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## Impact of animal saliva on the performance of rapid antigen tests for detection of SARS-CoV-2 (wildtype and variants B.1.1.7 and B.1.351)
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**Impact of animal saliva on the performance of rapid antigen tests for detection of SARS-CoV-2 (wildtype and variants B.1.1.7 and B.1.351)**

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**Abstract**

SARS-CoV-2 infects several animal species and SARS-CoV-2 variants of concern (VOCs) may even show (as in humans) enhanced inter- and intra-species transmission rates. We correlated sensitivity data of SARS-CoV-2 rapid antigen tests (RATs) to viral RNA genome equivalents assessed by real-time reverse transcriptase-polymerase chain reaction (RT-PCR). Further, we checked their suitability for testing animals by assessing saliva and VOC effects. Viral loads up to 2 logs (RNA copy number) under the hypothetical SARS-CoV-2 infectivity threshold were detected by most analyzed RATs. However, while saliva from various animal species showed generally no adverse effects on the RATs' analytical sensitivities, the detection of VOCs B.1.1.7 and B.1.351 was in some RATs inferior to non-VOC viruses.

**Keywords:** SARS-CoV-2; Real-time RT-PCR; Rapid Antigen Assays, Saliva**,** Variants of Concern (VOCs).

**Introduction**

Seven different coronaviruses are currently known to infect humans and all of them originate from animals (Su et al., 2016). In late 2019 a betacoronavirus of unknown origin, designated SARS-CoV-2 was identified and caused a worldwide pandemic. Apart from humans, SARS-CoV-2 can infect farm animals, hamsters, minks, ferrets, raccoons, cats and dogs (Abdel-Moneim and Abdelwhab, 2020). Infections of lions, tigers, pumas, snow leopards, cynomolgus macaques, rhesus macaques, treeshrew, gorilla and others were also frequently reported (OIE, 2021). Clinical signs in animals are usually mild, but infections can also be fatal (de Morais et al., 2020; Ferasin et al., 2021). Several SARS-CoV-2 variants of concern (VOCs) are circulating worldwide and may even be more transmissible to and pathogenic for domestic animals than the original strain (Ferasin et al., 2021). There is also a possibility that such infected animals can more easily spill the virus back to humans. To date, rapid antigen tests (RATs) receive much attention as they provide on-site results without the need for elaborate instrumentation and/or expertise (Igloi et al., 2020). RATs are therefore part of most national testing strategies for humans worldwide. Hence, the question arose whether such assays would also be suitable as point-of-care diagnostics for SARS-CoV-2 in animals and, if so, whether the currently circulating VOCs are detected by them just as well. A broad analytical sensitivity study of 122 RATs for use in humans has shown recently that the majority of the assays are detecting SARS-COV-2 viruses equivalent to about $10^5$ genome copies (Scheiblauer et al., 2021). Another study on 5 commercial assays proved their suitability for detecting VOCs (B.1.1.7 and B.1.351) in principle but also revealed differences in analytical sensitivities for the variants (Jungnick et al., 2021). In the aforementioned test, VOCs were better detected than the original SARS-CoV-2 strains. VOCs are primarily defined by differences in spike protein, even though mutations in other viral proteins also exist. Therefore, it is not surprising that variable recognition by RATs, most of which use the nucleoprotein as a target, is observed between VOCs and common SARS-

2

CoV-2 strains. In the described study here, we used saliva samples spiked with cell culture grown virus to show that RATs are also suitable tool for detecting animals shedding SARS-CoV-2. However, as it turned out limits of detection for VOC can also be substantially lower, calling for detailed assay validations prior to their use.

**Methods**

**Viruses and cells**

Vero E6 cells (ATCC CCL-81) were grown and maintained in Eagle's minimal essential medium (EMEM; Lonza) with 8% foetal bovine serum (PAA), and antibiotics (Sigma) at 37°C and 5% CO2 as previously described (de Wilde et al., 2013). Infection of Vero E6 cells with SARS-CoV-2 (strain 2019_nCoV Muc-IMB-1, GISAID accession number EPI_ISL_406862) and VOCs was performed as previously described (Case et al., 2020). Virus stocks (passage 3) were stored at -80°C and viral titres depicted as $TCID_{50}$/ml were calculated before use. All work with live SARS-CoV-2 and VOCs was performed in a biosafety-level 3 (BSL-3) laboratory at Friedrich-Loeffler-Institut, Germany. To perform downstream analysis under BSL-2 conditions, viruses were heat-inactivated at 95°C for 20 minutes.

