Introduction: The single cell gel electrophoresis (aka comet assay), is commonly used in the regulatory battery of genotoxicity testing for different industries. Therefore it is important to determine which parameters can influence the variability of the results and therefore can potentially impact the conclusion.

Objective: Due to the voltage gradient, slides positioned in different sections of the electrophoresis chamber could be subjected to different voltages. The present study was performed to evaluate how the position of the slide in the chamber impacted the median % tail intensity (% Tail DNA).

Material and Method: Slides were prepared using lung and duodenum cells from male Sprague Dawley rats. The negative control animals were untreated or dosed with water. The positive control animals were dosed with ethylmethanesulfonate (EMS). The route of administration was oral gavage. The electrophoresis chamber was divided in two sides (left, right) and three sections (top, middle and bottom). Slides of the negative and positive control animal were placed in each side or section of the chamber. The average % Tail DNA was determined for each side and the average % Tail DNA of all slides (total) for each animal was used as a standard for comparison between sections. To simulate the variability of a typical study design, permutations of the sides and sections were used to determine the minimum (min) and maximum (max) values, and the average deviation of the min and max values to the standard.

Results: A substantial difference was observed in the average % Tail DNA for the negative control slides positioned in different sections/sides of electrophoresis chamber. The percent deviation to the standard was between -34.9% and 30.5% for the lung and between -35.8% and 21.7% for the duodenum. For the positive control animal, the deviation to the standard was within ±10% for all areas for the lung and within ±3% for the duodenum. To determine if distributing the slides of a group through the chamber would suffice to minimize deviations, permutations with mixed regions were performed. The mixed groups of 3 slides consisted of one slide from each section and from one opposing side. The average deviation of the group to the standard was 1.7% (lung) and 2.1% (duodenum) for the negative control, and -3.6% (lung) and -1.5% (duodenum) for the positive control.

Conclusion: The variability of the results between positive control slides was sufficiently low to not impact the determination of a positive result. However, the variability of the negative control results (±35%) may deteriorate the confidence of negative or equivocal responses. Ensuring the heterogeneous distributions of slides in the electrophoresis chamber from the same treatment group was sufficient to reduce the deviation to acceptable levels and ensure high confidence in the determination of negative responses.

BIBLIOGRAPHY


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