Bacterial Stool Panel I PCR Kit

Multiplex real-time PCR assay for the direct, qualitative detection and differentiation of STEC, *Salmonella* spp., *Campylobacter* spp. and EIEC/*Shigella* spp. in human stool samples.

For Research Use Only. Not for Use in Diagnostic Procedures.

Catalog Number: 86-BS1HU-100
Size: 100 determinations
Version: 2018-06-08 - ALPCO 1.0

INTENDED USE

The Bacterial Stool Panel I is a multiplex real-time PCR assay for the direct, qualitative detection and differentiation of STEC, *Salmonella* spp., *Campylobacter* spp. and EIEC/*Shigella* spp. in human stool samples. For Research Use Only. Not for Use in Diagnostic Procedures.

PRINCIPLE OF THE TEST

The Bacterial Stool Panel I is a multiplex real-time PCR assay for the direct, qualitative detection and differentiation of STEC, *Salmonella* spp., *Campylobacter* spp. and EIEC/*Shigella* spp. in human stool samples.

After DNA isolation, amplification of gene fragments specific for STEC (stx1/stx2), *Salmonella* spp. (ttr), *Campylobacter* spp. (16s-rDNA) and EIEC/*Shigella* spp. (ipaH) occurs if present. The amplified targets are detected with hydrolysis probes, which are labeled at one end with a quencher and at the other end with a fluorescent reporter dye (fluorophore). In the presence of a target the probes hybridize to the amplicons. During the extension step the Taq-Polymerase breaks the reporter-quencher proximity. The reporter emits a fluorescent signal which is detected by the optical unit of a real-time PCR instrument. The fluorescence signal increases with the amount of formed amplicons. The Bacterial Stool Panel I assay contains an Internal Control DNA (ICD) as an internal control of sample preparation procedure and to determine possible PCR inhibition.

WARNINGS and PRECAUTIONS

- This test must only be carried out by trained laboratory personnel.
- Good laboratory practices must be used and the instructions for carrying out the test must be strictly followed.
- Do not mix reagents or coated microplate strips from kits with different lot numbers.
- Do not pipet samples or reagents by mouth. Avoid contact with bruised skin or mucosal membranes.
- During handling reagents or samples, wear appropriate safety clothing (appropriate gloves, lab coat, safety goggles) and wash your hands after finishing the test procedure.
- Do not smoke, eat, or drink in areas where samples or reagents are being used.
- Extraction, PCR preparation and the PCR run should be separated in different rooms to avoid cross-contaminations.
- Samples must be treated as potentially infectious as well as all reagents and materials being exposed to the samples and must be handled according to safety regulations.
- Do not use the kit after the expiration date.
- All reagents and materials used must be disposed properly after use. Please refer to the relevant regulations for disposal.

REAGENTS PROVIDED

Reagents provided in the kit are sufficient for 100 determinations.

Table 1. Reagents Provided

Kit Code	Reagent	Amount		Lid Color
1	Reaction Mix	2x	1050 µL	yellow
2	Taq-Polymerase	1x	80 µL	red
D	Internal Control DNA	2x	1700 µL	orange
N	No Template Control	1x	450 µL	white
P	Positive Control	1x	200 µL	blue

STORAGE INSTRUCTIONS

- Protect all reagents from light and store at -20°C. All reagents can be used until the expiration date. After expiry the quality guarantee is no longer valid.
- Carefully thaw reagents before use (e.g., in a refrigerator at 2 - 8°C).
- Reagents can sustain up to 5 freeze/thaw cycles without influencing the assay performance. After the initial thaw, separate into aliquots and freeze immediately.
- During PCR preparation, all reagents should be stored cold (2 - 8°C).

MATERIALS REQUIRED BUT NOT PROVIDED

The Bacterial Stool Panel I real-time PCR assay has been tested using manual nucleic acid extraction kits as well as the following extraction platforms and real-time PCR instruments. If using another extraction platform or real-time PCR instrument, refer to the settings for the tested instruments as a starting point and modify as necessary. Contact ALPCO technical support for more information.

Table 2. Extraction Platforms and PCR Instruments

Extraction platform	
Promega	Maxwell® RSC
Real-time PCR instrument	
Roche	LightCycler® 480II
Agilent Technologies	Mx3005P (with ATTO-Filter)

- Extraction platform if extraction not performed manually
- Real-time PCR instrument
- Real-time PCR consumables (plates, tubes, foil)
- Centrifuge with a rotor for the reaction vials
- Vortex mixer
- Pipettes (0.5 - 20 µL, 20 - 200 µL, 100 - 1000 µL)
- Filter tips
- Powder-free disposal gloves
- PCR Grade water (bioscience grade, nuclease-free)
- Color Compensation Kit IV (if running the LightCycler® 480II)

PREPARATION OF SAMPLES

For DNA isolation of human stool samples, use a commercially available nucleic acid extraction kit or nucleic acid extraction platform (e.g., Maxwell® RSC; Promega). Extract viral nucleic acid according to the manufacturer's instructions. It is recommended to dilute the stool sample 1:3 with PCR Grade water before extraction. Vortex intensely and centrifuge at 1000 x g for 30 seconds. Use the volume of supernatant indicated in the manufacturer's instructions.