**SARS-CoV-2 rapid antigen assays**

Rapid antigen assays were randomly selected from commercially available products. All assays are registered for testing human naso/oropharyngeal swab samples and to detect SARS-CoV-2 N-protein using dual anti-N-antibodies; a dye-labeled antibody in the sample pad and immobilized antibody fixed on a membrane. Upon adding a positive sample to the pad, the N-protein is bound by the labeled antibody and the complex migrates by capillary forces and is finally captured by the immobilized antibody. The test band gets visible by an accumulation of the dye and the control band gets visible by capturing a labeled unspecific antibody. Here, we aimed to evaluate limits of detection of SARS-CoV-2 rapid antigen assays by using serially

3

diluted heat-inactivated wild-type SARS-CoV-2 (Non-VOC; Wuhan-Hu-1 strain; $TCID_{50}$ $10^{3.8}$/mL) and VOCs B1.1.7 ($TCID_{50}$ $10^{6.75}$/mL) and B.1.351 ($TCID_{50}$ $10^{6.8}$/mL) in cell culture medium composed of carbonate buffered MEM with Hanks and Earls salts (50:50), pyruvate, non-essential amino acids and without antibiotics. Briefly, 25 µL from each viral dilution were applied to the manufacturer swab and processed as recommended and finally eluted in 300 µL from the supplied lysis buffer. After 120 µL from each swab suspension were applied to the lateral flow device and 140 µL were subjected to RNA isolation and subsequent RT-qPCR (Figure S1 A-B). We used these constant volumes in all assays for the comparability of experiments. Results were recorded after 15 minutes and a 4-grade scaling readout (+++/strong positive; ++/positive; +/weak positive; -/negative) was established to reflect the intensity of the target band, which was furtherly quantified using Image Lab (Image Lab, 6.0.1; BioRad). When results were dubious, the four or six-eye principle was used. Three independent and blinded experiments were performed. To analyze the sensitivity and specificity of an exemplary rapid antigen assay (BioNote), an additional double-fold dilution was performed to the borderline dilution (e.g.$10^{-3}$) to precisely reflect the borderline sensitivity and corresponding Ct values (Figure S1 C-D). Data were further analyzed by receiver operating analysis (ROC) using Sigmaplot (Systaat Software Inc., Chicago, USA).

**Detection of SARS-CoV-2 RNA by real-time RT-PCR**

Viral RNA from each swab suspension was extracted using QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) and subjected to real-time RT-PCR (SuperScript III Reverse Transcriptase, Invitrogen, Germany). An *envelope* (E) gene amplification was performed using CFX real-time PCR systems (Bio-Rad, Germany) and a set of primers and probes as previously described (Corman et al., 2020). Viral genome copy numbers were derived from the standard curves of an *in vitro* RNA transcript harboring the E gene.

**Spiking saliva with SARS-CoV-2**

To evaluate the use of animal saliva in rapid antigen assays, we collected saliva from different species and spiked it with defined amounts of virus diluted in a cell culture medium. While human saliva was self-collected, ferrets and bats saliva were obtained by swabbing animals' oral fluid using manufacturer's swabs. The saliva-soaked swabs obtained from ferrets were directly spiked with the diluted virus, whereas bat' saliva was eluted from the swabs in PBS and solution was spiked with the virus as described. Saliva from cat and farm animals as sheep, goats, and cattle were obtained by passive drool collection. For collecting substances of swine saliva, a chewing-rope was used as previously described (M Gutierrez et al., 2014) and the wet part of the rope was eluted before applying it in antigen assay and RT-qPCR. Of note, collected saliva was processed by centrifugation at 14,000 rpm/30 min and filtration by 0.2 μm filters to remove any debris. However, unprocessed saliva samples were also tested to exclude the inhibitory effect of saliva components such as mucin on RATs performance. Next, SARS-CoV-2 and VOCs were spiked, serially diluted into saliva, and 25 µL were applied on the manufacturer´s swab. Further, swabs were eluted in 300 µL buffer and 120 µL were applied on lateral devices. The percent of the target band intensity and corresponding viral gene copy numbers were reexamined as above described (Corman et al., 2020; Kennedy and Oswald, 2011). Importantly, an oropharyngeal swab from SARS-CoV-2-infected hamster was also used as an additional positive control, representing real-world clinical samples.

