Institution: Anglia Ruskin University

Unit of Assessment: 4

Title of case study: Applying novel measurement of hair and saliva biomarkers to behavioural change

Period when the underpinning research was undertaken: 2008 to 2020

Details of staff conducting the underpinning research from the submitting unit:

Name(s):	Role(s) (e.g. job title):	Period(s) employed by submitting HEI:
Matt Bristow	Associate Professor	2001 to present
Rachel Cook	Head of Psychology	1997 to 2018
Katy Parker	Laboratory Manager	2006 to present

Period when the claimed impact occurred: 2014 to 2020

Is this case study continued from a case study submitted in 2014? N

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

The ARU Biomarker Lab team are active psychology researchers with a background in developing innovative techniques for measuring hormones and immune markers within hair and saliva samples. This has led to:

- Development of state-of-the-art biomarker analysis and interpretation for researchers, clinicians and educators who would not normally have experience to apply biomarkers in behavioural research. This has enabled work which has impacted on non-academic awareness and understanding in eating disorders, suicidal behaviour, and smoking cessation behaviour that has been used by bodies including Public Health England, the World Health Organisation, and the US Department of Defense.
- Changes in the coaching strategies used by the Vitality Roses, the English national netball team, currently ranked third in the world.
- The development of a new business model for Stratech Ltd that led the company achieving double-digit annual percentage growth from 2014 and winning 17 European tenders.

2. Underpinning research (indicative maximum 500 words)

Biomarker analysis allows the measurement of chemicals in hair and saliva that allows us to better understand changes in the body that are linked to stress and other psychological processes. Cortisol is the most studied biomarker in saliva and hair with higher levels associated with increased stress and poorer health outcomes. A wide range of biomarkers are potentially measurable in saliva and hair, for example cytokines such as C-reactive protein (CRP) and interleukins IL-1β and IL-4, immune markers such as immunoglobulin A (IgA) and hormones such as testosterone and melatonin. The low levels of many saliva and hair biomarkers makes measurement technically very challenging. Since 2008 a unit of experienced scientists based at ARU led by **Dr Matt Bristow** have pioneered innovative research on the collection, analysis and interpretation of stress biomarkers extracted from saliva and hair samples.

Salivary Stress Biomarker Analysis

Salivary analysis offers an innovative approach to investigating stress biomarkers. While the levels of biomarkers in saliva are much lower and more challenging to measure than in more traditional blood-based measures, the relative ease of collecting saliva samples allows for a range of more powerful research designs. Bristow and Cook led a study in collaboration with Hodges at the Prince of Wales Medical Research Institute in Australia that examined levels of mucosal immunity (secretory IgA) assessed in saliva samples from carers of partners with frontal-temporal dementia

(**Reference 1)**. This was the first published study to examine mucosal immunity in participants experiencing enduring rather than transitory stress. In collaboration with O'Connor at the University of Leeds (**Reference 2**), Bristow then led the analysis and interpretation of salivary cortisol levels in a novel study examining the causal factors and function of the cortical awakening response (CAR; a steep rise in cortisol during the first 45 minutes after waking). This was the first study to investigate the effect of daily 'hassle appraisals' on next-day CAR and the consequences of CAR for same-day physical symptoms. In collaboration with van Paridon from ARU Sports Science (submitted to UoA24), Bristow (**Reference 3**) then conducted the first published meta-analysis of literature on cortisol reactivity during the anticipatory stress response, experienced by athletes who are anticipating the physiological demands of sport competition.

These studies led the ARU Biomarker Lab to participate in the first large-scale international comparison of measurement error of cortisol in saliva samples across six independent research laboratories in Europe, Canada and the United States, with Bristow co-authoring the first published study of the reproducibility of results in salivary cortisol analysis across different academic laboratories (**Reference 4)**. This paper represents an international benchmark in the reproducibility and precision of salivary cortisol measurement techniques in the field.

