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SARS-CoV-2 screening strategies for returning international travellers: Evaluation of a rapid antigen test approach

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ABSTRACT

Background: International travel poses the risk of importing SARS-CoV-2 infections and introducing new viral variants into the country of destination. Established measures include mandatory quarantine with the opportunity to abbreviate it with a negative rapid antigen test (RAT).
Methods: A total of 1,488 returnees were tested for SARS-CoV-2 with both PCR and RAT no earlier than 5 days after arrival. We assessed the sensitivity and specificity of the RAT. Positive samples were evaluated for infectivity in vitro in a cell culture outgrowth assay. We tracked if participants who tested negative were reported positive within 2 weeks of the initial test.
Results: Potential infectiousness was determined based on symptom onset analysis, resulting in a sensitivity of the antigen test of 89% in terms of infectivity. The specificity was 100%. All positive outgrowth assays were preceded by a positive RAT, indicating that all participants with proven in vitro infectivity were correctly identified. None of the negative participants tested positive during the follow-up.
Conclusions: RAT no earlier than the 5th day after arrival was a reliable method for detecting infectious travellers and can be recommended as an appropriate method for managing SARS-CoV-2 travel restrictions. Compliance to the regulations and a high standard of test quality must be ensured.

1. Introduction

The pandemic of COVID-19 has a massive and ongoing impact on daily life around the world, including travel. International travel has declined since the pandemic started in 2019 (Pearce, 2020). Many nations declared travel restrictions early on, hoping to prevent an introduction of the disease into their own country. Studies from March 2020 showed a significant reduction of international transmissions after the first bans were put in place (Chinazzi et al., 2020; Kraemer et al., 2020).

SARS-CoV-2 quickly proved to spread fast and wide, partly due to asymptomatic and presymptomatic transmissions (Harrison et al., 2020; Hoehl et al., 2020; Johansson et al., 2021; Oran and Topol, 2020). Measures were implemented to reduce the impact of travel on the spread of SARS-CoV-2 (Bundesregierung, 2021; Wells et al., 2020). Firstly, infectious people had to be identified before travel to prevent them from spreading the virus in the aircraft and introducing it into the country of destination. Travellers were not allowed to fly if they showed any symptoms of COVID-19 and were obligated to present a recent negative test. Secondly, infectious travellers had to be identified before re-entry into daily life in the country of destination. This was mostly based on quarantine as well as quarantine exit testing (Bundesregierung, 2021). A simulation study found routine testing before travel and an abbreviated quarantine paired with a negative PCR test on day 5 after arrival to be an effective strategy (Kiang et al., 2021). In Germany, obligatory travel requirements were introduced into legislation in November 2020 and have been updated repeatedly since then. Risk areas are determined using their official 7-day-incidence/100,000 inhabitants and other qualitative criteria (Robert-Koch-Institut et al.,

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2021). Depending on this, countries were classified as basic-risk, high-risk, or virus variant areas during the study period between February and May of 2021. Travellers coming to Germany from all risk areas had to present a negative rapid antigen test (RAT) performed no earlier than 48 hours before arrival or a negative PCR performed no earlier than 72 hours before arrival. The day of arrival was counted as day 0 (D0), with day 5 after arrival (D5) being the earliest possible date of quarantine exit testing. They also had to fill out the digital registration on entry. Travellers returning from virus variant areas were required to quarantine for 14 days with no option to leave quarantine early. Those returning from basic-risk or high-risk areas had to quarantine for 10 days but were able to shorten quarantine by presenting a negative RAT or PCR performed on D5. Regulations have been continuously updated as they adapted to new scientific knowledge and varying circumstances. Throughout the changes, the option of quarantine exit testing on D5 when returning from a high-risk area remained. All predeparture testing requirements were fulfilled by presenting a RAT with no demand for a PCR. RATs provide many advantages including fast results, easy handling, and low costs. Still, the sensitivity and quality of RATs vary depending on the manufacturer and quality of test execution (Baccani et al., 2021; Kohmer et al., 2021). In Germany, the high demand for SARS-CoV-2 tests caused the launch of many commercial test centers, with authorities struggling to ensure quality control.

Especially now, with the spread of the Omicron variant, entry requirements have gained renewed importance. This report focusses on the effectiveness of travel restrictions, in particular, the RAT as a testing method, and the practical utility of travel regulations. Furthermore, we would like to share our experience and handling of entry regulations.

