Goal

A previous validation determined the approximate sensitivity of the Spartacus Biomed SARS-CoV-2 rapid tester. However, it was noticed after this validation that a similar sensitivity with other patient samples could never be accomplished. It was therefore decided to examine the cause of this lowered sensitivity. Many different variables were examined to determine the cause of the lowered LOD, including: incubation time of the sample with buffer, a lowered temperature of the tester during transport, the origin of the buffer, and also batch to batch differences between different testers were tested. Nothing showed to have a positive effect on the LOD. Therefore it was decided to execute a new validation experiment with multiple patient samples to determine the LOD of the rapid tester once again.

Protocol

In this experiment four different highly positive SARS-CoV-2 samples were used:

Sample	**Previous determined Ct**	**Medium**
A	15,2	UTM
B	15,6	UTM
C	16,7	PBS
D	17,7	UTM

Each sample was initially diluted 1:10 (150µl + 1350µl) in the provided buffer. Here the samples were vortexed, incubated for at least 1 minute, vortexed and spun down, as described by the manufacturer. Next, a 9 step-dilution series was made in 1:3 manner (350µl + 700µl), again in the provided buffer. Of each dilution five Spartacus rapid testers were used to examine each sample. From this diluted sample, which was used on the rapid tester, another sample was taken to be tested with RT-PCR to directly compare the outcome of the rapid testers to the outcome of the PCR.

For all four samples, 80 µl of sample was added to each rapid test. A timer was set for 10 minutes after sample addition to the last rapid tester. Photos were taken of the results between 10 minutes after sample addition and before 25 minutes after sample addition. At this point a black dot was placed on the bottom right of the tester when the slightest line was observed. The following batches of testers were used:

Sample	Rapid tester Batch number
A1-1 to A5-3	10270
A6-3 to A9-5	10332
A10-5 to B4-3	10278
B4-5	10923
B5-3 to B10-5	10281
C1-1 to C4-3	10923
C5-3 to C9-5	10926
C10-5 to D4-3	
D5-3 to D9-5	10924
D10-5	10928

