

Assessing DNA repair ability in salivary leucocytes with the challenge-comet assay

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The challenge-comet assay provides a quantitative and functional determination of DNA repair ability, by combining a challenge treatment with a genotoxic agent and estimation of the remaining DNA damage with the comet assay after a certain period for repair. In addition, kinetics of the repair process can be monitored. Peripheral blood mononuclear cells (PBMC) are the cells most frequently employed in biomonitoring studies using the challenge-comet assay. However, a validated alternative of non-invasive biomatrix would be highly convenient for certain population groups and circumstances. Thus, the objective of this study was to validate the use of salivary leucocytes in the challenge-comet assay. Saliva samples were collected from 10 healthy volunteers, and leucocytes were isolated and challenged (either in fresh or after cryopreservation) with three genotoxic agents acting by different action mechanisms: bleomycin, methyl methanesulfonate, and ultraviolet radiation. DNA damage induced and remaining was evaluated by the comet assay just after treatment, and also at other three additional time points. The results obtained demonstrated that saliva leucocytes were as suitable as PBMC for assessing DNA damage of different nature that was efficiently repaired over the evaluated time points, even after 5 months of cryopreservation (after a 24 h stimulation with PHA). Furthermore, recommendations to perform the challenge-comet assay with salivary leucocytes depending on the type of DNA repair to be assessed are given. Validation studies are needed to verify whether the method is reproducible and results reliable and comparable among laboratories and studies.

FUNDING. This work was funded by Spanish Ministry of Science and Innovation: MCIN/AEI/10.13039/501100011033 (Grants PID2020-113788RB-I00 and PID2020-114908GA-I00), Ministry of Education, Culture and Sport [BEAGAL18/00142 to V.V.], and Ministry of Economy and Competitiveness, co-financed by the European Social Fund [RYC-2015-18394 to L.L-L].