Characterizing the Surface of HIV Virions in Plasma and Sera using Flow Virometry

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Introduction: Flow Virometry (FV) as a Tool to Study Viruses

- Flow virometry is a powerful emerging technique that can provide multiparametric analyses of individual virions in a high throughput manner[1].
- Advances in cytometry instrumentation have allowed for the visualization of virus particles through light scatter [1,2]. This advancement allows for the analysis of wild type viruses that are not fluorescently tagged.
- Recently, the development of computational calibration software has enabled quantitative estimates of individual protein number on virions through the use of antibody labelling paired with fluorescence reference beads [3] (Fig. 1).
- Interest in using FV as a diagnostic tool has become more prevalent [4,5], particularly for SARS-CoV-2, but little work has been done in this area.

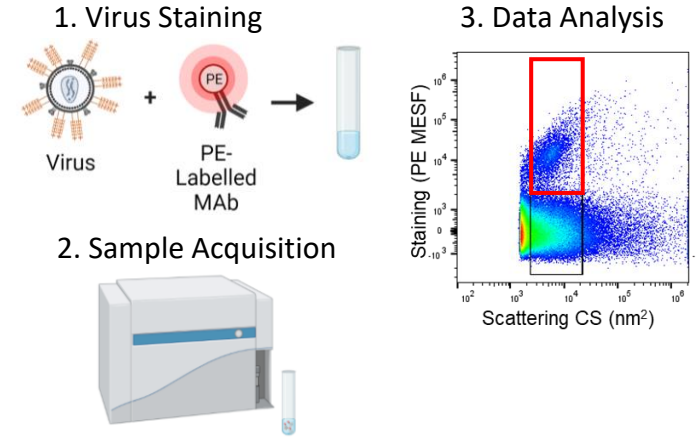

Fig. 1. Schematic displaying virus staining (1), sample acquisition of flow virometry samples (2) and a representative dot plot (3) showing antibody labelled virus (red gate).

Introduction: HIV as a Model Virus for FV Studies

- HIV provides an optimal target to use for FV optimization experiments since it is well characterized and is easily detectable through light scatter [2].
- The HIV envelope contains a broad range of cellular proteins that are acquired during budding from infected cells[6,7] (Fig. 2). These proteins which often outnumber the viral spike protein, can serve as high abundance targets on the virion surface for the optimization of flow virometry.
- These markers could serve as indicators of disease progression and may provide novel therapeutic targets.
- Developing this technique using HIV as a model virus may facilitate the use of flow virometry for more widespread study of other viruses using flow cytometry approaches.

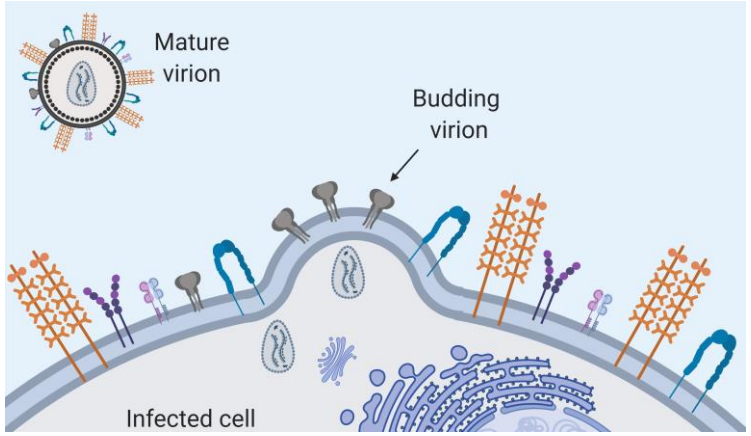

Fig. 2. Incorporation of host proteins (coloured) into a virion upon budding from an infected cell.

Research Goal

- To test the efficacy of antigen labelling on HIV spiked into plasma/sera as a proof of principle for staining viruses in clinical samples.

