Review of Point-of-Care / Collection Testing Devices for the Detection of Drugs of Abuse

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Executive Summary

General Comments

Relative to laboratory based Mass Spectrometry techniques, Point of Collection or Point of Care (PoCT’s) devices display poor analytical sensitivity, specificity and accuracy.

The high rates of false positive and negative results make their use unsuitable for “stand alone” drug testing programmes and restricting their application to a screening role. All presumptive positives must be confirmed by follow-up Mass Spectrometry techniques such as GC or LC-MS.

Sensitivity, Specificity and Accuracy of PoCT kits

The performance of PoCT devices is difficult to determine as the analytical specifications of individual products are often absent or inaccurate. Due to this, it is vital that a manufacturer’s claims be viewed with caution and, where possible, the devices accuracy is independently verified.

The preponderance of peer reviewed studies clearly report poor sensitivity and specificity in comparison to GC MS or other laboratory based testing techniques. Two major European studies, ROSITA and DRUID, both concluded that the PoCT devices tested within the study did not posses sufficient accuracy to be used as part of a legally defensible testing programme.

One of the largest studies of the immunoassay technique employed by PoCT devices was published by Melanson et al. in 2010. The study collated the qualitative results from six years of immunoassay proficiency testing by members of the College of American Pathologists (CAP). The test involved CAP members analysing a series of spiked standards. When using the positivity criteria stipulated by LGC Forensics (a leading UK forensics laboratory), the average detection rate was only 25% for Cannabis and 42% for Benzodiazepines and MDMA. Even at five times the threshold level, the detection rate for the synthetic opioid Oxycodone was only 17%.

The high probability of false negative results reported in this and many other studies means it would be unwise to use PoCT devices to test subjects that are employed in safety critical roles. In such circumstances it is essential that all testing is performed by high precision laboratory based techniques.
Thresholds

Typically a drug testing programme will set concentration thresholds to define what is “positive” or “negative”. In the UK and Europe there are no thresholds defined in law or by professional bodies. This means individual companies or programmes establish their own threshold criteria which in turn causes significant problems in the use of PoCT devices. This is because PoCT kits have a fixed detection threshold and only provide a qualitative result (i.e. positive or negative). Therefore, if the threshold level set by the organisation is different to the detection level of PoCT device, it is impossible to apply the positivity criteria of the testing programme.

It is also important to note that organisations using lower thresholds than recommended by the PoCT manufacturers will experience reduced accuracy in the reported results. Melanson et al. (2010) found that 88% of laboratories using PoCT immunoassay tests could identify samples with 50 ng / ml of the cannabis metabolite yet only 25% of laboratories could detect the metabolite at concentrations of 35 ng / ml. The threshold level set for cannabis by a leading UK forensic laboratory, LGC Forensics, is 25 ng per ml and is applied to N. Rail, Crossrail, London Underground and the National Grid. At these lower thresholds it is questionable whether PoCT devices are of any analytical use for the detection of cannabis. In such circumstances other more sensitive techniques should be employed.

Use of collection matrices other than urine

A wide range of PoCT devices designed to test Oral Fluids (OF) are currently available on the market. It is claimed that due to their ease of use and rapid response to drug administration they are superior to the urine based tests. It is clear that the OF devices will identify drug abuse more rapidly following drug administration but conversely the window of detection will be significantly shorter than using urine as the sample matrix.

The analytical accuracy of the OF tests has been closely studied due to their potential use for detecting Driving Under the Influence of Drugs (DUID). Two major studies have reviewed their analytical performance, “Road Side Testing Assessment” (ROSITA and ROSITA 2) and “Driving under the Influence of Drugs, Alcohol and Medicines” (DRUID). When reviewing OF devices, both the ROSITA and DRUID concluded that none of the commercially available kits had sufficient sensitivity to be used in the identification of DUID. Since publication, most of the OF kits reviewed in the studies have been removed from the market.
The OF approach has potential for the future, but current technology is clearly in the development phase and not ready for general use in a workplace and occupational testing programmes.

**Accreditation**

Typically, any company wishing to establish a drug testing programme would seek to attain some form of national or international accreditation. This allows the company to demonstrate a degree of competence in their abilities to potential clients. Of the most commonly used and well respected organisations are the International Standards Organisation (ISO) and The United Kingdom Accreditation Service (UKAS).

UKAS and ISO do offer an accreditation programme for medical PoCTs. However; there is no formal international accreditation for their use in workplace (occupational) testing programmes.

With the lack of any formal accrediting organisation it is possible for any individual to set up a company and supply PoCT devices. The accuracy of the devices claimed by the manufacturer will have no external verification or validation against external standards. This could permit less scrupulous organisations to supply substandard or poorly tested devices. Reliance on such instrumentation could result in serious legal implications if poorly functioning kits report false positives or false negatives.

It would seem prudent that any organisation wishing to use a PoCT based testing programme should ensure that, at a minimum, the company providing the service is accredited to the medical standard ISO 22870. This may appear excessive but could prevent costly litigation.

**Chain of Custody**

Maintaining an adequate Chain of Custody (CoC) for sample devices, test results and analytical procedures is essential for any testing programme. However, with the use of PoCT devices, it is often impossible to implement and maintain an adequate CoC.

The results of PoCT immunoassays are generally impermanent, with most results fading within an hour. Therefore, the only aspect of the analysis that remains is the data entry of the individual that carried out the testing. PoCT devices are also single use and typically do not permit collection of excess sample for subsequent confirmation testing.
With no permanent recording of data and no ability to retest the sample, it is impossible to produce a legally defensible CoC.

**Quality Control**

Due to the lack of a formal accreditation body or process, Quality Control (QC) varies between manufacturers and suppliers. Some of the cheaper PoCT devices have no formal QC, making their analytical precision at best uncertain. The most common form of visible QC comes in the form of a “reagent blank” visible on the PoCT device. This will produce a chromatic indicator if the PoCT device is not functioning correctly. This is an extremely limited form of QC and falls short of even the most basic requirements for forensic or drug testing applications.
List of Synonyms

BAC  Blood Alcohol Content
CoC  Chain of Custody
DUID Driving under the influence of drugs
DRUID Driving under the influence of drugs, alcohol and medicines
EMIT Enzyme Multiplied Immunoassay Technique
GC-MS Chromatography Mass Spectrometry
ISL International Standards for Laboratories
ISO International Standards Organisation
LC-MS Liquid Chromatography Mass Spectrometry
m/z Mass Charge Ratio
ml Millilitre
MS Mass Spectrometer
ng Nano-gram
OF Oral fluids
OTC Over-the-counter
PoCT Point-of-Care Testing Devices
QC Quality Control
ROSITA Road Side Testing Assessment
SAMHSA The Substance Abuse and Mental Health Services Administration
SOP Standard Operating Procedures
THC 1-nor-delta-9-tetrahydrocannabinol-9
μg Microgram
UKAS United Kingdom Accreditation Service
WADA World Anti-Doping Agency
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1 Introduction

1.1 Scope of work

The independent report, prepared by Dr Genevieve Boshoff (Protium Environ), Dr Simon Davis (Imperial College London) and Mr Paul Scott (Scott Analytics US) considers the analytical precision and reliability of Point-Of-Collection / Care Testing devices (PoCT) as part of a workplace / occupational drug testing programme.

The study compares the analytical properties of PoCT devices in relation to alternative / complementary laboratory based methodologies. In particular, the ability of PoCT devices to be used in workplace drug testing programmes without subsequent laboratory based confirmation of the devices’ findings.

The report also reviews how PoCT devices are accredited and whether their production and use conforms to Quality Control (QC) and Chain of Custody (CoC) requirements for similar laboratory based programmes.

1.2 Definition of drugs of abuse

The term "drugs of abuse" is often used as a “catch-all" term to encompass every illegal and illicit substance. However, this definition is more complex than might initially be apparent. Although some compounds can be defined solely on their legal classification (eg: heroin and cannabis), others have to be considered in relation to the amount and circumstances in which they are used. For example, alcohol is legal in the UK but would be considered a drug of abuse if imbibed in excessive amounts prior to driving a car².

Further complications arise when employment contracts and safety regulations are taken into consideration. Using alcohol as an example, a BAC of 0.04% would be considered legal when driving a car, but would result in dismissal if these levels where found in an employee of Network Rail ³(Network Rail Drug and Alcohol Policy (2011)).

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² A Blood Alcohol Content (BAC) of 0.08% or greater would be considered illegal in the UK if an individual was driving a vehicle on a public road.

³ Network Rail stipulates a maximum Blood Alcohol level of 0.029%. Levels above this result in dismissal (Network Rail Drug and Alcohol Policy (2011))
As such, a universal definition is not possible without defining the scope and circumstances within which a control programme is enforced. In relation to this report we will restrict the term “Drugs of Abuse” to those compounds listed in Table 1. This is not a fully inclusive table but reflects a standard suite of compounds which might commonly be screened for in a workplace testing programme (Source Express Medical pers.com. 2012).

**Table 1.** Drugs of abuse which are commonly screened in workplace testing programmes.

<table>
<thead>
<tr>
<th>Drug Of Abuse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
</tr>
<tr>
<td>Amphetamines (including ecstasy)</td>
</tr>
<tr>
<td>Barbiturates</td>
</tr>
<tr>
<td>Benzodiazepines</td>
</tr>
<tr>
<td>Cannabis</td>
</tr>
<tr>
<td>Cocaine</td>
</tr>
<tr>
<td>Ketamine</td>
</tr>
<tr>
<td>LSD</td>
</tr>
<tr>
<td>Methadone</td>
</tr>
<tr>
<td>Opiates</td>
</tr>
<tr>
<td>Propoxyphene</td>
</tr>
</tbody>
</table>

1.3  **Description and explanation of screening and confirmation testing**

Numerous analytical techniques can be employed to identify drug abuse. These range from rapid turnaround, low cost point-of-collection techniques to high precision and high complexity laboratory procedures. Although it would be ideal if every programme could employ techniques with the highest sensitivity and specificity, cost and time limitations make this impractical. Instead, it is common to use a low accuracy screen followed by a high accuracy confirmation method.

The purpose of the screening process is not to definitively identify drugs of abuse, but simply to identify any individuals whose chemical profile is abnormal and warrants further investigation.
Those samples that displayed atypical chemistry during the screening process can then be passed for further confirmation using high precision techniques such as Gas Chromatography Mass Spectrometry (GC-MS). These techniques provide definitive evidence of drug abuse and are legally defensible.

