Ultra-Sensitive and Specific Detection of SARS-CoV-2 Antibodies Using the ADAP STAR Assay Ready Workstation

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Introduction

As of April 16, 2020, there were more than two million confirmed infections of SARS-CoV-2, the virus responsible for Coronavirus Disease 2019 (COVID-19), around the world. In the battle against this rapidly expanding pandemic, successful COVID-19 community screening programs are essential for assessing infection prevalence, aiding identification of infected patients, and enacting appropriate treatment and quarantine protocols.

When initially screening for COVID-19 infections, many assay chemistries used Reverse Transcriptase PCR (RT-PCR) methods to detect live virus. This live virus method, however, has serious limitations that the healthcare community cannot ignore. RNA testing has high false negative rates with naso/oropharyngeal swab samples (30%–75% sensitivity during the first two weeks of infection) due to inconsistent swab technique or low viral titers in the sampled body compartments[1]. If an individual receives false negative results, they may relax quarantine precautions and unintentionally expose others to the virus. Additionally, if materials such as nasopharyngeal swabs and extraction kits are in short supply, they limit test capacity and cap the number of assays that a testing site can perform. Moreover, live virus RNA-based assays only detect acute infection and cannot be used to capture meaningful data on individuals who have cleared the virus or provide immunity information.

With these limitations, it is clear that there is an urgent, unmet need for a sensitive and specific liquid biopsy test to complement live virus RNA-based assays and improve disease identification and tracking.[1–7] Unlike live virus RNA-based assays, liquid biopsy DNA-based assays use qPCR to detect the individual's antibody response to the pathogen, and so may provide information critical for serum-based immunotherapies. Those assay methods employ ELISA and lateral-flow, which may have a relatively low sensitivity leading to a high degree of false negatives, especially when a large proportion of infected individuals are in the acute disease stage and have low antibody levels.[8]

Antibody Detection by Agglutination-PCR (ADAP) differs from traditional immunoassays in that it is a pure solution-phase assay, it is well suited to detecting conformationally challenging antibodies, and it requires little in terms of input sample volume. The assay uses a pair of antigen-DNA conjugates to probe the sample target antibodies. If present, agglutination between the antibody and antigen, and ligation, leads to formation of a specific DNA amplicon detected by qPCR.

Benefits-Based Highlights

- Enable sample-sparing analysis of precious samples.
- Achieve sensitive and specific antibody detection results.
- Eliminate time-consuming and repetitive manual pipetting.
- Reduce or eliminate risks of human error and variability to ensure consistency and quality of downstream results.

In this application note, we describe the development and validation of an ultra-sensitive and high-throughput liquid biopsy ADAP assay to detect antibodies to SARS-CoV-2. The ADAP SARS-CoV-2 Antibody Assay uses antigen-DNA conjugates to convert the presence of antibodies into a short DNA oligonucleotide, which is then amplified and quantified by real-time PCR.[9–12] ADAP has previously been used to create ultra-sensitive antibody assays for HIV,[11] Zika and dengue, Lyme disease, and Type 1 Diabetes;[12] detecting antibody markers earlier than standard immunoassays in a broad spectrum of substrates including saliva, whole blood, and serum.

The workflow is automated using the ADAP STAR assay ready workstation from Hamilton Company (see Workflow and Deck Layout at end). The ADAP STAR is based on the Company's Microlab® STAR™ liquid handling workstation, and is configured with CO-RE® gripper paddles and the iSWAP robotic transport arm for hands-free plate and lid movements. Microlab VENUS® software in ADAP STAR was optimized and pre-programmed for all steps. The ADAP SARS-CoV-2 Antibody Assay is amenable to self-collected sample types that may be transported via existing simple channels to ease the sample collection burden placed on healthcare professionals. The hands-free system enables high-throughput sample processing to reduce screening workload demands while helping to protect technicians from contacting biohazardous samples.

Materials and Methods

A cohort of 15 PCR-confirmed positive samples and 127 negative samples predating COVID-19 were analyzed once in order to generate timely data. Replication of results is ongoing. As saliva samples have the potential to contain high loads of virus, these samples were heated at 56°C for 30 minutes to inactivate it.

