

## Limit of Detection (LoD) Study Report

### 1. Purpose

Study the limit of detection (LoD) of the INVBIO COVID-19 Ag Rapid Saliva Test Device.

### 2. Scope

The INVBIO COVID-19 Ag Rapid Saliva Test Device produced by Innovation Biotech (Beijing) Co., Ltd

### 3. Materials

- (1) The INVBIO COVID-19 Ag Rapid Saliva Test Device (Lot: 2008251).
- (2) The inactivated SARS-CoV-2 virus was acquired from Vero E6 cell culture, which titers is  $6 \times 10^5$  TCID<sub>50</sub>/ml and the strains is BetaCoV/Wuhan/IPBCAMS-WH-01/2019;
- (3) The negative saliva swabs samples were collected from healthy person following the product instruction.
- (4) The negative matrix samples were acquired by mixture of the negative saliva swabs samples, use for dilute the inactivated SARS-CoV-2 virus.

### 4. Procedure

#### 4.1 Samples Preparation

Serial dilutions of samples were prepared as the method of Table 1.

**Table 1: Serial dilution of samples**

Dilution ratio	Method	Concentration TCID <sub>50</sub> /ml
NA	SARS-CoV-2	$6 \times 10^5$
1:10	200ul SARS-CoV-2 mix 1800ul negative matrix samples	$6 \times 10^4$
1:100	200ul SARS-CoV-2 (1:10) mix 1800ul negative matrix samples	$6 \times 10^3$
1:1000	200ul SARS-CoV-2 (1:100) mix 1.8ml negative matrix samples	$6 \times 10^2$
1:5000	400ul SARS-CoV-2 (1:1000) mix 1.6ml negative matrix samples	$1.2 \times 10^2$
1:10000	1ml SARS-CoV-2 (1:5000) mix 1ml negative matrix samples	60
1:50000	400ul SARS-CoV-2 (1:10000) mix 1.8ml negative matrix samples	12

#### 4.2 Test Serial Samples

INVBIO COVID-19 Ag Rapid Saliva Test Device (Lot: 2008251) was used to test the above samples, each specimen is tested 20 times according to IFU.

#### 4.3 Confirm the LoD

The inactivated SARS-CoV-2 virus ( $6 \times 10^5$  TCID<sub>50</sub>/ml) dilute in one determined concentration, then use the INVBIO COVID-19 Ag Rapid Saliva Test Device test the dilution samples in 20 times.

### 5. Acceptance Criteria

The LoD studies determine the lowest detectable concentration of inactivated SARS-CoV-2 at which approximately 95% of all 20 (true positive) replicates test positive.

### 6. Results

The test results of serial dilution samples are summarized in Table 2.

**Table2: Test results for serial dilution samples**

Dilution ratio	Concentration	Results					Ratio of positive
1:10	6×10 <sup>4</sup> TCID <sub>50</sub> /ml	10	10	10	10	10	100%
		10	10	10	10	10	
		10	10	10	10	10	
		10	10	10	10	10	
1:100	6×10 <sup>3</sup> TCID <sub>50</sub> /ml	9	9	9	9	9	100%
		9	9	9	9	9	
		9	9	9	9	9	
		9	9	9	9	9	
1:1000	6×10 <sup>2</sup> TCID <sub>50</sub> /ml	7	7	7	7	7	100%
		7	7	7	7	7	
		7	7	7	7	7	
		7	7	7	7	7	
1:5000	1.2×10 <sup>2</sup> TCID <sub>50</sub> /ml	4	4	4	4	4	100%
		4	4	4	4	4	
		4	4	4	4	4	
		4	4	4	4	4	
1:10000	60 TCID <sub>50</sub> /ml	3	3	3	3	3	95%
		3	3	3	3	3	
		3	3	3	-	3	
		3	3	3	3	3	
1:50000	12 TCID <sub>50</sub> /ml	-	-	-	-	-	0
		-	-	-	-	-	
		-	-	-	-	-	
		-	-	-	-	-	

Note: “-” indicate negative; “Number” indicate positive, the bigger the number, the darker the color.

The result shows that when the concentration of inactivated SARS-CoV-2 virus greater than 60 TCID<sub>50</sub>/ml, the ratio of positive is 100%, the concentration of inactivated SARS-CoV-2 virus is 60 TCID<sub>50</sub>/ml, the ratio of positive is 95%.

The inactivated SARS-CoV-2 virus (6×10<sup>5</sup>TCID<sub>50</sub>/ml) virus dilute in 1/10000 ratio in negative matrix samples. The INVBIO COVID-19 Ag Rapid Saliva Test Device(Lot: 2008251) was used to test the 1/10000 dilution samples in 20 times. The results were list in the Table3.

**Table3.The confirmation results for LoD**

Test1	Test2	Test3	Test4	Test5	Test6	Test7	Test8	Test9	Test10
3	3	3	-	3	3	3	3	3	3
Test11	Test12	Test13	Test14	Test15	Test16	Test17	Test18	Test19	Test20
3	3	3	3	3	3	3	3	3	3

Note: “Number” indicate positive, the bigger the number, the darker the color.

The LoD was then confirmed by testing 20 times with concentrations (60 TCID50/ml) at the tentative limit of detection. The final LoD resulting in positive detection of at least 19 out of 20 replicates.

#### 7. Conclusion

The LoD of INVBIO COVID-19 Ag Rapid Saliva Test Device is 60 TCID50/ml.

#### 8. Signatures

Performed by: Jiabin      Signature:       Date: 2020.08.28  
(Print Name)