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Guidance for Industry and FDA Review Staff

Collection of Platelets by Automated Methods

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For questions on the content of this guidance, contact the Division of Blood Applications, Office of Blood Research and Review at 301-827-3524.

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Contains Nonbinding Recommendations

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I. INTRODUCTION

This guidance provides you, blood establishments, and FDA staff with revised recommendations for the collection of Platelets by automated methods (plateletpheresis). This guidance is intended to help you ensure donor safety and the safety, purity, and potency of Platelets collected by an automated blood cell separator device. For the purpose of this document, Platelets collected by automated methods and resuspended in plasma will be referred to by the product name "Platelets, Pheresis." We consider the recommendations in this guidance document to provide appropriate criteria for a biologics license application or supplement for manufacturing Platelets, Pheresis, and provide guidance on preparing a manufacturing supplement for Platelets, Pheresis under Title 21 Code of Federal Regulations 601.12 (21 CFR 601.12).

This guidance applies only to the following Platelets, Pheresis components:

- Platelets, Pheresis (single, double, and triple collections);
- Platelets, Pheresis Leukocytes Reduced (single, double, and triple collections); and
- Platelets, Pheresis or Platelets, Pheresis Leukocytes Reduced collected concurrently with Plasma, Red Blood Cells (RBCs), and/or Source Plasma.[1]

This guidance replaces FDA's "Revised Guideline for the Collection of Platelets, Pheresis" dated October 1988. Also, this guidance finalizes the draft guidance, "Guidance for Industry and FDA Review Staff: Collection of Platelets by Automated Methods" dated September 2005.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited.

[1] This guidance does not apply to plateletpheresis components collected concurrently during apheresis granulocyte collection procedures or plasma reduced apheresis platelets, which are not currently licensed products, or to platelets prepared from plasmapheresis as described in 21 CFR 640.22(b).

The use of the word *should* in FDA's guidances means that something is suggested or recommended, but not required.

If you have any questions about the effect of any portion of this guidance on a regulatory requirement, contact the Center for Biologics Evaluation and Research (CBER), Office of Blood Research and Review, Division of Blood Applications, at 301-827-3524.

II. DISCUSSION

A. Background

Plateletpheresis is the routine collection of platelets using an automated blood cell separator device, which results in the product Platelets, Pheresis manufactured from a high yield of platelets from a single donor. Transfusion of Platelets, Pheresis is effective for treating patients with platelet related insufficiencies, while limiting the recipient's exposure to platelets from multiple donors. In recent years, many improvements have been made in automated blood cell separator device technology, platelet storage stability, and blood cell counting methods, including:

- collection process efficiency;
- storage container characteristics; and
- accuracy of methods for determining a donor's pre-donation platelet count and component yields.

Automated blood cell separator devices are now capable of various plateletpheresis collection procedures including but not limited to the following:

- collection of double and triple platelet components obtained during a single procedure;
- use of in-process leukocyte reduction (Ref. 1);
- collection of concurrent plasma components (Ref. 2); and
- collection of concurrent RBC components (Ref. 3).

This document includes the following recommendations:

- Published research indicates that there is poor recovery of viable platelets stored at a pH of less than 6.2 (Refs. 4 and 5). Therefore, your process validation and quality control (QC) testing for Platelets, Pheresis should assure a pH at or above 6.2, to rule out a pH less than 6.2 on the date the product is issued or on the date the product expires (outdates). Note that we recommend that you adopt a stricter pH standard than that currently specified in 21 CFR 640.25(b)(2).
- You should include additional deferral criteria for donors of Platelets, Pheresis who have taken certain medications (see section III.A.) (Refs. 6, 7, and 8).

- To protect the safety of the donor, seven days should elapse after collection of a double or triple Platelets, Pheresis before the donor is eligible to donate Platelets, Pheresis again. In addition, first-time donors without a pre-donation platelet count should not undergo collection of a triple Platelets, Pheresis.
- Because of similarities between plateletpheresis and Source Plasma donation, you should follow the donor weight provisions for Source Plasma donors under 21 CFR 640.63(c)(6) (see Section III.A.).
- QC testing, as prescribed in 21 CFR 640.25(b)(1) through (3) requires that, each month, four units prepared from different donors be tested at the end of the storage period for platelet count, pH of not less than 6.0 when measured at the storage temperature of the unit, and volume. In addition, 21 CFR 211.160(b) requires that laboratory controls include the establishment of scientifically sound and appropriate specifications, standards, sampling plans, and test procedures designed to assure that components, drug product containers, closures, in-process materials, labeling, and drug products conform to appropriate standards of identity, strength, quality, and purity.

We also note that bacterial contamination of blood components and associated transfusion risks is a continuing problem (Refs. 9 and 10). Bacterial contamination testing is a necessary part of process validation and quality assurance monitoring for Platelets, Pheresis.

B. Definitions

For purposes of the terms used in this guidance, the following definitions apply:

Actual platelet yield – The total platelet yield in the component, calculated by multiplying the platelet count of the sample times the volume of the component (platelet count x component volume = actual platelet yield).

Apheresis – Automated blood collection in which a device continuously or intermittently removes a small volume of whole blood, separates the components, collects certain components, and returns to the donor the uncollected remainder.

Automated blood cell separator – A device that uses a centrifugal or filtration separation principle to automatically withdraw whole blood from a donor, separate the whole blood into blood components, and return to the donor the remainder of the whole blood and blood components. The automated blood cell separator device is intended for routine collection of blood and blood components for transfusion or further manufacturing use.

Bacterial contamination testing – Testing conducted to determine whether a product contains viable contaminating bacteria.

Component – A part of a single donor's blood, such as platelets, separated from whole blood by physical or mechanical means. For Platelets, Pheresis, a component is a

transfusable product that may result from a single collection (resulting in one component), a double collection (resulting in two Platelets, Pheresis components), or a triple collection (resulting in three Platelets, Pheresis components).

Concurrent component – When a blood component, such as Platelets, is being collected during an apheresis procedure, a concurrent component is a different blood component (i.e., Plasma, RBCs) collected at the same time.

Dedicated donation – Platelets, Pheresis donated for a specific recipient.

Devices cleared or approved – Describes a device that has been cleared or approved by FDA pursuant to a 510(k) Premarket Notification (cleared device) or Premarket Approval Application (approved device). (See Title 21, United States Code, section 360c; Federal Food, Drug, and Cosmetic Act (FDCA), section 515 – Premarket Approval; and, FDCA, section 510(k)).

Donation frequency – Interval between a donor's collection procedures.

Process validation – Establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its pre-determined specifications and quality characteristics.

Qualification – A part of process validation that establishes confidence that a manufacturing device is capable of operating consistently (equipment installation qualification) and can be performed effectively and reproducibly (process performance qualification), and that the finished product meets all of the release requirements for functionality and safety (product performance qualification).

Residual White Blood Cell (WBC) count – The number of WBCs remaining in a Leukocytes Reduced component, calculated by multiplying the WBC count from a sample of the component times the volume of the component. In this document:

- references to residual WBC count testing apply when the Platelets, Pheresis will be labeled as Leukocytes Reduced.
- references to percent platelet retention apply to leukocyte reduction by filtration, provided there is access to a pre-filtration sample.

Rolling 12-month period – Continual assessment of a donor over a 12-month period. This is not a set 12-month period (i.e., calendar year).

Target platelet yield – The intended platelet yield programmed into an automated blood cell separator device, which may be based on the donor's platelet count and other factors.

Tolerance values – Minimum and maximum values (i.e., container volume; platelet concentration) described by the manufacturer as being acceptable. These values may also be described as specifications.

Weight/volume conversion – The total weight of the component minus the tare weight of the empty container divided by the specific gravity of the component equals volume of the component.

III. DONOR SELECTION AND MANAGEMENT

A. Donor Selection

Under 21 CFR 640.21(c), plateletpheresis donors must meet donor suitability criteria described in the biologics license application or supplement. These typically conform to donor suitability requirements (21 CFR 640.3) and recommendations applicable to donors of Whole Blood. In addition, we recommend:

- donor weight of at least 110 pounds (currently required for Source Plasma donors under 21 CFR 640.63(c)(6))
- Prior to the first donation, collect a sample for a platelet count.
- If you cannot test a sample for a platelet count prior to the first donation (for example, because the donor presents at a mobile collection site), you should collect a pre-donation sample and evaluate the donor's platelet count after the first collection.

You should not collect Platelets, Pheresis from donors who have ingested platelet inhibitory drugs recently enough to adversely affect platelet function in the product, or the safety of the donor. These recommendations include, but may not be limited to:

- Aspirin (ASA)/ASA-containing drugs/Feldene – two full medication free days prior to donation (Refs. 6 and 7)
- Plavix (Clopidogrel) and Ticlid (Ticlopidine) – 14 full medication free days prior to donation (Ref. 8).

When the drugs listed in this section are taken for a specific medical condition, donors should not discontinue taking drugs prescribed or recommended by their physicians in order to be eligible[2] to donate Platelets, Pheresis. However, we do not necessarily recommend deferral of such donors for all blood products, if the donors are in good health, and establishments may make eligibility determinations for donations of other products.

[2] We are using the terms "eligible" and "eligibility" in this guidance to refer to the donor suitability requirements described in 21 CFR 640.3 and 640.21(c).

B. Donor Management

1. Platelet Count

- You should collect a pre-donation sample from the donor for a platelet count. The device operator should enter that platelet count, or the one obtained immediately following initiation of the collection procedure, to more accurately set the target platelet yield parameters for each collection of Platelets, Pheresis. These steps should be consistent with the automated blood cell separator device manufacturer's directions for use.

- For any collection facility that cannot test a pre-donation sample for a platelet count (for example, a mobile collection site), you may use an average of previous historic platelet counts (as specified by the device manufacturer), or a default platelet count (either as recommended by the automated blood cell separator device manufacturer, or determined by using blood center specific values), to set the target platelet yield. You should not collect a triple Platelets, Pheresis from first-time donors who do not have a pre-donation platelet count available either prior to or immediately following initiation of the collection procedure. Concurrent components may be drawn if the donor meets eligibility requirements for those components.

- You should defer from donation donors whose platelet counts are less than 150,000 platelets/uL until a subsequent pre-donation platelet count indicates that the donor's platelet count is at least 150,000 platelets/uL.

2. Donation Frequency

To protect the safety of the donor:

- a donor should undergo no more than 24 Platelet, Pheresis collections in a rolling 12-month period.
- the interval between each collection of Platelets, Pheresis should be at least two days with no more than two procedures in a seven-day period.
- the interval between collection of a double or triple Platelets, Pheresis and any subsequent collection of Platelets, Pheresis should be at least seven days.
- the automated blood cell separator device should be set with a post-donation platelet count target of no less than 100,000 platelets/uL.

3. RBC Loss Prior to a Collection of Platelets, Pheresis

To protect the donor from significant RBC loss, we recommend that:

- you not allow a donor who has donated a unit of Whole Blood, a single unit of Red Blood Cells by apheresis, or a single unit of Red Blood Cells by apheresis concurrent with Platelets, Pheresis or Plasma in the previous 8

weeks to donate Platelets, Pheresis, unless the extracorporeal red blood cell volume during the Platelets, Pheresis collection is expected to be less than 100 mL (Ref 3).

- you not perform any collection procedure on a donor who has donated two units of Red Blood Cells by apheresis within the previous 16 weeks (Ref. 3).

4. Total Plasma Volume Loss Per Collection Procedure

The total plasma volume (excluding anticoagulant) of all blood components retained per collection of Platelets, Pheresis should not exceed:

- 500 mL (600 mL for donors weighing 175 lbs or greater), or
- the volume described in the labeling for the automated blood cell separator device (this volume may be more or less than the 500 mL or 600 mL volume stated in the above bullet).

IV. INFORMATION PROVIDED TO THE DONOR

Under 21 CFR 640.22(c), the collection procedure must be as described in the biologics license application or supplement. As part of the collection procedure, Platelets, Pheresis donors should receive information about the collection procedure and its associated risks. You should provide Platelets, Pheresis donors with the same information that is provided to a Whole Blood donor[3], plus the following information specific to the platelet collection:

- a description of the procedure for collection of Platelets, Pheresis and its associated risks.
- information about potential side effects of the procedure including possible effects as a result of solutions and/or treatment to reduce side effects such as treatment with a calcium replacement. Examples of side effects include anticoagulant effects (tingling and/or nausea), hypovolemia (decreased blood volume), fainting, and any other side effect as described by the automated blood cell separator device manufacturer.
- information indicating that there are limitations to the number and types of components that can be donated per year.

V. COMPONENT COLLECTION

Improvements in collection of Platelets, Pheresis have enabled blood establishments to obtain from a single collection procedure one, two, or three Platelets, Pheresis component(s) (and concurrent collection of Plasma, Source Plasma and/or RBC components).

[3] Refer to FDA regulations and guidance developed by FDA on this topic and available on the FDA website. http://www.fda.gov/cber/blood/bldpubs.htm

Under 21 CFR 640.22(c), the collection procedure must be as described in the biologics license application or supplement. In addition, the phlebotomy must be performed by a single uninterrupted venipuncture with minimal damage to, and minimal manipulation of, the donor's tissue (21 CFR 640.22(d)). A sterile connecting device may be used as described in the manufacturer's directions for the apheresis collection set. The automated blood cell separator device must perform in the manner for which it was designed (21 CFR 606.60(a)). Accordingly, your collection procedures should be consistent with the Operator's Manual, directions for use, and/or manufacturer's specifications. Specifications identified by the manufacturer may include, but not be limited to, the donor's platelet count, weight, height or hematocrit; the minimum/maximum volume of the storage container; platelet concentration per uL in the storage container, or actual platelet yield. In addition, supplies and reagents must be used in a manner consistent with instructions provided by the manufacturer (21 CFR 606.65(e)).

VI. VALIDATION OF THE COLLECTION PROCESS

The Current Good Manufacturing Practice (CGMP) regulations described in 21 CFR Parts 210 and 211 contain the minimum requirements for methods to be used in, and the facilities or controls to be used for, the manufacture, processing, packing or holding of a drug to assure that the drug meets the requirements of the FDCA as to safety, and has the identity and strength and meets the quality and purity characteristics that it purports or is represented to possess (21 CFR 210.1(a)). These CGMP regulations also apply to Whole Blood and blood components (21 CFR 210.2(a), 211.1(b)) and supplement the CGMP regulations for blood and blood components contained in 21 CFR Part 606. As an element of CGMP, process validation "establishes documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its pre-determined specifications and quality characteristics" (Ref. 11).[4] We recommend that establishing documentation of process validation include, but not be limited to, validation protocol development, installation qualification, process operator performance qualification, and product performance component qualification (Ref. 11).

Each device intended for the routine collection of Platelets, Pheresis must be cleared or approved by FDA for this purpose (see 21 CFR 864.9245). You should conduct validation of the collection process using each type of device used in your establishment prior to implementing routine collections.

In addition, your validation efforts should include the following manufacturing steps:

- cell counting
- pH measurement: we recommend that a pH meter or gas analyzer be routinely used rather than pH (nitrazine) paper.
- component weighing

[4] The requirement for process control is set forth in general terms in 21 CFR 211.100.

- sterile connecting method (Ref. 12)
- storage
- shipping

A. Equipment Installation Qualification

21 CFR 606.60(a) requires that equipment be observed, standardized and calibrated on a regularly scheduled basis as prescribed in the Standard Operating Procedures Manual and must perform in the manner for which it was designed. Upon initial installation, the automated blood cell separator device should be qualified as described in the Operator's Manual or manufacturer's directions for use.

B. Validation Protocol

An integral element of the performance and documentation of process validation is the development of a validation protocol. You should refer to FDA's "Guideline on General Principles of Process Validation" (Ref. 11) as an outline for developing your validation protocol. The validation protocol should include at least the following:

- a description of the equipment to be used
- minimum/maximum acceptable values for the Platelets, Pheresis collection and/or component as specified by the automated blood cell separator device manufacturer
  - total volume (after removal of samples for hematological testing and bacterial contamination testing), including per component (container) from double and triple collections
  - actual platelet yield
  - residual WBC count (if Leukocytes Reduced) for the collection and components (if multiple components are collected), and percent platelet retention when applicable
  - concurrent component volume (Plasma or RBC), if applicable
  - pH measurement
- manufacturer's specifications or recommendations for processing parameters (i.e., actual platelet yield and concentration, weight or volume collected)
- description of supplies used in the collection (e.g., collection/storage containers, anticoagulants, etc.)
- failure investigation criteria
- personnel training criteria
- standard operating procedures for performing each element of the collection process
- documentation of the validation protocol criteria (all of the above)

C. Process Performance Qualification (Operator)

Each person engaged in the collection of Platelets, Pheresis must have adequate education, training, or experience to assure competent use of the automated blood cell separator devices involved (21 CFR 211.25(a)). Establishments must maintain applicable proficiency test results (21 CFR 606.160(b)(5)(v)).

We recommend that personnel training include the successful, consecutive, performance under supervision of an appropriate number of procedures, as defined by your facility. These procedures should result in the collection of Platelets, Pheresis meeting relevant component specifications.

D. Product Performance Qualification for Component Collection Process

Various mechanical and biological factors may influence the plateletpheresis collection process (i.e., the optical qualities of a donor's plasma, the donor's platelet count and platelet size, vascular access, and procedure duration) (Ref. 14). The objective of collection performance qualification is to verify that the automated blood cell separator device performs according to the manufacturer's claims when used, and through appropriate testing establishes confidence that the finished product produced by the specified process meets all release requirements for functionality and safety (Ref. 11). All components collected during the validation process can be released for transfusion provided that they meet minimum specifications as defined by the manufacturer, are labeled appropriately, and are otherwise suitable.

Process performance qualification should include testing for the actual platelet yield, pH, and volume; residual WBC count and percent platelet retention (for Leukocytes Reduced components) (See Table 1). We recommend that you assess the following at each collection site:

- **actual platelet yield** (platelet count multiplied by the volume):
  - determine actual platelet yield at collection.
  - follow the platelet pre-donation count recommendations in section III.B.1., and set an appropriate target platelet yield as recommended by the automated blood cell separator device manufacturer to maximize the likelihood that each transfusable component contains $\geq 3.0 \times 10^{11}$ platelets and the target collection type (single, double, triple) is achieved.
- **pH** as a measurement of quality after storage:
  - determine pH on the date the product is issued or on the date the product expires (outdates).
  - each transfusable component should have a pH ≥ 6.2
- **percent platelet retention**
  - perform when the automated blood cell separator device or filtration method is first put into use at an establishment and/or as recommended by the automated blood cell separator device manufacturer.
  - if leukocytes are reduced by filtration and there is access to both a pre-filtration and post-filtration sample, calculate percent platelet retention using pre- and post-filtration volume and cell content.
- **residual WBC count**:
  - perform when the automated blood cell separator device or filtration method is first put into use at an establishment and/or as recommended by the automated blood cell separator device manufacturer.

