GLP REPORT

TEST FACILITY

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CONFIDENTIAL

STUDY TITLE

SC5b-9 Complement Activation Assay

TEST ARTICLE NAME

Occlusin 505 Artificial Embolization Device

TEST ARTICLE IDENTIFICATION

FL288

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Summary

The test article, Occlusin 505 Artificial Embolization Device, FL288, was evaluated for the potential to activate the complement system. The clinical significance of the results should be evaluated with respect to the use of the medical device and its likely potential for activation of the complement system in clinical use. This study was conducted *in vitro* by incubating the test article in normal human serum (NHS) and detecting the presence of SC5b-9 in the exposed serum by an enzyme immunoassay method. The SC5b-9 concentration from the test article sample was compared statistically to activated NHS and negative (low density polyethylene) controls.

Under the conditions of this assay, the concentration of SC5b-9 in the test article sample was 4,817 ± 311.9 ng/mL (mean ± standard deviation). The concentration of SC5b-9 in the test article sample was not statistically higher than the activated NHS control and was statistically lower than the negative control. As a result, the test article was not considered to be a potential activator of the complement system.

Supervisory Personnel:

Don R. Pohl, B.S.
Manager, Technical Sales and Services

Todd A. Festerling, B.S., M.S.
Manager, In Vitro Toxicology

Approved by:

Lesia M. Wasio, B.S.
Study Director

10-28-09
Date Completed

Statement of GLP Compliance

This study was conducted in accordance with the provisions of the FDA Good Laboratory Practice (GLP) Regulations (21 CFR, Part 58).

There were no deviations from the protocol, standard operating procedures or the GLP Regulations which were judged to have had any significant impact on the validity or interpretation of the data.

All laboratory data have been accurately recorded and verified, as indicated by the signature below.

Study Director: _____ 10-28-09

Lesia M. Wasio, B.S. Date

1. Introduction

Purpose
The purpose of this study was to determine the complement-activation potential of the test article identified below. The activation of complement system can be clinically significant. The study was conducted *in vitro* by incubating the test article in normal human serum and detecting the presence of SC5b-9 in the exposed serum by an enzyme immunoassay (EIA) method. The SC5b-9 complex is the soluble, non-lytic form of the Terminal Complement Complex (TCC) that is only formed when there is an activation of the complement system.

Dates
Test Article Receipt: October 2, 2009
Test Conducted Date: October 19, 2009

GLP Compliance
The study initiated by protocol signature on October 8, 2009 was conducted in accordance with the provisions of the FDA Good Laboratory Practice (GLP) Regulations, 21 CFR 58. A Statement of Quality Assurance Activities was issued with the report.

Duplication of Experimental Work
By signature on the protocol, the sponsor confirmed that the conduct of this study did not unnecessarily duplicate previous experiments.

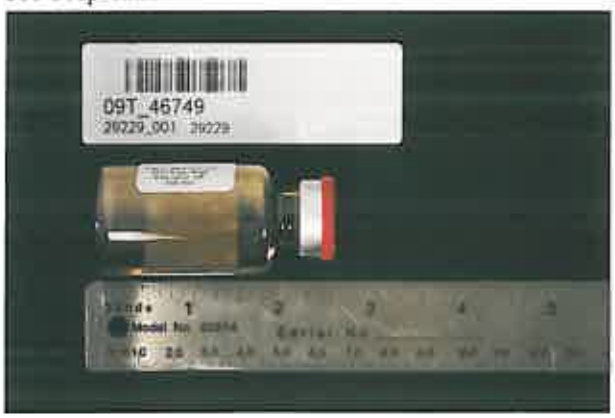
2. Materials

The test article provided by the sponsor was identified and handled as follows:

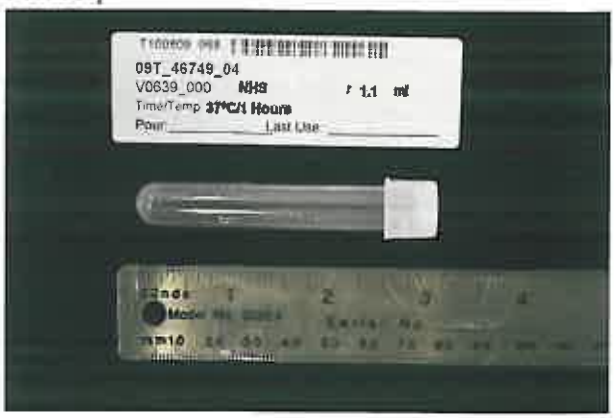
Test Article Name: Occlusin 505 Artificial Embolization Device

Test Article Identification: FL288

Pre-Preparation



Post-Preparation



Stability Testing: In progress

Expiration Date: Stable for duration of intended testing

Strength, Purity and Composition: Strength: Not applicable because no active ingredients are used to formulate a concentration; Purity: Not applicable because the test article is a multi-component device; Composition: Polylactide-co-glycolide and bovine collagen

Physical Description of the Test Article: Microspheres supplied as a dry powder in sterile sealed glass vials; 400mg/vial

Storage Conditions: Refrigerated

Complement Source:	Normal Human Serum (NHS), certified as negative for HIV (I and II) and Hepatitis B and C, was purchased from an outside source.
Test Article Preparation:	The test article was prepared based on the sponsor provided surface area of 6436 cm^2 per gram. A 0.001 g portion (6.436 cm^2) was included in each preparation. Based on the USP ratio of 3.0 cm^2:0.5 mL, a 6.4 cm^2 portion of the test article was covered with 1.1 mL of NHS. Triplicate preparations were incubated at 37°C for 60 minutes.
Positive Control:	Cobra Venom Factor (CVF) was used to confirm maximal activation of complement. Ten units of CVF were added to a 0.5 mL aliquot of NHS. Triplicate preparations were incubated at 37°C for 60 minutes.
Stability Testing:	Marketed product, stability characterized by its labeling.
Strength, Purity and Composition:	Strength and Purity: ≥95%; Composition: Cobra venom factor, water.
High Control:	Human plasma (supplied with the EIA kit) with a determined SC5b-9 range of 117.3 – 172.3 ng/mL served as an additional control measure.
Low Control:	Human plasma (supplied with the EIA kit) with a determined SC5b-9 range of 13.1 – 28.4 ng/mL served as an additional control measure.
Activated NHS Control:	Triplicate aliquots of NHS were incubated at 37°C for 60 minutes to serve as the activated baseline control.
Positive Biomaterial Reference Control:	Latex examination gloves were used as the positive biomaterial reference control. Based on the USP ratio of 120 cm^2:20 mL, a 3 cm^2 portion of the control material was covered with 0.5 mL of NHS. Triplicate preparations were incubated at 37°C for 60 minutes.
Stability Testing:	Marketed product, stability characterized by its labeling.
Strength, Purity and Composition:	Strength: Not more than 50 µg of total water extractable latex protein/gram; Purity: Not applicable, multi-component device; Composition: Natural rubber latex, zinc carbamate accelerators, zinc oxide, and titanium dioxide.
Negative Control:	Low density polyethylene (LDPE), a biomaterial with a low ability to activate complement, was used as a negative control. Based on the USP ratio of 60 cm^2:20 mL, a 1.4 cm^2 portion of the control material was covered with 0.5 mL of NHS. Triplicate preparations were incubated at 37°C for 60 minutes.
Stability Testing:	Marketed product, stability characterized by its labeling.
Strength, Purity and Composition:	Strength: Not applicable, no active components in the formulation; Purity: Meets FDA 21 CFR 177.1520; Composition: Low density polyethylene.
Inactivated NHS Control:	An aliquot of NHS was placed on ice for 60 minutes to verify the background SC5b-9 concentration.

