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Evaluation of the AESKU SARS-CoV-2 Antigen Rapid Test

Purpose of the Study

The objective of this performance study is to establish the diagnostic sensitivity and diagnostic specificity of the AESKU.RAPID SARS-CoV-2 Antigen Rapid Test Kit (REF: 840001) and to provide data to demonstrate the product is effective for its intended use.

Product Information

Manufacturer	AESKU.DIAGNOSTICS GmbH & Co. KG Mikroforum Ring 2 55234 Wendelsheim Germany Tel.: +49 6734 9622 0, info@aesku.com, www.aesku.com
Test Name	AESKU.RAPID SARS-CoV-2 Antigen Rapid Test Kit (REF: 840001)
Detection Method	Immunochromatographic Test using a colored polymer-labeled novel coronavirus monoclonal antibody
Intended Use	Qualitative Detection of the N protein antigen from SARS-CoV-2 in human nasal swab specimen
Specimen	human nasal swab
Content of Testkit	AESKU.RAPID SARS-CoV-2 antigen test cassette Specimen processing tube Specimen sampling swab
Storage Condition	4-30°C
Lot number	P202010005 (exp date 09.04.2020), P202011003 (exp date 09.05.2020)

Study Management

Sample Collection

Biomex GmbH, Siemensstr. 38, 69123 Heidelberg, Germany

Investigation

Hannes Deisel, Biomex GmbH, Siemensstr. 38, 69123 Heidelberg, Germany
Sabine Szczesny, Biomex GmbH, Siemensstr. 38, 69123 Heidelberg, Germany
Dr. Harald Depner, ESfEQA GmbH, Siemensstr. 38, 69123 Heidelberg, Germany

Study Coordinator and Author

Dr. Dieter Groche, ESfEQA GmbH, Siemensstr. 38. 69123 Heidelberg, Germany
Tel.: 06221 89466971, groche@esfeqa.eu

Testing Site

Biomex GmbH, Siemensstr. 38, 69123 Heidelberg, Germany

Timelines

The Study was performed in November and December 2020

Study Design

Samples

- 148 nasal swabs and 19 throat swabs from donors with known SARS-CoV-2 infection. 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. Samples were collected within 7 days after onset of symptoms.
- 164 nasal swabs and 50 throat swabs from healthy donors: Sex, age and date of sample collection were known (see annex "Comparison study SARS COV-2_AESKU Rapid Test and PCR Test").

The nasal and throat swabs were collected between October 26th and December 12th and were stored at or below -20°C before they were analyzed.

Analytical investigation

The study was performed on three separate days. Each sample was analyzed with the AESKU.RAPID SARS-CoV-2 Antigen Rapid Test kit and immediately afterwards with the R-Biopharm (Darmstadt, Germany) real-time RT-PCR kit (see below). Identical sample preparations were used for both analytical methods.

Sample swabs were extracted in the AESKU.RAPID SARS-CoV-2 specimen processing tube as described in the IFU of the rapid test. Three drops of the specimen (approximately 145 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 300 µL of the remaining liquid using the R-Biopharm RIDA Xtract (REF:PGZ001) lot QL2000033 and lot QL200009, expiry date April 2022, and analyzed with the R-Biopharm RIDA Gene SARS-CoV-2 RUO real-time PCR kit (REF:PG6815 RUO) lot 24110N, expiry date March 2022, lot 26160N, expiry date April 2022, and RIDA Gene SARS-CoV-2 real time PCR kit (REF:PG6815) lot 24450N, expiry date November 2022. The instructions of the real-time RT-PCR kit manufacturer were followed with the exception that 300 µl instead of 400 µl of the solution was used for the extraction due to the limited volume in the specimen processing tube.

Real-time RT-PCR analysis was performed in duplicate 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 above 36 (mean of the two replicates) were excluded from any statistical evaluation in this study.

Results

In total 381 samples were tested in parallel with the AESKU.RAPID SARS-CoV-2 Antigen Rapid Test and the R-Biopharm real-time RT-PCR assay and included in this study.

Definitions

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

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

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

False negative sample: sample that was determined negative using the AESKU.RAPID SARS-CoV-2 Antigen 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

Analytical Results for all samples with PCR result either negative or positive with a Ct value of less than 32:

		RT-PCR	
		positive	negative
AESKU.RAPID SARS-CoV-2 Antigen test	positive	105	4
	negative	4	218

Specificity of AESKU.RAPID SARS-CoV-2 Antigen Rapid Test Kit: 98% (218/222), CI: 95-99%
Sensitivity of AESKU.RAPID SARS-CoV-2 Antigen Rapid Test Kit (Ct < 32): 96% (105/109), CI: 91-99%

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

Ct value	Number of Samples	Number of true positive Rapid Test Samples	Number of false negative Rapid Test Samples	Sensitivity of AESKU.RAPID SARS-CoV-2 Antigen test (CI)
< 30	77	77	0	100 % (95-100)
< 32	105	101	4	96 % (91-99)
< 34	136	123	13	90 % (84-94)
< 36	157	133	24	85 % (78-90)

The correlation between the Ct-values of the analyzed samples and the sensitivity reveals a sensitivity of 100% for samples with a Ct-value of up to 30. 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 AESKU.RAPID SARS-CoV-2 Antigen Rapid Test. This is in line with expectations regarding viral detection by antigen rapid testing compared to PCR analysis.

Conclusion

The specificity and sensitivity of the AESKU.RAPID SARS-CoV-2 Antigen Rapid Test Kit was evaluated in this study with 381 samples collected as nasal or throat swabs. All samples were tested in parallel with the AESKU.RAPID SARS-CoV-2 Antigen Rapid Test Kit and a real-time RT-PCR assay. Samples with a Ct value below 32 were selected for the calculation of the sensitivity of the AESKU.RAPID SARS-CoV-2 Antigen Rapid Test Kit.

The specificity of the AESKU.RAPID SARS-CoV-2 Antigen Rapid Test Kit calculated from results of all samples was 98%, the sensitivity calculated from results of samples with a Ct-value less than 32 (105 samples) was 96% (95% CI: 91-99%). 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 (157 samples) the sensitivity is calculated as 85% (95% CI: 78-90%).

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

Approval

This study complements version 002 of the 'Evaluation of the AESKU SARS-CoV-2 Antigen Rapid Test' by including a larger number of samples.

Version 003 of the Evaluation Study Aesku Diagnostics SARS-CoV-2 Antigen Test was created and approved in December 2020 by the following persons: