BIOMEX

Clinical Performance Study Report CPSRC705

AESKU.RAPID SARS-CoV-2

Diagnostic sensitivity

SARS-CoV-2 Omicron variant B.1.1.529 (BA.4/BA.5)

AESKU.DIAGNOSTICS GmbH & CO.KG

Mikroforum Ring 2
55234 Wendelsheim,
Germany

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Table of Acronyms

Acronyms	Full name
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
BA.4, BA.5	Sublineages of the SARS-CoV-2 Omicron variant
CPS	Clinical Performance Study
CPSP	Clinical Performance Study Plan
CPSR	Clinical Performance Study Report
COVID-19	Coronavirus disease 2019
NAT	Nucleic acid test
PCR	Polymerase chain reaction
Real-time RT-PCR	real-time (quantitative) reverse transcription PCR
IFU	Instructions for Use
SRF	Study Record Form

1 Purpose of the Study

The purpose of this performance study is to establish the diagnostic sensitivity of the AESKU.RAPID SARS-CoV-2 in relation to the sublineages BA.4/BA.5 of SARS-CoV-2 Omicron (B.1.1.529) variant in order to demonstrate that the product is effective for its intended use.

2 Involved Parties

2.1 Sponsor:

AESKU.Diagnostics GmbH & Co. KG

Mikroforum Ring 2
55234 Wendelsheim
Germany
Tel: +49-6734-9622-6666
www.aesku.com
E-Mail: info@aesku.com.

2.2 Investigation:

Biomex GmbH

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

Dr. Wioleta Chmielewicz
Head of Laboratory
Siemensstr. 38
D-69123 Heidelberg
Germany
Tel.: +49 6221 4166 125
E-Mail: wioleta.chmielewicz@biomex.de

2.3 Study Coordination:

Biomex GmbH

Dr. Heike Lukhaup
Study coordinator/Principal Investigator
Siemensstr. 38
D-69123 Heidelberg
Germany
Tel.: +49 6221 4166 430
E-Mail: lukhaup@biomex.de

3 Use of Data

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4 Scope

4.1 Objectives

The objective of this performance study is to establish the diagnostic sensitivity of the AESKU.RAPID SARS-CoV-2 in relation to the sublineages BA.4/BA.5 of the SARS-CoV-2 Omicron (B.1.1.529) variant.

Samples included in the study:

- 37 positive specimens from persons with COVID-19 symptoms within seven days after onset of symptoms and with the confirmed SARS-CoV-2 infection by the preliminary external PCR analysis. The subline determination of BA.4/BA.5 was carried out by mass spectroscopy.

4.2 Study Design Type

This retrospective study on frozen dry swab samples from COVID-19 infected donors is an observational study which aimed to establish the diagnostic sensitivity of the AESKU.RAPID SARS-CoV-2 in relation to the sublineages BA.4/BA.5 of SARS-CoV-2 Omicron (B.1.1.529) variant.

4.3 Current state of the art

The following general acceptance criteria for diagnostic sensitivity in relation to positive samples:

- diagnostic sensitivity: >80% (rapid tests) relative to the SARS-CoV-2 real-time RT-PCR test.

4.4 Reference Test

An analysis was performed of the correlation between the antigen-positive/PCR-positive with the Ct-values of the real-time RT-PCR tests. It should be noted that the Ct-values may vary between PCR tests in case of a given concentration of the target RNA.

4.5 Expected Risk & benefits

There was no risk attributed to the donors since the evaluation is done retrospectively on frozen samples. The results obtained in this study were not used for donor or 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 was performed by professionals who are qualified and trained for conducting the clinical performance study.

5 Timelines

Testing was carried out between 08 Aug 2022 and 23 Aug 2022

6 Description Device

6.1 Identification

Product name: AESKU.RAPID SARS-CoV-2

6.2 Manufacturer if different from the sponsor

Not applicable.

