To: Ian Gibbons[igibbons@theranos.com]
Cc: Sunny Balwani[sbalwani@theranos.com]; Carolyn Balkenhol[cbalkenhol@theranos.com]
From: Elizabeth Holmes
Sent: Tue 6/1/2010 3:43:14 AM
Subject: RE: Assay integrity.GSK.ppt
Case Study_Assay Development Validation and Selected Results 4-28-10.ppt

Thanks Ian. FYI – here is the final JH ppt.

From: Ian Gibbons
Sent: Saturday, May 29, 2010 7:27 AM
To: Elizabeth Holmes
Cc: Sunny Balwani; Carolyn Balkenhol
Subject: Assay integrity.GSK.ppt

Some talking points on our 3.x capabilities to ensure and record that assays are valid.

File Produced in Native Format

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Assay Development, Validation, and Selected Clinical Results: Theranos Systems

Theranos, Inc.

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Confidential Information Notification

Please note that this presentation is **STRICTLY CONFIDENTIAL** and discloses Theranos information *not in the public domain*.

The technology described is the subject of pending U.S. and international patents, which Theranos will actively enforce.

The information can only be provided to those covered by validly executed Confidential Disclosure Agreements on a need-to-know basis.

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Theranos Assay Libraries

Theranos Systems are platforms on which any assay run in a conventional lab can be rapidly transferred and run at equivalent or superior performance specifications through robotic automation of protocols and tools that are currently manually operated.

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Theranos Assay Libraries Examples

Immunoassays

Enzyme assays

- Direct assay measuring enzyme product
  - Serum APase
  - ALT

Direct assays for metabolites

- Glucose, cholesterol etc.
  - Using coupled-enzyme-based chemistry

Antigen assays

Assays for cells and cellular markers

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Exemplary Range of Theranos' Automated Assays

Macromolecular biomarkers

Cells

Antibodies and Antigens

Drug assays

- Macromolecular drug
  - Humanized Antibodies
  - Small molecule drugs (proprietary method)

Small molecules

- Hormones
- Small molecule drugs

Enzymes

- For example, ALT, Lipoprotein panels, etc.
- Enzymes can be measured by immunoassays.
- Results correlate well with results of direct enzyme assays.
  - This approach was validated for CK and CKMB, among others.

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Case Study:
Immunoassay Development and Validation

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Stages of Development

Establish design goals for each multiplex.

Choose key reagents (antibodies).

Screen reagents/antibodies.

Develop assays within system.

Establish calibration scheme.

Produce, evaluate validation cartridge lots.

In-house validation exercise

Release product for customer use.

Field validation

Monitor performance of product in field.

Documentation

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Establish Design Goals for Each multiplex

Purpose of assays

Selection of analytes

Design of dilution scheme

Assay reportable range

Precision

Specificity

Other usual quality measures

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Choose Key Reagents (Antibodies)

Commercially available antibodies

- Vendor information on:
  - Specificity
  - Affinity (where available)
  - Utility in sandwich assay configuration (Proteins)

Proprietary antibodies

- Exclusive arrangements with vendors
- Theranos development programs

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General Design Goals

Reportable range

- Clinically relevant range
- Usually match predicate methods

Specificity

- Match predicate methods

Accuracy

- Match predicate methods

Precision

- CV < 10 % within most of reportable range

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Screen Reagents/Antibodies

Many candidate pairs are evaluated.

- Microtiterplate screens
  - Preliminary work
- Screens within the Theranos system
  - Sensitivity
  - Assay range
  - Stability
  - Clinical validity
  - Compatibility with plasma and blood
- As many as 30 candidate pairs are compared.

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Develop Assays Within System

Theranos system works at controlled, elevated temperature.

Assays are performed on proprietary “capture elements.”

- Equivalent to wells of MTPs

Development is performed using reagents and cartridges made and assembled by the operations group.

- Performance is representative of finished product.

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Establish Calibration Scheme

1. Instruments are calibrated in a one-time exercise (optical and thermal).
2. Assay calibration is performed on a cartridge lot-specific basis.
3. Cartridge lot calibration is traceable to either:
   - Standard materials in a defined matrix
   - Selected predicate assay
4. Calibration generally is done with “aqueous” calibrators.
   - Authentic analytes in serum-like matrix
   - Up to eight calibrators
   - All analytes present
   - Calibrators stored frozen (stable)

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Calibration Scheme

5. Where matrix effects are significant, plasma-based calibrators are used.
6. Matrix effects are evaluated and defined.
    - Spiked serum, plasma and blood samples
    - Endogenous analyte concentration measured
    - Spike recovery established (one-time exercise)
    - Blood vs. plasma relationship established
        - In some cases, a clinical study is performed where special issues exist.
7. Calibration is performed on a cartridge lot basis.
8. The calibration function is proprietary and analyte-specific.
9. Calibration stability
    - Ongoing lot-lot comparison
    - Re-assay of archived standard samples

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Instrument Calibration

Response to enzyme activity is measured in replicate at several levels over the linear range of the Detector.

Slope of the response characterizes the Detector and is used to convert the measured signal (counts) to that of the all-instrument average.

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Instrument Calibration

Instruments once released are optically calibrated (one-time exercise).

Function is essentially identical across all calibrated instruments.

Instrument function is continuously monitored with data stored on the Theranos server.

Occasional optics recheck

Cartridge control data

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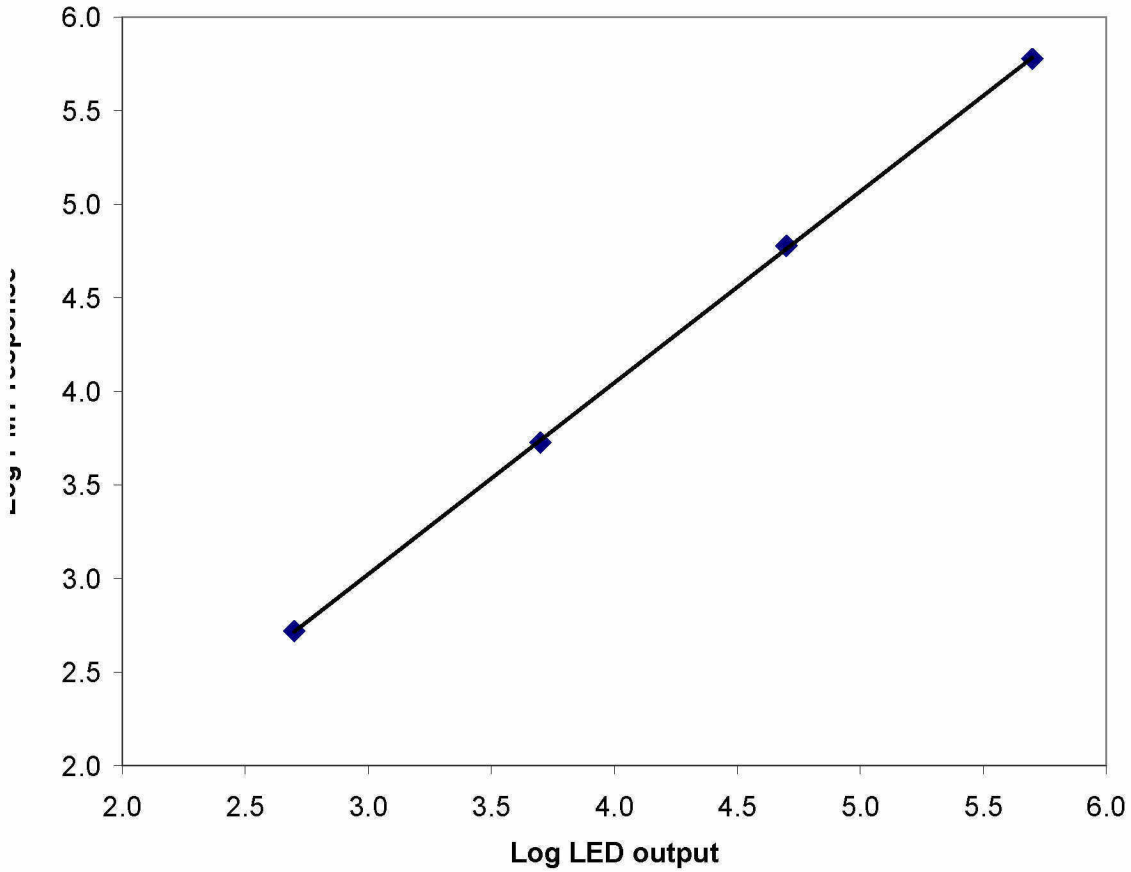
Instrument Calibration

Detector response is linear.



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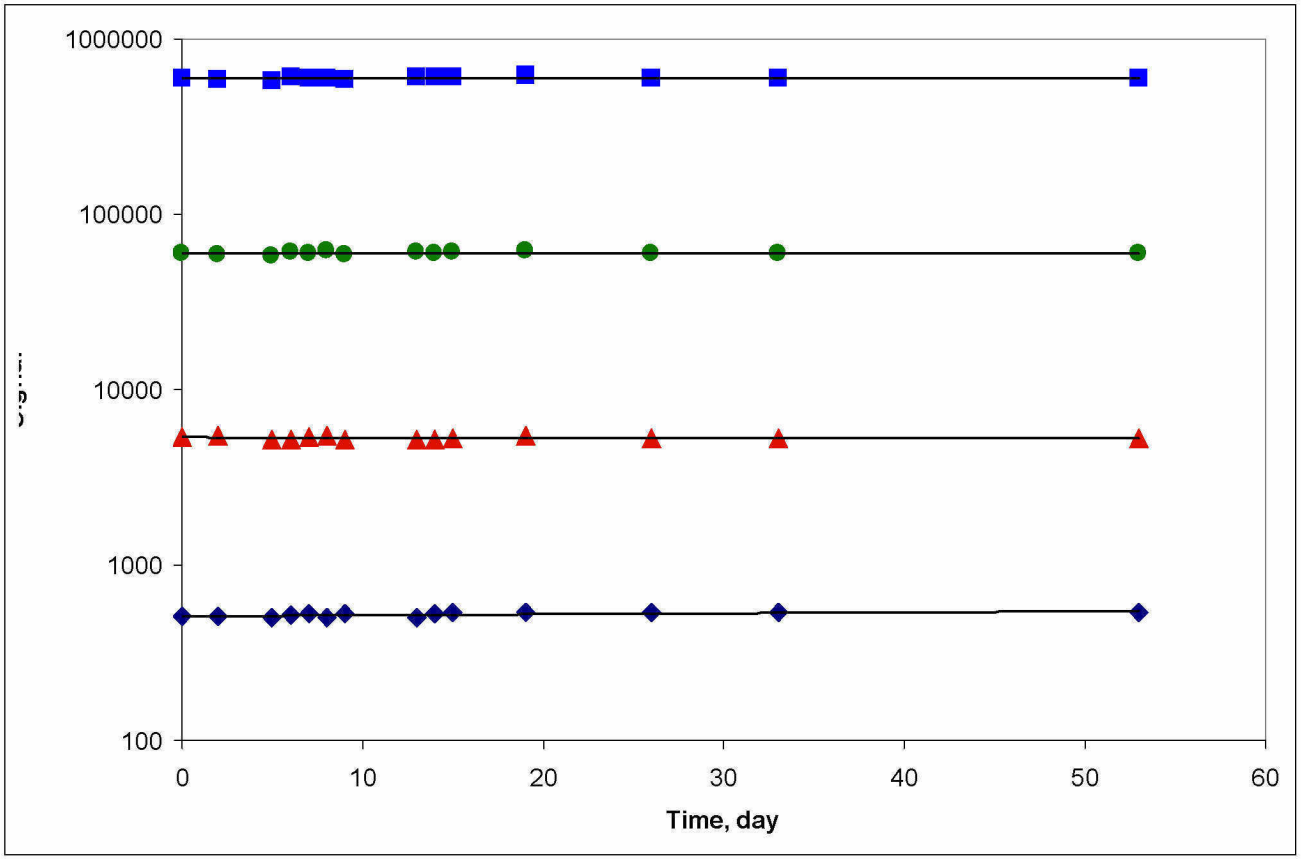
Instrument Calibration

Detector response is stable.



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Instrument Calibration

Temperature is stable.

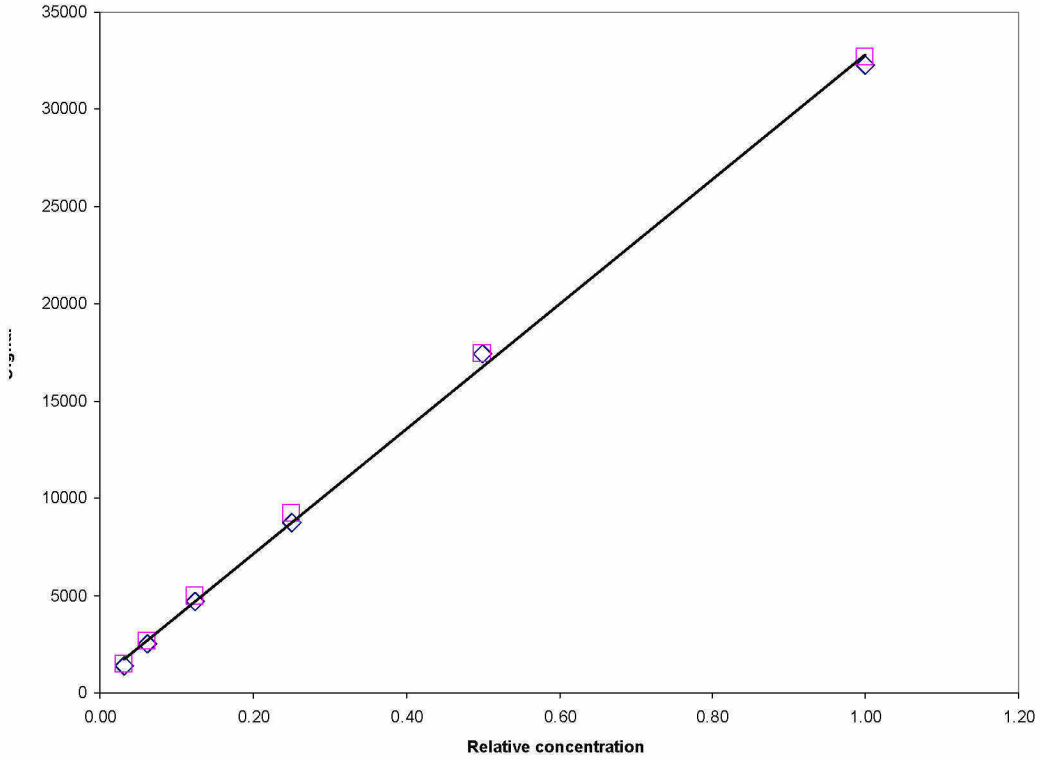
Temperature control example

Temperatures recorded at the time of assay were monitored for ten instruments at ten locations remote from Theranos laboratories (homes of clinical trial subjects) over a two-month period. The target temperature (34°C) was well maintained (114 measurements average 34.02°C +/- 0.22°C).

Dilution Accuracy

Enzyme product diluted within the instrument.

Two different dilution schemes gave identical results.



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Cartridge Lot Calibration Exercise

For each manufactured lot:

- Up to eight calibration levels
- Several instruments (usually three)
- Several replicates (usually three)
- About 60 cartridges/exercise
- Assays are typically done in duplicate within each cartridge and results averaged.

Data are fit to a calibration function.

Calibration is released against QC criteria.

Calibration is used for the measured life of the lot.

After lot expires, system will not use the cartridges.

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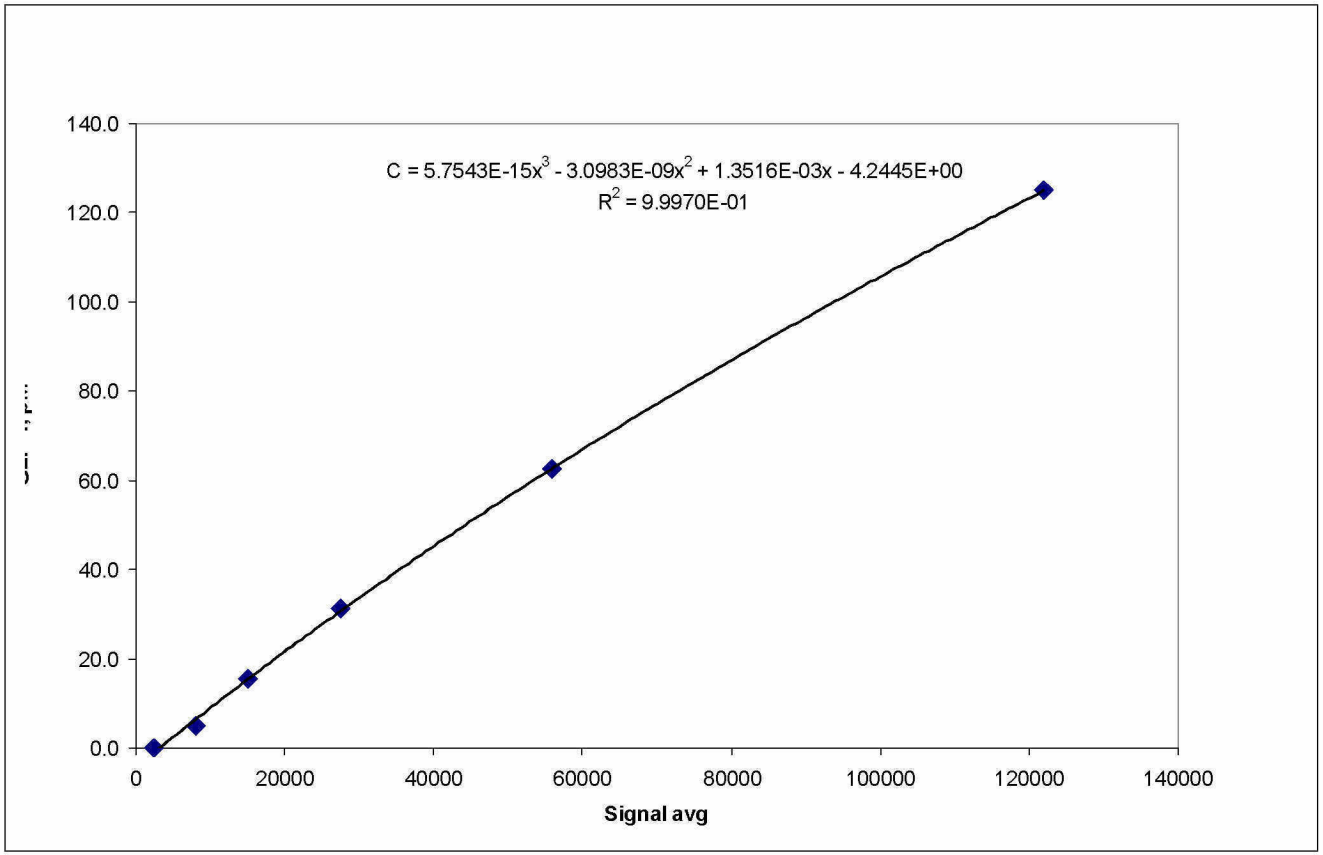
Cartridge Calibration Example

GLP-1 spiked in plasma (no endogenous analyte)



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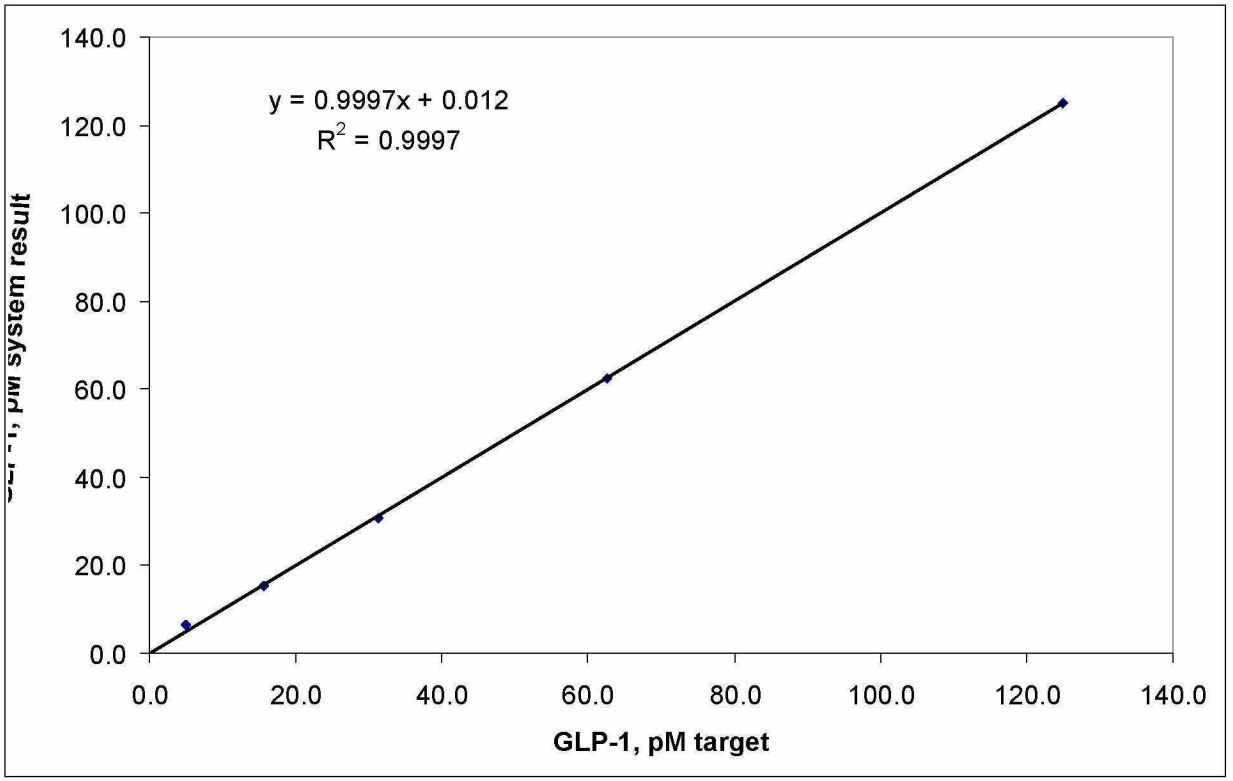
Calibration Verification



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Calibration for Plasma

Case with a matrix effect and/or endogenous analyte

If the sample dilution is large, we can usually ignore matrix effects and use “aqueous” (buffer-based) calibrators.

In a high sensitivity assay, which uses a low sample dilution, matrix effects are significant, and we calibrate by:

- selecting samples with low endogenous analyte.
- measuring endogenous analyte.
- calibrating against the sum of endogenous and exogenous (added) analyte.

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Calibration Example

(where endogenous analyte complicates calibration for plasma)

IL-6

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Solution

Measure endogenous IL-6 in plasma samples using reference method.

Select those with the lowest IL-6.

Plot signal versus sum of endogenous + spike IL-6 and extrapolate to S0.

Calibrate using IL-6 spiked plasmas (2) and plotting signal versus the sum of endogenous and spiked IL-6.

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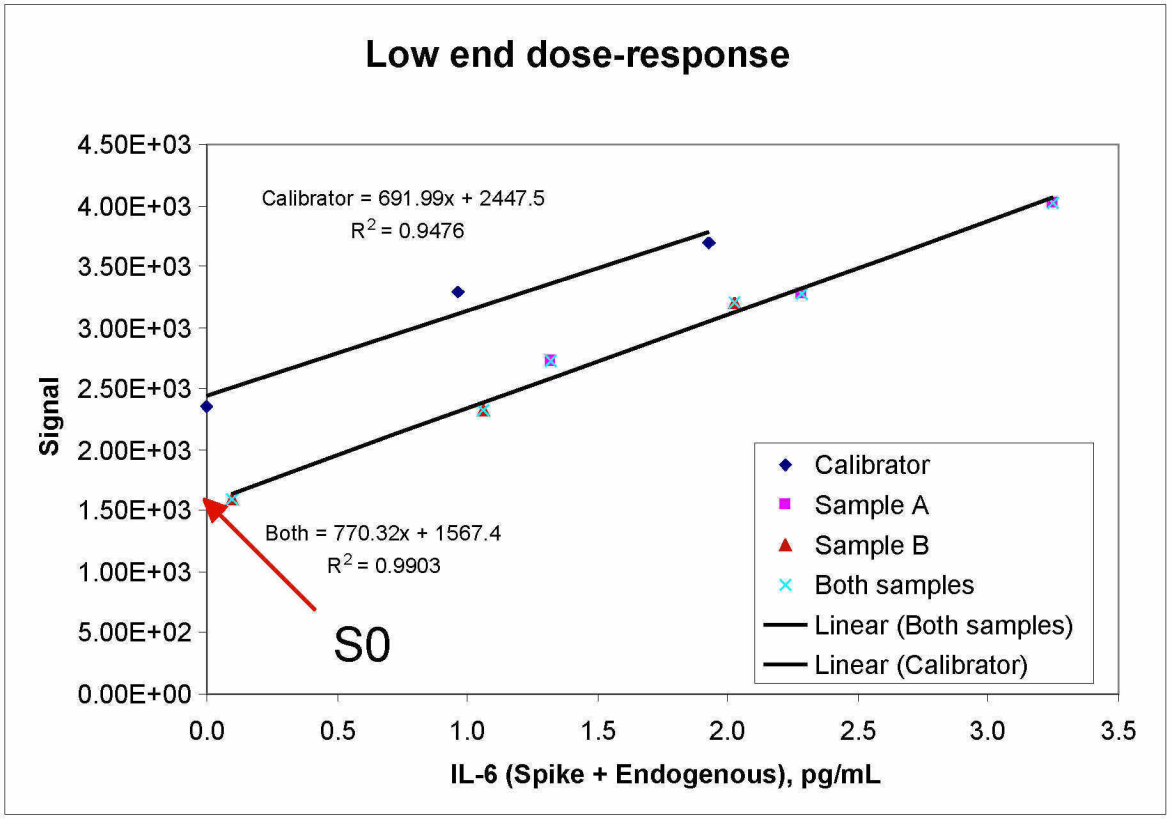
Dose Response

Spike at low [IL-6] and back extrapolation to zero analyte

Note: **Slope of D/R is the same for both samples**



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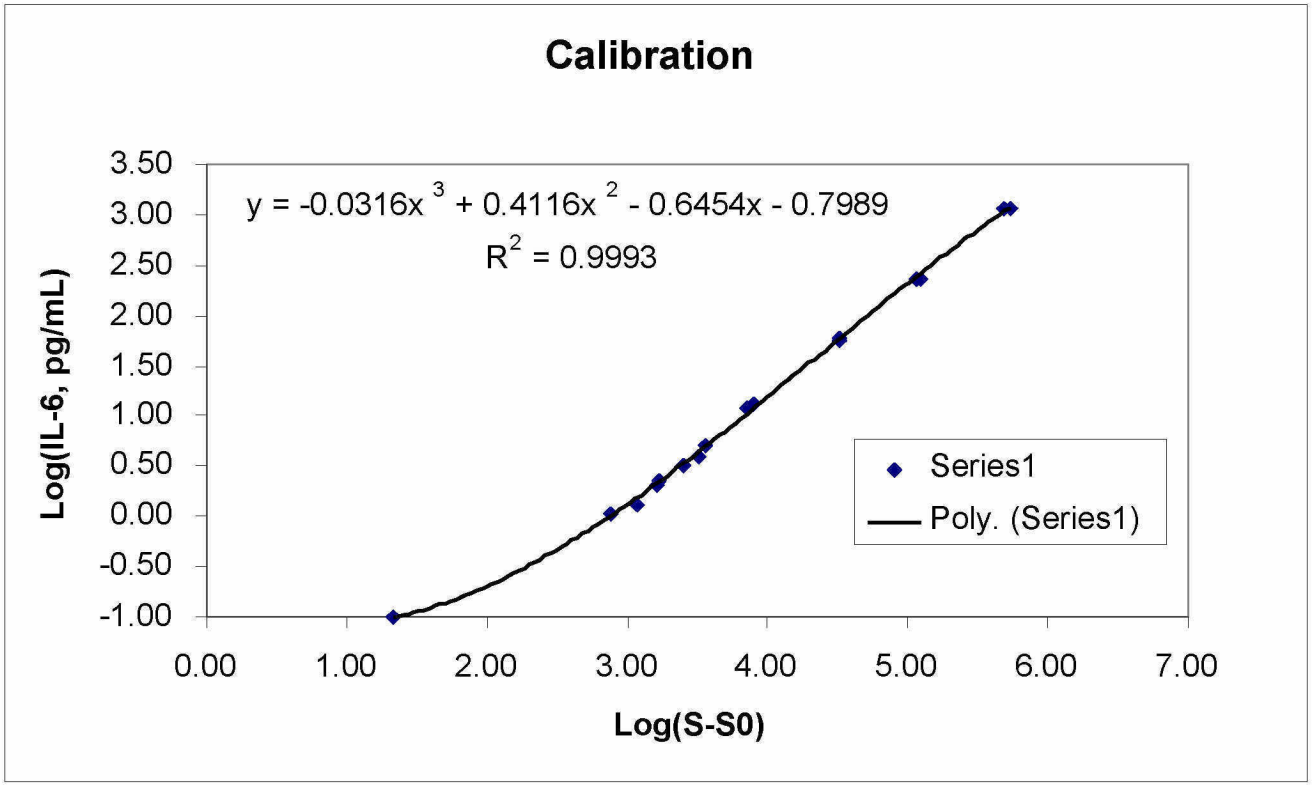
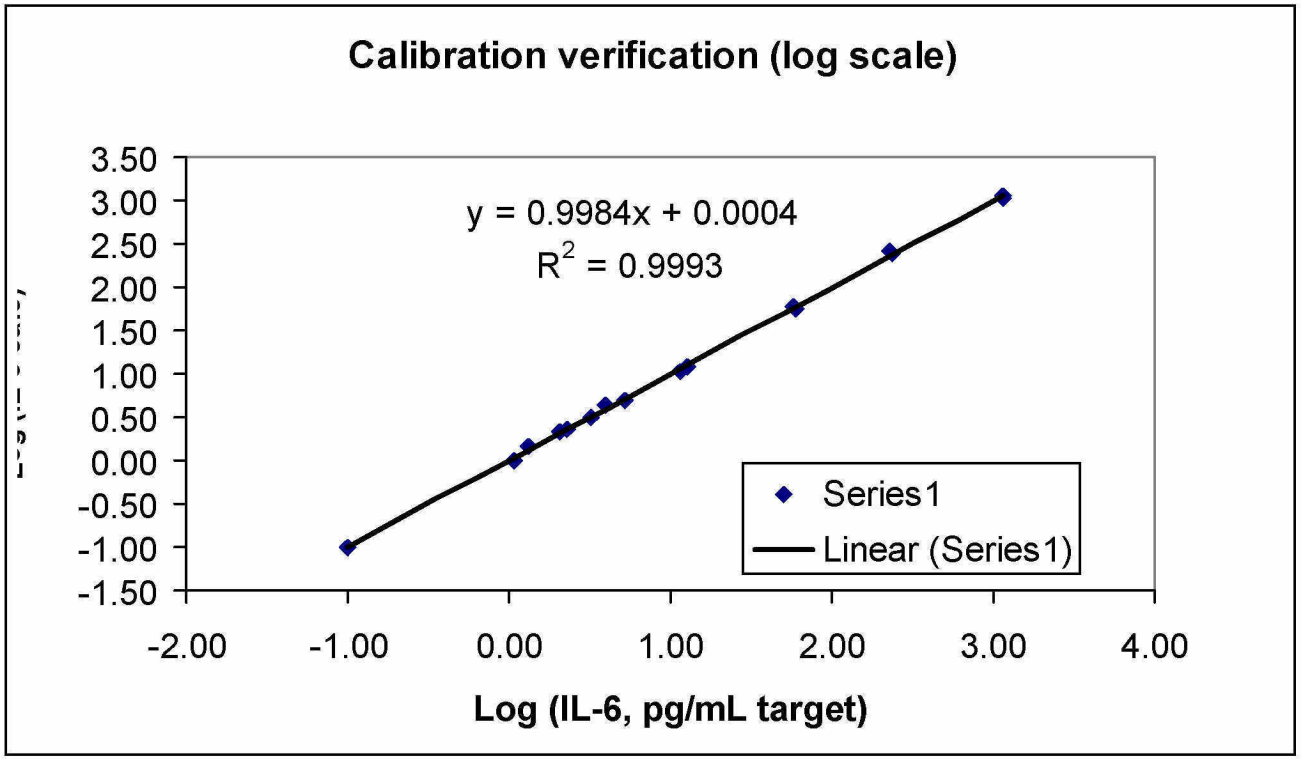
Calibration: Fit to Calibration Function



Calibration Verification



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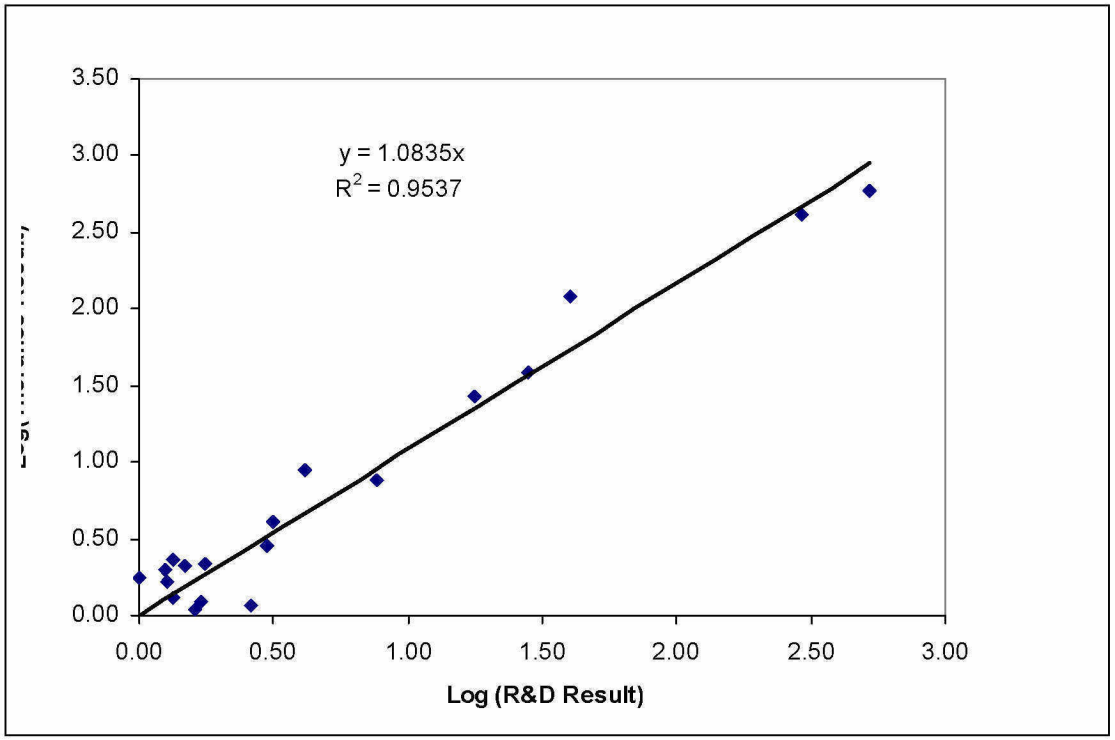
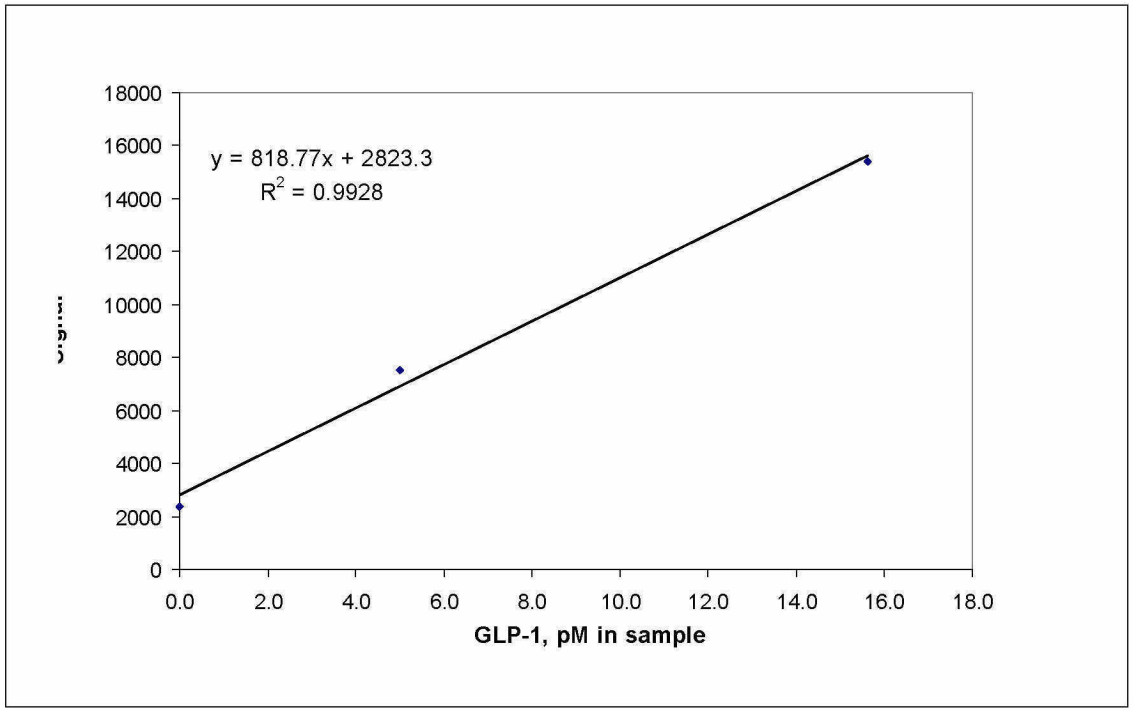
Validation of Calibration: Comparison of Methods

Log correlation to show low end

IL-6 Assay



Limit of Detection



Slope (DS/DC)	819	Counts/ pM
SD	213	Counts
Conf.	95	%
N	8	
LOD	0.52	pM

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Calibration for Blood

During the assay development process:

- Obtain several fresh blood samples.
  - Blood bank
- Measure endogenous analyte levels .
- Measure analyte spike recovery.
  - Mulwellle levels
- Determine extent of binding to formed elements.
  - Many analytes bind to RBCs.
  - Compare spike recovery in blood versus plasma separated from blood.

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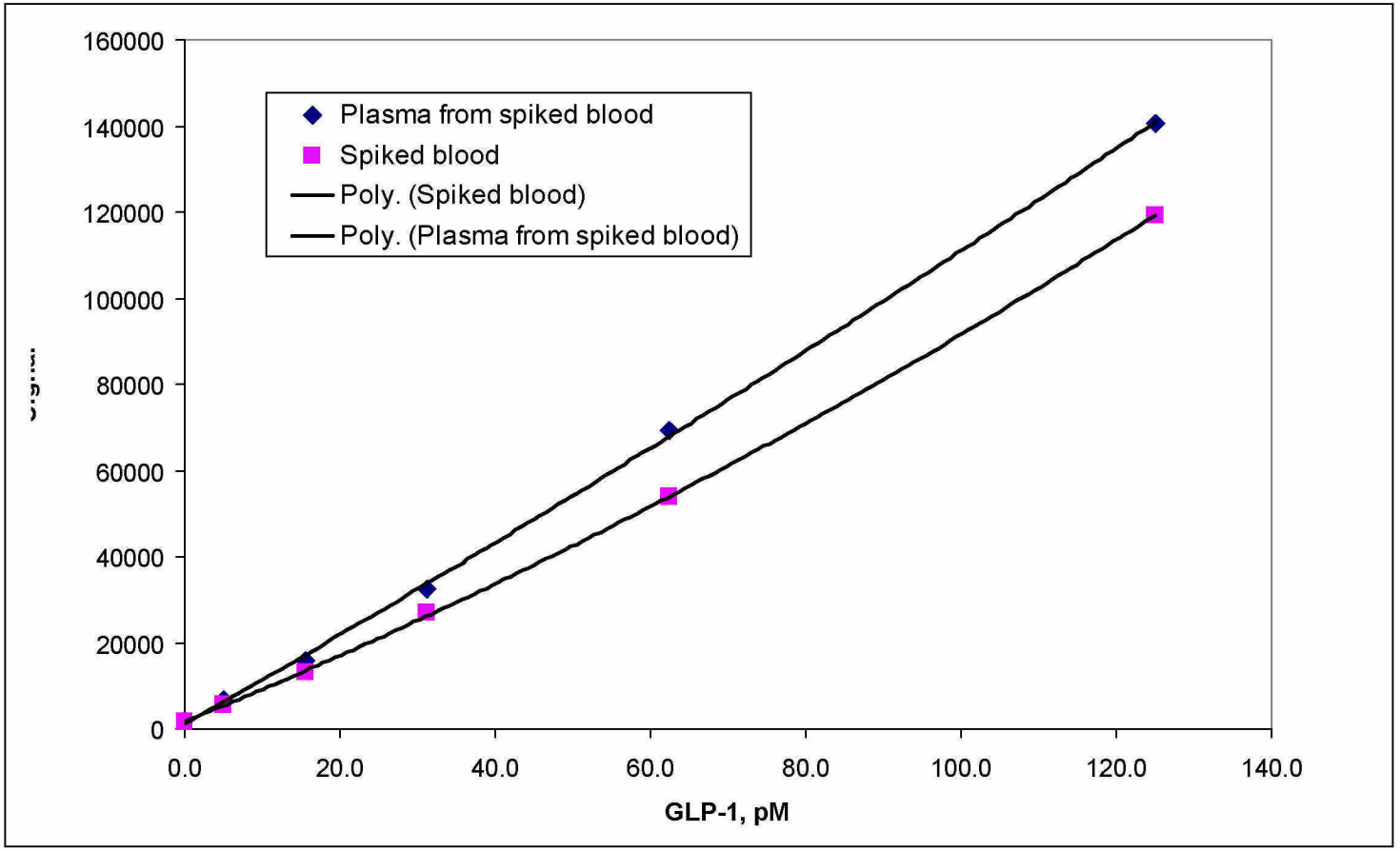
Example of Calibration for Blood



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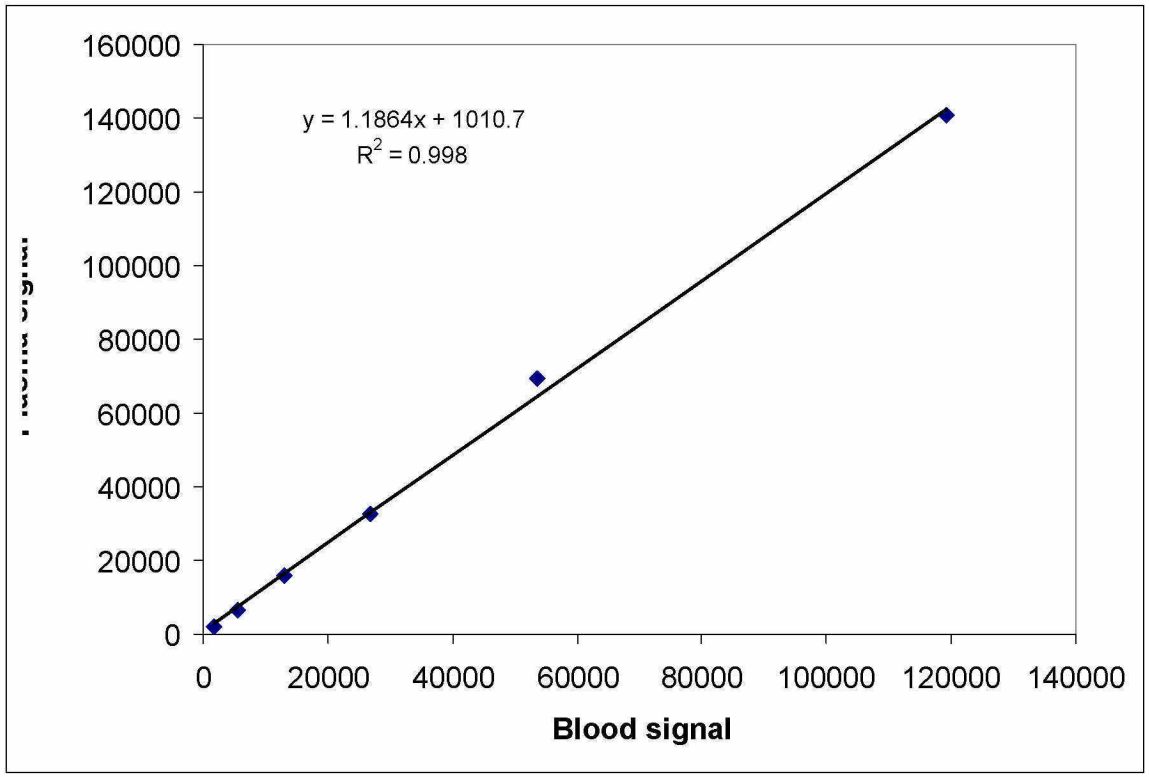
Calibration for Blood

A predictable relationship, which can be incorporated into calibration, exists.



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Produce, Evaluate Validation Cartridge Lots

Produce three lots with different batches of raw materials.

Make and release several sub-lots for each lot over several days.

Combine released sub-lots for calibration.

Calibrate.

Calibration stability is monitored.

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Establishing Reportable Ranges

Range achievable for all cartridge lots.

Range is fixed for assay, NOT for individual lots.

Ranges for plasma and blood may be different.

LOD varies lot to lot but always < specified minimum.

Analyte level well above the highest reportable level is tested to verify no hook.

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In-House Validation Exercise Examples

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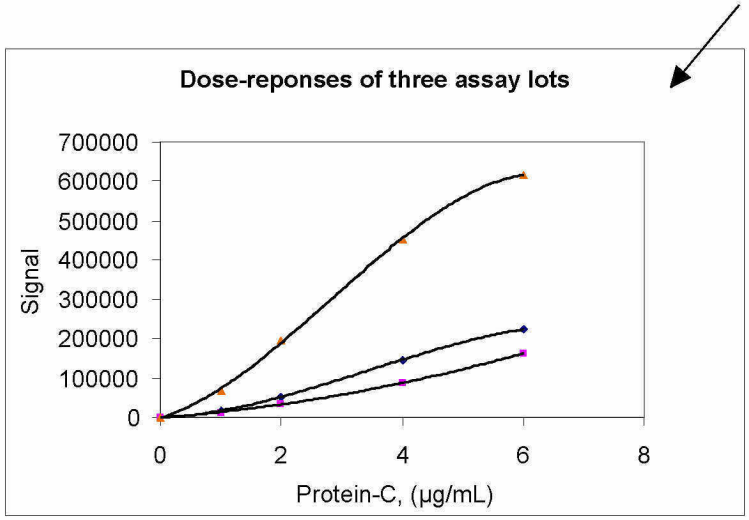
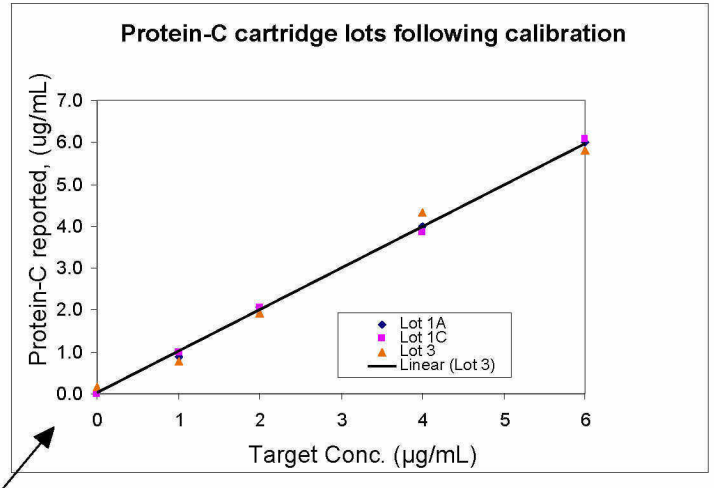
Cartridge Lots Calibrated to the Same Standard

Cartridge lots with different responses can be accurately calibrated to the same standard.

