Accurate Quantification of Viral Proteins to Support Reliable Viral Load Calculations

Yan Yan Beer[1,2], Cristian G. Arsene[1], Melanie M. Brinkmann[2,3], Gavin O'Connor[1,2]

[1]Physikalisch-Technische Bundesanstalt, Braunschweig, Germany [2]Technische Universität Braunschweig, Braunschweig, Germany [3]Helmholtz Centre for Infection Research, Braunschweig, Germany

LinkedIn

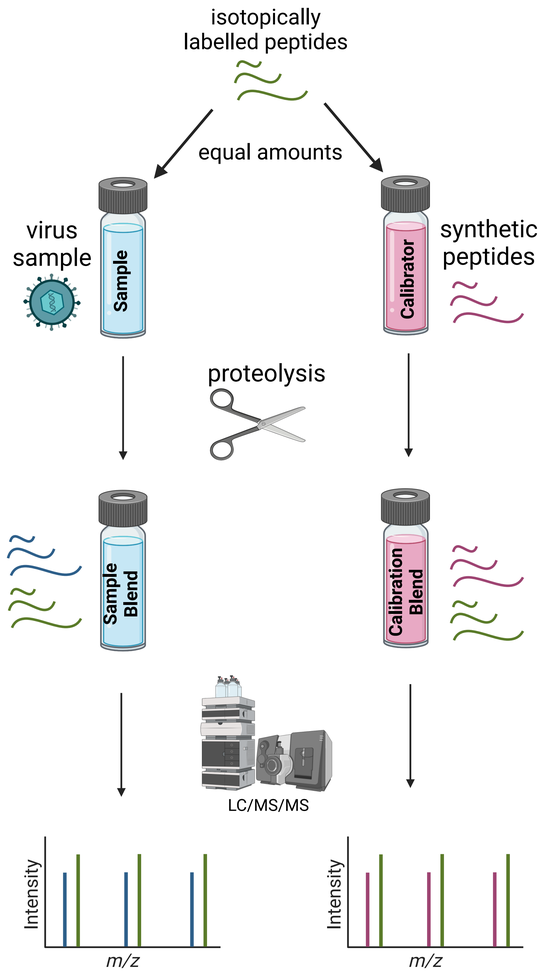
BACKGROUND

The SARS-CoV2 pandemic had shown that the determination of viral load is important to gain an insight into disease progression and treatment options in patients. However, difficulties in comparing measurement results between laboratories limits our understanding of the disease and reduces the effectiveness of clinical intervention and disease management.

The ability to determine the number of viral particles present in a representative biological sample, known as viral load, is essential in our continual quest to reduce the burden of infection on society. However, viral load is indirectly measured via the quantification of sequence specific-nucleic acids, or proteins.

