Supplemental Document

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# Small form factor flow virometer for SARS-CoV-2: supplement

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# Supplementary Information of

# A small form factor flow virometer for SARS-CoV-2

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## 1. Comparison of commercial and laboratory tests for SARS-CoV-2 detection

| Type of Test | Detection Method | Specimen | Time to Results | Sensitivity* | Specificity* | Assay Complexity** | Portability |
|---|---|---|---|---|---|---|---|
| RT-PCR | Molecular assay for qualitative/ quantitative detection of specific gene sequences of SARS-CoV-2 | Upper and lower respiratory specimens, sputum, saliva | 2-4 hours | 87.5% – 100% | 90% - 100% | Middle-to-High | Yes |
| RT-LAMP | Molecular assay for qualitative detection of specific gene sequences of SARS-CoV-2 | Anterior nasal/ nasopharyngeal swab, sputum, saliva | 15-60 minutes | 93% – 100% | 98% - 100% | Middle | Yes |
| Antibody/ Serology based tests | Enzyme linked immunosorbent assay (ELISA), Neutralization assay, Chemiluminescent immunoassay for qualitative/ quantitative detection of antibodies against the virus | Serum, plasma, whole blood | 10 min – 5 days (depending on detection method) | 14% (after 7 days of infection) - 100% | 93% - 100% | Middle-to-High | No |
| Rapid Antigen Tests (RAT) | Lateral flow immunoassay for qualitative detection of SARS-CoV-2 nucleocapsid protein | Anterior nasal/ nasopharyngeal swab | 15 – 30 min | 80% - 97% | 96.6% - 100% | Low | Yes |
| FVR | **Flow virometry for qualitative/ quantitative detection of SARS-CoV-2 spike protein** | **Saliva** | **25 – 30 min** | **92%** | **90%** | **Low-to-Middle** | **Yes** |

**Table S1. A comparison of commercial and laboratory tests for detection of SARS-CoV-2.[1]**

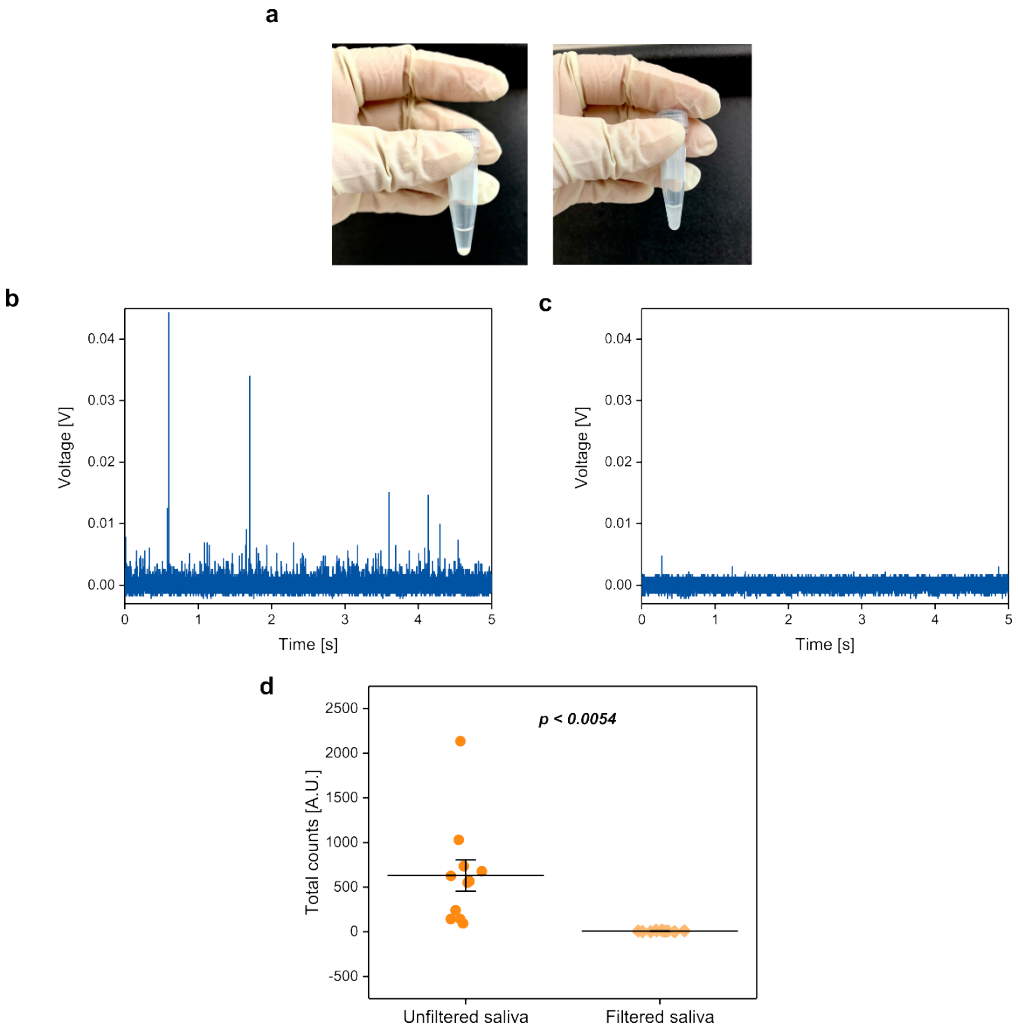
* Depending on manufacturer/ sample type/ collection

