**Supplement Table 1. Calibration curve parameters for all three assays**

1. free-AMB

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Run Number | Slope | Intercept | R-Squared | LLOQ | ULOQ | Regression |
| 1 | 0.000118 | -0.000029 | 0.9993 | 10.0 | 3000 | Resp. = Slope \* Conc. + Intercept |
| 2 | 0.000134 | -0.000057 | 0.9940 | 10.0 | 3000 | Resp. = Slope \* Conc. + Intercept |
| 3 | 0.000049 | -0.000006 | 0.9965 | 10.0 | 3000 | Resp. = Slope \* Conc. + Intercept |
|  |  |  |  |  |  |  |
| Mean | 0.000100 | -0.000031 | 0.9966 |  |  |  |
| S.D. | 0.000045 | 0.000026 | 0.0027 |  |  |  |
| %CV | 45.0 | -83.9 | 0.3 |  |  |  |
| n | 3 | 3 | 3 |  |  |  |

1. liposome-AMB

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Run Number | Slope | Intercept | R-Squared | LLOQ | ULOQ | Regression |
| 1 | 0.000051 | -0.000469 | 0.9954 | 100 | 50000 | Resp. = Slope \* Conc. + Intercept |
| 2 | 0.000022 | -0.000529 | 0.9949 | 100 | 50000 | Resp. = Slope \* Conc. + Intercept |
| 3 | 0.000050 | -0.000942 | 0.9916 | 100 | 50000 | Resp. = Slope \* Conc. + Intercept |
|  |  |  |  |  |  |  |
| Mean | 0.000041 | -0.000647 | 0.9940 |  |  |  |
| S.D. | 0.000016 | 0.000258 | 0.0021 |  |  |  |
| %CV | 39.0 | -39.9 | 0.2 |  |  |  |
| n | 3 | 3 | 3 |  |  |  |

1. total-AMB

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Run Number | Slope | Intercept | R-Squared | LLOQ | ULOQ | Regression |
| 1 | 0.000095 | -0.000840 | 0.9973 | 100 | 50000 | Resp. = Slope \* Conc. + Intercept |
| 2 | 0.000037 | -0.000679 | 0.9967 | 100 | 50000 | Resp. = Slope \* Conc. + Intercept |
| 3 | 0.000107 | -0.000849 | 0.9956 | 100 | 50000 | Resp. = Slope \* Conc. + Intercept |
|  |  |  |  |  |  |  |
| Mean | 0.000080 | -0.000789 | 0.9965 |  |  |  |
| S.D. | 0.000037 | 0.000096 | 0.0009 |  |  |  |
| %CV | 46.3 | -12.2 | 0.1 |  |  |  |
| n | 3 | 3 | 3 |  |  |  |

Supplement Table 2. Back-Calculated Concentrations of Calibration Standards for All Three Assays (ng/mL)

1. Back-Calculated Concentrations of Calibration Standards for free-AMB (Linear weighted 1/x2)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Run Number | 10.0 | 20.0 | 50.0 | 150 | 500 | 1500 | 2700 | 3000 |
| 1 | 10.4 | 19.6 | 49.2 | 152 | 512 | 1530 | 2700 | 3020 |
|  | 9.67 | 20.6 | 48.2 | 153 | 495 | 1510 | 2610 | 2970 |
| 2 | 10.7 | 19.1 | 53.2 | 137 | 545 | 1620 | 2810 | 3180 |
|  | 9.72 | 19.2 | 49.3 | 148 | 516 | 1520 | 2410 | 2670 |
| 3 | 10.7 | \*\*15.1 | 43.7 | 143 | 503 | 1580 | 2660 | 3080 |
|  | 9.51 | 19.9 | 50.3 | 159 | 520 | 1510 | 2760 | 2840 |
|  |  |  |  |  |  |  |  |  |
| Mean | 10.1 | 19.7 | 49.0 | 149 | 515 | 1550 | 2660 | 2960 |
| S.D. | 0.545 | 0.606 | 3.10 | 7.81 | 17.2 | 45.1 | 141 | 182 |
| %CV | 5.4 | 3.1 | 6.3 | 5.2 | 3.3 | 2.9 | 5.3 | 6.1 |
| %Bias | 1.0 | -1.5 | -2.0 | -0.7 | 3.0 | 3.3 | -1.5 | -1.3 |
| N | 6 | 5 | 6 | 6 | 6 | 6 | 6 | 6 |