The Bacterial Stool Panel I PCR assay contains an Internal Control DNA that detects PCR inhibition, monitors reagent integrity, and confirms that nucleic acid extraction was sufficient. The Internal Control DNA can either be used as PCR inhibition control or as both an extraction control for the sample preparation procedure and a PCR inhibition control.

If the Internal Control DNA is used only as a PCR inhibition control, 1 µl of the Internal Control DNA should be added to the Master Mix (see Table 4). If the Internal Control DNA is used as an extraction control for the sample preparation procedure and as a PCR inhibition control, 20 µl of the Internal Control DNA must be added during the extraction procedure. The Internal Control DNA should always be added to the sample-lysis buffer mixture and must not be added directly to the sample.

MASTER MIX PREPARATION

Calculate the total number of PCR reactions (sample and control reactions) needed. One positive control and one negative control must be included in each assay run. It is recommended to add a 10% volume overage to compensate for imprecision in pipetting (see Tables 3 and 4). Thaw, mix gently, and briefly centrifuge the Reaction Mix, Taq-Polymerase, Positive Control, No Template Control, and the Internal Control DNA before using. Keep reagents appropriately cold during working step (2 - 8°C).

Table 3. Calculation and pipetting example for 10 reactions of the Master Mix (ICD as extraction and PCR inhibition control)

Kit code	Master Mix Components	Volume per Reaction	10 reactions (+10% overage)
1	Reaction Mix	19.3 µL	212.3 µL
2	Taq-Polymerase	0.7 µL	7.7 µL
	Total Volume	20 µL	220 µL

Table 4. Calculation and pipetting example for 10 reactions of the Master Mix (ICD only as PCR inhibition control)

Kit code	Master Mix Components	Volume per Reaction	10 reactions (+10% overage)
1	Reaction Mix	19.3 µL	212.3 µL
2	Taq-Polymerase	0.7 µL	7.7 µL
D	Internal Control DNA	1.0 µL	11 µL
	Total Volume	21 µL	231 µL

Mix the components of the Master Mix gently and briefly spin down.

PREPARATION OF THE PCR MIX

1. Pipette 20 µL of the Master Mix into all reaction wells (tubes or plate wells).

2. Add 5 µL of the No Template Control to the negative control well.

Note: If the Internal Control DNA is being used as extraction control for the sample preparation procedure and as PCR inhibition control, add 1 µl of the Internal Control DNA to the negative control well.

3. Add 5 µL of DNA-Extract to corresponding sample wells.

4. Add 5 µL of Positive Control to the positive control well.

Note: If the Internal Control DNA is used as extraction control for the sample preparation procedure and as PCR inhibition control, add 1 µl of the Internal Control DNA to the positive control well.

5. Cover tubes or plate. Spin down and place in the real-time PCR instrument. The PCR reaction should be started according to the PCR instrument set-up (see Tables 5 and 6).

PCR INSTRUMENT SET-UP

Table 5. DNA Real-time PCR Profile

	LightCycler® 480II	**Mx3005P**
Initial Denaturation	1 min, 95°C	1 min, 95°C
Cycles	45 cycles	45 cycles
PCR Denaturation PCR Annealing / Extension	10 sec, 95°C 15 sec, 60°C	15 sec, 95°C 30 sec, 60°C
Temperature Transition Rate / Ramp Rate	Maximum	Maximum

Note: Annealing and Extension occur in the same step

Table 6. Universal Real-time PCR Profile

	LightCycler® 480II	**Mx3005P**
Reverse Transcription	10 min, 58°C	10 min, 58°C
Initial Denaturation	1 min, 95°C	1 min, 95°C
Cycles	45 cycles	45 cycles
PCR Denaturation PCR Annealing / Extension	10 sec, 95°C 15 sec, 60°C	15 sec, 95°C 30 sec, 60°C
Temperature Transition Rate / Ramp Rate	Maximum	Maximum

Note: Annealing and Extension occur in the same step
Note: The universal real-time PCR profile should only be used for DNA assays if ALPCO DNA and RNA real-time PCR assays are combined in one run.

DETECTION CHANNEL SET-UP

Table 7. Selection of Appropriate Detection Channels

Real-time PCR Instrument	Detection	Detection Channel	Note
Roche LightCycler® 480II	STEC	440/488	Color compensation kit IV is required.
	Salmonella spp.	465/510	
	ICD	533/580	
	EIEC/*Shigella* spp.	533/610	
	Campylobacter spp.	618/660	
Agilent Techn. Mx3005P	STEC	ATTO	Check that reference dye is none.
	Salmonella spp.	FAM	
	ICD	HEX	
	EIEC/*Shigella* spp.	ROX	
	Campylobacter spp.	Cy5	

QUALITY CONTROL

Sample analysis is done by the software of the real-time PCR instrument used, according to the manufacturer's instructions. Negative control and positive control must show correct results (see Table 8, Fig. 1, Fig. 2, Fig. 3, Fig. 4) to determine a valid run. The Positive Control for STEC, *Salmonella* spp., EIEC/*Shigella* spp. and *Campylobacter* spp. has a concentration of 10^3 copies/µl. In each PCR run it is used in a total amount of 5 x 10^3 copies, respectively.

