This Document is part of the Technical File, Annex A7	
Scope	To demonstrate that the performance of the assay (analytical performance and diagnostic performance) is in accordance with the directive 98/79/EC and the standard DIN EN 13612:2002.

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
Written by:	Dr. J. Bohne / Dr. Peter Rube / Dr. P. Jähde
Notified body:	- (under responsibility of the manufacturer)
Number of the notified body:	-
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1. Analytical Performance

1.1. Precision

Intra- and Inter-LOT variability studies were performed in order to determine the reproducibility and repeatability of results obtained with the nal von minden COVID-19 Ag test.

1.1.1 Intra-LOT Variability (Repeatability)

Aim

Determination of test performance variability within one LOT (Intra-LOT variations).

Testing procedure

The following controls were tested in replicates of 10 with tests cassettes from one final kit LOT (H02004) under the same conditions (operator, location, day)

- negative control
- low positive control
- high positive control

All tests were performed according to the procedure described in the package insert. Results with visible T-line (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

The results of the intra-LOT variability study are shown in table 1.

Table 1: Results of the intra-LOT variability study (repeatability)

Sample	Repetition									
	1	2	3	4	5	6	7	8	9	10
Negative control	-	-	-	-	-	-	-	-	-	-
Low positive control	+	+	+	+	+	+	+	+	+	+
high positive control	+	+	+	+	+	+	+	+	+	+

-: negative result, no visible T-line
+ positive result, T-line visible

All performed tests with high positive samples yielded positive results for all repetitions. Also all low positive controls showed consistently positive results. For the negative control, all repetitions showed the expected negative result with no T-line.

Conclusion
The study data show that the intra-LOT variability of the nvm COVID-19 Ag test performance is low. All results met the expectations. Therefore, intra-LOT variability is rated to be acceptable.

1.1.2 Reproducibility: Inter-LOT, between-user and day-to-day variability

Aim
Determination of test performance variability between different LOTs (Inter-LOT variations), different operators, different days and different sites.

Testing procedure
The following samples were used for the study:

- negative control
- low positive control
- high positive control

For the reproducibility study each control was tested in triplicate by three different operators on five different days at three different testing sites. All tests were repeated with test cassettes from three production lots (H02004, H02005, H02006). Test procedure and result interpretation were performed according to 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
The results of the inter-lot variability study are shown in table 2.

Table 2: Results of the inter-lot variability study (reproducibility)

LOT H02004, Day 1									
	Site 1			Site 2			Site 3		
Control	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3
Negative	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -
Low	3+	3+	3+	3+	3+	3+	3+	3+	3+
High	3+	3+	3+	3+	3+	3+	3+	3+	3+
LOT H02004, Day 2									
	Site 1			Site 2			Site 3		
Control	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3
Negative	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -
Low	3+	3+	3+	3+	3+	3+	3+	3+	3+
High	3+	3+	3+	3+	3+	3+	3+	3+	3+
LOT H02004, Day 3									
	Site 1			Site 2			Site 3		
Control	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3
Negative	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -
Low	3+	3+	3+	3+	3+	3+	3+	3+	3+
High	3+	3+	3+	3+	3+	3+	3+	3+	3+
LOT H02004, Day 4									
	Site 1			Site 2			Site 3		
Control	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3
Negative	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -
Low	3+	3+	3+	3+	3+	3+	3+	3+	3+
High	3+	3+	3+	3+	3+	3+	3+	3+	3+
LOT H02004, Day 5									
	Site 1			Site 2			Site 3		
Control	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3
Negative	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -
Low	3+	3+	3+	3+	3+	3+	3+	3+	3+
High	3+	3+	3+	3+	3+	3+	3+	3+	3+
LOT H02005, Day 1									
	Site 1			Site 2			Site 3		
Control	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3
Negative	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -
Low	3+	3+	3+	3+	3+	3+	3+	3+	3+
High	3+	3+	3+	3+	3+	3+	3+	3+	3+
LOT H02005, Day 2									
	Site 1			Site 2			Site 3		
Control	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3
Negative	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -
Low	3+	3+	3+	3+	3+	3+	3+	3+	3+
High	3+	3+	3+	3+	3+	3+	3+	3+	3+
LOT H02005, Day 3									
	Site 1			Site 2			Site 3		

Control	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3
Negative	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -
Low	3+	3+	3+	3+	3+	3+	3+	3+	3+
High	3+	3+	3+	3+	3+	3+	3+	3+	3+
LOT H02005, Day 4									
	Site 1			Site 2			Site 3		
Control	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3
Negative	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -
Low	3+	3+	3+	3+	3+	3+	3+	3+	3+
High	3+	3+	3+	3+	3+	3+	3+	3+	3+
H02005, Day 5									
	Site 1			Site 2			Site 3		
Control	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3
Negative	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -
Low	3+	3+	3+	3+	3+	3+	3+	3+	3+
High	3+	3+	3+	3+	3+	3+	3+	3+	3+
H02006, Day 1									
	Site 1			Site 2			Site 3		
Control	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3
Negative	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -
Low	3+	3+	3+	3+	3+	3+	3+	3+	3+
High	3+	3+	3+	3+	3+	3+	3+	3+	3+
H02006, Day 2									
	Site 1			Site 2			Site 3		
Control	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3
Negative	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -
Low	3+	3+	3+	3+	3+	3+	3+	3+	3+
High	3+	3+	3+	3+	3+	3+	3+	3+	3+
H02006, Day 3									
	Site 1			Site 2			Site 3		
Control	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3
Negative	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -
Low	3+	3+	3+	3+	3+	3+	3+	3+	3+
High	3+	3+	3+	3+	3+	3+	3+	3+	3+
H02006, Day 4									
	Site 1			Site 2			Site 3		
Control	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3
Negative	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -
Low	3+	3+	3+	3+	3+	3+	3+	3+	3+
High	3+	3+	3+	3+	3+	3+	3+	3+	3+
H02006, Day 5									
	Site 1			Site 2			Site 3		
Control	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3	Operator 1	Operator 2	Operator 3
Negative	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -
Low	3+	3+	3+	3+	3+	3+	3+	3+	3+
High	3+	3+	3+	3+	3+	3+	3+	3+	3+

-: negative result, no visible T-line
+: positive result, T-line visible

Number indicates the number of obtained results within the triplicate determination e.g. 3 - → 3 negative results ; 3+ → 3 positive results

Conclusion

For the negative, the low positive control and the high positive control, expected results were obtained for all determinations. The nvm COVID-19 Ag Test showed >99% agreement between experimentally obtained results and expected results. No significant variability between LOTs, operators, days or sites of testing were observed during the study. Therefore reproducibility is rated to be acceptable.

