The value of point-of-care tests for the detection of SARS-CoV-2 RNA or antigen in bronchoalveolar lavage fluid

Peer-reviewed author version

Van Slambrouck, Jan; Schoenaers, Charlotte; Laenen , Lies; Jin, Xin; Beuselinck, Kurt; Verdonck, Ann; Wauters, Joost; MOLENBERGHS, Geert; Vanaudenaerde, Bart M.; VOS, Robin; Mombaerts, Peter; Lagrou, Katrien & Ceulemans, Laurens J.
(2024) The value of point-of-care tests for the detection of SARS-CoV-2 RNA or antigen in bronchoalveolar lavage fluid. In: JOURNAL OF VIROLOGICAL METHODS, 323 (Art N° 114848).

DOI: 10.1016/j.jviromet.2023.114848
Handle: http://hdl.handle.net/1942/41984

Journal of Virological Methods *31 October 2023*

VIRMET-D-23-00305

Title

The value of point-of-care tests for the detection of SARS-CoV-2 RNA or antigen in bronchoalveolar lavage fluid

Authors and affiliations

Jan Van Slambrouck [a,b], Charlotte Schoenaers [a,b], Lies Laenen [c], Xin Jin [a], Kurt Beuselinck [c], Ann Verdonck [c], Joost Wauters [d,e] , Geert Molenberghs [f], Bart M. Vanaudenaerde [a], Robin Vos [b,g], Peter Mombaerts [h], Katrien Lagrou [c,i], Laurens J. Ceulemans [a,b,*]

[a] Department of Chronic Diseases and Metabolism, Laboratory of Respiratory Diseases and Thoracic Surgery (BREATHE), KU Leuven, Leuven, Belgium

[b] Department of Thoracic Surgery, University Hospitals Leuven, Leuven, Belgium

[c] Department of Laboratory Medicine & National Reference Center for Respiratory Pathogens, University Hospitals Leuven, Leuven, Belgium

[d] Department of Microbiology, Immunology and Transplantation, KU Leuven, Leuven, Belgium

[e] Medical Intensive Care Unit, University Hospitals Leuven, Leuven, Belgium

[f] Interuniversity Institute for Biostatistics and Statistical Bioinformatics, KU Leuven and UHasselt, Leuven, Belgium

[g] Department of Respiratory Diseases, University Hospitals Leuven, Leuven, Belgium

[h] Max Planck Research Unit for Neurogenetics, Frankfurt, Germany

[i] Department of Microbiology, Immunology and Transplantation, Laboratory of Clinical Microbiology, KU Leuven, Leuven, Belgium

[*] Corresponding author at: Department of Thoracic Surgery, University Hospitals Leuven, Herestraat 49, 3000 Leuven, Belgium. E-mail address: laurens.ceulemans@uzleuven.be (L. J. Ceulemans)

Abstract

Background: Transmission of SARS-CoV-2 from donor to recipient is a clinically relevant risk for developing severe COVID-19 after lung transplantation (LTx). This risk of iatrogenic transmission can be reduced by timely detection of viral RNA or antigen in samples of bronchoalveolar lavage (BAL) fluid obtained at the time of lung procurement. We aimed to retrospectively evaluate the detection of SARS-CoV-2 RNA or antigen in BAL fluid samples using three point-of-care tests (POCTs).

Methods: BAL fluid samples came from patients hospitalized in an intensive care unit during the SARS-CoV-2 pandemic. These pandemic samples were scored as positive or negative for SARS-CoV-2 by a comparator RT-qPCR assay for *orf1ab*. Three commercially available POCTs were then evaluated: cobas SARS-CoV-2 & Influenza A/B assay with the cobas Liat RT-qPCR system (Roche Diagnostics), ID NOW COVID-19 and COVID-19 2.0 (Abbott), and SARS-CoV-2 Rapid Antigen Test (RAT) (Roche Diagnostics). Samples from the pre-pandemic era served as negative controls.

Results: We analyzed a total of 98 BAL fluid samples, each from a different patient: 58 positive pandemic samples (*orf1ab* Ct<38), 20 putatively negative pandemic samples (*orf1ab* Ct≥38), and 20 pre-pandemic samples. Univariate logistic regression shows that the probability of detection was highest for cobas Liat, followed by ID NOW, and then RAT. Of clinical relevance, cobas Liat detected SARS-CoV-2 RNA in 30 of the 31 positive pandemic samples that were collected within ten days after RT-qPCR diagnosis of SARS-CoV-2 infection. None of the 20 pre-pandemic samples had a false-positive result for any POCT.

Conclusions: POCTs enable the detection of SARS-CoV-2 RNA or antigen in BAL fluid samples and may provide additional information to decide if donor lungs are suitable for transplantation. Detection of respiratory pathogens with POCTs at the time of donor lung procurement is a potential strategy to increase safety in LTx by preventing iatrogenic transmission and severe postoperative infections.