Early development stage cartridge lots can have good (but very different) dose-response.





After calibration, all lots report out the same result.

Once developed, each lot of cartridges have very similar dose-responses and provide identical results after factory calibration.

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Calibration Accuracy for Two Cartridge Lots

Analyte	Conc. Target µg/mL	Conc. Recovered µg/mL Lot 1a	Conc. Recovered µg/mL Lot 1c	Recovery % Lot 1a	Recovery % Lot 1c
Protein-C	6.0	5.9	6.1	99.0	101.6
Protein-C	4.0	4.0	3.9	101.1	96.6
Protein-C	2.0	2.0	2.1	98.8	103.2
Protein-C	1.0	1.0	1.0	100.2	98.8
Protein-C	0.0	0.0	0.0		
Protein-C Average				99.7	100.0
CRP	300.0	290.1	286.2	96.7	95.4
CRP	150.0	154.6	162.5	103.1	108.3
CRP	60.0	62.1	56.2	103.5	93.6
CRP	30.0	29.0	30.7	96.6	102.2
CRP	0.0	0.0	0.0		
CRP Average				100.0	101.4

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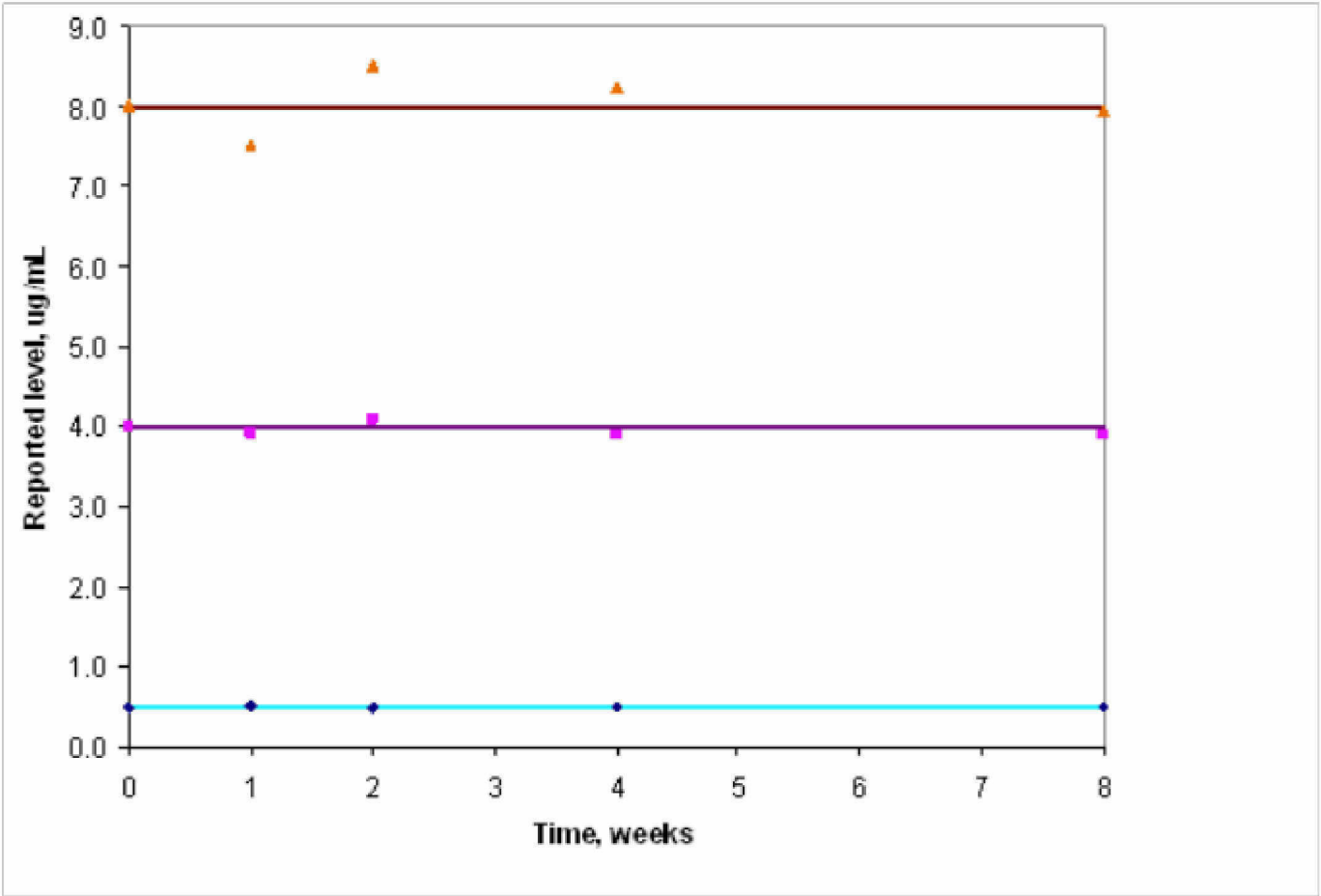
Calibration Stability

Protein-C Assay



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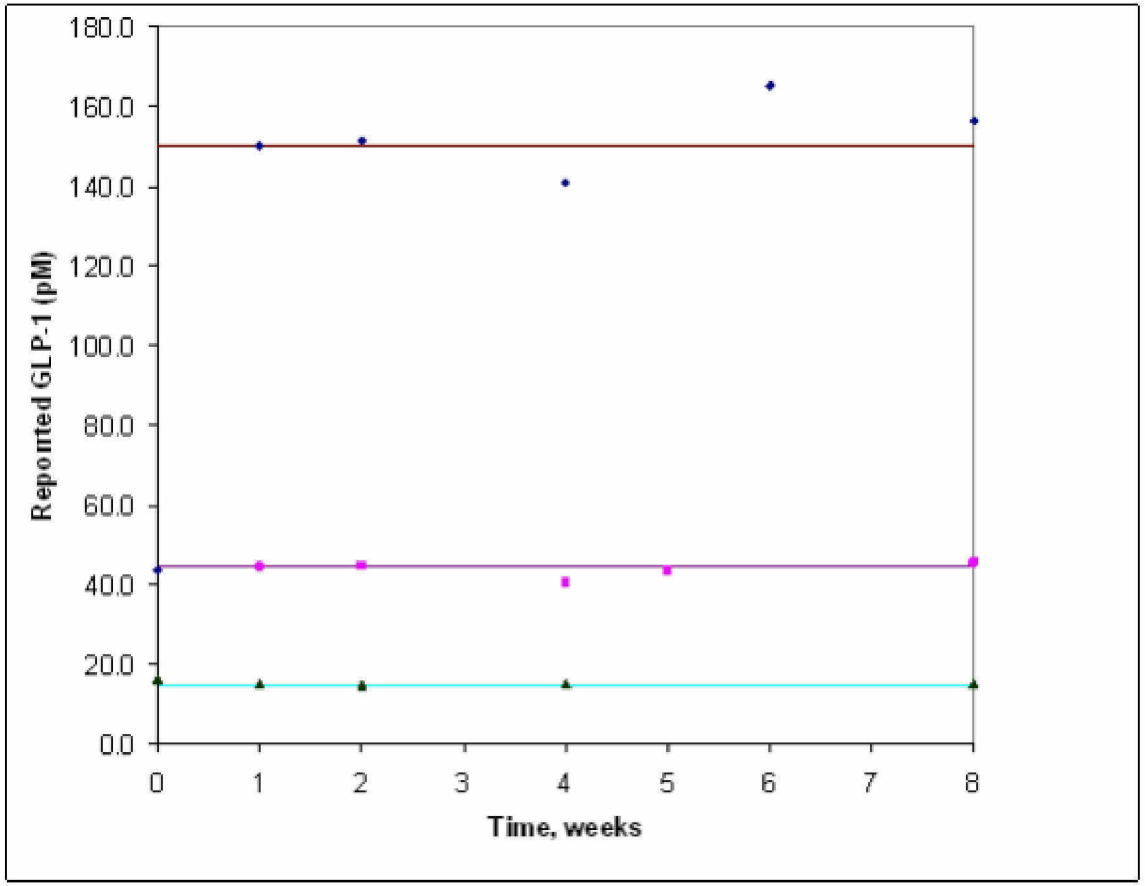
Calibration Stability

GLP-1 Assay



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Limits of Detection for Three Cartridge Lots

Analyte	Lot	LOD, µg/mL 95% conf
Protein-C	1a	0.10
Protein-C	1c	0.08
Protein-C	2	0.14
CRP	1a	0.12
CRP	1c	0.03
CRP	2	0.01

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Assay Precision (system wide)

Includes within cartridge lot and between instrument variation.

Three days, two instruments N = 6 cartridges at each analyte level.

Analyte	Conc. µg/mL	Precision % CV Lot 1a	Precision % CV Lot 1c
Protein-C	4.0	6.4	2.1
Protein-C	2.0		0.8
Protein-C	1.0	8.1	0.9
CRP	150.0	6.4	0.9
CRP	60.0		10.5
CRP	30.0	13.0	5.9

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Assay Accuracy
Method Correlations

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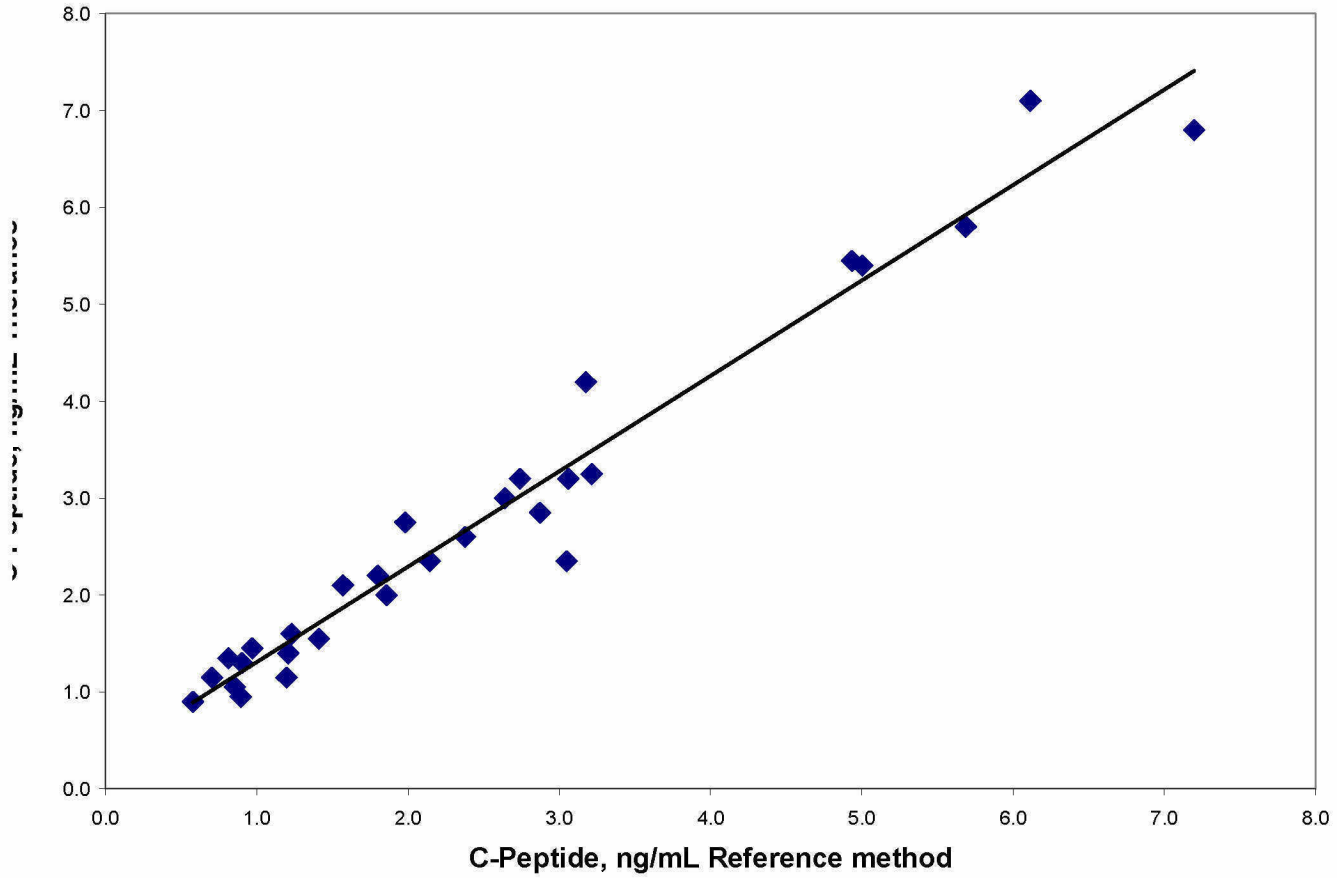
C-peptide

y = 0.985*x (ELISA) + 0.32 ng/mL; R^2 = 0.96; N = 29



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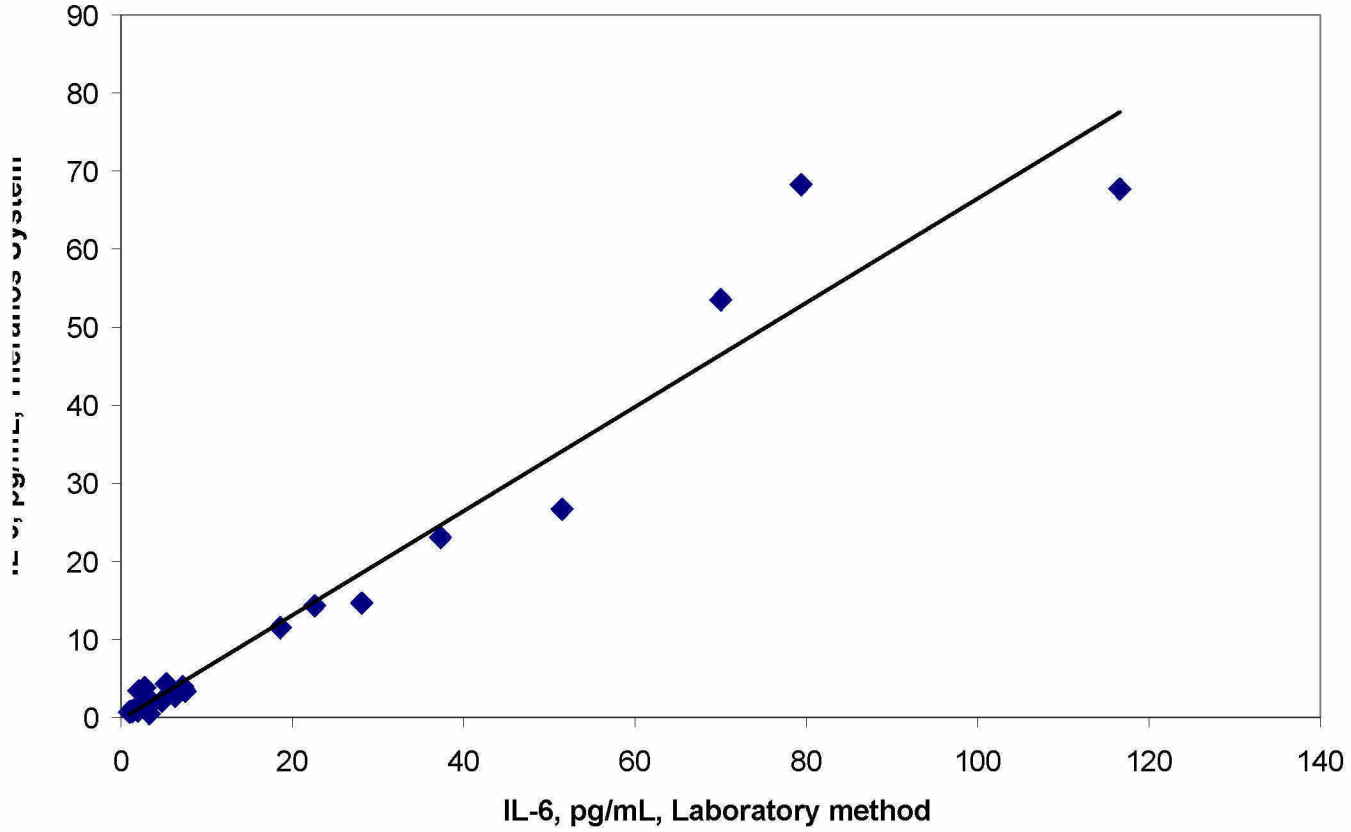
IL-6

y = 0.667x - 0.2488; R^2 = 0.95



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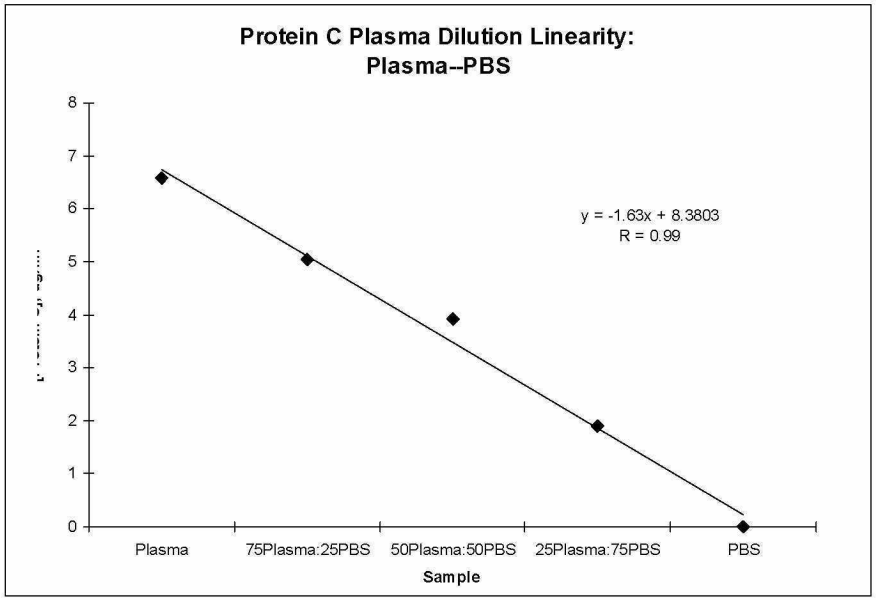
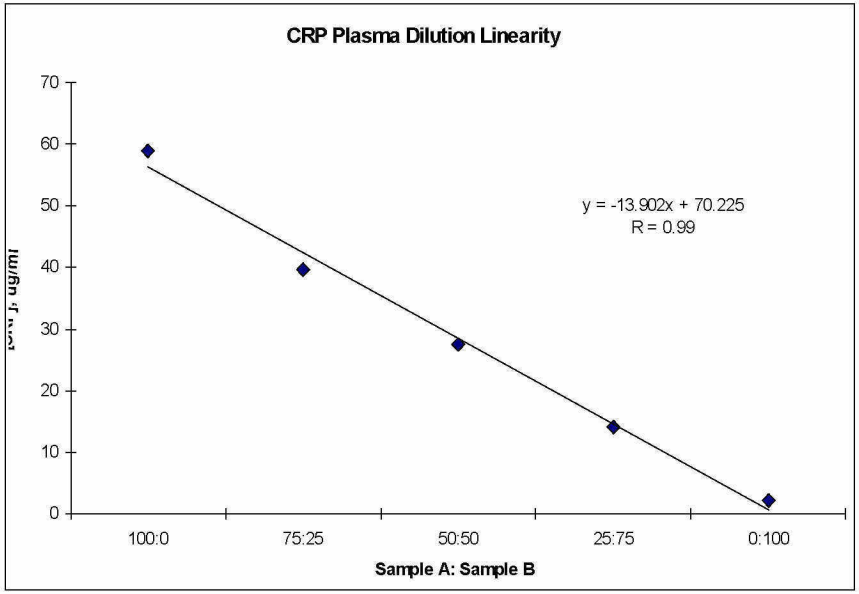
Sample Dilution Linearity





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Sample Dilution Linearity by Cartridge Lot

Repeat study with three cartridge lots.

If all lots give results within specifications, no further need to repeat the exercise, provided the chemistry is not changed.

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Specificity

Obtain a list of possible cross-reactants.

- Literature
- Package inserts
- 510ks

For multiplexed assays, combinations of cross-reactants may be used.

Define likely upper levels of cross reactants.

Measure change in reported analyte concentration due to cross-reactants.

Define levels of cross-reactants causing > 10% change in reported analyte level.

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Interfering Substances

Develop list of "usual suspects."

- Hemolysis, lipemia, icteria
- Rheumatoid factor, heterophile antibodies
- Any drugs or "unusual" substances likely to be in samples from particular clinical situations
  - For example, Avastin binds to VEGF

Determine likely upper level of interferents.

Use clinical samples with known high levels of interferents or spiked with interferent.

Determine level resulting in > 10% change in reported analyte level.

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Assay Validation Timeline

multiplex: three analytes

One Chemist: 12 Instruments

Three cartridge lots

Time required: 30 days excluding stability studies

Stability studies: 90 days minimum

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Release Product for Customer Use

Theranos server remains in contact with all field units for all assays.

Logs of assay and control performance are monitored. Temperatures are recorded.

Changes in performance can be observed and rectified as needed.

Instruments or cartridge lots with possible malfunction can be swapped out.

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Field Validation

Theranos Studies

- Informed consent
- IRB approval

Customers can also perform their own validation studies.

Archived clinical samples at company site

Controls

Fresh blood samples

- Fingerstick versus venous blood

Review of results with Theranos

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Monitor Performance of Product in Field

Performance across many sites can be compared.

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Documentation

Workbook with primary data

Theranos results recorded on server.

Development report (FDA/ICH guidelines)

Customer evaluation reports

510k submissions

Publications

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Regulatory Submissions

Approval for instrument

- Reader = Fluidics + Optical Detection + Temperature Control
- In effect, a micro-titration system

Approval for disposable

- Microtiterplate + Reagent kit equivalent

Specific assay multiplexes

- Where needed, a 510k will be filed for each

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Manufacturing Quality Oversight

1. All cartridge and reader manufacturing is in-house at Theranos.
2. The Theranos Quality System is in place, as documented by our Quality Manual QM-00001 and Quality Systems Procedures.
3. Production processes use Standard Operating Procedures and Manufacturing Batch Records.
4. Quality Control in-process inspections are throughout the production processes.
5. Final outgoing QC is performed on Readers and Cartridges.

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Manufacturing Quality Oversight

6. Inventory is controlled by a Quarantine and Release process.
7. The Purchasing SOP is used with lot traceability.
8. Employee training required for all production positions.
9. Formal Corrective And Preventive Action procedures in place.

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Product Development Quality Oversight

Theranos Quality System for product development in place, as documented by our Quality Manual QM-00001 and Quality Systems Procedures.

Phased approach to product development and introduction.

Product Data Management system in place (Agile) and in use for product development, product revision control, and enterprise change orders.

Reader product regulatory testing and compliance

- FCC, EC, CE, UL, etc.

Product reliability testing

- Temperature, shock, vibration, etc.

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Controls

System can measure commercially available controls.

Cartridges contain high and low internal controls.

Assay results are released only if control performance meets specifications.

Cartridge and instrument performance is monitored (100%) by a Theranos server.

Any trends or changes in calibration are detected and remedial action taken if needed.

Results are only released to customers if system performance is in control.

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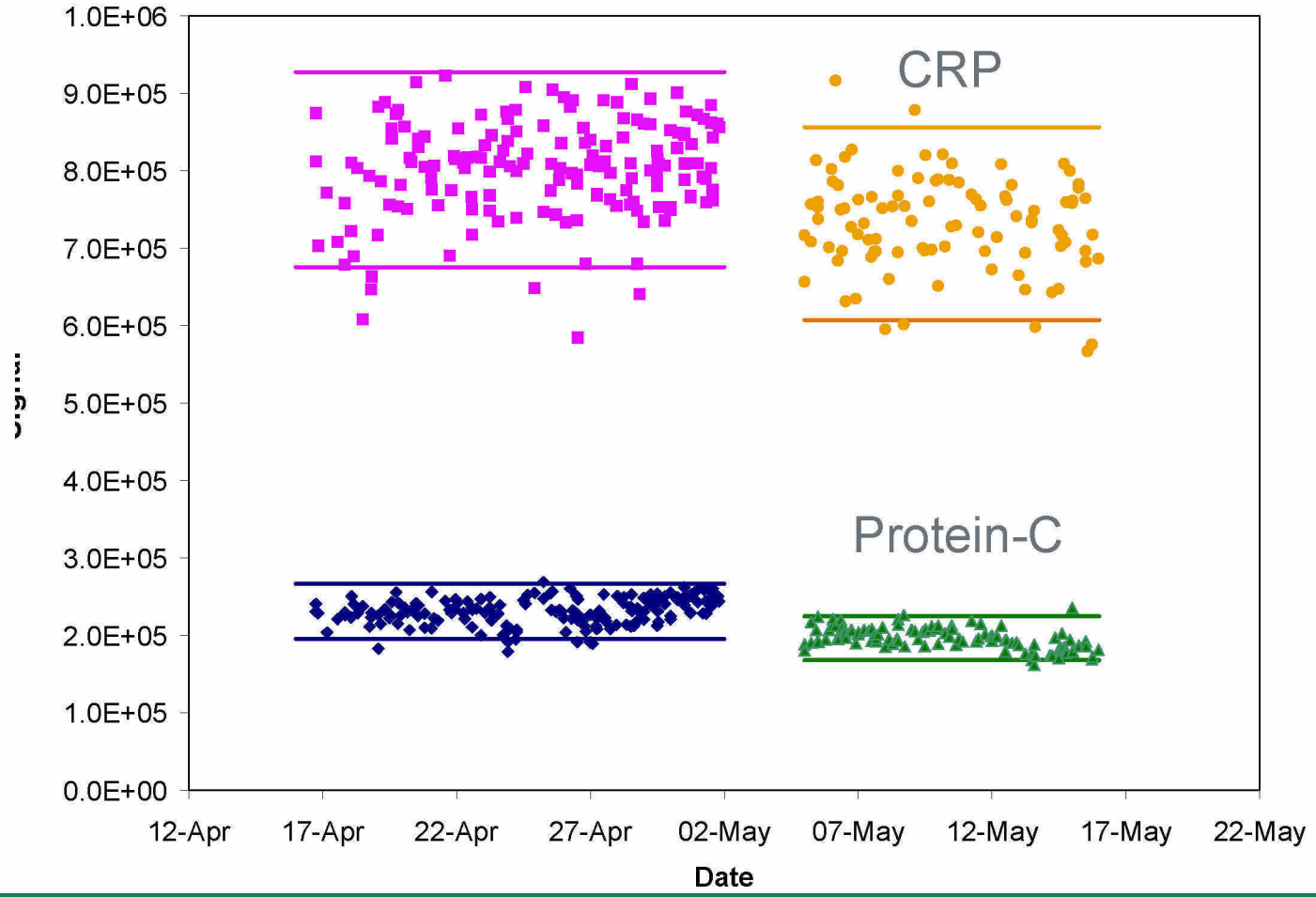
Internal Bi-Level Controls

Two cartridge lots – 30 instruments



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Validation of Multiplexes

No validation of Theranos multiplexed assays is required since:

- Reagents for each assay are physically separate.
  - No chemical cross-talk is possible.
- Each assay is read within a shielded optic.
  - No physical cross talk is possible.
- Dilution schemes are designed so that the diluent does not impact the assay if different multiplexed with any given assay are required.

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Validation of multiplexes

Analytes that cross react in samples

Example: VEGF and sVEGFR2

- VEGF + VEGFR2 <> VEGF:VEGFR2
- Assay response is determined for individual analytes and mixtures.
- Assays are calibrated with analyte mixtures.

Drugs that react with analytes

Example: VEGF and Avastin (an antibody)

- VEGF + AB <> VEGF:AB
- Impact of drug is established in field studies and/or assay validation.

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Sample Re-analysis

In customer validation exercises

- Sample archives are produced.
  - Samples stabilized by flash freezing.
  - In some cases stabilizing agent is added.
- Re-analysis at company and/or Theranos
  - Reference methods
    - In cases where there are discrepant results, more than one reference method is used.
  - Theranos system

Monitoring subjects over time

- Some anomalies can be seen as a discontinuity in the time course.

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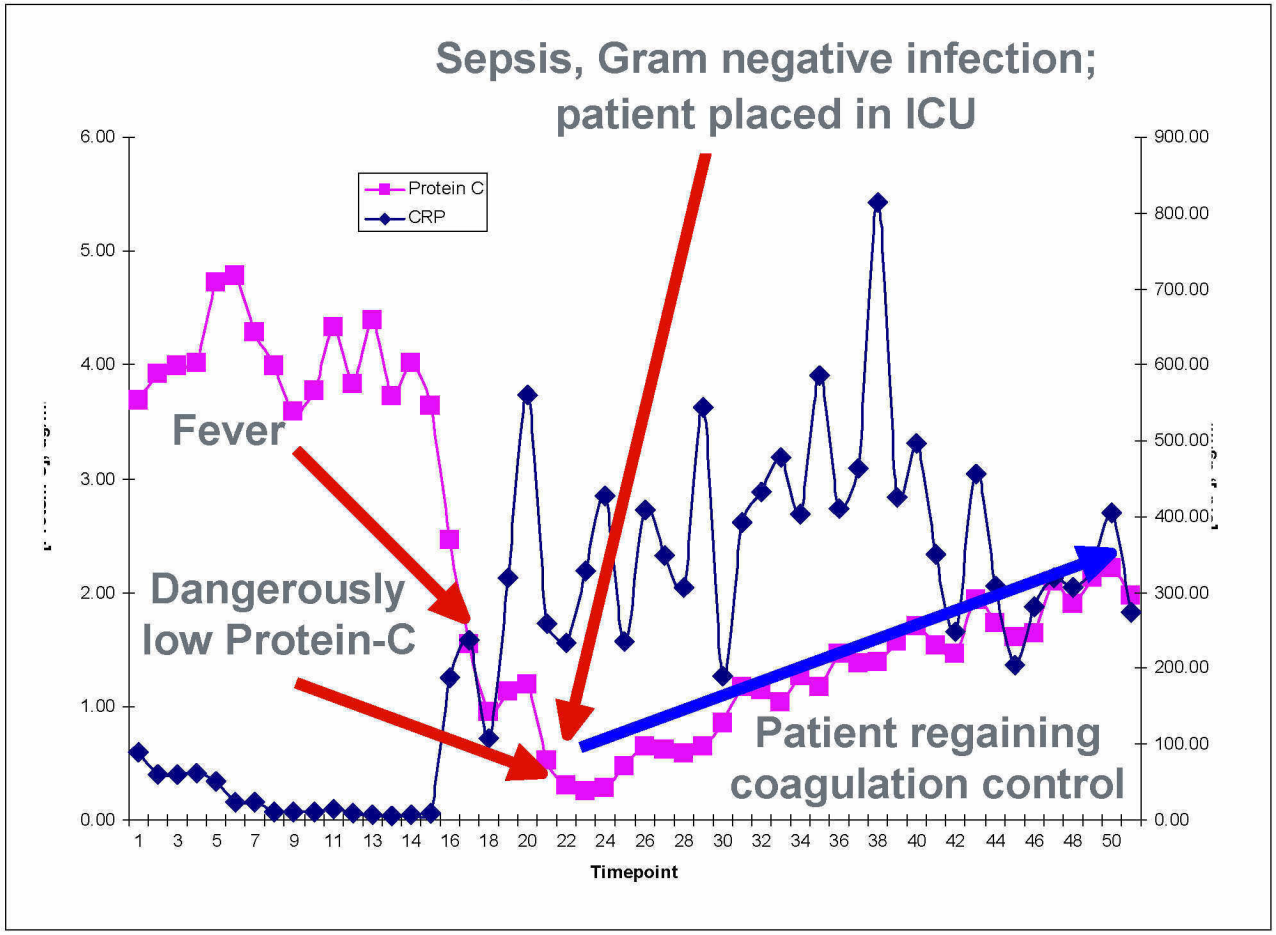
Use of Time Series Multiplexed Measurements

Example: Monitoring an AML patient for sepsis



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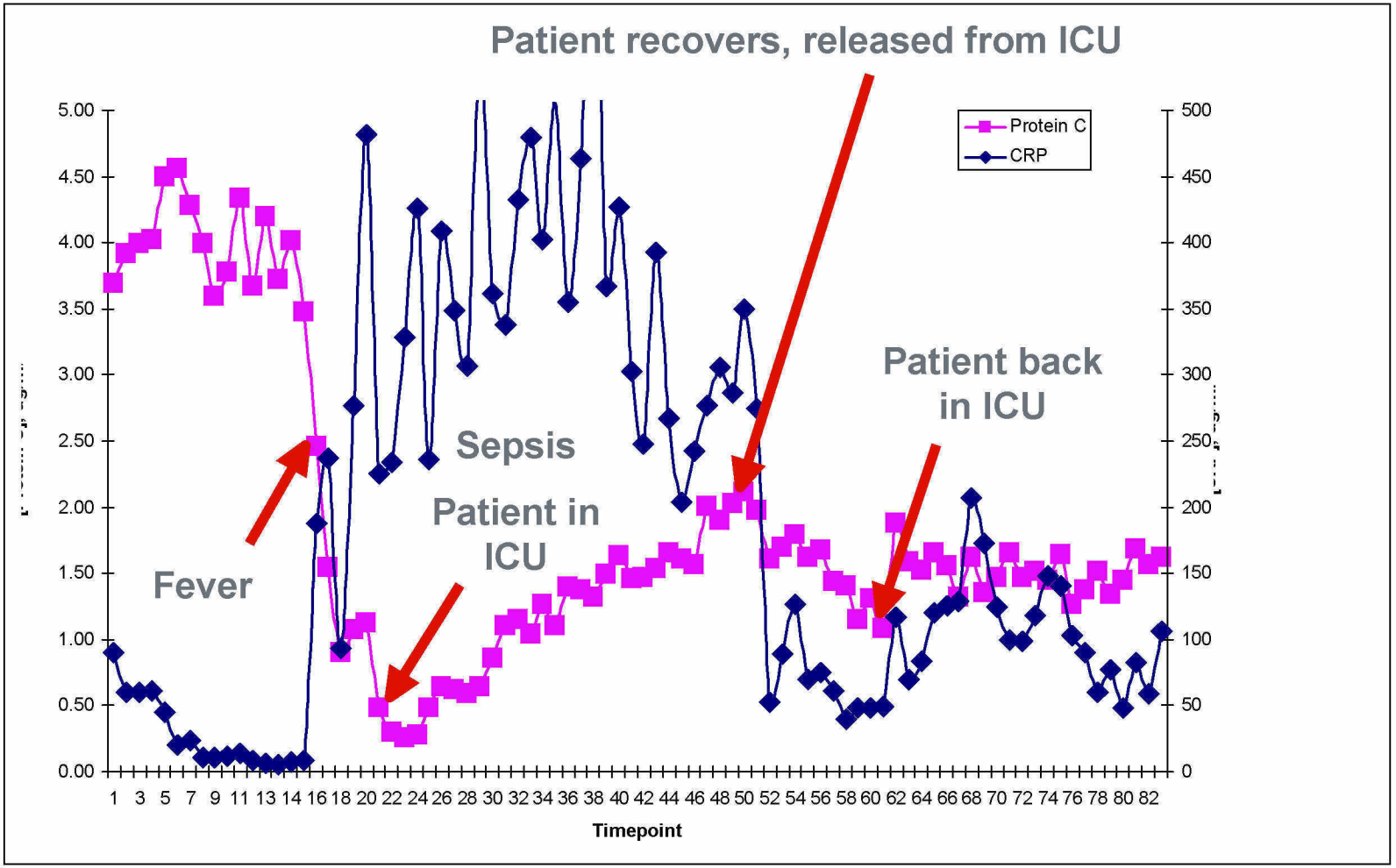
Patient Relapse



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Antibodies to Small Molecule Drugs for use as Reagents for Immunoassays

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Enabling Small Molecule Drug and Hormone Assays

Immunoassays can be set up.

Antibodies must be available or have to be created.

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Making Antibodies to Small Molecules

Landsteiner

Small molecules

- Covalently attached ("haptens")
- To "carrier" proteins
  - Albumin, IgG, KLH
  - High Hapten/Protein ratio
- Repeated immunization (hyperimmunization)
- Freunds' adjuvant

Evoke strong immune responses.

Monoclonal/Polyclonal antibodies can be made.

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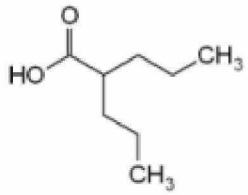
Anti-Drug Antibodies

Small molecule drugs are good haptens.

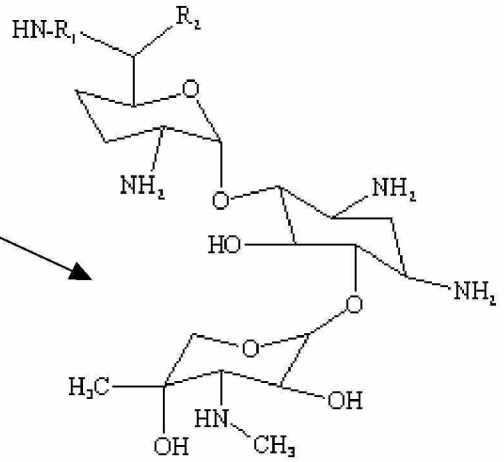
Molecules as small as valproic acid evoke a good response.



Rigid (carbamazepine)

Flexible (gentamycin)

No known case where antibodies cannot be made.



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Examples of Commercially Available Small Molecule Drug Immunoassays

Drugs of Abuse/Toxicology

Acetaminophen
Amphetamine/Methamphetamine
Barbiturates II U
Benzodiazepines
Benzodiazepines-Serum
Cannabinoids
Cocaine Metabolite
Methadone
Opiates
Phencyclidine II (PCP)
Propoxyphene
REA Ethanol
Salicylate
Tricyclic Antidepressants

Therapeutic Drug Monitoring

Amikacin
Carbamazepine
Cyclosporine
Digoxin
Gentamicin
Methotrexate
N-Acetylprocainamide
Phenobarbital
Phenytoin
Procainamide
Quinidine
Theophylline
Tobramycin
Valproic acid
Vancomycin

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Hapten Design

Inspect structures.

- Drug
- Metabolites, if known

Define which molecules are to be recognized by Ab.

- Do we want to see the metabolites?

Define structural elements likely to evoke Ab.

Select linking sites.

- Not important features
- Not in the middle of the molecule
- Best to have more than one

Design hapten or hapten precursor.

- Select linking chemistry
  - NHS-ester
  - Thiol-maleimide

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Why Two Haptens?

1. Antibody response is to protein-linker-hapten.
2. Anti-protein antibodies are screened out.
   - Screen against hapten conjugate of a protein different than that used as immunogen.
3. Some antibodies partly recognize linker.
   - Especially true in polyclonals.
4. Using second hapten to make reagent conjugates.
   - Enzyme-hapten in our case
   - No possibility of binding to linker in reagent.
   - For monoclonals, this is a lower concern.

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Why Two Haptens?

5. Making more than one hapten increases the chance of success.
   - Antibody production is a slow process.
   - By working in parallel with several options we greatly increase the success rate.
6. In many cases a single hapten can be used, however.

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Preparation of Haptens

Starting materials can be the drug or drug intermediates available from drug company.

Drug company may do some straightforward synthetic work.

Synthesis requiring only a few straightforward steps can be outsourced to local synthesis shops.

Activation (if needed) is done at Theranos.

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Activation of Hapten (if needed)

Thiol and maleimide haptens

- "Ready to go"

Carboxylic acid haptens

- Require activation as NHS-esters,
- If this is required, it is performed at Theranos.
- This must not destroy any antigenic features of the hapten.

Chemistry is:

- One-pot aqueous
- Performed in water miscible organic solvents.

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Hapten Synthesis

Consult with synthetic chemists.

Design synthetic routes (> 1 if possible).

Make hapten/activated haptens.

- Ideally, gram scale
- Need good purity

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Hapten Conjugation

R = hapten P = Carrier protein or Enzyme

NHS (N-Hydroxy-Succinimide)-ester

- R-CO2H + EDCI + NHS → R-CONHS
- R-CONHS + P-NH2 → R-CONH-P

Thiol: Maleimide (Michael addition)

Case 1

- P-NH2 → P-NH … Maleimide
- R-SH + P-NH … Maleimide → R-P

Case 2

- R-Maleimide + P-SH → R-P

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Steps in Antibody Production

Similar process to that for making antibodies to protein antigens.

- Outsourced from Theranos
- Polyclonal and monoclonal programs
- Early screening at vendor
- Selection of candidate animals
- Fusions and cloning
- Clonal selection
- Strong candidates screened at Theranos in specific assay format.
- Bulk production (MCs 100 mg; PCs 10s of mLs)
- Clones stabilized and archived (two locations).

All materials and IP owned by Theranos (and Partner).

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Timeline

Hapten Design: Weeks

Hapten Synthesis: Weeks to three months

Hapten Conjugation: Days

Antibody Production: Six months

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Assay of a Steroid Hormone

Example of a Small Molecule Immuno-Analyte

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The Target: Progesterone

Progesterone measurements are made to evaluate fertility and menstrual cycle.

During the menstrual cycle, levels of progesterone in blood are low prior to ovulation and then rise to a peak of about 10 ng/mL.

During pregnancy, levels progressively rise, reaching 55-255 ng/mL in the third trimester.

In males, levels are generally low (< 1 ng

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The Reagents

Surface: PS:aMIgG:Mouse α-Progesterone

Conjugate: Progesterone-labeled Apase

Antibody to: Progesterone

Immunogen: Progesterone-11-BSA

Progesterone binds to red cells and plasma proteins, including steroid-binding-globulin and albumin.

The clinical correlation shown below is good evidence of the assay specificity and lack of significant interferences.

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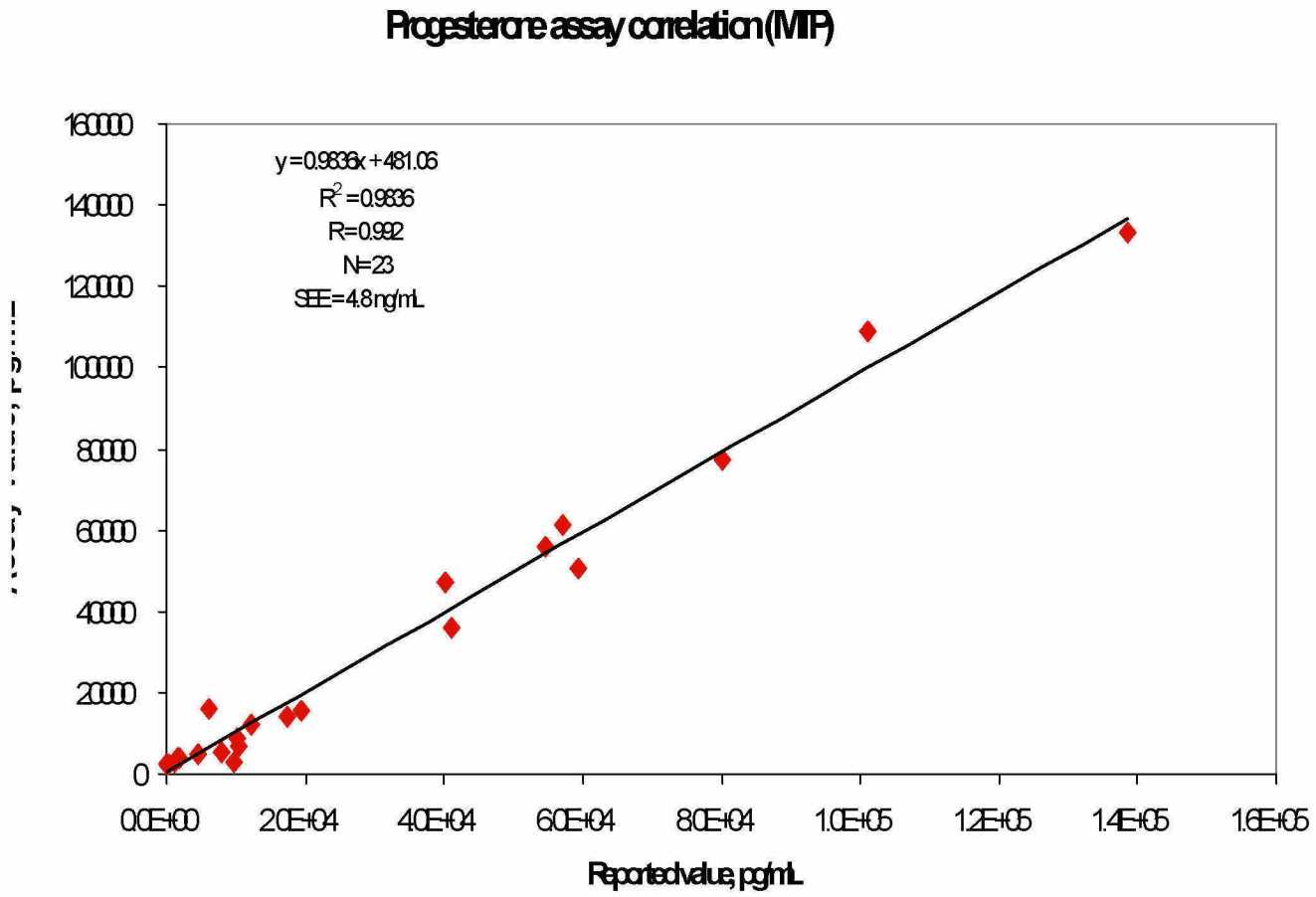
Assay Correlation: Theranos System vs. Reference



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Proprietary Assays: Three Examples

Antibody drug

- Humanized monoclonal IgG antibody

Proprietary assays for cell death and liver toxicity

- M30 (apoptosis)
- M65

Obviously each assay is case-specific.

