

## Assay Development Report

|               |                    |
|---------------|--------------------|
| Assay         | C-Reactive Protein |
| Developer     | Michael Chapman    |
| Date Released | 2-28-08            |

### 1. Analyte Background

C-Reactive Protein is an acute phase plasma protein produced by the liver and by adipocytes as part of the inflammatory response. It is a member of the pentraxin family of proteins. CRP is a 224 residue protein with a monomer molar mass of 25106 Da. Native CRP is a bit different as it has 10-subunits making two pentameric discs, with an overall molecular mass of 251060 Da.

CRP is a member of the class of acute phase reactants as its levels rise dramatically during inflammatory processes occurring in the body. This increment is due to a rise in the plasma concentration of IL-6, which is produced predominantly by macrophages as well as adipocytes. CRP binds to phosphorylcholine on microbes. It is thought to assist in complement binding to foreign and damaged cells and enhances phagocytosis by macrophages, which express a receptor for CRP. It is also believed to play an important role in innate immunity, as an early defense system against infections. CRP rises up to 50,000 fold in acute inflammation, such as infection. It rises above normal limits within 6 hours, and peaks at 48 hours.

### 2. Assay Specifications

The Theranos Assay for CRP is a sandwich ELISA and is usable in human whole blood, plasma and serum.

Reportable ranges are:

| Sample Type       | Low, µg/mL | High, µg/mL |
|-------------------|------------|-------------|
| Human whole Blood | 0.1        | 400         |
| Human Plasma      | 0.1        | 250         |
| Human Serum       | 0.1        | 300         |









### 3. Reference Assays

- A. R&D Systems Quantikine Human C-Reactive Protein Immunoassay;  
Catalog # DCRP00
- B. Helica Biosystems, Human C-Reactive Protein Detection ELISA kit;  
Catalog # 961CRP01H

#### 4. Antibody Screening for matched Pairs

Antibody screening was carried out in 384 well micro-titer plates, 10/10/10 assay time at room temperature and read on the M5

Table 1: Antibody Screen Summary

| Detection Ab                    |           | Capture Ab | Medix                                                                              | Biogenesis                                                                          | Biodesign                                                                           |
|---------------------------------|-----------|------------|------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| Vendor                          | Cat#      | Vendor     | 100058                                                                             | 0400-0006                                                                           | M86842M                                                                             |
| Medix                           | 100062    |            |  |                                                                                     |  |
| Biogenesis                      | 1707-0404 |            |                                                                                    |  |                                                                                     |
| Biodesign                       | M86284M   |            |  |                                                                                     |  |
| Number of Capture Ab's tested   |           |            | 3                                                                                  |                                                                                     |                                                                                     |
| Number of Detection Ab's tested |           |            | 3                                                                                  |                                                                                     |                                                                                     |
| Good Dose Response              |           |            |                                                                                    |    |                                                                                     |
| Some Modulation                 |           |            |                                                                                    |    |                                                                                     |
| No Response                     |           |            |                                                                                    |    |                                                                                     |

The matched pair from Biodesigns was used as they yielded the best dose response and they are widely available. The Medix Capture Ab with the Biodesign Detection Ab can be used as an alternate pair in the event of an emergency.

Table 2 Antibody screen on 384 well micro-titer plates, 10/10/10 assay at room temperature, Plate chemiluminescence read on M5, Assay Buffer standards, Stock CRP from Scripps, Dab at 5 ng/mL

