

## Biocompatibility

Jay McTaggart  
Email: JMCT@uswest.net

Biocompatibility is a mouthful of a word and can be intimidating to most of our colleagues.

Biocompatibility may be a daunting word but testing for it can be relatively simple. Why shouldn't it be? Let's keep in mind what it means. Biocompatibility simply means effects on life. So, prudent manufactures will simply follow a few simple steps to determine the effects of a medical device on life. This simple information is then used to understand the risk / benefit relationship in using a medical device. Many devices are not completely inert. Most things aren't. Drinking water is generally considered ok, but if we drink too much it is not ok at all.

We must first understand the intended clinical use of a medical device in order to determine the extent of biocompatibility testing required. Surface devices like bandages or pulse monitors are not generally tested as an implant. Conversely, it is very important to choose appropriate endpoints for the testing of implantable medical devices.

Approval of medical devices by regulatory agencies requires that biocompatibility testing and risk assessments be conducted to predict safety of the device. The aim of the risk assessment testing is to understand the safety and risks associated with the use of this device. This risk assessment usually involves functionality, biological safety, and packaging. This article will focus primarily on the biological safety testing of medical devices. MDRG has expertise in most of these areas from patents to patients. Our experts welcome each opportunity to provide support when needed.

Medical devices and their component materials may leach compounds, which may produce undesirable effects when used clinically. The selection and evaluation of materials and devices intended for use in humans requires a structured program of assessment to establish biocompatibility and safety. Current regulations, whether in accordance with the FDA, ISO, or MHLW require adequate safety testing of devices through pre-clinical and clinical phases as part of regulatory clearance.

The ISO, MHLW, OECD, and FDA guidelines provide a general framework to aid in the assessment of device biocompatibility. The number and type of specific safety tests required to assess product safety and compliance is dependent upon the individual characteristics of the device, its component materials, and its intended clinical use. Device Manufacturers must show due diligence in the risk assessment of any medical device. This due diligence involves testing for the safety of a medical device according to its IFU and the regulatory guidelines. Due diligence involves many complex steps but can be quite simple by using the expertise of MDRG.

During the development of the biological testing strategy of a product, it is necessary to discuss the regulatory requirements for the evaluation of the product. Risk assessments may be obtained by testing according to recognized guidelines, including those described in FDA G95-1, ISO 10993, and Japanese Guidelines for Basic Biological Tests for Medical Devices and Materials. Other testing guidelines may include, OECD, ASTM, USP, BP, etc.

