AMPLIRUN® STREPTOCOCCUS PNEUMONIAE DNA CONTROL

For *in vitro* diagnostic use

MBC070: Purified DNA of *Streptococcus pneumoniae* to be used to control *in vitro* diagnosis techniques based in nucleic acids amplification.

INTRODUCTION:
Streptococci are catalase negative, facultative anaerobic, Gram-positive coccal bacteria that grow in chains in liquid media. S. pneumoniae or pneumococcus is an α-haemolytic capsulated streptococcus that is found in the normal flora of the human oropharynx. It can cause pneumonia, sinusitis, otitis media, meningitis or endocarditis.

CHARACTERISTICS:
The lyophilized nucleic acid is included in a thermo-sealed foil pouch containing a silica gel bag. It is necessary to reconstitute it before use (refer to "Preparation of reagents").

Preparation: Grown in Blood agar culture medium.
Extract preparation: Commercial genomic DNA extraction method.

KIT CONTENTS:
1 VIRCELL SPN DNA CONTROL: 1 vial with lyophilized DNA of *Streptococcus pneumoniae,* (Jorgensen 262 strain), (10000-20000 copies/µl once reconstituted (see Table 1)). DNA quantification has been performed with real-time PCR instrument from Stratagene (ref. Mx3005P).
2 VIRCELL CONTROL RECONSTITUTION SOLUTION: 500 µl of molecular biology grade water, DNase, RNase free.

Lot number		
Concentration	copies/µl	

Table 1.

Materials required but not supplied:
Additional diagnosis kit.

STORAGE REQUIREMENTS:
Special transport conditions not required. Store the lyophilized vial at 2-8ºC inside the foil pouch. Once the pouch is opened, reconstitute the lyophilized vial immediately and store between -5ºC and -40ºC after reconstitution (temperature indicated on the label).

STABILITY AND HANDLING OF REAGENTS:
Handle reagents in aseptic conditions to avoid microbial contaminations.
Use only the amount of reagent required for the test.

After control resuspension DNA solution should be aliquoted in order to avoid multiple freeze-thaw cycles. 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 DNA CONTROL could include genetic material or substances of animal and/or human origin. VIRCELL DNA CONTROL could contain inactivated *Streptococcus pneumoniae* antigen. VIRCELL DNA CONTROL contains purified nucleic acids obtained from inactivated microorganism, nevertheless, it should be considered potentially infectious and handled with care. 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 clinical waste disposal.
7. Dilutions below 1000 copies/µl should be made immediately before use. Freezing of product dilutions containing less than 1000 copies/µl is not recommended as partial DNA degradation might occur.

PREPARATION OF THE REAGENTS:
1. Tear the foil pouch containing VIRCELL DNA CONTROL 1.
2. Centrifuge VIRCELL DNA CONTROL 1 1 minute at 1000 g.
3. Add 100 µl of VIRCELL CONTROL RECONSTITUTION SOLUTION 2 and mix until completely reconstituted. The concentration will be 10000-20000 copies/µl once reconstituted (see Table 1).
4. Shake with vortex for 30 seconds to dissolve and homogenize completely.
5. It is recommended to prepare VIRCELL DNA CONTROL aliquots. In case dilutions were to be prepared use VIRCELL CONTROL RECONSTITUTION SOLUTION 2 for this purpose.

TEST PROCEDURE:
Once nucleic acid is reconstituted, use it according to indications of additional diagnosis kit. Use resuspended VIRCELL CONTROL as an extracted clinical sample adding it directly to amplification reagents.

INTERNAL QUALITY CONTROL:
Each batch is subjected to internal quality control testing before releasing. Quality control analysis is performed by real-time PCR run in a LightCycler® 480 (Roche). Final quality control results for each particular lot are available.

INTERPRETATION OF RESULTS AND VALIDATION PROTOCOL FOR USERS:
Refer to indications of additional diagnosis kit.

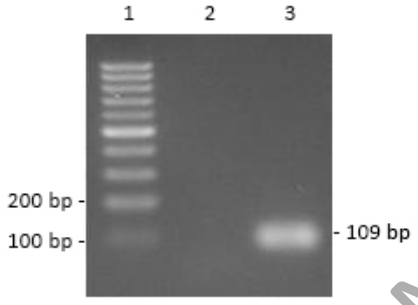
LIMITATIONS OF METHOD:
1. This reagent is intended to be used with methods of human diagnosis. This test has not been verified with other methods.

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. The identity test was carried out with some specific primers according to publicly available sequences of the microorganism. Changes in the sequences of the primers of the reaction may produce a range of different sizes or may not display product amplification.
5. This control does not substitute internal diagnostic kit controls.
6. The quantification was carried out by own brand qPCR against a standard used as a calibrator. Results may vary with the amplification conditions of the end user.

PERFORMANCES:

- **IDENTITY TEST**

PCR analysis of DNA control: PCR analysis was performed with a specific oligo pair on purified *Streptococcus pneumoniae* DNA. The reaction produced a 109 bp fragment. It was visualized on a 2% agarose gel using ethidium bromide staining. The gel photograph is shown below:



Line	Sample
1	Molecular size marker (100 bp)
2	Negative control
3	109 bp amplified fragment

- **QUANTIFICATION TEST**

A correlation test was performed between microorganism culture and *Streptococcus pneumoniae* extracted DNA. Less than 0.5 log variance was observed between both assays.

- **INTRA-ASSAY PRECISION**

3 replicas of 5 serial dilutions of 3 different vials of the product were performed by the same operator under identical qPCR conditions.
Less than 5% coefficient of variance was observed between all assays.

- **INTER-ASSAY PRECISION**

3 different replicas of 5 different serial dilutions of 1 vial of the product were individually amplified by 2 different operators on 2 consecutive days.
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)
	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
NO-OP	Do not open until use

BIBLIOGRAPHY:

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5. Stralin K, Backman A, Holmberg H, Fredlund H, Olcen P. (2005). Design of a multiplex PCR for *Streptococcus pneumoniae*, Haemophilus influenzae, Mycoplasma pneumoniae and Chlamydophila pneumoniae to be used on sputum samples. APMIS. 113:99-111.

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