Evaluation of Coagtrol N for Use as Normal Plasma in Mixing Tests

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Background: *The selection of normal plasma is an important factor in mixing tests used for the detection of lupus anticoagulant (LA), since residual platelets (phospholipids) can shorten clotting time and, affect mixing test results. Here, we investigated whether Coagtrol N (CoagN), a type of commercially available lyophilized plasma in Japan, can be used as normal plasma in mixing tests for widening the use of cross mixing tests.*

Methods: *Three lots of CoagN were evaluated using plasma samples from 23 LA-positive patients. CRYOCheck Pooled Normal Plasma (PBI) and homemade pooled normal plasma (HM) were used as controls. Mixing tests were performed with six mixing ratios (1:0, 4:1, 1:1, 1:4, 1:9, 0:1) of patient to normal plasma. The activated partial thromboplastin time (APTT) of the mixing test samples was measured, and the index of circulating anticoagulant (ICA) and the 4:1 mix percent correction (4:1%Co) were compared.*

Results: *The platelet count for all normal plasma, obtained using a platelet specific monoclonal mouse anti-human CD41 was < 10×10^9/L. The median ICA for patient plasma (n = 23) was 28.8, 27.9, 31.9, 23.8, 33.2 for CoagN (Lot.021), CoagN (Lot.022), CoagN (Lot.023), HM and PBI, respectively. All the CoagN lots had significantly higher ICA than HM and CoagN (Lot.022) alone had significantly lower ICA than PBI ($p < 0.001$). The proportions of patient plasmas assessed as inhibitor positive because of ICA ≥ 12 were respectively 100% CoagN (Lot.021), 96% CoagN (Lot.022), 100% CoagN (Lot.023), 96% HM and 100% PBI. The median 4:1%Co in patient plasma (n = 23) was 9.5, 12.5, 8.6, 12.2, 9.9 for CoagN (Lot.021), CoagN (Lot.022), CoagN (Lot.023), HM and PBI, respectively. All of the patient samples were inhibitor-positive with a 4:1%Co < 50 using normal plasma.*

Conclusion: *CoagN had no significant impact on the detection of inhibitors compared to PBI and HM; therefore, we conclude that CoagN can be used as a substitute for normal plasma in mixing tests. It indicates that the findings of this study will contribute to the widespread use of mixing tests.*

Key Words APTT, Coagtrol N, ICA, 4:1%Co, Mixing Test, Normal Plasma

INTRODUCTION

Mixing tests are a useful tool for examining the detection of lupus anticoagulant (LA). The CLSI H-60A guidelines recommend using homemade normal human pooled plasma (NHPP) derived from at least 20 healthy individuals (LA-negative, ≥ 80% activity for all coagulation factors, and platelet counts < 10×10^9/L) for mixing tests [1]. Furthermore, the LA detection guidelines published by the subcommittee of the International Society on Thrombosis and Haemostasis (ISTH) Scientific and Standardization Committee (SSC) recommend the use of commercially available lyophilized normal plasma or frozen normal plasma in addition to homemade NHPP [2].

Although we have prepared homemade NHPP ourselves, the process is time consuming and laborious. Moreover, the coagulation factor activity in our homemade NHPP was < 80% (76% and 77%) for factors V and XII (≥ 90% for all other factors). In Japan, there has been a report on the usefulness of commercially available frozen normal plasmas as substitutes for the recommended normal plasmas, for widening the use of cross mixing tests [3]. Although we agree with this report, it is difficult for many laboratories to procure frozen plasma after considering cost and purchase route.

Note: This article is translated and republished from the Sysmex Journal Web Vol.18 No.1, 2017. (Japanese)

Here, we evaluated the applicability of Coagtrol N (CoagN; Sysmex Corporation, Kobe, Japan) which is sold only in Japan, a commercially available lyophilized normal plasma used in many laboratories as a coagulation test calibrator, as the normal plasma source in mixing tests for widening the use of cross mixing tests. The use of Coagtrol N as a substitute for normal plasma will facilitate the performance of mixing tests, thus improving the LA detection rate.

MATERIALS AND METHODS

Three lots of CoagN (021, 022, and 023) were evaluated to rule out inter-lot differences. Controls included frozen CRYOCheck Pooled Normal Plasma (PBI; Precision BioLogic Inc., Dartmouth, Nova Scotia, Canada: Lot A1157) and homemade NHPP (HM). The platelet count for all normal plasma was $< 10 \times 10^9$/L and was, obtained using a platelet specific monoclonal mouse anti-human CD41(gpIIb) (5B12)/FITC antibody (DAKO, Glostrup, Denmark). PBI had the highest coagulation factor activity (≥ 100% for all factors), followed by CoagN (≥ 80% for all factors) (***Table 1***).

