Feidhmeannacht na Seirbhíse Sláinte
Health Service Executive

LÁRIONAD NÁISIÚNTA CÓIREÁLA DRUGAÍ

NATIONAL DRUG TREATMENT CENTRE

DRUG ANALYSIS LABORATORY

HSE National Drug Treatment Centre
Drug Analysis Laboratory

A Guide to Service Users

7th Edition
July 2018

Contents

Introduction

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

The Drug Analysis Laboratory provides a national drug analysis service to the HSE Addiction Services, general practitioners, hospitals (general, psychiatric and maternity), juvenile detention centres, voluntary organisations, and the Dublin Drug Court Probation Service.

I welcome the 7th publication of this user guide and I commend the hard work of the laboratory team for its compilation and distribution.

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General Information

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Samples for Drug Analysis

Many different types of biological samples can be tested for drugs including urine, blood, oral fluid and hair. Here at the NDTC we screen urine and Oral Fluid for drugs of abuse.

Table 1: Drug detection times in biological matrices

Sample type	Detection time range	Drug use detected
Oral fluid	0-48 hours	Acute, under the influence
Blood	0-48 hours	Acute, under the influence
Urine	Days - weeks	Recent

Urine

Urine is the most commonly used specimen due to its ease of collection and long window of detection (drugs can be detected for a number of days, and in some cases, weeks, after last use). It is the universally preferred sample to screen for the presence or absence of drugs. However, urine is unsuitable for determining drug levels due to the many factors which influence the composition and concentration of urine.

Blood

The only blood testing done in the NDTC is therapeutic monitoring of blood methadone levels. (see page 7 for further information).

Oral fluid (Saliva)

Oral fluid collection may be time consuming and the volume of oral fluid is small. Drug levels in oral fluid are low compared to levels found in urine and in addition levels of drugs and their metabolites have a shorter window of detection in comparison to urine. However collection of oral fluid can be supervised without any invasion of privacy.

Hair

Hair can demonstrate a historical record of drug use, however the NDTC do not provide this service.

Consent

The HSE NDTC 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 should be obtained from a parent or guardian.

Packaging and Transport

Samples should be packaged according to United Nations (UN) regulations. Under these regulations samples are divided into two groups:
1) Diagnostic: a specimen collected for the purpose of diagnosis.
2) Infectious: a specimen containing a viable microorganism that is known, or reasonably believed, to cause disease.

UN approved packaging consists of a **triple packaging** system:

- **Primary receptacle** – the urine bottle. This must be leak-proof, sealed securely, 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 the contents from outside influences such as physical damage and / or water while in transit.

Supply

UN3373 approved packaging is available commercially from third parties (please contact the laboratory directly if you require further information).

NOTE: It is the responsibility of the sender to ensure the correct designation, packaging, labeling and documentation of all specimens.

Sample Collection

The procedure for sample collection is detailed in Table 2 below (Standard precautions and procedures should be followed when sampling).

Table 2: Sample collection

Sample Type	Collection Details
Urine	• Use clean plastic container <u>without preservative</u>. Preferably 70 ml yellow lidded Sarstedt urine pots. • 20 - 30mls where possible. • Where collection bottle with temperature strip is used temperature of urine should be between 34-39^{0}C when freshly voided. • Sample should be stored in a cool, dry dark place (preferably refrigerated) pending dispatch to the laboratory • Sample should be dispatched to Laboratory as soon as possible
Oral Fluid	• Use Quantisal collection device (no other collection device will be accepted) • Observe donor 10-15mins without food or drink prior to collection. • Supervise collection (approx. 5 mins).
Blood (Serum Methadone only)	• Serum Red Cap Tube with 10 mls where possible

Sample Label Information

The following information is mandatory and must be included on the sample container;

1. Patients full name
2. Date of Birth
3. Name or assigned code of clinic or hospital
4. Date of sample collection

Request forms are not necessary: any extra testing can be requested by writing the test required on the sample label.

In addition the following information may be required on some samples

1. Name of Doctor
2. DAIS Code

Note: failure to include the correct information prevents the processing of a sample.

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 **cannot be analysed**. The laboratory will make every effort to obtain the correct sample identification in order to proceed with analysis.

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

Notification of non-compliances will be sent to the sender by means of a comment on report or a non-compliance form, detailing the nature of the non-compliance.

