BIOMEX

Clinical Performance Study Report **CPSR 2021_37**

HIGHTOP SARS-CoV-2 Antigen Rapid Test

Analytical/diagnostic specificity

Diagnostic sensitivity

Sponsor:

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HIGHTOP

Content

1 Purpose of the Study

The objective of this performance study is to establish the sensitivity and specificity of the HIGHTOP SARS-CoV-2 Antigen Rapid Test 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 15.01.2021.

2 Sponsor – investigation – study coordination

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3 Scope

3.1 Objectives

The objective of this performance study was to establish the diagnostic sensitivity and diagnostic and analytical specificity of the HIGHTOP SARS-CoV-2 Antigen Rapid Test 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 15.01.2021.

Samples included:

- 102 persons with COVID-19 symptoms within seven days after onset of symptoms.
  The collection of the swabs was carried out in Germany with European subjects, usually the samples have been collected in the patients' home environment. No samples have been collected in hospitals.
- 300 asymptomatic persons without a concrete risk of exposure in the rapid SARS-CoV2 antigen test. The collection of the swabs was carried out in Germany with European subjects
- Examination of samples including those with a high concentration of related human coronaviruses (e.g. human coronavirus 229E, human coronavirus OC43, human coronavirus NL63, MERS coronavirus).
- Examinations 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)

3.2 Study Design Type

This retrospective study on frozen dry swab samples from COVID-19 infected and healthy donors was an observational study which aims to establish the analytical/diagnostic specificity and sensitivity of the HIGHTOP SARS-CoV-2 Antigen Rapid Test (REF: CHR15).
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. All swabs were collected from anterior nasal cavity.
After collection all swabs (dry swabs) have been stored immediately 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 300 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 has been 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 Timelines

Starting date: 16th of July 2021
End-date: 23rd of July 2021

5 Description Device

5.1 Identification

HIGHTOP SARS-CoV-2 Antigen Rapid Test

5.2 Manufacturer if different from the sponsor

Not applicable.

5.3 Intended purpose

HIGHTOP SARS-CoV-2 Antigen Rapid Test is used for the detection of SARS-CoV-2 antigens in samples from the human anterior nasal cavity area. It is used to detect SARS-CoV-2 nucleoprotein antigens within 7 days of the onset of symptoms suspected of coronavirus infection. Positive test results can be used for early isolation and rapid treatment of suspected cases, but they cannot serve as a basis for a definitive diagnosis of coronavirus infection.

5.4 Analyte or marker

SARS-CoV-2 antigen (nucleocapsid protein)

5.5 Specimen Type

Nasal swab

5.6 Metrological Traceability

Not applicable.

5.7 Technical and Functional Features

According to the gold immunochromatographic test principle, the nitrocellulose membrane is coated with SARS-CoV-2 monoclonal antibody 2 and goat anti-mouse IgG antibody, the gold conjugate pad solid phase is fixed with SARS-CoV-2 monoclonal antibody 1. When the antigen is contained in the sample, the antigen binds with the corresponding gold labeled monoclonal antibody to form a compound, moving forward under the chromatography, then combines with the coated antibody in the test line to form Au-novel coronavirus (SARS-CoV-2) monoclonal

antibody 1-antigen-novel coronavirus (SARS-CoV-2) monoclonal antibody 2 complex to condenses into a red band (Test line, T), indicating a positive result. When the sample does not contain antigen, complex cannot be formed in the test line, and no red band appears, indicating negative result.

No matter whether the samples contain antigens or not, the gold labeled monoclonal antibody will combine with the coated goat anti-mouse IgG antibody at the quality control line to form a Au-novel Coronavirus (SARS-CoV-2) monoclonal antibody 1-goat anti-mouse IgG antibody complex and condenses into a red band (quality control line, C).

6 Study Design

6.1 Materials Supplied by the manufacturer.

6.1.1 Test Kits and Instructions for Use

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

6.1.2 Instrument

Not applicable.

6.2 Materials Supplied by the Investigator

6.2.1 Standard laboratory reagents and disposables.

These are supplied by the Investigator and must meet the specifications required to correctly carry out the test procedure.
HIGHTOP SARS-CoV-2 Antigen Rapid Test used:
Lot number: COV-1210527 Expiry date: 20220520

6.2.2 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: QL210010 Expiry date: 2022-07

R-Biopharm RIDA Gene SARS-CoV-2 real-time PCR kit used:
Lot number: 212001Z Expiry date: 2023-05

6.2.3 Samples

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

6.3 Study population

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

<u>Diagnostic sensitivity:</u>
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 300 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 %.

Required patient information:
- Collection date of swab
- Age, sex
- Date of onset of symptoms (if present)/time of infection
- Severity of symptoms (if known)
- Date of initial PCR testing (when patient was tested for the first time)
- Initial PCR result (i.e. positive or negative)

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.

6.4 Test procedure

Throughout the evaluation, all samples swabs were extracted in the HIGHTOP SARS-CoV-2 Antigen Rapid Test extraction buffer as described in the IFU of the rapid test. 2-3 drops of the treated sample (approximately 60-80 µL) were applied 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).

