TOXIKON

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Medical Device Testing Guide

A resource for sample submissions, test descriptions, sample requirements, and turnaround times for biocompatibility testing according to:

- FDA/ISO 10993
- MHLW-Japan
- USP
- Microbiology

15 Wiggins Avenue, Bedford, MA 01730 ✆ 781.275.3330 www.toxikon.com

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About Toxikon

For over thirty five years, Toxikon has provided product safety testing to the medical device, chemical, and pharmaceutical industries. Our professional expertise along with a recognized stature in the industry provides us unique capability to assist clients with their testing needs in a timely and cost-effective manner. Toxikon is ISO 17025 accredited and certified to perform studies according to FDA 21 CFR Good Laboratory Practices (GLP) and Good Manufacturing Practice (GMP). Toxikon is registered in the United States, Europe and Japan as a contract-testing laboratory.

Contact Information

Technical Sales and Service Account Managers
Toll-Free: 800-458-4141
E-mail: info@toxikon.com
Website: www.toxikon.com

USA
Toxikon Corporation
15 Wiggins Avenue
Bedford, MA 01730
Toll-Free: (800) 458-4141
Main: (781) 275-3330
Fax: (781) 271-1136

Europe
Toxikon Europe N. V.
Romeinsestraat 12
B-3001 Leuven, Belgium
Main: +32 (16) 400484
Fax: +32 (16) 401304

Asia
Toxikon India
21, Parmeshwar, 353/20
R.B. Mehta Marg, Ghatkopar (E)
Mumbai 400 077, India
Main: +91 (22) 6798 3604
Fax: +91 (22) 2511 9168

Toxikon Japan
Moriya Sangyo K.K
Shinjuku Kokusai Bldg. Annex
Nishi - Shinjuku 6 - 6 – 3
Shinjuku - ku, Tokyo 160, Japan
Main: + 81.353066267
Fax: + 81.353066268

Study Pricing
The Device Testing Guide includes a sampling of Toxikon's standard studies. Additional Standard Studies for microbiology, ETO Residual, analytical chemistry, and customized studies can be performed. For all quote and study requests, please contact your Account Manager or info@toxikon.com.

Turnaround Time:

Standard Processing: Tests are scheduled into the laboratory on a first-come, first-served basis. A pre-determined, standard time limit is assigned to each test at log-in. This timeline allows for sample receipt, sample log-in, performance of the test, raw data review, report preparation, review, signature, and transmission of results. If final results are needed within a critical timeframe, a Toxikon Associate in Technical Sales and Service can explain options prior to the start of your testing.

Expedited Study Requests (STAT): Sponsor requests for a test and/or report to be completed in less time than standard turnaround time, must be communicated to a Toxikon Service Representative. Toxikon will make every effort to accommodate all Sponsor requests; however, scheduling will be dependent on current lab capacity. Sponsor must provide a purchase order for STAT processing fees.

Instructions for Test Requests & Sample Submission

Studies are scheduled upon receipt of the signed Study Contract Documents and test material. Study contract documents include 1) Signed Quotation, 2) Test Requisition Form, and 3) GLP Protocol Acceptance Pages (when applicable).

Sample Submission Forms: To ensure proper and timely results of your testing please fill out the appropriate Test Requisition Form. Accurate and clear information will avoid delays in the testing process and report generation, as well as avoid possible amendment fees. Toxikon Sample Submission Forms are available on our web site at www.toxikon.com and can be downloaded in either PDF or Word Format. Available forms include:

- GLP Test Request
- Non-GLP Test Request
- Microbiology Test Request
- Accelerated Aging Test Request
- ETO Residual Testing Request

Instructions to Complete Requisition Forms

Please complete one form for each different test article type submitted. Requisition forms must include the following:

Section 1. Company/Contact information

- Your Company Name, Address, Phone and Fax Number
- Name of Sponsor Contact
- Email Address
- Billing Address
- **Purchase Order:** Please submit purchase orders to help expedite the processing of a work order. Standing purchase orders can be established for routine submissions to reduce repetitive paperwork. In some cases, your company policy may prohibit testing without prior P.O. approval.

Section 2. Test Article Information-*It is critical that all spelling and numberings are correct.*

- Test Article name *(entered exactly as you want reported)*
- Lot/Batch numbers
- CAS-code number *(as applicable for chemicals)*

Section 3. Test Selection/Extraction Conditions

- **Tests Names:** List each of the tests to be performed on the test article. Refer to the test names listed on the quotation for guidance.
- **GLP Protocol Number**: Protocol numbers and protocols will be issued to Sponsor for review and signature. When studies need to be performed according to the FDA Good Laboratory Practices (GLP) regulations, Sponsor should notify Toxikon at the time of quotation request.
- **Sample Preparation:** Devices may require either direct or indirect exposure, which must be noted on the request form. Various choices for extraction temperatures and times are listed on the table below, as well as on the test request form.

Selections must include both Vehicle and Temperature/Time

Vehicles	Temperature/Time
NaCl—Sodium Chloride	37 °C/24 hours
CSO—Cottonseed Oil	37 °C/72 hours
MEM—Minimum Essential Media	50 °C/72 hours
PEG—Polyethylene Glycol	70 °C/24 hours
EtOH—Ethanol	121 °C/1 hour
PBS—Phosphate Buffered Saline	Sponsor Specific
SWFI—Sterile Water for Injection	Not Applicable
PW—Purified Water	
PBS—Phosphate Buffered Saline	

Section 4. Storage & Dispensation of Test Material

- Storage Conditions: Please select (Room temperature, 2-8°C, -20°C or -80°C)
- Dispensation Instructions: Please indicate to **Dispose or Return** your sample(s). Samples will automatically be disposed of after 90 days if this information is not specified.

Section 5. Sponsor Signature: The final test request form submitted must include a signature. Forms with missing signature will delay scheduling of the entire project.

Section 6. Where to Send Samples and Forms

Fax Forms to: (781) 271-1138

Mail to: **Attn: Sample Log-in**
Toxikon Corporation
15 Wiggins Avenue
Bedford, MA 01730

TOXIKON
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Biocompatibility / Safety Evaluation Guidelines for Global Compliance

DEVICES

Toxikon Corp.
15 Wiggins Ave
Bedford, MA 01730
USA
800.458.4141
800.425.4044 (Midwest office)
info@toxikon.com

Toxikon Europe N.V.
Romeinsestraat 12
B-3001 Leuven
Belgium
+32.16.400484
info@toxikon.be

Toxikon India
21, Parmeshwar
353/20 R B Mehta Marg
Ghatkopar East
Mumbai 400 077
+91.22.67983604
rajiv.desai@toxikon.com

Toxikon Asia
Moriya Sangyo K.K.
3-36-7 Wada.Suginami-Ku
Tokyo, Japan 166-0012
+011.813.5306.6267
sangyo@morisan.com

FDA, ISO and Japanese MHLW Test Selection Chart

Device Categories		Biological Effects Initial Evaluation									Biological Tests Supplemental Evaluation			
Body Contact	Contact Duration	Cytotoxicity	Sensitization	Irritation/Intracutaneous Reactivity	Acute Systemic Toxicity	Pyrogenicity	Subacute/Subchronic Toxicity	Genotoxicity	Implantation	Hemocompatibility	Chronic Toxicity	Carcinogenicity	Reproductive/ Developmental Toxicology	Biodegradation
Surface Devices														
Skin	A	■	■	■										
	B	■	■	■										
	C	■	■	■										
Mucosal Membranes	A	■	■	■										
	B	■	■	■	●	●	●		●					
	C	■	■	■	●	●	■	■	●		●			
Breached/Compromised Surfaces	A	■	■	■	●	●								
	B	■	■	■	●	●	●		●					
	C	■	■	■	●	●	■	■	●		●			
External Communicating Devices														
Blood Path, Indirect	A	■	■	■	■	■				■				
	B	■	■	■	■	■	●			■				
	C	■	■	●	■	■	■	■	●	■	■	■		
Tissues/Bones/Dentin	A	■	■	■	●	●								
	B	■	■	□	□	□	□	■	■					
	C	■	■	□	□	□	□	■	■		□	□		
Circulating Blood	A	■	■	■	■	■		●		■				
	B	■	■	■	■	■	□	■	□	■				
	C	■	■	■	■	■	■	■	□	■	■	■		
Implant Devices														
Tissues/Bones	A	■	■	■	●	●								
	B	■	■	□	□	□	□	■	■					
	C	■	■	□	□	□	□	■	■		■	■		
Blood	A	■	■	■	■	■	◆		■	■				
	B	■	■	■	■	■	□	■	■	■				
	C	■	■	■	■	■	■	■	■	■	■	■		

A	Limited Exposure (less than 24 hours)
B	Prolonged Exposure (24 hours to 30 days)
C	Permanent Exposure (More than 30 days)
■	Evaluation Required by FDA, ISO, and MHLW
□	Evaluation Required by FDA and ISO
●	Evaluation Required by FDA
◆	Evaluation Required by ISO

Sample Requirements for Standard Studies

Cytotoxicity Tests	
Neutral Red Uptake (NRU)	30 cm² if < 0.5 mm thick, 15 cm² ≥ 0.5 mm, 1 gram
MEM Elution	USP: 120 cm² if < 0.5 mm thick; 4 grams
	ISO: 120 cm² if < 0.5 mm thick; 60 cm² if ≥ 0.5 mm thick; 4 grams
	MHLW: 8 grams; 120 cm2 or 240 cm²
Agar Diffusion	USP: 2 pieces, each 1 cm x 1 cm
	ISO: 3 pieces, each 100 mm²
Genetox/Mutagenicity Tests	
Ames Assay	ISO: 240 cm² if < 0.5 mm thick; 120 cm² if ≥ 0.5 mm thick; 8 grams
	MHLW: 20 mL; 8 grams; 120 cm²; 240 cm²
Mouse Micronucleus Assay	ISO: 600 cm² if < 0.5 mm, 300 cm² if ≥ 0.5 mm, 25 grams
Chromosomal Aberration Assay	ISO: 240 cm² if < 0.5 mm thick; 120 cm² if ≥ 0.5 mm thick; 8 grams
	MHLW: 8 grams; 120 cm² or 240 cm²
Mouse Lymphoma Forward Mutation Assay	ISO: 300 cm² if < 0.5 mm thick; 150 cm² if ≥ 0.5 mm thick; 10 grams
Hemocompatibility Tests	
Hemolysis Complete (ASTM Method)	ISO: 500 cm² if < 0.5 mm thick; 250 cm² if ≥ 0.5 mm thick; 40 grams
Hemolysis Direct (ASTM Method)	ISO: 250 cm² if < 0.5 mm thick; 125 cm² if ≥ 0.5 mm thick; 20 grams
Hemolysis Indirect (ASTM Method)	ISO: 250 cm² if < 0.5 mm thick; 125 cm² if ≥ 0.5 mm thick; 20 grams
	MHLW: 600 cm² if < 0.5 mm thick; 300 cm² if ≥ 0.5 mm thick; 25 grams
Complement Activation Direct	ISO: 75 cm² if < 0.5 mm thick; 50 cm² if ≥ 0.5 mm thick; 2.5 grams
Complement Activation Indirect	ISO: 10 cm² if < 0.5 mm thick; 5 cm² if ≥ 0.5 mm thick; 1 grams
In Vitro Hemocompatibility Direct	ISO: 75 cm² if < 0.5 mm thick; 50 cm² if ≥ 0.5 mm thick; 2.5 grams
In Vitro Hemocompatibility Indirect	ISO: 10 cm² if < 0.5 mm thick; 5 cm² if ≥ 0.5 mm thick; 1 grams
Unactivated Partial Thromboplastin Time Direct	ISO: 75 cm² if < 0.5 mm thick; 50 cm² if ≥ 0.5 mm thick; 2.5 grams
Unactivated Partial Thromboplastin Time Indirect	ISO: 10 cm² if < 0.5 mm thick; 5 cm² if ≥ 0.5 mm thick; 1 grams
Thrombogenicity in Dogs	ISO: 2 units
In Vivo Tests	
Kligman Maximization	ISO: 720 cm² if < 0.5 mm thick; 360 cm² if ≥ 0.5 mm thick; 24 grams
	MHLW: Inquire
Buehler Closed Patch	ISO: 120 pieces of 2.5 cm x 2.5 cm patches each, or 60 grams
Intracutaneous Injection/Irritation	ISO: 240 cm² ≤ 0.5 mm thick, 120 cm² ≥ 0.5 mm thick, or 8 grams
	MHLW: 16 grams; 240 cm² or 480 cm²
Primary Skin Irritation	ISO: 240 cm² < 0.5 mm thick, 120 cm² ≥ 0.5 mm thick, or 8 grams
Acute Systemic Injection	ISO: 240 cm² ≤ 0.5 mm thick, 120 cm² ≥ 0.5 mm thick, or 8 grams
	MHLW: 16 grams; 240 cm² or 480 cm²
Material Mediated Rabbit Pyrogen	USP: 600 cm² if < 0.5 mm thick; 300 cm² if ≥ 0.5 mm thick; 20 grams
	ISO: 600 cm² if < 0.5 mm thick; 300 cm² if ≥ 0.5 mm thick; 20 grams
	MHLW: 600 cm² if < 0.5 mm thick; 300 cm² if ≥ 0.5 mm thick; 20 grams
Class Tests per USP	Class I: 120 cm² < 0.5 mm thick, 60 cm² ≥ 0.5 mm thick, or 4 grams
	Class II: 240 cm² < 0.5 mm thick, 120 cm² ≥ 0.5 mm thick, or 8 grams
	Class III: 480 cm² < 0.5 mm thick, 1200 cm² ≥ 0.5 mm thick, or 16 grams
	Class IV: 360 cm² < 0.5 mm thick, 180 cm² ≥ 0.5 mm thick, or 12 grams
	Class V: 480 cm² < 0.5 mm thick, 240 cm² ≥ 0.5 mm thick, or 16 grams
	Class VI: 550 cm² < 0.5 mm thick, 275 cm² ≥ 0.5 mm thick, or 22 grams
Implants (Muscle, Subcutaneous, Bone, etc.)	ISO: 18 strips per time point, each strip 10 mm x 1mm. Sample should be supplied by sponsor in specified size, separately packaged and sterilized, and edges should be rounded and smooth.
▪ 2 Week	
▪ 4 Week	
▪ 6 Week	
▪ 8 Week	
▪ 12 Week	
▪ 13 Week	
▪ 26 Week	
▪ 52 Week	
▪ 1 & 4 Week Implant	MHLW: 24 pieces, each 1mm x 1mm x 10mm
General Chemistry Tests	
Physicochemical and other Compendial Tests	USP: 20 grams or 600 cm² / Inquire

CYTOTOXICITY TESTS—ISO 10993-5

PURPOSE: Cytotoxicity tests are in-vitro assays used to assess the possibility of a test article to cause the death of cells in culture or to prevent their multiplication.

Agar Diffusion – *Sample needed: 6 cm^2 or 1mL (at least 3 units if 1 unit <1 cm^2)*

The purpose of the study is to determine the potential biological reactivity of a mammalian cell culture (L929) in response to the test article extract. The cells are allowed to grow to approximately 80% confluency in cell culture dishes and then overlaid with an agarose layer. The test article is placed over the agar layer allowing the diffusion of leachables onto the cell layer. 3 replicates plates are prepared per article. The plates are incubated for 48 hours at 37 °C. The biological reactivity of the cells around the test article is visually observed, and graded on a scale of 0 (no reactivity) to 4 (severe reactivity). The test article is considered non-cytotoxic if none of the cultures exposed to the test article shows greater than mild reactivity (Grade 2). This assay is appropriate for screening purposes.

Approximate turnaround time is two to three weeks.

Agar Diffusion with Extraction – *Sample needed: 12 cm² < 0.5 mm thick, 6 cm² ≥ 0.5 mm thick, or 0.4 g*

The purpose of the study is to determine the potential biological reactivity of a mammalian cell culture (L929) in response to the test article extract. The cells are allowed to grow to approximately 80% confluency in cell culture dishes and then overlaid with an agarose layer. The test article extract is placed over the agar layer on a filter disc allowing the diffusion of extractables onto the cell layer. 3 replicates plates are prepared per article. The plates are incubated for 48 hours at 37 °C. The biological reactivity of the cells around the test article extract is visually observed, and graded on a scale of 0 (no reactivity) to 4 (severe reactivity). The test article is considered non-cytotoxic if none of the cultures exposed to the test article extract shows greater than mild reactivity (Grade 2). This assay is appropriate for screening purposes. This a specialized variation from the Agar Diffusion Test.

