#927

Diagnosis at the Point of Care with a Smartphone Dongle

Guo TW[1], Laksanasopin T[1], Nayak S[1], Sridhara AA[1], Xie S[1], Olowookere OO[1], Cadinu P[1], Meng F[1], Chee NH[1], Kim J[1], Chin CD[1], Munyazesa E[2], Mugwaneza P [3], Rai AJ[4], Mugisha V[2], Castro AR[5], Steinmiller D[6], Linder V[6], Justman JE[7], Nsanzimana S[3],Sia SK[1].
[1]Department of Biomedical Engineering, Columbia University; [2]ICAP–Rwanda, Mailman School of Public Health, Kigali, Rwanda; [3]Institute of HIV Disease Prevention and Control, Rwanda Biomedical Center, Kigali, Rwanda; [4]Department of Pathology and Cell Biology, Columbia University, New York, NY; [5]Centers for Disease Control and Prevention, Atlanta, GA; [6]OPKO Diagnostics, LLC, Woburn, MA; [7]ICAP, Mailman School of Public Health, Columbia University. New York, NY

Smartphones are rapidly being adopted worldwide and enable fast computing, friendly user interface, and connectivity, all within arms reach. We have leveraged these aspects to develop a smartphone accessory that can runa full laboratory-quality immunoassay. This low-cost dongle replicates all mechanical, optical, and electronic functions of lab-based enzyme-linked immunosorbent assay (ELISA) without requiring any stored energy; all necessary power is drawn from a smartphone. Testing in the field can exhibit markedly different performance from tests run in a laboratory. After a 30 minute training, Rwandan health care workers used the dongle to test whole blood obtained via fingerprick from 96 patients enrolling into care at prevention of mother-to-child-transmission clinics or voluntary counselling and testing centers. The dongle performed a triplexed immunoassay not currently available in a single test format: HIV antibody, treponemal-specific antibody for syphilis, and non-treponemal antibody for active syphilis infection. For the three markers, the dongle showed sensitivity of 92-100% and specificity of 79-92% when compared with the gold standard of lab-based HIV ELISA and rapid plasma reagin (a screening test for syphilis). Importantly, patient preference for the dongle was 97%compared to lab-based tests, with most pointing to the convenience of obtaining quick results with a single fingerprick. Simplifying laboratory equipment with the use of smartphone technology has the potential to broaden access to critical diagnostic information.

#930

Consistently Ultra-selective and Continuously Tunable Nucleic Acid Hybridization Technologies

Wang JS[1,2,3], Wu LR[1], Zhang DY[1,2,3].[1]Department of Bioengineering, Rice University, Houston, TX; [2]Systems, Synthetic, and Physical Biology, Rice University, Houston, TX; [3]Searna Technologies, Inc., Houston, TX

Hybridization of complementary sequences forms the bedrock of nucleic acid assays and technologies, such as primers binding to templates. However, Watson-Crick base pairing even at optimized temperatures typically only yields a 5- to 10-fold difference between single nucleotide variants. The unintended binding of closely related sequences limits the accuracy of nucleic acids detection or sequencing; improved hybridization selectivity bolsters the assay accuracy and reliability, informing better scientific interpretation and clinical guidance.

Our research group specializes in using nucleic acid molecular competitions to suppress unintended interactions. Key characteristics of our hybridization-based technologies are: 1) high hybridization selectivity against single-nucleotide variants (median 900-fold affinity difference); 2) robust performance across temperatures (15 – 67 ºC), buffers (0.15 – 1.5 M Na+), and denaturants (0% – 30% formamide); 3) finely tunable tradeoff between sensitivity/yield and specificity/selectivity.

As one example, we developed ultra-selective fluorescent probes capable of detecting 0.1% variant allele frequency in 44 different pairs of cancer-related mutant and wildtype sequences drawn from COSMIC. We have applied our technology to human genomic DNA samples in the form of PCR primers as well; these primers achieve 20 cycle ΔCq in discriminating single nucleotide variants. Finally, we have developed on-the-fly method of tuning probe affinity and selectivity via the stoichiometry of auxiliary species, and enforced uniform capture efficiency of 31 DNA molecules with G/C content ranging between 0% and 100%.

