# Ig_PLEX: A Novel Automated Platform for Bioanalytics

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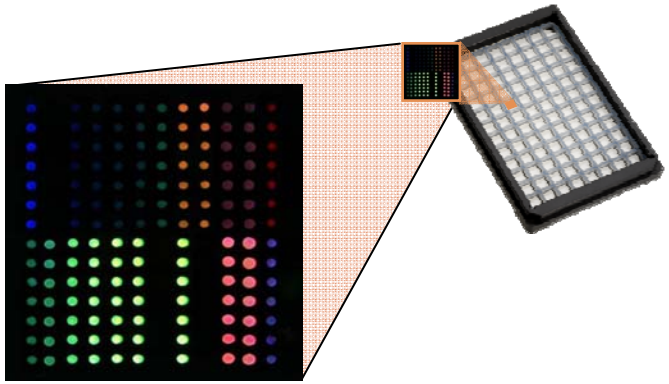
SQI Diagnostics Inc.
Nov 15, 2012

# INTRODUCTION TO SQI

**SQI Diagnostics** is a diagnostics systems company that enables antibody testing with proprietary technology in multiplexing, miniaturization and automation.

- Life sciences instruments and diagnostics company founded in 1999
- Engaged in providing multiplexed technologies to the IVD market
  - Development and marketing of tests for autoimmune, allergen, infectious disease and custom diagnostics
  - Development, manufacture, and marketing of fully-automated diagnostic instruments
  - Microarray printing and processing, manufacture of assay consumables
- Headquartered in Toronto, Canada

# Ig_PLEX™ PATENTED MICROARRAY ASSAY



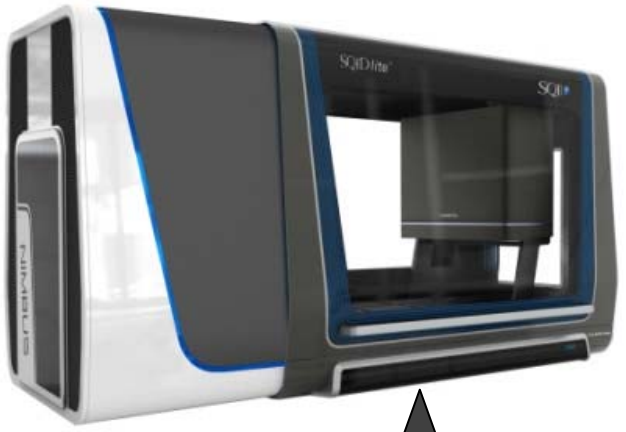
**Ig_ PLEX**
**96 Well Consumable Plate**

- 1 well
- 1 patient
- Multiple Results

Ig_PLEX assays measure the antibody response to multiple antigens, and differentiate the isotypes of the response in a single well.

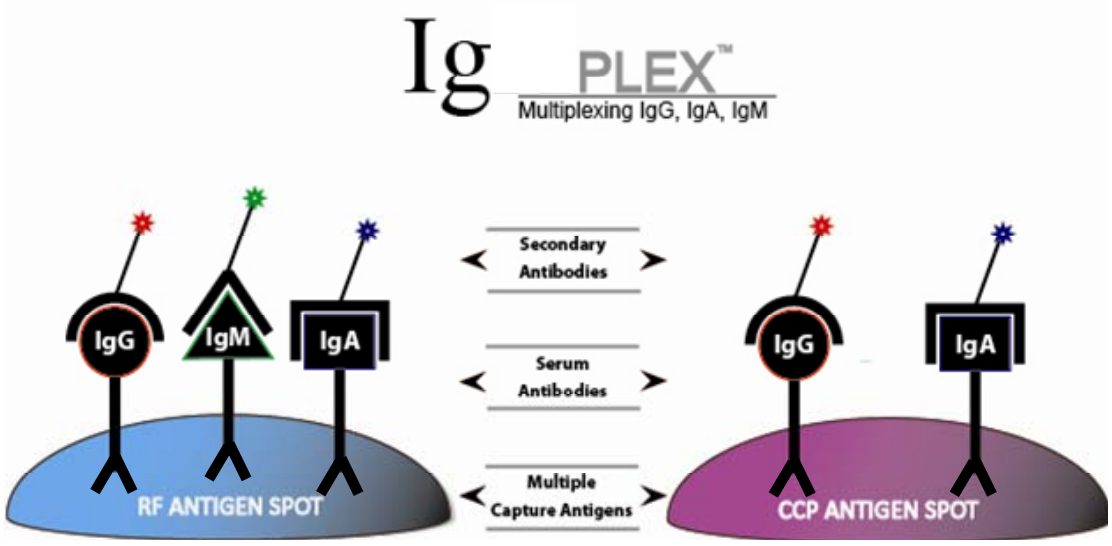
- Multiplexing at 2 levels
- Standard 96 well format
- QC and calibration controls in-well