Approximate turnaround time is two to three weeks.

L929 MEM Elution – *Sample needed: 120 cm² < 0.5 mm thick, 60 cm² ≥ 0.5 mm thick, or 4 g*

The purpose of the study is to determine the potential biological reactivity of a mammalian cell culture (L929) in response to the test article extract. The test article is extracted usually in Minimum Essential Medium (MEM) with 10% Fetal Bovine Serum for usually 24 hours at 37 °C. The cells are allowed to grow to sub-confluency in tissue culture plates and exposed to the test article extract at neat (100%) in 3 replicates. The plates are incubated for 48 hours at 37 °C. The biological reactivity of the cells following the exposure to the extracts is visually observed with a microscope, and graded on a scale of 0 (no reactivity) to 4 (severe reactivity). The test article is considered non-cytotoxic if none of the cultures exposed to the test article extract shows greater than mild reactivity (Grade 2). This assay is the default assay appropriate for screening purposes.

Approximate turnaround time is two to three weeks.

L929 MTT Cytotoxicity (1 Concentration)
L929 MTT Cytotoxicity (4 Concentrations) – *Sample needed: 30 cm² < 0.5 mm thick, 15 cm² ≥ 0.5 mm thick, or 1 g*

The purpose of the study is to determine the potential biological reactivity of a mammalian cell culture (L929) in response to the test article extract. The test article is extracted usually in Minimum Essential Medium (MEM) with 10% Fetal Bovine Serum for usually 24 hours at 37 °C. The cells are allowed to grow to sub-confluency in tissue culture plates and exposed to the test article extract at 1 (neat, 100%) or 4 (100%, 50%, 25%, 12.5%) concentrations in 6 replicates. The plates are incubated for 24 hours at 37 °C after which the viability is assessed via the reduction of the yellow water-soluble MTT by the mitochondrial reductases enzymes into a blue-violet insoluble formazan. The number of viable cells correlates to the color intensity determined by photometric measurements after dissolving the formazan. The test article is considered non-cytotoxic if the percentage of viable cell is equal to or greater than 70% of the untreated control. This assay is appropriate for biocompatibility testing.

Approximate turnaround time is two to three weeks.

CYTOTOXICITY TESTS—ISO 10993-5 *(cont.)*

Neutral Red Uptake (NRU) Cytotoxicity (1 Concentration)
Neutral Red Uptake (NRU) Cytotoxicity (4 Concentrations) – *Sample needed: 30 cm² < 0.5 mm thick, 15 cm² ≥ 0.5 mm thick, or 1 g*

The purpose of the study is to determine the potential biological reactivity of a mammalian cell culture (L929) in response to the test article extract. The test article is extracted usually in Minimum Essential Medium (MEM) with 10% Fetal Bovine Serum for usually 24 hours at 37 °C. The cells are allowed to grow to sub-confluency in tissue culture plates and exposed to the test article extract at 1 (neat, 100%) or 4 (100%, 50%, 25%, 12.5%) concentrations in 6 replicates. The plates are incubated for 24 hours at 37 °C after which the viability is assessed via the incorporation of Neutral Red (NR) by the lysosomes of the live cells. The number of viable cells correlates to the color intensity determined by photometric measurements after dissolving the NR. The test article is considered non-cytotoxic if the percentage of viable cell is equal to or greater than 70% of the untreated control. This assay is the default assay appropriate for biocompatibility testing.

Approximate turnaround time is two to three weeks.

SENSITIZATION TESTS—ISO 10993-10

PURPOSE: Sensitization tests are in-vivo assays used to assess the allergic or sensitizing capacity to the repeated or prolonged exposure of a test article. Sensitization is characterized by the fact that reactions are delayed, not localized, and independent of dose.

Kligman Guinea Pig Maximization Test (GPMT)
1 Extract – *Sample needed: 360 cm² < 0.5 mm thick, 180 cm² ≥ 0.5 mm thick, or 12 g*
2 Extracts – *Sample needed: 720 cm² < 0.5 mm thick, 360 cm² ≥ 0.5 mm thick, or 24 g*
Direct Exposure – *Sample needed: 12 g*

The purpose of the study is to determine the allergenic potential or sensitizing capacity of the test article in guinea pigs. The animal is exposed to the test article (or test article extract). Extracts of the test article are prepared in a polar (saline) and/or non-polar (cotton seed oil) solution. The assay begins with intradermal injections of Freund's Complete Adjuvant (FCA) and the test article. Seven days later the injection sites are covered with the test article/extract for a period of 48 hours. Fourteen days later a new site is challenged with a topical application of the test article/extract and scored out to 72 hours. A sensitization reaction to the test article is scored based on the defined evaluation criteria in ISO 10993-10.

Approximate turnaround time is seven to nine weeks.

Buehler Sensitization
Direct Exposure – *Sample needed: 120 pieces of 2.5 cm x 2.5 cm patches each, or 17 g*

The purpose of the study is to determine the allergenic potential or sensitizing capacity of the test article in guinea pigs. The test article is exposed to the assay system by direct application. In the "induction phase" the test article is applied topically to the skin as closed patches, for three exposures per week for three weeks. Fourteen days after the induction phase, the challenge assay will be performed by the application of a assay patch to new sites and observed for erythema and edema at twenty-four and forty-eight hours. A sensitization reaction to the test article (or test article extract) is scored based on the defined evaluation criteria in ISO 10993-10.

Approximate turnaround time is seven to nine weeks.

Murine Local Lymph Node Assay
1 Extract – *Sample needed: 120 cm² < 0.5 mm thick, 60 cm² ≥ 0.5 mm thick, or 4 g*
2 Extracts – *Sample needed: 240 cm² < 0.5 mm thick, 120 cm² ≥ 0.5 mm thick, or 8 g*

The purpose of the study is to determine the allergenic potential or sensitizing capacity of the test article in the lymph nodes of mice. This reaction is measured by an increase in proliferation of lymphocytes in the lymph nodes draining the site of administration. The assay procedure is used for screening contact allergens in mice and extrapolating the results to humans but does not establish the

SENSITIZATION TESTS—ISO 10993-10 *(cont.)*

actual risk of sensitization. The study involves three consecutive days of topical dosing to both ears followed by two days without treatment. On the sixth day, 5 hours before harvest, an injection containing 20 μCi of H-methyl thymidine is administered. The draining lymph nodes are subsequently harvested and a single cell suspension made. The Stimulation Index (SI) is obtained by comparing the proliferation of lymph node cells in assay animals versus that of the control animals. The test article is considered negative if the test article produces Stimulation Index less than 3.0.

Approximate turnaround time is four to five weeks.

IRRITATION TESTS—ISO 10993-10

PURPOSE: Irritation tests are in-vivo assays to assess the potential of test articles (or test article extracts) to cause irritation on the exposed part of the body. Standard studies are single exposure evaluations. Based on clinical product use, studies may be customized for evaluation of repeat exposures.

Intracutaneous Injection
1 Extract – *Sample needed: 120 cm² < 0.5 mm thick, 60 cm² ≥ 0.5 mm thick or 4 g*
2 Extracts – *Sample needed: 240 cm² < 0.5 mm thick, 120 cm² ≥ 0.5 mm thick or 8 g*
3 Extracts – *Sample needed: 360 cm² < 0.5 mm thick, 180 cm² ≥ 0.5 mm thick or 12 g*
4 Extracts – *Sample needed: 480 cm² < 0.5 mm thick, 240 cm² ≥ 0.5 mm thick or 16 g*
Direct Exposure – *Sample needed: 10 mL*

The purpose of the study is to determine local responses to solutions or extracts following intracutaneous injections into rabbits. The test article will be exposed to the assay system directly or through test article extracts. Extracts of the test article are prepared in a polar (saline) and/or non-polar (cotton seed oil) solutions. Two (USP) or three (ISO) rabbits are injected intracutaneously with the test article and control articles. The injected sites are examined over a seventy-two hour period for evidence of tissue reaction such as erythema, edema, or necrosis. Observations are scored according to the Classification System for Scoring Skin Reactions. At the end of the observation period the scores are used to determine an overall mean reaction score for the test article versus the corresponding control article. The requirements of the assay are met if the difference of the mean reaction score for the test article and the control article is 1.0 or less.

Approximate turnaround time is four to five weeks.

Primary Skin Irritation
1 Exposure/1 Extract – *Sample needed: 120 cm² < 0.5 mm thick, 60 cm² ≥ 0.5 mm thick or 4 g*
1 Exposure/2 Extracts – *Sample needed: 240 cm² < 0.5 mm thick, 120 cm² ≥ 0.5 mm thick or 8 g*
Direct Exposure – *Sample needed: 6 pieces at 2.5 cm x 2.5 cm, or 4 g*

The purpose of the study is to determine the potential of a test article to produce dermal irritation after a single topical exposure in rabbits. The test article may be exposed directly or through test article extracts. Extracts of the test article are prepared in a polar (saline) and/or non-polar (cotton seed oil) solutions. After preparing the skin sites, the test article is kept in contact with the skin by wrapping with an impervious bandage for a minimum exposure of four hours. After the exposure period, the test article is removed and the skin sites are observed typically over a seventy-two hour observation period for signs of irritation. Observations are scored according to the Classification System for Scoring Skin Reactions (Draize scale). The scores obtained are used to calculate the Primary Irritation Index

Approximate turnaround time is five to six weeks.

Primary Ocular Irritation
1 Exposure/1 Extract – *Sample needed: 120 cm² < 0.5 mm thick, 60 cm² ≥ 0.5 mm thick or 4 g*
1 Exposure/2 Extracts – *Sample needed: 240 cm² < 0.5 mm thick, 120 cm² ≥ 0.5 mm thick or 8 g*
Direct Exposure – *Sample needed: 5 mL*

The purpose of the study is to determine the potential of articles that will come in contact with the eye or eyelid. The test article will be exposed to the test system directly or through test article extracts.

Extracts of the test article are prepared in a polar (saline) and/or non-polar (cottonseed oil) solutions. Rabbits are treated by placing the test article into the lower conjunctival sac of the left eye of each animal. The right eye remains untreated and serves as a control. The eyes are examined over a period of 72 hours and scored according to the Classification System for grading ocular lesions. The test article is considered an eye irritant if more than one of the animals in the assay group exhibits a positive reaction.

Approximate turnaround time is five to six weeks.

Primary Buccal (Mucosal) Irritation
1 Exposure/1 Extract – *Sample needed: 120 cm² < 0.5 mm thick, 60 cm² ≥ 0.5 mm thick or 4 g*
1 Exposure/2 Extracts – *Sample needed: 240 cm² < 0.5 mm thick, 120 cm² ≥ 0.5 mm thick or 8 g*
Direct Exposure – *Sample needed: 4 g*

The purpose of the study is to determine the potential of articles or devices to produce irritation when in contact with the buccal tissue (cheek pouch) of three Syrian Golden Hamsters. The test article is exposed to the assay system directly or through test article extracts. Extracts of the test article are prepared in a polar (saline) and/or non-polar (cotton seed oil) solutions. The animals are exposed to the test article for a minimum of five minutes every hour for four hours. Test and control sites are scored macroscopically after the last dose and again at twenty-four hours. Following the twenty-four hour scoring, tissues are collected for microscopic evaluation by a pathologist. The scores obtained from the microscopic evaluation are used to determine the irritation level. The irritation level (group average) for the control is subtracted from the group average of the test article to obtain the Irritation Index. The Irritation Index for the test article is categorized as a non-, minimal, mild, moderate or severe irritant.

Approximate turnaround time is seven to eight weeks.

Bladder Irritation with Histology
1 Extract – *Sample needed: 800 cm² < 0.5 mm thick, 400 cm² ≥ 0.5 mm thick, or 32 g, or 150 mL*
2 Extracts – *Sample needed: 40 g*
Direct Exposure – *Sample needed: 150 mL*

The purpose of the study is to determine the potential of the test article to produce bladder irritation and is considered only for articles with intended contact with the bladder. The test article or extract is placed in the bladder of New Zealand White rabbits daily for five consecutive days. After the exposure period, assay and control sites are scored macroscopically, the tissues collected and preserved, and evaluated microscopically by a pathologist. The scores obtained from the microscopic evaluation are used to determine the irritation level then the irritation level (group average) for the control article is subtracted from the group average of the test article to obtain the Irritation Index. The Irritation Index for the test article is categorized as a non-, minimal, mild, moderate or severe irritant.

Approximate turnaround time is four to six weeks.

Vaginal Irritation with Histology
1 Extract – *Sample needed: 240 cm² < 0.5 mm thick, 120 cm² ≥ 0.5 mm thick, or 8 g*
2 Extracts – *Sample needed: 480 cm² < 0.5 mm thick, 240 cm² ≥ 0.5 mm thick, or 16 g*
Direct Exposure – *Sample needed: 20 mL*

The purpose of the study is to determine the potential of the test article to produce vaginal irritation after five consecutive days of exposure. This assay is considered only for articles with intended contact with the vagina. The test article is exposed to the assay system directly or through test article extracts. Extracts of the test article are prepared in a polar (saline) and/or non-polar (cotton seed oil) solutions. Animals will be observed for irritation and clinical signs 24 hours after the initial application and immediately prior to each daily treatment. The final observation will be made twenty-four hours after the final application. Observations will be scored for erythema and edema according to the Classification System for Oral, Penile, Rectal, and Vaginal Reaction. Animals will be necropsied twenty-four hours after the last dose and the tissue scored macroscopically. The tissue will be processed and histological slides of hematoxylin and eosin stained sections will be prepared and evaluated by a Veterinary Pathologist. The irritation level (group average) for the control article will be

IRRITATION TESTS—ISO 10993-10 *(cont.)*

subtracted from the group average of the test article to obtain the Irritation Index. The Irritation Index for the test article is categorized as non-, minimal, mild, moderate or severe irritant.

Approximate turnaround time is four to six weeks.

Rectal Irritation with Histology
1 Extract – *Sample needed: 120 cm² < 0.5 mm thick, 60 cm² ≥ 0.5 mm thick, or 4 g*
2 Extracts – *Sample needed: 2470 cm² < 0.5 mm thick, 120 cm² ≥ 0.5 mm thick, or 8 g*
Direct Exposure – *Sample needed: 20 mL*

The purpose of the study is to determine the potential of the test article to produce rectal irritation after application for five consecutive days. This assay is considered only for articles with intended rectal contact. The test article is exposed to the assay system directly or through test article extracts. Extracts of the test article are prepared in a polar (saline) and/or non-polar (cotton seed oil) solutions. Animals will be observed for irritation and clinical signs twenty-four hours after the initial application and immediately prior to each daily treatment. The final observation will be made 24 hours after the final test article application. Observations will be scored for erythema and edema according to the Classification System for Oral, Penile, Rectal, and Vaginal Reaction. Animals will be necropsied twenty-four hours after the last dose and the tissue scored macroscopically. The tissue will be processed and histological slides of hematoxylin and eosin stained sections will be prepared and evaluated by a Veterinary Pathologist. The irritation level (group average) for the control article will be subtracted from the group average of the test article to obtain the Irritation Index.

Approximate turnaround time is four to six weeks.

Penile Irritation with Histology
1 Extract – *Sample needed: 120 cm² < 0.5 mm thick, 60 cm² ≥ 0.5 mm thick, or 4 g*
2 Extracts – *Sample needed: 240 cm² < 0.5 mm thick, 120 cm² ≥ 0.5 mm thick, or 8 g*
Direct Exposure – *Sample needed: 20 mL*

The purpose of the study is to determine the potential of the test article to produce penile irritation after applications repeated every hour for four hours. This assay is considered only for articles with intended contact with the penis. The test article is exposed to the assay system directly or through test article extracts. Extracts of the test article are prepared in a polar (saline) and/or non-polar (cotton seed oil) solutions. Irritation observations will be made at 1, 24, and 48 hours after the final test article application. Observations will be scored for erythema and edema according to the Classification System for Oral, Penile, Rectal, and Vaginal Reaction. Animals will be necropsied 48 hours after the last dose and the tissue scored macroscopically. The tissue will be processed and histological slides of hematoxylin and eosin stained sections will be prepared and evaluated by a Veterinary Pathologist. The irritation level (group average) for the control article will be subtracted from the group average of the test article to obtain the Irritation Index. The irritation index for the test article is categorized as a non-irritant, minimal, mild, moderate or severe irritant.

Approximate turnaround time is four to six weeks.

SYSTEMIC TOXICITY TESTING—ISO 10993-11

PURPOSE: Systemic toxicity tests are in-vivo assays used to assess the impairment or activation of a system – rather than the impairment of individual cells or organs. Systemic toxicity assays evaluate a test article's potential to induce a systemic response after exposure to the test article. Categories are based on duration of exposure and dose route.

