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# Fresh frozen plasma kept at cryogenic temperatures in Bio Boxes during marine transportation

## Abstract

**Introduction:** It is difficult to maintain fresh frozen plasma (FFP) at cryogenic temperatures during marine transportation, even with a power supply.

**Methods:** In this study, without a power supply and at temperatures below -30 °C, we used BioBoxes used for transportation of coronavirus disease vaccines, for two demonstration experiments involving round-trip FFP transportation to Hachijojima Island (200 km from Tokyo) and Ogasawara (1,000 km from Tokyo).

**Results:** We successfully transported FFP at a temperature of -70 °C or lower. There was no change in the properties of FFP and minimal change in the activities of clotting factors.

**Conclusion:** We could transport FFP to an isolated island in BioBoxes in a passenger ship, without a power supply using only dry ice. Furthermore, we demonstrated that FFP does not get destroyed or thaw due to vibration of the passenger ship, even in an approaching typhoon, proving possibilities for stable supply of FFP.

**Keywords:** coronavirus disease-19, plasma, severe acute respiratory syndrome coronavirus 2, temperature, vaccines

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## Introduction

Surgical procedures often require replacement of red blood cells (RBCs) and clotting factors; this imposes the need to transport blood products and fresh frozen plasma (FFP). In Japan, RBCs for transfusion are usually transported by land in shipping boxes maintained at temperatures of 2–6 °C for 2–3 hours. An active transport refrigerator (ATR) can control the temperature for longer hours (ATR700; TOHO Pharmaceutical Co., Ltd., Tokyo, Japan). Additionally, drones are used in cases of unsafe roads; fixed-wing drones are currently under study in Rwanda.[1,2]

In Japan, passenger ships are used to transport RBC solutions to nearby islands, and aircrafts and helicopters are used in emergencies. Trial transportation of helicopters without a crew have done.[3] Marine transportation has been used to transport RBC solutions in ATRs to the remote island (Ogasawara).[4]

However, there is no report on a novel means of FFP transportation, given that FFP has to be transported in cryogenic temperature; that is, below -30 °C, in line with the recommendations of the Japanese Red Cross Society.[5,6] The coronavirus disease (COVID-19) vaccine needs cryogenic (below -70 °C) transportation using dry ice. Therefore we thought that we were able to use the same way of vaccine transport to the FFP transportation which recommended below -30 °C. Just checking this way, we performed an experiment using dry ice over a prolonged time period in the absence of power supply. We used BioBoxes to keep the temperature inside the chamber at approximately -70 °C; it was effective. Therefore, we planned two experiments using BioBoxes for transportation of COVID-19 vaccines. We transported FFP to and from Hachijojima (approximately 200 km from Tokyo) and Chichijima Island in the Ogasawara Islands (1,000 km from Tokyo) over the Pacific Ocean. We assessed transportation safety and clotting factor activity. In this pilot experiment, a BioBox Cell® (BBC; 340 × 260 × 340 mm, 3 kg, Sugiyamagen Co. Ltd., Tokyo, Japan) and BioBox Freezer® (BBF; 560 × 440 × 430 mm, 10.5 kg, Sugiyamagen Co. Ltd., Tokyo, Japan) of the BioBox series were each filled with dry ice and placed in our laboratory for four days and 11 days, respectively, to maintain the cryogenic temperature. We monitored the temperature inside the BBC and BBF. We observed that the boxes could maintain temperatures at <-70 °C. Based on these observations, without a power supply and at temperatures below -30 °C, we used BioBoxes for transportation of COVID-19 vaccines for two demonstration experiments involving round-trip FFP transportation to Hachijojima Island (200 km from Tokyo) and Ogasawara (1,000 km from Tokyo).

## Methods

Preparation of FFP packs

### Experiment 1

Three samples of whole blood were collected by phlebotomy from patients with polycythemia; the samples were separated into plasma and blood cells using the standard method recommended by the Japanese Red Cross Society. We divided each plasma sample into three unequal portions (30 mL, 100 mL, and 100 mL). We stored the 30-mL portion (the underlying FFP; FFP-A, FFP-B, FFP-C) in a pack (BB-TQ008J; Terumo Co. Lt., Tokyo, Japan) at -80°C

in an electric freezer in the laboratory, while waiting for the time duration of the experiment; the remaining two 100-mL portions (FFP-A1, FFP-B1, FFP-C1; and FFP-A2, FFP-B2, FFP-C2, respectively) were each stored in a pack (BB-TQ020CJ; Terumo Co. Lt., Tokyo, Japan) in the same manner.

We used one of the 100-mL packs (FFP-A1, FFP-B1, and FFP-C1) for transportation and the other (FFP-A2, FFP-B2, FFP-C2) as a control.

### Experiment 2

Using the same procedure as in Experiment 1, three blood samples were divided into FFP-D, FFP-E, and FFP-F as the underlying FFP; FFP-D1, FFP-E1, FFP-F1 as FFP for transportation, and FFP-D2, FFP-E2, FFP-F2 as controls.

Round-trip transportation of the blood products to islands in the Pacific Ocean

For both voyages, thermometers, a watch Logger (KT-265F, Fujita Electric Works, Ltd, Kanagawa, Japan), and vibrometers (CEM DT-178A USB 3-Axis Vibration Datalogger, Shenzhen Everbest Machinery Industry Co., LTD, Shenzhen, China) were attached to the top of the BBC and BBF; these instruments were used to measure the parameters of the outside environment.

### Experiment 1

We transported FFP-A1, FFP-B1, and FFP-C1 in the BBC to the passenger terminal in Takeshiba Pier, Tokyo, in a private car; this was approximately 10 km from the laboratory. They were then transported to and fro Hachijojima (290 km from Tokyo) by sea in Tachibana-maru (passenger ship, Tokai Kisen Co., Ltd.). We measured the temperature of the surface of the FFP-A1 pack using a watch logger (KT-155F/Ex, Fujita Electric Works, Ltd, Kanagawa, Japan).

The FFP packs left Takeshiba Pier at 11:00 pm on October 14, 2021 and returned to the pier at 3:00 pm on October 15, 2021—the following day. We transported the FFP packs to the laboratory by car.

### Experiment 2

We transported the FFP packs in a BBF to Chichijima (1,000 km from Tokyo). FFP-D1, FFP-E1, and the FFP-F1 was similarly transported in the BBF to Takeshiba Pier. They were shipped using ocean transportation in Ogasawara-Maru (passenger ship, Ogasawara Kaiun Co., Ltd.) to and from Chichijima. We measured the temperature of FFP-D1 as we did in Experiment 1.

The FFP packs left Takeshiba Pier at 10:00 am on November 26, 2021. The ship took 25 hours to get to Chichijima, where it anchored for three nights at the Chichijima Port. The FFP packs arrived Takeshiba Pier at 3:00 pm on December 6, 2021. The FFP packs were then transported to the laboratory by car.

### Macroscopic findings (Experiments 1 and 2)

We verified whether the transported FFP packs were damaged or whether the FFP started thawing.

### Measurement of activity levels of clotting factors (Experiments 1 and 2)

We evaluated the activities of the clotting factors in the FFP; the activity levels were measured before the experiment (underlying FFP), during FFP transportation by sea, and during FFP storage in the laboratory. In addition, in Experiment 1 we passed the FFP through a blood transfusion filter to replicate an actual blood transfusion. We also evaluated the activities of the clotting factors.

Clotting tests (plasma fibrinogen, activated partial thromboplastin time [aPTT], and prothrombin time) and activities of clotting factors (factors II, V, VII, IX, X, XI, XIII, and von Willebrand factor) were measured by BML Inc. (Tokyo, Japan).

### Numerical evaluation

The rate of deterioration of the clotting factors (%DCA) were calculated as follows: %DCA = (the activity of the FFP clotting factors after transportation or that of the control FFP) / the activity of the FFP clotting factors of the underlying FFP. We then calculated the mean and standard deviation of the DCA of the FFP transported to the island and that of the FFP left in the laboratory in Experiments 1 and 2, respectively. We assessed variance using an F-test in Excel version 3.0, with a risk rate of 5%.

## Results

Round-trip transportation to an island in the Pacific Ocean

### Experiment 1

During the voyage to Hachijojima, the outer envelope temperature of the BBC when placed in a cabin was approximately 22 °C (21.8 – 22.2 °C). The temperature of the inside surface of the BBC and the FFP pack remained below -70 °C. The external forces were calculated as follows: External force (g) = square root {X-axis force^2 + Y-axis force^2 + (absolute value of Z-axis force-1) ^2} (g). The vibrations were approximately 0.7 g.