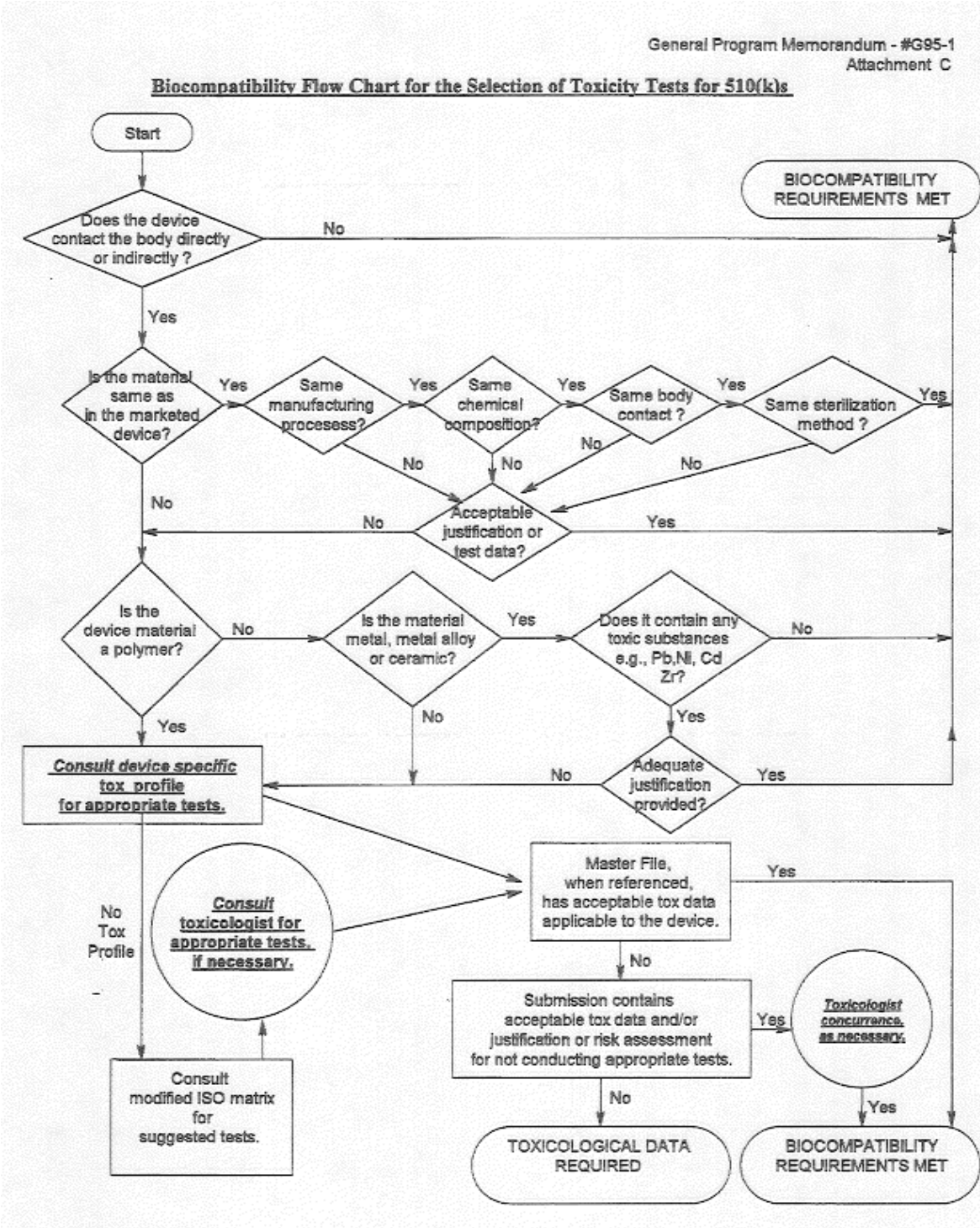
Risk assessments can most accurately be predicted through the diligent use of these methods. The testing strategy developed for the medical devices should be developed to immaculate the intended final use of the product and to utilize the most diligent pathway while minimizing the use of laboratory animals.

Manufacturers must understand the materials used in the product and the processes that those materials are encountering. Many times, manufacturers will use component materials that are not novel and have been previously used in manufacture of a predicate medical device. You may ask if additional testing is required. My standard answer is "Risk assessments have proven that inert component materials can have interactions".

I recommend some limited testing data simply to compare the data from the old device to that of the new device. Prudent manufacturers must realize this. ISO committees and regulatory bodies have noted this necessity for many years.

---

The decision tree below comes from the FDA's decision tree and is a great guide to help understand the point. There are other resources that can be sent to you upon request. MDRG members welcome any questions and can be an excellent resource for device manufacturers.



I am certain that you know that it may be impossible to be 100% sure that the new component material is 100% safe. Your goal is to show diligence in identifying the risk involved with using this material in your current product. I assure you that there are several ways to examine these risks and you may receive different advice from different scientists.

The purpose of biocompatibility testing is not simply to test the materials but also the processes that these materials go through and the intended nature of the product. Process variations and variations in use may cause the materials to leach differently.

The purpose of this article is to provide the groundwork for a biocompatibility study plan for manufacturers to use when determining the extent of biocompatibility to perform. You will find a table in Appendix 1. The table is a fill-in table from the FDA. This table should be completed with your 510K submissions. It is usually helpful to understand what reviewers will be looking for.

