Welcome to Clinical Device Group's web publications for medical devices. Today, we'll take a manager-level look at the introductory standards (Parts 1 and 2) and the standards dealing directly with biological effects (Parts 3, 4, 5, 6, 10, and 11). You can take the quiz as we go along to test your knowledge, then read the text for understanding.

The ISO 10993 series of standards describe how to evaluate the biological safety of medical devices. The standards are prepared by an international group of experts under the auspices of ISO Technical Committee 194 (TC 194). The Technical Committee, consisting of around 100 experts, is divided into 15 working groups. Each working group focuses its attention on specific standards. Standards are reviewed every five years to assure they reflect current technology and ethical norms.
ISO 10993

- 16 published standards.
- 2 drafts for new standards (not published).
- 3 work items for new standards.
- ISO Technical Committee 194.
- 15 working groups.

The approach to evaluating medical device biological safety is opposite from the approach taken by classical toxicologists in evaluating drug safety. Drug toxicologists work from the specific to the general, identifying each chemical component in a formulation and evaluating its biological effect, inductively reasoning the drug is safe. Device toxicologists work from the general to the specific, taking extracts of the material (or finished device), administering the extract to an animal, and looking for a response. If there is no response, they deduce the material is safe.

Only six of the standards address specific categories of biological effects (Parts 3, 4, 5, 6, 10, and 11), five address material degradation (Parts 9, 13, 14, 15, and 16), two standards give an overview of strategic and ethical principles (Parts 1 and 2), one deals with ethylene oxide residuals (Part 7), one deals with reference materials (Part 8), and one deals with sample preparation (Part 12).

The standards on degradation identify an important trend in how device toxicologists approach their work: looking more closely at the chemical make-up of a material, deliberately assessing possible degradation products, and thinking from the specific to the general. Our strategies are changing—moving toward the classical model of toxicology. This trend will require more technical expertise and strategic thinking on our parts.
Part 1: Evaluation and Testing

True or false: In evaluating biological safety...

[   ] In evaluating biological safety, we consider all the available information, we don’t just do a bunch of tests.

[   ] It’s the main material that counts, we can ignore any minor residues or degradation products that may be present.

[   ] All materials are evaluated equally, regardless of their application.

Part 1, Evaluation and Testing (1997), helps us build a strategy for evaluating the safety of a device. Note the emphasis on the word “evaluating”. We don’t have to newly conduct a test for every possible biological effect, instead a knowledgeable expert should consider all the possible biological effects, then develop a strategy for evaluation based on all available information and a knowledge of the materials’ chemical properties. The goal is to establish safety while doing as few new tests as possible: a “paper toxicology” approach.

We need to consider all of the device, including processing aids, additives, inadvertent contaminants, residues, coatings, degradation products, effects of the manufacturing and sterilization processes, and any other stray chemicals that might find their way in or on the device, as well as the primary materials themselves.
Part 1: Evaluation and Testing

True or false:

[   ] Devices are categorized by duration of contact and type of tissue contact, shown below.
[   ] The depth and breadth of an evaluation is based on the category of the device.

<table>
<thead>
<tr>
<th>Limited time</th>
<th>Prolonged time</th>
<th>Permanent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface contact</td>
<td>Surface contact</td>
<td>Surface contact</td>
</tr>
<tr>
<td>Limited time</td>
<td>Prolonged time</td>
<td>Permanent</td>
</tr>
<tr>
<td>External communicating</td>
<td>Externally communicating</td>
<td>Externally communicating</td>
</tr>
<tr>
<td>Limited time</td>
<td>Prolonged time</td>
<td>Permanent</td>
</tr>
<tr>
<td>Implant</td>
<td>Implant</td>
<td>Implant</td>
</tr>
</tbody>
</table>

We need to consider the duration of contact and type of tissue contact of the device. Limited exposure devices are those that come in contact with tissue for 24 hours or less, prolonged exposure devices for 24 hours to 30 days, permanent exposure devices for more than 30 days. We consider the total lifetime exposure to disposable devices that are used repeatedly, like wound dressings or urinary catheters.

