Automation of bead-based ELISA for SARS-CoV-2 neutralizing antibody on the Opentrons OT-2 with an on-deck Heater-Shaker Module

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ABSTRACT

A robust serological test to detect neutralizing antibodies to SARS-CoV-2 is a useful tool for public health and clinical purposes. Enzyme-linked immunosorbent assays (ELISAs) for detection of neutralizing antibodies have been issued Emergency Use Authorization (EUA) from FDA. Other than conventional ELISAs with the antigen (e.g., RBD) immobilized on the surface of a 96-well plate, antigen-conjugated magnetic bead-based ELISAs have emerged as an alternative with several advantages. We have developed a bead-based ELISA for detection of SARS-CoV-2 neutralizing antibodies. This assay requires separate instruments to perform major steps such as liquid transfer, washing and shaking. To streamline this assay on the Opentrons OT-2 platform equipped with Magnetic Module and Heater Shaker, a protocol was designed and tested to process serum samples collected from COVID-19 patients. RBD-coupled magnetic beads acquired from ACROBiosystems were also tested and compared using the same protocol.

Key findings

- The OT-2 equipped with a Magnetic Module and a Heater-Shaker Module demonstrated the ability to conduct bead-based ELISAs for SARS-CoV-2 neutralizing antibody detection with excellent precision.
- In a higher throughput setting, this protocol can handle up to 96 samples with minimal hands-on time.

INTRODUCTION

Upon recognition of viral components, cell-mediated immune responses trigger secretion of neutralizing antibodies against viral antigens, for example nucleocapsid (N) protein and spike (S) protein present on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). An antibody test can be used to detect the presence of these neutralizing antibodies in serum due to viral infection, providing information of resolving or past SARS-CoV-2 infection in a population to better understand the epidemiology of SARS-CoV-2. Such tests are common tools to determine vaccine efficacy during clinical trials and after large-scale vaccination.

An antibody test is typically performed using an enzyme-linked immunosorbent assay (ELISA), a technique that detects and quantifies a target molecule (e.g., an antibody) in fluid specimens of a patient through highly specific antibody-antigen interactions. For a typical sandwich ELISA for antibody detection, the first step is to attach a SARS-CoV-2 antigen to the solid surface of 96-well or 12-well stripes, which can be assembled on a frame compatible to a 96-well microplate reader. Then the samples are added to the wells, and the anti-SARS-CoV-2 antibody captured via binding to the antigen and immobilized. The detection antibody, usually an antibody that recognizes human IgG and carries horseradish peroxidase (HRP) is used, and with the presence of the enzyme's substrate, a colorimetric signal is generated and can be measured and quantified. The absorbance is proportional to the level of anti-SARS-CoV-2 neutralizing antibody in the sample.

Alternatively, magnetic beads can also serve as the solid surface in ELISAs. The advantages include an increased available surface area and an even distribution of beads throughout the sample processing, which more rapid and sensitive detection of low analyte concentrations can be achieved. We developed a bead-based ELISA for detection of SARS-CoV-2 virus neutralization antibodies. This assay utilizes the receptor binding domain (RBD) of SARS-CoV-2

S protein as the capture antigen and Thermo Fisher's Dynabeads (Waltham, MA, USA) as the solid surface.

Despite the variety of assay design, ELISAs typically follow a similar workflow. The general steps include reagent transfer, incubations, and repeated washes. The procedure for bead-based ELISAs requires separate instruments to perform these steps, which can potentially be automated on the Opentrons OT-2 platform with Magnetic Module and Heater Shaker. We developed a protocol to streamline this assay to circumvent the need of multiple instruments and to reduce hands-on time. The protocol was also validated using ACROBiosystems' RBD-conjugated magnetic beads (Newark, DE, USA) to confirm the quality of the OT-2' sample handling.

MATERIAL AND METHODS

Schematic of the ELISA tested on the Opentrons OT-2, the outline of steps to complete the assay, and the deck layout (Figure 1)

RBD-conjugated beads were prepared by attaching 60 µg of His-tagged RBD of SARS-CoV-2 S protein (ACROBiosystems, Newark, DE, USA) to 5 mg of Dynabeads (Thermo Fisher, Waltham, MA, USA), superparamagnetic spherical polymer particles for magnetic separation of biological materials, and then stored in 2.5 mL blocking buffer. The same protocol was also tested by using commercially available RBD pre-coated beads from ACROBiosystems (36 µg RBD per mg beads). Human anti-SARS-CoV-2 S1 antibody as the standard and mouse monoclonal anti-human IgG Fc with HRP conjugated as the detection antibody were purchased from Cell Sciences (Newburyport, MA, USA) and Abcam (Cambridge, UK), respectively. Custom formulated buffers for the assay were supplied by Teknova (Hollister, CA, USA).

