

# Using Ligand-Binding Assay Sensitivity for Improved Matrix Tolerance and Related Parameters by Tailored Sample Dilution.

*Dr. Mark Spengler, Chimera Biotec*

EBF 3rd Open Conference

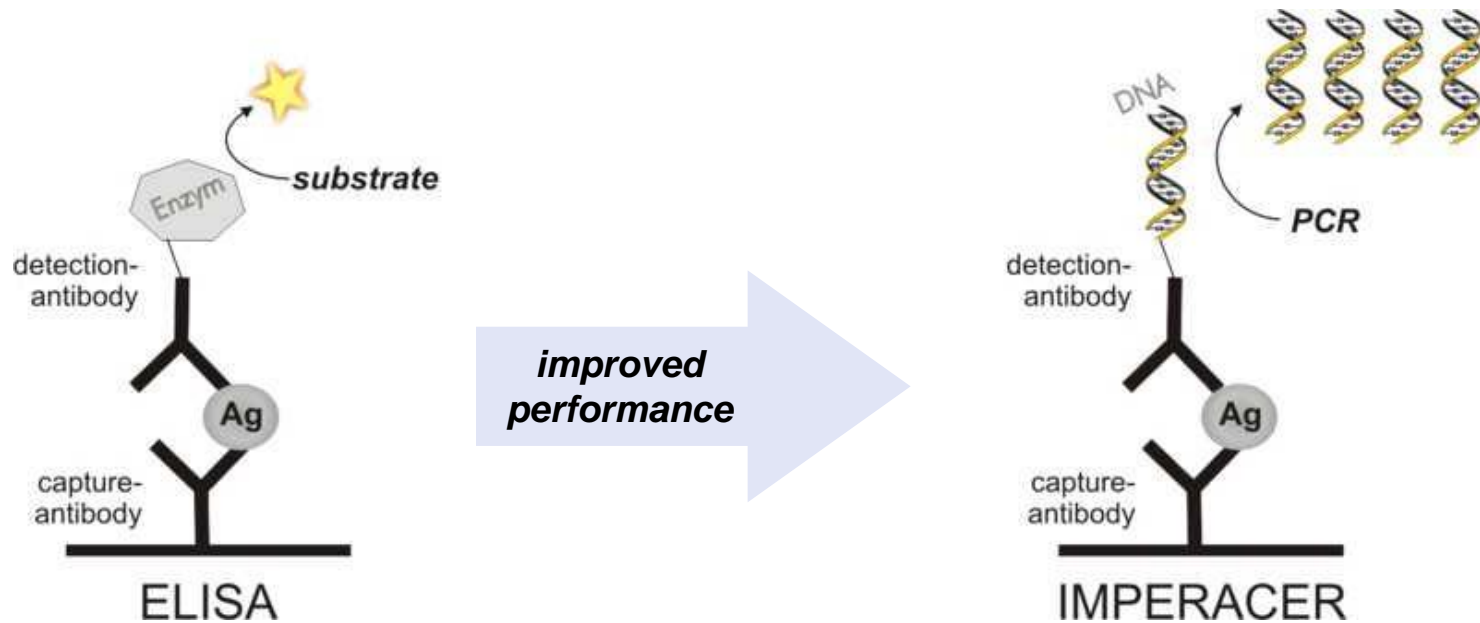
From Challenges to Solutions

EBF

European  
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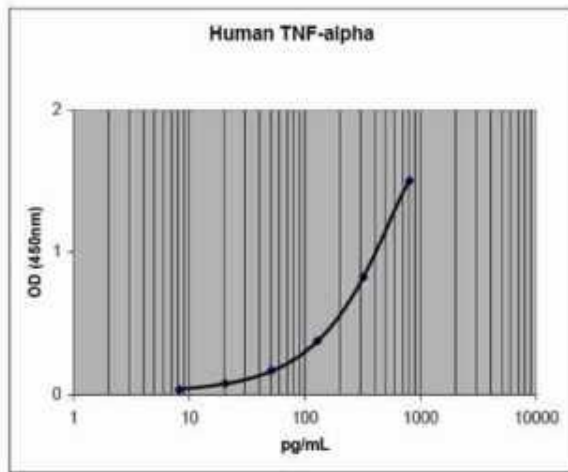
## Company Overview

- Bioanalytical CRO with proprietary platform
- Focus: Ultra-sensitive LBA's
- Founded 2000
- Marketing Imperacer<sup>®</sup> since 2004
- Collaboration with **PRA International** for GLP Studies
- Revenues: 50/50 (EU/US)



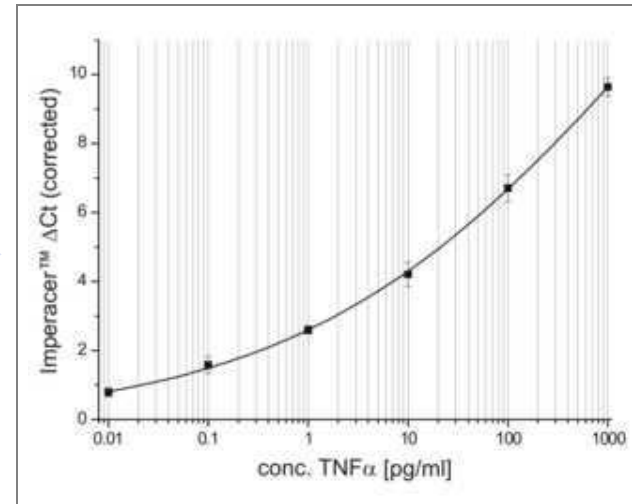
- ⇒ **Antibody-DNA Conjugate replaces Antibody-enzyme Conjugate**
- ⇒ **Processing & Read-out by real-time PCR**

## Biosource TNF $\alpha$ ELISA kit:



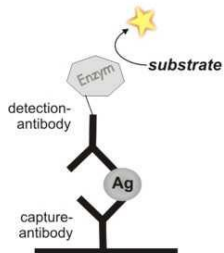
improved  
performance

## Chimera Biotech TNF $\alpha$ Imperacer<sup>®</sup> kit:

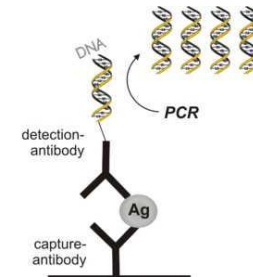


LOD: 10 pg/ml  
(40,000,000 molecules absolute)