The plasma samples from 23 LA-positive patients with diagnosed or suspected Antiphospholipid Syndrome (APS) were used in the study. LA-positive samples were defined has having a phospholipid concentration ratio (LA ratio) > 1.15 (the cut-off value); this value was derived from a total of 29 healthy individuals with the LA test reagent (Gradipore, MBL, Japan) used for measuring the dilute Russell viper venom time (dRVVT). The ratios of the 23 patient plasma samples ranged from 1.25 to 2.43 (***Table 2***).

Table 1 *Properties of pooled normal plasmas.*

	CoagN (Lot.021)	CoagN (Lot.022)	CoagN (Lot.023)	HM	PBI
Platelet Counts (/L)	0.00×10^9	1.00×10^9	0.01×10^9	0.02×10^9	0.03×10^9
PT (sec)	12.4	12.3	12.4	11.7	11.6
PT (%)	95	97	95	107	109
APTT (sec)	28.5	28.9	28.3	31.3	27.3
Factor XII (%)	87	83	82	76	112
Factor XI (%)	124	92	119	94	124
Factor IX (%)	106	98	107	90	122
Factor VIII (%)	94	85	97	96	121
Factor X (%)	90	87	87	92	104
Factor VII (%)	94	102	102	103	118
Factor V (%)	98	85	97	77	113
Factor II (%)	86	82	83	92	103
Fibrinogen (mg/dL)	294	269	283	235	283

The mixing tests were performed with six mixing ratios (1:0, 4:1, 1:1, 1:4, 1:9, 0:1) of patient plasma to normal plasma in a single measurement. The activated partial thromboplastin time (APTT) of the 23 patient plasma samples was measured using Thrombocheck APTT-SLA (Sysmex Corporation) which is sold only in Japan and ranged from 39.7 to 77.4 seconds (***Table 2***). The mixing of patient and normal plasma and APTT measurements were conducted using a fully automated process with the CS-2500 system (Sysmex Corporation). The index of circulating anticoagulant (ICA) of each LA-positive sample was calculated using the formula:

$$(b - c) / a \times 100$$

where a is the APTT of the patient plasma, b is the APTT of the 1:1 patient and normal plasma mixture, and c is the APTT of the normal plasma. The ICA of the normal plasma samples was compared and samples with an ICA ≥ 12 (the cut-off value) were considered as inhibitor positive. The 4:1%Co of each LA positive sample was calculated using the formula:

$$(a - d) / (a - c) \times 100$$

where a is the APTT of the patient plasma, c is the APTT of the normal plasma and d is the APTT of the 1:4 mixture of normal plasma and patient plasma. The 4:1%Co was compared among the normal plasmas. The cut-off value of the 4:1%Co was set at 50% following the previous report [4].

ICA and 4:1%Co were statistically compared with the Wilcoxon signed-rank test using JMP 11 (SAS Institute Inc, US). The level of significance was set at 0.05 and $p < 0.05$ was taken as statistically significant.

Table 2 *LA1/LA2 (dRVVT) and APTT of plasma samples.*

No.	LA1/LA2	APTT (sec)
1	1.94	71.8
2	1.92	52.2
3	1.55	74.9
4	2.02	77.4
5	1.86	49.9
6	2.05	43.2
7	1.40	67.6
8	1.67	46.0
9	2.43	63.7
10	2.29	72.1
11	1.70	52.6
12	2.39	56.2
13	1.77	54.2
14	2.28	44.9
15	2.39	46.8
16	2.07	73.5
17	1.45	52.1
18	1.53	43.9
19	1.48	39.7
20	1.35	42.2
21	1.25	52.8
22	1.97	56.1
23	1.72	52.1

RESULTS

The ICA of the 23 patient plasmas obtained in mixing tests is shown in ***Table 3*** and ***Fig. 1***. The median (IQR) ICA for the 23 patient plasma samples was 28.8 (21.2-41.8) CoagN (Lot.021), 27.9 (29.1-37.9) CoagN (Lot.022), 31.9 (20.9-42.7) CoagN (Lot.023), 23.8 (17.8-33.9) HM, and 33.2 (24.3-43.8) PBI, respectively. All CoagN lots exhibited significantly higher ICA than HM ($p < 0.001$), while CoagN (Lot 022) had a significantly lower ICA than PBI ($p < 0.001$).

The proportion of patient plasma samples assessed as inhibitor-positive (ICA ≥ 12) was 100% (23/23), 96% (22/23), 100% (23/23), 96% (22/23), and 100% (23/23) for CoagN (Lot 021), CoagN (Lot 022), CoagN (Lot 023), HM, and PBI, respectively. The ICA for patient sample 19 was < 12 for both CoagN (Lot 022) (11.9) and HM (9.1) and the dRVVT ratio and APTT of this sample were 1.48 and 39.7 seconds, respectively.