Request Forms

Request forms are available on the HSE NDTC web site (www.addictionireland.ie), however forms are NOT required for routine screening. Additional tests can be requested by writing the test code on sample bottle. See table of test abbreviations in Additional Non –Routine Testing Table 4.

Request forms for blood methadone testing are also available on the website and should accompany all blood methadone requests

Range of Testing

Testing is divided into several categories including:

- Testing for adulteration
- Routine urine screening
- Additional non-routine urine screening
- Confirmatory analysis
- Oral fluid screening
- Serum Methadone levels.

See details in the Table 3 below. (See page 5)

Testing for adulterants

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

Dilution is 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 dilution, therefore all samples received for analysis are tested for Creatinine.

'Normal' urine should have a Creatinine level in the range 80-200mg/dL.

'Dilute' urine is indicated by a Creatinine level of less than 20mg/dL.

'Abnormal' urine is indicated by a Creatinine level of <2mg/dL.
No test results will be reported on samples deemed to be 'abnormal'.

Abnormal **pH** readings will indicate tampering of a sample by the addition of or substitution by, another substance or liquid.

Table 3: Range of Testing

Sample type	Test Type	Method	Test	Window of Detection	Normal Turnaround Times	Included in Scope of Accreditation
Urine	Routine Screen	IA	**Opiate Class**	2-4 days[1]	24-48 hours	Yes
			6-AM	24 hours[2]		Yes
			Benzodiazepine Class	3-30 days[1]		Yes
			EDDP	Unknown		Yes
			Cannabis class	1-30 days[1]		Yes
			Cocaine	2-4 days[1]		Yes
			Amphetamine Class	1-2 days[1]		Yes
		Enzymatic	**Alcohol**	7-12 hours[1]		Yes
		Chemical	**Creatinine**	n/a		Yes
	Non-Routine	Enzymatic	**Pregnancy**	n/a		No
		Chemical	**pH**	n/a		Yes
			Glucose	n/a		No
		IA	**EtG – Alcohol marker**	Up to 48 hours[3]		Yes
			Buprenorphine	4-24 hours[4]		Yes
	Confirm	LC-MS	**Opiate Identification**		5-10 days	No
			Zopiclone			No
			'Headshop' products (psychoactive substances) Bathsalts only			No
			THC-COOH (Cannabis metabolite)			No
		LC-MS	**Benzodiazepine identification**			No
Oral Fluid	Screen	IA	**Opiate Class**	1-36 hours[3]	5-10 days	Yes
			6-AM	unknown		Yes
			Benzodiazepine Class	unknown		Yes
			Methadone	unknown		Yes
			Cannabis class	Up to 24 hours[3]		Yes
			Cocaine	1-36 hours[3]		Yes
			Amphetamine	1-48 hours[3]		Yes
Blood	Screen	IA	**Methadone levels**	n/a		No

Please Note: Turnaround time is measured from time of receipt of sample at the laboratory
Abbreviations:
6-AM: 6-acetylmorphine, primary metabolite of heroin; EtG – Ethyl Glucuronide (Alcohol biomarker)
EDDP: 2-ethylidine-1,5-dimethyl-3,3-diphenylpyrrolidine; primary metabolite of methadone
LC-MS: Liquid Chromatography Mass Spectrometry; IA: Immunoassay; Screen: Screening Test

1. Moeller et al Urine Drug Screening: Practical Guidelines for Clinician Mayo Clin Proc Jan 2008 83(1):66-76
2. Cedia Package Insert
3. Objective testing-Urine and other drug tests. Hadland and Levy Child Adolesc Psychiatr Clin N Am 2016 25(3) 549-565
4. Robert Kronstrand Journal of Analytical Toxicology, Vol. 32, October 2008

Routine Screening Analysis

The majority of drug testing performed by the laboratory falls 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. Tests included in the routine screen are detailed in table 3 above.

Immunoassay is a **qualitative method** which indicates only the presence or absence of a drug/drug class in a sample.

Each test by immunoassay has **a defined cut-off level**, above which the test is deemed positive indicating that the presence of a drug/drug class was detected above the cut-off level.
If a test result falls below the cut-off level, the result is deemed negative indicating that the drug/drug class was not detected above the cut-off.
Cut-off levels are detailed on every test report.

With the exception of alcohol, the current format used does not give any information about the level or concentration of the drug present.

