



BIOCOMPATIBILITY TESTING

Medical devices and their component materials may leach compounds or have surface characteristics that 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 U.S. Food and Drug Administration (FDA), the International Organization for Standardization (ISO), or the Japanese Ministry of Health, Labor and Welfare (JMHLW), require that manufacturers conduct adequate safety testing of their finished devices through pre-clinical and clinical phases as part of the regulatory clearance process.

The FDA, ISO, and JMHLW guidelines provide a general testing framework to aid manufacturers in the assessment of device biocompatibility. The number and type of specific safety tests required to assess product safety and compliance are dependent on the individual characteristics of the device, its component materials, and its intended clinical use.

Within the general safety testing framework, it remains the responsibility of the device manufacturer to select and justify the specific tests most appropriate for the establishment of product safety and compliance with FDA, ISO, and JMHLW requirements. It is recommended that testing be performed to comply with GLP regulations. WuXi AppTec, with many years of experience in biocompatibility testing, provides exceptional expertise to assist medical device manufacturers in designing thorough, well constructed testing programs to satisfy regulatory requirements.

IN THIS SECTION

NOTE: *All samples for biocompatibility testing should be sent to WuXi AppTec's St. Paul facility.*

As applicable, samples should be submitted after exposure to the Sponsor's sterilization process.

LIST OF TESTS IN THIS SECTION – See next page.

REFERENCE INFORMATION Pages B-30 – B-34

- Guide to Assessing Biocompatibility Testing Needs [Chart]
- Testing Requirements Under GLP Regulations
- Required Biocompatibility Tests for Classifications of Plastics (USP)
- Device Categories
- Initial Evaluation Tests for Consideration [Chart based on ISO 10993-1 and FDA G95-1 Guidelines]
- “Quick Guide” to Sample Requirements for Biocompatibility Tests [Chart]

IN THIS SECTION

- **Cytotoxicity Testing** *Pages B-3 – B-6*
 - Agarose Overlay
 - MEM Elution
 - Growth Inhibition Assay
 - Direct Contact Cytotoxicity
 - Cytotoxicity for Liquid Products
 - MTT Assay
- **Genotoxicology / Mutagenicity Testing** *Pages B-7 – B-9*
 - Bacterial Reverse Mutation Tests
 - In Vitro* Chromosomal Aberration Assay
 - In Vitro* Mouse Lymphoma Assay
 - In Vivo* Mouse Micronucleus Screen
 - In Vivo* Mouse Micronucleus Test
- **Hemocompatibility Testing** *Pages B-10 – B-12*
 - NIH and ASTM Hemolysis Tests
 - Complement Activation
 - Partial Thromboplastin Time
 - Platelet and Leukocyte Counts
 - Thrombosis (*In Vivo*)
- **Implantation Testing** *Pages B-13 – B-15*
 - USP / ISO Intramuscular Implantation
 - ISO Subcutaneous Implantation
 - ISO Intramuscular & Subcutaneous Implant Tests – 13-week Duration
 - Intramuscular or Subcutaneous Implant Screen Test
 - Histopathology Assessment
 - Surgical Implantation
- **Irritation/Intracutaneous Reactivity Testing** *Pages B-16 – B-17*
 - USP / ISO Intracutaneous Irritation Test
 - ISO Vaginal Mucosal Irritation Test
 - Ocular Irritation Test [Topical]
 - Intraocular Irritation Test
 - ISO Primary Skin Irritation Test
- **Pyrogenicity Testing (*In Vivo*)** *Page B-18*
 - USP Rabbit Pyrogen Test
 - ISO Rabbit Pyrogen – Materials Mediated
- **Sensitization Testing** *Pages B-19 – B-20*
 - Murine Local Lymph Node Assay (LLNA)
 - ISO Maximization Sensitization Test
 - Repeated Patch Dermal Sensitization Test
- **Subacute/Subchronic Toxicity Testing** *Page B-21*
- **Systemic (Acute) Toxicity Testing** *Page B-22*
- **Chronic Toxicity and Carcinogenicity Testing** *Page B-23*
- **Finished Product Release Testing** *Page B-23*
 - USP Safety Test in Mice

JMHLW TESTING *Pages B-24 – B-29*

Testing services designed specifically to meet the requirements of the Japanese Ministry of Health, Labor and Welfare (JMHLW)

For Analytical Chemistry and USP Physicochemical testing, see the “Chemistry” section of this catalog.

Additional test methods are available.
Contact your WuXi AppTec Account Manager for more information.

These tests involve the exposure of substances extracted from test material to one of two cell culture lines. Cell cultures are extremely sensitive to minute quantities of leachable chemicals and readily display characteristic signs of toxicity in the presence of potentially harmful leachables. The tests are frequently used during product planning stages to qualify the use of a material and as a periodic check for routinely used materials to ensure that no shift in quality has occurred. Cytotoxicity *in vitro* testing is also required in testing the biocompatibility of materials. Typical testing programs will utilize the ISO test method to meet international regulatory requirements. The USP test method is performed to meet the FDA's U.S. regulatory requirements. The screening test method can be performed to characterize materials or to evaluate new materials against established ones.

AGAROSE OVERLAY

SAMPLE REQUIREMENTS 1 mL liquid (sterile) • Sufficient material to produce 3 patches, 1cm x 1cm each
TURNAROUND TIME 7 days (non-GLP) • 14 days (GLP)

L-929 mouse fibroblast cells are overlaid with a permeable agar film. A solid sample or liquid saturated disc is then placed in triplicate containers on the agar surface. Cells are examined at 24 hours for signs of toxicity.

140150
(ISO) Agarose Overlay – L-929 Mouse Fibroblast Cells

L-929 mouse fibroblast cells are overlaid with a permeable agar film. A solid sample or liquid saturated disc is then placed in duplicate containers on the agar surface. Cells are examined at 24 hours for signs of toxicity.

140100
(USP) Agarose Overlay – L-929 Mouse Fibroblast Cells

L-929 mouse fibroblast cells are overlaid with a permeable agar film. A solid sample or liquid saturated disc is then placed in one single container on the agar surface. Cells are examined at 24 hours for signs of toxicity.

140000
(Screening) Agarose Overlay – L-929 Mouse Fibroblast Cells

MRC-5 human embryonic lung cells are overlaid with a permeable agar film. A solid sample or liquid saturated disc is then placed in triplicate containers on the agar surface. Cells are examined at 24 hours for signs of toxicity.

140220
(ISO) Agarose Overlay – MRC-5 Human Embryonic Lung Cells

MRC-5 human embryonic lung cells are overlaid with a permeable agar film. A solid sample or liquid saturated disc is then placed in one single container on the agar surface. Cells are examined at 24 hours for signs of toxicity.

140175
(Screening) Agarose Overlay – MRC-5 Human Embryonic Lung Cells

MEM ELUTION

SAMPLE REQUIREMENTS

All samples should be sterile and cut into approx. 5 x 0.3 cm sizes with total surface area as shown

| By Thickness | | By Weight | By Weight (low density material) | Liquids |
|--------------------|--------------------|-----------|-------------------------------------|---------|
| < 0.5mm thick | >0.5mm thick | | | |
| 30 cm ² | 15 cm ² | 1 g | 0.5 g | 3 mL |

***Tests are set up on Mondays, Thursdays and Fridays.
Samples should arrive at least one business day prior to testing.
Samples will not be set up on weekends or holidays.***

140320

**(ISO) MEM Elution –
L-929 Mouse Fibroblast Cells**

Solid materials are extracted in cell culture medium and the extracts are then placed in triplicate containers of L-929 mouse fibroblast cells. Cells are examined at 24, 48 and 72 hours for signs of toxicity.

TURNAROUND TIME 7 days (non-GLP) • 18 days (GLP)

140325

**(ISO) MEM Elution –
L-929 Mouse Fibroblast Cells
(Liquid Test Article)**

Liquid test articles are mixed with cell culture medium and then placed in triplicate containers of L-929 mouse fibroblast cells. Cells are examined at 24, 48 and 72 hours for signs of toxicity.

TURNAROUND TIME 7 days (non-GLP) • 18 days (GLP)

140300

**(USP) MEM Elution –
L-929 Mouse Fibroblast Cells**

Solid materials are extracted in cell culture medium and the extracts are then placed in duplicate containers of L-929 mouse fibroblast cells. Cells are examined at 24 and 48 hours for signs of toxicity.

TURNAROUND TIME 6 days (non-GLP) • 17 days (GLP)

140270

**(Screening) MEM Elution –
L-929 Mouse Fibroblast Cells**

Solid materials are extracted in cell culture medium and the extracts are then placed in a single container of L-929 mouse fibroblast cells. Cells are examined at 24 hours for signs of toxicity.

TURNAROUND TIME 5 days (non-GLP) • 16 days (GLP)

When a test material is shown to be toxic by the MEM elution test, eight 2-fold dilutions are made to determine the toxic endpoint. Performance of the test will result in an estimation of the relative "strength" of the cytotoxic substance in the material.

TURNAROUND TIME 7 days (non-GLP) • 18 days (GLP)

140350

**MEM Endpoint Dilution –
L-929 Mouse Fibroblast Cells**

Solid materials are extracted in cell culture medium and the extracts are then placed in triplicate containers of MRC-5 human embryonic lung cells. Cells are examined at 24, 48 and 72 hours for signs of toxicity.

TURNAROUND TIME 7 days (non-GLP) • 18 days (GLP)

140420

**(ISO) MEM Elution –
MRC-5 Human Embryonic Lung
Cells**

Solid materials are extracted in cell culture medium and the extracts are then placed in one container of MRC-5 human embryonic lung cells. Cells are examined at 24 hours for signs of toxicity.

TURNAROUND TIME 5 days (non-GLP) • 16 days (GLP)

140370

**(Screening) MEM Elution –
MRC-5 Human Embryonic Lung
Cells**

When a test material is shown to be toxic by the MEM elution test, eight 2-fold dilutions are made to determine the toxic endpoint. Performance of the test will result in an estimation of the relative "strength" of the cytotoxic substance in the material.

TURNAROUND TIME 7 days (non-GLP) • 18 days (GLP)

140450

**MEM Endpoint Dilution –
MRC-5 Human Embryonic Lung
Cells**

CYTOTOXICITY TESTING CONTINUED NEXT PAGE

CYTOTOXICITY TESTING

140500

Growth Inhibition Assay (ISO 9363)

The purpose of the test is to evaluate the cytotoxic potential of test articles or test article extracts according to ISO method (9363-1:1994(E)). Solid materials are extracted in cell culture medium and a series of dilutions of the test article extract are placed into triplicate containers of L-929 mouse fibroblast cells for 72 hours. The protein levels of all samples will be determined by using the Lowry protein determination method. The percentage of growth inhibition will then be determined.

