Review Article

iMedPub Journals
http://www.imedpub.com/

British Biomedical Bulletin
ISSN 2347-5447

2019
Vol.7 No.2:313

Micro-fluidics in Disease Diagnosis: Past, Present, and Future-An Overview

Madhav Gajananrao Raje[1*], Manasi Raje[2]

[1]Mahakali Polyclinic Psychology/Psychiatry, Ayodhya Nagar Square, Nagpur, Maharashtra, India

[2]Biomedical Engineer, Shield Diagnostics, CA,USA

[*]**Corresponding author:** Madhav Gajananrao Raje, Consultant Psychiatrist and Psychotherapist, Mahakali Polyclinic Psychology/Psychiatry, Ayodhya Nagar Square, Nagpur, Maharashtra, India, E-mail: drmadhavraje@gmail.com

Received date: February 13, 2019; **Accepted date:** July 23, 2019; **Published date:** July 30, 2019

Citation: Madhav Gajananrao Raje, Manasi Raje (2019) Micro-fluidics in Disease Diagnosis: Past, Present, and Future-An Overview. Br Biomed Bull Vol.7 No. 2:313.

Abstract

Micro-fluidics is a subspecialty or super-specialty of biomedical engineering. It has the potential to grow faster and influence the diagnostic field of medical practice. However, this information and utility of micro-fluidics is sparse among the medical fraternity. User and adviser of application of micro-fluidics and devices based on it is a medical fraternity. Hence, it is difficult for micro-fluidics to grow exponentially without strong demand from doctors and Para-medical personnel. Therefore, awareness among doctors, paramedical, and industrialists manufacturing devices in the diagnostic field is essential. More and more articles ought to be published to create such awareness.

Many students and their parents in India; looking for additional fields of career, look upon biomedical engineering with a lot of hopes, but with little information about the super-specialty fields like micro-fluidics. If they get information, then the growth of biomedical engineering and super specialties in biomedical engineering would spur in developing countries like India. Hence, written this overview/article to spread awareness among doctors, paramedical, engineers, scientists, industrialists, and aspiring students.

This overview throws light on past, present and future scenario of micro-fluidics. It summarizes how and why the growth of microfluidics has become complicated. It touches the complexities created by the role of different specialties and its interactions. Mechanical engineering, biological science, genetics, electronics, physical chemistry, a diagnostic field in medical practice, commercial industry, etc. play a significant role in the application of microfluidics. Interactions among these fields affect micro-fluidics in general. In the end, the authors express their opinion on how to expedite the growth of the micro-fluidics industry.

Keywords: Micro-fluidics; Micro-chip; Lab-on-chip; MEMS

Introduction

Micro-fluidics is a highly specialized field of biomedical engineering that focuses on manipulation and analysis of biological fluids (like blood, saliva, urine, etc.) through microchannels. Micro-fluidics broadly comprises of two parts, first part is about developing a device that deals with the flow of biological fluids through microchannels, and the second part is 'technology' that deals with detecting or sensing of biomarkers of biological fluids for analysis. Thus, the analysis of pathogens, toxins, and effects of disease like antigen-antibody reactions helps confirm the diagnosis. In this case, detection and analysis take place quite faster to offer faster or instant results than that of traditional laboratory testing of biological fluids. Since traditional testing including analysis of biological fluids in the laboratory is not so quick, thus delays the process of diagnosis and thereby delays the beginning of accurate treatment, which in turn affects the recovery of the patient. That is how the need for role of micro-fluidics is perceived all over the world by medical and diagnostic industry. This need was felt overwhelming in the time of epidemics or endemics. Because examination of biological fluids easily ascertains the diagnosis or detects the causative microorganism that helps to expedites the appropriate treatment. Thus, the spread of infectious epidemic if occurs can be controlled. Other than treatment, detection of microorganism at an early stage of the epidemics facilitates health authorities to plan and execute preventive measures. Thus, micro-fluidics can facilitate both treatment and prevention.

Tests based on micro-fluidics can provide instant results without the presence of specialist like pathologist or microbiologist, which expedites diagnosis and treatment to save the life of the patient in case of emergency. Thus, micro-fluidics has a diagnostic role to play along with or without the presence of physicians during routine medical emergencies and during epidemics or endemics of infectious diseases.

Thus, progress and future of the field of diagnostic medicine depend to some extent on micro-fluidics in order to improve clinical effectiveness and positively influence a patient's recovery. Market-research industry and beneficiaries predict

much higher dependence on micro-fluidics in years to come though it has some limitations like accuracy, universal applicability, technical advancement, etc.

Limitations like accuracy, speed of analytical assessment, acceptance, and validity by treating doctors are daunting and perhaps to some extent affect future progress of micro-fluidics. At a theoretical and conceptual level, micro-fluidics appears promising but manufacturing and wide acceptance by doctors all around the globe are a few issues, which affect future progress. Such aspects are discussed in the following overview.

Micro-fluidics is defined as science and technology that deals with the flow of fluids in microchannels [1]. It is a broad term comprising of two words, technology and science. To-gather it comprises of (i) a device that would deal with precise mechanical control and manipulation of movement of biological fluids through micro-channels, and (ii) various analytical processes. Analytical processed are conducted with the help of technologies that need to be implanted in the device to analyze the biological fluids in order to sense biomarkers present in the fluid or detect the effect of microorganism present in the fluid and further take appropriate decision with the help of technology to arrive at a diagnosis. Development of such chip started back in 1990 [2]. This chip functions like as if it is a Lab-on-Chip (LOC). This name indicates that there is a laboratory functioning on this chip. This technology or chip is also known as ' Miniature-Total-Analytical-System ' (μTAS), Micro-Electro-Mechanical-System (MEMS).

