Lab on a Chip

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CRITICAL REVIEW

Commercialization of microfluidic point-of-care diagnostic devices†

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A large part of the excitement behind microfluidics is in its potential for producing practical devices, but surprisingly few lab-on-a-chip based technologies have been successfully introduced into the market. Here, we review current work in commercializing microfluidic technologies, with a focus on point-of-care diagnostics applications. We will also identify challenges to commercialization, including lessons drawn from our experience in Claros Diagnostics. Moving forward, we discuss the need to strike a balance between achieving real-world impact with integrated devices *versus* design of novel single microfluidic components.

1. Introduction

A large part of the excitement behind microfluidics is in its potential for producing revolutionary but practical devices. It is, at heart, a technology for manipulating small volumes of fluids, with the potential to miniaturize complex laboratory procedures onto a small microchip. A variety of theoretical models and academic proof-of-concept studies have demonstrated tantalizing advantages of lab-on-a-chip (LOC) systems over laboratory tests.[1–4] These advantages include consumption of small volumes of reagents and sample, and delivery of results with fast turnaround time. This technology has also benefited from investments, from government funding agencies to venture capital in Europe and North America, directed specifically towards the development of practical devices.[5] Despite this convergence of factors, surprisingly few LOC-based diagnostic tests have been successfully introduced into the market. What factors account for this discrepancy, and what can be done to overcome these challenges?

In this review, we focus on the commercialization of point-of-care (POC) diagnostic devices, a much-discussed application of microfluidics. The review will not discuss other exciting applications of microfluidics, such as laboratory-based microfluidic technologies in genomics, sequencing, high-throughput screening, and separations, although some of the lessons drawn here may share similarities. We will first describe the landscape of successfully commercialized products in this field, and highlight a subset of companies currently developing the next generation of technologies (Section 2). Next, we will attempt to draw lessons from past development efforts to identify challenges to commercialization that were not obvious in the first round of serious development efforts during the 1990's (Section 3). We will also reflect on lessons based on our personal experiences with Claros Diagnostics (a company two of us co-founded with David Steinmiller, from a technology initially developed in George Whitesides' lab). Finally, we will conclude with summary thoughts as the field continues to develop (Section 4).

2. Past and current work

2.1 Past work on POC diagnostics, and evolution to LOC

A major class of POC diagnostic tests is the lateral flow test, which uses a membrane or paper strip to indicate the presence of protein markers such as pathogen antigens or host antibodies. On a membrane, addition of sample induces capillary action without user intervention. As the sample flows across the membrane, it gathers labeling reagents embedded in the membrane, and flows over an area that contains capture molecules; the labeled captured analytes are interpreted by eye to form a visible band. In the U.S., lateral flow tests are used for diagnosis in a small number of indications, most notably pregnancy as well as infections with streptococcus or flu; in developing countries, the lateral flow test is widely used to diagnose HIV. Although the test is simple to perform, the single-flow action does not mimic the multi-step procedures of laboratory-based assays that are crucial for producing highly reproducible, quantitative, and sensitive results. As the lateral flow test comprises a multibillion dollar market, with many of the technologies now consolidated at the company Alere (formerly Inverness Medical Innovations), there has been significant industry interest in trying to improve their performance over the last few decades, but so far without significant progress.

The other major class of successful POC tests is the blood glucose test, which serves as a textbook example for a high-impact POC diagnostics product that has improved diabetic patients' lives, and now acts as a pillar of the entire diagnostics

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industry. The glucose test is also performed on membranes, but is typically classified differently from lateral flow immunoassays as the rest of the analytical method is altogether different (for example, it uses signal amplification by a redox enzyme, typically ending in an electrochemical readout).[6,7] The glucose test, however, is by nature somewhat unique. On the technology side, the concentration of the analyte is in the mM range, which far exceeds the concentration of most diagnostics markers. On the market side, the frequency of testing, typically multiple times a day, outnumbers that of most other tests.

With the dearth of new POC diagnostic technologies being introduced into the market, there has recently been a re-surgence in interest to develop novel and clever methods to re-purpose both lateral flow immunoassays and the glucose test, to significantly improve their performance and expand their range of targets. Examples include marrying modern LOC concepts, such as sophisticated flow control, to diagnostic tests performed on paper and membranes,[8–11] as well as novel methods to extend the reach of glucose tests.[12–14] By and large, however, companies that have traditionally focused on membrane-based testing (such as Alere, Biosite, and Chembio) have been exploring in the other direction *via* integration of conventional LOC-based components and functions.

In the current conception of LOC-based devices, the iSTAT handheld system (now part of Abbott) was among the first commercially successful products. The iSTAT system combines miniature fluidics and electrochemical detection to perform clinical chemistry measurements, such as electrolyte levels and limited immunoassays. Another interesting hybrid of LOC technologies with lateral flow is the A1cNow test for diabetic patients formerly from Metrika (now Bayer Healthcare), which uses multiple strips integrated with detection optics in a single package.

As LOC-based technologies struggle to achieve real clinical impact, the clinical need for new POC diagnostic tests remains high, especially for tests that can detect low concentrations of target and with an ability to quantify. Currently, tests such as lateral flow immunoassays can detect analytes present at high native concentrations (μM to mM). For targets present at low native concentrations, the assay systems require amplification either of the signal or the target, which typically increases the complexity of the testing device (Table 1), and is absent in current POC tests. In addition, outside of a small number of targets that reside at high concentrations, current POC tests remain largely unreliable for targets that require quantitation. Finally, multiplexing continues to be a challenge for current POC tests. For nucleic-acid testing (commonly referred to as ''molecular diagnostics''), the availability of POC tests is even more limited than that for clinical chemistry and immunoassays. There exist some nucleic-acid testing devices for use in healthcare clinics, but the operation of the tests requires a trained technician and limited laboratory infrastructure, which precludes use outside of a laboratory.

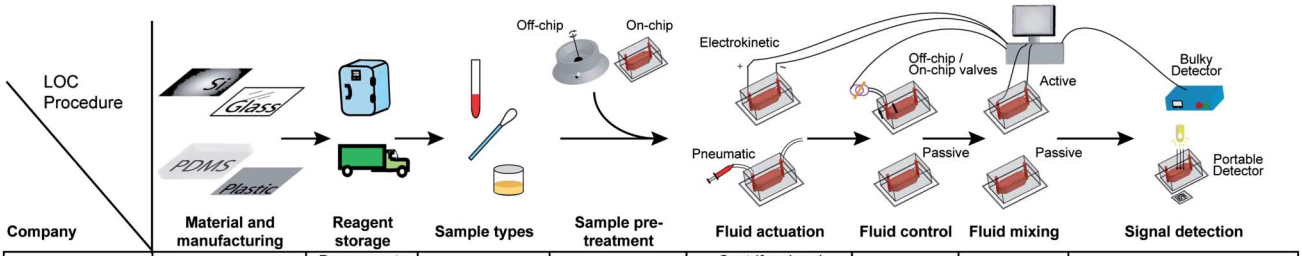
2.2 Current microfluidics companies developing POC diagnostics

LOC-based methods will continue to be the most likely technological driver to transform the POC diagnostics industry. The use of LOC spans a range of possibilities: adapting LOC components into an existing non-LOC POC technology, re-working current LOC concepts into a practical device, or developing completely new LOC concepts. In this section, we cover some of the current LOC-based work for developing next-generation POC diagnostics that has moved beyond the academic lab; the use of LOC concepts covers the range just mentioned. Moreover, no matter the specific technology, there are specific design choices that need to be made on a common set of technical challenges. We have previously outlined one version of these common technical challenges.[15] Below, and as summarized in Fig. 1, we also describe how each company has made its specific design choice to address the common technical challenges, grouped by the type of diagnostic marker. Additional information about the companies below, including financing, are shown in Table 2.

