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Specificity testing by point prevalence as a simple assessment strategy using the Roche Elecsys[®] anti-SARS-CoV-2 immunoassay



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ABSTRACT

Background: The detection of antibodies to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is mandatory for the diagnosis, retrospective assessment of disease progression, and correct evaluation of the current infection situation in the population. Many such assays have been launched by various manufacturers. Unfortunately, the new US Food and Drug Administration emergency use regulations have resulted in a situation where laboratories have to perform their own validation studies but many of these laboratories do not have the biobank needed to conduct the studies.

Methods: We introduce a method that allows institutions to quickly perform a verification study in a low-prevalence infection situation. As proof of concept, we used the Roche Elecsys[®] anti-SARS-CoV-2 electrochemiluminescence immunoassay and an SAP-based hospital information system. The Shenzhen YHLO Biotech IgM and IgG assay targeting other surface patterns was used as a confirmatory test.

Results: The Roche assay demonstrated a limit of detection of 0.069 cutoff index and successfully passed the performance validation according to Clinical and Laboratory Standards Institute EP15-A3. The study population of 627 inpatients has a median age of 64 years, and approximately 13% of the group were under intensive care at the respective time point. All patients included tested negative for SARS-CoV-2 infection by quantitative reverse transcription polymerase chain reaction (cobas[®] 6800, Roche, Mannheim, Germany). Only one false-positive result was obtained, resulting in a specificity for the Roche Elecsys anti-SARS-CoV-2 test of 99.84% and a negative predictive value of 99.98%.

Conclusions: The anonymized use of residual material enables quick evaluation of anti-SARS-CoV-2 immunoassays, as shown in this work with the Roche Elecsys assay. Comparison of the control population with economic data makes it possible to validate the sampling set and therefore to determine diagnostic specificity. By use of the approach chosen, it was shown that the Roche test achieved very good results in terms of diagnostic specificity, reproducibility, and limit of detection.

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Introduction

On March 11, 2020, the World Health Organization declared COVID-19, the disease caused by infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a pandemic. At the time of writing (October 28, 2020) the aggregated data show

almost 44 million confirmed infections and nearly 1.2 million deaths worldwide (Johns Hopkins Whiting School of Engineering, 2020). For pandemic control, the identification and isolation of infected individuals is crucial, as is the assessment of seroprevalence to guide social restriction measures. COVID-19 testing can be principally divided into two approaches: the direct quantitative reverse transcription polymerase chain reaction (qRT-PCR)-based detection of SARS-CoV-2 viral RNA, using nucleic acid amplification tests (NAATs), from respiratory specimens collected with, for example, nasopharyngeal swabs; and the indirect detection of virus-specific antibodies in peripheral blood as an indicator of a former infection. NAATs are limited mostly to the early phase of infection (Guo et al., 2020; Kucirka et al., 2020), and their diagnostic value is limited by false-negative test results due to

Abbreviations: CLSI, Clinical and Laboratory Standards Institute; COI, Cutoff index; CV, Coefficient of variation; ICD-10, International Statistical Classification of Diseases and Related Health Problems tenth revision; NAAT, nucleic acid amplification test; qRT-PCR, quantitative reverse transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

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either preanalytical or analytical obstacles (Tu et al., 2020; Wang et al., 2020b). The need to detect immunoreactivity against SARS-CoV-2 to diagnose a previous infection and to estimate seroprevalence at the population level places immunoassays in the spotlight. Although currently available tests for detecting anti-SARS-CoV-2 antibodies have high diagnostic sensitivity and specificity (Kohmer et al., 2020; Kontou et al., 2020), the positive predictive value is often very low because seroprevalence is still low (Bryant et al., 2020). For example, given the seroprevalence of 0.301% in Germany, a test with a sensitivity of 99.5% and a specificity of 99.78% will result in a positive predictive value of 58%. This means that a person has a 42% chance of actually having no immune response to SARS-CoV-2. Assessing a test's specificity is therefore crucial to ensuring the diagnostic benefit (Neumaier, 2020).

Currently, no studies assessing the specificity of serological assays in a large population are available; in particular, the rate of cross-reactivity with other coronaviruses or with autoimmune-comorbidities such as rheumatoid arthritis or psoriasis presents a potential problem (Vinyé Bausà et al., 2020; Wang et al., 2020a). Another limitation is an insufficient understanding of antibody kinetics over time to distinguish potential immunity thresholds (Winter and Hegde, 2020).