Acute systemic toxicity assaying is the most commonly performed, and includes a single exposure with a 72 hour observation period.

Repeat or continuous exposure periods are classified as Sub-acute, Sub-Chronic, and Chronic. The route of exposure should be chosen based on clinical relevance. Possible dose routes include implantation, intraperitoneal, intravenous, oral, and subcutaneous. Exposure to the test article may be in the form of an extract or direct exposure of the test article, as appropriate.

SYSTEMIC TOXICITY TESTING—ISO 10993-11 *(cont.)*

Unlike acute systemic toxicity, the sub-acute, sub-chronic, and chronic toxicity studies, include full clinical pathology (clinical chemistry, hematology, and coagulation), necropsy and organ weights, as well as full histopathology.

Study Design Comparisons

Study Type	Animal Number		Study Exposure Period	Body Weight	Clinical Observation	Clinical Pathology	Gross Pathology	Organ Weights	Histopathology
	Rodent	Non-Rodent							
Acute	5	3	Up to 24 hours	Daily	Daily	Not Typical	Not Typical	Not Typical	Not Typical
Sub-Acute	10 (5 per sex/group)	Minimum 6 (3 per sex/group)	Intravenous 1 to 14 days Other routes 14 to 28 days	Weekly	Daily	Yes	Yes	Yes	Yes
Sub-Chronic	20 (10 per sex/group)	Minimum 8 (4 per sex/group)	Intravenous 14 to 28 days Other routes 90 days in rodents Other routes, other species, <10% of lifespan	Weekly	Daily	Yes	Yes	Yes	Yes
Chronic	40 (20 per sex/group)	16 (8 per sex/group)	6 to 12 months Major portion of lifespan	Weekly	Daily	Yes	Yes	Yes	Yes

ACUTE SYSTEMIC TOXICITY TESTS

Acute Systemic Injection Assay
1 Extract – *Sample needed: 120 cm² ≤ 0.5mm thick, 60 cm² ≥ 0.5 mm thick, or 4 g*
2 Extracts – *Sample needed: 240 cm² ≤ 0.5mm thick, 120 cm² ≥ 0.5 mm thick, or 8 g*
3 Extracts – *Sample needed: 360 cm² ≤ 0.5mm thick, 180 cm² ≥ 0.5 mm thick, or 12 g*
4 Extracts – *Sample needed: 480 cm² ≤ 0.5mm thick, 240 cm² ≥ 0.5 mm thick, or 16 g*
Direct Exposure – *Sample needed: Minimum of 10 mLs*

The purpose of the study is to determine solutions and test article extracts for potential toxic effects as a result of a single-dose systemic injection in mice. The test article is exposed to the assay system directly or through test article extracts. Extracts of the test article are prepared in polar (saline) and/or non-polar (cottonseed oil) solutions. The test article is injected intravenously and/or intraperitoneally in groups of five mice. The animals are observed for 72 hours after administration for signs of biological reactivity. This assay is considered negative if none of the animals injected with the test article show a significantly greater biological reaction than the animals treated with the control article. If two or more mice die, or show signs of toxicity, or if three or more mice lose more than 2 g of body weight, the test article does not meet the requirements of the assay.

Approximate turnaround time is four weeks.

Article-Mediated Rabbit-Pyrogen Assay – *Sample needed: 600 cm² < 0.5 mm thick, 300 cm² ≥ 0.5 mm thick, or 20 g*

ACUTE SYSTEMIC TOXICITY TESTS *(cont.)*

The purpose of the study is to determine the risk of a fever reaction as a result of the administration of the test article solution or test article extract. The test article solution or test article extract is administered by intravenous injection into three New Zealand White rabbits. The rectal temperatures of the injected rabbits are compared with the temperature of a control rabbit similarly injected. The baseline temperatures of the rabbits, as determined no more than thirty minutes prior to injection of the test article, are used to exclude rabbits whose body temperatures vary by more than 1 °C from each other and whose temperatures are greater than 39.8 °C. Body temperatures are recorded in 30 minute intervals between 1 and 3 hours subsequent to injection. If no rabbit exhibits a rise in temperature of 0.5 °C or more above its baseline temperature, the product meets the requirements for the absence of pyrogens.

Approximate turnaround time is three to four weeks.

SUBACUTE SYSTEMIC TOXICITY TESTING

14-Day IV Injection Subacute Toxicity Study – *Sample needed: 336 g, or 10,080 cm² if < 0.5 mm thick, 5,040 cm² if > 0.5 mm thick*

Approximate turnaround time is ten to twelve weeks.

Abridged-14 Day IV Dose Toxicity Study – *Sample needed: 336 g, or 10,080 cm² if < 0.5 mm thick, 5,040 cm² if > 0.5 mm thick*

Approximate turnaround time is nine weeks.

Study Design Options—Intravenous Dose				
	ISO 10993-11:2006 Compliant		Abridged Design	
Groups	**Males**	**Females**	**Males**	**Females**
Test Article	5	5	5	5
Control	5	5	5	5
Total No.	*N=20*		*N=20*	
Species	Sprague Dawley Rats		Sprague Dawley Rats	
Dose Route	Intravenous tail vein injection		Intravenous tail vein injection	
Dose Frequency	Daily for 14 days		Daily for 14 days	
Dose Volume	Specified by Sponsor up to 40 mL/kg		Up to 40 mL/kg	
Clinical Observations	Daily		*Same*	
Body Weights	Weekly		*Same*	
Clinical Pathology	All groups terminal; Standard panels for clinical chemistry, hematology, and coagulation		*Same*	
Necropsy	Gross observations and tissue collection		*Same*	
Organ Weights	Standard tissues by ISO guidelines		*Same*	
Tissue Preservation & Storage	All tissues preserved and processed for histopathology (see below)		Approx. 36 tissues preserved in formalin and stored for up to 1 year; Additional charges will apply for future tissue analysis if requested by Sponsor	
Histology/Pathology	Approximately 45 tissues processed and evaluated by a board-certified pathologist		Up to 12 target organs processed and evaluated by a board-certified pathologist	
Reporting	Standard final report		Standard final report	

**Note: Modified designs for alternate dose routes, study duration, and study parameters are available upon request.*

SUBACUTE SYSTEMIC TOXICITY TESTING (Cont.)

28-Day Sub-Acute Toxicity Study – *Sample needed: To be determined*
Approximate turnaround time is fourteen to sixteen weeks

Abridged-28-Day Toxicity Study – *Sample needed: To be determined*
Approximate turnaround time is fourteen weeks.

Study Design Options—Other than Intravenous				
	ISO 10993-11:2006 Compliant		Abridged Design	
Groups	**Males**	**Females**	**Males**	**Females**
Test Article	5	5	5	5
Control	5	5	5	5
Total No.	*N=20*		*N=20*	
Species	Sprague Dawley Rats		Sprague Dawley Rats	
Dose Route	Routes other than intravenous (ie: intraperitoneal injection or implant)		Routes other than intravenous (ie: intraperitoneal injection or implant)	
Study Duration	28 days		28 days	
Clinical Observations	Daily		*Same*	
Body Weights	Weekly		*Same*	
Clinical Pathology	All groups terminal; Standard panels for clinical chemistry, hematology, and coagulation		*Same*	
Necropsy	Gross observations and tissue collection		*Same*	
Organ Weights	Standard tissues by ISO guidelines		*Same*	
Tissue Preservation & Storage	All tissues preserved and processed for histopathology (see below)		Approx. 33 tissues preserved in formalin and stored for up to 1 year; Additional charges will apply for future tissue analysis if requested by Sponsor	
Histology/Pathology	Approximately 45 tissues processed and evaluated by a board-certified pathologist		Up to 12 target organs processed and evaluated by a board-certified pathologist	
Reporting	Standard final report		Standard final report	

**Note: Modified designs for alternate dose routes, study duration, and study parameters are available upon request.*

SUBCHRONIC SYSTEMIC TOXICITY TESTING

28-Day IV Injection Sub-Chronic Toxicity Study – *Sample needed: To be determined, if < 0.5 mm thick 20,180 cm^2 or 672 g*

Approximate turnaround time is twelve to fourteen weeks.

Abridged-28 Day IV Dose Toxicity Study – *Sample needed: To be determined, if < 0.5 mm thick 20,180 cm^2 or 672 g*

Approximate turnaround time is eleven weeks.

Study Design Options—Intravenous Dose	ISO 10993-11:2006 Compliant		Abridged Design	
Groups	**Males**	**Females**	**Males**	**Females**
Test Article	10	10	10	10
Control	10	10	10	10
Total No.	*N=40*		*N=40*	
Species	Sprague Dawley Rats		Sprague Dawley Rats	
Dose Route	Intravenous tail vein injection		Intravenous tail vein injection	
Dose Frequency	Daily for 28 days		Daily for 28 days	
Dose Volume	Up to 40 mL/kg		Up to 40 mL/kg or as specified by Sponsor	
Clinical Observations	Daily		*Same*	
Body Weights	Weekly		*Same*	
Clinical Pathology	All groups terminal; Standard panels for clinical chemistry, hematology, and coagulation		*Same*	
Necropsy	Gross observations and tissue collection		*Same*	
Organ Weights	Standard tissues by ISO guidelines		*Same*	
Tissue Preservation & Storage	All tissues preserved and processed for histopathology (see below)		Approx. 33 tissues preserved in formalin and stored for up to 1 year; Additional charges will apply for future tissue analysis if requested by Sponsor	
Histology/Pathology	Approximately 45 tissues processed and evaluated by a board-certified pathologist		Up to 12 target organs processed and evaluated by a board-certified pathologist	
Reporting	Standard final report		Standard final report	

**Note: Modified designs for alternate dose routes, study duration, and study parameters are available upon request.*

SUBCHRONIC SYSTEMIC TOXICITY TESTING (Cont.)

90-Day Sub-Chronic Toxicity Study – *Sample needed: To be determined*
Approximate turnaround time is twenty-one to twenty-three weeks

Abridged-90-Day Toxicity Study – *Sample needed: To be determined*
Approximate turnaround time is twenty-one weeks.

Study Design Options—Other than Intravenous				
	ISO 10993-11:2006 Compliant		Abridged Design	
Groups	**Males**	**Females**	**Males**	**Females**
Test Article	10	10	10	10
Control	10	10	10	10
Total No.	*N=40*		*N=40*	
Species	Sprague Dawley Rats		Sprague Dawley Rats	
Dose Route	Routes other than intravenous (ie: intraperitoneal injection or implant)		Routes other than intravenous (ie: intraperitoneal injection or implant)	
Study Duration	90 days		90 days	
Clinical Observations	Daily		*Same*	
Body Weights	Weekly		*Same*	
Clinical Pathology	All groups terminal; Standard panels for clinical chemistry, hematology, and coagulation		*Same*	
Necropsy	Gross observations and tissue collection		*Same*	
Organ Weights	Standard tissues by ISO guidelines		*Same*	
Tissue Preservation & Storage	All tissues preserved and processed for histopathology (see below)		Approx. 33 tissues preserved in formalin and stored for up to 1 year; Additional charges will apply for future tissue analysis if requested by Sponsor	
Histology/Pathology	Approximately 45 tissues processed and evaluated by a board-certified pathologist		Up to 12 target organs processed and evaluated by a board-certified pathologist	
Reporting	Standard final report		Standard final report	

**Note: Modified designs for alternate dose routes, study duration, and study parameters are available upon request.*

CHRONIC SYSTEMIC TOXICITY

PURPOSE: Chronic systemic toxicity tests are in-vivo assays used to assess the impairment or activation of a system – rather than the impairment of individual cells or organs. In systemic toxicity assays, the test article extract is evaluated for systemic toxic effects of long-term exposures, a minimum of six months duration.

26-Week Chronic Toxicity Study – *Sample needed: To be determined*
Approximate turnaround time is thirty-four to thirty-six weeks.

Abridged 26-Week Toxicity Study – *Sample needed: To be determined*
Approximate turnaround time is thirty-four weeks.

Study Design Options—Other than Intravenous				
	ISO 10993-11:2006 Compliant		Abridged Design	
Groups	**Males**	**Females**	**Males**	**Females**
Test Article	20	20	20	20
Control	20	20	20	20
Total No.	*N=80*		*N=80*	
Species	Sprague Dawley Rats		Sprague Dawley Rats	
Dose Route	Routes other than intravenous (ie: intraperitoneal injection or implant)		Routes other than intravenous (ie: intraperitoneal injection or implant)	
Clinical Observations	Daily		*Same*	
Body Weights	Weekly		*Same*	
Clinical Pathology	All groups terminal; Standard panels for clinical chemistry, hematology, and coagulation		*Same*	
Necropsy	Gross observations and tissue collection		*Same*	
Organ Weights	Standard tissues by ISO guidelines		*Same*	
Tissue Preservation & Storage	All tissues preserved and processed for histopathology (see below)		Approx. 33 tissues preserved in formalin and stored for up to 1 year; Additional charges will apply for future tissue analysis if requested by Sponsor	
Histology/Pathology	Approximately 45 tissues processed and evaluated by a board-certified pathologist		Up to 12 target organs processed and evaluated by a board-certified pathologist	
Reporting	Standard final report		Standard final report	

**Note: Modified designs for alternate dose routes, study duration, and study parameters are available upon request.*

GENOTOXICITY TESTS—ISO 10993-3

PURPOSE: Genotoxicity tests are used to assess the potential of the test article (or test article extract) to induce gene mutations or chromosome damage using a battery of tests in bacterial, mammalian cells in vitro, and in vivo test systems. This information is of critical importance and constitutes an essential part of preclinical studies because genetic damage can cause an increase in the incidence of heritable diseases and cancer in human populations. Physical or chemical agents that induce such effects by interacting with genetic material and alter their structure are considered "genotoxic".

Gene Mutations: Ames Reverse Mutation Assay
1 Extract – *Sample needed: 36 cm² < 0.5 mm thick, 18 cm² ≥ 0.5 mm thick, or 1.2 g*
2 Extracts – *Sample needed: 72 cm² < 0.5 mm thick, 36 cm² ≥ 0.5 mm thick, or 2.4 g, and at least 2 units*

The purpose of the study is to determine the potential mutagenic effect of the test article extract on a panel of bacterial strains (4 strains of Salmonella typhimurium and 1 strain of E. coli). The test article is extracted in a polar and a non-polar vehicle. These bacteria are exposed to this extract via plate incorporation in the presence or absence of metabolic activation, and then selected for the generation of mutant cells via their lack of requirement for specific amino-acid (histidin or tryptophan). For each strain and condition, the numbers of colonies after exposure to the test article are compared to those of a negative control. The test article is considered not mutagenic if the difference is found not statistically significant ($p > 0.05$).

2 Extracts with Confirmation – *Sample needed: 108 cm² < 0.5 mm thick, 54 cm² ≥ 0.5 mm thick, or 3.6 g, and at least 2 units*

The purpose of the study is to determine the potential mutagenic effect of the test article extract on a panel of bacterial strains (4 strains of Salmonella typhimurium and 1 strain of E. coli). The test article is extracted in a polar and a non-polar vehicle. These bacteria are exposed to this extract via plate incorporation and with pre-incubation (confirmation) in the presence or absence of metabolic activation, and then selected for the generation of mutant cells via their lack of requirement for specific amino-acid (histidin or tryptophan). For each strain and condition, the numbers of colonies after exposure to the test article are compared to those of a negative control. The test article is considered not mutagenic if the difference is found not statistically significant ($p > 0.05$). The test with confirmation is described in OECD guidelines but is usually not necessary for medical device testing.

Approximate turnaround time is four weeks.

Chromosomal Aberration: Chromosomal Aberration Assay with CHO Cells
1 Extract – *Sample needed: 150 cm² < 0.5 mm thick,75 cm² ≥ 0.5 mm thick, or 5 g*
2 Extracts – *Sample needed: 300 cm² < 0.5 mm thick, 150 cm² ≥ 0.5 mm thick, or 10 g, and at least 2 units*

The purpose of the study is to determine the potential clastogenic effect (damages to chromosomes) of the test article extract on mammalian cells (CHO-K1) in culture in-vitro. The test article is extracted in a polar vehicle (cell culture medium or NaCl) and an alternative vehicle (DMSO, PEG, or Ethanol). The cells are exposed to this extract for 3 to 6 hours in the presence and absence of metabolic activation, and then harvested for analysis of their chromosomes integrity or aberrations by microscopic observation. For each condition, the proportion of aberrations after exposure to the test article is compared to that of a negative control. The test article is considered not clastogenic if the difference is found not statistically significant ($p > 0.05$).

