

Novel Corona Virus (SARS-CoV-2) Ag Rapid Test Kit

Limit of Detection (LOD) Study Report

1. Purpose

To evaluate the limit of detection for Novel Corona Virus (SARS-CoV-2) Ag Rapid Test Kit

2. Reference Standards

(1) ISO 15193:2009: In vitro diagnostic systems — Measurement of quantities in samples of biological origin — Requirements for content and presentation of reference measurement procedures;

(2) Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures;

Approved Guideline—Second Edition This. EP17-A2 Vol. 32 No. 8 Replaces EP17-A Vol. 24 No.

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3. Product Information

Product Name:	Novel Corona Virus (SARS-CoV-2) Ag Rapid Test Kit
Batch number:	20201101
Specifications:	20 tests/kit

4. References

Name	Description	Concentration
SARS-Related Coronavirus 2	Wuhan-Hu-1	2×10^5 PFU/mL
SARS-Related Coronavirus 2	Italy-INMI1	2×10^5 PFU/mL

5. Method

5.1 Dilution Matrix Preparation

10 nasopharyngeal swabs obtained from healthy donors were washed by 400 μ L of PBS, respectively. The eluates were mixed and confirmed as SARS-CoV-2 negative by RT-PCR to form the nasopharyngeal matrix.

5.2 LOD of Wuhan-Hu-1 strain

The Isolate strain was spiked into pooled human nasal swab matrix obtained from multiple healthy volunteers eluted in PBS. Sample extraction buffer was used for the subsequent diluting process.

The virus was diluted to 1×10^5 PFU/mL for the following evaluation.

5.2.1 LOD Screening of Wuhan-Hu-1 strain

An initial range finding study was performed testing devices in triplicate using a 10-fold dilution series. The lowest concentration at which all (3 out of 3 replicates) were positive was chosen for LOD Range finding.

SARS-CoV-2 tested (PFU/mL)	Test Result
1×10^5	<u>3</u> /3 positive
1×10^4	<u>3</u> /3 positive
1×10^3	<u>3</u> /3 positive
1×10^2	<u>3</u> /3 positive
1×10^1	<u>0</u> /3 positive
1×10^0	<u>0</u> /3 positive

5.2.2 LOD Range Finding of Wuhan-Hu-1 strain

Using the 1×10^3 PFU/mL concentration, the LOD was further refined using a 2-fold dilution series of the SARS-CoV-2 virus made in pooled negative human nasal matrix. These dilutions were tested in triplicate. The lowest concentration at which all (3 out of 3 replicates) were positive was treated as the tentative LOD for the Novel Corona Virus (SARS-CoV-2) Ag Rapid Test Kit.

SARS-CoV-2 tested (PFU/mL)	Test Result
1×10^2	<u>3</u> /3 positive
5×10^1	<u>2</u> /3 positive
2.5×10^1	<u>0</u> /3 positive
1.25×10^1	<u>0</u> /3 positive
7.5	<u>0</u> /3 positive

5.2.3 LOD Confirmation of Wuhan-Hu-1 strain

The LOD of the test was then confirmed by testing 20 replicates at the tentative, the last and the next concentration. The final LOD of the Novel Corona Virus (SARS-CoV-2) Ag Rapid Test Kit was determined to be the lowest concentration resulting in positive detection of at least nineteen (19) out of twenty (20) replicates.

SARS-CoV-2 tested (PFU/mL)	Test Result
2×10^2	<u>20</u> /20 positive
1×10^2	<u>19</u> /20 positive
5×10^1	<u>8</u> /20 positive

5.3 LOD of Italy-INMII strain

The Isolate Italy-INMII strain was spiked into pooled human nasopharyngeal swab matrix obtained from multiple healthy volunteers eluted in PBS. Sample extraction buffer was used for

the subsequent diluting process. The virus was diluted to 1×10^5 PFU/mL for the following evaluation.

5.3.1 LOD Screening of Italy-INMI1 strain

An initial range finding study was performed testing devices in triplicate using a 10-fold dilution series. The lowest concentration at which all (3 out of 3 replicates) were positive was chosen for LOD Range finding.

SARS-CoV-2 tested (PFU/mL)	Test Result
1×10^5	<u>3</u> /3 positive
1×10^4	<u>3</u> /3 positive
1×10^3	<u>3</u> /3 positive
1×10^2	<u>3</u> /3 positive
1×10^1	<u>3</u> /3 positive
1×10^0	<u>0</u> /3 positive

5.3.2 LOD Range Finding of Italy-INMI1 strain

Using the 1×10^2 PFU/mL concentration, the LOD was further refined using a 2-fold dilution series of the SARS-CoV-2 virus made in pooled negative human nasal matrix. These dilutions were tested in triplicate. The lowest concentration at which all (3 out of 3 replicates) were positive was treated as the tentative LOD for the Novel Corona Virus (SARS-CoV-2) Ag Rapid Test Kit.

SARS-CoV-2 tested (PFU/mL)	Test Result
1×10^1	<u>3</u> /3 positive
5×10^0	<u>1</u> /3 positive
2.5×10^0	<u>0</u> /3 positive
1.25×10^0	<u>0</u> /3 positive

5.3.3 LOD Confirmation of Italy-INMI1 strain

The LOD of the test was then confirmed by testing 20 replicates at the tentative, the last and the next concentration. The final LOD of the Novel Corona Virus (SARS-CoV-2) Ag Rapid Test Kit was determined to be the lowest concentration resulting in positive detection of at least nineteen (19) out of twenty (20) replicates.

SARS-CoV-2 tested (PFU/mL)	Test Result
2×10^1	<u>20</u> /20 positive
1×10^1	<u>19</u> /20 positive
5×10^0	<u>6</u> /20 positive

6. Conclusion

Project Name: Novel Corona Virus (SARS-CoV-2) Ag Rapid Test Kit

The Limit of Detection of the Novel Corona Virus (SARS-CoV-2) Ag Rapid Test Kit on Isolate Wuhan-Hu-1 strain is 1x10² PFU/mL

The Limit of Detection of the Novel Corona Virus (SARS-CoV-2) Ag Rapid Test Kit on Isolate Italy-INMI1 strain is 1x10¹ PFU/mL