Development and Optimization of Quantitative Image Analysis for Hepatocyte BrdU Labeling Indices Using NIS-Elements Software

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Introduction: NIS-Elements imaging software was used to determine hepatocyte BrdU (cell proliferation marker) labeling indices, establish optimal exclusion parameters of images previously evaluated using Image-Pro Plus software, and compare quantitative results between automated (NIS-Elements) and manual BrdU+ nuclei. **Experimental Design:** Images (6 images/animal) were collected from the left lateral liver lobe sections from twelve rats [Crl:CD (SD)] (6 males/6 females; 9-10 weeks) that received 0.8 mg/mL of BrdU in drinking water for 7 days prior to necropsy. Samples were fixed in 10% neutral buffered formalin, processed, sectioned and stained (anti-BrdU and Blue Feulgen/NOVA Red). **Methods:** NIS-Elements inclusion and exclusion criteria were developed to derive BrdU positive and negative hepatocyte nuclei object counts. Various features of NIS-Elements were tested to optimize contrast methods and selected features were applied to each image. Standard operating procedures were developed and BrdU labeling indices were calculated. Manual BrdU+ counts served as a positive control for NIS-Elements object count performance. Statistical measures to evaluate NIS-Elements software were derived. **Results:** NIS-Elements mean total, BrdU+, and manual hepatocyte nuclear counts were similar in both sexes. BrdU labeling indices of Image-Pro Plus and NIS-Elements software showed a positive correlation. Statistical measures in both sexes were unequivocal and statistically significant. **Conclusion:** NIS-Elements is a highly efficient, sensitive, and specific software program capable of determining hepatocyte BrdU labeling indices in images previously evaluated with Image-Pro Plus with marked reproducibility. **Impact statement:** Based upon our evaluation, NIS-Elements imaging software can be used to quantify rodent hepatotoxicity by evaluating BrdU labeling indices.