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Biomarker Measurement- Maximum Information from Limited Volume

Barcelona, Spain 16th November 2011

John Chappell, Immunoassay Services UK

Biomarker Services

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- Equipment :
  - MSD Sector Imager 2400 (3)
  - Gyros Gyrolab (3)
  - Luminex 100 and 200
  - Immulite 2000
  - Roche Elecsys
  - Randox Evidence
  - Beckman Access
  - Multimode UV/VIS/Chemiluminescence/Fluorescence plate readers (4)
  - Automated 96 well microplate assays







ICON Laboratory Services

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Global Laboratory Services

Bioanalytical Services

PK & Immunogenicity

Large and Small Molecules

LCMSMS
Immunoassay

UK and US laboratories

Specialty Biomarker Services

Immunoassays – Method Dev., Validation, S.A., Automated & Multiplex platforms

LCMSMS

Molecular Diagnostics – Flow Cytometry, CTC's, RTqPCR, HLA Typing, Chip based Genotyping, PBMC's Nucleic Acid Extraction, IHC*, Biobanking

UK & US laboratories for all, +India and Singapore for FC

Central Laboratories

Routine Safety:-
Clinical Chemistry
Haematology
Urinalysis
Toxicology
Coagulation
Histopathology
Microbiology

UK, US, India, China, Singapore laboratories

Biomarker Validation

Validation PK Guidelines to Support:

- Bioequivalence (BE)
- Bioavailability (BA)
- Pharmacokinetic Assessments
- Toxicokinetic Assessments

Sometime referred to as a "GLP Validation"

Biomarker Requirements are Different

- Same Levels of Documentation

Biomarker Validation

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First Consensus White Paper on Assay Validation Requirements

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Pharmaceutical Research (© 2006)
DOI: 10.1007/s11095-005-9045-3

Research Paper

Fit-for-Purpose Method Development and Validation for Successful Biomarker Measurement

Jean W. Lee,[1,16,17] Viswanath Devanarayan,[2] Yu Chen Barrett,[3] Russell Weiner,[3] John Allinson,[4] Scott Fountain,[5] Stephen Keller,[6] Ira Weinryb,[7] Marie Green,[8] Larry Duan,[9] James A. Rogers,[10] Robert Millham,[10] Peter J. O'Brien,[11] Jeff Sailstad,[12] Masood Khan,[13] Chad Ray,[14] and John A. Wagner[15]

Biomarker Validation

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Opportunities for Biomarker use in Drug Development

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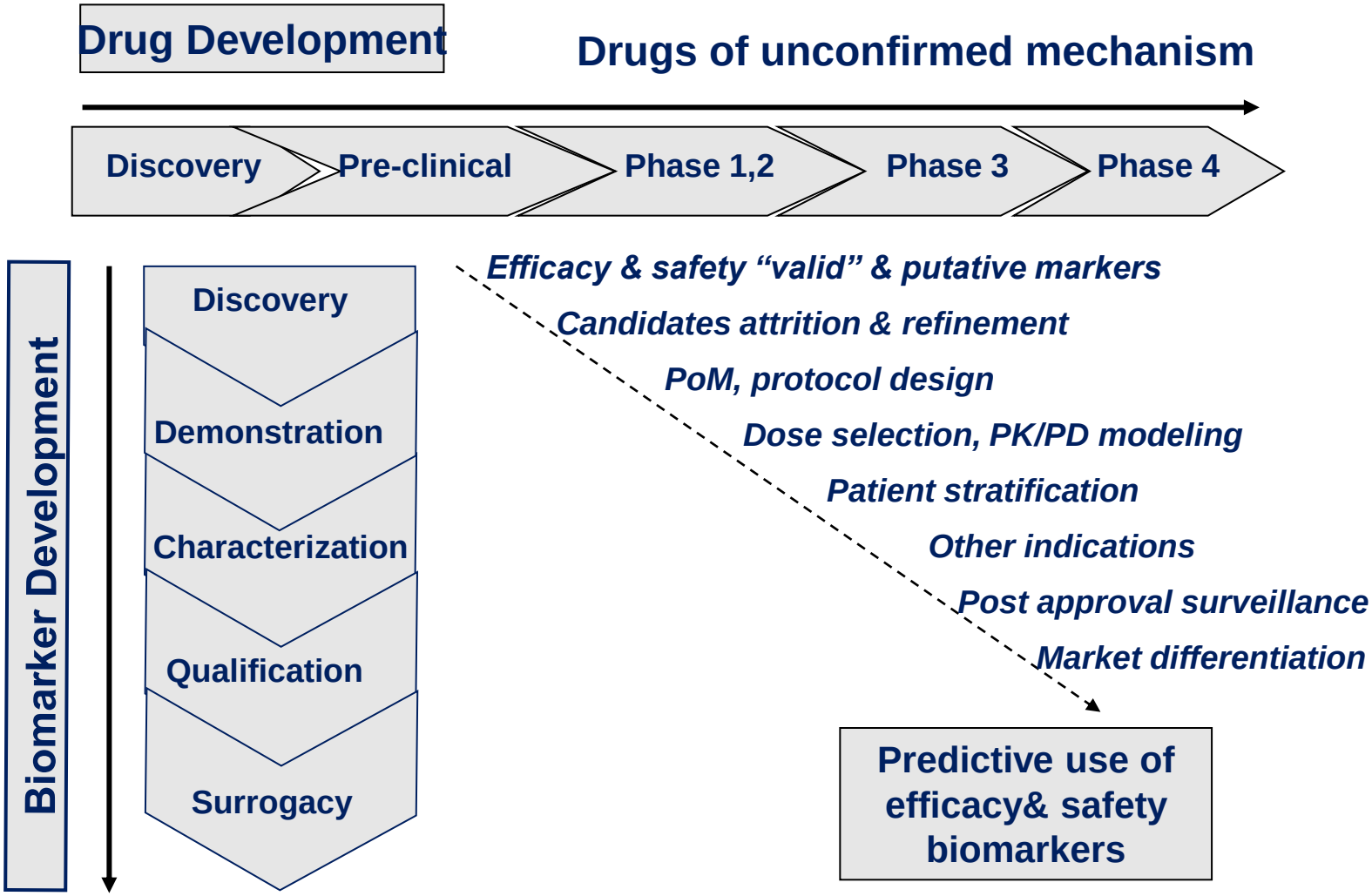

