SARS-CoV-2 infectivity correlates with high viral loads and detection of viral antigen and is terminated by seroconversion

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Conflict of interest: The authors declare no conflict of interest.

Funding: Bavarian Ministry of Science and Art within the pandemic responsiveness fund.

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Summary

Based on analyses in 79 respiratory samples from 59 patients, isolation of SARS-CoV-2 correlated significantly with high viral loads and detection of viral antigen, while it decreased significantly 10 days after symptom onset and with detection of SARS-CoV-2 antibodies.

Abstract

Background. From a public health perspective, effective containment strategies for SARS-CoV-2 should be balanced with individual liberties. **Methods.** We collected 79 respiratory samples from 59 patients monitored in an outpatient center or in the intensive care unit of the University Hospital Regensburg. We analyzed viral load by quantitative real-time PCR, viral antigen by point-of-care assay, time since onset of symptoms and presence of SARS-CoV-2 IgG antibodies in the context of virus isolation from respiratory specimen. **Results.** The odds ratio for virus isolation increased 1.9-fold for each $\log_{10}$ level of SARS-CoV-2 RNA and 7.4-fold with detection of viral antigen, while it decreased 6.3-fold beyond 10 days of symptoms and 20.0-fold with presence of SARS-CoV-2 antibodies. The latter was confirmed for B.1.1.7 strains. The positive predictive value for virus isolation was 60.0% for viral loads above 10^7 RNA copies/mL and 50.0% for the presence of viral antigen. Symptom onset before 10 days and seroconversion predicted lack of infectivity with 93.8% and 96.0%. **Conclusions.** Our data support quarantining patients with high viral load and detection of viral antigen, and lifting restrictive measures with increasing time to symptom onset and seroconversion. Delay of antibody formation may prolong infectivity.

Key words: SARS-CoV-2, infectivity, viral load, viral antigen, seroconversion, public health

Background

SARS-CoV-2 infection was first detected in Wuhan, Hubei Province, China, and has since rapidly become a global pandemic [1, 2]. High numbers of infections, protracted courses of disease in intensive care units [3, 4], and limited treatment options [5] have imposed a heavy burden on the health care system. In order to limit complications and deaths from COVID-19, non-pharmacological interventions like containment strategies are of key relevance [6-9]. Individual restrictions such as isolating infected persons are necessary to prevent uncontrolled transmission. For this, knowledge about the excretion of infectious viruses is crucial.

Infectivity is defined as period of time during which an infection can be transmitted. Virus isolation in cell culture is considered the best surrogate for infectivity, because SARS-CoV-2 propagation requires replication-competent virus. Detection of SARS-CoV-2 RNA by RT-PCR has become the gold standard for diagnosis of infection. In early phases of the illness, viral loads correlate with infectious virus [10-12]. As reported, shedding of infectious SARS-CoV-2 was most pronounced around the onset of symptoms until one week thereafter [11-13]. This kinetics of virus excretion contrasts with SARS-CoV infections and pandemic influenza, where viral loads use to peak 5-7 days or 2 days after symptom onset, respectively [14, 15]. However, SARS-CoV-2 RNA may remain positive in respiratory samples beyond the usual period of infectivity [10, 11, 16]. In individual cases, infectious virus has been detected beyond a two-week period in severely ill and immunocompromised subjects [10, 17].

Antigen point of care tests (AgPOCT) are provided by many suppliers and score with economic benefits, simplicity of use and rapid results compared to RT-PCR assays. These advantages contrast with lower sensitivity, resulting in positive test results only with high viral loads [18, 19]. Depending on the supplier, positive results may correlate with viral loads at which infectivity is likely to occur

[19]. However, studies that correlate antigen test results with virus isolation and thus allow direct statements about infectivity are rare [20].

