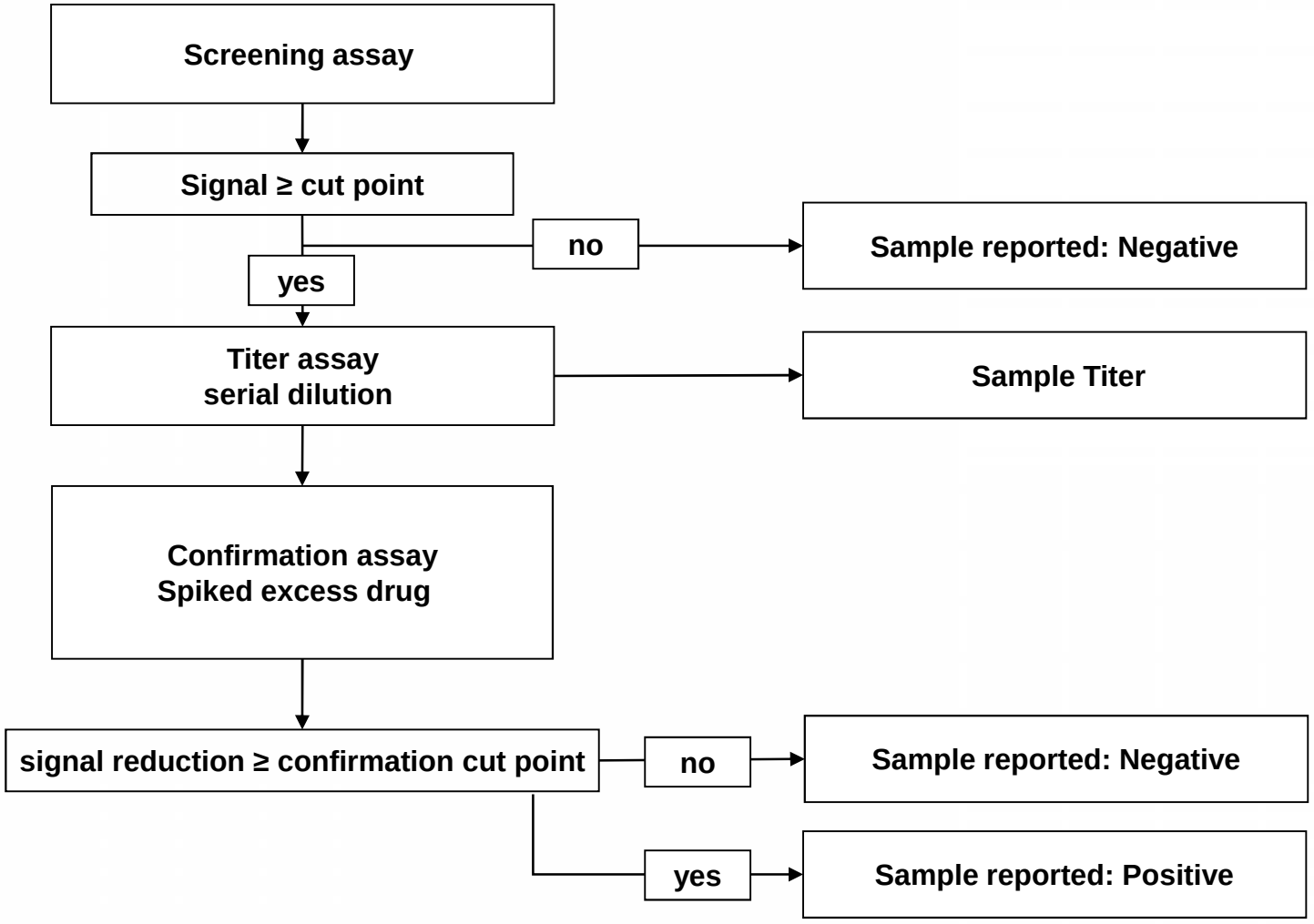
Immunogenicity Assay Considerations

Jochem Gokemeijer
Jochem.gokemeijer@bms.com
Bioassays and Bioanalytics Method Development, Berkley CA
October 8th 2013