Our hybridization technologies, being robust to conditions and not dependent on enzymes, may be easily incorporated in many nucleic acid assay platforms. We envision that our technologies have direct applications in detecting circulating (cell-free) nucleic acids, sequence-specific enrichment for targeted sequencing, and sequencing validation.

#932

Electrochemical Platform for Controlled Microscale pH Generation On Demand

Fomina N, Ahmad H, Johnson C, Maruniak A, Kavusi S, Lang C. Robert Bosch Research and Technology Center, Palo Alto, CA

Active pH control of solution in contact with an electrode surface has potential applications in modulating antibody-antigen interactions, DNA hybridization, controlling enzymatic processes, studies of protein aggregation/misfolding, cell manipulation, electrostatic assembly of molecules on charged surfaces, as a means for accelerating or inhibiting chemical reactions with high spatial and temporal resolution, or in any other process involving pH as a variable.

We describe an electrochemical platform that enables precise localized control over pH at an electrode surface. We utilize a benign, quinone-based additive in conjunction with current shaping to regulate pH across a broad range, with tight spatio-temporal control and with unprecedented precision. We demonstrate reversible pH switching at a rate of 0.1 pH/s in the range between 4.5 and 7.5 in buffered solutions. We show that the electroactive molecules are compatible with biological systems, such as proteins and enzymes. To that end we demonstrate an enzymatic assay where the activity of the enzyme is regulated via pH modulation.

Our platform is comprised of a standard lab glass slide (1”x3”) which is patterned with an array of Indium Tin Oxide (ITO) electrodes. ITO is the most commonly used transparent conducting oxide material, and is advantageous in that it provides reasonable mobilities while preserving the optical transparency of the underlying glass substrate; it is also amenable to many of the same chemical treatments commonly applied to standard glass (e.g., silane modification). This allows the platform to function as a drop-in replacement for existing protein and cellular assays that employ optical readout. For protein assays, we use a polyethylene glycol (PEG) hydrogel with reactive N-Hydroxysuccinimdyl ester (NHS) groups, to first enable protein immobilization and subsequently minimize non-specific fouling.

pH modulation is effected through an electroactive additive to the buffer which incorporates chemically-reversible proton release/uptake upon electrochemical stimulation. This is preferable to the commonly-employed water electrolysis approach, as the high voltage required for the latter can degrade ITO electrodes, reduce the integrity of surface-bound layers, and initiate undesirable side-reactions with protein/DNA analytes.

The ability to dynamically change pH of a standard biological solution without the need for additional steps or equipment will significantly expand the capabilities of many diagnostic tools, as well as open new avenues in basic biochemical research.

#933

Reducing Turnaround Time of Centrifugation Using a Processing Centrifuge.

Cobert ML, Smith BP, Ludington KT, Fritchie PP, Osikowicz GW, Petteway LD. Abbott Laboratories, Dallas, TX.

Background: Laboratory turnaround time is critical in making clinical decisions. Centrifugation time has become a bottleneck in the laboratory due to the batching of samples. A new device, being developed by Abbott, has the ability to continuously process samples without the batching process. This device significantly reduces sample turnaround time.

Methods: Batch centrifuge (BC) data was gathered from on market devices using internal device monitoring systems. Data points included rack insertion (RI) time, input-output module (IOM) input time, centrifuge module (CM) input and output time, and IOM output time. In order to simulate the continuous processing centrifuge (CPC), RI time and IOM input time were utilized in addition to the CPC centrifuge capacity (24 tubes), cycle time (12 seconds), and spin time (180 seconds). These values are derived from design requirements and working prototypes. Customer sites were evaluated based on centrifugation volume (tubes/day), number of CMs, and sample spin time. In addition to all centrifuged tubes, the subset of STAT tubes was evaluated for both systems to determine turnaround time differences.