Additionally, with its emergency use authorization, the US Food and Drug Administration has drastically reduced manufacturer requirements for using serological test systems in patient care. This implies that the laboratories must perform their own validation studies before offering a serological assay (U.S. Food & Drug Administration, 2020). The large number of immunoassays on the market, the absence of a sufficient biobank, the difficult working conditions due to the ongoing pandemic, and the limited number of peer-reviewed publications all mean that these studies are a huge task for laboratory physicians (Huang et al., 2020; Johns Hopkins Center for Health Security, 2020).

For introduction into routine care settings, tests must be of high throughput and have a short turnaround time. One such assay with Food and Drug Administration approval is the Roche Elecsys[®] anti-SARS-CoV-2 electrochemiluminescence immunoassay (Roche Diagnostics, 2020). Here we present a strategy for simple, fast-track validation and performance verification of SARS-CoV-2 serological assays that requires no extensive biobanking and uses standard-of-care clinical samples as a prerequisite for introducing serological assays into routine care. We do not claim this approach can replace extensive test validation as usually provided by the manufacturers, but it allows the laboratory to get a clear indication of the test performance in terms of specificity.

Materials and methods

Patient recruitment and specimen collection

All blood samples were collected on July 2, 2020, at the University Medical Center Mannheim, Medical Faculty Mannheim, University of Heidelberg, Germany, as clinically indicated. After determination of the required laboratory parameters, residual blood samples were anonymized before anti-SARS-CoV-2 antibody detection. To verify the representativeness of the study population, the medical history was evaluated by a hospital information system database query (Cerner, IS-H*med, version 7.2.1). The International Statistical Classification of Diseases and Related Health Problems tenth revision (ICD-10)-coded main condition and the number of secondary diagnoses from all patients who were treated in the hospital on the day of testing were examined.

Wards used as index wards for patients suspected of having COVID-19 and for treatment of confirmed infections were excluded from the assessment. The sex, age, and treating medical department of all study participants were recorded. Anonymized use of

residual patient material for test evaluation and method development was approved by the Institutional Review Board, and the study was conducted in accordance with the Declaration of Helsinki.

Sample collection

Clotted serum samples or lithium heparin blood samples were centrifuged at 2,000 g for 10 min at 18 °C within 4 h after sample collection. Residual blood samples were analyzed after examination of requested standard-of-care laboratory testing. To avoid compromising the requirements of follow-up patient testing, samples with a residual volume of less than 1 ml serum/plasma were excluded.

Detection of antibodies

All antibody detections were performed with the Elecsys anti-SARS-CoV-2 assay manufactured by Roche (Penzberg, Germany; reagent lot numbers 496298 and 495464). This electrochemiluminescence immunoassay detects anti-SARS-CoV-2 antibodies using a recombinant protein that represents the nucleocapsid antigen. This CE/IVD-marked assay is designed for detecting different antibody subclasses (IgM, IgA, and primarily IgG).

The results are reported as the cutoff index (COI), and results with a COI above 1.0 are interpreted as positive. The manufacturer does not specify a gray area or repeat range. Since no commercial controls were available at the time of the study, controls were prepared according to the manufacturer's instructions. The controls were analyzed in duplicate every 8 hours during testing. All analyses were performed with a cobas[®] e 411 analyzer (Roche, Mannheim, Germany).

For all samples with a COI above 0.9, a confirmatory test was performed with an iFLASH 1800 analyzer with use of Shenzhen YHLO Biotech's IgM and/or IgG assay, a magnetic chemiluminescence immunoassay for the detection of IgM and IgG against the SARS-CoV-2 nucleocapsid protein and spike protein. This confirmatory test detects surface structures different from those detected by the Roche assay and is offered only for the immunoglobulin subclasses and IgG and IgM.

Repeatability and determination of precision

Within-run precision and total precision were evaluated with use of controls provided by the manufacturer with predefined COI values and one pooled serum near the cutoff. The target value determination for this serum sample was performed with a tenfold serial measurement where the average value found was used as a target value. Precision evaluations were achieved by our measuring the controls and serum sample five times on five consecutive days, in line with the protocol (EP15-A3) of the Clinical and Laboratory Standards Institute (CLSI). The model is easy to use and enables one to verify the manufacturer's claims in terms of precision and reliability. The verification study was performed with five replicates per day for 5 days, whereupon imprecision estimates in terms of the within-run (repeatability) coefficient of variation (CV) and the within-laboratory CV were calculated and compared with the stated specifications.

