Message

**From**: Ian Gibbons [/O=THERANOS ORGANIZATION/OU=FIRST ADMINISTRATIVE GROUP/CN=RECIPIENTS/CN=IGIBBONS]
**Sent**: 2/18/2010 7:38:39 PM
**To**: Sunny Balwani [/O=THERANOS ORGANIZATION/OU=FIRST ADMINISTRATIVE GROUP/CN=RECIPIENTS/CN=Sbalwani]; Elizabeth Holmes [/O=THERANOS ORGANIZATION/OU=FIRST ADMINISTRATIVE GROUP/CN=RECIPIENTS/CN=Eholmes]
**CC**: Gary Frenzel [/O=THERANOS ORGANIZATION/OU=FIRST ADMINISTRATIVE GROUP/CN=RECIPIENTS/CN=Gfrenzel]
**Subject**: System 4.0 PPT
**Attachments**: System 4.0.v2.ppt

As requested …

theranos
redefining healthcare

# System 4.0

## System component requirements and selection

02/18/2010

theranos
redefining healthcare

# Overview

System 4.0 will be capable of performing any measurement required *in a distributed test setting*

It is envisaged that *several distinct measurement technologies* will be incorporated

The system will be broadly based on the *existing cartridge and reader concepts*

Open architecture for both reader and disposable

The number of total measurements per sample will be increased by 2 to 3-fold (*target: 15 assays?)*

theranos
redefining healthcare

# Assay Menu

- It is crucial to further refine/define which sample types/assays/design requirements are important
- In the field of immunoassay we have good information and experience
- In other fields, we have less secure information
- Specifically:
  - Which nucleic acid analytes?
  - What is the sensitivity requirement?
  - How many and which cell types and surface markers?

theranos
redefining healthcare

# Candidate technologies

- ELISA using chemiluminescence (current)
- ELISA using absorbence
- General chemistry using absorbence
- PCR and RT-PCR using fluorescence readout
- Cell marker assays using laser fluorescence + movement of detector relative to cells
- Electrochemistry for electrolytes and blood gasses
- Sample imaging using a camera (see Appendix 3)

theranos
redefining healthcare

# General system requirements

- Sample: Blood, plasma and control materials (other types?)
- Sample processing: Plasma from blood, Lysis of cells
- Sample volume: < 20 uL (preferably < 5 uL)
- (For some purposes (*e.g.* very high multiplex), volumes as high as 200 uL may be permitted.)
- Assay menu: All assays available for system 3.0/3.1 + TBD analytes in other assay classes
- Size, weight: TBD but not larger then System 3.0
- Assay times: TBD but not longer than System 3.1
- Other capabilities: TBD but not less than System 3.0

theranos
redefining healthcare

# Proposal for the basis of System 4.0

- Review available technologies: Done
- Define requirements
  - Needs versus wants
- First pass proposal
  - Based on current platform concept (cartridge + dispense)
  - Integrate thee optical detection means in one low sample volume device
    - Luminescence
    - Absorbence
    - Fluorescence
  - Add a separate system for cell counting
  - Add red cell removal technology
  - <Add sample integrity evaluation means>
  - Chemistries proposed all exist
- Engineering review

theranos
redefining healthcare

# Comparison of requirements, capabilities and limitations of available detection technologies

| Technology | Analytes | Sensitivity | Dynamic range | Detector Difficulty |
|---|---|---|---|---|
| Absorbance | 1,2,3,4,5,6,7 | High | 30 fold | Low |
| Absorbance spectroscopy | 1,2,3,4,5,6,7 | High | 30 fold | Moderate |
| Turbidimetry | 1,3,5 | Low | 30 fold | Low |
| Nephelometry | 1,3,5 | Moderate | 100 fold | Low |
| Fluorescence | 1,2,3,4,5,6,7 | High | 1000 fold | Moderate |
| Fluorescence spectroscopy | 1,2,3,4,5,6,7 | High | 1000 fold | High |
| Luminometry | 1,2,3,4,5,6,7 | V. high | 10,000 fold | Low |
| Cell counting | 9 | Good | 100 fold | Moderate |
| Cell imaging | 9, 10 | Moderate | 30 fold | High |
| PCR (and RT-PCR) | 8 | V. high | 10,000 fold | Moderate |
| Electrochemistry | 11, others? | Moderate? | ? | Low |

| Analyte class | Key |
|---|---|
| Biomarkers | 1 |
| Microbial antigens | 2 |
| Small molecules (drugs) | 3 |
| Small hormones | 4 |
| Antibodies | 5 |
| Metabolites | 6 |
| Enzymes | 7 |
| Nucleic acids, viral genomes | 8 |
| Cell surface markers | 9 |
| Intra cellular markers | 10 |
| Electrolytes and blood gases | 11 |

theranos
redefining healthcare

# Comparison of Physical Technologies used for Assays

| Physical Technology | Application | Mechanical difficulty | Chemistry difficulty | IP/Licensing |
|---|---|---|---|---|
| Separation | IA | Moderate | Low | |
| Non-separation | Clinical chemistry | Low | Low | |
| Non-separation | Nucleic acids | Low | Low | |
| Non-separation | Immunochemistry | Low | High | Required |
| Physical multiplex | Any | Moderate | Low | |
| Chemical multiplex | Immunochemistry | Low | High | |
| Flow/Movement | Cell counting | Moderate | Low | |
| Imaging | Cell counting | Low | Low | |

theranos
redefining healthcare

# Separation of red cells and washing

- There is a trade-off between simplicity of operation of cartridge and instrument versus requirements for (1) low sample volume, (2) using plasma as the sample and (3) washing the capture surface in immunoassays
- We were not able to obtain a sufficient plasma sample from blood for multiplexed immunoassays
- Solutions to this problem are:
  - (1) Use blood as the sample
    - Calibration becomes more complicated
  - (2) Use lysed blood as the sample
    - Calibration should be corrected for HCT
  - (3) Separate plasma *and* use a very low volume measurement technology (e.g. Nanodrop)
    - Will require reverse engineering and/or a license

theranos
redefining healthcare

# Special problem for blood samples

## Red cell separation means

- Glass fiber wick or pad + plasma extraction
  - Vogel patent has expired
- Magnetic particle agglutination
- …
- …
- Pump through a frit??
- …

theranos
redefining healthcare

# Separation versus non-separation technologies

- Theranos has used *separation-based* ELISA as core technology. This requires efficient washing of the assay capture surface to remove unbound label (time consuming).
- There are several immunoassay techniques that have *non-separation* technologies
  - Typically these are:
    - *Proprietary* (license would be needed)
    - Fast
    - Not as sensitive as separation methods
- There are three such technologies that are very sensitive and general
  - EMIT (small molecule drugs) [Syva now Siemens]
  - β-Galactosidase complementation (Biomarkers, receptors etc.) [DiscoveRx]
  - Alpha screen (General immunoassay) [Packard]
- These are reviewed in Appendix 1

theranos
redefining healthcare

# Compatibility and conflict between requirements for the different measurement technologies

- Each distinct detection technology compounds system (instrument and disposable) complexity and cost
  - Is a three-technology system 3x or 9x more difficult to develop?
- Temperature control
  - Thermo-cycler may compromise temperature control for the rest of the instrument
- Light shielding
  - Luminescence detector needs protection from high intensity light sources
- Time factors
  - Current Biomarker assay chemistry takes (say) 30 m
  - RT-PCR may take > 2 hours

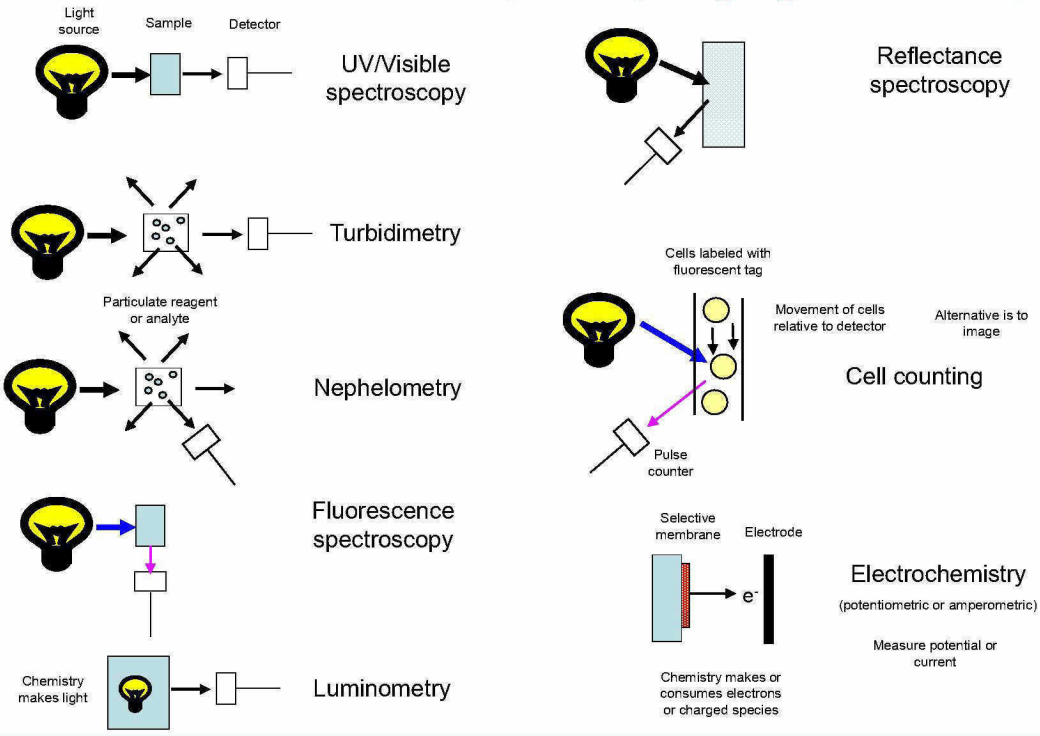
theranos
redefining healthcare

# Trade-off of sensitivity and time

- Luminescence ELISA can generally be complete in 15-30 m
- High sensitivity absorbance ELISA may need hours for equivalent sensitivity
- PCR sensitivity increases with # of cycles
  - 10 cycles: 10^3-fold
  - 20 cycles: 10^6-fold
  - 30 cycles: 10^9 fold

theranos
redefining healthcare

# Detection configurations compared (imaging not shown)



theranos
redefining healthcare

# Requirements, capabilities and limitations of candidate detection technologies and chemistries

theranos
redefining healthcare

# ELISA with Chemiluminescence readout

- Very sensitive with very wide dynamic range
- Capable of giving results rapidly
- Suitable for *immunoassay* of small and large analytes
- Weakness is that reagent stability (E-Ab) and substrate contamination are crucial

- Essential technology

theranos
redefining healthcare

# Absorbance

- Many applications
  - General chemistry (e.g. cholesterol)
  - Hemoglobin (and calculated HCT)
  - Albumin and total protein
  - TDM by licensed assays (EMIT/DiscoveRx etc.)
  - Enzyme assays (e.g. ALT)
  - ELISA with absorbance readout
    - Enables adaptation of existing assays?
  - Electrolytes (e.g. Ca2+)
    - Sensitive with moderate dynamic range
    - Take longer than those with chemiluminescent readout
- For full value wavelength range of 340 – 650 needed
- Essential technology

theranos
redefining healthcare

# Fluorescence

- Essential for nucleic acid assays
- Not needed for clinical chemistry or immunochemistry
- Good sensitivity and dynamic range
- Capable of multiplexing

