

Introduction

- SAB-142 is a fully human, multi-specific, targeted, Anti-Thymocyte Globulin (hATG) for delaying onset and progression of type 1 diabetes (T1D)
- There are challenges in developing a pharmacokinetic (PK) assay for monitoring SAB-142 exposure in clinical trials:
- Difficulty in distinguishing SAB-142 from endogenous human immunoglobulins due to its fully human nature.
 - High-sensitivity assay is required
 - Low dose level of SAB-142 requiring LLOQ of 0.25µg/mL
 - Very low concentration of unbound free active SAB-142 circulating in the bloodstream due to low dose administration. Contributing to this - after infusion, the active components of SAB-142 bind to the circulating lymphocytes.
- To address these challenges, a novel high-sensitivity PK assay was developed and validated for the quantitative measurement of the SAB-142 in human serum samples from SAB-142 clinical trials.

Methods

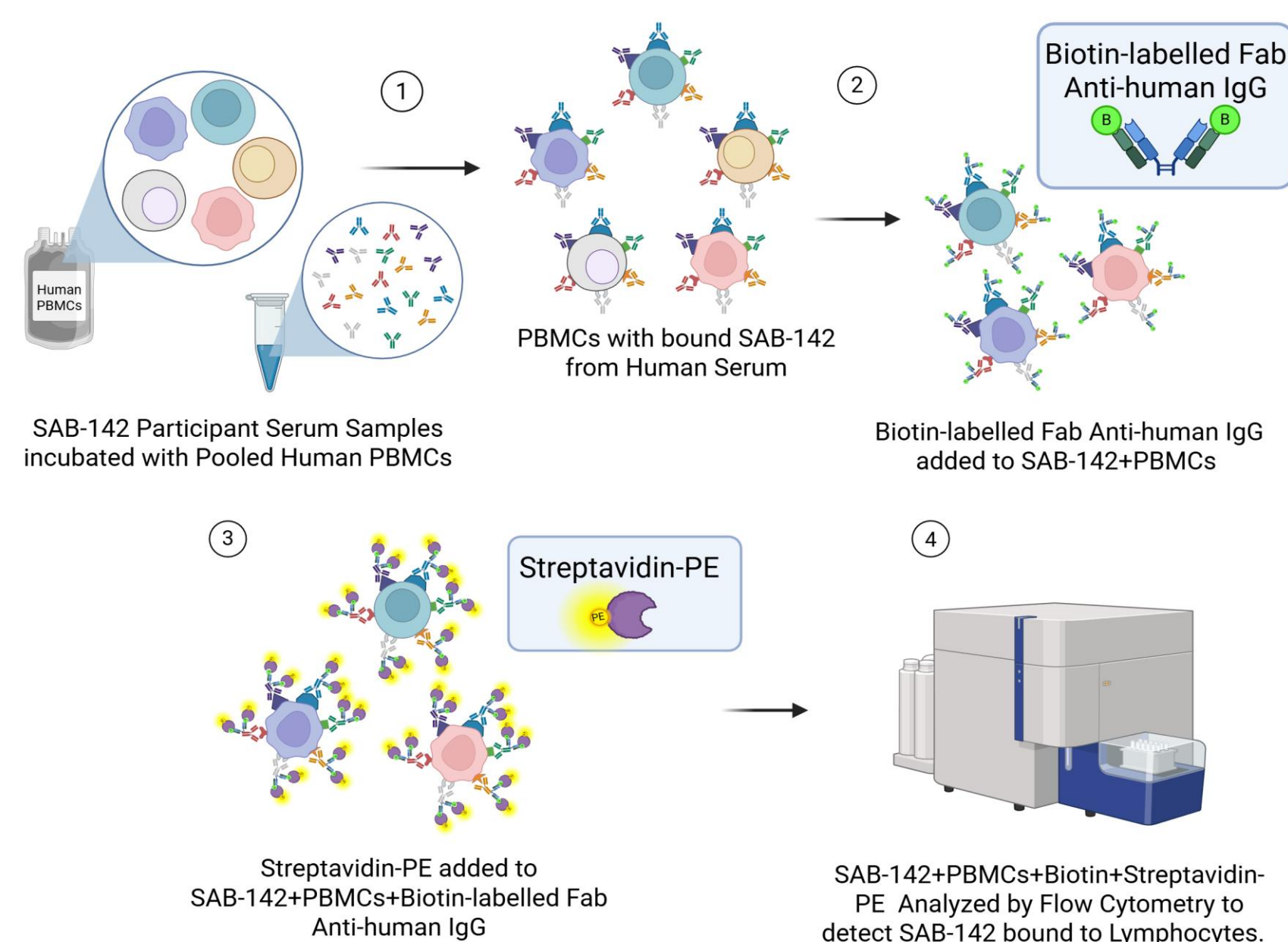


Figure 1. Schematic diagram for the methodology of a multi-specific, lymphocyte targeted, flow cytometry-based PK assay quantifying SAB-142 in human serum

- Pooled human PBMCs (5×10^5 cells) were incubated with human serum samples for 1 hr at RT.
- Cells were washed, incubated with biotin F(ab)₂ goat anti-human IgG-Fc (30 mins at RT), washed, and finally incubated with streptavidin-PE (15 mins at RT)
- Fluorescently-labeled PBMCs were gated for lymphocytes and analyzed via flow cytometry. SAB-142 concentration is determined by interpolating Median Fluorescence Intensity (MFI) against the standard.
- Assay was validated for accuracy, precision, selectivity and range.