Table 3 *Comparison of ICA obtained in cross mixing tests with different pooled normal plasmas.*

No.	ICA				
	CoagN (Lot.021)	CoagN (Lot.022)	CoagN (Lot.023)	HM	PBI
1	56.3	52.9	57.4	49.6	53.8
2	44.2	40.7	47.5	38.7	43.8
3	56.8	51.2	58.3	48.2	52.5
4	52.1	50.4	52.0	49.9	51.7
5	36.4	35.0	37.9	32.1	43.0
6	23.9	22.6	25.1	19.9	24.5
7	45.1	41.4	45.8	39.5	44.3
8	28.8	24.5	30.2	21.1	33.2
9	39.3	35.9	39.9	32.8	43.8
10	45.1	40.0	45.5	35.0	44.3
11	28.0	27.9	31.9	26.2	33.1
12	33.8	30.0	34.6	24.0	34.5
13	32.4	28.4	35.0	23.8	36.1
14	22.7	20.1	22.8	18.5	28.3
15	20.9	19.0	20.8	16.2	25.2
16	34.1	30.7	34.0	30.3	35.3
17	25.3	22.1	25.3	19.0	24.1
18	20.8	17.6	21.1	12.5	23.1
19	13.9	11.9	13.6	9.1	16.6
20	21.4	17.4	20.6	12.3	18.7
21	18.9	19.2	19.5	19.1	19.4
22	18.0	16.7	17.8	13.2	16.5
23	19.3	18.6	19.2	17.1	28.1
max	56.8	52.9	58.3	49.9	53.8
75th percentile	41.8	37.9	42.7	33.9	43.8
Median	28.8	27.9	31.9	23.8	33.2
25th percentile	21.2	19.1	20.9	17.8	24.3
min	13.9	11.9	13.6	9.1	16.5

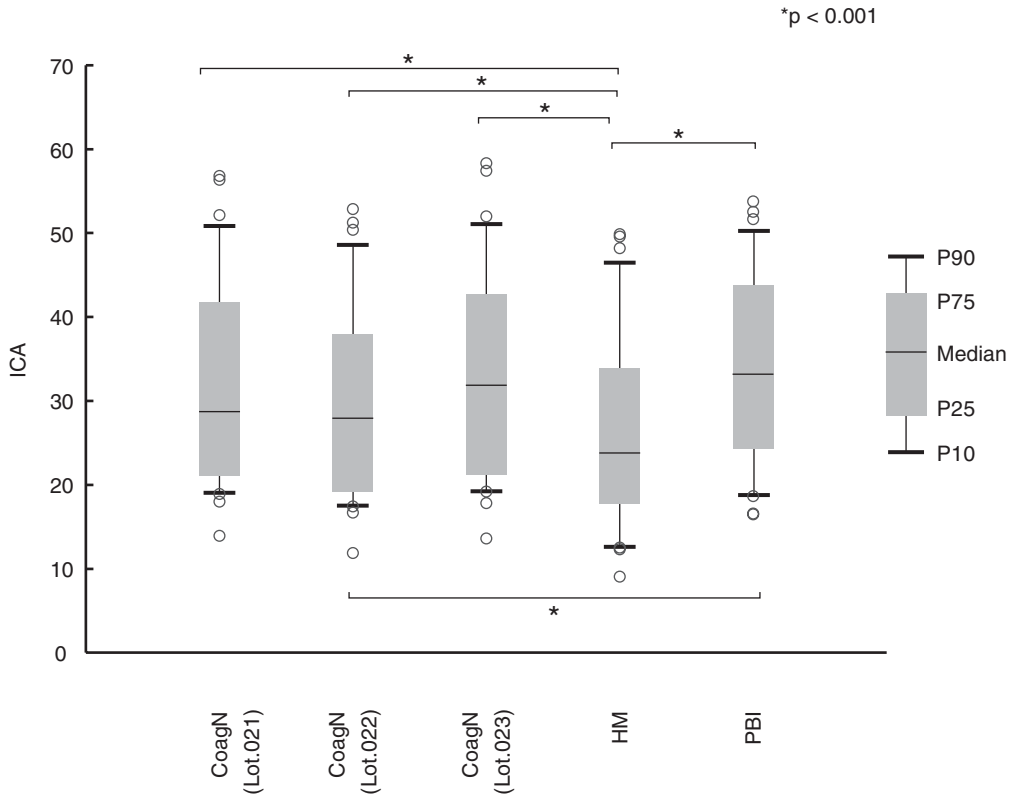

Fig. 1

*ICA values obtained in mixing tests with different pooled normal plasma sources and 23 LA-positive patient samples. The box represents the 25th-75th percentiles of the distribution, the bars extend to the 10th and 90th percentiles, and the central line signifies the median. *p < 0.001 for the between-group comparison.*

