

Stability Studies Part of TD, Annex A6			
Product: COVID-19 Ag Test (cassette, single pouched) Nasal, naso-, oropharyngeal swab	Revision: 1.3	Valid from: 2021-05-10	pages: 1 von 11

This Document is part of the Technical File, Annex A6	
Scope	To demonstrate that the stability of the assay (real time and/or accelerated stability, in use and transport stability) is in accordance with the directive 98/79/EC and the standard DIN EN 23640:2011.

Responsible manufacturer:	nal von minden GmbH Carl-Zeiss Str. 12 47445 Moers Germany
Product:	COVID-19 Ag Test Test for the qualitative detection of SARS-CoV-2 viral nucleoprotein antigens Format: Cassette Test, single pouched and derived variants (as listed in Annex B8)
Sample Material	human nasal, nasopharyngeal and oropharyngeal specimens* *sample collection with provided swabs; before application to the test cassette, swabs are extracted in the provided buffer
REF (Article#):	For main nal von minden Products*: 243103X-Y e.g. 243103N-20 X-Y = optional extension for different variants (X: optional letter code; Y: optional number code for kit size) *Customer specific variants, brand name variants or variants in language, kit sizes or kit-specific accessories are possible and might have deviating REF (refer to confidential Annex B8 for an overview of available product variants)
Classification: (according to IVDD 98/79/EC)	Other device (all devices except Annex II and self-testing devices)
Product Certification Conformity Assessment Route	IVDD 98/79/EC Annex III
EDMA-Code:	15-70-90-90-00
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1. Accelerated Stability Study

Aim

Determination of stability of the COVID-19 Ag Test (cassette, single pouched) with an accelerated stability testing. Tests are stored at elevated temperature in order to accelerate aging processes.

Testing procedure

Accelerated Stability of the COVID-19 Ag Test (cassette) was evaluated using tests from three different production LOTs. These tests were placed in an incubator with the temperature calibrated at 55 °C, with relative humidity (RH) calibrated at 60%. A series of stability tests were performed at 0, 5, 10, 15, 20, 25, 30 and 35 days*. At each time point, cassettes were brought to room temperature (25±5°C) before testing. Tests were assayed using following samples:

- Negative control
- SARS-CoV-2 Ag low positive control
- SARS-CoV-2 Ag high positive control

Each specimen was measured in triplicate for each production LOT.

The tests were performed according to the procedure described in the package insert. Results with visible T-line(s) (test line) and visible C-line (control line) were documented as positive results (positive = +). If only a visible C-line appeared but no T-line, results were documented as negative results (negative = -).

*The study refers to the "Standard Guide for Accelerated Aging of Sterile medical Device Packages", American Society for testing and Materials (ASTM) F1980-02 which complies with ISO 11607-1:2006. Accordingly, the necessary study duration to verify a shelf life of at least 24 months of the test cassettes is calculated based on the Arrhenius Reaction Rate Theory which states that "a rise in temperature of 10°C will roughly double the rate of a chemical reaction".

Calculating the necessary storage duration at 55°C to verify a 27 months (with Q₁₀ (aging factor) = 2.5, and TRT (ambient temperature) = 20°C), shelf life leads to a result of 32.8 days. Therefore, the duration of the accelerated stability study is set to 35 days.

Results

The results are presented in the following table.

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COVID-19 Ag Test (cassette): Results Accelerated Stability Study
Testing after storage at 55 °C