Results: Six sites were evaluated in the simulation, each site demonstrated results similar to the figure below. The figure displays the number of tubes processed during a given time point. Using the figure below, a majority of the tubes are processed (from rack insertion to centrifuge output) by the CPC after 7.3 ± 3.8 minutes, whereas the BC saw a majority of tubes processed after 15.3 ± 5.3 minutes. This represents a statistically significant difference ($p < 0.0001$). Overall, the difference in TAT between the CPC (12.5 ± 6.0 minutes) and BC (30.6 ± 8.5 minutes) for all sites was significant ($p < 0.0001$).

Conclusions: This simulation demonstrates that the turnaround time for the CPC is significantly greater than the current on market BC. The overall impact this has on total sample turnaround time, from sample draw to reported result, warrants further evaluation. The CPC is a promising technology poised to greatly reduce the ‘laboratory bottleneck’ and allow for faster clinical results.

#934

Rapid Phenotypic Methods for Diagnosing Infections and Antibiotic Susceptibility Testing

Sauer-Budge AF. Fraunhofer Center for Manufacturing Innovation, Brookline, MA

Traditionally, bacterial pathogens have been identified using culture-based methods that can take several days to obtain results. This can lead to physicians making treatment decisions based on an incomplete diagnosis. To decrease diagnosis time, we are developing novel devices and methods for isolating, concentrating, and detecting dilute pathogens and coupling these with novel downstream detection modalities. We have developed rapid methods to isolate bacterial pathogens from blood and urine, and then coupled that with rapid downstream ID (portable PCR, Surface enhanced Raman spectroscopy) and our own novel rapid antibiotic susceptibility method (shear stress enhanced microfluidic platform). The key to the development of truly rapid diagnostic tests is to obviate the need for growth steps. Our sample preparation and microfluidic-based methods are addressing this goal. We describe here integrated approaches and findings with clinical samples.

#936

Developing Point of Care Tests for Cardiovascular Diseases, Sepsis, and Pregnancy

Van Groll EJ[1], Hoo RS[1], Sidharta K[2], Sanz L[1], Fawver J[1], Meade TJ[2], Bao YP[1]. [1]Ohmx Corporation, Evanston, I; [2]Northwestern University, Evanston, IL.

With increasing demand for timely treatment decisions, the ability to provide accurate diagnosis near patients is highly desirable. Ohmx is developing a comprehensive platform aimed to provide rapid and accurate diagnostic tests at the Point of Care (POC).Using our proprietary bio-electrochemical detection technology, different types of target analytes, including proteins, small molecules, and nucleic acids, can be detected and quantified on a single platform. Cyclic voltammetry techniques produce a self-calibrating signal allowing for a rapid, fully quantitative determination of dose response over a broad, 3 log range of analyte concentration. Here we present further improvement to a number of assays for POC tests including high sensitivity troponin I/T (cTnI/T) assays for risk stratification of acute myocardial infarction (AMI), and two protein assays, procalcitonin (PCT) and C-reactive proteins (hsCRP/CRP), for monitoring sepsis. We also present a human chorionic gonadotropin (hCG) assay for confirmation of pregnancy. Given the fact that a quantitative hCG blood test may be ordered in an emergency setting to help a doctor make an informed decision on what tests or treatments to offer, an effort was made to develop such a test with our POC platform.

An alpha instrument has been developed for automated sample processing, with which small molecule or protein analytes are converted into a common mediator that is able to trigger changes in our proprietary electro-active molecules. Change of electrochemical signals in the absence or presence of the common mediator, which is proportional to target analyte concentration, is measured. Superior sensitivity and accuracy was demonstrated for the cTnI assay with LOD of 0.8 ng/L and 6.3% CV at the 99th percentile (10 ng/L). The assay was further validated with clinical samples, showing a good correlation with many predicate devices. The range of the CRP test is ~ 0.3-200 mg/L, achieving “one test for two” by spanning both normal and high sensitivity ranges. LOD for the PCT assay is 15 pg/mL. Good analytical performances of the hCG test were demonstrated with LOD of ~ 2 IU/L, dynamic range of 5-400 IU/L, precision of <10% in CV, and accuracy of 113-119% in recovery. Taken together, we have further confirmed that our assay platform is versatile and suitable for near patient care.

