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Exhaled breath SARS-CoV-2 shedding patterns across variants of concern

Joren Raymenants[1,8,*], Wout Duthoo[3], Tim Stakenborg[4], Bert Verbruggen[3], Julien Verplanken[5], Jos Feys[6], Joost Van Duppen[4], Rabea Hanifa[4], Elisabeth Marchal[4], Andy Lambrechts[3], Piet Maes[7], Emmanuel André[1,2], Nik Van den Wijngaert[3], Peter Peumans[4]

[1] *Laboratory of Clinical Microbiology, Department of Microbiology, Immunology and Transplantation, KU Leuven, 3000, Leuven, Belgium*
[2] *Department of laboratory medicine, University Hospitals Leuven, 3000, Leuven, Belgium*
[3] *Imec Solutions department, imec, 3001, Leuven, Belgium*
[4] *Life Science Technologies department, imec, 3001, Leuven, Belgium*
[5] *Enabling Digital Transformations department, imec, 9000, Ghent, Belgium*
[6] *Department of Clinical and Epidemiological Virology (Rega Institute), 3000, Leuven, Belgium*
[7] *KU Leuven, 3000, Leuven, Belgium*
[8] *Department of general internal medicine, University Hospitals Leuven, 3000, Leuven, Belgium*

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ABSTRACT

Objectives: We performed exhaled breath (EB) and nasopharyngeal (NP) quantitative polymerase chain reaction (qPCR) and NP rapid antigen testing (NP RAT) of SARS-CoV-2 infections with different variants.
Methods: We included immuno-naïve alpha-infected (n = 11) and partly boosted omicron-infected patients (n = 8) as high-risk contacts. We compared peak NP and EB qPCR cycle time (ct) values between cohorts (Wilcoxon-Mann-Whitney test). Test positivity was compared for three infection phases using Cochran Q test.
Results: Peak median NP ct was 11.5 (interquartile range [IQR] 10.1-12.1) for alpha and 12.2 (IQR 11.1-15.3) for omicron infections. Peak median EB ct was 25.2 (IQR 24.5-26.9) and 28.3 (IQR 26.4-30.8) for alpha and omicron infections, respectively. Distributions did not differ between cohorts for NP ($P = 0.19$) or EB ($P = 0.09$). SARS-CoV-2 shedding peaked on day 1 in EB (confidence interval [CI] 0.0 - 4.5) and day 3 in NP (CI 1.5 - 6.0). EB qPCR positivity equaled NP qPCR positivity on D0-D1 ($P = 0.44$) and D2-D6 ($P = 1.0$). It superseded NP RAT positivity on D0-D1 ($P = 0.003$) and D2-D6 ($P = 0.008$). It was inferior to both on D7-D10 ($P < 0.001$).
Conclusion: Peak EB and nasopharynx shedding were comparable across variants. EB qPCR positivity matched NP qPCR and superseded NP RAT in the first week of infection.

Introduction

Both virus characteristics and host response influence SARS-CoV-2 transmission. The more transmissible and immune evasive variants of concern (VOC) shape the former (Liu and Rocklöv, 2021; Nishiura *et al.*, 2022; Planas *et al.*, 2021a, Planas *et al.*, 2021b, Planas *et al.*, 2022; Willett *et al.*, 2022), whereas, vaccine-and natural infection-based immunity and their waning shape the latter (Feikin *et al.*, 2022).

Consecutive VOCs show increased intrinsic transmissibility and a shorter incubation period (; Homma *et al.*, 2021; Liu and Rocklöv, 2021; Nishiura *et al.*, 2022). However, the underlying mechanism is not completely understood. An increase in environmental stability is not thought to be a driver (Arav *et al.*, 2021). In contrast, alpha, delta and omicron VOCs all show enhanced infection of host cells (Ramanathan *et al.*, 2021; Syed *et al.*, 2021; Willett *et al.*, 2022). To assess whether increased shedding from the respiratory tract contributes to increased transmission, studies

* Corresponding author: Joren Raymenants, Herestraat 49, 3000 Leuven, Belgium.
E-mail address: Joren.raymenants@kuleuven.be (J. Raymenants).

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used quantitative polymerase chain reaction (qPCR) ct values from swab-based upper respiratory samples (Abu-Raddad *et al.*, 2021; Blanquart *et al.*, 2021; Thompson *et al.*, 2021; Kissler *et al.*, 2021; Kissler *et al.*, 2022; Levine-Tiefenbrun *et al.*, 2021; Pajon *et al.*, 2022; Pouwels *et al.*, 2021). Samples from patients with high viral loads in upper respiratory tract samples were significantly more infectious in one study (Marks *et al.*, 2021). There was a trend in another (Julin *et al.*, 2021). These studies, however, face several difficulties. First, ct values are a poor measure of the concentration of infectious viruses (Puhach *et al.*, 2022). Second, COVID-19 is mostly aerosol transmitted (Greenhalgh *et al.*, 2021; Tang *et al.*, 2021) and viral shedding patterns differ between respiratory compartments (Alsved *et al.*, 2022; Chen *et al.*, 2021b). Third, vaccination and natural infections are difficult to account for (Feikin *et al.*, 2022).

Most SARS-CoV-2 transmission takes place early in the infection (Ge *et al.*, 2021; Meyerowitz *et al.*, 2020). This is also when infectious virus is most frequently isolated (Puhach *et al.*, 2022). Unfortunately, current diagnostic tests do not reflect this pattern of contagiousness. Rapid antigen tests (RAT) lack sensitivity in the early phase of infection (Smith *et al.*, 2021). qPCR on upper respiratory samples remains positive long after contagiousness has subsided (Yan *et al.*, 2021). qPCR on exhaled breath (EB) may be a more effective physiological sampling method to discern contagiousness directly (Giovannini *et al.*, 2021). Several studies compared EB qPCR to other respiratory samples and tests (Alsved *et al.*, 2022; Ma *et al.*, 2021; Malik *et al.*, 2021; Ryan *et al.*, 2021; Sawano *et al.*, 2021). EB qPCR results were more often positive early in the infection (Alsved *et al.*, 2022; ; Ma *et al.*, 2021; Malik *et al.*, 2021), associated with lower respiratory involvement (Sawano *et al.*, 2021) and had diagnostic value complementary to swab-based upper respiratory samples (Ryan *et al.*, 2021). One study linked EB positivity to increased transmission among household members (Alsved *et al.*, 2022). EB qPCR sensitivity has thus far failed to match reference diagnostic methods. No study has compared SARS-CoV-2 viral shedding patterns across VOCs.