It is generally accepted that in order to submit a 510K to FDA, you may only need to claim substantial equivalence to a currently marketed product. This may be easier if you own the safety data of the predicate device. For this reason, Toxikon laboratories will generally recommend that you have full biocompatibility and safety data on the lead or predicate product in any product family. This may make future product revisions more efficient from a regulatory perspective.

Regulatory clearance based upon ISO, as well as FDA guidelines, requires that a biological assessment be documented on the finished device with the new material to indicate substantial equivalence. If testing is required the selection of tests should be based upon the test matrix outlined in ISO 10993-1.

Device manufacturers may decide to use a step or screening approach to evaluate the toxic potential of a can be most efficient. The step approach can be beneficial in understanding the biocompatibility of the medical device.

Lets work through an example.

You may have been making a product using a component material for many years. Suddenly the component manufacturer will not provide the same material for you. They say the new material is similar to the old material. The component manufacturer will likely have some test data for you to prove it. \*Remember that this test data will not likely have identical components attached, packaging or sterilization identical to yours. Manufacturers may simply provide a letter to file. Prudent manufacturers will also perform some basic screening tests to show due diligence.

Remember that the levels of safe exposure to leachables from the device correspond to the nature and duration of contact during clinical end use. Table 1 is a comprehensive list for testing of a guide wire or delivery catheter. It may also be helpful for many other types of devices. Table 2 is a detailed list of timelines and sample requirement.

A comprehensive test selection strategy allows for tests that challenge various in vitro non-animal and in vivo multi-animal models with device materials and polar and non-polar extracts to determine biocompatibility characteristics. Manufacturers may choose chemical characterization and or biological testing. Toxikon recommends a combination.

**Table 1**

ISO 10993 Requirement	Path	Test Code	Test Name	Rationales for the Selection
Cytotoxicity	I		L929-MEM	ISO 10993 – 5 This assay determines the biological reactivity of a mammalian cell culture (L929) in response to an extract of a test material. The cells are allowed to grow to confluency in petri plates. An extract of the test material is prepared which is transferred onto the cell layer. Both positive and negative controls are tested similarly. The degree of cytotoxicity is graded on a scale from no reactivity to severe reactivity. This study is a good indication of basic cellular responses to test material and extract of that material. If the study outcomes are not identical or better then further studies will be ordered. If these studies are identical then we feel

ISO 10993 Requirement	Path	Test Code	Test Name	Rationales for the Selection
				comfortable that cytotoxicity equivalence has been indicated for this product.
Sensitization	2		Kligman – GPMT	ISO 10993 – 10 This test evaluates the allergenic potential or sensitizing capacity of a test material. The test is used as a procedure for screening contact allergens in guinea pigs and extrapolating the results to humans, but it does not establish the actual risk of sensitization. The allergic potential is classified on a scale of 0 to 3.
Irritation	2		Intracutaneous Injection	ISO 10993 – 10 The screen tests materials or their extracts for potential toxic effects as a result of a single dose intracutaneous injection in rabbits. These toxic effects may be considered the first step in sensitization A primary irritation index is determined based on the defined evaluation criteria in ISO 10993-10. If primary irritation potential is observed then further studies will be ordered. If the study outcomes are not identical or better then further studies will be ordered. If outcomes of these studies are identical then we feel comfortable that substantial equivalence has been indicated for the biological effects of this new product.
Acute Systemic Toxicity	2		Systemic Injection	ISO 10993- 11 This <i>in vivo</i> test screens test materials or their extracts for potential toxic effects as a result of a single-dose systemic injection in mice. If the study outcomes are not identical or better then further studies will be ordered. If these studies are identical then we feel comfortable that equivalence has been indicated for systemic effects of this new product.
	3		Material Mediated Pyrogenicity Assay	ISO 10993- 11 This test is performed to limit to an acceptable level the risks of febrile reaction in a patient by the administration of the product concerned. The test involves measuring the rise in temperature of albino rabbits following the intravenous injection of a test material or its extract through the marginal ear veins of four rabbits. Body temperatures are recorded at 30-minute intervals between 1 and 3 hours subsequent to injection. If no rabbit shows an individual rise in temperature of 0.5 degrees C or more above the baseline temperature, the test material meets the requirements for the absence of pyrogens.
Genotoxicity	2		Salmonella Reverse Mutation Assay (Ames Mutagenicity Assay)	ISO 10993-3 (Screening for Genotoxicity Indications using in-vitro methodologies) If markers show adverse response then more conclusive In-vivo testing may be investigated. If markers in the two bacterial strains are normal we feel comfortable that the genetic safety of our product is acceptable.
	3		Mouse Lymphoma Assay	ISO 10993-3 (Screening for Genotoxic Indications using in-vitro methodologies) This assay measures the ability of a test material to induce forward mutations at the thymidine kinase locus in the presence and absence of a metabolic activation system. The principle and design of the assay is similar to the HGPRT assay except that the selective agent is trifluorothymidine and the colonies are isolated on agar. If Chromosomal markers in the In Vitro Cells are normal we feel comfortable with the genetic safety of our product.
	3		Rodent Bone Marrow Micronucleus Assay	ISO 10993-3 (Screening for Genotoxic Indications using in-vitro methodologies) If markers in the bone marrow are normal we feel comfortable with the genetic safety of our product.

