Point-of-care devices for pathogen detections: The three most important factors to realise towards commercialization

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ABSTRACT

The development of lab-on-a-chip technology and its applications in biochemical and biomedical analyses has, during the last two decades, led to the potential realisation of portable and on-site detection devices, the so-called point-of-care (PoC) detection systems. These are essentially cheap, easy-to-handle systems, offering rapid sample-to-answer results to non-technical operators. In this perspective, we do not review all the current advances of Lab-on-a-chip techniques for the realisation of PoC. Instead, we aim to provide insight into what we foresee as the three most important factors to play the essential roles for succeeding in making commercially viable PoC pathogen-detection devices. The three insights are namely: the utilizations of (i) disposable polymer (microfluidic) chips, (ii) the implementation of surface-bound (or solid-phase) nucleic-acid amplification techniques and (iii) relying (more) on open-source hardware and software.

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1. Introduction

Lab on a chip (LoC) technology has been developed since the work of Manz in 1990 [1] introduced the idea of a miniaturised total analysis system to obtain better chemical separation performance. The original aims of the technology are thus to pursuit analytical chemistry testing and experiment in a low volume of reagents (down to pico-litre) for rapid and efficient sensing and diagnostics. In the last three decades, the applications of LoC technology have been expanded to many other fields such as biophysics, bioengineering, medicine, energy, material sciences, etc. In general, those applications are divided into two categories: bio-applications and non-bio-applications. This perspective review focuses on the bio-applications aspect, especially in the detection of pathogens and towards the development of LoC technology into real-life, portable and on-site applications - the PoC devices.

The concept of PoC testing, where the test is conducted at, or near, to the patient, and performed by an operator without

laboratory training, is actually not new. Its origin can be traced back to the 15th century when urine was inspected and tested at the patient's home [2]. The PoC testing, however, has been renowned in the last 30 years due to the high demand for clinical diagnostic market and the extraordinary development of the biomedical and engineering technologies. The current market of PoC was estimated to be about 23.6 billion in 2016 and can be 39.96 billion in 2021 [3]. Nowadays, the development of technology has widened the concept of PoC devices, not just limiting it to healthcare institutions [4]. The PoC concept is therefore not only applied in the field of clinical diagnostics but also, e.g. biosensing, food safety, environmental sensing etc. For applications outside the healthcare institutions, PoC has also sometimes been referred to as point-of-need [5–7], point-of-use [8], or point-of-collection [9]. In this perspective, we define the PoC devices as devices that meet the following criteria: performing tests for the detection of target pathogens on, or at sites with limited infrastructure, the test should be easy-to-use, accurate, cost-efficient, rapid and preferably requiring only a non-technical operator to run.

Moreover, in this perspective, laboratories can be categorised as either: settings with no infrastructure, minimal infrastructure, or moderate-to-advanced infrastructure, based on the number of resources and capabilities available for testing. The argument of

laboratory categorization is made in the context of clinical diagnostics capabilities [10], and remains relevant even for small and medium-sized enterprises (SMEs) in the area of food, food processing industries, and food and environmental regulatory setups for monitoring pathogens. The need for PoC devices in the area of rapid diagnostics has been identified as one of the major priorities by the Bill and Melinda Gates Foundation and the National Institute of Health (NIH) in the "Grand Challenges for Global Health" [11]. The lacking of rapid, suitable diagnostic devices for remote resource-limited settings is still being the critical factor behind the devastating effects causing by infectious diseases.

PoC devices aimed at clinical diagnostics are a good example of an interface where biomedical and engineering disciplines meet. In addition, recently, nucleic acid-based molecular testing has become an important tool in the detection of pathogens and has shown potential for integration in PoC devices [12]. Development of such PoC devices requires multidisciplinary competencies that may include molecular biology, biochemistry, electronics, photonics, microfluidics and software engineering. The potential utility of such PoC devices is again in low infrastructure laboratories with limited resources available. Here, these devices can help to provide rapid actionable information for patient care at the time and site of an encounter, allowing timely therapeutic interventions and improving patients' clinical outcomes. In addition, it is also essential to remark, that, since fluorescence is the workhorse for analysis in the majority of LoC applications these days [13–15], in this perspective we hence constrain our focus on and discuss PoC devices primarily using fluorescence as the mean of signal detections.

The PoC devices generally consist of three components: (a) sample handling, (b) detecting and (c) signal reading (Fig. 1). The interconnection and interdependency of these three components will be discussed in this perspective. It is important to clearly outline the area which we will discuss in this insight paper. In this perspective, we do not aim to review all the current advance LoC techniques for PoC application. Here, we focus on the possible solutions for the three components (Fig. 1) and hence a suggestion for an opportunity to realise a commercial PoC device which satisfies the criteria aforementioned. In short, we believe that the three components of the PoC can be solved respectively by (a) The use of disposal polymer microfluidic chips for sample handling; (b) applying surface-bound nucleic acid amplification for detecting targets and (c) employing open-source hardware and software performing the test, e.g. signal reading, prompt the user, provide test results to the user, etc. Details of this discussion will be given in the next sections.

## 2. Polymer microfluidic chips for sample handling in PoC devices

In the early days of the LoC era, based on silicon technology and microelectronic processes, the microfluidic chips (from hereon referred to simply as chips) were made from silicon and glass such as borofloat and fused silica. In 1998, Whitesides group introduced the soft-lithography technique [16] which made use of (cheap) poly (dimethylsiloxane) (PDMS) material, giving rise to a burst of development in the LoC research, especially in the biology and medicine areas. Subsequently, using LoC systems for fluorescent biomedical-physical measurements gained tremendously great attention. The material used to fabricate micro- and nanofluidic chips for fluorescent-related measurements has mainly been fused silica, which is painfully costly and requires long processing time. There have been not many researchers in the lab-on-a-chip community being aware of the existence of the state-of-the-art of using polymer micro-, nanofluidic chips and the use of them in their research investigation in general and in investigating fluorescent-related experiments particularly. An excellent review on the microfabrication techniques available for fabricating polymer micro-, and nanofluidic chips was presented by Becker [17] et al., in 2008, which currently (as of May 2019) has been cited 472 times, equal to 43 citations per year on average, during the approximately 11 years that has passed since the review was published. This number is, however, still low compared to the tremendous growth the LoC community has seen, as a search using the keywords "Lab on a chip" in Web of Science, showing 23,465 citations just for 2018. This implies that not many people in the LoC community realised the availability of polymer microfabrication technologies for micro-, nano-fluidic systems. Another search in Web of Science using the keyword "Lab on a chip and polymer injection" shows the results of only 279 citations in 2018, which is consistent with our previous discussion point.

The interdependencies of application, fabrication methods and materials play a vital role in driving and directing the experimentalists when designing and fabricating their LoC devices [18]. Fluorescent-biomedical-physical measurements require that the materials used have excellent optical properties, i.e. are UV-transparent, stable, and inert with UV radiation, etc. Specifically for micro- and nanofluidics and LoC applications, the most suitable candidates for meeting these requirements are listed in Table 1.