In a series of cohort studies, we assessed whether increased EB shedding can explain the increased transmission of consecutive SARS-CoV-2 VOCs. We also assessed whether EB qPCR positivity can match that of nasopharyngeal (NP) qPCR and supersede that of NP RAT in the first week of infection.

Methods

Study design

This study compared SARS-CoV-2 shedding in the upper and lower respiratory tract for different VOCs. NP qPCR and NP RAT measured the viral shedding in the upper respiratory tract while shedding in the lower respiratory tract was measured using EB qPCR.

The alpha cohort consisted of 11 immuno-naïve patients, followed between April and June 2021. High-risk contacts of confirmed COVID-19 patients underwent EB qPCR and saliva qPCR (N = 68) once daily. NP qPCR and NP RAT tests were performed alternately. SARS-CoV-2 antibodies and prior vaccination lead to exclusion. After the first positive test, participants underwent saliva and NP qPCR testing once or twice daily. Inclusion within days after exposure and a negative qPCR test before inclusion ensured early detection of the infection (Stakenborg *et al.*, 2021; Raymenants *et al.*, 2022). Symptoms were not recorded. Follow-up stopped after patients tested negative for EB qPCR for at least two consecutive days.

The omicron cohort comprised eight fully vaccinated adults, of which two had received a booster, followed between December 2021 and January 2022. High-risk contacts of confirmed COVID-19 patients underwent NP qPCR, EB qPCR and NP RAT (N = 39) once a day until infection or exclusion. Inclusion within days after exposure ensured early detection of the infection. Follow-up of infected participants stopped as soon as the viral loads showed a clear downward trend in the first five participants and until EB qPCR showed negative results for at least two consecutive days in the rest.

The inclusion of vaccinated delta-infected patients (n = 18) took place only after diagnosis. Analyses involving delta patients are therefore discussed in supplementary material.

Sampling methods

Standard flocked cotton swabs were used for NP qPCR samples. They were collected in 1 and 5 ml zymo-medium (Zymo Research). Abbott Panbio™ COVID-19 Ag Rapid Test Devices were used for point-of-care NP RAT.

We used a silicon chip-based solid impactor with 85% capture efficiency for particles > 300 nm diameter at flow rate of 0.6 l/s to collect EB samples. Participants exhaled through the device for 1 min while vocalizing (producing the letter “e” or “u”). They exhaled into the mouthpiece and inhaled through the nose or away from the device. Subjects could stop at any moment during the process if they experienced discomfort. Vocalization was the most sensitive breathing protocol in a previous study (Stakenborg *et al.*, 2021). Samples were taken in duplicate. If both were positive, the ct values were averaged. If one was positive, that ct value was the result.

Analysis methods

A positive Abbott anti-N IgG antibody assay on Abbott Architect indicated a possible previous infection and consequent natural immunity. The RT-qPCR protocol for the nasopharyngeal, saliva and breath samples has been described (Stakenborg *et al.*, 2021). Whole genome sequencing was performed for VOC confirmation on at least one NP sample per participant using the ARTIC Network protocol v3.17 and with Oxford Nanopore Technologies ARTIC library preparation. Complete sequences were recovered using the ARTIC analysis pipeline and were typed using Pangolin and NextClade. A time-calibrated maximum likelihood phylogenetic tree was built using IQ-TREE v2.0.3.19 (GTR model) and TimeTree v0.8.4.22. If sequencing failed, typing was based on an epidemiological link and S-gene target failure (Cuypers *et al.*, 2022).

Study outcomes

Peak viral shedding measured by ct values, was compared between patients with alpha and omicron variants for both EB and NP.

We compared the viral proliferation phase, measured by time to peak shedding, between the upper (NP) and lower (EB) respiratory compartment in alpha and omicron patients combined.

In an exploratory analysis, the time to viral clearance was compared between alpha and omicron patients in EB only. It was computed by taking the time from first positive test to a second test with declining viral load.

Test positivity of EB qPCR, NP qPCR and NP RAT was compared against aggregate positivity in the alpha and omicron cohorts combined. Stratification by test day enabled comparison in different infection phases. Saliva samples were not considered. Positivity was compared between tests in three phases: D0-D1, D2-D6, D3-D10. Scarcity of data on day 11 and over led to exclusion of these data points. See supplementary material for an analysis including the delta cohort.