It offers an advantage over conventional research in disease diagnosis by providing quick, easy to read, cheap, error-free, investigations. It also offers other advantages like it can be applied in bio-sensing, bio-actuation, chemical synthesis, etc (sensor Dec. 2016). This chip detects biomarkers; analyze biological fluids through technologies like immunoassay, automation. To do so, it requires automation and integration of electrical, mechanical, photo-tonic sensing, flow control technologies.

Initially, the semiconductor industry used to manufacture such a device or LOC. Soon, Micro-Electro-Mechanical System (MEMS) took over this function of manufacturing LOC. There is another term, one needs to be familiar with to understand micro-fluidics, which is 'Point Of Care Test' (POCT).

Meaning LOC does its functioning by the side of the patient or point of care of the patient. For example, urine examined to detect the presence of pregnancy in OPD itself. Meaning, these tests carried out by the bedside of a hospitalized patient, or in the house of the patient, or in the OPD during a clinical examination. That also means a laboratory technician need not execute such tests every time. Unskilled personnel can carry out these tests with reliability. That is why micro-fluidics is an integration of science and technology.

Five groups of Biomarkers i.e. carbohydrates, proteins, lipids, nucleic acid, and cells are routinely analyzed to arrive at a diagnosis. Microchip analyses any of these biomarker/s and run immunoassay through automation. A most popular application of LOC at POCT until the date is 'pregnancy test'.

Other well-known and routinely used tests are HIV screening test, blood glucose level and flu detection test, etc. So now, one understands, here is a device that can run without the presence of skilled technician or doctor and can offer accurate, quick, and easy to read tests to ascertain the diagnosis of a single individual or group/s of affected individuals. Thus, expectations from the specialty field like micro-fluidics rise to diagnose the disease at the Point Of Care (POC). Such specialty can provide 'quantitative assessment of the health status of society' and 'qualitative assessment of individual's health' for multiple diseases at the point of examination of the patient, i.e. POC.

Background and Aim

This overview tries to comprehend the journey of the progress of the diagnostic technology of micro-fluidics to assess how technology evolved over the years. Time considered before 2010 is 'past' in this overview, from then until 2018 is considered 'present' and 2018 onwards is considered as 'future'. Since 1970, micro-fluidic research started contributing to the diagnostic field. However, commercialization of research has not reached far and wide until date. Indian and Chinese health care system who serves the world's half population still does not routinely use tools (Point of care tests/POCT) to diagnose infectious diseases like Dengue/malaria/TB in OPD. Doctors in many developing countries have not gone beyond measuring blood sugar, blood pressure, pregnancy in their OPD/clinics with the help of this technology.

This scenario desperately needs a change because undetected or delayed detection of diseases like dengue or falciparum malaria is fatal. With this fundamental concept, we searched for the attributing factors relevant to past, present, and future progress (and other-wise) of technology. The literature reviewed with the same intention to obtain an overall picture. Writing or repeating a comprehensive essay of published articles avoided to narrate the technology and field in detail, because it was not aimed. Outline of this overview includes past, present and future of the diagnostics industry through the timeline, data describing advances in technology, limitations, and future scope.

Past scenario: Before 2010

Past of micro-fluidics tools: Microbiology tools proceeded to the era of micro-fluidics tools. Before 1980 Enzyme Immunoassay (EIA) used to detect antigen-antibody. The breakthrough came when EIA detected HIV virus. This happened in 1980. However, screening of HIV by EIA was neither specific nor sensitive. To make screening specific indirect Immunofluorescence (IFA) developed. Since then the screening and confirmatory test of HIV became a protocol. EIA technology was later supported by Recombinant Immune-Blotting Assay (RIBA) which helped further to diagnose Hepatitis C (HCV). This technology could not support the need for counting viral load, so Polymerase Chain Reaction (PCR) was developed. PCR based technology helped identify the genetic profile of pathogens also. Later PCR based technology paved the way for nucleic acid analysis. Nucleic acid analysis technology revolutionized the diagnostic field. The nucleic acid analysis helped not only analysis of pathogens, finding sequences of pathogens, but also

contributed to the development of molecular epidemiology. Thus, microbiology tools evolved to perform 'molecular surveillance' during times of epidemics/outbreaks of infectious diseases, though in a centralized laboratory.

Simultaneously 'flow cytometry' that was developed during 1970, combined with newly invented 'monoclonal antibody' to detect low numbers and percentage of CD 4 lymphocytes. Since then the use of flow cytometry began in the diagnostic practice of HIV and Cancer. Flow cytometry was and is the most successful and simple diagnostic technology ever since [3].

Then in 1991, microarray technology developed. Microarray technology applied along with nucleic acid analysis to read the genetic expression in cancer. This combination of microarray and nucleic acid technology helped analyze the genome in cancer and inflammatory diseases. Further, 'gene chip' developed to identify resistant HIV and TB individuals.

Chronic diseases change the genome of host/patient. Evaluation of changing process of the host genome can help to identify the staging of disease. DNA microarray application identified sequencing of the genome of the host, which then helped not only to identify specific drug of treatment but also to develop future vaccines to prevent [3].

Microarray technology contributed hugely not only in diagnosis but also in the prevention of communicable diseases. However, unless this technology was decentralized, made it applicable at the Point Of Care (POC) for end-users and health professional it was not directly benefitting patients. Hence, the combination of microarray and micro-fluidics was imminent. Therefore, microarray with a microfluidic technology-based tool used as a POC assay, to diagnose infectious and non-infectious diseases was developed. These devices worked with a sample like a saliva, urine, stool, blood spots. Thus, a revolution in the diagnostic industry took flight. What so ever progress happened; integration of this POC technology at a large scale was still a distant reality to dawn. However, multiple hurdles attribute difficulty in transforming research of a lab to commercial and consumer product.

Micro-fluidic tools and system

LOC technology: LOC deals mainly with micro-fluidic and molecular biology. Microtechnology and microfabrication of LOC were born essentially way back in 1950 for the use of space/spacecraft. Laboratory in spacecraft needs to be in miniaturized size and shape. Therefore, LOC was developed. By 1960, MEMS developed for the use of Airbag and smartphones. Use of microchip developed in 1979 at Stanford University for diagnostic purpose. During 1979, the first analysis system of LOC developed by SC Terry at Stanford University [4]. Gas chromatography was applied to make it. Research on LOC got major thrust in 1980 when polymer chips were fabricated. Technology that fabricated polymer chip called 'soft-lithography'. Then onwards many researchers tried to make microchips since it was easy to fabricate microchip with polymer [5]. A major boost in research happened during mid 1990 when capillary electrophoresis and DNA microarray applied in genomic research [4]. Then in 1990, PCR technology came, which used for diagnostic purposes. PCR paved way from detection and analysis of fragmented DNA. Very small DNA/RNA amplified and sequences studied with the PCR technology. Simultaneously researchers felt the need for analysis of more than one analytes. To meet this demand, Micro-Total-Analytical System (μTAS) evolved during the same time that helped assessment of more than one analytes on a single chip. Martinez et al. developed the first paper microfluidic device [6]. This device used for urine analysis. Paper microfluidic devices used to detect pathogens and toxins. Most of these tests rely on calorimetric reactions. Hence, the results of these tests read by the change of color, that an unskilled person can also read. So these tests became easily popular, e.g. pregnancy test of urine. In the light of such a scenario, many experts like Dominic Eicher; Christopher A Merten projected significant growth and evolution of diagnostic devices with applications of micro-fluidics [7].

Point of care technology (POCT): Many technologies tried to develop POCT by different researchers' way back since 1956. Lateral Flow Immuno Assay (LFIA) which currently applied in almost every device developed by Singer and Plotz in 1956. However, it took more than a decade to make it suitable for POC platform. Taking a test from centralized laboratory to Place of Care (POC) is really quite an arduous task. In fact, era of POC tests began in 1962, but it was in the infantile stage by then.

Monoclonal antibodies developed by 1970, which combined with flow cytometry to identify the minimum/low number of CD 4 lymphocytes to diagnose HIV with low viral load. Since 1970, the use of flow cytometry began in diagnostic procedures to diagnose HIV, cancer. Because of this development by 1970, many POC tests were developed. Then era started of measuring blood glucose levels at the bedside.

During that period, (1977) urine pregnancy test also saw the light (commercial use), where lateral flow technology was applied. However, until then POC tests were not developed to diagnose many diseases. Even then, diseases like pneumonia, malaria, TB, and syphilis diagnosed during that initial phase. However, the significance of the impact of these initial not much developed POC tests highlighted from the fact that it could prevent 1.2 million deaths in developing countries then [8]. Eventually, many numbers of diseases diagnosed with evolved technology.

Present Scenario

Loc

Principle: Principle of technology of LOC differentiates it from microbiological tools. Micro-fluidic device integrates multiple technologies into a single miniature system format.

LOC technology: Traditionally tests advised by doctors bring results after 1 to 3 days or at times after 7 days. Therefore, diagnosis/treatment delayed.

This delay in initiation of treatment poses significant health issues, like infection (TB) spreads to threaten life or stage of cancer advances. Hopes ignited when micro-fluidic technology found to offer great help to expedite the diagnostic procedure

and reduce treatment-action taking time. Micro-fluidic technology could carry out this marvel only when LOC technology integrated with the POC system. Addition of POCT changed the picture of the diagnostic procedure.

LOC system/devices have three components built within, (i) fluid flow controlling devices, e.g. microchannels, micro pumps, microvalves, mixers, (ii) sensors e.g. optical or electrochemical sensors embedded in a chip and (iii) decision making capability technology, e.g. (a) micro-total-analytical-system (μTAS), (b) Micro-Electro-Mechanical-System (MEMS) [9].

Current LOC technology focuses on (i) large scale manufacturing of LOC to propagate commercial utility, which means fabrication process needs to be adapted at industrial level/for large production (ii) making design for specific surface treatment, and (iii) simultaneous application/processing of more than one analytes on a single chip [5]. Because of this focus, the self-integrated cartridge was developed. Self-integrated cartridge runs multiple reagent tests, automatically, minimize the time needed for test and analyze analytes automatically, e.g. washing/calibration, etc. carried out automatically. For example, Jing et al. [10] integrated air-born 'on-chip bacteria capturing system' and rapid bacteriological immunoassay into an automated micro-fluidic system. This system captures and process analytical tests on bacteria within 50 min and avoids the step of culture and time lag for culture. Immuno magnetic separation of bacteria takes place on such chips. However, making of such device/chip is yet not a usual/easy experience. Depending on the segment, LOC technology divided into various types, like instrument segment, cartridge, drug delivery segment, paper-based material, home care setting and others. Asia-pacific region is the most promising areas of LOC in present and in the future too.

Types of LOC technology tabulated in **Table 1**, which will help one, comprehend it.

Table 1: Segments of Lab-on-chip (LOC) and its details.

No.	Type of segment	Sub-types	Description of the segment
1	Product	*Instrument	: Micro-fluidic chip, Cartridge (49.1% share of product segment)
		*Reagent and consumable	: others
		*Software and Services	
2	Application	*Genomics, proteomics	:Peripheral vascular disease(On the rise)
		*Diagnostics	: wound care management (developing healthcare infrastructure)
		*Drug discovery	: Pharma and Biotech research(Rising geriatric population)
		*Others	: *In-vitro*-diagnostics
			: Drug delivery and: Others.
3	Material	*Silicon	: Paper based devices cut cost
		*Glass	: Easy to fabricate
		*Polymer	
		*Paper	
4	End user's	*Hospitals	: Bench-top instruments used
		*Academic and Research Institutes	: Less dependency of central lab
		*Diagnostic laboratories	: Authentic support/control from central lab
		*Home care settings	: Less no. of technical staff
		* Others	
5	Geography	*North America	: USA, Canada (37.4% share of geography segment market)
		*Europe	: Germany, France, UK, Italy, Spain, others
		*Asia-Pacific region	: Japan, China, India (half of the world population)
		*Latin America	: Mexico, Brazil, other Latin American countries.
		*The Middle East and Africa	: Saudi Arabia, South Africa, and the rest of countries