2. Methods

2.1. Study population

All travellers returning from a designated SARS-CoV-2 risk area with residence in Frankfurt, Germany were registered with the Public Health Department and contacted by telephone or email in line with the Ordinance on Coronavirus Entry Regulations (Bundesregierung, 2021). Amongst those, potential study participants were preselected solely based on the country they travelled from (see Supplemental Table 1). They were systematically tested for SARS-CoV-2 by the Public Health Department Frankfurt/Main between February and May of 2021. Three main groups were included: (1) travellers arriving by airplane who had departed from a list of selected countries (Supplemental Table 1); (2) travellers arriving by airplane from any country with a reported 7-day incidence of $\geq$200/100,000 cases of COVID-19; and (3) travellers arriving by car or bus from a list of selected countries (Supplemental Table 1).

People who had been flagged as exempt in their online registration form, minors, and members of the same household or travel group were not eligible to participate. Individuals fulfilling the inclusion criteria were invited by phone call to participate as part of the standard governmental control call to verify quarantine compliance. The only advantage that study participants had over nonparticipants was that they simultaneously received a PCR test free of charge. They were informed about the study, and written consent was obtained at the day of their test.

All participants were asked to complete an online questionnaire before their day of testing, where they stated their travel history, vaccination status and previous SARS-CoV-2 infections, previous tests, and any symptoms within the 2 weeks before arriving in Germany. Factors such as gender, age, immunity status, likelihood of infection, or clinical presentation, which were checked for as part of the regulatory phone call, did not affect the preselection of the study participants.

2.2. Virological testing

Two samples were collected from each study participant by a trained medical professional: for the PCR, 1 nasopharyngeal swab for the RAT and 1 oropharyngeal swab or a combined oro- and nasopharyngeal swab. All tests were performed by the same person using the same procedures to avoid any discrepancies.

2.3. Rapid antigen test

A nasopharyngeal swab was tested using an immunochromatographic SARS-CoV-2 Antigen Test by Roche Diagnostics, Basel, Switzerland according to the manufacturers' instruction (Diagnostics, 2021). The result was assessed after 15 minutes.

2.4. PCR testing

RT-PCR testing of a nasopharyngeal or oropharyngeal swab was done on the Cobas 6800 instrument (Roche diagnostics, Basel, Switzerland) or the Alinity® m instrument (Abbott Laboratories, Chicago, IL, USA) according to the manufacturers' instructions.

The result was declared borderline positive when only 1 of 2 PCR targets was detected, the E-gene or ORF-1 (open reading frame) (Roche Cobas), or the cycle threshold value (CT-value) was between 38.0 and 40.0 (Abbott Alinity m). For conversion to quantitative test results of the virus concentration, 3 quantitative comparison samples containing 10^5, 10^6, and 10^7 SARS-CoV-2 (BetaCoV/Munich/ChVir984/2020) RNA copies/mL were used. A standard curve was used to calculate the viral RNA copies/mL.

2.5. Cell culture

Caco-2 cells (human colon carcinoma cells) were maintained in minimum essential medium (MEM) supplemented with 10% fetal calf serum (FCS, Sigma-Aldrich; St. Louis, MO, USA), 100 IU/mL of penicillin, and 100 g/mL of streptomycin. Then, 500 µL of the swab dilution conceptual was mixed with 1.5 mL of MEM containing 1% FCS, 7.5 µg/mL Amphotericin B (Sigma-Aldrich), and 0.1 mg/mL Primocin (InvivoGen; San Diego, CA, USA) and cultivated with Caco-2 cells. Cellular monolayer was monitored daily for the appearance of cytopathogenic effect (CPE), indicating presence of infectious virus in tested swab. CPE was assessed daily for 7 days or until cell lysis occurred.

2.6. Infectiousness

The potential infectiousness of patients was determined using the same criteria as the Public Health Department, in accordance with guidelines of the Robert Koch Institute (RKI), the German federal agency for disease control. Infectiousness was linked to symptom onset, and it was assumed that infectiousness lasted a maximum of 14 days after the onset of symptoms or after a positive test, if the patient had recovered by day 12. A high CT-value was viewed as an indicator for a low viral load and a smaller risk of infectiousness, but it only factored into the termination of infectiousness in conjunction with the time of symptom onset or a follow-up PCR test. Typical symptoms such as fever, cough, dyspnoea, or loss of smell and taste were defined as symptom onset.