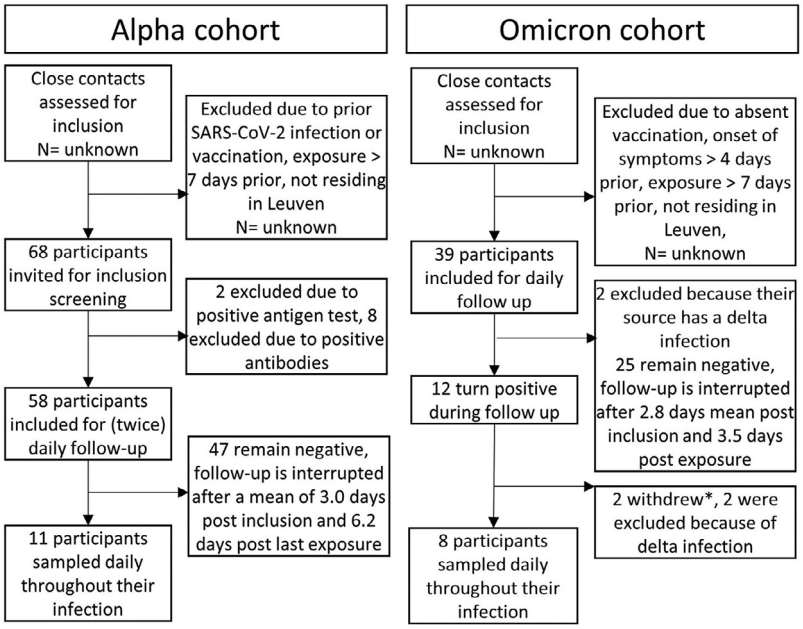


Figure 1. Inclusion flowchart of the alpha and omicron cohorts.
In the alpha cohort, 68 non-vaccinated high-risk contacts were included a mean of 3.1 days (range of 1 to 6) after last exposure. They underwent NP qPCR, NP RAT and EB qPCR (in duplicate), saliva qPCR and blood antibody testing. We excluded two subjects because they had a positive RAT and a history suggestive of late-stage infection. The presence of antibodies led to exclusion of eight. The remaining 58 subjects were followed daily for 5 to 7 days after exposure. A total of 11 tested positive during the follow-up. In the first six positive subjects, sampling of saliva and EB took place twice daily. NP sampling took place once daily. Intermediate data analysis showed very similar ct values in samples taken on the same day. This prompted a switch to sampling of subjects once daily for all diagnostic tests. In the omicron cohort, 39 vaccinated recent close contacts were included a mean of 1.6 days (range of 0 to 5) after last exposure. They underwent NP RAT, NP qPCR and EB qPCR (in duplicate) once daily. The detection of a delta infection in index cases led to the exclusion of their two participating contacts. The remaining high-risk contacts were excluded on average after 1-4 days (mean of 2.84) of follow-up because they remained negative. Twelve individuals tested positive. Two of them withdrew consent after one day. Two had a delta infection, which led to exclusion. The number of available breath samplers determined the size of the cohorts. *We included data from the two participants who withdrew consent in the analysis of positivity of different samples (Figure 5).
EB, exhaled breath; NP, nasopharyngeal; NP RAT, NP rapid antigen testing.

Statistical analysis

All statistical analyses were performed using either R Statistical Software version 4.1.1 or Python.

We used Wilcoxon-Mann-Whitney test to compare peak ct values of EB and NP between the alpha and omicron cohorts. Bootstrap method was used to calculate the median viral proliferation phase and the 95% confidence interval (CI) in EB and NP. A total of 1000 iterations of resampling took place with six participants per sample.

The same bootstrap method was also used to calculate the median time of the second day of viral load decline in EB in alpha and omicron patients separately, and the 95% CI.

To compare test positivity of in three phases, Cochran's Q test was first performed on all tests in all phases combined. Pairwise Cochran's Q test was performed for phases with a significant difference between tests. Bonferroni correction was applied for multiple hypothesis testing (alpha = 0.00625). Supplementary material shows the analysis including delta patients.

Results

Patient characteristics

Figure 1 shows the inclusion and exclusion of patients in the alpha and omicron cohorts. Table 1 lists the characteristics of included patients in both the cohorts. The mean age was lower in the omicron compared with the alpha cohort. The majority was male in both the cohorts. Natural immunity was not assessed in the omicron cohort but was the exclusion criteria for the alpha cohort. Complete vaccination led to exclusion in the alpha cohort. In contrast, it was a prerequisite in omicron patients. While symptoms were not assessed in the alpha cohort, they were present in nine of ten omicron patients at inclusion or during follow-up. No participants were excluded due to severe illness, and all remained ambulatory throughout follow-up. Extended Figures 2 and 3 show the individual viral shedding profiles. Extended Figures 1, 4 and extended Data table 1 show the inclusion and exclusion criteria of delta patients, their characteristics and individual shedding profiles.

Distinct viral shedding from the nasopharynx and lower respiratory compartment

Viral shedding patterns for the two cohorts show that ct values are generally higher in EB than in NP. Also, EB viral loads decrease before NP viral loads (Figures 2 and 3). The median time to peak viral shedding was similar in EB (median 1.0, lb 0.0 - 4.5) and NP (median 3.0, lb 1.5 - 6.0). However, the small sample size and resulting wide CIs limit the strength of this assessment (Figure 3).

Comparison of peak NP and EB viral shedding in the alpha and omicron cohorts

Peak viral shedding was similar in EB and NP in both cohorts. The median peak NP ct value was 11.5 (interquartile range [IQR] 10.1 to 12.1) in the alpha cohort, and 12.2 (IQR 11.1 to 15.3) in the omicron cohort. Their distribution did not differ significantly (Wilcoxon W = 27.5, $P = 0.19$; $P = 0.34$ after outlier exclusion). For EB, the median peak ct value was 25.2 (IQR 24.5 to 26.9) in

Table 1
Characteristics of the patients included in the alpha and omicron cohorts.
The alpha cohort consisted mostly of male young adults (mean age of 21). None of the included individuals had previously been vaccinated or had natural immunity. All had recent exposure to a confirmed case of COVID-19 and had a negative NP qPCR test in the 1 to 4 (mean 1.64) days before inclusion. Follow-up was 10 to 15 days (mean of 12 days) after the first diagnostic test turned positive. The omicron cohort consisted mostly of male adults (mean age of 37). All were vaccinated between 27-174 (mean 138) before inclusion and two had received a booster dose 2 and 17 days before inclusion. Natural immunity was not assessed. All had recent exposure to a confirmed case of COVID-19. Follow-up was 5-11 days (mean 7.8), excluding the two participants who withdrew after day 1. WGS was successful in all but two individuals, who were classified as being an omicron infection based on an epidemiological link and S-gene target failure. Nine presented with symptoms either at inclusion or during follow-up.