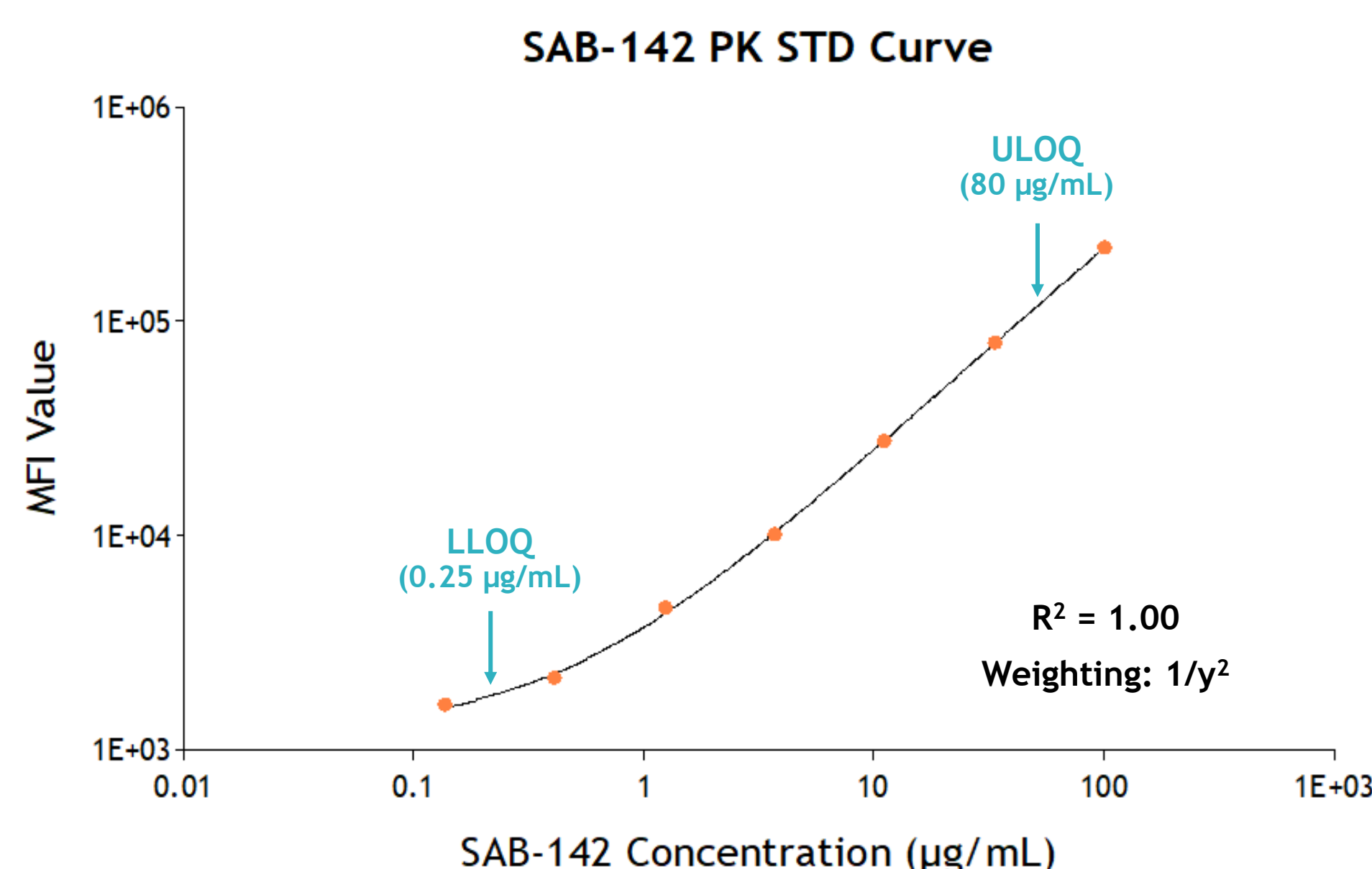


Figure 2. Standard curve between SAB-142 concentration and fluorescence intensity of flow cytometry in human serum

Results: Validation

Table 1. Accuracy in human serum samples spiked with SAB-142 at five QC concentrations

| Sample ¹ | Nominal (µg/mL) | Mean ² (µg/mL) | Mean Recovery ³ (%) | Passed/Failed ⁴ |
|---------------------|-----------------|---------------------------|--------------------------------|----------------------------|
| ULOQ | 80.0 | 85.9 | 107% | Passed |
| HQC | 15.0 | 14.8 | 99% | Passed |
| MQC | 5.00 | 5.29 | 106% | Passed |
| LQC | 1.00 | 1.03 | 103% | Passed |
| LLOQ | 0.25 | 0.19 | 76% | Passed |

¹ULOQ = Upper Limit of Quantitation; HQC = High Quality Control; MQC = Medium Quality Control; LQC = Lower Quality Control; LLOQ = Lower Limit of Quantitation
²Average of Sample ran in triplicate (3 wells/replicate)
³(Measured concentration/theoretical concentration)*100
⁴Acceptance Criteria: HQC, MQC & LQC = %Recovery between 80-120%; ULOQ & LLOQ = %Recovery between 70-130%

Table 2. Overall precision in human serum samples spiked with SAB-142 at five QC concentrations

| Sample ¹ | Nominal (µg/mL) | Mean ² (µg/mL) | CV ³ | Bias ⁴ (%) | Total Error ⁵ (%) | Passed/Failed ⁶ |
|---------------------|-----------------|---------------------------|-----------------|-----------------------|------------------------------|----------------------------|
| ULOQ | 80.0 | 82.4 | 5.8% | 3.0% | 8.7% | Passed |
| HQC | 15.0 | 14.7 | 5.9% | 2.0% | 7.9% | Passed |
| MQC | 5.00 | 5.18 | 12.8% | 3.6% | 16.4% | Passed |
| LQC | 1.00 | 1.05 | 5.4% | 5.0% | 10.4% | Passed |
| LLOQ | 0.25 | 0.26 | 21.6% | 2.4% | 24.0% | Passed |