- perform within 48 hours of collection or per the manufacturer's directions for the cell counting methodology used (Ref. 15).
- conduct testing on the collection (parent container) and on the individual components from double and triple collections

- **volume**:
  - determine the volume after removal of samples for testing (i.e., cell count, bacterial contamination testing).
  - fill each storage container consistent with the manufacturer's minimum/maximum specifications.
  - equilibrate storage containers for double or triple collections $\pm$ 10 mL, or per the manufacturer's directions if different.

You also should qualify devices and perform failure investigations as follows:

- **Devices**:
  - complete product performance qualification for apheresis devices from different manufacturers, and for each model.
  - obtain data from all automated blood cell separator devices at each site for initial product performance qualification. If additional devices of the same model are added at the facility after qualification, include qualification data in monthly QC only.
- **Failure investigation**: Conduct an investigation for all component qualification failures, and when appropriate, initiate corrective action and follow-up measures (see 21 CFR 211.192; 606.100(c)). We understand that some failures may occur due to conditions **not** resulting from a failure of the process (e.g., automated blood cell separator device failures, donor reactions). In addition, you should:
  - investigate as qualification failures residual WBC counts that exceed the following:
    - single collection: $\geq 5.0 \times 10^6$ (collection)
    - double collection: $\geq 8.0 \times 10^6$ (collection), **and** $\geq 5.0 \times 10^6$ (either or both components)
    - triple collection: $\geq 1.2 \times 10^7$ (collection), **and** $\geq 5.0 \times 10^6$ (one, two or all three components).

  - However, each transfusable component from a double or triple collection of Platelets, Pheresis may be labeled as Leukocytes Reduced provided the residual WBC count on the component is found to be $< 5.0 \times 10^6$. investigate collections that fail to meet the percent platelet retention, if performed. However, the component may be transfused if the actual platelet yield is determined subsequent to filtration, and the component is labeled appropriately.

Variation in the actual platelet count might be due to the platelet counter used and the type of platelet count used at the time of collection (pre-donation or historic average). However, you should select a statistically sound sample size, based on 95% confidence that 75% of components (platelet yield) will meet the recommended results (see Table 1). For pH and recommended residual WBC count, you should select a statistically

sound sample size, based on 95% confidence that 95% of components (pH) or collections (residual WBC count) will meet the recommended results. Using the binomial statistic for example, a minimum of 60 components/collections should be tested, with zero process failures (93 tested with one process failure, 124 tested with two process failures, etc.) to qualify the process. Determine the sample size selection before starting the qualification process. For example, if you test 60 samples and encounter a failure, you should not continue with the testing of an additional 33 components. If you select a sample size of 93 and encounter a failure during testing, you may continue to test but there should be no additional failures. Similarly, if you select a sample size of 124 and encounter two failures, you may continue to test, but there should be no additional failures.

Table 1. Product Performance Qualification Criteria for the Platelet Component Collection Process

Test	Recommended Results	Target[1]	Allowable Process Failures[2] to achieve recommended results for a set of N tests[3]		
Actual platelet yield of transfusable component	$\geq 3.0 \times 10^{11}$	95%/75% *	N=11 **	N=18 **	N=23 **
			0	1	2
pH	≥ 6.2	95% / 95% ***	N=60	N=93	N=124
			0	1	2
Percent component retention	$\geq$ 85% component retention if performed ****	95%/95%	N=60	N=93	N=124
			0	1	2
Residual WBC count *****	Single collection: $< 5.0 \times 10^6$	95% / 95%	N= 60 collections	N=93 collections	N=124 collections
			0	1	2
	Double collection: Collection: $< 8.0 \times 10^6$ or Components: $< 5.0 \times 10^6$	95%/95%	N=60 collections	N=93 collections	N=124 collections
			0	1	2
	Triple collection: Collection: $< 1.2 \times 10^7$ or Components: $< 5.0 \times 10^6$	95%/95%	N=60 collections	N=93 collections	N=124 collections
			0	1	2

[1,2] Process failures only; non-process failures should be excluded.

[3] Corrective actions for exceeding allowable process failures

- if you select a sample size of 11 and find one failure, 17 additional samples would need to be tested with no additional failures.
- if you select a sample size of 60 and find one failure, 91 additional samples would need to be tested with no additional failures. If you select a sample size of 93 and find two failures, 157 additional samples should be tested with no failures. If you select a sample size of 124 and find three failures, 127 additional samples should be tested with no failures.

* 95% confidence that greater than 75% of the components meet the standard.

** The sample size numbers can be used in a sampling plan that should be representative of products collected on each machine type in each facility.

*** 95% confidence that greater than 95% of the components meet the standard.

**** Or per the container/automated blood cell separator device manufacturer's specifications

***** The stratified recommended results should ensure that the individual transfusable units will be $< 5.0 \times 10^6$ even with a 25% error in equilibration of the volume for double and triple collections.

E. Re-Qualification/Re-Validation

- Exceeding the allowable **process** failures of the collection process qualification may indicate that the process is not in control. You must investigate and correct the source of this failure (see 21 CFR 211.192, 606.100(c)) and should repeat validation.

- The manufacturer may provide re-qualification requirements for the automated blood cell separator device to be followed.

VII. QUALITY ASSURANCE AND MONITORING

Quality assurance (QA) is the sum of activities planned and performed to provide confidence that all systems and system elements that influence the quality of the component are functioning as expected (Ref. 13). When this is demonstrated, the process is considered to be in a state of control. Whether a process is operating in a state of control is determined by analyzing the day-to-day process and the data for conformance with the manufacturer's specifications and for variability.

You must have a quality control (QC) unit that has the responsibility and authority to approve or reject all components, containers, closures, in-process materials, packaging material, labeling and drug products and the authority to review production records to assure that no errors have occurred or, if errors have occurred, that they have been fully investigated (21 CFR 211.22(a)). Thus, the QC unit's responsibilities include the review of production records, and the review of complaints involving the possible failure of a product to meet its specifications. (See, for example, 21 CFR 211.22, 211.192, 211.198, 606.100(c)). Please refer to FDA's "Guideline for Quality Assurance in Blood Establishments" (Ref. 13) for developing a QA and Monitoring program.

A. Standard Operating Procedures (SOPs) and Recordkeeping

1. Requirements for SOPs

- An automated blood cell separator device must "perform in the manner for which it was designed" (21 CFR 606.60(a)) during the collection or processing of apheresis components. Written SOPs must be maintained and must include all steps to be followed in the collection, processing, compatibility testing, storage, and distribution of blood and blood components (21 CFR 606.100(b)). Therefore, you must have written SOPs for each step in the collection of Platelets, Pheresis.

2. Additional Provisions Applicable to SOPs

- **Adverse reactions:** You must have a written SOP for investigating adverse donor and recipient reactions (21 CFR 606.100(b)(9)). In addition, you should have a written SOP for managing a cardiopulmonary emergency or

any other adverse reactions associated with donation, containing steps for contacting physicians, obtaining an emergency rescue squad response, and transporting the donor to the hospital.

- **Hematocrit**: If the final platelet collection contains more than 2 mL of packed RBCs, you should attach a sample of donor blood to the platelet storage container for compatibility testing to prevent the possibility of an adverse reaction during transfusion. In addition, you should hold the Platelets, Pheresis collection prior to distributing as Leukocytes Reduced until a residual WBC count of the transfusable component can be determined and found to be $< 5.0 \times 10^6$.

- **Component volume**: You should describe how to process components in the event the volume exceeds the automated blood cell separator device manufacturer's specifications. In addition, the volume in the storage containers from double or triple collections should be within ± 10 mL of each other or per the manufacturer's directions if different.

- **Samples for QC:** Containers for QC samples should be attached to the component/collection set using a sterile connecting device, to ensure the maintenance of the closed system.

- **Actual platelet yield**: The platelet yield from each collection of Platelets, Pheresis should be available to provide to the transfusion facility.

- **pH measurement**: Accurate pH measurement is time dependent, and samples should be tested within 1 hour of sampling, or as suggested by the manufacturer of the pH measurement system. We recommend that a pH meter or gas analyzer be routinely used rather than pH (nitrazine) paper. However, if you choose to determine pH measurements with nitrazine paper, the selected paper should read in increments of one-tenth units, or it may provide inaccurate measurements.

- **RBC loss**: You must have a written SOP for your collection procedure, including in-process precautions to measure accurately the quantity of blood removed from the donor (21 CFR 606.100(b)(5)). You should calculate the donor's RBC loss, which may include the residual RBCs remaining in the apheresis collection set after a collection of or discontinued collection of Platelets, Pheresis; the extracorporeal RBCs remaining in event of no RBC rinseback; the RBC loss from collection of tubes for testing; and/or collection of a concurrent RBC. You should record such RBC loss in the donor's record, in a manner that allows tracking of cumulative RBC loss over time.

- **Bacterial contamination testing**: You must maintain written SOPs and include all steps to be followed in the testing of blood and blood components (21 CFR 606.100(b)). Bacterial contamination testing should be performed using a culture based methodology, and using your established procedures.

- **QC failures**: You must thoroughly investigate any unexplained discrepancy or the failure of a batch to meet any of its specifications (21 CFR 211.192). You should define appropriate criteria for retesting of components, testing of additional components, final labeling, and disposition of components that fail to meet specifications.

- **Failure investigations**: (see 21 CFR 211.192; 606.100(c)) The criteria to assess in the performance of a thorough failure investigation (including the conclusions and followup) should include, but not be limited to: donor characteristics or specifications; operation and or performance of the collection device; adherence to SOPs; lot numbers of reagents or supplies; sample collection, handling, storage or shipping; operator performance, training or competency; and cell counting instrument performance including shifts or trends in controls.

- **Manufacturer's performance specifications**: You should state the acceptable tolerance specifications for the volumes, platelet concentration, and/or actual platelet yield for each storage container as described by the manufacturer. You should have a procedure addressing the handling of components that do not meet the manufacturer's performance specifications (e.g., use in research or further manufacture).

- **Labeling**:
  - The final component volume stated on the label should be determined after removal of samples for platelet count determination, QC, and/or bacterial contamination testing.
  - Platelets, Pheresis for transfusion should routinely contain $\geq 3.0 \times 10^{11}$ platelets. When special circumstances warrant their use, Platelets, Pheresis components containing less than 3.0×10^{11} platelets should be labeled with the actual platelet content.

- **Component Storage**:
  - If Platelets, Pheresis are stored at 20 to 24 °C, you must maintain a continuous gentle agitation throughout the storage period (21 CFR 640.25(a)). You should describe how temperature and agitation will be monitored, and the disposition of platelet components that are not stored properly.
  - You must follow the automated blood cell separator device manufacturer's directions for use (21 CFR 606.60(a)). If sterile connecting an additional container(s) is necessary, use a container(s)

designed to achieve and protect a sterile conduit. Because of differences in container specifications, you should use containers from the same manufacturer.

3. Recordkeeping

All recordkeeping requirements of 21 CFR Part 606, Current Good Manufacturing Practice for Blood and Blood Components, Subpart I (Records and Reports); Part 211, Current Good Manufacturing Practice for Finished Pharmaceuticals, Subpart J (Records and Reports); and applicable provisions of 21 CFR 640.20 through 640.27, must be met.

B. Donor Monitoring

1. Platelet Counts

If the platelet count is known, you should notify your Medical Director when a donor has a post collection platelet count less than 100,000/uL, and you should defer the donor until his/her platelet count has returned to at least 150,000/uL.

Transient decreases in platelet counts have been reported in donors undergoing multiple collections of Platelets, Pheresis (Ref. 16). You should periodically review a donor's records to monitor platelet counts.

2. Adverse Reactions in Donors

Records must be maintained of any reports of complaints of adverse reactions regarding each unit of blood or blood product arising as a result of blood collection or transfusion and a thorough investigation of each reported adverse reaction must be made (21 CFR 606.170(a)).

3. Red Blood Cell Loss

- **Per collection**:
  - If the collection procedure needs to be discontinued for any reason before completion, and if the Operator's Manual allows, you should attempt to return RBCs to the donor.
  - Donor eligibility based on RBC loss (with or without RBC rinseback, and including all other types of donation) is described in Table 2.

Table 2: Recommendations for donor eligibility based on RBC loss per collection

Donor's Initial packed RBC loss	**Donor's Second packed RBC loss within 8 weeks**	**Eligibility**
Less than 200 mL	No donation or total from initial and second loss less than 200 mL	No deferral of donor for packed RBC loss; frequency of donation of Platelets, Pheresis as discussed in section III.B.2
Less than 200 mL	More than 200 mL but less than 300 mL total	Donor is not eligible to donate for 8 weeks from 2nd loss
More than 200 mL but less than 300 mL	NA	Donor is not eligible to donate for 8 weeks from initial loss
Less than 200 mL	Total loss from initial and second loss of more than 300 mL	Donor is not eligible to donate for 16 weeks from the 2nd loss
300 mL or more	NA	Donor is not eligible to donate for 16 weeks from initial loss.

- **Per 12 months**:
  Under 21 CFR 640.3(b), a person may not serve as a source of Whole Blood more than once in 8 weeks. In any such assessment, and in assessing a donor's RBC loss during the past rolling 12-month period, the RBC loss associated with the collection of Platelets, Pheresis, and including any other donation type (i.e., Whole Blood, RBC by apheresis), should also be considered.
- **Total plasma volume loss per 12 months**:
  The maximum volume (excluding anticoagulant) collected from a donor during a rolling 12-month period, and including any other donation type (i.e. Whole Blood, plasmapheresis) should not exceed:
  - 12 liters (12,000 mL) for donors weighing 110 – 175 lbs
  - 14.4 liters (14,400 mL) for donors weighing more than 175 lbs

  (Ref. 2).

C. Component Testing

1. Component Specification Check

- Actual platelet yield (volume x platelet count) must be determined after each collection (21 CFR 211.103).
- Weight/volume conversion is necessary to determine the volume of each collection. To convert weight to volume, divide the weight of the collection (the total weight minus the weight of the bag) by the specific gravity (1.03).

- Bacterial contamination testing: You should perform bacterial testing as specified by the storage container manufacturer (i.e., 7-day storage of Platelets, Pheresis, Leukocytes Reduced).

2. QC Monitoring

Under 21 CFR 211.160(b), laboratory controls must include the establishment of scientifically sound and appropriate specifications, standards, sampling plans and test procedures to assure that components and products conform to appropriate standards. One example of a scientifically sound statistical sampling and analytic plan is based on a binomial approach (see Table 1: Product Performance Qualification Criteria for the Platelet Component Collection Process). The sampling sizes described in Table 1 will confirm with 95% confidence a < 5% non-conformance rate for pH and residual WBC count, and < 25% non-conformance rate for actual platelet yield.

However, other statistical plans may also be appropriate, such as the use of scan statistics.

As part of your QC protocol you should:

- define a plan for non-selectively identifying collections to be tested. This should ensure testing of components collected on each individual automated blood cell separator device, each collection type, and each location.
- define sampling schemes for actual platelet yield (including volume determination) and pH, and residual WBC. We recognize that these sampling schemes may be mutually exclusive. However, the platelet yield of the collection (and designation of single, double or triple) should be made prior to performing the residual WBC count QC.
- test actual platelet yield (platelet count times the volume) and pH at the maximum allowable storage time for the container system used (or representing the dating period). Title 21 CFR 640.25(b) specifies that QC testing, including platelet count and measurement of actual plasma volume, be performed at the end of the storage period. We believe that such testing may be conducted "at issue" or within 12 hours after expiration. In addition, actual platelet yield and pH testing may be conducted on one storage container of a double or triple collection.
- include the residual WBC count (Ref. 1) for Leukocytes Reduced collections, if manufacturing leukocytes reduced products.
    - Perform the residual WBC count on the collection. For the purpose of labeling as Leukocytes Reduced (see 21 CFR 606.121(c)(1)), you may also perform a residual WBC count on the transfusable units for double and triple collections that fail the collection acceptance criteria listed (see below in this section).

  - Test for the residual WBC count within 48 hours after collection (Ref. 15), or per the manufacturer's directions for the cell counting methodology, to reduce aberrant results due to cellular deterioration and clumping.
  - Test for percent platelet retention, if leukocytes reduced by filtration.
- describe the criteria for investigation of failures during QC, including the factors to consider in categorizing a failure as process or non-process.
- have a method to document all calculations and test results.

We recommend that you consider the following QC results to be acceptable:

- pH $\geq$ 6.2. If one component from a double or triple collection is found to have a pH < 6.2, the corresponding component(s) from the collection should be retrieved and/or quarantined until they are tested and found to be acceptable.
- transfusable Platelets, Pheresis components $\geq 3.0 \times 10^{11}$ platelets.
- residual WBC count:
  - Single collection: $< 5.0 \times 10^{6}$ WBC
  - Double collection: $< 8.0 \times 10^{6}$ WBC
    Note: If $\geq 8.0 \times 10^{6}$, **but** each transfusable component is $< 5.0 \times 10^{6}$, this is not considered a collection failure.
  - Triple collection: $< 1.2 \times 10^{7}$
    Note: If $\geq 1.2 \times 10^{7}$, **but** each transfusable component is $< 5.0 \times 10^{6}$, this is not considered a collection failure.
- percent platelet retention should be $\geq$ 85% or per the manufacturer's specifications. Components with < 85% platelet retention may be distributed, but a failure investigation should be performed.
- negative for bacterial contamination testing, when performed.

D. Equipment/Supplies

Equipment must be observed, standardized, and calibrated on a regularly scheduled basis as prescribed in the Standard Operating Procedures Manual (21 CFR 606.60(a)). Such equipment includes, but may not be limited to, the automated blood cell separator device, cell counting instrument(s), pH meter, scales and sterile connector.

All supplies (including containers) and reagents must meet all of the requirements described in 21 CFR 606.65.

E. Operator Training

Operators must have adequate training, education and experience, or combination thereof, to assure competent performance of their assigned functions (21 CFR 606.20(b)). We recommend that assessment of operators include scheduled

competency assessment and proficiency testing. In addition, we recommend that you develop and document appropriate training on component preparation and/or machine maintenance as updated information becomes available (Ref. 12).