Results: Validation of Flow Virometry Technique

- Pseudoviruses designed to overexpress host proteins (CD14, CD59, CD162) were stained overnight with PE-labelled monoclonal antibodies (MAb) targeting the host protein on the viral surface (Fig. 3). Unstained virus controls were run in parallel on the cytometer.

- Fig. 3 demonstrates that our virus labelling protocol[2] provides robust staining which results in clearly distinguishable labelled virus populations (red gates).

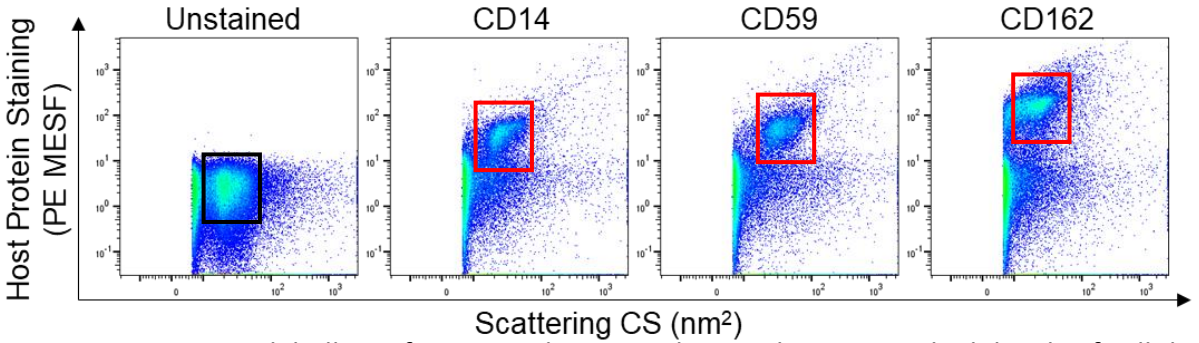

Fig. 3. Host protein labelling of HIV pseudoviruses designed to express high levels of cellular proteins in the viral envelope. Stained virus populations are gated in red with the unstained population gated in black.

Results: Validation of Antigen Staining on HIV Spiked into Plasma and Sera

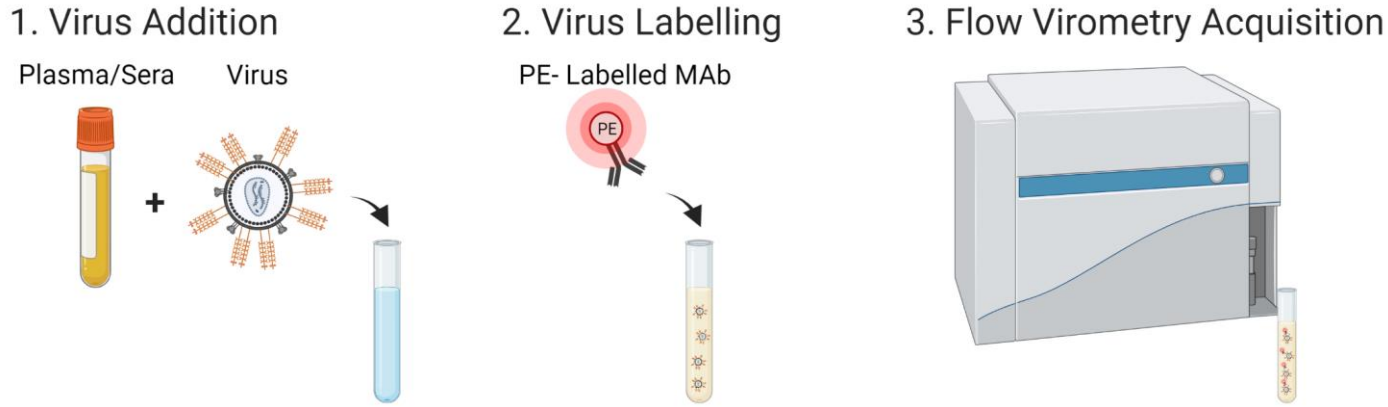

Fig. 4. Schematic depicting the workflow for a proof of principle virus staining protocol for preparing mock-clinical samples for flow virometry.