Tissue contact categories include surface contact (skin and mucous membranes), external communicating contact (indirect blood path, tissue/bone/dentin, and circulating blood), and implanted devices (tissue/bone and blood).

The longer the duration of exposure and the less protected the tissue by natural barriers (skin is a marvelous natural barrier), the broader and deeper we need to go in the safety evaluation.

For some devices it is more practical to consider individual materials. Endoscopes have a “business-end” that contacts internal tissues and a “handle-end” that only contacts the surgeon’s hands. The two parts of the device don’t need the same level of evaluation. So it’s a useful strategy to base evaluations on the individual materials, then step back for a global “device-look” at the end.
Part 1: Evaluation and Testing

True or false:

[ ] Twelve categories of biological effects pretty much cover anything a device can do to tissues, organs, or the body as a whole.

- Cytotoxicity.
- Sensitization.
- Irritation or intracutaneous reactivity.
- Acute systemic toxicity.
- Subacute toxicity.
- Genotoxicity.
- Implantation.
- Hemocompatibility.
- Chronic toxicity.
- Carcinogenicity.
- Reproduction and developmental toxicity.
- Biodegradation.

There are twelve major biological effects to be considered: 1) cytotoxicity—an effect on individual cells, 2) sensitization—an immune response, 3) irritation or intracutaneous reactivity—local cell effects, 4) acute systemic toxicity—immediate effects to body systems, such as the central nervous system, 5) subacute toxicity—organ or system effects that take weeks to months to appear, 6) genotoxicity—effects to the DNA, 7) implantation effects—effects to tissues surrounding an implant and body system responses to an implant, 8) hemocompatibility—blood effects, 9) chronic toxicity—organ and body system effects that take months to years to appear, 10) carcinogenicity—the effect of causing cancer, 11) reproductive and developmental toxicity—effects on the ability to bear offspring and the health of those offspring, and 12) biodegradation—the body’s effect on the device.

By considering these twelve effects, we pretty much cover anything a device can do to mammalian tissues, organs, or the body as a whole.
Part 2: Animal Welfare Requirements

True or false:

[ ] Animals shall be treated humanely.

[ ] In vitro tests should be performed before in vivo tests.

[ ] Testing should be conducted per a written experiment plan or protocol.

[ ] An animal should not be used in more than one experimental series.

[ ] It is unnecessary to repeat a test if the results can be confirmed by other means.

Part 2, Animal Welfare Requirements (1993), describes the ethical principles for conducting experiments in living animals. Animals should be treated humanely, according to good veterinary practices, and we should take every opportunity to minimize pain, anxiety, suffering, distress or lasting harm. Testing may not begin until a protocol describing the test article, details of any statistical methods, statement of scientific goals, procedures, species of animal, number of animals, method of euthanasia, and any controls or comparators has been written. Finally, the need to avoid undue suffering in any one animal takes precedence over the need to reduce the number of animals used.

Note, in the US the protocol must be approved by an Institutional Animal Care and Use Committee (IACUC). In Europe, test houses are accredited so no protocol review is required.
Part 3: Genotoxicity...

True or false:

[ ] Genotoxicity includes gene mutation effects, DNA effects and chromosomal aberrations.

[ ] Three gene toxicity tests are required, at least two in mammalian cells.

[ ] Two extraction media are required, a polar medium such as saline and a nonpolar medium such as DMSO.

[ ] An vivo test should be done if any one of the in vitro tests is positive.

[ ] No tests are necessary for materials known to show no genotoxicity.