### Experiment 2

During the voyage to Chichijima, the outer envelope temperature of the BBC when placed in a cabin was approximately 23 °C (20.7–23.8 °C). The temperature of the inside surface of the BBC and the FFP pack remained below -70 °C (Figure 1A).

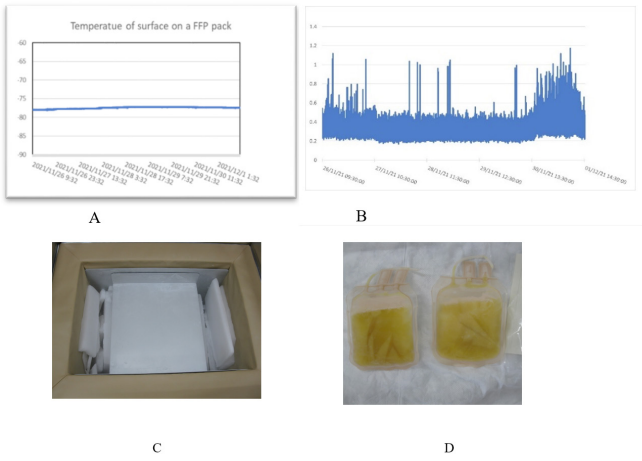


**Figure 1** Temperature, vibration, and macroscopic effects of transportation to Ogasawara,

Chichijima Island

A Shows that the surface temperature (e of the FFP-D1 pack in the BBF (BioBox Freezer) with 20

kg of dry ice during its transportation to Chichijima is below -75 °C. B shows that the external force

(g) of the vibration during the transportation of the packs to Chichijima voyage was maximum 1.2 g; even

with the approaching typhoon, it was about 1 g. C shows a decrease in dry ice in the BBF after the

Chichijima voyage. D shows an FFP pack with no signs of damage or thawing.

FFP, fresh frozen plasma

We thought that the vibrations would be substantial because of an approaching typhoon, but they were generally insignificant. However, the vibrations were approximately 1 g (Figure 1B). The reduced dry ice and BBF after the transport experiments are shown in Figure 1C.

### Macroscopic findings (Experiments 1 and 2)

In both experiments, we observed that the transported FFP packs were not damaged and there had been no shipping-related thawing. Figure 1D shows the state of the FFP packs after they were transported

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to Chichijima (Ogasawara) in Experiment 2; as in Experiment 1, there were no macroscopic changes.

Activity levels of the clotting factors

## Experiment 1

The results of the BML measurements of clotting factor activity in FFP-A, FFP-B, and FFP-C are in the first section of Table 1. The results after transportation to Hachijojima and during storage in the laboratory are shown in Subscript 1 (FFA-1, for example) and Subscript 2 (example: FFA-2, ), respectively. There was a most no decrease in activity values in both cases.

