Development of a Generic Anti-PEG Antibody Assay Using BioScale's Acoustic Membrane MicroParticle Technology

Robert Dodge[1] Huijin Dong[1], Johanna R. Mora[1], Catherine Brockus[1], Shannon D. Chilewski[1], Colin Merrifield[2], Matthew Dickerson[2], Renuka Pillutla, Binodh DeSilva[1]

[1] Bioanalytical Sciences Biologics, Bristol-Myers Squibb, Princeton, NJ, USA

[2] BioScale, Billerica, MA, USA

Bristol-Myers Squibb

Reagents for Assessing Assay Sensitivity

Commercial reagents are limited and are not human so we developed of two sets of anti-PEG antibody controls.

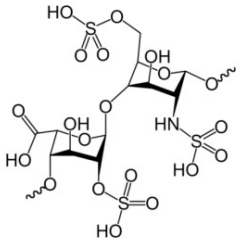
1. Monoclonal antibodies in mice and than chimeric with human Fc tail.
2. Polyclonal antibodies from transgenic cows (human immune system)

Anti-PEG Antibody Assay Objective

Bristol-Myers Squibb has numerous PEGylated drugs in the pipeline

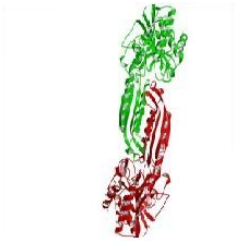
(protein drugs crosslinked to PEG

Humoral Immune Response is only to Proteins or to Non-Proteins crosslinked to a Protein (i.e. Heparin to PF4)



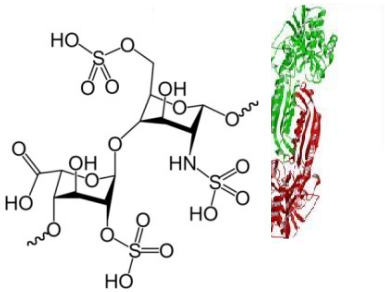
Heparin

+



Platelet Factor 4 (PF4)

=



Complex

Anti-PEG Antibody Assay Objective

Bristol-Myers Squibb has numerous PEGylated drugs in the pipeline

(protein drugs crosslinked to PEG

Humoral Immune Response is only to Proteins or to Non-Proteins crosslinked to a Protein (i.e. Heparin to PF4)

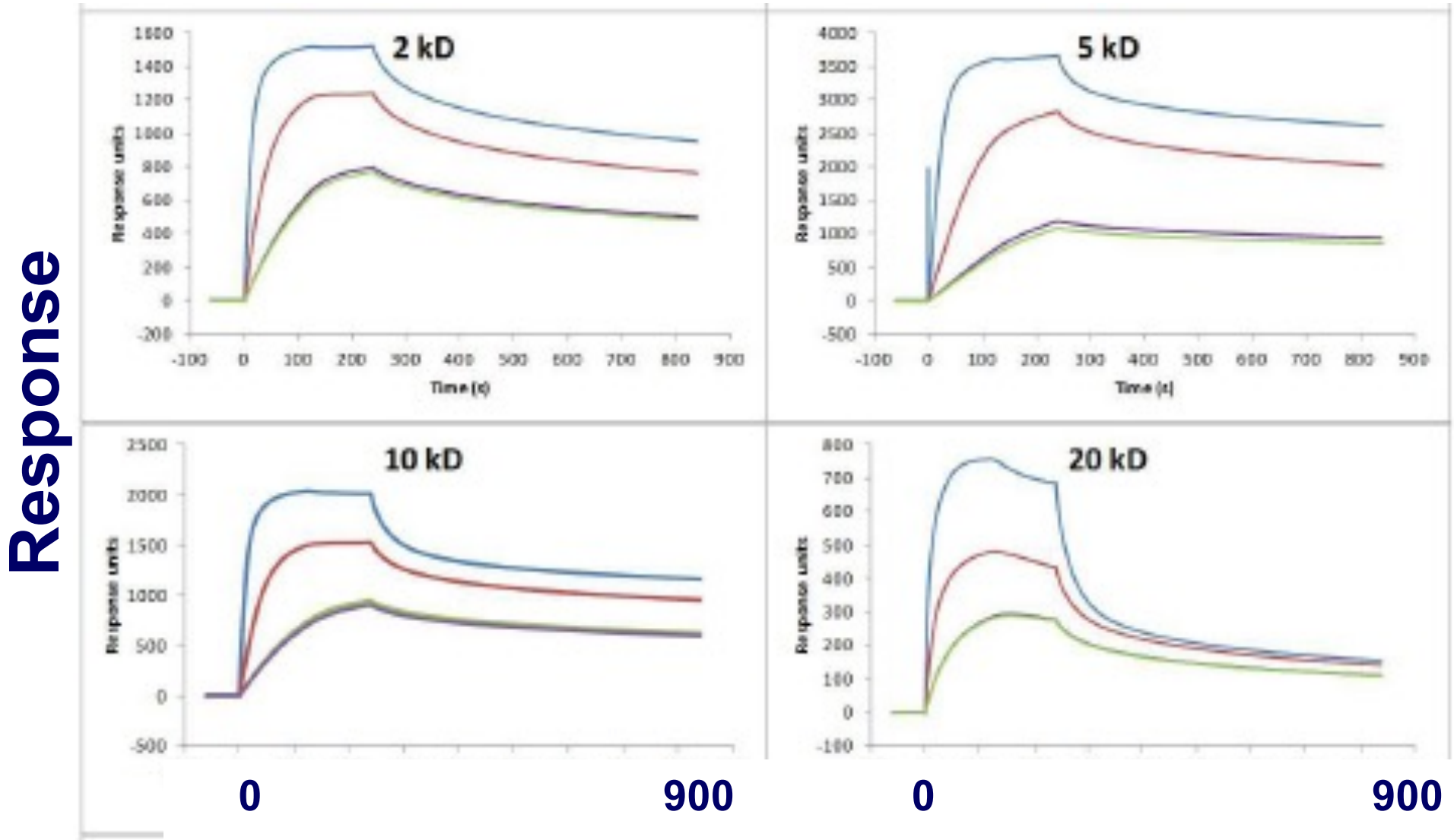
Key Question:

Can a PEG molecule crosslinked to drug (non-protein to protein) elicit a Humoral Immune response (i.e. anti-PEG antibodies)?

- Are there pre-existing anti-PEG antibodies in humans?
- Are is there a level of PEG in drug naïve humans.

Reagents for Assessing Assay Sensitivity

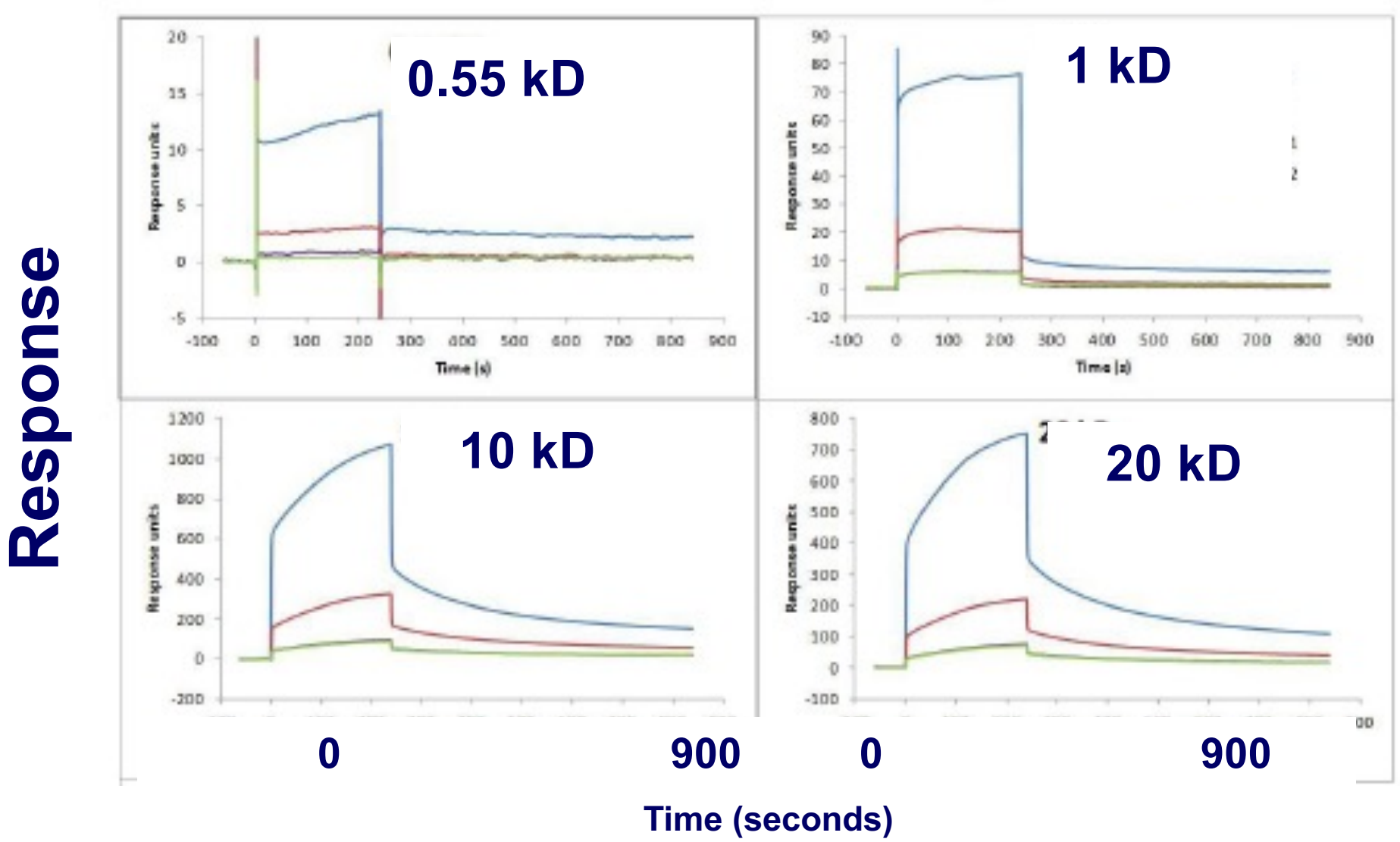
Characterisation Kon / Koff of polyclonal antibody



Submitted Bioanalysis Krishna et al.