1.4 Quantitative and Qualitative Analytical Reporting and their relevance to Thresholds

When a drug test is performed the presence or absence of a compound is not always sufficient to declare a positive result. This is due to a number of factors:

- The compound may be below the detection limit of the technique applied.
- Governmental or workplace legislation may set mandatory positivity criteria. This may require a given amount of the drug of abuse to be present in the sample before a positive is reported.
- Cross-reacting compounds could result in false positive or negative results.

Depending on the analytical test employed the results will be presented in either a quantitative or qualitative format.

1.4.1 Quantitative and Qualitative Reporting

Quantitative techniques, such as GC-MS, present results in units of drug relative to units of sample. For example, the concentration of the cannabis metabolite THC might be reported as 49 ng of THC per ml of urine.

In the case of qualitative testing the results can only be expressed as positive or negative. The detection limits of the technique will allow the user to determine if a compound is present at concentrations greater than the detection limit. However, no other information is provided.

1.4.2 Thresholds

Thresholds are the minimum concentration of a drug or metabolite that must be present in a specimen in order for a test to be reported as positive. Due to differences in metabolism and relative intoxication effects the positivity thresholds are not the same for all drugs of abuse.
There is no universal agreement on the concentrations that should be used to set positivity criteria. As a result, thresholds vary significantly between drug testing programmes and this prevents any meaningful comparisons being made. It is important for any organisation embarking on a workplace testing programme to ensure they have clear thresholds in place and that any testing device or method is calibrated to this threshold. If the devices are not correctly calibrated and only report qualitative results, this will result in a significant number of false positives or negatives.

For example, a PoCT device may be manufactured to report a positive cannabis result when urinary THC levels exceeded 30 ng per ml. However, if the testing programme stipulates a threshold of 50 ng per ml, then the PoCT device will report false positives for urinary THC levels between 31 and 49 ng per ml. As PoCT devices only provide qualitative results there is no way to determine the true THC urinary concentrations without further testing. This will make any prosecution of a cannabis positive legally indefensible.

In the USA legislation has led to a more unified and controlled setting with the implementation of threshold levels for workplace testing programmes. Here the thresholds are based upon the research and guidance of SAMHSA (The Substance Abuse and Mental Health Services Administration). Any device employed in federal workplace testing must meet the federal threshold for screening (Table 2). The use of these standard threshold concentrations ensures that all laboratory and PoCT devices screen at the same level and that results from different regions are comparable. No such statutory regulations apply in Europe or the United Kingdom and the thresholds that PoCTs employ vary between kit and manufacturer, as illustrated in Table 3. It should be noted that concentrations below the threshold do not necessarily infer an absence of the drug of abuse, but simply that the concentrations were less than the threshold.
Table 2. SAMHSA cut off thresholds for initial screening and confirmatory testing (Federal Register November 25 2008 (73 FR 71858)).

<table>
<thead>
<tr>
<th>Initial test analyte</th>
<th>Initial test cutoff concentration</th>
<th>Confirmatory test analyte</th>
<th>Confirmatory test cutoff concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marijuana metabolites</td>
<td>50 ng/mL</td>
<td>THCA&lt;sup&gt;1&lt;/sup&gt;</td>
<td>15 ng/mL</td>
</tr>
<tr>
<td></td>
<td>150 ng/mL</td>
<td>Benzoylcegonine</td>
<td>100 ng/mL</td>
</tr>
<tr>
<td>Cocaine metabolites</td>
<td></td>
<td>Codeine</td>
<td>2000 ng/mL</td>
</tr>
<tr>
<td>Opiate metabolites.</td>
<td></td>
<td>Morfine</td>
<td>2000 ng/mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6-AcetylMorphine</td>
<td>10 ng/mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phencyclidine</td>
<td>25 ng/mL</td>
</tr>
<tr>
<td>Codeine/Morphine&lt;sup&gt;2&lt;/sup&gt;</td>
<td>2000 ng/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-Acetylmorphine</td>
<td>10 ng/mL</td>
<td>Amphetamine</td>
<td>250 ng/mL</td>
</tr>
<tr>
<td>Phencyclidine</td>
<td>25 ng/mL</td>
<td>Methamphetamine&lt;sup&gt;5&lt;/sup&gt;</td>
<td>250 ng/mL</td>
</tr>
<tr>
<td>AMP/MAMP&lt;sup&gt;4&lt;/sup&gt;</td>
<td>500 ng/mL</td>
<td>MDMA</td>
<td>250 ng/mL</td>
</tr>
<tr>
<td>MDMA&lt;sup&gt;6&lt;/sup&gt;</td>
<td>500 ng/mL</td>
<td>MDA&lt;sup&gt;7&lt;/sup&gt;</td>
<td>250 ng/mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MDEA&lt;sup&gt;8&lt;/sup&gt;</td>
<td>250 ng/mL</td>
</tr>
</tbody>
</table>

<sup>1</sup> Delta-9-tetrahydrocannabinol-9-carboxylic acid (THCA).
<sup>2</sup> Morphine is the target analyte for codeine/morphine testing.
<sup>3</sup> Either a single initial test kit or multiple initial test kits may be used provided the single test kit detects each target analyte independently at the specified cutoff.
<sup>4</sup> Methamphetamine is the target analyte for amphetamine/methamphetamine testing.
<sup>5</sup> To be reported positive for methamphetamine, a specimen must also contain amphetamine at a concentration equal to or greater than 100 ng/mL.
<sup>6</sup> Methylenedioxyamphetamine (MDMA).
<sup>7</sup> Methylenedioxyamphetamine (MDA).
Table 3. Threshold concentrations for a range of PoCT kits commonly used compared to the SAHMSA threshold concentrations.

<table>
<thead>
<tr>
<th>Drug</th>
<th>SAHMSA EMI (ng/ml)</th>
<th>Fastect® II (ng/ml)</th>
<th>Reditest (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamines</td>
<td>500</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Barbiturates</td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>Cocaine</td>
<td>150</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>Marijuana</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Methadone</td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>Methamphetamines</td>
<td>500</td>
<td>500/1000</td>
<td></td>
</tr>
<tr>
<td>Opiates</td>
<td>2000</td>
<td>300</td>
<td>300/2000</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Phencyclidine</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

*The target analyte must be d-methamphetamine and the test must significantly cross-react with MDMA, MDA, and MDEA.
1.5 Analytical Methodologies used to Identify Drugs of Abuse

There are many analytical procedures available to any organisation wishing to implement a workplace drug testing programme. GC-MS and immunoassay-based point-of-collection techniques are the most common approaches, possibly due to the speed and cost of analysis and sample collection.

Of the two techniques the quantitative GC-MS approach provides the highest degree of accuracy. When this technique is applied correctly it will operate with near 100% sensitivity and specificity to the drugs being tested for.

The immunoassay techniques have considerably lower analytical accuracy but are economical and can be operated at the point of collection. Due to their use at the site of sample collection, they are commonly known as point-of-collections tests or PoCTs.

1.6 Principles of GC-MS

The workhorse of most analytical laboratories since the 1970s has been the combination of Gas Chromatography followed by Mass Spectrometry. This technique provides both conclusive evidence of the identification of a compound and precise quantification where required⁴.

The basic process of analysis is:

- **Sample preparation.** The sample matrix is physically and chemically cleaned to remove compounds that might interfere with the analysis. In certain circumstances, compounds may have a low volatility making their analysis difficult without further chemical preparation. In a simple process known as derivatisation, the volatility of a compound can be greatly increased by the addition of further chemical groups. Once this process is complete, samples can be injected into a chromatographic column for separation.

- **Chromatographic separation.** The separation of the compound is carried out in a long flexible glass tube known as a column. The column is lined or packed with a

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⁴ Semi quantitative and / or qualitative results are occasionally reported when using GC-MS if the GC-MS is used for screening purposes.
compound developed to differentially slow the passage of chemical groups as they are flushed through the column by a carrier gas (typically helium). The degree of flow degradation varies between compounds with different chemical structures. This results in compounds being separated from one another by retention times within the column. The separated compounds form “peaks” as they elute from the column.

Figure 1 shows a peak of the active compound THC, often used to identify cannabis abuse.

Although Gas Chromatography separates individual compounds, it does not provide definitive identification\(^5\). It is therefore necessary to perform Mass Spectral analysis to determine the composition of any peak.

- **Compound identification.** Following chromatographic separation, the compounds are passed to the Mass Spectrometer (MS) for identification and quantification. During this process the compound is exposed to a stream of electrons. This has two effects, firstly to ionize the compound and secondly to create ionized fragments of the parent compound. The mass \((m)\) and the charge \((z)\) of the fragments are then determined by the MS. The ratio of the mass and charge \(m/z\) of the parent molecule and the daughter fragments are then used to identify the compound in question. Figure 1 shows a mass spectrogram of the molecule THC used for the identification of cannabis. The parent molecule has an \(m/z\) of 488 and two prominent daughter fragment ions with an \(m/z\) of 473 and 371, respectively. The combination and relative abundance of the ions provide conclusive evidence that the compound being analysed is THC.

\(^5\) The time a compound elutes from the column (retention time) can permit identification in certain well controlled experiments. However, different compounds can share the same retention time and can co-elute. As such, in a sample whose chemical composition is unknown, it is essential that Mass Spectrometry is used for conclusive identification.
Figure 1. Mass spectrogram of THC displaying three diagnostic ions at m/z 488, 437, 371.
2 PoCT Devices

2.1 Introduction

PoCT devices are generally small portable devices that provide a quick and economic on-site testing solution. They are designed to be self-contained and simple to use, arguably reducing the need for highly qualified technical staff. Typically, a urine or saliva sample is taken from the subject and exposed to an immunoassay reagent. This will produce a visible indicator to determine if any drugs of abuse are present. Somewhat confusingly, in many of the early devices the absence of a visual indicator would mean the test was positive, whilst the presence of an indicator would mean the test was negative. Although some suppliers still sell these devices, the majority of modern PoCT’s display a bar if a drug of abuse is detected.

Although the indicator shows the presence of a compound the tests are qualitative and cannot provide detailed information on concentration. This generally limits their reporting detail to a positive or negative.

2.2 Principle of PoCT Operation

PoCT devices are typically based on immunoassay procedures. These depend on the fact that antibodies bind strongly only to a specific compound or a closely related compound with a specific shape. Antibodies specific to a particular drug are tagged with markers such as an enzyme (EIA) or fluorescent label. Reagents containing the labelled antibodies are introduced into the sample. If the analyte is present, a reaction will occur (Kapur, 1993). EIA permits detection of extremely small quantities of substances but only has the sensitivity to determine the class of drug and not the individual compound (Saxon et al. 1990).

