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High Throughput Ligand Binding Assay for Therapeutic Antibodies and Biomarkers Using GYROS Microfluidic Instrument

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Pharmacokinetics & Drug Metabolism

NBC Symposium: Advantages of Non-Traditional Technologies for Ligand-Binding Assay Development (#128)

May 19, 2010

Overview

- Platform selection
- Gyrolab technology
- Reagent
  - Generic human IgG assay
- GYROS PK application
  - Cyno PK of two fusion proteins

Analytical Methods For Protein Analysis

Technology	Pros	Cons
ELISA Colorimetric, Chemiluminesence	Universal, Simple, CRO	Sample volume, Throughput, Dynamic range
Single source and higher cost methods		
Gyrolab	Sample volume, Automated, Dynamic range	CRO
MSD	Universal, Dynamic range, CRO	Sample volume, Throughput
Luminex Technology	Multiplex	Complicated validation, Dynamic range
Biacore/ Kinexa/ Ultracentrifugation	Protein and reagent characterization	Low throughput
Alphalisa	Homogenous, Low affinity	Hook effect
Imperacer	Sensitive	Dynamic range, CRO
Octet	Universal	Sensitivity, CRO
LC-MS	Multi-analytes	Sensitivity, Lack of proper internal standard

Analysts have an increasing number of options to consider

Platform Selection Considerations

- **Platform Selection**
  - **Long term support**
    - **Supply source**
    - **Vendor support**
  - **Instrumentation**
    - **Validation and compliance**
    - **User friendly**
    - **Troubleshooting**
  - **Method development**
    - **Time**
  - **Method transfer (CRO)**
  - **Cost**

Bioanalytical method needs to be carefully selected

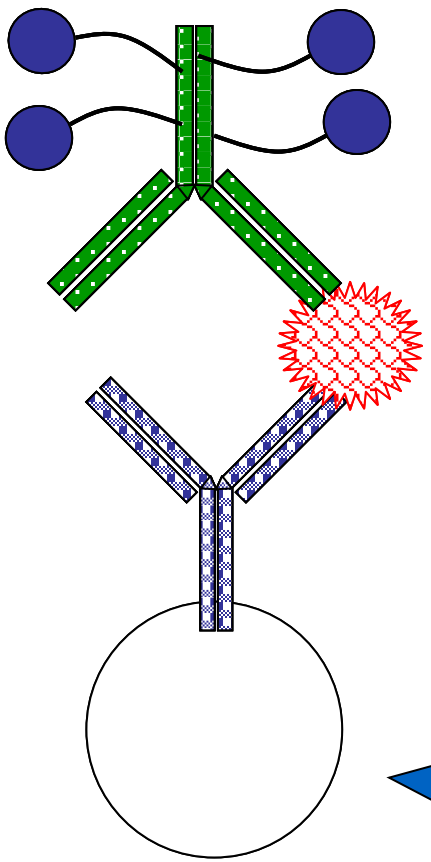
GYROS Instrument

Gyrolab Bioaffy CD

Bioaffy 200 and 20 have 112 microstructures in each CD.

Hossein Salimi-Moosavi, NBC10, May 19, 2010

Integrated Ligand Binding Assay on µ-Column

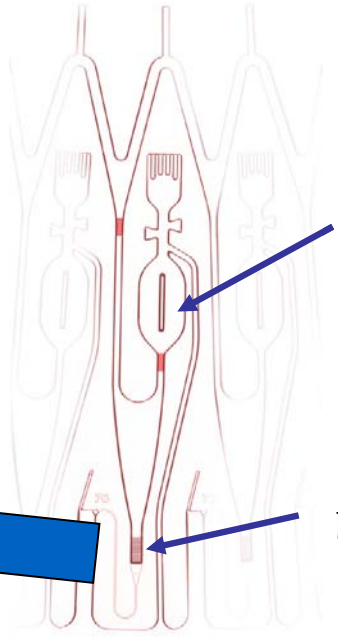


Streptavidin-
Coated Beads

Alexa Fluor647

Excitation at 633 nm

Emission: at 668 nm



Sample chamber
(200 nl)

µ-column (15 nl)

Gyrolab Technology Advantages

- Small sample volume (200 nL)
- Automated
  - High precision (low CV)
- Wide dynamic range
  - Less sample dilution
- Assay development time
- High throughput
  - Up to 5 CD run

Gyrolab Technology Disadvantages

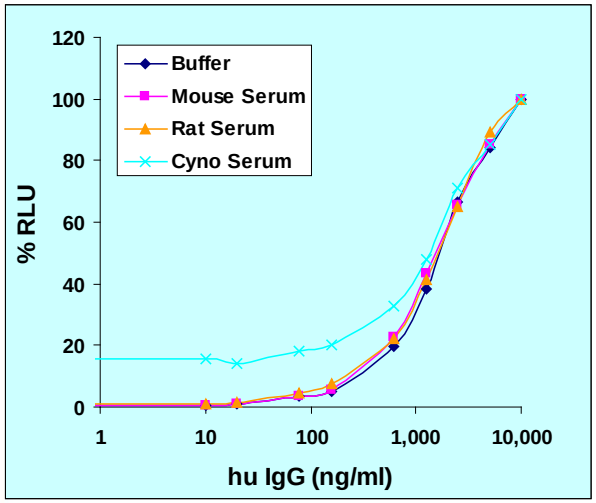
- Single source (Long term support)
- Software (limited applications)
- CRO assay transfer
- Troubleshoot
- Validation (only for research support)
- Cost
- Carryover