The ADAP STAR was manually loaded with the following consumables from Hamilton Company (Reno, NV):

- 96- and 384-well PCR Frame Plates (P/N 814302, 814305)
- 50 µL Conductive Non-Sterile Filter Tips (P/N 235948)
- 300 µL Conductive Non-Sterile Filter Tips (P/N 235903)
- PCR Comfort Lid (P/N 814300)
- Optically Clear Plate Seals (P/N 67765-01)

The ADAP SARS-CoV-2 Antibody Assay kit reagents (Enable Biosciences; P/N DK2-100 (SARS-CoV-2)) were manually prepared according to manufacturer instructions. Agglutination mix, ligation mix, pre-amplification mix, and one qPCR-primer mix from the Enable reagent kit were added to 1.5 mL conical microcentrifuge tubes on the CPAC, and ligation mix and dilution water were added to a chilled 12-column reagent reservoir (P/N 201256-100, Agilent Technologies, Santa Clara, CA).

The ADAP SARS-CoV-2 Antibody Assay was performed manually and also by automated means using the ADAP STAR assay ready workstation as follows:

1. 8 µL of Agglutination mix and 4 µL of the appropriate body fluid sample were added to a 384-well frame plate and transferred to the 384-well ODTC and incubated at 37°C for 30 minutes.
2. 4 µL of the resulting mix was added to a 96-well frame plate along with 116 µL of ligation mix and transferred to the 96-well ODTC and incubated at 30°C for 15 minutes.
3. 25 µL of the resulting mix was added to a 96-well frame plate along with 25 µL of pre-amplification mix and transferred to the 96-well ODTC and subjected to thermocycling (13 cycles of PCR, cycling between 95°C and 56°C for a total of approximately 40 minutes).
4. The amplified product was diluted 20-fold using molecular biology grade water (P/N 46-000-CM, Corning Corporation, Corning, NY), and 8.5 µL of the diluted product was added to 11.5 µL of each individual qPCR-primer mix.
5. The final solution was sealed using the plate sealer for subsequent qPCR quantification on a CFX384 Touch™ Real-Time Detection System (Bio-Rad Laboratories, Hercules, CA).

Results and Discussion

Assay Sensitivity/Specificity Using Serum Samples

The ADAP SARS-CoV-2 Antibody Assay contains S1 protein probes from SARS-CoV-2, which were used to analyze 15 PCR-confirmed serum samples and 127 negative control samples on the ADAP STAR workstation. The positive samples were collected primarily from acutely infected individuals (8-15 days post-onset). The negative samples were sourced from a biobank prior to the COVID-19 outbreak. Several of the negative control samples were positive for antibodies against other viruses, including HIV, CoV-HKU1, and CoV-NL63.

SARS-CoV-2 antibodies were detected in all of the samples tested with 100% sensitivity for samples collected after eight days post-COVID-19 symptom onset (Table 1). In the negative control cohort, 126 samples were designated as negative, with one false positive, to give 99.2% specificity. None of the related coronaviral samples (CoV-HKU1/CoV-NL63) were reactive (Table 1). Among the 15 samples, eight were collected from five donors across different time points during the acute infection phase (Figure 1). Together, these results substantiate that ADAP methodology is a highly sensitive and specific assay for detecting an immune response to SARS-CoV-2 without cross-reactivity to other coronaviruses.

Table 1: Sensitivity and Specificity of ADAP SARS-CoV-2 Antibody Assay

Sensitivity	Samples from PCR Confirmed COVID-19 Patients After Eight Days of Symptom Onset	**Specificity**	Control Samples Prior to Outbreak (N=127), Including Other Coronavirus Samples
Methods	ADAP COVID-19	**Methods**	ADAP COVID-19
Number of Ab+ Samples	15/15 (100%)	**Number of Ab– Samples**	126/127 (99%)

The ADAP SARS-CoV-2 Antibody Assay is highly sensitive and specific. Data for the sensitivity cohort, generated by automated means, was from PCR-confirmed COVID-19 patients. The automated assay identified all PCR+ individuals. The specificity cohort was collected prior to outbreak and included CoV-HKU and CoV-NL63 samples. Both sample types were Ab negative by ADAP. Blood samples from the two Ab+ patients were obtained from commercial biobanks, and they were fully de-identified.

Figure 1. Longitudinal samples from five patients tested using the ADAP SARS-CoV-2 Antibody Assay on the ADAP STAR showed positive results for all individuals. The assay identified patients as early as the eighth day following COVID-19 symptom onset.