Keywords

bronchoalveolar lavage, COVID-19, lung transplantation, point-of-care test, SARS-CoV-2

Abbreviations

BAL, bronchoalveolar lavage; CI, confidence interval; Ct, cycle threshold; DBI, diagnosis-to-BAL interval; ICU, intensive care unit; IQR, interquartile range; LRT, lower respiratory tract; LTx, lung transplantation; NAAT, nucleic acid amplification test; OR, odds ratio; POCT, point-of-care test; URT, upper respiratory tract; UTM, universal transport medium; RAT, rapid antigen test; RT-qPCR, reverse transcription quantitative polymerase chain reaction.

1. Introduction

As SARS-CoV-2 primarily resides in the respiratory tract, lung transplant recipients have been a highly vulnerable patient group during the COVID-19 pandemic (Van Lerberghe et al., 2023). A challenging scenario is donor-derived transmission of SARS-CoV-2 despite a negative reverse transcription quantitative PCR (RT-qPCR) result on upper respiratory tract (URT) samples (Mahmood et al., 2021). There are several reports of SARS-CoV-2 transmission during lung transplantation (LTx) that led to COVID-19 in the recipient (Van Slambrouck et al., 2022; Querrey et al., 2021; Ushiro-Lumb et al., 2021). LTx recipients require profound immunosuppression, which increases the chance of a severe course of disease in COVID-19 including a fatal outcome. Missed detection of SARS-CoV-2 in deceased donors has resulted in severe and fatal COVID-19 after LTx (Kaul et al.,2021; Kumar et al., 2021). A potential strategy to intercept viral presence in donor lungs is the use of portable point-of-care tests (POCTs) that enable rapid detection of SARS-CoV-2 RNA or antigen in bronchoalveolar lavage (BAL) fluid samples obtained at the moment of procurement (Van Slambrouck et al., 2022). However, data on the value of POCTs for this purpose are scarce (De Pace et al., 2021; Dinnes et al., 2022; Schildgen et al., 2021; Wolters et al., 2020). The aim was to determine the value of three commercially available POCTs for the detection of SARS-CoV-2 RNA or antigen in BAL fluid samples: cobas Liat, ID NOW and Rapid Antigen Test (RAT).

2. Methods

2.1 Study design, sample selection, patients and ethics

We performed a retrospective analysis with 98 residual BAL fluid samples stored at the Department of Laboratory Medicine (University Hospitals Leuven). Each sample was from a different patient treated in an intensive care unit (ICU). There were two sets of samples: 78 pandemic samples and 20 pre-pandemic samples. The pandemic samples were obtained between April 2020 and December 2021. Patients were eligible if they had a known SARS-CoV-2 infection, if the date of RT-qPCR diagnosis was available, and if the volume of the sample was sufficient to perform each POCT. The pre-pandemic samples were obtained in 2018, well before the emergence of the pandemic. Digital health records were retrospectively analyzed to collect data on demographics, comorbidities and COVID-19 course. This study was approved by the Ethics Committee UZ/KU Leuven (S66301).

2.2 SARS-CoV-2 *orf1ab* RT-qPCR comparator assay

The RT-qPCR comparator assay for SARS-CoV-2 *open reading frame 1 a and b (orf1ab)* RNA is part of a lab-developed PCR panel for the detection of 29 respiratory pathogens in BAL fluid samples. All 78 pandemic samples underwent analysis with this panel. RNA was extracted from 500 µl BAL fluid and tested with the panel as described (Raymenants et al., 2023). The detection of *orf1ab* RNA was used as comparator assay to evaluate the detection of SARS-CoV-2 in BAL fluid samples by the three POCTs. According to the cycle threshold (Ct) value, samples were classified as positive (Ct<38) or putatively negative (Ct≥38). Correlation of Ct values with viral copy numbers per volume was ≥7.0 log copies/ml or *very high* for Ct≤19.2; followed by ≥5.0-<7.0 log copies/ml or *high* for 19.2<Ct≤25.9; next, ≥3.0-<5.0 log copies/ml or *intermediate* for 25.9<Ct≤32.6; and finally, ≥1.0-<3.0 log copies/ml or *low* for 32.6<Ct<38 (Cuypers et al. 2022).

2.3 Point-of-care tests

The three commercially available POCTs were cobas SARS-CoV-2 & Influenza A/B assay on the cobas Liat system (Roche Diagnostics), ID NOW COVID-19 Test Kit for pandemic samples and COVID-19 2.0 Test Kit for pre-pandemic samples on the ID NOW system (Abbott), and a SARS-CoV-2 Rapid Antigen Test (RAT) (Roche Diagnostics).

BAL fluid samples stored at -20°C were thawed at 4°C. All analyses were carried out by the same researcher, who was blinded for the results of the comparator *orf1ab* RT-qPCR assay.

The cobas Liat platform entails a nucleic acid amplification test (NAAT) based on RT-qPCR detecting *orf1ab* and *nucleocapsid* (*N*) RNA in a compact and portable RT-qPCR instrument. A 1:6 dilution was prepared with 200 µl BAL fluid and 1000 µl universal transport medium (UTM; Copan, Cat#331C). Next, 200 µl of the mixture was transferred into the test cassette. The interface of cobas Liat reports either 'detected', 'not detected', or 'invalid'.

