

Chemitrace

**Novel monoclonal antibodies for 1-Hydroxypyrene
Glucuronide (1-OHPyrG), the production of a Lateral Flow (LF)
test and the development of on-site Polycyclic Aromatic
Hydrocarbon (PAH) biomonitoring**

Dr. Lathan Ball - CEO



Polycyclic Aromatic Hydrocarbon (PAH) Biomonitoring

Project Partners:

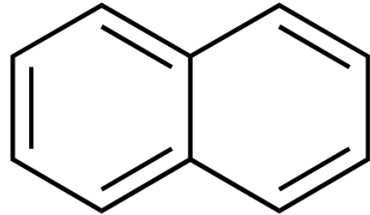


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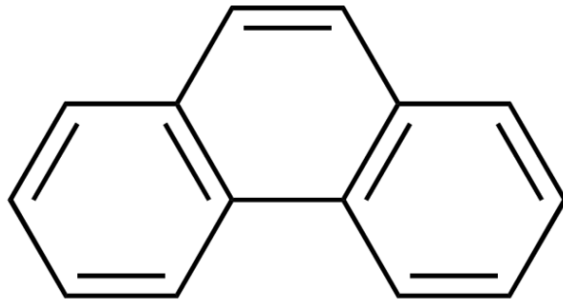
Polycyclic Aromatic Hydrocarbons - PAHs



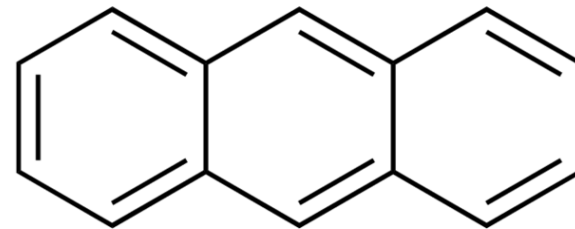
Naphthalene

PAHs are a class of more than 100 chemicals produced during the incomplete burning of organic materials.

PAHs contain two or more single or fused aromatic rings



Phenanthrene



Anthracene

Sources of PAHs

Sources of PAHs can be both natural and anthropogenic:

Natural sources

- forest fires
- oil seeps
- volcanoes
- green plants, fungi, and bacteria

Anthropogenic sources

- oil and petroleum refinery
- electricity generation
- waste incineration
- home heating
- production of coke, carbon black, coal tar, and asphalt
- vehicle exhaust

PAH Health Effects

PAHs have been classified as –

- Carcinogenic
- Mutagenic
- Immunosuppressent

PAH exposure occurs through -

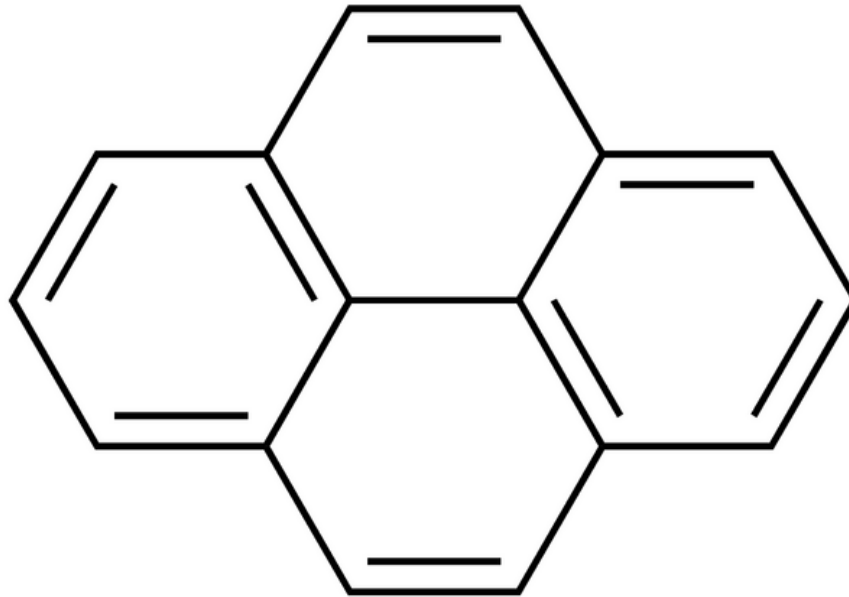
- Inhalation
- Absorption
- Ingestion

PAH exposure occurs on a regular basis

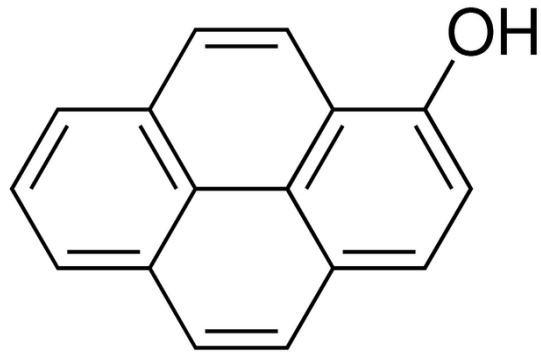
PAH BIOMONITORING

Pyrene is a common component of PAH mixtures.

