Hook Effect Studies

Product Name: Rapid SARS-CoV-2 Antigen Test Card
Catalog No.: 1N40C5

File No.	RR1N40016
Date	2020.06.17-2020.10.15
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Xiamen Boson Biotech Co., Ltd.

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Overview

The hook effect refers to the phenomenon of false low or negative result due to inappropriate antigen to antibody ratio. The hook effect is common in immunoassays, and detection of high-concentration samples can result in false low values or even false negative results, leading to wrong test results. The Rapid SARS-CoV-2 Antigen Test Card uses the double-antibody sandwich method to test samples, and thus it is necessary to study possible hook effects that are present in the test kit.

1. Purpose

To test SARS-CoV-2 recombinant antigens and viral cultures, and evaluate the hook effect of the test kit.

2. Personnel and Responsibility

Name	Position	Education	Responsibility
Haolong Shen	Management Representative	B.S.	Approval of study report
Zhijuan Jia	R&D Manager	M.S.	Review of study report
Kesai Liu	R&D Engineer	M.S.	Study implementation, recording, analysis of results, and report drafting
Mengjuan Wu	R&D Vice Manager	M.S.	Study implementation, recording, analysis of results, and report drafting

3. Materials

3.1. Evaluated Reagents

Rapid SARS-CoV-2 Antigen Test Card (1N40C5)		
	Lot Number	Manufacturer
1	H20061502	Xiamen Boson Biotech Co., Ltd.
2	H20061601	Xiamen Boson Biotech Co., Ltd.
3	H20061701	Xiamen Boson Biotech Co., Ltd.

3.2. Other Materials

	Name	Lot No. (Catalog No.)	Notes
1	SARS-CoV-2 recombinant antigen (N-protein)	R20050718	Shanghai Novoprotein Technology Co., Ltd.
2	SARS-CoV-2 viral culture 1#	NR-52284 (Italy-INMI1)	ZeptoMetrix Corporation
3	SARS-CoV-2 viral culture 2#	NR-52282 (Hong Kong/VM2000106/2020)	ZeptoMetrix Corporation
4	SARS-CoV-2 viral culture 3#	NR-52281 (USA-WA1/2020)	ZeptoMetrix Corporation

4. Hook Effect Studies on SARS-CoV-2 Recombinant Antigens

4.1. Methods

4.1.1. Sample Preparation

Use the sample extraction buffer to dilute the SARS-CoV-2 recombinant antigens with a concentration of 3.72 μg/mL, and prepare 4 serially-diluted test samples with concentrations of 3.72 μg/mL, 372 ng/mL, 37.2 ng/mL and 3.72 ng/mL. Use the sample extraction buffer as the negative control.

4.1.2. Sample Testing

Use three batches of the Rapid SARS-CoV-2 Antigen Test Card to test different concentrations of SARS-CoV-2 recombinant antigen samples. Perform 3 parallel tests for each sample.

Perform the test according to the instructions for use, and read results 15-20 min after sample addition.

4.2. Results

Table 1. Test results for different concentrations of recombinant antigens using three batches of product

Concentration	H20061502			H20061601			H20061701		
3.72 μg/mL	+++	+++	+++	+++	+++	+++	+++	+++	+++
372 ng/mL	+++	+++	+++	+++	+++	+++	+++	+++	+++
37.2 ng/mL	++	++	++	++	++	++	++	++	++
3.72 ng/mL	+	+	+	+	+	+	+	+	+
Negative control	+	+	+	+	+	+	+	+	+

Notes: “+~+++” indicates sequential increase in intensity of color rendering.

4.3. Analysis of Results

The test results for SARS-CoV-2 recombinant antigens with a concentration of 3.72 μg/mL were positive. The color intensity gradually decreased with increasing dilution, and no hook effect was found.

4.4. Conclusion

The Rapid SARS-CoV-2 Antigen Test Card did not show hook effect when testing recombinant antigens at a concentration of 3.72 μg/mL.

5. Hook Effect Studies on SARS-CoV-2 Viral Cultures

5.1. Methods

5.1.1. Sample Preparation

Use the sample extraction buffer to dilute SARS-CoV-2 viral culture 1# with viral titer of 1.02×10^8 $TCID_{50}$/mL, and prepare 6 serially-diluted test samples with viral titers of 1.02×10^8, 1.02×10^7, 1.02×10^6, 1.02×10^5, 1.02×10^4 and 1.02×10^3 $TCID_{50}$/mL. Use the sample extraction buffer as the negative control.