LOD: 0.01 pg/ml  
(12,000 molecules absolute)

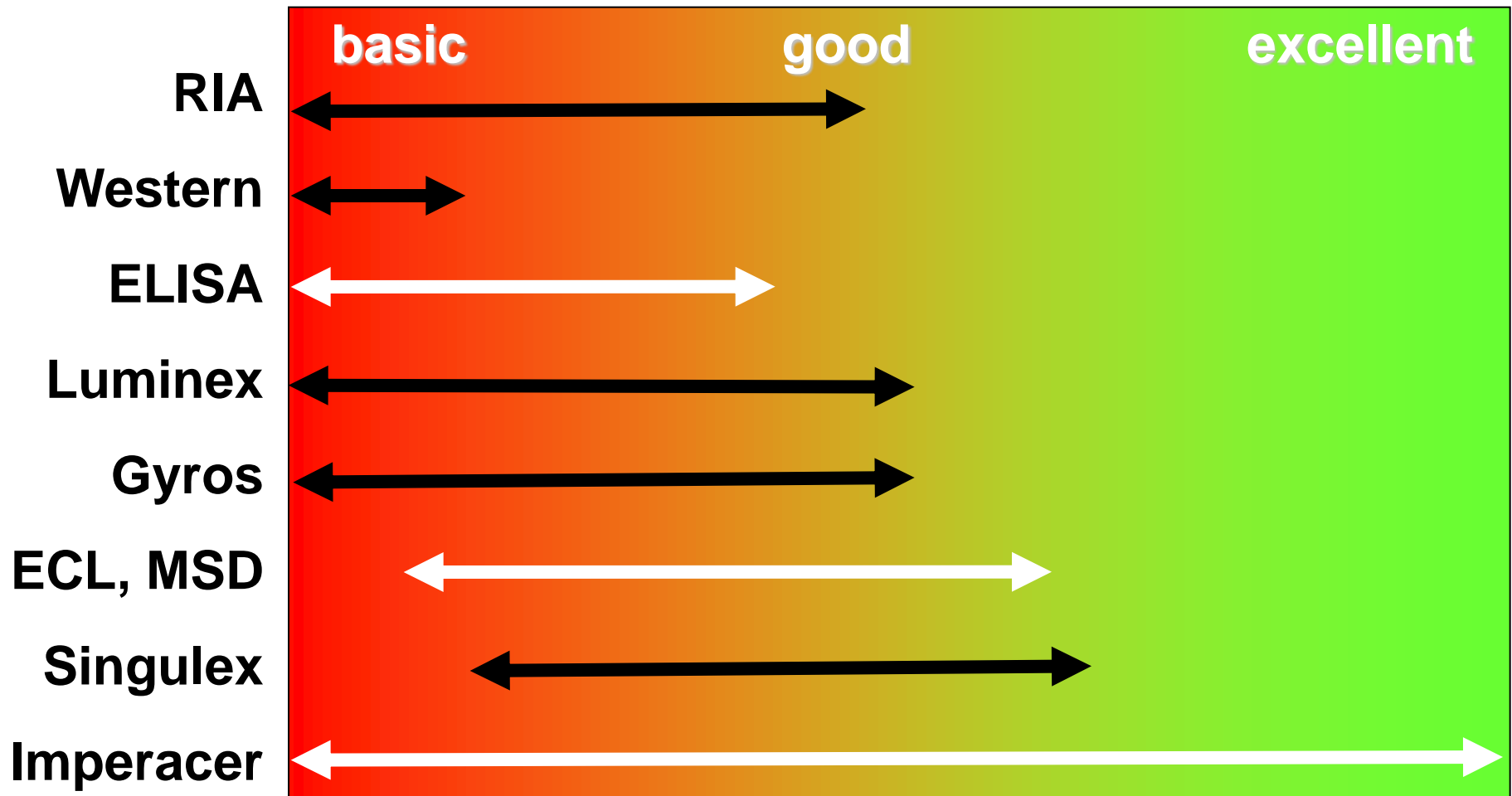


Using the same set of  
antibodies

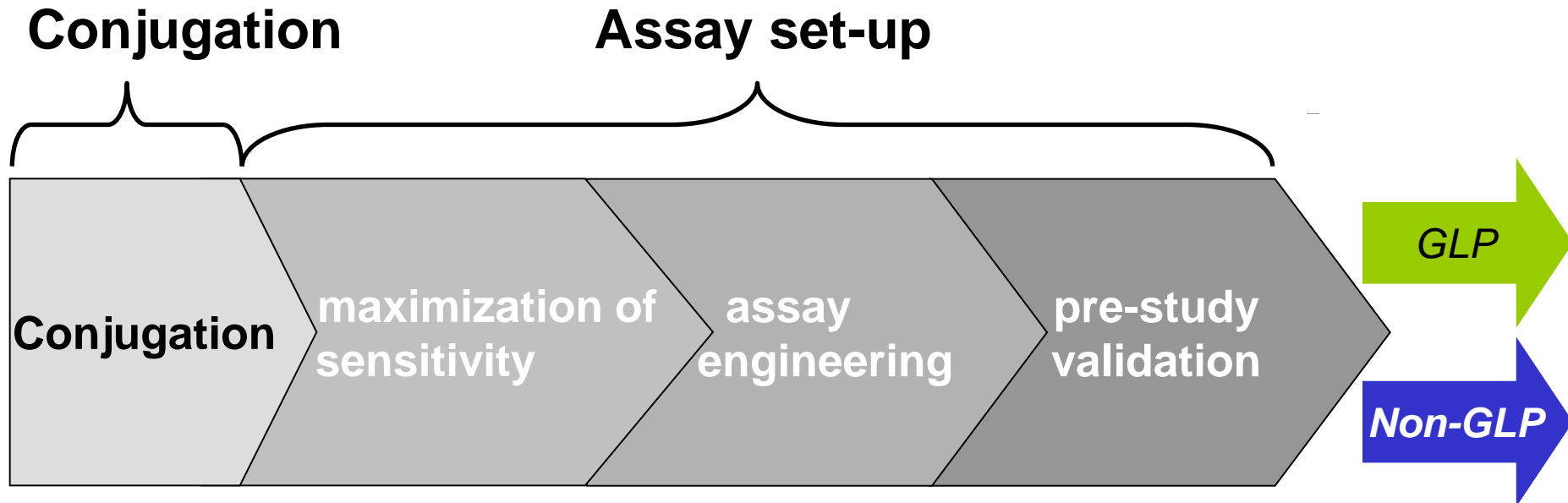


# Comparison of Platforms

## Assay Sensitivity



⇒ Choose the appropriate platform for your assay

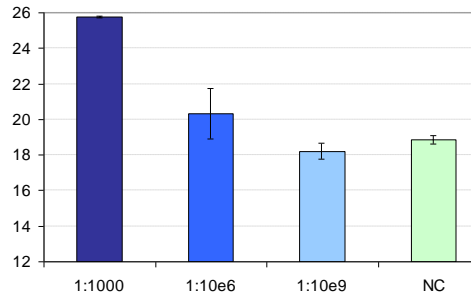
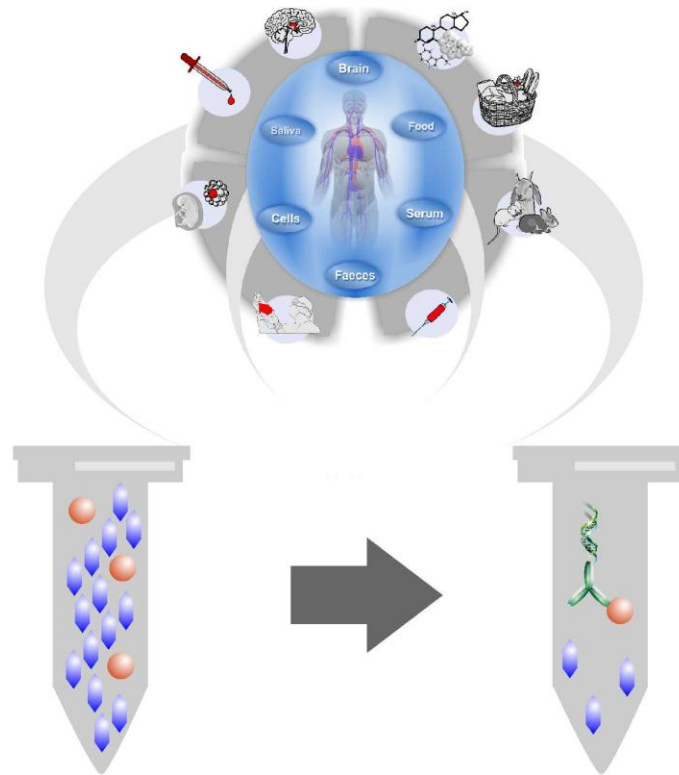


⇒ **Option 1: Preclinical BA @ Chimera Biotec (non-GLP)**

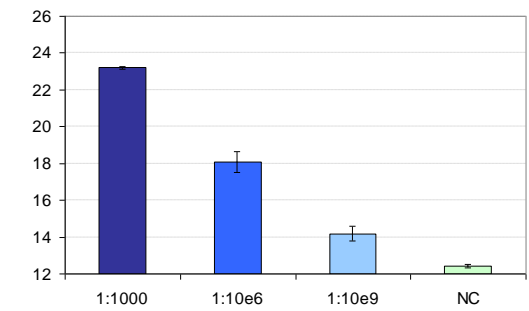
⇒ **Option 2: Clinical BA @ PRA-International (GLP)**

