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SMCxPRO™ Grant Accelerator Package

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Introduction and SMC™ Technology Overview

On behalf of the Immunoassay Platform Specialist team, thank you for your interest in SMC™ Immunoassay technology. We recognize that in many cases researchers, core facilities, and research and development groups will be applying for funding in support of adding this enhanced technology to their labs.

The purpose of this Grant Accelerator Package is to provide grant writers access to necessary technology and application information that can be easily incorporated into grant applications. Everything included in this document – from text to data – can be used in any funding application. Please contact your local Immunoassay Platform Specialist for pricing quotes, to schedule an onsite SMC™ demonstration, technical assistance, or for any additional information needed.

SMC™ technology, developed by Singulex, Inc in 2004, relies on a basic sandwich immunoassay format utilizing two antibodies specific to the analyte of interest: a capture antibody coated on a plate or magnetic bead and a detection antibody conjugated to a fluorescent protein. Only the SMC™ Immunoassay technology allows researchers to use both plate-based and bead-based assay designs, thus offering unmatched flexibility in assay design. Distinguishing SMC™ assays from traditional immunoassays, an elution buffer is used to break apart the immunoassay complex once constructed. The eluate containing the fluorescent reporter molecule is transferred to a 384-well plate, thus removing other assay components that contribute high background fluorescent signal, such as the magnetic beads and antibodies used for analyte capture. The plate is loaded into the SMCxPRO™ instrument, the second-generation SMC™ instrument, where a laser excites the fluorescent-labeled detection antibody as it passes through a narrow interrogation window. Individual photons are captured by an avalanche photodiode and the signal is recorded. This allows for digital quantification of individual molecules. Analyte concentrations in the unknown samples are calculated using the corresponding standard curve.

Additional Online Resources:

- SMC™ Learning Center
- SMC™ Publication Database

SMC™ Technology Workflow

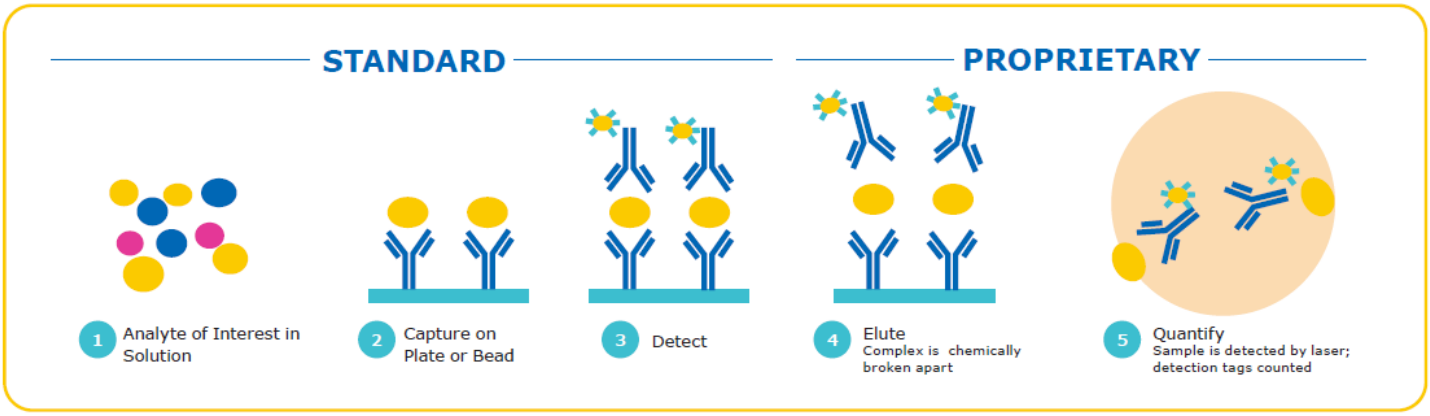

Figure 1: Single Molecule Counting (SMC™) immunoassays combine a familiar workflow with powerful SMC™ technology, providing femtogram/mL analyte quantification to accelerate scientific discovery and therapeutic development.
After following a traditional sandwich ELISA workflow, the proprietary SMC™ protocol steps concentrate the signal by disassociating the fluorescent-labeled detection antibody from the sandwich complex. The fluorescent-labeled detection antibody is the signal acquired in the SMCxPRO™ instrument. This results in reproducible signal, and improved quantification of proteins, particularly those at very low abundance.