SAMPLE REQUIREMENTS *Sample requirements are the same as for MEM tests.*

TURNAROUND TIME 7 days (non-GLP) • 18 days (GLP)

140250

Direct Contact Cytotoxicity – L-929 Mouse Fibroblast Cells

A sample is placed in direct contact with L-929 mouse fibroblast cells. Cells are examined at 24 hours for signs of toxicity.

SAMPLE REQUIREMENTS 3 samples of 1 cm² solid (sterile)

TURNAROUND TIME 4 days (non-GLP) • 18 days (GLP)

140260

Direct Contact Cytotoxicity – MRC-5 Human Embryonic Lung Cells

A sample is placed in direct contact with MRC-5 human embryonic lung cells. Cells are examined at 24 hours for signs of toxicity.

SAMPLE REQUIREMENTS 3 samples of 1 cm² solid (sterile)

TURNAROUND TIME 4 days (non-GLP) • 18 days (GLP)

140550

MTT Cytotoxicity Assay with L-929 Mouse Fibroblast Cells

Solid materials are extracted in cell culture medium. Multiple dilutions of the extract are prepared and added to triplicate wells. After incubation, the MTT reagent {3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide} is added to each well. After a second incubation, the amount of formazan formed is determined. Cytotoxicity is calculated based on the formazan levels.

SAMPLE REQUIREMENTS
(Per Extract)

| By Thickness | | By Weight | By Weight (lightweight) |
|--------------------|--------------------|-----------|----------------------------|
| <0.5mm thick | >0.5mm thick | | |
| 36 cm ² | 18 cm ² | 1.2 g | 0.8 g |

TURNAROUND TIME 19 days (non-GLP) • 26 days (GLP)

Genotoxicology (mutagenicity) tests evaluate the ability of a material to cause mutation or gross chromosomal damage. Any materials intended for implantation or long term exposure should be evaluated for mutagenic properties. Unpolymerized materials, additives, trace monomers or oligomers and biodegradative products can all be potential mutagens.

The International Organization for Standardization (ISO) 10993-3 outlines tests for genotoxicity, carcinogenicity and reproductive toxicity. The ISO guidelines for genetic toxicity testing require a minimum of two tests: one for gene mutation (Bacterial Mutagenicity Test), and one for DNA effects with chromosomal aberrations (Mouse Lymphoma Assay).

The FDA requires three tests. The Bacterial Reverse Mutation and the *in vitro* Mouse Lymphoma tests are the same as suggested by ISO. Currently, for the third test, some within the FDA are recommending an *in vivo* test, such as the Mouse Micronucleus test. (See Page B-9.)

Extracts are prepared using solutions that will extract both hydrophilic (polar) and lipophilic (non-polar) compounds possibly present in device materials. Generally, medical devices require the use of one dose (the undiluted extract) for regulatory submission, unless significant toxicity is anticipated.

**In addition to the tests listed on the following pages, other genotoxicology tests are available.
For more information, contact your WuXi AppTec Account Manager.**

Sample Requirements for Genotoxicology Testing

All material samples should be cut into approx. 5 x 0.3 cm sizes with total surface area as shown.

Note: For ISO, sample preparation complies with ISO10993-12.

EXTRACT OPTIONS: Polar Extracts (e.g., Normal Saline)
Non-polar Extracts (e.g., DMSO or Cottonseed Oil)

When ordering a test, specify one set of extraction conditions:

121°C/1hour 70°C/24 hours 50°C/72 hours 37°C/72 hours

[NOTE: 50°C/72 hours is recommended for genotoxicology testing.]

SAMPLE REQUIREMENTS FOR *IN VITRO* TESTING (PER EXTRACT)

| By Thickness | | By Weight | By Weight (low density material) | Liquids |
|--------------------|--------------------|-----------|-------------------------------------|---------|
| <0.5mm thick | >0.5mm thick | | | |
| 60 cm ² | 30 cm ² | 2 g | 1 g | Inquire |

SAMPLE REQUIREMENTS FOR *IN VIVO* TESTING (PER EXTRACT)

| By Thickness | | By Weight | By Weight (low density material) | Liquids |
|---------------------|--------------------|-----------|-------------------------------------|---------|
| <0.5mm thick | >0.5mm thick | | | |
| 120 cm ² | 60 cm ² | 4 g | 2 g | Inquire |

**For testing that meets Japanese regulatory requirements, call laboratory regarding sample size.
See also test listings for JMHLW testing starting on Page B-24.**

BACTERIAL REVERSE MUTATION TESTS

190800 5 *S. typhimurium*

190802 4 *S. typhimurium*
+ 1 *E. coli*

**Ames Mutagenicity Screen –
Saline (SCI) Extract**

The Ames Mutagenicity test is performed on extracts of solid materials. To perform this test, five tester strains of bacteria (five *Salmonella typhimurium* or four *Salmonella typhimurium* and one *Escherichia coli*), in which specific mutations can be induced, are exposed to the test material and to a number of positive controls. This test can only be used for screening purposes.

TURNAROUND TIME 21 days (non-GLP) • 28 days (GLP)

190860 5 *S. typhimurium*

190862 4 *S. typhimurium*
+ 1 *E. coli*

190863 5 *S. typhimurium*
+ 1 *E. coli*

**(ISO) Bacterial Mutagenicity
Test – Saline (SCI) and Dimethyl-
sulfoxide (DMSO) Extracts**

The ISO Bacterial Reverse Mutation test is performed according to ISO 10993-3 using OECD test method 471. Tester strains of bacteria (five *Salmonella typhimurium* or four *Salmonella typhimurium* and one *Escherichia coli* or five *Salmonella typhimurium* and one *Escherichia coli*) are exposed to extracts of the test material in the presence and absence of an exogenous metabolic activation system. One dose level of the test article per extract and both positive and negative controls are used.

TURNAROUND TIME 21 days (non-GLP) • 28 days (GLP)

190760 5 *S. typhimurium*

190762 4 *S. typhimurium*
+ 1 *E. coli*

190763 5 *S. typhimurium*
+ 1 *E. coli*

**Bacterial Mutagenicity Test –
One Extract**

This Bacterial Reverse Mutation test is performed according to ISO 10993-3 using OECD test method 471. Tester strains of bacteria (five *Salmonella typhimurium* or four *Salmonella typhimurium* and one *Escherichia coli* or five *Salmonella typhimurium* and one *Escherichia coli*) are exposed to an extract of the test material in the presence and absence of an exogenous metabolic activation system. One dose level of the test article extract and both positive and negative controls are used.

TURNAROUND TIME 21 days (non-GLP) • 28 days (GLP)

190810

**Ames Mutagenicity Confirmation
Test**

Test materials yielding positive results on the Ames Mutagenicity plate incorporation test should be repeated with the strains that showed positive results. The confirmation test is run with appropriate positive and negative controls and in the presence and absence of metabolic activation.

TURNAROUND TIME 21 days (non-GLP) • 28 days (GLP)

IN VITRO MAMMALIAN CELL TESTS

Mammalian cells are exposed to the test material or extract in the presence and absence of metabolic activation and blocked in metaphase using a spindle poison. Visualization of chromosomes is performed microscopically after hypotonic swelling, fixation and staining. Test code 190828 (two extracts) satisfies the ISO requirement for assessing *in vitro* chromosomal aberrations.

TURNAROUND TIME 55 days (non-GLP) • 62 days (GLP)

190820 1 extract
190828 2 extracts

***In Vitro* Chromosomal Aberration Assay**

Mouse Lymphoma cells are used to determine whether a test material has the capacity to induce either point mutations or clastogenic (chromosomal breakage) events in a cultured mammalian cell line. Mutants can be selected and mutant frequencies derived by including a thymidine analogue (trifluorothymidine, TFT) in the culture medium of cells after exposure to the test material. Test code 190850 (two extracts) satisfies the ISO requirement for assessing *in vitro* DNA genotoxic effects.

TURNAROUND TIME 32 days (non-GLP) • 39 days (GLP)

190851 1 extract
190850 2 extracts

***In Vitro* Mouse Lymphoma Assay**

IN VIVO TESTS

The mammalian *in vivo* micronucleus test is used for the detection of damage induced to the chromosomes or the mitotic apparatus of erythroblasts by analysis of erythrocytes sampled from bone marrow of mice. For this assay, male mice are injected with 1 dose level of the test article extract or positive or negative control. Cells are collected at 24 and 48 hours after dosing.

TURNAROUND TIME 48 days (non-GLP) • 55 days (GLP)

190700 1 extract

***In Vivo* Mouse Micronucleus Screen**

The mammalian *in vivo* micronucleus test is used for the detection of damage induced to the chromosomes or mitotic apparatus of erythroblasts by analysis of erythrocytes from bone marrow of treated mice. In this assay, male and female mice are injected with 1 dose level of the test article extract or positive or negative control. Cells are collected for analysis at 24 and 48 hours after dosing.

TURNAROUND TIME 48 days (non-GLP) • 55 days (GLP)

190852 1 extract
190853 2 extracts

***In Vivo* Mouse Micronucleus Test**

HEMOLYSIS TESTING

An important measure of hemocompatibility is the hemolysis test, which measures the ability of a material or material extract to cause red blood cells to rupture. Hemolysis testing should be performed on all materials directly contacting the bloodstream, or any materials used to form a fluid conduit to the bloodstream. The following tests are derived from well-established studies/standards and are useful in evaluating a variety of materials intended to contact blood or fluids entering the circulatory system. While both the NIH and ASTM methods are accepted by regulatory agencies, the ASTM method is the preferred method for ISO-compliant testing.

150100 NIH Method
150300 ASTM Method

Hemolysis Test – Direct Contact Method

This test, intended for materials that directly contact the bloodstream or compromised tissues, is performed in triplicate and uses rabbit blood in direct contact with the test material. The degree of hemolysis is measured spectrophotometrically.

NIH Method

SAMPLE REQUIREMENTS 3 x 2 g or 3 x 0.2 g (light weight)
(No comparison product required)

TURNAROUND TIME 2 days (non-GLP) • 21 days (GLP)
Tests set up on Tuesday.

