Supplementary Information

Influence of viral transport media and freeze-thaw cycling on the sensitivity of RT-qPCR detection of SARS-CoV-2

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Silica-coated high magnetisation beads

RNase Activity Assay

Silica-coated high magnetisation beads

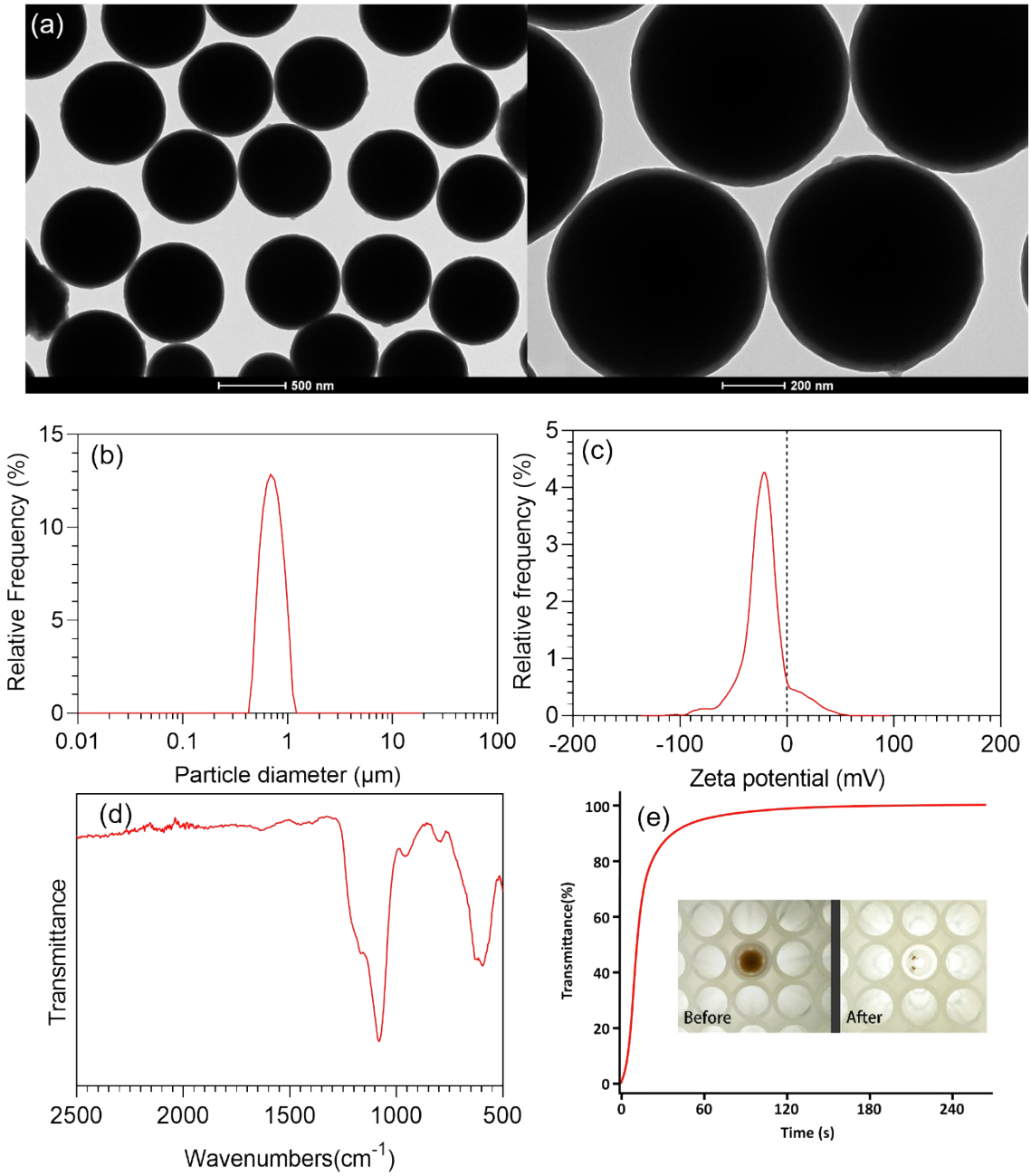

Figure S1. Characterization data of magnetic beads used for RNA extraction. (a) Transmission electron microscopy images of magnetic beads showing the Fe_3O_4 superparamagnetic core coated with a silica shell. Hydrodynamic size (b) and Zeta potential (c) of silica-coated magnetic beads obtained by dynamic light scattering. Mean hydrodynamic diameter: 0.77 µm, PDI:0.09, Zeta potential: -22.6 mV ± 0.11mV (mean ± SD). (d) FITR spectra of silica coated magnetic beads demonstrating characteristic silica peaks. FITR (ATR): 1080 cm^{-1} (Si-O), 955 cm^{-1} (Si-OH), 795 cm^{-1} (Si-O). (e) Separation kinetics of magnetic beads dispersed in PBS in a 96 deep-well plate placed on a M96D-400 magnetic separator expressed as an increase in the transmittance of white light through the well. More than 95 % of beads are captured within the first 60 seconds. Inset shows the image of the well loaded with magnetic beads before and after exposure to a magnetic field.

