BIOMEX

Clinical Performance Study Report - CPSR ACRO 2021_01

Evaluation of the SARS-CoV-2 Antigen Rapid Test (Swab)

REF. ISCO-ACO502

Analytical/diagnostic specificity

Diagnostic sensitivity

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Content

1 Purpose of the Study

The objective of this performance study is to establish the sensitivity and specificity of the SARS-CoV-2 Antigen Rapid Test (Swab) (REF: ISCO-ACO502) in order to meet the "Minimum criteria for SARS-CoV-2 antigen tests in the sense of §1 Abs. 1 Satz 1 TestVO: Antigen rapid tests" of the Paul-Ehrlich-Institut (PEI) dated 04.12.2020.

2 Sponsor – investigation – study coordination

2.1 Sponsor:

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3 Scope

3.1 Objectives

The objective of this performance study is to establish the diagnostic sensitivity and diagnostic and analytical specificity of the SARS-CoV-2 Antigen Rapid Test (Swab) (REF: ISCO-ACO502) in order to meet the "Minimum criteria for Rapid SARS-CoV-2 Antigen Tests Pursuant to Section 1 para 1 Sentence 1 TestVO (Statutory Test Regulation): Rapid Antigen Tests " of the Paul-Ehrlich-Institut (PEI) dated 04.12.2020.

3.2 Study Design Type

This retrospective study on frozen dry swab samples from COVID-19 infected and healthy donors is an observational study which aims to establish the analytical/diagnostic specificity and sensitivity of the SARS-CoV-2 Antigen Rapid Test (REF: ISCO-ACO502).
The swabs for the positive samples have been collected during the infectious phase of COVID-19 infected patients, the swabs of the negative samples have been collected from healthy donors. After collection all swabs (dry swabs) have been immediately stored at ≤-20°C.
As reference method all samples were tested with a RT-PCR system.

3.3 Current state of the art

The assays clinical performance is considered acceptable if the following requirements are met:

Diagnostic sensitivity:

Method: Parallel examination of diagnostic PCR tests and antigen tests in at least 100 persons with COVID-19 symptoms within seven days after onset of symptoms

Criterion: >80% of at least 100 unselected PCR-positive samples, positive in the SARS-CoV-2-rapid antigen test

Diagnostic specificity:

Method: Examinations of at least 100 asymptomatic persons without a concrete risk of exposure in the rapid SARS-CoV2 antigen test; clarification of any reactive samples by means of PCR.

Criterion: Specificity > 97 %

3.4 Reference Test

An analysis should be performed of the correlation between the antigen -positive/PCR-positive and the antigen-negative/PCR-negative samples with the Ct values of the PCR. The detection rate of the antigen test (e.g. detection rate >90%) should be observed in relation to the ct value. However, it should be noted that the Ct values vary between PCR tests in the case of a given concentration of the target RNA.

3.5 Expected Risk & benefits

There is no risk attributed to the patient since the evaluation is done retrospectively on frozen samples. The results obtained in this study will not be used for patient care decisions.

The risks related to the user have been reduced as far as possible by providing detailed instructions for use with the kits, including warning and precautions for the users and known limitations of the device. Furthermore, the study will be performed by professionals who are qualified and trained for conducting the clinical performance study.

4 Description Device

4.1 Identification

SARS-CoV-2 Antigen Rapid Test

4.2 Manufacturer if different from the sponsor

Not applicable.

4.3 Intended purpose

The SARS-CoV-2 Antigen Rapid Test (Swab) is a rapid chromatographic immunoassay for the qualitative detection of SARS-CoV-2 Nucleocapsid protein antigens in human nasopharyngeal swab, oropharyngeal swab and nasal swab specimen from individuals with suspected SARS-CoV-2 infection in conjunction with clinical presentation and the results of other laboratory tests.

Results are for the detection of SARS-CoV-2 Nucleocapsid protein Antigens. An antigen is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results indicate the presence of viral antigens, but clinical correlation with patient history and other diagnostic information is necessary to determine infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions. Negative results should be treated as presumptive and confirmed with a molecular assay, if necessary for patient management. Negative results should be considered in the context of a patient's recent exposures, history and the presence of clinical signs and symptoms consistent with SARS-CoV-2.