Cohort	Alpha (Apr - Jun 2021)	Omicron (Dec 2021 – Jan 2022)
Demographics		
Gender	4 F, 7M	3 F, 7M
Age	18-24 (mean 21)	28-56 (mean 37)
Immune status		
Natural infection	0/11	Not assessed
Fully vaccinated (single full course)	0/11	8/8 (7 BNT162b2, 3 mRNA-1273)
Time since full vaccination (days)*	NA	27-174 (mean 138)
Booster mRNA-1273	NA	2/8
Time since booster (days)	NA	7 and 17
Viral parameters		
SARS-CoV-2 phenotype*	All b.1.1.7	All b.1.1.529 BA.1
Clinical parameters		
Symptomatic during follow up	Not assessed	9/10 (feverishness 2, couch 4, breathing difficulties 3, sore throat 4, muscle/body ache 1, headache 4, new loss of smell 0, congestion or runny nose 4, nausea or vomiting 0, diarrhea 0, unusual fatigue 1)
Phase of infection monitored		
Negative test prior to inclusion	11/11	2/10
Last negative test to inclusion	1-4 (mean 1.64)	1 and 3
Symptom onset to inclusion	NA	3 before – 2 after (mean 0.57 after)
Risk contact identified	11/11	9/10
Time from exposure to inclusion	1-6 (mean 3.1)	0-5 (mean 2.7)
First positive test to last sample	10-15 (mean 12)	1-11 (mean 6.7)

NP, nasopharyngeal; WGS, Whole genome sequencing.

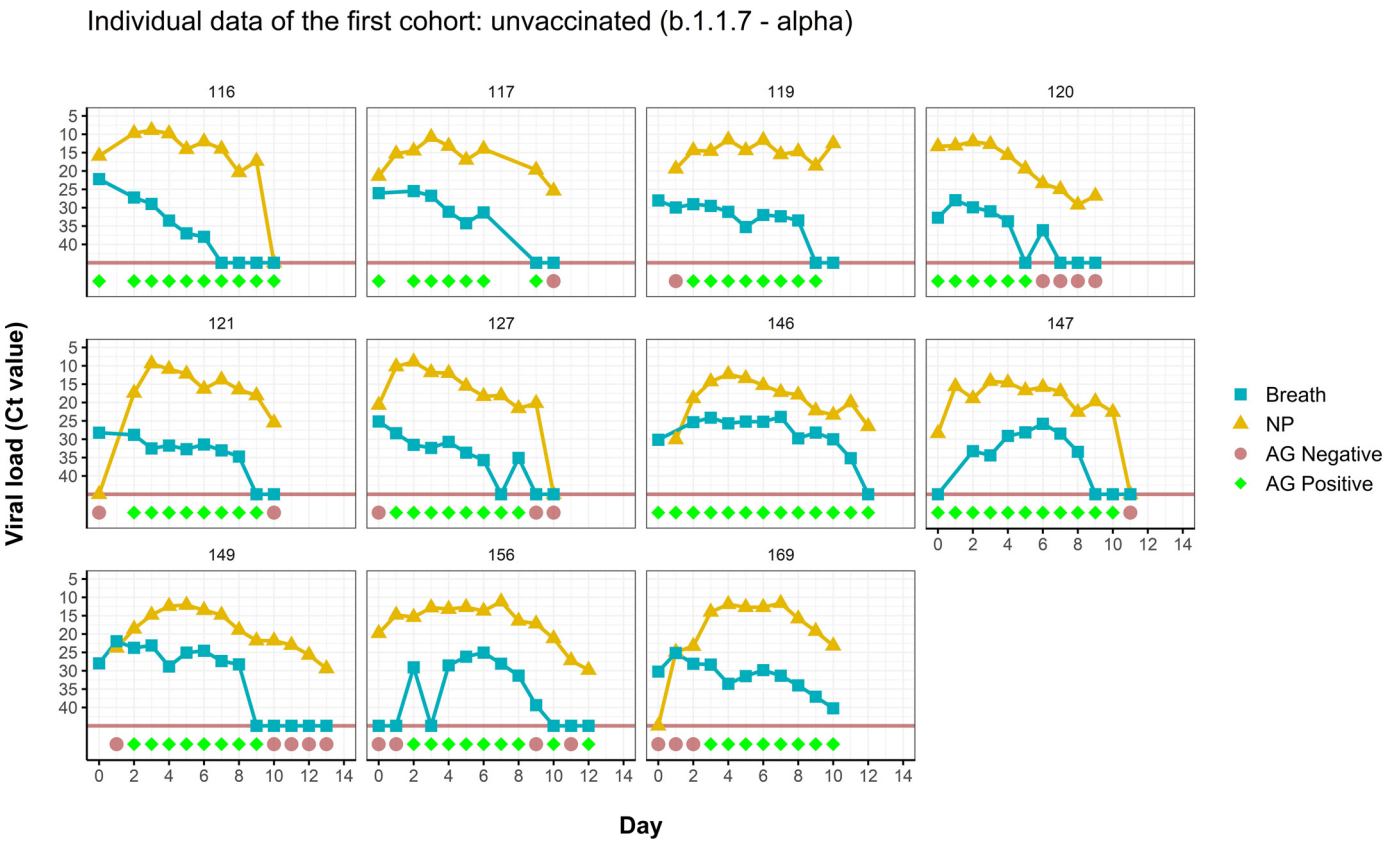


Figure 2. EB and NP shedding patterns, comparison between cohorts. The figure shows EB (top) and NP (bottom) qPCR ct values for the different cohorts (alpha vs. omicron patients). They are stratified by day since first positive test. The dotted line represents the day EB peaked, the dashed line the day the NP peaked. The horizontal red line represents negative tests.
EB, exhaled breath; NP, nasopharyngeal.