2.7. Follow up

Patients who had a borderline positive or weak positive PCR result (CT-values >30) and did not have a history of symptoms or

Table 1
Number of positive participants from the respective risk areas.

Designation of country of departure	Total number of study participants	Number of study participants with a positive SARS-CoV-2 PCR result	Prevalence and 95% confidence interval
Basic-risk area	461	19	4.1% (2.5–6.4)
High-incidence area	941	22	2.3% (1.5–3.5)
Virus variant area	86	3	3.5% (0.7–9.9)

previous positive tests were retested after 2 days. The progression of CT-values was used to determine the stage of infection (Bullard et al., 2020). If the initial CT-value was followed by an equally high or higher value, it was interpreted as the later stages of an infection, whereas a sudden drop indicated the beginning of an infection.

A list of all participants was compared with all cases of COVID-19 reported to the Public Health Department Frankfurt/Main in order to check if any participants who were tested negative on D5 were later reported positive within 14 days of entering the country.

2.8. Comparison between study participants and nonparticipants

Furthermore, a list of all individuals who met the inclusion criteria but did not participate in the study was compared with all positive cases reported in Frankfurt/Main within 2 weeks of their individual entry dates. Even though it was not always evident if the positive test was a result of mandatory entry requirements, a general prevalence could still be calculated and compared with the prevalence seen within the study. This way, the study can also be used to evaluate the effectiveness of the travel regulation in practice.

2.9. Statistical analysis

Confidence intervals were calculated using the software Bias.

2.10. Ethical approval

The retrospective analysis of the data collected by the Public Health Authority, City of Frankfurt, Germany, was approved by the ethics committee of the Medical Faculty of Goethe University Frankfurt, Germany (Vote number 2021-257). Written informed consent was obtained from all study participants.

3. Results

3.1. Study population

The study included 1,488 participants, aged between 18 and 85 (mean age 39.2) years. Approximately 53.3% (n = 793) were male, 46.0% (n = 685) were female, and 0.7% (n = 10) were nonbinary. A total of 1,435 (96.4%) study participants completed the survey. A total of 6,480 people were offered participation, of whom 4,992 declined the offer.

Approximately 14.7% (n = 222) were considered immune, either because they had been infected before or because they had been vaccinated (Robert-Koch-Institut 2021).

A total of 1,213 (81.5%) travelled by air, 179 (12.0%) by car, 42 (2.8%) by bus, and 2 by ship or truck.

The study participants returned from 90 different countries.

A total of 941 (63.2%) returned from high-incidence areas, 462 (41.6%) from basic-risk areas, and 86 (5.8%) from virus variant areas. The results showed the lowest prevalence among the travellers returning from high-risk areas (table 1). The basic-risk areas depicted in the study were mostly countries in the Middle East and Africa, some of which may be vulnerable to underreporting (Kobia and Gitaka, 2020; Loembé et al., 2020).

Positive samples were collected from participants returning from 17 different countries (Supplemental Table 2).

On average, the participants got tested on day 5.3 (median: day 5, range: day 5 to day 14).

3.2. Results of virological testing

Of the 1,488 participants, 26 (1.75%) had a positive RAT. We calculated a specificity of the RAT of 100%. Of the 1,462 study participants with a negative RAT, 18 tested positive for SARS-CoV-2 RNA by RT-PCR. Determined by PCR positivity, this amounts to a prevalence of 3% (44 of 1,488).

The overall sensitivity of the RAT, compared to PCR, was 59%. The cycle threshold (CT) values of all PCR-positive samples varied between 17.68 and 37.54 (PCR target: ORF-1 a/b nonstructural region). This corresponds to approximately 375 thousand copies/mL and 245 copies/mL, respectively. The median CT-value (ORF-1 if not otherwise specified) was 29.64 (standard deviation [SD]: 6.32).

Figure 1 and 3.

In 38 of the 44 PCR-positive samples, cell culture experiments were conducted. In 8 samples, the virus was cultured successfully (18.2% of PCR-positive samples). The CT-values of the sample that were successfully cultured ranged from 17.68–27.11 (mean value: 21.58; SD: 2.73). All samples with a positive cell culture also had a positive RAT.

