Evaluating the Performance of Nucleic Acid Amplification Tests that Detect *Mycoplasma genitalium* and Mutations Associated with Macrolide Resistance Using Novel Quality Controls Formulated on Swabs

#3474

S. Rivers [1], P. Zhelev [1], A. Alagic [1], J. Li[1], C. Randall [1], M. Luscher [1], K. Hughes [1]

[1]*Microbix Biosystems – Mississauga, ON, Canada*

ASM MICROBE, HOUSTON, TX. JUNE 15-19TH 2022

INTRODUCTION

Mycoplasma genitalium (Mgen) is classified as a Sexually Transmitted Infection that is prevalent among 3% of the population[1]. While most cases are asymptomatic, infection can cause non-gonococcal urethritis in men, and cervicitis, pelvic inflammatory disease, and infertility in women. Macrolide antibiotics are often prescribed to patients with Mgen infections; however, the emergence of macrolide resistance has resulted in a high proportion of treatment failure (between 44-90% of infected patients)[1]. Due to the slow-growing nature of the bacterium, nucleic acid amplification tests are used for clinical diagnosis, which not only allows for rapid detection of Mgen but also the molecular markers that predict macrolide-resistant genotypes and phenotypes. Molecular assays that incorporate detection of mutations associated with macrolide resistance are commercially available in Europe and Australia, and under evaluation in the United States. To support clinical use of these assays, quality control materials are required for External Quality Assessment, assay verification/validation, laboratory personnel training, and ongoing Quality Control (QC). Unfortunately, since Mgen is challenging to culture, the availability of such materials is limited.

MATERIALS & METHODS

Microbix designed five inactivated antimicrobial resistant Mgen whole-process controls that each contain a macrolide resistance marker in the 23S rRNA gene (A2058G, A2058C, A2058T, A2059G, A2059C; *E. coli* numbering). The external controls are designed to simulate patient specimens by containing a challenging mixture of wild-type and macrolide resistant strains to mimic the standard process of adaptive antimicrobial resistant evolution, along with human cells. The external controls are formulated on a Copan FLOQSwab® to ensure compatibility with all assay workflows and elution buffers, and stability at 2-30°C storage conditions. External control performance was evaluated using melting curve, dual primer, and PlexZyme® nucleic acid amplification test technologies, with the latter being the most advanced methodology for single nucleotide polymorphism detection.

Table 1: Samples Used in this Study

Product Name	Cat. #	Intended Use
M.genitalium A2059G Swab Positive Control	RED-S-63-02	RED FLOQ IVD Control
M.genitalium A2058G Swab Positive Control	RED-S-63-03	
M.genitalium A2059C Swab Positive Control	RED-S-63-04	
M.genitalium A2058T Swab Positive Control	RED-S-63-05	
M.genitalium A2058C Swab Positive Control	RED-S-63-06	
M.genitalium A2059G Swab Positive Sample	VP-S-63-02	PROCEED FLOQ Research Use Only
M.genitalium A2058G Swab Positive Sample	VP-S-63-03	
M.genitalium A2059C Swab Positive Sample	VP-S-63-04	
M.genitalium A2058T Swab Positive Sample	VP-S-63-05	
M.genitalium A2058C Swab Positive Sample	VP-S-63-06	

RESULTS

1. ddPCR

Table 2: The Macrolide Resistant Mgen Product Concentrations were Quantified via ddPCR

Product Name	Cat. #	Approximate Concentration (Copies/swab)
M.genitalium A2059G Swab Positive Sample	VP-S-63-02	3.67E+04
M.genitalium A2058G Swab Positive Sample	VP-S-63-03	3.67E+04
M.genitalium A2059C Swab Positive Sample	VP-S-63-04	1.84E+05
M.genitalium A2058T Swab Positive Sample	VP-S-63-05	3.74E+03
M.genitalium A2058C Swab Positive Sample	VP-S-63-06	6.25E+03