**SQiD**works™ Platform



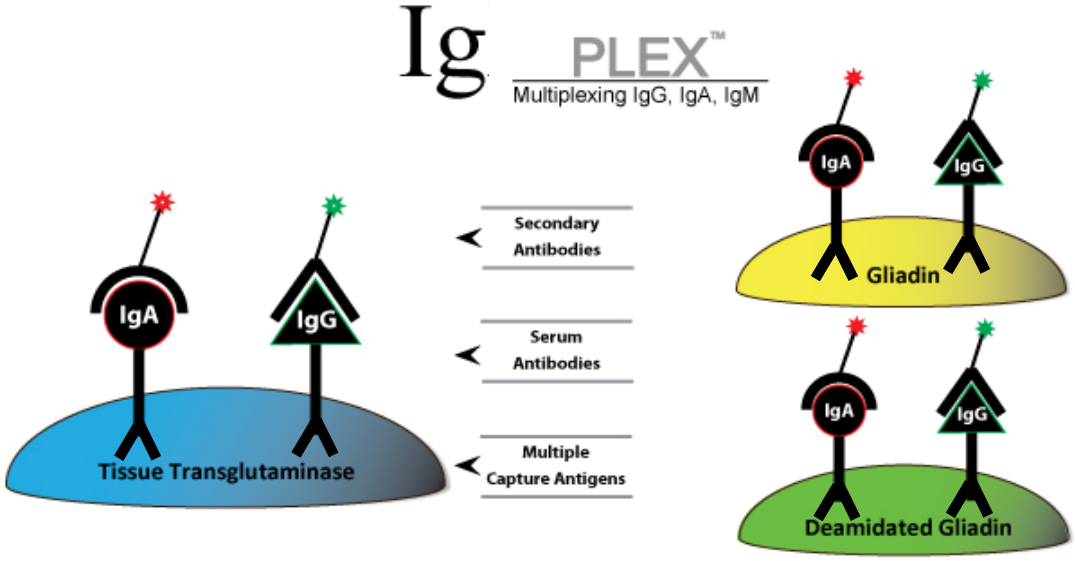
**SQiD**lite™ Platform

# Current IVD Assays for Autoimmune Diseases




Rheumatoid Arthritis

**Differentially labelled α-human 2º antibodies permit simultaneous measurement of patient immunoglobulin classes**



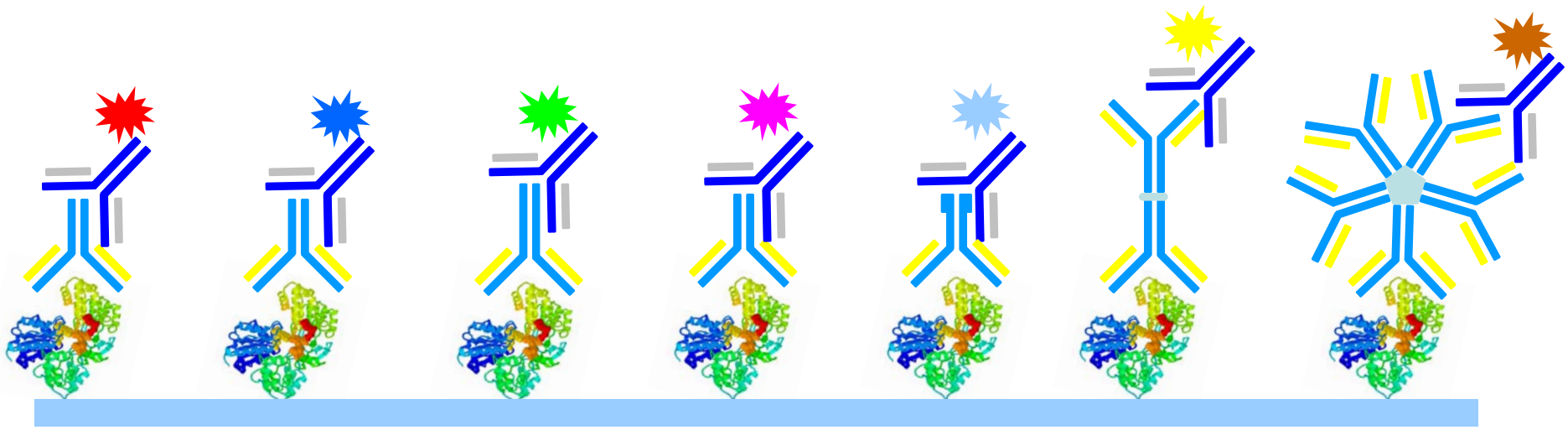

Celiac Disease
4/6 plex

# MULTIPLEXED ANTIBODY DETECTION IN A SIN

Ig_PLEX™ For Immunogenicity

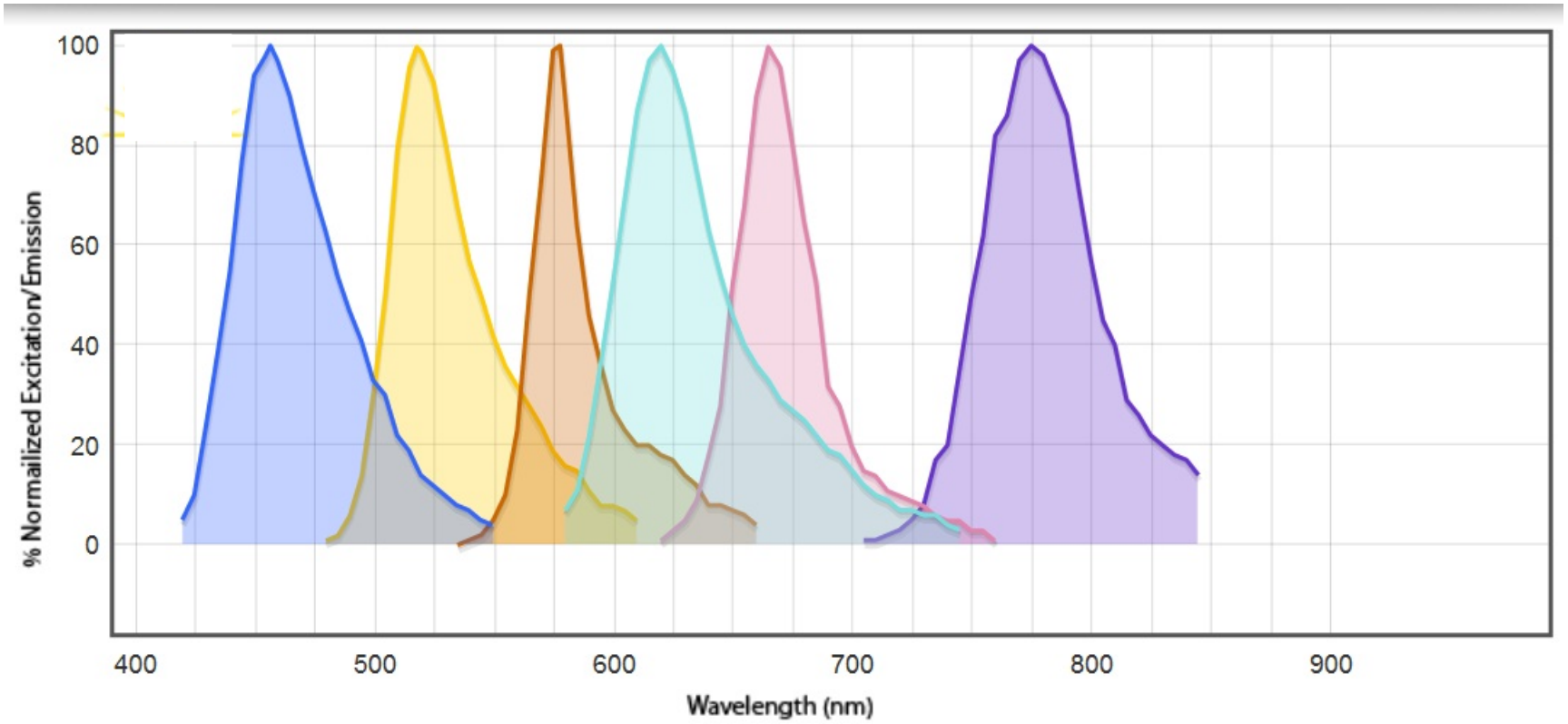
Multiplexing IgG, IgA, IgM. IgE

$IgG_1$, $IgG_2$, $IgG_3$, $IgG_4$



$IgG_1$ $IgG_2$ $IgG_3$ $IgG_4$ IgE IgA IgM

# DISCREET EMISSION SPECTRA UP TO 6 DETECTORS/\




Current scanner implementation is 3 wavelengths
IVD business unit moving forward with 6 wavelength scanner

# WHY IG_PLEX FOR BIOANALYTICS ?

- Automated analysis of ADAs including isotype & subclass
- Array multiple captures: Tx protein innovators, biosimilars, drug metabolites or recombinant protein subunits