** Low= does not require the use of pipette and it needs little operational steps.

Middle = it requires the use of pipette and might require the use of additional specialized equipment (other than the detection system).

High = it requires the use of trained personnel, pipette and additional specialized equipment (other than the detection system).

## 2. Filtration of raw saliva to reduce background noise

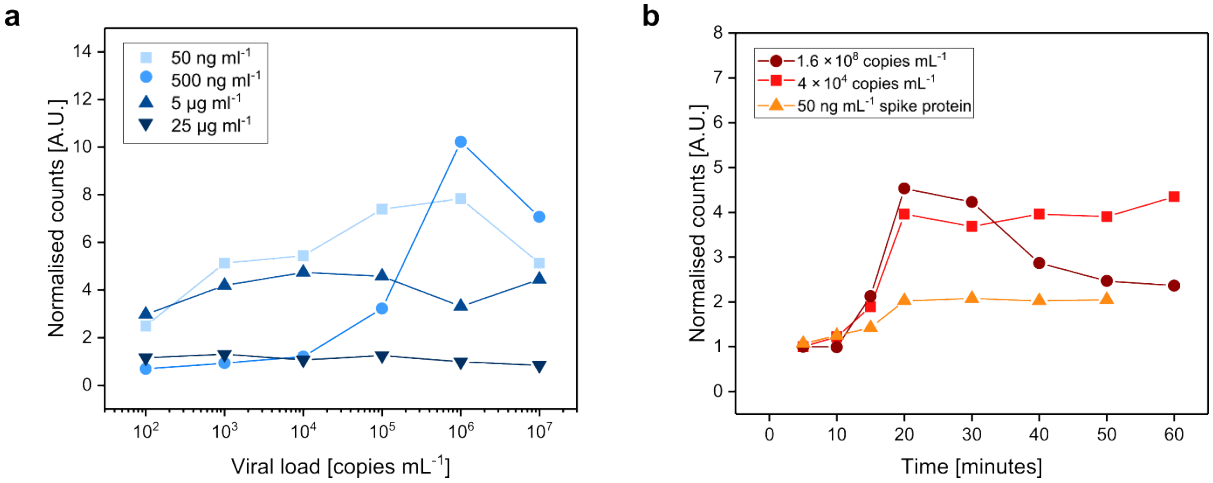



**Supplementary Figure 1. Noise associated to saliva samples. (a)** Picture of collected saliva as received (left) and after vortexing to re-suspend particles and heavy molecules (right); Voltage vs time signal of **(b)** unfiltered saliva sample and **(c)** filtered saliva sample; **(d)** difference in total counts between a population of 11 unfiltered (left) and filtered (right) saliva samples. The filtration process enables a 98% noise reduction. The two sample populations are statistically different with $p < 0.0054$, calculated with a two-tailed student t-test.

