

LCMS DRUG CONFIRMATION

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HISTORY		
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1	26/10/2005	New Edition
2	28/9/2007	Laboratory move to KCH, change to Pathnet
3	1/12/2008	Change method to LCMS
4	18/5/2009	Change LC to Agilent pumps/Aria OS
5	01/09/2010	Change to WinPath, change to Turboflow and change from Sprinter

CIRCULATION LIST		
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1	Toxicology Laboratory	Andrew Marsh

TABLE OF CONTENTS

PURPOSE OF THE TEST	3
PRINCIPLE OF THE TEST	3
SAMPLE COLLECTION AND PREPARATION	4
REAGENTS, MATERIALS AND EQUIPMENT	4
Stock Reagents	4
Reagent Preparation	5
Materials	6
Equipment	7
COSHH ASSESSMENT	7
HEALTH AND SAFETY PRECAUTIONS	8
QUALITY CONTROL	8
QUALITY ASSURANCE	9
Internal Quality Assessment	9
External Quality Assessment	9
METHOD	9
Worksheet Creation	9
Sample Preparation	9
Run Preparation	10
Starting the Run	11
Processing LCMS Data	12
REQUESTING AND REPORTING OF RESULTS	12
Entering Results	13
REFERENCE RANGES	14
LIMITATION OF THE ASSAY	15
INTERPRETATION OF RESULTS	15
REFERENCES	16
APPENDICES	<i>Error! Bookmark not defined.</i>
PROCEDURE AMENDMENT FORM	16
SIGNATURE PAGE	17

PURPOSE OF THE TEST

The first stage of a drug screen is to use an immunoassay method to screen samples. This method can identify samples that are positive for a class of substances but cannot distinguish between different drugs within the class, for example morphine and codeine for opiates or amphetamine and ecstasy for the amphetamine group.

In the second stage of a drug screen, all samples that are positive for a specified class of drugs are confirmed by Liquid Chromatography-Mass Spectrometry (LCMS). This procedure uses a different type of method from the screening process to identify the main classes of drug. The use of a different type of method allows non-specific interference to be reduced so that the accuracy of the final result is assured.

The predominant opiate found in the laboratory is morphine, which can either be present as the primary drug or more probably as a metabolite of heroin. Other main opiates detected are codeine and dihydrocodeine. There are a variety of opiate class drugs, some of which are available over the counter, some are prescription only medicines, and some are controlled or illicit drugs.

The main amphetamines found in the laboratory are amphetamine and ecstasy (methylene dioxy methyl amphetamine, MDMA), but there are also a large number of immunoassay positive results from interfering substances, principally mebeverine (Colofac ®)

Other drugs that can also be confirmed using this method include methadone and cocaine metabolites.

PRINCIPLE OF THE TEST

Urine samples that require confirmation are aliquoted into a HPLC vial containing internal standard, and sealed and loaded onto the HPLC system. Four different mobile phases are used to separate the components of the urine sample, such that they are eluted into the source of the mass spectrometer at known retention times.

The eluant from the Cohesive Technologies HPLC system is introduced into the LCQ mass spectrometer via the Electrospray Ionisation (ESI) probe. A combination of high voltage, heat and nitrogen gas drives off all of the solvent, leaving the ions to be drawn through the ion transfer capillary into the mass spectrometer. A series of lenses, including a quadrupole and octupole, guide the ions into the ion trap, where they can be collided or expelled and detected by the dynode.

Each analytical cycle consists of a number of stages or Scan Events. Initially a full scan of parent ions is performed in positive mode. Parent ion masses are compared with the mass list, and if an ion is present within the specified time window, a data dependent scan can be performed, producing an MS² spectrum of the parent ion. This scan event can be repeated with several ions within the list, or dedicated scan events for specific ions.

Once the analytical run is complete, the chromatogram is analysed by the ToxID software for a specified set of analytes. The parent masses, retention times and ion intensities on the chromatogram are compared with the ToxID configuration file. If a match is found and the most intense daughter ion matches that in the configuration file, a library search is performed on that spectrum. A library match greater than the hit threshold set up in ToxID is considered a positive result for that particular analyte. The configuration file is compared with a cutoff sample on a daily basis to ensure that retention times and ion intensities do not shift significantly.

SAMPLE COLLECTION AND PREPARATION

1. Urine is the only sample that is normally acceptable for drugs of abuse screening
2. Urine should be collected into a universal container without preservative
3. The volume of sample required for the test is at least 1mL.
4. No specific patient preparation is required
5. No specific timing of sample collection is required
6. Pre-analysis: Urine samples are received from the Olympus AU640
7. Post-analysis: The samples are stored in TOX6 for one week after receipt after which they are disposed of as clinical waste.

