

**SARS-CoV-2 Antigen Rapid Test Kit (Colloidal Gold
Immunochromatography)**

Limit of Detection (LOD) Investigation Report

Beijing Lepu Medical Technology Co., Ltd.

Contents

1 Overview	2
2 Information about the kit used in this investigation	2
3 Investigation Method.....	2
3.1 Test method	2
3.2 Information about the test.....	3
4 Test Results	3
4.1 Investigation results when sample processing solution is used as diluent.....	3
4.2 Investigation results when human nasal swable sample is used as diluent	4
4.3 Determination of LOD	6
5 Conclusion.....	8

1 Overview

The Report presents the LOD investigation data on the SARS-CoV-2 Antigen Rapid Test Kit (Colloidal Gold Immunochromatography) (“the kit” for short).

2 Information about the kit used in this investigation

For the SARS-CoV-2 Antigen Rapid Test Kit (Colloidal Gold Immunochromatography) used for LOD investigation, the specification is 25 tests/kit and the lot numbers are 20CG2704X-y, 20CG2705X-y and 20CG2706X-y.

The SARS-CoV-2 recombinant G protein used for LOD investigation was purchased from Fapon Biotech Inc.

The negative nasal swab samples of healthy subjects used in this investigation were provided by IPE Center for Clinical Laboratory (IPE).

3 Investigation Method

3.1 Test method

3.1.1 LOD when sample processing solution is used as diluent

LOD was investigated by serial dilution. The sample processing solution contained in this kit was used to dilute the SARS-CoV-2 N protein produced by Fapon Biotech Inc. Multiple dilution gradients were set up; each gradient was tested repeatedly for 10 times and the 100% detectable minimum concentration level was used as the preset LOD. A sample at several concentration gradients was prepared; the diluent at each gradient was divided into 3 parts and each part of diluted sample was tested repeatedly for 20 times; and the antigen level with 90%~95% positive rate was taken as the LOD.

3.1.2 LOD when human nasal swab sample is used as the diluent

LOD was investigated by serial dilution. The nasal swab sample of healthy subjects was used to dilute the SARS-CoV-2 N protein produced by Fapon Biotech Inc. Multiple dilution gradients were set up; each gradient was tested repeatedly for 10 times and the 100% detectable minimum concentration level was used as the preset LOD. A sample at several concentration gradients was prepared; the diluent at each gradient was divided into 3 parts and each part of diluted sample was tested repeatedly for 20 times; and the antigen level with 90%~95% positive rate was taken as the LOD.

3.2 Information about the test

Lot numbers: 20CG2704X-y, 20CG2705X-y, 20CG2706X-y

Testers: Zhang Bo, Liu Yanjuan

4 Test Results

4.1 Investigation results when sample processing solution is used as diluent

Sample	Dilution ratio	20CG2704X-y									
		1	2	3	4	5	6	7	8	9	10
SARS-CoV-2 N protein	1	+	+	+	+	+	+	+	+	+	+
	1/10	+	+	+	+	+	+	+	+	+	+
	1/10 ²	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ²)	+	+	+	+	+	+	+	+	+	+
	1/10 ³	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ³)	+	+	+	+	+	+	+	+	+	+
	1/10 ⁴	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ⁴)	+	+	+	+	+	+	+	+	+	+
	1/10 ⁵	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ⁵)	+	+	+	+	+	+	+	+	+	+
	1/10 ⁶	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ⁶)	+	-	-	+	-	+	+	-	+	+
1: 10 ⁷	-	-	-	-	-	-	-	-	-	-	
Sample	Dilution ratio	20CG2705X-y									
		1	2	3	4	5	6	7	8	9	10
SARS-CoV-2 N protein	1	+	+	+	+	+	+	+	+	+	+
	1/10	+	+	+	+	+	+	+	+	+	+
	1/10 ²	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ²)	+	+	+	+	+	+	+	+	+	+
	1/10 ³	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ³)	+	+	+	+	+	+	+	+	+	+

	1/10 ⁴	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ⁴)	+	+	+	+	+	+	+	+	+	+
	1/10 ⁵	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ⁵)	+	+	+	+	+	+	+	+	+	+
	1/10 ⁶	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ⁶)	+	-	+	-	+	-	-	+	+	+
	1: 10 ⁷	-	-	-	-	-	-	-	-	-	-
Sample	Dilution ratio	20CG2706X-y									
		1	2	3	4	5	6	7	8	9	10
SARS-CoV-2 N protein	1	+	+	+	+	+	+	+	+	+	+
	1/10	+	+	+	+	+	+	+	+	+	+
	1/10 ²	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ²)	+	+	+	+	+	+	+	+	+	+
	1/10 ³	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ³)	+	+	+	+	+	+	+	+	+	+
	1/10 ⁴	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ⁴)	+	+	+	+	+	+	+	+	+	+
	1/10 ⁵	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ⁵)	+	+	+	+	+	+	+	+	+	+
	1/10 ⁶	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ⁶)	-	-	+	+	-	+	-	-	+	+
	1: 10 ⁷	-	-	-	-	-	-	-	-	-	-

4.2 Investigation results when human nasal swab sample is used as diluent

Sample	Dilution ratio	20CG2704X-y									
		1	2	3	4	5	6	7	8	9	10
SARS-CoV-2	1	+	+	+	+	+	+	+	+	+	+
N protein	1/10	+	+	+	+	+	+	+	+	+	+

