Institution:
University of Cambridge

Unit of Assessment:
UoA1

Title of case study:
Meeting the diagnostic needs in resource-limited settings

1. Summary of the impact (indicative maximum 100 words)

Communicable diseases are a major health burden in the developing world. Early detection and accurate identification of infectious agents is key to their management. However, the complex procedures and logistics of current diagnostic tests often make them unsuitable for use in developing countries. Two technology platforms have been developed that have led to a new generation of simple and inexpensive rapid tests that can be applied in resource-limited settings. A spinout company was set up to allow translation of these platforms into new products. Three tests (Chlamydia, Hepatitis B and HIV) were launched since 2008, with test kits marketed, allowing patients to receive treatment for infections which would have previously gone unnoticed and untreated. The spinout company has raised >$30 million, of which >$20million is since 2008.

2. Underpinning research (indicative maximum 500 words)

The Diagnostics Development Unit (DDU) was established in 1996 by Dr Helen Lee, Reader in Medical Biotechnology, Dept of Haematology (since 1991), who left the diagnostic industry with the goal of developing simple, high-performance, robust yet affordable assays for resource-limited settings. A multi-disciplinary team was assembled to develop the diagnostic technologies and translate them into products. Key researchers, all in the Dept of Haematology include C Wisniewski (Senior Res Associate, engineering, 01/09/2007 to present), M Dineva (Senior Res Associate, nucleic acid chemistry, 01/10/2004 – 30/09/2010), A Ritchie (Res Associate, molecular biology, 11/09/2006 to present), C Michel (Res Assistant, pilot plant manufacturing, 02/02/1998 – 31/08/2011), N Goel (Res Assistant, quality control and probe synthesis, 23/04/2009 to present).

Two generic platform technologies that greatly improve the performance of rapid tests have since been developed: the **S**ignal **A**mplification **S**ystem (**SAS**) for protein-based targets (Lin et al, JCM 2008), and **S**imple **AM**plification **B**ased **A**ssay (**SAMBA**) for nucleic acid-based targets (Lee et al, JID 2010).

Platform 1 – SAS, the protein-based point-of-care platform (1996 - 2008)

Current membrane-based lateral flow tests are rapid because no complex reagent additions, washing or incubation steps are required. However, the ease-of-use and short assay time are achieved at the expense of sensitivity. Whereas antibody-based rapid tests tend to have equivalent sensitivity to the more complex and machine-dependent Enzyme Immunoassays (EIA), rapid tests for the detection of antigen usually suffer from lower sensitivity. This limitation is particularly evident in infectious disease diagnosis for which high sensitivity is required and yet a low analyte level may be present. Research in the DDU resulted in the development of SAS, which is based on multiplying a visual signal via an increase in the valency and size of the coloured immune complex in the assay, by chemically coupling multiple copies of a hapten moiety to the primary detection antibodies. The resulting lattice formed between the analyte, multiple hapten-labelled antibody and the anti-hapten colour conjugate yields a strong visual signal. This increases the valency and the size of immune complexes and thereby greatly increases the visual sensitivity of lateral flow-based rapid tests (Fig 1).

Fig 1 Effect of SAS on sensitivity of *Chlamydia* lipopolysaccharide (LPS) detection

Format	LPS (picogram) 0	420	125	40	15
Conventional Rapid test					
SAS-based Rapid test					

The sensitivity improvement of SAS for the detection of the Chlamydial lipopolysaccharide was used to develop a simple Chlamydia rapid test with sufficient sensitivity that enabled the use, for the first time of non-invasive samples such as self-collected vaginal swab from women and urine from men. To further improve the sensitivity of the Chlamydia Rapid test for testing male urine, a unique and innovative device, FirstBurst™, was developed by DDU for convenient collection of the

initial fraction of the urine stream containing 84% of the bacterial load (Wisniewski et al, JCM 2008). SAS was also the underpinning technology that allowed the development of a hepatitis B surface antigen (HBsAg) rapid test which became the first rapid test to receive the CE mark because it was able to meet the stringent sensitivity requirement (Lin et al, JCM 2008).