ASTM Method

SAMPLE REQUIREMENTS (No comparison product required)

| By Thickness | | By Weight | Liquids |
|------------------------|------------------------|-----------|---------|
| ≤0.5mm thick | >0.5mm thick | | |
| 3 x 42 cm ² | 3 x 21 cm ² | | |

TURNAROUND TIME 2 days (non-GLP) • 21 days (GLP)
Tests set up on Thursday.

150200 NIH Method
150500 ASTM Method

Hemolysis Test – Extract Method

This test, intended for materials through which fluids pass before entry into the body, is performed in triplicate and uses saline to extract leachable substances. The material is removed and rabbit blood is added to the extract. The degree of hemolysis is measured spectrophotometrically.

Specify one set of extraction conditions: 121°C/1hour 70°C/24 hours
37°C/72 hours 50°C/72 hours

NIH Method

SAMPLE REQUIREMENTS 3 x 2 g or 3 x 0.2 g (light weight)
(No comparison product required)

TURNAROUND TIME 2 days (non-GLP) • 21 days (GLP)
Tests set up on Tuesday.

ASTM Method

SAMPLE REQUIREMENTS (No comparison product required)

| By Thickness | | By Weight | Liquids |
|------------------------|------------------------|-----------|---------|
| ≤0.5mm thick | >0.5mm thick | | |
| 3 x 60 cm ² | 3 x 30 cm ² | | |

TURNAROUND TIME 2 days (non-GLP) • 21 days (GLP)
Tests set up on Thursday.

150505 ASTM (F756) Method

Hemolysis Test – Combination Extract / Direct Contact Method

The following tests are offered for evaluating the interaction of biomaterials, polymers and medical devices with circulating blood. The assays are designed to be compliant with ISO 10993-4. Additional studies are available. For more information, contact your Account Manager.

Note: Testing a sponsor-supplied comparison product is recommended when requesting these assays to aid in clarifying interpretation of test results.

The activation of complement resulting from the use of a medical device has been associated with many adverse clinical findings. An enzyme immunoassay is used to screen for complement component(s) in human serum that has been incubated with the test article. Elevated levels of complement components C3a and SC5b-9 indicate activation of the complement system. Both the C3a and SC5b-9 assays are available.

SAMPLE REQUIREMENTS PER ASSAY:
6 cm² (<0.5 mm thick) or 3 cm² (>0.5 mm thick)
or 0.2 g

An additional fee will apply for test articles with a surface area greater than required.

TURNAROUND TIME 18 days (non-GLP) • 25 days (GLP)

150600 C3a Assay
150620 SC5b-9 Assay
150630 C3a and SC5b-9

Complement Activation

150605 C3a Assay
150625 SC5b-9 Assay
150635 C3a and SC5b-9

Complement Activation Including Sponsor-Supplied Comparison Product

The PTT assay is a general screening test for the detection of coagulation abnormalities in the intrinsic coagulation pathway. The test determines the time it takes citrated human plasma to form a clot when it is exposed first to the test material, then to calcium chloride and finally to partial thromboplastin. (Partial thromboplastin is a phospholipid suspension extracted from rabbit brain cephalin.) Test results report the "partial thromboplastin time," which is the time it takes the recalcified citrated plasma to clot once the partial thromboplastin has been added. Shortening of the PTT following contact with a material under standardized conditions indicates activation of the contact phase of blood coagulation.

SAMPLE REQUIREMENTS 3 x 4 cm²

TURNAROUND TIME 18 days (non-GLP) • 25 days (GLP)

155200
Partial Thromboplastin Time (PTT)

155205
Partial Thromboplastin Time (PTT) Including Sponsor-Supplied Comparison Product

Platelet and leukocyte counts are evaluated before and after exposure to the test material in human blood. Counts are evaluated for changes that may indicate activation, adhesion, aggregation, or lysis.

SAMPLE REQUIREMENTS 3 x 12 cm²

TURNAROUND TIME 18 days (non-GLP) • 25 days (GLP)

155600
Platelet and Leukocyte Counts

155605
Platelet and Leukocyte Counts Including Sponsor-Supplied Comparison Product

800520

Thrombosis (*In Vivo*)

Test article (e.g., tubing or catheter) is implanted in the jugular veins of two (2) dogs. Usually the test article is implanted in the jugular vein on one side and a sponsor-supplied comparative control article on the contralateral side for up to 3 days. The test article and implant sites are removed and examined for the presence of thrombi, and the vein is examined for patency (occlusion). These observations are augmented with photographs.

SAMPLE REQUIREMENTS 2 test products and 2 control products
(Length: 8-15cm • Max. Diameter: ≤ 3mm)

TURNAROUND TIME 21 days (non-GLP) • 28 days (GLP)

These tests assess the local effects of material or finished product on contact with living tissue. Using a needle or surgical procedure, a sample is implanted into the tissue site appropriate for the intended use of the device. After the selected duration of contact, the tissue sites are evaluated for gross changes and – if requested – histopathology.

Biomaterial is implanted intramuscularly into rabbits to assess the reaction of the surrounding tissue. The implants remain in the muscle for the sponsor-designated time period. If the animals exposed to the test article do not show significant signs of irritation above that observed in the concurrent test control, the test article passes the test. Scoring of the sample and control reactions will be by gross observation. Histopathologic evaluation and photomicrographs (gross and histologic) will also be provided at the request of the sponsor. (Additional fees apply.)

SAMPLE REQUIREMENTS

USP: Sufficient material to produce 12 implants, approx. 10mm x 3mm

ISO: Sufficient material to produce 15 implants, approx. 10mm x 3mm

[Note: For ISO, sample preparation complies with ISO10993-12.]

TURNAROUND TIME

| Implant | Gross | With Histopathology |
|---------|----------|---------------------|
| 1 wk | 35 days | 49 days |
| 2 wk | 42 days | 56 days |
| 4 wk | 56 days | 70 days |
| 8 wk | 94 days | 98 days |
| 13 wk | 119 days | 133 days |

- 900100** 1 wk implantation duration
- 900200** 2 wk implantation duration
- 900300** 4 wk implantation duration
- 900400** 8 wk implantation duration
- 900505** 13 wk implantation duration

USP Intramuscular Implantation (2 Rabbits)

- 902100** 1 wk implantation duration
- 902200** 2 wk implantation duration
- 902300** 4 wk implantation duration
- 902400** 8 wk implantation duration
- 902505** 13 wk implantation duration

ISO Intramuscular Implantation (3 Rabbits)

A portion of the material or device is implanted subcutaneously into rabbits to assess the reaction of the surrounding tissue. The implants remain for the sponsor-designated time period. If the animals exposed to the test article do not show significant signs of irritation above that observed in the concurrent test control sites, the test article is considered equivalent to the comparator. The sample and control tissue sites will be evaluated grossly. Histopathologic evaluation and photomicrographs (gross and histologic) will also be provided at the request of the sponsor. (Additional fees apply.)

SAMPLE REQUIREMENTS

Sufficient material to produce 15 implants, approx. 10mm x 3mm

[Note: For ISO, sample preparation complies with ISO10993-12.]

TURNAROUND TIME

| Implant | Gross | With Histopathology |
|---------|----------|---------------------|
| 1 wk | 35 days | 49 days |
| 2 wk | 42 days | 56 days |
| 4 wk | 56 days | 70 days |
| 8 wk | 84 days | 98 days |
| 13 wk | 119 days | 133 days |

- 901310** 1 wk implantation duration
- 901320** 2 wk implantation duration
- 901340** 4 wk implantation duration
- 901380** 8 wk implantation duration
- 901430** 13 wk implantation duration

ISO Subcutaneous Implantation (3 Rabbits)

902605

**ISO Intramuscular Implant
Test (5 Rabbits) –
13-week Implantation
Duration**

[Includes Histopathology,
Clinical Chemistry, and
Hematology]

The purpose of this study is to evaluate the potential for local effects of a test article, in direct contact with skeletal muscle for an extended duration.

For the safety evaluation of biomaterials, the test article, or portions thereof, will be implanted in the paravertebral muscles of live rabbits. Blood will be drawn for clinical chemistry and hematology at defined intervals. At minimum, blood will be collected pre-implant and at termination. After a predetermined exposure period, the animals will be sacrificed and the implantation sites will be examined, harvested and fixed for histopathology. The final analysis of the local effect of the test article will be based on the clinical, gross and histopathologic data. Depending on the nature of the device microstructure, the specimens may be fixed to stabilize the tissue device interface and then oriented and cut-in for histology processing. Microscopic evaluation will include such things as: cell type, cell distribution, fibroplasia, and calcification. Control implants typically consist of similar dimensional pieces of USP high density polyethylene RS (HDPE) implanted contralaterally. Sponsor supplied, clinically marketed predicate materials may be more appropriate controls used in conjunction with or in replacement of HDPE.

Gross and histologic photomicrographs will also be provided at the request of the sponsor. (Additional fees apply.)

SAMPLE REQUIREMENTS

Sufficient material to produce 25 implants, approx. 10mm x 3mm

[Note: For ISO, sample preparation complies with ISO10993-12.]

TURNAROUND TIME 133 days

901555

**ISO Subcutaneous Implant
Test (5 Rabbits) –
13-week Implantation
Duration**

[Includes Histopathology,
Clinical Chemistry, and
Hematology]

The purpose of this study is to evaluate the potential for local effects of a test article, intended for human use, when implanted into the subcutaneous tissue of the rabbit for an extended duration.

For the safety evaluation of a test article, the entire device or appropriate samples and an appropriate reference control will be implanted in the subcutaneous tissues of live rabbits. Blood will be drawn for clinical chemistry and hematology at defined intervals. At a minimum, blood will be collected pre-implant and on study termination. After a predetermined exposure period, the animals will be sacrificed and the implantation sites will be examined, harvested and fixed for histopathology. The final analysis of the local effect of the test article will be based on the clinical, gross and histopathologic data. Depending on the nature of the device microstructure, the specimens may be fixed to stabilize the tissue device interface and then oriented and cut-in for histology processing. Microscopic evaluation will include such things as: cell type, cell distribution, fibroplasia, and calcification. Control implants typically consist of similar dimensional pieces of USP high density polyethylene RS (HDPE) implanted contralaterally. Sponsor supplied, clinically marketed predicate materials may be more appropriate controls used in conjunction with or in replacement of HDPE.

Gross and histologic photomicrographs will also be provided at the request of the sponsor. (Additional fees apply.)

SAMPLE REQUIREMENTS

Sufficient material to produce 25 implants, approx. 10mm x 3mm

[Note: For ISO, sample preparation complies with ISO10993-12.]

