IVD For *in Vitro* Diagnostic Use

CE

A.I.I. Screen Real-TM

Handbook

Real Time PCR test for the qualitative detection and differentiation of *Shigella Spp. E.coli, Salmonella spp., Campylobacter spp., Adenovirus F, Rotavirus A, Norovirus 2 genotype, Astrovirus*

REF **B45-50FRT**

Σ **50**

NAME

A.I.I. (Acute Intestional Infections) Screen Real-TM

INTRODUCTION

Acute Intestinal Infections (A.I.I) are one of the primary causes of hospitalization in infectious disease departments. In accordance with the data provided by the contemporary literature the following bacterial and viral agents are the most often detectable and generally spread etiological agents of A.I.I.:

Bacterial agents:

- Shigella species microorganisms and enteroinvasive E coli (EIEC);
- Salmonella species microorganisms;
- Thermophillic group of Campylobacter species microorganisms;
- Enteropathogenic E coli (EPEC) and enteroaggregative E coli (EAEC);

Viral agents:

- Group A rotaviruses;
- Genotype 2 noroviruses;
- Group F adenoviruses (Types 40 and 41);
- Astroviruses.

The following causative agents are less widely or not universally spread but are not less important for epidemic outbreaks:

- Vibrio cholerae;
- Yersinia pseudotuberculosis;
- Clostridium diffi cilae;
- Enterotoxigenic E. coli (ETEC), Enterohemorrhagic E. coli (EHEC);
- Genotype 1 Enteroviruses;
- Group C Rotaviruses.

INTENDED USE

Kit **A.I.I. Screen Real-TM** is a Real-Time test for the qualitative detection and differentiation of *Shigella Spp. E.coli, Salmonella spp., Campylobacter spp., Adenovirus F, Rotavirus A, Norovirus 2 genotype, Astrovirus* in the biological materials and in the environment. RNA/DNA is extracted from specimens, amplified using RT-amplification and detected using fluorescent reporter dye probes specific for A.I.I. and IC (Internal Control).

PRINCIPLE OF ASSAY

Kit **A.I.I. Screen Real-TM** is based on three major processes: isolation of RNA/DNA from specimens, reverse transcription of the RNA and Real Time amplification. Test contains an IC which serves as an amplification control for each individually processed specimen and to identify possible reaction inhibition

MATERIALS PROVIDED

Module No.1: Real Time PCR kit (B45-50FRT)

Part N° 1 – **"A.I.I. Screen Real-TM":**

- **PCR-mix-1 *Shigella* spp. / *Salmonella* spp.** 0,6 ml;
- **PCR-mix-1 *Campylobacter* spp. / Adenovirus,** 0,6 ml;
- **PCR-mix-1 *Rotavirus* / *Astrovirus,*** 0,6 ml;
- **PCR-mix-1 *Norovirus* / IC,** 0,6 ml;
- **RT-PCR-mix-2**, 5 x 0,3 ml;
- **TaqF Polymerase**, 4 x 0,03 ml;
- **M-MLV Revertase**, 4 x 0,015 ml;
- **RT-G-mix-2**, 4 x 0,015 ml.

Contains reagents for 55 reactions

Part N° 2 – **"Controls"**

- **Negative Control C-**, 1,6 ml; *
- **Internal Control (IC)**, 5 x 0,12 ml; **
- **Positive Control *Shigella sonnei* / *Salmonella* C+,** 0,1 ml;
- **Positive Control *Campylobacter jejuni* / *Adenovirus* C+,** 0,1 ml;
- **Positive Control *Rotavirus/ Astrovirus* C+,** 0,1 ml;
- **Positive Control *Norovirus* 2/IC C+,** 0,1 ml;
- **DNA-buffer**, 0,5 ml;

* *must be used in the isolation procedure as Negative Control of Extraction.*

** *add 10 µl of Internal Control during the RNA/DNA purification procedure directly to the sample/lysis mixture*

MATERIALS REQUIRED BUT NOT PROVIDED

Zone 1: sample preparation:

- RNA extraction kit
- Biological cabinet
- Vortex
- 65°C ± 2°C dry heat block
- Desktop microcentrifuge for “eppendorf” type tubes (RCF max. 16,000 x g)
- Tube racks
- Microcentrifuge tubes, 1,5 - 2,0 ml
- Pipettes with sterile, RNase-free filters tips
- Biohazard waste container
- Disposable gloves, powderless
- Refrigerator, Freezer

Zone 2: Real Time amplification:

- Disposable gloves, powderless
- Biohazard waste container
- Refrigerator, Freezer
- Real Time Thermal cycler
- Workstation
- Pipettes (adjustable)
- Sterile pipette tips with filters

PRODUCT USE LIMITATIONS

All reagents may exclusively be used in in vitro diagnostics. Use of this product should be limited to personnel trained in the techniques of DNA amplification (UNI EN ISO 18113-2:2012). Strict compliance with the user manual is required for optimal PCR results. Attention should be paid to expiration dates printed on the box and labels of all components. Do not use a kit after its expiration date.

QUALITY CONTROL

In accordance with Sacace’s ISO 13485-Certified Quality Management System, each lot is tested against predetermined specifications to ensure consistent product quality.

STORAGE INSTRUCTIONS

Part N° 1 – **“A.I.I. Screen Real-TM”** must be stored at -16°C or below.

Part N° 2 – **“Controls”** can be stored at 2-8°.

The kit can be shipped at 2-8°C but should be stored at 2-8°C and -16°C or below immediately on receipt.

STABILITY

A.I.I. Screen Real-TM Test is stable up to the expiration date indicated on the kit label. The product will maintain performance through the control date printed on the label. Exposure to light, heat or humidity may affect the shelf life of some of the kit components and should be avoided. Repeated thawing and freezing of these reagents should be avoided, as this may reduce the sensitivity.

WARNINGS AND PRECAUTIONS

IVD **_In Vitro_ Diagnostic Medical Device**

For _In Vitro_ Diagnostic Use Only

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local authorities' regulations.
- Specimens should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid sample or reagent contact with the skin, eyes, and mucous membranes. If skin, eyes, or mucous membranes come into contact, rinse immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be one-directional, it should begin in the Extraction Area and then move to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area in which the previous step was performed.

Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

A.I.I. Screen Real-TM can analyze RNA extracted from:

- *water:* centrifuge 10-20 ml for 10 min at maximum speed. Discard the supernatant and leave about 100 µl of solution for DNA extraction;
- *whole blood* collected in EDTA tubes*;*
- *feces:*
    - Prepare 10-20% feces suspension, for instance adding 4ml of Saline Solution and 1,0 gr (approx. 1,0 ml) of feces in 5 ml tube (the same can be done in 2,0 ml tube). The DNA/RNA purification must be done immediately, if it is not possible add 20% Glycerol sterile solution (cryoprotective agent that provides intracellular and extracellular protection against freezing) and store at -20°C.
    - Vortex to get an homogeneous suspension and centrifuge for 5 min to 7000-12000g. Use the supernatant for the extraction of the viral DNA/RNA and the bacterial fraction (white-yellowish line between the sediment and the supernatant) for the extraction of bacterial DNA.

Specimens can be stored at +2-8°C for no longer than 12 hours, or frozen at -20°C to -80°C.

Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

RNA/DNA ISOLATION

Any commercial RNA/DNA isolation kit, if IVD-CE validated for the specimen types indicated herein at the “SAMPLE COLLECTION, STORAGE AND TRANSPORT” paragraph, could be used.

Sacace Biotechnologies recommends to use the following kits:

- ⇨ **DNA/RNA-Prep** (Sacace, REF K-2-9);
- ⇨ **Ribo-Sorb-** (Sacace, REF K-2-1);

Please carry out RNA extraction according to the manufacture’s instruction.

Add 10 µl of Internal Control during DNA isolation procedure directly to the sample/lysis mixture.

RT AND AMPLIFICATION

Total reaction volume is **25 µl**, the volume of RNA sample is **10 µl**.

1 Prepare the reaction mix for required number of samples. For each clinical specimen it must be prepared 4 reaction mixes (with **PCR-mix-1 *Shigella* spp. / *Salmonella* spp.**, **PCR-mix-1 *Campylobacter* spp. / Adenovirus, PCR-mix-1 *Rotavirus* / *Astrovirus,* PCR-mix-1 *Norovirus* / IC),** each one in a new sterile tube.