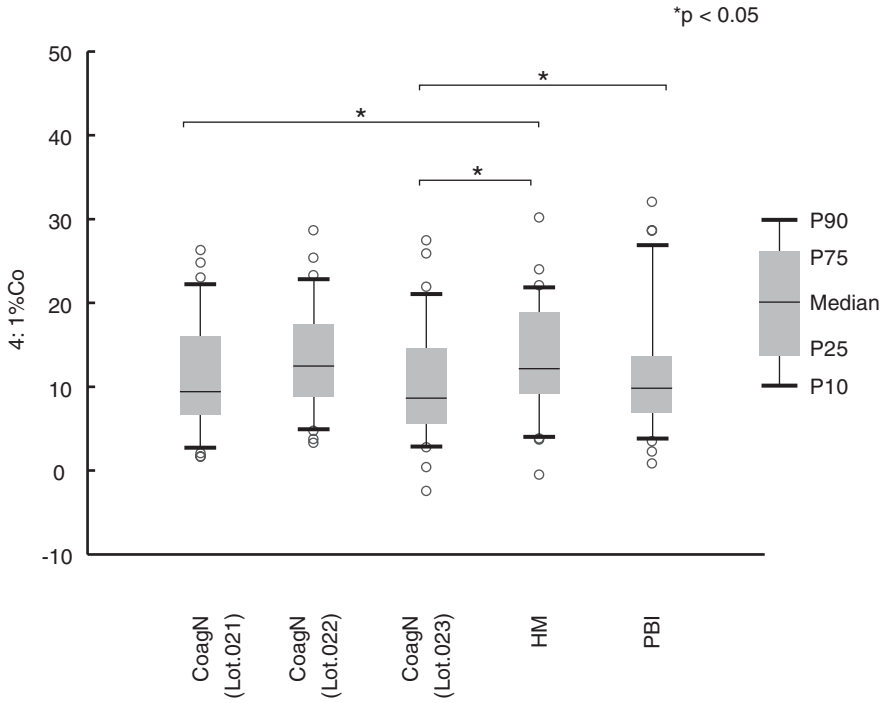

Fig. 2

*4:1%Co values obtained in mixing tests with different pooled normal plasma sources and 23 LA-positive patient samples. The box represents the 25th-75th percentiles of the distribution, the bars extend to the 10th and 90th percentiles, and the central line signifies the median. *p < 0.05 for the between-group comparison.*

The 4:1%Co of the 23 patient plasmas obtained in mixing tests are shown in ***Table 4*** and ***Fig. 2***. The median (IQR) 4:1%Co for the 23 patient plasma samples was 9.5 (6.6-16.1) CoagN (Lot.021), 12.5 (8.9-17.5) CoagN (Lot.022), 8.6 (5.6-14.9) CoagN (Lot.023), 12.2 (9.1-18.9) HM, and 9.9 (6.8-13.7) PBI, respectively. Samples with 4:1%Co of < 50 (the cut-off value) were judged as inhibitor positive. The CoagN (Lot 023) had significantly lower 4:1%Co than HM and PBI ($p < 0.05$). The proportion of patient plasmas assessed as inhibitor-positive was 100% (23/23) for all of the normal plasma.

The results of the mixing tests of all samples are shown in the Supplement.

Table 4 *Comparison of 4:1%Co obtained in cross mixing tests with different pooled normal plasmas.*

No.	4: 1%Co				
	CoagN (Lot.021)	CoagN (Lot.022)	CoagN (Lot.023)	HM	PBI
1	2.1	3.8	4.2	3.7	4.8
2	1.7	3.3	-2.4	-0.5	2.3
3	1.7	5.7	0.4	4.9	8.1
4	5.0	5.8	3.1	4.1	6.5
5	5.2	4.8	2.8	3.8	0.9
6	8.5	11.6	8.3	10.9	13.9
7	7.2	10.2	6.4	7.5	7.2
8	6.5	9.5	5.1	11.1	6.1
9	10.3	13.5	10.2	12.1	7.9
10	7.8	10.0	7.8	12.2	9.9
11	15.7	13.9	12.4	11.7	12.5
12	9.5	11.2	6.6	19.3	12.8
13	8.5	12.5	8.6	12.4	8.4
14	13.1	15.4	14.0	10.7	3.5
15	16.4	17.1	15.7	17.8	13.5
16	19.1	21.0	17.9	18.4	19.6
17	14.1	18.8	16.6	20.6	19.2
18	10.8	15.4	10.1	20.5	12.9
19	16.7	17.9	12.5	12.5	8.5
20	6.8	8.3	6.2	16.2	13.4
21	26.3	25.4	27.5	22.1	28.7
22	24.8	28.7	25.9	30.2	32.1
23	23.0	23.3	21.9	24.0	28.6
max	26.3	28.7	27.5	30.2	32.1
75th percentile	16.1	17.5	14.9	18.9	13.7
Median	9.5	12.5	8.6	12.2	9.9
25th percentile	6.6	8.9	5.6	9.1	6.8
min	1.7	3.3	-2.4	-0.5	0.9