**Statistical analysis**

GraphPad Prism (version 9; GraphPad Software, Inc., CA, USA) was used to analyse data. Normal distribution of data and analysis by Bonferroni's multiple comparisons tests was performed. Statistical analysis was performed using SPSS software (IBM Corp. Released 2011.

IBM SPSS Statistics for Windows, Version 20.0, IBM Corporation, Armonk, NY, USA). Significance was considered upon P values ≤0.05.

**Ethics statement**

Collected human saliva was collected after required consent and without using any person-related data.

**Results**

**Effective detection of high and moderate wild-type SARS-CoV-2 loads by rapid antigen assays**

Six assays showed detection limits down to $10^3 – 10^4$ viral RNA genome copies pro mL (Figure 1A), while for one assay virus dilutions equivalent to at least $10^5$ viral copies and another $10^6$ genome copies were needed to obtain positive results (Figure 1A). Therefore, the least sensitive RAT was excluded from the study (Figure 1A). Based on these results, all rapid antigen assays were ranked according to their sensitivity and detection limits as shown in Table 1. To determine the cut-off value and the overall sensitivity of RATs, one exemplary assay (BioNote) was evaluated. For this experiment, virus dilutions corresponding to high, low and near-to-detection-limit viral loads were used. All samples rated positive by RAT were also positive by RT-qPCR (16/16) and only four samples were considered as false negative (4/18) (Figure 1B). Therefore, a cut-off value of Ct 32,25 was determined as a limit of detection (LoD) of rapid antigen assay (Figure 1B). This achieved an overall sensitivity of 93,75% with a ROC curve area of 98,61% when compared to RT-qPCR (Figure 1C).

**Saliva from animal species has no adverse effect on the sensitivity of rapid immunoassays**

To test whether saliva could have an impact on the analytical sensitivity of rapid antigen assays, we collected plain saliva from human and animal species and spiked them with SARS-CoV-2.

6

Saliva was collected either by swabbing oral fluids (as in bats and ferrets), passive drool collection (as in human, cat, sheep, goats, and cattle), or by chewing-ropes in swine as previously described (M Gutierrez et al., 2014). SARS-CoV-2 non-VOC and VOC were serially diluted into saliva and applied on lateral devices. Interestingly, both target and control bands were detected on lateral devices using spiked animal saliva with no significant differences when compared to previous results without using saliva (control) (Figures 2A). This clearly demonstrates that saliva has no adverse effects on the sensitivity of antigen assays. Further, we examined the corresponding viral genome copies by real-time RT-PCR and found no significant impact on viral copy numbers upon using saliva as the detection limits were also about $10^3$ – $10^4$ viral copies (Figure 2B). Together, these results underline that saliva from the animal species studied did not adversely affect the sensitivity of rapid antigen assays and/or real-time RT-PCR.

**Lower performance of rapid antigen tests for SARS-CoV-2 variants of concern B.1.1.7 and B.1.351**

To examine the potential effect of SARS-CoV-2 variants of concern on the sensitivity of antigen assays, VOCs B.1.1.7 and B.1.351 were also included in this study. Surprisingly, the tested antigen assays showed a significant reduction by nearly 1 log in LoD when VOCs were used (Figure 3A-D). Further, we compared the corresponding SARS-CoV-2 Ct-values and gene copy numbers and found a significant reduction by 1-2 logs (p<0.0001, Figure 3E; p<0.001, Figure 3F). Next, we aligned N-protein from wild-type SARS-CoV-2 and B.1.1.7 and B.1.351 variants and found multiple amino acids substitutions in N-protein (Figure 3G). Whether these substitutions significantly hampered efficient immune-detection of such variants by RATs remained to be determined and worth future studies.

