PCR is a highly sensitive method, it is important that there is no risk within the automated workflow in regards to cross-contamination, which could lead to false positive results. This has to be ensured during:

1. The transfer of enrichment samples to the lysis buffer
2. The transfer of the lysates to the PCR mix

For testing the risk of cross-contamination during the OT-2 workflow, a challenge test will be performed in a so-called “checkerboard” approach, in which highly positive samples are arranged alternately with negative samples, see Figure 1 below. In total 48 positive and 48 negative samples were tested per plate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	+	-	+	-	+	-	+	-	+	-	+	-
B	-	+	-	+	-	+	-	+	-	+	-	+
C	+	-	+	-	+	-	+	-	+	-	+	-
D	-	+	-	+	-	+	-	+	-	+	-	+
E	+	-	+	-	+	-	+	-	+	-	+	-
F	-	+	-	+	-	+	-	+	-	+	-	+
G	+	-	+	-	+	-	+	-	+	-	+	C+
H	-	+	-	+	-	+	-	+	-	+	-	C-

Figure 1 Layout checkerboard, +: high positive sample, -: negative sample, C+: PCR positive control, C-: lysis buffer negative control

As positive samples pure *Salmonella* (*Salmonella enterica* subsp. *enterica* serovar Typhimurium, ATCC 14028) / *Listeria* (*Listeria monocytogenes*, ATCC 1911) cultures were used, which have been grown to approx. 1×10^9 CFU/mL in BHI overnight at 37 °C.

For passing the cross-contamination test, no amplification signals for *Salmonella* (BACGene *Salmonella* spp.) respectively *Listeria* (BACGene *Listeria* Multiplex, for both targets *L. spp.* and *L. mono*) are allowed in the negative samples and all positive samples have to be strong positive with Cq-values ≤ 20 .

One checkerboard for *Salmonella* and one for *Listeria* were performed.

3.4 Robustness

3.4.1 Evaporation during lysis

In contrast to the manual workflow, lysis tubes will not be closed during the lysis performed with the automated workflow on the OT-2. As the two lysis steps within the *BACGene* workflow (1. step: 20 min at 37 °C, 2. step: 10 min at 95 °C) are run on the same OT-2 heating module, it is not necessary to transfer the plate to the second temperature and therefore closing of the caps is no longer required. During lysis with open tubes, a certain amount of the lysate will evaporate, therefore it has to be shown, that there is no negative impact on the overall performance of the methods due to this evaporation. Two topics have to be considered during the evaporation test:

- I. Potential increase of inhibition due to up-concentration of lysates

Criterion:

- a. Evaporation rate $\leq 40\%$
- b. IPC not inhibited

- II. Potential cross-contamination

Criterion: no cross-contamination

- a. Evaluated by the negative controls in that test
- b. Additionally evaluated by surface swabs taken before and after the experiment to look for potential DNA contamination arising through evaporation during the open lysis procedure.

Divided a sample plate into single wells. Each lysis mixture is dispensed into 16 wells. Transfer the planned volume of the lysis mix into the wells using a single pipette. Then the wells are transferred into the OT-2, incubate them as in the lysis step. Upfront, the weight of the filled wells are measured versus weight of wells exposed to the lysis procedure in order to have a more accurate calculation of the weight loss – representing liquid loss during evaporation.

The test was performed 3 individual times. Evaluation was done based on mean values for every condition.

In terms of surface swabs taken, not only critical positions in the surrounding of the heating unit, but others are taken not only to evaluate potential DNA evaporation during lysis but also checking the overall handling workflow within the OT-2 automation system for potential cross-contamination.

3.5 Stability

3.5.1 Positive control stability

For the OT-2 workflow, the positive control (C+) will be placed to the reagents-deck from the beginning and is therefore exposed to air and ambient temperature for the whole process (approx. 1 h – 75 min). The stability of the C+ was analysed to ensure, that there is no negative impact on PCR-evaluation coming from that deviation compared to the manual workflow.

The C+ of the BACGene *Salmonella* spp. and the BACGene *Listeria* Multiplex kit was incubated at ambient temperature for up to 8 hours and compared to timepoint 0 (reference) without any incubation, tubes were left open for the whole duration of the test. Each condition was tested in duplicates, for evaluation mean values were calculated, acceptance criterion is the following: $Cq_{tx} - Cq_{ref} < 1.0$.

3.5.2 PCR mix stability

For the OT-2 workflow, the PCR plate containing the ready-to-be used mix will be placed to the reagents-deck from the beginning and is therefore exposed to air and ambient temperature for the whole process (approx. 1 h – 75 min). The stability of the mix was analysed to ensure, that there is no negative impact on PCR evaluation coming from that deviation compared to the manual workflow.

The mix of the BACGene *Salmonella* spp. and the BACGene *Listeria* Multiplex kit was incubated at ambient temperature for up to 4 hours and compared to timepoint 0 without any incubation, tubes were left open for the whole duration of the test. Each plate was tested over the official BACGene QC protocol for final batch release incl. LOD and sensitivity, for acceptance the official criteria have been applied.

3.5.3 PCR-plate stability after addition of samples

For the OT-2 workflow, it can happen that a mixed plate (*Salmonella* and *Listeria*) will be tested. As the two protocols will run one after the other, the respective first PCR plate has to stay at ambient temperature after samples are applied until the other protocol is finished (approx. 1 h – 75 min). The stability of the mix incl. samples was analysed up to 4 h to ensure, that there is no negative impact on PCR evaluation coming from that deviation compared to the manual workflow.

Each condition was tested in six replicates, for evaluation mean values were calculated, acceptance criterion is the following: $Cq_{tx} - Cq_{ref} < 1.0$.

3.6 Matrix testing

For the automated workflow on the OT-2, it is important to proof that there is no risk due to matrix particles clogging in pipette tips, which could negatively impact the transferred volume and therefore could lead to false negative results.

For this test, different food matrices from all categories in the respective methods scope have been selected with the focus on particle-rich sample types. Each matrix was analysed as blank, as well as spiked sample. Spiking was performed post enrichments by taking the blanks and inoculating them with different strains, see Table 4 below.

Table 4 Strains used for artificial inoculation.

Test kit	Strain	Source
BACGene <i>Salmonella</i> spp.	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium	ATCC 14028
	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Enteritidis	ATCC 13076
	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Heidelberg	ATCC 8326
BACGene <i>Listeria</i> Multiplex	<i>Listeria innocua</i>	DSMZ 20649
	<i>Listeria ivanovii</i> <i>ivanovii</i>	DSMZ 20750
	<i>Listeria welshimeri</i>	DSMZ 20650
	<i>Listeria monocytogenes</i>	ATCC 19111
BACGene <i>Listeria</i> spp.	<i>Listeria innocua</i>	DSMZ 20649
	<i>Listeria ivanovii</i> <i>ivanovii</i>	DSMZ 20750
	<i>Listeria welshimeri</i>	DSMZ 20650
	<i>Listeria monocytogenes</i>	ATCC 1911
BACGene <i>Listeria monocytogenes</i>	<i>Listeria monocytogenes</i>	ATCC 19111

For details regarding exact food item per type and category, spike strain and inoculation level see Annex (chapter 0),

Table 32 (*Salmonella*), Table 33 (*Listeria* spp.) and Table 34 (*Listeria monocytogenes*).

For evaluation, each sample was tested over the manual as well as over the automated workflow, evaluation was done using the respective BACGene Evaluation sheet, results are not allowed to differ between the two workflows. Furthermore, raw data in form of Cq values and EPF values was evaluated. Difference in Cq values between manual and automated workflow must be ≤ 1 Cq and EPF values ≤ 33 %.

Cultural confirmation according to the respective BACGene IFU was only done in case of deviating final results, which was not the case.

4 Test Results

4.1 Accuracy of the liquid transfer

All volumes which are transferred in the respective steps during BACGene lysis and PCR preparation have been proved to be in the acceptance criteria previously validated for the manual workflow.

Table 5 Results for accuracy of liquid transfer. For each combination 24 wells were dispensed. The pipetted volume was measured by weighing the pipetted liquid and calculation of the volume thereof.

Step	Dispensing mode	Volume transferred	Acceptance criterion	Mean measured volume	Deviation from the defined volume	Evaluation
Dispensing of lysis buffer to lysis plate	Single dispense	70 µL	± 15 %	70.4 µL	+ 0.6 %	✓
Dispensing of lysis buffer to lysis plate	Single dispense	90 µL	± 15 %	90.2 µL	+ 0.2 %	✓
Dispensing of enrichment samples to lysis buffer	Multichannel dispense (8-channel)	30 µL	± 20 %	30.7 µL	+ 2.3 %	✓
Dispensing of enrichment samples to lysis buffer	Multichannel dispense (8-channel)	10 µL	± 20 %	9.9 µL	- 1 %	✓
Dispensing of lysates to PCR mix	Multichannel dispense (8-channel)	5 µL	± 20 %	<i>Listeria</i> kits: 4.5 µL <i>Salmonella</i> kit: 4.2 µL	<i>Listeria</i> kits: - 10 % <i>Salmonella</i> kit: - 16 %	✓

4.2 Accuracy of incubation temperature

It has been shown, that the incubation temperatures of 37 °C and 95 °C in the OT-2 are within the acceptance criteria previously validated for the manual workflow.

Table 6 Results for accuracy of the incubation temperature. The temperature was measured with an external thermometer in a lysis plate filled with 100 µL water in different locations in the block (edges and center). Mean of four measurements was calculated.

Step	Temperature	Acceptance criterion	Mean measured temperature	Evaluation
1	37 °C	± 2 °C	36.3 °C	✓
2	95 °C	± 5 °C	91.2 °C	✓

4.3 Cross contamination

It has been shown, that during the transfer of the enrichment samples to the lysis buffer and the transfer of the lysates to the PCR mix, no contamination risk arises with the automated workflow.

Highly positive samples were arranged alternately with negative samples, see Figure 1 above. In total 48 positive and 48 negative samples were tested per plate.

As a result, no amplification curves of negative samples were observed in two checkerboard experiments conducted using BACGene *Salmonella* spp. with respective *S. Typhimurium* spiked samples and using BACGene *Listeria* Multiplex with respective *L. monocytogenes* spiked samples. All positive samples generated Cq values below 20, which indicated very high positive signals.

4.4 Robustness

4.4.1 Evaporation during lysis

It has been shown, that there is no negative impact on the overall performance of the method due to the evaporation of the lysate, which occurs during the incubation of the lysate in the open tubes.

Table 7 Results of the robustness tests in regards to the evaporation during the lysis. The evaporation rates were determined in 48 wells by weighing.

	Acceptance criterion	Mean measured result	Evaluation
<i>Listeria</i> lysate	≤ 40 %	26.0 %	✓
<i>Salmonella</i> lysate	≤ 40 %	26.6 %	✓

Furthermore, all surface swabs which have been taken from various locations within the OT-2 (see Figure 2) and analyzed for the respective target DNA have been completely negative.

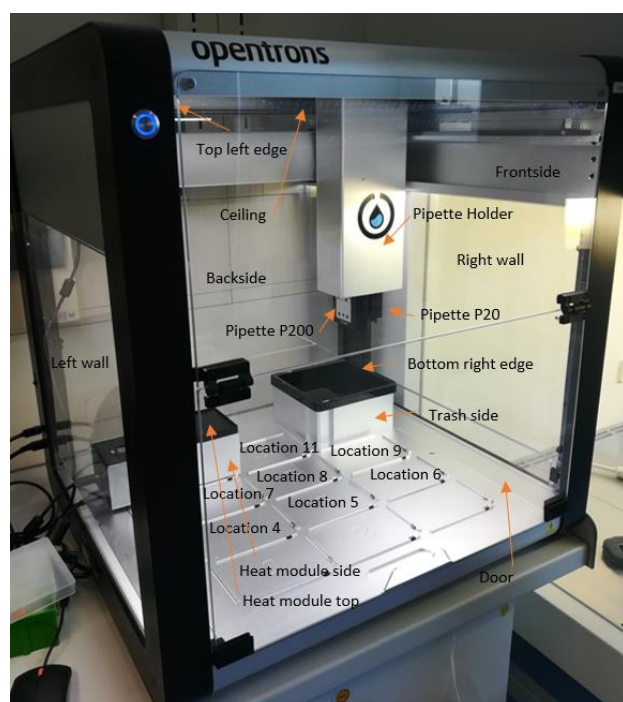


Figure 2 Locations in the robot tested on contamination. The shown areas were tested by swabbing the areas with a moistened swab and analyzing it on DNA contaminations. For detailed test results see Annex Table 31.

4.4.2 Positive control stability

It has been shown, that there is no negative impact on PCR-evaluation coming from the exposure to ambient temperature of the positive control for up to 8 hours.

Table 8 Results of the stability test of the BACGene *Salmonella* spp. positive control. The positive control has been left open for up to 8 hours and analyzed in the corresponding PCR. Results have been compared to the unincubated positive control and mean of duplicate results has been calculated.

Incubation time	Acceptance criterion	Measured delta mean Cq value ($Cq_{tx} - Cq_{ref}$)	Evaluation
1 h	Delta mean Cq ($Cq_{tx} - Cq_{ref}$) < 1.0	-0.20	✓
2 h	Delta mean Cq ($Cq_{tx} - Cq_{ref}$) < 1.0	-0.83	✓
4 h	Delta mean Cq ($Cq_{tx} - Cq_{ref}$) < 1.0	-0.82	✓
8 h	Delta mean Cq ($Cq_{tx} - Cq_{ref}$) < 1.0	-0.86	✓

Table 9 Results of the stability test of the BACGene *Listeria* Multiplex positive control. The positive control has been left open for up to 8 hours and analyzed in the corresponding PCR. Results have been compared to the unincubated positive control and mean of duplicate results has been calculated.

Incubation time	Acceptance criterion	Measured delta mean Cq value ($Cq_{tx} - Cq_{ref}$) <i>Listeria</i> spp.	Measured delta mean Cq value ($Cq_{tx} - Cq_{ref}$) <i>Listeria</i> mono.	Evaluation
1 h	Delta mean Cq ($Cq_{tx} - Cq_{ref}$) < 1.0	-0.07	-0.25	✓
2 h	Delta mean Cq ($Cq_{tx} - Cq_{ref}$) < 1.0	-0.20	-0.34	✓
4 h	Delta mean Cq ($Cq_{tx} - Cq_{ref}$) < 1.0	-0.09	-0.31	✓
8 h	Delta mean Cq ($Cq_{tx} - Cq_{ref}$) < 1.0	-0.74	-1.04	✓

4.4.3 PCR mix stability

It was shown, that there is no negative impact on PCR evaluation coming from the exposure of the ready-to-use PCR plate to air and ambient temperature for up to 4 hours.