Biomarker Validation

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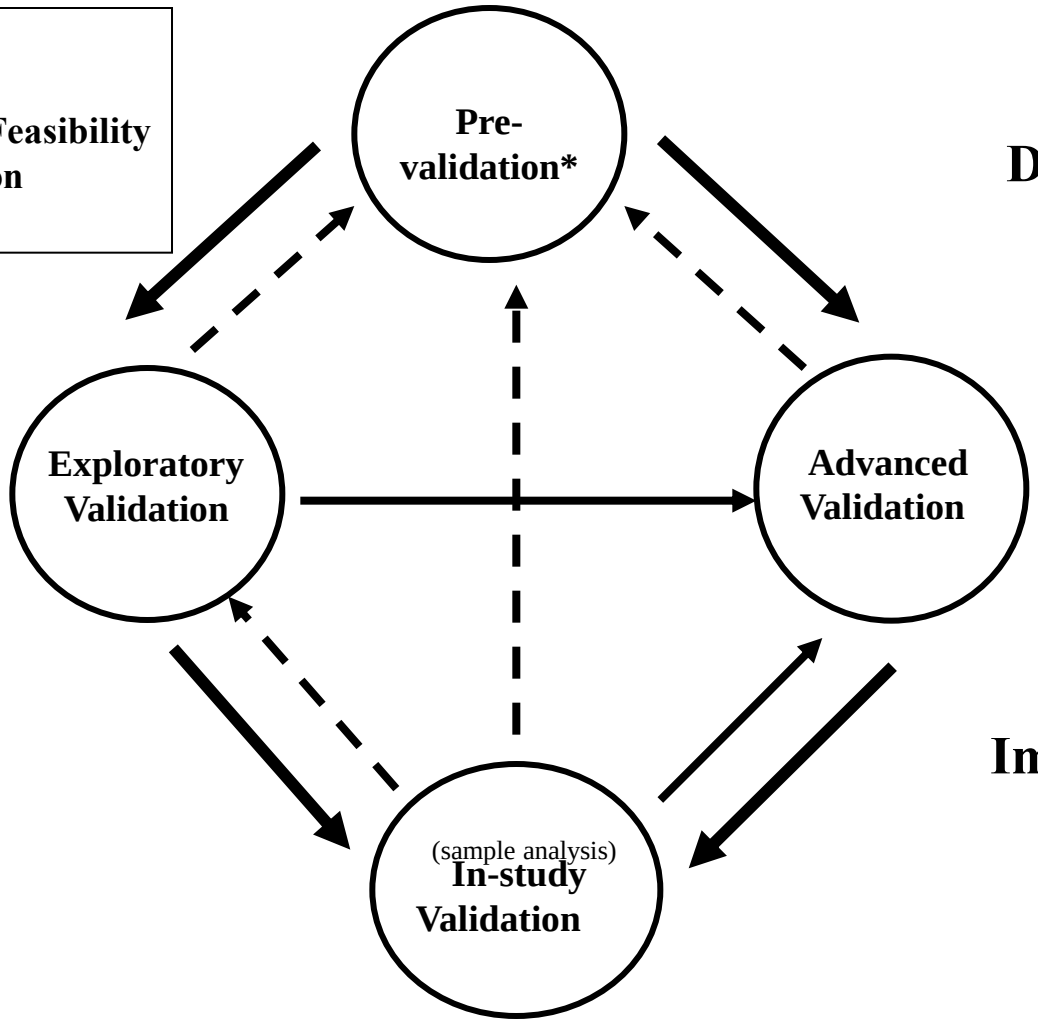
***Pre-validation:**
Pre-Analytical and
Analytical Method Feasibility
Method Optimization



Planning

Development

Validation

Implementation

Biomarker Validation

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	Diagnostic	PK Study	Biomarker study
Intended application	Distinguish diseased from healthy	PK parameters of BA & BE	PD, Safety & efficacy
Method & reagent source	Well established, from vendor	Specifically developed method	diagnostic kits, R&D kits and reagrents
Reference standard	Vendor consistent, conc. may change with time	Well characterized & pure	Inconsistent; research grade Stds often vary within and between vendors –
Calibrator matrix	Substituted matrix	Analyte-free biological matrix	Substituted matrix
Validation samples	From vendor. ?matrix, 2-3 levels same as QC	Spiked ref standard into bio matrix	Prepared by lab spiked ref standard and incurred samples. 4-5 levels.
QC samples	From vendor, common pool among labs. monitor trend.	Spiked ref standard into bio matrix	Prepared by lab for run acceptance, usually at 3 levels.

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ISSUES!!!

"Do you have an Assay for Biomarker "X" Validated?

Validated for what?

….what are the relevant details we need to consider from the study to ensure we validate it correctly?

- **Summary - issues to consider**
- **Clinical and study need**
  - *Safety / efficacy / PD/ exploratory?*
  - *Study specific targets / go or no-go decision criteria*
  - *Expected changes – appropriate analytical range**?***
- ***Previous data produced or methods used?***
- **Stress, posture, gender, ethnicity, circadian rhythm, fasting?,seasonal variation, any additional specific factors for this Biomarker?**
- **Stability …>>> construct collection/preparation procedure - ? biobank matrix required if BM not detectable in normal subjects, or use incurred samples**
- **Commercial kit or develop in-house?**
  - *Reagent source, QC?, third party reference material?*
  - *Alternatives – best fit for study requirements?*
- **Endogenous BM present in matrix?? Calibrators**
- **Choice of Analytical Platform**
- **Matrix of choice, Sample volume, Turn-around time, Throughput**
- **Biological biomarker variations in different diseases - underlying disease status - endogenous interference?**
- **Concomitent drug interference / T.A. interference**
- **Choosing the right Biomarker for the right Species**
- **Population and Intra-individual variation**
- **Method correlations /transfer. From lab-lab or method-method**
- **Microplate coating & edge effects?**
- **Multi-analyte validation requirements**
- **Parallelism / matrix interference / stability -?Consent for incurred samples**
- **Cost**