**Discussion**

Here most antigen assays were able to find positive results up to the level of Ct-value 32,25 and $10^3$-$10^4$ viral RNA genome copies respectively. However, there were also two assays with lower sensitivities, rendering them less suitable for field applications (Figure 1A-B and Table 1). Our data demonstrate that detection limits of rapid tests were up to 2 logs under the hypothetical infectivity threshold (Wölfel et al., 2020), underlining their safety margin for detecting SARS-CoV-2 infectious individuals and/or animals (Figure 1A). Initial reports on SARS-CoV-2 natural infections in animals as cats, dogs, and lions showed relatively high Ct values >30/35 for positive animals showing clinical signs, suggesting missed infections upon using RATs (Abdel-Moneim and Abdelwhab, 2020; de Morais et al., 2020; Segalés et al., 2020). Nonetheless, as the virus continues to evolve and adapt in hosts, natural infections with lower Ct values <25 were recently reported, for instance in lions, where a RAT was successfully used to detect the infection (Fernández-Bellon et al., 2021).

Hundreds of rapid tests from different vendors are now available on the market (FIND, 2020) and it is not surprising that some assays perform better than others. This, however, strongly underlines the need for a regular and rigorous evaluation of rapid antigen tests before their market approval. A leading-edge assay should be able to detect minimum SARS-CoV-2 and VOC loads equivalent to $10^3$-$10^4$ viral RNA copies (per mL). Overall, rapid antigen tests showed an analytical sensitivity of 93,75% when compared to RT-qPCR (Figure 1C).

SARS-CoV-2 rapid tests rely on antigen-antibody reactions that can be inhibited by different physiochemical properties of samples, e.g. viscosity and extreme pH values (Reverberi and Reverberi, 2007). It is well known that animal saliva possesses a variable viscosity and pH range (Reid and Huffman, 1949). Accordingly, whether animal saliva might affect the sensitivity of SARS-CoV-2 antigen assays was an intriguing question that has been answered

8

in this study. Neither saliva composition nor collection methods showed adverse effects on the sensitivity of rapid tests as interpreted qualitatively by lateral flows and quantitatively by relative band intensity (Figure 2A), and RT-qPCR (Figure 2B). Hence, antigen-antibody reactions can tolerate pH changes between 6.5-8.5 (Barnes, 1966; Hughes-Jones et al., 1964). Our data align with previous reports that recommended the use of saliva for detecting SARS-CoV-2 by antigen tests or RT-qPCR in humans (Basso et al., 2021; Wyllie et al., 2020).

B.1.1.7 and B.1.351 variants harbor mutations mainly in the S-gene that significantly impact S-gene targeting by RT-qPCR, a phenomenon known as S-gene dropout (Washington et al., 2020). Therefore, it is of utmost importance to detect whether mutations in other genes (e.g. N-gene) have an impact on the performance of these tests, especially commercial antigen assays that target N-protein (Figure 3G) (Azad, 2021). We observed that analytical sensitivities of RATs were significantly reduced especially at lower viral concentrations (Figure 3), which suggests a partial interaction between N-protein variants and lateral flow antibodies. The reason for that could be attributed to altered stability or epitopes of N-protein of B.1.1.7 and B.1.351 variants.

**Conclusion**

In summary, saliva of the species tested has no impact on the functionality and performance of SARS-CoV-2 RATs. Therefore, the use of RATs can be recommended as a point-of-care surveillance tool for SARS-CoV-2 infections in these species. However, the tests should be checked beforehand for their suitability to equally detect VOC B.1.1.7 and B.1.351.

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**Author contributions**

ITH, MHG, MK conceived and designed the experiments. ITH, SW, MK performed

experiments. ITH, SW, MHG, MK analyzed data. ITH, SW, MK contributed

reagents/materials/analysis tools. ITH, MK, MHG attained funding. ITH, MHG, MK wrote

the paper. All authors reviewed, edited, and approved the final version.

**Conflict of interest**

No conflict of interest is declared

**References**

Abdel-Moneim, A.S., Abdelwhab, E.M., 2020. Evidence for SARS-CoV-2 infection of

animal hosts. Pathogens 9, 529.

Azad, G.K., 2021. Identification and molecular characterization of mutations in nucleocapsid

phosphoprotein of SARS-CoV-2. PeerJ 9, e10666.

