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Article

Can primary care in extreme point-of-care settings benefit from CD-based microfluidics?

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Abstract: This is the abstract section. The abstract should be one section and count less than 200 words.

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1. Introduction

In resource-limited settings, for example in parts of Africa and India, access to everyday commodities such as clean water and electricity is restricted. This makes day-to-day living in the developing world vastly different from the experiences of first world settings, and even more so when it comes to healthcare. In these resource-limited settings, exposure to extreme environmental conditions is commonplace, including high levels of humidity, heat and dust. Electricity is often intermittent or non-existent, compounding the harshness of the environment and posing significant challenges for equipment and data connectivity. In addition, lack of trained staff makes it difficult to provide a high standard of diagnostic testing and throughput of patients. Clinics are remotely situated and patients need

to travel, often over great distances, to seek medical assistance. Samples collected at the clinics need to be sent to a centralized laboratory with a waiting period to receive test results, and frequently patients do not return to the clinic for the diagnoses as a result of travel time and prohibitive costs.

In addition to the lack of doctors, nurses, and hospital beds, primary care is limited to those living in third world settings in rural parts of India and Africa. In India, for example, approximately 30 % of the population does not have access to primary care [1](PwC 2011). This lack of primary care, coupled with absence of health education, leads to the accumulation of disease burden in society; and patients present in advanced disease states, requiring expensive secondary and tertiary care. 39 million people fall below the poverty line in India alone every year as a result of health-related expenses [1](PwC 2011). Communicable diseases such as Tuberculosis (TB), Malaria and Hepatitis are prominent in under-resourced settings [2](Peeling and Mabey, 2010, etc.), and timely diagnoses of these are urgently needed. Providing comprehensive primary care in under-resourced settings is a critical global challenge, and it is clear that the realization of innovative, effective point-of-care diagnostic technologies in these extreme environments is the solution.

Significant humanitarian, social and economic benefits can be derived from such point-of-care technology initiatives. Delivery of primary care in extreme point-of-care conditions close to peoples' homes makes it possible for women and children to benefit from care; and this has important implications. For example, it is estimated that about 20 % - 40 % of maternal deaths in India occur as a result of anaemia; India contributes to about 50 % of global maternal deaths due to anaemia, and prevalence of anaemia among pregnant women in India is upwards of 40 % (Ministry of Women and Child Development, Government of India - wcd.nic.in). This could be easily corrected through iron-folic acid supplementation through a primary care centre. Iron deficiency anaemia is estimated to cause 591 000 perinatal deaths globally. The associated loss of healthy life years amounts to more than 19 million disability-adjusted life years (DALYs) from perinatal causes. It has been concluded from large meta-analysis studies that there is significant reduction in perinatal risk estimates, concurrent with maternal iron-folic acid supplementation [3](Stoltzfus et al. 2004). Such examples emphasize the importance of combining socio-economic aspects with technology to ensure that primary care is comprehensive in under-resourced settings. Providing effective healthcare forms part of global millennium development goals [REF], as well as National Development Plans for South Africa (REF NDP 2030 (SA)) and India (REF Millenium Development Goals (MDG) Goals 4 & 5).

Advances in the development of point-of-care diagnostics have accelerated in recent years [4](Gubala et al. 2012, ETC.), with initial success seen in first world settings such as hospitals and doctor's offices, where skills and the environment pose fewer constraints than in the developing world. Emphasis is now shifting towards the development of point-of-care solutions for the developing world [5](Drain et al. 2013, ETC.)- also known as extreme point-of-care solutions - to address the striking demand for effective healthcare solutions where the need and the impact is highest.

Extreme point-of-care tests are required to address many challenges, including power limitations, lack of skills and environmental conditions. New technologies also need to be developed for point-of-care tests, as conventional lab-based diagnostic technologies are often too expensive and complex to operate and would not be feasible options at the point-of-care. This highlights the need for point-of-care systems to be developed along with developmental pathways for these emerging technologies.