Days	Sample	Lot 1	Lot 2	Lot 3	Pass or Fail
0	Negative control	3 -	3 -	3 -	Pass
	SARS-CoV-2 Ag low positive control	3 +	3 +	3 +	
	SARS-CoV-2 Ag high positive control	3 +	3 +	3 +	
5	Negative control	3 -	3 -	3 -	Pass
	SARS-CoV-2 Ag low positive control	3 +	3 +	3 +	
	SARS-CoV-2 Ag high positive control	3 +	3 +	3 +	
10	Negative control	3 -	3 -	3 -	Pass
	SARS-CoV-2 Ag low positive control	3 +	3 +	3 +	
	SARS-CoV-2 Ag high positive control	3 +	3 +	3 +	
15	Negative control	3 -	3 -	3 -	Pass
	SARS-CoV-2 Ag low positive control	3 +	3 +	3 +	
	SARS-CoV-2 Ag high positive control	3 +	3 +	3 +	
20	Negative control	3 -	3 -	3 -	Pass
	SARS-CoV-2 Ag low positive control	3 +	3 +	3 +	
	SARS-CoV-2 Ag high positive control	3 +	3 +	3 +	
25	Negative control	3 -	3 -	3 -	Pass
	SARS-CoV-2 Ag low positive control	3 +	3 +	3 +	
	SARS-CoV-2 Ag high positive control	3 +	3 +	3 +	
30	Negative control	3 -	3 -	3 -	Pass
	SARS-CoV-2 Ag low positive control	3 +	3 +	3 +	
	SARS-CoV-2 Ag high positive control	3 +	3 +	3 +	
35	Negative control	3 -	3 -	3 -	Pass
	SARS-CoV-2 Ag low positive control	3 +	3 +	3 +	
	SARS-CoV-2 Ag high positive control	3 +	3 +	3 +	

3- indicates negative test results for all 3 replicates

3+ indicates positive test results for all 3 replicates

Throughout the whole testing period, all tests from all LOTs showed correct negative results for the negative controls, and all positive controls were correctly determined as positive. No false results were obtained for the different LOTs or within the determinations.

Conclusion

The nal von minden COVID-19 Ag Test (cassette) is stable at 55 °C for 35 days. If plotted on an Arrhenius Plot, the shelf life of this product can be predicted to be at least 24 months. In addition, the study demonstrates that the tests tolerate moderately elevated temperature of 55 °C for 35 days without a loss in functionality.

The Accelerated Stability Study for the nal von minden COVID-19 Ag Test was continued for the time points 42, 49, 56, 63, 70, 77, 84 and 91 days. Here, it was demonstrated that the nal von minden COVID-19 Ag Test (cassette) is stable at 55 °C for 91 days. This extends the tolerance range when choosing the numerical values for the variables referring to "Standard Guide for Accelerated Aging of Sterile medical Device Packages", American Society for testing and Materials (ASTM) F1980-02 which complies with ISO 11607-1:2006. However, the intended shelf life of the test between 2 °C and 30 °C remains specified as 24 months.

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2. Real Time Stability Study

Aim

This study is for the determination of the real time stability of the product when stored under intended storage conditions (2 – 30 °C).

Testing procedure

Real Time Stability of the COVID-19 Ag Test (cassette, single pouched) was evaluated using tests from three different LOTs. These were either placed in a refrigerator with the temperature calibrated to appr. 2 – 8 °C or in an incubator with temperature set to 30 °C. A series of stability tests were performed at 0, 3, 6, 9 and 12 months. Studies are ongoing and will be continued for the time points 15, 18, 21, 24 and 27 months.

Tests were assayed using following samples:

- Negative control
- SARS-CoV-2 Ag low positive control
- SARS-CoV-2 Ag high positive control

Testing at each specific time point consisted of 3 replicates for each specimen. Testing procedure was performed as described for the accelerated stability testing and according to the package insert.

Results

All available data is summarized in the following table.

COVID-19 Ag Test (cassette): Real time stability study (ongoing)

Time point (month)	Stability panel member	LOT 1		LOT 2		LOT 3	
		2-8°C	30°C	2-8°C	30°C	2-8°C	30°C
0	Negative	3-	3-	3-	3-	3-	3-
	Low Positive control	3+	3+	3+	3+	3+	3+
	High Positive control	3+	3+	3+	3+	3+	3+
3	Negative	3-	3-	3-	3-	3-	3-
	Low Positive control	3+	3+	3+	3+	3+	3+
	High Positive control	3+	3+	3+	3+	3+	3+
6	Negative	3-	3-	3-	3-	3-	3-
	Low Positive control	3+	3+	3+	3+	3+	3+
	High Positive control	3+	3+	3+	3+	3+	3+
9	Negative	3-	3-	3-	3-	3-	3-
	Low Positive control	3+	3+	3+	3+	3+	3+
	High Positive control	3+	3+	3+	3+	3+	3+
12	Negative	3-	3-	3-	3-	3-	3-
	Low Positive control	3+	3+	3+	3+	3+	3+
	High Positive control	3+	3+	3+	3+	3+	3+