3. Test System

Test System and Justification

The test system measures the complement activating potential of a test article by detecting the presence of a complement protein, SC5b-9, in normal human serum following exposure to the test article in vitro. The level of SC5b-9 is quantified using the SC5b-9 Plus Enzyme Immunoassay (EIA) kit from Quidel Corporation (San Diego, CA). SC5b-9 is unique to the common terminal pathway of complement cascade. Activation of either the classical or alternative pathway leads to the formation of the Terminal Complement Complex (TCC) by the assembly of C5 through C9. The membrane attack complex (MAC), which is a form of TCC, is a stable complex which mediates the irreversible cell membrane damage associated with complement activation. Complexes formed in the absence of a target membrane bind to a naturally occurring regulatory serum protein, the S protein. The S protein binds to nascent C5b-9 complexes at the C5b-7 stage of assembly. The SC5b-9 is a stable, non-lytic form of the TCC. Since C5 is unique to the common terminal pathway of complement cascade, detection of SC5b-9 is proof of activation of the terminal pathway.

Sample Preparation

Each prepared test article, positive biomaterial reference control, and negative control were placed into separate, labeled polypropylene tubes and covered with the appropriate aliquots of NHS. The positive control (CVF) and NHS controls were also prepared in polypropylene tubes. All test article(s) and controls, except the inactivated NHS control, were incubated for 60 minutes in a 37°C water bath. The inactivated NHS control remained on ice for 60 minutes. The samples were agitated immediately before and immediately following incubation. The NHS was then placed in an ice bath until use. To perform the assay, the NHS was appropriately diluted with sample buffer. The standards, low control, high control, NHS controls, negative control, positive control and positive biomaterial reference control were prepared and diluted as appropriate. The NHS was removed from the test article and controls, and placed into cryogenic storage vials. Once the dilutions were completed, the samples were placed in a liquid nitrogen tank for storage.

4. Method

Manufacturer's Instructions

The Quidel SC5b-9 Plus Enzyme Immunoassay for the quantification of SC5b-9 in human serum was a three-step procedure using (1) a microassay plate coated with a mouse monoclonal antibody which binds specifically to human SC5b-9; (2) an HRP-conjugated goat antibody to antigens of SC5b-9; and (3) a chromogenic substrate. The Quidel test kit provided the SC5b-9 standards A, B, C, D, and E and the low and high controls. Each standard consisted of human serum containing known amounts of SC5b-9 in PBS, protein stabilizers and preservatives.

The microassay wells were rehydrated by adding approximately 300 µL of wash solution to each well and incubating for 2 minutes at room temperature. A 100 µL aliquot of the prepared standard series, blank, low control, high control and inactivated control were added to duplicate microassay wells precoated with anti-SC5b-9 monoclonal antibody. A 100 µL aliquot of each triplicate sample (positive biomaterial reference control, negative control, positive control, activated NHS control and test article) was added to a single well precoated with anti-SC5b-9 monoclonal antibody and the plate was incubated at room temperature for 60 minutes. The anti-SC5b-9 was specific for SC5b-9 and would not bind to any other complement fragment. The SC5b-9 present in the standards, controls, or test article dilutions was bound to the immobilized anti-SC5b-9. After the 1 hour incubation, the plate was washed with approximately 300 µL of the wash solution, incubated for 1 minute at room temperature, and then removed by inverting the plate by tapping firmly on absorbent paper. This wash procedure was repeated an additional 4 times without the 1 minute incubation.

A 50 µL aliquot of horseradish peroxidase (HRP) -conjugated goat anti-SC5b-9 was added to each well and incubated for 30 minutes at room temperature. In this step, the enzyme conjugated anti-SC5b-9 bound to SC5b-9 which was captured by the monoclonal anti-SC5b-9 on the surface of the microassay wells. After the incubation, the plate was washed with approximately 300 µL of the wash solution, incubated for 1 minute at room temperature, and then removed by inverting the plate by tapping firmly on absorbent paper. This wash procedure was repeated an additional 4 times without the 1 minute incubation to remove any unbound, excess conjugate.