⇒ **Option 3: BA in-house (non-GLP / GLP): Imperacer® Instrumentation**

# Minimizing Matrix Effects

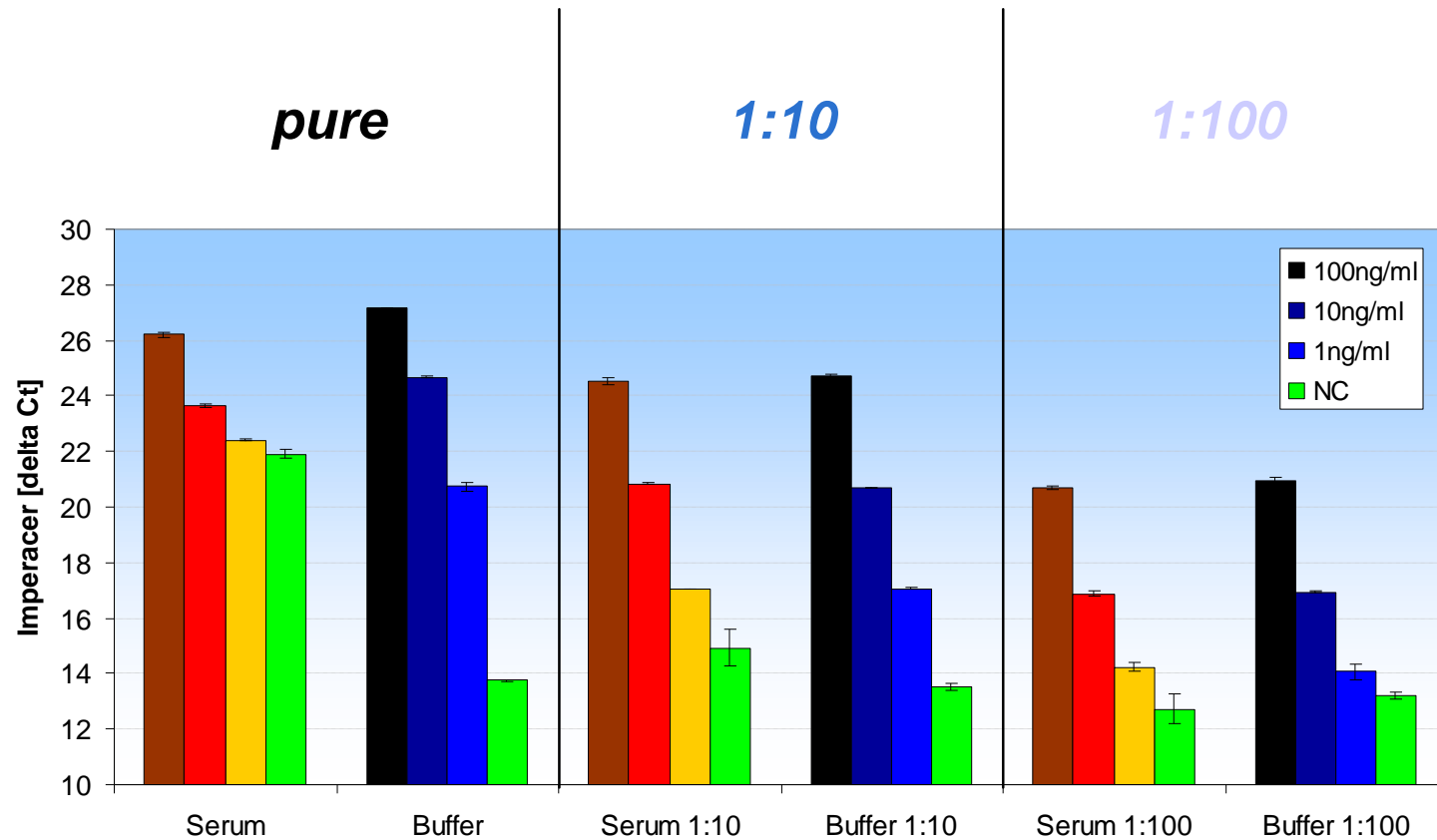


Biological Sample:  
Matrix compounds (♾) interfere