

Validation report rapid tester Spartacus Biomed.

7-1-21

Goal

A validation was done where the rapid test made by Spartacus Biomed was tested with a previously determined highly positive SARS-CoV-2 patient sample. The Ct of this sample was previously determined with RT-PCR to be 16.7. A theoretical approximation can be made to calculate this to around 8×10^6 DNA copies/ml of sample. A dilution series was made of this sample. The results were compared to a proven RT-PCR method.

Protocol

1:10 dilution series (N=1)

The sample was diluted 1:10 (150 μ l + 1350 μ l). This was incubated for 1 minute, vortexed and spun down. Next a dilution series was made where 100 μ l sample was each time put in 900 μ l of buffer. This was done 6 times leading to a total of 7 samples. Also a negative was included where only buffer was used. Of each sample 80 μ l of buffer with virus was added to the rapid test. A timer was set for 10 minutes. Photos were taken of the results between 10 minutes after sample addition and before 25 minutes after sample addition.

The dilutions that were made were also tested in the RT-PCR in the same concentration.

1:3 dilution series (N=10)

The sample was diluted 1:10 (150 μ l + 1350 μ l). This was incubated for 1 minute, vortexed and spun down. Next a dilution series was made where 500 μ l sample was each time put in 1000 μ l of buffer. This was done 6 times leading to a total of 8 samples. Also a negative was included where only buffer was used. Of each sample 80 μ l of buffer with virus was added to the rapid test. A timer was set for 10 minutes. Photos were taken of the results between 10 minutes after sample addition and before 25 minutes after sample addition.

The dilutions that were made were also tested in the RT-PCR in the same concentration.

Results

1:10 dilution series (N=1) Rapid tester results

The first two rapid testers were positive for SARS-COV-2. The other 5 and the negative control were negative.

1:10 Rapid tester (N=1)		
Sample	Dilution	Result
Negative control	N.A.	NEGATIVE
1	1:10	POSITIVE
2	1:10	POSITIVE
3	1:10	LOW POSITIVE
4	1:10	NEGATIVE
5	1:10	NEGATIVE
6	1:10	NEGATIVE
7	1:10	NEGATIVE

Table 1: The rapid tester determined 3 of the 7 samples positively.



Photo 1: An overview of the 1:10 dilution series results. Tester 1, 2 showed a positive outcome. A very dim line can be seen in tester 3.

1:10 dilution series, RT-PCR results

4 of the 7 samples were determined to be positive.

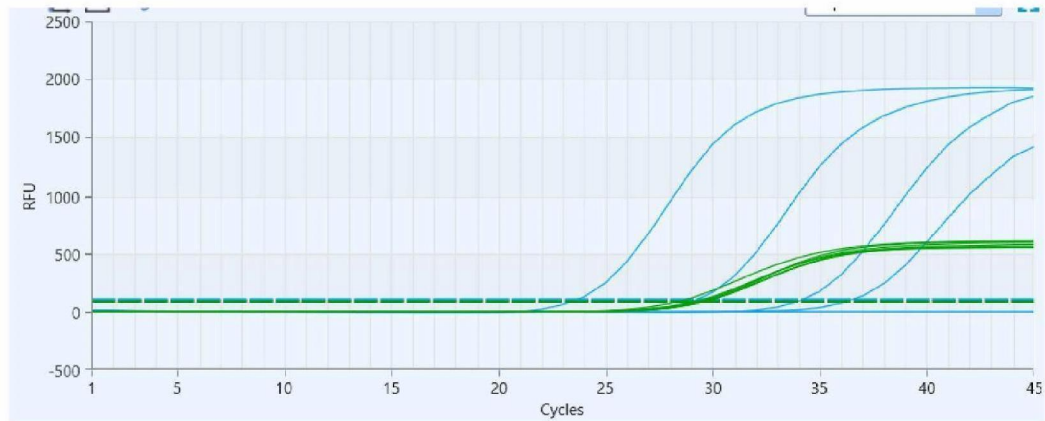


Figure 1: In blue the positive signal for SARS-CoV-2 can be seen. In green the internal control is visualized. This is to confirm the reaction.

1:10 RT-PCR (N=1)			
Sample	Dilution	Result RT-PCR	RT-PCR Ct
Negative control	N.A.	NEGATIVE	-
1	1:10	POSITIVE	23,6
2	1:10	POSITIVE	29,3
3	1:10	POSITIVE	34,2
4	1:10	POSITIVE	36,5
5	1:10	NEGATIVE	-
6	1:10	NEGATIVE	-
7	1:10	NEGATIVE	-

Table 2: The RT-PCR results found 4 of the 7 samples.