The World Health Organization (WHO) has set out the ASSURED criteria - Affordable, Sensitive, Specific, User friendly, Rapid and Robust, Equipment free and Deliverable to end users - to which point-of-care diagnostics should conform [6](Kettler et al. 2004). The ASSURED criteria are particularly relevant to extreme point-of-care settings, and have been examined with specific application to the developing world and under-resourced settings [2,5](Peeling and Mabey 2010 and Drain et al. 2014). The criteria have also been investigated for existing important tests such as CD4 counts for monitoring of HIV [7](Glynn et al. 2013) where commercial systems are evaluated according to their fit to the ASSURED principles. Glynn et al. also noted that an additional important requirement for emerging point-of-care technologies is their compatibility with concurrent emerging trends and technologies. The ASSURED principles provide an ideal to work towards, but practical limitations still exist, for example, equipment free solutions may require minimal instrumentation as a first step.

Even with the definition of the ASSURED criteria and the well-known need for POC solutions for under-resourced environments, particularly in parts of Africa and India, little commercial implementation has been achieved. The WHO and Gates Foundation have established various funding streams in support of research efforts to develop and deliver effective point-of-care diagnostics for under-resourced settings which have resulted in innovations such as the lab in a backpack [REF - Rice University], consisting of compact lab equipment including an oil immersion microscope, and a battery pack to last for up to 8 hours, for distribution in rural clinics.

In resource limited settings in South Africa, for example, a number of clinics have been set up and training provided to healthcare workers in an attempt to improve healthcare in under-resourced settings [8](Moleko et al. 2014). However, a number of challenges remain within the testing environments themselves in South Africa, and include specimen collection methods, lack of staff training, and inconsistent or absent quality assurance practices [9](Begg et al.). Point-of-care tests are also often perceived negatively by healthcare workers as being time-consuming technologies that require training and an increased workload [10](Blattner et al 2010). In addition, point-of-care tests are frequently not as reliable as conventional tests, compounding the negative perception of point-of-care testing [REFs for blood cell counts??]. These issues illustrate the importance of developing accurate point-of-care tests that are automated - with little user interaction or skill required - to overcome these barriers to widespread acceptance of point-of-care solutions for under-resourced settings. Adaptability to existing training, workflow and environmental restrictions in under-resourced settings need to be taken into account for point-of-care diagnostic systems to become an integrated part of technology-enabled primary care solutions.

Microfluidic systems have been applied to point-of-care diagnostics over the past few decades [11–16](Jung et al. 2015, Weaver et al. 2014, Gomez 2013 and Sia and Kricka 2008, Chin et al. 2007, Yager et al. 2006, ETC.) as they utilize small sample and reagent volumes implemented on disposable devices that can perform various controlled fluidic functions. More recently, there has been a drive towards the implementation of microfluidic systems for point-of-care diagnostics in resource-limited settings [17–19](Sharma et al. 2015, Lee et al. 2010, Mao and Huang, 2012, ETC.), but many challenges remain in producing viable commercial devices.

In this article, we focus on centrifugal microfluidic, or lab-on-a-disc systems. These systems make use of three pseudo forces present on a rotating platform, i.e. centrifugal, Coriolis and Euler forces to effectively propel and control fluids within disc-shaped devices. The centrifugal force, acting radially outward and proportional to the square of the angular velocity, is the primary force used to move fluid from the center to the edge of the disc, where the flow rate is dependent on fluidic properties such as density and viscosity, the angular velocity of the disc, channel geometry, and radial location of fluid on the disc. The average velocity of liquid on a disc is given by Equation 1:

$$\vec{\mathbf{U}} = \frac{D_b^2 \rho \vec{\boldsymbol{\omega}}^2 \bar{r} \Delta r}{32 \mu L} \tag{1}$$

where D_b is the hydraulic diameter of the channel, ρ is the liquid density, $\vec{\boldsymbol{\omega}}$ is the angular velocity, $\bar{r}$ is the average distance of the liquid from the center of the disc, Δr is the radial extent of the liquid, μ is the viscosity of the liquid, and L is the length of the liquid column in a channel or chamber on the disc.