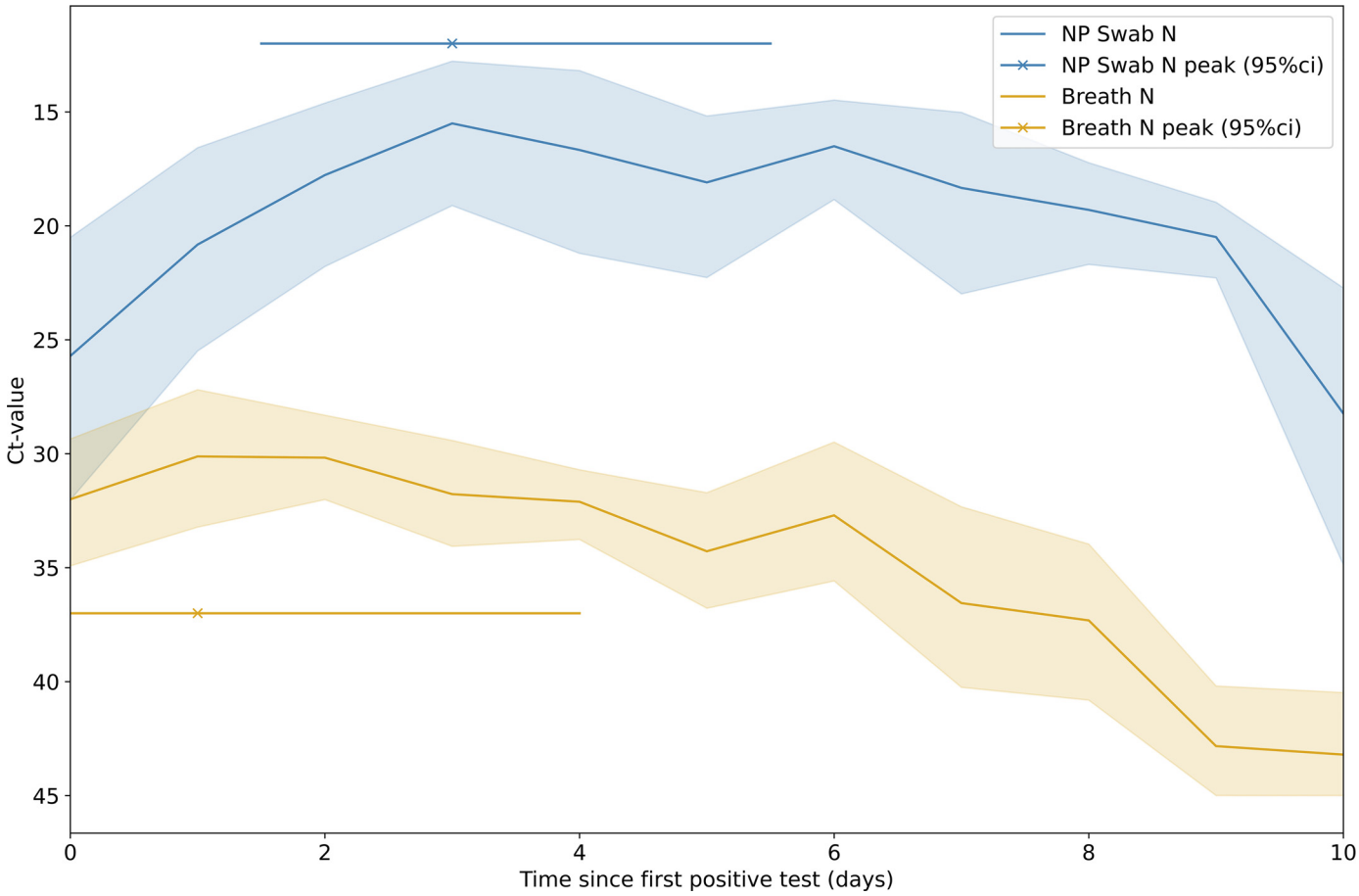


Figure 3. EB and NP shedding patterns, comparison of time to peak shedding across cohorts. This graph shows the average ct values and 95% CIs for the NP (top) and EB (bottom) samples of all participants in the alpha and omicron cohorts, stratified by day since first positive test. The median time to peak viral shedding was similar in EB and NP, as demonstrated by overlapping CIs.
CI, confidence interval; EB, exhaled breath; NP, nasopharyngeal.

the alpha cohort and 28.3 (IQR 26.4 to 30.8) in the omicron cohort. There was no significant difference between both (Wilcoxon W = 23, $P = 0.09$) (Figure 4). In an exploratory analysis, we compared the time from the first positive test until the second day of declining viral load in EB between the alpha and omicron cohorts. They had wide and overlapping CIs, with a median of 4.0 (CI 3.0-8.0) in the former and 3.0 days (CI 2.5-5.0) in the latter.

EB qPCR positivity equals that of NP qPCR and is superior to that of NP RAT in the first week of infection

The positivity of EB qPCR, NP qPCR and NP RAT was compared with aggregate positivity in three infection phases: D0-1, D2-6, D6-10 since first positive test. The analysis combined data from the alpha and omicron cohorts (*Figure 5*). Cochran's Q test revealed a significant difference in positivity between the three test types for the whole dataset ($P < 0.001$) and the three phases ($P = 0.002$ for D0-1, 0.001 for D2-6 and $P < 0.001$ for D6-10). Pairwise comparison between EB qPCR and NP qPCR showed superior positivity of NP qPCR across the whole dataset ($P < 0.001$). Yet, EB qPCR had equal positivity on D0-1 ($P = 0.44$) and D1-6 ($P = 1.0$). In contrast, its positivity was inferior in the days after ($P < 0.001$). Pairwise comparison between EB qPCR and NP RAT showed equal positivity across all periods ($P = 0.42$). However, EB qPCR had superior positivity on D0-1 ($P = 0.003$) and D1-6 ($P = 0.008$). Its positivity was inferior thereafter ($P < 0.001$) (Figure 5). Inclusion of the delta cohort led to similar results (see Extended Data Figure 5).

Discussion

In a series of cohort studies, we used EB samples to assess two questions about COVID-19 transmission. The first was whether increased EB shedding can explain the increased transmission of consecutive SARS-CoV-2 VOCs and the second was whether EB positivity can match the positivity of NP qPCR and supersede that of NP RAT in the first week of infection.

Peak exhaled breath viral shedding was similar across VOCs

Studies using swabs from upper respiratory samples have given a mixed picture. It is unclear whether increased viral shedding from the respiratory tract leads to higher transmission of more recent VOCs. When comparing both alpha and delta infections to preceding VOCs, both higher and equal peak viral loads have been detected in unvaccinated individuals (Blanquart *et al.*, 2021; Julin *et al.*, 2021; Kissler *et al.*, 2021; Singanayagam *et al.*, 2022; Wang *et al.*, 2021). A study showed that unvaccinated individuals have lower viral loads in delta versus pre-delta infections, even though individuals had higher titers of infectious viruses (Puhach *et al.*, 2022). Vaccination generally reduces the peak and duration of shedding immediately after an immunity boost. (Blanquart *et al.*, 2021; Julin *et al.*, 2021; Kissler *et al.*, 2021; Singanayagam *et al.*, 2022; Wang *et al.*, 2021). Time-dependent immune waning as well as increased transmissibility and immune escape counteract this effect (Blanquart *et al.*, 2021; Levine-Tiefenbrun *et al.*, 2021; Pouwels *et al.*, 2021). A report on omicron-infected patients

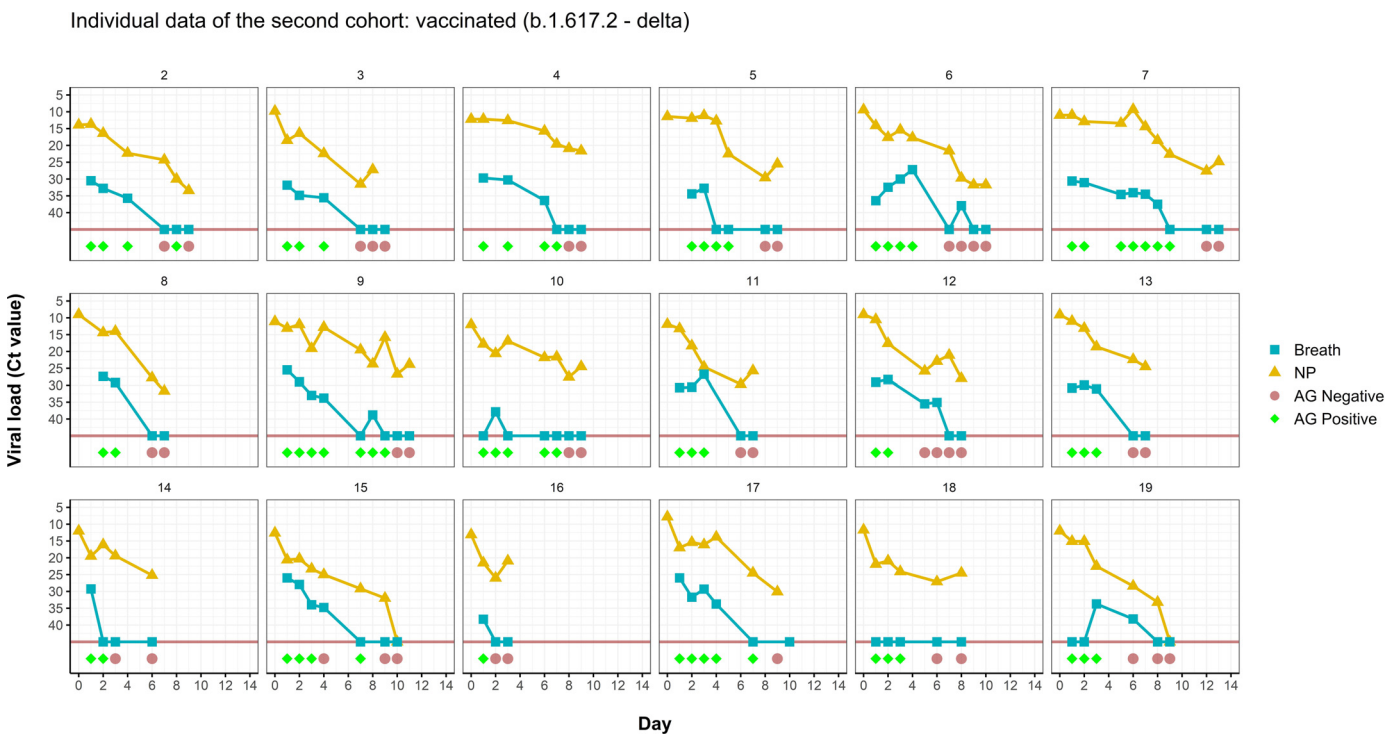


Figure 4. Comparing EB and NP peak viral loads between alpha and omicron cohorts, measured by qPCR ct values. This figure shows the peak viral loads expressed as qPCR ct values in both EB and NP in the immuno-naïve alpha-infected cohort (n = 11) and the partly boosted omicron-infected cohort (n = 8). Median peak viral loads are added. EB, exhaled breath; NP, nasopharyngeal.

showed significantly lower peak viral shedding and shorter clearance times compared to delta variant-infected patients. Unfortunately, the immune statuses of the patients were not accounted for (Kissler *et al.*, 2022).

This study is the first to compare EB and NP SARS-CoV-2 shedding between cohorts infected with different VOCs and immune statuses. Our results show equal peak shedding in partly boosted omicron-infected and an immuno-naïve alpha-infected patients as measured by both NP qPCR ($P = 0.19$; $P = 0.34$ after outlier exclusion) and EB qPCR ($P = 0.09$). The *P*-value of the latter suggests a trend toward lower peak EB SARS-CoV-2 shedding in omicron infections. Several possible explanations exist. First, omicron-infected individuals may shed a similar or lesser number of viral particles of more infectious viruses. Second, vaccination and/or natural infection may have been the cause of decline in viral shedding. Third, omicron-infected patients may have been included later in their infection. In an exploratory analysis, we compared the time from first positive test result to the second day of viral load decline in EB. CIs were wide and overlapped, but there was no trend toward longer shedding in the omicron cohort. These results provide evidence that reasons other than higher or longer viral shedding from the lower respiratory tract explain the increased transmission of recent VOCs. Enhanced infections of host cells likely play a role (Ramanathan *et al.*, 2021; Syed *et al.*, 2021; Willett *et al.*, 2022).

