Krishnaja. 24·3·08.

HUMN XL

International Collaborative Project on the Micronucleus Frequency in Human Exfoliated Buccal Cells

Laboratory n. (to be filled in by the HUMN$_{XL}$ committee).....

Name of the corresponding scientist: Dr. Mrs. Krishnaja, A.P.

Institution: .....

Department: .....

Address: .....

Tel: .....Fax: .....

Web SIte: .....

E-mail Address .....

GENERAL INFORMATION ABOUT YOUR LABORATORY:

1. What is your experience with the micronucleus assay in exfoliated buccal cells?
   - I never performed it ☐
   - I apply it routinely ☐
   - I have just started using it ☐
   - I would like to use it in the future ☐

2. In which type(s) of cells have you applied the MN assay ?
   - Buccal ☑
   - Urothelial ☑
   - Nasal ☐
   - Cervical smears ☐
   - Lymphocytes ☑
   - Other ☐ *Specify* .....

INFORMATION ABOUT LABORATORY METHODS:

1. **Cell collection**

- by wooden tongue-depressor ☑
- by metal spatula ☐
- by cytobrush moistened with water ☐
- by cytobrush moistened with buffer to swab ☐
- by toothpicks ☐
- by toothbrushes ☐
- by other methods (identify)..... .....

  - In which part of the mouth were cells collected?
    - Right cheek ☐
    - Left Cheek ☐
    - Both cheeks ☑
    - Sublingual (below tongue) area ☐
    - Tongue ☐

  - Were specific procedures (e.g. vigoruous or light scraping; repeated collection three times within 10 minutes; etc...) included in the sample collection protocol ?

    yes ☑ no ☐

If "yes" describe briefly the exact procedure used for cell collection:

.....

.....

- How many samples did you collect from an individual on a single day ?
  ..... one .....

- Do you collect separate samples from different areas of the mouth (i.e. left and right cheeks) ?

2. **Slide preparation**

- Was the instrument used to collect buccal cells shaken in a (container)centrifuge tube filled with collection buffer solution?

  yes ☑ no ☐

- If yes describe the collection buffer solution used.....

- Was the cell suspension centrifuged and washed ?

  yes ☑ no ☐

- If yes describe the washing solution used .....

- How were cells transferred to slides?
  1. directly ☐
  2. by cytocentrifugation ☐
  3. by dropping cell suspension on slides ☐
  4. other method ☐
     describe.....

- Were cells fixed before or after transferring to slides?
  Before ☐ After ☐

- What was the fixative used?.....

3. **Staining**

Which of the following staining methods was used:

- Feulgen-Fast Green (FFG) ☐
- DAPI ☐
- May-Grunwald Giemsa ☐
- Acridine orange ☐
- Acetorceine ☐
- Wrights stain ☐
- Hoechst ☐
- other ☐
  describe.....

Did you use FISH with pan-centromeric probes to assess origin of micronuclei in some of your studies?

yes ☐ no ☑

If yes what is the size of the study/studies in terms of numbers of subjects studied with this approach?.....

Did you use FISH with chromosome-specific centromeric probes to assess aneuploidy in nuclei and/or chromosome specific loss in micronuclei in some of your studies?

yes ☐ no ☑

If yes what is the size of the study/studies in terms of numbers of subjects studied with this approach?.....

C.	Dietary habit	yes____% no_____%
	Alcohol consumption ✓	yes____% no_____%
	Tea consumption	yes____% no_____%
	Coffee consumption	yes____% no_____%
	Fruit and vegetable consumption	yes____% no_____%
	Vitamin supplement intake	yes____% no_____%
	Body Mass Index	yes____% no_____%
D.	Genetic polymorphisms	yes____% no_____%
	Genomic/Proteomic data	yes____% no_____%
	Epigenetic data	yes____% no_____%
	Other biomarker data (CA, comet, adducts etc)	yes____% no_____%
E.	Frequency of basal cells	yes____% no_____%
	Frequency of basal cells with micronuclei	yes____% no_____%
	Frequency of normal differentiated cells	yes____% no_____%
	Frequency of differentiated cells with micronuclei	yes____% no_____%
	Frequency of any cell with micronuclei	yes____% no_____%
	Frequency of "broken egg" cells (nuclear bud cells)	yes____% no_____%
	Frequency of binucleated cells	yes____% no_____%
	Frequency of karyorrhectic cells	yes____% no_____%
	Frequency of fragmented nucleus cells	yes____% no_____%
	Frequency of condensed chromatin cells	yes____% no_____%
	Frequency of pyknotic cells	yes____% no_____%
	Frequency of karyolytic cells	yes____% no_____%
	Degenerated Cells Index*	yes____% no_____%
	Number of cells scored	.....
	Date when assay was performed	.....
	Date when sample was collected	.....

* frequency of all cells undergoing cell death including karyorrhexis, condensed chromatin, pyknosis, karyolysis.

5. Have you published data in peer reviewed journals?

   yes ☐ no ☐

6. If yes, please provide a list of publications:

3. Do you have data on MN frequency in buccal cells and lymphocytes in the same subjects?

Yes ☐ ✓ no ☐

4. Give an estimate of the number of subjects that you have examined using the micronucleus assay in exfoliated buccal cells during the specified years:

Before 1987_____	1994_____	2001_____
1988_____	1995_____	2002_____
1989_____	1996_____	2003_____
1990_____	1997_____	2004_____
1991_____	1998_____	2005_____
1992_____	1999_____	2006_____
1993_____	2000_____	2007_____

5. Please indicate whether your data base includes the following information.
If possible please also enter the proportion of the database for which this information is available by writing the percentage figure next to each response if the answer is yes:

A. Name/Surname of subjects	yes____% no____%
Date of birth	yes____% no____%
Age	yes____% no____%
Address	yes____% no____%
Gender	yes____% no____%
Ethnicity	yes____% no____%
B. Family history of cancer	yes____% no____%
Family history of neurodegenerative disease	yes____% no____%
Smoking status	yes____% no____%
Environmental tobacco smoke	yes____% no____%
Quantitative info. on cigarette consumption	yes____% no____%
Occupation	yes____% no____%
Occupational exposure to genotoxins	yes____% no____%
Previous or current disease states	yes____% no____%
Chronic conditions or diseases	yes____% no____%
Medical treatments/ drugs	yes____% no____%
Recreational drug use	yes____% no____%

4. Scoring Criteria

Which of the following criteria for identifying cells for inclusion into the MN frequency do you use *(see the references at the end of questionnaire)*?

- Basic (score MN in any cell only) ☐
- Countryman 1976 ☐
- Stich & Rosin 1983 ☐
- Sarto et al 1987 ☐
- Tolbert 1991 ☐
- Livingston 1990 ☐
- Titenko-Holland 1994 ☐
- Other ☐

Describe .....
.....

Which of the following cells do you score for micronuclei? *Please refer to figures 1 and 2 as a guide when answering this question*:

- Basal cells only (i.e. cells forming basal layer) ☐
- Normal Differentiated cells only (i.e. mainly cells from prickle cell layer and keratinised layer; i.e. not basal cells) ☐
- Basal cells and Normal Differentiated cells ☐
- All cell types including dead-dying cells (e.g. condensed chromatin, karyorrhectic, pyknotic, karyolitic cells) ☐

Describe any other criteria you use to select mononuclear cells for scoring micronuclei:
.....
.....
.....

5. Data collection from slide scoring (please refer to figures 1 and 2 as a guide when answering this question)

Which of the following information do you collect from the slides:

- frequency of basal cells ☐
- frequency of basal cells with micronuclei ☐
- frequency of micronuclei in basal cells ☐
- frequency of normal differentiated cells ☐
- frequency of normal differentiated cells with micronuclei ☐
- frequency of micronuclei in normal differentiated cells ☐
- frequency of cells with nuclear buds (broken eggs) ☐
- frequency of binucleated cells ☐
- frequency of karyorrhectic cells ☐
- frequency of fragmented nucleus cells ☐

6. **Design**

- How many slides per individual do you typically set up
  - one slide ☐
  - two slides ☐
  - three slides ☐
  - More than 3 ☐

- How many cells from each slide do you score to obtain the micronucleus frequency and what is a total number of cells scored per subject?

