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LETTER TO EDITOR

(MAY 29, 2012)

RESPONSE TO THE LETTER OF DR. C. CASSINI ET AL.
Cassini C, Calloni C, Bortolini G, Garcia SC, Dornelles MA, Henriques JAP, et al. *Letter to Editor*. Int J Occup Med Environ Health 2012;25(3):314–5

I acknowledge the response of Dr. Cassini and co-authors which is good for finding the truth in the results of scientific investigations.

They wrote that "We agree that to get robust results in MN (both in lymphocytes and exfoliated buccal mucosa cells), 2000 cells should be scored in a DNA-specific stain protocol. However, there are various works in the literature that used Giemsa stain for buccal cells and that counted 1000 cells for the micronucleus frequency analysis".

This argument of the authors is completely wrong. If somebody applied incorrect approach, one should not repeat it! As can be noted, all the mentioned papers were published after the appearance of our paper in which we analyzed the impact of stain on the outcome in the buccal cells MN assay and the modern protocol of this assay (articles indicated as numbers 12 and 13 in my Letter). In the recent paper of Bonassi et al. [1], a database of 5424 subjects with buccal MN values obtained from 30 laboratories worldwide was compiled and analyzed to investigate the influence of several conditions affecting MN frequency. The authors of this review paper stated that "The significant increase in MN observed with Giemsa staining is probably the most interesting finding concerning the role of methodological parameters on MN frequency […] Given the lack of DNA specificity of the Giemsa stain, the higher MN frequencies observed in those laboratories using this approach might be due to the scoring of non-nuclear fragments resembling MN, such as keratohyalin granules or bacteria".

Bonassi et al. [1] also stated that the number of 4000 cells scored per individual is essential to obtain reliable results in the buccal cell cytome assay (which is 4-fold higher than the number of cells evaluated by Cassini et al.).

Cassini et al. also wrote that "It is important to mention that to prevent false results, the cells were washed many times with a buffer". In this case, washing is not crucial for the MN outcome, but the stain is!

Hence, based on the results of the meta-analysis of the data obtained from 30 labs (5424 subjects) neither the stain nor the number of evaluated cells in the study of Cassini et al. correspond to the requirements for modern approaches to the buccal cell MN cytome assay.

REFERENCE

1. Bonassi S, Coskun E, Ceppi M, Lando C, Bolognesi C, Burgaz S, et al. *The human MicroNucleus project on exfoliated buccal cells (HUMN(XL)): the role of life-style, host factors, occupational exposures, health status, and assay protocol*. Mutat Res 2011;728:88–97.

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