A total of 56.8% (n = 25) of PCR-positive participants reported symptoms during the course of their infection. Average symptom onset was 4.6 days before the date of testing (SD: 5.37). Approximately 76.9% (n = 20) of all participants with a positive RAT presented symptoms with an average onset of 3.9 days before testing (SD 4.01, range: 10 days before testing to 2 days after testing). CT-values of PCR-positive samples with a negative RAT had a median of 34.65, ranging from 27.47–37.56 (figure 2).

Approximately 16.7% (n = 3) of participants who were PCR-positive with a negative RAT had already tested positive for SARS-CoV-2 at least a month before. If prolonged virus shedding after infection is assumed for all 3 patients, sensitivity of the RAT improves to a value of 63.4%. Approximately 27.8% (n = 5) showed a symptom onset more than 10 days ago and 38.9% (n = 7) had no history of symptoms but were tested negative or borderline positive with CT-value of >30 in a follow-up test 2–4 days later. Therefore, 83.3% (n = 15) of the patients presenting as false-negative in the RAT indeed showed a positive PCR test, but due to the low viral load, these participants were classified as likely not infectious (Bullard et al., 2020; Singanayagam et al., 2020).

The 3 remaining patients with a negative RAT showed a symptom onset 6 days before testing (CT-value: 27.47/27.32), 1 day before testing (CT-value: 32.36/31.96), and 2 days after testing (CT-value: 34.5/33.76) and were considered likely infectious at the time of the RAT. The RAT showed a sensitivity of 89.7% for detecting patients that were potentially infectious due to those criteria. All of these patients had a negative cell culture experiment.

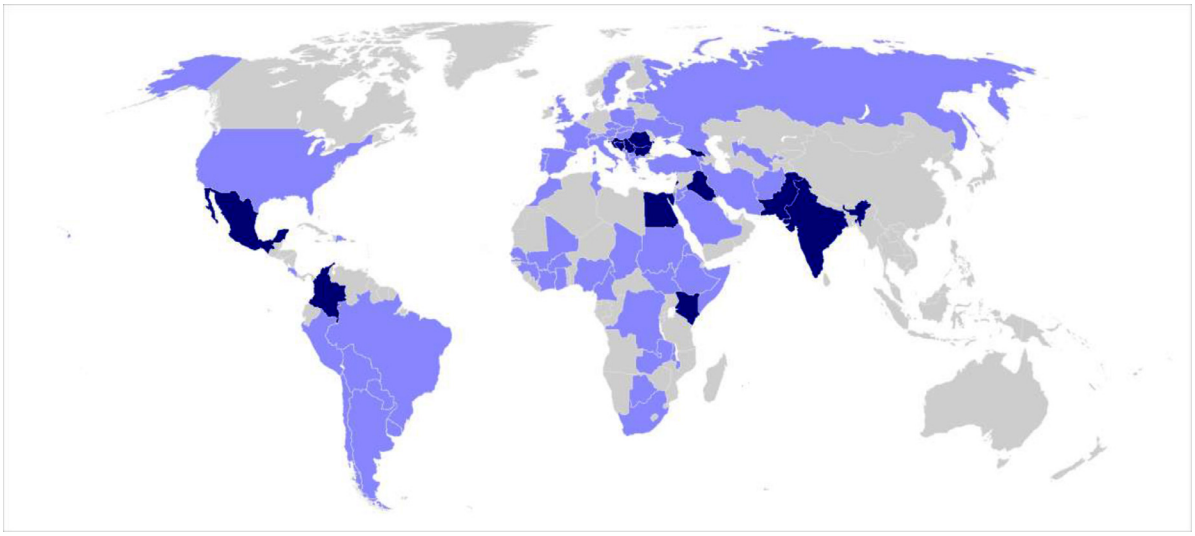

Figure 1. Simplified world map. Countries from which study participants departed before their arrival in Frankfurt are depicted light. Countries from which at least 1 study participant with a positive test departed are marked dark.