F. Quality Monitoring

You should assess the following:

- total component volume and equal distribution of volume in double and triple component collection containers. This assessment should include checking the performance of the scale; the use of the tare weight of the empty containers/tubing; and the weight/volume conversion.
- component bacterial contamination testing: Rates of bacterial contamination of plateletpheresis should be monitored, and bacterial contamination rates that exceed 1:3000 (Refs. 10 and 12) should be investigated.

VIII. PROCESSING AND TESTING

A. Processing

Platelets, Pheresis must be processed as described in 21 CFR 640, Subpart C – Platelets (21 CFR 640.20-640.27).

B. Communicable Disease Testing

Donations of Platelets, Pheresis must be tested for communicable diseases (21 CFR 610.40, 640.5(a) through (c), 640.23). Platelets, Pheresis may be released or shipped prior to completion of communicable disease testing in accordance with 21 CFR 610.40(g).

You must test donations of human blood and blood components from a donor whose donations are dedicated to and used solely by a single identified recipient except that, if the donor makes multiple donations for a single identified recipient, you may perform such testing only on the first donation in each 30-day period (21 CFR 610.40(c)(1)(i)).

C. Expiration Date

The dating period for Platelets, Pheresis collected using an FDA cleared or approved collection container under a closed or functionally closed system will be specified by the collection container manufacturer.

In accordance with such instructions and our recommendation, Platelets, Pheresis collected in an open system expire 24 hours from the termination of the procedure if the integrity of the hermetic seal is broken during processing.

If the integrity of the hermetic seal is broken after collection, the Platelets, Pheresis expire 4 hours from the time of the integrity violation, or at the original expiration date, whichever is earlier (21 CFR 606.122(l)(2)).

IX. LABELING

An instruction circular must be available for distribution if the product is intended for transfusion (21 CFR 606.122).

Your container labels must comply with 21 CFR 606.121 and 610.60.

In addition:

- The label should include the estimated amount of anticoagulant in the component container.
- Platelets, Pheresis components for transfusion, containing less than 3.0×10^{11} platelets per storage container, should be labeled with the actual platelet content.
- A component from a double or triple Platelets, Pheresis may accurately be labeled as Leukocytes Reduced when the residual WBC count of the collection is $\geq 8.0 \times 10^{6}$ (double) or $\geq 1.2 \times 10^{7}$ (triple) **IF** the transfusable component is tested and found to have a residual WBC count $< 5.0 \times 10^{6}$.
- Platelets, Pheresis may be labeled (i.e., tie-tag) with the residual WBC count if counted and found to contain $< 1.0 \times 10^{6}$.

X. REPORTING CHANGES TO AN APPROVED BIOLOGICS LICENSE APPLICATION (BLA)

Licensed establishments must report changes to their approved application(s) in accordance with 21 CFR 601.12. For assistance in reporting your changes see FDA's "Guidance for Industry: Changes to an Approved Application: Biological Products: Human Blood and Blood Components Intended for Transfusion or for Further Manufacture." The information below is intended to assist you in determining which reporting mechanism is appropriate for a change to your approved BLA, as it applies to the manufacture of Platelets, Pheresis. You should prominently label each submission with the reporting category under which you are reporting your change, e.g., "Prior Approval Supplement;" "Supplement - Changes Being Effected in 30 Days;" "Supplement - Changes Being Effected;" or "Annual Report."

A. Prior Approval Supplement (PAS): Changes Requiring Supplement Submission and Approval Prior to Distribution of the Product Made Using the Change (Major Changes) (21 CFR 601.12(b))

Under 21 CFR 601.12(b), changes that have a substantial potential to have an adverse effect on the identity, strength, quality, purity, or potency of the product as they may relate to the safety or effectiveness of the product must be reported to FDA in a Prior Approval Supplement (PAS).

Under this standard, the following kinds of manufacturing changes would fall within this category, warranting submission of your request to implement the following changes to your approved BLA as a PAS:

- if you currently hold an unsuspended, unrevoked BLA to manufacture blood components other than Platelets, Pheresis, and you intend to manufacture and distribute Platelets, Pheresis under that license.
- if you are currently approved to manufacture Platelets, Pheresis at a specific facility, and you intend to manufacture Platelets, Pheresis at a different facility, not under an approved Comparability Protocol. To submit a request for a Comparability Protocol see below.
- if you are approved to manufacture Platelets, Pheresis, but intend to change your manufacturing process in a manner that presents a substantial potential for an adverse effect on the product. FDA believes that such manufacturing changes include: change in storage conditions; change in anticoagulant; leukocyte reduction; and collection of an additional or different product.
- if you intend to collect Platelets, Pheresis using an automated blood cell separator device new to the market or new to your establishment.
- if you are requesting approval for a Comparability Protocol. The Comparability Protocol described in 21 CFR 601.12(e) is a supplement that describes the specific tests and validation studies and acceptable limits to be achieved to demonstrate the lack of adverse effect for specified types of manufacturing changes on the identity, strength, quality, purity, or potency of the product as they may relate to the safety or effectiveness of the product. A new Comparability Protocol, or a change to an existing one, requires approval from FDA prior to distribution of the product which, if approved, may justify a reduced reporting category for the particular change because the use of the protocol for that type of change reduces the potential risk of an adverse effect (21 CFR 601.12(e)).

  A Comparability Protocol is appropriate, but not required, if you wish to add multiple collection facilities under your direction and control, using the same process to manufacture Platelets, Pheresis. If you request approval for a Comparability Protocol, you should describe the procedures and processes that each new collection facility will implement to ensure conformance with the Comparability Protocol. You may identify one or more collection facilities for the purpose of validation and submission of the Comparability Protocol and supporting data to CBER for review. Approval of such a Comparability Protocol for future collection facilities justifies a reduced reporting category for the particular change because the use of the protocol for that type of change reduces the potential risk of an adverse effect.

  If you are using an approved Comparability Protocol, you should routinely review the procedures and specifications in the Comparability Protocol to assure that they remain current and consistent with the applicable application and current guidance. If modifications are required, you should contact FDA to discuss the change and to determine the appropriate reporting category.

- We consider the recommendations in this guidance document to provide appropriate criteria for a biologics license application or supplement for Platelets, Pheresis. You may use an alternative approach if such approach satisfies the requirements of the applicable statutes and regulations. Your alternative procedure(s) may be acceptable if you demonstrate that the resulting Platelets, Pheresis components meet applicable standards. We have determined that it may be adequate to determine the actual platelet yield at collection, and that re-determination of the actual platelet yield at issue or outdate is unlikely to provide additional relevant information. If you choose to discontinue determining the platelet count for QC testing as described under 21 CFR 640.25(b)(1), you must submit a request for an alternative procedure under 21 CFR 640.120.

You must not distribute in interstate commerce blood components made using a changed manufacturing process requiring a PAS until you have received our approval of your PAS (21 CFR 601.12(b)(3)).

B. Changes Being Effected in 30 Days (CBE-30) Supplement: Changes Requiring Supplement Submission at Least 30 Days Prior to Distribution of the Product Made Using the Change (21 CFR 601.12(c))

Under 21 CFR 601.12(c), changes that have a moderate potential to have an adverse effect on the identity, strength, quality, purity, or potency of the product as they may relate to the safety or effectiveness of the product must be reported to FDA in a Changes Being Effected in 30 days (CBE-30) supplement.

You must submit your request to implement manufacturing changes with a moderate potential for an adverse effect to your approved BLA as a CBE-30 supplement under 21 CFR 601.12(c). The manufacturing changes described below are examples of changes that we believe fall within this category:

- certain software and hardware upgrades provided by the manufacturer to your cleared or approved automated blood cell separator device
- addition of concurrent plasma collection
- implementation of a new collection facility under an approved Comparability Protocol

You may distribute your blood components made using the change requested in your CBE-30 supplement in interstate commerce 30 days after we receive your supplement, unless we notify you otherwise (21 CFR 601.12(c)(4)).

C. Submission Inclusion Documents

1. PAS: To comply with the requirements in 21 CFR 601.12(b)(3), the following must be included in the supplement:

- identification of the components involved (e.g., single plateletpheresis component, double plateletpheresis components, and/or triple plateletpheresis components) and manufacturing site(s) or area(s) affected, and a detailed description of the manufacturing change (including device collection technology and the collection protocol(s)) (21 CFR 601.12(b)(3)(i) through (iii)). We recommend that this information be documented in a cover letter and FDA Form 356h. To permit assessment of the manufacturing change we recommend including copies of the following SOPs:
  - collection
  - informed consent
  - labeling including labels
  - donor qualification, deferral and adverse event follow-up
  - a description of training (or an example of training documents)
  - component manufacturing
  - monitoring donor RBC and plasma loss
  - failure investigation
  - quality control including sampling scheme, sample handling, tracking and trending
  - equipment standardization/calibration
  - quarantine and disposition of unsuitable products

Additionally, we recommend that the following SOPs, if already approved for other blood collection activities and unrevised, would not need to be submitted:

  - sample preparation
  - component storage and shipping
  - donor arm preparation

- product labeling for each component, if changed (21 CFR 601.12(f)). We recommend submitting a Form FDA 2567 including Circular (unless already on file at FDA)
- a reference list of relevant SOPs (21 CFR 601.12(b)(3)(vii))
- relevant validation protocols and data (21 CFR 601.12(b)(3)(vi)). We recommend a summary of the validation protocol, including failure investigations.
- a description of the methods used and studies performed to evaluate the effect of the change and the data derived from such studies (21 CFR 601.12(b)(3)(iv) through (v)). We recommend submitting the following information and data:
  - the device manufacturer
  - the device type
  - blood unit number
  - component description (i.e., leukocytes reduced)
  - date of collection
  - date of testing
  - result interpretation(s)
  - the identity of the person performing the testing

- the identity of the collection facility
- evidence of QA oversight, and
- expected component specifications.

- Additionally, we recommend two months of QC data for actual platelet yield and volume, pH, and residual WBC count (if requesting approval for Leukocytes Reduced platelets).

We further recommend that you provide an agreement to summarize bacterial contamination testing results for the first two hundred and fifty (250) Platelets, Pheresis collections in your Annual Report.

2. Comparability Protocol: If you are an establishment with multiple manufacturing sites and wish to submit a comparability protocol to justify a reduced reporting category for a manufacturing change at multiple sites (see Section X.C.4 below), you must submit that protocol as a PAS (21 CFR 601.12(e)). In addition to the information listed in Section X.C.1 above , we recommend that you include the following:

- implementation plan
- proposed reporting category for changes made under proposed Comparability Protocol

3. CBE-30 submissions (excluding new facilities under an approved Comparability Protocol): Under 21 CFR 601.12(c)(3) and 601.12(b)(3)(i) through (vii), the following information must be included in your CBE-30 submission:

- identification of the Platelets, Pheresis components involved (e.g., single plateletpheresis component, double plateletpheresis components, and/or triple plateletpheresis components) and manufacturing site(s) or area(s) affected, and a detailed description of the proposed manufacturing change (including device collection technology and the collection protocol(s)). We recommend that you document this information in a cover letter and FDA Form 356h. To permit assessment of the documented manufacturing change, we recommend that you include copies of any new or revised SOPs.

- relevant validation protocols and data. We recommend that you submit a summary of the validation protocol, including failure investigation.

- the data derived from such studies. We recommend two months of QC data for actual platelet yield and volume, pH, and residual WBC count (if requesting approval for Leukocytes Reduced platelets).

4. CBE-30 submissions for new facilities under an approved Comparability Protocol: To comply with 21 CFR 601.12(c)(3) and 601.12(b)(3)(i) through (vii), the following information must be included:

- identification of the components involved (e.g., single plateletpheresis component, double plateletpheresis components, and/or triple plateletpheresis components) and new manufacturing site(s) or areas(s) affected, and a detailed description of the proposed implementation plan (manufacturing change including device collection technology and the collection protocol(s)). Additionally, we recommend that this information be documented in a cover letter and FDA Form 356h.

- relevant validation protocols and data. We recommend a summary of the validation protocol, including failure investigations to meet the requirement.

- the data derived from studies. We recommend two months of QC data for actual platelet yield and volume, pH, and residual WBC count (if requesting approval for Leukocytes Reduced platelets).

In addition, you should include the submission tracking number (STN) of the approved Comparability Protocol, or the STN(s) of changes to the SOPs associated with an approved Comparability Protocol.

D. Submission of Platelets, Pheresis Sample(s) to CBER

To obtain a biologics license under Section 351 of the Public Health Service Act for any biological product, the manufacturer must submit an application to CBER, and sample(s) representative of the product must be listed in the application (21 CFR 601.2(a)).

We recommend that:

- applicants with no prior experience in the collection of Platelets, Pheresis schedule submission of Platelets, Pheresis products to CBER.
- applicants who submit a CBE-30 for an additional facility under an approved Comparability Protocol generally would not need to submit Platelets, Pheresis products to CBER.

CBER may request the submission of product samples by other applicants, as necessary, during the review process or at any other time (21 CFR 610.2(a)).

E. Shipping Platelets, Pheresis Sample(s) to CBER

If CBER has requested you to submit a Platelets, Pheresis sample(s) to CBER, you should contact CBER Division of Hematology, Laboratory of Cellular Hematology at (301) 496-2577 to schedule delivery of the products to arrive prepaid. Platelets, Pheresis sample(s) should be shipped to the following address between 8:30 a.m. and 4:00 p.m. Monday through Friday, excluding Federal holidays:

Center for Biologics Evaluation and Research (CBER)
Food and Drug Administration
8800 Rockville Pike
Building 29, Room 323
Bethesda, Maryland 20892

We recommend that you enclose a pre-paid, self-addressed shipping label to allow return of shipping boxes and coolants, if desired.

We recommend that you ensure that the Platelets, Pheresis sample(s) arrives at CBER prior to the expiration time. The Platelets, Pheresis sample(s) should not expire on Friday or Saturday at midnight, or at midnight on the day before a Federal holiday.

Labeling and processing, including required testing for evidence of infection due to communicable disease agents (21 CFR 610.40), should be complete prior to shipment.

When shipping to us, you should follow your SOPs for collection, processing, storage and distribution of blood components intended for transfusion.

XI. CONTACT INFORMATION

You may direct questions specific to Platelets, Pheresis application submissions to the Division of Blood Applications. You may also direct questions to the Office of Communications, Training, and Manufacturers Assistance (OCTMA) as an initial general point of contact. Submit all registration forms (Form FDA 2830) and licensure applications/supplements to the Director, CBER.

Table 3: FDA Contact Information

Submissions: Registrations License Applications	Director, Division of Blood Applications Center for Biologics Evaluation and Research, HFM-370, Food and Drug Administration, c/o Document Control Center, HFM-99, 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448.
General Questions	Director, OCTMA, HFM-40, Food and Drug Administration, c/o Document Control Center, HFM-99, 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448, Voice (301) 827-2000; Fax (301) 827-3843.
Application Submission	Director, Division of Blood Applications, Center for Biologics Evaluation and Research, HFM-370, Food and Drug Administration, c/o Document Control Center, HFM-99, 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448, Voice (301) 827-3543; Fax (301) 827-3534.
Platelets, Pheresis Samples to CBER	Center for Biologics Evaluation and Research (CBER) Food and Drug Administration 8800 Rockville Pike Building 29, Room 323 Bethesda, Maryland 20892

XII. REFERENCES

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[原著]

事前検査におけるヘモグロビン測定の導入

香川県赤十字血液センター
内田 立身，窪田 明美，中西 幸美，安藤 浩子
西村 拓史，白井 隆，小河 敏伸，西尾由美子
細川 和浩，木村 史子，三枝 明子，本田 豊彦

Implementation of measuring hemoglobin concentration at pre-donation test

Kagawa Red Cross Blood Center
Tatsumi Uchida, Akemi Kubota, Yukimi Nakanishi, Hiroko Andoh,
Takuji Nishimura, Takashi Shirai, Toshinobu Ogoh, Yumiko Nishio
Kazuhiro Hosokawa, Humiko Kimura, Akiko Saigusa and Toyohiko Honda

抄　録

香川県赤十字血液センターでは2003年10月に，事前検査として血液比重にかわって，ヘモグロビン(Hb)測定法を導入した。Hb法の最大の利点はその定量性にあり，献血者にHb値を数字として提示することができ，Hb低値者，高値者に対する対応を明確にし得た。また，懸念されていたHb不足による献血不適格者数，VVR発症率も比重法施行時と大差がなかった。今回の検討で，Hb12.5g/dL以上がほぼ比重1.053以上に，12.0g/dL以上が1.052以上に相当すること，Hbと赤血球指数との関係から，赤血球が正色素性から小球性低色素性に変わるHb値が12.5～12.0g/dLであることから現行の採血基準は妥当であると考えられた。Hb法は測定装置がHbの表示まで時間を要すること，温度差による配慮が必要であるなどの欠点はあるが，定量性，均一性を重視するGMPからみても従来の比重法より優れていると結論した。

Key words: Pre-donation examination, Hemoglobin determination
Blood donation criteria, HemoCue hemoglobin analyzer

はじめに

香川県赤十字血液センターでは，2003年10月より，事前検査として硫酸銅法による比重測定にかわって，簡易ヘモグロビン(Hb)測定装置，ヘモキュウヘモグロビンシステム(以下Hb法)による方法に変更した。採血基準は，血液事業の根幹の一つであり，その判定には定量的なHb法が最も妥当と考えられるゆえである。自動血球算定装置がルーチン化したわが国において，貧血の診断はすべてHb，ヘマトクリット，赤血球数によっており，目視による比色法(ザーリ法)や比重法(硫酸銅法)は赤十字血液センターを除いて用いられていない。最近の献血の適否に関する世界の論文は，すべてがHb法を用いて判断しており[1)~3)]，比重法は

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検査法として教科書の記載すらない現状である[4]。

今回，比重法とHb法の比較，変更前後の献血不適格者の比率，副作用，とくに血管迷走神経反応(Vasovagal Reflex：以下VVR)の比率，また，200mL献血12.0g/dL以上，400mL献血12.5g/dL以上とされている採血基準の妥当性についても検討した。さらに，Hb法の有用性を生かして，不適格者のHb濃度別による個人指導のありかたについても検討したので，これらの成績を報告する。