Stress Biomarker Analysis in Hair

In 2010 the ARU Biomarker Lab developed and validated their own novel hair analysis method to provide an accurate, reliable and fast analysis of endocrine markers in human and animal hair samples. The advantage of hair analysis is that it provides researchers with access to longer-term indices of endocrine function, commonly cortisol, in comparison to traditional matrices, such as saliva, blood and urine. The unit developed a process that was able to grind more samples at one time that could be scaled as sample numbers increased. While other laboratories used grinding mills that required a number of different steps (e.g. sample washed and dried, then transferred to a ball mill for grinding; sample is weighed out into another tube for extraction; steel mill decontaminated and washed), the ARU Biomarker Lab's novel method minimized the number of steps involved, both improving throughput and reducing errors. Samples are cut and weighed into a polypropylene grinding tube as the first step, whereas other laboratories weigh after grinding. The Biomarker Lab's method is less wasteful of hair sample and substantially reduces the risk of transfer errors. The same polypropylene tube is used from the initial weighing stage through to the methanol extraction stage, which reduces the risk of error or sample being lost when transferred from one vessel to another. The methods and the underlying validation data were subsequently made publicly available (**Reference 5**). The advantages of this method were demonstrated in a collaboration between Bristow and Bhopal at UCL in a study examining hair and salivary cortisol measures of stress amongst infants in rural India (**Reference 6**). This is the largest study of hair cortisol in infants ever conducted, and the first to be carried out in a low- and middle-income country setting. Without the underpinning research of the ARU Biomarker Lab in relation to the novel collection and analysis of hair cortisol samples, it would not have been possible to conduct such a large-scale study in such a challenging rural environment.

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

1. Bristow, M., Cook, R., Erzinclioglu, S. & Hodges, J. (2008) Stress, distress and mucosal immunity in carers of a partner with fronto-temporal dementia. *Aging and Mental Health*, 12(5), 595 - 604. https://doi.org/10.1080/13607860802343076
2. Gartland, N., Connor, D. B. O., Lawton, R., & Bristow, M. (2014). Exploring day-to-day dynamics of daily stressor appraisals, physical symptoms and the cortisol awakening response. *Psychoneuroendocrinology*, 50, 130–138. https://doi.org/10.1016/j.psyneuen.2014.08.006
3. Paridon, K. N. Van, Timmis, M. A., Nevison, C. M., & Bristow, M. (2017). The anticipatory stress response to sport competition; a systematic review with meta-analysis of cortisol reactivity. *BMJ Open Sport & Exercise Medicine*, 1–12. https://doi.org/10.1136/bmjsem-2017-000261
4. Calvi, J. L., Chen, F. R., Benson, V. B., Brindle, E., Bristow, M., De, A., Walker, C. D.

(2017). Measurement of cortisol in saliva: a comparison of measurement error within and between international academic - research laboratories. *BMC Research Notes,* 1–6. https://doi.org/10.1186/s13104-017-2805-4

5. Open access hair validation and method protocol
   - Parker, K. & Bristow, M. (2020). Hair cortisol analysis protocol. https://dx.doi.org/10.17504/protocols.io.bqevmte6
   - Reid, J., Parker, K., Bristow, M. (2020): Hair cortisol concentration method validation study 1. Dataset. https://doi.org/10.6084/m9.figshare.13352822.v2
   - Bristow, M., Clemens, L. & Parker, K. (2020): Hair cortisol concentration method validation study 2. Dataset. https://doi.org/10.6084/m9.figshare.13359695.v2
6. Bhopal, S., Verma, D., Roy, R., Soremekun, S., Kumar, D., **Bristow, M.**, Bhanushali, A., Divan, G., & Kirkwood, Betty (2019) The contribution of childhood adversity to cortisol measures of early life stress amongst infants in rural India: findings from the early life stress sub-study of the SPRING cluster randomised controlled trial (SPRING-ELS). *Psychoneuroendocrinology, 107.* 241-250 (included in REF2).

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

The ARU Biomarker Lab's extensive research experience and innovative methods have enabled over 240 research initiatives for clients of the Lab during the impact period (Evidence 1). These initiatives have enabled the measurement biomarkers of stress in relation to the health and wellbeing of people and animal welfare in the UK and internationally, brought economic benefits for a UK commercial company that utilised the Lab's methodology, and enabled research and innovation, leading to policy recommendations on smoking cessation and changes in coaching recommendations for the England national netball team. These impacts could not have been achieved without the contribution of the Lab, which provided expertise in testing processes established by the research described in section 2, from study design and collection devices, through to research protocols, biomarker analyses and data interpretation.

The ARU Biomarker Lab team are active psychology researchers working within the Division of Psychology and, having developed the methods used in the laboratory to analyse both hair and salvia samples, **Bristow** and **Parker** are uniquely placed to advise clients on study design, collection protocols and the interpretation of the data within the context of behavioural research. The Biomarker Lab at ARU is the only research group operating in the UK to be externally certified by US company Salimetrics, the world leader in salivary biomarker development, as a Salivary Bioscience Center of Excellence (COE), and is one of only nine comparable research groups operating worldwide (Evidence 2). Since 2014 the team have provided 'Spit Camp' training courses to over 100 behavioural researchers wanting to work with saliva and hair biomarkers but who lacked relevant experience to design and implement the projects (Evidence 1). **Bristow** and **Parker** have been able to apply the knowledge gained developing methods and their own research to support complicated projects, such as collecting samples in rural India, working with saliva from infants in neonatal intensive care, and adults with special needs.

Salivary Biomarkers

The ARU Biomarker Lab has contributed with research expertise and techniques in salivary biomarker analysis to several projects investigating smoking behaviour that have featured in NHS, WHO and UK and Dutch Government policy recommendations (Evidence 3). This includes contributing analysis of cotinine levels (a salivary marker of having smoked recently) to a project led by Gilbert at University College London examining smoking cessation cited in strategic policy guidelines published by **Public Health England** (2018) and the **National Institute of Clinical Excellence** (NG183, 2020). The Lab also utilized saliva measures of C-Reactive Protein (CRP) on a vaping study led by Shahab at University College London cited in briefing notes (2017) and an evidence review on vaping (2018) published by the **UK Government**; briefing notes on e-cigarettes published by the **Dutch Government** (2020); and a report on tobacco product regulation published by the **World Health Organisation** (2019).

The ARU Biomarker Lab's research expertise in salivary biomarker analysis enabled a study conducted by **English Institute of Sport** with **England Netball**, the national governing body for England's biggest female team sport with over 97,000 affiliated members, to help inform effective strength and conditioning training. The results identified training-session order as a key consideration when planning the training of elite female athletes and directly led changes in England Netball's coaching practices. While previously strength training was completed prior to netball training, the biomarker findings demonstrated that strength training was more effective when completed after netball training. This research directly led to a change in the training programme of the Vitality Roses, the England national netball team, currently ranked third in the world (Evidence 4).

The ARU Biomarker Lab has contributed extensively to studies conducted by the **Laboratory for Stress and Health Research (STARLab)** at the University of Leeds, and the **Suicidal Behaviour Research Laboratory (SBRL)** at the University of Glasgow (Evidence 5). Without access to the research expertise and innovative assays provided by ARU Biomarker Lab, these studies would not otherwise have been completed. ARU's analyses enabled STARLab to demonstrate that high cortisol reactivity to stress is associated with increased food intake, a finding important in increasing awareness and understanding of markers of wellbeing. The ARU Biomarker Lab also contributed to innovative research by SBRL demonstrating that participants who made a previous suicide attempt exhibited lower cortisol response to acute stress, while lower levels of cortisol in response to stress were associated with higher suicidal ideation. This research changed awareness and understanding of the links between suicidal behaviour and response to acute stress and was presented annually to the **US Department of Defense** between 2016 and 2019**,** who were seeking to improve the wellbeing of combat personnel and veterans. A summary of the cortisol stress findings and their application in a military context was posted as a research highlight on the **Department of Defense CDMRP website** in 2020 (Evidence 6).