RNase Activity Assay

The RNaseAlert QC system V2 Kit from Thermo Fisher was used to test the solutions for RNase activity using the supplied protocol. Samples, positive and negative controls were tested in duplicates. RNase-free, filtered pipette tips were used throughout. Recommended fluorometer settings from the RNaseAlert QC system V2 protocol were followed. The temperature, focal height and gain settings were 37 ^{o}C, 3.4, and 50%, respectively. The excitation/emission wavelengths were 490/520nm. The well scan setting was set to orbital and a mixing step was selected for 5 seconds before plate reading at 500rpm. The time interval for data collection was set to 1.5 minute intervals for 20-30 cycles i.e. 30- 45 minutes.

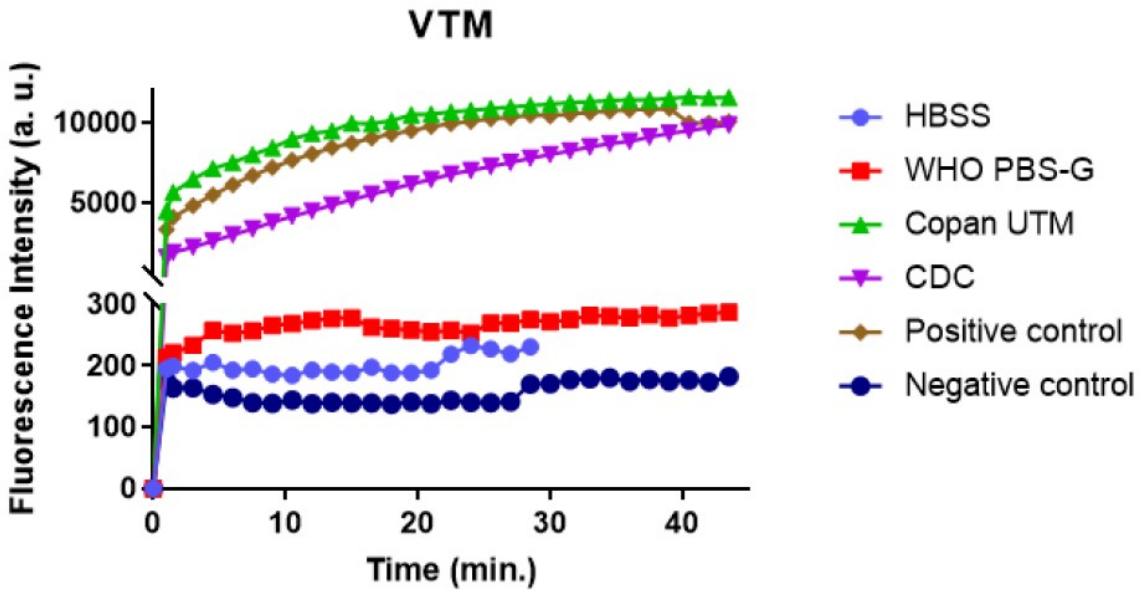

Figure S2. RNase activity assay (RNaseAlert QC system V2) performed on different VTM used in this study in comparison to positive and negative controls. UTM (green) and CDC (purple) displayed high levels of RNase activity that was comparable to positive control (brown). HBSS (blue) and PBS-G (red) presented as negative for RNase activity, comparable to negative control (navy). Positive control: RNase A, Negative control: nuclease-free water (black).

