# Heterophile Antibodies IgM Assay Development Report

**Theranos, Inc.**

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# 1. ASSAY INFORMATION[ TC "ASSAY INFORMATION" \F C \L "2" ]

## 1.1 Assay Specifications[ TC "Assay Specifications" \f C \l "3" ]

Heterophile antibodies are produced by the human immune system in response to EBV infection (infectious mononucleosis).

This assay is designed to qualitatively determine Heterophile antibodies (IgM) in human plasma and serum.

### 1.1.1 Reference Assays [ TC "Reference Assays and Standards" \f C \l "3" ]

The following commercial kits have been used in house as predicate methods:

- Genzyme OSOM Mono Test (Cat# GZM-145)
- Status Mono (Life Sign LLC) (Cat# 84M30)

(Qualitative Detection of Infectious Mononucleosis Heterophile Antibodies in Whole Blood, Serum or Plasma)

### 1.1.2 Materials and Methods[ TC "Materials and Methods" \f C \l "1" ]

Heterophile antigen coated surface serves as the capture surface for the Anti-Heterophile antibody assay. The sample (plasma or serum) is diluted and then incubated on the capture surface for 10 minutes, the surface is washed, and then an alkaline phosphatase(AP)-labeled anti-human IgM antibody is incubated on the surface for 10 minutes. After the detection antibody incubation, another washing cycle is performed and the alkaline phosphatase substrate is incubated on the surface for 10 minutes, and the resulting chemiluminescence is read in Relative Light Units (RLU).

**Table [ SEQ Table * ARABIC ]: Materials**

| Name | Supplier | Catalog # |
|---|---|---|
| Carbonate-Bicarbonate buffer | Sigma | C3041 |
| Alkaline Phosphatase Labeling Kit | Dojindo | LK13-10 |
| StabilZyme AP | Surmodics | SA01-1000 |
| Blocking Buffer<br>(3% BSA in TBS, 0.05% Sodium Azide) | Sigma (BSA, Fraction V, 99% Pure) | A3059-500G |
| In house Substrate | Theranos | |
| StatusFirst Mono Controls | Life Sign | 200014 |

**Table [ SEQ Table * ARABIC ]: Antigens**

| Antigen # | Vendor | Product Code | Description |
|---|---|---|---|
| 1 | East Coast Bio | L501L | Heterophile antigen |
| 2 | Fitzgerald | 30-AH71 | Mononucleosis protein antigen |

**Table [ SEQ Table * ARABIC ]: Detection Antibodies**

| DAb # | Supplier | Catalog # | Description |
|---|---|---|---|
| 1 | AbD Serotec | 5278-5159 | Mouse Anti Human IgM |
| 2 | Novus | NB7436 | Human IgM Antibody |
| 3 | Novus | NB500-468 | Human IgM, Fc Fragment Antibody (CH2) |

# 2 ASSAY DEVELOPMENT[ TC "ASSAY OPTIMIZATION" \F C \L "2" ]

## 2.1 Capture Surface: Antigen Screen (MTP)

Clinical samples were screened on the Genzyme and Status mono kits to determine positive and negative Heterophile Antibodies IgM samples. Commercially available antigens were then screened with these positive and negative samples on a microtiter plate (MTP). Each antigen was prepared in Carbonate-Bicarbonate buffer for direct coating on the Nunc-384 well plates. All antigens were coated at 10 ug/ml. A sample dilution of 1:25 was added to the surface followed by wash steps. The detection antibody (D#1, 2, 3) concentration was 100 ng/ml in 3% BSA in TBS blocking buffer. Both Antigens #1 and #2 showed good modulation between positive and negative samples but antigen # 1 had a better modulation and hence Antigen #1 was chosen for further development. Direct coating of raw antigen had much better modulation than the ultraavidin coating of the biotinylated antigen because the biotinylation of the antigen (with SH group) destroyed the antigen structure and hence there was no modulation with positive and negative control with biotinylated antigen. The data is summarized in Table 4 and 5

