Testing for sensitivity to chemical extractables from medical devices is a key element of the biocompatibility standards. Note: this is the fourth installment in an ongoing series of articles on ISO 10993. If you haven't done so already, you might like to read part one, ISO 10993: An Introduction to the Standard [5].

According to International Organization for Standardization (ISO) 10993-1—the first in the set of international standards covering the biological evaluation of medical devices and the basis for FDA's blue book memorandum on this subject (#G95-1)—all device materials must undergo cytotoxicity, sensitization, and irritation testing. Methods for performing such tests are described in other parts of ISO 10993, including ISO 10993-10, "Tests for Sensitization and Irritation."

This article focuses on those test methods currently being used to determine whether sensitization reactions are elicited by any chemicals that may be released from specific biomaterials and medical devices. Earlier articles addressed materials characterization [6] and cytotoxicity [7].

SENSITIZATION REACTIONS
Sensitization or hypersensitivity reactions usually occur as a result of repeated or prolonged contact with a chemical substance that interacts with the body's immune system. Because most such reactions to biomaterials have been of the dermal cell–mediated type, rather than the humoral or antigen-antibody type, the skin of laboratory animals is used in sensitivity testing. Dermal sensitization reactions in laboratory animals are marked by redness and swelling.

Biomaterials and devices that cause sensitization reactions do so by means of their extractable chemicals. In some cases, an individual may develop a reaction only after encountering a material repeatedly or after continuous, prolonged contact, such as with an implant. Or, after wearing natural latex gloves daily for several weeks or months, a previously unaffected person may develop a persistent rash on the hands and wrists (Figure 1). This sensitization may be caused by one of several chemical components of the gloves acting as an allergen.

![Figure 1. After wearing natural latex gloves daily for several weeks or months, a person may develop a rash.](image)

In other cases, when an individual has already become sensitized to a chemical, such as one present in the environment, he or she will experience a reaction when first exposed to a device that contains that chemical. Thus, an individual previously sensitized to nickel will develop a rash along the temples a few days after beginning to wear eyeglasses with nickel-plated frames.

**TEST METHODS**

Biomaterials and other device materials are tested for the presence of sensitizing chemicals using guinea pigs, a species known to be nearly as responsive to dermal sensitizers as human beings are. Guinea pig sensitization tests require six to eight weeks and thus take the longest time to complete of all the acute biocompatibility tests described in the 10993 standards.

The repeated-patch, or Buehler, test method involves exposing the shaved backs of guinea pigs directly to the test material under occlusive dressings for a minimum of six hours. This procedure is repeated as many as three times a week for three weeks. This part of the test is often referred to as the induction phase. Following a two-week rest or recovery period to allow for the development of a delayed response, the animals are challenged by a final exposure to a patch of the biomaterial. The repeated-patch model is used primarily for topical devices such as dermal electrodes and surgical gowns and drapes, since in these cases the method of applying test materials to the animals simulates clinical use.

In the maximization, or Magnuson-Kligman, test method, fluid extracts of the test material are prepared in saline and vegetable oil, and separate groups of guinea pigs are exposed repeatedly to the two types of extracts. The guinea pigs are first injected with an extract along with an adjuvant intended to enhance an immune response, then receive a topical application. Following a two-week rest or recovery, the animals
are covered with a topical patch containing the extract. Generally considered more sensitive than the
repeated-patch model, the maximization test is used for device materials that will contact areas other than
skin. The use of both a saline extract and an oil extract simulates extraction by bodily fluids and by
intravenous liquids and other pharmaceutical products that first contact the device and then the patient.

![Figure 2. Positive response to a positive-control substance in a guinea pig, seen in the maximization, or Magnuson-Kligman, test.](image)

In both techniques, the area of the challenge patches is examined for reactions (redness and swelling)
that are not present in negative-control animals. In addition, known sensitizing chemicals are used
periodically to validate the model and the technician (Figure 2).

**A CAVEAT AND FUTURE POSSIBILITIES**

The guinea pig sensitization tests described above are useful methods for eliminating the possibility that
patients will be exposed to strong sensitizing chemicals extracted from medical device materials.
However, these methods are far from perfect in their ability to detect weak sensitizers or chemicals, and
they do not detect chemicals that act as adjuvants, enhancing an immune response to other chemicals to
which a patient might be exposed. Nor are they able to detect responses to antigens such as the plant
proteins found in natural latex, which have been responsible for the severe, even life-threatening, systemic
immune responses that occasionally have been reported.

Additional test methods currently under development also may prove useful for evaluating biomaterials for
sensitization. One test that shows promise is the local lymph node assay in mice. In this method, the ears
of mice are treated and then the surrounding lymph nodes are examined for a lymphocyte proliferative
response as demonstrated by an accumulation of a radio-labeled marker.

Much effort also has been devoted to developing in vitro alternatives to the guinea pig sensitization
models, but thus far no suitable replacements have been identified.

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Continue to part 5 of this series, Irritation Testing [9].