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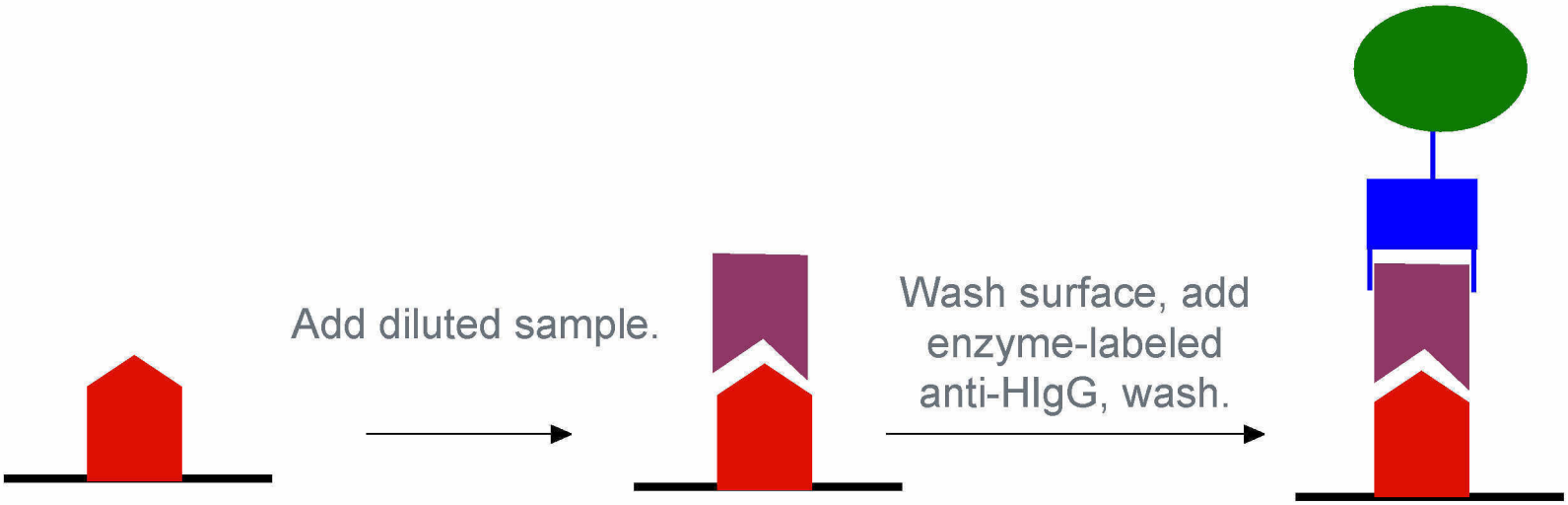
Antibody Drug: Assay Configuration



Attach proprietary anti-ideotope antibody to solid phase.

Antibody (drug) binds.

Quantity of enzyme bound to surface is directly proportional to drug concentration.

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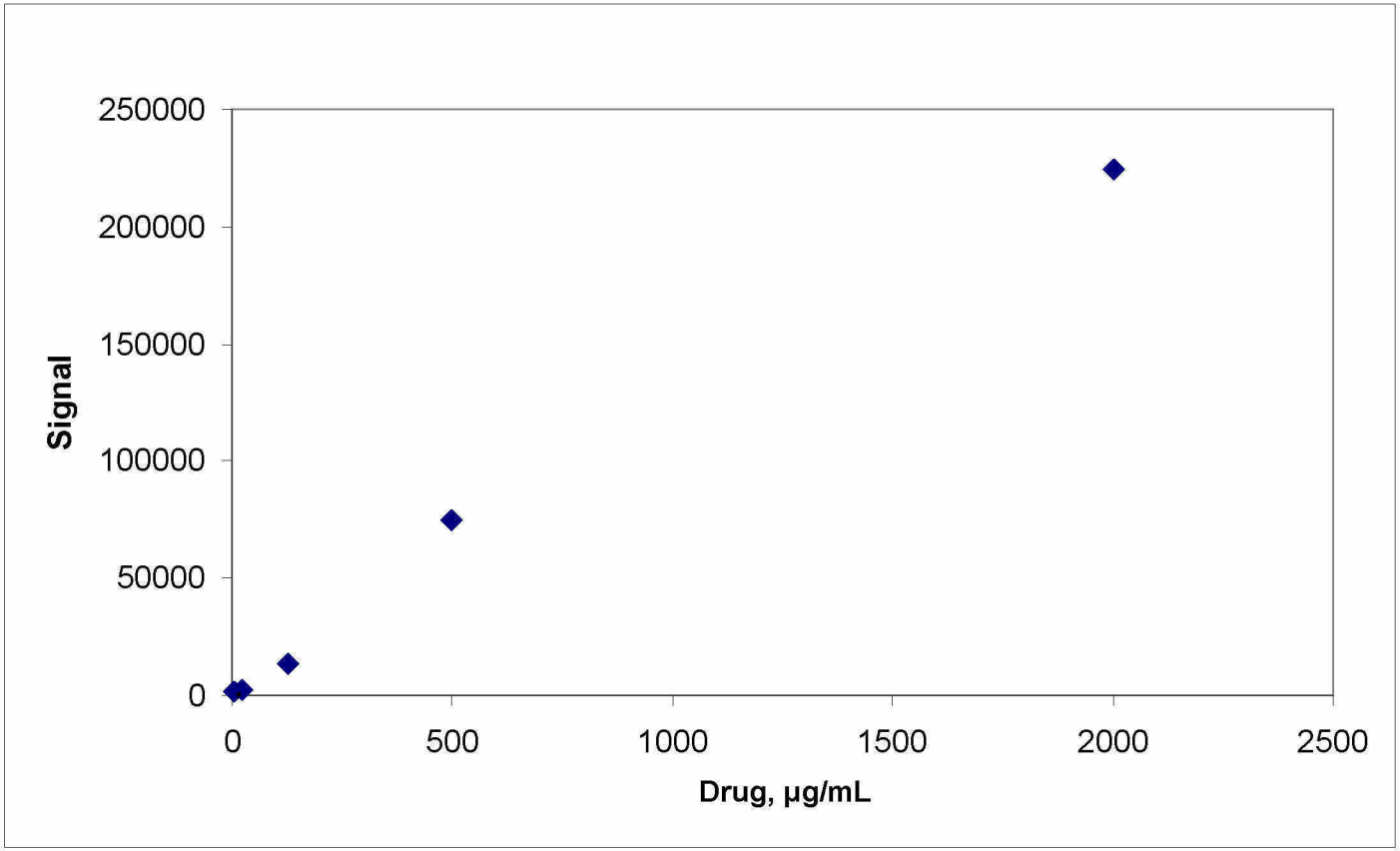
Assay Dose-Response



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Cell Death Assays

Apoptosis and liver toxicity

- M30

Cell Death and liver toxicity

- M65

Proprietary reagents

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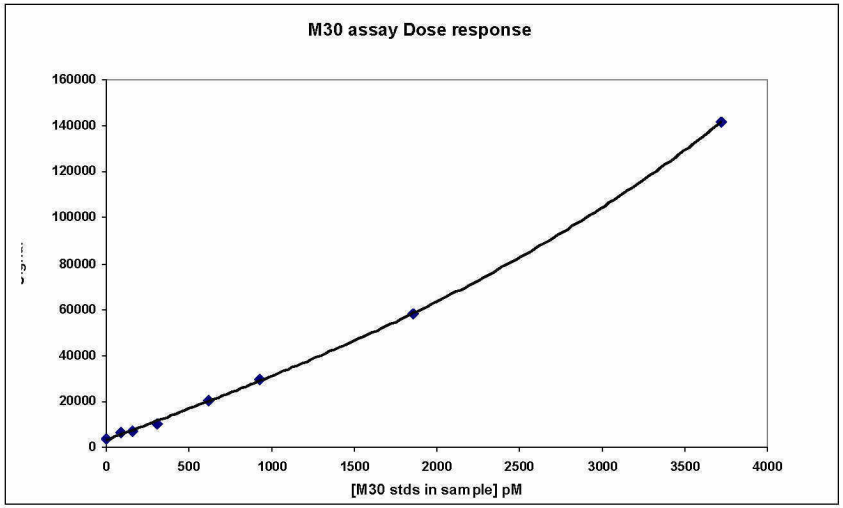
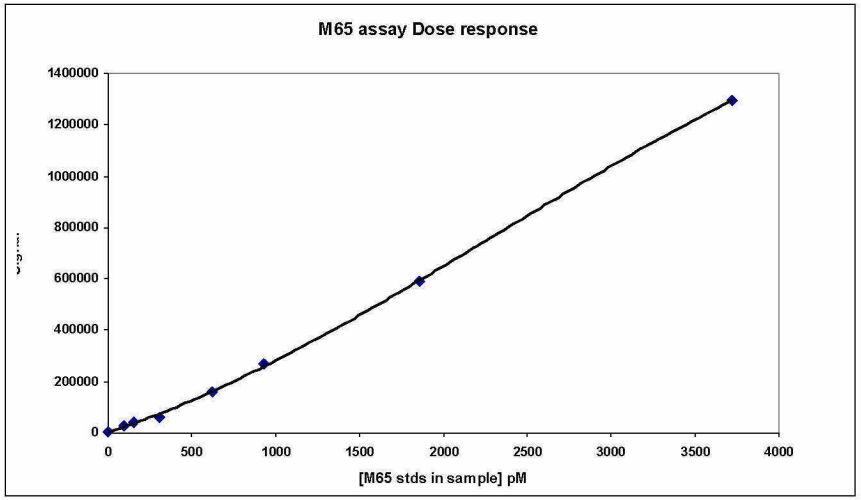
Cell Death and Liver Toxicity Assays: Dose-Responses

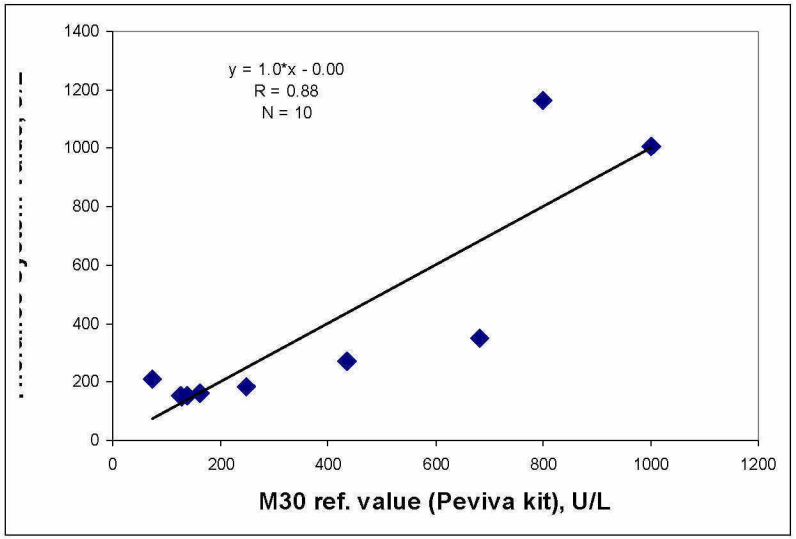




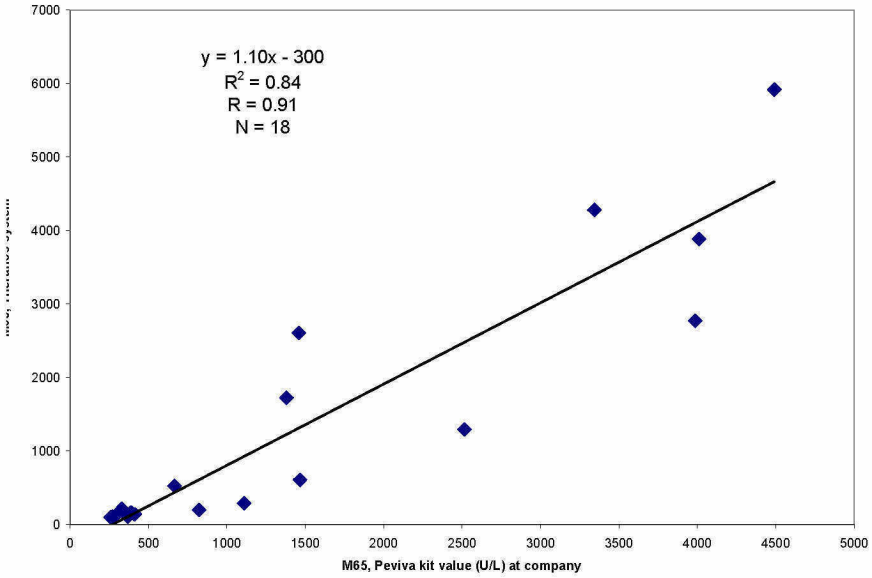
Pre-clinical Results

Archived plasma samples from drug company study

M30



M65



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Multiplexed Assay Development Case Study
PK and PD Assays

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Case Study Objective

Provide a clinical assay system for time PK/PD profiling in plasma and whole blood samples from Phase 1 studies of an asthma drug.

PD analytes selected:

- PD-1
- PD-2
- PD-3
- PD-4
- PD-5

Proprietary assay for drug

- Key reagents provided by company.

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Assay Formats

PD assays

- Two antibody sandwich ELISA
- Biotinylated capture antibody on the solid phase
- Analyte and detection antibody in solution phase
- Detection antibody is Alkaline Phosphatase labeled.

PK assay: Analyte: humanized antibody (IgG)

Capture reagent: anti-idiotype provided by Company

Detection antibody: anti-human IgG

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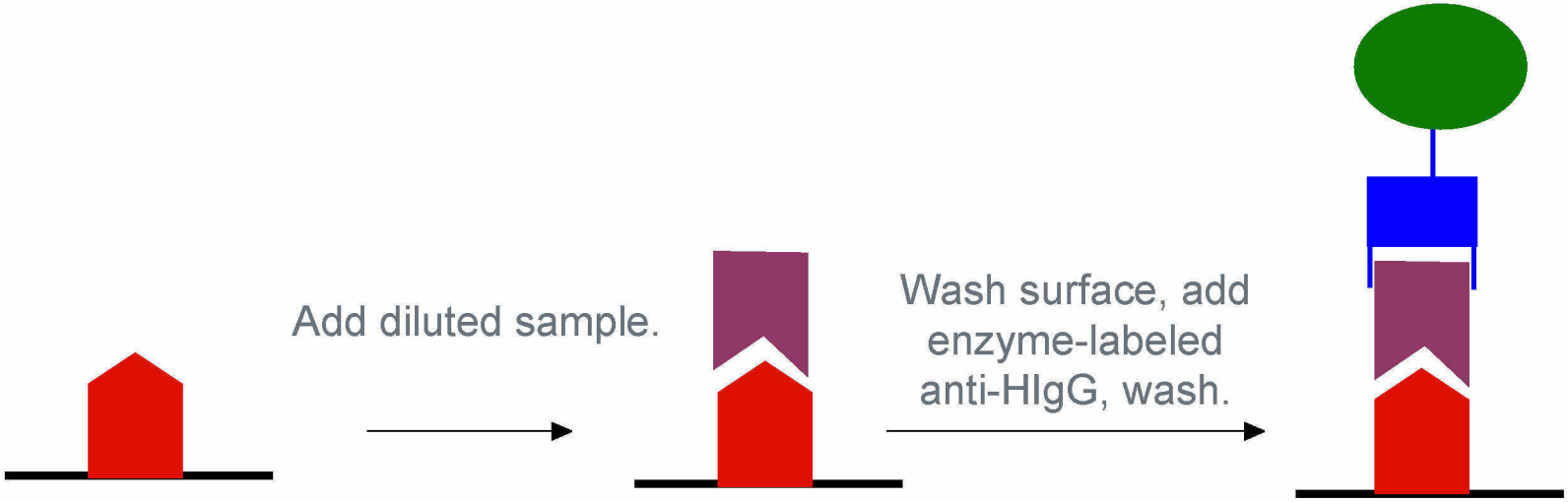
PK Assay Configuration



Attach proprietary anti-ideotope antibody to solid phase.

Antibody (drug) binds.

Quantity of enzyme bound to surface is directly proportional to drug concentration.

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Antibody Screening Summary

Antibody screening on 384 well MTP.

Each antibody is conjugated to test as capture (biotin) and detection (Alk. Phos.) reagents.

Each capture antibody is tested against all available detection antibodies with a 4 - 6 point standard curve spanning the assay range.

Initial testing in Assay buffer followed by finalization of the pair in Plasma/Whole blood.

Assay	# of Antibody Pairs Tested	# of Pairs Response Seen
PD-1	121	18
PD-2	11	11
PD-3	25	11
PD-4	36	2
PD-5	16	12
Drug	2	2

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Antibody Screening Example: PD-1

Number of Capture antibody tested : 11

Number of Detection antibody tested: 11

Total Number of antibody pairs tested: 121

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Assay Ranges

Theranos assay ranges are determined on the basis of

- normal and disease state levels from Literature
- reference assays
- customer requirements

	Assay	Range	Units
1	PD-1	15 - 2000	pg/mL
2	PD-2	23 - 6000	pg/mL
3	PD-3	25 - 1000	pg/mL
4	PD-4	0.5 - 500	ng/mL
5	PD-5	25 - 2400	ng/mL
6	Drug	0.1 - 2000	µg/mL

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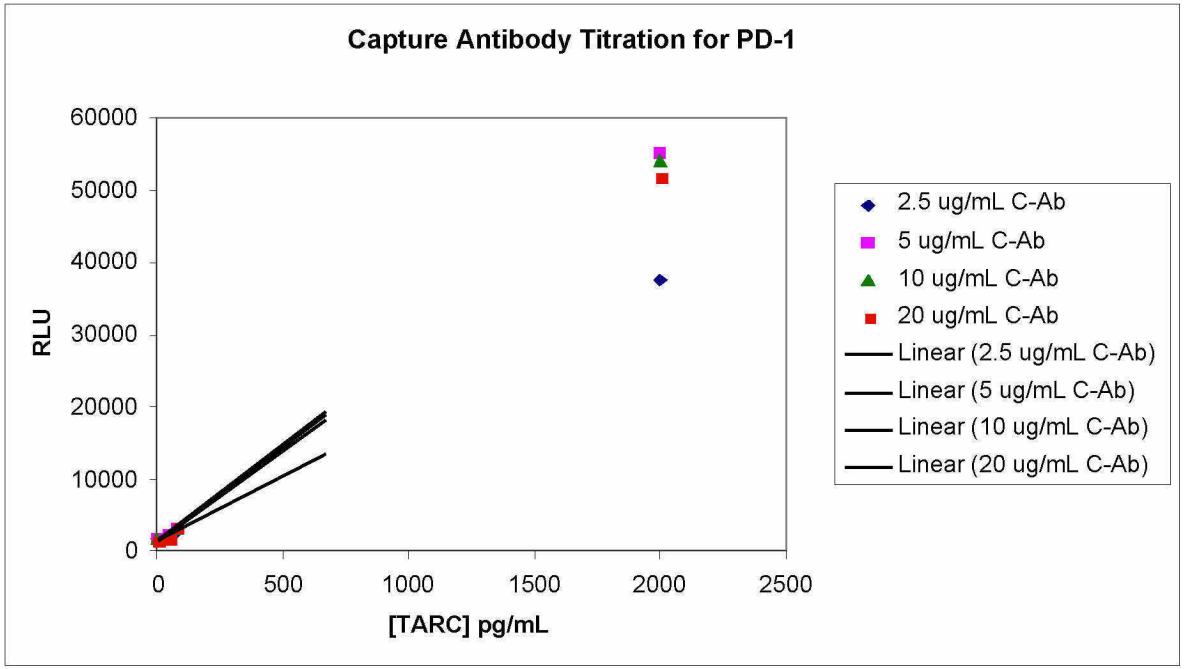
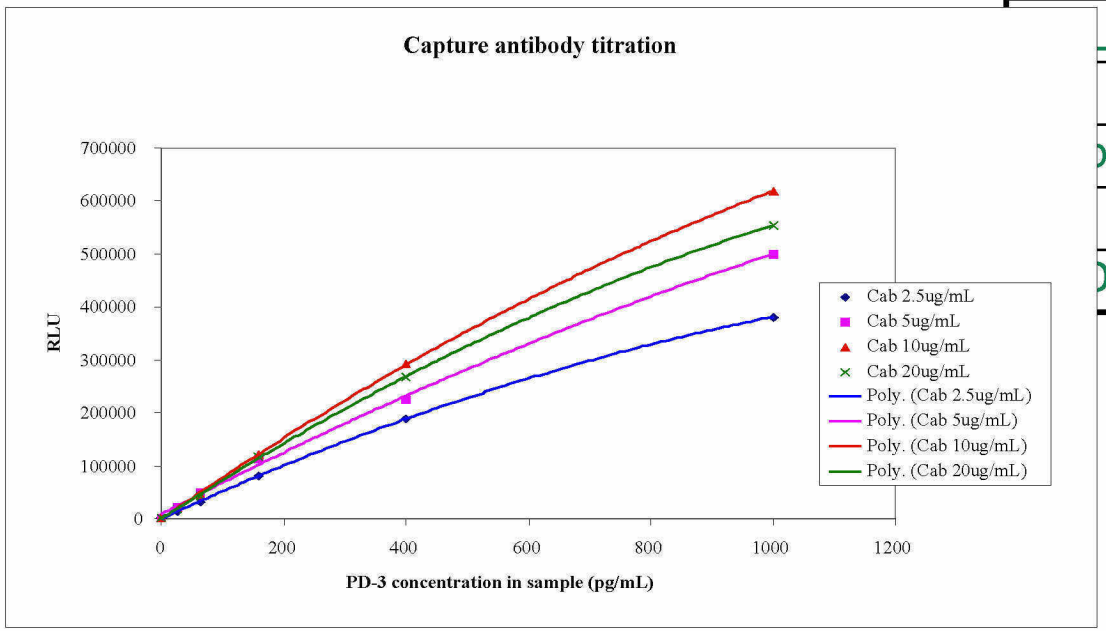
Capture Antibody Titration

Capture antibody is titrated on capture surface to determine optimal concentration. Typically, four concentrations of capture antibody are tested. In general, there is little change in assay response over a wide range of Capture Antibody concentration.



Capture Antibody Titration for PD-3

Generally, assay response is not very sensitive to capture Antibody load (Ab is in excess over Ag).



SUMMARY

CAB, µg/mL	20	10	5	2.5
S/B_StD 1/6	229	240	166	206
_StD 5/6	7.7	8.0	7.2	6.9
CV	9	11	11	11
be	626	653	739	483
StDev	1971	2761	1878	2216
, pg/mL	6.30	8.46	5.08	9.18

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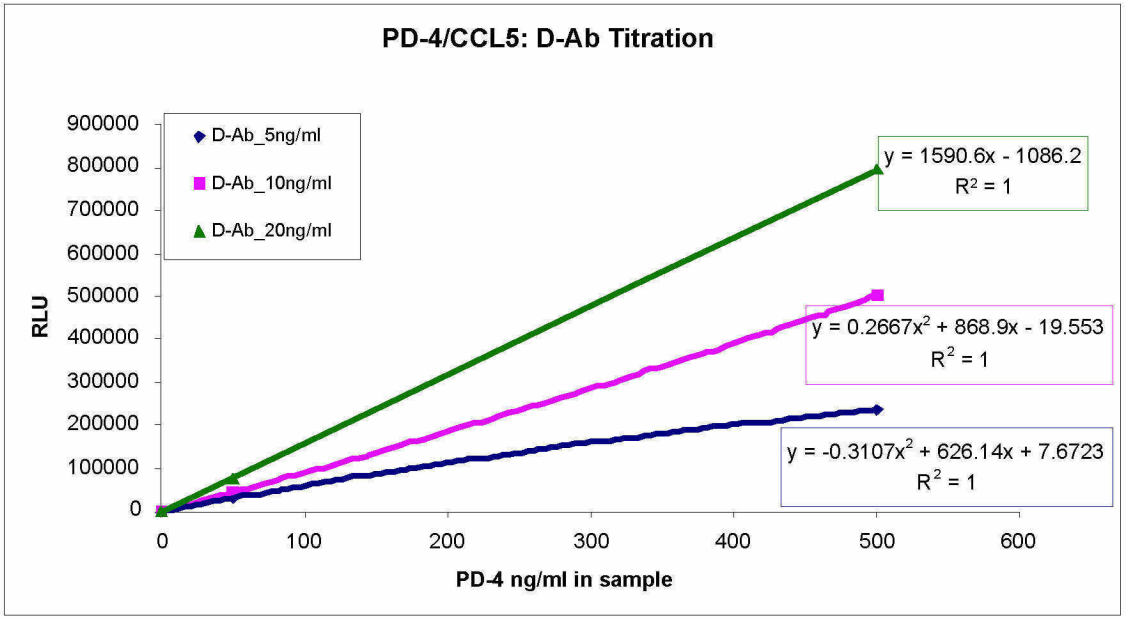
Detection Antibody Titration: PD-4

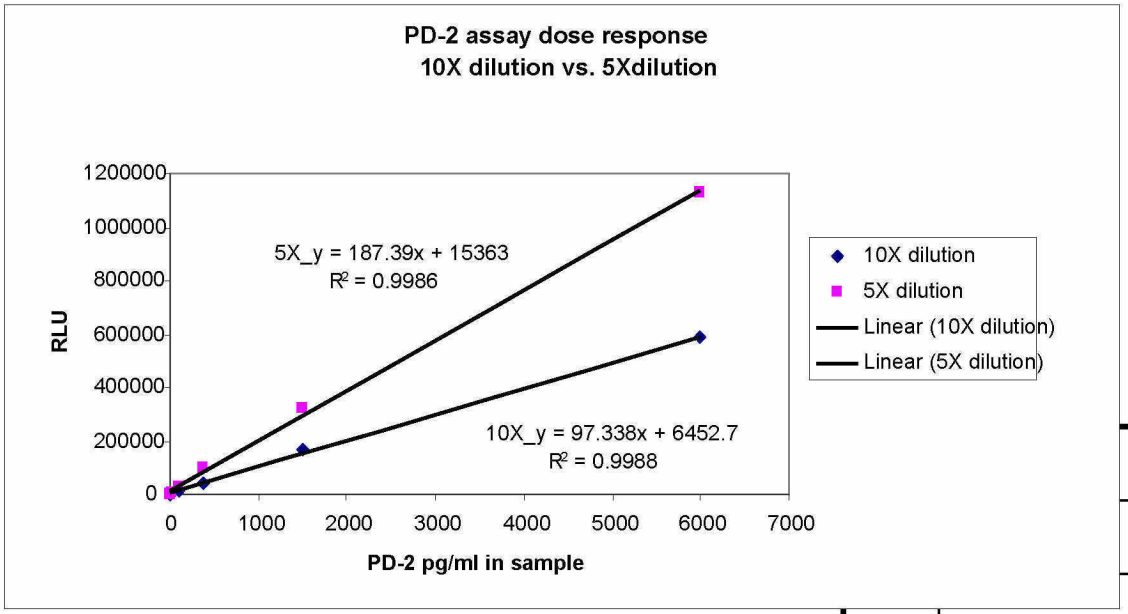
Response is typically directly proportional to reagent concentration.



SUMMARY

D-AB, ng/mL	5	10	20
S/B_StD 1/6	2096	4763	6910
S/B_StD 5/6	1.9	2.7	4.5
Avg CV	25	14	7
Slope	611	882	1526
Avg StDev	3893	2175	2550
LOD, ng/mL in sample	12.75	4.93	3.34

Protocol Optimization: Dilution



		10x dilution			5x dilution		
		Avg.	StD	CV	Avg.	StD	CV
		586592	27275	5	1131962	72859	6
2	1500	167840	9221	5	325304	44674	14
3	375	44736	2016	5	95784	20695	22
4	93.75	11752	1138	10	27269	2260	8
5	23.43	4361	931	21	7556	580	8
6	0	774	121	16	785	72	9
	Slope	114			282		
	Avg StDev	730			971		
	LOD, pg/mL	13			7		

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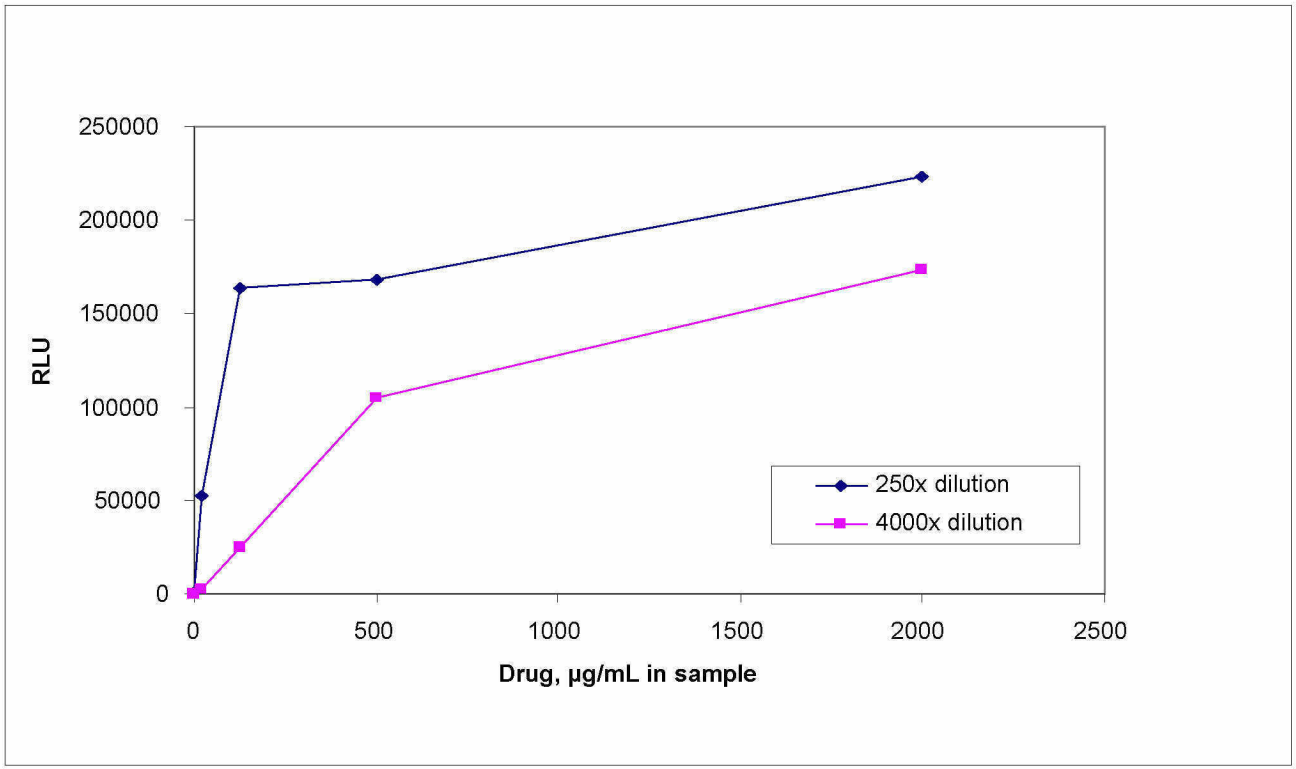
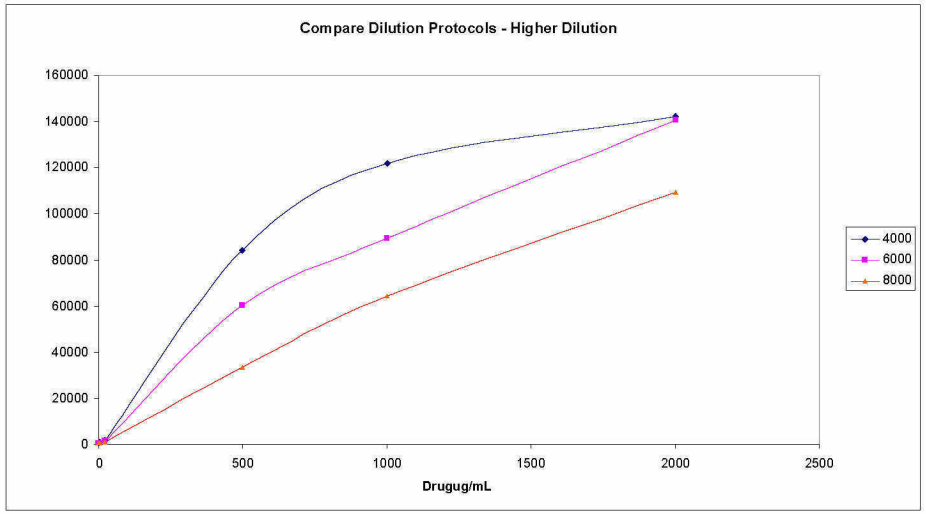
Dilution Optimization for Drug Assay

Need to evaluate different dilution levels since the actual range of drug concentration in patient samples was not known at the outset of assay development.

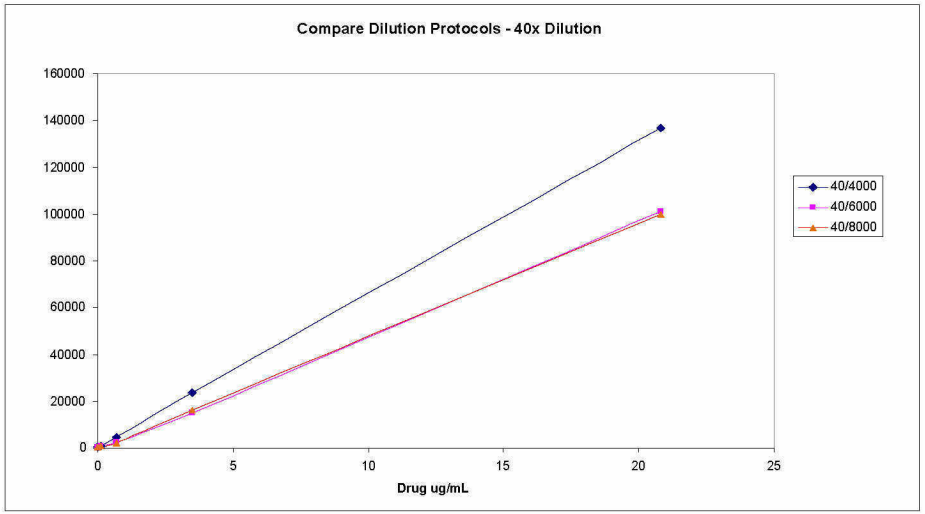


Drug Assay: Protocol Optimization, Sample Dilution

Response at Higher Dilutions



Response at 40x dilution



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Protocol Optimization

Effect of post sample wash: PD-1

In this case no benefit was seen.

Spiked Blood	No post sample wash			Post sample wash		
pg/mL	Mean RLU	StDev	CV %	Mean RLU	StDev	CV %
0	3751	560	14.9	3382	113	3.3
25	4149	148	3.6	3890	0	0.0
74	5200	9	0.2	4873	200	4.1
222	8904	974	10.9	8671	126	1.5
667	23728	2398	10.1	16892	1178	7.0
2000	69542	2053	3.0	52497	5049	9.6
Avg CV %	7			5		
Slope	20			20		
Avg StDev	239			157		
LOD, pg/mL	24			16		

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Whole Blood Screen

Whole blood and plasma are screened:

- to determine endogenous level of analyte
- to obtain low endogenous samples to use for spiking experiments

A minimum of 25 samples screened.

Assay Buffer Standard curve is used to calculate analyte level.

Blood spun down and plasma recovered and tested.

	Assay	Units	Assay Range	Blood Screen Range
1	PD-1	pg/mL	15 - 2000	20 - 266
2	PD-2	pg/mL	23 - 6000	270 - 1200
3	PD-3	pg/mL	25 - 1000	272 - 1070
4	PD-4	ng/mL	0.5 - 500	12.3 - 465
5	PD-5	ng/mL	25 - 2400	4 - 153
6	Drug	µg/mL	0.1 - 2000	0 – 0.14

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Whole Blood Spike Recovery

Since the asthma panel will be used for real-time profiling of whole blood and plasma samples, it is important to evaluate the following:

- Matrix effects due to whole blood and plasma
- Interaction of red blood cells with analyte
- Hematocrit effect

Analyte was spiked at mulwellle levels across the range of the assay into whole blood (with known endogenous level) and spike recovery calculated.

Plasma spun down from the spiked blood was tested for hematocrit effect.

An assay buffer calibration was used to estimate recovery.

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Analyte Recovery, %

For the PD assays, some low recovery values in plasma recovered from blood are due to analyte binding to formed elements.

In the PK assay, high recovery in plasma recovered from spiked blood is due to the impact of red cells occupying a significant fraction of the sample volume.

Note: These effects are accounted for in calibration.

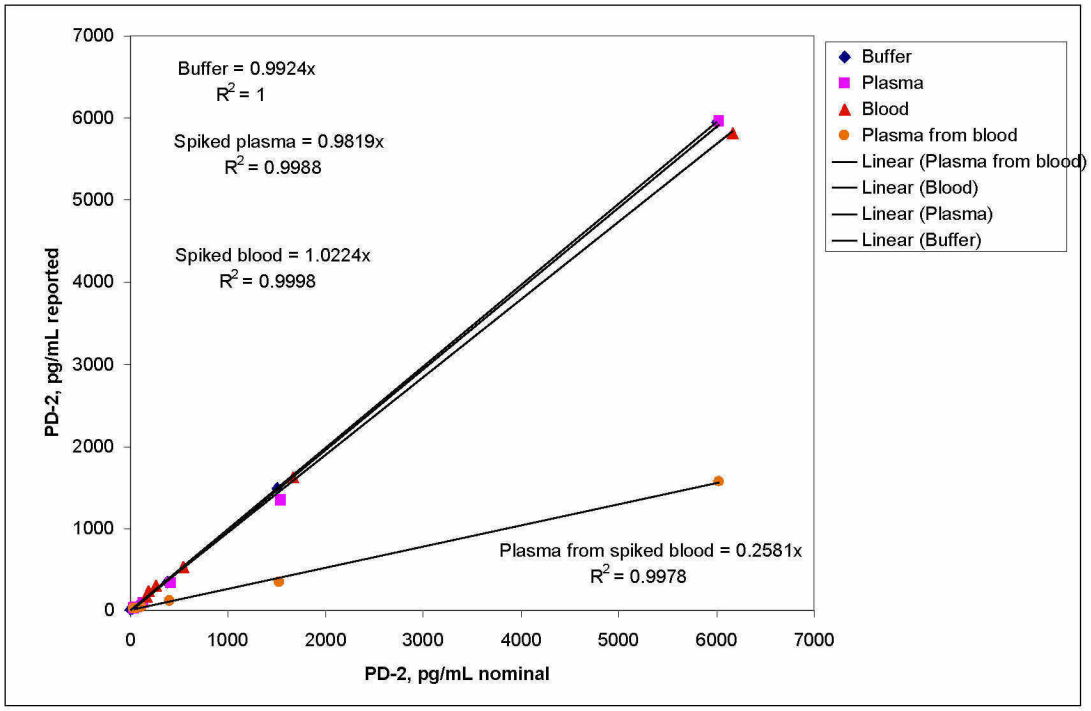
	Assay	Spiked Whole Blood	Plasma from Whole Blood	Spiked Plasma
1	PD-1	73	70	84
2	PD-2	91	35	80
3	PD-3	91	43	95
4	PD-4	76	24	67
5	PD-5	83		88
6	Drug	85	169	111

Spike Recovery: PD-2

Recovery is excellent in plasma and blood.

In plasma recovered from spiked blood, recovery is lower, since most of the analyte is bound weakly to formed elements.

This effect is accommodated in calibration.



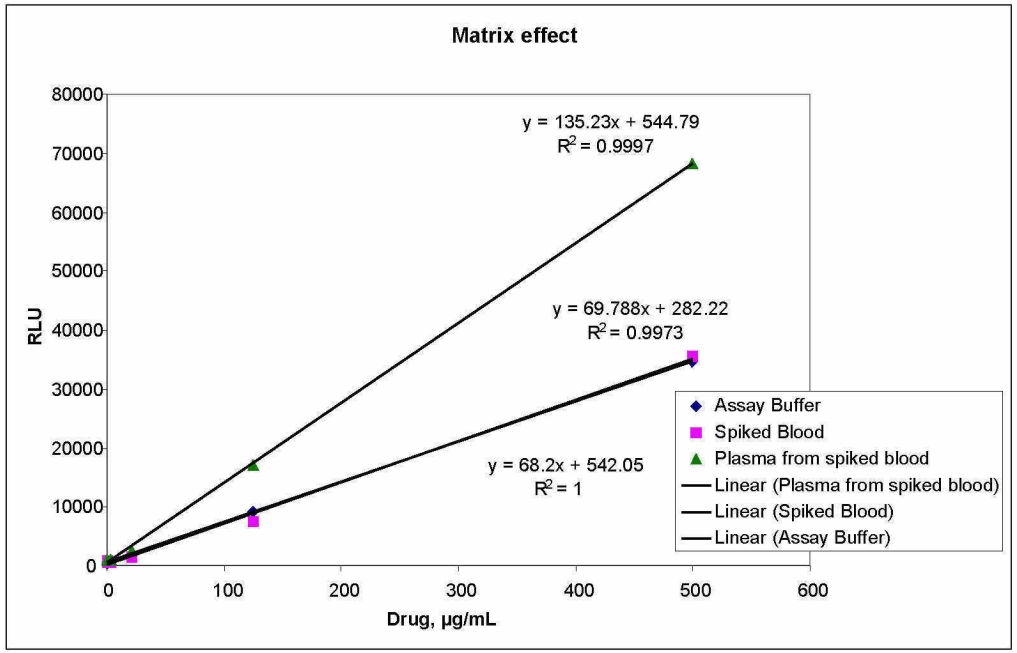
Spike Recovery: Drug

Blood and plasma have little to no effect on assay response.

Drug conc. in plasma from spiked blood is higher due to hematocrit (Cplasma = Cblood*(100/(100-%HCT)).

Drug does not bind to red cells.

This effect is accommodated in the calibration process.



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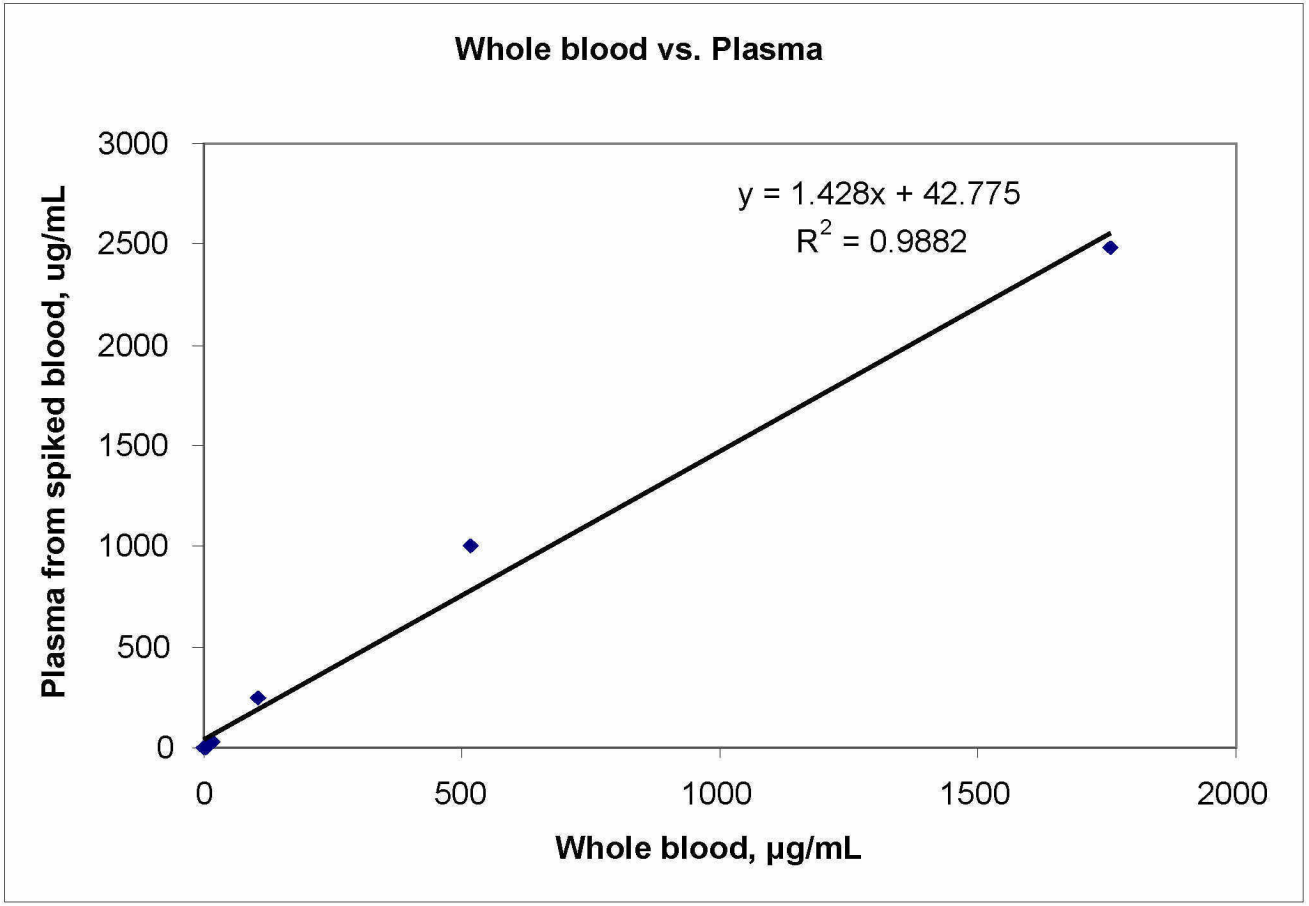
Hematocrit Effect: Drug Assay



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Assay Precision Within One Cartridge Lot for Mulwellle Instruments

One analyte level in the mid range of the assay was run on 24 instruments to determine the mid-range coefficient of variation (%CV).

Each assay can be performed in duplicate within each cartridge.

The results shown here apply to singlicate assays.

If two replicates per assay within each cartridge are used (as is usual), CV values are 1.3x lower.

Most CVs are then < 10%.

	Assay	% CV
1	PD-1	10.8
2	PD-2	13.5
3	PD-3	12.0
4	PD-4	11.7
5	PD-5	12.8
6	Drug	12.0

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Precision for Three Lots of Reagents

Three lots of cartridges were used to create a standard curve.

% CV of signal were determined.

Conc. CVs and signal CVs are almost equal for most of the response.

Samples were assayed in replicate on mulwellle instruments (N = 3).

CVs across the range of the assay (6 levels) were averaged.

If replicate assays are done within each cartridge, CVs are reduced by 1.3-fold.