Soft-lithography material, namely PDMS, can be used only in cases of low flow rate or low-pressure LoC measurements, e.g. electrophoresis [24], electro-osmosis [25], streaming potential [26], acoustophoresis [27], or in microfluidic mixers [28]. Furthermore, water can evaporate through permeable polymers such as PDMS [29], and PDMS is also not ideal for fabrication of high-aspect-ratio structures [30]. In addition, PDMS can be damaged from high energy light (for example wavelength of 266 nm, which is often used to excite tryptophan fluorescence) [30]. Furthermore, for some experiments, which demand highly applied pressure, such as ultra-rapid mixers, the bonding of the chips also becomes crucial, and consequently, PDMS cannot be used in this kind of experiments. For the silicon technology, microelectronic and cleanroom standard processes, these requirements have only a few addressing options, either glass or quartz (silicon is not transparent to UV light, hence unsuitable). Glass such as Pyrex has low signal-to-noise fluorescent intensity due to limited transmission of deep UV light for applications related to tryptophan fluorescence. In general, that leaves us the last option of using fused silica (also called quartz). Fused silica is transparent for a wide range of UV wavelengths [20]. This material is, however, expensive and difficult to process, for instance, bonding and etching steps [31–33], both in a lab and at industrial scale. To overcome this obstacle, we believe that using the simple and low-cost fabrication processes, such as polymer injection moulding, for mass production of Cyclic Olefin Copolymer (COC) micro- and nanofluidic chips suitable for fluorescent biomedical-physical experiments is a great solution. COC has a better UV transparency compared to PDMS, polymethamethylacrylate (PMMA) and polycarbonate (PC), and has extremely low water absorption [22,34,35]. Besides, polymer injection moulding is a high-throughput and easily scalable technique, hence providing faster and cheaper possibilities for production compared to fused silica and elastomer casting PDMS (Fig. 2). The inexpensive high throughput fabrication of LoC devices can yield broad distribution of the devices, enabling access to personalised diagnosis and hence PoC devices.

For instance, each working day (8 h), in our lab, we can produce approximately 720 COC chips, i.e. 40 s turn-around time per chip. Since the injection moulding is an automated process, approximately 30 min are needed to set up the machine, after which the production runs basically without supervision. The amount of copolymer used for each polymer chip (with microscope-slide size)
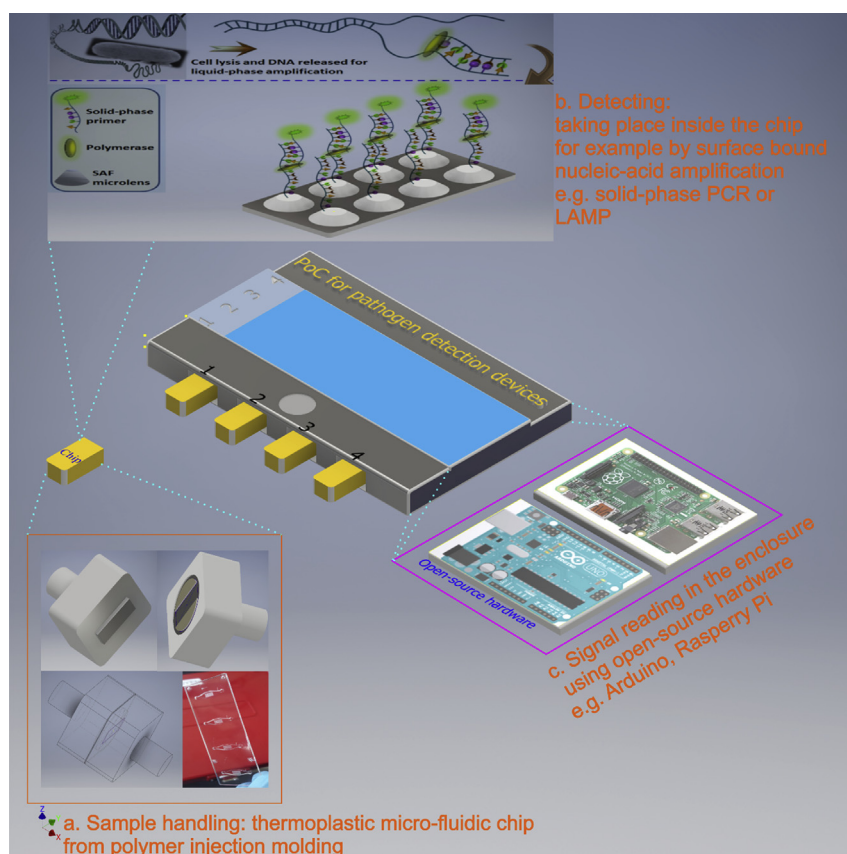
Fig. 1. The three essential components of PoC devices for (multiplex) pathogen detections and the proposed solutions, include (i) the use of polymer disposal microfluidic chip, (ii) surface-bound (or solid phase) nucleic acid amplification, (iii) using open source hard- (and soft-) wares for electrical readout and controlling.

Table 1
UV transmittance of materials used in fluorescent-biophysical measurements.

| Materials	LoC fabrication Processing	Transmittance, UV wavelength λ > 350 nm	References
Glass	H	80−85%	[19,20]
Fused silica	H	90−95%	[20]
PDMS	S	80−85%	[21]
COC	S	85−90%	[22]

H = hard micromachining [23]; S = Soft micromachining.

is ~7 cm^3, which means one kg of COC can produce 180 chips [107]. One kg of COC material costs only 20 USD [36]. On the other hand, the cost of a fused silica wafer (e.g. from PlanOptik, Germany) is 50 USD/wafer (525 μm thick) and 80 USD/wafer (170 μm thick). Of these two wafers, costing 130 USD in total, each fabrication experiment can maximally produce approximately ten working chips. The cost of materials in this comparison is, therefore by far favourable for the use of COC copolymer. Moreover, the cleanroom fabrication processes for using fused silica is also painfully costly and cumbersome. The total cost for materials and processing a batch of 10 fused silica chips could estimatedly cost ~1700 USD (shown in Table 2) and could take up to 3−4 days of processing for cases of successfully made, not to mention the development cost and fault during each fabrication step and batch. On the other hand, in case of polymer injection moulding, the shim fabrication process and materials costs around 500 USD to 1000 USD (depending on the requirement of the structures and patterns, see Table 3). In many cases, the fabrication cost can be less than 200 USD (if the chips do not possess complicated structures and nanochannels, i.e.

using a micro-milling shim for microfluidic structures with sizes larger than 10 μm). In both cases, the shim can be used multiple times (>300 replicas without any signs of failure [37]), which can result in the production of thousands of chips. Hence, injection moulding is, by far, more economical and less time consuming compared to using fused silica chips.

The other important feature of polymer injection moulding is that the resulting COC chips, after injection moulding, shows an extremely high replication rate, usually around ~98% even for nanochannels. For instance, experiments on COC chips at DTU showed that nanostructures with a depth dimension in the order of 70 nm, the largest depth deviation among the different polymer samples was 3 nm. The average polymer channel depth replication fidelity was shown to be 98% [38,39] (for 5 different measurements).

Moreover, in NanoTech department of DTU (as of January 2019 called DTU Health Tech), experiments conducted on fluorescent measurement using nanofluidic COC chips have been carried out since 2010 [40]. In these very first experiments, single-molecule DNAs were trapped and stretched inside the COC polymer nano-channels. The results were then compared with measurements using costly fused silica chips and found consistent and with similar performance. This again confirmed the advantages of using low-cost (and massive productions) COC polymer chip in the replacement of fused silica.

