Evaluation of proposed FDA criteria for the evaluation of radiolabeled red cell recovery trials

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BACKGROUND: FDA requirements for recovery of radiolabeled red blood cells (RBCs) 24 hours after autologous reinfusion in normal subjects have evolved over time. This study defined the ability of currently available RBC systems to satisfy the most recently proposed criteria.

STUDY DESIGN METHODS: RBC recoveries were collected from US laboratories participating in clinical trials for RBC systems that have received FDA approval. Data were stratified for analysis into liquid-stored, gamma-irradiated, and frozen components. With statistical software, 24 individual samples were randomly selected with replacement from each stratum, repeating this for 5000 sample groups per stratum, to simulate experimental outcomes for each population. The percentage of sample groups that passed each and all of the proposed FDA criteria was determined. This procedure was repeated for recovery success thresholds of 75, 70, and 67 percent.

RESULTS: A total of 941 RBC recoveries were obtained from 11 laboratories and 34 studies performed between 1990 and 2006 for 12 sponsors. While the criterion for the mean was almost always satisfied, the standard deviation (SD) criterion was more problematic. Causing most failures was the success threshold definition. Changing the success threshold from 75 to 70 percent or 67 percent increased the likelihood of meeting the requirement for all RBC types.

CONCLUSION: The probability of passing the FDA-proposed criteria for currently FDA-approved products was poor. Changing the success threshold for an individual RBC recovery from 75 to 67 percent resulted in improved ability to meet this criterion for all three RBC types. This change had no affect on the pass rates based on the mean and SD criteria.

United States Food and Drug Administration (FDA) approval of red blood cell (RBC) systems for use in the United States has traditionally required submission of satisfactory in vitro biochemical and hematologic characteristics and in vivo recovery data. Most notably among the former category is documentation of less than 1 percent hemolysis at the end of storage. Other indicators of the metabolic status of the RBC such as the concentrations of adenosine 5′-triphosphate (ATP), 2,3-diphosphoglycerate (2,3-DPG), glucose, and lactate as well as RBC morphology are required measurements, although there are no specific, absolute a priori acceptance criteria. These factors have been shown in various studies to be associated with in vivo recovery outcomes, but the specificity of these in vitro indicators has not been consistently demonstrated.[1] The standard for recovery of radiolabeled RBCs at 24 hours after autologous reinfusion in normal subjects on the last day of storage has evolved over time. Initially, 70 percent was regarded as adequate to avoid hemoglobinuria that could be confused with immunologic destruction of incompatible RBCs. Ross and colleagues[2] in their investigation of early candidates for RBC preservative solutions

ABBREVIATION: SLR = single-label recovery.

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Conflict of interest: The authors' research laboratory has been supported by participating sponsors listed in the manuscript and/or the authors have served as advisors to these sponsors.

Received for publication August 31, 2007; revision received November 26, 2007, and accepted November 26, 2007.

doi: 10.1111/j.1537-2995.2008.01642.x

TRANSFUSION 2008;48:1053-1060.

arbitrarily selected 70 percent as a minimum recovery. In the early 1980s, the expectation was raised to a mean recovery of 75 percent.[3] Additional expectations were later expressed that the sample mean from studies at two laboratories be at least 75 percent with a sample standard deviation (SD) not to exceed 9 percent. These evolving criteria have been applied by the agency in reviewing manufacturers' submissions. The most recent step in this evolution of requirements, proposed by the FDA at a meeting of the Blood Products Advisory Committee in July 2004, included an additional requirement that the one-sided, lower limit of the 95 percent confidence interval (CI) for the proportion of the population that would have a "successful" recovery must be greater than or equal to 70 percent. A successful individual recovery, the success threshold, was defined as a recovery greater than or equal to 75 percent. This new stipulation translated into a successful trial having no more than 3 of 24 (or 2 of 20) recoveries below 75 percent. Studies were to be carried out at a minimum of two laboratories with at least 20 evaluable subjects. These requirements are summarized in Table 1.

The objective of this study was to define the ability of currently available RBC collection and storage systems to satisfy new RBC recovery criteria proposed by the FDA for approval of RBC systems. We collected and analyzed RBC recovery data for RBC systems that have received FDA approvals and are currently in use in the United States. We then applied the proposed requirements and several variations of them to identify which aspects of the tripartite concept presented the greatest challenge for current RBC systems and the inherently variable biology of human clinical trials. From this work, we propose some concepts that may be useful in setting regulatory requirements for blood storage systems.

