Supporting Information

Sample-to-answer COVID-19 nucleic acid testing using a low-cost centrifugal microfluidic platform with bead-based signal enhancement and smartphone read-out

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Figure S1. Proof-of-concept of PCR amplicon enrichment on N-benzyl-N-methylethanolamine beads. Synthetic SARS-CoV-2 full genomic RNA (MN908947.3, Twist Bioscience) was spiked at a total of 50 to 50,000 copies per 25 μL reaction mix containing 1x TaqMan FAST Virus 1-step MM (Thermo Fisher Scientific), 0.6 μM forward primer (5’-GTG ARA TGG TCA TGT GTG GCG G-3’), 0.8 μM reverse primer (5’-CAR ATG TTA AAS ACA CTA TTA GCA TA-3’) and 0.2 μM of molecular beacon probe 1 (5’-FAM-CAG GTG GAA CCT CAT CAG GAG ATG C-BHQ_1-3’) and 2 (5’-FAM-CCA GGT GGW ACR TCA TCM GGT GAT GC-BHQ_1-3’). The mixture was first incubated for 5 min at 50 °C for reverse transcription, denatured for 20 s at 95 °C and subjected to a series of 45 cycles of 3 s at 95 °C and 30 s at 60 °C. The amplification generates a 100 bp long amplicon. A- RT-PCR with increasing RNA copy numbers in solution, measured using a micPCR magnetic induction cycler (BMS, Australia). Each
viral copy titer was measured in quadruplicate. B- Fluorescence intensity measured on Capto Adhere beads, or solution upstream of the beads after flowing 10 μL of the pre-amplified mixture (after the 45 amplification cycles in A) through the bead-packed microchannel. Both fluorescence intensity on the beads and in solution were measured using fluorescence microscopy and ImageJ software (NIH, USA) by measuring the grey scale intensity in both regions (16-bit images). The beads provide an increase of ~100-fold in fluorescence signal intensity. Vertical and horizontal error bars correspond to the standard deviation of 4 independent PCR amplification and bead-capture experiments. The inset plot shows the correlation between cycle threshold values of the tested viral loads and fluorescence intensity measured on the beads. C- Microscopy images of the beads with increasing copy numbers of SARS-CoV-2 genomic RNA (initial copy numbers before PCR).
**Figure S2.** Schematics of the bead capture selectivity in the presence of positive or negative samples amplified with LAMP or PCR. In the case of LAMP, the fluorescence is generated by a dsDNA fluorescent intercalator (e.g. SYBR green I). For PCR, the fluorescence is generated using a molecular beacon (e.g. FAM-BHQ-1 pair). In the tested setup, the PCR amplicon has a length of 100 bp and is able to effectively penetrate the pores of the agarose beads. Green droplets indicate strongly fluorescent solutions, while white droplets indicate non-fluorescent or weakly fluorescent solutions.
**Figure S3.** Detailed dimensions (parallel to the rotation axis) of the microchannels drilled on the discs. All values are in mm units. The depth of the channel is 400 μm except at the interface between the 0.5 mm and 6 mm wide regions, where the depth is 50 μm.
Figure S4. Exploded view of the centrifugal platform. The smartphone adapter design depends on the specific position of the camera being used and can be adapted to ensure an optimal focal distance when measuring the fluorescence signal on the microchannels. A Kapton™ 100 μm thick polyimide film is attached between the lens and the disc to block the excitation light from the laser diode.
Figure S5. Position and incidence angle of the laser light relative to the disc. The light from the laser has a perpendicular beam divergence of 25° (FWHM) and a parallel beam divergence of 11°. The higher perpendicular divergence ensures illumination of the entire solution and bead region along the microchannels.