LoC applications requiring the application of high pressure can also be attained using COC chips. It has been reported that COC chips bonded by ultrasonic welding can withstand up to 8.5 bar of applied pressure [41]. This opens up interesting possibilities, for instance, studies using ultra-rapid microfluidic mixers made from
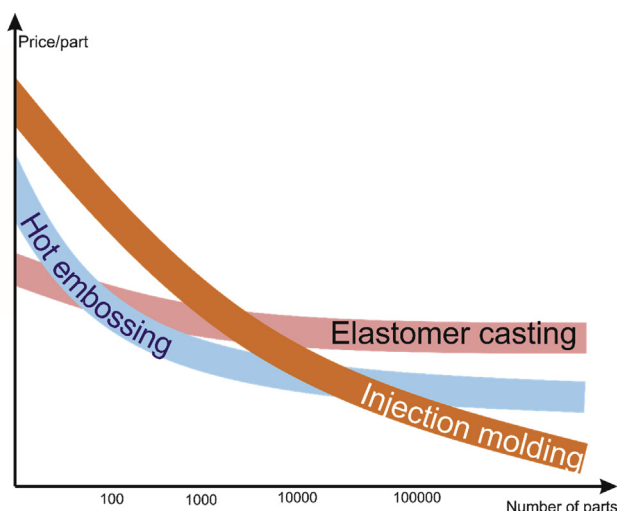
Fig. 2. Economic comparison among injection moulding, elastomer casting and hot embossing. Adapted and Inspired by Becker from Ref. [17]. The figure is included for the sake of argument, and the numbers are approximate.

Table 2
Estimated price for fabricating 10 fused silica chips.

Process for making 1 batch (1 top and 1 bottom fused silica wafers), resulting in approximately 10 chips.	Estimated cost for cleanroom use of equipment and chemicals (USD, price estimated in 2016, in Michigan, USA)
Mask cleaning	10
Wafer cleaning	10
Spin coater, primer, pre-exposure baking, post-exposure baking	40
Mask Aligner	20
Development	20
Etching	100
Stripping photoresist	20
Cleaning, inspecting using a microscope, coating protection layer	20
Sandblasting	20
Cleaning, Bonding	100
Dicing, inspecting using a microscope	100
Material fused silica	130
Clean room access	680
Labour of the experimentalist and transportation between the lab and the cleanroom.	400
Total (USD)	**1670**

Table 3
An estimated cost for fabricating an injection moulding shim using electroplating in our lab.

Process for making an injection moulding shim	Estimated cost (USD) for cleanroom use of equipment and chemicals, in NanoLab, DTU.
Silicon wafer	10
Spin Coater	20
Mask Aligner	20
Development	10
Sputtering	50
Electroplating	140
Cleanroom access	400
Injection moulding	20
Labor	200
Total (USD)	**970**

COC in the investigation of protein folding, instead of costly fused silica chips [33]. In the ultra-rapid microfluidic mixer, using fused silica, the maximal applied pressure was 80 psi, approximately 5.5 bar and normally 20 psi, or 1.37 bar was used in these experiments [32]. Furthermore, COC being a thermoplastic with high thermo-stability up to 130 °C makes it a perfect candidate for PCR based applications in PoC devices.

3. Advanced molecular techniques in PoC systems

3.1. Challenges in the detection of pathogens in PoC systems by molecular techniques

Revolution in molecular analysis of Nucleic Acids (NA) has focused the scientific research on the development of new miniaturised and easy-to-use technologies, such as PoC technologies, interfaced with advanced molecular techniques. Ideally, such PoC systems should allow rapid NA analysis in decentralised environments, with all the steps necessary for sample preparation and detection automated, to make the test accessible and manageable by unskilled personnel [12]. Recently, a number of the advanced molecular techniques such as Polymerase Chain Reaction (PCR), Loop-Mediated Isothermal Amplification (LAMP) etc., have been integrated into PoC systems for rapid detection of pathogens in food and clinical samples [42]. However, there is still a requirement for efficient sample preparation and its integration into the microfluidic-based PoC systems. Sample preparation is the greatest challenge in the rapid detection of pathogenic microorganisms in the area of clinical diagnostics. While developing a robust sample preparation method with capacity for automation and integration, one should keep in mind the complexities of the clinical and environmental samples. The complexity and heterogeneity of biological matrices, such as whole blood, urine, saliva, swab and fecal materials, in particular, the non-uniform distribution of pathogens, as well as the low abundance of target pathogens, are the main challenges for rapid and ultra-sensitive detection of pathogens. In addition, the analytical performance of advanced pathogen detection strategies also suffers from the interference from indigenous microflora [43]. Although microfluidic-based PoC systems have emerged as promising tools in recent years, limited measurement volumes and low abundance of target pathogens are an obvious bottleneck in the applications of such devices. In most of the cases, the microbial load can be even less than 1 CFU/mL in blood. The recoverable NA concentration from such samples may far exceed the capacity of most of the sample preparation methods [44]. The low operational sample volume in the microfluidic channels might render difficulties in attaining high sensitivity and required selectivity [45]. Hence, we believe that additional efforts are required to integrate efficient sample handling strategies with microfluidic systems to implement them for real-time pathogen detection in biological samples.

3.1.1. Sample preparation: a prerequisite step for PoC systems
As just mentioned in the previous section, accurate identification of pathogens directly from the blood, without proper sample preparation steps, is nearly impossible or at least highly challenging, to obtain in PoC systems. Therefore, sample concentration and growth-based enrichment of microorganisms have been the key step in current methodologies. Recently, several microfluidic platforms based on different principles have been reported to address the complexity of sample preparation [46–49]. Zhang et al., have reviewed a few microfluidic systems that work on the principle of physical, chemical and biochemical methods for the efficient sample preparation [50]. It is worth to mention that the selection of an ideal sample concentration principle should be compatible with the downstream analytical methodology. In general, sample concentration based on the biochemical principles is a more convenient and efficient way to attain higher selectivity. This may be due to the specific interactions between cell surface