2 For N samples prepare 4 mixes adding in a new tube:

	1	2	3	4
10 µl x N samples	**PCR-mix-1 *Shigella* spp. / *Salmonella* spp**	**PCR-mix-1 *Campylobacter* spp. / Adenovirus**	**PCR-mix-1 *Rotavirus* / *Astrovirus***	**PCR-mix-1 *Norovirus* / IC**
5.0 µl x N samples	**RT-PCR-mix-2**	**RT-PCR-mix-2**	**RT-PCR-mix-2**	**RT-PCR-mix-2**
0.5 µl x N samples	**TaqF Polymerase**	**TaqF Polymerase**	**TaqF Polymerase**	**TaqF Polymerase**
0.25 µl x N samples	**RT-G-mix-2**	**RT-G-mix-2**	**RT-G-mix-2**	**RT-G-mix-2**
0.25 µl x N samples	**M-MLV Revertase**	**M-MLV Revertase**	**M-MLV Revertase**	**M-MLV Revertase**

3 Vortex the tube, then centrifuge shortly. Add **15 µl** of prepared reaction mix into each tube.

4 Using tips with aerosol filter add **10 µl** of RNA samples obtained at the stage of RNA isolation and mix carefully by pipetting**.**

5 Prepare for each panel the following controls:

- add **10 µl** of **DNA-buffer** to the tube labeled Amplification Negative Control;
- add **10 µl** of ***Shigella sonnei* / *Salmonella* C+** to the tube with **PCR-mix-1 *Shigella* spp. / *Salmonella* spp**;
- add **10 µl** of ***Campylobacter jejuni* / *Adenovirus* C+** to the tube with **PCR-mix-1 *Campylobacter* spp. / *Adenovirus;***
- add **10 µl** of ***Rotavirus* / *Astrovirus* C+** to the tube with **PCR-mix-1 *Rotavirus* / *Astrovirus;***
- add **10 µl** of ***Norovirus* / *IC C+*** to the tube with **PCR-mix- *Norovirus* / IC**

Amplification:

Create a temperature profile on your Real-time instrument as follows:

	Rotor type instruments[1]				Plate type or modular instruments[2]			
Stage	**Temp, °C**	**Time**	**Fluorescence detection**	**Cycle repeats**	**Temp, °C**	**Time**	**Fluorescence detection**	**Cycle repeats**
Hold	50	30 min	–	1	50	30 min	–	1
Hold	95	15 min	–	1	95	15 min	–	1
Cycling 2	95	10 s	–	45	95	10 s	–	45
	60	25 s	FAM(Green), JOE(Yellow)		60	30 s	FAM, JOE/HEX/Cy3	
	72	10 s	–		72	10 s	–	

[1] For example Rotor-Gene™ 6000/Q (Qiagen)

[2] For example, SaCycler-96™ (Sacace), iQ5™/CFX™ (BioRad); Mx3005P™ (Agilent), ABI® 7300/7500/StepOne (Applied Biosystems), SmartCycler® (Cepheid)

Settings

<u>Rotor-type instruments</u> (Rotor-Gene 3000/6000, Q)

Channel	Calibrate/Gain Optimisation…	Threshold	More Settings/Outlier Removal	Slope Correct
FAM/Green	*from 4 Fl to 8 Fl*	*0.05*	*10%*	*On*
JOE/Yellow	*from 4 Fl to 8 Fl*	*0.05*	*10%*	*On*

<u>Plate- or modular type instruments</u> (SaCycler, iQ5, Mx300P, ABI 7500, SmartCycler)

The threshold line should cross only sigmoid curves of signal accumulation of positive samples and should not cross the baseline; otherwise, the threshold level should be raised. Set the threshold at a level where fluorescence curves are linear and do not cross curves of the negative samples.