Biomarker Validation

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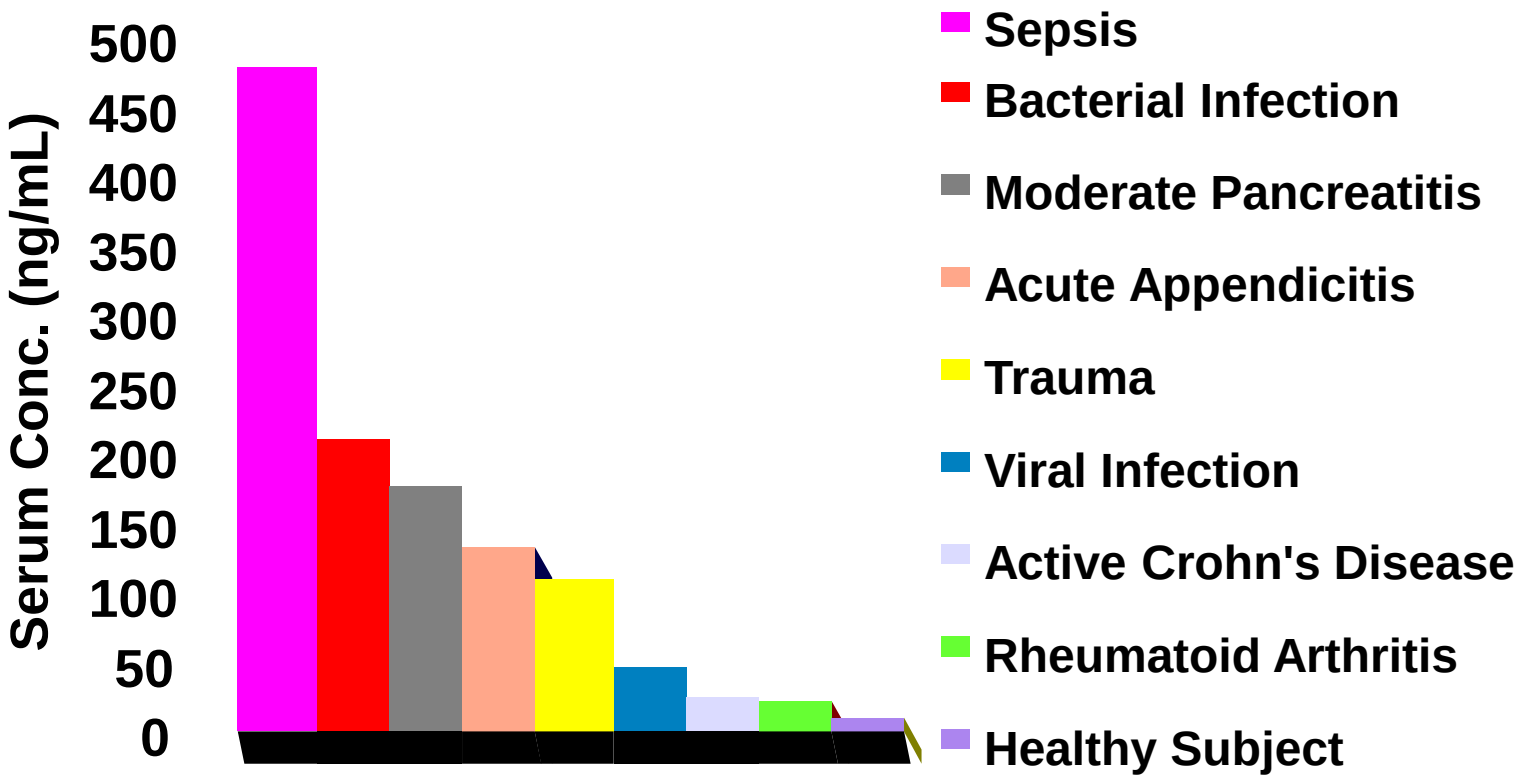

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	Analyte	Biological Variation		Desirable specification		
		CVw	CVg	I(%)	B(%)	TE(%)
S-	C-Reactive protein	52.6	84.4	26.3	24.9	68.3
S-	C-Telopeptide type I procollagen	8	28.8	4	7.5	14.1
S-	Dehydroepiandrosterone sulfate	11.6	49	5.8	12.6	22.2
U-	Deoxipyridinoline/creatinine, 24h	15.3	30.3	7.7	8.5	21.2
U-	Deoxipyridinoline/creatinine, morning spot	26.5	35.7	13.3	11.1	33
P-	Dipeptidyl-peptidase IV	8.2	14.5	4.1	4.2	10.9
P-	Elastase-PI	13.6	16.4	6.8	5.3	16.5
S-	Folate	24	73	12	19.2	39
S-	Follicle stimulating hormone	10.1	32	5.1	8.4	16.7
P-	Homocysteine	9	40.3	4.5	10.3	17.7
S-	Insulin	21.1	58.3	10.6	15.5	32.9
S-	Interleukin-1B	30	36	15	11.7	36.5
S-	Interleukin-8	24	31	12	9.8	29.6
S-	Osteocalcin	6.3	23.1	3.2	6	11.2
S-	Procollagen type 1 N-terminal	6.8	18.4	3.4	4.9	10.5
S-	Prolactin (men)	6.9	61.2	3.5	15.4	21.1
P-	Prolyl endopeptidase	16.8	13.9	8.4	5.5	19.3
S-	Protein C	5.8	55.2	2.9	13.9	18.7
P-	Protein S	5.8	63.4	2.9	15.9	20.7
S-	Prostatic specific antigen (PSA)	14	72.4	7	18.4	30
S-	Testosterone	9.3	23.7	4.7	6.4	14

Biomarker Validation

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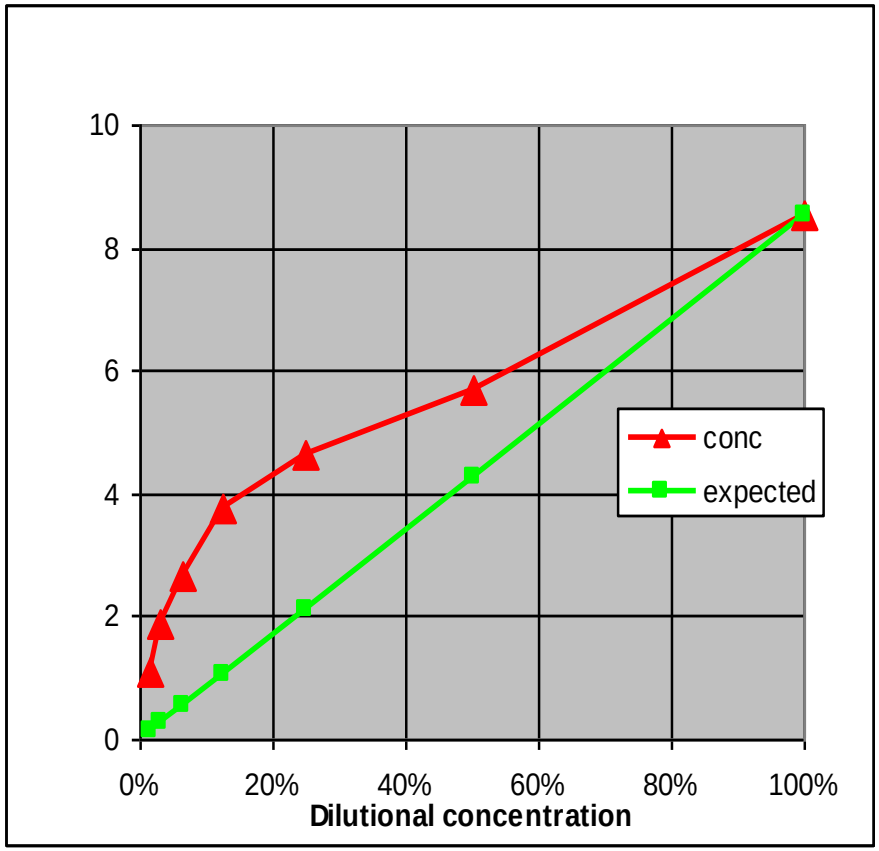
- Acceptance Criteria
  - 4-6-X not always applicable
  - Confidence Limits – tie the acceptance criteria to method performance
  - Physiological variation not normally known for novel biomarkers
  - Markers can only be “qualified” in actual clinical studies

