Diagnostics

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Pooled testing via magnetized droplets on a chip

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Pooled testing for the diagnosis of COVID-19 via isothermal nucleic acid amplification and detection can be automated by using electromagnetically actuated swarms of millimetric magnets to handle droplets of magnetized samples on a microfluidic chip.

In an epidemic, finding who is infected can be accelerated by sample pooling. In pooled testing for viral infections, the diagnostic test is performed once after mixing the collected specimens. If the test is negative, all samples are negative for the tested virus; if the test is positive, then each sample is re-tested (often, without additional sample pooling). Pooling strategies are typically designed to maximize the efficiency of screening of the population, according to the local prevalence of the virus[1]. Pooled testing can therefore be a fast and cost-effective way for triaging individuals suspected of infection. However, high-throughput pooled testing involves automated liquid handling via robotic instrumentation, which constrains its use to centralized laboratory settings. Now writing in *Nature*, Sam Emaminejad, Dino Di Carlo and colleagues report that pooled testing can be performed on a microfluidic chip[2]. The chip leverages swarms of electromagnetically actuated millimetric magnets to pool the liquid samples, to amplify viral genes via loop-mediated isothermal amplification (LAMP) and, by using specially designed algorithms, to detect which samples are positive for the virus. The chip provides a low-cost solution for the point-of-care adoption of pooled viral testing.

Emaminejad and co-authors' chip consists of a programmable printed circuit board with fluidic channels on top, and of individually addressable millimetric magnets (or 'ferrobots') underneath to programmatically manipulate magnetized droplets of sample (or 'ferro-droplets', as they are spiked with magnetic nanoparticles) containing the reagents for gene amplification (Fig. 1a,b). The authors show that tens of ferrobots can be individually addressed to direct the motions of ferro-droplets, so as to carry out the necessary steps — such as aliquoting and droplet merging, mixing or heating — for automated nucleic acid amplification. Aliquoting was carried out precisely by making use of corrugated structures along which ferrobots were directed to dispense a volume of aliquot. The volume could be adjusted by altering the corrugated structures, from 100 nl to 10 µl. Droplet merging was achieved by applying a relatively low voltage (0.3–1.5 V) across fluidic channels. Electromagnetic oscillation of ferrobots at 5 Hz led to the mixing and homogenization of the contents of the merged droplets. And resistive heater elements on the printed circuit board were used to carry out sample lysis and reverse transcription LAMP (RT-LAMP). The amplified genes were detected colorimetrically, either via naked eye or electronically via optical detectors integrated in the board. The on-chip assay was 100% concordant with a standard (off-chip) RT-LAMP assay, and for a set of 100 clinical samples it achieved 98% sensitivity and 100% specificity compared with gold-standard reverse transcription polymerase chain reaction (RT–PCR). Notably, the on-chip assay was robust and reliable; the authors show that operations of aliquoting, merging and transportation led to variations in droplet diameters of less than 1% over 800 repetition cycles.

To carry out assays at high throughput, Emaminejad and co-authors integrated, into a single chip, multiple microfluidic testing units with ferro-droplets and ferrobots. In particular, for pooled testing, the authors constructed 4^2 and 3^2 arrays of connected units (with 9 and 4 ferrobots in each array, respectively), with the RT-LAMP assay regions located at the outer sides of the chip (at the ends of each column and row). Then the authors implemented the following pooling scheme (Fig. 1c): (1) the samples are divided in aliquots at all the sample regions; (2) one aliquot of each sample region is electromagnetically transported and mixed into a single RT-LAMP assay region on one corner of the chip; (3) if all the tests on the side of the chip are negative, then all samples are negative, and the assay ends; (4) if any of the test regions gives a positive result, the next round of testing involves column pooling and row pooling. In this case, the second and third aliquots of all sample regions are transported to the end of each row and column, respectively. (5) Positive samples are identified as those at the intersections of columns and rows with positive RT-LAMP results. With this sample-pooling scheme, only two rounds of nucleic acid amplification are needed to identify all virus-infected samples. At low prevalence of the virus, the approach would substantially increase the efficiency of testing.

Before the multiplexed chip can be implemented as a point-of-care device for the screening or the triaging of patients, the technology would need to be optimized for accuracy, speed and portability. For instance, the chip could leverage recent ultrafast RT–PCR or CRISPR-based assays (CRISPR stands for clustered regularly interspaced short palindromic repeats) to increase speed and accuracy[3,4]. Also, adapting the chip to leverage lyophilized reagents would dispense the need for refrigeration and hence enhance its portability and usability. And the versatility of the chip's design could provide additional functionalities that allow for its fully automated use in resource-limited settings.