Multi Tiered Immunogenicity Assay Approach



Bristol-Myers Squibb

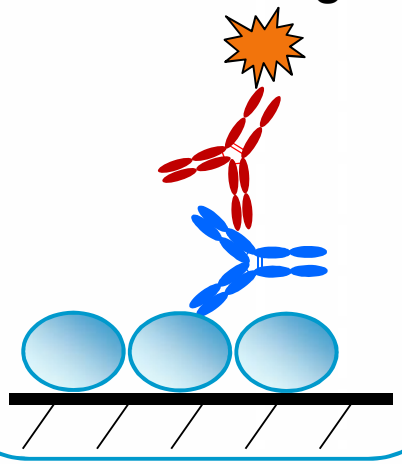
Immunogenicity Assay Considerations:

- **Detect relevant anti drug antibodies**
  - 250-500 ng/ml
  - Detect all isotypes (particularly IgG / IgM)
- **Drug Interference**
  - Wash out periods in tox study might not be available in clinical studies
  - Long T1/2 of biologics make washing out difficult
- **Pre Clinical to Clinic Assay**
  - Prefer optimization vs redevelopment
- **Positive control**
  - The positive controls should reflect the human immune response
    - NHP polyclonal not available for early development
    - → NHP ADA bind to Fc region vs human ADA bind to CDR region for majority of human Abs

Bristol-Myers Squibb

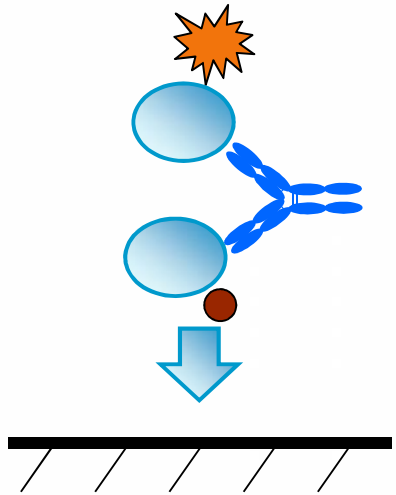
Immunogenicity Assay Formats:

Direct Binding



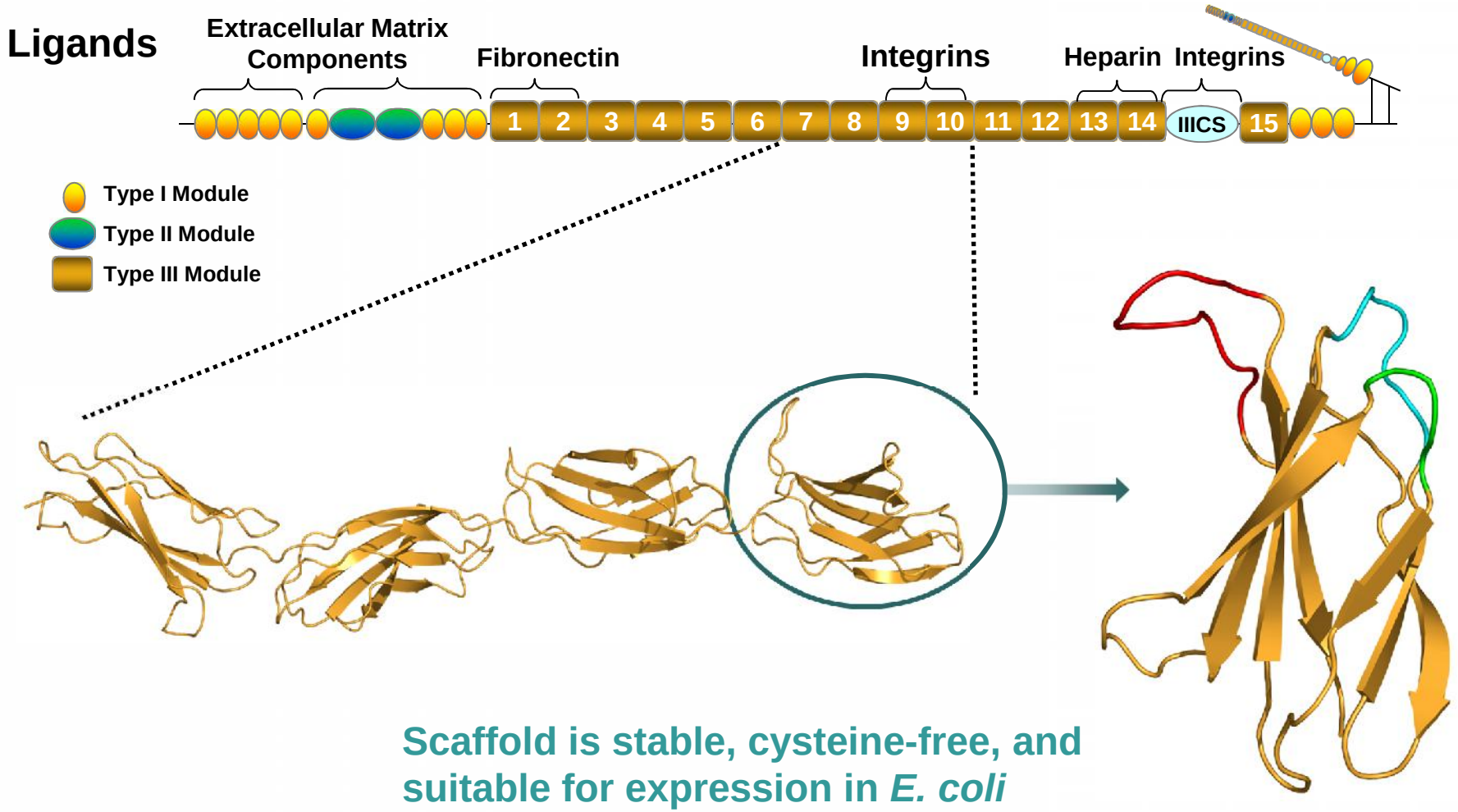
- Isotype depending on detection Ab
- Random orientation of drug absorbed to plate
- Local concentration and avidity
- Human mAbs drug pose a challenge