Limit of blank and the limit of detection

The multiassay diluent and the positive control provided by the manufacturer were used to determine the limit of blank and the limit of detection according to CLSI EP17-A2 with use of Abacus 2.0 (LABanalytics GmbH, Jena, Germany).

Evaluation of linearity

The linearity evaluation was performed with a 12-fold serial dilution (1 + 1) of one patient sample with a very high initial COI (125.4). A serum pool from participants who tested negative with an average COI of 0.0598 was chosen as the dilution medium. Data were evaluated according to CLSI EP-06A with use of Abacus 2.0.

Statistical analysis

All statistical analyses were performed with SPSS Statistics version 25 (IBM, Armonk, NY, USA), and *p* values below 0.05 were considered as significant. The assessment of test performance with CLSI models was done with the Excel plug-in Abacus 2.0. Diagrams and charts were plotted with Prism version 7.0d (GraphPad Software, San Diego, CA, USA).

Results

Assay verification

The results of the performance evaluation regarding repeatability and intermediate precision were obtained with the 5-day protocol set out in CLSI EP15-A3 and are summarized in Figure 1. As expected, the highest percentage variations occurred in the lower measurement range. However, the CV was in line with the acceptance criteria for all three levels, demonstrating very good overall performance (Clinical and Laboratory Standards Institute, 2012). There was no substantial difference between intrarun and interrun repeatability.

Evaluation in line with CLSI EP-17A revealed a limit of blank of 0.065 COI and a limit of detection of 0.069 COI. Both values are in agreement with the acceptance criteria (Clinical and Laboratory Standards Institute, 2012) (Figure 2).

Data obtained from the linearity assessment are displayed in Figure 3. The results deviated significantly from the expected values ($y = 0.1821x^3 - 2.0682x^2 + 6.5411x - 4.9006$; $R^2 = 0.9873$; $p < 0.05$); therefore, no linearity range can be defined.

Study population

All patient samples that met the aforementioned inclusion criteria were included in the study. A total of 627 samples were

analyzed, representing more than 95% (627/658) of the samples sent to our laboratory on the relevant day. Of the 627 samples, 276 (44%) were from female participants and 348 (55.5%) were from male participants. For three samples, no sex information was contained in the laboratory information system at the time of collection. The median age of the study population was 64 years. 80 (12.8%) patients were treated in intensive care units or intermediate care units. All patients included had tested negative for SARS-CoV-2 infection by qRT-PCR performed as part of standard-of-care diagnostics (cobas 6800, Roche Diagnostics, Mannheim, Germany) on admission to our hospital.

Data retrieval from the hospital information system

To protect the business data of our hospital, the query data can be displayed only in a highly abstracted form and mainly in proportions. The submitting departments were combined as far as possible. The internal medicine departments made up the largest share and were classified as “INM”. The departments of visceral surgery, orthopedic surgery, trauma surgery, and neurosurgery were combined and labeled as “SUR”. The general pediatric department, the pediatric surgery department, and the pediatric oncology department were merged under “KIDS”. The urology, gynecology, dermatology, and ophthalmology departments were classed as “UR/GY/DER/OPH”. The other submitters were not subdivided further and were summarized as “UDEF”.

Figure 4a/b are showing the data obtained from the hospital information system for all patients treated at our hospital at the time of the study, and the data from the patients who were included in our study. Here, a significant difference regarding age was revealed. Samples with a residual blood volume below 1 ml were excluded from anti-SARS-CoV-2 antibody testing, and this was most often the case for young children, which explains the age shift and the lower percentage of samples from KIDS.

The distribution of the ICD-10-coded main condition block is displayed in Figure 5 This shows a broad distribution with a focus on “C” (malignant neoplasms), “I” (circulatory system), and “Z” (factors influencing health status and contact with health services in the study’s collective). This highlights the variability of clinical symptoms and diseases of patients treated at our hospital and included in the assessment of test specificity with our point prevalence approach.