antigenic biomarkers of the pathogen and recognition ligands. Kant et al., reviewed the possible applications of bio-recognition ligands, such as antibodies, peptides, aptamers, Endolysin and Carbohydrate-binding proteins based-ligands for foodborne pathogen detection on microfluidic devices [45]. The basic intention of the sample preparation may not be limited to concentrate the target pathogens. It may also be desirable to eliminate the adverse effects of biological matrices on the analytical/bioanalytical performances and to reduce the heterogeneity of food or clinical samples. Although most of the physical techniques for sample preparation can concentrate the target pathogens non-selectively, they may fail to eliminate the biological matrices. These traces of biological matrices, if remained in the sample, may interfere or inhibit the downstream analytical performance. Biochemical principle of sample preparation has advantages in this regard, as the method can concentrate the target pathogens selectively, thereby eliminating the interference from indigenous microflora of the food samples. The possibility of separating and eliminating the biological matrices from the samples is an added advantage when the bio-recognition ligands used for the pathogen capturing are immobilised on a solid support, such as microfluidic channels or magnetic beads. In this direction, immunomagnetic separation strategy has been studied by several researchers, also in combination with microfluidic systems [51–56]. Magnetic bead-based sample preparation has an advantage that it increases the chance of pathogen bio-recognition due to their high surface-to-volume ratio. The strategy also enables simple mixing of the capturing ligand-functionalized magnetic beads with the suspected pathogen contaminated samples of various matrix categories (Blood, saliva, urine, food, feed and fecal samples), thus, ideally suited for PoC applications [57–60]. Magnetic beads functionalized with target-specific capturing ligands can bind to intended targets with high specificity even at very low pathogen concentration. In a previous report, our group has demonstrated the immunomagnetic concentration of *Salmonella enterica* from different food samples and evaluated the biocompatibility of magnetic beads in direct PCR [58]. In that study it was observed that magnetic bead-based approach had a capturing efficiency of >95% for *Salmonella* Typhimurium in food samples containing background microflora. The antibody functionalized magnetic beads did not pose any inhibitory effect during direct PCR. Direct PCR conducted on *Salmonella* captured magnetic beads had a relative PCR efficiency of >92% resulting in a limit of detection (LoD) of ~2-3 CFU/mL of the spiked food sample. Several such microfluidic systems combined with magnetic beads/ particles for efficient sample concentration were reviewed in detail by Kant et al., wherein the efficiency of pathogen concentration may be attributed to the combination of magnetic beads and pathogen-specific bio-recognition ligands [45]. Thus, a magnetic bead-based approach enables sample concentration and sample purification, simultaneously, by concentrating pathogens from large sample volumes besides eliminating matrix and PCR inhibitors. Therefore, this strategy is ideally suited for designing sample preparation integrated PoC devises.

3.1.2. PCR technologies and singleplex assays in PoC systems

Integration of molecular techniques such as PCR and LAMP into PoC systems has the potential of overcoming hurdles in rapid and sensitive diagnostics. Nevertheless, it is important to address challenges such as the capacity of multiplexing and PCR inhibitors in the microfluidic-based PoC platforms. The integration of qPCR in microfluidic devices is a promising approach that has the potential to result in miniaturised, portable and rapid detection platforms. However, PCR inhibitors can drastically reduce amplification efficiency and thereby affect the accuracy of such devices. In this direction, Phusion high-fidelity DNA polymerases (Pfu), having the

capacity to overcome the most common PCR inhibitors, could be a better choice for PCR-based PoC systems. The Pfu DNA polymerase exhibits good processivity, stability and increased tolerance to high salt concentration and PCR inhibitors. These features are attributed to the fused double-strand DNA-binding protein domain (Sso7d) that improves the performance of the Pfu DNA polymerase by guiding negative supercoiling [61]. Petralia and Conoci gave an insight into the progress and perspectives of PCR based technologies for PoC applications [12]. In the review, they have discussed the suitability of PCR amplification chemistry for PoC applications in detail and highlighted several fully integrated PoC prototypes and commercial PoC products available in the market based on PCR technologies. A droplets-platform developed by Pipper et al. (2007) to detect the avian influenza virus H5N1 from a throat swab is one of the examples of fully integrated PoC prototype [62]. As a unique approach, surface-functionalized superparamagnetic particles were emulsified in an immiscible liquid. The emulsification resulted in a 100-nL droplet spontaneously forming on a perfluorinated glass or polymer chip. Influenza (H5N1) viral RNA was concentrated up to 50,000% in a sequential process and subjected to ultrafast real-time RT-PCR. The bioassay was sensitive, 440% times faster and completed in less than 28 min as claimed by authors. A portable microfluidic system was reported by Qiu et al. (2011) for bacteria detection in saliva [63]. The device consists of a polycarbonate cassette with integrated fluidic components for DNA extraction and RT-PCR. This disposable cassette carries out cell lysis, nucleic acid isolation, concentration, thermal cycling, and either real-time or lateral flow (LF) based detection within an hour. In order to control the thermos-cycling precision during RT-PCR, a double-sided heating/cooling scheme with a custom feedforward, variable, structural proportional-integral-derivative (FVSPID) controller was used. The device was capable of detecting *B. cereus* from 100 μL saliva, thus, suited for resource-poor regions lacking centralized laboratory facilities and skilled personnel [63]. Several PoC platforms that work on the principle of PCR technology are commercially available. In general, a disposable cartridge prefilled with all the necessary reagents, microfluidic actuation, thermal actuation module and optical fluorescence detection module has been the key features in these platforms. Most of the platforms (GeneXpert by Cepheid, Cobas Liat System by Roche, FilmArray by Biomerieux, ML by Enigma) employ magnetic bead-based solid-phase extraction coupled with real-time PCR methodologies, however, use benchtop instruments. Petralia and Conoci [12] have compiled some of these commercially available platforms and summarized major features that include extraction/detection methods, system architectures, target pathogens, time of analysis and regulatory status. However, developing PoC devices with multiplexed detection capacity is much challenging and subjected to the discussion in the next section where the surface bound technique is required to solve this issue.

3.1.3. Multiplexed PCR assays in PoC systems

Detection and differentiation of pathogens at genus, sub-species or serotype level is very much important to analyse food, clinical or environmental samples accurately. However, as mentioned above, the capacity of online multiplexing with qPCR-based microfluidic systems is limited due to the interferences between different primer and probe sets. Replacement of conventional PCR with solid-phase PCR (SP-PCR) in the PoC systems may be a possible alternative solution. In the SP-PCR, the target nucleic acids are amplified on a solid support with one or both primers immobilised on a surface [108]. The SP-PCR provides much higher multiplexing capability since numerous primers can be arranged in an array. This strategy minimises undesirable interactions due to the spatial separation of the immobilised

primers. Hung et al. have demonstrated the integration of SP-PCR in combination with the supercritical angle fluorescence (SAF) microlens array embedded in a microfluidic chip [64]. It is also important to note that Hung et al. also tested the feasibility of a re-engineered Phusion high-fidelity DNA polymerases for rapid multiplexed detection of various *Salmonella* species in a microfluidic chip based on the SP-PCR [64]. Here, *Salmonella* subtype-specific DNA probes were immobilised directly on top of the cone-shaped SAF microlens array located at the bottom of a chamber of the microchip. During PCR, initial amplified products of the pathogen DNA in the liquid phase served as templates for the solid phase probe elongation. This results in the covalently attached PCR amplified products and fluorescence signals being generated by labelling one of the primers with a fluorescent dye. Thus, the pathogens were identified from the pattern of fluorescence signals on the SAF array on the microfluidic chip at a sensitivity of 1.6 copies/μL. This smart approach could be a better solution to improve the multiplexing capacity of the PoC systems. Attempts were made in recent past to integrate multiplexed-PCR assays with microfluidic devices. A segmented continuous-flow multiplex PCR (SCF-MPCR) was developed on a spiral-channel microfluidic device and reported by Shu et al. [65]. The microfluidic capillary channel made up of disposable polytetrafluoroethylene (PTFE) was coiled on three isothermal blocks with different heating zones to facilitate thermocycling. The system was a proof of concept for multiple identifications of DNAs of *S. enterica*, *L. monocytogenes*, *E. coli* O157:H7 and *S. aureus*. Purified DNA of specified targets mentioned above were isolated from spiked banana, milk, and sausage and identified in the SCF-MPCR devise with a detection limit of 100 copies/μL [65]. Droplet digital PCR (ddPCR) is yet another promising concept for multiplexed PCR based PoC applications. The ddPCR concept is based on the amplification of target DNA molecules in many separate droplets that follows Poisson statistics. Here, majority of reactions in the droplets contain either one or zero target DNA molecule. Therefore, number of PCR-positive reactions equals the number of template molecules, thus, easily quantifiable. As a proof of concept, a ddPCR platform was reported by Bian et al. for the simultaneous and sensitive detection of *E. coli* O157:H7 and *L. monocytogenes* in water [66]. In this platform, a mineral oil-saturated polydimethylsiloxane (OSP) chip was used that facilitates droplet generation, on-chip amplification and end-point fluorescence readout. This ddPCR platform had a limit of detection of 10 CFU/mL reaching a sensitivity of single-molecule resolution without significant cross-assay interference [66]. Despite real-time PCR being an advanced molecular technique, a requirement of precise and repeated thermocycling necessitates an instrumental design with complex thermal control mechanisms. The invention of isothermal amplification strategies has provided a positive advancement for overcoming the thermal limitations of a PCR based method. Craw and Balachandran (2012) reported an overview and critical assessment of such isothermal amplification techniques and their suitability for PoC systems [42].