Liquid transfer was performed by the 8-Channel Pipette. The ELISA plate was placed on the Magnetic Module and manually moved to the Heater-Shaker Module when agitation if needed. Serum samples previously tested as SARS-CoV-2 antibody positive or negative were obtained for the study. Immediately after the assay was completed, the absorbance of each sample was measured at 450 nm by a colorimetric plate reader off-deck. The runtime for each assay at different sample sizes was estimated **(Table 1)**.

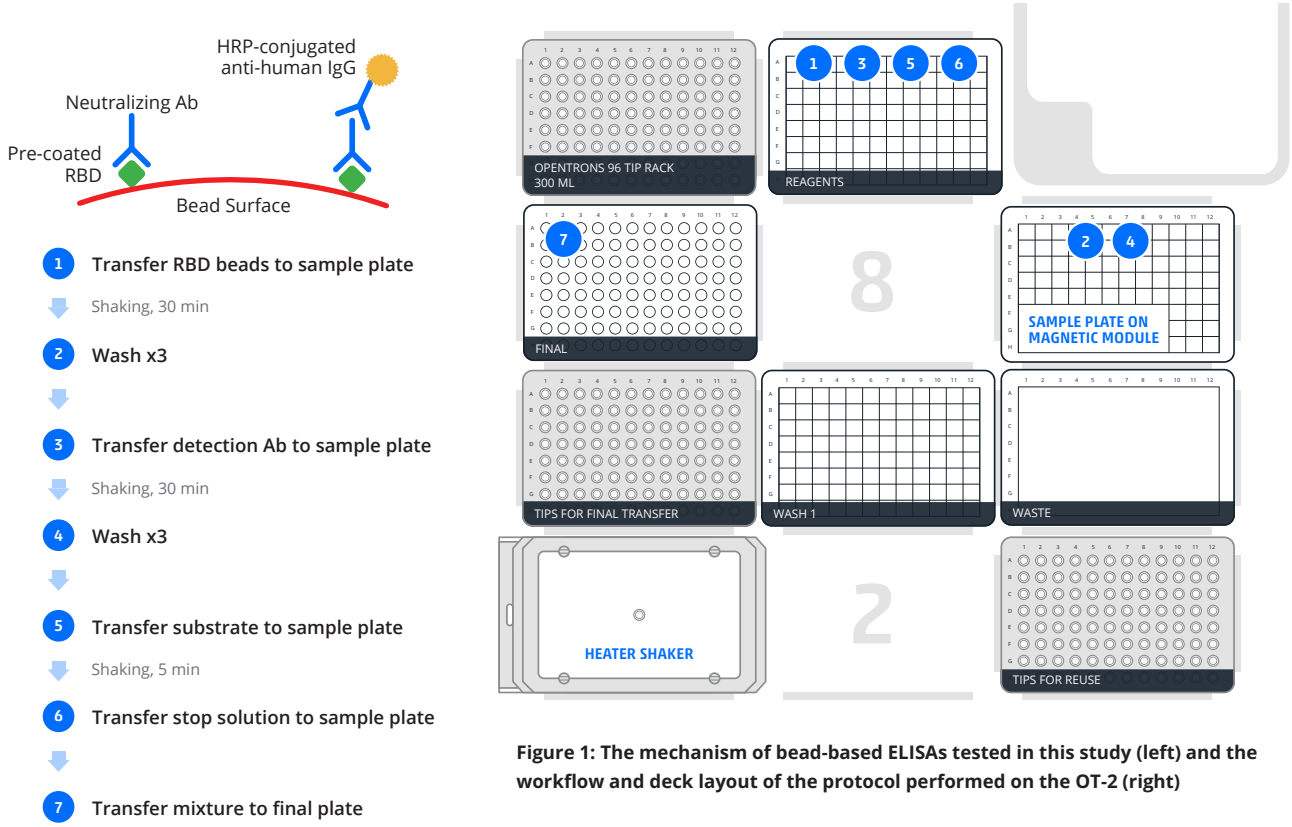

Figure 1: The mechanism of bead-based ELISAs tested in this study (left) and the workflow and deck layout of the protocol performed on the OT-2 (right)

HEATER SHAKER SETTINGS		
Incubation (target capturing)	Shaking (rpm)	1000
	Time (min)	30
Incubation (detection antibody binding)	Shaking (rpm)	1000
	Time (min)	30
Signal development	Shaking (rpm)	1000
	Time (min)	5
Estimated runtime		
24 samples (3 columns)		2 hrs
48 samples (6 columns)		2.5 hrs
96 samples (full plate)		3.5 hrs