#937

Integrating Polymer Microfabrication Techniques to Enable Complete Lab-on-a-Chip Functions

Ehrlich S, Podczerviensky J, Begolo S, Levine M. ALine, Inc., Rancho Dominguez, CA

Integrated microfluidic devices allow for precise fluid handling in the microliter and sub-microliter scale, enabling the implementation and automation of clinical laboratory protocols.

In this work, we report on the reproducible performance of a set of microfluidic components for metering, mixing, pumping, venting, and dispensing using a pneumatic instrument, the
ADEPT™ with a defined set of pneumatic inputs/outputs.

The performance of each microfluidic component separately and then integrated into a demonstration device, the M2D2 is shown. Through choice of materials and design constraints, we provide data on the repeatable performance of a device that meters, mixes, debubbles and dispenses (M2D2) with instrumented control using the ADEPT.

The microfluidic devices were produced using laser cutting and lamination technologies, with processes that are suitable for both prototyping and large scale manufacturing. Interface between the ADEPT and the devices can be achieved by using commercial connectors or by integrating custom 3D printed connectors into the laminated devices.

#938

Strain Specific Identification of Bacteremic Organisms in Whole Blood: From Sample to Answer within 7 hrs

Boardman AK[1], Wong W[2], Premasiri WR[3], Ziegler LD[3], Lee JC[4], Klapperich CM[2], Sharon A[1,5], Sauer-Budge AF[1,2]. [1]Fraunhofer USA - CMI, Brookline, MA,[2]Department of Biomedical Engineering, Boston University, Boston, MA,[3]Department of Chemistry and The Photonics Center, Boston University, Boston, MA,[4]Brigham and Women's Hospital, Harvard Medical School, Boston, MA,[5]Department of Mechanical Engineering, Boston University, Boston, MA

Traditionally, pathogens in the blood have been identified using culture-based methods that can take 24-48 hours to obtain results. This can lead to physicians making treatment decisions based on an incomplete diagnosis, which can increase the patient's mortality risk. To decrease time to diagnosis, we developed a novel technology that is able to identify microorganisms at a strain-specific level directly from whole blood in less than 7 hrs, which fits within a typical work shift. This technology combines two main components: a sample preparation process using a bacterial concentrator and a detection method using surface-enhanced Raman spectroscopy (SERS). The sample preparation and concentration step enriches viable microorganisms from 10 mL of whole blood into a 200-µL aliquot. After a short incubation period, strain-level identification of the microorganisms is attained using SERS.

This process was tested using a model of pooled human blood that was inoculated with bacteria. When using the bacterial concentrator independently, we achieved a lower limit of detection better than 1 CFU/mL for the inoculated *S. aureus* population in 10 mL of whole blood. By treating SERS as an independent recognition instrument, we correctly identified 17 different species important for bacteremia. When testing the technology as an end-to-end process (from sample preparation using the bacterial concentrator to identification using SERS analysis), we obtained sensitivities and specificities greater than 88% for *E. coli* and *S. aureus* at concentrations ranging from 10^1-10^4 CFU/mL. Of the inoculated samples, 97% were correctly identified as either *E. coli* or *S. aureus*. Using this novel technology, we hope to revolutionize clinical microbiology by providing a rapid, sensitive, and specific diagnostic for identifying the presence of bacteria in blood.

#939

Analytical Methods for Detecting *Mycobacterium tuberculosis* DNA on Oral Swabs

Lohmiller J[1], Nguyen F[1], Weigel K[1], Cangelosi G[1]. [1]University of Washington, Seattle, WA.

