FINAL STUDY REPORT

Clinical evaluation of the Biozek COVID-19 Antigen Rapid Test Cassette in nasopharyngeal swabs from symptomatic patients

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1. Introduction

In this study, the test characteristics of the Biozek COVID-19 Antigen Rapid Test Cassette was evaluated in clinical nasopharyngeal swab samples from 40 patients with PCR proven COVID-19 and 42 PCR negative patients with respiratory symptoms.

2. Adherence to Regulatory Standards

All portions of this study performed at the ISO certified testing facility adhered to the applicable standard operating procedures (SOPs) with any applicable protocol amendments specified in the study report, but was not conducted in compliance with international good laboratory practice (GLP) regulations.

3. Responsible personnel

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4. Material and Methods

SARS-CoV-2 Antigen Test

The presence of SARS-CoV-2 antigen was determined by performing the Biozek COVID-19 Antigen Rapid Test Cassette (Biozek) on 30 PCR positive and 42 PCR negative nasopharyngeal swabs collected in symptomatic patients on the same day and transported in M4RT transport medium (Remel). Although using a transport medium is not recommended by the manufacturer and directoly placing the swab in lysis buffer is preferred, further procedures where conducted following the manufacturer's instructions. From 10 PCR positive patients, a new sample was collected and the antigen test was performed by directly placing the swab in the lysis buffer of the test kit instead of transporting the swab in transport medium first.

SARS-CoV-2 PCR

The presence of SARS-CoV-2 RNA was determined by performing an in house PCR protocol according to the protocol as provided by the WHO, detecting the E-gene of the virus in the nasopharyngeal swab samples (Corman *et al.,* 2020).

Patient samples

All nasopharyngeal swab samples used in this study were routinely collected during treatment by their physician or screening by the public health offices as part of the diagnostic COVID-19 work up. For each sample, the PCR and antigen test were performed within 24 hours of collection.

Patient groups

PCR positive patients were 40 patients having at least one positive PCR for SARS-CoV-2. PCR negative patients were 42 (mildly) symptomatic patients tested by the Regional Public Health Offices as part of the screening policy of symptomatic persons having a negative SARS-CoV-2 PCR.

5. Results and Discussion

Sensitivity and specificity of the Biozek COVID-19 Antigen Rapid Test Cassette

In table 1, the test results of the 30 PCR positive and 42 PCR negative samples tested with the swab transported in M4RT medium is shown.

Table 1. Results of the Biozek COVID-19 Antigen Rapid Test on swabs transported in M4RT medium.

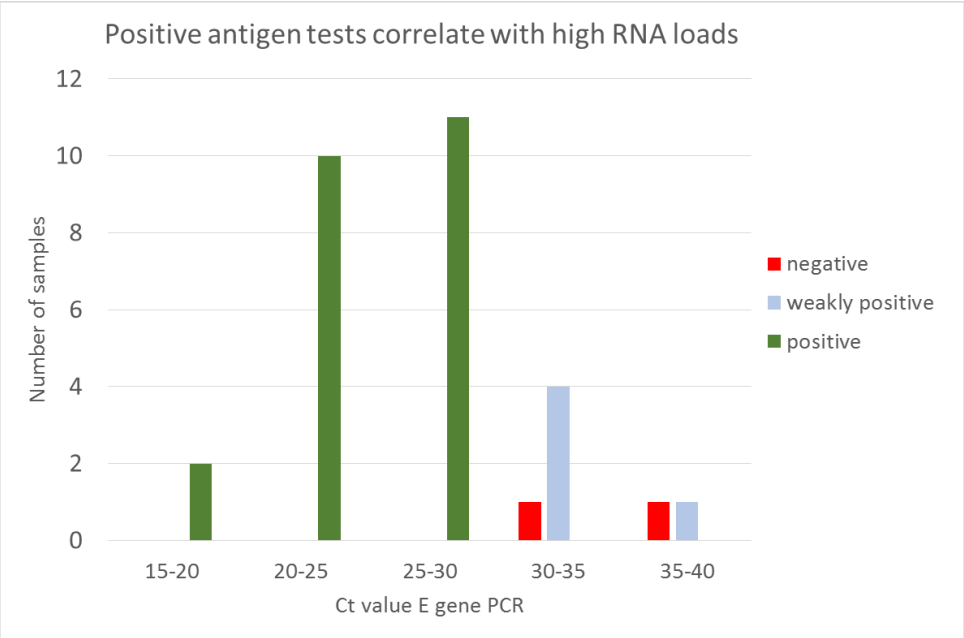
		Biozek COVID-19 Antigen rapid test		
		Positive	**Negative**	**Total**
PCR	**Positive**	25	5*	**30**
	Negative	0	42	**42**
	Total	**25**	**47**	**72**

* 3 of 5 samples tested weakly positive when testing the M4RT medium itself.

Of the 42 PCR negative samples, all tested negative in the antigen test, giving a specificity of 100% in these samples.

Of the PCR positive samples, 25/30 samples tested positive, giving a sensitivity of 83.3% in these samples. Of the 5 PCR positive, antigen test negative samples, 3 tested weakly positive when the test was repeated with the M4RT medium itself (75 microliter M4RT medium placed in the test cassette). Combining these results, 28/30 samples were positive in one of the two tests, giving a combined sensitivity of 93.3%.