The ID NOW platform entails an isothermal NAAT detecting *RNA-dependent RNA polymerase* (*RdRp*) RNA in a compact and portable instrument. Before the heating step, 500 µl of BAL fluid was added to 2500 µl of buffer in the test kit to reach a 1:6 dilution. The ID NOW interface reports either 'positive', 'negative', or 'invalid'.

RAT is a laminar flow test detecting nucleocapsid protein. A 1:2 dilution was prepared by adding 300 µl of BAL fluid to the 300 µl of buffer provided in the RAT kit. Four drops were added to the cassette. After 30 minutes, the appearance of a red band was scored as 'positive' if both the control and test band were visible, 'negative' if only the control band was visible, and 'invalid' if neither band was visible.

In case of an invalid result for one or multiple POCTs, the analysis was not repeated due to limited sample volumes.

2.4 Statistics

Dichotomous variables were summarized as numbers and percentage. Continuous variables were summarized as median and interquartile range (IQR). GraphPad Prism 9.5.0 was used for statistical analysis and graphs. For the pandemic samples, univariate logistic regression was performed with the *orf1ab* Ct value as independent variable and detection of SARS-CoV-2 with each POCT as dependent variable. Output was odds ratio (OR) with 95% confidence interval (CI) and p-value, considered significant when below 0.05.

3 Results

3.1 Bronchoalveolar lavage fluid samples

Pandemic samples with *orf1ab* RT-qPCR Ct<38 are referred to as the positive pandemic samples (n=58). The viral RNA load was very high (Ct≤19.2) in 4 of these 58 samples, high (19.2<Ct<25.9) in 21 samples, moderate (25.9<Ct<32.6) in 20 samples, and low (32.6<Ct<38) in 13 samples. Pandemic samples with *orf1ab* RT-qPCR Ct≥38 are referred to as the putatively negative pandemic samples (n=20). To assess POCT specificity, a set of 20 samples obtained in 2018 were selected and are referred to as the pre-pandemic samples.

Patient characteristics are summarized in **Table S1** and **Table S2**.

3.2 Detection of SARS-CoV-2 RNA or antigen with three point-of-care tests

The POCTs were carried out once on all 78 pandemic samples.

Fig. 1 shows the results of SARS-CoV-2 detection for each POCT with regard to the *orf1ab* RT-qPCR comparator assay. Ct values for *orf1ab* RT-qPCR are evenly distributed within a range of 16.8 to 38. Results were categorized as 'detected', 'not detected', or 'invalid'. Sample characteristics and results per assay are summarized in **Table S3**.

Cobas Liat detected SARS-CoV-2 in 61 of the 78 pandemic samples (78%). In 2 of the 58 positive pandemic samples, with *orf1ab* Ct values of 34.9 and 35.0, cobas Liat did not detect RNA. Cobas Liat was invalid in one positive pandemic sample with *orf1ab* Ct value of 37.5. Conversely, in 6 of the 20 putatively negative pandemic samples (30%), cobas Liat did detect viral RNA, with Ct values ranging from 29.0 to 35.8 (**Table S3**). Orthogonal analysis with the RT-qPCR assay Xpert Xpress plus confirmed the presence of SARS-CoV-2 RNA in 4 out of 5 putatively negative pandemic samples (80%) (**Table S4**). Cobas Liat was invalid for 5 of the 20 putatively negative pandemic samples (25%).

ID NOW detected SARS-CoV-2 in 37 of the 78 pandemic samples (47%). In seven positive samples (*orf1ab* Ct values ranging from 25.2 to 37.5), viral RNA was not detected. The analysis

was invalid for 3 negative samples and 15 positive samples (*orf1ab* Ct values ranging from 18.7 to 33.8). In one negative pandemic sample, ID NOW did detect viral RNA; viral RNA was also detected by cobas Liat Ct value of 29.0.

The RAT detected SARS-CoV-2 antigen in 33 of the 58 positive pandemic samples (57%) but not in the remaining 25 positive pandemic samples (*orf1ab* Ct values ranging from 24.2 to 37.5). No analyses of pandemic samples with the RAT were invalid (**Table S3**).

Fig. 2 shows the POCT results for the 20 pre-pandemic samples. Cobas Liat did not indicate SARS-CoV-2 detection and no analyses were invalid. ID NOW indicated no detection of viral RNA in 15 of the pre-pandemic samples and was invalid in five samples. RAT did not detect SARS-CoV-2 in 19 of the pre-pandemic samples and was invalid in one sample (**Table S5)**.

Figure 1: Detection of SARS-CoV-2 RNA or antigen in the 78 pandemic samples with the point-of-care tests in comparison to the *orf1ab* Ct value of the comparator assay

Each triangle or square represents a BAL fluid sample obtained during the COVID-19 pandemic.

RAT, rapid antigen test.