- Requires a high quality light source (laser[s])
- Compensation for light source instability is needed

theranos
redefining healthcare

# PCR and Reverse Transcriptase-PCR

- Essential for all nucleic acid assay targets
- Requires both elevated temperatures (90C) and temperature cycling
- Likely to require development of new disposable elements/surface chemistry
- Needs fluorescent readout

theranos
redefining healthcare

# Cell counting

- Questions:
- How many markers?
  - >> How many lasers and complexity of the optics
  - One laser gives three colors
- Is rare cell detection needed?
  - Problem is sample volume and the need for cell concentration prior to detection

theranos
redefining healthcare

# Rare cell analysis

- Circulating cancer cells
- Fetal cells in maternal blood

- Problem:
  - Cell numbers may be as low as (say) 10/mL
  - Blood drop = 20 uL
  - Cells/drop = 0.2: Impossible to detect reliably
- Solution is to pre-concentrate target cells from (say) 1 mL of blood then to detect
  - Not compatible with POC context
  - Technically demanding

theranos
redefining healthcare

# Technologies deemed unnecessary

Reflectance

Imaging

Electrochemistry

theranos
redefining healthcare

# Attractive potential add-on technologies
**(See appendix 1)**

- Non-separation, receptor-based assays with absorbance or luminescence readout
- Pulse oximetry

theranos
redefining healthcare

# Review of issues for technologies not yet familiar at Theranos

theranos
redefining healthcare

# Nucleic acid assays

- Polymerase Chain reaction (PCR)
  - Amplify DNA:  30 cycles = 10^9-fold amplification
  - Needed for sensitivity
- Reverse transcriptase (RT-PCR)
  - Measure RNA (transcribe to DNA then apply PCR
  - Needed for RNA virus detection
- Real time PCR
  - Semi quantitative
- All need temperature cycling and elevated temperatures
- Read-out by fluorescence

theranos
redefining healthcare

# PCR and RT-PCR:

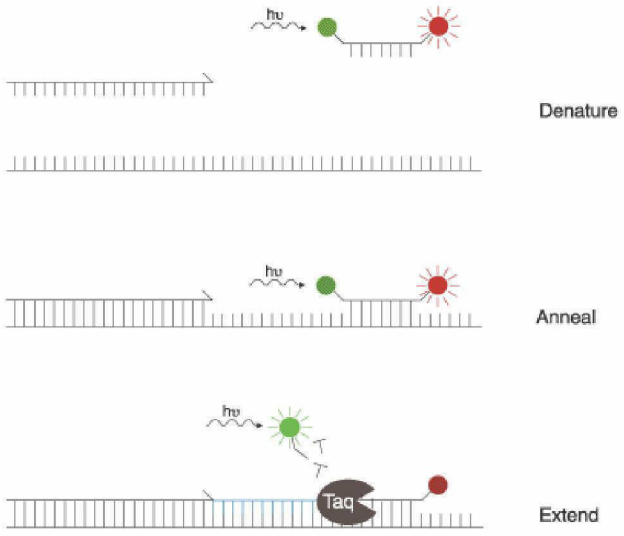
## Time and temperature requirements, typical

| Step | Temperature, C | Time, min |
|---|---|---|
| Denature | 95 | 3 |
| Anneal | 65 | 1 |
| Elongate | 72 | 3 |
| Cycle | # | |
| | 20<br>(10^6 fold amplification) | 140 |

theranos
redefining healthcare

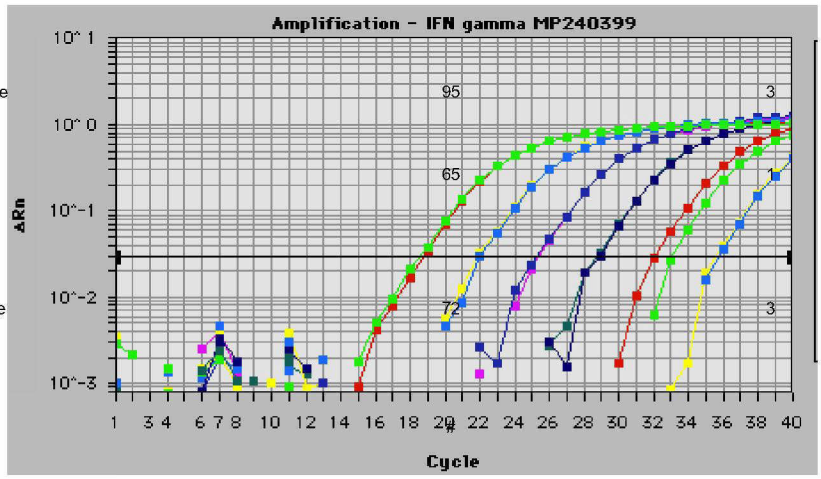
# Real-time PCR (TaqMan)

## One favored readout means (needs a license)



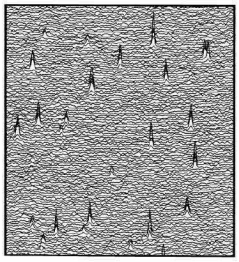
theranos
redefining healthcare

# Real-time PCR read-out




| | | |
|---|---|---|
| Denature | 95 | 3 |
| Anneal | 65 | 1 |
| Elongate | 72 | 3 |
| Cycles | 20 | 140 |

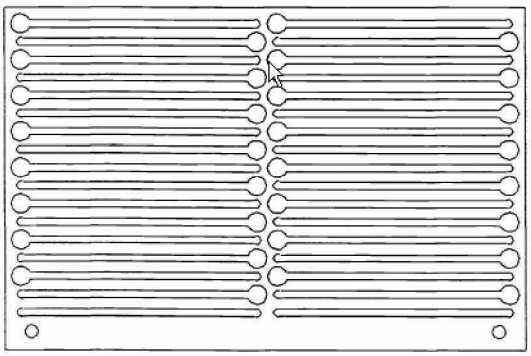
# Cell counting: option 1



**Microvolume Laser Scanning Cytometry Platform for Biological Marker Discovery**

Ian D. Walton, Louis J. Dietz, Gary Frenzel, Jerry Chen, Jim Winkler, Scott M. Norton, Aaron B. Kantor

SurroMed Inc., 1060 East Meadow Circle, Palo Alto, CA 94303.

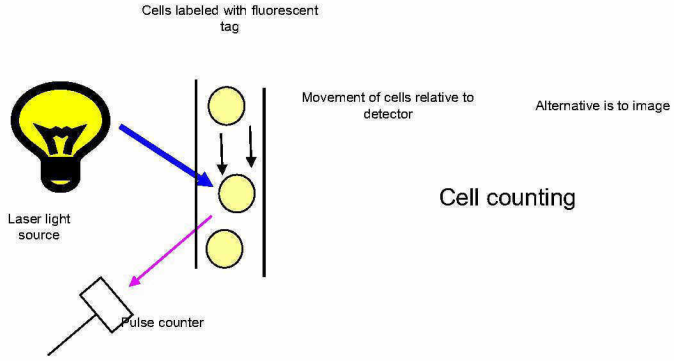


- Spatial array of stained cells
- Raster optics (move laser)
- Or, image
- Simple capillary cartridge (shown for analysis of 32 samples)

theranos
redefining healthcare

# Cell counting: Option 2

- Needs controlled flow
- Measure pulses over *time*



theranos
redefining healthcare

# Questions/Issues

Complexity versus cost and practicality

Nanodrop does not measure absorbance and fluorescence in the same unit

Laser: size/cost versus # of cell surface markers

Costs

theranos
redefining healthcare

# Do we need electrochemistry?

- Many analytes measured by electrochemical methods ($O_2$, $CO_2$, $HCO_3^-$, $Ca^{2+}$, $Mg^{2+}$) are only needed in emergency situations
- Many analytes such as $K^+$, $Ca^{2+}$, $Mg^{2+}$,Phosphorus ($PO_4^{2-}$) and pH can be measured by absorbence
  - Protocols are simple
    - Two or three liquid reagents
    - Mix with sample and incubate

theranos
redefining healthcare

# Small volume assay reader concept

02/18/2010
Development Team

theranos
redefining healthcare

# Strategic Issues For POC Assay Systems

- Performance equivalent to laboratory methods
- Multiplexed assay capability
- *Multiple assay type capability*
  - *Immunoassays*
  - *Direct assays (cholesterol etc.)*
  - *Enzyme assays*
  - *Nucleic acid assays*
  - *Electrolytes (and blood gasses??)*
- Simplicity and reliability of use
  - Non-technical users
  - No false results
- *Small sample volume*
  - < (say) 5 uL *blood*
  - *No hematocrit effect*
- Speed
  - *< (say) 15 m*
- *Ease of assay development*
- *Speed of assay development*
- *Low cost*
  - *Instrument*
  - *Disposable*

- Italics emphasize possible improvements/extensions on/of current system

# Small volume assay concept

- Blood sample: say 10 uL (= about 5 uL plasma)
- Sample capillary sucks plasma (say 3 uL; note filter does not need to be very efficient) from a (say) glass fiber filter.
  - Could also use a frit in a "tip" (?) + aspiration
- Plasma fills capillary and is then displaced into a dilution well. Mixing by repeated aspiration and re-expression >> (say) 30 uL diluted plasma
- Diluted sample (3 uL) is aspirated into standard Theranos tips (up to 8 assays = 24 uL diluted sample)
- Process assays with color forming chemistry (ELISA) generating (say) 3 uL colored product
  - Tip precludes evaporation
- Read in Nanodrop-style photometer
- Advantages
  - HRP chemistry enabled
  - Small sample volume
  - Red cell removal would be possible
  - Higher multiplex might be possible
  - Can also be used for clinical chemistry assays
    - Glucose, Cholesterol
    - Enzymes (ALT)
- Disadvantages
  - Light source required (can be very simple and inexpensive as can the detector, however)
  - Need to wash sample chamber (Nanodrop cleans up fine by just wiping +/- one wash)

theranos
redefining healthcare

# "Nanodrop" spectrometer


- Designed to measure protein and nucleic acid concentrations in very small volumes
- 200 – 800 nm
- 0.5 – 5 uL
- Measurement complete l a few seconds

theranos
redefining healthcare

# Use of Nanodrop

Pipette (say) 2 uL onto pedestal

Instrument automatically defines a precise optical pathlength (1 mm)

theranos
redefining healthcare

# Instrument Specs.

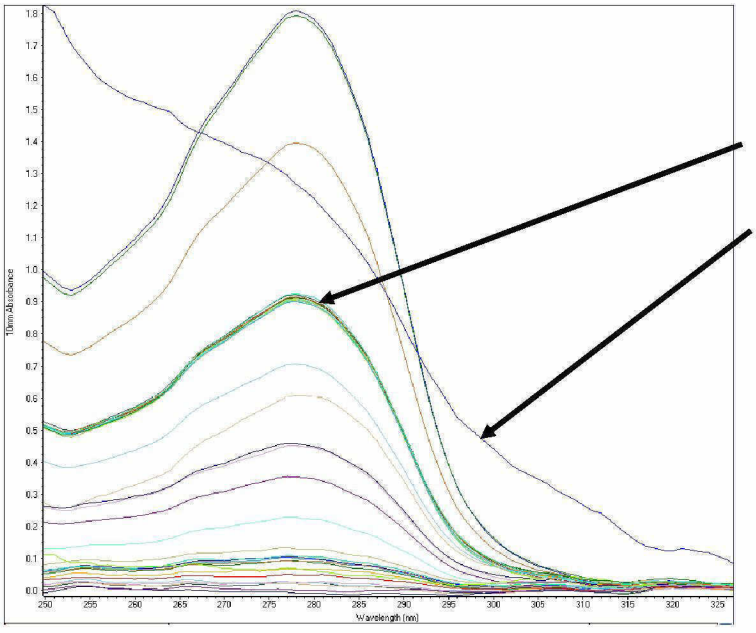
## Instrument Specifications

### NanoDrop 2000/2000c – pedestal mode

| | |
|---|---|
| Instrument Type: | Spectrophotometer |
| Minimum Sample Size: | 0.5 µL |
| Pathlength: | 1 mm (auto-ranging to 0.05 mm) |
| Light Source: | Xenon flash lamp |
| Detector Type: | 2048-element linear silicon CCD array |
| Wavelength Range: | 190-840 nm |
| Wavelength Accuracy: | ±1 nm |
| Spectral Resolution: | ≤1.8 nm (FWHM @Hg 253.7 nm) |
| Absorbance Precision: | 0.002 absorbance (1 mm path) |
| Absorbance Accuracy: | ± 2% (at 0.76 absorbance at 257 nm) |
| Absorbance Range: | 0.02 -300 (10 mm equivalent) |
| Detection limit: | 2 ng/µL dsDNA |
| Maximum Concentration: | 15,000 ng/µL (dsDNA) |
| Measurement Time: | < 5 seconds |
| Footprint: | 14 cm x 20 cm |
| Weight: | 2.0 kg |
| Sample pedestal Material of Construction: | 303 stainless steel and quartz fiber |
| Operating Voltage: | 12 VDC |
| Operating Power Consumption: | 12-18 W, (max 30 W) |
| Software Compatibility: | Windows® XP and Vista (32 bit) |

theranos
redefining healthcare

# Protein spectra




- Serial dilutions of a protein solution are shown
- Measurements are reproducible
- Spectral ratios make it easy to detect malfunctions (e.g. bubbles in the light-path)

theranos
redefining healthcare

# Nanodrop applications illustrated

Performance measures

Sample volume independence

Protein assay (UV measurements)

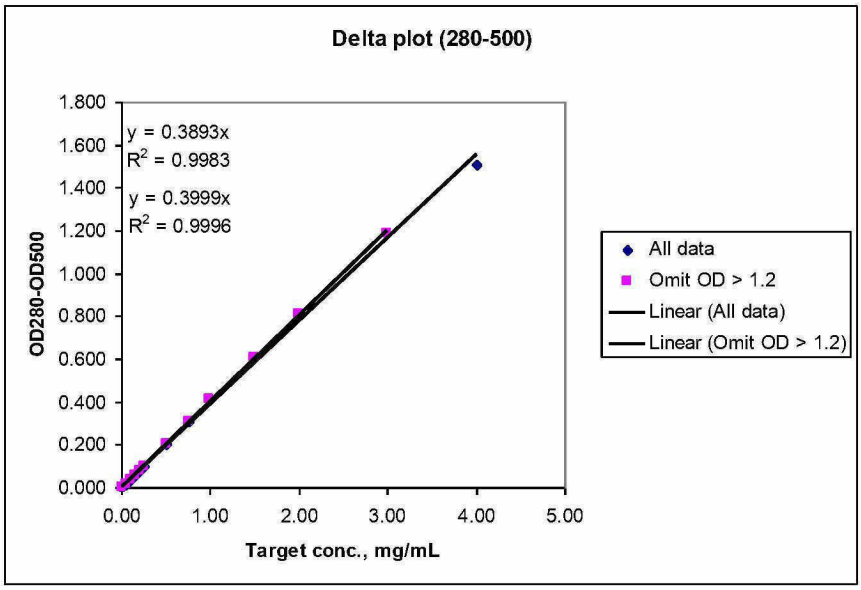
Measurements in the visible

ELISA readout

Hemoglobin/HCT measurement

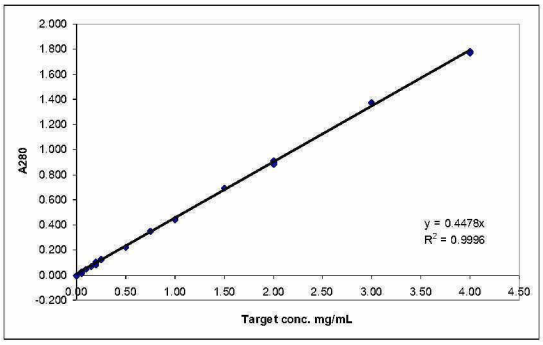
theranos
redefining healthcare

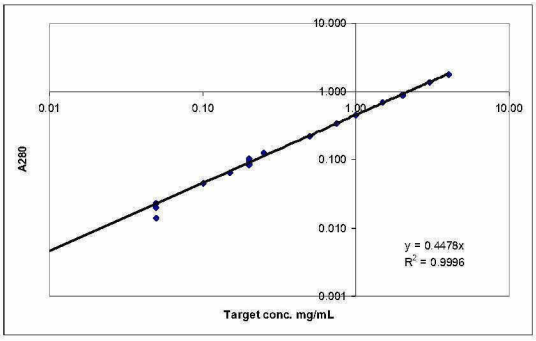
# Dose-response is linear up to about 1.5 OD



theranos
redefining healthcare

# Good linearity from low to high absorbance



theranos
redefining healthcare

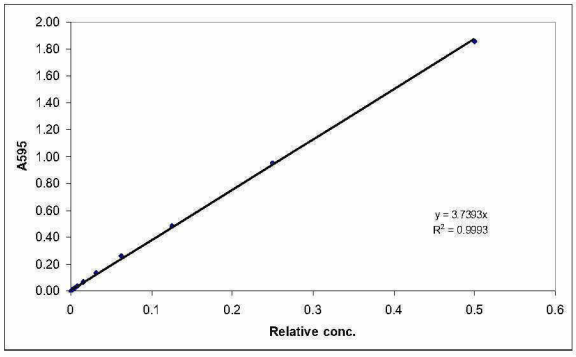
# Precision is good.
## Even at low A values

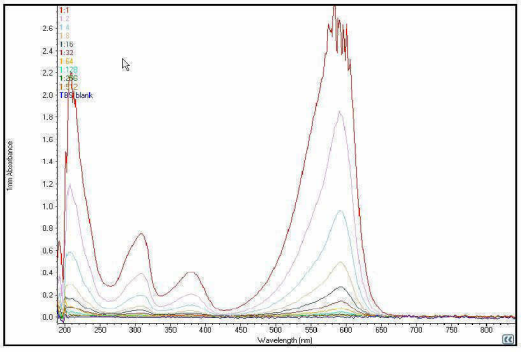
| BSA, mg/mL nominal | A, 1cm,280, avg | N | CV, % |
|---|---|---|---|
| 2.0 | 0.923 | 11 | 0.9 |
| 0.2 | 0.093 | 5 | 8.2 |
| 0.05 | 0.019 | 4 | 18.6 |

LOD (95% confidence) = 0.01 (Absorbance); 0.02 mg/mL (BSA conc.)

theranos
redefining healthcare

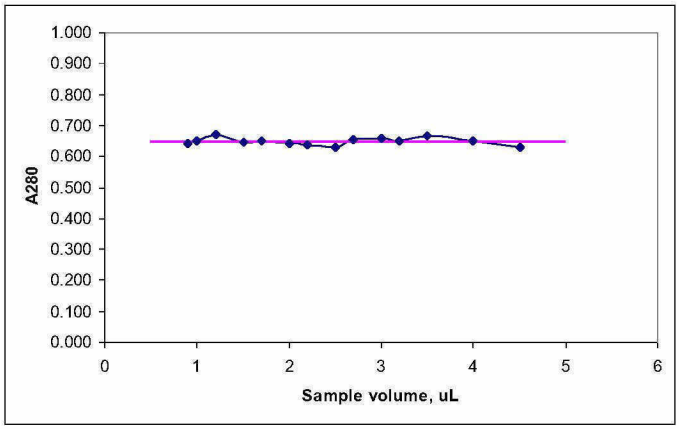
# System also works in the visible range



theranos
redefining healthcare

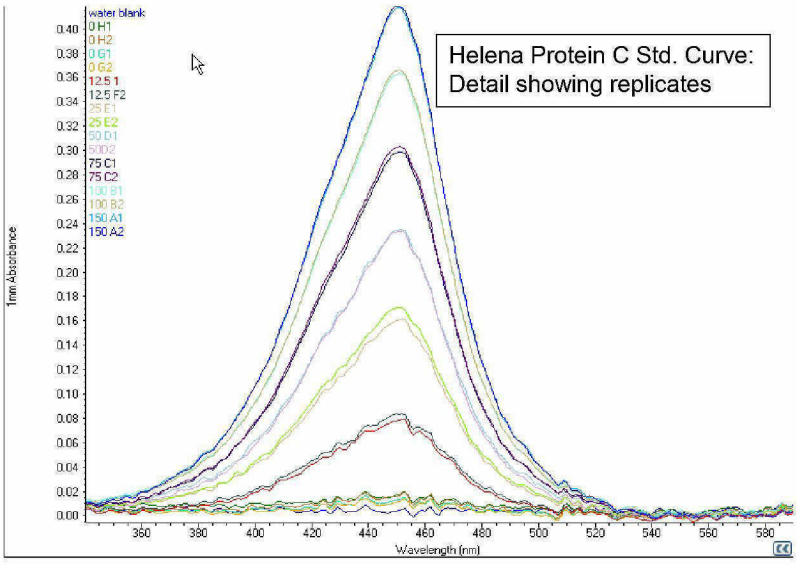
# System response is independent of sample volume




