Application note

Fluidic Analytics

Determination of antibody affinities and concentrations directly in clinical samples

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Abstract

The Seroaffinity and Concentration (SAffCon) Assay measures antibody affinity and concentration directly in clinical samples such as serum or plasma. In this application note, we explain that samples with similar antibody titers determined by ELISA can have dramatically different SAffCon fingerprints. Unlike ELISA, our assay can provide truly quantitative insights into the immune response to infection, vaccination, inflammation, transplants, or new drugs. The SAffCon Assay is very easy to perform, takes only 2–3 hours, requires 50 µL of serum, is immobilization-free, provides universally comparable K_D values and absolute antibody concentrations and importantly can be performed directly in clinical samples.

Introduction

The antibodies circulating in our blood provide a unique opportunity to track the immune response to infection, vaccination, inflammation, transplants, or new drugs. The complex, protein rich nature of serum or plasma make them ideal media for characterizing health and disease but also causes challenges for accurate characterization of protein interactions. In response to these challenges many approaches have employed techniques to minimize the impact of background noise, such as dilution in standard buffers or the addition of blocking agents. While this can be effective at reducing background, the resulting modification to the biological environment, reduction of detection sensitivity, and the potential introduction of handling errors can significantly affect the reliability and applicability of these modified assays. Being able to use serum or plasma to understand the progression and resolution of a disease depends on understanding the fundamental properties of the antibodies that will provide a protective immune response. Standard immunoassays (most importantly, ELISA assays) have been used routinely for decades. While ELISA assays are well established, automated, and low cost, the tests are often tedious to optimize, susceptible to non-specific binding, are difficult to compare between laboratories, and can only report a simplistic antibody titer (Figure 1).

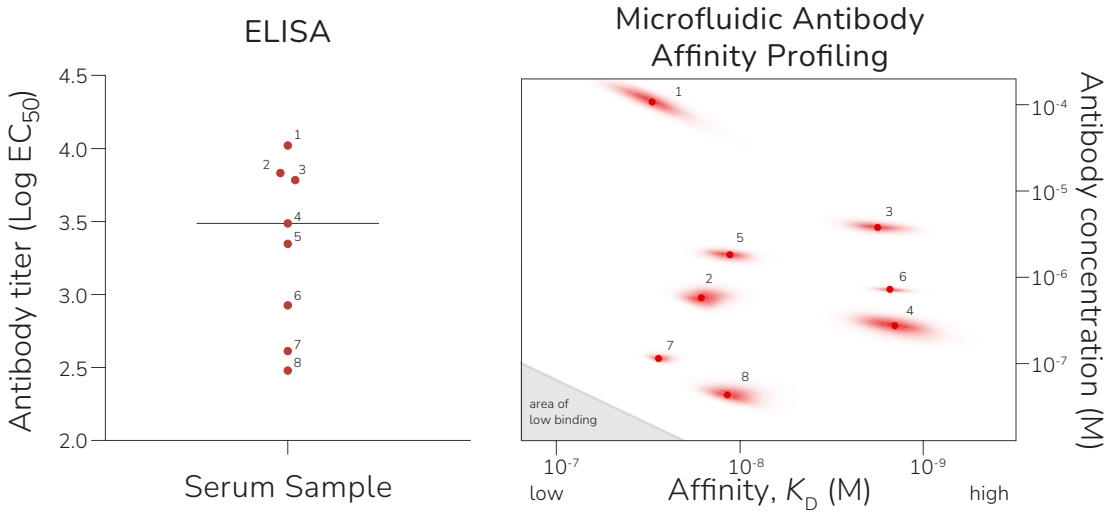


Figure 1. Unlike titers, the Seroaffinity and Concentration (SAffCon) Assay uniquely measures affinity and concentration directly in clinical samples. Both plots show the same serum samples measured by serological ELISA and by SAffCon Assay. In both experiments, samples were tested for the presence of antibodies specific to SARS-CoV-2 spike receptor binding domain (data taken from Emmenegger *et al.* 2022). Shaded areas in the SAffCon Assay data indicate parameter precision. The gray area indicates the affinity–concentration range of low/no binding.