**Table S1.** Nasopharyngeal swab samples analysed using Cepheid GeneXpert targeting E and N genes. “Average Ct” refers to the average of both E and N gene Ct values. “Medium” refers to the collection tube used to store the nasopharyngeal swab samples prior to analysis. Viocult transport tubes contained liquid Viocult medium and both Transwab and ESwar tubes contained liquid Amies medium. Sample ID codes refer to very high viral load (V), high viral load (H), medium viral load (M), low viral load (L), very low viral load (VL) and PCR-negative samples (N). All samples were collected at Karolinska University Hospital (Huddinge) from patients having symptoms attributable to a possible SARS-CoV-2 infection. Sample IDs identified with an asterisk were tested using the iLACO primer set. All remaining samples were tested with the As1e primer set.

| Sample ID | Ct E | Ct N2 | Average Ct | Medium |
|-----------|------|-------|------------|--------|
| V1*       | 11   | 13,5  | 12,3       | Viocult|
| V2*       | 12,2 | 14,5  | 13,4       | Viocult|
| V3*       | 13,5 | 15    | 14,3       | Transwab|
| V4*       | 13,3 | 15,8  | 14,6       | Transwab|
| V5*       | 13,7 | 15,4  | 14,6       | Transwab|
| H1*       | 14,3 | 16,3  | 15,3       | Viocult|
| H2*       | 15,6 | 17,8  | 16,7       | Viocult|
| H3*       | 16,9 | 18,8  | 16,9       | Transwab|
| H4*       | 17,1 | 18,9  | 18,0       | Transwab|
| H5*       | 17,6 | 19,8  | 18,7       | Viocult|
| H6        | 14,5 | 16,9  | 15,7       | Viocult|
| H7        | 14,5 | 17,2  | 15,85      | Viocult|
| H8        | 16,2 | 18,5  | 17,35      | Viocult|
| H9        | 16,3 | 18,4  | 17,35      | Viocult|
| H10       | 16,5 | 18,3  | 17,4       | Viocult|
| H11       | 17,4 | 19,2  | 18,3       | Viocult|
| H12       | 17,2 | 19,6  | 18,4       | Viocult|
| H13       | 17,3 | 19,5  | 18,4       | Transwab|
| H14       | 17,8 | 20,7  | 19,25      | Transwab|
| H15       | 18,4 | 20,7  | 19,55      | Transwab|
| H16       | 18,3 | 21,2  | 19,75      | Transwab|
| H17       | 19,3 | 20,3  | 19,8       | Viocult|
| M1*       | 19,2 | 21,2  | 20,2       | Viocult|
| M2*       | 19,4 | 21,6  | 20,5       | Viocult|
| M3*       | 20,1 | 22,3  | 21,2       | Transwab|
| M4*       | 20,1 | 22,5  | 21,3       | Transwab|
| M5*       | 20,6 | 22,7  | 21,7       | Viocult|
| M6*       | 20,8 | 23,2  | 22,0       | Viocult|
| M7*       | 21,3 | 24,2  | 22,8       | Viocult|
| M8*       | 21,6 | 24,2  | 22,9       | Transwab|
| M9*       | 22,2 | 24,2  | 23,2       | Viocult|
| M10*      | 22,1 | 24,7  | 23,4       | Transwab|
| M11*      | 22,5 | 24,8  | 23,7       | Transwab|
| M12*      | 23,4 | 25,3  | 24,4       | Transwab|
| M13       | 19,3 | 21,1  | 20,2       | Viocult|
| M14       | 19,5 | 21,5  | 20,5       | Viocult|
| M15       | 19,6 | 22,1  | 20,85      | Viocult|
| M16       | 19,7 | 22,2  | 20,95      | Transwab|
| M17       | 19,8 | 22,3  | 21,05      | Viocult|
| M18       | 20,6 | 22,7  | 21,65      | Viocult|
| M19       | 21   | 23,1  | 22,05      | Viocult|
| M20       | 21,4 | 23,8  | 22,6       | Viocult|
| M21       | 22,9 | 24,9  | 23,9       | Viocult|
| M22       | 23,1 | 25,5  | 24,3       | Viocult|
| M23       | 23,3 | 26    | 24,65      | Transwab|
| L1*       | 25,1 | 27,1  | 26,1       | Transwab|
| L2*       | 25,1 | 27,4  | 26,3       | Transwab|
| L3*       | 25,7 | 28,2  | 27,0       | Transwab|

* Sample IDs identified with an asterisk were tested using the iLACO primer set.