DISCUSSION

Here, we evaluated the feasibility of using CoagN which is sold only in Japan as normal plasma in mixing tests. The controls used in this study included PBI, which has been reported as an effective normal plasma substitute for mixing tests [3], and homemade NHPP, which is recommended by the lupus anticoagulant detection guidelines. The coagulation factor activity in homemade NHPP was < 80% for factors V and XII (76% and 77%, respectively). However, it should be noted that the process of preparing homemade NHPP involves collecting blood from 20 healthy individuals, performing two centrifugation steps, measuring PT/APTT/PLT counts, and then storing the samples at -75°C; 4-5 hours may elapse before the samples are actually stored at -75°C. Thus the labile factor, FV, may have decreased in the samples. In addition, Gordon et al. have reported that FXII activity is present at lower levels in the plasma of Asian individuals than in white American subjects [5]. The FXII calibrator used in this study was not prepared from Asian donors, therefore it is possible that the FXII in homemade NHPP may be < 80%. However, 76% FXII and 77% FV were considered sufficient for recovering these factors in the mixing tests.

The LA ratio and APTT of the 23 LA-positive patient samples showed a broad distribution, suggesting that these were suitable samples for mixing test evaluation. We used the ICA (Rosner Index) and 4:1%Co for objective evaluation of the comparative performance of different normal plasma samples. The ICA of CoagN was significantly different when compared to PBI and homemade NHPP; however, there was no significant effect on the detection of inhibitors. The ICA of patient sample 19 was < 12 for both CoagN (Lot 022) and homemade NHPP; however, these values did not deviate substantially from 12. The cut-off value setting and the use of PBI as the normal plasma are based on a previous study [6]. If we set a different cut-off value for each normal plasma source, the sample might have been assessed as inhibitor-positive. On the other hand, the median value of 4:1%Co was in the order of CoagN (Lot.023) < CoagN (Lot.021) < homemade NHPP < PBI < CoagN (Lot.022). The proportion of plasma samples assessed as inhibitor positive was 100% for all the normal plasmas tested, with no difference among them.

In summary, based on our results we conclude that Coagtrol N can be useful a substitute for normal plasma in mixing tests. It indicates that the findings of this study will contribute to the widespread use of mixing tests, since the utilization of Coagtrol N will eliminate the need to prepare or purchase normal plasma.