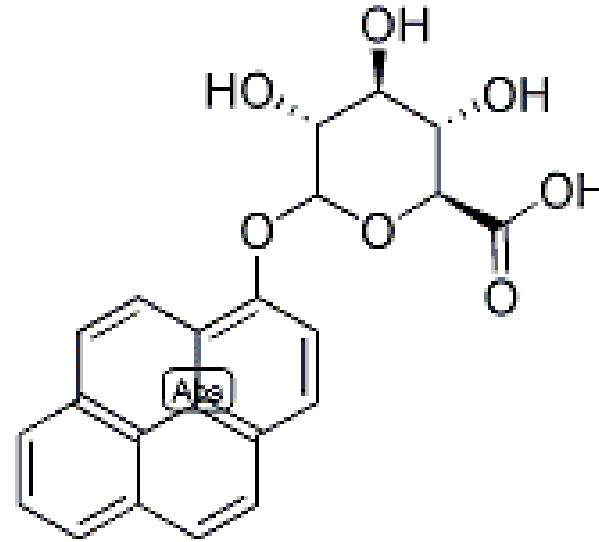
Pyrene



PAH BIOMARKERS



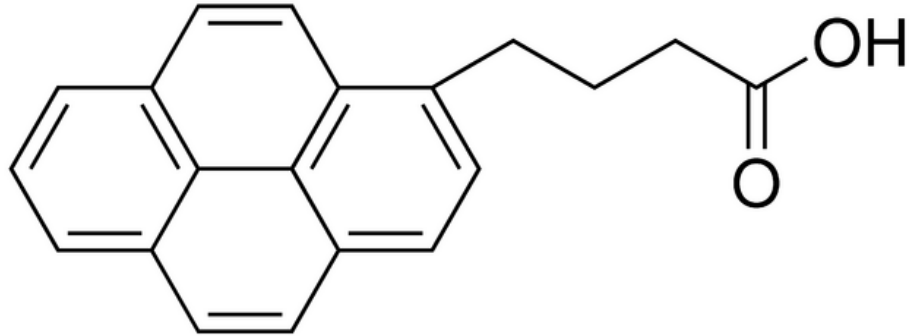
1-Hydroxypyrene



1-Hydroxypyrene Glucuronide

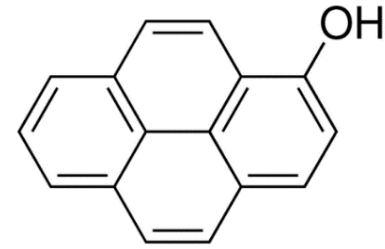
The metabolism of pyrene leads to the excretion of 1-hydroxypyrene and 1-hydroxypyrene glucuronide in urine

ANTIBODY PRODUCTION

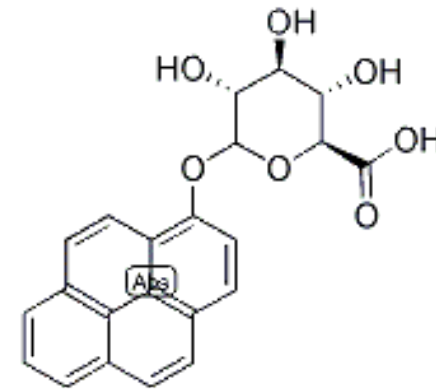


1-Pyrenebutyric

1-Pyrenebutyric acid was coupled to KLH with the cross-linker EDC (*N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide) and the conjugate used to induce antisera to OHPyr and OHPyrG in sheep

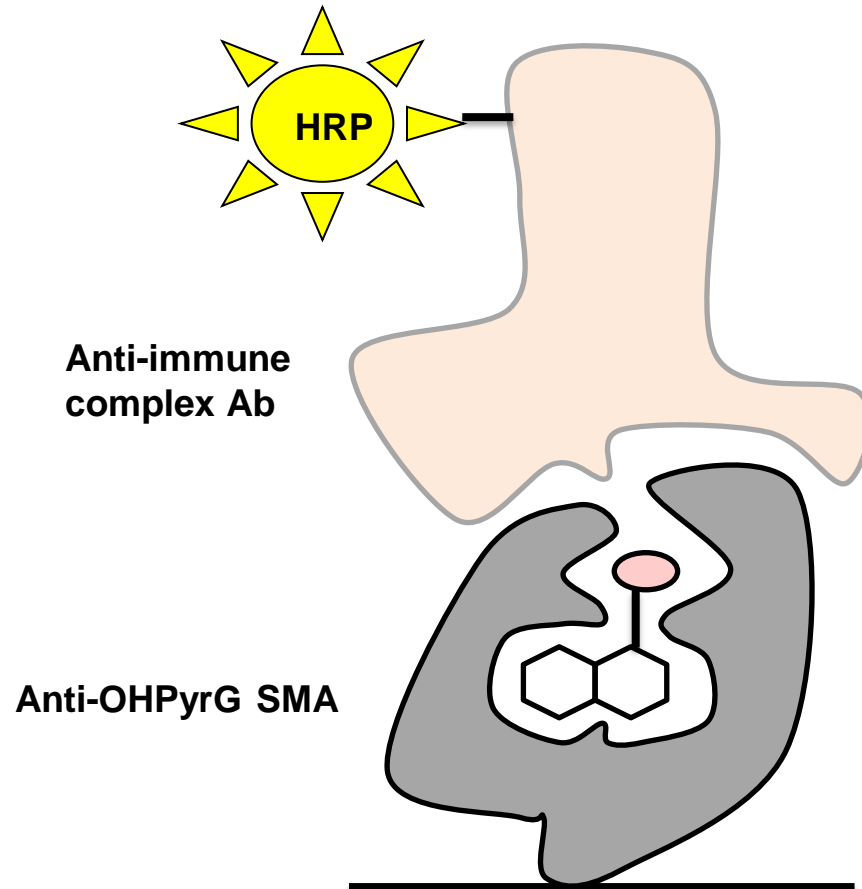


1-Hydroxypyrene



1-Hydroxypyrene Glucuronide

1-OHPyrG ELISA & Assay Kit



Format Advantages:

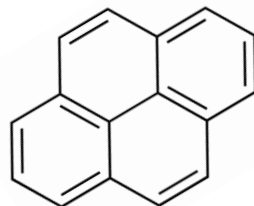
Sensitivity

Specificity

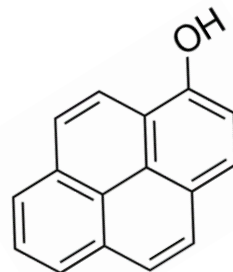
Positively correlated signal



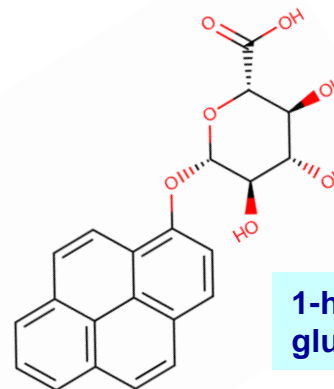
1-OHPyrG and Closely Related Pyrenes



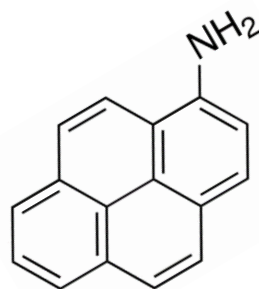
pyrene (pyr)



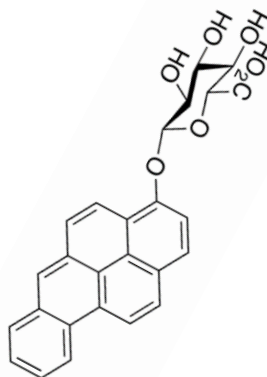
1-hydroxypyrene (1-OHPyr)



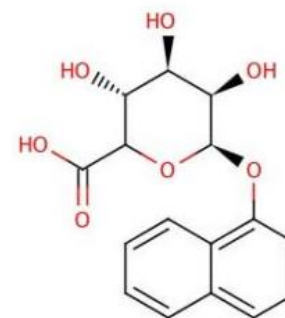
1-hydroxypyrene-glucuronide (1-OHPyrG)



