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# Magnetic particles for integrated nucleic acid purification, amplification and detection without pipetting

Yanju Chen [a], Yang Liu [b], Ya Shi [b], Jianfeng Ping [a], Jian Wu [a, c, *], Huan Chen [b, **]

[a] College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou, 310058, China
[b] Key Laboratory of Microbiol Technology and Bioinformatics of Zhejiang Province, Zhejiang Institute of Microbiology, Hangzhou, 310012, China
[c] Key Laboratory of on Site Processing Equipment for Agricultural Products, Ministry of Agriculture, Hangzhou, 310058, China

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ABSTRACT

Nucleic acid amplification based detection plays an important role in food safety, environmental monitoring and clinical diagnosis. However, traditional nucleic acid detection process involves transferring liquid from one tube to another by pipetting. It requires trained persons, equipped labs and consumes lots of time. The ideal nucleic acid detection is integrated, closed, simplified and automated. Magnetic particles actuated by magnetic fields can efficiently adsorb nucleic acids and promote integrated nucleic acid assays without pipetting driven by pumps and centrifuges. We will comprehensively review magnetic particles assisted integrated system for nucleic acid detection and hope it can inspire further related study.



## 1. Introduction

In recent years, nucleic acid amplification technologies have made great progress, which promotes the wide applications of nucleic acid detection [1,2]. The increasing number of sudden infectious diseases are great threats to public healthcare, for example, Ebola virus [3], Zika virus [4] and more recently SARS-CoV-2 [5]. In this case, point-of-care testing (POCT) for the pathogens is required. The on-site detection technologies of pathogens mainly include immunological detection and nucleic acid detection.

However, during outbreaks of infectious disease, compared with immunological methods, nucleic acid detection is a direct method with higher sensitivity and the preparation of kits for nucleic acid detection takes less time [6]. There are many amplification free [7] and amplification based [8] methods for nucleic acid detection. In traditional molecular testing labs, the experimenters need to move the liquid from one tube to another. The sensitivity of nucleic acid detection can increase after amplification. However, pipetting based nucleic acid assays have two disadvantages. One is that complex operating steps cannot meet the requirements of POCT [9]. Another is that target formed aerosol after amplification may contaminate the environment [10], especially some highly infectious viruses which may put technicians in danger. Quantitative polymerase chain reaction (qPCR) has become the standard of some infectious disease detection. Although the amplification and detection steps of qPCR are combined in one closed tube, making nucleic acid detection convenient and time-saving [11], the extraction and purification steps are not integrated.

To avoid the problems mentioned above, an integrated, closed, simplified and automated analysis system for POCT is urgently needed. Recently, microfluidic technology was well developed. It has been widely used in life science, environmental science and diagnosis. Integrated microfluidic chips are gradually moving from laboratory research to commercial application [12]. Microfluidics technology replicates testing functions of traditional biochemical laboratory on a chip but it is difficult to move small amounts of liquid in tight spaces. To solve it, most of microfluidic chips with narrow aisles require pumps, valves or centrifuges to control the liquid transfer, which limits their application and promotion in

*Abbreviations:* POCT, point-of-care testing; PCR, polymerase chain reaction; qPCR, quantitative PCR; ICP-AES, inductively coupled plasma atomic emission spectroscopy; TEM, transmission electron microscopy; DLS, dynamic light scattering; XRD, X-Ray diffraction; SQUID, superconducting quantum interference device magnetometer; FMR, ferromagnetic resonance; GTC, guanidinium thiocyanate; PEG, polyethylene glycol; ATP, adenosine triphosphate; IFAST, immiscible filtration assisted by surface tension; RPA, recombinase polymerase amplification; LAMP, loop-mediated isothermal amplification.

* Corresponding author. College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou, 310058, China.
** Corresponding author.
*E-mail addresses:* wujian69@zju.edu.cn (J. Wu), chenhuan7809@gmail.com (H. Chen).

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portable POCT. Some capillary flow assay based or paper based chips can avoid using external equipment to pipette when preventing and monitoring public health outbreaks [13,14]. However, if the analysis channel is long or the liquid composition is complicated, the detection sensitivity will be significantly affected by the liquid evaporation. In summary, an integrated nucleic acid purification, amplification and detection system without pipetting is needed.

The trend of nucleic acid detection is moving towards automation, efficiency and point-of-care detection. Magnetic particles are good tools for nucleic acid purification. Many magnetic bead extraction kits have become commercial products [15]. They can move under the magnetic field, avoiding removing the lid and pipetting, which contributes to the integration, portability and rapidity of nucleic acid assays [16]. There are several advantages of magnetic particles assisted nucleic acid assays. Firstly, it is a safe and environmentally friendly nucleic acid extraction method compared with some chemical methods [17]. Secondly, researchers can choose different types and sizes of magnetic particles according to their samples, which is beneficial to improve extraction efficiency and reduce costs [18]. Finally, the magnetic particles can be driven under the magnetic fields with no limitation of liquid volume. It is promising for the integrated nucleic acid purification, amplification and detection [19,20]. Electromagnet strategy can not only promote the automation of the integrated system, but also help the full mixing of magnetic particles and reaction liquids. The presence of electromagnet will contribute to the automation of integrated nucleic acid detection platform. When using the electromagnet, the magnetic field can be controlled by power-on and power-off. The strength of the magnetic field can be controlled by the intensity of current. In this way, we can control the speed of magnetic particles when passing through different reaction tanks by controlling the current intensity. Also, it will be convenient to accelerate the full mixing of magnetic particles with reaction liquids by electromagnetic field [21].

However, there are few review articles on the topic of applications of magnetic particles for the integration of nucleic acid purification, amplification and detection in the closed system. van Reenen et al. [22] and Yin et al. [23] discussed integrated nucleic acid detection microsystem. They just present pipetting based detection methods which need to move liquids under the help of pumps or centrifuges. They have no introduction about magnetic particles. Hejazian et al. [24] and Berensmeier [25] focused on the function of magnetic particles for the separation and purification of target but they did not involve nucleic acid amplification and detection. Jamshaid et al. [26] and Mauk et al. [27] introduced magnetic particles assisted integrated system but most of contents are based on immunoassay detection rather than nucleic acid. Above all, there is still a big room for our review to give a summary about applications of magnetic particles for integration of nucleic acid purification, amplification and detection avoiding pipetting. Our review will provide a comprehensive review on magnetic particles assisted integrated nucleic acid purification, amplification and detection without pipetting. The advantages and major challenges of it will be presented. We do hope our review can provide an overview for future researchers and inspire more studies on it.

## 2. Magnetic particles

### 2.1. The synthesis and characterization of magnetic particles

The magnetic particles with small size in diameter have some magnetic properties different from conventional materials. The superparamagnetic particles do not have magnetic interactions or aggregations without external magnetic field, so they can be well dispersed in solution and sufficiently adsorb the targets [28]. Magnetic particles can be divided into three categories: metal oxides, pure metals and magnetic alloys. Co, Fe and Ni based magnetic particles are commonly used in biomedical applications. Among these magnetic particles, magnetite $Fe_3O_4$ and maghemite $\gamma$-$Fe_2O_3$ are the most popular in nucleic acid assays because of their good biocompatibility, stability and fast separation under the external magnetic fields [29].