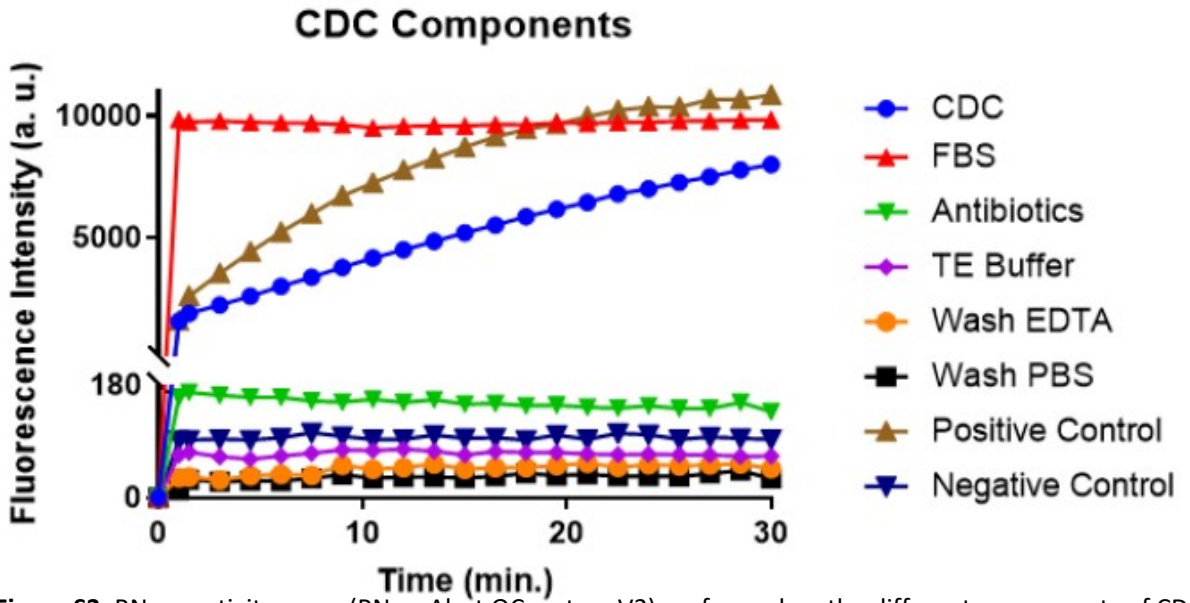

Figure S3. RNase activity assay (RNaseAlert QC system V2) performed on the different components of CDC (blue) that displayed a high RNase activity. All CDC components were RNase-free except for FBS (red), which displayed a higher RNase activity compared to the positive control (brown). Positive control: RNase A, Negative control: nuclease-free water (navy).

