

Chemitrace Limited

THE PRODUCTION OF ANTIBODIES TO THE BENZENE BIOMARKER S-PMA AND THE DEVELOPMENT OF TEST KITS FOR COST-EFFECTIVE LABORATORY ANALYSIS AND ON-SITE BENZENE EXPOSURE SCREENING.

Dr. Lathan Ball - CEO



BENZENE

Industrial Chemical & Environmental Pollutant

oil refineries, chemical manufacturing, petrol stations, vehicle exhaust, cigarette smoke

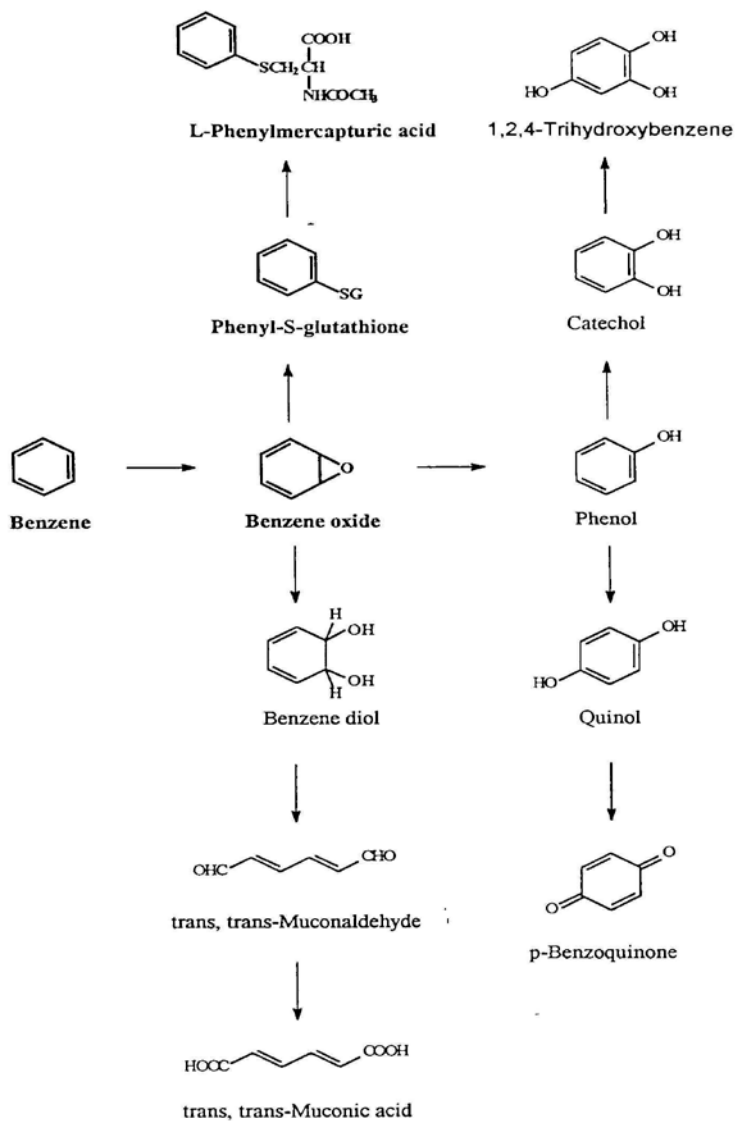
Human Carcinogen

Aplastic anaemia, leukaemia

Occupational Exposure Limits

US 1 ppm, Europe 1ppm (8hr/TWA)

Benzene Metabolism



The toxicity of benzene and its metabolism and molecular pathology in human risk assessment.

A Yardley-Jones, D Anderson, and D V Parke
Br J Ind Med. Jul 1991; 48(7): 437-444.