1.2. Analytical sensitivity

In order to assess the analytical sensitivity of the nvm COVID-19 Ag Test the detection limit of the test was determined with two different kind of sample materials namely inactivated SARS-CoV-2 virus and recombinant viral nucleoprotein.

1.2.3 Analytical sensitivity based on recombinant viral nucleoprotein

Aim

The aim of this study was to assess the minimum detection limit of the nvm COVID-19 Ag test for recombinant SARS-CoV-2 viral nucleoprotein.

Testing procedure

The analytical sensitivity study was conducted by testing recombinant SARS-CoV-2 viral nucleoprotein. Buffer was used as diluent, and a series of different concentrations ranging from $4x10^5$ ng/ml to 0.04 ng/ml were tested with the rapid test. Each concentration was tested in triplicates with three production lots (H02004, H02005, H02006). The limit of detection (LOD) was defined as the lowest concentration, where the detection rate was greater than 95%.

The detection rate was calculated by the formula: Detection rate= (number of positive tests)/ (number of total tests)

In order to confirm the LOD, testing was repeated with the lowest detectable concentration and the concentration below in replicates of 20 with 3 LOTs.

Results

The results are shown in tables 3 and 4.

Table 3: Results of the analytical sensitivity study for recombinant SARS-CoV-2 viral nucleoprotein

	LOT 1 H02004							
Conc. [ng/ml]	4×10^5	4×10^4	4×10^3	4×10^2	40	4	0.4	0.04
Replicate 1	+	+	+	+	+	+	+	-
Replicate 2	+	+	+	+	+	+	+	-
Replicate 3	+	+	+	+	+	+	+	-
Detection Rate	3/3	3/3	3/3	3/3	3/3	3/3	3/3	0/3

	LOT 2 H02005							
Conc. [ng/ml]	4 x 10^5	4 x 10^4	4 x 10^3	4 x 10^2	40	4	0.4	0.04
Replicate 1	+	+	+	+	+	+	+	-
Replicate 2	+	+	+	+	+	+	+	-
Replicate 3	+	+	+	+	+	+	+	-
Detection Rate	3/3	3/3	3/3	3/3	3/3	3/3	3/3	0/3
	LOT 3 H02006							
Conc. [ng/ml]	4 x 10^5	4 x 10^4	4 x 10^3	4 x 10^2	40	4	0.4	0.04
Replicate 1	+	+	+	+	+	+	+	-
Replicate 2	+	+	+	+	+	+	+	-
Replicate 3	+	+	+	+	+	+	+	-
Detection Rate	3/3	3/3	3/3	3/3	3/3	3/3	3/3	0/3

Table 4: Confirmation testing of LOD for recombinant SARS-CoV-2 viral nucleoprotein

Protein Conc.	**0.4 ng/ml**			**0.04 ng/ml**		
Replicate n =20	LOT 1 H02004	LOT 2 H02005	LOT 3 H02006	LOT 1 H02004	LOT 2 H02005	LOT 3 H02006
1	+	+	+	-	+	+
2	+	+	+	+	-	-
3	+	+	+	-	-	-
4	+	+	+	-	-	-
5	+	+	+	-	-	+
6	+	+	+	-	+	-
7	+	+	+	+	-	-
8	+	+	+	-	-	-
9	+	+	+	-	+	-
10	+	+	+	-	-	+
11	+	+	+	+	-	+
12	+	+	+	-	-	-
13	+	+	+	-	+	-
14	+	+	+	-	+	-
15	+	+	+	-	-	-
16	+	+	+	+	-	+
17	+	+	+	+	-	-
18	+	+	+	-	+	-
19	+	+	+	-	-	-
20	+	+	+	+	-	+
Detection Rate	20/20	20/20	20/20	6/20	6/20	6/20
	100 %	100 %	100 %	30 %	30 %	30 %

Conclusion

According to the study shown above, the lowest concentration tested that still led to a 100% SARS-CoV-2 viral nucleoprotein detection rate was 0.4 ng/ml.

1.2.4 Analytical sensitivity based on inactivated SARS-CoV-2 Virus

Aim

The aim of this study was to assess the limit of detection (LOD) for the nvm COVID-19 Ag test for inactivated SARS-CoV-2 Virus.

Testing procedure

Buffer was used as diluent to prepare a series of dilutions of inactivated SARS-CoV-2 virus (stock titer: 1x $10^{6.4}$ $TCID_{50}$/ml, Virus strain hCoV-19/China/ZJ-NB841/2020). Approximately 10µL of different concentrations of inactivated virus were spiked onto either negative naso-pharyngeal swabs or negative oropharyngeal swabs. Each concentration of inactivated SARS-CoV-2 virus was tested with the nvm COVID-19 Ag Test. Each concentration was tested in triplicates with three kit LOTs.

Testing procedure and result interpretation were performed according to the IFU.

In order to confirm the LOD, testing was repeated with the lowest detectable concentration and the concentration below in replicates of 20 with 3 LOTs.

Results

The results are shown in tables 5 and 6.

Table 5: Results of the analytical sensitivity study for inactivated SARS-CoV-2 virus