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SMCxPRO™ Instrument Specifications and Ancillary Equipment

The following table lists all recommended equipment for running successful, reproducible SMC™ assays. Due to the highly sensitive nature of SMC™ assays, protocol steps including incubation timing and temperature, and washing steps are tightly controlled to prevent variability using both a Boekel Scientific Jitterbug™ plate shaker and a BioTek® 405TSUVS plate washer for the SMC™ platform, respectively. Both pieces of equipment are very highly recommended for running SMC™ immunoassays and we offer the BioTek 405TSUVS plate washer for the SMC™ platform along with the SMCxPRO™ instrument as a bundle package to maximize cost savings. The Jitterbug™ shaker can be purchased separately.

Instrument	Specifications
SMCxPRO™ 	**Merck Part number: 95-0100-00** Automated System-wide Suite of Instrument Self Tests (ASSIST) Read plate format: 384-well plate Immunoassay background-suppressing optics - Rotating objective moves laser spot at 50 mm/sec through eluted analyte to scan - Single fluorochromes excite and emit fluorescence. Fluorescence is captured by high 0.83 NA optical system and imaged onto confocal stop - Low noise Avalanche Photodiode (APD) counts individual photons striking its active area Power requirement: - U.S.: 115 VAC, 50-60 Hz (op. range 90-125 V) - Int.: 230 VAC, 50-60 Hz (op. range 180-250 V) Network/included PC: - Microsoft Windows® 10 OS - Static IP address and FTO server - Integrated xPRO Software Dimensions: 14" H x 16" W x 17.5" D
BioTek® 405™TSUVS Washer for the SMC™ Platform 	**Merck Part number: 95-0004-05** Washer package includes MagPlate assembly and custom magnet, vacuum regulator, dispense and waste system (including vacuum pump) Pre-programmed for use with SMC™ assays Volume range: - 50-3,000 µL/well (96-pin manifold) Wash speed: - 96-well: 300 µL/well, 3 cycles; ≤30 sec Supply bottle: - 4 L Power requirement: - 100-240 V (50-60 Hz) Dimensions: 14" W x 17" D x 10"H
Boekel Scientific Jitterbug™ Microplate 2-plate incubator/shaker 	**VWR Part number: 35821-065 (115 VAC), 130000-2 (230 VAC)** Mixer speed: 575 to 1500 RPM Temperature range: - ambient to 40°C Power requirement: - US: 130000-150 VAC (50-60 Hz) - Int.: 130000-2-230 VAC (50-60 Hz) Dimensions: 10" x 10" x 4"
Additional suggested equipment	12-Channel pipet capable of transferring 10 µL -250 µL 2 µL and 200 µL Single-channel pipettes **Merck partners with Eppendorf® to offer these items. Ask your Immunoassay Platform Specialist for additional information.**

Applications

The following sections detail example applications for SMC™ Immunoassay technology. Please contact your Immunoassay Specialist and Field Application Scientist if information specific to your proposed study design is required. Please note that data which is based on our testing procedures may only be compared to testing following the same procedures. Data provided below are intended for informational purposes only and does not represent a binding statement with respect to the characteristics of the products delivered. All sales of our products referenced herein are subject to our Terms and Conditions of Sale or an agreement between the parties governing the purchase and sale of the products, if applicable.

Biomarkers

Cardiac Troponin-I (cTnI), a biomarker specific to cardiomyocytes, is released into the bloodstream following the occurrence of a heart-damaging event. Due to this correlation, serum cTnI measurement is the gold standard for diagnosis of acute myocardial events in humans and is increasingly being used as a measurement for the assessment of cardiotoxicity in drug safety studies. The median cTnI concentration observed in a healthy individual is 1.75 pg/mL; however, the limit of detection of most clinical analyzers is 30 pg/mL – the concentrations measured in individuals after a cardiac event. The sensitivity of the SMC™ cTnI immunoassay allows for baseline measurement of cTnI in healthy individuals so that disease progression can be monitored prior to a cardiac event or cardiotoxicity.