3.2. Loop-mediated isothermal amplification (LAMP) technology

Isothermal amplification techniques emerged in the 1990s and have been verified as a simple, rapid and efficient method of nucleic acid amplification. Unlike PCR, that requires distinct thermal steps for denaturation, annealing and extension, the entire process of isothermal amplification is conducted at a constant temperature. In the past decades, microfluidic systems and PoC devices that employed PCR as the detection technique have been extensively studied. However, the thermal cycling requirement for PCR remains the main challenge for designing handheld devices for on-site testing of pathogens. Beside the rapidity and high efficiency, the simple thermal requirement of isothermal amplification techniques was considered as a proper alternative for PCR for implementation in PoC device. Among the various isothermal amplification techniques currently available, LAMP has been frequently reported to be more sensitive, specific and faster. Since its invention in 2000, LAMP has been employed in many commercial diagnostic kits from different suppliers [42].

3.2.1. Role of inner loop primers in LAMP

LAMP, unlike PCR, uses a set of four target-specific primers, including two inner primers, termed forward inner primer (FIP) and backward inner primer (BIP), and two outer primers (F3 and B3). The inner primers of LAMP have a special 'fold back' configuration which forms stem-loops motifs with self-priming capability [67]. As shown in Fig. 3, each inner primer itself contains two functional sequences (F2 and F1c, B2 and B1c). Overall, the LAMP process can be distinguished in two steps, i.e. structure-producing step and cyclic step.

In contrast to PCR, LAMP takes advantages of using a DNA polymerase with a strand-displacing activity that eliminates the necessity of applying a complex thermal cycling step for dsDNA denaturation between each amplification cycles. The principle of priming and amplification from outer primers (F3 and B3) in LAMP are similar to conventional PCR. In contrast, the amplified products primed by F2 and B2 portions of the inner primers result in a DNA strand having complementary sequences for binding F1c and B1c portions of the inner primers respectively during the structure producing steps (Fig. 3). This unique stem-loops motif called "double dumbbell structure" enable self-priming during cyclic amplification steps that are independent of parent target DNA strand. As a result, LAMP produces amplified products that are several orders of magnitude higher in concentration (Fig. 3) compared to PCR, and within a shorter time [68]. In LAMP, the inner primers play a major role that has several advantages as explained below for the integration into the PoC devices. First, the strand displacement driven amplification that occurs at constant temperature (60−65 °C in general) eliminates the requirement of considering repeated thermocycling necessities during instrumental design. This fulfils the requirement for having simple design and robustness of PoC devises. Second, self-priming driven amplification of initial stem-loop structures, which is independent of parent target DNA strand, results in the accumulation of a high amount of amplicons allowing an 'amplification is detection' scheme. This provides the required rapidity for PoC devises to be able to detect targets within a short time. Third, the use of a minimum of 4−6 primers makes LAMP a highly specific reaction. Also, LAMP has been shown to have analytical sensitivity equivalent to, or sometimes even exceeding, that of the conventional PCR assays, with a limit of detection (LOD) as low as 5 copies per reaction [42,69]. Fourth, the involvement of multiple primers and stem-loop mediated self-priming capability provides LAMP with a higher tolerance to substances which would normally inhibit PCR amplification. Thus, a working PoC device with minimal sample pretreatment may be developed. This fulfils the requirement of having analytical precision and accuracy in the PoC device. Fifth, rapid amplification also releases insoluble pyrophosphate as a by-product during LAMP reaction. This insoluble pyrophosphate salt can be employed in a turbidimetric detection strategy for either qualitative visual indication or real-time quantification [69]. This could be an added advantage, that provides ultimate robustness and flexibility during PoC development, together with integrated custom required signal detection strategies such as turbidimetry, colourimetry or fluorimetry. These attributes make LAMP well
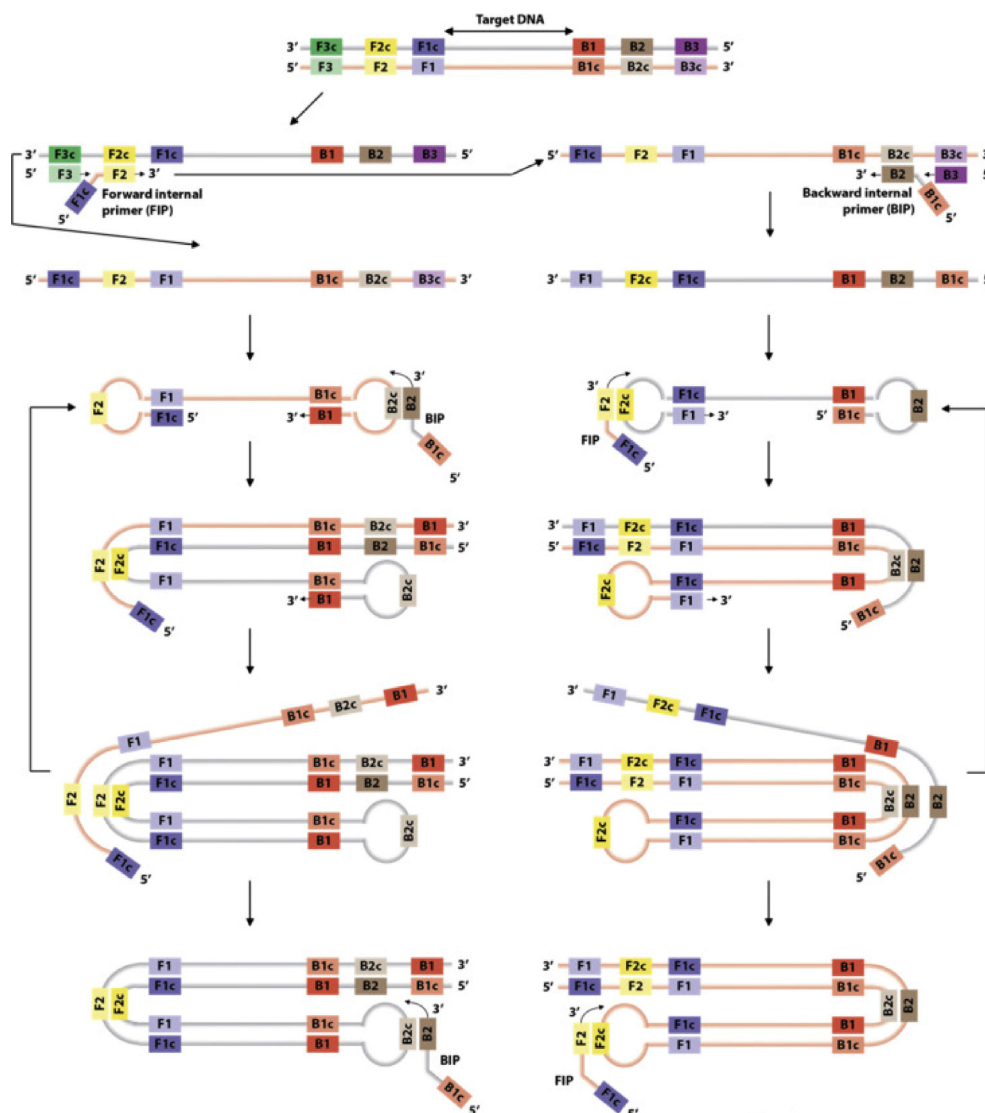
Fig. 3. Schematic representation of the principle of Loop-Mediated Isothermal Amplification (LAMP) method. Reprinted with the permission from Ref. [70].

