Institution: Cardiff University

Unit of Assessment: UoA3

Title of case study: Cardiff chemiluminescent technology underpinning global adoption of nucleic acid-based clinical diagnostic assays

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

Cardiff University research led to second-generation chemiluminescent technology. The invention allowed for internal amplification control in nucleic-acid based clinical diagnostic assays for infectious disease and produced results with greater accuracy and fulfilled previously unmet regulatory standards. Adopted by the market leader in nucleic acid diagnostics (a sub-licensee of Cardiff University) the Cardiff technology is used globally in more than 60 million *in vitro* diagnostic tests annually. Sales of the tests approach $500 million per year and the sub-licensee was subsequently sold for $3.8 billion.

2. Underpinning research (indicative maximum 500 words)

A family of dyes with distinct chemiluminescent profiles

Cardiff University School of Medicine staff Stuart Woodhead (L/SL/Reader 1971 to 1998 inclusive and Professor 2001-2004) and Ian Weeks (Lecturer 1985-1994 inclusive, and Professor 2009 to present) investigated from 1993 the chemiluminescent (light emitting) properties of a family of acridinium-ester (AE) dyes. Specifically, the emissions of various AE dyes that were distinguishable in terms of their light-emitting wavelengths (colour), quantum yield (light emitting efficiency) and significantly the kinetics or life time of emitted light signal, i.e. "flash" or "glow") [3.1,3.2,3.3]. The work involved a series of design-synthesis-characterisation rounds with chemical modifications to the AEs leading to novel molecules each possessing distinct chemiluminescent properties. It evolved to a 'reduction to practice' for the simultaneous, though independent, quantification of the light emissions of several different AE labelled oligonucleotide probes. The work also addressed AE dye protection during nucleic acid hybridization reactions and the effect of background signal. This required optimisation of the critical property of AE dye protection within a double stranded nucleic acid helix (such as is formed when an AE-labelled oligonucleotide probe binds to its specific nucleic acid target) as opposed to the deactivation of the AE dye (and its light emitting properties) in non-hybridized probes.

A number of patent families arose from the research for the use and development of modified AE dyes as molecular labels in *in-vitro* chemiluminescent-based nucleic acid diagnostic assays [3.1,3.2,3.4,3.5]. In particular the research underpinned Cardiff technology allowing for the concurrent use of two types of AEs distinguishable by kinetic emission, i.e. one that "flashes" and another that "glows". This innovation provided a solution to the unmet requirement for simultaneous detection and quantification of multiple targets, i.e. multiplex analysis, in nucleic acid hydridization assays.

The timeliness of the research was crucial since there was a burgeoning interest in nucleic acid diagnostics on which Cardiff's technology ultimately impacted. Namely, a vital element in highly sensitive nucleic acid based diagnostic tests is nucleic acid amplification, a process providing a high level of assay sensitivity but one prone to non-specific interferences potentially yielding incorrect results, particularly when conducted in automated systems. For clinical approval of nucleic acid based diagnostic tests the regulatory authorities required the amplification process in such assays to be internally controlled. The Cardiff technology provided a novel, patentable solution to this. For example, it allowed the amplification of the clinical nucleic acid target (slow light emission) to be internally controlled by including a parallel amplification reaction for a control sequence that could be independently monitored (fast light emission) [3.2,3.4,3.5]. The technology also allowed simultaneous detection of multiple screening or diagnostic targets within the same sample.

Patent landscape and research partnering

The initial research in Cardiff University was part-funded by the University and a University spin-out company (Molecular Light Technology Ltd; MLT) in which the University held equity and Board representation, and in which Weeks later became a director. The later developmental research in Cardiff University was also sponsored by GEN-PROBE Inc., a San Diego based company and one of the market leaders in nucleic acid diagnostic test systems. GEN-PROBE was also a sub-licensee of previous but completely unrelated Cardiff chemiluminescent 'first-generation' technology, which, though very successful, only permitted single analyte detection and quantitation. The crucial underpinning research of the University described here was the discovery of the

'second-generation' "flash" or "glow" chemiluminescent technology. The role of University researchers is evidenced by the initial granting to the University of background patents with PIs - Weeks and Woodhead named as sole inventors [3.1] and later [3.2,3.4,3.5] joint inventors. The commercial partner GEN-PROBE was ultimately assigned the patents for Cardiff's second-generation technology which formed GEN-PROBE's chemiluminescent Dual Kinetic Assay (DKA) approach, and which is used in their *in-vitro* blood screening and clinical diagnostics products (see section 4).