Barnes, A.E., 1966. The specificity of pH and ionic strength effects on the kinetics of the Rh

(D)-anti-Rh (D) system. The Journal of Immunology 96, 854-864.

Basso, D., Aita, A., Padoan, A., Cosma, C., Navaglia, F., Moz, S., Contran, N., Zambon, C.-

F., Cattelan, A.M., Plebani, M., 2021. Salivary SARS-CoV-2 antigen rapid detection:

a prospective cohort study. Clinica Chimica Acta 517, 54-59.

Case, J.B., Bailey, A.L., Kim, A.S., Chen, R.E., Diamond, M.S., 2020. Growth, detection,

quantification, and inactivation of SARS-CoV-2. Virology 548, 39-48.

10

Corman, V.M., Landt, O., Kaiser, M., Molenkamp, R., Meijer, A., Chu, D.K., Bleicker, T.,
Brünink, S., Schneider, J., Schmidt, M.L., 2020. Detection of 2019 novel coronavirus
(2019-nCoV) by real-time RT-PCR. Eurosurveillance 25, 2000045.

de Morais, H.A., Dos Santos, A.P., do Nascimento, N.C., Kmetiuk, L.B., Barbosa, D.S.,
Brandão, P.E., Guimarães, A.M.S., Pettan-Brewer, C., Biondo, A.W., 2020. Natural
infection by SARS-CoV-2 in companion animals: a review of case reports and current
evidence of their role in the epidemiology of COVID-19. Frontiers in veterinary
science 7, 823.

de Wilde, A.H., Raj, V.S., Oudshoorn, D., Bestebroer, T.M., van Nieuwkoop, S., Limpens,
R.W., Posthuma, C.C., van der Meer, Y., Bárcena, M., Haagmans, B.L., 2013. MERS-
coronavirus replication induces severe in vitro cytopathology and is strongly inhibited
by cyclosporin A or interferon-α treatment. The Journal of general virology 94, 1749.

Ferasin, L., Fritz, M., Ferasin, H., Becquart, P., Legros, V., Leroy, E.M., 2021. Myocarditis in
naturally infected pets with the British variant of COVID-19. bioRxiv.

Fernández-Bellon, H., Rodon, J., Fernández-Bastit, L., Almagro, V., Padilla-Solé, P., Lorca-
Oró, C., Valle, R., Roca, N., Grazioli, S., Trogu, T., Bensaid, A., Carrillo, J.,
Izquierdo-Useros, N., Blanco, J., Parera, M., Noguera-Julián, M., Clotet, B., Moreno,
A., Segalés, J., Vergara-Alert, J., 2021. Monitoring Natural SARS-CoV-2 Infection in
Lions (Panthera leo) at the Barcelona Zoo: Viral Dynamics and Host Responses.
Viruses 13, 1683.

FIND, 2020. SARS‐ CoV‐ 2 Diagnostic Pipeline. FIND.

Hughes-Jones, N., Gardner, B., Telford, R., 1964. The effect of pH and ionic strength on the
reaction between anti-D and erythrocytes. Immunology 7, 72.

Igloi, Z., Velzing, J., van Beek, J., van de Vijver, D., Aron, G., Ensing, R., Benschop, K.,
Han, W., Boelsums, T., Koopmans, M., 2020. Clinical evaluation of the Roche/SD

Biosensor rapid antigen test with symptomatic, non-hospitalized patients in a municipal health service drive-through testing site. medRxiv.

Jungnick, S., Hobmaier, B., Mautner, L., Hoyos, M., Haase, M., Baiker, A., Lahne, H., Eberle, U., Wimmer, C., Hepner, S., 2021. Detection of the new SARS-CoV-2 variants of concern B. 1.1. 7 and B. 1.351 in five SARS-CoV-2 rapid antigen tests (RATs), Germany, March 2021. Eurosurveillance 26, 2100413.

Kennedy, S., Oswald, N., 2011. PCR troubleshooting and optimization. Caister Academic Press.

M Gutierrez, A., J Ceron, J., Fuentes-Rubio, M., Tecles, F., A Beeley, J., 2014. A proteomic approach to porcine saliva. Current Protein and Peptide Science 15, 56-63.