The most common immunoassay methodology employed is the Enzyme Multiplied Immunoassay Technique (EMIT) (Neerman, 2006). The test was first introduced by the Syva Company in the 1970s. This is a competition based process in which the target compound competes for binding sites on drug specific antibodies with an enzyme-linked drug. The enzyme employed, glucose-6-phosphate dehydrogenase (G6P-DH), converts oxidized nicotinamide adenine dinucleotide (NAD+) to NADH resulting in an absorbance change that

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6 PoCT devices can provide semi-quantitative results, e.g. the concentration is 50 to 100 ng per ml, but cannot provide quantitative results e.g. the concentration is 56.3 ng per ml.
is measured spectrophotometrically. When the enzyme bound drug binds with the antibody, the enzyme loses its activity and is unable to react with a substrate which is mixed with the urine sample (glucose-6-phosphate). The drug in the urine competes for the limited number of antibody binding sites with the enzyme bound drug. If the free drug binds with the antibody, the enzyme bound drug is able to react with the substrate, facilitating a biochemical reaction. The ratio between the two is used to determine the presence or absence of the analyte in the sample. The enzymatic activity, and hence the extent of the biochemical reaction, is directly proportional to the concentration of the drug in the urine. The results from these tests are available within 5 to 10 minutes but rely on items of laboratory equipment to perform the analysis.

Since the EMIT test was introduced, a number of different rapid detection devices, or PoCT kits, have become commercially available. These devices are all based on the EMIT principle of competitive binding but use a lateral flow immunoassay format to remove the need for laboratory based procedures.

The lateral flow devices contain a membrane strip impregnated with a drug protein conjugate at a particular region (the test region). In addition, colloidal gold conjugated antibodies (coloured antibody conjugates) are coated on a pad (a conjugate pad). After the sample is brought into contact with the coated pad, the sample fluid begins to migrate up the membrane strip by capillary action. If molecules of a panel drug are present in the sample, they bind to the coloured antibody conjugates. This prevents the drug from flowing to the test region of the membrane and binding to the drug protein conjugate. In this process no colour is formed and the test is considered positive. In the absence of drug molecules in the sample, the coloured antibody conjugates will bind with the drug protein conjugates and form a coloured band (a negative test).

These devices come in a number of different formats, some of which are designed to identify a single specific compound and others which detect a range, or panel, of compounds. Results from these devices are based on a specific calibrator concentration (threshold) which is specified by the manufacturer\(^7\). A positive result reflects that the concentration is at (or above) the threshold, whilst a negative result reflects that the concentration is below the threshold.

\(^7\) Although the threshold is set by the manufacturer it may reflect a legislative threshold or a threshold set by a client.
Due to the obvious contradiction of a positive result being indicated by the lack of a coloured band, most of the modern PoCT devices have now developed a process where a positive visual signal indicates the presence of a drug.

These devices use a technique known as the ascending multi-immunoassay procedure. In this method a urine specimen is added to a well containing reagent beads. Drug molecules in the urine compete for limited binding sites with drug molecules labelled with colloidal gold. The mixture is then transferred to a test strip impregnated with specific antibodies in different test regions. In the absence of drugs in the sample, all colloidal gold particles are conjugated with antibodies present in the reagent beads and none are available to bind with immobilised antibodies on the test strip. Therefore no colour is formed. If drugs are present in the urine sample, the drug molecules are bound to the respective drug antibodies present on the reagent beads and drugs labelled with the colloidal gold are free in the solution. When mixed with the test strip, they are free to bind with the immobilised antibodies to produce a colour reaction.

2.3 How Analytical Results from PoCT Kits are Reported

In a drug testing programme the main objective is to determine if a drug of abuse is present or absent. When considering how to report analytical results, there are four possible outcomes that must be considered:

i. **True Positive (TP)**: The result of the test is positive and that drug is present in the sample at or above the threshold concentration of the test.

ii. **False Positive (FP)**: The result of the test is positive but the drug is not present in the sample or at concentrations below the threshold of the test.

iii. **True Negative (TN)**: The result of the test is negative and the drug is not present in the sample or is below the threshold concentration of the test.

iv. **False Negative (FN)**: The result of the test is negative but the drug is present in the sample above the threshold concentration of the test.

The range of possible outcomes is summarized in Table 4.
### Table 4. Possible range of outcomes from drugs of abuse analysis

<table>
<thead>
<tr>
<th>Drug Present</th>
<th>Positive Result</th>
<th>Negative Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP</td>
<td></td>
<td>FN</td>
</tr>
<tr>
<td>Drug Absent</td>
<td>FP</td>
<td>TN</td>
</tr>
</tbody>
</table>

#### 2.4 Calculation of Sensitivity and Specificity in PoCT devices

The relative rate of outcomes, described in Table 4, is used to determine the Sensitivity and Specificity of PoCT devices.

Sensitivity is defined as the test’s ability to identify true positives and is calculated as described in Equation 1:

\[
Sensitivity (\%) = \frac{TP}{TP + FN} \times 100
\]

The specificity of a PoCT device is its ability to detect true negatives and is calculated in the manner described in Equation 2:

\[
Specificity (\%) = \frac{TN}{TN + FP} \times 100
\]

The rates of false positives and negatives will be discussed later in this report, but it should be clear from this section that a simple positive or negative result should not be viewed in isolation from the sensitivity and specificity of the analytical technique used.

#### 2.5 Sensitivity in PoCT Immunoassays

Although it is sometimes necessary to use a PoCT device to identify a single compound, most kits are used to detect a range or panel of drugs. However, due to their poor sensitivity, PoCT Immunoassays generally lack the ability to identify all drugs present in a
given class (Saxon et al., 1990). For example, general opiate / opioid\(^8\) immunoassays are unable to detect the presence of the opioid methadone. The detection of methadone depends upon a separate PoCT device specifically designed for this purpose. Opiate / opioid immunoassays are also unable to detect the semi-synthetic opioids oxycodone, oxymorphine, buprenorphine, or hydromorphone even at high concentrations (Von Seggern et al., 2004).

While many benzodiazepines can be identified by PoCT devices, certain related compounds (such as clonazepam) are undetectable by all immunoassays techniques (Gourlay et al. 2006).

Detection of a particular drug by a drug-class-specific immunoassay depends on two factors:

- The structural similarity of that drug, or its metabolite(s), to the compound used for standardisation
- The sample concentration of that drug / metabolite compared with the standardising compound (Yang, 2001).

The standardisation compound refers to the material used to generate the antibodies employed in the manufacturing of the devices.

A PoCT device’s sensitivity is highly variable depending on the panel of drugs it is designed to detect. Also, there is variability between device manufacturers. Concerns have also been raised that there may be variability in sensitivity between batches of devices produced by the same manufacturer (Melanson et al. 2010). This makes any true estimate of sensitivity impossible.

2.6 Specificity of PoCT Immunoassays

Assay specificity refers to the ability of an antibody to produce a specific response to the analyte of interest, whilst not showing any response to other compounds. A highly specific test gives few false-positive results.

The specificity of PoCT device varies between drugs. For example, opiate / opioid immunoassays generally have a low specificity and cannot distinguish between morphine

\(^8\) An opiate is a drug derived from the poppy seed whilst an opioid is a synthetic or semi-synthetic compound.
(the urinary metabolite of heroin), codeine and other opioids, including opiates from poppy seeds used in baked goods (Thevis et al. 2003).

PoCT devices used to identify amphetamines also have low specificity and are highly cross reactive with sympathomimetic amines such as ephedrine and pseudoephedrine (Machikanti et al. 2008). As both ephedrine and pseudoephedrine are legal in many countries, including the UK, poor sensitivity in a PoCT device could result in a positive report for amphetamines after the use of an over the counter cold remedy.

In a study carried out by Hsu et al. (2003), four commercially available immunoassays for amphetamines (Dx-Amp, COBAS-Amp, OnLine-Amp/MDMA, CEDIA-Amp/MDMA) were evaluated for their effectiveness in serving as the preliminary test methodology for the analysis of 3,4-methylenedioxymethamphetamine / 3,4-methylenedioxyamphetamine (MDMA/MDA) and methamphetamine/amphetamine (MA/AM). As can be seen in Table 5, the false positive rate for the detection of MDMA/MDA ranged between 11% and 21% while the false positive rate for the detection of MA/AM ranged from 18% to 50%.

Table 5. Specificity of four amphetamine Immunoassay kits

<table>
<thead>
<tr>
<th>Cut-off, specimen no., analyte, pos./neg. rate</th>
<th>TDx-Amp</th>
<th>COBAS-Amp</th>
<th>OnLine-Amp/MDMA</th>
<th>MDMA</th>
<th>d-MA</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDMA/MDA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunocassay cutoff (ng/mL)</td>
<td>560</td>
<td>480</td>
<td>730</td>
<td>550</td>
<td>—</td>
</tr>
<tr>
<td>Specimens with valid data</td>
<td>28</td>
<td>28</td>
<td>27</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Number of false negatives</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Immunocassay positive/GC-MS positive</td>
<td>19/15</td>
<td>20/17</td>
<td>18/16</td>
<td>20/17</td>
<td></td>
</tr>
<tr>
<td>(% false positive)</td>
<td>(21%)</td>
<td>(15%)</td>
<td>(11%)</td>
<td>(15%)</td>
<td></td>
</tr>
<tr>
<td>MA/AM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunocassay cutoff (ng/mL)</td>
<td>1420</td>
<td>750</td>
<td>2590</td>
<td>—</td>
<td>1150</td>
</tr>
<tr>
<td>Specimens with valid data</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>—</td>
<td>32</td>
</tr>
<tr>
<td>Number of false negatives</td>
<td>6</td>
<td>10</td>
<td>7</td>
<td>—</td>
<td>6</td>
</tr>
<tr>
<td>Immunocassay positive/GC-MS positive</td>
<td>13/9</td>
<td>10/5</td>
<td>13/8</td>
<td>—</td>
<td>11/9</td>
</tr>
<tr>
<td>(% false positive)</td>
<td>(31%)</td>
<td>(50%)</td>
<td>(38%)</td>
<td>—</td>
<td>(18%)</td>
</tr>
</tbody>
</table>

* MDMA and d-MA were used as the calibrators for the analysis of MDMA and MA, respectively.

1 Out of the 28 specimens listed in Table IV, for the reasons stated in the text, only 20 were adopted for regression analysis to derive the immunoassay apparent MDMA concentrations (adapted as cutoffs for respective immunoassays) that are equivalent to 500 ng/mL MDMA. However, all valid data derived from each specific immunoassay are used to derive data in this table for that specific immunoassay.