| | |
|---|---|
| average | 0.648 |
| sd | 0.011 |
| cv, % | 1.744 |

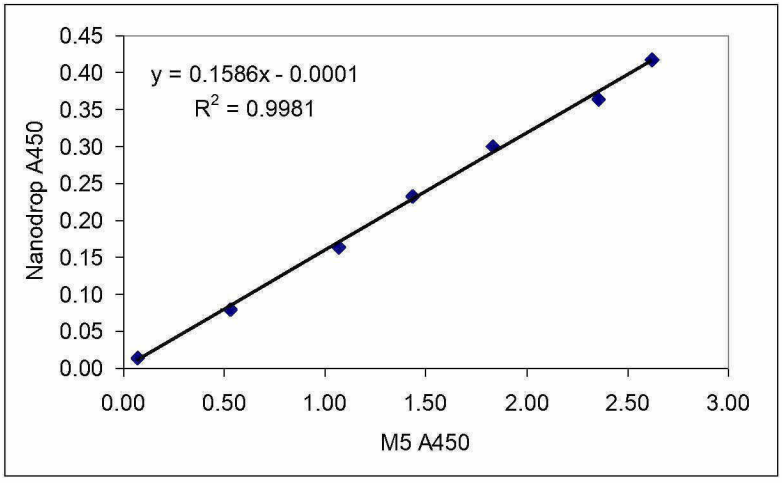
theranos
redefining healthcare

# ELISA: Visible spectra



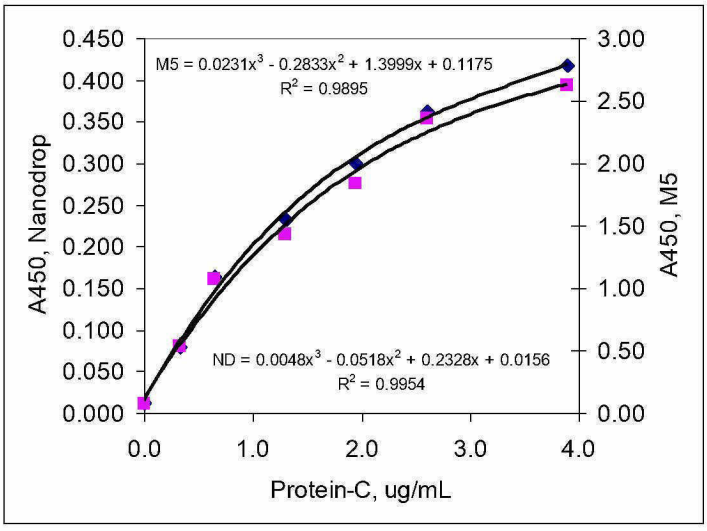
theranos
redefining healthcare

# ELISA assay comparison (signal)
# Helena Protein-C assay



theranos
redefining healthcare

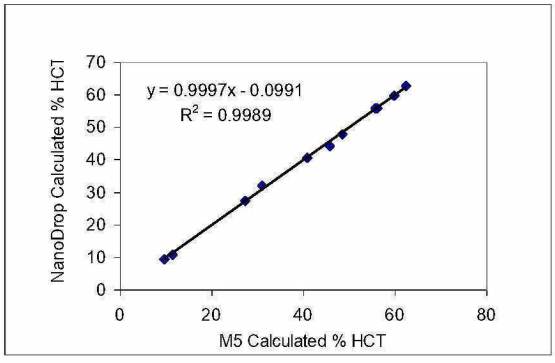
# Protein-C ELISA response comparison

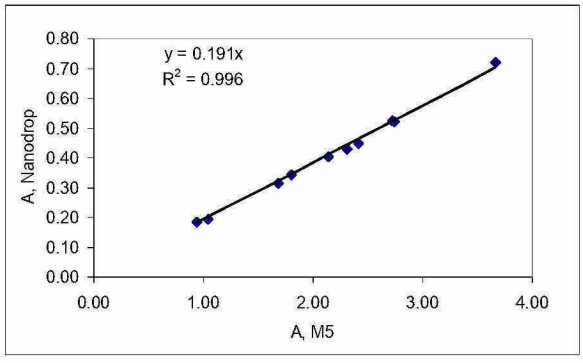


theranos
redefining healthcare

# Hematocrit/Hemoglobin assay

**Standard clinical assay for Hb (Drabkin's method)**
**(RBCs are lysed; Hb is converted to Cyan:Met-HB; read A540 nm)**



theranos
redefining healthcare

## Possible applications in a next generation Theranos Instrument

- Colorimetric ELISAs
  - HRP or APase labels
- Clinical chemistry analytes can be measured colorimetrically
  - Glucose
  - Cholesterol
  - HDL-cholesterol
  - Electrolytes
- Enzyme and other types of assays with NADH or NADPH readout
  - EMIT! (TDM Assays!)

theranos
redefining healthcare

# Signal is lower than for a standard spectrometer

- Path length is 1 mm
- A is about 10 – 20 % that of a MTP reader (path length 0.5 – 1.0 cm)
- ΔA (SD) 0.002 *i.e.* CV = 10% at A = 0.02 (OD 0.2)
- This can/may be compensated for by letting signal (A) values rise into the range (say) 0.2 – 5.0
  - Elisa assays: let enzyme work longer and use elevated temperature
  - General Chemistry: dilute less

theranos
redefining healthcare

# Nanodrop 3300: Spectroflorometer


**Q: Is the Thermo Scientific NanoDrop 3300 an upgrade to the NanoDrop™ 2000?**
A: No, the NanoDrop 3300 is used to measure fluorescence whereas the NanoDrop 2000 is used to measure absorbance.

theranos
redefining healthcare

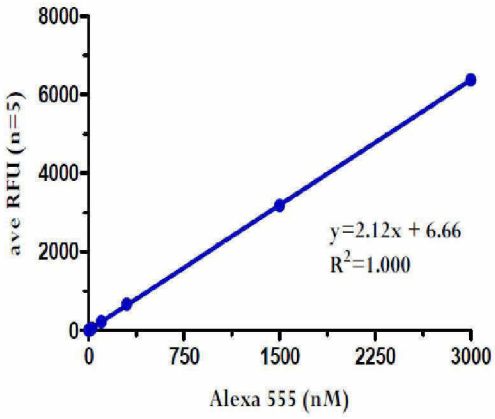
# Nanodrop Fluorimeter Specifications

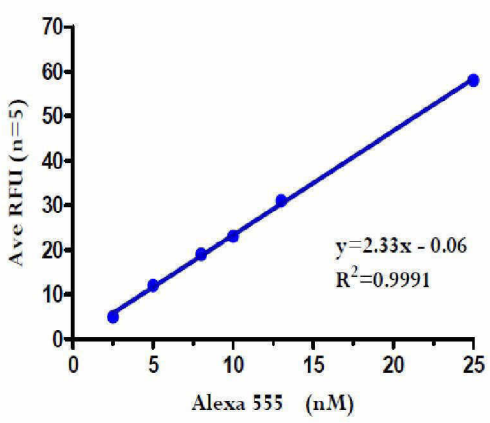
**Specifications**

| | |
|---|---|
| Instrument Type | Fluorospectrometer |
| Minimum Sample Size | 1 µl |
| Light Sources | 3 light emitting diodes (LEDs) |
| Excitation Maxima | UV: 365 nm<br>Blue: 470 nm<br>White 460-650 nm |
| Detector Type | 2048 - element linear silicon CCD array |
| Wavelength Range | 400-750 nm |
| Wavelength Accuracy | 1 nm |
| Spectral resolution | 8 nm (FWHM at Hg 546 nm) |
| Fluorescence Precision | < 5% CV (10 nM fluorescein) |
| Detection Limit | < 1 fmol fluorescein |
| Measurement Cycle Time | 2 - 10 seconds |
| Dimensions (footprint) | 14 cm X 20 cm |
| Weight | 1.5 kg |
| Sample Pedestal Material of Construction | 303 stainless steel and quartz fiber |
| Operating Voltage | 5 vdc (supplied by USB port, no external power supply) |
| Operating Power Consumption | 2 W |
| Standby Power Consumption | 1 W |
| Software Compatibility | Microsoft Windows® 2000, XP, and Vista (32 bit) |
| UL/CSA and CE | All units are approved to these standards |

theranos
redefining healthcare

# Nanodrop Fluorimeter:Sensitivity and dynamic range



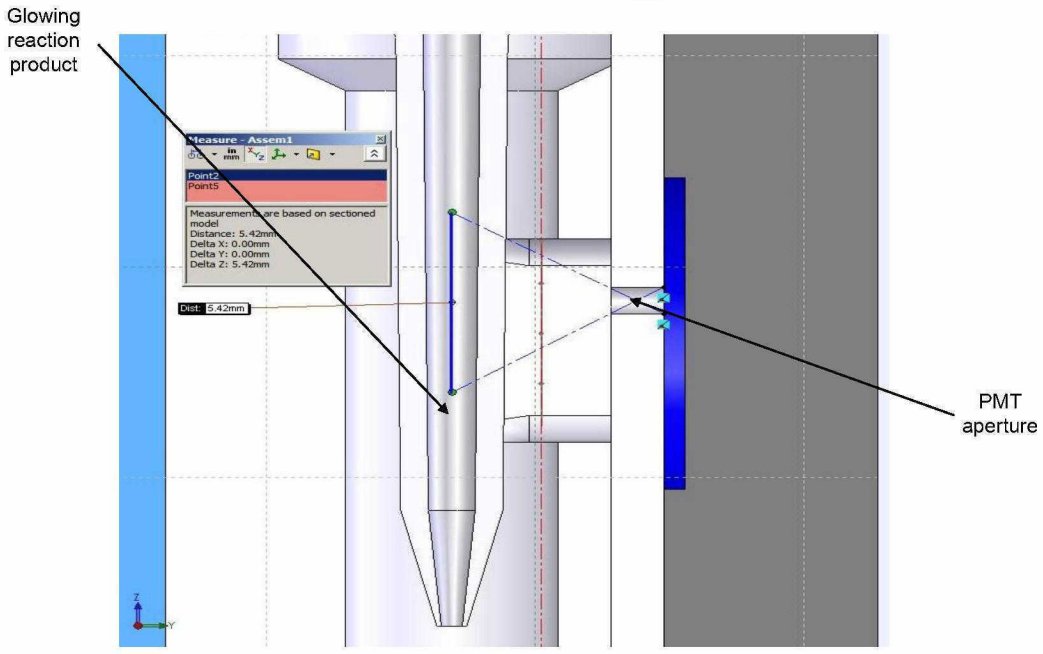
theranos
redefining healthcare

# Question and Idea

- Why not adapt the Nanodrop spectrometer to chemiluminescence?
- Develop a multi-detection mode platform
  - Absorbence
  - Fluorescence
  - Luminescence
- Problem might be smaller volume >> less signal
- The Nanodrop optic, however, appears to use a larger fraction of the sample volume than conventional spectrometers (and the Edison?)
  - Edison light collection volume has been estimated as about 5 uL but only a fraction of the light from that volume gets to the PMT.

theranos
redefining healthcare

# Current Instrument Light Collection



theranos
redefining healthcare

# IP

## US 6,628,382 Sept. 30 2003 CW Robertson

## Priority date: 08/1999

1. A photometric or spectrophotometric apparatus wherein a sample in the form of a liquid drop is contained by surface tension forces between two planar surfaces, one containing a photometric or spectrophotometric source and the other a photometric or spectrophotometric detector and an optical path is established through the sample between the two surfaces said apparatus comprising:

first and second anvil surfaces at least one being moveable relative the other to any one of three positions:

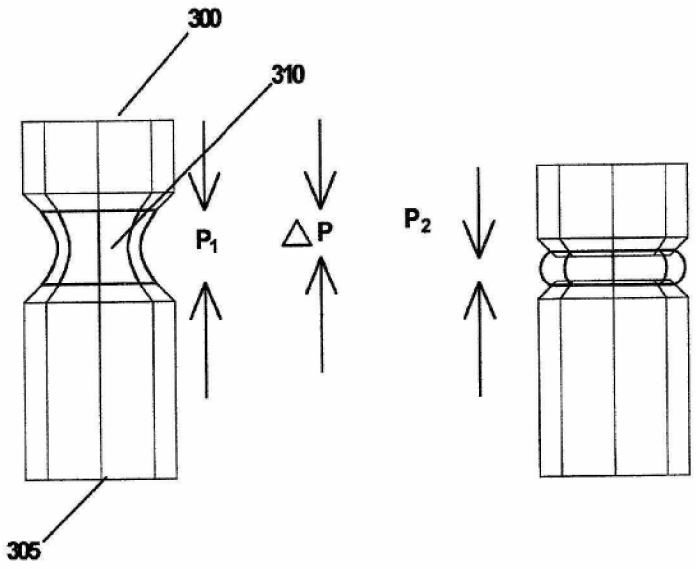
an adjustable sample loading position so selected that the at least one moveable surface and the other surface are so remotely spaced that a liquid drop can be placed on the first surface;

an adjustable sample compression position so selected that the surfaces are opposed and substantially parallel and proximally spaced so that the liquid wets and spreads upon both surfaces;

an adjustable sample measuring position so selected that the opposed substantially parallel surfaces are spaced apart to pull the sample into a column wherein it is contained by surface tension thereby providing an optical path for a photometric or spectrophotometric measurement.

theranos
redefining healthcare

# NanoDrop: Definition of light path



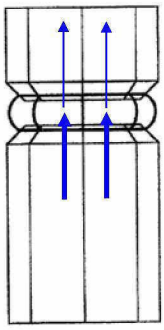

*Sample pathlength and illuminated volume are defined by the geometry of the pedestal*

*Volume in excess over 1 uL is expressed out of the light beam and contained by surface tension*

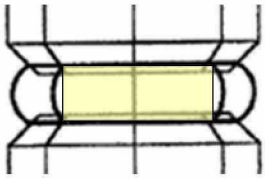
theranos
redefining healthcare

# Optics For Three Detection Technologies
# (1) Absorption

Detector



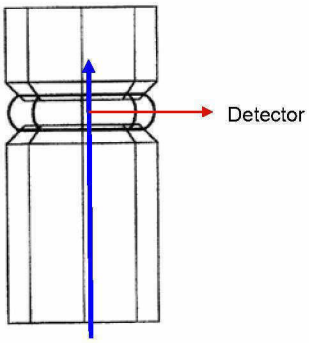
Light source producing a wide highly-collimated beam



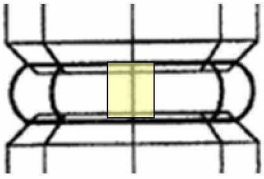
Defined illuminated volume

theranos
redefining healthcare

# Optics For Three Detection Technologies
# (2) Fluorescence




Light source producing a *narrow* highly-collimated beam

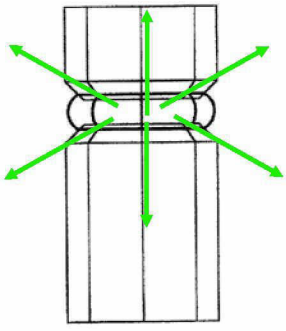


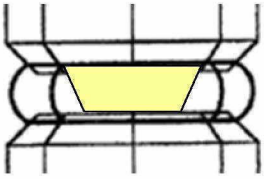
Defined illuminated volume

theranos
redefining healthcare

# Optics For Three Detection Technologies
# (3) Luminescence

Detector

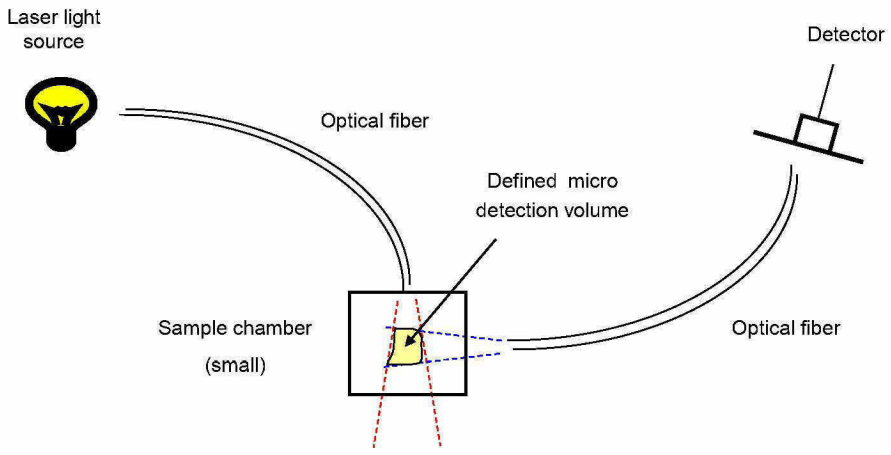




Defined volume for
light collection

theranos
redefining healthcare

# Alternative low volume system for optical detection of fluorescence using optical fibers

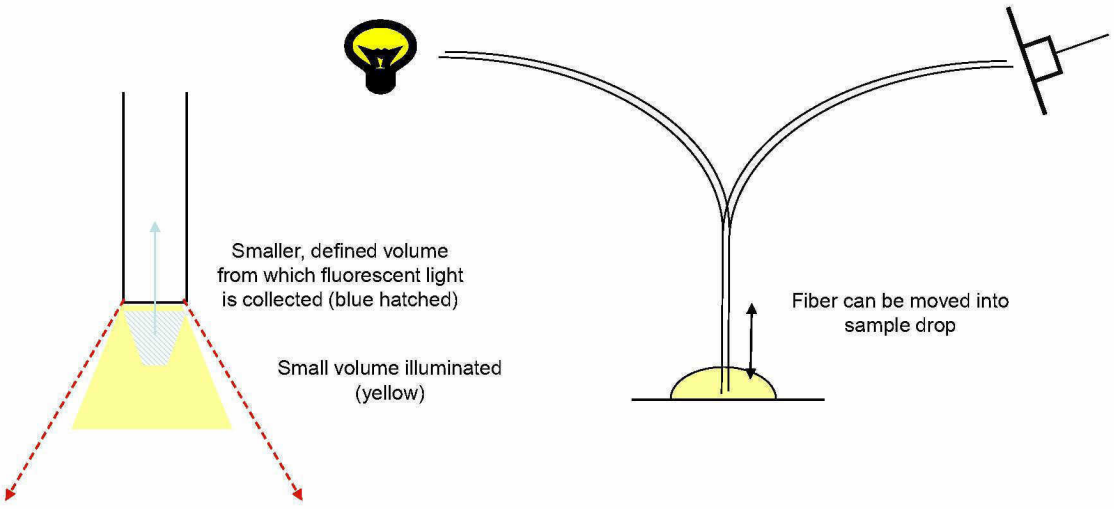



See also: US **4,676,640 (expired)**

*US4676640.pdf*

theranos
redefining healthcare

# Another version of the fiber approach for low volume fluorescence detection and cell counting




See: US **4,676,640 (expired)**

*US4676640.pdf*

theranos
redefining healthcare

## What would be needed to incorporate this technology in our system?

- Interface with liquid dispensing
  - Demo in progress
- Interface with RBC filtration
- Add a simple light source to the instrument
- Detector
  - PMT? or simple CCD (spectroscopy [many wavelengths]) or Photodiode (one color)
- IP: license or (better) reverse engineer

theranos
redefining healthcare

# Recommendations

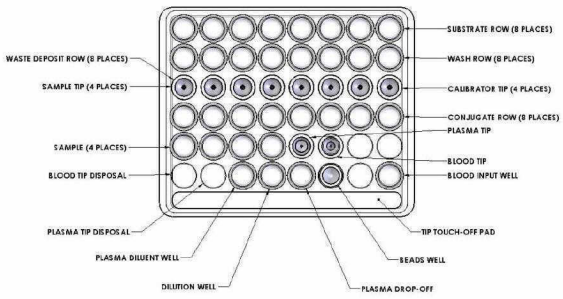
- Selected candidate technologies
  - Luminescence
  - Absorbence
    - Spectroscopy needed (multi-wavelength)
    - Wavelength range 340-650 nm
  - Fluorescence
  - RT-PCR
  - Cell counting
- Need to *consolidate* optics around a low volume detection means (*invention needed)*
  - Nanodrop-like?
- Need to *invent* and develop red cell separation technology

theranos
redefining healthcare

# Pipettor head multiplicity: Issues

- Holding liquid during incubations
  - "Tip" versus "well"
  - Gravity
    - Tip orientation
    - Small volume would likely remain fixed (even if tip is vertical)
  - Evaporation (very important if volume < 5 uL)
- Pressure transmission when picking up tip
  - Premature ejaculation
- Precise positioning
  - Nanodrop approach would require good precision in x, y and z
  - < 0.1 mm is OK and easy to implement
  - But multiple head compounds the problem
- Temperature control
  - Absolute requirement for immunoassays and PCR

# Cartridge layout and number of assays




- Current design completely fills the footprint (close packing of assay elements
- Footprint is dominated by *diameter* of reagent tubes and the tip boss
- Can these be smaller?
- Can some reagents be shared?
  - One head concept makes this easier
  - But, there is a time penalty

theranos
redefining healthcare

# Engineering evaluation

- Feasibility review
  - Complete analyte list
  - Complete chemistry review against all analytes
  - Are integrated optics feasible?
  - How to implement more assays on same footprint
    - Can footprint be bigger?
    - Cartridge height could be less for smaller volume assays
- Development plan
- Costs and Development Time

theranos
redefining healthcare

# Appendix 1

- Some alternative technologies including non-separation methodologies
- Review of Theranos requirements against possible licensable technologies

theranos
redefining healthcare

# Technology Review

April 2009

02/18/2010

theranos
redefining healthcare

# Theranos mission

## Instrumented system

To enable rapid, accurate, multiplexed POC measurements of key biomarkers and therapeutic agents

To replace laboratory methods in monitoring disease and therapy

theranos
redefining healthcare

# Capabilities (ideal)

**Sample types**
- Blood (F/S, venous), serum, plasma, urine (?)

**Marker types**
- Proteins
- Small molecules
- Metabolites
- Nucleic acids
- Cell markers (Surface + Internal)
- Electrolytes