**Table [ SEQ Table * ARABIC ]: Antigen Coating Methods (MTP)**

| C#1 at 10ug/ml and D# 1 at 100ng/ml | **Avidin Surface** | | **Direct Coat** | |
|---|---|---|---|---|
| **Sample ID** | **MeanValue** | **CV%** | **MeanValue** | **CV%** |
| Negative Control | 7035 | 5 | 12073 | 10 |
| Positive Control | 2905 | 21 | 1045635 | 2.8 |
| | | | | |
| Negative Sample | 575091 | 5 | 83432 | 9 |
| Positive Sample | 423953 | 6 | 1130514 | 6 |
| No Sample* | 737 | 9 | 726 | 19 |
| ***Positive control/negative control*** | 0 | | 87 | |
| ***Positive control/Mean normal*** | 0 | | 13 | |
| ***Mean positive /Mean normal*** | 1 | | 14 | |

*No Sample: Blocking buffer blank with detection antibody and substrate.

**Table [ SEQ Table * ARABIC ]: Capture Surface Screen (MTP)**

| **Antigen #** | **1** | | **2** | |
|---|---|---|---|---|
| **[Antigen], ug/ml** | **10ug/ml** | | **10ug/ml** | |
| **Sample#** | **Mean Value** | **CV%** | **Mean Value** | **CV%** |
| Negative: #1 | 75442 | 9 | 172559 | 3 |
| #2 | 91421 | 9 | 243502 | 9 |
| **Mean Negative** | **83432** | | **208031** | |
| Positive: #3 | 915185 | 11 | 982858 | 11 |
| #4 | 1345842 | 1 | 1410887 | 5 |
| **Mean Positive** | **1130514** | | **1196873** | |
| Negative Control | 12073 | 10 | 26772 | 5 |
| Positive Control | 1045635 | 2.8 | 1182337 | 11 |
| No Sample* | 726 | 19 | | |
| **Positive control/negative control** | 87 | | 44 | |
| **Positive control/ Mean normal** | 13 | | 6 | |
| **Mean positive /Mean normal** | 14 | | 6 | |

*No Sample: Blocking buffer blank with detection antibody and substrate.

## 2.2 Capture Antigen Surface Titration on Theranos System[ TC " Capture Antigen Surface Antigen " \f C \l "1" ]

The direct coat antigen surface was titrated at levels: 10, 5 and 1 µg/mL. Table 6 summarizes the results of Antigen #1 and Detection Antibody # 1 at 100ng/mL. 1 µg/mL provides the best modulation between the pooled positive and pooled normal clinical samples and was finalized as the capture antigen surface concentration.

**Table [ SEQ Table * ARABIC ]: Capture Antigen Surface Titration**

| | 10 µg/mL | | 5 µg/mL | | 1 µg/mL | |
|---|---|---|---|---|---|---|
| **Sample ID** | **Inter-Cartridge RLU** | | | | | |
| | **Mean** | **CV%** | **Mean** | **CV%** | **Mean** | **CV%** |
| **Positive Control** | 214917 | 18 | 190571 | 23 | 166526 | 18 |
| **Pooled Positive** | 2288791 | 9 | 2175271 | 12 | 2024979 | 8 |
| **Pooled normals** | 87461 | 17 | 66967 | 21 | 39811 | 14 |
| **Negative Control** | 2329 | 20 | 1889 | 9 | 1062 | 14 |
| **Positive control/negative control** | 92 | | 101 | | 157 | |
| **Positive control/ Mean normal** | 2 | | 3 | | 4 | |
| **Mean positive /Mean normal** | 26 | | 32 | | 51 | |

## 2.3 Effect of different Detection Antibodies on Theranos System

Antigen #1 was tested with the different detection antibodies. These antigens were screened on the Theranos system at 1 ug/ml direct coat. Three different detection antibody was being tested and the concentration was 100 ng/ml in 3% BSA Blocking buffer. Clinical samples were tested on the above mentioned commercial kits, and then used as the test set on the Theranos system. A sample dilution of 1:25 is manually done and the protocol was run at the 10, 10,10 minutes incubation time.