	Nasopharyngeal swab					
	LOT 1 H02004					
Dilution of Stock*	1/10	1/100	1/1000	1/2500	1/5000	1:10000
Conc. [$TCID_{50}$/ml]	$1x10^{5.4}$	$1x10^{4.4}$	$1x10^{3.4}$	$4x10^{2.4}$	$2x10^{2.4}$	$1x10^{2.4}$
Replicate 1	+	+	+	+	+	-
Replicate 2	+	+	+	+	+	+
Replicate 3	+	+	+	+	+	-
Detection Rate	3/3	3/3	3/3	3/3	3/3	1/3
	LOT 2 H02005					
Dilution	1/10	1/100	1/1000	1/2500	1/5000	1:10000
Conc. [$TCID_{50}$/ml]	$1x10^{5.4}$	$1x10^{4.4}$	$1x10^{3.4}$	$4x10^{2.4}$	$2x10^{2.4}$	$1x10^{2.4}$
Replicate 1	+	+	+	+	+	-
Replicate 2	+	+	+	+	+	-
Replicate 3	+	+	+	+	+	-
Detection Rate	3/3	3/3	3/3	3/3	3/3	0/3
	LOT 3 H02006					
Dilution	1/10	1/100	1/1000	1/2500	1/5000	1:10000
Conc. [$TCID_{50}$/ml]	$1x10^{5.4}$	$1x10^{4.4}$	$1x10^{3.4}$	$4x10^{2.4}$	$2x10^{2.4}$	$1x10^{2.4}$
Replicate 1	+	+	+	+	+	-
Replicate 2	+	+	+	+	+	-
Replicate 3	+	+	+	+	+	+
Detection Rate	3/3	3/3	3/3	3/3	3/3	1/3
	Oropharyngeal swab					
	LOT 1 H02004					
Dilution of Stock*	1/10	1/100	1/1000	1/2500	1/5000	1:10000
Conc. [$TCID_{50}$/ml]	$1x10^{5.4}$	$1x10^{4.4}$	$1x10^{3.4}$	$4x10^{2.4}$	$2x10^{2.4}$	$1x10^{2.4}$
Replicate 1	+	+	+	+	+	-
Replicate 2	+	+	+	+	+	-

Replicate 3	+	+	+	+	+	+
Detection Rate	3/3	3/3	3/3	3/3	3/3	1/3
	LOT 2 H02005					
Dilution	1/10	1/100	1/1000	1/2500	1/5000	1:10000
Conc. [$TCID_{50}$/ml]	$1x10^{5.4}$	$1x10^{4.4}$	$1x10^{3.4}$	$4x10^{2.4}$	$2x10^{2.4}$	$1x10^{2.4}$
Replicate 1	+	+	+	+	+	+
Replicate 2	+	+	+	+	+	-
Replicate 3	+	+	+	+	+	+
Detection Rate	3/3	3/3	3/3	3/3	3/3	2/3
	LOT 3 H02006					
Dilution	1/10	1/100	1/1000	1/2500	1/5000	1:10000
Conc. [$TCID_{50}$/ml]	$1x10^{5.4}$	$1x10^{4.4}$	$1x10^{3.4}$	$4x10^{2.4}$	$2x10^{2.4}$	$1x10^{2.4}$
Replicate 1	+	+	+	+	+	+
Replicate 2	+	+	+	+	+	-
Replicate 3	+	+	+	+	+	+
Detection Rate	3/3	3/3	3/3	3/3	3/3	2/3

*Stock titer: 1x $10^{6.4}$ $TCID_{50}$/ml, Virus strain hCoV-19/China/ZJ-NB841/2020

Table 6: Confirmation testing of LOD for inactivated SARS-CoV-2 virus

	Nasopharyngeal swab						**Oropharyngeal Swab**					
	$2x10^{2.4}$ $TCID_{50}$/mL			$1x10^{2.4}$ $TCID_{50}$/mL			$2x10^{2.4}$ $TCID_{50}$/mL			$1x10^{2.4}$ $TCID_{50}$/mL		
Replicate n =20	LOT 1	LOT 2	LOT 3	LOT 1	LOT 2	LOT 3	LOT 1	LOT 2	LOT 3	LOT 1	LOT 2	LOT 3
1	+	+	+	+	+	+	+	+	+	+	+	-
2	+	+	+	-	+	+	+	+	+	-	+	+
3	+	+	+	+	-	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+	+
6	+	+	+	-	+	-	+	+	+	+	-	-
7	+	+	+	-	-	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+	+	-	+	+
9	+	+	+	+	+	+	+	+	+	-	+	+
10	+	+	+	+	+	-	+	+	+	+	+	+
11	+	+	+	-	+	-	+	+	+	+	-	-
12	+	+	+	+	+	+	+	+	+	+	+	+
13	+	+	+	+	-	+	+	+	+	+	-	+
14	+	+	+	+	-	+	+	+	+	+	+	+
15	+	+	+	-	+	+	+	+	+	-	-	+
16	+	+	+	+	+	+	+	+	+	-	+	+
17	+	+	+	+	+	+	+	+	+	+	+	-
18	+	+	+	+	+	-	+	+	+	+	-	+
19	+	+	+	+	+	+	+	+	+	+	-	+
20	+	+	+	+	+	+	+	+	+	+	+	+
Detection Rate	20/20	20/20	20/20	15/20	16/20	16/20	20/20	20/20	20/20	15/20	14/20	16/20
	100%	100%	100%	75%	80%	80%	100%	100%	100%	75%	70%	80%

Conclusion

According to the study shown above, the lowest concentration that still led to a 100% detection rate was $2x10^{2.4}$ $TCID_{50}$/mL of inactivated SARS-COV-2 for both swab types (nasopharyngeal or oropharyngeal swab).

1.3. Analytical specificity

The analytical specificity of the nal von minden COVID-19 Ag Test was examined to show that it is unlikely to obtain false results caused by chemical substances, proteins or pathogens which might be present in the sample material.
The analytical specificity was determined in an interference testing and a cross reactivity study. In addition a possible matrix influence caused by swabtype (nasopharyngeal, oropharyngeal) was addressed in a specimen type equivalence study.

1.3.1 Interference testing

Aim
This study demonstrates that common substances that are naturally present or might be artificially introduced in the sample material do not interfere with correct result generation.

Testing procedure
The tested potentially interfering substances and their respective concentrations are shown in table 7. Each substance was tested for its influence on the generation of correct negative results (no viral protein in the sample) or its influence on the generation of correct weak positive results (samples spiked with recombinant viral protein to low positive). Each substance was tested in triplicates with three final kit LOTs (H02004, H02005, H02006).
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
The results of the interference study experiments are shown in table 7.