| Concentration | Wells | Values | MeanValue | Std.Dev. | CV%  |
|---------------|-------|--------|-----------|----------|------|
| 750           | A4    | 469297 | 468253    | 1475     | 0.3  |
|               | A5    | 467210 |           |          |      |
| 500           | B4    | 441806 | 444219    | 3413     | 0.8  |
|               | B5    | 446633 |           |          |      |
| 214           | C4    | 369806 | 383600    | 19508    | 5.1  |
|               | C5    | 397394 |           |          |      |
| 100           | D4    | 296687 | 306028    | 13210    | 4.3  |
|               | D5    | 315369 |           |          |      |
| 50            | E4    | 248115 | 243814    | 6083     | 2.5  |
|               | E5    | 239513 |           |          |      |
| 25            | F4    | 149317 | 161445    | 17152    | 10.6 |
|               | F5    | 173573 |           |          |      |
| 5             | G4    | 44659  | 47562     | 4105     | 8.6  |
|               | G5    | 50465  |           |          |      |
| 1             | H4    | 10010  | 10988     | 1382     | 12.6 |
|               | H5    | 11965  |           |          |      |
| 0             | I4    | 206    | 275       | 97       | 35.4 |
|               | I5    | 344    |           |          |      |

## 5. Assay Reagents

### A. Capture Antibody

|               |                                                                                                                                                                                                 |
|---------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Vendor        | Biodesign International                                                                                                                                                                         |
| Catalog #     | M86842M                                                                                                                                                                                         |
| Current lot # | 12L34106; Clone 2                                                                                                                                                                               |
| Type          | Monoclonal Antibody to Human C Reactive Protein                                                                                                                                                 |
| Specificity   | Human CRP. Recognizes antigen both in the presence and in the absence of Calcium.                                                                                                               |
| Stock Conc.   | Unlabeled: 5.3 mg/mL; Biotin conjugated: 0.5 mg/mL                                                                                                                                              |
| Data sheet    | [ <a href="Y:\\Common\\Assay Development Reports\\CRP Assay Development Report\\Data Sheets">HYPERLINK "Y:\\Common\\Assay Development Reports\\CRP Assay Development Report\\Data Sheets"</a> ] |

### B. Detection Antibody

|               |                                                                                                                                                                                                                                                                               |
|---------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Vendor        | Biodesign International                                                                                                                                                                                                                                                       |
| Catalog #     | M86284M                                                                                                                                                                                                                                                                       |
| Current lot # | 9K32606; Clone 6                                                                                                                                                                                                                                                              |
| Type          | Monoclonal Antibody to Human C Reactive Protein                                                                                                                                                                                                                               |
| Specificity   | Human CRP. Recognizes antigen both in the presence and in the absence of Calcium.                                                                                                                                                                                             |
| Stock Conc.   | Unlabeled: 6 mg/mL; Biotin conjugated: 0.5 mg/mL                                                                                                                                                                                                                              |
| Data sheet    | [ <a href="Y:\\Common\\Assay Development Reports\\CRP Assay Development Report\\Data Sheets\\CRP Detection Antibody Data Sheet.doc">HYPERLINK "Y:\\Common\\Assay Development Reports\\CRP Assay Development Report\\Data Sheets\\CRP Detection Antibody Data Sheet.doc"</a> ] |

### C. Analyte

|               |                                                                                                                                                                                                 |
|---------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Vendor        | Scripps                                                                                                                                                                                         |
| Catalog #     | C0125                                                                                                                                                                                           |
| Current lot # | 2235202                                                                                                                                                                                         |
| Stock Conc.   | 2.54mg/mL                                                                                                                                                                                       |
| Storage       | Aliquot stock and store at -20C, Avoid freeze thaw cycles                                                                                                                                       |
| Data sheet    | [ <a href="Y:\\Common\\Assay Development Reports\\CRP Assay Development Report\\Data Sheets">HYPERLINK "Y:\\Common\\Assay Development Reports\\CRP Assay Development Report\\Data Sheets"</a> ] |

## 6. Sample handling and storage

Store all reagents at 4°C. Frozen calibrators can be kept at room temperature for up to 12 hrs.