The SARS-CoV-2 Antigen Rapid Test (Swab) is intended for use by trained clinical laboratory personnel.

4.4 Analyte or marker

SARS-CoV-2 antigen.

4.5 Technical and Functional Features

The SARS-CoV-2 Antigen Rapid Test (Swab) is a qualitative membrane-based immunoassay for the detection of SARS-CoV-2 Nucleocapsid protein Antigens in human nasopharyngeal swab, oropharyngeal swab and nasal swab specimen.

SARS-CoV-2 Nucleocapsid protein antibody is coated in the test line region. During testing, the specimen reacts with SARS-CoV-2 Nucleocapsid protein antibody-coated particles in the test. The mixture then migrates upward on the membrane by capillary action and reacts with the SARS-CoV-2 Nucleocapsid protein antibody in test line region. If the specimen contains SARS-CoV-2 Antigens, a colored line will appear in test line region as a result of this. If the specimen does not contain antigens to SARS-CoV-2, no colored line will appear in the test line region, indicating a negative result. To serve as a procedural control, a colored line will always appear in the control line region, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

5 Study Design

5.1 Materials Supplied by the manufacturer.

5.1.1 Test Kits and Instructions for Use

Sufficient kits of the SARS-CoV-2 Antigen Rapid Test together with the Instructions for Use will be supplied free of charge to carry out the entire evaluation.

5.1.2 Instrument

Not applicable.

5.2 Materials Supplied by the Investigator

5.2.1 Standard laboratory reagents and disposables.

These are supplied by the Investigator and must meet the specifications required to correctly carry out the test procedure.

SARS-CoV-2 Antigen Rapid Test used:
Lot number: NCP20120044 Expiry date: 2022-12
Lot number: NCP20120052 Expiry date: 2022-12

5.2.2 Equipment/Instrumentation

Nucleic acid extraction will be performed with the R-Biopharm RIDA Xtract (REF: PGZ001) and analyzed with the R-Biopharm RIDA Gene SARS-CoV-2 real-time PCR kit (REF: PG6815), with the CFX96 Touch Real-Time PCR Detection System from Bio-Rad Laboratories (Hercules, USA).

R-Biopharm RIDA Xtract Kit used:
Lot number: QL200007 Expiry date: 2021-10

R-Biopharm RIDA Gene SARS-CoV-2 real-time PCR kit used:
Lot number:24450N Expiry Date: 2022-12
Lot number: 28510Z Expiry date: 2022-12

5.2.3 Samples

The samples used have been collected as dry swabs and are stored at -20°C.

5.3 Study population

According to the Minimum criteria for Rapid SARS-CoV-2 Antigen Tests the following sample numbers must be tested:

Diagnostic sensitivity:
Parallel examination of diagnostic PCR tests and antigen tests in at least 100 persons with COVID-19 symptoms within seven days after onset of symptoms

Criterion antigen test: >80% of at least 100 unselected PCR-positive samples, positive in the SARS-CoV-2-rapid antigen test.

Diagnostic specificity:
Examinations of at least 100 asymptomatic persons without a concrete risk of exposure in the rapid SARS-CoV2 antigen test; clarification of any reactive samples by means of PCR Devices shall have a specificity of > 97 %.

Analytical specificity

- *Potentially cross-reactive markers:*

Examination of samples including those with a high concentration of related human coronaviruses

  - *human coronavirus 229E*
  - *human coronavirus OC43*
  - *human coronavirus NL63*
  - *MERS coronavirus*

- *Potentially interfering substances:*

Examinations should also be performed on pathogen-positive samples in which the pathogen can cause analogous symptoms (e.g. influenza A, B; RSV), or could interfere with the test principle (e.g. protein A-positive Staphylococcus aureus in the case of nasal swabs as sample matrix

  - *influenza A*
  - *influenza B*
  - *RSV*

An analysis should be performed of the correlation between the antigen -positive/PCR-positive and the antigen-negative/PCR-negative samples with the Ct values of the PCR. In addition, the PCR protocol should be described. The mean Ct value should be determined for the antigen-positive samples. In another evaluation, the detection rate of the antigen test (e.g. detection rate >90%) should be observed in relation to the ct value. However, it should again be noted that the Ct values vary between PCR tests in the case of a given concentration of the target RNA.