Physical, chemical, and microbiological methods are the main methods for synthesizing magnetic particles. Methods are summarized in Fig. 1. Among the above three methods, chemical synthesis methods are the commonly used while physical methods are less used [30]. Physical synthesis methods, including gas-phase deposition [31] and electron beam lithography [32], do not require chemical reagents or complex manual operation. However, it is difficult to control the size of the synthesized particles, which is hard to meet the requirements of bioanalysis. Microbiological synthesis of magnetic particles is a green chemistry approach with low energy and high recovery rate. It was reported that various of magnetic particles can be synthesized by bacteria, fungi, yeast and virus [33]. Although some efforts have been made to optimize the microbial cultivation methods and the extraction techniques, the incubation of microorganisms and production of magnetic particles takes too much time [34]. Chemical methods are the most commonly used, such as oxidation method [35], chemical coprecipitation [36], sol-gel synthesis [37], aerosol/vaporphase method [38], hydrothermal reaction [39,40] and so on. Chemical methods can synthesize magnetic particles with controllable form and size. In order to improve the efficiency of synthesis, the chemical reactions should be performed under high temperature and high pressure [41], which consumes lots of energy. It is notable that the residuals of stabilizer or metal oxide during the preparation process may influence subsequent biological experiments. Therefore, the separation, washing and drying steps after the reaction are also important.

The synthesized magnetic particles are easily oxidized in the external environment. In order to improve their stability and dispersibility in the solution, surface coating can be applied to form a core/shell structure. According to the different applications, different chemical groups can be modified during the preparation process [42]. We will also introduce different coatings of magnetic particles for nucleic acid experiments in the below. Having prepared the magnetic particles, the characterization of the magnetic particles in nucleic acid experiments should be evaluated. Take $Fe_3O_4$ core/shell structure magnetic particles as an example, the concentration of total iron in the solution can be measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES). The morphology in the colloidal suspension can be observed by transmission electron microscopy (TEM). Dynamic light scattering (DLS) can be used to measure the hydrodynamic size distribution and zeta potential of magnetic particles. X-Ray diffraction (XRD) experiments can be performed to measure the non-coated magnetic particles. Magnetic characterization can be performed using superconducting quantum interference device magnetometer (SQUID) and ferromagnetic resonance (FMR) [43]. Apart from that, the recovery of the targets can be used for evaluating the separation and purification capacity of synthesized magnetic particles.

### 2.2. The types of magnetic particles for nucleic acid purification

The target extraction process before nucleic acid detection affects the sensitivity and reliability of the entire experiment. As a bridge, magnetic particles are connected to the integrated steps of separation, purification, amplification and detection. The magnetic

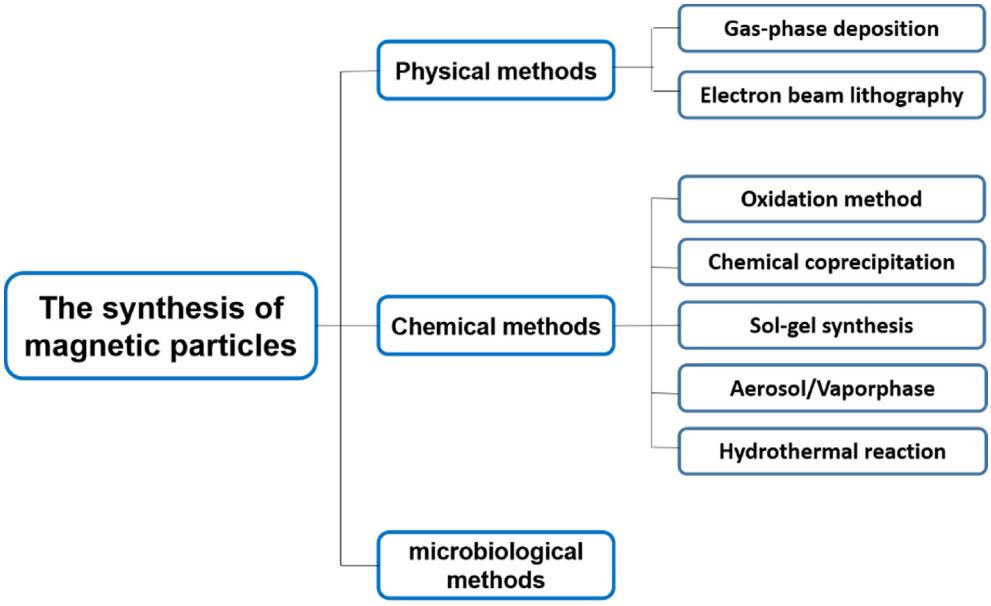


**Fig. 1.** Summary of synthesis methods of magnetic particles.

solid phase separation method uses magnetic particles to adsorb and purify nucleic acids under the external magnetic fields. The commonly used magnetic particles are silica coated, amino coated and carboxyl coated $Fe_3O_4$ or $\gamma$-$Fe_2O_3$ magnetic particles.

The silica coated magnetic particles are popular in various biochemical analysis. Nucleic acids can be bound to the surface of silica coated magnetic particles in a high concentration of salt and low pH solution while the targets can be eluted again at a low concentration of salt and high pH solution. The binding mechanism is most likely to be the electrostatic interactions between nucleic acids with silica. When the solution is at low pH and high salt concentration, the negatively charged phosphate backbone of nucleic acids bind to the positively charged surface of particles [44]. It was reported that silica coated magnetic particles could extract HBV and HCV nucleic acids from serum in 30 min. 1 g of prepared silica coated magnetic particles can adsorb up to 10.6 mg DNA or 7.7 mg RNA with 4 M guanidinium thiocyanate (GTC) at pH 5.5 [45]. Based on silica coated magnetic particles, total nucleic acids of RNA virus/viroid can be extracted from lily-leaf or grapevine-leaf samples for qPCR detection [46]. For many biological samples, both DNA and RNA need to be extracted for analysis. Silica coated magnetic particles were demonstrated to simultaneously extract DNA and RNA from *Hepatocellular Carcinoma*, which is convenient and low cost for medical diagnosis [47].

The positively charged magnetic particles are ideal for the attachment of phosphate backbone of nucleic acids. Within the particular concentration of salt in solution, the negatively charged phosphate groups of nucleic acids have interactions with positive charges on the surface of magnetic composite particles. The nucleic acids can be separated from solution and eluted from magnetic particles [48]. The DNA extracted from whole blood by amino coated particles were well performed in PCR. The concentration of extracted DNA increased with the identity of amino groups [49]. It was reported that the behaviors of DNA adsorption/desorption on amino coated magnetic particles could be influenced by phosphate. When the concentration of phosphate buffer is 1 M, the recovery of efficient DNA desorption is 85%, which provides a good guidance for the subsequent enzymatic experiments [50].

The negatively carboxyl coated magnetic nanoparticles can also extract nucleic acids. In high concentrations of polyethylene glycol (PEG) and sodium chloride solutions, nucleic acids can bind to the surface of carboxyl coated magnetic particles, the mechanism of which is complex. In general, in solution containing low concentrations of salt and PEG, DNA are at random-coil state. The increasing concentration of PEG and sodium chloride promotes the condensation and compaction of DNA. The negative charge on the condensed part of DNA is almost neutralized in PEG solution with a small part of negative charge remaining on the uncondensed part of DNA, eliminating electrostatic repulsion between nucleic acids and magnetic particles and promoting the DNA adsorption on the surface of magnetic particles [51]. The carboxyl coated magnetic particle method is expeditious for extracting of PCR-ready DNA from *staphylococcus aureus* bacteriophages without using toxic compounds [52]. Apart from isolation mRNA from mammalian cells, carboxyl coated magnetic particles can also be used to extract the supercoiled form of plasmid DNA from agarose gel. The method is simple and inexpensive with high quality of extraction [53].

The extraction methods mentioned above are based on the chemical properties of nucleic acids. They can be used to extract total nucleic acids rather than specific nucleic acids, for example, isolation of RNA from unwanted cellular components DNA and proteins. Sequence specific oligonucleotides immobilized on the magnetic particles can extract specific nucleic acid fragments by Watson-Crick pairing. To evaluate the function of silica coated, oligo coated and specific oligonucleotide sequence coated magnetic particles, these particles are incubated in the samples to target RNA for 1 min, the recovery of which are 75%, 71%, 7% respectively. When the incubation time increases to 180 min, the recovery of specific oligonucleotide sequence coated magnetic particles can recover 77% targets. It is expected that silica coated, oligo coated and specific oligonucleotide sequence coated magnetic particles extract total RNA, mRNA and specific gene mRNA respectively. It will be a good guidance for the next detection step of different tolerance of non-target nucleic acids [18]. Furthermore, ion-tagged oligonucleotides coupled with a magnetic liquid support can not only distinguish nucleotide mismatches, they can also extract target DNA with higher sensitivity than commercial magnetic kits [54]. Magnetite particle conjugated with pyrrolidinyl peptide nucleic acid might also be a potential support for base discrimination [55].