	Assay	Inter	Intra
1	PD-1	6	13
2	PD-2	6	16
3	PD-3	3	12
4	PD-4	9	14
5	Drug	7	11

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Dilution Linearity

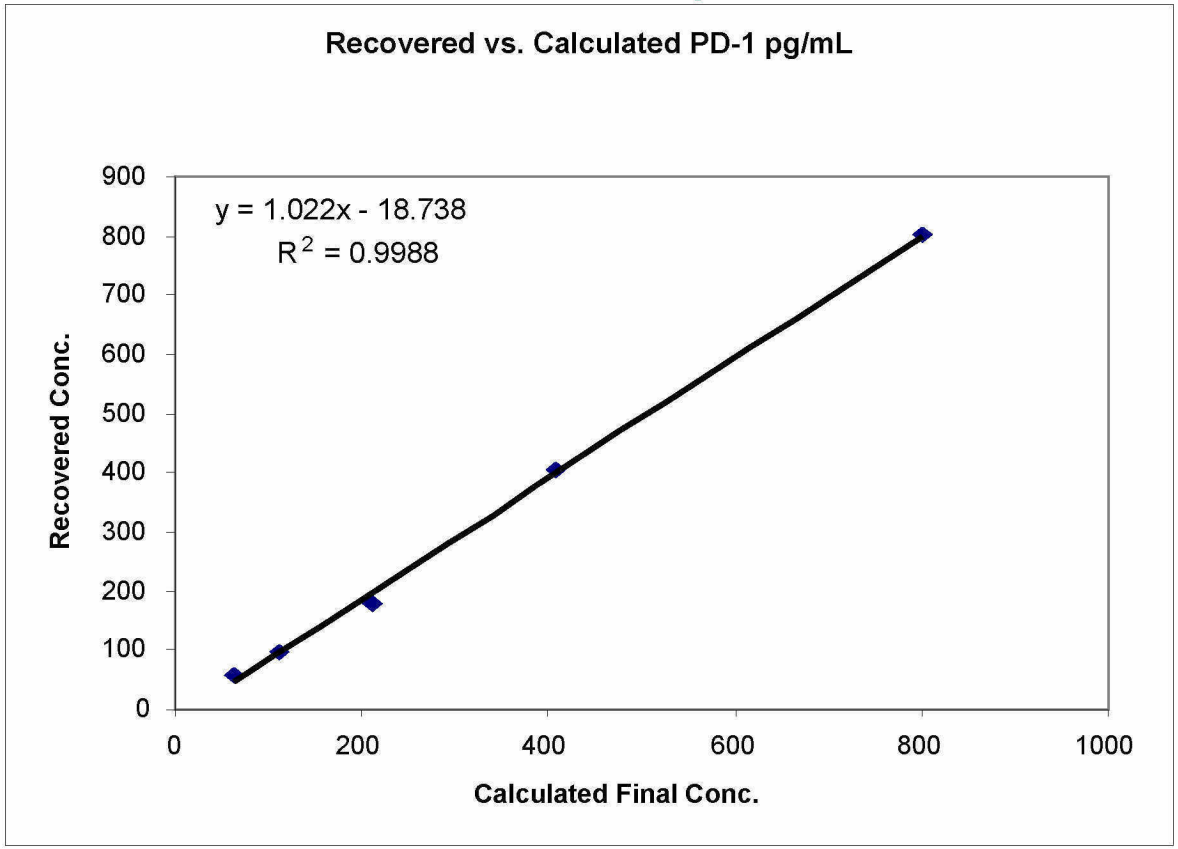
Dilution linearity of the assays was tested by either spiking in the analyte at the maximum level into plasma and serially diluting or using a low endogenous clinical sample to serially dilute a known (high) clinical sample.

Average recovery across the calibration is presented.

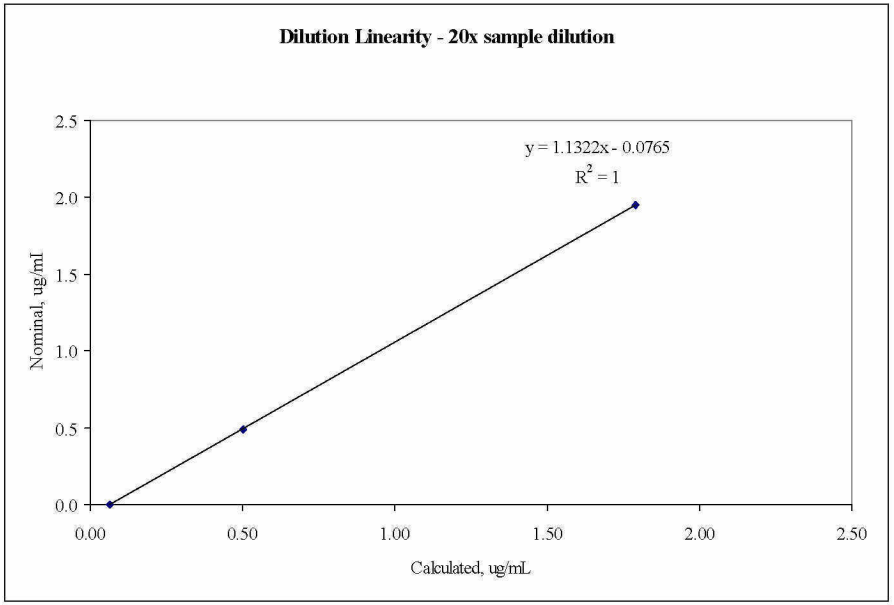
	Assay	% Recovery
1	PD-1	98
2	PD-2	116
3	PD-3	103
4	PD-4	120
5	PD-5	86
6	Drug	89

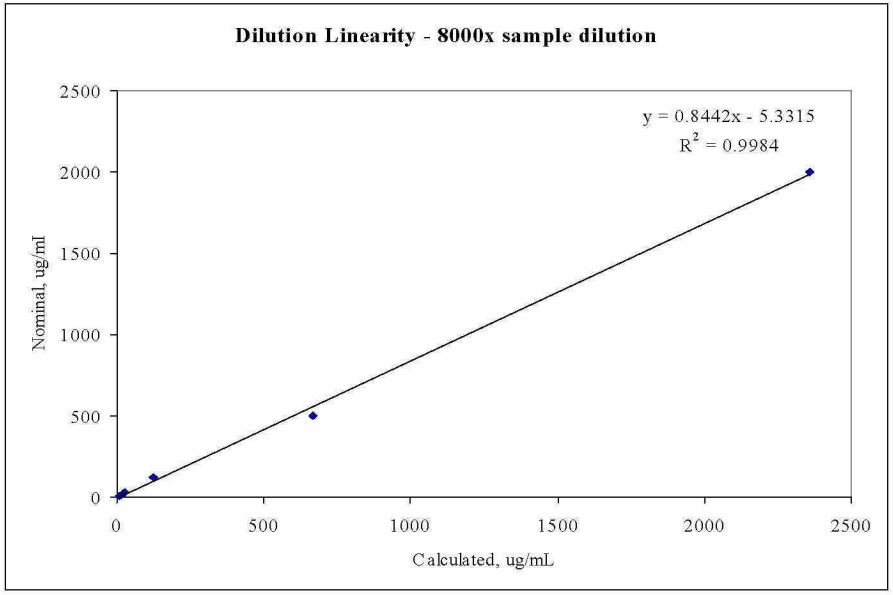
Dilution Linearity: PD-1

Clinical samples



Dilution Linearity: Drug





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Maximum Analyte Range

High levels of analyte *beyond the reported Theranos range* were spiked into either assay buffer, whole blood or plasma to determine the upper range of the assay and to check for hook effect.

None were observed.

	Assay	Units	Reported range	Highest level tested
1	PD-1	pg/mL	15 - 2000	51,200
2	PD-2	pg/mL	23 – 6000	24,000
3	PD-3	pg/mL	25 – 1000	6400
4	PD-4	ng/mL	0.5 – 500	2000
5	PD-5	ng/mL	25 – 2400	12,150
6	Drug	µg/mL	0.1 - 2000	4000

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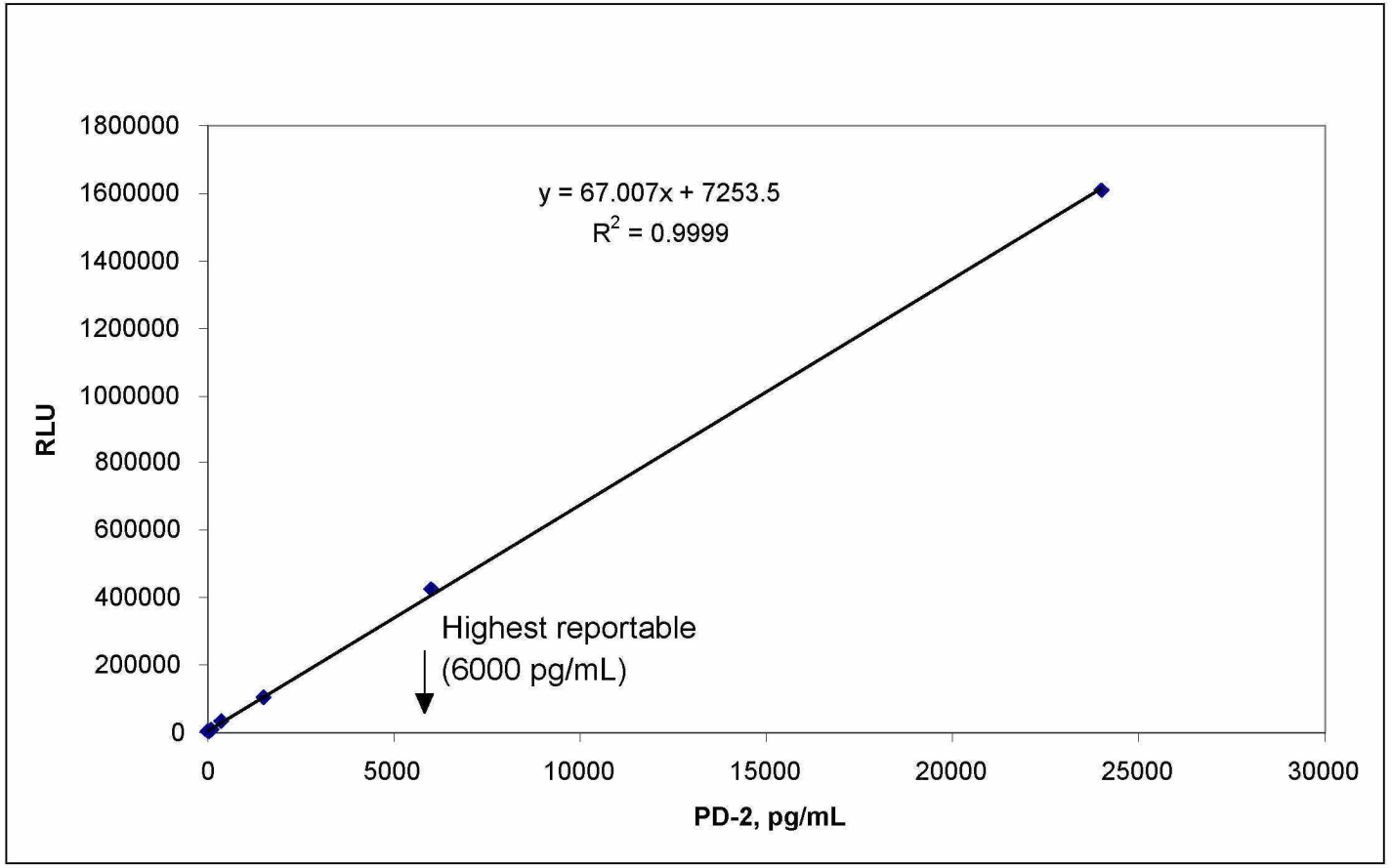
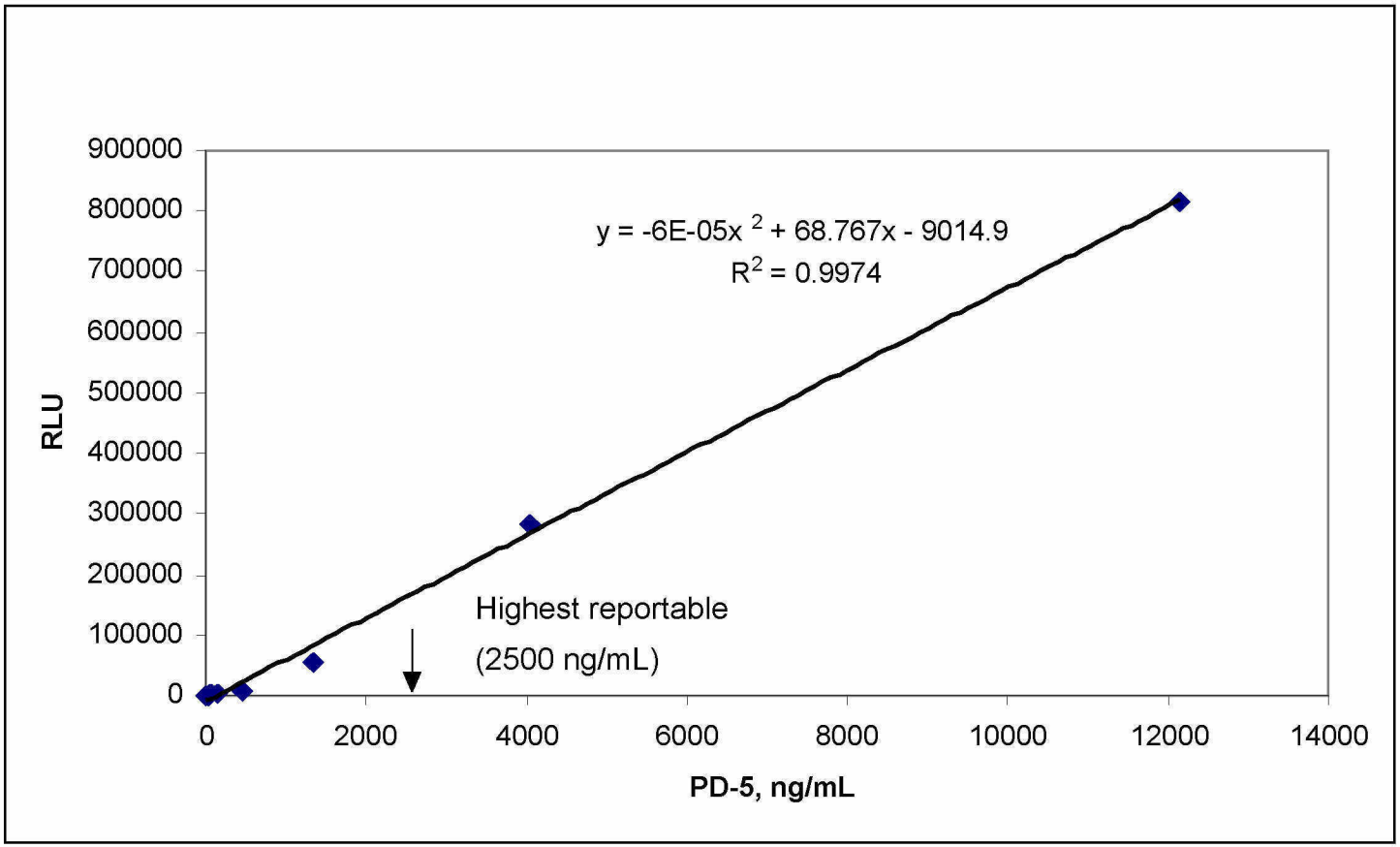
Maximum Range: PD-2



Maximum Range: PD-5



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Matrix Effects

The effect of spiking analytes into various potentially interfering matrices like hemolyzed blood and lipemic plasma was tested.

Mulwellle levels of analyte across the assay range were tested.

Percent recovery calculated against an assay buffer curve.

No impact of the matrices tested was seen.

	Assay	Hemolyzed blood	Lipemic serum
1	PD-1	100	119
2	PD-2	105	123
3	PD-3		90
4	PD-4	65	58
5	PD-5	104	83
6	Drug	99	94

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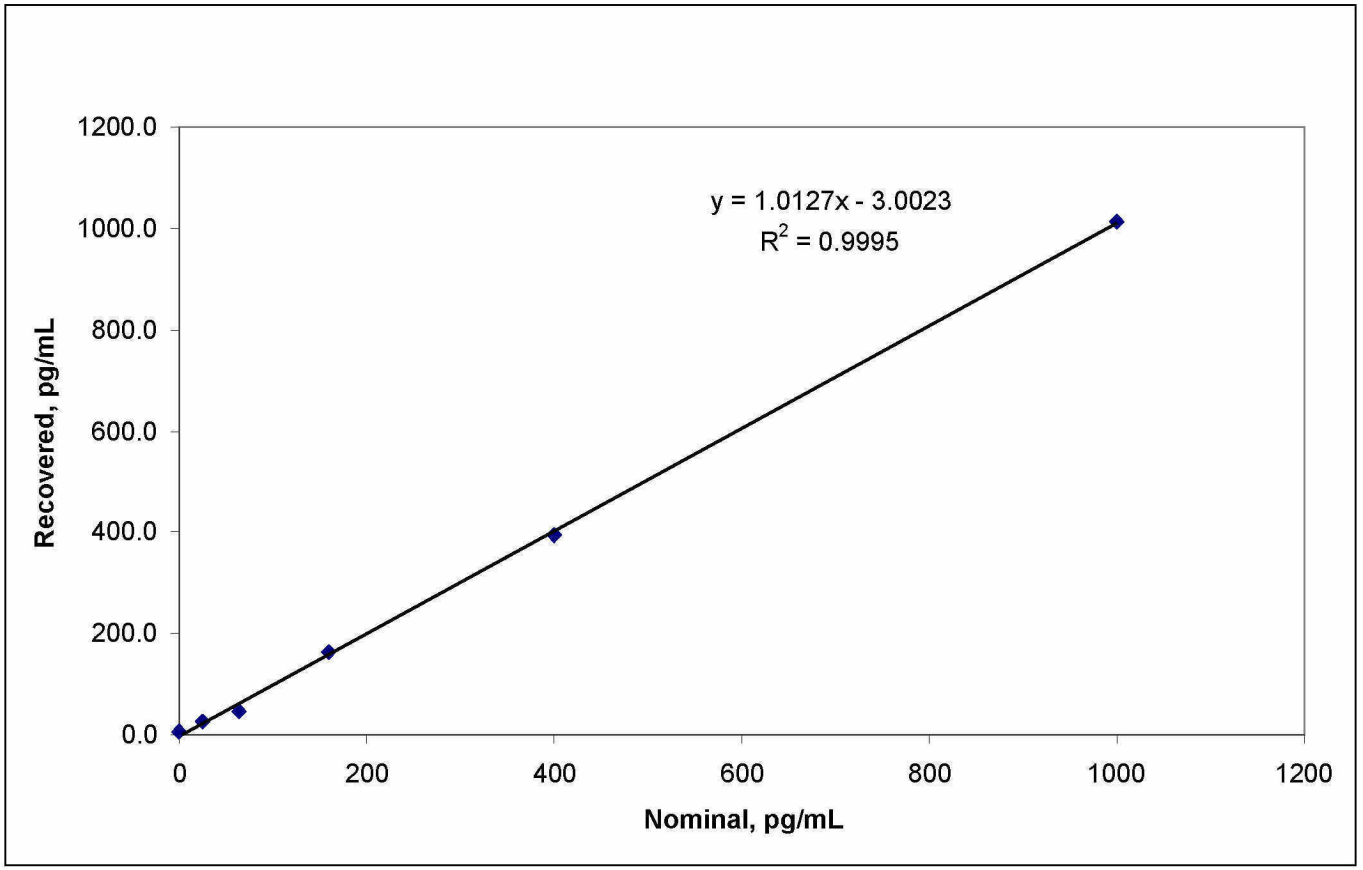
Spike Recovery in Lipemic Plasma: PD-3

Example of evaluation of interference due to matrix effects



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Cartridge/Reagent/System Stability

1. Stability for capture surfaces (reaction wells and reference) tested for a 12-week period for storage at 4°C and room temperature. wells are stored in sealed bags containing desiccant.
2. Stability for detector antibody in working concentration in Alkaline phosphatase stabilizer tested over a period of 12 weeks for storage at 4°C and room temperature.
3. A 4 point standard curve spanning the assay range is used.
4. Several instruments are used.
5. Analyte standards are pre-made for the entire study, aliquoted and flash frozen for single time use.

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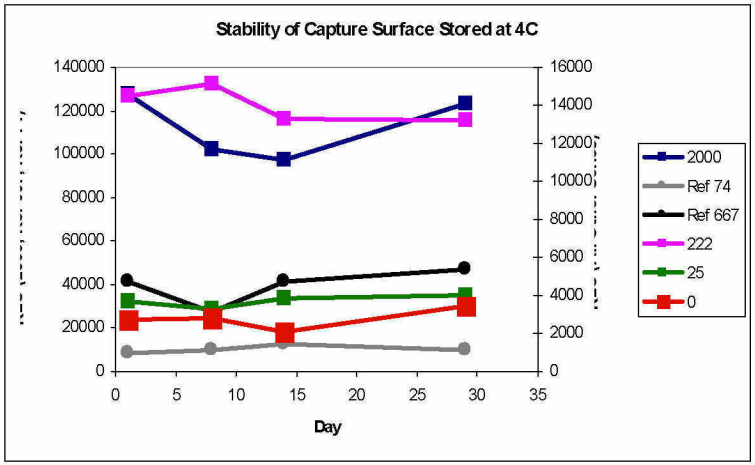
Cartridge/Reagent/System Stability

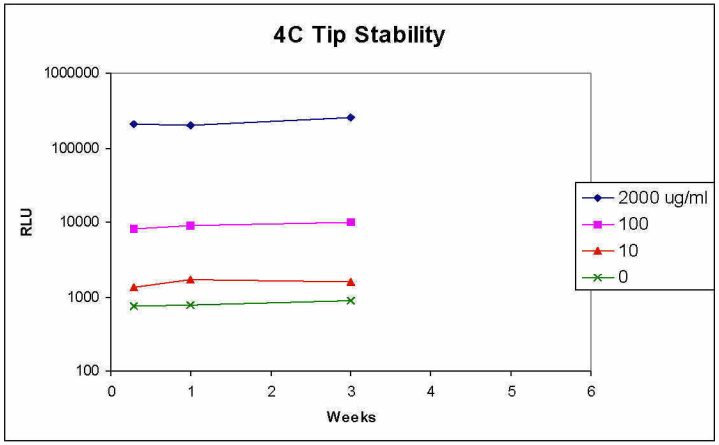
6. Integrated/multiplexed cartridges released to customers are monitored for stability over their useable life.
7. Studies are ongoing. No calibration instability has yet been observed. Examples are given below for a study in progress.
8. Log scale is used to allow visualization of the assay response over the very wide dynamic ranges.
9. Note: these programs demonstrate stability of reagents, cartridges and instruments.

Capture Antibody Stability: 4°C

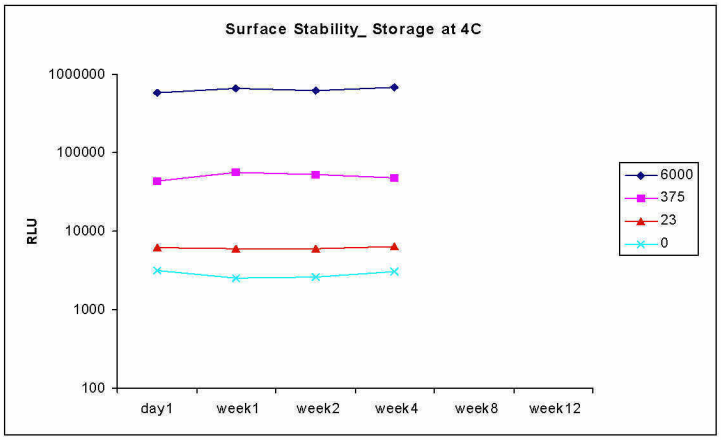
PD-1



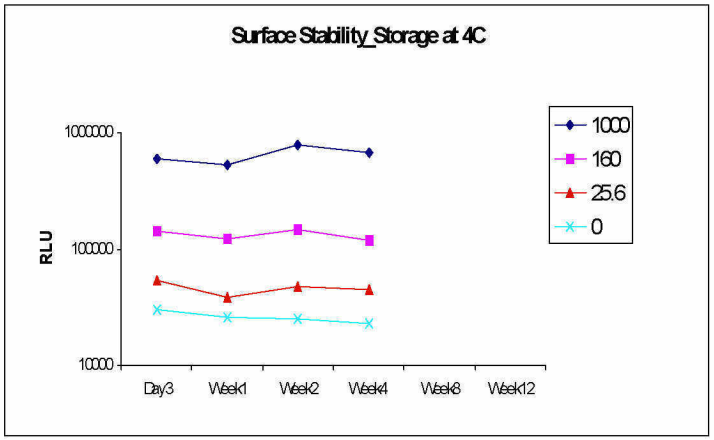
Drug Assay



PD-2

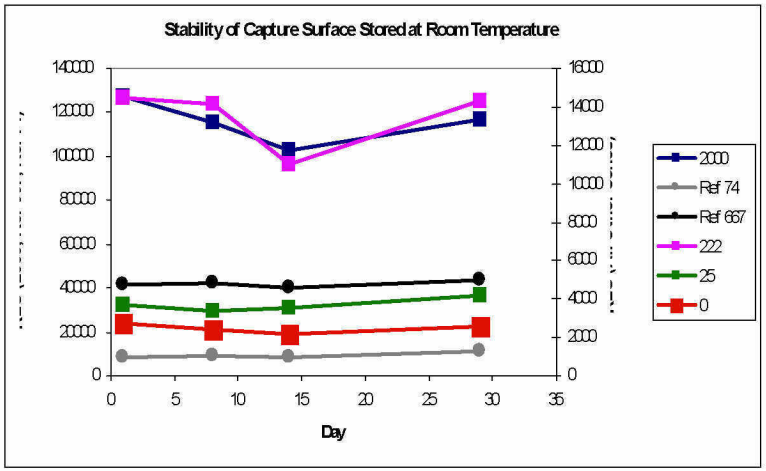


PD-3

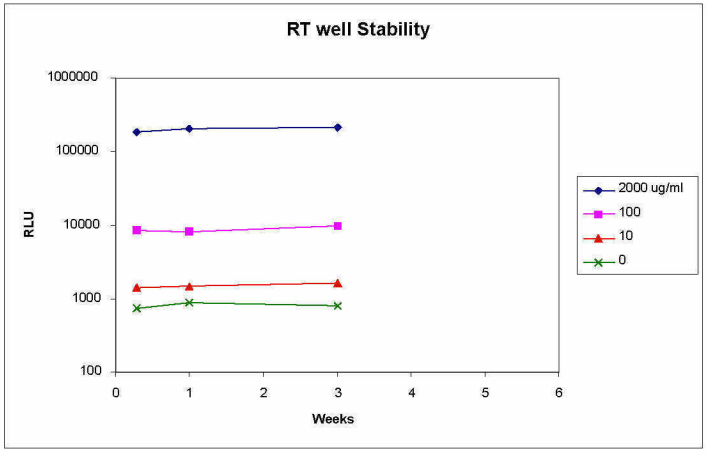


Capture Antibody Stability: RT

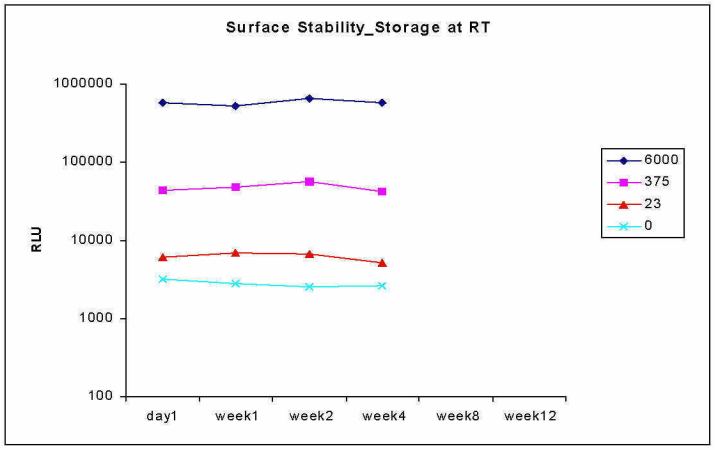
PD-1



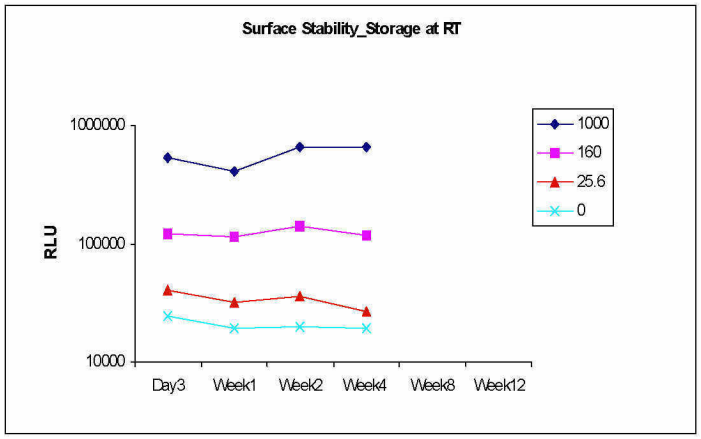
Drug Assay



PD-2

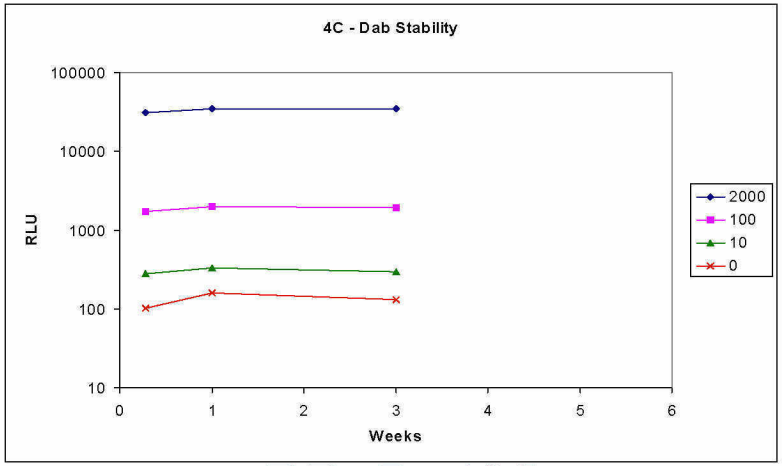


PD-3

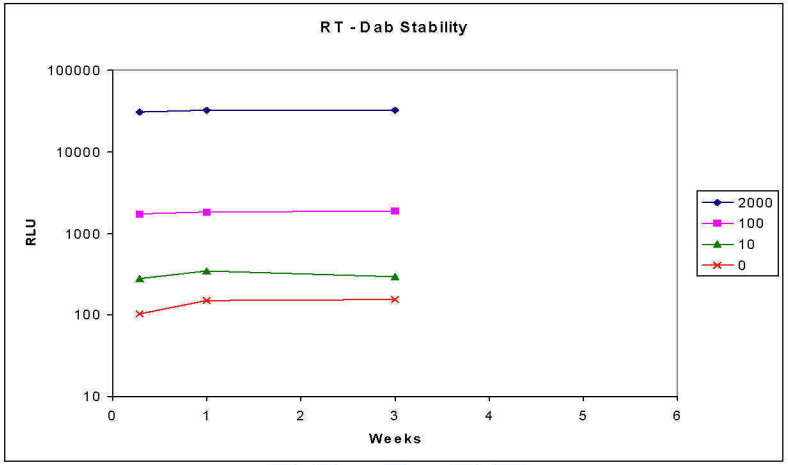


Detector-Antibody Stability

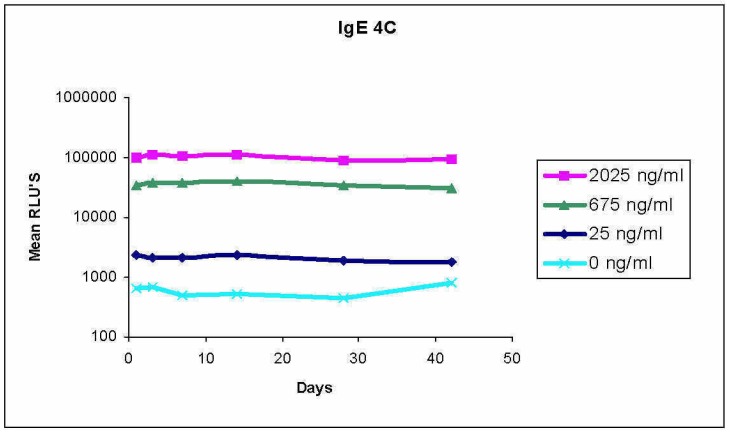
Drug Assay: 4°C



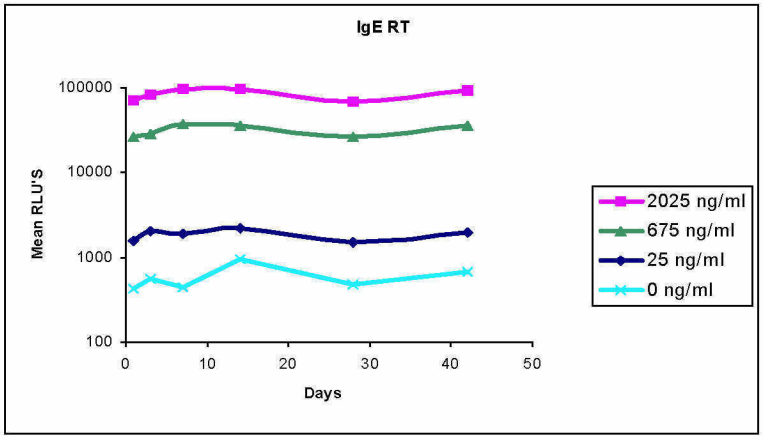
Drug Assay: RT



PD-5: 4°C

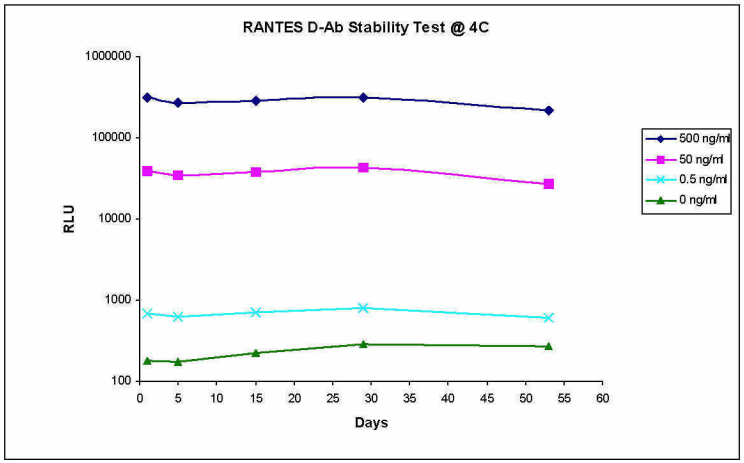


PD-5: RT

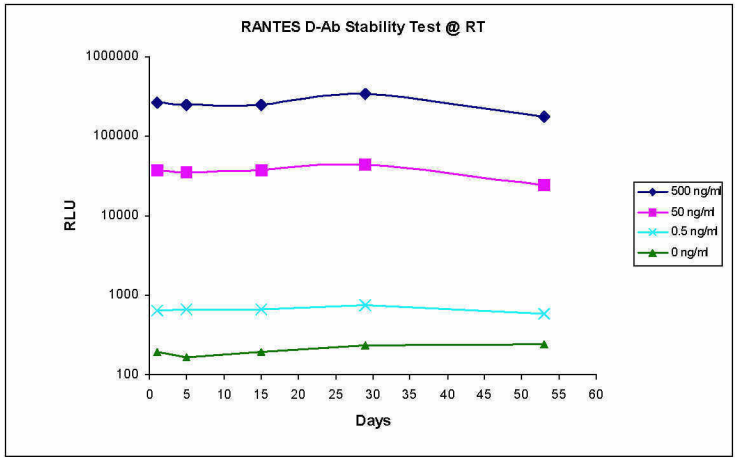


Detector-Antibody Stability

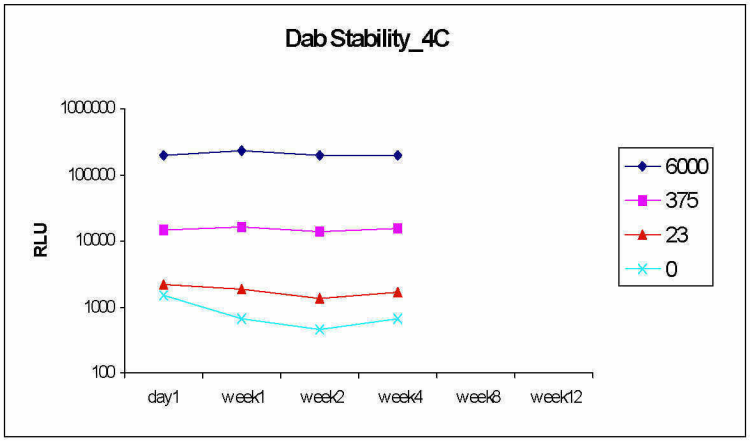
PD-4: 4°C



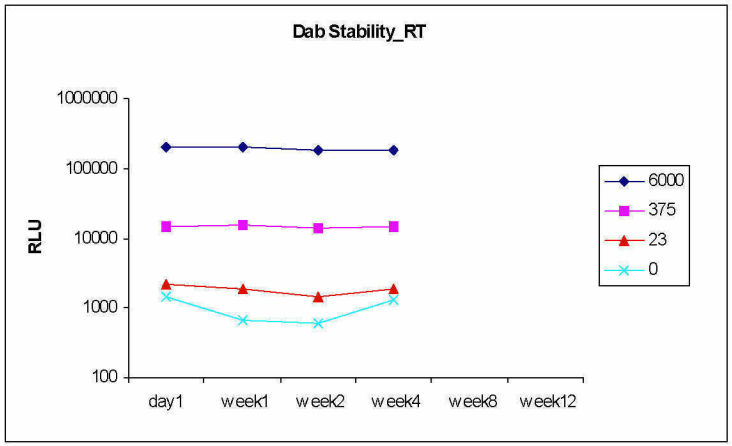
PD-4: RT



PD-2: 4°C



PD-2: RT



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multiplex: Combinations

The following multiplexes have been tested using duplicate assays (two wells) for each analyte.

Other configurations are possible including six analytes on each cartridge.

multiplex 1

	Assay	Sample dilution
1	PD-2	10x
2	PD-5	10x
3	Drug	8000x

multiplex 2

	Assay	Sample dilution
1	PD-3	5x
2	PD-1	5x
3	PD-4	200x

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Assay Validation: Correlation with Predicate Methods

PD Assays

- 20 or more serum samples
- Samples were from Asthmatic subjects
- Correlation against reference assays from R&D systems

PK Assay 10 samples

Drug was spiked into pooled serum from asthmatics.

Enzymatic Assay (Glucose)

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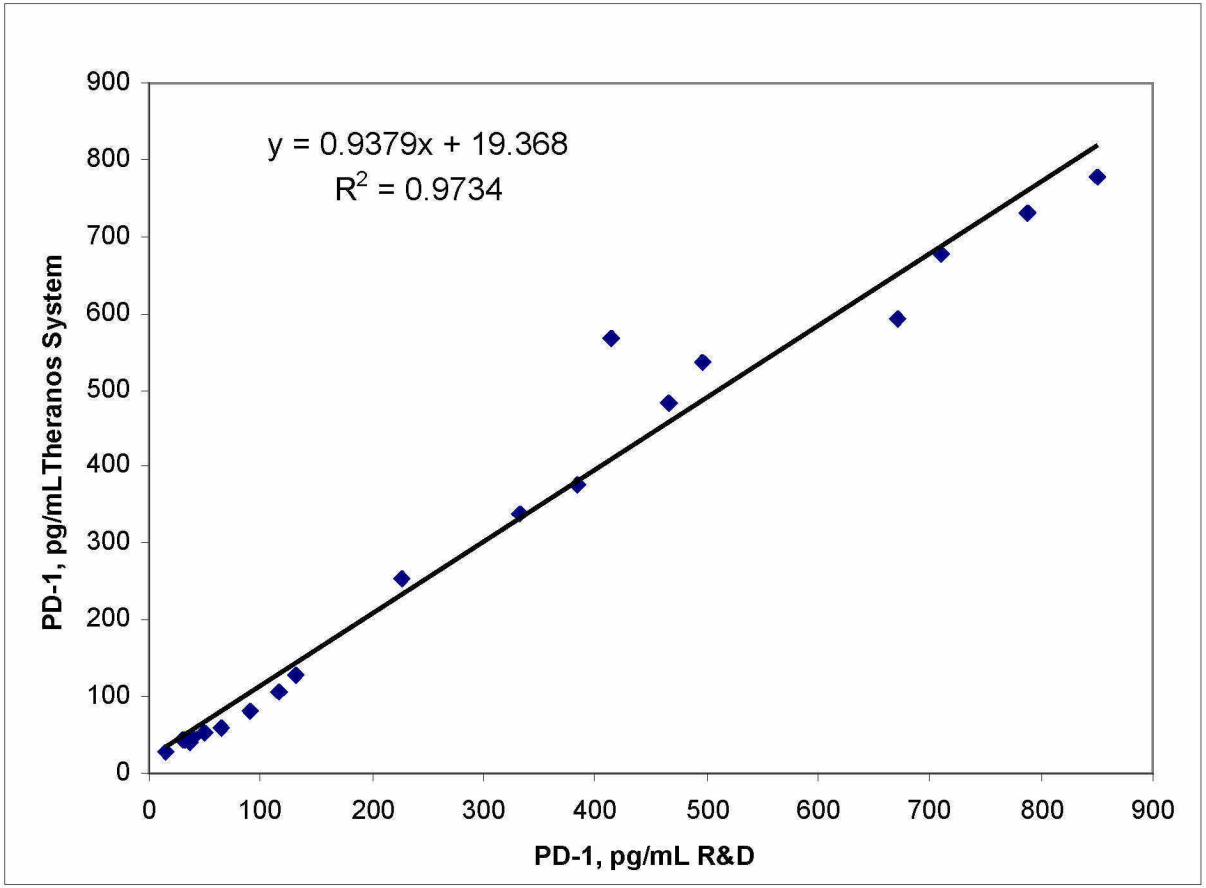
PD-1 Correlation



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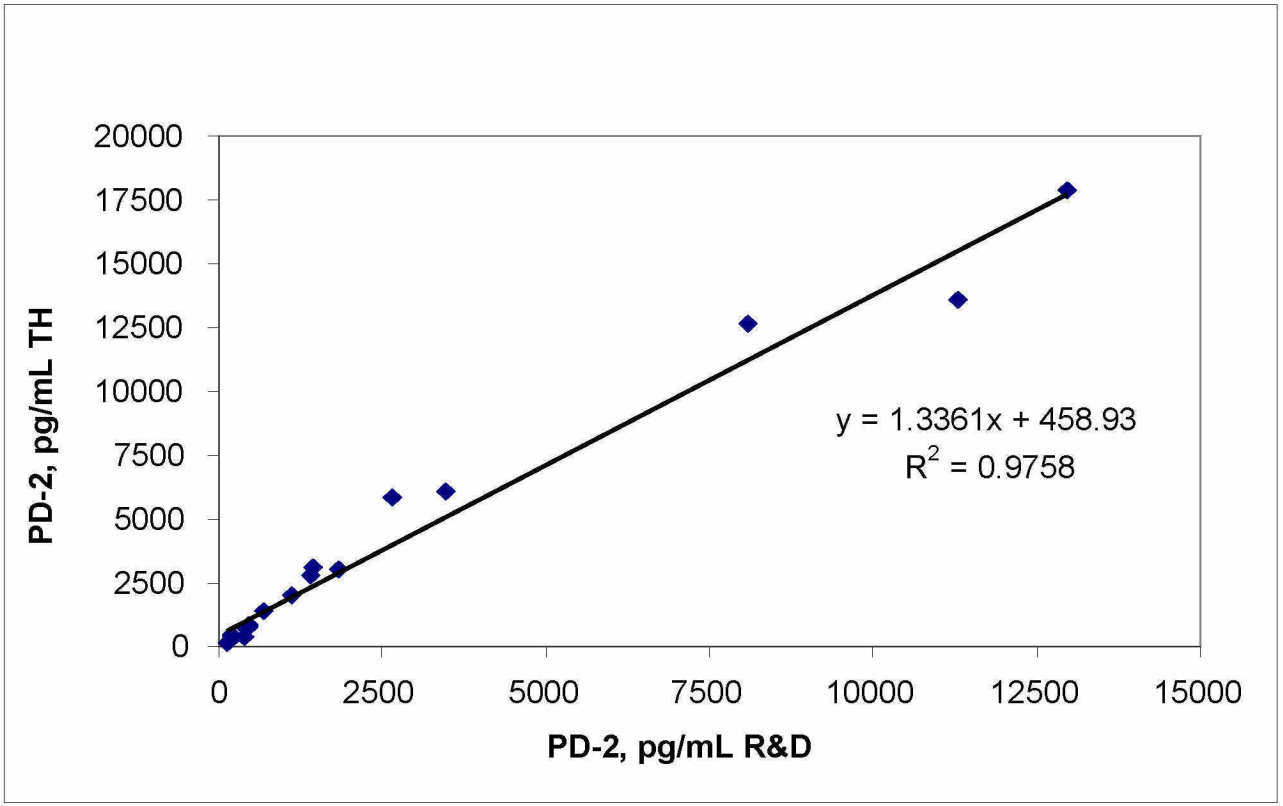
PD-2 Correlation



