図 1. 文献9より引用

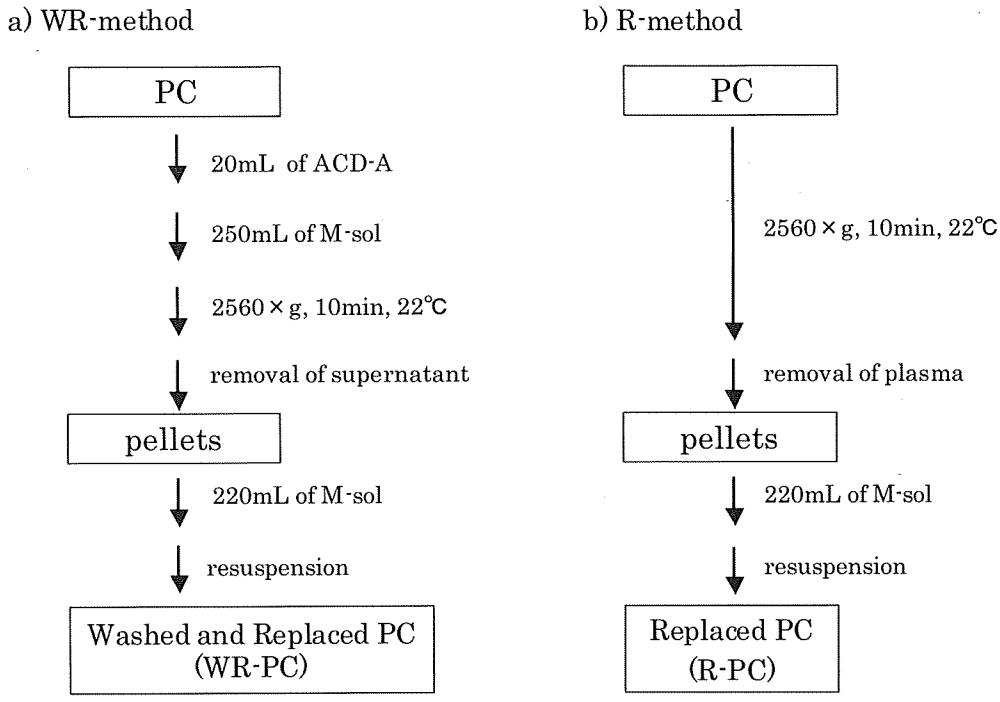


Fig. 1 Two methods of preparing W/R-PC

WR-method: Washing and replacement method.
R-method: Replacement method.