TURNAROUND TIME 133 days

WuXi AppTec's professional staff can assist in the design and implementation of studies to investigate medical device biocompatibility issues. These studies help determine whether device surface characteristics, polymeric composition and physical geometry may effect local tissue responses such as inflammation, tissue ingrowth, vascularization and fibroplasia. Gross and histologic photomicrographs can also be provided at the request of the sponsor.

For other custom implant studies, such as bone, intracranial and intraperitoneal implants, contact your Account Manager.

Custom Implant Studies**Intramuscular or
Subcutaneous Implant
Screen Test**

Histopathology assessment is conducted for muscle or subcutaneous tissues if part of the protocol. (Additional fees apply.)

Note: ISO guidelines assume histopathology will be conducted.

TURNAROUND TIME Inquire

800250**Histopathology Assessment**

Surgical implantation may be requested by client or may be required for larger sample sizes. (Additional fees apply.)

910100**Surgical Implantation**

IRRITATION TESTING

Irritation (reactivity) tests assess the localized reaction of tissues to device materials or extracts. The selection of a test method is based on the intended patient contact type. For breached tissue and blood contact, the intracutaneous test is usually selected and uses only extracts. The dermal irritation test usually involves direct contact with the test material. The mucosal irritation test can involve either direct contact or use of extracts. The ocular tests usually use extracts. Extracts are prepared using solvents that will extract either hydrophilic (polar) or lipophilic (non-polar) compounds that may be present in the device materials.

900600

USP Intracutaneous Irritation Test
(2 rabbits per extract or pair of extracts)

An extract of the device or biomaterial is prepared in up to four (4) standard USP extraction solutions and is injected intracutaneously into rabbits to assess the irritancy of extractable compounds that may exist in the biomaterial. The animals are observed for dermal reactions over a 72-hour period. If the animals exposed to the test article extract do not show significant signs of irritation above those observed in the concurrent test control groups, the test article passes the test. Gross photography may be requested. (Additional fees apply.)

EXTRACT OPTIONS

- Normal Saline
- 5% Ethanol in Saline
- Cottonseed Oil
- Polyethylene Glycol

Specify one set of extraction conditions:

| | |
|---------------|---------------|
| 121°C/1hour | 70°C/24 hours |
| 50°C/72 hours | 37°C/72 hours |

SAMPLE REQUIREMENTS
(Per Extract)

| By Thickness | | By Weight | By Weight (lightweight) | Liquids |
|--------------------|--------------------|-----------|-------------------------|---------|
| <0.5mm thick | >0.5mm thick | | | |
| 36 cm ² | 18 cm ² | 1.2 g | 0.6 g | 5 mL |

Note: For ISO, normal saline and/or cottonseed oil extracts are recommended. Sample preparation complies with ISO10993-12.

TURNAROUND TIME 22 days (non-GLP) • 29 days (GLP)

910790

ISO Vaginal Mucosal Irritation Test
(6 rabbits per extract)

Test material coming in direct or indirect contact with mucosal tissue can be assessed as to its irritation potential by repeated instillation of an extract into rabbit vaginas. Acute irritation is evaluated by gross observation and histopathology of the vaginal mucosa and submucosa. Usually an application is performed on each of five days unless longer treatment is indicated due to the use of the device. Final evaluation is based on histopathological evaluation.

EXTRACT OPTIONS

- Normal Saline
- Cottonseed Oil

Specify one set of extraction conditions:

| | |
|---------------|---------------|
| 121°C/1hour | 70°C/24 hours |
| 50°C/72 hours | 37°C/72 hours |

SAMPLE REQUIREMENTS
(Per Extract)

| By Thickness | | By Weight | By Weight (lightweight) | Liquids |
|-----------------------|------------------------|-----------|-------------------------|---------|
| < 0.5mm thick | >0.5mm thick | | | |
| 5 x 60cm ² | 5 x 30 cm ² | 5 x 2 g | 5 x 1 g | 30 mL |

Note: For ISO, normal saline and/or cottonseed oil extracts are recommended. Sample preparation complies with ISO10993-12.

TURNAROUND TIME 47 days (non-GLP) • 54 days (GLP)

Three rabbits receive a 0.1 mL dose of a polar (normal saline) or nonpolar (cottonseed oil) extract of the test material into one eye of each rabbit. After a 24-hour exposure, the eye is flushed and the conjunctiva, cornea and iris are evaluated for up to 72 hours for acute irritation or injury. Test materials should be prescreened using the Cytotoxicity Agarose Diffusion Test.

EXTRACT OPTIONS • Normal Saline • Cottonseed Oil

Specify one set of extraction conditions: 121°C/1hour 70°C/24 hours
50°C/72 hours 37°C/72 hours

SAMPLE REQUIREMENTS

(Per Extract)

| By Thickness | | By Weight | Liquids |
|--------------------|--------------------|-----------|---------|
| <0.5mm thick | >0.5mm thick | | |
| 60 cm ² | 30 cm ² | 2 g | 5 mL |

Note: For ISO, sample preparation complies with ISO10993-12.

TURNAROUND TIME 35 days (GLP)

910810

Ocular Irritation Test [Topical]
(3 rabbits per extract)

A 0.15 mL dose of test material extract is injected with a fine-gauge needle into the anterior chamber of one eye. At the same time, a vehicle control is injected in the same manner into the opposing eye. Over the 3-day exposure of the test, reaction to the injected substance is evaluated using a slit-lamp microscope and the degree of reaction is scored using a standard scoring system. The test material passes the test if the test article extract does not produce irritation to a significantly greater degree than the control material. Test materials should be prescreened using a cytotoxicity test.

EXTRACT Balanced Salt Solution (BSS)

Specify one set of extraction conditions: 121°C/1hour 70°C/24 hours
50°C/72 hours 37°C/72 hours

SAMPLE REQUIREMENTS

| By Thickness | | By Weight |
|--------------------|--------------------|-----------|
| <0.5mm thick | >0.5mm thick | |
| 60 cm ² | 30 cm ² | 2 g |

Note: For ISO, sample preparation complies with ISO10993-12.

TURNAROUND TIME 35 days (GLP)

910825

Intraocular Irritation Test
(3 rabbits)

910820

Intraocular Irritation Test
(6 rabbits)

This test is performed to assess the potential for topical irritation from acute exposure or use of the device material. The material is applied to intact skin of three (3) rabbits and left in contact for 4 to 24 hours. An estimate of irritation, erythema (redness) and edema (swelling) is made during the next 72 hours. Histopathologic evaluation and photomicrographs (gross and histologic) will also be provided at the request of the sponsor. Additional fees apply.

Note: Sponsor specifies contact duration on test request form.

SAMPLE REQUIREMENTS

Sufficient material to produce 7 patches, approx. 2.5cm x 2.5cm each • 5 mL of liquid

TURNAROUND TIME 23 days (non-GLP) • 30 days (GLP)

910699

ISO Primary Skin Irritation Test
(3 rabbits)

NOTE: For information on additional irritation tests, contact your WuXi AppTec Account Manager.

PYROGENICITY TESTING (IN VIVO)

Pyrogenicity tests determine the potential of materials, extracts, and/or a finished device to induce a pyrogenic (fever) response from sources in addition to endotoxin.

Note: Bacterial endotoxin (the most commonly encountered type of pyrogen) can be readily detected and quantified using the *in vitro* Limulus Amebocyte Lysate (LAL) Test. For information on LAL tests, see this catalog's "Endotoxin (LAL)" section.

900750

USP Rabbit Pyrogen Test (3 rabbits)

The test articles are prepared in a sterile solution, which is injected intravenously into three (3) rabbits to assess pyrogenicity. The animals are observed over a 3-hour period for an increase in body temperature. If the animals exposed to the solution do not show significant increase in body temperature, the test article passes the test. If any single animal of the three has a temperature increase above the acceptable range, the test can be continued with 5 additional animals at client's request. (See below.)

SAMPLE REQUIREMENTS

Transfusion / Infusion Assemblies and Similar Devices:
10 device assemblies to represent the lot under test.

Blood and Tissue Contact Devices:
10 devices to represent the lot under test.

TURNAROUND TIME 25 days (non-GLP) • 32 days (GLP)

900770

ISO Rabbit Pyrogen – Materials Mediated (3 rabbits)

An extract of the test article is prepared in a sterile saline solution and injected intravenously into three (3) rabbits to assess pyrogenicity. The animals are observed over a 3-hour period for an increase in body temperature. If the animals exposed to the test article extract do not show significant increase in body temperature, the test article passes the test. If any single animal of the three has a temperature increase above the acceptable range, the test can be continued with 5 additional animals at client's request. (See below.)

EXTRACT OPTION Normal Saline

Specify one set of extraction conditions: 121°C/1hour 70°C/24 hours
50°C/72 hours 37°C/72 hours

SAMPLE REQUIREMENTS

| By Thickness | | By Weight | By Weight (lightweight) |
|---------------------|---------------------|-----------|----------------------------|
| <0.5mm thick | >0.5mm thick | | |
| 900 cm ² | 450 cm ² | 30 g | 15 g |

TURNAROUND TIME 19 days (non-GLP) • 26 days (GLP)

900755

Rabbit Pyrogen Test Continuation (5 rabbits)

See USP Rabbit Pyrogen and ISO Rabbit Pyrogen – Materials Mediated tests above. Continuation of testing is at client's request. (Additional fees apply.)

EXTRACT OPTION Normal Saline

EXTRACTION CONDITIONS Same as original test

SAMPLE REQUIREMENTS

| By Thickness | | By Weight | By Weight (lightweight) |
|----------------------|---------------------|-----------|----------------------------|
| <0.5mm thick | >0.5mm thick | | |
| 1350 cm ² | 675 cm ² | 45 grams | 22.5 g |

TURNAROUND TIME 19 days (non-GLP) • 26 days (GLP)

Sensitization tests estimate the potential for contact sensitization of devices through the testing of appropriate materials or extracts.

For submissions to the FDA or JMHLW, the Guinea Pig Maximization Sensitization assay [see next page and Page B-28] should be chosen. For submission solely within the European Union, the Murine Local Lymph Node Assay (LLNA) [see below] can be used.

The LLNA, which is gaining acceptance as a standard methodology for sensitization studies, offers greater sensitivity and specificity than the Guinea pig assays, and – when combined with interpretation based on statistical data analysis between test and negative control groups – it has become a recognized model for determination of delayed-type hypersensitivity.

However, the effectiveness of the LLNA assay in detecting sensitizing metals is limited. For devices containing metals, the (Guinea pig) Maximization Sensitization assay should be used.