## 3. The open type of magnetic nucleic acid assays

Traditional nucleic acid extraction in laboratory relies on centrifuge tubes with an adsorption film, which requires lots of organic reagents and multiple centrifugation steps. This process is difficult to automate and the organic reagents are toxic to the operators. For large-volume samples, multiple batches of extraction are required. The traditional extraction method cannot meet the requirements of POCT with high speed, simple steps and high capture efficiency. To the contrary, the magnetic separation process is simple without centrifugation and precipitation steps. Commercial magnetic extraction kits and automated instruments greatly reduce the burden of manual operation [56]. However, as illustrated in Fig. 2, these are open type nucleic acid extraction assays, requiring magnetic particles transfer [57] or liquid transfer [25] from one tube to another. When performing pathogens experiments in an open system, it is difficult to avoid contamination of amplification products and infection of operators. For some highly infectious viruses, this open nucleic acid extraction method is not applicable.

Some companies have developed automated pipetting systems using robotic arms in an enclosed environment. To avoid cross-contamination, air flow systems supporting pipetting systems always keep running in order to take away aerosols. The bulky equipment with high prize is suitable for specialized laboratories and high-level hospitals rather than remote areas lacking of resources. To apply it to on-site detection, disposable cartridges with manual pipetting are studied. In the disposable cartridge, operators need to manually move the pipettor up and down, left and right. The whole operation process is very complicated. Also, it is difficult to transport the cartridges because of the stored liquid reagents in tubes [58]. Compared with pipetting based methods, non-pipetting methods will be easier to automate. In order to improve the universality and applicability of nucleic acid detection, there is an urgent need for a closed system integrating target extraction, signal amplification and final detection without pipetting. This will be helpful for controlling the outbreaks of infectious diseases and reducing the casualties of the epidemic.

## 4. The closed type of magnetic nucleic acid assays

### 4.1. The immiscible interface based nucleic acid purification

Traditional nucleic acid detection processes are performed in the open tubes. It is time-consuming and difficult to prevent cross-contamination. With the increasing demand for point-of-care nucleic acid detection, a series of technologies have been developed towards the integrated systems. Some companies have developed commercial chips or cassettes integrating nucleic acid purification, amplification and detection. However, these chips or cassettes are equipped with dedicated instruments, which is complicated and expensive. The magnetic particles can pass through the immiscible interface introduced above without pipetting. It is a good carrier for nucleic acid integrated detection. The immiscible interface can also provide a closed system for the transport of magnetic particles from one chamber to another and prevent aerosol contamination. In integrated system, purified nucleic acids should be prepared for amplification and detection. The purification process is important for the next detection. Thus, the integrated purification process that avoids multiple elution and centrifugation will be introduced initially.

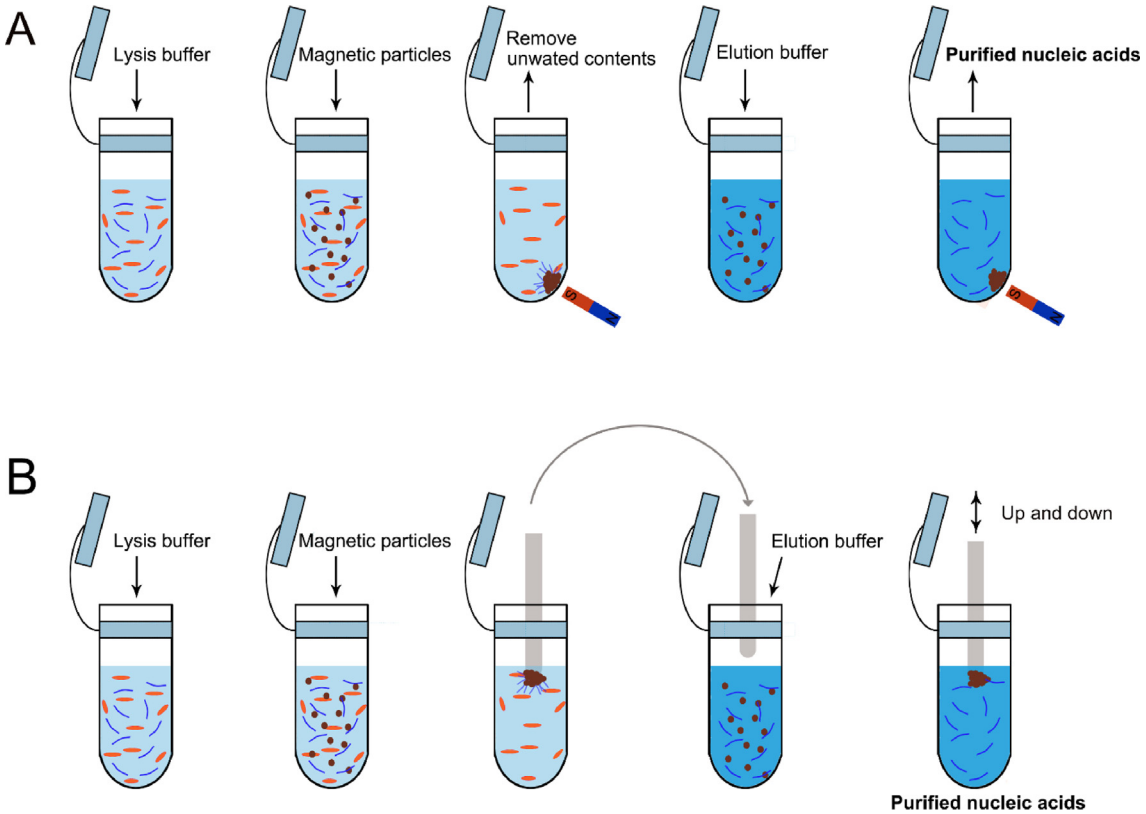


**Fig. 2.** The open type of magnetic nucleic acid purification system. (A) The open magnetic nucleic acid extraction assay requiring liquid transfer. (B) The open magnetic nucleic acid extraction assay requiring magnetic particles transfer.

Magnetic particles assisted extraction is a solid phase extraction method actuated by magnetic fields. Magnetic particles can 'swim' in a single passage through immiscible phase replacing multiple pipetting steps. The phase guide solid extraction passage includes water/oil interfaces and water/air interfaces.

*4.1.1. The water-oil interface based nucleic acid purification device*

In 2010, Sur et al. reported a molded cartridge equipped with two wells containing lysis/binding buffer and elution buffer respectively. The two wells are covered with a layer of liquid wax, which can connect the two channels and prevent the liquid from mixing. The clinical samples are added to the well of the lysis buffer and then nucleic acids are released from the cells. Magnetic particles that adsorb nucleic acids shuttle through water/oil interface (Fig. 3A). In addition to avoiding the bulky equipment and lowering the assay costs, the time required for nucleic acid purification has been shortened and the steps have been simplified [59]. This is the initial device for magnetic particles assisted nucleic acid assays. This device separates the solution in the wells from external environment through the liquid max which contacts with the external environment directly. Different from it, in T-junction device the oil only separates lysis buffer and elution buffer but cannot formed a closed environment. The T-junction device is composed of three wells connecting by a long channel and a short channel (Fig. 3B). The lysate well and elution well are connected at both ends of the long channel. If the oil is not enough, it can be added through the middle oil well. To make the magnetic particles travel through the channel smoothly, the relatively large passing area is required. This device is applied to extract influenza RNA from nasopharyngeal swab sample [60]. The overall pattern can be printed on a microfluidic chip by using three-dimensional print technology. The cellulose functionalized magnetic particles can recover 61% HPV plasmid concentrations in 15 min, which is promising for the point-of-care diagnosis [61].

