Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary Information

Portable self-flowing platform for filtration separation of samples

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Microfluidic channel

The manufacturing process of the microfluidic channel is shown in Fig. S1. The mold is made using the soft lithography process. The photoresist used is JSR-126N, which is a negative thick photoresist with high strength. If the spin-coating speed is controlled appropriately, the required microfluidic channel height can be formed easily. A brass substrate is cleaned and dried first and then spin-coated with JSR-126N. After soft baking and photolithography exposure, the photoresist is developed using KTD-1. Then, it is cleaned with deionized water and blow dried. The last procedure involves hard baking to form the mold. The PDMS is poured over the mold, cured by heating, and removed from the mold to obtain a PDMS microfluidic channel.

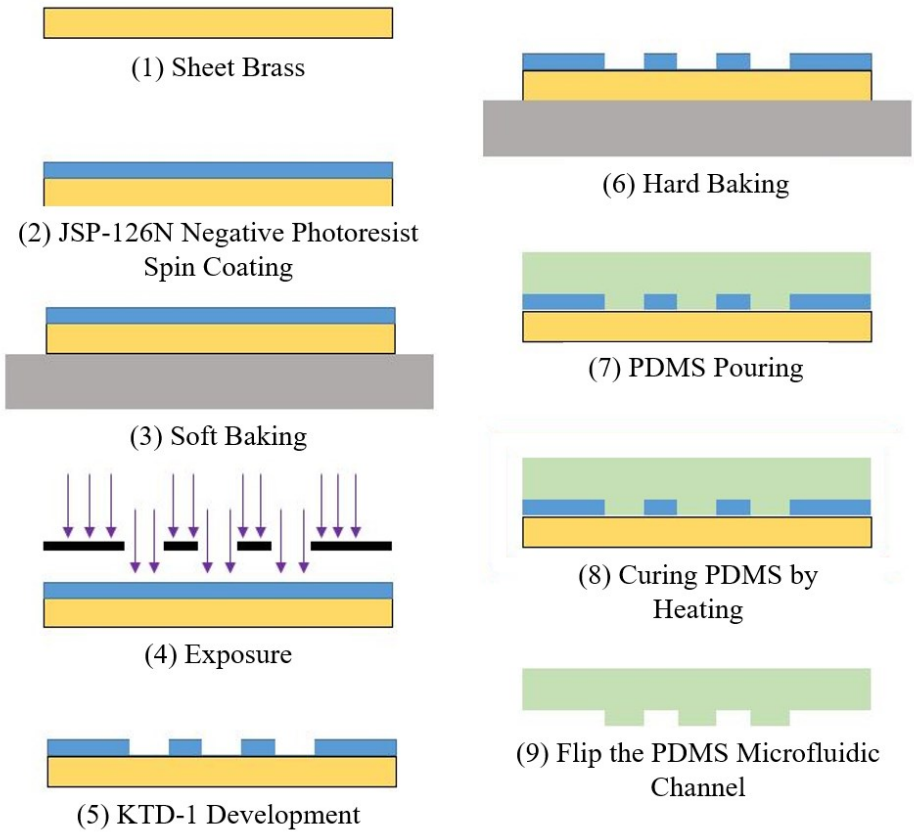

Fig. S1 Manufacturing process of the PDMS cover with microfluidic channels.

The photomask used in the manufacturing process of the microfluidic channel is first designed using SolidWorks software. The size of the microfluidic channel model must be designed well corresponding to the position of the electrode of the biosensing chip and converted to a 2D photomask pattern using AutoCAD software. The photomask was fabricated by the Taiwan Kong King Co., Ltd. (TKK) with an emulsion film material. This microfluidic channel contains the wafer detection zone and microfluidic channel pump, among others. Fig. S2 shows the design of the photomask for microfluidic channel manufacturing. The diameter of the mask is 4 inches. Four microfluidic channel patterns are arranged on the substrate, each of which is 23 mm long and 21 mm wide. The width of each microfluidic channel is 300 μm, while the width of the excess liquid removal channel is 500 μm. The excess liquid removal channel just after the separation zone is employed to remove the excess liquid of the bead solution once the beads are filled and centrifuged, thereby preventing this liquid from flowing into the wafer detection zone and affecting subsequent inspection. The width of the comb-shaped microfluidic channel pump is 400 μm.