- Detection cocktail of labeled secondary antibodies: anti-isotype or anti-IgG subclass (up to 6/assay)
- Qualitative screen, quantitative titer
- Automate sample/drug pre-incubation for specificity confirmation
- Non-cell based ligand binding assays for neutralizing antibodies
- IVD proven technology with precision, sensitivity and method concordance

# SQI`S TECHNOLOGY IN PRACTICE

- Multiplexing Isotypes with IgG subclasses on drug molecule analogs
- Demonstrate multiplexed serum antibody specificity
- System compatibility with dissociation of ADA/drug complexes
- Detection limits for serum antibodies
- Quantitate serum trough levels of Infliximab on printed TNF
- 8 plex Cytokine assay: antibodies as captures for biomarker or drug quantitation

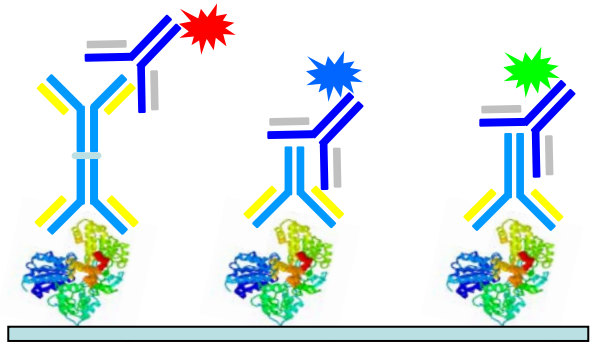
# 2 X 9 - PLEXED ISOTYPE AND SUBCLASS

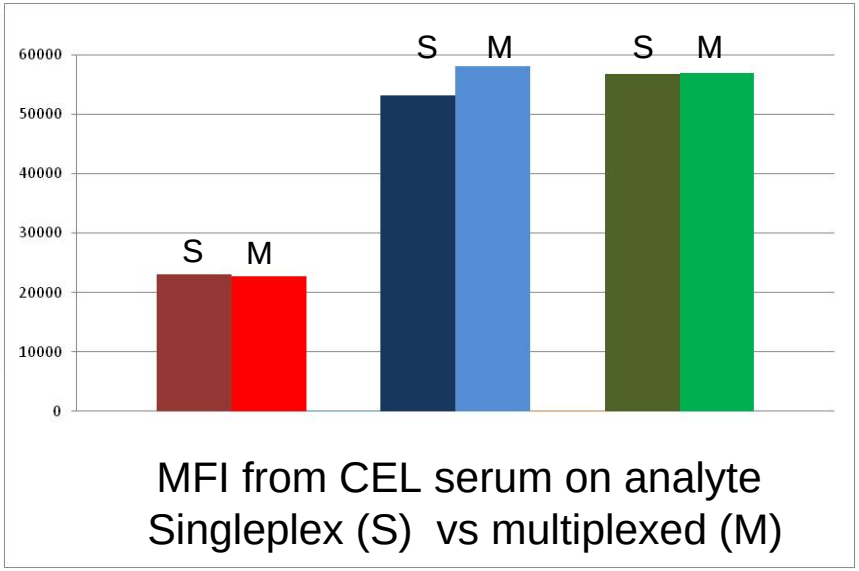
**Objective: Demonstrate equivalence between single plex and multiplex assays of serum antibody Isotypes with IgG subclasses on drug molecule analogs**

**Challenge Panel (17 Celiac + 7 normal)**
**Overall agreement to Single plex predicate**

| Multiplex 1 | | | Multiplex 2 | | |
|---|---|---|---|---|---|
| IgA (APC) | | | IgG2 (Dy649) | | |
| analyte 1 | analyte 2 | analyte 3 | analyte 1 | analyte 2 | analyte 3 |
| 100% | 95% | 90% | 90% | 89% | 94% |
| IgG3 (RPE) | | | IgG4 (RPE) | | |
| analyte 1 | analyte 2 | analyte 3 | analyte 1 | analyte 2 | analyte 3 |
| 90% | 100% | 95% | 90% | 95% | 100% |
| IgG1 (AF488) | | | IgE (FITC) | | |
| analyte 1 | analyte 2 | analyte 3 | analyte 1 | analyte 2 | analyte 3 |
| 100% | 100% | 100% | 88% | 94% | 100% |

**Screen Isotypes and Subclasses in a Single Well**

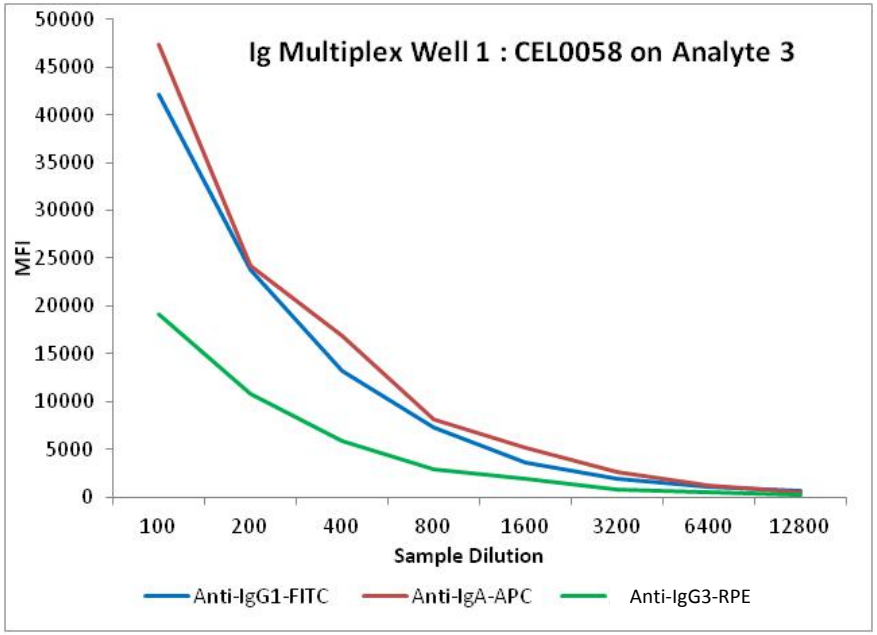


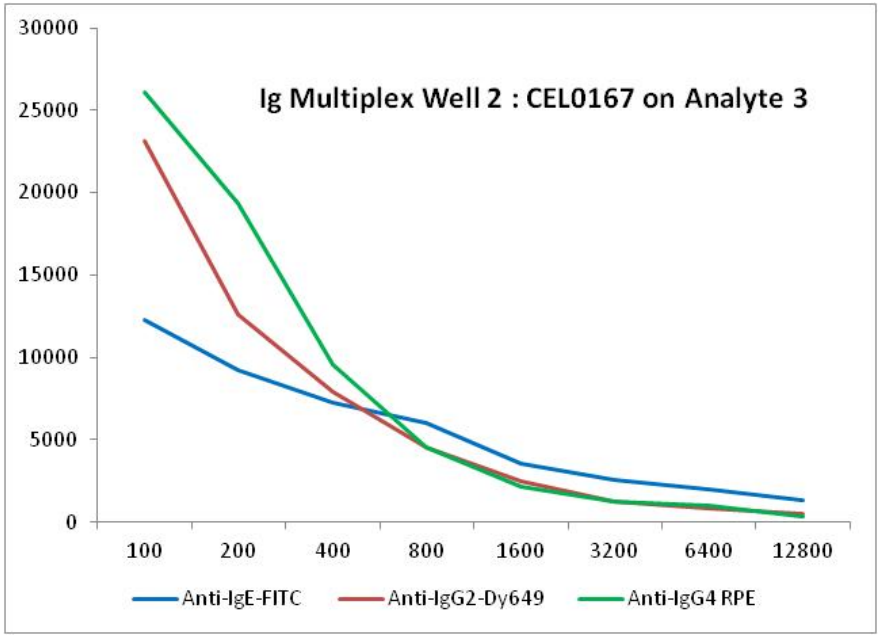



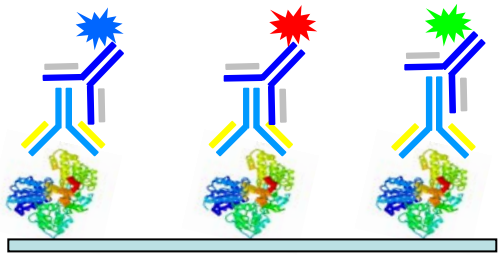

MFI from CEL serum on analyte
Singleplex (S) vs multiplexed (M)