Currently, the most advanced device of LOC is Polymerase Chain Reaction (PCR). Kary Mullis developed PCR in 1983. Nobel Prize was awarded to him in 1993. PCR is considered 'gold standard' in LOC. It amplifies a small quantity of DNA to copy its specific fragments. Because of this quality, it is used in labs to clone the gene. PCR detects pathogens (like TB, influenza) to diagnose diseases, and identify fingerprints. Prof. Michael Kubista evolved qPCR in 1991. Later on, RT-PCR, droplet-based

PCR were developed. Droplet-based PCR helps in system integration and automation, which is the need for the ongoing diagnostics market.

Examples of LOC and technology: Following are examples of LOC, (a) Curtis Chin et al. developed plastic/silicon-based device [11]; (b) Lee's group developed strip test, to detect Chlamydia and diagnose Trachoma; (c) MacDevitt and colleagues developed a device that measures C-reactive protein in saliva; (d) Enteric antigens can be detected in a stool sample; (e) Mirkin and co-workers developed a device to identify protein markers; (f) M-Chip (2004 to 2007, by Samuel Sia). Technologies like Flow cytometry, MEMS, µTAS, Lateral Flow Immunoassay (LFIA), PCR technology, soft lithography, optical or electrochemical sensors, immuno-chromatography, Pulse-Field-Gel-Electrophoresis (PFGE), etc. are applied in LOC.

Following are the major manufacturers of LOC in the world, Abbot (i Stat), F. Hoffman La-Roch, Thermo-fisher Scientific Inc., Danaher Corporation, etc (the list is not all-inclusive). In addition to these technologies, the following are the different technologies applied in LOC.

Different technologies applicable in LOC: Multiple fields like biosensors, biotechnology, VLSI, software like MEMS, NEMS, MATLAB, µTAS are integrated within a fully functioning LOC device. When added a sample, e.g. blood drop, all embedded technologies work synergistically to produce results. Such multiple complexities, however, are a big challenge while making of LOC. Various types of technologies are used for LOC. Following examples of varied technologies would reveal that making of LOC integrated with technology is quite an intricate process. ESCARGOT (creates micro channels), ELISA, Lateral Flow (LFIA), Vertical flow (MedMira), hydrodynamic flow switching, electrokinetic flow switching, di-electrophoresis, electrowetting assisted flow switching, etc. DNA microarray or microarray technology help analyze DNA.

Similarly many other like Photolithography, Cell sorting/ separation (Sulchek), high throughput droplet formation, Nucleic acid analysis, PCR technology, Surface Plasma Resonance (SPR), Poly Dimethyl Siloxane Processing (PDMS), are also in use to make LOC. There are some examples that need to be mentioned, though the list may appear quite long; like isothermal application (Gullikson and colleagues), Immunochromatography Strips (ICS), Enzyme Immunoassay (EIS), Indirect Immuno Florence (IFA), Recombinant Immuno Blotting Assay (RIBA), Pulse Field Gel Electrophoresis (PFGE) (to analyze genome of bacteria), etc. This advance in technology indicates that the growth of research in biomedical engineering.

Steps of LOC functioning: Following are the steps of LOC functioning, e.g. (Add sample, mix with reagent to produce mixture) Biological process, (system of sensing facilitates detection of analytes) Biosensors/Transducers, (Identify, amplify analytes which are sensed) Amplifier, (detecting amplifies data, processing data to arrive at diagnosis) microelectronics [4]. Therefore, every LOC functions through four steps, biological, biosensors, amplifier, and microelectronics.

Biggest Challenge is developing a chip: Major challenge is (a) chip preparation, (b) selection of technology to detect analytes/s in microchannels [4]. Process of miniaturization, automation, storage of multiple reagents, and fluid handling capacity to carry out complex protein assay are the hurdles in preparation of a chip. The second hurdle is developing a technology that can readily detect signal in the micro-fluidic system [12].

Integration of System engineering and device with that of user experience is the biggest challenge while developing a chip, Samuel Sia mentioned. High performance of chip with low cost increases the difficulties of researchers, he further states. So Samuel Sia suggests all to consider 'use-value' of the chip instead of cost-value of making a chip a more important criterion. A Limited Resource Setting (LRS) is another major hurdle to make application of LOC devices suitable [11]. Challenges come from not only technical or socio-economic fronts across the developing countries but also from 'priorities of developed countries', e.g. despite usefulness limited number of micro-fluidic projects are undertaken by industries of developing countries, so expansion, growth of micro-fluidic industry does not pace up as expected [12].

Though development begins from the research role of manufacturers of LOC at POC is important, because results can see the day of light only when manufacturers complete the job.

Poct

Need and demand of time is perceived across the globe to bring micro-fluidic technology to perform at bed side of the patient to produce reliable, perfect results during the time of clinical examination, i.e. clinical decision-making process. That means system and technology assist doctors to diagnose disease within 30-40 minutes during the clinical examination itself.

Hence, the need of the day is; to make a single device integrated with preparative and analytical technologies to produce fast, easy to read, perfect, cost-effective results.

Such technology is called "Point of care" (POC) technology. Traditionally centralized laboratory used to perform most of the POC tests at a low cost.

This is a set the trend of POC tests until this decade, though it demands a change of place where POC tests are performed [13]. This change of place of application of POC test invites challenges, discussed later. This trend is popular right now in biochemistry and hematology practices.

