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1.0 Introduction

1.1 CSA: Simian Panel C Overview

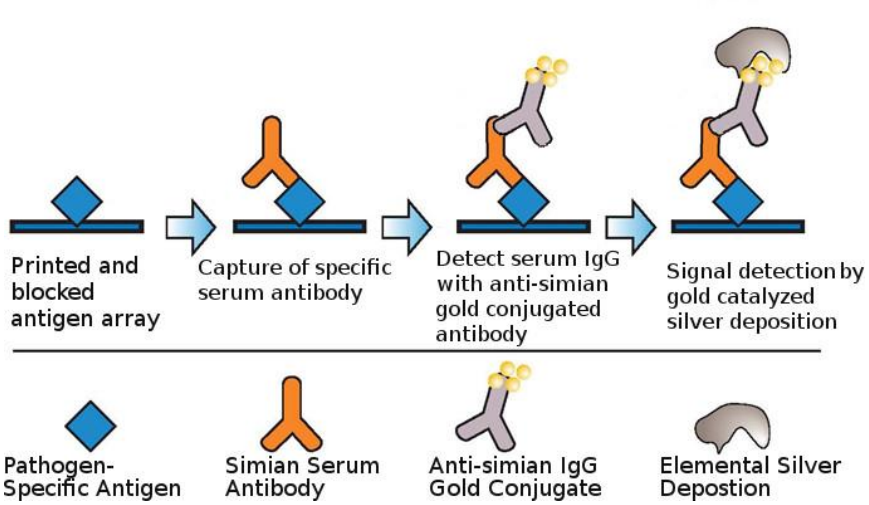
The health and well-being of your non-human primate (NHP) colonies are critical to the success of your research. The Colony Surveillance Assay™: Simian Panel C assay can provide a sensitive, robust and user-friendly alternative to ELISA or bead-based methods by combining multiplex detection technologies with compact and cost-effective analysis tools for colony health screening. This approach provides an indication of previous exposure to pathogenic agents present in NHP colonies, both to identify potential infectious threats to the colony and their human caretakers and to ensure your research remains uncompromised.

The CSA: Simian Panel C is intended to be used as a tool for the continual monitoring of your specific pathogen-free (SPF) colony. This method measures specific serum IgG in a multiplex format, providing you with data for tracking your colony's health. The use of CSA: Simian Panel C kit is intended for use as a profiling assay and not a definitive diagnostic assay. Cut-off values have been determined through robust validation methods to provide universal optimal sensitivity, and are to be used as suggested starting points for your analysis. Routine surveillance of your colony with the CSA: Simian Panel C assay will give you confidence in your colony health, and reduce the number of samples needing to be tested by more expensive and time-consuming methods.

NOTE: Intuitive Biosciences highly recommends using secondary methods (such as IFA or PCR) to validate your results, resolve equivocal results, and confirm positive results in your SPF colony.

The CSA: Simian Panel C kit consists of reagents sufficient to process up to ninety-two samples, and the included control sera. The CSA: Simian Panel C kit uses the same basic laboratory instruments as an ELISA. The SilverQuant® chromogenic reagents are used for signal generation on the CSA: Simian array products. Assay results are measured using the AthenaQuant® System, where data is easily generated using a scanner and quickly analyzed using the included AthenaQuant software.

Figure 1. CSA: Simian Panel C Schematic



1.2 SilverQuant Surface Chemistry

The CSA: Simian Panel C arrays are printed on Intuitive Biosciences' proprietary protein microarray slides and are specifically designed for multiplex immunoassays and deliver high signal-to-noise with high sensitivity for protein microarray applications.

2.0 Kit Contents

Component	Description	Quantity	Prod. No.
CSA: Simian Panel C Array	Array containing 24 subarrays consisting of antigens representing unique simian pathogens	4-pack	12-1027
5X Slide Wash Buffer	Buffer used to remove unbound protein	100 mL	2-1039
CSA Buffer	Buffer used to dilute samples and Gold Detection Reagent	100 mL	7-1037
Positive Control	Positive control sample	10 µL	12-1049
TB Positive Control	Positive control sample for TB	10 µL	12-1053
Negative Control	Negative control sample	10 µL	12-1051
SilverQuant Anti-simian IgG Gold Conjugate	Gold Detection Reagent	50 µL	10-2142
SilverQuant Reagent A	Development Reagent A	10 mL	10-2132
SilverQuant Reagent B	Development Reagent B	10 mL	10-2112
SIM*plex*™ Well Seals	Used to seal wells during sample incubation	1 each	4-1009
96 Deep Well Dilution Plate	Used for serum dilutions	1 each	12-1025
SilverQuant Array Tube	Used to hold slides during incubations	1 each	10-2151
CSA: Simian Panel C User Protocol			L741

