In vitro mammalian chromosome aberration test

1. Objective

The purpose of the *in vitro* chromosomal aberration test is to identify agents that cause structural chromosome aberrations in cultured mammalian cells.

2. Introduction

Structural aberrations may be of two types, chromosome or chromatid. With the majority of chemical mutagens, induced aberrations are of the chromatid type, but chromosome type aberrations also occur. An increase in polyploidy may indicate that a chemical has the potential to induce numerical aberrations. However, this method is not designed to measure numerical aberrations and is not routinely used for that purpose. Chromosome mutations and related events are the cause of many human genetic diseases and there is substantial evidence that chromosome mutations and related events causing alterations in oncogenes and tumour suppressor genes of somatic cells are involved in cancer induction in humans and experimental animals.

This test is used to screen for possible mammalian mutagens and carcinogens. Many compounds that are positive in this test are mammalian carcinogens; however, there is not a perfect correlation between this test and carcinogenicity. Correlation is dependent on chemical class and there is increasing evidence that there are carcinogens that are not detected by this test because they appear to act through mechanisms other than direct DNA damage.

3. Method

$5x10^4$ cells are sowed out per chamber of a quadriperm plate.

After 16 – 24 h the media will be removed and replaced by the solutions of the test item, negative and positive controls. Exposure of test systems will be performed without and with metabolic activation using 3 h of incubation. Afterwards, incubation mixtures have to be removed and Complete Medium containing 24.2 µM BrdU stock solution will be put on each chamber and the quadriperm plates are further incubated for 15 h.

After incubation the metaphase arresting substance colcemid is added for further 3 h. Afterwards, medium will be removed and replaced by prewarmed hypotonic buffer. Incubation for 20 min. at 37°C. The hypotonic buffer will be removed to a level that the slides are just covered and then the hypotonic treatment will be stopped by adding fixative. After 20 min. at room temperature the fixative will be removed and replaced by fresh fixative. This step has to be repeated after 20 min. again. After fixation the slides are air dried.

4-10 days after chromosome preparation the slides have to be Giemsa stained.

4. Evaluation

At least 200 well-spread metaphases have to be scored per concentration and control. This number could be reduced when a high number of aberrations are observed.
Since fixation procedures often result in the breakage of a proportion of metaphase cells with loss of chromosomes, the cells scored should therefore contain a number of centromeres equal to the modal number 22 ± 2 for all cell types..
Though the purpose of the test is to detect structural chromosome aberrations, it is important to record polyploidy and endoreduplication when these events are seen.

5. Results

The experimental unit is the cell, and therefore the percentage of cells with structural chromosome aberration(s) have to be evaluated. Different types of structural chromosome aberrations are listed with their numbers and frequencies for experimental and control cultures. Gaps are recorded separately and reported but generally not included in the total aberration frequency.

There are several criteria for determining a positive result, such as a concentration-related increase or a reproducible increase in the number of cells with chromosome aberrations. Biological relevance of the results should be considered first. Statistical methods may be used as an aid in evaluating the test results.
An increase in the number of polyploid cells may indicate that the test substance has the potential to inhibit mitotic processes and to induce numerical chromosome aberrations. An increase in the number of cells with endoreduplicated chromosomes may indicate that the test substance has the potential to inhibit cell cycle progression.

A test substance for which the results do not meet the above criteria is considered non-mutagenic in this system.

There is no requirement for verification of a clear positive response. Equivocal results should be clarified by further testing preferably using modification of experimental conditions. Modification of study parameters to extend the range of conditions assessed should be considered in follow-up experiments. Study parameters that might be modified include the concentration spacing and the metabolic activation conditions.

Although most experiments will give clearly positive or negative results, in rare cases the data set will preclude making a definite judgement about the activity of the test substance. Results may remain equivocal or questionable regardless of the number of times the experiment is repeated.