Individuals tested positive for SARS-CoV-2 are usually quarantined to interrupt the chain of SARS-CoV-2 transmission. In several countries, symptom-based strategies are implemented for the isolation of infected persons. For mild and moderate courses of disease, national public health institutes such as the CDC (USA) or the Robert Koch-Institute (Germany) as well as the WHO recommend isolation for at least 10 days, starting from the onset of symptoms or an initial positive test result in asymptomatic cases. The time of isolation required to safely prevent further infections is highly dependent on infectivity. The necessity of isolating SARS-CoV-2 positive cases based on the putative duration of infectivity, however, has implications for infection control as well as for social constraints of individuals and their environment. It is therefore highly relevant to better characterize the predictors of infectivity so as not to disproportionately restrict personal freedom while meeting the interests of infection control. As virus isolation is time-consuming and labor-intensive, this method is not applicable for large-scale analyses. The aim of this study was to determine whether time since onset of symptoms, viral load and rapid antigen detection as well as presence of SARS-CoV-2 antibodies are suitable predictors of infectivity.

Material & Methods

Selection of patients.

This study collected data from 25 adult patients hospitalized for a severe course of disease at the intensive care unit (ICU) of the University Hospital Regensburg (COVUR study) from March to May 2020, and from 34 patients at an early stage of disease, who visited an outpatient practice with a positive SARS-CoV-2 RT-PCR in the preceding 72 hours or were quarantined by the respective Public Health Departments between May and December 2020. Details of the two patient groups are

presented in **Table 1**. The occurrence of COVID-19-typical symptoms in the patients (e.g. fever, rhinitis, cough, shortness of breath, loss of smell and/or taste) was assessed as accurately as possible by the treating physicians in the outpatient clinic and in the emergency department of the University Hospital and documented in the patient file. All patients provided written informed consent. Patient samples were either used as fresh samples or stored at -80°C prior to analysing viral loads, antigen point of care test (AgPOCT), virus isolation, and SARS-CoV-2 IgG antibodies, as approved by the ethical commission of the Faculty for Medicine, University of Regensburg (ref. nos. 20-1918-101 and 20-1785-101, respectively).

RT-PCR testing

RNA was extracted from oropharyngeal swabs, throat washings, and tracheal aspirates using the EZ1 Virus Mini Kit v2.0 on the EZ1 Advanced XL platform (Qiagen, Hilden, Germany). The SARS-CoV-2 envelope gene was amplified using a published protocol [21] on the StepOnePlus Real-Time PCR System (ThermoFisherScientific, Schwerte, Germany). For absolute quantification, an *in-vitro* transcribed assay-specific RNA standard was used, as described previously [22]. The 95% limit of detection (LoD) was determined to be 300 RNA copies/mL. Bacteriophage MS2 served as an internal control for isolation, reverse transcription and amplification.

Identification of B.1.1.7 variants of concern (VOC)

Mutation analysis for B.1.1.7 (VOC Alpha) was performed by real-time RT-PCR and melting curve analysis using the VirSNiP SARS-CoV-2 Spike assays „del HV69/70“ and „N501Y“ (TIB MOLBIOL, Berlin, Germany) according to the manufacturer’s instructions.

Antigen point of care test

The Roche SARS-CoV-2 Rapid Antigen Test was used as point of care test (AgPOCT). Following a published protocol, 50 µl of the respiratory specimen were added to the extraction buffer tube [19]. Subsequent procedures were performed according to the recommendations of the manufacturer. The 50% and 95% LoDs of the AgPOCT were determined to be 3.4 x 10^5 and 4.6 x 10^6 SARS-CoV-2 RNA copies/mL, respectively **(Suppl. Fig. 1)**. Test results of the study were evaluated by two persons and were photo-documented.

SARS-CoV-2 antibodies

SARS-CoV-2 IgG antibodies were determined using a semi-quantitative in-house ELISA based on the SARS-CoV-2 receptor-binding domain as previously described [23].

Time of seroconversion

Time of seroconversion was defined as the day of the first detection of SARS-CoV-2 IgG antibodies in relation to the specimen collection.

Onset of symptoms and onset of disease

Onset of symptoms was defined as the day when COVID-19-typical symptoms were first noticed by the patient. In these patients, the onset of symptoms was identical with the onset of disease; in asymptomatic patients and patients without documented onset of symptoms, the day of the first detection of SARS-CoV-2 by RT-qPCR was used as onset of disease.

Virus isolation

SARS-CoV-2 was isolated from respiratory specimens using Vero cells. Cells were cultivated in Dulbecco's Modified Eagle's Medium supplemented with 10% heat-inactivated fetal calf serum (Sigma-Aldrich, Munich, Germany), 90 U/mL streptomycin, 0.3 mg/mL glutamine, 200 U/mL penicillin, and 2.5 µg/mL amphotericin B (PAN Biotech, Aidenbach, Germany). For virus isolation, 500-1000 µl of respiratory specimens were added to the cell cultures. One day after infection, supernatants were completely removed and replaced by fresh media. Seven days post infection, virus isolation was considered positive when viral loads in cell culture supernatants were above 10^6 RNA copies/mL using the RT-qPCR approach described above. Isolation of B.1.1.7 strains was performed accordingly using the colorectal carcinoma cell line CaCo-2 (CLS Cell Lines Service GmbH, Eppelheim, Germany).

Statistics

All variables were evaluated with respect to virus isolation. Viral loads were $\log_{10}$ transformed before statistical analysis. Undetectable viral loads were set to 10^0 RNA copies/mL before transformation. Asymptomatic cases were excluded from all analyses involving symptom onsets. Binary univariate logistic regression analysis was performed for all predictors of infectivity. Categorical variables were additionally evaluated using two-tailed Fisher's exact probability test. All analyses were performed using SPSS 26.0, defining statistical significance as $p < 0.05$. Figures were compiled using GraphPadPrism 8.4.2.

Results

Characterization of study cohort

This study included 79 respiratory specimens of 59 patients (29 male, 30 female). 34 samples were provided from patients in ambulatory care or who were monitored by the Public Health Department. 45 samples were provided by patients treated at the intensive care units of the University Hospital Regensburg. Median age of patients was 48 years (interquartile range, IQR, 29.50-59.75). Median viral load of respiratory specimens was 6.80 x 10^4 RNA copies/mL (IQR, 4.75 x 10^3 - 1.81 x 10^6); 24 (30.4%) samples had SARS-CoV-2 RNA levels above 10^6 copies/mL, while 12 specimens (15.2%) were below detection level. 14 samples each (17.7%) were positive for AgPOCT and virus isolation. SARS-CoV-2 antibody status was available for 41 samples; 25 and 16 of these were obtained from patients who were seropositive and seronegative at the time of respiratory specimen collection, respectively. 15 samples were obtained from patients with unknown onset of symptoms, and 5 samples were collected from patients who remained asymptomatic. In all patients with known onset of symptoms (n=59), the median number of days between onset of symptoms and collection of the respiratory samples was 11 days (IQR, 6.0-28.0). For further analyses, symptom and disease onset, SARS-CoV-2 RNA levels, detection of viral antigen, and IgG antibodies at the time of sample collection were correlated with SARS-CoV-2 isolation from respiratory specimens.

High viral loads in respiratory specimens were correlated with SARS-CoV-2 isolation

Previous reports indicated that high SARS-CoV-2 RNA levels were correlated with the detection of infectious viruses in airway samples [10-12, 24, 25]. We confirmed these data in our study cohort, showing 52-fold higher viral loads in samples with positive compared to negative SARS-CoV-2 isolation in cell culture (6.14 vs. 4.40 log10 RNA copies/ml; p=0.002) **(Fig. 1A)**. The median viral load

in the former group was 1.35 x 10^6 RNA copies/mL (IQR, 2.89 x 10^5 – 4.45 x 10^7 RNA copies/mL). Of note, we were able to isolate virus from samples with viral loads below this threshold, including one sample that contained 1.8 x 10^3 RNA copies/mL. On the other hand, several samples with viral loads above 1.0 x 10^7 RNA copies/mL did not result in successful virus isolation. Virus isolation was most likely to occur in the first 10 days after onset of clinical symptoms **(Fig. 1B)**. Surprisingly, SARS-CoV-2 was isolated from one respiratory specimen which was obtained 167 days after onset of severe COVID-19 infection. This patient had been treated with rituximab in the setting of malignant lymphoma and had remained symptomatic throughout the entire observation period.