## 7. Protocols

Tip coating and assay protocols can be found at

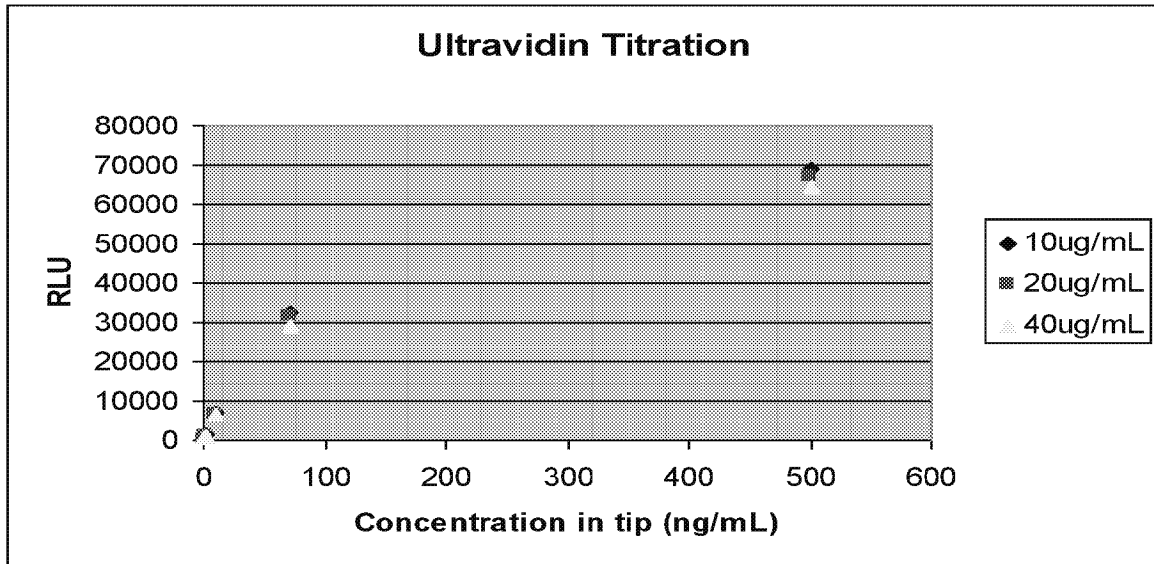
[ [HYPERLINK "Y:\\Common\\Assay Group Protocols\\CRP"](Y:\\Common\\Assay Group Protocols\\CRP) ]

