

AMPLIRUN® TOTAL GASTROINTESTINAL BACTERIAL PANEL CONTROL (STOOL)

For *in vitro* diagnostic use

MBTC021: A panel of six common pathogenic enteric bacteria pooled, inactivated to render them non-infectious and formulated in a matrix that mimics stool specimens. Table 1 lists strains and culture medium used for each bacteria included in this control. This kit is intended to validate and control sample processing, amplification and detection in nucleic acid assays based on the molecular identification of gastrointestinal bacteria, using the product as an external run control.

INTRODUCTION:

Bacterial gastroenteritis is a common disease worldwide, killing millions of individuals each year. It is usually the result of food poisoning and poor levels of sanitation and water hygiene. The typical symptoms are vomiting, diarrhoea, and abdominal discomfort. The identification of etiological agent of the bacterial gastroenteritis by conventional diagnostic procedures, such as culture and microscopy, is laborious and time-consuming. A major advantage of molecular methods is the reduced time to detection.

CHARACTERISTICS:

The content is lyophilized. It is necessary to reconstitute it before use (refer to "Preparation of the reagents"). Total Controls are designed for single use, excess material should be discarded. Nucleic acid detection requires an extraction step that releases DNA/RNA for amplification and detection.

PRODUCT DESCRIPTION:

Bacteria were grown in the laboratory (see Table 1 for culture medium used in each case), inactivated and diluted in a synthetic stool matrix. This matrix has been formulated to mimic real stool samples according to Bristol stool scale. This matrix is formulated at a Bristol scale of 7 resulting in a watery stool.

BACTERIA	STRAIN	CULTURE MEDIUM
<i>Campylobacter jejuni</i>	Martin AS-83-79	Campylobacter selective agar
<i>Clostridium difficile</i>	Type strain	Blood agar
<i>Escherichia coli</i>	EHEC	Blood agar
<i>Salmonella enteritidis</i>	CDC K-1891	Blood agar
<i>Shigella flexneri</i>	Sv 2b Gp B	Blood agar
<i>Yersinia enterocolitica</i>	Billups-1803-68	Blood agar

Table 1.

KIT CONTENTS:

1 VIRCELL TOTAL GASTROINTESTINAL BACTERIAL PANEL CONTROL (STOOL): 10 vials with a pooled of lyophilized gastrointestinal bacteria in a stool specimen. Each bacteria is in a concentration that ranges from 10000 to 25000 copies/vial. Batch concentration is provided in Certificate of Analysis.

Quantification validation was performed by real-time PCR.

Materials required but not supplied:

Molecular Biology grade water
Additional extraction and detection kit.

STORAGE REQUIREMENTS:

Special transport conditions not required. Store the lyophilized vial at 2-8°C. After reconstitution, suspension should be used on the same day. Unused product should be discarded.

STABILITY AND HANDLING OF REAGENTS:

Handle reagents in aseptic conditions to avoid microbial contaminations.

Use only the amount of reagent required for the test.

The product is stable until the expiry date indicated in the label, if the instructions for use are followed.

VIRCELL, S.L. does not accept responsibility for the mishandling of the reagents included in the kit.

RECOMMENDATIONS AND PRECAUTIONS:

1. This product is for *in vitro* diagnosis use only and for professional qualified staff.
2. Sterile tips with aerosol barrier are essential to prevent contamination.
3. Specimens should be handled as in the case of infectious samples using safety laboratory procedures. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.
4. In order to perform the test it is essential to have separate working areas.
5. Dispose of unused reagents and waste in accordance with all applicable regulations.
6. The component VIRCELL TOTAL CONTROL could include genetic material or substances of animal and/or human origin. VIRCELL TOTAL CONTROL contains inactivated microorganism, nevertheless, it should be considered potentially infectious and handled with care. Inactivation was verified by the absence of growth under same culture conditions used for each microorganism. No present method can offer complete assurance that these or other infectious agents are absent. All materials should be handled and disposed as of potentially infectious. Observe the local regulations for waste disposal.

PREPARATION OF THE REAGENTS:

1. Add 500 µl of Molecular Biology grade water to vial **1** and mix until completely reconstituted. The concentration will be approximately 35000 copies/ml for each bacterium in the panel once reconstituted.
2. Shake with vortex for 30 seconds to dissolve and homogenize completely.
3. Follow diagnostic kit instructions treating TOTAL CONTROL in an identical manner to a clinical specimen using recommended amount for extraction and detection.

INTERNAL QUALITY CONTROL:

Each batch is subjected to internal quality control testing before releasing. Quality control analysis is performed using a sample preparation kit and real-time PCR for quantification. Final quality control results for each particular lot are available.

INTERPRETATION OF RESULTS AND VALIDATION PROTOCOL FOR USERS:

Refer to indications of additional extraction and detection kit.



LIMITATIONS OF METHOD:

1. This reagent is intended to be used with methods of human diagnostics. This test has not been verified with other methods.
2. The user of this kit is advised to read carefully and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results.
3. Use of this product should be limited only to personnel trained in molecular techniques.
4. This external run control does not substitute internal diagnostic kit controls.
5. Quantification conclusions cannot be drawn from a single point sample of known concentration. Precise clinical sample quantification could only be achieved by the standard curve method using a calibrator.
6. AMPLIRUN® TOTAL has not been designed to be used with a particular diagnostic kit coming from a certain manufacturer. It is used to validate and control sample processing, analysis and detection of a diagnostic laboratory functioning procedure.

PERFORMANCE:**• IDENTITY TEST**

PCR analysis was performed after extraction with a specific oligonucleotide pair for each bacteria present in the panel. The reactions produced fragments of the expected sizes.

• QUANTIFICATION TEST

Quantification is based on Real-Time qPCR using the standard curve method. This method involves the use of multiple replicates of different serial dilutions of both the product and the standard of quantification.







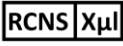


• INTRA-ASSAY PRECISION

3 vials of the product were extracted under identical extraction conditions and 3 replicas of each extraction were amplified by the same operator under identical qPCR conditions. Less than 15% coefficient of variance was observed between all assays.

• INTER-ASSAY PRECISION

1 vial of the product was extracted and 3 replicates from this vial were amplified by 2 different operators on 3 consecutive days. Less than 15% coefficient of variance was observed between all assays.

SYMBOLS USED IN LABELS:

	In vitro diagnostic medical device
	Use by (expiration date)
	Store at x-y°C
	Batch code
	Catalogue number
	Consult instructions for use
	Reconstitute in x µl
	Shipment temperature
	Storage temperature

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