This centrifugal pumping mechanism has been used to successfully pump a variety of liquids regardless of their physical and chemical properties in lab on a disc systems, demonstrating its effectiveness in biological applications where it is important to be able to handle a dynamic range of liquid types and volumes on the same disc [REF].

The Coriolis force, which is perpendicular to the velocity of a moving particle on the disc and directly proportional to both the mass and rotation speed of the disc, is frequently used for density based particle separation and sorting on the disc [REF]. Particles are sent in different path trajectories through an on disc chamber based on their differing masses allowing for effective separation of key biological components [REF].

The Euler force is present when there is a changing angular velocity with respect to time and acts perpendicular to the centrifugal force and opposite to the angular acceleration. In lab on disc systems, the primary function of the Euler force is in mixing to create lateral motion of the fluid during disc acceleration.

The combined pseudo forces, per unit volume, on a particle or liquid droplet on a disc, are shown in Equation 2:

$$\vec{\mathbf{F}_{\mathbf{tot}}} = \rho\vec{\boldsymbol{\omega}}(\vec{\boldsymbol{\omega}} \times \vec{\boldsymbol{r}}) - 2\rho\vec{\boldsymbol{\omega}} \times \frac{d\vec{\boldsymbol{r}}}{dt} - \rho\frac{d\vec{\boldsymbol{\omega}}}{dt} \times \vec{\boldsymbol{r}} \quad (2)$$

where ρ is the liquid density, $\vec{\boldsymbol{\omega}}$ is the angular velocity in rad/s, $\frac{d\vec{\boldsymbol{r}}}{dt}$ is the velocity vector of the particle moving on the disc, $\frac{d\vec{\boldsymbol{\omega}}}{dt}$ is the angular acceleration, and $\vec{\boldsymbol{r}}$ is the average distance of the liquid from the center of the disc. The first term represents the Centrifugal force, the second represents the Coriolis force, and the third represents the Euler force.

The use of these pseudo forces affords a wide range of control to the user in liquid manipulation with minimal outside hardware, making CD-based microfluidic systems well suited to point-of-care diagnostic applications [20,21] (Gorkin et al. 2010, Ducree et al. 2007, ETC.), and have the potential to address many of the challenges associated with delivering point-of-care diagnostics to extreme settings. Centrifugal microfluidic systems provide favorable point-of-care solutions as low cost integration of vast diagnostic functionality is achievable, as will be discussed in this paper. CD-based extreme point-of-care devices thus enable solutions towards effective primary care in under-resourced settings to be realized.

2. Advantages of centrifugal based systems for extreme point-of-care

In addition to the advantages of microfluidic systems in general for point-of-care applications, including small sample and reagent volumes, control of fluidic functionality, short diffusion distances, and compact, disposable devices, centrifugal systems provide some extra benefits suited to point-of-care applications. These include the simple and compact external instrumentation required - only a small rotating motor is required to perform a vast assortment of complex fluidic functionality, in contrast to bulky, expensive pumps or high voltage supplies that are often required to drive fluids in other microfluidic technologies. The disc format of centrifugal microfluidic devices lends itself to effective multiplexing of tests on one device as a result of radial symmetry, which also enables a high throughput of tests. Simple actuation principles are used for CD-based systems and thus clean, modular separation between the disposable disc and the drive or readout unit can be achieved [22](Burger et al. 2012).

