**Supplemental Table S1** Precision evaluation of the four evaluated PRA assays

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Lab | Level 1 |  |  |  | Level 2 |  |  |  | Level 3 |  |  |  |
|  | Mean, pmol/L/min | Intra-run CV, % | Inter-run CV, % | Total CV, % | Mean, pmol/L/min | Intra-run CV, % | Inter-run CV, % | Total CV, % | Mean, pmol/L/min | Intra-run CV, % | Inter-run CV, % | Total CV, % |
| B | 18.1 | 3.66 | 4.35 | 5.69 | 28.9 | 2.25 | 4.16 | 4.73 | 122.6 | 1.67 | 3.20 | 3.61 |
| C | 12.6 | 3.87 | 1.60 | 4.18 | 26.4 | 4.17 | 2.97 | 5.12 | 112.6 | 4.02 | 1.53 | 4.30 |
| D | 15.0 | 1.54 | 2.91 | 3.29 | 38.0 | 2.29 | 4.83 | 5.34 | 157.6 | 1.87 | 3.37 | 3.85 |
| E | 12.2 | 2.21 | 6.85 | 7.19 | 25.1 | 5.30 | 4.35 | 6.85 | 125.4 | 2.09 | 6.84 | 7.15 |

Imprecision was evaluated according to the CLSI document EP15-A3 [18]. Three levels of pooled human plasma samples with low (14.5 pmol/L/min), median (29.6 pmol/L/min), and high concentrations (129.6 pmol/L/min) of PRA were measured in triplicates for three runs on three days. The intra-, inter-, and total CVs were calculated by one-way analysis of variance (ANOVA) for each concentration. CV, coefficient variation.

**Supplemental Table S2** The homogeneity test for pooled human plasma samples

|  |  |  |  |
| --- | --- | --- | --- |
|  | F-test value | Critical value | P value |
| RM9 | 1.78 | 3.02 | 0.19 |
| RM11 | 0.97 | 3.02 | 0.51 |
| RM13 | 2.60 | 3.02 | 0.08 |

The sample testing was performed by Lab B. Ten aliquots of each RM were measured in triplicates within one run. All P values >0.05 indicated that the homogeneity of this batch of candidate RMs was acceptable at 95% confidence.

**Supplemental Table S3** Two-way variance analysis for the pool size and spiked contributing to the commutability rates

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Main effect and Interaction effect** | | | | | | | | | | | | |
|  | Large-size  (>1000) | Small-size  (<50) | Main effect of Spiked | | | | Main effect of Pool Size | | | Interaction effect of Spiked and Pool Size | | |
|  | Mean±SDa | Mean±SD | F test | P b | | partial η2c | F test | P | partial η2 | F test | P | partial η2 |
| Spiked | 0.945±0.110 | 0.643±0.055 | 2.833 | 0.118 | | 0.191 | 4.066 | 0.067 | 0.253 | 12.130 | **0.005** | 0.503 |
| Native | 0.846±0.125 | 0.928±0.127 |  |  | |  |  |  |  |  |  |  |
| **Simple effect** | | | | | | | | | | | | |
|  | Simple effect of Pool Size | | | |  | |  | Simple effect of Spiked | | | | |
|  | F test | P | partial η2 | |  | |  | F test | | P | partial η2 | |
| Spiked | 15.624 | **0.002** | 0.566 | |  | | Small-size | 11.818 | | **0.005** | 0.496 | |
| Native | 1.042 | 0.328 | 0.080 | |  | | Large-size | 1.859 | | 0.198 | 0.134 | |