A 100 µL aliquot of chromogenic enzyme substrate solution was added to each microassay well and the plate was allowed to stand at room temperature for 15 minutes. The bound HRP-conjugate reacted with the substrate. After the incubation, the enzyme reaction was stopped by the adding 100 µL of the Stop solution (2N sulfuric acid) and the absorbance was measured at 450 nm using a spectrophotometer.

All times and temperatures reported herein are approximate and are within ranges established by the external standards described in the References section of this report and/or NAMSA standard operating procedures.

5. Evaluation and Statistical Analysis

A standard curve was generated by plotting the blank subtracted absorbance values for the standards on the y-axis against the respective SC5b-9 concentration on the x-axis. The concentrations of SC5b-9 in the test samples and controls were calculated from the standard curve and reported in ng/mL. The concentration of SC5b-9 in the positive control and test article sample was compared statistically with the activated NHS and negative controls using t-tests. A p value of < 0.05 was considered statistically significant.

The study was considered valid since the following criteria were met:

a. The correlation coefficient for the standard curve was greater than 0.95.
b. The slope of the standard curve was between 0.0039 and 0.0123.
c. The y-intercept of the standard curve was between (-) 0.189 and (+) 0.201.
d. The concentration of SC5b-9 in the positive control was statistically higher than the activated NHS and negative controls.
e. The low and high controls demonstrated SC5b-9 content in the range indicated on the Certificate of Analysis for the kit used.
f. The SC5b-9 concentration in the inactivated NHS control was less than 3-fold the SC5b-9 value stated on the Certificate of Analysis for the NHS lot.

For the test article to be considered a potential activator of the complement system, the test article sample must be statistically higher than that of the activated NHS control and the negative control.

Currently, there is no regulatory guidance for evaluation of complement activation by medical devices. Interpretation of the results should be based on the clinical use of the device, which takes into account the degree of blood exposure. For example, medical devices such as dialyzers and by-pass circuits have a large surface area and prolonged blood exposure, and thereby pose a greater concern for complement activation. In contrast, devices such as catheters and guidewires with much smaller surface areas and blood exposure pose significantly less concern for complement activation. As a result, the results from complement assay should be evaluated with respect to the medical device's clinical use and its likely potential for complement activation under such clinical use.

6. Results

The controls performed as anticipated. The absolute SC5b-9 concentrations obtained for the test article and each control are presented in Table 1.

Table 1 - SC5b-9 Concentrations

	SC5b-9 Concentration of Sample 1 (ng/mL)	SC5b-9 Concentration of Sample 2 (ng/mL)	SC5b-9 Concentration of Sample 3 (ng/mL)	SC5b-9 Concentration ng/mL (mean ± S.D.)
Test Article	4,482	5,099	4,870	4,817 ± 311.9[a]
Positive Control	435,170	428,544	481,633	448,449 ± 28,929[bc]
Activated NHS Control	6,149	5,400	4,771	5,440 ± 689.9
Positive Biomaterial Reference Control	26,584	22,461	25,746	24,930 ± 2,179
Negative Control	6,434	7,162	6,195	6,597 ± 503.7
Inactivated NHS Control	1,365	Not Applicable	Not Applicable	Not Applicable
High Control	142,293	Not Applicable	Not Applicable	Not Applicable
Low Control	16,846	Not Applicable	Not Applicable	Not Applicable

S.D. = Standard deviation

[a] = The SC5b-9 concentration of the test article sample was statistically lower than the negative control ($p < 0.05$)
[b] = The SC5b-9 concentration of the positive control was statistically higher than the activated NHS control ($p < 0.05$)
[c] = The SC5b-9 concentration of the positive control was statistically higher than the negative control ($p < 0.05$)

7. Conclusion

Under the conditions of this assay, the concentration of SC5b-9 in the test article sample was 4,817 ± 311.9 ng/mL (mean ± S.D.). The concentration of SC5b-9 in test article sample was not statistically higher than the activated NHS control and was statistically lower than the negative control. As a result, the test article was not considered to be a potential activator of the complement system.