1-aminopyrene



3-Hydroxy benz-a-pyrene glucuronide



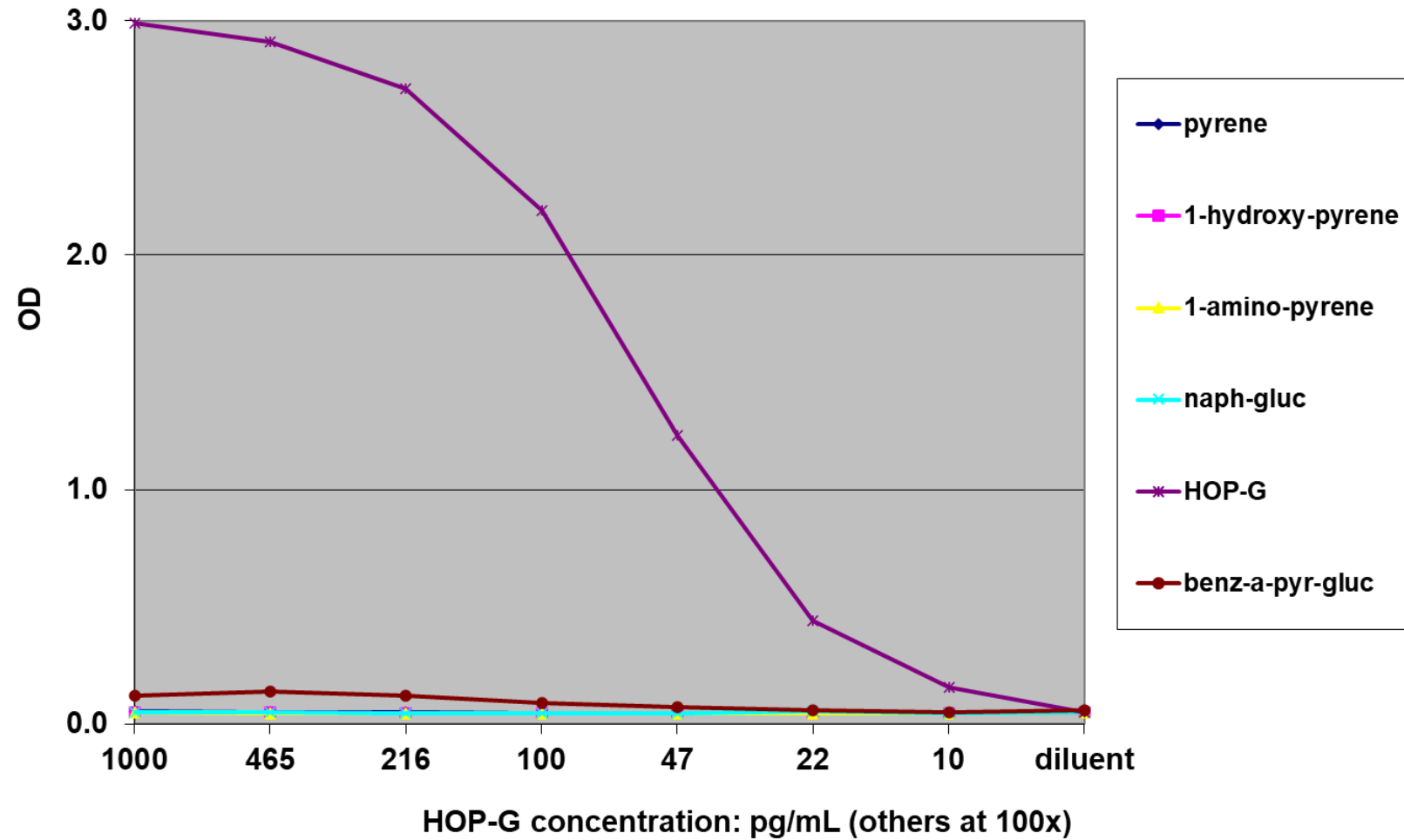
Naphthol-glucuronide



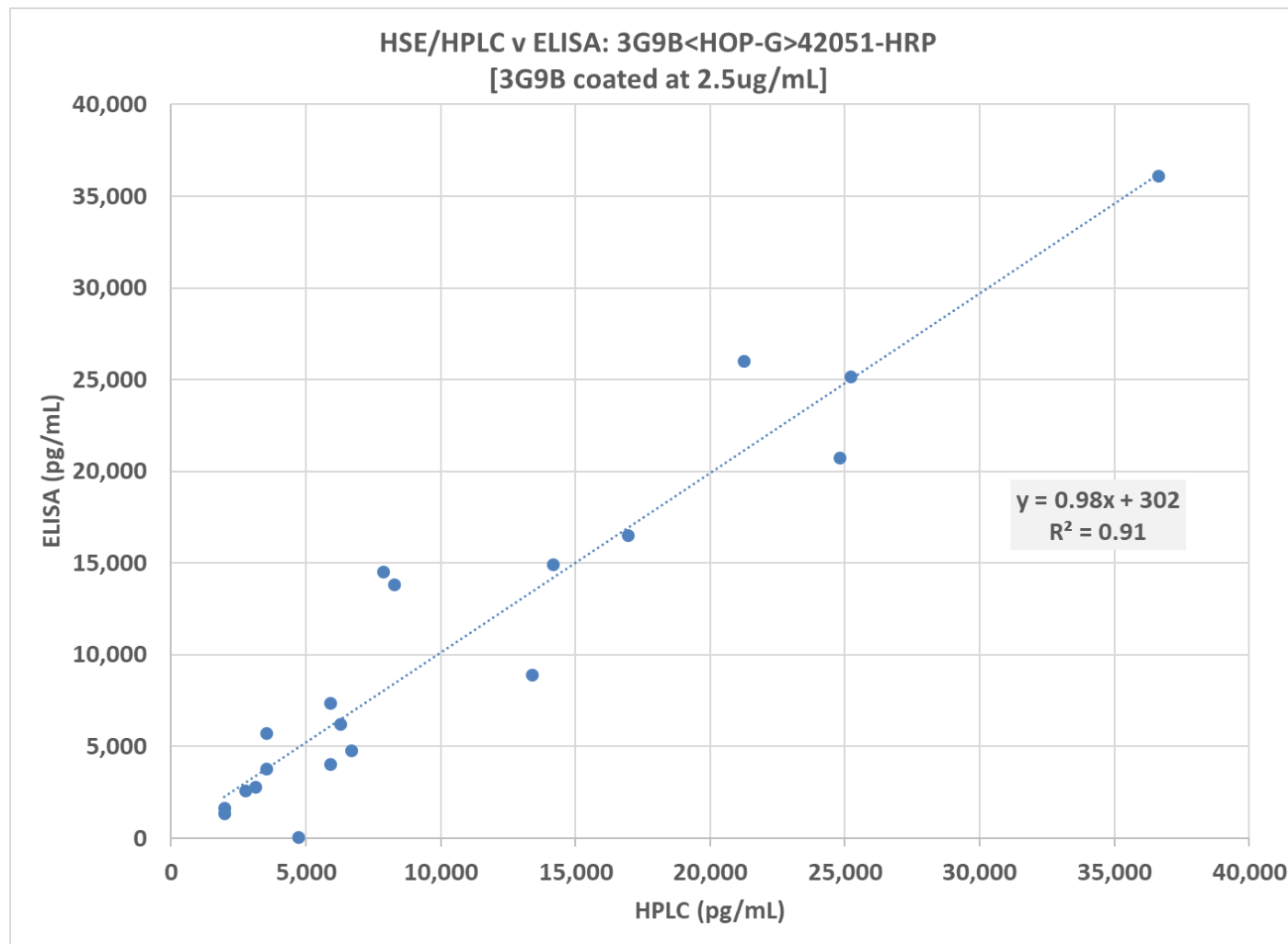
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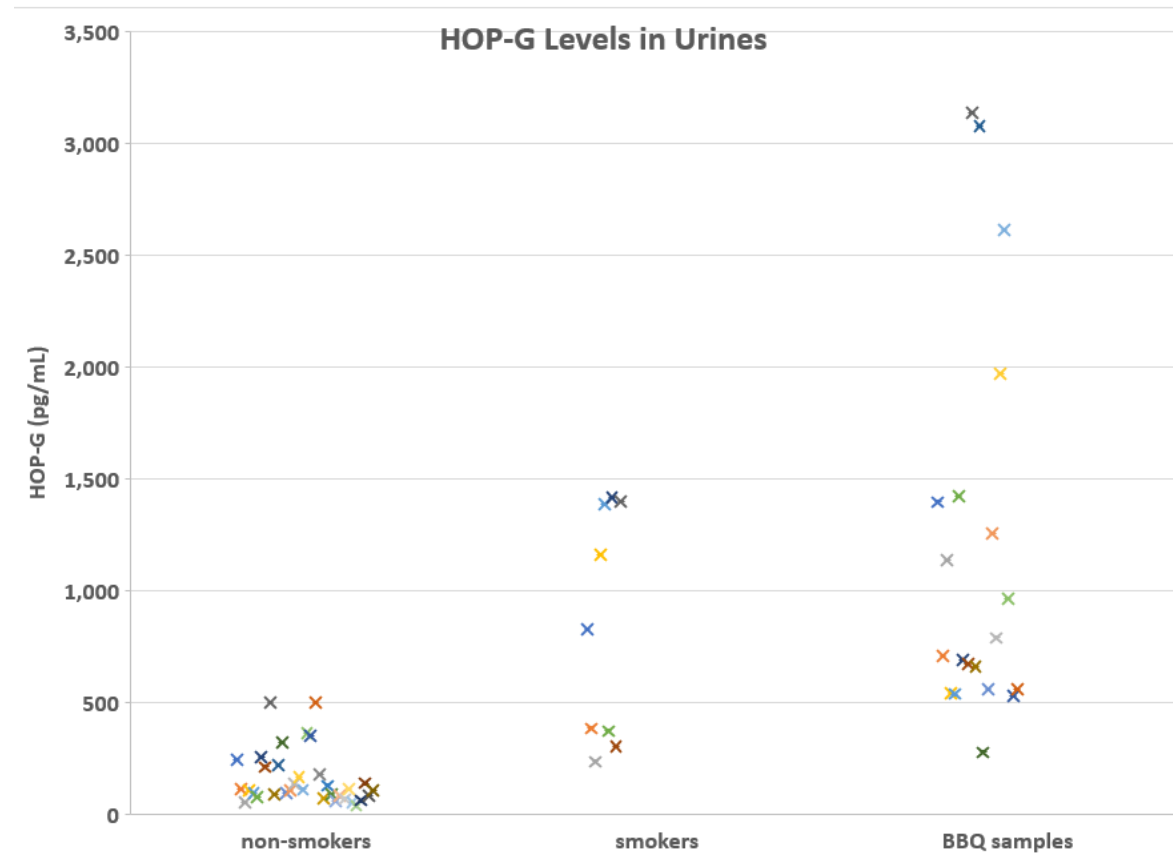
1-OHPyrG ELISA Sensitivity & Specificity