Reagents for Assessing Assay Sensitivity

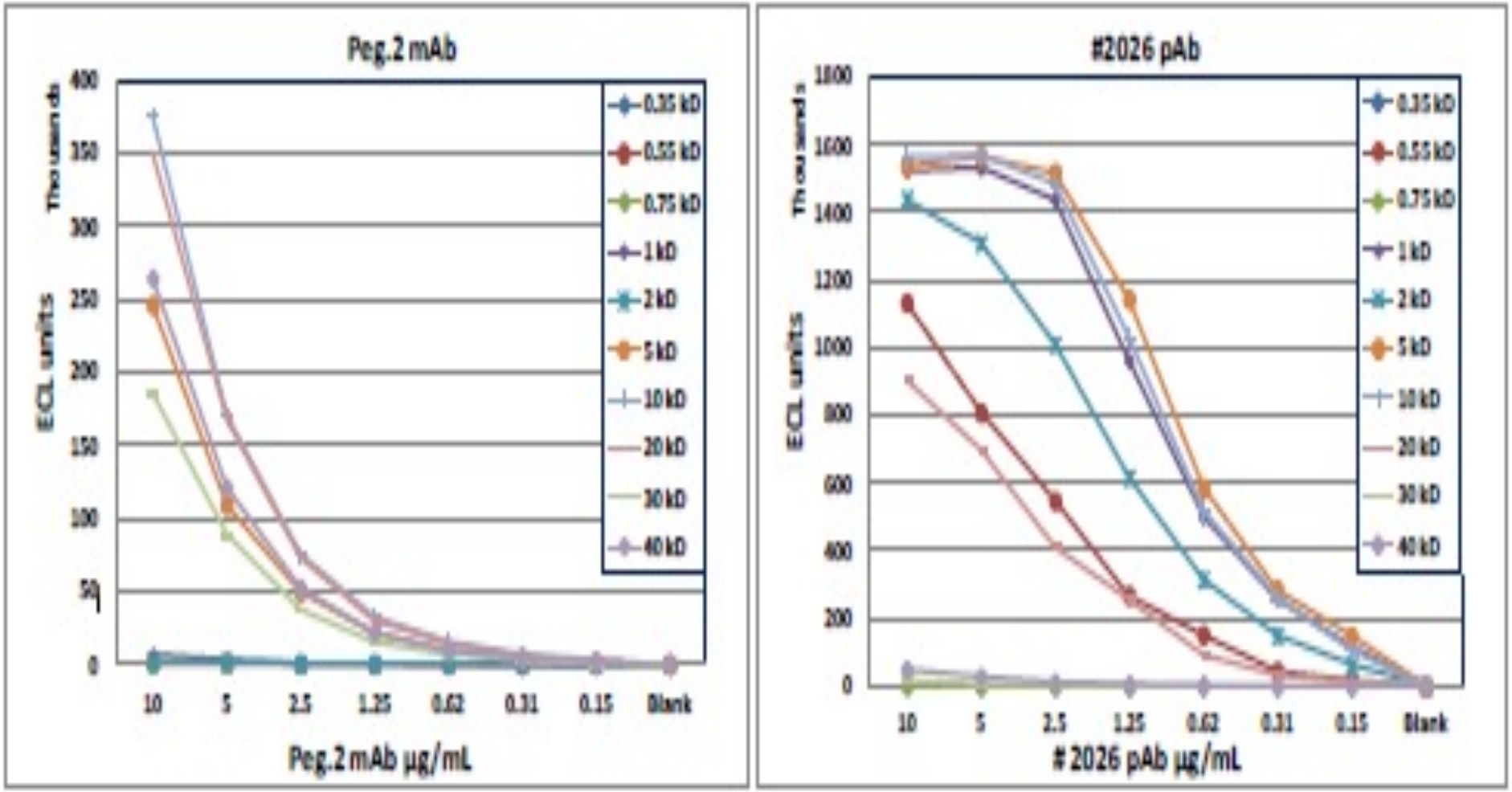
Characterisation Kon / Koff of monoclonal antibody



Reagents for Assessing Assay Sensitivity

Characterisation Kon / Koff of polyclonal antibody

A. Direct binding assay format on the MSD platform



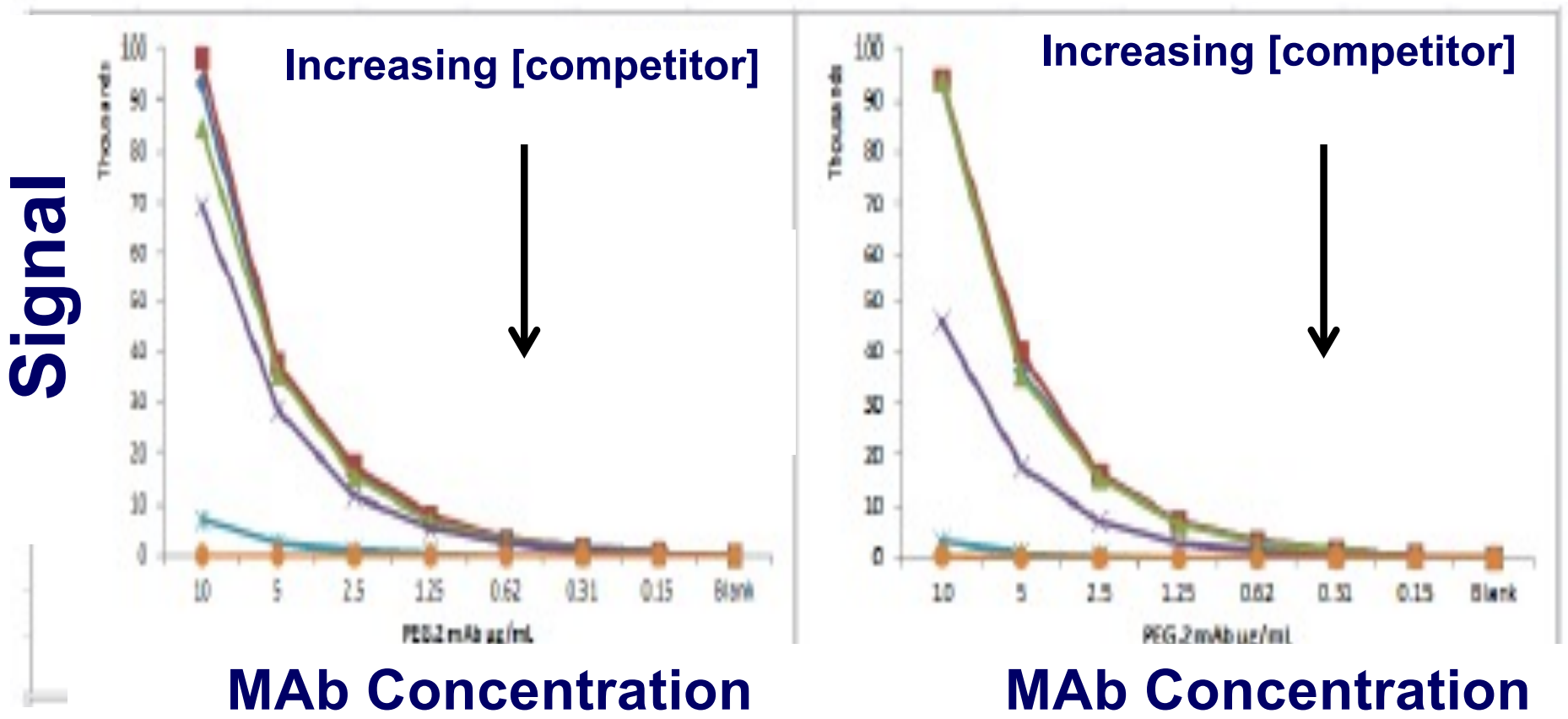
Submitted Bioanalysis Krishna et al.