Bridging Assay



- Detects all Isotype
- Need to label drug
- Solution binding
- Prone to hook effect

Adnectins: Fibronectin Domains Evolved for Binding



Scaffold is stable, cysteine-free, and suitable for expression in *E. coli*

Bristol-Myers Squibb

Adnectins Uniquely Mimic Antibody Domain Structure and Function

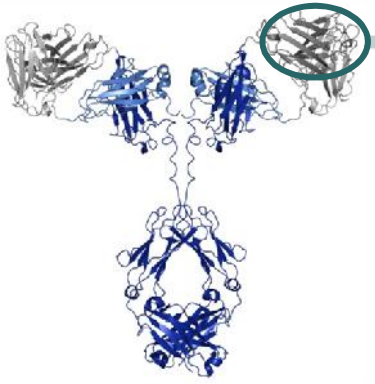
Bristol-Myers Squibb

Human fibronectin:

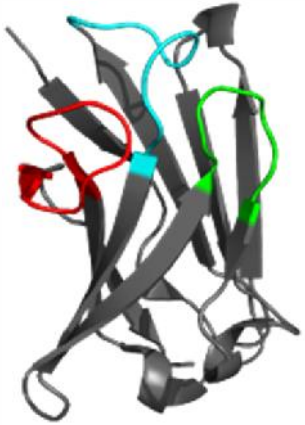
Naturally <u>multi-specific</u>

Adnectin:
Redirected, engineered targeting domain

Human antibody:

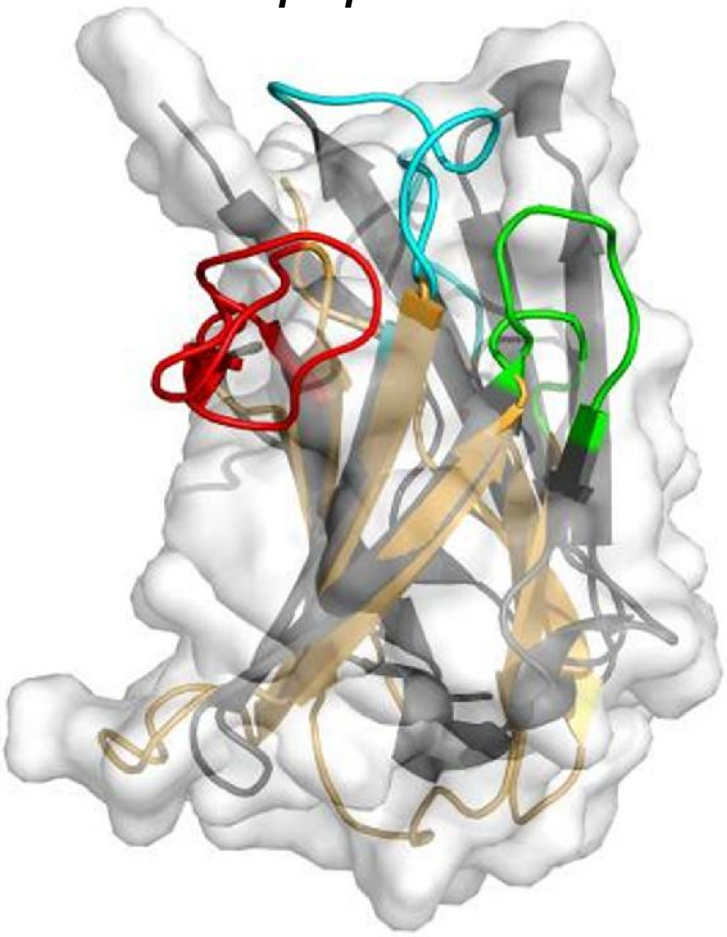


Naturally <u>mono-specific</u>



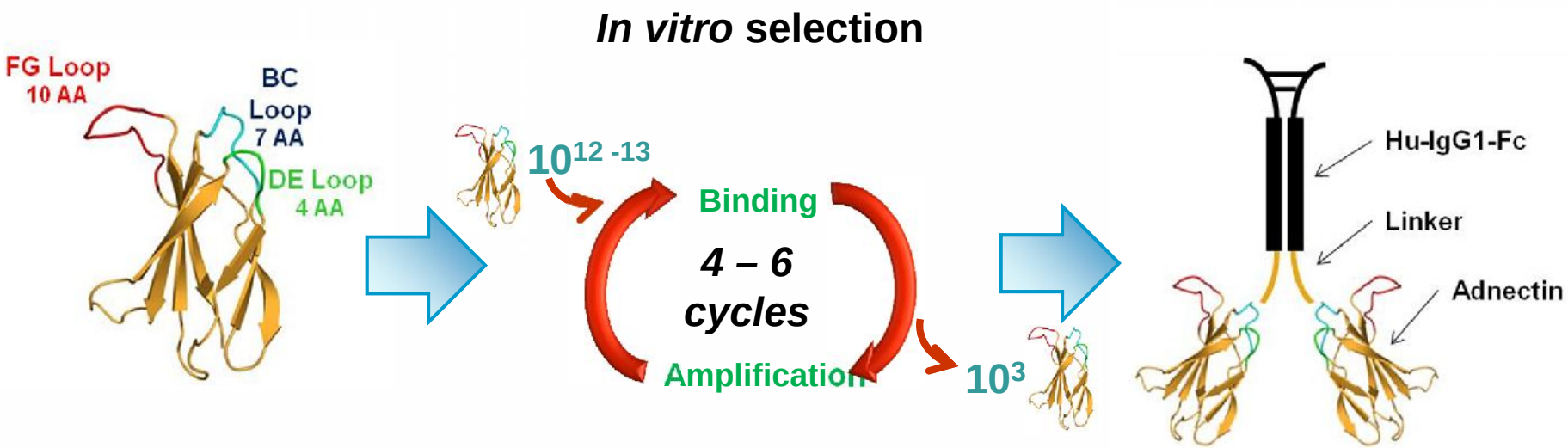
V_H Domain:
Targeting domain

Superposition



Bristol-Myers Squibb

Target Binding Adnectins are Culled from Diverse Libraries Using in vitro Selection



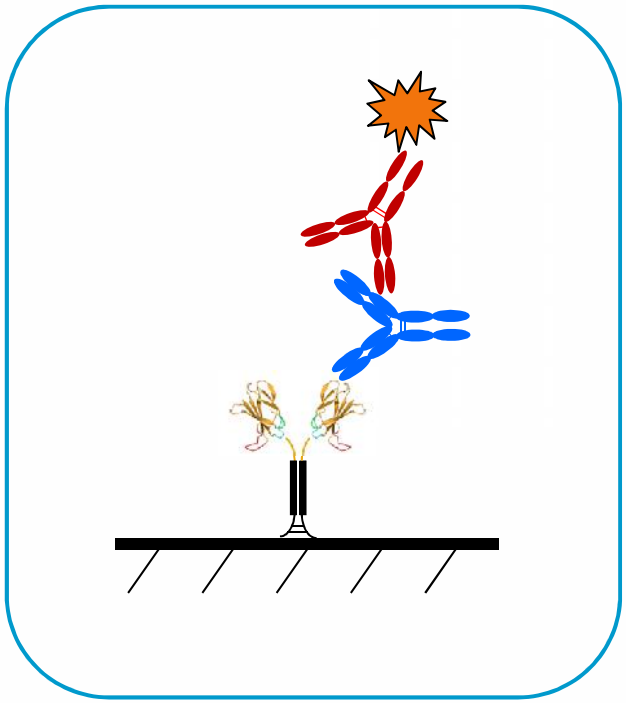
Library design
~ 10^{26} theoretical diversity