| Sample ID | Ct E | Ct N2 | Average Ct | Medium |
|-----------|------|------|------------|--------|
| L4        | 24.3 | 26   | 25.15      | Transwab |
| L5        | 24.2 | 26.3 | 25.25      | Transwab |
| L6        | 24.3 | 26.2 | 25.25      | Virocult |
| L7        | 24.1 | 26.4 | 25.25      | Virocult |
| L8        | 24.6 | 27.2 | 25.9       | Transwab |
| L9        | 24.4 | 27.5 | 25.95      | Virocult |
| L10       | 25.6 | 27.6 | 26.6       | Virocult |
| L11       | 25.4 | 27.9 | 26.65      | Transwab |
| L12       | 26   | 28   | 27         | Virocult |
| L13       | 25.6 | 28.4 | 27         | ESwab   |
| L14       | 26   | 28.1 | 27.05      | Virocult |
| L15       | 26.3 | 28.1 | 27.2       | Virocult |
| L16       | 26.3 | 28.1 | 27.2       | Transwab |
| L17       | 26.2 | 28.4 | 27.3       | Virocult |
| L18       | 26.1 | 28.6 | 27.35      | Virocult |
| L19       | 26.4 | 28.6 | 27.5       | Virocult |
| L20       | 26.5 | 29.9 | 28.2       | ESwab   |
| L21       | 27.1 | 29.6 | 28.35      | Transwab |
| L22       | 27.1 | 29.7 | 28.4       | Virocult |
| L23       | 27.1 | 29.7 | 28.4       | Virocult |
| L24       | 27.1 | 29.8 | 28.45      | Virocult |
| L25       | 27.3 | 30.1 | 28.7       | Transwab |
| L26       | 27.6 | 30.2 | 28.9       | Virocult |
| L27       | 28.2 | 30.4 | 29.3       | Transwab |
| L28       | 28.3 | 30.3 | 29.3       | Transwab |
| L29       | 28.5 | 30.4 | 29.45      | Virocult |
| L30       | 28.8 | 30.5 | 29.65      | Virocult |
| L31       | 28.3 | 31.2 | 29.75      | Virocult |
| L32       | 28.4 | 31.2 | 29.8       | Transwab |
| L33       | 28.8 | 30.9 | 29.85      | Transwab |
| L34       | 28.5 | 31.5 | 30         | Transwab |
| VL1       | 29.1 | 31.5 | 30.3       | Transwab |
| VL2       | 29.1 | 31.6 | 30.35      | Virocult |
| VL3       | 29.1 | 31.8 | 30.45      | Transwab |
| VL4       | 29.4 | 32.8 | 31.1       | Virocult |
| VL5       | 30   | 32.5 | 31.25      | Transwab |
| VL6       | 29.9 | 32.7 | 31.3       | Virocult |
| VL7       | 30.6 | 33.3 | 31.95      | Virocult |
| VL8       | 30.7 | 34.2 | 32.45      | Transwab |
| VL9       | 31.1 | 34.2 | 32.65      | Virocult |
| VL10      | 31   | 34.6 | 32.8       | Transwab |
| VL11      | 32.3 | 34.5 | 33.4       | Virocult |
| VL12      | 32   | 34.8 | 33.4       | Transwab |
| VL13      | 31.9 | 35.1 | 33.5       | Virocult |
| VL14      | 32.2 | 35   | 33.6       | Transwab |
| VL15      | 31.9 | 35.5 | 33.7       | Transwab |
| VL16      | 32.7 | 34.8 | 33.75      | Transwab |
| VL17      | 32.5 | 35.1 | 33.8       | Transwab |
| VL18      | 32.4 | 35.7 | 34.05      | Virocult |
| VL19      | 33.5 | 35.1 | 34.3       | Transwab |
| VL20      | 33.5 | 35.3 | 34.4       | Transwab |
| VL21      | 34.4 | 35.9 | 35.15      | Virocult |
| VL22      | 33.8 | 36.7 | 35.25      | Virocult |
| VL23      | 33.5 | 37.2 | 35.35      | Transwab |
| VL24      | 33.9 | 37.3 | 35.6       | Virocult |
| VL25      | 34.9 | 36.9 | 35.9       | Virocult |
| VL26      | 34.5 | 37.4 | 35.95      | Virocult |
| VL27      | 34.7 | 37.3 | 36         | Virocult |
| VL28      | 34.4 | 37.6 | 36         | Virocult |
| VL29      | 34.7 | 37.5 | 36.1       | Virocult |
| VL30      | 35.5 | 36.8 | 36.15      | Virocult |
| VL31      | 35.6 | 36.8 | 36.2       | Transwab |
| VL32      | 35.2 | 37.6 | 36.4       | Transwab |
| VL33      | 35.5 | 38   | 36.75      | Transwab |
| VL34      | 35.1 | 38.8 | 36.95      | Transwab |
| VL35      | 37.6 | 36.8 | 37.2       | Transwab |
| VL36      | 36.4 | 39.4 | 37.9       | Transwab |
