

Comet Assay

Like the name of the topic, the method of this assay engages ones attention too. Being linked to an analytical stream of science, this topic intrigued me as it has a lot to do with quantitation, it also tests lab skills, knowledge about instrumentation and quality work. It also includes application of principles that require critical thinking and basic understanding of concepts. Comet assay is a method to test genotoxicity. It is a very sensitive methodology. It detects the extent to which the DNA is damaged. This procedure adheres to the concept that DNA maintains a very organized association with the proteins present in the nucleus matrix. This well organized association is altered when the DNA is damaged. This damage is measured and assessed. Why 'comet'? The answer lies further when we explore the end of the procedure.

The procedure includes three main steps namely; encapsulation, lysis and electrophoresis. The cells to be analysed are suspended in a matrix on a slide. This matrix is made up of agarose which is a jelly like substance. This matrix constitutes of carbohydrate fibres in which the cells get enmeshed. In other words the cells get encapsulated, hence the term encapsulation for this step. The next step involves lysis of the cells in which the cells are destroyed so that the contents of the cells spill out and get embedded in the matrix. This is done by drenching the slide of cells in a lysing solution which includes a highly concentrated salt and a detergent. The salt disturbs the bonding pattern of the proteins in the cells and also the structure of the RNA. The detergent causes the cell membrane to break after which the contents are out in the agarose matrix. The DNA too disentangles and fills the cavity of the matrix. The slide is cleaned with distilled water so that the salts are washed off. After washing it off the slide is dipped in an electrophoresis solution. This causes the DNA to become denatured i.e. the double helix becomes single stranded.

An electric potential is applied to the solution because of which the negatively charged DNA gets attracted to the positively charged anode. However only the smaller DNA fragments which are broken and separated from the bigger strand moves out of the cavity of the matrix, leaving the larger fragments behind. The slide is stained with a DNA specific fluorescent stain and observed under a microscope. The microscope is in turn attached to a charged coupled device which resembles a digital camera. This device is connected to a computer having image analyses software. These devices constitute the instruments required for comet assay.

The image analyses software measures the intensity of the fluorescence of the entire DNA and of the DNA that has migrated to the anode. The two intensities are then compared which gives information of how much DNA has left the cavity thus giving an estimation of the amount of damage. The image resembles a comet where the head is the undamaged DNA and the tail resembles the damaged DNA which has migrated away from the cavity where the larger DNA is located. Hence the name 'comet'. As comet assay is a very sensitive technique, the alkalinity of the solutions, the pH of the solution and the temperatures have to be maintained at an appropriate level. This technique has its application in detecting sperm cell DNA damage, in the tests for cancer, etc. However attempts are made to develop this technique by bringing about variations in the methodologies. This technique is a boon in the field of research and science.