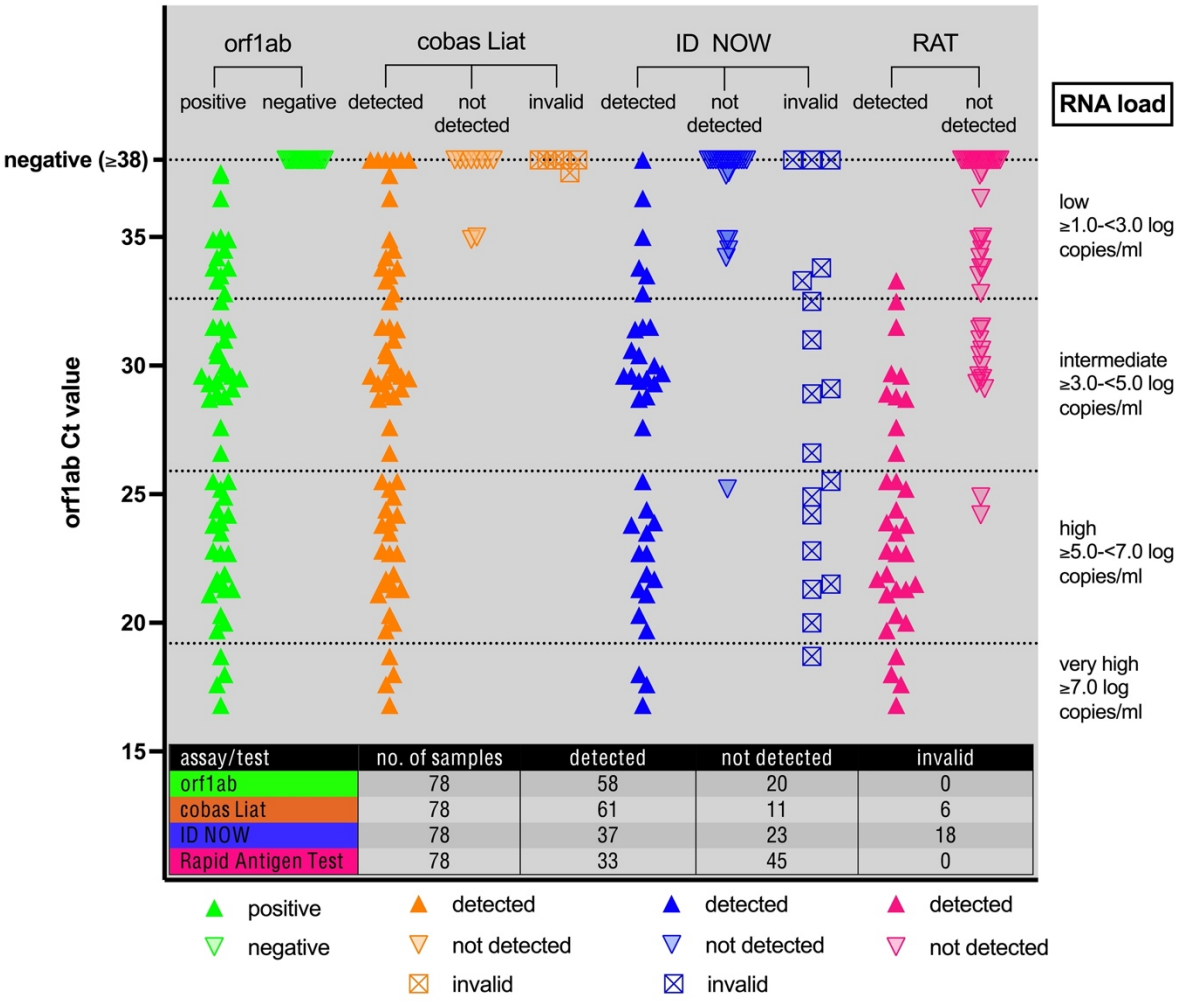


Figure 2: Detection of SARS-CoV-2 RNA or antigen in 20 pre-pandemic samples with the point-of-care tests

Each dot represents a BAL fluid sample obtained before the COVID-19 pandemic.

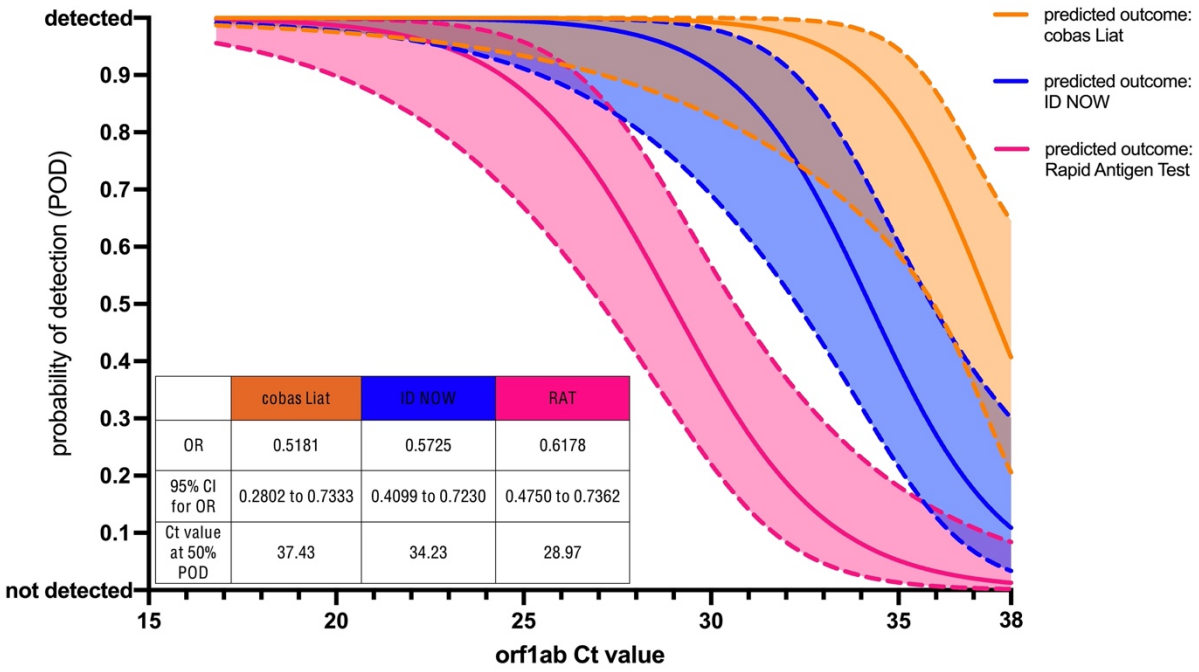
3.3 Univariate logistic regression for comparison of the point-of-care tests

To determine and visualize the performance of the POCTs, we carried out a univariate logistic regression with the *orf1ab* RT-qPCR as comparator assay. The analysis shows that each POCT can detect SARS-CoV-2 RNA or antigen in BAL fluid samples (**Fig. 3**).

The *orf1ab* Ct value with 50% probability of detection is 37.43 for cobas Liat, 34.23 for ID NOW and 28.97 for the RAT. The OR to detect SARS-CoV-2 in BAL samples is 0.5181 (95% confidence interval (CI)= 0.2802-0.7333, P<0.0001) for cobas Liat, 0.5725 (95% CI=0.4099-0.7230, P<0.0001) for ID NOW and 0.6178 (95% CI=0.4750-0.7362, P<0.0001) for the RAT.

Figure 3: Univariate logistic regression for SARS-CoV-2 RNA or antigen detection with POCTs

Each POCT is represented by a curve in the graph showing the relationship between the viral load in the pandemic samples represented by the *orf1ab* Ct value (x-axis) and the probability of SARS-CoV-2 detection (y-axis). The dashed lines represent the 95% confidence interval.



3.4 Detection of SARS-CoV-2 RNA or antigen within the course of SARS-CoV-2 infection

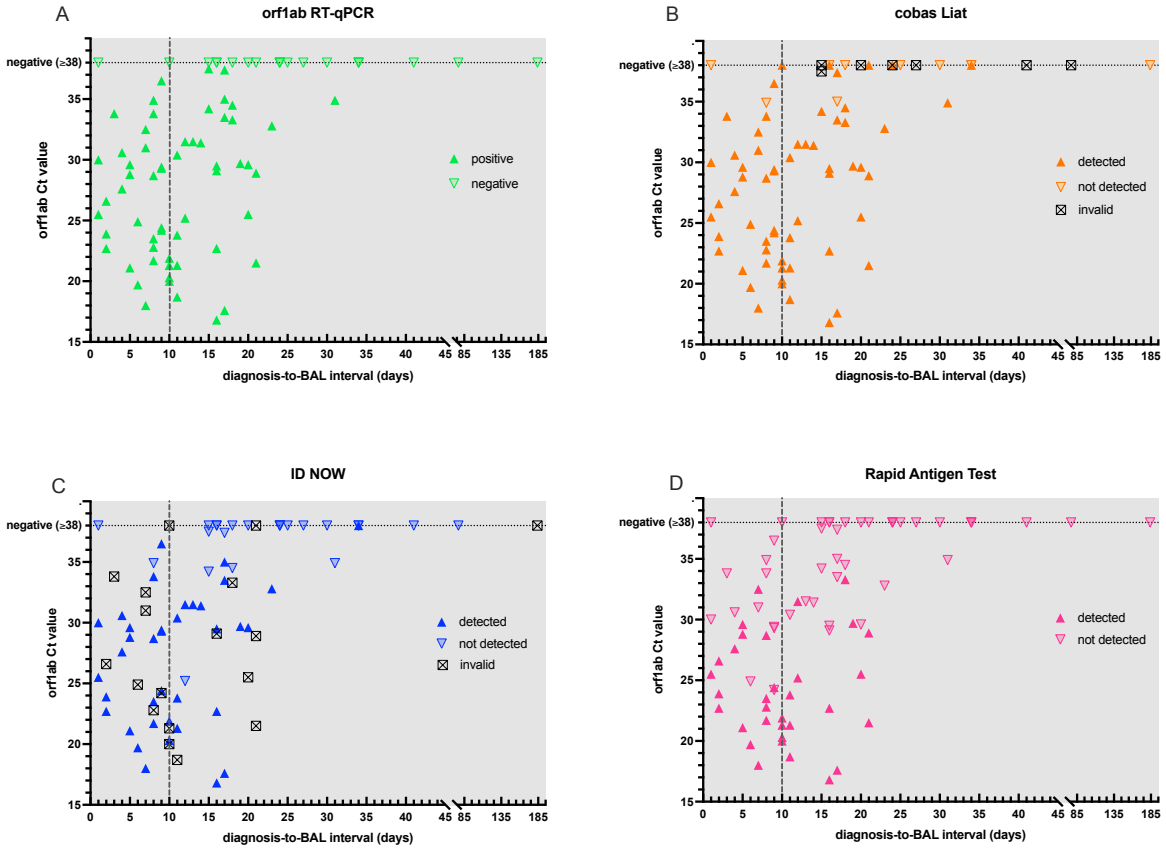
We chose to use the period in days between the time of RT-qPCR diagnosis and the time of BAL fluid sampling – the diagnosis-to-BAL interval (DBI) – as a reliable proxy for the disease duration of COVID-19. **Fig. 4** shows the relationship between DBI and the result of the reference *orf1ab* RT-qPCR and three POCTs. Infectious virus is most likely to be detected in the first 10 days of the disease course and we therefore chose a DBI of ≤10 days as a clinically relevant cut-off point (Bullard et al., 2020; Killingley et al. 2022; Puhach et al., 2023). With cobas Liat, SARS-CoV-2 was detected in 30 of 31 positive pandemic samples with a DBI ≤10 days (97%). For one negative sample with a DBI of 10 days, cobas Liat disagreed with the reference *orf1ab* RT-qPCR and did detect SARS-CoV-2. Viral RNA was detected in BAL as long as 34 days after diagnosis.