Use the sample extraction buffer to dilute SARS-CoV-2 viral culture 2# with viral titer of

1.15×10^7 $TCID_{50}$/mL, and prepare 5 serially-diluted test samples with viral titers of 1.15×10^7, 1.15×10^6, 1.15×10^5, 1.15×10^4 and 1.15×10^3 $TCID_{50}$/mL. Use the sample extraction buffer as the negative control.

Use the sample extraction buffer to dilute SARS-CoV-2 viral culture 1# with viral titer of 9.55×10^6 $TCID_{50}$/mL, and prepare 4 serially-diluted test samples with viral titers of 9.55×10^6, 9.55×10^5, 9.55×10^4 and 9.55×10^3 $TCID_{50}$/mL. Use the sample extraction buffer as the negative control.

5.1.2. Sample Testing

Use three batches of the Rapid SARS-CoV-2 Antigen Test Card to test different concentrations of SARS-CoV-2 viral culture and negative control samples. Perform 3 parallel tests for each sample.

Perform the test according to the instructions for use, and read results 15-20 min after sample addition.

5.2. Results

Table 2. Test results for different concentrations of viral culture 1# using three batches of product

Concentration ($TCID_{50}$/mL)	H20061502			H20061601			H20061701		
1.02×10^8	+++	+++	+++	+++	+++	+++	+++	+++	+++
1.02×10^7	+++	+++	+++	+++	+++	+++	+++	+++	+++
1.02×10^6	++	++	++	++	++	++	++	++	++
1.02×10^5	++	++	++	++	++	++	++	++	++
1.02×10^4	+	+	+	+	+	+	+	+	+
1.02×10^3	+	+	+	+	+	+	+	+	+
Negative Control	-	-	-	-	-	-	-	-	-

Notes: “+~+++” indicates sequential increase in intensity of color rendering.

Table 3. Test results for different concentrations of viral culture 2# using three batches of product

Concentration ($TCID_{50}$/mL)	H20061502			H20061601			H20061701		
1.15×10^7	+++	+++	+++	+++	+++	+++	+++	+++	+++
1.15×10^6	+++	+++	+++	+++	+++	+++	+++	+++	+++
1.15×10^5	++	++	++	++	++	++	++	++	++
1.15×10^4	+	+	+	+	+	+	+	+	+
1.15×10^3	+	+	+	+	+	+	+	+	+
Negative Control	-	-	-	-	-	-	-	-	-

Notes: “+~+++” indicates sequential increase in intensity of color rendering.

Table 4. Test results for different concentrations of viral culture 3# using three batches of product

Concentration ($TCID_{50}$/mL)	H20061502			H20061601			H20061701		
9.55×10^6	+++	+++	+++	+++	+++	+++	+++	+++	+++
9.55×10^5	+++	+++	+++	+++	+++	+++	+++	+++	+++
9.55×10^4	++	++	++	++	++	++	++	++	++
9.55×10^3	+	+	+	+	+	+	+	+	+
Negative Control	-	-	-	-	-	-	-	-	-

Notes: “+~+++” indicates sequential increase in intensity of color rendering.

5.3. Analysis of Results

The test results for SARS-CoV-2 viral cultures with a concentration of 1.02×10^8 $TCID_{50}$/mL were all positive. The color intensity gradually decreased with increasing dilution, and no hook effect was found.

The test results for SARS-CoV-2 viral cultures with a concentration of 1.15×10^7 $TCID_{50}$/mL were all positive. The color intensity gradually decreased with increasing dilution, and no hook effect was found.

The test results for SARS-CoV-2 viral cultures with a concentration of 9.55×10^6 $TCID_{50}$/mL were all positive. The color intensity gradually decreased with increasing dilution, and no hook effect was found.

5.4. Conclusion

The Rapid SARS-CoV-2 Antigen Test Card did not show hook effect when testing viral cultures at concentrations of 1.02×10^8 $TCID_{50}$/mL, 1.15×10^7 $TCID_{50}$/mL and 9.55×10^6 $TCID_{50}$/mL.