There was a significant improvement in terms of modulation between the three detection antibodies. Dab # 3 gave the best modulation with low background and was selected for further optimization. Dab # 1 is a possible back-up detection antibody, the data is summarized in Table 7

**Table [ SEQ Table * ARABIC ]: Detection Antibodies on Theranos System**

| | Dab #1 | | Dab # 2 | | Dab # 3 | |
|---|---|---|---|---|---|---|
| **Sample ID** | **Inter-Cartridge RLU** | | | | | |
| | **Mean** | **CV%** | **Mean** | **CV%** | **Mean** | **CV%** |
| **Positive Control** | 213192 | 17 | 1595161 | 18 | 56309 | 19 |
| **Pooled Positive** | 1929907 | 13 | 3490887 | 7 | 1061400 | 25 |
| **Pooled normals** | 26710 | 18 | 286941 | 12 | 7247 | 15 |
| **Negative Control** | 1658 | 11 | 12410 | 26 | 763 | 21 |
| **Positive control/negative control** | 129 | | 129 | | 74 | |
| **Positive control/ Mean normal** | 8 | | 6 | | 8 | |
| **Mean positive /Mean normal** | 72 | | 12 | | 146 | |

## 2.4 Effect of Assay Diluent

Three commercially available blockers (SuperBlock®, StartingBlock™ and SeaBlock) and one in-house blocking buffer were tested as diluents for the assay. Data was compared to the control diluent which was the blocking buffer consisted of 3% BSA and 0.05% sodium azide in TBS. There was a not a lot of difference in modulation between each diluent and hence In house 3% BSA in TBS was chosen for further optimization. The data is summarized in Table 8

**Table [ SEQ Table * ARABIC ]: Effect of Assay Diluent**

| Sample Type | Sample # | In House BB Mean RLU | CV% | Super Block Mean RLU | CV% | Starting Block Mean RLU | CV% | Sea Block Mean RLU | CV% |
|---|---|---|---|---|---|---|---|---|---|
| **Negative Samples** | 1 | 3535 | 15 | 4476 | 18 | 3481 | 24 | 6900 | 23 |
| | 2 | 5489 | 13 | 5026 | 18 | 4227 | 23 | 6187 | 18 |
| | 3 | 2489 | 11 | 3928 | 19 | 4210 | 22 | 5255 | 17 |
| | 4 | 57796 | 5 | 37458 | 20 | 42791 | 12 | 28411 | 14 |
| | 5 | 1442 | 17 | 2084 | 15 | 1845 | 9 | 2730 | 16 |
| | 6 | 1959 | 9 | 9889 | 20 | 10037 | 13 | 11722 | 13 |
| | 7 | 539 | 15 | 2557 | 14 | 2284 | 18 | 3381 | 18 |
| | Mean Negative | **10464** | | **9345** | | **9839** | | **9226** | |
| **Positive Samples** | 8 | 258955 | 24 | 139239 | 20 | 124808 | 28 | 153539 | 26 |
| | 9 | 817865 | 25 | 608016 | 6 | 573463 | 16 | 556823 | 21 |
| | 10 | 1631917 | 31 | 1579826 | 8 | 1714087 | 3 | 1821697 | 5 |
| | 11 | 1536862 | 21 | 1279139 | 17 | 1493682 | 1 | 1468091 | 19 |
| | Mean Positive | **1061400** | | **901555** | | **976510** | | **1000037** | |
| | Modulation | **101** | | **96** | | **99** | | **108** | |

## 2.5 HAMA and Rf Positive Sample Testing

5 HAMA positive and 5Rf positive sera obtained from a commercial source were tested on the Theranos Heterophile Antibody IgM Assay and on the two commercial kits. Out of the 10 samples tested, all were negative for Heterophile antibodies IgM showing that our assay has excellent correlation. Table is summarized in Table 9