Mixing can be accomplished in centrifugal microfluidic systems by exploiting Euler forces - acceleration and deceleration of the spinning disc - much simpler than mixing in traditional microfluidic systems where laminar flow dominates. Air bubbles are also not problematic as in the case of many other microfluidic systems, as they are pushed to the top of the microfluidic disc device. Robust, high performance liquid handling and use of unique artificial gravity conditions enable the integration of full bioanalytical process chains - from sample preparation through to assay implementation and detection - to be achievable on CD-based microfluidic systems. [DCU - please refine this paragraph, add references]

A number of excellent reviews on centrifugal microfluidic or lab-on-a-disc technologies provide insight into the mechanisms utilized in such microfluidic systems, highlighting the functional building blocks, integration and advantages of centrifugal microfluidics [23,24](Strohmeier et al. 2015, Zoval and Madou 2004) and with specific focus on biomedical applications [20,21] (Gorkin et al. 2010,

Ducree et al. 2007)[ADD REF HERE FOR Kong et al 2015].

In addition to the functional blocks of CD-based systems that are advantageous over existing microfluidic techniques for point-of-care applications, CD-based systems can emulate existing standard laboratory equipment. CDs are traditionally played using a Discman or a CD-ROM, and this surrounding CD infrastructure, including a servo-motor for spinning the disc, a laser and lens system for optical detection on the disc, and a tracking system which moves the laser to different radial distances along the disc, can be viewed as an all-in-one lab - an integrated system that can perform a number of standard laboratory procedures such as centrifugation, microscopy, vortexing, pumping (including reciprocating pumping), x,y-positioning system, etc. These ideas were first introduced 17 years ago by Madou, Kido and Kellog [ADD REFERENCES HERE]. Different laboratory equipment functions that can be realized by CD-based microfluidics and their surrounding infrastructure in one automated system are discussed in the following paragraphs and are summarized in Table 1. [UCI - please refine this paragraph, add references]. [ADD FIGURES HERE - CD-based MICROSCOPY ETC.]

[Please provide inputs on table: UCI, DCU, KIT, CSIR - add references] - suggestions on how to present this please. Also need to decide whether we will include paragraph descriptions too (as below the table) or combine everything into the table. Marc said Sasha should ADD PICTURES

CD microfluidics allows for automated centrifugation, and is thus particularly advantageous for application where separation and sedimentation of cells and particles are required [22,25](Burger et al. 2012a, Burger et al. 2012b). Large volume separation of plasma from whole blood was demonstrated by [26]Amasia et al. 2010, while more recently, capture of bacteria in v-shaped structures to detect urinary tract infections through optical counting of bacteria in a urine sample has been demonstrated [27](Schroder et al. 2015). Rare cell detection - magnets used to separate cells, incubation is integrated into the device, then dispensed into v cup array for counting of sorted rare cells [DCU - please refine and add references]. In other types of microfluidic systems, centrifugation is difficult to achieve, and includes methods such as gravity driven flow, which are often slow and not fully effective [CSIR - add references].

Valving for fluid handling can be implemented in many ways on a centrifugal microfluidic disc, and can either depend on the rotational speed of the disc (burst frequencies), or be implemented using more advanced methods including paraffin wax plugs [28](Abi-Samra et al. 2011a), and event-triggered valves using disposable films that are independent of spin speeds [29](Kinahan et al 2014). Assists with clock/timing of assay steps [DCU - please add to this, also references]. In other microfluidic systems, valving is often performed using external, commercial systems, adding complexity and size [CSIR - add references]. [Marc added: Distiction between active and passive valves comes here- also according to SamsungâĂŤwax valves stil problematic for reliability due to manufacturing problems]

Although unidirectional flow in centrifugal microfluidics is perceived as a limitation of these systems, many methods have been devised to overcome this issue, including pneumatic [ADD REFERENCE], thermopneumatic pumping [30](Abi-Samra et al. 2011b), electrochemical pumping [Noroozi et al ADD REFERENCE] and incorporation of paper on disc devices [31–33](Godino et al. 2012, Vereshchagina et al. 2012, Hwang et al. 2011). The latter hybrid paper-on-a-disc solutions combine capillary flow and