Part 3, Genotoxicity, Carcinogenicity and Reproductive Toxicity (2000), deals with the selection of tests for genotoxicity, carcinogenicity, and reproductive toxicology. There are three types of genotoxic effects: gene mutation effects, DNA effects, and chromosomal aberrations. Gene mutations are alternations in the base-pairing of the DNA helix. The alterations may occur because a nucleotide base has been deleted (deletion frameshift mutation), and extra nucleotide base has been added (insertion frameshift mutation), or one nucleotide base has been substituted for another (point mutation). Chemicals that cause genotoxic effects are called genotoxins. DNA effects are alterations that occur to the double helix, such as breaks in the strands, molecules intercalating between the strands, or the substitution of an aberrant base for the four accepted bases. Chromosomal aberrations are morphological alterations in a chromosome, such as deletions, duplications, inversions or translocations of chromosomal material.

We’re required to do least three assays in two solvents (six tests), usually according to OECD guidelines. A suggested strategy is to conduct a gene mutation assay in bacteria (Ames test in Salmonella typhimurium), a gene mutation assay in mammalian cells, and a test for DNA damage and repair in mammalian cells (clastogenicity test). If any of the in vitro tests is positive, an in vivo test in whole animals is required. No genotoxicity tests are necessary if the device is made from materials known to show no genotoxicity.

The third most commonly asked question by clients is “do I have to do genotoxicity testing”. You can understand why, a genotoxicity profile costs tens of thousands of dollars.
Part 3: ...Carcinogenicity...

True or false:

[ ] Carcinogenicity tests should be considered for devices having tissue contact lasting 30 days or more, when there is suggestive data from other sources.

[ ] Carcinogenicity prescreening may be done via in vitro cell transformation systems.

[ ] One animal species, tested for its lifetime, is sufficient.

[ ] Carcinogenicity and chronic toxicity testing may be combined into one test.

[ ] FDA may allow clinical evaluations to proceed parallel with carcinogenicity testing.

Carcinogenicity is the ability of a material or extract to cause cancer. Such tests last for the lifetime of the animal (usually a rodent) and may be combined with chronic toxicity tests. Carcinogenicity tests should be considered for permanently implanted materials, materials where the cumulative tissue contact time is greater than 30 days (including resorbables), or materials which have tested positive in in vitro and in vivo genotoxicity tests.

Two parallel tests are conducted, one in a group of animals receiving the maximum allowable dose, a second in a group of animals receiving a fraction of the maximum allowable dose. Additional animals for a reference group or a sham surgical or vehicle dose group may be needed. One-hundred and twenty (60 male and 60 female) animals are used per group. The animals are followed for their lifetime (18 months for mice, 24 months for rats), then autopsied. Up to 40 separate organ systems and tissues are examined for cancerous cells.

There is a phenomenon in rats called solid state carcinogenicity in which implanted solid materials cause tumors via tissue destruction related to their geometry (Oppenheimer effect). This is not true carcinogenicity and does not occur in humans.
Part 3: ...Reproductive Toxicity

True or false:

[  ] Reproductive toxicity tests should be considered for intrauterine devices or other devices with prolonged contact with reproductive tissues.

[  ] Reproductive toxicity tests are not necessary for energy-depositing devices such as X-ray machines or CT scanners.

Reproductive toxicity covers the areas of reproduction, fertility and teratogenicity. Many substances can affect fertility and reproduction, both in males and females, without other signs of toxicity. Effects can range from slightly decreased reproductive capability to complete sterility.

Prescreening and testing are done according to OECD protocols, with modifications to accommodate the dose, route of exposure, extraction media and exposure time of the device. These tests should be considered for devices that contact the reproductive tissues, embryo, or fetus, devices that deposit energy to the body, and devices with leachables or resorbables.
Part 4: Blood Interactions

True or False:

[ ] There are five major types of blood interactions: thrombosis, coagulation, platelet, hematology, and complement system.

[ ] In vitro, in vivo, and ex vivo test methods exist for all five types of blood interactions.

[ ] Patency is the most common measure of success or failure for implanted devices.