## 3. Optimal antibody concentration and incubation time for SARS-CoV-2 spike protein detection using FVR

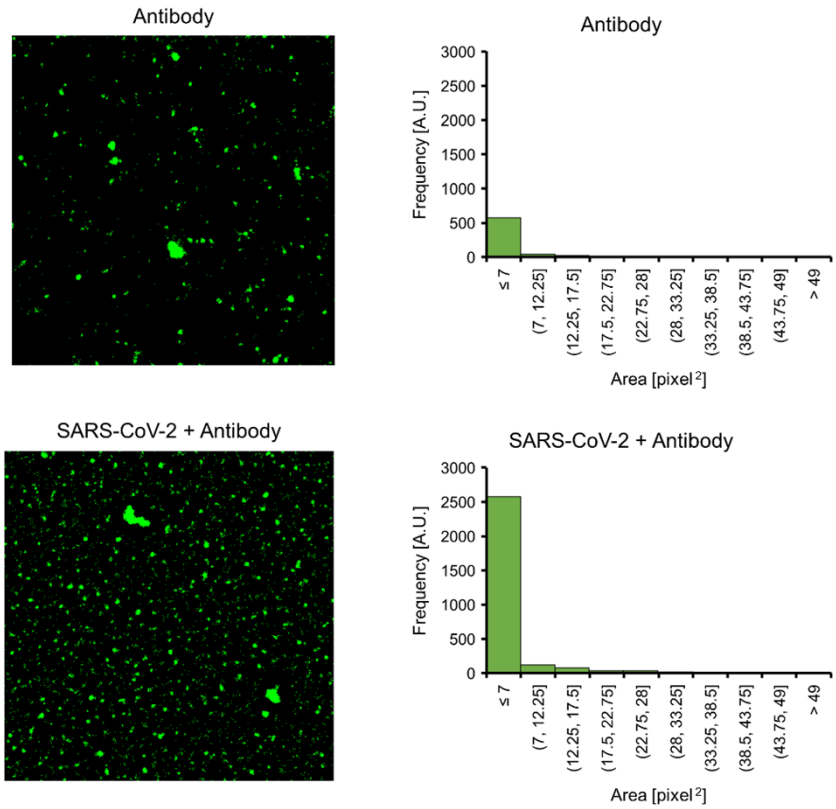
In order to determine the optimal antibody concentration, the SARS-CoV-2 positive saliva samples were tested by labelling with four different antibody concentrations. The signal obtained from the positive samples were normalised with respect to that of the corresponding antibody concentration in PBS. From **Supplementary Fig. 2a,** it can be seen that 50 ng $mL^{-1}$ antibody concentration showed the best response from viral load of $10^3$ to $10^6$ copies $mL^{-1}$. Thus, 50 ng $ml^{-1}$ was selected to be the optimal antibody concentration for the labelling protocol, because it provides close to optimal discrimination between positive samples and antibody signal. Next, using this antibody concentration we investigated the optimum incubation time with respect to signal increase and time-to-results by testing two positive SARS-CoV-2 saliva samples and one healthy donor sample spiked with SARS-

CoV-2 spike proteins were tested to establish the incubation time. From **Supplementary Fig. 2b** the optimum labelling time was found to be around 20 minutes.




**Supplementary Figure 2. Labelling protocol optimization**. **(a)** Determination of optimal antibody concentration. Four antibody concentrations, 25 µg $mL^{-1}$, 5 µg $mL^{-1}$, 500 ng $mL^{-1}$ and 50 ng $mL^{-1}$ were used for labelling SARS-CoV-2 positive samples with varying viral load from $10^7$ copies $mL^{-1}$ to $10^2$ copies $mL^{-1}$; **(b)** determination of optimal incubation time for labelling saliva samples with 50 ng $mL^{-1}$ anti-SARS-CoV spike antibodies.

## 4. Stimulated emission depletion microscopy (STED) for analysis of only anti-SARS-CoV spike antibodies and labelled SARS-CoV-2 positive saliva samples.

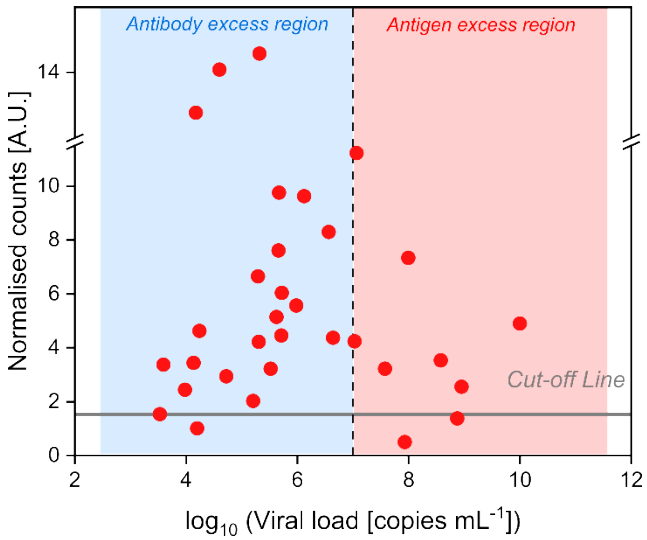



**Supplementary Figure 3. A super-resolution microscopy, STED images of only anti-SARS-CoV spike antibodies (top) and labelled SARS-CoV-2 positive saliva samples (bottom).** Corresponding plots of frequency vs area show (bottom) a 5-fold increase in fluorescence signal in labelled SARS-CoV-2 positive saliva samples, while (top) anti-SARS-CoV spike antibodies only 1-fold increase in fluorescence signal. Analysis were performed with ImageJ 1.53c.