3.0 Required Materials

Component	Description
SIM*plex* 96 Multiplexing Systems	A reusable 96 well device with well seals and gasket (Product No. 4-1060)
Bench-top microcentrifuge	Capacity to hold 1.5 mL microcentrifuge tubes
Nitrogen, or Purified Air Stream	With regulator at 80 psi for drying slides. **NOTE**: Alternative drying by tabletop centrifuge using 50 mL conical tubes @ 5 minute low g-force spin.
Vortex Mixer	Various
1.5 mL Microcentrifuge tubes	Various
15 mL conical tubes	Various
50 mL conical tubes	Various
Deionized or Ultrapure Water	Clean water
Microarray Scanner	AQ 101, AQ 500, or AQ 1000
Microarray Image Analysis Software	AthenaQuant software (included with Product AQ 101, AQ 500, AQ 1000)
CSA: Simian Panel C data analysis template	Included with AthenaQuant software
Micropipettes	Single, Repeat, and 8-channel; various capacities

4.0 Storage

The CSA: Simian Panel C Detection kit should be stored at 2-8˚C until used.

5.0 Safety and Handling

Use Universal Safety Precautions when handling animal body fluids. For all other materials, normal precautions exercised in handling laboratory materials should be followed. The material is not considered hazardous according to 29CFR1910.1200. The chemical, physical, and toxicological properties of this product may not, as yet, have been thoroughly investigated. We recommend the use of gloves, lab coats, and eye protection when working with any material.

6.0 Protocol Overview

The CSA: Simian Panel C kit contains sufficient reagents for qualitative analysis of up to 92 serum samples. However, only a single assay may be performed. Reagent B is air sensitive and once opened, it must be used within a day. Both Reagent A and Reagent B are light sensitive and should not be exposed to direct or excess light. CSA: Simian Panel C arrays should be handled with care (never touch the arrayed surface) and not allowed to dry once they have been wetted. Proper storage and handling of serum samples is critical for obtaining optimal data. Avoid repeated freeze-thaw cycles and aliquot and freeze samples at -80°C for long-term storage. Developed CSA array slides are stable and can be stored indefinitely in the slide holder they came in.

7.0 Procedure

7.1 Preparation of Buffers and Reagents

NOTE: Equilibrate entire kit to room temperature (18-30°C) prior to use for peak assay performance.

7.1.1 In a container capable of holding at least 500 mL, add 400 mL of ultrapure water. Add 100 mL of the 5X Slide Wash Buffer (Product No. 2-1039). Mix thoroughly. Store at room temperature.

7.2 Assembly of CSA: Simian Panel C Arrays using the SIM*plex* Multiplexing System

Refer to the SIM*plex* 96 Multiplexing System User Protocol (L140) for more detail.

7.2.1 Place the SIM*plex* device on a clean surface so that the well openings of the device are facing up and the etched well labels are visible. Loosen all four TIGHTENING SCREWS and remove the TOP PIECE of the device.

7.2.2 Loosen the POSITIONING SCREW on the HOLDER PIECE of the device, remove place holder slide (if present), and insert the CSA: Simian Panel C Array. **Make sure the slide is facing up**. The active surface of the array is facing up when the barcode serial number is legible. The array should be oriented with the barcoded end of the slide towards the POSITIONING SCREW.

7.2.3 Record the array serial number in the plate map below.

7.2.4 Using your fingers, gently tighten the POSITIONING SCREW to secure the array.

7.2.5 Repeat steps 7.2.2-7.2.3 for the other three slides.

7.2.6 While wearing gloves, place the gasket bottom side (thick walled) into the TOP PIECE of the device. The thin walled side of the gasket will now be facing up and will contact the slide. Confirm the gasket is fully reinserted by gently pressing it into the grooves.

7.2.7 Place the TOP PIECE of the device onto the HOLDER PIECE and gently tighten the four TIGHTENING SCREWS using your fingers.

7.3 Serum Dilution and Addition to the Array

NOTE: After step 7.3.3, do not allow the surface of the array to dry completely at any time before you are ready to scan the array.

7.3.1 Note the location of each sample to be loaded in the plate map below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Positive Control											
B	Negative Control											
C	TB Pos. Control											
D												
E												
F												
G	Negative Control											
H	Positive Control											
	Serial no. of array #1			Serial no. of array #2			Serial no. of array #3			Serial no. of array #4		

7.3.2 Ensure that each sample is completely thawed, and vortex briefly. Spin each sample at 5,000 rpm for at least 10 seconds to collect the material in the bottom of the tube.

7.3.3 Add 200 µL of CSA Buffer (Product No. 7-1037) to each well of the SIM*plex* (Product No. 4-1060) using a repeat or multi-channel pipettor. Tap the side of the SIM*plex* unit two times to ensure that the buffer covers each well completely.