Reagents for Assessing Assay Sensitivity

Characterisation Specificity of monoclonal antibody

$CH_3O - (CH_2CH_2O)_3 H$

$H_2N - O (CH_2CH_2O)_n - NH_2$



Submitted Bioanalysis Krishna et al.

Reagents for Assessing Assay Sensitivity

Summary of Regent Characterisation:

1.SPR (BiaCore) results on and off rate varied by PEG size.

2.Direct binding assay affinity / avidity consistent results with SPR data i.e. dramatic influence of PEG size.

3.Specificity results, no clear end cap – backbone

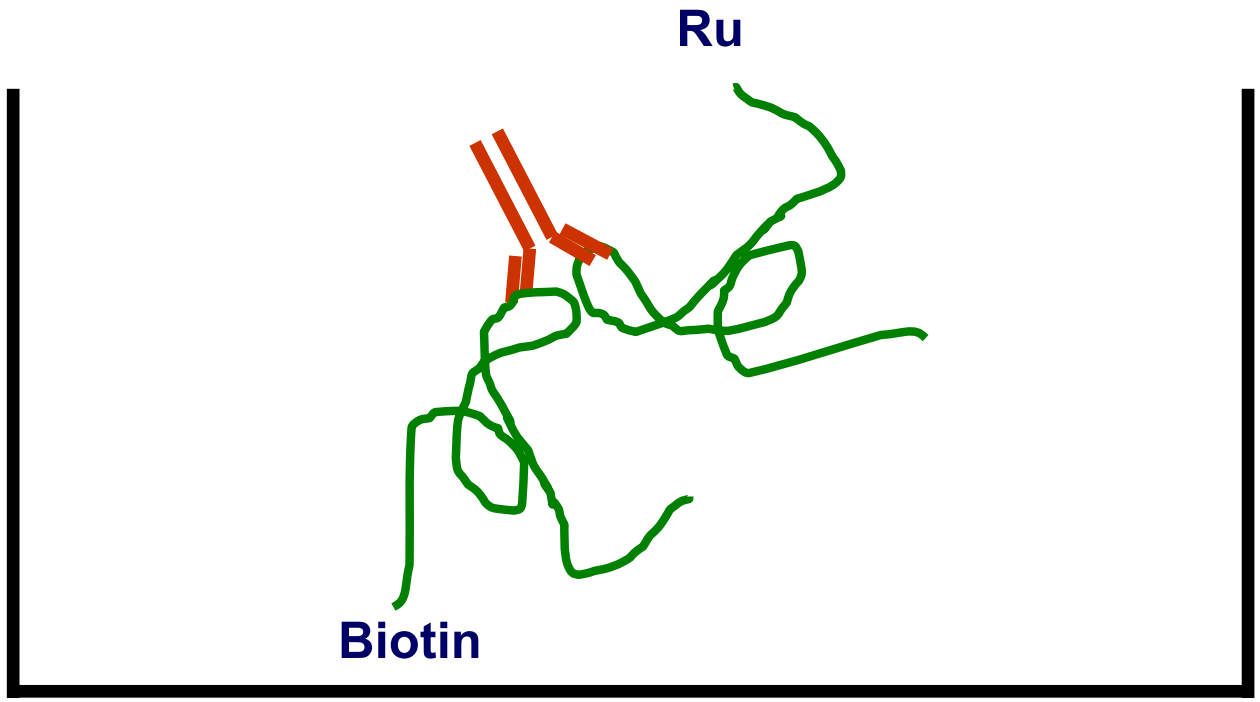
Conclusion: If our assay can detect these control antibodies, we will be confident that we are detecting most anti-PEG antibodies

Why do we need a new platform for Anti-PEG antibody testing?

Bridge Format Assays were not capable of detecting our positive control Ig antibodies to PEG in serum at reasonable sensitivity.