OIE: OIE technical fact sheet - INFECTION WITH SARS-COV-2 IN ANIMALS. Available online: https://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/COV-19/A_Factsheet_SARS-CoV-2.pdf (accessed on 25.05.2021).

Reid, J., Huffman, C., 1949. Some physical and chemical properties of bovine saliva which may affect rumen digestion and synthesis. Journal of Dairy Science 32, 123-132.

Reverberi, R., Reverberi, L., 2007. Factors affecting the antigen-antibody reaction. Blood transfusion 5, 227.

Scheiblauer, H., Filomena, A., Nitsche, A., Puyskens, A., Corman, V.M., Drosten, C., Zwirglmaier, K., Lange, C., Emmerich, P., Müller, M., Knauer, O., Nübling, C.M., 2021. Comparative sensitivity evaluation for 122 CE-marked SARS-CoV-2 antigen rapid tests. medRxiv, 2021.2005.2011.21257016.

Segalés, J., Puig, M., Rodon, J., Avila-Nieto, C., Carrillo, J., Cantero, G., Terrón, M.T., Cruz, S., Parera, M., Noguera-Julián, M., 2020. Detection of SARS-CoV-2 in a cat owned by a COVID-19− affected patient in Spain. Proceedings of the National Academy of Sciences 117, 24790-24793.

Su, S., Wong, G., Shi, W., Liu, J., Lai, A.C., Zhou, J., Liu, W., Bi, Y., Gao, G.F., 2016.

    Epidemiology, genetic recombination, and pathogenesis of coronaviruses. Trends in

    microbiology 24, 490-502.

Washington, N.L., White, S., Barrett, K.M.S., Cirulli, E.T., Bolze, A., Lu, J.T., 2020. S gene

    dropout patterns in SARS-CoV-2 tests suggest spread of the H69del/V70del mutation

    in the US. medRxiv.

Wölfel, R., Corman, V.M., Guggemos, W., Seilmaier, M., Zange, S., Müller, M.A.,

    Niemeyer, D., Jones, T.C., Vollmar, P., Rothe, C., 2020. Virological assessment of

    hospitalized patients with COVID-2019. Nature 581, 465-469.

Wyllie, A.L., Fournier, J., Casanovas-Massana, A., Campbell, M., Tokuyama, M.,

    Vijayakumar, P., Geng, B., Muenker, M.C., Moore, A.J., Vogels, C.B., 2020. Saliva is

    more sensitive for SARS-CoV-2 detection in COVID-19 patients than nasopharyngeal

    swabs. MedRxiv.

