Biocompatibility Safety Assessment of Medical Devices: FDA/ISO and Japanese Guidelines

Amid efforts at harmonization, important differences exist between U.S./FDA/ISO and Ministry of Health, Labour, and Welfare (Japan) medical device biocompatibility requirements.

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Submissions for approval of medical devices by regulatory agencies require that biocompatibility assessment be conducted to assure safety of the device or material. Safety data can be obtained by testing according to certain prescribed or recommended guidelines, including guidance documents developed by the International Organization for Standardization (ISO) and FDA. These guidelines include ISO 10993, "Biological Evaluation of Medical Devices," and the guidance document released by FDA in 1995, blue book memorandum #G95-1, "Use of International Standard ISO 10993, 'Biological Evaluation of Medical Devices'—Part 1: Evaluation and Testing."[1,2]

In the past, submissions to the European regulatory bodies and to FDA were accompanied by safety data that were different in procedures and references. Prior to 1995, biocompatibility testing was usually conducted by the use of the #G87-1 Tripartite Biocompatibility Guidance (1987). In 1995, FDA released the #G95-1 guidance document, which was an FDA-modified version of ISO 10993, "Biological Evaluation of Medical Devices—Part 1." This document is in concordance with the ISO testing matrix, with certain exceptions. The exceptions are additional assessments that may be required for FDA submissions for which standardized protocols are available based on guidelines published by the Organization for Economic Cooperation and Development (OECD) and others. The FDA document, therefore, made it possible for a medical device manufacturer to test simultaneously for European submissions for obtaining the CE mark, and for submissions to FDA.

Although it is a large medical device market, Japan has been an exception to such harmonization efforts. Japan is an active participant in ISO standards setting; however, its written guidelines differ from those of the ISO in several important ways. Marketing approval submissions for medical devices in Japan falls under the regulatory mandate of the Ministry of Health, Labour and Welfare (MHLW). MHLW has testing guidelines that specify testing protocols and requirements, including the "Testing Methods to Evaluate Biological Safety of Medical Devices," Notice from the Office Medical Devices Evaluation Number 36 (2003).[3] Other testing guidance documents are category-specific or vertical standards. These testing guidelines acquiesce to ISO/FDA testing protocols in some medical device categories. In others, however, the procedures are different and

warrant different protocols. Therefore, medical devices intended for Japanese and/or European Union (EU) or U.S. markets are generally tested using different study protocols. The ISO guidelines, for the most part, refer to the compendial methods described in the *U.S. Pharmacopoeia* (USP) or in the standards published by ASTM. In some instances, the MHLW guidelines also refer to ASTM procedures.

The requirements for ISO 10993 have been comprehensively reviewed in a previously published series of articles.[4] This article intends to describe the differences in some of the biological testing procedures and protocols required by the ISO, FDA, and MHLW guidelines. Tests in areas of biocompatibility not covered by this article would be based upon current guidelines, such as the OECD guidelines for testing of chemicals and others.[5,6]

The categorization of medical devices based on type and duration of contact is very similar between the ISO and MHLW guidelines, and specifies the areas of biocompatibility that should be investigated. Other device-specific Japanese standards define test procedures for certain devices. As can be seen from Tables I and II, the requirements of ISO 10993 and those of MHLW are essentially similar, with differences residing in specific test procedures and protocols.

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Table I. ISO/FDA test chart.