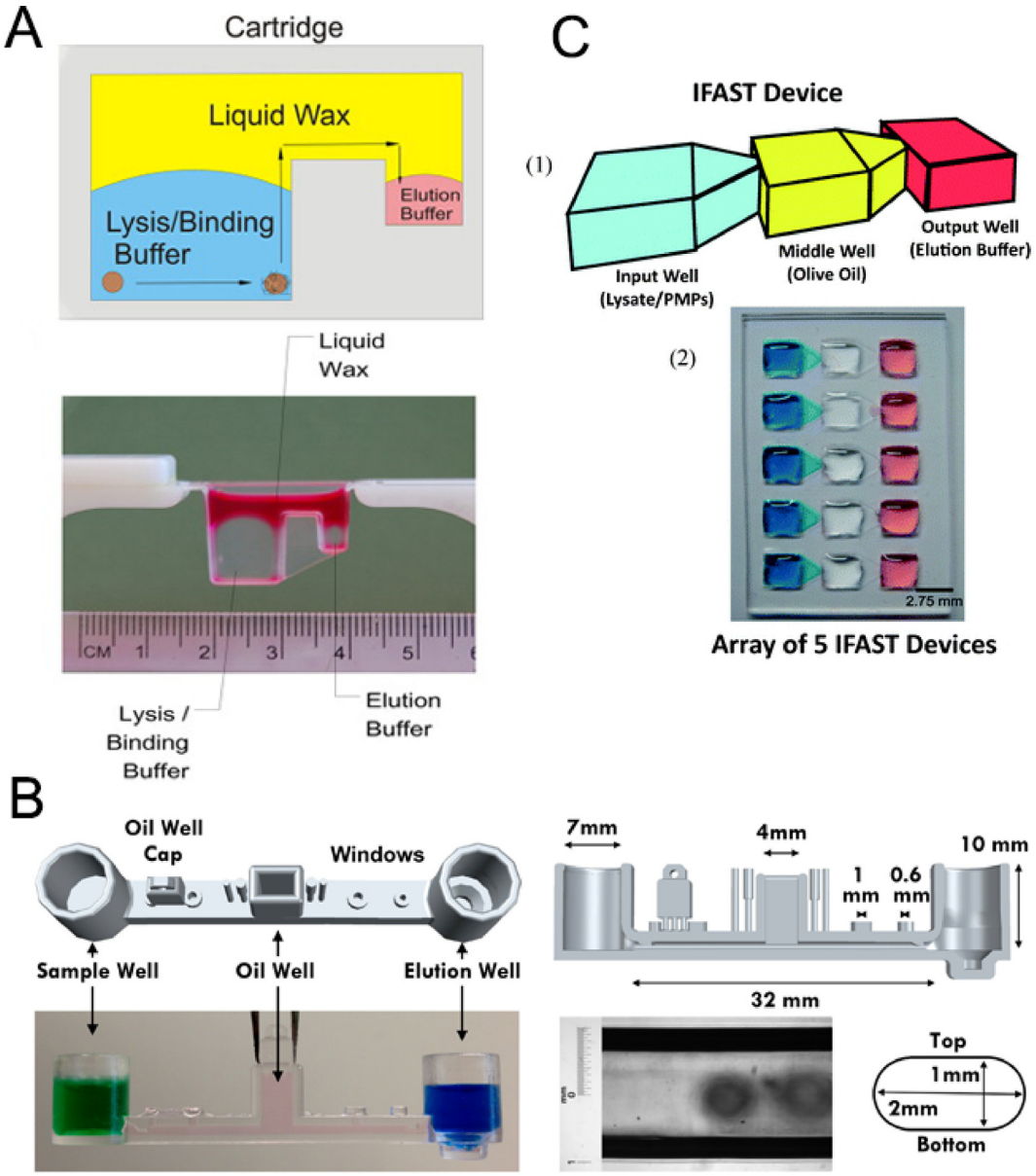


**Fig. 3.** The transport of magnetic particles by oil-water immiscible interface. (A) Hydrophilic liquid is covered with liquid wax acting as the separator. Reprinted from Ref. [59] with the permission from Elsevier. (B) The 3D printed T junction device that allows for the vortex and centrifugation free extraction of nucleic acids. Reprinted from Refs. [60] Open Access. (C) The horizontal immiscible filtration assisted by surface tension device. Reprinted from Ref. [62] with the permission from Royal Society of Chemistry.

Neither of the above-mentioned devices is totally closed, which is difficult for transportation. In 2012, the immiscible filtration assisted by surface tension (IFAST) device was reported. The reaction solutions are not in contact with the external environment. The horizontal IFAST device consists of three wells and two trapezoidal microfluidic conduits arranged in a line. The lysis well and elution well are separated by surface tension (Fig. 3C). The sensitivity of this device is comparable to the commercial magnetic extraction kits. Furthermore, the time it takes to accomplish extraction process is much less than conventional magnetic extraction kits with manual intensive. It was verified that nucleic acids of *Clostridium botulinum* type A in the food matrices could be extracted by the device, replacing tedious wash and centrifugation steps [62]. The IFAST device uses surface tension to separate different reaction liquids, greatly simplifying the design of the device for magnetic nucleic acid assays. The wells of IFAST device are designed in a straight line, which is easier to automate. Combined with an automated liquid handler and a custom-built magnet actuator, operation of multiple IFAST and multiple arrays of extraction are realized. The robotic operation gains higher capture and higher repeatability compared with manual operation [63].

This horizontal device is a total closed system and can reduce the processing time down to 5 min [64]. As illustrated in Fig. 4, to deal with large volume samples, the size of input well is adjusted and the nucleic acids of *Helicobacter pylori* from stool samples are extracted, requiring only 7 min [65]. However, the magnetic particles have to pass through the wells along the bottom of the horizontal device. Magnetic beads may carry some impurities deposited on the bottom when passing through different wells. Thus, a vertical device is designed. The magnetic particles pass through the middle part of the wells rather than the bottom, avoiding the risk of carrying unwanted components [66].

Furthermore, to better evaluate the results, sometimes to purify both RNA and DNA simultaneously is needed. The traditional techniques are cesium chloride step-gradient ultracentrifugation or the phase separation guanidinium thiocyanate-phenol-chloroform procedures, which requires complex operation and toxic reagents. Based on vertical IFAST device, Strotman et al. addressed this problem and developed an efficient method to purify RNA and DNA from a single sample [67]. Difference from the previous device, they present a vertical symmetrical structure. The improved IFAST (Fig. 5) has two output wells that are symmetrical but not interoperable.

Firstly, add the samples, lysis buffer/RNA binding buffer and oligonucleotide modified magnetic particles to bind RNA in the input well. Then pull the magnet in the front of the IFAST device and release RNA from magnetic particles in the front output well. Having extracted RNA, add the DNA binding buffer and silicon modified magnetic particles in the input well to bind DNA. Then pull the magnet in the back of the device and release DNA in the output well on the back. The RNA and DNA extracted from a low abundance of cells are demonstrated to be used as templates for Sanger sequencing. It is a fast, sensitive and low-cost improved device to extract DNA and RNA simultaneously.

The composition of samples collected from the site is complex. These samples may contain some compounds that can inhibit the nucleic acid amplification [68]. The magnetic nucleic acid extraction step can enrich targets and remove most inhibitors. However, in the process of travelling through water-oil immiscible phase, the magnetic particles will inevitably carry a small volume of aqueous liquid which may contain inhibitors of enzymatic reaction. It is reported that the volume of carriers increases with the surface tension of the immiscible interface until it reaches a critical value. When the surface tension rises to a critical value, the volume of carriers remains constant, which will provide a guidance for the size of magnetic particles and the value of surface tension [69]. If the oil was not involved, the magnetic particles assisted solid phase extraction process would be simpler. It was reported that the air gaps could replace the oil interface as the separator between different solutions.

