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Foreword

In November 2005 we published our first Laboratory Service Users Guide. The demand for copies of this document was high and the feedback from our customers has been very positive. As we are committed to continual improvement we are now publishing this updated version.

Some of the topics which have been updated include:

- Range of testing;
- Interpretation of confirmatory analyses;
- Cross-reactivity information;
- Information on ISO 17025 accreditation.
- It is now downloadable from our website at www.addictionireland.ie

Generally, we have tried to make the guide as clear and concise as possible.

I welcome the second publication of this user guide and I commend the hard work of our laboratory team for its compilation and distribution.

Sheila Heffernan
General Manager

March 2007

Introduction

This toxicology laboratory guide has been written for medical, nursing and support staff who avail of The Drug Treatment Centre Board (DTCB) drug analysis service. It provides general information on how to access this service and details the range of tests available.

The Drug Analysis Laboratory is the leading centre for drugs of misuse testing in Ireland. It provides a national drug analysis service to health areas, general practitioners, hospitals (general, psychiatric and maternity), juvenile detention centres, voluntary organisations and the Dublin drug court.

The laboratory uses techniques which include immunoassay and chromatography to analyse drugs such as Opiates, Benzodiazepines, Cannabis, Cocaine, Amphetamines, Alcohol and EDDP (the primary metabolite of methadone).

The laboratory has a full-time staff of twelve, including a Principal Biochemist, four Senior Biochemists, three Basic Grade Biochemists, four Laboratory Aides and a half-time administrative assistant.

General Information

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Trinity Court, 30-31 Pearse St., Dublin 2.

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Samples used in Drug Analysis

Suitable samples for drug testing include urine, blood, hair and oral fluid.

Urine is the most commonly used specimen because it is easy to collect and drugs can be detected for a number of days, and in some cases, even weeks, after last use. Urine is the universally preferred sample when testing for the presence or absence of drugs. Urine is unsuitable for determining drug levels due to the many factors which influence the composition and concentration of urine. Urine samples should be collected under supervised conditions where appropriate.

Blood is more difficult to obtain and the time during which the drug can be detected after use is in the order of hours as opposed to days. However it is more suitable than urine for determining drug levels.

Hair is easily collected and, depending on the length, can demonstrate a historical record of the client's drug use, over a period of time.

Oral fluid is also easily collected but like blood it has a short window of detection. Oral fluid is still the subject of much research.

Hair and oral fluid are not routinely tested by this laboratory.

Range of Testing

There are five categories of testing, these include - testing for adulterants, routine screening analysis, confirmatory analysis, therapeutic drug monitoring of methadone, and non routine testing.

Testing for Adulterants

Adulteration testing refers to tests carried out to determine whether a urine sample is genuine or if it has been tampered with in any way. Methods of urine adulteration include dilution with, addition of, or substitution by, a drug-free substance or solution.

Dilution is probably the most common method of adulteration used by drug users to evade detection of misuse. Creatinine levels in urine can indicate the extent of this adulteration. Normal urine should have a creatinine concentration of 80-200mg/dL. Research in our laboratory has shown that in order to achieve creatinine levels of less than 20mg/dL excessive amounts of water must be consumed. Therefore urine samples with less than 20mg/dL will be reported as dilute.

Urine samples with creatinine levels of $<2\text{mg/dL}$ are considered abnormal. These samples are reported as 'abnormal' and therefore no other test results will be reported.

Routine Screening Analysis

The majority of drug tests performed fall into this category. Routine screening is carried out by immunoassay, enzyme assay or chemical assay. These are rapid methods used for screening drugs of misuse. A routine screen includes opiates, benzodiazepines, EDDP (the primary metabolite of methadone), cannabis, cocaine, amphetamines, alcohol and creatinine.

Immunoassay is a method which indicates the presence or absence of a drug in a sample. The current format used does not give any information about the level or concentration of the drug (with the exception of alcohol). Results are reported as positive or negative. Care should be taken when interpreting screening results as many over the counter drugs can give true positive results which the healthcare worker might not expect. An example of this is Solpadeine® which will give a positive opiate result because it contains codeine which is an opiate. Some drugs and medications can produce false positive results using immunoassay and further confirmatory analysis may be necessary. An example would be pseudoephedrine which can give a false positive result for amphetamine. This cross reactivity occurs most commonly with opiates and amphetamines. However, benzodiazepine, cannabis, cocaine and the methadone metabolite immunoassay tests are relatively specific and rarely produce false positive results. Because of this phenomenon screening results by immunoassay alone are not legally defensible and further confirmation of the test result is required. See Appendix 1.

Confirmatory Analysis

All confirmatory testing must be specifically requested by a doctor. Confirmatory analysis is carried out for opiates and benzodiazepines. See Appendix 2 and Appendix 3 for details.

