Institution: The University of Edinburgh

Unit of Assessment: UoA5: Biological Sciences

Title of case study:

04. Blood donations are screened for malaria exposure with an immunoassay.

1. Summary of the impact

Impact on health and welfare: The malaria screening assay allows early re-admittance of malaria-risk donors to blood donation programmes whilst maintaining protection against transfusion-transmitted malaria. Increasing the availability of safe blood for donation through use of the malaria assay saves lives.

Impact on commerce: The malaria EIA is the front-line assay in at least ten countries today. Almost 2.5 million tests have been sold in the REF impact census period through a number of distributors, including Bio-Rad worldwide, [text removed for publication].

Beneficiaries: Individuals requiring blood transfusions, national blood transfusion services and hospitals; commercial companies marketing the malaria EIA.

Significance and Reach: Over 700,000 assays are now performed per year in the UK, France, Belgium, Portugal, Spain, Italy, Netherlands, New Zealand and Australia. In the UK alone, more than 345,000 blood donations from malaria-risk donors have been cleared for clinical use.

Attribution: All research was led by Dr Jana McBride, Dr David Cavanagh, and Eleanor Riley, at the University of Edinburgh (UoE), except output [6] which was an international consortium to which UoE contributed recombinant malaria antigens and technical expertise.

2. Underpinning research

Malaria is one of the world's most common diseases, affecting over 300 million people and is a major cause of morbidity and mortality. It is caused by *Plasmodium* protozoan parasites and is transmitted to humans through bites from infected mosquitos.

UoE research led by Jana McBride and David Cavanagh has investigated the nature of naturally-acquired human immune responses to the malaria parasite *Plasmodium falciparum.* By cloning and expressing recombinant antigens derived from the genes of *P. falciparum,* and using these antigens as screening tools, the research aimed to identify which of many malaria parasite antigens clinically-protective human antibodies were directed against. Seroepidemiological studies in malaria-exposed Africans were carried out to identify associations between the acquisition of antibodies to specific *P. falciparum* antigens and reduced incidence of clinical malaria episodes [1-4].

Recombinant antigens derived from several major merozoite proteins of the parasites were produced in McBride's laboratory [5]. These proteins included two major proteins from the surface coat of the parasite, merozoite surface proteins 1 and 2 (MSP-1 and MSP-2). The seroepidemiological studies conducted by Cavanagh and colleagues from UoE in collaboration [1] with the University of Khartoum, Sudan between 1996 and 1998 indicated that nearly all naturally-exposed individuals produced serum antibodies to either or both MSP-1 and MSP-2 after malaria infections [1,5]. Importantly, in observations of serological responses carried out up to 2001 in an international collaboration, it was noted that some individuals selectively responded by production of antibodies to only one of the two antigens after new malaria infections [6]. In a longitudinal study conducted by Cavanagh in 1996-7, antibodies to the C-terminal region of MSP-1, known as MSP-1_{19}, were found in >90% of a cohort of Sudanese villagers exposed to highly seasonal malaria transmission, but responses to this antigen were observed to be short-lived in the absence of parasitaemia [1]. Further research on both MSP-1 and MSP-2, by now both potential vaccine candidates, was undertaken with cohorts of African children between 1998 and 2006. These studies showed that serum IgG against both MSP-1 and MSP-2 antigens was strongly associated with a reduced risk of malaria [2,3,4]. Analysis of data from these West African cohorts and other

cohorts in Kenya from 1997 to 2008 also showed that only by combining antibody reactivity to MSP-1 and MSP-2 could the majority of malaria-exposed individuals be robustly detected.

Key personnel: all employed by UoE School of Biological Sciences on the dates stated: Dr Jana McBride, Senior Lecturer (1980 – retired 2010), Dr David Cavanagh, Senior Lecturer (1994-present), Professor David Arnot (1989–present) Eleanor Riley, Senior Research Fellow (1990-1998), Rachel Taylor, PhD student of JMcB and ER (1993-1996).

Prof. David Conway, LSHTM: long-time collaborator and co-author on MSP-1/MSP-2 work [2-4,6]; Wolfram Metzger, PhD student LSHTM: worked with Cavanagh on MSP-2 work in Edinburgh, testing Gambian sera against MSP-2 antigens [3]; Prof. Kevin Marsh, Head of KEMRI Wellcome Collaborative Research programme and Nuffield Department of Medicine, University of Oxford: collaborator and head of Kilifi labs, where immune responses to MSP-1/2 antigens were tested by Dr. Faith Osier [6].