REAGENTS, MATERIALS AND EQUIPMENT

Stock Reagents

Fluka Formic Acid

Cat. No. : 06440
Volume : 500 mL
Contents : Formic acid
Storage : Stored at room temperature
Location : Stored in acid cupboard in Bay 4

VWR Ammonium Carbonate

Cat. No. : 21217.260
Volume : 500 g
Contents : Ammonium carbonate
Storage : Stored at room temperature
Location : Stored in acid cupboard in Bay 4

Fluka Ammonium Formate

Cat. No. : 70221
Volume : 100 g
Contents : Ammonium formate
Storage : Stored at room temperature
Location : Stored in chemicals cupboard in Bay 2

Rathburn Acetonitrile

Cat. No. : RH1016
Volume : 2.5L
Contents : HPLC grade S acetonitrile
Storage : Stored at room temperature
Location : Stored in flammable cupboard in Bay 4, or in the outside solvent store

VWR Propan-2-ol

Cat. No. : 20842.330
Volume : 2.5L
Contents : Propan-2-ol
Storage : Stored at room temperature
Location : Stored in flammable cupboard in Bay 4

VWR Acetone

Cat. No.	:	20066.321
Volume	:	2.5L
Contents	:	Acetone
Storage	:	Stored at room temperature
Location	:	Stored in flammable cupboard in Bay 4.

Reagent Preparation

Preparation of reagents must be recorded in the reagent file in the confirmation laboratory, and the date of preparation should be written on the bottle.

HPLC Solvents

i) Loading Pump 1A (0.1 % formic acid (aq))

Preparation	:	Add 2 mL of formic acid to approximately 1500 mL of deionised water in a 2 L volumetric flask. Fill to the mark with deionised water. Filter before use using the HPLC eluant filtration apparatus
Stability	:	Stable for 2 months from date of preparation
Storage	:	Stored at room temperature
Labelling	:	Label with Harmful hazard label

ii) Eluting Pump 1A (10 mmol/L ammonium formate (aq))

Preparation	:	Weigh 1.26 g ammonium formate into a weighing boat and transfer to a 2 L volumetric flask. Fill to the mark with deionised water. Filter before use using the HPLC eluant filtration apparatus. Adjust the pH to pH* 6 using the Jenway pH meter.
Stability	:	Stable for 2 months from date of preparation
Storage	:	Stored at room temperature
Labelling	:	Label with Harmful hazard label

iii) Loading and Eluting Pump 1B (0.1 % formic acid in acetonitrile)

Preparation	:	Add 2 mL of formic acid to approximately 1500 mL of acetonitrile in a 2 L volumetric flask. Fill to the mark with acetonitrile. Filter before use using the HPLC eluant filtration apparatus
Stability	:	Stable for two months from the date of preparation.
Storage	:	Stored at room temperature
Labelling	:	Label with Harmful Flammable hazard label

iv) Loading Pump 1D (10 mmol/L ammonium carbonate (aq))

Preparation	:	Weigh 3.14 g ammonium carbonate into a weighing boat and transfer to a 2 L volumetric flask. Fill to the mark with deionised water. Filter before use using the HPLC eluant filtration apparatus. Adjust the pH to pH* 9 using the Jenway pH meter.
Stability	:	Stable for two months from the date of preparation.
Storage	:	Stored at room temperature
Labelling	:	Label with Harmful Flammable hazard label

v) Wash 1 (10 mmol/L ammonium formate and 0.1 % formic acid (aq))

Preparation	:	Weigh 1.26 g ammonium formate into a weighing boat and transfer to a 2 L volumetric flask. Fill with approximately 1500 mL of deionised water and add 2 mL of formic acid. Fill to the line with deionised water.
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Stability : Stable for two months from the date of preparation.
Storage : Stored at room temperature
Labelling : Label with Harmful Flammable hazard label

vi) Magic Mix (Wash 2) (45 % acetonitrile, 45 % propan-2-ol and 10 % acetone)

Preparation : Add 450 mL acetonitrile to 1 L 'magic mix' bottle. Add 450 mL propan-2-ol (isopropanol) and 100 mL acetone. Mix before use.
Stability : Stable for two months from the date of preparation.
Storage : Stored at room temperature
Labelling : Label with Harmful Flammable hazard label

vii) Internal Standard Solution (20 mg/L morphine-D₆, 20 mg/L benzoylcegonine-D₃ and 20 mg/L methadone-D₃)

Preparation : Add 1 mL methadone-D₃ (1 mg/mL), 1 mL benzoylcegonine-D₃ (1 mg/mL) and 1 mL morphine-D₃ to a 10 mL volumetric flask. Fill to line with deionised water. Take 10 mL of this stock solution and add to a 50 mL volumetric flask. Fill to line with deionised water.
Stability : Stable for six months from the date of preparation.
Storage : Stored in Tox 10 fridge
Labelling : Label with Harmful Flammable hazard label

Materials

HPLC Vials

Supplier : Kinesis
Cat. No. : SNV12-02
Contents : 10 x 100, 11 mm, 2 mL glass vials (32x12mm)
Storage : Stored at room temperature
Location : Stored in cupboard in Bay 4

HPLC Caps

Supplier : Kinesis
Cat. No. : SNC11-04S
Contents : 1000 x caps
Storage : Stored at room temperature
Location : Stored in cupboard in Bay 4

Membrane filters (0.45 µm)

Supplier : Phenomenex
Cat. No. : Nylon 66 AFO-0504 or equivalent
Contents : 100/pack
Storage : Stored at room temperature
Location : Stored in cupboard in Bay 2