In this assay, the ability of a material to potentially elicit a delayed-type hypersensitivity response is evaluated by the ability to cause mitotic proliferation of lymphocytes within the draining auricular lymph nodes.

The standard assay utilizes three groups of mice – test article, positive control and negative control (n=15 total). Adult female CBA strain mice are treated topically with solutions or extracts applied to the dorsum of the ears bilaterally. The response is compared to appropriate concurrent positive and negative controls. Measurement of the degree of cell proliferation is quantified by incorporation of ³H-thymidine into DNA of replicating lymph node lymphocytes. Interpretation is based on a statistically significant difference in the stimulation index (SI) between the test and negative control groups. Assay performance requires that the positive control's SI be greater than 3.0.

190856 Standard Assay

Murine Local Lymph Node Assay (LLNA)

EXTRACT OPTIONS • Normal Saline • Dimethyl Sulfoxide/Polyethylene Glycol

Specify one set of extraction conditions: 121°C/1hour 70°C/24 hours
50°C/72 hours 37°C/72 hours

SAMPLE REQUIREMENTS
(Per Extract)

| By Thickness | | By Weight | By Weight (lightweight) | Liquid |
|------------------------|-------------------------|-----------|-------------------------|--------|
| <0.5mm thick | >0.5mm thick | | | |
| 3 x 30 cm ² | 30 x 15 cm ² | 3 x 1 g | 3 x 0.5 g | 3 mL |

TURNAROUND TIME 25 days (non-GLP) • 32 days (GLP)

SENSITIZATION TESTING CONTINUED NEXT PAGE

SENSITIZATION TESTING

900850

ISO Maximization Sensitization Test

Guinea pigs are exposed to the extract twice within a 2-week period (Inductions I and II). The animals are re-exposed (challenged) 10-14 days after Induction II by placing fresh extract in contact with previously unexposed skin. Over a 72-hour period, the animals are observed for signs of a delayed allergic response when compared to a control group. If the test results are equivocal, a re-challenge can be conducted within 7-10 days of the initial challenge.

NUMBER OF ANIMALS 10 test • 5 irritant controls (if needed) • 5 negative controls

EXTRACT OPTIONS • Normal Saline • Cottonseed Oil

Specify one set of extraction conditions: 121°C/1hour 70°C/24 hours
50°C/72 hours 37°C/72 hours

SAMPLE REQUIREMENTS
(Per Extract)

| By Thickness | | By Weight | By Weight (lightweight) | Liquid |
|------------------------|------------------------|-----------|-------------------------|--------|
| <0.5mm thick | >0.5mm thick | | | |
| 3 x 60 cm ² | 3 x 30 cm ² | 3 x 2 g | 3 x 1 g | 15 mL |

Note: For ISO, sample preparation complies with ISO10993-12.

TURNAROUND TIME 47 days (non-GLP) • 54 days (GLP)

900899

Repeated Patch Dermal Sensitization Test

[Buehler method modified for medical devices]

Guinea pigs are patched with the test material 3 times per week for 3 weeks during the Induction Phase. After 2-week recovery period, animals are topically challenged with similar patches of test material to assess for delayed contact sensitization.

NUMBER OF ANIMALS 10 test / 5 controls

SAMPLE REQUIREMENTS

- Sufficient material to produce 105 patches, approx. 2.5cm x 2.5cm each
- 50 mL of liquid

TURNAROUND TIME 50 days (non-GLP) • 57 days (GLP)

Subacute toxicity is assessed after single or multiple exposures to extracts of device materials. The exposure period is longer than typical acute toxicity tests, but not exceeding 10% of animal lifespan. Subchronic toxicity is assessed after repeated intravenous injections of the test article. These studies involve expanded evaluations and can include systemic changes, local irritation, body weight, blood values and tissue changes as part of the protocol. The length of time for the test and the parameters evaluated will depend on the end use of the device. WuXi AppTec will assist in the test program design.

Multiple extracts of a device are prepared with standard polar and/or nonpolar vehicles and injected into 6 male and 6 female mice or rats over the test time period. Two similar control groups are also injected with control vehicle. The animals are observed during the test time period for signs of toxicity and are subjected to a gross observation at study termination. The test article passes if the test parameters (weight, survival, clinical observations and gross necropsy) are not significantly different from the concurrent control animal parameters. Other parameters evaluated include clinical chemistry and hematology.

Tests **800560**, **800570**, **800540** and **800550** are recommended for medical devices categorized as “prolonged contact” (24 hours to 30 days).

Tests **800565**, **800575**, **800545**, **800555** and **800595** are recommended for medical devices categorized as “permanent contact” (more than 30 days).

EXTRACT OPTIONS • Normal Saline • Cottonseed Oil

Specify one set of extraction conditions: 121°C/1hour 70°C/24 hours
50°C/72 hours 37°C/72 hours

SAMPLE REQUIREMENTS
(Per Extract)

| | By Thickness | | By Weight | By Weight (lightweight) |
|---|--------------------------|-------------------------|-----------|----------------------------|
| | <0.5mm thick | >0.5mm thick | | |
| 800560 • 800570 • 800565 800575 • 800550 | 5 x 30 cm ² | 5 x 15 cm ² | 5 x 1 g | 5 x 0.5 g |
| 800555 • 800595 | 14 x 30 cm ² | 14 x 15 cm ² | 14 x 1 g | 14 x 0.5 g |
| 800540 | 5 x 240 cm ² | 5 x 120 cm ² | 5 x 8 g | 5 x 4 g |
| 800545 | 14 x 240 cm ² | 14 x 120cm ² | 14 x 8 g | 14 x 4 g |

Note: For ISO, sample preparation complies with ISO10993-12.

TURNAROUND TIME Inquire

OPTIONS

Additional fees apply for the following services. For further information, contact your Account Manager.

800210 Histopathology for 14-Day Toxicity Studies

800215 Tissue Storage

14-Day Toxicity Studies

800570
Subacute Intraperitoneal Toxicity
in Mice (5 repeat dose)

800550
Subacute Intraperitoneal Toxicity
in Rats (5 repeat dose)

800560
Subchronic Intravenous Toxicity
in Mice (5 repeat dose)

800540
Subchronic Intravenous Toxicity
in Rats (5 repeat dose)

800575
Subacute Intraperitoneal Toxicity
in Mice (14 repeat dose)

800555
Subacute Intraperitoneal Toxicity
in Rats (14 repeat dose)

800565
Subchronic Intravenous Toxicity
in Mice (14 repeat dose)

800545
Subchronic Intravenous Toxicity
in Rats (14 repeat dose)

The above tests include:
Clinical Chemistry and Hematology

28-Day Subchronic Toxicity

800595
Subchronic Intravenous Toxicity
in Rats (28 repeat dose)

Includes:
Clinical Chemistry, Hematology and
Histopathology

For special subacute/subchronic toxicity testing involving dermal, intramuscular, subcutaneous, mucosal or oral dosing programs, contact your Account Manager.

See Page B-23 for a note on Chronic Toxicity and Carcinogenicity testing.

SYSTEMIC (ACUTE) TOXICITY TESTING

Acute systemic toxicity tests estimate the potential harmful systemic effects from a single exposure to polar or nonpolar extracts of device materials.

900700

USP Acute Systemic Toxicity Test

An extract of the device or biomaterial is prepared in up to four (4) standard USP extraction solutions and injected into mice (5 per extract, 5 per control) to assess the toxicity of extractable compounds that may exist in the biomaterial. The animals are observed over a 72-hour period. If the animals exposed to the test article extract do not show signs of toxicity greater than the concurrent control groups, the test article passes the test.

EXTRACT OPTIONS

- Normal Saline
- 5% Ethanol in Saline
- Cottonseed Oil
- Polyethylene Glycol

901770

ISO Acute Systemic Toxicity Test

Specify one set of extraction conditions:

| | |
|---------------|---------------|
| 121°C/1hour | 70°C/24 hours |
| 50°C/72 hours | 37°C/72 hours |

SAMPLE REQUIREMENTS
(Per Extract)

| By Thickness | | By Weight | By Weight (lightweight) |
|--------------------|--------------------|-----------|----------------------------|
| < 0.5mm thick | >0.5mm thick | | |
| 48 cm ² | 24 cm ² | 1.6 g | 0.8 g |

Note: For ISO, normal saline and/or cottonseed oil extracts are recommended. Sample preparation complies with ISO10993-12.

TURNAROUND TIME 23 days (non-GLP) • 27 days (GLP)

OTHER BIOCOMPATIBILITY TESTS

CHRONIC TOXICITY CARCINOGENICITY

These tests are often long-term studies that can extend for a period of up to two years or longer. If the device involves new chemistry that (from material characterization and exposure assessments) indicate a high enough risk, one or more of these studies may be necessary. If this is the case, contact your Account Manager for assistance in designing an appropriate long-term study.

FINISHED PRODUCT RELEASE

This assay is designed to serve as a safety evaluation for lot release. A single unit or device is extracted with sterile saline solution and injected intravenously into five mice to assess the acute toxicity of the device. The animals are observed over a 48-hour period. If the animals survive the injection with no signs of toxicity, the device passes the test.

EXTRACT Normal Saline

Specify one set of extraction conditions:

| | |
|---------------|---------------|
| 121°C/1hour | 70°C/24 hours |
| 50°C/72 hours | 37°C/72 hours |

SAMPLE REQUIREMENTS One (1) device to represent the lot under test.

TURNAROUND TIME 22 days (non-GLP) • 29 days (GLP)

800510

**USP Safety Test in Mice
(5 mice)**

A comprehensive range of additional finished product release testing is available, including Rabbit Pyrogen tests [see Page B-18] and Bacterial Endotoxin (LAL) tests [see "Endotoxin" section].

Please contact your WuXi AppTec Account Manager for more information.

JMHLW TESTING

Following are descriptions of WuXi AppTec testing services designed specifically to meet the requirements of the Japanese Ministry of Health, Labor and Welfare (JMHLW).

CYTOTOXICITY

140470

Japanese Cytotoxicity Test Colony Microassay By Elution (JMHLW)

The purpose of this procedure is to evaluate the cytotoxic response of a specified mammalian culture cell line when exposed to an extract of the test article. This assay utilizes the sensitivity of low cell density to evaluate the cytotoxicity of medical devices.

Test articles and controls will be prepared and extracted for 24-25 hours at 37 ± 1 °C. Appropriate dilutions of the extracts will be prepared using E-MEM +10% FBS. After removing spent medium, 2.0 ml of the extract will be added to the plates. The cells will be incubated at 37 ± 1 °C for 7-9 days before fixation, staining, and counting.