It should be noted that all analytical results are subject to **Uncertainty of Measurement (UoM),** *see section on Uncertainty of Measurement (page 11). The performance of qualitative test results around the cut-off concentration is routinely monitored by the use of quality controls which are run with every batch of samples. Clinical consideration and judgment should be applied to all immunoassay test results. Confirmatory analysis may be requested for a positive drug screening result if required. (See page 8)

Additional non routine testing

Additional tests not routinely performed on samples include 6-Acetylmorphine (6-AM) the primary metabolite of Heroin, Zopiclone, Buprenorphine, 'Headshop' drug analysis (New Psychoactive substances, see Appendix 4), pregnancy testing, pH, glucose and Ethyl Glucuronide (EtG).

These tests are carried out on request only. These requests can be made by writing the test abbreviation on the sample bottle/label.

Table 4 Test abbreviations for additional tests

Drug	Abbreviation
Heroin Metabolite	6-AM
Ethyl Glucuronide	EtG
Suboxone/Buprenorphine	Bupn
Zopiclone/Zimmovane	ZOP
Opiate ID	OPIA ID
Tramadol	Tram
Pregnancy	hcG
Headshop	Hshop
Full Screen	FS
Oxycodone	Oxy

The full range of analyses available is indicated in Table 3 (page 5).

'True and 'False' Positives

Care should be taken when interpreting immunoassay screening results, as some over the counter drugs will give 'true' positive results. An example of this is Solpadeine® which will give a positive opiate result because it contains codeine, which is also classified as an opiate type drug.
Some drugs and medications can also produce **'false' positive results** when tested using immunoassay, due to cross reactivity and further confirmatory analysis may be necessary. This cross reactivity occurs most **commonly** with **opiate** and **amphetamine** drug classes.

A study carried out in our laboratory showed that for the **CEDIA® Amphetamine/Ecstasy assay**, cross reaction was occurring with other compounds particularly new psychoactive substances such as cathinones **('Headshop' Drugs/Bath Salts/Legal Highs).** If you have any queries about an amphetamine positive result please contact the laboratory. Confirmatory analysis can be carried out on request. Benzodiazepines, cannabis, cocaine and EDDP immunoassay tests are relatively specific and rarely produce false positive results. See **Appendix 1 for table of cross-reactivity for urine kits.**
Because of cross-reactivity, screening results by immunoassay alone are not legally defensible and further confirmation of the test result is required depending on the purpose of the testing.

Therapeutic drug monitoring of Methadone

Therapeutic drug monitoring of methadone is performed to identify rapid Methadone metabolisers and can **help with titrating Methadone dosages**, provided other factors are considered when interpreting the result, e.g. sex, weight, time of dose, time of sampling etc. (It is always 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:

CRITERIA

- 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** +/- 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 +/- 30 minutes.
- Samples must be collected into a **serum tube**.
- The sample must be accompanied by a **request form**/ letter 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.

Therapeutic levels of Methadone:
'With chronic administration of 100-200 mg daily oral doses to tolerant subjects, the plasma concentration peaked at 4 hours, with an average value of 0.83ug/ml (range, 0.57 -1.06) and declined to 0.46mg/L (range, 0.28-0.79) 24 hours after last dose (average plasma half life of 25 hours).'
'It has been estimated that trough plasma methadone levels should be at least 0.05 - 0.10 mg/L to prevent withdrawal systems in narcotic maintenance patients (i.e. 50-100ng/ml)'.
[Baselt 2004, Disposition of Toxic Drugs and Chemicals in man, 6th edition, p. 642 – 643]

Confirmatory Analysis

Confirmatory analysis is carried out using liquid chromatography mass spectrometry (LC-MS).
See Table 3 (page 5) and Appendices 2, 3 and 4 for further details.

Oral Fluid testing

Oral Fluid tests include Opiates, Benzodiazepines, Methadone, Amphetamine, Cocaine, Cannabis and 6-Acetylmorphine (the primary metabolite of heroin). The methods are accredited for using the **Quantisal® collection devices** and no other collection device will be accepted (product code QS-0025) available from Alere Toxicology PLC (Concateno) 0044-1235861483.

Depending on the drug used, dosage and route of administration, a drug may be detected in oral fluid in less than one hour after use and remain detectable up to 48 hours after last use.
Substances such as **food, beverages**, over-the-counter **medication**, and mouthwash can affect the oral fluid drug test results. For this reason, before collection of saliva or oral fluid, the collector should observe the donor for a **10-15 min period** in which **the donor should not smoke, consume food or drink**.

The full range of analyses available is indicated in Table 3 (page 5).

