

COMMENTARY

Harmonisation of the micronucleus assay in human buccal cells—a Human Micronucleus (HUMN) project (www.humn.org) initiative commencing in 2007

Michael Fenech^{1,*}, Claudia Bolognesi²,
Micheline Kirsch-Volders³, Stefano Bonassi⁴,
Errol Zeiger⁵, Siegfried Knasmüller⁶ and Nina Holland⁷

¹CSIRO Human Nutrition, Adelaide, Australia, ²Unit of Environmental Carcinogenesis, National Cancer Research Institute, Genoa, Italy, ³Laboratory for Cell Genetics, Vrije Universiteit Brussel, Brussel, Belgium, ⁴Unit of Molecular Epidemiology, National Cancer Research Institute, Genoa, Italy, ⁵Errol Zeiger Consulting, Chapel Hill, NC, USA, ⁶Institute of Cancer Research, Medical University, Vienna, Austria and ⁷School of Public Health, University of California, Berkeley, CA, USA

The buccal cell micronucleus (MN) assay, first proposed by Stich *et al.* (1,2), is useful as a biomarker of genetic damage caused by life-style habits (e.g. consumption of tobacco products and alcohol, micronutrient deficiency), exposures to environmental pollutants (e.g. pesticides, arsenic, formaldehyde), medical procedures (e.g. radio- and/or chemotherapy), as well as, inherited genetic defects in DNA repair (1–18).

The non-invasive nature of this technique makes it an attractive candidate for the bio-monitoring of human populations or individuals. However, the increased use of the MN assay in buccal cells, has led to a wide diversity in techniques, timing for cell collection relative to exposure period, cell and nuclear staining procedures, numbers and types of cells analysed, and scoring criteria for micronuclei (MNi) and other nuclear anomalies (e.g. karyorrhexis, condensed chromatin, pycnosis, karyolysis, ‘broken egg’ or nuclear buds; 4,5). Additionally, a lack of knowledge regarding the biological and biochemical bases of these various nuclear anomalies often seen in buccal cells may lead to confusion in distinguishing normal homeostatic responses from pathological effects at the genome or chromosome level. This confusion is compounded by the use of various staining methods that may or may not be DNA-specific and may or may not lead to the staining of structures that resemble MNi (e.g. keratohyalin granules, clumps of bacteria), and could lead to inclusion of false positive MNi in the MN frequency value (19). Furthermore, the issue of whether MNi should be scored solely in the basal layer of buccal epithelia, or in terminally differentiated exfoliated cells, and whether the scoring should be limited to cells with an intact ‘normal’ nucleus and/or ‘degenerated’ cells (i.e. differentiated cells showing other nuclear anomalies) remains unresolved. The number of scored cells required to determine statistically meaningful changes in MN frequency also needs to be carefully examined (20,21). These uncertainties in procedure and interpretation may potentially hinder the wider use of this method in bio-monitoring and preclude efforts to perform meta-analysis of data collected from different laboratories.

Consequently, in an attempt to resolve the important outstanding issues outlined above, the HUMN project (www.humn.org) has decided to focus its attention on the human buccal MN assay. The strategy will be similar to that developed

for the cytokinesis-block MN assay in lymphocytes (22–24) and has been planned to occur in the following stages:

- (i) A review of the buccal MN assay (to be completed in 2007) aimed at identifying: (a) the most important gaps of knowledge regarding the biology and biochemistry underlying the expression of MNi and other nuclear anomalies in buccal epithelial cells, and (b) the key technical issues that need to be resolved to ensure harmonious application of this method world-wide and thus allow reliable comparison of data among laboratories and populations.
- (ii) A HUMN project workshop on the buccal MN assay will be held at the Fifth International Conference on Environmental Mutagenesis in Human Populations, in Antalya, Turkey in May 2007 (www.environmentmutagen2007.org) to discuss the current state of knowledge regarding the buccal MN assay and to decide on an appropriate plan of action to resolve the key methodological issues.

It is the intention of the HUMN project to eventually provide a clear directive on the most appropriate procedure for preparation of slides and visual scoring of buccal cells for MNi and other nuclear anomalies. In the interim, until an appropriate validation of the various alternative procedures is performed, it is prudent to suggest that Feulgen/Fast green staining and the scoring criteria of Tolbert *et al.* (4,5) be adopted as the basic method. Other staining methods and scoring criteria should be validated against these techniques, which are the most widely used to date. Expressions of interest for participation in this HUMN project workshop on the buccal MN assay should be sent by email to Dr Michael Fenech (michael.fenech@csiro.au).

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*To whom correspondence should be addressed. Email: michael.fenech@csiro.au

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