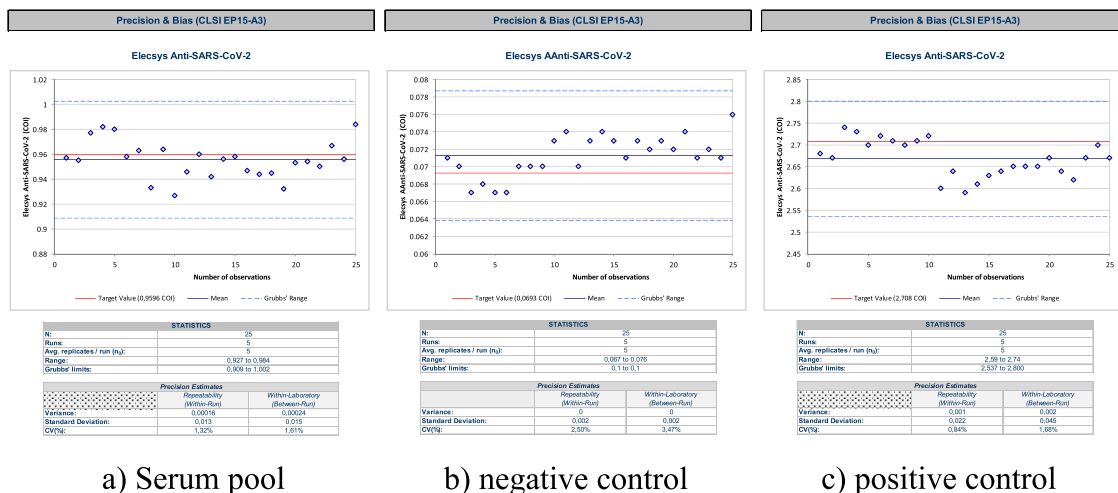
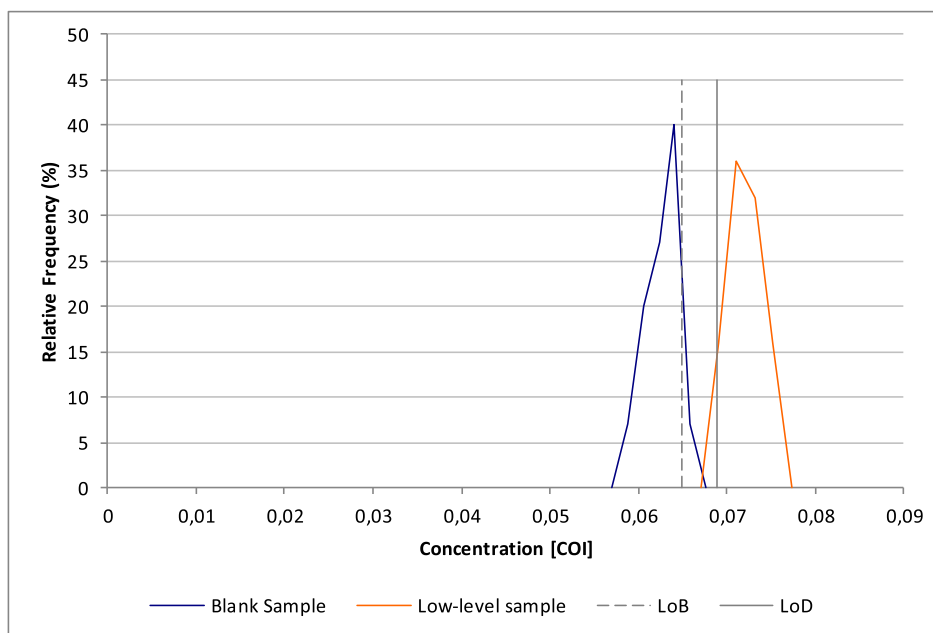


Figure 1. Precision and diagnostic accuracy determined on the basis of the Clinical and Laboratory Standards Institute (CLSI) EP15-A3 protocol with the quality control material provided by the manufacturer and an internally prepared serum pool near the cutoff point. COI, cutoff index; CV, coefficient of variation; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Limit of Detection (CLSI EP-17A)

Ant-SARS-CoV-2



STATISTICS		
	Blank Sample	Low-level Samples
n:	30*	25*
No. of Samples	1**	1**
Mean:	0,0618	0,0713
Median:	0,062	0,071
Std. Deviation	0,002	0,0024
Anderson Darling (z):	0,698	0,535
p-Value:	0,0683 ($\alpha = 0,05$)	0,1712 ($\alpha = 0,05$)
Method:	Non-parametric	Parametric
LoB ($\alpha = 0,05$)	0,0650 COI	
LoD ($\beta = 0,05$)	0,0689 COI	

Figure 2. Evaluation of detection capability performed according to the Clinical and Laboratory Standards Institute (CLSI) EP17-A protocol. The limit of blank (LoB) was determined by the 30 times serial measurement of the manufacturer’s dilution medium. Subsequently, a series measurement with a very low concentration was performed and the limit of detection (LoD) was calculated accordingly. COI, cutoff index.

