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U.S. DEPARTMENT OF COMMERCE

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Reference Material® 8038
H5N1 (Avian Influenza) Synthetic RNA Fragments
REFERENCE MATERIAL INFORMATION SHEET

Purpose: The non-certified values delivered by this Reference Material (RM) are intended for assessing performance characteristics of influenza A/H5 subtyping diagnostic assays for the detection of H5 avian influenza.

Description: A unit of RM 8038 consists of one vial each of three RNA materials: Part A: H5_HA, Part B: N1_NA, and Part C: MP. Each vial contains approximately 100 µL of RNA in a background of 5 ng/µL Jurkat RNA in a buffered solution. Sequences for each RNA were derived from the A/American Wigeon/South Carolina/22/2021 virus isolate and encode the H5 hemagglutinin (H5_HA), N1 neuraminidase (N1_NA), and matrix proteins 1 and 2 (MP) gene segments.

Non-Certified Values: Non-certified values are suitable for use in method development, method harmonization, and process control but do not provide metrological traceability to the International System of Units (SI) or other higher-order reference system [1]. Non-certified genome copy number values expressed as copies per µL are provided below.

Part	Material	Concentration (copies/µL)	95 % Credible Interval[(a)] (copies/µL)	Standard Uncertainty (copies/µL)
A	H5_HA	1.738×10^6	1.414×10^6 to 2.210×10^6	1.866×10^5
B	N1_NA	2.615×10^6	1.755×10^6 to 3.595×10^6	4.387×10^5
C	MP	1.182×10^6	8.562×10^5 to 1.830×10^6	2.452×10^5

[(a)] The true concentration value of the material is expected to be within the given credible interval with about a 95 % level of confidence.

Additional Information: Concentration measurements were determined by reverse transcription droplet digital polymerase chain reaction (RT-ddPCR) using the One-Step RT-ddPCR Advanced Kit for Probes and the QX200 System . The sequence information for each RNA fragment can be found in Appendix A. Primer and probe sequences for each assay and thermal cycling conditions can be found in Appendix B. The sequence information can also be found at the NIST storefront at https://shop.nist.gov/ccrz__ProductDetails?sku=8038.

Period of Validity: The non-certified values are valid within the measurement uncertainty specified until 05 January 2031. 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 verify they have the most recent version of this documentation, available through the NIST SRM website (https://www.nist.gov/srm).

Rebecca A. Zangmeister, Acting Chief
Biomolecular Measurement Division

Steven J. Choquette, Director
Office of Reference Materials

Safety: This is a human-source material. It should be handled as a Biosafety Level 1 material, according to applicable federal, state, and/or local regulations, and according to the policies and procedures of the recipient's organization.

Storage: The original unopened vials of RM 8038 should be stored frozen between –60 °C and –80 °C. An open vial can be refrozen and thawed up to three times without affecting concentration measurements.

Use: RM 8038 IS INTENDED FOR RESEARCH USE. Before use, the contents of the unopened vials should be thawed and mixed by brief vortexing. Centrifuge briefly before opening vials.

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.; Molloy, J.; Nelson, M.A.; Phinney, K.W.; Polakoski, M.; Possolo, A.; Sander, L.C.; Schiel, J.E.; Sharpless, K.E.; 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 260-136, 2021 edition; National Institute of Standards and Technology, Gaithersburg, MD (2021); available at https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2021.pdf (accessed Jan 2026).

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; or the Internet at https://www.nist.gov/srm.

