Using Analytical Methods and Simulation to Estimate the Magnitude of Errors in Calculations for Recovery in Washed Red Blood Cells

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Abstract: Canadian Blood Services produces a modified blood product, called washed red blood cells. RBCs are washed to reduce potential transfusion reactions in vulnerable populations. Quality control standards require that at least 75% of the red cells in a unit are retained through the washing process. However, field reports suggest that cell recovery values greater than 100% can be observed. The purpose of this study is to analyse the propagation of error in the washing process and to determine if values exceeding 100% are reasonable, given the accuracy of the equipment in use. Employing analytical techniques and simulation methods, it was found that recovery rates in excess of 100% are possible, but that any calculated value exceeding 102% is unlikely and should be investigate for process errors.

1 BACKGROUND

Red blood cells (RBC) are cells that are responsible for oxygenating a person's cells. In general, most patients receiving a transfusion are supplied with production standard RBC. However, in patients with potential for severe anaphylactic reactions, RBC are washed to remove plasma, plasma protein, micro-aggregates, cytokines, and unwanted antibodies from a blood product (Hansen, Turner, Kurach, & Acker, 2015). Washed RBCs reduce the incidence of unwanted, and potentially dangerous, transfusion related reactions in certain vulnerable recipient populations.

Canadian Blood Services (CBS) is the not-for-profit agency responsible for the collection, production, testing, and distribution of blood and blood products in all of Canada, outside of the Province of Quebec, which maintains its own agency (Blake & Hardy, 2013). As a regulated blood agency, CBS maintains an extensive quality control program to ensure the viability of its products and to monitor its processes. For example, quality control standards dictate that production/distribution sites providing washed RBCs to customers must perform a monthly audit of their procedures to ensure that the equipment and practices employed result in products with acceptable characteristics. These standards dictate that the amount of recovered red cells in the output product must be ≥ 75% of the red cells in the input product (Canadian Blood Services, 2021).

However, it has been observed in the field that in some instances of washed process audit the percentage of recovered cells identified in the output product exceeded 100%. Since the percent recovery is based on the number of red cells in the output bag divided by the number of cells in the input bag and cells cannot be added to the output product via the washing process, ratios greater than 1.0 are physically impossible and must, therefore, be due either to errors in method or the accuracy of equipment used to measure values used in the calculation.

2 OBJECTIVE

This study provides a method for evaluating the degree of error associated with the accuracy of the equipment used to measure parameters used in the percent recovery calculation at Canadian Blood Services and to estimate the range of error in practice. The purpose of this study is to identify when a calculated percent recovery can be considered *reasonable,* given known or estimated, error in the process and when a calculated value must be

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considered anomalous, indicating that a cause for the exception must be identified.

3 METHOD

The range of potential values for percent recovery was evaluated using analytical and simulation methods. Monte Carlo simulation, either on its own or combined with analytical methods, is a common method for estimating error propagation in complex systems. It has been used to estimate uncertainty in digital elevation models for geospatial applications (Temme, Heuvelink, Shoorl, & Claessens, 2009), data corruption in high performance computing (Li, et al., 2021) and air pollution modelling (Evans, Cooper, & Kinney, 1984), amongst other applications. Our method follows the same general plan as Evans, Cooper and Kinney (1984), but tailored for a process of washing red blood cells. We believe that this is the first application of Monte Carlo methods to support error propagation analysis in a red cell washing process.

The operational calculation used to determine percent recovery was analyzed and, using algebra combined with assumptions regarding typical values, the potential range of values was identified. A sensitivity analysis was performed on the assumed values. A simulation was then employed to evaluate the likely range of errors and to confirm the analytic results.

3.1 Analytical Analysis

Percent recovery is a ratio of cells post-wash to cells pre-wash. Since the number of cells in both the input and output product bags cannot be measured directly, they must be estimated. To estimate the number of cells, the volume of product in a bag is multiplied by the product hematocrit, or percent of a blood product composed of red blood cells, as determined by a cell analyzer, based on a small sample taken from the product or an associated segment. Percent recovery is thus calculated as:

$$\%\ Recovery = \frac{v_O h_O}{v_R h_R} \tag{1}$$

Where:

v_O is the volume of the product (post-wash)
h_O is the hematocrit of the product (post-wash)
v_R is the volume of the product (pre-wash)
h_R is the hematocrit of the product (pre-wash)

However, the volume of the input (pre-wash) and output (post-wash) products also cannot be directly measured. Instead, the volume is calculated by multiplying the net weight of the product in the bag by the specific gravity of blood as follows:

$$\%\ \text{Recovery} = \frac{n_O sg_B h_O}{n_R sg_B h_R} \tag{2}$$

Where:

n_O is the net weight of the product (post-wash)
h_O is the hematocrit of the product (post-wash)
n_R is the net weight of the product (pre-wash)
h_R is the hematocrit of the product (pre-wash)
sg_B is the specific gravity of blood.

