



# Increase in Serum Potassium Levels After Refrigerated Storage: A Component of Blood Clot Contaminates the Serum Layer Over the Separator Gel

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This is the accepted version of the following article: [Increase in Serum Potassium Levels After Refrigerated Storage: A Component of Blood Clot Contaminates the Serum Layer Over the Separator Gel. Kohsuke Yoshida, Hiromi Tani, Sae Nobuhara, and Shinji Yamamoto. Biopreservation and Biobanking], which has now been formally published in...

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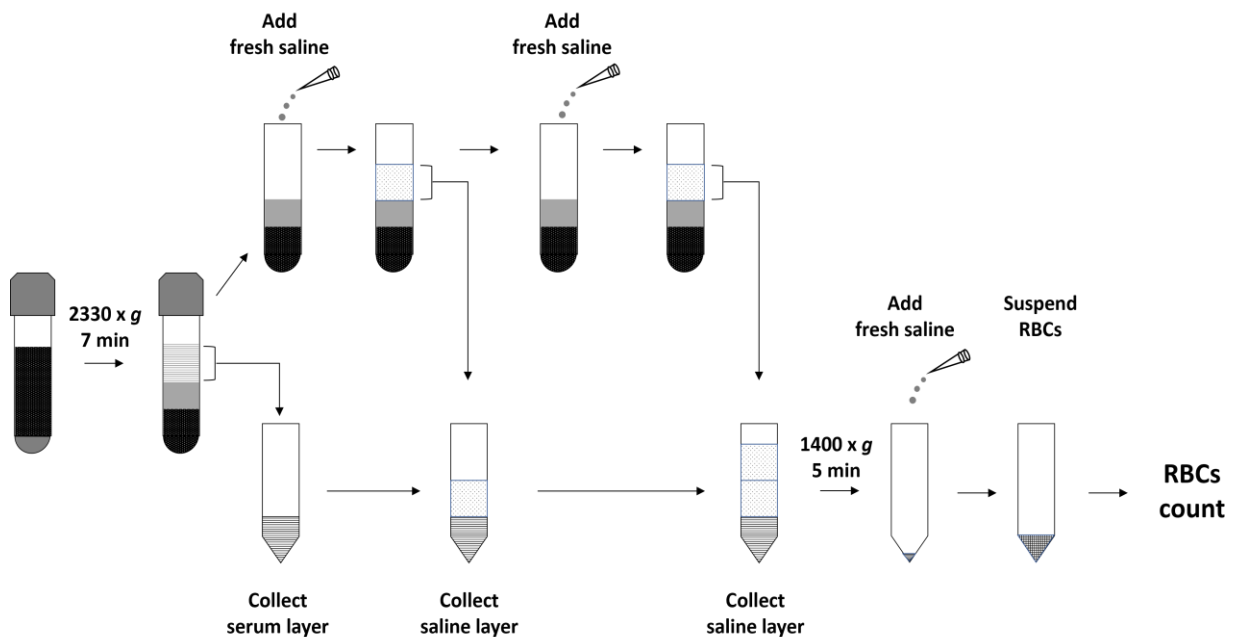
Supplementary Table 1. Profile of the healthy volunteers in Figure 4.

No	Sex	Age (years old)	Total amount of blood (mL)		
			For Fig 4A	For Fig 4C	For Fig 4E
1	M	37	28 (4mL x 7)	NE	NE
2	M	38	28 (4mL x 7)	NE	NE
3	M	39	28 (4mL x 7)	16 (4mL x 4)	NE
4	M	39	28 (4mL x 7)	16 (4mL x 4)	NE
5	M	40	28 (4mL x 7)	NE	13 (4mL + 4 + 5)
6	M	40	28 (4mL x 7)	16 (4mL x 4)	13 (4mL + 4 + 5)
7	M	55	28 (4mL x 7)	NE	13 (4mL + 4 + 5)
8	M	41	28 (4mL x 7)	16 (4mL x 4)	13 (4mL + 4 + 5)
9	M	39	NE	16 (4mL x 4)	13 (4mL + 4 + 5)
10	F	26	NE	NE	13 (4mL + 4 + 5)
11	F	50	NE	NE	13 (4mL + 4 + 5)

M, male. F, female. Venous blood samples for each experiment were taken on independent days.

NE, Not Examined.

