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**Immunogenic Evaluation Method of Medical Devices -
Part 1: T Lymphocyte Transformation Test in Vitro**

医疗器械免疫原性评价方法 第1部分：体外T淋巴细胞转化试验

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Immunogenic Evaluation Method of Medical Devices - Part 1: T Lymphocyte Transformation Test in Vitro

1 Scope

This Part gives the MTT method and CFSE method for the T lymphocyte transformation test in vitro; it is applicable to evaluate the effect of medical devices/materials on the T lymphocyte immune function.

2 Normative References

The following documents are essential to the application of this document. For the dated documents, only the versions with the dates indicated are applicable to this document; for the undated documents, only the latest version (including all the amendments) are applicable to this document.

GB/T 16886.1 Biological Evaluation of Medical Devices - Part 1: Evaluation and Testing

GB/T 16886.2 Biological Evaluation of Medical Devices - Part 2: Animal Welfare Requirements

GB/T 16886.12 Biological Evaluation of Medical Devices - Part 12: Sample Preparation and Reference Materials

GB/T 16886.20 Biological Evaluation of Medical Devices - Part 20: Principles and Methods for Immunotoxicology Testing of Medical Devices

3 Terms and Definitions

For the purposes of this document, the terms and definitions given in GB/T 16886.1 and GB/T 16886.20 apply.

4 Abbreviation

The following abbreviations are applicable to this document.

NOTE: The immunogens that may be present in the medical devices/materials are mostly macromolecular substances; therefore, the effectiveness of the preparation method for the selected test samples shall be explained.

7 MTT Method

7.1 Principle

MTT is a pale-yellow cell dye; it enters into the living cells and forms water-insoluble blue-purple crystalline formazan particles in mitochondria under the role of succinate dehydrogenase and deposits in the cells; dissolve the formazan in the cells, and determine its absorbance value by an enzyme-linked immunosorbent assay; then it can indirectly reflect the number and/or metabolic activity of the living cells. When stimulated by an antigen or mitogen, the lymphocytes may undergo a specific or non-specific immune response, proliferate and differentiate; the number or metabolic activity of the cells may increase. Inspect the ability of lymphocytes to proliferate by MTT dyes, then it can reflect the effects of immunogens in the medical devices/materials on the lymphocyte immune function.

NOTE 1: MTT is a commonly used staining marker for detecting the cell proliferation. Other suitable staining markers such as BrdU, MTS and CCK-8 can also be used. $^3\text{H-TdR}$ is a highly sensitive marker for detecting the cell proliferation; but the laboratory to use it shall have the qualification to use radioactive materials and the testing staffs shall be trained and qualified.

NTOE 2: This Method is suitable for detecting the proliferation of T lymphocytes in vitro; does not rule out the interference of the proliferation of other types of cell subsets on the results of this experiment.

7.2 Lymphocyte preparation

The BALB/c mice were sacrificed by taking the cervical vertebrae off; the single cell suspension was prepared by grinding; collect the cell suspension; take 400g to centrifuge for 5min; discharge the supernatant; add 5mL of red blood cell lysate; mix up; incubate at 4°C for 5min; add RPMI1640 nutrient solution containing 10% FCS to stop the reaction; take 400g to centrifuge for 5min; discharge the supernatant; add RPMI1640 nutrient solution containing 10% FCS to adjust the cell concentration into 2×10^6 cells/mL.

7.3 Test grouping

- a) Control group for cell nutrient solution (blank control);
- b) Negative control group (cell control);
- c) Positive control group (cell + ConA, 5 $\mu\text{g}/\text{mL}$);

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