CA 15129, DiMoPEx
Diagnosis, **Mo**nitoring, **P**revention of **Ex**posure
Related Non-Communicable Diseases

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WG5

Optimizing Urine derived Cells staining for the

Human Micronucleus assay

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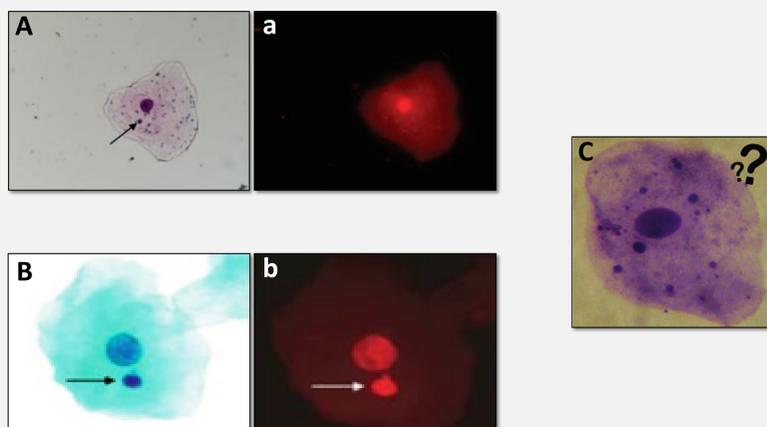Cooperation with: Claudia Bolognesi (WG 5)

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 a 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.

(**A-**) Epithelial cell stained with Giemsa (**a-**) epithelial cell stained with Feulgen (Nersesyan et al., 2006, *Cancer Ep Biom Prev* 15(10):1835-40); (**B**) and (**b-**) epithelial cells stained with Light Green and Feulgen (Thomas et al., 2011, *Methods Mol Biol* 682:235-48); **C-** Cell stained with Giemsa containing fragmented micronucleus (Khlifi et al., 2013, *Biomed Rest Int*, 2013;2013:905252).

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.

Urine sample   Washing, isolation and UDC fixation   Staining   Counting

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