Small molecules. One of the most successful examples of point-of-care diagnostics using microfluidics and microfabricated devices is the iSTAT device. Founded in 1983 and acquired by Abbott in 2004, iSTAT Corp. developed novel microsensor technology in microfabricated thin-film electrodes on silicon chips for detecting a range of blood chemistries (sodium, potassium, chloride, glucose, hematocrit, gases), coagulation and cardiac markers (B-type natriuretic peptide or BNP, troponin I). The handheld and battery-powered analyzer is targeted primarily for emergency and critical care settings, and relies on pneumatic actuation to handle drops (~65 to 100 μL) of whole blood (Fig. 1 and 2A). No sample dilution or pretreatment is needed. The analyzer consists of a motor that controls flow of calibrant and sample within the cartridges, an electrical connector to receive signals from the cartridges, an electronic system to measure and monitor signals from the microfabricated thin-film electrodes, and an LCD display as user interface.[16,17] The disposable plastic test cartridges contain a silicon microchip with a microfabricated array of thin-film electrodes coated with specific ionophores or enzymes. Modalities of electrochemical detection vary depending on the analyte: potentiometry for direct measurements of sodium,

Table 1 Classes of assays for POC testing

Class of assays	Examples	Typical concentration range	Method of detection	Availability of POC products	
				Qualitative	Quantitative
Chemistries	Glucose, HbA1c	μM - mM	Direct detection	Widespread	Widespread
Immunoassays	Troponin, PSA	fM-nM	Signal amplification	Widespread	Limited
Nucleic acid testing	HIV viral load	aM	Target amplification followed by signal amplification	Limited	Limited



Company	Material and manufacturing	Reagent storage	Sample types	Sample pre-treatment	Fluid actuation	Fluid control	Fluid mixing	Signal detection
Abaxis	Plastic disc	Dry reagents on disc	Whole blood	On disc	Centrifugal and capillary	Passive	Passive	Absorbance
Advanced Liquid Logic	Glass, insulated electrodes	On- and off-chip reservoirs	Whole blood	On chip	Electrokinetic (electrowetting)	Electrokinetic	Electrokinetic	Fluorescence, chemiluminescence
Alere (formerly Inverness Medical)	Plastic and elastically deformable materials	On cartridge	Whole blood (capillary)	On cartridge	Mechanical (peristaltic), capillary	Passive	Passive	Fluorescence
Biosite (Alere)	Strip with textured microstructures	On test strip	Whole blood (venous), plasma	Centrifugation	Capillary	Passive	Passive	Fluroescence
Cepheid	Disposable plastic cartridge	On test cartridge	Whole blood, sputum	Sample dilution buffer, shaking	Pneumatic (syringe plunger)	Rotary valves	Passive, manual	Fluorescence (with molecular beacons)
Claros Diagnostics (Opko)	Plastic cassette	On cassette	Whole blood, urine	On cassette	Pneumatic	Passive	Passive	Absorbance
Daktari Diagnostics	Plastic cartridge	On cartridge blister packs	Whole blood	On cartridge	Pneumatic	Passive	Passive	Electrochemical (impedance spectroscopy)
Diagnostics For All	Paper	Dry reagents on paper	Whole blood, urine	On stamp	Capillary	Passive	Passive	Colorimetric
Epocal (Alere)	Film, epoxy laminates	Dry reagents	Whole blood	On chip	Capillary, pneumatic, electrokinetic	Electrokinetic	Electrokinetic	Electrochemical, chemiluminescence
Focus Dx (Quest)	Plastic (polypropylene)	PCR reagent prep needed	Nasal & pharyngeal swabs	Nucleic acid extraction	Centrifugal	Passive	Passive	Fluroescence
HandyLab (BD)	Disposable cartridges	Dry reagents	Vaginal, rectal, nasal swabs	Broth enrichment, cell lysis (MRSA)	Electrokinetic, thermal pneumatics	Valves	Passive	Fluorescence (with molecular beacons)
i-STAT Corp (Abbott)	Plastic cartridge with silicon microchip	On cartridge	Whole blood, urine	On cartridge	Pneumatic (air bladder), capillary	Passive	Passive	Electrochemical (potentiometry, amperometry, conductivity)
Micronics (Sony)	Plastic, laminates, paper	On cartridge	Whole blood, stool	On cartridge	Pneumatic, capillary	Passive, valves	Passive	Absorbance, colorimetry
MBio Diagnostics, Inc.	Plastic, transparent planar components	On chip	Whole blood	On chip	Pneumatic	Passive	Passive	Fluorescence (with planar waveguides)
Philips	Plastic cartridge	Dry reagents on cartridge	Whole blood, saliva	On cartridge	Pneumatic	Passive	Passive	Optical (frustrated total internal reflectance)
TearLab	Polycarbonate	None	Tear	On chip	Capillary	Passive	Passive	Electrochemical
Zyomyx	Glass, plastic	Dry reagents	Whole blood	Bead-conjugated Ab incubation	Gravitational	Passive	Passive	Colorimetric (cell stacking)

Fig. 1 Technology map of design choices made by selected microfluidics companies for a set of common LOC procedures central for building an integrated POC diagnostics device. Adapted from ref. 15 with permission from the Royal Society of Chemistry.

potassium, chloride, pH, pCO_2, and for indirect measurements of blood urea nitrogen, which is hydrolyzed in presence of urease to ammonium ions; amperometry for measuring glucose by detecting hydrogen peroxide formed by reaction with glucose oxidase and of pO_2 by reaction with a thin film oxygen reducing electrode; and conductive measurement of hematocrit.[18] The plastic cartridges also contain pre-loaded calibrant solution, a sample-handling system to avoid need to add measured amount of sample, and conductivity pads that make electrical contact with the analyzer. To actuate fluid movement, a motor in the analyzer depresses an air bladder in the cartridge which forces air to push all fluids along defined fluidic paths within the cartridge.[19,20] The pneumatic actuation consecutively moves the calibration solution, air bubble (positioned by the air chamber in the cartridge), and then blood sample over the sensing electrodes. In 1992, the analyzer was approved and introduced into the U.S., and has since been considered one of the gold standards of POC diagnostics given its long history and commanding share of the market for POC diagnostics. The device continues to be improved, and can conceivably adapt modern LOC components to expand its range of targets and sensitivity.

Epocal (acquired by Alere in 2010) has also developed a portable blood chemistry analyzer which was launched in the U.S. in 2007 (Fig. 1 and 2B). Fluids are actuated and manipulated using a combination of electroosmotic and pneumatic pumps and capillary flow. Self-contained cards (called ''Flexcards'') have fluidic circuits that can be fabricated either in a thin film or thick film, depending on whether high or low degree of multiplexing is desired.[21] Microporous elements are either laminated or deposited on planar substrates with patterned electrodes. To achieve high-scale production at low cost, biosensor arrays are manufactured in 35-mm tape-on-reel format, and membranes or reagents are deposited by in-line micro-dispensing. An interesting feature of Epocal is the ''SmartCard'' technology and wireless communication capability of the reader and its associated mobile computer, which can interface with a laboratory information system *via* standard HL7 interface.

Abaxis has taken a compact disc-based approach to blood-chemistry analysis, using injection-molded plastic discs to test for small-molecule and protein markers for metabolic, lipid, liver, and renal diseases. This system is FDA-approved and CLIA-waived. The analyte panel discs contain an aqueous diluent in the center and dry reagents beads in cuvettes around the disc periphery. Plasma separation, mixing, and volumetric measurements are driven by centrifugal and capillary forces on the disc (Fig. 1 and 2C).[22] The compact analyzer (''Piccolo express'') weighs about 5 kg and is roughly the size of a shoebox.

Table 2 Unofficial list of companies working on microfluidics-based POC test. Technology features, financing, and other information were based primarily on publicly available information

Company[a]	Analytes[b]	Diseases or applications	Highlights of technology suited for point-of-care	Stage of product(s)[c]	Financing sources	Funding prior to approval of 1st product[d]	Year founded
Abaxis	SM, P	Blood chemistries (*e.g.* metabolites, lipid, electrolytes, gases)	Compact analyzer (Piccolo® xpress), injection-molded plastic discs, no sample pre-processing	FDA approved (CRP, metabolic, lipid, liver, renal panels)	Private	$40 4M	1989
[f]Advanced Liquid Logic	SM, P, NA	HIV/AIDS, Iysosomal storage disease, MRSA	Compact, benchtop analyzer, manipulation of nano- and micro-droplets (digital microfluidics)	In development	Public (NIAID, NIJ)	$6.2M	2004
Alere (formerly Inverness)	P, C	HIV/AIDS, clotting time	Disposable cartridge, portable analyzer, automated image-based immune hematology test	CE marked (CD4 counts), FDA approved (prothrombin)	Private	N/A	2001
Atonomics	P, NA	Cardiovascular disease, maternal health, prostate cancer	Porable analyzer v/ith disposable card; integration of optics, microcapillaries, target amplification	In development	Private	€9.0M	2001
Biosite (Alere)	SM, P	Cardiovascular disease, drugs of abuse, waterborne parasites	Porable reader (Triage® meter); disposable, capillary-driven microfluidic test strips	FDA approved (BNP, cardiac tests, intestinal parasites)	Private	$34.1M	1988
Biosurfit	P, NA	Viral infections cardiac markers and blood chemistries	Disposable plastic discs (spinit®); label-free, portable surface plasmon resonance analyzer	In development	Private	€1 6M	2006
Cepheid	NA	Respiratory infections (bacterial and viral), cancer	Disposable cards with benchtop analyzer (GeneXpert®), on-card sample processing (sputum)	FDA approved (MRSA, CDF, flu), WHO approved (MDR-TB)	Private, public (US Army, DARPA, USPS, NIAID, CDC)	$84.3M	1996
[f]Claros Diagnostics (Opko)	P	Urological maladies, infectious diseases	Portable analyzer, injection-molded plastic cassette, low-cost optical detection of silver films	CE marked (PSA)	Private	$11.8M	2005
Daktari Diagnostics	C	HIV/AIDS	Handheld instrument, label-free electrochemical sensing of captured cell lysate	In development	Private, public (Gates Foundation, PATH)	$9.1M	2008
[f]Diagnostics For All	SM, P	Liver damage from HIV/AIDS medication	Instrument-free tests based on paper, capillary-drvien microfluidics, colorimetric readout	In development	Public (Gates, DFID/UK gov., USAID)	$3.0M+	2007
DNA Electronics	NA	Single nucleotide polymorphisms	Disposable cartridges, low-cost silicon biosensors for label-free pH-mediated detection	In development	Public (UK gov.)	£1.2M	2003
Epocal (Alere)	SM	Blood chemistries	Self-contained cards (Flexcards™), patterned electrodes for sensing, wireless data transmission	FDA approved	Private, public (Canada gov.)	$42.7 M	2001
FluimediX	NA	Warfarin metabolism	Fully automated tabletop system (NanoCycler™), disposable cartridges for handling saliva	In development	Private	$1.1M	2003
Focus Dx (Quest)	NA	Flu, intestinal pathogens	Portable detector (3M™ Integrated Cycler), discs with on-board extraction	FDA approved (flu, RSV), CE marked (EBV, BK virus, CDF)	Private	N/A	2008

