Optimizing Urine derived Cells staining for the Human Micronucleus assay

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Background

- The formation of micronucleus has been widely used in toxicology as a biomarker of chromosomal damage, genomic instability and carcinogenic events.
- This biomarker is commonly used in epidemiological studies since it can predict certain diseases that may be caused either by lifestyle or genetic and environmental factors.
- Most cancers are of epithelial origin and therefore micronucleus test (MN) in urine derived cells (UDC) is of great importance. These cells are collected via a minimally invasive procedure, an important advantage for human biomonitoring studies.
- Giemsa and Feulgen are the most commonly used stains on MN.
- Giemsa staining allows a quick preparation of slides, however it may favour false positive readings.
- Feulgen staining is more time-consuming but is DNA specific and allows a good contrast between nucleus and cytoplasm.
- The main problem regarding UDC is the lack of standardization of MN protocol in particular the staining. Current data show a variety of methods which may lead to bias.
- The use of different stains and protocols may contribute to the large inter-laboratory variations and inconsistencies found in studies.

Aims

→ Apply different staining techniques in UDC from human samples;
→ Establish a detailed set of criteria for scoring all of the biomarkers in UDC.

Methods

- Urine samples will be collected from a group of individuals;
- Cells will be isolated and fixed;
- Giemsa staining and Feulgen staining technique will be performed for each sample;
- Reading will be performed using both light and fluorescence microscopy.

Expected Results

→ Standardize the application of the MN assay in UDC;
→ Select the most reliable method to stain UDC;
→ Characterize the cell types and nuclear anomalies in UDC;
→ Establish criteria for scoring.

Acknowledgments

This work was supported by Project NORTE-01-0145-FEDER-00010 - Health, Comfort and Energy in the Built Environment (HEBE), cofinanced by Programa Operacional Regional do Norte (NORTE2020), through Fundo Europeu de Desenvolvimentos Regionais (FEDER).