# 2 X 9 - PLEXED ISOTYPE AND SUBCLASS

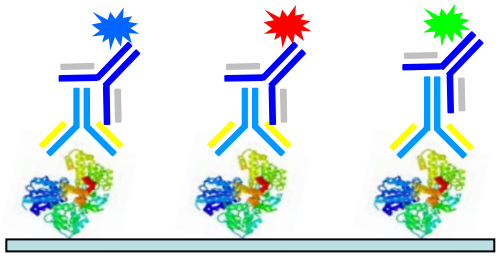
## Multiplexed dilution response



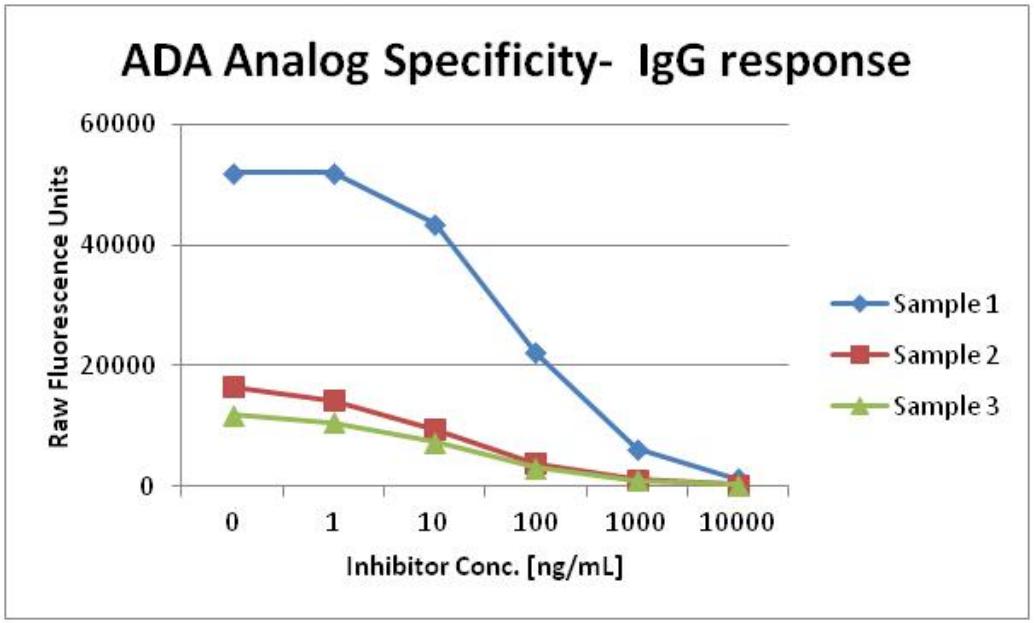



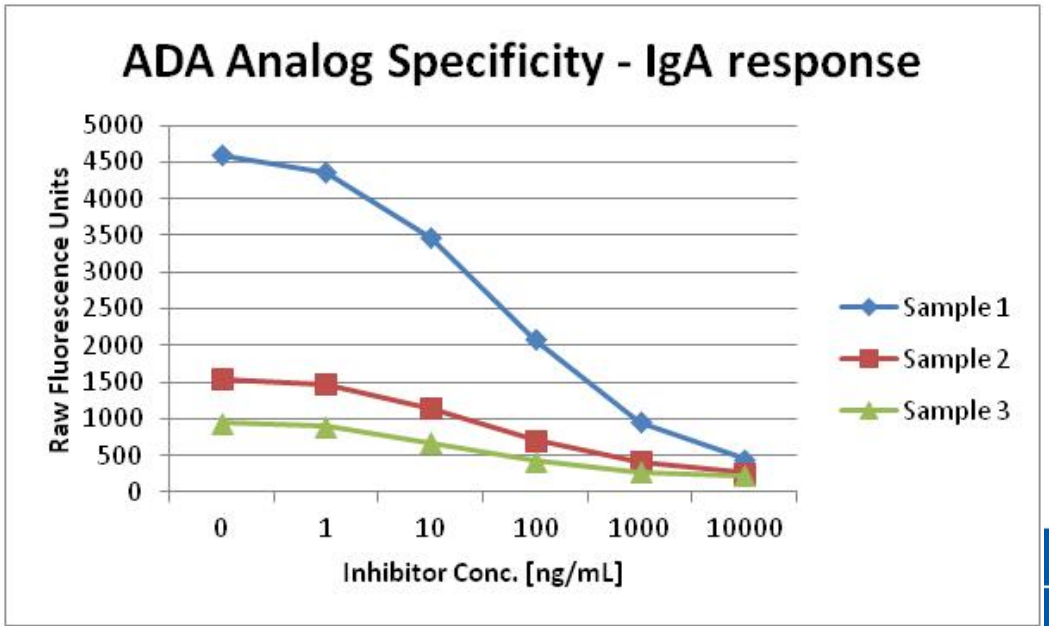
$IgG_1$ IgA $IgG_3$



IgE $IgG_2$ $IgG_4$

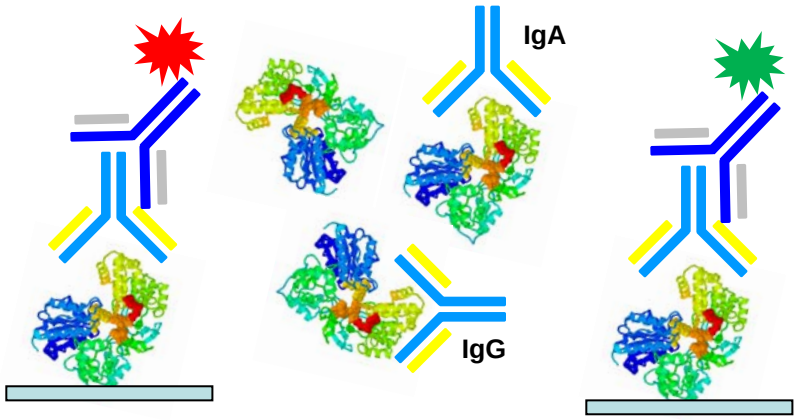
# CONFIRM MULTIPLE ADA ISOTYPES SPECIFICITY IN SIN




**Objective: Demonstrate multiplexed serum antibody specificity**

3 IgG/A positive serum samples pre-incubated with serial dilutions of drug molecule analog before microarray assay