Part 4, Selection of Tests for Interactions with Blood (1993), tells us there are five important categories of blood interactions: 1) thrombosis—clot formation in major vessels independent of the clotting factor cascade, 2) coagulation—clot formation resulting from activation of the clotting factor cascade, 3) platelet effects—activation, inactivation, or interference with maturation, 4) hematology—effects on red cells and other cellular and plasma components of blood, and 5) complement system effects—usually activation.

We must evaluate all five categories of blood interactions. Evaluations may consist of simple in vitro assays, ex vivo experiments, or in vivo experiments. In ex vivo experiments, the circulating blood in a living animal is shunted out of the body, through the device, then back into the body. Ex vivo experiments are used for externally communicating devices and sometimes for implanted devices which won’t fit into the animal’s body. In in vivo experiments, the device or material is implanted into circulatory system of the body, mimicking actual use. Patency is the most common measure of success or failure for implanted devices.
Part 4: Interactions with Blood

True or False:

[ ] Blood compatibility evaluations vary depending on whether the device is: non-blood contacting, externally communicates indirectly with blood, externally communicates with circulating blood, or is implanted.

[ ] Whenever possible, devices should be tested under conditions which simulate their intended clinical use.

[ ] The pig’s hematology and circulatory systems are similar to man’s, making it a suitable animal model for study.

Devices may be: 1) non-blood contacting (such as in vitro diagnostic devices), 2) externally communicating indirectly with blood (such as administration set tubing, which does not contact blood directly, but fluids flowing through the tubing do enter the bloodstream), 3) externally communicating with circulating blood (such as guidewires or intravascular endoscopes), or 4) implanted devices. Whenever possible, devices should be tested under conditions which simulate their intended clinical use.

Pigs (porcine) and dogs (canine) are the two most commonly used animal systems. The pig is favored because its hematology and circulatory systems are similar to man’s.
Part 5: Cytotoxicity

True or False:

[ ] Cytotoxicity tests evaluate biological effects on mammalian cells, test articles are placed on top of cells and the cells monitored for health.

[ ] Cytotoxicity tests were originally developed as a substitute for implantation tests.

[ ] Test articles are scored on a scale of 0-3, noncytotoxic to severely cytotoxic.

Part 5, Tests for In Vitro Cytotoxicity (1999), describes various methods for testing devices for effects on mammalian cells. The tests were originally designed to replace implantation tests, although this has not occurred. Three replicates of either whole devices, intact materials, or extracts may be tested; extraction media may be culture medium with serum, culture medium without serum, saline, or other suitable solvent. The extraction temperature should challenge the material without causing it to fuse, melt, soften, or degrade.

Briefly, cells are grown in a culture dish, then the intact material or extract is placed over the cells. After a suitable incubation period, the cells are examined for their physical appearance (dead cells pop open and spill their contents) and other measures of cell health. The cytotoxicity of a material or extract is a function of the toxicity, the diffusion rate, and the concentration of the molecules that leach out. Cytotoxicity is measured on a scale of 0-3, rated from noncytotoxic to severely cytotoxic.
Part 6: Implantation

■ True or False:

[  ] Implantation tests evaluate the effect of materials on mammalian cells in a whole animal situation.
[  ] Tests are also intended to evaluate mechanical or functional loading.
[  ] Tests may be short-term or long-term, so long as the cells reach a steady state.
[  ] Tests may be performed in mice, rats, guinea pigs, rabbits, dogs, sheep, goats, or pigs.
[  ] Materials are usually implanted into muscle, but may be implanted into bone or subcutaneous tissue.

Part 6, Tests for Local Effects after Implantation (1995), describes several strategies for evaluating the biological effect of materials on cells in whole animal situations. The tests are not intended to evaluate mechanical or functional loading. Solid material may be implanted as fabricated coupons, rods or sheets; liquids, pastes and particulates may be mixed, allowed to set, and/or contained in tubes to facilitate insertion. Grooved materials may be evaluated.

