b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for HM-1296

Streptococcus sp., Strain CMW7705B

Catalog No. HM-1296

Product Description: *Streptococcus* sp., strain CMW7705B is a vaginal isolate obtained in 2014 from a pregnant woman in St. Louis, Missouri, USA. [HM-1296 was deposited to BEI Resources as *Streptococcus mitis*, however, digital DNA-DNA hybridization (dDDH) analysis, performed at BEI Resources, could not confirm the species-level classification.]

Lot[1,2]: 70006888 **Manufacturing Date: 28JUL2017**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive cocci	Gram-positive cocci
Colony morphology[3]	Report results	Circular, low convex, entire, smooth and gray (Figure 1)
Motility (wet mount)	Report results	Non-motile
VITEK® MS (MALDI-TOF)	*Streptococcus* sp.	*Streptococcus mitis/Streptococcus oralis* (99.9%)
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 1490 base pairs)	≥ 99.9% sequence identity to *Streptococcus* sp., strain CMW7705B (GenBank: LRQR01000051.1)	99.9% sequence identity to *Streptococcus* sp., strain CMW7705B (GenBank: LRQR01000051.1)
Digital DNA-DNA hybridization (dDDH)[4]	≥ 70% for species identification	*Streptococcus mitis* (53.8%)[5]
Purity (post-freeze)[6]	Consistent with expected colony morphology	Consistent with expected colony morphology
Viability (post-freeze)[3]	Growth	Growth

[1]Quality control of HMP material is only performed to demonstrate that the material distributed by BEI Resources is identical to the deposited material. It should not be considered a complete characterization of the deposited organism.

[2]*Streptococcus* sp., strain CMW7705B was deposited by Amanda Lewis, Ph.D., Assistant Professor, Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri, USA. HM-1296 was produced by inoculation of the deposited material into Tryptic Soy broth, which was used to inoculate Brucella agar with hemin (5 µg/mL) and vitamin K1 (10 µg/mL) supplemented with 5% defibrinated sheep blood and grown for 1 day at 37°C in an aerobic atmosphere. Colonies from the plate were then suspended in Tryptic Soy broth and preserved in 10% glycerol. HM-1296 was produced by inoculation of the preserved material into Tryptic Soy broth and grown for 1 day at 37°C in an aerobic atmosphere. The material from the initial growth was passaged once in Tryptic Soy broth for 1 day at 37°C in an aerobic atmosphere to produce this lot.

[3]1 day at 37°C in an aerobic atmosphere on Brucella agar with hemin (5 µg/mL) and vitamin K1 (10 µg/mL) supplemented with 5% defibrinated sheep blood

[4]Relatedness between bacterial strains has traditionally been determined using DDH. For additional information refer to Auch, A. F., et al. "Digital DNA-DNA Hybridization for Microbial Species Delineation by Means of Genome-to-Genome Sequence Comparison." Stand Genomic Sci. 2 (2010): 117-134. PubMed: 21304684.

[5]*Streptococcus* sp., strain CMW7705B has the highest similarity to *Streptococcus mitis*, however, the similarity is below the 70% threshold for species identification. The lack of complete type strain genome coverage across the entire genus prevents a definitive determination of species.

[6]Purity of this lot was assessed for 7 days at 37°C in an aerobic atmosphere with 5% CO_2 on Tryptic Soy agar with 5% defibrinated sheep blood.

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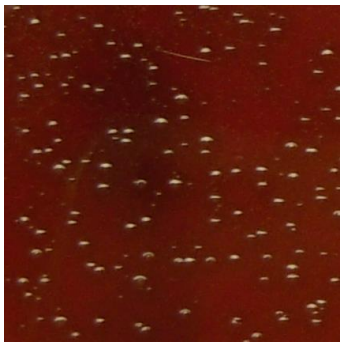
Figure 1: Colony Morphology



Date: 07 NOV 2017 **Signature:**

BEI Resources Authentication

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected by ATCC® to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.