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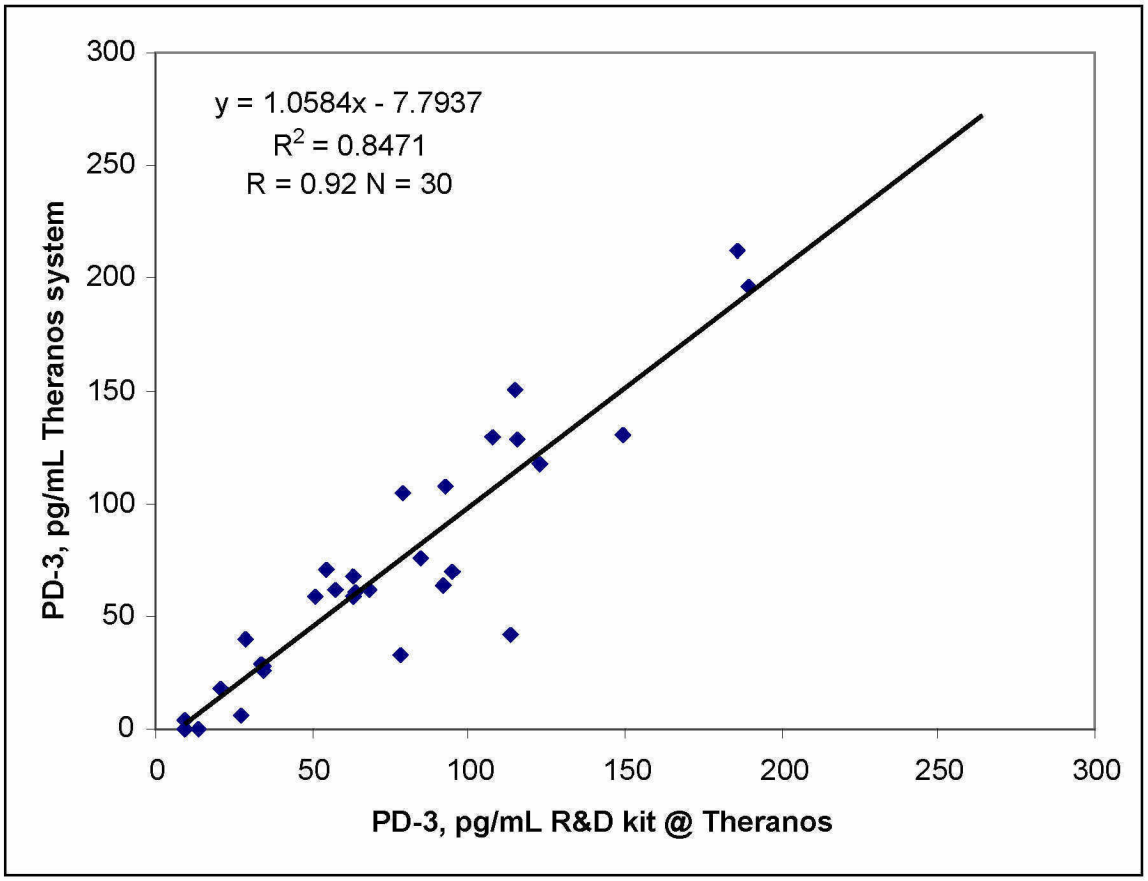
PD-3 Correlation



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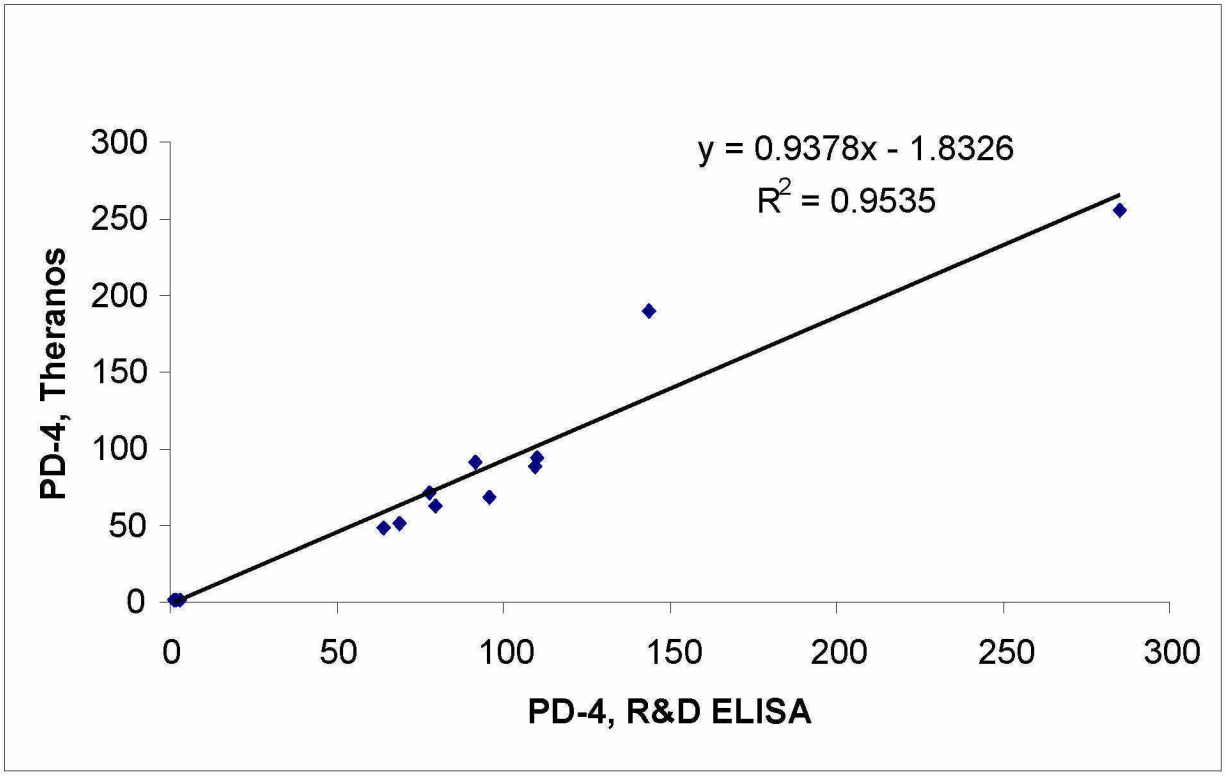
PD-4 Correlation



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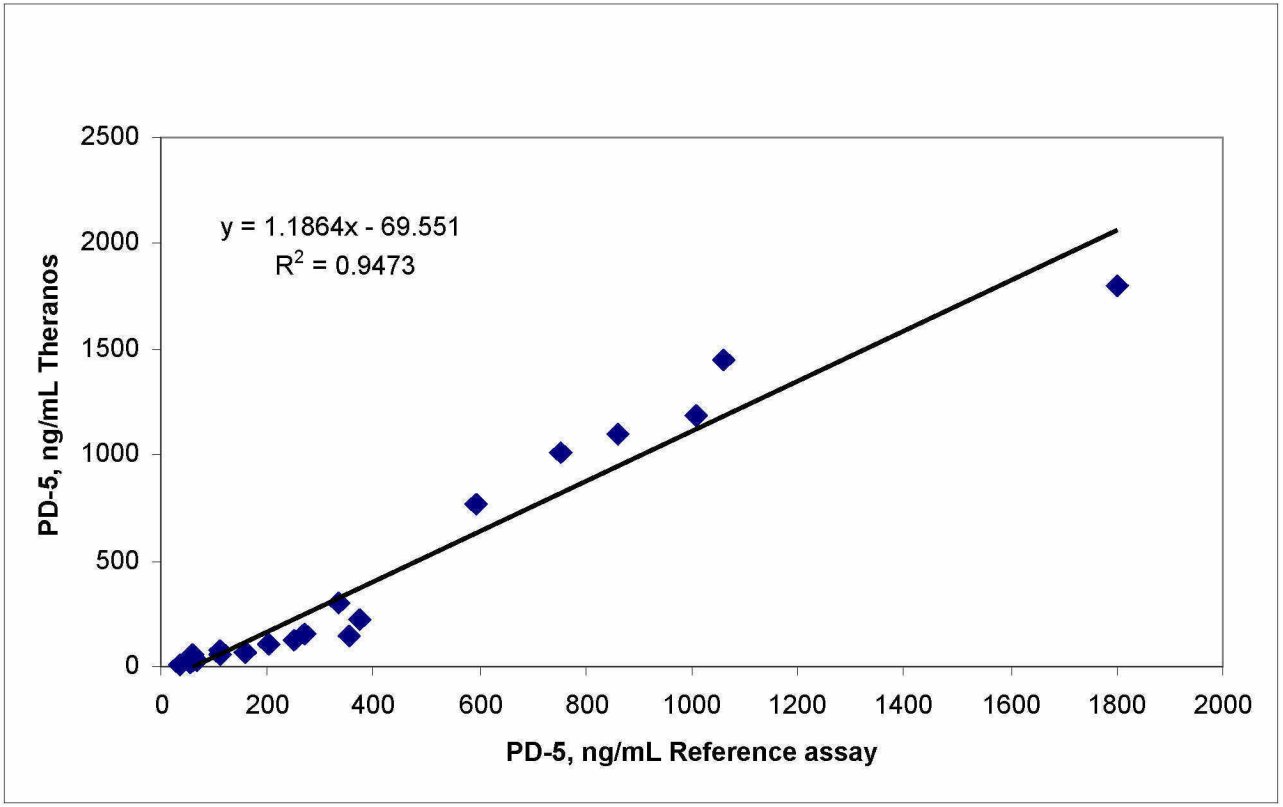
PD-5 Correlation



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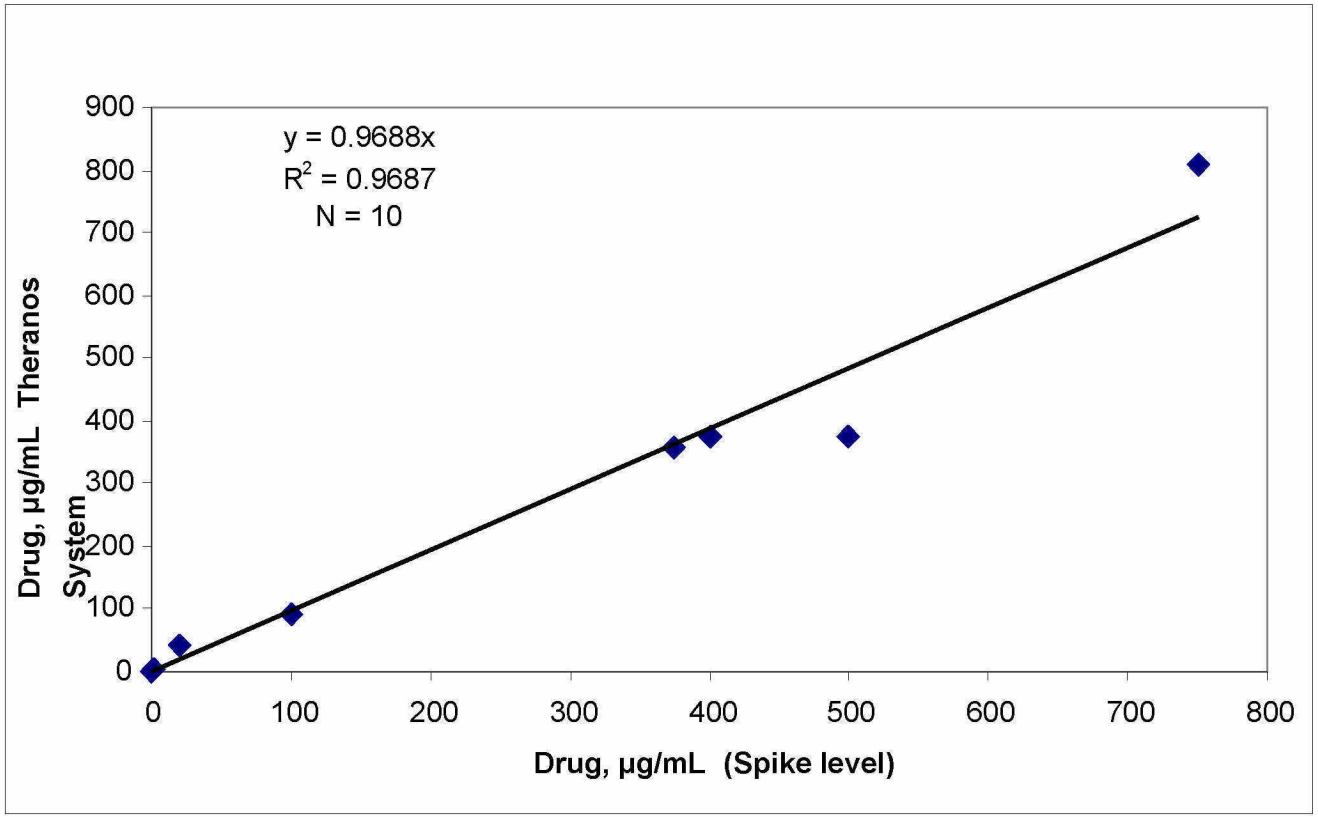
Drug Assay Correlation



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Theranos approach to integrated, distributed clinical testing

General Chemistry

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System Concept

Integrate laboratory capabilities into a system that can be deployed in distributed (non-laboratory) situations

Component modules

- State-of the-art immunoassay
- General Chemistry Assays (equivalent to standard laboratory SMAC panels)
- Cell Counting (CBC and cell markers)
- Measurement of nucleic acid markers
  - Viral RNA
  - Infectious disease organism genes

Two-way communications with Theranos Server for data analysis and control of the instrumented system, Medical Professionals and Patients (already developed)

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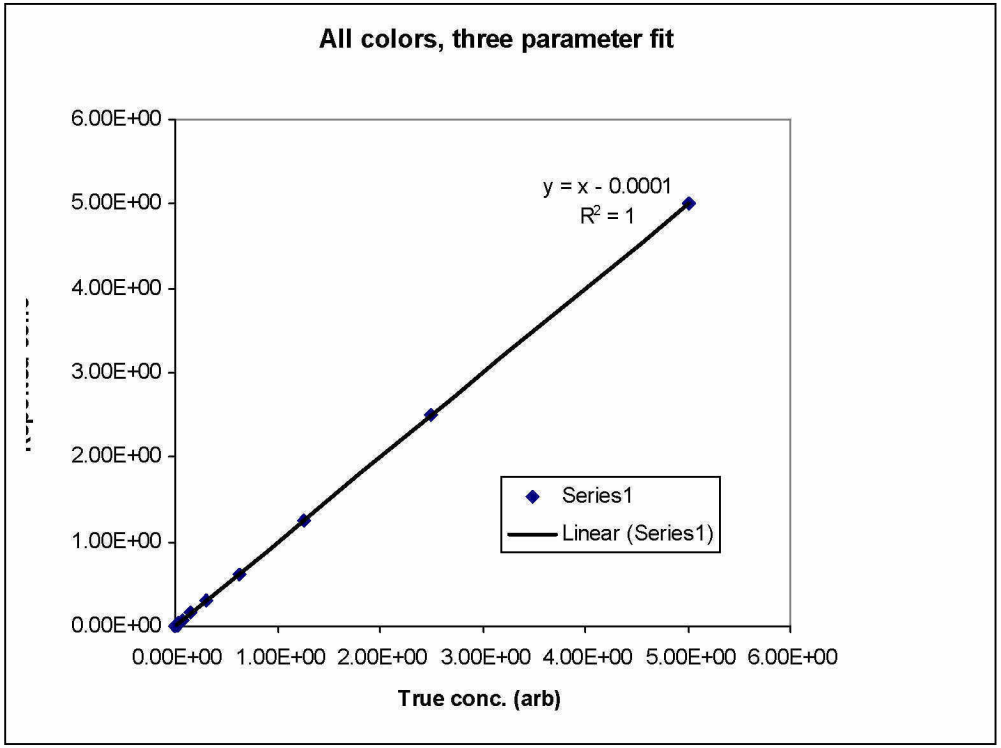
Calibration algorithm (proprietary) fits the data



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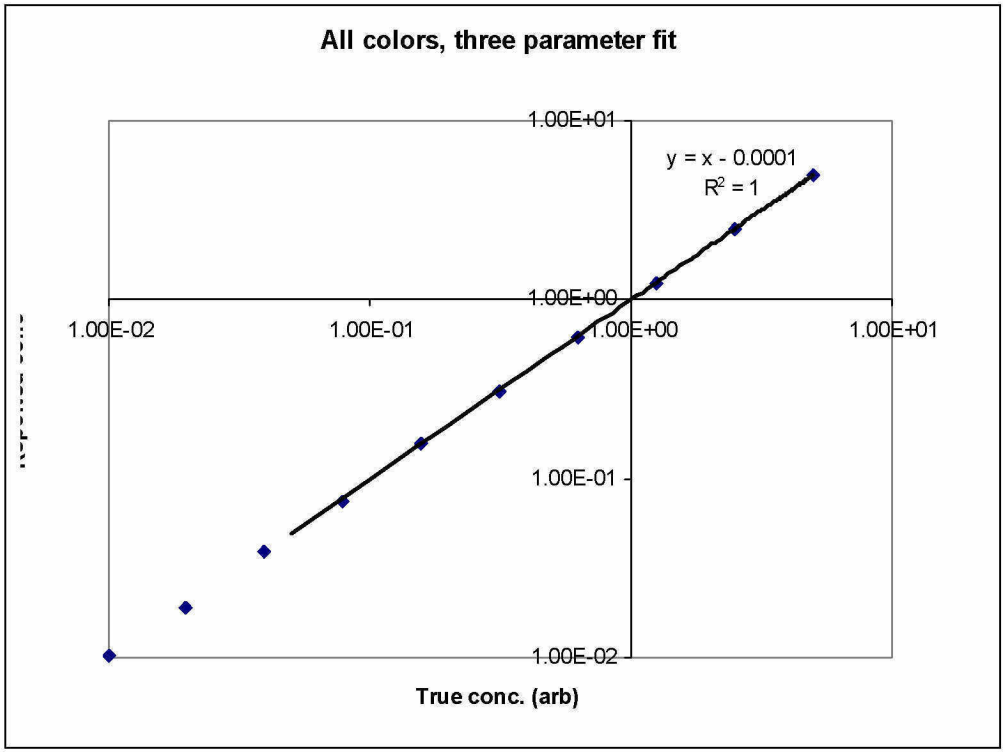
Linearity of signal transform with concentration over a very wide dynamic range

Use of three color channels permits reading of very high OD samples



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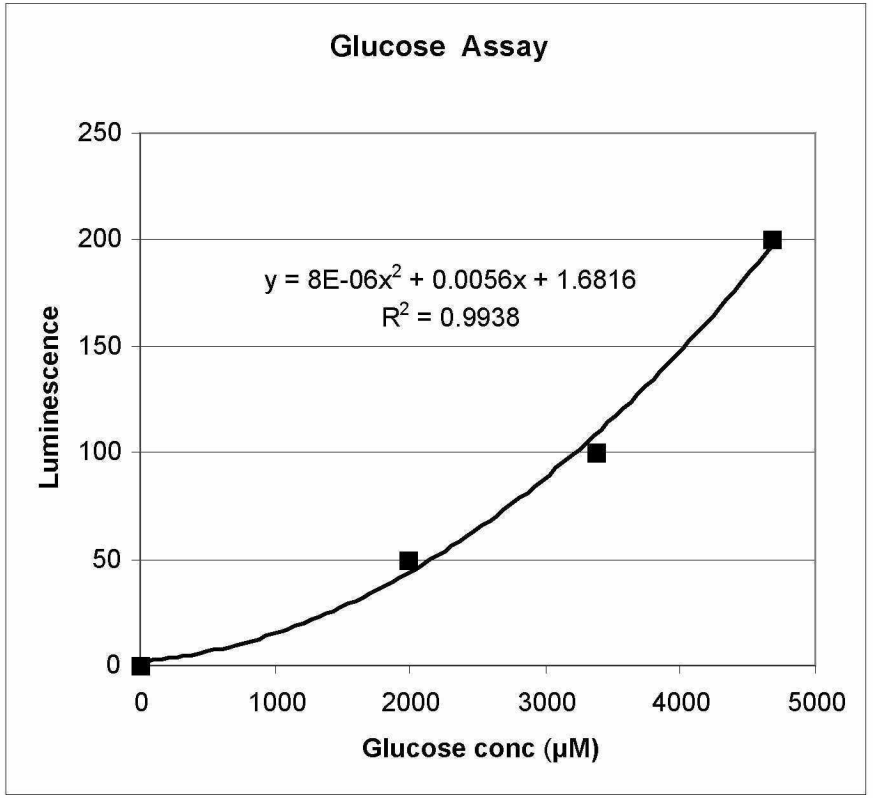
Glucose Assay

Coupled enzyme-based

- Theranos Proprietary

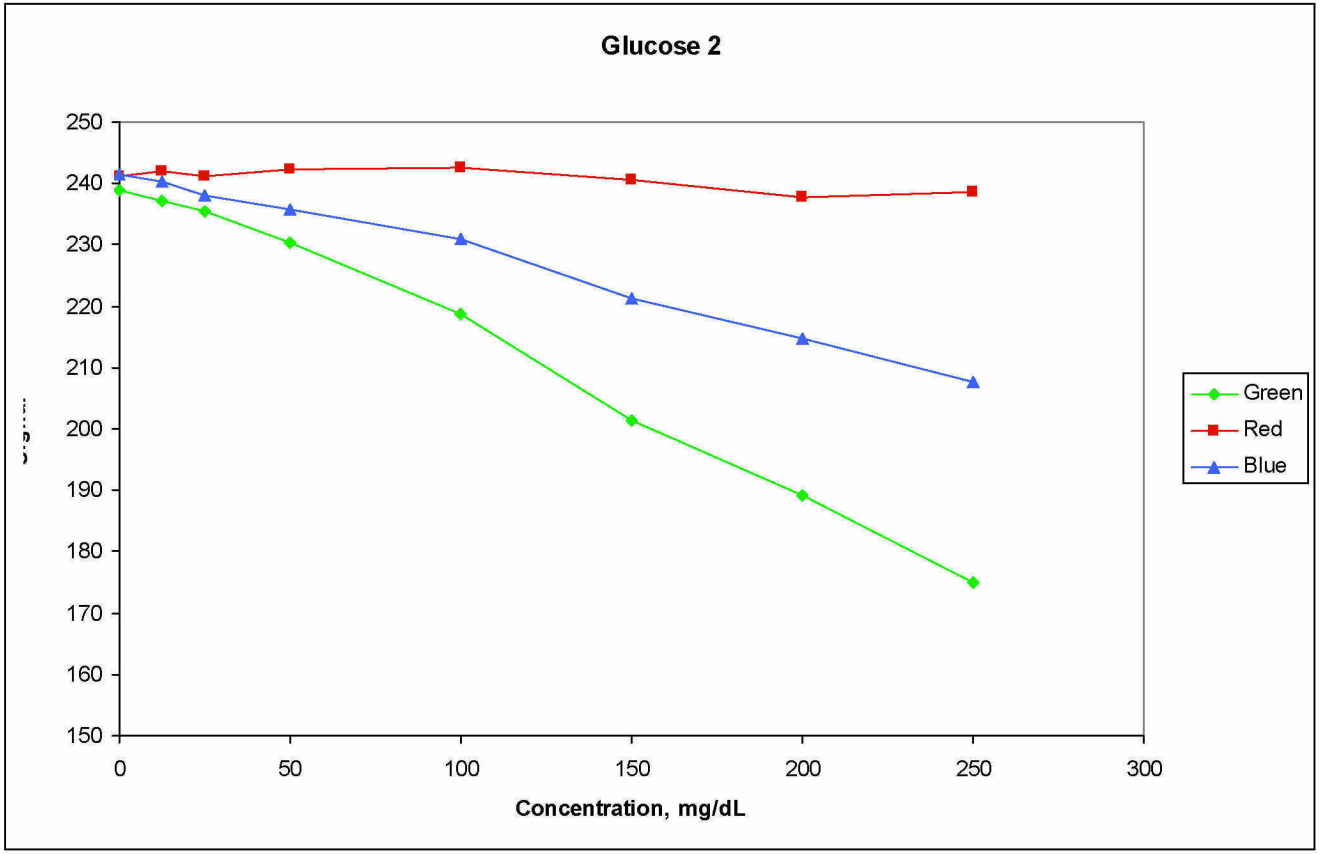
Can be multiplexed with immunoassays in the same cartridge.

Works with blood and plasma.



Signals as a function of [analyte]

Assay in which color *increases* with analyte



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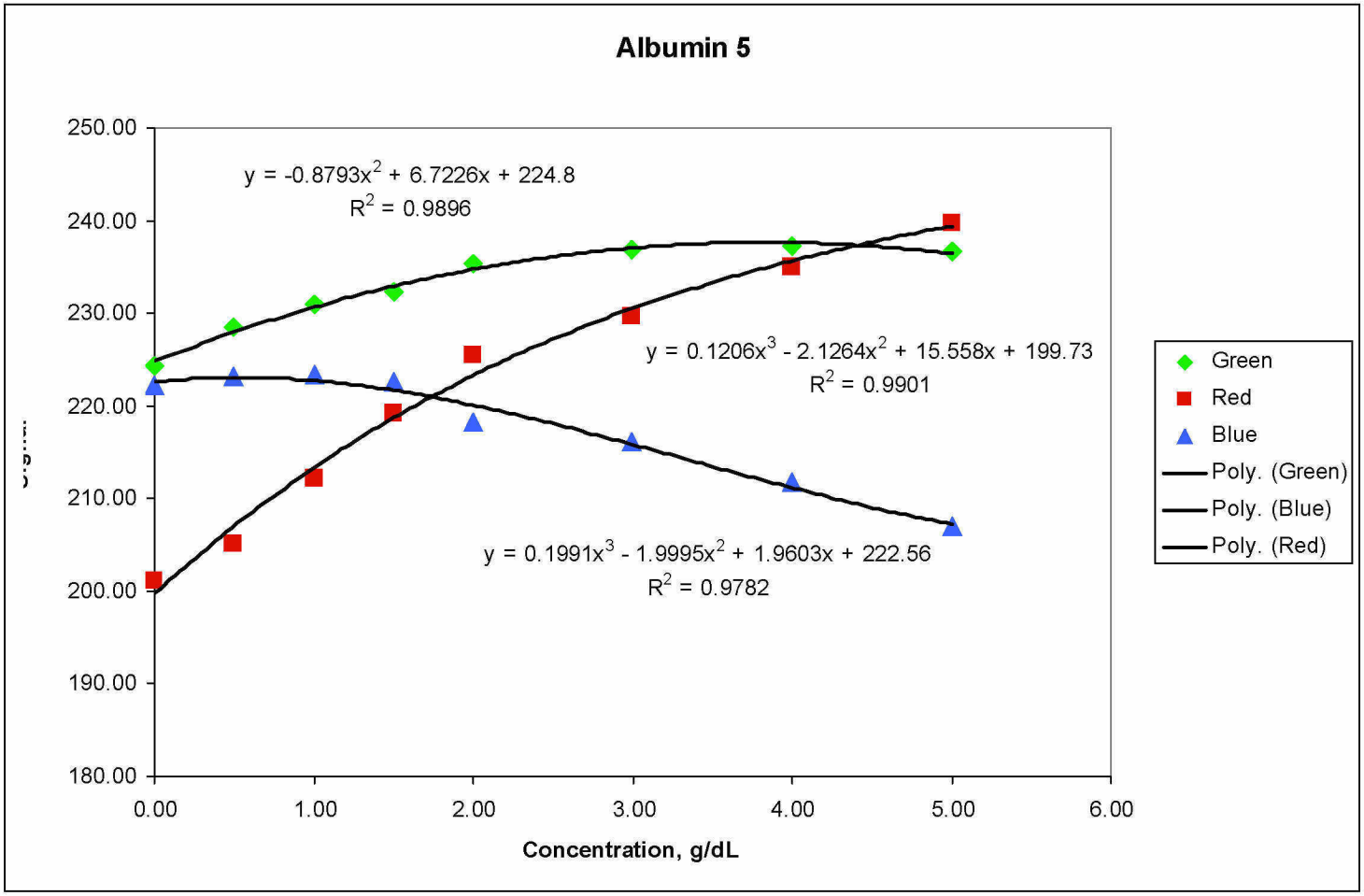
Signals as a function of [analyte]

Assay in which color *changes* with analyte



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Selected Results

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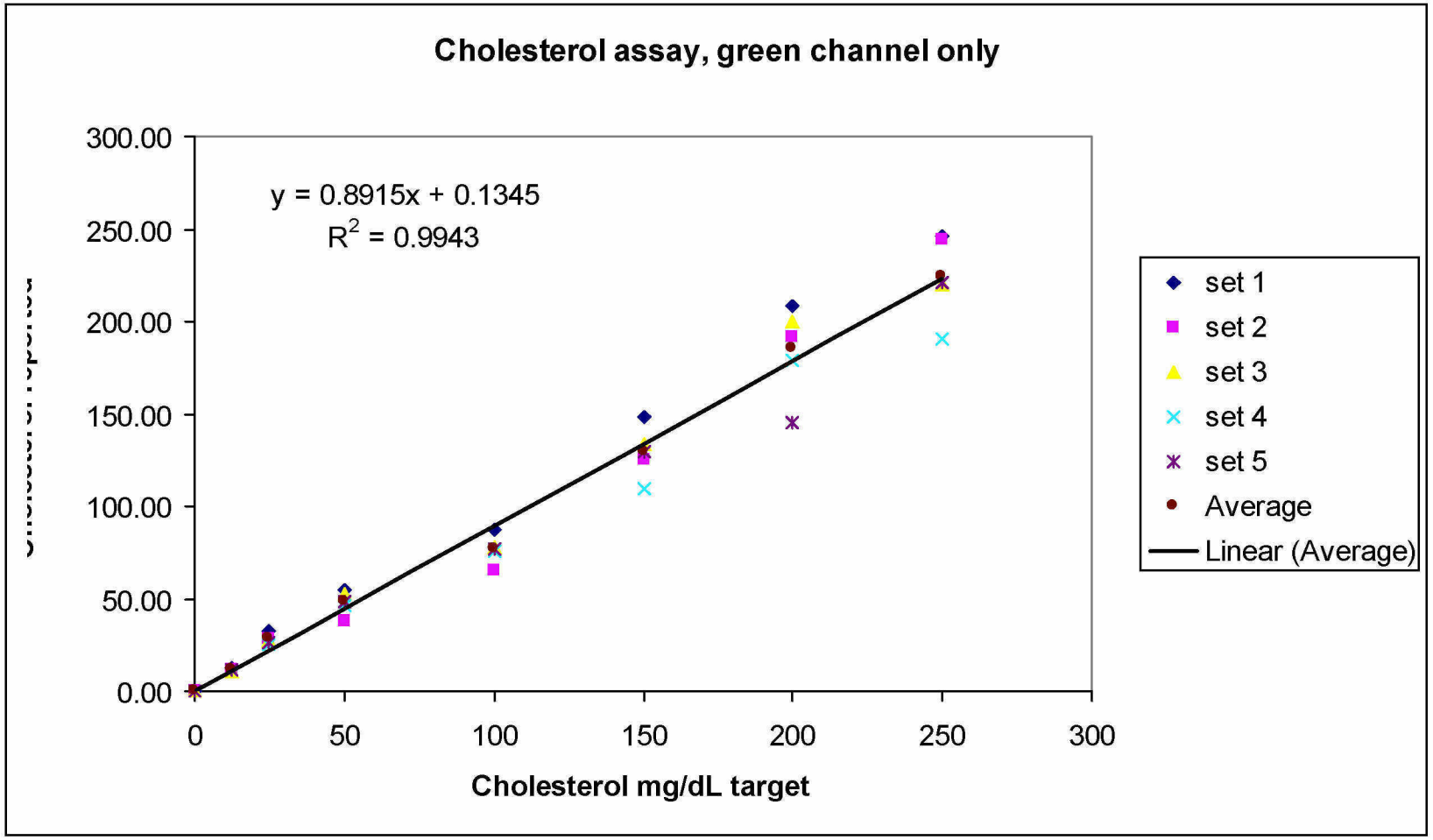
Reproducibility of calibration (example)

Calibration of an assay with one data set applied to four others



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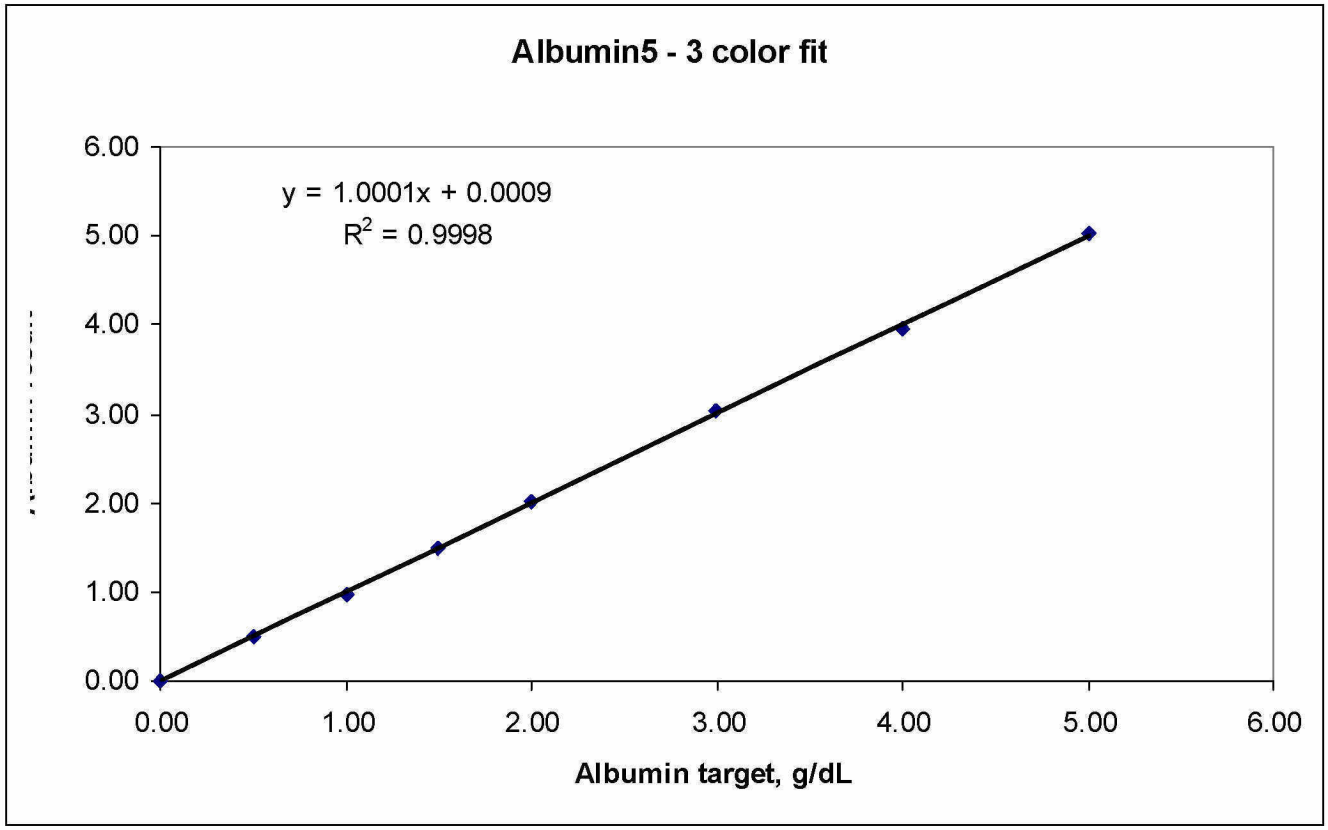
Fitting algorithm within a data set



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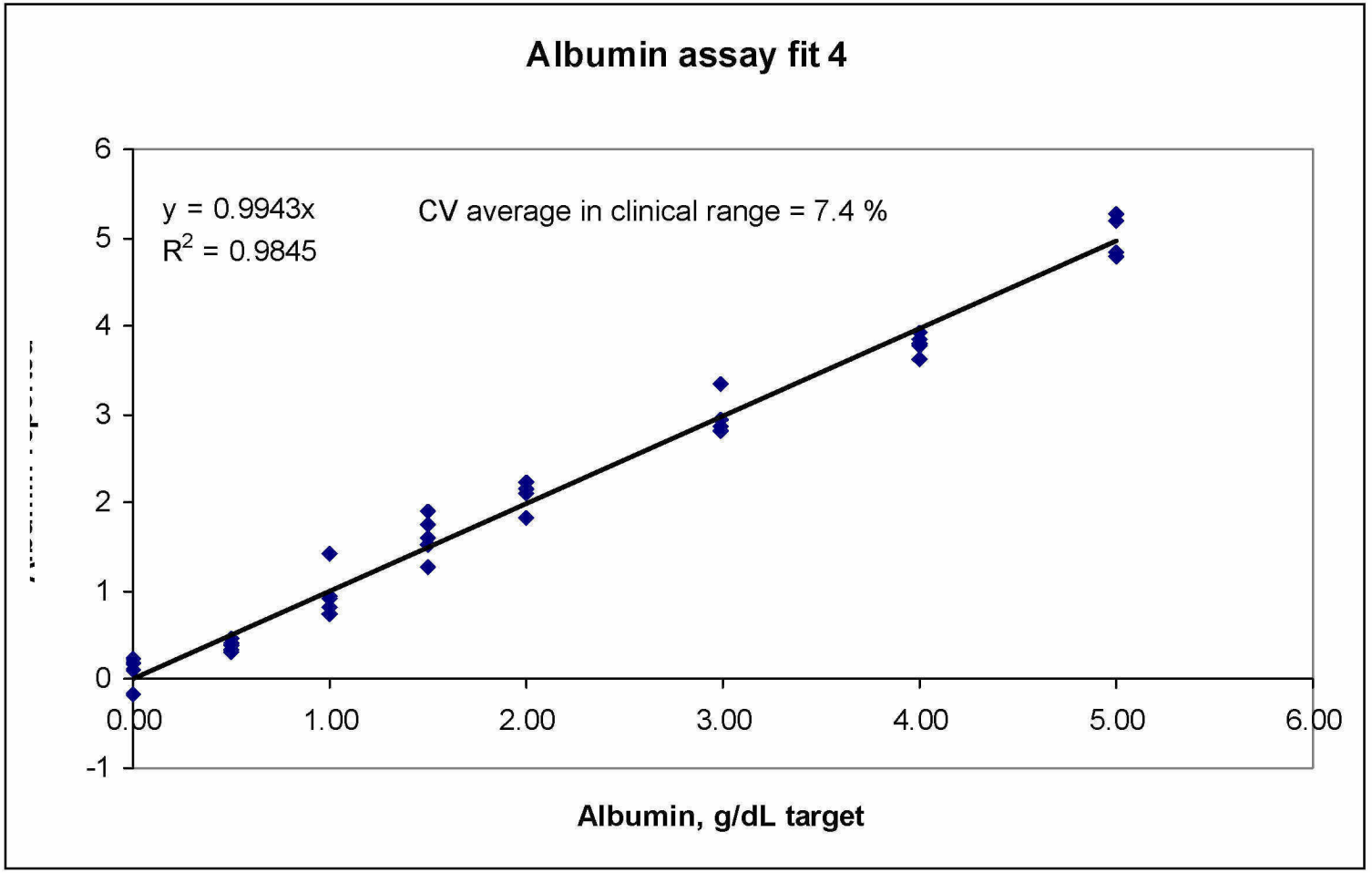
Replicate measurements



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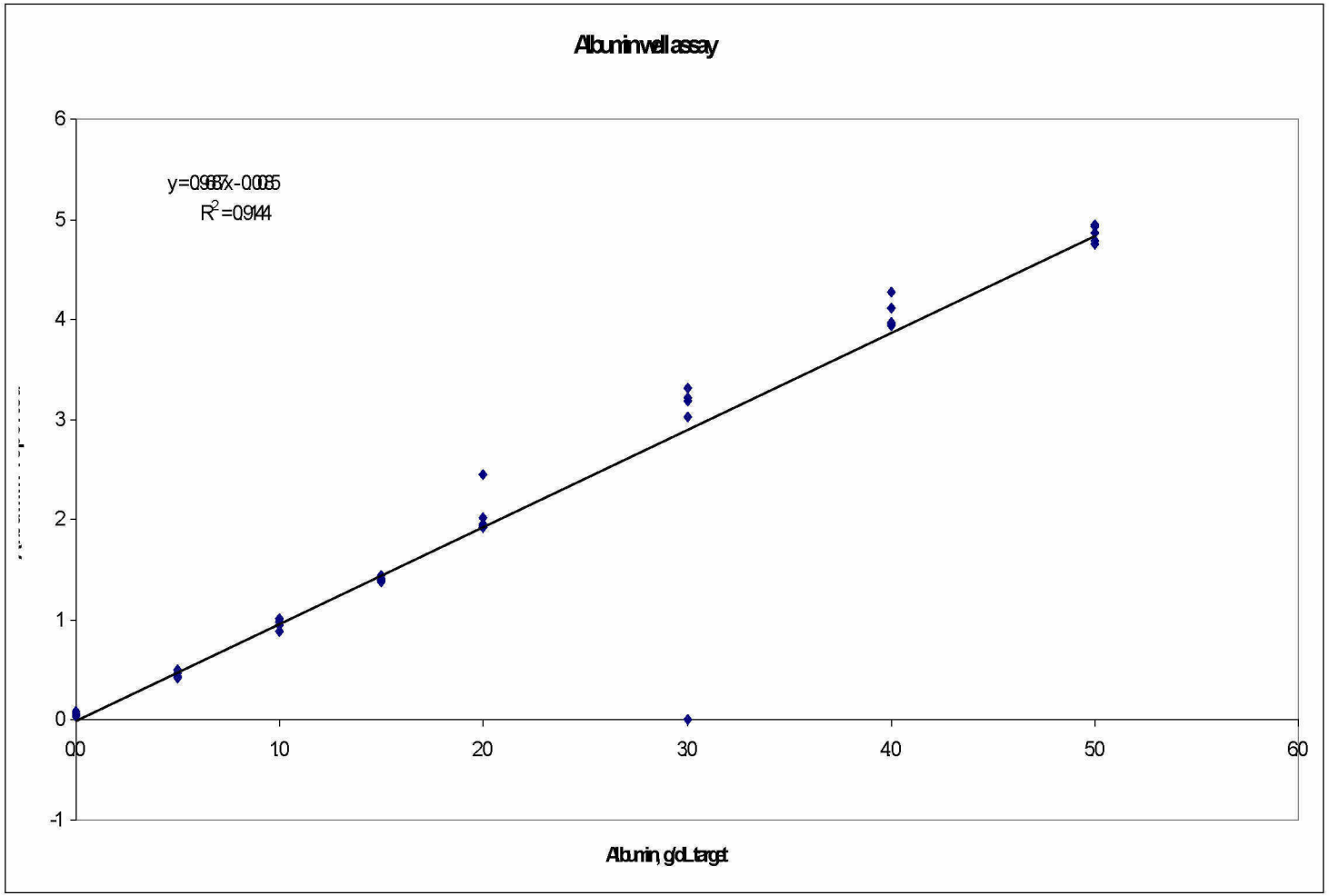
Performance of Albumin Assay



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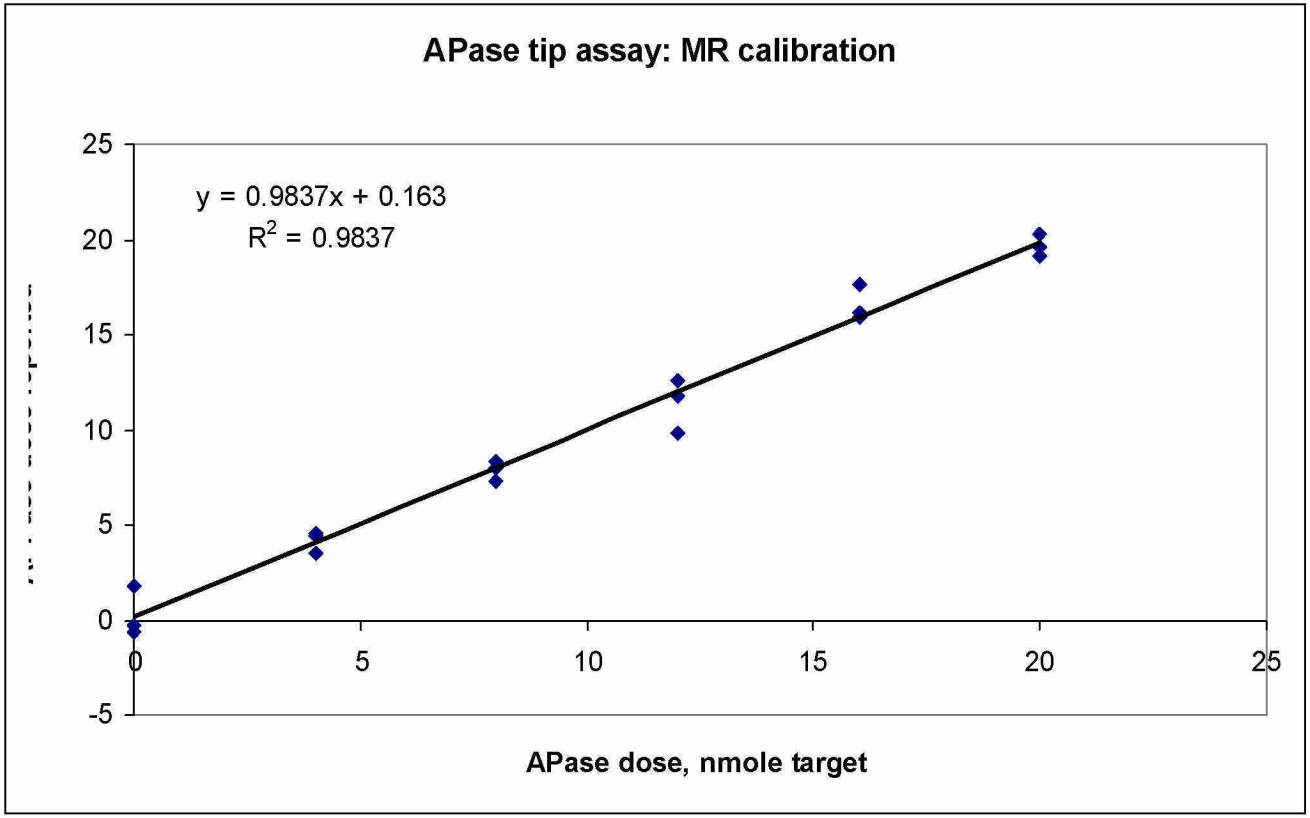
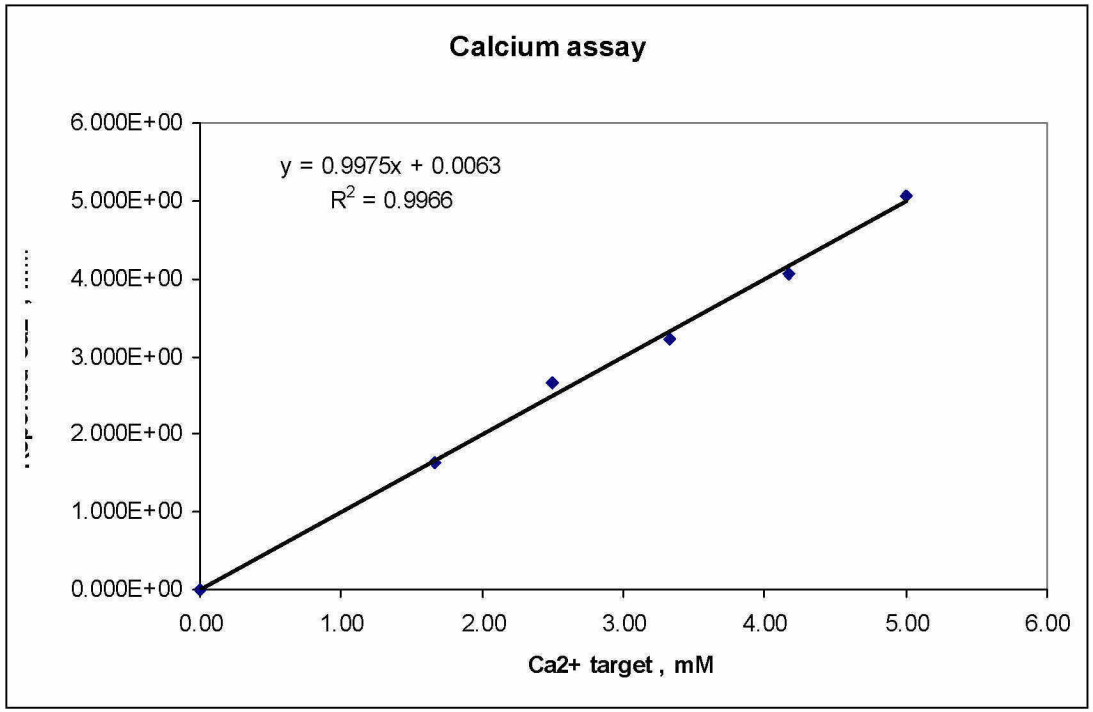
Alkaline Phosphatase assay



Assay for calcium



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General Chemistry Summary

Assays for major classes of analytes Metabolites (Glucose, Cholesterol, Lactate)

- Electrolytes (Calcium)
- Enzymes (Alkaline Phosphatase)
- Proteins (Albumin)

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Cell Analysis and Counting

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Cell enumeration method (example)

Blood is diluted accurately and precisely by our existing liquid handling technology

- For example, 1.0 uL blood is diluted 1:3

Staining reagent (for example fluorescent- antibody) is added to the diluted blood and mixed to provide a well-defined reaction mixture

- For example, 1 uL diluted blood is mixed with 2 uL reagent to a final dilution of 1:9 relative to undiluted blood.