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15	Negative	[]	[]	[]	[]	[]	[]
	Low Positive control	[]	[]	[]	[]	[]	[]
	High Positive control	[]	[]	[]	[]	[]	[]
18	Negative	[]	[]	[]	[]	[]	[]
	Low Positive control	[]	[]	[]	[]	[]	[]
	High Positive control	[]	[]	[]	[]	[]	[]
21	Negative	[]	[]	[]	[]	[]	[]
	Low Positive control	[]	[]	[]	[]	[]	[]
	High Positive control	[]	[]	[]	[]	[]	[]
24	Negative	[]	[]	[]	[]	[]	[]
	Low Positive control	[]	[]	[]	[]	[]	[]
	High Positive control	[]	[]	[]	[]	[]	[]
27	Negative	[]	[]	[]	[]	[]	[]
	Low Positive control	[]	[]	[]	[]	[]	[]
	High Positive control	[]	[]	[]	[]	[]	[]

3- indicates negative test results for all 3 replicates

3+ indicates positive test results for all 3 replicates

[] indicates open results in this ongoing study

Tests generated expected correct results for the time period from month 0 to month 12. Transport stability studies are ongoing and will be continued for the time points 15, 18, 21, 24 and 27 months. This document will be updated when the transport stability study is finished.

Conclusion

Based on the results of the real time stability studies, the stability of the COVID-19 Ag Test (cassette, single pouched) was validated for up to 12 months. The real time stability study for the COVID-19 Ag Test is still ongoing to confirm stability for at least 24 months. This document will be updated in regular intervals.

Based on the accelerated stability study (see section 1), the shelf life of the COVID-19 Ag Test can be predicted to be at least 24 months.

3. In-use stability Study

Aim

Depending on the product, In-use stability might comprise two issues:

1. The stability of the test from the time of opening of the primary container to the time of reading the test result ("on board stability").
2. Stability in actual routine use for tests that are packed in a way that makes a repeated opening and closing of the primary container necessary. Here, it is necessary to determine if a repeated exposure to environmental influences during opening/closing affects the stability.

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Testing Procedure

Sealed foil pouches were opened and test cassettes were stored at 10 °C and 35 °C combined with 20% humidity or 80% humidity. Tests were evaluated after 0, 1, 2, 3, and 4 hours (h) using following samples:

- Negative control
- SARS-CoV-2 Ag low positive control
- SARS-CoV-2 Ag high positive control

The whole set of experiments was repeated with three production Lots. The tests were performed according to the procedure described in the package insert. Results with visible T-line(s) (test line) and visible C-line (control line) were documented as positive results (positive = +). If only a visible C-line appeared but no T-line, results were documented as negative results (negative = -).

Results

All available data is summarized in the following tables. Since results were identical for all three production Lots, only results for lot 1 are shown.

COVID-19 Ag Test (cassette): In-use stability study

Testing after storage at 10 °C, Lot 1

Time after opening pouch Samples	10 °C									
	20% humidity					80% humidity				
	0 h	1 h	2 h	3 h	4 h	0 h	1 h	2 h	3 h	4 h
Negative control	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -
SARS-CoV-2 Ag low positive control	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +
SARS-CoV-2 Ag high positive control	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +

3- indicates negative test results with 3 replicates

3+ indicates positive test results with 3 replicates

Testing after storage at 35 °C, Lot 1

Time after opening pouch Samples	35 °C									
	20% humidity					80% humidity				
	0 h	1 h	2 h	3 h	4 h	0 h	1 h	2 h	3 h	4 h
Negative control	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -
SARS-CoV-2 Ag low positive control	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +
SARS-CoV-2 Ag high positive control	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +

3- indicates negative test results with 3 replicates

3+ indicates positive test results with 3 replicates

Throughout the whole testing period with opened pouch under the different conditions, where temperature and humidity were varied, all test cassettes showed correct negative results for the negative controls, and all positive controls were correctly determined as positive.

Conclusion

After opening the primary packaging of the nal von minden COVID-19 Ag Test (cassette,

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single pouched), the test is stable for up to 4 hours. To avoid any risk and to obtain best results, the assay should be performed immediately after opening the foil pouch as stated in the package insert.

4. Transport Stability Study

Aim

Determination of the transport stability of the product: This study evaluates the impact of extreme conditions (e.g. elevated temperature) on test performance and shelf life using simulated transport conditions combined with real time stability studies.