In 2011, the US market for POC tests was $ 15 billion, with the prediction of annual growth at 4%. POC market is of two types, (a) over the market, where tests are sold over the counter and applied by unskilled/end users. (b) Professional market, where tests advised by doctors get carried out by skilled health care workers. Such tests conducted in ICU or hospitals for patients of critical care, cardiac care, diabetes, hematological diseases, and infectious diseases.

Definition of POC: POC is a Medical test conducted at the time of making clinical examination and treatment action/s that leads to improved health care.

To explain further, if rapid HIV antibody test is performed by a technician at centralized laboratory it would not be label as POC.

However, if the same test conducted at the bedside or at the scene of clinical examination (near the bed) then it is called POC test. Similarly, if the same test performed at 'Limited Resource Setting' (LRS) then also it is a POC test. LRS means lack of resources e.g. lack of trained personnel, lack of laboratory and facilities associated with laboratory, limited transportations, undeveloped country terrains, distant military areas, etc.

'Aim of POC test is to improve health care' by facilitating clinician's decision to diagnose and expedite treatment. This aim shadows over accuracy of the test [8]. However, clinicians do get 'help' from even compromised standard of accuracy to expedite diagnosis and treatment action. Hence, excessive emphasis on standard of accuracy is unwarranted. This information may ease burden over engineers who try to fabricate/develop highly accurate POC tests.

However, accuracy is indispensible. For same reason, International Council for Standardization in Hematology (ICSH) offers directives to improve accuracy of the POCT. ICSH laid out standardized procedure to apply POCT that is mandatory while being implemented.

Thus, whosoever unskilled or skilled he/she has to shoulder the responsibility to carry out test as per guidelines. ICSH advise central laboratory to train such persons who will carry out POCT and maintain regulatory constraints to achieve standards of accuracy to maintain validity.

POCT technology: POCT technology is divided into two categories, (i) Small handheld devices. These devices provide quantitative as well as qualitative results. Such a device may detect one or multiple analytes, e.g. glucose biosensor strips, lateral flow strips which detect cardiac markers and pathogens. (ii) A large device used near the bedside e.g. small hematological and immunology analyzers.

There are broadly 4 steps in devices of POC, (i) sample collection, sample pre-preparation; (ii) reagent mixing; (iii) running of the analytical assay (Lateral flow/flow-through); and (iv) detection of the result. Miniature portable electrochemical are analyzers integrated into micro-fluidic devices at POC platform.

Most of such devices do have a self-contained cartridge which bears dry lyophilized reagent, sample chamber, calibration solution, the waste chamber, and multiple sensors (either battery operated or passively regulated).

Ideal POC test: WHO declared criteria of ideal POCT **(Table 2)** WHO calls it (mnemonic) ASSURED. It is affordable, sensitive, specific, user-friendly, rapid, equipment-free, delivered directly to needy people.

However, Samuel Sia of Claros diagnostics opines that criterion should be 'ASSURD'. The equipment-free criterion may be skipped Sia feels. S AB Hermsen et al. finds an essential criterion of POCT are its portability, small size (handheld), less weight, fully automotive and applicability of multiple parameters [13].

Table 2: Example of POCT: (Not all comprehensive lists).

Qualitative POC tests	Quantitative POC tests
hCG (Pregnancy test)	Glucose test
Flu POC test	Blood Glucose test
Strepto. A test	HbA1c
Drug abuse test	Creatinine
Influenza test	Electrolytes
Urine Analysis test	D-Dimer
Fast check POC 20 (Allergy test)	Troponin
Clotting time (BT CT)	Malaria screening
Platelet function	HIV screening
HIV confirmatory	CBC

Following diseases can be diagnosed by POCT: T.B., Malaria, Flu, HIV, HCV, HBV, Dengue, Influenza, respiratory infections, Streptococcal infections, Chlamydia, Trachoma, Enteric fever, Rheumatoid Arthritis **(Table 3).**

Table 3: Some of Manufacturers of POCT with name and some details of POCT: (Not all-inclusive lists).

Sr.	Manufacturer	Test name	Test advise
1	Abaxis	The Piccolo	Blood chemistry
2	Alere (Biosite)	Alere Triage meter pro	Blood/urine chemistry with waived lipid-liver panels
3	Epocal	The POC blood analysis system	Blood chemistry
4	Focus Dx	Simplex	Flu A/B
5	Handy lab (BD)	BD Max TM GBS assay	Group B Strept.
6	Abbot (istat)	Istat analyzer	Blood chemistry, coagulation, cardiac marker
7	Idaho Technologies	Film array R P	Respiratory pathogens

Challenges of POCT: Technical: Accuracy of test results depends not only on the system of POCT but also on the personnel performing it. Most of the users of POCT, even if they include technicians, nurses, lab assistants, homemakers or even doctors running private clinics need to undergo training.

Next of kin or unskilled person must undergo this training. International Council for Standardization (ICS) has set up such rules to maintain authentication of POCT.

Training to carry out the standard operative procedure, to read the results without error, to document results, to collaborate with the central laboratory for back up to maintain quality control has become mandatory [14].

In fact, concerns of standardization, universality, repeatability, authenticity, accuracy have made POCT vulnerable to grow.

Collaboration/integration of LOC and POC technology has limitations **(Table 4)** It would be possible when 'benchtop fabrication methods' were applied and used universally [15]. Such limitation/s affect negatively on the pace of development of more/ new LOC technology. Therefore, the commercialization of new POC tests does not catch up speed [16].

Table 4: Difficult collaboration of LOC and POCT.

Micro-fluidics advances with difficult Collaboration of two pillars, LOC and POCT	
LOC offers	POCT offers
Use of small volume sample	Miniaturization e.g. micro-camera detect results.
Faster analysis , Low cost	Integration of complex functions
Drug delivery system	Usage in limited-resource settings.

Future

Projections

Projections of the market: Micro-fluidic market- Global micro-fluidic market will grow with the rate of (CAGR) 19.4% as against 2011 report of 4% CAGR (Coherent market insight 30.11.2017). Strongly indicate robust growth during these 6 years (from 2011 to 2017) **(Table 5).**

LOC market: PRNewswire writes that in 2014 market was 1.886 B $ which is likely to become 6.4 B $ by 2020(29.7.2015).

