Nelson Labs
A Sotera Health company

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ASTM Hemolysis (Extract Method) Final Report

Test Article:	Coated pannel
Purchase Order:	ZB-PO-6955
Study Number:	1619266-S01
Study Received Date:	01 Jun 2023
Test Started Date:	08 Jun 2023
Test Finished Date:	13 Jun 2023
Testing Facility:	Nelson Laboratories, LLC 6280 S. Redwood Rd. Salt Lake City, UT 84123 U.S.A.
Test Procedure(s):	Standard Test Protocol (STP) Number: STP0093 Rev 16
Deviation(s):	None

Summary: The difference between the hemolytic indexes of the test article and the negative control equals 0.31 percent. This places the test article in the non-hemolytic range according to the grade outlined below.

Hemolytic Index and Grade:

Hemolytic Index	Hemolytic Grade
0-2	Non-Hemolytic
2-5	Slightly Hemolytic
>5	Hemolytic

All test method acceptance criteria were met. Testing was performed in compliance with US FDA good manufacturing practice (GMP) regulations 21 CFR Parts 210, 211 and 820. The test procedure(s) listed above were followed without deviation.

Alyssa Staley electronically approved	15 Jun 2023 22:41 (+00:00)
Study Director Alyssa Staley	Study Completion Date and Time

Results:

Test Article/Control	Optical Density	Average Optical Density	Hemolytic Index	Average Hemolytic Index (% Hemolysis)	Corrected Hemolytic Index (% Hemolysis)
Test Article	0.003	0.003	0.833	0.91	0.31
	0.004		1.068		
	0.003		0.833		
Negative Control	0.003	0.002	0.833	0.60	0.00
	0.002		0.597		
	0.001		0.361		
Positive Control	0.051	0.307	12.148	72.4	71.8
	0.458		108.094		
	0.411		97.014		
Phosphate Buffered Saline (PBS) Blank	0.002	0.002	0.597	0.60	N/A
	0.002		0.597		
	0.002		0.597		

Hemoglobin Standard:

Regression Output	
Constant	0.00074
Standard Error of Y Estimate	0.00534
R^2	0.99970
Degrees of Freedom	6
X Coefficient(s)	1.39554
Standard Error of Coefficient	0.00993

Hemoglobin Totals	
Total Plasma Free Hemoglobin	0.322 mg/mL
Total Final Hemoglobin Present	9.472 mg/mL

Test Method Acceptance Criteria: The negative control must produce a corrected hemolytic index of less than 2%. The positive control must produce a corrected hemolytic index of greater than 5% above the negative control.

Test Article Preparation: The test article tested did not include the product packaging. The amount of material tested was based on both ASTM and ISO surface area recommendations or by weight.

Extract Solvent	Extraction Ratio	Test Article/ Extraction Solvent Amount (Per Extraction)	Extraction Parameters	
			Temperature	Time
PBS	6 cm^2/mL	1) 203.06 cm^2/33.8 mL 2) 201.44 cm^2/33.6 mL 3) 208.85 cm^2/34.8 mL	37 ± 1°C	72 ± 2 hours
			With Agitation	

The extract fluid was held at room temperature for less than four hours before testing. The extract fluids were not filtered, diluted, centrifuged or manipulated in any way following the extraction process.

Test Article Post Extract Appearance	
Post extract	No Changes

Pre and Post Extract Appearance		
Test Article(s)	Pre extract	Clear with no particulates present
	Post extract	Clear with no particulates present No color change noted
Controls	Pre extract	Clear with no particulates present
	Post extract	Clear with no particulates present No color change noted

Procedure: The procedure follows the principles outlined in ASTM F 756. The PBS used in testing was calcium and magnesium free. The ASTM method has been validated using human blood, which is in compliance with ISO 10993-4, which states that due to differences in blood activity, human blood should be used where possible.

Blood Draw: An equal amount of blood from 3 donors was drawn into vacutainers containing 0.1 M sodium citrate at a ratio of 9:1 (3.2% anticoagulant to blood). The collected blood was refrigerated until testing was performed. The blood was pooled and used in testing within four hours of the draw.

Hemoglobin Standard: A hemoglobin standard was diluted with Drabkin's reagent to give solutions at concentrations of 0.80, 0.60, 0.40, 0.30, 0.20, 0.10, 0.02, and 0.01 mg/mL. The solutions were allowed to stand at room temperature for a minimum of 15 minutes. The absorbance was read on a spectrophotometer at 540 nanometers (nm). A standard curve was determined using linear regression with the absorbance values and the standard concentrations of hemoglobin.