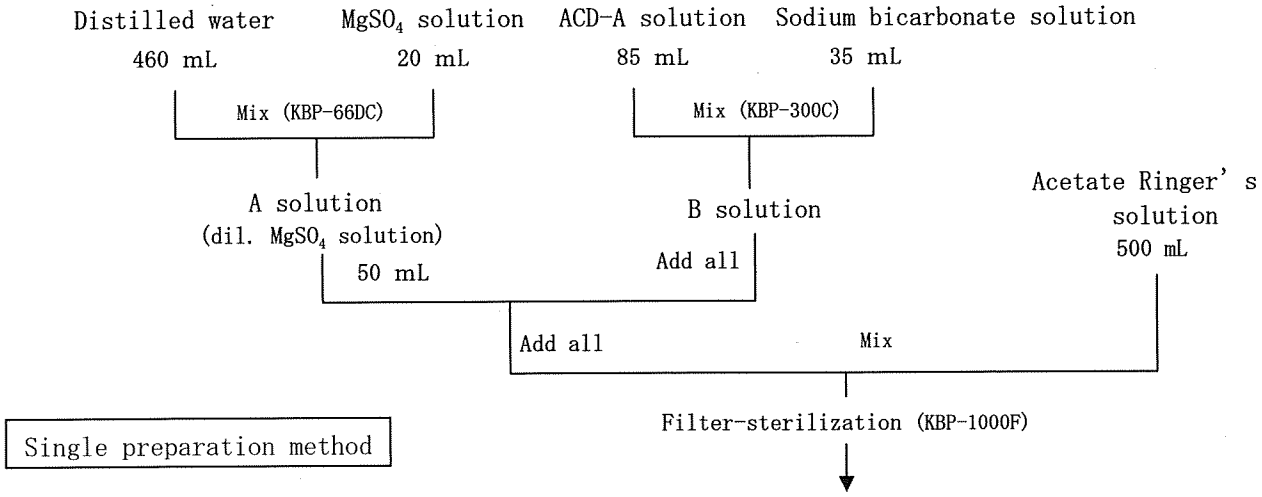
表 1. 文献9より引用

Table 1 In vitro variables for PCs

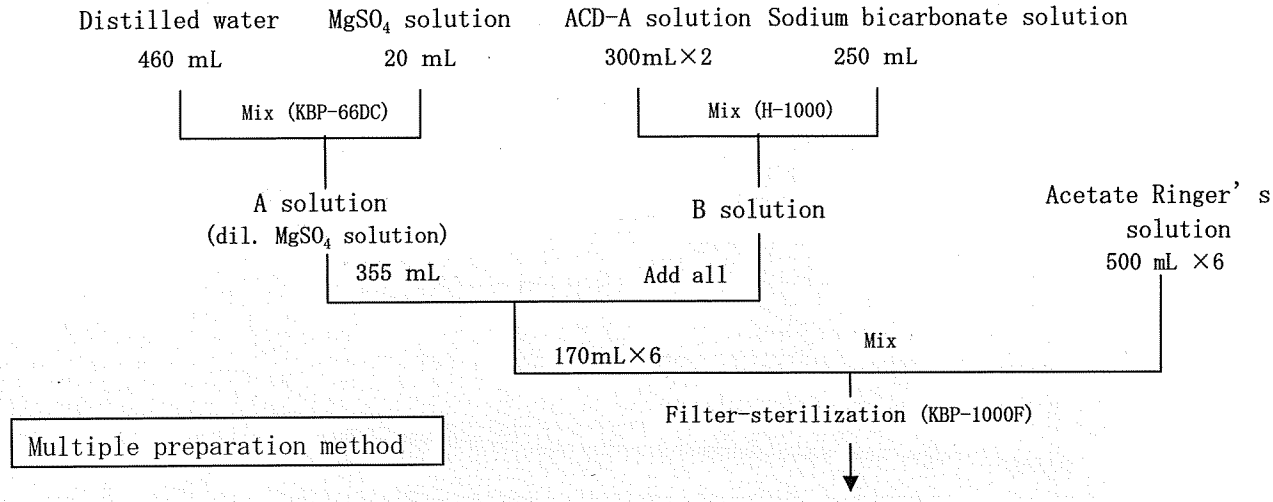
Measurement	Method/Machine
Platelet count	Automated blood cell counter (K-4500、Sysmex)
Supernatant protein	Bicinchoninic acid method (BCA Protein Assay Reagent Kit、PIERCE)
pH	Automatic blood gas analyzer (248 pH/blood gas analyzer、Chiron Diagnostics Ltd.)
Aggregation	Induction with ADP 5 μM and collagen 1 μg/mL (Aggrepack, Arkray Co., HEMA TRACER 313M aggregometer)
HSR	Holme's[8] method
P-selectin	Hagberg's[9] flow cytometric method
Morphology	Percentage of normal discoid PLTs, discriminated from non-discoid PLTs following Kunichi's[10] method
Swirling	Visual inspection of swirling under the light

図 2. 文献10より引用

Fig. 1 Preparation of Single and Multiple M-sols



Single preparation method-derived M-sol



Multiple preparation method-derived M-sol

図3. 文献10より引用

Fig. 3 pH changes in vacuum packed M-sols under various preservation temperatures

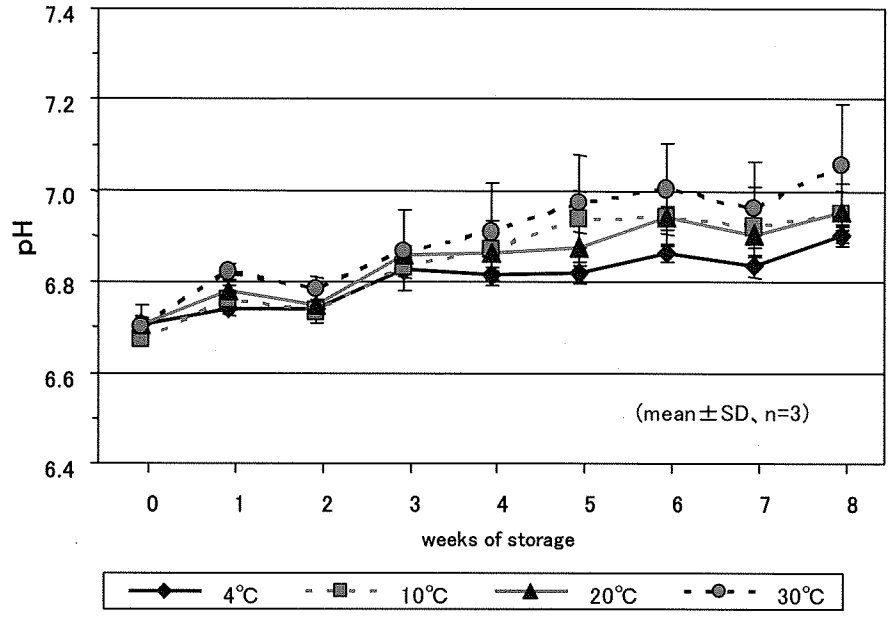


Fig. 4 pH changes in M-sols during preservation for one year

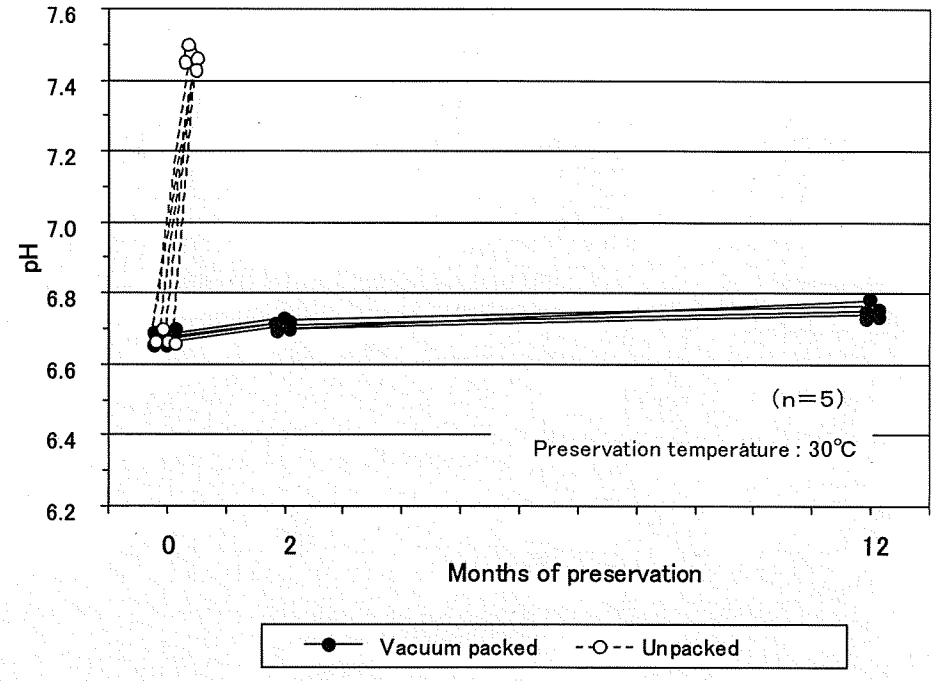


表 2. 文献10より引用

Table 2 In vitro variables of single-use and multiple-use M-sols

	Single-use	Multiple-use
pH	6.8±0.1 (6.7～7.1)	6.8±0.1 (6.8～7.0)
Na^+ (mmol / L)	150.0±2.4 (144～156)	152.3±1.0 (151～155)
K^+ (mmol / L)	2.7±0.1 (2.5～2.7)	2.7±0.1 (2.7～2.8)
Cl- (mmol / L)	80.5±1.6 (76～83)	81.4±1.2 (78～83)
Mg^{2+} (mmol /L)	1.6±0.1 (1.5～1.9)	1.6±0.03 (1.5～1.6)

n=30、mean±SD (MIN～MAX)

表 3. 文献10より引用

Table 3 In vitro qualities of R-PC prepared by fresh M-sol and stored M-sol

	M-sols	Storage period of R-PC*		
		24 hrs	48 hrs	120 hrs
Total PLT count ($\times 10^{11}$/bag)	Fresh †	3.1±0.8	NT	NT
	Stored‡	3.1±0.8	NT	NT
MPV (fL)	Fresh	7.8±0.6	7.9±0.7	8.1±0.3
	Stored	7.8±0.5	7.8±0.5	8.1±0.3
pH	Fresh	7.58±0.06	7.65±0.06	7.67±0.03
	Stored	7.61±0.05	7.65±0.05	7.68±0.03
P-selectin (%)	Fresh	2.6±2.0	1.6±1.4	3.9±1.3
	Stored	2.7±2.5	1.5±1.5	3.7±1.6
Aggregation (%) 10 μg/mL collagen	Fresh	79.0±1.4	88.5±6.4	84.0±5.2
	Stored	82.0±0.0	88.0±8.5	85.7±6.4
%HSR (%)	Fresh	71.7±4.5	72.1±6.4	73.9±3.8
	Stored	70.4±7.1	72.1±3.7	74.1±5.8
Glucose ($mmol/10^{12}$plts)	Fresh	10.3±2.7	9.9±2.5	7.0±2.2
	Stored	10.9±2.7	10.1±2.5	7.4±2.1
Lactate ($mmol/10^{12}$plts)	Fresh	2.1±0.4	3.4±1.0	6.2±0.9
	Stored	2.0±0.5	3.3±0.9	6.4±0.9
LDH (IU/L)	Fresh	64.8±27.7	83.0±24.6	119.1±32.1
	Stored	58.8±20.9	81.8±27.4	122.0±35.8

表4. 文献9より引用

Table 2 Comparison of in vitro qualities of W/R-PCs prepared by the WR-method and R-method

Method	PCs	W/R-PCs	
Total platelets ($\times 10^{11}$/bag)			Recovery of platelets (%)
WR-method	3.23 ± 0.17	2.92 ± 0.16	90.5 ± 1.4
R-method	3.21 ± 0.17	2.87 ± 0.15	89.5 ± 1.8
Plasma proteins (mg/bag)			Removal of plasma protein (%)
WR-method	13568 ± 506	428 ± 95]**	96.9 ± 0.7]**
R-method	13499 ± 535	627 ± 130]	95.4 ± 0.9]

** $p < 0.01$ (Wilcoxon t-test) mean ± S.D. n = 7

WR-method: Washing and replacement method.
R-method: Replacement method.

表 5.　文献9より引用

Table 3　Changes of in vitro qualities of W/R-PCs prepared by the WR method and R method

		PCs	W/R-PCs	after 24hrs	after 48hrs
pH					
	WR-method	7.15±0.10	6.95±0.06	7.37±0.03	7.53±0.05
	R-method	7.15±0.10	6.98±0.06	7.40±0.02	7.52±0.02
	control	7.15±0.09	—	7.05±0.04	7.00±0.03
Aggregation (%) (ADP+collagen)					
	WR-method	60.4 ± 3.2	55.0 ± 14.0	59.2 ± 4.9	57.4 ± 4.0
	R-method	60.4 ± 3.2	54.7 ± 14.5	58.9 ± 6.2	57.0 ± 5.5
	control	60.4 ± 3.2	—	65.6 ± 2.4	61.8 ± 2.6
HSR (%)					
	WR-method	80.8 ± 4.7	73.4 ± 7.1	75.5 ± 8.9	73.9 ± 4.4
	R-method	80.8 ± 4.7	73.8 ± 9.0	75.5 ± 5.7	76.3 ± 5.4
	control	80.8 ± 4.7	—	80.6 ± 3.7	80.6 ± 3.6
P-selectin (%)					
	WR-method	1.9 ± 0.8	4.1 ± 3.4	3.1 ± 1.7	3.1 ± 1.4
	R-method	1.9 ± 0.8	3.0 ± 2.4	3.5 ± 2.5	3.3 ± 1.5
	control	1.9 ± 0.8	—	1.9 ± 0.9	2.7 ± 0.9
Morphology (%disk)					
	WR-method	68.0 ± 6.4	63.6 ± 6.7	62.0 ± 4.7	60.6 ± 5.7
	R-method	68.0 ± 6.4	63.1 ± 11.2	65.1 ± 6.9	62.3 ± 7.7
	control	68.0 ± 6.4	—	67.4 ± 5.5	63.3 ± 5.3
Swirling (++; double positive、+ ; positive、-; negative)					
	WR-method	++	++	++	++
	R-method	++	++	++	++
	control	++	—	++	++