ELISA Correlation with HPLC/Fluorescence

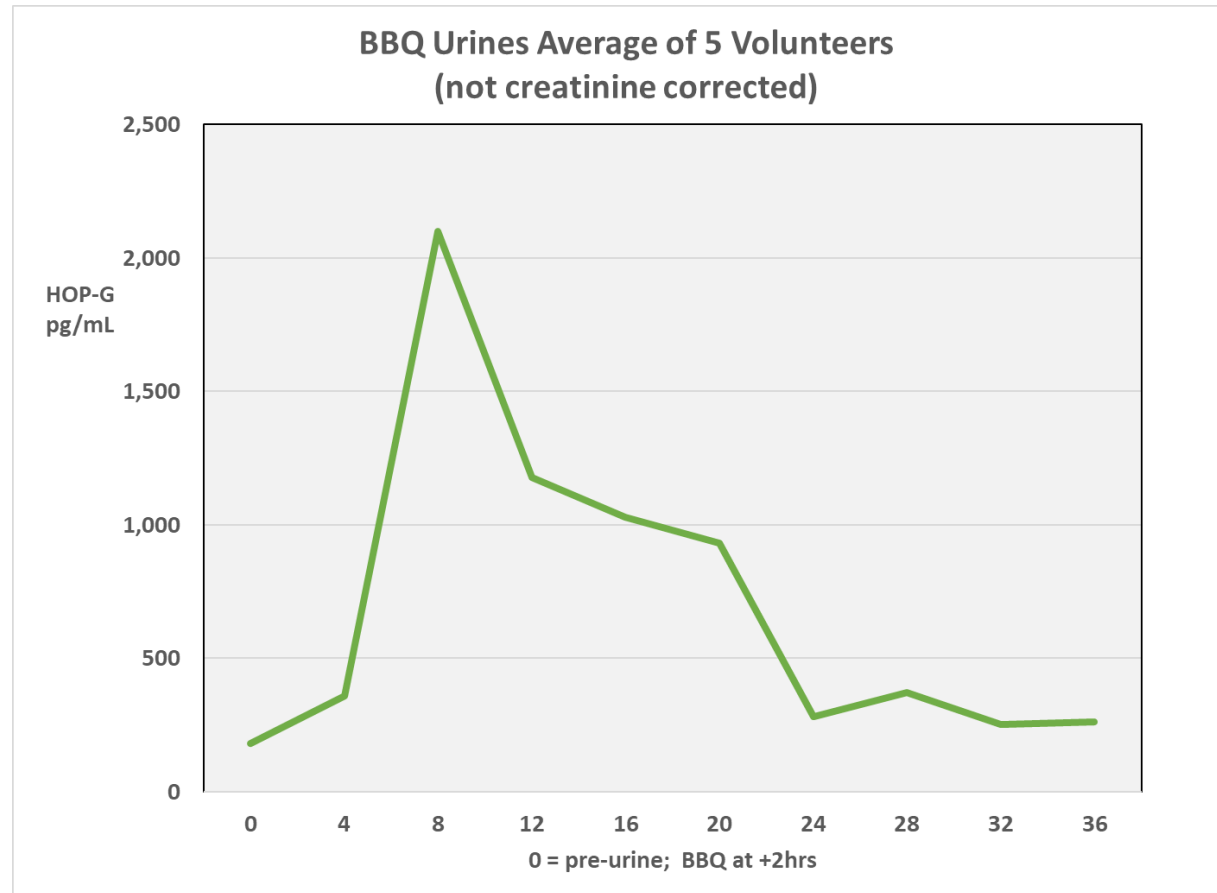


ELISA: Data Example 1



- Levels found in volunteer groups agrees with literature data

ELISA: Data Example 2



- Time-course of HOP-G in urines of volunteers exposed to crispy/burnt BBQ food in line with literature references