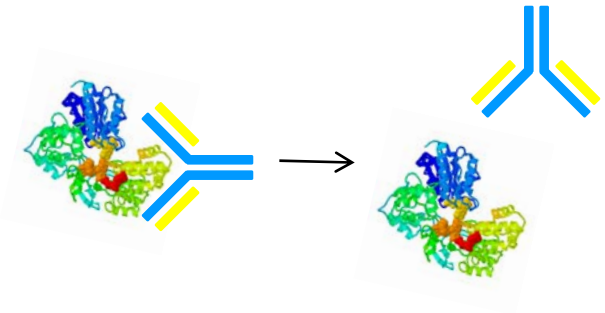



**Serum IgG and IgA antibody specificity for a drug molecule analog demonstrated in a <u>single</u> inhibition assay.**

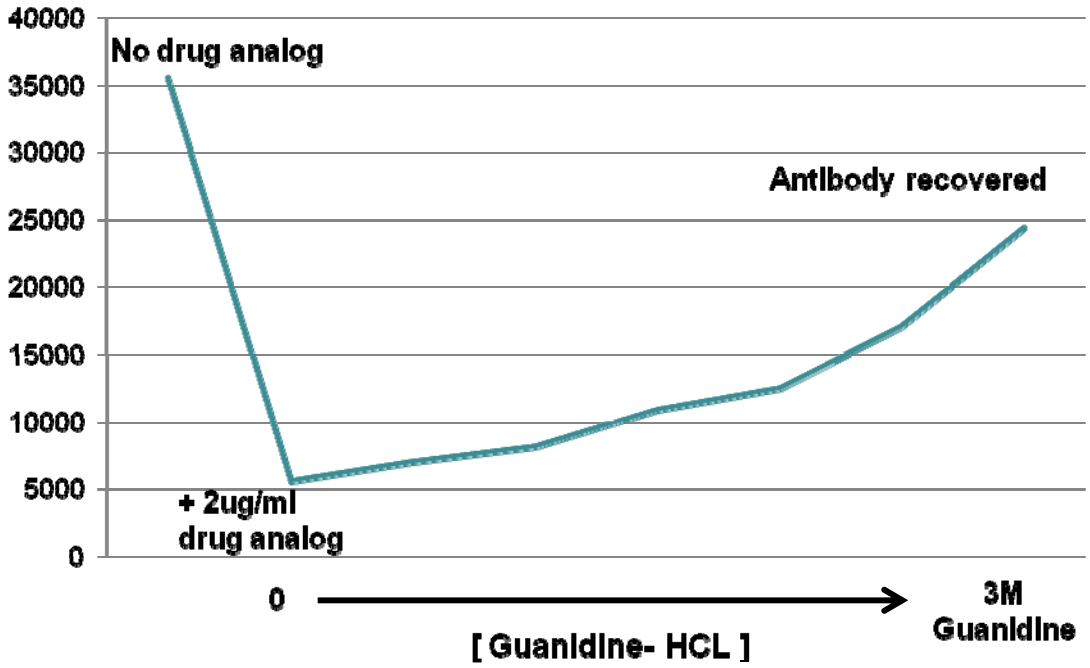
# IMMUNE COMPLEX DISSOCIATION WITH

**Objective:** **Bioanalytic assays may require a dissociation step prior to measurement of ADAs**

IgG pos serum + Analog
+ 2 fold dilutions of Guanidine
Neutralized with 1N NaOH



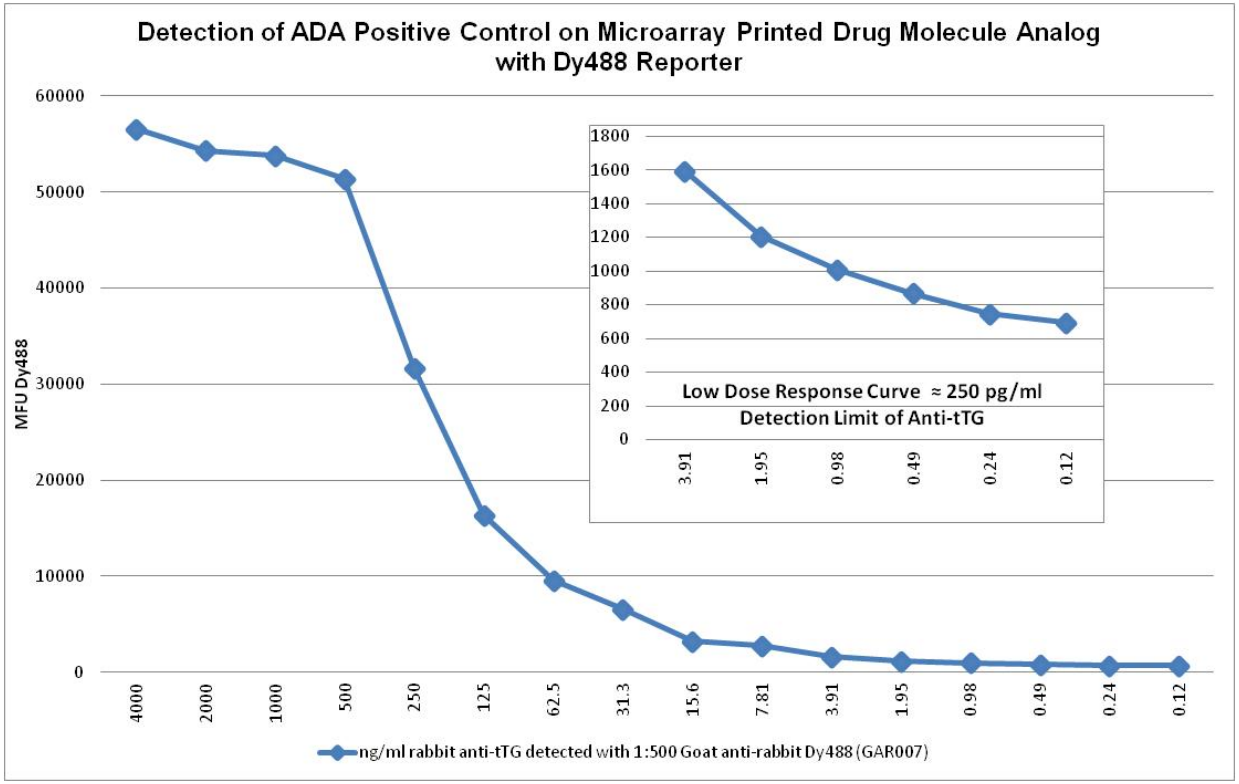