Table 7: Results of the substance interference study

Substance	Concentration	Negative (without recombinant viral nucleoprotein)			Low positive (with recombinant viral nucleoprotein)		
		H02004	H02005	H02006	H02004	H02005	H02006
3 OTC nasal sprays*	10%	3-	3-	3-	3+	3+	3+
3 OTC mouthwashes*	10%	3-	3-	3-	3+	3+	3+
3 OTC throat liquids*	10%	3-	3-	3-	3+	3+	3+
4-acetamidophenol	10 mg/ml	3-	3-	3-	3+	3+	3+
Acetylsalicylic acid	20 mg/ml	3-	3-	3-	3+	3+	3+
Albuterol	20 mg/ml	3-	3-	3-	3+	3+	3+
Chlorpheniramine	5 mg/ml	3-	3-	3-	3+	3+	3+
Dexamethasone	5 mg/ml	3-	3-	3-	3+	3+	3+
Dextromethorphan	10 mg/ml	3-	3-	3-	3+	3+	3+
Diphenhydramine	5 mg/ml	3-	3-	3-	3+	3+	3+

Substance	Concentration	Negative (without recombinant viral nucleoprotein)			Low positive (with recombinant viral nucleoprotein)		
		H02004	H02005	H02006	H02004	H02005	H02006
Doxylamine succinate	1 mg/ml	3-	3-	3-	3+	3+	3+
Flunisolide	3 mg/ml	3-	3-	3-	3+	3+	3+
Guaiacol glyceryl ether	20 mg/ml	3-	3-	3-	3+	3+	3+
Mucin	1%	3-	3-	3-	3+	3+	3+
Mupirocin	250 µg/ml	3-	3-	3-	3+	3+	3+
Oxymetazoline	10 mg/ml	3-	3-	3-	3+	3+	3+
Phenylephrine	10 mg/ml	3-	3-	3-	3+	3+	3+
Phenylpropanolamine	20 mg/ml	3-	3-	3-	3+	3+	3+
Relenza®(zanamivir)	20 mg/ml	3-	3-	3-	3+	3+	3+
Rimantadine	500 ng/ml	3-	3-	3-	3+	3+	3+
Tamiflu® (oseltamivir)	100 mg/ml	3-	3-	3-	3+	3+	3+
Tobramycin	40 mg/ml	3-	3-	3-	3+	3+	3+
Triamcinolone	14 mg/ml	3-	3-	3-	3+	3+	3+

-: negative result, no visible T-line
+ positive result, T-line visible
Number indicates the number of obtained results within the triplicate determination e.g. 3 - → 3 negative results ; 3+ → 3 positive results
* OTC= over the counter

Conclusion

No interference was observed with any of the tested potentially interfering substances. The negative results yielded correct negative results for all LOTs and determinations. The low positive samples yielded consistent positive results. The nvm COVID-19 Ag Test was not affected by the substances in the study indicating that the test shows a good robustness towards potentially interfering substances.

1.3.2 Cross reactivity and microbial/viral interference

Aim

The purpose of this study was to test for cross reactivity and interference with other viruses or microorganisms that might be present in sample material. It should evaluate if the test specifically detects SARS-CoV-2 antigen or if there is an unwanted cross reactivity or interference with other pathogens.

Testing procedure

Cross-reactivity: Potential cross-reacting organisms were spiked onto negative nasopharyngeal swabs and extracted according the procedure described in the package insert. Each spiked sample was tested in replicates of 10 with three independent LOTs (see Table 8).

Microbial interference: Each potentially interfering organism combined with inactivated SARS-COV-2 was diluted with nasopharyngeal matrix. Mixtures were transferred onto swabs. Each spiked sample was tested in replicates of 3 with three independent LOTs (see Table 9). Results were documented either as negative (no visible test result line) or as positive (visible test result line) after 15 minutes. In addition to the "defined microorganisms" also a pool of nasal washes was tested in order to evaluate if the normal microbiological flora would show a cross reactivity with the test.

Results

The results of the cross reactivity- and microbial/viral interference experiments are shown in following tables.

Table 8: Results of the cross reactivity study

Pathogen type	Concentration	Spiked nasopharyngeal swab (negative for SARS-COV-2)		
		LOT 1	LOT 2	LOT 3
Virus	**$TCID_{50}$/ml**	**H02004**	**H02005**	**H02006**
Source: Zeptometrix				
HCoV-OC43	2×10^5	10-	10-	10-
HCoV-NL63	2×10^5	10-	10-	10-
HCoV-229E	2×10^5	10-	10-	10-
Source: First Affiliated Hospital of Guangzhou Medical University				
HCoV-HKU1	-*	10-	10-	10-
Source: Zhejiang Provincial Center for Disease Control and Prevention				
Measles virus	2×10^5	10-	10-	10-
Epstein-Barr virus	2×10^5	10-	10-	10-
Influenza A (H1N1)pdm09	3×10^5	10-	10-	10-
Influenza A (H3N2)	3×10^5	10-	10-	10-
Influenza A (H5N1)	3×10^5	10-	10-	10-
Influenza A (H7N9)	3×10^5	10-	10-	10-
Influenza A (H7N7)	3×10^5	10-	10-	10-
Influenza B Victoria lineage	3×10^5	10-	10-	10-
Influenza B Yamagata lineage	3×10^5	10-	10-	10-
Respiratory syncytial virus	3×10^5	10-	10-	10-
Adenovirus	4×10^5	10-	10-	10-
Parainfluenza 1 virus	2×10^5	10-	10-	10-
Parainfluenza 2 virus	2×10^5	10-	10-	10-
Parainfluenza 3 virus	2×10^5	10-	10-	10-
Parainfluenza 4 virus	2×10^5	10-	10-	10-
Human metapneumovirus	2×10^5	10-	10-	10-
Rhinovirus	2×10^5	10-	10-	10-
Coxsackie virus A16	2×10^5	10-	10-	10-
Norovirus	2×10^5	10-	10-	10-
Mumps virus	2×10^5	10-	10-	10-
Bacteria/Fungi	**cfu/ml**			
Bordetella parapertussis	2×10^6	10-	10-	10-
Bordetella pertussis	2×10^6	10-	10-	10-
Haemophilus influenzae	1×10^6	10-	10-	10-

Pathogen type	Concentration	Spiked nasopharyngeal swab (negative for SARS-COV-2)		
		LOT 1	LOT 2	LOT 3
Virus	$TCID_{50}$/ml	H02004	H02005	H02006
Candida albicans	1×10^6	10-	10-	10-
Mycobacterium tuberculosis	1×10^6	10-	10-	10-
Source: ATCC				
Legionella pneumophila	2×10^6	10-	10-	10-
Mycoplasma pneumoniae	2×10^6	10-	10-	10-
Chlamydia pneumoniae	2×10^6	10-	10-	10-
Streptococcus pyogenes	2×10^6	10-	10-	10-
Streptococcus agalactiae	2×10^6	10-	10-	10-
Group C Streptococcus	2×10^6	10-	10-	10-
Staphylococcus aureus	2×10^6	10-	10-	10-
Streptococcus pneumoniae	2×10^6	10-	10-	10-
Source: in-house				
Pooled human nasal wash of presumably negative employees (assumed to be representative of normal microbial flora)	N/A	10-	10-	10-

