



Research and Development

NAME: Marta Skwarecka

DATE: 27.11.2020

PROPOSED PRODUCT: SAVD

1. Title (*The title tells what has been done. Should be short (preferably up to ten words) and describe the main point of the research.*)

SAVD Maturity test

2. Purpose and scope (*explain what the research is in a long sentence (be specific!)*)

The purpose of the test is to determine the stability of the SAVD test. The lyophilisate is stored at room temperature in a place protected from light and moisture. The analysis is performed at approximately 7-day intervals for the first month using the same concentrations of RNA templates suspended in SAVD buffer. After the first month the analysis is performed at approximately monthly intervals.

3. Method

Date of the test:	27.11.2020-30.10.2021
Place of the test:	ul. Szybowcowa 8a, 80-298 Gdańsk, Poland
Test conditions (temperature, humidity):	Temp 20,2°C Humidity 21%
The person performing the tests:	Dr Marta Skwarecka
LOT of reagents analyzed:	SAVD test: LOT 9961711201C SAVD Buffer: LOT 1232912201 RNA SARS-CoV-2: LOT RNA101220J
LOT of reference reagents and trade name:	N/A

Description of the tested method:

- 1. 10-fold dilutions of RNA SARS-CoV-2 in SAVD buffer were prepared and 50 µl of RNA was added to the well of the SAVD assay.*
- 2. One well was used as negative control by adding 50 µl of SAVD buffer.*
- 3. The tubes prepared in this way were placed in a thermal cycler and reacted according to the temperature-time profile of the SAVD test.*
- 4. The test was carried out at regular intervals on the lyophilized SAVD test with the same LOT number.*

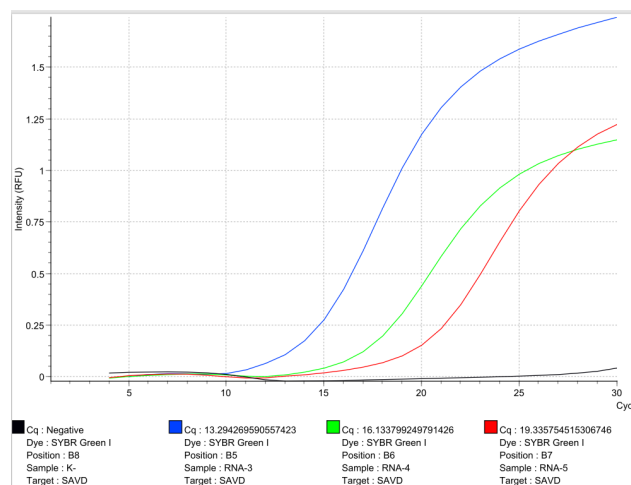
5. The obtained results were and the summary table with the Ct values are presented in section 5 The results of the following report.

4. Tested samples (enter here what samples were tested)

Sample number	Name	Supplier	Producer (like commercial material)	Concentration (as commercial material)
1.	SAVD test	GeneMe	GeneMe	1x
2.	SAVD Buffer	GeneMe	GeneMe	1x
3.	RNA SARS-CoV-2	GeneMe	GeneMe	1x

5. Results (tables with results, tables with comparative results, charts, data repository)

Day 0 (27.11.20):



Pos.	Note	Sample	SYBR Green I	Type	Cq	+
B5		RNA-3	SAVD	U	13.29	✓
B6		RNA-4	SAVD	U	16.13	✓
B7		RNA-5	SAVD	U	19.34	✓
B8		K-	SAVD	N		

Figure 1. Dependence of the fluorescence level on the duration of the SAVD reaction, table of Ct values for individual RNA templates



Figure 2. The photo of the lyophilized mixture.