Table 1. Lab functionality of CD-based systems

Laboratory equipment/function	**Implementation method**	**Example applications**
Centrifuge	Motor for spinning disc driven at high RPMs [REFs]	Blood plasma separation [26]
Vortex		Rotaprep
Lysis machine	Magnets incorporated, glass beads rupture cells [REFs]	Lysis of bacteria [REFs]
Microscope	CD or DVD-player laser diode used as laser scanning microscope	Detection of biomolecules [ADD REF Ramachandraiah et al. 2013, KIDO AND ZOVAL]
x-y table/spotter	Rotational and linear positioning motors of CD-player used	
Sample concentration	Reciprocation pump - analyte flushed back and forth	
Low-cost consumables	Polycarbonate disc used	
Mixer	Spin protocol combined with ceramic mixing beads	Mixing of two different reagents
Cell counter	Laser in a CD drive detects errors on a CD where cells or biomolecules are located in channels	Counting of microparticles, labelled cells. [ADD REFERENCE Imaad et al. 2011]
Thermal Cycler	Peltier elements for heating and cooling of small chambers	DNA amplification via PCR [Amasia ADD REFERENCE]
Hypergravity simulation	Centrifugal force to simulate high G-forces on live samples trapped in fluidic chambers	C. elegans stress response cultivation platform [Kim ADD REFERENCE]

centrifugal forces to act in opposing directions, providing added fluidic handling capabilities.

Optical read-out or detection technologies for centrifugal microfluidics have been reviewed in detail by [34] King et al (2014). Detection techniques that have been implemented on CD-based microfluidics include PEDD - optical absoption measurement using 2 LEDs - one as a light source and one as a detector (reverse bias) - these have been integrated with Bluetooth technology on a 3D printed disc reader [DCU - please edit and add brief info and add references][Marc said: Make sure to add HoracioâĂŹs microscopy here. I actually think that Kido should be an author]

For extreme point of care applications one might want to avoid a bulky and expensive optical setup. Electrochemical detection methods, which are inexpensive, portable, and have a low equipment footprint, are favoured in this respect. A fluorescent sensing scheme on the CD also requires more expensive

optical grade polycarbonate discs, something that can be avoided when using an electrochemical detection scenario. Furthermore, the latest electrochemical sensors , such as amperometric sensors featuring redox amplification (see below-Figure), have sensitivities and very low limits of detection (LODs) comparable to optical detection schemes, making them an attractive option for application in future point of care systems. The advantages of electrochemical detection vs optical in point of care applications in general are summarized in Table II [Discuss Table II]. Amperometric detection has already been successfully integrated onto the LoD platform [was LoD introduced already in this paper] using a slip ring-and-brush setup [REF] or a low-noise slip ring with liquid mercury [really? REF?]. Electrochemistry has been used in centrifugal microfluidic systems for glucose sensing [1972 Beckman reference], to detect proteins in bodily fluid [REF], perform whole blood fractionation [REF], for pumping through electrolysis [] and for flow monitoring [REF]. Future schemes can integrate electrochemical sensors into total analysis discs for detection in common immunoassays such as ELISA and even as electrochemical DNA biosensors [REF].

[Electrochemical sensors can be classified as potentiometric, conductometric, and amperometric sensors. In potentiometric sensing, the potential difference between a working electrode and a reference electrode is measured with respect to the analyte concentration before any current flows. Conductometric sensors measure the conductivity of an electrolyte, which is directly proportional to the ion concentration in analyte. In amperometric techniques, analysis is performed by monitoring the current. In these techniques, a potential difference is established between the working electrode and the reference electrode. The applied potential drives a redox reaction which generates a current directly proportional to the concentration of the analyte to be detected. Instrument wise, electrochemical analysis experiments can be performed using a simple potentiostat. A reliable potentiostat has a wide electric potential range, high current resolution, high scan rate, and multiple working electrode inputs. A combination of low-cost miniaturized potentiostat2, inductive power transfer, and wireless data transfer3 is ideal for point of care applications.] TOO DETAILED, NOT NEEDED IN MY OPINION

