Effect of Test Parameters on Material-mediated Hemolysis Using the ASTM F756-17 Standard Test Method

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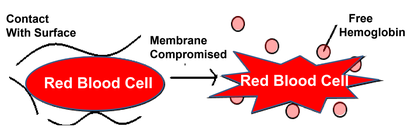
Abstract

Background: To ensure the safety of blood-contacting medical devices (e.g., catheters, blood pumps), FDA works with industry to develop standard procedures for pre-market hemocompatibility testing. **Purpose**: To improve the ASTM F756-17 testing standard for assessing damage to red blood cells (hemolysis) by device materials, we investigated the impact of multiple test parameters: positive control materials, test volume (8 mL vs 2 mL), and incubation time. **Methods**: Rabbit blood pooled from 3 donors was diluted with Ca/Mg-free phosphate buffered saline (CMF-PBS) to a total hemoglobin concentration of 125 mg/dL in vials containing materials. Test materials, including possible positive (nitrile gloves, Buna-N rubber, latex) and negative controls (high-density polyethylene [HDPE]), at a surface area to CMF-PBS volume ratio of 3 or 6 cm^2/mL (based on material thickness per ASTM F756) were exposed to either 2 or 8 mL of the diluted blood and tested in triplicate. Dimethyl sulfoxide (DMSO) at various concentrations in CMF-PBS was also tested with diluted blood. Vials were incubated at 37°C for up to 3 hrs. Treated blood was centrifuged and the supernatant assayed for free hemoglobin released from damaged red blood cells using a cyanmethemoglobin reagent and spectrophotometer. **Results**: Average %hemolysis was <1% for the negative control HDPE, 5% to 89% for nitrile gloves (varying by brand), and 4-7% for Latex and Buna-N rubbers. Reducing the test fluid volume and material size by a factor of 4 did not significantly change the %hemolysis results for any sample type. The %hemolysis increased with increasing concentration of DMSO and test incubation time for all tested materials. **Conclusions**: HDPE met the ASTM F756 criterion as a negative control material as it reproducibly caused <2% hemolysis, while only DMSO (at concentration ≥ 15%) and one brand of nitrile glove were suitable positive controls consistently causing >5% hemolysis. Importantly, reducing the test sample volume by a factor of 4 allows less material to be required in the test without impacting the results. As hemolysis is time-dependent, incubation should adhere to the standard specified duration of 3 hrs. **These study results will help to revise the ASTM F756-17 testing standard.**

Introduction

- Hemolytic materials in blood-contacting devices can lead to hemoglobin being released from compromised red blood cell membranes (***Figure 1***).



- Free, unbound hemoglobin is vulnerable to oxidation reactions that can cause vascular and renal damage.
- The ASTM F756-17 standard has been used for over 20 years to determine the hemolytic potential (% hemolysis) of various materials by quantifying released supernatant hemoglobin.

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

- As medical device manufacturers and testing labs sometimes deviate from the standard ASTM F756-17 protocol, variations to the test methods are being assessed to determine factors that may impact the hemolysis levels and to improve the standard.

Materials and Methods

ASTM F756-17 Standard Protocol

Blood and test article preparation:

- Citrate anticoagulated rabbit blood pooled equally from 3 donors was diluted to 10 mg/mL total hemoglobin using calcium and magnesium-free phosphate buffered saline (CMF-PBS).
- Solid test articles with a surface area of 42 cm^2 or 21 cm^2 (depending on thickness) were dip-rinsed in PBS before being added to test tubes.
- For DMSO, stock solution was diluted with PBS and added to test tubes.
- 7 mL of PBS and 1 mL of diluted rabbit blood were pipetted into test tubes.

***Table 1. Test* Materials**

Nitrile Gloves (NG) from 4 manufacturers
Buna-N (Nitrile Rubber)
Latex Sheet
DMSO (Dimethyl Sulfoxide)
HDPE (High-density Polyethylene)

Incubation and sample collection:

- Samples incubated in water bath at 37 °C for 3 hrs with 3 gentle inversions every 0.5 hrs. Afterwards, treated blood samples centrifuged and supernatant collected.

Analysis:

- Cyanmethemoglobin method was used by diluting supernatant with Drabkin's reagent.
- Absorbance measured on plate reader at 540 nm.
- A hemoglobin concentration calibration curve was used to quantify the free hemoglobin in the supernatant.

Figure 2. *Test materials in 8 mL standard volume. (Left to right: NG, Buna-N, HDPE)*

Variations to Standard Testing Protocol

- *Effect of PBS/blood volume reduction:* Reduced the PBS/blood volume and surface area of test articles by a factor of 4 (i.e., 8 ml to 2 ml).
- *Time course of hemolysis:* Measured hemolysis in 45-min increments up to 3 hrs.
- *Effect of glove sample washing:* Washed NG in ultrapure water at 60 RPM, 37 °C for 48 hrs (changed water at 24 hrs).