Plasma Hemoglobin Determination: The blood was centrifuged at 700-800 x g for 15 minutes. A 1 mL aliquot of the plasma was added into 1 mL of Drabkin's reagent and placed at room temperature for a minimum of 15 minutes. The absorbance was read on a spectrophotometer at 540 nm. The hemoglobin concentration was determined from the standard curve and then multiplied by a factor of 2 to obtain the plasma free hemoglobin concentration. The plasma free hemoglobin concentration was less than 2 mg/mL.

Hemoglobin Present and Dilution: The total amount of hemoglobin present was determined by adding a 20 µL aliquot of the blood to 5 mL of Drabkin's reagent, in duplicate, and allowing the solution to stand at room temperature for a minimum of 15 minutes. The absorbance was read on a spectrophotometer at 540 nm. The hemoglobin concentration was determined from the standard curve and then multiplied by a factor of 251 to account for the dilution.

Based on the total hemoglobin present, the blood was diluted to 10 ± 1 mg/mL in PBS. To verify the blood dilution, a 300 µL aliquot of the diluted blood was added to 4.5 mL of Drabkin's reagent in triplicate and allowed to stand at room temperature for a minimum of 15 minutes. The absorbance was read on a spectrophotometer at 540 nm. The hemoglobin concentration was determined from the standard curve and then multiplied by a factor of 16 to account for the dilution.

Controls: A non-hemolytic negative control, a hemolytic positive control and a PBS blank were included and extracted at the same time and temperature as the test articles. Three of each control and the PBS blank were prepared. Refer to the test article preparation table for extraction details.

Incubation: Glass test tubes were labeled appropriately. To each test tube, 7 mL of each test article, control or PBS blank extract, and 1 mL of diluted blood was added. The tubes were then incubated at 37 ± 2°C for a minimum of 3 hours. The tubes were gently inverted twice at 30 minute intervals throughout the incubation period.

Centrifugation and Calculations: After incubation, the test articles and controls were centrifuged at 700-800 x *g* for 15 minutes and 1 mL of the supernatant fluid was combined with 1 mL of Drabkin's reagent and allowed to stand at room temperature for a minimum of 15 minutes. Following the centrifugation phase, the test article, the PBS blank and the negative control supernatant visually appeared clear and were particulate free. The supernatant of the positive control visually appeared red and were particulate free. The test articles and controls were then read at 540 nm in a spectrophotometer.

The Hemolytic index (Percent Hemolysis) was interpreted using the following equation:

$$\text{Hemolytic Index} = \frac{\text{Hemoglobin Released (mg/mL)}}{\text{Hemoglobin Present (mg/mL)}} \times 100$$

Where: Hemoglobin Released (mg/mL) = (Optical Density x X Coefficient + Constant) x 16
Hemoglobin Present (mg/mL) = Diluted Blood 10 ± 1 mg/mL

The corrected hemolytic index was calculated by subtracting the hemolytic index of the PBS blank solution from the hemolytic index of the test article and controls.

The test article is compared to the negative control by subtracting the hemolytic index of the negative control from the hemolytic index of the test article.

Test Parameters:

Blood Type Used:	Human, Citrated
Positive Control:	Nitrile Glove Material, tested at 3 cm^2/mL
Negative Control:	Polypropylene Pellets tested at 0.2 grams/mL
Total Hemoglobin Kit:	Pointe Scientific
Incubation Time:	Minimum of 3 hours
Incubation Temperature:	37 ± 2°C

References:

ASTM F756-17, 2017, *Standard Practice for Assessment of Hemolytic Properties of Materials*. ASTM International, West Conshohocken, PA

ISO 10993-12:2021. *Biological evaluation of medical devices - Part 12: Sample preparation and reference materials*. International Organization for Standardization, Geneva, Switzerland

ISO 10993-1:2018. *Biological evaluation of medical devices – Part 1: Evaluation and testing within a risk management process*. International Organization for Standardization, Geneva, Switzerland

ISO 10993-4:2017. *Biological Evaluation of Medical Devices - Part 4: Selection of tests for the interaction with blood.* International Organization for Standardization, Geneva, Switzerland