  .....

- Are the slides for each subject scored by:
  - one person only ? ☐
  - two persons? ☐
  - three persons? ☐

7. **If you have any additional comments please write them in the space below:**

- frequency of condensed chromatin cells ☐
- frequency of pyknotic cells ☐
- frequency of karyolytic cells ☐

Features of Healthy Oral Mucosa

The oral epithelium is a stratified squamous epithelium. It consists of four layers:

- The keratinised layer at the surface
  The prickle cell layer (or stratum spinosum)
  The basal layer (or stratum basale)
  Rete pegs
  Lamina propria (connective tissue)

Figure 1. Different cell layers in healthy oral mucosa

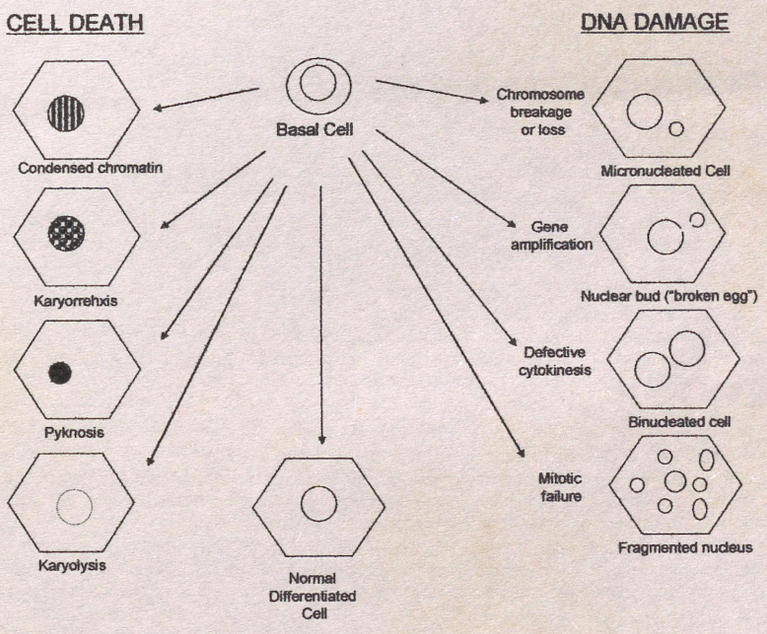

Figure 2. Schematic Diagram of various cell types in the buccal micronucleus assay. Important note: cells with a fragmented nucleus are also sometimes classified as karyorrhexis cells.

References to Scoring Criteria

- Countryman PI, Heddle JA The production of micronuclei from chromosome aberrations in irradiated cultures of human lymphocytes .Mutat Res. **1976** Dec;41(2-3):321-32.
- H.F. Stich, R.H. San and M.P. Rosin Adaptation of the DNA-repair and micronucleus tests to human cell suspensions and exfoliated cells, Ann N Y Acad Sci 407 (**1983**) 93-105.
- F. Stich and M.P. Rosin Quantitating the synergistic effect of smoking and alcohol consumption with the micronucleus test on human buccal mucosa cells, Int J Cancer 31 (**1983**) 305-308.
- F. Sarto, S. Finotto, L. Giacomelli, D. Mazzotti, R. Tomanin and A.G. Levis The micronucleus assay in exfoliated cells of the human buccal mucosa, Mutagenesis 2 (**1987**) 11-17.
- G.K. Livingston, R.N. Reed, B.L. Olson and J.E. Lockey Induction of nuclear aberrations by smokeless tobacco in epithelial cells of human oral mucosa, Environ Mol Mutagen 15 (**1990**) 136-144
- P.E. Tolbert, C.M. Shy and J.W. Allen Micronuclei and other nuclear anomalies in buccal smears: a field test in snuff users, Am J Epidemiol 134 (**1991**) 840-850.
- Titenko-Holland, L.E. Moore and M.T. Smith Measurement and characterization of micronuclei in exfoliated human cells by fluorescence in situ hybridization with a centromeric probe, Mutat Res 312 (**1994**) 39-50.