TABLE 1. FDA proposed acceptance criteria for RBC products

In vivo: 24-hour autologous transfusion recovery in normal subject
1. Total of 20 or more evaluable 24-hour recoveries
2. Minimum of two laboratories
3. Sample mean $\geq$ 75%
4. Sample SD $\leq$ 9%
5. One-sided 95% lower confidence limit for the proportion of successes must be greater than 70%, with success threshold for individual recovery $\geq$ 75%

In vitro:
1. Hemolysis < 1% at the end of storage*
2. Residual WBCs $< 5 \times 10^6$ per unit*†
3. Postfiltration RBC recovery > 85% of original RBC mass*†
4. Comparison of the difference between control and test:‡
   i. ATP concentration
   ii. 2,3-DPG concentration
   iii. Product weight
   iv. WBC concentration
   v. RBC concentration
   vi. Platelet concentration
   vii. MCV
   viii. Morphology
   ix. pH
   x. Glucose concentration
   xi. Lactate concentration

* 95% confidence that at least 95% of the population meets or exceeds the specification.
† Applies only to leukoreduced RBC products.
‡ Acceptable differences not specified.

MATERIALS AND METHODS

We obtained permission to access clinical trial data from the sponsoring manufacturers of several US-based autologous RBC-radiolabeled recovery studies. We then contacted US laboratories known to have engaged in clinical trials utilizing radiolabeled RBC recoveries requesting their participation in this study. Each was asked to submit to a coordinating center the results of single-label RBC (24 hr) recovery studies performed since 1990 in a blood collection and processing system either that was already FDA-approved (such as might have been used as a "control" arm system in a trial) or that was subsequently approved (that is, was the "test" system in the clinical trial but was later FDA-approved). We provided participants an Excel spreadsheet and directions to enter these data: corresponding laboratory, year of the study, paired laboratory (if any), study sponsor, collection method, use of RBC additive solution (AS), storage duration, secondary treatment such as gamma irradiation or leukoreduction, study arm (control/test), study laboratory, study subject identifier, and 24-hour RBC autologous radiolabeled single-label recovery (SLR). Where conducted, double-label recovery results were also captured but were not analyzed as part of this study. Methods for single-label, radioisotopic labeling and recovery determination are published elsewhere.[4] Manufacturer sponsors of the studies verified data from their studies against their official internal files for completeness and accuracy and to ensure that the data were identical to what had ultimately been submitted to the FDA for regulatory approvals. We resolved database queries with study laboratories, sponsors, and published records of the studies as indicated during the course of finalizing the database. Data records with unresolved queries were dropped from further analysis. Laboratory and sponsor links to data sets are coded and will remain confidential due to the proprietary and confidential aspects of the information.

Data were accumulated on a trial-by-trial basis, pairing data from laboratories that had been involved as separate sites for a particular trial in the same manner that they would have been accumulated for submission for regulatory review. The original intention was to then analyze the data on a trial-

by-trial basis in a manner analogous to how a regulatory agency would apply the criteria to determine the proportion of trials that met the proposed criteria. Past studies, however, were not uniformly designed with 10 to 12 subjects per site in at least two laboratories, resulting in insufficient data for such an approach. After consultation with the BEST Collaborative and FDA contacts, an alternative approach was developed.

Data were stratified for analysis into liquid-stored—RBCs stored 42 days at 1 to 6°C with an approved RBC AS (AS-1, AS-3, or AS-5); gamma-irradiated—liquid-stored as above plus gamma-irradiated with 25 Gy either on Day 14 of storage with the unit held through 42 days before labeling or on Day 1 followed by labeling on Day 28 of storage; and frozen—RBCs frozen in glycerol, thawed, and deglycerolized by standard methods.[5-8] With a computer, we randomly selected 24 individual samples with replacement from each stratum separately, repeating this for 5000 sample groups per stratum, to simulate experimental outcomes for each of these three populations (PROC SURVEYSELECT, SAS 9.1.3, SAS Institute, Inc., Cary, NC). Such an approach may be referred to as a Monte Carlo simulation or a bootstrapping method, commonly applied to estimate CIs and for statistical inference.[9] We calculated the mean 24-hour recovery, SD, and the number of individual samples with 24-hour recovery of at least 75 percent (success threshold) for each sample group of 24. The percentage of sample groups that passed the current FDA criteria was determined (PROC SURVEYSELECT, PROC FREQ, SAS). This analysis was repeated with success thresholds of 67 and 70 percent. The results of the approach were verified by comparing to inferences with the binomial expansion.

