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Biological Evaluation of Nanomaterial Medical Devices - Test for Genotoxicity - In Vitro Mammalian Cell Micronucleus Test

纳米医疗器械生物学评价 遗传毒性试验

体外哺乳动物细胞微核试验

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1 Scope

This document provides an in vitro mammalian cell micronucleus test method for the evaluation of nanomaterial medical devices or nanomaterials used in medical devices. By determining the number of micronucleus-containing cells after the cells are exposed to nanomaterial medical devices or nanomaterial test solutions used in medical devices, it is evaluated whether there are potential genotoxic risks.

This document is applicable to the micronucleus test using immortalized cell cytokinesis blocking method to evaluate the genotoxicity of nanomaterial medical devices or nanomaterials used in medical devices.

NOTE: others, for example, human peripheral blood primary cell culture method, may take this method as a reference, but methodological verification should be carried out.

2 Normative References

The contents of the following documents constitute indispensable clauses of this document through the normative references in the text. In terms of references with a specified date, only versions with a specified date are applicable to this document. In terms of references without a specified date, the latest version (including all the modifications) is applicable to this document.

GB/T 16886.3 Biological Evaluation of Medical Devices - Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity

YY/T 0870.6-2019 Test for Genotoxicity of Medical Devices - Part 6: In Vitro Mammalian Cell Micronucleus Test

3 Terms and Definitions

For the purposes of this document, the terms and definitions given in GB/T 16886.3 and YY/T 0870.6 and the following apply.

3.1 nanomaterial medical devices

Nanomaterial medical devices refer to medical devices incorporating or containing nanomaterials.

[source: YY/T 0993-2015, 3.1.2, modified]

3.2 positive control

Positive control means reproducible positive results can be obtained in the test system when materials and / or substances (including positive control reagents and positive control materials) are thoroughly characterized and proven for use in in vitro micronucleus test.

[source: GB/T 16886.12-2017, 3.12, modified]

3.3 negative control

Negative control means reproducible negative results can be obtained in the test system when materials and / or substances (including medium negative control and negative control materials) are thoroughly characterized and proven for use in in vitro micronucleus test.

NOTE: the leaching medium or release medium of the test sample can be used as a medium negative control; polystyrene nanospheres can be used as a negative control material.

[source: GB/T 16886.12-2017, 3.11, modified]

3.4 test sample

Test Sample refers to a test solution of nanomaterial medical devices or nanomaterials used in medical devices.

4 Cells, Reagents and Main Equipment

4.1 Cells

Chinese hamster lung cell line (CHL), Chinese hamster ovary cell (CHO) and Chinese hamster lung cell line (V79) have stable karyotypes and have accumulated a large amount of background data. They are routine in vitro micronucleus test cell lines. However, due to defects in the function of the p53 gene in rodent cells, the repair function after DNA damage is reduced, resulting in a relatively high false positive rate in in vitro micronucleus tests based on CHL, CHO and V79. In order to avoid false positive results, the human lymphocyte cell line (TK6) with intact p53 gene function can also be used in the in vitro micronucleus test.

The ability of cells to uptake nanomaterials is the key to examining whether nanomaterials are thoroughly exposed to the cell test system. Appropriate techniques should be used to verify that the cell line used has a certain ability to uptake the test sample (nanomaterials contained) under the conditions of this test.

NOTE: when selecting the cell line, nanomaterials with different properties and physicochemical characterization are recommended to provide evidence that the nanomaterials can be taken up by the cells. CHL and TK6 cells have been confirmed to have the ability to uptake silver nanoparticles (Ag40).

5 Preparation of Test Sample and Control

The preparation of test sample and control is as follows:

a) Nanomaterial medical devices: refer to YY/T 0870.6-2019, and in combination with the release characteristics and usage mode of nanomaterials in nanomaterial medical devices, prepare the test sample;

NOTE 1: it is recommended to simultaneously use nanomaterial raw materials for evaluation, and refer to 5 b) for preparation.

NOTE 2: in order to ensure that the state of nanomaterials in the biological and physical environment in the in vitro test system is comparable to the state during in vivo application, the existing methods can be used to characterize the nanomaterials in the cell culture medium before the test sample is exposed to the cells (leaching solution) and after the test sample is exposed to the cells. For details, refer to GB/T 39261 for characterization. The characterization data can be used in the interpretation of test results.

b) Nanomaterials used in medical devices, negative control materials and positive control materials: the concentration range of the nanomaterials used in the test is related to the cytotoxicity and uptake by cells. The appropriate medium shall be selected based on the characteristics of the nanomaterials and the biological environment, in which, they are applied, to ensure that the nanomaterials remain well dispersed and stable in the test solution;

c) The medium negative control uses the medium used to prepare the test sample. For commonly used positive reagent controls, see Table 1 in YY/T 0870.6-2019;

d) Blank cell control: cells for testing.