Pulmonary tuberculosis (TB) is typically diagnosed by analysis of sputum samples. Sputum is a reliable specimen for TB diagnosis, but it has limitations. Requiring ill patients to cough up sputum can put health care workers at risk. The viscosity of sputum makes it difficult to work with. There are also patients, such as young children, who have difficulty producing sputum on demand.

Oral swabs could provide a quick, simple, non-invasive alternative to sputum sampling. In a pilot study, swabs were collected from 20 adult TB cases confirmed by Cepheid's GeneXpert (PCR) analysis of sputum. Eighteen of these subjects yielded swabs that were positive for *Mycobacterium tuberculosis*(MTB) DNA in a manual quantitative PCR (qPCR) analysis. Healthy control samples were 100% negative.

In order to improve the sensitivity and/or ease of use of oral swab analysis, the current study evaluated alternatives to the fully manual qPCR method used in the pilot study. Swabs were "spiked" with cultured MTB cells. Three systems were compared for their abilities to detect MTB and ease of use: Qiagen's DNA Mini Kit followed by manual qPCR, Claremont BioSolution's Purelyse followed by manual qPCR, and Cepheid's automated GeneXpert.

Qiagen's DNA Mini Kit kit is the fully manual DNA extraction used in the pilot study. Changes to the precipitation and elution steps of the protocol improved analytical sensitivity, resulting in stronger signals (2- to 4-Ct values) with an estimated limit of detection below 100 cfu per spiked swab. The fully automated Cepheid GeneXpert system was readily adaptable to swab analysis, yielding analytical sensitivity approaching or equaling that of the optimized Qiagen protocol. The PureLyse kit is battery powered and is designed for use as a point-of-care device. The Whatman OmniSwabs used in the pilot study produced poor results with the PureLyse kit, however an alternative swab brand (Puritan PurFlock) worked well with this system, delivering sensitivities comparable to those of the Qiagen and Cepheid systems.

Overall, the results show that *M. tuberculosis* can be detected on laboratory-spiked oral swabs using a variety of popular molecular analytical platforms. Non-invasive oral swab analysis has the potential to transform TB care and control.

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#940

Diazyme Vitamin B12 Assay: a Novel Vitamin B12 Assay for General Chemistry Analyzers

Saida FB, Chen X, Tran K, Dou C, Yuan CS. Diazyme Laboratories, Poway, CA.

BACKGROUND

Assays that measure vitamin B12 require very low detection limits and must include dissociation steps. Vitamin B12 assays currently available on the market are restricted to enzyme-immunosorbent, fluorescence and chemiluminescence methods. These assays cannot be adapted to colorimetric general chemistry analyzers.

METHOD

Diazyme Vitamin B12 Assay is the first colorimetric vitamin B12 assay for general chemistry analyzers. The assay is based on the principle of an improved version of the CEDIA® (technology (iCEDIA) in which α-complementation of the enzyme β-galactosidase and competition between an enzyme donor-vitamin B12 conjugate and vitamin B12 in a serum sample for an Intrinsic Factor take place.

Specimens are first diluted on-board then subjected to the addition of reagents R1, R2 and R3. Upon competition, a β-galactosidase signal is developed. A nitro-phenyl-β-galactoside derivative is used as the enzyme substrate. Its absorbance is proportional to the concentration of vitamin B12 in a particular specimen.

RESULTS

The performance of the Diazyme Vitamin B12 Assay has been evaluated on Roche's Modular P chemistry analyzer.

Precision: Simple precision were carried out in 20 replicates at three different levels of vitamin B12: 201.3 pg/mL, 386.9 pg/mL and 680.2pg/mL. %CV were 8.7%, 5.3% and 4.6%respectively. Sensitivity: The limit of quantitation (LOQ) of the Diazyme vitamin B12 assay was estimated at 75 pg/mL. Accuracy: The performance of the Diazyme assay was compared to that of legally marketed vitamin B12 enzyme immunoassays. 45 serum samples were used in the study. Linear regression of the data yielded a slope of 0.9019, a y intercept of 60.261 pg/mL and an $R^2 = 0.9423$. Linearity: Eleven levels of linearity were prepared by diluting a high serum sample (2200 pg/mL) with vitamin B12-depleted serum. Measurements were done in triplicates. Linear regression of the obtained data produced a slope of 1.0498, a y intercept of +5.9114 pg/mL and an $R^2 = 0.9937$.

CONCLUSION

The Diazyme Vitamin B12 Assay is the first colorimetric assay for vitamin B12 designed to run on general chemistry analyzers. The Diazyme assay aims at making vitamin B12 testing accessible to most clinical diagnostic laboratories. The Diazyme Vitamin B12 Assay can be adapted to general chemistry analyzers such as Modular P, Horiba's Pentra 400 and Alfa-Wasserman's ACE/Alera and some other analyzers capable of handling three reagents.

#942

Diazyme CUBE PCT Test for Diagnosis of Sepsis in Critical Care Environments

McBurney-Lin J[1],Gong XM[1], Dou C[1], Datta A[1] and Yuan CS[1]. [1]Diazyme Laboratories, Poway, CA.

Background: Procalcitonin (PCT) is one of the most effective and specific biomarkers for diagnosis of bacterial infections, sepsis, and septic shock. In healthy subjects, the PCT levels in circulation are<0.05 ng/mL.PCT levels of >0.50 ng/mL generally indicate a systemic bacterial infection, and levels of >10.0 ng/mL are a very strong indicator of septic shock. We recently developed a CUBE PCT test that can be used in critical care environments.

Methods: The CUBE uses a single cartridge containing two reagents separated into the cap and cuvette. The PCT assay is a latex enhanced immunoturbidimetric format measuring concentration of the PCT via agglutination of latex particles. Each test takes less than 10 minutes, and is completely automated via a pre-programmed RFID card.

Results:

Precision - Three samples containing 0.65 ng/mL, 0.90 ng/mL, and 9.00 ng/mL PCT were tested in 12 replicates, and found to have %CVs of < 8%, <7%, and <4.5% respectively.

Sensitivity - The limit of blank, detection, and quantitation was determined to be 0.07 ng/mL, 0.12 ng/mL, and 0.17 ng/mL respectively.

Linearity: Linearity was evaluated by testing eleven levels prepared by diluting a high sample containing 11.00 ng/mL of PCT with a PCT depleted matrix in triplicates. The linear regression results are as follows: R^2=0.999, slope=1.01.

Accuracy: The accuracy was tested with 40 individual serum samples and run in parallel with VIDAS® BRAHMS PCT. The linear regression results are as follows: R^2 = 0.9888, slope = 1.004, intercept = 0.012.

Conclusion: The Diazyme CUBE PCT is an effective and accurate device for diagnosing sepsis in critical care environments with capability of integrating the test results into modern eHealth systems.

#943

A Rapid, Fully Automated Sample Preparation Method of *Clostridium difficile* in Stool Using the SimplePrep™ X8 Instrument

FergusonTM[1], Chan E[1], Yale K[1], Cantera J[2], White H[2], Kumar P[3], Nguyen VB[1], Brown M[1], Herrington K[1], Talbot R[1], Boyle D[2], Niemz A[3], Doebler R[1]. [1]Claremont BioSolutions LLC, Upland, CA, [2]Program for Appropriate Technology in Health, Seattle, WA, and [3]Keck Graduate Institute of Applied Life Sciences, Claremont, CA.

