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 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
Notified body:	- (under responsibility of the manufacturer)
Number of the notified body:	-
Rev# replaced version **(also refer to History)**	- (start of Annex A7)

Table of Contents

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 variances within one LOT (Intra-LOT variations).

Testing procedure

The following controls were tested in replicates of 10 with tests cassettes of one final kit LOT 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 variances between different LOTs (Inter-LOT variations) between-operators and day-to-day

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 planned operator at planned time and site. 3 LOTs were tested by 3 operators at 3 different labs over 5 days. The operators were numbered as operator 1 to 3. Each result interpretation was performed by the 3 operators. 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 1, 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 2, Day 1								
	Site 1			Site 2			Site 3		
	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 3, Day 1								
	Site 1			Site 2			Site 3		
	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 2, Day 1								
	Site 1			Site 2			Site 3		
	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 2, Day 2								
	Site 1			Site 2			Site 3		
	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 2, Day 3								
	Site 1			Site 2			Site 3		
	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 3, Day 1								
	Site 1			Site 2			Site 3		
	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 3, Day 2								
	Site 1			Site 2			Site 3		
	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 3, Day 3								
	Site 1			Site 2			Site 3		

	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 address 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 LOTs. 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 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]	4×10^5	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 3 H02006							
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

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 was spiked onto either nasopharyngeal swabs or 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 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.
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)		
		LOT 1	LOT 2	LOT 3	LOT 1	LOT 2	LOT 3
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+
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

Aim

The purpose of this study was to test for cross reactivity 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 with other pathogens.

Testing procedure

Potential cross-reacting organisms were spiked onto negative nasopharyngeal swabs and extracted according the procedure described in the package insert. Each sample was tested in replicates of 10 with three independent LOTs. 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 experiments are shown in table 8.

Table 8: Results of the cross reactivity study

Pathogen	**Concentration**	**Spiked nasopharyngeal swab**		
		LOT 1	**LOT 2**	**LOT 3**
Virus	**$TCID_{50}$/ml**	**H02004**	**H02005**	**H02006**
Source: Zeptometrix				
HCoV-HKU1	2×10^5	10-	10-	10-
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: 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 1/2/3 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-
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-
Source: in-house				
Pooled human nasal wash of presumably negative employees (assumed to be representative of normal microbial flora)	N/A	10-	10-	10-

Conclusion

No cross reactivity was observed with any of the tested pathogens. Even the non-SARS human Cornaviruses showed no cross-reactivity in the listed concentrations. Consistent negative results were obtained for all determinations and LOTs. 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 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

2 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 9.

Table 9: Results of the sample equivalence study

		Nasopharyngeal swab			**Oropharyngeal Swab**		
Donor ID	RT-PCR result	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.

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, 348 respiratory samples were collected and tested with the nvm COVID-19 Ag test. 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. For some of the patients, the days between onset of symptoms and testing was known ranging from 1 day to 31 days. 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).

The 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

According to RT-PCR, 187 samples presented positive results and 161 samples presented negative results. There were 123 positive samples with Ct<30, and 64 positive samples with Ct≥30.

With the nvm COVID-19 antigen test, 150 of the samples were rated to be positive and 198 of the samples were rated to be negative. 120 of the positive results matched PCR results with C_t values <30, whereas 30 of the positive results matched PCR samples with C_t values of ≥30.

The results are summarized in the table 10.

Table 10: Summary of results of the clinical performance study

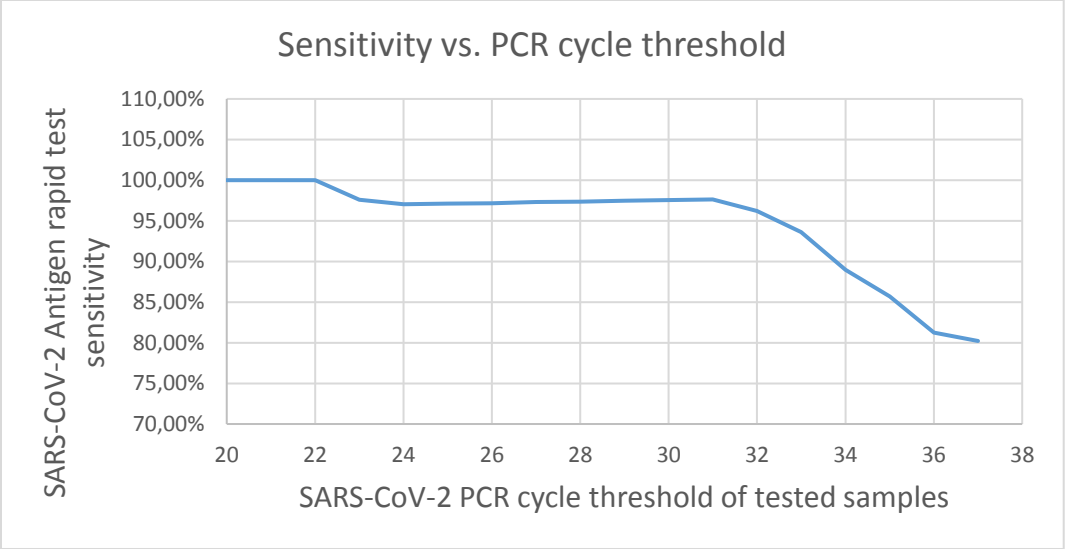
Method		**RT-PCR**		Total Results
	Results	Positive	Negative	
nvm	Positive	150 (tp)	0 (fp)	**150**
COVID-19 Ag Rapid Test	Negative	37 (fn)	161 (tn)	**198**
Total Results		**187**	**161**	**348**

		Calculation [x100]:
Diagnostic sensitivity:	80.2% (73.9% - 85.3%)*	150/(150+37)
Diagnostic specificity:	>99.9% (97.7% - 100%)*…	161/(161+0)
Overall agreement:	89.4% (85.7% - 92.2%)*	(150+161)/(150+37+161+0)

* 95% confidence interval

If the relative diagnostic sensitivity for strongly positive PCR samples with C_t values ≤30 as threshold was calculated, the relative diagnostic sensitivity for these samples was 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 (calculation: 120/(120+3)x100).

The following graph shows the correlation of the diagnostic sensitivity of the rapid test from 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.



Note

A more detailed clinical report listing each result of the clinical study can be made available on request to authorities.

Conclusion

The diagnostic performance study demonstrates that the nvm COVID-19 test showed an excellent relative diagnostic specificity. 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 80.2 %. As expected the rapid test was not as sensitive as RT-PCR. False negative results were predominantly obtained for samples with high C_t values (> 30) indicating that the original viral burden in the sample material was low. If the relative sensitivity 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 (n = 123). 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 showed a good reliability in the detection of samples with high virus burden (C_t of corresponding PCR ≤30) and showed a very good diagnostic specificity. However for 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. Product insert Rev 1.1 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.
- 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