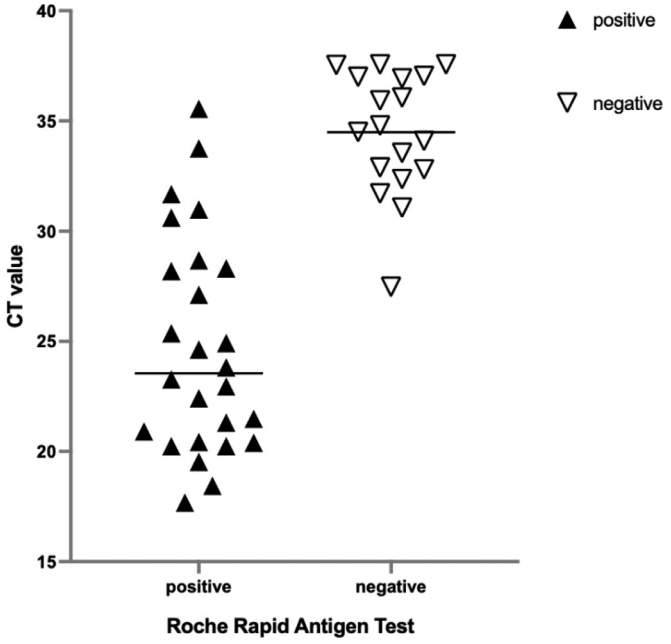

Figure 2. Rapid antigen test analysis results for rRT-PCR-positive samples. Positive (filled data point symbols) and negative (empty data point symbols) Ag-RDT results and corresponding CT-values (ORF-1). Ag-RDT, antigen rapid diagnostic test; CT, cycle threshold; rRT-PCR, real-time reverse-transcriptase PCR.

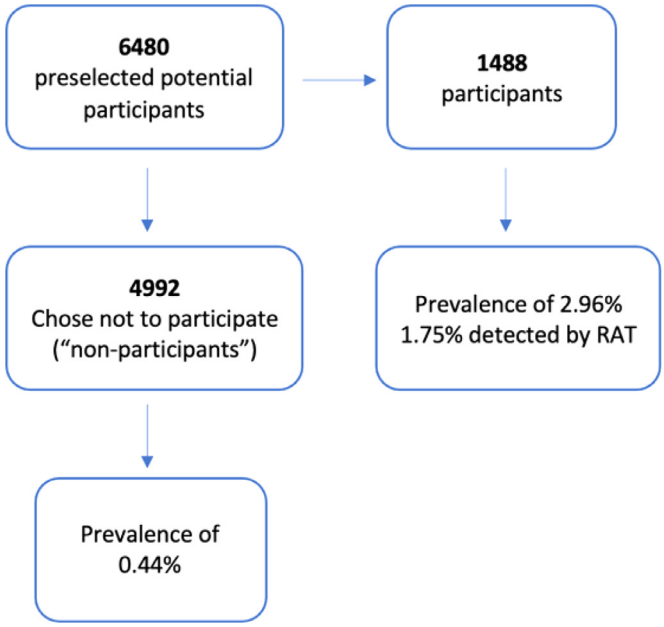

Figure 3. Flow diagram of the distribution of participants and nonparticipants.

3.3. Results of follow-up

None of the study participants that were tested negative for SARS-CoV-2 by either method tested positive within the following 14 days.

3.4. Comparison between study participants and nonparticipants

Of the 4,992 nonparticipants meeting the same criteria as the participants, 22 tested positive for SARS-CoV-2 within 2 weeks of arriving in Germany. On average, they were tested on day 6 after arrival (median: 5.5, range: day 1–day 13). A total of 11 of them (50%) were tested before day 5 or after day 10, indicating that their test was not performed to shorten quarantine. The overall prevalence among this group was 0.44%, as opposed to the prevalence of 2.97% among the study participants. The odds ratio is 4.49 (95% CI: 2.57, 7.84, p <0.00001), a highly significant difference between both groups.

4. Discussion

We evaluated SARS-CoV-2 testing with a RAT after at least 5 days of quarantine on arrival in Germany. By performing PCR-assays and cell culture experiments in parallel as markers of in vitro infectivity, we were able to determine the sensitivity and specificity of the RAT. This allowed us to draw a conclusion about its validity as a suitable method for detecting infectious travellers in the interest of public health.

Of the 1,488 study participants, 1,462 had a negative RAT. In accordance with current procedural practices, these participants could end mandatory quarantine early. Eighteen of them (1.2%)

had a positive PCR and would have been missed by a RAT only. To determine whether they were potentially infectious and consequently posed a public health risk, we analyzed PCR-positive and RAT-negative samples according to the result of the outgrowth assay, determined viral load, and analyzed the time since symptom onset. None of these samples showed a positive outgrowth assay; thus, infectivity could not be detected in vitro. The viral load of the RAT-negative samples was significantly lower than that of the RAT-positive samples (Figure 2) and was above a CT-value of 30 in all but 1 case, which is commonly associated with potential infectivity (Toptan et al., 2021).