Measuring an antibody titer is a blunt tool for characterizing protein interactions since they cannot independently provide information on the binding affinity and concentration for an antigen–antibody interaction. By contrast, the Seroaffinity and Concentration (SAffCon) Assay circumvents this challenge by being able to determine, directly in clinical samples, the affinity of antibodies to a target as well as the calibration-free concentration of target-specific antibodies without the need for immobilization or antibody purification. As shown in Figure 1, samples that show very similar titer values in ELISA can have dramatically different affinity–concentration profiles as measured by SAffCon Assay on the Fluidity One-M. In this application note, we explain the principle of the SAffCon Assay including a workflow, timings, and sample consumption when using a pooled convalescent standard plasma in conjunction with three SARS-CoV-2 RBD variants (wild-type, delta, and omicron).

Crucially, the assay only takes 2–3 hours, is immobilization-free and provides universally comparable K_D values and calibration-free antibody concentrations.

Results

The SAffCon assay measures the binding of a fluorescently labeled probe to antibodies in a serum or plasma sample. The assay utilizes microfluidic diffusional sizing on the Fluidity One-M which detects the size increase experienced by the probe upon antibody binding. For accurate quantification of both antibody affinity and concentration, at least two different probe concentrations need to be measured. Thus, we tested all three variants (wild-type, delta, and omicron) of fluorescently labeled SARS-CoV-2 spike receptor binding domain (RBD) at two different concentrations (Figure 2). The RBD probes were mixed with untreated plasma from convalescent donors (working reagent anti-SARS-CoV-2 immunoglobulin NIBS 21/234) at seven different concentrations. For analysis, the data was uploaded to the Fluidity Cloud application which determined the affinity and concentration of RBD-specific antibodies (Figure 3).

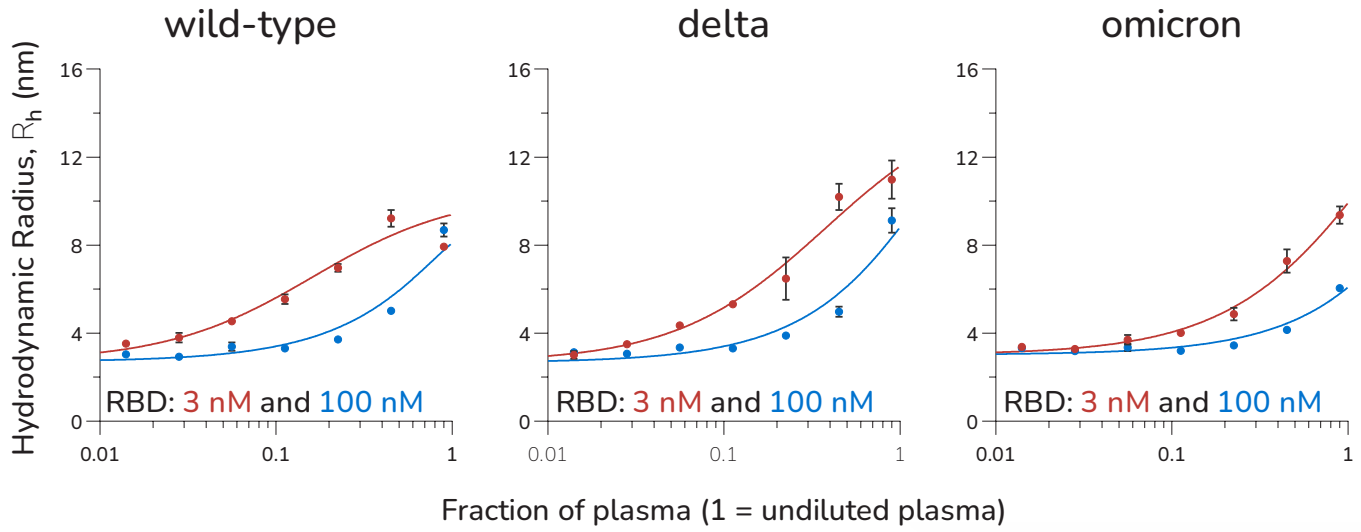


Figure 2. To determine affinities and concentration of RBD-specific antibodies, serially diluted SARS-CoV-2 plasma (highest concentration 90%) was incubated with the three fluorescently labeled RBD variants at two different concentrations (3 nM and 100 nM).