2 The following example illustrates how the number of "immunoassay positive", "GC-MS positive", and "% false positive" are derived. 19/25 (76%) of the 19 specimens found positive by the immunoassay, 15 were confirmed positive by GC-MS positive; thus, 21/4 (52%) of the 4 positive specimens were false positive. Since different immunoassays may identify different specimens and different number of specimens positive, the number of "GC-MS positive" out of the specimens, that have been identified as positive by respective immunoassays, may vary.

3 Out of the 32 specimens listed in Table V, for the reason stated in the text, only 24 were adopted for regression analysis to derive the immunoassay apparent MA concentrations (adapted as cutoffs for respective immunoassays) that are equivalent to 500 ng/mL MA. However, all valid data derived from specific immunoassay are used to derive data in this table for that specific immunoassay.

Conversely, urine tests for cocaine display a high specificity (a low rate of false negatives) reacting principally with cocaine and / or its primary metabolites, benzoylecgonine. Urine drug tests for marijuana have moderate specificity but can produce false positive results with, for example, marinol and Protonix and other hemp products (ElSohly et al. 2001).
2.7 Cross-reactivity

As described above, PoCT devices with poor specificity are known to produce false positives. This occurs when antibodies to a drug of interest react to compounds other than the target analyte. This process is known as cross-reactivity (Reisfeld et al. 2009).

All PoCT kits are immunoassays. As such, PoCT devices are subject to the risk of non-specific binding and cross-reactivity. There are several well documented circumstances where PoCT devices report adverse findings when no drugs of abuse have been administered by the test subject.

These include, but are not limited to:

- Codeine administration causing an adverse finding for opiates.
- Ingestion of poppy seeds causing an adverse finding for opiates.
- Papaverine (available as a topical treatment for erectile dysfunction) causing an adverse finding for opiates.
- Antibiotics causing an adverse finding for opiates.
- L-methamphetamine (nasal spray) causing an adverse finding for methamphetamine.
- HIV medication causing an adverse finding for THC (Cannabis).
- Pantoprazole causing an adverse finding for THC (Cannabis).
- Over the counter cold medications causing an adverse finding for amphetamine, etc.

All PoCT kits experience this problem to some extent, but the assays for amphetamine, methamphetamine and opiates are the most significantly affected. A survey of several studies using different matrices and different PoCT devices shows the agreement with GC-MS for adverse samples to range from 40% to 100%. The issue with cross-reactivity in PoCT kits is thus significant. As a result, it would never be appropriate to use a PoCT device for anything other than a screening test, where the result of an adverse finding must be considered a presumptive positive at best.
2.7.1 Opiate / Opioid Drug Testing

Opiate / Opioid PoCTs generally detect the presence of morphine, morphine glucuronide\(^9\) and codeine in a sample. However, PoCT tests are unable to distinguish between the individual compounds and can only report a positive for the class of drugs, i.e. a positive for opiates / opioids rather than a positive for heroin. This can result in significant analytical problems. One example of this is when codeine is given to a patient as a legal painkiller. The codeine would be detected by the PoCT device as a positive for opiates / opioids. Even if a separate test for codeine was performed, codeine is metabolised to morphine, which would again causes a false positive (Wolff \etal\. 1999; Brathwaite \etal\. 1995).

Opiate / opioid PoCT devices also report false positives if the subject is taking a range of legal medications. This includes the synthetic opioid hydromorphone, meperidine (an analgesic) and nalorphine (a narcotic antagonist). Dextromethorphan has also been implicated in cross-reacting with immunoassays for opioids, presumably due to structural similarities (Smith \etal\. 2000). Hydrocodone bitartate (cough syrups) and oxycodone HCl are the most common narcotic analgesics that, unfortunately, can generate false positives using immunoassays due to their structural similarities (Osterloh and Becker, 1995).

Cross-reacting compounds can be structurally unrelated to the standardising compound. Several quinolone antibiotics (eg, levofloxacin, ofloxacin) can potentially cause false-positive results for opiates by some common immunoassays, despite no obvious structural similarity with morphine (Baden \etal\. 2001; Zacher and Givone, 2004).

Certain foods can also cause false positives. For example, ingested poppy seeds may cause positive opiate results (Neerman, 2006) due to morphine and codeine being present on the surface of some seeds. In fact, morphine concentrations as high as 10,000 ng per ml have been found in the urine of volunteers who ingested poppy seed bakery products (Thevis \etal\. 2003).

2.7.2 Cannabinoid Drug Testing

The cannabinoid 1-nor-delta-9-tetrahydrocannabinol-9 (THC) is the principal psychoactive ingredient in marijuana (\textit{Cannabis sativa} L.). The compound THC is quickly and effectively absorbed by inhalation or from the gastrointestinal tract and is almost completely

\[^9\text{Morphine glucuronide is the major metabolites of heroin.}\]
metabolized by liver enzymes. Peak plasma levels of THC occur within 10 minutes of inhalation and approximately 1 hour after ingestion. Excretion of urinary and faecal metabolites begins within 72 hours after exposure. Cannabinoid assay PoCT kits detect the major metabolite of THC, 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THCA), in human urine.

THC has been prepared synthetically and marketed under the trade name Marinol® for the control of nausea and vomiting in cancer patients receiving chemotherapy and as an appetite stimulant for AIDS patients (ElSohly et al. 2001). The synthetic cannabinoid called nabilone (Cesamet®) is marketed in Canada for the same indications. PoCT devices are likely to report a positive result after therapy with Marinol®, which is identical to the THC from marijuana and it is possible that an immunoassay may cross-react with nabilone (Gourlay, 2005 and Gustafson et al. 2003)

Another drug currently available in Canada is Sativex® which contains THC and a cannabidiol extracted from Cannabis sativa L.. It is indicated as adjunctive treatment for the symptomatic relief of neuropathic pain in multiple sclerosis in adults. Sativex® will produce positive screening results for the metabolite THCA (Gourlay et al. 2006).

The increased availability of cannabinoid-based therapies has highlighted the need to identify other biologic markers or metabolites from the mix of cannabinoids in naturally occurring products that will accurately distinguish between ingestion of natural and synthetic cannabinoids (Gustafson et al. 2003 and ElSohly et al. 2001).

There have been reports of false positive urine immunoassay tests for cannabinoids in patients receiving proton pump inhibitors, such as pantoprazole (Protonix®) (Wyeth-Ayerst, 2005).

In extreme situations passive inhalation of marijuana smoke can produce positive results with low threshold cannabinoid assays (Niedbala et al. 2004 & 2005). These tend to be restricted to subjects confined to small unventilated areas over long time periods with high atmospheric THC concentrations. Non-smoking subjects exposed to cannabis smoke in more realistic settings are unlikely to have sufficient concentrations of THC to produce a positive result (Mule et al. 1988).

The probability of passive smoking causing a false positive can be greatly reduced by using urine as the sampling medium and applying a quantitative analytical technique such as GC-
MS. This will enable the laboratory staff to identify those samples with borderline THC levels that may be indicative of passive smoking.

2.7.3 Benzodiazepine Drug Testing

The Benzodiazepine immunoassay primarily detects drugs that produce oxazepam glucuronide as a major urinary metabolite. These assays test for benzodiazepines and its metabolites but can also detect related compounds such as alprazolam and midazolam.

The PoCT devices are unlikely to detect the presence of flurazepam, flunitrazepam or triazolam because of poor cross-reactivity and low concentrations of the urinary metabolite (Wild, 2005). False positive results have been shown in samples containing the non-steroidal anti-inflammatory drug oxaprozin (Daypro).

2.7.4 Cocaine Drug Testing

Cocaine PoCT kits are intended for use in the qualitative analysis of benzoylecgonine (the metabolite of cocaine) in human urine. Immunoassays can also detect cocaine and ecgonine. There is no structural similarity between other topical anaesthetics that end in “caine” (eg, procaine, lidocaine) and cocaine or benzoylecgonine. Therefore, cross-reaction does not appear to occur (Gourlay et al. 2006).

2.7.5 Barbiturate Drug Testing

Barbiturate PoCTs can identify long and short acting compounds. The devices typically use the presence of secobarbital above a given threshold to identify a positive result. Cross-reactivity means that most other barbiturates can be identified using this method10.

2.7.6 Methadone Testing

Methadone immunoassays detect the parent molecule (methadone) in both urine and saliva but suffer from cross-reactivity with other opioids.

A study by Collins et al. (2012) found cross-reactivity between the methadone EIA and the prescription painkiller tapentadol and its urinary metabolites. False positives were observed

10 In the case of barbiturates, cross-reactivity is a benefit as it permits the identification of a broad range of barbiturates in addition to secobarb.
when concentrations of tapentadol reached 6,500ng per ml within the sample. The metabolites, tapentadol glucuronide, tapentadol sulfate and N-desmethyltapentadol also caused false positives at concentrations of 25,000, 3,000 and 20,000 ng/mL, respectively. No cross-reactivity was observed with the methadone metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine EIA.

Individual concentrations indicated that separate or combined urinary concentrations of tapentadol and its conjugates may produce false-positive methadone screens through cross-reactivity with the methadone immunoassay (Collins et al. 2012).

Methadone immunoassays also detect high concentration of the antihistamine doxylamine, thereby producing false positive results. This assay does not detect the metabolites of the long acting form of methadone, 1-α-acetylmethadol (LAAM), in concentrations that would be found in the urine of patients on LAAM therapy.

2.7.7 Amphetamine / Methamphetamine Testing

Tests for amphetamine / methamphetamine are highly cross-reactive, and may detect other sympathomimetic amines (e.g. ephedrine and pseudoephedrine) and therefore are frequently unreliable and may lack predictive or diagnostic value. The interpretation of positive amphetamine and methamphetamine results could be challenging because of the structural similarities of many prescription and OTC products, including dietary agents, decongestants and selegiline used in the treatment of Parkinson’s disease (Gourlay et al. 2006). The OTC Vicks® Inhaler marketed in the United States contains L-desoxyephedrine, the L-form of methamphetamine, and could cause a false positive result (Shults, 2002).

Therapeutic doses of the following drugs may produce false positive results with this assay: chloroquine (Aralen), chlorpromazine (Thorazine), methoxyphenamine, quinicrine, phentermine, ranitidine (Zantac), Procainamide and its metabolite N-acetylprocainamide (NAPA). As benzphetamine (Didrex) metabolizes to amphetamine and methamphetamine, therapeutic doses of this drug may also produce a false positive result. Fenfluramine and phentermine are weight loss medications that can also produce false positive results for amphetamines using the immunoassay screens due to cross-reactivity (Osterloh and Becker, 1995)
2.7.8 Phencyclidine Testing

As with methadone, phencyclidine immunoassays detect the parent molecule at a given threshold concentration to identify abuse. Dextromethorphan, a common cough suppressant found in cough syrups, has been implicated in cases of false positives for phencyclidine (PCP) (Schier, 2000).