**Table [ SEQ Table * ARABIC ]: HAMA and Rf positive sample screen**

| Samples | Inter-Cartridge Mean | CV% | Theranos Assay | Osom Mono Test | Status Mono |
|---|---|---|---|---|---|
| **HAMA positive** | | | | | |
| H2 | 9669 | 19 | NEG | NEG | NEG |
| H4 | 7211 | 11 | NEG | NEG | NEG |
| H5 | 6460 | 13 | NEG | NEG | NEG |
| H6 | 10041 | 22 | NEG | NEG | NEG |
| H7 | 15238 | 13 | NEG | NEG | NEG |
| | | | NEG | NEG | NEG |
| **RF Positive** | | | NEG | NEG | NEG |
| RF 8 | 8062 | 25 | NEG | NEG | NEG |
| RF 9 | 2780 | 14 | NEG | NEG | NEG |
| RF 10 | 3941 | 13 | NEG | NEG | NEG |
| RF 11 | 3788 | 9 | NEG | NEG | NEG |
| RF 12 | 5665 | 12 | NEG | NEG | NEG |
| **Mean Negative+2SD** | **52328** | | | | |

## 2.6 Specificity (Cross Reactivity Sample Testing)

Literature mentioned that Heterophile antibody assay could have cross reactivity with other infectious diseases like rubella, toxoplasma, HBsAg, Hep C, HIV1 and 2. Positive sera or QC controls of various infectious diseases were tested on the Theranos Heterophile Antibody IgM Assay and on the two commercial kits. All Samples came out negative showing that the Heterophile antibody assay has high specificity.

**Table [ SEQ Table * ARABIC ]: Cross reactivity testing with various infectious disease positive samples**

| Samples | Inter-Cartridge Mean | CV% | Theranos Assay | Osom Mono Test | Status Mono |
|---|---|---|---|---|---|
| Virotrol Mumz | 1378 | 19 | NEG | NEG | NEG |
| Hep B Antibody | 5183 | 17 | NEG | NEG | NEG |
| Anti HSV-1 | 2128 | 16 | NEG | NEG | NEG |
| Total Anti HBC | 3652 | 12 | NEG | NEG | NEG |
| HBsAg | 4643 | 5 | NEG | NEG | NEG |
| Hep C Antibody | 756 | 19 | NEG | NEG | NEG |
| Anti Rubella | 2061 | 9 | NEG | NEG | NEG |
| Anti Toxoplasma | 3434 | 20 | NEG | NEG | NEG |
| Anti CMV | 2989 | 19 | NEG | NEG | NEG |
| HIV 1 | 4136 | 23 | NEG | NEG | NEG |
| HIV 2 | 4067 | 15 | NEG | NEG | NEG |
| **Mean Negative+2SD** | **52328** | | | | |

## 2.7 Effect of Detection Conjugate Stabilizer

Two commercial and one in house formulated alkaline phosphatase stabilizers were tested as detection antibody diluents, with the anti-human IgM DAb at 100 ng/mL. The samples were diluted 1:25 into 3% BSA in TBS Blocking Buffer . Signal modulation was best with StabilZyme. Table 11 summarizes the results of running the tests on 20 negative clinical samples and 5 positive clinical samples.

**Table [ SEQ Table * ARABIC ]: Effect of Detection Conjugate Stabilizer**

| Sample Type | Theranos AP Conjugate Stabilizer | | BioStab | | StabilZyme | |
|---|---|---|---|---|---|---|
| **Negative Samples** | Mean RLU | CV% | Mean RLU | CV % | Mean RLU | CV % |