Since the term sg_B appears in both the numerator and denominator of we can simplify the calculation in (2):

$$\%\ \text{Recovery} = \frac{n_O h_O}{n_R h_R} \tag{3}$$

The net weight of the product, both pre- and post-wash is determined by weighing the product and the container holding it and then subtracting from this weight an assumed tare weight (i.e. the weight of the empty container). If we define w_O and w_R to be the gross weight (i.e., total weight of the product and bag) of the output and input products respectively, and t_O and t_R to be the tare weights of the empty bags, then:

$$\begin{aligned} n_O &= (w_O - t_O) \\ n_R &= (w_R - t_R) \end{aligned} \tag{3a}$$

and Equation (3) can be written as:

$$\%\ \text{Recovery (SOP)} = \frac{(w_O - t_O)h_O}{(w_R - t_R)h_R} \tag{4}$$

Where:

w_O is the weight (measured) of the bag and blood (post-wash)
w_R is the weight (measured) of the bag and blood (pre-wash)
t_O is the tare weight (assumed) of the bag (post-wash)
t_R is the tare weight (assumed) of the bag (pre-wash)
h_R is the hematocrit (measured) of the product (pre-wash)
h_O is the hematocrit (measured) of the product (post-wash)

Equation 4 is the calculation specified at CBS for calculating percent recovery in washed RBCs. However, this calculation assumes that all values are known with certainty. In reality, of course, there are

errors in quantities measured due to accuracy limitations of the equipment used to determine the parameters of weight and hematocrit. Accordingly, if one were to assume that the calculated value was equal to the true value plus a randomly distributed error term, then equation (4) becomes:

$$\%\ \text{Recovery} = \frac{\left((w_O + w'_O) - (t_O + t'_O)\right)(h_O + h'_O)}{\left((w_R + w'_R) - (t_R + t'_R)\right)(h_R + h'_R)} \quad (5)$$

Where:

w_O, is the error in the weight of the bag and blood (post-wash)

w_R, is the error in the weight of the bag and blood (pre-wash)

t_O' is the error in the tare weight of the bag (post-wash)

t_R, is the error in the tare weight of the bag (pre-wash)

h_O, is the error in the hematocrit of the product (post-wash)

h_R, is the error in the hematocrit of the product (pre-wash)

Note that while the error is shown as additive in Equation (5), it should be understood that the error may be plus or minus from the true values and thus the error quantities themselves are defined as real numbers. With some algebra, the terms in (5) can be re-arranged:

$$\%\ \text{Recovery} = \frac{(w_O - t_O)h_O + (w'_O - t'_O)h_O + (w_O - t_O)h'_O + (w'_O - t'_O)h'_O}{(w_R - t_R)h_R + (w'_R - t'_R)h_R + (w_R - t_R)h'_R + (w'_R - t'_R)h'_R} \quad (6)$$

Equation (6) shows that the % Recovery calculation is comprised of three terms: a term derived from the measured values, a term arising from the errors in measurement and a mixed term that depends both on the measured values and the errors in measurement.

Figure 1: Classification of terms in % recovery calculation.

Because there are mixed terms in Equation (6), it is not possible to obtain an absolute estimate of experimental error; the weight and the hematocrit of the product pre- and post-wash influence the percent recovery calculation and so no absolute error can be calculated analytically.

However, it is possible to provide an estimate of the *average* magnitude of error that might be expected by assuming typical values for the required parameters. See Table 1 for data used in this analysis, which was obtained from a sample of washed red blood cells at a Canadian Blood Services production centre.

3.2 Data

Table 1: Values for % Recovery Calculation.

Parameter	Assumed Value	Source
w_O	351.375 g	Sample of 8 washes from collection centre A
t_O	89 g	Assumed tare weight of output bag
h_O	0.7820	Sample of 8 washes from collection centre A
w_R	401.75	Sample of 8 washes from collection centre A
t_R	35	Assumed tare weight of collection bag
h_R	0.6589	Sample of 8 washes from collection centre A
w'_O	+/- 1gm	Accuracy of scale
t'_O	+/-1 gm	Accuracy of scale
h'_O	+/- 0.006	Based on a sample of 20 washes.
w'_R	+/- 1gm	Accuracy of scale
t'_R	+/-1 gm	Accuracy of scale
h'_R	+/- 0.012	Based on a sample of 20 washes.