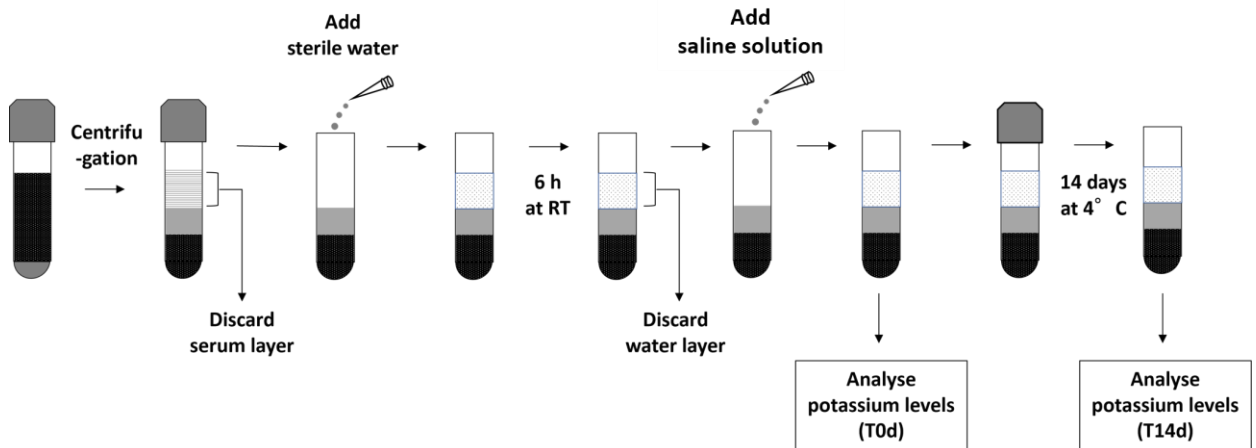
# Suppl. Fig 1



## Supplementary Figure 1.

Venous blood samples were obtained using Insepac II tubes. The serum was separated by centrifugation using the method described above ( $2330 \times g$  for 7 min), poured directly into a new conical tube. To collect all RBCs remaining above the separator, 2mL of fresh saline was added to their Insepac II tube, mixed by gently inverting, and then poured into the conical tube. The above steps were repeated, and the sample's total volume (almost 6mL) in conical tubes was centrifuged at  $1400 \times g$  for 5 min. The supernatant was discarded, the pellet was suspended in 1 mL of saline, and the number of cells was counted using a Fuchs-Rosenthal counting chamber.

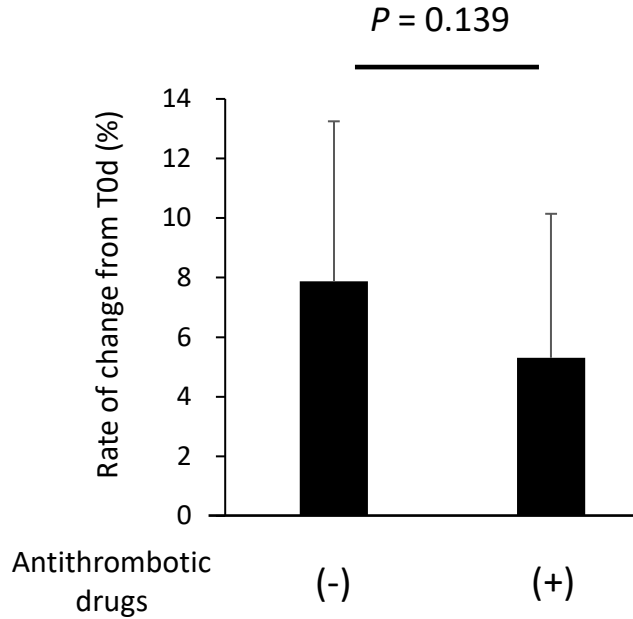
## Suppl. Fig 2



### Supplementary Figure 2.

Blood was collected intravenously into a blood collection tube containing a separator and centrifuged under the specified conditions ( $1710 \times g$  for 10 min, and  $2330 \times g$  for 7 min, 10 min, and 15 min). Because the RBCs in the serum that remain at the top of the separator must be completely haemolyse, the serum layer was discarded, replaced with sterile water, mixed, and allowed to stand for 6 h at room temperature. The upper layer was discarded, and then the saline solution was added to the tubes. The upper layer was used as the sample. The samples were then stored at  $4^{\circ} \text{C}$  for 14 days, and potassium levels were measured using TBA-120FR