PEG

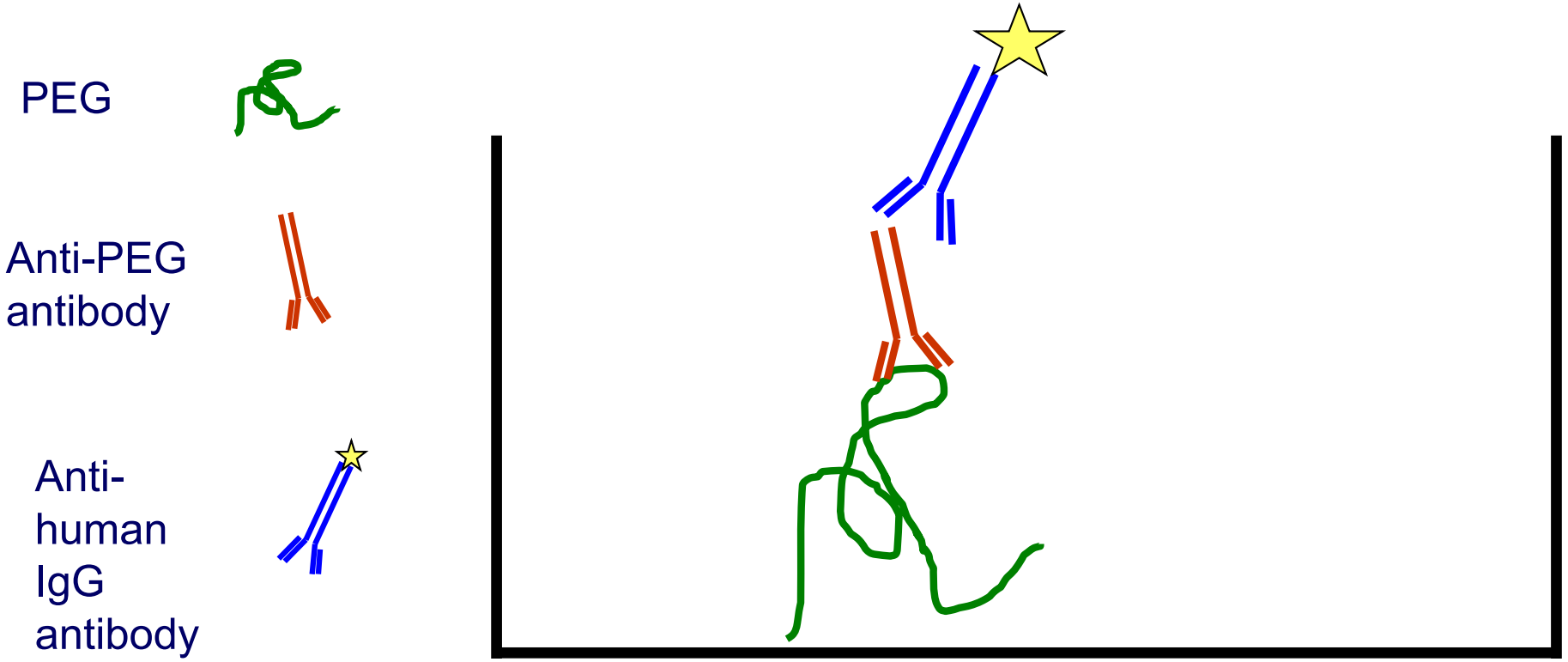
Anti-PEG antibody



Bristol-Myers Squibb

Why do we need a new platform for Anti-PEG antibody testing?

2. Direct assays were not capable of detecting our positive control IgG antibodies to PEG in serum at reasonable sensitivity.



Bristol-Myers Squibb

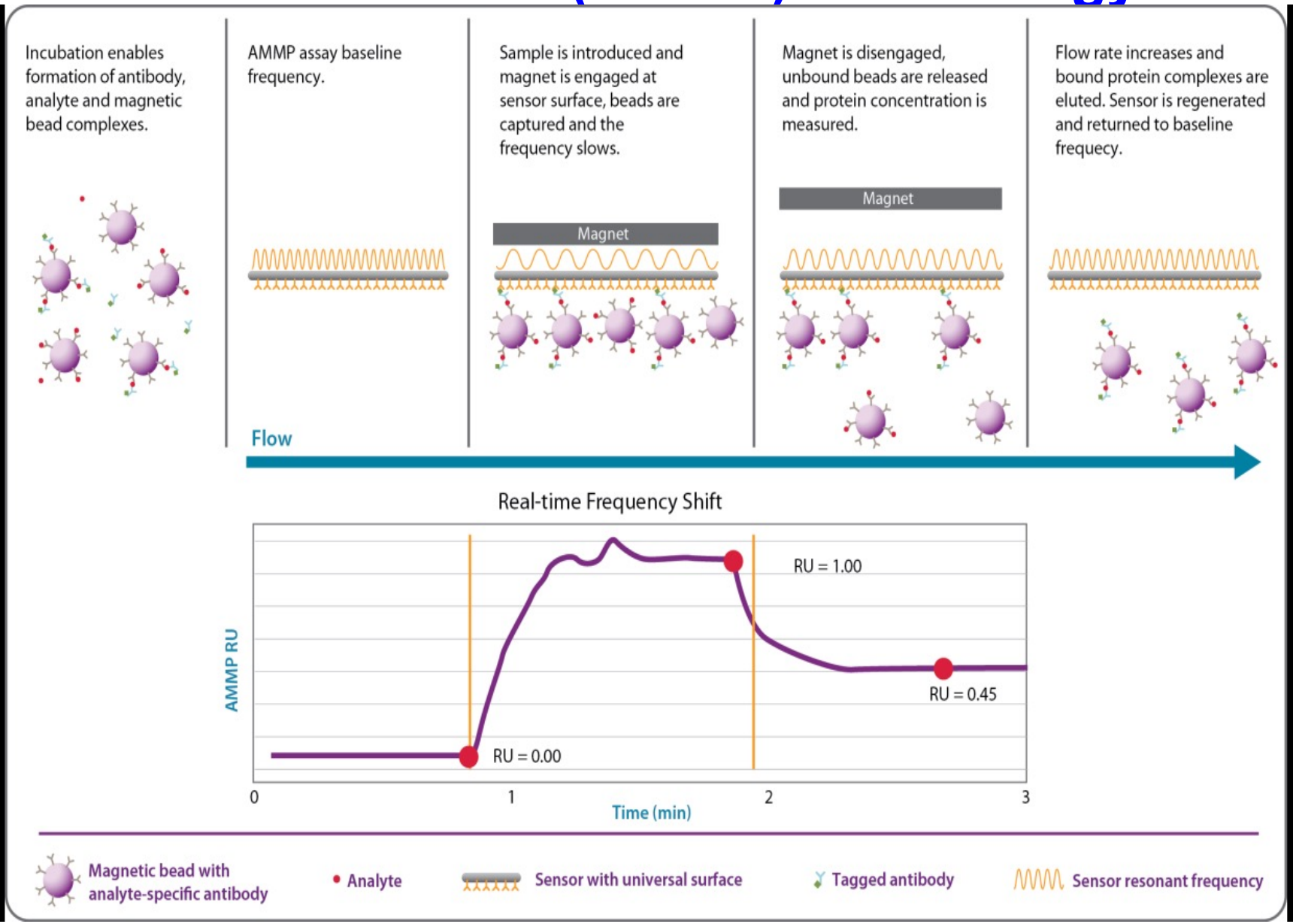
Why do we need a new platform for Anti-PEG antibody testing?

3. Other formats:

- AlphaLISA
- SPR
- Phadia ImmunoCAP

No assay had suitable sensitivity for our lower affinity anti-PEG IgG antibodies in human serum