The methodology involved is gas chromatography mass spectrometry (GC-MS). Confirmatory test results are legally defensible provided sample integrity can be proven (see page 8 for sample collection and chain of custody information).

Therapeutic drug monitoring of methadone

Therapeutic drug monitoring of methadone in serum is assayed using an enzyme linked immunosorbent assay (ELISA). This test will identify rapid methadone metabolisers and can help with titrating methadone dosages provided other factors are taken into account. It is important to treat the patient, not the level.

Blood samples should only be submitted for serum methadone level testing if the following criteria have been adhered to:

- A minimum of 3 days supervised methadone consumption prior to the day of blood collection.
- The time of dosing on each day should be the same plus or minus 30 minutes.
- The blood sample must be taken immediately before the next dose on day 4.
- The time must be the same as the previous 3 days plus or minus 30 minutes.
- Samples must be collected into a serum tube.
- The sample must be accompanied by a request form which includes time of dose on previous day, time of dose on day of sampling and time of blood collection.

Failure to adhere to these guidelines will result in unreliable data and defeat the purpose of carrying out the procedure.

It has been suggested that 150ng/ml of Methadone or greater should be adequate to maintain dose.

Non-routine testing

Non routine tests include 6-Acetylmorphine (6-AM), Tricyclic antidepressants, Zopiclone, Buprenorphine, pregnancy testing, pH, specific gravity and glucose. These tests are carried out on request only.

The full range of analyses available is indicated opposite in Table 1.

Service Users Guide

Test	Method	Sample Type	Test Type	Levels	Window of Detection	Turnaround time*
Opiate	IA	Urine	Screen	n/a	3-10 days	48 hrs
Opiate	GC-MS	Urine	Confirm	n/a	3-10 days	10 days
6-AM	IA	Urine	Screen	n/a	24 hours	48 hrs
Benzodiazepine	IA	Urine	Screen	n/a	2-28 days	48 hrs
Benzodiazepine	GC-MS	Urine	Routine	yes	2-28 days	10 days
EDDP	IA	Urine	Screen	n/a	Unknown	48 hrs
Serum methadone level	IA	Blood	Screen	yes	n/a	Tested twice weekly (Tues & Thurs)
Cannabis	IA	Urine	Screen	n/a	2-28 days	48hrs
Cocaine	IA	Urine	Screen	n/a	2-4 days	48hrs
Amphetamine	IA	Urine	Screen	n/a	1-2 days	48hrs
Tricyclic antidepressant	IA	Urine	Non-Routine	n/a	2-4 days	48hrs
Alcohol	Enzymatic	Urine	Screen	yes	1-2 days	48hrs
Zopiclone	GC-MS	Urine	Confirm	n/a	unknown	10 days
Burprenorphine	IA	Urine	Screen	n/a	2-4 days	Contact Lab
Creatinine	Chemical	Urine	Screen	yes	n/a	48hrs
Pregnancy	Enzymatic	Urine	Non-Routine	n/a	n/a	48hrs
pH	Chemical	Urine	Non-Routine	n/a	n/a	48hrs
Specific Gravity	Chemical	Urine	Non-Routine	n/a	n/a	48hrs
Glucose	Chemical	Urine	Non-Routine	n/a	n/a	Contact Lab

Table 1: Range of testing carried out in the Drug Analysis Laboratory of the Drug Treatment Centre Board.

** Please Note: Turnaround time is measured from time of receipt of sample at the laboratory*

Abbreviations:

6-AM: 6- acetylmorphine, metabolite of heroin

Benzo: Benzodiazepines

Chemical: Chemical Assay

Confirm: Confirmatory Test

EDDP: 2-ethylidine-1,5-dimethyl-3,3-diphenylpyrrolidine, primary metabolite of methadone

GC-MS: Gas Chromatography Mass Spectrometry

IA: Immunoassay

Screen: Screening Test

Consent

The DTCL Drug Analysis Laboratory does not take responsibility for obtaining “consent to test” for samples received for drug testing.

Consent should be obtained by the doctor or organisation requesting the test prior to sending samples to the laboratory. If the client is under 18 years old consent to screen should be obtained from a parent or guardian.

Packaging and Transport

The laboratory will only accept samples packaged according to ADR regulations, which have been enacted into Irish law. ADR is a European agreement concerning the international carriage of dangerous goods by road. Under these regulations samples are divided into two groups, diagnostic and infectious.

- A diagnostic sample is defined as a specimen collected for the purpose of diagnosis.
- An infectious sample is defined as a specimen containing a viable micro-organism that is known or reasonably believed to cause disease.

