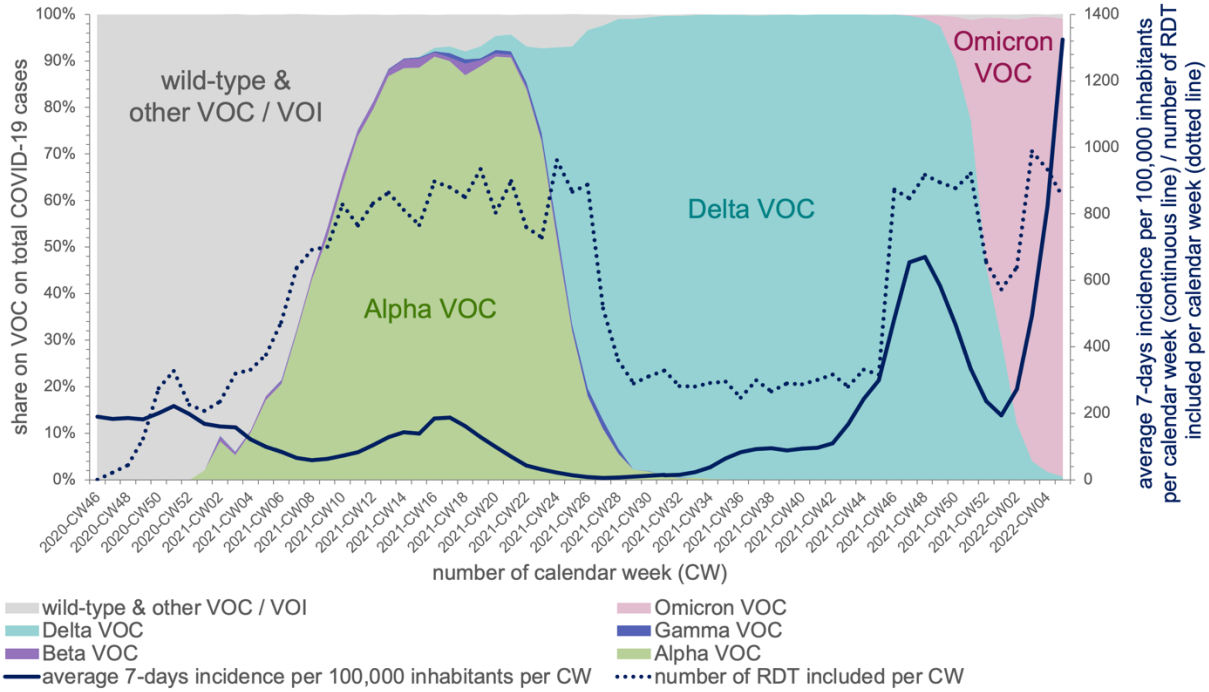
Supplementary Figure



Supplementary Fig. S1: Share of SARS-CoV-2 VOC on total COVID-19 cases in Germany during the study period.

At the beginning of the study period in November 2020 wild-type SARS-CoV-2 dominated, suppressed by Alpha (B1.1.7) VOC in spring and summer 2021, followed by Delta (B.1.617.2) VOC with main pandemic share in autumn and winter 2021. From the beginning of 2022, Omicron (B.1.1.529) established as dominating VOC. The average 7-days incidence per 100,000 inhabitants per calendar week in the federal state of Bavaria pictures the second and third wave of the COVID-19 pandemic in Germany was followed by low incidence over the summer months of 2021 as well as unprecedented high prevalence levels due to the fourth and fifth pandemic wave in winter 2022 with the spread of Delta and Omicron VOC (continuous dark blue line).

After RDT implementation in November 2020 the weekly average number of performed RDT included to the study could be enrolled to predominantly the total of clinical departments for all patients on admission, employees, and accompanying individuals. From June 30 to November 4, 2021, RDT performance as SARS-CoV-2 patient admission screening was restricted to critical areas such as emergency departments and the delivery room as reaction to the meanwhile low SARS-CoV-2 incidence setting (dotted dark blue line).

Data source: Robert Koch-Institut, Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, Bayerisches Landesamt für Statistik. [1-3]

VOC: Variant of Concern

Supplementary methods

Quantitative reverse transcription polymerase chain reaction (RT-qPCR)

RT-qPCR diagnostics were conducted in the hospitals' virological diagnostic laboratories based on utilisation of several RT-qPCR methods and performed in accordance with manufacturers' instructions.

The following RT-qPCR tests and analytical devices were used for viral load determination:

(I) FTD SARS-CoV-2-PCR, MagNaPure 96 / 7500 Real-Time PCR System (target N/ORF1ab-gene, Siemens Healthineers, Munich, Germany / Roche Diagnostics, Rotkreuz, Switzerland / Thermo Fisher Scientific, Waltham MA, USA)

(II) NeuMoDx SARS-CoV-2 Assay™, NeuMoDx™ 96 Molecular System / NeuMoDx™ 288 Molecular System (target N/Nsp2-gene, Qiagen, Hilden, Germany)

(III) Alinity m SARS-CoV-2 assay, Alinity m (target RdRp/N-gene, Abbott Laboratories, Abbott Park IL, USA)

(IV) QIAstat-Dx® Respiratory SARS-CoV-2 Panel, QIAstat-Dx® Analyzer (target RdRp/E-gene, Qiagen, Hilden, Germany)

(V) Xpert® Xpress SARS-CoV-2/Flu/RSV, GeneXpert® IV (target E/N2-gene, Cepheid, Sunnyvale CA, USA)