Reagents

- **Reagent quality**
  - **Assay quality depends on reagents**
- **Reagent screening**
  - **Specificity**
  - **High affinity**
  - **Low non-specific binding**

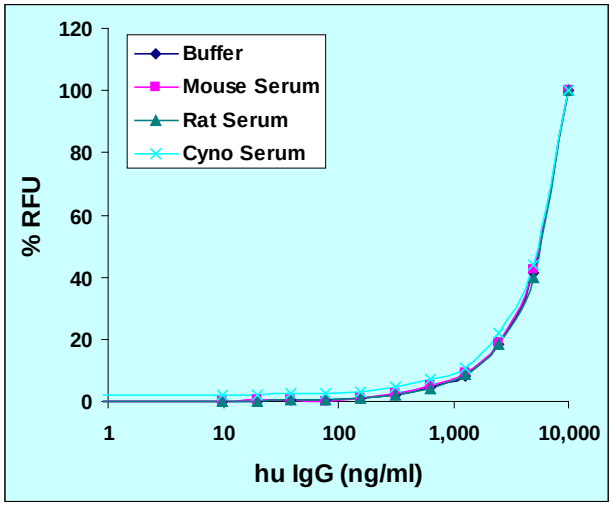
Assay can only be as good as the reagents.

Human IgG Assay in Different Platforms



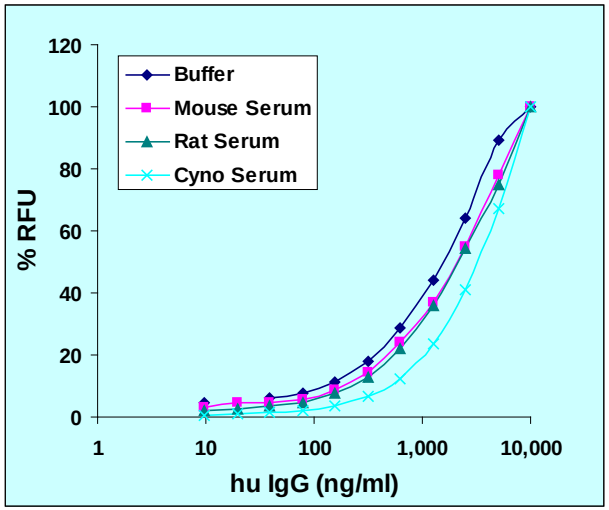
ELISA

	Assay Range
Buffer	10 to 10,000 ng/ml
Mouse	10 to 10,000 ng/ml
Rat	10 to 10,000 ng/ml
Cyno	40 to 10,000 ng/ml



GYROS

	Assay Range
Buffer	20 to 10,000 ng/ml
Mouse	20 to 10,000 ng/ml
Rat	20 to 10,000 ng/ml
Cyno	40 to 10,000 ng/ml

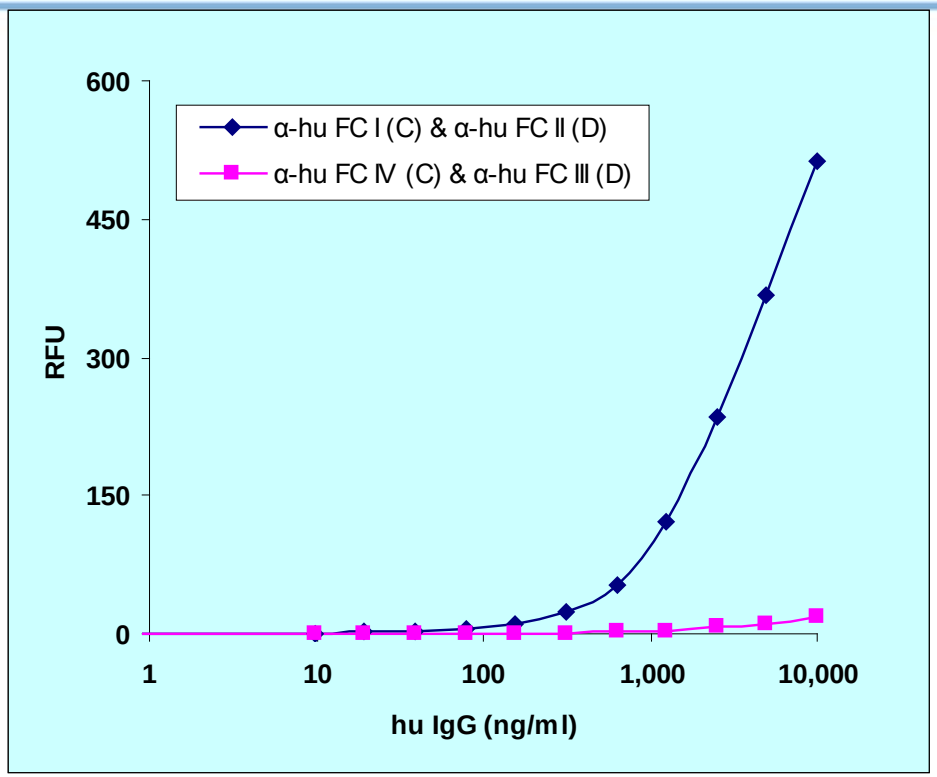


Alphalisa

	Assay Range
Buffer	10 to 10,000 ng/ml
Mouse	10 to 10,000 ng/ml
Rat	20 to 10,000 ng/ml
Cyno	10 to 10,000 ng/ml

Assay performance depends on more to reagents than method.