Results

Sample A

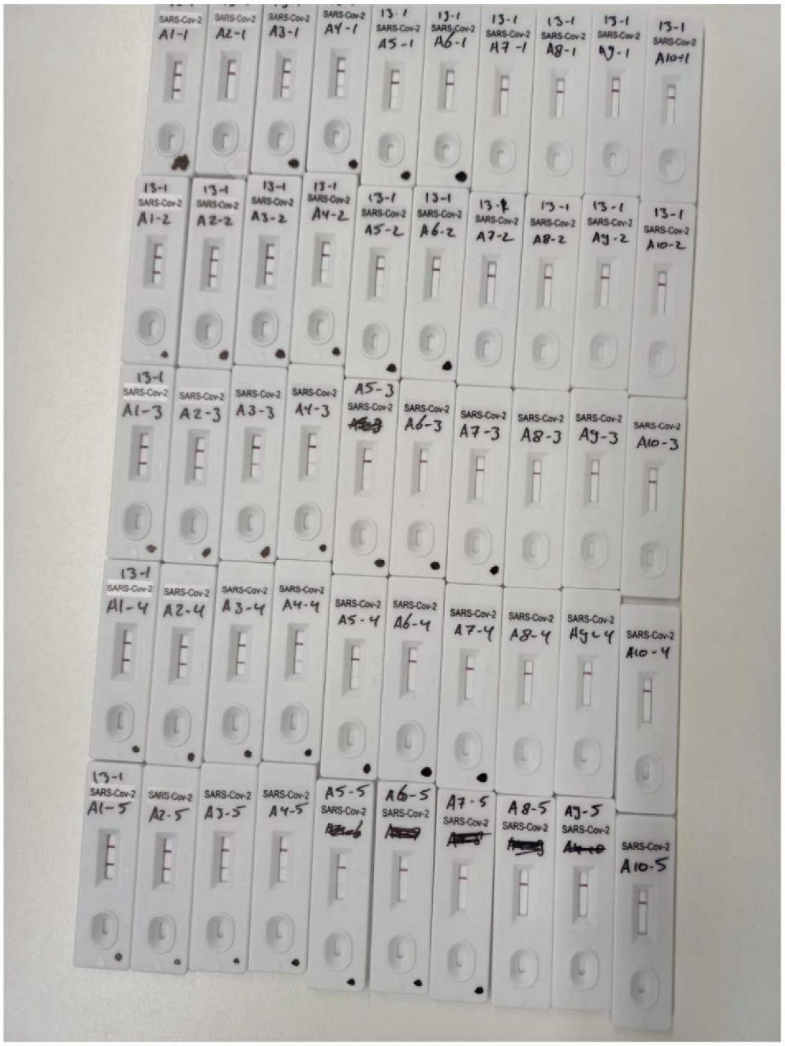

Figure 1| All rapidtesters with the dilution of sample A. The LOD lies between dilution 6 and 7. Note that rapidtester A2-1 is a false negative. A dot indicates a positive test.

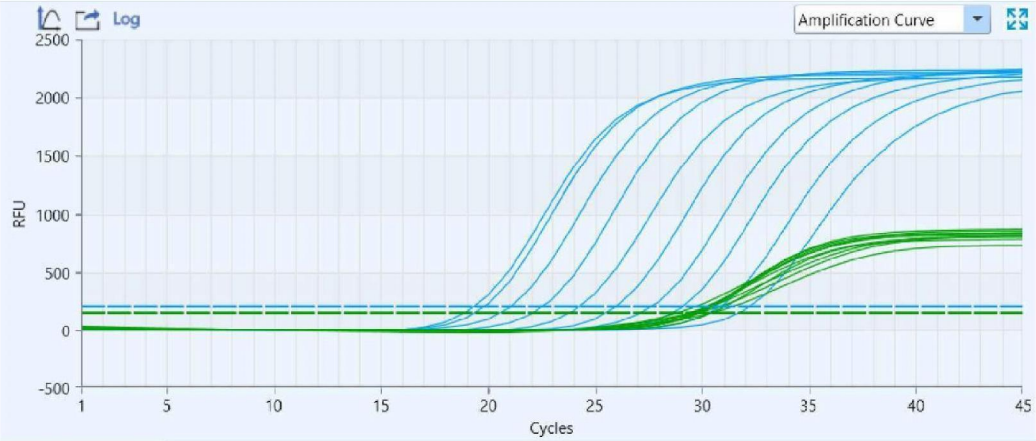

Figure 2| The RT-PCR results of sample A.

Sample	Dilution	Final dilution	Positive rapid testers	RT-PCR (Ct)
A1	1:10	1:10	5/5	19,3
A2	1:3	1:30	4/5	19,8
A3	1:3	1:90	5/5	21,1
A4	1:3	1:270	5/5	22,6
A5	1:3	1:810	5/5	24,3
A6	1:3	1:2430	5/5	26,0
A7	1:3	1:7290	3/5	27,7
A8	1:3	1:21.870	0/5	29,1
A9	1:3	1:65.610	0/5	30,8
A10	1:3	1:196.830	0/5	32,3