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

In light of its commercially sensitive nature the underpinning research was initially published as patents (Weeks and/or Woodhouse as Cardiff University leads) with any research articles relating back to this earlier work. Note patents underlined are referred to in sections 4 and 5.

[3.1] **Batmanghelich, S**., **Woodhead, J.S.** and **Weeks, I.** Detecting or quantifying multiple analytes using labelling techniques US5656207 (filing 1995, publication 1997), US5756011 (filing 1997, publication 1998).
http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=US&NR=5656207A&KC=A&FT=D&ND=3&date=19970812&DB=EPODOC&locale=en_EP

[3.2] Nelson, N.C., **Woodhead, J.S., Weeks, I**. and Cheikh, A.B. Compositions and methods for the simultaneous detection and quantification of multiple specific nucleic acid sequences. WO9613612 (filing 1995, published 1996) also published as US5658737 (filing 1996, published 1997), US5840873 (filing 1997, published 1998), US5756709 (filing 1996, published 1998), US5827656 (filing 1996, published 1998).
http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=WO&NR=9613612A2&KC=A2&FT=D&ND=3&date=19960509&DB=EPODOC&locale=en_EP

[3.3] Smith, K*., Li, Z., Yang, J.J., **Weeks, I.** and **Woodhead, J.S.** Synthesis and properties of novel chemiluminescent biological probes: substituted 4-(2-succinimidylcarbonylethyl)phenyl 10-methylacridinium-9-carboxylate trifluoromethane sulphonate. J. Photochem. Photobiol. (2000) 132: 181-191. *undertook contract chemistry synthesis for Woodhead and Weeks who are inventors on earlier patents. http://dx.doi.org/10.1016/S1010-6030(00)00209-4

[3.4] Browne, K.A. and **Weeks, I**. Chemiluminescent probes for multiplex molecular quantification and uses thereof. US20120231459 A1 (filing 2012, published 2012).
http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=US&NR=2012231459A1&KC=A1&FT=D&ND=3&date=20120913&DB=EPODOC&locale=en_EP

[3.5] Browne, K.A. **Weeks, I.** and Brown, R.C. Spectrally-resolved chemiluminescent probes for sensitive multiplex molecular quantification. US20130040859 A1 (filing 2013, published 2013).
http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=US&NR=2013040859A1&KC=A1&FT=D&ND=3&date=20130214&DB=EPODOC&locale=en_EP

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

Nature of impact

Cardiff University's research has led to the development of *in-vitro* blood screening and clinical diagnostic nucleic-acid amplification assays that have been adopted worldwide for the detection of infectious agents. These assays have been brought to the market by GEN-PROBE Inc. (San Diego, USA) and offer improved detection sensitivities and importantly possess internal control for the nucleic acid amplification ensuring earlier disease detection and a reduced incidence of incorrect results. The distinct contribution of Cardiff research to the products is evident in the respective 'product inserts' [5.1] which cite patents (underlined in references [3.1] and [3.2] of Section 3 above) upon which Cardiff University staff (Weeks and/or Woodhead) are named inventors. The Cardiff technology has led to year on year benefits to commerce and clinical practice.

Clinical adoption and commercial outcomes

GEN-PROBE's key nucleic acid detection assays use the Dual Kinetic Assay (DKA) technology with more than 60 million clinical tests performed worldwide each year. In April 2012 Hologic Inc. (a global healthcare company seeking to strengthen its diagnostic portfolio) acquired GEN-PROBE for $3.8 billion [5.2] with GEN-PROBE realising a doubling in its value from 2008 (NASDQ share

price of $42) to the point of acquisition (share price $83). GEN-PROBE as a wholly-owned subsidiary of Hologic continues to market and develop nucleic acid tests. The Hologic 2012 annual report [5.2] states "*Diagnostics product sales increased 25% in fiscal 2012 compared to fiscal 2011 primarily due to the inclusion of GEN-PROBE, which contributed $86.7 million in revenue*".