Table 8. For a valid run, the following conditions must be met:

Sample	Assay result	ICD Ct	Target Ct
Positive Control	Positive	NA *1	See Quality Assurance Certificate
Negative Control	Negative	Ct > 20	0

*1 *No Ct value is required for the ICD to make a positive call for the Positive Control*

If the positive control is not positive within the specified Ct range but the negative control is valid, prepare all new reactions including the controls.

If the negative control is not negative but the positive control is valid prepare all new reactions including the controls.

If the required criteria are not met, the following items should be checked before repeating the test:

- Expiry of the used reagents
- Functionality of the used instrumentation
- Correct performance of the test procedure

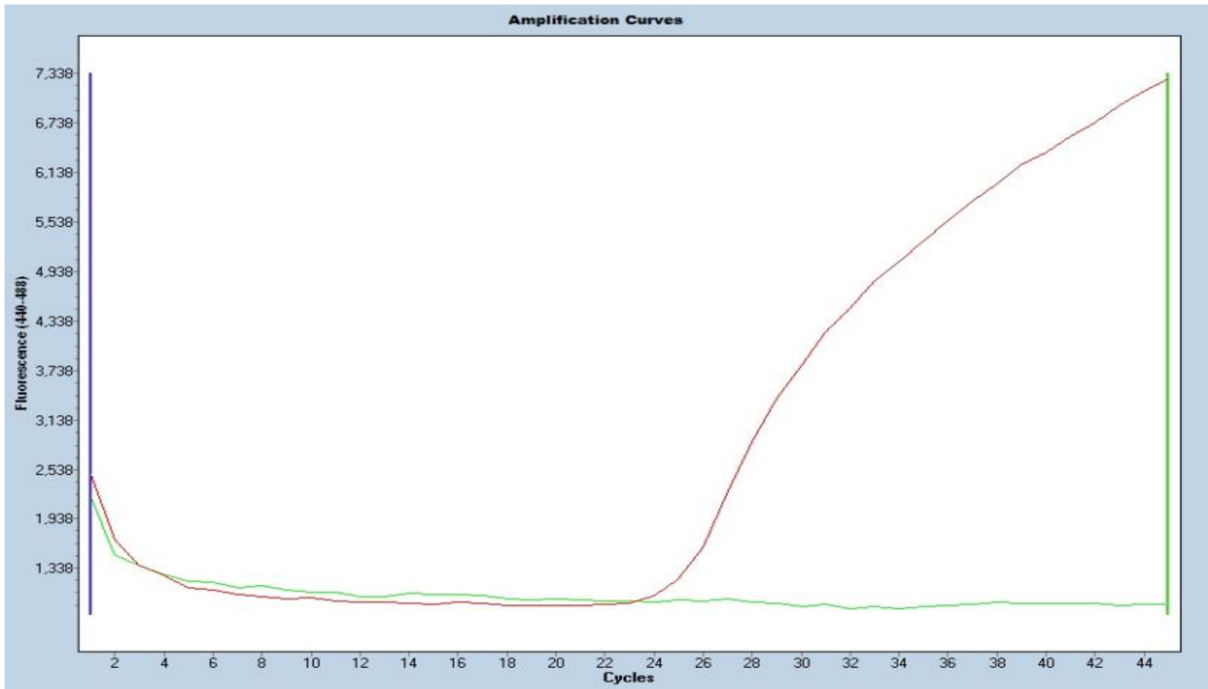

Fig.1: Correct run of the positive and no template control (STEC) on the LightCycler® 480II

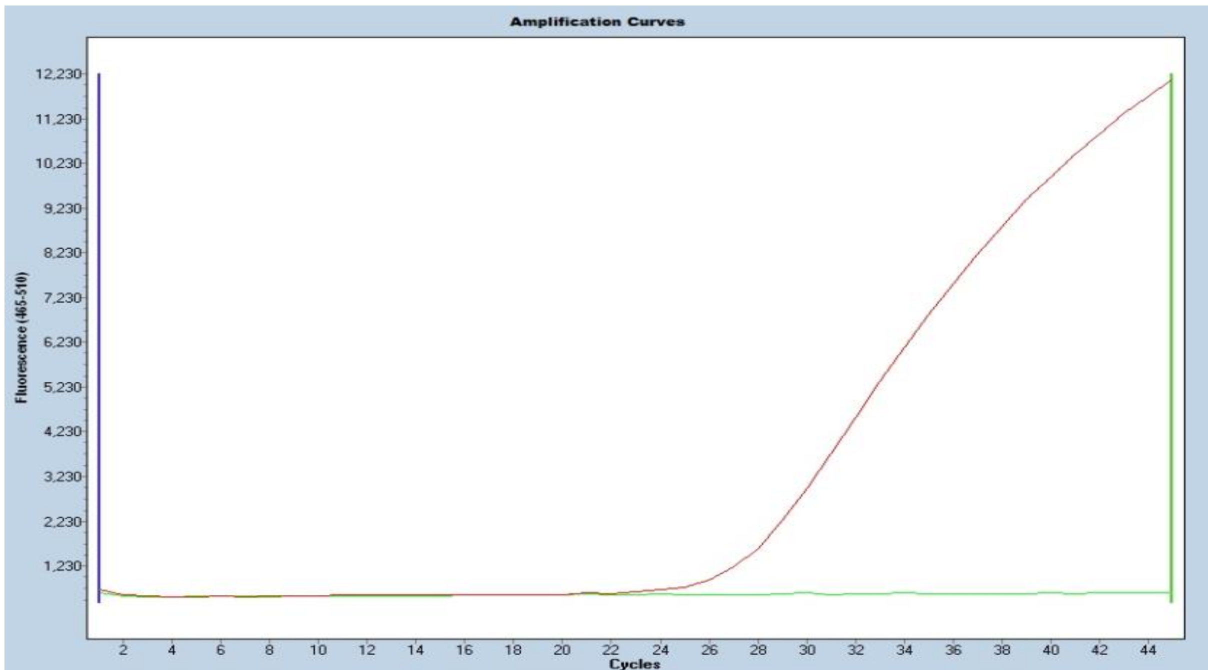

Fig.2: Correct run of the positive and no template control (*Salmonella* spp.) on the LightCycler® 480II

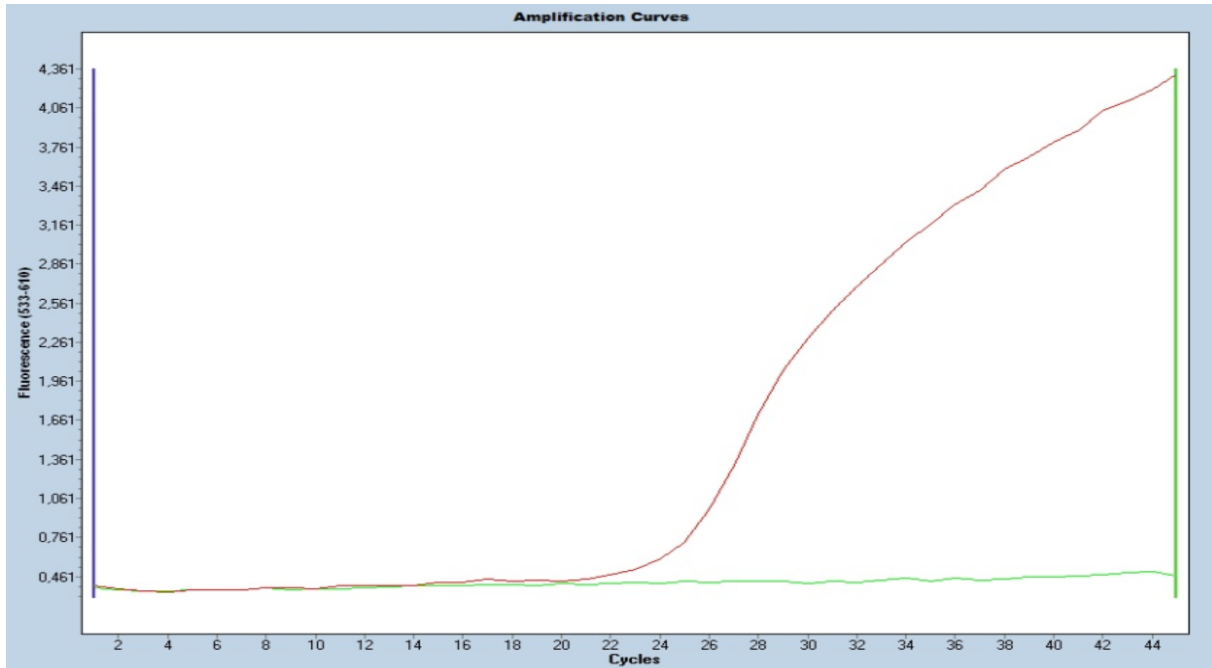

Fig. 3: Correct run of the positive and no template control (EIEC/*Shigella spp.*) on the LightCycler® 480II