Day 3. (30.11.20)

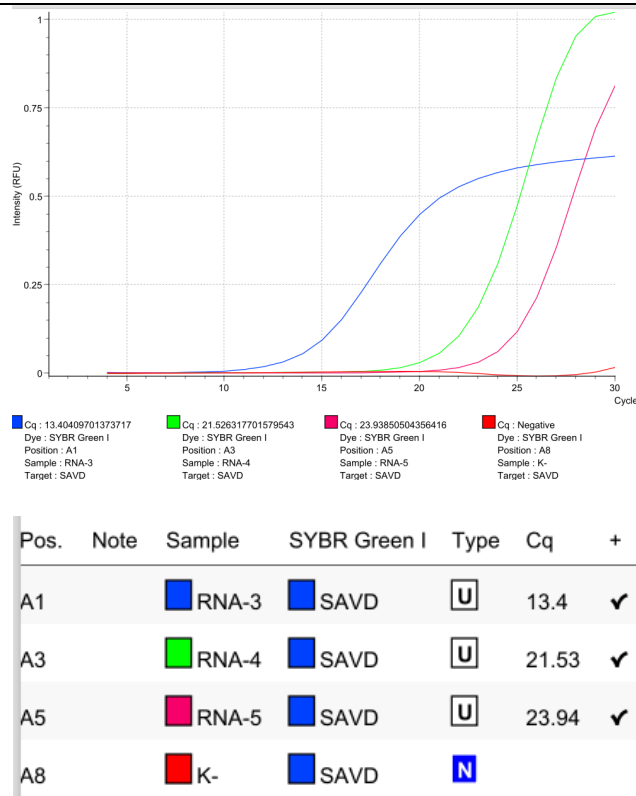
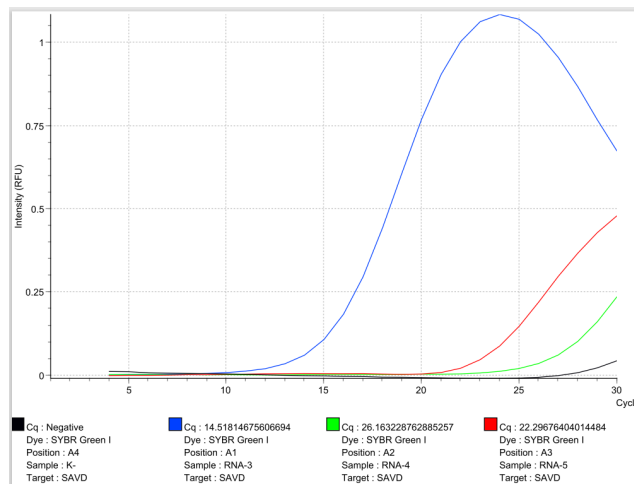


Figure 3. Dependence of the fluorescence level on the duration of the SAVD reaction, table of Ct values for individual RNA templates



Figure 4. The photo of the lyophilized mixture after 3 days of storage.

Day 7. (04.12.20)



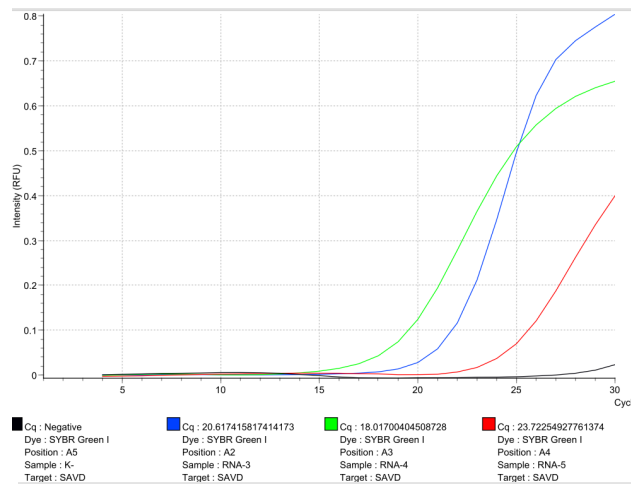
Pos.	Note	Sample	SYBR Green I	Type	Cq	+
A1		RNA-3	SAVD	U	14.52	✓
A2		RNA-4	SAVD	U	26.16	✓
A3		RNA-5	SAVD	U	22.3	✓
A4		K-	SAVD	U		

Figure 5. Dependence of the fluorescence level on the duration of the SAVD reaction, table of Ct values for individual RNA templates



Figure 6. The photo of the lyophilized mixture after 7 days of storage.