3. References to the research

1. Cavanagh DR, Elhassan IM, Roper C, Robinson VJ, Giha H, Holder AA, Hviid L, Theander TG, Arnot DE, McBride JS. (1998). A longitudinal study of type-specific antibody responses to Plasmodium falciparum merozoite surface protein-1 in an area of unstable malaria in Sudan. J Immunol **161,** 347–359. PubMedID: 9647243 [available on request]
   112 Scopus citations at 19/09/2013
2. Conway DJ, Cavanagh DR, Tanabe K, Roper C, Mikes ZS, Sakihama N, Bojang KA, Oduola AMJ, Kremsner PG, Arnot DE, Greenwood BM, McBride JS. (2000). A principal target of human immunity to malaria identified by molecular population genetic and immunological analyses. Nat Med **6,** 689–692. doi: 10.1038/76272.
   163 Scopus citations at 19/09/2013
3. Metzger WG, Okenu DMN, Cavanagh DR, Robinson JV, Bojang KA, Weiss HA, McBride JS, Greenwood BM, Conway DJ. (2003). Serum IgG3 to the Plasmodium falciparum merozoite surface protein 2 is strongly associated with a reduced prospective risk of malaria. Parasite Immunol **25,** 307–312. doi: 10.1046/j.1365-3024.2003.00636.x.
   68 Scopus citations at 19/09/2013
4. Polley SD, Conway DJ, Cavanagh DR, McBride JS, Lowe B, Williams TN, Mwangi TW, Marsh K. (2006). High levels of serum antibodies to merozoite surface protein 2 of Plasmodium falciparum are associated with reduced risk of clinical malaria in coastal Kenya. Vaccine **24,** 4233–4246. doi: 10.1016/j.vaccine.2005.06.030.
   59 Scopus citations at 19/09/2013
5. Taylor RR, Smith DB, Robinson VJ, McBride JS, Riley EM (1995). Human antibody response to Plasmodium falciparum merozoite surface protein 2 is serogroup specific and predominantly of the immunoglobulin G3 subclass. Infect Immun **63**, 4382–4388. PubMedID: 7591074
   115 Scopus citations at 19/09/2013
6. Osier FHA, Fegan G, Polley SD, Murungi L, Verra F, Tetteh KKA, Lowe B, Mwangi T, Bull PC, Thomas AW, Cavanagh DR, McBride JS, Lanar DE, MacKinnon MJ, Conway DJ, Marsh K. (2008). Breadth and magnitude of antibody responses to multiple Plasmodium falciparum merozoite antigens are associated with protection from clinical malaria. Infect Immun **76,** 2240–2248. doi: 10.1128/IAI.01585-07.
   104 Scopus citations at 19/09/2013

4. Details of the impact

Through collaboration between Lab21 and UoE, a **malarial antibody detection EIA** (Enzyme ImmunoAssay) has been developed and is now marketed commercially to screen blood donations from identified malaria-risk individuals. Malarial antibody screening is considered to be an effective strategy for dealing with donations from malaria-risk donors in non-endemic countries. The use of this assay enables the collection and release of blood donations from malaria-risk donors who otherwise would have to be deferred either for at least 12 months in the case of travellers, or from 5 years up to permanently in the case of previous residents of endemic areas. This approach has

been adopted by at least 10 national blood authorities, including England, Scotland, Wales, France, Italy, Netherlands, Belgium, Portugal, Spain, Australia and New Zealand.

Impact on health and welfare:

The UK has the highest incidence of travel-related malaria compared to other non-endemic countries; several thousand cases are imported into the UK every year. This constitutes an on-going risk of transfusion-transmitted malaria (TTM). The risk of transmitting malaria is complicated by the long and relatively asymptomatic period during which infectious organisms can remain in the blood. Because of this, most blood transfusion services have a policy of deferring 'at risk' donors. Prior to the introduction of the EIA screening test by the NHS Blood and Transplant authority in 2002, UK blood transfusion guidelines recommended that blood donation by travellers to malarial areas were deferred by at least 12 months and, in the case of previous residents of such areas, up to 5 years. This led to permanent inaccessibility to some donor groups, substantial loss of blood donations, and the unnecessary permanent deferral of many donors. The number of blood donations lost as a result of the lengthy deferral period of malaria-risk blood donors was significant. In England alone, up to 60,000 donations per year were lost [a]. This figure was rising year-on-year with the increase in travel to, and immigration from, endemic areas. An increasing number of donors are also deferred for a wide range of other possible infection risks, and this led to challenges in maintaining a sufficient blood supply.

It became essential that blood transfusion services devised strategies that ensure protection from TTM and also minimise the loss of donors and blood donations. In March 2004, the European Union issued Commission Directive 2004/33/EC for blood and blood components. Annex 2.2.1 details the deferral periods for four categories of malaria-risk individuals for blood donation. There is a reduced deferral or no deferral period if "an immunologic or molecular genomic test is negative". Such a screening test for malaria-risk individuals has to be sensitive enough to identify all infected donations and have high specificity. It also has to be low cost and straightforward to perform routinely in the laboratory.

In 2001 we started working with Newmarket Laboratories Ltd to develop a screening assay. We provided two purified recombinant antigens of MSP-2 and also alerted them to the existence of two MSP-1 proteins produced by National Institute for Medical Research. Our advice was that only by combining these four antigens in a screening assay could all malaria parasite antibody-positive individuals be detected. Several important results from our sero-epidemiological studies were essential in deciding which antigens to include in the assay [b]. Firstly, the high frequency (>90%) of recognition of MSP-1_{19} by African sera demonstrated in 1998 [1] provided supporting evidence for its inclusion in any antibody screening assay. Secondly, one of the MSP-2 antigens used in previous studies (Type A T9/96 antigen) [3,5] proved to have unacceptable cross-reactivity with sera from malaria unexposed individuals, and was substituted by another MSP-2 (Type A CH150/9 antigen) in subsequent research studies [4] and in the screening assay.