2 Extracts with Confirmation – *Sample needed: 450 cm² < 0.5 mm thick, 225 cm² ≥ 0.5 mm thick, or 15 g and at least 2 units*

This in-vitro cytogenetic assay is performed with CHO-K1 cells exposed to the test article extract(s) for an exposure period of 3-6 hours in the presence and absence of mammalian metabolic activation employing appropriate positive controls. Each condition will be tested in duplicate. The CHO cell line may be replaced by cultured human lymphocytes for an additional charge. The confirmatory assay is performed with prolonged exposure (18-21 hours).

Approximate turnaround time is seven to eight weeks.

Chromosomal Aberration: Chromosomal Aberration with Human Lymphocytes
1 Extract – *Sample needed: 150 cm² < 0.5 mm thick, 75 cm² ≥ 0.5 mm thick, or 5 g*
2 Extracts – *Sample needed: 300 cm² < 0.5 mm thick, 150 cm² ≥ 0.5 mm thick, or 10 g*

The purpose of the study is to determine the potential clastogenic effect (damages to chromosomes) of the test article extract on freshly isolated human lymphocytes in-vitro. The test article is extracted in a polar vehicle (cell culture medium or NaCl) and an alternative vehicle (DMSO, PEG, or Ethanol). The cells are exposed to this extract for 3 to 6 hours in the presence and absence of metabolic activation, and then harvest for analysis of their chromosomes integrity or aberrations by microscopic observation. For each condition, the proportion of aberrations after exposure to the test article is compared to that of a negative control. The test article is considered not clastogenic if the difference is found not statistically significant ($p > 0.05$).

2 Extracts with Confirmation – *Sample needed: 450 cm² < 0.5 mm thick, 225 cm² ≥ 0.5 mm thick, or 15 g*

The purpose of the study is to determine the potential clastogenic effect (damages to chromosomes) of the test article extract on freshly isolated human lymphocytes in-vitro. The test article is extracted in a polar vehicle (cell culture medium or NaCl) and an alternative vehicle (DMSO, PEG, or Ethanol). The cells are exposed to this extract for 3 to 6 hours in the presence and absence of metabolic activation (main assay) and for a period of 18 to 21 hours in absence of metabolic activation (confirmation assay). The cells are then harvested for analysis of their chromosomes integrity or aberrations by microscopic observation. For each condition, the proportion of aberrations after exposure to the test article is compared to that of a negative control. The test article is considered not clastogenic if the difference is found not statistically significant ($p > 0.05$). The test with confirmation is described in OECD guidelines but is optional for medical device testing.

Approximate turnaround time is seven to eight weeks.

Rodent Bone Marrow Micronucleus Assay in Mice
1 Extract – *Sample needed: 120 cm² < 0.5 mm thick,60 cm² ≥ 0.5 mm thick, or 4 g*
2 Extracts – *Sample needed: 240 cm² < 0.5 mm thick, 120 cm² ≥ 0.5 mm thick, or 8 g*

The purpose of the study is to determine the potential clastogenic effect (damages to chromosomes) of the test article extract on bone marrow cells (polychromatic erythrocyte) in mice. The test article is extracted in a polar and non-polar vehicles, typically NaCl and CSO, and injected IV or IP. Groups of mice are sacrificed at 24 and 48 hours, and their bone marrow is analysed by microscopic observation for the frequency of micronucleus in the polychromatic cells. For each condition, the proportion of micronuclei after exposure to the test article is compared to that of a negative control. The test article is considered not clastogenic if the difference is found not statistically significant (t-test, $p > 0.05$).

Approximate turnaround time is ten to twelve weeks.

Rodent Blood Micronucleus Assay in Mice
1 Extract – *Sample needed: 120 cm² < 0.5 mm thick,60 cm² ≥ 0.5 mm thick, or 4 g*
2 Extracts – *Sample needed: 240 cm² < 0.5 mm thick, 120 cm² ≥ 0.5 mm thick, or 8 g*

The purpose of the study is to determine the potential clastogenic effect (damages to chromosomes) of the test article extract on erythrocyte lineage cells in mice. The test article is extracted in a polar and non-polar vehicles, typically NaCl and CSO, and injected IV or IP. Groups of mice are sacrificed at 48 and 72 hours, and their blood is analysed by flow cytometry for the frequency of micronucleus in the red blood cells. For each condition, the proportion of micronuclei after exposure to the test article is compared to that of a negative control. The test article is considered not clastogenic if the difference is found not statistically significant (t-test, $p > 0.05$).

Approximate turnaround time is eight to ten weeks.

Mouse Lymphoma Forward Mutation

1 Extract – *Sample needed: 300 cm² < 0.5 mm thick, 150 cm² ≥ 0.5 mm thick, or 10 g*

2 Extracts – *Sample needed: 600 cm² < 0.5 mm thick, 300 cm² ≥ 0.5 mm thick, or 20 g*

The purpose of the study is to determine the potential mutagenic effect of the test article extract on mammalian cells (mouse lymphoma cells LY5178 $TK^{+/-}$). The test article is extracted in a polar vehicle (cell culture medium or NaCl) and an alternative vehicle (DMSO, PEG, or Ethanol). The cells are exposed to this extract for 3 to 6 hours in the presence and absence of metabolic activation, and then selected for the generation of mutant cells via their resistance to the cytotoxic agent TFT (Trifluoro-thymidine). For each condition, the numbers of colonies after exposure to the test article are compared to those of a negative control. The test article is considered not mutagenic if the difference is found not statistically significant ($p > 0.05$).

2 Extracts with Confirmation - *Sample needed: 900 cm² < 0.5 mm thick, 450 cm² ≥ 0.5 mm thick, or 30 g*

The purpose of the study is to determine the potential mutagenic effect of the test article extract on mammalian cells (mouse lymphoma cells LY5178 $TK^{+/-}$). The test article is extracted in a polar vehicle (cell culture medium or NaCl) and an alternative vehicle (DMSO, PEG, or Ethanol). The cells are exposed to this extract for 3 to 6 hours in the presence and absence of metabolic activation main assay) and for a period of 18 to 21 hours in absence of metabolic activation (confirmation assay) The cells are then selected for the generation of mutant cells via their resistance to the cytotoxic agent TFT (Trifluoro-thymidine). For each condition, the numbers of colonies after exposure to the test article are compared to those of a negative control. The test article is considered not mutagenic if the difference is found not statistically significant ($p > 0.05$). The test with confirmation is described in OECD guidelines but is optional for medical device testing.

Approximate turnaround time is nine to ten weeks.

IMPLANTATION TESTS—ISO 10993-6

PURPOSE: Implantation tests are in-vivo assays used to assess the local pathological effects on living tissue, at both the gross and microscopic level of a test article that is implanted into an appropriate implant site. Standard implant sites include muscle, bone, subcutaneous, intraperitoneal, and brain. The assay is typically performed in albino rabbits but other species are available. Studies can be customized based on species, animal number, implant site, duration and evaluation criteria.

For resorbable/degradable articles, time points selected for evaluation in animals should be related to the estimated degradation time of the test article as determined from *in vitro* degradation studies. In addition, pilot studies in rodents should be done to determine the expected rate of degradation prior to studies in larger animals. Implant studies of resorbable/degradable articles, should evaluate local tissue responses relative to the overall degradation process as represented by multiple time points including: a) When there is minimal or no degradation, b) When degradation is occurring, and c) When a steady state has been reached, resulting in tissue restoration or degradation nearing completion. The Sponsor is responsible for determining degradation and specifying relevant time points for evaluation in animals. When notified of resorbable/degradable articles requiring implantation, sites are implanted with a suitable marker (i.e. non-absorbable suture) to aid in identification of the implant site at the end of the designated time periods and addition cost changed to Sponsor. A predicate control article, if available, is recommended to be specified/supplied by the Sponsor.

Sample requirements listed are based on one test article. Test articles with multiple components require evaluation of each selected or specified patient contacting component.

Muscle Implant
2 Week Implant
4 Week Implant
6 Week Implant
8 Week Implant
12 Week Implant
13 Week Implant
26 Week Implant
52 Week Implant – *Sample needed per material: 18 strips at 1mmx1mmx10mm*

The purpose of the study is to evaluate the material or device for local effects on living tissue when implanted typically in the paravertebral muscles of the rabbit. The bioreactivity of a material is determined through macroscopic and microscopic through a comparison of the tissue response caused by the material or device as compared to an appropriate control material (i.e. negative control plastic or predicate control). At the end of the observation period, gross observations of the implant sites will be recorded. The area of the tissue surrounding the center position of each implant strip will be processed and a pathologist will process the implanted sites for histopathological evaluation. Inflammation, fibrosis, hemorrhage and necrosis are evaluated on a scale and compared to the control article sites.

Approximate turnaround time is duration of implant period + four to five weeks.

Subcutaneous Implant
2 Week Implant
4 Week Implant
6 Week Implant
8 Week Implant
12 Week Implant
13 Week Implant
26 Week Implant
52 Week Implant – *Sample needed per material: 18 strips at 1mmx1mmx10mm*

The purpose of the study is to evaluate the material or device for local effects on living tissue when implanted subcutaneously in rabbits. The bioreactivity of a material is determined through macroscopic and microscopic through a comparison of the tissue response caused by the material or device as compared to an appropriate control material (i.e. negative control plastic or predicate control). At the end of the observation period, gross observations of the implant sites will be recorded.

IMPLANTATION TESTS—ISO 10993-6 *(cont.)*

The area of the tissue surrounding the center position of each implant strip will be processed and a pathologist will process the implanted sites for histopathological evaluation. Inflammation, fibrosis, haemorrhage and necrosis are evaluated on a scale and compared to the control article sites.

Approximate turnaround time is duration of implant period + four to five weeks.

Bone Implant
2 Week Implant
4 Week Implant
6 Week Implant
8 Week Implant
12 Week Implant
13 Week Implant
26 Week Implant
52 Week Implant – *Sample needed per material: 18 strips at 1mmx1mmx10mm*

The purpose of the study is to evaluate the material or device for local effects on living tissue when implanted in rabbit femurs. Test material is implanted at the distal, medial, and proximal locations of the femur. The bioreactivity of a material is determined through macroscopic and microscopic comparison of the tissue response caused by the material or device as compared to an appropriate control material (i.e. negative control plastic or predicate control). At the end of the observation period, gross observations of the implant sites will be recorded. The area of the tissue surrounding the center position of each implant strip will be processed and a pathologist will process the implanted sites for histopathological evaluation. Inflammation, fibrosis, haemorrhage and necrosis are evaluated on a scale and compared to the control article sites.

Approximate turnaround time is duration of implant period + four to five weeks.

HEMOCOMPATIBILITY TESTS—ISO 10993-4

PURPOSE: Hemocompatibility tests are in-vitro assays used to assess the possibility of a test article to cause adverse effects on red blood cells (hemolysis), thrombosis, coagulation, platelets and complement system.

(Autian Method, similar to NIH Method)
Hemolysis - Indirect in Rabbit Blood (Autian Method) – *Sample needed: 360 cm² ≤0.5mm thick, 180 cm² ≥0.5 mm thick, or 12 g*

The purpose of the study is to determine the potential hemolytic effect (destruction of red blood cells with subsequent release of hemoglobin) of the test article extract on rabbit blood. The test article is extracted in 0.9% NaCl. Rabbit blood is added to the extract and incubated in 3 replicates for 1 hour at 37 °C. The supernatant is then decanted and its optical density measured with a spectrophotometer. The test article is considered non-hemolytic if the percentage of hemolyzed blood is equal to or lower than 5% of the untreated control. This is a default assay appropriate for screening purposes.

Approximate turnaround time is three weeks.

Hemolysis - Direct in Rabbit Blood (Autian Method) – *Sample needed: 360 cm² ≤0.5mm thick, 180 cm² ≥0.5 mm thick, or 12 g*

The purpose of the study is to determine the potential hemolytic effect (destruction of red blood cells with subsequent release of hemoglobin) of the test article on rabbit blood. The test article is added to 0.9% NaCl, followed by the addition of rabbit blood for incubation in 3 replicates for 1 hour at 37 °C. The supernatant is then decanted and its optical density measured with a spectrophotometer. The test article is considered non-hemolytic if the percentage of hemolyzed blood is equal to or lower than 5% of the untreated control. This is a default assay appropriate for screening purposes.

Approximate turnaround time is three weeks.

Hemolysis - Indirect in Human Blood (Autian Method) – *Sample needed: 360 cm² ≤0.5mm thick*

The purpose of the study is to determine the potential hemolytic effect (destruction of red blood cells with subsequent release of hemoglobin) of the test article extract on human blood. The test article is extracted in 0.9% NaCl. Human blood is added to the extract and incubated in 3 replicates for 1 hour at 37 °C. The supernatant is then decanted and its optical density measured with a spectrophotometer. The test article is considered non-hemolytic if the percentage of hemolyzed blood is equal to or lower than 5% of the untreated control. This assay is appropriate for screening purposes.

Approximate turnaround time is three weeks.

Hemolysis - Direct in Human Blood (Autian Method) – *Sample needed: 360 cm² ≤0.5mm thick, 180 cm² ≥0.5 mm thick, or 12 g and at least 3 units, or multiples of 3, if units are less than 21 cm^2, 42 cm^2 or 1.4 g.*

The purpose of the study is to determine the potential hemolytic effect (destruction of red blood cells with subsequent release of hemoglobin) of the test article on human blood. The test article is added to 0.9% NaCl, followed by the addition of human blood for incubation in 3 replicates for 1 hour at 37 °C. The supernatant is then decanted and its optical density measured with a spectrophotometer. The test article is considered non-hemolytic if the percentage of hemolyzed blood is equal to or lower than 5% of the untreated control. This assay is appropriate for screening purposes.

Approximate turnaround time is three weeks.

Hemolysis - Indirect in Rabbit Blood (ASTM Method) – *Sample needed: 360 cm² ≤0.5 mm thick, 180 cm² ≥0.5 mm thick, or 12 g, and at least 3 units, or multiples of 3, if units are less than 21 cm^2, 42 cm^2 or 1.4 g.*

The purpose of the study is to determine the potential hemolytic effect (destruction of red blood cells with subsequent release of hemoglobin) of the test article extract on rabbit blood. The test article is extracted in triplicate in Phosphate Buffer Saline. Blood from three rabbits is added to the 3 extracts and incubated for 3 hour at 37 °C. The supernatant is then decanted and mixed with methemoglobin reagent (to convert all isoform of hemoglobin into a single component) and its optical density measure with a spectrophotometer. The test article is considered non-hemolytic if the percentage of hemolyzed blood is equal to or lower than 2% of the negative control, slightly hemolytic if between 2 and 5%, and hemolytic if above 5%. This assay follows ASTM guideline F756 and is the recommended assay for test article in indirect contact with blood.

Hemolysis - Direct in Rabbit Blood (ASTM Method) – *Sample needed: 360 cm² ≤0.5 mm thick, 180 cm² ≥0.5 mm thick, or 12 g, and at least 3 units, or multiples of 3, if units are less than 21 cm^2, 42 cm^2 or 1.4 g.*

The purpose of the study is to determine the potential hemolytic effect (destruction of red blood cells with subsequent release of hemoglobin) of the test article on rabbit blood. The test article is added to Phosphate Buffer Saline, followed by the addition of blood from three rabbits for incubation in 3 replicates for 3 hour at 37 °C. The supernatant is then decanted and mixed with methemoglobin reagent (to convert all isoform of hemoglobin into a single component) and its optical density measure with a spectrophotometer. The test article is considered non-hemolytic if the percentage of hemolyzed blood is equal to or lower than 2% of the negative control, slightly hemolytic if between 2 and 5%, and hemolytic if above 5%. This assay follows ASTM guideline F756 and is the recommended assay for test article in direct contact with blood.

Approximate turnaround time two to three weeks.

Hemolysis – Complete (Direct and Indirect) in Rabbit Blood (ASTM Method) – *Sample needed: 540 cm² ≤0.5 mm thick, 270 cm² ≥0.5 mm thick, or 18 g, and at least 6 units (or multiples of 6), if units are less than 21 cm^2, 42 cm^2 or 1.4 g.*

The purpose of the study is to determine the potential hemolytic effect (destruction of red blood cells with subsequent release of hemoglobin) of the test article on rabbit blood. The test article is tested in parallel in a direct and an indirect way (with extract). The test article in Phosphate Buffer Saline, and its extract, are mixed with the blood from three rabbits for incubation in 3 replicates for 3 hour at

37 °C. The supernatant is then decanted and mixed with methemoglobin reagent (to convert all isoform of hemoglobin into a single component) and its optical density measure with a spectrophotometer. The test article is considered non-hemolytic if the percentage of hemolyzed blood is equal to or lower than 2% of the negative control, slightly hemolytic if between 2 and 5%, and hemolytic if above 5%. This assay follows ASTM guideline F756 and is the recommended assay for all test articles.