SAMPLE REQUIREMENTS 120cm² or 2g

TURNAROUND TIME 24 days (non-GLP) • 31 days (GLP)

GENOTOXICOLOGY

190812

Bacterial Reverse Mutation Assay Using Four Salmonella Strains And One Escherichia Coli Strain (JMHLW)

The purpose of this study is to evaluate the mutagenic potential of the test article (or its metabolites) by measuring its ability to induce back mutations at selected loci of four strains of *Salmonella typhimurium* and one strain of *Escherichia coli* in the presence and absence of microsomal enzymes.

The test article is tested at five dose levels along with appropriate vehicle and positive controls. The test article dilutions are analyzed using tester strains TA98, TA100, TA1535, TA97a, and WP2-uvrA⁻ with and without microsomal enzymes. The test article doses, negative vehicle, and positive controls will be plated in triplicate. Following incubation of approximately 48-72 hours at 37 ± 2 °C, revertant colonies per plate will be enumerated.

SAMPLE REQUIREMENTS Pretest: 2g
Method 1: Inquire* **or** Method 2: 3g

* During the pretest, the test article is extracted with methanol and acetone. The extracts are then rotary evaporated to a residue. The extract solvent that produces a greater residue (by weight) is used for the extraction for the main study (Method 1). Sample requirements are based on amount of residue obtained. If neither solvent produces a sufficient evaporation residue, then Method 2 (extraction) shall be used.

TURNAROUND TIME 33 days (non-GLP) • 40 days (GLP)

This assay is used to evaluate the potential of the test article to induce chromosome aberrations in the Chinese Hamster Ovary (CHO) cell line with and without *in vitro* metabolic activation (S9).

CHO-K₁ cells will be incubated in the presence of the test article extract, negative, or positive control for three hours in the presence and absence of metabolic activation. After this incubation period, the cells will be washed, fresh medium added, and returned to the incubator. Two hours prior to the end of incubation, colcemid will be added to all flasks to stop cells in metaphase. The cells will be collected from the flasks by mitotic shake-off, swollen, fixed, and dropped onto slides. The slides will be stained and permanently mounted prior to microscopic observation.

SAMPLE REQUIREMENTS Pretest: 2g
 Method 1: Inquire* or Method 2: 3g

* During the pretest, the test article is extracted with methanol and acetone. The extracts are then rotary evaporated to a residue. The extract solvent that produces a greater residue (by weight) is used for the extraction for the main study (Method 1). Sample requirements are based on amount of residue obtained. If neither solvent produces a sufficient evaporation residue, then Method 2 (extraction) shall be used.

TURNAROUND TIME 55 days (non-GLP) • 62 days (GLP)

190827

***In Vitro* Chromosome Aberration Analysis in Chinese Hamster Ovary (CHO) Cells (JMHLW)**

HEMOLYSIS

The purpose of this study is to evaluate the hemolytic potential of test articles according to the JMHW Notice 0213001, number 36.

Three healthy, mature New Zealand White rabbits of either sex, from a certified commercial vendor, will be selected as donors for this study. Three - five mL of blood will be collected from each rabbit using appropriate venipuncture, employing evacuated tubes containing 3.2% sodium citrate. The time and date of blood collection and rabbit ear tag number will be recorded directly on the vial, and the blood will be used within 6 hours of collection. Blood will be stored under refrigeration until time of use.

Test articles and controls will be prepared and extracted as requested. A total of 3 replicates will be prepared for extraction. Each test article replicate will be obtained from one device. Blood will then be added to the test and control materials. These mixtures will be incubated for 1, 2, or 4 hours at 37 ± 1°C. After this incubation, the mixtures will be transferred to centrifuge tubes and centrifuged to remove cellular materials. A cyanmethemoglobin reagent will be added to the supernatant and these samples will be read at 540 nm on a spectrophotometer. The hemolysis will be calculated based on these results.

SAMPLE REQUIREMENTS

| By Thickness | | By Weight | By Weight (low density) |
|------------------------|------------------------|-----------|----------------------------|
| < 0.5mm thick | >0.5mm thick | | |
| 3 x 90 cm ² | 3 x 45 cm ² | 3 x 3 g | 3 x 1.5 g |

TURNAROUND TIME 25 days (non-GLP) • 32 days (GLP)

150400

Hemolysis Assay (JMHLW)

JMHLW TESTING

IMPLANTATION

902515 1 wk implantation duration
902520 2 wk implantation duration
902525 4 wk implantation duration
902530 8 wk implantation duration
902533 13 wk implantation duration

Intramuscular Implantation Test (JMHLW)

The purpose of this study is to evaluate the local effects of a test article in direct contact with living skeletal muscle tissue.

For the safety evaluation of a test article, representative portions of the device or the entire device are implanted in the paravertebral muscles of live rabbits. A maximum of 4 test samples and 4 control samples will be implanted into each of four rabbits for each time point or group. After a predetermined exposure period, the animals will be sacrificed and the implantation sites exposed. Gross necropsy observations will be made and implant sites harvested and fixed. The specimens will be fixed to stabilize the tissue device interface and then oriented transversely and cut-in for histology processing. Microscopic evaluation will include such things as: cell type, cell distribution, fibroplasia, and neovascularization. Histopathology is necessary to meet the requirements.

SAMPLE REQUIREMENTS Sufficient material to produce 20 implants, approx. 10mm x 3mm

TURNAROUND TIME

| GLP: | Implant | |
|------|---------|----------|
| | 1 wk | 49 days |
| | 2 wk | 56 days |
| | 4 wk | 70 days |
| | 8 wk | 98 days |
| | 13 wk | 133 days |

Non-GLP: Subtract 7 days from GLP TATs shown above.

PYROGENICITY

900775

Material Mediated Rabbit Pyrogen Test (JMHLW)

The purpose of this study is to determine if a saline extract of the test article causes a febrile response in rabbits.

The test article will be extracted in 0.9% saline solution and injected into the marginal ear vein of each of three animals. All animals will receive a 10 mL/kg dose of the test article. Rectal temperatures will be recorded for each animal prior to injection and between 1 and 3 hours post-injection (at minimum 30 minute intervals). The test article will be considered negative if none of the animals has a temperature increase ≥ 0.6 °C and the cumulative temperature increase of the three animals is less than 1.4 °C.

If any rabbit has an individual temperature rise ≥ 0.6 °C, the test may be continued, per the Sponsor's request, using five additional rabbits [extra fees will apply]. If not more than two of the five animals, from the continued test, have a temperature increase ≥ 0.6 °C the test article under examination will meet the JMHLW requirements for the absence of pyrogens.

SAMPLE REQUIREMENTS

| By Thickness | | By Weight | By Weight Low density |
|---------------------|---------------------|-----------|--------------------------|
| < 0.5mm thick | >0.5mm thick | | |
| 900 cm ² | 450 cm ² | 30 g | 15 g |

TURNAROUND TIME 19 days (non-GLP) • 26 days (GLP)

IRRITATION

This test is designed to verify the existence of substances, which can irritate skin, in the extracts taken from test samples.

Test and negative control extracts will be prepared using normal saline (NS) and cottonseed oil (CSO). Twelve (12) rabbits will be shaved (6 rabbits per extract). The test and control extracts (0.5 mL per site) will be applied to both scratched and unscratched skin. Following application, the extracts will be covered with taped-backed gauze.

After the 24-hour exposure period, the patches will be removed. Observations for skin irritation will be conducted at 60 ± 6 minutes after unwrapping, and at 24 ± 2 , and 48 ± 2 hours. The test will be evaluated by an overall collection and interpretation of the data, using the dermal scores as reference marks, and based on the nature and reversibility of the skin lesions.

SAMPLE REQUIREMENTS (per extract)

| By Thickness | | By Weight | By Weight (low density) |
|--------------------|--------------------|-----------|----------------------------|
| < 0.5mm thick | >0.5mm thick | | |
| 48 cm ² | 24 cm ² | 1.6 g | 0.8 g |

TURNAROUND TIME 23 days (non-GLP) • 30 days (GLP)

910695

**Primary Skin Irritation
(JMHLW)**

The purpose of this test is to determine if chemicals that may leach or be extracted from the test material are capable of causing local irritation in the dermal tissues of the rabbit.

For safety evaluation of a biomaterial sample, rabbits will be injected intracutaneously (Dose = 0.2 mL x 5 sites) with extracts of the test article and associated vehicle controls. Injection sites will be examined and scored at 24 ± 2 , 48 ± 2 , and 72 ± 2 hours after treatment for signs of skin reactions. In addition, the sites will be photographed following injection and at all scoring periods. If the difference between the average scores for the extract of a test article and the control is less than or equal to 1.0, the test article passes the test.

SAMPLE REQUIREMENTS (per extract)

| By Thickness | | By Weight | By Weight (low density) |
|--------------------|--------------------|-----------|----------------------------|
| < 0.5mm thick | >0.5mm thick | | |
| 24 cm ² | 12 cm ² | 0.8 g | 0.4 g |

TURNAROUND TIME 22 days (non-GLP) • 29 days (GLP)

9107003

**Intracutaneous Reactivity Test
(JMHLW)**

SENSITIZATION

900851

Guinea Pig Maximization Sensitization Test (JMHLW)

This test is designed to evaluate the allergenic potential or sensitizing capacity of a test article by a method compliant with the requirements specified by the Japanese Ministry of Health, Labor, and Welfare test Guidelines for Basic Biological Tests of Medical Materials and Devices. The test is used as a procedure for the screening of contact allergens in guinea pigs and extrapolating the results to humans, but it does not establish the actual risk of sensitization.

After the material has been appropriately extracted, eleven (11) test and six (6) irritant control animals will be exposed to the extract in a series of two inductions, followed approximately two weeks later by a challenge exposure at which time the animals are evaluated for a sensitization response.

SAMPLE REQUIREMENTS Pretest: 20g
Method 1: Inquire* or Method 2: 15g

* During the pretest, the test article is extracted with methanol and acetone. The extracts are then rotary evaporated to a residue. The extract solvent that produces a greater residue (by weight) is used for the extraction for the main study (Method 1). Sample requirements are based on amount of residue obtained. If neither solvent produces a sufficient evaporation residue, then Method 2 (extraction) shall be used.