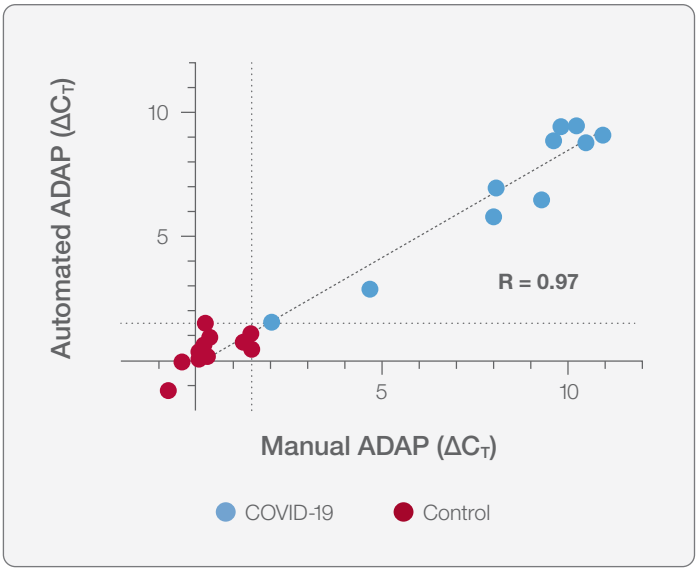

Figure 2. Both the manual and automated ADAP SARS-CoV-2 Antibody Assay correlated strongly with one another. Ten positive and nine negative samples (N=19) that were randomly selected and tested by manual and automated procedures were re-analyzed, and the data are shown here.

Next, we used the manual assay to re-analyze a portion of the same samples to determine the correlation between manual and automated versions of the assay. High correlation was observed, with Pearson correlation coefficient of 0.97 (Figure 2).

Intra-Assay Precision

As can be seen in Table 1, the intra-assay precision was calculated for automated ADAP assays. Mean, standard deviation, and coefficient of variation (%CV) of serum samples positive for SARS-CoV-2 antibodies were calculated for an N=5 for the automated ADAP assay. ΔC_T is defined as the difference in cycle threshold (C_T) between blank sample and actual sample and used as a performance characteristic. Due to the nature of this assay, the %CV of ΔC_T for negative samples is not representative of assay variation and not considered as an indicator of performance characteristics. The intra-assay variations were in-line with the industry standard for method validation developed by the US Department of Health and Human Services, Food and Drug Administration.[13]

Inter-Assay Precision

The inter-assay precision for the automated method is presented in Table 2. Mean, standard deviation, and coefficient of variation (%CV) of serum samples positive for SARS-CoV-2 antibodies were calculated by testing five replicates of samples on three different days using the automated ADAP assay. ΔC_T is defined as the difference in cycle threshold (C_T) between blank sample and actual sample and used as the performance characteristic. Due to the nature of this assay, the %CV of ΔC_T for negative samples is not representative of assay variation and not considered as an indicator of performance characteristics. The intra-assay variations were in-line with the industry standard for method validation developed by the US Department of Health and Human Services, Food and Drug Administration.[13]

Assay Performance Using Spiked Saliva Samples

To evaluate whether the ADAP SARS-CoV-2 Antibody Assay kit could detect SARS-CoV-2 antibodies in saliva, we spiked two COVID-19 positive serum samples at 1:50 dilution into saliva samples collected prior to the outbreak. The two positively spiked saliva samples were correctly identified as positive. Un-spiked saliva samples collected from individuals prior to the outbreak were used as negative controls (Figure 3). Given that saliva samples potentially contain high viral loads, we investigated the effect of a heat inactivation protocol used to destroy the infectivity of the SARS1 virus (heating the sample to 56°C for 30 minutes).[14] We observed no change of signals with and without such inactivation, paving a way for staff to safely handle saliva samples in large scale SARS-CoV-2 testing. Inactivation does not appear to perturb the assay signals.

Table 2: Inter-Assay Precision of the Automated ADAP SARS-CoV-2 Antibody Assay Run on the ADAP STAR