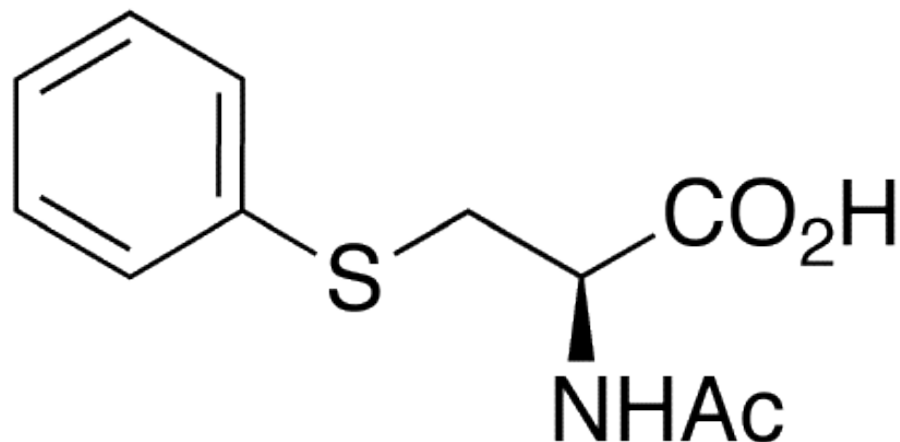
S-PMA is the marker of choice

Recommended:

UK HSE

US ACGIH

German DFG



Biological Guidance Values:

Germany (DFG) 45ug S-PMA/ug creatinine

USA (ACGIH) 25ug S-PMA/ug creatinine

Benzene Biomonitoring

Traditional Analytical Methods

- Require a complicated work-up prior to analysis
- Need skilled technical operators
- Restricted to larger laboratories with sophisticated analytical equipment
- Not routinely available so slow sample turnaround
- Expensive

Immunoanalytical Methods

- Development of simple and easy to perform immunoassays
- Can be performed in many laboratories
- Rapid sample analysis and result reporting
- Cost-effective
- Allow the development of test kits for large sample numbers
- Allow the development of “point of care” tests

Point of Care Testing

Allows “Real-time” Analysis

- Monitor immediate impact of Good Working Practice
- Allow immediate removal of potentially exposed workers
- Provides on the job reassurance

Low Cost

- Eliminate negative samples
- Selection of positive samples for further analysis
- Encourages increased testing