7.3.4 Immediately remove the CSA Buffer from the wells by inverting over a sink three times. Remove excess solution from the top of the SIM*plex* well unit by blotting dry with a lint-free wipe.

7.3.5 Add 75 µL of fresh CSA Buffer to each well of the SIM*plex*.

7.3.6 Pipette 500 µL of CSA Buffer into the 96 Well Dilution Plate (Product No. 12-1025), to all wells EXCEPT A1 and H1 or the Positive Control wells.

7.3.7 Pipette 250 µL of CSA Buffer to wells A1 and H1, or the Positive Control wells.

7.3.8 Add 5 µL of Positive Control serum (12-1049) to wells A1 and H1 of the 96-well dilution plate. Mix by pipetting up and down 3 times.

7.3.9 Add 5 µL Negative Control (12-1051) to wells B1 and G1 of the 96-well dilution. Mix by pipetting up and down 3 times.

7.3.10 Add 5 µL of TB Positive Control serum (12-1053) to well C1 of the 96-well dilution plate. Mix by pipetting up and down 3 times

7.3.11 Add 5 µL of each serum sample to be tested to remaining wells of the 96-well dilution plate as indicated in the table above. Mix by pipetting up and down 3 times.

NOTE: If your serum sample has been diluted, please adjust the dilution accordingly.

7.3.12 Set multichannel pipette to 25 µL. Mix each sample by pipetting up and down 5 times prior to drawing up 25 µL.

7.3.13 Dispense 25 µL of diluted sample into each well of the SIM*plex* keeping the locations within the plate the same, taking care not to touch the bottom of the well of the array (pipette into a corner or side of the well).

7.3.14 Cover the wells with the provided plate seal (Prod. No. 4-1009) to prevent evaporation and incubate at room temperature for **1 hour**.

7.4 Wash 1 and Add Gold Conjugate Reagent

7.4.1 Briefly spin the SilverQuant Anti-Simian IgG Gold Conjugate (Product No. 10-2139) using a bench top microcentrifuge to collect all the material into the bottom of the tube and gently vortex to mix.

7.4.2 Prepare the Gold Conjugate Reagent by adding 60 µL of SilverQuant Anti-Simian IgG Gold Conjugate to 12 mL of CSA Buffer. Mix gently and thoroughly.

7.4.3 Remove the serum solutions from wells by covering the SIM*plex* with a paper towel or other absorbent paper, invert, and, while holding the paper towel, tap down three times. Dispose of the adsorbent paper into a biohazardous waste container. Add 200 µL of 1X Slide Wash Buffer (from **Step 7.1.1**) to each well using a repeat or multi-channel pipettor.

7.4.4 Repeat 200 µL of 1X Slide Wash Buffer 4 times, for a total of 5 washes in the SIM*plex*.

NOTE: Leave the Wash Buffer in the wells of the SIM*plex* until **Step 7.4.6.**

7.4.5 Open the SilverQuant Array Tube (Product No. 10-2151).

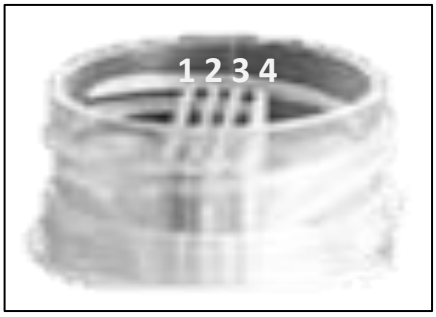
7.4.6 Stand the Array Tube up and fill with 1X Slide Wash Buffer, approximately 15 mL when working with 4 slides. The volume needed will increase when fewer slides are in the Array Tube. Leave the cap off.

7.4.7 Invert the SIM*plex* over a waste container and shake out the Slide Wash Buffer. While still inverted, dry the top of the SIM*plex* with a paper towel.

7.4.8 Place the SIM*plex* on a benchtop, loosen all four top tightening screws and remove the top piece of the SIM*plex*. Loosen the positioning screw on the bottom slide holder piece.

7.4.9 Taking care not to touch the top surface of the array, transfer the CSA: Simian Array(s) to the Array Tube. Handle the array only by the edges near the barcode. Make sure the active side of the slide (barcode readable facing up) is facing the proper direction, as shown in Figure 2.

Figure 2. Schematic showing the directions the slide surface faces in the Array Tube during incubation.



Slide Position	Slide Surface Faces
4	Towards slide 1
3	Towards slide 4
2	Towards slide 4
1	Towards slide 4

NOTE: If only processing 2 slides, place in positions 1 and 4.

7.4.10 After all arrays have been transferred to the Array Tube, replace the pink cap on the Array Tube and gently mix by inverting the tube two or three times.