Formatting

Bristol-Myers Squibb

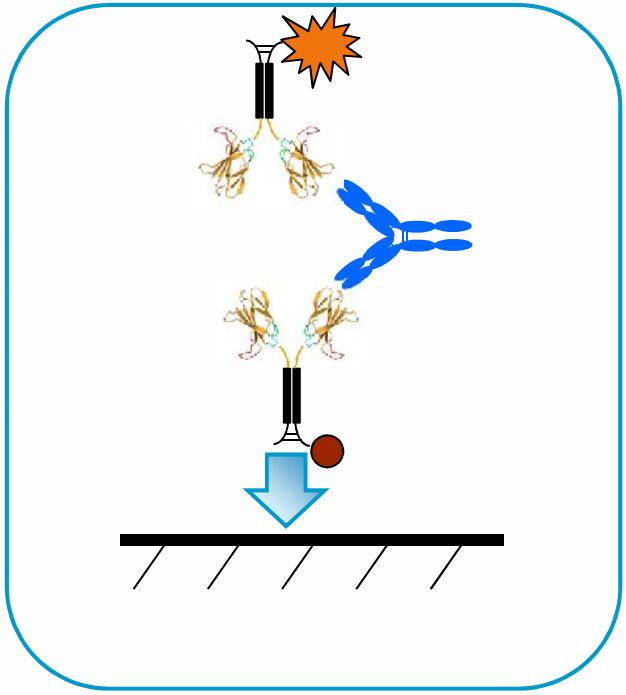
Immunogenicity Assay Formats for Adnectin Fc

Direct Binding



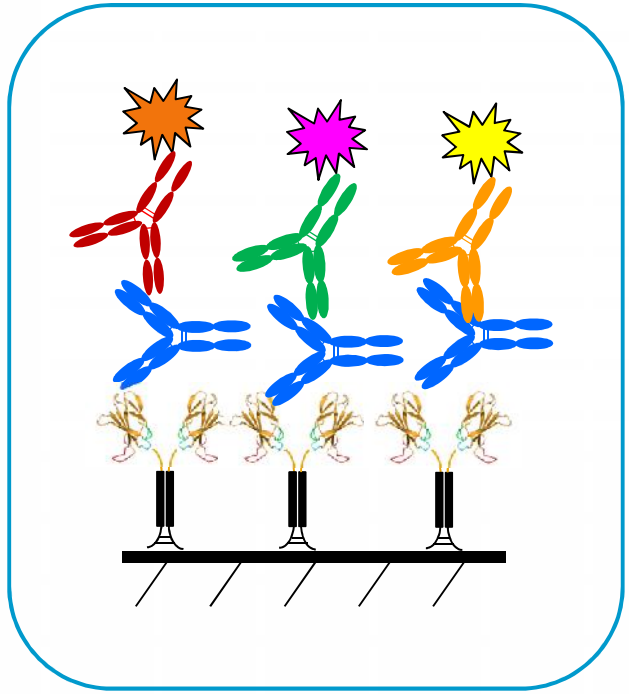
- Anti fab detection (IgG/IgM)
- Coating @ 2 ug/ml
- ELISA