Device Categories			Initial Evaluation								Supplemental Evaluation	
	Body contact	Contact duration	Cytotoxicity	Sensitization	Irritation or intracutaneous reactivity	Systemic toxicity (acute)/pyrogenicity	Subchronic toxicity	Genotoxicity	Implantation	Hemocompatibility	Chronic toxicity	Carcinogenicity
Surface Devices	Skin	A	•	•	•							
		B	•	•	•							
		C	•	•	•							
	Mucosal membrane	A	•	•	•							
		B	•	•	•	0	0		0			
		C	•	•	•	0	•	•	0		0	
	Breached/ compromised surface	A	•	•	•	0						
		B	•	•	•	0	0		0			
		C	•	•	•	0	•	•	0		0	
External Communicating Devices	Blood path indirect	A	•	•	•	•				•		
		B	•	•	•	•	0			•		
		C	•	•	0	•	•	•	0	•	•	•
	Tissue/bone dentin communicating	A	•	•	•	0						
		B	•	•	0	0	0	•	•			
		C	•	•	0	0	0	•	•		0	•
	Circulating blood	A	•	•	•	•		0		•		
		B	•	•	•	•	0	•	0	•		
		C	•	•	•	•	•	•	0	•	•	•
Implant Devices	Bone/tissue	A	•	•	•	0						
		B	•	•	0	0	0	•	•			
		C	•	•	0	0	0	•	•		•	•
	Blood	A	•	•	•	•			•	•		
		B	•	•	•	•	0	•	•	•		
		C	•	•	•	•	•	•	•	•	•	•

A = Limited exposure (≤ 24 hours) B = Prolonged exposure (24 hours – 30 days) C = Permanent contact (> 30 days)
• = FDA and ISO evaluation tests 0 = Additional tests for FDA

Table II. Japanese MHLW test chart.

Device Categories			Initial Evaluation								Supplemental Evaluation	
	Body contact	Contact duration	Cytotoxicity	Sensitization	Irritation or intracutaneous reactivity	Systemic toxicity (acute)/pyrogenicity	Subchronic toxicity	Genotoxicity	Implantation	Hemocompatibility	Chronic toxicity	Carcinogenicity
Surface Devices	Skin	A	•	•	•							
		B	•	•	•							
		C	•	•	•							
	Mucosal membrane	A	•	•	•							
		B	•	•	•	0	0		0			
		C	•	•	•	0	•	•	0		0	
	Breached/ compromised surface	A	•	•	•	0						
		B	•	•	•	0	0		0			
		C	•	•	•	0	•	•	0		0	
External Communicating Devices	Blood path indirect	A	•	•	•	•				•		
		B	•	•	•	•	0			•		
		C	•	•	0	•	•	•	0	•	•	•
	Tissue/bone dentin communicating	A	•	•	•	0						
		B	•	•	0	0	0	•	•			
		C	•	•	0	0	0	•	•		0	•
	Circulating blood	A	•	•	•	•		0		•		
		B	•	•	•	•	0	•	0	•		
		C	•	•	•	•	•	•	0	•	•	•
Implant Devices	Bone/tissue	A	•	•	•	0						
		B	•	•	0	0	0	•	•			
		C	•	•	0	0	0	•	•		•	•
	Blood	A	•	•	•	•			•	•		
		B	•	•	•	•	0	•	•	•		
		C	•	•	•	•	•	•	•	•	•	•

A = Limited exposure (≤ 24 hours) B = Prolonged exposure (24 hours – 30 days) C = Permanent contact (> 30 days)
• = FDA and ISO evaluation tests 0 = Additional tests for FDA

EXTRACTION, SAMPLE PREPARATION

Sample preparation is dealt with at length by ISO 10993-12, "Sample Preparation and Reference Materials" (1996) and by "Testing Methods to Evaluate Biological Safety of Medical Devices," Notice from the Office Medical Devices Evaluation Number 36 (2003). The procedures are in concordance with the compendial methods described in USP or ASTM standards. Extracts provide suitable samples of potentially hazardous substances that may leach out from the device into the surrounding tissue or may be released from the device during its use. The choice of extraction medium is based upon the use and nature of the device and the predictability of the test method used. Test devices or materials are extracted by incubating the device or representative portions at a surface-area-to-extractant volume ratio. The most common ratios depend on the thickness of the device or material. The ratio is 60 cm^2 per 20-ml extraction vehicle if device or material thickness is greater than or equal to 0.5 mm; 120 cm^2 per 20-ml extraction vehicle if device or material thickness is less than or equal to 0.5 mm. A weight-to-

volume ratio (4 g per 20-ml extraction vehicle) is utilized for extraction at a specific temperature and period of time if the surface area of the device or material cannot be determined. The conditions of extraction should maximize the amount of extractable substances and subject the test device or material to the extreme conditions it may be exposed to, without causing significant degradation. A common extraction procedure is to incubate the test device or material at 37°C for 24 hours at a ratio of either 60 cm^2 , 120 cm^2 , or 4 g per 20 ml of cell culture medium. The extraction conditions, ratios, and extraction vehicles to be used are generally similar between the ISO and MHLW guidelines. Any differences are addressed in the individual sections.