suited for its integration into simple, robust and user-friendly miniaturised PoC devices.

3.2.2. LAMP integrated PoC devises: current perspectives

Various LAMP integrated PoC devices have been reported for on-site pathogen detection [71,72]. Oh et al. reported a centrifugal microfluidic device that is capable of identifying multiple foodborne pathogens using LAMP and colourimetric based rapid and simple test, without the need for an additional instrument for detection [73]. The chip was capable of analysing 25 samples simultaneously, with a LOD of 380 copies genomic DNA of *Escherichia coli* O157: H7 within 60 min. By adding indicator Eriochrome Black T (EBT), the initial purple colour of LAMP mixture would change to sky blue colour once the target gene was amplified. The use of EBT detection could detect the presence of target by the naked eye. However, in this system, sample preparation has to be performed off-chip. Another centrifugal microfluidic device integrated with the complete procedure from sample preparation for the detection of *Salmonella* by naked eye was reported by Ahmed et al. [74]. This microfluidic platform consisted of 16 amplification chambers and could analyse four samples simultaneously. Here, SYBR Green I was used as a DNA intercalating dye that enabled detection by the naked eye, as well as fluorescence. The sensitivity was found to be 5×10^{-3} ng/μL DNA. Tourlousse et al. have demonstrated LAMP on a chip containing 15 interconnected reaction wells with dehydrated primers. The chip tested with purified genomic DNA and could detect multiple foodborne pathogens, including *Salmonella, Campylobacter, Shigella* and *Vibrio choleae*, in less than 20 min [75]. A LOD of 10−100 gene copies per reaction was achieved. Duarte et al. also reported a LAMP on a silicon chip containing 4 arrays of 6×6 wells with dehydrated primer mixture for multiplexed detection of *Escherichia coli* O157, *Listeria monocytogenes* and *Salmonella* spp. [76]. A LOD of 10^5 CFU/mL was achieved. However, in both systems, the sample preparation and nucleic acid purification processes were performed off-chip before the LAMP amplification. Yi et al. have reported a LAMP-based LOC system for real-time quantitative detection of *Salmonella*. The system was integrated with a magnetic bead for sample preparation. LAMP was employed for rapid and quantitative detection of *Salmonella* in enriched pork meat samples [77]. The whole process, including DNA isolation, isothermal amplification and real-time detection, was completed in a single chamber. The system was able to perform eight samples

simultaneously with a LOD of 50 cells per test within 40 min. Several of such assays have been developed by Eiken Chemical Company Ltd., for in-vitro diagnostic applications that show the popularity of LAMP as the next generation amplification technology for PoC applications (http://www.eiken.co.jp/en/product/index.html). These examples demonstrated the advantages of the integration of LAMP into microfluidic chips and PoC for rapid real-time or at site pathogen detection.

3.2.3. Multiplexed analysis with LAMP

Although LAMP is a well-developed method, multiplexed analysis using LAMP is challenging because LAMP amplicons are very complicated in structure. Unlike PCR, LAMP amplicons do not have a defined structure and target gene amplicon specific definite molecular size. Normally, LAMP generates amplicons with various lengths irrespective of the length of the template. Therefore, in order to develop multiplexed LAMP, it is necessary to discriminate target-specific amplicons from the mixture of LAMP products originating from simultaneous amplification of multiple target genes [78]. Attempts were made in this direction in the recent past by several researchers and unique approaches such as lateral flow concept [79], sequence-based barcodes [78] and microfluidics with dedicated micro-channels for each target [80] have been reported. Wang et al. (2017) have reported a multiple-LAMP (mLAMP) assay in combination with lateral flow biosensor to detect *Enterococcus faecalis* and *Staphylococcus aureus* in blood samples. *Ef0027* gene (*E. faecalis*-specific gene) and *nuc* gene (*S. aureus*-specific gene) were initially amplified simultaneously via mLAMP reaction using FITC and digoxin-modified forward loop primers respectively for each target. Forward inner primers (FIP) of both targets were modified with biotin at the 5′-end. After LAMP, target specific amplicons bearing either biotin-FITC or biotin-digoxin respectively were spatially separated in a lateral flow format immobilised with *anti*-FITC and anti-digoxin antibodies as different target lines. Binding of gold nanoparticles capped with streptavidin to biotinylated amplicons facilitated visual identification of respective targets at pre-defined target lines in the lateral flow strip. The limit of detection achieved with this method was 710 *E. faecalis* CFU/mL and 680 *S. aureus* CFU/mL of blood sample that was 10- and 100-fold more sensitive than qPCR and PCR technologies for detection of two target pathogens in spiked blood samples. The entire procedure, including blood samples processing (30 min), mLAMP reaction (40 min) and result reporting (within 2 min) was completed within 75 min [79].

Sun et al. (2017) have demonstrated multiplexed LAMP assay using a microfluidic chip-based platform in combination with smartphone assisted fluorescence detector to identify four equine respiratory pathogens [80]. In this approach, a silicon-based microfluidic chip with 10 parallel flow channels and a shared sample inlet facilitates multiplexed LAMP reaction. The chip had a dimension of 25 mm × 15 mm and each channel are 10 mm in length, 500 μm in width and 200 μm in depth that can hold 1 μL sample volume. These channels are pre-loaded with dedicated dried LAMP primers of target pathogens in duplicates. A custom-designed optics module having an overall dimension of ~90 mm × 70 mm × 95 mm was interfaced with microchip and smartphone. The optical module comprised 525-nm long-pass filter selected according to the emission wavelength of the DNA intercalating fluorescent dye (EvaGreen), 12.5x macro lens and eight 485-nm blue LEDs as light source. The multiplexed LAMP assay was demonstrated with this set up targeting *Streptococcus equi*, *Streptococcus zooepidemicus*, Equine herpesvirus type 1 and type 4 as representative examples. This smartphone-based compact, rapid and multiplexed detection system was sensitive enough to detect 50 genome copies of *Streptococcus equi* and

Streptococcus zooepidemicus in 1 μL and 5 genome copies of Equine herpesvirus type 1 and type 4 in 1 μL sample within an hour. In summary, the major limiting factor in developing multiplexed LAMP-based PoC systems may be in the complex engineering challenges. Integrating and automating LAMP assays with both upstream sample processing and downstream detection schemes is the bottleneck in developing multiplexed LAMP-based PoC devices.