方　法

簡易Hb法(ヘモキュウ)によるヘモグロビン測定は，あらかじめ試薬が充填された専用マイクロキュベットに10μLの末梢血をサンプリングしアナライザーにセットして，表示されるHb量を読み取る。Hb測定はアザイドメトヘモグロビン法により570nmと880nmからなる2波長様式によっている。

200mL献血申込者63名，400mL献血申込者62名において，血液比重測定と同時に自動血球計数装置(STKS)によるHb測定を行い両法の比較を行った。次に，平成14年4月1日から15年3月31日の間に比重法によって判定した献血者と平成16年4月1日から17年3月31日の間にHb法で判定した献血者において，本社採血基準による献血不適格者の比率，VVRの発症比率を比較検討した。また，献血申込者男性1,472名，女性771名のHb法によるHb濃度別度数分布を作成した。次に，STKSによって得られたMCV，MCH，MCHCとHb値の関係をみることにより，Hb法採用時の採血基準の妥当性を検討した。

Hb法(ヘモキュウ)を導入して1年6カ月経過した時点で，献血バスで実際に使用している看護師17名にアンケート調査を行った。

結　果

1．比重法とHb法の関係

400mL献血申込者のうち，血液比重1.053以上を示した献血者62名のHb値は12.6～17.3g/dLの範囲になり，その平均値±1SDは14.96±1.12g/dLであった。同様に比重1.052以上の200mL献血申込者63名は12.1～16.4の範囲で平均値は13.64±1.16g/dLであった。以上から，400mLの採血基準1.053以上またはHb12.5g/dL以上，200mLの採血基準1.052以上または12.0g/dL以上は両者ともcut off値として妥当であると考えられた。また，比重法の結果はHb値で幅広い範囲に分布し，定量性がないことも明らかとなった。

2．簡易Hb法と自動血球計算装置との相関

簡易Hb法(ヘモキュウ)と自動血球算定装置(Coulter STKS)によって測定した結果の相関を図1に示した。相関係数0.951(Y＝0.8893X＋1.59)の高い相関がみられた。

3．Hb法による献血者ヘモグロビンの度数分布

Hb測定の定量性を生かして献血者ヘモグロビンの度数分布が得られた（図2）。献血申込者の男性1,472名，女性771名の解析で最も頻度が高いのは，男性15.0～15.5g/dL，女性12.5～13.0g/dLであった。

4．比重法およびHb法による献血不適格者の比較

表1に比重法(平成14年4月1日～15年3月31日)とHb法(16年4月1日～17年3月31日)で判定した比重あるいはHb不足による献血不適格者の比率を示す。両者の年齢区分毎不適格率で大きな差異は認めなかった。200mL，400mLの合計において比重法の男性申込者は23,985名，うち不適格者数(率)151名(0.6%)，女性申込者は21,715名，うち不適格者4,404名(20.3%)，Hb法の男性申込者22,749名，不適格者数(率)151(0.6%)，女性申込者20,504名，不適格者数3,958名(19.3%)で，いずれも差異を認めなかった。400mL申込女性で40歳代では，多数の(26～30%)不適格者がみられた。また，400mL申込女性でHb12.5g/dL未満431名のうち10.0g/dL未満が43名(10.0%)，8g/dL未満も4名みられ，治療を必要とすると考えられた。

5．献血時副作用の比較

輸血副作用のうち採血基準が関係すると思われるvaso-vagal reaction(VVR)の発症率を比較した。ヘモキュウが用いられる献血バス200mL，400mL採血のVVRはHb法で男性が減少していたが，女性での頻度の差は認められなかった（表2）。いずれにしてもHb法を導入してVVRが増加することはなかった。

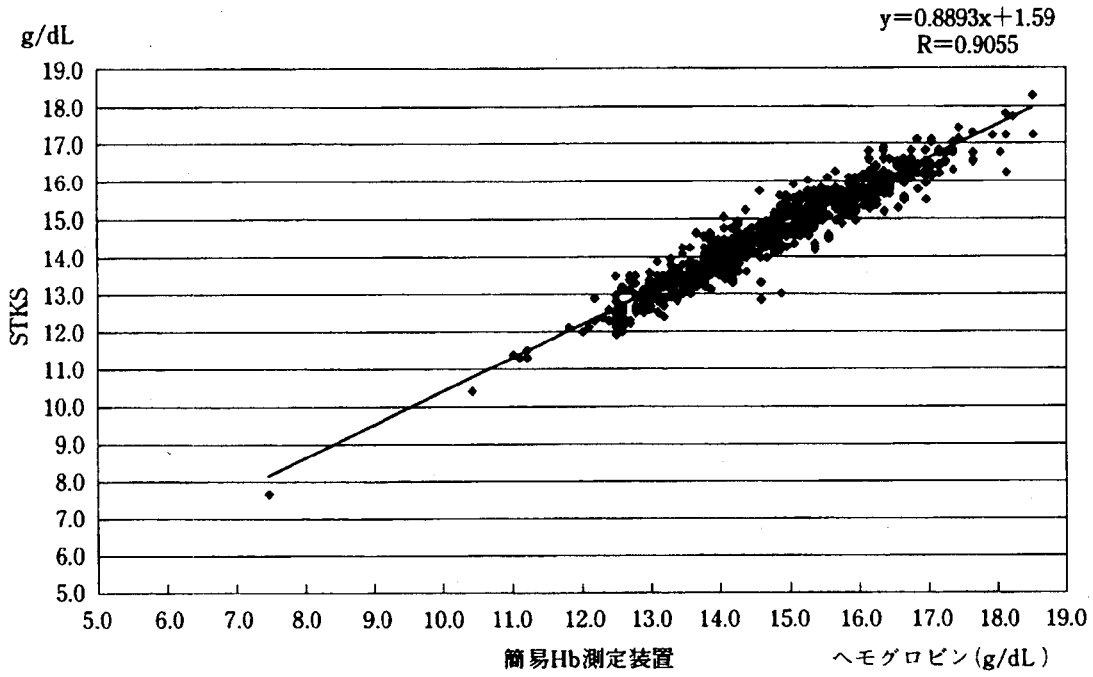
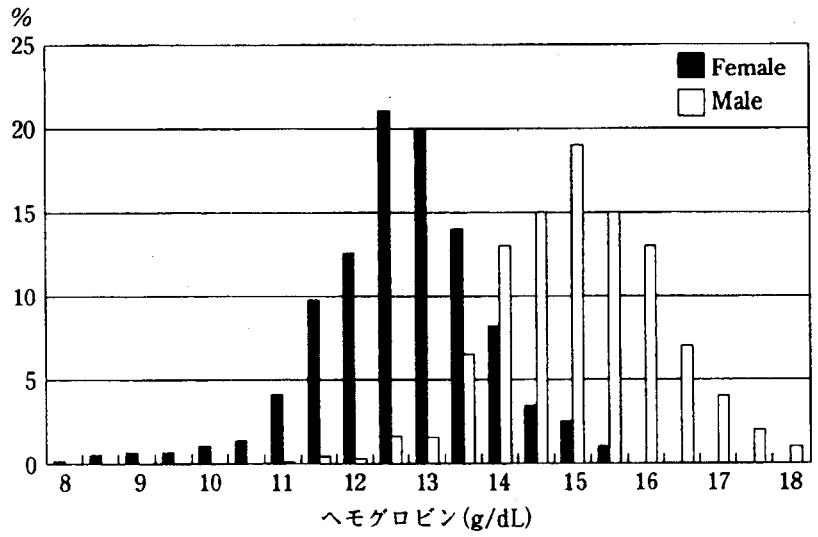


図1　簡易Hb測定装置(ヘモキュウ)と自動血球算定装置(STKS)との比較



献血申込者，男性1,472名，女性771名のヘモグロビン分布。男性で最も多いのは15.0～15.5g/dL，女性で最も多いのは12.5～13.0g/dLであった。

図2　献血申込者のヘモグロビン値の分布

6．ヘモグロビンと赤血球指数の関係

Hb値と赤血球指数(MCV，MCH，MCHC)の平均値の関係を表3に示す。Hbの低下に伴って赤血球指数も低下してくる。低下傾向が認められるのは男性で，MCV，MVH，MCHCともHb12.5g/dL未満から，女性12.0g/dL未満からであり，小球性低色素性の傾向が認められるのは男性が0.5g/dL高かった。以上から，Hbの低下にともなって赤血球は12.5～12.0g/dLで正色素性から小球性低色素性に変わることが判明した。

7．Hb低値による献血不適格者への対応

Hb測定の定量性を生かして献血者のHb値に応じた指導を行うこととした。Hb値10g/dL未満の献血者には医療機関を受診し治療を受けるよう医

表1　比重法およびHb法による献血不適格者の比較

			年齢区分	19～19	20～29	30～39	40～49	50～59	60～69	計
比重法	男性	200	申込数	1,091	286	346	550	517	210	3,000
			不適数	8	0	5	5	15	1	34
			不適率	0.7	0	1.4	0.9	2.9	0.5	1.1
		400	申込数	1,040	4,464	5,683	5,198	3,659	941	20,985
			不適数	5	14	21	29	30	18	117
			不適率	0.5	0.3	0.4	0.6	0.8	1.9	0.6
	女性	200	申込数	2,240	3,139	2,938	1,976	1,904	689	12,877
			不適数	399	602	689	448	239	67	2,444
			不適率	17.8	19.2	23.5	22.8	12.6	9.7	19.0
		400	申込数	601	1,923	2,097	1,923	1,771	523	8,838
			不適数	110	446	588	582	198	36	1960
			不適率	18.3	23.2	28.0	30.3	11.2	6.9	22.2
Hb法	男性	200	申込数	1,050	298	340	421	448	224	2,781
			不適数	7	1	1	4	5	8	26
			不適率	0.7	0.3	0.3	1.0	1.1	3.6	0.9
		400	申込数	1,147	4,183	5,510	4,832	3,373	923	19,968
			不適数	2	9	17	24	31	18	101
			不適率	0.2	0.2	0.3	0.5	0.9	2.0	0.5
	女性	200	申込数	2,422	2,579	2,825	1,762	1,510	612	11,710
			不適数	461	425	593	386	140	64	2,069
			不適率	19.0	16.5	21.0	21.9	9.3	10.5	17.7
		400	申込数	601	2,038	2,286	1,786	1,584	499	8,794
			不適数	176	454	596	467	163	33	1,889
			不適率	29.3	22.3	26.1	26.1	10.3	6.6	21.5

表2　比重法およびHb法によるVVR発症率の比較

		男　性	女　性
比重法	軽症	83	53
	重症	1	1
	計	84	54
	発症率（%）	0.44	0.43
Hb法	軽症	44	50
	重症	3	2
	計	47	52
	発症率（%）	0.27	0.44

師が指導し，12g/dL未満，10g/dL以上の献血者には食事指導用のパンフレットを作成し配布すると同時に，月に1度栄養士会による個別栄養指導も開設した。

8．Hb高値の献血者の頻度

採血可能であった男性1,472名，女性771名について（図2），Hb17.0g/dL以上の比率は，17.5＞Hb≧17.0：30例（3.0%），18.0＞Hb≧17.5：3例（0.3%），18.5＞Hb≧18.0：3例（0.3%），19.0＞Hb≧18.5：1例（0.1%）の計37例で，いずれも男性で女性にはみられなかった。また，赤血球指数は正常であった。

9．ヘモキュウ使用者のアンケート結果

ヘモキュウを使用している看護師のアンケート結果は以下のとおりであった。まず，利点としては①感染性廃棄物としての後始末が簡単になった（100%），②測定法が簡単である（74%），③献血者にHb値を示すことで説得力がある（63%），などであった。欠点としては①外気温や光線の影響

表3　Hbと赤血球指数の関係

Hb(g/dL)	男性			女性		
	MCV (fl)	MCH (pg)	MCHC (g/dL)	MCV (fl)	MCH (pg)	MCHC (g/dL)
16.0>Hb≧15.5	93±4	32±2	34±0			
15.5>Hb≧15.0	93±5	32±2	34±1	93±4	32±2	35±1
15.0>Hb≧14.5	92±3	32±2	34±1	92±3	32±1	35±0
14.5>Hb≧14.0	92±5	32±2	34±1	91±3	31±1	35±0
14.0>Hb≧13.5	92±4	32±2	35±1	91±1	32±1	35±0
13.5>Hb≧13.0	92±6	32±2	34±0	90±4	31±2	35±1
13.0>Hb≧12.5	92±5	32±2	34±1	90±3	31±1	34±0
12.5>Hb≧12.0	84±6	28±3	34±1	91±6	31±2	34±0
12.0>Hb≧11.5	83±5	28±2	34±0	87±5	30±2	34±1
11.5>Hb≧11.0*	77±0	25±0	33±0	83±5	28±2	34±0
11.0>Hb≧10.5				83±6	27±2	34±1

n=20 (*n=2)

を受けやすい(94%)，②測定に時間がかかる(94%)，③新たに精度管理が必要になった(69%)，などであった。

考　案

従来から採血基準として用いられている硫酸銅法による血液比重は，献血者を1.052未満，1.052以上(200mL)，1.053以上(400mL)と3区分して可否を判定するもので，各区分内に様々なヘモグロビン濃度が含まれる定性法であり，血液事業が始まって以来半世紀あまりずっと用いられている。しかしながら，比重法は測定者により±0.001程度のバラツキがあることが指摘されている[5]。一般に，赤血球沈降速度は，高温で促進，低温で遅延し補正が必要とされている[6]。佐野らの検討では，10℃で20℃に比し，0.001～0.002低い値，30℃で0.001～0.002高い値が得られるとしている[5]。また，Jamesら[7]は比重法の方がHb法よりも偽の適判定(false-pass)が多いことを証明した。以上から，現在のGMPに準拠した血液事業の理念からすれば，いつ，誰が，どう行っても一定した数値が得られるHb法の方が理想的であることは明白である。今回，簡易ヘモグロビン測定装置(ヘモキュウ)を導入して2年あまりになるので，従来の比重法との比較を様々の面から試みた。

ヘモキュウによるHb測定は，自動血球計算装置との相関で高い相関があり，とくに問題がないことが示された。これは過去の報告のとおりである[8)-10)]。また，比重法とHb法で献血不適格者の比率が異なるか否かを検討した。比重法とHb法の比較検討では，時期が異なるため厳密な比較ではないが，献血不適格者の増減はなく，現行の採血基準で有意の差はないと思われた。男性のVVRは，軽症でHb法の方が少なくヘモグロビン値以外の原因が考えられる。

Hb法の利点は，献血者のHb値を数字として表示できることであり，度数分布を知ることができる。この度数分布によって，女性献血申込者の中に，10g/dL未満の要加療者が不適格者の10%近くみられることが判明した。従来の比重法では，低比重以外の情報がなくそのまま放置されるわけであるが，Hb法ではHb値を提示できるので医療機関への受診を勧めることができた。また，10.0～12.5g/dLの方には栄養指導や食事のアドバイスができた。すなわち，貧血の予防と治療の双方を区別して指導することが可能である。

採血基準では，真性赤血球増加症(多血症)は採血しないことになっているが，比重法ではHb高値者を除外することができない。Hbを測定することによって，17g/dL以上は男性で3.7%にみられ，女性にはみられなかった。また，これらは白血球数，血小板数，赤血球指数が正常で，相対的(ストレス)赤血球増加症と考えられた。真性赤血

球増加症は白血球増加，血小板増加，小球性低色素性赤血球の傾向を示すことから，今回の検討で，Hb19.0g/dL未満で白血球数，血小板数，赤血球指数が正常であれば，採血可能と判断した．

今回Hb測定の定量性を生かして，従来の採血基準の妥当性を検討した。まず，比重法とHb法の比較で，1.052以上はHb12.1g/dL以上を，1.053以上はHb12.6g/dL以上を示した。また，Hb値の低下に伴って赤血球指数が低下してくるが，平均値の低下開始に相当するHb値は，小球性低色素性赤血球に移行する点で，女性の成分採血の際の可否判定に用いられているところである。低下開始点は男性12.5g/dL，女性12.0g/dLで，男性が0.5g/dL高かった。また，12.5g/dL以下の男性献血申込者の比率は0.6%と少なく，あえて男性の採血基準を引き上げる必要はないと考えられる。以上および米国FDAの基準[11]を勘案して，私たちはHb法の判定に男女差を設けず，従来の採血基準を用いることで問題がないと考えた。

今回用いたヘモキュウによるHb測定法は，英国のNational Quality Assessment Schemeの精度管理で正確性の保証が得られている[11]。また，静脈血採血と耳朶あるいは指尖毛細血管穿刺との間に差異があるとの議論がある。これは，サンプリングが不適切な場合で，血流が十分保たれ，穿刺が正確に行われた場合は有意の差がないとの見解が一般的である[12]。また，指尖穿刺の方が，静脈穿刺より正確性を欠くとの報告もある[13]。

献血の可否を決定する検査は，大別して，血液学的検査，生化学検査，感染症関連検査が行われている。生化学，感染症関連検査は1953年血液事業が開始されて以来，次々と改良，改善が加えられ，NAT検査の導入によって世界的水準を保つにいたっている。一方，採血基準の根幹である貧血の有無判定については，当初の硫酸銅による比重法が現在にいたるも用いられ，一向に改良の気配がない。その間，比重不足による献血不適格者は増加の一途であり，女性の400mL献血で本社の調査で，1990年 9.9%，2000年 18.1%，2003年21.3%である[14),15]。輸血によるウイルス性肝炎が激減したのと極めて対照的である。いうまでもなく比重法は測定者の目視による定性的判定法であり，温度・湿度の影響，使用滴下回数や蒸発，観察者の主観を無視できない。臨床の場においても，かつては比重法や比色法(ザーリ法)が用いられたが，現在はHb,ヘマトクリットに統一され，比重，比色によっている医療機関は皆無である。したがって，血液センターと医療機関の間で貧血に関するかぎり整合した議論が全くできていない。国は献血者の確保の推進として，献血の検査結果を健康診査，人間ドック，職場検診で活用するとともに，地域の保健指導に用いるよう求めているが[16]，比重で表示される献血不適格者の成績は利用し得ない状況である。以上から，血液センターにおいてもHb法を早急に導入し，定量的な評価によって献血者の健康を守る配慮をすべきである。

結　論

1．献血の可否判定にHb法を導入した。従来の比重法に比して，不適格者率，副作用発症率とも差異はなかった。

2．Hbおよび赤血球指数の度数分布から，従来の採血基準(400mL：12.5g/dL以上，200mL：12.0mg/dL以上)を用いて差し支えないことが判明した。

3．Hb低値の献血申込者に対して，Hb値に応じた栄養指導，医療機関への受診指導を行うことができた。

4．Hb法は定量性，客観性において比重法に優っており，Hb法に統一すべきであることを提言した。

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(12)

Statistical analysis of inappropriate results from current Hb screening methods for blood donors

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BACKGROUND: The objective was to apply statistical analysis to the false passes and fails that occur with the primary and secondary Hb-screening methods used at blood-donor sessions.