6 Micronucleus Test Using Cytokinesis Blocking Method

6.1 Overview

Under appropriate conditions, the micronucleus test using cytokinesis blocking method can detect the genotoxic risk of nanomaterials. Routine test procedures include pre-test, contact treatment, cell harvesting and preparation, and result observation, etc., as shown in YY/T 0870.6-2019.

6.2 Special Procedures for Nanomaterials

6.2.1 Preparation concentration

In accordance with the following requirements, set the preparation concentration.

a) At least 3 preparation concentrations shall be included, with at least 2 replicates for

each concentration.

b) The highest reaction concentration shall have no obvious precipitation in the culture medium and have no significant impact on the pH and osmotic pressure of the culture fluid.

c) In accordance with the cytotoxicity, set the range of preparation concentration. The cytotoxicity adopts cytokinesis-block proliferation index (CBPI) or replication index (RI) for evaluation.

d) If there is no obvious cytotoxicity in the original leaching solution or under the highest concentration that can be provided (compared with the blank cell control group, the relative cell viability is not lower than 70%), the concentration interval is usually set at 2 ~ 3 times; when cytotoxicity exists in the test sample, the test sample or original leaching solution shall be used as the highest concentration for gradient dilution. In other words, when harvesting cells, the cytotoxicity incidence rate at the highest concentration used for testing shall be controlled at (55 ± 5)%. Each treatment group is set at an interval of 5 ~ 10 times. The selected concentration range shall include non-cytotoxic concentrations to concentrations that generate moderate cytotoxicity, so as to obtain a better dose-effect relation. If the concentration effect curve is relatively steep, the concentration interval can be reduced and more than 3 concentrations can be used for testing.

6.2.2 Culture system

Various components in cell culture fluid can affect the ability of cells to uptake nanomaterials. For example, serum-containing culture fluid or human albumin can increase the size of nanoparticles, reduce their potential and change the ability of CHL and TK6 cells to uptake nanomaterials. Therefore, it is not recommended to use nanomaterials coated with human serum albumin for testing.

The addition of cytochalasin B (CytoB) in the micronucleus test using cytokinesis blocking method can interfere with the formation of the cytoskeleton, thereby affecting the uptake of nanomaterials by cells. The method of applying CytoB can adopt the method of post-treatment (after the cells are co-cultured with the test sample, replace the CytoB-containing culture liquid and continue to act for about 2 cell doubling times) or delayed co-treatment (after the cells are co-cultured with the test sample, replace the culture liquid simultaneously containing the test sample and CytoB and continue to act for about 2 cell doubling times), so as to ensure that the nanomaterials and cell culture system are thoroughly exposed without the application of CytoB. The dosage concentration of CytoB may take YY/T 0870.6-2019 as a reference.

6.2.3 Reaction time

The reaction time may have a certain impact on the detection rate of nanomaterial micronuclei. In order to ensure that the nanomaterials are thoroughly taken up by cells, it is necessary to set up a 3 h ~ 6 h treatment group (without and with metabolic activation), a 24-h treatment group (without metabolic activation) and a 48-h treatment group (without metabolic activation) to

2) The percentage of medium in the volume of the final cell culture fluid.

c) Cells:

1) Type, source and passage number of cells used;

2) Regular inspection results on mycoplasma contamination and cell doubling time;

3) Cell culture conditions and methods.

NOTE: if the test of the cells' ability to uptake nanomaterials is completed, corresponding data should be provided.

d) Test conditions:

1) Components of cell culture fluid;

2) Cell culture temperature, CO_2 volume fraction, humidity and culture time;

3) Cell density during inoculation / sample addition treatment;

4) Final concentration range of test sample / negative control / positive control in the test system;

5) Reaction time of test sample / negative control / positive control;

6) Whether a cytokinesis blocking agent is used, the reagent information, concentration, reaction time, and whether it is added simultaneously or respectively with the test sample;

7) Type and composition of metabolic activation system (S9 source, S9 mixture preparation method and final volume of S9 mixture, etc.);

8) Production method;

9) Staining method;

10) Cytotoxicity detection method and analysis of cell numbers;

11) Micronucleus-containing cell counting criteria and analysis of cell numbers;

12) Result judgment criteria.

e) Results:

1) Whether there are any abnormal conditions, for example, precipitation, in the test system;

2) Cytotoxicity test results, for example, CBPI or RI count results, etc.;

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