

EVALUATION OF AMPLIRUN® TOTAL MACROLIDE RESISTANT MGE CONTROL PANEL (SWAB)

1. Introduction

AMPLIRUN® TOTAL MACROLIDE RESISTANT MGE CONTROL PANEL (SWAB) (MBTC029) has been evaluated using **ResistancePlus®** MG FlexiBle kit (SpeedX) on the GeneXpert® Platform (Cepheid, USA). This kit is a qualitative multiplexed *in vitro* diagnostic real-time PCR test for the identification of *M. genitalium* and detection of 4 mutations that are associated with resistance to azithromycin (macrolide antibiotic). The purpose of this document is to summarize the results obtained in this verification study.

PRODUCT DESCRIPTION:

MBTC029: Inactivated *Mycoplasma genitalium* (MGE) cells formulated to mimic a genital specimen and intended to control sample processing, analysis and detection of MGE nucleic acids and genetic markers for macrolide resistance using the product as an external run control. The panel is a 3-member panel; 1 sensitive and 2 macrolide resistant strains.

KIT CONTENTS:

- 1 VIRCELL TOTAL MGE CONTROL (SWAB): 4 vials with lyophilized cells of *M. genitalium* sensitive type strain (20000-50000 copies/vial). Batch concentration is provided in Certificate of Analysis.
- 2 VIRCELL TOTAL MGE RESISTANT (A2059G) CONTROL (SWAB): 3 vials with lyophilized cells of *M. genitalium* (20000-50000 copies/vial) harboring the A2059G mutation in 23S rRNA gene that confers macrolide resistance. Batch concentration is provided in Certificate of Analysis.
- 3 VIRCELL TOTAL MGE RESISTANT (A2058G) CONTROL (SWAB): 3 vials with lyophilized cells of *M. genitalium* (20000-50000 copies/vial) harboring the A2058G mutation in 23S rRNA gene that confers macrolide resistance. Batch concentration is provided in Certificate of Analysis.

2. Objectives

The aims of this study were:

- 1) To develop a procedure for creating “mock” MGE positive specimens using AMPLIRUN® MYCOPLASMA GENITALIUM DNA CONTROL (MBC085) and DNA from mutant strains 23S rRNA (A2058G & A2059G) and qualify performance with the real-time PCR test.
- 2) To qualify performance of a panel of AMPLIRUN® TOTAL MACROLIDE RESISTANT MGE CONTROL PANEL (SWAB) (MBTC029) (4x wild type, 3x A2058G, 3x A2059G) with the real-time PCR test.

3. Study design

Material used:

- 1x kit of MGE wild-type DNA (MBC085).
- 1x kit of MGE mutant DNA, developed for the purpose of this evaluation using DNA from 23S rRNA A2058G & 23S rRNA A2059G mutant strains.

- 1x kit of MGE Total Process Controls (Total process controls consisting of *M. genitalium* cells in a matrix that simulates a genital swab) (MBTC029).
- 10x MGE negative urine specimens.
- 10x MGE negative vaginal swab in UTM™ specimens.
- Real-time PCR test:
 - 3x **ResistancePlus®** MG Flexible kits (Cat No. S2A-2000410*). Each kit contains 10 cartridges (30 cartridges used in total for the study).
- Controls:
 - 1x **ResistancePlus®** MG S2A Positive Control kit (Cat No. 95001)
 - Negative clinical specimens (urine and vaginal swab in UTM™)

Method:

Vircell AMPLIRUN® DNA controls:

1. Preparation DNA Control (MBC085) and DNA Controls from mutant strains 23S rRNA A2058G & 23S rRNA A2059G according to Vircell's instructions.
2. Create 'mock' MGE positive specimens by adding 5 µl of prepared DNA control to 1 ml of negative clinical specimen (urine or vaginal swab in UTM™). Test each mock MGE positive specimen using the **ResistancePlus®** MG Flexible test (table 1).

AMPLIRUN® DNA control (MBC085)	Urine samples	Vaginal swab in UTM™ samples	Expected results with ResistancePlus® MG Flexible	
			<i>M. genitalium</i>	23S rRNA mutation
MGE, 23S rRNA wild type	3	3	DETECTED	NOT DETECTED
MGE, 23S rRNA A2058G	3	3	DETECTED	DETECTED
MGE, 23S rRNA A2059G	3	3	DETECTED	DETECTED
	9	9		

Table 1. AMPLIRUN® DNA control testing. Specimen types and number.

Vircell AMPLIRUN® TOTAL process controls:

Run each control following the same procedure as though it were a clinical sample with the **ResistancePlus®** MG Flexible test (Table 2).

AMPLIRUN® TOTAL controls (MBTC029)	Number	Expected results with ResistancePlus® MG Flexible	
		<i>M. genitalium</i>	23S rRNA mutation
MGE, 23S rRNA wild type	4	DETECTED	NOT DETECTED
MGE, 23S rRNA A2058G	3	DETECTED	DETECTED
MGE, 23S rRNA A2059G	3	DETECTED	DETECTED
TOTAL	10		

Table 2. AMPLIRUN® TOTAL positive control and clinical samples required to create the 'mock' MGE positive specimens.

Results:

A summary of the AMPLIRUN® DNA controls and number of copies is presented in the following table 3.