**Number of multiplexed markers**
- Up to 20

**Number of assays available**
- >100

**Specificity, accuracy, precision and sensitivity**
- Match current state of the art

**Size and speed**
- Match current POC state-of-art
  - Time < 15 min

**Stability**
- > One year at RT

**Theranos cost per result**
- < $3 equivalent to $18/six plex; Price = > $100 at > 80% gross margin

**Disposables/year**
- > 1,000,000

**FDA, CLIA cleared, ISO-9xxx**

**Patent protected**

**Sample integrity and volume measurement**

**Sample pre-treatment (including removal of red cells)**

theranos
redefining healthcare

## Comparison of current Theranos and Competing technology

| | |
|---|---|
| **Sample types**<br>• Blood (F/S, venous), serum, plasma, urine | Match |
| **Marker types** | Match |
| • Proteins | Better |
| • Small molecules | Match |
| • Metabolites | Worse |
| • NOT Nucleic acids | N/A |
| • Cell markers (Surface + Internal) | Worse |
| **Number of multiplexed markers**<br>• Up to six | Worse (30-plex available) |
| **Number of assays available**<br>• 100 | Worse |
| **Specificity, accuracy, precision and sensitivity**<br>• Match current state of the art | Match |
| **Size and speed**<br>• Match current POC state-of-art<br>  • Time < 15 min | Worse |
| **Stability**<br>• > One year at RT | Worse |
| **Theranos cost per result**<br>• < $3 equivalent to $18/six plex;<br>• Price = > $100 at > 80% gross margin | ? |
| **Disposables/year**<br>• > 1,000,000 | ? |
| **FDA cleared** | Worse |
| **Patent protected** | Worse (Situation improving this year) |

theranos
redefining healthcare

# Current capabilities

| Attribute | Current | Possible |
|---|---|---|
| Sample types | YES | |
| • Blood (F/S, venous), serum, plasma, urine | | |
| Marker types | | |
| • Proteins | YES | |
| • Small molecules | YES | |
| • Metabolites | NO | YES |
| • Ions (Ca2+ etc) | NO | ? |
| • NOT Nucleic acids | N/A | ? |
| • Cell markers | NO | ? (NEW SYSTEM) |
| Number of multiplexed markers | NO | YES |
| • Up to six | | |
| Number of assays available | NO | YES |
| • 100 | | |
| Specificity, precision and sensitivity | YES | |
| • Match current state of the art | | |
| Size and speed | NO | MAYBE |
| • Match current POC state-of-art | | |
| • Time < 15 min | | |
| Stability | NO | YES |
| • > One year | | |
| Theranos cost per result | NO | MAYBE |
| • < $3 equivalent to $18/six plex; | | |
| • Price = > $100 at > 80% gross margin | | |
| Disposables/year | | |
| • > 1,000,000 | NO | YES |
| Instruments/year | YES | |
| • 1,000 | | |
| FDA, CLIA etc. cleared | NO | YES |
| Patent protected | NO | YES |

theranos
redefining healthcare

# Existing technology capabilities

## Sensitivity limit (Can we do better for biomarkers? NO)

| Calculations | Chemiluminescence EIA | | |
|---|---|---|---|
| **Specific signal** | | | |
| Antigen conc. | 5.00E-01 | pg/mL | Current best |
| Dilution | 3.00E+00 | fold | |
| Diluted antigen conc. | 1.67E-01 | pg/mL | |
| Effeciency of binding | 1.00E+01 | % | |
| Enzyme molecules/antigen | 2.00E+00 | mole/mole | Guess |
| Enzyme turnover | 5.00E+04 | sec^-1 | |
| Enzyme conc. | 3.33E-02 | pg/mL | Apase |
| Interogated volume | 1.00E+00 | uL | |
| Enzyme quantity | 3.33E-05 | pg | Only see part of liquid column |
| Enzyme MW | 1.20E+05 | | |
| Avogadro's # | 6.04E+23 | | |
| Vmax | 8.39E+06 | molecules of product/sec | |
| S/K | 5.00E-02 | | |
| v | 4.19E+05 | molecules of product/sec | Substrate is not saturating |
| Quantum yield | 5.00E-01 | % | |
| Photon production | 2.10E+03 | counts/sec | Guess |
| Detection efficiency | 5.00E+01 | % | |
| Signal | 1.05E+03 | counts/sec | |
| Enzyme molecules | 1.68E+02 | | |
| **Background signal** | | | |
| Signal | 100 | counts/sec | |
| Signal | 300 | counts/sec | Substrate blank |
| S/B | 5.59E-01 | | NSB (best case) |

theranos
redefining healthcare

## Some current potentially competing technology systems

The clinical laboratory ***

Service providers ***

Platforms for customer assay development

ELISA
- Multiplexed Imaging (Searchlight)

Lateral flow (Biosite)

Electrochemiluminescence (MSD)

Time resolved fluorescence (TRF)

Non-separation methods
- Alpha screen (Packard)
- Enzyme complementation (DiscoveRx)
- Homogeneous time-resolved fluorescence (HTRF)
- EMIT (small molecules)
- FP (small molecules)
- Surface plasmon resonance ("Reagent-free", Biacor, Forte Bio)
- Evanescent wave

FACS screen
- Guava

Bead-based multiplexes (Luminex, BD etc.)

POC niche
- Cholestech (Lipids)
- Abaxis (General chemistry + low sensitivity immunoassay)
- Coagucheck *etc.* (PT)
- Hemocue (Hemoglobin)
- Many (blood glucose)
- Metrika (HbA1c)
- Ions (ISE) + IA (I-Stat)

theranos
redefining healthcare

# Existing competitive technology limitations

Factory calibration

Validation problems (multiplexes)

Sample handling (blood)

- Stability

Sample volume (blood)

- Too large

theranos
redefining healthcare

## Potential improvements to our existing system

Up to 12 assays

Smaller sample 10 uL at 6-plex?

Faster (assays complete in < 15min?)

Smaller (1/4 th current size?)

More precise (< 5 %CV?)

More reliable (< 1/1000 failures?)

Add selected general chemistries

Add other types of marker

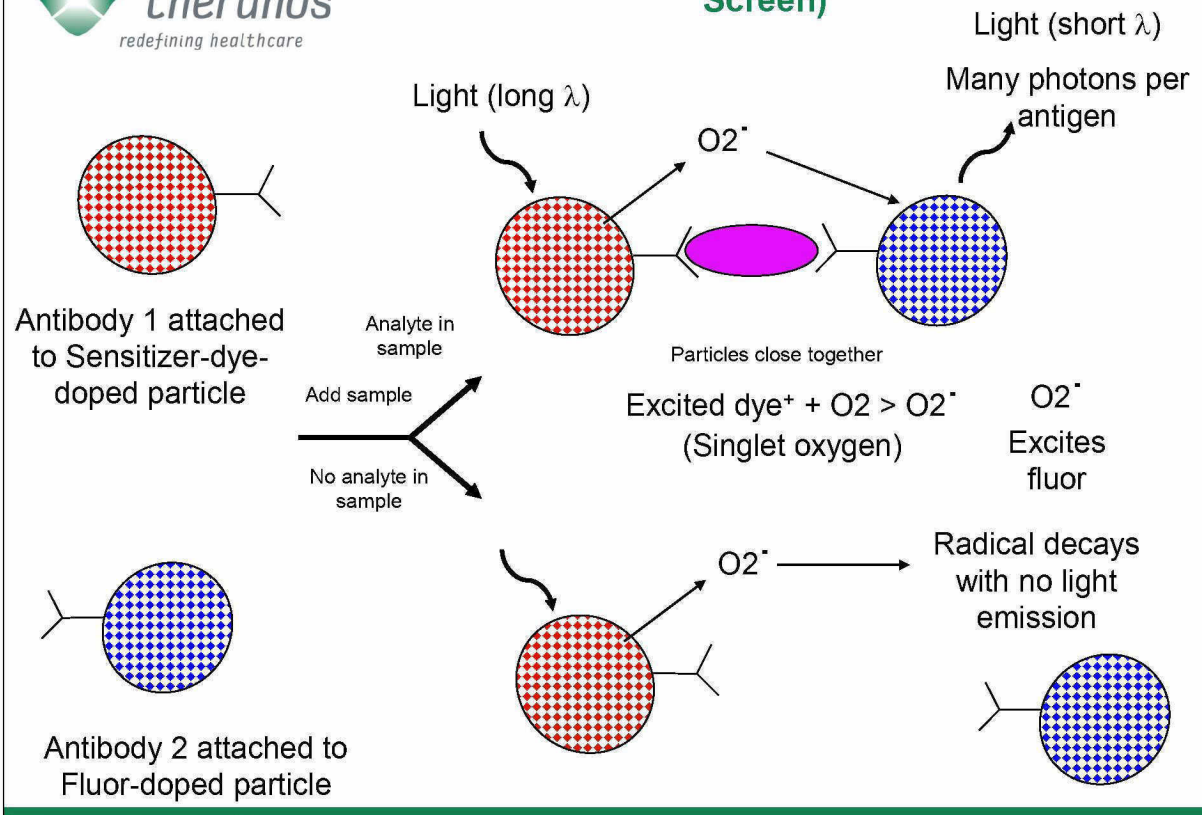
theranos
redefining healthcare

# Candidate supplemental/alternate technologies

## Examples

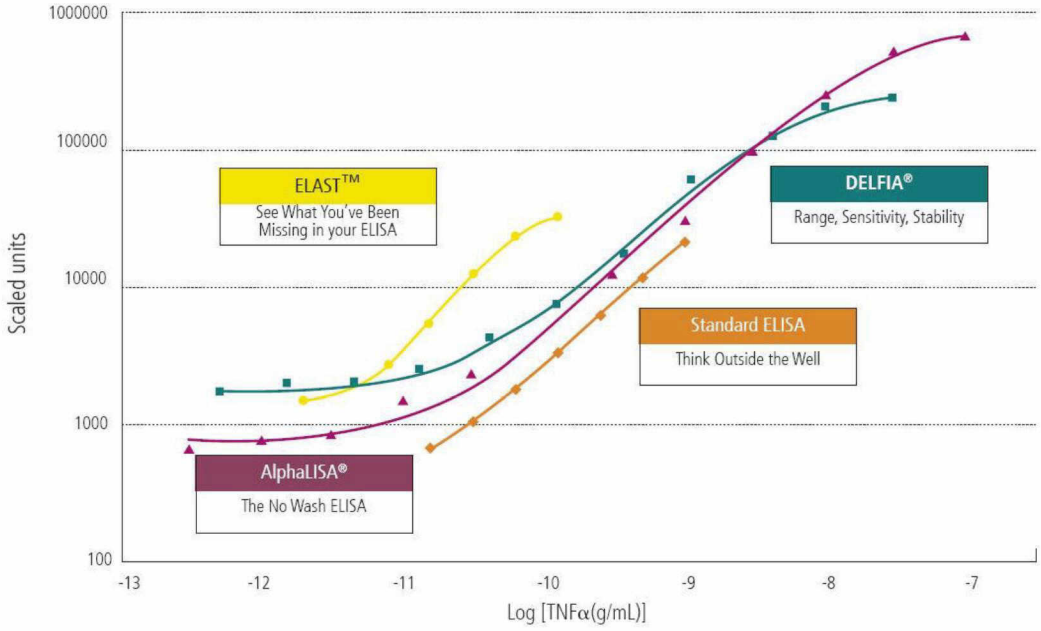
- DiscoveRx
- AlphaLISA
- EMIT
- HTRF
- ELAST
- …

# Luminescence Oxygen Channeling IA (LOCI, Alpha Screen)



theranos
redefining healthcare

# Technology comparison



theranos
redefining healthcare

# Enzyme complementation (DiscoveRx)

## β-Galactosidase

Native β–Galactosidase polypeptide

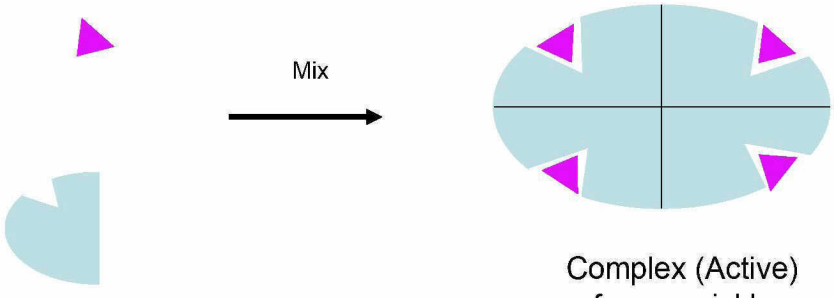
Mutant, inactive β–Galactosidase polypeptide (EA)