Benzene Biomonitoring



Colour coded standards for easy recognition



High and Low Quality Controls



Coloured dyes aid pipetting

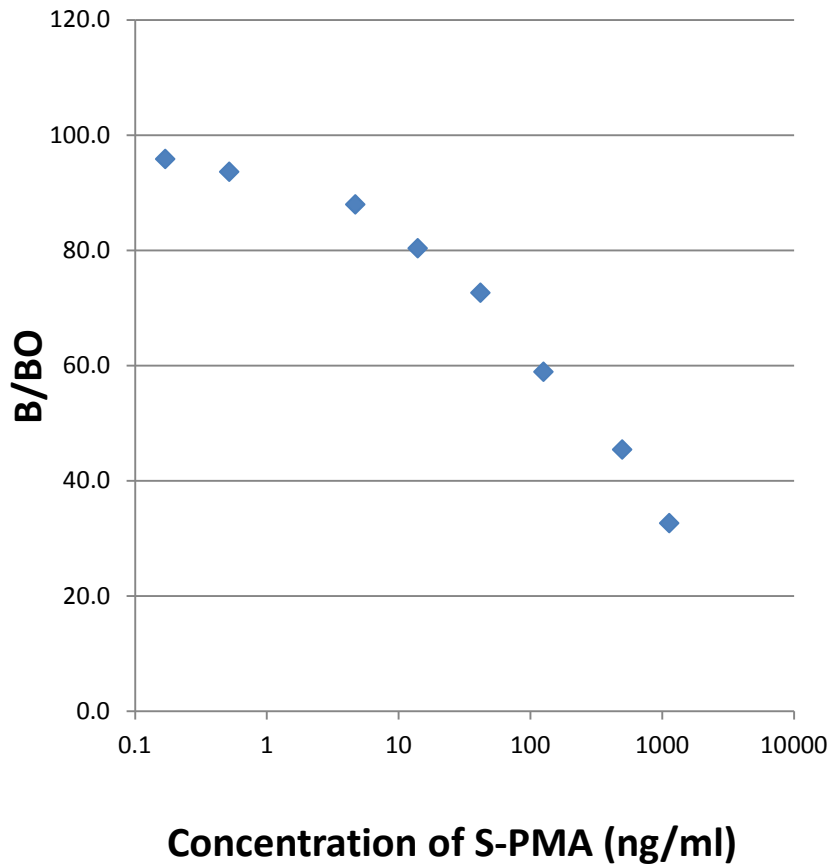


Re-sealable for future use

S-PMA ELISA

Pipette	10ul Standards, Controls and Samples in to wells
Pipette	50ul Assay Diluent in to wells
Pipette	100μL of Anti-S-PMA 1° ab. into all wells.
Mix & incubate	at room temperature for 120 minutes .
Wash wells	x4 with Wash Solution.
Pipette	100μL of Anti-Sheep-HRP into all wells.
Mix & incubate	at room temperature for 30 minutes .
Wash wells	x4 with Wash Solution.
Pipette	100μL TMB Substrate reagent into all wells.
Mix & incubate	at room temperature for 30 minutes .
Pipette	100μL Acid Stop Solution 1 into all wells.
Mix & read	wells at 450nm wavelength
Calculate	S-PMA results for all Controls/Samples.

Urinary S-PMA Immunoassay - Dose Response



Test Kit:

Measuring range – 1.2 -1000ng/ml

Samples – 40 in duplicate

Time – 4 hours

Intra-assay variance:

Mean: 160ng/ml, SD: 11, CV 5%, N: 6

Inter-assay variance:

Mean: 18.1ng/ml, SD: 1.9. CV 10.9%, N: 9

Mean: 44.5ng/ml, SD: 5.4. CV 12.1%, N: 9

Correlation with LC-MS/MS = 0.9

Range = 0 – 1130 ng/ml. N = 39

A “Point of Care Test” POCT for Benzene Biomonitoring

Chemitrace is working in collaboration with BBI Solutions to develop a simple to use, rapid and cost-effective POCT.

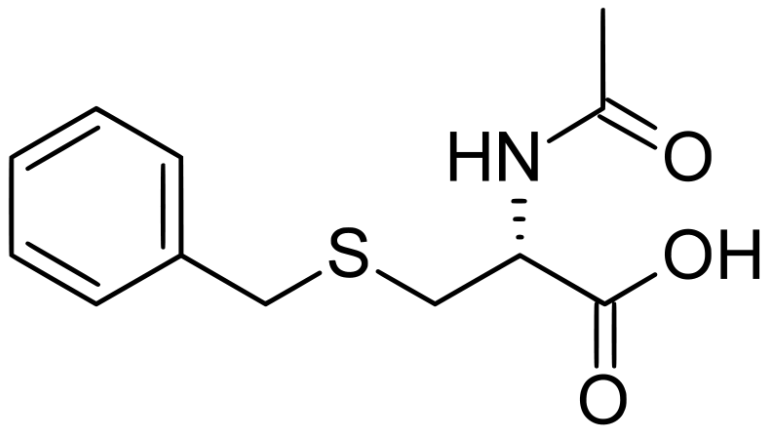


Fab Fragments and Assay Development

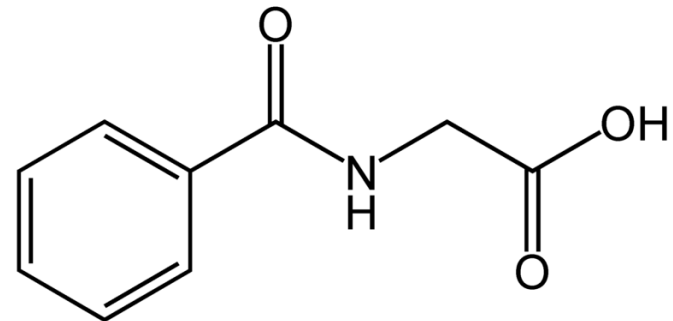
- **Exceptional sensitivity - picomolar affinities**
- **Fast and reliable bulk production in E. coli**
- **Engineered free of Cysteine residue**
- **More antigen binding sites per milligram of antibody**
- **Reduced non-specific binding**
- **His tag**

Antibody Specificity

Cross-reactivity with structurally similar metabolites:



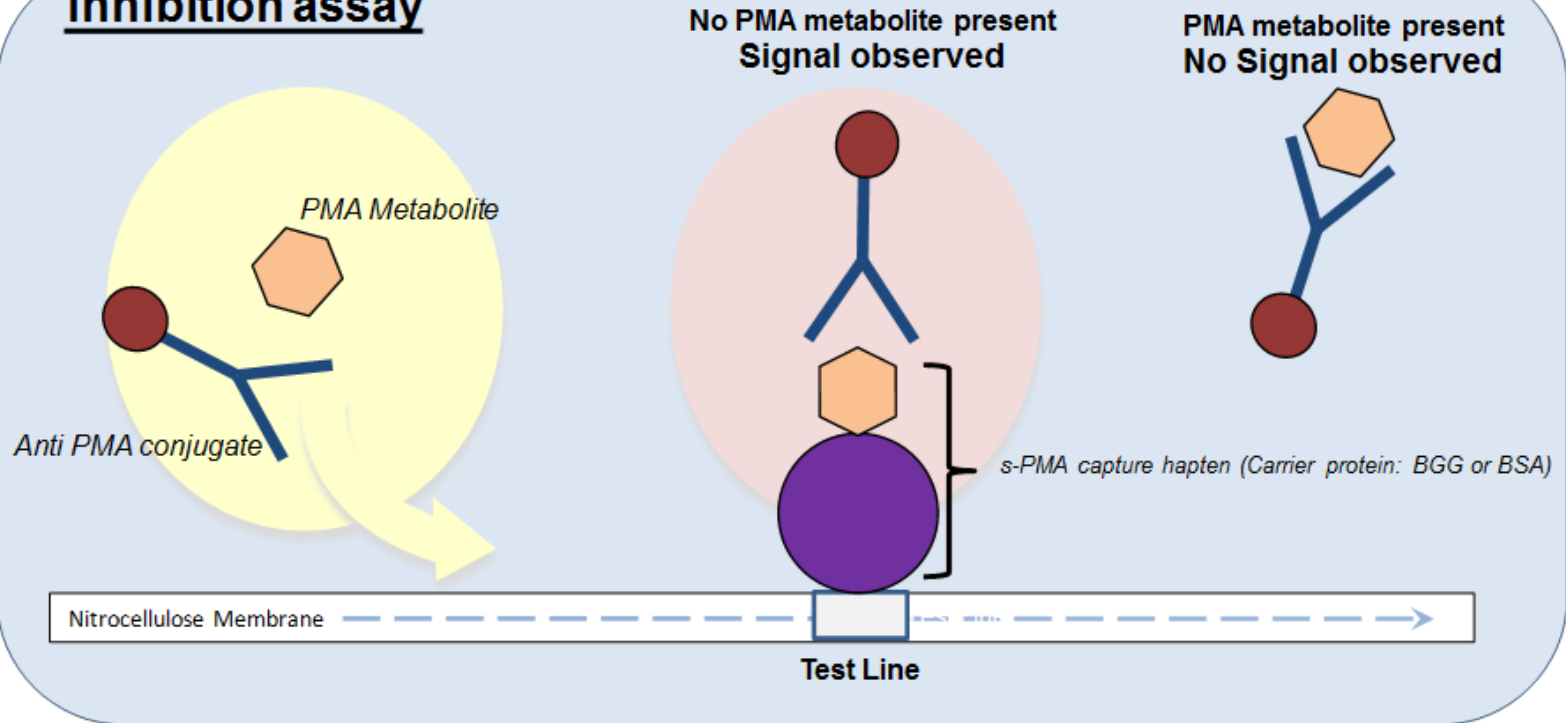
Benzylmercapturic Acid



Hippuric Acid

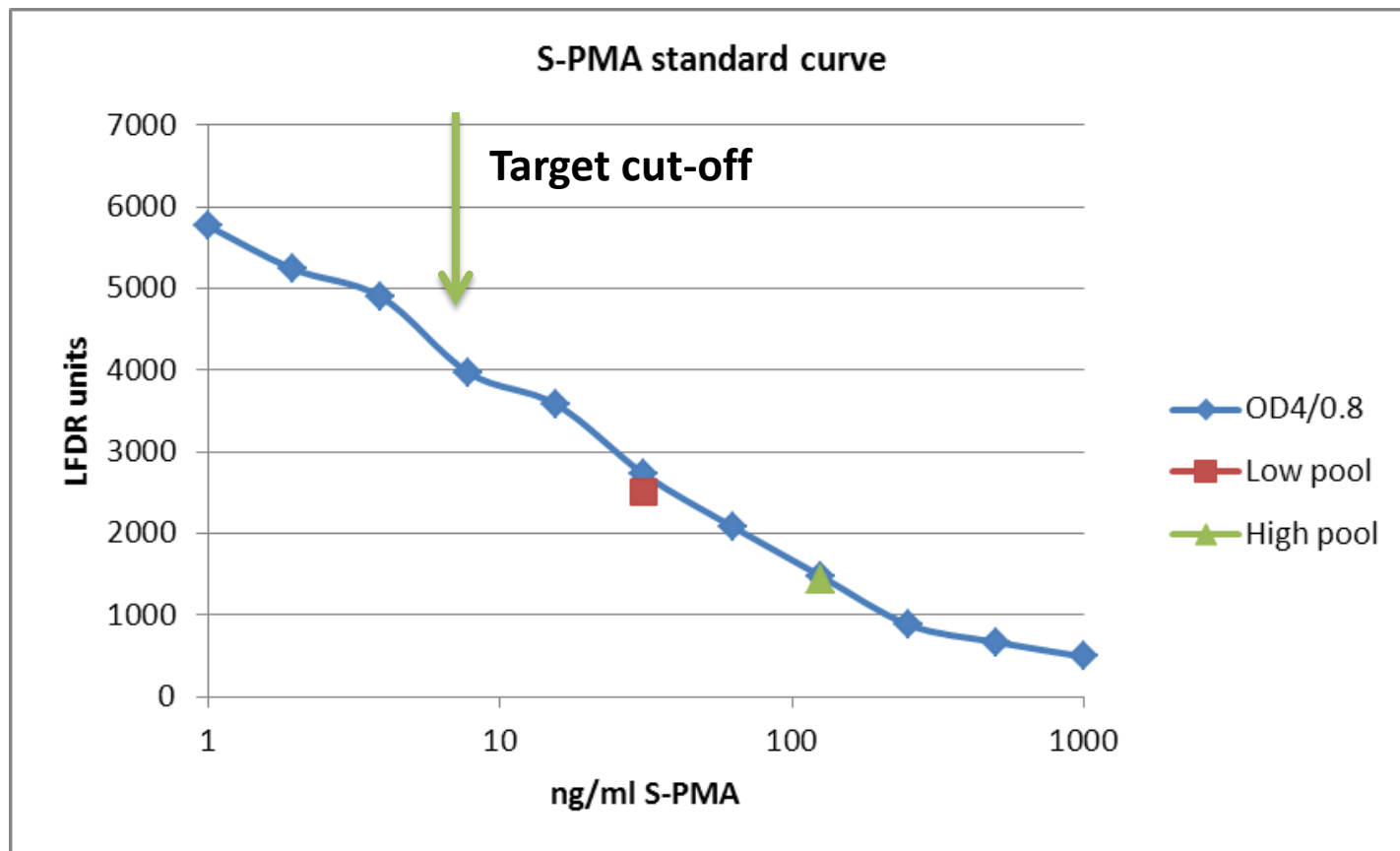
Development and validation of a competitive immunoassay for urinary S-phenylmercapturic acid and its application in benzene biological monitoring. Aston JP, Ball RL, Pople JE, Jones K, Cocker J. Biomarkers. 2002 Mar-Apr;7(2):103-12.

Inhibition assay



S-PMA "POCT" FORMAT

A Benzene Biomonitoring POCT



S-PMA Concentration v Hydration Status

	Dil.	–	Normal	–	Conc.
Background Non-smokers	0.60	-	2.98	-	5.97
Background Smokers	1.08	-	5.41	-	10.83
0.5ppm (ACGIH BEI)	7.50	-	37.5	-	75.00
1.0ppm (German BEI)	13.5	-	67.5	-	135

A cut-off of 7.5 – 10 ug/l would result in approximately 12% of samples screening positive (UK HSL)

Conclusion - Future

Immunoassay can play a significant role in the development of biomonitoring

An ELISA test kit for benzene biomonitoring has been developed

- cost-effective screening by occupational and environmental laboratories.**

We have demonstrated the feasibility of a POCT

- allowing real-time monitoring and on the job intervention.**

- significantly reduce the costs**



Thank you

**Dr Karen Whiting, Dr Amanda Harris
from BBI Solutions**

**Dr John Cocker and Dr Kate Jones
UK Health and Safety Laboratory.**

Chemitrace Limited

Simple and cost-effective
benzene biomonitoring test kits.

lathanball@chemitrace.com