*Virus titer not determined. RT-PCR C_t values: 29.9 and 27.2

Table 9: Results of the microbial/viral interference study

Pathogen type	Concentration	Spiked nasopharyngeal swab (positive for SARS-COV-2)		
		LOT 1	LOT 2	LOT 3
Virus	$TCID_{50}$/ml	H02004	H02005	H02006
Source: Zeptometrix				
HCoV-OC43	2×10^5	3+	3+	3+
HCoV-NL63	2×10^5	3+	3+	3+
HCoV-229E	2×10^5	3+	3+	3+
Source: First Affiliated Hospital of Guangzhou Medical University				
HCoV-HKU1	-*	3+	3+	3+
Source: Zhejiang Provincial Center for Disease Control and Prevention				
Measles virus	2×10^5	3+	3+	3+
Epstein-Barr virus	2×10^5	3+	3+	3+
Influenza A (H1N1)pdm09	3×10^5	3+	3+	3+
Influenza A (H3N2)	3×10^5	3+	3+	3+
Influenza A (H5N1)	3×10^5	3+	3+	3+
Influenza A (H7N9)	3×10^5	3+	3+	3+
Influenza A (H7N7)	3×10^5	3+	3+	3+
Influenza B Victoria lineage	3×10^5	3+	3+	3+
Influenza B Yamagata lineage	3×10^5	3+	3+	3+
Respiratory syncytial virus	3×10^5	3+	3+	3+
Adenovirus	4×10^5	3+	3+	3+
Parainfluenza 1 virus	2×10^5	3+	3+	3+
Parainfluenza 2 virus	2×10^5	3+	3+	3+
Parainfluenza 3 virus	2×10^5	3+	3+	3+
Parainfluenza 4 virus	2×10^5	3+	3+	3+
Human metapneumovirus	2×10^5	3+	3+	3+
Rhinovirus	2×10^5	3+	3+	3+
Coxsackie virus A16	2×10^5	3+	3+	3+

Pathogen type	Concentration	Spiked nasopharyngeal swab (positive for SARS-COV-2)		
		LOT 1	LOT 2	LOT 3
Virus	**$TCID_{50}$/ml**	**H02004**	**H02005**	**H02006**
Norovirus	2×10^5	3+	3+	3+
Mumps virus	2×10^5	3+	3+	3+
Bacteria/Fungi	**cfu/ml**			
Bordetella parapertussis	2×10^6	3+	3+	3+
Bordetella pertussis	2×10^6	3+	3+	3+
Haemophilus influenzae	1×10^6	3+	3+	3+
Candida albicans	1×10^6	3+	3+	3+
Mycobacterium tuberculosis	1×10^6	3+	3+	3+
Source: ATCC				
Legionella pneumophila	2×10^6	3+	3+	3+
Mycoplasma pneumoniae	2×10^6	3+	3+	3+
Chlamydia pneumoniae	2×10^6	3+	3+	3+
Streptococcus pyogenes	2×10^6	3+	3+	3+
Streptococcus agalactiae	2×10^6	3+	3+	3+
Group C Streptococcus	2×10^6	3+	3+	3+
Staphylococcus aureus	2×10^6	3+	3+	3+
Streptococcus pneumoniae	2×10^6	3+	3+	3+
Source: in-house				
Pooled human nasal wash of presumably negative employees (assumed to be representative of normal microbial flora)	N/A	3+	3+	3+

*Virus titer not determined. RT-PCR C_t values: 29.9 and 27.2

Conclusion

No cross reactivity or interference was observed with any of the tested pathogens. Even the non-SARS human Corona viruses showed no cross-reactivity or interference in the listed concentrations. For all determinations and LOTs, expected results were generated. From the obtained study data, it can be assumed that the tests shows a high specificity for SARS-CoV-2 virus and that a cross-reactivity or interference with the listed pathogens is unlikely.

1.3.3 Specimen type equivalence study

Aim

The purpose of this study was to determine if naso- and oropharyngeal swabs obtained in parallel from SARS-CoV-2 negative or positive patients (confirmed by RT-PCR) generated comparable results with the antigen test

Testing procedure

Two sample types – one nasopharyngeal swab and one oropharyngeal swab – were collected in parallel from 30 patients confirmed to be positive or negative for SARS-CoV-2 infection by RT-PCR. One half of the donors were negative, the other half was positive. The samples were labelled and randomized so that the operator was blind for the PCR status of the sample and

did not know which samples were obtained from the same patient. Samples were then extracted according to the procedure described in the package insert. The extract was tested in duplicates with the test. Results were documented either as positive result (T-line visible after 15 minutes) or as negative results (no T-line visible after 15 minutes).

Results

The results of the sample equivalence study are summarized in the table 10.

Table 10: Results of the sample equivalence study

Donor ID	RT-PCR result	Nasopharyngeal swab			Oropharyngeal Swab		
		Sample# after randomizing	Rapid Test Result 1	Rapid Test Result 2	Sample# after randomizing	Rapid Test Result 1	Rapid Test Result 2
D01	Positive	39	+	+	38	+	+
D02	Positive	7	+	+	30	+	+
D03	Negative	13	-	-	57	-	-
D04	Positive	44	+	+	8	+	+
D05	Negative	28	-	-	36	-	-
D06	Positive	59	+	+	15	+	+
D07	Negative	40	-	-	41	-	-
D08	Negative	54	-	-	1	-	-
D09	Positive	45	+	+	53	+	+
D10	Positive	22	+	+	32	+	+
D11	Positive	60	+	+	3	+	+
D12	Negative	10	-	-	23	-	-
D13	Negative	18	-	-	46	-	-
D14	Negative	16	-	-	12	-	-
D15	Positive	55	+	+	47	+	+
D16	Negative	31	-	-	14	-	-
D17	Negative	37	-	-	5	-	-
D18	Positive	48	+	+	11	+	+
D19	Positive	24	+	+	9	+	+
D20	Negative	33	-	-	27	-	-
D21	Negative	6	-	-	17	-	-
D22	Negative	29	-	-	35	-	-
D23	Negative	21	-	-	56	-	-
D24	Negative	43	-	-	51	-	-
D25	Negative	58	-	-	34	-	-
D26	Positive	20	+	+	52	+	+
D27	Positive	49	+	+	50	+	+
D28	Positive	42	+	+	4	+	+
D29	Positive	19	+	+	2	+	+
D30	Positive	26	+	+	25	+	+

Conclusion

The results obtained with either oropharyngeal swabs or nasopharyngeal swabs matched the RT-PCR results. This indicates that both kind of swabs are suitable to detect SARS-CoV-2 antigen. Operators should keep in mind that a good sample collection technique is essential

in order to obtain optimal results. The respective sections in the instructions should be observed.

