EVALUATION OF PARAMETERS AFFECTING BOVINE BLOOD HEMOLYSIS TESTING

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INTRODUCTION

Evaluation of blood damage caused by medical devices is a critical parameter needed to determine the safety of medical devices submitted to the FDA for approval. Determination of red blood cell damage, or hemolysis, is done by measurement of the amount of hemoglobin released from red blood cells into the plasma, or plasma free hemoglobin (PFHg, mg/dL). Our goals for this work were to 1) evaluate the variability of bovine blood fragility by mechanical testing, 2) evaluate how well a bench-top hemolysis test might be used to predict pump flow hemolysis and 3) evaluate the usefulness of a historical equation used for normalizing blood damage level (the Normalized Index of Hemolysis, NIH).

MATERIALS AND METHODS

NIH equation

The NIH equation has been derived as a means to normalize parameters known to affect blood damage [1]. The equation has the following form:

$$NIH = \left(\frac{\Delta PFHg}{\Delta t}\right) \bullet \frac{(1 - Ht/100)\cdot V}{\dot{Q}} \quad \text{[mg/dL]} \qquad (1)$$

where $\Delta PFHg$ is the change in plasma free hemoglobin (mg/dL), Δt is the change in time (min), Ht is the hematocrit (%), V is the volume (L), and $\dot{Q}$ is the flow rate (LPM). In order to evaluate the validity of the NIH equation, each parameter was varied independently to determine the individual effect on PFHg during pump blood testing. This was achieved by using the following form of the NIH equation:

$$Slope = \frac{\Delta PFHg}{\Delta t} \propto \frac{\dot{Q}}{(1 - Ht/100)\cdot V} \quad \text{[mg/dL/min]} \qquad (2)$$

Pump testing

A clinical roller pump (Stöckert, Sorin Biomedical Inc.) was used for all pump testing in identical circuits (1/4" medical grade tubing). Each circuit consisted of: pressure monitors upstream and downstream of the test section (plate clamp or orifice), ultrasonic flow probe, a blood reservoir bag covered with a cooling blanket connected to a heat exchanger, and an inline temperature probe (see Figure 1). The plate clamp (tapered entrance/exit model) and orifice (sudden contraction model) have pressure drops (ΔP) in bovine blood of ~300 mmHg and ~500 mmHg, respectively measured at 3 LPM and 35% Ht.

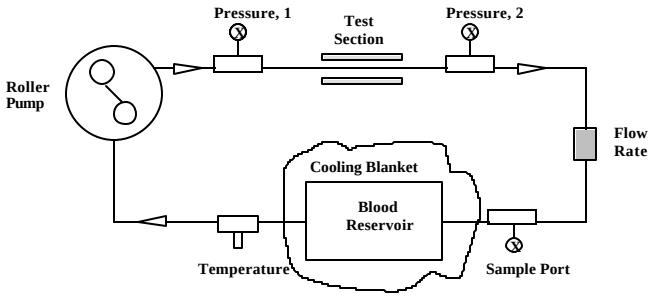

Figure1. Standard Pump Test Loop

The following standard test methods were followed [2]:

(1) Bovine blood was collected from a local slaughterhouse using a custom large bore needle and 2L blood collection bag filled with heparin/phosphate buffered saline (PBS) solution. This blood was considered to be at the Native Ht.

(2) All pump testing was performed on fresh filtered blood.

(3) The standard pump test condition set the following parameters: $\dot{Q}$ = 3 LPM, Temperature = 25 ± 2 degrees C, Volume = 350 mL, and Ht = 35% (PBS dilution).

(4) Using blood from one cow on each test day, one parameter from the NIH equation ($\dot{Q}$, V, or Ht) was tested through a range of values by pumping fresh undamaged blood through the circuit for one hour at each parameter set point. Typical parameter set points were: $\dot{Q}$ (LPM) = 0.25, 0.7, 1.5, 2.25, 3; Ht (%) = 20, 25, 30, 35, Native; V (mL) = 150, 250, 350, 500, 1000.

(5) Triplicate blood samples were drawn from the pump loop at the sample port both at the start (baseline) and end of each hour test period. One sample was used for measuring Ht, glucose and blood gases and was then discarded. The remaining two samples were refrigerated for the remainder of the testing that day.
(6) The duplicate blood samples were centrifuged twice at ~1025 relative centrifugal force (RCF) for 20 minutes and the supernatant plasma was drawn off after each centrifugation. All samples were frozen at –80 degrees C for subsequent analysis of PFHg.

Rocker bead testing

Our standard bench-top rocker bead test is a modification of a test procedure used by Dr. Marina Kameneva at the University of Pittsburgh. Our test was done on both undiluted Native Ht and 35% Ht PBS diluted blood using the following test procedure:

(1) 8mL polystyrene test tubes were filled with eight 1/4" diameter stainless steel beads and 3 mL of filtered blood was added to each of 8 vials.
(2) 5 test tubes were rocked for one hour at room temperature on a standard test tube rocker with an angle of inclination of 24 degrees at 20 RPM.
(3) 3 test tubes were kept in a static condition in a test tube rack.
(4) All test tubes were centrifuged twice at ~1025 RCF for 20 minutes, the supernatant plasma was drawn off, and all samples were frozen at –80 degrees C.
(5) $\Delta PFHg$ was determined from the rocked minus static value.