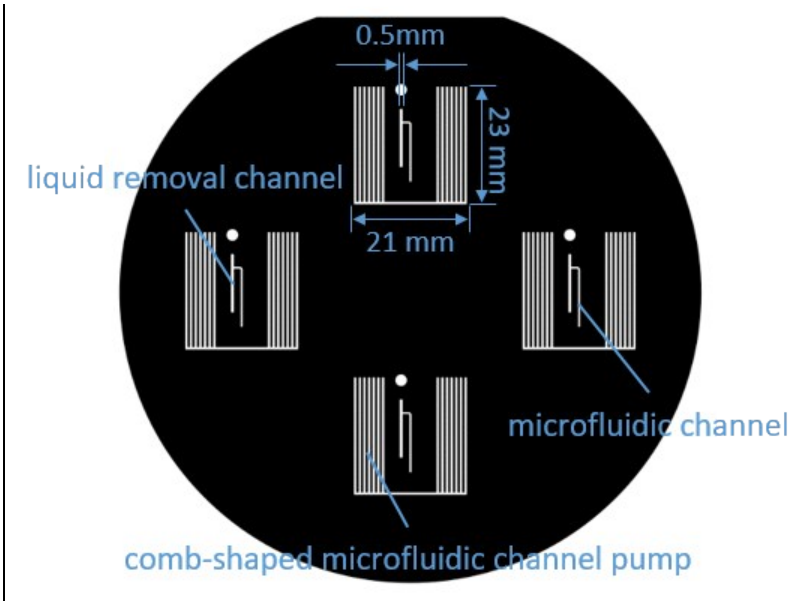

Fig. S2 Photomask design for the microfluidic channel manufacturing process.

The microfluidic channel is made of PDMS combined with the polymethylmethacrylate (PMMA) substrate of the chip. When the separation platform is placed in a vacuum packaging machine, the pressure in the microfluidic channel decreases. As the PDMS is gas-permeable, when the device is degassed and placed in air, pressure can be generated in the microchannel before use. Because of the porosity of PDMS, it can maintain the negative pressure state for a longer duration and drive the liquid into the microfluidic channel to flow toward the detection zone. Once the mold of the microfluidic channel is complete, the acrylic fixture is placed on the brass sheet mold, as shown in Fig. S3, and the PDMS is poured over the fixture. Then, the device is heated in an oven at 70 °C for 1 h to cure the PDMS. Finally, the PDMS microfluidic channel is removed from the mold, as shown in Fig. S4.

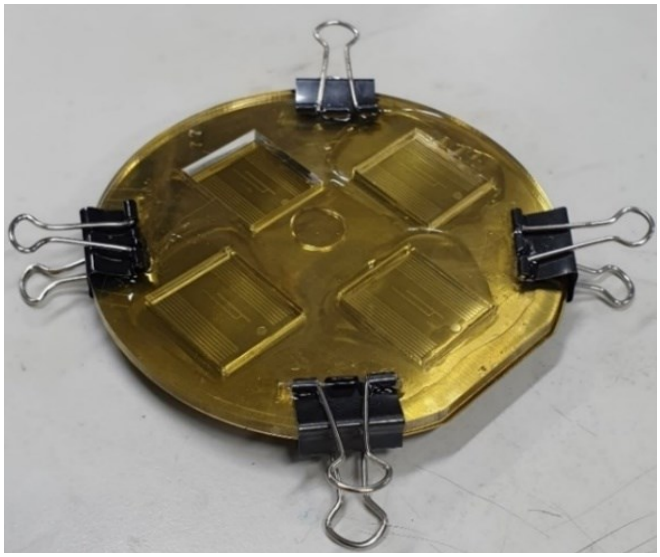

Fig. S3 Microfluidic channel mold.

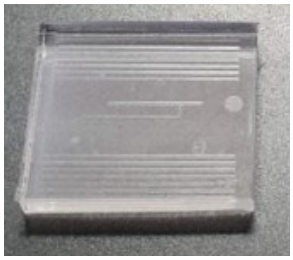

Fig. S4 Microfluidic channel made of PDMS.

