BIOMEX

Clinical Performance Study Plan **CPSP 2021_37**

HIGHTOP SARS-CoV-2 Antigen Rapid Test

Analytical/diagnostic specificity
Diagnostic sensitivity

Sponsor:

Qingdao Hightop Biotech Co., Ltd.
No.369 Hedong Road,
Hi-tech Industrial Development Zone,
Qingdao, Shandong,266112
P.R. China

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 and to provide data to demonstrate the product is safe and effective for its intended use. The data obtained will be used in the application for CE certification.

2 Sponsor – investigation – study management

2.1 Sponsor

Qingdao Hightop Biotech Co., Ltd.
No.369 Hedong Road,
Hi-tech Industrial Development Zone,
Qingdao, Shandong,266112
P.R. China

2.2 Investigation

Biomex GmbH
Laboratories of Biomex GmbH Heidelberg
Siemensstr. 38
D-69123 Heidelberg
Germany
www.biomex.de

Mr. Hannes Deisel
Project Lead
Biomex GmbH Heidelberg
Siemensstr. 38
D-69123 Heidelberg
Germany
Tel.: +49 6221 894669 23
e-mail: deisel@biomex.de

2.3 Study coordination

Biomex GmbH
Dr. Heike Lukhaup
Head of validation
Principal Investigator
Siemensstr. 38
D-69123 Heidelberg
Tel.: +49 6221 894669 43
e-mail: lukhaup@biomex.de

3 Scope

3.1 Objectives

The objective of this performance study is 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:

- At least 100 persons with COVID-19 symptoms within seven days after onset of symptoms
- At least 100 asymptomatic persons without a concrete risk of exposure in the rapid SARS-CoV2 antigen test.
- 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 is 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. After collection all swabs (dry swabs) have been immediately stored at ≤-20°C.

As reference method all samples will be 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 Timelines

Envisaged starting date: July 2021
Envisaged end-date: 2-3 weeks later

5 Description Device

5.1 Identification

HIGHTOP SARS-CoV-2 Antigen Rapid Test (Immunochromatography)

5.2 Manufacturer if different from the sponsor

Not applicable.

5.3 Intended purpose

SARS-CoV-2 Antigen Rapid Test (Immunochromatography) 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 will be supplied free of charge to carry out the entire evaluation.

As the kits may be from more than one lot of finished materials, do not mix the reagents from more than one batch.

Information on the kits received must be recorded on the Product Accountability log in Annex.

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.

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).

6.2.3 Samples

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

6.3 Study population and selection criteria

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 200 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.

<u>Diagnostic specificity:</u>
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 %.

<u>Required patient information:</u>

- 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 will be extracted in the HIGHTOP SARS-CoV-2 Antigen Rapid Test extraction buffer as described in the IFU of the rapid test. Results obtained with the rapid test device are visually read-out by two operators between 15 and 20 minutes after the sample has been applied onto the test. Digital images are taken from used rapid test cassettes after visual read-out.

Total RNA will be 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 are 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 is 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 are obtained as Ct values.

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 management plan

The data entered in the database, that will be used for analysis, will be verified against source data by the CPS Coordinator. The sample information and reference results of the samples will be 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'

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 HIGHTOP 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.

All data will be summarized in a final report in the English language by the CPS coordinator, including material and methods section, results tables, discussion and conclusion per item. The final report will be approved and signed by the Investigator.

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 will be performed:

The diagnostic sensitivity of the HIGHTOP SARS-CoV-2 Antigen Rapid Test will be 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 will be 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 will be reported together with a 2-sided 95% confidence interval.

8 Evaluation phases

8.1 Study Site Initiation

An initiation meeting shall be conducted and documented by the Study coordinator at the beginning of the clinical performance study.

Names, initials, signatures and functions shall be documented on the training log.

Prerequisites for initiation:

a) Signed clinical performance study plan, copies shall be provided to all parties involved.
b) Instructions for use of the IVD product.
c) Required number of IVD devices are available at the investigation site and documented on the Product Accountability log.

d) Any financial arrangements between the Investigator and the sponsor are documented.
e) Any required application(s) to begin the clinical performance study in a given country have been submitted to the appropriate regulatory authority(ies) for review, acceptance or permission.

8.2 Training

Before starting the study, the CPSP should be clearly understood by all members of the study team.

Training of all involved parties shall be documented in the training log. A template for the training log can be found in Annex II.

8.3 Conduct of the study

Testing of the samples must be done by trained persons.

Accountability of the devices during testing shall be logged in the Product Accountability log.

Any issues observed during the testing phase shall be reported to the CPS Coordinator and discussed.

8.4 End of the study

The following documentation shall be available at end of the study:

- Completed and signed SRFs, pictures of the developed devices and study database (including reference data and subject information).
- Clinical performance study report signed by the investigator.
- Any unused devices handled as agreed upon with the sponsor (returned, destroyed, …).
- Completed Product Accountability log.

9 Study Plan Amendments and Deviations

Any deviation from the clinical performance study plan will be reported to the Study coordinator and documented in the clinical performance study report.
Amendments to the clinical performance study plan shall be agreed upon between the sponsor and the Investigator. The amendment will not be implemented before the approval is obtained from all involved parties.

10 Statements of Compliance and Ethical Principles

The Investigator will perform the clinical performance study in accordance with the applicable laws and regulations, including the applicable privacy laws and recognized Ethical principles laid down in the Declaration of Helsinki. The study will be carried out in accordance with ISO 20916:2019 – *In vitro* diagnostic medical devices – Clinical performance studies using specimens from human subjects – Good study practice.

The investigator informs that no approval of the study plan (and each amendment (if any)) and related documents, if any, is required by the Ethics Committee.

The results obtained in this clinical performance study will not be used for patient care decisions.

11 Adverse events, Adverse device effects and device deficiencies

Any adverse device effect or observed device deficiency as defined below, encountered during the laymen study will be recorded and reported to the Study coordinator, and documented in the clinical performance study report.

Any serious adverse device effect or device deficiency that could have led to a serious adverse device effect, shall be reported, without unjustified delay, to the Study coordinator who will inform the sponsor.

12 Suspension or premature termination of the Clinical Performance Study

The Study coordinator, whether or not in consultation with the sponsor, can decide to immediately suspend or prematurely terminate the clinical performance study in case of:

- occurrence of serious adverse device effects/device deficiencies
- suspicion of unexpected risk to the users or other persons involved in the study

The Study coordinator will inform the sponsor and justify the decision for suspension or premature termination of the CPS in writing to the Investigator. All routine end of study activities shall be conducted.

After risk assessment performed by the sponsor in collaboration with the Study coordinator, it can be concluded to resume the suspended CPS.

13 Clinical Performance Study Report

The Clinical Performance study report will be written by the Study coordinator and shall contain documented information on the study plan, results and conclusions of the Clinical Performance study, including negative findings.

The results and conclusions shall be transparent, free of bias and clinically relevant. The report shall contain sufficient information to enable it to be understood by an independent party without reference to other documents. The report shall also include as appropriate any study plan amendments or deviations, and data exclusions with the appropriate rationale.

The final study report will be approved and signed by the Investigator. The Investigator grants Qingdao Hightop Biotech Co., Ltd. the use of the results of this study for regulatory and marketing purposes, for example in the design dossier for the CE-self-testing certification of the HIGHTOP SARS-CoV-2 Antigen Rapid Test. The Investigator shall treat the results of the study confidentially and only disclose them after written agreement of Qingdao Hightop Biotech Co., Ltd.

14 Unused devices

Reconciliation of kits received, used and returned to the sponsor must be recorded on the Product Accountability log in Annex.
After completion of the study, all remaining materials must be returned or destroyed according to the sponsor's decision.
The Product Accountability log should be completed and returned to the Study coordinator upon the finalization of the evaluation and provided to the sponsor.

15 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

16 Annexes

Annex I Product Accountability log
Annex II Training log

17 Approval

Author

Biomex GmbH

Name: Dr. Heike Lukhaup
Function: Study coordinator/Principal Investigator

Date: 15.07.2021 Signature: 

Approval

Qingdao Hightop Biotech Co., Ltd.

Name: Zoe Cui
Function: Management Representative

Date: 16.07.2021 Signature: 

Biomex GmbH

Name: Oliver Bošnjak
Function: CEO

Date: 15.07.2021 Signature: 

Annex I - Product Accountability log

IVD name:		**REF:**	
Received			
Date	Batch number/expiry date	Number of kits / devices	
Storage			
Temperature and location			
Used			
Date	Batch number	Number of kits / devices taken	Number of kits / devices left
Returned ☐ **Destroyed ☐**			
Date	Batch number	Number of kits / devices	

Annex II - Training log

<table>
<tr><td colspan="2">Trainee
Name: …… Initials: ……

Function: ……..</td></tr>
<tr><td>Date:</td><td>Subject:
IFU ☐
CPSP ☐

Other: ……</td></tr>
<tr><td>Name, Date and Signature:
Trainer:</td><td>Date and Signature:
Trainee:</td></tr>
</table>