Table 2 (*Continued*)

[f]Genefluidics	P, NA	Pathogen identification, antimicrobial susceptibility, cancer	Benchtop and portable systems for multiplexed electrochemical quantification of biomarkers	In development (clinical), approved (open lab system)	Private, Public (NIBIB)	$12.0M	2000
HandyLab (BD)	NA	Bacterial infections and drug susceptibility testing	Disposable cards with integrated heating, detection, sample processing in a portable instrument	FDA approved, CE marked (GBS, MRSA, CDF)	Private	$46.0M	2000
i-STAT Corp (Abbott)	SM	Blood chemistries, coagulation, cardiac markers	Porable analyzer (i-STAT®), capillary-driven microfluidics, thin-film electrodes for detection	FDA approved	Private	$51.6M	1983
Idaho Technologies	NA	Upper respiratory tract infections (viral), bioterrorism agents	Automated analysis on FilmArray™ instrument, disposable pouch with freeze-dried reagents	FDA approval (Q fever, influenza A/B, and respiratory panel)	Private, public (DHHS, DOD)	N/A	1990
[f]IQuum	NA	Infectious diseases (respiratory pathogens, HIV/AIDS), genotyping	Lab-in-a-tube platform for automated analysis in compact benctop instrument, results < 30 min	FDA approved, CE marked (flu)	Private, public (NIH, DOD CDC, Homeland Security)	N/A	1998
LabNow	NA, C	infectious diseases, cancer, cardiovascular disease	Compact tabletop analyzer utilizing membrane-based nano-bic-chip and quantum dot detection	In development	Private, public (NIH)	$40.0M	2005
[f]LeukoDx	P, C	Sepsis, urinary tract infections, HIV/AIDS	Compact tabletop fluoro cytometry instruments with disposable, single-use cards; handheld in development	In development	Private, public (NASA, Israeli gov.)	$3M	2009
[f]MBio Diagnostics, Inc.	P, NA, C	HIV/AIDS, hepatitis, flu	Integrated fluidic cartridge and low-cost, low-power fluorescence imaging using planar waveguides	In development	Public (NIAID, NIST, PATH)	$7.2M	2009
[f]Medimate	SM	Bipolar disorder (lithium ions), chronic kidney disease, heart failure	Portable reader (Medimate Multireader®) measuring fluid conductivity in disposable card	In development	Private, public (Dutch gov., European Union)	€4.5M	2006
[f]Micronics (Sony)	P, NA	Malaria, shiga toxin-producing E. coli, ABO blood typing	Disposable cartridges composed of thin-film laminates and injection-molding	FDA approved (ABO blood typing)	Private, public (Gates, NIAID, NIJ, DOD. US Army)	$28.8 M	1996
[f]Molecular Vision	SM, P	Cardiovascular and kidney disease	Low-cost, instrument-free, disposable cards using light emitting and detecting polymers	In development	Private, public (UK gov., European Union)	£5.2M	2001
Nanomix	SM, P	Cardiac damage (troponin I) and asthma (nitrous oxide)	Handheld reader with disposable cards, electrochemical detection using carbon nanotubes	In development	Private, public (NSF, Homeland Security)	$27.0M	2000
Philips	SM, P, NA	Cardiac damage, drugs of abuse, hormones	Handheld reader with self-contained cards, concentration of magnetic nanoparticles for rapid analysis	In development	Private	N/A	1891
Rheonix	NA	HPV detection, warfarin dosing, sepsis, waterborne pathogens	Disposable card (Rheonix CARD®), on board reagents, workstation for electrochemical detection	In development	Private, public (NIAID, NSF, EPA)	$14.7M	2008
SlipChip LLC	NA	Rare cells, mutations	Digital PCR without mechanical valves or pumps	In development	Private, public (NIH)	N/A	2011
[f]Sphere Medical	SM	Blood chemistry	Microanalyser with silicon chips and functionalized electrodes for rapid electrochemical detection	FDA approved	Private	$30.0M[e]	2002

Table 2 (*Continued*)

[f]TearLab	SM	Dry eye disease (tear osmolarity), ocular allergy (IgE antibodies)	Porable osmolarity reader with disposable cards; capillary-driven flow, gold electrodes for detection, results in 5 s	FDA approved, CE marked (dry eye disease)	Private	$7.0M	2002
Vivacta	SM, P, NA	Endocrine imbalances, infections, sepsis, stroke	Self-contained care with dry reagents and piezofilm sensors, capillary-driven flow, portable reader	In development	Private	$27.9 M	2005

[a] Acquisitions of POC microfluidics companies by other companies are noted in parentheses. [b] SM = small molecules, P = protiens, NA = nucleic acids, C = cells. [c] BNP, B-type Natriuretic Peptide; CRP, C-Reactive Protein; MRSA, Methicillin-Resistant S. aureus; CDF, C. difficile; MDR-TB, Multi-Drug Resistant Tuberculosis; GBS, Group B Streptococcus; RSV, Respiratory Syncytial Virus; EBV, Epstein-Barr Virus. [d] Funding raised and received prior to launch of first test (if approved), or raised to date (if test not yet approved); N/A, not available. [e] Does not include funding from initial public offering. [f] received feedback from company to clarify aspects of the information.