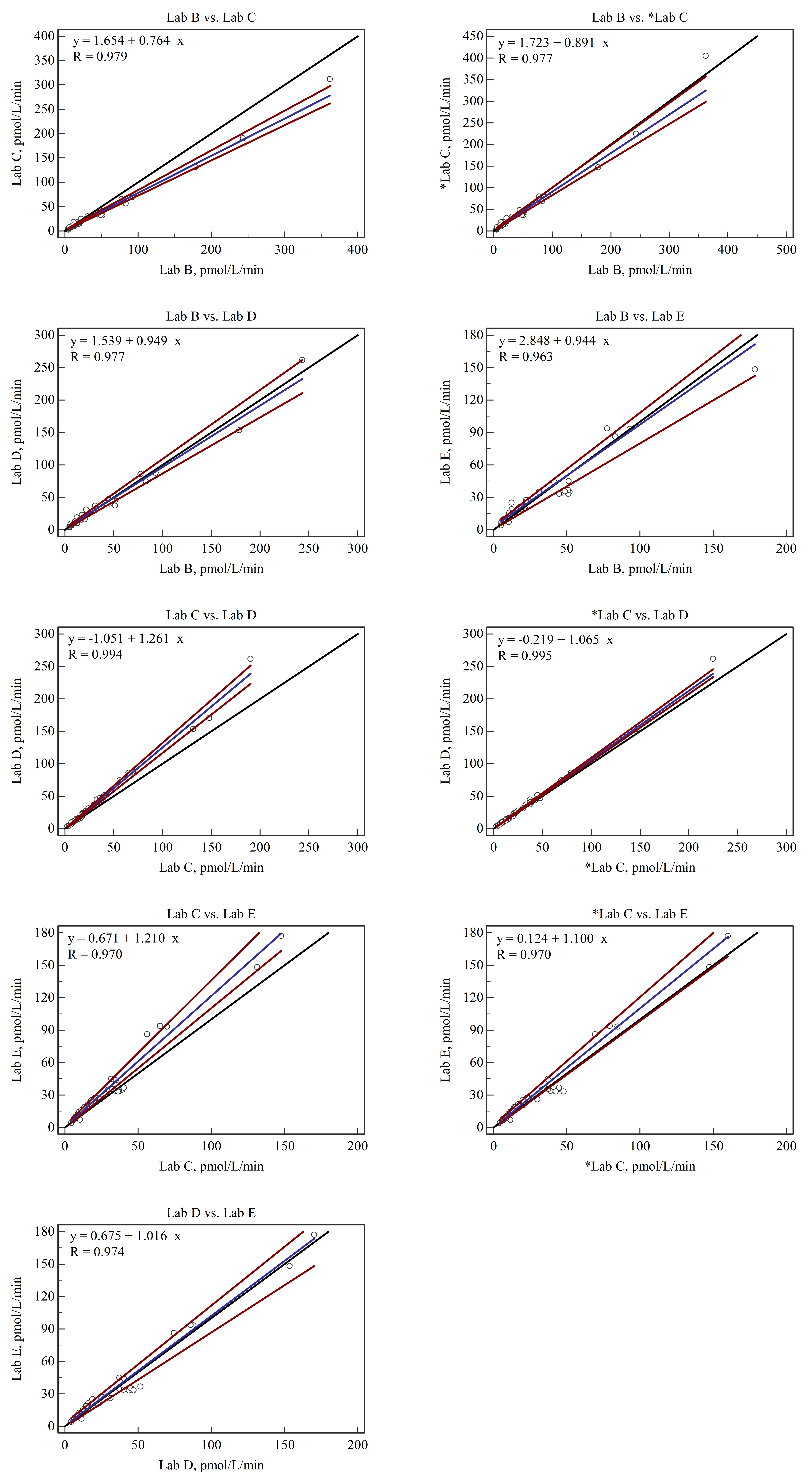
a represents the mean and standard deviation for commutability rates (i.e., for each candidate RMs, the proportion of commutable pairs to total pairs).

b represents the significance of the F test. When P value <0.05 indicates that the main effect/interaction effect of spiked or pool size is significant.

c represents an indicator of the impact intensity of a factor.