| | | | | | | |
|---|---|---|---|---|---|---|
| 1 | 2842 | 14 | 5577 | 20 | 1559 | 12 |
| 2 | 5503 | 20 | 9237 | 15 | 2425 | 13 |
| 3 | 3005 | 11 | 5538 | 23 | 1764 | 17 |
| 4 | 60102 | 6 | 78745 | 7 | 29791 | 9 |
| 5 | 4859 | 9 | 5783 | 19 | 3625 | 18 |
| 6 | 23567 | 17 | 31106 | 14 | 11325 | 10 |
| 7 | 12764 | 13 | 22162 | 17 | 4895 | 11 |
| 8 | 13314 | 18 | 17406 | 14 | 6448 | 6 |
| 9 | 3597 | 16 | 6705 | 8 | 2067 | 13 |
| 10 | 14781 | 9 | 18039 | 16 | 7255 | 15 |
| 11 | 11776 | 6 | 6039 | 19 | 2343 | 4 |
| 12 | 7599 | 12 | 10479 | 11 | 4252 | 6 |
| 13 | 3107 | 19 | 6647 | 9 | 2374 | 12 |
| 14 | 43749 | 9 | 50904 | 11 | 18927 | 5 |
| 15 | 1648 | 9 | 3620 | 20 | 952 | 15 |
| 16 | 4539 | 17 | 6519 | 13 | 2088 | 19 |
| 17 | 2239 | 14 | 4489 | 14 | 1098 | 11 |
| 18 | 21360 | 11 | 30756 | 9 | 8845 | 8 |
| 19 | 9370 | 12 | 14549 | 10 | 3582 | 17 |
| 20 | 5661 | 4 | 11364 | 12 | 2195 | 18 |
| **Mean Negative** | **12769** | | **17283** | | **5890** | |
| **Positive Samples** | Mean RLU | CV% | Mean RLU | CV % | Mean RLU | CV % |
| 1 | 273650 | 10 | 326918 | 7 | 123846 | 11 |
| 2 | 371538 | 7 | 529704 | 8 | 208698 | 2 |
| 3 | 871938 | 10 | 1019256 | 3 | 519362 | 3 |
| 4 | 1997198 | 18 | 2233970 | 5 | 1655136 | 5 |
| 5 | 1722123 | 8 | 1805897 | 13 | 1232998 | 14 |
| **Mean Positive** | **1047289.267** | | **1183149.01** | | **748008** | |
| **Mean Negative +2 SD** | **42765** | | **54793** | | **20096** | |
| **Modulation (Mean Pos/ Mean Neg+2SD)** | **24** | | **22** | | **37** | |

## 2.8 Detection antibody Titration

The AP conjugated detection antibody was titrated in StabilZyme. The best modulation between the positive and negative control was achieved with 25 ng/mL of the anti-IgM Dab. Data is summarized in Table 12

**Table [ SEQ Table * ARABIC ]: Detection Conjugate Titration**

| Sample Type | 25ng/ml | | 50ng/ml | | 100ng/ml | |
|---|---|---|---|---|---|---|
| **Negative Samples** | Mean RLU | CV% | Mean RLU | CV % | Mean RLU | CV% |
| 1 | 913 | 20 | 1828 | 21 | 3066 | 11 |
| 2 | 14572 | 5 | 31144 | 8 | 52669 | 10 |
| 3 | 4044 | 9 | 8033 | 7 | 13339 | 9 |
| 4 | 13056 | 6 | 26847 | 6 | 45922 | 7 |
| 5 | 501 | 20 | 1045 | 35 | 1184 | 15 |
| 6 | 561 | 14 | 1265 | 19 | 2074 | 16 |
| **Mean Negative** | **5608** | | **11694** | | **19709** | |
| **Positive Samples** | Mean RLU | CV% | Mean RLU | CV % | Mean RLU | CV% |
| 1 | 67356 | 14 | 135858 | 18 | 237651 | 11 |
| 2 | 268678 | 8 | 510962 | 12 | 806501 | 7 |
| 3 | 949499 | 6 | 1520675 | 10 | 2035020 | 10 |
| **Mean Positive** | **428511** | | **722498** | | **1026390** | |
| **Mean Negative +2 SD** | **18226** | | **39129** | | **66573** | |
| **Modulation (Mean Pos/ Mean Neg+2SD)** | **24** | | **18** | | **15** | |