7.4.11 Remove the Slide Wash Buffer from the Array Tube by uncapping, **placing your finger over the top of the slides**, and inverting and flicking in a sink. Fill the tube with 1X Slide Wash Buffer (~15 mL). Cap the tube and gently mix by inverting the tube two or three times.

7.4.12 Repeat **Step 7.4.10** twice.

7.4.13 Remove the final wash and fill the Array Tube with Gold Conjugate Reagent (prepared in **Step 7.4.2**). Allow to sit for **1 hour** at room temperature.

7.5 Wash 2 and Development

7.5.1 Remove the Gold Conjugate Reagent from the tube by removing the pink lid, **placing your finger over the top of the slides**, then flicking the tube into a sink.

7.5.2 Fill the Array Tube with 1X Slide Wash Buffer. Cap the tube and gently mix by inverting the tube twice. Remove the Slide Wash Buffer from the Array Tube by uncapping, **placing your finger over the top of the slides**, and inverting and flicking in a sink.

7.5.3 Repeat **Step 7.5.2** four times, for a total of five washes.

NOTE: SilverQuant Reagent A (Product No. 10-2132) and SilverQuant Reagent B (Product No. 10-2112) are sensitive to light. Be sure to perform the following steps out of direct sunlight. It is also important to add the reagent solution swiftly to the tube because the reaction is time dependent. Once Reagent A and B have been mixed and added to the tube, tap gently to ensure reagent coverage and immediately cap the tube. Be sure to read and understand **Steps 7.5.4 - 7.6.1** and have all the needed equipment prepared and ready.

7.5.4 Set a timer to 3 minutes. Prepare the SilverQuant Development Reagent by directly pouring SilverQuant Reagent A (Product No. 10-2132) into SilverQuant Reagent B bottle (Product No. 10-2112). Cap the bottle and shake vigorously for ~3 seconds. Immediately fill the Array Tube with the SilverQuant Development Reagent.

7.5.5 Quickly cap the Array Tube, mix by inversion several times then tap the side of the tube twice to remove air bubbles and ensure that the Development Reagent covers the entire slide(s). Immediately start the timer and incubate for exactly 3 minutes. Place a cover (i.e. the lid of a box) over the Array Tube to protect it from light.

7.6 Final Rinse

7.6.1 When the incubation time expires, transfer the Development Reagent from the Array Tube into a proper chemical waste container and immediately fill the Array Tube with ultrapure water. Empty the tube by pouring the water into a sink. Repeat the water flush twice to ensure all the Development Reagent is rinsed out.

NOTE: Be sure not to touch the printed slide surface while removing the slide. Also, powder-free gloves should be worn during this step to avoid contaminating the slides.

7.6.2 Remove the slide locking pin as before and remove the slide(s) from the Array Tube. Rinse both sides of each slide with ultrapure water using a squirt water bottle to ensure there are no particulates on either side of the slide. **Wipe the back of the slide (barcode is readable when protein surface is facing up)** with a lint-free wipe and **immediately** dry the slide by either spinning in a centrifuge using a slide centrifuge, a fresh 50 mL tube in a centrifuge, or by gently blowing off any remaining liquid using only purified air or nitrogen stream at approximately 80 psi.

7.6.3 Empty any unused Development Reagent into a chemical waste container.

8.0 Ordering Information

Telephone: +1 608.561.8730

Email: orders@intuitivebio.com or

support@intuitivebio.com

website: www.IntuitiveBio.com

9.0 Appendix A: Troubleshooting

Problem: Weak Signal

Suggested Causes & Solutions:

1. Incorrect assay temperature – Reaction must occur at 18- 30°C for optimal results.
2. Protein degradation – use freshly prepared samples.
3. Slow addition of the Development Reagent – immediately add the Development Reagent to reaction tube, and cap without hesitation.
4. Incorrect assay incubation time – follow protocol for proper incubation times.

Problem: High Background

Suggested Causes & Solutions:

1. SilverQuant chromogenic reagents were exposed to light for an extended period - SilverQuant Reagents A and B should have minimal exposure to direct light. Cap the SilverQuant Array Tube immediately after the Development Reagent is added.

Problem: No signal from detection controls

Suggested Causes & Solutions:

1. A step in the protocol was skipped or a reagent was mishandled.
2. If a low signal is seen in the positive controls, the anti-simian IgG gold conjugate solution was possibly missing or mishandled.

Problem: Heterogeneous Background

Suggested Causes & Solutions:

1. Washing/Drying artifact – Salts in the wash buffer may leave "streaks" in the array image. Briefly rinse slide again with purified water and immediately dry by centrifugation or by applying an air or nitrogen stream at approximately 80 psi.
2. Dust may adhere to the slide after it has been dried. It may be necessary blow/wipe off any dust that may have settled on the slide or the scanner's glass surface.