Specificity testing results

The Elecsys anti-SARS-CoV-2 assay determined that 626 of the 627 samples were nonreactive. The mean value of these samples was 0.068 COI (median 0.069 COI, standard deviation 0.031, and CV 0.463%). None of the negatively measured samples were close to the manufacturer’s cutoffs.

Only one sample of the 627 tested was identified as Positive/reactive, with a COI of 63.47. The confirmatory test with the Shenzhen YHLO Biotech assay showed a result below the manufacturer’s cutoff of 10 AU/ml (IgM 5.3 AU/ml, IgG 5.06 AU/ml) for the detected antibody classes. This can be rated as a false-positive test result in our study population. With these data, a specificity of 99.84% (95% confidence interval 99.11–100%) was determined. This would give a negative predictive value of 99.69% with use of the current prevalence of 0.314% for the state of Baden-Württemberg. Using prevalence data for Germany as a whole (0.301%), it would give a negative predictive value of 99.98%.

Since the study design prevents the occurrence of truly positive cases in the test population, no statement can be made about positive predictive values or the sensitivity of the test method used.

Discussion

The diagnostic performance of the Elecsys anti-SARS-CoV-2 assay has been evaluated in various studies (Chan et al., 2020; Haselmann et al., 2020; Hubbard et al., 2020; Tan et al., 2021; Trabaud et al., 2020). It has demonstrated high overall diagnostic sensitivity and specificity, which range from 68.8% to 97.2% and from 99.05% to 100%, respectively. However, these clinical validation studies described so far rely on extensive blood sampling of infected individuals and especially of healthy volunteers, who are used as negative controls to assess the diagnostic specificity. In addition, there are also the validation data of more than 10,000 samples that are provided by Roche in the