## 2.9 Effect of Sample Dilution[ TC “Effect of Sample dilution" \f C \l "1" ]

The effect of sample dilution was tested with final sample dilution factors of 1:25, 1:50 and 1:100 PSW into 3% BSA in TBS blocking buffer. Modulation between pooled positive and negative sera was best at 100 fold sample dilution. However, 50 fold sample dilution is also reasonably good. We can observe of a greater reduction in the signal from negative samples compared to the reduction in signal from the positive samples. Results are summarized in Table 13.

**Table [ SEQ Table * ARABIC ]: Effect of sample dilution**

| **Sample Type** | **25x_PSW** | | **50x_PSW** | | **100x_PSW** | |
|---|---|---|---|---|---|---|
| **Negative Samples** | Mean RLU | CV% | Mean RLU | CV% | Mean RLU | CV% |
| 1 | 1444 | 22 | 660 | 18 | 485 | 12 |
| 2 | 13720 | 8 | 6640 | 24 | 3475 | 7 |
| 3 | 3018 | 14 | 1924 | 3 | 1315 | 5 |
| 4 | 12214 | 17 | 5118 | 16 | 3934 | 8 |
| 5 | 570 | 23 | 387 | 21 | 328 | 15 |
| 6 | 537 | 25 | 338 | 24 | 363 | 12 |
| Mean Negative | **5250** | | **2511** | | **1650** | |
| **Positive Samples** | Mean RLU | CV% | Mean RLU | CV% | Mean RLU | CV% |
| 1 | 51798 | 15 | 24070 | 5 | 12211 | 11 |
| 2 | 245055 | 7 | 132160 | 8 | 67462 | 5 |
| 3 | 889851 | 13 | 868038 | 16 | 666996 | 20 |
| **Mean Positive** | **395568** | | **341423** | | **248890** | |
| **Mean Negative +2 SD** | **17377** | | **7940** | | **4927** | |
| **Modulation (Mean Pos/ Mean Neg+2SD)** | **23** | | **43** | | **51** | |

## 2.10 Effect of changing reagent incubation time [ TC "Effect of changing reagent incubation time" \f C \l "1" ]

The effect of shorter reagent incubation times was tested with sample, detection conjugate and substrate incubation times respectively of 10, 10, 10 and 5, 5, 5 minutes. Eventhough 5,5,5 gave a better modulation than 10, 10, 10 ; it was not chosen because it gave a higher CV%. However, in case of multiplex, 5, 5, 5 can also be an option. There was not much of Assay modulation between the two incubation time and 10, 10, 10 minute incubation protocol was chosen as the final condition (lover CV%).

**Table [ SEQ Table * ARABIC ]: Effect of reagent incubation time**

| **Sample Type** | **10, 10, 10** | | **5, 5, 5** | |
|---|---|---|---|---|
| **Negative Samples** | Mean RLU | CV% | Mean RLU | CV% |
| 1 | 485 | 12 | 310 | 36 |
| 2 | 3475 | 7 | 1005 | 15 |
| 3 | 1315 | 5 | 359 | 14 |
| 4 | 3934 | 8 | 1101 | 4 |
| 5 | 328 | 15 | 224 | 35 |
| 6 | 363 | 12 | 238 | 20 |
| Mean Negative | **1650** | | **539** | |
| **Positive Samples** | Mean RLU | CV% | Mean RLU | CV% |
| 1 | 12211 | 11 | 3462 | 14 |
| 2 | 67462 | 5 | 14914 | 11 |
| 3 | 666996 | 20 | 262970 | 41 |
| **Mean Positive** | **248890** | | **93782** | |
| **Mean Negative +2 SD** | **4927** | | **1343** | |
| **Modulation (Mean Pos/ Mean Neg+2SD)** | **51** | | **70** | |

## 2.11 Clinical Sample Correlation and Cut off Determination

Normal donor plasma (N=40) were obtained and tested in the 2 commercial strip kits and in the Theranos System. The Theranos cutoff value was determined by taking the mean RLU of the normal samples plus 5 times the standard deviation of the 40 normal samples (Table 15). The sample RLU divided by the cutoff value yields the Antibody Index. The following criteria was applied to categorize the result as positive (red), negative (green) or borderline (yellow).