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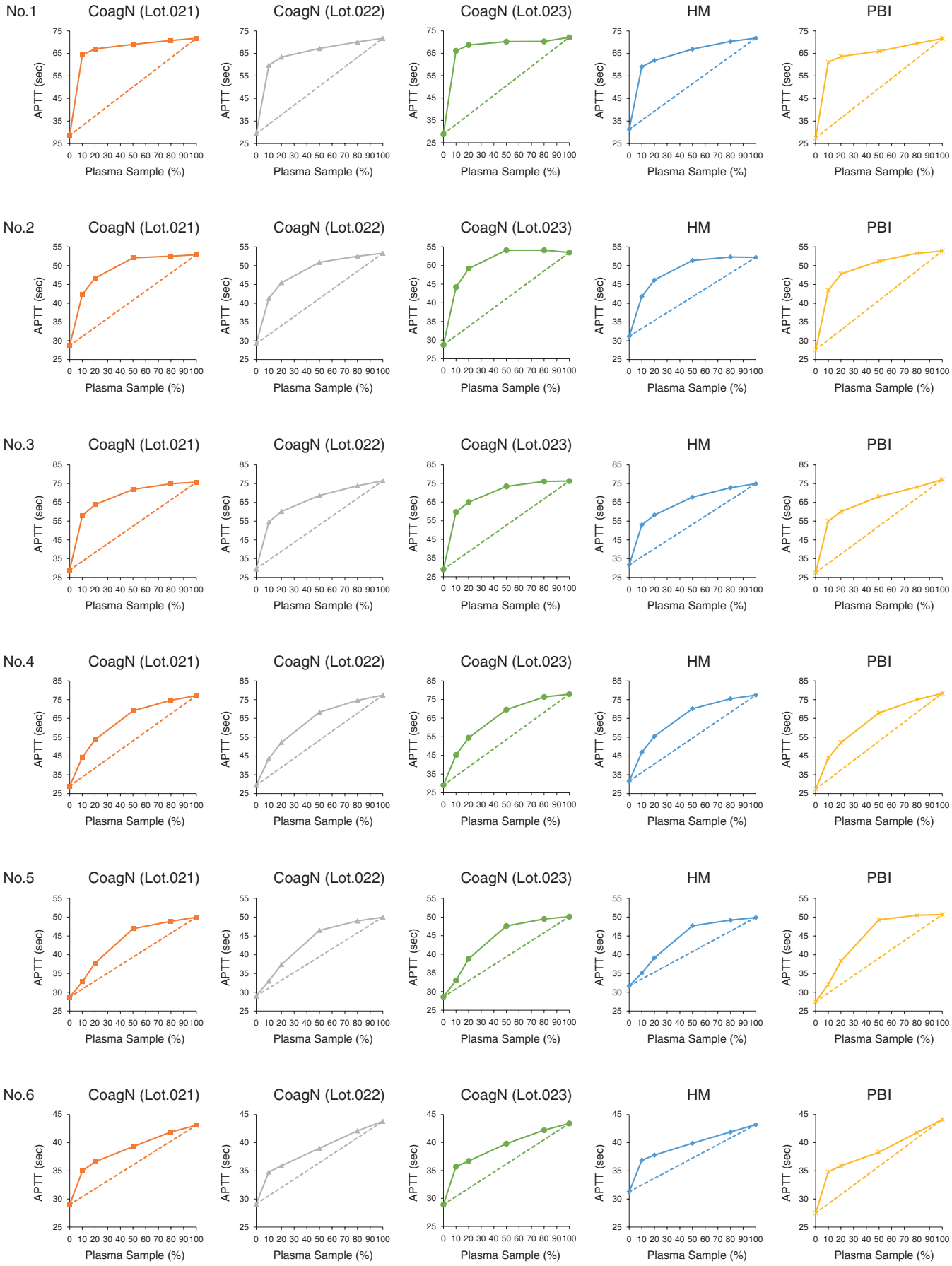