Subcontracted testing

When a request is received from a customer for a test which is not performed, the laboratory may as a service to the customer, subcontract the testing, if required. In this instance, the laboratory will endeavour to subcontract the testing request to a competent external laboratory which complies with ISO 17025 or equivalent. The Laboratory does not subcontract tests within the scope of its accreditation.

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 HSE NDTC laboratory can provide information on chain of custody collection kits and sampling procedures. All **positive immunoassay** screening test results **must be confirmed** by a second analysis using a confirmatory analytical method.

For further information, please contact Laboratory Customer Services.

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 samples are stored in a refrigerator at 4ºC, or if refrigeration is not available, in a cool dark place. Post analysis, the laboratory will **retain samples for 14 days in refrigerated conditions**, after which they will be **disposed of safely**. Should further testing be required outside of this period (e.g. for Zopiclone analysis) samples will be stored in refrigerated conditions until testing is complete.

Unless otherwise agreed, all **Chain of Custody samples will be frozen** and retained for **12 months** and all **Probation samples will be frozen and retained for 12 months** post analysis.

Reporting of Results

The **front page** of each report **details the customer** name and address, date on which each report is generated and **scope of INAB accreditation** (for accredited tests only).

Each **patient** is identified by name, date of birth, clinic code and chart number.

Each **sample** is identified by a unique barcode and sample date.

If a drug/drug class is detected in a sample, the result will appear as a "**+**" (positive), indicating the presence of the drug.

When no drug/drug class has been detected, it will be reported as a "**-**" (negative), indicating that the drug/drug class has not been detected above the cut-off level or concentration.

A **blank space** indicates that **no test was carried out**. Screening results will be available within **24-48 hours of receipt of samples** in the laboratory. Confirmatory testing usually takes longer to perform due to the complexity of the methodology.

Mode of reporting
The method of report transmission used must be **agreed in advance** with Laboratory Customer Services. Routine modes of reporting available are post or electronically (LER, DAIS).

Post:
Reports sent by post will be dispatched as soon as possible after completion of analysis.

Fax:
The laboratory no longer faxes reports as per HSE policy.

Electronic Reporting

Drugs Aids Information System (DAIS): HSE Addiction Service defined user's access laboratory results via DAIS. This is completed once the laboratory authorises the samples.

Laboratory Electronic Reporting (LER): The LER is a web-based system developed for NDTC customers which allows authorised users to access results electronically. Sample results are available in the system as soon as the analysis is complete.

Verbal reporting:
Verbal reporting can only be accommodated in the case of an emergency.

Measurement Uncertainty

When interpreting laboratory reports, consideration should always be given to the Measurement Uncertainty (MU) associated with the test result, because no measurement is absolutely exact.

When a quantity is measured, the outcome depends on the measuring system, e.g. test procedure, environmental conditions, volumetric effects, reference values, sampling matrix, operator etc. Therefore **all measurements are subject to uncertainty** and this should be taken into account in the interpretation of laboratory results. This can have a bearing on immunoassay test results which are close to their cut-off point and therefore within the range of measurement uncertainty for the test cut-off.

Clinical consideration and judgment should be applied to any immunoassay test result. Repeat testing or confirmatory analysis may be requested if required. The tests reported are qualitative with the exception of Alcohol which is quantitative.

Measurement Uncertainty

The figures for each test are given in brackets

URINE TESTS

OPIATE (+/-19.5%)[a], 6-AMOR (+/-21.6%)[a], BENZ (+/-13.5%)[a], EDDP (+/-12.2%)[a], CANN (+/- 20.0%)[b], AMPH (+/-16.6%)[a], COCA (+/-9.2%)[a], ALCO (+/-12.4%)[a], ETG (+/-17.6%)[a], BUP (+/-27.6%)[a], pH (+/- 6.5%)[a]

a = based on all 2017 QC data b= based on 6 months 2018 QC data

ORAL FLUID TESTS

OPIATE (+/-14.9%)[c], 6-AMOR (+/-27.9%)[c], BENZ (+/-28.3%)[c], CANN (+/-39.4%)[c], AMPH (+/-7.2%)[c], COCA (+/-25.1 %)[c], METH (+/-34.0%)[c]

c = based on 2018 validation data

Quality Control and Quality Assurance

To ensure **the highest confidence** in test results, the laboratory adheres to strict quality control (QC) and quality assurance (QA) standards. (Approx. 3% of all samples run are quality controls).

