

Validation of the Monocyte Activation Test with Three Therapeutic Monoclonal Antibodies

Supplementary Data

Tab. S1: Measured EE/mL concentrations of FLA samples with 3 PBMC lots

| Sample FLA | PBMC lot | Measured EE/mL concentration | | | mean | %CV |
|------------|----------|------------------------------|--------------------|--------------------|--------------------|------|
| | | Repl1 | Repl2 | Repl3 | | |
| 3.13 ng/mL | #1 | 0.056 ^a | 0.052 ^a | 0.045 ^a | 0.051 ^a | 10.9 |
| 6.25 ng/mL | #1 | 0.065 ^a | 0.067 ^a | 0.076 ^a | 0.069 ^a | 8.5 |
| 12.5 ng/mL | #1 | 0.109 | 0.115 | 0.099 | 0.108 | 7.5 |
| 25 ng/mL | #1 | 0.172 | 0.162 | 0.190 | 0.175 | 8.1 |
| 3.13 ng/mL | #2 | 0.041 ^a | 0.037 ^a | 0.035 ^a | 0.038 ^a | 8.1 |
| 6.25 ng/mL | #2 | 0.077 ^a | 0.065 ^a | 0.060 ^a | 0.067 ^a | 13.0 |
| 12.5 ng/mL | #2 | 0.096 | 0.085 | 0.104 | 0.095 | 10.0 |
| 25 ng/mL | #2 | 0.152 | 0.146 | 0.168 | 0.155 | 7.3 |
| 3.13 ng/mL | #3 | 0.041 ^a | 0.044 ^a | 0.043 ^a | 0.043 ^a | 3.6 |
| 6.25 ng/mL | #3 | 0.079 ^a | 0.075 ^a | 0.071 ^a | 0.075 ^a | 5.3 |
| 12.5 ng/mL | #3 | 0.115 | 0.117 | 0.119 | 0.117 | 1.7 |
| 25 ng/mL | #3 | 0.179 | 0.189 | 0.231 | 0.200 | 13.8 |

^a below LOQ of endotoxin standard (0.08 EE/mL), but above overall LOD

Tab.S2: Measured EE/mL concentrations of PAM samples with 3 PBMC lots

| Sample PAM | PBMC lot | Measured EE/mL concentration | | | Mean | %CV |
|-------------|----------|------------------------------|--------------------|------------------------|--------------------|------|
| | | Repl1 | Repl2 | Repl3 | | |
| 0.625 ng/mL | #1 | 0.066 | 0.089 | 0.107 | 0.087 | 23.5 |
| 1.25 ng/mL | #1 | 0.088 | 0.117 | 0.103 | 0.103 | 14.1 |
| 2.5 ng/mL | #1 | 0.134 | 0.168 | 0.133 | 0.145 | 13.7 |
| 5 ng/mL | #1 | 0.487 ^a | 0.401 ^a | 0.562 ^a | 0.483 ^a | 16.7 |
| 0.625 ng/mL | #2 | 0.195 | 0.170 | 0.180 | 0.182 | 6.9 |
| 1.25 ng/mL | #2 | 0.320 | 0.374 ^a | 0.335 ^a | 0.343 ^a | 8.1 |
| 2.5 ng/mL | #2 | 0.531 ^a | 0.548 ^a | 1.187 ^a | 0.755 ^a | 49.5 |
| 5 ng/mL | #2 | Not valid ^b | 0.724 ^a | Not valid ^b | 0.724 ^a | n.d. |
| 0.625 ng/mL | #3 | 0.352 ^a | 0.343 ^a | 0.400 ^a | 0.365 ^a | 8.4 |
| 1.25 ng/mL | #3 | 0.406 ^a | 0.387 ^a | 0.354 ^a | 0.382 ^a | 6.9 |
| 2.5 ng/mL | #3 | 0.457 ^a | 0.488 ^a | 0.440 ^a | 0.462 ^a | 5.3 |
| 5 ng/mL | #3 | 0.580 ^a | 0.626 ^a | 2.355 ^a | 1.187 ^a | 85.2 |

^a above range to adequately measure endotoxin (0.32 EE/mL). n.d.: not determined, only one valid result