ISO 10993 Requirement	Path	Test Code	Test Name	Rationales for the Selection
Hemocompatibility	2		IVH	ISO 10993 – 4 This is a study that Most efficiently examines multiple components of blood after challenge to our test material. This study not only studies basic hematology but also studies Platelets, hematocrit, erythrocyte indices, plasma-free hemoglobin and concentration; mean cell hemoglobin and cell volume as well as differential.
	2		Lee White	ISO 10993 – 4 This study is an analysis of the clotting time and thrombogenic indications using human blood upon direct exposure to our test material. This is a study that is widely used for Japanese Submission; the information will be useful at a later time.
	1		Hemolysis	ISO 10993-4 This study measures the effects of contact with test material on Red blood cells
	3		Complement Activation	ISO 10993-4 This is an <i>in vitro</i> assay designed to measure complement activation in human plasma as a result of exposure of the plasma to the test material. The measure of complement actuation indicates whether a test material is capable of inducing a complement induced inflammatory immune response (including production of anaphylatoxins) in humans. The assay is evaluated by measuring the quantity of the protein complements C3a and C5a in human blood plasma that has been exposed to the test material $37 \pm 2$ degrees C for 2,4 and 6 hours. Plasma control tubes are similarly incubated. A standard curve is obtained using controlled standards. The concentrations of C3a and C5a are determined against this standard curve. The mean concentration of C3a and C5a in plasma exposed to the test material should not significantly differ from the mean of the corresponding untreated plasma controls.
	3		In Vivo Thromboresistance	ISO 10993-4 This study evaluates material(s) intended for blood contact for thrombogenicity properties <i>in vivo</i> . This test is appropriate for comparing materials to each other in the same animal. Historically, dogs have been used in thrombogenicity studies to assess possible human toxicity caused by material that is intended for use in contact with human blood. The ISO 10993-4 guidelines have no published alternative (non-animal) methods. The extent of clot formation and damage to the vein intima is recorded and photographed. If requested by the sponsor, clot material is accumulated, dried and weighed in order to quantify differences between the test and control samples. The extent of thrombi formation is scored on a scaled of 0 to 5.
Reproductive / Developmental	4			
Biodegradation	2		Physical-Chemical properties	CFR 176
USP Physical / Chemical Characterization	1	1	Re-agent Test	Test performed on finished product USP 28

**Testing Matrix**

Base on the testing strategy above, detailed time lines and sample requirements have been estimated as following.

