KRISS COVID-19 Response

Part 2 | July 2020

1. Development of SARS-CoV-2 Reference Material

Contribution to the resolution of the COVID-19 pandemic through R&D

▶ Research Activities

- **SARS-CoV-2 RNA reference material**
  (Developed in June 2020, currently available to industries)
  - nucleic acid-based diagnostics
  - high accuracy
  - can be applied for evaluating diagnostic laboratories

- **SARS-CoV-2 virus-like particle reference material**
  (Expected to be completed in July-August 2020)
  - RNA packaged with an envelope
  - high stability
  - mimicking real patient samples

- **Recombinant protein reference material**
  (Expected to be completed in 2021)
  - SARS-CoV-2 antigens and antibodies
  - Immuno-based diagnostics
  - COVID-19 therapeutics

▶ Future Plans

- **Expand research on virus reference materials and measurement methods to combat emerging infectious diseases**

▶ Property Report of SARS-CoV-2 RNA RM

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PROPERTY REPORT OF REFERENCE MATERIAL

· RM Description: SARS-CoV-2 RNA

· RM No.: 111-10-506 · Serial No.: 1

· Specification: 50 μL

· Producer: Korea Research Institute of Standards and Science, 267 Gajeong-ro, Yuseong-gu, Daejeon 34113, Republic of Korea

This reference material (RM) contains a mixture RNA fragments of the SARS-CoV-2 virus. The copy number concentration of each fragment ranges from 6.0×10^6 to 1.3×10^7 copy number/μL.

Detailed Description

- Intended Use: This RM is intended
  -to check quantitative PCR based nucleic acid amplification test for SARS-CoV-2 nucleic acid amplification test
  - to support developing new nucleic acid amplification test for SARS-CoV-2
  - to provide reference values for internal quality assurance programs for SARS-CoV-2 nucleic acid amplification test
- Instructions for Storage and Use: This RM shall be stored in −70 ℃ freezer until use.
- Measurement Methods: One-step reverse transcription digital PCR
- Reference Values: Please refer to the detailed information.
- Stability: Please refer to the detailed information.
- Homogeneity: Please refer to the detailed information.
- Additional Information: Please refer to the detailed information.

Safety Information: Material Safety Data Sheet(MSDS).

Date of Report: Valid until: 7 Days from Supplied Date

· Reported by: Bae, Young-Kyung Sign · Date of Issue:

· Approved by: Kim, Seil Sign

President of KRISS Seal

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DETAILED INFORMATION

1. Reference Value

The measurands for this RM are copy number concentrations of five representative gene regions in SARS-CoV-2. The reference values are measured by one-step reverse transcription digital PCR. Between-bottle homogeneity, repeatability of measurements, partition volume [1-4], thresholding, and gravimetric dilution are considered as the components of uncertainty.

Target genes	Reference value (copy number/μL)	Homogeneity (%)	Expanded uncertainty (copy number/μL)	*k* (95% level of confidence)
RdRP	7.2×10^6	3.3	1.1×10^6	2.3
N	7.3×10^6	3.5	1.1×10^6	2.3
E	7.7×10^6	3.9	1.2×10^6	2.2
nsp3	6.3×10^6	4.0	0.97×10^6	2.2
nsp6-11	13×10^6	4.4	2.1×10^6	2.2

Table 1. Reference values, homogeneity and expanded uncertainty of RM

The reference genome sequence is SARS-CoV-2 strain Wuhan-Hu-1 (Genbank: MN908947). The templates of reference material are the target gene with T7 promoter (5' TAATACGACTCACTATAGGG 3') at the 5' end. The nucleotide sequence of the target gene is the same as the reference sequence unless specifically marked otherwise.

The composition of reference material isolate are followings;

(1) The nucleotide sequence including the RdRp gene

The nucleotide sequence including full coding sequence of the RdRp gene in table 1 (13442 - 16237)

(2) The nucleotide sequence including the N, E, and M genes

The nucleotide sequence including full coding sequences of the N, E, and M genes in table 1(26125 - 29560), The difference from the reference sequence: 28144, T>C

(3) The nucleotide sequence including the NSP1 and NSP2 genes

The nucleotide sequence including full coding sequences of the NSP1 and NSP2 genes in table 1 (266 - 2719)

(4) The nucleotide sequence including the NSP3 gene

The nucleotide sequence including full coding sequence of the NSP3 gene in table 1 (2720 - 8554), The difference from the reference sequence: 5062, G>T

(5) The nucleotide sequence including the NSP4 and NSP5 genes

The nucleotide sequence including full coding sequences of the NSP4 and NSP5 genes in table 1 (8555 - 10972), The difference from the reference sequence: 8782, C>T