Platform 2 - SAMBA, the nucleic acid-based point-of-care platform (2002-2008)

Existing nucleic acid tests are expensive, complex and time-consuming, requiring sophisticated instrumentation and highly-trained personnel. Thus, even in developed countries, only a small minority of clinical laboratories are capable of performing nucleic acid amplification tests (NAT's). The lack of simple and inexpensive nucleic acid extraction methods from complex biological samples with high efficiency is an important bottleneck for the application of NAT's in resource-limited settings. To address this critical issue, DDU developed SAMBA: a point-of-care molecular diagnostics platform (Fig 2) which uses novel chemistry to enable the visual detection of nucleic acid hybridisation at a sensitivity and specificity equal to that of complex methods, underpinned by a robust and simplified isothermal amplification process. The SAMBA sample preparation protocol takes <25 min, without requiring alcohol or chaotropic salts, and the cartridge provides ready-made unit dose reagents (Lee et al, JID 2010). Key advantages of the SAMBA system include simplicity, low technical complexity and the use of freeze-dried reagents that are extremely stable in high temperature and high humidity, thus circumventing the need for cold chain storage or transport. This simplification of complex chemistry without the need of expensive instrumentation or highly-trained personnel is essential to moving nucleic acid testing beyond sophisticated clinical laboratories to the point-of-care environment in resource-limited settings (Wu et al, JCM 2010).

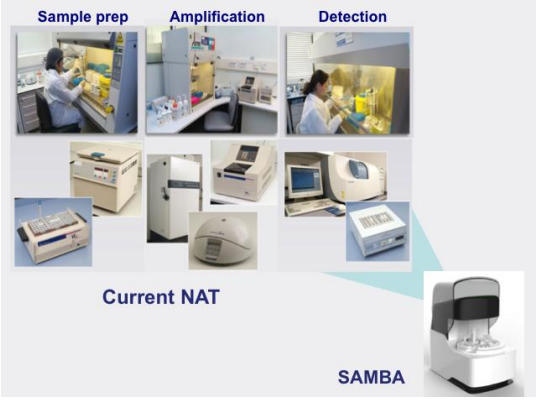

Fig 2: Simplifying NAT with SAMBA

3. References to the research (indicative maximum of six references)

A. Publications

1. **C.A. Wisniewski**, White JA, Michel CE, et al. Optimal method of collection of first-void urine for diagnosis of Chlamydia trachomatis infection in men. **J. Clin. Microbiol.** 2008. 46:1466-1469.
2. **Y-H. Lin**, Y. Wang, A. Loua et al. Evaluation of a new sensitive HBsAg rapid test with improved sensitivity. **J. Clin. Microbiol.** 2008. 46: 3319-3324.
3. **H.H. Lee**, M.A. Dineva, Y.L. Chua et al. Simple amplification-based assay: a nucleic acid-based point-of-care platform for HIV-1 testing. **J Infect Dis**. 2010. 201: Suppl 1:S65-72.
4. **L.T. Wu**, M.D. Curran, J. Ellis, S. et al. 2010. Nucleic acid dipstick test for molecular diagnosis of pandemic H1N1. **J. Clin. Microbiol**. 2010. 48(10): 3608-3613.

