Assessment of Factors Impacting Material-mediated Hemolysis Results in the ASTM F756-17 Testing Standard

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Statement of Purpose: Blood-contacting medical devices, such as vascular stents, hemodialyzers, and circulatory assist pumps, are vital for sustaining patients. To ensure the safety of these devices, FDA collaborates with manufacturers and testing labs to develop standardized methods for device hemocompatibility testing. The goal of this project is to assess factors to improve the in vitro evaluation of material-mediated hemolysis (damage to red blood cells) caused by medical devices, based on procedures detailed in ASTM F756-17 (*Standard Practice for the Assessment of Hemolytic Properties of Materials)*. The study included evaluation of possible new positive control materials, reduced test fluid volume (8 mL to 2 mL) to use less test material (same surface area to volume ratio), and test incubation time.

Methods: Citrate-anticoagulated rabbit blood pooled from 3 donors was diluted to a total hemoglobin concentration of 10 mg/mL using calcium and magnesium-free phosphate buffered saline (CMF-PBS). For the 8 mL fluid volume testing, 1 mL diluted blood and 7 mL CMF-PBS were added to each test tube. The same blood to CMF-PBS volume ratio was used for the 2 mL testing. Solid test materials (Table 1) prepared in triplicate were added to the test tubes at a surface area to CMF-PBS volume ratio of 3 or 6 cm^2/mL (depending on material thickness per ASTM F756-17). For dimethyl sulfoxide (DMSO), a stock solution was diluted with CMF-PBS to the specified concentration(s) prior to the addition of diluted rabbit blood. Samples were incubated at 37°C for 3 hrs with 3 gentle inversions every 0.5 hrs. Treated blood was transferred to a new tube, centrifuged, and the supernatant assayed for free hemoglobin using a cyanmethemoglobin reagent and comparison to a standard curve established at a 540 nm absorbance wavelength.

Table 1: Test materials used in the hemolysis evaluation

Material	Justification
High-density Polyethylene (HDPE)	Negative control material (ASTM F756-17)
Buna-N (Nitrile Rubber)	Positive control material (ASTM F756-17)
Nitrile Gloves (NG) from 3 sources	Used as positive control materials by some testing labs
Latex	Possible positive control material
Dimethyl Sulfoxide (DMSO)	Possible positive control liquid

% Hemolysis was calculated as follows:

$$\%\ \text{Hemolysis} = \frac{\text{Supernatant free hemoglobin}}{\text{Total hemoglobin}} \times 100\%$$

Results: The average %hemolysis was consistently less than 1% for the negative control HDPE, 5% to 89% for NG materials (varying by brand), and 4-7% for Latex and Buna-N rubbers (Fig. 1a). Reducing the test fluid volume and the corresponding test material by a factor of 4 did not significantly change the %hemolysis results for any sample type (Fig. 1a). The %hemolysis increased with increasing concentration of DMSO (Fig. 1b), and over the 3-hr test duration for all tested materials (Fig. 1c).

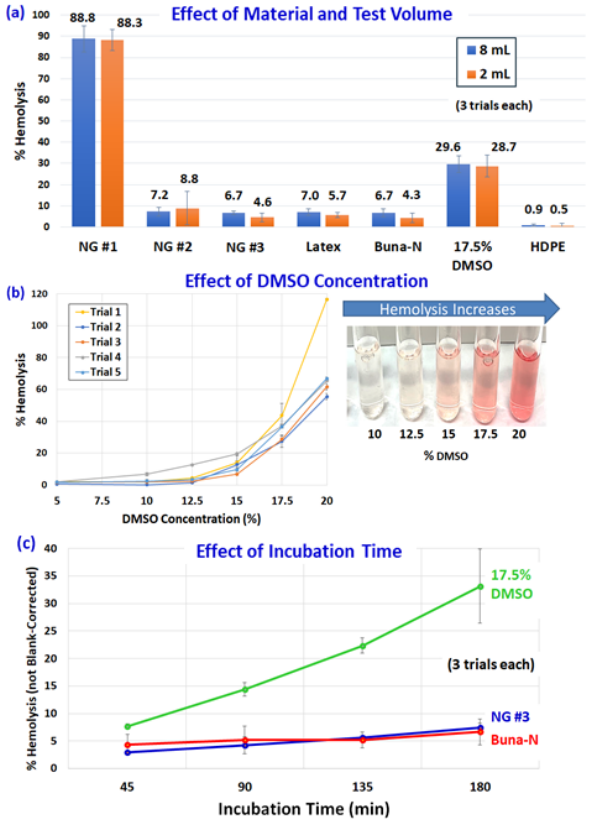

Figure 1. Impact on %hemolysis for (a) test materials and fluid volume, (b) DMSO concentration, and (c) sample incubation duration (for 3 test materials).

Conclusions: HDPE met the ASTM F756-17 criterion as a negative control material as it reproducibly caused < 2% hemolysis, while only NG#1 and DMSO (≥ 15%) were suitable positive controls consistently causing >5% hemolysis. Importantly, reducing the required test sample volume by a factor of 4 will allow less device materials to be used in the test without impacting the hemolysis results. However, incubation of materials with blood should remain at the standard specified duration of 3 hrs.

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