Day 14. (11.12.20)



Pos.	Note	Sample	SYBR Green I	Type	Cq	+
A2		RNA-3	Sso7dPfu	U	20.62	✓
A3		RNA-4	Sso7dPfu	U	18.02	✓
A4		RNA-5	Sso7dPfu	U	23.72	✓
A5		K-	Sso7dPfu	N		

Figure 7. Dependence of the fluorescence level on the duration of the SAVD reaction, table of Ct values for individual RNA templates



Figure 8. The photo of the lyophilized mixture after 14 days of storage.

Day 21. (18.12.20)

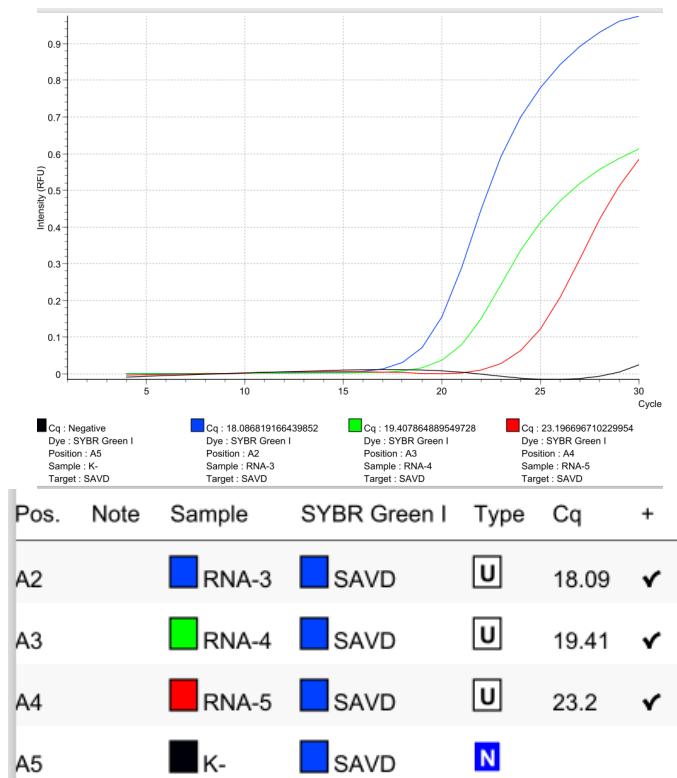
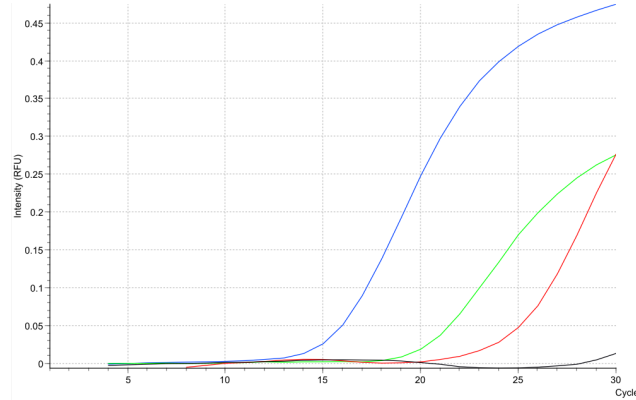


Figure 9. Dependence of the fluorescence level on the duration of the SAVD reaction, table of Ct values for individual RNA templates



Figure 10. The photo of the lyophilized mixture after 21 days of storage.

Day 33. (20.01.21)



■ Cq : Negative
 Dye : SYBR Green I
 Position : D8
 Sample : K-
 Target : A 33 B 34 C 35 D MOJ

■ Cq : 14.700848467758536
 Dye : SYBR Green I
 Position : D5
 Sample : RNA-3
 Target : A 33 B 34 C 35 D MOJ

■ Cq : 19.383936310607307
 Dye : SYBR Green I
 Position : D6
 Sample : RNA-4
 Target : A 33 B 34 C 35 D MOJ

■ Cq : 24.353109292169284
 Dye : SYBR Green I
 Position : D7
 Sample : RNA-5
 Target : A 33 B 34 C 35 D MOJ