^b OD was above highest point of the standard curve

Tab. S3: Measured EE/mL concentrations of PGN samples with 3 PBMC lots

| Sample PGN | PBMC lot | Measured EE/mL concentration | | | mean | %CV |
|------------|----------|------------------------------|--------------------|--------------------|--------------------|------|
| | | Repl1 | Repl2 | Repl3 | | |
| 1.25 µg/mL | #1 | 0.056 ^a | 0.070 ^a | 0.046 ^a | 0.057 ^a | 21.0 |
| 2.5 µg/mL | #1 | 0.058 ^a | 0.096 | 0.065 ^a | 0.073 ^a | 27.7 |
| 5 µg/mL | #1 | 0.085 | 0.091 | 0.069 ^a | 0.082 | 13.9 |
| 7.5 µg/mL | #1 | 0.076 ^a | 0.077 ^a | 0.080 | 0.078 ^a | 2.7 |
| 1.25 µg/mL | #2 | 0.155 | 0.190 | 0.188 | 0.178 | 11.1 |
| 2.5 µg/mL | #2 | 0.194 | 0.228 | 0.222 | 0.215 | 8.5 |
| 5 µg/mL | #2 | 0.219 | 0.212 | 0.248 | 0.226 | 8.4 |
| 7.5 µg/mL | #2 | 0.314 | 0.261 | 0.260 | 0.278 | 11.1 |
| 1.25 µg/mL | #3 | 0.152 | 0.134 | 0.126 | 0.137 | 9.7 |
| 2.5 µg/mL | #3 | 0.198 | 0.203 | 0.179 | 0.193 | 6.5 |
| 5 µg/mL | #3 | 0.215 | 0.209 | 0.239 | 0.221 | 7.2 |
| 7.5 µg/mL | #3 | 0.257 | 0.231 | 0.254 | 0.247 | 5.8 |

^a below LOQ of endotoxin standard (0.08 EE/ml), but above LOD

Tab. S4: Measured EE/mL concentrations of HKSA samples with 3 PBMC lots

| Sample HKSA cells/mL | PBMC lot | Measured EE/mL concentration | | | mean | %CV |
|-----------------------|----------|------------------------------|--------------------|------------------------|--------------------|------|
| | | Repl1 | Repl2 | Repl3 | | |
| 0.06x10 ⁶ | #1 | 0.101 | 0.102 | 0.104 | 0.102 | 1.5 |
| 0.125x10 ⁶ | #1 | 0.143 | 0.132 | 0.141 | 0.139 | 4.2 |
| 0.25x10 ⁶ | #1 | 0.200 | 0.201 | 0.187 | 0.196 | 4.0 |
| 0.5x10 ⁶ | #1 | Not valid ^d | 0.443 ^c | Not valid ^d | 0.443 ^c | n.d. |
| 0.06x10 ⁶ | #2 | 0.020 ^b | 0.020 ^b | 0.018 ^b | 0.019 ^b | 6.0 |
| 0.125x10 ⁶ | #2 | 0.029 ^b | 0.035 ^a | 0.032 ^a | 0.032 ^a | 9.4 |
| 0.25x10 ⁶ | #2 | 0.070 ^a | 0.080 | 0.075 ^a | 0.075 ^a | 6.7 |
| 0.5x10 ⁶ | #2 | 0.122 | 0.123 | 0.109 | 0.118 | 6.6 |
| 0.06x10 ⁶ | #3 | 0.061 ^a | 0.056 ^a | 0.070 ^a | 0.062 ^a | 11.4 |
| 0.125x10 ⁶ | #3 | 0.121 | 0.111 | 0.124 | 0.119 | 5.7 |
| 0.25x10 ⁶ | #3 | 0.147 | 0.178 | 0.188 | 0.171 | 12.5 |
| 0.5x10 ⁶ | #3 | 0.233 | 0.247 | 0.300 | 0.260 | 13.6 |

^a below LOQ of endotoxin standard (0.08 EE/mL), but above LOD; ^b LOD of MAT assay (0.03 EE/mL);

^c above range to adequately measure endotoxin (0.32 EE/mL). n.d.: not determined, only one valid result;

^d OD was above highest point of the standard curve

Mathematical equations: Relative bias (%) = [(GM (Measured potency))/(Target potency)-1]x100%.