Recommended Edison Protocols

Inflammation 2\_5k\_10k svn 1768

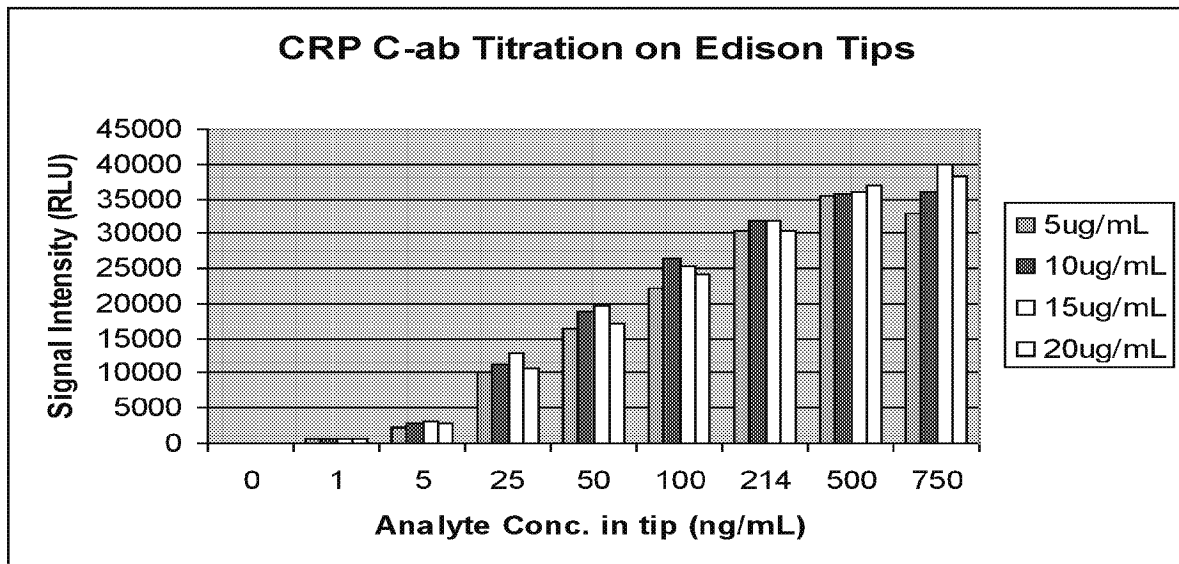
### 8. Avidin Titration

Figure 1: Ultravidin Titration on Edison tips; M5 readings



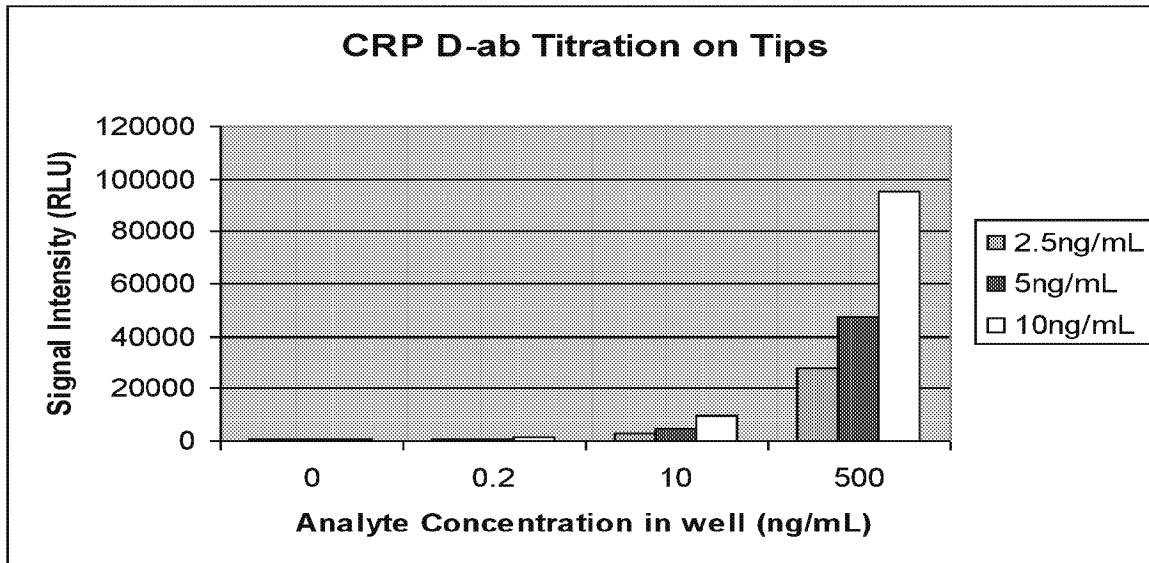
### 9. Capture Antibody Titration

Figure 2: Capture Antibody Titration; M5 readings



### 10. Detection Antibody Titration

Figure 3: Detection Antibody Titration; M5 readings



### 11. Spike into Whole Blood, Plasma from spiked blood.

Figure 4. Whole Blood spike and recovery in Plasma.