** $p < 0.01$ (Wilcoxon t-test with Bonferroni correction)　mean ± S.D. n = 7 each

・PCs were washed and replaced after 72hrs storage.

表6.　学会発表12より引用

非溶血性輸血副作用

Non-hemolytic transfusion reactions per bag from 2003 to 2008.

Blood products	Reaction products	Transfusion products	% of reactions
Red cell concentrates (RCC)	90 (14.8%)	23,658 (35.6%)	0.38
Fresh frozen plasma (FFP)	111 (18.3%)	32,655 (49.1%)	0.34
Platelet concentrates (PC)	402 (66.2%)	9,719 (14.6%)	4.14
Washed red cell concentrates (W-RCC)*	3 (0.5%)	271 (0.4%)	1.12
Washed platelet concentrates (W-PC)**	1 (0.2%)	192 (0.3%)	0.52
Total	607	66,495	0.91

* With or without leukocyte-depleted products;
** Over 90% of plasma is removed from PC as W-PC in secondary processing in the institute. In this process, PC is washed and replaced with G-sol containing acetate Ringer solution with glucose, sodium hydrogen carbonate, ACD-A, and distilled water.
The product is transfused within 24h.

表 7.　文献9より引用

Table 4　Corrected count increment (CCI) after W/R-PCs transfusion

		WR-method (WR-PCs)	R-method (R-PCs)
CCI ($\times 10^4/\mu L$)	1 hr	2.27 ± 0.99 (n = 44)	2.39 ± 0.74 (n = 14)
	24 hr	1.53 ± 0.82 (n = 51)	1.59 ± 0.78 (n = 18)

mean ± S.D.

表 8. 文献9より引用

Table 5 Adverse reactions after W/R-PCs transfusion

	WR-method (WR-PCs)	R-method (R-PCs)
adverse reaction/transfusion	0 / 75	0 / 31

表 9-1. 熊本県下 H21 年度(4 月～11 月)と H20 年度洗浄PC調製数

	H21年度(11 月まで)		H20年度(1年間)	
	洗浄PC	一般PC	洗浄PC	一般PC
熊本大学病院	16	1,159	10	1,615
A 病院	5	1,939	0	2,950
B 病院	3	792	5	1,221
洗浄PC合計	24		15	
県内PC10 使用総本数		7,838		11,851

表 9-2. 熊本県下 H20 年度洗浄赤血球の使用比率

本数

	洗浄血	赤血球	洗浄/赤血球
C 病院	36	38	94.7
D 病院	48	53	90.6
E 病院	18	26	69.2
F 病院	4	6	66.7
G 病院	28	72	38.9
H 病院	2	11	18.2
I 病院	1	7	14.3
J 病院	1	13	7.7
K 病院	4	56	7.1
L 病院	1	18	5.6
M 病院	1	22	4.5
N 病院	9	206	4.4
O 病院	2	53	3.8
P 病院	2	93	2.2
Q 病院	1	305	0.3
B 病院	8	4338	0.2
R 病院	5	3250	0.2
熊本大学病院	5	3802	0.1
A 病院	7	5671	0.1
S 病院	1	1116	0.1
洗浄血合計	184		
赤血球使用総本数		54,666 本	

別紙 1

Attachment 1-1

Indication guidance for washed and replaced platelets and their preparation (Version II) (Provisional)

Original version, February 1, 2008
Revised version presented, February 1, 2009

Purpose

In clinical settings, platelets are washed before administration to patients for the purpose of preventing side effects such as uticaria, fever, respiratory distress, decreased blood pressure and anaphylaxis from transfusion of platelet preparations. However, there are no clear guidelines for their indications, judgment of effects, composition of fluids used in washing, or washing methods, and no means of uniform evaluation for the prevention of side effects. The transfusion formulation subcommittee of our Society conducted a questionnaire survey on the use of washed and replaced platelet preparations in 2005 and 2006, and showed the effectiveness of washing and replacement of platelets in preventing side effects. These guidelines were prepared based on the results of that questionnaire survey, with the aim of safer and more appropriate preparation and use of washed and replaced platelets.