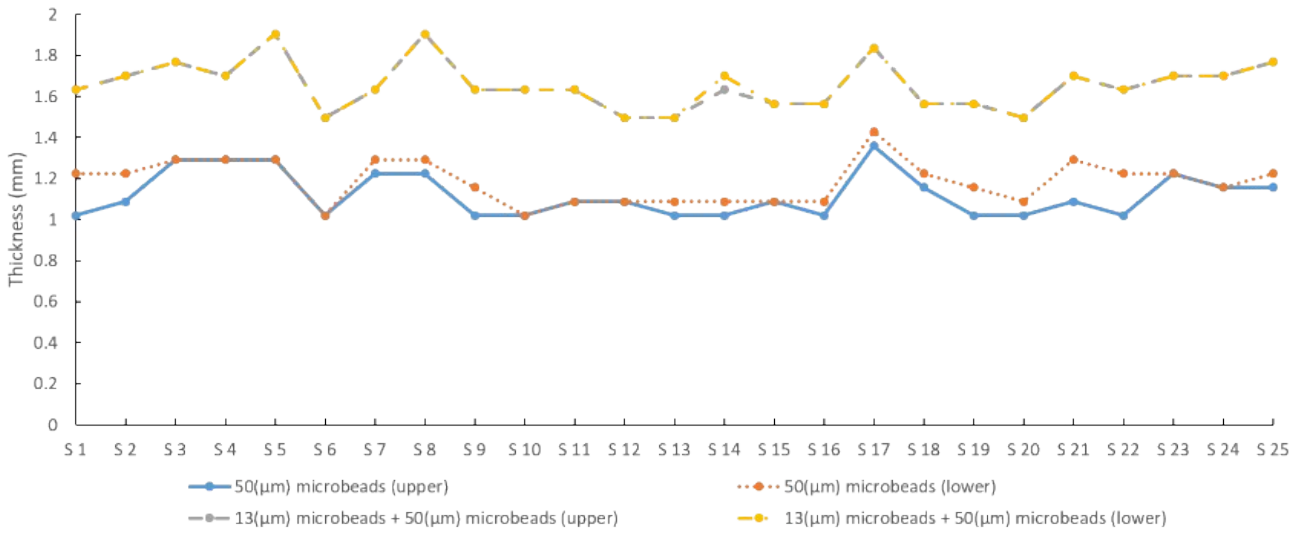

Fig. S5 Experiment on the optimal filling amount of microbeads (S1–S25 on the X-axis indicate the sample numbers).

Package of the portable separation platform

The portable separation platform is divided into three parts, including the biosensing chip package, PDMS package, and vacuum package, as follows:

1. Biosensing chip package: Commercially available AB adhesives or injection molding methods are used to embed the chip in the PMMA substrate before connecting the chip to the gold fingers of the signal output zone on the printed circuit board via wire bonding and using commercially available AB adhesive to protect the wire bonded part.
2. PDMS package: The molded PDMS microfluidic channel is pasted on the acrylic substrate. When pasting, the air between the PDMS and substrate is squeezed out with tweezers. PDMS can stick to the acrylic substrate owing to its adhesiveness. We then use AB adhesives to seal the four sides of the PDMS to ensure that the microfluidic channels are not easily separated from the substrate during transportation or use and to prevent air leakage.
3. Vacuum package: The microfluidic separation platform is placed in a protective box and then inserted into a vacuum bag; the vacuum bag is then placed in the Fuserjoy (VA420, Taiwan) vacuum packaging machine for sealing. The total vacuum time is 50 minutes and sealing time is 1 s; the cooling time is 3 s and vacuum used is 760 Torr.

Discussion on sample separation time and detection zone filling

Fig. S6A is an isometric view of the separation platform, and Fig. S6B is the front view. When the gap between the PDMS cover and chip is 0.2 mm, as shown in Fig. S6C, the average time required for placing the blood sample in the separation zone, separation, and flow out from the separation zone is about 56 s; then, the filling time of the detection zone is about 319 s. When the gap is 0.5 mm, as shown in Fig. S6D, the corresponding average time is about 80 s, and the filling time is about 3,487 s, as shown in Fig. S7. Therefore, for a smaller gap or volume between the PDMS cover and chip, the total time consumed is lesser. However, if the gap is 0.1 mm, the PDMS cover easily contacts the chip during or after vacuuming and cause the chip surface to be damaged. Therefore, the gap between the PDMS cover and chip should not be too small; an approximate size of 0.2 mm is optimal.