#### 4.1.2. The water-air interface based nucleic acid purification device

The water/oil interface can prevent the reaction solution from mixing. It was reported that water/air interface in the glass capillary with a small diameter can also achieve the same goal. Bordelon et al. developed the RNA extraction cassette for low-resource areas that are limited to specialized equipment and personnel (Fig. 6A) [70]. The prototype extraction cassette is composed of glass capillary chambers (each chamber 80 mm length; 2 mm inter diameter) and pipet tips (1000 μL) acted as spacers between each capillary chamber. The capillary chamber is used to place the samples to be detected and the other capillary chambers are preloaded with reaction reagents. To make the design simplifier, the prototype extraction cassette was transformed into the continuous tube (61 cm length; 1.6 mm inter diameter) in which each solution chamber is separated by the 2 mm air gap. The silica coated magnetic particles can connect the entire extraction process as a whole and finish the whole process in 15 min. However, the extraction recovery of RNA remains to be improved. Another disadvantage is that the extraction tube is a little long which is inconvenient in practical application. The surface tension assisted by water/air interface provides a new idea and extends the way for magnetic nucleic acid assays.

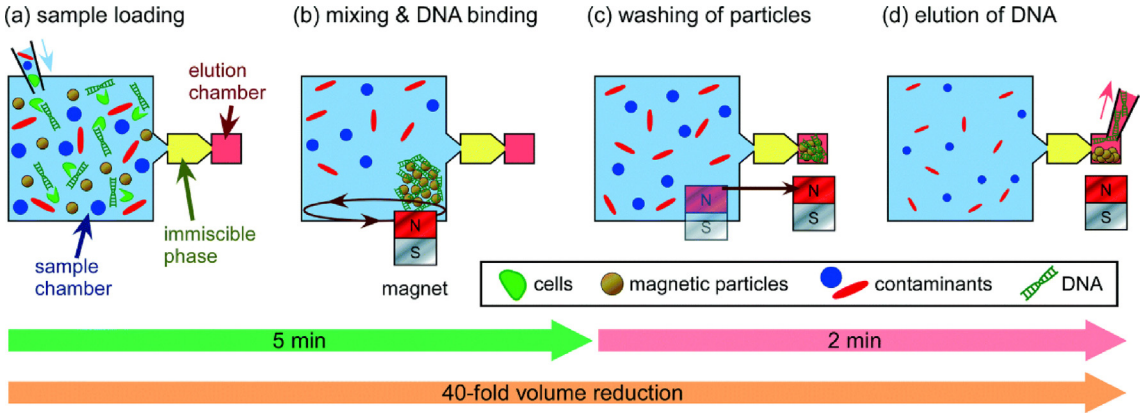


**Fig. 4.** The horizontal device for integrated nucleic acids extraction of *Helicobacter pylori* from stool samples in 7 min. Reprinted from Ref. [65] Open Access.

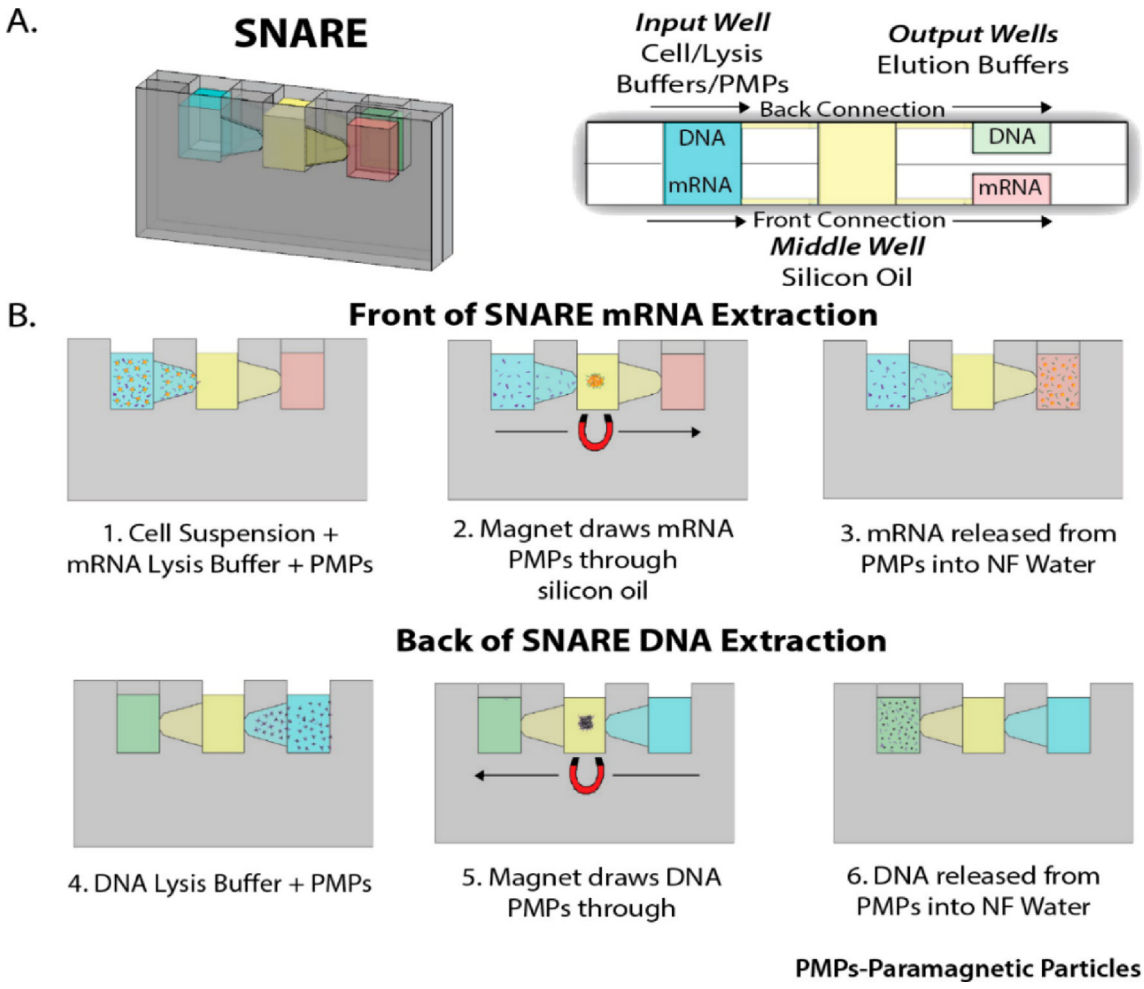


**Fig. 5.** The improved vertical device SNARE (Selective Nucleic Acid Removal via Exclusion) can capture RNA and DNA from a single sample simultaneously. (A) Picture of SNARE device with dimensions (left) and top down schematic of SNARE device (right). (B) Operation of SNARE for mRNA and DNA purification from a single sample. Reprinted from Refs. [67] with the permission from American Chemical Society.

Here, we give an example to illustrate the effectiveness of water-air interface based extraction method. The whole nucleic acid extraction processes are completed in a small diameter tubing 'extraction cassette' in which solutions are separated by air gaps. The bulb of a transfer pipette containing magnetic particles and lyophilized adsorption buffer are used to take human urine sample. Having mixed thoroughly, depress the bulb of the pipette to transfer the liquids into the thin tube in which all wash and elution steps are finished. In the results, 50% of spiked DNA was recovered by qPCR evaluation while no DNA could be detected in spiked samples without extraction [71]. The reason is that the magnetic extraction assays remove the PCR inhibitors in the samples. For practical application, the sample collection and extraction processes are simple. However, the recovery of target nucleic acids is low, which affects the sensitivity of downstream detection.