¹ULOQ= Upper Limit of Quantitation; HQC = High Quality Control; MQC = Medium Quality Control; LQC = Lower Quality Control; LLOQ = Lower Limit of Quantitation
²Average across all precision runs
³Coefficient of Variation; (Standard Deviation/Mean)*100; Average across all precision runs
⁴(Mean measured concentration-nominal concentration)/nominal concentration
⁵Sum of the absolute bias and CV%
⁶Acceptance Criteria: CV%: HQC, MQC, LQC ≤20%; ULOQ & LLOQ ≤ 30%; Total Error: HQC, MQC, LQC ≤30%; ULOQ & LLOQ ≤ 40%

Table 3. Selectivity in healthy volunteer serum spiked with SAB-142 at high and LLOQ concentrations

| Healthy Volunteer | | | | | | | |
|---------------------|------|------|------|--------------|--------------------------------|-----------------|----------------------------|
| Sample ¹ | 1 | 2 | 3 | Mean (µg/mL) | Mean Recovery ² (%) | CV ³ | Passed/Failed ⁴ |
| HQC | 12.1 | 12.9 | 13.7 | 12.9 | 86% | 6.2% | Passed |
| LLOQ | 0.27 | 0.28 | 0.22 | 0.26 | 103% | 13.0% | Passed |

¹ HQC = High Quality Control (15.0 µg/mL); LLOQ = Lower Limit of Quantitation (0.25 µg/mL); Individual samples ran in triplicate
²(Actual concentration/theoretical concentration)*100
³Coefficient of Variation; (Standard Deviation/Mean)*100
⁴Acceptance criteria: HQC %Recovery = 80-120% and CV% ≤25%; LLOQ %Recovery = 70-130% and CV% ≤30%

Table 4. Selectivity in Type 1 Diabetes serum spiked with SAB-142 at HCQ and LLOQ concentrations

| Type 1 Diabetes | | | | | | | | | | |
|---------------------|------|------|------|------|------|------|--------------|--------------------------------|-----------------|----------------------------|
| Sample ¹ | 1 | 2 | 3 | 4 | 5 | 6 | Mean (µg/mL) | Mean Recovery ² (%) | CV ³ | Passed/Failed ⁴ |
| HQC | 14.7 | 16.3 | 13.1 | 15.1 | 16.3 | 12.6 | 14.7 | 98% | 11% | Passed |
| LLOQ | 0.32 | 0.20 | 0.26 | 0.32 | 0.27 | 0.20 | 0.26 | 104% | 24% | Passed |

¹ HQC = High Quality Control; Lower Limit of Quantitation (0.25 µg/mL); Individual samples ran in triplicate
²(Actual concentration/theoretical concentration)*100
³Coefficient of Variation; (Standard Deviation/Mean)*100
⁴Acceptance criteria: HQC %Recovery = 80-120% and CV% ≤25%; LLOQ %Recovery = 70-130% and CV% ≤30%