Approximate turnaround time two to three weeks.

In-vitro Hemocompatibility Assay (Direct Contact)
Indirect – *Sample needed: 10 cm² < 0.5 mm thick, 5 cm² ≥ 0.5 mm thick, or 1 g*

The purpose of the study is to determine the potential effect of the test article on the cellular components of blood. The following parameters are measured: White Blood Cells, Red Blood Cells, Platelets and erythrocyte indices. The test article is extracted in 0.9% NaCl. The test article extract is added (10%) to human blood for incubation in 3 replicates for 1 hour at 37 °C. The blood is then analysed on an automated hematology analyser. For each parameter, the results after exposure to the test article are compared with those of a negative and an untreated controls. The test article is considered not having an effect on those parameter if the difference is found not statistically significant ($p > 0.05$).

Approximate turnaround time is two to three weeks.

In-vitro Hemocompatibility Assay (Direct Contact)
Direct – *Sample needed: 75 cm² < 0.5 mm thick, 50 cm² ≥ 0.5 mm thick, or 2.5 g, and at least 3 units, or multiples of 3*

The purpose of the study is to determine the potential effect of the test article on the cellular components of blood. The following parameters are measured: White Blood Cells, Red Blood Cells, Platelets and erythrocyte indices. The test article is added to human blood for incubation in 3 replicates for 1 hour at 37 °C. The blood is then analysed on an automated hematology analyser. For each parameter, the results after exposure to the test article are compared with those of negative and untreated controls. The test article is considered not having an effect on those parameter if the difference is found not statistically significant ($p > 0.05$).

Approximate turnaround time is two to three weeks.

In-vivo Thrombogenicity in Dogs – *Sample needed: 2 units, at least 2" in length*

The purpose of the study is to determine article(s) intended for direct blood contact for thrombogenic properties in-vivo. Historically, dogs have been used in thrombogenicity studies to assess possible human toxicity caused by an article that is intended for use in contact with human blood. ISO 10993-4 guidelines have no published alternative (non-animal) methods. The assay is carried out in two animals and the results are analysed by comparing the level of thrombus formation on the test and control articles. It is preferred that the Sponsor provide a predicate control although Toxikon can provide negative control HDPE plastic. Test articles are inserted into the jugular vein, femoral vein, or vena cava and the control articles are inserted into an opposing vein. Heparinization is carried out after approximately four hours of insertion, and exsanguination occurs soon thereafter. The veins are explanted and damage to the implanted veins is examined and the veins with the implants in-situ are photographed. The extent of the thrombus formation on the test and control articles is scored on a scale from zero to five.

Approximate turnaround time is five to six weeks.

Complement Activation
Indirect – *Sample needed: 10 cm² < 0.5 mm thick, 5 cm² ≥ 0.5 mm thick, or 1 g*

The purpose of the study is to determine the potential for the test article to activate the Complement system in human plasma. The test article is extracted in 0.9% NaCl. The test article extract is added (10%) to human plasma for incubation in 3 replicates for 1.5 hour at 37 °C. The concentration in two components of the Complement (C3 and C5) in the plasma is then analysed by ELISA. The results

after exposure to the test article are compared with those of negative and untreated controls. The test article is considered not having activated the Complement if the difference for both C3 and C5 is found not statistically significant ($p > 0.05$).

Approximate turnaround time is three to four weeks.

Complement Activation
Direct – *Sample needed: 36 cm² < 0.5 mm thick, 18 cm² ≥ 0.5 mm thick, or 1.2 g and at least 3 units, or multiples of 3*

The purpose of the study is to determine the potential for the test article to activate the Complement system in human plasma. The test article is exposed to human plasma for incubation in 3 replicates for 1.5 hour at 37 °C. The concentration in two components of the Complement (C3 and C5) in the plasma is then analysed by ELISA. The results after exposure to the test article are compared with those of negative and untreated controls. The test article is considered not having activated the Complement if the difference for both C3 and C5 is found not statistically significant ($p > 0.05$).

Approximate turnaround time is three to four weeks.

The Prothrombin Time Assay (PT)
Indirect – *Sample needed: 10 cm² < 0.5 mm thick, 5 cm² ≥ 0.5 mm thick, or 1 g*

The purpose of the study is to determine the potential for the test article to inhibit the coagulation of human plasma. The test article is extracted in 0.9% NaCl. The test article extract is added (10%) to human plasma for incubation in 3 replicates for 15 minutes at 37 °C. The Prothrombin Time (= coagulation time in response to thromboplastin after addition of calcium) is measured on a semi-automated instrument. The PTs after exposure to the test article are compared with those of negative and untreated controls. The test article is considered not having inhibited the coagulation if the difference is found not statistically significant ($p > 0.05$).

The Prothrombin Time Assay (PT)
Direct – *Sample needed: 36 cm² < 0.5 mm thick, 18 cm² ≥ 0.5 mm thick, or 1.2 g and at least 3 units, or multiples of 3*

The purpose of the study is to determine the potential for the test article to inhibit the coagulation of human plasma. The test article is exposed to human plasma for incubation in 3 replicates for 15 minutes at 37 °C. The Prothrombin Time (= coagulation time in response to thromboplastin after addition of calcium) is measured on a semi-automated instrument. The PTs after exposure to the test article are compared with those of negative and untreated controls. The test article is considered not having inhibited the coagulation if the difference is found not statistically significant ($p \geq 0.05$).

Approximate turnaround time is two to three weeks.

The Unactivated Partial Thromboplastin Time Assay (UPTT = PTT)
Indirect – *Sample needed: 10 cm² < 0.5 mm thick, 5 cm² ≥ 0.5 mm thick, or 1 g*

The purpose of the study is to determine the potential for the test article to increase the speed of coagulation of human plasma. The test article is extracted in 0.9% NaCl. The test article extract is added (10%) to human plasma for incubation in 3 replicates for 15 minutes at 37 °C. The UPTT (= coagulation time in response to phospholipids after addition of calcium) is measured on a semi-automated instrument. The UPTTs after exposure to the test article are compared with those of negative and untreated controls. The test article is considered not having increased the speed of coagulation if the difference is found not statistically significant ($p > 0.05$).

The Unactivated Partial Thromboplastin Time Assay (UPTT = PTT)
Direct – *Sample needed: 36 cm² < 0.5 mm thick, 18 cm² ≥ 0.5 mm thick, or 1.2 g and at least 3 units, or multiples of 3*

The purpose of the study is to determine the potential for the test article to increase the speed of coagulation of human plasma. The test article is exposed to human plasma for incubation in 3

replicates for 15 minutes at 37 °C. The UPTT (= coagulation time in response to phospholipids after addition of calcium) is measured on a semi-automated instrument. The UPTTs after exposure to the test article are compared with those of negative and untreated controls. The test article is considered not having increased the speed of coagulation if the difference is found not statistically significant ($p > 0.05$).

Approximate turnaround time is two to three weeks.

The Activated Partial Thromboplastin Time Assay (APTT)
Indirect – *Sample needed: 10 cm² < 0.5 mm thick, 5 cm² ≥ 0.5 mm thick, or 1 g*

The purpose of the study is to determine the potential for the test article to inhibit the coagulation of human plasma. The test article is extracted in 0.9% NaCl. The test article extract is added (10%) to human plasma for incubation in 3 replicates for 15 minutes at 37 °C. The APTT (= coagulation time in response to phospholipid and an activator such as Kaolin after addition of calcium) is measured on a semi-automated instrument. The APTTs after exposure to the test article are compared with those of negative and untreated controls. The test article is considered not having inhibited the coagulation if the difference is found not statistically significant ($p > 0.05$). This assay is not typically used to test medical devices as the activator may mask any intrinsic activator capacity of the test article.

Approximate turnaround time is two to three weeks.

The Activated Partial Thromboplastin Time Assay (APTT)
Direct – *Sample needed: 36 cm² < 0.5 mm thick, 18 cm² ≥ 0.5 mm thick, or 1.2 g and at least 3 units, or multiples of 3*

The purpose of the study is to determine the potential for the test article to inhibit the coagulation of human plasma. The test article is exposed to human plasma for incubation in 3 replicates for 15 minutes at 37 °C. The APTT (= coagulation time in response to phospholipid and an activator such as Kaolin after addition of calcium) is measured on a semi-automated instrument. The APTTs after exposure to the test article are compared with those of negative and untreated controls. The test article is considered not having inhibited the coagulation if the difference is found not statistically significant ($p > 0.05$). This assay is not typically used to test medical devices as the activator may mask any intrinsic activator capacity of the test article.

Approximate turnaround time is two to three weeks.

Lee & White Clotting Time
Indirect – *Sample needed: 72 cm² < 0.5 mm thick, 36 cm² ≥ 0.5 mm thick, or 2.4 g*

The purpose of the study is to determine the potential for the test article to affect the coagulation of human blood. The test article is extracted in 0.9% NaCl. The test article extract is added (10%) to human blood without any anti-coagulant at 37 °C. The clotting time is determined based on visual observation of the clot formation. The coagulation times after exposure to the test article are compared with those of negative and untreated controls. The test article is considered not having affected the coagulation if the difference is found not statistically significant ($p > 0.05$).

Lee & White Clotting Time
Direct – *Sample needed: 72 cm² < 0.5 mm thick, 36 cm² ≥ 0.5 mm thick, or 2.4 g, and at least 3 units, or multiples of 3*

The purpose of the study is to determine the potential for the test article to affect the coagulation of human blood. The test article is exposed to human blood without any anti-coagulant at 37 °C. The clotting time is determined based on visual observation of the clot formation. The coagulation times after exposure to the test article are compared with those of negative and untreated controls. The test article is considered not having affected the coagulation if the difference is found not statistically significant ($p > 0.05$).

Approximate turnaround time is two to three weeks.

HEMOCOMPATIBILITY TESTS—ISO 10993-4 (cont.)

Platelet Aggregation
Indirect – *Sample needed: 72 cm² < 0.5 mm thick, 36 cm² ≥ 0.5 mm thick, or 2.4 g*

The purpose of the study is to determine the potential effect of test article on ability of the platelets to aggregate. The test article is extracted in 0.9% NaCl. The test article extract is added (10%) to platelet rich human plasma for incubation in 3 replicates for 1 hour at 37 °C. The aggregation of the platelet is monitored via the increase of clarity of the plasma. This aggregation is monitored both as a spontaneous event and in response to the addition of ADP. The aggregation after exposure to the test article are compared with those of negative and untreated controls. The test article is considered not having affected the ability of the platelet to aggregate if the difference is found not statistically significant ($p > 0.05$).

Platelet Aggregation
Direct – *Sample needed: 72 cm² < 0.5 mm thick, 36 cm² ≥ 0.5 mm thick, or 2.4 g, and at least 3 units, or multiples of 3*

The purpose of the study is to determine the potential effect of test article on ability of the platelets to aggregate. The test article is exposed to platelet rich human plasma for incubation in 3 replicates for 1 hour at 37 °C. The aggregation of the platelet is monitored via the increase of clarity of the plasma. This aggregation is monitored both as a spontaneous event and in response to the addition of ADP. The aggregation after exposure to the test article are compared with those of negative and untreated controls. The test article is considered not having affected the ability of the platelet to aggregate if the difference is found not statistically significant ($p > 0.05$).

Approximate turnaround time is three to four weeks.

EO RESIDUAL TESTS—ISO 10993-7

PURPOSE: Testing for ethylene oxide (EO) residuals is a requirement for quality system validation and routine monitoring of sterilization of medical products with gaseous ethylene oxide. When determining the suitability of EO for sterilization of medical devices, it is important to ensure that the levels of residual EO and ethylene chlorohydrin (EC) pose a minimal risk to the patient in normal product use. Reference ANSI/AAMI/ISO 10993-7:2008.

Exhaustive Recovery Method for EO, EC, EG – *Sample needed: Lot size dependent*

If these lifetime use devices, if they are then we need one set for exhaustive recovery and then average daily dose can be calculated from EO and EC results. If prolonged use but less than 30 days: one set for the exhaustive recovery and report the results as average daily dose. The medical devices or materials to be tested are extracted in purified water for 24 hours at sponsor specified temperature. The extract is analyzed and re-extracted with fresh extraction solution until the analyses shows that the concentrations of EO, EC, and ethylene glycol (EG) are less than 10% of the initial extraction results, or are non-detectable. The number of extractions depends on the results of each day. All of the values obtained for residuals on each subsequent analysis are combined to reflect the entire residual content of the device.

Approximate turnaround time is one week –post final extraction.

Simulated Extraction Method – *Sample needed: Lot size dependent*

Since it is necessary to evaluate the residue levels available to the patient or other end-user from devices during their routine use, extraction methods that simulate use are required. Simulated use extraction shall be carried out under conditions that provide the greatest challenge to the intended use. For example, many blood containing and parenteral devices can be extracted with water or other aqueous fluids by filling or flushing the blood or fluid path (whichever is appropriate). Samples shall be extracted for a time equivalent to or exceeding the maximum time for single use (or that ensures total extraction) and at temperatures that provide the greatest simulated challenge (or sponsor specified temperature). The number of extractions depends on the results of the first day

Approximate turnaround time is one week post extraction.

CYTOTOXICITY TEST—MHLW

PURPOSE: Cytotoxicity in-vitro screening assays are used to assess – in a fast and sensitive way – the biocompatibility of the test material (extract) when in contact with a specific cell culture.

V79 Colony Assay – *Sample needed: 240 cm²≤ 0.5 mm thick, 120 cm² ≥0.5 mm thick, 8 g*

This test is a cytotoxicity assay for evaluating the ability of the test article to reduce the colony formation of V79 cells. Extracts of the test article are prepared in MEM at a ratio of 1 g per 10 mL and 100 cells per dish are exposed to the extract in triplicate. The number of colonies formed is visualized after 6-7 days of incubation and cytotoxicity is evaluated as the number of colonies in test article plates compared to untreated control group. The test is based on MHLW Administrative note IRYOKIKISHINSA # 36.

Direct Contact V79 Colony Assay – *Sample needed: 240 cm²≤ 0.5 mm thick, 120 cm² ≥0.5 mm thick, 8 g*

This test evaluates the ability of a flat test article to support the growth of cells. Cells are seeded on top of the test article and the number of colonies that were able to grow and form. Colonies are counted after 6-7 days. The colony formation rate for the test article is compared to the ones for the negative control.

Approximate turnaround time is three to four weeks.

SENSITIZATION TEST—MHLW

PURPOSE: The sensitization tests are used to determine the allergic or sensitizing capacity to the repeated or prolonged exposure of a test material. Sensitization is characterized by the fact that reactions are delayed, not localized, and independent of dose.

Japanese Skin Sensitization – *Sample needed: Up to 5 kg*

This test evaluates the test concentration that will cause a mean allergenic response of 1 in guinea pigs. The test article is typically extracted using organic solvents to create a residue, which is combined with a vehicle to create dosing solutions. A "primary irritation" phase is conducted to determine the lowest concentration of test article residue, which will cause erythema and edema. In the "induction phase", the dosing solution is applied via intradermal injections, followed by topical applications one week later. During the "challenge phase", serial dilutions of the dosing solution are applied to virgin skin sites and observed for erythema and edema at 24, 48 and 72 hours post challenge. A sensitisation reaction to the test article (extract) at a virgin site is scored based upon the defined evaluation criteria in the Japanese MHLW guidelines and the mean response of 1 is calculated.

Approximate turnaround time is ten to twelve weeks.

IRRITATION ASSAY—MHLW

PURPOSE: Irritation tests are in-vivo assays used to assess the potential of test articles – or their extracts – to cause irritation on the exposed part of the body.

Intracutaneous Injection – *Sample needed: 120 cm² <0.5 mm thick, 60 cm² ≥0.5 mm thick, or 4 g*

The purpose of the study is to determine the potential to produce irritation after injection of extracts of the test article. Extracts of the test article are prepared in an aqueous (saline) and lipophilic (cotton seed oil) solution. A minimum of three rabbits are injected intracutaneously with the assay and control articles and observed over a 72 hour period. The requirement of the assay are met if the difference between the assay mean score and the control is less than 1.0 as defined by the evaluation criteria in ISO 10993-10 and MHLW Notice Assessment of Medical Devices #36

Approximate turnaround time is three to four weeks.