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(6) The nucleotide sequence including the NSP6, NSP7, NSP8, NSP9, NSP10, and NSP11 genes

The nucleotide sequence including full coding sequences of the NSP6, NSP7, NSP8, NSP9, NSP10, and NSP11 genes in table 1 (10973 - 13480)

(7) The nucleotide sequence including the NSP13 and NSP14 genes

The nucleotide sequence including full coding sequences of the NSP13 and NSP14 genes in table 1 (16237 - 19620)

(8) The nucleotide sequence including the NSP15 and NSP16 genes

The nucleotide sequence including full coding sequences of the NSP15 and NSP16 genes in table 1 (19621 - 21552)

Gene Symbol	Protein product name	Start	End
NSP1	leader protein	266	805
NSP2	nsp2	806	2719
NSP3	PLpro	2720	8554
NSP4	nsp4	8555	10054
NSP5	3CLpro	10055	10972
NSP6	nsp6	10973	11842
NSP7	nsp7	11843	12091
NSP8	nsp8	12092	12685
NSP9	nsp9	12686	13024
NSP10	nsp10	13025	13441
NSP11	nsp12	13442	13480
NSP12(RdRp)	RdRp	13442	16237
NSP13	Helicase	16238	18040
NSP14	3'-to-5' exonuclease	18041	19620
NSP15	endoRNAse	19621	20658
NSP16	2'-O-ribose methyltransferase	20659	21552
S	Surface glycoprotein	21563	25384
ORF3a	ORF3a protein	25393	26220
E	envelope protein	26245	26472
M	membrane glycoprotein	26523	27191
ORF6	ORF6 protein	27202	27387
ORF7a	ORF7a protein	27394	27759
ORF7b	ORF7b protein	27756	27887
ORF8	ORF8 protein	27894	28259
N	nucleocapsid phosphoprotein	28274	29533
ORF10	ORF10 protein	29558	29674

Table 2. Genomic structure of SARS-CoV-2 strain Wuhan-Hu-1

2. Informative Value

The informative values are approximate C_q values from one-step reverse transcription quantitative PCR using 5 μL of diluted (10^{-4}) RM. For each five gene region (RdRP, N, E, nsp3, nsp6-11), the C_q values are 24 - 30.

3. Intended Use and Applications

This RM can be applied to the following.

(1) To provide reference values for SARS-CoV-2 nucleic acid amplification test

(2) To verify the newly developed SARS-CoV-2 nucleic acid amplification test

(3) To provide internal quality control for SARS-CoV-2 nucleic acid amplification test

4. RM production and Analysis

This RM is produced by mixing RNA fragments that are prepared by *in vitro* transcription. These RNA fragments are stored in RNA storage solution containing human total RNA at 5 ng/μL. This RM contains all SARS-CoV-2 coding regions except the Spike gene. The reference values are obtained by one-step reverse transcription digital PCR that uses optimized PCR assays with cycling conditions [5].

5. Storage and Instruction for users

This RNA RM must be transferred in dry ice and stored below -70 °C upon arrival. The frozen RM must be thawed at 4 °C. All experiments using this RM should be done with Nuclease-free plastic wares and water. Additionally, users should minimize temperature changes and limit freeze-thaw cycles to two.

6. Stability

The reference values of SARS-CoV-2 RNA RM are applicable only if the RM is stored and handled as specified in this report. While the RM is stored in -70 °C, the reference value is assured up to seven days from receipt. Any additionally diluted RM solution may not stably maintain the specified reference values.

7. Homogeneity

The homogeneity of this RNA RM is tested to be acceptable. For the five target gene regions, the values of relative standard deviations for between-bottle homogeneity range from 3.31 - 4.33 %.

8. Reference

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[3] P. Corbisier, L. Pinheiro, S. Mazoua, A.M. Kortekaas, P.Y.J. Chung, T. Gerganova, G. Roebben, H. Emons, K. Emslie, DNA copy number concentration measured by digital and droplet digital quantitative PCR using certified reference materials, Anal. Bioanal. Chem. 407 (2015) 1831-1840.

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[4] L. Dong, Y. Meng, Z. Sui, J. Wang, L. Wu, B. Fu, Comparison of four digital PCR platforms for accurate quantification of DNA copy number of a certified plasmid DNA reference material, Sci. Rep. 5 (2015). doi:10.1038/srep13174.

[5] WHO, Novel coronavirus (2019-nCoV) technical guidance: laboratory testing for 2019-nCoV in humans Available online: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance (accessed on June 24, 2020)

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