Generic Human IgG Assay Using GYROS



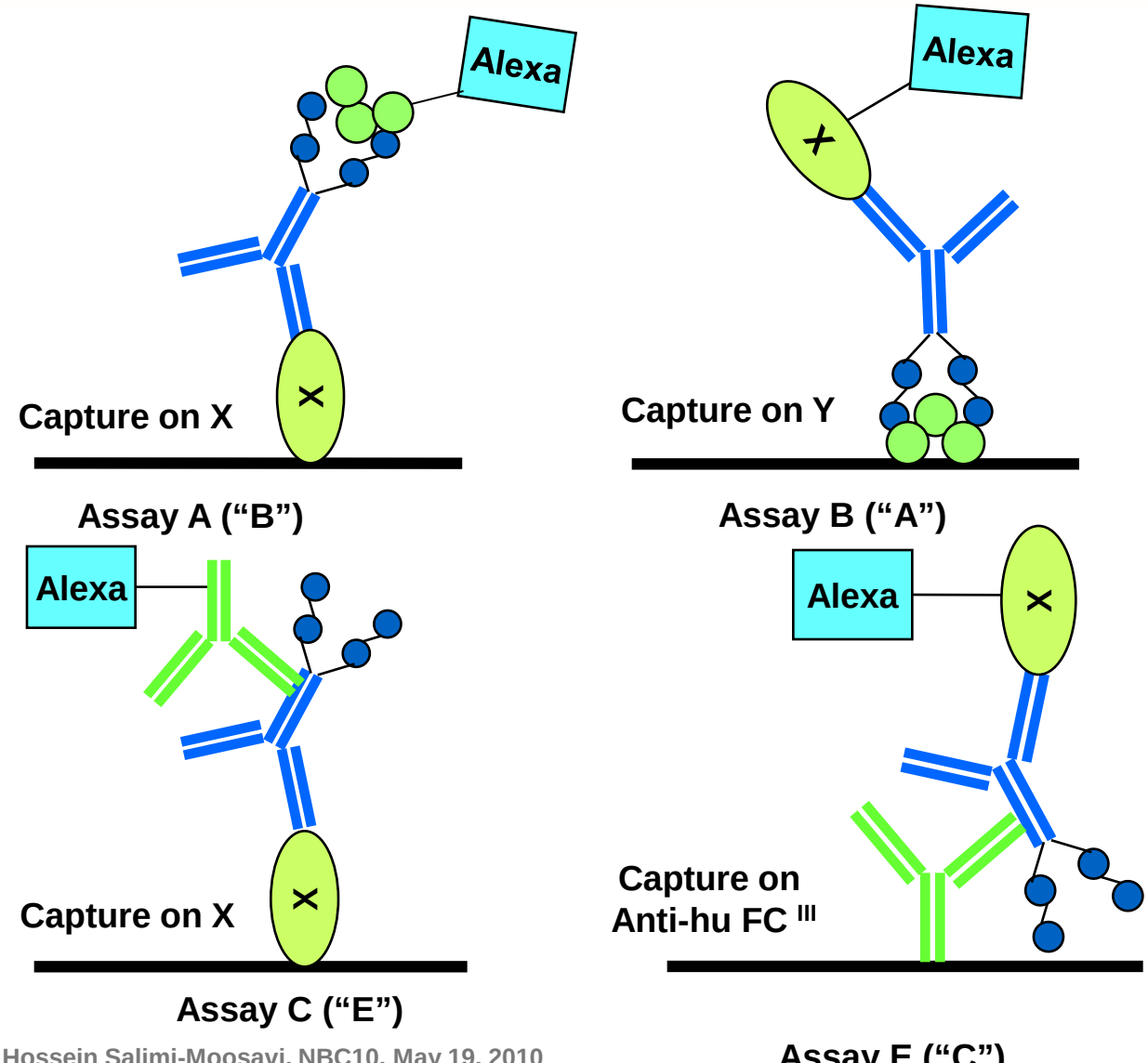
α-hu FC I	High affinity with minimal cross reactivity with Cyno IgG
α-hu FC II	Good affinity with some cross reactivity with Cyno and Rat IgG
α-huFC III	Cross reactivity with Cyno and Rat IgG
α-hu FC IV	Cross reactivity with Cyno and Rat IgG

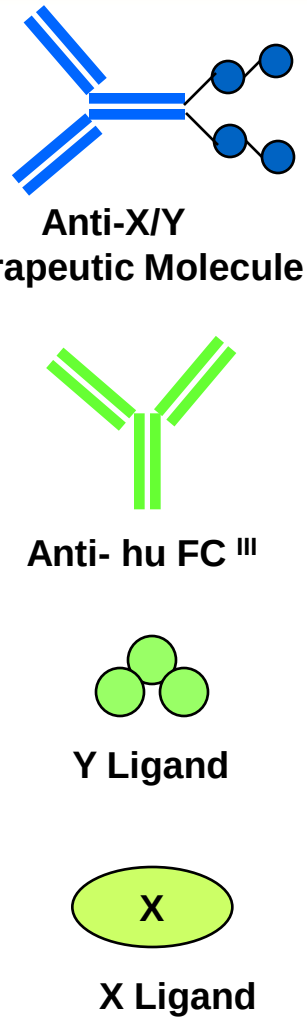
Assay can only be as good as the reagents.