IgG Signal on Printed Drug Analog

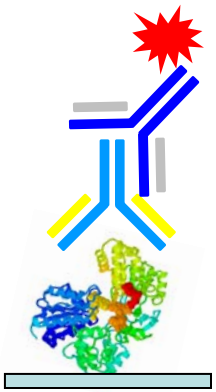



**Conclusion: Arrays are compatible with immune complex dissociation**

# SENSITIVITY : AB CAPTURE ON PRINTED DRUG ANALO

## Objective: Determine detection limit for serum antibodies



2º ab

Pos Control Ab

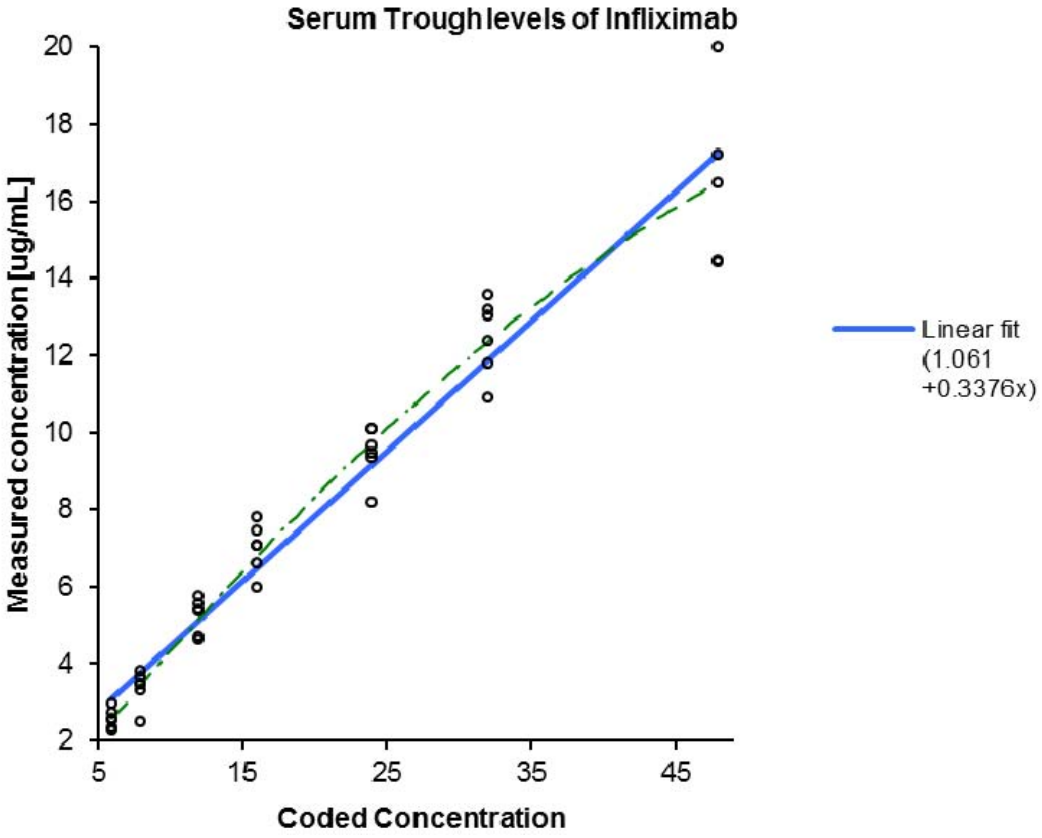
Drug Analog

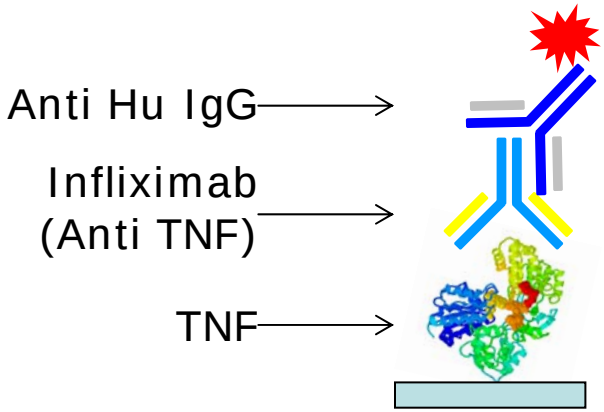
200 micron diameter spots at 550ug/ml
Serial dilutions of Rabbit anti-DMA as ADA pos control
+ Goat α-Rabbit-Dy488 (1:500)