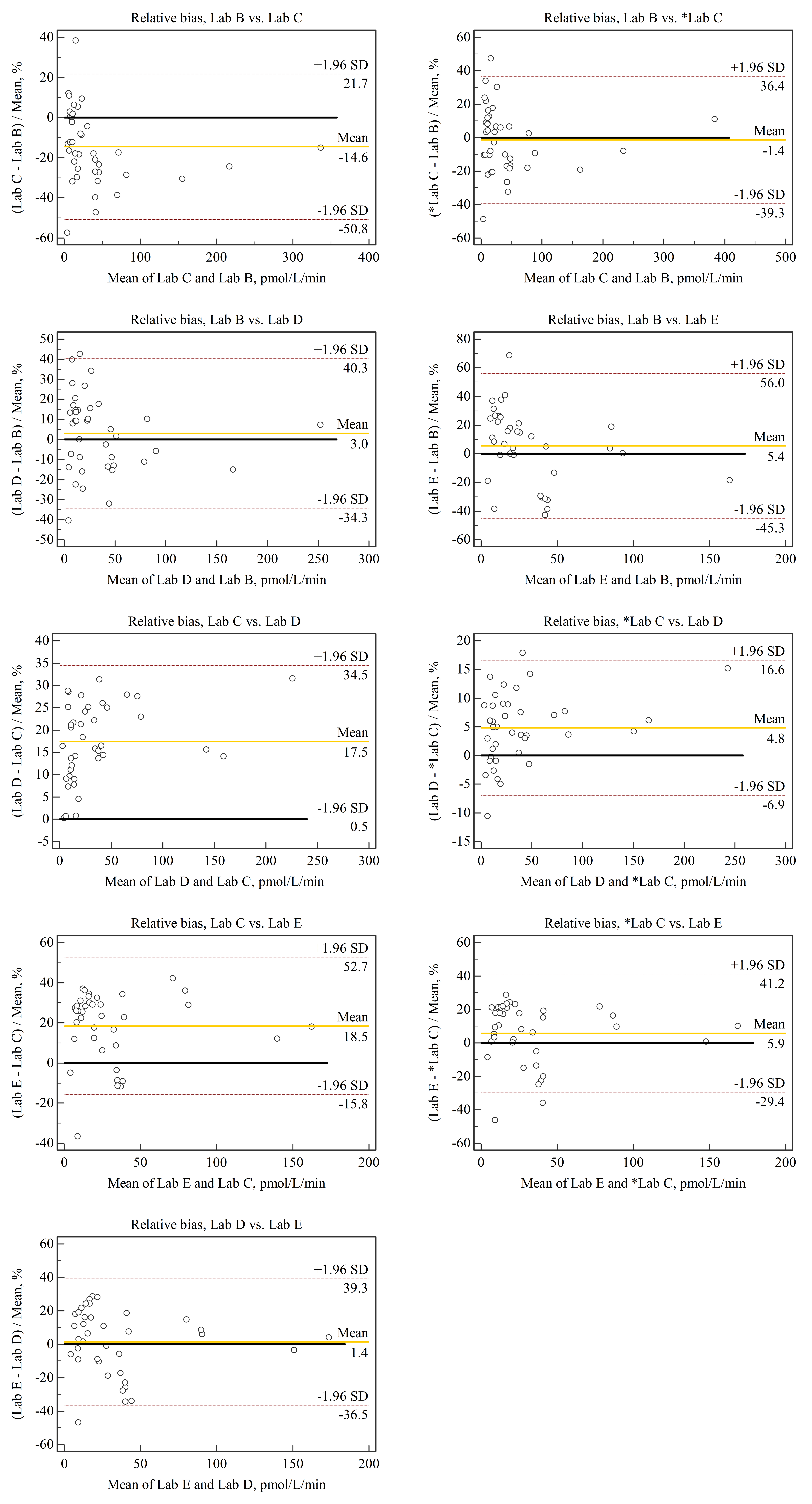
Herein p <0.05 for the interaction effect of spiked and pool size. Further, according to the analysis of simple effect, the simple effect of pool size on the commutability was significant when spiked (P=0.002) but not native (P=0.328); the simple effect of spiked on the commutability was significant for the small-size pool (P=0.005) but not large-size (P=0.198).

The data on commutability rates were derived from the CLSI EP30-A approach.



