

COMET ASSAY- A Sensitive Genotoxicity Test

Who And When?

In the Year 1984, Ostling O. and Johansson K.J. developed the Single Cell Gel electrophoresis (SCGE) Assay. Their research paper entitled "Microelectrophoretic study of radiation induced DNA damages in individual mammalian cells" was published in Biochemical Biophysical research communications. Singh and his coworkers, in the year 1988, published the modified alkaline version of SCGE assay, also called comet assay in the research article entitled "A simple technique for quantitation of low levels of DNA damage in individual cells" in the journal Experimental Cell Research.

What?

The Concept - Comet Assay is a simple yet sensitive method for measuring genotoxicity in terms of breaks in the DNA strands in cells. This is the only in-vitro technique to measure DNA damage in individual cells. This assay is capable of detecting and quantifying a wide range of DNA damage including single stranded breaks in DNA backbone, alkali labile sites, DNA-DNA cross linking, DNA-Protein cross linking, etc. It is a vital tool in the studies of mutagenicity and carcinogenicity.

How?

The methodology of comet assay is relatively simple and can be carried out within few hours. In this microgel electrophoresis, the cells to be analysed for DNA damage are embedded in low melting agarose gel on a slide. Low melting point agarose is specifically used for the encapsulation of the cells because it melts at 37°C. The slides are dipped in an alkaline lysis solution containing high concentration of salt and a detergent. This breaks the cell membrane and nuclear membrane of the cells so as to expose the contents of the cell to the agarose matrix. The DNA of the cell remains as a nucleoid with supercoiled loops of DNA in the agarose. After treatment of the slide with the lytic solution, electrophoresis is carried out after a brief incubation of the slides in the electrophoretic solution (buffer). Comet like structures are formed where the intact undamaged DNA forms the head and the broken DNA strands forms the tail. The length of the comet tail is directly proportional to the number of DNA breaks. This is followed by neutralization of the slides to pH 7. The final step in the assay is staining of DNA with a fluorescent stain and slide analysis using image software.

The instrumentation required for the comet assay includes a good electrophoretic set up with the power pack, a microscope and computer loaded with image analysis software. Comet Assay IV is an accurate analytical software which scores the comets from live video with high reproducibility

Why? Comet assay is a very simple yet powerful tool with high sensitivity, used in genetic toxicology, human bio monitoring, exogenotoxicology, mechanistic studies, molecular epidemiology, etc. It is a very vital analytical tool which is economical in terms of both money and time.