In 1 of the patients with negative RAT and positive PCR (CT-value: 34.5), symptoms were observed 2 days after the test. They were likely early in the infectious stage. The other 2 described a symptom onset 6 days and 1 day before quarantine exit testing, and therefore might have been infectious at the time of test. Overall, the sensitivity of the RAT on infectious samples was 89.7%, which is consistent with the comparative literature (Diao et al., 2020; Krüttgen et al., 2020; Pray et al., 2021).

At the time of the study, the incidence in Frankfurt was around 150/100,000, leading to an estimated prevalence of 2.85% (Phillips et al., 2021). This is comparable to the prevalence of 2.96% observed among travellers in this study.

Surprisingly, we found the highest rate among returnees from basic-risk areas (prevalence of 4.1%, CI: 2.5–6.4). Returnees from high-incidence areas had a prevalence of 2.3% (CI: 1.5–3.5). The difference is not significant (OR: 1.8, CI: 0.96–3.4). However, the result is unexpected. A plausible explanation is that official data do not reflect the true incidence in many countries, even though health authorities attempt to classify different areas accordingly. This is particularly interesting, as travellers from basic-risk areas have been exempt from entry regulations since mid-2021.

Comparison of prevalence in study participants and nonstudy participants within 2 weeks of arrival revealed concerning discrepancies, as reporting of SARS-CoV-2 in nonparticipants resulted in a rate nearly 4.5 times lower than that seen among participants (0.44% RAT-positive nonparticipants vs 1.95% RAT-positive participants). A possible explanation for the low prevalence of SARS-CoV-2 amongst the nonparticipant group is that most of them completed their quarantine without shortening it by quarantine exit testing, leading to an underestimation of their true prevalence. This seems unlikely, however, as our experience with entry regulations in Frankfurt/Main has shown that most people prefer a quarantine exit test.

Considering that some commercial test centers in Frankfurt/Main have been closed due to low testing quality, false-negative tests, and lack of reporting of positive results may contribute to the lower rates seen among nonparticipants. Finally, only a PCR-confirmed result met the case definition of COVID-19 in Germany. The obligation to perform PCR confirmation after a positive RAT may not have been followed in all cases.

Our study has some limitations: only travellers who entered from countries on the predefined list for this study were included. Although only 1 member of a travel group or household ought to be tested, it is likely that some participants omitted their relationship to each other in order to also be tested free of charge, thereby confounding their relative risk of infection. Furthermore, health departments relied heavily on patient honesty regarding subjective symptom onset to determine isolation duration. Returning travellers got tested between day 5 and 10 of quarantine. This variance might have affected the comparison of participants and nonparticipants.

Overall, we conclude that a RAT no earlier than day 5, when performed under study conditions, is an appropriate method for early termination of mandatory quarantine after travel. The fact that none of the negative participants tested positive during the following 14 days indicates that day 5 presents a suitable time to test (Kiang et al., 2021). However, the comparison between study participants and nonparticipants with respect to the case finding rate shows that the method is efficacious but not efficient. It is of the utmost importance that travellers comply with official regulations. Many public health authorities in Germany are overwhelmed with the task of contacting all inbound travellers and monitoring their compliance. In order to reduce the workload of the health authorities and in accordance with our results, it could even be considered to replace the 10-day quarantine and optional RAT with a 5-day quarantine and mandatory RAT.

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The overarching aim is to avoid inefficient use of general and intensive care facilities by providing adequate processes for the allocation of resources and for guiding both hospital and outpatients along a prototypical patient pathway. egePan Unimed is funded by the German Federal Ministry of Education and Research as part of the Netzwerk Universitätsmedizin (NUM) initiative (Grant-No.: 01KX2021). The egePan project leads are Prof. Dr. Jochen Schmitt and Dr. Michael von Wagner.

Conflict of Interest

The authors have declared no conflicts of interest.

Authors contribution

EL, SC, SH, RG, and UG conceptualized the study. EL was responsible for sample collection. EL, SH, and UG did the formal data analysis and assessed and verified the data. SH, SC, DB, and MW did the laboratory work. SC, SH, and UG oversaw the project. SC, SH, and UG acquired funding. TW, BB, and JS sourced and oversaw the software. EL wrote the initial draft of the manuscript. SH, UG, SC, MW, and DB reviewed and edited the manuscript. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2022.02.045.

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