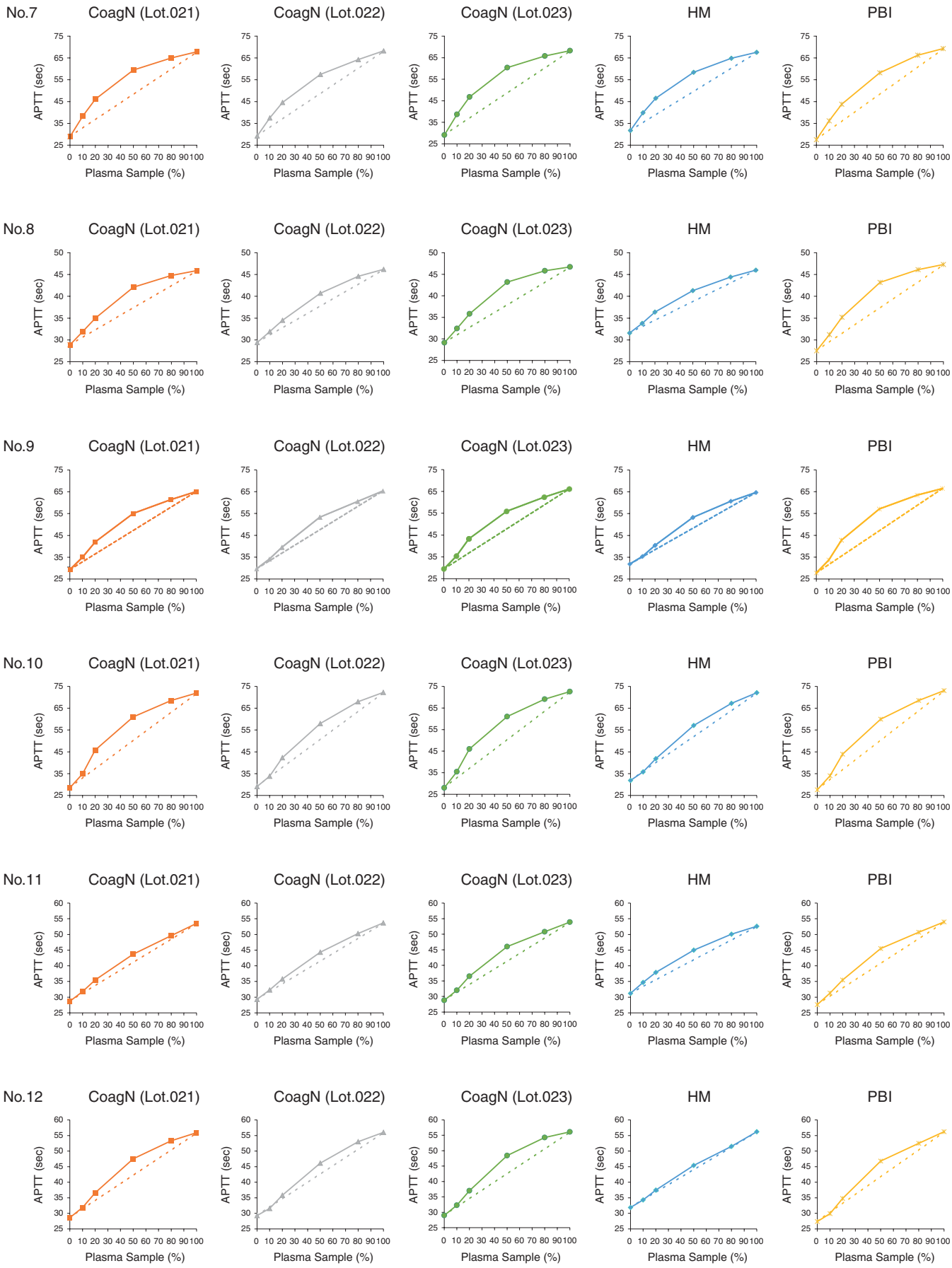
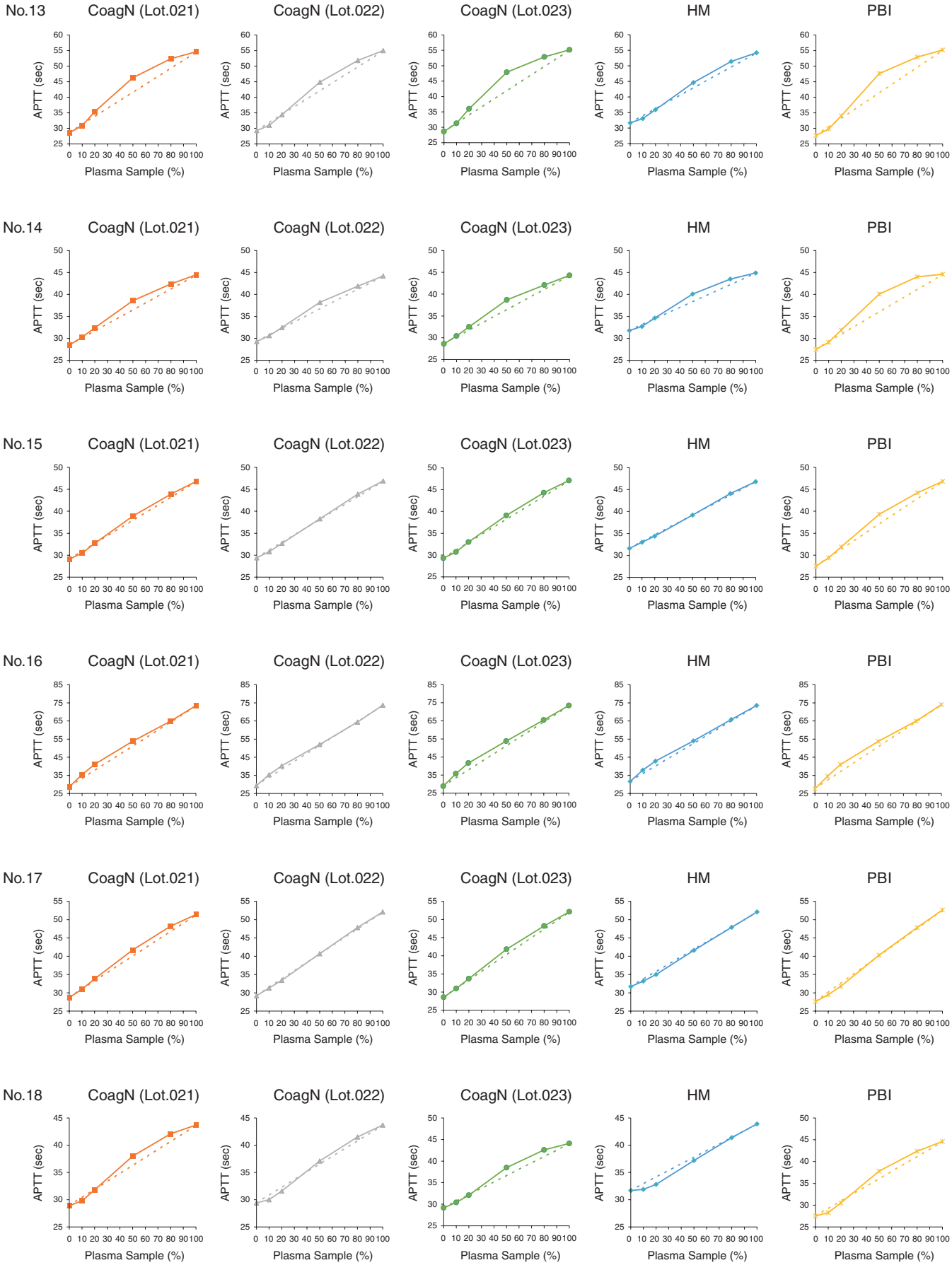