\*\*Exceed acceptance criteria

(2) Back-Calculated Concentrations of Calibration Standards for liposome-AMB (Linear weighted 1/x2)

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Run Number |  | 50.0 | 100 | 300 | 1500 | 5000 | 15000 | 45000 | 50000 |
| 1 |  | \*No Value | 211 | 522 | 1540 | 6370 | 16600 | 45400 | 52000 |
|  |  | 101 | 189 | 449 | 1460 | 5960 | 13400 | 43600 | 48700 |
| 2 |  | 103 | 186 | 520 | 1610 | 6150 | \*\*18100 | \*\*57700 | 53100 |
|  |  | 103 | 197 | 456 | 1320 | 5720 | 14900 | 47200 | 52200 |
| 3 |  | 107 | 213 | 530 | 1550 | 5760 | 14600 | 49700 | 56300 |
|  |  | 88.4 | \*\*159 | \*\*369 | \*\*1210 | 5350 | 14700 | 41200 | 46400 |
|  |  |  |  |  |  |  |  |  |  |
| Mean |  | 100 | 199 | 495 | 1500 | 5890 | 14800 | 45400 | 51500 |
| S.D. |  | 7.10 | 12.4 | 39.4 | 112 | 358 | 1150 | 3260 | 3470 |
| %CV |  | 7.1 | 6.2 | 8.0 | 7.5 | 6.1 | 7.8 | 7.2 | 6.7 |
| %Bias |  | 0.0 | -0.5 | -1.0 | 0.0 | -1.8 | -1.3 | 0.9 | 3.0 |
| N |  | 5 | 5 | 5 | 5 | 6 | 5 | 5 | 6 |

\*No internal standard response

\*\*Exceed acceptance criteria

(3) Back-Calculated Concentrations of Calibration Standards for total-AMB (Linear weighted 1/x2)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Run Number | 100 | 200 | 1000 | 5000 | 15000 | 30000 | 45000 | 50000 |
| 1 | 96.9 | 205 | 1010 | 5180 | 16200 | 30100 | 45700 | 51300 |
|  | 106 | 182 | 1010 | 4920 | 14200 | 30600 | 41800 | 48500 |
| 2 | 107 | 183 | 1030 | 5170 | 16000 | 31500 | 45400 | 51100 |
|  | 97.4 | 200 | 983 | 4890 | 15500 | 29100 | 39900 | 48800 |
| 3 | 110 | 201 | 1060 | 5270 | 16100 | 31200 | 47100 | 50000 |
|  | 95.4 | 177 | 981 | 4760 | 14900 | 28200 | 44700 | 46500 |
|  |  |  |  |  |  |  |  |  |
| Mean | 102 | 191 | 1010 | 5030 | 15500 | 30100 | 44100 | 49400 |
| S.D. | 6.26 | 12.0 | 29.8 | 202 | 794 | 1270 | 2700 | 1810 |
| %CV | 6.1 | 6.3 | 3.0 | 4.0 | 5.1 | 4.2 | 6.1 | 3.7 |
| %Bias | 2.0 | -4.5 | 1.0 | 0.6 | 3.3 | 0.3 | -2.0 | -1.2 |
| n | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |

**Supplement Table 3. Matrix Factors for All Three Assays**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Free-AMB | | Liposome-AMB | | Total-AMB | |
|  | Low | High | Low | High | Low | High |
| Lot 1 | 0.949 | 0.891 | 1.11 | 1.02 | 0.946 | 0.998 |
| Lot 2 | 0.910 | 0.890 | 1.03 | 1.02 | 0.920 | 1.03 |
| Lot 3 | 0.883 | 0.915 | 1.07 | 1.00 | 0.948 | 1.03 |
| Lot 4 | 0.885 | 0.904 | 1.04 | 0.957 | 0.996 | 0.999 |
| Lot 5 | 0.897 | 0.903 | 1.01 | 1.02 | 0.932 | 0.998 |
| Lot 6 | 0.901 | 0.903 | 1.04 | 1.01 | 0.936 | 0.985 |
| Mean | 0.904 | 0.901 | 1.05 | 1.00 | 0.946 | 1.01 |
| STDEV | 0.0242 | 0.00932 | 0.0352 | 0.0246 | 0.0264 | 0.0118 |
| %CV | 2.7 | 1.0 | 3.4 | 2.5 | 2.8 | 1.9 |

**\***Normalized Matrix Factor = Analyte matrix factor/IS matric factor;

Analyte Matrix Factor = (Peak area ratio of analyte in the presence of matrix) / (average peak area of analyte in the absence of matrix (Neat samples));

IS Matrix Factor = (Peak area ratio of IS in the presence of matrix) / (average peak area of IS in the absence of matrix (Neat samples)).

Note: The post-spiked sample for encapsulated and total assays is from free API.

**Supplement Table 4. Whole Blood Stability of liposome-AMB in liposome-AMB Assay**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| No | LQC (300 ng/mL) | | | | HQC (40000 ng/mL) | | | |
| Control | Ice bath 0.5hr | Ice bath 1hr | Ice bath 2hr | Control | Ice bath 0.5hr | Ice bath 1hr | Ice bath 2hr |
| 1 | 0.029686 | 0.030446 | 0.028086 | 0.030926 | 4.625864 | 4.524350 | 4.401941 | 4.617610 |
| 2 | 0.032561 | 0.029333 | 0.030664 | 0.031692 | 4.335374 | 4.149938 | 4.460687 | 4.345031 |
| 3 | 0.031009 | 0.030501 | 0.031767 | 0.027654 | 4.569392 | 4.534661 | 4.408298 | 4.091420 |
| 4 | 0.030202 | 0.030234 | 0.028627 | 0.027924 | 4.384823 | 4.528516 | 4.584622 | 4.068368 |
| 5 | 0.030447 | 0.028402 | 0.031283 | 0.029193 | 4.557911 | 4.513460 | 4.405312 | 3.900770 |
| 6 | 0.030669 | 0.032253 | 0.031947 | 0.030467 | 4.176771 | 4.318087 | 4.111351 | 4.384325 |
| Mean | 0.030762 | 0.030195 | 0.030396 | 0.029643 | 4.441689 | 4.428169 | 4.395369 | 4.234587 |
| SD | 0.000988 | 0.00129 | 0.00165 | 0.00165 | 0.172 | 0.160 | 0.156 | 0.261 |
| %CV | 3.2 | 4.3 | 5.4 | 5.6 | 3.9 | 3.6 | 3.5 | 6.2 |
| %Bias | N/A | -1.8 | -1.2 | -3.6 | N/A | -0.3 | -1.0 | -4.7 |
| n | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |

Note: Test samples were prepared by spiking liposomal injection solution into whole blood. The samples were set in ice bath up to 2 hours. The plasma samples were harvested and treated with cryoprotectant. The samples were then extracted and analyzed using liposome-AMB method and reported as the concentration of liposome-AMB.