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 to workers

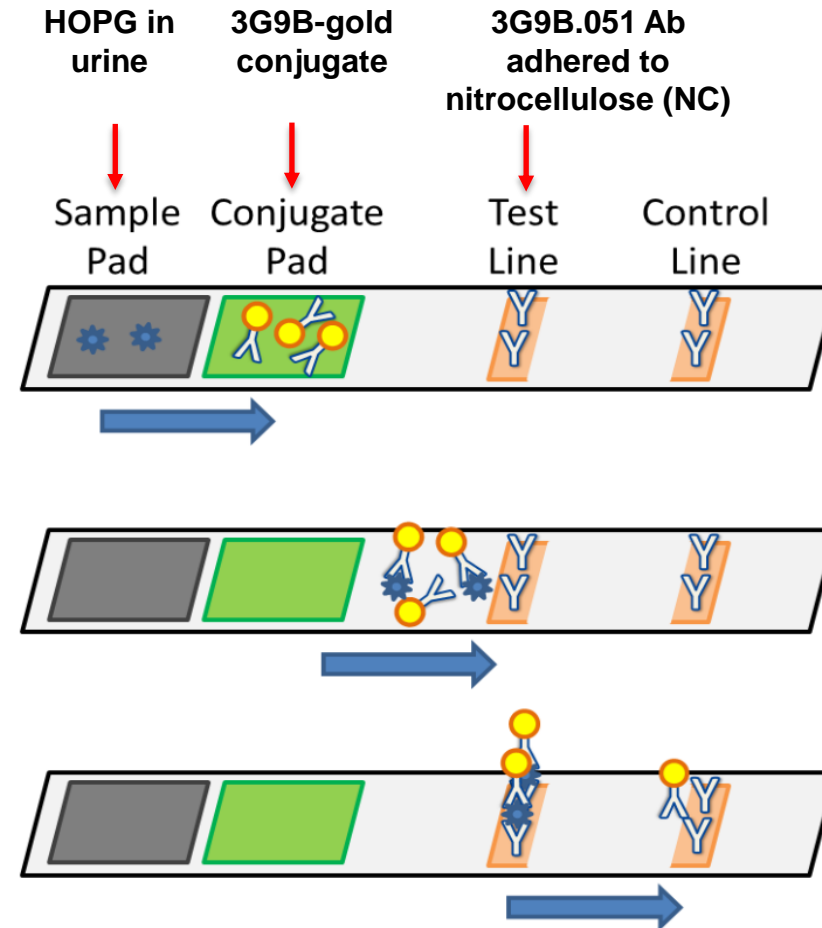
Low Cost

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

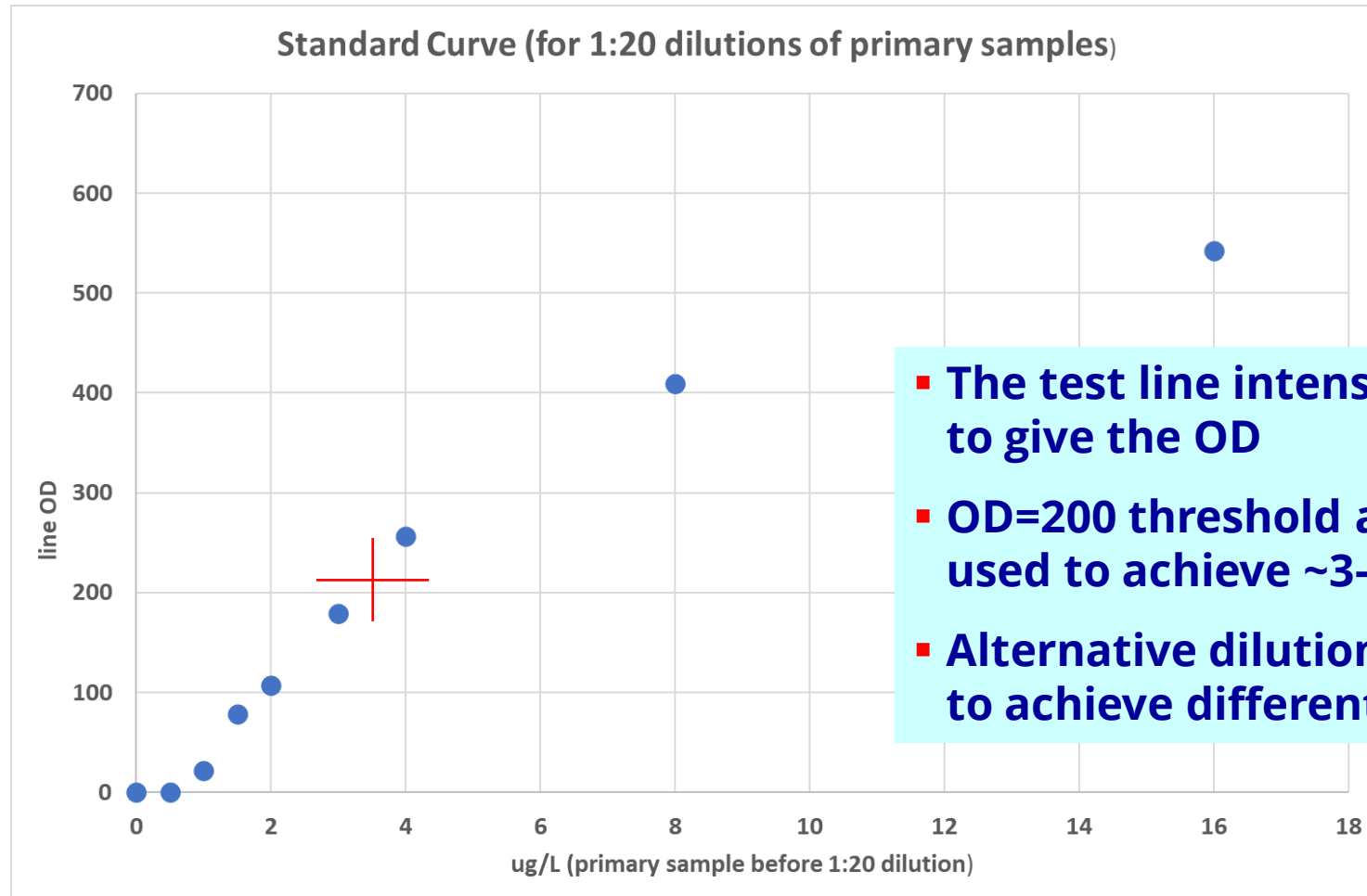


Lateral Flow Format

- Conventional “sandwich” format also works in lateral flow format



Lateral Flow Standard Curve