Results: SAB-142 Phase 1 PK Profile

SAB-142 Serum Concentration in Healthy Volunteers and T1D Patients

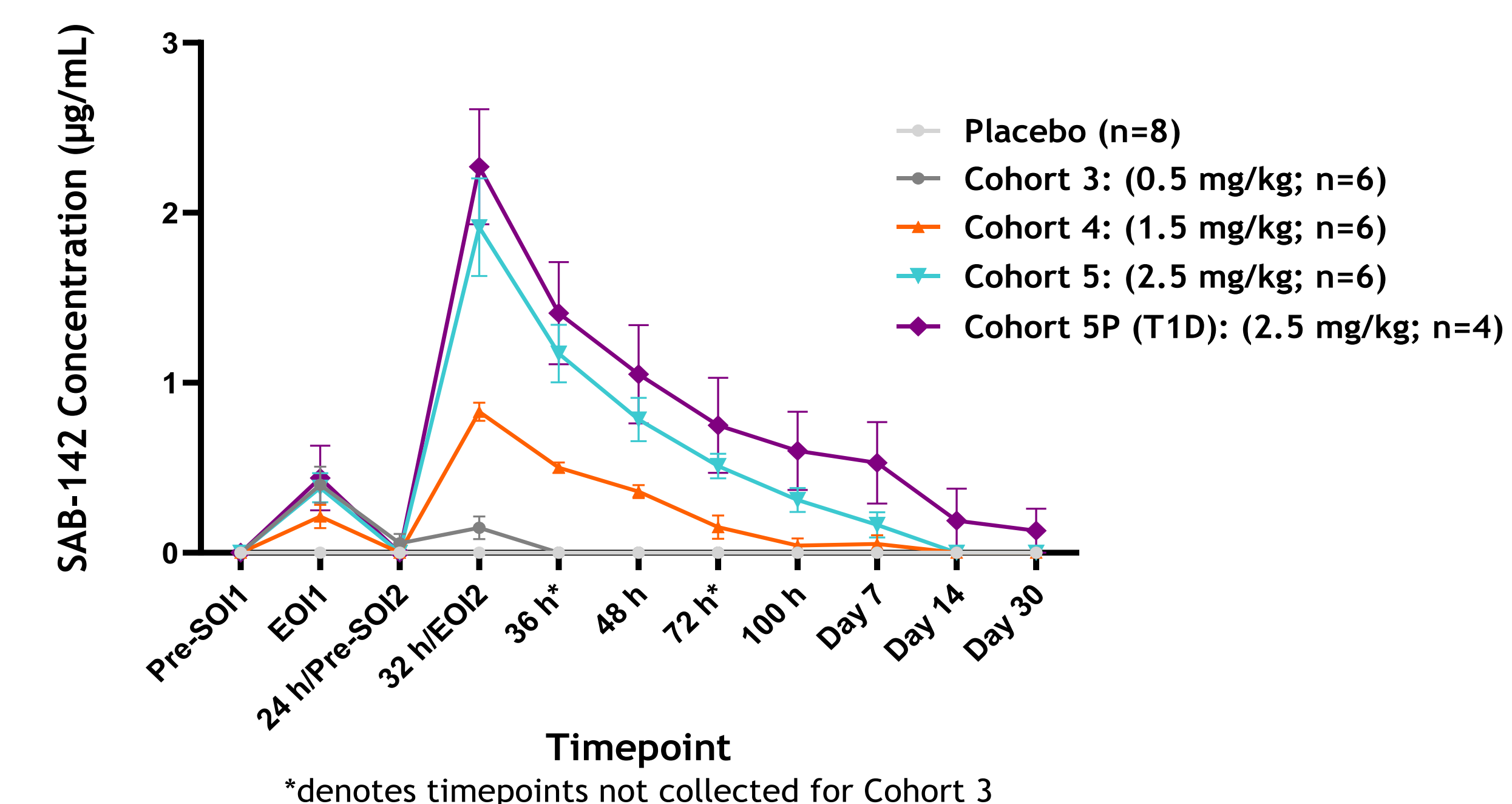


Figure 3. SAB-142 concentration (mean ± SEM) over time in healthy volunteer and Type 1 Diabetes serum following a single IV infusion of SAB-142 in Cohorts 3, 4, 5, 5P from Phase 1 study, SAB-142-101

Conclusion

- The novel PK assay developed was validated for accuracy, precision, selectivity, and range and exhibited high enough sensitivity to detect free unbound SAB-142 at very low circulating levels down to 0.25µg/mL.
- The utility of this assay was successfully demonstrated where SAB-142 had a dose-proportional PK profile measured in the SAB-142-101 Phase 1 Study.
- No major differences were observed in systemic exposure to SAB-142 between HVs and T1D patients dosed at 2.5mg/kg.