STUDY DESIGN AND METHODS: Venous samples from 1513 potential donors who had undergone primary $CuSO_4$ screening using capillary blood (Hb cut-offs: women, 125 g/L; men, 135 g/L) were tested at the session by a secondary method (HemoCue; cut-offs: women, 120 g/L; men, 130 g/L) and again at the base laboratory using another system (Beckman Coulter General S system), which generated the "true" Hb value.

RESULTS: False-pass and -fail rates for women and men, respectively, were 11.2 and 6.3 percent (women) and 5.2 and 1.8 percent (men) for $CuSO_4$; 1.9 and 3.7 percent (women) and 1.5 and 0.4 percent (men) for HemoCue; and 2.7 and 2.4 percent (women) and 1.8 and 0.2 percent (men) for a combined procedure that mimicked current practice of only testing $CuSO_4$ fails by HemoCue.

CONCLUSION: $CuSO_4$ Hb screening gives large numbers of false passes, particularly in women. Using venous samples, the majority correctly pass at the lower HemoCue cut-offs. The current dual-testing policy appears convenient for donor sessions, but because small percentages of false passes and fails represent large numbers of donors, every effort should be made to improve the accuracy of Hb screening.

Potential blood donors who attend donor sessions in the Trent Region (situated in the East Midlands, UK) initially undergo a health-screening survey. After passed this survey, they are subjected to primary Hb screening by the $CuSO_4$ gravimetric method carried out on finger-prick capillary blood, the cut-off levels for donation being set to correspond to Hb values of 125 g per L for women and 135 g per L for men.[1-3] To optimize blood-collection rates, UK regulations allow individuals who fail the primary $CuSO_4$ test to continue with the donation process if they pass the secondary Hb screening performed on a predonation venous sample using the HemoCue system.[2,4,5] With this method, donor acceptance or rejection is set at lower Hb levels: 120 g per L for women and 130 g per L for men.

We have recently become concerned that some donors are being bled inappropriately with these screening methods, whilst others with an acceptable Hb level are failing the tests. The purpose of this study is to determine whether this is the case and how to quantitate the problem by applying statistical analysis to the primary and secondary Hb-screening procedures used at our donor sessions, comparing them with a standard Hb measurement.

MATERIALS AND METHODS

Studies were carried out on potential volunteer blood donors attending routine donor sessions held throughout the Trent Region. All participants were fully informed of the purpose of the project and gave signed consent. The

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study had been formally approved by the Trent Multicentre Research Ethics Committee.

To avoid bias when selecting individual subjects for the study, a simple systematic sampling scheme was used at each donor session. Before screening, every n^{th} potential donor was approached for consent to enroll in the trial. If an individual declined, each subsequent person was approached until one consented. Subsequently, the next n^{th} individual was approached and so on. The value of n was controlled by the transfusion service staff at the screening station.

During quiet periods, n could be set at 1 so that every potential donor could be approached. During busier periods a larger value of n could be set, and at exceptionally busy times, sampling could be discontinued completely to avoid delaying the session.

Venous blood samples were collected from 730 women and 783 men who were potential donors who had undergone the primary $CuSO_4$ gravimetric Hb-screening test. All the venous samples, which included those from individuals who passed and failed $CuSO_4$ screening, were taken before any blood donation and tested at the donor session by the HemoCue method. These machines are calibrated to the International Council for Standardization in Haematology standard. The HemoCue results were used to construct a hypothetical screening test and were expressed as either a pass or fail in respect to cut-off Hb values of 120 g per L for women and 130 g per L for men.

A combined procedure that followed current practice was also applied. Thus, respondents were initially screened on the standard $CuSO_4$ test; those who passed were deemed to have passed the combined procedure. Those who failed the $CuSO_4$ test were considered to have passed the combined procedure if a subsequent HemoCue result was at least 120 g per L for women and 130 g per L for men.

The venous samples were tested again at the base laboratory with the Beckman Coulter General-S system (Beckman Coulter, High Wycombe, UK). These results were deemed to be the "true" Hb values against which the results of the $CuSO_4$, HemoCue and combined procedures could be compared.

Statistical methodology

In view of the known differences in Hb levels between men and women, data for the different sexes were analyzed separately. Because donor characteristics would be likely to vary considerably between individual donor sessions, any sampling biases with respect to donor age were adjusted by stratifying data for both men and women into quinquennial age bands and then testing to determine whether reweighting of the age-stratified data was necessary. This was achieved by chi-squared tests, comparing test and whole donor population data, and by a one-way ANOVA conducted for each of the women and men data sets with various Hb counts as the dependent variable and age category as the factor of interest.

The need to reweight was confirmed by both tests. A chi-squared value of 54.88 ($p < 0.0001$, $df = 10$) in respect to age distribution for women indicated that the test sample was severely under-represented in the 17 to 30 years age range, whereas for the age distribution for men, a chi-squared value of 18.60 ($p < 0.046$, $df = 10$) showed the test sample was under-represented in the 20-and-under ages. For the ANOVA, F values of 3.00 ($df = 10,724$, $p = 0.001$) for women and 2.23 ($df = 10,782$, $p = 0.015$) for men confirmed that in each case, Hb varied with age.

Reweighting to give reasonable donor population estimates was therefore carried out by calculating the stratified sample proportion of individuals possessing the appropriate attribute, together with its SE. This proportion is an unbiased estimator of the true population proportion possessing the desired attribute.[6,7] All values and standard errors were obtained using a statistical software package (SAS, SAS Institute, Cary, NC), and all proportions and standard errors were converted to percentages by multiplying them by 100.

The results of each screening test were compared to baseline Beckman Coulter Hb values of 125 g per L (women) and 135 g per L (men) for the $CuSO_4$ test and 120 g per L (women) and 130 g per L (men) for the HemoCue and combined procedures. The "false-pass" rates (i.e., the percentages of potential donors who would pass the relevant screening test but would fail the baseline Beckman Coulter test) were of particular interest.

RESULTS

Table 1 shows the results of the $CuSO_4$ Hb screening compared with the baseline Beckman Coulter values of 125 g per L (women) and 135 g per L (men). Table 2 (women)

TABLE 1. Results of $CuSO_4$ screening test compared with Beckman Coulter baseline at Hb levels of 125 and 135 g per L for women and men, respectively: population percentage estimates, stratum weighted by age

		Women		Men	
$CuSO_4$ result	Beckman Coulter result	Estimated percentage	SE	Estimated percentage	SE
Fail	Fail	12.4	1.3	3.9	0.7
Fail	Pass	6.3	0.9	1.8	0.5
Pass	Fail	11.2	1.3	5.2	0.8
Pass	Pass	70.1	1.8	89.0	1.1
Correct classification (%)		82.5		93.0	

TABLE 2. Results of screening tests for women compared with Beckman Coulter baseline Hb level of 120 g per L: population percentage estimates, stratum weighted by age

Screening test result	Beckman Coulter test result	$CuSO_4$ Estimated percentage	SE	HemoCue Estimated percentage	SE	Combined Estimated percentage	SE
Fail	Fail	6.0	1.0	6.0	0.9	5.3	0.9
Fail	Pass	12.7	1.3	3.7	0.7	2.4	0.6
Pass	Fail	1.9	0.6	1.9	0.6	2.7	0.7
Pass	Pass	79.4	1.6	88.4	1.3	89.6	1.2
Correct classification (%)		85.4		94.4		94.9	

TABLE 3. Results of screening tests for men compared with Beckman Coulter baseline Hb level of 130 g per L: population percentage estimates, stratum weighted by age

Screening test result	Beckman Coulter test result	$CuSO_4$ Estimated percentage	SE	HemoCue Estimated percentage	SE	Combined Estimated percentage	SE
Fail	Fail	2.2	0.5	2.0	0.5	1.7	0.5
Fail	Pass	3.6	0.6	0.4	0.2	0.2	0.2
Pass	Fail	1.3	0.4	1.5	0.4	1.8	0.5
Pass	Pass	93.0	0.9	96.2	0.7	96.3	0.7
Correct classification (%)		95.3		98.2		98.0	

and Table 3 (men) give the results of the individual $CuSO_4$ and HemoCue screening tests and of the combined procedures, comparing them with Beckman Coulter baseline values of 120 g per L for women and 130 g per L for men.

DISCUSSION

The UK requires a predonation Hb screening to be carried out on all potential donors, and only individuals with an Hb level at or greater than 120 g per L for women or 130 g per L for men proceed to donate.[8,9] However, accuracy of Hb-screening procedures at blood-donor sessions may be a problem, and our study, by quantitating this, provides data for informed debate (Tables 1-3). It also shows how such studies may be approached in the future. In the present case, statistical analysis without the need to reweight would have required an even larger sample size. This would have been impractical because the length of time it took to obtain the informed consent required by the Ethics Committee had a deleterious effect on the efficient running of many donor sessions, particularly busy ones. As a result, the test sample was not representative of the donor population as a whole. This, and because of clustering of sessions, made it important to reweight the data so that the test population truly reflected the whole donor population with regard to factors that affect screening outcomes, such as age and sex. Reweighting necessitated expressing the results in proportions (percentages) rather than as raw figures.

The primary purpose of Hb screening is donor protection, preventing an anemic individual from exacerbating their condition with potential ill effects. The secondary purpose is to ensure the patient receives a minimum infused Hb dose per RBC transfusion. Screening also acts as a nonspecific measure of the general health of the donor and may identify some conditions which could potentially be harmful to the recipient.[2]

Protocols with set cut-offs are not without problems: they cause administration and quality control costs, donor inconvenience, expense and anxiety as a result of medical follow-up of deferrals, as well as permanent loss of donors. Additionally, cut-offs need to be set to maximize donor safety but be balanced against the system's ability to collect an adequate blood supply, a particular concern when trying to exclude women with iron deficiency. Hb reference ranges vary with age, race, and sex, and are affected by altitude, smoking, and the site from which the sample is taken.[2,10] It has been suggested that, rather than having set cut-off values, a standard should be established whereby blood donations contain a "minimum Hb dose" of 50 g; this would allow individual blood centers to evaluate the appropriate safe Hb cut-off for their donors.[2]

The $CuSO_4$ gravimetric test has been the method of choice in the UK for primary Hb screening of potential blood donors for many years. It is fast, inexpensive, does not require a venous sample, and, although rigorous training and constant monitoring of session staff is necessary, does not need trained laboratory personnel. It does not, however, give a quantitative result, has a subjective endpoint, is difficult to quality control, and presents problems with the disposal of biohazardous material.[2] Although very anemic donors can, on occasion, pass the $CuSO_4$ test,[11] early reports suggested that the $CuSO_4$ method tended to give inappropriate failures, and thus significant numbers of such failed donors could be recovered with a revised Hb range or if an alternative screening method was applied.[2]

This is the rationale for the primary and secondary Hb-screening tests used in the UK. It is supported by several studies that show that many units of blood can be collected that would otherwise be lost. Figures of between 11 and approximately 50 percent recovery of donations with secondary screening are quoted.[2,12-14] The lowering of the cut-off Hb values for the secondary screening also helps. In one study, 29 percent of failed

donors passed the secondary test (HemoCue) at Hb cut-offs of 125 and 135 g per L (women and men, respectively); but with the cut-offs reduced to 120 and 130 g per L, this figure increased to over 44 percent.[14]

Initially there was concern that such a high proportion of donors, 11.2 percent of women and 5.2 percent of men in the present study, inappropriately pass the $CuSO_4$ screening test (Table 1); and, it should be noted that at these higher baselines, a HemoCue screening test would have considerably reduced the false-pass rates. Thus, the high false-pass rates in Table 1 do not mean that there is a similar proportion of donors being bled inappropriately. Examination of Tables 2 and 3 show that at baselines of 120 and 130 g per L, the $CuSO_4$ screening tests exhibit conservative false-pass rates similar in magnitude to the HemoCue procedure; only 1.9 percent of women and 1.3 percent of men who pass the $CuSO_4$ test have Hb levels less than 120 and 130 g per L, respectively, and should have been rejected as donors, indicating that, in practice, the current $CuSO_4$ cut-off levels can be tolerated. (The higher false-fail rates with the $CuSO_4$ test in Tables 2 and 3 are due to the higher cut-off settings.)

Tables 2 and 3 show that, had it been used in isolation, the HemoCue procedure would have classified 94.4 percent of women and 98.2 percent of men correctly at Hb levels of 120 and 130 g per L, respectively. Although this would appear to offer an improvement on the $CuSO_4$ test (set at 125 and 135 g/L for women and men, respectively), at present, the HemoCue procedure would be difficult to apply as a primary screening test on every potential donor because venous samples are preferred at our sessions. (HemoCue can be used on finger-prick blood, but capillary samples are known to give unreliable results[12,15] with all technologies and are thus unsuitable for secondary screening of blood donors.) Taking a venous sample from each person before donation could prove unacceptable to donors, slow down the donation process, as well as increase costs. Many studies have shown the excellent correlation between HemoCue and standard photometric methods in the laboratory,[14-18] and indeed we found the same in a prestudy evaluation of the analyzers used in this project. (In addition, HemoCue has a theoretic advantage over other photometric methods in that it incorporates a turbidity control, allowing more accurate results on lipemic samples.[2]) However, previous work has shown that accurate measurement of Hb level using the HemoCue system is difficult to achieve in the field.[19,20] There are several possible reasons for this; they include inadequate mixing of specimens,[19] sampling techniques, and operator performance,[20] rather than problems inherent to the methodology, and studies have shown that meticulous attention to sample mixing, mode of filling the cuvette, and continuous monitoring and training of staff can help to improve performance.[20]

Tables 1 through 3 show that the $CuSO_4$ and HemoCue screening tests are less accurate, compared with Beckman Coulter values, for women than men, with false-pass and -fail rates being higher for women than males. This has been recognized previously, and it was suggested that such differences in screening-test performance can be explained by the distribution of women and men donor Hb levels relative to the cut-off values for acceptance.[21] A comforting factor in our study, in spite of its relatively small sample size, is that the lowest false-pass levels were 109 g per L for women and 123 g per L for men. Although it was inappropriate to collect blood from such individuals by our current guidelines, these figures are not alarming; there were no clinical sequelae, as far as we are aware, in the donors, and the recipients would have obtained an adequate amount of Hb. The donors who had been inappropriately bled were contacted and informed.

The results of the "combined" screening procedures (Tables 2 and 3), which mimic current practice at donor sessions, respectively, show false-pass and false-fail rates of 2.7 and 2.4 percent, respectively, for women and 1.8 and 0.2 percent, respectively, for men. The false-pass rates for the combined procedure slightly exceed those for the HemoCue alone: 95-percent CIs for these differences in rate are approximately 1.6 and 0.8 percent for women and men, respectively. On the other hand, the false-fail rates on the combined procedures are slightly smaller than for HemoCue alone, with 95-percent CIs for these differences in rate of approximately 2.3 and 0.6 percent for women and men, respectively. It should be noted here that any false pass on HemoCue alone would also pass the combined procedure, regardless of the $CuSO_4$ test result. Consequently, the false-pass rate for the combined procedure must be at least as great as that for HemoCue alone.

In summary, compared with HemoCue alone, current practice trades off a slightly higher false-pass rate against a slightly lower false-fail rate, and so is still reasonable in spite of the error rates in the initial $CuSO_4$ screen, and they need not be changed until the problems of accurately measuring Hb in the field can be reduced or eliminated. Because approximately 2 million donations are collected annually in the UK, even small percentages of false passes and false fails at the Hb-screening stage represent a large number of individuals, and, consequently, any improvement in accuracy of Hb screening will be welcome.