2.7.9 Oxycodone Testing

The oxycodone immunoassay detects oxycodone and oxymorphone. This assay uses the signal from oxycodone as a cut-off to distinguish positive from negative samples. The assay uses specific antibodies that can detect oxycodone and oxymorphone without any significant cross-reactivity to other opiate compounds.

2.8 Other Factors Impacting on PoCT Results

An individual's physical condition can affect test sensitivity and specificity. Urine testing is not feasible for people with renal failure (e.g., those on dialysis) or other bladder control impairments. George and Braithwaite (1999) found that variations in metabolism and excretion could affect urine concentrations of methadone or its metabolites. Moolchan et al. (2001) noted that renal methadone clearance varies for subjects with certain medical conditions (e.g., renal disease) and those taking other prescribed or illicit drugs.
3 The use of Oral Fluids as a sampling matrix for PoCT

Oral Fluids (OF) provide a number of advantages over urine as a medium for workplace drug testing. In particular, detectable signs of drug abuse will appear in the saliva of a test subject within a few minutes of a drug being administered. Conversely, it can take a number of hours before drug abuse can be detected in the subject’s urine. This is important because a person who has taken a drug may experience the intoxicating effects of a compound long before it could be detected in the urine. This would enable a person to drive or operate machinery in an intoxicated state and still pass a random drug test.

OF sampling also facilitates ease of collection, without the need for private toilet facilities and possible hygiene problems faced by urine collection. The sampling process is also considered the least invasive process of collecting bodily fluids.

Evidence also exists indicating drugs of abuse may become concentrated in OF relative to the plasma. This is due to the change in pH between the plasma (typically pH 7.4) and the OF (pH 4 to 6). The decrease in pH results in ionisation of the compound which prevents the drug or metabolite from passing through the membrane walls within the mouth. This process is commonly referred to as “Ion Trapping” and results in increased OF concentrations of the drug and / or its metabolite(s). In turn, this aids the detection of any compound during subsequent analysis (Bosker & Huestis, 2009). Despite this, drug concentration in OF are still typically less than observed in urine (Verstraete, 2005).

3.1 Oral Fluid Sample Collection

Despite the advantages of OF sampling, there remain a number of issues that make its use more complicated than the commonly used urinary matrix. One of the major issues with OF sampling is the volume of fluid that can be extracted. Although MS analysis can be performed on relatively low volumes of fluid (<20 µl) the immunoassay based PoCT kits require between 0.5 to 1.5 ml of undiluted OF.

The method of OF collection has to be considered carefully when assessing the effectiveness of a testing technique. Ideally, the subject should provide the sample by the “Passive Drool”. In other words, the OF should be allowed to flow out of the mouth and into the collection vessel under the force of gravity. This will provide a sample that truly represents the composition of the OF in the mouth of the subject. However, this process can be difficult, time consuming and distasteful to the person providing the sample.
To aid sample collection, most PoCT suppliers use some form of wipe or swab. Some suppliers add proprietary “Aromas” which are special coatings added to the surfaces of collection devices. Such coatings are designed to stimulate salivation and facilitate rapid OF collection (Table 6). One problem with this approach is that when saliva flow is stimulated it increases pH due to increased bi-carbonate excretion. It is possible that the change in pH may reduce the beneficial effect of Ion Trapping. More importantly, it has been reported that stimulated flow has lower concentrations of drugs than would be found in pre-stimulated OF (Crouch, 2005). Boshker & Huestis (2009) reported that salivary stimulation with citric acid decreased amphetamine concentrations by 1.5 times when compared to individuals without stimulation. The implications of this are clear with the potential for increased false negative rates.

Table 6. Methods of oral fluid collection by a range of PoCT products

<table>
<thead>
<tr>
<th>PoCT product</th>
<th>Oral Fluid collection method</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIOSENS® Dynamic</td>
<td>Wipes or swabs</td>
</tr>
<tr>
<td>Cozart® DDS</td>
<td>Wipes or swabs</td>
</tr>
<tr>
<td>DrugWipe®</td>
<td>Wipes or swabs</td>
</tr>
<tr>
<td>OraLab ® 6 Expresser vials</td>
<td></td>
</tr>
<tr>
<td>OrAlert™</td>
<td>Sucrose and citric acid coated collector stick</td>
</tr>
<tr>
<td>RapidStat®</td>
<td>“Aroma”* coating</td>
</tr>
</tbody>
</table>

*Aroma is a proprietary coating designed to stimulate saliva production

One issue in OF sample collection is controlling the amount of fluid collected. Kauert et al. (2006) observed that OF collection varied between 0.38 to 1.53 g when using the Orasure Intercept® Drugs Of Abuse kit. Another study reported variation in OF collection of 1.05 to 1.67 g in a further 3 sample collection devices (Dickson et al. 2007). Such high variations are significant as they may produce false positives if insufficient sample is available for the immunoassay to function correctly. It should be noted that certain PoCT kits do have
indicators to determine when sufficient OF has been collected\textsuperscript{11}, although this facility is not universal.

Once the sample has been collected the OF must be transferred from the collection device / module to the immunoassay cell. This process has caused significant problems in the design of PoCT devices due to drug residues sticking to the surface of the sample container (Gallardo & Queiroz, 2008). Significant development has occurred to minimise this problem resulting in the use of a range of proprietary buffers and surfactants to “flush” the sample out of the collection device. The buffers have in themselves caused issues as they can interfere with subsequent MS confirmation analysis. They also act to dilute the OF, thereby increasing the effective detection threshold of the kits.

Although the use of buffers and surfactants has improved extraction efficiency, the extraction rates remain highly variable. This is apparent when looking at Table 7 where reported efficiencies range from 12.5\% to 100\%. It is also unclear whether these extraction efficiencies remain constant between different batches of a given collection device. The manufactures of PoCT kits don’t generally report the drug extraction efficiency of their devices. This results in poor knowledge and control of extraction rates that could lead to the reporting of false positive results.

\textsuperscript{11} Cozart® DDS has a sample presence indicator that turns blue when sufficient OF has been collected.
**Table 7.** Observed recovery rates of drugs of abuse from a range of Oral Fluid collection devices. Modified from Bosker & Huestis (2009).

<table>
<thead>
<tr>
<th>Collection Device</th>
<th>Drug Classes</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>THC</td>
<td>OPA</td>
</tr>
<tr>
<td>Acro Biotech</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Salicule</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cozart</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Cozart DDS</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Draeger DCD 5000</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Greiner Bio-One</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Saliva Collection System</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunalysis</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>StatSure Saliva Sampler</td>
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<td>Varian OraTube</td>
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</table>

THC = Cannabis, OPA = Opiates, AMP = Amphetamines, COC = Cocaine, BENZO = Benzodiazepines,
3.2 Contamination of Oral Fluids leading to false positive / negative results

OF contamination can be viewed in three ways:

3.2.1 Contamination from a legal product that results in a false positive / negative result

As discussed previously, there are a number of problems with cross-reactivity during immunoassay analysis, e.g. poppy seeds causing false positives for opiates. However, there is currently little evidence of increased reporting of false positives / negative results due to the use of OF as opposed to urine. No cross-reactivity was found during limited testing of specified foods, drinks, mouthwash, toothpaste, lipstick, gum and cigarettes (Cooper et al. 2004, Cooper et al. 2005, Cooper et al. 2006, Wong et al. 2005). It should, however, be noted that only five PoCT devices were tested in these studies, making the results at best limited. Further studies are clearly needed.

3.2.2 Elevated drug levels in Oral Fluid resulting in positive samples when urinary concentrations are below threshold levels.

One of the complications of OF drug testing is that concentrations of drugs in OFs are often different to plasma concentrations. As described previously, this can occur due to ion trapping but can also occur due to environmental or passive contamination.

Where legislation or an employer sets a threshold concentration to define a positive result, it is difficult to determine what would happen if the plasma concentration of the drug were below this threshold whilst the OF concentration were to be above the threshold. Strictly speaking plasma levels should be used as the true measure of drug abundance as this relates directly to intoxication and impairment. However, the authors of this report are not aware of any thresholds reported in peer review publications that discriminate between OF, urine or plasma. As such, the known elevated concentrations in OF could lead to legal challenges due to the lack of applicable thresholds. This area requires further research.

3.2.3 False positive results caused by passive smoking

Niedbala et al. (2004) observed positive results in four non-smokers after spending 30 minutes in an enclosed room with five smokers. A further study reported positive results for individuals who had not smoked cannabis but were exposed to secondary cannabis smoke in an enclosed vehicle (Niedbala et al. 2005).
This study also identified another significant risk relating to the use of PoCT devices. Kits exposed to the atmosphere within the vehicle showed a 3-14 μg per litre concentration of THCs. Thus, it is clear that environmental compounds can contaminate the sampling devices prior to OF collection. The implications are that an individual test can produce false positives if the collection devices are stored incorrectly. This emphasises the need for a formal and rigorous chain of custody for all kits, including location and handling of the devices prior to sample collection.

3.3 Overall Sensitivity and Specificity of Oral Fluid PoCT Devices

The precise sensitivity and specificity of OF PoCT devices is difficult to determine due to a number of factors:

- The devices normally contain proprietary information and full analytical specifications are not always available (Bosker & Huestis, 2009). To determine the exact performance it is always necessary to carry out independent testing and dosing experiments. Due to the expense, time constraints and medical ethics involved in such procedures, this approach is not always feasible.

- Development of kits is ongoing and improvements may occur after publication of performance criteria. This was highlighted in the review of OF PoCT devices between the ROSITA and DRUID studies. Here performance for the same devices improved significantly when compared to previous studies (Strano-Rossi et al. 2012).

- The performance of OF PoCT devices may vary between batches, making past performance of OF PoCT an unreliable predictor of future performance.

To provide an overview of OF PoCT performance, Bosker & Huestis (2009) reviewed the available literature on analytical precision of devices prior to 2009. A summarised version of the results of this review can be seen in Table 8. Here we see a large range in sensitivity and specificity (“x” indicates the drugs within the devices detection panel). The presence or absence of the drug was determined by either GC or LC-MS, the precise technique used is reported in Table 8 under “Confirmation Test”.

The sensitivity results demonstrate that no device was capable of identifying all positive samples over the range of drugs reported in the kits detection panel.
Of particular concern is the variable sensitivity reported in all devices to different classes of drugs within the devices panel\textsuperscript{12}. For instance, many of the kits performed well when identifying opiate abuse, with a sensitivity of around 90\%\textsuperscript{13}, yet the same device performed poorly when identifying cannabis use (sensitivities were typically less 50\%). One extreme example of this was the AmericanBio-MedicaOralStat device that displayed sensitivity for drugs within the manufactures reported panel that ranged from 0\% to 100\%. The device with the highest specificity was the LifePointImpact® which was able to identify between 70 and 100\% of the positive samples depending on the drug in question. However, if the positives were for one of the less sensitive drugs, such as cannabis, this test would still fail to identify three out of every ten positives.