CYTOTOXICITY TESTS

The in vitro tests for cytotoxicity assess the response of cells in culture to direct contact with devices or to their extracts. ISO 10993-5 (1999): "Tests for In Vitro Cytotoxicity" specifies procedures for testing devices by direct or indirect contact, extracts of devices, and filter diffusion. Extracts of test devices and materials are tested by exposure to the cell culture system (e.g., L929 mouse fibroblast cell line). The presence of cytotoxic leachates is indicated by loss of cell viability. To test cytotoxicity by direct contact, the agar diffusion or overlay assay is conducted by placing the test device or a representative portion directly on a mammalian cell layer that is protected from mechanical damage by a layer of agar. Cytotoxic leachates diffuse into the cell layer via the agar, and toxicity is indicated by a loss of viable cells around the test device. The direct contact assay involves the placement of the test material directly on the cell culture medium, without the agar layer. The filter diffusion test involves the exposure of cells grown on filters to test and control materials that are applied to the filters on the side opposite the cells.

Following an exposure period of about 2 hours, the cytotoxicity of the materials to the cells on the filter can be assessed by using appropriate stains. In general, in these tests, approximately one-half million to one million cells are present in each culture dish, and toxicity is verified after a period of exposure (typically 24–72 hours) of the cells to the extract or device. Cytotoxicity is evaluated by qualitative and quantitative means. Positive control materials (e.g., organo-tin-impregnated polyvinyl chloride material) and negative control materials (e.g., USP-grade high-density polyethylene RS) are similarly tested alongside to validate the test results.

The cytotoxicity procedure specified by the MHLW guidelines for testing extracts employs mammalian cell lines at a density of about 40–200 cells per dish. This test prescribes specific positive controls (polyurethane film containing 0.1% zinc diethyldithiocarbamate, and 0.25% dibutyldithiocarbamate) and the range of responses they should elicit in order to validate the assay. The extraction procedure and the ratio of test material to extractant also differ from that recommended by ISO 10993-5. The ratio is either 5.0 cm^2/ml or 1.0 g/10 ml. The cell cultures are exposed to the neat extract and several dilutions of it for a period of 6 to 7 days, the surviving colonies of cells are counted, and the results are expressed as a percent of the negative control. If cytotoxicity is observed at the neat extract concentration, the concentration that would cause 50% cytotoxicity (IC50; IC-inhibitory concentration) is calculated.

Table III

ISO 10993-5	MHLW 2003
Number of cells per dish: 0.5–1 million cells	40 to 200 cells per dish
Extraction ratio: 25 cm^2 per 20 ml if thickness ≥1.0 mm, 60 cm^2 per 20 ml if thickness ≥0.5 mm 120 cm^2 per 20 ml if thickness ≤0.5 mm or 4 g per 20 ml	6 cm^2/ml or 1 g/10 ml
Exposure period: Typically 24–72 hours (2 hours for filter diffusion test)	6–7 days
Toxicity determination: Visual grading and/or quantitative assessments	Quantification of surviving colonies
Positive controls: Materials providing a reproducible cytotoxic response (e.g., organo-tin-impregnated polyvinyl chloride)	Segmented polyurethane films containing 0.1% zinc diethyldithiocarbamate and 0.25% zinc dibutyldithiocarbamate

Table III. Differences between cytotoxicity test procedures specified by ISO 10993-5 and the MHLW guidelines (MHLW 2003).

The major differences between the test procedures are illustrated in Table III. MHLW also recommends a direct contact test where the cells are allowed to grow for a period of 6 to 7 days in contact with the material or device. This test is recommended for leachables that may be inactivated during extraction and for devices or mateials that come in direct contact with tissue (e.g., eye contact).