1.4. Robustness

1.4.1 Reading time flex study

Aim

The aim of this study was to estimate the influence of the time between sample application and reading of the result on the test outcome.

Testing procedure

The following samples were used for the study:

- SARS-CoV-2 antigen low positive control
- SARS-CoV-2 antigen high positive control
- SARS-CoV-2 antigen negative control

The samples were tested with test cassettes from production lot H02004. As reading times, a range of 5 to 60 minutes was chosen (see result table for used time points). For each sample and reading time, ten replicates were tested. Besides the differing reading time, all tests were performed according to the procedure described in the package insert.

Results

Table 11 shows the results of the reading time flex study.

Table 11: Results of the reading time flex study

Specimen		Reading time (Minutes)					
		5	10	15	30	45	60
Negative	1	-	-	-	-	-	-
	2	-	-	-	-	-	-
	3	-	-	-	-	-	-
	4	-	-	-	-	-	-
	5	-	-	-	-	-	-
	6	-	-	-	-	-	-
	7	-	-	-	-	-	-
	8	-	-	-	-	-	-
	9	-	-	-	-	-	-
	10	-	-	-	-	-	-

Specimen		Reading time (Minutes)					
		5	10	15	30	45	60
Low positive	1	-	-	+	+	+	+
	2	+	+	+	+	+	+
	3	+	+	+	+	+	+
	4	-	+	+	+	+	+
	5	+	+	+	+	+	+
	6	+	+	+	+	+	+
	7	+	+	+	+	+	+
	8	-	-	+	+	+	+
	9	+	+	+	+	+	+
	10	+	+	+	+	+	+
High positive	1	+	+	+	+	+	+
	2	+	+	+	+	+	+
	3	+	+	+	+	+	+
	4	+	+	+	+	+	+
	5	+	+	+	+	+	+
	6	+	+	+	+	+	+
	7	+	+	+	+	+	+
	8	+	+	+	+	+	+
	9	+	+	+	+	+	+
	10	+	+	+	+	+	+
Acceptable:		No	No	Yes	Yes	Yes	Yes

Conclusion

The results of the reading time study show that with the tested control samples, correct results are obtained within a range of 15 to 60 minutes. Therefore, a reading time of 15 minutes is recommended to the user.

1.4.2 Sample volume flex study

Aim

The aim of this study was to estimate the influence of sample volume variation on the test performance.

Testing procedure

The following samples were used for the study:

- SARS-CoV-2 antigen low positive control
- SARS-CoV-2 antigen high positive control
- SARS-CoV-2 antigen negative control

The samples were tested with test cassettes from production lot H02004. Sample volumes of 40µL, 80µL, 120µL and 160µL were added to the test cassettes with a transfer pipette. For each sample type and volume, three replicates were tested. Besides the differing sample volumes, all tests were performed according to the procedure described in the package insert.

In addition, the sample volume robustness was also evaluated when samples were applied with the dropper caps of the sample extraction tubes. The volume of one sample drop added via the dropper cap of the extraction tubes was measured with 30 replicates. The result was an average volume of 40±10µL per drop (data not shown).

Results

Tables 12 and 13 show the results of the sample volume flex study.

Table 12: Sample volume study, sample added via transfer pipette

Specimen	Repetition	Sample volume			
		40µL	80µL	120µL	160µL
Negative	1	Lateral flow failure	-	-	-
	2	-	-	-	-
	3	-	-	-	-
Low Positive	1	+	+	+	+
	2	Lateral flow failure	+	+	+, flooding
	3	+	+	+	+
High Positive	1	+	+	+	+, flooding
	2	Lateral flow failure	+	+	+
	3	+	+	+	+
Detection rate		**66.7%**	**100%**	**100%**	**77.8%**

Table 13: Sample volume study, sample added via dropper cap

Specimen	Repetition	Sample volume			
		1 drop	2 drops	3 drops	4 drops
Negative	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
Low Positive	1	+	+	+	+, flooding
	2	Lateral flow failure	+	+	+
	3	+	+	+	+
High Positive	1	+	+	+	+
	2	Lateral flow failure	+	+	+, flooding
	3	+	+	+	+
Detection rate		**66.7%**	**100%**	**100%**	**77.8%**

Conclusion

Based on the study results, the nvm COVID-19 antigen test can be performed with sample volumes of 80 and 120µL (transfer pipette) or 2 and 3 drops (dropper cap). Lower sample volumes can lead to lateral flow failure, whereas larger sample volumes can lead to flooding of the test.

2. Diagnostic performance: Accuracy of detection compared to RT-PCR with clinical samples

Aim

The aim of the study was to assess the performance of the nal von minden COVID-19 Ag test with clinical specimens via comparison to RT-PCR.

Testing procedure

To assess the anti-SARS-CoV-2 Ag detection performance of the test device, four studies were performed.

In the first study, 348 naso- and oropharyngeal samples were collected and tested with the nvm COVID-19 Ag test. 168 of these samples were nasopharyngeal swabs, and 180 were oropharyngeal swabs. Only individuals who were suspected of COVID-19 were enrolled in this study (including those who had symptoms, had contacted a confirmed patient, or had visited a disease outbreak area). At the time point of sample collection, some people exhibited mild (e.g. low fever, cough, fatigue) or severe (e.g. high fever, chest tightness, weakness) symptoms, while some people had no symptoms. RT-PCR was used as reference method to determine the status of each patient sample. In order to estimate the relative virus concentration, the C_t value of the PCR results was documented (cycle in which the PCR result becomes positive: the lower the C_t value the more virus RNA was present in the sample; it should be noted that C_t values might not be transferrable between different systems).

In the second study, 565 samples from RT-PCR confirmed SARS-CoV-2-negative test subjects were collected. 508 of these samples were oropharyngeal swab samples and 57 were nasopharyngeal swab samples. Only test subjects were enrolled who were suspected of a SARS-CoV-2 infection. At the time of sample collection, some test subjects exhibited symptoms (e.g. fever, cough, nasal congestion, headache, chest pain, tiredness) whereas others were asymptomatic.