5.4 Test procedure

Throughout the evaluation, all samples swabs were extracted in the SARS-CoV-2 Antigen Rapid Test extraction buffer as described in the IFU of the rapid test. Three drops of the specimen (approximately 100 µL) were added to the sample well of the test cassette. Results obtained with the rapid test device were visually read-out by two operators between 15 and 20 minutes after the sample had been applied onto the test cassette. Digital images were taken from used rapid test cassettes after visual read-out.

Total RNA was extracted from 50 µL of the remaining liquid using the R-Biopharm RIDA Xtract (REF: PGZ001), and analyzed with the R-Biopharm RIDA Gene SARS-CoV-2 real time PCR kit (REF:PG6815). The instructions of the real-time RT-PCR kit manufacturer were followed with the exception that 50 µl instead of 400 µl of the solution was used for the extraction due to the limited volume in the specimen processing tube.

According to a validation of different extraction volumes of 50 µl, 200 µl and 400 µl an average value of 3.14 Ct was calculated as difference between the used 50 µl and the requested 400 µl. Therefore, a Ct-value of 3.14 was subtracted from the PCR results received with 50 µl for each sample.

Real-time RT-PCR analysis was performed in singlicate analysis for all samples that were collected from infected donors and conducted using a CFX96 Touch Real-Time PCR Detection System from Bio-Rad Laboratories (Hercules, USA). The real-time RT-PCR results were obtained as Ct values. Samples with a Ct value of 36 (mean of the two replicates) or below were included in the calculation of the sensitivity of the SARS-CoV-2 Antigen Rapid Test.

6 Data management

Data management entails the planning for the creation, identification, verification, storage, transfer and archiving of data pertinent to the study, by means of the format of the study records, as well as associated responsibilities.

6.1 Data and results recording

The sample information and reference results of the samples will be recorded in the Study Record Forms (SRFs) in excel.
SRF completion:

- The sample ID recorded in the SRF must be exactly the same as the sample ID recorded by the instrument.
- Each item on the SRF must be completed
- No blanks can be left
- If an item is missing or not available, the entry shall be completed with 'NA'

Upon completion of the SRF, the study coordinator reviews the recorded data for completeness, accuracy and legibility.
To protect the subject or patient's privacy, no personal data shall appear anywhere on the SRF.

The data obtained with the SARS-CoV-2 Antigen Rapid Test will be recorded on a sample sheet and as digital images taken within the prescribed time frame. The results are transferred to the SRF.

The completed SRF with sample information and reference results will be made available upon finalization of the testing.

All data will be filed both as a hard copy and in electronic files by Biomex. Data will be stored for a time period as defined in the lab's QMS procedures but at least 5 years. All laboratory results are strictly confidential.

6.2 Data analysis

The following analyses will be performed:

The diagnostic sensitivity of the SARS-CoV-2 Antigen Rapid Test was calculated as the number of identified positive samples compared to the total number of positive samples tested in parallel on the reference RT-PCR-assay in correlation to the Ct-value.

The diagnostic specificity of the SARS-CoV-2 Antigen Rapid Test was calculated as the number of negative samples on the total number of negative samples tested with the RT-PCR-test.

The diagnostic sensitivities and specificities are reported together with a 2-sided 95% confidence interval.

7 Results

7.1 Definitions

True positive sample: sample that was determined positive both using the SARS-CoV-2 Antigen Rapid Test and by RT-PCR.

False positive sample: sample that was determined positive using the SARS-CoV-2 Antigen Rapid Test, but negative by RT-PCR.

True negative sample: sample that was determined negative both using the SARS-CoV-2 Antigen Rapid Test and by RT-PCR.

False negative sample: sample that was determined negative using the SARS-CoV-2 Antigen Rapid Test but positive by RT-PCR.

Specificity (%): # true negative samples/(# true negative samples + # false positive samples) x 100

Sensitivity (%): # true positive samples/(# true positive samples + # false negative samples) x 100

7.2 Diagnostic sensitivity

In total 137 swabs (50 nasal swabs and 87 throat swabs) from donors with known SARS-CoV-2 infection were tested with the SARS-CoV-2 Antigen Rapid Test.