B. Intellectual properties: Eight patents have been issued in territories including Australia, France, Germany, India, Italy Spain, UK and USA and include:

1. Improved capture and detection format: versatility for Dipstick Assays (PCT/GB01/03030). GB0016833.6,7 Jul 2000. **Granted: EU** Validating: FR, DE, IN, UK, USA
2. Signal enhancement system with multiple labeled-moieties (PCT/GB01/05325). GB0029154.2, 30 Nov 2000 **Granted: CN, FR, DE, IT, ES, UK, USA**

C. Peer-reviewed funding: During the period between 1st Jan 1993 and July 2013, over £17 million funding was received by DDU with selected funding listed below:

1. **World Health Organization**, Nucleic acid based dipstick assay for the detection of *C. trachomatis* infection from urine. Awarded Sep 1995, **$ 315,250**
2. **National Institutes of Health, USA,** Nucleic acid dipstick for *Chlamydia trachomatis* detection. Awarded Jan 1996, **£ 1,356,985**
3. **Sentinel Biosciences, Inc.**, Discovery of new HIV related viruses in 3 West African countries. Awarded Apr 1996, **£ 1,764,138**
4. **Wellcome Trust Programme Grant**: Development of a rapid DNA dipstick for detection of

Chlamydia trachomatis. Awarded Mar 1997, **£ 2,224,916**
5. **Wellcome Trust Translation Award**: Evaluation of *Chlamydia trachomatis* infection in clinical settings. Awarded Feb 2005, **£ 1,256,963**
6. **Wellcome Trust Strategic Award:** Meeting the diagnostic needs of resource-poor settings: development of a simple amplification test, Awarded Jun 2007, **£ 2,885,434**
7. **National Institutes of Health, USA** SAMBA HIV Point-of-Care Nucleic Acid System for Resource Limited Settings. Awarded Nov 2009, **$4,654,673**
8. **UK Technology Strategy Board,** Point-of-care nucleic acid-based tests for detection of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhea* (NG), Awarded Oct 2010, **£841,327**
9. **Children's Investment Fund Foundation,** Point of care nucleic acid test for detection of HIV infections in infants. Awarded Jul 2011, **$4,917,586**

4. Details of the impact (indicative maximum 750 words)

The two technology platforms (SAS for protein targets and SAMBA for nucleic acid targets) have been successfully commercialised by DDU's spinout company, Diagnostics for the Real World (DRW) established in 2002, with the Wellcome Trust and the University of Cambridge as corporate shareholders (Ref 1 in section 5). To meet the diagnostic needs of the developing world, the company limits its profit to 10%. In the past 10 years, the spinout company has raised >$30 million using a diversified funding strategy (Ref 2 in section 5), of which >$20million is since 2008 (including $8million from NIH, $12million from the Children's Investment Fund Foundation and £0.5million from the UK Technology Strategy Board).

Both the female and the male Chlamydia SAS-based rapid tests have undergone clinical trial in 3 UK clinics and in the Philippines (Refs 3 & 4 in section 5).). Since their launch in 2008 at the national OB-GYN conference in the Philippines, >150,000 tests have been provided to diverse geographic regions through its distribution partner (Oxoid Thermo-Fisher) and direct sales: France, Italy, the Czech Republic, Slovakia, Senegal, Madagascar, the Republic of Niger, the Ivory Coast, Morocco, Algeria, Tunisia, Kenya, Malaysia, the Seychelles, Vanuatu, Samoa, the Falkland Islands, Ghana, and St. Vincent and the Grenadines. They were used as a diagnostic tool for Chlamydia infection in symptomatic individuals as well as a screening tool in pregnant women (e.g. Samoa where Chlamydia is endemic), military personnel in the Falklands and in asymptomatic populations in general. The Chlamydia rapid test was funded by the UK Technology Strategy Board in 2012 as a tool to generate a cost effective model for 'test and treat' at the point of care vs. centralised testing in 3 UK sexual health clinics. The FirstBurst™ first void urine collection device received the 2003 Medical Futures Best Diagnostic Innovation Award and continues to be used in the DRW Chlamydia test with on-going impact in studies such the UK national survey for sexually transmitted diseases in the general population.