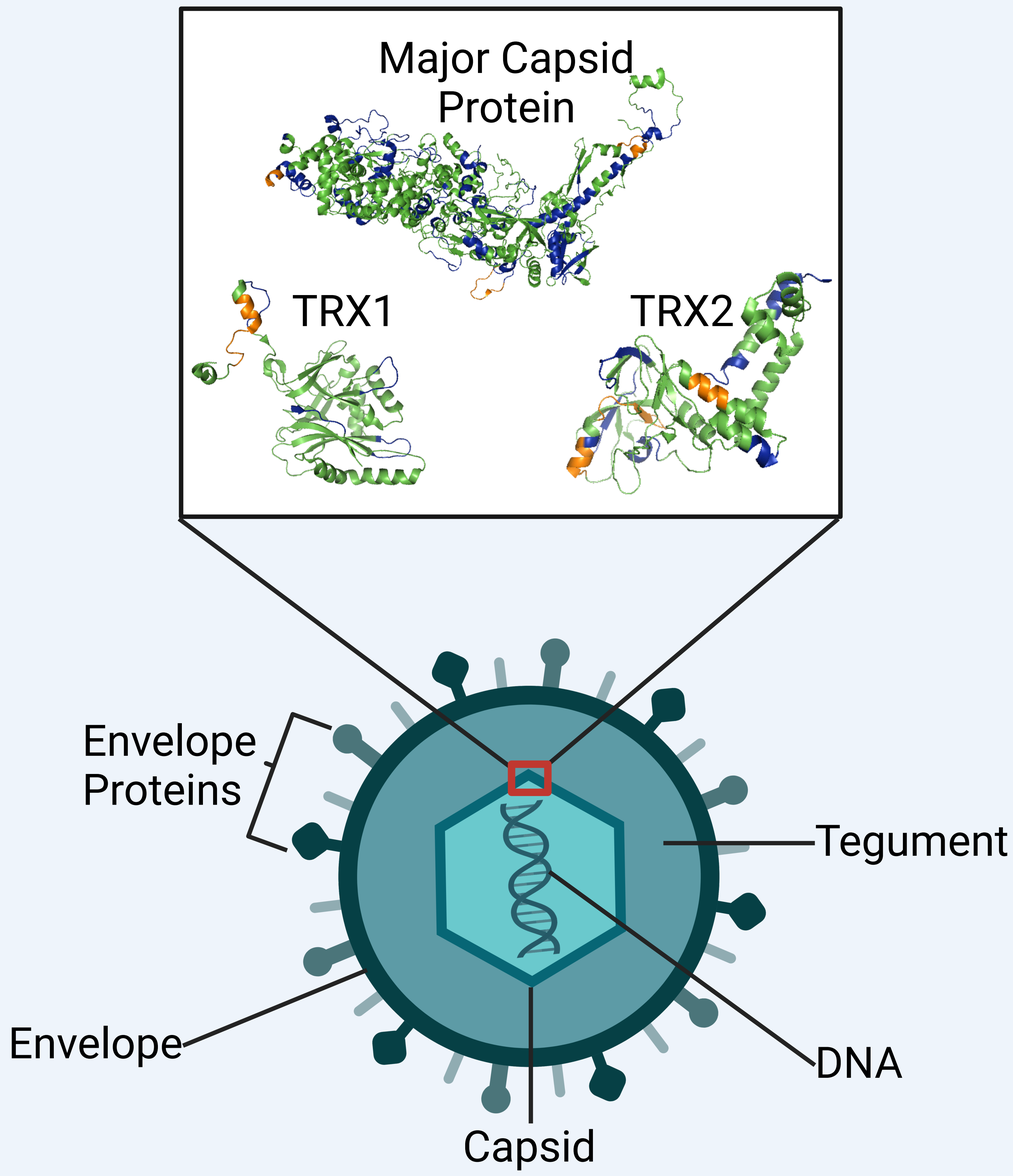
We are interested in assessing the comparability of viral load measurements using these different approaches with the aim of reducing the measurement uncertainty, improving the accuracy and standardizing viral load measurements to facilitate overall measurement comparability.

METHODS



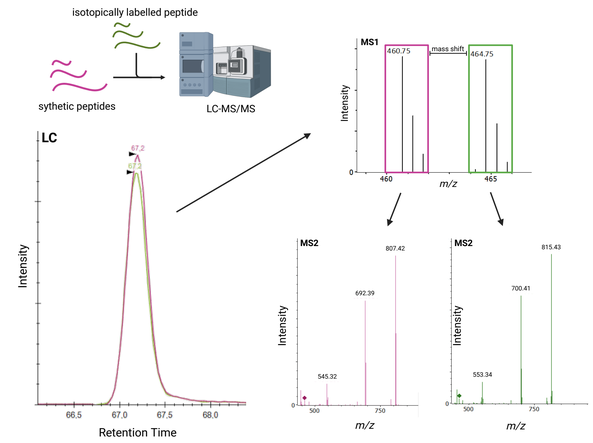
Method workflow for the quantification of viral proteins by isotope dilution mass spectrometry (IDMS). Tryptic cleavage fragments of viral proteins were quantified using synthetic peptides as calibrator peptides of known concentration and isotopically labelled analogues as internal standard. The amount of protein was calculated based on comparison of the signal ratio obtained for the virus sample and the calibration solution.