ACUTE SYSTEMIC TOXICITY ASSAYS—MHLW

PURPOSE: Acute systemic toxicity tests are in-vivo assays used to assess the impairment or activation of a system – rather than the impairment of individual cells or organs. The test article is evaluated for systemic toxic effects as a result of a single, acute exposure.

Japanese MHLW Systemic Injection Assay – *Sample needed: 240 cm² < 0.5mm thick, 120 cm² ≥ 0.5 mm thick, or 8 g*

The purpose of the study is to determine that there are no substances with acute toxicity in the extracts taken from assay samples. Solutions extracted from assay samples using physiological saline and vegetable cottonseed oil are administered to a group of 5 mice via intravenous (physiological saline extract) or intraperitoneal injection oil extract. The animals are observed for up to 72 hours after administering the solutions and evaluated for the presence/absence of toxicity (clinical signs, weight changes, gross pathologic abnormalities), compared to the group with control solution administration.

Approximate turnaround time is three to four weeks.

Japanese MHLW Rabbit Pyrogen Assay – *Sample needed: 600 cm² < 0.5 mm thick, 300 cm² ≥ 0.5 mm thick, or 20 g*

This in-vivo assay involves measuring the rise in temperature of three albino rabbits following the intravenous injection of a test article (or test article extract) through the marginal ear veins of rabbits. Body temperatures are recorded three times at 1-hour intervals subsequent to injection. If there is no rise of temperature of 0.6°C or more above baseline, the test article meets the requirements for the absence of pyrogens.

Approximate turnaround time is three to four weeks.

GENOTOXICITY TEST—MHLW

PURPOSE: Genetic toxicology testing is conducted to determine the potential of the test article to induce mutations or chromosome damage using a battery of tests in bacterial, mammalian cells in vitro, and in vivo test systems. This information of is of critical importance because genetic damage can cause an increase in the incidence of heritable diseases and cancer in human populations. Physical or chemical agents that induce adverse effects by interacting with genetic material and alter their structure/function are considered "genotoxic".

Ames Reverse Mutation Assay – *Sample needed depends on test article extractability: 120 cm² or 4 g minimum*

The purpose of the study is to determine the potential mutagenic effect of the test article extract on a panel of bacterial strains (4 strains of Salmonella typhimurium and 1 strain of E. coli). The test article is extracted according to MHLW PFSB/ELD/OMDE Notification No. 0301-20. These bacteria are exposed to this extract via plate incorporation in the presence or absence of metabolic activation, and then selected for the generation of mutant cells via their lack of requirement for specific amino-acid (histidin or tryptophan). For each strain and condition, the numbers of colonies after exposure to the test article are compared to those of a negative control. The test article is considered not mutagenic if the difference is found not statistically significant ($p > 0.05$).

Approximate turnaround time is three weeks.

Chromosomal Aberration – *Sample needed depends on test article extractability: 240 cm², or 8 g*

The purpose of the study is to determine the potential clastogenic effect (damages to chromosomes) of the test article extract on freshly isolated human lymphocytes in-vitro. The test article is extracted according to MHLW PFSB/ELD/OMDE Notification No. 0301-20. The cells are exposed to this extract for 3 to 6 hours in the presence and absence of metabolic activation, and then harvest for analysis of their chromosomes integrity or aberrations by microscopic observation. For each condition, the proportion of aberrations after exposure to the test article is compared to that of a negative control. The test article is considered not clastogenic if the difference is found not statistically significant ($p > 0.05$).

Approximate turnaround time is eight weeks.

IMPLANTATION TESTS—MHLW

PURPOSE: Implantation tests are in-vivo assays used to assess the local pathological effects on living tissue, at both the gross and microscopic level of a test article that is implanted into an appropriate implant site.

Japanese Short Term Muscle Implant
1 Week Implant
4 Week Implant – *Sample needed: for each time point – 18 strips 1mmx1mmx10mm*

The purpose of the study is to determine a test article for the potential to induce local inflammatory and bioreactivity effects after short-term exposure in the muscle tissue of albino rabbits. Five male rabbits are used per time point. MHLW requires 2 rabbits have histology performed but to also comply with ISO, 3 animals are used for histology.

Approximate turnaround time is duration of implant period + six weeks.

HEMOCOMPATIBILITY TESTS—MHLW

PURPOSE: Hemocompatibility tests are in-vitro assays used to assess any adverse effects of blood contacting materials on hemolysis, thrombosis, coagulation, platelets and complement system. Testing for hemocompatibility requires different testing strategies depending upon the type of device. Specific hemocompatibility tests may also be needed, which simulate the geometry, contact conditions and flow dynamics of the device or material during use.

Japanese Hemolysis Indirect (MHLW) – *Sample needed: 600 cm² < 0.5 mm thick, 300 cm² ≥ 0.5 mm thick, or 25 g*

The purpose of the study is to determine the potential hemolytic effect (destruction of red blood cells with subsequent release of hemoglobin) of the test article extract on human blood. The test article is extracted in triplicate in 0.9% NaCl. Human blood is added to the extract and incubated in 3 replicates for 1, 2 and 4 hours at 37 °C. The supernatant is then decanted and its optical density measure with a spectrophotometer. The test article is considered non-hemolytic if the percentage of hemolyzed blood is equal to or lower than 2% of the untreated control.

Approximate turnaround time is four weeks.

CYTOTOXICITY TESTS—USP <87>

PURPOSE: Cytotoxicity tests are in-vitro assays used to assess the possibility of a test article to cause the death of cells in culture or to prevent their multiplication.

L929 MEM Elution USP – *Sample needed: 120 cm² ≤ 0.5 mm thick, 60 cm² ≥ 0.5 mm thick, or 4 g*

The purpose of the study is to determine the potential biological reactivity of a mammalian cell culture (L929) in response to the test article extract. The test article is extracted usually in Minimum Essential Medium (MEM) with 10 Fetal Bovine Serum for usually 24 hours at 37 °C. The cells are allowed to grow to sub-confluency in tissue culture plates and exposed to the test article extract at neat (100%) in 2 replicates. The plates are incubated for 48 hours at 37 °C. The biological reactivity of the cells following the exposure to the extracts is visually observed with a microscope, and graded on a scale of 0 (no reactivity) to 4 (severe reactivity). The test article is considered non-cytotoxic if none of the cultures exposed to the test article extract shows greater than mild reactivity (Grade 2).

Approximate turnaround time is two to three weeks.

Agar Diffusion USP – *Sample needed: 3 cm^2, 1 g, or 1mL*

The purpose of the study is to determine the potential biological reactivity of a mammalian cell culture (L929) in response to the test article. The cells are allowed to grow to approximately 80% confluency in cell culture dishes and then overlaid with an agarose layer. The test article is placed over the agar layer allowing the diffusion of leachables onto the cell layer. 2 replicates plates are prepared per article. The plates are incubated for 48 hours at 37 °C. The biological reactivity of the cells around the test article is visually observed, and graded on a scale of 0 (no reactivity) to 4 (severe reactivity). The test

CYTOTOXICITY TESTS—USP <87> *(cont.)*

article is considered non-cytotoxic if none of the cultures exposed to the test article shows greater than mild reactivity (Grade 2).

Approximate turnaround time is two to three weeks.

CLASS ASSAY FOR PLASTICS—USP <88>

PURPOSE: USP Class tests are assays designed to determine the biological response of animals to direct and/or indirect contact with a test article.

Class	Systemic Toxicity				Intracutaneous Reactivity				Implant
	NaCl	CSO	EtOH	PEG	NaCl	CSO	EtOH	PEG	
I	X	–		–	X	–	–	–	–
II	X	–	X	–	X	–	X	–	–
III	X	X	X	X	X	–	X	–	–
IV	X	X	X	–	X	X	X	–	X
V	X	X	X	X	X	X	X	X	–
VI	X	X	X	X	X	X	X	X	X

**Note: "X" indicates testing required*

Class I – *Sample needed: 120 cm^2 < 0.5 mm thick, 60 cm^2 ≥ 0.5 mm thick, or 4 g*
Approximate turnaround time is four weeks.

Class II – *Sample needed: 240 cm^2 < 0.5 mm thick, 120 cm^2 ≥ 0.5 mm thick, or 8 g*
Approximate turnaround time is four weeks.

Class III – *Sample needed: 480 cm^2 < 0.5 mm thick, 1200 cm^2 ≥ 0.5 mm thick, or 16 g*
Approximate turnaround time is four weeks.

Class IV – *Sample needed: Extraction-360 cm^2 < 0.5 mm thick, 180 cm^2 ≥ 0.5 mm thick, or 12 g*
Implant – 12 pieces at 1mm x 1mm x 10 mm
Approximate turnaround time is four to five weeks.

Class V – *Sample needed: 480 cm^2 < 0.5 mm thick, 240 cm^2 ≥ 0.5 mm thick, or 16 g*
Approximate turnaround time is four weeks.

Class VI – *Sample needed: Extraction-480 cm^2 <0.5 mm thick, 240 cm^2 ≥ 0.5 mm thick, or 16 g*
Implant – 12 pieces at 1mm x 1mm x 10 mm
Approximate turnaround time is four to five weeks.

COMPENDIAL PHYSICOCHEMICAL TESTS

Compendial tests are designed to determine the physical and chemical properties of a test article and of its impurities that are extracted from test article under specified conditions.

Physicochemical Test for Plastics, USP <661>-*Sample needed: 600 cm^2 or 20 grams*

The purpose of the study is to measure defined properties of impurities in plastics. First, the test article's surface is rinsed with Purified Water. Next it is extracted with Purified Water for 24 hours at 70 °C. The extract solution is analyzed for nonvolatile residue, residue on ignition, heavy metals, and buffering capacity.

Approximate turnaround time is two to three weeks.

Physicochemical Test for Elastomeric Closures, USP <381> – *Sample needed: 300 cm^2/extract*

The purpose of the study is to measure the physicochemical properties of impurities extracted from elastomeric closures. The test article is initially treated with boiling Purified Water for 5 minutes, followed by 5 water rinses. Next it is extracted with Purified Water for 30 minutes at 121 °C. The extract solution is analyzed for non-volatile residue, turbidity, acidity/alkalinity, reducing substances, heavy metals, color, zinc, optical absorbance, ammonium, and volatile sulfides.

Approximate turnaround time is three to four weeks.

Silicone Elastomer for Closures and Tubing, EP 3.1.9 – *Sample needed: 300 cm²*

The test article is extracted with 250 ml Purified Water for 5 hours under reflux conditions. A control (blank sample) is similarly processed. After cooling, extract solutions are decanted and analyzed for relative differences in visual appearance, acidity/ alkalinity, reducing substances, relative density, volatile matter, mineral oil, substances soluble in hexane, and phenylated compounds.

Approximate turnaround time is three to four weeks.

Polyethylene without Additives for Containers for Parenteral Preparations and for Ophthalmic Preparations, EP 3.1.4 – *Sample needed: 300 cm²*

Samples are extracted with Water for Injection for 5 hours under reflux conditions. Additional samples are extracted with toluene under reflux for 1.5 hour and others with 0.1M hydrochloric acid under reflux for 1 hour. The extract solutions are analyzed for acidity/alkalinity, optical absorbance, reducing substances, substances soluble in hexane, additives, and extractable heavy metals. A sample of the solid test article is also analyzed for sulfated ash.

Approximate turnaround time is three to four weeks.

Rubber Closure for Containers for Aqueous Parenteral Preparations, for Powders and for Freeze Dried Powders, EP 3.2.9 – *Sample needed: 300 cm²*

The purpose of the study is to measure the properties of impurities extracted from elastomeric closure materials. Prior to extraction, rubber closures with an exposed surface area of 100 cm^2 are covered with Water for Injection and boiled for 5 minutes. Closures are then rinsed 5 times with cold Water for Injection and extracted with 200 mL Water for Injection for 30 minutes at 121 °C in an autoclave. The extract solution is analyzed for visual appearance, acidity/alkalinity, and optical absorbance, reducing substances, ammonium, zinc, heavy metals and residue on evaporation. Test articles are also analyzed for volatile sulfides.

Approximate turnaround time is three to four weeks.

Total Extractables - Rubber Articles Intended for Repeated Use, **CFR 177.2600** – *Sample needed: 150 cm²/extract*

The purpose of the study is to analyze total extractables. Set of test articles with specified surface area is extracted with 150 mL Purified Water and another set with same volume of hexane, both extractions lasting 7 hours. Extract solutions are decanted and test articles are re-extracted with fresh 150 mL Purified Water and hexane for 2 hours. Extract solutions are evaporated to dryness and residues are weighed. Residue per surface area is calculated.

Approximate turnaround time is two to three weeks.

Residue on Ignition, USP <281> – *Sample needed: 5 grams*

The purpose of the study is to determine content of inorganic impurities in an organic substance. A 1–2 g sample is placed in a prepared crucible, moistened with sulfuric acid and heated until complete charring is achieved. Charred material is cooled, moistened with additional sulfuric acid, and heated until white fumes disappear, at which point content is ignited and incinerated at ~600 °C. Weight of the cooled residue is used to calculate percent residue in starting material.

Approximate turnaround time is two to three weeks.

METALS ANALYSIS

Heavy Metals—USP <231> Method I – *Sample needed: 20 grams*

The purpose of the study is to measure whether metallic impurities in a test article exceed the Heavy Metals limit for lead. Analysis is based on the color reaction between lead and organically bound sulfur. Individual color-comparison tubes are filled with test-article extract, reference standard lead solution and mixture of the two. The solutions are acidified and then mixed with thioacetamide. The color of the reaction products is compared.

Approximate turnaround time is two to three weeks.

Heavy Metals Analysis for 8 elements – *Sample needed: depends on testing requirements*

The purpose of the study is to determine the level of 8 common heavy metals (arsenic, barium, cadmium, chromium, lead, mercury, selenium and silver) in liquid, biological tissue, solid material, or extract of a solid material. Tissues or digestible solids are first acid digested to eliminate interference from proteins and other compounds. Procedure makes use of internal standards. Sample processing for mercury is separate from that used for the other metals, because mercury is analyzed by atomic absorption spectroscopy and the others by inductively-coupled-plasma spectroscopy using either emission spectrometer or mass spectrometer as detector. Per request, analytical service for quantifying analyte(s) includes validation of assay method.

Approximate turnaround time is two to three weeks.

Metals Analysis – Screening (ICP) – *Sample needed: 10 mL*
Metals Analysis – Screening (ICP/MS) – *Sample needed: 25 mL*

The purpose of the study is to determine the amount and types of metallic elements present in a liquid, biological tissue, solid material or extract of a solid material. Sample preparation involves acid digestion of tissues and digestible solids to eliminate interference from proteins and other compounds. Procedure makes use of internal standards. Final solution, be it an extract or filtered acid digest, is analyzed by inductively-coupled-plasma spectroscopy using either emission spectrometer or mass spectrometer as detector. Following metals are measured in a standard test: aluminum, antimony, arsenic, barium, beryllium, boron, cadmium, calcium, chromium, cobalt, copper, iron, lead, magnesium, molybdenum, manganese, nickel, potassium, selenium, silver, sodium, thallium, titanium, vanadium and zinc. Analyses of other elements are also available.

Approximate turnaround time is two to three weeks.

Mercury Analysis – *Sample needed: depends on testing requirements*

The purpose of the study is to determine the level of mercury in a liquid, solid material or extract of a solid material. Samples are digested in acid and then oxidized with persulfate or permanganate. Mercury is measured by atomic absorption spectroscopy.

Approximate turnaround time is two to three weeks.

MATERIAL ANALYSIS

Conductivity USP<645> – *Sample needed: 50 mL or per specified extraction ratio*

Conductivity is a test for the presence of dissolved ions. When dissolved in water, most inorganic and some organic compounds dissociate into ions. Ions are charged and, being mobile, they are electrical conductors. Conductivity testing is performed on either a supplied water sample or water extract of a solid test article. In the latter case, test article is extracted with an aqueous matrix under Sponsor-specified conditions. Conductivity results are reported in units of μS/cm.

Approximate turnaround time is one to two weeks.

Total Organic Carbon (TOC) USP<643> – *Sample needed: 45 mL or per specified extraction ratio*

The purpose of the study is to determine the amount of carbon present in organic compounds within an aqueous sample. The test article may be an aqueous solution, or the aqueous extract of a solid test article, for which extraction is Sponsor specified. The analytical process involves converting organic

compounds to carbon dioxide by a sequence of acidification and oxidation reactions. Liberated carbon dioxide is quantified with an infrared detector.

