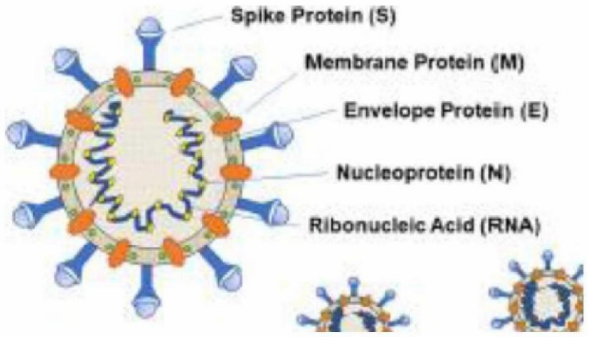
Dear Sir,

We thank you for having selected the Spartacus-Biomed COVID-19 antigen rapid test for further evaluations. We would like to provide scientific and technical explanations for Spartacus-Biomed COVID-19 antigenic tests developments and the "apparent failure" when you tested its sensitivity.

In the report it is mentioned:
"The antigen test was technically evaluated by diluting SARS-CoV-2 stock provided by Erasmus MC in 10-fold series (10-1 to 10-8) viral transport medium (Mediaproducts B.V., Groningen) in page 1"

Viral Transport Medium (VTM) used in the evaluation is incompatible with Spartacus-Biomed test:

Unlike many tests detecting the S protein, Spartacus-Biomed COVID-19 antigen rapid test is detecting the nucleocapsid N protein of the SARS-Cov-2. The N protein is internal within the virus. And the S is found on the surface of the Virus. Please see the attached review (Naqvi et al. BBA 2020) and the following picture:



We have a specially formulated buffer in the Spartacus-Biomed kit to release the N protein before testing. Dilution of the kit buffer over 10% with VTM leads to **poor release of the N protein and consequently a reduced sensitivity** as you noticed.

Your ranked 3rd the Spartacus-Biomed antigenic test in laboratory sensitivity which is not surprising because your laboratory test was done with addition of a large volume (~50%) of the wrong buffer (VTM) to the Spartacus-Biomed buffer. Indeed, we have previously tested that the serial addition of VTM volumes in Spartacus-Biomed buffer strongly lowers the Spartacus-Biomed test sensitivity when VTM is added at over 10% of our buffer volume (please see the tables below). If we were told that you were about to do this kind of test, we would have sent you a large vial of Spartacus-Biomed buffer. As seen below addition of VTM leads to "invalid" test (no C band) that we never seen with the proper buffer.

Volume of virus	Volume of VTM solution	Volume of Extraction buffer	Concentration of SARS-Cov-2 antigen	VTM Percent of final solution
0ul	2000ul	0ul	0 $TCID_{50}$/ml	N-100%
0ul	1800ul	200ul	0 $TCID_{50}$/ml	N-90%
0ul	1600ul	400ul	0 $TCID_{50}$/ml	N-80%
0ul	1400ul	600ul	0 $TCID_{50}$/ml	N-70%
0ul	1200ul	800ul	0 $TCID_{50}$/ml	N-60%
0ul	1000ul	1000ul	0 $TCID_{50}$/ml	N-50%
0ul	800ul	1200ul	0 $TCID_{50}$/ml	N-40%
5ul	1800ul	195ul	2.5×10^3 $TCID_{50}$/ml	P-90%
5ul	1600ul	395ul	2.5×10^3 $TCID_{50}$/ml	P-80%
5ul	1400ul	595ul	2.5×10^3 $TCID_{50}$/ml	P-70%
5ul	1200ul	795ul	2.5×10^3 $TCID_{50}$/ml	P-60%
5ul	1000ul	995ul	2.5×10^3 $TCID_{50}$/ml	P-50%
5ul	800ul	1195ul	2.5×10^3 $TCID_{50}$/ml	P-40%
5ul	600ul	1395ul	2.5×10^3 $TCID_{50}$/ml	P-30%
5ul	400ul	1595ul	2.5×10^3 $TCID_{50}$/ml	P-20%
5ul	200ul	1795ul	2.5×10^3 $TCID_{50}$/ml	P-10%
5ul	100ul	1895ul	2.5×10^3 $TCID_{50}$/ml	P-5%

Result of Lot 1

Dilution	Test result					Rate
	1	2	3	4	5	
N-100%	invalid	invalid	invalid	invalid	invalid	/
N-90%	invalid	invalid	invalid	invalid	invalid	/
N-80%	invalid	+	+	invalid	invalid	0%
N-70%	invalid	+	invalid	+	invalid	0%
N-60%	+	+	+	+	+	100%
N-50%	-	-	-	-	-	100%
N-40%	-	-	-	-	-	100%
P-90%	invalid	invalid	invalid	invalid	invalid	/
P-80%	invalid	invalid	invalid	invalid	invalid	/
P-70%	-	-	-	-	-	0%
P-60%	-	-	-	-	-	0%
P-50%	-	-	invalid	-	-	0%
P-40%	-	-	-	-	-	0%
P-30%	-	-	-	-	-	0%
P-20%	-	-	-	(+)	-	20%
P-10%	+	+	+	+	+	100%
P-5%	+	+	+	+	+	100%