Small N-terminal polypeptide (ED)

Enzyme Donor "ED" (Inactive)

Mix

Native enzyme is a tetramer



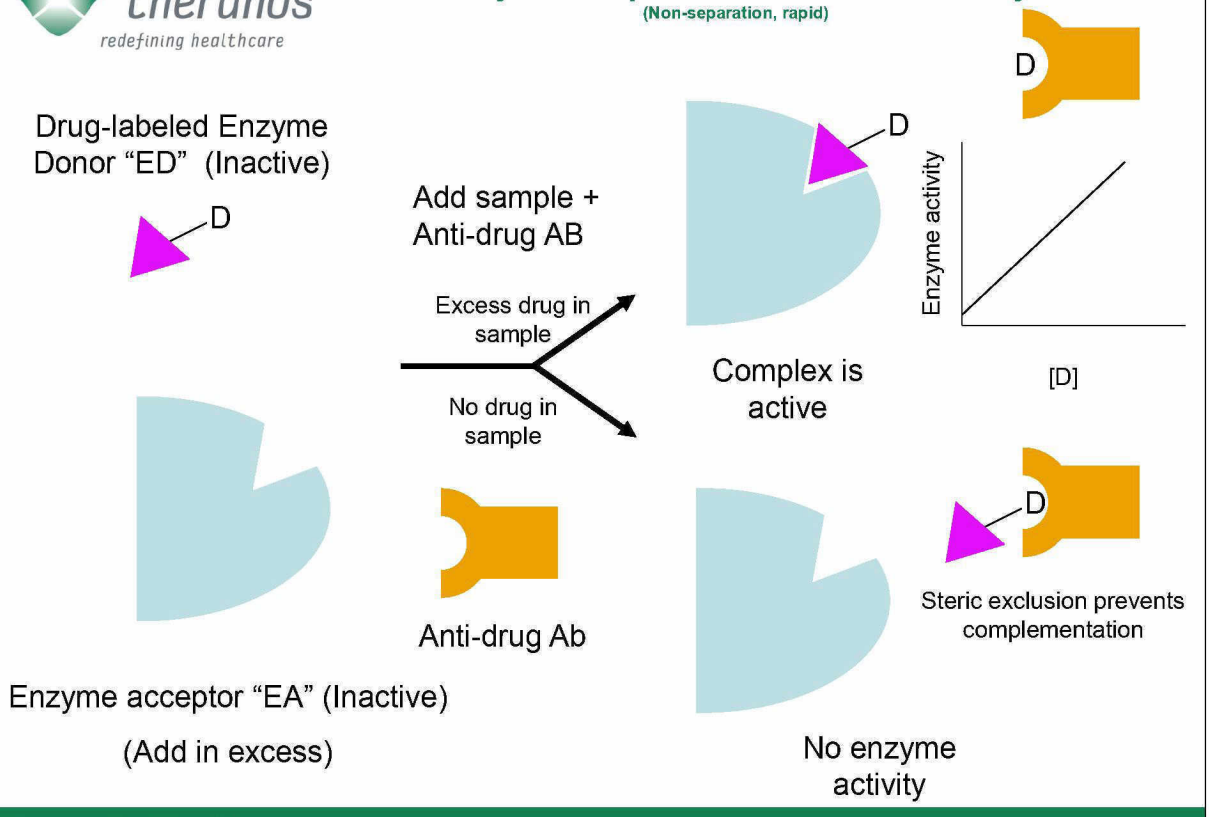
Complex (Active) forms quickly

Enzyme acceptor "EA" (Inactive)

theranos
redefining healthcare

# Enzyme complementation immunoassay

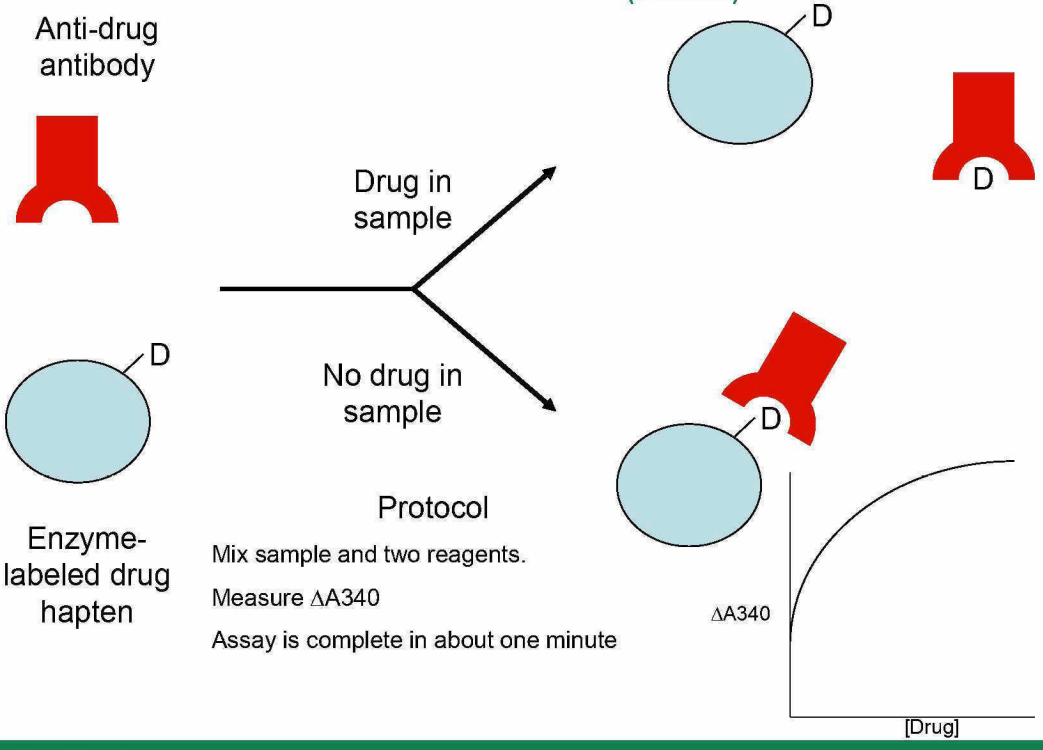
(Non-separation, rapid)



theranos
redefining healthcare

# EMIT (Enzyme multiplied immunoassay technique)

## Rapid, non-separation measurement of small molecule drugs (Siemens)




### Protocol

Mix sample and two reagents.

Measure ΔA340

Assay is complete in about one minute

theranos
redefining healthcare

# HTRF

- "Homogeneous time-resolved fluorometry"
- No separation required

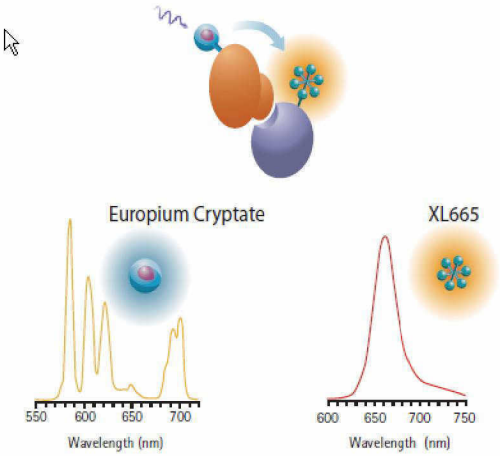



Fig.1 : HTRF principle with $Eu^{3+}$ cryptate and XL665 as respectively donor and acceptor. When the two entities come into close proximity and upon excitation, FRET occurs and XL665 re-emits a specific long-lived fluorescence at 665 nm.

theranos
redefining healthcare

# DELFIA

- Dissociation-enhanced lanthanide fluorescence immunoassay
- Europium chelate labeling
- Fluorescence enhanced by release of Eu and shielding on a micelle

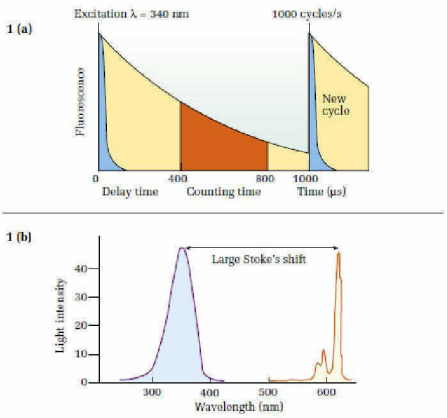



*Figure 1. Unique fluorescence properties of lanthanides, (a) long fluorescence decay times, (b) large Stokes' shift.*

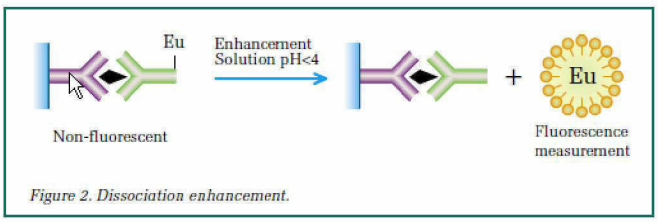
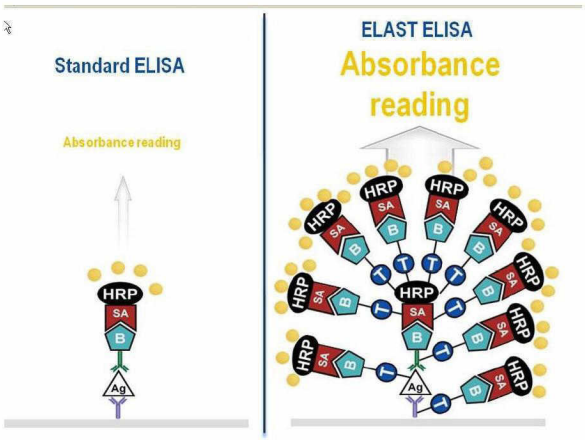



*Figure 2. Dissociation enhancement.*

theranos
redefining healthcare

# ELAST

- ELISA amplification system
- Signal amplification
- Generally not effective because of background amplification (Contrast PCR)



theranos
redefining healthcare

# Attributes (good and bad) of selected competing technology

theranos
redefining healthcare

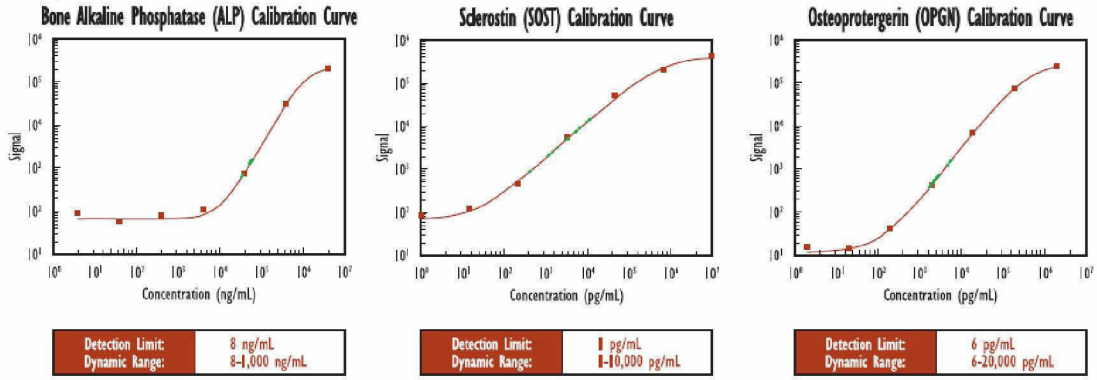
# MSD (Electrochemiluminescnece) Clumsy protocol

