



The Challenge to Global Acceptance of Part 3 of ISO 10993

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ISO 10993, Part 3: Testing for Genotoxicity, Carcinogenicity and Reproductive Effects, is not a globally recognised standard. The differences in these testing requirements in Europe, the United States and Japan are explained together with guidance on when to test and efficient strategies to adopt when marketing products in these regions.

International variations

The intent of ISO 10993 Biological Evaluation of Medical Devices¹ series of standards has been to provide global harmonisation of the biocompatibility testing of medical devices. The guidance document, ISO 10993, Part 3: Testing for Genotoxicity, Carcinogenicity and Reproductive Effects, was developed in 1992, adopted in 1993 and then revised in 2003, which is the current version. Unfortunately for medical device manufacturers, global harmonisation has not been achieved for ISO 10993, Part 3. Europe has fully accepted the testing requirements of Part 3, but the United States (US) Food and Drug Administration (FDA) and Japan's Ministry of Health, Labour and Welfare (MHLW) have testing requirements different from those described in Part 3 to address concerns about the genotoxicity potential of a device. Consequently, testing for genotoxicity varies depending on where the device is submitted for regulatory clearance. Although this guidance document also covers carcinogenicity and reproductive effects, a limited number of medical devices are required to have actual testing in these areas and it is not clear when this testing is required for the different geographical markets. Thus, the primary focus of this article will relate to genotoxicity testing.

When to test for genotoxicity

The need for genotoxicity testing is based on how the device is categorised, that is, the

type of body contact and contact duration for the device's intended application. In general, surface devices with permanent contact and externally communicating and implant devices with prolonged exposure or permanent contact would require genotoxicity testing. The chemical nature of possible leachables is a factor that is considered in genotoxicity testing. As with other parts in the ISO 10993 series, Part 3 recommends chemical characterisation of the medical device as outlined in Part 18. The results of the chemical characterisation may modify the requirement for genotoxicity testing. For example, if the leachables present are well known and non-mutagenic, a risk assessment could be conducted to address potential genotoxicity.

Test regime

There are a wide variety of well-established test methods for the detection of genotoxic effects from chemicals and pharmaceuticals. These methods can be conducted in non-mammalian (bacteria and yeast) and mammalian in vitro systems or in vivo (typically rodents). A single test or type of test may not be capable of detecting all genotoxic materials or effects. Therefore, regulators often require a test battery of two or more tests for genotoxicity. These classical methods have been adapted for use with medical devices and typically involve testing device extracts. In general, both polar solvents (saline or culture media) and nonpolar

solvents (dimethylsulphoxide or ethanol) are used to extract potential genotoxic, leachable chemicals from the device to use for testing.

If genotoxicity testing is required, ISO 10993, Part 3 describes two testing options. The test methods referenced are Organisation for Economic Cooperation and Development (OECD) test methods that generally have global acceptance.² Option 1 lists the following:

- (a) a test for gene mutations in bacteria (OECD 471, commonly referred to as the Ames or Bacterial Reverse Mutation test)
- (b) a test for gene mutations in mammalian cells (OECD 476, the mouse lymphoma assay)
- (c) a test for clastogenicity in mammalian cells (OECD 473, chromosomal aberration assay).

Option 2 is similar except for the mammalian assays; two endpoints are covered in one test. The required tests for option 2 are:

- (a) a test for gene mutations in bacteria (OECD 471)
- (b) a mouse lymphoma assay incorporating colony number and size determination to cover both endpoints, gene mutations and clastogenicity.