ID NOW detected SARS-CoV-2 in 21 of 31 positive samples with a DBI ≤10 days (68%). The analysis was invalid in 9 of 31 positive samples with a DBI ≤10 days (29%). Viral RNA was detected in BAL as long as 34 days after diagnosis.

RAT detected SARS-CoV-2 in 20 of 31 positive samples with a DBI ≤10 days (65%). For positive samples with DBI≤10 days and a high or very high viral load (*orf1ab* Ct≤25.9) RAT detected virus in 14 of 16 samples. For samples with a DBI ≤10 days and an intermediate or low viral load (25.9<*orf1ab* Ct <38), RAT detected antigen in 6 of 15 samples (40%). Viral protein was detected in BAL as long as 21 days after diagnosis.

Figure 4: SARS-CoV-2 detection within the course of SARS-CoV-2 infection

Each panel represents the detection of SARS-CoV-2 RNA or antigen with *orf1ab* RT-qPCR (A), cobas Liat (B), ID NOW (C), and RAT (D) as a function of the number of days between the time of RT-qPCR diagnosis of the SARS-CoV-2 infection and the time of BAL fluid sampling.



4 Discussion

To evaluate the value of POCTs for detection of SARS-CoV-2 RNA or antigen in BAL fluid, we subjected 78 pandemic samples and 20 pre-pandemic samples of unique ICU patients to analysis with cobas Liat, ID NOW, and a RAT.

Univariate logistic regression confirmed that each POCT can detect SARS-CoV-2 in BAL fluid. Cobas Liat provided the highest probability to detect SARS-CoV-2, with few invalids. Interestingly, cobas Liat detected SARS-CoV-2 RNA in 6 of the 20 putatively negative pandemic samples – these are thus samples with a Ct value ≥38 of the *orf1ab* RT-qPCR comparator assay. Explanations, which are not mutually exclusive, are: false-positive results with cobas Liat; an uneven distribution of SARS-CoV-2 RNA within the sample; or a higher sensitivity of cobas Liat compared to the *orf1ab* RT-qPCR comparator assay. ID NOW detected viral RNA in samples with both high and low RNA loads, but analyses were frequently invalid. ID NOW reported detection of SARS-CoV-2 in one negative pandemic sample. The RAT was only able to detect SARS-CoV-2 protein in samples with a high RNA load and invalid analysis occurred in one pre-pandemic sample. RAT did not report antigen detection in none of the negative pandemic samples.

Infectious virions are most likely to be detected in the URT within the first ten days after onset of symptoms (Bullard et al., 2020; Killingley et al., 2022; Puhach et al., 2023). Importantly, transferability of these data to lower respiratory tract (LRT) samples has not been studied. Within the first ten days after RT-qPCR diagnosis of SARS-CoV-2 infection, cobas Liat detected SARS-CoV-2 RNA in all but one positive pandemic sample; this sample with DBI of 8 days had an *orf1ab* Ct value of 34.9 and is therefore unlikely to have a clinical impact. Interestingly, ID NOW also did not detect viral RNA in this same sample. ID NOW analyses were invalid in 9 of 31 samples with DBI ≤10d. RAT failed to detect SARS-CoV-2 antigen in a third of the positive pandemic samples with DBI ≤10d.