Results and conclusions apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility. All procedures were conducted in conformance with good manufacturing practices and certified to ISO 13485:2003.

8. Quality Assurance

Inspections were conducted at intervals adequate to assure the integrity of the study in conformance with 21 CFR 58.35(b)(3). The final report was reviewed for conformance to Section 58.185, Subpart J, of the GLP Regulations. A Statement of Quality Assurance Activities was issued with the report.

9. Records

All raw data pertaining to this study and a copy of the final report are to be retained in designated NAMSA archive files.

10. References

Code of Federal Regulations (CFR), Title 21, Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies (2008).

SC5b-9 Enzyme Immunoassay Package Insert, Quidel Corporation, San Diego, CA.

Rapid Testing of Biomaterials for Complement Activation Using In Vitro Complement Immunoassays, Publication from Quidel Corporation, San Diego, CA, Jan. 1995.

NAMSA Validation study, 06G_35697.

Statement of Quality Assurance Activities

Phase Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management
Activation	October 19, 2009	October 19, 2009	October 19, 2009
Study Data Review	October 20, 2009	October 20, 2009	October 20, 2009
Final Report Review	October 22, 2009	October 22, 2009	October 22, 2009

Based on a review of this study, it has been concluded that this report accurately describes the methods and standard operating procedures, and that the reported results accurately reflect the raw data of the study. This study has been reviewed in accordance with the provisions of the FDA Good Laboratory Practice Regulations (21 CFR, Part 58).

QA Representative: Heatherbea L. Weirich 10-27-09

Heatherbea L. Weirich, B.S.
Auditor, Quality Assurance

Date

GLP PROTOCOL

TEST FACILITY:

NAMSA
6750 Wales Road
Northwood, OH 43619

SPONSOR:

NAMSA Ohio
6750 Wales Road
Northwood, OH 43619

STUDY TITLE:

SC5b-9 Complement Activation Assay

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Approvals

Sponsor Representative (Sponsor): _____

Date Approved: _____

Study Director (NAMSA): _____

Date Initiated: _____

1. Introduction

Purpose
The purpose of this study is to determine the complement activation potential of a biomaterial or a medical device using an *in vitro* test system. The activation of complement system can be clinically significant. The study will be conducted *in vitro* by incubating the test article in normal human serum and detecting the presence of SC5b-9 in the exposed serum by an enzyme immunoassay (EIA) method. The SC5b-9 complex is the soluble, non-lytic form of the Terminal Complement Complex (TCC) that is only formed when there is an activation of the complement system.

GLP Compliance
Good Laboratory Practice – This nonclinical laboratory study will be conducted in accordance with the United States Food and Drug Administration Good Laboratory Practice Regulations, 21 CFR Part 58.

2. Materials

Test Article
The sponsor will submit the test article to be evaluated. Detailed information about the test article was provided to NAMSA by the sponsor and is listed below:

Test Article Name:	Occlusion 505 Artificial Embolization Device
Test Article Identification:	FL288
Test Article Physical Description:	Microspheres supplied as a dry powder in sterile sealed glass vials/ 400mg/vial
Test Article Intended Clinical Use:	Embolotherapy
Test Article Stability:	Stability testing is in progress and sponsor affirms that test article is stable for duration of intended testing.
Test Article Strength:	Strength is not applicable because no active ingredients are used to formulate a concentration.
Test Article Purity:	Purity is not applicable because the test article is a multi-component device.
Test Article Composition:	The test article is composed of the following materials: polylactide – co-glycolide and bovine collagen
Test Article Mixture Analysis:	Analysis is not necessary because test article is a solid, powder, gel, or liquid being extracted or being tested as received (will not be mixed with a carrier).
Test Article Disposition:	Discard unused test article.