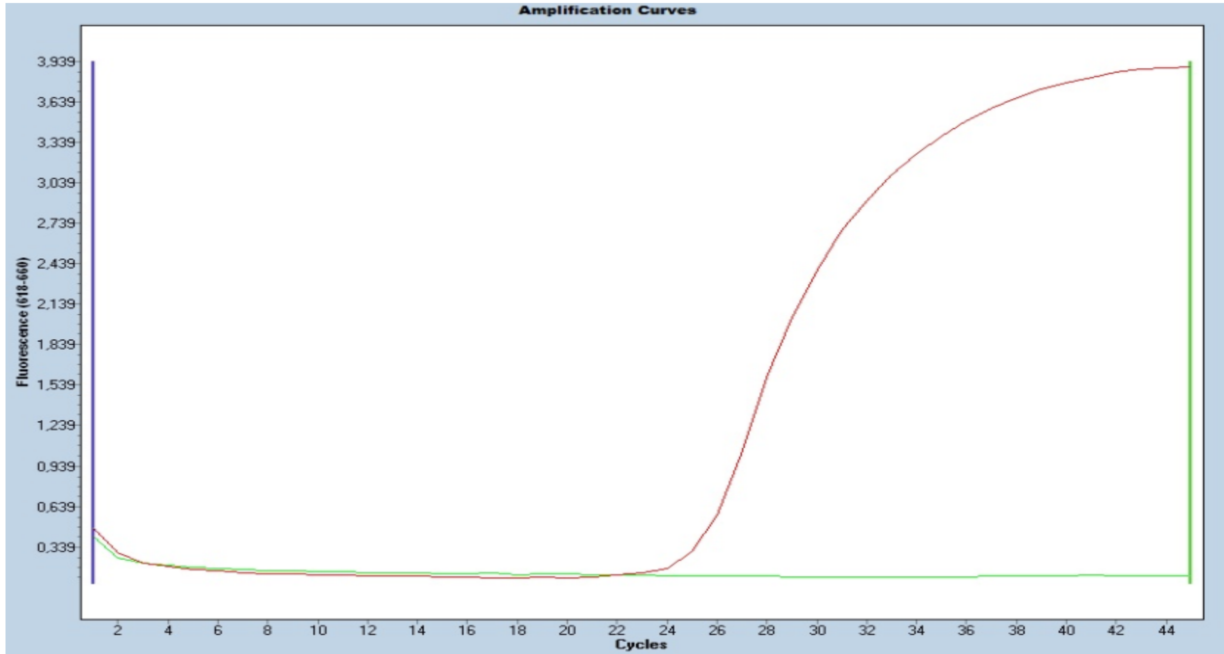

Fig. 4: Correct run of the positive and no template control (*Campylobacter* spp.) on the LightCycler® 480II

Table 9: Sample interpretation

Target Genes					
STEC	***Salmonella* spp.**	**EIEC/*Shigella* spp.**	***Campylobacter* spp.**	**ICD**	**Result**
positive	negative	negative	negative	positive/negative	STEC detected
negative	positive	negative	negative	positive/negative	*Salmonella* spp. detected
negative	negative	positive	negative	positive/negative	EIEC/*Shigella* spp. detected
negative	negative	negative	positive	positive/negative	*Campylobacter* spp. detected
positive	positive	negative	negative	positive/negative	STEC, *Salmonella spp.* detected
positive	negative	positive	negative	positive/negative	STEC, EIEC/*Shigella* spp. Detected
positive	negative	negative	positive	positive/negative	STEC, *Campylobacter* spp. Detected
positive	positive	positive	negative	positive/negative	STEC, *Salmonella* spp., EIEC/*Shigella* spp. detected
positive	negative	positive	positive	positive/negative	STEC, EIEC/*Shigella* spp., Campylobacter spp. detected
positive	positive	negative	positive	positive/negative	STEC, *Salmonella* spp., *Campylobacter* spp. detected
negative	positive	positive	negative	positive/negative	*Salmonella* spp., EIEC/*Shigella* spp. Detected
negative	positive	negative	positive	positive/negative	*Salmonella* spp., *Campylobacter* spp. Detected
negative	positive	positive	positive	positive/negative	*Salmonella* spp., EIEC/*Shigella* spp., *Campylobacter* spp. detected
negative	negative	positive	positive	positive/negative	EIEC/*Shigella* spp., *Campylobacter* spp. detected
positive	positive	positive	positive	positive/negative	STEC, *Salmonella* spp., EIEC/*Shigella* spp., *Campylobacter* spp. Detected
negative	negative	negative	negative	positive	Target genes not detected
negative	negative	negative	negative	negative	Invalid

A sample is evaluated positive if the sample DNA and the Internal Control DNA show an amplification signal in the detection system. A sample is also evaluated positive if the sample DNA shows an amplification signal but none for the Internal Control DNA in the detection system. The detection of the Internal Control DNA is not necessary because high concentrations of the amplicon can cause a weak or absent signal of the Internal Control DNA.

A sample is evaluated negative if the sample DNA shows no amplification signal, but an amplification signal for the Internal Control DNA in the detection system. An inhibition of the PCR reaction can be excluded by the detection of the Internal Control DNA.

A sample is evaluated invalid if the sample DNA and Internal Control DNA show no amplification signal in the detection system. The sample contains a PCR inhibitor, or a failure occurred in the extraction procedure. The extracted sample needs to be further diluted with PCR water (1:10) and reamplified, or the isolation and purification of the sample must be improved.

LIMITATIONS OF THE METHOD

1. This assay is For Research Use Only. It is not for use in diagnostic procedures.
2. The Bacterial Stool Panel I multiplex real-time PCR assay is only validated for stool samples.
3. Inappropriate sample collection, transport, storage and processing or a viral load in the sample below the analytical sensitivity can result in false negative results.
4. The presence of PCR inhibitors may cause invalid results.
5. Mutations or polymorphisms in primer or probe binding regions may affect detection of new variants resulting in a false negative result with the Bacterial Stool Panel I PCR assay.
6. As with all PCR based tests, extremely low levels of target below the limit of detection (LoD) may be detected, but results may not be reproducible.
7. A positive test result does not necessarily indicate the presence of viable organisms. However, a positive result is indicative for the presence of the target genes (stx1/2, ttr, 16s-rDNA, ipaH).