In order to assess performance, the laboratory is involved in five external Quality Assurance schemes:

- **LGC** – Drugs of abuse in urine, Ethanol in urine and Drugs in Oral Fluid
- Irish External Quality Assessment Scheme (**IEQAS**) – Drugs of abuse in urine.
- **Arvecon** – Ethylglucuronide in urine

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

Accreditation

Accreditation is the third party confirmation of an organisation's competence and integrity to perform testing services. Accreditation is objective proof that an organisation has the competence to comply with best practice. It is the internationally recognised system that is used to develop and sustain high standards of performance. It is often a prerequisite for eligibility to tender for international projects. Accreditation is provided by the national accreditation body for each Member State and in Ireland this is the Irish National Accreditation Board (INAB).

The HSE National Drug Treatment Centre Laboratory is accredited by the Irish National Accreditation Board (INAB) to undertake testing as detailed in the Schedule bearing the Registration number 169T which is available at:

http://www.inab.ie/Directory-of-Accredited-Bodies/Laboratory-Accreditation/Testing/HSE-National-Drug-Treatment-Centre.html

See also Table 3. (Page 5)

Testing in the scope of accreditation is carried out in conformity with ISO/IEC 17025:2005 2nd Edition ´General requirements for the competence of testing and calibration laboratories'**.**

ISO 17025 is the standard used by testing and calibration laboratories globally. The laboratory is assessed annually for compliance with International standard ISO 17025 by a team of Irish and international external auditors. This includes assessment of the organisations quality management system and the technical competence of the laboratory to perform the tests applicable to its scope of accreditation.

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:-
ACBI – Association of Clinical Biochemists of Ireland
TIAFT- The International Association Forensic Toxicologists
UKIAFT - UK and Ireland Association of Forensic Toxicologist

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

Appendix 1
Urine Immunoassay Cross Reactivity Tables

(Ref: Thermofisher Scientific CEDIA Drugs of abuse documentation)

CEDIA® Amphetamine/Ecstasy Assay (cut – off 1000ng/mL)* Drugs producing positive results		
Amphetamine Methamphetamine	N-Methylbenzodioxazolylbutanamine (MBDB) 3,4-Methylenedioxyamphetamine (MDA)	3,4-Methylenedioxyethylamphetamine (MDEA) 3,4-Methylenedioxymethamphetamine (MDMA)

CEDIA® Opiate Class (cut-off conc.300 ng/ml) Drugs producing positive results		
6-Monoacetylmorphine Diacetylmorphine Hydrocodone Hydromorphone Morphine	Morphine Sulfate Nalorphine HCl Naloxone	Naltrexone HCl Oxycodone Oxymorphone Pholcodine Thebaine

CEDIA® Benzodiazepine Class (cut-off conc. 300ng/ml) Drugs producing positive results		
Alprazolam Bromazepam Chlordiazepoxide Citalopram Clobazam Clonazepam Delorazepam Demoxepam	Diazepam Estazolam Flunitrazepam Flurazepam Halazepam Lormetazepam Medazepam Midazolam	Nimetazepam Nitrazepam Nordiazepam Oxazepam Prazepam Temazepam Tetrazepam Triazolam

CEDIA® 6-Acetylmorphine (10 ng/ml) Drugs producing positive results
6-Acetylmorphine

CEDIA® Cannabis (cut-off conc.50 ng/ml) Drugs producing positive results		
11-Nor- Δ -9-THC-COOH 11-Nor Δ 8-THC-COOH Δ 9-THC (Dronabinol)	11-Hydroxy- Δ- 9-THC 1- Δ -9-THC-Glucuronide 8-OH- Δ -9-THC	8β,11-di-OH- Δ -9-THC Cannabinol

CEDIA® Cocaine (cut-off conc. 300 ng/ml) Drugs producing positive results	
Benzoylecgonine Cocaethylene	Cocaine

DRI® Ethyl Alcohol (mg/dL) Drugs producing positive results
Ethanol

CEDIA® EDDP (cut-off conc. 100ng/mL) Drugs producing positive results
2-Ethylidin-1,5-dimethyl-3,3-diphenylpyrrolidin (EDDP)

Immunanlysis® Buprenorphine (cut-off conc. 5ng/mL) Drugs producing positive results
Norbuprenorphine

DRI® Ethyglucuronide (cut-off conc. 500ng/mL) Drugs producing positive results
Ethyglucuronide

NB. The above lists are not exhaustive – Please contact the laboratory if confirmation of a positive result is required

We have found that CEDIA® Amphetamine/Ecstasy assay may cross-react with other compounds particularly **new psychoactive substances ('Headshop' Drugs/Bath Salts/Legal Highs)**. If you have any queries about an amphetamine positive result please contact the laboratory. Confirmatory analysis can be carried out on request.