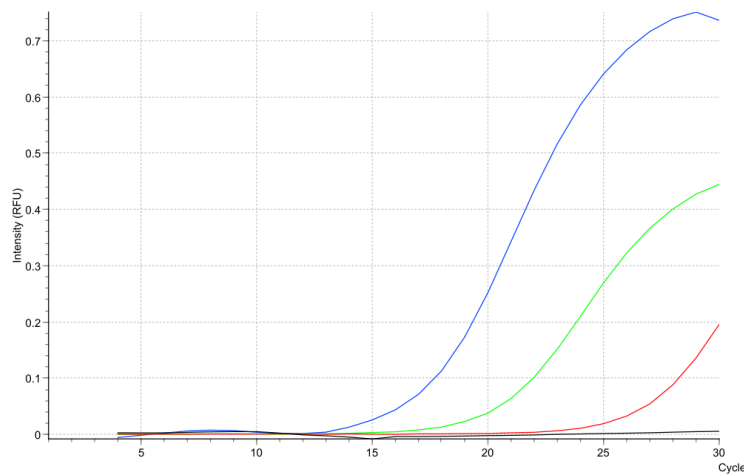
Pos.	Note	Sample	SYBR Green I	Type	Cq	+
D5	■	RNA-3	■ A 33 B 34 C 35 D MOJ	U	14.7	✓
D6	■	RNA-4	■ A 33 B 34 C 35 D MOJ	U	19.38	✓
D7	■	RNA-5	■ A 33 B 34 C 35 D MOJ	U	24.35	✓
D8	■	K-	■ A 33 B 34 C 35 D MOJ	N		

Figure 11. Dependence of the fluorescence level on the duration of the SAVD reaction, table of Ct values for individual RNA templates



Figure 12. The photo of the lyophilized mixture after 33 days of storage.

Day 53. (09.02.21)



■ Cq : Negative
 Dye : SYBR Green I
 Position : B8
 Sample : K-
 Target : SAVD

■ Cq : 16.57594881097266
 Dye : SYBR Green I
 Position : B5
 Sample : RNA-3
 Target : SAVD

■ Cq : 19.746814752893044
 Dye : SYBR Green I
 Position : B6
 Sample : RNA-4
 Target : SAVD

■ Cq : 26.13057040306391
 Dye : SYBR Green I
 Position : B7
 Sample : RNA-5
 Target : SAVD

Pos.	Note	Sample	SYBR Green I	Type	Cq	+
35		RNA-3	SAVD	U	16.58	✓
36		RNA-4	SAVD	U	19.75	✓
37		RNA-5	SAVD	U	26.13	✓
38		K-	SAVD	N		

Figure 13. Dependence of the fluorescence level on the duration of the SAVD reaction, table of Ct values for individual RNA templates

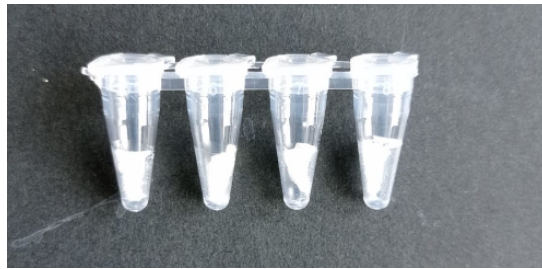
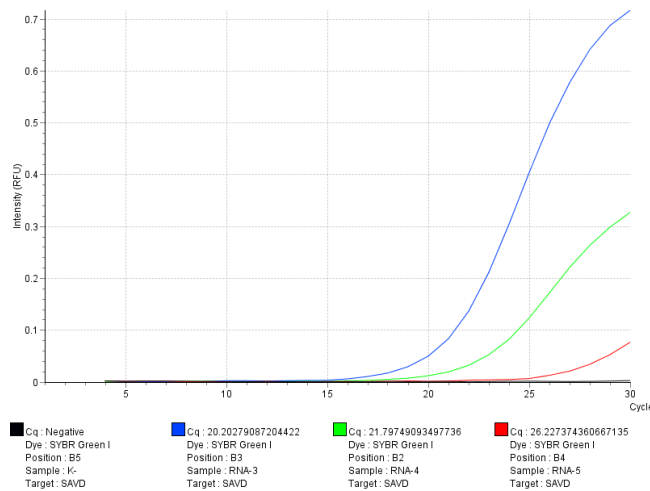


Figure 14. The photo of the lyophilized mixture after 53 days of storage.

Day 93. (23.03.21)



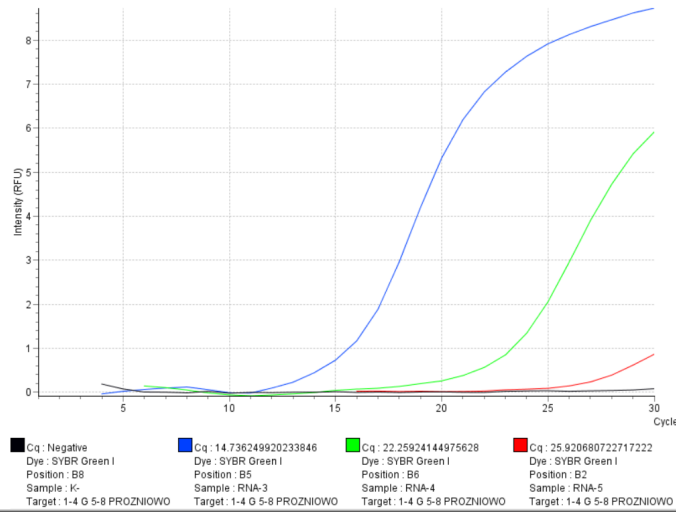
Pos.	Note	Sample	SYBR Green I	Type	Cq	+
B2		RNA-4	SAVD	U	21.8	✓
B3		RNA-3	SAVD	U	20.2	✓
B4		RNA-5	SAVD	U	26.23	✓
B5		K-	SAVD	N		

Figure 15. Dependence of the fluorescence level on the duration of the SAVD reaction, table of Ct values for individual RNA templates



Figure 16. The photo of the lyophilized mixture after 93 days of storage.

Day 121. (15.04.21)



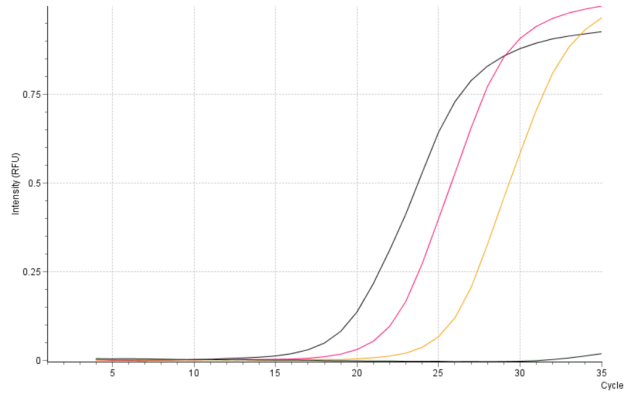
Pos.	Note	Sample	SYBR Green I	Type	Cq	+
B8		K-	SAVD	N		
B5		RNA-3	SAVD	U	14.74	✓
B6		RNA-4	SAVD	U	22.26	✓
B2		RNA-5	SAVD	U	25.92	✓

Figure 17. Dependence of the fluorescence level on the duration of the SAVD reaction, table of Ct values for individual RNA templates



Figure 18. The photo of the lyophilized mixture after 121 days of storage.

Day 146. (10.05.21)



Cq: 18.582839465465 Cq: Negative Cq: 21.196318978207376 Cq: 24.74954452070941
 Dye: SYBR Green I Dye: SYBR Green I Dye: SYBR Green I Dye: SYBR Green I
 Position: B2 Position: C5 Position: B3 Position: B4
 Sample: RNA-3 Sample: K- Sample: RNA-4 Sample: RNA-5
 Target: SAVD Target: SAVD Target: SAVD Target: SAVD

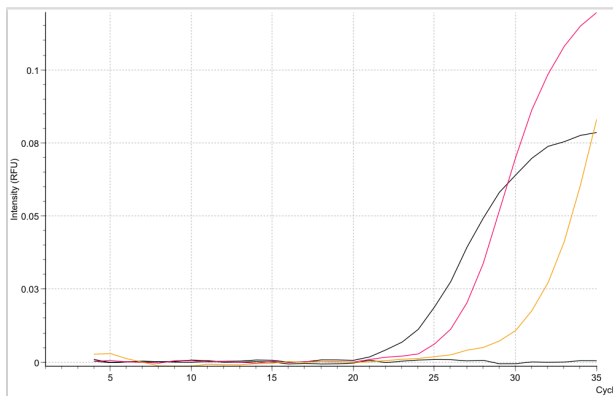
Pos.	Note	Sample	SYBR Green I	Type	Cq	+
B2	■ RNA -3	■ SAVD	■	U	18.58	✓
B3	■ RNA -4	■ SAVD	■	U	21.2	✓
B4	■ RNA -5	■ SAVD	■	U	24.75	✓
C5	■ K-	■ SAVD	■	N		

Figure 19. Dependence of the fluorescence level on the duration of the SAVD reaction, table of Ct values for individual RNA



Figure 20. The photo of the lyophilized mixture after 146 days of storage.

Day 197. (30.06.21)



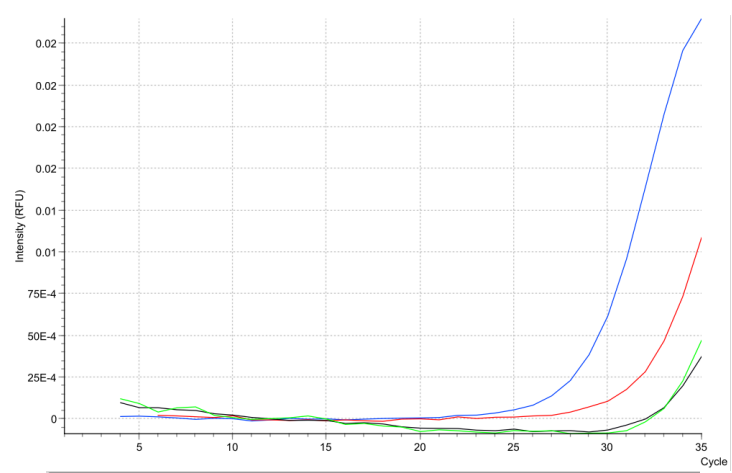
Pos.	Note	Sample	SYBR Green I	Type	Cq	+
B1		K-	SAVD	N		
B2		RNA -3	SAVD	U	22.61	
B3		RNA -4	SAVD	U	25.31	
B4		RNA -5	SAVD	U	31.19	

Figure 21. Dependence of the fluorescence level on the duration of the SAVD reaction, table of Ct values for individual RNA



Figure 22. The photo of the lyophilized mixture after 197 days of storage.

Day 259. (31.08.2021)



Pos.	Note	Sample	SYBR Green I	Type	Cq	+
B2		RNA -3	SAVD	U	28.14	
B3		RNA-4	SAVD	U	30.97	
B4		RNA-5	SAVD	U		
B5		K-	SAVD	N		

Figure 23. Dependence of the fluorescence level on the duration of the SAVD reaction, table of Ct values for individual RNA



Figure 24. The photo of the lyophilized mixture after 259 days of storage.

Day 289. (30.09.2021)

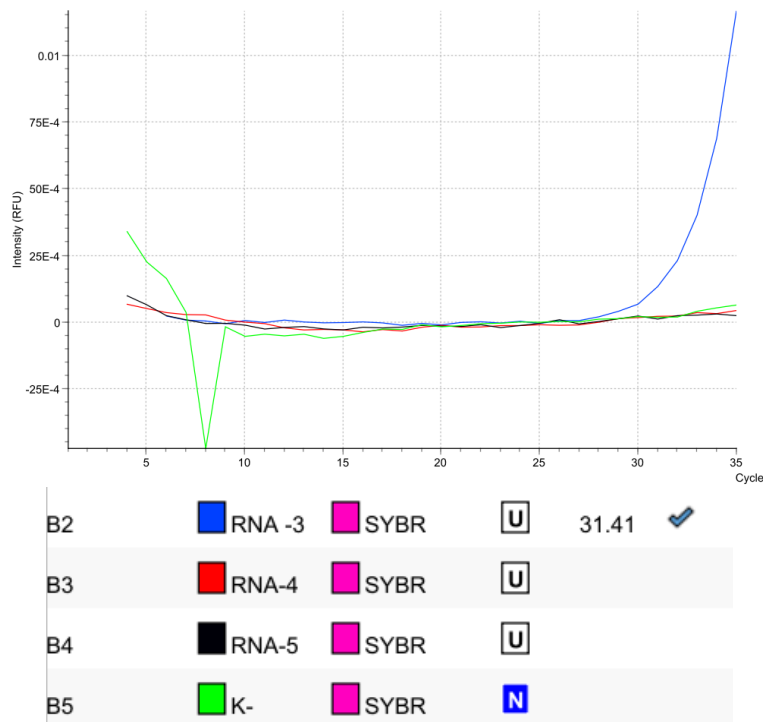


Figure 25. Dependence of the fluorescence level on the duration of the SAVD reaction, table of Ct values for individual RNA



Figure 26. The photo of the lyophilized mixture after 289 days of storage.

Day 320. (30.10.2021)

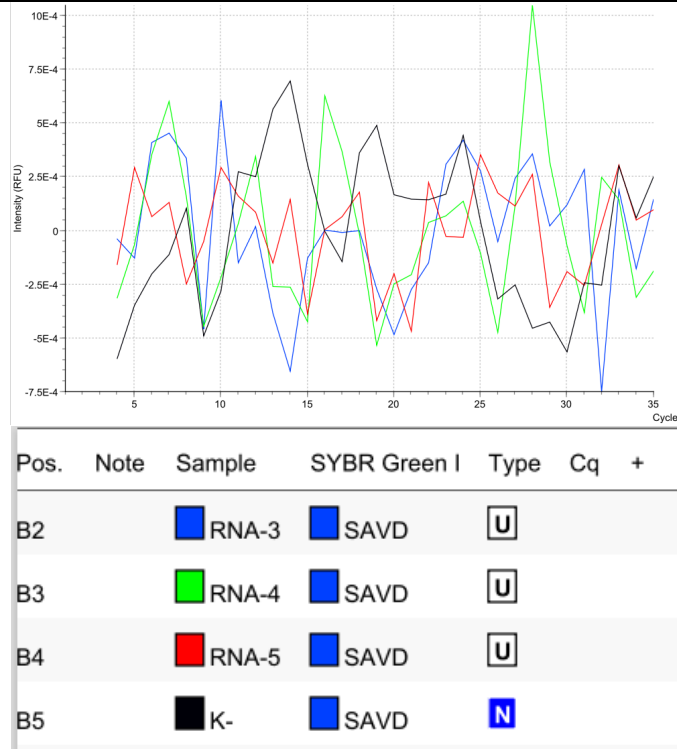


Figure 27. Dependence of the fluorescence level on the duration of the SAVD reaction, table of Ct values for individual RNA



Figure 28. The photo of the lyophilized mixture after 320 days of storage.

Link to the data repository kept in the cloud: --

6. Conclusions (logical interpretation of the results (what happened, what didn't, why?), Identify the limitation of the study (why something did not work))

The lyophilized SAVD test remained active and appropriate sensitivity after a storage time of 197 days (6 month).

After 259 days, the test is still active, but the sensitivity of the test decreases 10 times. Due to the rising negative control, the test should be shortened to 33 cycles.

After 289 days, the test is still active, but the sensitivity of the test decreases 100 times

After 320 days, the test is completely inactive. **The study is concluded.**

7. References (if there is a reference to the literature, please enter it here).

None

Approved for external release by Sabina Żołędowska, CQO

Date of approval: 30th October 2021

Signature: Sabina Żołędowska