RESULTS ANALYSIS:

1. The results are interpreted by the device software through the presence of crossing of fluorescence curve with the threshold line:
   - Internal Control (IC) is detected on the FAM (Green) channel and *Norovirus* on the JOE (Yellow) channel with PCR-mix- *Norovirus* / IC;
   - *Rotavirus A* is detected on the FAM (Green) channel and *Astrovirus* on the JOE (Yellow) channel with PCR-mix-1 *Rotavirus* / *Astrovirus;*
   - *Shigella Spp.* and *E. coli* is detected on the FAM (Green) channel and *Salmonella* spp on the JOE (Yellow) channel with PCR-mix-1 *Shigella* spp. / *Salmonella spp;*
   - *Campylobacter* spp.is detected on the FAM (Green) channel and *Adenovirus* on the JOE (Yellow) channel with PCR-mix-1 *Campylobacter* spp. /*Adenovirus*.
2. The sample is considered to be positive if the value of **Ct** is lower than boundary value (see table below).
3. The sample is considered to be negative if the result is positive only on the channel Fam with PCR-mix-1 *Norovirus* / IC and the Ct value is lower than boundary value (see table below).

Interpretation of results:

Ct channel	PCR-mix-1 *Shigella* spp. / *Salm.* spp.	PCR-mix-1 *Campylobacter* spp. / *Adenovirus*	PCR-mix-1 *Rotavirus* / *Astrovirus*	PCR-mix-1 *Norovirus* / IC
FAM/ Green	**Pos** (≤ boundary value*) **–** *Shigella* spp. DNA is **detected** **Neg –** *Shigella* spp. DNA is **not detected****	**Pos** (≤ boundary value*) **–** *Campylobacter* spp. DNA is **detected** **Neg –** *Camp.* spp. DNA is **not detected****	**Pos** (≤ boundary value*) **–** *Rotavirus* grA RNA is **detected** **Neg –** *Rotavirus* grA RNA is **not detected** **	**Pos** (≤ boundary value*) for **IC – valid results** **Neg – invalid results** ***
JOE/ Yellow/HEX	**Pos** (≤ boundary value*) **–** *Salmonella* spp. DNA is **detected** **Neg –** *Salmonella* spp. DNA is **not detected** **	**Pos** (≤ boundary value*) **–** *Adenovirus* grF DNA is **detected** **Neg –** *Adenovirus* grF DNA is **not detected** **	**Pos** (≤ boundary value*) **–** *Astrovirus* grA RNA is **detected** **Neg –** *Astrovirus* grA RNA is **not detected** **	**Pos** (≤ boundary value*) **–** *Norovirus* G2 RNA is **detected** **Neg –** *Norovirus* G2 RNA is **not detected** **

* For boundary values, see the table below.

** If the Ct value in the FAM/Green channel for PCR-mix-1 *Norovirus* / IC is not more than the boundary value, the result is correct.

*** If the Ct value in the FAM/Green channel for PCR-mix-1 *Norovirus* / IC is absent or exceeds the boundary value, the negative result for other PCR-mixes-1 is considered to be invalid. It is necessary to repeat PCR starting from the extraction stage.

Boundary Ct values for clinical material

PCR-mix-1	Ct value detected in the channel	
	FAM/Green	JOE/Yellow/HEX
PCR-mix-1 *Shigella* spp. / *Salmonella* spp.	*38*	*38*
PCR-mix-1 *Campylobacter* spp. / *Adenovirus*	*38*	*38*
RT-PCR-mix-1 *Rotavirus* / *Astrovirus*	*38*	*38*
RT-PCR-mix-1 *Norovirus* / *IC*	*38*	*38*

_Boundary Ct values for environmental samples

PCR-mix-1	Ct value detected in the channel	
	FAM/Green	JOE/Yellow/HEX
PCR-mix-1 *Shigella* spp. / *Salmonella* spp.	*40*	*40*
PCR-mix-1 *Campylobacter* spp. / *Adenovirus*	*40*	*40*
PCR-mix-1 *Rotavirus* / *Astrovirus*	*40*	*40*
PCR-mix-1 *Norovirus* / *IC*	*40*	*38*

PERFORMANCE CHARACTERISTICS

Analytical specificity

The analytical specificity of the primers and probes was validated with negative samples. They did not generate any signal with the specific *Shigella Spp. E. coli, Salmonella spp., Campylobacter spp., Adenovirus F, Rotavirus A, Norovirus 2 genotype, Astrovirus* primers and probes. The specificity of the kit **A.I.I. (Acute Intestinal Infectious) Screen Real-TM** was 100%. The potential cross-reactivity of the kit **A.I.I. (Acute Intestinal Infectious) Screen Real-TM** was tested against the group control. It was not observed any cross-reactivity with other pathogens.