Introduction to Acoustic Membrane MicroParticle (AMMP) Technology

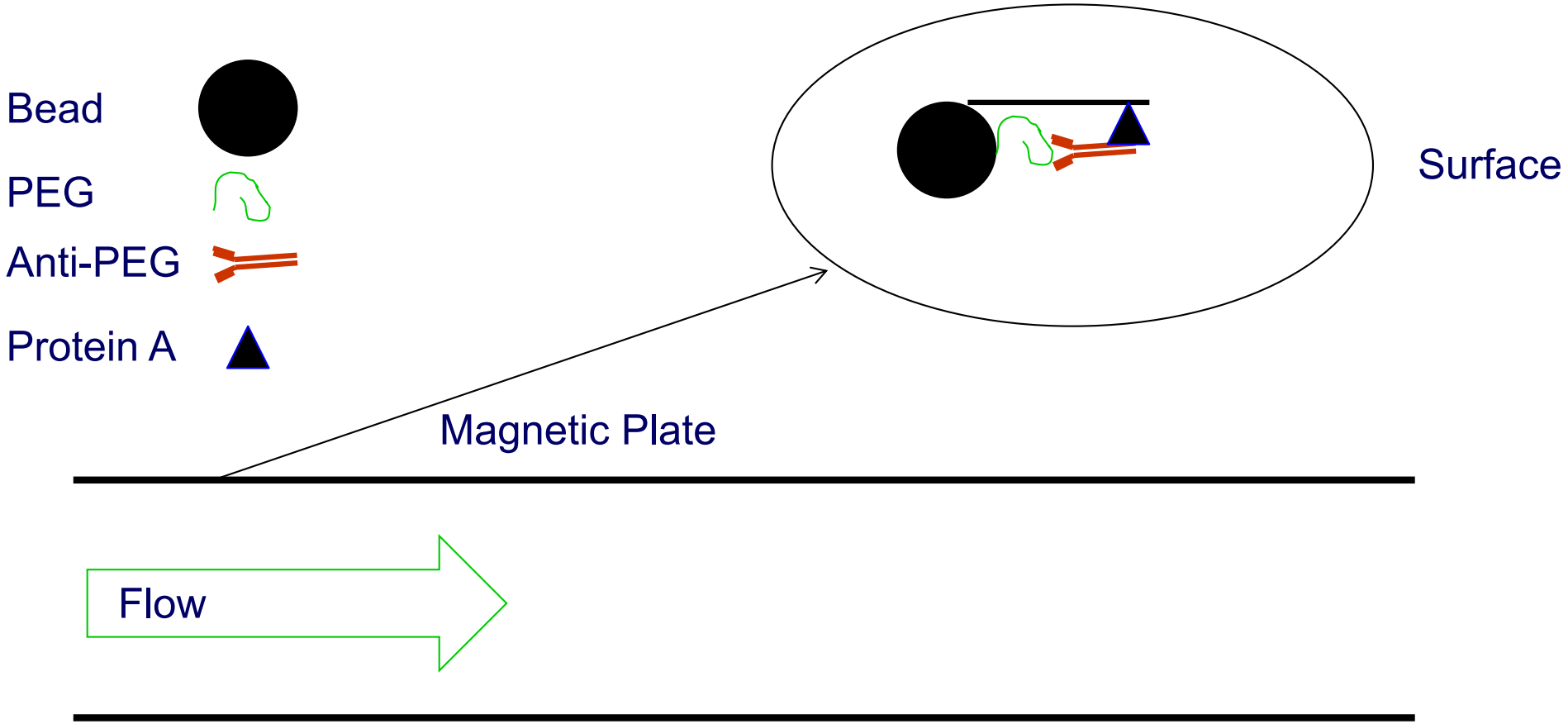


Instrument Advantages

- Good precision
- Short assay time
- Load and go
  - Automated assay steps
  - Time controlled reagent addition
  - Run 1 – 3 plates in an experiment unattended
- Low reagent consumption
- Detect low affinity or weak protein-protein interactions

Bristol-Myers Squibb

Essentially a direct assay but interference from non-specific IgG in serum in minimal at surface



Assay Method Development:
Labeling Magnetic Beads

Label Magnetic Beads

1. Label beads with PEGylated protein (epoxy chemistry)

Pro:

Easy to work with; solubility, characterization, concentrations defined for single site labels of PEG. Labeling options with amino acids.

Con:

Cross reactivity to drug sequence. Non-general assay format for multiple compounds. Specificity subject to subject not able to be assessed.

Assay Method Development:
Labeling Magnetic Beads

Label Magnetic Beads

2. Label beads with PEG directly (streptavidin beads)

Pro:

No protein cross reactivity possible. General format assay used across programs.

Con:

Solubility or non-covalent binding issues. Blocking buffer (albumin, casein) optimization complicated. Requires biotinylation of PEG

Optimized Assay Procedure

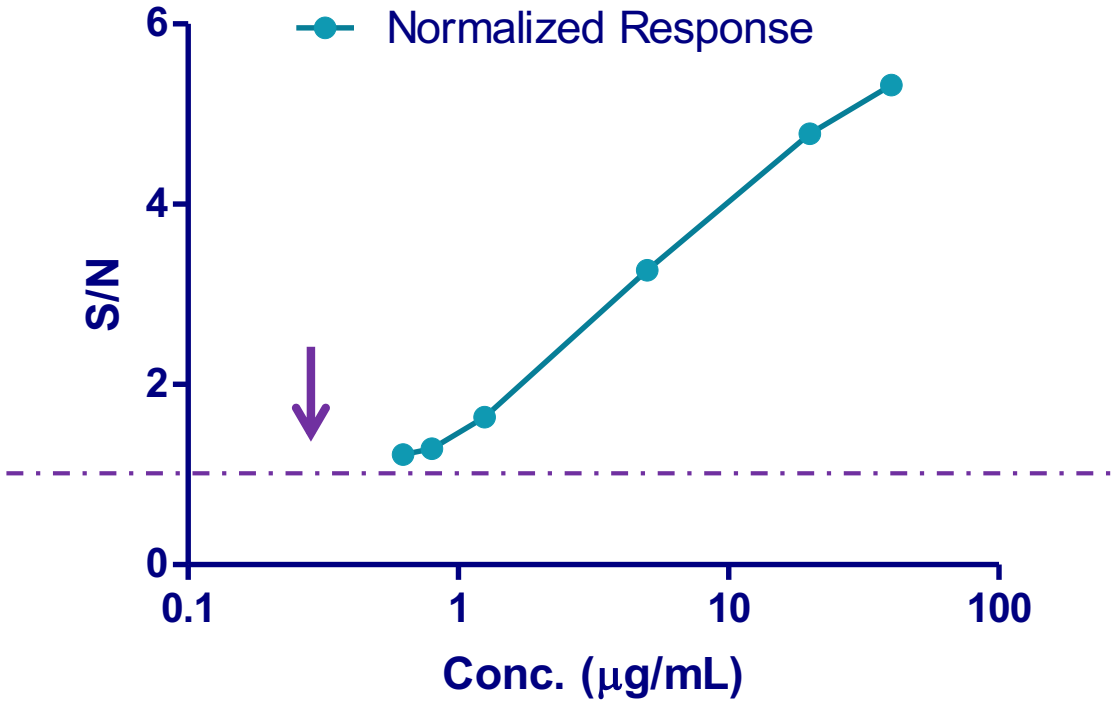
- Calibrators were prepared by spiking PEG.2 positive control into the normal human serum pool at 0.625 to 40 µg/mL and stored at -70° C for 24 hr prior to use. The spiked samples were thawed and diluted 10-fold in Blocker Casein in PBS.