(VI) cobas® SARS-CoV-2, cobas® 6800 (target ORF1ab/E-gene, Roche Diagnostics, Rotkreuz, Switzerland)

Viral loads were calculated from Cycle threshold (C_t) values based on viral loads and C_t values of two reference standards S_1 and S_2 as described earlier using the following formula:[4]

$$ViralLoad\ (Sample) = ViralLoad\ (S_1) \times \left(\sqrt[C_t(S_1)-C_t(S_2)]{\frac{ViralLoad(S_2)}{ViralLoad(S_1)}} \right)^{(C_t(S_1)-C_t(Sample))}$$

In case of multiple targets with different C_t values on a single RT-qPCR test (cobas® SARS-CoV-2, NeuMoDx SARS-CoV-2 Assay ™, Xpert® Xpress SARS-CoV-2/Flu/RSV), the viral load was calculated as geometric mean of the estimates derived from the two single genes.

Analysis by symptomatology

Patients were categorised into three groups referring to COVID-19 symptomatology according to the comparable COVID-19 case definition of the CDC[5] and the ECDC[6]:

A) typical COVID-19 symptomatology (e.g. fever, dry cough, shortness of breath, new anosmia or ageusia)
B) asymptomatic individuals
C) atypical symptomatology possibly caused by COVID-19 (e.g. deterioration of general condition, falls, diarrhoea, or seizures)

Supplementary Results

Relation of RDT sensitivity to days after symptom onset

In 107 of 224 symptomatic RT-qPCR positive cases, information on the date of symptom onset was available. Days after symptom onset (DSO) ranged from -2 (sampling two days before symptom onset) to 21 (sampling 21 days after symptom onset, median: 2 DSO, IQR: 1 to 6 DSO). Maximum geometric mean viral load was detected on day 1 after symptom onset ($6.71x10^6$ SARS-CoV-2 RNA copies per ml). Sensitivity increased from 0.00% (0/2) for -2 DSO over 43.59% (17/39, 95% CI 29.30%–59.02%) for 0-1 DSO up to 48.65% (18/37, 95% CI 33.45%–64.11%) for 2-5 DSO and decreased as representing the course of the SARS-CoV-2 infection over 31.81% (7/22, 95% CI 16.36%–52.68%) for 6-9 DSO to 28.57% (2/7, 95% CI 8.22%–64.11%) for at least 10 DSO.

Comparison of RDT manufacturers

Sensitivity ranged from 36.79% (78/212, 95% CI 30.59%–43.47%) for MEDsan® over 37.65% (61/162, 95% CI 30.56%–45.32%) for Panbio™ to 48.08% (25/52, 95% CI 35.10%–61.31%) for NADAL®. Specificity ranged from 99.60% (9,049/9,985, 95% CI 99.45%–99.71%) for NADAL® over 99.66% (8,789/8,819, 95% CI 99.51%–99.76%) for MEDsan® to 99.71% (17,099/17,149, 95% CI 99.62%–99.78%) for Panbio™.

RDT sensitivity by symptomatology

RDT sensitivity was significantly lower in asymptomatic individuals (29.25%, 43/147, 95% CI 22.50%–37.06%) or individuals with atypical COVID-19 symptoms (32.73%, 18/55, 95% CI 21.81%–45.90%) compared with RDT sensitivity on typical symptomatology presenting individuals (45.98%, 103/224, 95% CI 39.58%–52.52%, p=0.001, Fisher's exact test).

This is compatible with slightly higher viral loads in case of typically symptomatic cases (median: $8.75x10^5$ SARS-CoV-2 RNA copies per ml) in comparison to atypically symptomatic

(median: 4.84×10^5 copies per ml) or asymptomatic individuals (median: 1.56×10^5 copies per ml, p=0.029, Kruskal-Wallis test).

Supplementary References

1. *Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit: Übersicht der Fallzahlen von Coronavirusinfektionen in Bayern, https://www.lgl.bayern.de/gesundheit/infektionsschutz/infektionskrankheiten_a_z/coronavirus/karte_coronavirus/ (Accessed 17 March 2022).*
2. *Bayerisches Landesamt für Statistik: Bevölkerung: Gemeinden, amtliche Einwohnerzahl aktuell (jährlich, vierteljährlich)*. Available from: https://www.statistikdaten.bayern.de/genesis/online?operation=abruftabelleBearbeiten&levelindex=1&levelid=1647519965087&auswahloperation=abruftabelleAuspraegungAuswaehlen&auswahlverzeichnis=ordnungsstruktur&auswahlziel=werteabruf&code=12411-000&auswahltext=&nummer=7&variable=7&name=GEMEIN&werteabruf=Werteabruf#abreadcrumb (Accessed 17 March 2022)
3. *Robert Koch-Institut (RKI): Anzahl und Anteile von VOC und VOI in Deutschland* Available from: https://www.rki.de/DE/Content/InfAZ/N/Neuartiges_Coronavirus/Daten/VOC_VOI_Tabelle.html (Accessed 5 May 2022).
4. Wagenhäuser, I., et al., *Clinical performance evaluation of SARS-CoV-2 rapid antigen testing in point of care usage in comparison to RT-qPCR.* EBioMedicine, 2021. **69**.
5. *Centers for Disease Control and Prevention (CDC): Interim Guidelines for Collecting and Handling of Clinical Specimens for COVID-19 Testing, https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html (Accessed 3 March 2021).*
6. *European Centre for Disease Prevention and Control (ECDC): Case definition for coronavirus disease 2019 (COVID-19), as of 3 December 2020*.