Analytical Sensitivity Studies

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

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Table of Contents

Overview

Analytical sensitivity is the lowest concentration that can be detected by a test kit. It refers to the lowest value that can be measured with a certain probability that's different from zero. For colloidal gold test reagents, it is denoted as the limit of detection. Determination of the limit of detection is important for evaluating the effectiveness of colloidal gold products on the market. This study aims to study the limit of detection of the Rapid SARS-CoV-2 Antigen Test Card to evaluate product performance.

1. Purpose

To evaluate the limit of detection of the Rapid SARS-CoV-2 Antigen Test Card by testing SARS-CoV-2 recombinant antigens and viral cultures.

2. References

	Document No.	Document
1	EP17-A	Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline
2	BS EN 13612:2002	Performance evaluation of in vitro diagnostic medical devices

3. 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

4. Statistical Analysis Methods

The test results should be negative for negative samples, and positive for weak positive and positive samples. Calculate the positive detection rate, and preset the limit of detection at the lowest concentration level with 100% detection rate. Prepare several serially diluted samples around the preset limit of detection, and repeat at least 20 tests at each concentration. Set the limit of detection at the lowest concentration level with 95% positive detection rate.

5. Materials

5.1 Evaluated Reagent

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.

5.2 Other Materials

	Name	Lot No. (Catalog No.)	Notes
1	SARS-CoV-2 recombinant	R20050718	Shanghai Novoprotein

	antigens (N-protein)		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

6. Limit of Detection Study for SARS-CoV-2 Recombinant Antigens

6.1 Preliminary study on the limit of detection for SARS-CoV-2 recombinant antigens

6.1.1 Sample Preparation

1) Negative Samples

Collect the nasopharyngeal samples with the nasopharyngeal swabs from healthy individuals, add into the extraction tubes with sample extraction buffer, mix well and use as the SARS-CoV-2 antigen negative samples.

2) SARS-CoV-2 Recombinant Antigen Samples

Use the negative sample to dilute SARS-CoV-2 recombinant antigens with a concentration of 1 μg/mL, and prepare 5 serially diluted test samples with concentrations of 1 μg/mL, 100 ng/mL, 10 ng/mL, 1 ng/mL and 0.1 ng/mL.

6.1.2 Sample Testing

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

Carry out the test according to the instructions for use. Read and record results 15-20 min after sample addition.

6.1.3 Results

Table 1. Test results for different concentrations of SARS-CoV-2 recombinant antigens

No.	Concentration	H20061502	H20061601	H20061701	Total Agreement
1	1 μg/mL	3/3	3/3	3/3	9/9 (100%)
2	100 ng/mL	3/3	3/3	3/3	9/9 (100%)
3	10 ng/mL	3/3	3/3	3/3	9/9 (100%)
4	1 ng/mL	3/3	3/3	3/3	9/9 (100%)
5	0.1 ng/mL	0/3	0/3	0/3	0/9 (0%)

6.1.4 Analysis of Results

Detection of different concentrations of SARS-CoV-2 recombinant antigens showed that a concentration of 1 ng/mL gave an 100% positive agreement. A concentration of 0.1 ng/mL gave a 0% positive agreement. Therefore, the concentration of 1 ng/mL was used as the preset limit of detection. Further testing near this concentration is required to determine the final limit of detection.

6.2 Determination of the limit of detection for SARS-CoV-2 recombinant antigens

6.2.1 Sample Preparation

1) Negative Samples

Collect the nasopharyngeal samples with the nasopharyngeal swabs from healthy individuals, add into the extraction tubes with sample extraction buffer, mix well and use as the SARS-CoV-2 antigen

negative samples.

2) SARS-CoV-2 Recombinant Antigen Samples

Use the negative sample to dilute SARS-CoV-2 recombinant antigens, and prepare 5 serially diluted test samples with concentrations of 1 ng/mL, 0.75 ng/mL, 0.5 ng/mL, 0.25 ng/mL, 0.1 ng/mL.