Table 2 Test Turn-Around Time And Sample requirements

Part	Test Name	Test Model	Test Duration	Measurement	Test Outcome	Cost	Turnaround Time	Sample Requirement
1	L-929 MEM Elution	In-Vitro	72 Hours	Cellular Response	Scale 0-4		1-4 Weeks	4 gm or 120 cm <sup>2</sup>
2	Kligman Maximization Sensitization Assay	Guinea Pig	28 Days	Sensitization Potential	Scale 0-3		6-8 weeks	32 gm or 960 cm <sup>2</sup>
2	Intracutaneous Injection Assay	Rabbit	3 Days	Irritation Potential	Scale 0-3		2-4 weeks	8 gm or 240 cm <sup>2</sup>
2	Systemic Injection Assay	Mouse	3 Days	Systemic Response	Pass / Fail		2-4 weeks	8 gm or 240 cm <sup>2</sup>
3	Material Mediated / Rabbit Pyrogen Test	Rabbit	3 Hours	Febrile Response	Pass / Fail		1-2 weeks	20 gm or 600 cm <sup>2</sup>
2	Salmonella Reverse Mutation (Ames) Assay	Salmonella typhimurium & Escherichia coli	14 Days	Statistical Increase in Genetic Mutations	Positive / Negative		3-4 weeks	8 gm or 240 cm <sup>2</sup>
3	Mouse Lymphoma Assay	Cells		Statistical Increase in Genetic Mutations	Pass / Fail		10-12 Weeks	4 gm or 120 cm <sup>2</sup>
3	Rodent Bone Marrow Micronucleus Assay	Cells		Statistical Increase in Genetic Mutations	Positive / Negative		10-12 weeks	4 gm or 120 cm <sup>2</sup>
1	Hemolysis - Autian	Blood	1 Day	Hemoglobin Response	Pass / Fail		1-4 weeks	15 gm
1	Hemolysis - Direct	Blood	1 Day	Hemoglobin Response	Pass / Fail		1-4 weeks	15 or at least 3 units
3	Prothrombin Time Test	Blood	1 Day	Clotting Time	Pass / Fail		1-4 weeks	1 unit
2	Lee & White Clotting Time Test	Blood	1 Day	Clotting Time / Thrombogenic Potential	Pass / Fail		1-4 weeks	1 unit
2	In Vitro Hemocompatibility Assay	Blood	3 Days	Statistical Changes in various Blood components	Pass / Fail		1-4 weeks	3 units
3	In Vivo Thromboresistance Assay	Dog	4 Hours	Thrombus Formation	Scale 0-5		1-4 weeks	2 units
	USP 28 Physico/Chemical Test	GMP Markers	5 Days	Level of Residual	Pass / Fail		2-4 weeks	30 gm or at least 3 units

Let's say that the materials incorporated into the new product have an extensive clinical history and the new product has only slight modifications from the old product. The test selection should be based on the intended use of the finished device as outlined in ISO 10993-1. You will find that in pulling tests from 10993-1, you will need approximately 2 months simply for biocompatibility testing. This timeline may not suit many manufacturers but is the most vigilant testing strategy.

I must advise that it would be best and simpler to identify risks involved with the clinical use of a medical device if all of the testing recommended by regulatory agencies is performed. The benefit of this strategy would be that you would be closer to 100% sure that the finished medical device is 100% safe. With these data points it allows for a spectrum of analysis of an "Improved" product. Your objection to this strategy may be that it is quite costly, or the strategy will take up to three months to complete, or that it will require the more laboratory animals than is actually necessary to show due diligence with minor modifications to your predicate device.

Since it is our primary focus in this scenario is to show substantial biological equivalence of the new product to the predicate one, we will be examining and comparing test outcomes from the two products after completing the form in Appendix 2.

The manufacturer in the above scenario may choose an alternative testing strategy that may be completed within 2 weeks. This strategy uses biological screening tests and supplier data as a major factor to determine the biocompatibility of the device. Screening tests are typically very sensitive and relatively quick. This strategy may be used in order to minimize the use of laboratory animals while comparing the biological results of the two products.