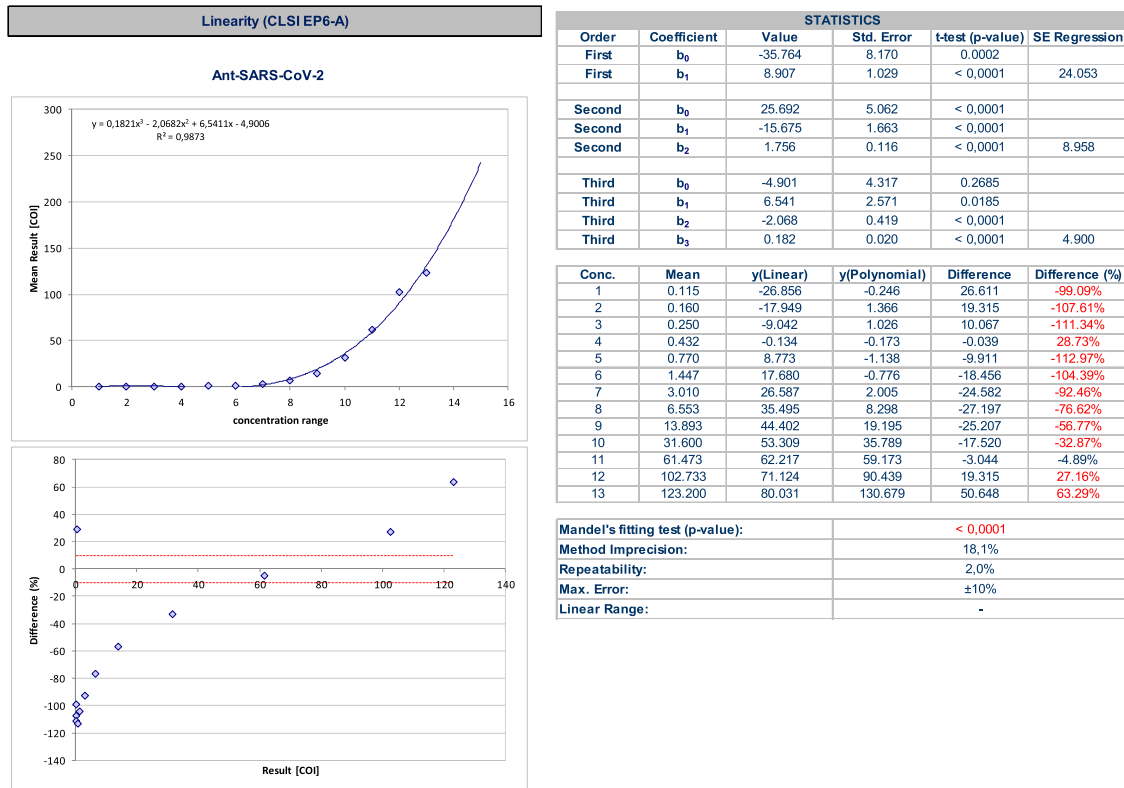


Figure 3. Linearity evaluation performed with a 12-fold serial dilution (1 + 1) of one sample with an initial cutoff index (COI) of 125.4. A serum pool from participants who tested negative with an average COI of 0.0598 was used as the diluent. CLSI, Clinical and Laboratory Standards Institute; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SE, standard error.

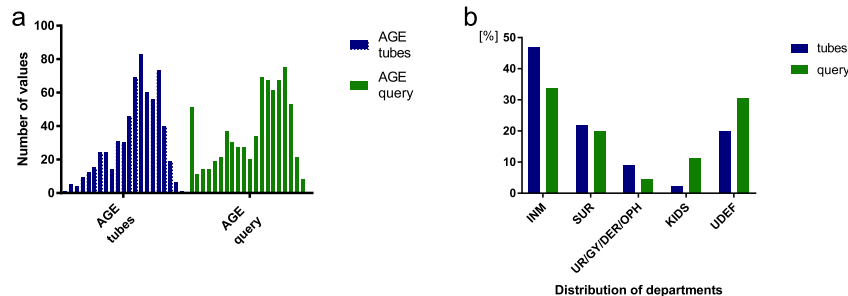


Figure 4. a) Comparison of the number of submissions of the departments and their occupancy (based on the controlling query) on the key date. b) Analysis of the age distribution of the submitted samples and age distribution of the departments on the key date. Pediatric surgery, and pediatric oncology, urology, gynecology, dermatology, and ophthalmology.

assay instructions (Roche Diagnostics, 2020). The validation approach for assessing diagnostic specificity in our study is the first that can be easily performed in any large-scale laboratory without the need for extensive blood sampling. It is a simple strategy that can be implemented promptly. This is of the utmost importance, given the huge societal and economic demand for fast, reliable, minimally invasive, and cost-efficient tests to safely detect a previous SARS-CoV-2 infection and immunity. Examining large sample sizes is the only way to detect individuals who were exposed to the virus and experienced only mild symptoms or were asymptomatic – these individuals represent the majority of cases (Kimball et al., 2020; Lavezzo et al., 2020; Mizumoto et al., 2020; Oran and Topol, 2020; Sutton et al., 2020). The approach described allows the laboratory to conduct its own verification study within a short time. This allows the assessment of the test performance on its own and the initiation of troubleshooting if there are major differences between the data obtained and those from the

literature or provided by the manufacturer. A large-scale application of high-performance assays might make it possible to predict the “true prevalence” of the disease, since most patients develop detectable antibodies once the virus is no longer traceable in the upper respiratory tract by a NAAT (Guo et al., 2020). There still remains a diagnostic gap until the formation of antibodies, which is reported to be 2 weeks after symptom onset (Deeks et al., 2020). Here it is important to remember that even with high specificities, the significance of a test result depends on the positive/negative predictive value, and the calculation of this presupposes that the prevalence has been correctly determined. Irrespective of this, a false-positive result would possibly give the individual a false sense of security regarding his or her humoral immune response status.