REWORK!!! [This section is a start of explaining Table II but it needs to be better written] For example, miniaturization, essential for low real estate lab on disc systems, enhances electrochemical sensors, improving their lower limit of detection and sensitivity by increasing the signal to noise ratio through reduction of capacitive current.Furthermore, the use of the latest three-dimensional carbon microelectrodes developed by Kamath et al. not only has a wide stability window and low fabrication cost, but has further increased sensitivity by using a technique known as redox cycling, a recurring electrochemical reaction of a reversible redox couple between two adjacent microelectrodes. This technique significantly amplifies the current generated in the electrochemical cell up to 40 times and allows these electrochemical sensors to operate at sensitivities comparable to optical detection. [This only needs to introduce REDOX amplification with a Figure !!]

Table 2. Comparison of optical vs electrochemical detection techniques

	Optical Sensors	**Electrochemical sensors**
Instrument cost and size	Often expensive and bulky	Always inexpensive and compact
Sensor cost	Fair	Low
Optically transparent substrate	Required	Not required
Selectivity	Good	Fair
Limit of detection (LOD)	Very good	Good and very good (redox amplification
Response time	Long (up to tens of seconds)	Less than a second
Simplicity of the method	Often simple	Simple
Analysis of turbid solutions	Sometimes problematic	Not problematic
Electromagnetic interface	No	Yes
Resistance to radiation and corrosion	Yes	No
Cross-talk	No	Yes
Ambient light	Problematic	Not problematic
Response curve	Sigmoidal	Nernstian (potentiometric or linear)
Sensitivity enhancement	Complicated	Simply possible by miniaturization

Lab-on-a-disc set-ups can be designed in such a way that they encompass all components required for obtaining measurement results, including the necessary actuators, sensors and analytes. However, this capability comes at a high cost in the context of extreme point-of-care situations which are remotely situated. Since the lab-on-a-disc equipment is integrated into one device, it contains specialized components which can only be maintained and repaired by the manufacturer. The high cost of transporting a technician to remote settings to service such devices implies that this would only be a feasible solution if enough devices are deployed in a specific region. To address this issue, an idea would be to reduce the complexity of these lab-on-a-disc devices and manufacture them with integrated self-test and calibration routines. In addition, these systems would be built in a modular way that allows maintenance to be performed by trained locals, with low-cost replacement modules readily available.

To date, lab-on-a-disc applications use only passive elements on the spinning disc, while all interactions with the disc are performed using stationary sensors and actuators. This presents challenges, such as bridging the gap between the instruments and the disc, as well as managing small duty cycles as a result of the rotation of the disk, which leads to expensive, highly sensitive and powerful device requirements. With new advances in wireless power and signal transfer, steps to overcome these limitations have been made, allowing for power and data connectivity to be integrated into centrifugal microfluidic systems [35](HÃűfflin et al. 2015). Among other applications, this would enable the operation of electrochemical electrodes on a spinning disc.

In the context of remote point-of-care applications, low-cost and maintenance free continuous operation is of paramount importance, and the approach of incorporating power and a microcontroller onto the spinning disc assists in addressing these needs. The on board logic, sensing and actuation capabilities allow for smaller, encapsulated, and hence more reliable components to be realized. The availability of a microcomputer also facilitates the implementation of test and calibration routines, which could aid local staff with minimal training to carry out repairs. In addition, the necessity for high accuracy speed control of the spinning motor would decrease, since the actual centrifugal forces on the disk could be measured directly, and the propagation of the fluidic interface could be used as a control trigger signal directly. To reduce cost and training efforts the system could be built using standard elements such as an Arduino microcontroller, wireless inductive electrical power - Qi - for energy transfer, and Bluetooth for data transfer. With these elements incorporated, Table 3 shows the energy budget obtainable, providing more than 3500 mW for the application.