1:3 dilution (N=10)

An overview of the results can be seen in table 1:

Test 1:3 Rapid tester (N=10)			
Sample	Dilution	Positive	Negative
Negative	N.A.	0/10	10/10
1	1:3	10/10	0/10
2	1:3	10/10	0/10
3	1:3	10/10	0/10
4	1:3	10/10	0/10
5	1:3	6/10	4/10
6	1:3	0/10	10/10
7	1:3	0/10	10/10
8	1:3	0/10	10/10

Table 3: The outcome of the rapid tester with a dilution series of 1:3. Each sample was tested on 10 different rapid testers.

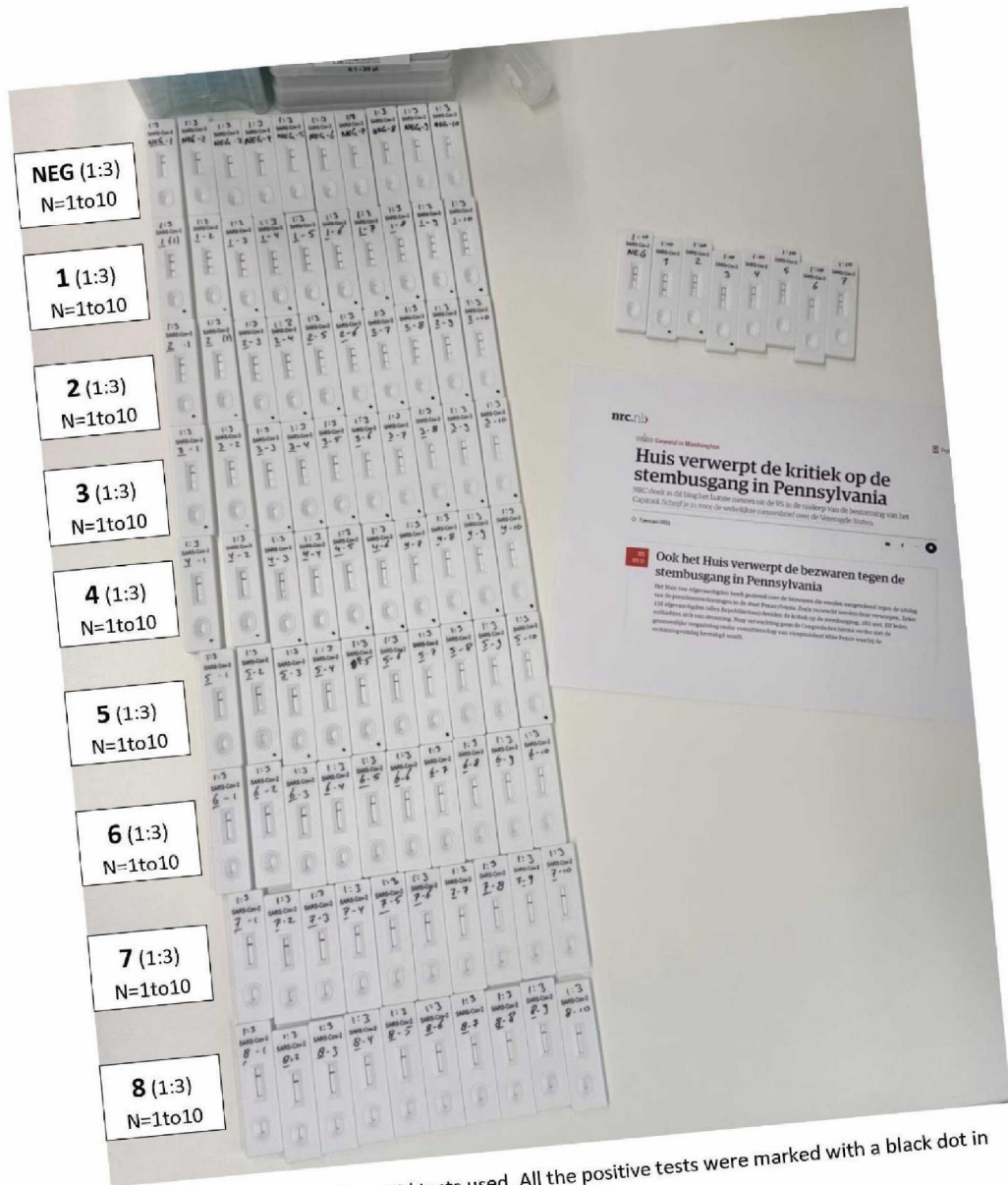


Photo 2: An overview of the rapid tests used. All the positive tests were marked with a black dot in the lower right corner.

1:3 dilution series, RT-PCR results (N=1)

8 of the 8 samples were determined to be positive.