Bridging Assay



- Labeled drug
- ECLA

SQI Multiplex



- Anti IgG /IgA/IgM detection
- Coating @ 125 ug/ml
- Multiple spots per well

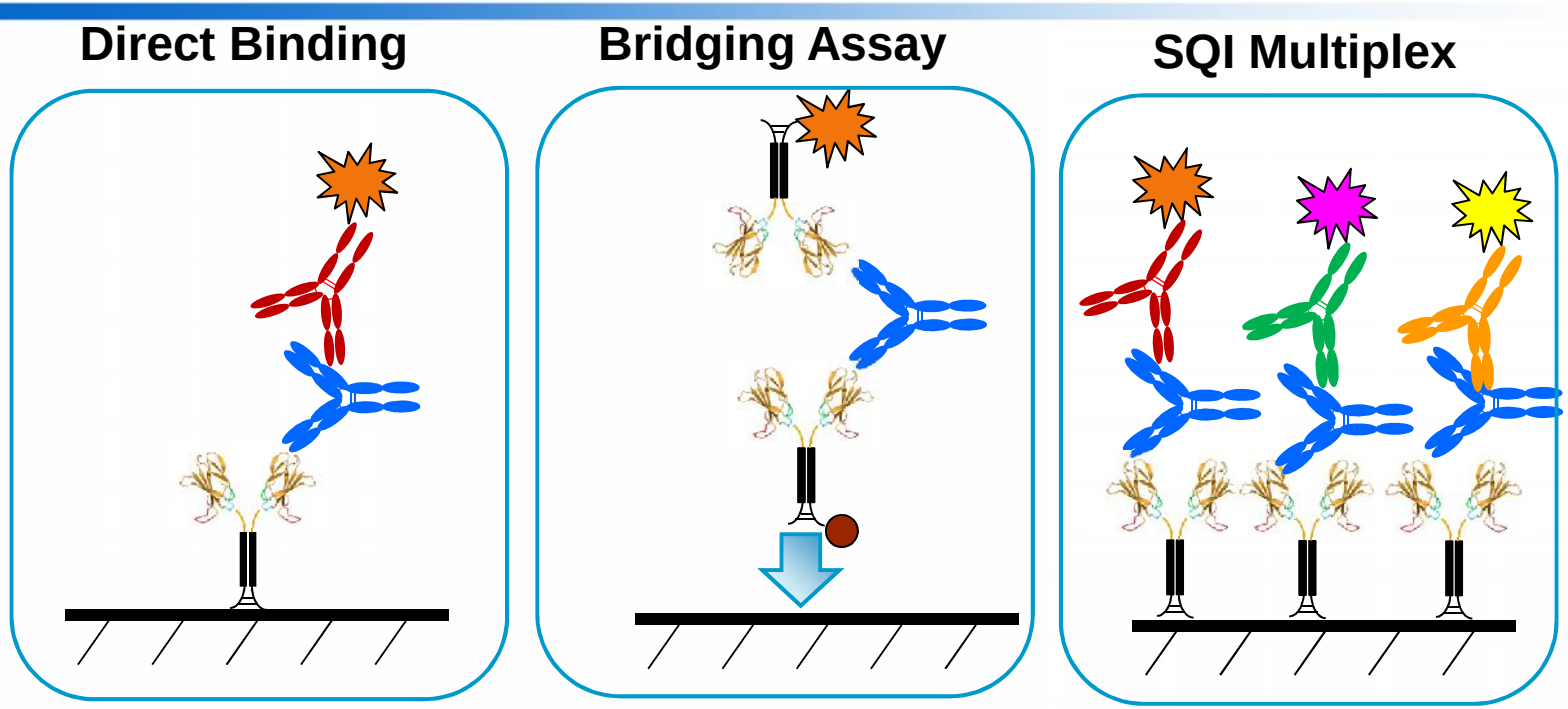
Assay Format Considerations

- **Labeling of drug**
  - Biotin / Ruthenium
  - Lysine's in binding loops / CDR's?
  - Use potency assay to assess activity

- **Dilution Background and Sensitivity**
  - High dilution minimizes background signal
  - Low dilution increases sensitivity
  - High dilution can decreases drug interference

→ highest dilution while still maintaining ~250 ng/ml sensitivity

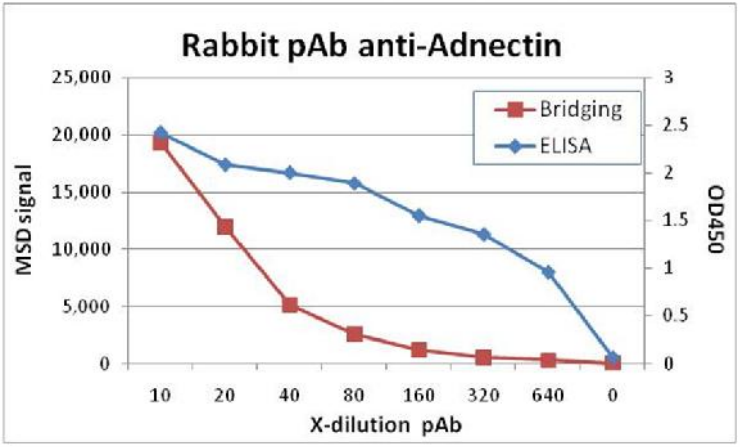
Immunogenicity Assay Formats for Adnectin Fc