PK Support of Early Discover Program Using Gyrolab Technology (Case Study)

- PK analysis of two fusion proteins in cynomolgus monkeys
- Molecule I
  - Anti-X/Y1: Anti-X antibody fused with Y1 binding protein
- Molecule II
  - Anti-X/Y2: Anti-X antibody fused with Y2 protein
- X is a soluble ligand
- Y is a soluble ligand
- Y1 binds to ligand Y
- Y2 is a cell surface protein and binds to ligand Y

Schematic Diagram for Anti-X/Y Assays





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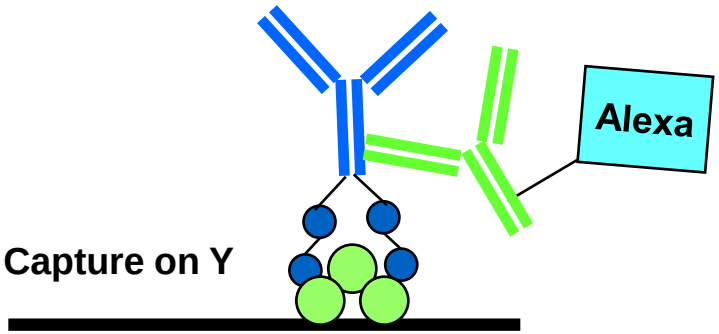
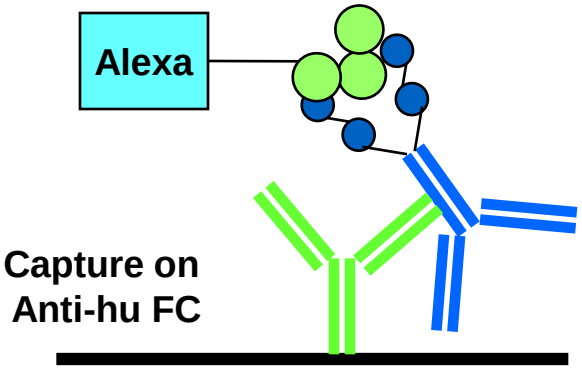
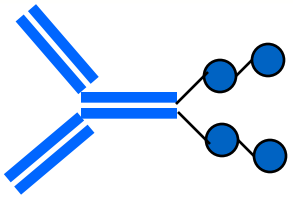
Schematic Diagram for Anti-X/Y Assays



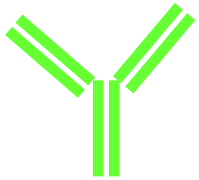
Assay D (“F”)



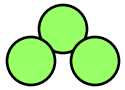
Assay F (“D”)



Anti-X/Y
Therapeutic Molecule



Anti- hu FC III



Y Ligand

X Ligand

Assay A	Intact Assay (Similar to B)
Assay B	Intact Assay (Similar to A)
Assay C	Total Assay (Similar to E)
Assay D	Intact Fused Protein (Similar to F)
Assay E	Total Assay (Similar to C)
Assay F	Intact Fused Protein (Similar to F)

PK Design of Anti-X/Y1 and Anti-X/Y2 in Cynomologus Monkey

- **Anti-X/Y1**
  - **IV administration of 0.1, 0.3, 1, and 3 mg/kg of Anti-X/Y1 in Cyno (n=3)**
  - **Serum samples were collected at the following time points: 0, 0.083, 1, 4, 24, 48, 120, 144, 168, 240, 336, 432, 504, 648, 672, 696 h**
  - **Total of (16 x 3 x 4) of 192 samples per assay**
  - **Total of 1152 samples for 6 assays**
- **Anti-X/Y2**
  - **IV administration of 0.1, 1, and 3 mg/kg of Anti-X/Y2 in Cyno (n=3)**
  - **Serum samples were collected at the following time points: 0, 0.083, 1, 4, 24, 48, 120, 144, 168, 240, 336, 432, 504, 648, 672, 696 h**
  - **Total of (16 x 3 x 3) of 144 samples per assay**
  - **Total of 864 samples for 6 assays**

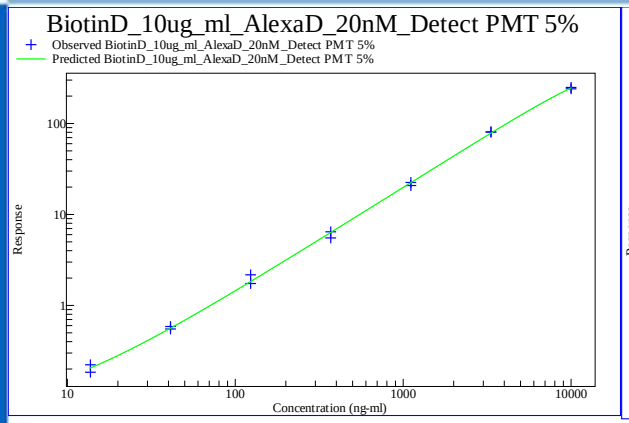
Comparison of ELISA and GYROS

- **ELISA**
  - Assay range: 8 to 2,000 ng/ml
  - Lower LLOQ
  - ~ 2 log dynamic range
  - More sensitive
  - Higher sample dilution
  - Manual assay
- **GYROS**
  - Assay range: 14 to 10,000 ng/ml
  - ~ 3 log dynamic range
  - Less sample dilution
  - Automated
  - Higher throughput
  - Faster assay development and analysis time

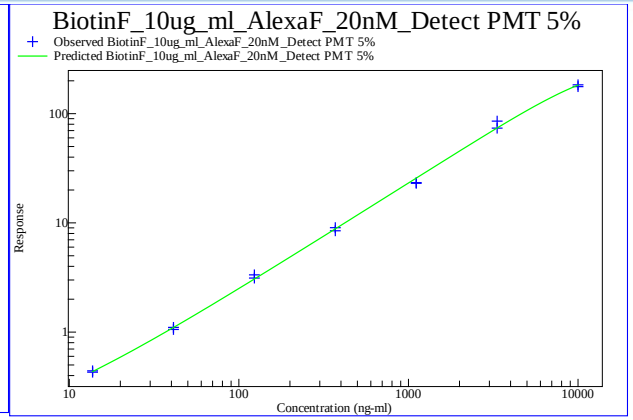
Parameters that need to be optimized	
ELISA	GYROS
Capture Ab	Capture Ab
Detection Ab	Detection Ab
Coating buffer	NA
Incubation time	NA
Stav-HRP	NA
Substrate	NA

Assay development easier with GYROS.

