

## Reference Material 8376

# Microbial Pathogen DNA Standards for Detection and Identification

## REFERENCE MATERIAL INFORMATION SHEET

**Purpose:** This reference material (RM) is intended for harmonizing measurements of abundance and identity using next-generation sequencing-based metagenomics.

**Description:** A unit of RM 8376 consists of 20 tubes (components) containing either bacterial (19 tubes) or human (1 tube) genomic DNA in an aqueous buffer (1 × Tris-EDTA). Each component contains approximately 100 µL of solution. The bacterial components are at a nominal concentration of 50 ng/µL, while the human DNA is nominally 100 ng/µL.

**Non-Certified Values:** Non-certified values are best estimates based on currently available information. However, they do not meet NIST's criteria for certification. Non-certified values do not provide metrological traceability to the International System of Units (SI) or other higher-order reference system [1]. Non-certified values for chromosomal copy number concentration are provided below. The non-certified values are metrologically traceable to the materials and procedures used in their determination.

Part	Name	Organism	Chromosomal Copy Number Concentration ×10 <sup>6</sup> (copy/µL) <sup>(a)</sup>
A	<i>E. coli</i> ATCC <sup>®(b)</sup> 43895 <sup>TM</sup> DNA	<i>Escherichia coli</i> O157:H7	8.84 ± 0.38
B	<i>E. coli</i> ATCC <sup>®</sup> BAA-2309 <sup>TM</sup> DNA	<i>Escherichia coli</i> O104:H4	8.89 ± 0.28
C	<i>S. enterica</i> ATCC <sup>®</sup> 700720 <sup>TM</sup> DNA	<i>Salmonella enterica</i> subsp. <i>enterica</i>	9.72 ± 0.38
D	<i>S. enterica</i> ATCC <sup>®</sup> 12324 <sup>TM</sup> DNA	<i>Salmonella enterica</i> subsp. <i>arizonae</i>	10.84 ± 0.52
E	<i>S. aureus</i> ATCC <sup>®</sup> BAA-44 <sup>TM</sup> DNA	<i>Staphylococcus aureus</i>	16.49 ± 0.76
F	<i>S. aureus</i> ATCC <sup>®</sup> 12600 <sup>TM</sup> DNA	<i>Staphylococcus aureus</i>	17.38 ± 0.68
G	<i>S. epidermidis</i> ATCC <sup>®</sup> 12228 <sup>TM</sup> DNA	<i>Staphylococcus epidermidis</i>	15.99 ± 0.60
H	<i>P. aeruginosa</i> ATCC <sup>®</sup> BAA-47 <sup>TM</sup> DNA	<i>Pseudomonas aeruginosa</i>	8.27 ± 0.34
I	<i>A. baumannii</i> ATCC <sup>®</sup> 19606 <sup>TM</sup> DNA	<i>Acinetobacter baumannii</i>	12.01 ± 0.56
J	<i>N. meningitidis</i> ATCC <sup>®</sup> 13077 <sup>TM</sup> DNA	<i>Neisseria meningitidis</i>	21.67 ± 0.94
K	<i>S. pyogenes</i> ATCC <sup>®</sup> 12344 <sup>TM</sup> DNA	<i>Streptococcus pyogenes</i>	22.55 ± 0.86
L	<i>E. faecalis</i> ATCC <sup>®</sup> 19433 <sup>TM</sup> DNA	<i>Enterococcus faecalis</i>	14.75 ± 0.50
M	<i>A. xylosoxidans</i> ATCC <sup>®</sup> 27061 <sup>TM</sup> DNA	<i>Achromobacter xylosoxidans</i>	7.28 ± 0.36
N	<i>A. hydrophila</i> ATCC <sup>®</sup> 35654 <sup>TM</sup> DNA	<i>Aeromonas hydrophila</i>	9.97 ± 0.34
O	<i>K. pneumoniae</i> ATCC <sup>®</sup> 13883 <sup>TM</sup> DNA	<i>Klebsiella pneumoniae</i>	7.68 ± 0.36
P	<i>S. sonnei</i> ATCC <sup>®</sup> 25931 <sup>TM</sup> DNA	<i>Shigella sonnei</i>	9.67 ± 0.36
Q	<i>V. furnissii</i> ATCC <sup>®</sup> 35016 <sup>TM</sup> DNA	<i>Vibrio furnissii</i>	9.70 ± 0.36
R	<i>L. monocytogenes</i> ATCC <sup>®</sup> 19115 <sup>TM</sup> DNA	<i>Listeria monocytogenes</i>	17.39 ± 0.64
S	<i>L. pneumophila</i> ATCC <sup>®</sup> 33152 <sup>TM</sup> DNA	<i>Legionella pneumophila</i>	13.63 ± 0.46
T	<i>Homo sapiens</i> GM24385 DNA	Human	0.0323 ± 0.0015

<sup>(a)</sup> The values are expressed as  $x \pm 2u(x)$ , where  $x$  is the value and  $u(x)$  is the standard uncertainty of  $x$ . The standard uncertainty combines reaction volume, unit, and repetition. While the best estimate value lies within the interval  $x \pm 2u(x)$ , this interval may not include the true value. For guidance in using and propagating this uncertainty, see reference 2.

<sup>(b)</sup> American Type Culture Collection (ATCC).

**Period of Validity:** The non-certified values are valid within the measurement uncertainty specified until **01 January 2025**. The value assignments are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

**Maintenance of Non-Certified Values:** NIST will monitor this material to the end of its period of validity. If substantive technical changes occur that affect the non-certified values during this period, NIST will update this Reference Material Information Sheet. Before making use of any of the values delivered by this material, users should obtain the most recent version of this documentation, available free of charge through the <https://www.nist.gov/srm> website.

**Safety:** RM 8376 IS INTENDED FOR RESEARCH USE. This is a bacterial and human DNA source material. Since there is no consensus on the infectious status of extracted DNA, handle RM 8376 components as Biosafety Level 1 materials capable of transmitting infectious disease, as recommended by the Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) [3]. RM 8376 components and derived solutions should be disposed of in accordance with local, state, and federal regulations.

This RM was developed after an appropriate human subjects research determination.

**Storage:** For optimal longevity, RM 8376 material should be stored at 4 °C in the dark. The material will remain stable at room temperature for extended periods of time (approximately 22 °C, 11 weeks) if the tubes remain sealed. **Do not freeze** the material, as this can alter the concentration and structure of the DNA. Diluted material is unstable, and not suitable for storage. Do not expose any DNA solution to direct sunlight.

**Use:** Prior to removing sample aliquots for analysis, the vial should be **mixed by horizontal vortexing for 5 seconds** and briefly centrifuged without opening the vial cap. For the reference values to be valid, materials should be withdrawn soon after opening the vials and processed without delay. Dilutions of aliquots of these materials may be made as appropriate for each application, but they must be used immediately.

**Additional Information:** The ATCC trademark and trade name, and any and all ATCC catalog numbers are trademarks of the American Type Culture Collection.

## REFERENCES

- [1] Beauchamp, C.R.; Camara, J.E.; Carney, J.; Choquette, S.J.; Cole, K.D.; DeRose, P.C.; Duewer, D.L.; Epstein, M.S.; Kline, M.C.; Lippa, K.A.; Lucon, E.; Phinney, K.W.; Polakoski, M.; Possolo, A.; Sharpless, K.E.; Sieber, J.R.; Toman, B.; Winchester, M.R.; Windover, D.; *Metrological Tools for the Reference Materials and Reference Instruments of the NIST Material Measurement Laboratory*; NIST Special Publication (NIST SP) 260-136; U.S. Government Printing Office: Washington, DC (2020); available at <https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2020.pdf> (accessed Jun 2021).
- [2] Possolo, A.; *Evaluating, Expressing, and Propagating Measurement Uncertainty for NIST Reference Materials*; NIST Special Publication (NIST SP) 260-202; U.S. Government Printing Office: Washington, DC (2020); available at <https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-202.pdf> (accessed Jun 2021).
- [3] CDC/NIH: *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed.; HHS publication No. (CDC) 21-1112; Chosewood, L.C.; Wilson, D.E.; Eds.; U.S. Government Printing Office: Washington, DC (2009); available at <https://www.cdc.gov/biosafety/publications/bmb15/> (accessed Jun 2021).

*Certain commercial equipment, instruments, or materials may be identified in this Reference Material Information Sheet to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.*

*Users of this RM should ensure that the Reference Material Information Sheet in their possession is current. This can be accomplished by contacting the Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, MD 20899-2300; telephone (301) 975-2200; e-mail [srminfo@nist.gov](mailto:srminfo@nist.gov); or the Internet at <https://www.nist.gov/srm>*

\*\*\*\*\* End of Reference Material Information Sheet \*\*\*\*\*