

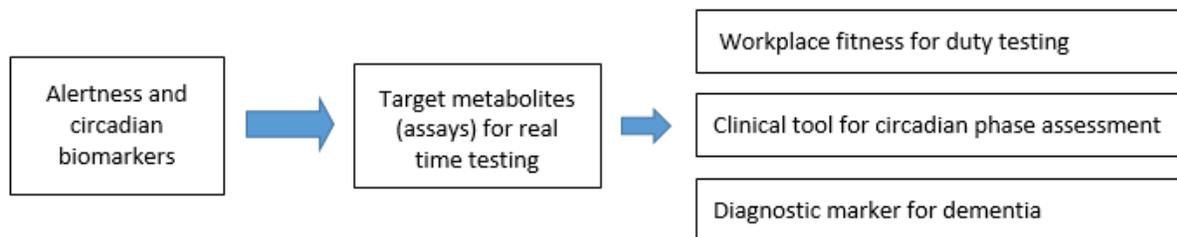
## Project Output Profile: Metabolite Biomarkers

The Alertness CRC has been working to identify a sleep biomarker with biological characteristics that can be objectively measured to determine how long an individual has been awake, or whether an individual has been awake for a specified period (Patent Pending).

The CRC method of identifying cases of sleep deprivation through analysis of samples for particular biomarker metabolites. Our team at Monash University have established a set of biomarkers that accurately predicts the likelihood an individual has been awake for more than 24 hours. This set of biomarkers also accurately predicts individuals who have been awake for between 18-24 hours.

The target metabolites are also being assessed as an accurate marker of circadian phase with current work providing early stage proof of concept for a robust dual time point in clinic measure and an updated algorithm currently being developed.

In addition to the value of the identified metabolites as objective markers of alertness and circadian phase, the body of work has been extended to further develop the application of these markers in relation to sleep health and dementia risk (additional confidential information available under NDA) .



### Alertness Testing

The Alertness CRC is currently able to provide an accurate assessment of sleep deprivation, which has in certain embodiments obtained an accuracy of 99.7% in distinguishing cases of base-line (0-16 hours' time since wake) compared to sleep deprivation (24-38 hours' time since wake).

A total of 23 metabolites have been identified as being important for determining how long an individual has been awake. The team has developed metabolomic signatures (e.g., mass, retention time) for all these metabolites. Of these 23, six have been classified (at either level 1 or level 2).

Using those **23** metabolites, a blood sample can provides a prediction as to whether an individual has been awake for >24 hours (compared to <16hours) with high accuracy:

- Training set model prediction accuracy: **98.9%** (98.9% and sensitivity is 97.7%)
- Testing set model prediction accuracy: **97.1%** (97.6% and sensitivity is 92.8%)

Analysis using the **six** classified metabolites only provides high accuracy in predicting 24 hours awake:

- Training set model prediction accuracy: **95.6%** (97.8% and sensitivity is 94.4%)
- Testing set model prediction accuracy: **91.6%** (specificity is 92.9% and sensitivity is 84.5%)

High levels of accuracy are also maintained when varying levels of sleep loss (e.g. 22 hours) are assessed. The threshold of 24 hours was chosen because it is the 'legal limit' in the US for many states, and is consistent with double the legal limit of alcohol from a performance impairment perspective.

### Applications

There are many potential applications for a method of objectively identifying how long a person has been awake or the extent of a person's sleep deprivation. These would naturally include the ability to regulate sleep deprivation in car/motorcycle/heavy vehicle operators (random roadside or post-accident testing); and of employees and others performing safety critical roles (fitness for duty testing).

In addition, other applications include:

- (a) testing for sleep deprivation in healthcare settings where inadequate sleep may impact disease diagnosis or prognosis (e.g. diabetes, dementia, stroke), or where inadequate sleep may affect the performance of a particular treatment (e.g. chemotherapy);
- (b) testing for sleep deprivation in the context of competitive sports to optimize or otherwise measure performance during events, as well in relation to sports training and preparation;
- (c) analyzing the effects of sleep deprivation in safety critical operations (e.g. space travel or military); and,
- (d) accurate testing of sleep deprivation or deficiency in scientific and research studies (e.g. pharmacological trials, sleep and circadian medicine).

*Alertness CRC is exploring a range of options to further the use of its research, technology and products and is open to speaking with a range of interested entities from investors to licensees and commercialization partners. Additional public information is available at: <https://mjkpartners.com/opportunities/alertnesscrc/> or contact Myron Kassaraba, MJK Partners, LLC, Tel. 617-902-0639, [myron@mjkpartners.com](mailto:myron@mjkpartners.com).*