In the third study, 245 nasal swab samples were collected from test subjects exhibiting symptoms of COVID-19. All samples were collected within 7 days after onset of disease symptoms. Two anterior nasal swabs were collected from each test subject and one of the swabs was tested with the nvm COVID-19 Ag test directly after collection. The other swab was used for RT-PCR confirmation.

To further test the specificity of the test device, 105 nasal samples from asymptomatic, presumably negative test subjects without known exposure to SARS-CoV-2 were collected.

All tests were performed according to the procedure described in the package insert. Operators did not receive any extra training before performing the test. Test results were documented as negative when no T-line was visible after 15 minutes. Results with a visible T-line after 15 minutes were documented as positive.

From the resulting data, relative diagnostic sensitivity, specificity and overall agreement were calculated as follows. Samples with positive result for RT-PCR were defined as true positive samples. Samples with negative results for RT-PCR were defined as true negative samples. Deviating results counted either as false positive or as false negative results.

Relative diagnostic sensitivity: $\frac{tp}{tp+fn} \times 100\ [\%]$

Relative diagnostic specificity: $\frac{tn}{tn+fp} \times 100\ [\%]$

Relative overall agreement: $\frac{tp+tn}{tp+tn+fp+fn} \times 100\ [\%]$

Results, nasal swabs

According to RT-PCR, 107 of the nasal swab samples were rated as positive and 138 samples were rated as SARS-CoV-2 negative.
With the nvm COVID-19 antigen test, 98 of the samples created positive results and 138 of the samples created negative results. The results are summarized in table 14.

Table 14: Summary of results of the clinical performance study, nasal swabs with C_t≤30 (full range)

Method		**RT-PCR**		Total Results
nvm	Results	Positive	Negative	
COVID-19 Ag Rapid Test	Positive	98	0	**98**
Nasal swabs	Negative	9	138	**147**
Total Results		**107**	**138**	**245**

		Calculation [x100]:
Diagnostic sensitivity:	91.6% (84.8% - 95.5%)*	98/(98+9)
Diagnostic specificity:	>99.9% (97.4% - 100%)*	138/(138+0)
Overall agreement:	96.3% (93.2% - 98.1%)*	(98+138)/(98+138+0+9)

* 95% confidence interval

With the tested nasal swab samples, the nal von minden COVID-19 Ag test reaches a sensitivity of 91.6%. Specificity compared to RT-PCR is >99.9%.

Five of the positive samples showed C_t values within the cutoff region of the comparator PCR (30-31). The following table summarizes the results if the calculations are restricted to positive samples with C_t values <30.

Table 15: Summary of results of the clinical performance study, positive nasal swab samples with C_t<30

Method		**RT-PCR (C_t < 30)**		Total Results
nvm	Results	Positive	Negative	
COVID-19 Ag Rapid Test	Positive	96	0	**96**
Nasal swabs	Negative	6	138	**144**
Total Results		**102**	**138**	**240**

		Calculation [x100]:
Diagnostic sensitivity (C_t<30):	94.1% (87.6% - 97.8%)*	96/(96+6)
Diagnostic specificity:	>99.9% (97.4% - 100%)*	138/(138+0)
Overall agreement:	97.5% (94.6% - 99.1%)*	(96+138)/(96+138+0+6)

* 95% confidence interval

Reducing the sample pool to samples with C_t values < 30 therefore increases the sensitivity of the test to 94.1%.

The additionally tested 105 nasal swab samples from asymptomatic test subjects were all detected as negative by the nvm COVID-19 Ag test (>99.9% sensitivity, 95% confidence interval: 96.5% - 100.0%).

Results, naso- and oropharyngeal swabs

According to RT-PCR, 187 of the naso- and oropharyngeal swab samples from the first study were rated as positive and 162 samples were rated as SARS-CoV-2 negative. There were 123 positive samples with C_t≤30, and 64 positive samples with Ct>30.
With the nvm COVID-19 antigen test, 150 of the samples created positive results and 198 of the samples created negative results. 120 of the positive results corresponded to PCR results with C_t values ≤30, whereas 30 of the positive results corresponded to PCR samples with C_t values of >30. The results are summarized in the table 16.

Table 16: Summary of results of the clinical performance study, nasopharyngeal and oropharyngeal swabs, studies one and two

Method		**RT-PCR**		Total Results
nvm	Results	Positive	Negative	
COVID-19 Ag Rapid Test	Positive	150 (tp)	0 (fp)	**150**
Naso-/oropharyngeal	Negative	37 (fn)	726 (tn)	**763**
Total Results		**187**	**726**	**913**

		Calculation [x100]:
Diagnostic sensitivity:	80.2% (73.9% - 85.3%)*	150/(150+37)
Diagnostic specificity:	>99.9% (99.5% - 100%)*	726/(726+0)
Overall agreement:	96.0% (94.5% - 97.1%)*	(150+726)/(150+37+726+0)

* 95% confidence interval

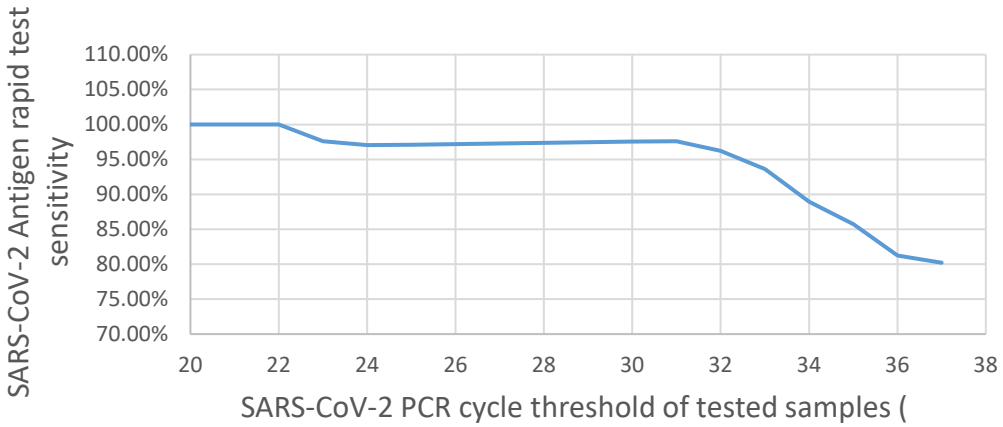
If the relative diagnostic sensitivity for strongly positive PCR samples with C_t values ≤30 as threshold is calculated, the relative diagnostic sensitivity for these samples is 97.6% (95% confidence interval 93.1% - 99.2%). From 123 positive PCR samples with C_t values ≤ 30, 120 samples were detected with the rapid test. For these strong positive samples, only 3 false negative results were obtained.
Results restricted to positive samples with C_t values from 20 to 30 are summarized in the following table.