Table 10 Results of the stability test of the BACGene *Salmonella* spp. PCR mix. The BACGene *Salmonella* spp. mix was incubated for 4 h at 25 °C and then tested using the official BACGene QC protocol for final batch release (including a LOD and sensitivity test). As sample a plasmid carrying the *Salmonella* spp. target was used. Results are compared to a fresh, not incubated PCR mix.

Sample	Number of replicates	Acceptance criteria	Incubated PCR mix (4 h at 25 °C)	Fresh PCR mix	Acceptance Criteria	
			FAM™ channel		Incubated PCR mix (4 h at 25 °C)	Fresh PCR mix
0.693 cp/rxn	5	25 – 75 % positive (3 - 9 reactions positive)	10/12	8/12	✗	✓
5 cp/rxn	20	95 % positive (≥ 19 reactions positive)	20/20	20/20	✓	✓
10 cp/rxn	4	100 % positive	4/4	4/4	✓	✓
C+	5	100 % positive (Cq values >30 and <37)	5/5	5/5	✓	✓
NTC	7	100 % negative	7/7	7/7	✓	✓

Table 11 Results of the stability test of the BACGene *Listeria* Multiplex PCR mix. The BACGene *Listeria* Multiplex PCR mix was incubated for 4 h at 25 °C and then tested using the official BACGene QC protocol for final batch release (including a LOD and sensitivity test). As sample a plasmid carrying the *Listeria* spp./*Listeria monocytogenes* target was used. Results are compared to a fresh, not incubated PCR mix.

Sample	Number of replicates	Acceptance criteria	Incubated PCR mix (4 h at 25 °C)		Fresh PCR mix		Acceptance Criteria	
			HEX™ channel	ROX™ channel	HEX™ channel	ROX™ channel	Incubated PCR mix (4 h at 25 °C)	Fresh PCR mix
0.693 cp/rxn	5	25 – 75 % positive (3 - 9 reactions positive)	4/12	4/12	6/12	6/12	✓	✓
5 cp/rxn	20	95 % positive (≥ 19 reactions positive)	20/20	20/20	19/20	19/20	✓	✓
10 cp/rxn	4	100 % positive	4/4	4/4	4/4	4/4	✓	✓
C+	5	100 % positive (Cq values >24 and <32)	5/5	5/5	5/5	5/5	✓	✓
NTC	7	100 % negative	7/7	7/7	7/7	7/7	✓	✓

For the 0.693 copies/reaction test for the BACGene *Salmonella* spp., the mix incubated for 4 hours at ambient temperature showed a slightly higher overall value. As the fresh mix shows less positives (test was done using the same DNA dilution), this is most likely coming from the handling and is not an effect due to the mix incubation.

4.4.4 PCR-plate stability after addition of samples

It has been shown, that there is no negative impact on PCR evaluation resulting from the exposure of the prepared PCR reaction (mix + sample) to ambient temperature for up to 4 hours.

Table 12 Results of the PCR plate stability after addition of the samples. As samples lysates of 1:1000 diluted overnight cultures of *Salmonella* Typhimurium or *Listeria monocytogenes* respectively have been used. Each condition has been tested in eight replicates and mean values of deviations to unincubated PCR mixture have been calculated.

Incubation time of PCR plate after addition of the samples	Delta mean Cq value ($Cq_{tx} - Cq_{ref}$)				
	BACGene <i>Salmonella</i> spp.		BACGene <i>Listeria</i> Multiplex		
	<i>Salmonella</i> spp.	IPC	<i>Listeria</i> spp.	<i>Listeria</i> <i>monocytogenes</i>	IPC
1 h	-0.28	-0.16	-0.30	-0.25	0.46
2 h	-0.27	-0.66	-0.85	-0.89	-0.83
3 h	0.44	0.14	-1.01	-1.12	-1.06
4 h	0.40	0.27	-1.05	-1.06	-0.74

4.5 Matrix test

To proof that the automated workflow shows a similar performance compared to the manual workflow, relevant food matrices from the six certified categories (with focus on particle - rich matrices) were tested with the respective BACGene kits (BACGene *Salmonella* spp., BACGene *Listeria monocytogenes*, BACGene *Listeria* spp. and BACGene *Listeria* Multiplex). Each matrix was analyzed as blank (analysis in the Cy5® channel), as well as spiked sample (analysis in the FAM™, ROX™ or HEX™ channel, depending on the respective BACGene kit). Spiking was performed post enrichment by taking the blanks and inoculating them with different *Salmonella* spp. and *Listeria* spp. strains with a low and a high spike level.

In total, 762 test portions have been tested across all three inoculation levels (Blank, Low spike and higher spike) and 4 kits, with no differences in final results for the IPC (valid), as well as for the respective targets *Salmonella* or *Listeria* (positive and negative)

Detailed analysis of the raw data (Cq-values and EPF-values), which are both used for final result evaluation shows, that a majority of samples show earlier Cq-values and higher EPF-values for the automated workflow on the OT-2, compared to manual handling.

This is a direct effect on the slight evaporation during open lysis and shows, that there is no negative effect regarding an increased inhibition rate, but that running the analysis on the OT-2 is slightly increasing the sensitivity of all BACGene methods.

4.5.1 BACGene *Salmonella* spp.

For the BACGene *Salmonella* spp. kit 42 different food matrices and environmental samples were analyzed (as blanks and spiked with a low and a high spike level) with the automated and the manual workflow and evaluated with the BACGene evaluation sheet. The Cq and EPF values of the blanks and spiked matrices were comparable for the majority of the matrices (see Figure 3).

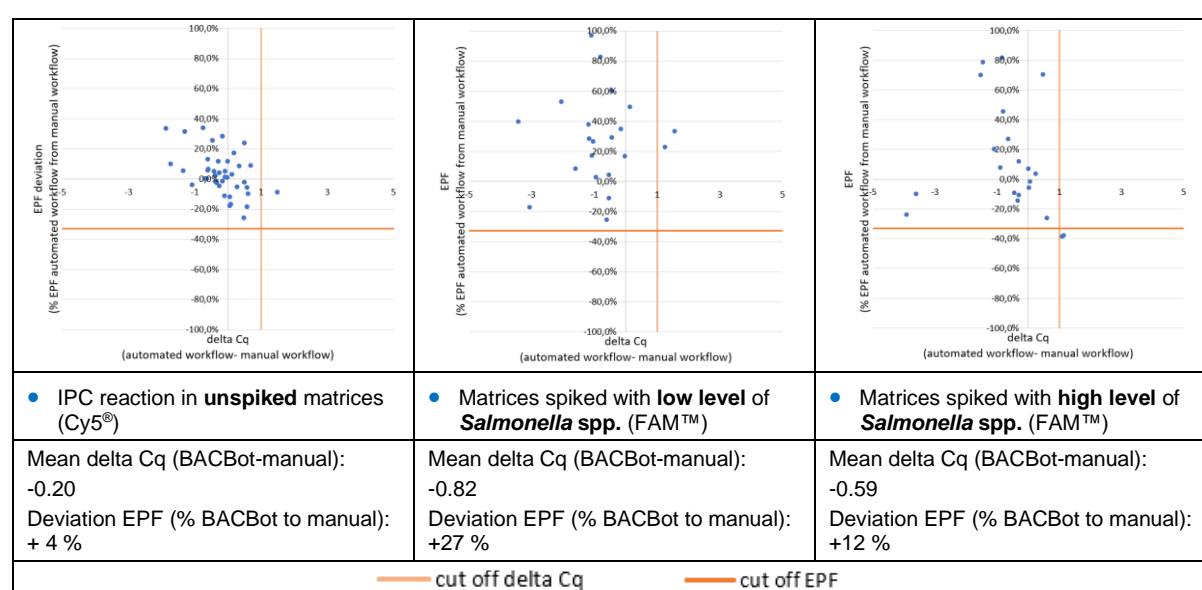


Figure 3 Overview of the results of the blank matrices (left) as well as for the *Salmonella*-spiked (low (middle) and high level (right)) matrices run with the automated and manual workflow and tested with the BAC Gene *Salmonella* spp. PCR kit. 42 relevant broad range of food matrices covering the certified categories were spiked with three

different *Salmonella* strains (*S. enteritidis*, *S. Heidelberg*, *S. typhimurium*). The spiked samples as well as the corresponding blanks were run with the manual and the automated workflow of the lysis and PCR and analyzed with the BACGene *Salmonella* spp. kit on the Bio-Rad CFX96 Touch™ Standard. Cq values and EPF values of the *Salmonella* target (FAM™ channel) and the internal positive control (Cy5® channel) were evaluated with the BACGene evaluation sheet. Results of the manual and the automated workflow were compared and are shown as delta Cq (automated - manual) and % EPF (automated vs. manual). The cut-offs for the delta Cq (horizontal) and % EPF (vertical) are shown as orange lines.

4.5.1.1 Blanks

For the sample whole liquid egg (fresh and pasteurized) inhibitory effects were observed in general and have therefore higher deviations in Cq and EPF values (see 13). The fresh whole liquid egg has a slightly earlier Cq (-1.5) in the IPC (Cy5®) in the unspiked sample in the manual workflow compared to the automated workflow (EPF not affected). The pasteurized whole liquid egg showed inhibition in the manual workflow in the unspiked sample (Cy5®). For the automated workflow no inhibition was detected.

When analyzing the mean Cq and EPF values over all blank matrices, the automated and manual workflow show similar mean Cq and EPF values (see 13).

Table 13 Results of the Blank matrices run with the automated and manual workflow and tested with the BACGene *Salmonella* spp. PCR Kit. 42 relevant broad range of food matrices covering the certified categories were tested. The lysis and PCR were performed with the manual and the automated workflow and analyzed with the BACGene *Salmonella* spp. kit on the Bio-Rad CFX96 Touch™ Standard. Cq values and EPF values of the internal positive control (Cy5® channel) were evaluated with the BACGene evaluation sheet. Results of the manual and the automated workflow were compared and are shown as delta Cq (automated - manual) and % EPF (automated vs. manual). Cq values of the internal positive control were between ~26 – 33.

Item	Result manual workflow	Result BACBot	Delta Cq value ¹⁾	% EPF value ²⁾
			(BACBot – manual workflow)	(BACBot of manual workflow)
Cy5® channel				
Marinated chicken	valid	valid	0.50	124 %
Blue mold cheese	valid	valid	0.60	90 %
Frozen spinach	valid	valid	0.06	82 %
Whole liquid egg (fresh)	valid	valid	1.50	91 %
Frozen Cod fillet	valid	valid	-0.26	104 %
Smoked trout	valid	valid	-0.35	98 %
Sponge soaked with NB buffer	valid	valid	-0.17	129 %
Sliced turkey	valid	valid	0.58	94 %
Raw milk	valid	valid	0.05	88 %
Sprouts	valid	valid	-0.17	98 %
RTRH vegetables	valid	valid	-1.36	105 %
Egg custard	valid	valid	0.33	109 %
Surimi	valid	valid	-0.42	105 %
Rinse water	valid	valid	-1.73	110 %
Minced pork	valid	valid	0.50	98 %
Ox cheek	valid	valid	-0.61	106 %
Ice cream with walnut inclusions	valid	valid	0.68	109 %

Cucumber salad	valid	valid	-0.27	96 %
Whole egg powder	valid	valid	-0.75	134 %
Shrimps	valid	valid	-1.30	132 %
Wipe dust	valid	valid	-1.08	96 %
Beef Trim	valid	valid	0.09	83 %
Raw milk cheese	valid	valid	-1.87	134 %
Ice cream with chocolate inclusions	valid	valid	-0.08	105 %
Fresh baby spinach	valid	valid	-0.01	112 %
Egg salad	valid	valid	-0.10	89 %
Hering salad	valid	valid	-0.37	98 %
Rinse water	valid	valid	-0.38	103 %
Frozen Carpaccio	valid	valid	-0.29	112 %
Goat cheese	valid	valid	-0.64	100 %
ready to eat green mixed salad	valid	valid	-0.60	107 %
Egg white powder	valid	valid	0.11	103 %
Frozen Cod fillet	valid	valid	-0.47	126 %
Wipe dust	valid	valid	-0.03	101 %
Swab soaked with Dey-Engley buffer	valid	valid	-0.62	113 %
Burger patties	valid	valid	0.48	74 %
Whole Milk Powder	valid	valid	-0.10	101 %
Romana salad	valid	valid	-0.68	100 %
Whole egg liquid (pasteurized)	Invalid	valid	-3.89	149 %
Mayonnaise	valid	valid	0.27	95 %
Smoked salmon	valid	valid	0.18	117 %
Sponge soaked with Lethen buffer	valid	valid	0.58	82 %
Mean (invalid excluded)			-0.20	104 %
Standard Deviation (invalid excluded)			0.67	14.2 %

¹⁾ Negative Cq values: indicate that the automated workflow results in earlier Cq values compared to the manual workflow. Positive Cq values: indicate that manual workflow results in earlier Cq values compared to the automated workflow.

²⁾ EPF values <100: indicate that the manual workflow results in higher EPF compared to the automated workflow. EPF values >100: indicate that the automated workflow results in higher EPF values compared to the manual workflow.

4.5.1.2 Matrices spiked with *Salmonella* spp.

For the spiked matrices, fresh whole liquid egg shows a complete inhibition in the *Salmonella* specific channel (FAM™) when analyzed with the manual workflow (see Table 14). The pasteurized whole liquid egg sample showed a slightly earlier Cq value and slightly higher EPF value in the *Salmonella* specific channel (FAM™) of the spiked sample when analyzing with the manual extraction (see

Table 15). This result indicates that the automated workflow leads to an improvement in the analysis of this matrix type due to a constant pipetting height.

Two other matrices (ice cream with walnut inclusions and romana salad) have slightly later Cq values (+ 1.57 and + 1.1) with the automated workflow in the *Salmonella* specific channel (FAM™) compared to the manual workflow. Also, the EPF is slightly dropped for the matrix romana salad (39 %), the ice cream with walnut inclusions is not affected.

When analyzing the mean Cq values over the low and high spike level, the automated workflow shows earlier mean Cq values compared to the manual workflow (0.82 earlier for the low spike level and 0.59 for the high spike level). Mean EPF values are comparable for the automated workflow and the manual workflow.

Table 14 Results of the artificially *Salmonella* - spiked (low level) matrices run with the automated and manual workflow and tested with the BACGene *Salmonella* spp. PCR kit. 42 relevant broad range of food matrices covering the certified categories were spiked with three different *Salmonella* strains (*S. enteritidis*, *S. Heidelberg*, *S. typhimurium*). The lysis and PCR were performed with the manual and the automated workflow and analyzed with the BACGene *Salmonella* spp. kit on the Bio-Rad CFX96 Touch™ Standard. Cq values and EPF values of the *Salmonella* target (FAM™ channel) were evaluated with the BACGene evaluation sheet. Results of the manual and the automated workflow were compared and are shown as delta Cq (automated - manual) and % EPF (automated vs. manual). Cq values of the *Salmonella* target were between ~30 – 37.