6.3 Intended use

According to the IFU of AESKU.RAPID SARS-CoV-2:

The AESKU.RAPID SARS-CoV-2 rapid antigen test is an immunological method that uses SARS-CoV-2 specific antibodies to provide qualitative evidence of coronavirus components in human nasal swab samples. The test is optimized for self-testing and is designed to provide evidence of SARS-CoV-2 antigens during the acute phase of an infection.

6.4 Analyte or marker

SARS-CoV-2 antigen

6.5 Specimen Type

Anterior nasal swab

6.6 Metrological Traceability

Not applicable.

6.7 Technical and Functional Features

According to the IFU of AESKU.RAPID SARS-CoV-2:

The AESKU.RAPID SARS-CoV-2 is based on immunochromatographic polymer technology combined with the sandwich principle for the qualitative detection of the nucleocapsid protein antigen in human nasal swab samples. The sample is mixed with colored polymer-labeled SARS-CoV-2 monoclonal antibody 1 in the test device's sample well and chromatographed along the nitrocellulose membrane. If SARS-CoV-2 antigens are present in the sample, they will bind to SARS-CoV-2 antibody 1, and the mixture will bind to immobilized SARS-CoV-2 antibody 2 on the nitrocellulose membrane. The resulting complex of antibody 1, antigen and antibody 2 forms the colored test line. The test device's control line is coated with secondary antibodies, resulting in a colored result during a standard test procedure.

7 Study Design

7.1 Parameters of clinical performance to be determined

The study focused on demonstrating the performance of the AESKU.RAPID SARS-CoV-2 in detecting the sublineages BA.4/BA.5 of SARS-CoV-2 Omicron variant in NAT positive samples from early infection within the first 7 days after symptom onset, when compared to the original diagnostic/known status of the sample. The antigen test result was verified using the TaqPath™ COVID-19 CE-IVD RT-PCR reference results.

7.2 Materials Supplied by the Manufacturer

7.2.1 Test Kits and Instructions for Use

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

AESKU.RAPID SARS-CoV-2 used:

Lot number: P20210431b Expiry date: 2023-04-19

7.2.2 Instrument

Not applicable.

7.3 Materials Supplied by the Investigator

7.3.1 Standard laboratory reagents and disposables

These were supplied by the Investigator and met the specifications required to correctly carry out the test procedure.

7.3.2 Equipment/Instrumentation

cobas® SARS-CoV-2 Qualitative -Nucleic acid test for use on the cobas ® 5800 systems.

7.3.3 Samples

The swabs for the positive samples have been collected during the infectious phase of COVID-19 infected patients. The collection of the swabs was carried out in Germany with European subjects, usually the samples have been collected in the patients' home environment All swabs were collected from anterior nasal cavity. After collection all swabs (dry swabs) have been stored immediately at ≤-20°C. The confirmation of the sub-lineage of SARS-CoV-2-Omicron samples was done externally through single nucleotide polymorphism (SNP) screening with the mass-spectrometry. Omicron BA.4/BA.5 lineage was assigned by the combination of H69_V70del-deletion, T859N, V1176F, N856K, N501Y, L452QR, E484KQ mutations. BA.4 and BA.5 could not be differentiated.

7.4 Study population

According to the MDCG guidelines from the document: "Guidance on performance evaluation of SARS-CoV-2 in vitro diagnostic medical devices", the following sample numbers will be tested:

Diagnostic sensitivity (positive specimens):

- at least 30 NAT positive samples from early infection within the first 7 days after symptom onset with the confirmed SARS-CoV-2 Omicron subvariant BA.4/BA.5 variant; samples should represent naturally occurring viral loads
- general acceptance criteria: detection of >80% (rapid tests) relative to the SARS-CoV-2 real-time RT-PCR test.
- Exclusion criteria:
    - sample has a negative RT-PCR against SARS-CoV-2;
    - sample does not represent sublineages BA.4/BA.5;
    - sample was collected from the donor more than after 7 days since the symptom onset.

Required donor/patient information:

- Collection date of swab
- Date of testing
- Age, sex
- Days since the symptom onset
- Severity of symptoms (if known)

An analysis of the correlation between the antigen -positive/PCR-positive with the Ct-values of the real-time RT-PCR tests should be performed.