**Detection limit of 250 pg/ml**

# DEVELOPMENT STAGE ASSAY FOR INFLIXIMAB PK

- Objective – determine trough levels in patients treated with the drug Infliximab
- Early discovery work was driven by clinically relevant measurement range of 2-20 ug/mL; (Values below 2ug/mL reported as <2ug/mL)



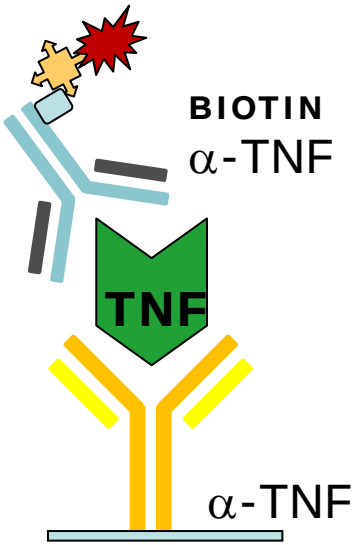

34 patient serum compared with Prometheus as predicate

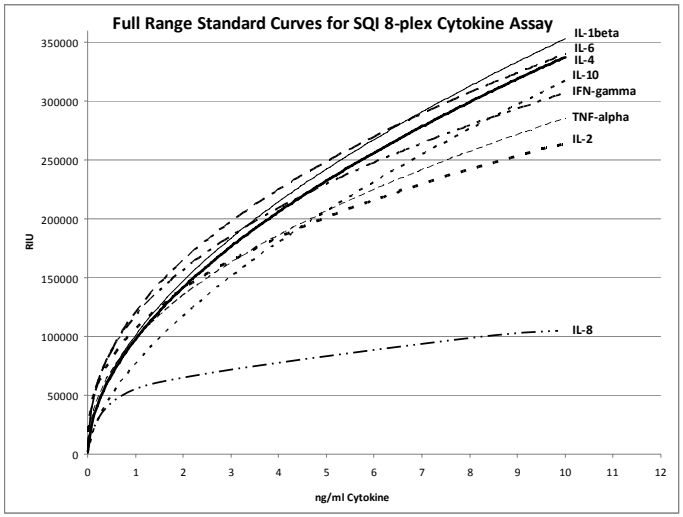
**Overall agreement > 80%**

All normals reported as negative

**Specificity of 100%**

# SQI 8-PLEX CYTOKINE MICROARRAY




SQI platform can also measure Cytokine Responses
or
Incorporate a sandwich assay for protein quantitation

| | IL-2 | IL-4 | IL-6 | IL-8 | IL-10 | TNF Alpha | INF Gamma | IL-1 Beta |
|---|---|---|---|---|---|---|---|---|
| LLOD (pg/mL) | 0.75 | 0.68 | 0.63 | 0.75 | 0.75 | 0.84 | 0.88 | 0.47 |
| LLOQ* (pg/mL) | 0.88 | 1.75 | 0.88 | 0.98 | 1.49 | 0.88 | 1.25 | 1.5 |
| ULQ* (ng/mL) | 7.5 | 1.72 | 7.5 | 10.0 | 7.5 | 6.25 | 6.25 | 10.0 |
| ULD (ng/mL) | 10.0 | 6.25 | 8.75 | 10.0 | 8.75 | 7.5 | 7.5 | 10.0 |
| CV% | 8.1 | 11.8 | 11.0 | 4.8 | 11.1 | 10.2 | 7.9 | 6.8 |
| % Recovery (Serum/ Plasma) 25pg/ml | 85/95 | 102/89 | 82/88 | 116/103 | 127/120 | 95/82 | 88/87 | 85/84 |

*precision (CV) <15%, accuracy (bias) <20%

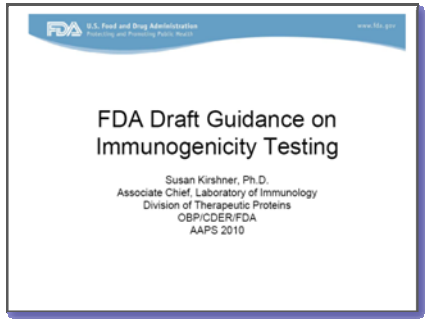
# SQI TECHNOLOGY : REGULATORY

**In vitro Diagnostic (IVD) Devices**:

- SQI cleared for market in the US, CE marked in EU and licensed in Canada
- SQI is ISO 13485 compliant
- FDA draft guidance: *Assay Development for Immunogenicity Testing of Therapeutic Proteins* (Dec 2009) builds from the FDA IVD regulations
- EMEA guidance: *Guideline on Bioanalytical Method Validation* (July 2011) uses similar framework to guide validation efforts

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

EMEA Guidelines




- ✓ Same serum dilutions for screening
- ✓ Use high, med, low QC controls
- ✓ Use a negative control
- ✓ Screening assays should detect all isotypes including IgE
- ✓ Systematic determination of assay cut point
- ✓ Compare using the same testing technology
- ✓ Sub class assays should be able to quantitate IgG4
- ✓ Robust and reproducible Assays

# IN SUMMARY

- SQI microarrays IVD level performance: validated for sensitivity, specificity and precision
- Assay kinetics and fluidics equivalent to ELISA
- A technology borne from IVD that provides:
  - Multiplexing isotypes with IgG subclasses (IgE and $IgG_4$)
  - Multiplexing ADA specificity confirmation
  - Sub nanogram detection limits for serum antibodies
  - Quantitation of drug serum trough levels
- **Acknowledgements:**

  Jeff Terryberry

  Stuart Carmichael, Sasi Mudumba, Shi-Fa Ding, Pieter Biessels

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