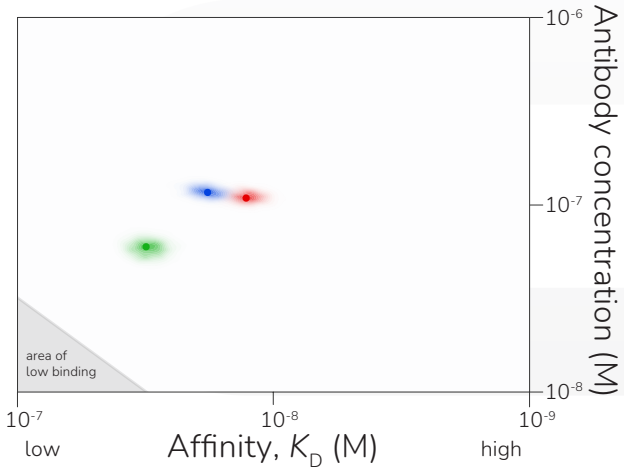


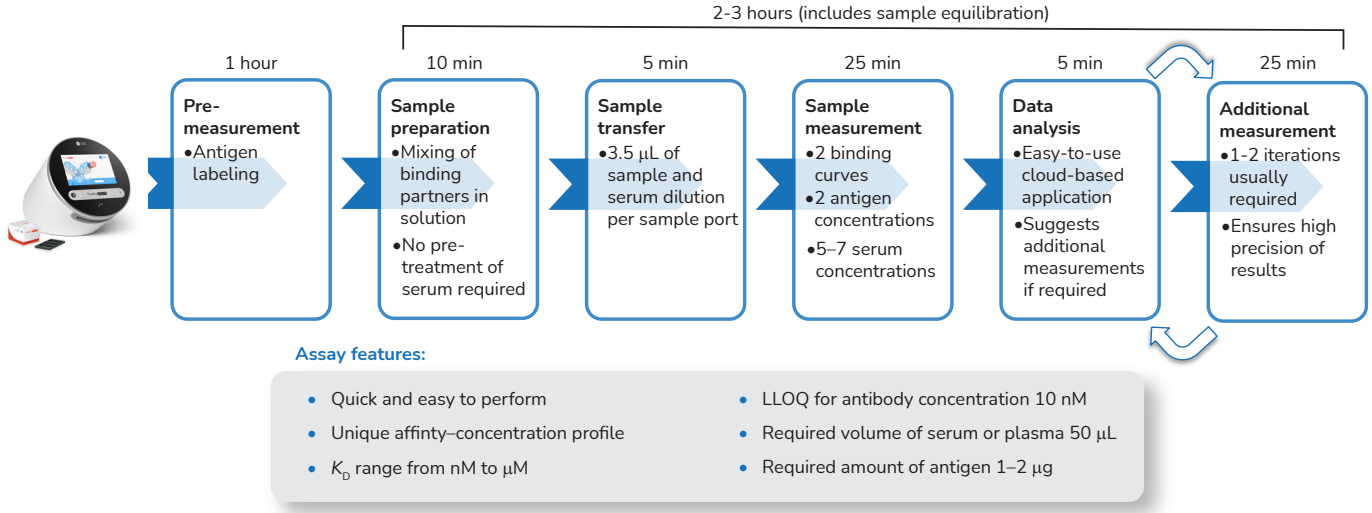
Figure 3. SAffCon fingerprints of SARS-CoV-2 RBD-specific antibodies (wild-type (red), delta (blue), and omicron (green) in pooled convalescent plasma. The gray area indicates the affinity–concentration range of low/no binding where antibody concentrations are below K_D.

As shown in Figure 3, the convalescent plasma contained RBD-specific antibodies with affinities in a range of 10 nM to 30 nM, and concentrations between 60 nM and 120 nM. This simple proof-of-concept experiment clearly reveals the benefit of determining affinities and concentrations of plasma antibodies rather than just an antibody titer. The SAffCon fingerprints indicated that lower binding of RBD delta in comparison with RBD wild type is caused by a loss of affinity as indicated by a slight shift parallel to the x-axis. On the other hand, reduced binding of RBD omicron in comparison to the other two variants is caused by a combination of both reduced concentration and affinity. As mentioned earlier, such granular information is not available by ELISA, which would likely only show an overall reduction in antibody titer for both scenarios.

Conclusion

The Seroaffinity and Concentration Assay is a novel serological assay to accurately determine affinity and concentration of antibodies directly in clinical samples. This enables researchers to quantify the immune response to infection, vaccination, inflammation, transplants, or new drugs. Crucially, our assay does not require sample pre-treatment like dilution, blocking reagents or any other form of purification and works directly in solution.

