AMPLIRUN® TOTAL CT/NG CONTROL (URINE)

For *in vitro* diagnostic use

MBTC003: Inactivated *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) cells formulated to mimic human urine specimen and intended to validate and control sample processing, analysis and detection in nucleic acid assays using the product as an external run control.

INTRODUCTION:

Chlamydiae are nonmotile, obligate intracellular bacteria with a unique life cycle that includes two phases: reticulate and elementary bodies. *Chlamydia trachomatis* is comprised of two human biovars: the lymphogranuloma venereum, remarkable for its tropism for lymphoid cells and its ability to cause systemic disease, and the trachoma biovar, limited primarily to epithelial cells of mucous membranes and able to cause trachoma, sexually transmitted disease, and neonatal inclusion conjunctivitis and pneumonia.

Neisseria gonorrhoeae or gonococcus is a Gram-negative, oxidase-positive, aerobic, nutritionally fastidious, coccal bacterium that appears microscopically under diplococcal arrangement. Humans are the only natural hosts for gonococcus, which is transmitted by sexual intercourse. Infections are generally limited to mucous surfaces that are lined with columnar epithelium cells, involving the urethra, cervix, rectum, pharynx, and conjunctiva.

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:

CT: Elementary bodies purified from supernatants of McCoy infected cells by differential centrifugation. Bacteria are inactivated rendering them non-infectious and diluted in a synthetic human urine solution.

NG: Grown in chocolate agar culture medium. Once purified, the cells are inactivated rendering them non-infectious and diluted in a synthetic human urine solution.

KIT CONTENTS:

1 VIRCELL TOTAL CT/NG CONTROL (URINE): 10 vials with lyophilized cells of *Chlamydia trachomatis* (2500-10000 copies/vial) and *Neisseria gonorrhoeae* (2500-10000 copies/vial).

Lot number			
Concentration	copies/vial	CT	
		NG	

Table 1.

C. trachomatis quantification was performed by inoculation on cell culture and counting of fluorescent focus forming units.

N. gonorrhoeae quantification was performed by direct counting in a Neubauer chamber.

Quantification validation was performed using a real-time PCR instrument from Stratagene (ref. Mx3005P).

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 2-8ºC. 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 1000 µl of Molecular Biology grade water to each vial 1 and mix until completely reconstituted. The cellular concentration will be approximately 5000 copies/ml 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 QIAamp Mini Kit (Qiagen) for sample preparation and real-time PCR run in a LightCycler® 480 (Roche) 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 as MBC075 AmpliRun® NEISSERIA GONORRHOEAE DNA CONTROL or MBC012 AmpliRun® CHLAMYDIA TRACHOMATIS DNA CONTROL.

PERFORMANCE:
- **IDENTITY TEST**

Performance analysis of TOTAL CONTROL:
Performance analysis was carried out by sample preparation and PCR analysis with a specific oligo pair for *C. trachomatis*. The reaction produced a 71 bp fragment. It was visualized on a 2% agarose gel using ethidium bromide staining.

Performance analysis was carried out by sample preparation and PCR analysis with a specific oligo pair for *N. gonorrhoeae*. The reaction produced a 414 bp fragment. It was visualized on a 2% agarose gel using ethidium bromide staining.

- **QUANTIFICATION TEST**

A correlation test was performed between culture and microorganism extracted nucleic acid. Less than 0.5 log variance was observed between both assays.

- **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 5% coefficient of variance was observed between all assays.

- **INTER-ASSAY PRECISION**

1 vial of the product was extracted by 2 different operators on 2 consecutive days. 3 replicas of each extraction are individually amplified on the same day the extraction was performed. Less than 5% coefficient of variance was observed between all assays.

SYMBOLS USED IN LABELS:

IVD	*In vitro* diagnostic medical device
	Use by (expiration date)
Y°C / X°C	Store at x-yºC
LOT	Batch code
REF	Catalogue number
	Consult instructions for use
RCNS | X µL	Reconstitute in x µl
SHIP	Shipment temperature
STORE	Storage temperature

BIBLIOGRAPHY:
1. Boel CH, van Herk CM, Berretty PJ, Onland GH, van den Brule AJ. (2005). Evaluation of conventional and real-time PCR assays using two targets for confirmation of results of the COBAS AMPLICOR *Chlamydia trachomatis*/*Neisseria gonorrhoeae* test for detection of *Neisseria gonorrhoeae* in clinical samples. J Clin Microbiol. 2005 May; 43(5):2231-5.
2. Bustin SA. (2000). Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. J Mol Endocrinol. 25(2):169-193.
3. Bustin SA. (2002). Quantification of mRNA using real-time reverse transcription PCR (RT-PCR): trends and problems. J Mol Endocrinol. 29(1):23-39.
4. Freeman WM, Walker SJ, Vrana KE. (1999). Quantitative RT-PCR: pitfalls and potential. Biotechniques. 1999 26(1):112-122, 124-125.
5. Hjelmevoll SO, Olsen ME, Sollid JU, Haaheim H, Unemo M, Skogen V. (2006). A fast real-time polymerase chain reaction method for sensitive and specific detection of the *Neisseria gonorrhoeae* porA pseudogene. J Mol Diagn 8(5):574-581.
6. Jaton K, Bille J, and Greub G. (2006). A novel real-time PCR to detect *Chlamydia trachomatis* in first-void urine or genital swabs. 55,1667-1674.
7. Larionov A, Krause A, Miller W. (2005). A standard curve based method for relative real time PCR data processing. BMC Bioinformatics. 6:62.
8. Madico G, Quinn TC, Boman J, Gaydos CA. (2000). Touchdown enzyme time release-PCR for detection and identification of *Chlamydia trachomatis*, *C. pneumoniae*, and *C. psittaci* using the 16S and 16S-23S spacer rRNA genes. J Clin Microbiol. 2000 38(3):1085-10.

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REVISED: 06/2014