\textsuperscript{12} The drug panel was determined from the manufacturer’s specifications.

\textsuperscript{13} Cross-reactivity makes most PoCT devices insensitive to synthetic Opioids such as Oxycodone. Many of the reports on Opiate screens do not test for Oxycodone which means that the devices’ sensitivity could be over-reported.

<table>
<thead>
<tr>
<th>PoCT device</th>
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<th>A</th>
<th>C</th>
<th>Confirmation</th>
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SN (%) | SP (%) | Failures (%) |
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<td>42.1–100</td>
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<td>ELISA98.6GC-MS91.3</td>
<td>ELISA/GC-MS:98.1/98.9</td>
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<td>70.0–100</td>
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<td>10.0–100</td>
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| THC = Cannabis, OPA = Opiates, AMP = Amphetamines, COC = Cocaine, BENZO = Benzodiazepines, SN = Sensitivity, SP = Specificity. |

The review shows a similar problem with specificity (the ability to identify true negatives), with high variability between devices and within devices among different drugs listed in their screening panel (inter and intra device specificity). Some devices do show 100% specificity,

14 SunOraLine achieved 100% sensitivity in one study (Walsh et al. 2007) but at significantly higher concentration of the study target detection threshold. At lower concentrations false positives and negatives were reported for Benzodiazepines, THC and TCH-COOH.
but these are limited to PoCT kits that only tested for one or two classes of drug and / or at high drug threshold detection levels. It should also be noted that testing programmes that look at real world populations will generally find large numbers of true false positives. This has the effect of skewing specificity results making them appear better than may actually be the case. Because of this, specificity should be viewed in conjunction with the overall accuracy of the devices (Strano-Rossi, 2012).

Another concern is the general failure rate of the OF devices. A failure is defined as the inability of the device to determine a positive or negative due to a fault in the device. Nearly a quarter of the devices listed in Table 8 reported device failure, with VarianOraLab displaying the highest rates with one in four devices failing.

Although the data reviewed in Table 8 provides a good overview, many of the studies were carried out in ideal conditions. It is important to determine how the devices perform in real world drug testing programmes.

3.4 Real World Use of Oral Fluid PoCT devices for the detection of drugs of abuse

The major advantages of OF PoCT devices are the apparent ease of deployment and use in real world applications. It is claimed that the kits do not require specialist facilities and that non-technical staff can be quickly trained to perform the testing procedures. The testing procedure can also be carried out in a matter of minutes compared to laboratory based procedures that can take over 24 hours. For this reason the OF PoCT approach has generated significant interest in the field of detecting Driving Under the Influence of Drugs (DUID). Two major studies have looked at the use of PoCT devices by police forces to identify and prosecute DUID offences. These were the ROADSIDE Testing Assessment (ROSITA and ROSITA – 2) and Driving under the influence of drugs, alcohol and medicines (DRUID). The devices used in these studies were assessed for sensitivity, specificity and accuracy. The DRUID project also assessed operational performance (i.e. ease of use in a field situation).

The ROSITA 2 project was carried out between 2003 and 2005 by a range of European and US based research, medical and criminology organisations\textsuperscript{15}. This project was followed

\textsuperscript{15} The study was carried out by National Institute for Criminalistics and Criminology in Brussels, Belgium, the National Public Health Institute in Helsinki, Finland, the Institute for Legal Medicine in
shortly afterwards by the DRUID project that reported in 2012. Both projects used subjects stopped by the local police force at areas where drug abuse was considered prevalent. The ROSITA project carried out roadside tests on 2046 subjects whilst the DRUID project carried out tests on 1025 subjects. All subjects where tested using a range of OF PoCT devices which were validated by subsequent laboratory based MS analysis.

The results from the ROSITA 2 study showed that none of the available PoCT devices where suitable for DUID detection. This resulted in many of the kits used in the study being modified or removed from sale (Crouch, 2005; Verstraete & Raes; 2009). The quality of the devices was so poor it led to the following comment by a leading researcher in the field:

“The promise of worldwide OF testing spurred commercial research and development of PoCT devices, and commercial devices were rushed to market before much of the basic science of drug excretion into OF was known.” (Bosker & Huestis, 2009).

As can be seen in Figure 2, the sensitivity of the devices in the ROSITA study is highly variable between different classes of drugs within the same testing panel. This conforms to the results from previous OF PoCT studies (Bosker & Huestis, 2009). The results also follow the same pattern with the devices displaying the highest sensitivity for Opiates and lowest sensitivity for Cannabis.

Strasbourg, France, the Institute for Legal Medicine in Homburg/Saar, Germany, the Division of Forensic Toxicology and Drug Abuse, Norwegian Institute of Public Health, Oslo, Norway and Institute of Legal Medicine, University of Santiago de Compostela, Spain. It was coordinated by Ghent University, Ghent, Belgium.

The study was performed in cooperation with the United States, where it is funded by The National Institute on Drug Abuse (NIDA), National Institutes of Health, US Department of Health and Human Services, the National Highway Traffic Safety Administration (NHTSA), US Department of Transportation and the Office of National Drug Control Policy Executive Office of the President. The US part was coordinated by The Walsh Group (Bethesda, Maryland). The study is carried out in the following states: Florida (Hillsborough County Sheriff’s Office, Florida Department of Law Enforcement, Manatee County Sheriff’s Office), Washington (Washington State Police, Washington State Toxicology Lab), Utah (Salt Lake City Police Department, Center for Human Toxicology) and Wisconsin (12 Police Jurisdictions, Wisconsin State Lab of Hygiene).
The DRUID project appeared to show some improvement in analytical performance when compared to the results from the ROSITA 2 project, although it should be noted that the acceptance criteria for the devices was reduced from > 90% sensitivity and 95% specificity to >80% sensitivity in the DRUID project (Schulze et al., 2012). Despite this, the devices performed poorly when detecting cannabis abuse and none of the devices were deemed sufficiently robust for use in roadside testing (Strano-Rossi et al., 2012).

A further study attempted to use four of the top performing OF PoCT devices from the ROSITA 2 and DRUID projects (Strano-Rossi et al., 2012). This confirmed that three out of the four devices did not meet the specification required for detection of DUID. Figure 2 shows that the sensitivity of two devices for cannabis was below 50%\(^{16}\) and for a third around 90%. The fourth device (DrugTest™) appeared to be within the DRUID criteria for use as in DUID detection (90% sensitivity and 95% specificity). Despite this, the results contradict those of the DRUID project and the error bars (Figure 3) show that the margin of error is such that the true analytical performance could fall outside the required criteria. It should also be noted that the device suffered from a number of failures. These included false positive results for cannabis due to haematic contamination of the swab interfering with

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\(^{16}\) When using the kit manufacturers recommended detection thresholds.
the band reading of the device. Strano-Rossi et al. (2012) also reported that some of the devices gave false positives for amphetamines during cold weather.

Figure 3. PoCT sensitivity to Cocaine and Cannabis as reported in (Strano-Rossi et al. 2012). Positivity criteria were based on the kit manufacturer’s recommendation (CUTOFF KIT) and those used in the DRUID study (CUTOFF DRUID).

The clear advantages of OF PoCT devices make the progress in this field interesting. However, the sensitivity and specificity of the kits still compares poorly to laboratory based MS techniques. As such, confirmation of PoCT positives by laboratory based MS is universally recommended by the referenced studies.

It could be argued that OF PoCT devices are desirable due to their flexibility, low cost and fast turn-around. It could also be argued that their poor accuracy could be compensated for by providing laboratory based confirmation. However, the poor specificity of the devices will result in false negative results which will not be identified by confirmation testing. Both ROSITA and DRUID demonstrate that OF PoCT testing is in many cases less reliable than traditional psychophysical methods employed by trained personnel, such as nystagmus.
testing. (Citek et. al, 2003). This will result in drivers and other safety critical workers who are under the influence of drugs continuing to drive, operate machinery & plant and carrying out potentially hazardous jobs. If the poor specificity became public knowledge, this could reduce the effectiveness of any drug testing programme as a deterrent.

Although OF PoCT devices show great promise, they clearly do not have the sensitivity or specificity required for legally defensible drug testing programmes without subsequent MS confirmation. Their use as a screening programme is possible but will result in a significant number of false positives and false negatives. The consequences of the false readings will depend on the workplace and the function of the operative involved. However, they could be extremely serious.
4 Administration and Application of Workplace Drug Testing Procedures

Although the scientific validity of a drug testing programme is essential, the administration of the programme is equally important. As such it is essential that any programme follows strictly laid out and precise protocols, including:

- Written and approved Standard Operating Procedures (SOP).
- Formal validation and review of the procedures described in the SOP.
- Where possible inter-laboratory / organisation validation.
- Where possible, approval of procedures by international accreditation organisations.
- Strict sample collection and sample transportation records i.e. a Chain of Custody (CoC).
- Quality control measures to ensure that minimum testing standards are followed and achieved.

4.1 Accreditation

Typically, any company or organisation wishing to establish a drug testing programme will seek some form of national or international accreditation. This allows the company to demonstrate a degree of competence in their abilities to any potential clients. Two of the most commonly used and well respected organisations are the International Standards Organisation (ISO) and The United Kingdom Accreditation Service (UKAS). It should be noted that UKAS often acts as the licensing body for ISO accreditation and many companies may display both UKAS and ISO accreditation whilst having met only the ISO requirements.

Care should be taken by any potential customer viewing accreditation claims as they may not always relate to a particular testing programme. For example, a company providing PoCT devices and subsequent laboratory validation analysis may hold ISO17025 accreditation (General Requirements for the Competence of Testing and Calibration Laboratories). This accreditation will cover the procedures occurring in the laboratory, but will not relate to any procedures performed outside the facility with the PoCT devices. It should also be noted that many accreditations simply confirm that certain procedures are carried out in a uniform and traceable manner. This simply means that standard procedures have been followed and any deviations from that procedure have been recorded and can be traced and rectified at a later date. This does not ensure that the procedures are good or fit
for purpose. For example, ISO9001 is a quality management accreditation and would not address an issue such as low specificity in PoCT kits to THC.