| VL37      | 37   | 40.2 | 38.6       | Virocult |
| VL38      | 38.7 | 40.1 | 39.4       | Transwab |
| VL39      | 38.8 | 40.7 | 39.75      | Virocult |
| VL40      | 42   | 38.2 | 40.1       | Virocult |
| Sample ID | Ct E | Ct N2 | Average Ct | Medium  |
|-----------|------|-------|------------|---------|
| VL41      | 40.1 | 41.1  | 40.6       | Transwab|
| VL42      | 44.6 | 42.3  | 43,45      | Transwab|
| N1*       | NA   | NA    | NA         | Virocult|
| N2*       | NA   | NA    | NA         | Virocult|
| N3*       | NA   | NA    | NA         | Virocult|
| N4*       | NA   | NA    | NA         | Virocult|
| N5*       | NA   | NA    | NA         | Transwab|
| N6*       | NA   | NA    | NA         | Transwab|
| N7        | NA   | NA    | NA         | Transwab|
| N8        | NA   | NA    | NA         | Transwab|
| N9        | NA   | NA    | NA         | Transwab|
| N10       | NA   | NA    | NA         | Transwab|
| N11       | NA   | NA    | NA         | Transwab|
| N12       | NA   | NA    | NA         | Transwab|
| N13       | NA   | NA    | NA         | Transwab|
| N14       | NA   | NA    | NA         | ESwab   |
| N15       | NA   | NA    | NA         | Transwab|
| N16       | NA   | NA    | NA         | Virocult|
| N17       | NA   | NA    | NA         | Transwab|
| N18       | NA   | NA    | NA         | Transwab|
| N19       | NA   | NA    | NA         | Virocult|
| N20       | NA   | NA    | NA         | Transwab|
| N21       | NA   | NA    | NA         | ESwab   |
| N22       | NA   | NA    | NA         | Transwab|
| N23       | NA   | NA    | NA         | Transwab|
| N24       | NA   | NA    | NA         | Transwab|
| N25       | NA   | NA    | NA         | Virocult|
| N26       | NA   | NA    | NA         | Virocult|
| N27       | NA   | NA    | NA         | Virocult|
| N28       | NA   | NA    | NA         | Transwab|
| N29       | NA   | NA    | NA         | Transwab|
| N30       | NA   | NA    | NA         | Transwab|
| N31       | NA   | NA    | NA         | Virocult|
| N32       | NA   | NA    | NA         | Transwab|
| N33       | NA   | NA    | NA         | Transwab|
| N34       | NA   | NA    | NA         | Virocult|
| N35       | NA   | NA    | NA         | Transwab|
| N36       | NA   | NA    | NA         | Virocult|
| N37       | NA   | NA    | NA         | Virocult|
| N38       | NA   | NA    | NA         | Virocult|
| N39       | NA   | NA    | NA         | Transwab|
| N40       | NA   | NA    | NA         | Transwab|
| N41       | NA   | NA    | NA         | Transwab|
**Figure S6.** Detection of SARS-CoV-2 in heat inactivated nasopharyngeal swab samples using the iLACO primer set. The samples in Table 1 were grouped in 5 categories, namely (1) very high – average Ct <15 (red), (2) high – Ct between 20 and 15 (yellow), (3) medium – Ct between 20 and 25 (green), (4) low – Ct > 25 (blue) and (5) negative (dark blue). Color coding applies to A, B and C. A- RT-LAMP analysis of clinical samples using a benchtop real-time thermocycler (micPCR). All samples were measured in duplicate. The inset plot shows the correlation between average PCR Ct value of each sample and time to positivity (TtP) measured for RT-LAMP. TtP was determined as the required incubation time at 65°C to increase the fluorescence intensity above the threshold. The threshold was fixed as the highest background fluorescence (before amplification) measured among all tested samples. The $r$ value is the Pearson correlation coefficient. B- Measurement of 2 samples in each Ct range using the integrated on-chip platform with an incubation time of 25 or 30 min at 65°C. Scale bars: 5 mm. C- Relative signal measured at pixel 50 for each nasopharyngeal swab sample. Relative values below the threshold are considered positive for SARS-CoV-2 RNA. The threshold and relative signal values in B and C were determined as previously described in section 2.3.