- Biotin-PEG 20 kDa labeled beads at 20 µg/mg were first diluted in Blocker Casein in PBS to a concentration of 4.5×10^5 beads/mL and incubated for 1.5 hour at room temperature on a Hula Mixer.

- 80 µL of each calibrator in 10% serum was combined with 40 µL of bead solution in a 96-well polypropylene plate and incubated for 1 hour on the ViBE instrument integrated shaker.

- Once the incubation was complete, the online assay steps initiated and data were collected by the ViBE software version 0.7.4.14126.

Results: Response curve (Mab) and relative assay sensitivity



- Data were normalized to the negative control value for each standard.
- The observed sensitivity was 800 ng/mL based on cut point calculation

  with the formula of ($1.645 \times SD_{Mean} + Pool_{mean}$).

Bristol-Myers Squibb

Results: Assay Reproducibility

Assay Reproducibility

	Nominal Concentration (µg/mL)						
Statistic	Std1 40.0	Std2 20.0	Std3 5.0	Std4 1.3	Std5 0.8	Std6 0.6	Std7 0.0
N	12	12	12	12	12	12	12
Mean AMMP Signal	0.822	0.725	0.461	0.218	0.182*	0.159	0.14
SD	0.017	0.026	0.065	0.048	0.023	0.042	0.019
Normalized Signal	5.87	5.18	3.29	1.56	1.30	1.14	1.0
Intrabatch (%CV)	0.0	0.1	0.7	1.2	1.2	1.8	1.7
Interbatch (%CV)	0.0	0.1	1.3	3.9	2.9	6.7	6.4
* Cut point = 0.171							

- The calibrators were run a total of 12 times using one plate per day containing six replicate sets for each of two days.
- Inter-plate and intra-plate variability for the replicates were within 6.7 % CV and 1.8% CV, respectively.

Assay Method Optimization:
What is a Negative / Naive Sample?

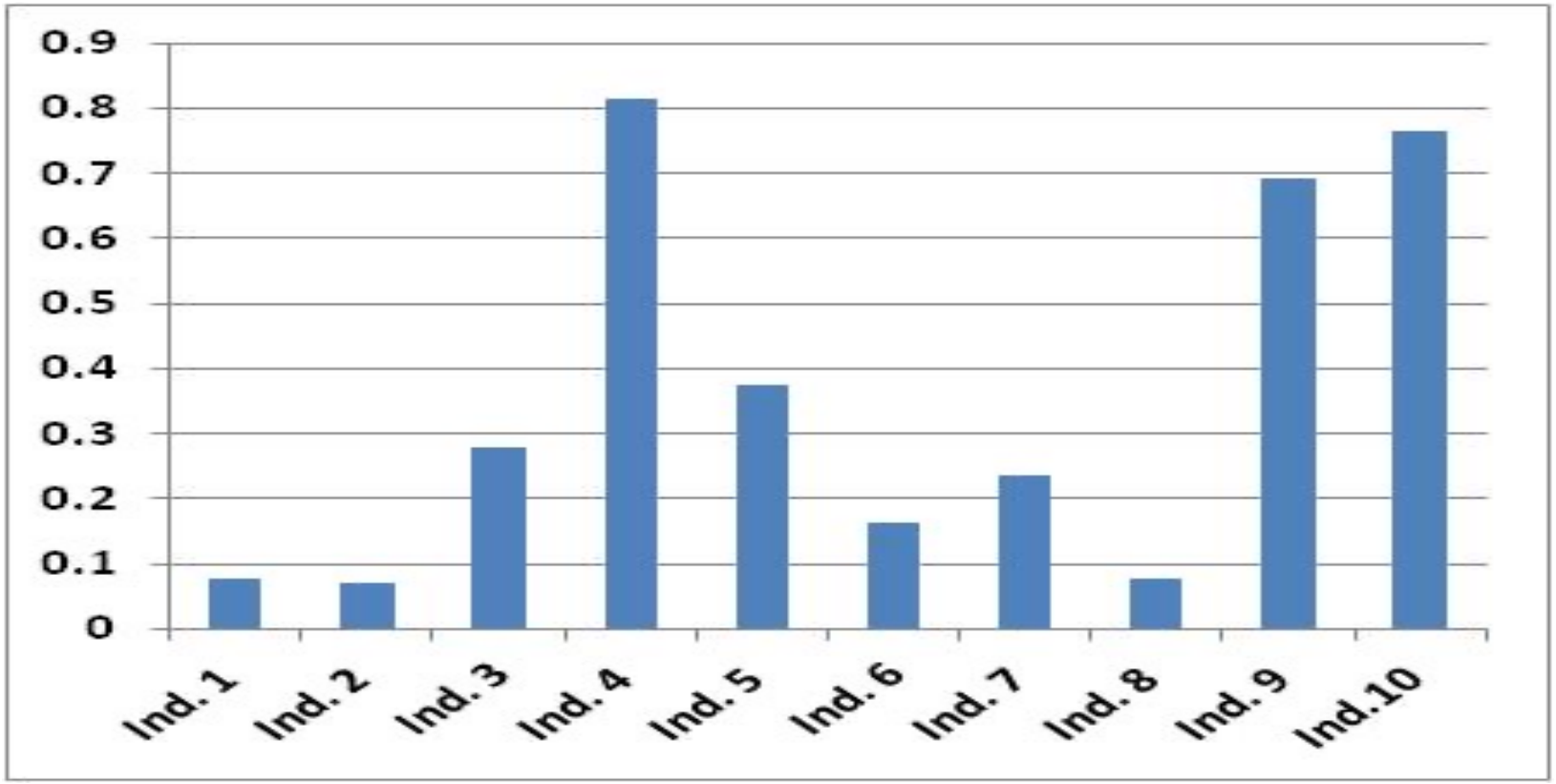
For a Drug, this may be easy question to answer: Any subject not previously exposed to drug.