246 POCTs can be an additional safety barrier to prevent donor-derived transmission of SARS-
247 CoV-2 in the setting of LTx. Transmission occurs when infectious virions are still present in
248 the donor lungs at the time of procurement (Kaul et al., 2021; Kumar et al., 2021). Therefore,
249 candidate lung donors are screened with RT-qPCR assays on URT samples and if available, on
250 LRT samples (Booker et al., 2023). However, a negative screening result does not always
251 preclude presence of infectious virions in the lungs, for at least three reasons (Mahmood et al.,
252 2021). First, donors are often only screened with URT samples, which might not contain
253 SARS-CoV-2, but infectious virions may well reside in the LRT (Kaul et al., 2021; Kumar et
254 al., 2021). Second, typically only a single test result is available, thereby providing only a
255 snapshot in the course of a possible infection. Third, as there is often an interval of several days
256 between screening and lung procurement, early infections with low viral load can be missed
257 and may lead to a scenario where, unknowingly, lungs are transplanted at or near the peak of
258 infection. POCTs can help in preventing the scenario where missed detection may result in
259 severe or fatal COVID-19 after LTx (Kaul et al., 2021; Kumar et al., 2021).
260 On the other hand, POCTs can also contribute to an unnecessary decision to decline life-saving
261 donor lungs. NAATs such as cobas Liat and ID NOW can detect the presence of low RNA
262 loads in BAL fluid samples and low RNA loads may reflect either an early phase of infection
263 or, conversely, the persistence of *SARS-CoV-2* RNA during convalescence (Puhach et al., 2023;
264 Ceuelemans et al., 2020; Ceulemans et al., 2021; Goetz et al., 2023; Jyothula et al., 2023). The
265 decision to decline lungs should therefore be based on the medical history, earlier screening
266 results, and the type of POCT that was used (Querrey et al., 2021).
267 POCT implementation during procurement relies on several practical issues. First, POCTs
268 must provide sufficient probability of detecting SARS-CoV-2 in BAL fluid samples - which
269 we here showed to be highest with cobas Liat. Second, the number of invalid results must be
270 low to avoid needles repetition of the test and inadvertent delays. In positive pandemic samples,
271 RAT analyses were never invalid, but ID NOW suffered from many invalid results. The high

fraction of invalid results for ID NOW could be explained by the incompatibility of BAL fluid and the buffer that is preloaded in the test cassette. Third, extensive logistical requirements for remote lung procurement must be avoided. The requirements for an electricity source, the set-up time, the cassette storage temperature, and the time to run the POCT must be considered. Another portable POCT assay with a favorable profile is the Vivalytic (Bosch) (De Pace et al., 2021).

Our study has several limitations. The number of frozen BAL fluid samples that were available for secondary analysis and re-testing was limited. The POCT analyses were not performed on fresh samples. We did not re-test all samples with the *orf1ab* RT-qPCR comparator assay. We evaluated the performance of POCT assays that were not designed or validated for BAL fluid samples, and the mucous aspect of the samples may affect the results and fraction of invalids. In case of an invalid analysis, we did not re-test samples, but re-testing with the same POCT or testing with another POCT is likely to be done in a real-life setting. SARS-CoV-2 is heterogeneously distributed within BAL fluid and analysis of different aliquots of the sample may have affected results of each test in this study. Parallel URT samples were not available to provide insight into the correlation with SARS-CoV-2 detection in LRT samples.

In summary, we have here shown that POCTs can be used for detection of SARS-CoV-2 RNA or antigen in BAL fluid samples. The highest probability of detection without invalids within the first 10 days after diagnosis was provided by cobas Liat. The high fraction of invalid results for ID NOW and low probability of detection with RAT render these assays less ideal for the setting of lung procurement. POCTs can be used as an additional safety barrier to intercept donor-derived SARS-CoV-2 transmission but results must be interpreted carefully, taking into account all available clinical information and previous screening results (Querey et al., 2021; Goetz et al., 2023). During the emergency phase of the COVID-19 pandemic, POCTs played

298 an important role in limiting the spread of SARS-CoV-2 among the general population.
299 Validated POCTs that can detect existing or emerging respiratory pathogens in BAL fluid
300 samples could be an additional safeguard in the prevention of donor-derived postoperative
301 infections after LTx.
302

Declaration of competing interest

The authors declare they have no competing interests.

Author Contributions

Conceptualization: JVS, AV, KL, LJC

Methodology: JVS, CS, LL, XJ, KB, AV, PM, KL, LJC

Formal analysis: JVS, CS, GM, LJC

Supervision: LJC

Visualization: JVS, CS

Writing - original draft: JVS, CS, LJC

Writing - review & editing: LL, KB, AV, JW, GM, BMV, RV, PM, KL

Acknowledgements

The authors thank Nicole Jannis (KU Leuven, Belgium) and Yo Vande Broeck (University Hospitals Leuven, Belgium) for their help with collecting and processing of samples.

Funding statement

This work was supported by the Fund Academic Research (KOOR) of University Hospitals Leuven.