Statistical analysis

Descriptive statistics and histograms of RBC recovery were computed with software (PROC UNIVARIATE. SAS 9.1.3). The probability of RBC recovery of at least 75 percent in 21 or more individual samples from a sample of 24 from these distributions was calculated with the binomial expansion (NCSS, Keysville, UT). The minimum proportion of successful recoveries to support a passing result for a sample was determined for a noninferiority hypothesis test assuming sample sizes of 20 and 24, the smallest acceptable proportion of successful recoveries (i.e., equivalent proportion) of 70 percent (note: this is not the minimum acceptable RBC recovery), alpha risk of 0.05, and power of 80 percent (one proportion noninferiority power analysis, NCSS).

RESULTS

A total of 11 laboratories submitted data from 38 studies performed between 1990 and 2006 for 12 sponsors (Table 2). Data from 3 studies were eliminated because the medical devices were not approved by FDA or there was nonstandard processing. One study was eliminated because queries were not satisfactorily resolved with the study laboratory or study sponsor. From data derived from 34 different studies, a total of 941 SLR results were available for analysis across several different conditions (Table 3).

The reported RBC recoveries for the three groups are summarized in Table 4. All RBC units stored 42 days in FDA-approved AS were pooled into one population (n = 641) as representative of RBC units presently in routine clinical use in the US (Fig. 1). RBC units that had

TABLE 2. Participating laboratories and sponsors

Laboratories
- American Red Cross, Louisville, KY
- Blood Center of Wisconsin, Milwaukee, WI
- Dartmouth-Hitchcock Medical Center, Lebanon, NH
- Hoxworth Blood Center, Cincinnati, OH
- Mayo Clinic, Rochester, NY
- American Red Cross, Norfolk, VA
- University of North Carolina, Chapel Hill, NC
- University of New Mexico, Albuquerque, NM
- University of Virginia, Charlottesville, VA
- Walter Reed Army Institute of Research
- Yale University, New Haven, CT

Sponsors
- American Red Cross
- Baxter
- Baxter/Army
- Cutter/Pall
- Gambro BCT, Inc.
- Haemonetics/Transfusion Technologies
- Hemasure
- MacoPharma/UnitedPharma
- Terumo
- Vitex

TABLE 3. Evaluable RBC recoveries*

	Leukocyte-reduced	Nonleukoreduced	Total
Automated collection			
Liquid-stored	205	9	214
Gamma-irradiated	67	0	67
Subtotal	272	9	281
Manual collection			
Liquid-stored	291	136	427
Gamma-irradiated	24	32	56
Subtotal	315	168	483
Frozen	140	37	177
Total	727	214	941

* A total of 941 evaluable RBC recoveries were entered into the analysis: liquid-stored—641 RBC units were stored in AS 42 days before radiolabeled RBC recovery studies; gamma-irradiated—gamma irradiation (n = 123) was with 25 Gy either on Day 14 of storage with the unit held through 42 days before labeling or on Day 1 followed by labeling on Day 28 of storage; and frozen—units frozen for 15 to 30 days totaled 177. Leukoreduction was with FDA-cleared filtration devices.

TABLE 4. RBC recovery by component type

RBC type	Number	RBC recovery (%)			Frequency of RBC recovery			Mean* (%)	SD* (%)
		Minimum	Lower 5% quantile	Median	≥75%	≥ 70%	≥ 67%		
42-day liquid-stored	641	36	70.7	82.4	88.3	95.5	98.1	82.1	6.71
Gamma-irradiated	123	47.1	63.6	79.3	69.1	81.3	91.1	77.9	8.36
Frozen	177	52.2	74.2	88.0	94.4	97.2	98.8	86.6	7.41

* All distributions failed tests for normality.

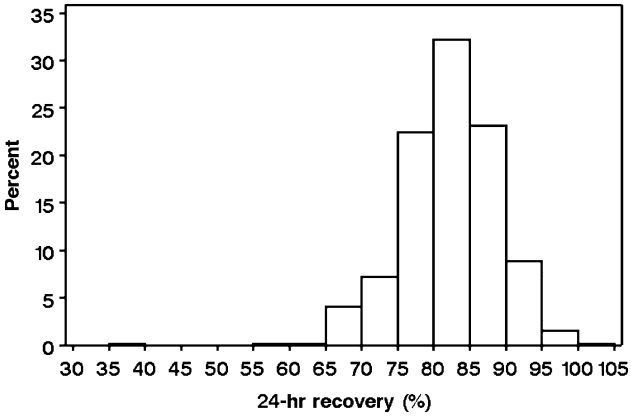

Fig. 1. Frequency distribution of 24-hour RBC recovery for RBCs stored for 42 days in AS. n = 641.

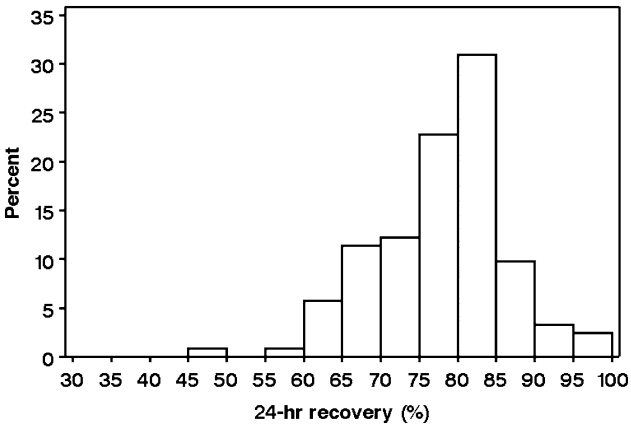

Fig. 2. Frequency distribution of 24-hour RBC recovery for gamma-irradiated RBCs. n = 123.

been collected by manual or automated techniques were irradiated either within 24 hours of collection and reinfused on Day 28 of liquid storage (n = 29) or irradiated on Day 14 and reinfused on Day 42 (n = 94); these two approaches yielded indistinguishable results, and the data were pooled (Fig. 2). Units were held frozen between 15 and 30 days (15 days, n = 21; 24 days, n = 9; and 30 days, n = 47) before thawing and deglycerolization by either manual or automated methods (Fig. 3).

Of the recoveries for additive system RBCs stored in liquid for 42 days, 95.5 percent had 24-hour recoveries of at least 70 percent, and 88.3 percent were at least

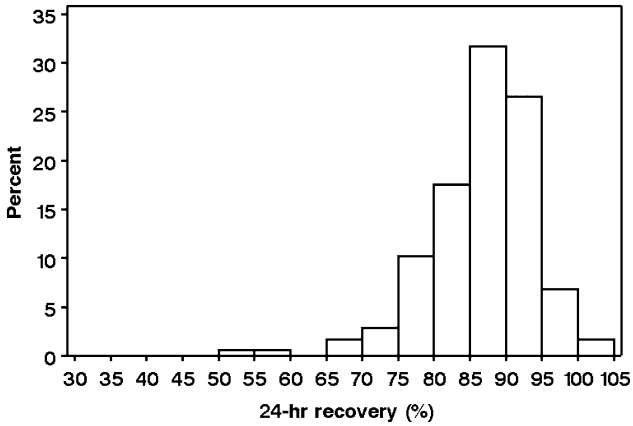

Fig. 3. Frequency distribution of 24-hour RBC recovery for RBCs stored frozen. n = 177.

75 percent (Table 4). A binomial expansion of the latter with sample size of 24 provides an expected performance when judged against the FDA-proposed criteria with an individual recovery defined as a success if greater than 75 percent. This returned a probability of 0.693 of having at least 21 of 24 recoveries in the sample with a recovery of at least 75 percent, that is, a passing result could be expected 69.3 percent of the time (Table 5). The resampling of 5000 data sets of 24 resulted in 3364 sample groups (67.3%) passing the FDA-proposed criteria with 21 or more units with recoveries of at least 75 percent (Fig. 4). All of the data sets passed the mean criteria being at least 75 percent, and 4758 of 5000 sample groups (95.2%) passed the SD criteria being no higher than 9 percent (Table 5). The gamma-irradiated group had a very low expected probability of passing the individual success threshold of 75 percent recovery, which was confirmed with the resampling having only 3.5 percent of sample groups pass (Fig. 5). The mean and SD criteria were met in 95.5 and 71.4 percent of the sample groups, respectively. The frozen RBCs had the best performance against the FDA-proposed criteria with 96.0 percent of sample groups passing the individual 75 percent success threshold, 100 percent passing the mean criterion, and 83.7 percent passing the SD criteria (Fig. 6).