Only insoluble or solid materials can be tested. Gels and liquids cannot be analyzed. If the material is composed of multiple parts, each part must be supplied and tested separately.

USP guidelines for extraction will be used to determine the amount of test article per unit volume of normal human serum (NHS). The NHS employed in this test is not an extraction vehicle but rather the complement source. NHS that has been tested negative for HIV I & II, and Hepatitis B and C surface antigens will be purchased from Quidel Corporation.

Preparation
The following information was completed based on the sponsor providing the information to NAMSA. Further instructions may be attached to the protocol. The sample will be prepared as follows:

Ratio of test article to NHS
Material thickness less than 0.5 mm, use ratio of 3.0 cm^2 : 0.5 mL (based on the USP ratio 120 cm^2 : 20 mL)

Note: Due to testing constraints, the volume of NHS used for incubation will be limited to 0.5 mL. If a volume greater than 0.5 mL of NHS is needed, the price will be adjusted.

Test Article Preparation Instructions
Prepare based on the following: Use 0.001g of sample. 0.001g of sample has a surface area of 6.4375cm^2.

Special Laboratory Instructions
Following the extraction procedure, it is acceptable to centrifuge the extract with the sample in it to be able to remove enough extract for testing

Control Article
Positive Biomaterial Reference Control: Latex examination gloves, a biomaterial with a high capacity to activate complement, will be prepared using a ratio of 3.0 cm^2 : 0.5 mL.

Negative Control: Low density polyethylene, a biomaterial with a low ability to activate complement, will be prepared using a ratio of 1.5 cm^2 : 0.5 mL.

Positive Control: 10 units of Cobra Venom Factor (CVF) will be used to confirm maximal complement activation. CVF has been shown to be a strong activator of the complement system.

Low Control: Human plasma (supplied with the EIA kit) with a low SC5b-9 content (as specified in the Certificate of Analysis) will be used as an additional control measure.

High Control: Human plasma (supplied with the EIA kit) with a high SC5b-9 content (as specified in the Certificate of Analysis) will be used as an additional control measure.

Activated NHS Control: NHS incubated at 37°C for 60 minutes will serve as the activated baseline control.

Inactivated NHS Control: NHS placed on ice for 60 minutes will serve as the inactivated baseline control.

The lot number and expiration date for the materials and control articles will be recorded.

3. Test System

Test System and Justification
Some biomaterials have the potential to activate the complement system via the alternative pathway when they come in contact with blood, producing undesirable results. Thus, for blood contacting medical devices, it is important to evaluate their potential for activating the complement system.

The test system measures the complement activating potential of a test article by detecting the presence of a complement protein, SC5b-9, in normal human serum following exposure to the test article in vitro. The level of SC5b-9 is quantified using the SC5b-9 Plus Enzyme Immunoassay (EIA) kit from Quidel Corporation (San Diego, CA). SC5b-9 is unique to the common terminal pathway of complement cascade. Activation of either the classical or alternative pathway leads to the formation of the Terminal Complement Complex (TCC) by the assembly of C5 through C9. The membrane attack complex (MAC) which is a form of TCC is a stable complex which mediates the irreversible cell membrane damage associated with complement activation. Complexes formed in the absence of a target membrane bind to a naturally occurring regulatory serum protein, the S protein. The S protein binds to nascent C5b-9 complexes at the C5b-7 stage of assembly. The SC5b-9 is a stable, non-lytic form of the TCC. Since C5 is unique to the common terminal pathway of complement cascade, detection of SC5b-9 is proof of activation of the terminal pathway.