6.2.2 Sample Testing

Use the Rapid SARS-CoV-2 Antigen Test Card to test different concentrations of SARS-CoV-2 recombinant antigen samples. Repeat 20 tests for each sample.

Carry out the test according to the instructions for use. Read and record results 15-20 min after sample addition.

6.2.3 Results

Table 2. Test results for different concentrations of SARS-CoV-2 recombinant antigens for the determination of the limit of detection

No.	Concentration	H20061502	H20061601	H20061701	Total Agreement
1	1 ng/mL	20/20	20/20	20/20	60/60 (100%)
2	0.75 ng/mL	18/20	19/20	18/20	55/60 (91.7%)
3	0.5 ng/mL	0/20	0/20	0/20	0/60 (0%)
4	0.25 ng/mL	0/20	0/20	0/20	0/60 (0%)
5	0.1 ng/mL	0/20	0/20	0/20	0/60 (0%)

6.2.4 Analysis Results

Detection of different concentrations of SARS-CoV-2 recombinant antigens showed that the concentration of 1 ng/mL gave an 100% total positive agreement, which is greater than the 95% positive detection rate. At the concentration of 0.75 ng/mL, the total positive agreement was 91.7%, which is less than the 95% positive detection rate.

6.3 Conclusion

The study tested serially diluted samples of SARS-CoV-2 recombinant antigens. The limit of detection was chosen at the antigen concentration level with 95% positive detection rate. Based on the test results, the limit of detection of SARS-CoV-2 recombinant antigens for the Rapid SARS-CoV-2 Antigen Test Card was determined to be 1 ng/mL.

7. Limit of Detection Study for SARS-CoV-2 Viral Cultures

7.1 Preliminary study on the limit of detection for SARS-CoV-2 viral cultures

7.1.1 Sample Preparation

1) Negative Samples

Collect the nasopharyngeal samples with the nasopharyngeal swabs from healthy individuals, add into the extraction tubes with sample extraction buffer, mix well and use as the SARS-CoV-2 antigen negative samples.

2) SARS-CoV-2 Viral Culture Samples

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

7.1.2 Sample Testing

Use the Rapid SARS-CoV-2 Antigen Test Card to test different concentrations of SARS-CoV-2 viral culture samples. Repeat 3 tests for each sample.

Carry out the test according to the instructions for use. Read and record results 15-20 min after sample addition.

7.1.3 Results

Table 3. Test results for different concentrations of SARS-CoV-2 viral cultures

No.	Concentration ($TCID_{50}$/mL)	H20061502	H20061601	H20061701	Total Agreement
1	9.55×10^{6}	3/3	3/3	3/3	9/9 (100%)
2	9.55×10^{5}	3/3	3/3	3/3	9/9 (100%)
3	9.55×10^{4}	3/3	3/3	3/3	9/9 (100%)
4	9.55×10^{3}	3/3	3/3	3/3	9/9 (100%)
5	9.55×10^{2}	3/3	3/3	3/3	9/9 (100%)
6	9.55×10^{1}	2/3	2/3	2/3	6/9 (66.7%)

7.1.4 Analysis of Results

Detection of different titers of SARS-CoV-2 viral cultures showed that a concentration of 9.55×10^{2} $TCID_{50}$/mL gave an 100% positive agreement. A concentration of 9.55×10^{1} $TCID_{50}$/mL gave a 66.7% positive agreement. Therefore, 9.55×10^{2} $TCID_{50}$/mL was used as the preset limit of detection. Further testing near this concentration is required to determine the final limit of detection.

7.2 Determination of the limit of detection for SARS-CoV-2 viral cultures

7.2.1 Sample Preparation

3) Negative Samples

Collect the nasopharyngeal samples with the nasopharyngeal swabs from healthy individuals, add into the extraction tubes with sample extraction buffer, mix well and use as the SARS-CoV-2 antigen negative samples.