Background	**++**	**-**	**-**
S/N	**+**	**-**	**+**
Assay			
Dilution	1:100	1:2	1:100
Sensitivity (mAb)	250 ng/ml	250 ng/ml	62.5 ng/ml

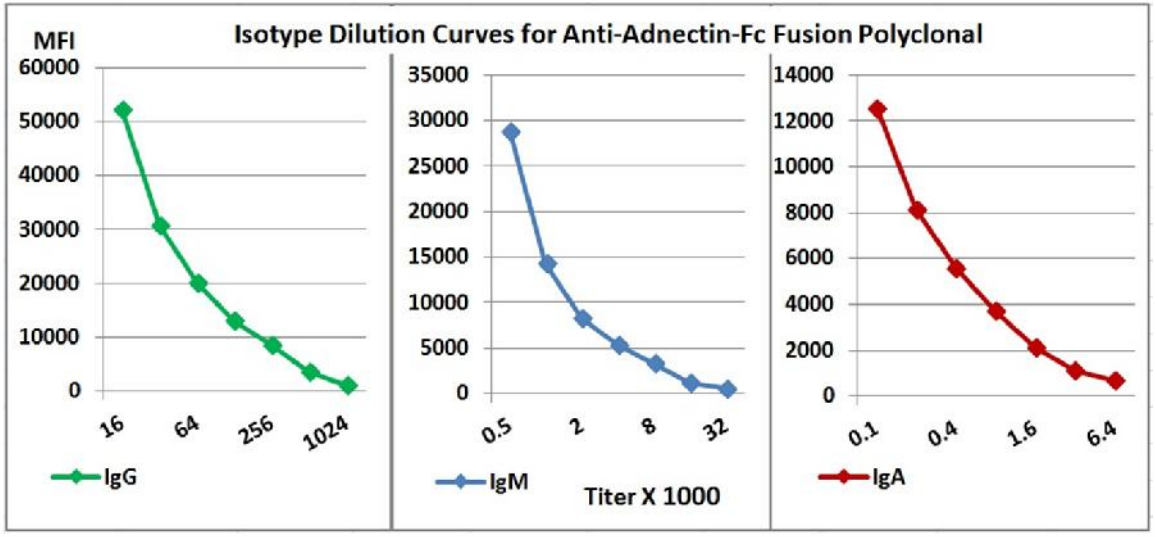
Assay Performance: Sensitivity Polyclonal Ab

ELISA / Bridging



Polyclonal anti serum shows significant higher sensitivity for plate based assays vs bridging assay

SQiD (IgG/IgA/IgM)



Drug Tolerance

Anti-Adnectin-Fc-Fusion			
	LOD (Monoclonal)	Drug Tolerance (monoclonal)	Drug Tolerance (Polyclonal)
ELISA	125 ng/mL	0.1 mg/mL	200 mg/mL @ 1:64K
Bridging Assays	125 ng/mL	0.1 mg/mL	0.1 mg/mL @ 1:160
SQI	62.5 ng/mL	0.1 mg/mL	250 mg/mL @ 1:128K

SQI Assay Development:

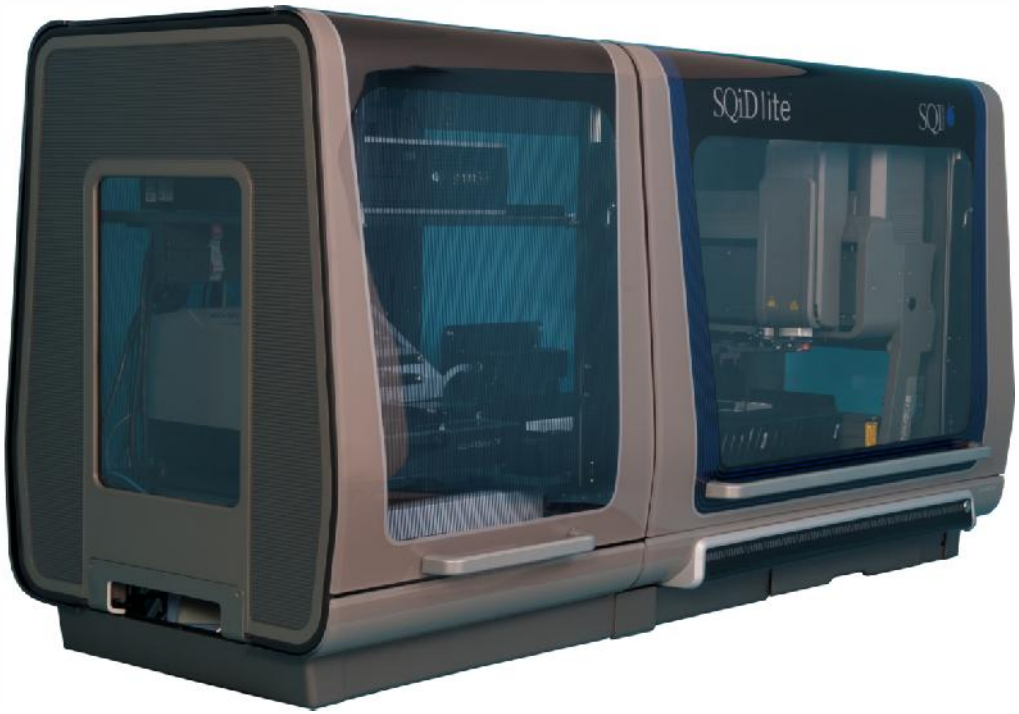
- Immunogenicity Assay was developed for Rat Mouse and Cyno at SQI Diagnostics : 6 Weeks
- Cross-over SQI format study vs ELISA results
- Two week, multiple dose study design
- Samples analyzed: Day 0 / Day 10 / Day 22

Species	Monkey		Mouse		Rat	
Isotype	IgG/M	IgA	IgG/M	IgA	IgG/M	IgA
% Positive Agreement	100 (8/8)	8/8	80 (8/10)	8/10	50 (5/10)	1/10
% Negative Agreement	100 (9/9)	9/9	87.5 (7/8)	4/8	100 (8/8)	8/8
% Overall Agreement	100	NA	83.3	NA	72.2	NA

Immunogenicity Assay Automation Considerations

- **Automate in house assay vs proprietary platform**
- **Quick development time**
- **Walk away capability and scalability**
  - Tox to Clinical studies
- **Software**
  - 21CFR compatible
  - LIMS System compatibility
- **System flexibility for liquid handling: intermediate dilution plate**
  - Automated on-deck dilution; sample in tubes
  - Automated on-deck titration; sample in tubes
  - Low sample volume conservation; off-deck dilution with manual sample load

Automation Detail, SQiDlite System*



- Load & Go capability for significant reduction in labor and supplies
- Flexible, configurable, automated workflow
- Bench-top size
- Full bi-directional LIS-integration
- <3 hours per plate
- CFR part 11
- User friendly assay development and run software included to report only what is required

*Info Provided by SQI Diagnostics

Bristol-Myers Squibb

Assay Reproducibility:

- Five (5) samples (four different titers of rabbit PAb-2 and one titer of PAb-6) in replicates of twelve (12) on each kit to report different concentration levels per isotype
- Three kits per lot from two lots were tested by two operators on one system to report precision reproducibility for intra-assay and inter-assay – between operators, days, and lots.
- Data analysis includes signal intensity

		Intensity				
	Sample	PAb2-1/200	PAb2-1/800	PAb2-1/25600	PAb2-1/51200	PAb6-1/400
Mean (n=72)	IgA	7993	3987	944	635	9431
	IgG	62465	63866	59613	40432	62779
	IgM	35733	27002	5693	3161	23383
% C.V.	IgA	14.2%	11.9%	14.7%	17.8%	21.7%
	IgG	2.7%	1.7%	6.8%	12.5%	2.9%
	IgM	14.3%	11.1%	14.7%	11.8%	11.3%

Summary and Conclusions

- Adnectin Fc Immunogenicity assay developed focused on high sensitivity and drug tolerance
- Plate based assay format showed significant increase in drug tolerance
- Assay was successfully developed at SQI with increased sensitivity and drug tolerance while providing additional isotyping data
- SQI assay was automated and qualified with high reproducibility

Acknowledgments:

Bristol Myers Squibb, Waltham MA

Sylvie Nesman

SQI Diagnostics, Toronto ONT

Jeff Terryberry

Jaymie R. Sawyer