**Supplemental Figure S1** Passing–Bablok regression analysis among the four PRA assays.

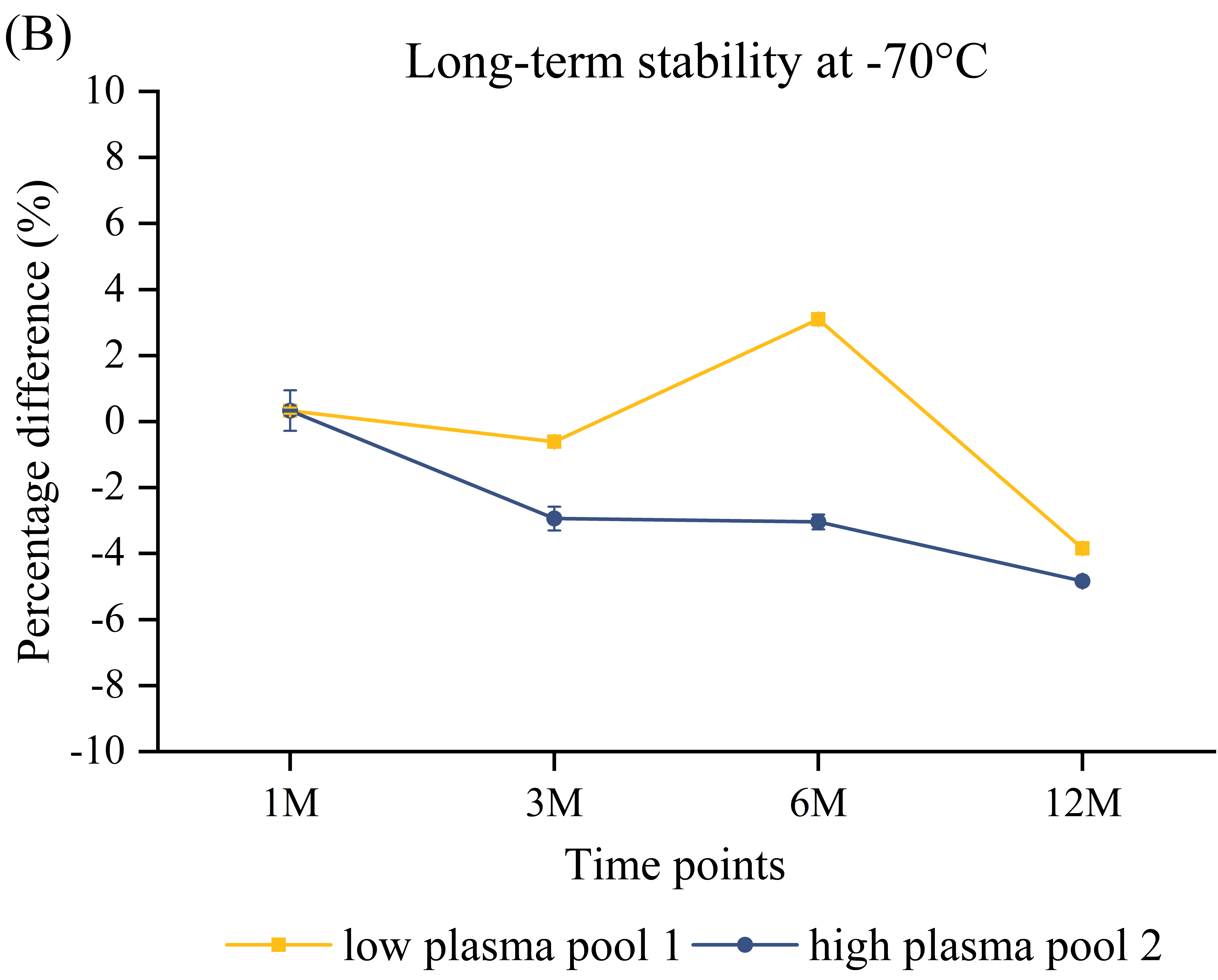
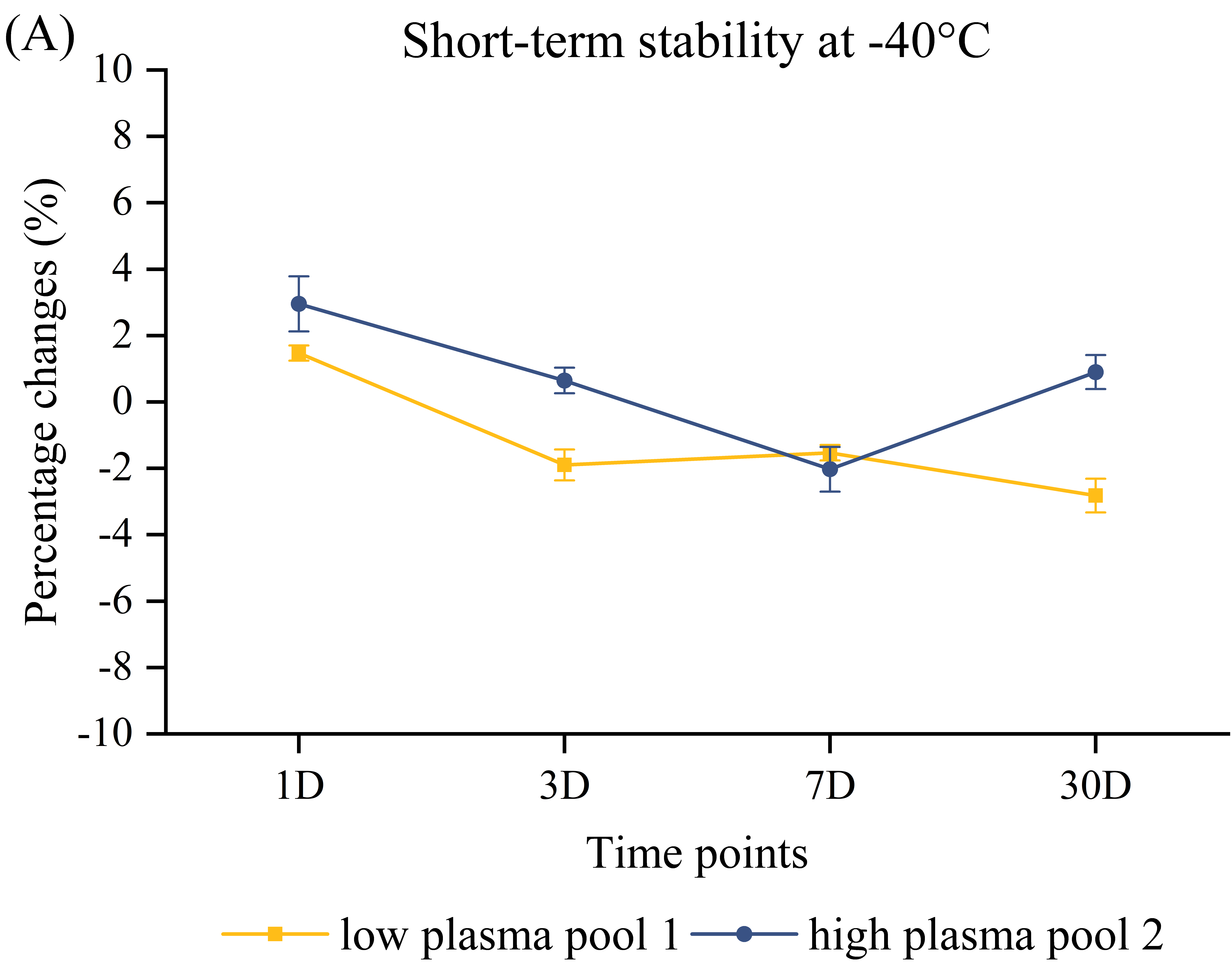
\*Refers to the final PRA results of Lab C were re-calculated without subtracting the blanks. Solid blue line: Passing–Bablok regression line; solid red line: 95% CI for the regression line; solid black line: identity line (x=y). R, Spearman correlation coefficient; CI, confidence interval.



**Supplemental Figure S2** Bland‒Altman plots among the four PRA assays.

\*Refers to the final PRA results of Lab C were re-calculated without subtracting the blanks.

Solid black line: zero line; solid yellow line: mean relative bias; red dotted lines: mean relative bias ± 1.96 SD. SD, standard deviation.



**Supplemental Figure S3** The stability during (A) short-term (-40°C) and (B) long-term (-70°C) storage. Percentage differences of 10% from baseline concentration were considered as the stability threshold.