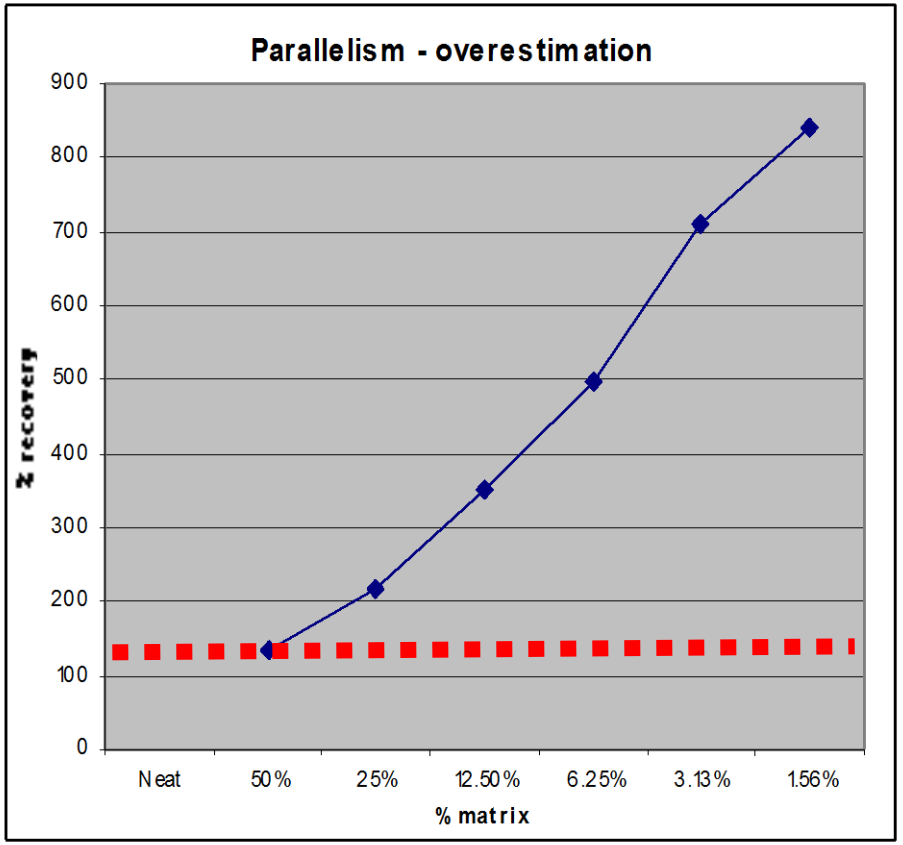






Matrix interference -- Parallelism, incomplete validation & mis-interpretation





AT 1/64 dilution overestimate = 900%

Case History 1

- **The challenge:**
  - 6 DELFIA assays
    - Manual
    - 3 day assays
    - Edge effects = small batch size
    - Expensive
    - Scarcity of antibody
    - Limited sample volume (<1mL)
  - Can we automate and multiplex
    - Shorter analytical time
    - Smaller sample and antibody volumes
    - More cost effective
    - ?improve performance

Case History 1

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- The challenge:
  - 6 DELFIA assays
    - CSF + PLASMA:-
      - Abeta Amyloid 1-40
      - Abeta Amyloid 1-42
      - Total Abeta Amyloid

Case History 1

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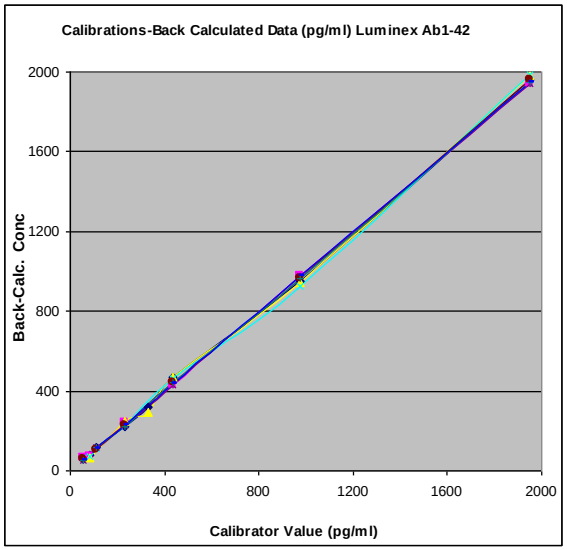
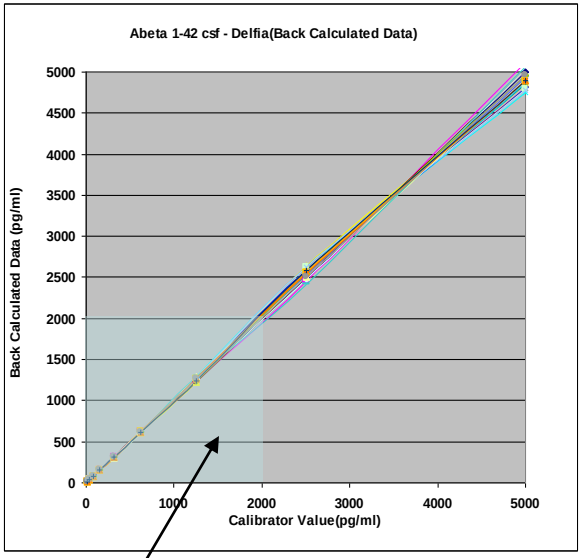
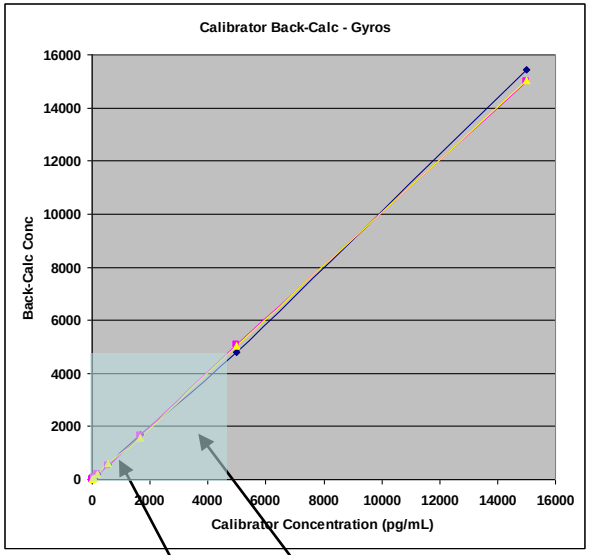
Abeta 1-42 (CSF)







