



POSTER PRESENTATION

Open Access

# Digital droplet PCR for precise quantification of human T-lymphotropic virus 1 proviral loads

Giovanna S Brunetto\*, Raya Massoud, Joan Ohayon, Kaylan Fenton, Irene Cortese, Steven Jacobson

From 16th International Conference on Human Retroviruses: HTLV and Related Viruses  
Montreal, Canada. 26-30 June 2013

Elevated HTLV-1 proviral load (PVL) is thought to be the major risk factor for developing HAM/TSP in HTLV-1 infected subjects, and a high cerebrospinal fluid (CSF) to peripheral blood mononuclear cells (PBMCs) PVL ratio might be diagnostic of the condition. However, the standard method for quantification of HTLV-1 PVL, Real time PCR, has multiple limitations: the inter-assay variability increases at low PVL and low cell numbers in CSF often precludes accurate quantification. Thus, we are evaluating a novel technique, Digital Droplet PCR (ddPCR), as a potentially more reliable tool. For ddPCR, DNA samples are partitioned into thousands of nanoliter-sized droplets, amplified on a thermocycler, queried for fluorescent signal and normalized to a housekeeping gene. Due to the high number of DNA molecules and number of "independent" events (droplets), Poisson algorithms are used to determine absolute copy numbers and are independent of a standard curve. Our results suggest that ddPCR is very accurate: Intraassay variability evaluated by calculating the coefficient of variation of ten replicates of three samples of DNA in three different ranges of PVL (low < 5%, medium 5-10%, and high > 10%) was 13.0%, 7.1% and 9.5%, respectively. Interassay variability, was evaluated by calculating the CV of duplicates of PVL from three independent runs and three independent extractions was 4.5% with a standard deviation of 0.008. Additionally, ddPCR is reliable in quantifying PVL in the CSF where we have confirmed and extended previous observations of increased HTLV-1 PVL in CSF of HAM/TSP compared to the periphery.

Published: 7 January 2014

Viral Immunology Section, Neuroimmunology Branch, National Institutes of Neurological Disorders and Stroke, Bethesda, MD, USA

doi:10.1186/1742-4690-11-S1-P13

**Cite this article as:** Brunetto *et al.*: Digital droplet PCR for precise quantification of human T-lymphotropic virus 1 proviral loads. *Retrovirology* 2014 **11**(Suppl 1):P13.

**Submit your next manuscript to BioMed Central and take full advantage of:**

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at  
[www.biomedcentral.com/submit](http://www.biomedcentral.com/submit)



© 2014 Brunetto et al; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.