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原　著

短期間の術前自己血貯血法の検討

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はじめに

医療レベルの向上に伴いその質が問われる現在，手術における同種血輸血の回避は患者の当然の選択肢となりつつある。心大血管手術においては，早くから多くの施設が積極的に自己血輸血を導入することにより，無輸血達成へ向けて努力している。無輸血達成率は自己血貯血量および貯血期間に比例するのは周知の事実である。しかし心大血管手術においては，長期の待期期間を設けられる場合がそれほどなく，術前の長期入院や通院も患者の負担が大きい。そこで当科では可及的に貯血期間を短縮し，貯血量を最大限に準備できる方法として，術前8日からの貯血開始を基本的に施行してきた。今回この貯血法を施行した186例を貯血期間が9日以上であった群と7日以下であった群とで比較検証し，また同種血輸血に至った例と無輸血例とを要因別に比較し，その成績と限界について検討した。

対象・方法

当科で1996年9月から2003年2月までに人工心肺を使用した心大血管手術例は427例であった。そのうち自己血貯血を施行したのは258例で，すべての人工心肺使用例中60.4％，全待期手術中73.5％あった。対象手術は冠動脈バイパス術，弁膜症手術，胸部大動脈瘤手術，先天性心疾患手術，その他であった。自己血貯血の適応は，原則として年齢が80歳以下で入院時Hbが10.0 g/dl以上の待期手術としており，非適応は感染性心内膜炎患者，透析患者，高度心不全患者，左主幹部病変を伴う不安定狭心症患者としている。貯血は全例入院中としている。自己血採血のプロトコールは，毎回採血前にHb値を測定し，10.0 g/dl以上であれば1週間ごとに400 ml採血している。保険適応内であればエリスロポエチン製剤（EPO）を6,000単位静脈投与を隔日投与，もしくは24,000単位の皮下注投与を隔週に投与した。また，鉄剤としてフマル酸第一鉄305 mgを毎日内服投与した。ここで論ずる貯血期間とは，初回自己血貯血開始日より手術前日までの日数とした。待期手術の患者は8日前に入院し，入院日に400 mlの貯血を行い，1週間後の手術前日にも400 ml貯血する（EPO投与は皮下注の場合は初回の1回のみ，静注の場合は計3回投与となる）という貯血法を186例に施行した（M群）。準緊急手術症例や心房中隔欠損症などの軽症例では貯血期間が7日以下で，400 mlのみの貯血で手術に臨み，これらは44例であった（S群）。術前の精査などで術前8日以前より入院可能であった患者においては，手術が決定した時点から貯血を開始した。このような症例で9日以上の貯血期間が得られたのは28例であった（L群）。これらの3群の無輸血率を比較するとともにM群において同種血輸血に至った例と無輸血例を性差，年齢，体重，EPO使用量，入院時Hb値，手術直前Hb値，人工心肺時間，手術時間，術式についておのおの要因別に比較した。検討において，

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術後から退院まで同種血輸血を施行しなかったものを無輸血例とした。手術時は全例回収洗浄式自己血輸血装置を用い，術後約12時間はドレーン排液も回収した。人工心肺は無血体外循環で手術終了時回路内血液を返血した。各群の数値は平均値±標準偏差で表し，統計学的検定はstudent-t，χ^2，分散分析を用い，p値＜0.05を有意差ありとした。

結果

各群の手術術式の内訳，およびその無輸血率は表1に示した。冠動脈バイパス術に貯血期間が短い傾向がみられたが，手術を急ぐ必要のある例が多かったためと思われた。おのおの3群間に有意差は認めなかったが，冠動脈バイパス術の無輸血率が低く，貯血期間の短い群にその傾向が強かった。各群の性差，年齢，体重，貯血期間，総貯血量，EPO使用量，入院時Hb値，手術直前Hb値，人工心肺時間，手術時間，無輸血率を表2，表3に示した。S群の貯血期間は1～7日，平均5.5±1.6日で，L群が9～28日，平均15.8±5.6日であった。総貯血量はM群で400～800 ml，平均770±103 ml，S群がすべて400 ml，L群が800～1,600 ml，平均1,029±249 mlであった。性差，

表1　対象手術と無輸血率

術式	例数			無輸血率		
	M群	S群	L群	M群	S群	L群
CABG	72 (63.7%)	29 (25.7%)	12 (10.6%)	72.2%	55.2%	91.7%
VD	76 (78.4%)	8 (8.2%)	13 (13.4%)	90.8%	87.5%	92.3%
TAA	14 (87.5%)		2 (12.5%)	78.6%		100 %
CHD	12 (63.2%)	7 (36.8%)		100 %	100 %	
その他	12 (92.3%)		1 (7.7%)	66.7%		100 %

CABG:冠動脈バイパス術，VD:弁膜症手術，TAA:胸部大動脈瘤手術
CHD:先天性心疾患手術

表2　対象群の比較1

	例数	性差 (M/F)	年齢 (years)	体重 (Kg)	貯血期間 (days)	総貯血量 (ml)	EPO投与量 (×1000 IU)
M群	186	119/67	63.1±12.9	56.3± 9.1	8.0±0.0	770±103	20.9± 5.9
S群	44	28/16	62.7±10.4	57.3±10.9	5.5±1.6	400± 0	3.8± 7.4
L群	28	18/10	61.6± 9.1	59.6± 9.6	15.8±5.6	1029±249	29.4±15.3

表3　対象群の比較2

	入院時Hb (g/dl)	手術直前Hb (g/dl)	人工心肺時間 (min.)	手術時間 (min.)	無輸血率	p value
M群	13.0±1.4	11.0±1.4	114±70	246±124	81.7%	0.047*
S群	12.9±1.7	11.4±1.4	99±49	242±155	68.2%	
L群	13.5±1.3	11.2±1.4	109±35	223± 53	92.9%	0.231

年齢，体重，入院時Hb値，手術直前Hb値，人工心肺時間，手術時間において3群間に有意差は認めなかった。M群の無輸血率は81.7%で，S群の68.2%と比べ有意に高く（p=0.047），L群の92.9%と比べ低いものの有意差はなかった。M群において同種血輸血例と無輸血例を，性差，年齢，体重，貯血量，EPO使用量，入院時Hb値，手術直前Hb値，人工心肺時間，手術時間の各要因で比較したところ（表4），年齢，体重，入院時Hb値，手術直前Hb値，人工心肺時間，手術時間において有意差を認めた。M群の内で，2回目の採血前にHb値が10.0 g/dl以下，もしくは全身状態不良，採取困難な例で800 ml貯血できなかった例は15例（8.1%）あり，その無輸血率は66.7%と低い傾向にあったが，800 ml貯血例の無輸血率と有意差は認めなかった。また，術後出血再開胸や再手術を施行した例は9例あり，その無輸血率は44.4%と有意に低かった。術式では冠動脈バイパス術と弁膜症手術を比較すると前者で無輸血率が有意に低値であった（表5）。なお，全例において自己血廃棄例はなかった。

考察

心臓血管外科領域においては，他の領域に先がけて早くより同種血輸血回避に対する努力が試みられ，年々手術成績が向上するに伴い無輸血手術に対する関心は広がりつつある。無輸血達成へのもっとも効果的な方法として，術前貯血式自己血輸血が施行されるようになり[1]，人工心肺を使用する心大血管手術においては，現在ほぼ一般的な手法とされている[2]。しかしその適応や貯血期間

表4　M群における輸血例と無輸血例の要因別比較

要因		輸血例	無輸血例	P値
男女比	(M/F)	17/17	102/50	0.060
年齢	(years)	69.4 ± 8.2	61.7 ± 13.3	0.002 *
体重	(Kg)	51.7 ± 8.5	57.3 ± 9	0.001 *
貯血量	(ml)	741 ± 144	777 ± 91	0.067
EPO使用量	(×1000IU)	21.9 ± 4.6	20.6 ± 6.1	0.269
入院時Hb	(g/dl)	12.5 ± 1.5	13.1 ± 1.4	0.032 *
手術直前Hb	(g/dl)	10.0 ± 1.1	11.2 ± 1.4	<0.001 *
人工心肺時間	(min.)	173 ± 123	101 ± 42	<0.001 *
手術時間	(min.)	381 ± 211	216 ± 64	<0.001 *

表5　M群における無輸血率に影響する因子

	例数	輸血例	無輸血率	p value
800ml未完遂	15 (8.1%)	5	66.7%	
800ml完遂	171 (91.9%)	29	83.0%	0.221
再開胸(+)	9 (4.8%)	5	44.4%	
再開胸(-)	177 (95.2%)	29	83.6%	0.012 *
冠動脈バイパス術	72 (38.7%)	20	72.2%	
弁疾患手術	76 (40.9%)	7	90.8%	0.003 *

に関しては，施設間で一定していないのが現状である。施設間で手術方法，成績，麻酔科の方針，病院での輸血に対する取り組み，マンパワー等，あらゆる面で異なるので，自己血貯血に対する方針にも若干差が見られて当然である。長期の貯血期間を設け，多量の貯血量を準備できれば，無輸血率が飛躍的に向上するのは当然のことである。しかし心大血管手術においては，それほど長期の待期期間を経て手術となる症例は少ない。また病院の稼働率を考慮した場合，術前の入院期間は制約を受けるのが現状である。外来通院での貯血は理想的であるが，輸血部のようなユニットが独立している大規模な施設以外では，マンパワーの制限があったり，心疾患患者での外来採血は不安も多く，患者の術前の精神的負担も大きい。したがって，当施設もそうであるが，入院後の自己血貯血が原則となる。自己血貯血にEPO投与が効果的であることは多く報告され[3,4]，ほとんどの施設で使用されているが，保険基準で貯血量が800 ml以上で1週間以上の貯血期間が必要と定められている。この基準を満たし，かつ最短の貯血期間を設けるため，当科では術前8日からの入院および貯血開始を施行してきた。無輸血率は81.7％とある程度許容される成績ではあるが，やはり貯血期間の長い症例と比較すると，有意差はないものの低い傾向にあった。しかし貯血期間が1週間以内で，400 mlしか貯血できなかった症例（S群）よりは有意に良好な無輸血率であった。開心術にあえて貯血式自己血輸血をせず，良好な結果を示した報告もある[5]。しかし同種血輸血の安全性が100％確立されていない現在，多少とも自己血貯血やEPO投与の機会があり，無輸血の可能性が1％でも増えるならば，その選択肢は提供されるべきであろう[6]。この貯血法で同種血輸血に至った症例は，無輸血例に比べ，高齢で低体重，術前のHb値が低いという結果は当然考えられ，人工心肺時間および手術時間の長い例ほど輸血率が高いという結果も他の報告と同様であった[7]。この短期間で800 mlの採血は手術直前のHb値が他の報告に比べ著しく低く，平均が11.0 ± 1.4 g/dlであった。つまりEPO投与で，十分な造血効果が発揮されるには期間が短すぎるかもしれない。エリスロポエチンによる造血刺激を促すには最低3週間必要という報告も見られる[8]。しかし，われわれは以前1週間でも造血効果は有意に上がっている結果を報告している[4]。初回の開心術における貯血量は800 mlが至適であるという報告も見られる[9]が，その800 mlを採血した後の手術直前Hb値がどれだけ保たれているかも重要な要因と思われる。これは貯血期間と造血能に依存し，この術直前Hb値の低さはこの貯血法の限界であろうと考える。しかし術前の患者の全身状態に影響がない限り，手術前日でも400 mlの貯血は無輸血手術に有効と考える。出血再開胸や他の再手術を要した症例の無輸血率は著しく低かったが，これらの症例は貯血期間，量に関係なく同種血輸血を要したと考えられるので，初回手術に限れば無輸血率はもう少し良好と思われた。また，冠動脈バイパス術の無輸血率が弁膜症手術に比べ有意に不良であったのは，前者の方がバイパスグラフト採取などで有意に手術時間が長いこと，術前に抗凝固剤が投与されている例も多く，出血量が多いためと考えられた。この貯血法の妥当性を検討した場合，単独弁膜症手術，心房中隔欠損閉鎖術など，比較的人工心肺時間や手術時間の短い症例であれば，ほぼ満足すべき結果が得られる方法と思われた。少量の貯血量で十分と予想されても予想外に侵襲，出血が多くなることもあり，無輸血手術を第一義的に考えれば「最大限の貯血期間を設け，できうる限り多量の貯血を行う」ということに尽きると思われる。しかし，同種血無輸血を目指すあまり，患者に術前の負担を過剰にかけたくないという方針で，当科ではこのような貯血法を基本とした。すべての開心術に有効とはいえないまでも，長期の待期期間が設けられない症例に対し，比較的短期間の術前入院および貯血期間でほぼ良好な無輸血率を達成できる一手法として，今後も活用したいと考える。

結語

人工心肺を用いる心大血管手術において，貯血期間8日で800 mlを貯血する自己血貯血法を186例に施行し，無輸血率81.7％と比較的良好な成績を得られた。

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原　著

自己血400 ml採血後2週間のヘモグロビン値の回復度に与える影響因子の検討

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はじめに

我々は顎矯正手術の一つである上下顎同時移動術に対して，400 ml採血の貯血式自己血輸血と希釈式自己血輸血を併用し，同種血輸血を100 %回避している。本手術の対象となる患者は若くて健康であるが，低体重の女性が多いという特徴がある。当院では，自己血採血によって低下するヘモグロビン値（Hb値）をはじめとする種々の因子の回復を考慮し，予定手術日の3週間前に採血することを原則にしている[1]。しかし，患者の都合などにより術前2週間の採血を余儀なくされる症例もある。今回，手術2週間前に採血を行った患者のヘモグロビン値の回復度に影響を与える因子について検討した。

対象・方法

福岡歯科大学付属病院において，文書と口頭にて自己血輸血の説明を行い同意が得られ，手術の約2週間前に400 mlの自己血採血を施行した患者47名（男性13名，女性34名）を対象とした。自己血採血当日の採血前に検査血を採取し，Hb値，血清鉄値，フェリチン値，総鉄結合能（TIBC），不飽和鉄結合能（UIBC），血清総蛋白量（TP），血小板数（Plt），白血球数（WBC）を測定した。400 mlの自己血採血を行った後，フェジン® 80 mgを加えた1000 mlの晶質液輸液または300 mlの膠質液輸液を行い，さらに採血翌日から2週間，200 mg/dayの鉄剤を経口投与した[2]。入院後，手術前日に検査採血を行い，この時のHb値を術直前Hb値とした。循環血液量は，体格や性別によって大きく異なる[3]が，当院では，一律400 mlの採血を行っているため，採血後のHb値低下の程度も異なると考えられる。そこで，400 mlの自己血採血後，1000 mlの晶質液輸液または300 mlの膠質液輸液を行った時のHb値を予測できる，当科で用いている計算方法[4]を用いて採血後予測Hb値を算出した。循環血液量（CBV）をOgawa式[5]にて求め，図1に示す式にて採血後予測Hb値および予測Hb値の採血前Hb値に対する割合（α）を算出した。また，実測した術直前Hb値の採血前Hb値に対する割合（β）を求めた。さらに採血前の各検査データとβとの間の相関関係の有無を検討した。統計処理には分散分析（多重比較検定：Scheffe法），χ^2検定およびピアソンの相関係数の検定を用い，危険率1 %未満を有意差有りとした。

結果

対象患者全員の予測Hb値を算出したところ，その平均値は12.4 g/dlであった。採血前の平均Hb

福岡歯科大学診断・全身管理学講座麻酔管理学分野

・ 循環血液量(CBV) (ℓ) ＝ $\begin{cases} \text{男性：}0.168\times(\text{身長(m)})^3+0.05\times\text{体重(kg)}+0.444 \\ \text{女性：}0.25\times(\text{身長(m)})^3+0.063\times\text{体重(kg)}-0.662 \end{cases}$

・ 採血後予測Hb値 (g/dl) ＝ 採血前Hb値 (g/dl) × $\dfrac{(\text{CBV}-0.4)}{\text{CBV}}$

・ α ＝ $\dfrac{\text{採血後予測Hb値 (g/dl)}}{\text{採血前Hb値 (g/dl)}}$

・ β ＝ $\dfrac{\text{術直前Hb値 (g/dl)}}{\text{採血前Hb値 (g/dl)}}$

図1　αおよびβの算出方法
α：採血後予測Hb値の採血前Hb値に対する割合
β：実測した術直前Hb値の採血前Hb値に対する割合

表1　A群およびB群の患者背景と採血前検査値

		A群 (n=22)	B群 (n=25)
男女比	(男:女)	5 : 17	8 : 17
年齢	(歳)	23.1±6.9	24.2±3.9
身長	(cm)	162.1±7.9	163.8±11.1
体重	(kg)	53.9±7.3	57.7±13.3
血清鉄	(μg/dl)	88.1±31.1	84.8±27.3
フェリチン	(ng/ml)	51.6±37.5	49.9±57.6
総鉄結合能(TIBC)	(μg/dl)	281.5±38.8	288.4±31.1
不飽和鉄結合能(UIBC)	(μg/dl)	193.4±53.9	205.0±40.7
血漿総蛋白量(TP)	(g/dl)	7.1±0.4	7.1±0.4
血小板数	($\times10^4$/μl)	21.4±5.6	23.4±5.3
白血球数	($\times10^2$/μl)	54.4±11.2	62.2±14.2
採血前Hb値	(g/dl)	13.3±1.2 ＊	14.3±1.3

＊ $p<0.01$　(Mean±SD)

A群：αが0.035以上増加した患者
B群：αの増加が0.035未満であった患者

値は13.8 g/dlであったので，αの平均は0.894となる。また，実測の術直前Hb値は12.8 g/dlであったのでβの平均は0.929となり，対象全員の（β－α）は，採血後2週間で平均0.035上昇していたことになる。このことからαが0.035以上増加した患者をA群とし，0.035未満であった患者をB群として比較検討した。A群は22名，B群は25名であり，群間の男女比，年齢，身長，体重には有意差は認められなかった。両群間の血清鉄値，フェリチン値，TIBC，UIBC，TP，Plt，WBCに

表2　C群およびD群の患者背景と採血前検査値

		C群 (n=5)		D群 (n=42)
男女比	(男:女)	2 : 3		11 : 31
年齢	(歳)	24.2±11.4		23.6±4.6
身長	(cm)	164.6±8.0		162.8±9.9
体重	(kg)	56.6±4.4		55.8±11.5
血清鉄	(μg/dl)	106.0±46.2	*	84.0±26.0
フェリチン	(ng/ml)	65.2±41.4		48.9±49.9
総鉄結合能(TIBC)	(μg/dl)	278.8±45.6		285.9±33.8
不飽和鉄結合能(UIBC)	(μg/dl)	172.8±86.3	*	202.7±41.0
血漿総蛋白量(TP)	(g/dl)	7.0±0.4		7.1±0.4
血小板数	($\times 10^4/\mu l$)	20.2±8.1		22.8±5.1
白血球数	($\times 10^2/\mu l$)	49.8±11.0		59.5±13.4
採血前Hb値	(g/dl)	13.2±1.8		13.9±1.3

* $p<0.05$　(Mean±SD)

C群：βが1以上であった患者
D群：βが1未満であった患者

有意差は認められなかったが，採血前Hb値はA群で有意に低い値を示した（表1）。しかし，採血前Hb値とβの相関関係については，決定係数（0.069），相関係数（－0.093）と共に低く，相関関係は認められなかった。

次に，採血後2週間の術直前Hb値が採血前Hb値以上に増加したC群（$\beta \geqq 1$）とそれ以下にしか回復しなかったD群（$\beta < 1$）に分配し，比較検討した。C群は5名，D群は42名であった。両群間の患者背景に有意差は認められなかったが，血清鉄およびUIBCに有意差を認めた（表2）。しかし，採血前Hb値を含むその他の採血前検査値に差は認められなかった。血清鉄およびUIBCとβとの相関を見ると，相関係数はそれぞれ0.356，－0.359と低く，両者の間には，弱い相関関係しか認められなかった（表3）。

表3　患者背景および採血前検査値とβとの相関関係

	相関係数	p値
年齢	- 0.199	0.226
身長	0.235	0.150
体重	0.135	0.414
血清鉄	0.356	0.026
フェリチン	0.227	0. 166
総鉄結合能(TIBC)	- 0.207	0. 208
不飽和鉄結合能(UIBC)	- 0.359	0. 024
血漿総蛋白量(TP)	- 0.047	0. 780
血小板数	- 0.096	0. 564
白血球数	- 0.301	0. 062
採血前Hb値	- 0.093	0. 574

考察

上下顎同時移動術時には輸血が必要となるような出血が起こる場合があり，患者のQOLを考慮すると有効かつ安全な自己血輸血が望まれる。当院における本術式の出血量は，大部分の症例で600～800 mlであるが，1,000 ml以上出血する症例もあるため[1]，確実に同種血輸血を回避するために