Positive AgPOCT in respiratory samples was correlated with SARS-CoV-2 isolation

Antigen detection in airway samples was reported to be associated with high viral loads and successful virus isolation [26, 27]. In line with these data, samples of our study cohort contained 589-fold higher viral loads with positive compared to negative AgPOCT (7.17 vs. 4.40 log10 RNA copies/mL; $p<0.001$) **(Fig. 1C)**. SARS-CoV-2 was successfully isolated from 7 of 14 samples with positive AgPOCT, while the others remained negative. Viral antigen was not detected beyond 16 days after onset of symptoms **(Fig. 1D)**.

Detection of SARS-CoV-2 antibodies was associated with lack of virus isolation

As shown above, viral RNA loads and antigen detection in airway samples of our study were correlated with virus isolation. However, 'outliers' occurred with successful virus isolation despite low RNA levels and lack of virus isolation despite high viral loads. Most striking was the isolation of SARS-CoV-2 in an airway sample nearly six months after onset of disease, as described above. This patient had not developed SARS-CoV-2 IgG, probably due to treatment with rituximab, which prompted us to investigate the role of these antibodies in infectivity. Viral loads in airway samples

were 28-fold higher when SARS-CoV-2 IgG was absent vs. present in the serum (6.36 vs. 4.92 log10 RNA copies/mL, respectively; p=0.004) **(Fig. 2A)**. In seven patients with follow-up samples, SARS-CoV-2 was preferentially isolated from the earliest specimen, while later samples were consistently negative **(Fig. 2B)**. This situation was reflected in the timeline, showing that, with one exception, virus isolation was not successful in samples with documented IgG seroconversion prior to collection of respiratory samples **(Fig. 2B)**. In conclusion, successful virus isolation clustered with high viral loads, sampling shortly after onset of symptoms, and seroconversion after collection of respiratory specimens **(Suppl. Fig. 2)**.

To find out whether the presence of SARS CoV-2 antibodies similarly limited the infectivity of VOCs, we investigated 22 respiratory samples that harbored B.1.1.7 strains with a deletion at position 69/70 and mutation N501Y in the viral spike protein according to real-time PCR and melting curve analysis. We were able to isolate virus from 10 of 12 samples obtained from patients without SARS-CoV-2 antibodies, but in none of 10 specimen from seropositive donors (p<0.001, Fisher's Exact Test) **(Fig. 3)**. These data confirmed that SARS-CoV-2 antibodies similarly restricted VOC Alpha infectivity.

Prediction of virus isolation

To date, high viral loads and the presence of viral antigen in airway samples, recent onset of symptoms, and lack of seroconversion have been confirmed as determinants of virus isolation in our study cohort. However, the extent to which each of these factors contributed to infectivity was still unclear. For these purposes, we analyzed all parameters using a binary logistic regression model **(Table 2)**. In this analysis, the likelihood of virus isolation was increased 1.9-fold for each $\log_{10}$ level of SARS-CoV-2 RNA. The probability increased 3.8-fold, 6.0-fold and 11.4-fold with viral loads above 1.0×10^5, 1.0×10^6 and 1.0×10^7 RNA copies/mL compared to viral loads equal to or below these

thresholds. Detection of viral antigen increased the odds ratio 7.4-fold. By contrast, it was reduced by 79% if the onset of symptoms was more than 10 days ago. When SARS-CoV-2 IgG antibodies were present, the probability was reduced by 91%.

Furthermore, we examined the sensitivity and specificity with which each parameter predicted virus isolation from respiratory samples **(Table 3)**. Sensitivities decreased and specificities increased with increasing SARS-CoV-2 RNA concentrations **(Suppl. Fig. 3A)**. A threshold of 1.0×10^7 RNA copies/mL resulted in positive and negative predictive values of 60.0% and 88.4%, respectively. Antigen test results were associated with a positive predictive value of 50.0% and a negative predictive value of 88.1%. An interval of 10 or more days between the onset of symptoms and the collection of airway samples resulted in a negative predictive value of 93.8% **(Table 3, Suppl. Fig. 3B)**. SARS-CoV-2 IgG antibodies were associated with a negative predictive value of 96.0%. In summary, best predictors for infectivity were viral loads above 1.0×10^7 RNA copies/mL and detection of viral antigen, while absence of infectivity was best predicted by the presence of SARS-CoV-2 IgG antibodies and a long time interval from the onset of symptoms.