## Assay Protocols

- Block MSD MULTI-SPOT plate for 1 hour, wash
- Add 25 µL of assay diluent solution to each well
- Add 25 µL of calibrator or sample (undiluted for Panel I; 20-fold diluted for Panel II; 250-fold for PICP assay; and 20-fold for TGFβ1 assay) to each well
- Incubate with shaking for 120 minutes, wash
- Add 25 µL of labeled antibody solution to each well
- Incubate with shaking for 60 minutes, wash
- Add MSD Read Buffer
- Read plate on MSD Reader

theranos
redefining healthcare

# MSD assay performance




http://mesoscale.com/CatalogSystemWeb/WebRoot/literature/applications/pdf/humanBONE.pdf

theranos
redefining healthcare

# Chemical multiplexing

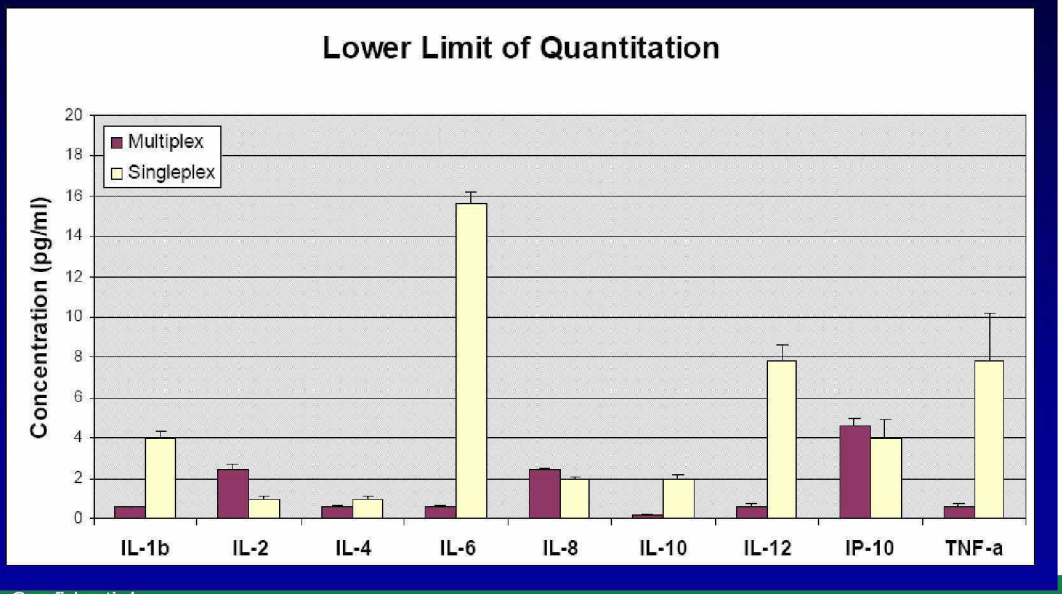
- Coded beads (Luminex, BD, BioRad)
- eTags (Monogram)
- The main problem is complexity and reliability
- In the case of chemical multiplexing background can be a problem

theranos
redefining healthcare

## Perils of non-separation multiplexing

http://www.luminexcorp.com/planetxmap/07_Proceedings/Paul%20Rhyne.pdf

# Luminex: Multiplex vs Singleplex



theranos
redefining healthcare

# Biosite

- Multiplexed (3) IA
- Good sensitivity
- Blood samples (50 -200 uL)
- Precision may not be good enough
- Small menu
- FDA cleared

theranos
redefining healthcare

## Requirements for other types of assay

General chemistry: glucose, lipids

Enzymes: ALT etc.

Electrolytes and blood gasses

theranos
redefining healthcare

## Extending the capability of the current system
### Glucose assay chemistry

### Current approach is chemiluminescent

- Compatible with current instrument
- Glucose + ATP > Glucose-phosphate + ADP (Hexokinase)
- ATP + D-Luciferin + O2 → Oxyluciferin + AMP + PPi + CO2 + Light (560nm) (Luciferase)
- Reverse reading assay; measures ATP remaining after ATP is converted to ADP

### If this approach works

- Lipid assays and enzyme assays may be feasible

theranos
redefining healthcare

# What additional critical analytes do we need/want?

## Clinical chemistry

- Glucose (WIP)
- HbA1c (Method proposed; Ab availability is an issue)
- Lipids
  - Cholesterol, HDL-C, LDL-C, Triglycerides
- ALT

## White cell markers ??

- CD4, CD8 etc.

## Small molecules with no IA chemistry compatible with current system and possible modifications

- Ions (detection means)
- Serotonin (organic chemistry required)

theranos
redefining healthcare

# Appendix 2
# Selected, existing POC systems for comparison

theranos
redefining healthcare

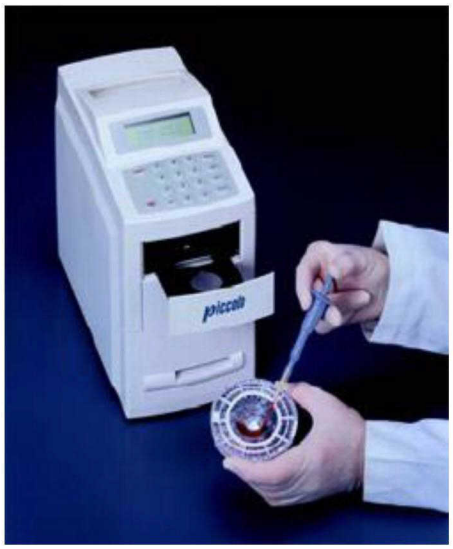
# Biosite: Multiplexed Immunoassay

theranos
redefining healthcare

# Cholestech: Multiplexed lipids and very limited other analytes

theranos
redefining healthcare

# Abaxis: Multiplexed general chemistry and very limited immunoassay menu

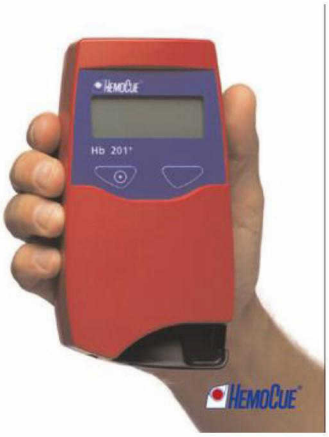


theranos
redefining healthcare

# HemoSense: PT/INR (only)

theranos
redefining healthcare

# Hemocue: Hemoglobin and glucose



theranos
redefining healthcare

# I-Stat: Multiplexed electrolytes and limited immunoassay

theranos
redefining healthcare

# Pulse Oximeter

- Measures Hb saturation with O2
- Non-invasive
- Could easily be added/attached to our instrument


theranos
redefining healthcare

# Appendix 3:
# Imaging Samples

Measurement of sample volume

Determination of sample integrity

02/18/2010

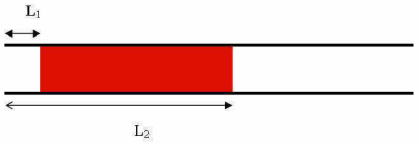
theranos
redefining healthcare

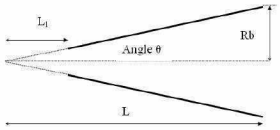
# Imaging enables sample volume measurement

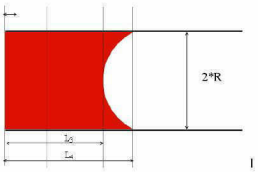
- Verification that the sample has the correct volume
- Verification that sample delivery was good
- Measurement of sample volume
  - Enables result correction for improper volume
- Inexpensive camera will work for this purpose

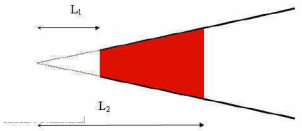
theranos
redefining healthcare

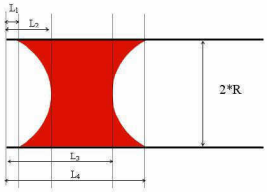
# Some examples

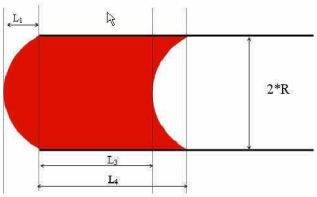


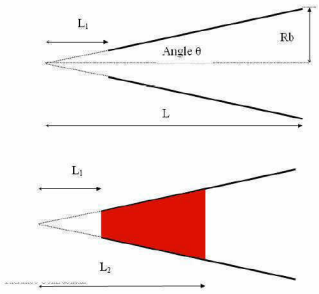



theranos
redefining healthcare

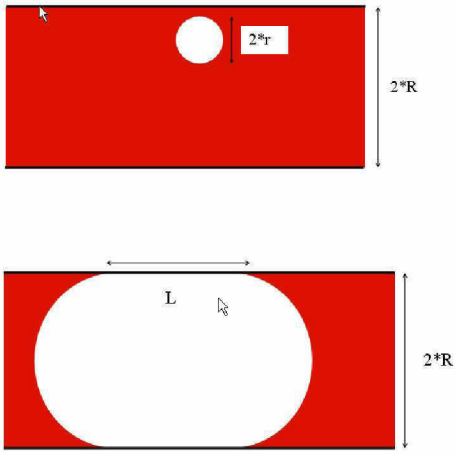
# Calculation of volume (example)

Example 4: Geometry of measurement in a conical capillary (often used for tips)
Rb = radius at base of cone
L = length
L1 = distance from (projected) top of the cone to lower sample meniscus
L2 = distance from (projected) top of the cone to lower upper meniscus
Volume introduced = p* (Rb/L)^2*[(L1)^3 - (L2)^3]/3
Tan θ = Rb/L



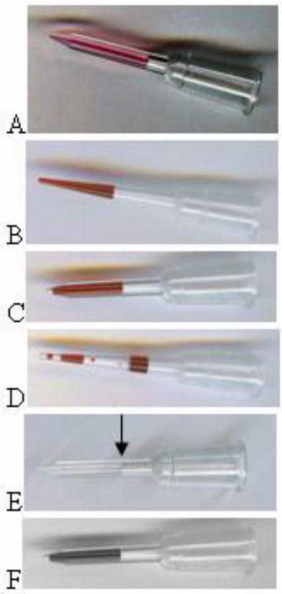
theranos
redefining healthcare

# Bubbles



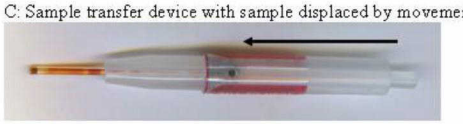
theranos
redefining healthcare

# Images




B: Sample transfer device with its capillary filled with sample. The "fill to" location is indicated.


C: Sample transfer device with sample displaced by movement of the plunger



D: Sample transfer device with sample incompletely displaced

theranos
redefining healthcare

# Measurement from image of conical capillaries