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## The Application and Benefits of Comet Assay in Biomonitoring Studies

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### Abstract

This article evaluates the benefits, principle and importance of COMET ASSAY, the most common assay which can be applied in the field of genotoxicology and ecotoxicology. Considering the fact that today's urban living is highly influenced by the intense growth of many industries, one of the goals of the cytogenetic monitoring is to focus on the ability of various chemical compounds to change the structure of chromosomes and DNA. In this article we explain the practical and experimental aspects of COMET ASSAY as a test which is accepted by World Health Organisation (WHO) and specified in the list of standard short-term tests for genotoxicological screening of human, animal or plant cells. I hope, that this article will be a precious source of knowledge for scientists and researchers in the field of biomedicine and other science which include biomonitoring.

**Keywords:** comet assay; genotoxicology; ecotoxicology; biomonitoring; cells.

### 1. Introduction

Genotoxic substances or genotoxins that are present in various manufacturing processes mainly represent a direct hazard to the workers involved in the process, as well as to local population in these industrial areas. Agents that are produced during manufacturing processes and that eventually enter the composition of those products, represent a potential health risk for a larger population, which include the consumers of these products.

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Genotoxins are mutagens that can cause genotoxicity leading to the damage of chromosome or DNA structure thus causing mutation. The harmful effects of genotoxic agents for induction of mutations do not necessarily manifest in the organism that is exposed to them, sometimes not even in the first generation. Instead, these harmful effects can be manifested in the next generations.

## **2. Methods**

The genotoxicity tests can identify or detect if a drug or other substance have the potential to cause mutation and genotoxicity. For that reasons, COMET ASSAY as a test can help us to identifying the hazards in the early stage of drug development itself. Also, it helps in detecting the DNA damage and detects a broad variety of primary DNA lesions. DNA damage detected by the COMET ASSAY showed to be sensitive and effective in the evaluation of the impact of human exposure to environmental mutagens and carcinogens. Several studies found a positive association between high level of air pollutants and DNA damage detected by COMET ASSAY. Most of these studies, especially in human study used lymphocytes.

COMET ASSAY is a single-cell gel electrophoresis and it has been used for more than 30 years in genotoxicity testing, human biomonitoring and ecotoxicology. COMET ASSAY obviously use fluorescent microscopic technique to examine DNA damage and repair at individual cell level. As a single-cell gel electrophoresis was first carried out by Ostling and Johanson in 1984 [1] on the base of single cell damage quantification proposed by Rydberg and Johanson in 1978 [2]. Singh in 1988 [3] combines the simplicity of biochemical techniques for detecting DNA single strand breaks, alkali-labile sites and crosslinking with the single cell approach typical of cytogenetic assays.

COMET ASSAY is rapid and sensitive procedure for quantification of damage and repair of DNA molecules at the level of individual cells [4,5]. It can be applied to a wide variety of tissues or any special cell types. The assay, does not require a large number of cells per sample, is inexpensive, relatively easy to apply, and is widely used as a test for genotoxicity. Indeed, it is considered optimal for detection of genotoxic effects of various agents. COMET ASSAY is performed on individual cells in agarose gel [6]. Cells embedded in agarose on a microscope slide are lysed with detergent and high salt to form nucleoids containing supercoiled loops of DNA linked to the nuclear matrix. Nucleoids are subjected to electrophoresis, resulting in formation of structures which resemble comets. The former cell nucleus which does not migrate, and the broken fragments stretched in the electric field, form the "head" and the "tail" of the comet, respectively. Indeed, the method itself was named after the characteristic appearance of the nucleoids. The intensity of the comet tail relative to the head reflects the number of DNA breaks. Whereas the standard alkaline version of the comet assay is able to detect single and double strand breaks and alkali-labile sites. But in certain conditions, this assay can also be used in combination with lesion-specific enzymes in order to detect certain other classes of DNA damage. Several versions of the comet assay are in use in research laboratories, and there are also commercial kits. There are two forms of COMET ASSAY: the neutral method for the detection of DNA double-strand breaks, and the alkaline method, which detects DNA single-strand breaks and alkali-labile lesions [7]. COMET ASSAY has however the potential to be used as a tool in genotoxicity testing and regulatory submissions for new chemicals and mixtures [8-12].

Basal human cells are the second choice of cells used in COMET ASSAY, especially in biomonitoring of humans occupationally exposed to different agents. Alterations caused in basal cells' nuclei by genotoxic events can be observed three weeks later in exfoliated buccal cells. Considering that a high number of cancers have an epithelial origin, the COMET ASSAY is considered suitable to assess occupational exposure to potentially genotoxic substances, as volatile organic compounds [13].

The most common way to visualise the comets is by using a fluorescent dye such as ethidium bromide, DAPI, acridine orange, propidium iodide, etc. These dyes are easy to use, as they do not require special treatment of gel or time-consuming procedures, but the use of fluorescent microscope represent for less developed laboratories a serious limitation for the use of the assay. Silver stain offers the possibility to stain comets and visualise it using conventional microscope. The modifications of the silver staining protocol allow the use of the software CASP combined with conventional microscope to measure the most common parameters used in comet assay to express DNA damage [14-17]. It is particular important, the possibility to measure % of DNA in tail probably the most useful parameter in the COMET ASSAY. This procedure offers an opportunity for those working with the assay without fluorescent microscopy and should contribute to a more extensive use of the assay.

Giemsa staining is a suitable staining method for the COMET ASSAY. Imaging and quantification of Giemsa-stained DNA comets required only a conventional bright-field microscope equipped with a regular CCD camera and freely available software [15].

### **3. Discussion and Conclusion**

The researchers can find the trial provided free-of-charge strictly for non-commercial evaluation purposes only. <http://www.scorecomets.com/comet-scoring/get-comet-assay-iv/free-trial>. But, you must agree not to use the demonstration for collation of any data used as part of a research project or commercial test or study.

Unlike silver staining, another staining method suitable for bright-field microscopy, which is very sensitive to experimental conditions and may produce high non-specific signal, Giemsa staining is very simple and more specific to DNA. Giemsa stain for the COMET ASSAY, at no significant cost to sensitivity, will facilitate new research in biomedicine [15, 16].

The advantages of COMET ASSAY is confirmed in chemotherapy. Prediction of a chemotherapy effectiveness and development of hematological complications is important task for any oncologist. For that reason, COMET ASSAY allow evaluating DNA damage in individuals undergoing chemotherapy [17]. Also, with COMET ASSAY can be confirmed oxidative damage and rapid apoptosis of the cells.

### **4. Recommendations**

In this paper I apprise the theoretical and experimental aspects of commonly used test for assessment of genotoxicity in human cells. Also, COMET ASSAY as a basic tests for genotoxicity can be applied as tools for biomonitoring in human, animal and plant study. One of the most important advantages of the COMET ASSAY is that the DNA damage can be measured in any (nucleated) cell type. The COMET ASSAY is rapid, relatively

easy to perform and inexpensive, it can detect a broad variety of primary DNA lesions which cannot be identified by other tests. It is sensitive to very low levels of DNA damage, and requires a small amount of cells per sample.

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