Sample B

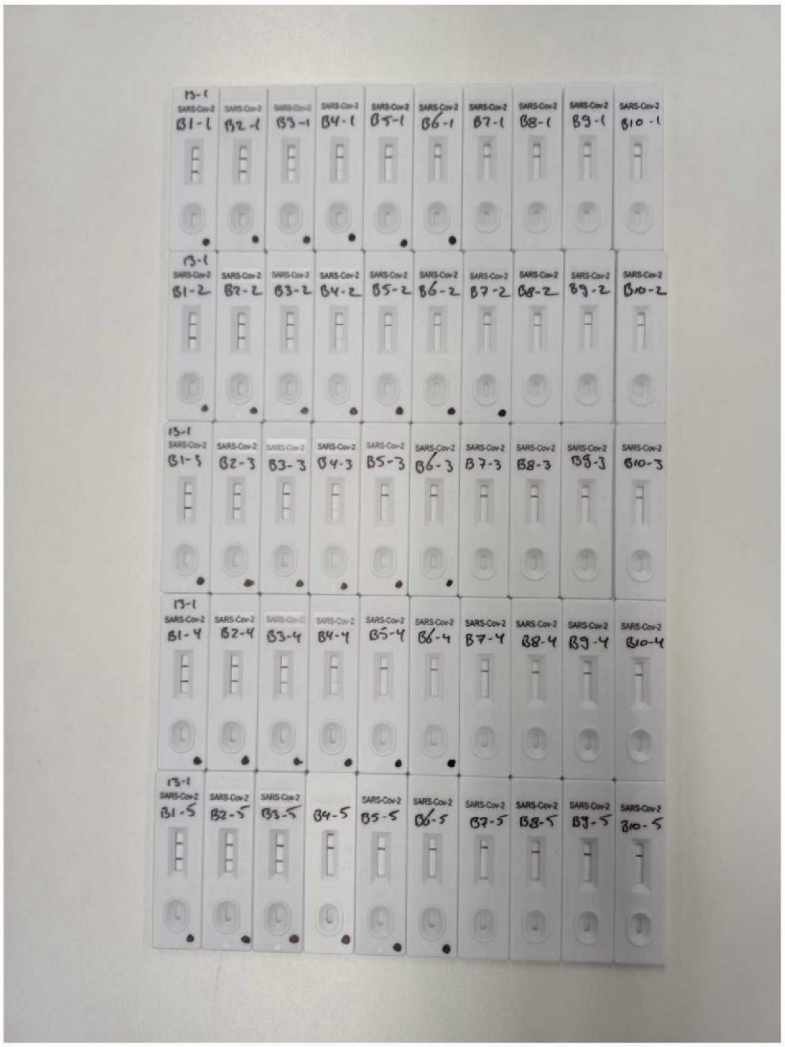

Figure 3| All rapidtesters with the dilution of sample B. The LOD lies between dilution 6 and 7. A dot indicates a positive test.

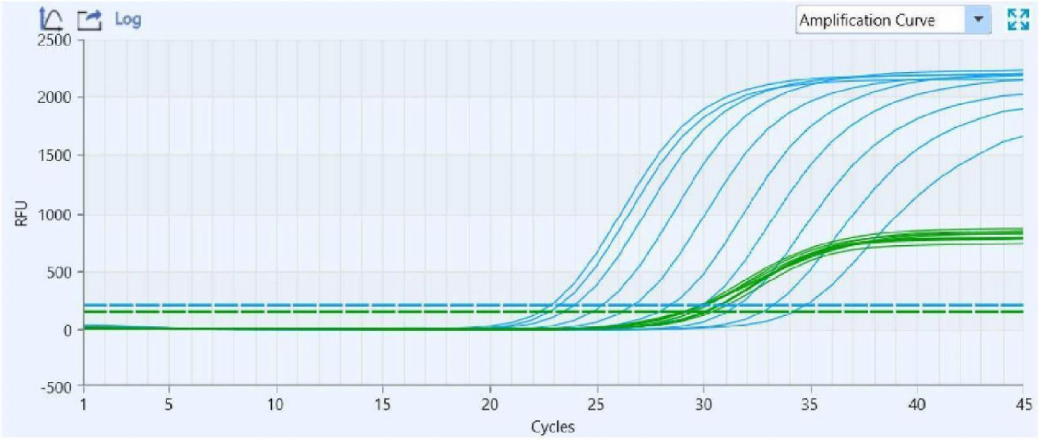

Figure 4| The RT-PCR results of sample B.

Sample	Dilution	Final dilution	Positive rapid testers	RT-PCR (Ct)
B1	1:10	1:10	5/5	23,3
B2	1:3	1:30	5/5	22,9
B3	1:3	1:90	5/5	24,0
B4	1:3	1:270	5/5	25,3
B5	1:3	1:810	5/5	26,7
B6	1:3	1:2430	5/5	28,5
B7	1:3	1:7290	1/5	30,0
B8	1:3	1:21.870	0/5	31,6
B9	1:3	1:65.610	0/5	33,3
B10	1:3	1:196.830	0/5	34,8