TURNAROUND TIME 74 days (non-GLP) • 81 days (GLP)

900852

Adjuvant and Patch Sensitization Test (JMHLW)

This test is designed to evaluate the allergenic potential or sensitizing capacity of a test article by a method compliant with the requirements specified by the Japanese Ministry of Health, Labor, and Welfare test Guidelines for Basic Biological Tests of Medical Materials and Devices. The test is used as a procedure for the screening of contact allergens in guinea pigs and extrapolating the results to humans, but it does not establish the actual risk of sensitization.

The Adjuvant and Patch Sensitization Test should be used as an alternative method to the Guinea Pig Maximization Sensitization Test (Method for MHLW, Japan) in the event that it is not appropriate to extract the test material and/or the extract cannot be injected intradermally, or when a sufficient residue (0.1% w/w of test sample) has been produced but it will not dissolve or disperse in the appropriate solvent (e.g. acetone, dimethylsulfoxide (DMSO), vegetable oil).

After the material has been appropriately extracted and prepared, eleven (11) test and six (6) irritant control animals will be exposed to the extract in a series of five inductions on scratched skin, followed approximately two weeks later by a challenge exposure at which time the animals are evaluated for a sensitization response.

SAMPLE REQUIREMENTS Inquire

TURNAROUND TIME 74 days (non-GLP) • 81 days (GLP)

ACUTE SYSTEMIC TOXICITY

The purpose of this test is to screen solutions or test article extracts for potential toxic effects as a result of a single-dose systemic injection in mice by a method compliant with the requirements specified in Part VI Acute Toxicity Test - Japanese Ministry of Health and Welfare, 2003.

For the safety evaluation of a test article, mice will be injected intraperitoneally or intravenously with either extracts or solutions of the test article or control (vehicle without test article). The animals will be observed for signs of toxicity immediately after injection and at 4 ± 0.75 hours and 24, 48, and 72 ± 2 hours post-injection. Body weights will be measured prior to injection and 24, 48, and 72 ± 2 hours post-injection. If none of the animals treated with the test article extract or solution show a greater adverse reaction than the animals treated with the blank, the test article will pass the test.

9017715

Japanese Acute Systemic Injection Test (JMHLW)

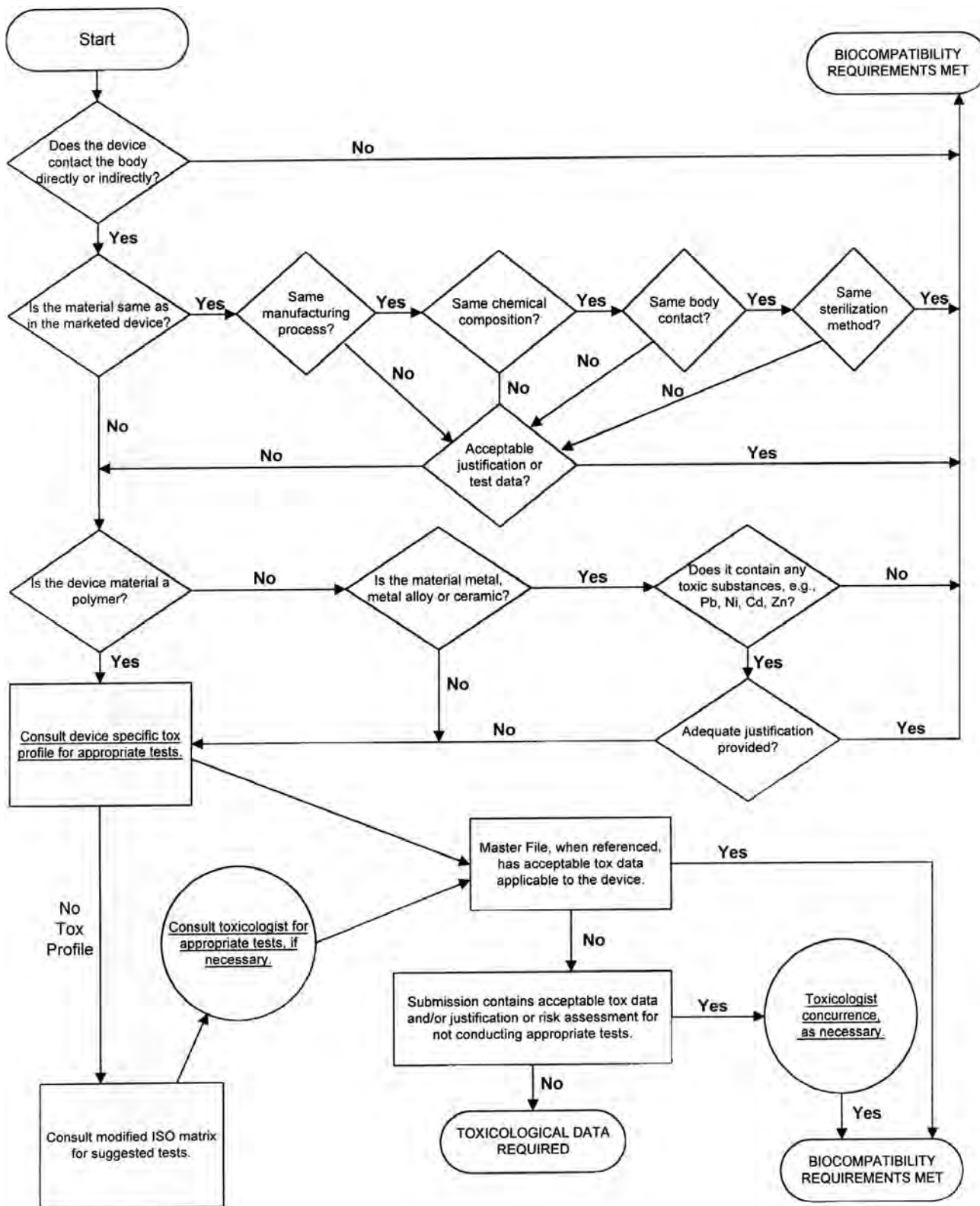
SAMPLE REQUIREMENTS (per extract)

| By Thickness | | By Weight | By Weight (low density) |
|--------------------|--------------------|-----------|-------------------------|
| < 0.5mm thick | >0.5mm thick | | |
| 48 cm ² | 24 cm ² | 1.6 g | 0.8 g |

TURNAROUND TIME 23 days (non-GLP) • 27 days (GLP)

For additional information on testing services to meet JMHLW requirements, contact your WuXi AppTec Account Manager.

GUIDE TO ASSESSING BIOCOMPATIBILITY TESTING NEEDS



FROM: May 1, 1995 FDA General Program Memorandum - #G95-1 / Attachment C

TESTING UNDER GLP REGULATIONS

Quality Assurance-Audited Testing Requirements Under Good Laboratory Practices (GLP) Regulations

- Protocol Development
- Study Director
- Testing Communications
- QA Master Schedule
- Test Schedule Notification
- Test Article Control
- QA Inspections
- Audited Final Report
- Raw Data Archive

NOTE: For GLP testing, clients will receive notification as to the date they can expect to receive their final reports.

BIOCOMPATIBILITY TESTS FOR CLASSIFICATIONS OF PLASTICS (USP)

PLASTICS CLASSES

Acute Systemic Toxicity [Mice]

| Extract of sample in: | I | II | III | IV | V | VI |
|--|---|----|-----|----|---|----|
| Sodium chloride injection | • | • | • | • | • | • |
| 1 in 20 solution of alcohol in sodium chloride injection | | • | • | • | • | • |
| Polyethylene glycol 400 | | | • | | • | • |
| Vegetable oil (cottonseed oil) | | | • | • | • | • |

Intracutaneous Irritation Test [Rabbit]

| Extract of sample in: | I | II | III | IV | V | VI |
|--|---|----|-----|----|---|----|
| Sodium chloride injection | • | • | • | • | • | • |
| 1 in 20 solution of alcohol in sodium chloride injection | | • | • | • | • | • |
| Polyethylene glycol 400 | | | | | • | • |
| Vegetable oil (cottonseed oil) | | | | • | • | • |

Intramuscular Implantation Test [Rabbit]

| Length of test: | I | II | III | IV | V | VI |
|-----------------|---|----|-----|----|---|----|
| 5-day | | | | • | | • |
| 7-day | | | | | | • |

NOTE: These tests are for material qualification, not for regulatory submission.

DEVICE CATEGORIES

DEVICE CATEGORIES BY NATURE OF CONTACT

Medical devices fall into one of four categories based on the nature of patient contact:

| | | |
|----------|---------------------------------------|--|
| 1 | Non-Contact Devices | Devices that do not directly or indirectly contact the patient are not required to undergo biocompatibility testing. |
| 2 | Surface Devices | <ul style="list-style-type: none"> • Contacting Intact Skin <i>(e.g., electrodes, external prostheses, fixation tapes, compression bandages)</i> • Contacting Mucous Membranes <i>(e.g., contact lenses, urinary catheters, colonoscopes, endotracheal tubes)</i> • Contacting Breached or Compromised Surfaces <i>(e.g., wound dressings, occlusive patches, healing devices)</i> |
| 3 | External Communicating Devices | <ul style="list-style-type: none"> • Contacting Blood Path Indirectly <i>(e.g., solution administration sets, I.V. extension sets, blood transfusion sets)</i> • Contacting Tissue, Bone or Dentin <i>(e.g., laparoscopes, arthroscopes, draining systems, dental cements, skin staples)</i> • Contacting Circulating Blood <i>(e.g., intravenous and delivery catheters, temporary pacemaker electrodes, dialyzers, dialysis tubing, hemadsorbants, immuno-adsorbants)</i> |
| 4 | Implant Devices | <ul style="list-style-type: none"> • Contacting Tissue and/or Bone <i>(e.g., orthopedic pins and plates, pacemakers, breast implants, replacement tendons, ligation clips, drug supply devices)</i> • Contacting Blood <i>(e.g., pacemaker electrodes, heart valves, vascular grafts, ventricular assist devices, internal drug delivery devices, stents)</i> |

DEVICE CATEGORIES BY DURATION OF CONTACT

Devices generally are placed in one of three categories based on expected duration of contact with patient:

| | |
|--|---|
| | Limited [≤ 24 hours] |
| | Prolonged [> 24 hours and ≤ 30 days] |
| | Permanent [> 30 days] |