Figure S7. Comparison of positive to negative fluorescence ratio for each As1e and iLACO primer sets in the presence and absence of NBNM beads. All images were acquired on the disc (at room temperature) using a smartphone with a constant ISO of 800. In the case of the channels without the beads, the signal is quantified as average fluorescence, whereas for the channels with beads the fluorescence is quantified using a line profile at the bead-liquid interface as described in the manuscript. All signal quantifications were performed using only the green channel. The negative and positive samples were composed of RNase/DNase free water without or with $10^4$ copies of SARS-CoV-2 full length synthetic RNA (Twist Bioscience, CA, USA, Control 2, GenBank: MN908947.3).
Figure S8. Detection of SARS-CoV-2 RNA in heat inactivated nasopharyngeal swab samples on the integrated platform (As1e primer set). Relative signal values were measured at pixel 50 according to section 2.3. Relative signal values above the gray band are considered negative while values below the gray band are considered positive. Values falling on the gray band are considered inconclusive. The upper and lower ranges of the grey band are calculated as 3.29σ (99.9% confidence interval threshold) of 9 independent measurements of negative and positive controls, respectively. Green and red lines indicate the mean relative signal of the negative and positive controls, respectively. The negative and positive controls were composed of RNase/DNase free water without or with $10^4$ copies of SARS-CoV-2 full length synthetic RNA (Twist Bioscience, CA, USA, Control 2, GenBank: MN908947.3).
Figure S9. Representative raw smartphone photos taken on the integrated platform for five negative and five positive nasopharyngeal swab samples.
Table S2. Cost estimate of the key components required to assemble the integrated platform. PMMA sheets required to assemble the box and fabricate disposable discs are not included since prices are highly dependent on scale and expected to represent a small fraction of total production costs.

| Item                                           | Price ($) | Quantity | Total ($) |
|------------------------------------------------|-----------|----------|-----------|
| Silicone Heater Mat                            | 50        | 2        | 100       |
| Type K Thermocouple                           | 6.8       | 2        | 13.6      |
| Thermocouple Amplifier                       | 18.3      | 2        | 36.6      |
| IRF540PBF N-Channel MOSFET                    | 1.3       | 2        | 2.6       |
| Osram Opto PL 450B Blue Laser Diode           | 22        | 1        | 30        |
| LM217LZ-TR Linear Voltage Regulator           | 0.4       | 1        | 0.4       |
| Polyimide absorption filter                   | 75 (5 sheets) | 1/10 sheet | 1.5 |
| Bipolar Hall Effect Sensor                    | 2.5       | 1        | 2.5       |
| Arduino UNO                                   | 24        | 1        | 24        |
| EMax 2204 + ESC (Motor)                       | 24        | 1        | 24        |
| **Grand Total**                               | **235.2** |          |           |