26.Jan.87 JOE/SZA MMR42DIVA21

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V79 Flow Cytometry (not yet authorized preliminary title)

Objective:

to replace the current method for determination of the replication cycle of V79 cells

Current method:

the current method classifies the replication cycles into 3 different cycles according to the staining pattern of the chromosomes observed microscopically (see TABLE A)

REPLICATION CYCLE	STAINING PATTERN
.LE.1	both chromatids are darly stained
2	chromosomes are differentially stained (1 chromatid darkly, 1 chromatid lightly stained)
.GE.3	both chromatids are lightly stained

TABLE A

REPLICATION CYCLE

Advantages of the current method:

The current method uses the same slides used for SCE evaluation. There is no need for additional cultures for the exposure and processing.

Drawbacks of the current method:

The current method is slow and subjective. The classification of metaphases into replication cycle .LE.1 or .GE.3 is hamperred by variation in staining intensity. The proportion of metaphases with undefined replication cycle varies and can be as many as 90 percent.

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Proposed method:	The method of BrdU/Hoechst flow cytometry is proposed to replace the current method. The proposed method utilizes a flow cytometer to analyze a suspension of single cells or nuclei for the amount of BrdU incorporated into DNA. The ratio BrdU to DNA is measured directly using 2 different fluorescence staining.
Advantages of the proposed method:	The method is fast and objective. More cells can be analyzed to give a quantitative results concerning the different stages of the cell cycle
Drawbacks of the proposed method:	Additional cultures are needed for the exposure and processing for the analysis. The method has to be established.