Table 17: Summary of results of the clinical performance study restricted to positive PCR samples with C_t values ≤30, naso- and oropharyngeal swabs, studies one and two

Method		**RT-PCR**		Total Results
nvm COVID-19 Ag Rapid Test	Results	Positive	Negative	
	Positive	120 (tp)	0 (fp)	**120**
	Negative	3 (fn)	726 (tn)	**729**
Total Results		**123**	**726**	**849**

Diagnostic sensitivity (C_t 20–30):	97.6% (95%CI: 93.1% – 99.2%)	120/(120+3)
Diagnostic specificity (C_t 20–30):	>99.9% (95%CI: 99.5% - 100%)	726/(726+0)
Overall agreement (C_t 20–30):	99.7% (95%CI: 99.0% - 99.9%)	(120+726)/(120+726+3+0)

The following graph shows the relation between the diagnostic sensitivity of the rapid test and the PCR cycle threshold. The PCR cycle threshold comprises all samples equal or below to the depicted number and is not meant as "isolated" C_t value.



The resulting data clearly shows a decrease of the rapid test sensitivity when more samples with higher C_t values are included in the sample pool. Nevertheless, the test still reaches a very high sensitivity of 97.6% up to C_t values of 31 in this study and only starts to show reduced sensitivity when samples with very low virus concentrations are included in the calculation.

Conclusion

The diagnostic performance study demonstrates that the nvm COVID-19 test showed an excellent relative diagnostic specificity for all sample types. No false positive results were obtained in the study so that the relative diagnostic specificity was calculated to be >99.9%.

The relative diagnostic sensitivity of the test was calculated to be 91.6% percent for nasal swab samples with C_t≤30 (94.1% for nasal swab samples with C_t<30). For the tested naso-/oropharyngeal swabs, the test reached a sensitivity of 80.2% for samples with C_t values between 20 and 37, and 97.6% for samples with Ct values between 20 and 30.

As expected, the rapid test was not as sensitive as RT-PCR. False negative results were predominantly obtained for nasopharyngeal and oropharyngeal samples with high C_t values (> 30) indicating that the original viral burden in the sample material was low. If the relative sensitivity for these samples was calculated with a threshold and only strong PCR samples with C_t values of ≤ 30 were included in the calculation, the relative diagnostic sensitivity was 97.6 %. These samples were reliably detected and only 3 false negative results were obtained for this kind of samples. This implies that the test would be suitable to identify individuals with high virus burden, which would presumably be persons with high infectiousness e.g. so called “superspreaders”.

Summarizing, the test reliably detected the SARS-CoV-2 nucleoprotein antigen in nasal samples and naso-/oropharyngeal samples with high virus burden (C_t of corresponding PCR ≤30). It showed excellent diagnostic specificity for negative samples of all types.

However, for naso-/oropharyngeal swab samples with low viral burden, PCR remains the most reliable method to detect infection. Therefore, the section “intended use” of the PI informs the user that a possible infectiousness of test subjects cannot be ruled out based on negative test results. This limitation clearly implies that no critical patient management decisions that depend on the knowledge of infectiousness of a patient should be based on the test result. Test results should only be used as an aid in diagnosis. Users are advised to observe the section LIMITATIONS that emphasizes that the rapid test results must not be used as sole criterion of diagnosis. Package insert Rev 7.0 lists the following limitations that should be taken into account by the user for result interpretation and diagnosis:

The NADAL® COVID-19 Ag Test is for professional *in-vitro* diagnostic use only. It should be used for the qualitative detection of SARS-CoV-2 viral nucleoprotein antigens in human nasopharyngeal and oropharyngeal specimens only. Neither the quantitative value nor the rate of increase/decrease in the concentration of SARS-CoV-2 viral nucleoprotein antigens can be determined with this qualitative test.

The NADAL® COVID-19 Ag Test only detects the presence of SARS-CoV-2 viral nucleoprotein antigens in specimens and should not be used as the sole criterion for a diagnosis of COVID-19.

Both viable and non-viable SARS-CoV-2 viruses can be detected using the NADAL® COVID-19 Ag Test.

The sections ‘Specimen Collection and Preparation’ as well as ‘Test Procedure’ must be followed closely while testing. Failure to follow them may lead to inaccurate test results because the antigen concentration in the swab is highly dependent on the correct procedure.

As with all diagnostic tests, all results should be interpreted in conjunction with other clinical information available to the physician.

In the course of SARS-CoV-2 infection, the concentration of viral nucleoprotein antigens may fall below the detection limit of the test.

If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of a SARS-CoV-2 infection and should be confirmed via molecular assay.

Document History

Product	Revision	Editor	Changes/Reason for Changes	Released (date)
COVID-19 Ag Test (cassette, single pouched)	1.0	JuBo	new Annex A7 because of new TD	2020-08-28
COVID-19 Ag Test (cassette, single pouched)	1.1	PhJä	Added robustness studies	2020-09-30
COVID-19 Ag Test (cassette, single pouched)	1.2	PeRu	- Section 1.3.2: Cross reactivity study was extended by microbial interference study. Samples spiked with SARS-CoV-2 and potentially interfering pathogens were tested for correct positive result generation. Correction in result table. -Section 2: Additional table (positive samples restricted to Ct values ≤30). Additional limitations	2020-10-20
COVID-19 Ag Test (cassette, single pouched)	1.3	PhJä	-Extended cross reactivity- and microbial interference data -Added clinical study for nasal swab samples	2021-03-01
COVID-19 Ag Test (cassette, single pouched)	1.4	PhJä	-Added nasal swab samples	2021-03-04
COVID-19 Ag Test (cassette, single pouched)	1.5	PhJä	-Updated clinical study	2021-03-09
COVID-19 Ag Test (cassette, single pouched)	1.6	PhJä	-Updated clinical study	2021-03-11
COVID-19 Ag Test (cassette, single pouched)	1.7	PhJä	-Corrected typing errors - clarified C_t values in clinical study for nasal samples	2021-06-16