Importance of false positive and false negative results obtained from urine samples analysed for Drugs of Abuse, using data obtained from the AXIO Drugs of Abuse in Urine (DAU) PT scheme

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Introduction

Screening urine for the presence of drugs is undertaken to detect recent drug use or misuse. This may be undertaken for a variety of reasons including healthcare, occupational monitoring, insurance screening, legal and forensic purposes. Any errors in the analysis could have severe consequences for the individual whose urine is being analysed. These include, but are not restricted to, dismissal from work or a potential miscarraige of justice.

The AXIO Drugs of Abuse in Urine (DAU) Proficiency Testing scheme offers four testing rounds per year, each containing 3 samples of lyophilised human urine. Assessment of the results returned by participants are undertaken according to the participant's choice of reporting thresholds. It includes the drugs that have thresholds dictated by the European Workplace Drug Testing Society (EWDTS) and the Substance Abuse and Mental Health Services Administration (SAMHSA). Additional substances are also included within the annual schedule to both cover current additional drugs of abuse and also to more broadly test the laboratories capabilities.

Incorrect results may be reported for a variety of reasons. Commonly, these reasons include 'non-analytical errors' such as transcription errors or sample mix-ups and 'analytical errors' such as method effects, sample/matrix interferences and methodological sensitivity. False positive and false negative findings can have serious consequences for individuals and/or criminal proceedings. Results obtained from the DAU scheme were examined including: fentanyl cross-reaction with LSD screening tests, the use of amfetamine screening tests and their limitations, and the failure of some barbiturate screening tests to detect the presence of certain barbiturates. The results obtained demonstrate the potential risks of relying on screening tests alone, with the possible consequences explained.

False Positive Findings

It is recommended and is standard practise in a Forensic environment that any positive screening test result should then confirmed using an additional confirmatory technique such as LC-MS, in order to unequivocally identify the presence or absence of the substance in question. If this process is not followed there is a possibility that an individual may be accused or subjected to the repercussions of a positive result when no substance (or an alternative substance) was present. This may occur when other substances, additional to those for which the assay is designed, react with the assay to produce a positive response.

An example of this type of error is the presence of fentanyl in urine specimens and the positive screening results that may be encountered with certain LSD Screening Tests. The manufacturers of the screening tests that have been identified below do include details regarding the cross reactivity of fentanyl with the LSD Screening tests within their literature. Fentanyl has been included in the DAU scheme for a number of samples at different concentrations and the following observations noted. Figure 1 shows the Fentanyl information from various historical samples.

Round and sample	Fentanyl concentration (µg/L)	Number of false positive screening tests results	Comments and confirmatory analysis
DU133-2	44	Nine false positive results (Eight CEDIA, one EMIT)	Only three laboratories undertook confirmatory analysis for LSD and obtained negative results. Six laboratories may have reported an incorrect LSD positive finding.
DU139-3	30	Three false positive results (Two EMIT and one "Other")	All three labs undertook confirmatory analysis and excluded the presence of LSD
DU144-3	23	Five false positive results (Three CEDIA, one EMIT and one "Other")	None of these labs further tested for LSD so all may have reported a LSD false positive finding
DU148-1	258	Ten false positive results (Seven CEDIA, one EMIT, one POCT (Point of Care Testing) and one "Other")	Only two laboratories undertook confirmatory analysis for LSD and excluded its presence, therefore eight laboratories have reported an incorrect LSD positive finding
Manufacturers include the following details:			
Siemens: EMIT, the LSD assay states that the presence of Fentanyl at a concentration of 3 ng/ml (3 µg/L) will provide a positive response.			
ThermoFisher Scientific: The CEDIA Assay insert states that the presence of Fentanyl at a concentration of 40 ng/mL (40 µg/L) will give a positive response.			
Reference: False-Positive Lysergic Acid Diethylamide Immunoassay Screen Associated with Fentanyl Medication, Gagajewski et al, Clinical Chemistry, January 2002.			

Figure 1: Fentanyl information

It is interesting to note that only in one round (DU139) all the laboratories who reported a positive LSD Screening Test result undertook a confirmatory analysis that excluded the presence of LSD. For all other rounds identified, a significant number of the laboratories did not exclude the presence of LSD and therefore there is the possibility that in a real case a report detailing the positive finding for the LSD Screening test may have been issued.

False Negative Findings

A false negative result may have serious consequences, as a negative screening test result would not necessarily be followed up with the confirmatory analysis unless there was other evidence/information to suggest that a substance had been ingested. Therefore, there is the possibility of the presence of a substance being missed entirely and the repercussions that may be associated with that scenario.

A false negative result may be encountered due to the varying cross reactivities of an assay designed for a particular screening group to various substances that are within that class of substances. Examples of this are Benzodiazepine Assays and the various benzodiazepines such as diazepam, temazepam, nitrazepam and many others too numerous to list, opiate assays, amphetamine assays, barbiturate assays and many others.

The DAU scheme has identified this issue in numerous samples, of which the Barbiturate Screening Test is a specific example. In round 139- Sample 2, distributed in 2021, Phenobarbitone was included at a spiked concentration of 503 µg/L. Laboratories are assessed upon the detection of a substance but may also submit the concentration detected if they wish. The Assigned Value determined from the confirmatory analytical results was 489.3 µg/L. This concentration is significantly greater than both the EWDTS reporting threshold of 200 µg/L and the Clinical (LGC) reporting threshold of 300 µg/L. A number of laboratories received unsatisfactory assessments for the barbiturate screening results. Figure 2 shows the assessments obtained by laboratories (referred is where tha laboratory stated they would refer the analsys for further testing), for all reporting thresholds. A laboratories which obtained an unsastisfactory assessment for the screening test, may risk missing the presence of phenobarbitone in a case sample if further investigation was not undertaken.