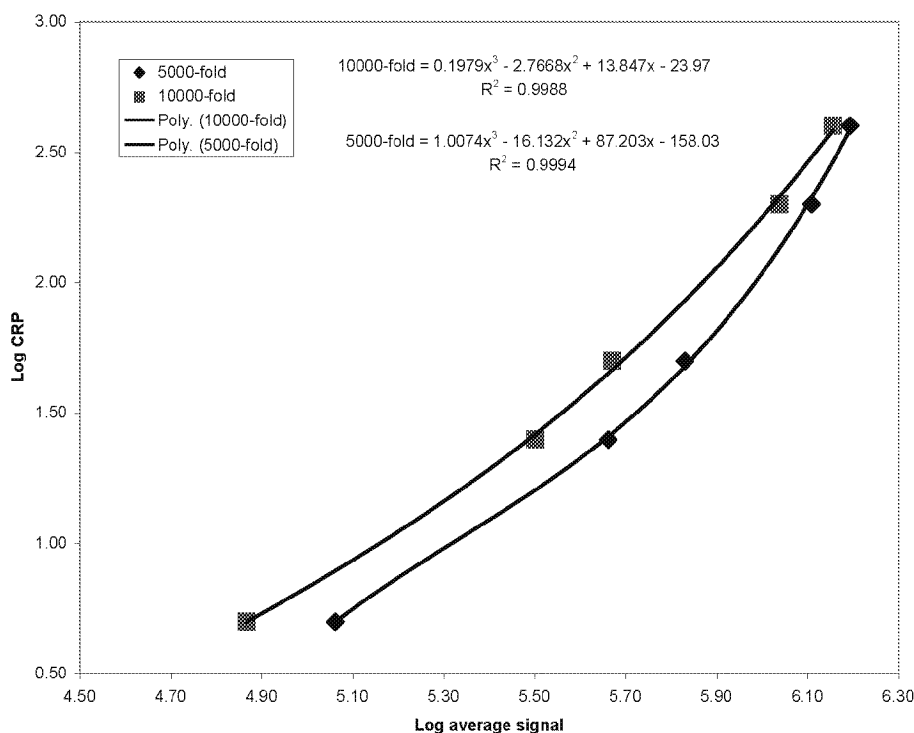
| Whole Blood 50ug/mL Spike          |                     |                  |        |       |     | Endogenous          | 24                         | in blood |            |
|------------------------------------|---------------------|------------------|--------|-------|-----|---------------------|----------------------------|----------|------------|
| Dilution                           | Theo. Conc. (ng/mL) | Signal           | Avg    | Stdev | CV  | Calc. Conc. (ng/mL) | Sample Calc. Conc. (ng/mL) | ug/mL    |            |
| 500x                               | 100                 | 277852<br>268000 | 272926 | 6966  | 2.6 | 162.3<br>6          | 81178                      | 81       |            |
| 2000x                              | 25                  | 130199<br>115551 | 122875 | 10358 | 8.4 | 33.40               | 66804                      | 67       |            |
|                                    |                     |                  |        |       |     |                     | average spike              | 74       |            |
|                                    |                     |                  |        |       |     |                     | recov., %                  | 121.7    |            |
|                                    |                     |                  |        |       |     |                     | overall recovery, %        | 102.9    |            |
| Plasma from WB 50ug/mL Blood Spike |                     |                  |        |       |     |                     |                            | HCT corr |            |
| Dilution                           | Theo. Conc. (ng/mL) | Signal           | Avg    | Stdev | CV  | Calc. Conc. (ng/mL) | Sample Calc. Conc. (ng/mL) | ug/mL    |            |
| 500x                               | 100                 | 292539           | 277836 | 20793 | 7.5 | 168.7<br>3          | 84367                      | 84       | 40<br>50.6 |

|       |    |                             |        |     |     |       |                           |      |        |
|-------|----|-----------------------------|--------|-----|-----|-------|---------------------------|------|--------|
| 2000x | 25 | 263133                      |        |     |     |       |                           |      |        |
|       |    | 143938                      | 143791 | 208 | 0.1 | 44.65 | 89294                     | 89   | 53.6   |
|       |    | 143644                      |        |     |     |       |                           |      |        |
|       |    |                             |        |     |     |       | average spike recovery, % |      | 52.1   |
|       |    |                             |        |     |     |       |                           |      | 60.4   |
|       |    |                             |        |     |     |       |                           | 40   | hct, % |
|       |    |                             |        |     |     |       |                           | 13.1 | blood  |
|       |    | Spiked Plasma 50ug/mL Spike |        |     |     |       | Endogenous                | 22   | plasma |

Recovery of sample when spiked into either Whole blood or plasma is very good... approximately 100%. Although when samples are spiked into whole blood and than plasma is collected from the blood, we see a sample loss of almost 40%. The thought is that some of the CRP is binding to the red bloods and other debris during centrifugation and is lost with the RBCs.