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

The Bacterial Stool Panel I multiplex real-time PCR assay has a detection limit of ≥ 10 DNA copies per reaction for STEC, *Salmonella* spp., EIEC/*Shigella* spp. and *Campylobacter* spp.

The following figures 5, 6, 7 and 8 show dilution series of STEC, *Salmonella* spp., EIEC/*Shigella* spp. and *Campylobacter* spp. (each 10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II.

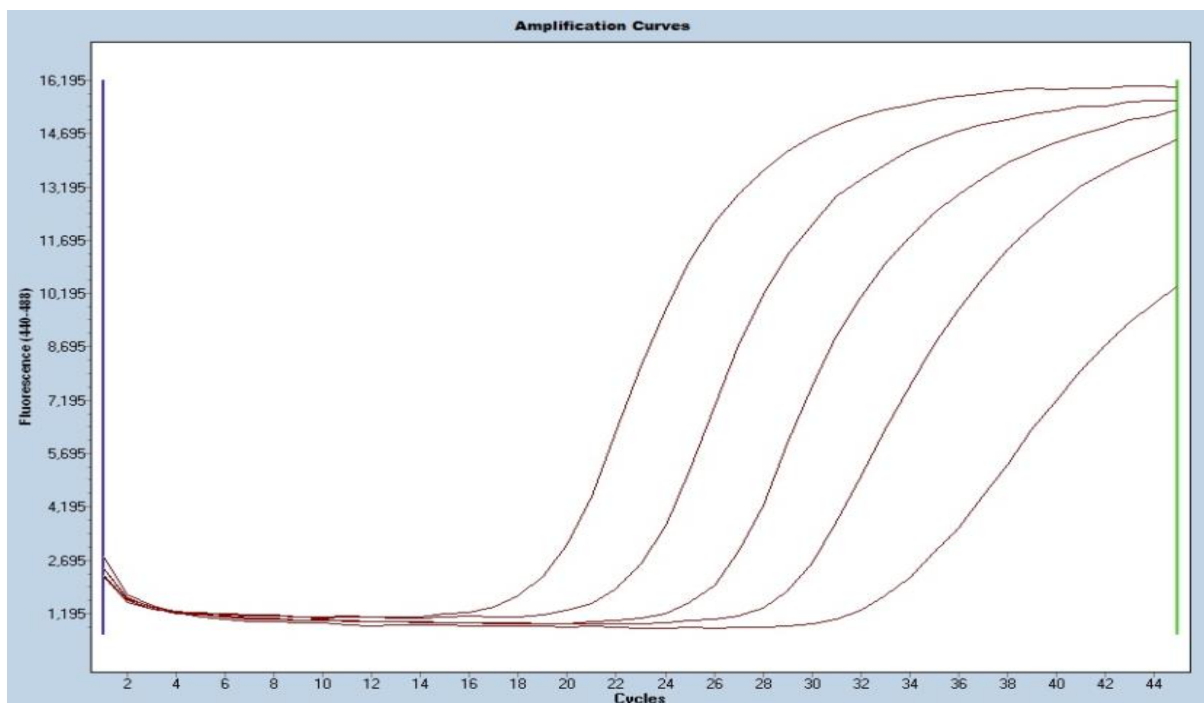

Fig. 5: Dilution series STEC (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