- The test line intensity is measured to give the OD
- OD=200 threshold at 1:20 dilution used to achieve ~3-4ug/L cut-off
- Alternative dilutions could be used to achieve different cut-offs

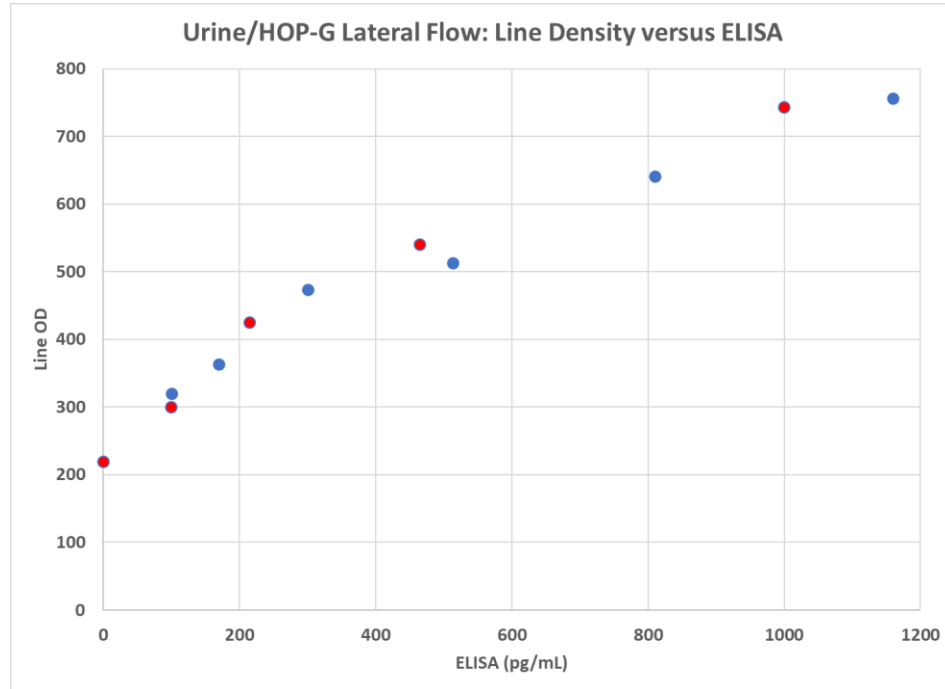
HOP-G LF Sensitivity & Specificity

Interference Testing

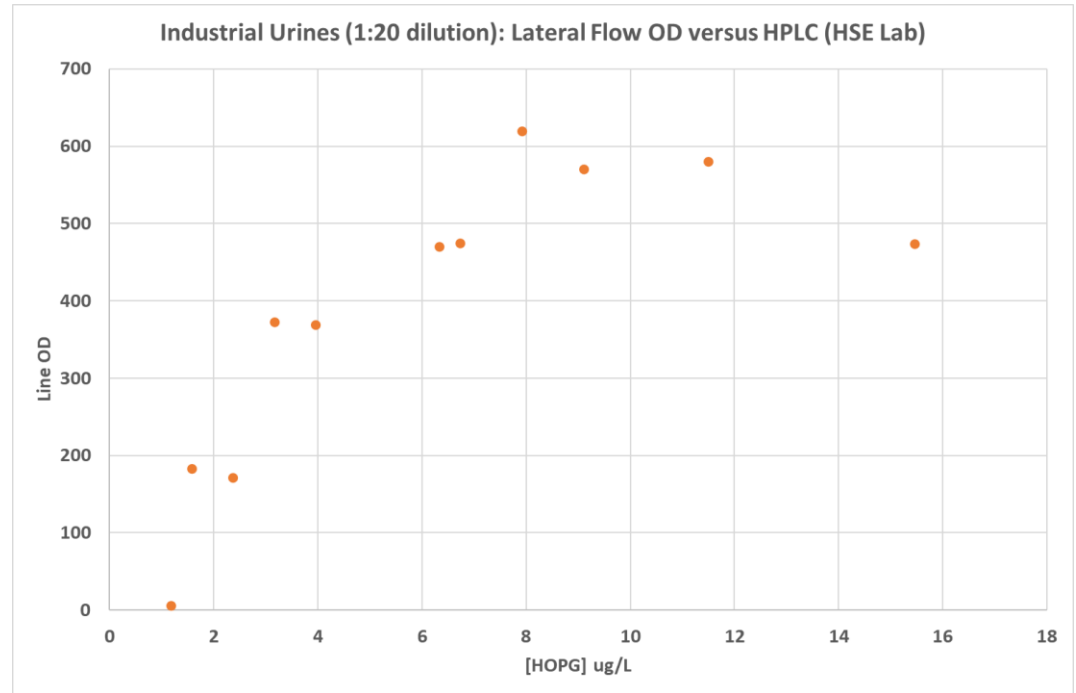
The following common substances were tested (0.2mg/ml)