**Figure captions**

**Figure 1: Limits of detection (LoD) of SARS-CoV-2 rapid antigen assays.** (A) Comparison of the rapid antigen test results with the corresponding viral RNA genome copy numbers (per mL) determined by RT-qPCR. * Antigen kits (WuHan UNscience) did not meet the requirements (weak positive at 3.12 E+06 per mL), as the LoD was significantly different from the other tests (*p<0.05; Bonferroni's multiple comparisons). The dashed line refers to the hypothetical infectivity threshold ($10^6$/mL) (12). (B) Interactive dot analysis for an exemplary antigen assay (BioNote) results compared to RT-qPCR Ct values. The solid and dashed lines refer to the estimated Ct cut-off value 32.25 and the arbitrary limit of RT-qPCR Ct LoD, respectively. (C) ROC curve for the analysis of sensitivity and specificity of the leading rapid antigen tests (AUC 98.6; P<0.001). No significance was observed between assays (i-vii) (p-value ≤0.05). Three to four experiments were evaluated. One-way analysis of variance (ANOVA) with Bonferroni's multiple comparison test was used to analyse the data. Standard deviations (SD±) are represented by error bars.
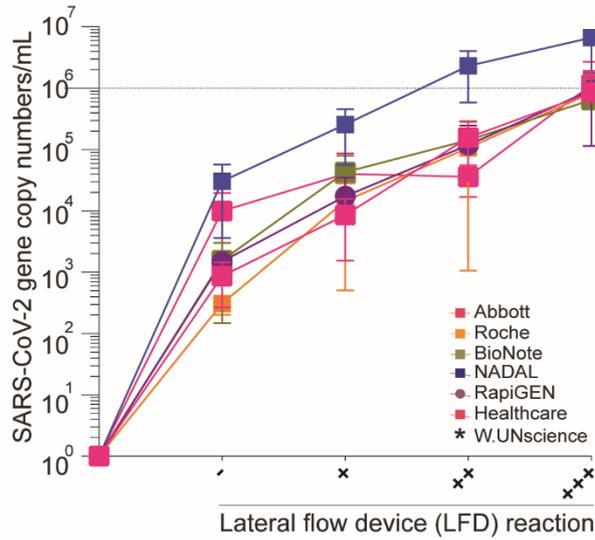
**Figure 2: Saliva from animal species has no adverse effect on the analytical sensitivity of rapid antigen assays. (A)** SARS-CoV-2 target band intensity (%) in lateral flow device after normalization to the control band. **(B)** SARS-CoV-2 copy numbers from spiked human and animal species saliva with correspondence to the results of lateral flow device using real-time RT-PCR. Controls blue and black respectively represent non-saliva sample and an oropharyngeal swab from SARS-CoV-2-infected hamster (Positive clinical sample). Non-applicable data (test band missing, therefore no calculation possible) were referred to as 'na'. Three to four average data sets were analysed. Standard deviations (SD±) are represented by error bars. No significant differences were detected (Control vs. human, feline, ferrets, cattle, sheep, swine, and bats; Bonferroni's multiple comparisons test; Significance level was set at p-value ≤0.05).
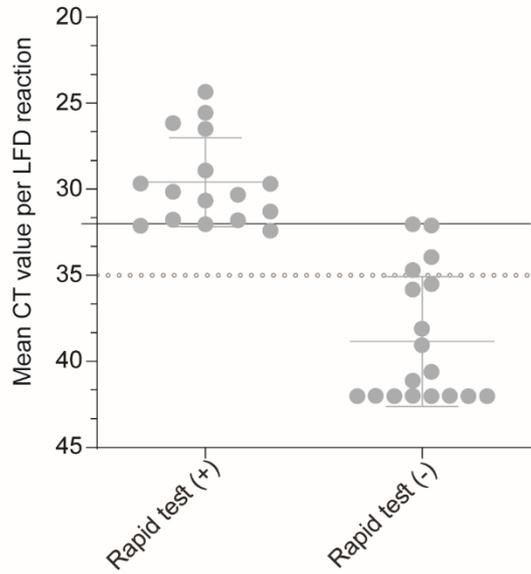
14

**Figure 3: Partial impairment of analytical sensitivity of antigen assays by B.1.1.7 and B.1.351 VOC. (A), (B),** and **(C)** Qualitative results of an exemplary antigen assay (BioNote) using serially diluted cell culture supernatants from SARS-CoV-2 and VOC B.1.1.7 and B.1.351 (Fig. S2). **(D)** Quantitative comparison of band intensities (%) of SARS-CoV-2 and VOCs. **(E)** and **(F)** Comparison of borderline detection limit (+) of antigen assay (BioNote/NowCheck) in case of eluted SARS-CoV-2 and VOCs using Ct-values and gene copy numbers (****$p<0.0001$; **$p<0.01$; *$p<0.05$). **(G)** Alignment of N-protein sequences of SARS-CoV-2 and VOC, highlighting changes in amino acids residues. Three to four average data sets were analysed. Standard deviations (SD±) are depicted by error bars. The one-way analysis of variance (ANOVA) with Bonferroni's multiple comparisons test was used to analyse data.
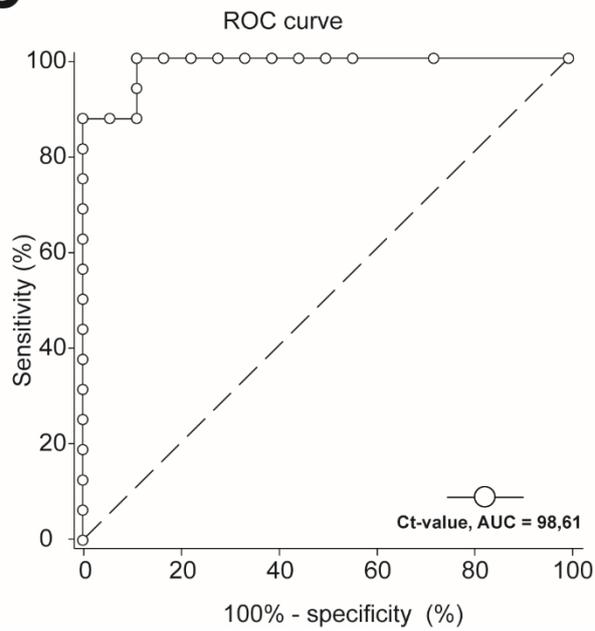
**Figure 1**

