

Following are descriptions of tests offered by WuXi AppTec that are in compliance with Japanese Ministry of Health, Labour, and Welfare: Notice from the Office Medical Device Evaluation No. 36. March 19, Heisei 15 or 2003. Pharmaceutical and Food Safety Bureau Notice No. 0213001; and are adapted from Testing Methods to Evaluate Biological Safety of Medical Devices as issued by the Director of Evaluation and Licensing Division of the Ministry's Pharmaceutical and Food Safety Bureau, Japan.

For **cytotoxicity**, **sensitization**, **genotoxicology**, and **blood compatibility** testing the JMHLW generally prefers the methods described below. For **skin reactivity**, **implant**, **systemic toxicity**, and **pyrogenicity**, they have in the past accepted ISO methods as an alternative to the tests described on the reverse of this sheet. **WuXi AppTec can perform either method as deemed appropriate by your specific test plan and regulatory considerations. For more information, please contact a WuXi AppTec Account Manager at (+1) 651-675-2000 or 888-794-0077.**

CYTOTOXICITY

Extract Colony Assay [JMHLW]

The purpose of this procedure is to evaluate the cytotoxic response of a specified mammalian culture cell line when exposed to the extract of the materials under test. The Extract Colony Assay Cytotoxicity Test utilizes the sensitivity of low cell density to evaluate material/device cell toxicity. Test material is extracted for 24 to 25 hours in Eagle's Minimal Essential Media (E-MEM) with 10% heat-inactivated fetal bovine serum (FBS). Extracts are placed in contact with L-929 cells seeded at a low cell density. The resulting cell colony growth is fixed, stained, and counted. The colony growth is expressed as a percentage of the cell control. These percentages are then plotted on a semilogarithmic graph against the dilution of the test article extract. The dilution that inhibits colony formation to 50% is determined from the resulting inhibition curve. Three (3) reference controls are run in parallel with the test article to confirm the validity of the assay run.

SENSITIZATION

Maximization Sensitization Test [JMHLW]

Exaggerated extracts of the test article are performed using solvents to remove any leachable materials. The extract is then concentrated using a rotary evaporator to produce a residue. If a residue is not recovered, the alternative pathway will be used. This includes a solvent extraction and concentration of compiled extractants. After the material has been appropriately extracted, 11 test and 6 negative control animals are exposed to the extract in a series of two inductions, followed approximately two weeks later by a challenge exposure at which time the animals are evaluated for a sensitization response. A concurrent positive control group utilizing six animals is employed. The rationale for this test method is based on the generally accepted notion that the guinea pig is the best animal model for the human allergic contact dermatitis. The use of Freund's complete adjuvant and sodium lauryl sulfate tend to enhance the ability of this test to detect weak sensitizing agents. While this test does not ensure that test materials are completely non-allergenic, it is the most sensitive animal test in common use today.

GENOTOXICOLOGY

Bacterial Reverse Mutation Assay Using Four Salmonella Strains and One E. coli Strain [JMHLW]

Exaggerated extracts of the test article are performed using solvents to remove any leachable materials. Extract is then concentrated using a rotary evaporator to produce a residue. If residue is not recovered, the alternative pathway will be used. In this case, another strong solvent is used for a room temperature extract. Test article is tested at 5 dose levels along with appropriate vehicle and positive controls. Test article dilutions are analyzed using tester strains TA98, TA100, TA1535, TA1537, and WP2-uvrA- with and without microsomal enzymes. The test article doses, negative vehicle, and positive controls are plated in triplicate. Following incubation of approximately 48-72 hours at 37±2°C, revertant colonies per plate are enumerated.

In Vitro Chromosome Aberration Analysis in Chinese Hamster Ovary (CHO) Cells [JMHLW]

Exaggerated extracts of the test article are performed using solvents to remove any leachable materials. The extract is then concentrated using a rotary evaporator to produce a residue. If a residue is not recovered, the alternative pathway will be used. In this case, another strong solvent is used for a room temperature extract. CHO-K1 cells are incubated in the presence of the test article extract, negative, or positive control for three hours in the presence and absence of metabolic activation. After this incubation period, the cells are washed, fresh medium added, and returned to the incubator. Two hours prior to the end of incubation, colcemid is added to all flasks to stop cells in metaphase. The cells are collected from the flasks by mitotic shake-off, swollen, fixed, and dropped onto slides. Slides are stained and permanently mounted prior to microscopic observation.