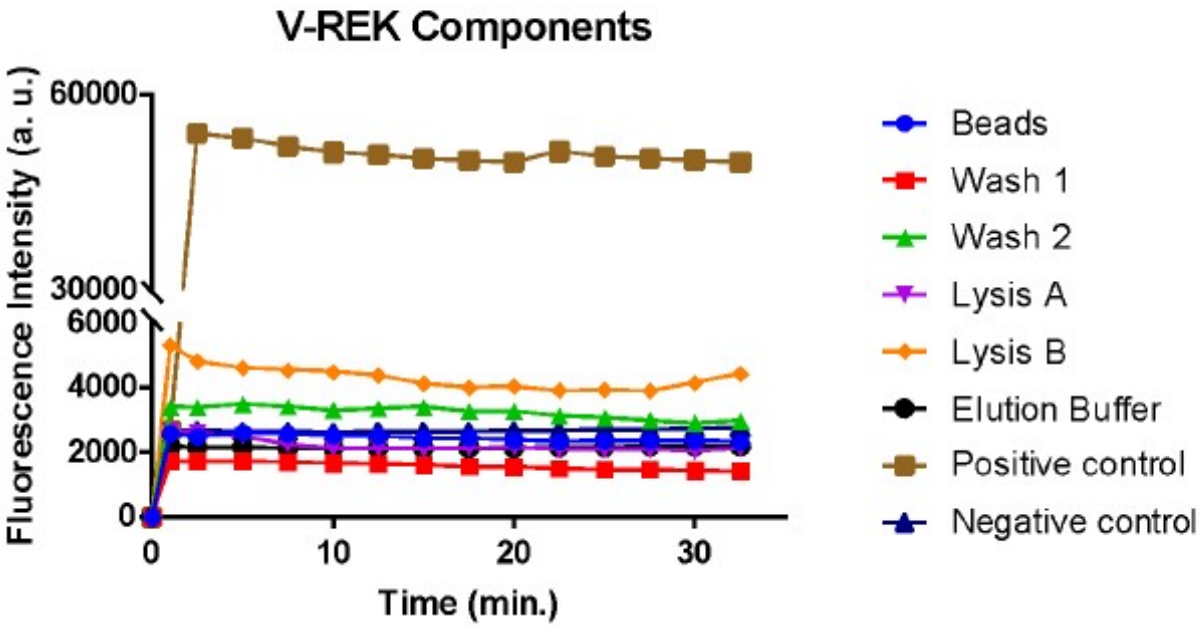

Figure S4. RNase activity assay (RNaseAlert QC system V2) performed on the various components of V-REK nucleic acid extraction kit used in this study in comparison to positive (brown) and negative (navy) controls. All components tested negative for RNase activity. Positive control: RNase A, Negative control: nuclease-free water.

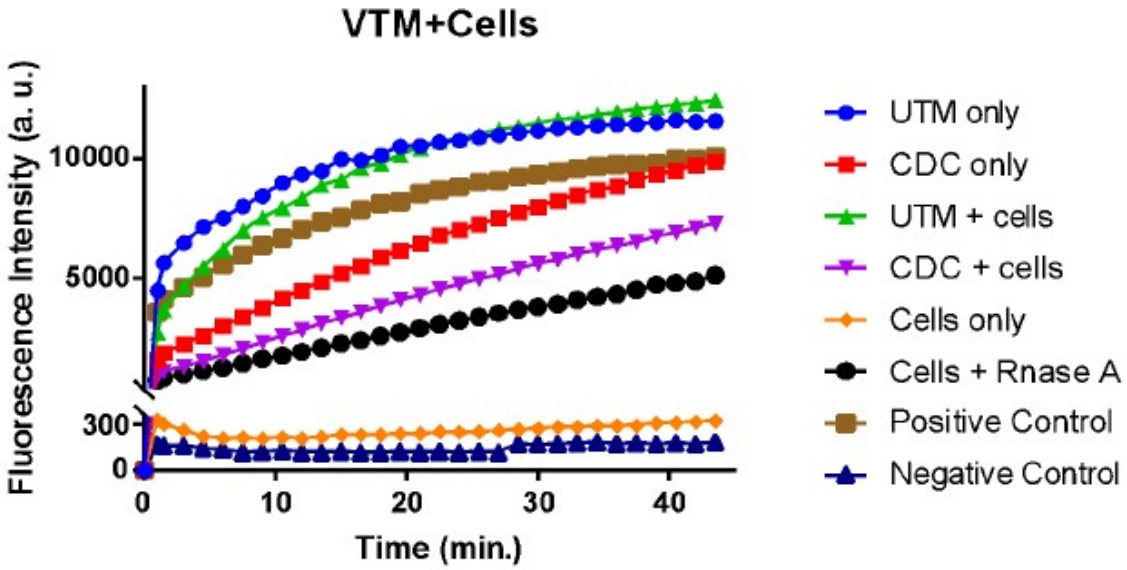

Figure S5. RNase activity assay (RNaseAlert QC system V2) performed on the RNase-positive VTMs in the presence of A375 cells. The RNase activity in UTM (blue) and CDC (red) decreased in the presence of cells (UTM+cells, green; CDC+cells, purple). The positive control RNase A (brown) also displayed a reduced activity when tested in the presence of A375 cells (Cells+Rnase A, black). RNase activity of A375 cells (Cells only, orange) was comparable to the negative control (nuclease-free water, navy).