Emaminejad and colleagues' work is exemplary of how technological integration across fields of research — microfluidics, nanotechnology, biochemical assays and robotic actuation, in particular — can be leveraged for real-world needs in health care. Automated liquid handling at high throughput has been challenging to realize by using pressurized flows in conventional microfluidic systems. The use of magnetic nanomaterials in liquid droplets is a breakthrough in this regard, as it enables the asynchronous transportation of microdroplets programmatically and at scale. The technology could constitute a platform for the handling of liquid droplets across a wide range of

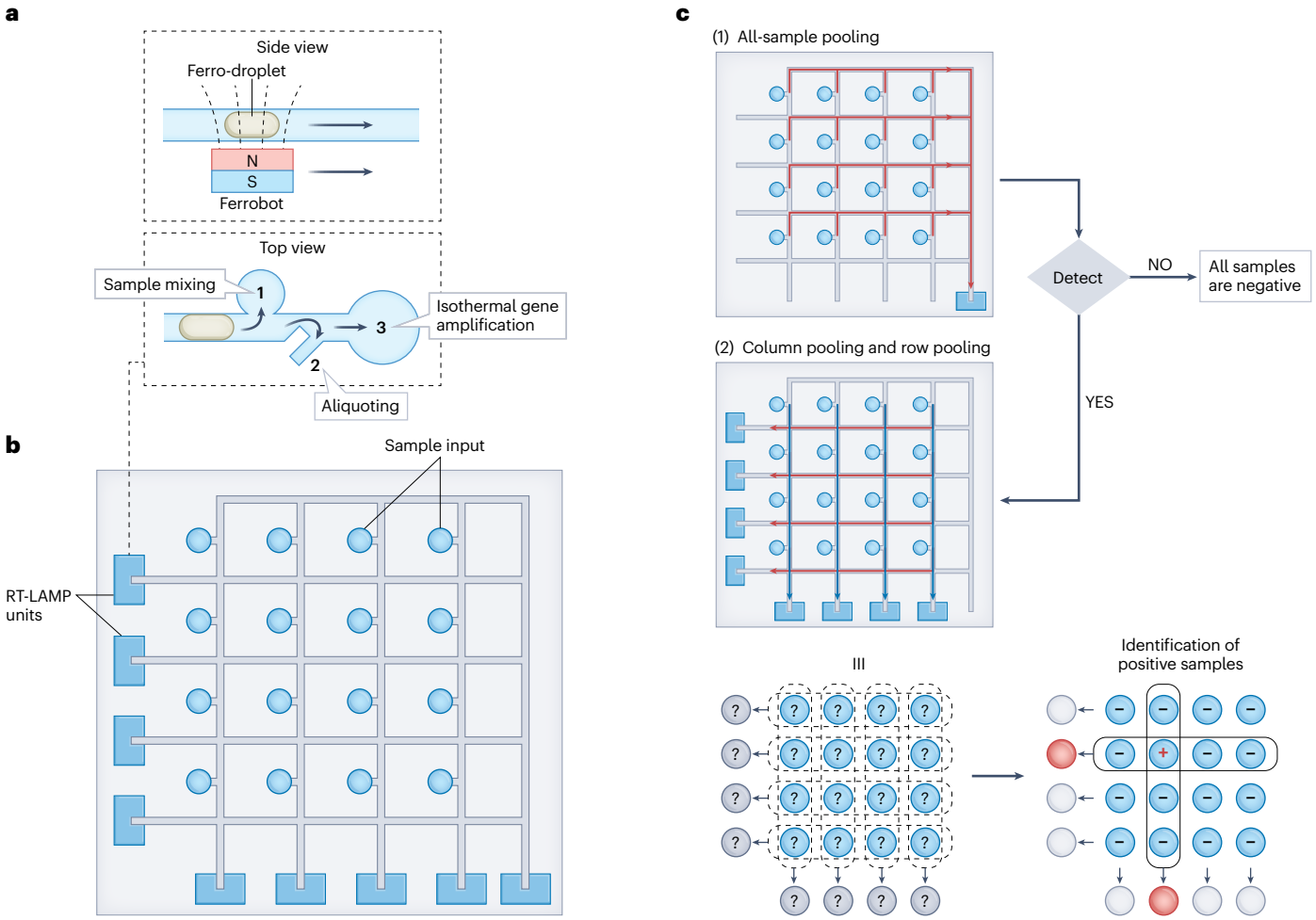

Fig. 1 | Automated testing of pooled samples. a, Millimetric magnets ('ferrobots') are used to electromagnetically move microdroplets spiked with magnetic nanoparticles ('ferro-droplets') within channels in a microfluidic chip. Compartments in the testing units are designed to perform sample mixing, ferro-droplet aliquoting, and RT-LAMP. **b**, A 4^2 chip array for performing multiple RT-LAMP assays simultaneously. **c**, Pooled-testing scheme implemented in a 4^2 chip array, and involving 2 rounds of RT-LAMP assays: (1) all-sample pooling; and (2) column pooling and row pooling. If the RT-LAMP result from testing all pooled samples is positive, the infected samples (indicated with a '+') correspond to those at the intersections between rows and columns with positive results (red circles). Figure adapted with permission from ref. 2, Springer Nature Ltd.

biomedical applications in, for example, diagnostics, nucleic acid sequencing and drug discovery.

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Competing interests

The authors declare no competing interests.