- Paracetamol (acetaminophen)
- Caffeine
- Acetylsalicylic acid
- Ascorbic acid
- Gentisic acid

Lateral Flow Comparative Method Study



Lateral Flow v ELISA



Lateral Flow v HPLC Fluorescence

- Lateral flow OD gives good correlation with established methods

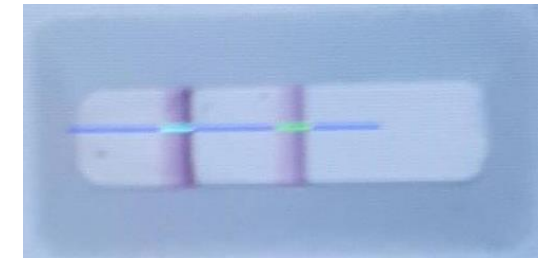
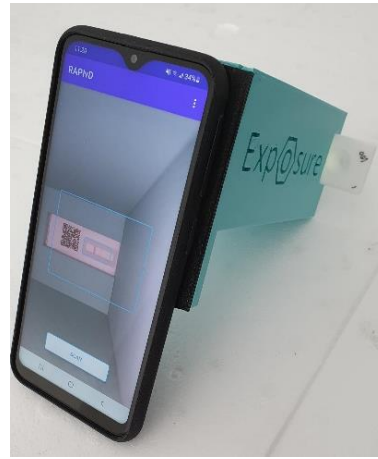
Phone Reader and 'App'





high medium low

← control
← test



3. Phone camera detects the presence of the control line and scans the test line

1. Run 1-OHPyrG lateral flow

2. Insert cassette into top-box after 15-60 minutes



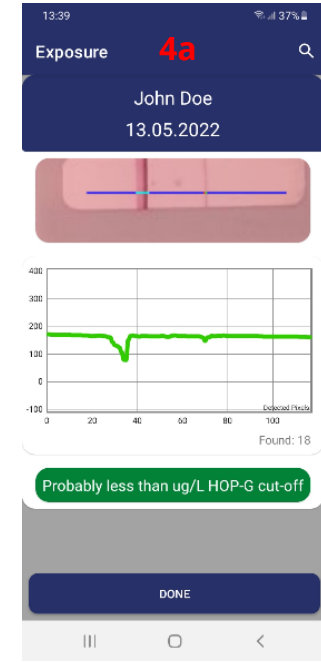
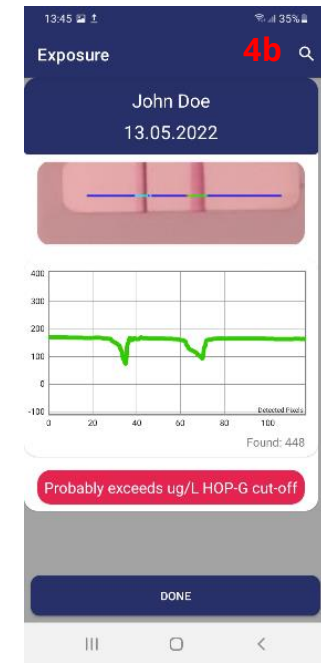
Phone Reader and 'App'

5. Data automatically stored on server

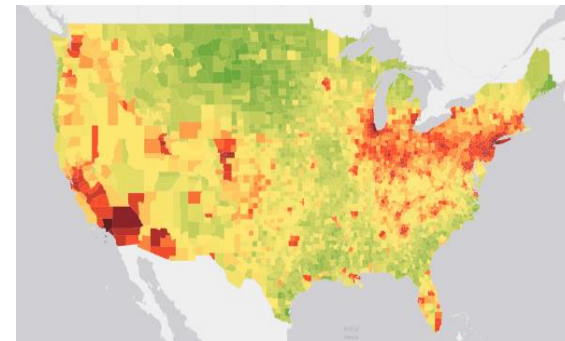
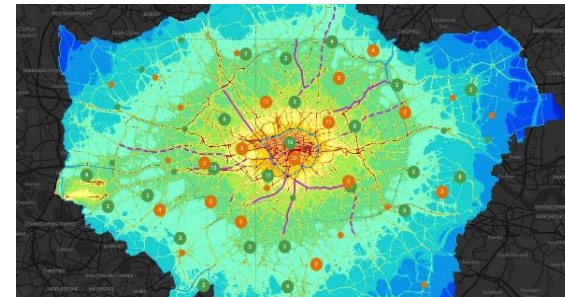
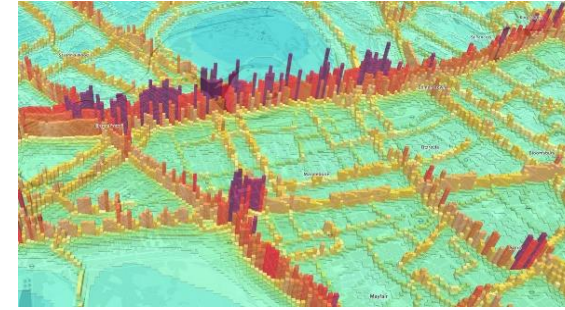


4a. Below cut-off

4b. Above cut-off (sample to be sent to central lab for confirmation or retest)



Phone Reader and 'App' Supports Remote Application



1. Urine test measures OHPyrG

2. Phone app quantifies levels and opens up local and global gateway

3. Data can be uploaded and visualised in the context of local, regional and global levels



POCT FIELD TRIAL

The field trial will:

- **Confirm the validity of the approach**
- **Support the incorporation of end-user requirements**
- **Allow the launch of a fit-for-purpose product.**



Thank you

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Pyrene (HOP-G)