Determination of PFHg and plasma protein

After thawing to room temperature and centrifugation, PFHg was determined from measurement of supernatant absorbance with a spectrophotometer using the Cripps method [3]. Plasma protein level was determined using a clinical refractometer.

RESULTS

Bovine population variables were: Age = 18.11 ± 2.4 months, N=18; Sex = 3 Females, 15 Males; Native Ht = 38.75 ± 2.6 %, N=20; 35% Ht dilution = 34.99 ± .53 %, N=15; Glucose (35% Ht) = 49.88 ± 11.08 mg/dL, N=16; Glucose (Native Ht) = 56.22 ± 17.49 mg/dL; Plasma Protein (35% Ht) = 5.09 ± .44 g/dL, N=15; Plasma Protein (Native Ht) = 6.16 ± .48 g/dL, N=16.

We found the variability between cows was rather low as evidenced by data from our standard tests done both in the pump (plate clamp) and the rocker bead models (see Figure 2). Comparison between rocker and pump $\Delta PFHg$ showed no correlation ($r^2 = 0.002$, N = 14). Results for each parameter's affect on the slope (Equation 2) for both the plate clamp and orifice (sudden contraction) are shown in Figure 3. In order to correct for the variation of total hemoglobin concentration with Ht, the parameter $Ht/(1-Ht)$ was added to the results in Figure 3 [4]. Additional tests were performed to determine the relationship between pressure drop and flow rate for the plate clamp, $\Delta P \propto \dot{Q}^{1.36} (r^2 = 0.995)$, and the orifice (sudden contraction), $\Delta P \propto \dot{Q}^{1.94} (r^2 = 0.999)$, in 35% Ht bovine blood.

DISCUSSION

A series of blood pump and rocker bead tests were performed to evaluate the variability of bovine blood fragility by mechanical testing. We found that when pump testing is rigorously controlled, the variability in blood damage level among cows of ~ 26% CV is obtainable. Testing was also performed to evaluate how well a bench-top test (rocker bead test) predicts pump flow hemolysis. Although the rocker $\Delta PFHg$ did not correlate with pump $\Delta PFHg$, the rocker data provides a reproducible measure of blood mechanical fragility (CV = 16 % per animal). Bovine blood pump testing was performed to evaluate the usefulness of a historical equation (NIH) used for normalizing blood damage level. The NIH equation was valid for flow rate and volume parameters in the plate clamp. However, the utility of the NIH equation for the orifice (sudden contraction) is less apparent. The flow rate effect seems to be consistent with ΔP versus $\dot{Q}$ trends for the two devices tested in bovine blood. It is evident that Ht is not effectively accounted for in the NIH equation, and therefore a parameter such as $Ht/(1-Ht)$ must be used to account for variation in total hemoglobin content with Ht.

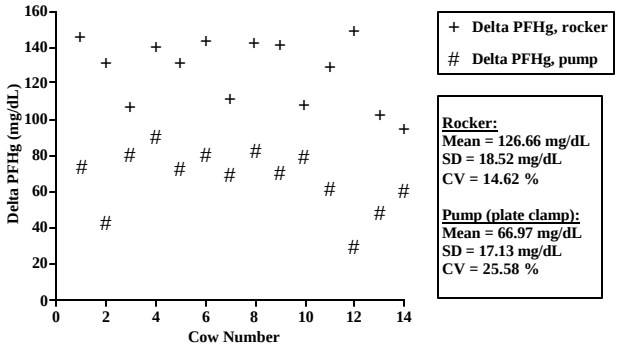

Figure 2. Variability of Pump (Plate Clamp) and Rocker Bead Data for 14 Cows

Plate Clamp	Orifice
$Slope \propto \dot{Q}^{1} (r^2 = 0.97)$	$Slope \propto \dot{Q}^{1.78} (r^2 = 0.87)$
$Slope \propto (1-Ht)^{-4.30} (r^2 = 0.61)$	$Slope \propto (1-Ht)^{-10.26} (r^2 = 0.88)$
$Slope \propto V^{-0.977} (r^2 = 0.93)$	$Slope \propto V^{-0.773} (r^2 = 0.83)$
$Slope \propto \left(\frac{Ht}{(1-Ht)}\right)^{1.34} (r^2 = 0.65)$	$Slope \propto \left(\frac{Ht}{(1-Ht)}\right)^{3.00} (r^2 = 0.84)$

Ideal NIH: $Slope \propto \dot{Q}^{1}$, $\propto (1-Ht)^{-1}$, $\propto V^{-1}$

Figure 3. NIH Equation Slope as a Function of Test Parameters (2 Cows per Parameter per Test Device)

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