Table 1: Heater Shaker Module settings for the bead-based ELISA protocol and the estimated runtime at different sample sizes

RESULTS

To generate the standard curve for the test, on the OT-2, 50 µL of RBD-conjugated bead suspension was transferred to each well of a 96-well 2 mL deep-well plate pre-loaded with serial 2-fold dilutions of human anti-SARS-CoV-2 S1 antibody, and the assay proceeded as described as in MATERIAL AND METHODS.
The absorbances were measured, and a dose-response curve plotted. The results demonstrated the precision of the OT-2 on performing this assay (CV < 10%) **(Figure 2A)**, and an excellent linear association of the assay readout versus antibody concentration between 100 ng/mL to 12.5 ng/mL (R-squared > 0.99) **(Figure 2B)**.

RBD pre-coated beads from ACROBiosystems were then reconstituted (1 mg beads per mL blocking buffer) and tested using the same protocol on the OT-2. Serial 2-fold dilutions of human anti-SARS-CoV-2 S1 antibody were prepared and exposed to 20 µL bread suspension. The data were used to plot a dose-response curve for further analysis. The consistency of sample handling on the OT-2 (CV < 10%) **(Figure 3A)** and the linearity of the assay readout versus antibody concentration were both confirmed (100 ng/mL to 6.25 ng/mL, R-squared > 0.99) **(Figure 3B)**.

To perform the bead-based ELISA to determine the level of SARS-CoV-2 S1 neutralizing antibodies, the protocol designed for RBD-conjugated beads prepared in-house or ACROBiosystems RBD-conjugated breads was performed to process serum samples previously tested by conventional plate-based, FDA-certified ELISA (positive: *). The results again showed the precision of OT-2's liquid handling (CV < 10%), and the antibody concentrations were obtained according to the standard curves shown in **Figure 4**.

Antibody neutralization assay on the OT-2

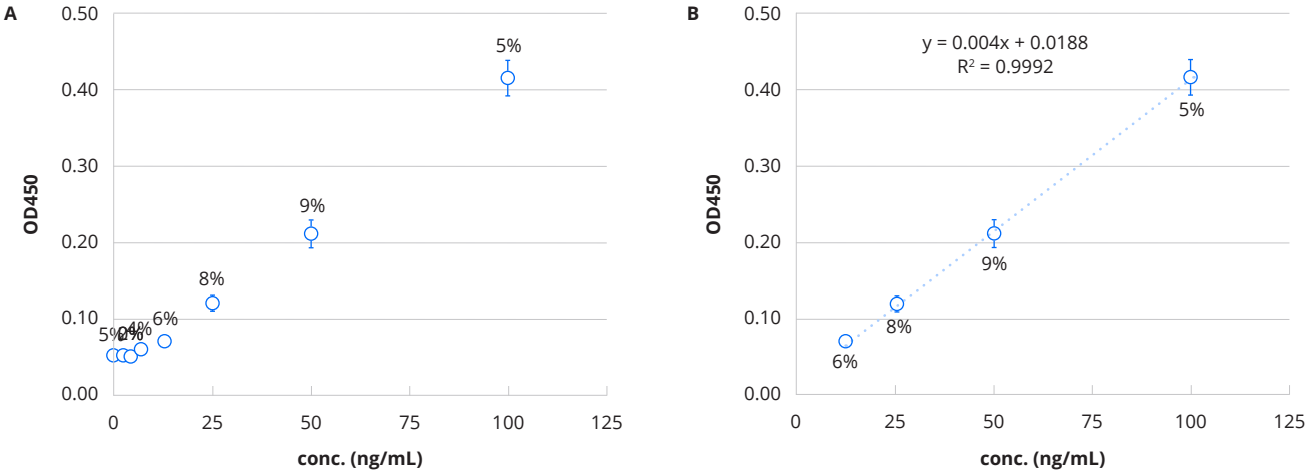

Figure 2: SARS-CoV-2 virus neutralization test developed in-house was successfully performed on the OT-2 with highly consistent sample handling (A, CV obtained by processing each concentration in triplicate), and the dose-response curve obtained with linearity between 100 ng/mL to 12.5 ng/mL (B)

