

This is the peer reviewed version of the following article:

- Lavado-García J, Jorge I, Boix-Besora A, Vázquez J, Gòdia F, Cervera L. Characterization of HIV-1 virus-like particles and determination of Gag stoichiometry for different production platforms. *Biotechnol Bioeng*. 2021 Jul;118(7):2660-2675. doi: 10.1002/bit.27786. Epub 2021 Apr 23. PMID: 33844274.

which has been published in final form at: <https://doi.org/10.1002/bit.27786>

Abstract

The importance of developing new vaccine technologies towards versatile platforms that can cope with global virus outbreaks has been evidenced with the most recent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic. Virus-like particles (VLPs) are a highly immunogenic, safe, and robust approach that can be used to base several vaccine candidates on. Particularly, HIV-1 Gag VLPs is a flexible system comprising a Gag core surrounded by a lipid bilayer that can be modified to present diverse types of membrane proteins or antigens against several diseases, like influenza, dengue, West Nile virus, or human papillomavirus, where it has been proven successful. The size distribution and structural characteristics of produced VLPs vary depending on the cell line used to produce them. In this study, we established an analytical method of characterization for the Gag protein core and clarified the current variability of Gag stoichiometry in HIV-1 VLPs depending on the cell-based production platform, directly determining the number of Gag molecules per VLP in each case. Three Gag peptides have been validated to quantify the number of monomers using parallel reaction monitoring, an accurate and fast, mass-spectrometry-based method that can be used to assess the quality of the produced Gag VLPs regardless of the cell line used. An average of 3617 ± 17 monomers per VLP was obtained for HEK293, substantially varying between platforms, including mammalian and insect cells. This offers a key advantage in quantification and quality control methods to characterize VLP production at a large scale to accelerate new recombinant vaccine production technologies.

Keywords: Gag; HIV-1; mass spectrometry; parallel reaction monitoring; virus-like particle.