Luminex Analytical range

Luminex Analytical range

Delfia Analytical range

The importance of looking at raw data

Case History 1

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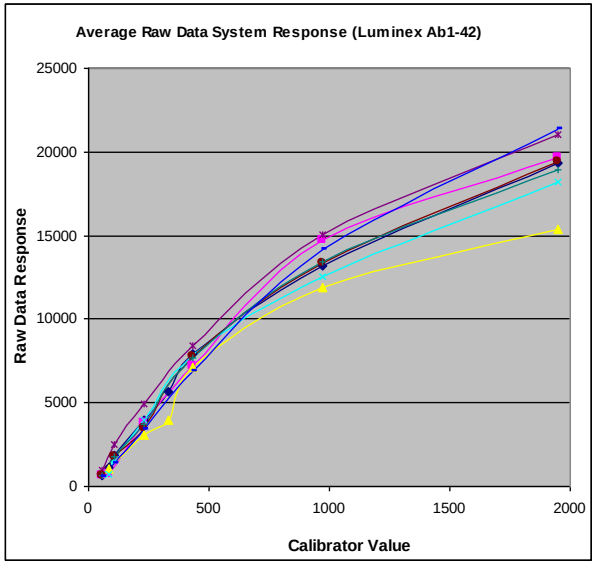
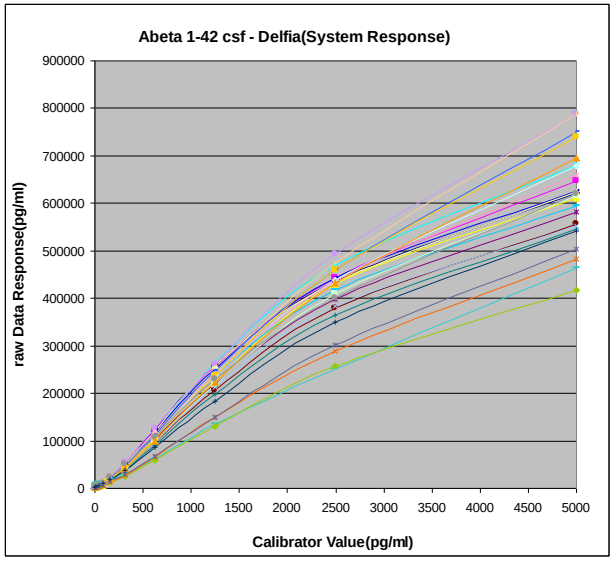
Abeta Amyloid 1-42 (CSF)

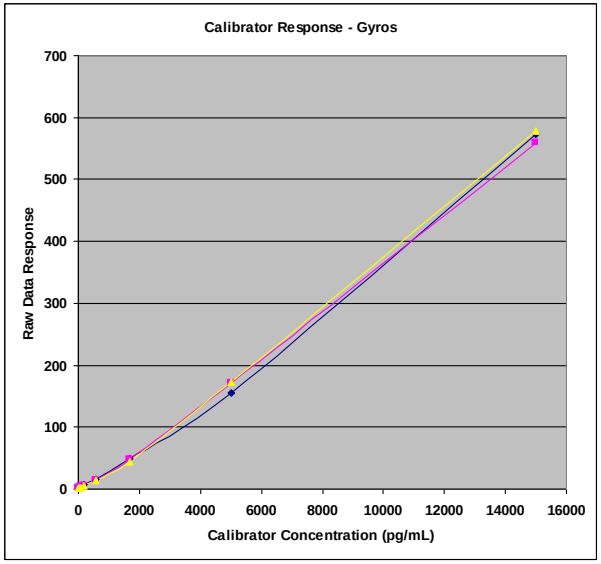
Luminex*



Delfia



Gyros



***Part of 3-plex assay**

Case History 1

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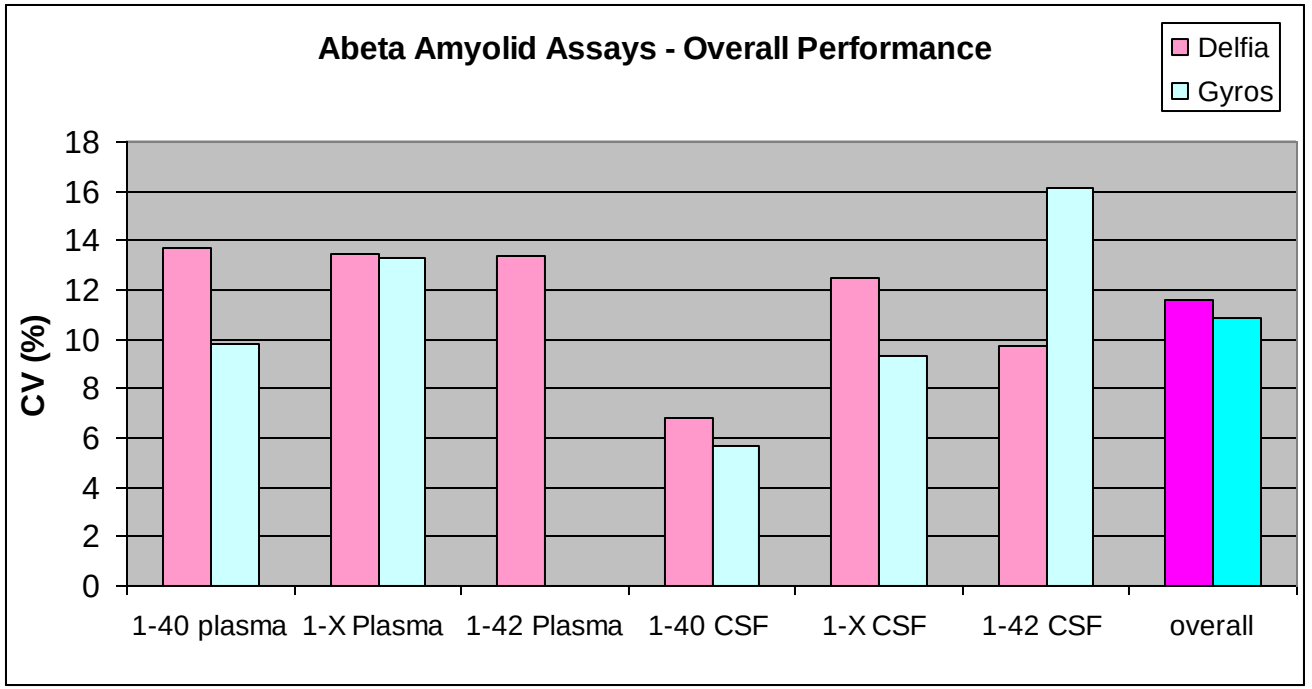
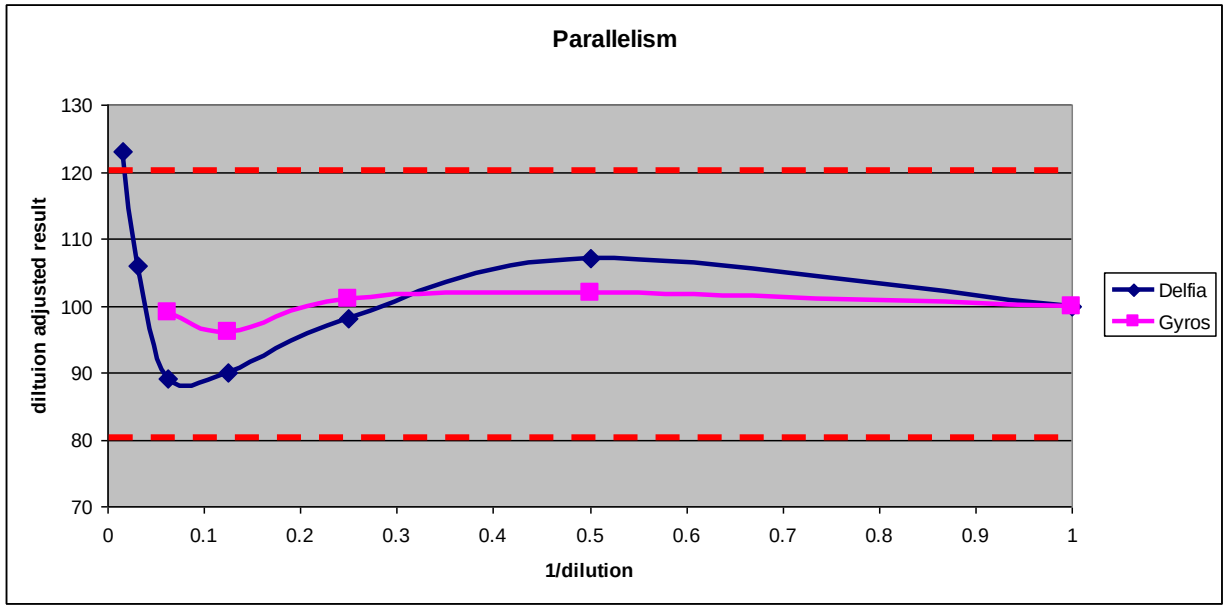
Abeta Amyloid Assay- Overall Performance								
Assay		1-40 plasma	1-X Plasma	1-42 Plasma	1-40 CSF	1-X CSF	1-42 CSF	overall
DELFIA	Mean CV	13.7	13.5	13.4	6.8	12.5	9.7	11.6
Gyros		9.8	13.3	TBC*	5.7	9.3	16.1	10.8