with target (●) detection



Sensitive IPCR target detection after sample dilution

... through simple sample dilution

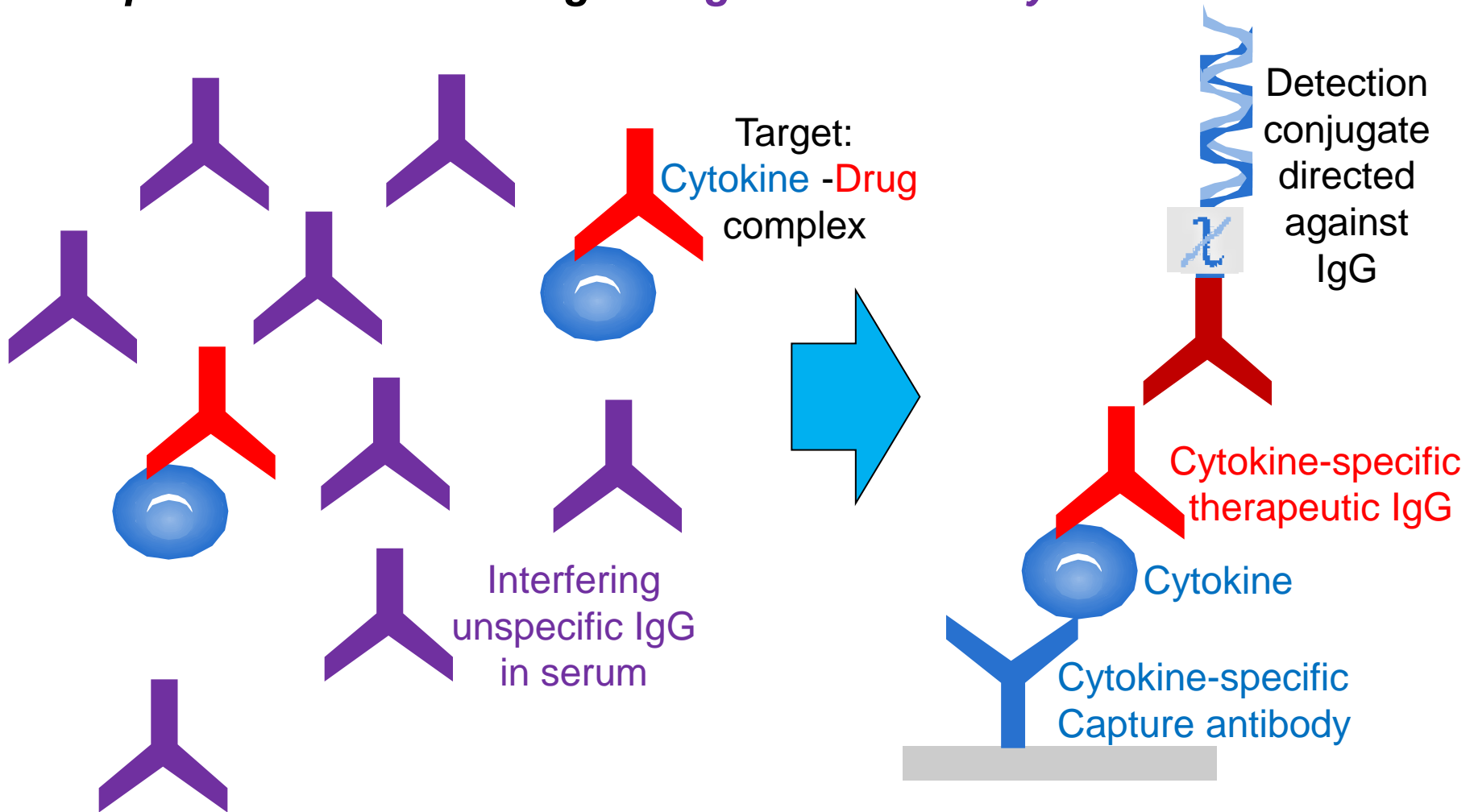


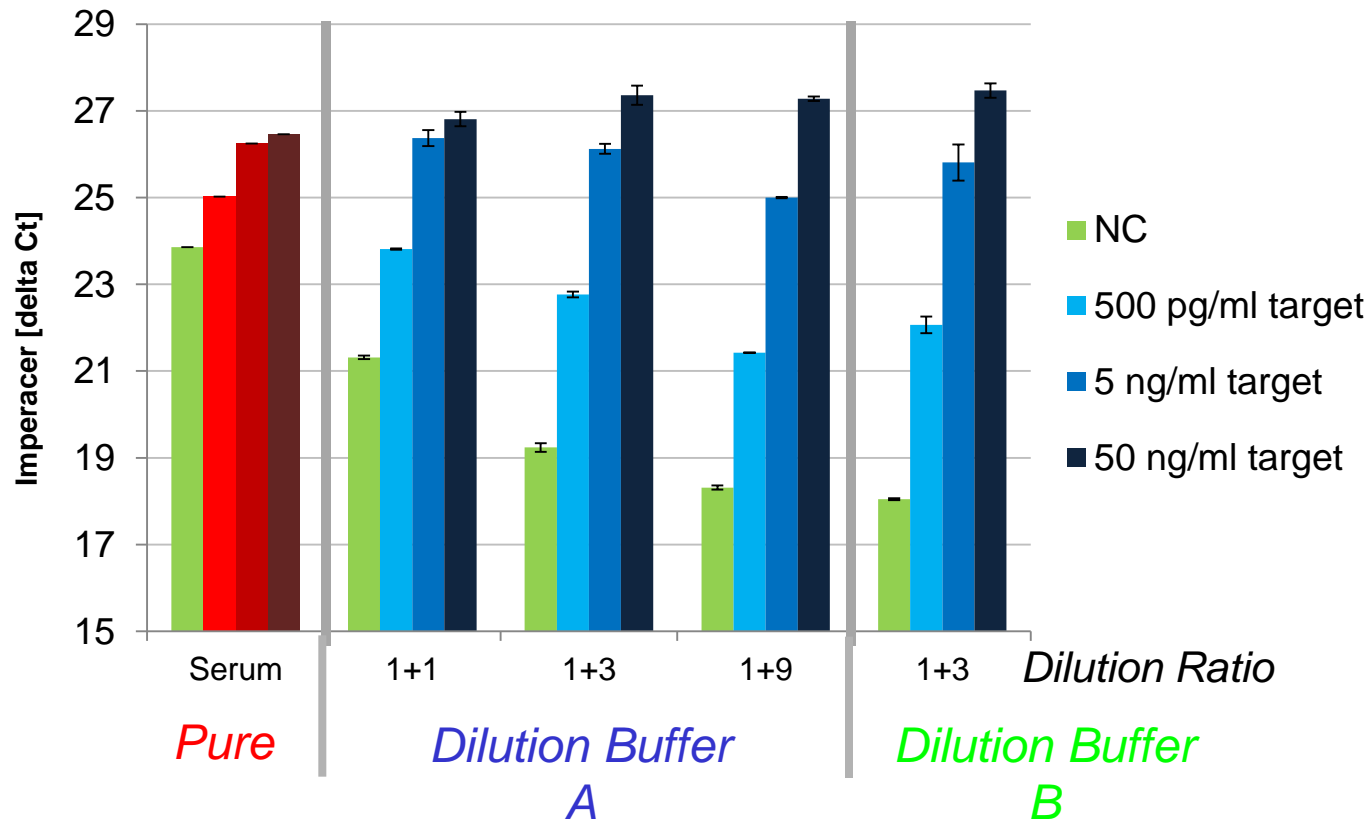