Cytotoxicity and hemolysis are good examples of these screening tests. Cytotoxicity tests are generally performed in vitro on cells that are extremely sensitive to minute quantities of leachable chemicals. These cells readily display characteristic signs of toxicity. Hemolysis is another screening test that can provide useful information. The hemolysis test is usually performed in rabbit blood and studies a product's effects on red blood cells. The results of these tests are often an indicator of biological responses. These tests may provide an adequate touchstone for comparison of two devices. See Appendix 2.

This is my recommended strategy if no additional regulatory requirements are necessary and you will simply need to identify that the "Improved" product is "Substantially Equivalent" to the product that has been previously tested. This strategy will look at key endpoints and allow for the analysis of these endpoints. The endpoint data (Numerical Score in the raw data or scientific conclusion) from the Cytotoxicity testing and Hemocompatibility testing will be compared in Appendix 2. Cytotoxicity scores are graded from 0-4. If your old score was 1 and your new score is 1 then you may conclude that they are "Substantially Equivalent". Hemolysis testing is scored on a Pass/Fail basis with a numerical value. If your old product caused less than 1.7 % hemolysis and your new product caused 1.7% hemolysis then you may conclude again that the two products are "Substantially Equivalent" in this area as well. I would personally recommend performing the In-Vitro Hemocompatibility assay with this strategy since it gives much data of what is happening with various blood components after contact with the test material. The more data points for comparison, the better understanding you will have.

The benefit of this strategy is that it will support a claim of "Substantial Equivalence". This strategy will take less than 30 days to complete (if the endpoints are clearly equivalent), it will cost much less, and it will minimize the use of laboratory animals. Your objection to this strategy is that it does create a slight risk of "Missing something" in the analysis of the "Improved" product. IF substantial equivalence cannot be shown then additional time and investment will become necessary to better understand any biological differences in the endpoint responses. The next group of tests would be those labeled Path 2 – 4 in tables 1 and 2.

Appendix 1:

### Biocompatibility Certification

Fill out the table for all applicable testing that will be performed based on the nature (category and contact type) and duration of body contact prior to marketing your device.

If testing will be performed on each component/material, fill out a separate table for each material. If test(s) are deemed not applicable, provide rationale for each omitted. Provide details of extraction (i.e., will the entire device be extracted, or only a small piece, and will all the components be included in the extraction, will the extraction be done on the FINISHED device subjected to all processing methods and sterilization, and according to GLPs)

Body Contact Classification: External Communicating Device with Limited contact to Circulating Blood

<b>Test to be performed</b> (literature citation, standard reference*)	<b>Extract(s)</b> if used (polar, non-polar), or animal model/cell line	<b>Extract conditions</b> (time, temperature, area or mass to volume ratio compared to in-use conditions)	<b>Test and Control(s)</b> used.	<b>Pass/Fail Criteria</b> (units when appropriate)
<b>Cytotoxicity</b>	MEM	37°/24hrs 10993-12	L-929 Cells	Scale 0-4
<b>Sensitization</b>	Polar/Non-Polar	37°/72hrs 10993-12	Guinea Pig	Scale 0-3
<b>Irritation/Intracutaneous Toxicity</b>	Polar/Non-Polar	37°/72hrs 10993-12	Rabbit	Scale 0-3
<b>Systemic toxicity</b>	Polar/Non-Polar	37°/72hrs 10993-12	Mouse	Pass / Fail
<b>Sub-chronic toxicity</b>	N/A	N/A	N/A	10993-1
<b>Genotoxicity</b>				
Gene mutation	Polar/Non-Polar	37°/72hrs 10993-12	Salmonella, E coli	Positive / Negative
Chromosome aberration	If genetic markers in the In Vitro Salmonella and E. coli are normal with both Polar and Non- Polar extractions we feel comfortable with the genetic safety of our product.			
DNA damage				
<i>in vivo assay</i>				
<b>Implantation</b>	N/A	N/A	N/A	10993-1
<b>Hemocompatibility</b>				
Hemolysis	Direct/NaCl	Direct/37°/24hrs	Blood	Pass/Fail – Hemoglobin Response
Thrombogenicity (Lee & White/ IVH)	NaCl	37°/72hrs 10993-12	Blood	Pass/Fail – Clotting Time, Thrombogenic Potential/Statistical Changes in varied Blood Components
Complement activation	N/A	N/A	N/A	10993-4
<b>Pyrogenicity</b>	NaCl	37°/72hrs 10993-12	Rabbit	Pass/Fail – Febrile Response
<b>Chronic toxicity</b>	N/A	N/A	N/A	10993-1
<b>Carcinogenicity</b>	N/A	N/A	N/A	10993-1
<b>Biodegradation</b>	WFI	37°/24hrs 10993-12	WFI	10993-1
<b>Reproductive/Developmental toxicity</b>	N/A	N/A	N/A	10993-1