Materials may be implanted surgically in muscle, bone, or subcutaneous tissue for short-term (12 weeks or less) or long-term (12 weeks or more) evaluations. The test should be long enough for the cell's biological reaction to reach a steady state. At the end of the evaluation period the animals are sacrificed and the implantation site evaluated with low magnification to record the nature and extent of any tissue reaction. Then, sections of tissue samples embedded in plastic are sectioned and examined under a microscope. The tissue is evaluated for fibrosis, fibrous capsule, inflammation, degeneration, number and type of inflammatory cells, necrosis, material debris, fatty infiltration, granuloma, and quality and quantity of tissue ingrowth into porous materials. We must test at least three animals and ten specimens, but happily, we can combine implantation testing with chronic toxicity testing.
Part 10: Irritation

True or False:

[ ] Irritation is a local response recognized by redness and swelling.

[ ] You should not do in vivo tests on materials already known to be severe irritants.

[ ] You may do skin irritation tests in humans on materials known to be non-irritating in animals.

[ ] Before testing, you should identify all processing aids, additives, contaminants, or residuals that may be present in the material.

Part 10, Tests for Irritation and Delayed-type Hypersensitivity (1995), describes tests for irritation and sensitization. We are all familiar with irritation: imagine battery-acid or hot peppers on your skin. Irritation is a local response measured by redness (erythema) and swelling (edema).

Approach the testing in a step-wise fashion: 1) identify all the processing aids, additives, contaminants or residues that are present in the material, 2) review the literature to determine if any of them are known irritants, 3) check to see if in vitro tests are available (there are several in development, but none are validated today), and 4) test the material. You may do skin irritation testing in humans—useful for marketing purposes of dermal contact devices—only after determining the material is non-irritating in animals.
Part 10: Irritation

True or False:

[ ] Albino rabbits are preferred for irritation testing.

[ ] If you expect irritation, test only one animal so as to minimize pain and suffering. If there’s no irritation, you can conclude the material is non-irritating.

[ ] Irritation is reported as negligible, slight, moderate or severe.

The preferred animal is the white rabbit, because its response profile is similar to man’s; three animals are required. If a material has a pH below 2 or above 11.5 you should assume it is an extreme irritant and not test it. If you suspect the material is an irritant, test only one animal first. If the irritation level is low, test the other two animals to complete the test. If the irritation level is high, stop the test.

Irritation is measured by scoring the erythema and edema at each site of exposure, and reported as negligible, slight, moderate or severe.
Part 10: Delayed-Type Hypersensitivity

True or False:

[ ] Delayed-type hypersensitivity is a type of immune response; it is systemic, involving the whole body.

[ ] There are two types of tests: guinea pig maximization and closed-patch (Buehler), the maximization test is more sensitive.

[ ] The ability of a chemical to cause hypersensitivity is independent of dose.

Part 10 also describes tests for delayed-type hypersensitivity, a specific type of immune reaction (sensitization). The reaction is termed “delayed” because symptoms don’t appear until about 24 hours after the initial exposure. In humans, the reaction is characterized by redness, swelling, even weeping and oozing, spreading from the site of exposure. Hives may cover the entire body. The symptoms do not resolve until exposure to the offending device is stopped.

There are two types of tests, both involving the guinea pig: 1) guinea pig maximization test and 2) closed-patch (Buehler) test. The maximization test is more sensitive and is preferred by FDA. The closed-patch test is used when you want to test intact samples rather than extracts.

Hypersensitivity is dose dependent. Just because a material tests positive, doesn’t mean you can’t use it in a device—you might be using the material well-below the sensitizing dose. On the other hand, a material that tests negative might still be a sensitizer, but the dose tested was too low to detect it. The highest possible dose that will not cause skin irritation is the correct dose to use for testing.
Part 10: Delayed-Type Hypersensitivity

True or False:

[ ] The tests involve an induction phase and a challenge phase, mimicking the delayed-type hypersensitivity process in real situations.