SENSITIZATION TESTS

Sensitization is the allergic response caused by the activation of complex cellular and humoral immunological mechanisms following exposure to an allergenic substance. Sensitization can occur after either single or multiple exposures. Typically, the allergen is capable of penetrating the skin and biochemically reacts with proteins, thereby becoming proallergenic. Cellular components in the skin act as memory cells, and during reexposures, these cells initiate adverse reactions in response to the challenge. Sensitization tests, including the Buehler closed-patch test and the Magnusson-Kligman guinea pig maximization test, simulate such exposures to allow the expression of the sensitization potential of test materials. The protocol typically includes an initial induction phase followed by a challenge phase. The maximization test includes intradermal injections of the test material or its extract, whereas the closed-patch test uses topical applications of the test materials or their extracts. In addition, the concurrent administration of Freund's complete adjuvant in the maximization test enhances the potential sensitization capacity of test materials or their extracts.

ISO 10993-10, "Tests for Irritation and Sensitization," recommends either of these tests. If the test material or its extract is amenable to intradermal injection, the maximization test is recommended. The closed-patch test is the assay of choice for non-extractable, or when the extract or material may be topically applied.

Table IV

ISO 10993-10	MHLW 2003
Sample preparation: Extraction in polar and/or nonpolar solvents.	Two extraction solvents, methanol and acetone, recommended
Extraction ratio: Extraction ratio is dependent on thickness of device or representative portion.	Specific extraction ratios: 10:1 (volume solvent:weight sample)
Extract used for testing. If extraction is not possible, the Buehler (Closed Patch) test is typically utilized.	Residue obtained from extraction is redissolved and used for testing. (If the residue is not able to be injected the residue is to be used in the adjuvant and patch assay)

Table IV. Differences between sensitization test procedures required by ISO 10993-10 and the MHLW guidelines.

MHLW recommends the maximization test for organic and inorganic materials. In the maximization test, organic test residue is obtained by extraction separately in methanol and acetone. The residues are combined and redispersed in dimethylsulfoxide (DMSO) or vegetable oil, and administered by intradermal injection. If the obtained residue is not

dispersable in DMSO or vegetable oil, the adjuvant and patch test is utilized, and the residue is administered topically. In both tests, adjuvant is used to enhance the potential sensitization capacity of the test material. The MHLW guidelines require the calculation of the MR1 (the concentration that induces a mean response of 1). The major differences between the ISO and MHLW guidelines are described in Table IV.

INTRACUTANEOUS REACTIVITY TEST

The irritation potential of a test device or its leachates when administered to human patients can be extrapolated from the response obtained by injecting the extract of the test device intracutaneously to test animals. This test is a generic irritation test and does not obviate the need for more-specific product end use–oriented tests. Because body tissues differ in vascularization, composition, and response, irritation potential needs to be tested using the same contact conditions as those encountered during actual usage (e.g., eye irritation tests for eye products).

ISO 10993-10 requires that two animals and the MHLW requires that three animals be administered the test extract intracutaneously at five sites of the skin. A control vehicle is similarly injected at five other adjacent sites. Typically, both polar (e.g., physiological saline or water) and nonpolar (e.g., vegetable oil) extractants are used. The appearance of the test sites is compared to that of the control sites over a 72-hour period. Grades assigned to the observed responses are normalized to provide quantitative comparisons with the control responses.

Table V

ISO 10993-10	MHLW 2003
Number of test animals: Two Rabbits for each extract	Three rabbits for each extract
Number of test/control injections per extract: Five test and five control injections	Five test and five control injections
Evaluation of responses: Quantitative comparison of responses of test and control responses	Quantitative comparison of responses of test and control responses With Photographs

Table V. Differences in intracutaneous reactivity test procedures required by ISO 10993-10 and the MHLW guidelines.

The appearance of the test and control sites are observed over a 72-hour period, and a qualitative comparison of the severity of the responses at the test sites to those at the

control sites is conducted by a trained technician. The major differences between the ISO and MHW procedures are described in Table V.