It is obvious that the adjacent solutions separated by immiscible interfaces will mix if the diameter of the continuous tube is too large. Thus, the physical parameters of the tube and the magnetic particles are significant for the stability transport, solution carryover and extraction efficiency. It is found that fluid stability is maximized if the inter diameter of the tube is below 0.8 mm. When using less than 0.2 mg of magnetic particles, the amount of solution carryover is minimized [72]. To transport more magnetic particles, relatively wider tube and water-air interface with stronger surface tension can be chosen. The designing parameters of low-resource magnetic assays will be useful for other researchers. To better apply water-air valve, the magneto-capillary valve technologies that magnetic particles travel through discrete liquids separated by water-air immiscible phase are summarized and compared, including the patterned air valve, the geometrical air valve and the paraffin valve. The structure of air valve is simple and of good pinch-off in the narrow tube. The paraffin valve is a robust solid structure but the pinch-off is not strong because of the poor surface tension in the wider tube [73]. The magneto-capillary valve technology is versatile in biological application and promising in biomarkers such as nucleic acid and protein for 'sample in and answer out' diagnosis.

The water-air passage is convenient for magnetic particles to transport. There are some problems in the practical application. In the traditional assays with pipetting, it is easy to realize thorough mixing by vortex or sucking in and out by pipette. Indeed, in the closed type of magnetic nucleic acid assays, it will be a little difficult to realize thorough mix between magnetic particles and reaction liquids due to the gravity of the magnetic particles. Particularly, in narrow tubes, magnetic particles could not interact with samples thoroughly. In water-air passage, magnetic particles have a large specific surface area but are easily deposited in the liquids due to the gravity. To improve the mixing and capture efficiency of targets from the liquids in water-air passage, the two-magnet strategy is applied. Compared with the single-magnet strategy, the capture time of two-magnet is three times faster and the capture efficiency

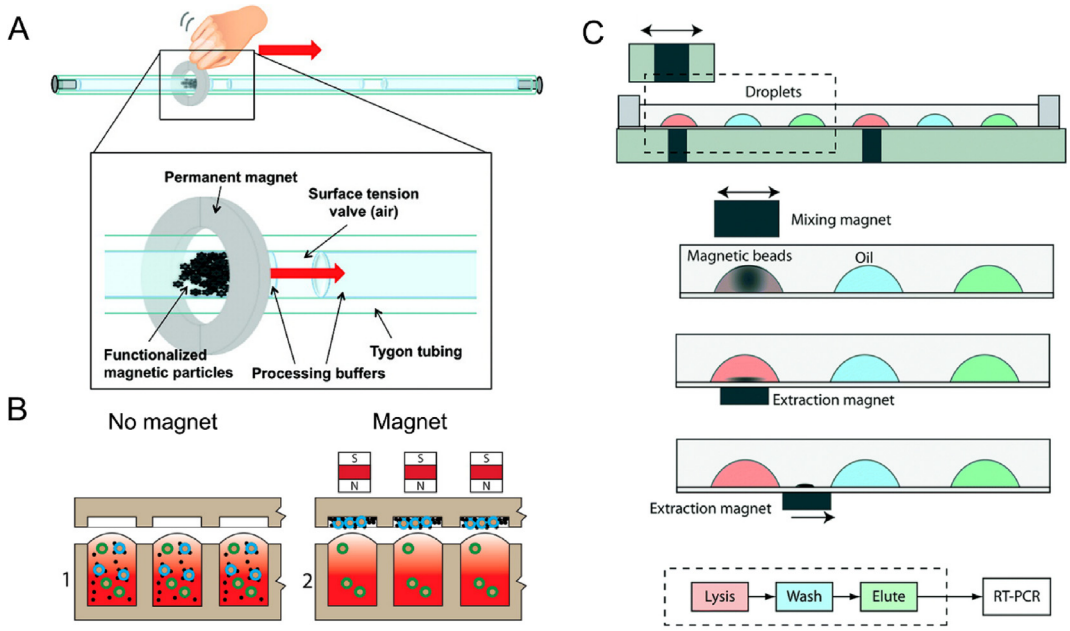


**Fig. 6.** The transport of magnetic particles by air-water immiscible interface. (A) The tubing extraction cassette containing individual solutions separated by air-water surface tension valves. Reprinted from Ref. [70] with the permission from American Chemical Society. (B) The magnetic particles can jump out of the solution when the magnetic force is stronger than surface tension. Reprinted from Refs. [76] Open Access. (C) The array of surface-adhering droplets used for magnetic particles assisted integrated purification. Reprinted from Ref. [77] with the permission from American Chemical Society.

is 2.5 fold higher [74]. Also, the coaxial channel that can be applied to the multiring high-gradient magnetic field is designed. The coaxial channel is composed of an inner quartz capillary and an outer quartz capillary. In this situation, the magnetic particles can be captured against both walls with more uniform distribution [75]. Inspired by magnetic stirrer, we can use magnetic force, generated by electromagnet instead of permanent magnet, to promote stirring and mixing in closed type of magnetic nucleic acid assays. According to the different type of closed devices designed for nucleic acid assays, different strengths of electromagnetic force can be applied. The electromagnetic force generating device will be a very useful tool in the integrated nucleic acid assays. Through these methods, the efficiency of water-air interface assisted nucleic acid extraction will greatly increase.

In addition to transferring magnetic particles in capillaries by water-air interfaces, lots of research have been done to realize integrated nucleic acid detection by water-air interfaces without capillaries. The water-air interface assisted magnetic nucleic acid assay is simple and cheap. In medical institutions and public health agencies, large number of samples need to be processed in the short term. It is also good to realize extraction of multiple arrays and samples simultaneously. The magnetic particles bound to targets can 'jump' through the air-liquid interface when magnetic force is greater than the surface tension force (Fig. 6B). The microarray experiment can be performed simultaneously by the magnetic "air-jump" with high target yield, excellent reproducibility and shorten time [76]. It effectively avoids the blocking problem of magnetic particles when passing through the slender tube. Similarly, to conveniently and efficiently extract nucleic acids, magnetic particles and droplet array based parallel extraction methods are explored. The array of surface-adhering droplets was used for the transportation of magnetic particles through buffer solutions. Suspended magnetic particles are placed above the droplet array to mix the liquid [77] (Fig. 6C). When using the sliding lid for droplet extractions with two magnets, the collection, release and resuspension of magnetic particles can pass through multi-wash and elution wells regardless of proximity [78].

Based on the water-oil and water-air interface, lots of integrated devices are designed. In the below, the integrated nucleic acid detection device will be introduced and analyzed.

### 4.2. The integrated magnetic nucleic acid detection assays

Magnetic technology for nucleic acid extraction is well developed. The integrated methods of nucleic acid purification for the downstream analysis are reviewed. The immiscible interface assisted devices have many versatile functions. It plays an important role in RNA or DNA extraction, protein expression analysis, circulating tumor cells capture and quantification [79]. It is a powerful tool supporting and promoting integrated assays. The Group B *Streptococcus* from artificial urine samples can be rapidly detected based on it. The target pathogens are adsorbed by immune-magnetic particles and detected using an adenosine triphosphate (ATP) bioluminescence assay [80]. From the examples given above, we know that there are many magnetic particles based integrated systems. A lot of studies are exploring the integration of nucleic acid extraction and promoting the improvement of this technology. Some designed magnetic integrated devices can be used in immunoassay or cell staining.

Currently, the key point is how to realize the integrated magnetic particles based detection in a designed device that is closed, cheap and transportable. Compared with integrated immunoassays, the magnetic particles assisted integrated system for nucleic acid extraction, amplification and detection without pipetting is a little more difficult. There are several reasons. Firstly, it is difficult to link extraction and amplification step because there is no way to transfer nucleic acids eluted from magnetic particles into amplification solution. Secondly, enzymes required for nucleic acid amplification are generally stored at low temperatures. The storage of the reagents placed in advance and the temperature control of enzymatic reactions remains to be solved. The final problems are the testing instruments and the transport of integrated devices. In the following, we will give a few examples to illustrate the above problems.

