4.1.1   Limit of Detection

4.1.1.1   Objective
The study was performed to evaluate the limit of detection of NanoRepro SARS-CoV-2 Antigen Rapid Test.

4.1.1.2   Parameter
Based on the Technical Key Points for Coronavirus (COVID-19) Antigen-antibody Detection Reagent Registration Review (Trial), the limit of detection (LOD) for the SARS-CoV-2 Antigen Rapid Test Kit was established using limiting dilutions of the viral sample inactivated by gamma irradiation.

Detection limit reference material (L): SARS-CoV-2 inactivated viral sample (BetaCoV/Wuhan/IPBCAMS-WH-01/2019) was purchased from Biocome Co, Ltd.

The viral sample is supplied at a concentration of 4.8×10^5 TCID $_{50}$/ml as the detection limit reference material L. In this study, designed to estimate the LOD of the assay when using anterior nasal swab sample, the starting material is spiked into a volume of pooled human anterior nasal swab sample obtained from healthy donors and confirmed negative for SARS-CoV-2. Using diluent to dilute reference materials L for 1:10, 1:100, 1:1000, 1:0000 and 1:20000, then marked as L1~L5. Test with three bathes of kits and each dilution was tested in three replicates. Record the results. The positive result was represented by '+', and the negative result was represented by '-'. The test results are shown in the table below.

LoD samples preparation

Samples	Dilution Ratio	Virus Concentration (TCID 50/mL)
L	Original	4.8×10^5
L1	1:10	4.8×10^4
L2	1:100	4.8×10^3
L3	1:1000	4.8×10^2
L4	1:10000	4.8×10^1
L5	1:20000	2.4×10^1

Result

Lot No. / Sample	20200601			20200605			20200608		
	1	2	3	1	2	3	1	2	3
L	5+	5+	5+	5+	5+	5+	5+	5+	5+
L1	4+	4+	4+	4+	4+	4+	4+	4+	4+
L2	3+	3+	3+	3+	3+	3+	3+	3+	3+
L3	2+	2+	2+	2+	2+	2+	2+	2+	2+
L4	2+	2+	2+	2+	2+	2+	2+	2+	2+
L5	-	-	-	-	-	-	-	-	-

The sample L1, L2, L3, L4 all showed positive results and sample L5 gave 3 negative results. As a result, the concentration of L4 was chosen for further dilution. Using diluent to dilute L4 for 1:1.3, 1:1.6, 1:1.9, then marked as L6~L8. Test with three bathes of kits and each dilution was tested in three replicates. Record the results. The positive result was represented by '+', and the negative result
was represented by '-'. The test results are shown in the table below.

LOD samples preparation

Samples	Dilution Ratio	Virus Concentration (TCID 50/mL)
L4	1:10000	4.8×10^1
L6	1:13000	3.7×10^1
L7	1:16000	3.0×10^1
L8	1:19000	2.5×10^1

Lot No. / Sample	20200601			20200605			20200608		
	1	2	3	1	2	3	1	2	3
L4	2+	2+	2+	2+	2+	2+	2+	2+	2+
L6	1+	1+	1+	1+	1+	1+	1+	1+	1+
L7	1+	1+	1+	1+	1+	1+	1+	1+	1+
L8	-	-	-	-	-	-	-	-	-

Results：The sample L4. L6 and L7 showed positive results. Sample L8 gave all negative results.

Conclusion:
Therefore, sample L7 (diluting the detection limit reference sample L by 16000 times) was determined as the minimum detection limit concentration level.

4.1.1.3   Verification of limit of detection

Using diluent to dilute the detection limit reference material L ($4.8×10^5$ $TCID_{50}$/mL) to 1:16000 and marked as L7 for the verification of the limit of detection. Sample L7 was tested with three bathes of kits and repeat the test 20 times for each batch. Test and record the result. The positive detection rate should be above 90%. The positive result was represented by '+', and the negative result was represented by '-'. The test results are shown in the table below.

Lot No. / Sample	20200601 No. Positive/Total			20200605 No. Positive/Total			20200608 No.Positive/Total			The total positive rate %
	1	2	3	1	2	3	1	2	3	
L7	1+ 20/20	1+ 20/20	1+ 20/20	1+ 20/20	1+ 20/20	1+ 20/20	1+ 20/20	1+ 20/20	1+ 20/20	100%

4.1.1.4   Results

The results showed that the positive detection rate of sample L7 in three batches was 100%, which meets the requirements.

Conclusion:
Based on the evaluation of experiments, sample L7 which is diluted by the detection limit reference sample L ($4.8 x 10^5$ $TCID_{50}$/mL) by 16000 times was determined as the minimum detection limit concentration level.

Reference sample **L**	LOD concentration	No. Positive/Total	The total positive rate%
$4.8 x 10^5$ TCID $_{50}$/ml	30TCID $_{50}$/ml	180/180	100%