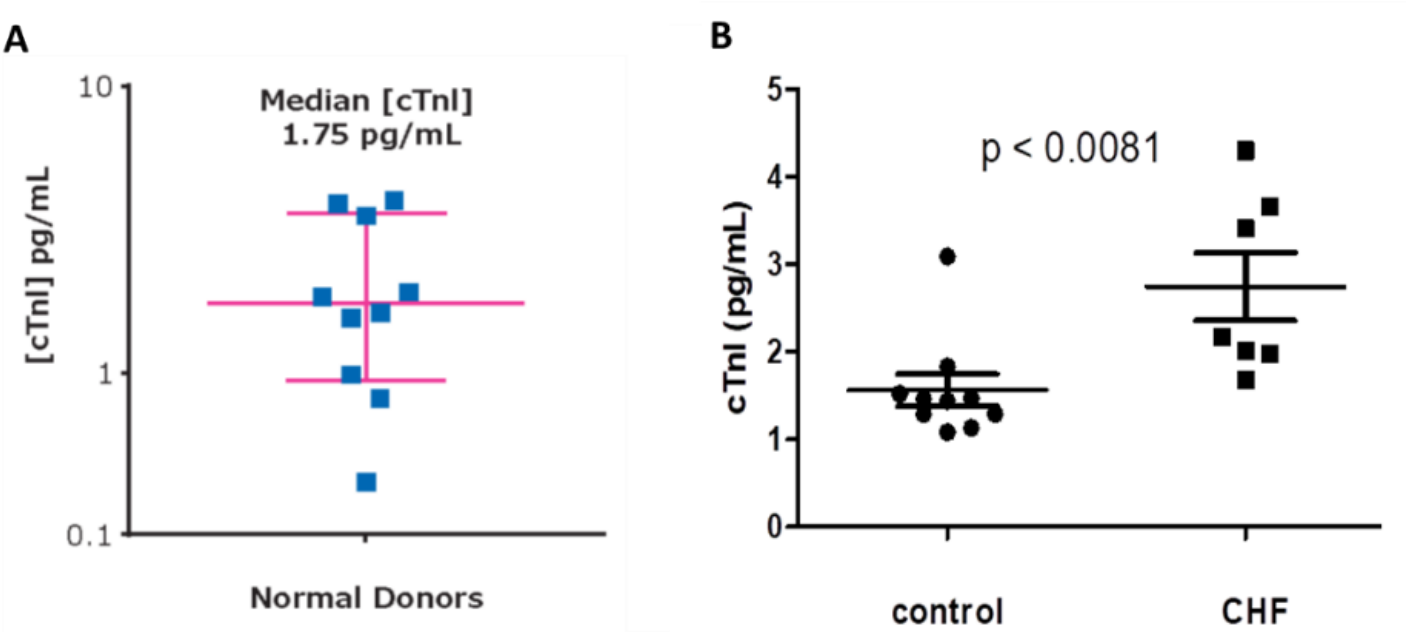

Figure 2: Measurement of human serum Cardiac Troponin-I (cTnI) in healthy vs congestive heart failure (CHF) patients using SMC™ assay technology.
(A) The SMC™ Human cTnI High Sensitivity Immunoassay uses a quantitative fluorescent sandwich immunoassay technique to measure biomarkers such as cTnI in human serum and plasma. With a Lower Limit of Quantitation (LLoQ) of 0.69 pg/mL, the SMC™ Human cTnI High Sensitivity Immunoassay accurately quantitates endogenous cTnI levels in healthy subjects. (B) Plasma samples (250 µL) from 10 healthy control subjects and 7 CHF patients were assayed using the SMC™ High Sensitivity Human cTnI kit, showing a significant difference in cTnI concentrations between healthy and CHF patients. Data was collected and analyzed using a SMCxPRO™ instrument.

Interferons in Biology and Disease

Interferons are involved in mediating protective immune effector functions. While the antiviral immune function of Type I interferons such as IFN-α2 and IFN-β1 make them candidate therapies for infectious diseases, the ability of the Type II IFN-γ to function in both adaptive and innate immunity led to its utility in oncology and autoimmunity research. The high sensitivity of our SMC™ kits enhances applications in both basic immunology research as well as pre-clinical studies, including topics such as acute infection and pharmacokinetic profiling of interferon-based therapies.