Item	Inoculation level CFU/mL	Spike Strain	Result manual workflow	Result BACBot	Delta Cq value ¹⁾ (BACBot – manual workflow) FAM™ channel	% EPF value ²⁾ (BACBot of manual workflow)
Marinated chicken	5.2 x 10 ⁴ CFU/mL	<i>S. enteritidis</i>	+	+	-2.05	153 %
Blue mold cheese			+	+	0.14	149 %
Frozen spinach			+	+	-1.09	197 %
Whole liquid egg (fresh)			-	+	n/a	n/a
Frozen Cod fillet			+	+	-0.80	182 %
Smoked trout			+	+	-0.44	160 %
Sponge soaked with NB buffer			+	+	-0.15	135 %
Sliced turkey	4.5 x 10 ⁴ CFU/mL	<i>S. Heidelberg</i>	+	+	-1.18	138 %
Raw milk			+	+	-0.54	104 %
Sprouts			+	+	-0.53	89 %
RTRH vegetables			+	+	-1.04	126 %
Egg custard			+	+	-1.60	108 %
Surimi			+	+	-1.16	128 %
Rinse water			+	+	-1.07	117 %
Minced pork	5.1 x 10 ⁴ CFU/mL	<i>S. typhimurium</i>	+	+	-0.94	103 %
Ox cheek			+	+	-0.45	129 %
Ice cream with walnut inclusions			+	+	1.57	133 %
Cucumber salad			+	+	-0.02	116 %
Whole egg powder			+	+	-3.43	140 %
Shrimps			+	+	-3.07	83 %
Wipe dust			+	+	-0.61	74 %
Mean					-0.82	127 %
Standard Deviation					1.16	30.4 %

¹⁾ Negative Cq values: indicate that the automated workflow results in earlier Cq values compared to the manual workflow. Positive Cq values: indicate that manual workflow results in earlier Cq values compared to the automated workflow.

²⁾ EPF values <100: indicate that the manual workflow results in higher EPF compared to the automated workflow. EPF values >100: indicate that the automated workflow results in higher EPF values compared to the manual workflow.

Table 15 Results of the artificially *Salmonella* - spiked (high level) matrices run with the automated and manual workflow and tested with the BACGene *Salmonella* spp. PCR kit. 42 relevant broad range of food matrices covering the certified categories were spiked with three different *Salmonella* strains (*S. enteritidis*, *S. Heidelberg*, *S. typhimurium*). The lysis and PCR were performed with the manual and the automated workflow and analyzed with the BACGene *Salmonella* spp. kit on the Bio-Rad CFX96 Touch™ Standard. Cq values and EPF values of the *Salmonella* target (FAM™ channel) were evaluated with the BACGene evaluation sheet. Results of the manual and the automated workflow were compared and are shown as delta Cq (automated - manual) and % EPF (automated vs. manual). Cq values of the *Salmonella* target were between ~30 – 35.

Item	Inoculation level CFU/mL	Spike Strain	Result manual workflow	Result BACBot	Delta Cq value ¹⁾ (BACBot – manual workflow) FAM™ channel	% EPF value ²⁾ (BACBot of manual workflow)
Beef Trim	1.04 x 10 ⁵ CFU/mL	<i>S. enteritidis</i>	+	+	-0.29	112 %
Raw milk cheese			+	+	-0.63	127 %
Ice cream with chocolate inclusions			+	+	0.47	170 %
Fresh baby spinach			+	+	-1.44	179 %
Egg salad			+	+	-0.80	145 %
Hering salad			+	+	-0.85	181 %
Rinse water			+	+	-1.53	170 %
Frozen Carpaccio	0.9 x 10 ⁵ CFU/mL	<i>S. Heidelberg</i>	+	+	-0.89	108 %
Goat cheese			+	+	-1.08	120 %
ready to eat green mixed salad			+	+	-0.30	89 %
Egg white powder			+	+	-3.61	90 %
Frozen Cod fillet			+	+	0.61	74 %
Wipe dust			+	+	0.25	104 %
Swab soaked with Dey-Engley buffer			+	+	0.00	107 %
Burger patties	1.0 x 10 ⁵ CFU/mL	<i>S. typhimurium</i>	+	+	-0.45	91 %
Whole Milk Powder			+	+	-3.91	76 %
Romana salad			+	+	1.10	61 %
Whole egg liquid (pasteurized)			+	+	1.15	62 %
Mayonnaise			+	+	-0.34	86 %
Smoked salmon			+	+	0.02	94 %
Sponge soaked with Lethen buffer			+	+	0.07	98 %
Mean					-0.59	112 %
Standard Deviation					1.28	37.4 %

¹⁾ Negative Cq values: indicate that the automated workflow results in earlier Cq values compared to the manual workflow. Positive Cq values: indicate that manual workflow results in earlier Cq values compared to the automated workflow.

²⁾ EPF values <100: indicate that the manual workflow results in higher EPF compared to the automated workflow. EPF values >100: indicate that the automated workflow results in higher EPF values compared to the manual workflow.

4.5.2 BACGene *Listeria monocytogenes*

For the BACGene *Listeria monocytogenes* kit 42 different food matrices and environmental samples were analyzed (as blanks and spiked with a low and a high spike level with *Listeria monocytogenes*) with the automated and the manual workflow and evaluated with the BACGene Evaluation sheet.

The Cq and EPF values of the blanks and spiked matrices were comparable for the majority of the matrices (see Figure 4).

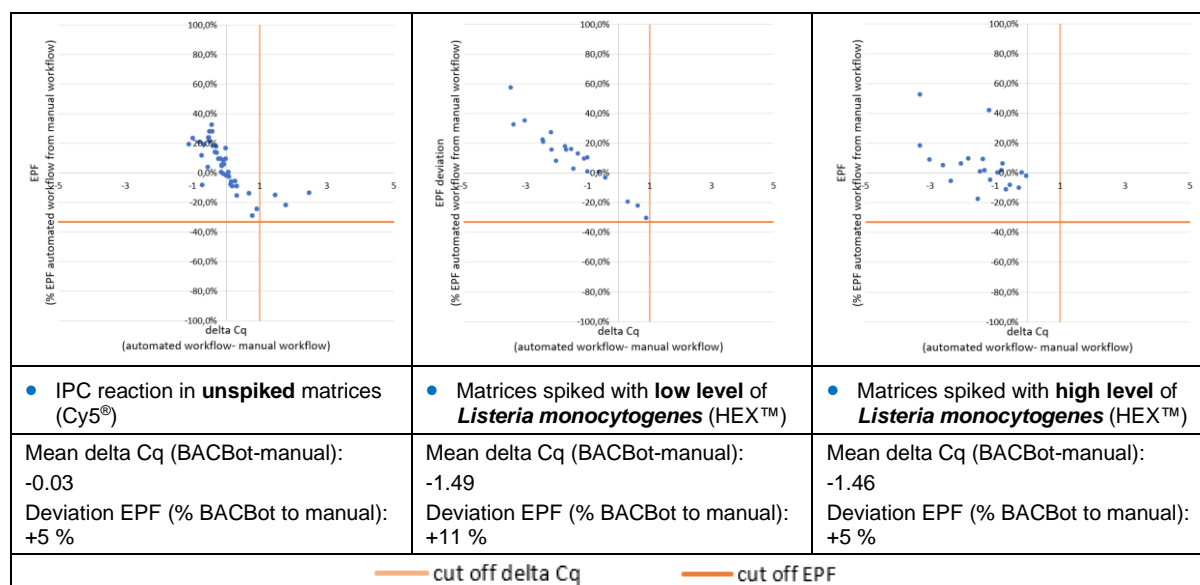


Figure 4 Overview of the results of the blank matrices (left) as well as for the *Listeria monocytogenes*-spiked (low (middle) and high level (right) matrices run with the automated and manual workflow and tested with the BACGene *Listeria monocytogenes* PCR kit. 42 relevant broad range of food matrices covering the certified categories were spiked with *Listeria monocytogenes*. The spiked samples as well as the corresponding blanks were run with the manual and the automated workflow of the lysis and PCR and analyzed with the BACGene *Listeria monocytogenes* kit on the Bio-Rad CFX96 Touch™ Standard. Cq values and EPF values of the *Listeria monocytogenes* target (HEX™ channel) and the internal positive control (Cy5® channel) were evaluated with the BACGene evaluation sheet. Results of the manual and the automated workflow were compared and are shown as delta Cq (automated - manual) and % EPF (automated vs. manual). The cut-offs for the delta Cq (horizontal) and % EPF (vertical) are shown as orange lines.

4.5.2.1 Blanks

For three matrices (wipe dust, deli salad and soft raw milk cheese) the Cq values of the blanks (IPC Cy5® channel) are slightly later in the automated workflow compared to the manual workflow (1.46, 1.78 and 2.46 respectively), see Table 16. EPF values are within the acceptance criteria for the automated workflow. These samples do not show any significant deviation for the *L. monocytogenes* target (HEX™) when analyzed with *Listeria monocytogenes* spiked matrices (see Table 17 and Table 18). This indicates that these three matrices are not generally leading to a later Cq value in the automated workflow compared to the manual workflow and is therefore not seen as critical.

However, the mean Cq value as well as mean EPF values over all blanks are comparable for the automated and manual workflow.

Table 16 Results of the Blank matrices run with the automated and manual workflow and tested with the BACGene *Listeria monocytogenes* PCR kit. 42 relevant broad range of food matrices covering the certified categories were tested. The lysis and PCR were performed with the manual and the automated workflow and analyzed with the BACGene *Listeria monocytogenes* kit on the Bio-Rad CFX96 Touch™ Standard. Cq values and EPF values of the internal positive control (Cy5® channel) were evaluated with the BACGene evaluation sheet. Results of the manual and the automated workflow were compared and are shown as delta Cq (automated - manual) and % EPF (automated vs. manual). Cq values of the internal positive control were between ~27 – 32.

Item	Result manual workflow	Result BACBot	Delta Cq value ¹⁾ (BACBot – manual workflow)	% EPF value ²⁾ (BACBot of manual workflow)
			Cy5® channel	
Omelet	valid	valid	-0.42	128 %
Burger patties	valid	valid	-0.51	124 %
Raw milk	valid	valid	-1.00	123 %
Frozen spinach	valid	valid	-0.29	114 %
Frozen Cod fillet	valid	valid	-0.50	121 %
Tuna	valid	valid	-0.80	121 %
Sponge soaked with NB buffer	valid	valid	-0.09	108 %
Frozen lasagna	valid	valid	-1.11	120 %
Minced turkey	valid	valid	-0.71	92 %
Raw milk cheese	valid	valid	-0.02	117 %
Blue mold cheese	valid	valid	-0.02	109 %
Cantaloupe melon	valid	valid	-0.73	112 %
Smoked trout	valid	valid	-0.24	110 %
Rinse water	valid	valid	-0.33	114 %
Deli salad	valid	valid	1.78	79 %
Cookies	valid	valid	-0.13	105 %
Frozen Carpaccio	valid	valid	0.07	100 %
Whole Milk Powder	valid	valid	0.32	85 %
Cucumber	valid	valid	0.18	91 %
Shrimps	valid	valid	0.09	98 %
Wipe dust	valid	valid	-0.09	99 %
Sandwich	valid	valid	-0.67	120 %
Minced pork	valid	valid	-0.16	110 %
Ox cheek	valid	valid	-0.51	128 %
Goat cheese	valid	valid	-0.53	124 %
Mixed leafy greens	valid	valid	-0.41	118 %
Smoked salmon	valid	valid	-0.44	132 %
Rinse water	valid	valid	-0.30	118 %
Whole egg powder	valid	valid	0.69	86 %
chicken wings	valid	valid	-0.06	106 %
Raw milk cream cheese	valid	valid	-0.54	104 %
Brokkoli	valid	valid	0.27	95 %
Frozen Cod fillet	valid	valid	-0.14	101 %
Wipe dust	valid	valid	1.46	85 %
Swab soaked with Dey-Engley buffer	valid	valid	-0.13	10 %
Pizza	valid	valid	0.31	91 %
Beef Trim	valid	valid	0.13	92 %
Soft raw milk cheese (Camembert)	valid	valid	2.46	87 %
Romana Salad	valid	valid	0.78	71 %
Sprouts	valid	valid	0.90	76 %
Surimi	valid	valid	0.15	94 %

Sponge soaked with Lethen buffer	valid	valid	-0.01	98 %
Mean			-0.03	105 %
Standard Deviation			0.70	15.5 %

¹⁾ Negative Cq values: indicate that the automated workflow results in earlier Cq values compared to the manual workflow. Positive Cq values: indicate that manual workflow results in earlier Cq values compared to the automated workflow.

²⁾ EPF values <100: indicate that the manual workflow results in higher EPF compared to the automated workflow. EPF values >100: indicate that the automated workflow results in higher EPF values compared to the manual workflow.

4.5.2.2 Matrices spiked with *Listeria monocytogenes*

For the spiked matrices (low and high spike level) there is no matrix, which does not meet the acceptance criteria (see Table 17 and Table 18).

When analyzing the mean Cq values over the low and high spike level, the automated workflow shows earlier mean Cq values compared to the manual workflow (1.49 earlier for the low spike level and 1.46 for the high spike level). Mean EPF values are comparable for the automated workflow and the manual workflow.

Table 17 Results of the artificially *Listeria monocytogenes* - spiked (low level) matrices run with the automated and manual workflow and tested with the BACGene *Listeria monocytogenes* PCR kit. 42 relevant broad range of food matrices covering the certified categories were spiked with *Listeria monocytogenes*. The lysis and PCR were performed with the manual and the automated workflow and analyzed with the BACGene *Listeria monocytogenes* kit on the Bio-Rad CFX96 Touch™ Standard. Cq values and EPF values of the *Listeria monocytogenes* target (HEX™ channel) were evaluated with the BACGene evaluation sheet. Results of the manual and the automated workflow were compared and are shown as delta Cq (automated - manual) and % EPF (automated vs. manual). Cq values of the *Listeria monocytogenes* target were between ~30 – 39.

