A Practical Guide to ISO 10993-10: Irritation

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ISO 10993-10

The international standard's four-tier approach to irritation testing protects patients and minimizes animal studies.

Three types of testing—cytotoxicity, sensitization, and irritation are mandated for all medical device materials by the international biocompatibility standard ISO 10993-1, "Guidance on the Selection of Tests," and its FDA counterpart, blue book memorandum #G95-1. Specific tests that can be used to satisfy these requirements are then provided in ISO 10993-5, which covers cytotoxicity, and ISO 10993-10, which addresses both sensitization and irritation. This article focuses on that part of ISO 10993-10 devoted to irritation tests. (Earlier articles in this MD&DI series outlined the other two types of required testing—cytotoxicity and sensitization.)

IRRITATION RESPONSES

The chemicals released from device materials that contact the body may produce skin, mucosal, or eye irritation. In general terms, such irritation is a local tissue response characterized by the usual signs of inflammation—redness and swelling—and sometimes accompanied by heat and pain (Figure 1). Numerous chemicals are capable of causing irritation, either immediate or delayed, and some of these
may be present in materials as additives, processing or manufacturing aids, or inadvertent contaminants. For example, the organotin stabilizers used in nonmedical vinyl formulations are corrosive chemicals capable of causing irritation and necrosis when applied to mucosal surfaces; residual concentrations of ethylene oxide present in gas-sterilized devices can produce an irritant response if they are not reduced to acceptable levels before the device is used; and residues of such contaminants as chemical detergents in a particular batch of materials or devices can cause unexpected irritation responses in users or patients.

Figure 1. Some individuals exhibit a marked response to the chemicals present in such products as latex gloves. This case was judged to be an irritation, not a sensitization, response.

With regard to irritation testing, the ISO standard defines irritation as a "localized inflammatory response to single, repeated, or continuous application of the test substance, without involvement of an immunological mechanism." ISO 10993-10 also proposes a four-tier approach to assessing the potential of a material to cause irritation. A device manufacturer should first conduct a review of the literature to determine whether others have reported that the chemical or material under consideration, or structurally related chemicals or materials, can cause irritation. It is essential that the chemical or material of interest already be sufficiently characterized that it can be correlated to those described in the literature. The second step is to use available, validated in vitro tests (such as cytotoxicity assays using mammalian cells in culture) to identify, whenever possible, severely irritating materials without using test animals. When materials have not been ruled out by the first two steps, they should be evaluated using the in vivo tests described in the standard. The final step is the use of noninvasive clinical studies in human subjects, but this is not presented in the standard as a routine part of an irritation testing program. Rather, it is presumably reserved for samples with unusual characteristics or those with equivocal test results.

IN VIVO TEST METHODS

The intracutaneous, primary skin, and ocular irritation tests are the three in vivo, nonclinical tests commonly used to evaluate materials for possible contact hazards. The intracutaneous test has been described in the United States Pharmacopeia (USP) for more than 30 years as a means to assess the suitability of plastic pharmaceutical containers for their intended use. The test is conducted much like allergy testing in human patients. Fluid extracts of the test material are prepared under controlled conditions of temperature, time, and ratio of the material surface area to the volume of extraction fluid. Using small-bore needles, a volume of fresh extract is then injected intracutaneously (that is, into the skin) at multiple sites on the shaved backs of two albino rabbits. An equal number of control sites are injected with an untreated volume of the extraction vehicle. At 24, 48, and 72 hours after injection, the test and control sites are observed and scores are given for the severity of any redness (erythema) or swelling (edema) (Figure 2). Extracts that produce a significantly greater response than controls are considered irritants. The USP method describes the use of up to four extraction fluids—saline, saline-alcohol, polyethylene glycol 400, and vegetable oil—for evaluating pharmaceutical containers. For medical device materials, saline and vegetable oil are used to ensure extraction of both water-soluble and fat-soluble chemicals.

Figure 2. In intracutaneous irritation tests using albino rabbits, the raised
blebs caused by injecting extracts of test materials are either resolved without causing any visible changes or produce inflammation marked by redness and swelling in the 24 to 72 hours following injection.

The intracutaneous reactivity test is aggressive in that it makes use of extracts prepared under exaggerated conditions and places them directly into the skin of the test animal, thereby maximizing the chance of finding irritant chemicals if they are present. The primary skin irritation test is less aggressive in that portions of the test material itself are simply placed on the shaved backs of albino rabbits. The samples are then covered with an occlusive dressing and left in place for at least 4 hours but more commonly for 24. The contact sites are observed for an additional period of up to 72 hours and scored for erythema and edema. After these scores are totaled, they are compared with known values for primary skin irritation (available in table format), and the response is categorized as negligible, slight, moderate, or severe. When necessary, the method may be modified to use fluid extracts rather than the material itself on the contact sites.

Generally reserved for eye-contact products, the ocular irritation test is usually performed with fluid extracts prepared as described above, although some materials may be tested directly as solids or powders. A small volume of fresh extract (or solid) is placed directly into the pocket formed by withdrawing the lower eyelid of an albino rabbit. The rabbit's other eye is left untreated as a control. Observations are made at regular intervals for up to 72 hours, and scores are compiled for redness and swelling of the eye's conjunctiva, response of the iris to light, corneal opacity, and presence of discharge. These scores are then compared with a classification table to determine which test materials are considered eye irritants.

In addition to the intracutaneous, primary skin, and ocular irritation tests, oral, rectal, penile, and vaginal irritation tests are described in annex D of the standard as complements to, not replacements for, the primary tests. They are considered relevant for medical devices intended to be applied to these respective mucosal areas of the body. Of the four, the vaginal irritation test in the rabbit is of particular importance for several reasons. Extracts applied to the vaginal mucosa remain in contact with the tissue for an extended time, exaggerating exposure; the vaginal epithelium of the rabbit is only one cell thick and thus particularly sensitive to irritants; and a microscopic scoring system is available, providing a cellular basis for judging the irritant potential of a material.

CONCLUSION

Considerable effort has been expended during the past 20 years to identify suitable in vitro alternatives to animal tests for irritation. However, none of the methods reported on thus far has duplicated the complex physiology of the animal model, so the search continues. Meanwhile, the tiered approach described in ISO 10993-10 offers a means of minimizing both the potential for exposure of human patients to irritating medical devices and the amount of animal testing that must be conducted.

Continue to the next article in the series, systemic effects [7].
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