For PEG, virtually impossible question to answer: Huge number of products (lip balm, shampoo, cosmetics, toothpaste, ink jet printers, food grade anti-foam). Virtually everyone in a modern society has been exposed to PEG.

Assay Method Optimization:
What is a Negative / Naive Sample?

Ten purchased normal healthy individuals (serum)

Note: Instrument read out is ratio and 1 is maximum response



Bristol-Myers Squibb

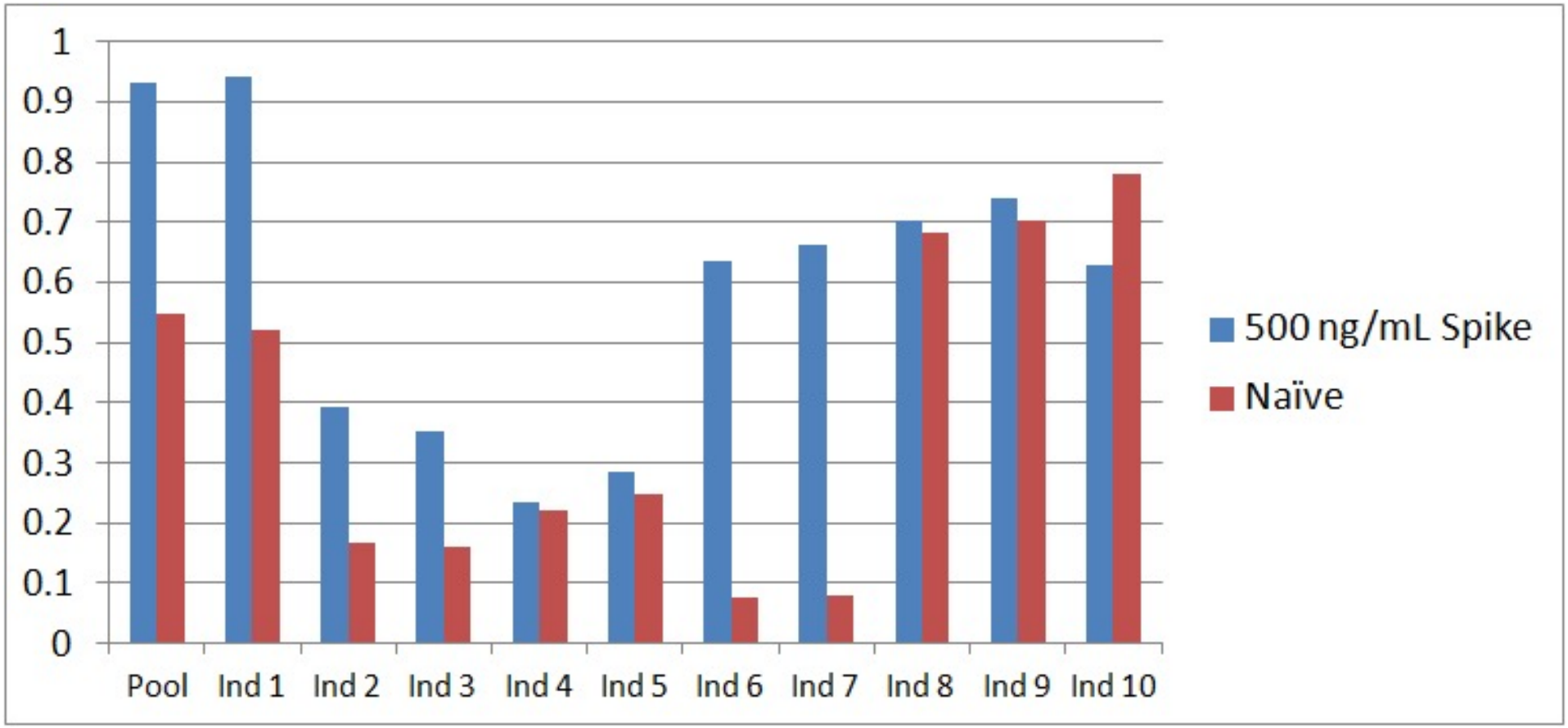
Assay Method Optimization: Interference (Drug Tolerance)

For a Drug, this may be easy question to answer: Pre-treatment of purchased subject serum will not have drug. For enhancement therapy of constitutively expressed protein, levels may be low as to not interfere with IgG detection.

For PEG, virtually impossible question to answer: Huge number of products (lip balm, shampoo, cosmetics, toothpaste, ink jet printers, food grade anti-foam). Virtually everyone in a modern society has been exposed to PEG.

Assay Method Optimization: Interference (Drug Tolerance)

Purchased Pool and 10 individuals



Bristol-Myers Squibb

Assay Method Optimization: Interference (Drug Tolerance)

Purchased Pool and 10 individuals

Bristol-Myers Squibb

Conclusions

- A generic Acoustic Membrane MicroParticle assay to detect anti-PEG antibodies in human serum has been successfully developed
  - Observed sensitivity in human serum sample is 800 ng/mL with our lowest avidity positive control.
  - Inter-plate and intra-plate variability for the replicates are within 6.7 % CV and 1.8% CV, respectively.
  - Assay is specific to detect anti-PEG antibody. Assay signal is depleted when increased concentration of free PEG is added to sample.
- Benefits of ViBE assay over conventional ELISA
  - The assay reaction is in homogeneous environment. No off line wash step to wash off low affinity antibodies.
  - In the detection step, antibody complexes are magnetically captured on sensor surface and separated from other matrix components present in the sample, therefore reducing matrix interference and non-specific binding.

Future Work

- Scheme to positively identify samples with pre-exisisting anti-PEG antibodies
- Obtain pool / group of subjects with low levels of PEG or anti-PEG antibodies to use as controls
- Develop senstivity PEG assay to determine subjects PEG level in serum
- Validate assay (with existing limitations) and test clinical samples.