	Neg	Low	Medium
Mean	-0.34	3.36	6.56
SD	0.19	0.12	0.29
%CV	–	4.5%	3.7%

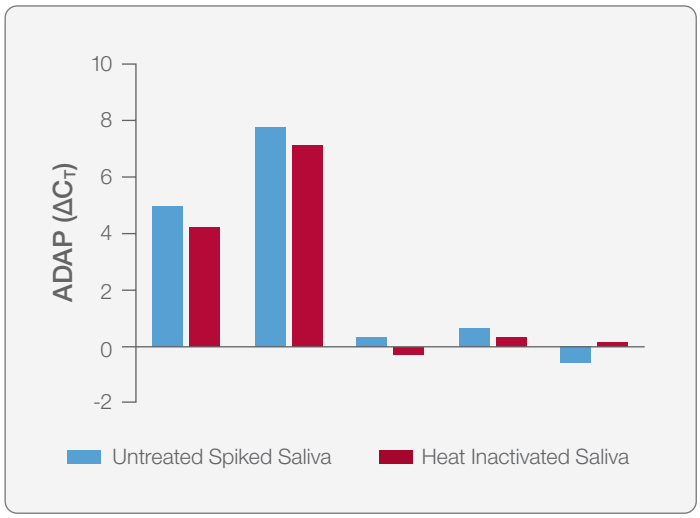

Figure 3. COVID-19 serum samples were spiked into saliva and analyzed by ADAP (two positive on left, three negative on right). Heat treatment did not significantly change signal intensity.

Conclusion

We constructed an ultra-sensitive antibody assay and evaluated its performance both manually and on an automated liquid handling platform by analyzing 15 PCR-confirmed serum samples and 127 negative control samples. In our initial trial utilizing PCR-verified samples, we demonstrated that the automated ADAP SARS-CoV-2 Antibody Assay correctly identified all COVID-19 positive individuals tested. Many samples were collected between eight to fifteen days after symptom onset, highlighting the strong sensitivity of ADAP platform. Furthermore, among 127 control specimens collected prior to COVID-19, ADAP correctly assigned 126 of them as antibody negative. Notably, these control specimens included samples from individuals with other human coronavirus infected samples such as HKU1 and NL63. ADAP did not react with samples derived from other coronaviral infections.

The ADAP STAR assay ready workstation can be readily deployed in labs on a high throughput basis, analyzing up to 921 samples per day with high correlation to manual analysis and minimal technician input.

The high-throughput ADAP STAR assay ready workstation has been provisionally validated for the sensitive and specific detection of SARS-CoV-2 antibodies in serum samples. ADAP's strong past performance with saliva samples also positions ADAP well for COVID-19 testing using other easily-collected sample types. The use of ADAP STAR in COVID-19 analytical workflows has the potential to greatly improve the reach, efficacy and impact of vital viral screening programs.

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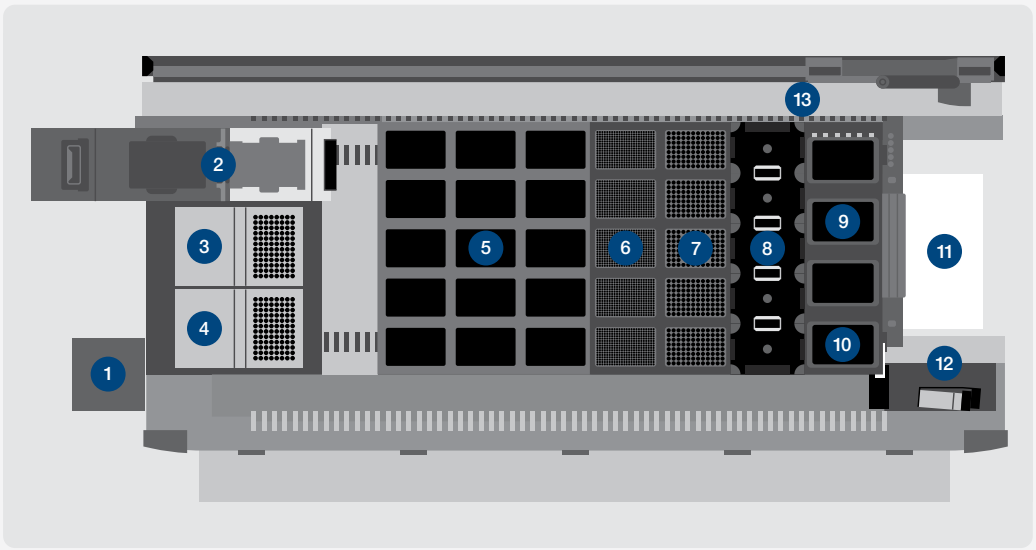
ADAP STAR Antibody Detection Workflow

ADAP STAR Deck Layout for the ADAP SARS-CoV-2 Antibody Assay



1. 96 MPH Waste
2. Plate Sealer
3. 96-well Thermal Cycler
4. 384-well Thermal Cycler
5. 50 µL and 300 µL Pipette Tips
6. 384-well PCR Plate Positions
7. 96-well PCR Plate Positions
8. Sample Plate Carrier
9. Cold Plate Air Cooled (CPAC) Devices
10. Comfort Lid Stack
11. Waste
12. Autoload
13. iSwap

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