SYSTEMIC TOXICITY AND PYROGENICITY TESTING

The release of the chemical constituents of a medical device, either by leaching or breakdown of the device, into the body has the potential for systemic toxicity. Systemic toxicity tests are generally conducted by administering the extracts (polar and nonpolar in most cases) as a single dose to test animals, and the health status of the animals is verified periodically—typically 24, 48 and 72 hours after dosing. Control animals are administered the extraction vehicle. ISO 10993-11, "Tests for Systemic Toxicity," is a melding of ASTM F750, "Standard Practice for Evaluation of Material Extracts by Systemic Injection in Mice (Method A: Intravenous)," and the USP test procedure "<88> Biological Reactivity Tests, In Vivo," to determine systemic toxicity.[7,8] Differences between the procedures are minor, mainly in the grading scale used for evaluating the test and the requirement for necropsy and photographs for the MHLW guideline. Systemic toxicity test grading scales for scoring responses of test animals are slightly different and are specified within the documents (see Table VI). ISO defines the clinical observation descriptors to which would be evaluated for severity ranging from respiratory, motor activities, convulsion, reflexes, ocular signs, cardiovascular signs, salivation, Piloerection, analgesia, muscle tone, gastrointestinal and skin.

Table VI

	MHLW	ISO/USP
Response	Description	
Normal, no symptoms	Mouse exhibits no adverse physical symptoms after injection	
Slight	Mouse exhibits slight loss of motor function, slight difficulty breathing, and symptoms of irritation in the abdominal cavity are observed	
Moderate	Mouse exhibits irritation in the abdominal cavity, difficulty breathing, loss of motor function, dropping of eyelids, and diarrhea clearly observed	
Marked	Mouse exhibits prostration, cyanosis, and trembling, or a sever case of irritation in the abdominal cavity, diarrhea, drooping of the eyelids, and difficulty of breathing are observed	
Dead, expired	Mouse dies after injection.	
	Interpretation The test is considered negative if none of the animals injected with the test article extracts shows a significantly greater biological reaction than the animals treated with the control article. If two or more mice show either marked signs of toxicity or die, the test article does not meet the requirements of the test. If any animals treated with a test article shows slight signs of toxicity, and not more than one animal shows marked signs of toxicity or dies, a repeat test using freshly prepared extract should be conducted using groups of 10 mice each. A substantial decrease in body weight for all animals in the group, even without other symptoms of toxicity, requires a retest using groups of 10 mice each. In the repeat test, the requirements are met if none of the animals injected with the test article shows a substantially greater reaction than that observed in the animals treated with the control article.	*Interpretation* The test is considered negative if none of the animals injected with the test article shows a significantly greater biological reaction than the animals treated with the control article. If two or more mice die, or show signs of toxicity such as convulsions or prostration, or if three or more mice lose more than 2 g of body weight, the test article does not meet the requirements of the test. If any animal treated with a test article shows only slight signs of biological reaction, and not more than one animal shows gross signs of biological reaction or dies, a repeat test should be conducted using groups of 10 mice. On the repeat test, all 10 animals must not show a significantly greater biological reaction than the animals treated with the control article.

Table VI. Comparison of grading scales used to score responses of test animals to MHLW and ISO/USP procedures.

Pyrogens are substances in devices that cause a febrile reaction. Bacterial endotoxin contamination is most commonly associated with such an adverse effect; however, leachates of materials can cause similar febrile responses (material-mediated pyrogenicity). Material-mediated pyrogenicity assessment is typically included in biocompatibility assessment, while bacterial contamination and endotoxin-mediated pyrogenicity are issues related more to manufacturing processes than to biocompatibility of the device or material.

ISO 10993-11 recommends testing the pyrogenicity potential of extractable substances derived from material leaching. ISO 10993 includes the pyrogen test, based on USP methodology, in the category of systemic toxicity testing. The baseline temperature of the rabbits prior to injection provides a basis for comparison.