3.3 Sensitivity Analysis

The value of h_O listed in Table 1 is derived from sample wash data provided by the collection centre A. However, if one were to assume 100% recovery, the necessary hematocrit for the output product can be calculated as follows. If 100% recovery is achieved, then:

$$(w_O - t_O)h_O = (w_R - t_R)h_R \quad (7)$$

and thus, the h_O that would be necessary to achieve 100% recovery can be calculated as:

$$h_O^{Perfect} = \frac{(w_R - t_R)h_R}{(w_O - t_O)} \quad (8)$$

For example, using the average values listed in Table 1, the $h_O^{Perfect}$ that would be associated with 100% recovery can be calculated as 0.9210.

To calculate the maximum value that could be observed in Equation (6), given the typical values listed in Table 1 it is assumed that the error terms listed in the equation take on the signs listed in Table

2. The resulting values of % recovery can be found in Table 3.

Table 2: Error Sign Necessary to Maximize % Recovery Calculation.

Error Parameter	Sign
w'_O	+
t'_O	-
h'_O	+
w'_R	-
t'_R	+
h'_R	-

Table 3: Expected and Maximum Values of Percent Recovery Calculations.

	Assume h_O from Data	Assume $h_O^{Perfect}$
Expected value	84.91%	100.00%
Maximum value	89.81%	105.57%
Difference	4.91%	5.57%

From Table 3 it can be observed that the difference between the expected value of the calculation (i.e., the value if all errors are 0) and the maximum value of the calculation (i.e., the value if all errors contribute towards maximizing Equation (6)) is between 4.91% and 5.57%. Of course, this value depends on the actual weights of the products pre- and post-wash. To give an idea of the range of potential difference between expected and maximum values in the percent recovery calculation, a sensitivity analysis was conducted on the assumed product weight and hematocrit values used in the calculation. See Tables 4 and 5.

Table 4: Sensitivity Analysis Product Weight.

	Product Weight -20%		Product Weight +20%	
	Assume h_O	Assume $h_O^{Perfect}$	Assume h_O	Assume $h_O^{Perfect}$
Expected value	79.61%	100.00%	88.30%	100.00%
Maximum value	84.57%	105.95%	93.16%	105.35%
Difference	4.96%	5.95%	4.86%	5.35%

Table 5: Sensitivity Analysis Product Hematocrit.

	Product Hematocrit -20%		Product Hematocrit +20%	
	Assume h_O	Assume $h_O^{Perfect}$	Assume h_O	Assume $h_O^{Perfect}$
Expected value	84.91%	100.00%	84.91%	100.00%
Maximum value	90.80%	106.68%	89.17%	104.84%
Difference	5.89%	6.68%	4.26%	4.84%

From Tables 3-5 it may be observed that the maximum error in the percent recovery ranges from 4.26% to 5.95% across the sensitivity analysis. Error, moreover, increases inversely to increases in both product weight and product hematocrit. Thus, the smaller the value of either weight or hematocrit, the larger the potential for error in the calculation. Finally, it should be noted that the percent recovery calculation is more sensitive to errors in the determination of hematocrit than product weight.

Measurement error for percent recovery is normally distributed, since repeated measurements made of the same quantity are, by definition, normally distributed (Miller & Miller, 1988). Further, for the maximum errors as listed in Tables 3-5 to be observed it is necessary that all errors be in the correct direction and at their extreme values at the same time. If one assumes that individual errors are independent of one another, the probability of seeing all errors at their extreme value and with the requisite sign to maximize the total error is unlikely, but calculable under the assumption of normality. Thus, while the maximum values listed in Tables 3-5 are *possible*, they may not be *likely* values. To estimate the likely range of values that could be observed, given the typical values assumed in Table 1, a simulation reproducing the measurement process was run. The simulation assumes that that errors in weight measurement (w_O', w_R', t_O', t_R', h_O', h_R') are uniformly distributed since these values are dependent on the accuracy of the equipment used to take measures. The magnitude of the errors in measurement of weight was assumed to be +/-1 gram, based on the accuracy of the scales (i.e. the number of significant digits displayed by the equipment). Hematocrit errors were calculated from sample data such that the observed error would have a mean of 0 and a standard deviation that would yield a coefficient of variation (CV=σ/μ) equal to 0.0080 for h_R' and 0.0186 for h_O'. The estimates of coefficient of variation were derived from a sample of 20 washes using the standard operating procedure. Since hematocrit error is related to the product mass, coefficient of variation, rather than standard deviation is used for simulation calculations.

Based on an average hematocrit of 0.6589 for a pre-washed unit and 0.7820 for a post-washed unit, error estimates of 0.012 for the pre-wash measurement and 0.0063 for the post-wash measurement can be calculated. Using these values, a simulation was then executed for a total of 500,000 replications and the resulting percent recovery was recorded. The simulation yielded the following results:

Table 6: Simulation Output.

