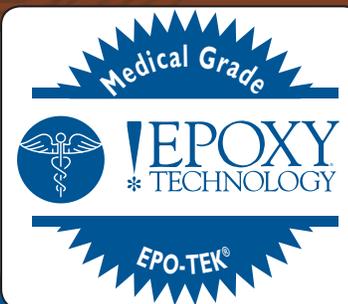


Biocompatibility Testing of Adhesives*

Biocompatibility testing of adhesives is used to determine how compatible a material is within a biological environment. It is also used to predict any potentially harmful effects on the human body. The testing for this falls either under ISO 10993 or USP Class VI.



ISO 10993 Testing

ISO 10993-4 Hemocompatibility Testing

Hemolysis Complete (Direct and Indirect)

The Hemolysis test is an in-vitro assay that determine the ability of a test article or its extract, to destroy red blood cells with the subsequent release of the hemoglobin. Rabbit blood with hemoglobin concentration adjusted to standards is added in triplicate to the test article in phosphate buffer (Direct) or its extract (Indirect). Following 3 hours incubation at 37°C, the hemoglobin present in the supernatant is made to react with Drabkin's reagent converting all the forms of hemoglobin into cyanmethemoglobin. The hemoglobin content of the supernatant is then assayed spectrophotometrically using a hemoglobin standard curve. A hemolysis index above the negative control of 0 to 2% will be considered non-hemolytic, while values between 2 and 5% will be considered slightly hemolytic, and above 5% will be called hemolytic. This assay is in conforms to ASTM Guideline F756.

ISO 10993-5 Cytotoxicity Testing

MEM Elution Cytotoxicity

The "in-vitro" biological reactivity of the L929 mouse fibroblast cell culture is qualitatively determined in response to an extract of the test material in triplicate. The cells are allowed to grow to sub-confluency in tissue culture plates. An extract of the test material is prepared in Minimum Essential Media (MEM), which is transferred onto the cell layer in triplicate. The plates are incubated for forty-eight hours at 37°C in a 5% CO₂ incubator, and scored for reactivity at twenty-four and forty-eight hours on a scale from Grade 0 (no reactivity) to Grade 4 (severe reactivity). The test item is considered non-cytotoxic if none of the cultures exposed to the test item shows greater than mild reactivity (Grade 2). This assay is appropriate for screening purposes.

ISO 10993-6 Implantation Testing

Implant/Muscle/2 weeks

The muscle implantation test assesses the material or device for local effects on living tissue when implanted typically in the paravertebral muscles of the rabbit. The bioreactivity of a material is evaluated both macroscopically and microscopically through a comparison of the tissue response caused by the material as compared to a control material (i.e. negative control plastic). At the end of the observation period, gross observations of the implant sites will be recorded. The area of the tissue surrounding the center position of each implant strip will be processed and a pathologist will process the implanted sites for histopathological evaluation. Inflammation, fibrosis, haemorrhage and necrosis are evaluated on a scale and compared to the control sites.

ISO 10993-10 Sensitization Testing

Kligman Maximization/2 Extracts/Concurrent (+) Controls

The Kligman Maximization Test evaluates the allergenic potential or sensitizing capacity of the test article in guinea pigs. The test article is exposed to the test system directly or through test article extracts. Extracts of the test material are prepared in a polar (saline) and/or non-polar (cotton seed oil) solution. The test begins with intradermal injections of Freund's Complete Adjuvant (FCA) and the test article. Seven days later the injection sites are covered with the test article/extract for a period of forty-eight hours. Fourteen days later a new site is challenged with a topical application of the test article/extract and scored out to forty-eight hours. A sensitization reaction to the test article is scored based on the defined evaluation criteria in ISO 10993-10.

ISO 10993-10 Irritation/Intracutaneous Reactivity Testing

Intracutaneous Injection/2 Extracts

The Intracutaneous Injection Test is designed to evaluate local responses to solutions or extracts following intracutaneous injections into rabbits. The test article will be exposed to the test system directly or through test article extracts. Extracts of the test material are prepared in a polar (saline) and/or non-polar (cotton seed oil) solutions. A minimum of three rabbits are injected intracutaneously with the test article and control materials. The injected sites are examined over a seventy-two hour period for evidence of tissue reaction such as erythema, edema, or necrosis. Observations are scored according to the Classification System for Scoring Skin Reactions (Draize scale). At the end of the observation period the scores are used to determine an overall mean reaction score for the test article versus the corresponding control article. The requirements of the test are met if the difference of the mean reaction score for the test article and the control article is 1.0 or less.