Discussion

Our study investigated the relevance of four different parameters for the isolation of SARS-CoV-2 in cell culture, namely SARS-CoV-2 RNA levels and viral antigen in respiratory specimens in addition to time after onset of symptoms and IgG antibodies in the serum. We confirmed previously reported findings that the risk of infectivity increases with increasing viral load [10, 11]. However, virus load as unique predictor for infectivity allows only limited conclusions, because infectivity usually terminates after 10 days [10-12, 24, 28], while SARS-CoV-2 RNA is detectable in respiratory samples for an average of 17 days [16]. Furthermore, conversion of ct values into viral loads is dependent on

laboratory-specific validation [29, 30]. In line with these data, we were unable to define a threshold for infectivity. We could isolate virus from a sample harboring only 1.8 x 10^3 RNA copies/mL at an early stage of infection as evident from the absence of SARS-CoV-2 IgG antibodies in the serum. On the contrary, several samples with viral loads above 1 x 10^7 RNA copies/mL failed to support virus isolation, most likely due to the presence of antibodies. This may be particularly important for patients treated in intensive care units who received convalescent plasma. Therefore, viral load can only be considered as a surrogate parameter, which should be interpreted together with clinical information.

Point-of-care testing of viral antigen captures the samples with the highest viral load. In our study, a positive AgPOCT was associated with a 7.4-fold increased likelihood of virus isolation, while a negative test resulted in a low probability of infectivity even with positive PCR. In the predictive model, AgPOCT missed half of the samples with positive virus isolation, but on the other hand detected the second half, providing the chance to quarantine these individuals and limit viral spread. It needs to be investigated how much this proportion contributes to the incidence of infection at the population level in particular because the result of an AgPOCT is available within minutes, whereas a PCR test (and thus the measures to be taken) take much longer. Therefore, broad AgPOCT testing with the limitation of missing low viral loads, especially at the onset of infectivity, may be combined with targeted PCR in high-risk situations and for confirmation. A considerable proportion of infections are transmitted before diagnosis, as viral shedding begins before symptoms appear. This situation calls for vaccination and hygiene as the main primary prevention approach; the contribution of broad PCR or antigen screening remains to be further investigated [31].

Furthermore, our study revealed a significant decrease of virus isolation 10 days after the onset of symptoms. This time interval is slightly longer than previously reported [11], possibly caused by the inclusion of 45 samples from patients treated in the intensive care unit during the first COVID-19 wave in Regensburg. These patients did not receive regular dexamethasone but had a severe course of disease. 15 patients received convalescent plasma and two patients each were treated with anakinra, remdesivir, and hydroxychloroquin. A total of 10 patients were immunosuppressed, suffering from rheumatological diseases, lymphomas or leukemia, were transplanted, or had received corticosteroids for more than four weeks before the onset of disease. Of note, we observed prolonged virus shedding in one patient after B-cell depletion, which resolved after administration of convalescent plasma. On the contrary, we were not able to isolate virus from all samples collected in the first days after symptom onset. This was still the case when all samples were excluded where the exact onset of symptoms was unknown. Thus, “days after onset of symptoms” as unique predictor for infectivity allowed only limited conclusions in our study. From a public health perspective, it seems important to differentiate between the majority of "uncomplicated" courses and those with pre-existing conditions or immunosuppressive medication [32]. Not immunosuppression per se, but the lack of SARS-CoV-2 antibodies appears to be crucial for prolonged virus excretion. This goes in hand with the fact that the majority of immunosuppressed patients received convalescent plasma during the ICU stay.

