

## Certificate of Analysis for NR-51701

### Mycobacterium ulcerans, Strain S4018

### Catalog No. NR-51701

#### **Product Description:**

*Mycobacterium ulcerans (M. ulcerans)*, strain S4018 was isolated in December 2006 from the cutaneous lesion of an active Buruli ulcer on a male child in Benin. NR-51701 was produced by the depositor and was harvested and vialed at ATCC<sup>®</sup>.

Lot: 70029957 Manufacturing Date: 30SEP2019

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis <sup>1</sup>		
Cellular Morphology (pre-vial)	Report results	Gram-positive rods
Colony morphology	Report results	Irregular, raised, undulate, rough
28 days at 30°C in an aerobic atmosphere on		and yellow (Figure 1) <sup>2</sup>
Lowenstein-Jensen agar		
Motility (wet mount)	Report results	Non-motile
Growth rate	≥ 7 days	28 days
Growth at 45°C	Negative	Negative
Growth at 55°C	Negative	Negative
Acid-fast stain	Positive (red colonies)	Positive (red colonies)
Pigmentation in the dark (Scotochromogen)	Negative (no pigment)	Negative (no pigment)
Photoinduction for 1 hour (Photochromogen)	Negative	Negative
Nonchromogen (no pigment)	Positive	Positive
Biochemical tests <sup>3,4</sup>		
Catalase	Report results	Positive <sup>5</sup>
Catalase (semiquantitative)	Report results	Negative
Catalase (68°C)	Report results	Positive <sup>5</sup>
Iron uptake	Report results	Negative
Nitrate reduction	Negative	Negative
Tween 80	Negative	Negative
Urease	Report results	Negative <sup>6</sup>
Growth in the presence of 5% sodium chloride	Negative	Negative
Growth in the presence of thiophene-2-carboxylic	Negative	Negative
acid hydrazide (TCH)	-	
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene	≥ 99% sequence identity to	99.5% sequence identity to
(~ 1430 base pairs)	M. ulcerans, strain S4018	M. ulcerans, strain S4018
, ,	(GenBank: MDUB01000259.1)	(GenBank: MDUB01000259.1) <sup>7</sup>
Sequencing of Heat Shock Protein 65 gene	≥ 99% sequence identity to	99.8% sequence identity to
(~ 440 base pairs)	M. ulcerans, strain S4018	M. ulcerans, strain S4018
, ,	(GenBank: MDUB01000482.1)	(GenBank: MDUB01000482.1)
Purity (post-freeze)	,	, , , , , , , , , , , , , , , , , , ,
Middlebrook 7H10 agar with OADC enrichment	Growth consistent with expected	Growth consistent with expected
28 days at 30°C in an aerobic atmosphere	colony morphology	colony morphology
Tryptic Soy agar	Report results	No growth
28 days at 37°C in an aerobic atmosphere with 5% CO <sub>2</sub>		
Viability		
28 days at 30°C in an aerobic atmosphere on	Growth	Growth
Middlebrook 7H10 agar with OADC enrichment		
1 Information on Musehosterium testing is sucilable from Dibán M	<u> </u>	<u> </u>

¹Information on *Mycobacterium* testing is available from Ribón, W. "Biochemical Isolation and Identification of Mycobacteria, Biochemical Testing" <u>Biochemical Testing</u>. (2012) Jose C. Jimenez-Lopez (Ed.), InTech, Available from: <a href="http://www.intechopen.com/books/biochemical-testing/biochemical-isolation-and-identification-of-mycobacteria">http://www.intechopen.com/books/biochemical-testing/biochemical-isolation-and-identification-of-mycobacteria</a> and Lévy-Frébault, V. V. and F. Portaels. "Proposed Minimal Standards for the Genus *Mycobacterium* and for Description of New Slowly Growing *Mycobacterium* Species." <u>Int. J. Syst. Bacteriol.</u> 42 (1992): 315-323. PubMed: 1581193. <sup>2</sup>Yellow colonies indicate the presence of the virulence plasmid expressing the toxin mycolactone. Repeated passaging may result in white colonies, indicating the loss of the virulence plasmid. For more information, please see Nakanaga, K., et. al. "Naturally Occurring Loss of a Giant Plasmid from *Mycobacterium ulcerans* subsp. *shinshuense* Makes it Non-Pathogenic." <u>Sci. Rep.</u> 8 (2018): 8218. PubMed: 29844323.
³Negative tests are observed for >7 days.

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<sup>4</sup>Biochemical test results rule out *M. marinum, M. pseudoshottsi* and *M. shottsi*.

<sup>7</sup>Also consistent with *M. basiliense*, *M. liflandii*, *M. marinum*, *M. pseudoshottsi* and *M. shottsi* 

Figure 1: Colony Morphology



/Heather Couch/ Heather Couch

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Program Manager or designee, ATCC Federal Solutions

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<sup>&</sup>lt;sup>5</sup>Most strains of *Mycobacterium ulcerans* are negative for this test; however, up to 15% of strains are positive.

<sup>&</sup>lt;sup>6</sup>Most strains of *Mycobacterium ulcerans* are negative for this test; however, 15% to 49% of strains are positive.