Maximum % Recovery	88.04%
Average % Recovery	84.91%
Standard Deviation	1.03%

The simulation validates the maximum error calculations made in Table 3-5 (i.e., the range is ~ 85% +/-3%). However, the simulation also provides information on the likelihood of seeing extreme values. Since errors from repeated measurements are normally distributed and the observed standard deviation over the 500,000 replications of the simulation was 1.03%, the likelihood of seeing an error of a particular magnitude can be estimated from the properties of a normal distribution as follows:

Table 7: Probability of Observing Error of a Particular Size.

Magnitude of Error	Probability Error <= Value	Probability Error >= Value
1.03%	84.134%	15.866%
1.69%	95.002%	4.998%
2.06%	97.725%	2.275%
3.09%	99.865%	0.135%
4.12%	99.997%	0.003%

Accordingly, it may be seen that while the maximum error possible could be as large as 5.57%, error values exceeding +/- 1.69% are unlikely. Thus, calculated percent recovery calculations exceeding 101.69% are not likely to be due to random fluctuations in measurement and other sources of error should be suspected in such situations.

It is also possible to extrapolate from Table 7 a lower tolerance limit for the percent recovery calculation. Since current quality standards dictate that the amount of recovered red cells in the output product must be ≥ 75% of the cells in the input product, there may be an advantage in setting a lower tolerance level for the wash process above 75%. Doing so would reduce the likelihood that an unacceptable unit would incorrectly be assumed to meet the quality standard. Consider, for instance, a unit that is found to have exactly 75% recovery, post-wash. Based on the assumption that errors are normally distributed, there is only a 50% chance that the unit actually achieves the quality standard and thus a 50% chance that the unit will be incorrectly labelled as positive proof of the quality standard. (A 95% prediction interval would suggest a true range between 72.7% and 77.9%, with 50% of all observations falling below the nominal target threshold.) Accordingly, if the minimum observation for declaring a sample acceptable were to be increased, a corresponding decrease in false positives could be expected. Table 8 shows the expected probability of a false positive for a given measurement of percent recovery, under the assumption of a measurement process with a normally distributed error of N(0,0.0103). For instance, if the nominal QC cut-off value was increased to 77.78%, only 2.3% of the completed units would fail to have the requisite minimum requirement of 75% of the pre-wash cells preserved through the washing process.

Table 8: Probability of False Positive, Given a Measured % Recovery.

Measured % Recovery	Probability of False Positive
75.0%	50.0%
75.93%	15.9%
76.53%	5.0%
77.78%	2.3%
78.81%	0.1%

4 CONCLUSION

Errors in the accuracy of the equipment used to measure the necessary parameters to estimate percent recovery in washed red cells can reasonably give rise to calculated values more than 100%. It was determined that no absolute figure for accuracy could be given that would be applicable in all cases, since the error terms in the calculation interact with both the product weight and hematocrit of the pre- and post-wash products. However, using average values obtained from a sample of data provided by collection centre A, it was determined that values between 4.91% and 5.57% more than the true value of percent recovery are possible, given the accuracy of the scales and cell analyzers used to estimate parameters. Thus, it is possible that all calculated values of percent recovery less than 105.57% *could* potentially be valid. However, since process errors are normally distributed in aggregate, the more the value deviates from the expected value, the lower the likelihood of the event being truly due to error in machine accuracy and the greater the probability that other factors (i.e., process or operator error) may be involved. Using a simulation to estimate the likely range of errors it was noted that all calculated values of percent recovery in washed red blood cells exceeding 101.69% should be

regarded as suspicious. Similarly, it was determined that, if QC minimums were increased to 77.78%, errors in processes that provided less than a 75% yield could be identified more often. See Figure 2 for a diagram of the acceptable bounds for percent recovery calculations.

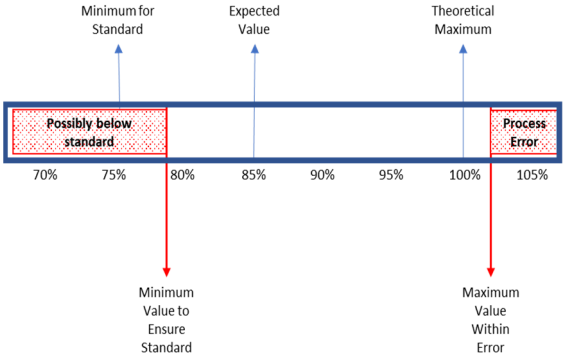

Figure 2: Process limits derived from the simulation and the analytical results.

We conclude by noting that information taken from this study was used to inform standard operating procedures used at Canadian Blood Services when conducting quality control audits for washed red cells.

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