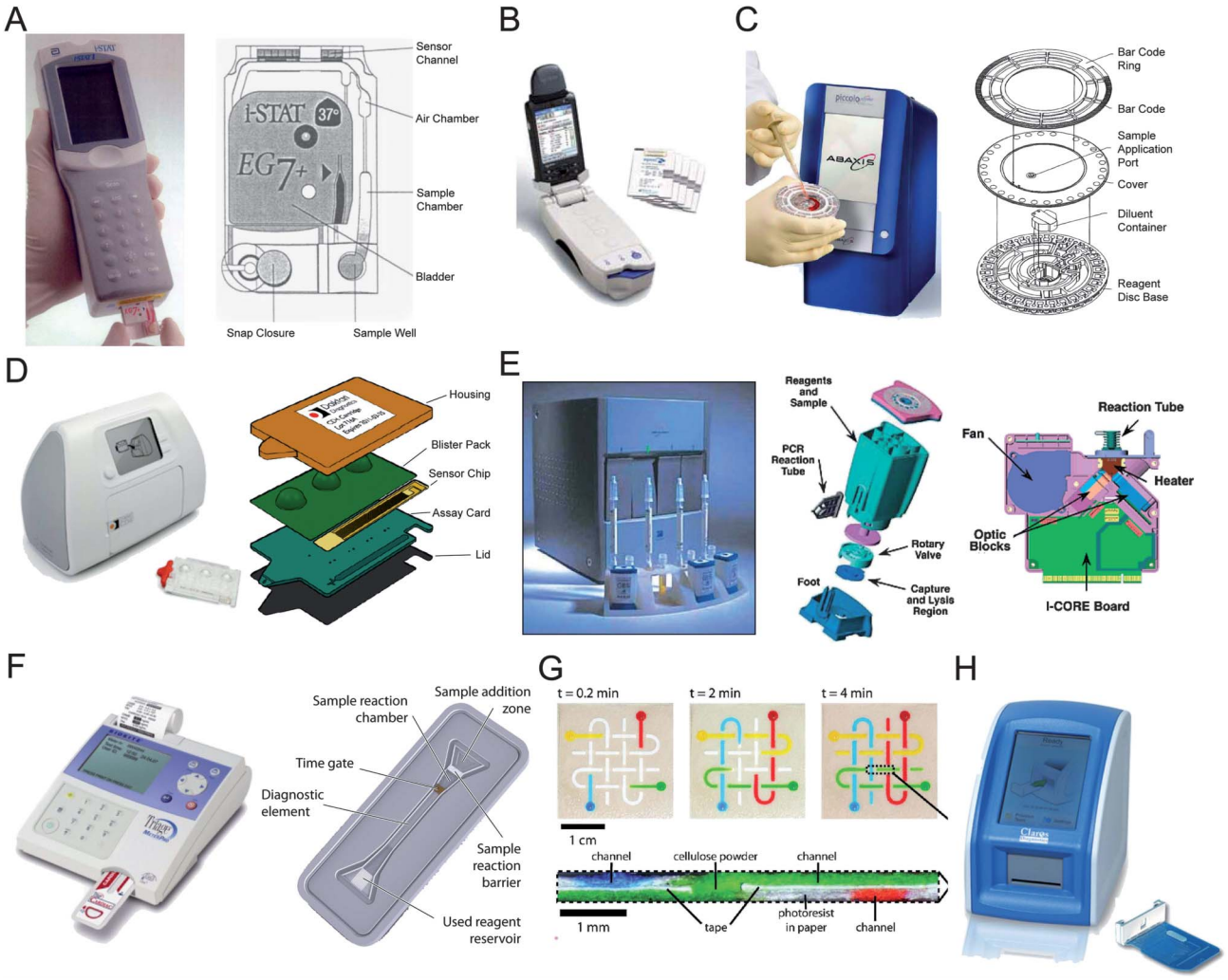


Fig. 2 Images and schematics of selected microfluidics-based POC tests: (A) i-STAT (Abbott). Adapted from ref. 18 with permission from Pure and Applied Chemistry. (B) Epocal. Taken from www.epocal.com with permission from Epocal. (C) Abaxis. Taken from www.abaxis.com and adapted from ref. 84 with permission from Abaxis. (D) Dakari Diagnostics. Adapted from www.daktaridx.com and ref. 85 with permission from Daktari. (E) Cepheid. Taken from ref. 86 with permission from Sensors Magazine. (F) Biosite. Taken from www.alere.com with permission from Alere and adapted with permission from ref. 87. Copyright (2011) Annual Reviews. (G) Diagnostics for All. Adapted with permission from ref. 88. Copyright (2008) National Academy of Science, U. S. A. (H) Claros Diagnostics. Taken from www.clarosdx.com with permission from Claros.

Other companies have also developed centrifugal-based platforms for POC diagnostics, including Focus Diagnostics (acquired by Quest Diagnostics in 2006) (Table 2 and Fig. 1), which has developed several FDA-approved and CE-marked tests for detection of nucleic acid signatures for respiratory pathogens.

Cells. Several companies are developing novel microfluidic CD4 + T-cell counters for monitoring HIV/AIDS, a disease with particularly high prevalence in developing countries. Alere (formerly Inverness Medical), through its subsidiary ClonDiag, is developing the Pima CD4 counter, which employs the same static image analysis and counting principles as flow cytometry, but in a compact, portable and robust package, which does not require extensive laboratory training or equipment, requires only 25 μL of capillary blood, and provides absolute CD4 T-cell counts within 20 min (Table 2).[23–25] Alere uses a disposable cartridge with sealed dry reagents to avoid need for refrigeration and no manual sample processing is required (Fig. 1). Peristaltic movement transports the sample first to an incubation compartment with fluorescent monoclonal anti-CD3 and anti-CD4 antibodies, and then transfers the stained sample into a detection microchannel where the analyzer detects signals with miniaturized multi-colour fluorescent imaging optics and camera. The computer analyzes fluorescent signals with proprietary software algorithms. The Pima CD4 system received CE mark in 2009.

An alternative approach to image-based CD4 cytometry is being pursued by Daktari Diagnostics. Daktari uses affinity chromatography to selectively capture CD4 + T-cells in a controlled shear stress microfluidics design.[26,27] (This technical concept is also being developed by the company On-Q-Ity in detection of circulating tumor cells). Unlike traditional flow cytometry, which relies on optical detection of fluorescently labeled cells, Daktari detects lysate of captured T-cells by impedance spectroscopy, thereby avoiding need for expensive and bulky optics, filters and lenses (Fig. 1 and 2D).[28,29] Daktari has also explored multi-chamber devices combined with high viscosity rinsing solution to deplete monocytes from whole blood prior to CD4 counts.[30] Dakari is currently collaborating with thinsXXS Microtechnology, a company specializing in developing and producing microfluidic devices from plastics, to develop the Daktari CD4 system.[31] A clever solution to storing liquid reagents is their development of coated aluminum blister packs or pouches on-cartridge, which can deliver controlled microfluidic volumes with very low dead volume.[32]

Another interesting alternative to cell detection by traditional flow cytometry principles is in development of improved waveguide sensors, a strategy pursued by MBio Diagnostics, Inc. for point-of-care CD4 counts as funded by NIAID.[33] Their technology uses low-power diode lasers for fluorescence evanescent illumination at assay surface, and incorporates integrated lens as part of the planar waveguide component to improve light coupling reproducibility (Fig. 2).[34–36] Evanescent illumination by the planar waveguide also avoids the need for wash steps and line-of-sight optical requirements, thereby reducing complexity of both assay and microfluidic design of their injection-molded cartridges. MBio is seeking to develop their platform for other applications such as multiplexed detection of protein and nucleic acid signatures for HIV, hepatitis, syphilis, and influenza (Table 2).

An instrument-free microfluidics approach to CD4 counting is being developed by Zyomyx with support from the Gates-funded Imperial CD4 initiative (Fig. 1).[24] The test relies on separation by sedimentation and visual interpretation of bead-bound cell stacks, thereby avoiding reporter reagents and signal intensity calibration.[37,38] In a first-generation device, whole blood collected by fingerprick is labeled off-device with antibodies conjugated with large, dense particles (1 μm mean diameter, density > 10 g mL^{-1}), and then injected in a closed-end high precision glass capillary (80 μm inner diameter) filled with a high density medium. Time to result is expected within 30 min.

Nucleic acids. There is increasing clinical demand for detection of DNA and RNA signatures for diagnosis and monitoring of patients at the point of care. The landscape of microfluidic POC diagnostics companies reflects this trend, with over half of the companies in our expanded list developing nucleic acid-based assays (Table 2). Nucleic acid tests are arguably some of the most challenging assays to develop due to additional steps required for sample pre-treatment (*e.g.* cell sorting, isolation, and lysis, as well as nucleic acid extraction), signal amplification (due to low physiological concentrations) (Table 1), and target contamination and instability.[15]

One company which pioneered the integration of multiple microfluidics procedures for nucleic acid targets was Handylab (founded in 2000 and acquired by BD in 2009).[39] Handylab developed disposable cartridges with on-board dry reagents, and a benchtop instrument with integrated heating, mechanical valves for fluid control, and fluorescence detection using molecular beacons (Fig. 1). The device can be potentially used for near-patient diagnosis. Handylab has released multiple tests for common hospital-acquired infections, with FDA approvals of their *S. aureus* (and methicillin-resistant *S. aureus*, MRSA), *C. difficile*, and group B streptococcus tests in 2008, 2009, and 2010, respectively (although the tests are not CLIA-waived)) (Table 2). All three tests, are also approved for use in Europe, along with a test for vancomycin-resistant enterococci. Noteworthy is the ability of the device to handle complex specimen matrices such as nasal swabs. Some procedures, however, are done off-chip, such as broth enrichment and cell lysis for MRSA testing.

Another early pioneer of integrated molecular diagnostics is Cepheid (founded in 1996), which has developed an integrated benchtop analyzer ("GeneXpert") for detection of MRSA, *C. difficile*, and influenza (all FDA-approved), and tuberculosis (WHO-qualified). The analyzer performs ultrasonic lysis of filter-captured organisms in disposable plastic cartridges and then mixes DNA molecules with on-board PCR reagents, performs seminested real-time amplification, and detects amplicons using molecular beacons (Fig. 1 and 2E).[40] A reagent is used to liquefy and inactivate sputum samples (for tuberculosis). Incubation and mixing of the solution mixture is manually performed by the user prior to transfer onto the test cartridge. Once sample mixture is loaded, however, fluids are automatically driven by pneumatic actuation *via* a syringe plunger, and a

rotary valve controls fluid movement among multiple reagent chambers, nucleic-acid purification valve assembly body, and PCR tube.[41] The analyzer performs ultrasonic lysis of filter-captured organisms in disposable plastic cartridges, mixes DNA molecules with on-board PCR reagents, performs semi-nested real-time amplification, and detects amplicons using molecular beacons.[40] Considering the cost and bulk of the analyzer and cartridges, the system in its current version may be best suited for moderate-infrastructure settings.

Micronics (founded in 1996 and acquired by Sony in 2011) is developing a battery-powered instrument for multiplexed detection of malaria and shiga toxin-producing *E. coli* (Table 2). Micronics was one of the first companies to recognize the importance of plastics in developing point-of-care diagnostics, and has developed strategies for fabricating thin-film laminate-based and injection-molded plastic devices.[42,43] Besides nucleic-acid tests, Micronics has also developed an FDA-approved blood typing test (ABO Card) that can be used in transfusion centers. This test relies on agglutination or visible interpretation of colored lines, but can be performed without the aid of an instrument; pneumatic actuation of fluids is achieved by applying a finger on the surface of the device (Fig. 1).

An alternative to continuous-flow microfluidics, which constitutes the majority of current microfluidics systems, is digital microfluidics, which involves the manipulation of discrete droplets. Control of the droplets is typically attained through electrowetting, dielectrophoresis or immiscible-fluid flows. One advantage of digital microfluidics is its potential for flexible and scaleable fluidic configurations.[44–46] The company Advanced Liquid Logic is developing a digital microfluidics platform for detection of nucleic acids, proteins and small molecules (Table 2 and Fig. 1). In one demonstration of the platform, a real-time PCR test was performed in a closed-loop flow-through format through transport between different temperature zones within an oil-filled cartridge.[47] The platform was capable of performing multiplexed detection of MRSA, *Mycoplasma pneumoniae*, and *Candida albicans*.