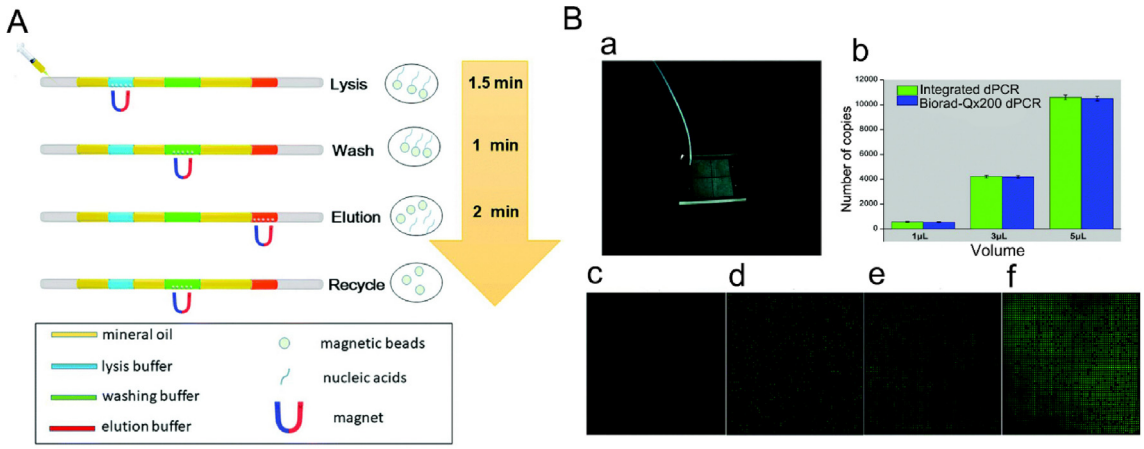


**Fig. 7.** The oil-water immiscible interface assisted integrated nucleic acid detection. (A) The integrated extraction system can be directly linked to the digital PCR chip by the automatic pump. (B) The performance can achieve almost the same as commercial Bio-Rad QX200 digital PCR system. Reprinted from Refs. [82] with the permission from Royal Society of Chemistry.

To reduce costs and increase operability, Tian et al. developed a negative pressure assisted integrated system for DNA isolation and digital PCR detection. The integrated chip is divided into DNA purification zone and digital PCR zone. In DNA purification zone, the magnetic particles in the sample lysate are captured and kept still by the magnet. The tube connected with lysis zone is pre-loaded wash buffer and PCR mix. The pre-loaded wash buffer can flow into the chamber and exclude contaminations by negative pressure from the injection syringe. Then the nucleic acids can be eluted by PCR mix and pulled into digital PCR region [81]. In this study, the role of magnetic particles is to capture and restrict target DNA in the certain chamber when the wash buffer flowing through. The negative pressure provided by the injection syringe play the main role in integrating the nucleic acid purification with amplification. The nucleic acid extraction reagents are not compatible so two steps and outlets are applied to pull the reagents. The PCR mix is comprised of RNase-free water and necessary enzymatic reaction reagents so the elution effect may not be as good as the commercial dedicated elution buffer.

The digital PCR mentioned above is mainly completed by the negative pressure generated by the syringe and assisted by magnetic extraction. It has been reported that water-liquid immiscible interface can assist magnetic particles to extract nucleic acids. Based on it, Yin et al. proposed droplet digital PCR integrating with a fully closed extraction system [82]. In the polytetrafluoroethylene tube (an inner diameter of 1 mm) which has the lowest surface tension and does not adhere to any substances, 10 μL mineral oil are used to separate lysis buffer, wash buffer and elution buffer. The small volume of sample is injected into the tiny tube and mixed with magnetic particles. As you can see in the (Fig. 7), having completed the extraction process, the eluent is directly pulled into digital PCR chip by an automatic pump. The above two methods

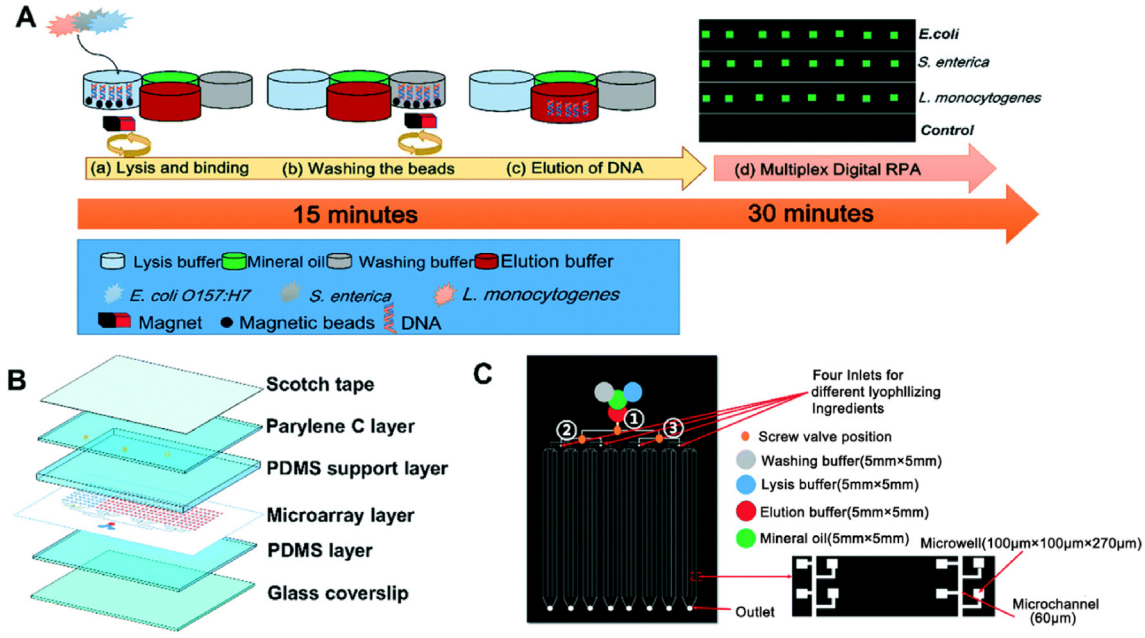


**Fig. 8.** (A) The whole nucleic acid extraction and digital RPA process can be completed on the integrated multiplex digital RPA chip in 45 min. (B) Multilayer structure of the chip. (C) The nucleic acid extraction region and digital RPA region is separated by the screw valves. Reprinted from Refs. [84] with the permission from Royal Society of Chemistry.