3.2.4. Merits, limitation and future perspectives of LAMP in PoC systems

Distinguished merits of LAMP in comparison to PCR for application in PoC devices can be summarized in the following points: 1) the LAMP process is performed at a constant temperature (60−65 °C) eliminating the necessity of temperature ramping and the demanding of sophisticated thermal control instrumentation. This also leads to shorter testing time. 2) LAMP produces a tremendous amount of amplicons, (up to 0.8 μg/μL DNA) detectable even by the naked eye. 3) LAMP is a highly specific test due to the use of four/six primers. 4) LAMP has been proven to be less affected by inhibitors those commonly present in biological samples due to the combination of multiple primers and high tolerant strand displacing polymerase [81−84]. Despite many brilliant advantageous, LAMP shows some weaknesses yet to be addressed. Although employing four to six primers for detecting a particular target increase the specificity of the test, the process of defining four to six priming sites can be a challenge, especially for the short target sequence containing high mutation spots like RNA viruses. Another significant drawback of LAMP for using in PoC diagnostic devices is the lack of multiplex detection capability due to the requirement of primers for each particular target of interest.

LAMP can be considered as a potential technique in future generations of PoC devices due to its sensitivity (10−100-fold greater than conventional PCR and 500−1000 times more sensitive than antigen detection), rapidness, robustness and specificity, as well as simplicity in practical implementations. Besides traditional optical and electrochemical-based detection modalities, novel techniques, e.g. giant magnetoresistance (GMR) [85] have been adapted for LAMP in PoC devices. The next generation of PoC may be fully integrated systems which are portable, fast, low cost, easy to use, sensitive and specific. To integrate LAMP as a powerful detection tool in PoC devices, it needs to be combined with an appropriate monitoring method. In general, monitoring methods are defined as the methods adapted for real-time or endpoint recognition of signals from a biochemical reaction [69]. The field of integration of monitoring methods within PoC devices is currently an ongoing developing field. The feasibility of integrating LAMP in PoC devices and the feasibility of adopting miniaturised detection components such as electronics and cost-effective readouts (e.g. photodiodes) interfaced through operating microcontrollers, will be discussed in the next section.

4. Open-source hardware

Definition of open-source hardware will here be defined simply from a practical standpoint: As hardware to which the design and instructions needed to duplicate the part in question made freely available (schematics, PCB design, CAD/CAM files, etc.). It is thus closely linked to the whole movement of "Makers", where the point is that everything is freely available to anyone who could benefit from making a similar solution, or potentially even builds on and expand it. In return, the inventor gets valuable feedback on the developed solution, as well as all the further development to the hardware provided by other users.

While open-source hardware principally encompasses everything, from very simple tools, to DIY 3D printers [86], CNC [87], or

even advanced robotic solutions [88], the open hardware solutions that are relevant for PoC systems generally consists of two things: mechanics, for sample handling and interaction, and electronics, for controlling functions, and possibly, for running user interaction/interface processes [89]. Furthermore, once the desired hardware solution that fulfils the task, e.g. performing the desired PoC test, has been developed, the system needs to be packaged, that is, put in an enclosure. The packaging is important, both for protecting the internal mechanics and electronics, but also to make it user-friendly and functional.

A note about developing new solutions/hardware: It is imperative to stress, that the creative process of developing new solutions cannot be short-circuited/avoided, and that the developer needs a keen interest in the process. Developing new hardware and solutions will often imply stepping out of a comfort zone/area, and be willing to run the risk that, at least in the beginning, might feel like constant failures. However, the right mindset, which involves a willingness to "do" and then learn from the process, is essential (also implying not to label these necessary steps as "failures"). Fast prototyping is about trying out ideas, not forcing out the "perfect" idea in the first attempt, as almost all good solutions come to mind while the mind is occupied with "making" and building something, constantly learning from the behaviour of the material as it is being tooled and assembled into shape and function. This cyclic way of thinking about developing (mechanical hardware) solutions is probably best known from software development, where it is usually referred to as "iterative development" [90,91], (in contrast to incremental development). Transferred to hardware development, it can visually be represented as the cycle shown in Fig. 4.

4.1. Open-source hardware in PoC systems

As mentioned earlier, the development of open-source hardware solutions for PoC systems generally comprises of (at least) two parts, mechanics and electronics, which needs fundamentally different tools and strategies to develop. Custom made parts for the mechanics of the system, as well as the enclosure, is usually designed using CAD software, and fabricated using fast-prototyping tools, e.g. tabletop laser cutters, CNC's, and/or 3D printers. Interestingly, even the fast prototyping tools such as 3D printers and CNCs needed to fabricate these custom-made parts can also be made from readily available open-source hardware resources, provided by the ever-growing community of makers [93–95]. It is here important to remember again, that no open-source hardware solutions generally need to be developed from scratch, but can also be adapted or tweaked from existing solutions, this is one of the biggest advantages of the open-source resources.
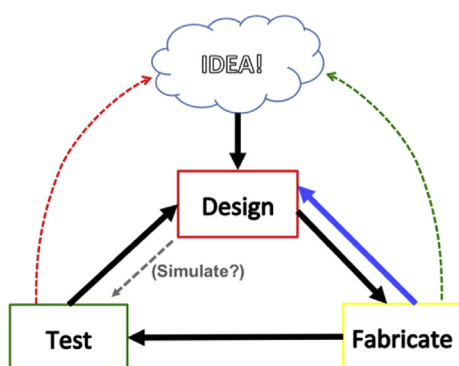
To be able to perform any tests with PoC systems, these systems need to include some microcontrollers or other control circuitry, to run the necessary procedure for the test [89]. Obvious choices, especially for developing prototypes, are open-source microcontroller-based development boards, for instance, the ones from the popular family of Arduino products, or the Raspberry Pi. Other brands and open-source platforms exist, for example, Teensy, Beaglebone, Nucleo, etc., however, here we will focus mainly on applications using either the Raspberry Pi or the very simple to use Arduino's, as we believe that these platforms, apart from being commonly available, have the biggest community of users (e.g. "Makers"), and therefore most resources available. Both platforms are relatively cheap and very accessible for new users, and the vast amount of resources available makes it possible to prototype electronics and electronic circuitry, even for people with little or no prior experience in electrical engineering. Choosing an appropriate development board depends very much on the requirements of the finished device. The family of Atmel microcontrollers featured in the range of Arduino boards generally runs what is called a Real-Time Operating System (RTOS), which means that they can run and perform only one task at a time. The processing is designed to be extremely robust, though, and is thus useful in applications that require high precision in the timing of procedures, with minimal maintenance over time. The Raspberry Pi, on the other hand, is based on a General-Purpose Operating System (GPOS), which means that it is basically a mini-computer, capable of performing multiple tasks and advanced operations and calculations, e.g. image processing and/or recognition, implementations of machine learning algorithms, or interfacing and controlling of large touchscreens, etc. [96,97] However, the advanced processing capabilities of a GPOS might be at the expense of robustness in time-critical applications, as opposed to microcontrollers running a RTOS. Another consideration is that the Arduino platform features a very user-friendly and simple to use Integrated Development Environment (IDE, see https://www.arduino.cc/en/main/software), while the Raspberry Pi is generally coded indirectly via high-level programming tools such as Python (https://www.raspberrypi.org/documentation/usage/python/).