**Table 1** Clotting activity levels and Rate of change for marine transport.

| | aPTT (sec) | %PT | INR | Fib (mg/dL) | II (%) | V (%) | VII (%) | VIII (%) | IX (%) | X (%) | XI (%) | XIII (%) | vWF (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Clotting activity levels in the FFP transported to Hachijojima Island** | | | | | | | | | | | | | |
| FFP-A | 30.2 | 105.2 | 0.97 | 261 | 106.4 | 100 | 99.5 | 66.5 | 108.7 | 113.7 | 88.2 | 113 | 62.9 |
| FFP-A1 | 30.3 | 105.1 | 0.97 | 255 | 106.4 | 93 | 98 | 61.4 | 107.6 | 113.7 | 87.5 | 106 | 62.4 |
| FFP-A2 | 30.6 | 105.1 | 0.97 | 255 | 102 | 87.9 | 99.5 | 60.6 | 105.6 | 112.5 | 89.8 | 107 | 62.5 |
| TF-A1 | 29.6 | 105.1 | 0.97 | 255 | 102 | 95.7 | 98 | 61.4 | 103.6 | 112.5 | 89.8 | 108 | 61.4 |
| TF-A2 | 29.6 | 111.1 | 0.95 | 261 | 103.4 | 94.3 | 98 | 61 | 107.6 | 113.7 | 88.2 | 111 | 61 |
| FFP-B | 32.6 | 93.7 | 1.04 | 313 | 109.5 | 90.4 | 96.6 | 105.5 | 111.9 | 118.4 | 106.3 | 162 | 103.5 |
| FFP-B1 | 32.5 | 93.8 | 1.04 | 303 | 107.9 | 75.6 | 90.9 | 111.5 | 105.6 | 113.7 | 105.3 | 144 | 105.9 |
| FFP-B2 | 33 | 90.9 | 1.05 | 303 | 107.9 | 76.6 | 93.7 | 110.6 | 106.6 | 116 | 106.3 | 146 | 105.4 |
| TF-B1 | 32.7 | 95.1 | 1.03 | 314 | 107.9 | 76.6 | 93.7 | 108.9 | 108.7 | 116 | 107.2 | 140 | 103.7 |
| TF-B2 | 32.4 | 92 | 1.05 | 310 | 109.5 | 78.7 | 93.7 | 106.3 | 110.8 | 118.4 | 109.2 | 148 | 104.4 |
| FFP-C | 35.3 | 105.2 | 0.97 | 246 | 91.4 | 97.1 | 130.5 | 154.6 | 92.4 | 124.8 | 67.7 | 121 | 171.2 |
| FFP-C1 | 35.9 | 105.1 | 0.97 | 241 | 90.2 | 91.7 | 132.7 | 148.2 | 89.8 | 122.2 | 66.5 | 120 | 166.3 |
| FFP-C2 | 35.7 | 105.1 | 0.97 | 239 | 92.6 | 94.3 | 128.4 | 137.6 | 90.7 | 122.2 | 66.5 | 118 | 166.6 |
| TF-C1 | 35.6 | 106.9 | 0.96 | 239 | 91.4 | 94.3 | 135 | 145.8 | 91.5 | 123.5 | 65.4 | 114 | 176.3 |
| TF-C2 | 35.7 | 106.9 | 0.96 | 243 | 91.4 | 95.7 | 135 | 143.4 | 90.7 | 120.9 | 63.3 | 111 | 171.8 |
| **Average of % DCA and SD after trip Hachijojima Island (%)** | | | | | | | | | | | | | |
| Transport, average | 100.6 | 100 | 100 | 97.5 | 99.1 | 90.4 | 98.1 | 98 | 96.8 | 98 | 98.8 | 94 | 99.6 |
| SD | 0.8 | 0.1 | 0 | 0.5 | 0.7 | 4.8 | 3.1 | 5.7 | 1.9 | 1.6 | 0.4 | 4.2 | 2.1 |
| Stored, average | 101.2 | 98.9 | 100.3 | 97.2 | 98.6 | 89.9 | 98.5 | 95 | 96.9 | 98.3 | 100 | 94.1 | 99.5 |
| SD. | 0.1 | 1.4 | 0.5 | 0.4 | 2.2 | 5.3 | 1.2 | 7 | 1.2 | 0.5 | 1.5 | 3 | 1.8 |
| **Average of % DCA and SD after passage via transfusion filter** | | | | | | | | | | | | | |
| Transport,average | 99.7 | 101 | 99.3 | 98.4 | 98.1 | 92.5 | 99.6 | 96.6 | 97.2 | 98.6 | 99.8 | 92.1 | 100.3 |
| SD | 1.2 | 0.8 | 0.5 | 1.4 | 1.7 | 5.5 | 2.8 | 4.7 | 1.5 | 0.5 | 2.3 | 4 | 2.2 |
| Stored, average | 99.5 | 101.8 | 99.3 | 99.3 | 99.1 | 93.3 | 99.6 | 95.1 | 98.7 | 99 | 98.7 | 93.8 | 99.4 |
| SD. | 1.3 | 3 | 1.3 | 0.5 | 1.3 | 4.7 | 2.8 | 4 | 0.4 | 1.5 | 3.9 | 3.2 | 1.7 |
| **Clotting activity levels in the FFP transported to Chichijima Island (Ogasawara)** | | | | | | | | | | | | | |
| FFP-D | 33.5 | 88.1 | 1.07 | 433 | 122 | 90.2 | 97.1 | 78.7 | 130.4 | 100 | 114 | 132 | 95.9 |
| FFP-D1 | 31.5 | 90.7 | 1.05 | 463 | 120.9 | 89 | 97.1 | 76.9 | 140.1 | 104.3 | 112.4 | 130 | 95.2 |
| FFP-D2 | 31.7 | 89.3 | 1.06 | 471 | 119 | 90.3 | 95.6 | 77.5 | 140.1 | 105.4 | 113.5 | 132 | 95 |
| FFP-E | 36.2 | 98.4 | 1.01 | 265 | 84.8 | 71.6 | 97.1 | 64.2 | 82.1 | 82.5 | 68.5 | 121 | 77.6 |
| FFP-E1 | 35.7 | 98.4 | 1.01 | 277 | 85.6 | 73.9 | 98.7 | 60.3 | 89 | 86 | 71.6 | 110 | 82.5 |
| FFP-E2 | 35.2 | 98.4 | 1.01 | 263 | 86.9 | 76 | 98.7 | 62.1 | 86.6 | 86 | 71 | 111 | 77.9 |
| FFP-F | 30 | 100 | 1 | 277 | 79.9 | 86.3 | 101.9 | 148.9 | 105.9 | 73.6 | 89.1 | 126 | 157.8 |
| FFP-F1 | 29.1 | 103.4 | 0.98 | 286 | 83.3 | 90.3 | 97.1 | 137.1 | 116.6 | 78.1 | 91.4 | 120 | 158.1 |
| FFP-F2 | 29.1 | 103.4 | 0.98 | 280 | 79.8 | 90.3 | 98.7 | 141.7 | 115.5 | 75.9 | 93.8 | 122 | 161.8 |
| **Average of % DCA and SD after trip to Chichijima Island (Ogasawara) (%)** | | | | | | | | | | | | | |
| Transport, average | 96.5 | 102.1 | 98.7 | 104.9 | 101.4 | 102.2 | 99 | 94.6 | 108.6 | 104.9 | 101.9 | 94.9 | 101.9 |
| SD | 1.9 | 1.5 | 0.9 | 1.5 | 2.1 | 2.5 | 2.7 | 2.3 | 1.1 | 0.9 | 2.5 | 3.1 | 3.1 |
| Stored, average | 96.3 | 101.6 | 99 | 103 | 100 | 103.6 | 99 | 96.8 | 107.3 | 104.3 | 102.8 | 96.2 | 100.7 |
| SD | 1.2 | 1.4 | 0.8 | 4.1 | 2 | 2.6 | 2 | 1.4 | 1.5 | 0.9 | 2.4 | 3.4 | 1.4 |

