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 analysis 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 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 and Results

Antisera were raised to 1-OHPyrG and a competitive urinary ELISA developed (Fig. 1). Variations in the urine matrix were overcome with a carefully formulated assay diluent. The ELISA has a Limit of Detection of 0.5nM. The assay is specific (Fig. 2) and robust (Fig. 3).

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.

A field trial is ongoing. The assay can detect elevated levels of 1-OHPyrG in the urine of workers exposed to PAHs. Samples determined by ELISA and HPLC-fluorescence are in good agreement (Corr. Coeff. 0.9, range 0-1410nM, N=60) . A biomonitoring app is under consideration. This will support data collection, presentation, comparison and interpretation of test results.

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 will enable on-site analysis. On-site analysis increases the utility of biomonitoring and allows employers to demonstrate the immediate impact of good working practice. A biomonitoring app is being designed which will support occupational health professionals and provide individuals with easy to view results and interpretation. These developments will increase the utility of PAH and improve the protection of workers health.

⬇ **Pipette**	50µl Assay Diluent into wells
⬇ **Pipette**	10µl Standards, Controls & Samples into wells
⬇ **Pipette**	100µl of Anti-OHPyrG 1°ab into all wells.
⬇ **Incubate**	R/T for **120 minutes.**
⬇ **Wash**	Wash x3
⬇ **Pipette**	100µl of Anti-Sheep-HRP into all wells.
⬇ **Incubate**	R/T for **30 minutes.**
⬇ **Wash**	Wash x5
⬇ **Pipette**	100µl TMB Substrate reagent into all wells.
⬇ **Incubate**	R/T for **30 minutes.**
⬇ **Pipette**	100µl Acid Stop Solution into all wells.
⬇ **Read**	**450nm** wavelength.
⬇ **Calculate**	1-OHPyrG results for all Controls/Samples.

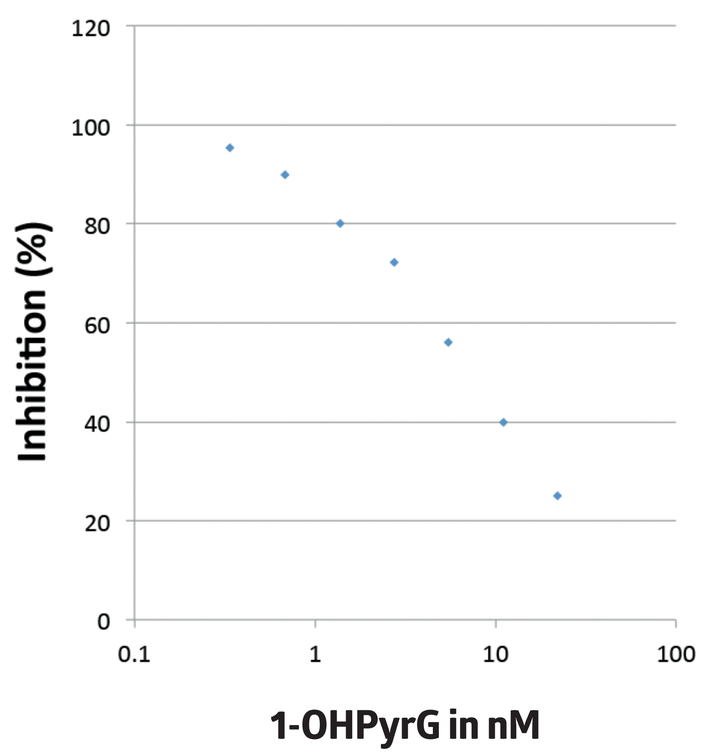

Fig. 1 An ELISA for the determination of urinary 1-OHPyrG

	Cross Reactivity	Urine Background
OH-Pyrene	0.5-50nM	90ng/l (0.4 nM)
1-Naphthol	<10 mM	2680 ng/l (18.6 nM)
2-Naphthol	<10 mM	2470 ng/l (17.1 nM)
9-Phenanthol	0.05 mM	267 ng/l (0.37 nM)

Fig. 2.1 OHPyr G assay cross-reactivity

Within Assay Variation			Between Assay Variation		
Mean	17.8	4.9	Mean	28	4.5
SD	0.8	0.4	SD	2.6	1.0
CV	4.7%	8.5%	CV	9.6%	22.2%
N	4	4	N	4	4

Fig. 3 Intra and inter urinary 1-OHPyr G assay variation.