**Figure 2**

17

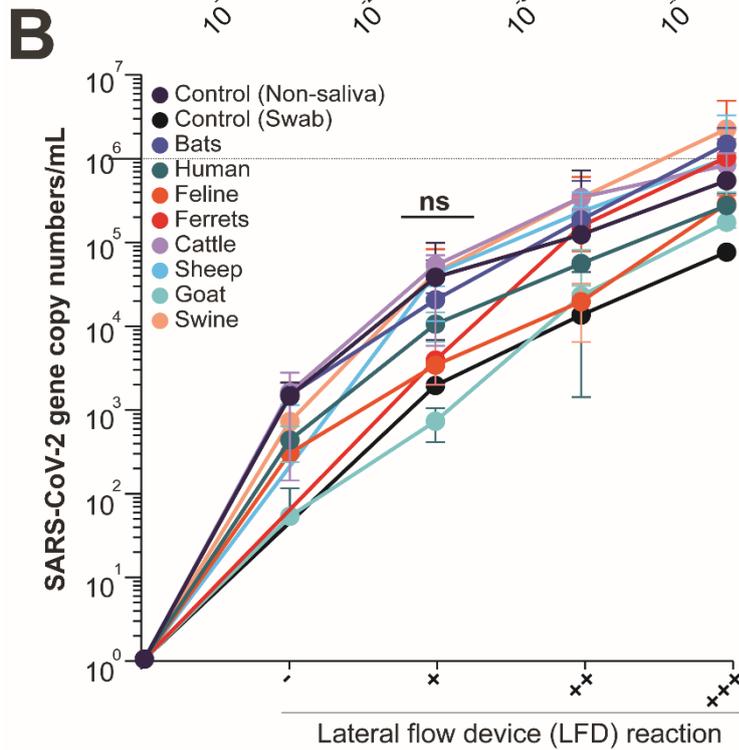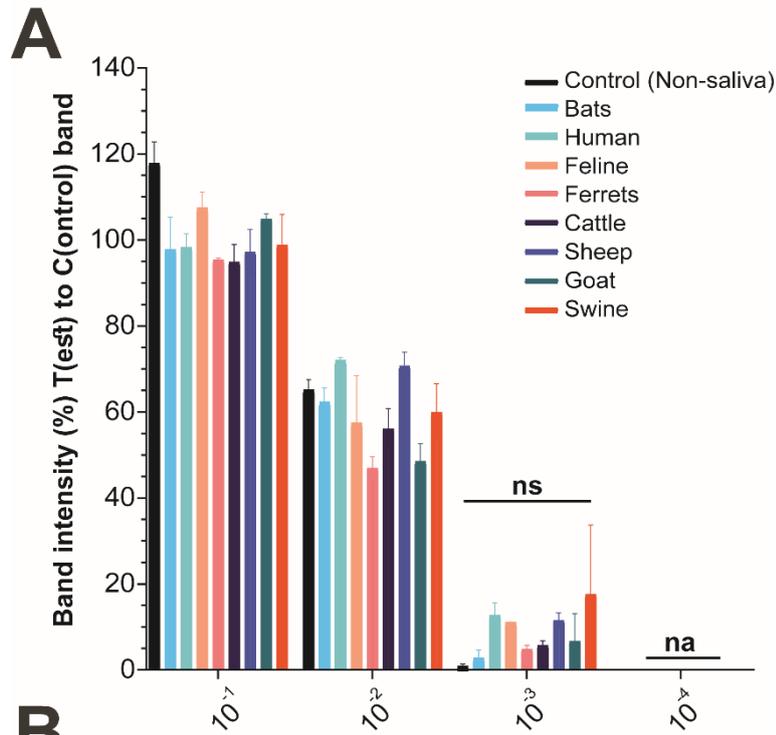**Figure 3**