Table. 1 Panel of tested pathogens.

Strain ID	Organism	Strain ID	Organism
K2033	*Salmonella* Ser. Grumpensis	K2015	*Salmonella* Ser. Oranienburg
K1806	*Salmonella* Ser. Newport	AM01144	*Salmonella* Ser. Newport
K2077	*Salmonella* Ser. Enteriditis	K1810	*Salmonella* Ser. Anatum
83-99	*Salmonella* Ser. Typhimurium	K1991	*Salmonella* Ser. Typhimurium
PS505	*Shigella boydii*	K1898	*Salmonella* Ser. Heidelberg
PS408	*Shigella sonnei*	PS555	*Shigella boydii*
B4003	*Shigella sonnei*	F6446	*Shigella dysenteriae*
PS801	*Shigella dysenteriae*	S821X1	*Shigella dysenteriae* type 1
C898	*Shigella dysenteriae* type1	S177X1	*Shigella dysenteriae* type 1
F2035	*Shigella flexneri*	S3314	*Shigella dysenteriae* type 2
E2539-C1	Enterotoxigenic *Escherichia coli* (ETEC)	PS071	*Shigella flexneri*
H10407	Enterotoxigenic *Escherichia coli* (ETEC)	PS050	*Shigella flexneri*
F1008	Enterotoxigenic *Escherichia coli* (ETEC)	F7862	*Shigella flexneri*
EDL 933	Shiga-toxin *E. coli* (STEC)	TX1	Enterotoxigenic *Escherichia coli* (ETEC)
3543-01	Shiga-toxin *E. coli* (STEC)	3525-01	Shiga-toxin *Escherichia coli* (STEC)
4752-71	*Proteus vulgaris*	25922	*Escherichia coli* O6:H1
QA/QC	*Citrobacter freundii*	621-64	*Citrobacter freundii*
QA/QC	*Aeromonas*	3910-68	*Aeromonas spp.*
3043-74	*Serratia marcescens*	E9113	*Vibrio cholerae*
QA/QC	*Serratia marcescens*	501-83	*Edwardsiella spp.*
F7894	*Vibrio vulnificus*	587-82	*Providencia stuartii*
F8515	*Yersinia enterocolitica*	27853	*Pseudomonas aeruginosa*
F8510	*Yersinia enterocolitica*	D4989	*Helicobacter cineadi*
K4299	*Vibrio parahaemolyticus*	D6827	*Helicobacter pullorum*
F9835	*Vibrio parahaemolyticus*	D5127	*Helicobacter pylori*
K2023	*Salmonella* Ser. Kentucky	D2686	*Arcobacter butzleri*
K1684	*Salmonella* 4,12:1:-		

Analytical sensitivity

The kit **A.I.I. (Acute Intestional Infectious) Screen Real-TM** allows to detect *Shigella Spp. E. coli, Salmonella spp., Campylobacter spp., Adenovirus F, Rotavirus A, Norovirus 2 genotype, Astrovirus* RNA/DNA in 100% of the tests with a sensitivity of not less than 500-1000 copies/ml. The detection was carried out on the control standard and its dilutions by negative sample.

KEY TO SYMBOLS USED

REF	List Number		Caution!
LOT	Lot Number		Contains sufficient for <n> tests
IVD	For *in Vitro* Diagnostic Use	VER	Version
	Store at	**NCA**	Negative Control of Amplification
	Manufacturer	**NCE**	Negative control of Extraction
	Consult instructions for use	**C+**	Positive Control of Amplification
	Expiration Date	**IC**	Internal Control

* SaCycler™ is a registered trademark of Sacace Biotechnologies
* CFX™ and iQ5™ are registered trademarks of Bio-Rad Laboratories
* Rotor-Gene™ is a registered trademark of Qiagen
* MX3005P® is a registered trademark of Agilent Technologies
* ABI® is a registered trademark of Applied Biosystems
* SmartCycler® is a registered trademark of Cepheid

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