4. Method

Preparation
Each test article, positive biomaterial reference control, activated NHS control, positive control and negative control will be prepared in triplicate in order to be compared statistically. The inactivated NHS will be prepared as a single sample. The low and high controls supplied with the kit will be plated per kit instructions. The inactivated NHS and the low and high controls will only be used for quality control of the system. All test article(s) and controls will be covered with the appropriate amount of NHS and incubated for 60 minutes in a 37°C water bath with the exception of the inactivated NHS control. The inactivated NHS control will be placed on ice for 60 minutes. The activated NHS control provides a baseline value to evaluate the activation of complement by the positive control and test article. The samples will be agitated immediately before and immediately following incubation.

Following the 60 minute incubation, the tubes will be placed on ice to hinder further activation of complement. The serum will be withdrawn from all controls and test articles, and placed into labeled polypropylene tubes. The test article sera and controls will be diluted, as needed, with sample buffer. All serum samples and dilutions will be kept on ice to hinder further activation of complement. Each prepared dilution will be tested using the complement activation kit according to the manufacturer's instructions.

Manufacturer's Instructions
The Quidel SC5b-9 Plus Enzyme Immunoassay for the quantification of SC5b-9 in human serum is a three-step procedure using (1) a microassay plate coated with a mouse monoclonal antibody which binds specifically to human SC5b-9; (2) horseradish peroxidase (HRP)-conjugated goat antibodies to antigens of SC5b-9; and (3) a chromogenic substrate. The Quidel test kit will provide the SC5b-9 standards A - E. Each standard consists of human serum containing known amounts of SC5b-9 in PBS, protein stabilizers and preservatives.

Standard series, blank, low control, high control and inactivated control will be added to duplicate microassay wells precoated with anti-SC5b-9 monoclonal antibody. All triplicate samples (positive biomaterial reference control, negative control, positive control, activated NHS control and test article will be added to single wells precoated with anti-SC5b-9 monoclonal antibody. The anti-SC5b-9 monoclonal antibody is specific for SC5b-9 and will not bind to any other complement fragment. The SC5b-9 present in the standards, controls, or test article dilutions will bind to the immobilized anti-SC5b-9. After 1 hour of incubation, a wash cycle will be used to remove unbound material.

HRP-conjugated goat anti-SC5b-9 will be added to each well. In this step, the enzyme conjugated anti-SC5b-9 will bind to SC5b-9 which was captured by the monoclonal anti-SC5b-9 on the surface of the microassay wells. After 30 minutes of incubation, a wash cycle will be used to remove unbound, excess conjugate.

A chromogenic enzyme substrate will be added to each microassay well. The bound HRP-conjugate will react with the substrate forming a green color. After 15 minutes of incubation, the enzyme reaction will be chemically stopped by the addition of the Stop solution (2N sulfuric acid) and the color intensity will be measured spectrophotometrically at 450 nm. The color intensity of the reaction mixture will be proportional to the concentration of SC5b-9 present in the test specimens, standards, and controls.

If the absorbance reading (A_{450}) of the test article activated sera is greater than that of the SC5b-9 Standard E, or less than 0.150 A_{450} units above the blank reading, the test article sera will be reassayed at a different dilution so that the absorbance reading will be between these limits. In all repeat assays, the standards and controls will also be retested.

5. Evaluation and Statistical Analysis

A standard curve will be generated by plotting the blank subtracted absorbance values for the standards on the y-axis against the respective SC5b-9 concentration on the x-axis. The concentration of SC5b-9 in the test samples and controls will be calculated from the standard curve and reported in ng/mL unit. The concentration of SC5b-9 in the positive control and test article extract will be compared statistically with the activated NHS and negative control using t-tests. A p value of < 0.05 will be considered statistically significant.