Immunoassays. Despite the huge investment in lateral flow devices, they are still limited in several important aspects: low sensitivity, inability to quantify the target (relying instead on user interpretation of a yes/no signal), and inability to detect multiple targets at once.

Several companies are leveraging capillary forces for fluid actuation, one of the most attractive features of the lateral flow test, in their microfluidics systems. One notable company is Biosite (founded in 1998, acquired by Alere in 2007), which uses test strips with textured microstructures to test for a range of protein and small-molecule markers for cardiovascular disease, drugs of abuse, and waterborne parasites (Table 2). A portable reader (“Triage” meter) measures fluorescence signals from the disposable test strips (Fig. 1 and 2F). The device does not yet work with finger-pricked whole blood, and for most of its applications, still requires centrifugation of blood sample. Biosite has several FDA-approved tests, some of which are CLIA-waived. A more recent company is Diagnostics for All, which uses paper and patterned hydrophobic regions to direct movement of microfluidic volumes driven by capillary forces (Fig. 1 and 2G). Their tests are designed to be instrument-free, with initial prototypes designed to assess liver damage from HIV medication (*e.g.* albumin, transaminases, and lactate dehydrogenase). A unique aspect of Diagnostics for All is their non-profit business model for targeting diseases of greatest concern in the developing world.

Philips is developing a handheld Magnotech™ system with self-contained, disposable cartridges for rapid detection (within 1 to 5 min) of protein analytes at low concentrations (pM or below) (Fig. 1). The technology is based on the manipulation of functionalized superparamagnetic nanoparticles which capture and detect protein analytes in the sample fluid, such as blood or saliva.[48,49] The nanoparticles are dried and pre-loaded in the cartridge. To achieve fast binding kinetics, an electromagnet situated underneath the disposable cartridge concentrates the superparamagnetic nanoparticles on a sensor surface which is also functionalized with capture molecules. A second magnetic field is used to pull away unbound nanoparticles. Optical detection is based on frustrated total internal reflection. Proof-of-concept assays have included sandwich assays for intraoperative parathyroid hormone (used in predicting success of minimally invasive parathyroidectomies)[50] and cardiac troponin I,[51] as well as inhibition assays for drugs of abuse such as morphine.[48] Philips is currently collaborating with bioMérieux for expected commercial launch of tests using Magnotech platform in 2013.[52]

Claros Diagnostics (founded in 2004, received venture-capital funding in 2007, and bought by Opko Health in 2011) focuses on multiplex and quantitative immunoassays for use in remote locations (Fig. 1 and 2H). From the beginning, the technology was designed to be used in developing countries;[53] later, the technology was also leveraged to address medical needs in western countries. The Claros test uses a silver enhancement chemistry to develop an amplified signal which can be measured by an absorbance reader to determine optical density. Multiple reagents can be stored in the assay cartridge and delivered in a controlled manner to run multi-step protocols.[54] For example, in an ELISA test, the multiple incubation and washing steps over the immunocapture surface that are important in achieving the precision, sensitivity and specificity required for clinical applications can be replicated inside a Claros cartridge.[53] The plastic Claros cartridges are made in high volumes using injection-molding. At the end, an ELISA-quality result can be generated 10 min after a finger prick. A device for prostate cancer testing obtained CE Mark in 2010,[55] and is on track for FDA approval with CLIA waiver for the U.S. market. In parallel, a minimalist version of the device has been field-tested in sub-Saharan Africa on over 400 patient samples for diagnosis of HIV and sexually transmitted diseases.[53]

2.3 Summary

For the companies listed above, the technology design choices for overcoming the critical LOC challenges are summarized in Fig. 1. The diversity of design choices reflects the diversity of targets as well as end-use settings. The term “POC” encompasses many possible end-use settings outside of a centralized testing facility, including regional health clinics, physicians’ offices, emergency settings, at-home use, or mobile use in resource-limited settings.

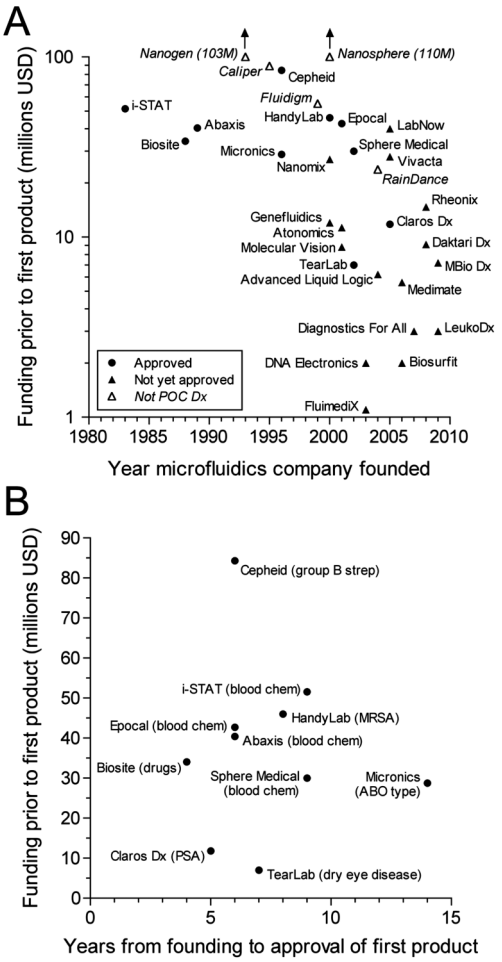


Fig. 3 Unofficial chart showing funding and development time of microfluidics companies prior to approval or launch of first product. (A) Funding (millions USD) prior to regulatory approval of first microfluidics-based product *versus* year of company founding. The symbols group the companies by stage of development of the technology: filled circles indicate companies which have at least one microfluidics-based product approved by regulatory agency (FDA, European Commission); filled triangles indicate companies whose first microfluidics-based product is not yet approved; open triangles indicate companies which have developed microfluidics-based products but are not point-of-care diagnostic tests. Since all funding data were taken and compiled from public sources, funding estimates are likely to be conservative. Whenever possible, we used numbers verified by company officials. The following 10 year average exchange rates were used to approximate funding posted in non-US currency: €0.80 = $1.00; £0.59 = $1.00. (B) Funding (millions USD) prior to regulatory approval of first microfluidics-based product *versus* time to development. We acknowledge Bill Rodriguez (Daktari Diagnostics) for first producing a different version of the figure.

In Table 2, we have provided more information about these and other companies (31 in total) in the microfluidics POC diagnostics space: the information includes the classes of analyte targets, initial diseases or applications, characteristics of company's technology which make its diagnostic test particularly well-suited for use at POC, development stage of diagnostic test with regard to its regulatory approval or market availability, sources of financing and estimated funding raised prior to launch of first test, and the founding year of the company. We compiled this information from public sources. We have tried to verify the information with the companies themselves, and have noted in the table the 12 cases where the company provided additional clarifications to us. We recognize this list is not comprehensive, but we hope it would serve as a useful source of information for the community.

In Fig. 3, we present the level of financing (prior to approval or launch of first product), the year of founding, and time to reach regulatory approval of first product (when applicable) in chart forms, for selected companies over the past decade and half (Fig. 3). We emphasize that the financing amounts reported are those prior to the approval or launch of the first product; some companies have raised additional financing (not reported here) after approval or launch of the first product. For comparison, we also plot data for selected microfluidics companies that are not in the POC diagnostics space. The funding amounts are estimates only: most likely, they are conservative as we only used information available from public sources. In addition, when appropriate, we estimated currency conversion rates for financing from non-U.S. sources using ten-year averages. Nevertheless, one notes that companies founded and operating prior to year 2000 were able to secure larger investments (prior to approval of first product) than companies operating more recently (Fig. 3A; note the *y*-axis is in log scale). (This trend still holds even after taking into account the longer time-scale available for the older companies to raise money.) In addition, the amount of money raised by most POC microfluidics diagnostics companies has been lower than for some microfluidics companies in non-POC diagnostics applications (Fig. 3A). With one exception, most companies have raised between 10 to 50 million U.S. dollars by the time their first POC diagnostics product has been approved (Fig. 3B).

3. Challenges and lessons learned

3.1 Technology: dividing does not conquer

Many academic groups, along with a number of startup companies, have developed methods for fluid delivery and control, signal detection, and microfabrication that have potentially transformative capabilities. However, successful operation of technically complex assays necessitates the ability to perform all the assay procedures *altogether* and *at once*, not separately, in a seamless and automated fashion. For the purposes of meeting milestones and conducting short-term research projects, it may be convenient to treat integration of components as an after-thought. However, such an approach has not led to successful development of real-world products in the past.