⇒ **Minimizing Matrix Effects through Dilution**



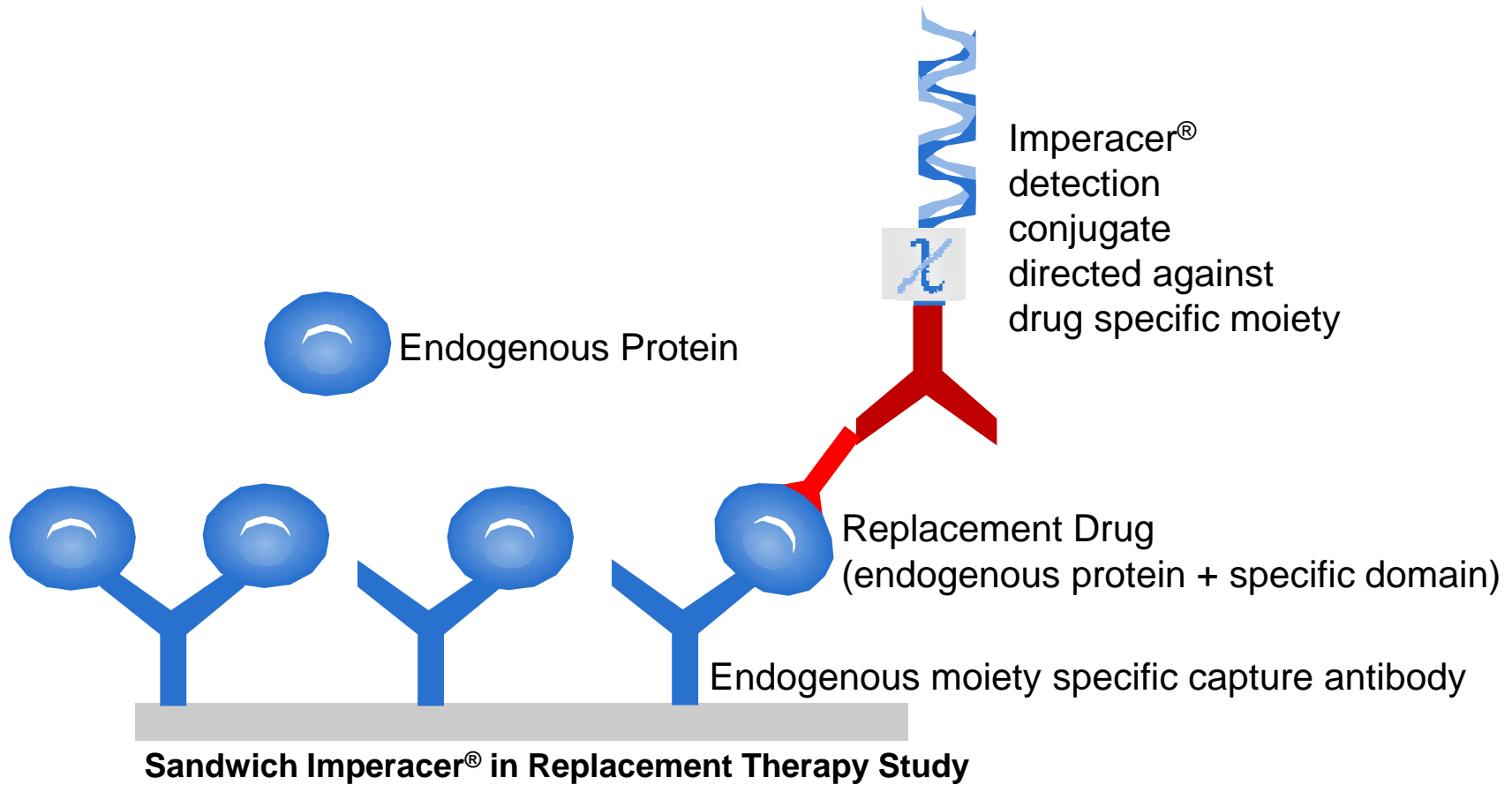
1. Anti-cytokine Drug
2. Replacement Therapy
3. Immunogenicity
4. Clinical Study enrollment