Clostridium difficile (*C.diff*) is the leading cause of healthcare-associated infections (HAIs) in the United States, with an estimated 0.5 million infections in 2011 and 29,000 associated deaths within the first 30 days of initial diagnosis[1]. With the rapidly growing nucleic acid amplification testing (NAAT) market, particularly in clinical and hospital settings, rapid diagnosis of *C.diff* infections is possible. Current NAAT-based *C.diff* systems (i.e., Cepheid XPert® C.difficile/Epi and Meridian BioSciences' Illumigene® *C.diff)* require expensive and large instrumentation and/or multiple cumbersome manual stool sample preparation steps and are therefore less amenable to point-of-care testing (POCT). The complete automation of NAAT-based systems continues to be hindered by ineffective nucleic acid sample preparation due to complexities relating to sample matrices (i.e. consistency, inhibitors, background human genomic DNA) and fluidics integration. The primary objective of this study is to demonstrate fully automated sample preparation (up to 8 samples at once), including nucleic acid extraction, of *C.diff* in stool in approximately 6 minutes using Claremont BioSolution's (CBio's) SimplePrep™X8 instrument. The SimplePrep™ disposable cartridge incorporates CBio's core OmniLyse® and PureLyse® technologies for mechanical lysis and nucleic acid extraction of pathogens, particularly hard-to-lyse organisms, in the absence of inhibitory reagents (i.e., chaotropes, alcohols) or extra upfront manual steps. A mechanism for removing inhibitors introduced by the sample (i.e. stool, sputum, soil, blood) is also incorporated into the cartridge.

Using the SimplePrep™ instrument, we have successfully extracted *C.diff* DNA from spiked *C.diff* negative human stool ($2x10^3$-$2x10^5$ cfu/mL) and from blinded clinically positive human stool specimens, in approximately 6 minutes. Amplification and detection of extracted *C.diff* gDNA was conferred using an in-house qPCR assay of the toxin B (*tcdB)* gene, as well as with internally developed real-time isothermal Cross Priming Amplification (CPA) assays, with preliminary data suggesting CPA is more sensitive and more robust against inhibitors than the qPCR assay. Our real-time CPA assays utilize novel strand displacing DNA polymerases and molecular beacons (CPA-MB) to accurately detect the wildtype *C.diff tcdB* gene and hypervirulent *C.diff* strains, which are increasingly emerging as the cause of *C.diff*-related HAI outbreaks with more severe disease outcomes. Amplification and detection of the extract by the CPA-MB assays occur within 40 minutes at 60^OC and can be performed in real-time PCR machines or much lower cost isothermal real-time amplification and detection systems, proving to be more beneficial in POCT systems.

The SimplePrep™X8 instrument offers great utility as a standalone sample preparation instrument or within a NAAT-based integrated platform. We are currently moving toward the integration of *C.diff* DNA extraction via SimplePrepX8 in conjunction with our real-time CPA-MB *C.diff* wildtype and hypervirulent assays, with minimal user involvement.

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#944

Towards Integrated Nucleic Acid Testing for Dengue Diagnosis in Peripheral Settings

Daniel Pak[1], Hsiang Wei Lu[1], Leomar Patam[1], Pranav Kumar[1], Brianna Watson[1], Jordan Jeorge[1], Kevin Leung[1], Arthur Young[1], Diana Chao[1], Tanya M. Ferguson[2], Mark Brown[2], Robert Unger[1], Morgan Pike[1], Angelika Niemz[1]. [1]Keck Graduate Institute, Claremont, CA, [2]Claremont BioSolutions, Upland, CA

Dengue virus (DENV) infection is a major health problem in endemic subtropical and tropical regions, with rapidly increasing incidence, accounting for ~ 390 million infections and ~96 million symptomatic cases per year globally. Early accurate diagnosis of DENV infection is important to monitor patients for warning signs of severe complications that occur in ~5-10% of cases. Diagnosis of acute DENV infection via nucleic acid amplification testing (NAAT) is more sensitive and specific than immunoassays, but practical implementation has been hampered by the incompatibility of existing NAAT technologies with remote settings.

Our goal is to develop a portable, robust, easy to use, integrated nucleic acid testing device that enables early, rapid, and sensitive diagnosis of acute DENV infection. In this effort, we are leveraging an existing device developed by our group for TB diagnosis from sputum, consisting of a disposable cartridge and compact, battery operated instrument that automates and integrates sample preparation, isothermal amplification, and endpoint detection.