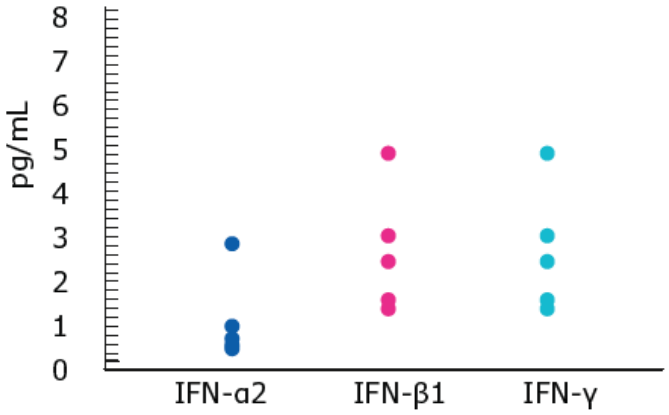

Figure 3: Measurement of interferons in Human plasma.
Normal human plasma samples were analyzed using the SMC™ High Sensitivity Immunoassay Kits for IFN-α2 (●), IFN-β1 (●), and IFN-γ (●). The endogenous concentrations ranges were determined to be 0.04-0.14, 0.15-3.37, and 0.41-6.53 pg/mL for IFN-α2 (●), IFN-β1 (●), and IFN-γ (●) respectively.

The Assay performance for SMC™ IFN-α2, IFN-β1, and IFN-γ High Sensitivity Immunoassay Kits. The lower limit of quantification (LLoQ) for the three SMC™ kits were 0.62 pg/mL for IFN-α2, 0.148 pg/mL for IFN-β1, and 0.033 pg/mL for IFN-γ. Merck R&D data 2020.

SMC™ Assays	IFN-α2	IFN-ß1	IFN-γ
Endogenous Serum Sample Range (pg/mL)	0.04-0.14	0.15-3.37	0.41-6.53
Standard Curve Range (pg/mL)	0.015-80	0.070-170	0.016-25
Lower Limit of Detection (pg/mL)	0.004	0.031	0.010
Lower Limit of Quantification (pg/mL)	0.062	0.148	0.033
Intra-Assay CV% / Inter-Assay CV %	<15/<20	<10/<15	<15/<20
% Recovery in Plasma / Serum	96/102	93/88	98/104
Sample Volume Required (µL)	100	50	25

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Homebrew Assay Development

Certain cases require having the ability to develop custom SMC™ assays, such as when working with proprietary reagents or when there is a need for an SMC™ immunoassay beyond what is offered for off-the-shelf assays. Furthermore, bioanalytical applications such as pharmacokinetics (PK), pharmacodynamics (PD), and anti-drug antibody (ADA) studies often require custom homebrew immunoassays. The SMCxPRO™ platform is "open", allowing researchers to easily develop and specifically tailor an application-specific immunoassay in either bead- or plate-based formats for non-diagnostic research and development purposes. Our Immunoassay Field Application Scientists support hands-on, onsite assay development training, in addition to in-house assay development services.