Ab Index > 1.1
Ab Index > 0.9, < 1.1
Ab Index < 0.9

**Table [ SEQ Table * ARABIC ]: Heterophile antibody assay : Cut off Determination**

| Samples | Inter-Cartridge Mean | CV% | Ab Index |
|---|---|---|---|
| 1 | 1309 | 15 | 0.14 |
| 2 | 926 | 10 | 0.10 |
| 3 | 980 | 8 | 0.11 |
| 4 | 458 | 8 | 0.05 |
| 5 | 689 | 9 | 0.08 |
| 6 | 1274 | 12 | 0.14 |
| 7 | 832 | 16 | 0.09 |
| 8 | 1150 | 11 | 0.13 |
| 9 | 893 | 22 | 0.10 |
| 10 | 4029 | 21 | 0.44 |
| 11 | 1128 | 24 | 0.12 |
| 12 | 1012 | 22 | 0.11 |
| 13 | 839 | 14 | 0.09 |
| 14 | 584 | 16 | 0.06 |
| 15 | 788 | 17 | 0.09 |
| 16 | 487 | 21 | 0.05 |
| 17 | 3875 | 15 | 0.42 |
| 18 | 345 | 12 | 0.04 |
| 19 | 1134 | 14 | 0.12 |
| 20 | 1048 | 19 | 0.11 |
| 21 | 981 | 19 | 0.11 |
| 22 | 1813 | 12 | 0.20 |
| 23 | 1487 | 20 | 0.16 |
| 24 | 436 | 16 | 0.05 |
| 25 | 1251 | 6 | 0.14 |
| 26 | 934 | 22 | 0.10 |
| 27 | 1130 | 16 | 0.12 |
| 28 | 1855 | 16 | 0.20 |

| | | | |
|---|---|---|---|
| 29 | 346 | 21 | 0.04 |
| 30 | 760 | 21 | 0.08 |
| 31 | 1383 | 17 | 0.15 |
| 32 | 926 | 10 | 0.10 |
| 33 | 980 | 8 | 0.11 |
| 34 | 1347 | 13 | 0.15 |
| 35 | 1750 | 22 | 0.19 |
| 36 | 2286 | 17 | 0.25 |
| 37 | 1529 | 12 | 0.17 |
| 38 | 1639 | 24 | 0.18 |
| 39 | 8587 | 11 | 0.93 |
| 40 | 5487 | 4 | 0.60 |
| MEAN | 1517 | | |
| CUT OFF | 9186 | | |

Out of the 40 normals tested 39 were negative and 1 was equivocal on the Theranos assay based on the aforementioned cutoff computation. These same samples were all negative except for 1 on one of the 2 Strip kits and the data showed excellent correlation with the Theranos result (Table 16).

For Clinical sample correlation, a total of 58 clinical samples were tested on Theranos assay and on 2 FDA approved commercial kits and the results are summarized in table 16. Excellent correlation was seen for all 58 samples.