UKAS and ISO do have accreditation programmes for medical PoCTs, and have awarded accreditations to a number of UK-based companies (ISO 22870:2006 (Point of Care Testing – Particular Requirements for Quality and Competence)). However, there is no formal international accreditation for PoCT use in workplace (occupational) drug testing programmes. It should be noted that ISO 22870 does require confirmation testing of presumptive PoCT positives using MS procedures. It also requires sample collection and results interpretation to be carried out by qualified medical staff.

To help provide some guidance to companies performing workplace testing programmes a professional organisation known as the European Workplace Drug Testing Society (EWDTS) was formed in 1998. This organisation is a private body that is not affiliated to the EU or any other governmental body. The society is funded by subscriptions and private sponsorship.

The EWDTS has helpfully produced a guideline document to assist companies or organisations wishing to implement workplace drug testing programmes entitled:


However, this guidance document does not provide detailed protocols on the use of PoCT kits. The document does refer to an appendix describing the use of PoCT devices. However, this section of the report was never completed (EWDTS pers. com. 2012.).

The report does state that:

“[w]here immediate test results are required Point of Collection test (PoCT) can be utilized, but the principals and procedure for specimen collection outlined in these guidelines still apply.”

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17 In the United States, the FDA does have a clearance process for PoCT devices. However, this approval does not apply to ensuring manufacturing processes. Nor does it review of the application of the kit by a testing provider. (Walsh 2008).
From this statement it would appear that the EWDTS recommends that all users of PoCT devices should follow the same procedures as would be expected in laboratory analysis and sample collection.

U.S. Federal guidelines also require that the results and quality controls of PoCT Kits be “like” those from a laboratory. Specifically;

“Regardless of the design of the device, it must perform drug and validity tests “like” a laboratory, with the same precision, accuracy, and reliability for each analyte around the cutoff. Additionally, if the validity of a PoCT is separate from the PoCT device, the validity tests must also perform “like” a laboratory by testing for creatinine, oxidizing adulterants, and pH. All results generated through PoCT must be equal to results generated from an HHS-certified laboratory.” (Bush, 2008).

Even these requirements, however, are not a standard or accreditation in themselves. The federal guidelines attempt to relate, in unspecified ways, PoCT kit results to laboratory methods, yet provide no specific guidance.

With the lack of any formal accrediting organisation it is possible for any individual to set up a company and supply PoCT devices. The accuracy of the devices claimed by the manufacturer will have no external verification or validation against external standards. This could permit less scrupulous organisations to supply substandard or poorly tested devices. Reliance on such instrumentation could result in serious legal implications if poorly functioning devices report false positives or false negatives.

The issue of poor quality control has been addressed in a number of studies and prompted one author to state that:

“... manufacturer’s claims should be interpreted with caution and should be verified in each organisation’s patient population, if possible.” (Melanson et al. 2010)

It would seem prudent that any organisation wishing to use a PoCT led testing programme should ensure that, at a minimum, the company providing the service is accredited to the medical standard ISO 22870. This may appear excessive but could prevent costly litigation. Working to ISO 22870, however, does put an onus on an organisation because it requires that all collectors are medically qualified. In effect, it requires that collectors (who will presumably interpret the “instant” results) are trained medical staff with a knowledge base, in
respect of drug testing matters, beyond that required by collectors who send all samples for laboratory testing.

4.2 Chain of Custody

Even without formal accreditation, some organisations may wish to proceed with PoCT testing in the workplace. One of the first considerations for of any testing programme is to ensure that the sample collected is from the intended subject. This requires a Chain of Custody or CoC.

US government regulations are clear:

“All urine specimens must be collected using chain of custody procedures to document the integrity and security of the specimen…” (Bush, 2008).

The World Anti-Doping Agency (WADA) considers CoC to have been appropriately conducted when:

“[t]he external record is initiated at the collection site and ensures that the Samples and the results generated by the Laboratory can be unequivocally linked to the [donor].” (WADA TD2009LCOC).

The EWDTS provides similar language requiring that:

“…the results reported relate beyond a doubt to that specimen.” (EWDTS (2011)).

The importance of this step from both a scientific and legal perspective cannot be understated. The consequences of a failure of the CoC were recently demonstrated in the case of Ryan Braun, a high profile US baseball player. A somewhat technical, but clear, deviation from the CoC rules set forth by Major League Baseball invalidated an adverse drugs finding. The consequences of this failure resulted in a ban being overturned and collection procedures of the entire Baseball drug testing programme having to be reviewed. The possibility of further legal action in the case has not been ruled out.

4.2.1 Specific Problems Relating to the Chain of Custody of PoCT Devices

To understand the problems encountered when attempting to establish a CoC for PoCT devices it is necessary to review the basic processes behind the testing procedure. As
described previously, PoCT kits depend on competitive immunoassays to identify the presence or absence of the drug of abuse in the sample.

Once the sample has been voided, a wick draws up the fluid and mixes the sample with a labelled antibody and assay reagents. The combined sample / assay mixture continues up the wick until it reaches a testing area containing an antibody. The labelled antibody is captured and a chromatic signal developed, typically in the form of a visible bar. Crucially, this bar will only remain visible for as long as the solution remains aqueous, usually only for one hour. The impermanence of the test result data results in a significant problem. The “original” results are rapidly lost and cannot be retrieved at a later date. There are ways to document the results, ranging from photocopying the result to simply having the technician record them with a pen and paper. However, all these methods introduce the possibility of transcription error and none would meet international standards for a CoC. Other organisations that carry out drug testing require that the initial test results are retained in case the findings are later challenged.

WADA requires that:

“[t]he raw data supporting all analytical results shall be retained in secure storage for at least eight (8) years.”

ISO 17025 requires a laboratory to:

“retain records of original observations, derived data and sufficient information to establish an audit trail…” (ISO 17025 2005).

As the initial result is lost, any legal challenge could depend upon the testimony of a poorly qualified operative and his / her recollection of the testing procedure and results. As the operative may have carried out a significant number of tests on the day in question and the legal challenge may occur sometime after the date of collection (months or even years), it is unlikely the individual could provide an accurate testimony of his / her actions.

Further problems may ensue if the operative has since left the company or is unwilling to appear in a court to provide testimony.
4.2.2 Comparison of PoCT Chain of Custody with Laboratory Based Chain of Custody

Contrasted against standard laboratory methods, CoC with PoCT devices is poor. An ISO 17025 accredited laboratory is required to maintain the original data, potentially indefinitely. All laboratory methods, including GC-MS and laboratory immunoassays, provide electronic or printed original data.

Laboratory CoC standards, as exhibited by the WADA ISL, requires that the laboratory

“record the Analytical Testing process and the traceability of the Sample during Analytical Testing.” (WADA TD2009LCOC).

WADA further requires that

“[t]he Laboratory shall have Laboratory Internal Chain of Custody procedures to maintain control of and accountability for Samples from receipt through final disposition of the Samples.” (WADA ISL 2012).

CoC documentation must also be included with any documentation package reporting an adverse result. (WADA ISL. 2012). This should also be presented as the evidential package at an industrial or employment tribunal, something that is not possible for PoCT kits and which may result in legal challenges.

4.3 Quality Control Standards

Due to the lack of a formal accreditation body or process, Quality Control (QC) varies between manufacturers and suppliers. Some of the cheaper PoCT devices have no formal QC making their analytical precision at best uncertain. The most common QC control is a visible bar that appears if the device is not functioning correctly. This is an extremely limited form of QC and falls short of even the most basic requirements of a forensic or drug testing application.

Standard QC practice for laboratories requires that:

“[e]ach assay batch must include a minimum total of 10% open and blind positive and negative quality control samples in an appropriate urine matrix. External blind quality control samples are not required, but are highly recommended.” (Goldberger, 1997).
This, however, represents only a minimum acceptable standard. Accredited laboratories have more rigorous requirements. ISO 17025 requires:

“The laboratory shall have quality control procedures for monitoring the validity of tests and calibrations undertaken. The resulting data shall be recorded in such a way that trends are detectable and, where practicable, statistical techniques shall be applied to the reviewing of the results. This monitoring shall be planned and reviewed and may include, but not be limited to, the following:

a) Regular use of certified reference materials and/or internal quality controls using secondary reference materials;

b) Participation in inter-laboratory comparison or proficiency-testing programmes;

c) Replicate tests or calibrations using the same or different methods;

d) Retesting or recalibration of retained items;

e) Correlation of results for different characteristics of an item.”

(ISO 17025 (2005))

These quality controls are in place not only to ensure the reliability of the results, but also to serve the related goal of providing a legal defence of those results. PoCT kits, because they are designed to be inexpensive and self-contained, cannot provide a robust in-assay quality control. Therefore, the results cannot be relied upon without a laboratory based confirmation procedure.
Discussion

How good are PoCT devices at detecting drug abuse?

5.1.1 False negative results

The ability of PoCT devices to identify the presence of a drug of abuse when present in a sample (device sensitivity) is far from perfect. The sensitivity of the device varies between manufacturer, target compound and the threshold set for detection. For example, an oral fluid tested by a DrugTest® device will correctly identify 95% of the positive samples with high concentrations of cocaine. However, when using an Oralab 6® device to identify low concentrations of cannabis, less than 20% of the positives will be correctly identified (i.e. a false negative rate of > 80%).

Ignoring the high variability, PoCT device sensitivity compares poorly to other techniques such as GC or LC MS. This makes their application to any programme that requires legally defensible analysis problematic. Two major European studies, ROSITA and DRUID, highlighted this problem when investigating the use of PoCT devices to identify individuals driving under the influence of drugs. Both studies concluded that the PoCT devices tested had insufficient sensitivity for legally defensible DUID detection.

Further complexity is added when considering the format in which the PoCT devices are sold. Typically a device will be marketed as being capable of identifying a panel of compounds. For example, a device may claim the ability to identify “Opiates”. However, this will not include the semi-synthetic opioids oxycodone, oxymorphone, buprenorphine, methadone or hydromorphone, even at high concentrations. The detection limitations of PoCT devices are rarely described in detail in the product specifications and any organisation wishing to use PoCT devices should seek expert advice prior to implementation of the programme.

5.1.2 Implications of false negative PoCT results

As demonstrated in the case of cannabis, false negative rates for PoCT devices can exceed 80%. The consequences of this are simple; a subject can pass a test yet still be intoxicated by a drug of abuse. If that subject is undertaking safety critical duties such as operating plant & machinery or flying a plane or driving a train or lorry, then the consequences of a false negative result could be catastrophic.
5.1.3 False positive results

In general PoCT devices perform better in relation to false positives than they do to false negatives. However, cross-reactivity in certain classes of drugs can result in poor specificity and a high rate of false positives. Some of the more common compounds that can result in false positives are listed below:

- Codeine administration causing an adverse finding for opiates.
- Pseudo-ephedrine causing adverse findings for amphetamines
- Ingestion of poppy seeds causing an adverse finding for opiates.
- Papaverine (available as a topical treatment for erectile dysfunction) causing an adverse finding for opiates.
- Antibiotics causing an adverse finding for opiates.
- L-methamphetamine (nasal spray) causing an adverse finding for methamphetamine.
- HIV medication causing an adverse finding for THC (Cannabis),
- Pantoprazole causing an adverse finding for THC (Cannabis).