は自己血貯血は必須である。我々は，本法に対して400 mlの自己血貯血を行っているが，他施設においても術前の貯血量は400 mlが主流となっている[6]。800 ml以上の貯血を行わないとエリスロポエチンは健康保険の適応外となるため使用できず，採血による貧血を回復させるためには，十分な期間をとる必要性がある。顎矯正外科手術は待機手術であり，大部分の患者は若く，健康状態は良好であるため外来採血が可能で，通常は比較的長く術前貯血期間をとることができるが，患者の時間的な都合や手術日の決定が遅延することなどにより期間を短縮せざるをえない場合もある。他領域の手術においては術前貯血量が800 ml以上必要となるような症例ではエリスロポエチンを併用して手術1週間前まで採血を行い，Hb値の低下もほとんど認められなかったという報告がある[7]。一方で，多少の貧血があっても術前400 ml貯血をした胃全摘術において100 %術中の同種血輸血が回避できたという報告[8]もあり，上下顎同時移動術を受ける患者では400 mlの貯血と術前貯血期間を十分とることで同種血回避率100 %をより確実に維持できると考えられる。

幹細胞の分化が始まって末梢血中に網状球として出現するのに要する期間は，約8日であり[9]，健康成人の生理的赤血球産生量は，全血量に換算すると1日30～40 mlである[10]。さらに，有効な造血刺激が加わると赤血球産生予備能は最大5～6倍まで亢進する[11]。これらのことからは，2週間の貯血期間は貧血回復には十分な期間であるように考えられる。しかし，今回検討した47例中400 ml採血後2週間で完全に元のHb値に回復したものは5例のみであったことから，臨床的には，採血から手術までの期間が2週間以上あることが望ましいと考えられる。症例数が少なかったこともあり，予測因子を明確にすることはできなかったが，採血前のHb値の低い症例の方がβが高かったことから，採血前Hb値が低いほど赤血球造血能が亢進する可能性が示唆された。これは，鉄欠乏性貧血患者は，貯血開始1～2週の早期から著明な造血能の亢進がみられるという新名主らの報告[12]と一致する。この理由として貧血患者では，貧血のない患者と比較して採血後の内因性エリスロポエチン濃度が高い[11]ことが考えられる。しかし，造血には，エリスロポエチンとともに材料となる鉄が必要である。C群の5症例は，D群の42症例と比較して，採血前Hb値には差がなく，血清鉄およびUIBCに有意差を認めた。フェリチン値には有意差は認められなかったが，その平均値はD群が48.9 ng/mlであったのに対しC群では65.2 ng/mlと高い傾向にあった。これは，貧血患者の方が早期のHb値の回復は速いが，完全に回復するためには貯蔵鉄量が関係する可能性がある。採血後全症例に量的には十分な鉄剤を投与しているので，採血前の貯蔵鉄量が関係する可能性は少ないように思われるが，鉄の吸収には個人差があり，採血前に貯蔵鉄量の多い患者の方が鉄の吸収度が高かったことが要因の一つとして考えられる。また，C群の採血前Hb値はD群のそれと有意差がなく，貯蔵鉄量は多かったことから考えて鉄を有効にHb生成に利用できている可能性が考えられる。しかし，今回は鉄剤投与後の貯蔵鉄量を測定していないため明らかにはできなかった。さらに検討を進めることで，顎矯正外科手術に対するより有効な術前貯血を行うことが可能となるものと考える。

結語

術前2週間に400 mlの採血を行った症例においてHb値回復に影響をおよぼす因子について検索した。採血前Hb値と貯蔵鉄量がHb値回復程度に影響を与えている可能性が示唆された。

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Daily doses of 20 mg of elemental iron compensate for iron loss in regular blood donors: a randomized, double-blind, placebo-controlled study

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BACKGROUND: A considerable number of regular blood donors develops an iron deficiency, and the exact amount of iron required to compensate for the iron loss from whole-blood donation in males and females is still unknown.

STUDY DESIGN AND METHODS: A total of 526 regular blood donors (289 male and 237 female) were randomly assigned to treatment with either 40 mg, 20 mg, or 0 mg per day of elemental iron as ferrous gluconate for a period of 6 months, during which one unit of whole blood was collected on four occasions (males) or three occasions (females). Hemoglobin level, serum ferritin, and soluble transferrin receptor levels were measured before each donation.

RESULTS: Daily doses of either 40 mg or 20 mg of elemental iron adequately compensated for iron loss in males, who gave blood at 2-month intervals, but did not result in a positive iron balance or an increase in storage iron as reflected by the logarithm of the ratio of transferrin receptor to ferritin concentration. In females, who donated at 3-month intervals, the same daily doses not only restored the iron balance but also led to an increase in storage iron. The number of gastrointestinal side effects due to iron supplementation (12%) was only slightly higher in both iron groups than in the placebo group.

CONCLUSION: The results of this study indicate that 20 mg of elemental iron per day can adequately compensate for iron loss in males and females who donate whole blood up to four (females) or six times per year (males).

The major side effect of whole-blood donation is iron depletion. In Germany, men are generally allowed to donate whole blood every 8 weeks and women every 12 weeks. However, the normal diet is usually unable to compensate for the resulting iron loss.[1,2] Consequently, a considerable number of regular blood donors develops a negative iron balance that may eventually progress to iron deficiency anemia.[3-7] Menstruating female donors are at a particularly high risk for chronic iron deficiency. Although this is well-known, only a few controlled, double-blind studies have dealt with the question of whether iron supplementation can prevent iron depletion in menstruating female blood donors.[8-11] There is evidence suggesting that daily doses of 40 mg of elemental iron as ferrous sulfate can sufficiently compensate for iron loss resulting from whole-blood donation and can improve iron status.[10,11] However, the question of whether a lower dose of iron is sufficient to compensate for iron loss in female donors is still open. In addition, controlled studies on iron supplementation in male donors are lacking. Most importantly, no valid measure of iron storage was used in early studies.[12,13] Today, serum ferritin and soluble transferrin receptor levels can be routinely measured and iron status can be much better assessed than previously.[14-17] The logarithm of the ratio of

ABBREVIATIONS: Fe^{2+} = elemental iron as ferrous gluconate; log(TfR/F) = logarithm of ratio of the soluble transferrin receptor to ferritin concentration.

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the soluble transferrin receptor to ferritin concentration (log[TfR/F]), which was shown to have a highly linear correlation to body storage iron, is currently the most precise measure of body storage iron available.[14,15] Here, we present the results of a double-blind study in which we randomly assigned regular male and female blood donors to treatment with 40 mg, 20 mg, or 0 mg (placebo) per day of elemental iron for 6 months.

MATERIALS AND METHODS

Selection of donors and study design

A total of 526 regular blood donors (289 male and 237 female) were enrolled in this study, which was approved by the Ethics Committee of Charité University Medical Center. Written informed consent was obtained from all volunteers. In accordance with the German guidelines for blood donor selection, all donors were determined to be healthy based on their history and had hemoglobin (Hb) concentrations of no less than 13.5 g per dL (males) or 12.5 g per dL (females). The investigational products consisted of identical capsules in blister packs containing 1.5 mg pyridoxal-phosphate, 2.25 µg cyanocobalamine, 400 mg ascorbic acid, 200 µg folic acid, and 75 µg biotin without (placebo) or with 20 mg of elemental iron as ferrous gluconate (Fe^{2+}) (Phyt-Immun GmbH, Homburg, Germany). Ascorbic acid was added to enhance iron absorption. Because most people believe in beneficial effects of vitamin supplements, the other selected vitamins were added for improved compliance. The form of iron used meets the European Community criteria for dietary foods for special medical purposes. The participants were randomized to one of three groups receiving either 40 mg Fe^{2+}, 20 mg Fe^{2+}, or 0 mg Fe^{2+} in two capsules once daily for 6 months. Hb, serum ferritin, and soluble transferrin receptor levels were determined before blood collection at each initial and follow-up visit. Each male volunteer was scheduled for a total of four visits, including a randomization visit before the first donation at Week 0 and three subsequent predonation visits at 2-month intervals. The females were scheduled for a total of three visits: a randomization visit at Week 0 and two predonation visits at 3-month intervals (Fig. 1). The intervals were chosen in accordance to the German guidelines, which allow six donations per year for male and four donation per year for female volunteers. Volunteers with hemoglobin concentration less than 13.5 g per dL (males) or 12.5 g per dL (females) were deferred, but not excluded from study. Compliance, which was defined as the ingestion of at least 90 percent of the capsules as prescribed, was checked by counting the returned capsules between blood donations.

Laboratory methods

Hemoglobin concentrations in fingerstick blood samples were determined by the acid methemoglobin method using a photometer (HemoCue B-Hemoglobin photometer, HemoCue, Großostheim, Germany). Ferritin and soluble transferrin receptor concentrations in serum were

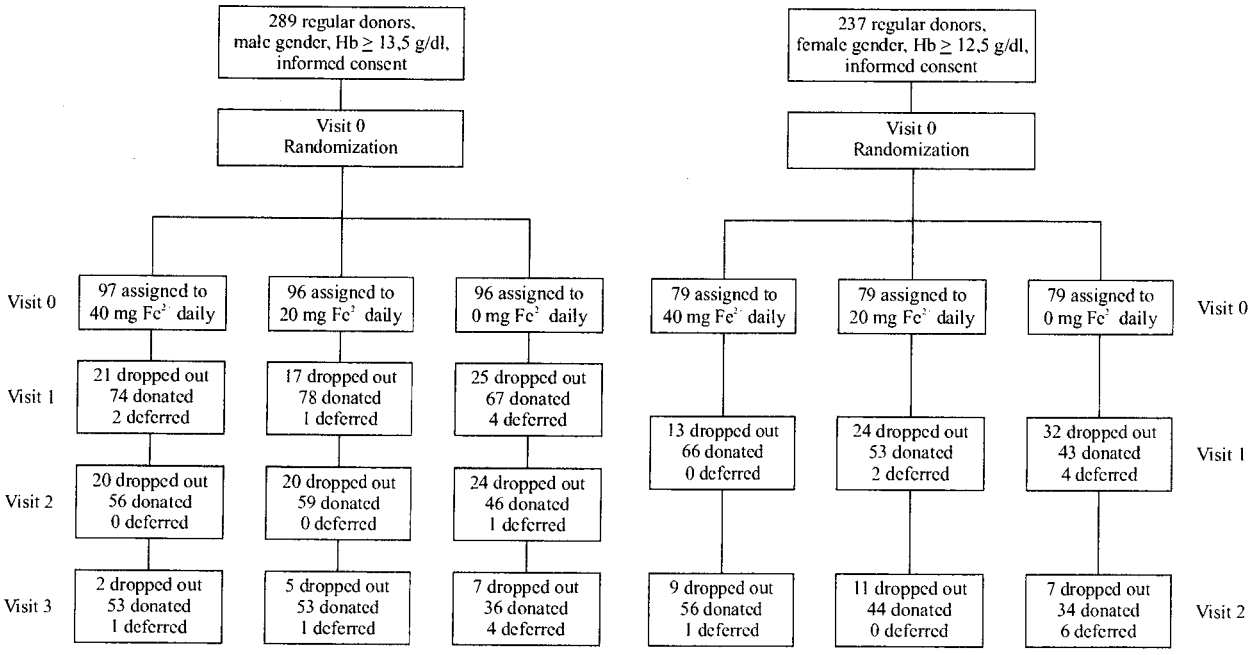

Fig. 1. Flow of participants during study.

determined by nephelometry using an automatic analyzer (BN Prospec, Dade Behring, Marburg, Germany).

Statistics

Sample-size calculation, randomization, and statistical analyses were performed using software (Stata for Windows, Stata Corp., College Station, TX). Based on the serum ferritin concentration, the required sample size was determined to be 49 males and 40 females per group, assuming a power of 0.9, a significance level of 0.0167 (Bonferroni adjustment for three groups), a smallest meaningful ferritin difference of 10 µg per L between groups, three (males) or two (females) follow-up measurements, a within-subject correlation coefficient of 0.8, and a standard deviation (SD) of 26 µg per L (males) or 22 µg per L (female) for serum ferritin. Assuming a dropout rate of 50 percent, we arrived at a final sample size of 98 males and 80 females per group.

The randomization plan was generated using block randomization with variable block length. Statistical analyses were performed as an intent-to-treat analysis for all participants coming for more than one visit using a linear regression model for longitudinal data (cross-sectional time-series regression model with generalized estimating equation analysis).[18] The logarithm of the ratio of transferrin receptor to ferritin concentration, an accepted measure of storage iron, was used as the outcome variable. To model the change in storage iron over time, we applied the difference values for log(TfR/F) and included the iron supplement as the predictor variable.

RESULTS

Males

Of the 289 male volunteers (age range, 19-67 years) enrolled in the study, 141 (49%) dropped out, yielding a dropout rate of 44 percent in the 40 mg of Fe^{2+} group, 44 percent in the 20 mg of Fe^{2+} group, and 58 percent in the placebo group (p = 0.075; Fisher's exact test). A total of 63 (45%) of the male dropouts withdrew before their second visit (Table 1). The mean interval between visits was 60 days. Deferral from donation because of unacceptable hemoglobin concentration values (<13.5 mg/dL) occurred in 14 of 825 visits (1.7%). This was more frequently the case in the placebo group than in the 20 mg and 40 mg iron groups (n = 9 vs. 2 vs. 3, p = 0.022; Fisher's exact test). Compliance was poor in roughly one-third of the male participants.

In the male placebo group, the mean serum ferritin concentration decreased from 35 µg per L at baseline to 21 µg per L at the final visit, the number of males with depleted iron stores (ferritin <12 µg/L) increased from 20 percent to 54 percent, and the mean concentration of soluble transferrin receptors rose slightly from 1.6 mg per L to 1.7 mg per L (Table 2, Fig. 2). In the male 20 mg iron group, serum ferritin decreased from 35 µg per L to 25 µg per L, whereas the median ferritin value changed only slightly (Table 2, Fig. 2); both the number of males with depleted iron stores (25%) and the transferrin receptor concentration (1.5 mg/L) remained nearly constant. In the male 40 mg iron group, the ferritin (33 µg/L) and transferrin receptor levels (1.5 mg/L) remained constant, whereas the number of individuals with iron depletion dropped from 26 percent to 13 percent.

The log(TfR/F) remained nearly constant in both iron groups, but rose continuously in the placebo group

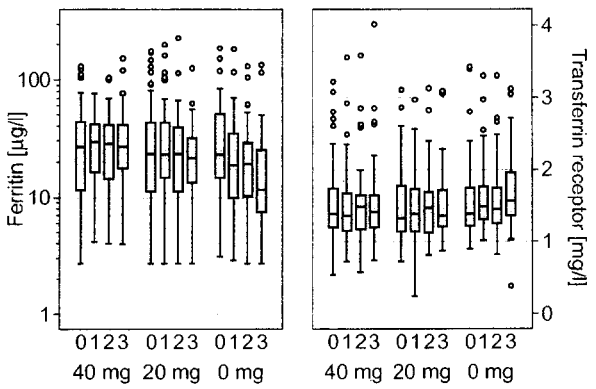

Fig. 2. Box-plot for the concentration of serum ferritin and soluble transferrin receptor in male donors.

TABLE 1. Reasons and numbers of dropouts during study

	Unknown		Gastrointestinal complaints		Poor compliance		Other	
Reason	(%)	(n/total)	(%)	(n/total)	(%)	(n/total)	(%)	(n/total)
Male donors								
40 mg iron	15.5	15/97	5.2	5/97	12.4	12/97	13.4	13/97
20 mg iron	18.8	18/96	6.3	6/96	16.7	16/96	3.1	3/96
0 mg iron (placebo)	20.8	20/96	6.3	6/96	21.9	21/96	11.5	11/96
Female donors								
40 mg iron	8.9	7/79	2.5	2/79	10.1	8/79	6.3	5/79
20 mg iron	20.3	16/79	6.3	5/79	11.4	9/79	6.3	5/79
0 mg iron (placebo)	24.1	19/79	3.8	3/79	10.1	8/79	11.4	9/79

TABLE 2. Serum ferritin concentration, number of donors with depleted iron stores (ferritin concentration <12 µg/L), and logarithm of the ratio of transferrin receptor to ferritin concentration (log[TfR/F]) for all donors with at least one follow-up visit

Visit number	Ferritin (µg/L) (mean ± SD)	Depleted iron stores (%)	Depleted iron stores (n/total)	log(TfR/F) (mean ± SD)
Male donors				
40 mg iron				
0	32.7 ± 27.5	26.3	20/76	1.54 ± 0.51
1	31.4 ± 18.8	16.2	12/74	1.47 ± 0.49
2	30.2 ± 20.8	17.9	10/56	1.50 ± 0.51
3	33.2 ± 26.7	13.0	7/54	1.52 ± 0.55
20 mg iron				
0	34.7 ± 36.3	25.3	20/79	1.48 ± 0.48
1	33.1 ± 33.3	21.8	17/78	1.46 ± 0.44
2	30.2 ± 32.7	25.4	15/59	1.47 ± 0.45
3	25.0 ± 19.8	24.5	13/53	1.52 ± 0.47
0 mg iron (placebo)				
0	35.1 ± 32.4	19.7	14/71	1.55 ± 0.50
1	27.5 ± 27.9	30.9	21/68	1.61 ± 0.45
2	24.9 ± 24.7	29.8	14/47	1.60 ± 0.52
3	21.4 ± 27.5	53.9	21/39	1.67 ± 0.53
Female donors				
40 mg iron				
0	19.3 ± 15.0	39.4	26/66	1.43 ± 0.65
1	28.5 ± 19.8	15.2	10/66	1.26 ± 0.49
2	31.4 ± 19.4	14.0	8/57	1.29 ± 0.54
20 mg iron				
0	20.0 ± 32.3	54.6	30/55	1.38 ± 0.46
1	23.3 ± 27.9	45.1	23/51	1.36 ± 0.42
2	23.5 ± 26.1	34.1	15/44	1.35 ± 0.49
0 mg iron (placebo)				
0	17.7 ± 15.0	48.9	23/47	1.39 ± 0.65
1	17.6 ± 14.5	44.2	19/43	1.40 ± 0.42
2	15.1 ± 12.3	48.7	19/39	1.55 ± 0.66

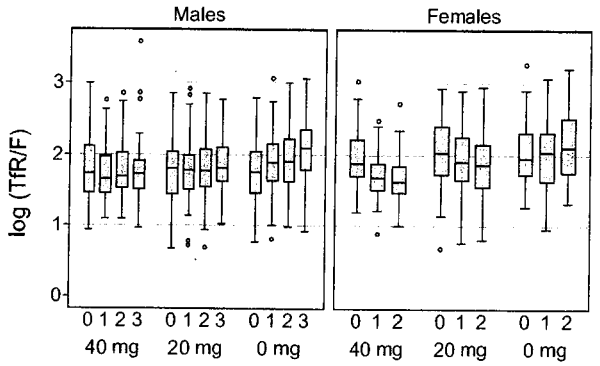

Fig. 3. Box-plots for the logarithm of the ratio of soluble transferrin receptor to ferritin concentration in male and female donors.

(Fig. 3), as was clearly demonstrated in the regression analysis (Table 3). The log(TfR/F) value increased by nearly 0.09 per donation in the placebo group, but changed only marginally in the two iron groups. Both iron groups differed significantly from the placebo group with respect to log(TfR/F).

Females

Of the 237 female volunteers (age range, 19-65 years) enrolled in the study, 96 (41%) dropped out, yielding a dropout rate of 28 percent in the 40 mg iron group, 44 percent in the 20 mg iron group, and 49 percent in the placebo group ($p = 0.015$; Fisher's exact test). A total of 69 (72%) of the female dropouts withdrew before their second visit (Table 1). The mean interval between visits was 88 days. Deferral from donation because of unacceptable dropout concentration values (<12.5 mg/dL) occurred in 13 of 546 visits (2.4%). This was the case more frequently in the placebo group than in the 20 mg and 40 mg iron groups (n = 10 vs. 2 vs. 1, $p = 0.001$; Fisher's exact test). Compliance was poor in roughly one-quarter of the female participants.

In the female placebo group, the mean concentration of serum ferritin decreased from 18 µg per L at baseline to 15 µg per L at the final visit, the number of females with depleted iron stores (ferritin <12 µg/L) remained constant (49%), and the mean soluble transferrin receptor concentration rose from 1.4 mg per L to 1.6 mg per L (Table 2, Fig. 4). In the female 20 mg iron group, serum ferritin increased from 20 µg per L to 24 µg per L, the number of individuals with depleted iron stores decreased from 55 percent to 34 percent, and the transferrin receptor concentration remained nearly constant (1.4 mg/L). In the female 40 mg iron group, ferritin concentration rose from 19 µg per L to 31 µg per L, transferrin receptor level fell slightly from 1.4 mg per L to 1.3 mg per L, and the number of individuals with iron depletion decreased from 39 percent to 14 percent.

The log(TfR/F) dropped in both iron groups, but rose continuously in the placebo group (Table 2, Fig. 3), as demonstrated by the regression analysis. The log(TfR/F) value increased by nearly 0.09 per donation in the placebo group (Table 3), but decreased by roughly 0.06 and 0.12, respectively, in the 20 mg and the 40 mg iron groups.

Side effects

Most donors (approx. 60%) did not report any side effects. There was no significant difference in the incidence of adverse effects between the three groups. In particular, the frequency of gastrointestinal complaints was low (11% in the 40 mg iron group, 13% in the 20 mg iron group, and 11% in the placebo group).

TABLE 3. Regression models for the change in log(TfR/F)

Predictor	Coefficient	95-percent confidence interval	p value
Male donors			
20 mg Fe^{2+}	−0.074	−0.121 to −0.028	0.002
40 mg Fe^{2+}	−0.118	−0.168 to −0.068	<0.001
Constant	0.091	0.058 to 0.123	<0.001
Female donors			
20 mg Fe^{2+}	−0.150	−0.238 to −0.061	0.001
40 mg Fe^{2+}	−0.209	−0.292 to −0.127	<0.001
Constant	0.086	0.018 to 0.153	0.012

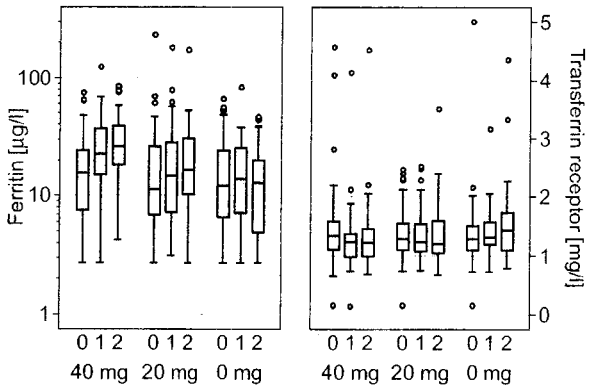

Fig. 4. Box-plot for the concentration of serum ferritin and soluble transferrin receptor in female donors.

DISCUSSION

Regular blood donation frequently leads to iron depletion, and it has been shown that iron supplementation can prevent this complication.[8,10,11] However, the exact dose needed to compensate for this type of iron loss remains unclear, and there is uncertainty as to whether iron supplementation is required in both male and female donors. Attempting to elucidate this complex issue more precisely, we monitored the logarithm of the TfR/F ratio as a measure of body storage iron in regular male and female whole-blood donors. The donors were randomly assigned to receive daily supplements containing selected vitamins plus 40 mg, 20 mg, or 0 mg of elemental iron. Dropout rates were marginally (male) or significantly (female) higher in the placebo group than in both iron groups. The reason for this finding is obscure.

Daily doses of 40 mg and 20 mg of elemental iron resulted in both a positive iron balance and an increase in storage iron in female donors and compensated for iron loss in males. This indicates that 20 mg of elemental iron per day is indeed sufficient to compensate for iron loss in both males and females. The differences in storage iron responses may be due to the shorter donation intervals in males (every 2 months) compared to females (every 3 months). It is likely that the ascorbic acid in the capsules may have increased the iron absorption by roughly 50 percent.[19] The question of whether the other vitamins may play any role in this context is speculative. The only reason for including these vitamins in the investigational products was our desire to improve the compliance rate.

In the present study, we monitored ferritin and soluble transferrin receptor levels as well as the logarithm of the TfR/F ratio. The latter variable, which was shown to have a highly linear correlation with body storage iron, is the most precise measure of body storage iron available.[14,15] Until now, body iron of blood donors was assessed mainly by measuring serum ferritin.[1,3,5-7] However, this variable is somewhat unspecific and may give false-high results in the presence of various underlying diseases.[2] In fact, if ferritin had been the only variable used for assessment of body storage iron, the effects of 20 mg elemental iron in males would have been underestimated in our study.

Interestingly, the number of side effects in the two groups treated with iron(II)-gluconate was only slightly higher than the number observed in the placebo group. In particular, the incidence of gastrointestinal side effects in the iron groups was very low (12%). Due to the slight risk of poisoning in children, iron capsules should be delivered in individual packages. Elemental iron preparations like carbonyl iron are preferred as an alternative by many experts due to the much higher lethal doses.[9,10,20,21] However, carbonyl iron is not available in the European countries. In comparison, bioavailabity of carbonyl iron is slightly lower than that of ferrous salts,[21] but side effects seem to be comparable: The incidence of gastrointestinal complaints for both preparations was reported much higher in two previous studies, probably due to the supplementation with higher doses of iron.[9,21] The utility of iron supplements for prevention of iron deficiency in menstruating female blood donors is currently being discussed.[20,22] However, others and we prefer a supplementation of iron for a short-term period after blood donation but not in general.

In conclusion, our results indicate that daily doses of 20 mg Fe^{2+} can adequately compensate for iron loss resulting from whole-blood donation in males who donate up to six times a year and in females who donate up to four times a year.

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2. 貧血と採血基準を考える
～血液学的立場から～

香川県赤十字血液センター
内田 立身

1．貧血の定義

貧血の定義について血液学の代表的な教科書をみると、①a reduction below normal in the concentration of hemoglobin or red blood cells in the blood[1] ②anemia is functionally best characterized by a hemoglobin concentration below normal[2] などの記載があり、健常人のヘモグロビンの下限値から判断するのが一般的である。米国人においては表1のような数字が用いられている[1) 2) 3) 4)]。この際、健常人として選ばれる対象のうち特に鉄欠乏状態の多い女性では血液学的に正常でない人が含まれ、下限域が低く算定される可能性があった。

表1 米国健常人のヘモグロビン(g/dL)下限値

	男性	女性	文献番号
WHO	13.0	12.0	3
Beutler E	14.0	12.3	1
Lee GR	13.2	11.6	2
NHANESⅢ	13.5	12.0	4

最近、Beutlerら[5] は米国人の貧血の定義としてNHANES-Ⅲ(The Third US National Health and Nutrition Examination Survey)[4] が行なったように、トランスフェリン飽和率16%以上、血清フェリチン10ng/mL以上の人を健常人として正常域の5%値未満を貧血としている(表2)。血液学的な貧血の定義として妥当な決め方である。

日本人の貧血の頻度について、私たちは「1981年～1991年」までの鉄欠乏の頻度を検索したことがあるが[6]、このデータをもとに鉄欠乏のない健常人を対象としてヘモグロビン値を求めたところ表3のとおりとなった。同じ方法で求められた斎藤ら[7] の成績とあわせると、鉄欠乏のない日本人のヘモグロビン下限値は男性12.8～13.2g/dL、女性11.8～12.1g/dLとなり、日本人成人の貧血の定義は男性13.0g/dL未満、女性12.0g/dL未満が妥当と考えられた。最近の日本人については鉄欠乏に関する正確なデータがなく、厚生労働省が行なっている「国民健康・栄養調査報告」などから鉄欠乏のない健常人のヘモグロビン値を求め、日本人の貧血の定義を定める必要がある。

表2 健常米国人のヘモグロビン(g/dL)下限値
(Beutler, 2006)

	男性(20～59歳)	女性(20～49歳)
白 人	13.7 (6,907人)	12.1 (2,966人)
アフリカ系	12.8 (434人)	11.1 (205人)

表3 鉄欠乏のない健常日本人のヘモグロビン値

	平均ヘモグロビン値	1標準偏差	5%正常分布値	文献
男性(284例)	14.8	1.0	12.8	6
女性(390例)	13.9	0.9	12.1	
男性 (26例)	15.0	0.9	13.2	7
女性(134例)	13.4	0.8	11.8	

2．日本人の貧血の頻度

私たちは、1981～1991年にかけて3,015名の女性で貧血の調査を行なった。その成績は、健常者43.6%、貯蔵鉄欠乏33.4%、潜在性鉄欠乏8.4%、鉄欠乏性貧血8.5%、その他6.5%

表4　日本人の貧血の頻度(%)（平成16年度国民健康・栄養調査報告から）

年齢	男性			女性		
	平均Hb±SD	Fr<10(%)	Hb下限値	平均Hb±SD	Fr<10(%)	Hb下限値
20～29	15.1±1.0	1.6	13.1	12.9±1.0	30.5	10.9
30～39	15.1±0.8	1.2	13.5	12.7±1.2	36.5	10.3
40～49	15.2±1.0	1.2	13.2	12.5±1.6	37.5	9.3
50～59	14.9±1.2	1.8	12.5	13.2±1.1	10.0	11.0
60～69	14.5±1.4	2.5	11.7	13.1±1.0	3.9	11.1
70≦	14.0±1.5	2.8	11.0	12.6±1.2	5.6	10.2
計	14.6±1.4	2.1	11.8	12.9±1.2	17.3	10.5

男性1,537名、女性2,634名の調査。

で40歳台前半では17.2％の鉄欠乏性貧血がみられた[6]。

その後、日本人についての詳細なデータがなく、特に女性の鉄欠乏性貧血の頻度をみるには毎年厚生労働省が行なっている国民健康・栄養調査から類推するのがよいと思われる[8]。表4はその成績である。高齢者を除くと男性の貧血は5.8％以下、鉄欠乏の頻度も2.5％以下であるが、女性は16.8％が貧血であり血清フェリチン低値(鉄欠乏)の頻度も高率であることから、ほとんどが鉄欠乏性貧血である。40歳台では25.0％に貧血があり同年代の半数（47.5％）が鉄欠乏状態にある。

また、香川県赤十字血液センターにおいて平成17年度に400mL献血を申し込んだ女性のうちヘモグロビン不足(Hb12.5g/dL未満)で献血ができなかった女性の比率[9]を表5に示すが、30～40歳台女性の約35％が献血できていない。また、日本赤十字社による全国的な調査によると[10]、平成17年に比重不足で献血できなかった人は485,746人で、これは東京都で1年間に献血できた人の数407,235人をはるかに凌駕するほどである。

表5　ヘモグロビン不足で献血できない女性の割合
（平成17年：香川県赤十字血液センター）

年齢	Hb<12.5g/dL
16～19	28.6%
20～29	32.6%
30～39	35.6%
40～49	35.3%
50～59	18.9%
60～69	17.5%
全体平均	19.4%（申込者数 9,963人）

わが国の女性の貧血の頻度は欧米に比して高い。米国の国民健康・栄養調査報告によると、20～40歳台の女性の鉄欠乏性貧血の頻度は5％、鉄欠乏状態は11％[11]、米国24血液銀行における2003年度の女性ヘモグロビン不足(12.5g/dL未満)の割合は平均で6.6％(1.3～13％)、Wisconsin州において17～49歳では21～23％である[12]。わが国のこれに対応する成績は400ml献血ができなかった女性が該当し、16～19歳で28.6％、20～29歳で32.6％、30～39歳で35.6％、40～49歳で35.3％であり[13]、どの調査をみても頻度は高いといわざるを得ない。

わが国で鉄欠乏の多い原因は鉄摂取量の不足にある。平成16年国民健康・栄養調査によると、男性の1日平均鉄摂取量は8.1mg、女性の1日平均は7.7mg（20～39歳で6.9～7.0mg)で必要量に比して少ない[8]。日本人の必要鉄摂取量は男性10mg、月経のある女性12mgであるが、その差2mgは全血にして10～12mLにしか相当せず、平均的月経量を30～40mLとして外国並に15～18mgは必要であろう。となるとわが国の月経のある女性は必要量の半分の鉄しか摂取していない。しかも鉄摂取量は過去の上記の調査によると年々減少してきている。

他方、米国における調査によると、白人男性で1日あたり17.2±0.3mg、女性で13.4±0.4mgで相当の開きがある[8]。採血基準を考える際には、以上のようなわが国の事情を勘案して決める必要がある。

3．採血基準をどう決めるか

日本の現状を踏まえて、わが国の採血基準をどう決めたらよいかについて以下に私見をまじえて述べたい。

代表的な国の採血基準を表6に示す。このうちEU諸国とオーストラリアは男女差があるが、米国とわが国は男女差がない。わが国の採血基準は1986年に改定され、200mL献血と400mL献血に分け、比重法かヘモグロビン法で判定するようになっている。現在、貧血の定義はヘモグロビンで記載されており、わが国の医療機関のすべてがヘモグロビン法で貧血を診断しているので、ヘモグロビン法に統一することが望ましい。また献血も400mL献血が主流になりつつあるので諸外国に倣い200mL、400mLを一本化して表記するのがよいと考えられる。

表6　各国の採血基準（400mL相当）

	男性	女性
Council of EU	13.5	12.5
Australia	13.0	12.0
U.S.A	12.5	12.5
日本	12.5	12.5

1)　ヘモグロビンの正常範囲から決める

鉄欠乏のない健常者から正常分布域を定め、5％正常値を求めると男性13.0g/dL、女性12.0g/dLとなり、これ以上を採血基準とする方法はわかりやすく貧血の定義とも一致する。

2)　貧血状態にない人から採血する

赤血球は鉄欠乏の進展に伴い、小赤血球化、低色素性化する。図1、図2は男性および女性におけるヘモグロビンと赤血球恒数との関係で、MCV・MCHが低下するのは男性で12.5g/dL、女性で12.0～12.5g/dLである[14]。また、鉄欠乏性貧血82例の私達の検討から、ヘモグロビンの分布域の上限は13.0g/dLであることをみると、現行の米国やわが国の基準である12.5g/dLは矛盾しない数字となってくる。

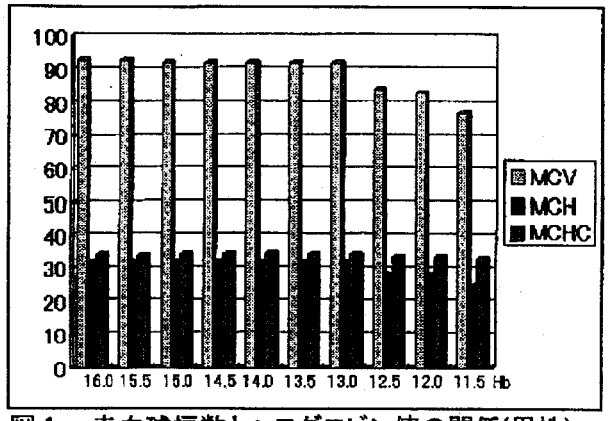

図1　赤血球恒数とヘモグロビン値の関係(男性)

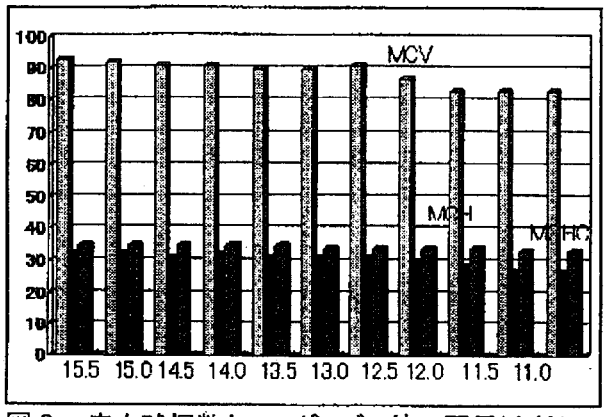

図2　赤血球恒数とヘモグロビン値の関係(女性)

3)　現在考えられる適切な採血基準は

上記を踏まえて採血基準について考察すると、わが国では鉄欠乏状態にある女性の頻度が高く、抜本的対策の見出せない現状では、貧血のない鉄欠乏からの採血をできるだけ避けるために女性の基準は12.0g/dLよりは12.5g/dLのほうが妥当と思われる。また、男性については貧血のない鉄欠乏はほとんどないが、12.5～13.0g/dLは貧血の人から採血することになり矛盾を生ずるので、13.0g/dLが妥当ではないかと思われる。

いずれにしても、採血基準の改定には正確なデータに基づく議論が必要である。それには、日本人の鉄欠乏性貧血、貧血のない鉄欠乏、鉄欠乏のない健常人の頻度（これは現行の国民健康・栄養調査の個々のデータから算出可能である）、献血申込者のヘモグロビン不足による男女別、年齢別不適格者の頻度などの解析によって決められるべきであろう。

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