## Detection of a **Cytokine-Drug** complex in the presence of interfering **endogenous antibody**



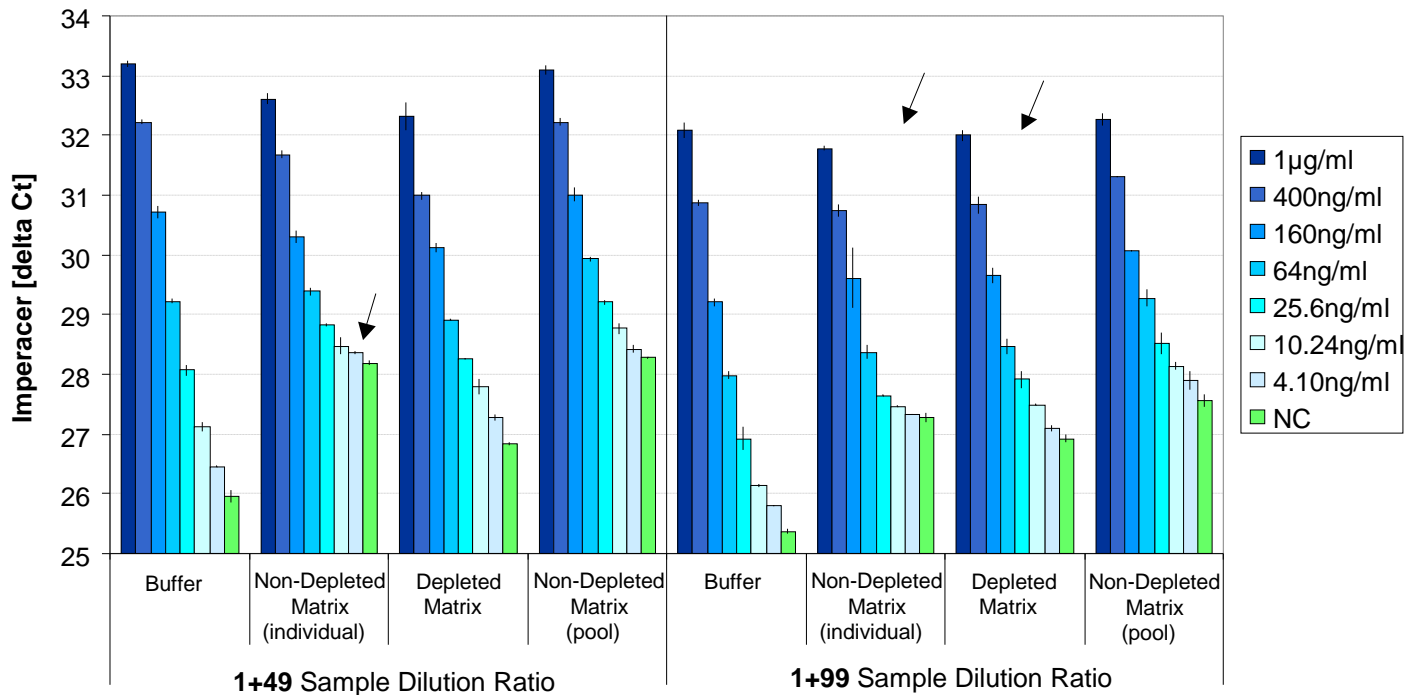


- ⇒ Tailored sample dilution enables massive background reduction.
- ⇒ Maximizing Performance by appropriate sample dilution buffer
- ⇒ Current LOD: 1 pg/ml; aiming at sub pg/ml levels of the Cytokine



## The Challenge:

At Standard dilution ratio either endogenous protein or unspecific antibodies saturate the capture plate