Some of the tests that have seen first-user adoption in the assessment period are described below.

Blood screening assays: GEN-PROBE's blood screening assays are marketed by Novartis Diagnostics under the PROCLEIX® trade mark [5.3.,5.4], with those approved for the Tigris® platform shown in Table 1; Tigris® is a fully automated robotic instrument enabling high-throughput sample processing and sample containment. Worldwide more than 80 million units of blood are donated annually with screening performed for infectious agents at major reference laboratories. The most common screens are for HIV, hepatitis C (HCV), hepatitis B (HCB) and West Nile Virus (WNV). The PROCLEIX assays for these indications use the Cardiff DKA technology and importantly have been designed and approved for use on Tigris®. Since 2008 the number of Tigris® platforms in worldwide use at major blood testing centres has doubled from 200 units (2008) to 390 units in 2011 [5.5]; typically a single Tigris® unit is sufficient for a single testing centre.

Table 1: PROCLEIX Tigris® assays	PROCLEIX WNV	PROCLEIX ULTRIO	PROCLEIX ULTRIO Plus	PROCLEIX ULTRIO Elite
Infectious agent	WNV	HIV-1, HCV, HBV	HIV-1, HCV, HBV	HIV-1, HCV, HBV, HIV-2
First approval date on Tigris® platform	CE marked and FDA approved in March 2007	CE marked and FDA approved to include HBV testing in 2008	CE marked in 2009 and FDA approved 2012	CE marked in 2012 under further development for other regions
Country approvals at 2013 (new approvals since 21/12/2009)[5.4]	28 EU USA 6 other (2)	28 EU USA 26 other (9)	28 EU 19 other (19 inc. USA)	28 EU
Notes: (a) CE = marked compliant for Europe (b) PROCLEIX ULTRIO Plus provides improved detection sensitivity to the ULTRIO assay				

The PROCLEIX assays have a ca. 32% share of the global blood screening market (i.e. 25 million assays per year) and are used in ca. 80% (14.5 million tests) of US blood screens [5.3,5.4,5.6]. The PROCLEIX assays yield product sales to GEN-PROBE of ca. $200 million per year [5.3,5.6] with ca. $16 million p.a. representing "at cost sales" of the Tigris® instrumentation platform [5.6]; in 2012 the FDA approved GEN-PROBE's low- to mid-volume throughput testing platform (Panther®) which will use the DKA-based assays. The DKA-based assays represent a clinical advance to blood screening services. For example, in 2011 the South African National Blood Service showed PROCLEIX ULTRIO to identify 9744 contaminated blood donation units from 3.8 million tested, with 447 of these positive samples not otherwise detected by existing approaches [5.7]. In adopting PROCLEIX ULTRIO the Australian National Blood Authority (2010) showed the risk of transmission of HBV from blood donations was decreased by 33% relative to HBsAg serology [5.8].

Clinical diagnostic assays for sexually transmitted disease (STD): GEN-PROBE's APTIMA family of clinical diagnostic STD assays all utilise Cardiff technology. Placement of Tigris® platforms worldwide for APTIMA STD diagnostics has increased 66% from 150 units (2008) to 250 units in 2011 [5.5]. APTIMA Tigris® products approved 2008 onward include:

- APTIMA *Trichomonas vaginalis* (CE marked 2010 and FDA approved 2011). The only FDA-approved nucleic acid amplification test to specifically detect this parasite, which causes trichomoniasis, the most common curable STD (the Center for Disease Control estimates 7.4 million cases p.a. in USA).
- APTIMA HPV (CE marked 2008 and FDA approved 2011) which detects the presence of one or more of the high risk 14 subtypes of human papillomavirus (HPV) associated with cervical cancers; HPV is the etiological agent responsible for more than 99% of all cervical cancers.
- APTIMA HPV Genotype 16, 18/45 (FDA approved 2012). Detects the presence of the very high risk HPV genotypes 16 and 18 or 45 associated with 80% of invasive cervical cancers.

In 2012 USA healthcare professions and USA government recommended HPV screening every 5 years in women over 30 years. In clinical studies involving approximately 45,000 women the APTIMA HPV assay (detecting certain mRNAs) has consistently shown similar sensitivity and

better specificity than the most frequently used DNA-based tests. Based on a clinical evaluation of more than 10,000 US women with normal cytology results, the APTIMA HPV assay had 24% fewer false positives than the leading FDA-approved DNA test [5.9].