These ISO test requirements can be met using all in vitro assays. In vivo assays are only indicated in the cases when positive genotoxic results are seen in the in vitro assays. The in vivo assay selected (gene mutation or clastogenicity) should be based

Table 1:

Item	ISO 10993, 3 (Europe)	FDA (US)	MHLW (Japan)
In vitro test	a) gene mutation in bacteria b) gene mutations in mammalian cells c) clastogenicity in mammalian cells or a) gene mutation in bacteria b) mouse lymphoma assay covering both gene and clastogenic endpoints	a) gene mutation in bacteria b) chromosomal aberration or mouse lymphoma assay in mammalian cells c) mouse micronucleus assay	a) gene mutation in bacteria b) chromosomal aberration or mouse lymphoma assay in mammalian cells
In vivo test	Not required unless positive results with in vitro test	Required as part of battery of tests	Not required or discussed
Extraction vehicle	Refer to, ISO 10993,12 Polar and nonpolar vehicles	Same as ISO	Methanol and acetone (exaggerated extraction)
Extraction conditions	Ratios, time and temperatures as described in ISO 10993,12 Conditions should not cause significant degradation of the device	Same as ISO	Extract at room temperature at a ratio of 10:1 (solvent:material) and obtain residue, redissolve in appropriate solvent and test residue If unable to obtain sufficient residue, follow extraction methods described for each assay

on the type of in vitro assay showing positive results, thereby determining whether a similar positive result still occurs in an in vivo system. Commonly used in vivo assays are:

- (a) micronucleus test in rodents (OECD 474)
- (b) metaphase analysis in rodent bone marrow (OECD 475)
- (c) unscheduled DNA synthesis test with mammalian liver cells (OECD 486).

FDA requirements

Although FDA has accepted or recognised many of the various parts of ISO 10993 as “consensus” documents, it has not accepted Part 3 as a consensus document for genotoxicity testing. The FDA’s Center for Devices and Radiological Health currently follows the International Conference on Harmonisation “Guidelines on Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals.”³ This document calls for a three-test battery with

- (a) a test for gene mutation in bacteria
- (b) an in vitro test with cytogenetic evaluation of chromosomal damage with mammalian cells or an in vitro mouse lymphoma assay

phoma assay

- (c) an in vivo test for chromosomal damage using rodent hematopoietic cells.

As with ISO 10993, Part 3, a multiple test battery is required, but as part of that battery, an in vivo (that is, rodent micronucleus assay) is required. Because this battery of three tests including an in vivo assay goes beyond the requirements in Part 3, it is generally considered to meet or exceed ISO requirements.

Requirements in Japan

For Japan, a two-test battery is required consisting of:

- (a) a test for gene mutation in bacteria, and
- (b) an in vitro test with cytogenetic evaluation of chromosomal damage with mammalian cells or an in vitro mouse lymphoma assay.

These test requirements are based on the Japanese MHLW guideline: “Test methods for biological safety evaluation of medical devices, Part 3, Genotoxicity Test.” The primary difference between the Japanese MHLW requirements and ISO 10993, Part 3, is the nature of the extract used. The MHLW

guidelines have a specific extraction process using organic solvents (methanol or acetone) to generate a “residue.” This exaggerated extraction process is intended to pull more leachables from the medical device than standard extraction methods as described in ISO 10993, Part 12, Sample Preparation and Reference Materials. In the event that sufficient residue cannot be obtained, the MHLW guidelines call for regular, that is, nonexaggerated extraction methods using extraction vehicles, ratios and time/temperature specific to this standard.

Selecting the right strategy

Table I summarises the major differences among requirements. So what testing guidelines should the device manufacturer follow? This decision should be based on where the manufacturer intends to market the device initially and in the future. The company should consider the ISO requirements as the starting point. If submitting to Europe and the US, following FDA guidelines will cover both. If contemplating the Japanese market as well as Europe and the US, using the Japanese (exaggerated extraction) methods for the in vitro assays together with conducting the mouse micronucleus assay will meet all the different test requirements. It is incumbent upon device manufacturers to know where they plan to submit their medical device so that the optimum test programme can be conducted, thereby avoiding repetitious or unnecessary testing.

References

1. www.iso.org/iso/en/prods-services/ISOstore/store.html
2. www.oecd.org/document
3. This document is located on the FDA web site: www.fda.gov/Cder/guidance/1856fnl.pdf [mdt](#)

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