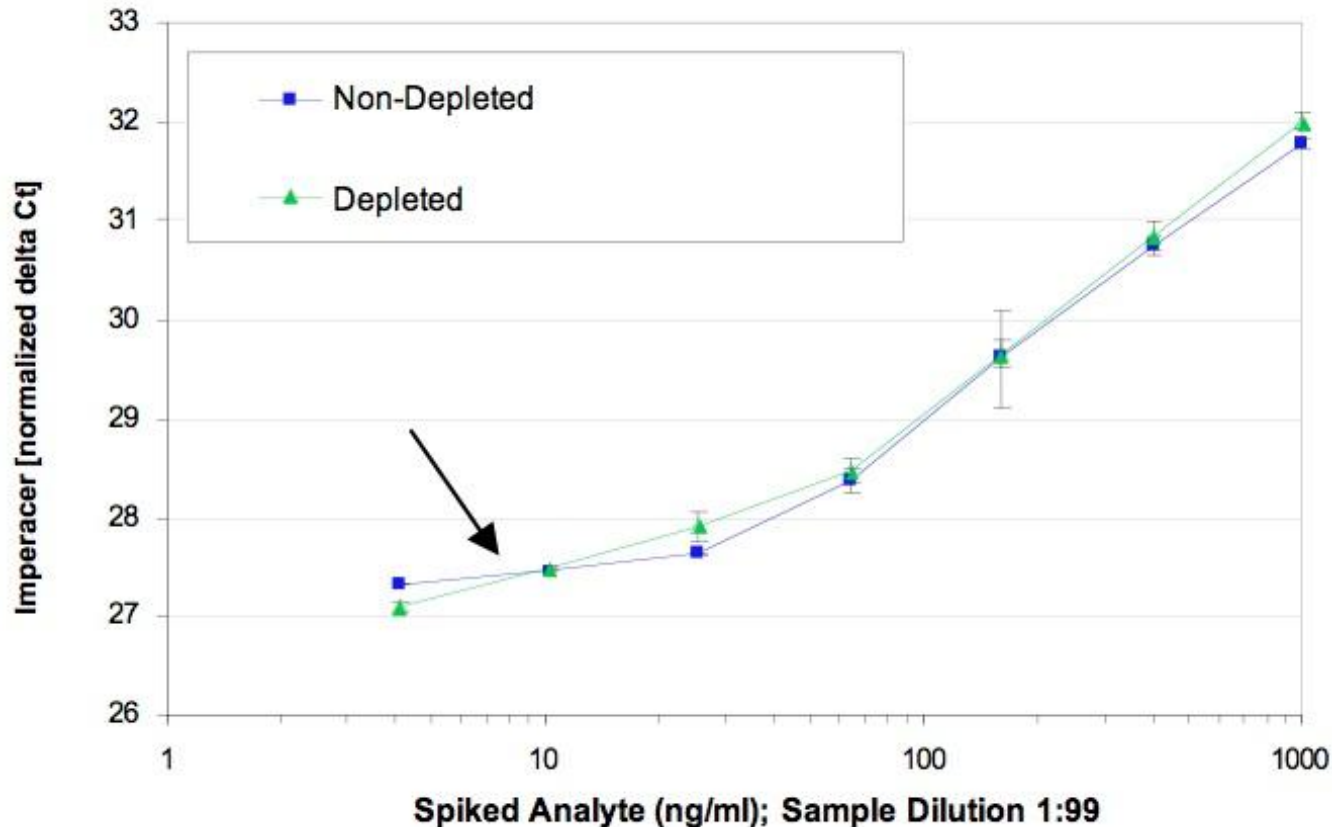


1 : 50

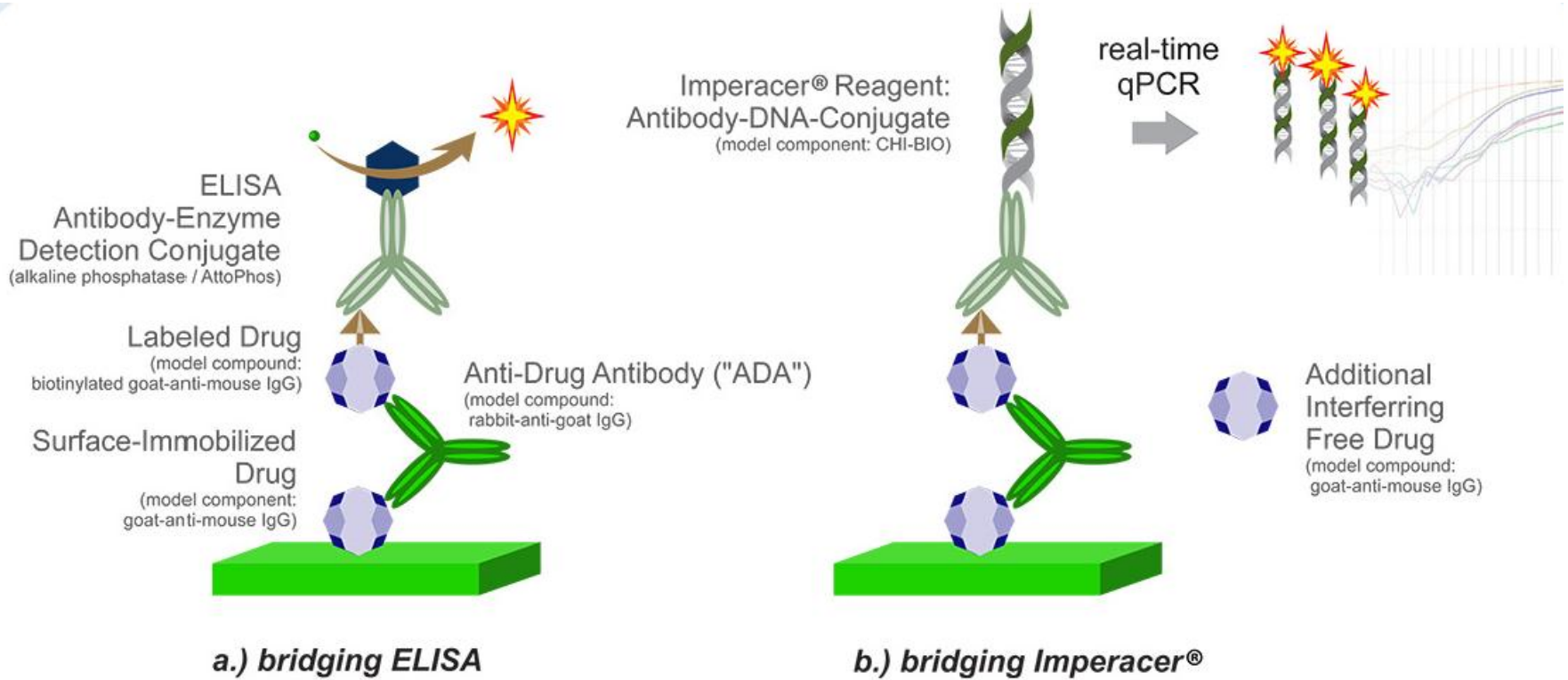
1 : 100

*Dilution Ratio*

⇒ High sample dilution in appropriate sample dilution buffer neutralizes interference by endogenous counterpart.