Approximate turnaround time is two to three weeks.

pH USP <791> – *Sample needed: 25 mL or per specified extraction ratio*

This is a test for the presence of acidic or basic components in a solution. Test is performed with either an aqueous sample or aqueous extract of a solid test article, for which extraction is Sponsor specified.

Approximate turnaround time is one to two weeks.

Fourier Transform InfraRed (FTIR) Scan, USP<197> – *Sample needed: 5 g or 10 cm^2*

FTIR is an instrument for analyzing chemical composition of many organic materials. Because each type of covalent bond has a specific absorption band in the infrared, FTIR is useful for identifying materials and the quality or consistency of samples. The spectra of solid test articles are recorded using attenuated total reflectance. Spectral analysis can include comparison between recorded spectrum and reference spectra of known compounds that are available in a reference-library database. Such analysis confirms identity of a test article and its variance from other materials.

Approximate turnaround time is one to two weeks.

Sample Analysis by UV/Vis, USP<197> – *Sample needed: 10 mL or per specified extraction ratio*

Ultraviolet/Visible spectroscopy (UV/ Vis) is used to measure photon absorption by the electronic shell of atoms. It is useful to characterize the absorption, transmission, and reflectivity properties of any material, be it solid or liquid, organic or inorganic. UV/Vis spectroscopy is routinely used to quantify the concentration of photon-absorbing molecules in a sample. It has limited use for identification of samples that have mixtures of absorbers, because electronic absorption bands are relatively broad and overlap each other. Most UV/Vis instruments are capable of scanning the spectrum from 200 to 1000 nm, which wavelengths respectively correspond to Far UV and Near InfraRed.

Approximate turnaround time is one to two weeks.

Water Determination by Karl Fischer USP<921> – *Sample needed: depends on water content, normally 5 g or 10 cm^2*

This is the standard test for determining the water content of solid or liquid samples. Analysis is based on the reaction between free iodine, water and sulfur dioxide in the presence of an alcohol (the Karl Fischer reaction). Analytical service employs validated assay method.

Approximate turnaround time is one to two weeks.

Loss on Drying USP<731> – *Sample needed: 5 g*

The purpose of the test is to determine the amount of volatile matter in a sample. A known mass of sample is placed in a pre-dried container and heated for 2 hours at ~105 °C (conditions can vary). Resulting non-volatile component is cooled to room temperature in desiccators before its mass is weighed.

Approximate turnaround time is one to two weeks.

Residual Solvents USP<467> – *Sample needed: Depends on testing requirements*

The purpose of the study is to determine the volatile solvents left in a product as a result of its manufacture. Product may be drug substance, excipient or device. Using head space sampling, volatile compounds are driven from product with heating and injected into gas chromatography instrument equipped with flame ionization detector. Samples are analyzed for target compounds known by sponsor to have been used during manufacture. If sponsor lacks such information, samples are screened for potential solvents. To confirm the identity of any detected compound, analysis requires demonstration that chromatography parameters of detected compound and of its reference standard are identical.

Per request, analytical service for quantifying analyte(s) includes development/qualification and validation of assay method.

Approximate turnaround time is one to two weeks.

Analysis for di(2-ethylhexyl) phthalate (DEHP) – *Sample needed: 10 g or cm^2*

DEHP is a plasticizer used in the manufacture of certain plastics. This test determines the amount of DEHP that can be extracted from a test article. Plastic is extracted with 95% ethanol (or alternative solvent) for a given time interval and temperature, which conditions are representative of product life and storage (or as specified by Sponsor). Extract solution is analyzed for DEHP levels by Gas Chromatography/Mass Spectrometry (GC/MS). Analytical service includes extending Toxikon's validated assay method for DEHP to new matrices.

Approximate turnaround time is two to three weeks.

Analysis for bisphenol A (BPA) – *Sample needed: 10 g or cm^2*

BPA is another plasticizer used in the manufacture of certain plastics. This test determines the amount of BPA that can be extracted from a test article. Plastic is extracted with 95% ethanol (or alternative solvent) for a given time interval and temperature, which conditions are representative of product life and storage (or as specified by Sponsor). The extract solution is analyzed for BPA levels by GC/MS. Analytical service includes extending Toxikon's validated assay method for BPA to new matrices.

Approximate turnaround time is two to three weeks.

Head-Space Analysis for Volatile Organic Compounds (VOC) by GC/MS – *Sample needed: Depends on testing requirements*

This is a test for volatile organic chemicals. Test article (solid or solution) is placed in vial, which is inserted into head-space sampler. Content of vial is heated using a predefined program that controls temperature and time duration. Procedure makes use of internal standards. Volatilized gas phase is delivered to the GC/MS for analysis and identification. Per request, analytical service for quantifying analyte(s) includes development/qualification and validation of assay method.

Approximate turnaround time is two to three weeks.

Analysis for Volatile Organic Compounds (VOC) by GC/MS – *Sample needed: Depends on testing requirements*

This test is generally performed with solutions, as it involves purge-and-trap rather than head-space sampling. An aliquot of test solution or extract of a solid article is purged with ultra pure helium which then flows through an activated-carbon column where volatiles are trapped by adsorbing to the solid phase. Procedure makes use of internal standards. The trapped volatiles are subsequently desorbed by heating and delivered to the GC/MS for analysis and identification. Per request, analytical service for quantifying analyte(s) includes development/qualification and validation of assay method.

Approximate turnaround time is two to three weeks.

Analysis for Semi-Volatile Organic Compounds (SVOC) by GC/MS – *Sample needed: Depends on testing requirements*

This is a test for partially volatile chemicals. Test article (solid or solution) is first extracted with an appropriate solvent. Procedure makes use of internal standards. Aliquots of the extract are injected into the GC/MS for analysis and identification. Per request, analytical service for quantifying analyte(s) includes development/qualification and validation of assay method.

Approximate turnaround time is two to three weeks.

Accelerated Aging – *Sample needed: Depends on testing requirements*
Accelerated Aging at 25 °C – *Sample needed: Depends on testing requirements*
Accelerated Aging at 40 °C – *Sample needed: Depends on testing requirements*
Accelerated Aging at 45 °C – *Sample needed: Depends on testing requirements*

MATERIAL ANALYSIS *(cont.)*

Accelerated Aging at 50 °C – *Sample needed: Depends on testing requirements*
Accelerated Aging at 55 °C – *Sample needed: Depends on testing requirements*
Accelerated Aging at 60 °C – *Sample needed: Depends on testing requirements*
Accelerated Aging at 65 °C – *Sample needed: Depends on testing requirements*
Accelerated Aging at 70 °C – *Sample needed: Depends on testing requirements*
Accelerated Aging at 75 °C – *Sample needed: Depends on testing requirements*

Accelerated aging is usually performed in conjunction with toxicological and stability studies to ascertain integrity of test article sooner than would be achievable with real-time testing. For accelerated aging, test article is stored in an environmental chamber under specified and controlled humidity, time duration and elevated temperature. Accepted mathematical algorithms are used to calculate the effect of accelerated aging on analyte stability, integrity and the corresponding equivalent times for storage at lower temperatures.

Approximate turnaround time is one week after study ends.

GENERAL MICROBIOLOGY

Heterotrophic Plate Count USP <61> – *Sample needed: 100 mL*

The Heterotrophic Plate Count assay is a procedure for estimating the number of live Heterotrophic bacteria (the load) present in an aqueous sample. Heterotrophic organisms are organisms that are not able to synthesize cell components from carbon dioxide as a sole carbon source. During most manufacturing processes, treated water is used to make reagents and for the routine cleaning of equipment. Using treated water to minimize the amount of microbes exposed to a product during manufacturing, it is required to have that treated water analyzed on a regular basis.

The membrane filtration method is the method of choice when analyzing aqueous sample. The sample is filtered through a sterile filter membrane and the filter is rinsed with three 20–30 mL portions of sterile Phosphate Buffer to remove bacteriostatic / fungistatic contaminants that might be present. Then the filter is aseptically transferred to plated Heterotrophic agar media, and incubated for the appropriate period of time. The visible colonies are quantified as total Colony Forming Units (CFU) and reported in CFU/mL.

Approximate turnaround time is one to two weeks.

Incubate and Read-USP <1116>

Plates are submitted by the Sponsor for environmental monitoring evaluation. The plates are incubated aerobically for 5 days, then evaluated and reported as CFUs per plate.

Approximate turnaround time is one week.

Gram Stain & Identification

The purpose of a Gram Stain and Identification study is to determine the biochemical profile of an isolate. A Gram stain can be conducted on all bacteria and yeasts. A Gram stain is used to differentiate between Gram positive and Gram negative, cocci and bacilli organisms.

Approximate turnaround time is one week.

BIOBURDEN TESTING

Purpose: The Bioburden test is carried out in order to determine the total number of viable microorganisms on a medical device. Bioburden are performed prior to sterilization after all in-process steps are completed. The dose used for effective radiation sterilization is determined by the resulting bioburden counts. Routine bioburden testing acts a monitoring system which may alert possible production problems which could lead to inadequate sterilization or possible product recall.

Bioburden Validation – Repetitive (Exhaustive) Recovery Method – *Sample needed: 3-10 non-sterile units*

BIOBURDEN TESTING *(cont.)*

The repetitive (exhaustive) recovery method uses the naturally occurring bioburden of the product to determine the efficiency of the recovery of the naturally occurring bioburden on a test article. The extraction method (stomaching, ultrasonicating, shaking, flushing, etc) is repeated on each test article until no significant increase in the number of recovered microorganisms is observed. The goal is to recover all viable microorganisms by washing the test article several times. The recovery fluid can be enumerated by membrane filtration, pour plating, spread plating, and incubated under specific conditions (aerobic bacteria and spores: 30-35°C for 2 to 5 days, Fungi (mold and yeast): 20-25 °C for 5 to 7 days, Anaerobic bacteria: 30-35 °C for 3 to 5 days). The number of viable microorganisms (colony forming units (CFU)) recovered from the first extraction are compared to the total CFU recovered from the sum of all washes to calculate a percent recovery. A correction factor is then calculated which is applied to the Bioburden test numbers for the product. The resulting CFU recovered from one extraction is multiplied by the correction factor to determine the total bioburden of a product. This bioburden value is used to determine the required gamma radiation sterilization dose for the test article. (Refer to Table B.1 of the ANSI/AAMI/ISO 11137 guidelines for dose determination).

Approximate turnaround time is one week.

Bioburden Validation – Spore Inoculation – *Sample needed: 3-10 non-sterile units*

The spore inoculation method determines the efficiency of recovering the naturally occurring bioburden on a test article by creating an artificial bioburden and establishing recovery efficiencies and a correction factor. Each test article is inoculated with a known number of organisms, typically 1×10^2 CFU of *Bacillus subtilis*, and allowed to dry. The recovery method best suited for the particular test article (stomaching, ultrasonicating, shaking, flushing, etc) is used to remove the microorganisms. The recovery fluid can be enumerated by membrane filtration, pour plating, spread plating, and incubated under specific conditions (Aerobic bacteria and spores: 30-35°C for 2 to 5 days, Fungi (mold and yeast): 20-25 °C for 5 to 7 days, Anaerobic bacteria: 30-35 °C for 3 to 5 days). Using the recovered titer to initial inoculum count, the recovery efficiency and correction factor for the test article is calculated. This bioburden value is used to determine the required gamma radiation sterilization dose for the test article. (See Table B.1 of the ANSI/AAMI/ISO 11137 guidelines for dose determination).

Approximate turnaround time is one week.

Bioburden Testing – *Sample needed: Ten samples with each quarterly audit, per AAMI Radiation Sterilization guideline (AAMI/ANSI/ISO 11137)*

Aerobic
Anaerobic
Aerobic & Anaerobic
Aerobic & Yeast/Mold
Aerobic & Heat Shock
Bioburden Aerobic, Yeast/Mold & Heat Shock
Total Bioburden (Aerobic, Anaerobic, and Yeast/Mold)
Total Bioburden & Heat Shock

Bioburden tests may include aerobic bacteria, spores, aerobic fungi, and anaerobes or any combination of these. Testing for both aerobic bacteria and fungi should be considered at minimum. The method (extraction and enumeration procedures) used for the bioburden validation of the test article is used for the routine bioburden testing. The correction factor for the test article determined in the validation is used to calculate the actual bioburden in routine studies.

Approximate turnaround time is one week.

STERILITY TESTING

Purpose: Central to all sterility assurance programs is the sterility testing of medical devices and/or biological indicator spore strips (BIs) exposed to a sterilization process. The release of medical devices for distribution is based on negative sterility test results.

Bacteriostasis & Fungistasis (B&F) Sterility Validation
Membrane Filtration Method per USP <71> – *Sample needed: 6 units*
Direct Transfer Method per USP <71> – *Sample needed: 6 units*
Membrane Filtration Method per AAMI – *Sample needed: 3 units*
Direct Transfer Method per AAMI – *Sample needed: 3 units*

In the Bacteriostasis & Fungistasis (B&F) test, low numbers of viable microorganisms are added to an appropriate medium (TSB per AAMI, TSB & FTM per USP) containing the sterile test article to confirm that the test article does not have any bacteriostatic or fungistatic properties, i.e. does not interfere with organism growth. Such properties may lead to a false negative sterility test (invalid sterility test); therefore this test is required (USP and FDA). This test needs to be performed on a test article prior to a sterility test. This assay must reflect the actual sterility test methodology that will be used on the sample. It needs to be performed on all new products and when any material changes are made during its manufacturing. A sterility test is not valid unless this test has been performed on the test article.

Approximate turnaround time is two weeks.

Product Sterility Testing – *Sample needed: Lot size dependent, recommended 10 minimum*
USP <71> Sterility by Direct Transfer Method
USP <71> Sterility by Membrane Filtration Method
AAMI Sterility by Direct Transfer Method
AAMI Sterility by Membrane Filtration Method

Sterility tests may be carried out via direct transfer method (solid products) or via membrane filtration method (liquid products and extracts from solid products). Using the direct transfer method, products are aseptically transferred directly into the appropriate media. Using the membrane filtration method, products are agitated for at least 15 seconds, the extract fluid filtered through a filter membrane, and then the membrane is transferred into the appropriate media. The media used are Trypticase Soy Broth (TSB) per AAMI guidance or both TSB and Fluid Thioglycollate Media (FTM) per USP guidance. The test articles and/or filters are incubated for 14 days with observations for growth at day 3, day 7 and day 14 at a minimum. A passing sterility test requires no growth over the entire incubation period.

Approximate turnaround time is three weeks.

Inoculated Product Sterility – *Sample needed: Lot size dependent, recommended 10 minimum*

USP Sterility by Direct Transfer Method
USP Sterility by Membrane Filtration Method
AAMI Sterility by Direct Transfer Method
AAMI Sterility by Membrane Filtration Method

The medical device is inoculated with a known amount of spore suspension that is used as a biological indicator. This test is most suited to verify steam or EO penetration into an area of a medical device that is too small to be monitored with a spore strip.

Approximate turnaround time is three weeks.

USP Biological Indicator (BI) Strip Sterility – *Sample needed: 10 to 20 spore strips to monitor a cycle (ISO 11135)*
AAMI Biological Indicator (BI) Strip Sterility – *Sample needed: 10 to 20 spore strips to monitor a cycle (ISO 11135)*

Validated EO sterilization loads are monitored with spore strips placed inside or outside the product depending on the type of spore strip testing performed during the validation. Sterility tests performed on spore strips only is appropriate for devices sterilized by steam or EO in a validated cycle. Medical devices containing the spore strips are used for fractional and half cycles in ethylene oxide and steam

STERILITY TESTING *(cont.)*

sterilization validations. Spore strips from both these tests are cultured in appropriate media and incubated for at least 7 days. A positive control should be included with all spore strip sterility tests.

BI Strip Population Verification – *Sample needed: Based on lot size*

The microbial population recovery from a biological indicator is determined via a population verification test. Each BI strip is aseptically transferred into 100 mL of chilled USP Sterile Water for Injection (SWFI) and homogenized. At the end of homogenization, an additional 5 mL of chilled SWFI is added. Based upon the label claim population, appropriate dilutions are prepared to yield 30-300 CFU per plate. 1 mL of each dilution is pour plated, in duplicate, on Trypticase Soy Agar (TSA) and incubated at 30-35 °C for up to 4 days. A standard plate count is utilized for verification of spore population. This test is routinely performed on incoming BI shipments to verify that the population meets specification before use. At least three BIs should be tested and the percent recoveries of the Label Claim Populations are reported.

Note: The identification of the BI organism and expected titer needs to be provided by Sponsor/manufacturer.