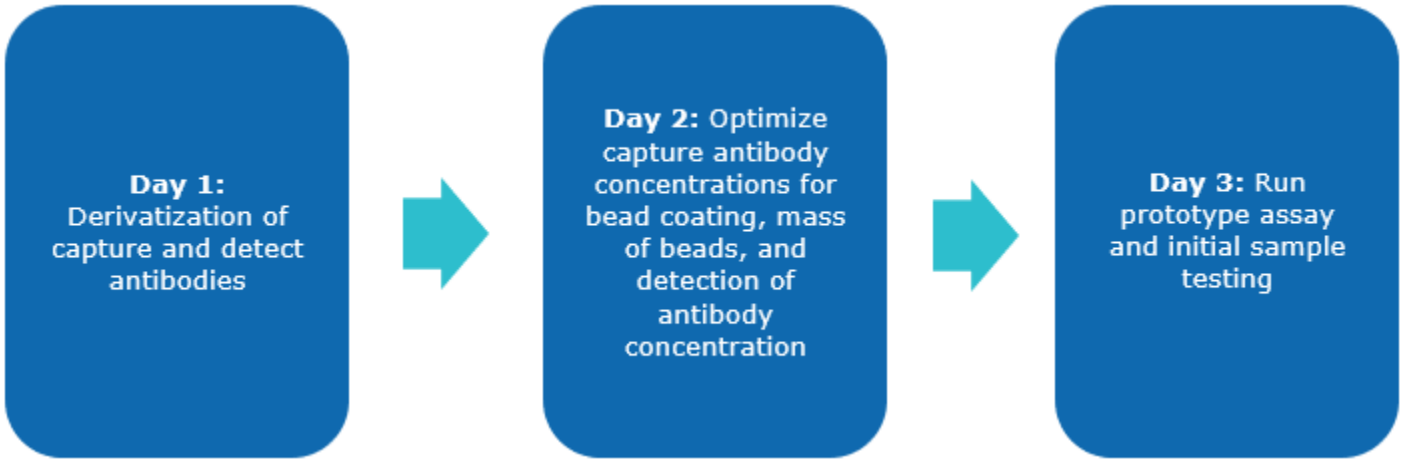

Figure 4: The "open" SMC™ technology platform allows for custom immunoassay feasibility development in 3 days.
Day 1: Assay development involves derivatization of capture and detection antibodies; specifically, biotin labeling of the capture antibody and fluorescent labeling of the detection antibody. Paramagnetic microparticles are coated with 2 titers of biotinylated capture antibody and blocked. Day 2: Assay feasibility is determined employing a checkerboard study assessing optimal amounts of coated capture antibody, mass of magnetic beads, and detection antibody titrations using a known analyte spike and "zero" to determine the signal:background. Day 3: Based on the results of this study, the lower limit of quantitation (LLoQ) is confirmed using a 12-point standard curve and preliminary sample testing. Further assay optimization and validation are performed as necessary.

Bioanalytical Pharmacokinetics (PK) and Pharmacodynamics (PD)

Traditional immunoassay methodologies offer limited capacity for PK and PD profiling and are often unable to show the full clearance profile of the biotherapeutic agents. In addition, these methodologies often have a limited ability to measure low concentrations of a given biotherapeutic such as those used in micro-dosing studies.

Pharmacokinetics

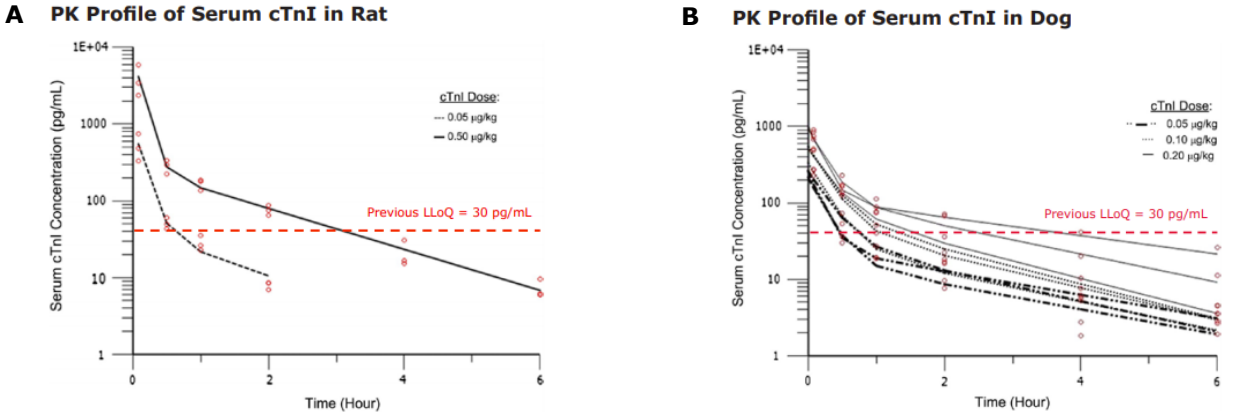

Figure 5: Complete PK profile of serum cTnI in rat and dog is achievable using SMC™ immunoassay technology.
(A) The pharmacokinetics of cTnI in the Wistar Han rat was analyzed with a two-compartment model. Open circles are serum cTnI concentrations from individual animals. Rat blood samples (300 µL) were collected at multiple time points and cTnI concentrations measured using SMC™ technology. (B) The pharmacokinetics of cTnI in the beagle dog was analyzed with a two-compartment model. Open circles are serum cTnI concentrations as measured using SMC™ technology. Each data line tracks changes in serum cTnI concentration post-dosing. Values are reported as means and SD. Dunn, et al. Toxicological Sciences 123(2), 368-373 (2011)