The most striking result of our study was the pronounced effect of seroconversion on virus isolation. Once SARS-CoV-2 IgG antibodies directed against the receptor-binding domain of SARS-CoV-2 were present in the serum, virus isolation was no longer possible – with the exception of one patient, who was borderline positive for SARS-CoV-2 IgG and had recently seroconverted. The presence of IgG antibodies corresponding to neutralizing activity [33] was associated with a high negative predictive value and therefore seems to be a suitable marker for the decision to suspend quarantine [10].

Notably, we were able to confirm these findings for VOC Alpha. Further studies are needed for VOCs Beta and Gamma, which harbor mutations that contribute to virus escape from neutralizing antibodies (K417N, E484K). We attempted to include and calculate all data in a multivariate model. However, the predictions of the univariate models were not improved, likely due to the small sample size of our cohort [34] and multicollinearity, which prevented the identification of independent variables through close correlation of all variables. In addition, the exact day of seroconversion remained unclear in our study, because SARS-CoV-2 antibody status was not analyzed on a regular basis. Another limitation is that virus isolation in cell culture, although considered a surrogate for natural infection, may underestimate true infectivity.

What can be recommended based on our data and the data of other groups? Patients with high viral loads and/or a positive AgPOCT (confirmed by PCR) should be considered infectious and quarantined, regardless of whether they are symptomatic or not [35]. In uncomplicated cases, isolation can be lifted no earlier than seven to nine days after the onset of symptoms, when the patient has recovered. This interval fits well with a recent review that considers infectivity beyond 10 days after onset of symptoms to be very unlikely [36] and another study that failed to detect replicative viral intermediates beyond eight days after onset of illness [37]. Measurement of SARS-CoV-2 IgG antibodies appears to be most useful in complicated cases with prolonged viral shedding, especially if the patient has developed a severe infection, is immunosuppressed, or has conditions that delay antibody formation. The pandemic is most likely driven by virus transmission through pre- and asymptomatic carriers when the infected person is not yet aware of the infection [13, 38, 39]. As the disease subsides, infectivity will be much lower, also because those affected are encouraged to continue to adhere to hygiene measures. The data in our study originate from the first COVID-19 wave in Germany, so the relevance of these recommendations remains to be seen for the current variants of concern, which are apparently characterized by increased infectivity [40] and reduced susceptibility to neutralizing antibodies [41, 42]. With all due caution, the detection of SARS-CoV-2 IgG antibodies can be expected to mark loss of infectivity here as well.

Acknowledgments

The authors thank the Virology Molecular Diagnostics team at the Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, for expert technical assistance, the colleagues of the Public Health Service in the Upper Palatinate and Lower Bavaria for collaboration, and Andre Gessner for continuous support. We acknowledge support by the Bavarian Ministry of Science and Art within the pandemic responsiveness fund.

Figure legends.

Fig. 1. Correlation of SARS-CoV-2 RNA and antigen loads in respiratory specimens of patients with the timeline of infectivity. A, Comparison of viral loads obtained by real-time qPCR in samples with and without successful SARS-CoV-2 isolation in cell culture. **B,** Distribution of samples with positive and negative virus isolation as a function of days after onset of symptoms. **C,** Comparison of SARS-CoV-2 RNA levels in respiratory specimens with positive and negative antigen point-of-care test (AgPOCT). **D,** Distribution of samples with positive and negative AgPOCT as well as positive and negative virus isolation as a function of days after onset of symptoms. Box plots show median and interquartile ranges plus maximal and minimal values of viral loads, which were compared after logarithmic transformation using Student's t-test. Samples with undetectable viral loads were set to 10^0 RNA copies/mL.

Fig. 2. Correlation of SARS-CoV-2 IgG antibody status with the timeline of infectivity of wild-type strains. A, Comparison of SARS-CoV-2 RNA concentrations in respiratory samples obtained from seropositive and seronegative patients. Box plots show median and interquartile ranges plus maximal and minimal values of viral loads, which were compared after logarithmic transformation using student's t-test. **B,** Intraindividual courses of virus isolations in seven patients as a function of days after onset of symptoms and SARS-CoV-2 IgG serostatus. **C,** Time of seroconversion with respect to sampling of respiratory specimen and virus isolation. Samples marked with an asterisk (*) indicate the last negative SARS-CoV-2 IgG measurement in patients without seroconversion during the observation period.