Table VII

ISO 10993-11	MHLW 2003
Number of animals: Three rabbits required; comparison of febrile response in test animals to baseline temperature for evaluation of pyrogenicity potential	Three rabbits (test) required; comparison to baseline temperature is evaluated as index of pyrogenicity potential
Test duration: Test measurement intervals: every 30 minutes for 3 hours	Test measurement intervals: every hour for 3 hours
Evaluation: Cutoff for positive febrile response: 0.5°C	Cutoff for positive febrile response: 0.6°C

Table VII. Comparison of pyrogen test procedures required by ISO 10993-11 and the MHLW guidelines.

MHLW holds the pyrogen test as a separate test category. Material-mediated pyrogenicity is tested in a manner similar to ISO 10993 by injecting extracts of the test device into rabbits, and measuring the temperature rise at intervals over a 3-hour period. The major differences between the ISO and MHLW recommendations for testing systemic toxicity and pyrogenicity potential are outlined in Table VII.

TESTS FOR GENOTOXICITY

ISO 10993-3, "Tests for Genotoxicity, Carcinogenicity, and Reproductive Toxicity," recommends that the potential for genetic toxicity be assessed using a series of at least three assays. Two of these assays should use mammalian cells as the test system, and the tests should cover the three levels of genotoxic effects: DNA effects, gene mutations, and chromosomal aberrations. The International Conference on Harmonization (ICH)

"Guidelines on Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals, "are currently being applied to medical device assessments also. These guidelines recommend a three-test battery. These tests include reverse mutation assay using *Salmonella typhimurium* and *Escherichia coli* strains of bacteria, the in vitro chromosomal aberration assay or the mouse lymphoma $tk^{+/-}$ mutation assay, and the in vivo rodent bone-marrow micronucleus assays.

MHLW notification no. 99 recommends a test battery including the reverse mutation assays using *Salmonella typhimurium* and *E. coli* strains of bacteria and the in vitro chromosomal aberration assay.

ISO 10993-3 recommends the testing of either the test material extract or dissolved material, whereas the MHLW guideline recommends a specific process for obtaining residue from the test materials. Residue is obtained by incubating the test material in methanol and acetone, evaporating the solvents and redispersing the residue in an appropriate vehicle, such as DMSO, and testing. If sufficient residue is unobtainable, MHLW allows the test material to be extracted at 37°C for 48 hours in ethanol, acetone, or DMSO for the Ames mutagenicity assay and in cell culture medium for the chromosomal aberration assay, and the extract tested.

Table VIII

ISO 10993-3	MHLW 2003
Extraction vehicles: A physiological medium is used and, where appropriate, a solvent (e.g. dimethylsulfoxide)	Recommends methanol and acetone as extracting vehicles
Extraction: Extract test material and test the extract or dissolve material in solvent and conduct test. The conditions of extraction should maximize the amount of extractable substances, as well as subject the test device or material to the extreme conditions it may be exposed to, without causing significant degradation. Extraction ratio is dependent on thickness of test material.	Extract at room temperature at a ratio of 10:1 (solvent material) and obtain residue (at least 0.1–0.5% [weight of residue/weight of test material]), redissolve in appropriate solvent and test residue. If sufficient residue is unobtainable, extract test material (in ethanol, acetone, or DMSO at 10 g of test material per 20 ml for the Arnes mutagenicity assay, and in cell culture medium at 120 cm^3 or 4 g/20 ml for the chromosomal aberration assay), at 37°C for 48 hours and test extract. The Ames mutagenicity assay is conducted with a volume of 200 µl per plate.

Table VIII. Differences in genotoxicity testing procedures required by ISO 10993-3 and the MHLW guidelines.

Differences in the procedures required by ISO 10993 and the MHLW for genotoxicity testing are illustrated in Table VIII.

IMPLANTATION

The local effects of an implantable device or a material on tissue are assessed by implantation procedures that introduce the material or device, or a representative portion, into tissue. The implanted tissue region is allowed to heal, then explanted and examined for macroscopic and microscopic tissue responses. The evaluation of the effects of the implantation test is by assessment of several macroscopic and microscopic parameters, including fibrosis, degeneration; presence of phagocytic cells, necrosis, fatty infiltration, and foreign debris.