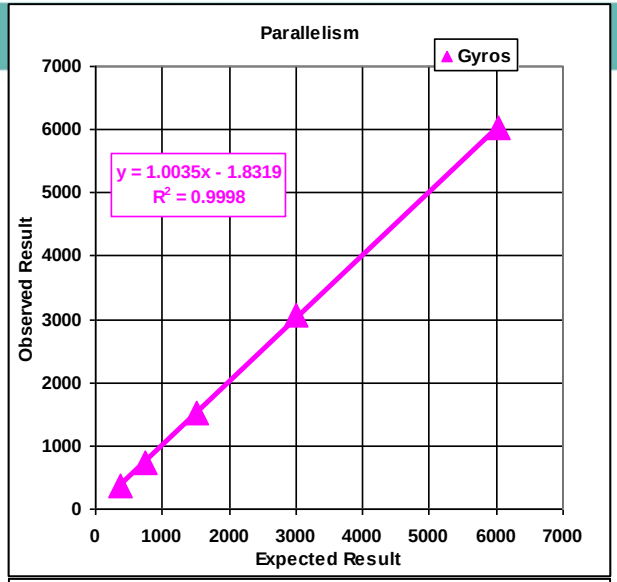


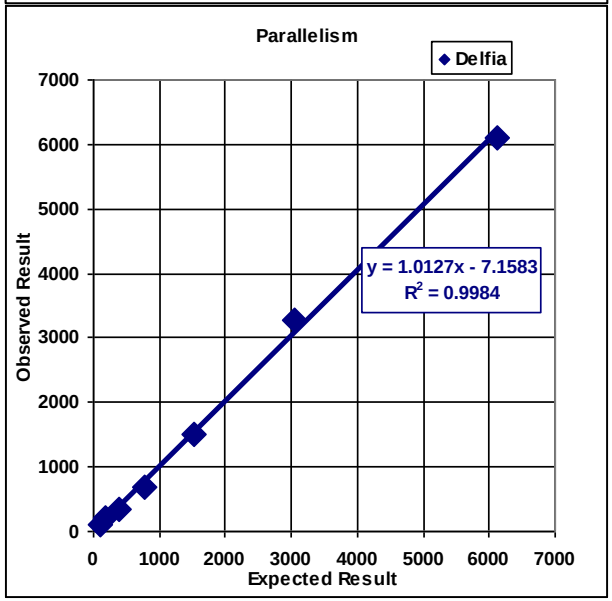
Case History 1

Parallelism- No Matrix effects

Abeta 1-40 CSF







Case History 1

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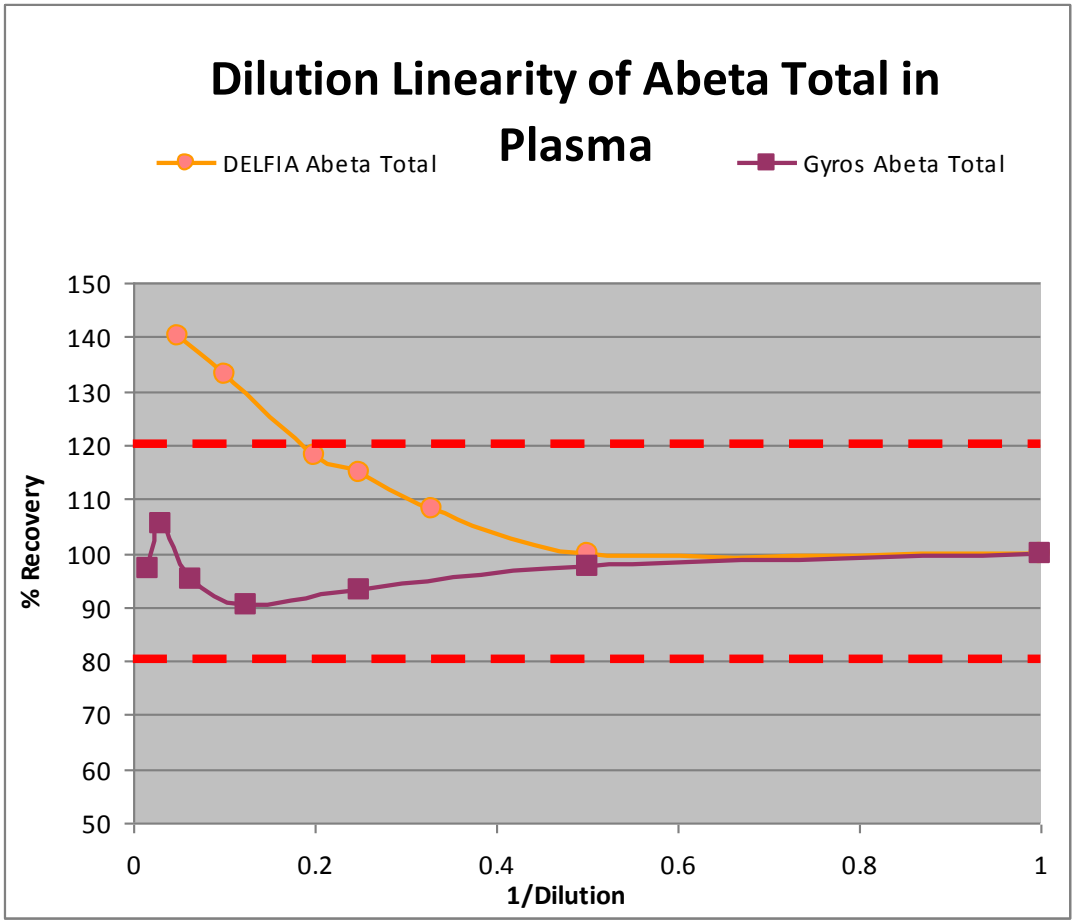
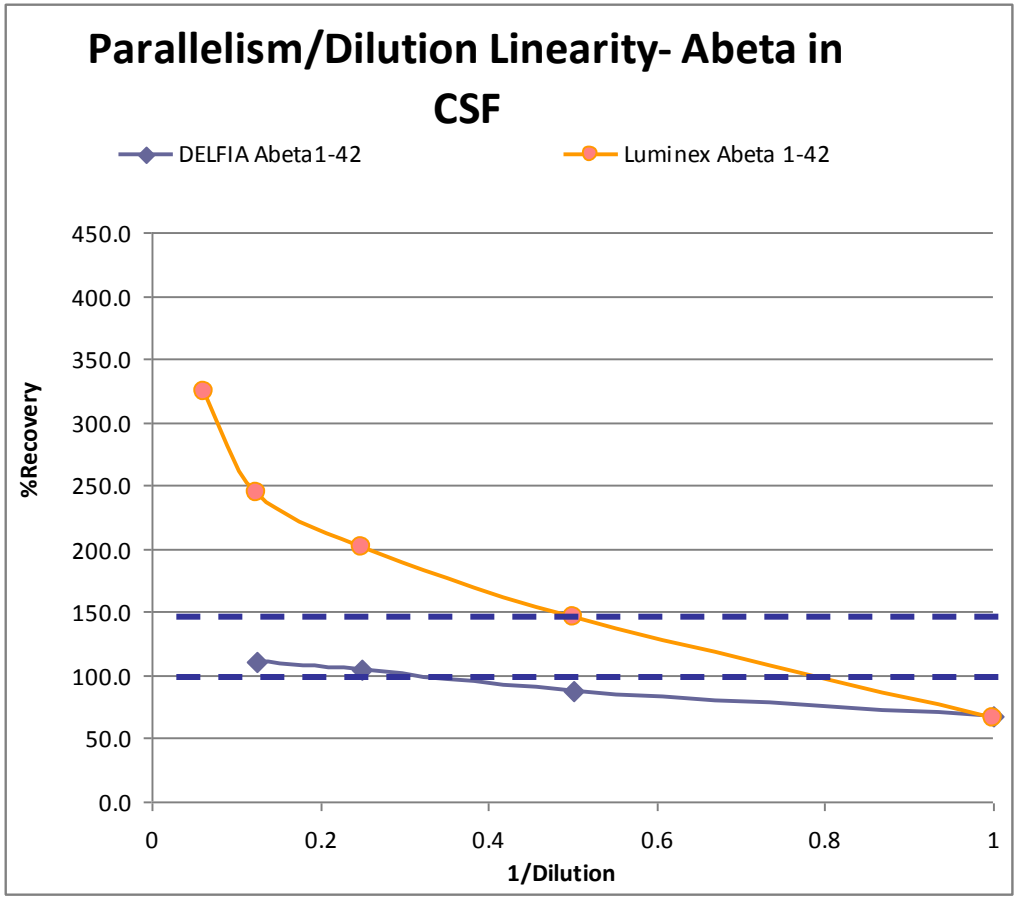
Total Abeta (Plasma)



Case History 1

- **Abeta Amyloid 1-42 (CSF)**



Case History 1

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Stability of Amyloid Beta in CSF

AB1-42 No tween	Baseline		24 hours at RT		24 hours at 4°C	
	CSF1	CSF2	CSF1	CSF2	CSF1	CSF2
Mean Con.	542	866	171	320	289	314
%Difference	-	-	**-68.4**	**-63.1**	**-46.8**	**-63.8**

Ab1-42 Tween	Baseline		24 hours RT		24 hours at 4°C	
	CSF1	CSF2	CSF1	CSF2	CSF1	CSF2
Mean Con.	858	543	813	515	799	512
%Difference	-	-	**-5.3**	**-5.0**	**-7.0**	**-5.6**

Summary- Case History 1

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- 3 day assays converted to 1.5h assays
- Batch size increased from 19 to 32 samples
- 32 samples per CD, up to 5 assays = 160 results
- Comparable Performance (Abeta 1-42 Plasma)
- Wider analytical range for some assays
- Reduced matrix interference effects
- Reduced sample volume requirement
- Reduced Antibody requirement

Case History 2

- **The challenge:**