References: USP 31, NF 26, 2008. <55> Biological Indicators Resistance Performance Tests

BACTERIAL ENDOTOXIN (LAL) TESTING

Purpose: The Limulus Amebocyte Lysate (LAL) test is an *in vitro* assay used for detection and quantitation of bacterial endotoxin. LAL is an aqueous extract of the blood cells of horseshoe crabs which forms a clot or change in color, depending on the technique, in the presence of bacterial endotoxin. Validated test methods include both the gel clot and chromogenic methods. Inhibition/Enhancement testing is performed prior to routine LAL testing to demonstrate validity of the LAL test.

Inhibition and Enhancement Testing, Gel Clot Method – *Sample needed: Units from 3 lots*

This test involves testing a liquid sample or a sample extract with Limulus Amebocyte Lysate (LAL). The liquid sample or extract is spiked with varying concentrations of Control Standard Endotoxin (CSE) in quadruplicate. LAL is then added to each tube. All tubes are incubated in a 37 ± 1 °C heat block for 60 ± 2 minutes. After incubation all tubes are examined for agglutination. The test samples are compared to a standard series of Control Standard Endotoxin (CSE) dilutions.

Approximate turnaround time is one week.

Inhibition and Enhancement Testing, LAL Kinetic Chromogenic Method – *Sample needed: Units from 3 lots*

The test article is reconstituted in USP Sterile Water for Injection (SWFI) and assayed in duplicate at various concentrations. A standard curve of endotoxin is prepared with concentrations of 0.005, 0.05, 0.5, and 5 EU/mL. LAL Reagent (endotoxin–free) water and SWFI serve as the negative controls. The microtiter plate is pre–incubated in the plate reader at 37 ± 1 °C for ≥ 10 minutes. After incubation, LAL (0.1 mL) is added to each well and the absorbance of each well at 405 nm is read every 150 seconds for a total of 40 data points or until the concentration reaches 0.2 absorbance units. The Kinetic QCL reader uses the initial reading of each well as its own blank. The absolute value of the correlation coefficient (r) must be ≥ 0.980 in order for the test to be valid.

Approximate turnaround time is one week.

Gel Clot Limulus Amebocyte Lysate Test – *Sample needed: Lot size dependent*

The endpoints of these dilutions are used to calculate the amount of endotoxin present in the sample. All tests are performed at least in duplicate. A positive product control using the sample and negative control using non-pyrogenic water are also performed. The endotoxin limit for devices used intrathecally (cerebrospinal fluid contact) is 2.15 EU/device (Endotoxin Units/device). The limit for other

BACTERIAL ENDOTOXIN (LAL) TESTING *(cont.)*

medical devices is 20.0 EU/device. For drugs and liquids, refer to the LAL Guideline published by US FDA (December 1987).

Approximate turnaround time is one week.

Kinetic Chromogenic Limulus Amebocyte Lysate Test – *Sample needed: Lot size dependent*

The test articles are either immersed in or flushed with USP Sterile Water for Injection (SWFI) at room temperature for 60 ± 2 minutes. Test article extracts are assayed in duplicate at the neat concentration. A standard curve of endotoxin is prepared with concentrations of 0.005, 0.05, 0.5, and 5 EU/mL. A positive product control (PPC) is prepared containing 0.09 mL of the test article and 0.01 mL of a 5 EU/mL endotoxin standard to give a final concentration of 0.5 EU/mL. LAL Reagent (endotoxin–free) water and SWFI serve as the negative controls. The microtiter plate is pre-incubated in the plate reader at 37 ± 1 °C for ≥ 10 minutes. After incubation, LAL (0.1 mL) was added to each well and the absorbance of each well at 405 nm was read every 150 seconds for a total of 40 data points or until the concentration reaches 0.2 absorbance units. The Kinetic QCL reader uses the initial reading of each well as its own blank. The absolute value of the correlation coefficient (r) must be ≥ 0.980 in order for the test to be valid.

Approximate turnaround time is one week.

CUSTOM STUDIES, MICROBIOLOGY

Bi Directional Ingress / Penetration, FDA – *Sample quantity varies per study*

Toxikon offers three categories of testing for bi directional ingress: microbial, viral, and dye. These studies are recommended for Intravascular Administration Sets (FDA, July 11, 2008) because a device that facilitates bi-directional fluid flow may increase the patient's risk of infection. These features may allow the entry of microorganisms into the sterile fluid path. This testing is intended to simulate repeated access, should involve a worst-case challenge scenario, and ideally also include a failure-mode condition. A summary of the three categories are presented below.

Microbial Ingress Testing

Methodology involves applying microorganism onto device surface and performing the access procedure. If device includes disinfection Instructions for Use (IFU) a validation of the described IFU procedure is included. The aim of this study is to establish fluid path barrier capability and microbial ingress into the fluid pathway from the device exterior. Example devices include: extension sets, IV stopcocks and manifolds, in-line filters, flow regulators, fluid delivery tubing, vial adapters, IV transfer sets, subcutaneous administration sets, blood administration sets, & transfusion filters.

Viral Ingress Testing

This test is employed for Single-Use Intravascular Administration Sets which interface with a Multi-Use component. To date there is no industry standard for testing of HIV/HBV specific to medical devices. Toxikon has modified a bacteriophage model routinely used for liquid proofness (i.e. blood) of textiles described by ASTM. The Toxikon model offers approximately 8 Log_{10} sensitivity and is many orders of magnitude greater than detection methods for HIV/HBV. The bacteriophage Toxikon utilizes is based on size, morphology, environmental stability, and non-human infectivity. The purpose of this study differs from the microbial ingress test in that it serves to establish whether virus from an infected patient is capable of penetrating barriers and ingress into a multi-use component. Example devices include multi-use fluid delivery systems such as infusion pumps.

Dye Ingress Testing

This model is used in parallel to the Viral Ingress test as a secondary and unrelated method to the bacteriophage model. This test simulates a chronically infected asymptomatic hepatitis patient (10^8 virion per mL blood). The methodology is non-biological and does not require culture. Mathematically the detection is equivalent to ~100 virion and therefore ~10-100× more sensitive than most HBV detection methods. Example devices include multi-use fluid delivery systems such as infusion pumps.

CUSTOM STUDIES, MICROBIOLOGY *(cont.)*

Approximate turnaround time is based upon study specific parameters.

Sanitization of Clean Room / Pharmaceutical Facilities, USP – *Sample quantity varies per study*

Cleanrooms are designed to minimize and control environmental contamination. Effective pharmaceutical manufacturing maintenance requires appropriate disinfectants and/or sterilants capable of inactivating contaminant environmental microorganisms. Toxikon offers a cleanroom surface cleaning and disinfection validation. These studies utilize the surfaces present in a client's cleanroom and the various cleaners and disinfectants used to decontaminate those surfaces. Common surfaces include: epoxy resins, vinyl sheets, stainless steel, and tempered glass. The end point is Log_{10} reduction of microorganism. An expanded test matrix can include analysis residual of cleaner/disinfectant.

Approximate turnaround time is based upon study specific parameters.

MEDICAL DEVICES, MICROBIOLOGY

REUSABLE MEDICAL DEVICES, FDA – *Sample quantity varies per study*

Toxikon offers five testing categories for reusable medical devices: Lifecycle conditioning, cleaning validations, disinfection validations, and sterilization with dry-time validations. Medical Devices in this category must have adequate labeling and directions for use (21 CFR Part 801) related to device preparation, Instructions For Use (IFU), and reprocessing. These studies apply worst-case challenge conditions and are required to ensure a reusable device is appropriately prepared in order to minimize the risk of residuals transferred to subsequent patients. A summary of the categories are presented below.

Life Cycle Conditioning AAMI/ISO/IEC

The purpose of this study is to simulate a lifetime of device usage. Methodology may involve multiple cycles of soiling, microbial challenge, mechanical stressing, cleaning, disinfection, and sterilization in a manner consistent with the device IFU. The aim of the study is to condition devices for the intended lifecycle. Conditioning is required to demonstrate device functionality over the lifespan of the product. It is also recognized that devices nearing the end of their usable lifecycle may be more difficult to reprocess as the accumulation of residual matter may interfere with biocompatibility, cleaning, disinfection, and sterilization.

Cleaning Validation ANSI/AAMI/ISO

The purpose of this study is to evaluate the recommended cleaning procedure as outlined in the reusable device IFU according to AAMI TIR12 and AAMI TIR30. A successful validation is required to assure a device can be properly cleaned after clinical use. Methodology involves soiling the device with an appropriate artificial test soil and cleaning the device according to the manufacturer's IFU. Conditions employed are typically worst-case and may involve excessive soil challenge and a minimized cleaning procedure. Visual inspection of all devices is performed and exhaustive recovery of residual carbohydrate, hemoglobin (HGB), protein, total organic carbon (TOC) and endotoxin are quantitatively determined and evaluated against the appropriate acceptance criteria.

Disinfection Validation ANSI/AAMI/ISO

The purpose of this study is to determine the efficacy of the disinfection procedure as outlined in the reusable device IFU according to AAMI TIR12, ANSI/AAMI ST81, and FDA guidance documents. Methodology involves inoculating the medical device with an appropriate titer of microorganism. The contaminated device is subjected to the manufacturer's disinfecting instructions and the presence and

MEDICAL DEVICES, MICROBIOLOGY *(cont.)*

quantity of surviving organisms is determined. There are three disinfection levels with distinct acceptance criteria.

High Level – 6 $\log_{10}$ reduction of *Mycobacterium spp*.

Intermediate Level - 6 $\log_{10}$ reduction of *Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli* as well as a 3 $\log_{10}$ reduction of *Mycobacterium spp*.

Low Level - 6 $\log_{10}$ reduction of *Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli.*

Sterilization and Dry-Time Validation ANSI/AAMI/USP

The purpose of this study is to validate the sterilization procedure described in the reusable medical device IFU. Sterility is the absence of viable microorganisms and typically requires a sterility assurance level (SAL) of 10^{-6}. Methodology involves challenging the medical device with a suspension of > 10^6 bacterial spores of *Geobacillus stearothermophilus*, *Bacillus atrophaeus*, or other robust Biological Indicators (BIs) and subjecting the contaminated device to the sterilization procedure. Exhaustive recovery of contaminated devices is performed on devices to determine the presence or absence of surviving organisms.

Some sterilization methods use steam and sterilized devices that retain moisture may be subject to subsequent contamination upon environmental exposure. To ensure the integrity of the sterilization process, devices must be sufficiently dry and a dry time validation is often performed in parallel.

Approximate turnaround time is based upon study specific parameters.

ANTIMICROBIAL STUDIES, MICROBIOLOGY

Toxikon offers a suite of compendial and specialized antimicrobial testing services. Our staff is proficient with USP, AOAC, ASTM, AATCC, JIS, and CLSI test methodologies. Testing services span distinct product categories including cosmetics, textiles, disinfectants, sanitizers, topical antiseptics, antibiotics, and antimicrobial and anti-adherent medical device. A selective summary of standard antimicrobial assays are presented below.

Preservative Efficacy – USP

The purpose of this study is to characterize the efficacy of preservatives contained within compendial products including injections or other parenterals, topicals, oral products, and antacids. This assay can be modified for other product categories and applications and is commonly used for antimicrobial catheters. Methodology involves challenging the product with bacteria, yeast, and mold and incubating for up to 28 days. The population levels of the challenge organisms are determined over multiple time points. The acceptance criterion varies by product category. In all cases, microbial populations cannot exceed the initial inoculum levels and typically, a 3$\log_{10}$ reduction of bacterial challenge is required.

Zone of Inhibition – USP/AATCC

The purpose of this study is to determine the antimicrobial activity of diffusible antimicrobial agents. Methodology involves inoculating an agar plate with a test organism and introducing an antimicrobial device, antibiotic, or product onto the inoculated agar surface. During incubation, the antimicrobial agent diffuses into the surrounding media and may inhibit the growth of the target organism, producing a zone of inhibition. Typically, the zone of inhibition is measured for a quantitative assessment of

activity. Antimicrobial medical devices are often conditioned to simulate use conditions and subjected to the zone of inhibition assay as a simple screen to assess the potential decrease in antimicrobial activity over the course of a device lifespan.

Time Kill – ASTM

The purpose of this study is to determine the reduction of microorganisms within a specified time period upon exposure to an antimicrobial item or substance. Methodology is application specific and involves introduction of an aerobic microbial population into a test product or onto a test item, thoroughly mixing, and quantitatively determining the kill kinetics of microbial reduction over time. Time Kill is often performed as a Shake Flask method and neutralization validation is required for each antimicrobial product and strain combination.

Disinfectant Efficacy Testing – AOAC/ASTM/USP

The purpose of this study is to validate the antimicrobial efficacy of sanitizing agents against target microorganisms according to AOAC, ASTM, and USP Guidelines. Methodology involves applying a small volume aliquot of target culture, often supplemented with 5% blood serum, to a substrate, allow to dry, and disinfectant application. Exposure times vary but typically do not exceed 10 minutes. Contaminated and disinfectant-exposed substrates are transferred to an appropriate neutralizer where surviving microorganisms are harvested, enumerated, and compared to untreated controls. Methodology is versatile and applicable for bactericidal, fungicidal, tuberculocidal, virucidal, and sporicidal efficacy claims. Neutralization validation is required for each disinfectant and microorganism combination to ensure any log reductions observed are a result disinfectant efficacy and not from inhibition of microbial growth due to residual disinfectant transfer during enumeration.

MIC, MBC, and FIC – CLSI

Minimum Inhibitory Concentration (MIC)
The purpose of this study is to determine the quantitative *in vitro* activity of an antimicrobial agent against a specific bacterial isolate and can be applied to bacteria that grow aerobically, anaerobically, or fastidious bacteria. Methodology involves inoculation of a standardized microorganism into serial, two-fold dilutions of an antimicrobial agent. After incubation, microorganism growth is observed. The lowest concentration of antimicrobial in which no growth is observed is the minimum inhibitory concentration (MIC). MIC measures growth inhibition.

Minimum Bactericidal Concentration (MBC)
The purpose of this assay is to determine the lowest concentration of an antimicrobial agent required for cidality. Methodology involves sub-culturing aliquots in which no growth was observed from the MIC assay and observing for the presence or absence of surviving microorganisms. Antimicrobial concentrations in which no bacterial growth was observed both the MIC and MBC demonstrate cidality against the test strain.

Fractional Inhibitory Concentration (FIC)
Some compounds may improve the antimicrobial efficacy of other compounds and act synergistically to inhibit or kill bacterial isolates. Methodology follows a checkerboard format and involves inoculation of a standardized microorganism into serial, two-fold dilutions of an antimicrobial agent as well as a second compound. Compound combinations are observed for their effect on inhibition or cidality, compared to their effect as a monotherapy, and may be classified as indifferent, antagonistic or synergistic.

Anti-Adherance

The purpose of this study is to determine the anti-adherent properties of a material coating to microbial colonization. These studies are distinct from antimicrobial efficacy studies in that a reduction in the

ANTIMICROBIAL STUDIES, MICROBIOLOGY *(cont.)*

population of a challenge suspension is not required, only a reduction in adherent microorganisms compared to an untreated control. Methodology involves microbial challenge, appropriate incubation, and elimination of non-adherent or weakly-adherent microorganisms. Attached organisms are either removed by sonication and quantified or directly visualized by electron microscopy and compared to untreated controls.

Bacterial Anti-Virulence Studies

Bacteria possess multiple mechanisms that contribute to virulence and include multidrug efflux pumps (MDR), Type III Section (TTSS), Quorum Sensing (QS), persister formation, iron acquisition by siderophores, and antibiotic modifying enzymes (AGMEs or ESBLs). Many compounds inhibit these virulence processes and while they may not possess intrinsic antimicrobial activity, they can improve the efficacy of existing antibiotics or delay virulence and allow a successful immune defense. These studies are highly variable and methodology may involve phenotypic observation (growth, motility, biofilm formation) and biochemical and fractional inhibition concentration (FIC) assays.

Approximate turnaround time is based upon study specific parameters.

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Animal Models of Infection

Toxikon offers a state-of-the-art animal facility. Animal models of infection are an invaluable resource for translational research and development and offer efficacy data not available through strictly *in vitro* approaches. Administration of the challenge, site of infection, and microbial challenge vary according to the product and target claims. The methodology involves inoculating model animals with an indicator microorganism(s) or promoting infection from resident microorganisms and applying product or drug regimen. The result of the microbial injury may be characterized directly by visualization with electron microscopy or quantitative recovery. Additionally, injury may be characterized by the histological effects on surrounding tissues or by quantitation of immune response indicators and compared to untreated controls.

Approximate turnaround time is based upon study specific parameters.