TMR projects that 3.88 B $ market in 2016 will grow to 8.7 B $ in 2024 (Aug 2013).

TMR projects that PCR based Instrument segment of Product type of LOC would grow from 6.06 B $ in 2013 to 9.6 B in 2020 (Aug. 2013).

Table 5: POCT market-all figures in USD, Billion (B).

Yr.	**2013**	**2015**	**2016**	**2017**	**2018**	**2018**	**2020**	**2023**
$ Bn	1.6	18	2.8	19.6	3.6-5.7	10.06	4	27.91
Author	Volpatti	Kalorama	Coherent	Kalorama	Volpatrti	Marketsand	PR	Marketsand
	L R	information	Market insight	information	L R	Market	Nweswire	Market

Future Projections of Technological Advances: (Groups are Formed Randomly)

Group A

Technology-wise: Future growth is expected in the following technologies, sample droplet production technology, micro-fluidic sensors, micro-fluidic enabled microscopes, micro-fluidic valve controller (TMR 2017).

Region-wise: North America will lead the market of micro-fluidics.

Material wise: Polymer-based microfluidic will have a major share of the market.

Application wise: Drug delivery device segment of micro-fluidic will grow most.

Product-wise: Cartridge and reagent segment will rise above other products.

Group B: Demand for developing countries

• Paper-based micro-fluidic devices that can be used in the future to detect heavy metals in contaminated water. This application would help reduce environmental toxicity in future, very useful for developing countries

• Single-stranded Oligonucleiotids, molecular Beacons may be used in LOC made for developing countries

• Pharmaco-genetic studies will expedite personalized medicine in developing the world

• Micro-fluidics would be used to control epidemics and endemics of infectious diseases, in developing countries [17]

• Digital PCR (dPCR) would bring out results within 10 min at POC. A genomic study carried out by dPCR at POC would be highly appreciated in developing countries. Advances in PCR like dPCR, qPCR, RT-PCR, etc. would accelerate growth. Reverse transcription-PCR (RT-PCR) test is also used to diagnose Dengue Virus Disease (DVD). It offers diagnosis within 2 hours. The rapid diagnosis could reduce the mortality of Dengue in developing countries like India

• In the near future, LOC and POCT will be used for Epidemiological surveillance mostly in developing countries [18]

Group C: Diagnostic tests facilitated by LOC

• Future advances would assist doctors in the emergency room to diagnose poisoning and type of poison

• Application of micro-fluidic devices for radiological diagnosis would be seen in near future [17]

• Advanced technology would diagnose neuropsychiatric diseases like epilepsy, depression, substance abuse [4]

• A diagnostic test to identify biomarkers for concussion, a heart attack would become routine POCT

• Micro-fluidic based radiological tests (X-ray) to detect Alzheimer's disease may soon be a reality

• POCT Blood tests to identify circulating Tumor markers to detect cancer in a very early stage are becoming reality

• New diagnostic tests: A few are mentioned here, not all-inclusive

• 'Nadia' developed by Dolomite Bio in Nov. 2017. Nadia is a single cell platform for sequencing single-cell RNA. This instrument can generate 6000 single-cell libraries per sample within 15 min. Droplet micro-fluidic base is applied. Nadia can run parallel multiple samples (TME Nov. 2017). 'Claros 1 immunoassay analyzer' is another diagnostic instrument that generates results within 10 min

• Chembio's fever panel: It is available at some of the places. It can diagnose (9 Tests) various causes of fever, malaria, dengue, Ebola virus fever, Chikungunya, Zika, Laptospirosis, *R. typhi*, *B. psudomallei*, and *Orentia tsutsugamushi*

• Cobas Liat system-is a rapid reverse transcription (RT-PCR) PCR diagnostic test for Influenza A and B

• *C. elegans* would be used for biomedical research and for the screening of drugs (B G Gupta Micromechanics 2016) [19]

Group D: future medical treatment facilitated by LOC

• Advanced wound care management would be more efficient with the involvement of micro-fluidics. Advanced wound dressings like foam dressing, Alginate dressing, Hydrocolloid dressing, hydro fiber dressing, collagen dressing, antimicrobial dressing, etc. would be available for diabetic foot ulcers, venous leg ulcers, pressure ulcers, surgical and traumatic wounds, and burn wounds. In this way, wound care management would be immensely impacted in the near future

• Specific treatment like Gene therapy for hemophilia expected to hit the market in the near future [2]. Use of recombinant biological agent as replacement therapy for factor VIII and IX in hemophilia would become a reality in the near future [20]

• Instruments with a micro-fluidic system would soon be used for controlled drug delivery while treating patients [18]

Group E: Other future applications

• Combination of oligonucleotides conjugated nano-particles (now commercialized by Nanosphere) is useful in analyzing nucleic acid in the future

• Helicase dependent DNA amplification may be integrated into LOC devices in future

• Role of micro-fluidic to study kinate receptors in synaptogenesis would be a future application. Prasanna Sakha of University of Helsinki (22.4.2016) is studying it presently

• Micro-fluidic devices would be used in forensic DNA analysis with some limitations. LOC analyses DNA material from contaminated DNA material