The study will be considered valid if the following criteria are met:

- The correlation coefficient for the standard curve must be above 0.95.
- The slope of the standard curve must be between 0.0039 and 0.0123.
- The y-intercept of the standard curve must be between (-) 0.189 and (+) 0.201.
- The concentration of SC5b-9 in the positive control must be statistically higher than the activated NHS and negative controls.
- The low and high controls should demonstrate the SC5b-9 content in the range indicated on the Certificate of Analysis for the kit used.
- The SC5b-9 concentration in the inactivated NHS control should be less than 3-fold the SC5b-9 value stated on the certificate of analysis for the NHS lot.

For evaluation of the test sample results, the following criteria will be considered:

- If the SC5b-9 concentration of the test sample is statistically higher than both the activated NHS and negative controls, then the test article is considered a potential activator of the complement system. The data will be reviewed by the appropriate scientific personnel.
- If a positive response (statistically higher as compared to both the activated NHS control and negative control) is noted for the test sample, then the SC5b-9 concentration of the test sample will be compared with the SC5b-9 concentration of the positive biomaterial reference control based on the following formula:

$$\frac{(Average\ \ SC5b\text{-}9\ Conc.of\ Test\ Article - Average\ SC5b\text{-}9\ Conc.of\ \ Activated\ NHS)}{(Average\ SC5b\text{-}9\ Conc.of\ \ Pos.\ Biomaterial\ \ Ref.Control\ -\ Average\ SC5b\text{-}9\ Conc.of\ \ Activated\ NHS} * 100$$

If a positive response is noted for the test article, the SC5b-9 concentration of the test article will be compared to the historical range of the activated NHS and negative controls to determine biological relevance. If the SC5b-9 concentration for the test

article is within the mean ± 1 standard deviation of both the activated NHS and negative historical controls, then the article will be considered a "low potential activator" of the complement system.

Currently, there is no regulatory guidance for evaluation of complement activation by medical devices. Interpretation of the results should be based on the clinical use of the device, which takes into account the degree of blood exposure. For example, medical devices such as dialyzers and by-pass circuits have a large surface area and prolonged blood exposure, and thereby pose a greater concern for complement activation. In contrast, devices such as catheters and guidewires with much smaller surface areas and blood exposure pose significantly less concern for complement activation. As a result, the results from complement assay should be evaluated with respect to the medical device's clinical use and its likely potential for complement activation under such clinical use. Other data and/or information may be utilized in reaching a final conclusion regarding the results of this assay.

6. Report

The final report will include a description of the methods employed, acceptability of controls and a table listing SC5b-9 concentrations of triplicate samples, as well as the mean concentration and standard deviation for the test article, positive control, activated NHS control, negative control and positive biomaterial reference control, and the SC5b-9 concentration of the inactivated NHS control, low control, and high control.

7. Quality Assurance

Inspections will be conducted at intervals adequate to assure the integrity of the study in conformance with 21 CFR 58.35(b)(3). The final report will also be reviewed for conformance to Section 58.185, Subpart J, of the GLP Regulations. A Statement of Quality Assurance Activities will be provided with the final report.

8. Proposed Dates

The study dates will be finalized by the study director following receipt of the sponsor-approved protocol and appropriate material for the study. Initiation of the study will be the date on which the study director signs the GLP protocol. Projected dates for starting the study (first treatment) and for the completion of the study (final report release) will be provided to the sponsor (or representative of the sponsor).

9. Records

All raw data pertaining to this study and a copy of the final report will be retained in designated NAMSA archive files.

10. References

Code of Federal Regulations (CFR), Title 21, Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies (2008).

SC5b-9 Plus Enzyme Immunoassay Package Insert, Quidel Corporation, San Diego, CA.

Rapid Testing of Biomaterials for Complement Activation Using In Vitro Complement Immunoassays, Publication from Quidel Corporation, San Diego, CA, Jan. 1995.

NAMSA Validation study, 06G_35697.

11. Protocol Changes

Any necessary changes to the protocol after sponsor approval or study initiation will be documented and approved by the study director as protocol amendments. Copies will be distributed to the sponsor, the raw data file, and the NAMSA Quality Assurance department.