INITIAL EVALUATION TESTS FOR CONSIDERATION

BIOCOMPATIBILITY TESTING

[Based on ISO 10993-1:2009 and FDA G95-1 Guidelines]

| DEVICE CATEGORIES | | BIOLOGICAL EFFECT | | | | | | | | | | | | | | |
|--------------------------------|--|---------------------------|--|-----------------------------|--------------|---------------|------------|---------------------------|--------------------------------|----------------|--------------|-------------------|------------------|-----------------|---|--|
| | | Initial | | | | | | | Other ⁴ | | | | | | | |
| Body Contact | Contact Duration | A – Limited [≤ 24 hrs] | B – Prolonged [>24 hrs to ≤30 days] | C – Permanent [>30 days] | Cytotoxicity | Sensitization | Irritation | Systemic Toxicity (Acute) | Subchronic Toxicity (Subacute) | Genotoxicity | Implantation | Hemocompatibility | Chronic Toxicity | Carcinogenicity | | |
| | | SURFACE DEVICES | Skin | A | B | C | ● | ● | ● | | | | | | | |
| | | | | A | B | C | ● | ● | ● | | | | | | | |
| | | | | A | B | C | ● | ● | ● | ◇ | ◇ | | ◇ | | | |
| Mucosal Membranes | A | | B | C | ● | ● | ● | ◇ | ● | ● | ◇ | | ◇ | | | |
| | A | | B | C | ● | ● | ● | ◇ | ◇ | | ◇ | | | | | |
| | A | | B | C | ● | ● | ● | ◇ | ● | ● | ◇ | | ◇ | | | |
| EXTERNAL COMMUNICATING DEVICES | Blood Path, Indirect ³ | | A | B | C | ● | ● | ● | ● | | | | ● | | | |
| | | | A | B | C | ● | ● | ● | ● | ◇ | | | ● | | | |
| | | | A | B | C | ● | ● | ◇ | ● | ● | ● | ◇ | ● | ◇ | ◇ | |
| | Tissue ¹ /Bone/Dentin Communicating | A | B | C | ● | ● | ● | ◇ | | | | | | | | |
| | | A | B | C | ● | ● | ● | ● | ● | ● | ● | | ◇ | ◇ | | |
| | | A | B | C | ● | ● | ● | ● | | ◇ ² | | ● | | | | |
| | Circulating Blood ³ | A | B | C | ● | ● | ● | ● | ● | ● | ● | ● | | | | |
| | | A | B | C | ● | ● | ● | ● | ● | ● | ● | ● | ◇ | ◇ | | |
| | | A | B | C | ● | ● | ● | ◇ | | | | | | | | |
| IMPLANT DEVICES | Tissue / Bone | A | B | C | ● | ● | ● | ◇ | | | | | | | | |
| | | A | B | C | ● | ● | ● | ● | ● | ● | ● | | ◇ | ◇ | | |
| | | A | B | C | ● | ● | ● | ● | ● | ● | ● | | | | | |
| | Blood ³ | A | B | C | ● | ● | ● | ● | ● | ● | ● | ● | | | | |
| | | A | B | C | ● | ● | ● | ● | ● | ● | ● | ● | | | | |
| | | A | B | C | ● | ● | ● | ● | ● | ● | ● | ● | ◇ | ◇ | | |

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¹ "Tissue" includes tissue fluids and subcutaneous spaces.

² For all devices used in extracorporeal circuits.

³ Pyrogenicity / Materials Mediated should be considered.

⁴ Based on the risk assessment, additional tests may be necessary.

● – ISO Evaluation Tests for Consideration

◇ – Additional tests that the FDA considers may be applicable.

For reproductive and biodegradation tests, contact your WuXi AppTec Account Manager.

This chart is designed as a convenient quick guide for some of our most commonly ordered tests (and does not list all available tests). Turnaround times for GLP testing are shown in brackets with each test listing. For non-GLP testing, turnaround times would be approximately 7 days shorter. **If you have questions, before submitting samples, contact Client Services at WuXi AppTec's St. Paul facility: 651-675-2000 or 888-794-0077.**

| Test | < 0.5mm thickness (120cm ² /20mL ratio) [film, sheet, tubing wall] | ≥ 0.5mm thickness (60cm ² /20mL ratio) [tubing wall, slab, molded items] | Irregularly Shaped (4g/20mL ratio) [powder, pellets, foam, non- absorbent molded items] | Membranes (2g/20mL ratio) (low-density materials) | Liquids |
|---|--|--|--|---|---------|
| CYTOTOXICITY | | | | | |
| MEM Elution Using L-929 Cells – ISO/USP [18 days] | 1 x 30cm ² | 1 x 15cm ² | 1 x 1g | 1 x 0.5g | 3mL |
| Extract Colony by Elution – JMHLW [31 days] | 120cm ² or 2g | | | | 10mL |
| Agarose Overlay Using L-929 Cells – ISO/USP [14 days] | Sufficient material to produce 3 patches, 1cm x 1cm each | | | | 1mL |
| MTT Cytotoxicity Assay with L-929 Mouse Fibroblast Cells [26 days] | 1 x 36cm ² | 1 x 18cm ² | 1 x 1.2g | 1 x 0.8g | NA |
| HEMOCOMPATIBILITY / BLOOD COMPATIBILITY | | | | | |
| Hemolysis: ASTM – Direct Contact [21 days] | ≤ 0.5mm thick: 3 x 42cm ² • > 0.5mm thick: 3 x 21cm ² • Irregularly shaped: 3 x 1.4g | | | | 21mL |
| Hemolysis: ASTM – Extract [21 days] | ≤ 0.5mm thick: 3 x 60cm ² • > 0.5mm thick: 3 x 30cm ² • Irregularly shaped: 3 x 2g | | | | NA |
| Hemolysis: NIH – Direct Contact [21 days] • NIH – Extract [21 days] | 5g/10mL ratio: 3 x 2g • 0.5g/10mL ratio (low density materials): 3 x 0.2g | | | | NA |
| Hemolytic Toxicity – JMHLW [32 days] | 3 x 90cm ² | 3 x 45cm ² | 3 x 3g | 3 x 1.5g | NA |
| Complement Activation C3a and SC5b-9 [25 days] | 1 x 6cm ² | 1 x 3cm ² | 1 x 0.2g | NA | NA |
| Partial Thromboplastin Time [25 days] | 3 x 4cm ² (test samples) and 3 x 4cm ² (comparison samples) | | | | |
| Platelet and Leukocyte Count [25 days] | 3 x 12cm ² (test samples) and 3 x 12cm ² (comparison samples) | | | | |
| In Vitro Hemocompatibility [25 days] | 3 x 12cm ² (test samples) and 3 x 12cm ² (comparison samples) | | | | |
| Thrombosis (In Vivo) – 2 Dog [28 days] Other animal models available. | 2 test samples and 2 comparison samples [Length: 8-15cm, Maximum Diameter: ≤ 3mm] | | | | |
| GENOTOXICOLOGY | | | | | |
| Bacterial Mutagenicity (Ames) [28 days] | 2 x 24cm ² | 2 x 12cm ² | 2 x 0.8g | 2 x 0.4g | 4mL |
| Bacterial Reverse Mutation – JMHLW [40 days] | 5g [Sample amounts will increase if residue is obtained during pretest.] | | | | |
| In Vitro Chromosome Aberration [62 days] | 2 x 42cm ² | 2 x 21cm ² | 2 x 1.4g | 2 x 0.7g | 8mL |
| In Vitro Chromosome Aberration – JMHLW [62 days] | 5g [Sample amounts will increase if residue is obtained during pretest.] | | | | |
| In Vitro Mouse Lymphoma [39 days] | 2 x 42cm ² | 2 x 21cm ² | 2 x 1.4g | 2 x 0.7g | 7mL |
| In Vivo Mouse Micronucleus [55 days] | 2 x 120cm ² | 2 x 60cm ² | 2 x 4g | 2 x 2g | 18mL |
| IRRITATION / STIMULATION | | | | | |
| Intracutaneous Reactivity – ISO/USP/JMHLW [29 days] | 2 x 36cm ² | 2 x 18cm ² | 2 x 1.2g | 2 x 0.6g | 5mL |
| Vaginal Mucosal Irritation – ISO [54 days] | 10 x 60cm ² | 10 x 30cm ² | 10 x 2g | 10 x 1g | 30mL |
| Primary Skin Irritation – ISO [30 days] | Sufficient material to produce 7 patches, 2.5cm x 2.5cm each | | | | 5mL |
| Primary Skin Stimulatory – JMHLW [30 days] | 2 x 48cm ² | 2 x 24cm ² | 2 x 1.6g | 2 x 0.8g | NA |
| PYROGENICITY | | | | | |
| Material Mediated Rabbit Pyrogen – ISO and JMHLW [26 days] | 900cm ² | 450cm ² | 30g | 15g | 150mL |
| SENSITIZATION | | | | | |
| Murine Local Lymph Node Assay (LLNA) [32 days] | 6 x 30cm ² | 6 x 15cm ² | 6 x 1g | 6 x 0.5g | 3mL |
| Maximization Sensitization (Guinea Pig) – ISO [54 days] | 6 x 60cm ² | 6 x 30cm ² | 6 x 2g | 6 x 1g | 15mL |
| Maximization Sensitization – JMHLW [81 days] | 35g [Sample amounts will increase if residue is obtained during pretest.] | | | | |
| Repeated Patch Dermal Sensitization Test – Buehler [57 days] | Sufficient material to produce 105 patches, 2.5cm x 2.5cm each | | | | 50mL |
| SYSTEMIC TOXICITY | | | | | |
| Acute Systemic Test – ISO/USP/JMHLW [27 days] | 2 x 48cm ² | 2 x 24cm ² | 2 x 1.6g | 2 x 0.8g | 7mL |
| Subacute/Subchronic Toxicity (Mice) – 5 Dose Exposure [89 days] | 5 x 30cm ² | 5 x 15cm ² | 5 x 1g | 5 x 0.5g | Inquire |
| Subacute/Subchronic Toxicity (Mice) – 14 Dose Exposure [89 days] | 14 x 30cm ² | 14 x 15cm ² | 14 x 1g | 14 x 0.5g | Inquire |
| IMPLANTATION [Note: TAT is in addition to implant duration.] | | | | | |
| Intramuscular/Subcutaneous Implantation – ISO/JMHLW [42 days] | Sufficient material to produce 15 implants, approx. 10mm x 3mm | | | | |
| Intramuscular Implantation – USP [21 days] | Sufficient material to produce 12 implants, approx. 10mm x 3mm | | | | |
| CHEMISTRY | | | | | |
| Physicochemical Test for Plastics – USP [7 days] | 10g or 600cm ² | | | | |
| FTIR [7 days] | 5g | | | | |