A known volume of this reaction mixture is imaged.

- For example, 1.8 uL is placed in a chamber in the disposable with dimensions:
  - 3x3x0.2 mm
- In this volume there will be about 140 cells of a sub-type representing 10 % of the total white cells
- Count precision will therefore be about 8% CV [100/(#^(-0.5))]

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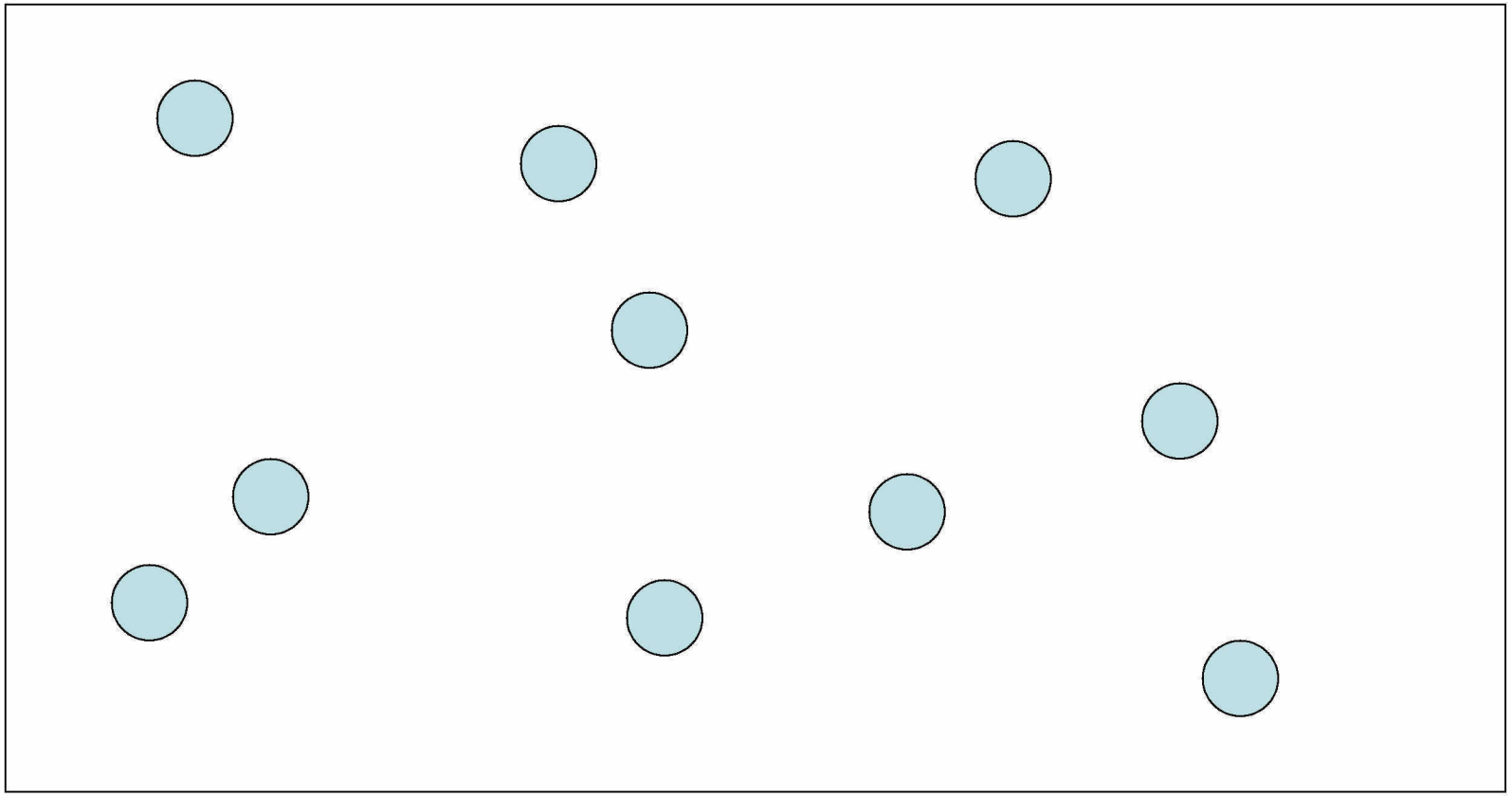
Cartoon of cell counting concept

Impression of about 10% of the imaged field, showing approximate density of stained cells



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Theranos System Cytometry Methodology

1. Theranos System meters and dilutes an exact volume of blood
2. System imaging interrogates a known, exactly defined fraction of the diluted sample within a field
   - The number of fields imaged is determined by the target cell abundance
     - Rare cell type: Several fields
     - Abundant cell type: Only one field
   - Cells are recognized by size and shape
3. Cells are identified by a combination of:
   - Fluorescence intensity
   - Fluorescence color
   - Size

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Theranos System Cytometry Methodology (cont.)

4. Imaging software accurately counts cells within each field (as shown in examples)
   - Sample is diluted to a level where overlapping cells in the image are a small proportion of the total
   - Software can even recognize overlapping cells
   - Sufficient cells for a statistically reliable count are counted
5. Result is computed from cell counts in image/known sample volume and reported as #/uL
6. As shown in the following slides, the image analysis software counts all cells in the image fields with an average system level accuracy of 99%.

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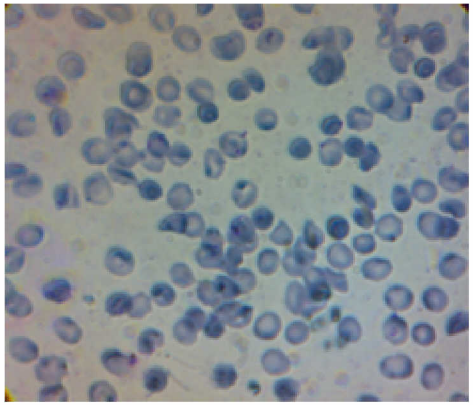
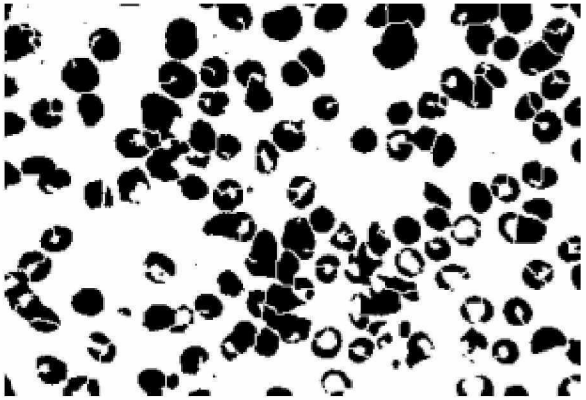
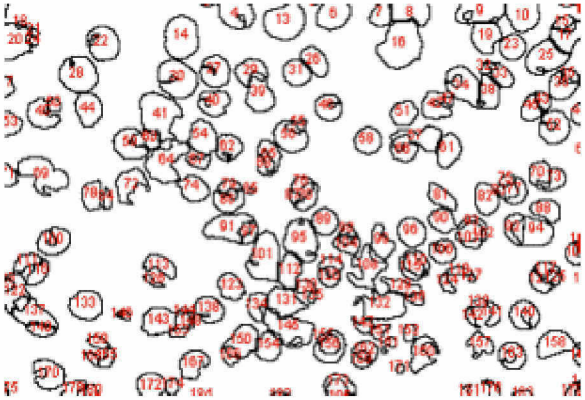
Red blood cells stained with dye and counted by rapid, automated image analysis software



Original image



Grey-scale image



Counting of cells

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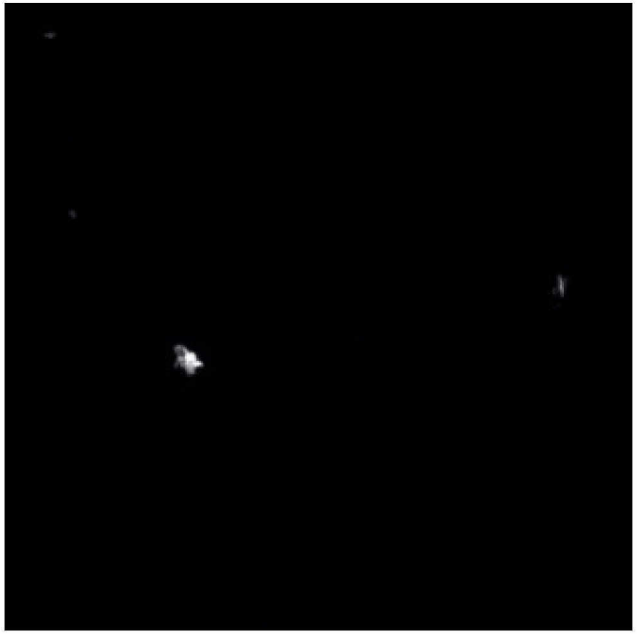
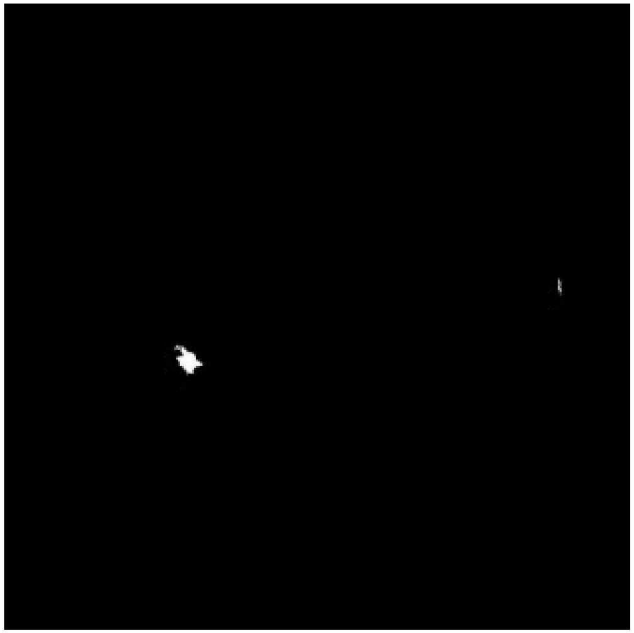
Fluorescent antibody-stained white cells: conversion of image to grey scale

White Blood Cell Differential: Basophil Count

Raw image



Thresholded image



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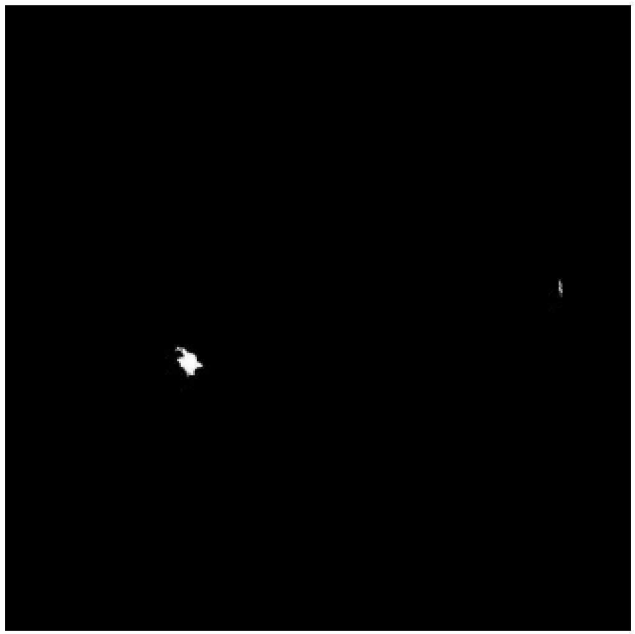
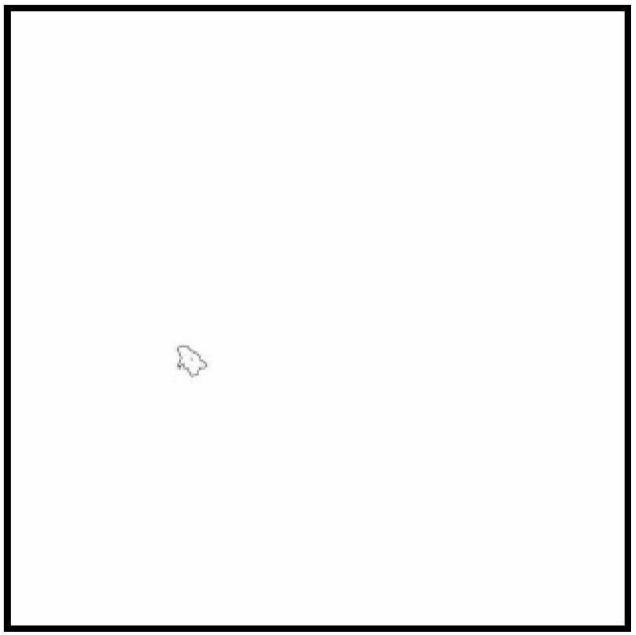
Fluorescent antibody-stained white cells: enumeration by image analysis

White Blood Cell Differential: Basophil Count

Thresholded image



Counted



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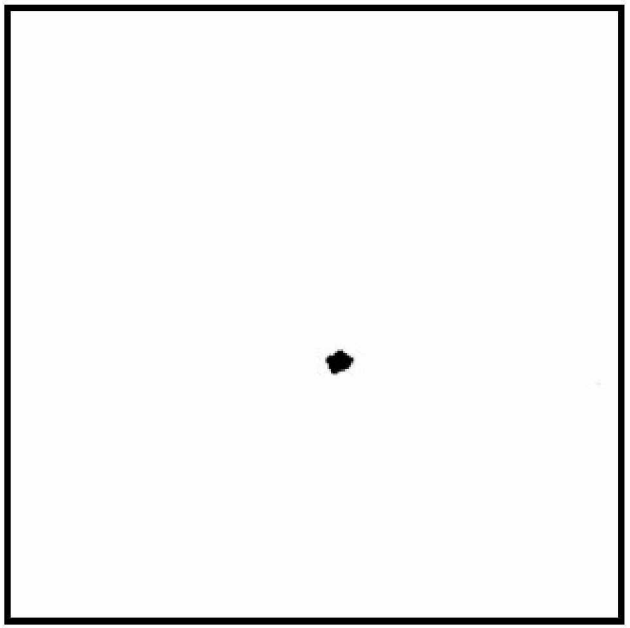
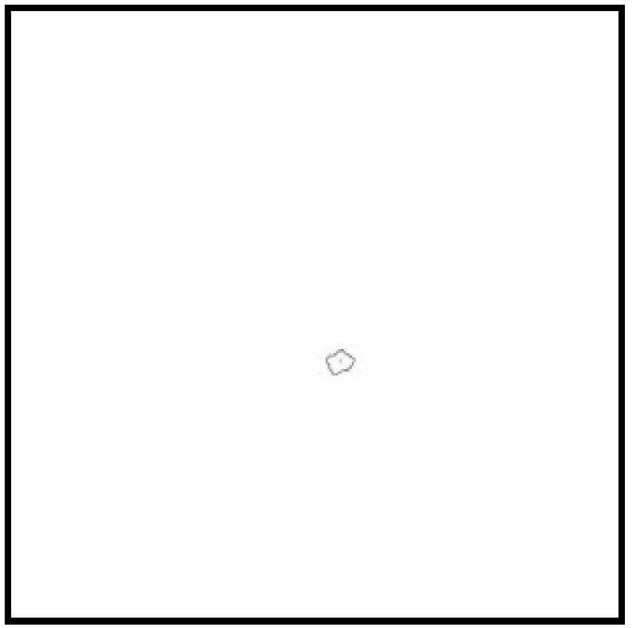
Fluorescent antibody-stained white cells: enumeration by image analysis

White Blood Cell Differential: Monocyte Count

Thresholded image



Counted



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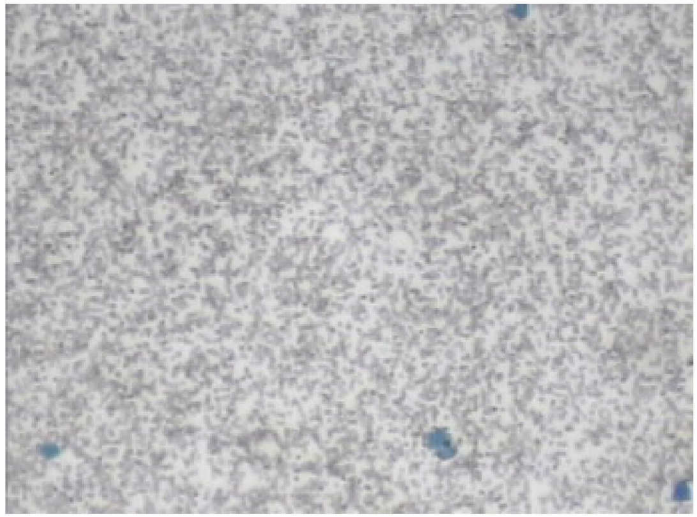
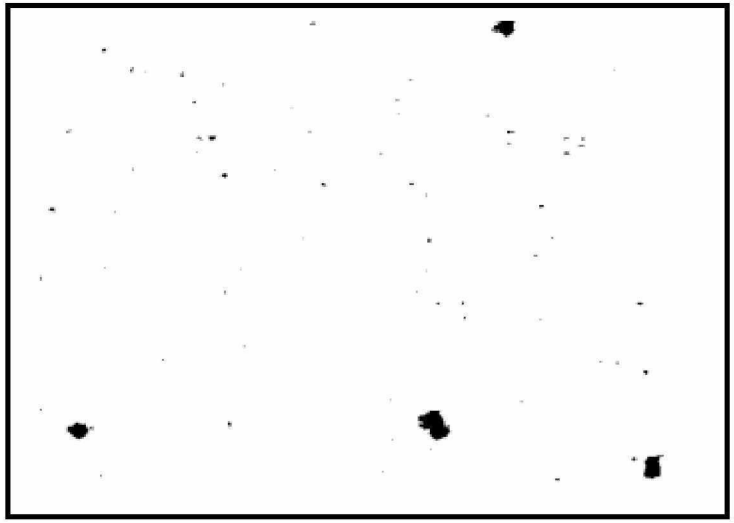
Trypan blue-stained white cells: conversion of image to grey scale

Raw image



Thresholded image



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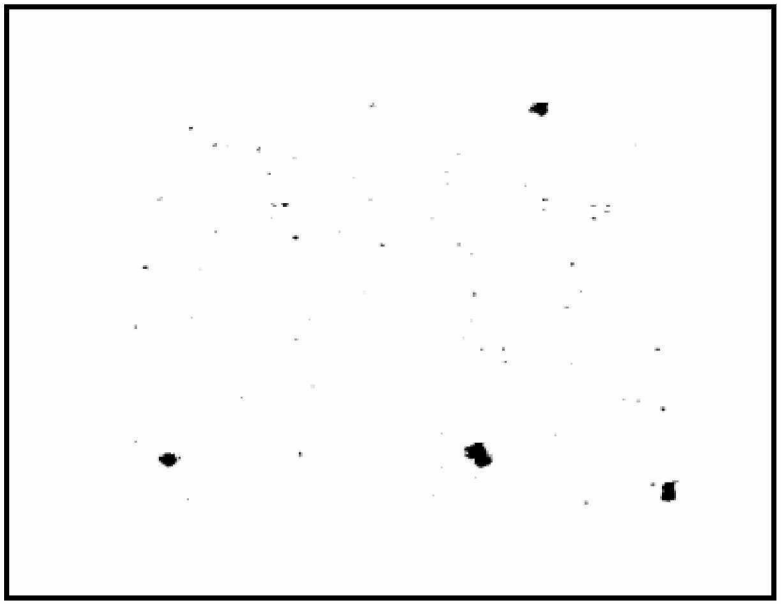
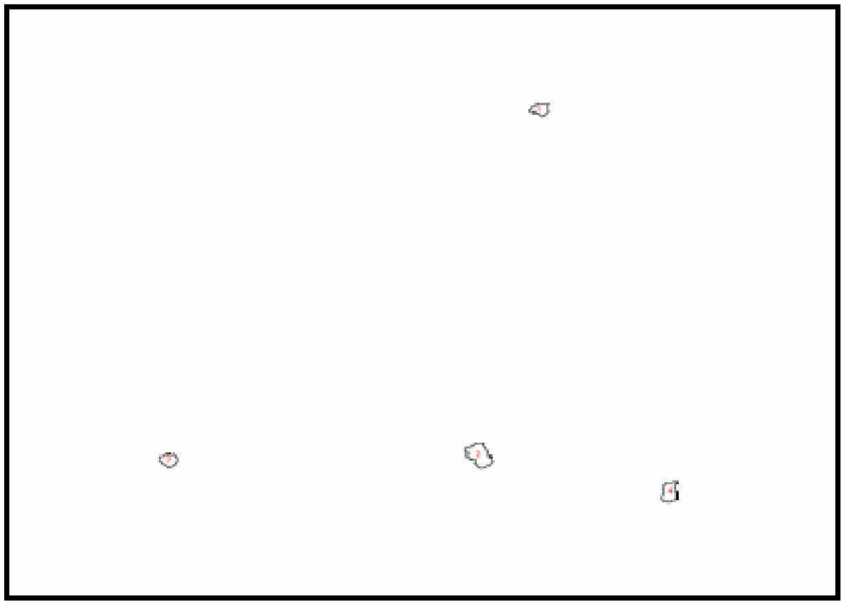
Trypan blue-stained white cells: counting the cells

Thresholded image



Counted



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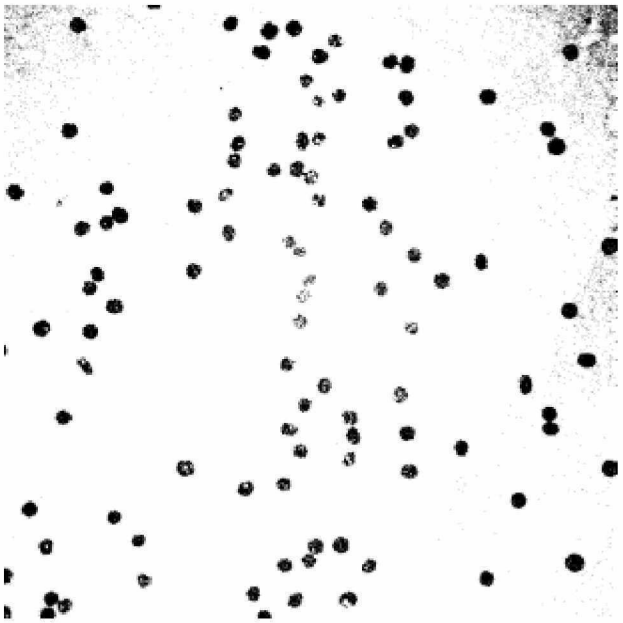
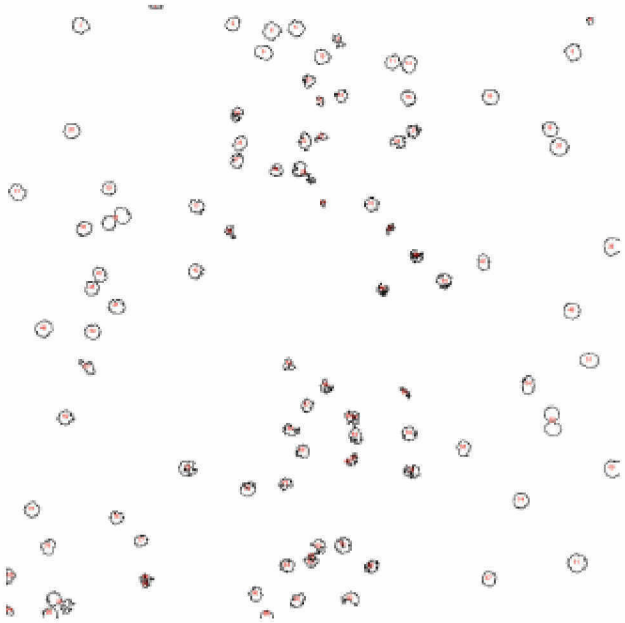
Enumeration of RBCs

Every cell is counted using Theranos proprietary software. Red blood cell in whole blood concentration can then be directly calculated.





~ 100 cells in image view → 3.1 x 10^6 RBC/µL blood

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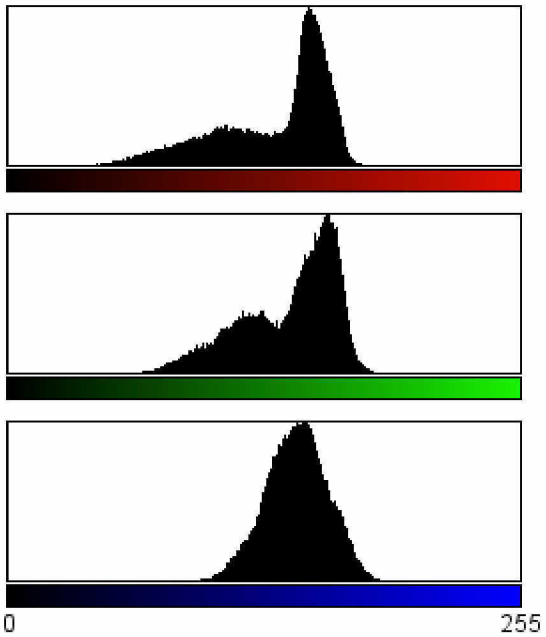
Analysis of image by three-color intensity:

Different cell populations can be distinguished and their distribution defined



Count: 106959

rMean: 132.45	rSD: 28.93	rMode: 150
gMean: 139.70	gSD: 24.05	gMode: 159
bMean: 143.55	bSD: 16.05	bMode: 148

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Data from Selected Clinical Studies

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Clinical Studies

- Monitoring AML patients in chemotherapy
- Monitoring osteoporosis therapy
- Monitoring metabolic processes and therapy using hormone assays
- Monitoring cancer therapy
- Fertility marker study

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Monitoring AML Patients in Chemotherapy

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Monitoring AML Patients in Chemotherapy

Hospitalized patients

All become febrile.

Many progress to sepsis.

Anticipating progression to sepsis would be of great value.

Use of broad spectrum antibiotics is dangerous.

- Development of drug resistance in hospital
- Side effects in patients

Seventeen patients were monitored using the Theranos System over about 30 days.

- Four became septic.

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Monitoring AML Patients in Chemotherapy

Biomarker measurements enabled prediction of progression to sepsis.

Multiplexed measurements of Protein-C, and CRP were made in about 1000 blood samples.

Multiplexed IL-6, TNFα and CRP were made on a subset of plasma samples.

In some cases, laboratory assays of other analytes were made in archived plasma to identify other useful markers.

Going forward, an optimal candidate set of markers has been selected for large-scale clinical studies of prediction of sepsis.

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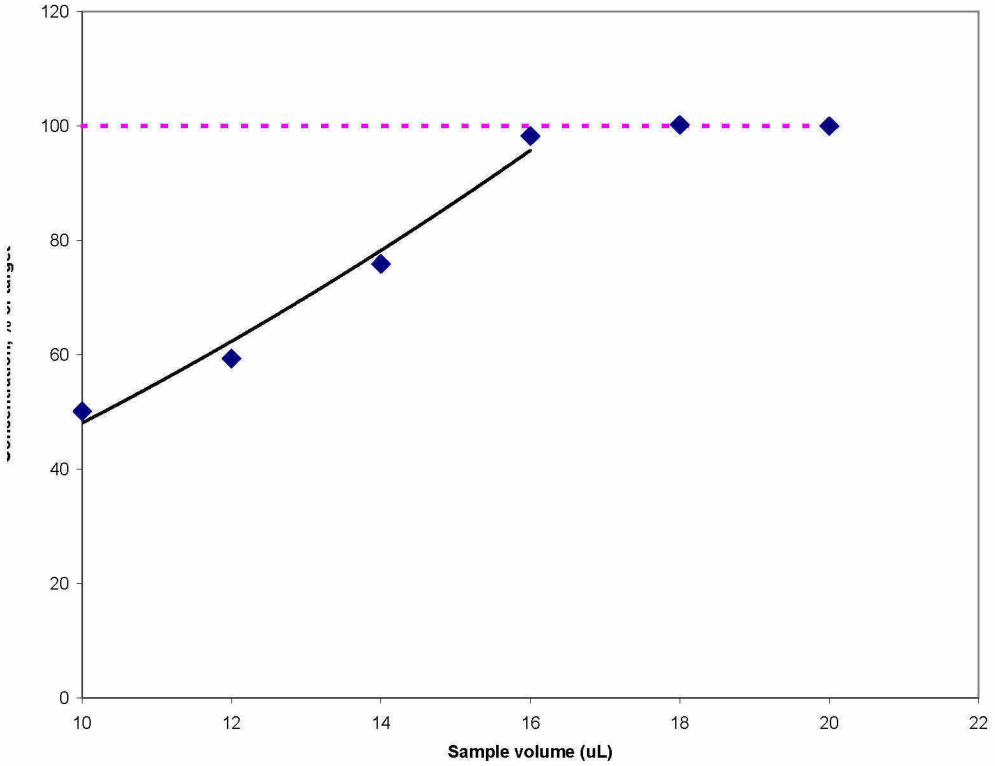
Results Independent of Blood Sample Volume > 15 µL

System is designed to use a 25 µL sample.

Sample transfer device automatically collects and delivers a 25 µL sample (blood, plasma, serum) to the disposable.

System then uses a smaller (precisely metered) volume.

System works even if the sample volume delivered to the disposable is less than the target volume.



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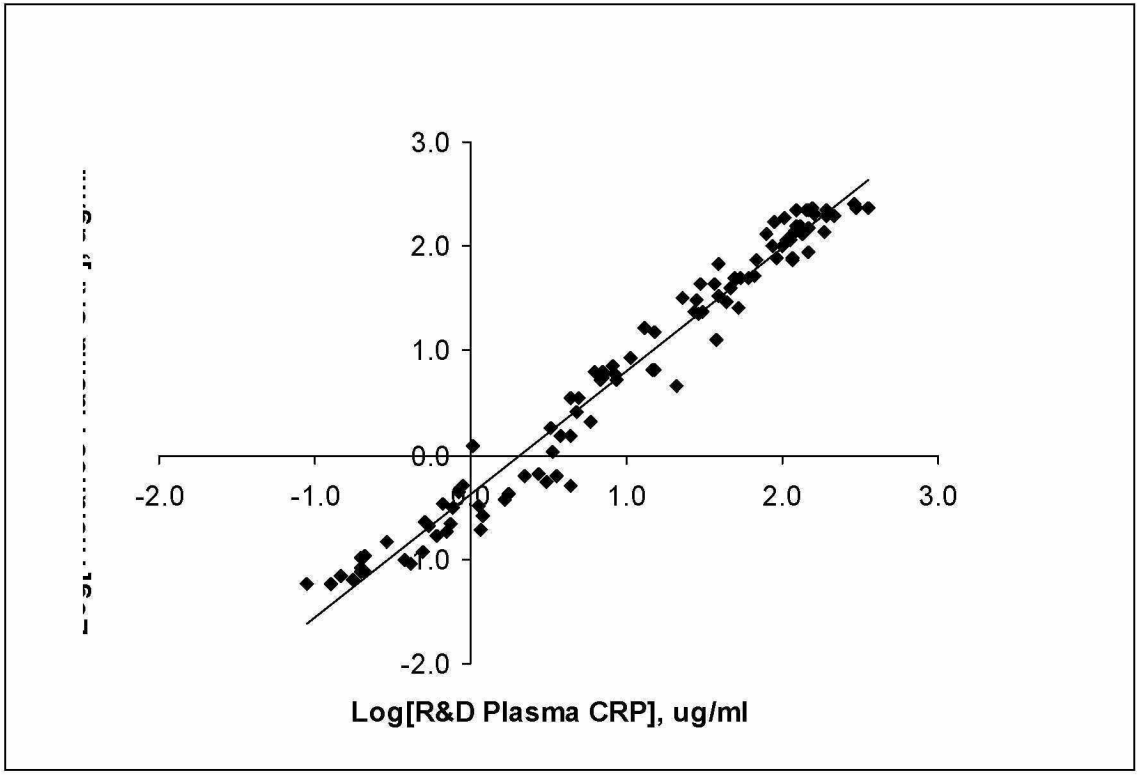
CRP Assay Correlation Over 10,000-fold Range

y = 1.179x - 0.3746, r = 0.99, N = 104



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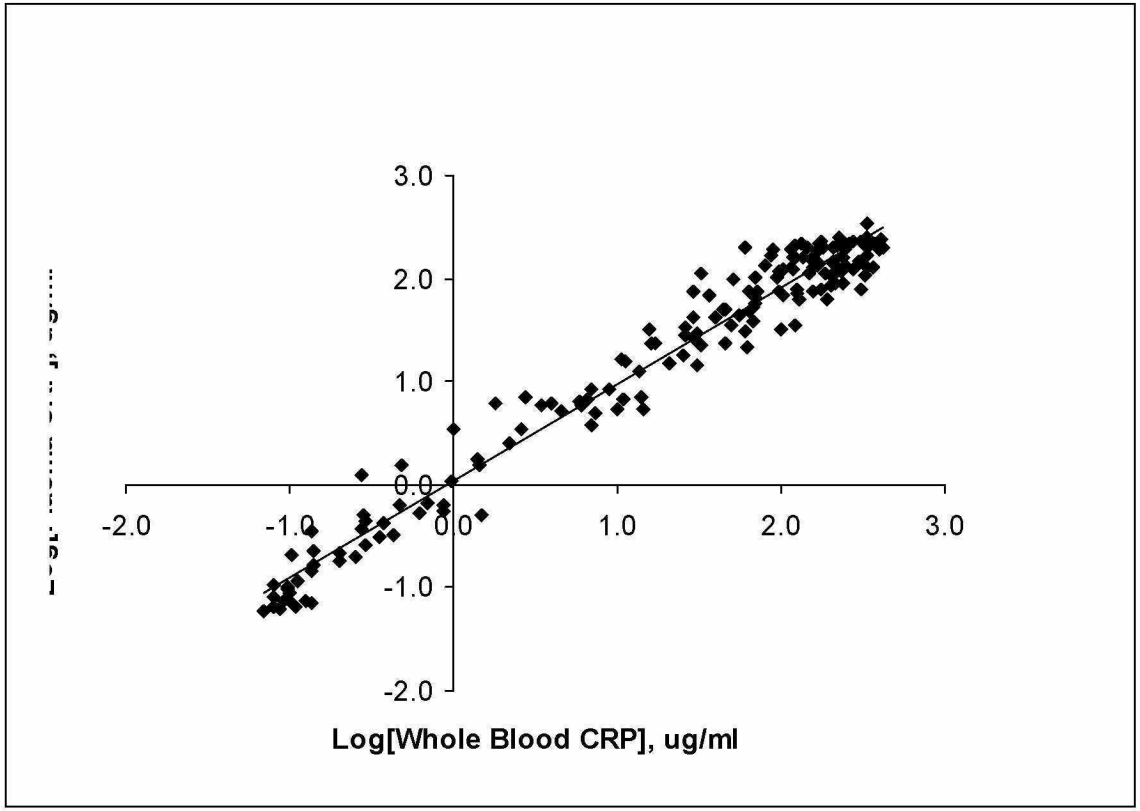
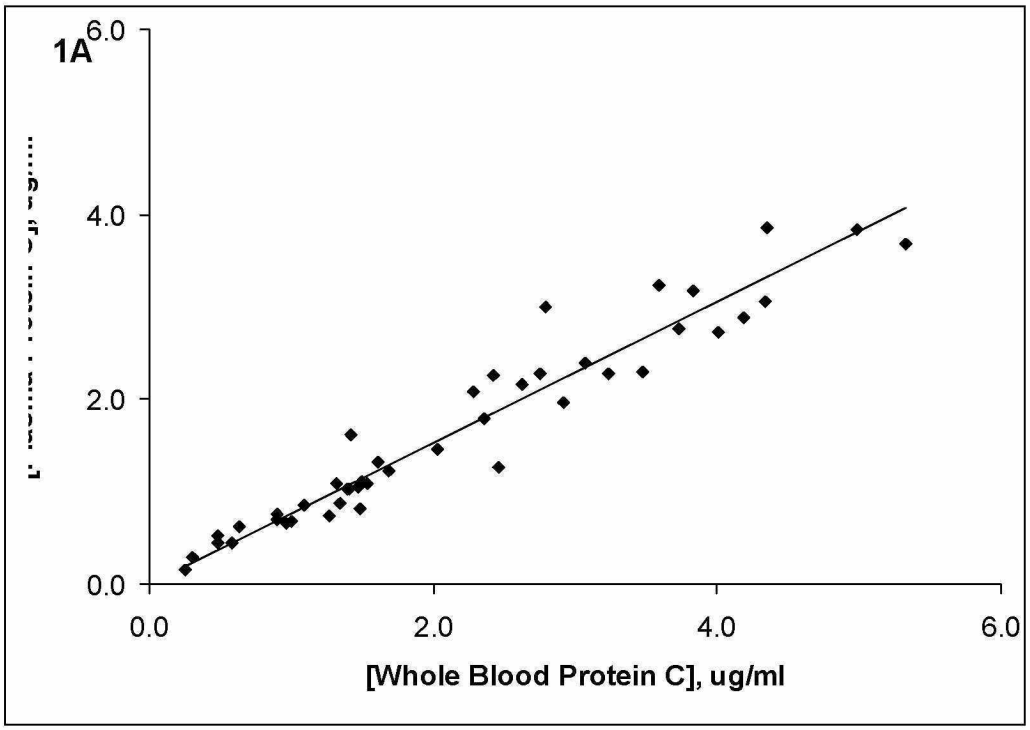
CRP Assay: Blood Versus Plasma



Protein-C: Blood Versus Plasma

System calibrated for blood.

Clinical data is then used to calibrate for plasma (as a one-time exercise).



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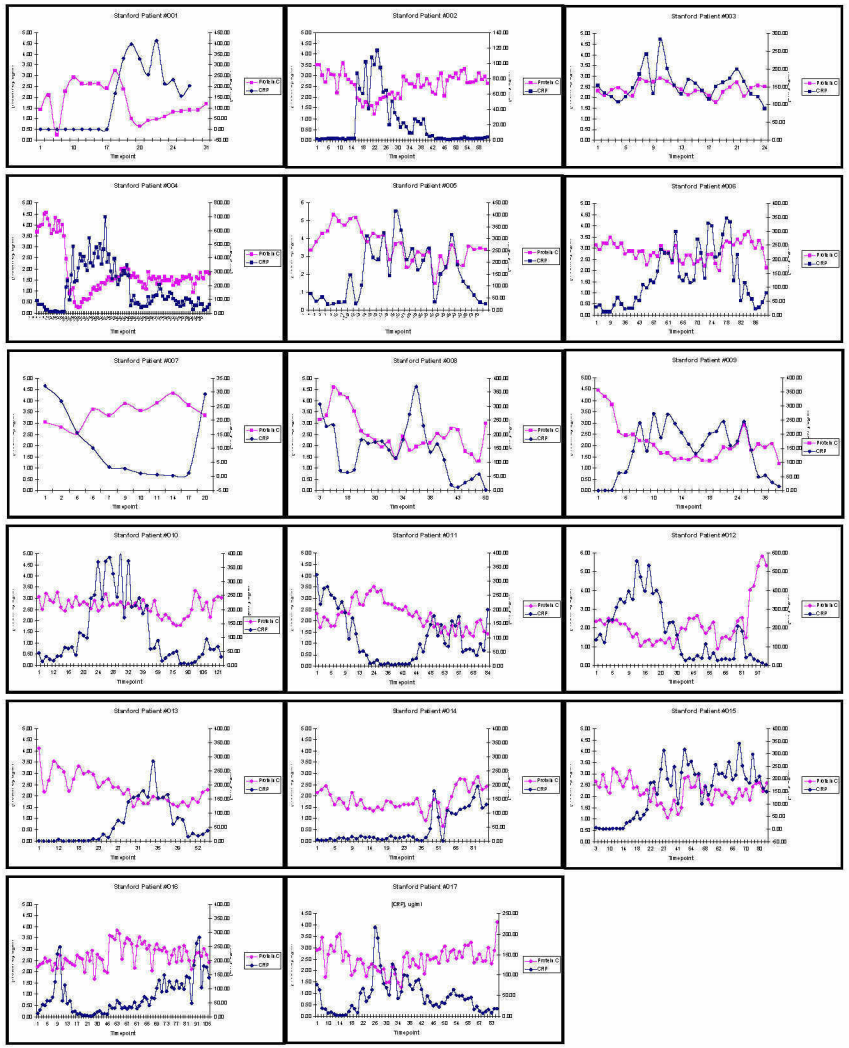
Monitoring AML Therapy and Sepsis

Protein-C/CRP multiplex

- Time courses for 17 patients
- Protein-C declined significantly prior to or at Sepsis diagnosis but not in non-septic patients.
- CRP levels fluctuated wildly.
- CRP levels were as high as 500 …g/mL.



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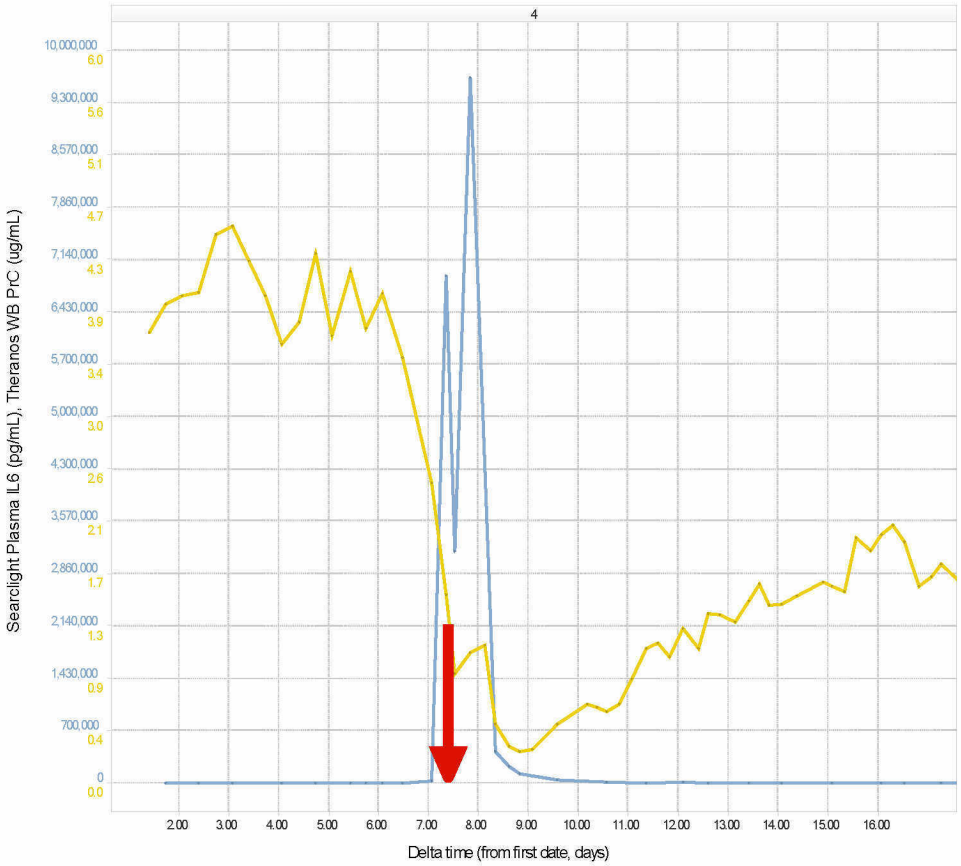
Predicting Sepsis: Biomarker Spikes or Changes

For one patient, IL-6 and Protein-C levels are shown surrounding the time of sepsis diagnosis.

Protein-C declined prior to diagnosis, and IL-6 exhibited a massive rapid spike at the time of diagnosis.



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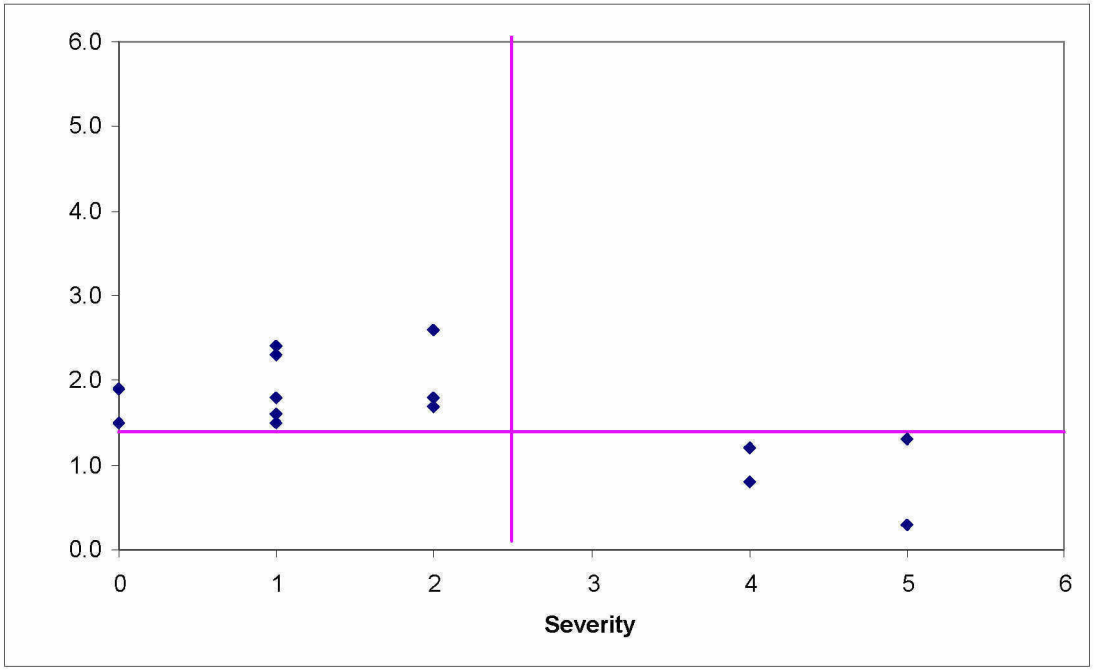
Diagnosis of Sepsis

Significant decline in Protein-C correlated with sepsis.

