REF TB44-50 FRT
VER 11.04.09

Shigella/Salmonella/Campylobacter Real-TM

Real Time Kit for use with Rotor-Gene™ 3000/6000 (Corbett Research)

Key to symbols used

REF	List Number		Store at 2-8°C
RUO	For Research Use Only		Caution!
LOT	Lot Number	VER	Version
	Expiration Date		Consult instructions for use
	Contains reagents		Manufacturer

NAME
Shigella/Salmonella/Campylobacter Real - TM

INTENDED USE
The **Shigella/Salmonella/Campylobacter Real-TM** is a "Real-Time Amplification" test for the qualitative detection and differentiation of Shigella spp and Enteroinvasive E.Coli (EIEC)/Salmonella spp/Campylobacter spp in the water and feces. DNA is extracted from samples, amplified using real time amplification with fluorescent reporter dye probes specific for Shigella spp and Enteroinvasive E.Coli (EIEC)/Salmonella spp/Campylobacter spp and Internal Control IC. Test contains an (IC) which serves as an amplification control for each individually processed specimen and to identify possible reaction inhibition.

MATERIALS PROVIDED
Part N° 1 – **"DNA-Sorb-B":** isolation of DNA from clinical specimens;
Part N° 2– **"Shigella/Salmonella/Campylobacter Real-TM":** Real Time Amplification;

Part N° 1 – **"DNA-Sorb-B":**
- Lysis Solution, 15 ml;
- Washing Solution 1, 15 ml;
- Washing Solution 2, 50 ml;
- Sorbent, 1,25 ml;
- DNA-eluent, 5,0 ml.

Contains reagents for 50 extractions

Part N° 2– **"Shigella/Salmonella/Campylobacter Real-TM":**
- **PCR-mix-1 *Shigella* spp. / *Salmonella* spp.**55 ready-to-use single-dose test tubes;
- **PCR-mix-1 *Campylobacter* spp. /Internal Control** 55 ready-to-use single-dose test tubes;
- **PCR-mix-2-Flu**, 0,77 ml;
- **Positive Control *Shigella sonnei* C+,** 0,1 ml;
- **Positive Control *Salmonella* C+,** 0,1 ml;
- **Positive Control *Campylobacter jejuni* C+,** 0,1 ml;
- **Negative Control C-**, 1,6 ml;
- **Internal Control IC,** 1,0 ml;
- **DNA-buffer**, 0,5 ml;

Contains reagents for 55 tests.

MATERIALS REQUIRED BUT NOT PROVIDED
Zone 1: sample preparation:
- Biological cabinet
- Desktop microcentrifuge for "eppendorf" type tubes (RCF max. 16,000 x g); Eppendorf 5415D or equivalent
- 60°C ± 2°C dry heat block
- Vortex mixer
- Pipettors (capacity 5-40 µl; 40-200 µl; 200-1000 µl) with aerosol barrier
- 1,5 ml polypropylene sterile tubes (Sarstedt, QSP, Eppendorf)
- Disposable gloves, powderless
- Biohazard waste container

Zone 2: RT and amplification:
- Real Time Thermalcycler
- Workstation
- Pipettors (capacity 0,5-10 µl; 5-40 µl) with aerosol barrier
- Tube racks

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WARNINGS AND PRECAUTIONS

1. Lysis Solution contains guanidine thiocyanate. Guanidine thiocyanate is harmful if inhaled, or comes into contact with skin or if swallowed. Contact with acid releases toxic gas. (Xn; R: 20/21/22-36/37/38; S: 36/37/39).
2. Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.
3. Do not pipette by mouth.
4. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
5. Do not use a kit after its expiration date.
6. Dispose of all specimens and unused reagents in accordance with local regulations.
7. Biosafety Level 2 should be used for materials that contain or are suspected of containing infectious agents.
8. Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0,5% sodium hypochlorite, or other suitable disinfectant.
9. Avoid contact of specimens and reagents with the skin, eyes and mucous membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately.
10. Material Safety Data Sheets (MSDS) are available on request.
11. Use of this product should be limited to personnel trained in the techniques of amplification.
12. Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where you performed previous step.