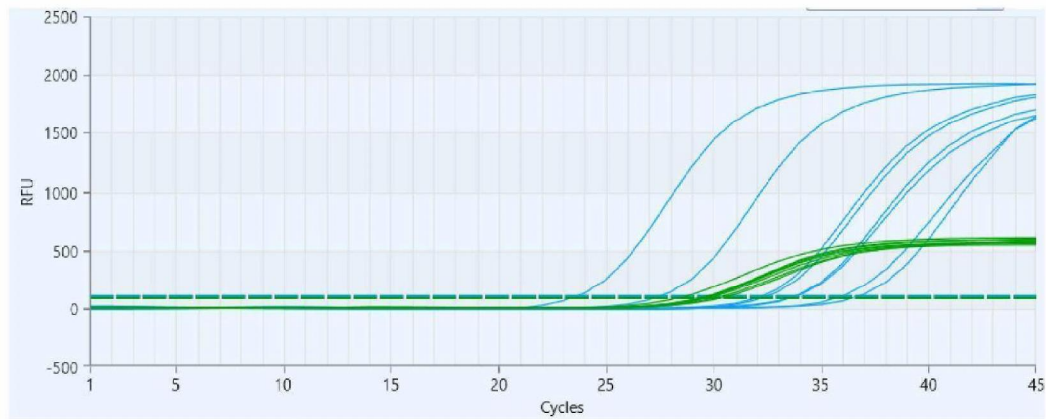


Figure 2: In blue the positive signal for SARS-CoV-2 can be seen. In green the internal control is visualized. This is to confirm the reaction.

1:3 RT-PCR (N=1)			
Sample	Dilution	Result RT-PCR	RT-PCR Ct
Negative control	N.A.	NEGATIVE	-
1	1:3	POSITIVE	23,6
2	1:3	POSITIVE	27,5
3	1:3	POSITIVE	32,2
4	1:3	POSITIVE	32,5
5	1:3	POSITIVE	33,4
6	1:3	POSITIVE	33,8
7	1:3	POSITIVE	36,0
8	1:3	POSITIVE	36,7

Table 4: The RT-PCR determined 8 of the 8 samples correctly.

Conclusion

When comparing the RT-PCR results with the rapid tester the following can be determined. (Table 5) In the 1:10 dilution 3 of the 7 testers showed a positive outcome where the RT-PCR showed 4 out of 7. The rapid tested could correctly determine the outcome of the sample to a Ct value of approximately 32.

In the 1:3 dilution a similar trend can be observed. Here the rapid tester correctly identified 4 out of the 8 positive samples with certainty. The fifth dilution showed to be positive 6 out of 10 times. Similar to the 1:3 dilution series the Rapid tester showed to accurately determine the outcome of the samples up to a Ct value of around 32. The RT-PCR determined all samples to be positive when diluted 1:3.

Sample	Dilution	Result Rapid tester	Result RT-PCR	RT-PCR Ct
NEG Control	N.A.	NEGATIVE	NEGATIVE	-
1	1:10	POSITIVE	POSITIVE	23,6
2	1:10	POSITIVE	POSITIVE	29,3
3	1:10	LOW POSITIVE	POSITIVE	34,2
4	1:10	NEGATIVE	POSITIVE	36,5
5	1:10	NEGATIVE	NEGATIVE	-
6	1:10	NEGATIVE	NEGATIVE	-
7	1:10	NEGATIVE	NEGATIVE	-
NEG Control	N.A.	NEGATIVE	NEGATIVE	-
1	1:3	POSITIVE	POSITIVE	23,6
2	1:3	POSITIVE	POSITIVE	27,5
3	1:3	POSITIVE	POSITIVE	32,2
4	1:3	POSITIVE	POSITIVE	32,5
5	1:3	6/10 POSITIVE	POSITIVE	33,4
6	1:3	NEGATIVE	POSITIVE	33,8
7	1:3	NEGATIVE	POSITIVE	36,0
8	1:3	NEGATIVE	POSITIVE	36,7

Table 5: Comparison of the outcome of the rapid tester compared to the outcome of the RT-PCR.

Outlook - Spartacus-Biomed Rapid tester

The validation of the SARS-CoV-2 rapid tested made by Spartacus-Biomed, which was performed by our lab on 7-1-21 showed very promising results. In this experiment the rapid tester was directly compared to a diluted positive patient sample which was tested also on RT-PCR, which is the golden standard in the field up to this day. The sensitivity of the rapid tester showed to correctly determine samples up to a Ct of 33, which is very high for a rapid tester. Because of this promising results, it is advised to test more positive patient material. For this our lab could test every positive sample around the LOD of the rapid tester (Ct 28 to 34) which they encounter on both RT-PCR and the rapid tester. This way a better insight in the clinical application of the rapid tester can be given.