**Supplement Table 5. Dilution Integrity (DF10) Evaluation for All Three Assays**

|  |  |  |  |
| --- | --- | --- | --- |
| Sample No. | free-AMB | liposome-AMB | total-AMB |
| DQC (12000 ng/mL) | DQC (200000 ng/mL) | DQC (200000 ng/mL) |
| 1 | 10700 | 190000 | 205000 |
| 2 | 10400 | 196000 | 206000 |
| 3 | 10500 | 205000 | 200000 |
| 4 | 10400 | 198000 | 195000 |
| 5 | 10400 | 198000 | 198000 |
| 6 | 11000 | 195000 | 185000 |
| Mean | 10600 | 197000 | 198000 |
| SD | 242 | 4900 | 7680 |
| %CV | 2.3 | 2.5 | 3.9 |
| %Theoretical | 88.3 | 98.5 | 99.0 |
| %Bias | -11.7 | -1.5 | -1.0 |
| n | 6 | 6 | 6 |

|  |
| --- |
|  |

**Supplement Table 6. Autosampler Reinjection Reproducibility Stability for All Three Assays**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| No | total-AMB  (4°C, 181.2 hr) | | | | free-AMB  (4°C, 168.5 hr) | | | | liposome-AMB  (4°C, 170.6 hr) | | | |
| LQC  300 ng/mL | L-MQC  2500 ng/mL | MQC  20000 ng/mL | HQC  40000 ng/mL | LQC  30.0 ng/mL | L-MQC  200 ng/mL | MQC  1200 ng/mL | HQC  2400 ng/mL | LQC  300 ng/mL | L-MQC  2500 ng/mL | MQC  20000 ng/mL | HQC  40000 ng/mL |
| 1 | 278 | 2460 | 20500 | 38900 | 30.7 | 222 | 1350 | 2400 | 322 | 2630 | 20600 | 41700 |
| 2 | 303 | 2360 | 19800 | 41700 | 31.5 | 219 | 1120 | 2260 | 296 | 2270 | 19800 | 39300 |
| 3 | 286 | 2550 | 21200 | 37500 | 29.8 | 217 | 1290 | 2290 | 297 | 2390 | ~23100 | 40700 |
| 4 | 274 | 2470 | 19300 | 39100 | 30.1 | 215 | 1260 | 2240 | 325 | 2560 | 20500 | 40900 |
| 5 | 307 | 2430 | 19800 | 37400 | 31.9 | 210 | 1280 | 2200 | 300 | 2450 | 17900 | 36700 |
| 6 | 286 | 2410 | 21300 | 38300 | 30.7 | 201 | 1250 | 2220 | 263 | 2470 | 19100 | 43700 |
| Mean | 289 | 2450 | 20300 | 38800 | 30.8 | 214 | 1260 | 2270 | 301 | 2460 | 20200 | 40500 |
| SD | 13.3 | 64.1 | 818 | 1580 | 0.801 | 7.54 | 76.3 | 71.7 | 22.4 | 127 | 1750 | 2360 |
| %CV | 4.6 | 2.6 | 4.0 | 4.1 | 2.6 | 3.5 | 6.1 | 3.2 | 7.4 | 5.2 | 8.7 | 5.8 |
| %Theoretical | 96.3 | 98.0 | 101.5 | 97.0 | 102.7 | 107.0 | 105.0 | 94.6 | 100.3 | 98.4 | 101.0 | 101.3 |
| %Bias | -3.7 | -2.0 | 1.5 | -3.0 | 2.7 | 7.0 | 5.0 | -5.4 | 0.3 | -1.6 | 1.0 | 1.3 |
| n | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |

**Supplement Table 7. Hemolysis Effect for All Three Assays**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| No. | free-AMB | | liposome-AMB | | total-AMB | |
| LQC  30.0 ng/mL | HQC  2400 ng/mL | LQC  300 ng/mL | HQC  40000 ng/mL | LQC  300 ng/mL | HQC  40000 ng/mL |
| 1 | 29.9 | 2300 | 331 | 40900 | 320 | 40600 |
| 2 | 30.5 | 2250 | 287 | 42000 | 278 | 40300 |
| 3 | 29.7 | 2440 | 283 | 43500 | 294 | 40300 |
| 4 | 32.0 | 2420 | 320 | 38700 | 271 | 42100 |
| 5 | 28.4 | 2360 | 299 | 40200 | 289 | 40800 |
| 6 | 25.7 | 2420 | 321 | 41500 | 292 | 40800 |
| Mean | 29.4 | 2370 | 307 | 41100 | 291 | 40800 |
| SD | 2.14 | 76.4 | 19.9 | 1630 | 16.9 | 668 |
| %CV | 7.3 | 3.2 | 6.5 | 4.0 | 5.8 | 1.6 |
| %Theoretical | 98.0 | 98.8 | 102.3 | 102.8 | 97.0 | 102.0 |
| %Bias | -2.0 | -1.3 | 2.3 | 2.8 | -3.0 | 2.0 |
| n | 6 | 6 | 6 | 6 | 6 | 6 |

**Supplement Table 8. Hyperlipidemia Effect for All Three Assays**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| No. | free-AMB | | liposome-AMB | | total-AMB | |
| LQC  30.0 ng/mL | HQC  2400 ng/mL | LQC  300 ng/mL | HQC  40000 ng/mL | LQC  300 ng/mL | HQC  40000 ng/mL |
| 1 | 29.9 | 2330 | ~352 | 42400 | 297 | 40700 |
| 2 | 28.9 | 2310 | 308 | 42900 | 285 | 41300 |
| 3 | 31.5 | 2280 | 286 | 41300 | 306 | 42000 |
| 4 | 33.0 | 2330 | 294 | 43000 | 301 | 41700 |
| 5 | 31.9 | 2310 | 327 | 43700 | 286 | 41900 |
| 6 | 34.0 | 2320 | 333 | 46000 | 313 | 41600 |
| Mean | 31.5 | 2310 | 317 | 43200 | 298 | 41500 |
| SD | 1.90 | 18.6 | 25.1 | 1580 | 11.1 | 476 |
| %CV | 6.0 | 0.8 | 7.9 | 3.7 | 3.7 | 1.1 |
| %Theoretical | 105.0 | 96.3 | 105.7 | 108.0 | 99.3 | 103.8 |
| %Bias | 5.0 | -3.8 | 5.7 | 8.0 | -0.7 | 3.8 |
| n | 6 | 6 | 6 | 6 | 6 | 6 |

**Supplement Table 9. Extraction Recovery for all Three Assays**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Low QC | | Med QC | | High QC | |
| %Recovery | %CV | %Recovery | %CV | %Recovery | %CV |
| Free-AMB | 114 | 8.4 | 112 | 10.2 | 107 | 2.6 |
| Liposome-AMB | 96.9 | 9.1 | 105 | 5.4 | 99.3 | 6.2 |
| Total-AMB | 111 | 3.3 | 108 | 1.9 | 108 | 2.6 |

Analyte recovery were calculated from n=6, IS recovery were calculated from n=18.

Note: The post-spiked sample for encapsulated and total assays is from free API.