\* Describe all deviations from the standard/protocol n a separate sheet.

Appendix 2:

Below you will find the modified FDA

Fill out the table for all applicable testing that has been performed on the predicate device and on the new device based on the nature (category and contact type) and duration of body contact prior to marketing your device. Results will be clear and easy to correlate. If the results of biological tests are not identical then consider the next test pathway. If results are equivalent after the first pathway, then supplier's data and finished device testing may be adequate touchstones risk assessment of the new device.

Body Contact Classification: External Communicating Device with Limited contact to Circulating Blood

Test to be performed (literature citation, standard reference*)	Extract(s) if used (polar, non-polar), or animal model/cell line	Extract conditions (time, temperature, area or mass to volume ratio compared to in-use conditions)	Test and Control(s) used.	Pass/Fail Criteria (units when appropriate)	Predicate Product Results	New Product Results
<b>Cytotoxicity</b>	MEM	37°/24hrs 10993-12	L-929 Cells	Scale 0-4		
<b>Sensitization</b>	Polar/Non-Polar	37°/72hrs 10993-12	Guinea Pig	Scale 0-3		
<b>Irritation/Intracutaneous Toxicity</b>	Polar/Non-Polar	37°/72hrs 10993-12	Rabbit	Scale 0-3		
<b>Systemic toxicity</b>	Polar/Non-Polar	37°/72hrs 10993-12	Mouse	Pass / Fail		
<b>Sub-chronic toxicity</b>	N/A	N/A	N/A	10993-1		
<b>Genotoxicity</b>						
Gene mutation	Polar/Non-Polar	37°/72hrs 10993-12	Salmonella, E coli	Positive / Negative		
Chromosome aberration	If genetic markers in the In Vitro Salmonella and E. coli are normal with both Polar and Non- Polar extractions we feel comfortable with the genetic safety of our product.					
DNA damage						
<i>in vivo assay</i>						
<b>Implantation</b>	N/A	N/A	N/A	10993-1		
<b>Hemocompatibility</b>						
Hemolysis	Direct/NaCl	Direct/37°/24hrs	Blood	Pass/Fail – Hemoglobin Response		
Thrombogenicity (Lee & White/ IVH)	NaCl	37°/72hrs 10993-12	Blood	Pass/Fail – Clotting Time, Thrombogenic Potential/Statistical Changes in varied Blood Components		
Complement activation	N/A	N/A	N/A	10993-4		
<b>Pyrogenicity</b>	NaCl	37°/72hrs 10993-12	Rabbit	Pass/Fail – Febrile Response		
<b>Chronic toxicity</b>	N/A	N/A	N/A	10993-1		
<b>Carcinogenicity</b>	N/A	N/A	N/A	10993-1		
<b>Biodegradation</b>	WFI	37°/24hrs 10993-12	WFI	10993-1		
<b>Reproductive/Developmental toxicity</b>	N/A	N/A	N/A	10993-1		

\* Describe all deviations from the standard/protocol on a separate sheet.

For additional information, please contact:

Toxikon  
13911 Ridgedale Drive  
Minneapolis, MN. 55305  
Phone: 800-425-4044  
Fax: 952-847-9007  
[www.toxikon.com](http://www.toxikon.com)

Jay McTaggart  
Email: [JMCT@uswest.net](mailto:JMCT@uswest.net)