⇒ Samples with unknown conc. of endogenous protein can be quantified against STD curve in depleted matrix or native matrix pool.



Source: Spengler et al., BBRC (2009)

## Comparison of Assay Platforms

|  | <b>ELISA</b>            | <b>MSD</b>              | <b>IMPERACER<sup>®</sup></b> |
|--|-------------------------|-------------------------|------------------------------|
| <b>Sample Volume</b>                                     | 100 µl                  | 100 µl                  | 30 µl                        |
| <b>Sensitivity<br/>Using mAB</b>                         | 70 ng/ml                | 20 ng/ml                | 40 pg/ml                     |
| <b>Drug Tolerance<br/>(mAB : Drug)</b>                   | 1 : 1                   | 1 : 40                  | 1: 1000                      |
| <b>Sample Pre-<br/>Treatment<br/>(Acid Dissociation)</b> | Increased<br>Background | Increased<br>Background | NA                           |
| <b>Matrix Interference</b>                               | +++                     | +                       | NA                           |

⇒ **Sponsor in-house ELISA & ECL assays compared to Imperacer<sup>®</sup>**

⇒ **Same Antibodies & Reagents used**

⇒ **No sample pre-treatment was used in Imperacer<sup>®</sup> procedure**

**From ELISA to a novel Immuno-PCR (i-PCR) based immunogenicity assay in quest to improve the drug tolerance**  
*Poster presentation at the AAPS NBC 2010,*

Darshana Jani<sup>1</sup>, Joyce Sobolowski<sup>1</sup>, Jeannine Keefe<sup>1</sup>, Michael Adler<sup>2</sup>, and Jaya Goyal<sup>1</sup>

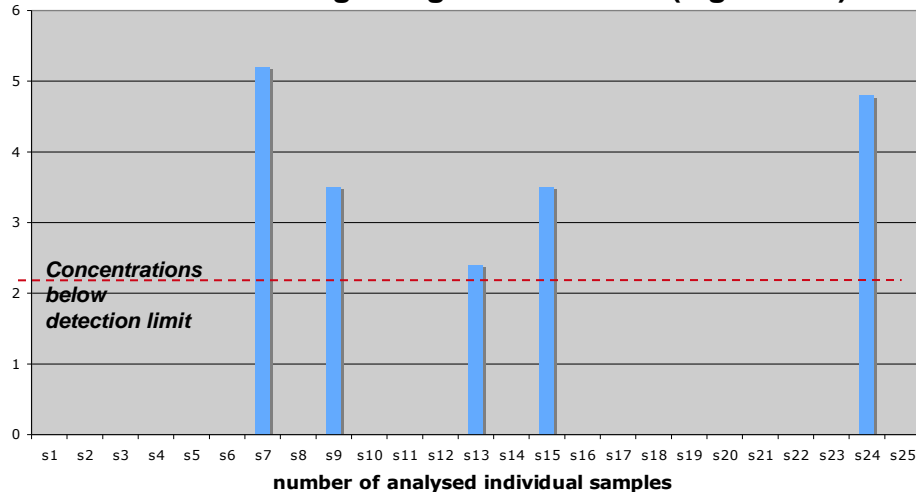
Clinical Science and Technology, Preclinical and Clinical Development Sciences, Biogen Idec Inc. Cambridge, MA<sup>1</sup>

and Chimera Biotec, Dortmund Germany<sup>2</sup>



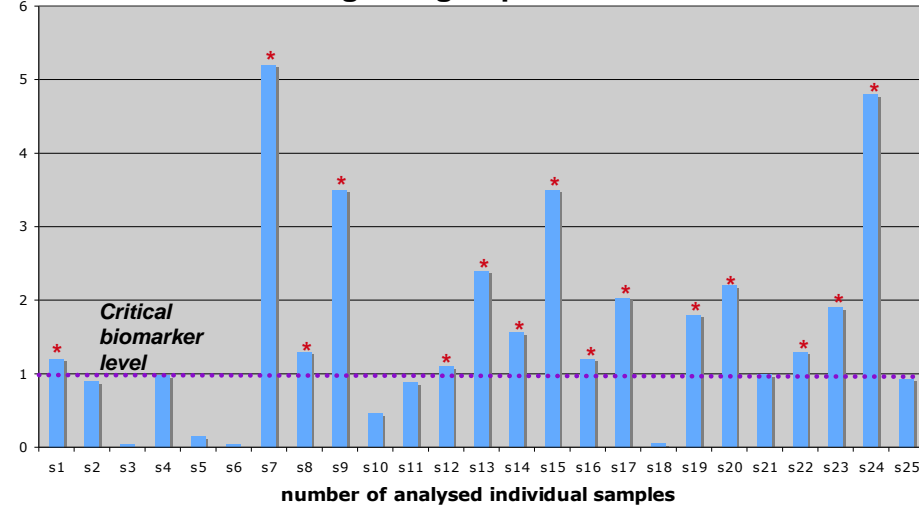
# Study Group Profiling

### Biomarker screening using classical LBA (e.g. ELISA)



- Most concentrations are below detection limit

### Biomarker screening using Imperacer®



- Detection of base level concentrations
- Identification of patients with critical biomarker level

⇒ **Ultra-sensitive patient screening enables dramatic increase in drug responder rates**

⇒ **Option: Companion diagnostics**

**Imperacer<sup>®</sup>**

*Dive deeper into the Proteome*

**Thank You!**



**chimera biotec**  
*ultra sensitive protein detection*

*Contact:*

**Chimera Biotec GmbH**

**Mark Spengler**

*Director of Project Management*

email: [spengler@chimera-biotec.com](mailto:spengler@chimera-biotec.com)

[www.chimera-biotec.com](http://www.chimera-biotec.com)

**PRA International**

**Martin Nemansky**

*Scientific Director*

email:

[NemanskyMartin@PRAintl.com](mailto:NemanskyMartin@PRAintl.com)

[www.prainternational.com](http://www.prainternational.com)