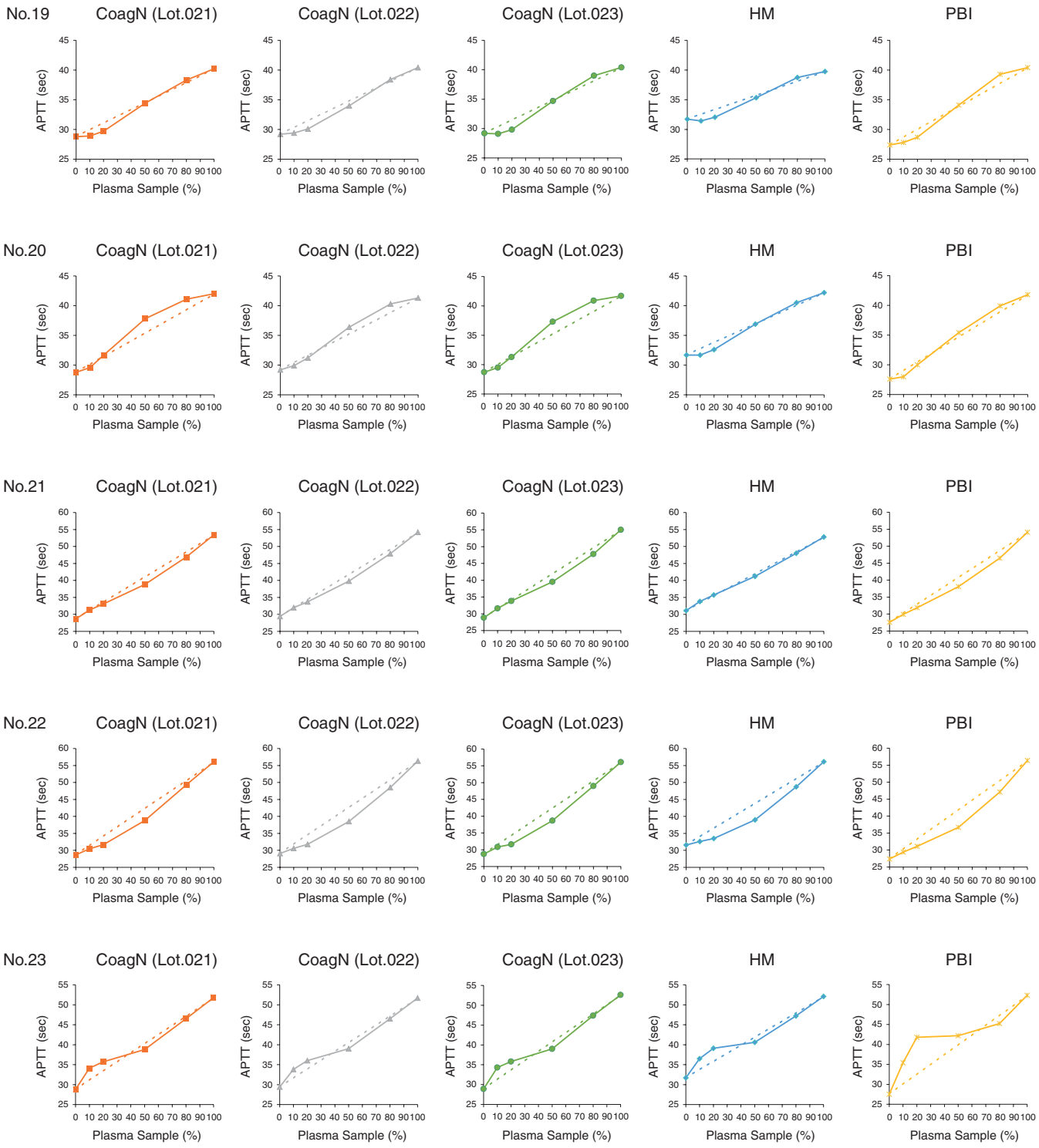
Supplement (1) *The results of the mixing tests (No.1 - No.6).*



Supplement (2) *The results of the mixing tests (No.7 - No.12).*



Supplement (3) *The results of the mixing tests (No.13 - No.18).*



Supplement (4) *The results of the mixing tests (No.19 - No.23).*