Sex, age and symptoms of the donors as well as date of onset of symptoms were known. The date of infection was presumed from indications by the donor. Date of swab collections were documented (see annex "SRF Main Evaluation SARS-CoV-2 Antigen Rapid Test").

Analytical Results with correlation to Ct-values of the positive samples:

Ct value	Number of Samples	Number of true positive Rapid Test Samples	Number of false negative Rapid Test Samples	Sensitivity of SARS-CoV-2 Antigen Rapid Test (CI)
≤ 30	74	72	2	97.3 % (91-99)
≤ 32	103	99	4	96.1 % (90-99)
≤ 34	126	107	19	84.9 % (78-90)
≤ 36	137	111	26	81 % (74-87)

The correlation between the Ct-values of the analyzed samples and the sensitivity reveals a sensitivity of 96% for samples with a Ct-value of up to 32. Samples with a higher Ct value in the real-time RT-PCR and consequently less viral RNA copies as well as viral antigen in the samples result in lower sensitivity values for the SARS-CoV-2 Antigen Rapid Test. This is in line with expectations regarding viral detection by antigen rapid testing compared to PCR analysis.

7.3 Diagnostic specificity

Samples included:
100 nasal swabs from healthy donors: Sex, age and date of sample collection were known (see annex "Evaluation study SARS-CoV-2 Antigen Rapid Test and PCR Test").

Analytical Results with correlation to Ct-values of the negative samples:

Number of Samples	Number of true neg. Rapid Test Samples	Number of false positive Rapid Test Samples	Sensitivity of SARS-CoV-2 Antigen Rapid Test (CI)
100	100	0	100 % (96-100)

Diagnostic Specificity of SARS-CoV-2 Antigen Rapid Test: 100% (100/100), Wilson 95% CI: 96-100%

Analytical Results (Total Accuracy) for all samples with PCR result either negative or positive with a Ct value of less than 32 in this study:

		RT-PCR	
		positive	negative
SARS-CoV-2 Antigen Rapid Test	positive	103	0
	negative	4	100

Total accuracy of SARS-CoV-2 Antigen Rapid Test: 98,0% (199/203), Wilson 95% CI: 95-99%
Sensitivity of SARS-CoV-2 Antigen Rapid Test (Ct <32): 96 % (99/103), CI: 90-99%
Specificity of SARS-CoV-2 Antigen Rapid Test: 100 % (100/100), CI: 96-100%

7.4 Analytical specificity

Samples included:

The following heat inactivated viruses were purchased from ZeptoMetrix Corporation, 878 Main Street, Buffalo, NY 14202:

Virus	Strain	Lot #	Exp. Date	Titer ($TCID_{50}$)
Coronavirus	229E	325111	24/09/2023	$1{,}41 \times 10^5$
Coronavirus	NL63	325222	15/10/2023	$4{,}68 \times 10^4$
Coronavirus	OC43	325491	16/11/2023	$5{,}01 \times 10^5$
MERS-CoV	Florida/USA-2_Saudi Arabia_2014	325281	20/10/2023	$1{,}17 \times 10^5$
RSV-A	2006 Isolate	324924	25/08/2023	$5{,}01 \times 10^5$
RSV-B	CH93-18(19)	325289	22/10/2023	$1{,}55 \times 10^4$
Influenza A	H1N1 New Caledonia	320943/522670	Man. 09/2018	$1{,}15 \times 10^7$
Influenza B	Yamagata/16/88	323828	25/02/2023	$5{,}62 \times 10^4$
Influenza B	Victoria/2/87	325078	23/09/2023	$1{,}70 \times 10^5$

The above listed samples were diluted with the extraction buffer provided in the SARS-CoV-2 Antigen Rapid Test.