Item	Inoculation level CFU/mL	Spike Strain	Result manual workflow	Result BACBot	Delta Cq value ¹⁾ (BACBot – manual workflow) HEX™ channel	% EPF value ²⁾ (BACBot of manual workflow)
Omelet	7.1 x 10 ⁴	<i>L. monocytogenes</i>	+	+	-1.69	116 %
Burger patties			+	+	-1.53	116 %
Raw milk			+	+	-1.11	110 %
Frozen spinach			+	+	-2.18	116 %
Frozen Cod fillet			+	+	-2.19	128 %
Tuna			+	+	-2.44	121 %
Sponge soaked with NB buffer			+	+	-1.73	118 %
Frozen lasagna			+	+	-2.45	123 %
Minced turkey			+	+	-2.02	108 %
Raw milk cheese			+	+	0.30	81 %
Blue mold cheese			+	+	0.62	78 %
Cantaloupe melon			+	+	-1.32	113 %
Smoked trout			+	+	-0.44	97 %
Rinse water			+	+	-1.01	101 %
Deli salad			+	+	-1.46	103 %
Cookies			+	+	-3.49	158 %
Frozen Carpaccio			+	+	-3.39	133 %
Whole Milk Powder			+	+	-3.03	135 %
Cucumber			+	+	-0.65	101 %
Shrimps			+	+	-1.02	111 %

Wipe dust	+	+	0.89	70 %
Mean			-1.49	111 %
Standard Deviation			1.20	20 %

¹⁾ Negative Cq values: indicate that the automated workflow results in earlier Cq values compared to the manual workflow. Positive Cq values: indicate that manual workflow results in earlier Cq values compared to the automated workflow.

²⁾ EPF values <100: indicate that the manual workflow results in higher EPF compared to the automated workflow. EPF values >100: indicate that the automated workflow results in higher EPF values compared to the manual workflow.

Table 18 Results of the artificially *Listeria monocytogenes* - spiked (high level) matrices run with the automated and manual workflow and tested with the BACGene *Listeria monocytogenes* PCR kit. 42 relevant broad range of food matrices covering the certified categories were spiked with *Listeria monocytogenes*. The lysis and PCR were performed with the manual and the automated workflow and analyzed with the BACGene *Listeria monocytogenes* kit on the Bio-Rad CFX96 Touch™ Standard. Cq values and EPF values of the *Listeria monocytogenes* target (HEX™ channel) were evaluated with the BACGene evaluation sheet. Results of the manual and the automated workflow were compared and are shown as delta Cq (automated - manual) and % EPF (automated vs. manual). Cq values of the *Listeria monocytogenes* target were between ~29 – 39.

Item	Inoculation level CFU/mL	Spike Strain	Result manual workflow	Result BACBot	Delta Cq value ¹⁾ (BACBot – manual workflow) HEX™ channel	% EPF value ²⁾ (BACBot of manual workflow)
Sandwich	1.8 x 10 ⁵	<i>L. monocytogenes</i>	+	+	-0.17	100 %
Minced pork			+	+	-1.82	109 %
Ox cheek			+	+	-1.32	102 %
Goat cheese			+	+	-1.38	109 %
Mixed leafy greens			+	+	-2.60	105 %
Smoked salmon			+	+	-3.31	118 %
Rinse water			+	+	-0.27	90 %
Whole egg powder			+	+	-2.37	95 %
chicken wings			+	+	-3.02	109 %
Raw milk cream cheese			+	+	-1.53	83 %
Broccoli			+	+	-3.31	153 %
Frozen Cod fillet			+	+	-2.06	106 %
Wipe dust			+	+	-1.18	142 %
Swab soaked with Dey-Engley buffer			+	+	-1.46	101 %
Pizza			+	+	-0.81	102 %
Beef Trim			+	+	-0.04	98 %
Soft raw milk cheese (Camembert)			+	+	-0.54	92 %
Romana Salad			+	+	-0.66	89 %
Sprouts			+	+	-1.15	95 %
Surimi			+	+	-0.93	100 %
Sponge soaked with Letheen buffer			+	+	-0.77	106 %
Mean					-1.46	105 %
Standard Deviation					1.00	16 %

¹⁾ Negative Cq values: indicate that the automated workflow results in earlier Cq values compared to the manual workflow. Positive Cq values: indicate that manual workflow results in earlier Cq values compared to the automated workflow.

²⁾ EPF values <100: indicate that the manual workflow results in higher EPF compared to the automated workflow. EPF values >100: indicate that the automated workflow results in higher EPF values compared to the manual workflow.

4.5.3 BACGene *Listeria* spp.

For the BACGene *Listeria* spp. kit 42 different food matrices and environmental samples were analyzed (as blanks and spiked with a low and a high spike level) with the automated and the manual workflow and evaluated with the BACGene evaluation sheet. For the BACGene *Listeria* spp. kit artificially spiked matrices with *L. monocytogenes* as well as three *Listeria* species were analyzed.

The Cq and EPF values of the blanks and spiked matrices were comparable for the majority of the matrices (see Figure 5 and Figure 6).

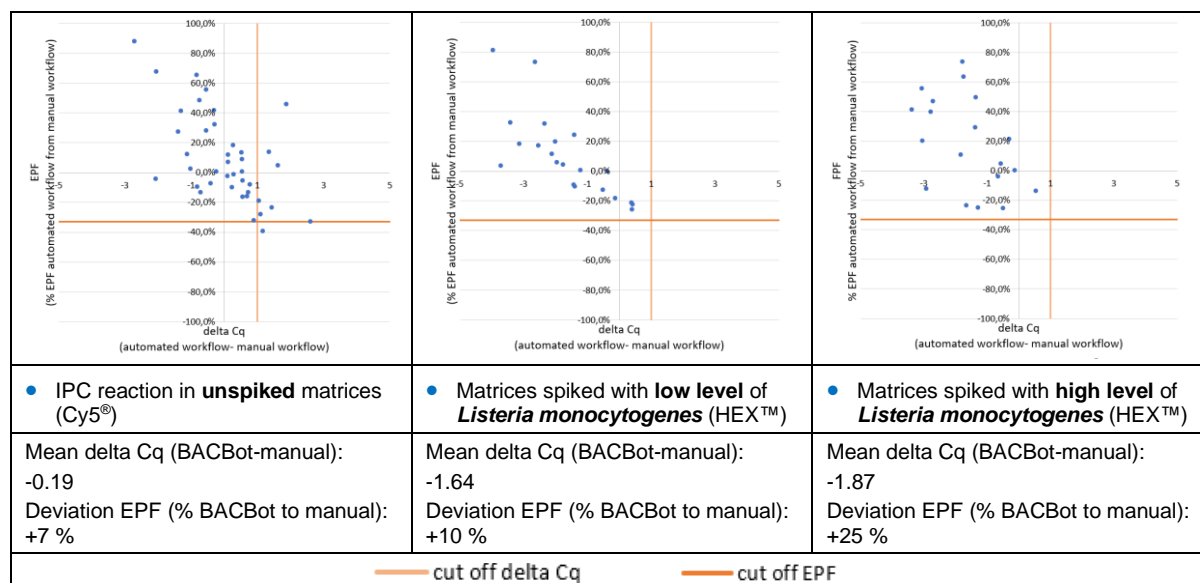
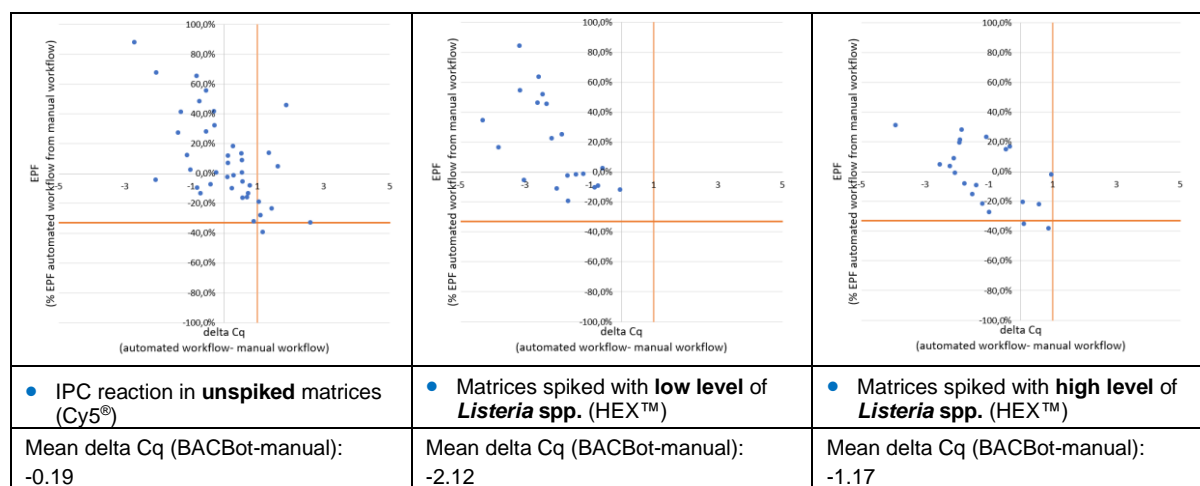


Figure 5 Overview of the results of the blank matrices (left) as well as for the *Listeria monocytogenes*-spiked (low (middle) and high level (right)) matrices run with the automated and manual workflow and tested with the BACGene *Listeria* spp. PCR kit. 42 relevant broad range of food matrices covering the certified categories were spiked with *Listeria monocytogenes*. The spiked samples as well as the corresponding blanks were run with the manual and the automated workflow of the lysis and PCR and analyzed with the BACGene *Listeria* spp. kit on the Bio-Rad CFX96 Touch™ Standard. Cq values and EPF values of the *Listeria* spp. target (HEX™ channel) and the internal positive control (Cy5® channel) were evaluated with the BACGene evaluation sheet. Results of the manual and the automated workflow were compared and are shown as delta Cq (automated - manual) and % EPF (automated vs. manual). The cut-offs for the delta Cq (horizontal) and % EPF (vertical) are shown as orange lines.



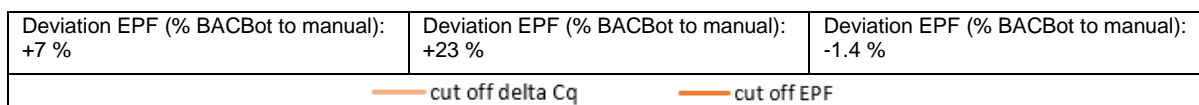
Deviation EPF (% BACBot to manual): +7 %	Deviation EPF (% BACBot to manual): +23 %	Deviation EPF (% BACBot to manual): -1.4 %
		

Figure 6 Overview of the results of the blank matrices (left) as well as for the *Listeria* spp.-spiked (low (middle) and high level (right)) matrices run with the automated and manual workflow and tested with the BACGene *Listeria* spp. PCR kit. 42 relevant broad range of food matrices covering the certified categories were spiked with three different *Listeria* strains (*L. innocua*, *L. ivanovii* and *L. welshimeri*). The spiked samples as well as the corresponding blanks were run with the manual and the automated workflow of the lysis and PCR and analyzed with the BACGene *Listeria* spp. kit on the Bio-Rad CFX96 Touch™ Standard. Cq values and EPF values of the *Listeria* spp. target (HEX™ channel) and the internal positive control (Cy5® channel) were evaluated with the BACGene evaluation sheet. Results of the manual and the automated workflow were compared and are shown as delta Cq (automated - manual) and % EPF (automated vs. manual). The cut-offs for the delta Cq (horizontal) and % EPF (vertical) are shown as orange lines.

4.5.3.1 Blanks

For one matrix (tuna) of the unspiked sample an inhibition in the manual workflow was observed, whereas no inhibition in the automated workflow was detected (see Table 19). However, in both workflows the IPC (Cy5® channel) of the spiked tuna samples with *L. innocua* (see Table 22) and *L. monocytogenes* (see Table 20), no inhibition was observed, indicating a failure in the manual extraction. For the blank matrix frozen spinach, a later Cq (+1.88, Cy5® channel) was observed for the automated workflow compared to the manual workflow. EPF was not affected. In addition, there are two wiped dust samples showing a delta Cq of +5.85 and +2.60 as well as a drop in the EPF values (-64 % and -33 %) indicating for a slightly higher inhibition of the automated workflow compared to the manual workflow (see Table 19).

However, when the two dust samples are spiked with *Listeria*, no inhibition effect could be observed in the IPC (Cy5® channel), see Table 20 to Table 23.

There are some blank matrices showing a slightly later Cq value in the IPC (Cy5® channel) for the automated workflow compared to the manual workflow. Matrices with a Cq value between 1 and 1.5 Cq values later are not listed in detail. All blank matrices which were analyzed in the BACGene *Listeria* spp. kit showing a slight Cq shift were also analyzed in the BACGene *Listeria* multiplex kit and did not show a Cq shift >1 in the automated workflow compared to the manual workflow (see chapter 4.5.4.1). This indicates that the automated workflow does not in general leads to later Cq values compared to the manual workflow.

Table 19 Results of the Blank matrices run with the automated and manual workflow and tested with the BACGene *Listeria* spp. PCR kit. 42 relevant broad range of food matrices covering the certified categories were tested. The lysis and PCR were performed with the manual and the automated workflow and analyzed with the BACGene *Listeria* spp. kit on the Bio-Rad CFX96 Touch™ Standard. Cq values and EPF values of the internal positive control (Cy5® channel) were evaluated with the BACGene evaluation sheet. Results of the manual and the automated workflow were compared and are shown as delta Cq (automated - manual) and % EPF (automated vs. manual). Cq values of the internal positive control were between ~29 – 36.

Item	Result manual workflow	Result BACBot	Delta Cq value ¹⁾ (BACBot – manual workflow)	% EPF value ²⁾ (BACBot of manual workflow)
	Cy5® channel			
Omelet	valid	valid	-0.55	128 %

Burger patties	valid	valid	0.72	87 %
Raw milk	valid	valid	-1.02	102 %
Frozen spinach	valid	valid	1.88	146 %
Frozen Cod fillet	valid	valid	-0.54	156 %
Tuna	Invalid	valid	n/a	n/a
Sponge soaked with NB buffer	valid	valid	-0.83	165 %
Frozen lasagna	valid	valid	-0.81	90 %
Minced turkey	valid	valid	-1.39	127 %
Raw milk cheese	valid	valid	0.79	92 %
Blue mold cheese	valid	valid	1.43	77 %
Cantaloupe melon	valid	valid	-2.07	96 %
Smoked trout	valid	valid	1.06	81 %
Rinse water	valid	valid	-1.30	141 %
Deli salad	valid	valid	-0.41	93 %
Cookies	valid	valid	-0.24	101 %
Frozen Carpaccio	valid	valid	0.53	101 %
Whole Milk Powder	valid	valid	-0.75	148 %
Cucumber	valid	valid	0.12	112 %
Shrimps	valid	valid	0.54	109 %
Wipe dust	valid	valid	2.60	67 %
Sandwich	valid	valid	-2.05	168 %
Minced pork	valid	valid	0.56	94 %
Ox cheek	valid	valid	1.17	61 %
Goat cheese	valid	valid	-0.72	87 %
Mixed leafy greens	valid	valid	1.10	72 %
Smoked salmon	valid	valid	1.36	114 %
Rinse water	valid	valid	0.89	68 %
Whole egg powder	valid	valid	0.13	107 %
chicken wings	valid	valid	0.69	84 %
Raw milk cream cheese	valid	valid	-2.72	188 %
Broccoli	valid	valid	0.24	90 %
Frozen Cod fillet	valid	valid	-1.11	112 %
Wipe dust	valid	valid	5.85	36 %
Swab soaked with Dey-Engley buffer	valid	valid	0.56	84 %
Pizza	valid	valid	0.52	113 %
Beef Trim	valid	valid	0.28	99 %
Soft raw milk cheese (Camembert)	valid	valid	1.63	105 %
Romana Salad	valid	valid	-0.30	132 %
Sprouts	valid	valid	-0.30	142 %
Surimi	valid	valid	0.10	97 %
Sponge soaked with Lethen buffer	valid	valid	0.27	118 %
Mean (invalid excluded)			0.19	107 %
Standard Deviation (invalid excluded)			1.44	31.7 %

¹⁾ Negative Cq values: indicate that the automated workflow results in earlier Cq values compared to the manual workflow. Positive Cq values: indicate that manual workflow results in earlier Cq values compared to the automated workflow.

²⁾EPF values <100: indicate that the manual workflow results in higher EPF compared to the automated workflow. EPF values >100: indicate that the automated workflow results in higher EPF values compared to the manual workflow.

4.5.3.2 Matrices spiked with *Listeria monocytogenes* and *Listeria spp.*

When analyzing the spiked samples, two matrices (raw milk cream cheese spiked with *L. monocytogenes* (see Table 21) and whole milk powder spiked with *L. welshimeri* (see Table 22)) had a significantly earlier Cq value (-5.9 and -4.3 respectively) in the *Listeria* specific channel (HEX™ for *L. monocytogenes* and HEX™ for *L. welshimeri*) in the automated workflow compared to the manual workflow which indicates for a failure in the manual workflow. This shows that the automated workflow is more robust, as human handling errors are excluded.

When analyzing the mean Cq values over the low and high spike level, the automated workflow shows earlier mean Cq values compared to the manual workflow (1.64 earlier for the low *Listeria monocytogenes* spike level (see Table 20) and 1.87 for the high *Listeria monocytogenes* spike level (see Table 21) and 2.12 earlier for the low *Listeria spp.* spike level (see Table 22) and 1.17 for the high *Listeria spp.* spike level (see Table 23) respectively). Mean EPF values are comparable for the automated workflow and the manual workflow.

Table 20 Results of the artificially *Listeria monocytogenes* - spiked (low level) matrices run with the automated and manual workflow and tested with the BACGene *Listeria spp.* PCR kit. 42 relevant broad range of food matrices covering the certified categories were spiked with *Listeria monocytogenes*. The lysis and PCR were performed with the manual and the automated workflow and analyzed with the BACGene *Listeria spp.* kit on the Bio-Rad CFX96 Touch™ Standard. Cq values and EPF values of the *Listeria spp.* target (HEX™ channel) were evaluated with the BACGene evaluation sheet. Results of the manual and the automated workflow were compared and are shown as delta Cq (automated - manual) and % EPF (automated vs. manual). Cq values of the *Listeria spp.* target were between ~27 – 38.

Item	Inoculation level CFU/mL	Spike Strain	Result manual workflow	Result BACBot	Delta Cq value ¹⁾ (BACBot – manual workflow) HEX™ channel	% EPF value ²⁾ (BACBot of manual workflow)
Omelet	7.1 x 10 ⁴	<i>L. monocytogenes</i>	+	+	-2.64	173 %
Burger patties			+	+	-1.40	90 %
Raw milk			+	+	-1.42	91 %
Frozen spinach			+	+	-2.12	112 %
Frozen Cod fillet			+	+	-2.34	132 %
Tuna			+	+	0.39	79 %
Sponge soaked with NB buffer			+	+	-1.75	104 %
Frozen lasagna			+	+	-3.13	118 %
Minced turkey			+	+	-2.53	117 %
Raw milk cheese			+	+	0.40	74 %
Blue mold cheese			+	+	-3.71	104 %
Cantaloupe melon			+	+	-2.00	120 %
Smoked trout			+	+	-0.51	87 %
Rinse water			+	+	-1.40	124 %
Deli salad			+	+	-1.95	106 %

Cookies	+	+	-3.41	132 %
Frozen Carpaccio	+	+	-0.12	82 %
Whole Milk Powder	+	+	-3.95	181 %
Cucumber	+	+	-0.36	100 %
Shrimps	+	+	-1.21	100 %
Wipe dust	+	+	0.43	77 %
Mean			-1.64	110 %
Standard Deviation			1.34	28 %

¹⁾ Negative Cq values: indicate that the automated workflow results in earlier Cq values compared to the manual workflow. Positive Cq values: indicate that manual workflow results in earlier Cq values compared to the automated workflow.

²⁾ EPF values <100: indicate that the manual workflow results in higher EPF compared to the automated workflow. EPF values >100: indicate that the automated workflow results in higher EPF values compared to the manual workflow.

Table 21 Results of the artificially *Listeria monocytogenes* - spiked (high level) matrices run with the automated and manual workflow and tested with the BACGene *Listeria spp.* PCR kit. 42 relevant broad range of food matrices covering the certified categories were spiked with *Listeria monocytogenes*. The lysis and PCR were performed with the manual and the automated workflow and analyzed with the BACGene *Listeria spp.* kit on the Bio-Rad CFX96 Touch™ Standard. Cq values and EPF values of the *Listeria spp.* target (HEX™ channel) were evaluated with the BACGene evaluation sheet. Results of the manual and the automated workflow were compared and are shown as delta Cq (automated - manual) and % EPF (automated vs. manual). Cq values of the *Listeria spp.* target were between ~26 – 36.

Item	Inoculation level CFU/mL	Spike Strain	Result manual workflow	Result BACBot	Delta Cq value ¹⁾ (BACBot – manual workflow) HEX™ channel	% EPF value ²⁾ (BACBot of manual workflow)
Sandwich	1.8 x 10 ⁵	<i>L. monocytogenes</i>	+	+	-0.31	121 %
Minced pork			+	+	-2.71	147 %
Ox cheek			+	+	-1.29	75 %
Goat cheese			+	+	-3.07	156 %
Mixed leafy greens			+	+	-3.38	141 %
Smoked salmon			+	+	0.54	86 %
Rinse water			+	+	-1.78	174 %
Whole egg powder			+	+	-1.66	76 %
chicken wings			+	+	-2.92	88 %
Raw milk cream cheese			+	+	-5.90	160 %
Broccoli			+	+	-3.04	120 %
Frozen Cod fillet			+	+	-2.77	140 %
Wipe dust			+	+	-0.57	105 %
Swab soaked with Dey-Engley buffer			+	+	-1.82	111 %
Pizza			+	+	-1.37	129 %
Beef Trim			+	+	-0.13	100 %
Soft raw milk cheese (Camembert)			+	+	-1.35	150 %
Romana Salad			+	+	-1.74	164 %
Sprouts			+	+	-2.80	213 %
Surimi			+	+	-0.50	75 %
Sponge soaked with Lethen buffer			+	+	-0.65	96 %

Mean	-1.87	125 %
Standard Deviation	1.45	37.3 %

¹⁾ Negative Cq values: indicate that the automated workflow results in earlier Cq values compared to the manual workflow. Positive Cq values: indicate that manual workflow results in earlier Cq values compared to the automated workflow.

²⁾ EPF values <100: indicate that the manual workflow results in higher EPF compared to the automated workflow. EPF values >100: indicate that the automated workflow results in higher EPF values compared to the manual workflow.

Table 22 Results of the artificially *Listeria* - spiked (low level) matrices run with the automated and manual workflow and tested with the BACGene *Listeria spp.* PCR kit. 42 relevant broad range of food matrices covering the certified categories were spiked with three different *Listeria* strains (*L. innocua*, *L. ivanovii* and *L. welshimeri*). The lysis and PCR were performed with the manual and the automated workflow and analyzed with the BACGene *Listeria spp.* kit on the Bio-Rad CFX96 Touch™ Standard. Cq values and EPF values of the *Listeria spp.* target (HEX™ channel) were evaluated with the BACGene evaluation sheet. Results of the manual and the automated workflow were compared and are shown as delta Cq (automated - manual) and % EPF (automated vs. manual). Cq values of the *Listeria spp.* target were between ~31 – 38.

Item	Inoculation level CFU/mL	Spike Strain	Result manual workflow	Result BACBot	Delta Cq value ¹⁾ (BACBot – manual workflow) HEX™ channel	% EPF value ²⁾ (BACBot of manual workflow)
Omelet	4.15 x 10 ⁴	<i>L. innocua</i>	+	+	-2.60	146 %
Burger patties			+	+	-1.84	125 %
Raw milk			+	+	-2.58	164 %
Frozen spinach			+	+	-2.45	152 %
Frozen Cod fillet			+	+	-3.16	184 %
Tuna			+	+	-2.96	201 %
Sponge soaked with NB buffer			+	+	-2.31	146 %
Frozen lasagna	8.5 x 10 ³	<i>L. ivanovii</i>	+	+	-2.16	122 %
Minced turkey			+	+	-0.58	102 %
Raw milk cheese			+	+	-0.03	88 %
Blue mold cheese			+	+	-1.18	99 %
Cantaloupe melon			+	+	-0.83	90 %
Smoked trout			+	+	-1.68	98 %
Rinse water			+	+	-0.73	91 %
Deli salad	8.4 x 10 ³	<i>L. welshimeri</i>	+	+	-1.65	81 %
Cookies			+	+	-2.01	89 %
Frozen Carpaccio			+	+	-3.02	95 %
Whole Milk Powder			+	+	-4.31	134 %
Cucumber			+	+	-1.41	98 %
Shrimps			+	+	-3.81	116 %
Wipe dust			+	+	-3.14	155 %
Mean					-2.12	123 %
Standard Deviation					1.10	35 %

¹⁾ Negative Cq values: indicate that the automated workflow results in earlier Cq values compared to the manual workflow. Positive Cq values: indicate that manual workflow results in earlier Cq values compared to the automated workflow.

²⁾ EPF values <100: indicate that the manual workflow results in higher EPF compared to the automated workflow. EPF values >100: indicate that the automated workflow results in higher EPF values compared to the manual workflow.

Table 23 Results of the artificially *Listeria* - spiked (high level) matrices run with the automated and manual workflow and tested with the BACGene *Listeria spp.* PCR kit. 42 relevant broad range of food matrices covering the certified categories were spiked with three different *Listeria* strains (*L. innocua*, *L. ivanovii* and *L. welshimeri*). The lysis and PCR were performed with the manual and the automated workflow and analyzed with the BACGene

Listeria spp. kit on the Bio-Rad CFX96 Touch™ Standard. Cq values and EPF values of the *Listeria spp.* target (HEX™ channel) were evaluated with the BACGene evaluation sheet. Results of the manual and the automated workflow were compared and are shown as delta Cq (automated - manual) and % EPF (automated vs. manual). Cq values of the *Listeria spp.* target were between ~28 – 36.

Item	Inoculation level CFU/mL	Spike Strain	Result manual workflow	Result BACBot	Delta Cq value ¹⁾ (BACBot – manual workflow) HEX™ channel	% EPF value ²⁾ (BACBot of manual workflow)
Sandwich	2.1 x 10 ⁵	<i>L. innocua</i>	+	+	-1.84	128 %
Minced pork			+	+	-1.92	119 %
Ox cheek			+	+	-1.76	92 %
Goat cheese			+	+	-0.47	115 %
Mixed leafy greens			+	+	-2.09	109 %
Smoked salmon			+	+	-0.34	117 %
Rinse water			+	+	-1.07	123 %
Whole egg powder	4.3 x 10 ⁴	<i>L. ivanovii ivanovii</i>	+	+	0.89	62 %
chicken wings			+	+	-2.54	105 %
Raw milk cream cheese			+	+	-2.22	104 %
Broccoli			+	+	-0.98	73 %
Frozen Cod fillet			+	+	-1.51	85 %
Wipe dust			+	+	0.10	65 %
Swab soaked with Dey-Engley buffer			+	+	-1.20	79 %
Pizza	4.2 x 10 ⁴	<i>L. welshimeri</i>	+	+	-1.90	121 %
Beef Trim			+	+	-1.40	91 %
Soft raw milk cheese (Camembert)			+	+	-2.06	99 %
Romana Salad			+	+	-4.05	174 %
Sprouts			+	+	-3.93	131 %
Surimi			+	+	0.97	98 %
Sponge soaked with Letheen buffer			+	+	0.57	78 %
Mean					-1.17	98.6 %
Standard Deviation					1.24	21.1 %

¹⁾ Negative Cq values: indicate that the automated workflow results in earlier Cq values compared to the manual workflow. Positive Cq values: indicate that manual workflow results in earlier Cq values compared to the automated workflow.

²⁾ EPF values <100: indicate that the manual workflow results in higher EPF compared to the automated workflow. EPF values >100: indicate that the automated workflow results in higher EPF values compared to the manual workflow.

4.5.4 BACGene *Listeria* Multiplex

For the BACGene *Listeria* Multiplex kit 42 different food matrices and environmental samples were analyzed (as blanks and spiked with a low and a high spike level) with the automated and the manual workflow and evaluated with the BACGene evaluation sheet. For the BACGene *Listeria* Multiplex kit artificially spiked matrices with *L. monocytogenes* as well as three *Listeria* species were analyzed.

The Cq- and EPF values of the blanks and spiked matrices were comparable for the majority of the matrices (see Figure 7 and Figure 8).

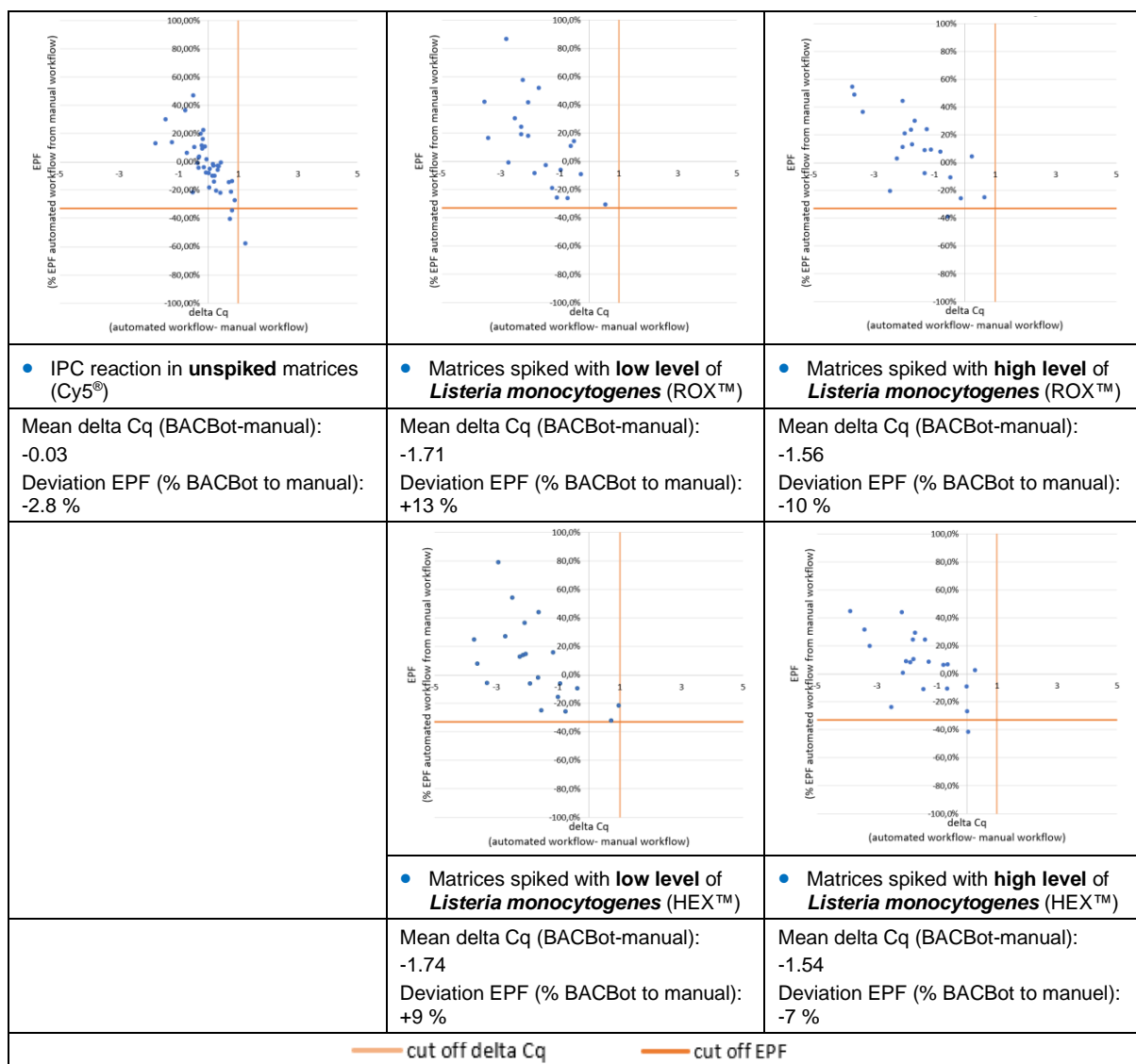


Figure 7 Overview of the results of the blank matrices (left) as well as for the *Listeria monocytogenes*-spiked (low (middle) and high level (right)) matrices run with the automated and manual workflow and tested with the BACGene *Listeria* Multiplex PCR kit. 42 relevant broad range of food matrices covering the certified categories were spiked with *Listeria monocytogenes*. The spiked samples as well as the corresponding blanks were run with the manual and the automated workflow of the lysis and PCR and analyzed with the BACGene *Listeria* Multiplex kit on the Bio-Rad CFX96 Touch™ Standard. Cq values and EPF values of the *Listeria monocytogenes* target (ROX™ channel), the *Listeria* spp. target (HEX™) and the internal positive control (Cy5® channel) were evaluated with the BACGene evaluation sheet. Results of the manual and the automated workflow were compared and are shown as delta Cq (automated - manual) and % EPF (automated vs. manual). The cut-offs for the delta Cq (horizontal) and % EPF (vertical) are shown as orange lines.

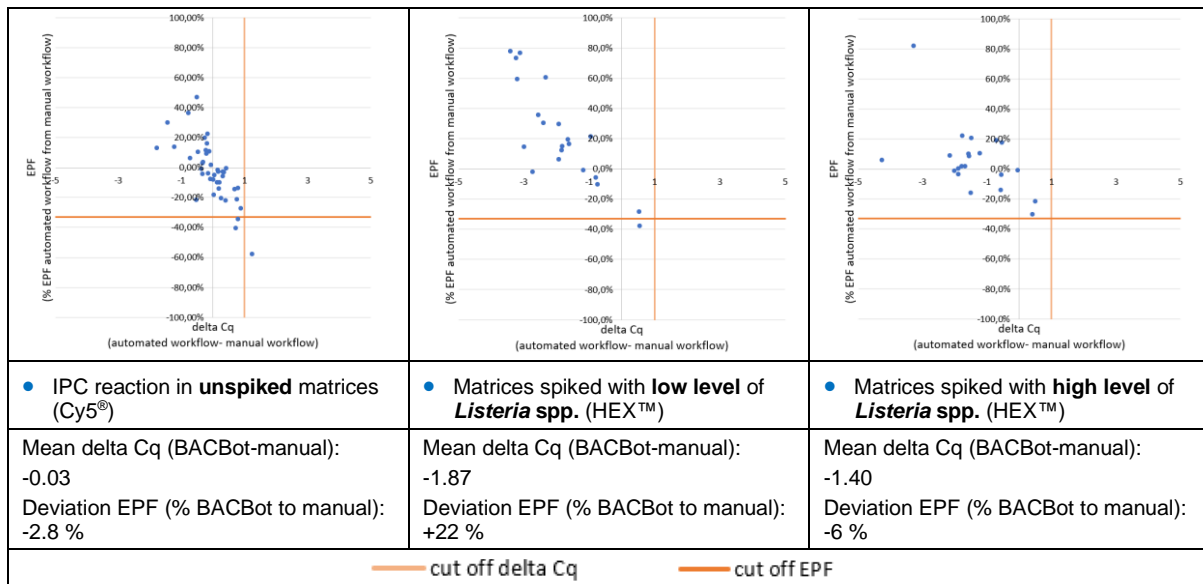


Figure 8 Overview of the results of the blank matrices (left) as well as for the *Listeria* spp.- spiked (low (middle) and high level (right)) matrices run with the automated and manual workflow and tested with the BACGene *Listeria* Multiplex PCR kit. 42 relevant broad range of food matrices covering the certified categories were spiked with three different *Listeria* strains (*L. innocua*, *L. ivanovii* and *L. welshimeri*). The spiked samples as well as the corresponding blanks were run with the manual and the automated workflow of the lysis and PCR and analyzed with the BACGene *Listeria* Multiplex kit on the Bio-Rad CFX96 Touch™ Standard. Cq values and EPF values of the *Listeria* spp. target (HEX™) and the internal positive control (Cy5® channel) were evaluated with the BACGene evaluation sheet. Results of the manual and the automated workflow were compared and are shown as delta Cq (automated - manual) and % EPF (automated vs. manual). The cut-offs for the delta Cq (horizontal) and % EPF (vertical) are shown as orange lines.

4.5.4.1 Blanks

For one matrix (burger patties) in the unspiked sample a lower EPF (Cy5® channel) in the manual workflow was observed, whereas the EPF in the automated workflow was not affected and also Cq values were similar (see Table 24). For the matrices minced pork, whole egg powder and chicken wings in the unspiked sample a slight drop in EPF values (Cy5® channel) compared to the manual extraction was observed (40 %, 58 %, 35 % respectively). The Cq values were comparable for the automated and the manual workflow.

However, the mean Cq value as well as mean EPF values over all blanks are comparable for the automated and manual workflow (see 24).

Table 24 Results of the Blank matrices run with the automated and manual workflow and tested with the BACGene *Listeria* Multiplex PCR kit. 42 relevant broad range of food matrices covering the certified categories were tested. The lysis and PCR were performed with the manual and the automated workflow and analyzed with the BACGene *Listeria* Multiplex kit on the Bio-Rad CFX96 Touch™ Standard. Cq values and EPF values of the internal positive control (Cy5® channel) were evaluated with the BACGene evaluation sheet. Results of the manual and the automated workflow were compared and are shown as delta Cq (automated - manual) and % EPF (automated vs. manual). Cq values of the internal positive control were between ~29 – 32.

Item	Result manual workflow	Result BACBot	Delta Cq value ¹⁾ (BACBot – manual workflow)	% EPF value ²⁾ (BACBot of manual workflow)
Cy5® channel				
Omelet	valid	valid	-0.78	136 %

Burger patties	valid	valid	0.89	723 %
Raw milk	valid	valid	-0.08	92 %
Frozen spinach	valid	valid	-0.17	122 %
Frozen Cod fillet	valid	valid	0.16	98 %
Tuna	valid	valid	0.18	86 %
Sponge soaked with NB buffer	valid	valid	-0.47	110 %
Frozen lasagna	valid	valid	-1.23	114 %
Minced turkey	valid	valid	-1.44	130 %
Raw milk cheese	valid	valid	-1.78	113 %
Blue mold cheese	valid	valid	0.14	90 %
Cantaloupe melon	valid	valid	-0.73	106 %
Smoked trout	valid	valid	0.68	86 %
Rinse water	valid	valid	0.41	78 %
Deli salad	valid	valid	-0.52	78 %
Cookies	valid	valid	0.77	79 %
Frozen Carpaccio	valid	valid	0.26	80 %
Whole Milk Powder	valid	valid	0.42	100 %
Cucumber	valid	valid	0.04	82 %
Shrimps	valid	valid	-0.15	96 %
Wipe dust	valid	valid	0.32	94 %
Sandwich	valid	valid	-0.33	103 %
Minced pork	valid	valid	0.72	60 %
Ox cheek	valid	valid	-0.12	111 %
Goat cheese	valid	valid	-0.51	147 %
Mixed leafy greens	valid	valid	-0.32	103 %
Smoked salmon	valid	valid	-0.25	120 %
Rinse water	valid	valid	-0.20	109 %
Whole egg powder	valid	valid	1.24	42 %
chicken wings	valid	valid	0.80	65 %
Raw milk cream cheese	valid	valid	0.21	90 %
Broccoli	valid	valid	0.80	86 %
Frozen Cod fillet	valid	valid	-0.06	102 %
Wipe dust	valid	valid	0.16	97 %
Swab soaked with Dey-Engley buffer	valid	valid	0.31	97 %
Pizza	valid	valid	0.05	95 %
Beef Trim	valid	valid	-0.32	96 %
Soft raw milk cheese (Camembert)	valid	valid	-0.36	99 %
Romana Salad	valid	valid	-0.18	116 %
Sprouts	valid	valid	0.36	97 %
Surimi	valid	valid	-0.23	111 %
Sponge soaked with Letheen buffer	valid	valid	0.01	92 %
Mean			-0.03	97.2 %
Standard Deviation			0.61	19.9 %

¹⁾ Negative Cq values: indicate that the automated workflow results in earlier Cq values compared to the manual workflow. Positive Cq values: indicate that manual workflow results in earlier Cq values compared to the automated workflow.

²⁾ EPF values <100: indicate that the manual workflow results in higher EPF compared to the automated workflow. EPF values >100: indicate that the automated workflow results in higher EPF values compared to the manual workflow.

4.5.4.2 Matrices spiked with *Listeria monocytogenes*

For the spiked samples with *L. monocytogenes* (low and high spike level) none of the samples was showing a Cq shift >1 in the automated workflow compared to the manual workflow (see Table 25 to Table 28). Only for the sample whole egg powder the EPF dropped in the automated workflow (41 %, HEX™ channel/ 39 %, ROX™) compared to the manual workflow, see Table 26 and Table 28. Whole egg powder is known as a difficult matrix. When analyzing the mean Cq values over the low and high spike level, the automated workflow shows earlier mean Cq values compared to the manual workflow (1.54 earlier for the low spike level and -1.71 for the high spike level). Mean EPF values are comparable for the automated workflow and the manual workflow.

Table 25 Results of the artificially *Listeria monocytogenes* - spiked (low level) matrices run with the automated and manual workflow and tested with the BACGene *Listeria* Multiplex PCR kit. 42 relevant broad range of food matrices covering the certified categories were spiked with *Listeria monocytogenes*. The lysis and PCR were performed with the manual and the automated workflow and analyzed with the BACGene *Listeria* Multiplex kit on the Bio-Rad CFX96 Touch™ Standard. Cq values and EPF values of the *Listeria spp.* target (HEX™ channel) were evaluated with the BACGene evaluation sheet. Results of the manual and the automated workflow were compared and are shown as delta Cq (automated - manual) and % EPF (automated vs. manual). Cq values of the *Listeria spp.* target were between ~26 – 36.

Item	Inoculation level CFU/mL	Spike Strain	Result manual workflow	Result BACBot	Delta Cq value ¹⁾ (BACBot – manual workflow) HEX™ channel	% EPF value ²⁾ (BACBot of manual workflow)
Omelet	7.1 x 10 ⁴	<i>L. monocytogenes</i>	+	+	-2.49	154 %
Burger patties			+	+	-1.62	144 %
Raw milk			+	+	-1.16	116 %
Frozen spinach			+	+	-2.93	179 %
Frozen Cod fillet			+	+	-2.13	114 %
Tuna			+	+	-2.23	113 %
Sponge soaked with NB buffer			+	+	-2.08	136 %
Frozen lasagna			+	+	-2.71	127 %
Minced turkey			+	+	-2.05	115 %
Raw milk cheese			+	+	-3.72	125 %
Blue mold cheese			+	+	-3.30	94 %
Cantaloupe melon			+	+	-1.65	98 %
Smoked trout			+	+	-0.93	94 %
Rinse water			+	+	-0.76	74 %
Deli salad			+	+	-1.53	75 %
Cookies			+	+	-1.90	94 %
Frozen Carpaccio			+	+	-3.62	108 %
Whole Milk Powder			+	+	0.72	68 %
Cucumber			+	+	-0.36	90 %
Shrimps			+	+	-0.99	84 %
Wipe dust			+	+	0.98	78 %
Mean					-1.74	109 %
Standard Deviation					1.25	28.6 %

¹⁾ Negative Cq values: indicate that the automated workflow results in earlier Cq values compared to the manual workflow. Positive Cq values: indicate that manual workflow results in earlier Cq values compared to the automated workflow.

²⁾ EPF values <100: indicate that the manual workflow results in higher EPF compared to the automated workflow. EPF values >100: indicate that the automated workflow results in higher EPF values compared to the manual workflow.

