**Abstract**

In 2015 80 million Europeans tried an illicit drug. We have developed a multiplexed, enzyme and paper-based biosensor to semi-quantitatively measure heroin purity, detect PMA (p-methoxyamphetamine) in ecstasy and DNP (2,4-Dinitrophenol) in unregulated diet pills. This will contribute to harm reduction efforts related to illicit drug use. Drug Consumption Rooms and pill testing services have agreed to use our biosensor in their attempts to lessen morbidity and mortality.

**Introduction**

**Heroin**
Drug deaths are mostly due to varying purity (13.5-98%). Scotland has the highest rate of 112.5 drug-induced deaths per million citizens.

**PMA in ecstasy**
The use of ecstasy, commonly composed of MDMMA, has risen. MDMA production can result in the formation of the acutely toxic compound PMA.

**DNP in Diet Pills**
DNP is often found in unregulated weight loss pills. There are many adverse side effects of DNP, leading to a Global Health Warning.

**Detection Methods**

**Heroin Esterase (BBa_K1615045)**

**NADPH Production in the Presence of Heroin**
Heroin Esterase + Morphine Dehydrogenase

**MDMA**

**Amplex® Red**

**Monamine Oxidase A**

**Laccase**

**ABTS**

**DNP**

**Rate of Reaction (V)**

**Time (seconds)**

**Application Zone**

**Bioactive Zone**

**Laccase Activity over Time**

**Results**

A demonstration of the PMA biosensor detecting H2O2 with a 3 minute time to detection. HRP and Amplex® Red were applied to each bioactive zone. 20µl of 10 mM H2O2 solution was added to the application zone.

**Implementation - Harm Reduction**

**Drug Consumption Rooms (DCRs)** provide a safe space to consume drugs in the presence of trained social workers. Our visit to a DCR in Amsterdam confirmed that the staff and clients would use our biosensor to test their samples.

**Where do you think you are?**

**Market biosensor online where a majority of diet pills are purchased.**

**Outreach**
Our biohack crowdsourced the making of a smart phone app to detect the intensity of the colour for our biosensor.

**Enzyme Immobilisation**
After production the enzymes diffused on the paper, undermining the results. This led to us fusing the enzymes to a suite of 5 Cellulose Binding Domains (CBDs), on both the N and C terminus to ensure functional folding leading to submitting 72 parts.

**Legislation**
The UK Misuse of Drugs Act of 1971 does not ban the possession of drug paraphernalia. Thus, possession of drug testing kits is not a crime. Under this Act, Schedule 2 substances (such as heroin) are permitted in universities for research purposes.

**Design Development**
When designing this paper-based biosensor we considered our stakeholders’ input.

**Stakeholders’ Input**

**Prototype 1:** Simple design. Easy to use.

**Prototype 2:** Multiplexed

**Prototype 3:** Contained colour

**Final:** Small sample size

Individuals should have the choice to consume drugs with absolute impunity.

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Note how the N terminal fusion of cenA folds incorrectly and does not fluoresce.

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