Indications

Cases in which side effects that cannot be prevented with preadministration of various drugs or other measures are observed two or more times.
Cases in which serious side effects such as anaphylactic shock are observed once.

Washing and replacement (preparation)

Acetate Ringer solution and physiological saline are the main solutions used in washed and replaced platelet preparations. However, there is a difference in platelet function at 24 hours after washing and replacement,[1,2,3] and so it is desirable to use a preparation depending on the time since preparation. The composition of washing and replacement solutions actually used is described in Document 1, and the method of preparation in Document 2. Refer to Document 3 for actual replacement procedures.

1) Selection of washing and replacement solution

- M-sol is preferable when the transfusion is performed on the day following preparation. However, washing weakens the antimicrobial/microbicidal activity in plasma, and so when administering on the day following preparation, the start of administration should

not be unnecessarily prolonged.

- When transfusion is conducted on the day of preparation, a solution other than M-sol that may be used is glucose acetate Ringer solution (G-sol).
- When used soon after preparation, in cases when neither M-sol nor G-sol are used, physiological saline with added anticoagulant (S-sol) or frozen blood wash solution No. 3 with added anticoagulant (B-sol) can also be used.

2) Washing method:

- Plasma replacement alone is sufficient.
- With plasma replacement, clearing of no less than 90% of plasma is possible, and sufficient side effect prevention effect may be expected.

Determination of transfusion effects

If the washed and replaced platelets are concentrated, a roughly equivalent effect can be expected. In judging the effects of transfusion, it is desirable to use the CCI value, of which an objective determination can be made.

According to "Use of blood preparations" (edited by the Ministry of Health, Labor and Welfare), when a platelet concentrate is transfused, the CCI value at 1 hour is normally $\geq$7,500/μl, and on the following day or after 24 hours $\geq$4,500/μl.

Informed consent

It is necessary to obtain informed consent in all cases prior to administration of washed and replaced platelets prepared in hospital.

Observation of clinical symptoms at time of transfusion

Close attention is needed for side effect prevention and whether there are adverse events.

References

1) Sasaki D, Kosunago S, Komiyama Y, Suzuki K, Urano S, Nakano T, Shimizu T, KamiyaT, Hiranuma T, Nishioka K, Ida Y. Comparison of platelet quality in washed platelet concentrates prepared with different synthetic storage solutions. Japanese Journal f Transfusion Medicine 47: 777-782, 2001

2) Hirayama J, Azuma H, Fujihara M, Homma C, Yamamoto S, Ikeda H. Storage of platelets in a novel additive solution (M-sol), which is prepared by mixing solutions approved for clinical use that are not especially for platelet storage. Transfusion, 47: 960-965, 2007

3) Hirayama J, Azuma H, Fujihara M, Akino M, Homma C, Yamamoto, Kato T, Ikeda H.

Platelet storage in M-sol, a novel additive solution comprised of a mixture of solutions approved for clinical use. S, Ikeda H. Japanese Journal of Transfusion and Cell Therapy;54: 17-22, 2008

Attachment 1-2

Electrolyte solution for preparation of M-sol	
1. Solacet F (acetate ringer solution)	500 ml
2. Meylon (7% sodium biocarbonate solution)	35 ml
3. ACD-A	85 ml
4. Diluted Mediject Mg (MgSO4 solution)	50 ml
(distilled water 460 ml+Mediject Mg 20ml)	

Electrolyte solution for preparation of G-sol	
1. Veen D (glucose acetate ringer solution)	100 ml
2. Meylon (7% sodium biocarbonate solution)	8 ml
3. ACD-A	30 ml
4. Distilled Water for Injection	102 ml