Minimum values were plotted against a measure of the severity of infection.



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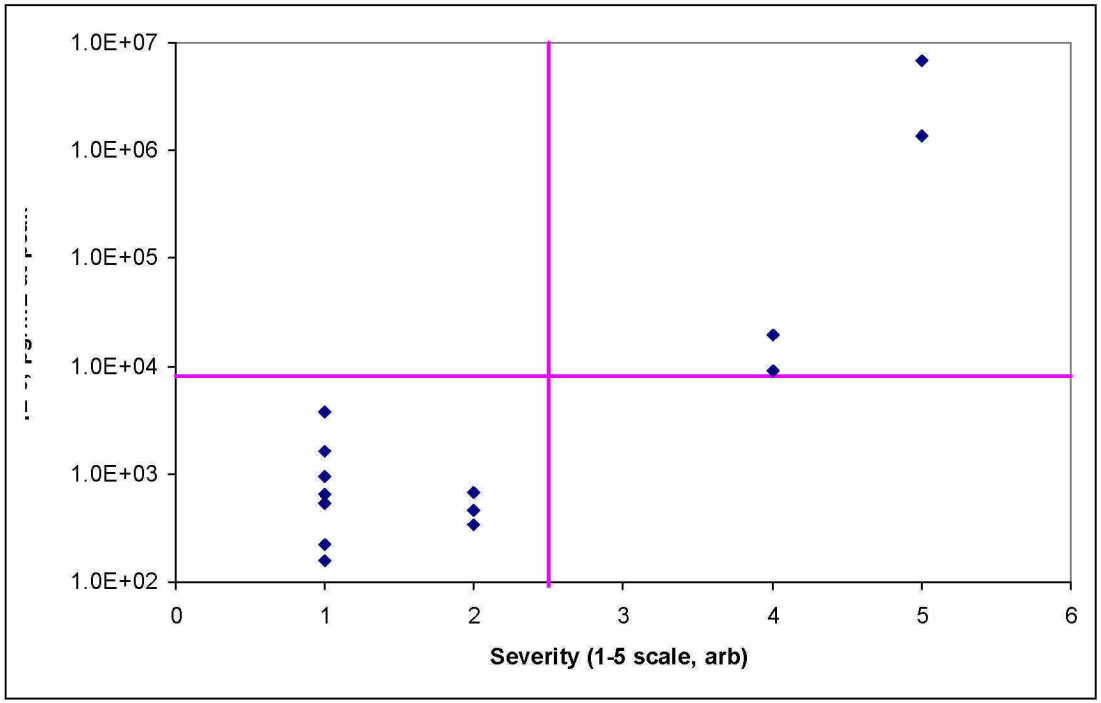
Diagnosis of Sepsis

IL-6 > 5 ng/mL was correlated with severity of infection.

Note: some IL-6 levels were in the µg/mL range.

IL-6 spikes were sometimes so rapid they would have been missed on a regular blood sampling schedule (twice per day).

Theranos system facilitates more frequent analysis.



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Anticipating Sepsis

Using a combination of changes in three markers, which can be measured in a Theranos multiplex, sepsis can be predicted in 4/4 patients.

0/11 non-septic subjects were negative by the proprietary algorithm used. (2 subjects were not evaluable.)

Patient	Criterion	Days (Marker to Diagnosis)
1	Marker 1	2.2
1	Marker 2	0.8
1	Fever	0.0
4	Marker 1	0.2
4	Marker 2	1.1
4	Marker 3	0.9
4	Fever	0.0
12	Marker 2 + Fever	2.0
12	Fever	2.0
15	Marker 1	0.5
15	Fever	0.1

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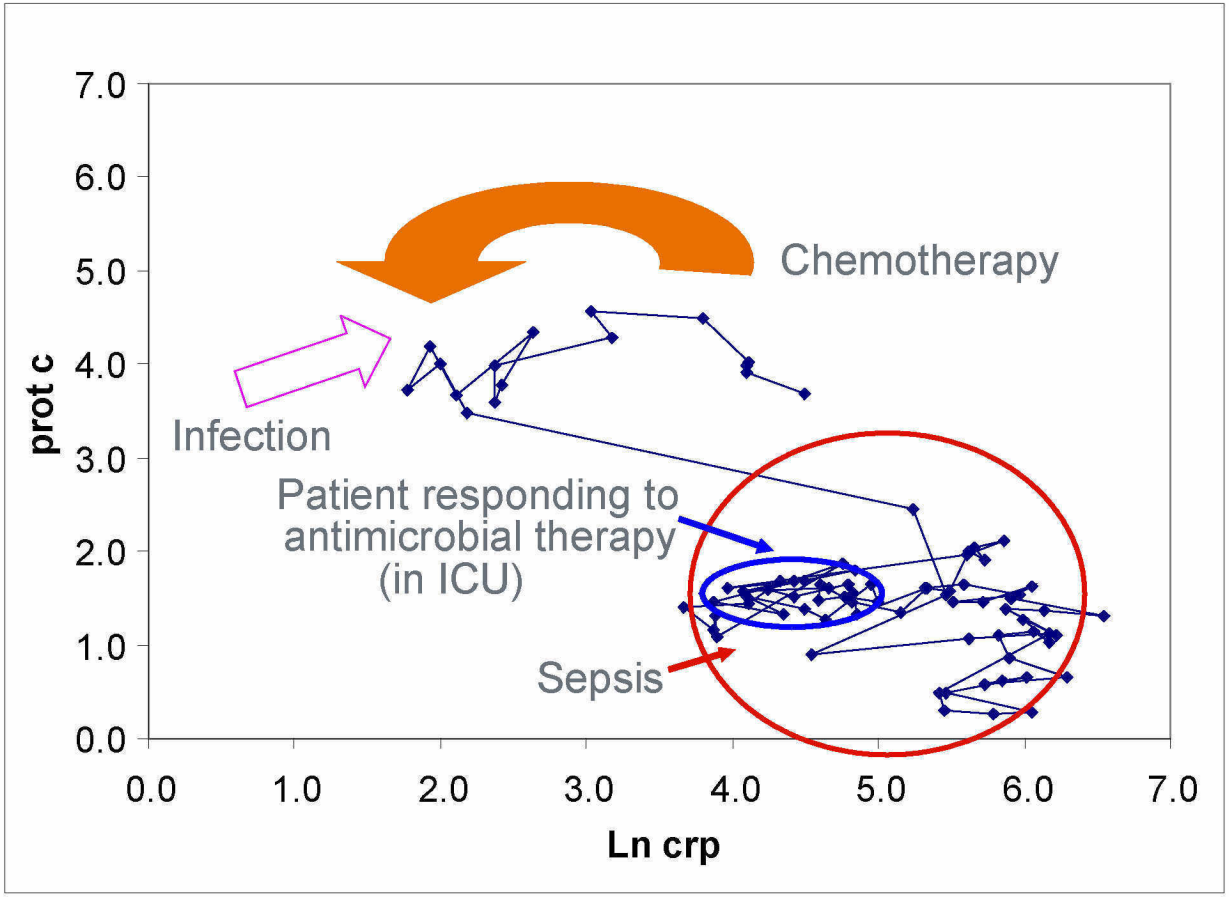
Trajectory to Sepsis

Mulwellle assays used to follow disease and therapy.

Sequential time points connected.

Protein-C and CRP as a two-dimensional plot clearly show a series of ”events” and processes in progression to and recovery from Sepsis.



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Monitoring Osteoporosis Therapy

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Monitoring Osteoporosis Therapy

Patients were given an osteoporosis drug.

Samples were collected prior to and after the drug administration.

Retrospective study using a Theranos inflammatory marker multiplex (IL-6, TNFα, and CRP) using archived plasma samples from the drug company.

All assays showed good correlation with predicate methods and demonstrated a lower limit of detection.

Patients could be classified according to the results.

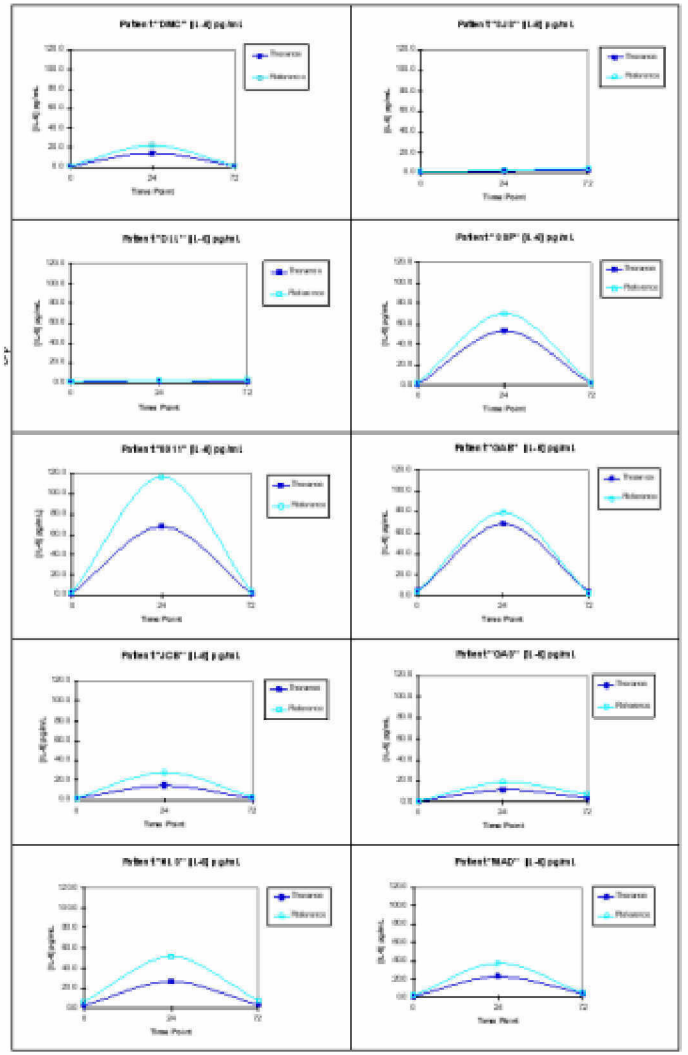
Inflammatory Marker 1 – IL-6

For each subject, the time course of biomarker changes was monitored.

Results from the Theranos System tracked well with those of a predicate method.

There were very great differences in the magnitude of the peak response between patients.

LOD = 0.2 pg/mL
Range = 0.2 pg/mL-1500 pg/mL



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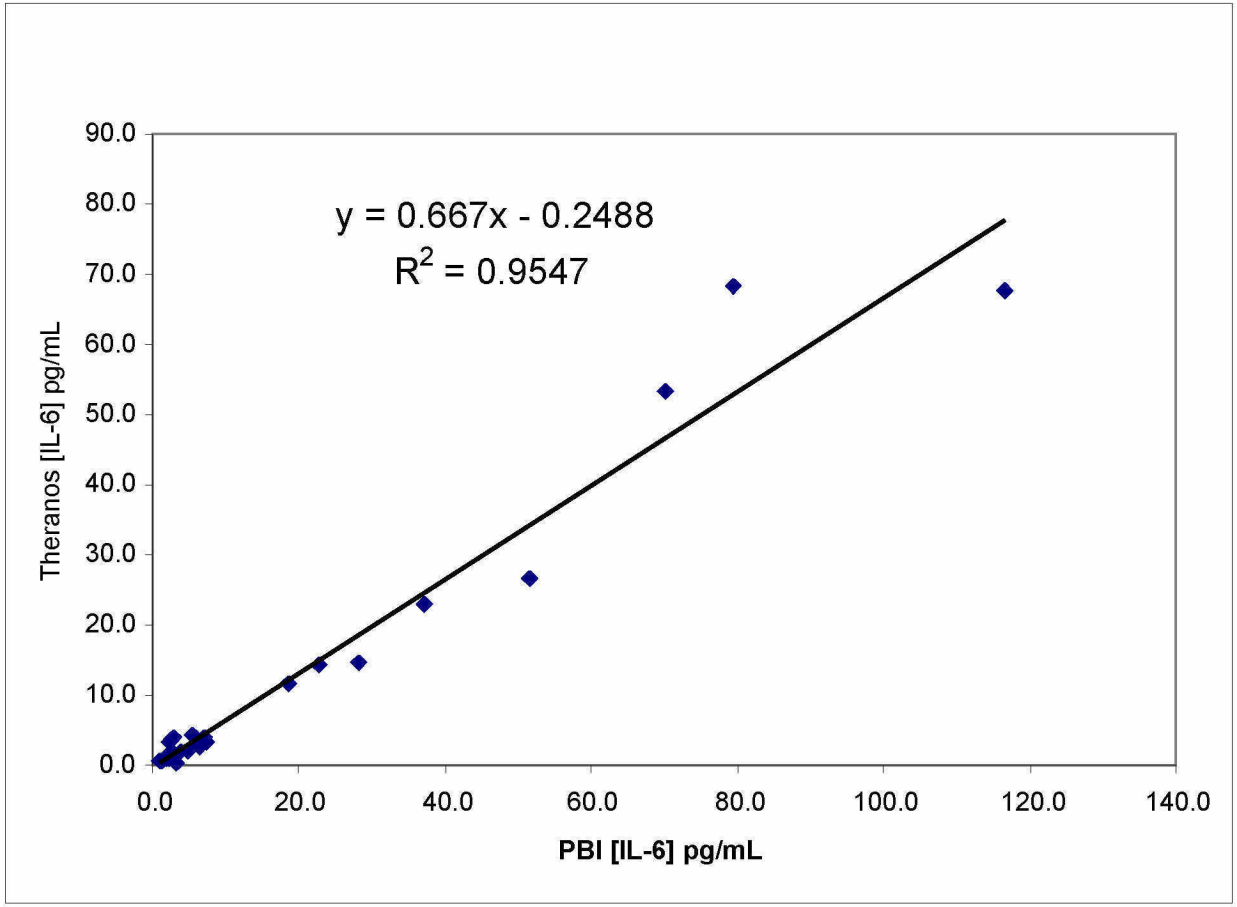
IL-6 Assay Method Correlation



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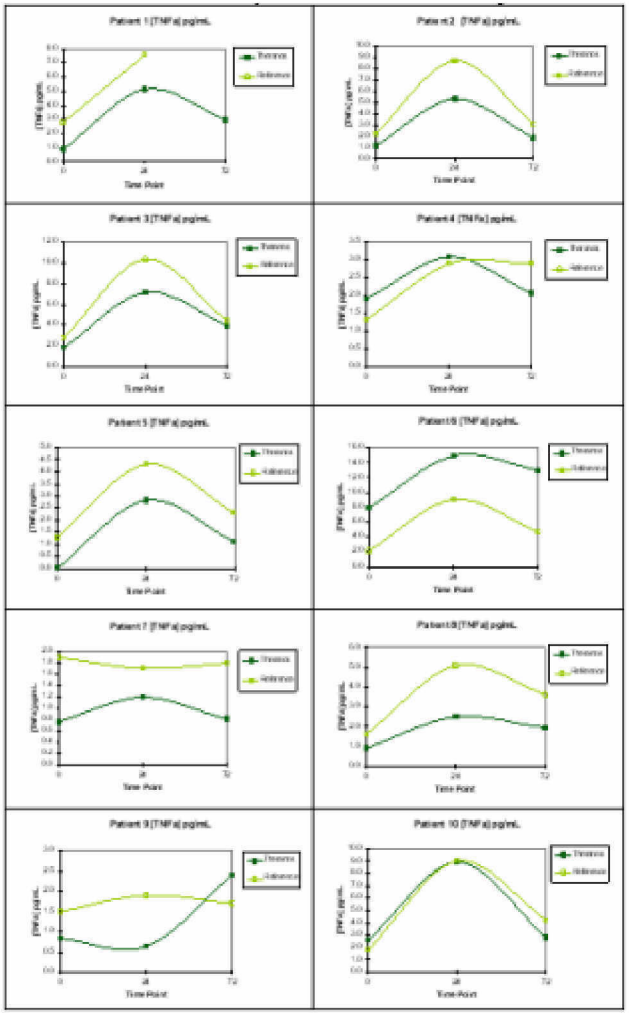
Inflammatory Marker 2 – TNFα

For each subject, the time course of biomarker changes was monitored.

Results from the Theranos System tracked well with those of a predicate method.

There were very great differences in the magnitude of the peak response between patients.

LOD = 0.65 pg/mL
Range = 0.65 pg/mL-15,000 pg/mL



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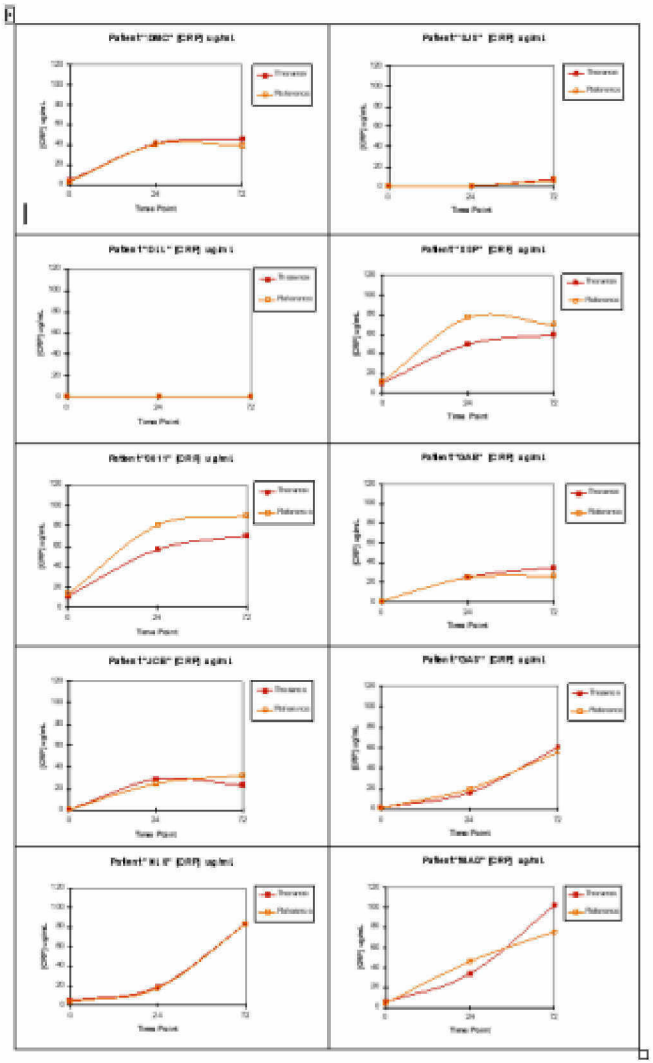
Inflammatory Marker 3 – CRP

For each subject, the time course of biomarker changes was monitored.

Results from the Theranos System tracked well with those of a predicate method.

There were very great differences between patients in both the kinetics and magnitude of the response.

LOD = 0.06 ..g/mL
Range = 0.06 µg/mL-250 µg/mL



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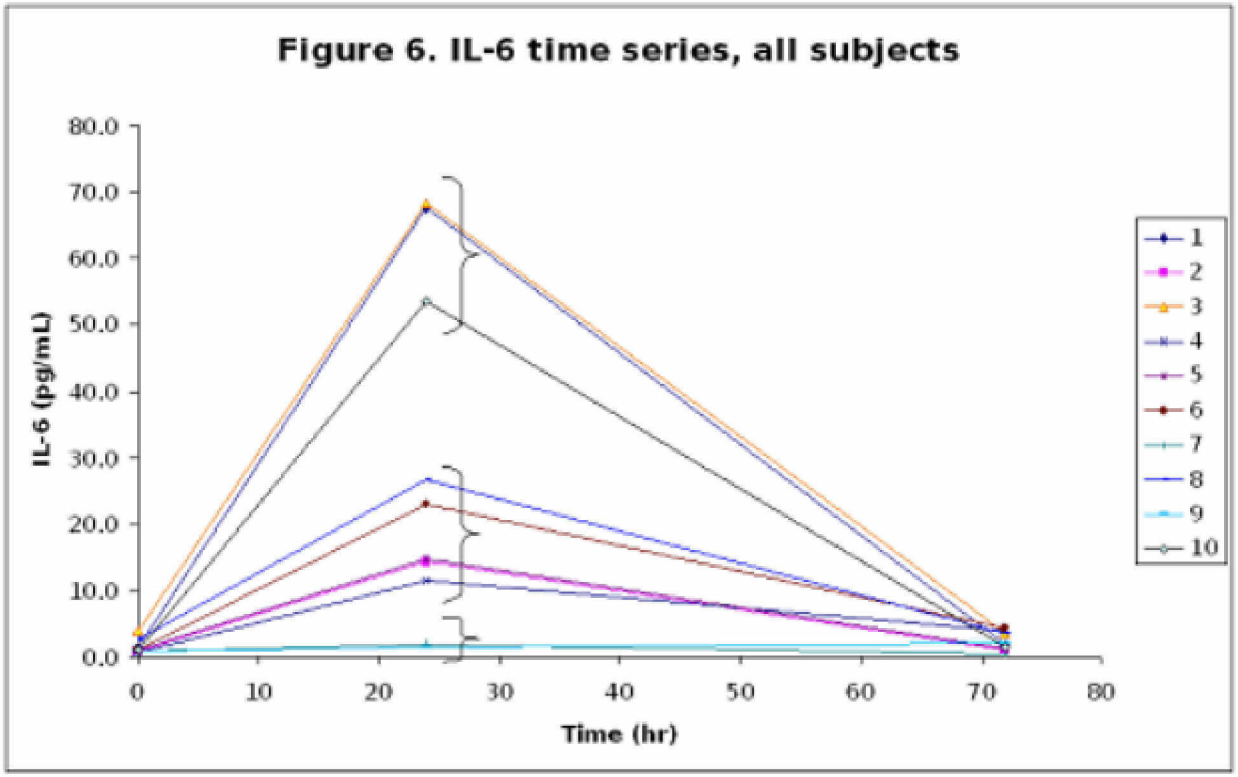
Classification of Patients Using One Biomarker

Patients produced inflammatory marker IL-6 in amounts differing by more than an order of magnitude.



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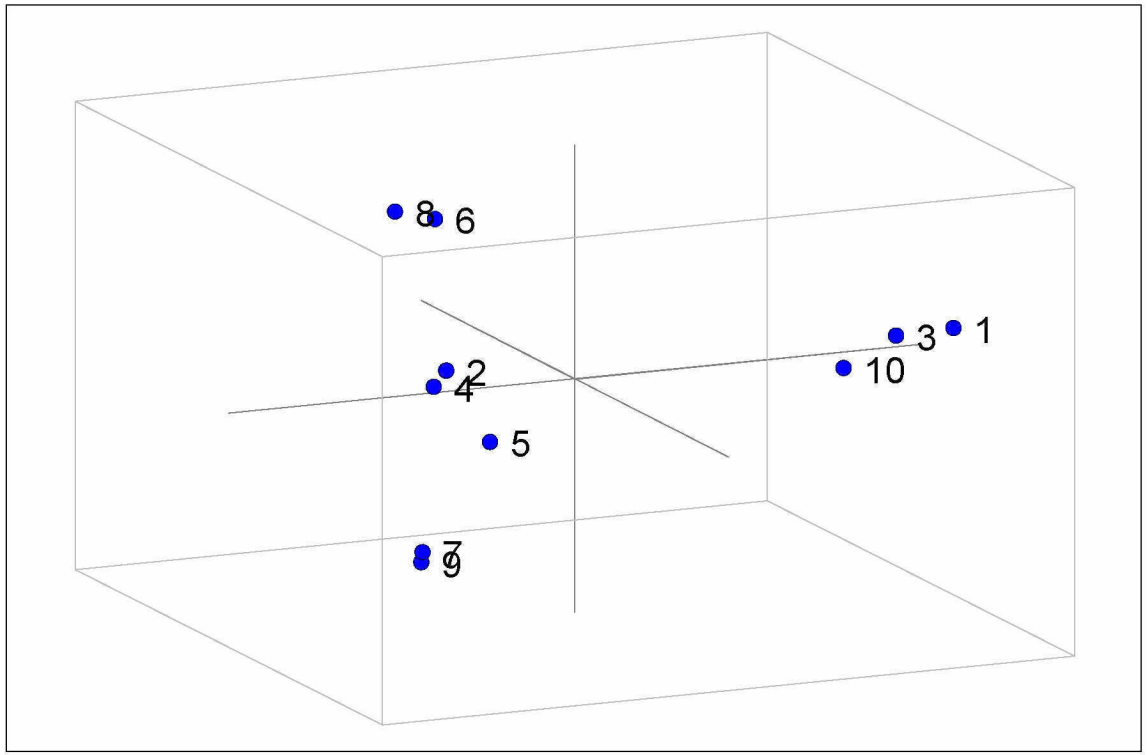
Classification of Patients Using Three Biomarkers

Canonical discriminate analysis was performed on the output clusters.

These data are plotted in a three dimensional space, clearly verifying that four clusters can be distinguished.



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Monitoring Metabolic Processes and Therapy Using Hormone Assays

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Monitoring Metabolic Processes and Therapy

In several validation studies, GLP-1(active), GLP-1 (inactive) and C-Peptide were measured by the Theranos System.

- Glucose challenge
- Glucose clamp + two levels of GLP-1 infusion

Results

- Theranos GLP-1 (active) results correlated with the most sensitive predicate method but not with the most well-known assay, which was too insensitive for comparisons to be made.
- Theranos C-peptide results correlated well with those of a predicate method.
- No predicate GLP-1 (inactive) assay which is sensitive enough was available.
- Major differences in magnitude and kinetics of the biomarker responses among patients were observed, enabling classification.

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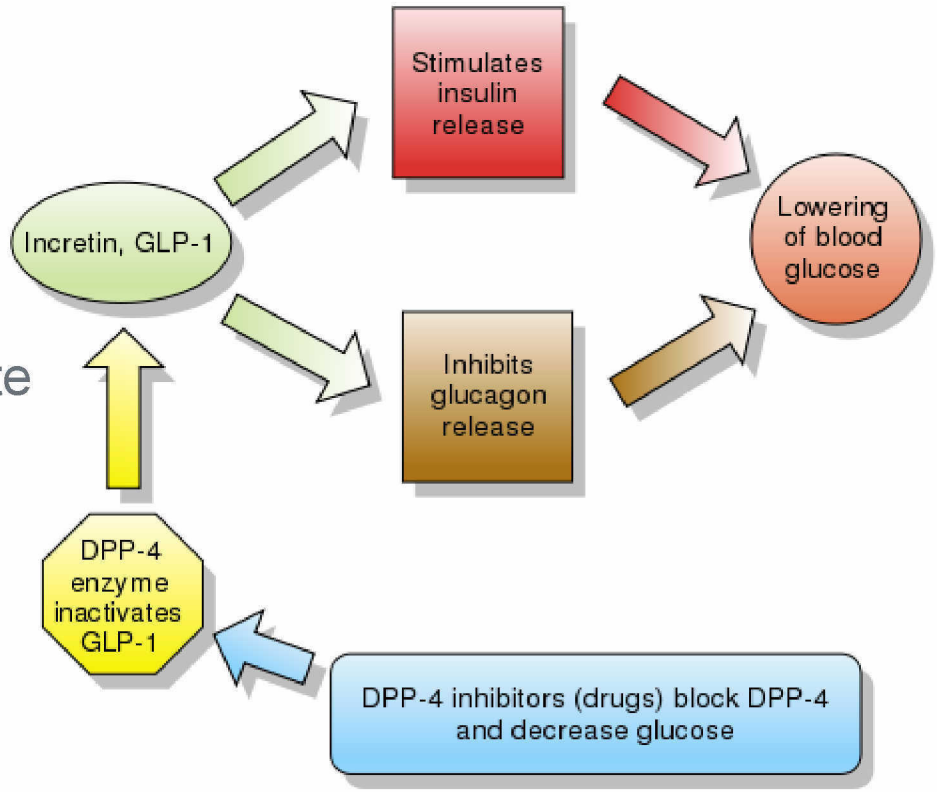
Characterizing GLP-1 in Metabolism

Key hormone in carbohydrate metabolism

- Active GLP-1 is released into blood when food is ingested.
- Rapidly inactivated (t1/2 = about 2 minutes) by DPP-4.
- Active and inactive forms are also eliminated from blood through the kidney etc.
- DPP-4 inhibitors are candidate diabetes drugs.
- Humans differ greatly due to genetic and other factors in their response to food challenge and hormone production and elimination.



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Characterizing C-Peptide in Metabolism

1. C-peptide is a peptide that is made when proinsulin is split into insulin and C-peptide. They split before proinsulin is released from endocytic vesicles within the pancreas – one C-peptide for each insulin molecule.
2. C-peptide binds to a receptor at the cell surface and activates signal transduction pathways that result in stimulation of Na+, K+ATPase and endothelial nitric oxide syntheses (eNOS), both of which are enzymes with reduced activities in type I diabetes.
3. C-peptide functions in repair of the muscular layer of the arteries.

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Characterizing C-Peptide in Metabolism

4. C-peptide also exerts beneficial therapeutic effects on many complications associated with diabetes mellitus, such as diabetic neuropathy and other diabetes-induced ailments.
5. In the kidneys, C-peptide prevents diabetic nephropathy, and in the heart, blood flow is improved in diabetic patients.

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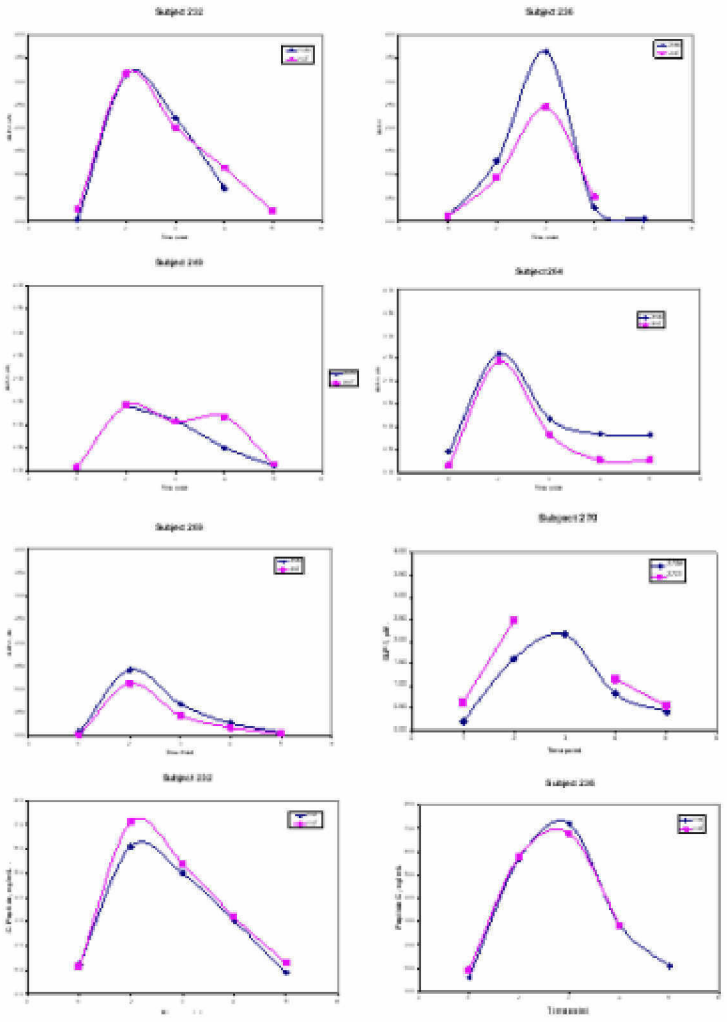
Monitoring GLP-1 Release: Food Challenge

Food challenge at time zero (after time point 1).

Theranos System and predicate method results shown.

Significant variation between subjects in kinetics and magnitude of the response.



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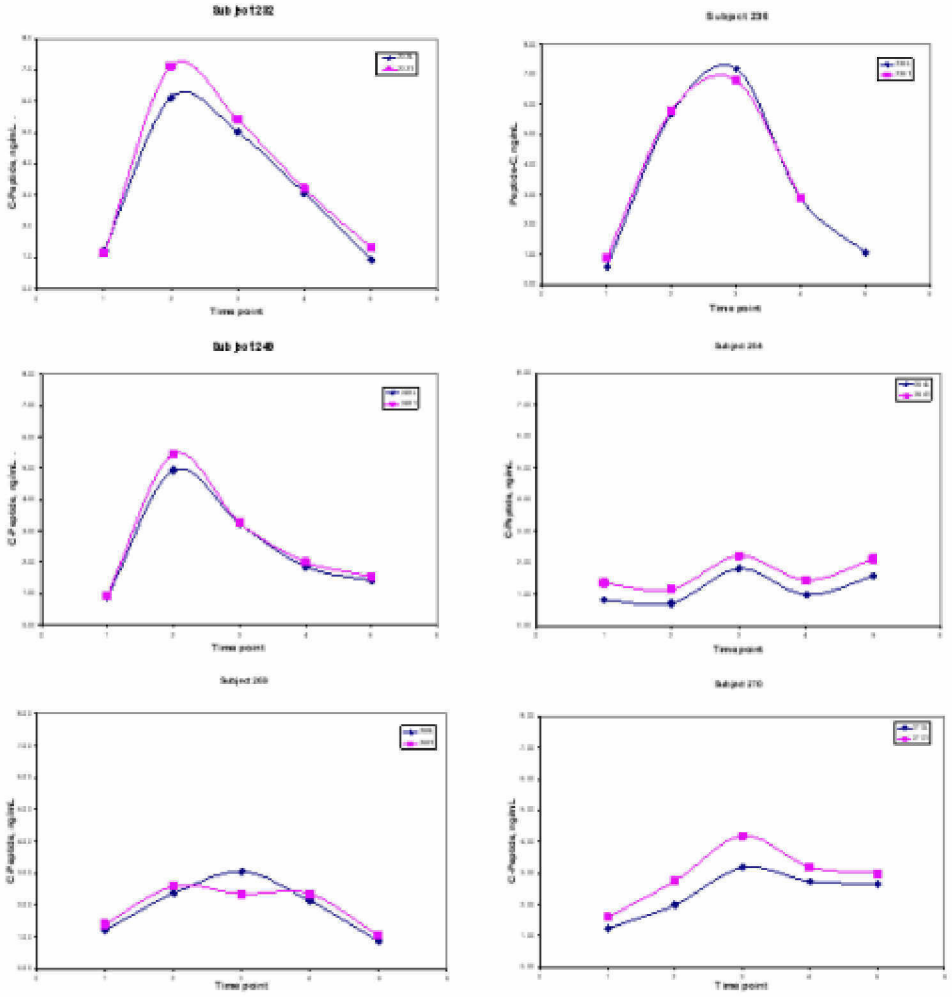
Monitoring C-Peptide Release: Food Challenge

Food challenge at time zero (after time point 1).

Theranos System and predicate method results shown.

Significant variation between subjects in kinetics and magnitude of the response.



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Monitoring Hormone Levels

May enable classification of subjects

- Propensity to diabetes
- Response to food
- Response to drugs

Serial measurements provide superior classification means.

Theranos System is a powerful, convenient means for serial measurements of several biomarkers in non-laboratory settings.

Large-scale studies of variation in human response to hormone or glucose challenge may lead to screening method for diabetes risk and monitoring of novel drug therapies for diabetes.

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Effect of Diabetes-Associated Gene Variation on Glucose Metabolism in Humans

Study conducted in collaboration with a major academic medical institution.

- 190 normal, non-diabetic patients not on drug therapy
- 7 timepoints per patient (-5,10,20,30,60,90,120 minutes)
- 75g oral glucose tolerance test (OGTT)
  - Administered at T = 0 min

Samples assayed in the Theranos System for both active and inactive GLP-1 levels.

- Multiplexed assays

Major differences between subjects were observed in both the speed and magnitude of responses.

Analysis of response patterns enables classification of subjects.

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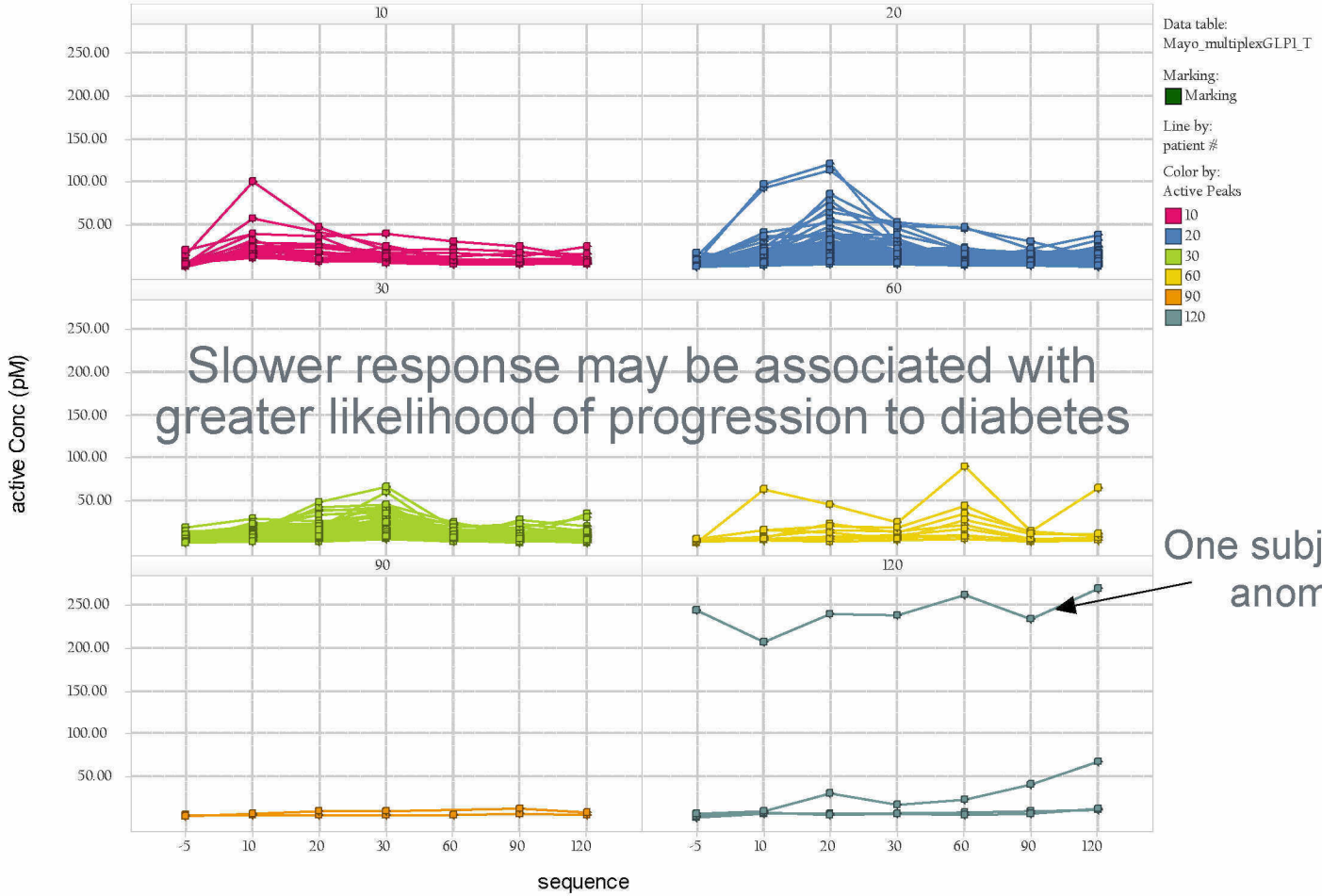
Oral Glucose Tolerance Test

Classified by time of active GLP-1 peak



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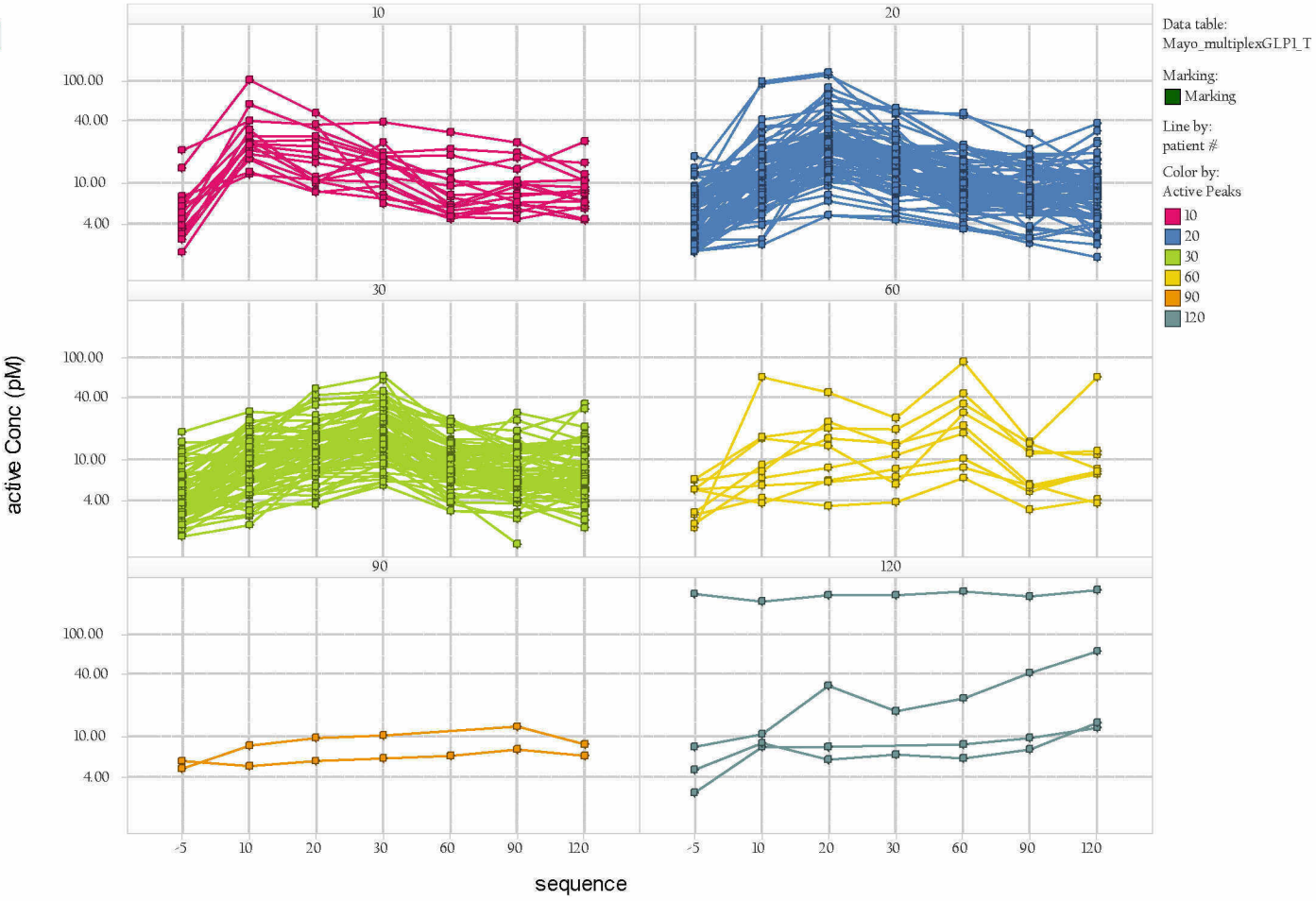
Oral Glucose Tolerance Test

Response on log scale to show huge variation between subjects within each group.