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APPENDIX A

Fragment sequence for Part A H5_HA:

GGTTCACTCTGTCAAAATGGAGAACATAGTACTACTTCTTGCAATAGTTAGCCTTGTTAAAAGTGATC
AGATTTGCATTGGTTACCATGCAAACAATTCGACAGAGCAAGTTGACACGATAATGGAAAAGAACGT
CACTGTTACACATGCCCAAGACATACTGGAAAAAACACACAACGGGAAGCTCTGTGATCTAAATGG
GGTGAAGCCTCTGATTTTAAAGGATTGTAGTGTAGCTGGATGGCTCCTCGGAAACCCAATGTGCGAC
GAATTCATCAGAGTGCCTGAATGGTCCTACATAGTGGAGCGGGCTAACCCAGCTAATGACCTCTGTT
ACCCAGGGAGCCTCAATGACTATGAAGAACTGAAACACATGTTGAGCAGAATAAATCATTTTGAGA
AGATTCTGATCATCCCCAAGAGTTCCTGGCCAAATCATGAAACATCACTAGGGGTGAGCGCAGCTTG
TCCATACCAGGGAGCGCCCTCCTTTTTCAGAAATGTGGTGTGGCTTATCAAAAAGAACGATGCATAC
CCAACAATAAAGATAAGCTACAATAATACCAATCGGGAAGATCTCTTGATACTGTGGGGGATTCATC
ATTCCAACAATGCAGAAGAGCAGACAAATCTCTACAAAAACCCAACCACCTACATTTCAGTTGGAAC
ATCAACTTTAAACCAGAGGTTGGCACCAAAAATAGCTACTAGATCCCAAGTAAACGGGCAACGTGG
AAGAATGGACTTCTTCTGGACAATCTTAAAACCAGATGATGCAATCCATTTCGAGAGTAATGGAAAT
TTCATTGCTCCAGAATATGCATACAAAATTGTCAAGAAAGGGGACTCAACAATTATGAAAAGTGGAG
TGGAATATGGCCACTGCAACACCAAATGTCAAACCCCAGTAGGTGCGATAAATTCTAGTATGCCATT
CCACAACATACATCCTCTCACCATTGGGGAATGCCCCAAATACGTGAAGTCAAACAAGTTGGTCCTT
GCGACTGGGCTCAGAAATAGTCCTCTACGAGAAACGCGAGGCCTGTTTGGGGCGATAGCAGGGTTTA
TAGAGGGAGGATGGCAGGGAATGGTTGATGGTTGGTATGGGTACCATCATAGCAATGAGCAGGGGA
GTGGGTACGCTGCGGACAAAGAATCCACCCAAAAGGCAATAGATGGAGTTACCAATAAGGTCAACT
CAATCATTGACAAAATGAACACTCAATTTGAGGCAGTTGGAAGGGAGTTTAATAACTTAGAAAGGA
GGATAGAGAATTTGAACAAGAAAATGGAAGACGGATTCCTAGATGTCTGGACCTATAATGCTGAACT
TCTAGTTCTCATGGAAAACGAGAGGACTCTAGATTTCCATGATTCAAATGTCAAGAACCTTTACGAC
AAAGTCAGATTACAGCTTAGGGATAATGCAAAGGAGCTGGGTAACGGCTGTTTCGAATTCTATCACA
AATGTGATAATGAATGTATGGAAAGTGTGAGAAATGGGACGTATGACTACCCTCAGTATTCAGAAGA
AGCAAGATTAAAAAGAGAAGAAATAAGCGGAGTGAAATTAGAATCAGTAGGAACTTACCAGATACT
GTCAATTTATTCAACAGCGGCAAGTTCCCTAGCACTGGCAATCATGATGGCTGGTCTATCTTTATGGA
TGTGCTCCAATGGGTCGTTACAGTGCAGAATTTGCATTTAGATTTGTGAGCTCAGATTGTAGTTAAAA
ACAC

Fragment sequence for Part B N1_NA:

AGTTCAAAATGAATCCAAATCAAAAGATAACAACCATTGGATCAATCTGTATGGTAATTGGGATAGT
CAGCTTGATGCTGCAAATTGGGAACATAATCTCAATATGGGTTAGCCATTCAATCCAAACAGGGAAC
CAATACCAGCCTGAACCATGCAATCAAAGCATCATTACCTATGAGAACAACACCTGGGTAAATCAGA
CGTATGTCAACATCAGCAATACCAATTTTCTTGCTGAGCAGGCTGTTACTTCGGTAACATTAGCGGGC
AATTCATCTCTTTGCCCTATTAGTGGGTGGGCAATATACAGTAAGGACAACGGTATAAGAATTGGGT
CCAAGGGGGATGTGTTTGTTATAAGAGAACCGTTCATCTCATGCTCCCACTTGGAATGCAGAACCTTT
TTCCTGACCCAGGGAGCTCTGCTGAATGACAAACATTCTAATGGGACCGTTAAGGATAGAAGCCCTT
ATAGAACTTTGATGAGTTGTCCCGTGGGTGAGGCTCCTTCCCCGTACAATTCAAGATTTGAGTCTGTT
GCTTGGTCGGCAAGTGCTTGTCATGATGGCATCAGTTGGTTGACAATCGGTATTTCTGGTCCAGACAA
TGGAGCTGTGGCTGTATTGAAGTACAATGGCATAATAACGGATACTATCAAGAGTTGGAGAAACAAC
ATTTTGAGAACTCAAGAATCTGAATGTGCGTGCGTAAATGGCTCTTGCTTCACCGTAATGACTGATGG
ACCAAGCAATGGGCAGGCCTCATATAAAATCTTCAAGATAGATAAAGGGAAAGTTGTCAAATCAGTT
GAATTGAATGCCCCTAATTACCACTACGAGGAATGCTCCTGTTATCCTGATGCGGGTGATATTATGTG
TGTGTGCAGGGACAATTGGCATGGCTCAAACCGGCCGTGGGTATCTTTTAATCAAAATCTGGAGTAT
CAAATAGGATATATATGCAGTGGGGTTTTCGGGGACAATCCCCGCCCCAATGATGGAACAGGCAGTT
GCAGTCCAATGTCCTCTAATGGGGCATATGGGGTAAAAGGGTTTTCATTTAAGTACGGTAATGGGGT
TTGGATCGGAAGAACAAAAAGCACTAGTTCCAGAAGCGGCTTTGAGATGATTTGGGATCCGAATGG
GTGGACTGAGACGGACAGTAGTTTCTCAGTGAAGCAAGACATTGTAGAAATAACTGACTGGTCAGG
ATATAGTGGGAGTTTTGTCCAGCATCCAGAACTGACAGGATTAGATTGCATGAGGCCTTGTTTCTGG
GTTGAGCTAATTAGAGGGAGGCCCAAAGAGAACACAATTTGGACTAGCGGGAGCAGCATATCCTTTT
GTGGTGTAAATAGTGACACTGTGGGTTGGTCTTGGCCAGACGGTGCTGAGTTGCCATTCACCATTGA
CAAGTAGTTTGTTCAAAAAACT

Fragment sequence for Part C MP:

ATGAGTCTTCTAACCGAGGTCGAAACGTACGTTCTCTCTATCGTCCCGTCGGGCCCCCTCAAAGCCGA
GATCGCGCAGAGACTTGAAGATGTCTTTGCAGGGAAGAACACCGATCTTGAGGCTCTCATGGAATGG
CTAAAGACAAGACCAATCCTGTCACCTCTGACTAAGGGGATTTTGGGATTTGTGTTCACGCTCACCGT