**Supplement Table 10. Whole Blood Stability of Free-AMB in Analytical QC in Free-AMB Assay**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| No | Cross LQC (30.0 ng/mL) | | | | Cross HQC (2400 ng/mL) | | | |
| Control | Ice bath 0.5hr | Ice bath 1hr | Ice bath 2hr | Control | Ice bath 0.5hr | Ice bath 1hr | Ice bath 2hr |
| 1 | 0.005675 | 0.005096 | 0.004343 | 0.004319 | 0.464580 | 0.423012 | 0.456647 | 0.440610 |
| 2 | 0.005523 | 0.005014 | 0.005053 | 0.004277 | 0.450406 | 0.466500 | 0.461028 | 0.462586 |
| 3 | 0.005714 | 0.004765 | 0.004550 | 0.004167 | 0.452084 | 0.458003 | 0.455851 | 0.436110 |
| 4 | 0.005635 | 0.004866 | 0.004133 | 0.004224 | 0.462962 | 0.464675 | 0.469015 | 0.464334 |
| 5 | 0.005800 | 0.004920 | 0.004986 | 0.004039 | 0.472807 | 0.449008 | 0.449165 | 0.410438 |
| 6 | 0.005664 | 0.004800 | 0.004636 | 0.003844 | 0.471059 | 0.458067 | 0.460511 | 0.415690 |
| Mean | 0.005669 | 0.004910 | 0.004617 | 0.004145 | 0.462316 | 0.453211 | 0.458703 | 0.438295 |
| SD | 0.0000913 | 0.000127 | 0.000358 | 0.000177 | 0.00937 | 0.0160 | 0.00661 | 0.0227 |
| %CV | 1.6 | 2.6 | 7.8 | 4.3 | 2.0 | 3.5 | 1.4 | 5.2 |
| %Bias | N/A | -13.4 | -18.6 | -26.9 | N/A | -2.0 | -0.8 | -5.2 |
| No | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |

Note: Test samples were prepared by spiking free API solution into whole blood. The samples were set in ice bath up to 2 hours. The plasma samples were harvested and treated with cryoprotectant. The samples were then extracted and analyzed using free-AMB method and reported as the ratio of analyte peak area/IS peak area. The %Bias = (Mean peak area ratio of the respective testing time point)/ (Mean peak area ratio of the control).

**Supplement Table 11. Plasma Collection Stability of Free-AMB in Analytical QC in Free-AMB Assay**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| No | LQC (30.0 ng/mL) | | | | HQC (2400 ng/mL) | | | |
| Control | Ice bath 0.5hr | Ice bath 1hr | Ice bath 2hr | Control | Ice bath 0.5hr | Ice bath 1hr | Ice bath 2hr |
| 1 | 0.004355 | 0.003652 | 0.003618 | 0.003765 | 0.325697 | 0.327428 | 0.322035 | 0.325899 |
| 2 | 0.003856 | 0.003797 | 0.003924 | 0.004208 | 0.321850 | 0.319172 | 0.312222 | 0.329896 |
| 3 | 0.004116 | 0.003918 | 0.003931 | 0.004041 | 0.299540 | 0.333071 | 0.323933 | 0.310076 |
| 4 | 0.004011 | 0.003636 | 0.004035 | 0.004023 | 0.320091 | 0.325274 | 0.317702 | 0.321178 |
| 5 | 0.004221 | 0.003868 | 0.004167 | 0.004005 | 0.319781 | 0.324438 | 0.329759 | 0.309511 |
| 6 | 0.003992 | 0.003876 | 0.004186 | 0.003986 | 0.327330 | 0.309678 | 0.313699 | 0.312343 |
| Mean | 0.004092 | 0.003791 | 0.003977 | 0.004005 | 0.319048 | 0.323177 | 0.319892 | 0.318151 |
| SD | 0.000178 | 0.000121 | 0.000208 | 0.000142 | 0.0100 | 0.00800 | 0.00664 | 0.00873 |
| %CV | 4.3 | 3.2 | 5.2 | 3.5 | 3.1 | 2.5 | 2.1 | 2.7 |
| %Bias | N/A | -7.4 | -2.8 | -2.1 | N/A | 1.3 | 0.3 | -0.3 |
| No | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |

Note: Test samples were prepared by spiking liposomal injection solution into plasma. The samples were set in ice bath up to 2 hours. After reaching each time point, cryoprotectant was added into the samples proportionally. The samples were then extracted and analyzed using free-AMB method and reported as the ratio of analyte peak area/IS peak area. The %Bias = (Mean peak area ratio of the respective testing time point)/ (Mean peak area ratio of the control).