The results obtained with our method matched the manufacturer's stated test performance in terms of specificity. Evaluation of the control query in conjunction with the study

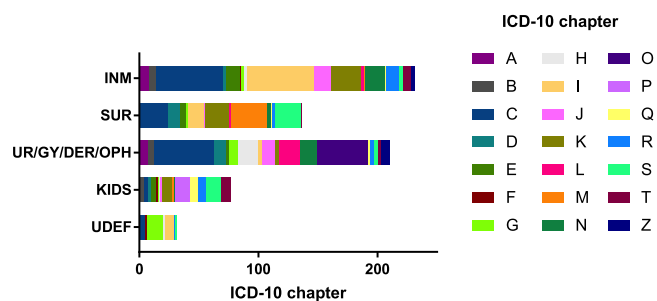


Figure 5. Distribution of the International Statistical Classification of Diseases and Related Health Problems tenth revision (ICD-10)-coded main diagnosis block based on the controlling query on the key date, INM, internal medicine department; SUR, visceral surgery, orthopedic surgery, trauma surgery, and neurosurgery departments; KIDS, pediatric, pediatric surgery, and pediatric oncology Udepartments; UR/GY/DER/OPH, urology, gynecology, dermatology, and ophthalmology departments; UDEF, other departments.

results demonstrates that the Roche Elecsys anti-SARS-CoV-2 assay provided an outstanding performance in “real-life” clinical conditions. While the vast majority of samples used by the manufacturer to determine specificity originate from routine diagnostics ($n = 6,305$) and from blood donors ($n = 4,148$), this study is the first to evaluate such a large sample size in a maximum-care hospital setting that therefore includes any type of comorbidity (Roche Diagnostics, 2020). Despite an extensive literature research, we could not identify any studies that used a similarly large population to determine the specificity of the Roche assay.

Although the Roche assay predominantly detects IgG antibodies, other antibody classes are also detected. This is due to the test technique. Several authors claim that it would be preferable not to use IgA and IgM for diagnostic purposes, as this can lead to false positives and therefore lower specificity (Van Elslande et al., 2020; Wang et al., 2020a). We do not agree with this with regard to the Roche assay. It seems that its electrochemiluminescence immunoassay technology is capable of reproducing, under real conditions, the 99.81% specificity indicated by the manufacturer (Roche Diagnostics, 2020).

Nevertheless, our study approach has two major limitations. First, the design chosen means that we cannot provide a statement about the sensitivity of the test. However, this problem has already been sufficiently addressed by other authors and can be clarified only by longitudinal observations. Another limitation is that data privacy laws in Germany make it impossible to process personal data without a written declaration of consent. As this would have prevented the study from being conducted, the samples were processed anonymously. The validation of our samples was verified by matching with the control data. Since all inpatient samples from the day that met the inclusion criteria were processed, it was not possible to achieve a higher number of cases. This could be overcome by a multicenter approach.

Another limitation concerns the negative predictive value. Since this was determined on the basis of a hospital population, it can be applied only to a comparable one. In addition, the study was conducted in summer, which results in a lower incidence of infections with other respiratory viruses and consequently of a lower probability of cross-reactivities.

One constraint we were unable to overcome because of the anonymous study design was a dedicated follow-up of the false-positive test result. Of course, it must be considered that this particular patient could also have had a false-negative NAAT result. However, this would seem unlikely because of the assay used, which has one of the highest sensitivities (Pujadas et al., 2020).

In conclusion, this study proves that the Roche Elecsys anti-SARS-CoV-2 assay is not compromised by most comorbidities occurring in hospital settings. Despite the current low prevalence of SARS-CoV-2, this test procedure seems to allow the implementation of high-throughput epidemiological investigations and provides a complementary diagnostic approach to overcome the limitations of qRT-PCR tests for viral detection from respiratory material.

The procedure presented in this study enables quick evaluation of these test methods in laboratories without biobanks. The anonymized use of residual material and comparison of the control population with abstracted economic data makes it possible to assess the validity of the random sampling and therefore to determine diagnostic specificity with the recommended sample size of 500 (Fang et al., 2020). Important in implementing this ubiquitous rapid test validation strategy is the use of one or two independent confirmatory tests addressing different surface antigens. However, the limitation of double false-positive results remains, and increases with multiple preexisting conditions and a higher number of people with previous infection.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

CRediT authorship contribution statement

Maximilian Kittel: Conceptualization, Methodology, Validation, Investigation. **Peter Findeisen:** Investigation, Writing - review & editing. **Maria-Christina Muth:** Investigation, Writing - review & editing. **Margot Thiaucourt:** Investigation, Writing - review & editing. **Catharina Gerhards:** Writing - review & editing, Writing - original draft, Resources, Visualization. **Michael Neumaier:** Supervision, Writing - review & editing. **Verena Haselmann:** Conceptualization, Methodology, Supervision, Writing - review & editing.

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