- Viruses designed to overexpress PSGL-1 (CD162) through transfection were spiked into plasma and sera and were stained with an anti-PSGL-1 MAb (Fig. 4). Control viruses produced without PSGL-1 were stained in parallel.

- Fig. 5 demonstrates that our FV protocol can label viruses in biological fluids with a similar level of efficacy as seen in a media control, suggesting that virus labelling in clinical samples could be possible with the same parameters.

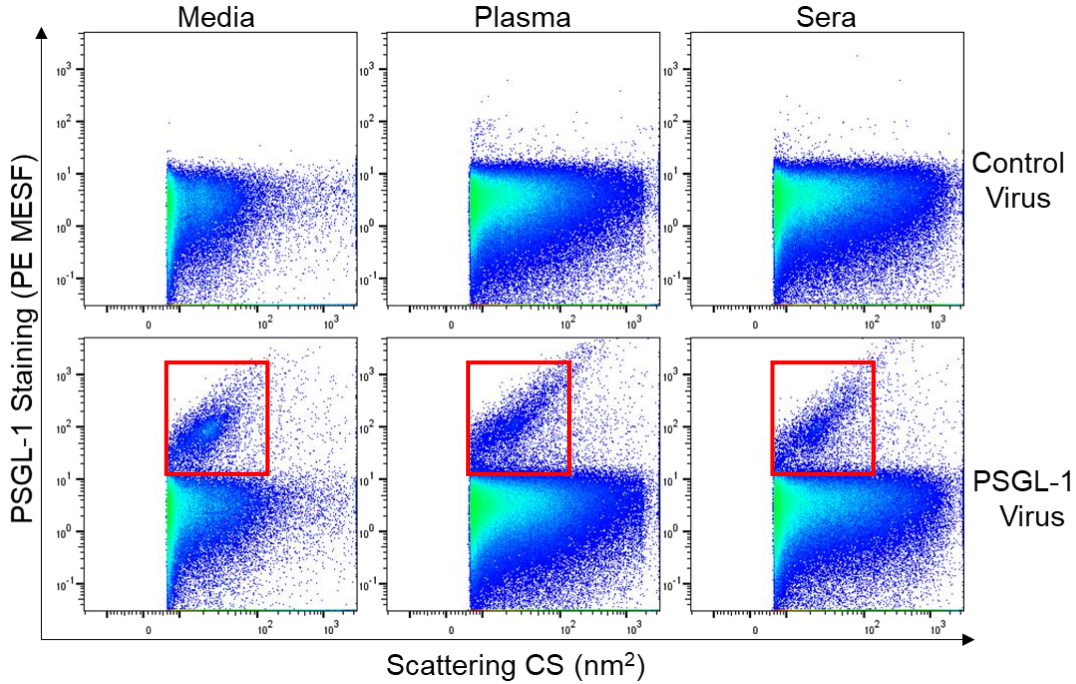

Fig. 5. PSGL-1 staining on pseudoviruses with (PSGL-1+) or without (control) PSGL-1 in the viral envelope on viruses spiked into media, plasma or sera. Data are representative of 5 independent donors.

Results: Evaluation of the Sensitivity of FV to Assess Protein Levels on HIV in Biological Fluids

- After showing labelling overexpressed proteins on the virion surface was possible, we next tested staining of a host protein (CD44) that is endogenously present on virions[6] at a lower abundance compared to PSGL-1 on our pseudoviruses (Figs. 2-3).

- For this purpose, viruses produced in the H9 T cell line were spiked into plasma and sera and stained.

- All stained plots show a defined population (Fig. 6; red gate), indicating that endogenous proteins on the virion surface can be labelled in biological fluids.