Supplementary material

Tables S1-5

References

Booker SE, Jett C, Fox C, Anesi JA, Berry GJ, Dunn KE, et al. OPTN required SARS-CoV-2 lower respiratory testing for lung donors: Striking the balance. Transpl Infect Dis. 2023;25:e14048.

Bullard J, Dust K, Funk D, Strong JE, Alexander D, Garnett L, et al. Predicting infectious Severe Acute Respiratory Syndrome Coronavirus 2 from diagnostic samples. Clin Infect Dis. 2020;71:2663-2666.

Ceulemans LJ, Khan M, Yoo S-J, Zapiec B, Van Gerven L, Van Slambrouck J, et al. Persistence of SARS-CoV-2 RNA in lung tissue after mild COVID-19. Lancet Respir Med. 2021;9:315-318.

Ceulemans LJ, Van Slambrouck J, De Leyn P, Decaluwé H, Van Veer H, Depypere L, et al. Successful double-lung transplantation from a donor previously infected with SARS-CoV-2. Lancet Respir Med. 2020;9:315-318.

Cuypers L, Bode J, Beuselinck K, Laenen L, Dewaele K, Janssen R, et al. Nationwide harmonization effort for semi-quantitative reporting of SARS-CoV-2 PCR test results in Belgium. Viruses. 2022;14:1294.

De Pace V, Caligiuri P, Ricucci V, Nigro N, Galano B, Visconti V, et al. Rapid diagnosis of SARS-CoV-2 pneumonia on lower respiratory tract specimens. BMC Infect Dis. 2021;21:926.

Dinnes J, Sharma P, Berhane S, van Wyk SS, Nyaaba N, Domen J, et al. Rapid, point-of-care antigen tests for diagnosis of SARS-CoV-2 infection. Cochrane Database Syst Rev. 2022;7:Cd013705.

Goetz RL, Nellore A, Geer JT, Rusanov V, Wille K, Scullin D, et al. Bilateral lung transplantation with donor positive for COVID-19 infection on bronchoalveolar lavage: A case report. Transplant Proc. 2023;55:540-542.

Jyothula S, Patel J, Dhand A. Successful bilateral lung transplantation from a recent SARS-CoV-2 PCR positive donor. Transpl Infect Dis. 2023;25:e14031.

Kaul DR, Valesano AL, Petrie JG, Sagana R, Lyu D, Lin J, et al. Donor to recipient transmission of SARS-CoV-2 by lung transplantation despite negative donor upper respiratory tract testing. Am J Transplant. 2021;21:2885-2889.

Killingley B, Mann AJ, Kalinova M, Boyers A, Goonawardane N, Zhou J, et al. Safety, tolerability and viral kinetics during SARS-CoV-2 human challenge in young adults. Nat Med. 2022;28:1031-1041.

Kumar D, Humar A, Keshavjee S, Cypel M. A call to routinely test lower respiratory tract samples for SARS-CoV-2 in lung donors. Am J Transplant. 2021;21:2623-2624.

Mahmood K, Abbott M, Van Nostrand K, Bechara R, Gonzalez AV, Brucker A, et al. Low utilisation of bronchoscopy to assess COVID-19 respiratory infection: a multicenter experience. BMJ Open Respir Res. 2021;8:e000962.

Puhach O, Meyer B, Eckerle I. SARS-CoV-2 viral load and shedding kinetics. Nat Rev Microbiol. 2023;21:147-61.

Querrey M, Kurihara C, Manerikar A, Garza-Castillon R, Lysne J, Tomic R, et al. Lung donation following SARS-CoV-2 infection. Am J Transplant. 2021;21:4073–8.

Raymenants J, Geenen C, Budts L, Thibaut J, Thijssen M, De Mulder H, et al. Indoor air surveillance and factors associated with respiratory pathogen detection in community settings in Belgium. Nat Commun. 2023;14:1332.

Schildgen V, Demuth S, Lüsebrink J, Schildgen O. Limits and Opportunities of SARS-CoV-2 Antigen Rapid Tests: An Experienced-Based Perspective. Pathogens. 2021;10:38.

Ushiro-Lumb I, Callaghan C, Parmar J, Olsburgh J, Berman M, Currie I, et al. Screening for SARS-CoV-2 in potential deceased organ donors. Am J Transplant. 2021;21:3204-5.

Van Slambrouck J, Van Raemdonck D, Wauters J, Vos R, Mombaerts P, Ceulemans LJ. Lung donation and SARS-CoV-2 transmission: Missed detection versus missed opportunity? Immunity, Inflammation and Disease. 2022;10:e603.

Vanlerberghe B, Vrij C, Bogaerts K, Vermeersch P, Lagrou K, Molenberghs G, et al. SARS-CoV-2 vaccine antibody response and breakthrough infections in transplant recipients. J Med Virol. 2023;95:e28736.

Wolters F, Van De Bovenkamp J, Van Den Bosch B, Van Den Brink S, Broeders M, Chung NH, et al. Multi-center evaluation of cepheid xpert® xpress SARS-CoV-2 point-of-care test during the SARS-CoV-2 pandemic. J Clin Virol. 2020;128:104426.