UN approved packaging for diagnostic and infectious samples is a triple packaging system consisting of:

- Primary receptacle – the urine bottle. This must be leak-proof, clearly labeled and wrapped in enough absorbent material to absorb all fluid in case of breakage.
- Secondary receptacle - used to enclose the primary receptacle. This must be durable and leak-proof. Several primary receptacles may be placed in one secondary receptacle. Sufficient additional absorbent material must be used to cushion multiple primary receptacles.
- Outer packaging – the secondary receptacle is placed in an outer package which protects it and its contents from outside influences such as physical damage and / or water while in transit.

This type of packaging is available commercially or through HSE Eastern Region Shared Services, Procurement and Materials Management division.

Most urine samples are generally considered to be diagnostic, however if you are unsure, please contact the laboratory for advice. It is the responsibility of the sender to ensure the correct designation, packaging, labeling and documentation of all infectious and diagnostic specimens.

Request Forms

Request forms for routine screening, confirmatory analysis and serum methadone levels are available on our website or from Laboratory Customer Services.

Information required by the laboratory prior to analysis

The following information must be included on the sample container and request form when submitting a sample for analysis.

- Patient's full name
- DAIS code (where appropriate)
- Date of birth
- Name of clinic or hospital
- Name of Doctor
- Date of sample collection
- Sample type

Failure to include the above could hinder the processing of a sample.

Sample Collection

Urine samples should be of approx. 20mls to 30mls in volume, collected in a clean plastic container without preservative. Care should be taken to ensure that the sample is authentic i.e. it has been freshly voided by the patient under supervision and not subsequently adulterated or substituted for a “drug free” specimen. It should then be sent as soon as possible to the Laboratory. Samples received more than 14 days after the collection date will not be tested due to the possibility of breakdown products generated on prolonged storage, which could result in false results.

In the absence of direct supervision, a temperature strip on the collection bottle can help determine if a sample is genuine. The temperature of urine should be between 34-39 °C when freshly voided.

If blood is the required matrix, then ideally 10mls of blood is required, collected in a serum tube. Standard precautions and procedures should be followed when sampling.

Information on the collection of hair or oral fluid samples can be obtained from Laboratory Customer Services.

Chain of Custody

In order for test results to be defensible in a court of law or professional hearing, chain of custody procedures must be followed. Chain of custody involves fully documenting who donated, collected and handled the sample thereafter. The DTCLB laboratory can provide information on chain of custody collection kits and sampling procedures. For further information please contact Laboratory Customer Services.

Non-Compliant Samples

Non-compliant samples are samples which do not demonstrate the mandatory information required to identify a particular sample, i.e. full name, date of birth, sample date and location from which the sample was sent. Samples missing any of the aforementioned data may not be analysed. The laboratory will make every effort to obtain the correct sample identification in order to proceed with analysis.

Leaking samples are also considered to be non-compliant samples. Leaking samples will not be analysed and the sample will be disposed of immediately.

Notification of non-compliant samples will be sent in writing to the sender by means of a non-compliance form detailing the nature of the non-compliance and requesting the correct information, before analysis can take place.

Storage and Retention of Samples

Samples should be sent to the laboratory at the earliest opportunity. If there is any delay, it is recommended that they are stored in a refrigerator at 4°C or in a cool dark place if refrigeration is not available. Post analysis, the laboratory will retain samples for 10 days, after which they will be disposed of safely. Should further testing be required outside of this period samples will be frozen.

All positive Chain of Custody samples will be frozen and retained for 36 months. All negative Chain of Custody samples will be frozen and retained for 3 months.

Quality Control and Quality Assurance

To ensure high confidence in test results, the laboratory adheres to strict quality control (QC) and quality assurance (QA) standards. Approx. 3% of all samples are quality controls. In order to assess performance, the laboratory is involved in two external quality assurance schemes, the United Kingdom National External Quality Assessment Scheme (UKNEQAS) and the Australian Urine Toxicology Proficiency Programme (AUSTOX).

Viewing of quality control data, proficiency testing data, and testing procedures will be accommodated on request by arrangement with the laboratory.

Accreditation

Our laboratory is accredited by the Irish National Accreditation Board (INAB) to ISO 17025. We are assessed annually by external auditors in order to maintain this high standard. The scope of our accreditation can be viewed at www.inab.ie/pdf/169T.pdf

Reporting

The front page of each report details the customer name and address, drug cut-off concentrations (above or below which samples are deemed positive or negative) date on which each report is generated and scope of INAB accreditation.

Each patient is identified by name, date of birth, clinic code and chart number. A sample is identified by a unique barcode and sample date.

If a drug is detected, the result will appear as a "+" (positive). When no drug has been detected, it will be reported as a "-" (negative). A blank space indicates that no test was carried out. Results will be available within 24-48 hours of receipt of samples in the laboratory. Confirmatory testing usually takes longer to perform (see Table1).