Pharmacodynamics

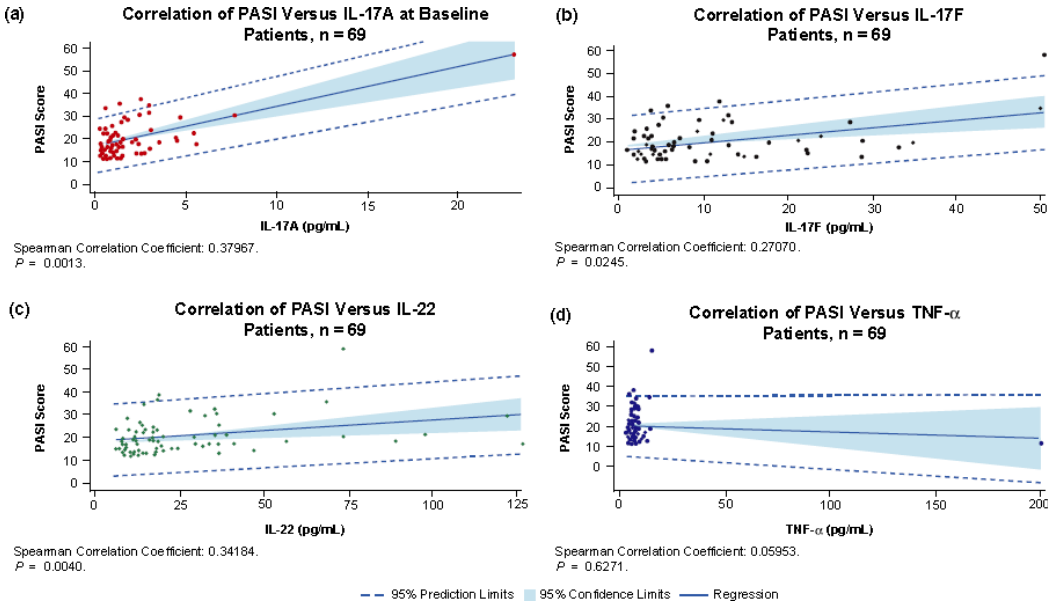

Figure 6: PD analysis using SMC™ assays.
Correlation between Psoriasis Area and Severity Index (PASI) score and (a) IL-17A, (b) IL-17F, (c) IL-22, and (d) TNF-α. Imafuku et al, The Journal of Dermatology (2020)

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Anti-Drug Antibody (ADA)/Immunogenicity

All biotherapeutics have the potential to induce an immune-mediated response ranging from benign to severe adverse effects. It is important to assess the immunogenicity risk of biotherapeutics for producing neutralizing and non-neutralizing ADA, which can result in diminished therapeutic efficacy, hypersensitivity, allergic reaction, and cytokine storms.

Additionally, ADA often impact pharmacokinetic and pharmacodynamic profiles, and possibly patient safety. The SMC™ platform supports all aspects of ADA testing by allowing for easy assay development, superior assay sensitivity, the ability to detect all ADA isotypes including IgM, IgA, IgE, and IgG, and the potential for high tolerance for matrix interference. Additionally, this can be achieved without the need for the acid-dissociation steps commonly required by other immunoassay platforms.