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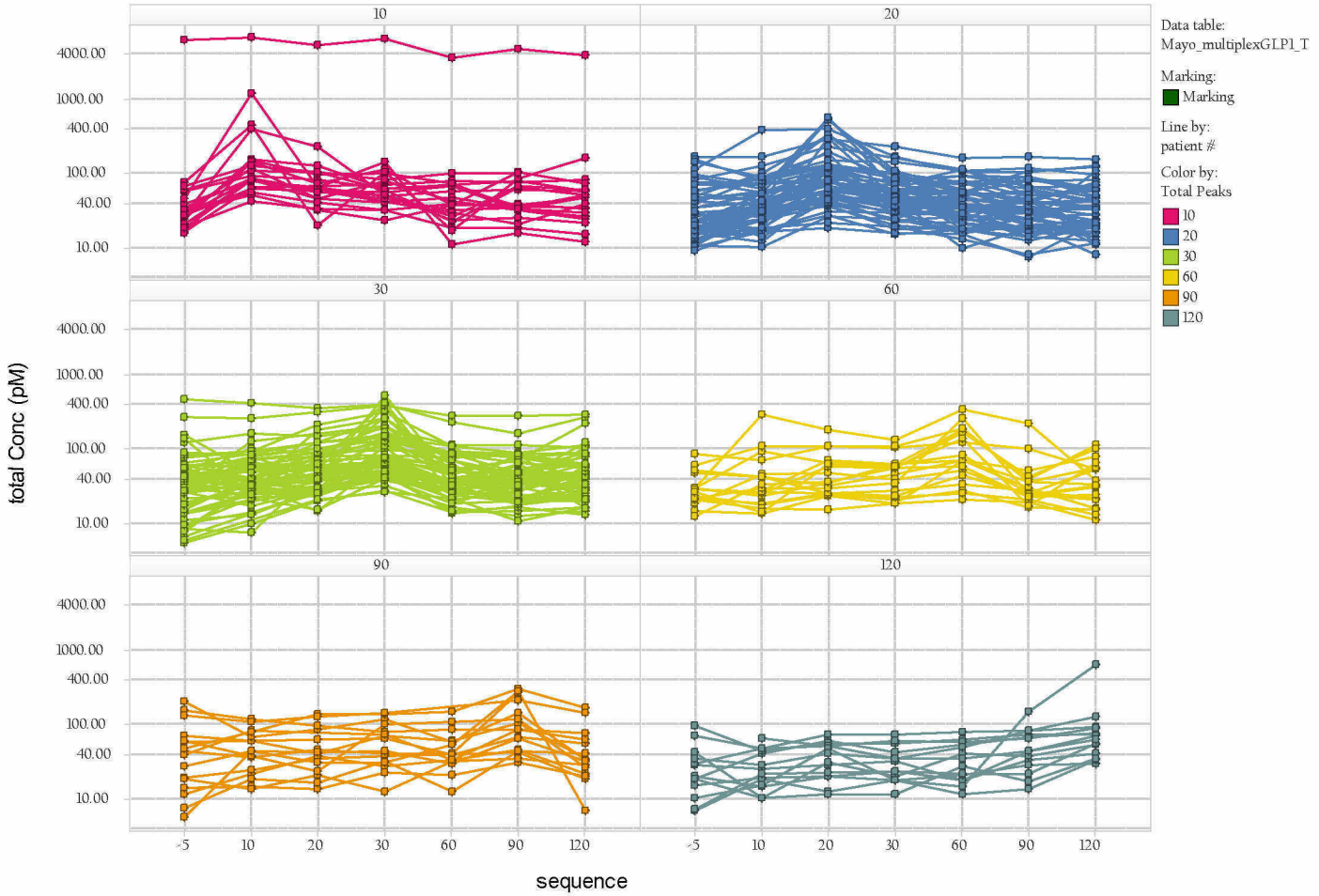
Oral Glucose Tolerance Test

Classified by time of total (sum of active and inactive) GLP-1 peak



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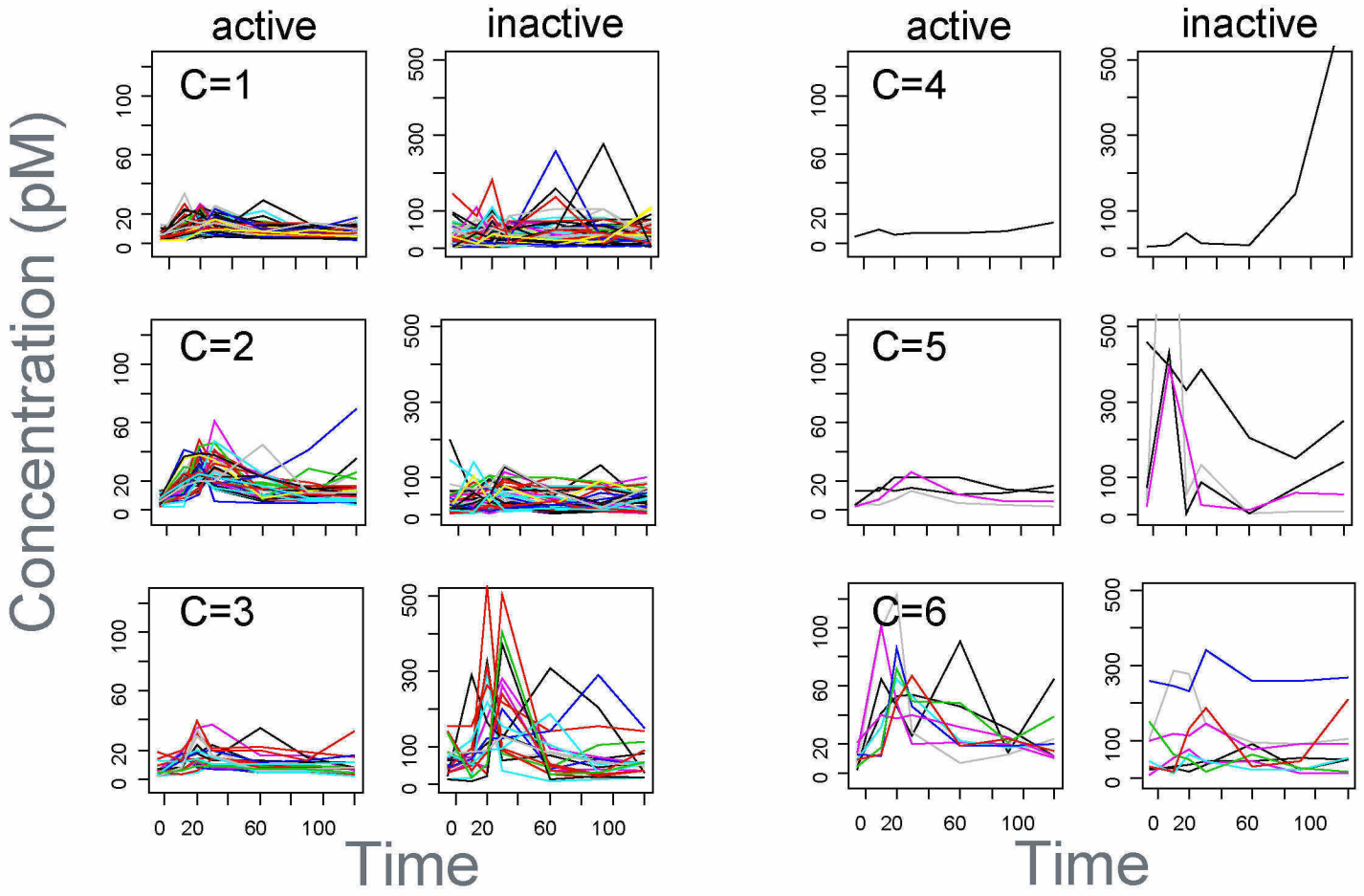
Classified Patients into Six Clusters

Used Two Marker Profile Vectors



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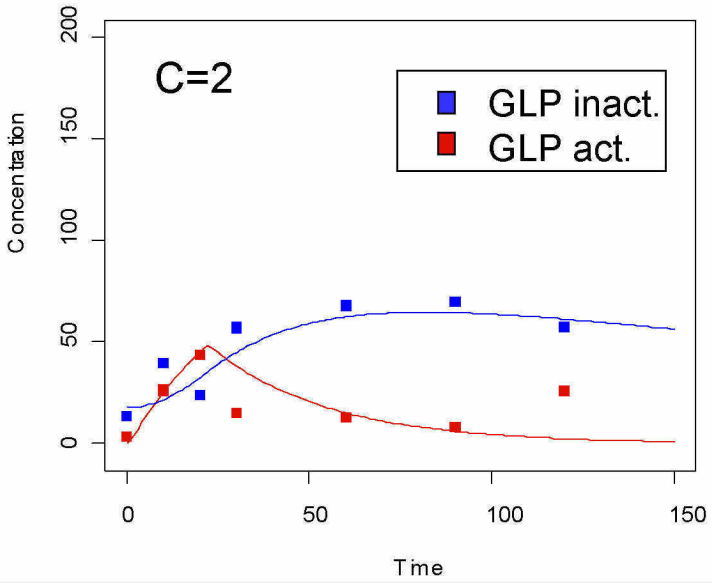
Characterized Response and Risk of Diabetes

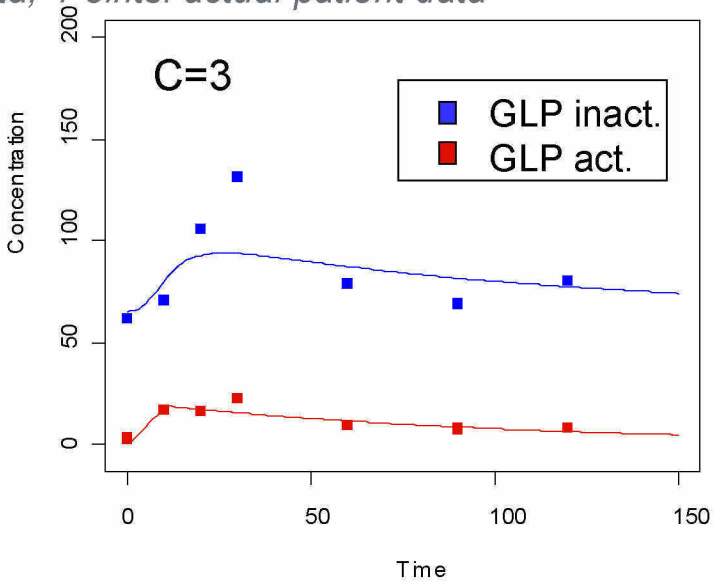
Simple mathematical model represents dynamic physiologic functions.

Identified functional physiologic differences between patients:

- Differences in glucose sensitivity
- Clearance rates of hormones

Solid lines: model simulated data; Points: actual patient data





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Impact of Genetic Variation in GLP-1R on Response to Active GLP-1

Study conducted with Academic Partner.

- 100 patients (normal, healthy, not on drug therapy)
- 12 time points per patient
  - 0,10,30,60,90,120,130,150,180,190,220,240 min
- Glucose clamp: 150 mg/dL
- Active GLP-1 infusions
  - 120-180 min 0.75 pmol/kg/min
  - 180-240 min 1.55 pmol/kg/min

Samples assayed for active GLP-1 on the Theranos System (3,740 cartridges total).

Subjects differed significantly in kinetics and magnitude of response.

Analysis correlating response characteristics with genotype is being published.

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Active GLP-1 Challenge

Each GLP-1 infusion results in an elevation of GIP-1, which peaks or reaches a plateau.

The magnitude of the elevation varies by more than an order of magnitude between subjects.

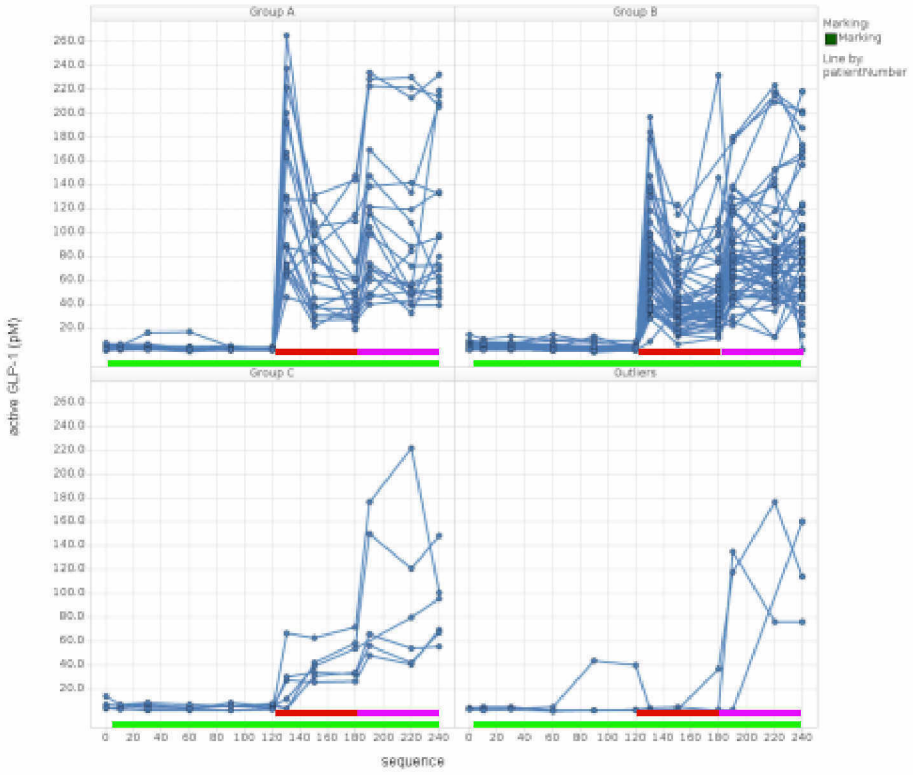
Significant differences in rate of elevation are evident between subjects.

Subjects are grouped by relative elevation.

- A: Peak 1 is bigger
- B: Peak 2 is bigger
- C: No initial peak

Infusions

- Green: Glucose clamp
- Red 0.75 pmole GLP-1/kg/min
- Pink 1.55 pmole GLP-1/kg/min



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Conclusions

Theranos Systems enable classification of subjects based on response profiles.

Studies enabled groups of subjects to be identified by kinetic differences.

Major differences in the intensity of the response to glucose or active GLP-1 were noted between subjects.

Hormone response to glucose in study 1 has been modeled.

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Monitoring Cancer Therapy

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Monitoring Cancer Therapy

Patients undergoing outpatient anti-angiogenic chemotherapy for solid tumors

Several different drug combinations were used.

Study used systems placed in clinics and patient homes (Tennessee).

Finger-stick and venous blood samples

Plasma samples

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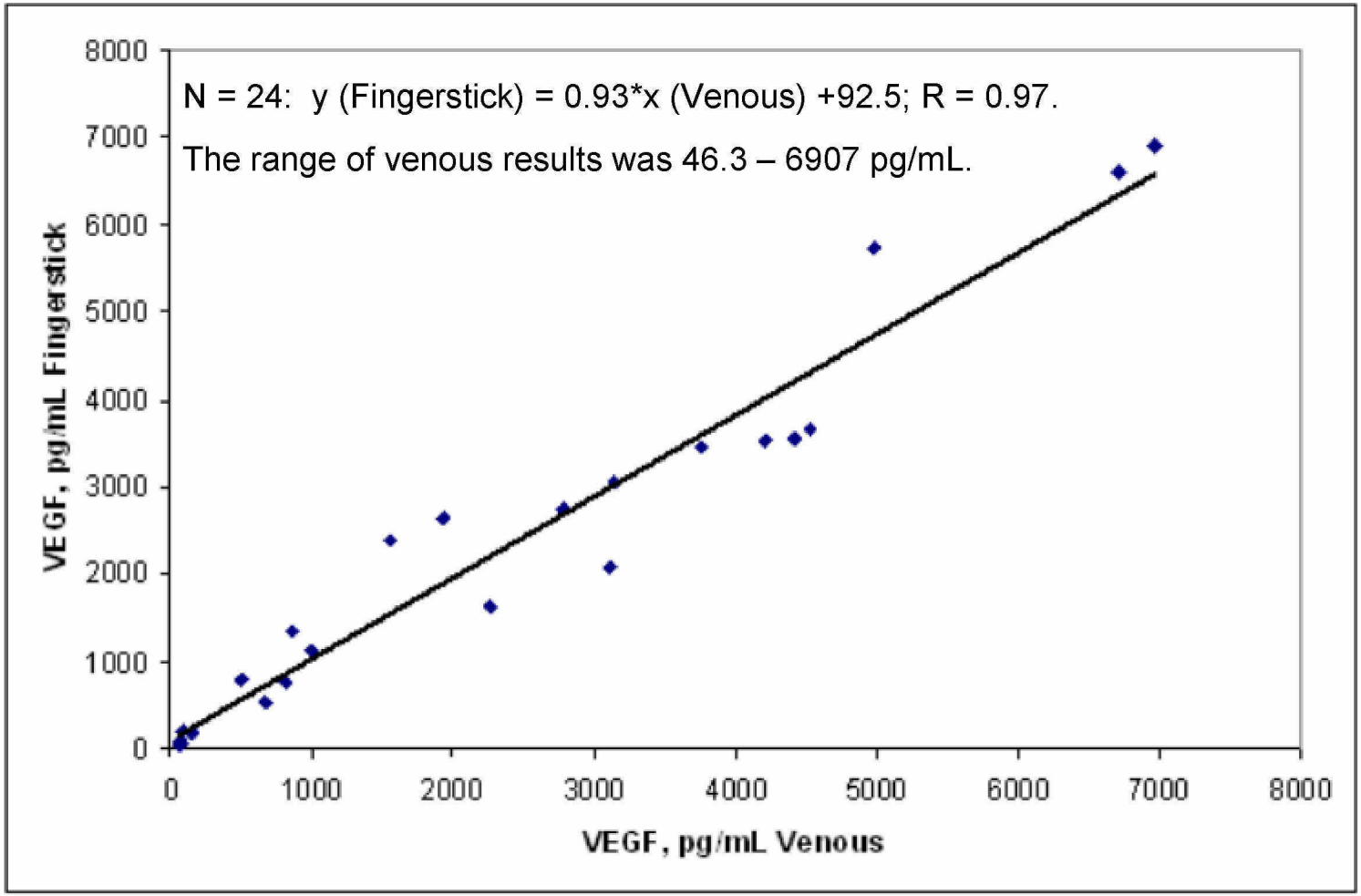
Finger-stick Versus Venous Samples



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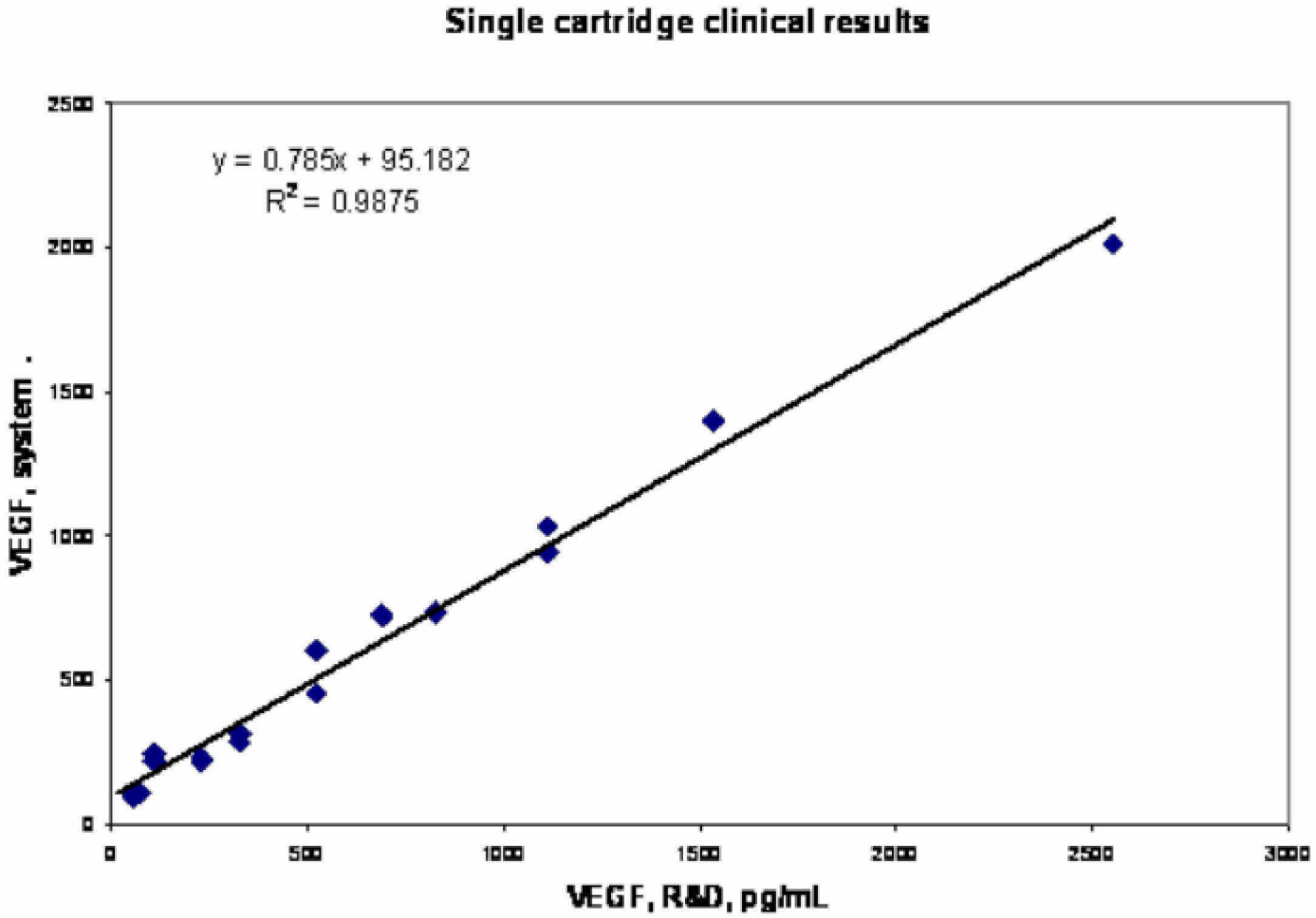
VEGF Assay Method Correlation



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Accuracy of Calibration and LOD

VEGF pg/mL	Recovery %	VEGFR2 pg/mL	Recovery %	PLGF pg/mL	Recovery %
10000	(100)	10560	(100)	780	100
2970	102	7920	93	312	88
990	95	5280	101	156	103
297	105	3960	105	47	106
100	109	2640	98	16	92
30	150	1320	101	5	99
10	101				

Analyte	LOD, pg/mL
VEGF	< 20
VEGFR2	< 200
PLGF	< 20

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Analytes That Bind to Other Species

One drug used for some patients in the study was Avastin which binds to one of the analytes: VEGF.

The Theranos assay is not greatly impacted by Avastin, whereas the reference assay response was almost completely blocked, as shown by the spike recovery study below.

Theranos assays can be configured to respond to both free and bound forms of analytes.

Avastin Present	VEGF average, pg/mL Ref	VEGF average, pg/mL Theranos	VEGF spike recovery, %
N	149	588	66.5
Y	136	8359	-1.3

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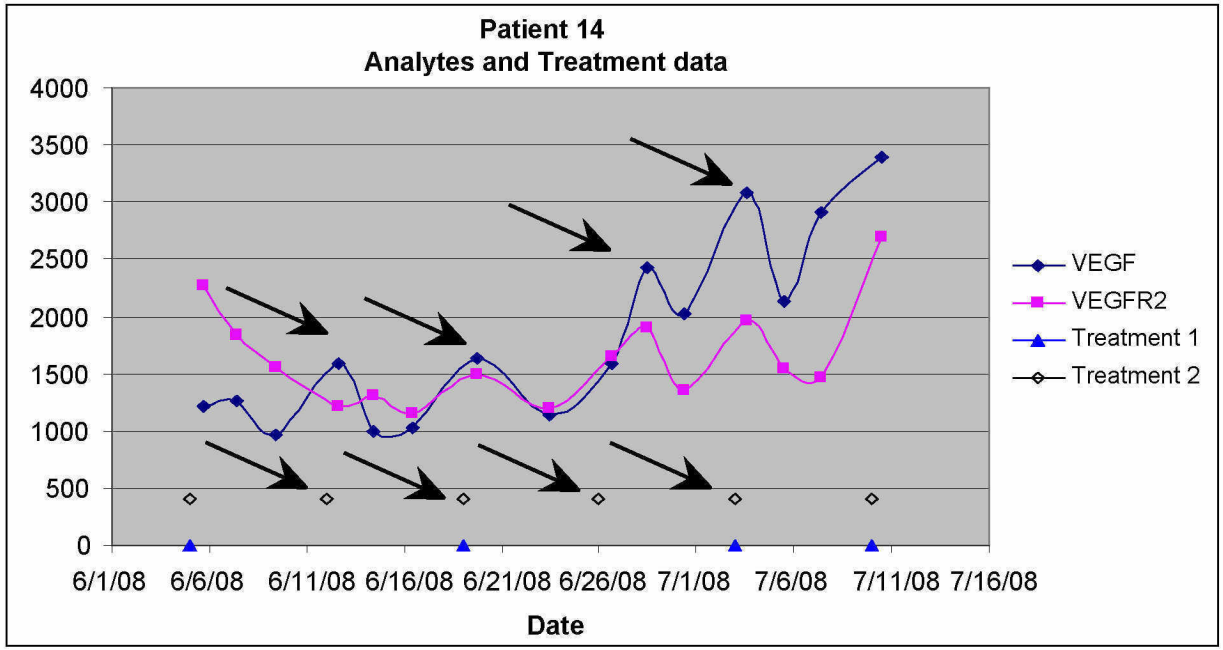
Characterizing Response to Therapy

Time Series analysis of real-time profiling for VEGF, PlGF, VEGFR2 and two concomitant treatments. ARIMA modeling, CCF analyses, and PACF analyses suggest fluctuations in biomarkers are highly correlated with treatment points.



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Studies of Cell Death

Drug company study as a preliminary to monitoring cancer therapy

Fresh blood samples

Normal subjects were monitored for M30 and M65.

Males monitored:

- before exercise
- after exercise

Females monitored:

- pre-menopause, including impact of cycle
- post-menopause

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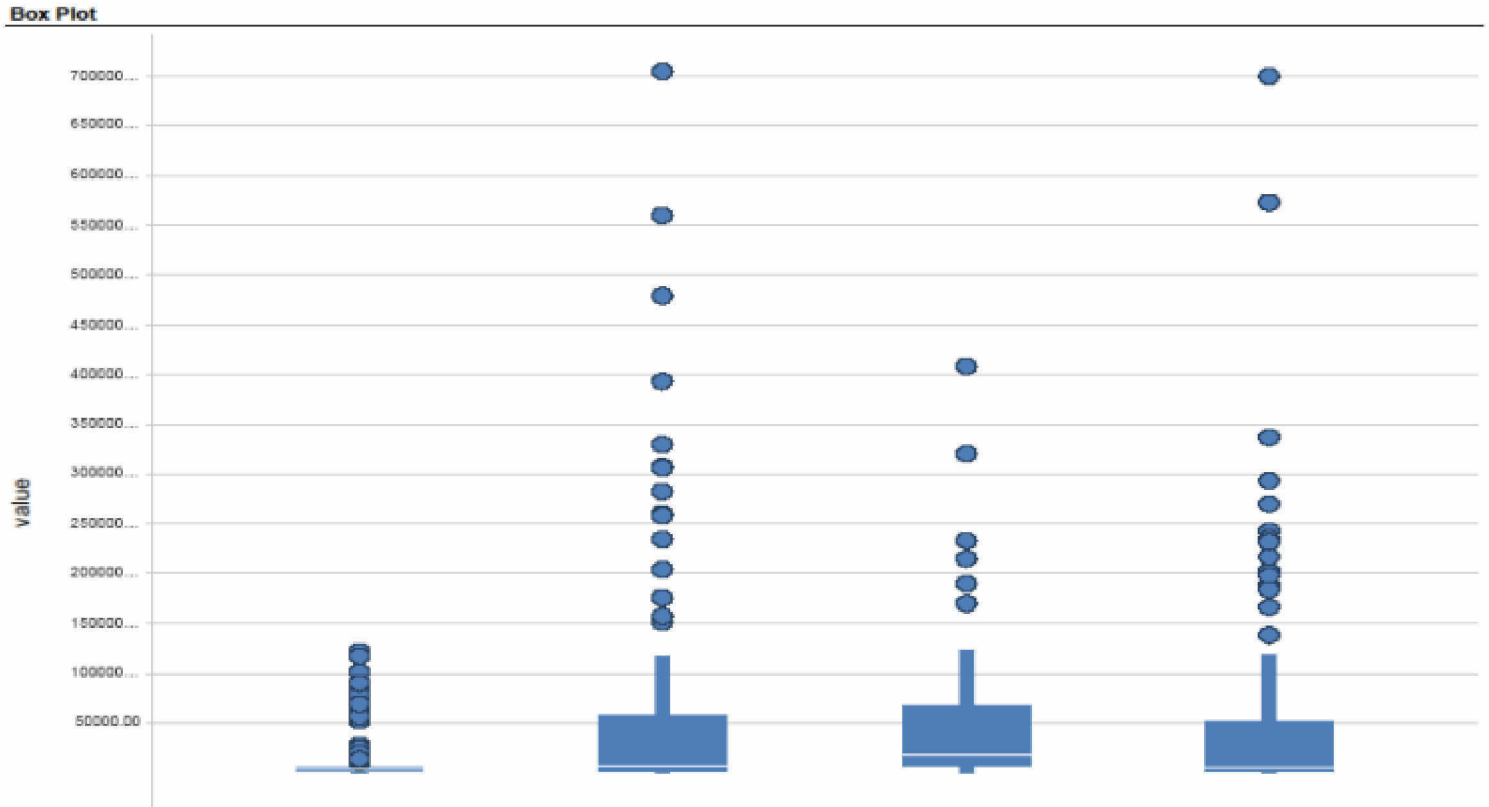
Cell Death Effects of Sex and Menopause: M30



	RLU M30			
	Male	Female		
	N/A	Pre-menopause		Post-menopause
	N/A	Menstruation cycle		N/A
StdDev	1.86E+004	1.21E+005	8.40E+004	1.03E+005
Median	2.70E+003	6.46E+003	1.75E+004	4.64E+003
Count	219	110	53	126

units, analyte, Sex, Pre/Post Menopause, Menstruation

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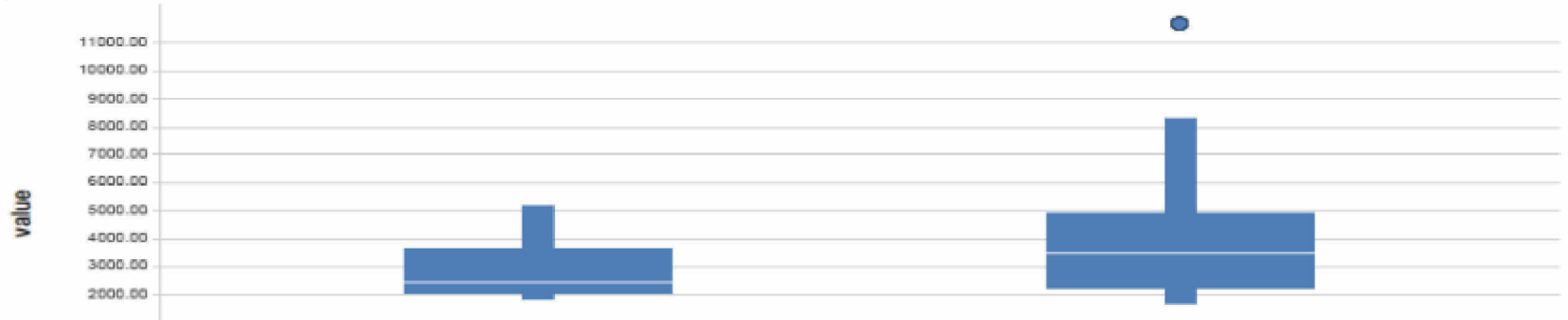
Cell Death Effects of Exercise: M30

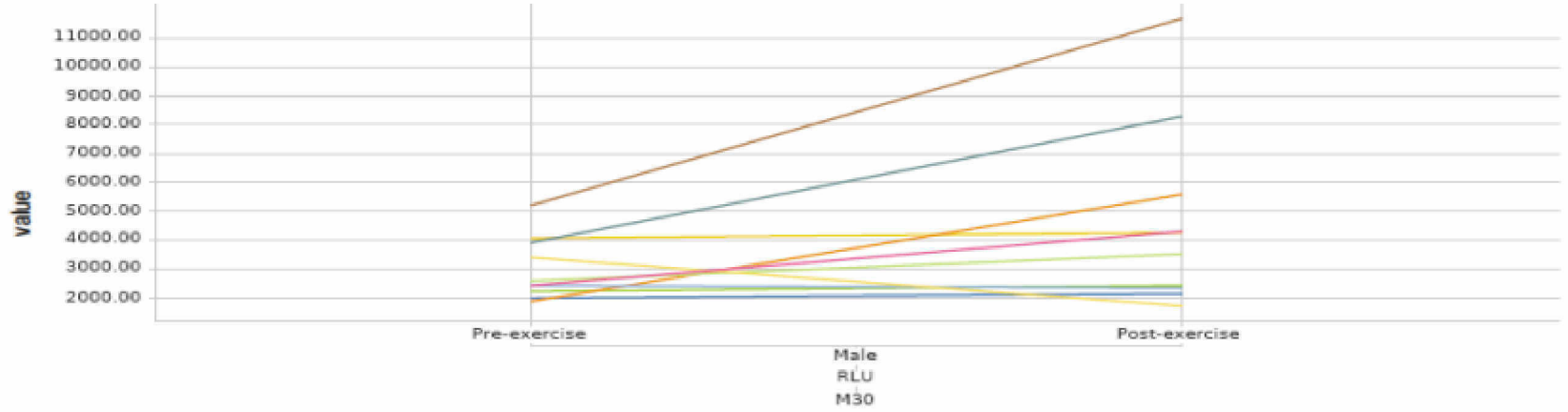
Box Plot



	Male	
	Pre-exercise	Post-exercise
Median	2.43E+003	3.49E+003
StdDev	1.09E+003	3.14E+003
Count	11	11

Sex, Pre/Post Exercise

Line Chart



analyte, units, Sex, Pre/Post Exercise

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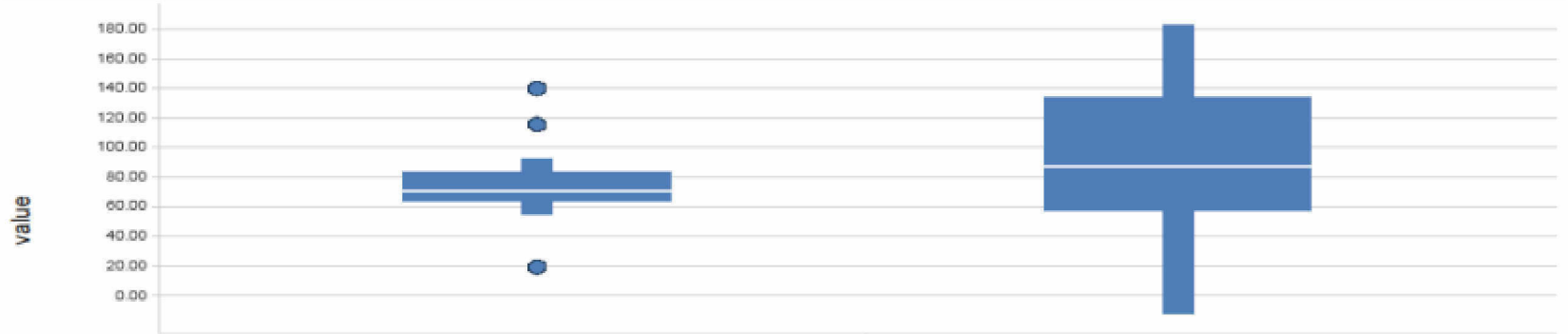
Cell Death Effects of Exercise: M65

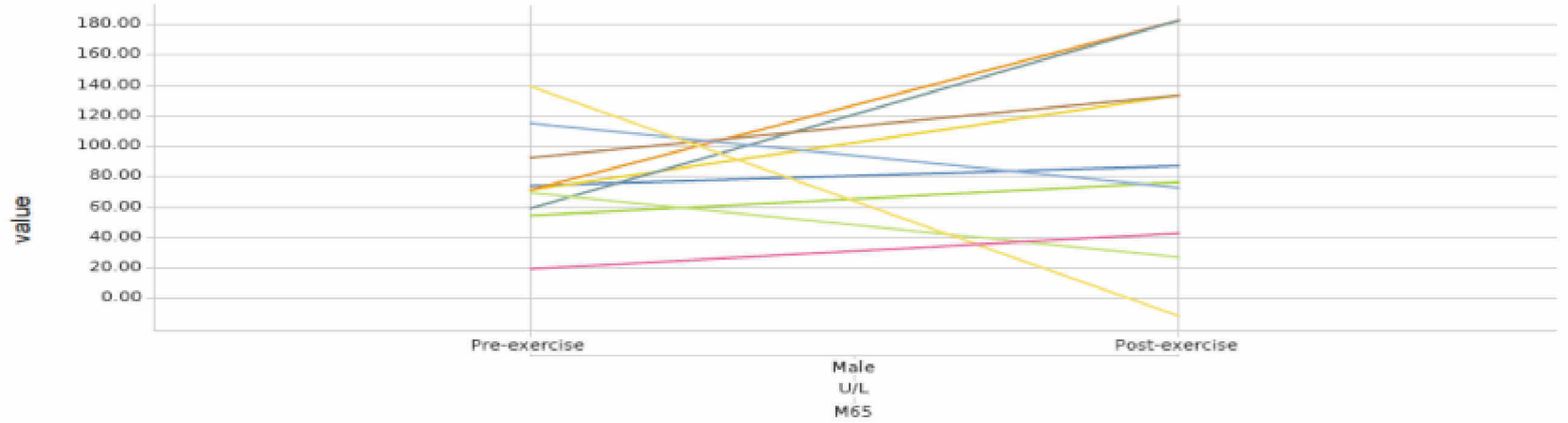
Box Plot



	Male	
	Pre-exercise	Post-exercise
Median	7.08E+001	8.72E+001
StdDev	3.16E+001	6.18E+001
Count	11	11

Sex, Pre/Post Exercise

Line Chart



analyte, units, Sex, Pre/Post Exercise

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Fertility Marker Study

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Fertility Marker Study

PlGF

Progesterone

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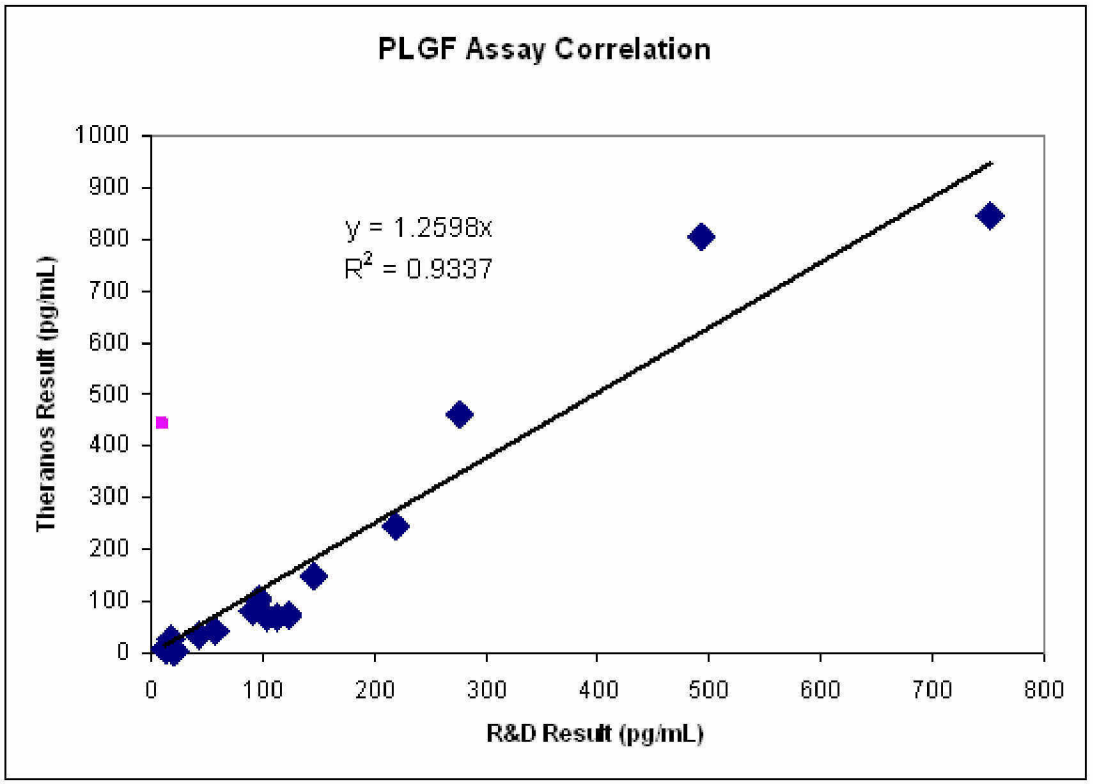
PlGF: Assay Method Correlation

Archived serum samples taken during pregnancy



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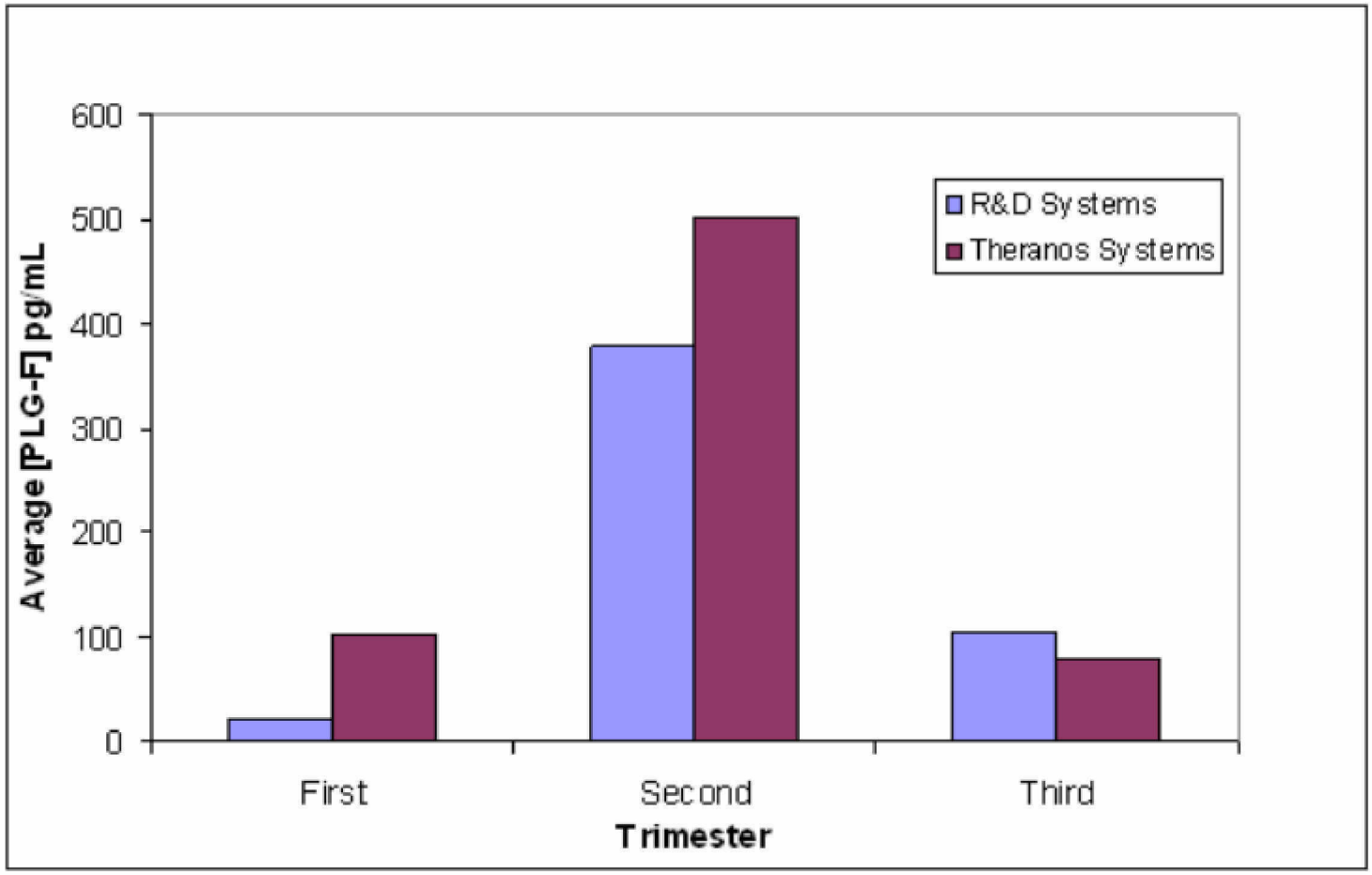
Concordance of Results



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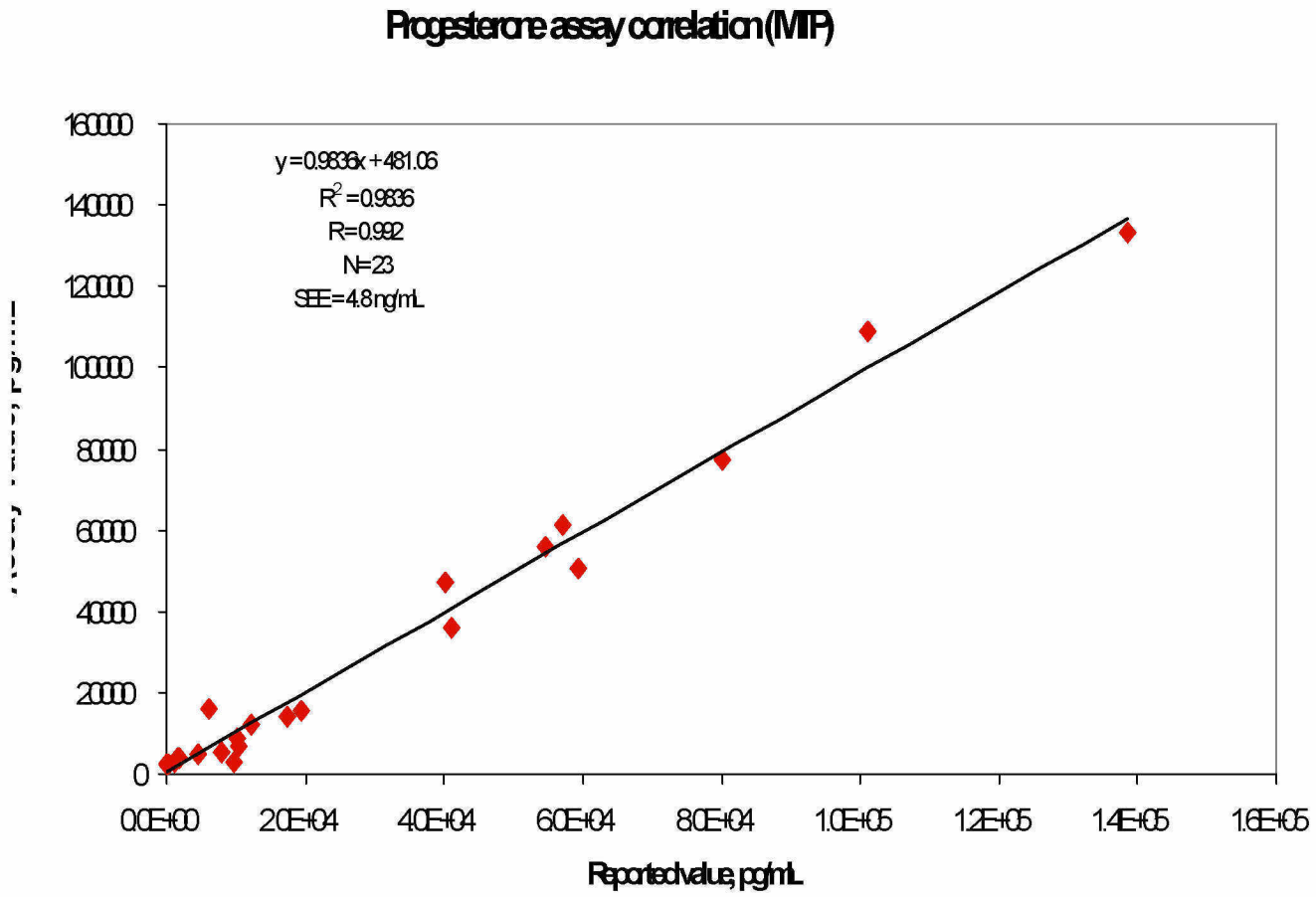
Progesterone: Assay Method Correlation



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Conclusions

1. The assay system enables up to six multiplexed assays in a single cartridge.
2. Assays have a very wide dynamic range.
3. Assay precision is good.
4. Blood, plasma, serum and controls can be used.
5. Sample volume required is small.
6. Many types of analytes can be measured.
7. Many assays have been developed.
8. Assay performance matches best clinical assays.

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Conclusions

9. Results correlate well with predicate methods.
10. Instrument calibration is stable.
11. Factory calibration is stable over cartridge lot lifetime.
12. The system has been successfully tested in many clinical studies.
13. Assays have been validated under FDA/ISH guidelines.