[ ] Tests results are variable between labs for a variety of reasons, including sample preparation and animal variability.

The tests are designed with an induction phase and a challenge phase. During the induction phase, the animal is exposed to the material or extract in such a way as to optimize the chance for sensitization. Then a two-week rest period is observed so that antibodies can accumulate in the body. Finally, during the challenge phase, the animal is exposed to the material or extract in such a way as to optimally illicit symptoms. If symptoms—redness, swelling, loss of function—occur the material is said to test positive.

Test results are known to vary from laboratory to laboratory for a variety of possible reasons, including sample preparation, animal preparation, and animal variability. I recommend finding a laboratory you trust and sticking with them.

Delayed-type hypersensitivity tests are expensive and take about three months from scheduling to report. No wonder the fourth most common question asked by clients is “do I have to do sensitization testing?”
Part 11: Systemic Toxicity

True or False:

[   ] Systemic toxicity is an effect on an entire body system, usually the central nervous system.

[   ] There is usually a minimum level of exposure that will cause a reaction, called the threshold level.

[   ] The NOEL is the highest dose of material that does not cause a reaction, the no observed effect level.

Part 11, Tests for Systemic Toxicity (1993), describes several methods for evaluating a material or its extracts for effects on a body system, most commonly the central nervous system. Systemic effects usually exhibit a threshold level, below which no symptoms are observed. The NOEL, or no-observed-effect level, is the highest dose of material (or extract) that does not cause a reaction. This is an extremely useful concept, because once the NOEL is established for a material, you can argue that a dose less than that will be safe for a user.

To confuse you, sometimes the NOEL is called the NOAEL or no-observed-adverse-effect level.
Part 11: Systemic Effects

True or False:

[ ] Oral, intravenous, dermal or inhalation exposure routes may be used.

[ ] Mice, rats, rabbits, guinea pigs, dogs, pigs, ferrets, or non-human primates may be used.

[ ] Test durations are categorized as acute, subacute, subchronic, or chronic.

[ ] Animals are monitored for behavior, mortality/morbidity, weight loss, and gross pathology.

Exposure may be by any of a number of routes—oral, intravenous, dermal or inhalation—obviously chosen to reflect actual use. Usually mice or rats are used, but rabbits, guinea pigs, dogs, pigs, ferrets, or non-human primates have been used also. You’ll need to scientifically justify using any animal other than mice or rats.

Acute tests are defined as lasting up to 1 day, subacute from 1-14 days, subchronic from 14-90 days or up to 10% of the animal’s lifespan, and chronic tests usually last from 6-12 months.

Animals are monitored for clinical observations (behavior changes), mortality/morbidity, changes in body weight, and gross pathology. Organ weights and histopathology are assessed in subacute, subchronic, and chronic tests.
The Commercial

True or False:

[   ] You can learn more about strategic planning for biological safety by taking the seminar “Biocompatibility Testing & Management” from Clinical Device Group.

[   ] You can learn more about managing biological safety by buying the book of the same title.

[   ] You can get assistance with your safety evaluations by contracting with our experts who specialize in device biological safety.

If you found this slide presentation useful, you should consider taking the two-day seminar “Biocompatibility Testing & Management” from Clinical Device Group, or consider purchasing the book by the same name. Both will help you with strategically planning your biological safety evaluations. On the other hand, you can get personal assistance with your safety evaluations by contracting with our experts.
More Information

- Visit [www.clinicaldevice.com](http://www.clinicaldevice.com).
- Email [njstark@clinicaldevice.com](mailto:njstark@clinicaldevice.com).
- Phone 1-773-489-5721.
- Fax 1-773-489-4281.

For more information (or the answers to the quiz), visit our website at [www.clinicaldevice.com](http://www.clinicaldevice.com), send me an email at njstark@clinicaldevice.com, or call me at 1-773-489-5721.