Fig. 3. Correlation of SARS-CoV-2 IgG status with the isolation of B.1.1.7 viruses from respiratory samples (n=22). A, Comparison of SARS-CoV-2 RNA levels in the respiratory specimens of these patients, stratified according to SARS-CoV-2 IgG status. **B,** Frequency of B.1.1.7 isolation in patients with or without SARS-CoV-2 IgG antibodies.

Table 1. Basic characteristics of patients treated at the outpatient center or at the intensive care unit (ICU) of the University Hospital Regensburg.

Parameters	Outpatient (n=34)	ICU (n=25)
Female	20 (58.8%)	10 (40.0%)
Age (median – IQR)	29.0 (24.0-47.8)	53.0 (47.0-63.0)
Immunosuppression	1 (2.9%)	9 (36.0%)
Days hospitalized	0	35.0 (24.0-49.0)
Convalescent plasma transfusion	1	14 (56.0%)
Pulmonary infiltration on chest CT[1]	n.a.	22 (91.7%)
Oxygen therapy	0	23 (92.0%)

[1] as evaluated by specialists in radiology

Table 2. Contribution of viral load, duration of symptoms, (timing of) seropositivity, and presence of SARS-CoV-2 antigen in respiratory specimen to isolation of infectious virus, (univariate binary logistic regression model).

Parameters	Number of samples	Odds ratio (95% CI)	P value
SARS-CoV-2 RNA	79		
Continuous (log_{10} copies/mL)		1.93 (1.23-3.02)	0.004
Categorical (≤/>4 log_{10}/mL)		7.61 (0.94-61.86)	0.058
Categorical (≤/>5 log_{10}/mL)		3.75 (1.06-13.24)	0.040
Categorical (≤/>6 log_{10}/mL)		6.00 (1.74-20.65)	0.004
Categorical (≤/>7 log_{10}/mL)		11.44 (2.65-49.46)	0.001
AgPOCT	73		
Categorical (positive/negative)		7.43 (2.00-27.58)	0.003
Days since onset of disease (all)	79		
Continuous: days		1.00 (0.98-1.02)	0.914
Categorical (≤/>7 days)		0.44 (0.14-1.42)	0.169
Categorical (≤/>10 days)		0.21 (0.06-0.81)	0.024
Days since onset of symptoms (in patients with known date of onset)	59		
Continuous (days)			
Categorical (≤/>7 days)		1.00 (0.98-1.02)	0.942
Categorical (≤/>10 days)		0.44 (0.11-1.75)	0.245

		0.16 (0.03-0.83)	0.029
IgG-antibodies at the time of respiratory specimen	41		
Categorical (yes/no)		0.05 (0.01-0.50)	0.010
Immunosuppression	79		
Categorical (yes/no)		1.57 (0.46-5.34)	0.471

Table 3. Sensitivity, specificity, and predictive values of defined categories of viral load, duration of symptoms, (timing of) seropositivity, and presence of SARS-CoV-2 antigen to predict infectivity of respiratory specimens (Fisher's exact test).

Parameters	Number of samples	P value	Sensitivity	Specificity	Positive predictive value	Negative predictive value
SARS-CoV-2 RNA						
≤/>4 log_{10}/mL	79	0.031	92.9%	36.9%	24.1%	96.0%
≤/>5 log_{10}/mL	79	0.041	71.4%	60.0%	27.8%	90.7%
≤/>6 log_{10}/mL	79	0.008	64.3%	76.9%	37.5%	90.9%
≤/>7 log_{10}/mL	79	0.002	42.9%	93.9%	60.0%	88.4%
AgPOCT						
positive/negative	73	0.004	50.0%	88.1%	50.0%	88.1%
Days since onset of disease (all)						
≤/>7 days	79	0.231	57.1%	63.1%	25.0%	87.2%
≤/>10 days	79	0.020	78.6%	56.9%	28.2%	92.5%
Days since onset of symptoms (in patients with known date						

of onset)						
≤/>7 days	59	0.283	50.0%	69.3%	25.0%	87.2%
≤/>10 days	59	0.033	80.0%	50.9%	29.6%	93.8%
IgG-antibodies at the time of respiratory specimen						
yes/no	41	0.003	87.5%	72.2%	43.8%	96.0%