## Suppl. Fig 3

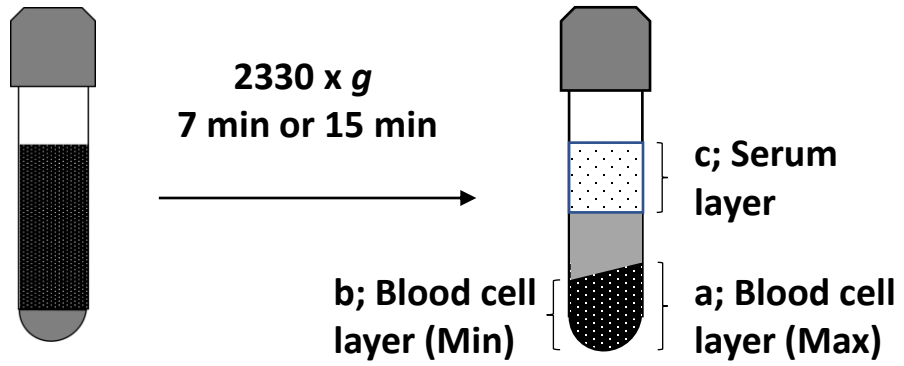


### Supplementary Figure 3.

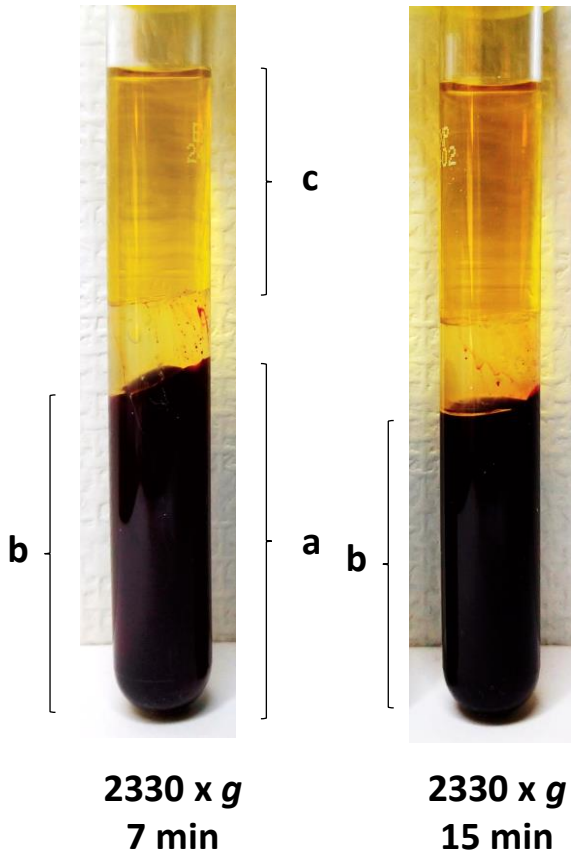
Rate of change (%) in serum potassium levels from the day of blood collection (T0d) to T14d after refrigeration storage in patients on antithrombotic drugs (N = 15) or not (N = 42).

# Suppl. Fig 4

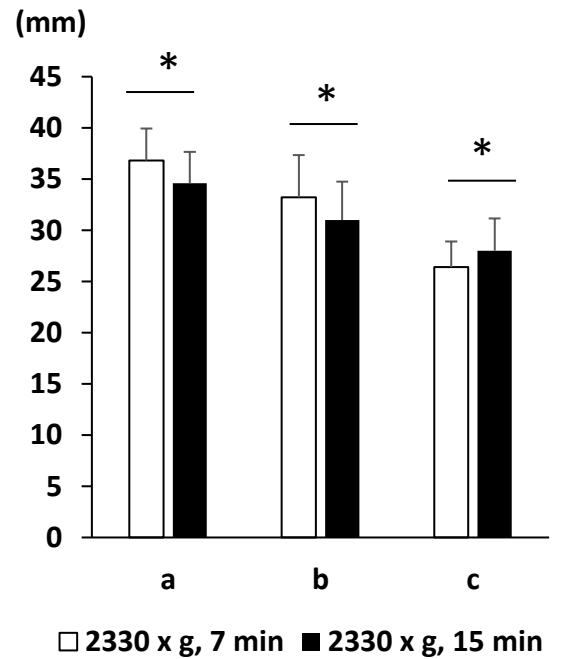
**A**



**B**



**C**



#### **Supplementary Figure 4.**

Differences in the separation of serum and blood cell layers under different centrifugal conditions (N = 5).

(A) Schematic image in this experiment. We centrifuged venous blood at  $2330 \times g$  for 7 min, and 15 min: the maximum blood cell layer diameter was (a, mm), and the minimum blood cell layer diameter was (b, mm), and the serum layer diameter was (c, mm),

(B) Representative data in blood collection tube after centrifugation at  $2330 \times g$  for 7 min, and 15 min.

(C) The length of the serum layer was significantly greater when centrifuged at  $2330 \times g$  for 15 min than at  $2330 \times g$  for 7 min, and conversely, it was significantly less in the clot layer. Values shown are means  $\pm$  standard deviation (SD). Statistical significance was determined by using the Student t test. \* $P < 0.05$ .