ISO 10993-11 Acute Systemic Toxicity

Acute System Injection/2 Extracts

The purpose of this assay is to evaluate the test article extracts for potential toxic effects as a result of single dose systemic injection in mice. Extracts of the test article are prepared in polar (saline) and/or non-polar (cottonseed oil) solutions. The test article is injected intravenously and/or intraperitoneally in groups of five mice. The animals are observed for 72 hours after administration for signs of biological reactivity. This assay is considered negative if none of the animals injected with the test article show a significantly greater biological reaction than the animals treated with the control article. If two or more mice die, or show signs of toxicity, or if three or more mice lose more than 2 g of body weight, the test article does not meet the requirements of the assay.



USP CLASS VI Testing

In-vivo Biological Reactivity Testing

There are three (3) biological mechanisms evaluated for the USP Class VI Test:

1. **Acute Systemic Toxicity** is evaluated via a single-dose systemic injection of the test article or test article extracts in mice. The test article will be exposed to the test system directly or through test article extracts. Extracts of the test material are prepared in a total of four (4) vehicles representing polar and non-polar solutions (NaCl, CSO, PEG, and EtOH). The test article is injected intravenously and/or intraperitoneally in groups of five mice. The animals are *observed* for seventy-two hours after administration for signs of biological reactivity. This test is considered negative if none of the animals injected with the test article show a significantly greater biological reaction than the animals treated with the control article. If two or more mice die, or show signs of toxicity, or if three or more mice lose more than 2g of body weight, the test article does not meet the requirements of the test.
2. **Intracutaneous Reactivity** is evaluated via intracutaneous injections of the test article or test article extracts into rabbits. The test article will be exposed to the test system directly or through test article extracts. Extracts of the test material are prepared in a total of four (4) vehicles representing polar and non-polar solutions (NaCl, CSO, PEG, and EtOH). A minimum of two rabbits are injected intracutaneously with the test article and control materials. The injected sites are examined over a seventy-two hour period for evidence of tissue reaction such as erythema, edema, or necrosis. Observations are scored according to the Classification System for Scoring Skin Reactions (Draize scale). At the end of the observation period the scores are used to determine an overall mean reaction score for the test article versus the corresponding control article. The requirements of the test are met if the difference of the mean reaction score for the test article and the control article is 1.0 or less.
3. **Local Tissue Reactivity** is evaluated via muscle implantation of the test article for local effects on living tissue when implanted typically in the paravertebral muscles of the rabbit. The bioreactivity of a material is evaluated macroscopically through a comparison of the tissue response caused by the material or device as compared to an appropriate control material (i.e. negative control plastic or predicate control). At the end of the observation period, gross observations of the implant sites will be recorded.

In-vitro Biological Reactivity Testing

MEM Elution for Cytotoxicity

The "in-vitro" biological reactivity of the L929 mouse fibroblast cell culture is determined in response to an extract of the test material. The cells are allowed to grow to sub-confluency in tissue culture plates. An extract of the test material is prepared in Minimum Essential Media (MEM), which is transferred onto the cell layer in duplicate. The plates

are incubated for forty eight hours at 37°C in a 5% CO₂ incubator, and scored for reactivity at twenty four and forty eight hours on a scale from Grade 0 (no reactivity) to Grade 4 (severe reactivity). The test item is considered non-cytotoxic if none of the cultures exposed to the test item shows greater than mild reactivity (Grade 2).

*** We would like to acknowledge TOXIKON Corporation Bedford, MA as the source for our Biocompatibility testing information. This independent testing laboratory is where Epoxy Technology has our adhesives tested. www.toxikon.com • 1.800.458.4141**

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Please consult our **Application Experts** at Epoxy Technology to find the most suitable adhesives for your specific technical challenges at: techserv@epotek.com.

EPOXY
* TECHNOLOGY

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Epoxy Technology Inc. • 14 Fortune Drive • Billerica, MA 01821
phone 978-667-3805 fax 978-663-9782 techserv@epotek.com

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