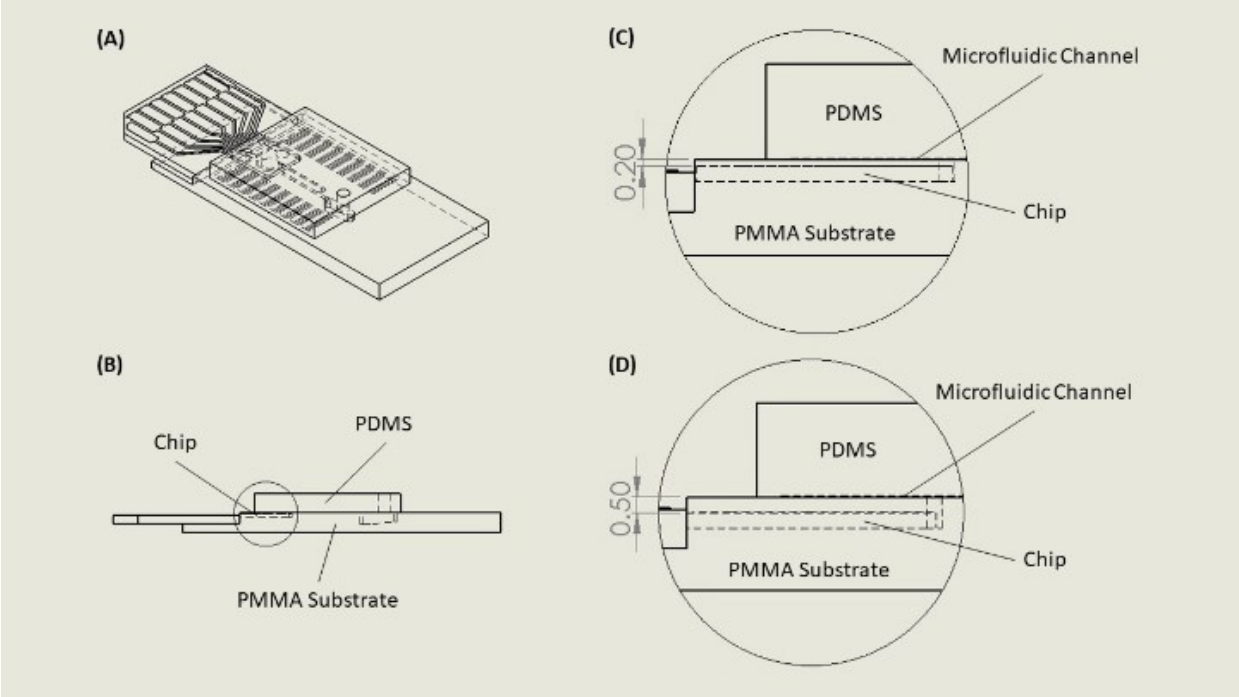

Fig. S6 Technical drawing of the separation platform: (A) isometric view of the separation platform; (B) front view of the separation platform; (C) gap of 0.2 mm between the PDMS cover and chip (5:1 drawing of the partially enlarged selected area in front view); (D) gap of 0.5 mm (5:1 drawing of the partially enlarged selected area in front view). The thickness of the PDMS is 3 mm, and the chip size in the detection zone is 7.4 mm × 4.1 mm.

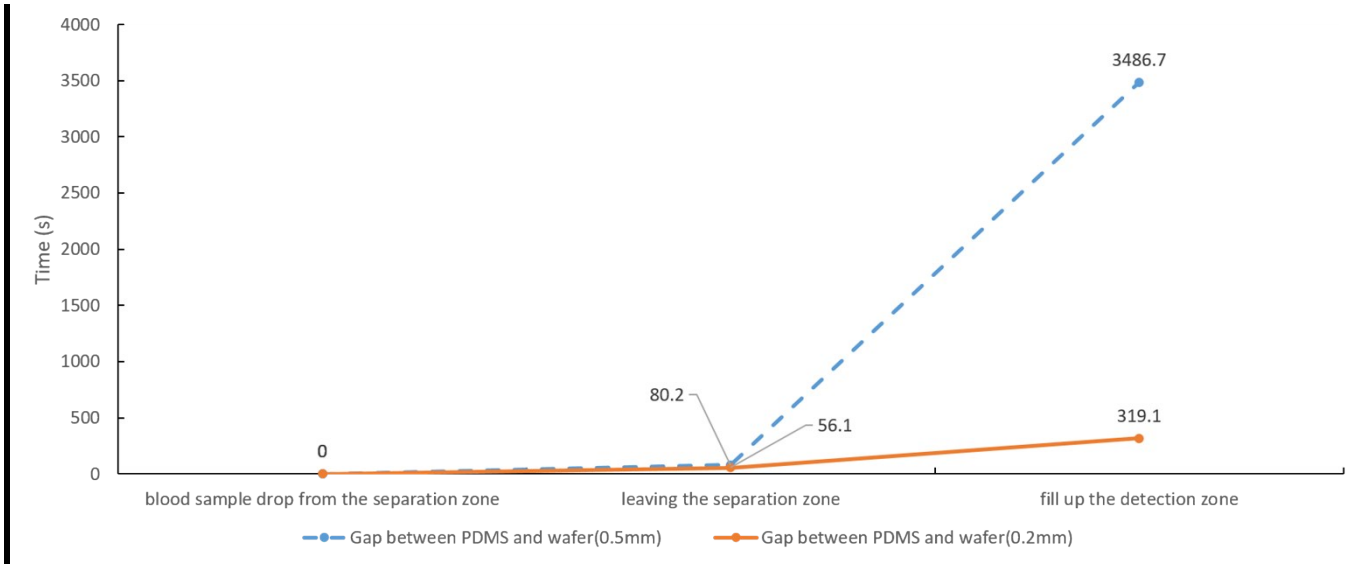

Fig. S7 Average time required for the blood sample dropped on the separation zone to undergo separation, leave the separation zone, and fill the detection zone.

The raw milk samples used in the experiments were obtained from the Taiwan Advance Bio-Pharmaceutical Inc. and stored at -20 °C after subassembly; the samples were defrosted only when needed. The casein and fat globules of raw milk will interfere with the electrochemical sensing electrodes on the chip. Therefore, it is necessary to separate these components at the front end. The samples separated using the platform in this study are transparent and colorless. Only biomarkers, such as proteins, viruses, and small bacteria are left in the sample after separation; the sample can be quickly separated anytime and anywhere without the need for traditional laboratory separation equipment.

As shown in Fig. S8, five separation platform samples were used in this research for testing. The platforms were all placed in vacuum for 50 min and packaged. The average time required to place a raw milk sample on the separation zone, separation, and leaving the separation zone is about 45.8 s, and the filling time of the detection zone is about 246.4 s.

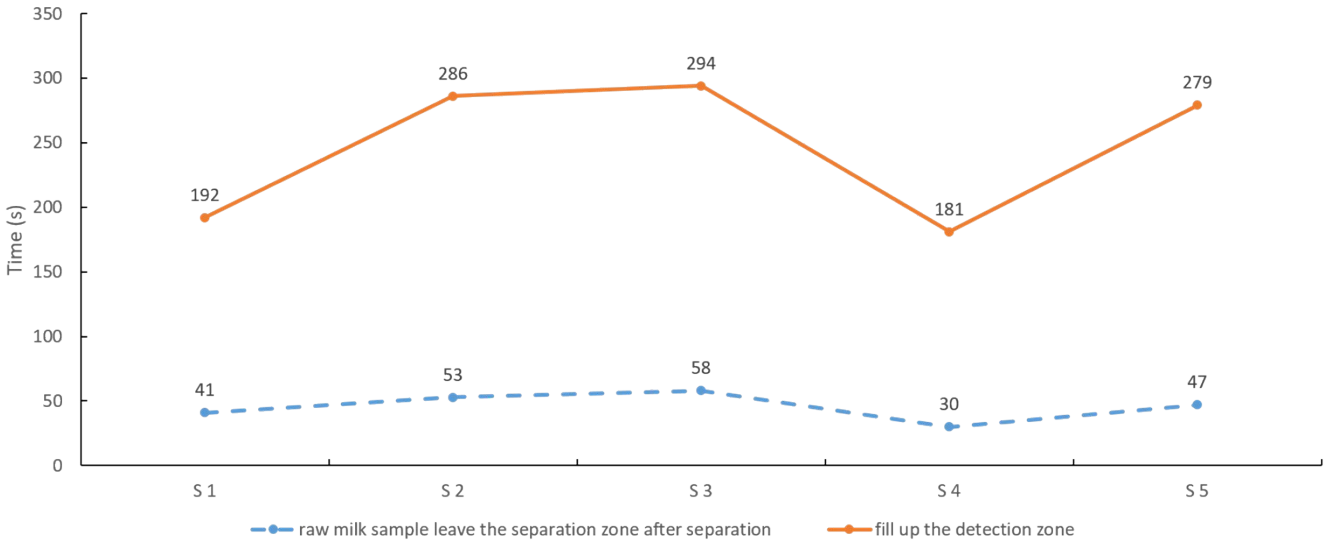

Fig. S8 Time required for placing the raw milk sample on the separation zone, separation, leaving the separation zone, and filling the detection zone (S1–S5 on the X-axis indicate the sample numbers).