	1/10 ²	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ²)	+	+	+	+	+	+	+	+	+	+
	1/10 ³	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ³)	+	+	+	+	+	+	+	+	+	+
	1/10 ⁴	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ⁴)	+	+	+	+	+	+	+	+	+	+
	1/10 ⁵	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ⁵)	+	+	+	+	+	+	+	+	+	+
	1/10 ⁶	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ⁶)	-	-	+	+	-	+	-	-	+	-
	1: 10 ⁷	-	-	-	-	-	-	-	-	-	-
Sample	Dilution ratio	20CG2705X-y									
		1	2	3	4	5	6	7	8	9	10
SARS-CoV-2 N protein	1	+	+	+	+	+	+	+	+	+	+
	1/10	+	+	+	+	+	+	+	+	+	+
	1/10 ²	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ²)	+	+	+	+	+	+	+	+	+	+
	1/10 ³	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ³)	+	+	+	+	+	+	+	+	+	+
	1/10 ⁴	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ⁴)	+	+	+	+	+	+	+	+	+	+
	1/10 ⁵	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ⁵)	+	+	+	+	+	+	+	+	+	+
	1/10 ⁶	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ⁶)	+	-	-	+	-	+	+	-	+	+
1: 10 ⁷	-	-	-	-	-	-	-	-	-	-	
Sample	Dilution ratio	20CG2706X-y									
		1	2	3	4	5	6	7	8	9	10

SARS-CoV-2 N protein	1	+	+	+	+	+	+	+	+	+	+	+
	1/10	+	+	+	+	+	+	+	+	+	+	+
	1/10 ²	+	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ²)	+	+	+	+	+	+	+	+	+	+	+
	1/10 ³	+	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ³)	+	+	+	+	+	+	+	+	+	+	+
	1/10 ⁴	+	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ⁴)	+	+	+	+	+	+	+	+	+	+	+
	1/10 ⁵	+	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ⁵)	+	+	+	+	+	+	+	+	+	+	+
	1/10 ⁶	+	+	+	+	+	+	+	+	+	+	+
	1/(5×10 ⁶)	+	-	+	-	-	-	-	+	-	+	+
	1: 10 ⁷	-	-	-	-	-	-	-	-	-	-	-

The results show that the two dilution systems are almost identical, so the sample processing solution contain in this kit can be directly used to dilute the N protein. Moreover, it can be seen from the above results that the preset LOD of SARS-CoV-2 N protein is 1/10⁶.

4.3 Determination of LOD

Determination of LOD

Lot number: 20CG2704X-y	First dilution	Dilution ratio	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
		1/(7×10 ⁵)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		1/(8×10 ⁵)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		1/(9×10 ⁵)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		1/10 ⁶	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		1/(2×10 ⁶)	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
		1/(3×10 ⁶)	+	-	+	+	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+
	Second dilution	1/(7×10 ⁵)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
		1/(8×10 ⁵)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
		1/(9×10 ⁵)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
		1/10 ⁶	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

		1/(2×10 ⁶)	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
		1/(3×10 ⁶)	-	+	+	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+
	Third dilution	1/(7×10 ⁵)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		1/(8×10 ⁵)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		1/(9×10 ⁵)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		1/10 ⁶	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		1/(2×10 ⁶)	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
		1/(3×10 ⁶)	+	-	+	+	+	+	-	+	+	-	+	+	+	+	+	-	+	+	+	+	+
Lot number: 20CG2705X-y	First dilution	Dilution ratio	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
		1/(7×10 ⁵)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		1/(8×10 ⁵)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		1/(9×10 ⁵)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		1/10 ⁶	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		1/(2×10 ⁶)	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
		1/(3×10 ⁶)	+	+	+	-	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
	Second dilution	1/(7×10 ⁵)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		1/(8×10 ⁵)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		1/(9×10 ⁵)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		1/10 ⁶	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		1/(2×10 ⁶)	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+
		1/(3×10 ⁶)	+	+	+	+	+	+	+	-	-	+	+	-	+	+	+	+	+	+	+	+	+
	Third dilution	1/(7×10 ⁵)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		1/(8×10 ⁵)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		1/(9×10 ⁵)	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		1/10 ⁶	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
		1/(2×10 ⁶)	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
1/(3×10 ⁶)		-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	
Lot number: 20CG2706X-y	First dilution	Dilution ratio	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
	1/(7×10 ⁵)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

		1/(8×10 ⁵)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
		1/(9×10 ⁵)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		1/10 ⁶	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		1/(2×10 ⁶)	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
		1/(3×10 ⁶)	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
	Second dilution	1/(7×10 ⁵)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
		1/(8×10 ⁵)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
		1/(9×10 ⁵)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
		1/10 ⁶	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
		1/(2×10 ⁶)	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
		1/(3×10 ⁶)	+	-	+	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	
	Third dilution	1/(7×10 ⁵)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
		1/(8×10 ⁵)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
		1/(9×10 ⁵)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
		1/10 ⁶	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
		1/(2×10 ⁶)	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
		1/(3×10 ⁶)	+	+	+	+	-	+	-	+	+	-	+	+	+	+	+	-	+	+	+	+	

According to the results above, at the gradient of 1/10⁶, the positive rate is 98.33%; at the gradient of 1/(2×10⁶), the positive rate is 92.22%, and at the gradient of 1/(3×10⁶), the positive rate is 81.67%. Therefore, it is determined that the dilution ratio at LOD is 1/(2×10⁶).

5 Conclusion

Through gradient dilution of SARS-CoV-2 recombinant N protein, the results showed that there was almost no difference between the two dilution systems (sample processing solution and human nasal swab sample), so the sample processing solution contained in this kit can be used to dilute the N protein directly. Through validation of the sample in several concentration gradient near the preset LOD concentration, the results showed that the positive rate was 92.22% under the condition of 1/(2 × 10⁶) dilution, which ranged from 90% to 95%. Therefore, the LOD dilution ratio was 1/(2 × 10⁶).