Workflow and assay features



Supporting literature

List of scientific publications

Emmenegger et al., Both COVID-19 infection and vaccination induce high-affinity cross-clade responses to SARS-CoV-2 variants. *iScience* **2022**, 25, 104766; DOI: 10.1016/j.isci.2022.104766

Fiedler et al., Serological fingerprints link antiviral activity of therapeutic antibodies to affinity and concentration. *Accepted in Scientific Reports* **2022**; DOI: 10.1101/2022.02.03.478946

Fiedler et al., Mutations in two SARS-CoV-2 variants of concern reflect two distinct strategies of antibody escape. *bioRxiv* **2021**; DOI: 10.1101/2021.07.23.453327

Denninger et al., Microfluidic Antibody Affinity Profiling Reveals the Role of Memory Reactivation and Cross-Reactivity in the Defense Against SARS-CoV-2. *ACS Infect. Dis.* **2022**, 8, 790–799; DOI: 10.1021/acsinfecdis.1c00486

Schneider et al., Microfluidic characterization reveals broad range of SARS-CoV-2 antibody affinity in human plasma. *Life Science Alliance* **2021**, 5, e202101270; DOI: 10.26508/lsa.202101270

Fiedler et al., Antibody Affinity Governs the Inhibition of SARS-CoV-2 Spike/ACE2 Binding in Patient Serum. *ACS Infect. Dis.* **2021**, 7, 2362–2369; DOI: 10.1021/acsinfecdis.1c00047

Schneider et al., Microfluidic Antibody Affinity Profiling for In-Solution Characteri-zation of Alloantibody - HLA Interactions in Human Serum. *bioRxiv*, **2020**; DOI: 10.1101/2020.09.14.296442

Getting started guide

Microfluidic antibody affinity profiling on the Fluidity One-M,

Assay data sheet

SARS-CoV-2 RBD Microfluidic Antibody Affinity Profiling assay

Methods

Fluorescent labeling of proteins

Prior to labeling, wild-type (Sino Biological, 40592-V08H), delta (Sino Biological, 40592-V08H121), and omicron (Sino Biological, 40592-V08H90) RBD were reconstituted according to the manufacturer's instructions using PBS (pH 7.4), and the concentration was measured by absorbance at 280 nm (NanoDrop One™). For labeling, the RBD proteins were diluted into labeling buffer (0.2 M NaHCO3, pH 8.3) and mixed with Alexa Fluor™ 647 NHS ester (Thermo Fisher Scientific) at a dye-to-protein ratio of 3:1. Following incubation overnight at 4 °C or 30 min at ambient temperature, the labeled RBD proteins were purified on an ÄKTA pure system (Cytiva) using a Superdex 75 Increase 10/300 column (Cytiva). All labeled RBD proteins were stored at -80 °C in PBS (pH 7.4) containing 10% (w/v) glycerol as cryoprotectant.

Seroaffinity and Concentration Assay

The working reagent for anti-SARS-CoV-2 immunoglobulin (National Institute for Biological Standards and Control 21/234) is a calibrated product equivalent to the high concentration samples (NIBSC 20/150) from the WHO working standard for anti-SARS-CoV-2 immunoglobulin (NIBSC 20/268). NIBSC 21/234 consists of pooled plasma from individuals who recovered from COVID-19 and was collected between April and May 2020. The MAAP assay was performed by mixing fluorescently labelled spike RBD from wild type, delta, and omicron with various dilutions of convalescent plasma and incubating on ice for at least 30 min. A buffer matching the viscosity of human plasma was used for plasma dilutions. Equilibrium binding of RBD to plasma antibodies was assessed by monitoring hydrodynamic radii (R_h) on the Fluidity One-M using a size range of 3–14 nm and viscosity setting 3. The measurement protocol involved adding 3.5 µL of the plasma sample and ViscoMatch buffer to the sample and flow buffer ports of the Fluidity One-M chip plate, respectively. The Fluidity Cloud was used to determine K_D and binding site concentrations from the mode of the joint posterior distribution obtained by Bayesian Inference.

Equations

$$f_d = \rho_f + \frac{[B]}{[L]}(\rho_b - \rho_f)$$

f_d	fraction of labeled species that diffused into the axillary flow of the microfluidic chip
[L]	total concentration of labeled species
[B]	equilibrium concentration of bound labeled species
ρ_f	fraction of free labeled species
ρ_b	fraction of bound labeled species

$$[B] = \frac{[L] + \alpha[U] + K_D - \sqrt{([L] + \alpha[U] + K_D)^2 - 4\alpha[L][U])}}{2}$$

[U]	total concentration of binding sites in the unlabeled species
α	α is the fractional concentration of unlabeled species in the binding measurement
K_D	dissociation constant (affinity)

About us

We envision a world where information about proteins and their behavior transforms our understanding of how the biological world operates, and helps all of us make better decisions about how we diagnose diseases, develop treatments and maintain our personal well-being.