What does "integration" mean? The integrated POC product must link a patient to a result, encompassing sample collection, sample pre-treatment (if applicable), analyte-specific reaction, signal production, signal detection and reporting of result. Different components must be designed such that each component is compatible with all the others. This mutual compatibility can prove to be extremely challenging as more components are being integrated, because the technical requirements can vary greatly for each component or assay step. Consider a hypothetical device which integrates ten different

components, for which there are five design decisions to be made for each component. In this scenario, the vast majority of the roughly one million combinations of individual components are mutually incompatible; only a very small number of combinations is successful.

We will give two examples from the design of the Claros device. First, we observed that if the plastic microfluidic cartridges were to be produced by injection molding, *all* fluidic features should be moldable, and there should be a single thermoplastic material that shares the chemical, mechanical and optical requirements for all the assay steps. Such decisions require clarity on the design inputs across diverse disciplines, from injection molding to microfluidic design to protein chemistry to optical measurement. Second, we adapted the use of silver enhancement from immunohistology to produce an amplified optical density signal.[56] This choice of signal amplification greatly reduced the cost and complexity of the optical detection hardware. The low cost enabled us to use multiple optical detectors for a multiplexed test, rather than a mechanical system for moving the cartridge relative to a single detector; the absence of a mechanical system, in turn, reduces the power consumption and increases the robustness of the device. The low power consumption and compact optical components allowed us to develop a handheld version of the detector (whereas traditional LOC technologies rely on microscopes or complex optics to read the signal[57]). The recording of the kinetics of optical density development also reduced the need for extensive external calibration, since the Beer–Lambert law ($absorbance = -\log(I/I_0)$) can be applied to every detection zone by comparing the intensity of light reaching the detector before (I_0) with the intensity after (I) development of the signal, regardless of day-to-day fluctuations in the performances of the optical components. This ''self-calibration'' is attractive for attaining CLIA-waiver during FDA regulatory approval. In this case, the very initial choices of amplification chemistry, hardware instrumentation, and analytical chemistry as it relates to regulatory approval requirements could not be uncoupled from one another. In a scenario where the vision for the final device is ill-defined at the initial stages of design, the integrated device may never be achievable, no matter how attractive the individual components may be on their own.

3.2 Fundraising from investors: understanding the ''market''

As a healthcare technology, POC diagnostics requires significant capital for development. Hence, fundraising is at the top of a researcher's challenge to bringing a promising technology to the market. We founded Claros Diagnostics in mid-2004, and obtained a first round of venture-capital investment of $7.8 million at the beginning of 2007. During this fundraising period, out of the many questions from investors, perhaps the single most discussed one was in the choice of initial market (we considered over 80 different possible protein markers, many of which remain attractive). This analysis was time-consuming because the value propositions of a POC device differed depending on the application: POC diagnosis of cardiac markers during the ''golden hour'' of a heart-attack patient can result in early life-saving treatment; for pregnancy tests, consumers value the convenience. A POC test needs to offer concrete value over centralized-lab testing, such as improved quality of care, improved convenience, or cost reduction. The centralized-lab test also sets the baseline analytical performance, as well as the initial cost structure of the test (as reflected by re-imbursement rates). The benefits of the POC test need to outweigh its drawbacks, such as: the need to change how a clinical practice is performed; the need for in-house sample collection and test operation, including personnel to run the test (especially for non CLIA-waived tests); and compliance with local regulations.

The other important factor is market size. Tests that are already performed in high quantities by centralized laboratories are attractive. However, markets with high growth rates can justify disproportionally large investments. For example, POC testing constitutes 31% of the diagnostics market (18% glucose testing, 11% professional POC products, and 2% over-the-counter), with 9% annual growth projected between 2007 and 2012. This growth is driven by the shift of testing of infectious diseases and cardiac markers from the centralized lab to POC (by comparison, the glucose segment is projected to grow by 1% annually during the same period). Another example is the nucleic-testing segment, which represents only 8% of the current diagnostics market but is growing 7% annually.[58] Development of a new diagnostics market can also be attractive if the product can establish a dominant position in the market, but will possibly require lengthy clinical validation studies, a complex regulatory pathway, and slow clinical adoption.

During fundraising, the ''market'' that needs to be understood is not just the clinical market, but also the market of investor sentiment. Although investor interest in specific sectors tends to wax and wane over time, the current climate exhibits a bias against healthcare investments. For example, within the last year, the FDA has been perceived by some in the medical device industry as ''adverserial'', with long delays, inconsistency, and unpredictability.[59] While we or others in the industry may or may not agree with this viewpoint, this sentiment continues to constrain interest in investors at healthcare opportunities, right from the seed stage.[60] We also found very early on that ''microfluidics'' on its own is not necessarily exciting in the investment community, due to a perception that past investments into POC microfluidics companies have not yielded significant returns. By contrast, our analysis of past microfluidics companies suggests that most grant funding for POC has been (and continues to be) focused on innovation, and that most investment in microfluidics companies has not been focused on delivering a POC product as much as demonstrating a platform product with many possible markets. In addition, investment from institutional investors may not always be the most attractive financing mechanism: whereas the garnering of large grants in an academic environment fuels laboratory research, in a startup company, raising large investment capital could dilute founders' shares and control of the company.

3.3 Business strategy: focus or perish

Government grants that are directed towards translational research (such as SBIR's and Department of Defense funding) are an alternative and potentially attractive source of financing,

especially if the goals of the funds are aligned with the company's mission. However, because it is unlikely that a single grant would be sufficient to bring the product to market, the disparate demands of different funding opportunities, as well as additional grant reporting requirements, are often misaligned with a focused product development effort. (In fact, few SBIR-funded companies have successfully brought products to market.)

Anticipating that the arguably single greatest roadblock for previous POC companies was a lack of focus, we set a clear goal to complete the development of our first product through regulatory clearance. Reflecting the sentiment of some investors at the time, a potential investor rejected our investment in 2005 with a written comment, "I think Claros is grossly underestimating the time and cost of developing a commercial product", and cited previous efforts of over $100 million without success. Although the Claros product is not yet on the market, its first product obtained CE marking in 3.5 years after financing with less than $12 million in investment. We found that along the course of product development, there existed many attractive opportunities could add value to the company, but more importantly, would have also diluted our limited resources: 1) *Performing proof-of-principle detection for different markers* came from numerous sources, including potential industry collaborations, grant funding opportunities, and inquiries from investors and acquisition targets. It is tempting to work towards disparate applications in order to demonstrate the full range of a powerful platform technology. However, we also judged that such efforts would significantly distract the development team, which would slow down development of the first product, which would result in missed milestones and in turn pressure the company to pursue even less attractive short-term funding opportunities, leading to an unrecoverable vicious cycle. We firmly believed that the way to show the technology can be used for many products is to start with one successful product: it is relatively easy to implement a second assay *after* completion of the platform validation. 2) *Technical publications* could be highly beneficial for the research community. However, we believed the primary goal of a startup company with limited resources should be to build a product rather than engaging in academic research. In addition, while the overcoming of technical challenges required a number of microfluidics innovations, the emphasis in academic journals on novel microfluidic concepts may also discourage the pursuit of such publications. The publication of clinical and performance data, however, could be an important milestone. 3) *Licensing of original intellectual property* from universities is a key first milestone for a startup company, but we learned to be patient and persistent with the negotiations if we were to focus on securing terms that will ensure success of the company until the first product and beyond.

The turn of the millennium was marked by the internet bubble, but this first decade has produced profitable "Web 2.0" companies that learned valuable lessons from the pioneers. In the microfluidics space, we similarly believe that if the product development is properly focused, one need not be unreasonably cynical by projecting long development cycles for diagnostic devices. By nature, their development takes place under bounded terms and occurs much more quickly and with less capital investment than development of drugs or vaccines. This supposition is supported by analysis of the capital requirements and development times of POC diagnostic devices that have successfully garnered regulatory approval or been introduced into the market (Fig. 3).

3.4 Manufacturing: paper or plastic, and the quantum leap to high-volume production

Up until recently, the majority of manufacturing methods published in the LOC community have been micromachining on glass or silicon, and soft lithography on PDMS. However, for POC applications, there is increasing recognition that other mass-production methods would be needed.[61,62] Currently, two leading materials for achieving high-volume, low-cost production are plastic (which can be produced by laminate, embossing, or injection molding) and paper or membranes. Both approaches represent a significant leap in the way microfluidics is done, either by greatly simplifying traditional high-performing microfluidics, or by increasing the performance of an inherently simple method (Fig. 4).

There is increasing recognition that lowering the "cost" of the material is a significant consideration. However, until the product is actually on the market, "cost" estimates are likely to be inaccurate. Examining each class of material:

• **Plastic.** There are many ways to microfabricate plastic-based materials, such as laminate, embossing, or injection molding. While each method has a different cost structure, we will discuss injection molding, which can be used on plastic such as cyclic olefin copolymer or polystyrene, and is the method used for producing the Claros cartridges. A breakdown of the cost of goods for the disposable Claros cartridge is shown in Fig. 5.[63] The production of the microfluidic components (which includes the cost of the plastic material, quality control, labor, and facilities) accounts for only 14% of the total cost (Fig. 5A).