Sample C

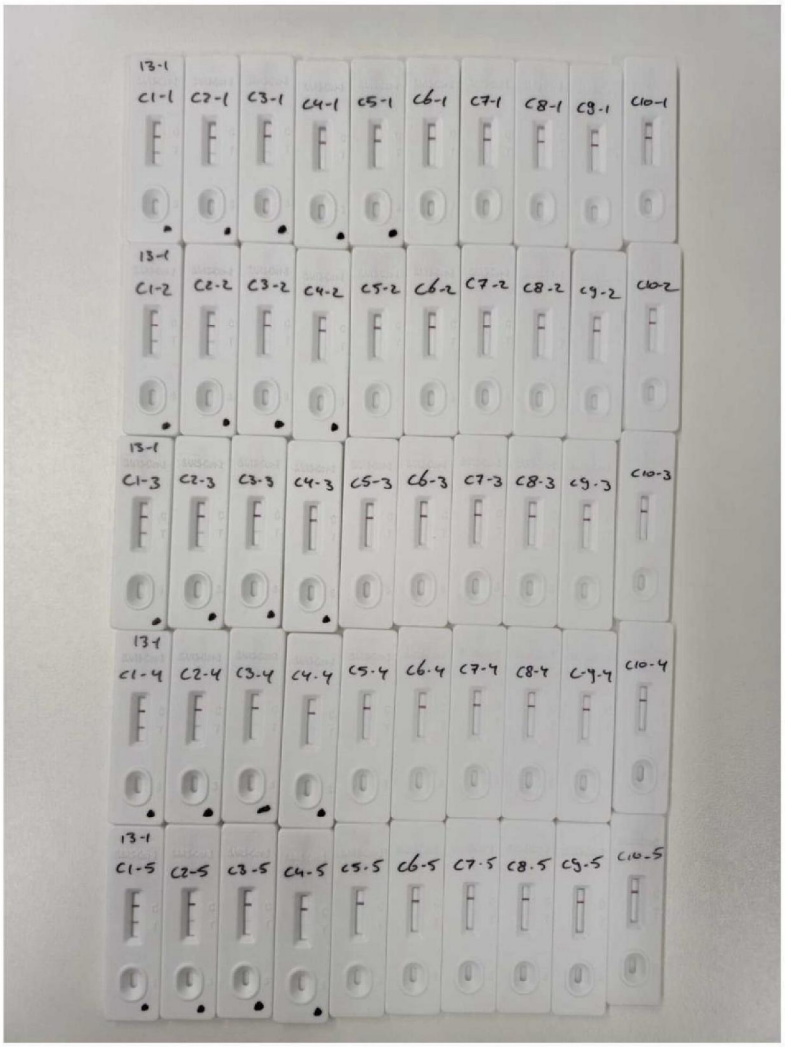

Figure 5| All rapidtesters with the dilution of sample B. The LOD lies between dilution 4 and 5. A dot indicates a positive test.

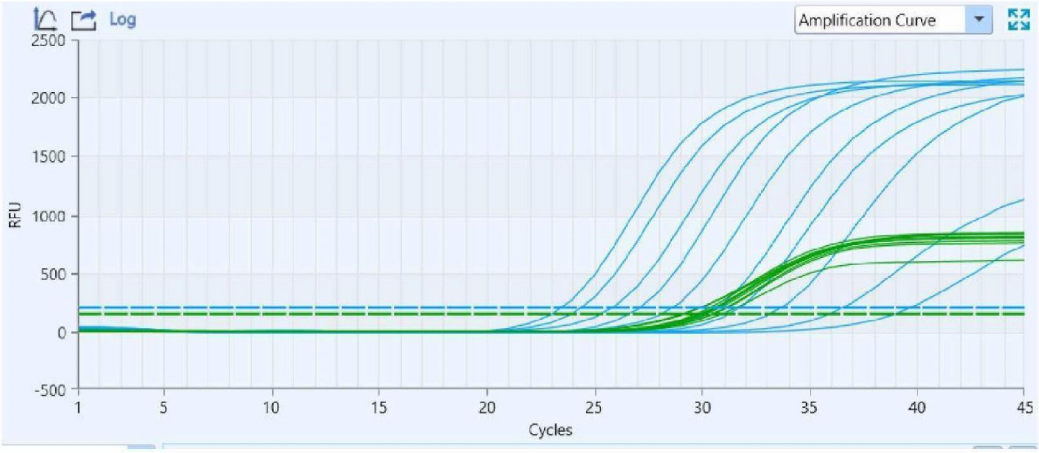

Figure 6| The RT-PCR results of sample C.