This list is by no means exhaustive and can be further complicated by interactions with other compounds and legal medications. This means that a full history of a test subject’s medication and dietary intake is essential to minimise the risk of a legal compound causing a false positive. It therefore follows that sampling staff must have sufficient training to identify any legal medications, compounds or foodstuffs that might cause a false positive.

It is also possible that a test subject might be able to mask drug abuse by claiming they were taking one of the compounds that are known to cause false positives.

Due to the real possibility of false positives, it is essential that all actionable results be confirmed by GC or LC MS.

**The effect of Thresholds on POCT accuracy**

Most drug testing programmes set concentration thresholds to define what is “positive” or “negative”\(^\text{18}\). In the case of the US these are set in law, but in the UK and Europe thresholds are defined by the organisation controlling the testing programme. This

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\(^\text{18}\) Refer to section 1.4.2 for a full description of thresholds.
means that positivity thresholds vary between different organisations and testing programmes which, in turn, cause significant problems in the use of PoCT devices. This is because PoCT kits have a fixed detection threshold and only provide a qualitative result (i.e. positive or negative). Therefore, if the threshold level set by the organisation is different to the detection level of a PoCT device, it is impossible to apply the positivity criteria of the testing programme.

As it is difficult to purchase PoCT devices with custom detection levels, it is likely that many testing programmes set their positivity criteria based on the detection thresholds of the device. This is clearly not ideal and may result in detection thresholds higher than would be desirable.

It is also important to note that if an organisation wishes to use lower thresholds this will affect the accuracy of the PoCT device. Melanson et al. (2010) found that 88% of laboratories using PoCT immunoassay tests could identify samples with 50 ng per ml of THC, yet only 25% of laboratories could detect THC at concentrations of 35 ng per ml. The threshold level for cannabis set by Network Rail, Cross Rail, London Underground and the National Grid is 25 ng per ml. At these lower thresholds it is questionable whether the use of PoCT devices is of any analytical benefit. In such circumstances other more sensitive techniques should be employed.

**Accreditation**

Some of the most commonly used and well respected accreditation organisations are the International Standards Organisation (ISO) and The United Kingdom Accreditation Service (UKAS).

UKAS and ISO do have an accreditation programme for medical PoCTs, but there are no formal international standards for their use in workplace (occupational) drug testing programmes.

With the lack of any formal accrediting organisation it is possible for any individual to set up a company and supply PoCT devices. The accuracy of the devices claimed by the manufacturer will have no external verification or validation against external standards. This could permit less scrupulous organisations to supply substandard or poorly tested devices. Reliance on such instrumentation could result in serious legal implications if poorly functioning kits report false positives or false negatives.
It would seem prudent that any organisation wishing to use a PoCT based testing programme should ensure that the company providing the service is accredited to the medical standard ISO 22870. Although this may appear overly cautious, it will certainly, nonetheless, provide protection from costly litigation.

With the use of PoCT devices, it is impossible to implement and maintain an adequate CoC. This is because the results of PoCT immunoassays are not generally permanent, with results fading within an hour. Therefore, the only record of the test results is the data recorded by the individual performing the test. PoCT devices are also single use and typically do not provide the ability to collect extra fluid for confirmation testing. As a result, the initial test cannot be verified without the collection of a second sample.

With no permanent recording of data and a limited ability to retest the sample, it is impossible to produce a legally defensible CoC.

**Quality Control**

In the absence of a formal accreditation body, Quality Control (QC) varies between manufacturers and suppliers. Some of the cheaper PoCT devices have no formal QC, making their analytical precision, at best, uncertain. Any company wishing to use PoCT kits as part of their testing programme should seek independent validation of the devices’ performance on a regular basis to ensure that proper QC is maintained.

**Do POCT devices offer an economic advantage over laboratory based procedures?**

One of the reported benefits of PoCT devices is their onsite use by operatives with only minimal training, avoiding the need for highly qualified and expensive laboratory staff. However, due to poor specificity and cross-reactivity it is necessary to complete a detailed and complex test subject history. This will include a list of medications and food ingested prior to the test. The operator / collector must have sufficient training to identify and record any substance that would result in cross-reactivity producing a false positive or negative result. It is well known that in clinical settings immunoassay tests are more accurate when the results are interpreted by clinicians rather than non-technical staff (Melanson et al., 2010). Therefore, the use of poorly trained individuals is likely to increase the false positive / negative rates. This will have a direct economic cost as it will increase the number of confirmation tests required. Although there are no studies quantifying the costs of false positives, it is likely to be significant.
Can POCT devices reliably detect drug abuse?

Although PoCT devices have many benefits, poor sensitivity and specificity result in an unacceptably high rate of false positive and negative results. This limits their use to a screening role which identifies individuals that warrant further testing by more reliable methodologies, such as GC-MS. This process is mandatory in the US for all workplace testing programmes and clinical applications. Currently there is no legislation in the UK and Europe controlling the use of PoCT devices.

It is also clear that any legal challenge to PoCT test results is indefensible without subsequent confirmation testing. It should be noted that many PoCT manufacturers and distributors accept the need for confirmation testing and offer this facility to their customers. A leading manufacturer clearly states on their website that PoCT devices should only be used as a screening tool:

“Screening tests

The first test on a sample is typically a screening test based on immunoassay technology [such as POCT devices]. This can be carried out using an instant test or in a laboratory.

Screening tests are used to identify negative results. Positive or non-negative screening results may require a more detailed confirmation test.

Confirmation testing

Confirmation testing, using GCMS (gas chromatography/mass-spectrometry) or LCMS (liquid chromatography/mass-spectrometry), provides fully defensible results for a court of law or industrial tribunal. They can only be carried out in the laboratory and are the ‘Gold Standard’ in drug testing.” (Concateno web site, 2012).
Conclusions

Even a cursory review of the literature reveals an unacceptably high rate of erroneous results reported by PoCT devices. The published accuracy is so poor that results cannot be assumed to be correct unless validated by techniques such as GC or LC MS.

Detection rates for certain compounds, such as cannabis, are so low that even limited application of PoCT devices is questionable.

Due to the limitations of PoCT devices, workplace testing programmes must be limited to a screening role with presumptive positives being confirmed by GC-MS or similar gold standard techniques. Even this limited role must be considered carefully as GC-MS confirmation will only identify false positive, and not false negative, results. As we have seen in the case of cannabis, false negative rates can be very high. It is, therefore, clearly unwise to use PoCT devices where the test subject is involved in a safety critical role. In such circumstances a high sensitivity / specificity approach is essential.

Due to the absence of an accreditation body for PoCT manufacturers, there is no independent guarantee on the quality and reproducibility of devices sold. Due to this, it is advisable that any organisation considering the use of PoCT devices seeks independent testing to ensure the specifications reported by the manufacturers are correct. In the absence of an accreditation body, it is also advisable that all testing programmes seek compliance with the clinical PoCT standard ISO 22870:2006 (Point of Care Testing – Particular Requirements for Quality and Competence). It should be noted that this standard requires that all sample collections and results interpretations are carried out by medically qualified staff.

One of the arguments in favour of using PoCT devices is removal of the need to employ highly skilled and costly sampling officers. It is, however, clear that PoCT sampling / analysis should be carried out by competent individuals who understand the performance specifications of these devices and threshold requirements of the testing programme. An understanding of how medications and food products can result in false positives and negatives is also essential. It is thus necessary that all PoCT collectors will need to be medically trained. The average PoCT collector will certainly have to be more highly (and specifically) trained than the average collector whose samples will always be sent to a laboratory for professional analysis and interpretation.
PoCT devices have a limited role to play in occupational drug testing and, due to a preponderance of analytical limitations, they cannot be used in isolation without further confirmation testing. The legal and safety implications resulting from erroneous results must be considered carefully before implementing a testing programme designed around PoCT devices.
7 About the Authors

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Simon currently holds the position of Post Doctoral Research Officer at Imperial College London. Simon manages the Mass Spectrometry facilities in the faculty of Earth Science and Engineering.

His research interests are focused on developing new and novel Mass Spectrometry and analytical techniques. Simon has held similar positions at Berkeley, Queens and Liverpool University. He also worked for Micromass UK (now Waters Inc.) as an instrument development engineer. During this period he helped design a range of Mass Spectrometry products now used for drug detection in many laboratories throughout the world.

Simon has also acted as an expert witness on analytical drug detection in a number of high profile sports doping cases. These included the Merlene Ottey (Jamaican Sprinter) and Floyd Landis (Tour de France cyclist) cases.

Mr. Paul Scott BSc. JD.

Paul is the founder and Chief Science Officer (CSO) of Scott Analytics, Inc. In this role he identifies and develops new and novel analytical techniques to detect abuse of both sporting and recreational drugs.

Prior to this Paul acted as Director of Clients at the UCLA Olympic Analytical Laboratory and a founder and the Chief Science Officer of the Agency for Cycling Ethics, Inc. For the last five years he has been at the forefront of drug detection science.

Before joining the UCLA laboratory, Paul was a practicing attorney at the New York offices of Sidley, Austin, Brown and Wood where his practice was primarily focused at the biochemical and pharmaceutical industries.

Paul holds a Bachelor of Science degree in Chemistry and Biology and a Juris Doctorate in Law.
Dr. Genevieve Boshoff PhD., BSc., CSci.

Genevieve is currently Technical Director of ProtiumMS. In this position she is in charge of analytical and instrumental product development, in particular Mass Spectrometry and novel laser Ratiometry techniques.

She has extensive experience in analytical techniques ranging from immunochemistry to techniques such as GC-MS and IRMS. She has been involved in setting up analytical laboratories and commissioning and testing analytical equipment for laboratory use both in the UK, and Europe.

She also has extensive involvement in academia, most recently as lecturer at Keele University and previously at Queens University. She has also been responsible for developing and delivering a number of lecture modules including offerings on biotechnology and analytical techniques and developing training materials for regulatory staff.

Genevieve also has close links with national and international technical organisations and maintains a presence in the industry through diverse activities including regular presentations at conferences and workshops and writing articles for journals and trade magazines.

Genevieve holds a BSc in Zoology and Microbiology, and a BSc (Honours) and PhD in Biotechnology and has been appointed as a Chartered Scientist (CSci).
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