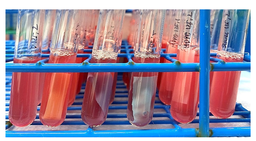


Figure 3. *Test materials in 2 mL reduced volume testing. (Left to right: DMSO, latex, NG #4, NG #2, HDPE, CMF-PBS).*

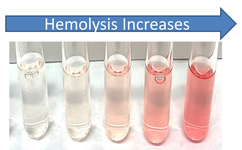


Figure 4. *Supernatant containing free hemoglobin after exposure to DMSO at various concentrations (Left to right: 10%, 12.5%, 15%, 17.5%, 20%).*

Results and Discussion

Positive Control Identification

According to ASTM F756-17, positive controls should reproducibly cause >5% hemolysis, while negative controls should cause <2% hemolysis. Different brands of NG produced different levels of hemolysis. Increasing concentration of DMSO caused corresponding increases in % hemolysis. HDPE was a consistent negative control material producing <2% hemolysis in all test conditions.

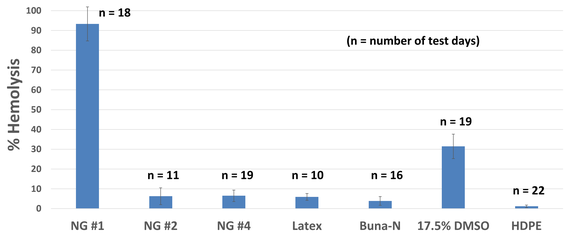


Figure 5. *%Hemolysis comparison for all tested materials. Samples tested in triplicate on each day.*

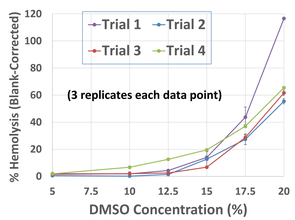


Figure 6. *%Hemolysis for different DMSO concentrations.*

Table 2. *Summary of material %hemolysis (mean ± SD).*

Material	% Hemolysis
NG #1	93.3 ± 8.6
NG #2	6.3 ± 4.2
NG #4	6.5 ± 2.9
Latex	5.9 ± 1.7
Buna-N	3.9 ± 2.2
17.5% DMSO	31.4 ± 6.2
HDPE	1.2 ± 0.6

Effect of CMF-PBS/Blood Volume Reduction

No significant difference in %Hemolysis test results for any material (p>0.05) when reducing the CMF-PBS/blood volume by a factor of 4.

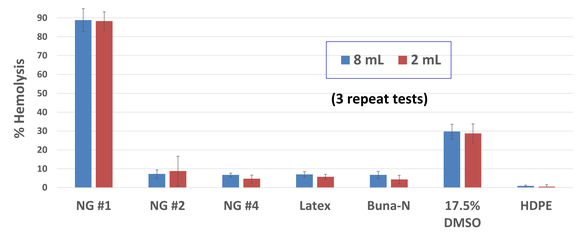


Figure 7. *Comparison of %hemolysis when using 8 mL (standard) versus 2 mL (reduced) CMF-PBS/ blood volume.*

Effect of Incubation Time

Rates of hemolysis varied for different materials tested, which supports the 3-hr study endpoint in the standard.

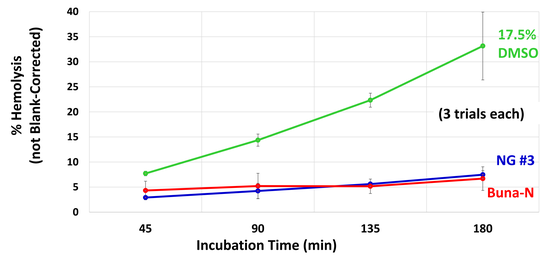


Figure 8. *Non-blank-corrected %hemolysis at 45-min increments during incubation (average from 3 separate trials). Each material was tested in 3 replicates at the 3-hr endpoint and 2 replicates at all other timepoints.*

Effect of Washing Nitrile Gloves

Hemolysis caused by washed nitrile gloves was lower compared to their unwashed counterparts.

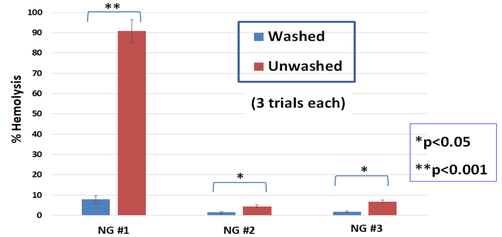


Figure 9. *%Hemolysis comparison for washed and unwashed NG.*

Conclusions

- HDPE was a consistent negative control (%hemolysis < 2%) for all conditions. Only DMSO (at concentration ≥ 15%) and one nitrile glove (NG #1) were suitable positive controls consistently causing >5% hemolysis.
- Use of reduced CMF-PBS/Blood volume of 2 mL (instead of standard 8 mL) did not impact the hemolysis results and could benefit manufacturers by decreasing the amount of device materials required for the test.
- As hemolysis is time-dependent, incubation should adhere to the standard specified duration of 3 hrs.
- Hemolysis caused by nitrile gloves is likely due to water-soluble chemicals, since washed gloves had lower hemolysis compared to unwashed ones.

Acknowledgement

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