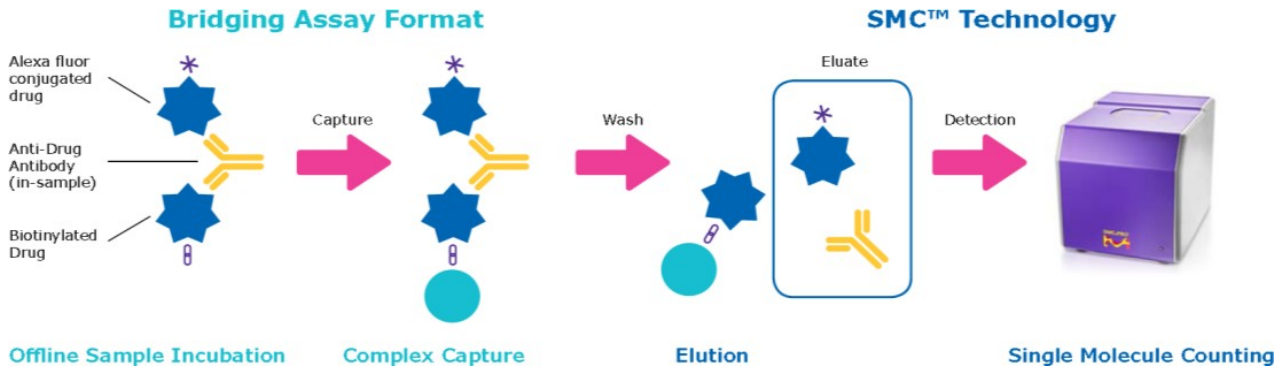

Figure 7: Immunogenicity assay workflow summary.
The presence of ADA in sample generates a bridged complex between biotinylated and fluorescently labeled biotherapeutic and is captured on a streptavidin paramagnetic bead or plate. An elution step disassociates the complex, and the final eluate containing the fluorescently labeled drug and ADA is transferred to the read plate. The data is then acquired on the SMCxPRO™ instrument.

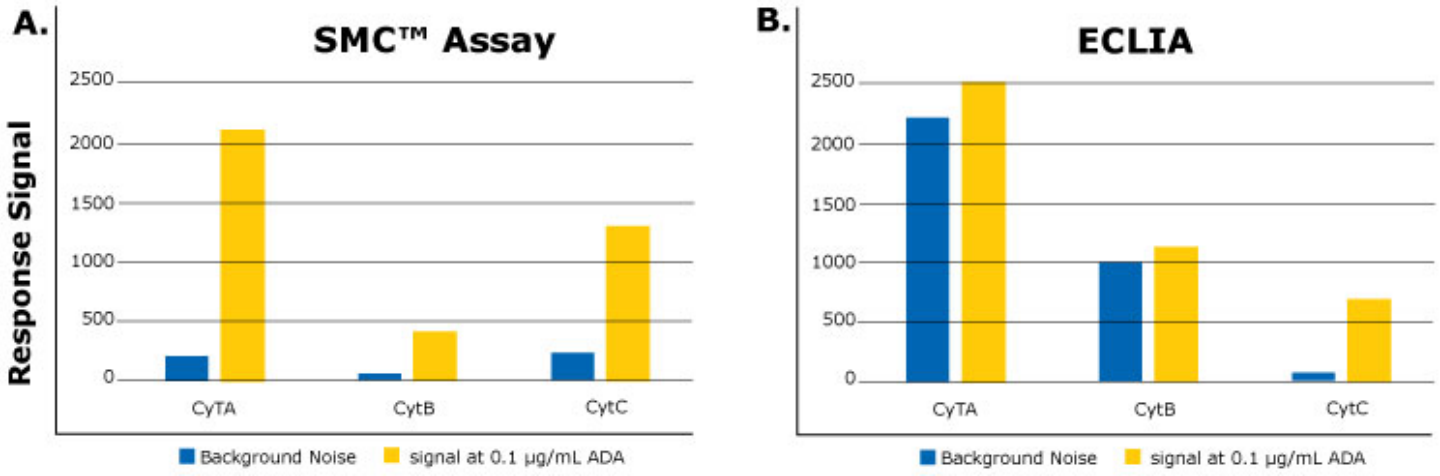

Figure 8: SMC™ ADA assays show significant improvement in sensitivity over traditional ADA assay methods.
(A) Immunogenicity assay for 3 cytokines, CytA, CytB, and CytC, developed using SMC™ technology demonstrating reduced background for all 3 cytokines – CytA, CytB, and CytC, thus improving assay sensitivity with improved signal:background. (B) ECLIA technology demonstrating high background for CytA and CytB, impacting assay sensitivity and reduced signal:background. Data presented at the European Immunogenicity Platform. Lisbon, 2020. Data used with permission by Sanofi Montpellier, France.

Frequently Cited Advantages of SMC™ Technology

A. Single Molecule Counting (SMC™) technology provides maximum immunoassay performance while following a workflow very similar to traditional ELISA technology.

1. Traditional ELISA methodologies demonstrate limitations in sensitivity and dynamic range, typically require high sample volumes, and are susceptible to matrix effects. Combined, these factors reduce the utility of traditional ELISAs for detection of low abundant proteins and endogenous biomarker levels in healthy subjects, thus hampering statistical analysis among study groups. By adapting an ELISA workflow, SMC™ technology achieves improved signal-to-noise ratios over traditional immunoassay technologies, thus providing quantification at both low and high levels of expression in one complete system. Digital counting of fluorescent events improves the assay sensitivity and extends the assay dynamic range beyond what can be achieved with traditional immunoassays.
2. Because SMC™ immunoassay technology can reach fg/mL sensitivity ranges, this platform offers the ability to dilute pre-clinical samples, when only low sample volumes are available.

