

Novel Coronavirus (COVID-19) Antigen Test Kit (Colloidal Gold)

Determination of Limit of Detection

Objective: To determine the limit of detection of Lyher Novel Coronavirus (COVID-19) Antigen Test Kit (Colloidal Gold)

Materials:

- 1) Lyher Novel Coronavirus (COVID-19) Antigen Test Kit Test Kit (Colloidal Gold) (Hereinafter referred to as “Lyher”)
- 2) Novel coronavirus positive swab specimens: spiked inactivated SARS-CoV-2 virus (purchased from Changzhou Ouji Biotech Co.,Ltd) into nasal specimens (collected from healthy individuals, purchased from Hangzhou KingMed Diagnostics Laboratory Co.,Ltd.)
- 3) Timer

Method: An initial Range-finding study was performed in triplicates using a 10-fold dilution series. Starting from the initial concentration, an addition of 50 µL of inactivated SARS_COV-2 virus was added to negative NP at each dilution prior to testing with Lyher kits. Between 2.7×10^2 and 27, the result shifted from “positive” to “negative”. Within this range, concentrations were further refined with a 2-fold dilution series. The final LoD demonstrating 100% positivity was confirmed with another 60 replicates.

Table 1: Range Finding Study

Concentration Of Inactivated virus (TCID ₅₀ /mL)	Lot:2006001-A			Lot:2006002-A			Lot:2006003-A		
	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
2.7×10^5 (initial concentration of the virus)	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
2.7×10^4	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
2.7×10^3	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
2.7×10^2	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
27	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

RESULTS

Table 2: Confirmation of the final LoD value (Lot:2006001-A)

Testing No.	0.675×10 ² TCID ₅₀ /mL			1.35×10 ² TCID ₅₀ /mL			2.7×10 ² TCID ₅₀ /mL		
	P1	P 2	P 3	P1	P2	P 3	P1	P 2	P 3
1	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive
2	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive
3	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive
4	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive
5	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive
6	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive
7	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive
8	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive
9	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive
10	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive
11	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive
12	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive
13	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive
14	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive
15	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive
16	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive
17	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive
18	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive
19	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive
20	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive

Table 3: Confirmation of the final LoD value (Lot:2006002-A)

Testing No.	0.675×10 ² TCID ₅₀ /mL			1.35×10 ² TCID ₅₀ /mL			2.7×10 ² TCID ₅₀ /mL		
	P1	P 2	P 3	P1	P2	P 3	P1	P 2	P 3
1	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive
2	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive
3	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive
4	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive
5	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive

**LYHER[®] Novel Coronavirus (COVID-19) Antigen Test Kit
(Colloidal Gold) (Nasal)**

16	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive
17	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive
18	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive
19	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive
20	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive

CONCLUSION

After using a series of 2-fold dilution series, the tentative LoD was determined to be 1.35×10^2 TCID₅₀/mL, which was further confirmed by showing positive in 60/60 replicates.