STORAGE INSTRUCTIONS

Shigella/Salmonella/Campylobacter Real-TM must be stored at 2-8°C.

STABILITY

Shigella/Salmonella/Campylobacter Real-TM Test is stable up to the expiration date indicated on the kit label.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

Shigella/Salmonella/Campylobacter Real-TM can analyze DNA extracted with **DNA-Sorb-B** 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 20% feces suspension by adding in 5 ml tube of 4ml of Saline Solution and 1,0 gr (approx. 1,0 ml) of feces. Vortex to get the homogeneous suspension and centrifuge for 5 min to 7000-12000g and using a micropipette with a plugged aerosol barrier tip transfer in a new sterile 1,5 ml tube 100 µl of the bacterial fraction (white-yellowish line between the sediment and the supernatant)
  - Add 800 µl of PBS or Saline Solution. Vortex to get the homogeneous suspension and centrifuge for 5 min to 7000-12000g. Remove and discard the supernatant
  - Resuspend the pellet in 0,3 ml of PBS or Saline Solution.

It is recommended to process samples immediately after collection. Store samples at 2–8 °C for no longer than 24 hours, or freeze at –20/80°C. Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

SPECIMEN AND REAGENT PREPARATION

1. **Lysis Solution** and **Washing Solution** (in case of their storage at +2-8°C) should be warmed up to 60°C until disappearance of ice crystals.
2. Prepare required quantity of 1.5 ml polypropylene tubes.
3. Add to each tube **300 µl** of **Lysis Solution** and **10 µl** of **IC**.
4. Add **100 µl** of **Samples** to the appropriate tube.
5. Prepare Controls as follows:
   - add **100 µl** of **C–** (**Negative Control**) to labeled C*neg*.
6. Vortex the tubes, incubate 5 min at 65°C and centrifuge for 5 sec.
7. Vortex vigorously **Sorbent** and add **25 µl** to each tube.
8. Vortex for 5-7 sec and incubate all tubes for 10 min at room temperature. Vortex periodically
9. Centrifuge all tubes for 1 min at 5000g and using a micropipette with a plugged aerosol barrier tip, carefully remove and discard supernatant from each tube without disturbing the pellet. Change tips between tubes.
10. Add **300 µl** of **Washing Solution 1** to each tube. Vortex vigorously and centrifuge for 1 min at 5000g and using a micropipette with a plugged aerosol barrier tip, carefully remove and discard supernatant from each tube without disturbing the pellet. Change tips between tubes.
11. Add **500 µl** of **Washing Solution 2** to each tube. Vortex vigorously and centrifuge for 1 min at 10000g and using a micropipette with a plugged aerosol barrier tip, carefully remove and discard supernatant from each tube without disturbing the pellet. Change tips between tubes.
12. Repeat step 11.
13. Incubate all tubes with open cap for 5 min at 65°C.
16. Resuspend the pellet in **50 µl** of **DNA-eluent.** Incubate for 5 min at 65°C and vortex periodically.
17. Centrifuge the tubes for 2 min at maximum speed (12000-16000 g). The supernatant contains DNA ready for amplification. The amplification can be performed on the same day of extraction.

PROTOCOL (Reaction volume 25 µl):

1. Prepare required quantity of **PCR-mix-1 *Shigella* spp. / *Salmonella* spp** and **PCR-mix-1 *Campylobacter* spp. /Internal Control** tubes for samples and controls.
2. Add **7 µl** of **PCR-mix-2 Flu** into each tube.
3. Add **10 µl** of **extracted DNA** sample to appropriate tube.
4. Prepare for each panel 4 controls:
   - add **10 µl** of **DNA-buffer** to the tube labeled Amplification Negative Control;
   - add **10 µl** of ***Shigella sonnei* C+** to the tube labeled. $C+_{Shigella}$;
   - add **10 µl** of ***Salmonella* C+** to the tube labeled . $C+_{Salmonella;}$
   - add **10 µl** of ***Campylobacter jejuni* C+** to the tube labeled . $C+_{Campylobacter}$**;**

Real Time Amplification with Rotor-Gene 2000/3000/6000

1. Close PCR-mix-1 tubes and transfer them into the Rotor-Gene 2000/3000.
2. Program Rotor-Gene 2000/3000 as follows:
   1. Hold 95 °C - 15 min
   2. Cycling 95 °C - 10 s
      60 °C - 25 s - detection
      72 °C -10 s
      Cycle repeats – 45 times.