**Table [ SEQ Table * ARABIC ]: Clinical Samples on Theranos vs. Commercial kits**

| Sample ID | Inter-Cartridge Mean | CV% | Theranos Ab Index | Osom Mono Test | Status Mono |
|---|---|---|---|---|---|
| 1 | 1309 | 15 | 0.14 | NEG | NEG |
| 2 | 926 | 10 | 0.10 | NEG | NEG |
| 3 | 980 | 8 | 0.11 | NEG | NEG |
| 4 | 458 | 8 | 0.05 | NEG | NEG |
| 5 | 689 | 9 | 0.08 | NEG | NEG |
| 6 | 1274 | 12 | 0.14 | NEG | NEG |
| 7 | 832 | 16 | 0.09 | NEG | NEG |
| 8 | 1150 | 11 | 0.13 | NEG | NEG |
| 9 | 893 | 22 | 0.10 | NEG | NEG |
| 10 | 4029 | 21 | 0.44 | NEG | NEG |
| 11 | 1128 | 24 | 0.12 | NEG | NEG |
| 12 | 1012 | 22 | 0.11 | NEG | NEG |
| 13 | 839 | 14 | 0.09 | NEG | NEG |
| 14 | 584 | 16 | 0.06 | NEG | NEG |
| 15 | 788 | 17 | 0.09 | NEG | NEG |
| 16 | 487 | 21 | 0.05 | NEG | NEG |
| 17 | 3875 | 15 | 0.42 | NEG | NEG |
| 18 | 345 | 12 | 0.04 | NEG | NEG |
| 19 | 1134 | 14 | 0.12 | NEG | NEG |
| 20 | 1048 | 19 | 0.11 | NEG | NEG |
| 21 | 981 | 19 | 0.11 | NEG | NEG |
| 22 | 1813 | 12 | 0.20 | NEG | NEG |
| 23 | 1487 | 20 | 0.16 | NEG | NEG |
| 24 | 436 | 16 | 0.05 | NEG | NEG |
| 25 | 1251 | 6 | 0.14 | NEG | NEG |
| 26 | 934 | 22 | 0.10 | NEG | NEG |
| 27 | 1130 | 16 | 0.12 | NEG | NEG |
| 28 | 1855 | 16 | 0.20 | NEG | NEG |
| 29 | 346 | 21 | 0.04 | NEG | NEG |
| 30 | 760 | 21 | 0.08 | NEG | NEG |
| 31 | 1383 | 17 | 0.15 | NEG | NEG |
| 32 | 926 | 10 | 0.10 | NEG | NEG |
| 33 | 980 | 8 | 0.11 | NEG | NEG |
| 34 | 1347 | 13 | 0.15 | NEG | NEG |

| | | | | | |
|---|---|---|---|---|---|
| 35 | 1750 | 22 | 0.19 | NEG | NEG |
| 36 | 2286 | 17 | 0.25 | NEG | NEG |
| 37 | 1529 | 12 | 0.17 | NEG | NEG |
| 38 | 1639 | 24 | 0.18 | NEG | NEG |
| 39 | 8587 | 11 | 0.93 | NEG | NEG |
| 40 | 5487 | 4 | 0.60 | POS | NEG |
| 41 | 320948 | 8 | 34.94 | POS | POS |
| 42 | 88399 | 12 | 9.62 | POS | POS |
| 43 | 143173 | 11 | 15.59 | POS | POS |
| 44 | 49020 | 13 | 5.34 | POS | POS |
| 45 | 31491 | 22 | 3.43 | POS | POS |
| 46 | 14957 | 8 | 1.63 | POS | POS |
| 47 | 15771 | 6 | 1.72 | POS | POS |
| 48 | 119201 | 10 | 12.98 | POS | POS |
| 49 | 156506 | 10 | 17.04 | POS | POS |
| 50 | 23409 | 13 | 2.55 | POS | POS |
| 51 | 144954 | 21 | 15.78 | POS | POS |
| 52 | 121523 | 11 | 13.23 | POS | POS |
| 53 | 11028 | 9 | 1.20 | POS | POS |
| 54 | 17138 | 21 | 1.87 | POS | POS |
| 55 | 32667 | 4 | 3.56 | POS | POS |
| 56 | 74602 | 7 | 8.12 | POS | POS |
| 57 | 986040 | 3 | 107.35 | POS | POS |
| 58 | 502621 | 9 | 54.72 | POS | POS |

## 2.12 Stability Studies

Stability monitoring is ongoing for the the assay reagents stored at 4°C and protected from light for 12 weeks