aPTT, activated partial thromboplastin time; %PT, % prothrombin time; INR, international normalized ratio; Fib, Fibrinogen; vWF, von Willebrand factor; FFP, fresh frozen plasma; % DCA, the deterioration of clotting factor activity (%); SD, standard deviation

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### Experiment 2

The results for FFP-D, FFP-F, and FFP-E after transportation to Chichijima are comparable to those in the second part of Table 1.

%DCAs with sea transport

### Experiment 1

We compared the %DCAs to the values in the second part of Table 1. The analysis of variance showed that the aPTT of stored FFP was longer than that of transported FFP, but there were no other changes. In addition, the same test was performed using FFP after its passage through the transfusion filter, and the aPPT remained unchanged.

### Experiment 2

There was no difference in the %DCAs between this experiment and those in the second part of Table 1.

## Discussion

We successfully transported FFP at cryogenic temperatures using the BioBox series of low-temperature transport boxes that were used recently to transport COVID-19 vaccines. The FFP was transported to an isolated island, about 1000 km from Tokyo, using a passenger ship and without a power supply. Dry ice was used to maintain the temperature. Furthermore, we showed that FFP does not deteriorate or disintegrate with vibration of the ship. This process can also be managed under approaching typhoon conditions.

There was a significant difference in the %DCA of aPTT during the transportation to Hachijojima; however, the longer aPTT was as a result of storage in the laboratory and not as a result of transportation.

Our findings reveal the possibility of supplying FFP in isolated islands using passenger ships without a special low-temperature freezer with power supply.

In addition, FFP has an extended shelf life, but there is always the challenge of expiry dates in relation to residual FFP kept on islands. The Japanese Red Cross Society states that the expiration date is 30 days.[5] RBC solutions are supplied to isolated islands using ATR to control the temperature. RBCs can also get expired. To reduce wastage, RBC solutions are transported to Tokyo in ATRs when their expiration date is approaching.

## Conclusion

Based on our results, we hypothesize that FFP in a BioBox can be used for blood product rotation.

So far, this has been prevented by financial constraints that come with transportation and the need for cryogenic refrigerators with a power supply.

## Acknowledgments

None.

## Conflicts of interest

The authors declare no conflicts of interest.

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