A Universal Planar Assay Format for High Sensitivity Cytokine Quantification in Human Serum and Plasma

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ABSTRACT

Quantifying prospective biomarkers of immune signaling in biological samples requires high assay sensitivity. The Simoa™ Planar Array provides greater sensitivity than ELISA, but has a finite assay menu. This study aimed to develop a universal 'homebrew' planar immunoassay format for development of high sensitivity assays for any analyte, by using an anchor antibody/peptide tag system to develop a universal capture plate. Anchor antibody specific for a peptide tag was microprinted in microwell plates. The peptide tag was conjugated to analyte-specific capture antibodies via maleimide chemistry, enabling high affinity binding of capture to anchor antibody. Bound sample antigen was sandwiched between the peptide-tagged capture and biotinylated detector antibodies; chemiluminescent detection was used to quantify signal. This 'homebrew' approach successfully detected sub picomolar levels of cytokines and chemokines in human or mouse serum and plasma, including IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-22, ITAC, MIP1a and MIP3b. When compared to commercially available planar assays the universal planar assay format provided comparable LLOQ and LOD with sample correlation (R2) >0.97 for all analytes tested. The universal planar assay was used to successfully screen multiple antibody pairs for human and mouse biomarkers and to develop robust assays using mouse, rabbit or goat monoclonal and polyclonal antibodies. This universal planar homebrew assay format combines the accuracy, reproducibility and high sensitivity of Simoa Planar Array technology with total analyte flexibility, and provides assay developers a simple and versatile method to rapidly develop robust high-sensitivity assays for any analyte of their choice.

METHODS

Simoa planar array technology enables high levels of immunoassay sensitivity and multiplexing via high-precision deposition of up to twelve capture antibody spots in each well of a microwell plate. In this study we aimed to develop a 'Simoa Planar Homebrew' assay - a planar array format assay which can be used with any analyte-specific capture antibody. As a first step, we developed a universal capture plate. Assay plates were spotted with an immobilized Anchor antibody, with one capture spot deposited in each of 96 microwells. This Anchor antibody is highly specific for a peptide tag (referred to as Simoa Planar Array Homebrew Tag). Assay workflow is shown in Figure 1 below. Capture antibodies were conjugated to the peptide tag via an SMCC linker. This capture antibody-peptide complex binds efficiently to the Anchor antibody spot. To capture analyte, the peptide-tagged capture antibody was added to the well in order to bind the plate-bound anchor antibody, forming an analyte specific capture spot. The remainder of the assay workflow followed a familiar sandwich immunoassay format. Antigen was sandwiched between the peptide-tagged capture and biotinylated detector antibodies. Streptavidin-HRP and a chemiluminescent HRP substrate were used to signal immunocomplex formation. Intensity of this signal is directly proportional to the quantity of analyte in the Standard or sample of interest. Immunocomplexes were imaged and quantified using image analysis algorithms via the SP-X™ Imaging and Analysis platform. We demonstrated proof of concept for this 'Planar Homebrew' assay format by screening antibody pairs and comparing immunoassay performance in 'Planar Homebrew' format versus directly printed Simoa Planar Arrays, in which capture antibody is directly deposited into microwells.