We quantify viral **capsid proteins** to determine **how many virus particle** are present in a biological sample.

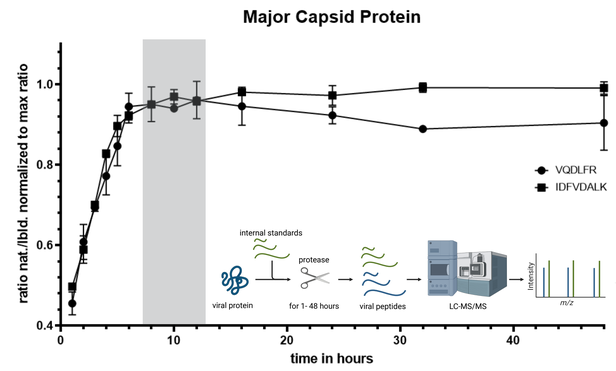


RESULTS

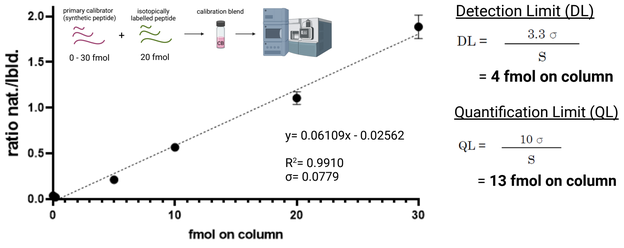
- Peptide-based protein quantification
- Isotope Dilution Mass Spectrometry
- 3 capsid proteins with at least 2 peptides per protein
- specificity ensured through 3 transitions per precoursor
- complete release of signature peptides from viral particles within 10 hours of tryptic digestion
- LOD= 4 fmol
- LOQ=13 fmol



Example chromatogramm, MS1 und MS2 spectra of peptide IDFVDALK (from major capsid protein). MS2 spectra were used for quantification.



Time course experiment of the major capsid protein - Release of peptides during proteolysis. All peptides were released from viral particles within 10 hours.



Calibration curve for DL and QL calculation. Both were calculated according to the ICH guideline.

Outlook, Challenges and Future Perspectives

- Viral DNA copy measurements
- Viral load calculations by combining protein and DNA measurements
- Measurement uncertainty estimation
- Commutability - in vitro vs. in vivo

REFERENCES:
Arsene, Cristian G., et al. "Protein Quantification by Isotope Dilution Mass Spectrometry of Proteolytic Fragments : Cleavage Rate and Accuracy." *Analytical Chemistry*, vol. 80, no. 11, 2008, pp. 4154–60.; Burkitt, William I., et al. "Toward Système International d'Unité-Traceable Protein Quantification: From Amino Acids to Proteins." *Analytical Biochemistry*, vol. 376, no. 2, 2008, pp. 242–51, doi:10.1016/j.ab.2008.02.010.; BIPM. Evaluation of Measurement Data – Guide to the Expression of Uncertainty in Measurement. 2008. ; Yu X, Jih J, Jiang J, Zhou ZH. Atomic structure of the human cytomegalovirus capsid with its securing tegument layer of pp150. Science. 2017;356(6345):eaam6892.; Hoofnagle AN, Whiteaker JR, Carr SA, Kuhn E, Liu T, Massoni SA, et al. Recommendations for the Generation, Quantification, Storage, and Handling of Peptides Used for Mass Spectrometry–Based Assays. Clinical Chemistry. 2016;62(1):48-69.; Turner DL, Korneev DV, Purdy JG, de Marco A, Mathias RA. The host exosome pathway underpins biogenesis of the human cytomegalovirus virion. Elife. 2020;9.