Hair Cortisol Biomarkers

The unique method described in Section 2 that the ARU team have developed provides a highly reliable and accurate hair cortisol assay that can be scaled up. As a consequence, it is now possible to utilise measurement of hair cortisol as a biomarker for stress in areas where it has not previously been practical. Since 2014, 45 different research initiatives from the UK, Ireland, Norway, Sweden, and the United States (Evidence 1) have made use of the method. For example, animal welfare is better understood now as applications of the hair cortisol technique has been used to establish the lifetime welfare status of pigs at time of slaughter. Pig bristle is considerably different to the human hair that the labs hair analysis method was developed with and the lab team needed to develop new protocols to prepare the hair for analysis. The research concluded that "Hair cortisol appears to be a useful physiological measure of lifetime welfare status in pigs" (Evidence 7), validating the importance of the ARU Biomarker Lab's work for the understanding of animal welfare. ARU's expertise has also had a pivotal role in studies showing the relationship between acute stress and exposure to natural environments, and work-related stress amongst private sector professionals (Evidence 7).

Economic Benefit

Between 2014 and 2016, the ARU Biomarker Lab was in a formal relationship with **Stratech Ltd** to provide research expertise and innovative methods for biomarker analyses directly to Stratech's national and international clients. Stratech entered the collaboration with the specific objective of using the Lab's expertise to help grow their business model, including promoting the analysis of hair cortisol as a commercial service to their clients. During this period, Stratech increased its sales from £600k to £800k and won its first European tender in 2014, growing to 17 tenders by 2019. From 2017 to the present, the ARU Biomarker Lab has continued to work closely with Stratech and provides expertise, research support and high-quality analysis to Stratech clients (Evidence 8).

Media Engagement (Evidence 9)

Dr. Bristow and the ARU Biomarker Lab team have facilitated the work for several television projects, raising public awareness and understanding of links between stress and wellbeing. The laboratory team have provided extensive expertise to the television production teams in order to accurately measure and interpret saliva and hair biomarkers. This work includes: saliva cortisol analysis to determine if singing in choirs lowers stress levels conducted with the University of Westminster (**Live Well for Longer, Channel 4**) that features as a health benefit of singing on the website **Sing Your Pain Away!**; saliva analysis of cortisol levels in pre- and post- wellbeing activities for 13- to 16-year olds on behalf of the University of Westminster for a feature in **Operation Ouch! (CBBC)** promoted by the **Teen Yoga Foundation**. Saliva from dogs as a measure of their stress levels was tested as part of the show **Dogs: Their Secret Lives (Channel 4),** in collaboration with the University of Bristol, and the laboratory and laboratory team were filmed as part of the series. In the show **Married at First Sight (Channel 4)**. the Biomarker Lab provided very innovative testing of hair samples for a range of biological markers (cortisol, estradiol and testosterone) in hair samples for couples. As far as ARU Biomaker Lab team is aware, they were the first laboratory to analyse estradiol in hair samples. The analysis for Married at First Sight allowed for an examination of the underlying biological nature of relationship formation and was a central part of how couples were matched in the show.

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

1. Contact details for ARU Biomarker Lab Manager (Business Development) to corroborate impact
2. Confirmation of Biomarker Lab's status as Salimetrics-approved Centre of Excellence
3. Details of impact from vaping and smoking cessation projects
4. Testimonial from English Institute of Sport on impact on England Netball coaching methods
5. Testimonial for work with STARLab and SBRL
6. Coverage of cortisol work by US Department of Defense
7. Examples of studies using ARU Biomarker Lab's hair cortisol assays
8. Testimonial and company data from Director of Stratech Scientific
9. Links to examples of media engagement