## 5. Quantification of SARS-CoV-2 in saliva samples – clinical validation.

### FVR Immunoassay: blind test

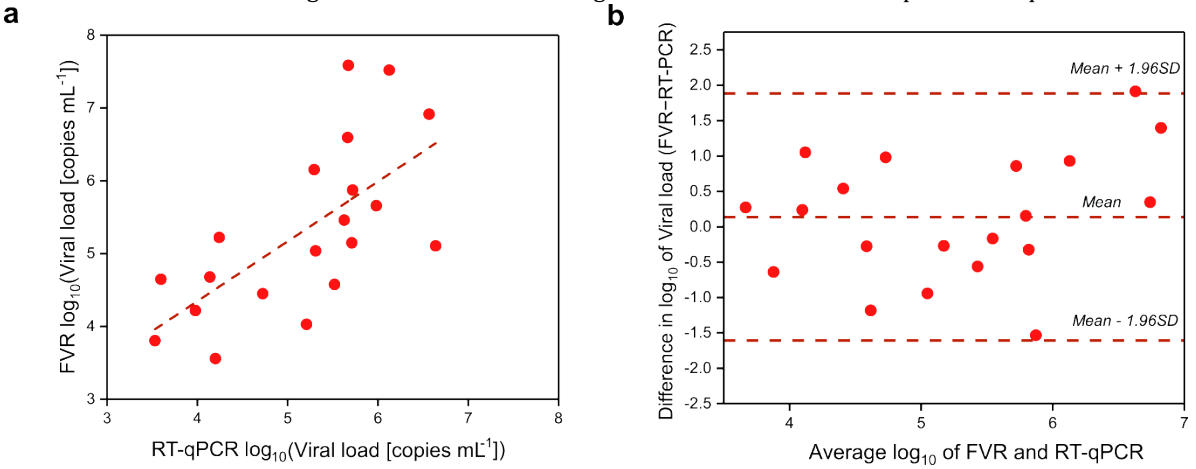
When comparing the signal obtained from the FVR with respect to the RT-PCR quantified viral load, the response registered is not linear over the full viral load range of the samples tested. More specifically, the response is linear and monotonically increasing for low concentrations. For concentrations higher than $10^7$ copies $mL^{-1}$, the signal start decreasing, almost linearly. A possible explanation for this behaviour is that the FVR response is dependent on the antigen concentration. Taking a closer look at the shape of the curve in **Supplementary Fig. 4**, we can identify two different regions: (i) antibody excess region and (ii) antigen excess region. In the antibody excess region, the antigens on the viral particles act as a bridge and coagulate several labelled antibodies, thus the increase of the signal with respect to the blank solution and the increase of the signal as the viral particle concentration increases. The signal starts decreasing in the antigen excess region probably because the bridging process is unfavoured and the signal from the labelled antibodies is masked by the presence of viral particle in excess.




**Supplementary Figure 4. FVR Normalized Counts vs RT-qPCR.** The red dots represent the normalized counts from positive SARS-CoV-2 saliva samples measured with FVR plotted against the log of the viral load determined from RT-qPCR. Grey line indicates the cut-off line. Shaded blue area is the antibody excess region. Shaded red area is the antigen excess region. Vertical black dashed line divide the antibody and antigen excess region.

**FVR vs. RT- qPCR**

The viral load quantification was performed using the calibration curve with equation y=2.1742x-2.3842. The obtained viral load value was multiplied by 100 in order to take into account the 1:100 dilution factor used to prepare the blind test samples. We calculated the statistical relationship between the viral load measured with RT-qPCR and with our FVR, by performing a Pearson`s correlation test (**Supplementary Fig. 5a**). We found a good correlation with a coefficient r of 0.66 and a p value of 0.001 for the blind samples falling into our analytical measuring range (<$10^7$ copies $mL^{-1}$). For samples with viral load above the analytical range of the FVR, we were not be able to quantify the viral load correctly but were still able to determine if the tested sample was positive or negative. To further understand the correlation between the FVR and the RT-qPCR we analysed the agreement of the two methods with the Bland-Altman plot[2] (**Supplementary Fig. 5b**). Given the normal distribution of the difference of the measurement of the two methods, we set the limit of the agreement as the mean of the difference of the measurements plus 1.96 time its standard deviation. The average of the differences is 0.138. This means that FVR measures on average 0.138 times more the log10 of the viral load with respect to RT-qPCR.




**Supplementary Figure 5. FVR vs RT-qPCR. (a)** Red dots represent the viral load quantification with the FVR reader versus the RT-qPCR viral load. Dashed red line is the linear regression of the quantification curve. The equation of the linear regression is y=0.8216x + 1.0612 ($R^2$=0.43); **(b)** difference in the viral load prediction between the FVR reader and the RT-qPCR. The normalized root-mean-square deviation NRMSD associated to the FVR predicted viral load is 9.2%.

**References**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2. Bland, J. M. & Altman Douglas G. Correlation, Agreement, and Bland-Altman Analysis: Statistical Analysis of Method Comparison Studies. *Lancet* 307–310 (1986). doi:10.1016/j.ajo.2008.09.032