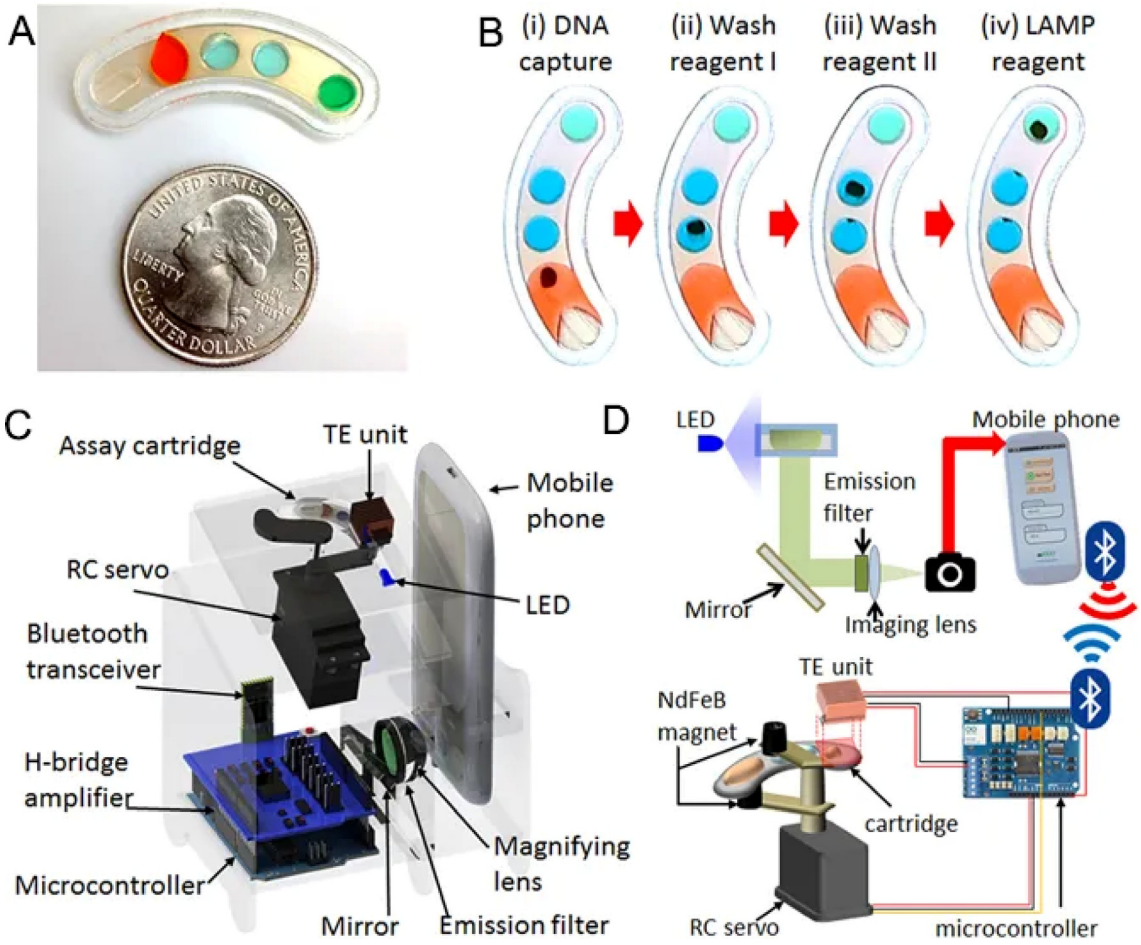


**Fig. 9.** (A) The size of magneto-fluidic cartridge next to US quarter for comparison. (B) Magnetic particles that adsorb nucleic acids move directly to the amplification region without passing isolated elution area. (C) Auxiliary modules can promote the magnetic particle manipulation, thermal control and optical signal acquisition using a mobile phone. (D) Schematic diagram of mobile phone receiving optical signals and controlling magnetic particles and thermal blocks. Reprinted from Refs. [87] Open Access.

integrated nucleic acid extraction with digital PCR. They both use negative pressure to drive liquid transfer and connect the extraction and amplification processes. However, the former uses a syringe for pipetting in the whole process and can handle larger sample volumes. The latter can just handle micro-volume samples and the automatic pump is only used in the interface for extraction and amplification. Depending on the type of samples, the appropriate method can be chosen.

In a thin channel, magnetic particles may block the channel and make the movement difficult. Under the premise of ensuring phase guide filtration, a relatively large space will facilitate the movement of the magnetic particles. If there is no oil or air gaps, the integrated operation might be difficult to propel [83]. The integrated chip combines nucleic acid extraction, digital recombinase polymerase amplification (dRPA) and fluorescence detection. As illustrated in Fig. 8, there are four chambers in the integrated chip. The centered one is filled with mineral oil and connected with other three uniformly distributed reagent chambers. The nucleic acids absorbed on the magnetic particles shuttle between the chambers driven by the magnet field. The screw valves can separate solution in different regions and prevent mixing. Having extracted the nucleic acids in almost 10 min, the extracted nucleic acid and mineral oil can be driven into the micro-wells ready for the digital RPA when opening the screw valves [84]. This 'sample in and answer out' chip provide a simple way, screw microvalve, to control the isolation and integration of extraction and amplification. They effectively used the mineral oil in only one chamber to separate other reaction chambers, which is promising for the POCT in the future.

In addition to transferring elution buffer containing nucleic acid eluted from magnetic particles to the amplification region by centrifugal force [85] or punching-press mechanism [86], it is a new idea to move the magnetic particles to the amplification area. The mobile nucleic acid amplification testing platform use the mobile phone and droplet magnetic fluidics to deliver target nucleic acid. In Fig. 9, at first, magnetic particles function as the mobile substrate for nucleic acid capture and transport from lysis buffer to wash buffer and loop-mediated isothermal amplification (LAMP) reagents. The LAMP reaction produces a superabundance of pyrophosphate, coupling this reaction with colorimetric indicators. The mobile phone can not only control the assay initiation and optical signal acquisition, but also collect the signal by the camera sensor [87]. To demonstrate the performance in practical detection, the platform was used to test 30 potentially *Chlamydia trachomatis*-infected patients in a hospital and the results showed 100% concordance with laboratory-based detection, which represents its great potential in diagnosis.

## 5. Conclusion and prospects

This review focuses on magnetic particles assisted nucleic acid purification, amplification and detection integrated system. Different from the microfluidic chips that requires pumps or

centrifuges, we stress the liquid transfer free system. Magnetic particles are good carriers for nucleic acid adsorption and transfer driven by magnetic fields. Immiscible interface can effectively separate different reaction solutions but allow magnetic particles to pass through, which creates conditions for integrated detection. In the review, we first introduce the basic information of magnetic particles, including preparation, characterization and types, which is important for the nucleic acid purification. Secondly, to explain the disadvantages of the open detection system and the advantages of the closed system, the steps of traditional open detection system are briefly described. The open nucleic acid detection system has disadvantages of complicated operation, consuming too much time, cross-contaminations, which is difficult to apply to point-of-care detection. The transport of magnetic particles assisted by immiscible interface in the closed system can avoid these problems. Then the integrated nucleic acid purification assisted by magnetic particles through water-oil interface and air-water interface is described, which lays the foundation for integrated detection. Finally, the integrated nucleic acid detection system is introduced. It greatly simplifies the steps of nucleic acid assays and promotes the integrated detection. On this basis, the improved integrated nucleic acid purification, amplification and detection system is discussed in detail.

At present, the integrated extraction of magnetic particles based immiscible interfaces is well developed and can achieve the same efficiency as the commercial extraction kits. The existing problem is how to transfer the eluent containing nucleic acids to the amplification region. There are two solutions to the problem. One method is based on pipetting, which transfers the eluent to the amplification region by negative pressure or withdrawal of physical obstruction; The second method is to integrate the amplification solution with elution buffer, which directly transfers the magnetic particles adsorbing nucleic acids from wash buffer to the amplification solution. Both of the two points deserve further study. For point of care detection, it is better to minimize the operating steps. The reagents required for nucleic acid detection should be pre-loaded on the chips. The storage of the reagents needs to be considered. Lyophilization and chitosan encapsulation are now commonly used to store reagents. It has been shown that the reagents stored for several days behaves well. With the development of cold chain transportation, we believe that storage will not be the main problem hindering the development of POCT. Furthermore, immiscible phase assisted integrated purification and detection devices need to ensure that the solution separated by oil or air gaps should not be mixed when experiencing a certain intensity of shock during transportation.

Nucleic acid enzymatic reactions have high requirements for temperature control. Compared with PCR requiring repeated heating and cooling, isothermal amplification reactions are obviously more suitable for point-of-care detection. Some researchers also carry out nucleic acid amplification experiments with some household products, such as coffee cups and thermos bottles. A portable thermal block designed to fit the integrated detection device should be considered. For detection, it is better to use colorimetric methods which are visible to the naked eye so that users can intuitively judge the results. With the popularization of intelligent mobile phones, making good use of equipped cameras will be very useful.

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