Sample	Dilution	Final dilution	Positive rapid testers	RT-PCR (Ct)
C1	1:10	1:10	5/5	23,5
C2	1:3	1:30	5/5	24,4
C3	1:3	1:90	5/5	25,8
C4	1:3	1:270	5/5	27,1
C5	1:3	1:810	1/5	28,6
C6	1:3	1:2430	0/5	30,7
C7	1:3	1:7290	0/5	31,7
C8	1:3	1:21.870	0/5	33,7
C9	1:3	1:65.610	0/5	36,6
C10	1:3	1:196.830	0/5	39,6

Sample D

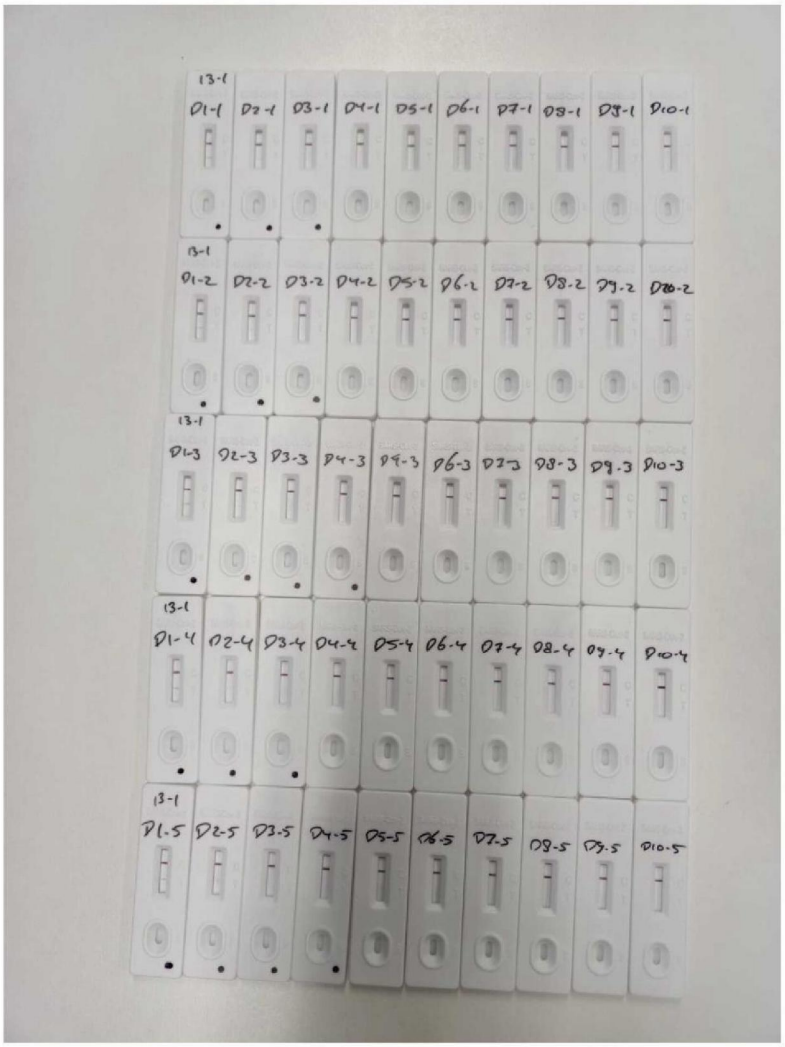

Figure 7| All rapidtesters with the dilution of sample B. The LOD lies between dilution 3 and 4. A dot indicates a positive test.

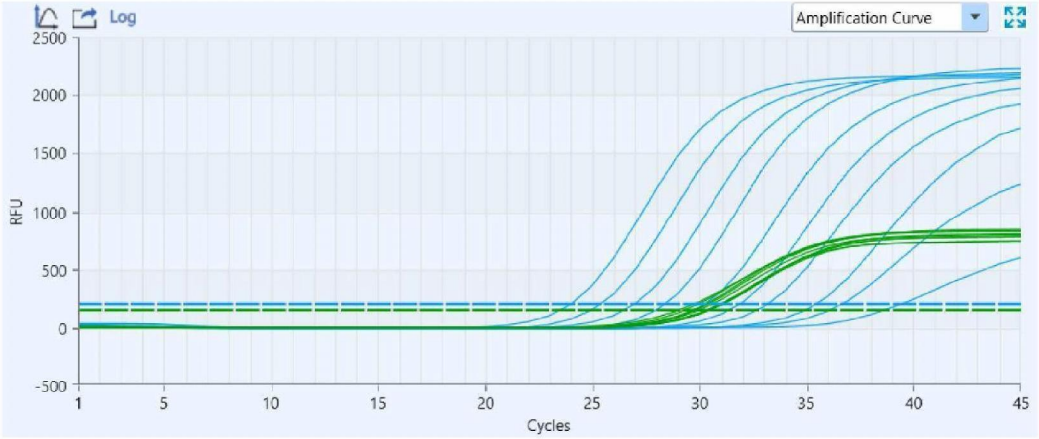

Figure 8| The RT-PCR results of sample B.

