On December 31, 2019, the first case of pneumonia of unknown etiology was reported in the Wuhan province of China, and within a few weeks, had evolved into a global outbreak, affecting over 200 countries and territories.1 A novel strain of coronavirus of zoonotic origin—SARS-CoV-2—was identified as the cause of “coronavirus disease 2019” (COVID-19), which has affected more than 5.5 million people globally and caused more than 353,000 deaths as of May 28, 2020.

According to estimates from the European Centre for Disease Prevention and Control (ECDC), more than 1.8 million cases of COVID-19 were identified in the European nations, with the United Kingdom having the highest number of confirmed cases—above 269,000 as of May 29, 2020.2 The World Health Organization (WHO) developed “Operational Planning Guidelines to Support Country Preparedness and Response” to assist UN country teams in scaling up their country’s preventive measures in response to COVID-19. This document suggested a coordinated global response plan to control the COVID-19 epidemic, which primarily involved prevention of human-to-human transmission of the virus, caring for and monitoring infected patients, and rapid diagnosis of the disease, followed by effective infection control measures.3

On January 10, 2020, the viral genome sequence for SARS-CoV-2 was released for immediate public health support, and since then, several diagnostic assays have been developed both commercially and in-house by individual labs for the rapid detection of the novel coronavirus (Wuhan-Hu-1, GenBank accession number MN908947).4

We interviewed Dr. Thomas Juretzek of the Carl-Thiem-Klinikum (CTK) Hospital in Cottbus, Germany, who developed an in-house screening assay using Luminex’s ARIES® System for SARS-CoV-2, and implemented it as part of a two-step diagnostic algorithm at the hospital.

Please tell us about yourself and your institution.

I started working at the Carl-Thiem-Klinikum (CTK) Hospital in Cottbus more than 16 years ago as a Scientific Assistant at the Center for Laboratory Medicine, Microbiology, and Hospital Hygiene (Zentrum für Laboratoriumsmedizin, Mikrobiologie und Krankenhaushygiene), in Dr. Heidrun Peltroche’s unit. With more than 1,200 beds, the CTK is the largest hospital in the federal state of Brandenburg. Our laboratory carries out diagnostic tests for patients at the CTK, as well as for more than 100 medical outpatient practices, resulting in over 100,000 tests on approximately 15,000 samples each year. We offer a broad spectrum of different diagnostic assays for detecting bacteria, viruses, and parasites; the main targets are respiratory viruses, herpesviruses, Chlamydia, hepatitis viruses, and mycobacteria. In addition to managing the diagnostic laboratory, I also teach forensics at the Brandenburg University of Technology.

What are the current challenges associated with diagnostic testing for SARS-CoV-2?

There are several challenges and bottlenecks when it comes to diagnostic testing for SARS-CoV-2. The first challenge to overcome is the need for rapid identification of people who need to be tested. Due to the lack of available tests, testing priority is currently given to symptomatic individuals. However, as the epidemiological data shows, asymptomatic carriers can also initiate community spread.

The second challenge involves the design of a rapid diagnostic test that can provide accurate answers within hours instead of one or more days. We need to develop highly sensitive and specific tests so that we can accurately capture true cases of COVID-19 to ensure that patients are assigned correctly to their specific cohorts. Moreover, test sensitivity and specificity are important for identifying individuals who actually carry SARS-CoV-2, and for preventing false-positive results in cases where the virus is not present.
How is the Center for Laboratory Medicine, Microbiology, and Hospital Hygiene managing COVID-19 diagnostics?

Our mandate for COVID-19 testing extends to the CTK and the Sana Heart Clinic in Cottbus, as well as to the surrounding hospitals and local health officials. This testing includes screening of all newly admitted patients, employee testing, and postmortem diagnostics for SARS-CoV-2.

Our laboratory wanted to develop a rapid, automated SARS-CoV-2 real-time RT-PCR assay as part of a two-step diagnostic algorithm—using an E gene assay as the first line screening tool and an RdRp gene assay as the confirmatory test. To this end, we developed the screening assay for the E gene on Luminex’s ARIES® automated sample-to-answer platform and the confirmatory assay using the RdRp gene on the Roche LightCycler® 480 II Instrument (LC480 II), and implemented this two-step diagnostic algorithm in our facility for STAT SARS-CoV-2 testing.

How do the assay and diagnostic algorithm work?

We developed our assay based on the recent study by Corman et al., who established and validated an RT-PCR assay and diagnostic workflow for primary screening and specific confirmation of SARS-CoV-2. Their diagnostic algorithm used the E gene assay as the first-line screening tool and the RdRp gene assay as the confirmatory test. A negative call in the E gene assay indicated absence of SARS-CoV-2, whereas a positive result was further confirmed using the RdRp gene assay. The probe used in the RdRp assay, RdRP_SARSr-P2, is highly specific and can therefore differentiate between SARS-CoV-2 RNA and SARS RNA. This approach allows rapid screening, ruling out non-infected patients, and identification of presumptive positive patients, of which the latter are then confirmed by the second assay. However, since patients are admitted to the hospital on a 24/7 basis, there is a need for a STAT test as well. Therefore, we adapted this assay concept to two separate platforms—we ran the E gene screening assay on the ARIES® system and then confirming results on the LC480 II instrument following the manufacturer’s instructions. For the ARIES® System, the primers and probes were added to the Exo+ Master Mix tube, the tube was attached to the ARIES® Extraction Cassette, and 200 µL of the sample at each copy number was added directly into the sample chamber of the cassette.