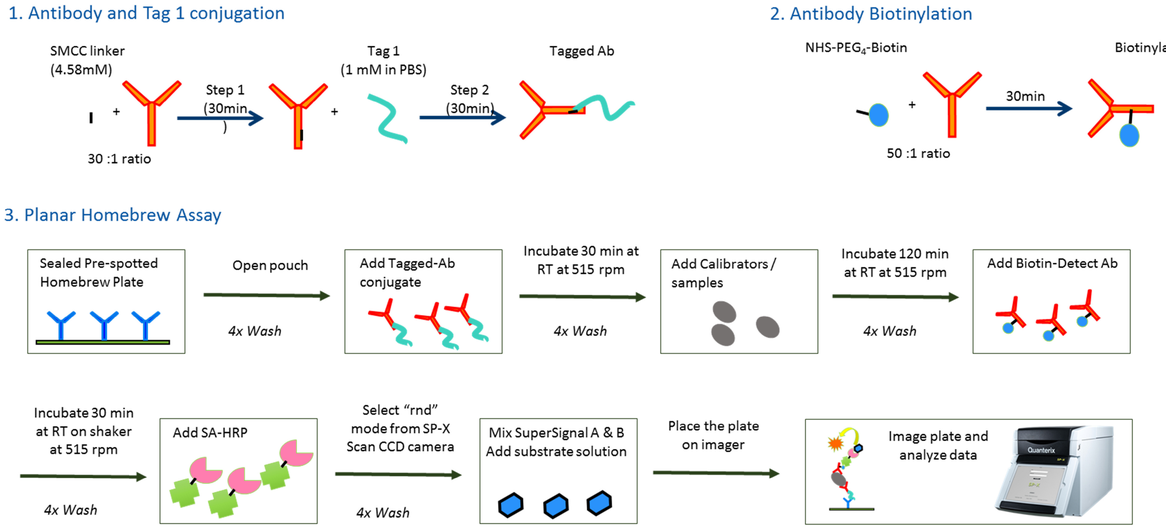

Figure 1 . 'Simoa Planar Homebrew' Assay Workflow

RESULTS

Case Study 1. Comparison of human IL-6 assay performance in Planar Homebrew and Standard Formats

Performance of a Simoa Planar Homebrew human hIL-6 assay was compared with performance of the commercially available Simoa Planar human hIL-6 assay kit (Figure 2). In the homebrew assay format, tagged hIL-6 capture antibody was incubated with immobilized anchor antibody in a pre-spotted Homebrew plate. Then, hIL-6 calibrators were incubated with the anchored hIL-6 capture antibody. In the standard assay format, hIL-6 calibrators were incubated with hIL-6 capture antibody pre-spotted in the plate. For the rest of the assay steps, the same biotinylated detect antibody reagent, SA-HRP reagent and Chemiluminescent substrate were applied. Both hIL-6 assay formats showed the same lower limit of quantification (LLOQ= 27.83 fg/mL) and similar limit of detection (LOD = 9.7 fg/mL for Standard format and 6.8 fg/mL for Homebrew format).

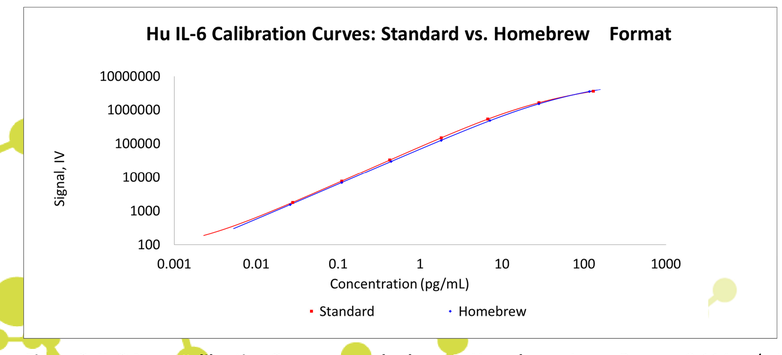

Figure 2. IL-6 Assay Calibration Curves – standard versus Homebrew. Assay Range = 0-114 pg/mL

Case Study 2. Successful IL-22 antibody pair screening and sample detection using Simoa Planar Homebrew assay

Antibody Candidate	Host Species	Monoclonal/ Polyclonal
Ab1	Rat	Mono
Ab2	Rat	Mono
Ab3	Mouse	Mono
Ab4	Rat	Mono
Ab5	Mouse	Mono

Table 1. Human IL-22 antibodies used for screening

To demonstrate the utility of Simoa Planar Homebrew for antibody pair screening in immunoassay development, we compared antibody screening data for a recently developed directly-spotted human IL-22 planar array with data from the same antibody pairs generated using the Planar Homebrew Array. Five monoclonal antibody candidates reported as specific for hIL-22 were selected (Table 1) and screened in various capture/detect orientations using Planar Homebrew Array or directly spotted Standard Array format.

		Standard Format		Homebrew Format	
Detect Antibody		Ab3	Ab4	Ab3	Ab4
Capture Antibody	Ab1	10.6	123.8	3.5	381.5
	Ab2	1.0	1.0	0.8	1.0
	Ab5	0.9	1.0	1.1	1.2

Table 2. Signal to Background Ratio of 2 pg/mL IL-22

As an indicator of antibody pair performance, Table 2 shows ratios of signal-to-background for hIL-22 standard (2 pg/mL), and demonstrates that in both Homebrew and Standard assay formats, Ab1 as capture and Ab4 as detector represent the antibody pair generating highest signals (highlighted by green). These data demonstrate the value of the Planar Array Homebrew assay as an antibody screening tool.

To further demonstrate the utility of the Simoa Planar Homebrew assay, detection of endogenous hIL-22 in serum and plasma samples was performed using hIL-22 Homebrew and Standard Assay formats. Sample reading data are shown in Figure 3, and indicate extremely close correlation between sample reading data generated via Planar Array Homebrew assay and Standard Planar Array, with R^2 value = 0.97.