Discussion

Having considered past, present and future scenario of micro-fluidics one comprehend the major role of micro-fluidics is in diagnostics and research relevant to the healthcare system [5]. However, growth of micro-fluidics does not solely depend on research/technology but the integration of micro-fluidics with that of enabling health care system, Dr. Madhukar Pai of Mac Grill University says. As long as the Health care system does not demand or value the role of LOC or POCT real boost to micro-fluidics market remains awaited. This overview points to un-uniform market and low application of POCT across the globe. Market and application of POCT both are comparatively quite robust in the USA (region-wise, P R Newswire 29th July 2015). However, its actual need is in developing countries, like the Asia-pacific region and African-middle-east countries. Despite the need, its application is scarce. To some extent, reasons identified behind this picture are as follows, (i) presumptive(not precise) method of treatment by doctors [20], many general doctors treat patients intuitively or treat on the basis of their clinical judgment in developing countries [21]), (ii) stringent rules for operative procedure of POCT, (iii) lack of faith of govt. authorities and doctors on reliability and accuracy of POCT [14], (iv) lack of awareness of end-users (TMR Aug. 2013), (v) limited funding and govt. initiatives to facilitate the health care system. Days of POCT to become routine practice while treating patients will delude unless these reasons are dealt with. Most effective reason among them could be a lack of awareness of persons involved in the health care system. Awareness could be made by publications of articles in medical or biomedical journals (not only engineering, chemical, research journals), main news media, and workshops for doctors/nurses/lab assistants/hospital owners, etc. Clinicians should be involved in training and use of POCT more often [13] but in reality, they are not. For exam. 'WONCA-Global family doctor' recently (2014) organized a workshop titled 'how to set up and manage POCT in your family practice' at Kuching, Malaysia. A total number of delegates was 30, who participated from Malaysia, Indonesia, Brunei, Japan, Hong-Kong, Australia, and Nigeria. (www.globalfamilydoctor.com). No delegate was from India or China, which comprises half of the world's population. This picture is indicative of the lack of involvement of doctors in POCT training. The message needs to spread far and wide about the use of POCT to and by the health care system. For an exam,

private G Ps of Australia, Korea, and Japan take more interest in using POCT in their day-to-day practice. Lateral flow-based Immuno chromatography reagents are widely used in Japanese G P clinics for diagnosis of measles [22]. Another example that demands mention; is that of PHCs of Uppsala (Europe) where the use of POCT based CBC test is routinely applied in their outpatient department [14]. In Australia, there are three networks where POCT is used under strict control for want of concerns of accuracy, reliability, repeatability; errors of unskilled health workers, etc. Despite these issues, POCT is currently applied in Australia (Rosy Tirimacco, 2010). Such news needs to be spread far and wide among doctors, pathologists, lab assistants, corporate hospitals, govt. health dept. or health ministry or doctor's organizations like IMA in India. NGOs in the health care system may take this issue up. For example, FIND (est. 2003) partners with more than 1000 organizations to develop POCT. FIND is developing diagnostics tests for TB, Malaria, HIV, Ebola, sleeping sickness, Hepatitis C, Leishmaniasis, Chagas disease (4.4.2017) (www.finddx.org). FIND can contribute more to spread awareness. New organizations like FIND if developed would sure propagate this issue. At present, there are only five funding agencies operating in this field. They are (1) Gates foundation; (2) U S National Institute of Health; (3) Welcome trust; (4) UK department of international development; (5) European Commission. More organizations need to come forward to fund research, training, and marketing. There is a distinct disparity between the amount of funding done for the development of a new drug and that of the development of a new diagnostic test like POCT [20]. This scenario needs to be changed. There are some intricate issues, which can affect the growth of the market negatively. These issues noted as follows.

• End users should get an operational advantage over older devices. Manufacturers need to realize and improvise accordingly for the demand of POCT to increase. Thus, the diffusion of consumer product gets limitations [23]

• Integration of multiple fields (interdisciplinary dependence) is required to form full-functioning LOC/POCT. Fields like bio-sensors, biotechnology, microtechnology, molecular biology, microfabrication, tools or software (like MEMS, NEMS, uTAS, Metlabs, etc.), automation engineering to design, amplifiers, droplet movement electrophoresis, chemistry, use of biological samples (saliva, blood, urine, etc.) [4]. The appropriate collaboration of many fields is key for the foundation of POCT. On a similar note, Lux research too expects two types of companies would lead the micro-fluidic market, (i) chip manufacturers, and (ii) device integrators. Chip manufacturers like mini FAB, Sony DADC and device integrators like Agilent and Fluidigm do have a major role to play

• Development of test is quite an arduous process. It takes around five to ten years to develop a test at the cost of $ 2 to 10 million [21]

• Major bottleneck decelerators are stringent regulatory approvals from FDA, EMA, and reliability of POCT (TMR Aug. 2013)

• Western micro-fluidic setups do have a lukewarm interest to design a LOC for developing countries [11]. Most of the micro-fluidic set ups/labs fabricate or develop small-scale devices, systems, or techniques of advance applicability and use this research for publications/conferences. Such effort/development is of no use for large-scale manufacturing or integration. So large-scale manufacturing do not get direct impetus from various micro-fluidic labs (Tsun-Ming-Tseng)

Future Challenges/Limitations

Future limitations revolve around (a) Lack of standardization of micro-fluidic manufacturing and; (b) *Orentia tsutsugamushi* Transformation of micro-fluidics into viable commercial technology. This is difficult because micro-fluidic is an R and D tool and conversion of R and D tool to consumer product itself has its own inherent limitations; (c) Continuous production of manually designed and prepared micro-fluidics for large-scale production appears impracticable as of today (2017); (d) Traditional POC technology based on Lateral Flow Immunoassay (LFIA) may compromise with accuracy. Similarly, a large Coefficient of Variation (CV) limits the reproducibility of POCT. Hence, the performance of these tests varies from time to time and sample to sample.

Author's View

There are limitations in the growth of LOC/POCT/micro-fluidics in both directions. Vertical growth needs evolved technology, biomedical engineering achieved in research labs [24]. Vertical growth will expedite if research or inventions pace up. However, horizontal growth can expedite the overall growth. Horizontal growth means widening the reach of the micro-fluidic market. Horizontal growth can be achieved by focusing on the 'end user's and allies' market'. End users alone who include hospitals, dispensaries, specialist's clinics, pathology labs, health care facilities (Primary health centers/PHC), next of kin, would not provide adequate boost unless allies who include service industry i.e. pharmacists, lab technicians, nurses, community health workers, are involved extensively. Allies of the health service sector can boost the impeded growth in developing countries because their livelihood would depend on it. Lab assistants like Diploma in Medical Laboratory Technology (DMLT in India) who are not as capable as a pathologist can now compete and benefitted by advance technology to run their own lab independently. Thus, this service sector can extend the horizontal reach of micro-fluidics from the research lab to the house.