EB qPCR is highly sensitive in the first week of infection

SARS-CoV-2 transmission occurs almost exclusively in the first week of infection. A test for infectiousness should therefore detect the infection early and return a negative result after the first week (2020; Ge *et al.*, 2021; Meyerowitz *et al.*, Puhach *et al.*, 2022). While a RAT lacks the former (early detection), qPCR on a swab-based upper respiratory sample lacks the latter (Smith *et al.*, 2021; Yan *et al.*, 2021). Several studies have compared EB qPCR to other respiratory samples as it may be a more effective physiological test to detect infectiousness (Alsved *et al.*, 2022; Malik *et al.*, 2021; Ma *et al.*, 2021; Ryan *et al.*, 2021; Sawano *et al.*, 2021). In these studies, EB was positive only during early infection (Alsved *et al.*, 2022; Ma *et al.*, 2021; Malik *et al.*, 2021) and was associated with higher household transmission (Alsved *et al.*, 2022). Unfortunately, EB qPCR sensitivity lagged behind reference tests (Alsved *et al.*, 2022; Ryan *et al.*, 2021; Ma *et al.*, 2021; Malik *et al.*, 2021; Sawano *et al.*, 2021). This could be because of technical aspects of sample collection and analysis, clinical presentation and timing of follow-up and/or the use of tidal breathing. Tidal breathing is known to generate a lower number and volume of aerosols than other maneuvers (Shen *et al.*, 2022). It was the least sensitive of four maneuvers in Alsved *et al.* (2022) a previous comparison that used the same sampling and analysis methods as the current study (Stakenborg *et al.*, 2021). Our findings are in accordance with the previous studies showing that viral loads are generally lower in EB than NP. We did not see a difference in time of peak viral shedding between EB and NP, which might be due to the limited sample size. Our results were consistent with previous results showing that viral loads were highest in several respiratory samples shortly before and after the symptom onset (Chen *et al.*, 2021a). For the first time however, we showed that EB qPCR positivity can match that of NP qPCR and supersede that of NP RAT against aggregate positivity in the first week of infection. After the first week, positivity declined for both EB qPCR and NP RAT, while it remained for NP qPCR. This unique pattern of EB qPCR is compatible with a test for infectiousness (Figure 6). This study demonstrates that the design flexibility, portable nature, and sensitivity of a silicon chip-based solid impactor improves the quantitative assessment of EB bioaerosol loads. This may also improve transmission models incorporating such assessments for COVID-19 and other pathogens (Chen *et al.*, 2021a; Shen *et al.*, 2022; Stakenborg *et al.*, 2021).

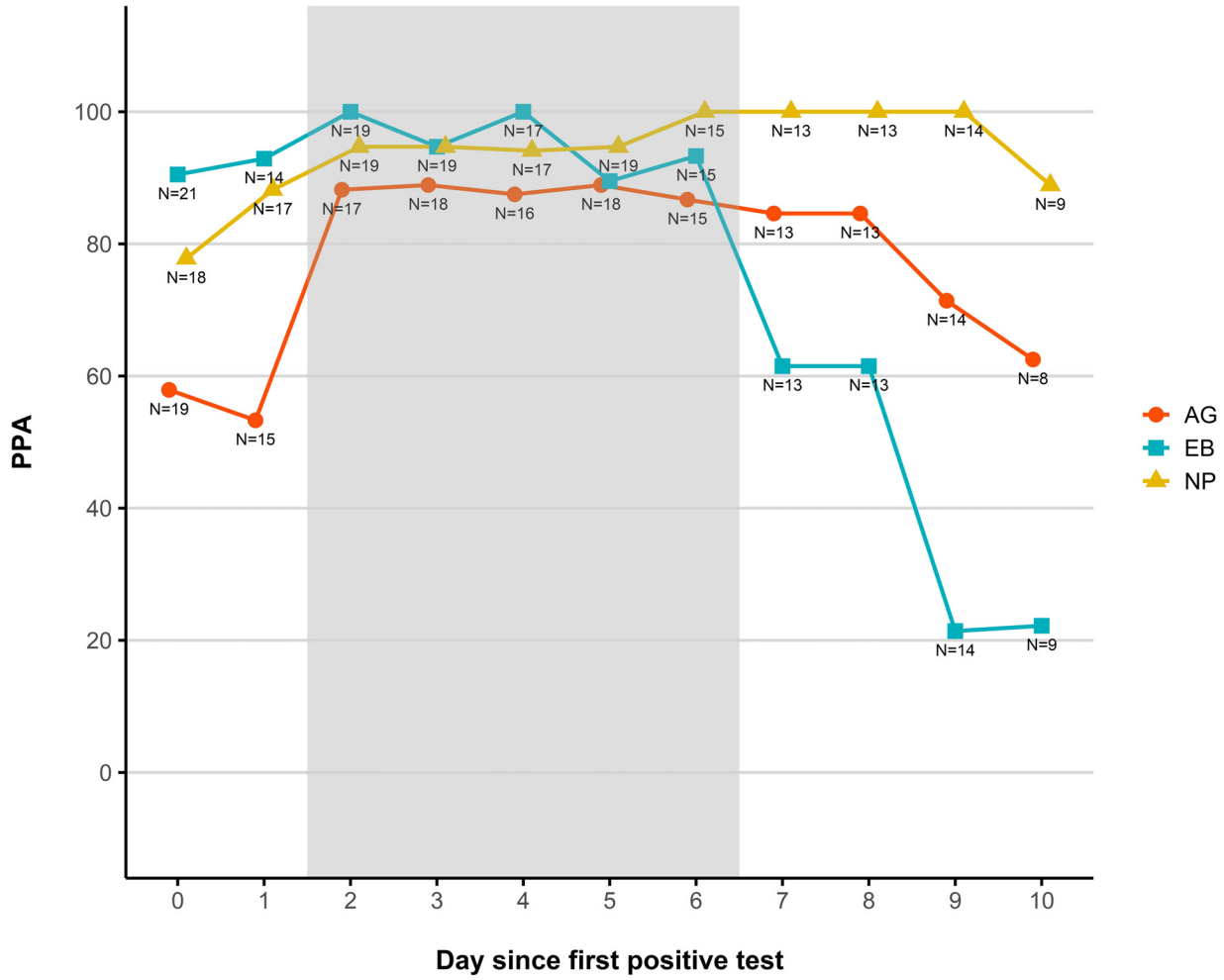
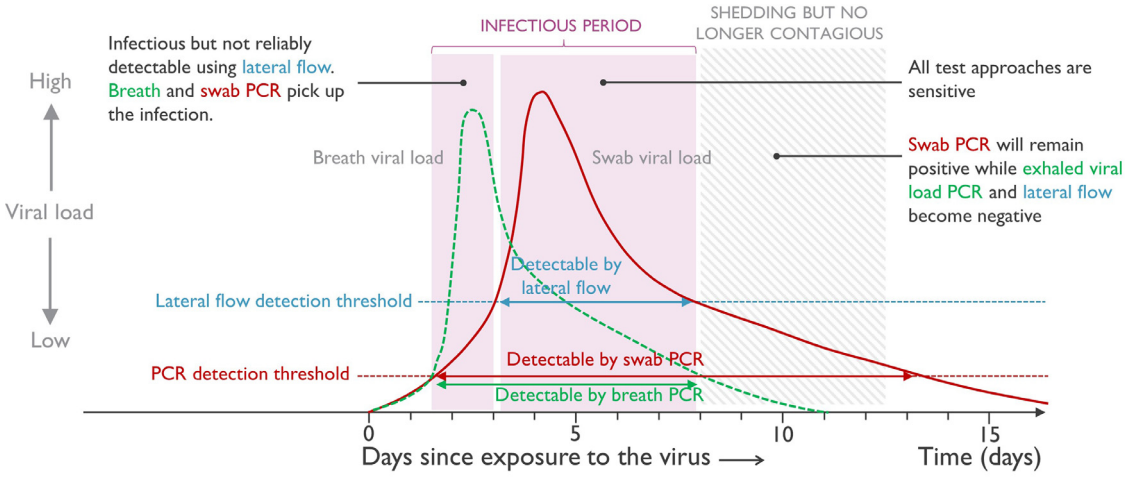