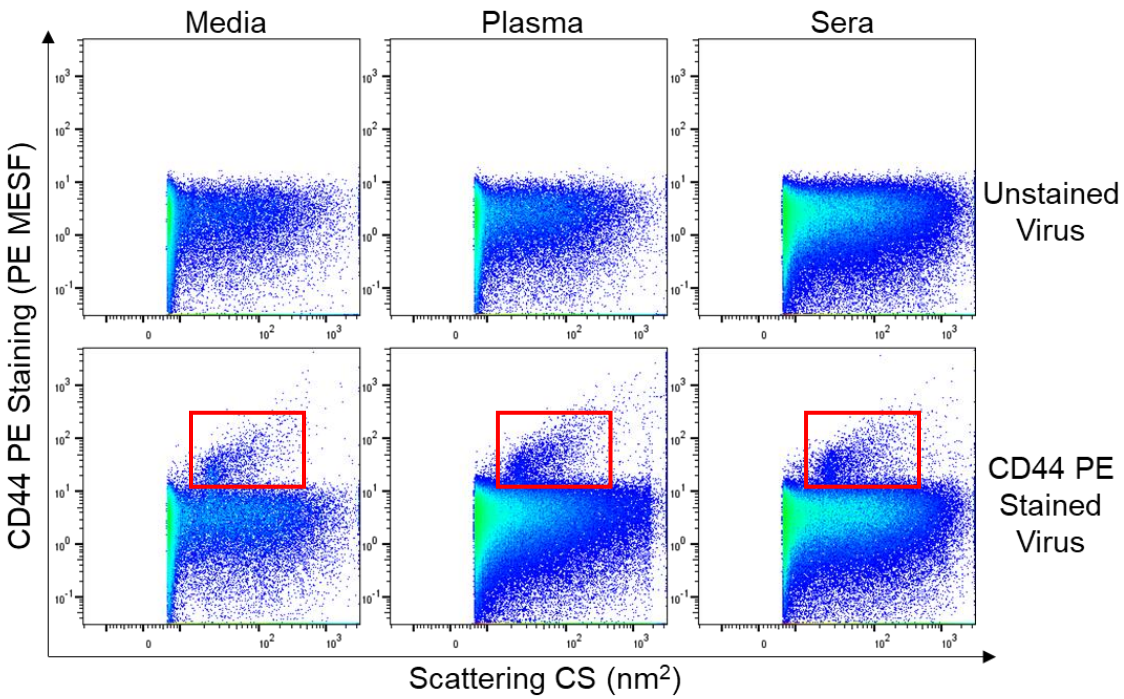

Fig. 6. CD44 labelling of HIV-1 IIIB produced in the H9 cells in media, plasma and sera. Positive staining (virus population) is indicated in red gates.

- To determine how sensitive the FV protocol was for labelling proteins on the viral surface in biological fluids, serial dilutions of virus were spiked into plasma and sera and stained at a range of 0.5 – 35 ng/mL of p24 (viral capsid protein).

- Differential staining efficacy was demonstrated in both plasma and sera compared to the media control (Fig. 7) with higher levels of labelling observed in plasma compared to sera at lower virus concentrations.