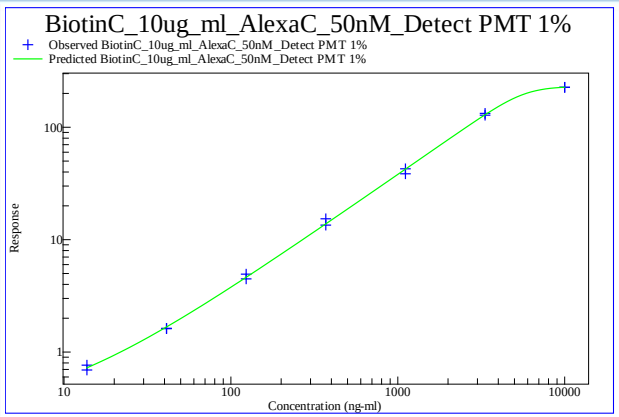
Standard Curves for Six Assays Using GYROS



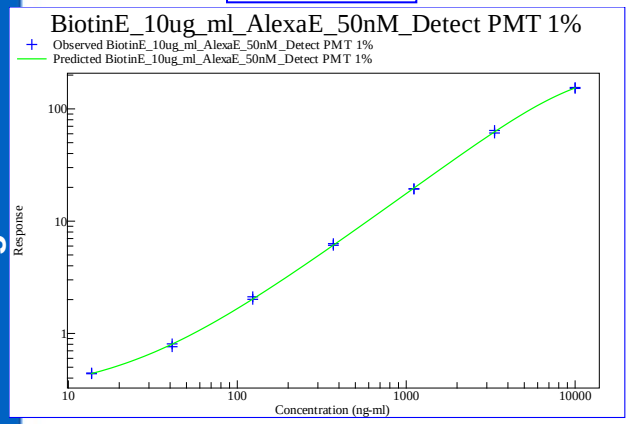
Assay A



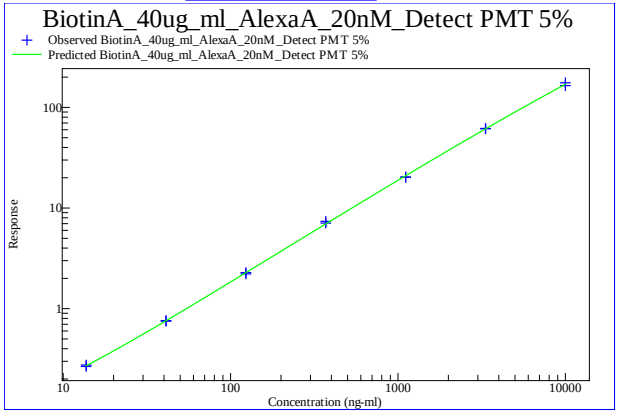
Assay B



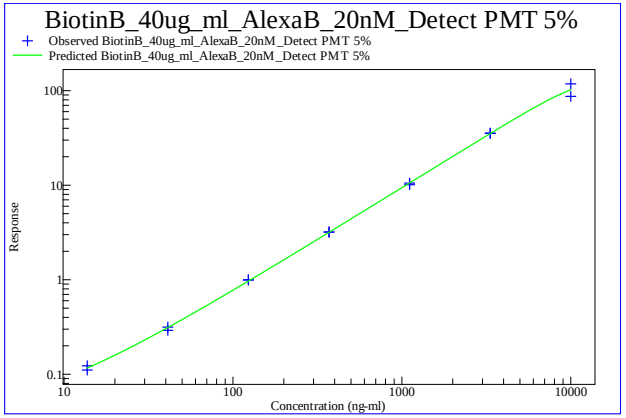
Assay C



Assay D



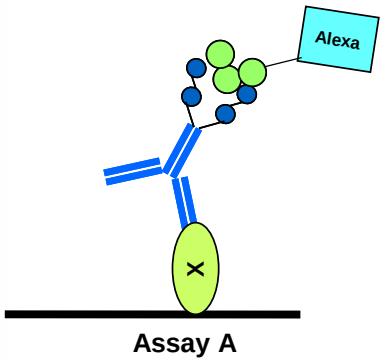
Assay E

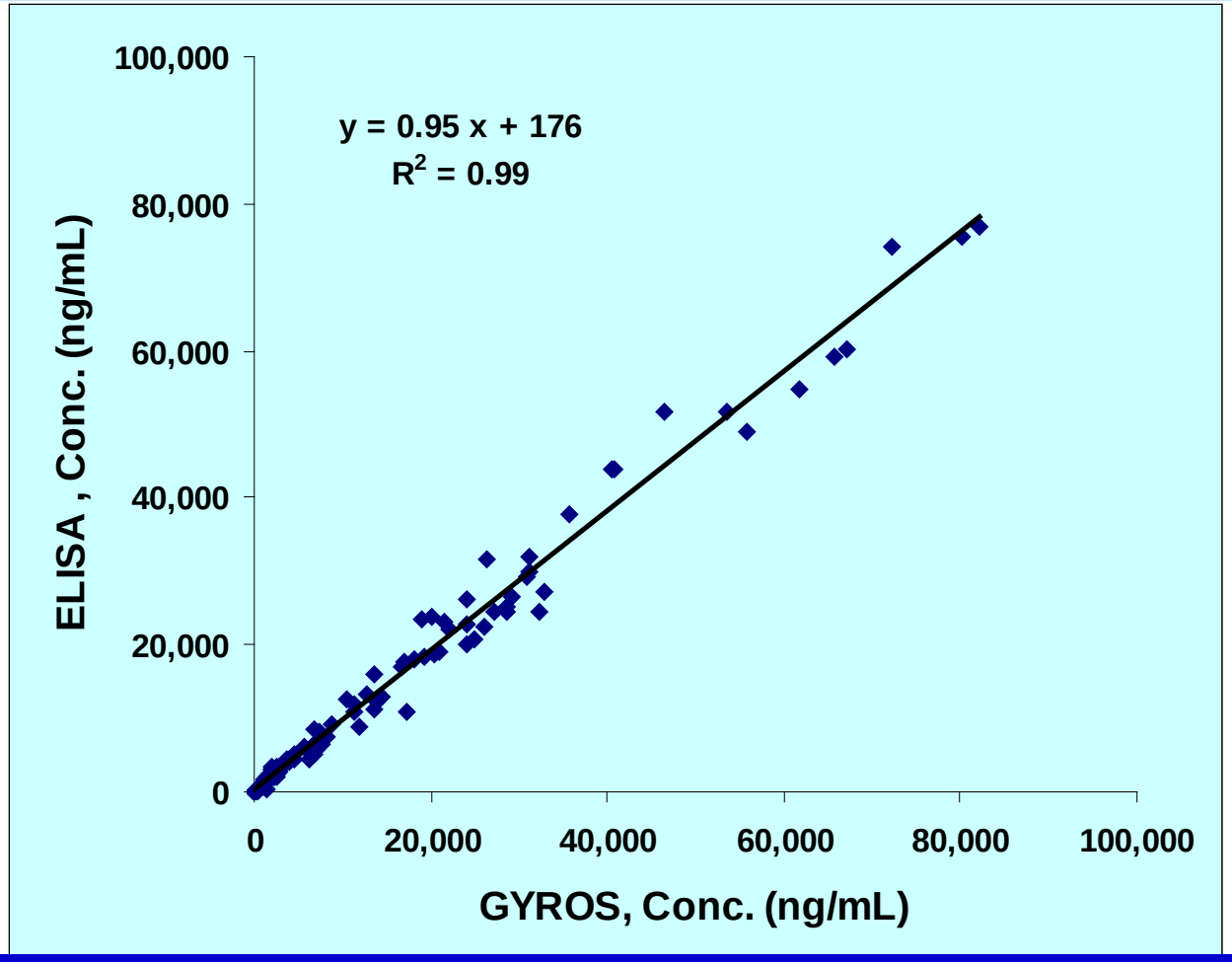


Assay F

~3 log dynamic range with good precision (CV < 10%).

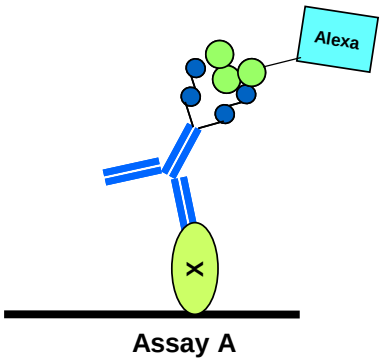
Correlation of Concentration of Anti-X/Y Measured by ELISA and GYROS Using Cyno PK Samples

