Limits and opportunities of SARS-CoV-2 antigen rapid tests – an experience based perspective

Jessica Lüsebrink¹,a, Oliver Schildgen¹, and Verena Schildgen¹, a,#

1. All Authors’ affiliation: Kliniken der Stadt Köln gGmbH, Institut für Pathologie, Klinikum der Privaten Universität Witten/Herdecke, Ostmerheimer Str. 200, 51109 Köln

a) authors contributed equally to this manuscript

# Send correspondence to:
Priv.-Doz. in. Dr. rer. nat. Verena Schildgen
Kliniken der Stadt Köln gGmbH
Klinikum der Privaten Universität Witten/Herdecke
Institut für Pathologie
Ostmerheimer Str. 200
D-51109 Köln (Cologne)
Germany
Tel.: +49(0)221-890718887
Fax.: +40(0)221-89073542
E-Mail: schildgenv@kliniken-koeln.de

verena.schildgen@uni-wh.de

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Abstract

Due to the currently increasing case numbers of SARS-CoV-2 infections worldwide there is an increasing need for rapid diagnostic devices in addition to existing PCR-capacities. Therefore, rapid antigen assays including lateral flow assays are discussed as an alternative method. In comparison to an established RT-PCR protocol, however the novel lateral flow assay unfortunately lowered the expectations set in these assays.

Brief Communication

In December 2019 the public became aware of the new betacoronavirus SARS-CoV-2 due to an outbreak in Wuhan, China (1). Very soon it turned out that spreading of the virus cannot be prevented and Covid-19 was declared a pandemic in March 2020 (https://www.euro.who.int/en/health-topics/health-emergencies/coronavirus-covid-19/news/news/2020/3/who-announces-covid-19-outbreak-a-pandemic). In order to minimize the risk of infection different undirected as well as targeted tracking strategies were developed, whose success is dependent on extensive testing of the largest possible number of people (2). For SARS-CoV-2 detection different PCRs are used in routine diagnostic. Although these PCRs are actually the “gold” standard (3), valuable time passes until the result is available (https://www.nytimes.com/2020/08/04/us/virus-testing-delays.html). Since it was shown that rapid antibody screenings are not suitable to evaluate chains of infection or their interruption, other kinds of rapid on-site tests are needed to perform the requested mass testing.

One of these is the BIOCREDIT COVID-19 Ag test of RapiGENJNC (Korea). This lateral flow test is based on immunochromatography and shows SARS-CoV-2 antigen presence by staining with colloid gold conjugate as a black test line. According to the manufacturer’s instruction it should support early diagnosis of Covid-19 infections in patients with clinical symptoms. Sampling should be performed in the nasopharynx with the supplied swab, but UTM/VTM media is also appropriate if diluted 1:2 with assay buffer. Moreover it has been shown that specimen other than nasopharyngeal swaps have comparable diagnostic sensitivity (4)(5). For this reason we tested a pilot sample panel of 11 BALFs (bronchoalveolar lavage fluid) and 70 throat washing samples with confirmed PCR results (RealStar® SARS-CoV-2 RT-PCR Kit, Altona, Germany), as well as 9 throat washes invalid by PCR. First, we evaluated if the BIOCREDIT assay is able to detect SARS-CoV-2 Ag in these specimens. For this reason we applied 150 µl of either diluted (d) or original (o) specimen on the device and checked for the presence of the red control line (C) and the black test line (T). This pilot approach showed that the specimens used allow a proper test
performance and that SARS-CoV-2 antigen can principally be detected in throat washes (I and II) and BALF (III) (figure 1).