Table 26 Results of the artificially *Listeria monocytogenes* - spiked (high level) matrices run with the automated and manual workflow and tested with the BACGene *Listeria* Multiplex PCR kit. 42 relevant broad range of food matrices covering the certified categories were spiked with *Listeria monocytogenes*. The lysis and PCR were performed with the manual and the automated workflow and analyzed with the BACGene *Listeria* Multiplex kit on the Bio-Rad CFX96 Touch™ Standard. Cq values and EPF values of the *Listeria spp.* target (HEX™ channel) were evaluated with the BACGene evaluation sheet. Results of the manual and the automated workflow were compared and are shown as delta Cq (automated - manual) and % EPF (automated vs. manual). Cq values of the *Listeria spp.* target were between ~24 – 36.

Item	Inoculation level CFU/mL	Spike Strain	Result manual workflow	Result BACBot	Delta Cq value ¹⁾ (BACBot – manual workflow) HEX™ channel	% EPF value ²⁾ (BACBot of manual workflow)
Sandwich	1.8 x 10 ⁵	<i>L. monocytogenes</i>	+	+	0.27	102 %
Minced pork			+	+	-1.89	108 %
Ox cheek			+	+	-2.16	144 %
Goat cheese			+	+	-1.78	110 %
Mixed leafy greens			+	+	-3.42	131 %
Smoked salmon			+	+	-3.89	145 %
Rinse water			+	+	-1.73	129 %
Whole egg powder			+	+	0.05	59 %
chicken wings			+	+	-2.52	76 %
Raw milk cream cheese			+	+	-2.02	109 %
Broccoli			+	+	-3.24	120 %
Frozen Cod fillet			+	+	-2.14	101 %
Wipe dust			+	+	-0.01	91 %
Swab soaked with Dey-Engley buffer			+	+	-1.79	124 %
Pizza			+	+	-0.66	89 %
Beef Trim			+	+	-0.64	107 %
Soft raw milk cheese (Camembert)			+	+	-0.78	106 %
Romana Salad			+	+	-1.39	124 %
Sprouts			+	+	-1.45	89 %
Surimi			+	+	-1.27	109 %
Sponge soaked with Lethen buffer			+	+	0.01	73 %
Mean					-1.54	107 %
Standard Deviation					1.16	22.5 %

¹⁾ Negative Cq values: indicate that the automated workflow results in earlier Cq values compared to the manual workflow. Positive Cq values: indicate that manual workflow results in earlier Cq values compared to the automated workflow.

²⁾ EPF values <100: indicate that the manual workflow results in higher EPF compared to the automated workflow. EPF values >100: indicate that the automated workflow results in higher EPF values compared to the manual workflow.

Table 27 Results of the artificially *Listeria monocytogenes* - spiked (low level) matrices run with the automated and manual workflow and tested with the BACGene *Listeria* Multiplex PCR kit. 42 relevant broad range of food matrices covering the certified categories were spiked with *Listeria monocytogenes*. The lysis and PCR were performed with the manual and the automated workflow and analyzed with the BACGene *Listeria*

Multiplex kit on the Bio-Rad CFX96 Touch™ Standard. Cq values and EPF values of the *Listeria monocytogenes* target (ROX™ channel) were evaluated with the BACGene evaluation sheet. Results of the manual and the automated workflow were compared and are shown as delta Cq (automated - manual) and % EPF (automated vs. manual). Cq values of the *Listeria monocytogenes* target were between ~29 – 39.

Item	Inoculation level CFU/mL	Spike Strain	Result manual workflow	Result BACBot	Delta Cq value ¹⁾ (BACBot – manual workflow) ROX™ channel	% EPF value ²⁾ (BACBot of manual workflow)
Omelet	7.1 x 10 ⁴	<i>L. monocytogenes</i>	+	+	-2.25	158 %
Burger patties			+	+	-1.70	152 %
Raw milk			+	+	-0.63	111 %
Frozen spinach			+	+	-2.81	186 %
Frozen Cod fillet			+	+	-2.31	119 %
Tuna			+	+	-2.07	118 %
Sponge soaked with NB buffer			+	+	-2.06	142 %
Frozen lasagna			+	+	-2.51	131 %
Minced turkey			+	+	-2.30	124 %
Raw milk cheese			+	+	-3.54	142 %
Blue mold cheese			+	+	-2.73	99 %
Cantaloupe melon			+	+	-1.47	97 %
Smoked trout			+	+	-0.96	94 %
Rinse water			+	+	-0.72	74 %
Deli salad			+	+	-1.08	74 %
Cookies			+	+	-1.85	92 %
Frozen Carpaccio			+	+	-3.42	116 %
Whole Milk Powder			+	+	0.55	69 %
Cucumber			+	+	-0.28	91 %
Shrimps			+	+	-1.25	81 %
Wipe dust			+	+	-0.51	114 %
Mean					-1.71	113 %
Standard Deviation					1.06	30.6 %

¹⁾ Negative Cq values: indicate that the automated workflow results in earlier Cq values compared to the manual workflow. Positive Cq values: indicate that manual workflow results in earlier Cq values compared to the automated workflow.

²⁾ EPF values <100: indicate that the manual workflow results in higher EPF compared to the automated workflow. EPF values >100: indicate that the automated workflow results in higher EPF values compared to the manual workflow.

Table 28 Results of the artificially *Listeria monocytogenes* - spiked (high level) matrices run with the automated and manual workflow and tested with the BACGene *Listeria* Multiplex PCR kit. 42 relevant broad range of food matrices covering the certified categories were spiked with *Listeria monocytogenes*. The lysis and PCR were performed with the manual and the automated workflow and analyzed with the BACGene *Listeria* Multiplex kit on the Bio-Rad CFX96 Touch™ Standard. Cq values and EPF values of the *Listeria monocytogenes* target (ROX™ channel) were evaluated with the BACGene evaluation sheet. Results of the manual and the automated workflow were compared and are shown as delta Cq (automated - manual) and % EPF (automated vs. manual). Cq values of the *Listeria monocytogenes* target were between ~26 – 39.

Item	Inoculation level CFU/mL	Spike Strain	Result manual workflow	Result BACBot	Delta Cq value ¹⁾ (BACBot – manual workflow) ROX™ channel	% EPF value ²⁾ (BACBot of manual workflow)
Sandwich	1.8 x 10 ⁵	<i>L. monocytogenes</i>	+	+	0.24	104 %
Minced pork			+	+	-2.05	111 %

Ox cheek	+	+	-2.05	144 %
Goat cheese	+	+	-1.75	113 %
Mixed leafy greens	+	+	-3.36	136 %
Smoked salmon	+	+	-3.64	149 %
Rinse water	+	+	-1.65	130 %
Whole egg powder	+	+	-0.56	61 %
chicken wings	+	+	-2.47	79 %
Raw milk cream cheese	+	+	-1.99	121 %
Broccoli	+	+	-3.71	155 %
Frozen Cod fillet	+	+	-2.24	103 %
Wipe dust	+	+	0.65	75 %
Swab soaked with Dey-Engley buffer	+	+	-1.77	124 %
Pizza	+	+	-0.50	89 %
Beef Trim	+	+	-0.81	108 %
Soft raw milk cheese (Camembert)	+	+	-1.11	109 %
Romana Salad	+	+	-1.25	124 %
Sprouts	+	+	-1.33	92 %
Surimi	+	+	-1.32	109 %
Sponge soaked with Lethen buffer	+	+	-0.13	74 %
Mean			-1.56	110 %
Standard Deviation			1.17	25.4 %

¹⁾ Negative Cq values: indicate that the automated workflow results in earlier Cq values compared to the manual workflow. Positive Cq values: indicate that manual workflow results in earlier Cq values compared to the automated workflow.

²⁾ EPF values <100: indicate that the manual workflow results in higher EPF compared to the automated workflow. EPF values >100: indicate that the automated workflow results in higher EPF values compared to the manual workflow.

4.5.4.3 Matrices spiked with *Listeria* spp.

For the spiked samples with the three *Listeria* spp. strains (low and high spike level) none of the samples was showing a Cq shift >1 in the automated workflow compared to the manual workflow (see Table 29 and Table 30). Only for the sample whole milk powder the EPF dropped slightly in the automated workflow (38 %, HEX™ channel) compared to the manual workflow. The matrix raw milk cream cheese had a significantly earlier Cq value (HEX™ channel) with the automated workflow compared to the manual workflow, again indicating for a failure in the manual extraction, which did not occur in the automated workflow.

When analyzing the mean Cq values over the low and high spike level, the automated workflow shows earlier mean Cq values compared to the manual workflow (1.87 earlier for the low spike level and 1.41 for the high spike level). Mean EPF values are comparable for the automated workflow and the manual workflow.

Table 29 Results of the artificially *Listeria* - spiked (low level) matrices run with the automated and manual workflow and tested with the BACGene *Listeria* Multiplex PCR kit. 42 relevant broad range of food matrices covering the certified categories were spiked with three different *Listeria* strains (*L. innocua*, *L. ivanovii* and *L. welshimeri*). The lysis and PCR were performed with the manual and the automated workflow and analyzed with the BACGene *Listeria* Multiplex kit on the Bio-Rad CFX96 Touch™ Standard. Cq values and EPF values of the

Listeria spp. target (HEX™ channel) were evaluated with the BACGene evaluation sheet. Results of the manual and the automated workflow were compared and are shown as delta Cq (automated - manual) and % EPF (automated vs. manual). Cq values of the *Listeria spp.* target were between ~31 – 37.

Item	Inoculation level CFU/mL	Spike Strain	Result manual workflow	Result BACBot	Delta Cq value ¹⁾ (BACBot – manual workflow) HEX™ channel	% EPF value ²⁾ (BACBot of manual workflow)
Omelet	4.15 x 10 ⁴	<i>L. innocua</i>	+	+	-2.56	136 %
Burger patties			+	+	-1.65	119 %
Raw milk			+	+	-0.74	90 %
Frozen spinach			+	+	-3.12	177 %
Frozen Cod fillet			+	+	-3.22	173 %
Tuna			+	+	-2.39	131 %
Sponge soaked with NB buffer			+	+	-3.41	178 %
Frozen lasagna	8.5 x 10 ³	<i>L. ivanovii</i>	+	+	-1.61	117 %
Minced turkey			+	+	-2.33	161 %
Raw milk cheese			+	+	-0.95	121 %
Blue mold cheese			+	+	-0.79	94 %
Cantaloupe melon			+	+	-1.84	112 %
Smoked trout			+	+	-1.92	130 %
Rinse water			+	+	-1.92	106 %
Deli salad	8.4 x 10 ³	<i>L. welshimeri</i>	+	+	0.53	72 %
Cookies			+	+	-1.18	99 %
Frozen Carpaccio			+	+	-2.72	98 %
Whole Milk Powder			+	+	0.55	62 %
Cucumber			+	+	-1.83	115 %
Shrimps			+	+	-2.99	115 %
Wipe dust			+	+	-3.20	160 %
Mean					-1.87	122 %
Standard Deviation					1.14	32.8 %

¹⁾ Negative Cq values: indicate that the automated workflow results in earlier Cq values compared to the manual workflow. Positive Cq values: indicate that manual workflow results in earlier Cq values compared to the automated workflow.

²⁾ EPF values <100: indicate that the manual workflow results in higher EPF compared to the automated workflow. EPF values >100: indicate that the automated workflow results in higher EPF values compared to the manual workflow.

Table 30 Results of the artificially *Listeria* - spiked (high level) matrices run with the automated and manual workflow and tested with the BACGene *Listeria* Multiplex PCR kit. 42 relevant broad range of food matrices covering the certified categories were spiked with three different *Listeria* strains (*L. innocua*, *L. ivanovii* and *L. welshimeri*). The lysis and PCR were performed with the manual and the automated workflow and analyzed with the BACGene *Listeria* Multiplex kit on the Bio-Rad CFX96 Touch™ Standard. Cq values and EPF values of the *Listeria spp.* target (HEX™ channel) were evaluated with the BACGene evaluation sheet. Results of the manual and the automated workflow were compared and are shown as delta Cq (automated - manual) and % EPF (automated vs. manual). Cq values of the *Listeria spp.* target were between ~28 – 35.

Item	Inoculation level CFU/mL	Spike Strain	Result manual workflow	Result BACBot	Delta Cq value ¹⁾ (BACBot – manual workflow) HEX™ channel	% EPF value ²⁾ (BACBot of manual workflow)
Sandwich	2.1 x 10 ⁵	<i>L. innocua</i>	+	+	-1.57	110 %
Minced pork			+	+	-1.68	102 %
Ox cheek			+	+	-1.78	102 %

Goat cheese	4.3 x 10 ⁴	<i>L. ivanovii ivanovii</i>	+	+	-0.04	99 %
Mixed leafy greens			+	+	-1.77	122 %
Smoked salmon			+	+	-0.70	119 %
Rinse water			+	+	-1.56	109 %
Whole egg powder			+	+	-0.55	96 %
chicken wings			+	+	-1.89	96 %
Raw milk cream cheese			+	+	-4.25	106 %
Broccoli			+	+	-1.47	121 %
Frozen Cod fillet			+	+	-3.27	182 %
Wipe dust			+	+	0.50	78 %
Swab soaked with Dey-Engley buffer			+	+	-1.22	111 %
Pizza	4.2 x 10 ⁴	<i>L. welshimeri</i>	+	+	-0.58	86 %
Beef Trim			+	+	-1.49	84 %
Soft raw milk cheese (Camembert)			+	+	-2.01	99 %
Romana Salad			+	+	-1.89	100 %
Sprouts			+	+	-2.15	109 %
Surimi			+	+	-0.53	118 %
Sponge soaked with Lethen buffer			+	+	0.41	70 %
Mean					-1.40	106 %
Standard Deviation					1.12	22.3 %

¹⁾ Negative Cq values: indicate that the automated workflow results in earlier Cq values compared to the manual workflow. Positive Cq values: indicate that manual workflow results in earlier Cq values compared to the automated workflow.

²⁾ EPF values <100: indicate that the manual workflow results in higher EPF compared to the automated workflow. EPF values >100: indicate that the automated workflow results in higher EPF values compared to the manual workflow.

5 Annex

Table 31 Results of surface swab. All listed location of the OT-2 were swabbed and analyzed in the BACGene *Salmonella* spp. and the BACGene *Listeria* Multiplex kit.