Table 3. NEED A CAPTION and explanation of table here - KIT to fill in

Component	**Energy [mW]**	**Component**	**Energy [mW]**
Qi transfer module	5000	Bluetooth and SD card	-200
Stabilized power on disc	4000	Arduino microcontroller	-190

Digital - centrifugal microfluidic 'transistor' and logical conditions: AND, OR all at a single spin rate [DCU - please add brief info and edit and add references]

Requirements of microsystems for low-income point-of-care were discussed at length by [36]Chin et al. 2013 and include the ability of these systems to utilize materials of a very low cost while maintaining robust functionality to cope with harsh handling, storage and transportation conditions in temperature ranges of 4 - 40 °C. All fluidic functionality should be automated and detection should be low-cost, portable and part of a self-contained system. CD-based microfluidic systems have the potential to conform to these requirements, with many aspects addressed in more detail in the sections to follow.

Advances and challenges of centrifugal systems, with emphasis on commercialization aspects, have been discussed extensively [37](Siegrist et al. 2010). System integration of microsystem technologies was investigated by [38]Sin et al. 2011 and shows many advantages of centrifugal microfluidic systems , also when compared to other microfluidic technologies. Centrifugal microfluidic systems are also favourably positioned for mass production and commercial roll-out, as they can make use of existing equipment (laboratory centrifuges, etc.), meaning that the instrumentation required is accessible and widely accepted [39](Mark et al. 2012), making for more efficient fall-in with existing technologies and mindsets in the healthcare industry.

3. Examples of centrifugal systems for point-of-care

Centrifugal microfluidic systems were first introduced in the 1960's? [CSIR - reference]. Research-based centrifugal microfluidic platforms have been established at many universities and

institutes around the world [CSIR - add references], and more recently in developing world settings, for example in South Africa [40](Hugo et al. 2014). A number of research and development based centrifugal microfluidic systems have showcased integrated systems towards point-of-care diagnostic applications, and includes a fully integrated system for analysis of biochemistry and immunoassays using whole blood [41](Lee et al. 2011). Recently, a fully automated bacterial pathogen detection using PCR and DNA extraction on a disc was presented [42](Czilwik et al. 2015).

Nwankire et al. 2014 [43] describe a portable centrifugal system for liver function testing that was deployed successfully in a lab environment in Nigeria, showing the potential of centrifugal microfluidic systems to perform well in under-resourced clinical settings. The company Abaxis started its centrifugal-based blood chemistry analysis system development more than 20 years ago [44](Schembri et al. 1995) and is one of the few commercial centrifugal-based systems available today. The Abaxis Picollo Xpress blood chemistry analyzer has recently been used to support testing of Ebola [45,46](Hill et al. 2014 and Owen et al. 2015). Sharma et al. 2015 [17] also explores this system as a point-of-care diagnostic suited to low-resourced settings. Other commercial centrifugal systems for diagnostics include the Samsung LABGEO, which is similar to the Picollo Xpress for blood chemistry gas analysis. GenePOC is in the process of developing products and POC Medical are working on a product for breast cancer detection. The potential for centrifugal microfluidic systems to provide point-of-care diagnostic solutions for extreme environments and specific life-threatening diseases has been demonstrated [17](Sharma et al. 2015). LaMotte WaterLink Spin is a commercially successful CD-based system for swimming pool water analysis.

4. Approaches to implementing extreme point-of-care on centrifugal platforms

The advantages of having a comprehensive primary care solution that leverages advanced technology are manifold. In addition to diagnosing and treating infectious diseases, effective management of non-communicable diseases (NCDs), such as Diabetes, Hypertension, Arthritis, etc. is also achievable. This has a far-reaching impact on DALYs saved, improvement in major health indices (eg. MMR, IMR, Life Expectancy), societal productivity, reduced expenses through judicious use of drugs (due to better diagnosis), and most importantly, reduction of healthcare-induced poverty.