Bead-based ELISA on the OT-2

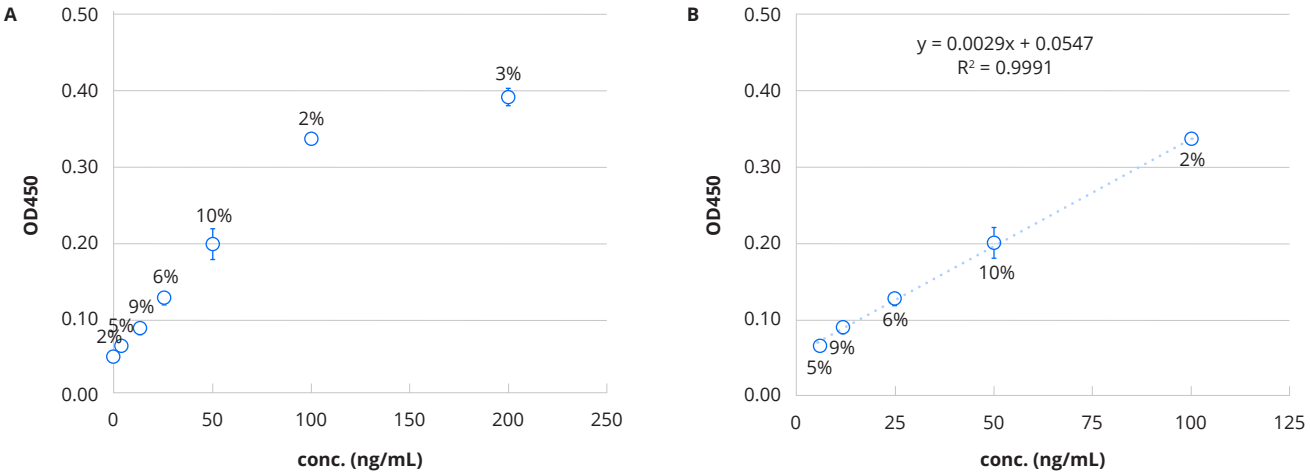

Figure 3: RBD pre-coated beads from ACROBiosystems was tested for the bead-based ELISA protocol on the OT-2. The quality of sample handling (A, CV obtained by processing each concentration in triplicate) and the linear relationship between assay readout and antibody concentration (100 ng/mL to 6.25 ng/mL, B) were confirmed.

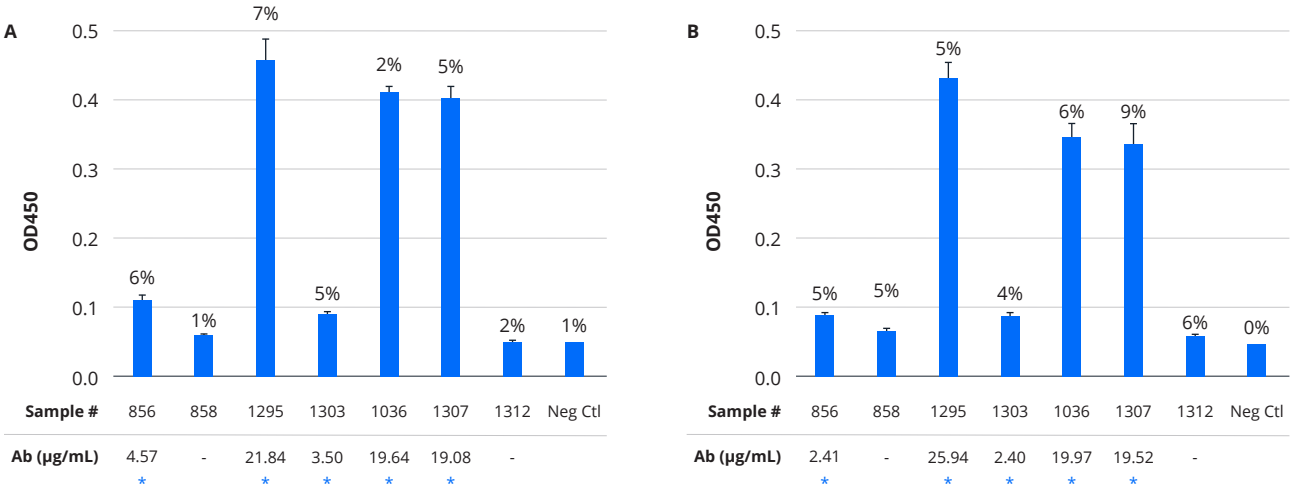

Figure 4: Bead-based ELISAs using RBD-conjugated beads prepared in-house (A), and ACROBiosystems RBD-conjugated beads (B) performed on the OT-2 measured neutralizing antibodies present in the human serum samples with excellent precision (CV < 10%). The antibody concentrations were also determined. Samples previously tested positive by FDA-certified ELISA (*) were confirmed by the assay.

CONCLUSION

Bead-based ELISAs can be successfully conducted on the OT-2 platform equipped with Magnetic Module and Heater Shaker Module. The results demonstrated an excellent quality of sample handling, and the protocol is suitable to process up to 96 samples with minimal hands-on time.