## 12. Calibration and Validation

Figure 5: Calibration of CRP cartridge lot using Inflammation2\_5k\_10K protocol.



### Calibration Algorithm for CRP

S5000 = Average corrected signals 5000-fold dilution

$C5000 = 10^{(a+b*(\text{Log } S5000)+c*(\text{Log } S5000)^2+d*(\text{Log } S5000)^3)}$

S10000 = Average corrected signals tips 3,4

$C10000 = 10^{(a+b*(\text{Log } S10000)+c*(\text{Log } S10000)^2+d*(\text{Log } S10000)^3)}$

$C = \text{Average}(C5000, C10000)$

Figure 6: Calibration verification

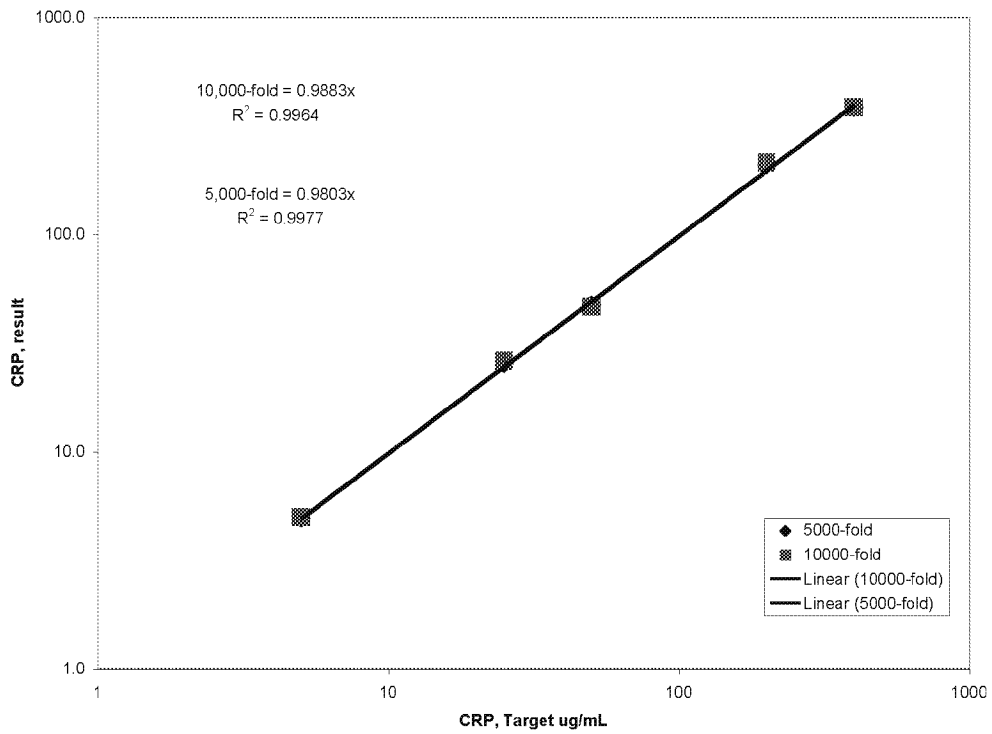


Figure 7: Test of calibration using clinical serum samples