Testing Procedure

Tests were assayed using following samples:

- Negative control
- SARS-CoV-2 Ag low positive control
- SARS-CoV-2 Ag high positive control

One LOT was tested in the study. Three groups of stress tests were performed for simulating transport conditions:

- 1) 3x FT: 3 freeze/thaw cycles were performed. For each cycle, complete test kits (including buffer and sample collection swabs) were frozen to -20°C for 12 hours, then thawed and kept at room temperature for 12 hours. After the last thaw phase, tests were transferred to storage temperature for the remainder of the study.
- 2) 2 Days at 55 °C: Test kits were placed at 55 °C for 2 days and then transferred to storage temperature for the remainder of the study.
- 3) Reduced atmospheric pressure: Test kits were placed under vacuum to a level of 500 mTorr for 24 hours and then transferred to storage temperature for the remainder of the study.

After simulated transport conditions, devices were divided into two groups: one group was stored at 2 – 8 °C, the other at 30 ± 2 °C. Testing was conducted at 0, 3, 6, 9 and 12 months. At each time point, test devices were brought to room temperature and evaluated using the stability panel: negative, low positive and high positive controls (panel members). Testing procedure and result interpretation were performed according to package insert. Testing was performed in triplicate for each panel member under each condition.

Studies are ongoing and will be continued for the time points 15, 18, 21, 24 and 27 months.

Results

The following tables summarize the results of the study:

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Transport Stability after Stressed by 3x FT (ongoing)

Month	Specimen	2 – 8°C			30°C		
		1	2	3	1	2	3
0	Negative	-	-	-	-	-	-
	Low Positive control	+	+	+	+	+	+
	High Positive control	+	+	+	+	+	+
3	Negative	-	-	-	-	-	-
	Low Positive control	+	+	+	+	+	+
	High Positive control	+	+	+	+	+	+
6	Negative	-	-	-	-	-	-
	Low Positive control	+	+	+	+	+	+
	High Positive control	+	+	+	+	+	+
9	Negative	-	-	-	-	-	-
	Low Positive control	+	+	+	+	+	+
	High Positive control	+	+	+	+	+	+
12	Negative	-	-	-	-	-	-
	Low Positive control	+	+	+	+	+	+
	High Positive control	+	+	+	+	+	+
15	Negative	[]	[]	[]	[]	[]	[]
	Low Positive control	[]	[]	[]	[]	[]	[]
	High Positive control	[]	[]	[]	[]	[]	[]
18	Negative	[]	[]	[]	[]	[]	[]
	Low Positive control	[]	[]	[]	[]	[]	[]
	High Positive control	[]	[]	[]	[]	[]	[]
21	Negative	[]	[]	[]	[]	[]	[]
	Low Positive control	[]	[]	[]	[]	[]	[]
	High Positive control	[]	[]	[]	[]	[]	[]
24	Negative	[]	[]	[]	[]	[]	[]
	Low Positive control	[]	[]	[]	[]	[]	[]
	High Positive control	[]	[]	[]	[]	[]	[]
27	Negative	[]	[]	[]	[]	[]	[]
	Low Positive control	[]	[]	[]	[]	[]	[]
	High Positive control	[]	[]	[]	[]	[]	[]

“-“ indicates a negative test result
 “+“ indicates a positive test result
 [] indicates open result in this ongoing study

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Transport Stability after Stressed by 2 Days at 55 °C (ongoing)