Result of Lot 2

Dilution	Test result					Rate
	1	2	3	4	5	
N-100%	invalid	invalid	invalid	invalid	invalid	/
N-90%	invalid	invalid	invalid	invalid	invalid	/
N-80%	invalid	+	invalid	invalid	invalid	/
N-70%	+	+	+	+	invalid	0%
N-60%	+	+	+	+	+	0%
N-50%	-	-	-	-	-	100%
N-40%	-	-	-	-	-	100%
P-90%	invalid	invalid	invalid	invalid	invalid	/
P-80%	invalid	invalid	invalid	invalid	invalid	/
P-70%	-	-	-	-	-	0%
P-60%	-	-	-	-	invalid	0%
P-50%	-	-	-	-	-	0%
P-40%	-	-	-	-	-	0%
P-30%	-	-	-	-	-	0%
P-20%	-	-	-	-	-	0%
P-10%	+	+	+	+	+	100%
P-5%	+	+	+	+	+	100%

Result of Lot 3

Dilution	Test result					Rate
	1	2	3	4	5	
N-100%	invalid	invalid	invalid	invalid	invalid	/
N-90%	invalid	invalid	invalid	invalid	invalid	/
N-80%	invalid	+	invalid	invalid	invalid	/
N-70%	+	+	+	+	+	0%
N-60%	+	+	+	+	+	0%
N-50%	-	-	-	-	-	100%
N-40%	-	-	-	-	-	100%
P-90%	invalid	invalid	invalid	invalid	invalid	/
P-80%	invalid	invalid	invalid	invalid	invalid	/
P-70%	-	-	-	-	-	0%
P-60%	invalid	-	-	-	-	0%
P-50%	-	-	invalid	-	-	0%
P-40%	-	-	-	-	-	0%
P-30%	-	-	-	-	-	0%
P-20%	-	-	-	-	-	0%
P-10%	+	+	+	+	+	100%
P-5%	+	+	+	+	+	100%

Insufficient incubation/shaking (40 seconds/10 seconds) has consequences:

Decreasing the release of the protein and therefore also of decreasing the measured sensitivity of the kit.

Why Spartacus-Biomed test detects the N protein of the virus instead of the S protein?

The N protein binds the viral genome and nsp3, a key component of the replicase complex, and the M protein. These protein interactions likely help tether the viral genome to the replicase-transcriptase complex (RTC), and subsequently package the encapsidated genome into viral particles (Anthony R et al. 2015). Therefore, the N protein has a much higher selection pressure to allow for virus replication and accordingly is very much less subject to mutations Whereas the S protein has a high mutation rate (please see for review Dutta et al., 2020, attached).

We believe our strategy is good because. Indeed, very recently the United Kingdom (UK) has faced a rapid increase in COVID-19 cases in South East England, leading to enhanced epidemiological and virological investigations. Analysis of viral genome sequence data identified a large proportion of cases belonged to a new single phylogenetic cluster. The new variant is defined by **multiple S protein mutations** (deletion 69-70, deletion 144, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H).

It is anticipated that this new virus variant with increased infectiousness will soon or later spread over Europe and will not be detected by concurrent tests targeting the S protein.

In the report it is mentioned:

Ease of use
The assay has, compared to other SARS-CoV-2 rapid antigen test, some disadvantages concerning ease of use. The swab in the test kit (Model 905101, Taizhou Sun Trine Biotechnology Co.) has a longer, less flexible head compared to the swab used for PCR (Type A-04, Jiangsu Hanheng Medical Technology Co) in the testlane in Bussum, causing discomfort for the participants when NP swabs were taken.

We can propose a more flexible swab at no extra cost.

The buffer is not pre-filled per tube.
We have changed this. In the new protocol the buffer is supplied in single use dropper containers containing the correct volume. just empty it into the tube (takes 2 seconds). No need to count – just empty it.

The material of the tubes and buffer vials are quite hard to squeeze.
In the protocol we have provided December 2th , unlike other tests which detect the S protein it is enough to squeeze the tube 3 times (3 seconds) and incubate 40s.
Please watch the video at this link.

Tube has to be shaken for 10 seconds after attachment of the nozzle.
Please replace 10 seconds by 10 times (which corresponds to 3 seconds)

The test kit does not contain a positive control (swab). The test is less suitable to perform high volumes of tests, as is the case for large testlanes.

With the protocol provided on December 2th there is no longer any problem.

Conclusion
Based on the data presented the assay is not in agreement with the criteria proposed by WHO (1); sensitivity ≥80% and specificity ≥97% for detection of SARS-CoV-2 infected cases with RT-PCR as a reference. The technical lab evaluation confirms the low sensitivity of the assay (sensitivity level 3).
According to the Ease of use remarks and the conclusion, The protocol provided by email December 2th seems not to have been followed correctly which resulted in a decrease in the release of the protein targeted by the Spartacus-Biomed test. In addition, and as explained above, the use of the VTM to dilute the virus inactivated the test.

Conclusion:
According to our laboratory's research, our product is not suitable for using VTM samples to detect products, which will greatly reduce the sensitivity of the product. Our research conclusion is that when the volume of the VTM solution is less than 10% of the final processing solution, it will not affect Product detection sensitivity. We ask you for a new evaluation using the protocol (IFU) provided by email on December 2th and using the buffer from the Spartacus-Biomed kit to make the dilutions.

Thank you very much,
Best regards,

5.1.2e

Anthony R. Fehr and Stanley Perlman.Coronaviruses: An Overview of Their Replication and Pathogenesis.Methods Mol Biol. 2015