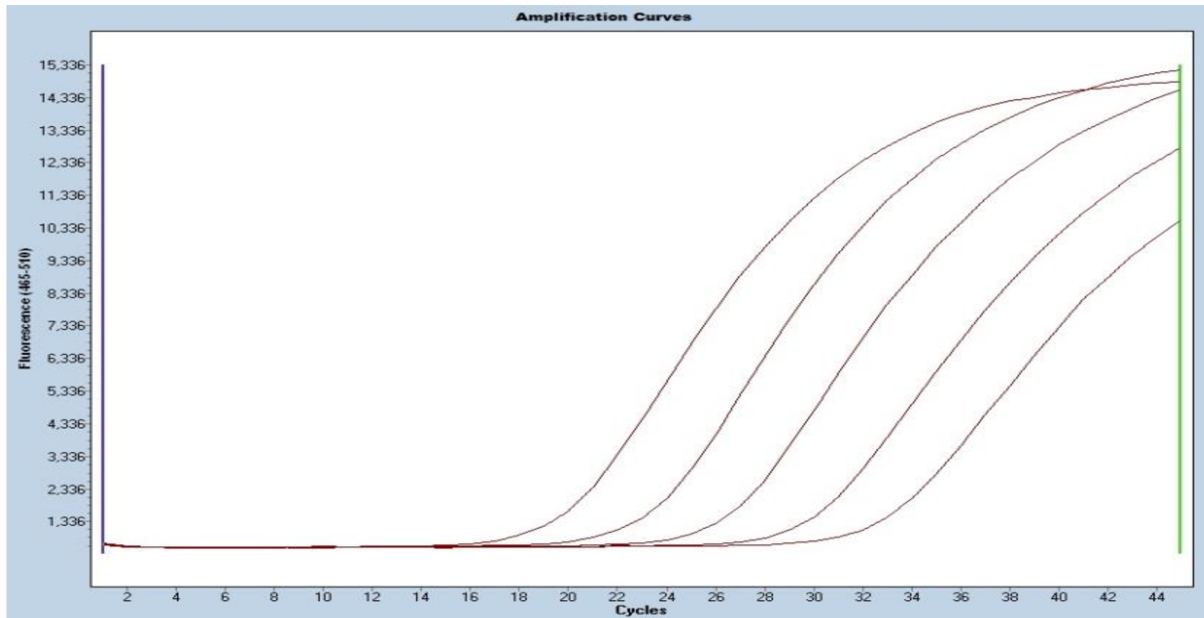

Fig. 6: Dilution series *Salmonella* spp. (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

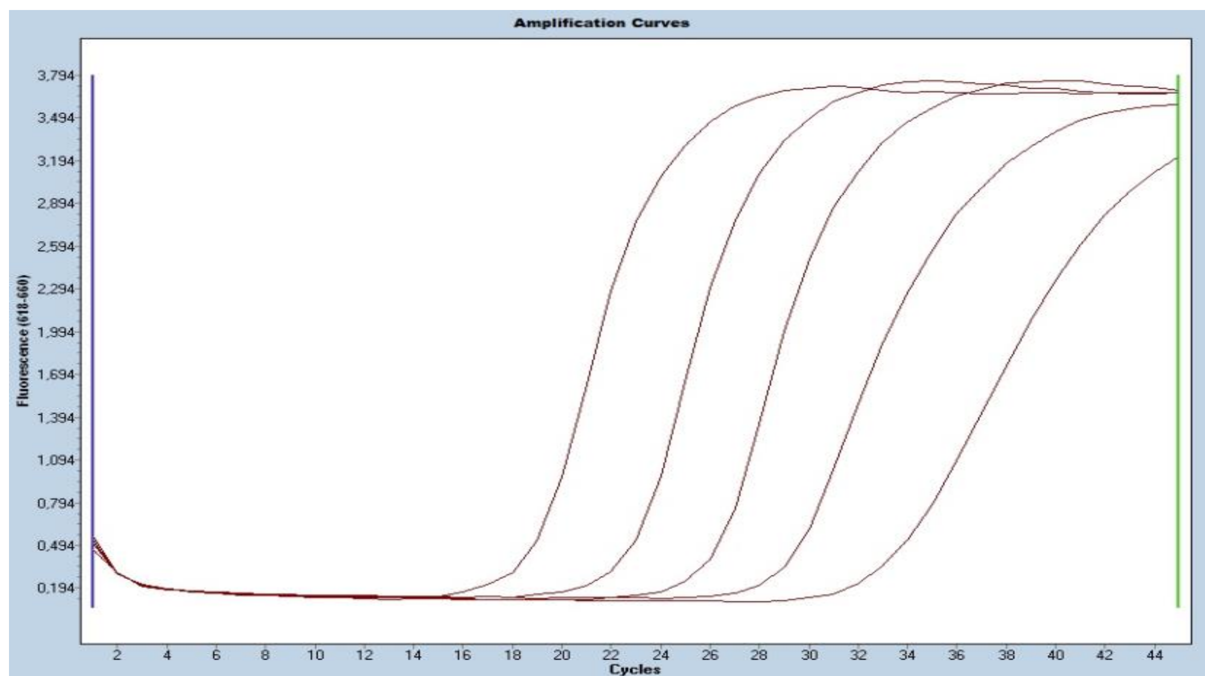

Fig.7: Dilution series EIEC/*Shigella* spp. (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

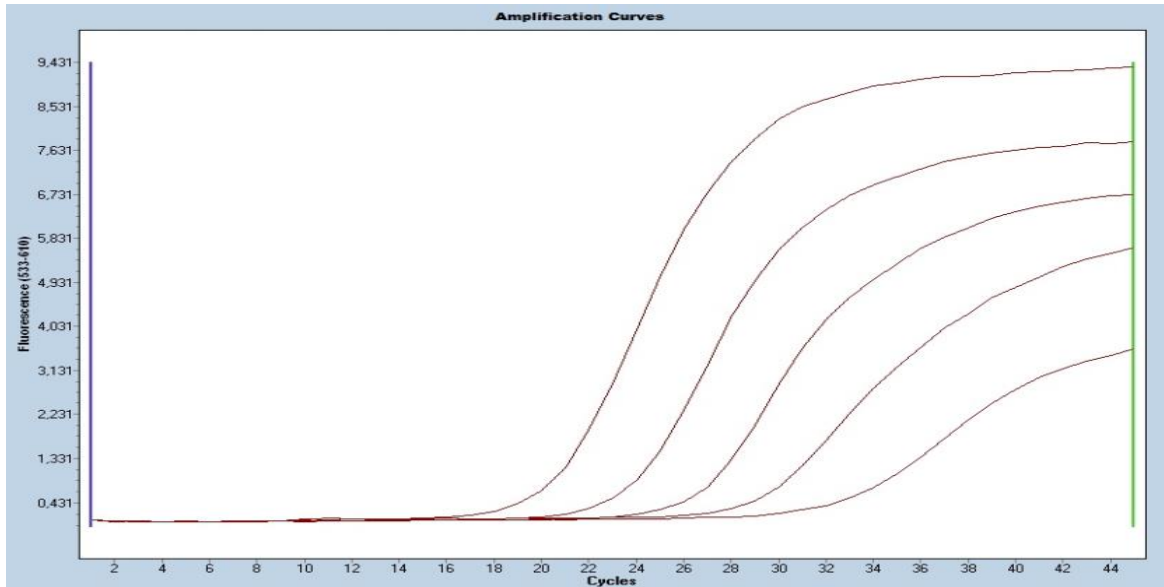

Fig. 8: Dilution series *Campylobacter* spp. (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

The detection limit of the whole procedure depends on the sample matrix, DNA extraction and DNA concentration.

Analytical Specificity

The analytical specificity of the Bacterial Stool Panel I multiplex real-time PCR assay is specific for STEC, *Salmonella* spp., EIEC/*Shigella* spp. and *Campylobacter* spp. No cross-reaction could be detected for the following species (see Table 10).

Table 10: Cross-reactivity Testing

Adenovirus 1, human, strain Adenoid 71	-	*Candida albicans*	-	*Cryptosporidium parvum*	-	Norovirus GG I	-
Adenovirus 7, human, strain Gomen	-	*Citrobacter freundii*	-	*E. coli* (O26:H-)	-	Norovirus GG II	-
Adenovirus 40, human, strain Dugan	-	*Clostridium bifermentans*	-	*E. coli* (O6)	-	*Proteus vulgaris*	-
Adenovirus 41, human, strain Tak	-	*Clostridium difficile*	-	*Entamoeba histolytica*	-	*Pseudomonas aeruginosa*	-
Aeromonas hydrophila	-	*Clostridium novyi*	-	*Enterobacter cloacae*	-	Rotavirus	-
Arcobacter butzleri	-	*Clostridium perfringens*	-	*Enterococcus faecalis*	-	*Serratia liquefaciens*	-
Astrovirus	-	*Clostridium septicum*	-	*Giardia intestinalis* Portland 1	-	*Staphylococcus aureus*	-
Bacillus cereus	-	*Clostridium sordellii*	-	*Giardia intestinalis* WB Clone 6	-	*Staphylococcus epidermidis*	-
Bacteroides fragilis	-	*Clostridium sporogenes*	-	*Giardia lamblia*	-	*Vibrio parahaemolyticus*	-
Campylobacter fetus subsp. *Fetus*	-	*Cryptosporidium muris*	-	*Klebsiella oxytoca*	-	*Yersinia enterocolitica*	-
Campylobacter upsaliensis	-						

Analytical Reactivity

The reactivity of the Bacterial Stool Panel I multiplex real-time PCR assay was tested against multiple stx1/stx2 subtypes, *Campylobacter* species, *Salmonella* serotypes and EIEC/*Shigella* spp. (see Table 11). All STEC genogroups, *Campylobacter* species, *Salmonella* serotypes and EIEC/*Shigella* spp. were detected by the Bacterial Stool Panel I multiplex real-time PCR assay.

Table 11: Analytical Reactivity Testing

stx1-Subtype					
stx1a	+	stx1c	+	stx1d	+
stx2-Subtype					
stx2a	+	stx2d	+	stx2g	+
stx2b	+	stx2e	+		
stx2c	+	stx2f	+		
***Campylobacter*-Species**					
C. coli	+	*C. jejuni*	+	*C. lari*	+
***Salmonella*-Serotype**					
S. augustenbourg	+	*S. ealing*	+	*S. muenchen*	+
S. abony	+	*S. enteritidis*	+	*S. newport*	+
S. agona	+	*S. essen*	+	*S. nottingham*	+
S. amsterdam	+	*S. glostrup*	+	*S. ohio*	+
S. anatum	+	*S. gloucester*	+	*S. oranienburg*	+
S. arizonae	+	*S. goldcoast*	+	*S. paratyphi A*	+
S. bareilly	+	*S. hadar*	+	*S. poona*	+
S. berta	+	*S. haifa*	+	*S. pullorum*	+
S. blegdam	+	*S. heidelberg*	+	*S. rostock*	+
S. bongori	+	*S. infantis*	+	*S. saintpaul*	+
S. bovismorbificans	+	*S. javiana*	+	*S. schwarzengrund*	+
S. brandenburg	+	*S. kedougou*	+	*S. senftenberg*	+
S. caracas	+	*S. kentucky*	+	*S. typhimurium*	+
S. chloeraesius	+	*S. kiel*	+	*S. virchow*	+
S. derby	+	*S. livingston*	+	*S. wernigerode*	+
S. diarizonae	+	*S. mississippi*	+	*S. wilhelmsburg*	+
S. dublin	+	*S. montevideo*	+	*S. worthington*	+
S. duesseldorf	+	*S. moscow*	+		
Shigella					
S. boydii	+	S. dysenteriae	+	*S. flexneri*	+
S. sonnei	+				

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