In the USA, there are such experiments already implemented where pharmacy or community pharmacy is involved in POCT [25]. It is experienced there that younger age group (20-34) is willing to pay at community pharmacy at Nashville, TN, USA [26]. Similarly, younger age group people in developing countries too would respond.

Conclusion

Despite limitations, this continuous on-going demand from the health care sector may offer micro-fluidics a mainstay in health care diagnostic field. Vertical growth of micro-fluidics justifies it. However, horizontal growth appears sluggish. To make micro-fluidic one among household utility health care sector, it may need to join hands with research. The ultimate outcome depends on the cost of manufacturing, ease of doing these tests, acceptance from medical clinicians/technicians, and patients in general.

Limitations

Fore-most limitation of this overview is that it is a short review of the past and present of micro-fluidics. Diagnostic part of micro-fluidics discussed in short. Details of many POCTs, the technology of microchips/cartridges, methods of making LOC, mention of an exhaustive list of researchers/manufacturers/NGOs, could not be included. Because this is not an all-inclusive review, many details were not included. Purpose of this review is to throw light on the overall scenario of micro-fluidics, befitting the title and to promote awareness.

References

1. Christian H, Sebastian A, Weisse, Marcel V (2017) Commercial value and challenges of drop-based microfluidic screening platform-An opinion. Microma 8: 193.
2. Jayson VP, Vishal S, Adam TW (2015) Application of microfluidics and microchips Electrophoresis for potential clinical biomarkers analysis. Anal Bioanal chem 407: 6911-6922.
3. Shikha S, Julia ZR, Pedro E, Richard O (2015) Point of care diagnostics in Low resource setting: Present status and future role of microfluidics. Biosensor 5: 577-601.
4. Shagun G, Ramesh K, Ahmed S, Vippan k (2016) Lab on chip technology: A review on design trends and future scope in biomedical applications. Int J Biosci and Biotechnol 8: 311-322.
5. Guilhem VC, Timothee H (2015) Introduction to Lab on chip 2015: Review, History and future.
6. Paper microfluidic devices: A review 2017.
7. Dominic E , Christof AM (2011) Microfluidic devices for diagnostic applications. J Expert Rev Mol Diagn 11: 5.
8. Drain PK, Emily PH, Ingrid VB (2014) Evaluating diagnostic POC tests in Resource limited settings (RLS). Lancet Infect Dis 14: 239-249.
9. Book: Lab on chip technology: Bimolecular separation and analysis (2009), Editor-K. E. Herold, Avraham Rasooly, Horizon Scientific press. ISBN 1904455476, 9781904455479.
10. Wenmen J, Xiran J, Wang Z, Guodong S (2014) Microfluidic platform for direct capture and analysis of air born Mycobacterium tuberculosis. Anal Chem 86: 12.
11. Curtis DC, Vincent L, Samuel KS (2007) Lab on chip devices for global health: Past studies and future opportunities. RSC 7: 41-57.
12. Kenyon SM, Meighan MM, Hayes MA (2011) Recent development in electrophoretic separation on microfluidic device. Electrophoresis 32: 482-493.
13. Hermsen SAB, Roszek B (2013) Lab on chip device for clinical diagnostics, RIVM report, National Institute for Public Health and Environment, Ministry of health, welfare and sport.
14. Anders L, Roman GP, Albert H (2015) State of POCT: A European perspective. Ups J Med Sci 120: 1-10.
15. Posthuma TGA, Korf J, Amerongen A (2009) Lateral flow immunoassay: Its strength, weakness, opportunity and threats-A literature survey. Anal Bioanal Chemistry 393: 569.
16. Cutis DC, Vincent L, Sia SK (2012) Commercialization of micro fluidic (POC) point of care diagnostic devices. LAB Chip 12: 2118-2134.
17. Ghazal A, Josaine P, Kell M, Jorg PK, Lise A, et al. (2016) Recent advances in X-ray compatible with micro-fluidics for application in soft materials and Life sciences. LAB Chip 16: 4263-4295.
18. Sanjay ST, Zhou W, Dou M, Tavakoli H, Ma L, et al. (2017) Recent advances in control drug delivery using microfluidic platform. Adv Drug Deliv Rev 128: 3-28.
19. Tay A, Pavesi A, Yazdi SR, Lim CT, Warkiani ME, et al. (2016) Advances in microfluidics in combating infectious diseases. Biotechnol Adv 34: 404-421.
20. Larry J, Dan LL (2015) Precision medicine: Personlized, problematic, and promising. N Engl J Med 372: 2229-2234.
21. Peeling RW, Mabey D (2010) POCT for diagnosing infections in developing world. Clin Microbial Infect 16: 1062-1069.
22. Kei Numazaki (2012) A study on clinical application of rapid diagnostic reagent for measles. Eur Jr Gen Med 9: 39-44.
23. Lisa RV, Yetisen AK (2014) Commercialization of microfluidic devices. Trends Biotechnol 32: 347-350.
24. Paula J, Santrach MD. Mayo Clinic, Rochester, MN.
25. Hohmeir KC, McDonough SLK, Wang J (2017) Co-creation of market expansion in POCT in US: Industry leadership perspective on community pharmacy segment. Res Social Adm Pharm 13: 746-753.
26. Hohmeir KC, Loomis B, Gatwood J (2018) Consumer perception and willingness to pay for POCT services in community pharmacy. Res Social Adm Pharm 14: 360-366.