B. SMC™ users are fully supported by onsite Merck Immunoassay Field Application Scientists and Specialists, as well as dedicated technical support teams.

We understand the SMC™ platform is an important investment for research labs and is committed to ensuring the success of SMC™ users. Regardless of the types of assays being used, all SMC™ users are fully supported by onsite Immunoassay Field Application Scientists and Specialists who have experience working with researchers from a broad range of lab types, including academic, government, biotech, pharma, CRO, and regulated labs.

C. The SMC™ platform is a completely "open" immunoassay platform, making it a versatile system that can be used in multiple study types, including biomarker assessment, bioanalytical work such as pharmacokinetic and pharmacodynamic studies, and immunogenicity testing.

Complimenting a menu of off-the-shelf, verified assay kits, we offer several options for homebrew custom assay development for research and development activities, including on-site assay development training. In addition, the SMC™ Custom Assay and Sample Testing scientific team can be contracted to perform custom assay development, verification services, and sample testing at their site in St. Louis, MO.

D. SMC™ assays are available - or can be developed – in both plate-based and bead-based formats.

1. The proprietary SMC™ technology allows scientists to measure proteins with increased precision, enabling unparalleled quantification at low and high abundance levels of expression. The flexible SMC™ immunoassay system acquires data from both plate-based assays and bead-based assays, providing a choice of format depending on budget and quantification requirements.
2. The SMC™ assay read plate is a 384-well plate; the entire plate can be read in less than three hours. This high-throughput platform allows researchers to perform an entire SMC™ assay run from sample prep through data analysis in one day.

E. SMCxPRO™ system can be integrated with a Hamilton Microlab® STARlet liquid handling system, increasing assay throughput.

In certain environments, automation of SMC™ immunoassays is desirable so that researchers can focus on other high-value activities to increase overall efficiency. The Hamilton Microlab® STARlet liquid handling workstation offers a hands-free option providing a robust, reproducible SMC™ workflow eliminating sources of error and variability. This technology is routinely used by the Custom Assay and Sample Testing team, and programming scripts are available for free.

REFERENCE: Verification of the Hamilton Microlab® STARlet for use with the SMCxPRO™ and Erenna® Immunoassay Systems Powered by Single Molecule Counting (SMC™) Technology.

F. Both data acquisition and analysis are performed within a single software package.

1. The SMCxPRO™ software package was developed in-house, thus affording full transparency to data processing algorithms. The software is user-friendly and allows end users to set up the instrument, read the plate, and analyze results quickly and easily. The software enables easy data curation, including manual outlier removal.
2. For labs operating in a regulated environment, the SMCxPRO™ instrument generates one signal data stream that can be imported into Laboratory Information Management Systems, such as WATSON, or other software. 21 CFR Part 11 compliance features can also be enabled.

Comparison to ELISA

SMC™ Assays	Traditional ELISA	Comparison
Assay Format Bead-based or plate-based sandwich immunoassay	**Assay Format** Stationary plate-based sandwich immunoassay	The SMCxPRO™ platform provides greater flexibility in assay configurations to address assay sensitivity needs
Sensor Technology 642 nm laser to scan approximately 275 µm above the surface of a 384-well flat bottom plate	**Sensor Technology** Single data point used to represent all molecules in the sample	SMCxPRO™ platform's 647 nm Alexa-labeled detection antibodies are excited as single molecules pass through the confocal-focused read laser area
Sensitivity LLoQ = fg/mL	**Sensitivity** LLoQ > 10 pg/mL	The bead-based capture antibody of the SMCxPRO™ platform allows for better ligand binding efficiency, improving the sensitivity vs the traditional immobilized plate-based ELISA
Sample Volume 5-100 µL	**Sample Volume** ~100 µL	The SMCxPRO™ platform's increased sensitivity is achieved by using a simple elution step to concentrate the signal using a final read volume of 20 µL
Dynamic Range >4 logs	**Dynamic Range** 4 logs	
Detector Single fluorescent events pass through a numeric aperture objective with a sub-4 µm spot size and are digitally counted by an avalanche photodiode within the instrument	**Detector** Absorbance	
Year invented 2004	**Year invented** 1971	

Merck Contact Information

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