• **Paper and membrane.** Nitrocellulose or cellulose are often used in lateral flow tests and more recent microfluidic paper-based tests. Like plastic, the paper or membrane substrate is very low-cost. The cost of manufacturing will include removing static from the substrate, physical processing to control its geometry, shape, or size, micropatterning of the paper to control hydrophilic and hydrophobic areas, and/or aligning and stacking multiple substrates to form three-dimensional networks.

For both molded plastic or paper strips, *ancillary materials* and *processing steps* are needed to produce a functional POC system. These steps could rival or exceed the cost of producing the microfabricated cassettes themselves:

• **Plastic.** For the Claros cartridge, the cost of goods for ancillary materials and processing steps is also shown in Fig. 5A. These ancillary materials include reagents, a cover (applied onto the molded grooves to form channels), and an absorbing pad. A small sample collection device that forms a fluidic connection with the cartridge is also included to meter the sample, ensuring precise quantitative measurements. By avoiding the need for any type of microscopic alignment

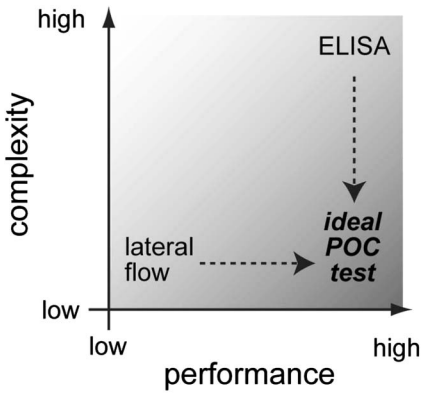


Fig. 4 Diagram of two conceptual approaches to achieving simple-to-use and high-performing POC diagnostics devices.

throughout assembly, the labor costs for assembling a microfluidic plastic cartridge is kept to only about 8% of the overall costs in our current design.

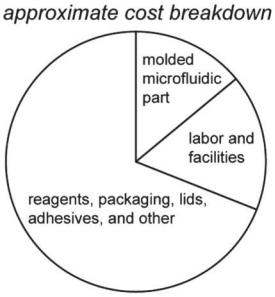
• **Paper and membrane.** Taking a product in final form as example, a typical lateral flow test kit is shown in Fig. 5B. Although we do not have a similar breakdown for cost-of-goods of future paper-based tests, future products will likely require processing of the substrate with biological and chemical reagents using deposition techniques. Other possible processing steps include application of absorbing pad to prevent fluids from escaping or blood filtration membranes, assembly of paper or membrane materials using either finely deposited glue or lamination, and packaging inside a plastic housing. Additional materials or processing steps (such as sample volume metering) may be needed to produce tests that can detect multiple diseases, be quantitative or exhibit higher sensitivity. In our plastic cartridge, ancillary costs and processing steps (rather than the cost of substrate material itself) dominate the final cost of goods; such costs will likely also have to be considered carefully in determining the final cost of goods for future paper- based products. Finally, as we have accounted for the plastic cartridge, labor and depreciation considerations will also apply to the cost of paper-based tests.

At the other end of the cost-consideration spectrum is what final price would be acceptable to the market. Although many conjectures could be made, the best available benchmark may be the HIV lateral flow test which has already been widely accepted in the developing world. The market prices for the most widely purchased HIV lateral flow tests in the developing world show that the market will pay for tests up to several dollars per test (Fig. 5B). The HIV test has some unique qualities: it is possibly the simplest type of lateral flow test to manufacture as it detects a marker at high concentrations with yes/no accuracy, it benefits extremely well from scale as tens of millions are purchased per year (the prices in Fig. 5B are bulk prices), and it is priced extremely low compared to tests in the U.S. and Europe markets (such as the pregnancy test). FDA-approved rapid HIV antibody screening tests are priced at $8–26 per device,[64] and average reimbursement for a CLIA-waived rapid HIV-1 antibody test (Current Procedural Terminology [CPT] code 86701QW) is $12 and for a CLIA-waived rapid HIV-1/2 antibody test (CPT 86703QW) is $19.[65] For future POC tests that can provide additional clinical value, or for tests that do not satisfy the unique conditions of the HIV lateral flow test, there are not yet solid data on whether more or less than several dollars a test will be an acceptable price to the market.

No matter which manufacturing approach, we found it is crucial to implement the final mass-manufacturing technique, and not a simplified manufacturing procedure, as early as possible in the product development process. At Claros Diagnostics, we built a manufacturing facility and obtained ISO 13485 certification in 2.5 years after financing. In our device, artifacts arose from the physical-chemical properties of PDMS and the surface finishes of machined plastic; optimization of our method based on such materials would have been a wasted effort upon the moment of switching to injection-molded thermoplastics. From the other direction, we identified features early on that were specific to injection molding, as well as specific

A

Claros plastic microfluidics cassette: approximate cost breakdown



B

HIV lateral flow test

HIV rapid test	Manufacturer	Market price (US$)
Capillus	Trinity Biotech	2.20
Determine	Abbott Diagnostics	1.20
First Response	Premier Medical Corp	1.15
OraQuick	OraSure Technologies	4.00
SD Bioline	Standard Diagnostics	1.10
Uni-Gold	Trinity Biotech	2.34

Fig. 5 Cost considerations for plastic- and paper-based tests. (A) Approximate breakdown of cost of goods of current Claros plastic product. (B) Current HIV lateral flow tests. (Left) Image of OraQuick ADVANCE® Rapid HIV-1/2 Antibody Test kit components (by OraSure Technologies), consisting of test strip with plastic casing, developer solution vial, test stand, specimen collection loop, and absorbent packet.[89] (Right) Market prices (at bulk pricing) of current HIV lateral flow tests in the developing world, as compiled by WHO, UNICEF, UNAIDS and MSF.[90]

methods for solid-phase preparation, application of lids and covers, and packaging which were pertinent to the performance of the final product. Identifying and implementing mass-manufacturing conditions could be expensive at an early stage of product development, but ultimately expedited the overall product development by guiding decisions for investment in capital-intensive manufacturing equipment. We also leveraged existing manufacturing techniques and processes whenever possible to reduce the overall final cost, resulting in a final combination of specific processes that were custom designed and selected for the needs of our test.

3.5 Regulatory approval

We will focus our discussion here on the regulatory pathway in the U.S. (In Europe, a diagnostics product can be commercialized with a CE mark, which is obtained through compliance to the Directive of *In Vitro* Diagnostic Medical Devices, 98/79/EC, without necessarily specific regulations for POC use; device performance and usability characteristics will drive adoption in the POC environment. Approvals in other countries require either country-specific regulations or prior FDA approval or CE Mark.)

In the U.S., there are a set of common requirements[66] for approval of diagnostics devices, such as monitoring of product quality, analytical studies, clinical validation of the product through trials, and studies that demonstrate robustness of the test under different user conditions (*i.e.* "flex studies"). Generally, the approval takes place through either a 510(k) pathway (for markers or claims that compare to existing FDA-approved methods) or a PMA (Premarket Approval for new markers or claims; in POC diagnostics, it applies for selected infectious diseases such as HIV, even if there are predicate devices on the market). The 510(k) pathway is preferred, as PMA takes longer and is more expensive. Although approval *via* 510(k) or PMA allows the device to be used in laboratories with trained personnel, an additional regulatory requirement called "CLIA waiver" is required for use in locations without laboratory-trained personal and equipment. (Not all POC diagnostic products require CLIA waiver; in settings such as intensive care units, surgery room, and emergency rooms which contain appropriately trained staff, devices that are simple-to-use will be favored but will not necessarily require CLIA waiver.) The requirements for CLIA waiver are defined in a guideline,[67] and impose additional constraints that could alter important design decisions at the start of the product development effort. Some of the CLIA-waiver requirements that have direct technical implications include:

• **Self-contained and fully-automated test, and use of unprocessed specimen.** In case of blood markers, this requirement bars the user to centrifuge blood to produce serum or plasma. Hence, the test must either use whole blood as sample or include an automated blood separation step without user intervention.

• **No specialized training.** Any user shall be able to operate the test without technical training, based on reading instructions written in English at the 7th-grade level.

• **Easily interpreted results.** The readout should be directly usable for a clinical decision, without the need for additional calculation or calibration.

• **Robust method.** The result must be reliable when the device is operated under real-world conditions that account for user-based variations (such as sample collection, timing in user actions, and storage conditions).

3.6 Global health

Perhaps nowhere is the potential public health impact greater for a POC diagnostics device than in the developing world.[15,68] Design of POC devices for developing countries presents a daunting array of additional design challenges: extreme low cost, low power consumption, poor public health infrastructure, and challenging environmental conditions for transportation and storage.[15,68] For diagnostics in resource-poor settings, Peeling and colleagues at WHO/TDR coined a set of criteria called "ASSURED" (Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free or minimal equipment and Deliverable to end-users).[69,70] In fact, these criteria apply equally to many POC diagnostics applications in the U.S. and Europe. While the theoretical capabilities for POC diagnostics have been touted for a number of years, research and development efforts for all microfluidics-based POC diagnostics have surged in recent years from interest and funding in developing diagnostics for resource-limited settings.