The thinking is to include a 'case study' where Satadal could mention things that can be treated at the POC through proper education and management and then discuss the CD technology that could be implemented to support it - (need to combine management with diagnostics to be successful and effective)

To explore this potential impact, the magnitude of mortality of children under 5 years in India can be considered. Three causes accounted for 78 % (0.79 M/1.01 M) of all neonatal deaths in India: 1) prematurity and low birthweight (0.33 M; 99 % CI 0.31-0.35 M), 2) neonatal infections (0.27 M; 99 %CI 0.25-0.29 M) and 3) birth asphyxia and birth trauma (0.19 M; 99 % CI 0.18-0.21 M). Two causes accounted for 50 % (0.67 M/1.34 M) of all deaths at ages 1-59 months: 1) pneumonia (0.37 M; 99 %CI 0.35-0.39 M) and 2) diarrhoeal diseases (0.30 M; 99 %CI 0.28-0.32 M). (www.ncbi.nlm.nih.gov

Table 4. Panel of tests required for effective POC diagnosis in extreme settings

Critical:
Complete blood count
Blood group (ABO and Rhesus)
Blood sugar (F and PP), Urea, Creatinine, Uric Acid
Serum Sodium, Potassium
Pregnancy test
Liver Function Test - Bilirubin, Liver enzymes (SGOT, SGPT, SAKP)
HbA1C
Thyroid Function Test (T3, T4, TSH)
Urine - routine and microscopic, culture
Stool - Occult blood and microscopic for cysts, etc., culture
Widal Test (Typhoid Fever)
HbsAG
HIV
Essential:
Lipid profile
Serum Calcium
Serum Vit D3
Serum Albumin, Globulin
Troponin - T
Urine Microalbuminuria
TB PCR
Malaria - Antigen/Antibody/Parasite Detection
Dengue
Coagulation profile (PT, APTT)
Desirable:
Rheumatoid Factor, Anti-nuclear factor, HLA B27
Fevel panel - Pyrexia of unknown origin - Viral/Bacterial/Parasitic - how to identify
Serum Iron, Vitamin B12

- Satadal, is there a specific reference from the website that we could use please?). Prematurity and low birth weight issues can be addressed by correction of maternal anaemia; neonatal infections, birth asphyxia and trauma can be reduced through health education regarding institutional delivery; pneumonia and diarrohoeal diseases can be diagnosed and treated at primary care centres equipped with appropriate POC diagnostics. The cascade effect is dramatic. Primary care together with POC diagnostic technologies will have a multiplier effect on the population and society as a whole.

For many of the important diagnostic tests as listed in Table 4, effective point-of-care solutions can potentially be realized by CD-based mircofluidic technologies, hand-in-hand with primary care. As an initial panel of tests, a complete blood count, TB, HIV, Malaria, etc. can be considered.

As part of a complete blood count, haemoglobin measurement is an important parameter. Centrifugal microfluidic systems have been implemented for automated haemoglobin measurement using low-cost optical techniques [47](Stiegert et al 2006), etc

Other tests that make up a valuable panel should then be described here, and for each it should be justified and how a CD-based solution could contribute and what is still missing define a few tests to be used as a case study - Satadal

Possible ideas that could be included to address the panel of tests (to be decided once Satadal gives inputs):

- Biomimetic systems - cocoons for environmental regulation, 'weather station', etc [UCI]
- Sensing - electrochemical, optical - how each can be optimized for extreme POC - pros and cons [UCI, DCU]
- Reagent storage, lyophilizing of reagent [UCI]
- Advanced flow control - advanced valving, dissolvable films, multiphase valving, etc. [DCU]
- Addressing issue of power in rural settings - intermittent, non-existent - battery technologies, solar power, hand-operated centrifuges [KIT] - a normal car battery and maybe a solar charger should be enough
- Coupling power to a CD - energy harvesting - spinning disc itself could provide power [KIT] - hard to get sufficient energy and Qi is cheap, reliable and powerful
- Reduction of RPMs needed (for power reduction) by using latex balloons. Elastic chamber roof instead of rigid polymer means that a lower RPM can be used for all tests [UCI]

5. Summary and conclusion

Main text paragraph.

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Author Contributions

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Conflicts of Interest

Required. State any potential conflicts of interest here or "The authors declare no conflict of interest".

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