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WS

**INDUSTRY STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

WS/T 615-2018

**Estimation of radiation biological dose - analyzing premature
chromosome condensation ring
(English Translation)**

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Foreword

This standard was drafted in accordance with the rules given in GB/T 1.1-2009.

This standard was proposed by the National Health Standards Committee Radiological Health Standards Committee.

This standard was drafted. Radiation Protection and Nuclear Safety Medical Institute of

China Center for Disease Control and Prevention, Radiation Medical Research, Chinese Academy of Medical Sciences Institute, Suzhou University.

1 Scope

This standard specifies the method for estimating the biological dose of irradiated external exposure to ionizing radiation by analyzing the early maturing chromosome agglutination ring.

This standard applies to X or gamma-rays, acute whole body uniformity occurring within 1 month after exposure, absorbed doses ranging from 4 Gy to 20 Gy.

Or biological dose estimation of an approximately uniform externally illuminated person.

2 Normative references

The following documents are indispensable for the application of this document. For dated references, only the dated version applies to this document.

For undated references, the latest edition (including all amendments) applies to this document.

GB/T 28236-2011 chromosomal aberration estimation biological dose method

3 Terms and definitions

The following terms and definitions as defined in GB/T 28236-2011 apply to this document.

Premature chromosome condensation; PCC Using chemical or biological methods, the interphase lymphocyte nucleus is induced to enter the mitosis phase in advance, making the interphase lymphocyte nuclear extreme the chromatin in the scattered state is condensed into a fibrillar-like chromosome-like structure.

Premature chromosome condensation ring; PCC-R when the premature chromosome agglutination breaks, the two broken ends of one chromosome recombine to form a circular chromosome.

Dose-effect curve

There is a quantitative relationship between the response of a biological system after exposure and the dose of radiation, which can be described by appropriate mathematical models.

As described, a scale curve is prepared for the relationship between the corresponding reaction effect and the dose to be irradiated, and the dose to be used can be estimated.

4.1 Main instrument and reagent preparation

See Appendix A for the main instrument and reagent preparation.

4.2.1 Sample collection

Human venous blood was extracted from 1 mL to 2 mL, and heparin anticoagulation tube was injected.

4.2.2 Sample inoculation and cell culture

Add 0.5 mL of heparin anticoagulated whole blood to 4.5 mL of whole medium (RPMI 1640) containing 20% fetal bovine serum, set 2 for each sample.

A parallel sample, cultured in a constant temperature incubator at 37 ° C for 46 h to 47 h, and then added to the final concentration of 50 nmol/L of Calyx spp.

(Calyculin A) or Okadaic acid with a final concentration of 500 nmol/L, continue to culture for 1 h to 2 h.

4.2.3 Harvesting cells

Blow the culture with a pipette, transfer to a 15 mL graduated centrifuge tube, centrifuge at 250 g for 10 min in a horizontal centrifuge, discard the supernatant, leaving approximately 1 mL.

The remaining precipitate.

4.2.4 Low permeability

Add 75 ° C pre-warmed 0.075 mol/L potassium chloride (KCl) to 8 mL ~ 10 mL per centrifuge tube, pipette evenly with a pipette, set 37 ° C Low permeability 10 min ~ 30 min.

4.2.5 Pre-fixation

Add 1 mL to 2 mL of freshly prepared fixative [methanol/glacial acetic acid = 3/1 (volume ratio)] to each centrifuge tube, gently pipette and mix with a pipette.

Evenly, centrifuge at 250 g for 10 min, discard the supernatant.

4.2.6 Fixed

Add 8 mL of fixative solution to each centrifuge tube, mix gently by pipetting, fix at room temperature for 20 min, centrifuge at 250 g for 10 min, and discard the supernatant.

4.2.7 Second fix

Add 8 mL of fixative, mix gently by pipetting, fix at room temperature for 15 min, centrifuge at 250 g for 10 min, discard most of the supernatant, and leave the supernatant is measured.

4.2.8 Preparation of cell suspension

The remaining supernatant and the precipitate were mixed by pipetting to obtain a cell suspension.

4.2.9 Production

Drop 2 to 3 drops of the cells onto the pre-cooled slide, over-fire for 2 s to 3 s, and dry naturally at room temperature.

4.2.10 Dyeing

Diluted Giemsa dye solution (Giemsa) to 8% solution in phosphate buffer pH 6.8, stained for 8 min to 10 min, rinse with tap water Dry it out.

5.1 Optical microscopic reading

Under the low magnification of the optical microscope (x10 times), find the appropriate premature chromosome agglutination splitting phase; then in the oil mirror (x100 times) the premature chromosome agglutination and division phase is adjusted to the middle of the field of view, and the number of precocious chromosome agglutination rings is counted.

5.2 Judging criteria and counting criteria for early maturing chromosome agglutination

The precocious chromosome agglutination ring is divided into a hollow ring and a solid ring. The hollow ring is a hollow circular or irregular annular structure. Solid ring is present the solid spherical structure is slightly dense and has a diameter larger than the transverse diameter of the early-maturing agglutination dyeing monomer. The solid ring is not counted during analysis. Precocious chromosome condensation See Appendix B for an example of a ring.

The split phase in which the two sister chromatids are lined up is called the G2/M premature chromosome agglutination and splitting phase, and the ring term appears in this split phase.

As a G2/M precocious chromosome agglutination ring. Two similar ring structures in the G2/M phase precocious chromosome agglutination and splitting phase Count 1 premature chromosome agglutination ring. The split phase in which the two chromatids are clearly