The basis for developing a PoC device could, for instance, be some newly developed technology, e.g. an experimental setup in a lab such as a sensor or lab-on-a-chip system. An overall tactic for the development of such an (open source) PoC device would be: 1) Identifying the core function(s) of the system and how they could be automated, and 2) replacing any commercial tools necessary to run the function with self-made solutions, either developed from scratch, or open-source resources. For instance,
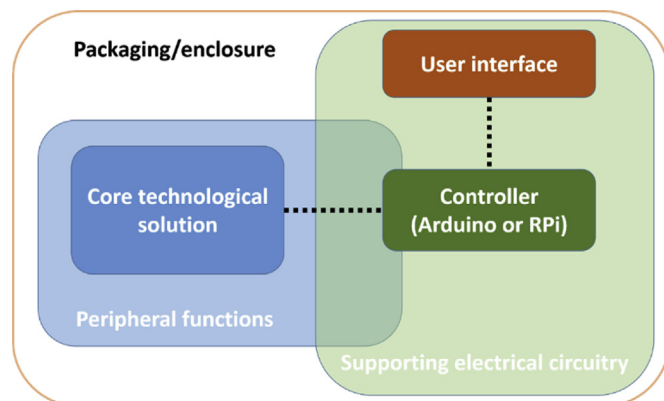

Fig. 4. Example of iterative product development of hardware solutions. It is reprinted with permission from Andreasen 2017 [92].

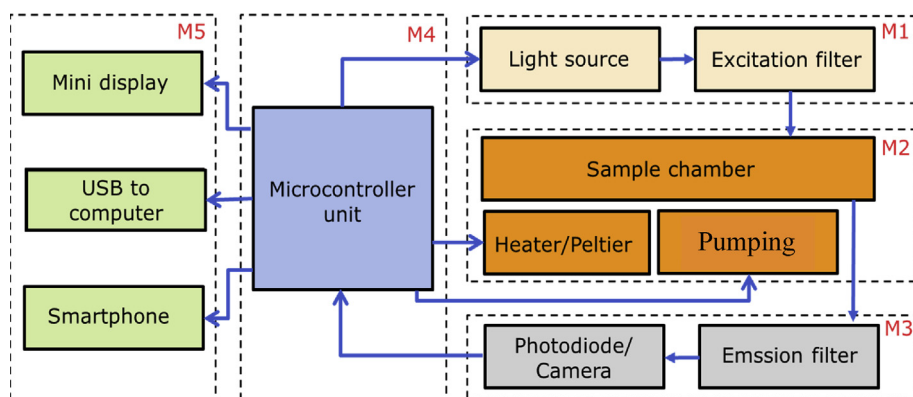**Fig. 5.** Schematic showing how the fundamental parts of a PoC system prototype generally correlate.

Fig. 6. Functional block diagram of the general fluorescence detection system.

a wide range of solutions has already been developed for microfluidic pumping (with feedback control of flow-rate) [98], heating/cooling and temperature control algorithms [99], or camera control and the image acquiring (see next section for an example of tactic for realizing fluorescence microscopy) [100]. 3) The third step is to develop a user interface for the system, as well as the necessary functions to control and operate the system, and finally, 4) figuring out how the developed solution(s) could be packaged into a fully integrated device. A schematic representation of how to think about the integration process can be seen below in Fig. 5:

4.2. Fluorescent PoC detection using open-source hardware

To help the beginners to get started on building a prototype from scratch easily, we propose the function block diagram of the general fluorescence detection platform (see Fig. 6). In general, the platform includes (1) An excitation module (M1) consisting of a light source (LEDs or Laser) and excitation filters; (2) A sample chamber module (M2, that accommodates a microscope slide, nitrocellulose membrane, microfluidic chip or cassette). Different kinds of a heater can be used for different purpose of applications such as a resistive heater, which is sufficient for employing a loop-mediated isothermal amplification (LAMP) [101], and Peltier elements used for the building of a thermocycler, for instance for running a polymerase chain reaction (PCR) [102]. An optional regulator for pressure-controlled microfluidic flows or positional motor can be included to the sample chamber (M2) for liquid-based fluorescence detection [98,103] or for controlling camera position movements in order to observe/focus on the samples [100]. (3) An emission filter and signal readout module (M3), consisting of emission filters, photodiodes, and charge integration readout electronics, for multiplexed fluorescence detection [104,105] or a camera, for fluorescence image array detection [100,103,105,106]. (4) A microcontroller module (M4), for control of the sequence, power, and data processing. (5) A display and connectivity module (M5), including an optional mini display for prompting and presenting results to the user. Alternatively, a smartphone, with a user interface, and established a connection via Bluetooth or Wi-Fi, could be used to show device status and displays results. Other possibilities are uploading of information for cloud processing and providing interpretation feedback to the operator, i.e. a primary care physician [104].

5. Conclusion and outlook

In this perspective review, we discuss the three most important factors to realise which could potentially lead to commercialization with PoC pathogen detection devices based lab-on-chip technology. They include: (i) the use of disposal polymer biochips, (ii) the application of surface-bound nucleic acid amplification techniques and (iii) implementing and building on open-source hardware and software whenever possible. We foresee that those combinations enable the development of specialized PoC solutions, with the potential to be a major breakthrough in the future development of PoC diagnostic tools.

Despite being a potential research area in the last two decades, the realisation of an ideal PoC device for pathogen detection is yet to revolutionise the market. Major discrepancy in this direction was neither the engineering challenges nor the biochemical/bio-analytical technologies but the integration of both engineering and biochemical technologies, which is a complex multidisciplinary problem. This obstacle must be overcome by choosing an ideal biochemical/bioanalytical technique to facilitate a simple, robust and portable device with "sample-in, answer-out" functionality. LAMP, being an isothermal amplification method displaying high speed, high amplification power, and high diagnostic specificity and sensitivity, is ideally suited for the development of this next generation of PoC devices. The application of surface-bound nucleic acid amplification techniques could be a direction that has the potential to provide multiplexing capability to the next generation of PoC devices in the near future. Open-source hardware and the possibility of implementing disposable polymer biochips, instead of expensive and cumbersome to fabricate silicon- and silica-based chips, has the potential to overcome the existing design complexities and the engineering hurdles in to be successful in developing simple, yet robust, PoC devices. Thus, the new frontiers in the future PoC devices could be fully integrated, simple, compact devices, providing analytical performance superior to that of the current state-of-the-art technologies.

Declaration of competing interest

The authors declare no conflict of interest.

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