Vircell AMPLIRUN® DNA controls	Number of copies per μL
AmpliRun® MGE DNA Control (Wild-type) - (MBC085)	16,000 cps/ μL
<i>Mycoplasma genitalium</i> Resistant DNA Control (A2058G) – not commercially available	13,000 cps/ μL
<i>Mycoplasma genitalium</i> Resistant DNA Control (A2059G) - not commercially available	11,000 cps/ μL

Table 3. DNA control material and expected number of copies.

Vircell AMPLIRUN® DNA controls: The Vircell AMPLIRUN® DNA controls were then spiked into negative clinical specimens (either urine or vaginal swab in UTM™) to create mock MGE positive samples. The mock samples were run with **ResistancePlus®** MG Flexible kit. All mock samples produced the expected results. These results are presented in the following table 4.

Sample ID	Test Result		Ct values		
	<i>M. genitalium</i>	23S rRNA mutation	MGE	23S rRNA	IC
wt_Urine_1	DETECTED	NOT DETECTED	19.3	33.6	17.4
wt_Urine_2	DETECTED	NOT DETECTED	18.9	33.5	16.8
wt_Urine_3	DETECTED	NOT DETECTED	19.2	32.9	17.5
wt_UTM_1	DETECTED	NOT DETECTED	18.4	32.3	17.3
wt_UTM_2	DETECTED	NOT DETECTED	18.9	33.2	17.6
wt_UTM_3	DETECTED	NOT DETECTED	18.3	31.8	17.3
58G_Urine_1	DETECTED	DETECTED	19.2	24.4	17.5
58G_Urine_2	DETECTED	DETECTED	19.3	24.4	17.8
58G_Urine_3	DETECTED	DETECTED	19.5	24.4	18.2
58G_UTM_1	DETECTED	DETECTED	19.1	23.6	16.8
58G_UTM_2	DETECTED	DETECTED	18.6	23.4	16.4
58G_UTM_3	DETECTED	DETECTED	18.9	23.7	16.6
59G_Urine_1	DETECTED	DETECTED	19.5	21.1	17.4
59G_Urine_2	DETECTED	DETECTED	20.2	21.6	18.5
59G_Urine_3	DETECTED	DETECTED	19.6	21.2	17.7
59G_UTM_1	DETECTED	DETECTED	21.4	21.7	17.6
59G_UTM_2	DETECTED	DETECTED	21.2	21.9	17.1
59G_UTM_3	DETECTED	DETECTED	21.3	21.9	17
Pos control (MGE 23S wild-type)	DETECTED	NOT DETECTED	24.4	0	20.2
UTM neg	NOT DETECTED	NOT DETECTED	0	0	17.2
Neg_control-urine	NOT DETECTED	NOT DETECTED	0	0	17.1

Table 4. **ResistancePlus®** MG Flexible results using AMPLIRUN® DNA controls. MGE (*M. genitalium*); 23S rRNA (gene region); IC (internal control).

Vircell AMPLIRUN® Total process controls: The Vircell AMPLIRUN® Total process controls were prepared and run with **ResistancePlus®** MG Flexible kit. One of the reactions aborted due to an error. All other samples produced the expected results (table 5).

Sample ID	Test Result		Ct values		
	<i>M. genitalium</i>	23S rRNA mutation	MGE	23S rRNA	IC
wt_1	DETECTED	NOT DETECTED	20	32.5	19.2
wt_2	DETECTED	NOT DETECTED	19.4	33.3	19
wt_3	DETECTED	NOT DETECTED	19.9	33.4	19.5
wt_4	DETECTED	NOT DETECTED	19.6	33.3	19.1
58G_1	DETECTED	DETECTED	19.7	24.6	19.1
58G_2	DETECTED	DETECTED	19.3	24.1	18.9
58G_3	DETECTED	DETECTED	19.4	24.3	19.1
59G_1	DETECTED	DETECTED	19.9	21.4	19
59G_2	DETECTED	DETECTED	20	21.2	19.4
59G_3	ERROR*	ERROR*	0	0	0
Neg control - urine	NOT DETECTED	NOT DETECTED	0	0	22.1
Pos control (MGE 23S wild-type)	DETECTED	NOT DETECTED	24.7	0	20.4

Table 5. **ResistancePlus®** MG Flexible results using AMPLIRUN® total control. MGE (*M. genitalium*); 23S rRNA (gene region); IC (internal control).

* Mechanical error related to the GeneXpert® cartridge

Conclusions

The results from this qualification study demonstrate that the AMPLIRUN® TOTAL MACROLIDE RESISTANT MGE CONTROL PANEL (SWAB) (MBTC029) of Vircell is suitable for use with the **ResistancePlus®** MG Flexible test from SpeedX. The 'mock' samples created using the AMPLIRUN® DNA control (MBC085) as well as the AMPLIRUN® Total process controls produced the expected results. Vircell AMPLIRUN® TOTAL controls may be used as part of verification of the commercial kits for simultaneous detection of *M. genitalium* and 4 mutations associated with resistance to azithromycin (macrolide-based antibiotic). This feature represents a great advantage where laboratories have limited access to known MGE positive clinical samples.

***ResistancePlus®** MG is distributed exclusively by Cepheid and may not be available in all countries. For more information, contact your Cepheid representative or email cepheid@cepheideurope.fr