

## The Development of Novel Immunoassays and the production of Biomonitoring Test Kits for the cost-effective laboratory analysis and on-site screening of Benzene Exposure.

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### Objectives

Benzene is a significant industrial chemical, a ubiquitous environmental pollutant and a known human carcinogen. S-phenylmercapturic acid (S-PMA) is a urinary metabolite of benzene and a specific and sensitive biomarker of benzene exposure. Measurement of urinary S-PMA has allowed the introduction of occupational and environmental biomonitoring programmes. However, current methods of laboratory analysis are laborious, expensive and provide slow sample turnaround. Immunoassays overcome these limitations, enable the development of simple to employ test kits and support the introduction of routine screening programmes.

### Methods and Results

The production of an antisera specific for S-PMA has allowed the development of a competitive ELISA for urinary S-PMA. The assay (Fig. 1) has a measuring range of 1.2-1200ng/ml and was validated in a comparative study against an LC-MS/MS procedure.

Test kits have been manufactured (Fig. 2) and used to detect elevated levels of S-PMA in the urine of workers exposed to benzene. Occupational samples determined by ELISA and LC-MS/MS were in good agreement ( $R=0.9$ , range 0-1130ng/ml,  $n=39$ ). Each test kit allows up to 40 samples to be determined in duplicate and an assay can be completed in less than 4 hours.

A biomonitoring app is now under consideration. This will support data collection, presentation, comparison and interpretation of test results.

### Conclusion

Immunoassays enable the production of cost-effective benzene biomonitoring tests. An ELISA kit has been produced which will allow laboratories to provide a routine test service. A point of care test is being developed which will enable on-site testing and provide immediate confirmation of good working practice. Mobile technology will enable data capture, transfer and the centralised provision of consultancy and support services. A biomonitoring app is being designed which will support occupational health professionals and provide individuals with easy to view results, comparisons (tracking and trending) and interpretation. These developments will increase the utility of benzene biomonitoring and improve the protection of workers health.

- ↓ **Pipette** 50µl Assay Diluent into wells
- ↓ **Pipette** 10µl Standards, Controls & Samples into wells
- ↓ **Pipette** 100µl of Anti-S-PMA 1<sup>st</sup> 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** S-PMA results for all Controls/Samples.

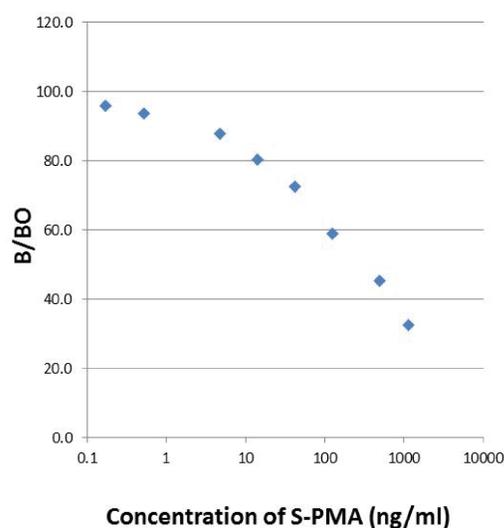


Fig. 1 An ELISA for the determination of urinary S-PMA



Fig. 2 Biomonitoring test kits contain all the reagents needed to determine urinary S-PMA.