Calculation of the pass rate of *E. coli*

After passing the mixed stool sample and *E. coli* solution through the filter device, the pass rate of *E. coli* is calculated.

Since the concentration of the *E. coli* solution before passing through the filter can be measured first by spectrophotometer,

subtract the "'Difference in OD before and after passing' of 'Stool sample' "[1,2,3] from the "'Difference in OD before and after passing' of 'Stool sample and *E. coli* solution mixture'"; then calculate the concentration of *E. coli* after passing through the filter. From this, the pass rate of *E. coli* is known.

Consider one of the samples with (volume of *E. coli* solution): (volume of stool sample) = 1:1 as an example:

- OD_{600} (stool sample inlet)
  - OD_{600} (stool sample outlet)

  = 0.218 244 625
- OD_{600} (stool sample + *E. coli* solution inlet)
  - OD_{600} (stool sample + *E. coli* solution outlet)

  = 0.241 5
- Thus, the concentration change of the *E. coli* solution is
  = 0.241 5 - 0.218 244 625/2 = 0.132 377 687 5
- Hence, the concentration of the *E. coli* solution at the outlet
  = 1.408 9/2 (*E. coli* solution inlet) - 0.132 377 687 5 (concentration change of the *E. coli* solution)

  = 0.572 072 312 5
- Pass rate of E. coli (η) = $(\frac{C_o}{C_i}) \times 100\%$
  =0.572 072 312 5/ (1.408 9/2) = 81.21%

(C_i is the *E. coli* concentration at the inlet; C_o is the *E. coli* concentration at the outlet)

Instructions for using the portable sample separation platform

Fig. S9 shows the instructions for using the proposed portable sample separation platform. If the sample is blood, first clean the fingertip from which the sample is to be collected with an alcohol swab, prick the fingertip using a lancet, and squeeze the a drop of blood. The next steps can be applied to all four types of samples examined in this work. The first step involves drawing the sample with a medical syringe; the second step involves placing a required amount of the samples in the included solution cup and gently stirring with the internal solution, after which the sample is drawn with a syringe upon mixing evenly; the third step involves removing the vacuum packaging of the sample separation platform; the fourth step involves injecting the mixed sample into the inlet port of the separation zone; the final step involves waiting for the sample to separate and fill the detection zone. The device is very easy to operate; hence, its operation can be transferred to nurses, non-professionals, or even the patient's family members or patients themselves.

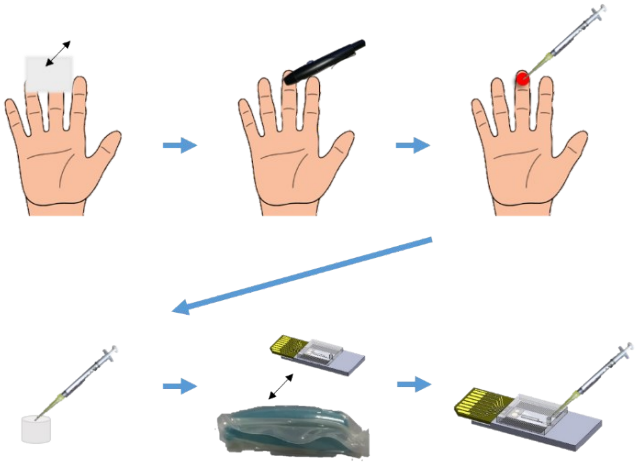

Fig. S9 Instructions for using the portable sample separation platform.

References

[1] T. Shimizu, T. Fujii, H. Haruna, H. Shoji, T. Kudo and Y. Yamashiro, *J. Paediatr. Child Health*, 2003, **39**, 286-288.

[2] D. Raguza, C. F. H. Granato and E. Kawakami, *Dig. Dis. Sci.*, 2005, **50**, 453-457.

[3] M. Matsuda, Y. Noda and Y. Takemori, *J. Gastroenterol.*, 2003, **38**, 222-228.