7.5 Test procedure

Throughout the evaluation, all the relevant swabs content was extracted in the AESKU.RAPID SARS-CoV-2 extraction buffer as described in the Instructions for Use (IFU) of the rapid test. The content of the swab was extracted in **~400** µL of the test's extraction buffer. **3** drops (approximately **~100** µL) of the treated sample were applied to the sample well of the test cassette. Results obtained with the rapid test device were visually read out by two operators 15 minutes after the sample had been applied to the test cassette. Digital images were taken from used rapid test cassettes after visual read-out.

For this study, nasal swabs have been used. A dry frozen swab was extracted in the extraction buffer of the antigen test, the antigen test was performed, and with the remaining volume after the extraction, the PCR test was carried out. This method ensures that the antigen-test and the PCR could be carried out of exactly the same sample.

The initial diagnosis of each patient was performed with a nasopharyngeal or oropharyngeal swab.

50 µL of the liquid remaining after the antigen testing was mixed with 550 µL of cobas® PCR medium and subjected to the reference real-time RT-PCR testing. The RNA-extraction and the PCR testing were done automatically and user-independently in the cobas® 5800 system using the cobas® SARS-CoV-2 qualitative RT-PCR detection assay (P/N 09448870190). This CE assay for *in vitro* diagnostics targets the *ORF1ab* genomic region unique to SARS-CoV-2 as well as a highly conserved region in the E-gene of pan-Sarbecoviridae (including SARS-CoV-2).

8 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.

8.1 Data and results recording

The sample information and reference results of the samples were recorded in the Study Record Forms (SRFs) in Excel.

SRF completion:

- Each item on the SRF was completed
- No blanks were left
- If an item was missing or not available, the entry was completed with "*n.a.*"

Upon completion of the SRF, the study coordinator reviewed the recorded data for completeness, accuracy and legibility.

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

The data obtained with the AESKU.RAPID SARS-CoV-2 were recorded on a sample sheet and as digital images taken within the prescribed time frame. The results were transferred to the SRF.

The results reported here refer exclusively to the evaluation performed for the AESKU.RAPID SARS-CoV-2.

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

All data were 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 AESKU.RAPID SARS-CoV-2 results are for performance evaluation only and must not be used for diagnostic purposes.

8.2 Data analysis

The following analyses have been performed:

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

The diagnostic sensitivity is reported together with a 2-sided Wilson 95% confidence interval.

9 Results

9.1 Definitions

- True positive sample: sample that was determined positive both using the SARS-CoV-2 AESKU.RAPID SARS-CoV-2 and by real-time RT-PCR.
- False negative sample: sample that was determined negative using AESKU.RAPID SARS-CoV-2 but positive by real-time RT-PCR.
- Sensitivity (%): # true positive samples/(# true positive samples + # false negative samples) x 100
- Wilson method for calculating 95% confidence intervals:

$$\frac{N}{N+z_{\alpha/2}^2}\left[\hat{p}+\frac{z_{\alpha/2}^2}{2N}-z_{\alpha/2}\sqrt{\frac{\hat{p}\hat{q}}{N}+\frac{z_{\alpha/2}^2}{4N^2}}\right]\le p\le\frac{N}{N+z_{\alpha/2}^2}\left[\hat{p}+\frac{z_{\alpha/2}^2}{2N}+z_{\alpha/2}\sqrt{\frac{\hat{p}\hat{q}}{N}+\frac{z_{\alpha/2}^2}{4N^2}}\right]$$

(α = 0.05 (5 %), 1- α = 0.95 (95 %); N – total number of samples; $z_{\alpha/2}$ = 1.96 (for α= 5%); p^ = true pos rate; q^ = false neg rate; p^+q^=1)

9.2 Diagnostic sensitivity

9.2.1 Swabs from the donors with known SARS-CoV-2 infection of BA.4/BA.5 variants.

37 nasal swabs from donors with known SARS-CoV-2 infection and confirmed BA.4 variant were tested with the AESKU.RAPID SARS-CoV-2 and compared with the real-time RT-PCR testing:

Ct	total	true pos	false neg	Sens., %	95% CL	95% HL
≤30	10	10	0	100	72.3	100
≤32	19	19	0	100	83.2	100
≤34	33	33	0	100	89.6	100
≤37	37	35	2	94.6	82.3	98.5

35 out of 37 RT-PCR-positive samples yielded positive antigen test result. This equals to the sensitivity of 94.6% with false positive occurring for the sample with high Ct value of over 34. The diagnostic sensitivity meets the common requirement of >80%.

Below is the breakdown of antigen test results depending on the days since the symptom onset:

days since symp onset	total	true pos	false neg	Sens., %	95% CL	95% HL
≤ 1	6	6	0	100	n.a.	n.a.
≤ 2	19	19	0	100	n.a.	n.a.
≤ 3	27	25	2	92.6	n.a.	n.a.
≤ 4	32	30	2	93.8	76.6	97.9
≤ 5	34	32	2	94.1	79.9	98.3
≤ 6	34	32	2	94.1	80.9	98.4
≤ 7	37	35	2	94.6	80.9	98.4

35 out 37 RT-PCR-positive samples within 7 days since the symptom onset yielded positive antigen test result. AESKU.RAPID can detect positive BA.4/BA.5 sample within 7 days since the symptom onset.

10 Conclusion

The aim of this study was to evaluate the diagnostic sensitivity of the AESKU.RAPID SARS-CoV-2 relation to the sublineages BA.4/BA.5 of SARS-CoV-2 Omicron (B.1.1.529) variant. For that, samples collected as anterior nasal swabs were analyzed. All samples were tested simultaneously with the AESKU.RAPID SARS-CoV-2 and the real-time RT PCR assay.

The overall diagnostic sensitivity of the AESKU.RAPID SARS-CoV-2 in relation to BA.4/BA.5 variant was **94.6**%.

In conclusion, the results from this study reveal a very high diagnostic sensitivity for AESKU.RAPID SARS-CoV-2 in relation to SARS-CoV-2 Omicron (BA.4/BA.5) variant, which meets the requirements of >80% (rapid tests) relative to the SARS-CoV-2 real-time RT-PCR test. AESKU.RAPID can detect all samples collected within 7 days since the symptom onset. It can be concluded with a high degree of certainty, that the AESKU.RAPID SARS-CoV-2 can reliably qualitatively detect the antigen of the SARS-CoV-2 Omicron (BA.4/BA.5) variant in human swab specimens.

11 Bibliography

- WHO Document "Instructions and requirements for Emergency Use Listing (EUL) submission: In vitro diagnostics detecting SARS-CoV-2 nucleic acid and rapid diagnostics tests detecting SARS-CoV-2 antigens".
- WHO Document "Methods for the detection and characterisation of SARS-CoV-2 variants"
- Medical Device Coordination Group Document MDCG 2021-21 "Guidance on performance evaluation of SARS-CoV-2 *in vitro* diagnostic medical devices"
- "COVID-19 rapid antigen self-tests. Performance requirements and risk mitigation strategies" for SARS-CoV-2 Antigen Self-testing devices from Australian TGA
- 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-Institute (PEI)
- Food and Drug Administration FDA Document with the "Recommendations for the developers of the antigen test"
- CLSI EP17-A2 Document "Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures"
- EU Regulation 2017/746 on *in vitro* Diagnostic Medical Devices
- ISO 20916 *in vitro* Diagnostic Medical Devices – Clinical Performance Studies using specimens from human subjects – Good Study Practices
- EU Guidance on the management of clinical trials during the COVID-19 pandemic.
- European Commission working document of Commission services "Current performance of COVID-19 test methods and devices and proposed performance criteria"

12 Annexes

Annex I SRF AESKU C705_BA.4-BA.5

Annex II Set of images with antigen tests of positive samples

Annex III RT-PCR Data