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ASTM Hemolysis (Direct Contact and Extract Methods) GLP Report

Test Article:	Test Article Name: TM9MEP Lot # / Batch #1175200
Purchase Order:	TE202442
Study Number:	1334079-S01
Study Received Date:	22 Aug 2020
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 15
Deviation(s):	None

Summary: All test method acceptance criteria were met.

Direct Contact Method: The difference between the hemolytic indexes of the test article and the negative control equals 0.00 percent, which places the test article in the non-hemolytic range according to the grade outlined below.

Extract Method: The difference between the hemolytic indexes of the test article and the negative control equals 0.00 percent, which 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

Tracy Hubertz electronically approved — 03 Sep 2020 20:59 (+00:00)

Study Director Tracy Hubertz — Study Completion Date and Time

These results apply to the samples as received and relate only to the test article listed in this report. Reports may not be reproduced except in their entirety. Subject to NL terms and conditions at www.nelsonlabs.com.

Results:

Direct Contact Method:

Test Article / Control	Optical Density	Average Optical Density	Hemolytic Index	Average Hemolytic Index (% Hemolysis)	Corrected Hemolytic Index (% Hemolysis)
Test Article	0.003	0.003	1.061	0.99	0.15
	0.002		0.833		
	0.003		1.061		
Negative Control	0.004	0.003	1.288	1.14	0.30
	0.003		1.061		
	0.003		1.061		
Positive Control	0.527	0.514	120.243	117.3	116.5
	0.497		113.419		
	0.518		118.196		
Phosphate Buffered Saline (PBS) Blank	0.002	0.002	0.833	0.83	N/A
	0.002		0.833		
	0.002		0.833		

Extract Method:

Test Article / Control	Optical Density	Average Optical Density	Hemolytic Index	Average Hemolytic Index (% Hemolysis)	Corrected Hemolytic Index (% Hemolysis)
Test Article	0.002	0.002	0.833	0.91	0.00
	0.002		0.833		
	0.003		1.061		
Negative Control	0.004	0.003	1.288	0.99	0.08
	0.002		0.833		
	0.002		0.833		
Positive Control	0.377	0.427	86.126	97.5	96.6
	0.450		102.729		
	0.454		103.639		
Phosphate Buffered Saline (PBS) Blank	0.003	0.002	1.061	0.91	N/A
	0.002		0.833		
	0.002		0.833		

Hemoglobin Standard:

Regression Output	
Constant	0.00241
Standard Error of Y Estimate	0.01173
R^2	0.99853
Degrees of Freedom	6
X Coefficient(s)	1.44874
Standard Error of Coefficient	0.02268

Hemoglobin Totals	
Total Plasma Free Hemoglobin	0.318 mg/mL
Total Final Hemoglobin Present	10.191 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.

Procedures: The procedure uses 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 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.

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

Direct Contact Method: Glass test tubes were labeled appropriately. To each test tube, 21 cm^2 of the test article, 7 mL of PBS and 1 mL of the diluted blood were added. A non-hemolytic negative control, a hemolytic positive control and a PBS blank were included. The controls consisted of the appropriate amount of control material, 7 mL of PBS and 1 mL of the diluted blood. Three tubes were prepared for each test article and control.

Extract Method:

Extract Solvent	Extraction Ratio	Test Article/ Extraction Solvent Amount	Extraction Parameters	
			Temperature	Time
PBS	3 cm^2/mL	75 cm^2/25 mL	50 ± 2°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, centrifuged or manipulated in any way following the extraction process.

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

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. Refer to the test article preparation table for extraction details.

After the extraction, 7 mL of test article or control extract and 1 mL of diluted blood was added to test tubes. Three tubes were prepared for each test article and control.

Incubation: The tubes were 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. A non-hemolytic negative control, a hemolytic positive control and a PBS blank were included.

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. The appearances of the supernatants are found in the table below. The test articles and controls were read at 540 nm in a spectrophotometer.

Supernatant Appearance	
Test Article (Direct Contact Method)	Clear; particulate free
Test Article (Extract Method)	Clear; particulate free
PBS Blank (Direct Contact and Extract Method)	Clear; particulate free
Negative Control (Direct Contact and Extract Method)	Clear; particulate free
Positive Control (Direct Contact and Extract Method)	Red; particulate free

The hemolytic index (percent hemolysis) was interpreted using the following equation:

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

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

Test Article Preparation:

References:

ASTM F756-17, 2017, *Standard Practice for Assessment of Hemolytic Properties of Materials*. ASTM International, West Conshohocken, PA (CRD217)

ANSI/AAMI/ISO 10993-12:2012. *Biological Evaluation of Medical Devices - Part 12: Sample preparation and reference materials*. Association for the Advancement of Medical Instrumentation. Arlington, VA. (CRD023)

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. (CRD027)

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. (CRD613).

Quality Assurance Statement

Compliance Statement: The test was conducted in accordance with the USFDA (21 CFR Parts 58, 210, 211, and 820) Regulations. This final report reflects the raw data.

Activity	Date
Study Initiation	25 Aug 2020
Phase Inspected by Quality Assurance: Hemoglobin Present	31 Aug 2020
Audit Results Reported to Study Director	01 Sep 2020
Audit Results Reported to Management	01 Sep 2020

Scientists	Title
Chad Summers	Supervisor
Tracy Hubertz	Study Director

Data Disposition: The study plan, raw data and final report from this study are archived at Nelson Laboratories, LLC or an approved off-site location.

Nicole Widmer electronically approved
Quality Assurance

03 Sep 2020 16:18 (+00:00)
Date and Time