To adapt this system for DENV diagnosis from blood, we have developed early proof of principle demonstration for the required processes. To separate plasma from 500 μL of whole blood, we have developed a custom, non-instrumented, inexpensive, filtration based device suitable for integration into the cartridge. RNA extraction from plasma was demonstrated using an RNAexpress™ protocol developed by Claremont BioSolutions, which utilizes a low pressure, flow-through solid phase column to rapidly isolate RNA without centrifugation.

We are using reverse transcription loop-mediated isothermal amplification (RT-LAMP) for serotype common DENV detection, and have demonstrated coupling of RT-LAMP with upstream sample preparation and downstream detection. Endpoint detection can be accomplished through nucleic acid lateral flow with visual readout. However, such visual detection does not create an electronic record, may be ambiguous, and is prone to user error. We have therefore demonstrated electrochemical detection of LAMP amplicons on inexpensive screen printed electrodes via chronoamperometry, similar to the method used in glucose sensors.

Suitable integration and automation of these processes into a compact, integrated, automated, field-deployable system that does not require trained personnel or laboratory infrastructure can provide significant value by enabling sensitive and specific diagnosis of acute DENV infections in endemic resource-limited settings.

#945

Integrated Nucleic Acid Testing for TB diagnosis in Peripheral Settings

Hsiang Wei Lu[1], Leomar Patam[1], Tanya M. Ferguson[2], Keith Herrington[2], Pranav Kumar[1], Murray Learmonth[3], Anna Hickerson[1], Kris M. Weigel[4,5], Gerard A. Cangelosi[4,5], Robert Doebler[2], Angelika Niemz[1]. [1]Keck Graduate Institute, Claremont, CA, [2]Claremont BioSolutions, Upland, CA, [3]Leardon Solutions, San Diego, CA, [4]University of Washington, Department of Environmental and Occupational Health Sciences, Seattle, WA; [5]Seattle Biomedical Research Institute, Seattle, WA

There is an urgent need for methods that enable rapid and affordable diagnosis of tuberculosis (TB) accessible to affected populations in regions with high TB incidence, to facilitate effective treatment and limit disease transmission. We have developed an easy-to-use fully integrated nucleic acid testing device for TB diagnosis from sputum, consisting of a disposable cartridge approximately the size of a mobile phone, with an associated compact, inexpensive, battery operated instrument. This system automates pathogen lysis, nucleic acid extraction, isothermal DNA amplification and lateral flow detection. To date, we have demonstrated fully integrated process execution using sputum spiked with *Mycobacterium tuberculosis* H37Ra. This sample is liquefied and disinfected, using inexpensive and stable reagents, which reduces the viable *Mycobacterium tuberculosis* (*M.tb*) load in sputum ≥1e6 fold, thus mitigating biohazard concerns. The liquefied and disinfected sample is injected into the cartridge, where sample preparation is performed in the SimplePrep™ valve (Claremont BioSolutions), a single-use, inexpensive, disposable six port active valve that integrates the PureLyse® miniature bead blender for cell disruption and DNA extraction. The DNA-containing eluate is pumped into a reaction chamber containing dry thermostable mastermix reagents. This reaction chamber entails an innovative double-pouch design that enables effective heat transfer and facilitates liquid handling, in conjunction with custom designed check valves. After isothermal amplification, the sample is pumped out of the reaction chamber and onto a lateral flow strip for visual detection. All required reagents are stored inside the cartridge in a thermo-stable form. Miniaturized and inexpensive electrolytic pumps (ePumps) integrated into the cartridge facilitate liquid handling during sample preparation, amplification and detection. The closed system cartridge mitigates amplicon carryover contamination. Automated process execution is facilitated by a compact inexpensive instrument, which is designed using low power electronics to enable battery operation. This system can provide a result in <1.5h sample to answer, is suitable for use by minimally trained personnel, and does not require additional laboratory equipment, regular maintenance, or temperature controlled facilities. Such a device can enable TB diagnosis and treatment initiation in the same clinical encounter in near-patient low-resource settings of high TB burden countries.