The Malaria EIA containing all four antigens was developed at Newmarket Laboratories Ltd [b]. The assay detects IgG, IgM and IgA isotype antibodies to all four human malaria strains, *P. falciparum, P. vivax, P. ovale* and *P. malaria*. It is reported to be 98% and 100% sensitive for *P. falciparum* and *P. vivax* respectively with 98-100% specificity [c]. This makes it the most successful malaria antibody screening assay and is more sensitive than the previous "gold standard" test, the IFAT, which is time-consuming and depends to some extent on operator interpretation of antibody reactivity.

The Newmarket Labs malaria EIA was trialled at the English National Transfusion Microbiology Reference Laboratory alongside the in-house IFAT. The results, published in 2004 [d], represented the actual outcomes of screening samples from malaria-risk donors. The data showed that the EIA was indeed a sensitive assay and as a consequence, since 2004 donation screening in England and Wales has been performed successfully using only the Malaria EIA derived from UoE research. It is also in use as the primary screening assay in Scotland and in several other European countries and was adopted by Australia and New Zealand in 2005.

This has a significant ongoing impact during the REF census period 2008-13. In England, ~3% of all donations require malaria antibody screening. In the first 5 months of 2008, the English National Blood Transfusion Service (NBTS) screened 24,400 donations for malaria antibodies. Of these,

98% tested negative and so 23,919 donations were cleared immediately for clinical use. These percentages are fairly constant over time and so we estimate that over 320,000 donations were enabled in England during the REF census period. The Scottish NBTS screened 6210 donors using the malaria assay in a two-year period from October 2010. 96.3% of these were non-reactive and so over 5,900 donors were returned to the donation pool [e,f]. By extrapolation, we can estimate that some 16,470 donors were enabled in Scotland during the REF census period. The Welsh NBTS data shows that in 2008, 95.24% of donations screened were non-reactive, enabling an estimated 13,600 donations to be used during the census period. So, for these 3 UK countries alone, more than 345,000 blood donations have been retained by the early re-admittance of donors, thanks to use of the assay from 1st January 2008 to 31st July 2013.

Prior to the routine use of the assay, the deferral period for malaria-risk individuals in Australia was 12 months. Assessment of the assay's performance using Australian blood donor samples from malaria-risk individuals showed that over 40,000 donations are now retained annually after screening [g], so for the REF impact census period, c. 220,000 donations were cleared for clinical use. Data from New Zealand shows that 90.2% of donations screened for malaria antibodies are non-reactive, releasing approx. 30,000 donations for clinical use during the REF census period.

Impact on commerce:

In 2006 Newmarket was acquired by Lab21, a global provider of state-of-the-art diagnostic products and services. The Malaria EIA was sold by Lab 21 throughout the whole of the REF impact census period and distributed worldwide through Bio-Rad, a global life science and clinical diagnostics product supplier [h,i]. Trinity Biotech purchased the relevant operational arm of Lab21 in July 2013 and now markets the Malaria EIA. [text removed for publication].

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

The Tiny URLs provide a link to archived web content, which can be accessed if the original web content is no longer available

a. Kitchen A, Mijovic A, Hewitt P (2005) Transfusion-transmitted malaria: current donor selection guidelines are not sufficient. Vox Sang 88: 200–201. doi:10.1111/j.1423-0410.2005.00610.x. [available on request]

b. Corroboration of UoE contribution: Lead scientist responsible for development of the malaria EIA at Newmarket Laboratories Ltd and Lab21.

c. Seed CR, Cheng A, Davis TME, Bolton WV, Keller AJ, Kitchen A, Cobain TJ. The efficacy of a malarial antibody enzyme immunoassay for establishing the reinstatement status of blood donors potentially exposed to malaria. Vox Sang 2005; 88(2):98-106. [available on request]

d. Kitchen AD, Lowe PHJ, Lalloo K, Chiodini PL (2004) Evaluation of a malarial antibody assay for use in the screening of blood and tissue products for clinical use. Vox Sang 87: 150–155. doi:10.1111/j.1423-0410.2004.00561.x. [available on request]

e. Corroboration of impact of malaria EIA on blood donor inclusion rates in England: Head of National Transfusion Microbiology Reference Laboratory, National Blood Service

f. Corroboration of impact of malaria EIA on blood donor inclusion rates in Scotland: Head of National Microbiology Reference Unit, Scottish National Blood Transfusion Service.

g. Seed, C.R., Kee, G., Wong, T., Law, M., Ismay, S. (2009). Assessing the safety and efficacy of a test-based, targeted donor screening strategy to minimize transfusion transmitted malaria. *Vox Sanguinis*, 98 (3 A), pp. e182-e192. [available on request]

h. Malaria EIA sales material and product information: http://tinyurl.com/ku7gggr and http://tinyurl.com/m3qelrj

i. Marketing Director, Trinity Biotech (UK) Ltd, formerly Lab21 will corroborate number of assays/year and countries to which it is sold.