We validated our ARIES® assay by running several control specimens on the ARIES® System and then confirming results on the LC480 II prior to implementing the assay and diagnostic algorithm to run patient samples in our center. All ARIES® positive results are confirmed on the LC480 II.

Why did you choose the ARIES® System as a platform for your screening assay?

Almost two years ago, we began using the ARIES® System to run samples that arrived late in the day or on the weekend. Initially, we started with the CE-IVD assays, including Flu A/B & RSV, HSV 1&2, C. difficile, and norovirus. It was very easy to implement ARIES®-based testing in our workflow, as shown by the norovirus study we did initially, which was presented by Dr. C. Lynen at the 2018 European Society for Clinical Virology (ESCV) conference.

With the ability to run lab-developed tests (LDTs) on ARIES® and the analyte-specific reagents (ASRs) provided by Luminex for many different targets, we started a project on herpesvirus testing. Our aim was to create sensitive, low-plex assays for herpesviruses, where we combined the detection of several different viruses in one PCR assay. In case of a positive call, we were able to administer the appropriate antiviral, such as acyclovir for HSV 1&2 and VZV, ganciclovir for CMV, and cidofovir for adenovirus. To this end, we developed and validated three low-plex assays combining different resistant strains of these viruses, and successfully implemented them for routine use in our center, much of which was presented by Daniel Berger in his bachelor’s thesis at the Brandenburg University of Technology. Considering our experience with the sample-to-answer ARIES® System and its benefits—such as ease of use and rapid turnaround time—it was only logical to establish the SARS-CoV-2 testing on ARIES® as well.

Can you describe your assay procedure on the Luminex ARIES® System?

We obtained positive control material consisting of RNA extracted from SARS-CoV-2 Frankfurt-1 strain cell culture and the E gene RNA from Wuhan coronavirus 2019 from the National Consultant Laboratory for Coronavirus at Charité Institute for Virology. Control materials were standardized to 50 genomes per µL. The SARS-CoV Frankfurt 1 strain was obtained at a stock concentration of 1.0 x 10^4 copies/µL. 5 µL of this stock solution was serially diluted in PCR-grade water to prepare dilutions at 1:10 (5,000 copies/reaction), 1:100 (500 copies/reaction), and 1:1,000 (50 copies/reaction). The Wuhan coronavirus 2019 stock solution was obtained at a concentration of 1.0 x 10^5 copies/µL. 5 µL of this stock solution was serially diluted in PCR grade water to prepare dilutions at 1:100 (5,000 copies/reaction), 1:1,000 (500 copies/reaction), and 1:10,000 (50 copies/reaction). Samples and control material used on the LC480 II assay were extracted using the QIAGEN EZ1 extraction system and the assay was performed on the LC480 II instrument following the manufacturer’s instructions. For the ARIES® System, the primers and probes were added to the Exo+ Master Mix tube, the tube was attached to the ARIES® Extraction Cassette, and 200 µL of the sample at each copy number was added directly into the sample chamber of the cassette.

For a STAT test as well. Therefore, we adapted this assay concept to run patient samples in our center. All ARIES® positive results are confirmed on the LC480 II.
What are the benefits of rapid first-line screening?

We need to obtain results quickly with minimal hands-on time in order to operate as efficiently as possible. We are testing approximately 300–400 patient samples using the LC480 II and about 20–60 samples on the ARIES® each day. A rapid screening assay is vital for triage and quarantine protocols. It is the basis of effective hospital infection prevention and control measures, which aim to keep non-infected patients separate from a COVID-19 cohort when admitted to the hospital and to preserve sufficient bed capacity for COVID-19 patients. Currently, we are using the E gene assay on ARIES® as the screening assay for emergency cases, off-hour patient samples, and for testing employees. We are using the E gene assay on the LC480 II as the screening assay for all inpatient testing, pre-testing before hospitalization, and testing requested by local health authorities. All confirmatory testing is done with the RdRp assay on the LC480II.

Using an automated, sample-to-answer platform like the ARIES® System as the first-line of screening during an outbreak has a number of clear clinical benefits, as described here. The ARIES® System is user-friendly, scalable for higher throughput, and has guided workflows that reduce the chance for user error. Further, automated workflows and sample-to-answer analysis enable 24/7 and emergency testing. When it comes to responding to a pandemic, rapid turnaround times help labs run samples quickly, and provide the prompt clinical guidance needed to cope with the evolving needs of the healthcare community at large.

Recently, Dr. Juretzek completed validation of Luminex’s NxTAG® CoV Extended Panel, which was implemented in parallel with the NxTAG Respiratory Pathogen Panel (RPP). This allows for comprehensive testing for respiratory pathogens at CTK, as it is especially requested for pediatric patients.

REFERENCES