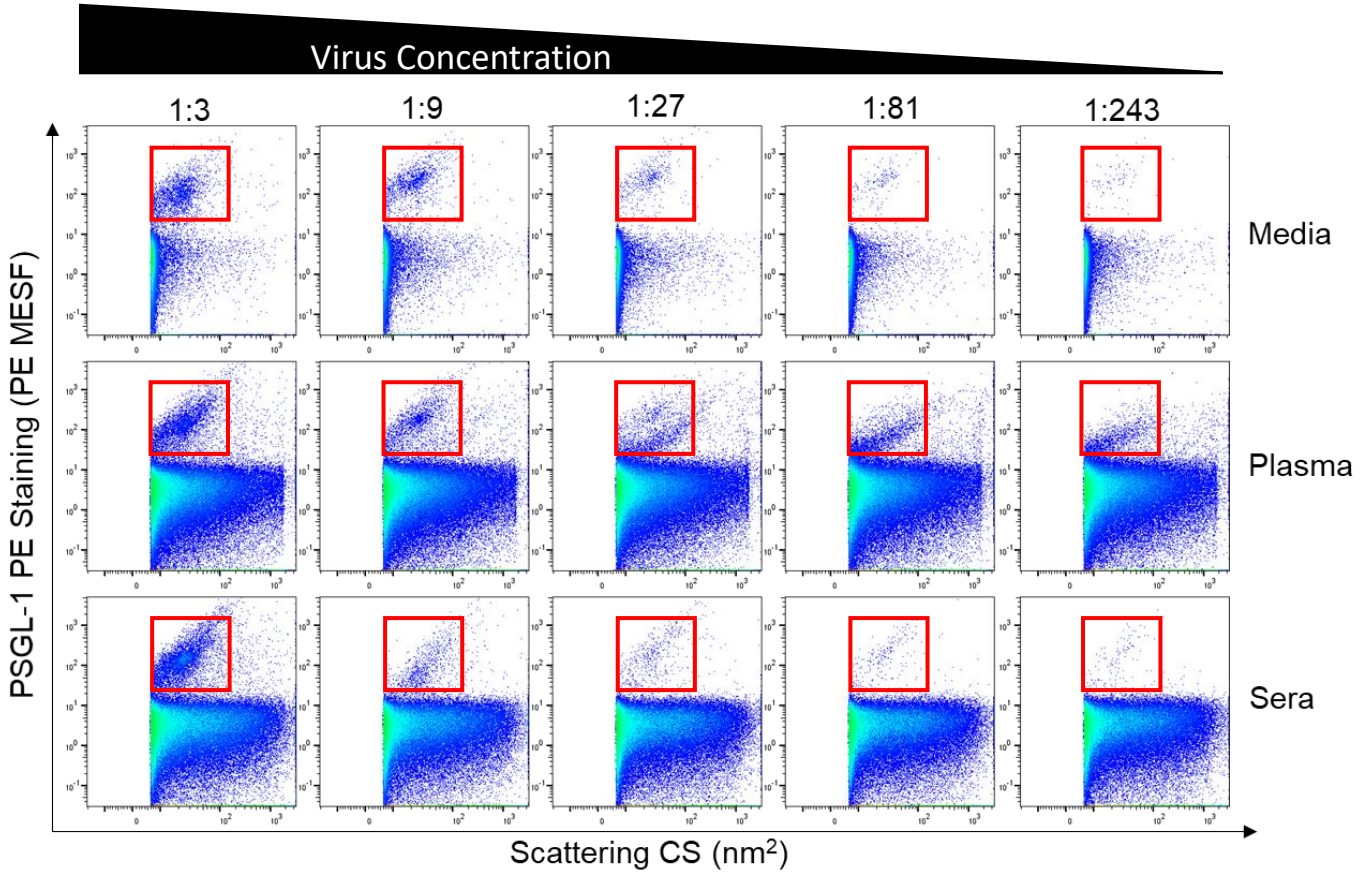

Fig. 7. PSGL-1 staining on serial dilutions of a pseudovirus designed to overexpress PSGL-1 on the viral surface. Positive staining (virus population) is indicated in red gates.

Conclusions

- In this proof of principle study, we demonstrate that flow virometry can be effectively applied to label proteins that are on the viral surface in human plasma and sera.
- We show that successful labelling of virion-incorporated proteins can occur at viral titres as low as 0.5 ng/mL p24 in plasma and sera. We estimate that this is equivalent to ~10^7 viral copies/mL[8,9].
- Since this corresponds to the high range of viral load in PLWHIV without ART, these data suggest that this FV labelling protocol may be directly adaptable to banked clinical specimens.

Significance

- Flow virometry is a high throughput technique that can provide detailed information on single virions using a quick labelling protocol. This work supports the idea that FV could be used to assess clinical samples or for diagnostic purposes since we were able to analyze virions in biological fluids without the need for virus purification steps.

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Figures

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