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Posttransfusion red blood cell (RBC) survival determined using biotin-labeled RBCs has distinct advantages over labeling with ^{51}Cr

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We are writing regarding the following comment by Oliver and colleagues¹ in the August issue of **TRANSFUSION**. The authors state in the Introduction that “Radioisotope chromium 51 (^{51}Cr) labeling of blood, despite its disadvantages, is still the only reliable method to predict the intravascular success of an incompatible transfusion. Alternative techniques such as direct biotinylation of red blood cells (RBCs), despite their promise, have not been routinely adopted.”

We concur that biotinylated red blood cells (RBCs) have not yet been routinely adopted for quantitation of RBC survival, due in part to the current Food and Drug Administration requirement that an investigational new drug is required for studies that include transfusion with biotin-labeled RBCs. Nonetheless, several factors lead us to predict that measurement of RBC survival using the biotin label will be considered to serve as the reference method eventually. More recent publications than those cited by Oliver and colleagues indicate that the biotin method for labeling RBCs is now reaching its promise.

Human RBCs may be reliably biotinylated *ex vivo*. Ten to 50 mL of labeled RBCs administered to a 70-kg adult yield 0.4% to 2% biotinylated RBCs in the bloodstream; this initial percentage is adequate to permit accurate tracking by flow cytometry for greater than 85% of RBC survival.^{2,3} In addition to basic measures of RBC recovery and life span, the biotin method has been used in humans for studies to compare survival of multiple RBC populations concurrently in the same individual and to assess age-dependent changes in RBCs, including the following:

1. The survival characteristics of two populations of RBCs that were labeled with different biotin densities and were distinguishable by flow cytometry were simultaneously studied in the same study subject.² The ^{51}Cr RBC label can be used for only a single population of RBCs at any one time and, consequently, studies of

CONFLICT OF INTEREST

The authors declare no conflicts of interest relevant to this letter.

multiple RBC populations must be done sequentially—possibly, introducing biologic and/or temporal variability because studies were not done concurrently.

2. Time-dependent changes in the properties of sickle RBCs have been determined, including the survival of biotinylated RBCs containing HbF versus those without detectable HbF.⁴
3. The rate of formation of glycated Hb (e.g., HbA_{1c}) in controls and patients with diabetes determined using magnetic isolation of aging biotin-labeled RBCs has been helpful in interpreting the biologic and clinical significance of glycated Hb as an indicator of high blood glucose levels and long-term complications of diabetes.⁵

In studies such as those by Mock and colleagues² and Franco,³ understanding intrasubject variability in RBC survival is important. The accuracy of the ⁵¹Cr method is limited due to dissociation from the beta-chain of Hb in the RBCs at rates that vary substantially among individuals and even more among species. This loss of label has a direct effect on accuracy of RBC life span quantitation determined with ⁵¹Cr. The situation is different with biotinylated RBCs, even though up to 40% of the covalently linked biotin labels on the outward facing membrane of the RBC are lost during the first few weeks in the circulation. Because the biotinylated RBCs are examined and enumerated individually by flow cytometry and continue to fall within the designated region for a positive cell, they are counted accurately. Accordingly, RBC recovery and life span can be quantitated until nearly all of the biotin-labeled RBCs are removed from the circulation, that is, until the biotin-labeled RBCs fall below 0.06% of the circulating RBCs. The biotin RBC label has been used successfully in all mammals tested thus far and is thus useful in preclinical as well as clinical studies.

Current information indicates that biotinylated RBCs may be used safely. In adult humans, the biotin RBC label has been associated with the development of transient agglutinating antibodies to biotin-labeled RBCs in approximately 15% of adult study subjects infused with 50 mL of biotinylated RBCs.⁶ In the 12 years since the publication of these observations, we have observed only one of eight (13%) additional adult participants who developed a transient positive direct antibody result (Table 1).² Of note, in this more recent report, subjects were infused with four times the volume of biotinylated RBCs (200 mL) containing an estimated sevenfold greater biotin density per RBC without an increase in the percentage of individuals developing antibodies. The development of antibodies to biotin-labeled RBCs had no effect on the survival of biotin-labeled RBCs. The four adult subjects who developed antibodies to biotinylated RBCs have not developed an identifiable change in the rate of elimination of biotin-labeled RBCs,^{2,6} clinical signs, or symptoms (personal follow-up communication with study subjects). However, we note that none of these four individuals have been rechallenged with biotinylated RBCs.

We write to call these more recent studies utilizing biotin labeling of RBCs to the attention of the authors and other investigators performing RBC kinetic studies so that the advantages of biotin-labeled RBCs can be exploited.

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TABLE 1

Time course after transfusion of antibodies to biotinylated RBCs

BioRBC antibody titer*	Sampling time relative to transfusion with biotin-labeled RBCs						
	Before	1 month	2 months	3 months	4 to 5 months	6 months	10 to 12 months
Ref. 2 Subject 1	0	ND	ND	ND	+ / + +	+	0
Ref. 2 Subject 2	0	ND	ND	ND	+ / + +	+ / + +	0
Ref. 2 Subject 3	0	ND	ND	ND	+ weak	0	0
Ref. 6 Subject 1	0	0	0	+ / -	+ weak	ND	0

* 0 = no visible agglutination; + / = weaker than "+, weak"; + weak = minute clumps visible macroscopically; + = many small clumps, turbid, reddish background; ++ = medium-sized clumps, clear background; +++ = several large clumps, clear background; ++++ = one large clump, clear background.