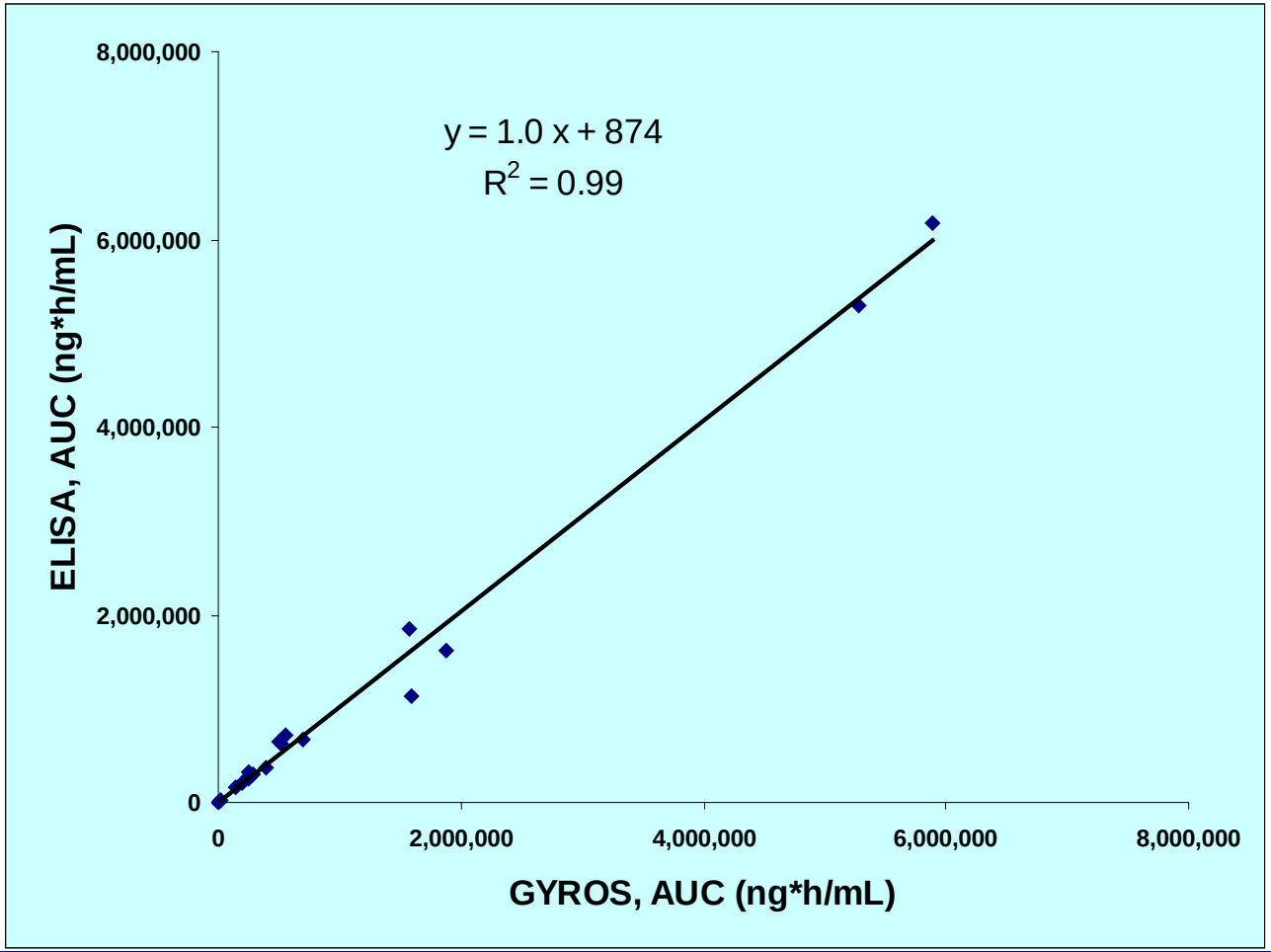




A good correlation was observed between ELISA and GYROS.

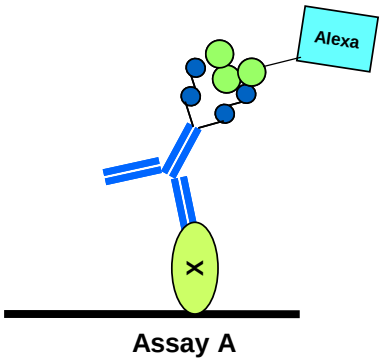
Correlation of PK Parameter (AUC) Between ELISA and GYROS Using Cyno PK Samples

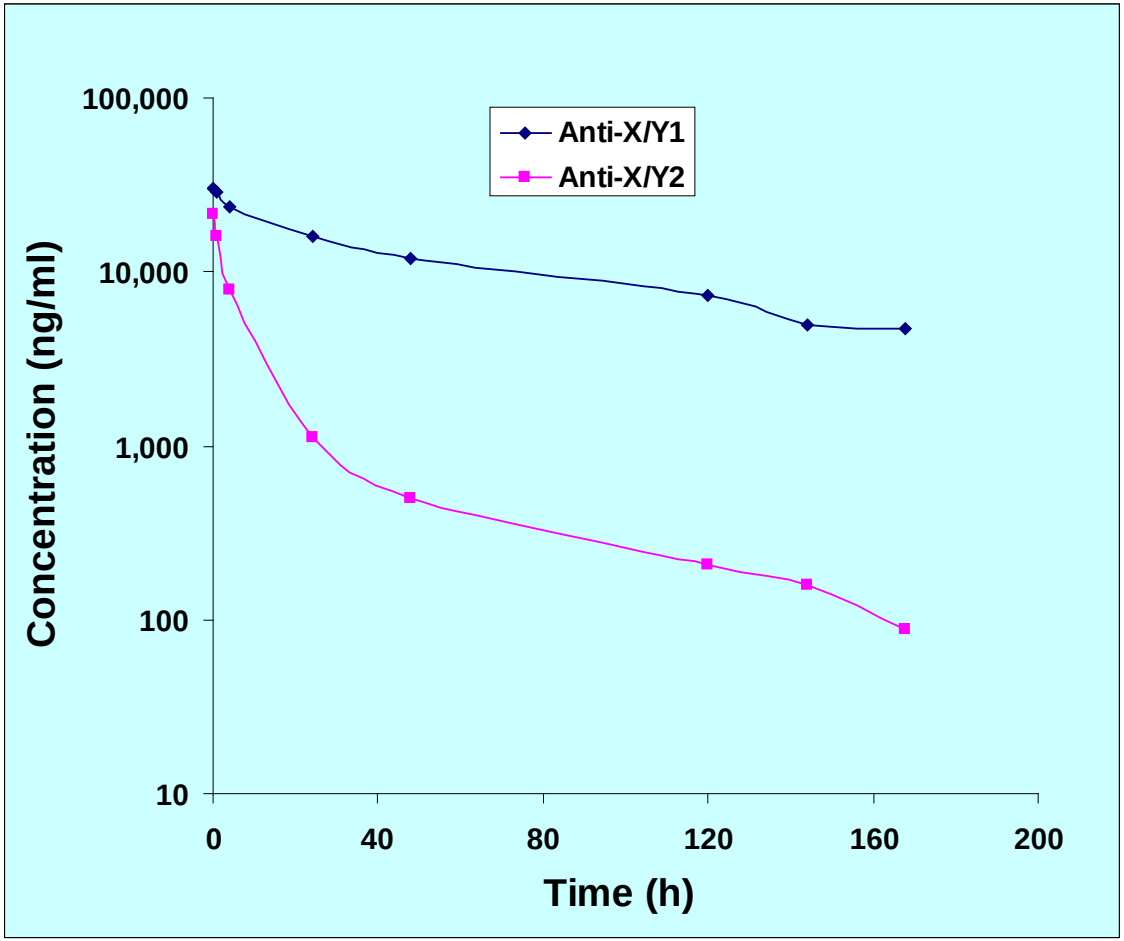




A good correlation was observed between ELISA and GYROS.