GCCCAGTGAGCGAGGACTGCAGCGTAGACGCTTTGTCCAAAATGCTCTAAATGGAAATGGAGACCC
AAACAACATGGACAGGGCAGTCAAGTTGTACAGGAAATTGAAGAGAGAGATAACATTCCATGGGGC
TAAAGAAGTTGCACTCAGTTACTCAACCGGTGCACTTGCCAGTTGTATGGGTCTCATATACAACAGG
ATGGGGACGGTGACCGCAGAAGTGGCATTGGGCCTAGTGTGTGCCACCTGTGAGCAGATTGCTGATT
CACAGCATCGGTCTCACAGACAGATAGCTACCACCACCAACCCACTAATCAGACATGAAAACAGAA
TGGTGTTGGCCAGTACTACAGCTAAGGCTATGGAGCAGATGGCTGGATCGAGTGAGCAAGCAGTGG
AAGCCATGGAGGTTGCTAGTCAGGCTAGGCAGATGGTGCAGGCGATGAGGACCATTGGAACTCATC
CTAGCTCCAGTGCCGGTCTGAGAGATGATCTCCTTGAAAATTTGCAGGCCTACCAAAAACGGATGGG
AGTGCAACTGCAGCGATTCAAGTGATCCTCTCGTTATTGCCGCAAGTATCATTGGGATCTTGCACTTG
ATATTGTGGATTCTTGATCGCCTTTTCTTCAAATGCGTTTATCGTCGCCTTAAATACGGTTTGAAAGG
AGGGCCTTCTACGGAAGGAGTACCTGAGTCCATGAGGGAAGAGTACCGGCAGGAACAGCAGAGTGC
TGTGGATGTTGACGATGGTCATTTTGTCAACATAGAGCTGGAGTAA

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APPENDIX B

Table B1. Primers and probes sequences used for non-certified value determination by RT-ddPCR.

Primer/Probe Name	Sequence (5′ to 3′)
NA #1 Forward Primer	GGGTGGGCAATATACAGTAAGG
NA #1 Reverse Primer	CAAGTGGGAGCATGAGATGAA
NA #1 Probe	FAM/ACAACGGTATAAGAATTGGGTCCAAGGG/MGB-NFQ
NA #3 Forward Primer	GACGTATGTCAACATCAGCAATAC
NA #3 Reverse Primer	CACCCACTAATAGGGCAAAGA
NA #3 Probe	FAM/TTACCGAAGTAACAGCCTGCTCAGC/MGB-NFQ
HA #2 Forward Primer	AGGGAATGGTTGATGGTTGG
HA #2 Reverse Primer	TGCCTTTTGGGTGGATTCTT
HA #2 Probe	FAM/TGTCCGCAGCGTACCCACTCCCC/MGB-NFQ
HA G Forward Primer	GGCTCAGAAATAGTCCTC
HA G Reverse Primer	GTACCCATACCAACCATC
HA G Probe	FAM/TATAAACCCTGCTATCGCCC/MGB-NFQ
MP #2 Forward Primer	GCGCAGAGACTTGAAGATG
MP #2 Reverse Primer	CTTAGTCAGAGGTGACAGGATTG
MP #2 Probe	FAM/TGCAGGGAAGAACACCGATCTTGA/MGB-NFQ
MP #3 Forward Primer	GCGTTTATCGTCGCCTTAAATAC
MP #3 Reverse Primer	GTCAACATCCACAGCACTCT
MP #3 Probe	FAM/ACGGAAGGAGTACCTGAGTCCATGA/MGB-NFQ

Note: For non-certified value measurements, primers and probes were used at final concentrations of 900 nmol/L and 250 nmol/L, respectively.

Table B2. Thermal cycling conditions for RT-ddPCR.

Stage	**Step**	**Temperature (°C)**	**Cycles**	**Time**
1	1	50	1	1 h
2	1	95	1	10 min
3	1	94	60	30 s
3	2	58	60	1 min
4	1	98	1	10 min

Analysis: Non-certified value assignment of the genome copies/µL of Parts A, B, and C of RM 8038 was based on RT-ddPCR measurements on eight vials per RNA fragment over two days. Four vials per RNA were thawed at room temperature, vortexed, and centrifuged briefly each day. A 1:200 dilution was prepared for each sample by pipetting 2 µL stock RM 8038 RNA into 398 µL of THE RNA Storage Solution (Invitrogen). Diluted samples were heated to 65 °C for 5 minutes, then placed on ice. RT-ddPCR was performed on ice on diluted samples using the primers and probes listed in Table B1, the cycling conditions listed in Table B2, and the Bio-Rad One-Step RT-ddPCR Advanced Kit for Probes to obtain concentration measurements in copies/µL on a QX200 (Bio-Rad).

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