Oral Fluid Immunoassay Cross Reactivity

We have limited information on cross-reactivity for Oral Fluid tests. Triazolam (Halcion) causes false positive results for 6-Acetylmorphine (6-AM) oral fluid test in patients who are prescribed this drug. Patients on Halcion are therefore unsuitable for oral fluid testing. Urine testing should be used for these patients.

Appendix 2
Benzodiazepine Identifications

The routine immunoassay screening method for benzodiazepines is unable to distinguish between metabolites, therefore urinary benzodiazepine identifications are carried out where required using a more complex technique called LC/MS which can specifically target and unambiguously identify the drug or metabolite present.

Benzodiazepines can be short-acting or long acting and depending on the drug taken. They can persist for an extended time in the urine of habitual users even after all use has ceased. A further complication is that the metabolic pathways of benzodiazepines can often result in common metabolites (the most significant being Oxazepam) and this means that in many cases it may not be possible to determine the parent drug. Therefore it can be difficult to unambiguously identify which parent drug(s) was originally consumed.

See Table 5 below.
Many factors such as how much fluid has been consumed prior to giving the sample, the time since the drug was taken, the physical condition and metabolism of the patient etc. may influence the dilution of a urine sample and therefore the drug level present. Therefore drug levels in urine may be subject to large fluctuations. If urine is dilute, drug levels will be lowered. Consequently urinary levels of benzodiazepines are not performed in the NDTC Laboratory

Table 5 Benzodiazepines and their metabolites

Parent Drug	Target Drug/Metabolite(s) Tested
Alprazolam	α–hydroxyalprazolam, Alprazolam
Bromazepam	Bromazepam
Chlordiazepoxide	Chlordiazepoxide, Nordiazepam, Oxazepam, demoxepam
Clobazam	Clobazam
Clonazepam	Clonazepam, 7-aminoclonazepam
Diazepam	Diazepam,Nordiazepam,Oxazepam,Temazepam
Estazolam	Estazolam
Flunitrazepam	Flunitrazepam, 7-Aminoflunitrazepam
Flurazepam	2-Hydroxyethylflurazepam
Lorazepam	Lorazepam
Midazolam	Midazolam
Nitrazepam	Nitrazepam
Oxazepam	Oxazepam
Prazepam	Oxazepam
Temazepam	Temazepam, Oxazepam
Triazolam	Triazolam, a-hydroxytriazolam

Appendix 3
Opiate Identifications

The routine screen by immunoassay will be positive when certain opiates are present in the urine sample at a concentration above the cut-off concentration of 300ng/ml

- Heroin, Morphine, Codeine and Dihydrocodeine will cause a positive result in the screening assay when present at levels above the cut-off.
- Naloxone (present in Suboxone) can also cause an opiate positive on the routine screen at very high levels. Not all patients on Suboxone will have opiate positive urines, it usually occurs in patients that are on high levels of Suboxone
- Oxycodone has also been known to cause a positive at extremely high levels, at therapeutic levels it should not cause a positive.
- Tramadol does NOT cause a positive on the routine screen

If the routine test is opiate positive and further analysis is requested in order to determine the source of the opiate positive, a 6-AM screening test can be performed on the sample. As 6-AM is a unique metabolite of Heroin it is only present in urine after recent ingestion of Heroin, a positive 6-AM result indicates that Heroin has been taken in the past 10 to 24 hours. A 6-AM negative result may indicate either that the ingestion of heroin is not recent **or** that the individual has not taken Heroin and that the sample is opiate positive due to a non heroin opiate. An opiate positive, 6 -AM negative test can be subjected to further testing by LC/MS in order to determine what opiate has been taken.

Metabolism of Opiates
Because of the similar metabolic pathways of some opiates it can be difficult to distinguish between the use of Heroin, Morphine or Codeine, or the use of a combination of more than one of these because both Morphine and Codeine may be present after Heroin use, Morphine use or after Codeine use. If more Codeine than Morphine is present in a sample it would indicate that Codeine has been ingested. If more or similar levels of morphine and codeine are present in a sample it is not possible to definitively determine which drug was taken.