2. Evaluate Product Performance Using PlexZyme® Technology

SpeeDx

Table 3: Product Performance with ResistancePlus® MG Assay

Product Name (RED FLOQ)	Target Analytes						Outcome
	MgPa	A2058G	A2059G	A2058C	A2059C	A2058T	
M.genitalium A2059G Swab Positive Control	+	-	+	-	-	-	Positive for Mgen DNA and A2059G mutation
M.genitalium A2058G Swab Positive Control	+	+	-	-	-	-	Positive for Mgen DNA and A2058G mutation
M.genitalium A2059C Swab Positive Control	+	-	-	-	+	-	Positive for Mgen DNA and A2059C mutation
M.genitalium A2058T Swab Positive Control	+	-	-	-	-	+	Positive for Mgen DNA and A2058T mutation
M.genitalium A2058C Swab Positive Control	+	-	-	+	-	-	Positive for Mgen DNA and A2058C mutation

3. Evaluate Product Performance Using Dual Primer Technology

Seegene

Table 4: Product Performance with Allplexó MG&AziR Assay

Product Name (RED FLOQ)	Target Analytes						Outcome
	WT	A2058G	A2059G	A2058C	A2059C	A2058T	
M.genitalium A2059G Swab Positive Control	+	-	+	-	-	-	Positive for Mgen DNA and A2059G mutation
M.genitalium A2058G Swab Positive Control	+	+	-	-	-	-	Positive for Mgen DNA and A2058G mutation
M.genitalium A2059C Swab Positive Control	+	-	-	-	+	-	Positive for Mgen DNA and A2059C mutation
M.genitalium A2058T Swab Positive Control	+	-	-	-	-	+	Positive for Mgen DNA and A2058T mutation
M.genitalium A2058C Swab Positive Control	+	-	-	+	-	-	Positive for Mgen DNA and A2058C mutation

RESULTS CONTINUED

4. Evaluate Product Performance Using Melting Curve Technology

Table 5: Product Performance with Real-Time PCR

Product Name (RED FLOQ)	Target Analytes						Outcome
	MgPa	A2058G	A2059G	A2058C	A2059C	A2058T	
M.genitalium A2059G Swab Positive Control	+	-	-	-	-	-	Positive for Mgen DNA. Negative for A2059G mutation
M.genitalium A2058G Swab Positive Control	+	+	-	-	-	-	Positive for Mgen DNA and A2058G mutation
M.genitalium A2059C Swab Positive Control	+	-	-	-	+/-	-	Positive for Mgen DNA, but non-reproducible results for A2059C mutation
M.genitalium A2058T Swab Positive Control	+	-	-	-	-	-	Positive for Mgen DNA. Negative for A2058T mutation
M.genitalium A2058C Swab Positive Control	+	-	-	+	-	-	Positive for Mgen DNA and A2058C mutation

CONCLUSION

In conclusion, the macrolide resistant Mgen positive swabs successfully performed when tested with PlexZyme® and Dual Primer nucleic acid amplification test methodologies. The melting curve technology could not consistently differentiate between wild-type and macrolide resistant strains, which is likely due to the ratio of wild-type:macrolide resistant strains included in the formulation. While it is possible to adjust the formulations, the results suggest that melting curve technology may not be the most suitable approach for identifying antimicrobial resistant genotypes and phenotypes in practice – particularly in cases where patient samples are comprised of a mixture of wild-type and resistant strains as part of the adaptive evolution of antimicrobial resistant types. Instead, advanced technologies, such as PlexZyme® and Dual Primer, should be the preferred methods for single-nucleotide polymorphism detection in the challenging cases of mixed wild type and muti-drug resistant patient samples.

Overall, Macrolide resistant Mgen samples desiccated on a Copan FLOQSwab® are advantageous QC materials that support the clinical use and accuracy of emerging Mgen molecular assays. The products showed acceptable performance on multiple platforms, thereby demonstrating their potential use as cross-platform compatible quality controls, verification panels, and External Quality Assessment samples.

ACKNOWLEDGEMENTS

We would like to acknowledge our collaboration with SpeeDx Pty Ltd

REFERENCES

[1] CDC. (2021, July 22). *Mycoplasma Genitalium - STI Treatment Guidelines*. Centers for Disease Control and Prevention. Retrieved January 16, 2023, from https://www.cdc.gov/std/treatment-guidelines/mycoplasmagenitalium.htm

CONTACT INFORMATION

Pavel Zhelev, Director, Product Management
Email: pavel.zhelev@microbix.com

Microbix Biosystems Inc.
+1-800-794-6694
+1-905-361-8910
www.microbix.com