Obesity and insulin resistance are on the rise and are a leading cause of a variety of illnesses, including Non-Alcoholic Fatty Liver Disease (NAFLD) and Non-Alcoholic Steatotic Hepatitis (NASH), which can further lead to the development of liver cancer. A histological assessment of a liver biopsy is considered as the gold standard for classification of liver steatosis (mild, moderate, or severe) (Figure 1). However, the observations are made visually and therefore, are prone to observer bias and inter-observer variation. These classifications are used to determine suitability of donor livers for transplantation and to determine stage of NAFLD and NASH.

The aim of the project was to develop a simple quantitative test to measure the fat content in isolated hepatocytes.

Nile Red was used to stain lipids in human hepatocyte batches (14 steatotic and 8 non-steatotic). Cryopreserved human hepatocytes were used. Cells were thawed, filtered, centrifuged, resuspended and counted. 10,000 cells were placed per well in a black 96-well plate and 10 µg/l diluted Nile red solution was added. Plates were incubated (37°C, 10 min), then read using a fluorescence plate reader. Data were recorded and compared to the reported fat content from histology findings.

Cytospins of 10,000 cells per slide were allowed and the slides were then air-dried for 24h. They were stained with Nile Red (humidity chamber at 37°C, 10 min), cell nuclei were counterstained with DAPI, and slides were visualised under a fluorescence microscope. Images were taken. [In both techniques, unstained cells were used as negative controls.]

No reliable technique is available for grading steatotic hepatocytes as transplantation requires the use of “good” quality cells. Our study shows that Nile Red staining was an easy, quick, and reliable technique for grading steatotic hepatocytes isolated from a liver biopsy of a patient with NAFLD or NASH, or from a donor liver to be used in transplantation. These results can be combined with a FibroScan to give a useful quantitative measure of steatosis and fibrosis in a sample of hepatocytes.

References: