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ROLE OF PLATELET CONTAMINATION IN ASSESSING mtDNA CONTENT IN PERIPHERAL BLOOD MONONUCLEAR CELLS

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OBJECTIVE: To evaluate the influence of platelet contamination in the quantification of peripheral blood mononuclear cells (PBMC) mitochondrial DNA (mtDNA) content.

METHODS: PBMC were separated from the same donations using four different protocols; DNA was extracted by standard techniques. The number of mtDNA copies per cell was quantified using a novel Real Time PCR assay that measures mtDNA and nuclear DNA (nDNA), and allows the normalization of the results by a dual plasmid. Platelets were counted by a haemocytometer.

RESULTS: The number of mtDNA copies changed considerably depending on the protocol used for PBMC isolation ranging, for example, from 532 to 1164 copies/cell in a donor, and from 172 to 1230 copies/cell in another. mtDNA content was always directly proportional to the number of platelets contaminating the samples. Plasma contained high amounts of mtDNA, but not nDNA, and its amount was also proportional to the number of platelets.

DISCUSSION: The effects of antiretroviral drugs on mitochondria are under investigation. Different techniques have been developed to quantify mtDNA in white blood cells, but different results are emerging. In fact, mtDNA reductions and restoration with therapy cessation in buffy coats from HIV-positive patients with hyperlactatemia ([N Engl J Med 2002 Mar 14;346\(11\):811-20](#)) is in contrast to data obtained in platelet-free, purified lymphocytes in HIV-infected children with lipodystrophy ([J Infect Dis 2002 Feb 1;185\(3\):299-305](#)), or in leukocytes from adults with lipodystrophy or lactic acidosis

([AIDS 2002 Mar 8;16\(4\):513-8](#)). We found that sample processing affects platelet quantity, and thus mtDNA content, even during routine isolation of PBMC or buffy coat preparation, suggesting that some reported changes could be due to factors other than therapy. Platelet removal is technically simple, as cells purified by magnetic sorting can minimise platelet contamination. Appropriate interpretation of mtDNA quantification should rely on clearly defined and validated methodology, particularly when it may influence therapeutic choices.

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