The positivity of the antigen test correlated with the Ct value of the PCR test, showing that positive samples with a high RNA load (Ct <30) all were positive, showing a 100% sensitivity in samples with a high RNA load (graph).



The weakly positive results with the M4RT medium in the negative swabs suggests that the flocked Remel swab used, had transferred the antigen to the transport medium, leading to a lower antigen amount placed in the lysis buffer by the swab than using a swab that was directly placed in the lysis buffer. This could explain the false negative test results due to a diminished sensitivity when using a flocked swab with transport medium. Therefore, 10 PCR positive patients were swabbed a second time, and the antigen test was performed on swabs directly placed in the lysis buffer. Simultaneously, a second Remel swab was collected to perform PCR.

Table 2 shows the results when the swab was placed directly in the lysis buffer. Of the 10 patients that were PCR positive a few days before, 3 now had a negative PCR result. Of the 3 PCR negative patients, one still was positive in the antigen test.
All PCR positive patients were antigen positive, irrespective of the Ct value, ranging from Ct 28-38, showing a sensitivity of 100%.

Table 2. Results of the Biozek COVID-19 Antigen Rapid Test on swabs directly placed in lysis buffer.

		Biozek COVID-19 Antigen rapid test		
		Positive	**Negative**	**Total**
PCR	**Positive**	7	0	**7**
	Negative	1*	2	**3**
	Total	**8**	**2**	**10**

* 1 sample of a previously PC Rpositive patient that was negative in samples tested weakly positive when testing the M4RT medium itself.

Conclusion

In conclusion, the Biozek COVID-19 Antigen Rapid Test Cassette was able to detect infections accurately when using the swab directly in the lysis buffer, as recommended by the manufacturer. When flocked swabs in transport medium like the Remel M4RT medium are used, a loss of sensitivity is observed, probably due to dissolving and dilution of the antigen to the transport medium.
The specificity in all conditions tested was 100%.

Appendix 1 – Results of individual swabs samples in transport medium from SARS-CoV-2 PCR positive patients tested with Biozek COVID-19 Antigen Rapid Test Cassette

Sample	Biozek antigen test	Reapeted Biozek antigen test with M4RT medium	Ct E-gen (PCR)
937C9001918	neg	neg	32.7
937C0036507	pos		25
937C0159019	neg	weak	35.1
937C0036629	pos		28.2
937C7003678	pos		21
937C7006355	pos		24.1
937C9002870	pos		26.2
937C9002869	neg	weak	34
937C7006301	pos		28.8
937C0159244	weak		30.2
937C7006350	pos		22.6
937C7004141	pos		20.3
937C0158951	pos		27.7
937C9005855	pos		24.8
937C9005232	neg	neg	38.1
937C9005272	pos		24.5
937C8005418	pos		21.5
937C0036463	pos		27.2
937C7006059	pos		29
937C7003941	weak		30.7
937C7004010	pos		24.9
937C0156816	pos		25.9
937C7006333	pos		23.6
937C9005897	pos		19.3
937C0158979	pos		26.3
937C0157041	pos		25.6
937C0157039	neg	weak	32.7
937C9005255	pos		19.1
937C9005985	pos		27.5
937C9002875	pos		22.8

Appendix 2 – Results of individual swabs samples in transport medium from SARS-CoV-2 PCR negative patients tested with Biozek COVID-19 Antigen Rapid Test Cassette

Sample	Biozek antigen test	Reapeted Biozek antigen test with M4RT medium	Ct E-gen (PCR)
937C0039215	neg		neg
937C0048071	neg		neg
937C0162134	neg		neg
937C0041240	neg		neg
937C9018030	neg		neg
937C0041202	neg		neg
937C9003891	neg		neg
937C0041215	neg		neg
937C0041248	neg		neg
937C0048018	neg		neg
937C9011022	neg		neg
937C9003940	neg		neg
937C0041194	neg		neg
937C9018107	neg		neg
937C0039192	neg		neg
937C9003894	neg		neg
937C0039185	neg		neg
937C9004005	neg		neg
937C0039196	neg		neg
937C9011029	neg		neg
937C9011786	neg		neg
937C0039205	neg		neg
937C9018103	neg		neg
937C0041213	neg		neg
937C9018095	neg		neg
937C0039456	neg		neg
937C9018040	neg		neg
937C9003994	neg		neg
937C9003999	neg		neg
937C0156982	neg		neg
937C9003139	neg		neg
937C9003902	neg		neg
937C0154261	neg		neg
937C0048036	neg		neg
937C9003933	neg		neg
937C9004002	neg		neg
937C9018058	neg		neg
937C9003995	neg		neg
937C9003985	neg		neg
937C0048031	neg		neg
937C0154607	neg		neg
937C9018568	neg		neg

Appendix 3 – Results of individual swabs samples placed directly in lysis buffer from proven COVID-19 patients tested with Biozek COVID-19 Antigen Rapid Test Cassette

Sample	Biozek antigen test	Ct E-gen (PCR)
1	pos	35
2	pos	36
3	weak	38
4	pos	28
5	neg	neg
6	neg	neg
7	pos	31.9
8	pos	neg
9	pos	28.9
10	weak	34.9