  - To Measure 11 Biomarkers In Limited Volume
    - From Rare Tissue
    - Limited sample volume (60µL) after extraction
    - Cells extracted using RIPA Buffer
    - Potential Proof of concept

Case History 2

- Why Multiplex?
  - Sample volume restrictions
  - Potential for more analytical data
  - Cost
- Limitations:
  - In “qualitative” or research work – very good
  - For fully validated quantitative work – how many assays?
  - Cross-reactivity, Cross-talk, specificity, sensitivity, robustness

Case History 2

	Luminex / MSD	Grifols Triturus	Gyros Gyrolab
Multiple assays	YES	YES	YES
Method	microplate	microplate	“CD”
Sample vol	reduced	unaffected	much reduced
Reagent vol	unaffected	unaffected	vastly reduced
Analysis Time	unaffected	unaffected	often reduced

Case History 2

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- Assay Developed Individually rather than using Traditional multiplex
- Panel Not Available on either MSD or Luminex
- Allows Development of Individual Assay ranges
- 11 Cytokine Assays
  - 7 Gyros Assays
  - 3 ELISA Assays
  - 1 Activity Assay
  - 1 Protein Assay for Correction

Case History 2

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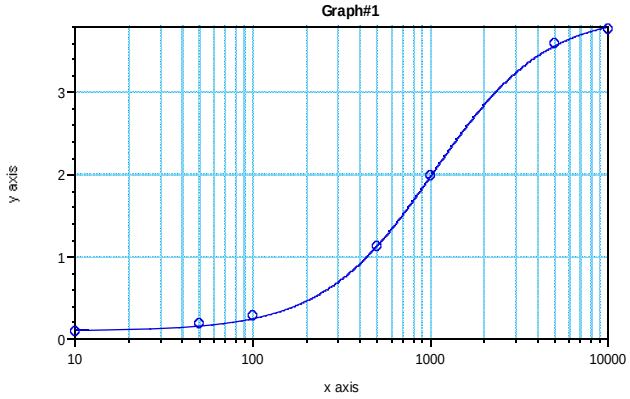
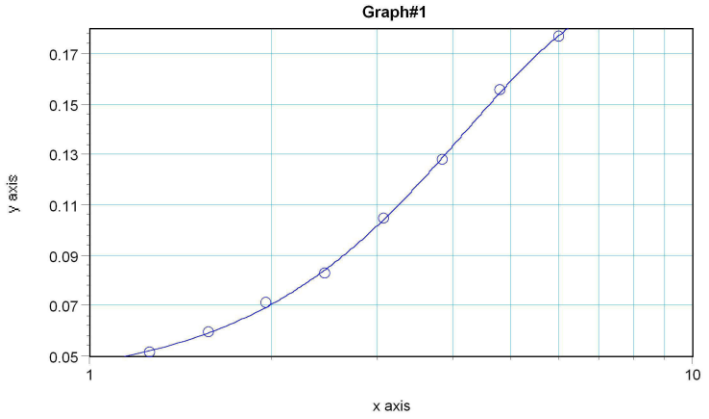