Coposition of additive solution

	G-sol (mM)	M-sol (Mm)
Sodium chloride	42.8	77.0
Potasium chloride	1.7	3.0
Calcium chlorideCl2	0.8	1.4
Calcium acetate	19.3	34.7
Glucose	115.7	15.3
Trisodium citrate	9.4	9.4
Citrate	5.2	5.2
Sodium hydrogen carbonate	27.8	41.7
Magnesium sulfate	0.0	1.6

別紙 2

Attachment 2-1

Processing standards and guidelines for washed platelet preparations (version 2.0) (provisional)

1. Purpose
   1.1 The purpose of these guidelines is to standardize and spread safe practices in washed and replaced platelet preparation methods. Compliance standards are needed, even for washed platelets prepared in hospitals in order to prevent human error and contamination and to ensure quality.
2. Target
   2.1 Conforms to the indications in the February 1, 2009, revision of the *Indication guidance for washed and replaced platelets and their preparation (Version II) (provisional).*
   2.2 After informed consent for blood transfusions, preparations are made in a hospital's blood transfusion department at the request of the physician in the department treating the patient, based on the system of responsibility on page 3, and the patient's consent is obtained for the risks accompanying preparation in the hospital and the side effect prevention effects.
3. System of persons responsible and workers
   3.1 A person with overall responsibility is appointed. This person is the head of the section or department in which the treatment using washed platelets is conducted.
   3.2 Persons responsible for production control and quality control are appointed. The person responsible for production control is the principle physician in the transfusion department.
   3.3 The person responsible for quality control is not the same as the person responsible for production control, and is a physician or technician in the transfusion department or clinical laboratory.
   3.4 The workers who wash the platelets are physicians or technicians of sufficient experience, and who have received training under the supervision of the production control director.
   3.5 These workers have undergone adequate education and training with regard to washed platelet preparation, and their training records are on file.
4. Facilities, equipment, and instruments for washed platelet preparation
   4.1 Preparations are made in an area within the transfusion department (fractionation room or cell processing room, etc.) to which outside personnel have restricted admittance.

4.2 Procedures are established for admittance to the preparation area.

4.3 Regular validation and sanitation is conducted for equipment and instruments.

5. Cell preparation work

5.1 Cell preparation is performed in compliance with the *Indication guidance for washed and replaced platelets and their preparation (Version II) (provisional).*

5.2 Washed platelet standard operating procedures (SOP) and work sheets are developed.

5.3 Procedures using aseptic junction devices for transfusion tubes are possible even without the use of a safety cabinet. Corresponding isolation bags and bags with sterilization filters are used. Multiple lots cannot be handled simultaneously.

5.4 Platelet count is confirmed from washed platelet final product quality tests performed in the hospital or by an outside vendor. If possible, the washing and replacement fluids should be subjected to sterilization tests and endotoxin sampling. Quality test records are made.

5.5 Use of Pharmacopoeia of Japan drugs and reagents is preferable in preparations and quality tests. A record of reagents is made.

5.6 Product specifications are determined for delivery of washed platelets. The final decision for delivery is the responsibility of the person in charge of production quality.

5.7 Work sheets are recorded during and after work, and signed by the workers and person responsible for production quality. After reporting the test results, quality test records are made and signed by workers and the person responsible for quality control.

5.8 The manufacturing number of the raw platelet formulation used in preparation, similar to other blood formulations for transfusion, is recorded in use records, as specified in the Pharmaceutical Affairs Act, and kept on file for 20 years.

6. Labels

6.1 As a rule, special labels, not hand-written ones, are attached to prepared bags.

6.2 Manufacturing numbers for raw platelet formulations are made so as to be traceable.

7. Samples

7.1 Plasma components isolated from raw platelet preparations are stored if possible.

7.2 Special labels are attached to samples.

7.3 Special records (stored sample records) are kept for stored samples.

8. Transfusion

8.1 Washed platelets are administered to patients soon after the preparation is completed. The time until transfusion conforms to the *Indication guidance for washed and replaced platelets and their preparation (Version II) (provisional).*

8.2 Hospital transfusion procedures are followed when performing a transfusion.

8.3 After transfusion, empty bags are returned to the transfusion department, stored or

disposed of appropriately.

8.4 A side effect report is made to the transfusion department regardless of whether there were any side effects from washed platelets.

8.5 When side effects occur from washed platelet transfusions, the transfusion department is contacted immediately, a report is made to the person responsible for production control, and measures are taken.

8.6 When problems such as contamination are discovered as a result of quality tests, the person responsible for production control conducts a verification inspection of the manufacturing process. The person responsible for production control confers with the person with overall responsibility, or physicians in the department treating the patient in whom the problem occurred, to confirm the status of the patient. Measures are taken in cooperation with the medical safety control office in the hospital.

9. Disposal

9.1 Standards are set for cases that do not meet production specifications.

10. Other

10.1 Material costs required for preparation in the hospital are determined in the hospital.

11. Attachments

11.1 Standard operating procedure (SOP)

11.2 Work sheet (Form)

11.3 Replacement solution product master formula

11.4 Replacement platelet product master formula

12. Reference

12.1 Indication guidance for washed and replaced platelets and their preparation (Version II) Revised version updated on February 1, 2009

12.2 Processing guideline for hospital preparation of cellular therapy product (Version 0.21). a proposal from a Small Committee for Cell Processing Standard, Cell Therapy Committee, Japan Society of Transfusion Medicine and Cell Therapy presented on April 23, 2008.

12.3 FACT-JACIE International Standards for Cellular Therapy Product Collection, Processing and Administration. 3rd Edition.

Date of issue: February 1, 2009

Attachment 2-2

Standard Operating Procedure SOP: 01	Hospital Transfusion dept.	
M-sol preparation using replacement platelets (R-PC) (provisional)		
Written by: _____ Confirmed by: _____ Person responsible: _____	Issue date: 2009/02/01	Page / Version 1

1 Purpose

1.1 To describe the method of preparing M-sol using replacement platelets (R-PC).

2 Definition

2.1 M-sol is a preparation reagent for R-PC prepared in hospital using acetated Ringer's solution, 7% sodium hydrogen carbonate solution, injection solvent, 0.5 M magnesium sulfate, and ACD-A solution from the Japanese Pharmacopoeia.

3 Required articles

3.1 Equipment and instruments

3.1.1 Antiseptic junction device

3.1.2 Tube sealer

3.1.3 Electronic pan balance with calibration

3.1.4 Benchtop wrapper and sealer

3.1.5 Dedicated label printer

3.1.6 Clamps

3.1.7 Kocher

3.2 Consumables

3.2.1 High calorie transfusion set, 1000-mL bag

3.2.2 Blood isolation bag, 600-mL double bag

3.2.3 Blood isolation bag, 1000-mL bag (with sterilization filter)

3.2.4 Aluminum Lamizip bag

3.2.5 Operation adapter

3.2.6 JMS injection needle, 18 G

3.2.7 Special labels

3.2.8 Nonsterile rubber gloves

3.2.9 Trash bags for infectious waste

3.3 Reagents

3.3.1 Acetated Ringer's solution, 500 mL

3.3.2 7% sodium hydrogen carbonate solution, 250 mL

3.3.3 Injection solvent, 500 mL

3.3.4 Magnesium sulfate infusion, 20 mEq

3.3.5 ACD-A solution, 300 mL

4 Methods

4.1 General policy

4.1.1 Specimens are prepared in conformity with the operational procedures in 4.2 so that the specimens do not become contaminated, on the assumption that the total amount will be administered to the transfusion recipient after processing.

4.1.2 An aseptic junction device is used in the connection operations following filtration in 4.2.4.3, and so all operations are closed systems.

4.1.3 From 4.1.2, a safety cabinet or clean bench is not necessary.

4.1.4 Necessary information (date, content details, etc.) is written on all containers used during processing.

4.1.5 Special labels are attached to M-sol that fulfills the M-sol product master formula. M-sol that deviates from the M-sol product master formula or does not have a special label affixed is disposed of.

4.1.6 Each specimen is double-checked in all cases by two transfusion department technicians.

4.1.7 The confirmation seal of the physician responsible is needed for final confirmation.

4.2 Operations

4.2.1 Solution A is prepared.

4.2.2 Solution B is prepared.

4.2.3 All of solution A is added to solution B, and gently agitated with the two bags still connected.

4.2.4 M-sol is prepared.

5 Records

5.1 SOP Form: 01 M-sol for replacement platelets (R-PC)

6 Reference