Drug Taken	**Metabolites/Drugs found**
Heroin	6-AM and Morphine, Codeine (as impurity)
Codeine	Codeine and Morphine
Dihydrocodeine	Dihydrocodeine
Morphine	Morphine
Oxycodone	Oxycodone and Oxymorphone
Naloxone	Naloxone

If Tramadol or Oxycodone analysis is requested this must be specifically requested as Opiate ID for **Tramadol** or **Oxycodone**.

Appendix 4
New Psychoactive substances (NPS)

The terms 'Legal highs', 'Head Shop products' or 'New Psychoactive Substances' refer to a new drugs with stimulant or psychoactive effects which had not been encountered as drugs of abuse or recreational drugs until recent years. Initially these were sold as 'legal' highs in so-called 'Head Shops' in Ireland and via the internet. Various legislative changes led to these being controlled drugs and the so called 'Head Shops' were closed down. Unfortunately the controls put in place have not eliminated the use of these substances and they are still in use in the illicit drug market.

The Laboratory of the HSE-NDTC is currently testing in the order of 500 samples annually for New Psychoactive Substances (on request only). The profile of drugs detected over the years has changed over time and the panel of drugs screened is updated periodically to include more recent variants in the compounds tested and as reference standards become available.

The parent drug is looked for in these analyses, as little is known about the metabolism of these drugs and in general, drug standards of the metabolites are not yet commercially available. There may be metabolites of these compounds that are present in higher concentrations than the parent in the urine and present for a longer time than the metabolite. It is not known how long any of these compounds are present in the urine.

The laboratory does not test for the synthetic Cannabinoids (Spice compounds) it only tests for the powders or "bath salts". A negative result for the 'Headshop 'test does not mean that an individual has not taken a so called 'Headshop' drug, it just means that they have not taken one of the compounds tested for in our assay.

For the latest information on these products and the legislation relating to them, refer to www.drugs.ie and the Irish Legislation website http://www.irishstatutebook.ie
For the latest information on NPS in Europe see the EMCDDA annual report and other publications on the EMCDDA website http://www.emcdda.europa.eu/

NDTC Laboratory Journal Articles

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2. "Head Shop" Compound abuse amongst attendees of The Drug Treatment Centre Board; S McNamara, S Stokes, N Coleman; The Drug Treatment Centre Board, 30-31 Pearse St, Dublin 2., The Irish Medical Journal, May 2010 Vol. 103, No. 5

3. "Quantitative evidence of a heroin drought" Siobhan Stokes, Drug Net Ireland, Issue 40, Winter 2011

4. "Screening of Stimulants Including Designer Drugs in Urine Using a Liquid Chromatography Tandem Mass Spectrometry System" Paul M. O'Byrne; Pierce V. Kavanagh; Sinead M. McNamara; Siobhan M. Stokes, Journal of Analytical Toxicology 2013; doi: 10.1093/jat/bks091

5. Eurosurveillance, Volume 20, Issue 40, 08 October 2015
   Rapid communication "Injection of new psychoactive substance snow blow associated with recently acquired HIV infections among homeless people who inject drugs in Dublin, Ireland, 2015"

   C Giese [1 2], D Igoe [2], Z Gibbons [3], C Hurley [4], S Stokes [3], S McNamara [3], O Ennis [4], K O'Donnell [2], E Keenan [3], C De Gascun [5 6], F Lyons [7], M Ward [4], K Danis [1 8], R Glynn [4], A Waters [5], M Fitzgerald [4], on behalf of the outbreak control team [9]

6. Contributed to EMCDDA–Europol Joint Report on a new psychoactive substance: 1-phenyl-2-(1-pyrrolidinyl)-1-pentanone (α-PVP) EMCDDA, Lisbon, September 2015

7. "Pregabalin Abuse amongst Opioid Substitution Treatment Patients" S McNamara, S Stokes, R Kilduff, A Shine, HSE National Drug Treatment Centre, McCarthy Centre, 30-31 Pearse Street, Dublin 2; The Irish Medical Journal, November December 2015 Vol. 108 No. 10

8. "New Psychoactive Substances in Europe Conference, Poznan" Siobhan Stokes, Drug Net Ireland, Issue 60, Winter 2017

9. "Increase in cocaine use among OST patients". Stokes, Siobhan Drugnet Ireland, Issue 63, Autumn 2017