BLOOD COMPATIBILITY

Hemolytic Toxicity Test [JMHLW]

The test article, as well as known negative and positive control materials, is exposed to defibrinogenated rabbit blood. After the exposure period (1 hr, 2 hrs and 4 hrs.), the mixture is carefully mixed and the blood solution transferred to new tubes. The exposed blood is centrifuged for 5 minutes to isolate the supernatant. These samples are then scanned on a spectrophotometer. (This is referred to as Method 1.) Drabkin's reagent is added to any sample showing preliminary signs of hemolysis. (This reagent is used to bind hemoglobins.) Finally, the supernatant is re-evaluated for the presence of released hemoglobins. The hemolytic index is then calculated based on these results. (This is referred to as Method 2.)

CONTINUED ON REVERSE.

STIMULATION

Intracutaneous Irritation Test [JMHLW]

For safety evaluation of a biomaterial sample, rabbits are injected intracutaneously (Dose = 0.2 mL x 5 sites) with extracts of the test article and associated vehicle controls. Injection sites are examined and scored at 24 ± 2 , 48 ± 2 , and 72 ± 2 hours after treatment for signs of skin reactions. If the difference between the average scores for the extract of a test article and the control is less than or equal to 1.0, the test article passes the test. The Japanese guidance provides that the test articles should be extracted at the highest temperature that can be tolerated by the test sample.

Primary Skin Stimulatory Test [JMHLW]

This test involves the use of 6 rabbits for each solvent extract. The extracts will consist of polar and non-polar solvents. Each extract will be applied to the shaved shin of the rabbit with scratched and unscratched areas. The extracts will be removed 24 hours post exposure and irritation will be scored at 1, 24 and 48 hours after the extracts were removed.

IMPLANT

Intramuscular Implant [JMHLW]

For the safety evaluation of a test article, representative portions of the device or the entire device are implanted in the paravertebral muscles of live rabbits. A minimum of 8 test samples and 8 control samples are implanted across a total of at least 4 rabbits for each time point or group. After a predetermined exposure period, the animals are sacrificed and the implantation sites exposed. Gross necropsy observations are made and implant sites harvested and fixed. The specimens are fixed to stabilize the tissue device interface and then oriented transversely and cut-in for histology processing. Microscopic evaluation includes: cell type, cell distribution, fibroplasia, and neovascularization. Histopathology is required.

SYSTEMIC TOXICITY

Acute Systemic Injection Test [JMHLW]

For the safety evaluation of a test article, mice are injected intraperitoneally or intravenously with either extracts or solutions of the test article or control (vehicle without test article). The animals are observed for signs of toxicity immediately after injection and at 4 ± 0.75 hours and 24, 48, and 72 ± 2 hours post-injection. Body weights are measured prior to injection and 24, 48, and 72 ± 2 hours post-injection. If none of the animals treated with the test article extract or solution shows a greater adverse reaction than the animals treated with the blank, the test article passes the test. Necropsy is required.

Subchronic Toxicity Test [JMHLW]

Twenty rats (10 test / 10 control) are administered intravenously (Dose = 20mL / kg x 28 intervals) a 0.9% normal saline extract of the test article or vehicle control 28 times over a 28-day test period. Animals are observed once daily for signs of toxicity. Animal weights are recorded twice a week for four weeks (28 days). On Day 28, the animals are euthanized and blood samples for hematology and clinical chemistry analysis are collected. A gross necropsy is performed, tissues and any lesions are collected, and histopathology is performed by WuXi AppTec.

PYROGENICITY

Materials Mediated Pyrogenicity [JMHLW]

The test article is extracted in 0.9% saline in solution and injected into the marginal ear vein of each of three (3) animals. Rectal temperatures are recorded for each animal prior to injection and between one (1) and three (3) hours post injection (at one (1) hour intervals). The test article is considered negative if none of the animals individual temperature difference is ≥ 0.6 °C and the cumulative temperature increases of the three animals is less than 1.4 °C, when the baseline temperature is subtracted from the maximum temperature between one (1) and three (3) hours post injection. If one rabbit has an individual temperature increase ≥ 0.6 °C or if the cumulative temperature increase of the three (3) animals is greater than 1.4 °C, the test may be continued, per Sponsor's request, using 5 additional rabbits (a continued test). If not more than three (3) of the eight (8) rabbits (from the initial test and continued test) have a temperature increase ≥ 0.6 °C, the test article under examination meets the requirements for the absence of pyrogens.

NOTE: For the tests listed above, JMHLW and ISO protocols are technically similar. If ISO-compliant tests have been conducted, it may not be necessary for the tests to be repeated to meet JMHLW submission requirements. Sponsors should discuss options with their regulatory department.