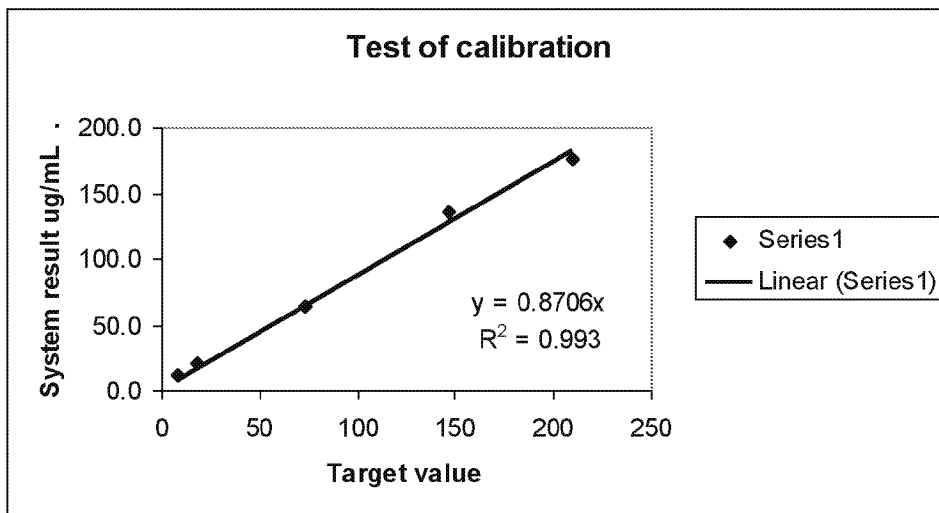
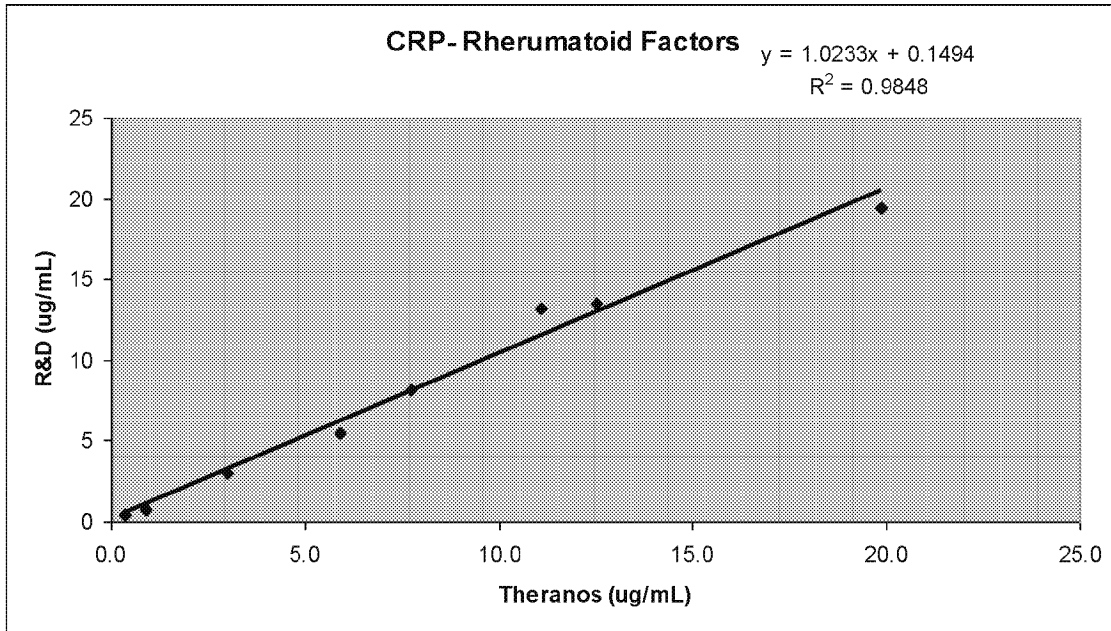


Figure 8: Effects of Rheumatoid factors on the Theranos CRP assay vs. commercial ELISA.



The CRP R&D commercial Elisa and the Theranos system correlate very well indicating that rheumatoid factors do not play a significant interfering role in this assay.

**13. Some of the raw data of the assay development can be found at**

[ [HYPERLINK "Y:\\Common\\Assay Development Reports\\CRP Assay Development Report\\Data" \]](#) ]

**14. Literature**

[ [HYPERLINK "Y:\\Common\\Assay Development Reports\\CRP Assay Development Report\\Literature" \]](#) ]