GEN-PROBE APTIMA STD kits using the Cardiff technology hold a 49% global market share with ca. 29 million tests p.a. [5.5] and revenue of ca. $200 million (2008) rising to over $350 million in 2011 [5.3]. In 2013 Hologic entered into a strategic alliance with Quest Diagnostics (the world's largest clinical diagnostics testing company) who will use APTIMA STD assays in diagnostic services [5.10].

The Cardiff research was transformative in providing a solution to the unmet regulatory need for the internal control of nucleic acid amplification in high sensitivity *in-vitro* screening and diagnostic assays. The technology has been adopted by the market leader in nucleic acid diagnostics in a range of clinical assays for infectious agents. The technology has achieved worldwide reach and underpinned significant commercial and clinical practice uptake [5.3].

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

[5.1] Patents underlined (references [3.1] and [3.2] in Section 3 above) refer to FDA approved package inserts for GEN-PROBE products PROCLEIX Ultrio, PROCLEIX Ultrio Plus, APTIMA Combo 2 Chlamydia/Gonorrhoea, APTIMA Trichomonas vaginalis, APTIMA HPV amongst others, e.g.: p 53. http://www.fda.gov/downloads/biologicsbloodvaccines/bloodbloodproducts/approvedproducts/licensedproductsblas/blooddonorscreening/infectiousdisease/ucm092120.pdf

[5.2] Hologic 2012 annual report showing: revenue from diagnostic products from GEN-PROBE acquisition (p 50); GEN-PROBE acquisition for $3.8 billion (p 65). http://www.sec.gov/Archives/edgar/data/859737/000119312512482840/d393079d10k.htm#tx393079_10

[5.3] Statement from former Vice-President Business Development, GEN-PROBE Inc. Corroboration of the distinct significance of Cardiff discoveries to the development and regulatory approval of GEN-PROBE's amplified nucleic acid screening and clinical diagnostic assays. Marketing of assays and income generation from the assays.

[5.4] Novartis Diagnostics PROCLEIX Assays. Shows range of assays and links to worldwide product approvals. Impact through PROCLEIX Assays triangulates through this to 5.1,5.3,5.5,5.6,5.7. http://www.novartisdiagnostics.com/products/procleix-assays/index.shtml

[5.5] GEN-PROBE presentation from Carl Hull, Chairman and CEO. 'An innovative growth company in molecular diagnostics' at 30th Annual JP Morgan Healthcare Conference. January 10th, 2012. Slide 8 -placement of Tigris systems; Slide 11 - APTIMA 49% share of market. http://media.corporate-ir.net/media_files/IROL/13/135117/120110_230pm_gpro_jp_morgan_final2.pdf

[5.6] GEN-PROBE Annual Report 2011 highlighting: key products using the DKA technology (pp 9-13); revenue from clinical (APTIMA STD) diagnostics of $350M in 2011 (p 46); PROCLEIX assays used in 80% of USA blood tests (p 8) and yielding sales ca. $200 million per year with ca. $16 million per annum representing "at cost sales" of the Tigris® instrumentation platform (p 46-50). http://www.gen-probe.com/pdfs/2011%20Annual%20Report.pdf

[5.7] Success of PROCLEIX preventing HIV and hepatitis transmission from donated blood (South African National Blood Service, 2011). http://en.prnasia.com/pr/2011/07/25/110712511.shtml

[5.8] Adoption of ULTRIO for blood screening by the Australian National Blood Authority (p 2). http://www.transfusion.com.au/sites/default/files/Medilink May10 FIN.pdf

[5.9] HOLOGIC/GEN-PROBE Clinical Diagnostics webpage listing clinical findings for its assays. In this link the clinical diagnostic benefits of APTIMA HPV assay. http://www.gen-probe.com/products-services/aptima-hpv-assays

[5.10] Quest Diagnostics enter into strategic alliance with HOLOGIC to use APTIMA STD family of products in its diagnostic services. http://ir.questdiagnostics.com/phoenix.zhtml?c=82068&p=irol-newsArticle_pf&id=1827821

All documents, testimony and webpages saved as PDFs are available from the HEI on request.