4) SARS-CoV-2 Viral Culture Samples

Use the negative sample to dilute SARS-CoV-2 viral culture 3#, and prepare 7 serially diluted test samples with viral titers of 9.55×10^{2}, 6.4×10^{2}, 4.3×10^{2}, 2.9×10^{2}, 1.9×10^{2}, 1.3×10^{2} and 8.7×10^{1} $TCID_{50}$/mL.

7.2.2 Sample Testing

Use the Rapid SARS-CoV-2 Antigen Test Card to test different concentrations of SARS-CoV-2 viral culture samples. Repeat 20 tests for each sample.

Carry out the test according to the instructions for use. Read and record results 15-20 min after sample addition.

7.2.3 Results

Table 4. Test results for different concentrations of SARS-CoV-2 viral cultures for the determination of the limit of detection

No.	Concentration ($TCID_{50}$/mL)	H20061502	H20061601	H20061701	Total Agreement
1	9.55×10^{2}	20/20	20/20	20/20	60/60 (100%)
2	6.4×10^{2}	20/20	20/20	20/20	60/60 (100%)

3	4.3×10^2	20/20	20/20	20/20	60/60 (100%)
4	2.9×10^2	20/20	20/20	20/20	60/60 (100%)
5	1.9×10^2	20/20	20/20	20/20	60/60 (100%)
6	1.3×10^2	20/20	20/20	20/20	60/60 (100%)
7	8.7×10^1	12/20	13/20	12/20	37/60 (61.7%)

7.2.4 Analysis Results

Detection of different concentrations of SARS-CoV-2 viral cultures showed that the concentration of 1.3×10^2 $TCID_{50}$/mL gave an 100% total positive agreement, which is greater than the 95% positive detection rate. At the concentration of 8.7×10^1 $TCID_{50}$/mL, the total positive agreement was 61.7%, which is less than the 95% positive detection rate.

7.3 Conclusion

The study tested serially diluted samples of SARS-CoV-2 viral cultures. The limit of detection was chosen at the viral titer level with 95% positive detection rate. Based on the test results, the limit of detection of SARS-CoV-2 viral cultures for the Rapid SARS-CoV-2 Antigen Test Card was determined to be 1.3×10^2 $TCID_{50}$/mL.

8. Verification of the Limit of Detection

8.1 Sample Preparation

1) Negative Samples

Collect the nasopharyngeal samples with the nasopharyngeal swabs from healthy individuals, add into the extraction tubes with sample extraction buffer, mix well and use as the SARS-CoV-2 antigen negative samples.

2) SARS-CoV-2 Viral Culture Samples

Add three different strains of SARS-CoV-2 viral cultures into the negative sample, and separately prepare test samples at the limit of detection (1.3×10^2 $TCID_{50}$/mL).

8.2 Sample Testing

Use the Rapid SARS-CoV-2 Antigen Test Card to separately test the three samples at the limit of detection. Repeat 20 tests for each sample.

Carry out the test according to the instructions for use. Read and record results 15-20 min after sample addition.

8.3 Verification Results

Table 5. Test results for SARS-CoV-2 viral cultures for the verification of the limit of detection

No.	Concentration ($TCID_{50}$/mL)	H20061502	H20061601	H20061701	Total Agreement
1#	1.3×10^2	20/20	20/20	20/20	60/60 (100%)
2#	1.3×10^2	20/20	20/20	20/20	60/60 (100%)
3#	1.3×10^2	20/20	20/20	20/20	60/60 (100%)

8.4 Analysis of Results

Three strains of SARS-CoV-2 viral cultures at the limit of detection were tested, with 20 tests repeated for each sample. Based on the verification, the positive detection rate for the Rapid SARS-CoV-2 Antigen Test Card was 100%.

8.5 Conclusion

Based on verification with three strains of SARS-CoV-2 viral cultures, the limit of detection for the Rapid SARS-CoV-2 Antigen Test Card is 1.3×10^{2} $TCID_{50}$/mL.