   *fluorescence detection on the channels Fam (Green) and Joe (Yellow) on the 2-nd pass (60°C)

Make the adjustment of the fluorescence channel sensitivity: *Channel Setup →Calibrate (Gain Optimisation for RG6000) → Perform Calibration (Optimisation) Before 1-st Acquisition.* For *Fam/Sybr (Green)* channel indicate *Min Reading* **10**, *Max Reading* **20** *and Joe (Yellow)* channel indicate *Min Reading* **5**, *Max Reading* **10**. In the column *Tube position* program position of the tubes in the carousel of the Rotor-Gene 2000/3000/6000 (the 1st position must contains reaction tube with reagents). Close the window *Auto Gain Calibration Setup.* Press *Analysis* then select button *Quantitation*. Perform the operation for the channel Fam (*Cycling A FAM),* then for the channels Joe (*Cycling A JOE),* For the channel Fam select *Dynamic Tube*, *More Setting (*Outlier Removal *) 10%, Threshold*: 0,05. For the channel Joe select *Dynamic Tube*, *Slope Correct*, *More Setting (*Outlier Removal*) 15%, Threshold*: 0,05

RESULTS ANALYSIS:

1. The results are interpreted with the software of **Rotor-Gene 3000/6000** through the presence of crossing of fluorescence curve with the threshold line.

   - ***Campylobacter spp* is detected on the FAM (Green) channel and *IC DNA* on the JOE (Yellow) channel with PCR-mix-1 *Campylobacter* spp. /Internal Control**
   - ***Shigella spp and Enteroinvasive E.Coli (EIEC)* are detected on the FAM (Green) channel and *Salmonella spp* on the JOE (Yellow) channel with PCR-mix-1 *Shigella* spp. / *Salmonella* spp.**

2. The sample is considered to be positive for *Campylobacter spp.* if in the channel Fam the value of **Ct** is different from zero (Ct < 33).
3. The sample is considered to be positive for *Shigella spp/Enteroinvasive E.Coli (EIEC)* if in the channel Fam the value of **Ct** is different from zero (Ct < 33).
4. The sample is considered to be positive for *Salmonella spp.* if in the channel Joe the value of **Ct** is different from zero (Ct < 33).
5. The sample is considered to be negative if in the channels Fam (or Joe *Salmonella spp.*) the Ct value is not determined (the fluorescence curve does not cross the threshold line) or Ct>33 and in the results table on the channel Joe the Ct value for Internal Control is lower than 31.
6. Occurrence of any value Ct in the table of results for the negative control sample (on channels Fam/Joe with PCR-mix-1 *Shigella* spp. / *Salmonella* spp. and on the Fam channel with PCR-mix-1 *Campylobacter* spp. /Internal Control) and for negative control of amplification (DNA-buffer) (on any of channels) testifies contamination of reagents or samples. In this case results of the analysis for all tests are considered invalid. It is required to repeat the analysis of all tests, and also to take measures to detect and eliminate the source of contamination.
7. No signal with Positive Controls indicates incorrect programming of the Real Time instrument: repeat the amplification with correct setting.
8. If the Ct value of the Internal Control is absent or higher than 31 a retesting of the sample is required.

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* primers and probes. The specificity of the kit was 100%. The potential cross-reactivity of the kit was tested against the group control. It was not observed any cross-reactivity with other pathogens.

Analytical sensitivity

The kit **Shigella/Salmonella/Campylobacter Real-TM** allows to detect *Shigella Spp. E.coli, Salmonella spp., Campylobacter spp* DNA in 100% of the tests with a sensitivity of not less than 1000-5000 copies/ml. The detection was carried out on the control standard and its dilutions by negative sample.

Target gene: *Shigella* – IpaH, *Salmonella* – TtrB, *Campylobacter* - 23SrRNA

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