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Figure 1

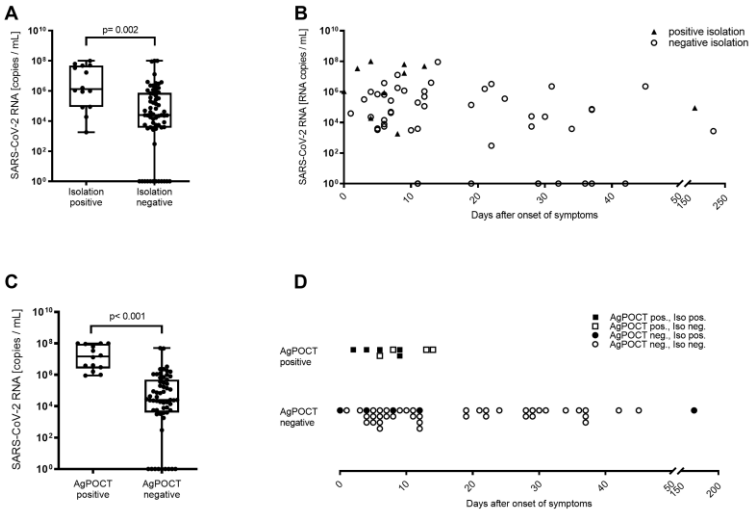
A
p= 0.002
SARS-CoV-2 RNA [copies / mL]
Isolation positive
Isolation negative
B
positive isolation
negative isolation
SARS-CoV-2 RNA [RNA copies / mL]
Days after onset of symptoms
C
p< 0.001
SARS-CoV-2 RNA [copies / mL]
AgPOCT positive
AgPOCT negative
D
AgPOCT pos., Iso pos.
AgPOCT pos., Iso neg.
AgPOCT neg., Iso pos.
AgPOCT neg., Iso neg.
AgPOCT positive
AgPOCT negative
Days after onset of symptoms

Figure 2

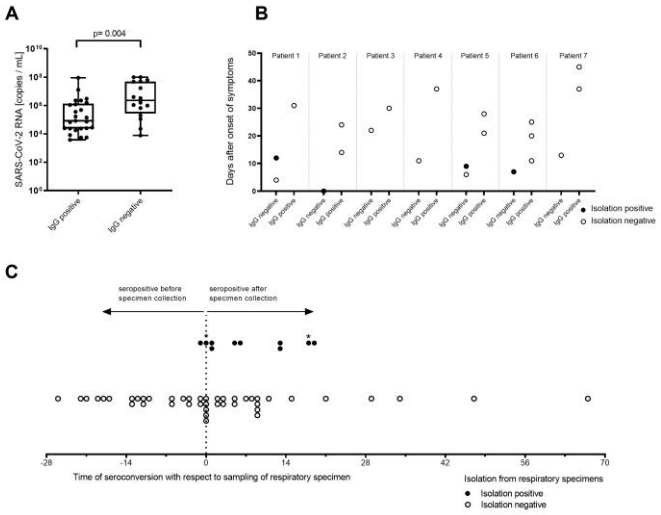
A
p= 0.004
SARS-CoV-2 RNA [copies / mL]
IgG positive
IgG negative
B
Days after onset of symptoms
Patient 1
Patient 2
Patient 3
Patient 4
Patient 5
Patient 6
Patient 7
Isolation positive
Isolation negative
C
seropositive before specimen collection
seropositive after specimen collection
-28
-14
0
14
28
42
56
70
Time of seroconversion with respect to sampling of respiratory specimen
Isolation from respiratory specimens
Isolation positive
Isolation negative

Figure 3