Sample	Dilution	Final dilution	Positive rapid testers	RT-PCR (Ct)
D1	1:10	1:10	5/5	24,1
D2	1:3	1:30	5/5	25,4
D3	1:3	1:90	5/5	27,0
D4	1:3	1:270	2/5	28,4
D5	1:3	1:810	0/5	30,3
D6	1:3	1:2430	0/5	31,9
D7	1:3	1:7290	0/5	33,2
D8	1:3	1:21.870	0/5	35,5
D9	1:3	1:65.610	0/5	36,7
D10	1:3	1:196.830	0/5	39,5

Conclusion

Below an overview of the region of interest can be seen. Up to a Ct of 27.1 all rapid testers successfully determined the positivity of the sample. Above a Ct of 30,0 there were no positive rapid testers. Therefore it can be concluded that the LOD of the Spartacus Biomed Rapid testers lies within a Ct of 27,1 and 30,0 when compared to RT-PCR. The exact LOD partially depends on the patient sample.

Based on our experience in the Netherlands this rapid tester test shows an above average LOD. Mozand B.V. will share these results with the RIVM (The national Dutch institute for public health) and will ask for advice how to proceed the validation of this SARS-CoV-2 rapid tester.

RT-PCR CT value	Sample	Positive Rapid testers
26,0	A6	5
26,7	B5	5
27,0	D3	5
27,1	C4	5
27,7	A7	3
28,4	D4	2
28,5	B6	5
28,6	C5	1
29,1	A8	0
30,0	B7	1
30,3	D5	0
30,7	C6	0

Table 1| An overview of the collected data around the LOD.

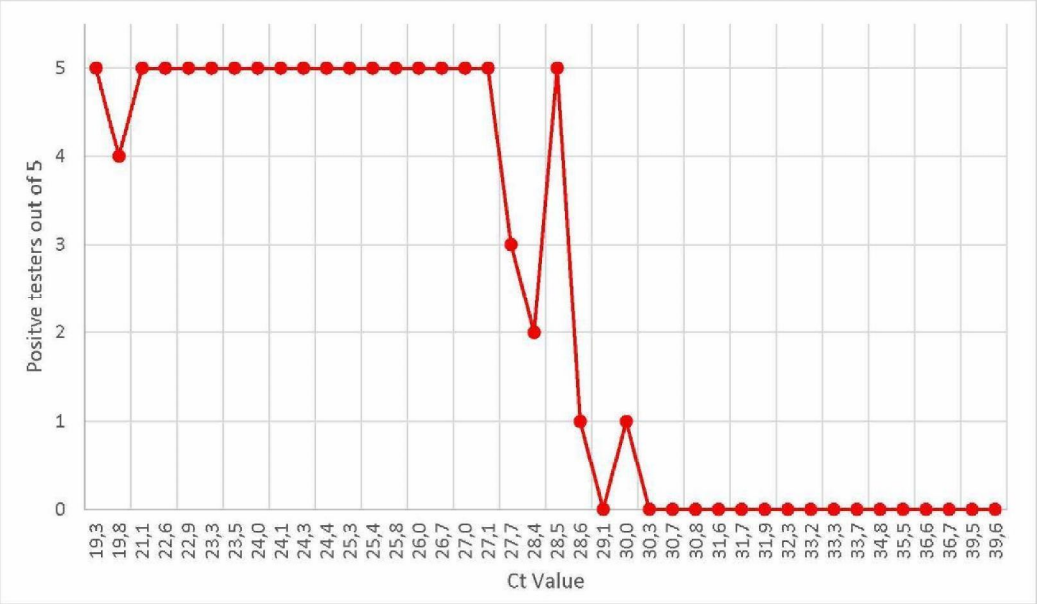

Figure 9| A graphical representation of the LOD of the rapid tester. Up to a Ct of 27.1 all samples could be diagnosed correctly.