ISO 10993-6, "Tests for Local Effects after Transplantation" requires that the selected implant tissue region be appropriate for the evaluation of biocompatibility of the test material. Responses of the test implant sites are generally compared with the responses of similar sites implanted with materials for which biocompatibility and suitability for use in medical devices has been verified (e.g., USP-grade negative control plastic). ISO 10993-6 categorizes implant tests as subchronic (short-term, meaning less than 12 weeks) or chronic (long-term, meaning more than 12 weeks). The choice of test period is dictated by the need to ascertain that a steady state of response to continued exposure to the leachates from the implant has been reached prior to assessing the effect of the implant. It is apparent that the invaded tissue will be in a state of enhanced recuperative activity following implantation—a normal tissue response to invasive procedures. In general, tissue activity is expected to be at increased levels during the early period following the implant procedure (less than 1 week). The assessment of tissue responses during this time period, therefore, may reflect not the biological response to the implanted material, but rather the sequelae of generalized recuperative processes. Typical periods for subchronic implantation are 1, 3, 4, 9, and 12 weeks. A common study design uses 2, 4, and 12 weeks of implantation prior to assessment. Chronic implantation time periods range from 12, 26, 52, 78, and 104 weeks. For intramuscular implantation procedures, ISO 10993-6 recommends use of a minimum of three rabbits per time period, and sufficient test and control implants to provide at least eight implant specimens for each implantation period. Subcutaneous and bone implantation methods are also recommended by ISO for assessing differences in various treatments or modifications of a material.

Representing an instance of harmonization, the MHLW guidelines recommend that the chronic (long-term) effects of implantation be assessed by the procedures described in ISO 10993-6. For the short-term effects, however MHLW suggests a different protocol. Short-term effects are assessed by evaluating tissue responses to the implant at 1 and 4 weeks following the procedure. At least four rabbits per time period are recommended, and each rabbit is implanted with at least four test and two negative control materials. The explant sites, both test and control, are evaluated for macroscopic and microscopic tissue responses. Evaluated responses include inflammatory reactions and the area thus affected. If at least two of the four test sites exhibit a significant response compared with the control sites, an adverse or positive effect can be assumed.

Table IX

ISO 10993-3	MHLW 2003
Time point(s) of assessment: Sufficient to achieve steady state (e.g. 2, 4, 6, and 12 weeks)	7 days and 4 weeks
Number of animals: At least three per time period of assessment	At least four per time period
Number of samples for evaluation At least eight per time period for test and control	No minimum number specified
Evaluation criteria: Comparative evaluation of responses to test and control materials	If more than two of the four test sites in each animal exhibit a significant response compared control sites, the test is considered positive

Table IX shows the differences between ISO 10993-6 and the MHLW guidelines for assessing the effects of device or material implantation.

The major differences between the ISO and MHLW implantation guidelines are found in the procedures used to assess short-term effects of implantation. For long-term implant effects, MHLW concurs with ISO methodology. Major differences between the guidelines are illustrated in Table IX.

EYE IRRITATION TEST

Tests for assessing potential for eye irritation of test devices are required by ISO 10993-10, "Tests for Irritation and Sensitization," and Japan's MHLW. The protocols specified by the ISO standardand the MHLW guidelines are essentially similar and recommend that at least three rabbits be used per extract. A volume of 0.1 ml of the test material or its extract (polar or nonpolar) is instilled in one eye of each animal. The other eye receives the control vehicle or extractant. The test and control eyes are assessed for biological responses at 1, 24, 48, and 72 hours after instillation. The observation period need not exceed 21 days. The reactions of the test and control eyes are recorded and assessed using a specific classification scale described in the ISO 10993-10 standard.

Table X

ISO 10993-10	MHLW 2003
Time of exposure: 1 second	30 seconds
Grading scale: Classification system for grading ocular lesions	Draize or McDonald-Shadduck scale

Table X shows differences in eye irritation testing procedures outlined in ISO 10993-10 and the MHLW guidelines.

The MHLW procedure is essentially similar to that of ISO10993-10, except that the grading criteria utilized can be either that of Draize or that of McDonald-Shadduck.[10,11] The major differences in the two guidelines are represented in Table X.