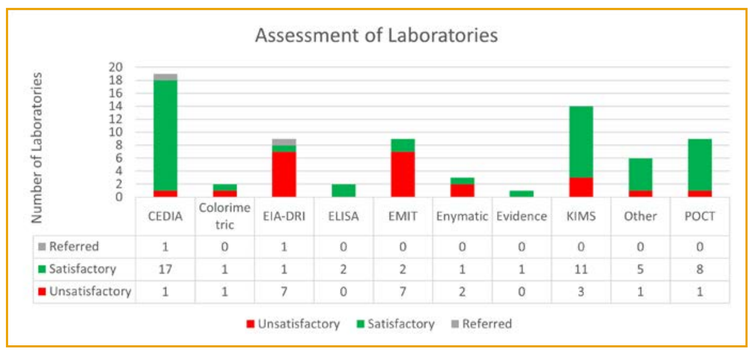

Figure 2: Total assessment for the various Barbiturate Screening Assays for Round 139- Sample 2

A number of laboratories also included quantitative data for the screening assays. Figure 3 shows the comparison of the median concentrations reported by the various assays with the assigned value from the confirmatory analysis and the two reporting threshold. It is clear that the lack of sensitivity of certain assays to phenobarbitone explains why laboratories participating in the DAU scheme may report a false negative finding.

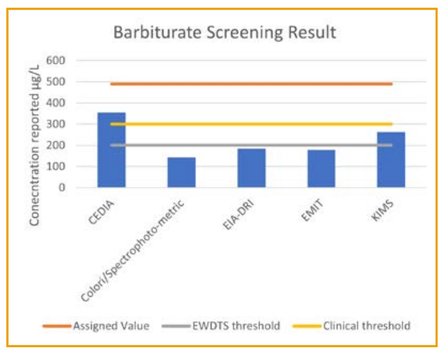

Figure 3: Concentrations and reporting threshold for Phenobarbitone and the methodologies.

It is important to note however, that the majority of the assays are raised to secobarbitone, which has been used previous within the DAU schene, with no observed issues with 'false' reporting of results below regulatory thresholds. The manufacturers of the assays include the cross reactivity information and therefore, it is down to the laboratory to ensure that the methods that they are using are fit for their purpose.

Amfetamines and Screening Tests

There are numerous screening tests and methodologies used to detect the presence of amfetamine and amfetamine-type drugs (including amfetamine, methamfetamine, MDMA screening tests). Since not all laboratories use or are able to use the MDMA or methamfetamine Screeng tests, LGC requests that for assessment purposes everything should be reported under the amphetamine screening group (and then additionally using the methamfetamine and/or MDMA Screening Group, if applicable). Metabolites also cross react to the Screening tests to varying degrees e.g. MDA.

We are not going to look at the responses to the presence of amfetamine as that is what the amfetamine screening tests are raised to detect and no issues have been observed where amfetamine has been present in the DAU test samples, as amfetamine screeinng tests are typically raised agaisnt this molecule

Figure 4 shows the assessments for Round 148, Sample 1 which contained MDMA (1300 µg/L) and MDA (507 µg/L). Figure 5 shows the assessments for Round 148, Sample 2 which contained Methamfetamine (1230 µg/L). The concentrations of each of these drugs are greater than the Clinical (1000 µg/L) and SAMHSA and EWDTS (500 µg/L) reporting thresholds.

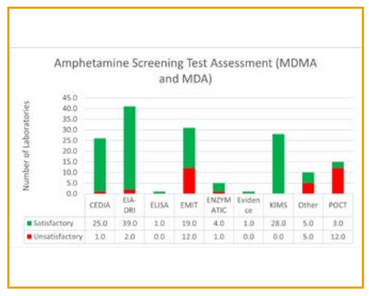

Figure 4: Assessments for Round 148- Sample 1

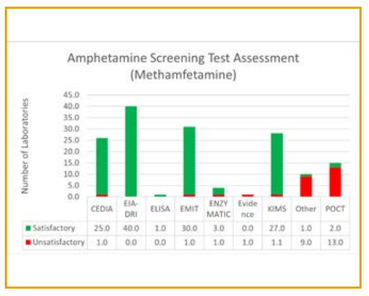

Figure 5: Assessments for Round 148- Sample 2

Conclusion

Urine drug screening may be undertaken using screening tests such as Point of Care Tests (POCT) or immunoassays. These test are generally fast, inexpensive and may be used to screen for the possible presence of a substance. It is then recommended where there are implications for the individual concerned that any positive screening result is then confirmed by a confirmatory analysis such as LCMS. It is also worth noting that it is increasingly common for 'confirmatory' analysis methods, such as LC-MS/MS to be used for screening.

It is extremely important that users are aware of the limitations of screening tests which include the possibility of false negatives due to sensitivity issues and false positives due to the potential of other substances to cross-react with the assays and when any unexpected findings are received from both case samples and PT schemes that these potential causes are investigated. Users should also be aware of the potential issues and repercussions that may be experienced for the individuals whose samples are being analysed if incorrect results are being issued.

A reference point should be the information from the manufacturers of the method used as the cross reactivity information is often readily available, however, it is our experience that not all users are aware. In addition, it is important to note that an assessment is provided based upon the presence or absence of a substance and not whether a method is able to detect it. Therefore, it is up to the laboratory to determine whether a methodology is suitable for their purpose.