Specimen	Dilution	Titer ($TCID_{50}$)
Coronavirus 229E	1:10	$1{,}41 \times 10^4$
Coronavirus 229E	1:100	$1{,}41 \times 10^3$
Coronavirus 229E	1:1.000	$1{,}41 \times 10^2$
Coronavirus NL63	1:10	$4{,}68 \times 10^3$
Coronavirus NL63	1:100	$4{,}68 \times 10^2$
Coronavirus OC43	1:10	$5{,}01 \times 10^4$
Coronavirus OC43	1:100	$5{,}01 \times 10^3$
Coronavirus OC43	1:1.000	$5{,}01 \times 10^2$
MERS CoV Florida/USA-2_Saudi Arabia_2014	1:10	$1{,}17 \times 10^4$
MERS CoV Florida/USA-2_Saudi Arabia_2014	1:100	$1{,}17 \times 10^3$
MERS CoV Florida/USA-2_Saudi Arabia_2014	1:1.000	$1{,}17 \times 10^2$
RSV-A 2006 Isolate	1:10	$5{,}01 \times 10^4$
RSV-A 2006 Isolate	1:100	$5{,}01 \times 10^3$
RSV-A 2006 Isolate	1:1.000	$5{,}01 \times 10^2$
RSV-B CH93-18(19)	1:10	$1{,}55 \times 10^3$
RSV-B CH93-18(19)	1:100	$1{,}55 \times 10^2$
Influenza A H1N1 New Caledonia	1:10	$1{,}15 \times 10^6$
Influenza A H1N1 New Caledonia	1:100	$1{,}15 \times 10^5$
Influenza A H1N1 New Caledonia	1:1.000	$1{,}15 \times 10^4$
Influenza A H1N1 New Caledonia	1:10.000	$1{,}15 \times 10^3$
Influenza A H1N1 New Caledonia	1:100.000	$1{,}15 \times 10^2$
Influenza B Yamagata/16/88	1:10	$5{,}62 \times 10^3$
Influenza B Yamagata/16/88	1:100	$5{,}62 \times 10^2$
Influenza B Victoria/2/87	1:10	$1{,}70 \times 10^4$
Influenza B Victoria/2/87	1:100	$1{,}70 \times 10^3$
Influenza B Victoria/2/87	1:1.000	$1{,}70 \times 10^2$

The $TCID_{50}$ value is converted to plaque forming units by the equation 0.69 PFU = 1 $TCID_{50}$. Example: a $TCID_{50}$ value of $1{,}15 \times 10^3$ corresponds to 794 PFU.

All dilutions were tested with the SARS-CoV-2 Antigen Rapid Test and found to be negative.

8 Conclusion

The specificity and sensitivity of the SARS-CoV-2 Antigen Rapid Test was evaluated in this study with 237 samples collected as nasal or throat swabs. All samples were tested in parallel with the SARS-CoV-2 Antigen Rapid Test and a real-time RT-PCR assay. Samples with a Ct value at or below 36 were selected for the calculation of the sensitivity of the SARS-CoV-2 Antigen Rapid Test.

The specificity of the SARS-CoV-2 Antigen Rapid Test calculated from results of all samples was 100 %, the sensitivity calculated from results of samples with a Ct-value less than 32 (103 samples) was 96 % (95% CI: 90-99 %). As expected, the sensitivity decreases by including samples with higher Ct value. Thus, by including all samples with a Ct value of or below 36 (137 samples) the sensitivity is calculated as 81 % (95% CI: 74-87 %).

In conclusion, the results from this study confirm that the SARS-CoV-2 Antigen Rapid Test can be used for the qualitative detection of antigen from SARS-CoV-2 in human nasal swab and throat swab specimens.

No cross-reactivity was detected with various tested viruses in the SARS-CoV-2 Antigen Rapid Test.

9 Bibliography

- Minimum criteria for SARS-CoV-2 antigen tests in the sense of §1 Abs. 1 Satz 1 TestVO: Antigen rapid tests" of the Paul-Ehrlich-Institut (PEI) dated 04.12.2020

10 Annexes

Annex I	SRF Main Evaluation SARS-CoV-2 Antigen Rapid Test
Annex II	Pictures of positive samples
Annex III	Pictures of negative samples

11 Approval

Author

Dr. Heike Lukhaup Study coordination Date: 07.02.2021 Signature: 

Approval

ACRO Biotech Name: Function: Date:	Signature:
Biomex Maximilian Maschler Project Lead Date: 07.02.2021	Signature: