THE PRODUCTION OF ANTIBODIES TO THE POLYCYCLIC AROMATIC HYDROCARBON (PAH) BIOMARKER 1-HYDROXYPYRENE GLUCURONIDE (1-OHPyrG) AND THE DEVELOPMENT OF TEST KITS FOR COST-EFFECTIVE LABORATORY ANALYIS AND ON-SITE PAH EXPOSURE SCREENING.

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Objectives

PAHs are complex mixtures with known adverse health effects. PAHs are produced during the incomplete burning of organic materials. Pyrene is a common component of PAH mixtures and metabolism of pyrene leads to the excretion of 1-OHPyrG (Fig. 1) in urine. The determination of urinary 1-OHPyrG has allowed the development of PAH biomonitoring programmes. The production of antibodies to 1-OHPyrG will allow the development of novel biomonitoring tests, facilitating laboratory analysis and on-site screening.

Methods

A high affinity sheep monoclonal was raised to 1-OHPyrG and a urinary ELISA developed. An anti-complex conjugate is used to develop the assay in a "sandwich" format and provide a positively correlated signal (Fig. 2). Variations in the urine matrix were overcome with a carefully formulated assay diluent.

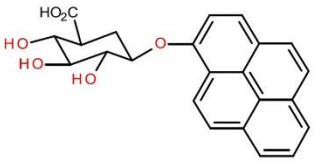

Fig. 1. 1-OHPyrG Biomarker

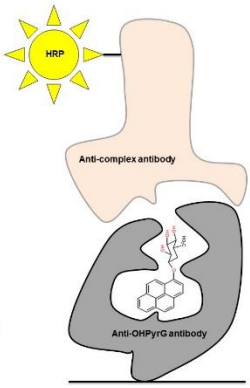

Fig. 2. An anti-complex conjugate develops the ELISA

Results

A sensitive (LOD 1.2pg/ml) and specific ELISA was developed (Fig. 3). The assay was used to detect elevated levels of 1-OHPyrG in the urine of workers exposed to PAHs. Samples determined by ELISA and HPLC-fluorescence (UK HSL) were in good agreement (correlation = 0.9, range 0-1140nM, n=19. Fig. 4). Urinary concentrations determined by ELISA agree with literature values (NHANES 95th percentile = 569ng/L, smokers x2, Fig. 5). Careful consideration was given to assay presentation and a test kit has been manufactured. An assay can be completed in less than 4 hours and 1 kit allows 40 samples to be determined in duplicate.

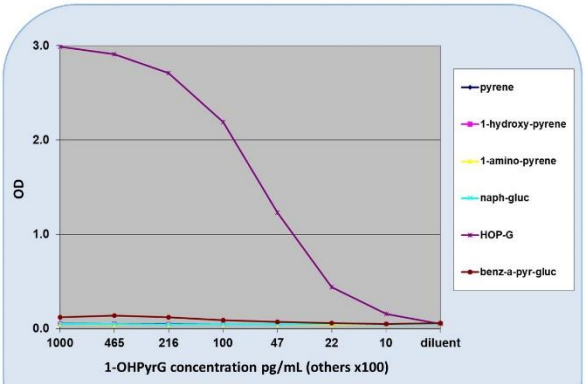

Fig. 3. 1-OHPyrG Sensitivity and Specificity

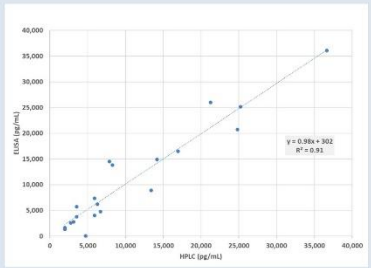

Fig. 4. 1-OHPyrG ELISA v HPLC Fluorescence Correlation Study

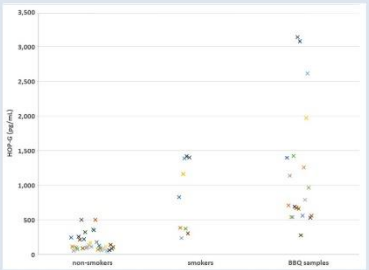

Fig. 5. Urinary 1-OHPyrG in background populations.

Conclusion

The development of an ELISA for 1-OHPyG has allowed the production of a PAH biomonitoring test kit. Test kits increase the accessibility of biomonitoring and facilitate the introduction of routine screening. Alternative test formats are being investigated. A lateral flow test is nearing completion. This will enable on-site analysis and allow employers to demonstrate the immediate impact of good working practice. A biomonitoring 'app' has been designed which will support occupational health professionals and provide individuals with easy to view results and interpretation. These developments will increase the utility of biomonitoring and improve the protection of workers health.

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