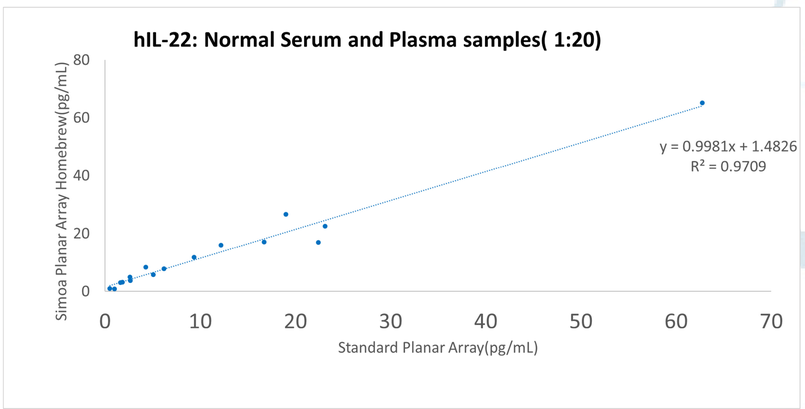

Figure 3. hIL-22 sample detection correlation: Standard vs. Homebrew (8 serum and 8 plasma samples)

Case Study 3. Conversion of an ultrasensitive p24 immunoassay to planar format using Planar Homebrew approach

p24 is a component of the HIV particle capsid, and is used as an early biomarker in detection of HIV infection. An ultrasensitive bead-based immunoassay for this analyte is commercially available from Quanterix. This case study aimed to determine if performance of a planar homebrew version of this assay could provide similar analytical performance as the well-established bead based assay. The same capture antibody as used in the bead-based assay was conjugated to the Planar Homebrew Tag in order to form a planar capture plate. The same detector antibody, recombinant HIV-p24 calibrator and sample diluent used in the bead-based p24 assay were also utilized in the Planar Homebrew assay format.

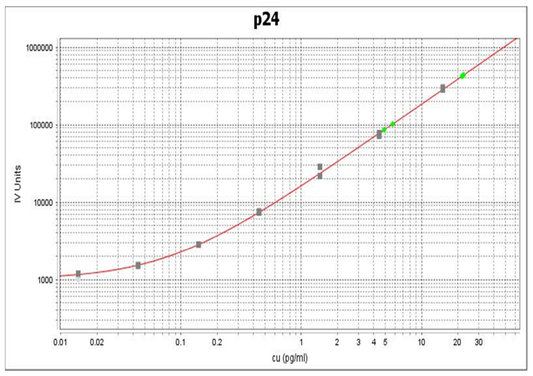

Figure 4. Calibration curve for HIV-p24 Planar Homebrew assay

We observed that in the planar homebrew assay format, a calibration curve was readily generated and the assay exhibited a high signal to background ratio (Figure 4). Excellent sensitivity in the femtogram per milliliter range, comparable to a commercially available bead-based Simoa p24 assay was achieved (Limit of Detection 1.1 fg/mL; Lower Limit of Quantification 14 fg/mL), shown in Table 3. Average spike recovery in serum was 108%. Furthermore in the planar assay format, smaller sample volumes were required (50 µL versus 125 µL for bead-based assay).

HIV-p24	Planar Homebrew	Bead-Based
LOD	1.1 fg/mL	2.7 fg/mL
LLOQ	14 fg/mL	10 fg/mL
Spike Recovery	108%	84.1%
Sample Volume	50 µL	125 µL

Table 3. Comparison of planar homebrew and bead-based p24 assays

Host	Mono/Poly	Capture	Detect
Mouse	Monoclonal	✓	✓
Rabbit	Monoclonal	✓	✓
Rat	Monoclonal	✓	✓
Goat	Polyclonal	✓	✓

Table 4. Antibody types successfully utilized with Simoa Planar Array Homebrew assay to date

The Simoa Planar Array Homebrew assay has been utilized to successfully screen multiple antibody pairs for human and mouse analytes and to establish robust homebrew immunoassays using antibodies from multiple species. Examples of these are shown in Table 4.

CONCLUSIONS

- We have developed a universal planar assay format which enables access to the high sensitivity immunoassay performance of Simoa planar arrays without the need for specialized array printing equipment.
- The Simoa Planar Homebrew assay enables a simple, versatile, and robust workflow for antibody pair screening and development of high-sensitivity immunoassays targeting any analyte for which antibodies are available.
- This assay has been successfully used to develop immunoassays using multiple antibody types and analytes.
- Performance of immunoassays developed using this Simoa Planar Homebrew assay approach is comparable to commercially available ultrasensitive immunoassays.