DRW has also launched the first CE marked HBsAg rapid test kit on the market due to its improved sensitivity and has begun its launch activities in north African countries and in France after a successful evaluation. The test generated a large number of internet-based articles during the World Hepatitis Day in 2010, including comments from Prof Baruch Blumberg, the Nobel Prize laureate for the discovery of the Hepatitis B virus and invention of the HBV vaccine: "Approval of the new Hepatitis B Rapid Test is positive news for the estimated 400 million HBV carriers worldwide. Being able to identify carriers, initiate immediate treatment of appropriate candidates, and vaccinate family members and close contacts, has the potential to greatly accelerate the programme to control HBV infection and spread. The Hepatitis B Rapid Test developed by Diagnostics for the Real World can make a significant contribution to the solution" (Ref 5 in section 5).

The first assay based on the SAMBA platform is the HIV-1 Semi-Quantitative Test for the monitoring of patients on antiretroviral therapy. Having been successfully trialled by Medecins sans Frontieres (MSF), the first batch of 4,000 tests and 10 SAMBA instruments were shipped to 3 MSF clinics in Malawi and Uganda in Q2, 2013. This is the first time a nucleic acid amplification test is implemented in a point-of-care setting. MSF has already committed to screening >30,000 patients for treatment efficacy using SAMBA. Product registration for the SAMBA HIV-1 Semi-Q Test is currently being sought in 6 other high-burden African countries (Cameroon, Kenya, Nigeria, South Africa, Zambia, Zimbabwe). Given the recent WHO recommendation to monitor all HIV infected patients at least once a year, the SAMBA HIV test will be an effective tool for the monitoring of HIV infected individuals in sub Saharan Africa (estimated to be 15 million in 2015).

Currently, HIV-exposed babies in sub-Saharan Africa can only be tested by nucleic acid amplification methods at centralised laboratories. This requires the transport of dried blood spots from peripheral clinics and leads to unacceptable delays to treatment due to long turn-around times and loss to follow up ranging from 30 to 70% depending on the country. A quantitative SAMBA HIV test has been developed for the early infant diagnosis at the point of care, and access to this simple, rapid and effective molecular test at peripheral clinics where mothers can receive the results in one visit will fill the current diagnostic gap. Ethical approvals have been obtained in Malawi, Uganda, South Africa and Kenya for clinical trials in Q3-Q4 2013. A Memorandum of Understanding was signed with the Ministry of Health, Zimbabwe for implementation of the SAMBA EID test.

5. Sources to corroborate the impact (indicative maximum of 10 references)

1. **Wellcome Trust Technology Transfer Showcase**
http://www.wellcome.ac.uk/Funding/Technology-transfer/Technology-transfer-showcase/WTX052911.htm
2. **Global Health Innovation Insight Series,** Diagnostics for the Real World – Raising funds for a niche solution (2012)
http://www.google.co.uk/url?sa=t&rct=j&q=&esrc=s&source=web&cd=6&ved=0CFIQFjAF&url=http%3A%2F%2Fwww.gsb.stanford.edu%2Fsites%2Fdefault%2Ffiles%2Fdocuments%2FDRWII-RaisingFunds.pdf&ei=T_95UrHQLfGM7Ab6y4GABA&usg=AFQjCNGSjqIxsnFJ3QZCMkZ-2fBPYuvBRA&sig2=oDcHxIjV7IAoSRRxHrLBRg&bvm=bv.55980276,d.ZGU
3. L. Mahilum-Tapay, V. Laitila, J.J. Wawrzynia et al. New point of care Chlamydia Rapid Test - bridging the gap between diagnosis and treatment: performance evaluation study. **British Medical Journal** 2007. 335: 1190-1194.4.
4. E-C. Nadala, B. T Goh, J-P. Magbanua, et al. Performance evaluation of a new rapid urine test for chlamydia in men: prospective cohort study **British Medical Journal** 2009. 339:b2655; doi:10.1136/bmj.b2655.
5. **Wellcome Trust Press Release,** 2010 **"**EU gives green light for while-you-wait Hepatitis B test" http://www.wellcome.ac.uk/News/Media-office/Press-releases/2010/WTX059435.htm