Figure 5. Comparing the positivity of EB qPCR to NP qPCR and NP RAT throughout infection.
This figure shows the positivity of the different tests in comparison to aggregate positivity. Data from the alpha and omicron cohorts are combined and stratified by day of testing. The positivity of EB qPCR is equal to that of NP qPCR and superior than NP RAT on days 0-1 and 2-6 and inferior to other tests on the days after. Due to scarcity of data, days 11 and over were excluded. The number of observations is indicated below each point in the graph.
EB, exhaled breath; NP, nasopharyngeal; NP RAT, NP rapid antigen testing.



Source: Adapted from Michael Mina et al., "Rethinking Covid-19 test Sensitivity – A Strategy for Containment", N Engl J Med 2020; 383:e120
Note: lateral flow test = rapid antigen test

Figure 6. EB qPCR as an infectiousness test.
This figure summarizes the infectious period, shedding patterns in the upper and lower respiratory tract, and the detection of SARS-CoV-2 through EB qPCR, NP qPCR and NP RAT. Our results show equal positivity of EB qPCR and NP qPCR in the first week of infection, both of which have superior positivity than NP RAT. EB qPCR does not over-diagnose COVID-19 when the patient is no longer infectious. This unique pattern of EB qPCR positivity is compatible with a test for infectiousness.
EB, exhaled breath; NP, nasopharyngeal; NP RAT, NP rapid antigen testing.

Limitations

Our study has several limitations. First, the small number of participants limits the power of certain calculations (*e.g.*, time to peak shedding and time to the second day of reducing viral load). Second, the young age of participants limits extrapolation to the general population. Third, the correlation between a positive EB qPCR and presence of infectious viruses is currently unknown. This is a key data point to confirm that a positive EB equates infectiousness. Fourth, all alpha-infected individuals had a negative NP qPCR test in the days before inclusion two out of eight omicron patients. This resulted from changed national testing guidelines. It may reduce the likelihood that the diagnosis of omicron infections was as early as alpha infections. Fifth, the only samples taken in duplicate were EB. They were deemed positive if virus was detected in either. This may have biased EB qPCR positivity upwards.

Conclusion

This study is the first to compare the shedding pattern of SARS-CoV-2 in EB and NP (through both qPCR and RAT) across infections with different VOCs. The results contradict that increased viral shedding from the lower respiratory tract drives the higher infectiousness of omicron. Also, this study is the first to show a positivity of EB qPCR which matches NP qPCR and supersedes NP RAT in the first week of infection. Contrary to NP qPCR, EB qPCR does not show prolonged positivity. This unique pattern is compatible with a test for infectiousness. The silicon chip-based solid impactor used in this study brings the diagnosis of a respiratory infectious diseases through EB qPCR much closer to clinical practice.

Author contributions

JR, WD, TS, BV, AL, EA, NVDW and PP conceived and designed the analysis. JR, WD, BV, JVD, RH and EM collected the data. PM contributed data. WD, JR, JV, JF, BV and TS performed the analysis. JR, WD and TS wrote the paper. BV, JV, JF, JVD, RH, EM, AL, PM, EA, NVDW and PP critically reviewed the paper.

Declaration of Competing Interest

The authors have no competing interests to declare.

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Author contributions

Ethical approval statement

Clinical studies were set up via the Ethical Commission of UZ Leuven/KU Leuven. Study references are S65005 and S65924.

Data availability statement

The data underlying the main analyses in this manuscript are available in the article and in its online supplementary material. The data that is not released with the paper can be made available on request from the corresponding author (Joren Raymenants) in case of a demonstrable affiliation with an academic or health institution, a legitimate research question and a commitment to not attempt to de-anonymize.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2022.07.069.

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