Overview of Urinary Pyrrole/Mauve Factor Analysis

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Urinary pyrrole (hydroxyhemopyrroline-2-one or HPL, Mwt; 123gmol$^{-1}$) is a labile intermediate lactam generated during the oxidative degradation of heme and its derives (biliverdin, biliruben and urobilinogen) in situ$^{12}$. 

Increased excretion of HPL can result from a range of factors including (but not limited to) a genetic disorder affecting haemoglobin synthesis, the accelerated oxidative degradation of heme and its derivatives (a form of oxidative stress), or from the disruption of this endogenous cycle and has been described as a common feature of many behavioural disorders (also referred to as Pyrroluria)$^{3,4,5}$. HPL is detectable in human urine, faeces, blood, and cerebrospinal fluid and can be eliminated by dialysis$^6$. A practical and reliable blood test for HPL is not possible due to the myriad of interfering substances$^{12}$, thus urine remains the preferred testing substrate. In fact, the most common (and very high) false positive results arise from the presence of blood in the specimen.

HPL is commonly mis-represented as kryptopyrrole (Figure 1), a chemically similar compound used as the standard for colorimetric HPL assay$^7$. This distinction has been confirmed by synthesis$^{8,9,10}$, Gas-Liquid chromatography (GC)$^{10}$ and liquid chromatography-mass spectrometry (LC-MS)$^{12}$. These methods compared favourably (linear r=0.98) with the colorimetric method used in this laboratory$^{12}$ (urinary HPL levels are measured quantitatively by solvent extraction, reaction with a developing reagent, and spectrophotometric measurement at 540nm)$^{4,7}$. Second morning void (or random – providing it’s not the first) specimens are collected into vials containing preservative, and snap frozen (-30°C). Samples are transported on dry ice (-30°C) to ensure the temperature remains constant and they remain frozen and protected from light until analysis.

Figure 1 – HPL and kryptopyrrole chemical structures.
HPL is very reactive and decomposes as soon as it leaves the body. It has a half-life of 10-12 hours\textsuperscript{11} (which means that if not frozen, after the first 24 hours, the level of HPL in the sample will be approximately 20% of the original value and 5% of the original value after 48 hours – which is also dependent on ambient temperature and other factors). In terms of results, this property results in enormous variability (which is not desirable for a reliable diagnostic test). In early development work on the urinary pyrrole test in Australia by this author, it was also found that exposure of samples to direct sun-light (or collection in a room illuminated by direct sunlight) resulted in almost instantaneous elimination of all HPL activity. In almost all cases in this laboratory, no detectable HPL has been found in samples that have thawed during transit (or arrived unfrozen). As a laboratory providing a national diagnostic service, it is imperative to provide the most reliable, accurate, precise, and consistent results as possible and as a result we have developed a uniform collection and transport protocol for all samples. The freezing of samples (and for them to remain frozen until analysis) is crucial to the successful and accurate analysis of urinary HPL at this time. In the future, Applied Analytical Laboratories will be introducing a technique (currently under development) for room temperature stabilization of the analyte using a novel process. This technique has shown promise, however more validation is required before roll-out.

The working ranges for urinary HPL levels using the method employed at Applied Analytical Laboratories are as follows:

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[HPL] < 10 \mu g/dL: \text{Normal} \\
10 \mu g/dL < [HPL] < 20 \mu g/dL: \text{Borderline} \\
[HPL] > 20 \mu g/dL: \text{Elevated}
\]

These ranges have been validated by clinical trial where mental health in-patients (150) and controls (50) were tested.

References
5. William J Walsh, Laura B. Glab, Mary Haakenson; Reduced violent behaviour following biochemical therapy, Physiology & Behaviour 82, 2004 835 -839.

Author’s Brief History.

Brett Lambert is a highly qualified scientist with over 25 years’ experience in research & development (pharmacology, pharmaceutical lead discovery, separation science and structural elucidation), as well as commercial laboratory management.

Brett was approached in 2003 by the Bio-balance Health Group to determine the feasibility of urinary pyrrole analysis in Australia. Following adaptation and development, the test was introduced to and used by medical practitioners undergoing specific training in the field of treating mental health.

Since its introduction, Brett has performed approximately 30,000 urinary pyrrole tests and is the most experienced scientist in Australia performing this work.

In 2013, Brett was acknowledged by the Bio-Balance Health group for his support and contribution to medical practitioner training in Australia.

Applied Analytical Laboratories provides a urinary pyrrole analysis service for medical practitioners through national pathology collection agencies and its analysis method has been developed to provide a reliable and consistent testing service to all regardless of geographic location.