To estimate the performance criteria that would fit the existing data, we first calculated the minimum proportion of successful recoveries (success threshold not

TABLE 5. Application of proposed criteria to data sets*

	1†	2†	3‡	4†	5§	6‡	7†	8§	9‡	10†	11§
			Success: recovery ≥ 75%			Success: recovery ≥ 70%			Success: recovery ≥ 67%		
RBC type	Data sets with mean ≥75%	Data sets with SD ≤9%	Probability (number of successes ≥21)	Data sets with successes ≥21	Probability (number of successes ≥18)	Probability (number of successes ≥21)	Data sets with successes ≥21	Probability (number of successes ≥18)	Probability (number of successes ≥21)	Data sets with successes ≥21	Probability (number of successes ≥18)
42-day liquid-stored	100%	95.2%	0.693	67.3%	0.580	0.979	95.5%	0.941	0.999	99.9%	0.994
Gamma-irradiated	95.5%	71.4%	0.035	3.5%	0.030	0.318	31.1%	0.249	0.840	82.7%	0.739
Frozen	100%	83.7%	0.957	96.0%	0.902	0.996	99.6%	0.982	0.9998	99.99%	0.998

* 5000 replicate data sets n = 24 were sampled with replacement from each of the three types of RBC data sets. The frequency (%) of sample means and SDs meeting the FDA proposed criteria for mean and SD are shown in Columns 1 and 2. The probabilities of a sample passing the FDA-proposed population criteria of 95 percent confidence that at least 70 percent of the population must have a successful recovery result are shown for success thresholds of 75, 70, and 67 percent.
† 5000 replicate data sets n = 24 were sampled with replacement.
‡ Binomial expansion based on n = 24 and frequency of recovery ≥ success threshold (Table 4).
§ Binomial expansion based on n = 20 and frequency of recovery ≥ success threshold (Table 4).

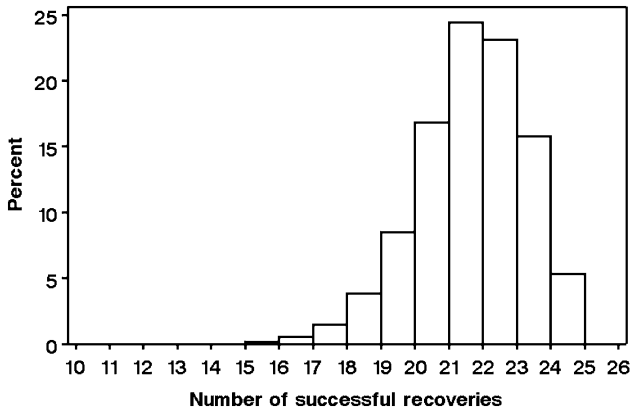

Fig. 4. 42-day liquid-stored RBCs—frequency distribution for 5000 random groups with sample sizes of 24 showing the percentage of units with 24-hour RBC recovery of at least 75 percent in a sample.

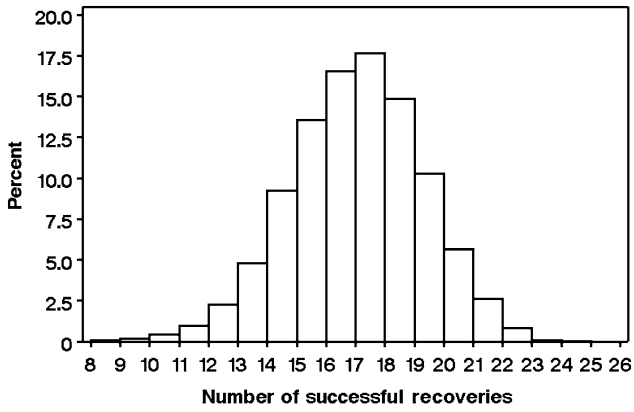

Fig. 5. 42-day liquid-stored irradiated RBCs—frequency distribution for 5000 random groups with sample sizes of 24 showing the percentage of units with 24-hour RBC recovery of at least 75 percent in a sample.

defined at this juncture) in a sample of 24 for a noninferiority hypothesis test assuming 70 percent as the smallest acceptable proportion of successful recoveries, an alpha risk of 0.05, and a power of 80 percent. In this case, the minimum proportion of successful recoveries to support a passing result in a sample of 24 was 90.2 percent. With the reported distributions (Figs. 1-3), we then determined the corresponding recovery value for the poorest performing RBC type, gamma-irradiated, as 67 percent. That is to say, with the distribution of in vivo RBC recoveries shown for the gamma-irradiated group, 90.2 percent are expected to be greater than 67 percent RBC recovery. We next repeated the evaluation of the 5000 data sets for each RBC type based on two new definitions of a success for an individual RBC recovery, 67 and 70 percent. Replacing the success threshold for SLR of 75 percent with 67 percent resulted in much greater likelihood of the success criterion being met for all three RBC types, with pass rates of

99.9, 82.7, and 99.99 percent for liquid-stored, gamma-irradiated, and frozen, respectively (Table 5). There were no effects on the pass rates for the mean and SD criteria.

We explored the effect of sample size on the chance of meeting this passing criteria by calculating the probability of 18 or more successes in a sample size of 20. As seen in Table 5, the chance of a pass is slightly reduced with the reduction of sample size to 20. We also explored the sensitivity of sample size requirement to the proportion of successes in a population with the noninferiority power analysis for an alpha of 0.05, a power of 80 percent, and an equivalent proportion of successes of 70 percent. Figure 7 demonstrates the increasing sample size requirements as the test population proportion of successes approaches the equivalent proportion of 70 percent.

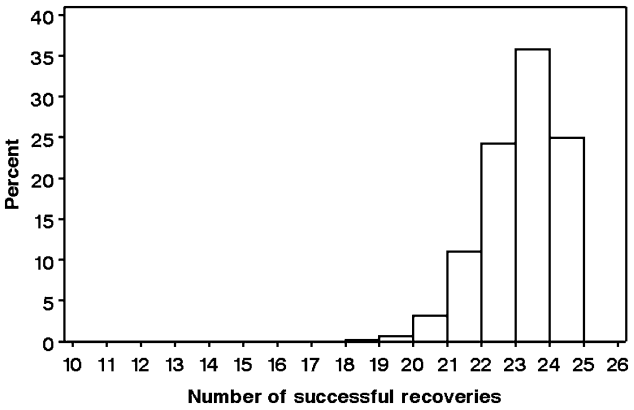

Fig. 6. RBCs stored frozen—frequency distribution for 5000 random groups with sample sizes of 24 showing the percentage of units with 24-hour RBC recovery of at least 75 percent in a sample.

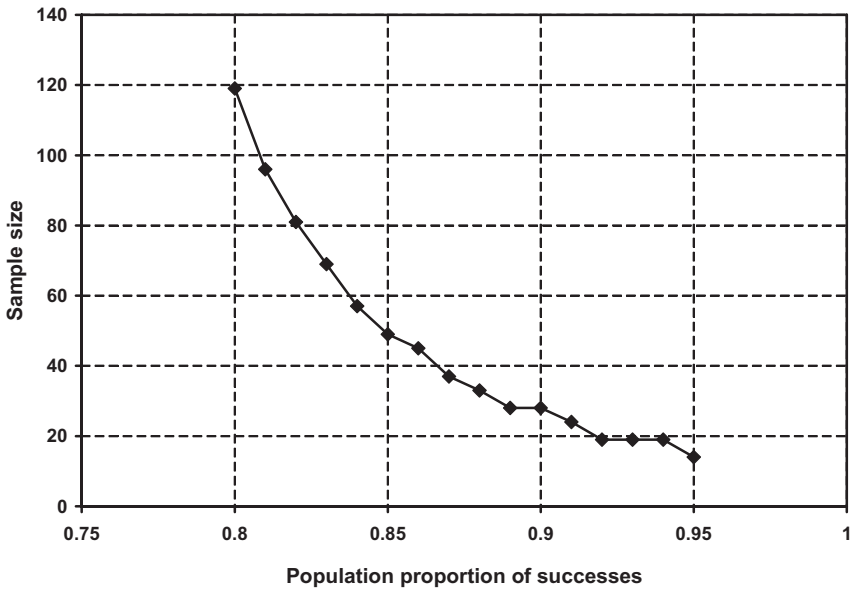

Fig. 7. Sample size sensitivity—sample size for a noninferiority test ($\alpha = 0.05$; power, 80%) increases as the true proportion of successes in the test population approaches the equivalent proportion of 70 percent. This is independent of absolute RBC recovery value and dependent on the definition of an RBC recovery success threshold.

CONCLUSIONS

Performance standards for pharmaceuticals and medical devices serve several important purposes. They provide minimum requirements that can be referenced by designers and manufacturers during product development and are available to users and practitioners as points of reference, and regulatory bodies can use these standards as criteria for approval and licensing of new or modified products. In the end, the primary intent is to provide a reasonable level of assurance to the patient and practitioner of the safety and efficacy of the drug or device. These requirements may also serve to spur improvements in future generations of the drug to provide even better treatment. For example, restating requirements to require greater effectiveness or a greater degree of confidence in achieving a previously established standard might prompt the development of a better drug formulation so as to increase the chance that the new drug would meet the stiffened requirements. It is critically important, however, that new, demanding requirements are solidly based on clinical outcomes as well as the current state of the art. Simply raising or stiffening requirements in and of itself does not translate into better drugs and devices.

FDA has proposed a panel of performance criteria for methods and devices that prepare RBC for transfusion (Table 1). Key among these requirements is the 24-hour recovery of radiolabeled autologous RBCs, the focus of this investigation. In FDA's progress toward stating and clarifying performance standards in statistically sound and state-of-the-art quality assurance terms, they have added requirements for CIs on various parameters, for example, the requirement to demonstrate with 95 percent confidence that at least 70 percent of the products being evaluated will have successful RBC recoveries.

The CI requirement has the effect that RBCs prepared by a device with 70 percent successful recoveries will have only a 5 percent chance of passing these criteria. In quality control terms this is often referred to as the consumer's risk or alpha risk. From the manufacturer's perspective, the device performance will need to have a success rate much greater than 70 percent to have a reasonable chance of passing the test, as illustrated in Fig. 7. Here it

becomes a tradeoff of sample size (money, time, and risks to human subjects) and the likelihood of passing the test. It is generally accepted that a study in normal human volunteers of 20 to 24 subjects is a safe and reasonable number for these types of evaluations. Given this constraint, this translates to a requirement that the RBC product will need to have at least 90.3 percent success rate to pass 80 percent of the time. Therefore, the effect of this part of the tripartite proposed requirement is to significantly drive up the overall performance expectation of new products.

FDA has further selected a 75 percent recovery as the minimum value for success based on rationale presented by Grindon.[10] Grindon proposed that RBCs stored for 30 days, 25 percent of the expected RBC lifetime of 120 days, should have a "24-hour survival" of 75 percent. He also suggested that a 75 percent target would ensure adequate recovery and survival of the transfused RBCs in clinical transfusions. These proposals are useful for initiating theoretical considerations, but they are arbitrary and were not developed with the support of clinical evidence (A.J. Grindon, personal communication, 2007).

Until now, this criterion plus that for the sample mean and SD have not been tested against data representing RBCs in actual clinical use. A critical component of the FDA-proposed requirements is the definition of a successful RBC recovery. When we evaluated the FDA proposal of 75 percent, the probability of passing for currently FDA-approved products was poor (3.5% for gamma-irradiated and 67.3% with liquid-stored). This appeared overly aggressive to us since the general clinical performance for these products is adequate as proved over years of clinical practice and certainly represents the current state of the art. Improvements in RBC preparation and storage methods are desirous and should be encouraged. The nature and target values for those improvements should be driven by clinical data, however. Arbitrarily raising a performance criterion could even act as a disincentive for product development investments. Based on the actual performance distribution of 941 RBC recoveries, we would suggest a reasonable recovery minimum in the range of 67 to 70 percent. When applied to these data with a large resampling approach of 5000 data sets of 24, we found the acceptance rate improved to 82.7 percent for gamma-irradiated products and 99.9 percent for liquid-stored RBCs. This adjustment had no affect on the proposed mean and SD criteria. We believe that the mean and SD criteria are useful guides, but these parameters should not be used as acceptance criteria primarily because these distributions do not meet assumptions regarding statistical normality (Table 6).

TABLE 6. Authors' proposed in vivo acceptance criteria for RBC 24-hour autologous transfusion recovery in normal subjects

1. Total of 20 or more evaluable 24-hour recoveries
2. Minimum of two laboratories
3. One-sided 95 percent lower confidence limit for the proportion of successes must be greater than 70 percent, with success threshold for individual recovery $\geq$ 67%

It is possible that the present data set used for our analyses did not include all RBC recovery studies performed with currently available collection and preparation methods. Either investigator-initiated trials or studies with poor outcomes may not have been submitted to FDA and/or were not captured in our inquiries. Such an effect would tend to bias high the performance numbers reported here. We feel that this is quite unlikely, however, in that the investigators who perform these studies are well known to each other, and our queries were directly to the laboratories that executed the studies.

The results of our analyses and inferences on the performance of RBC preparation and storage methods currently used in clinical medicine today are based on data from a 17-year time frame. This large data set provides much better estimates of the population of RBC being transfused today than analyses that may be performed with only a few recently completed studies.

Studies evaluating new or modified RBC collection or storage methods are sometimes designed as paired, crossover studies. This design presents the advantage of removing much of the interdonor variability in this measurement as well as allow rational elimination of data points for subjects who have inherently poor autologous recoveries due to a metabolic or other physiologic characteristic. Because of cost and time constraints, however, study sponsors often find it desirable to evaluate only the test arm and judge it against a RBC recovery standard as described in this work. The cost and time advantage are sometimes diminished with this approach if the study subject with inherently poor RBC recovery is encountered. Recently, some have advocated a Bayesian approach to this problem where initial testing is with a small number of subjects, and if the number of RBC recovery successes does not meet the standard (e.g., fewer than 18 successes of 20), then additional subjects are entered with all data considered as cumulative. This approach may have some advantages, but, as shown in Fig. 7, the sample size requirement tends to get large rather quickly as the test population proportion of successes approaches the equivalent proportion.

The criterion for a minimum acceptable recovery, success threshold, should be viewed as a product performance minimum. A true patient safety or efficacy minimum has not been established for RBC recovery, and this would require a large clinical outcomes study to determine. It is likely that the patient efficacy minimum, based on clinical experience with current RBC preparations, is much less than the 67 to 70 percent proposed here.

ACKNOWLEDGMENTS

The authors thank the following individuals for their efforts in gathering and verifying the data: Lauren Clark, Jose A. Cancelas, Tammy Corda, M. Dean Elfath, John Hess, Stein Holme, Jaime Houghton, Mike McAteer, Jeff Miripol, Ed Nelson, Leslie Rose, Neeta Rugg. Sherrie Sawyer, Yariv Sivan, Edward Snyder, Tania VandenBroeke, and Pamela Whitley. The authors also express their appreciation to individuals at the FDA/CBER Cellular Hematology Laboratory and Biostatistics for data review and helpful discussions.

REFERENCES

1. Klein HG, Anstee DJ, editors. Mollison' blood transfusion in clinical medicine. 11th ed. Malden: Blackwell Publishing; 2005. p. 365-8.
2. Ross JF, Finch CA, Peacock WC, Sammons ME. The in vitro preservation and post-transfusion survival of stored blood. J Clin Invest 1947;26:687-703.
3. FDA workshop on red cells stored in additive solution systems; 1985 Apr 25, 1985; Bethesda, MD.
4. Moroff G, Sohmer PR, Button LN. Proposed standardization of methods for determining the 24-hour survival of stored red cells. Transfusion 1984;24:109-14.
5. Bandarenko N, Hay SN, Holmberg J, Whitley P, Taylor HL, Moroff G, Rose L, Kowalsky R, Brumit M, Rose M, Sawyer S, Johnson A, McNeil D, Popovsky MA. Extended storage of AS-1 and AS-3 leukoreduced red blood cells for 15 days after deglycerolization and resuspension in AS-3 using an automated closed system. Transfusion 2004;44:1656-62.
6. Cancelas JA, Bandarenko N, Snyder E, Rugg N, Hay SN, Baril L, Corda T, Rose M, Kowalsky R, Foley J, Rose L, Popovsky M. Cryopreservation of AS-5-collected red blood cells in PVC or EVA bags by the closed ACP®215 system allows extended storage for 14 days post-thawing. Transfusion 2006;46:17A.
7. Snyder EL, Baril L, Dincecco D, Corda T, Carrano D, Greenwalt T, Rugg N, Gormas J, Pratt G, Adamson JW, Kagen L, Graminske S, Janik S, Lee W, Brantigan B, Elfath M. Evaluation of frozen ACD-A/Adsol (AS-1) RBC products. Transfusion 2004;44:61A.
8. Meryman HT, Hornblower M. A method for freezing and washing RBCs using a high glycerol concentration. Transfusion 1972;12:145-6.
9. Hogg RV, McKean JW, Cray AT, editors. Introduction to mathematical statistics. 6th ed. Upper Saddle River: Pearson Prentice Hall; 2005. p. 286-309.
10. Grindon AJ. Blood collection. In: Mcclatchey KD, editor. Clinical laboratory medicine. Philadelphia: Lippincott Williams & Wilkins; 2002. p. 1525-8.