HEMOLYSIS

The interaction of devices or biomaterials with blood is an immediate and serious concern during safety assessment. The effects of the biomaterial may be broadly classified into cellular and humoral effects. The interaction of biomaterials with cellular elements of blood includes thrombosis, embolism, lysis, and inflammation. The humoral effects include the activation of the coagulation, kinin, complement, and fibrinolytic systems. The cellular and humoral responses are tightly interwoven in their effects and each influences or elicits the other's response.

Blood, as is the case of the entire human or organismal mass, is composed of 90–95% water. The rest is composed of the various cellular elements of blood, including the red blood cells (RBCs), white blood cells (WBCs), platelets, etc. RBCs are the cells that harbor hemoglobin, the protein responsible for oxygen transport and cellular respiration. Lysis of RBCs would result in leakage of free hemo-globin into the plasma, potentially leading to severe hepatic and renal injury among other effects.

ISO 10993-4, "Selection of Tests for Interactions with Blood" (1992), suggests areas of hemocompatibility that should be tested, including thrombosis, coagulation, platelets and platelet function, hematology, and immunology. Specific tests are available to assess a biomaterial's potential to elicit adverse effects in these categories of blood function. More than one test is usually conducted to allow a broad assessment of the hemocompatibility of a biomaterial. The use of human blood is generally recommended in in vitro tests for hemocompatibility.

Although other investigations are generally required for a more comprehensive evaluation, the assessment of hemolytic potential has emerged as one of the commonly used screening tests for assessing the hemocompatibility of biomaterials because of the

short time and low cost for the test. The MHLW guidelines suggest that hemocompatibility be assessed by the methods outlined in ISO 10993-4, with the exception of the hemolysis test procedure. The hemolysis test specified by MHLW is similar to that of the method elaborated in the U. S. Department of Health, Education, and Welfare/National Institutes of Health document "Evaluation of Hemodialyzers and Dialysis Membranes" (1977) and Autian's *Authorized Toxicity Testing Protocols*, vol. I (ATTP-I).[12,13] This commonly performed test uses rabbit or human blood, and assays hemoglobin by recording absorbance of the extract to which blood has been added and incubated at 37°C for a 60-minute period, at a specific wavelength. The absorbances of the negative and positive controls are also assayed, and used to normalize the test absorbance, so that hemolysis can be expressed as a percentage of the positive control (assumed to be 100% hemolysis). A less than 5% hemolysis value is generally considered nonhemolytic. ISO 10993-4 and the MHLW as an alternate method recommends the hemolysis test procedure described in ASTM F756, "Standard Practice for Assessment of Hemolytic Properties of Materials."[14] This procedure is currently undergoing revision. Current revisions to ISO 10993-4 (committee draft N318) include device-specific test recommendations for assessing various hemocompatibility parameters, and the inclusion of a decision tree.

The MHLW guidelines specify that the test described above be conducted in similar replicates following incubation periods of 1, 2, and 4 hours. Because the assessment of hemolytic potential using rabbit blood as the test system may not be extrapolated to human blood exposure, other procedures that utilize human blood in similar procedures must be used. Notable differences in hemolysis test procedures required by ISO 10993-4 and MHLW 1995 are described in Table XI.

Table XI

ISO 10993-4	MHLW 2003
Hemolysis can be assessed by any of several validated methods to assay hemoglobin in plasma.	Hemolytic index is assessed by measuring hemoglobin at 1, 2, and 4 hours by spectrophotometric methods. The hemolysis over this period is expressed as a percentage of positive control.

Table XI. Differences in hemolysis test procedures recommended by ISO 10993-4 and the MHLW guidelines.

CONCLUSION

The MHLW standards and the ISO/FDA requirements have minor but important differences in the test procedures for assessing biocompatibility of a test device or material. The goal of current medical device regulatory efforts is towards harmonization

of such testing requirements and guidelines as described in this article. In general, harmonized standards and guidelines augment the confidence level in a safety assessment procedure, wherever it is conducted. The endeavor in all safety assessment, as in all scientific investigations, is toward good scientific practices and rationale that will be applied toward protecting the population. The rest of the details will generally fall into place on their own.

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