Position	Result <i>Salmonella</i> spp.	Results <i>Listeria</i> spp.
Positive control	Positive	Positive
Negative control	Negative	Negative
Left wall of OT-2	Negative	Negative
Backside of OT-2	Negative	Negative
Right wall of OT-2	Negative	Negative
Frontside of OT-2	Negative	Negative
Ceiling of OT-2	Negative	Negative
Pipette P20 side	Negative	Negative
Pipette P200 side	Negative	Negative
Pipette holder	Negative	Negative
Heat module side	Negative	Negative
Heat module Top side	Negative	Negative
Trash side	Negative	Negative
Top left edge	Negative	Negative
Bottom right edge	Negative	Negative
Door	Negative	Negative
Location 4	Negative	Negative
Location 5	Negative	Negative
Location 6	Negative	Negative
Location 7	Negative	Negative
Location 8	Negative	Negative
Location 9	Negative	Negative
Location 10	Negative	Negative
Location 11	Negative	Negative

Table 32 Details samples spiked with different *Salmonella* strains and tested with the BACGene *Salmonella* spp. kit after manual and automated sample lysis.

Category		Type	Item	Aimed inoculation level in CFU/mL	Spike Strain
1	Meat and meat products	Fresh and frozen meats	Minced pork	5 x 10 ⁴	<i>S. Typhimurium</i>
			Beef Trim	1 x 10 ⁵	<i>S. Enteritidis</i>
			Sliced turkey	5 x 10 ⁴	<i>S. Heidelberg</i>
		Ready-to-reheat, Ready-to-eat, ready-to-cook, and ready-to-reheat meat preparations	Burger patties	1 x 10 ⁵	<i>S. Typhimurium</i>
			Marinated chicken	5 x 10 ⁴	<i>S. Enteritidis</i>
		Delicatessen (cured, fermented and cooked)	Frozen Carpaccio	1 x 10 ⁵	<i>S. Heidelberg</i>
			Ox cheek	5 x 10 ⁴	<i>S. Typhimurium</i>
2	Milk and dairy products	Raw milks, fermented milks and cheeses milk powders, pasteurized milk and cheeses	Raw milk cheese	1 x 10 ⁵	<i>S. Enteritidis</i>
			Raw milk	5 x 10 ⁴	<i>S. Heidelberg</i>

		Milk powders, pasteurized milk and cheeses	Whole Milk Powder	1 x 10 ⁵	<i>S. Typhimurium</i>
			Blue mold cheese	5 x 10 ⁴	<i>S. Enteritidis</i>
			Goat cheese	1 x 10 ⁵	<i>S. Heidelberg</i>
		Dairy desserts (Panna cotta, ice cream Tiramisu etc.)	Ice cream with walnut inclusions	5 x 10 ⁴	<i>S. Typhimurium</i>
			Ice cream with chocolate inclusions	1 x 10 ⁵	<i>S. Enteritidis</i>
3	Produce (vegetables, fruits and related products)	Leafy greens and sprouts (spinach lettuce, baby leaves, sprouts)	Sprouts	5 x 10 ⁴	<i>S. Heidelberg</i>
			Romana salad	1 x 10 ⁵	<i>S. Typhimurium</i>
			Frozen spinach	5 x 10 ⁴	<i>S. Enteritidis</i>
		RTE foods (composite deli-salads with fresh produce, fruit salads...)	ready to eat green mixed salad	1 x 10 ⁵	<i>S. Heidelberg</i>
			Cucumber salad	5 x 10 ⁴	<i>S. Typhimurium</i>
		Heat treated products Ready-to-cook and ready-to-reheat preparations	Fresh baby spinach	1 x 10 ⁵	<i>S. Enteritidis</i>
			RTRH vegetables	5 x 10 ⁴	<i>S. Heidelberg</i>
4	Eggs and egg products	Liquid eggs	Whole egg liquid (pasteurized)	1 x 10 ⁵	<i>S. Typhimurium</i>
			Whole liquid egg (fresh)	5 x 10 ⁴	<i>S. Enteritidis</i>
		Egg powders	Egg white powder	1 x 10 ⁵	<i>S. Heidelberg</i>
			Whole egg powder	5 x 10 ⁴	<i>S. Typhimurium</i>
		RTE foods (Desserts, Pastries such as custard-based products, Mayonnaises, ...)	Egg salad	1 x 10 ⁵	<i>S. Enteritidis</i>
			Egg custard	5 x 10 ⁴	<i>S. Heidelberg</i>
			Mayonnaise	1 x 10 ⁵	<i>S. Typhimurium</i>
5	Fish and seafood products	Fish and seafood	Frozen Cod fillet	5 x 10 ⁴	<i>S. Enteritidis</i>
			Frozen Cod fillet	1 x 10 ⁵	<i>S. Heidelberg</i>
			Shrimps	5 x 10 ⁴	<i>S. Typhimurium</i>
		Ready to eat, ready to cook, and ready to reheat fish preparations	Herring salad	1 x 10 ⁵	<i>S. Enteritidis</i>
			Surimi	5 x 10 ⁴	<i>S. Heidelberg</i>
		smoked and cured fishes	Smoked salmon	1 x 10 ⁵	<i>S. Typhimurium</i>
			Smoked trout	5 x 10 ⁴	<i>S. Enteritidis</i>
6	Environmental samples	Dusts and Residues	Wipe dust	1 x 10 ⁵	<i>S. Heidelberg</i>
			Wipe dust	5 x 10 ⁴	<i>S. Typhimurium</i>
		Cleaning and Process Waters	Rinse water	1 x 10 ⁵	<i>S. Enteritidis</i>
			Rinse water	5 x 10 ⁴	<i>S. Heidelberg</i>
		Surface samples	Sponge soaked with Lethen buffer	1 x 10 ⁵	<i>S. Typhimurium</i>
			Sponge soaked with NB buffer	5 x 10 ⁴	<i>S. Enteritidis</i>

Table 33 Details samples spiked with different *Listeria* spp. strains and tested with the BACGene *Listeria* spp. and BACGene *Listeria* Multiplex kits after manual and automated sample lysis.

Category		Type	Item	Aimed inoculation level in CFU/mL	Spike Strain
1	Composite foods/ Ready-to-eat and ready-to-reheat	Ready-to-eat	Deli salad	1 x 10 ⁴	<i>L. welshimeri</i>
			Sandwich	5 x 10 ⁴	<i>L. innocua</i>
		Ready-to-reheat	Frozen lasagne	1 x 10 ⁴	<i>L. ivanovii</i>
			Pizza	5 x 10 ⁴	<i>L. welshimeri</i>
			Omlett	1 x 10 ⁴	<i>L. innocua</i>
		Confectionaries, pastries and egg products	Whole egg powder	5 x 10 ⁴	<i>L. ivanovii</i>
			Cookies	1 x 10 ⁴	<i>L. welshimeri</i>
2	Meat products	Raw products (frozen or fresh)	Minced pork	5 x 10 ⁴	<i>L. innocua</i>
			Minced turkey	1 x 10 ⁴	<i>L. ivanovii</i>
			Beef Trim	5 x 10 ⁴	<i>L. welshimeri</i>
		Meat based products ready-to-reheat	Burger patties	1 x 10 ⁴	<i>L. innocua</i>
			chicken wings	5 x 10 ⁴	<i>L. ivanovii</i>
		Raw and cooked delicatessen	Frozen Carpaccio	1 x 10 ⁴	<i>L. welshimeri</i>
			Ox cheek	5 x 10 ⁴	<i>L. innocua</i>
3	Milk and dairy products	Raw milk cheeses	Raw milk cheese	1 x 10 ⁴	<i>L. ivanovii</i>
			Soft raw milk cheese (Camembert)	5 x 10 ⁴	<i>L. welshimeri</i>
		Other products based on raw milk	Raw milk	1 x 10 ⁴	<i>L. innocua</i>
			Raw milk cream cheese	5 x 10 ⁴	<i>L. ivanovii</i>
		Heat treated products	Whole Milk Powder	1 x 10 ⁴	<i>L. welshimeri</i>
			Goat cheese	5 x 10 ⁴	<i>L. innocua</i>
			Blue mold cheese	1 x 10 ⁴	<i>L. ivanovii</i>
4	Vegetables	Raw products (frozen or fresh)	Romana Salad	5 x 10 ⁴	<i>L. welshimeri</i>
			Frozen spinach	1 x 10 ⁴	<i>L. innocua</i>
		Pre-cooked vegetables, vegetables under modified atmosphere	Broccoli	5 x 10 ⁴	<i>L. ivanovii</i>
			Cucumber	1 x 10 ⁴	<i>L. welshimeri</i>
		Ready to eat	Mixed leafy greens	5 x 10 ⁴	<i>L. innocua</i>
			Cantaloupe melon	1 x 10 ⁴	<i>L. ivanovii</i>
			Sprouts	5 x 10 ⁴	<i>L. welshimeri</i>
5	Seafood and fishery products	Raw Products (frozen or fresh)	Frozen Cod fillet	1 x 10 ⁴	<i>L. innocua</i>
			Frozen Cod fillet	5 x 10 ⁴	<i>L. ivanovii</i>
			Shrimps	1 x 10 ⁴	<i>L. welshimeri</i>
		Cured and smoked	Smoked salmon	5 x 10 ⁴	<i>L. innocua</i>
			Smoked trout	1 x 10 ⁴	<i>L. ivanovii</i>
		Ready-to-eat and ready-to-reheat	Surimi	5 x 10 ⁴	<i>L. welshimeri</i>
			Tuna	1 x 10 ⁴	<i>L. innocua</i>
6	Environmental samples	Dusts and Residues	Wipe dust	5 x 10 ⁴	<i>L. ivanovii</i>
			Wipe dust	1 x 10 ⁴	<i>L. welshimeri</i>
		Cleaning and Process Waters	Rinse water	5 x 10 ⁴	<i>L. innocua</i>
			Rinse water	1 x 10 ⁴	<i>L. ivanovii</i>

		Surface samples	Sponge soaked with Letheen buffer	5 x 10 ⁴	<i>L. welshimeri</i>
			Sponge soaked with NB buffer	1 x 10 ⁴	<i>L. innocua</i>
			Swab soaked with Dey-Engley buffer	5 x 10 ⁴	<i>L. ivanovii</i>

Table 34 Details samples spiked with *Listeria monocytogenes* and tested with the BACGene *Listeria monocytogenes*, BACGene *Listeria* spp. and BACGene *Listeria* Multiplex kits after manual and automated sample lysis.

Category		Type	Item	Aimed inoculation level in CFU/mL	Spike Strain
1	Composite foods/ Ready-to-eat and ready-to-reheat	Ready-to-eat	Deli salad	1 x 10 ⁴	<i>L. monocytogenes</i>
			Sandwich	5 x 10 ⁴	<i>L. monocytogenes</i>
		Ready-to-reheat	Frozen lasagne	1 x 10 ⁴	<i>L. monocytogenes</i>
			Pizza	5 x 10 ⁴	<i>L. monocytogenes</i>
			Omelet	1 x 10 ⁴	<i>L. monocytogenes</i>
		Confectionaries, pastries and egg products	Whole egg powder	5 x 10 ⁴	<i>L. monocytogenes</i>
			Cookies	1 x 10 ⁴	<i>L. monocytogenes</i>
2	Meat products	Raw products (frozen or fresh)	Minced pork	5 x 10 ⁴	<i>L. monocytogenes</i>
			Minced turkey	1 x 10 ⁴	<i>L. monocytogenes</i>
			Beef Trim	5 x 10 ⁴	<i>L. monocytogenes</i>
		Meat based products ready-to-reheat	Burger patties	1 x 10 ⁴	<i>L. monocytogenes</i>
			chicken wings	5 x 10 ⁴	<i>L. monocytogenes</i>
		Raw and cooked delicatessen	Frozen Carpaccio	1 x 10 ⁴	<i>L. monocytogenes</i>
			Ox cheek	5 x 10 ⁴	<i>L. monocytogenes</i>
3	Milk and dairy products	Raw milk cheeses	Raw milk cheese	1 x 10 ⁴	<i>L. monocytogenes</i>
			Soft raw milk cheese (Camembert)	5 x 10 ⁴	<i>L. monocytogenes</i>
		Other products based on raw milk	Raw milk	1 x 10 ⁴	<i>L. monocytogenes</i>
			Raw milk cream cheese	5 x 10 ⁴	<i>L. monocytogenes</i>
		Heat treated products	Whole Milk Powder	1 x 10 ⁴	<i>L. monocytogenes</i>
			Goat cheese	5 x 10 ⁴	<i>L. monocytogenes</i>
			Blue mold cheese	1 x 10 ⁴	<i>L. monocytogenes</i>
4	Vegetables	Raw products (frozen or fresh)	Romana Salad	5 x 10 ⁴	<i>L. monocytogenes</i>
			Frozen spinach	1 x 10 ⁴	<i>L. monocytogenes</i>
		Pre-cooked vegetables, vegetables under modified atmosphere	Broccoli	5 x 10 ⁴	<i>L. monocytogenes</i>
			Cucumber	1 x 10 ⁴	<i>L. monocytogenes</i>
		Ready to eat	Mixed leafy greens	5 x 10 ⁴	<i>L. monocytogenes</i>
			Cantaloupe melon	1 x 10 ⁴	<i>L. monocytogenes</i>
			Sprouts	5 x 10 ⁴	<i>L. monocytogenes</i>
5	Seafood and fishery products	Raw Products (frozen or fresh)	Frozen Cod fillet	1 x 10 ⁴	<i>L. monocytogenes</i>
			Frozen Cod fillet	5 x 10 ⁴	<i>L. monocytogenes</i>
			Shrimps	1 x 10 ⁴	<i>L. monocytogenes</i>
		Cured and smoked	Smoked salmon	5 x 10 ⁴	<i>L. monocytogenes</i>

6			Smoked trout	1×10^4	<i>L. monocytogenes</i>
		Ready-to-eat and ready-to-reheat	Surimi	5×10^4	<i>L. monocytogenes</i>
			Tuna	1×10^4	<i>L. monocytogenes</i>
	Environmental samples	Dusts and Residues	Wipe dust	5×10^4	<i>L. monocytogenes</i>
			Wipe dust	1×10^4	<i>L. monocytogenes</i>
		Cleaning and Process Waters	Rinse water	5×10^4	<i>L. monocytogenes</i>
			Rinse water	1×10^4	<i>L. monocytogenes</i>
		Surface samples	Sponge soaked with Letheen buffer	5×10^4	<i>L. monocytogenes</i>
			Sponge soaked with NB buffer	1×10^4	<i>L. monocytogenes</i>
			Swab soaked with Dey-Engley buffer	5×10^4	<i>L. monocytogenes</i>

Gold Standard Diagnostics Freiburg GmbH
Engesserstraße 4
D-79108 Freiburg im Breisgau
GERMANY
Phone: +49 (0)160 967 399 98
Website: www.goldstandarddiagnostics.com