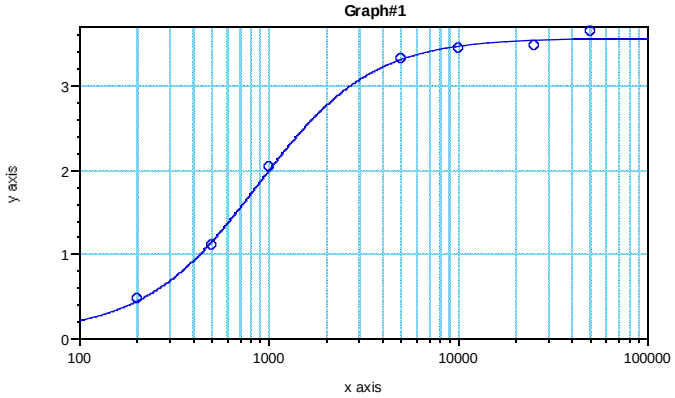
4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
Plot#1 (Std 0: Concentration vs MeanValue)	0.103	1.38	1.03e+03	3.95	1

Curve Fit Option - Fixed Weight Value



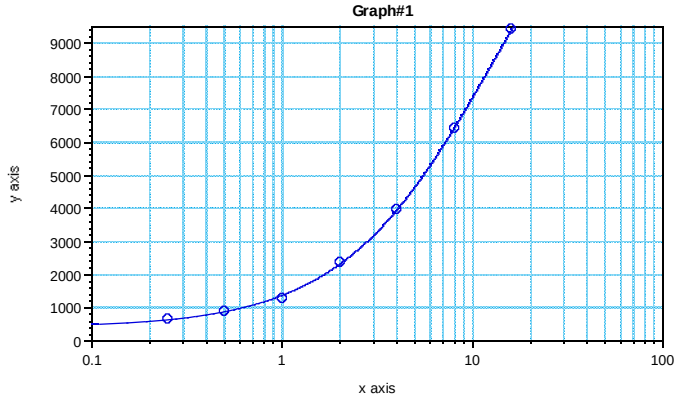
5-P Fit: y = (A - D)/((1 + (x/C)^B)^G) + D:	A	B	C	D	G	R^2
Plot#1 (Standards: NomConc vs MeanResponse)	0.0392	2.03	1.42e+03	0.197	1.4e+05	1

Curve Fit Option - Fixed Weight Value



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
Plot#1 (Std 0: Concentration vs MeanValue)	0.0671	1.44	860	3.57	0.999

Curve Fit Option - Fixed Weight Value



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
Plot#1 (Group02: Concentration vs MeanValue)	411	1.08	12.8	1.65e+04	1

Curve Fit Option - Fixed Weight Value

Case History 2

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Analyte	Method	*Curve range to be validated	Anticipated LLOQ (ULOQ to be determined)	Expected Literature Ranges	Assay Status	Buffers Used	QC Recovery (%) (+/- 20% nominal)
1	Gyros	20 - 1000pg/mL	16pg/mL	20 - 600pg/mL	Good	PBS/RIPA/PI for both Standard and QC preparation	Good recovery at 100 and 1000pg/mL
2	Gyros	1000 - 10000pg/mL	4000pg/mL (may achieve at 3000pg/mL - will need to test)	3000-10000pg/mL	Good	PBS/RIPA/PI for both Standard and QC preparation	Good recovery at 1000 and 10000pg/mL
3	Enzyme Assay	0.25 to 16ng/mL	0.25ng/mL (lowest standard point)	TBC	Good	Kit Diluent for standard preparation and PBS/RIPA/PI for QCs	Good recovery at 3.2 and 6.4ng/mL
4	In house ELISA		1.26 - 7.5 pg/mL	1-140pg/mL	Assay Being Redeveloped	PBS/RIPA/PI for both Standard and QC preparation	Poor sensitivity/ Poor QC recovery.
5	Gyros	50 - 5000pg/mL	80pg/mL	100-3000pg/mL	Good	PBS/RIPA/PI for both Standard and QC preparation	Good recovery at 100 and 1000pg/mL
6	Gyros		approx 1000pg/mL	2-100pg/mL	Assay Not Sufficient in sensitivity	PBS/RIPA/PI for both Standard and QC preparation. Different % of Rexxip H added to PBS/RIPA/PI curve to improve chances of curve	Assay needs redeveloping. Best we can do on the Gyros. Poor sensitivity.
7	Kit based		125 - 1000pg/mL	200-4000pg/mL	Under Development - poor QC results	PBS/RIPA/PI for both Standard and QC preparation	Poor QC recovery - assay under development
8	Gyros	100 - 1000pg/mL	400pg/mL	100 - 1000pg/mL	Good	PBS/RIPA/PI for both Standard and QC preparation	Good recovery at 1000pg/mL. -20.1% at 100pg/mL
9	Gyros	200 - 2000pg/mL	80pg/mL	500-2000pg/mL	Good	PBS/RIPA/PI for both Standard and QC preparation	Good recovery at 1000pg/mL and 200pg/mL.
10	Gyros	100 - 1000pg/mL	between 80 and 400pg/mL (actual value to be tested)	30-300pg/mL	Good	PBS/RIPA/PI for both Standard and QC preparation	Good recovery at 1000pg/mL. -24.2% at 100pg/mL
11	In house ELISA	200 - 100000pg/mL	200pg/mL	5000-50,000pg/mL	Good	PBS/RIPA/PI for both Standard and QC preparation	Good recovery at 5000pg/mL - need to assess higher QC level

Summary- Case History 2

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- 3 Assay Validations Complete
- 6 Validation Underway and due to complete Dec 11
- 2 Assays Being Re-developed
- 1000 Samples to be analysed

ICON Development Solutions Immunoassay Group

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- John Allinson (VP Biomarker Services)
- Paula Jardieu (Senior VP/GM Immunoassay Services)
- Elizabeth Thomas (Senior VP/GM Bioanalytical Services UK)
- Mike Anderson (Associate Director, Immunoassay Services US)
- Anthony Upton (Associate Director, Immunoassay Services UK)
- David Lane (Laboratory Manager)
- Ranga Pinnamaneni (Project Manager)