PK Profile of Anti-X/Y1 and Anti-X/Y2 in Cynomolgus Monkey (1 mg/Kg, IV)

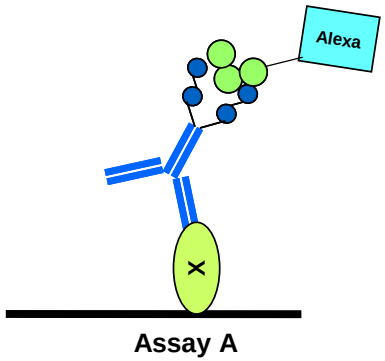




Faster clearance of anti-X/Y2 was observed.

PK Parameters Summary

Compound		Anti-X/Y1	Anti-X/Y2	% (Y2/Y1)
Assay ID		A	A	
SC Dose	(mg/kg)	1	1	100
MRT	(h)	112	18	16
$T_{1/2(eff)}$	(h)	78	12	16
Cmax	(ng/mL)	32,733	28,983	89
CL	(mL/h/kg)	0.432	4.326	1,002
Vss	(mL/kg)	48	78	161
AUC_{0-t}	(ng·h/mL)	1,789,189	230,132	13
AUC_{0-inf}	(ng·h/mL)	2,316,268	231,179	10



Anti-X/Y1 had superior PK attributes. Anti-X/Y2 had ~10 times higher clearance and ~60% higher volume of distribution.

PK Parameters of Anti-X/Y1 from Six Assays

Compound	Assay ID	SC Dose (mg/kg)	MRT (h)	Cmax (ng/mL)	CL (mL/h/kg)	Vss (mL/kg)	AUC_{0-t} (ng·h/mL)
Anti-X/Y1	A	1	112	32,733	0.432	48	1,789,189
Anti-X/Y1	B	1	118	30,917	0.476	56	1,749,929
Anti-X/Y1	C	1	106	33,717	0.399	42	1,989,004
Anti-X/Y1	D	1	102	24,333	0.507	52	1,596,705
Anti-X/Y1	E	1	108	30,167	0.467	50	1,833,853
Anti-X/Y1	F	1	99	35,867	0.427	42	2,059,127

% Ratio to Assay A

A	100	100	100	100	100
B	105	94	110	116	98
C	94	103	93	87	111
D	91	74	117	106	89
E	96	92	108	104	102
F	88	110	99	87	115

Anti-X/Y1 was mainly intact and no truncation was apparent from the multi-assay results

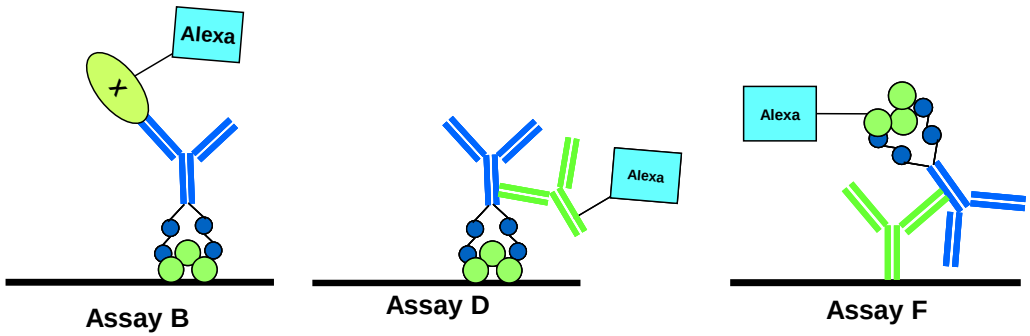
PK Parameters of Anti-X/Y2 from Six Assays

Compound	Assay ID	SC Dose (mg/kg)	MRT (h)	Cmax (ng/mL)	CL (mL/h/kg)	Vss (mL/kg)	AUC_{0-t} (ng·h/mL)
Anti-X/Y2	A	1	18	28,983	4.326	78	230,132
Anti-X/Y2	B	1	26	27,233	3.758	98	262,717
Anti-X/Y2	D	1	12	23,841	4.326	78	230,132
Anti-X/Y2	F	1	19	26,717	4.259	81	233,221

% Ratio to Assay A

B	100	100	100	100	100
D	144	94	87	125	114
F	65	82	100	100	100

Anti-X/Y2 was mainly intact and no truncation was apparent from the multi-assay results



Conclusions

- Reagents are the most important tools for any analytical platform.
- Multiple immunoassays for measuring intact and total of therapeutic provide critical information regarding therapeutic stability.
- Multi-immunoassays can be easily developed in GYROS
  - Low sample volume
  - Automated
  - Up to 250 samples in a 5 CD run (~4 h)
- GYROS data correlate well with ELISA data
- GYROS assays have been used only for research

Acknowledgment

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