Method of reporting is by prior arrangement with Laboratory Customer Services. Reports are usually sent by post, alternatively they can be faxed provided the confidentiality of the information can be guaranteed. Verbal reporting can only be accommodated in emergencies. Some HSE Addiction Service users may be able to access results electronically via the Drugs Aids Information System (DAIS).

Membership and Representation

To ensure best practice and to keep up to date with the latest developments and trends in drug misuse, laboratory staff have professional membership and attend meeting of various international societies, these include:-
TIAFT- The International Association of Forensic Toxicologists
SOFT- The Society of Forensic Toxicologist
EWDTS- European Workplace Drug Testing Society

The laboratory is also represented at the Early Warning and Emerging Trends (EWET) committee of the National Advisory Committee on Drugs (NACD).

Appendix 1 - Cross Reactivity Table

Medications which give true positive Opiate results	Medications which give false positive Opiate results	Medications which do not interfere with Opiate results	Medications which give false Amphetamine results	Medications which give false Tricyclic Antidepressant results
Benlylin with Codeine (codeine)	Quinolones	Diconal (Dipipanone)	Colofac (Mebeverine)	Tolvon (Mianserin)
Codeine Phosphate (codeine)		Distalgesic (Dextropropoxyphene)	Ephedrine	
Codis (codeine)		Doloxene (Dextropropoxyphene)	Pseudoephedrine	
Cyclimorph (morphine)		Equagesic (Meprobamate)		
DF118 (dihydrocodeine)		Pethidine		
Dimotane - Co (pseudoephedrine and codeine)		Temgesic (Buprenorphine)		
Feminax (codeine)				
Migraleve (codeine)				
Morphine Sulphate (MST) (morphine)				
Nurofen Plus (codeine)				
Oramorph (morphine)				
Panadeine (codeine)				
Paracodin (dihydrocodeine)				
Sevredol (morphine)				
Solpadol (codeine)				
Solpadeine (codeine)				
Tylox (codeine)				
This list is not exhaustive	This list is not exhaustive	This list is not exhaustive	This list is not exhaustive	This list is not exhaustive

Active ingredients are shown in brackets where appropriate



Appendix 2

Interpretation of Benzodiazepine Identifications

Metabolic pathways of benzodiazepines can often result in common metabolites, (the most significant is Oxazepam), therefore it can be difficult to absolutely identify which parent drug(s) was originally consumed. The routine immunoassay screening procedure for benzodiazepines is unable to distinguish between metabolites, therefore urinary benzodiazepine evaluations are carried out using more sophisticated techniques which can specifically target these metabolites. See Table 2 below.

Parent Drug	Target Metabolite
Diazepam / Valium	Nordiazepam; Oxazepam; Temazepam
Normison	Temazepam
Dalmane	2-hydroxy Flurazepam
Librium	Chlordiazepoxide

Table 2

There is no international data regarding benzodiazepine levels in urine. In 1999, a small study was carried out by our laboratory to monitor urinary benzodiazepines from seven detoxifying patients in a closed ward setting. This study suggested that resulting levels of < 3ug/ml Oxazepam and/or <3ug/ml Temazepam were frequently seen in cases of some commonly prescribed therapeutic dose benzodiazepines.

More recently, studies here have shown that levels of >10ug/ml Oxazepam and/or >10ug/ml Temazepam are frequently found in cases of suspected benzodiazepine abuse. However, many factors influence the dilution of a urine sample and consequently the drug concentration, therefore levels reported should be interpreted with caution.

Appendix 3

Interpretation of Opiate Identifications

In the body, heroin is metabolised firstly to 6-acetylmorphine (6-AM) and then to morphine. The presence of 6-AM proves the use of heroin, however, 6-AM has a short half-life and cannot be detected in urine 24 hours after last use. Therefore morphine is the most commonly detected metabolite in heroin abuse. A small amount of morphine also results from the metabolism of codeine (which includes over the counter painkillers). Because of this, it can be difficult to distinguish between the use of codeine or morphine alone or a combination of both.

Acetylcodeine is an impurity also detected in heroin which breaks down to codeine.

The routine immunoassay screening procedure for opiates is specific for morphine, codeine and dihydrocodeine but it cannot distinguish between them. Therefore a positive opiate result by immunoassay will be obtained if any one, or a combination of the above is present above the cut-off concentration of 300ng/ml.

In order to identify which opiate is present, further testing is required. Firstly, a 6-AM test is carried out and if positive, heroin use is confirmed. If negative, further testing by GC-MS must be performed.

See table below for interpretation of GC-MS results.

GC-MS Result	Interpretation
Morphine	Confirmation of use of formulated morphine or heroin
Codeine	Confirmation of codeine use
Dihydrocodeine	Confirmation of Dihydrocodeine use
Opiate Use	An opiate was confirmed, but cannot distinguish which one was taken

Table 3

NOTES