Our initial technology, as published in 2003, was invented with an explicit focus on the developing world.[56] Upon investor interest, the company Claros Diagnostics focused on applications of interest in western countries. At the same time, we have leveraged a separate set of collaborators to pursue a POC test for the developing world.[53] We have encountered some unique challenges, which we describe below, in developing microfluidics-based POC technologies for both domestic and global health markets.

Coordination of research and development pathways. To drive the development of a global health diagnostics device, an often-heard suggestion is to identify an overlap in market with the developed countries so that an essentially identical product can be developed, and that first-world sales will "subsidize" third-world deployment. This idea is difficult to implement in practice. On the technical side, unlike the development of drugs (where the identical compound can be used in all markets), development of a device rests on design choices that depend on precise end-use settings. On the business level, investors into a first-world product are generally reluctant to agree with an unprofitable subsidization strategy. Instead of combining the product development efforts, we have conducted the research and development pathways for U.S./Europe and for global health on parallel tracks, in both fundraising (with the former market from private investment, the latter from grants) as well as in technical development. Our work in industry has provided expertise in microfluidic chips, high-quality chemical reagents, and robust quality control in the instrumentation to support the global health work. The global health work, however, has taken place at an academic level in

collaboration with a host of other partners, with an emphasis on a final device of small footprint, low power consumption, and simplicity of use (with the same cost, simplicity of use, and power requirements as mobile phones which are prevalent in the developing world).

Lack of harmonization in regulatory pathways. The disparate regulatory approval processes among U.S., Europe, and developing countries also argue against combining the product development efforts into one pathway. Currently, there is no single regulatory authority (similar to an FDA) for approving diagnostics products in developing countries. The closest effort is the World Health Organization pre-qualification process, which offers to perform clinical evaluations and a bulk procurement scheme for products that perform well in the evaluations. Most (if not all) rapid infectious-disease tests currently used in the developing world do not have FDA approval at all.

Lack of financing. The difficulty in translating promising technologies to the market for global health diseases is well-recognized. Currently, there still lacks a clear and accessible financing source for bringing products to the market. Funding from NIH, NSF, philanthropies, and Department of Defense all continue to emphasize innovative technological concepts, rather than clinical validation to bring technologies at a proven stage of development to the market. This gap is usually bridged by investment capital, but the gap remains largely unfilled for neglected-disease markets. (Daktari Diagnostics, which has raised private investments for its CD4-counting product to be sold in the developing world, is a notable counterexample.) An interesting new funding mechanism to address this gap is ''prizes'' (such as the X-Prize), where a monetary award is made after a set of technical milestones are achieved. We believe this innovative model would have to propose realistic and achievable milestones, as well as a mechanism to guarantee that companies that achieve the target will be paid the prize, before researchers could justify the risk of making the initial research investment. Another model in public policy discussions is ''demand-driven'' financing,[71] but it is probably best suited for technologies without the upfront development cost required by POC diagnostics.

3.7 Cheaper and faster, but is it *better*?

It would seem provocative to question how cheaper and faster would not *always* be better. But exactly what would be a ''better'' test in the interest of public health? According to the WHO and the Disease Control Priorities Project, the relevant criterion isn't which test is cheapest, but rather the cost of the test needed to provide the desired clinical benefit.[72] If this cost-to-benefit ratio (with the benefit being measured as the disability-adjusted life years, or DALYs, avoided) is three times below the GDP of a country, the intervention is deemed very *cost-effective*.[73] Hence, the acceptable cost of a POC test depends on the expected clinical benefit. For example, a POC test that can avert the prescription of months of expensive, ineffective, and potentially toxic therapy (such as a POC test for multidrug resistant tuberculosis), or a POC test with sufficiently improved accuracy to avoid expensive and routine confirmatory testing, would justify a higher price than a POC test that only marginally improves public health. We have previously performed a cost-effectiveness analysis, with the aid of independent analysts, on our mChip device for a HIV-syphilis test targeted at improving antenatal care in India (as well as other developing countries)[15] (Table 3); in our case, because of the large benefit in preventing stillborn deaths, the cost-effectiveness matched that of immunizations or oral rehydration therapy. The acceptable ''price'' of a POC test must be weighed against its clinical benefit, and then against the ability of the public to pay as measured by the specific country's productivity.

Table 3 Cost-effectiveness analysis of an HIV-syphilis test in India.[53] The health economics model was taken from methodologies published by RAND Corporation and Gates Foundation.[83]

Projected Benefits and Costs	India
Deaths avoided	162 936
DALYs avoided	5 229 389
Cost per DALY avoided	$6.81
GDP per capita (2012–2016)	$1497–$2002

In addition, we propose a second criterion for consideration, which is the cost of ''discounting''. This concept, well-known in economics and finance, states that the availability of a good or service today has greater value than the same good or service in the future. It has been shown (by among others, Gates Foundation and RAND, and WHO/TDR[74]) that millions of people have died last year, this year, and will die every year in the future due to lack of accessible diagnostics for infectious diseases that are largely treatable (with analysis done on acute lower respiratory infections, HIV/AIDS, diarrhoeal diseases, malaria, TB, and sexually transmitted infections[74]). This analysis has shown that even an imperfect POC test, if available today, would save millions of lives per year, and that waiting for a perfect test that will not be available for decades will cost millions of lives in the meanwhile. Health workers are desperately seeking a POC test that can be used now.[75]

Similar to cost, it would seem advantageous to invest in new technologies that can provide results as quickly as possible (ideally, with real-time results). However, the clinical environment should again be considered carefully. For example, even for detection of cardiac markers in life-and-death situations, a 30-minute turnaround time has been deemed by the National Academy of Clinical Biochemistry (NACB)[76] to be sufficiently useful to make a large clinical impact.

From the perspective of improving public health, considerations of cost and speed do not exist in a vacuum: they must be balanced against what real clinical benefits can be derived, as well as the development time needed to achieve the stated specifications. At an academic level, there is a tendency to propose completely novel components which could ultimately enable a test to be done more cheaply or faster. While invention of additional technologies is undoubtedly valuable at an academic level (for example, we have developed methods to operate the powerful elastomeric microvalves in field settings, based on the work from Quake and Takayama[77–79]), the field

of microfluidics is already ripe with concepts at different stages of development. With end users familiar with the promises of microfluidics but unfamiliar with real devices in the market, there remains a treasure trove of existing microfluidics concepts – and with it an enormous potential – to deliver POC diagnostic devices in the near term to transform public health care.

4. Conclusion

Microfluidics is, at its heart, a technology, with a primary goal of improving the performances of end products. For building POC diagnostic devices, there have been dazzling advances in the development of individual LOC components, but few microfluidic technologies have made the leap to fully functioning integrated devices that provide real clinical value.[80] We list some of the central challenges towards achieving this practical goal: 1) *Development of practical LOC components and procedures*. These areas include new methods for sample collection, world-to-chip interfaces, sample pre-treatment, improvement of long-term stability of reagents, and working with complex sample specimens (such as whole blood, stool, swabs, sputum, tears, and saliva).[81,82] Such components and procedures are necessary for delivering an integrated product with clinical relevance; 2) *Postponement of system integration*. For the last two decades, the major approach has been to encourage invention and development of individual LOC components. Without a clear vision and dedicated effort towards building a coherent and fully functional integrated device, the design decisions for improving individual components may prove to be fundamentally mutually incompatible. With a clear vision and focused effort, life-saving diagnostics devices can be brought to market in the near term and with a modest investment. 3) *Difficulty in access to specimens*, once an integrated device is built. 4) *Uneven regulatory requirements* constrain investors' enthusiasm at the seed stage of investment, and could hamper the actual introduction of the technology to the market; 5) *Seamless data connectivity* to electronic medical record systems, as POC devices become increasingly used in remote settings.

There has been a dearth of integrated microfluidic devices that work from start to finish on a real clinical specimen. Because POC use can be beneficial for a range of indications reimbursed at different price points, should more POC devices be built, approved, and become available, it is likely that an appropriate clinical application can be identified. Even in the most resource-limited settings, the HIV lateral flow test (coupled with access to antiretroviral drugs) has stemmed the AIDS crisis at market prices of one or several dollars per test. Rather than allowing market forces to determine the prices, artificial demands of low prices could be counterproductive by discouraging investors and private companies from developing the product. In the U.S., POC tests (such as iSTAT, Biosite Triage, and others) have dramatically improved public health at price points of tens or even hundreds of dollars per test.

Moving forward, it may be worthwhile to consider how to balance basic-science innovation with crossing the bridge towards commercialization. If that balance is not found, impact on real patients' lives will remain elusive. As a maturing technology with novel concepts continuing to be developed, as well as a treasure trove of innovative ideas already in development, the prospects have never been brighter for capturing the panoply of transformative LOC-based technologies into clinically useful POC products. A number of such tests are already on the market, including single-marker POC tests for clinical chemistry and immunoassays. Future applications include multiplexed testing, POC tests with high sensitivity or quantitation, and more technically complex tests such as nucleic acid tests and combinations of different classes of tests. With the hunger for novel POC diagnostic tests unabated from end users, it is inevitable that more LOC-based devices will eventually be brought to the market to bring true lab-based testing capability to the POC.

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