The analysis of the complete test cohort reveals an overall sensitivity of 29.4% and an overall specificity of 86.7% (figure 2A). This means a PPV of 79.0% and a NPV of 41.9%. Next, we analyzed test sensitivity and specificity in 55 samples of confirmed symptomatic (58.2%) and asymptomatic (41.8%) individuals with regard to the respective PCR result, which leads to sensitivity values of 22.2% (asymptomatic) and 30% (symptomatic) with specificities of 92.9% (asymptomatic) and 76.9% (symptomatic) (figure 2B & C). Regarding the BALF specimens the test shows 54.6% sensitivity, but 100% specificity with a PPV of 100% and a NPV of 28.6% (figure 2B & C). These results damp the expectation that PCR invalid samples could reliably be analyzed with the rapid antigen test, although three of nine PCR invalid samples are SARS-CoV-2 antigen positive, whereas 6 did not show antigen presence.

When checking any correlation of viral RNA load and presence of SARS-CoV-2 antigen we were able to monitor two asymptomatic individuals, who were PCR positive for more than five weeks before recovering in week six. Ct values ranged from 23.8 to 35.2 in patient A (blue data series) and from 27.9 to 34.3 in patient B (green data series), but SARS-CoV-2 antigen was only detected in three samples with Ct values >30 (figure 3). The fact that SARS-CoV-2 antigen is detected in samples of individuals ranging from asymptomatic + PCR negative and symptomatic + PCR negative, to asymptomatic + PCR positive and symptomatic + PCR positive (figure 2), suggest that this test is not suitable for routine diagnostics as long as the complex relationships between viral RNA load, SARS-CoV-2 antigen detection, and clinical symptoms remain unsolved.

Regarding the correlation of PCR and the BIOCREDIT SARS-CoV-2 antigen test, the significance of the BIOCREDIT test cannot be evaluated only based on RNA presence, because both the intra-individual and the inter-individual course of viral replication kinetics with prolonged viral RNA shedding and the unknown quality and quantity of accompanying antigen, as also observed in the present study, make it impossible to estimate the diagnostic utility of rapid antigen tests. Moreover, the limited information on host and viral factors influencing shedding of SARS-CoV-2 antigens and their correlation to infectious viruses impede any prognosis on infectivity. So far it also remains unclear if further common phenomena such as defective interfering particles, antigen drift or antigen shift occur during the current pandemic influencing the assay performance of any SARS-CoV-2 test.

Although it seems that exclusive antigen tests actually cannot replace PCR assays, they could additionally be used to gain deeper insights into infectivity and the course of infection to develop more advanced testing strategies.
Figure 1: SARS-CoV-2 antigen detection. To evaluate the suitability of specimen others than nasopharyngeal swaps 150µl of two PCR positive throat washes (I: CtE-Gen=15,6, CtS-Gen=14,8; II: CT E-Gen=14,7, CtS-Gen=14,9) and one BALF (III: CtE-gen=13,1, CtS-Gen=12,6) were used. The BIOCREDIT Covid-19 Ag test detected SARS-CoV-2 antigen in all samples, but dilution in assay buffer (d) decreases sensitivity compared to original fluid (o). As shown by the control line the specimens allowed a proper test performance and did not contain any inhibitory substances. d=1:2 diluted, o= original.
Figure 2: Specificity and sensitivity of SARS-CoV-2 antigen detection in PCR tested specimen. (A) Analysis of the complete test cohort (n=90), consisting of PCR positive (n=51), PCR negative (n=30) and PCR invalid samples (n=9), reveals an overall sensitivity of
29.4% and an overall specificity of 86.7%. 55 samples confirmed symptomatic or asymptomatic have been divided into (B) PCR positive (n=28) and (C) PCR negative (n=27) subgroups resulting in sensitivity values of 22.2% (asymptomatic) and 30% (symptomatic) and specificities of 92.9% (asymptomatic) and 76.9% (symptomatic). BALF= bronchoalveolar lavage fluid, TW= throat wash, BC=BIOCREDIT Covid-19 Ag test

**Figure 3: Correlation of SARS-CoV-2 antigen detection with RNA load.** This figure shows longitudinal SARS-CoV-2 detection by PCR in two asymptomatic individuals. In both cases SARS-CoV-2 RNA can be detected for about five weeks before recovering in week six. Antigen is only detected in one individual in three samples with Cts >30 (indicated by arrows).

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