

The Low Molecular Weight (LMW) DNA Diffusion Assay

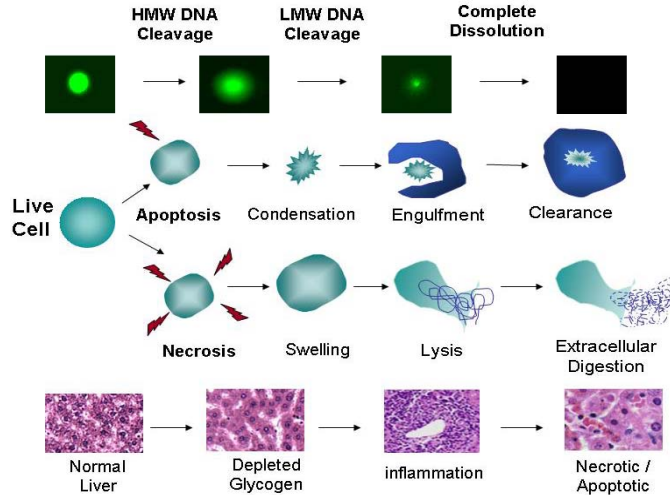
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The *in vivo* comet assay can be highly sensitive to the effects of single cell cytotoxicity that are mostly undetectable by histopathology. Low molecular weight (LMW) DNA fragments produced by the cleavage of high molecular weight (HMW) DNA in the initial or early stages of cell death can contribute to increases in DNA migration. However as cell death progresses, DNA migration can be decreased as the progressively smaller DNA fragments and even all evidence of the cells' existence are lost to phagocytosis, extracellular digestion, and/or advanced diffusion of the fragments during lysis and electrophoresis. The LMW DNA diffusion assay (diffusion assay) is a sensitive and single-cell measurement of this cytotoxicity-induced DNA fragmentation that can influence the interpretation of comet assay results.

Easily incorporated into the comet assay, the diffusion assay is conducted by preparing an extra and identical replicate comet slide that is removed from lysis after only 1 hour and fixed without electrophoresis. After fixation, the LMW slides are stained with SYBR® Gold (Molecular Probes) and 100 cells per slide are scored visually for the percentage of diffused versus condensed cells. After only 1 hour of lysis and in the absence of electrophoresis, the nuclear DNA of live cells will appear mostly condensed under microscopic evaluation. But cells with extensive DNA degradation caused by endonuclease activity during apoptosis or necrosis can quickly exhibit a progressively diffuse pattern as increasing amounts of low molecular weight DNA diffuse through the agarose matrix and away from the nucleus.

Note: Using slow-fading, high signal/background stains like SYBR® Gold and an immediate first assessment scoring technique are the best methods for ensuring consistent and accurate diffusion scores. The use of low signal/high background (e.g. ethidium bromide) or quickly fading (e.g. SYBR® GREEN) stains and/or a slow deliberate scoring technique can mask or introduce diffusion during scoring contributing to inconsistent scores.

Although there are overlaps and variations in cellular events and the order in which they can occur in different tissues and cells, the general trend in LMW DNA diffusion in single cells and the potential corresponding whole tissue histopathological findings s are depicted below.



Before cellular swelling and/or condensation are visible by gross examination or light microscopy of a tissue, the earliest detectable measurements of cell death can include an increase in the percentage of individual cells with LMW DNA diffusion and/or evidence of depleted glycogen (e.g. decreased cytoplasmic pallor) in tissue sections. With the lysis of the necrotic cell membranes, more advanced LMW DNA diffusion and/or cytoplasmic eosinophilia (i.e. inflammation) in the tissue may be detected. However, the stage at which tissue necrosis/apoptosis and/or post-necrotic effects (e.g. compensatory hyperplasia/hypertrophy) can be detected by histopathology is too advanced to be detected by the LMW DNA diffusion assay OR the comet assay as complete digestion and clearance of the individual dead and heavily damaged cells has already occurred.

Unlike viability assays such as those that measure ATP levels, membrane permeability, or metabolic competency, the LMW diffusion assay does not provide a definitive measurement by which "acceptable limits" (e.g. >70%) may be established. And unlike histopathology evaluation that is limited to detecting whole tissue effects, the LMW DNA diffusion assay also does not provide a definitive marker for cell death by which the exclusion of certain data may be justified. Nor does it distinguish between apoptosis and necrosis. But rather, it provides single cell data about the pre-processed condition of the nuclear DNA with which detected DNA migration patterns in the comet assay may be interpreted and/or qualified as demonstrated below.