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 as single determination 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 HIGHTOP SARS-CoV-2 Antigen Rapid Test.

7 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.

7.1 Data and results recording

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

- 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'

To protect the subject or patient's privacy, no personal data shall appear anywhere on the SRF.

The data obtained with the HIGHTOP SARS-CoV-2 Antigen Rapid Test are 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.

The HIGHTOP SARS-CoV-2 Antigen Rapid Test results are for performance evaluation only and must not be used for diagnostic purposes.

7.2 Data analysis

The following analyses have been performed:

The diagnostic sensitivity of the HIGHTOP 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 HIGHTOP 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.

8 Results

8.1 Definitions

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

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

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

False negative sample: sample that was determined negative using the HIGHTOP 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

8.2 Diagnostic sensitivity

In total 102 nasal swabs from donors with known SARS-CoV-2 infection were tested with the HIGHTOP 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 HIGHTOP SARS-CoV-2 Antigen Rapid Test").

The collection of the swabs was carried out in Germany with European subjects.

The age distribution of the infected donors was between 8 and 56 years, the gender distribution was 49.02 % (50) female and 50.98 % (52) male. This represents very well the average distribution of the target population.

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 HIGHTOP SARS-CoV-2 Antigen Rapid Test (CI)
≤ 30	77	77	0	100 % (95.25-100.0%)
≤ 32	87	87	0	100 % (95.77-100.0%)
≤ 34	100	98	2	98.00 % (93.00-99.45%)
≤ 36	102	100	2	98.04 % (93.13-99.46%)

The correlation between the Ct-values of the analyzed samples and the sensitivity reveals a sensitivity of 98.04 % for samples with a Ct-value of up to 36. This means that the sensitivity of the viral detection by the HIGHTOP SARS-CoV-2 Antigen Rapid Test can be compared with the sensitivity of PCR analysis.
10 samples out of the 102 have been from patients with the UK mutant B.1.1.7. All samples have been detected positive with the HIGHTOP SARS-CoV-2 Antigen Rapid Test, so that one can conclude that this variant is detected by this test with a very high sensitivity.

8.3 Diagnostic specificity

Samples included:
300 nasal swabs from healthy donors: Sex, age and date of sample collection were known (see annex "SRF Main Evaluation HIGHTOP SARS-CoV-2 Antigen Rapid Test_Annex_I").

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 HIGHTOP SARS-CoV-2 Antigen Rapid Test (CI)
300	300	0	100 % (98.74-100.0%)

Diagnostic Specificity of HIGHTOP SARS-CoV-2 Antigen Rapid Test: 100% (300/300), Cl 95% CI: 98.74-100.0%

Analytical Results (Total Accuracy) for all samples with PCR result either negative or positive with a Ct-value of ≤ 36 in this study:

		RT-PCR		
		positive	negative	Total
HIGHTOP SARS-CoV-2 Antigen Rapid Test	positive	100	0	100
	negative	2	300	302
	total	102	300	402

Total accuracy of HIGHTOP SARS-CoV-2 Antigen Rapid Test: 99.5 % (400/402), Cl 95% CI: 98.20-99.86%
Sensitivity of HIGHTOP SARS-CoV-2 Antigen Rapid Test (Ct ≤36): 98.04 % (100/102), CI: 93.13-99.44%)
Specificity of HIGHTOP SARS-CoV-2 Antigen Rapid Test: 100 % (300/300), CI: 98.74-100.0%

8.4 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 HIGHTOP SARS-CoV-2 Antigen Rapid Test.

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

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 HIGHTOP SARS-CoV-2 Antigen Rapid Test and found to be negative.

9 Conclusion

The specificity and sensitivity of the HIGHTOP SARS-CoV-2 Antigen Rapid Test was evaluated in this study with 402 samples collected as anterior nasal swabs. All samples were tested in parallel with

the HIGHTOP 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 HIGHTOP SARS-CoV-2 Antigen Rapid Test.

The specificity of the HIGHTOP 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 up to 36 (102 samples) was 98.04 % (95% CI: 93.13-99.44 %). The sensitivity was very good with only 2 negatives out of 102 samples.

In conclusion, the results from this study confirm that the HIGHTOP SARS-CoV-2 Antigen Rapid Test can be used for the qualitative detection of antigen from SARS-CoV-2 in human anterior nasal swab with a very high sensitivity and specificity.

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

10 Bibliography

- EU Regulation 2017/746 on *in vitro* Diagnostic Medical Devices
- 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 15.01.2021).
- ISO 20916 In vitro Diagnostic Medical Devices – Clinical Performance Studies using specimens form human subjects – Good Study Practices
- EU Guidance on the management of clinical trials during the COVID-19 pandemic version 3. April 2020.
- European Commission, Working document of Commission services – Current performance of COVID-19 test methods and devices and proposed performance criteria, 16 April 2020

11 Annexes

Annex I	SRF Main Evaluation HIGHTOP
Annex II	Pictures of positive samples
Annex III	Pictures of negative samples
Annex VI	Pictures of cross reactive samples

12 Approval

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