Month	Specimen	2 – 8°C			30°C		
		1	2	3	1	2	3
0	Negative	-	-	-	-	-	-
	Low Positive control	+	+	+	+	+	+
	High Positive control	+	+	+	+	+	+
3	Negative	-	-	-	-	-	-
	Low Positive control	+	+	+	+	+	+
	High Positive control	+	+	+	+	+	+
6	Negative	-	-	-	-	-	-
	Low Positive control	+	+	+	+	+	+
	High Positive control	+	+	+	+	+	+
9	Negative	-	-	-	-	-	-
	Low Positive control	+	+	+	+	+	+
	High Positive control	+	+	+	+	+	+
12	Negative	-	-	-	-	-	-
	Low Positive control	+	+	+	+	+	+
	High Positive control	+	+	+	+	+	+
15	Negative	[]	[]	[]	[]	[]	[]
	Low Positive control	[]	[]	[]	[]	[]	[]
	High Positive control	[]	[]	[]	[]	[]	[]
18	Negative	[]	[]	[]	[]	[]	[]
	Low Positive control	[]	[]	[]	[]	[]	[]
	High Positive control	[]	[]	[]	[]	[]	[]
21	Negative	[]	[]	[]	[]	[]	[]
	Low Positive control	[]	[]	[]	[]	[]	[]
	High Positive control	[]	[]	[]	[]	[]	[]
24	Negative	[]	[]	[]	[]	[]	[]
	Low Positive control	[]	[]	[]	[]	[]	[]
	High Positive control	[]	[]	[]	[]	[]	[]
27	Negative	[]	[]	[]	[]	[]	[]
	Low Positive control	[]	[]	[]	[]	[]	[]
	High Positive control	[]	[]	[]	[]	[]	[]

“-” indicates a negative test result
 “+” indicates a positive test result
 [] indicates open result in this ongoing study

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Transport Stability after Stressed by Reduced Atmospheric Pressure (ongoing)

Month	Specimen	2 – 8°C			30°C		
		1	2	3	1	2	3
0	Negative	-	-	-	-	-	-
	Low Positive control	+	+	+	+	+	+
	High Positive control	+	+	+	+	+	+
3	Negative	-	-	-	-	-	-
	Low Positive control	+	+	+	+	+	+
	High Positive control	+	+	+	+	+	+
6	Negative	-	-	-	-	-	-
	Low Positive control	+	+	+	+	+	+
	High Positive control	+	+	+	+	+	+
9	Negative	-	-	-	-	-	-
	Low Positive control	+	+	+	+	+	+
	High Positive control	+	+	+	+	+	+
12	Negative	-	-	-	-	-	-
	Low Positive control	+	+	+	+	+	+
	High Positive control	+	+	+	+	+	+
15	Negative	[]	[]	[]	[]	[]	[]
	Low Positive control	[]	[]	[]	[]	[]	[]
	High Positive control	[]	[]	[]	[]	[]	[]
18	Negative	[]	[]	[]	[]	[]	[]
	Low Positive control	[]	[]	[]	[]	[]	[]
	High Positive control	[]	[]	[]	[]	[]	[]
21	Negative	[]	[]	[]	[]	[]	[]
	Low Positive control	[]	[]	[]	[]	[]	[]
	High Positive control	[]	[]	[]	[]	[]	[]
24	Negative	[]	[]	[]	[]	[]	[]
	Low Positive control	[]	[]	[]	[]	[]	[]
	High Positive control	[]	[]	[]	[]	[]	[]
27	Negative	[]	[]	[]	[]	[]	[]
	Low Positive control	[]	[]	[]	[]	[]	[]
	High Positive control	[]	[]	[]	[]	[]	[]

“-” indicates a negative test result
 “+” indicates a positive test result
 [] indicates open result in this ongoing study

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After simulating transport conditions, tests generated expected correct results. This was shown for different time points. Transport stability studies are ongoing and will be continued for the time points 15, 18, 21, 24 and 27 months. This document will be updated when the transport stability study is finished.

Conclusion

The test showed a very good tolerance towards extreme temperature, temperature shifts and pressure conditions as they might occur during shipping. Exposure to these stress conditions did not interfere with the generation of correct results. Until now, testing was conducted at 0, 3, 6, 9 and 12 months. Studies are ongoing and will be continued for the time points 15, 18, 21, 24 and 27 months to validate tolerance to stress exposure until the expiry date.

Document History

Product	Revision	Editor	Reason/Changes	Released (date)
COVID-19 Ag Test (cassette, single pouched)	1.0	PhJä	Initial Annex A6 document	2020-08-21
COVID-19 Ag Test (cassette, single pouched)	1.1	PhJä	Added ongoing transport stability study	2021-01-25
COVID-19 Ag Test (cassette, single pouched)	1.2	PhJä	Fixed typing error in in-use stability study results	2021-01-26
COVID-19 Ag Test (cassette, single pouched)	1.3	PeRu	- Extended information on Accelerated stability study - Extended result tables of ongoing real time stability studies and transport stability studies.	2021-05-10