PFP Hypersil Gold HPLC Column

Supplier : Fisher Scientific
Cat. No. : HSA-615-130a (50 x 2.1)
Contents : One PFP Hypersil Gold HPLC Column
Storage : Stored at room temperature
Location : Stored in cupboard in Bay 4

TurboFlow HTLC Cyclone MAX Column

Supplier : Thermo-Fisher (Cohesive Technologies)

Cat. No. : 952980 (50 x 0.5 mm)
Contents : One Cyclone MAX TurboFlow column
Storage : Stored at room temperature
Location : Stored in cupboard in Bay 4

LCQ Fleet Consumables

Supplier : Thermo-Fisher
Cat. No. : See various in Equipment Folder or Desktop under LCQ Consumable

Plastic Pasteur pipettes

Supplier : Elkay
Cat. No. : 127P511000
Contents : 100 pipettes/box
Storage : Stored at room temperature
Location : Stored in cupboard in Bay 2

Cutoff Calibrator and IQCs

To make up cut-off calibrator; add 490 µL Biorad C2, 490 µL Biorad C3 and 25 µL internal standard to an HPLC vial and cap.

Validate cut-off calibrator by running alongside existing cut-off calibrator and ensuring that retention times and sensitivities are similar. Record date of preparation on HPLC vial.

Prepare IQCs as per patient samples, i.e. 975 µL IQC + 25 µL internal standard.

Equipment

HPLC Eluant filtration apparatus

COSHH ASSESSMENT

Substance	Data Sheet Reference	Handling requirements	Risk Rating
Acetone	VWR 1000300	Highly flammable. Irritating to eyes. Repeated exposure may cause skin dryness or cracking. Vapours may cause drowsiness or dizziness. Wear gloves and safety glasses when handling.	M
Acetonitrile	VWR 1038600	Toxic and Flammable. Wear gloves and safety glasses when handling. Obtain medical attention if ingested.	M
Ammonium Carbonate	Sigma-Aldrich S7795	Irritant. Wear gloves and safety glasses when handling. Obtain medical attention if ingested.	L
Ammonium Formate	Sigma-Aldrich 55674	Irritant. Wear gloves and safety glasses when handling. Obtain medical attention if ingested.	L
Formic acid	Sigma-Aldrich 94318	Corrosive and Flammable. Toxic by inhalation. Wear gloves and safety glasses when handling. Obtain medical attention on eye contact or after ingestion.	M
Propan-2-ol	VWR 1022400	Highly flammable. Irritating to eyes. Vapours may cause drowsiness and dizziness. Wear gloves and safety glasses when handling.	M
Standard Solutions	Cerilliant various	Flammable, toxic and irritant.	M

HEALTH AND SAFETY PRECAUTIONS

Laboratory coats must be worn when performing any part of this procedure
Gloves and goggles must be worn at all times while handling urine samples, methanol or solvents.

The BIORAD urine controls are lyophilised material of human source, which have been tested for hepatitis B surface antigen (HBsAG), antibody to hepatitis C (HCV) and antibody to HIV and found to be non-reactive. However as no method can offer complete assurance as to the absence of infectious agents, this material and all patient samples should be handled as though capable of transmitting infectious disease.

QUALITY CONTROL

Quality control samples are run with each batch of samples.

Bio Rad DAU Negative Control

Cat. No. : 460
Volume : 10x20mL
Contents : Drug free human urine
Storage : Stored at 2-8°C
Location : Stored in fridge TOX5
Preparation : Control is supplied ready to use
Stability : 30 days at 2-8°C after opening or until the expiry date on the package, whichever comes first

Bio Rad DAU Toxicology Control C2

Cat. No. : 442
Volume : 10x20mL
Contents : Human urine containing drugs and metabolites
Storage : Stored at 2-8°C
Location : Stored in fridge TOX5
Preparation : Control is supplied ready to use
Stability : 30 days at 2-8°C after opening or until the expiry date on the package, whichever comes first

Bio Rad DAU Toxicology Control C3

Cat. No. : 469
Volume : 10x20mL
Contents : Human urine containing drugs and metabolites
Storage : Stored at 2-8°C
Location : Stored in fridge TOX5
Preparation : Control is supplied ready to use
Stability : 30 days at 2-8°C after opening or until the expiry date on the package, whichever comes first

Bio Rad DAU Toxicology Control C4

Cat. No. : 444
Volume : 10x20mL
Contents : Human urine containing drugs and metabolites
Storage : Stored at 2-8°C

Location : Stored in fridge TOX5
Preparation : Control is supplied ready to use
Stability : 30 days at 2-8°C after opening or until the expiry date on the package, whichever comes first

QUALITY ASSURANCE

Internal Quality Control

When samples are analysed for compounds where no IQC is available, a sample known to contain the analyte will be run as an IQC sample.

External Quality Assessment


Three external quality assurance samples are received every three months.

Results are faxed or e-mailed to the organisers, and repeat samples may also be obtained from there.

Samples are received lyophilised and require reconstitution with 15mL of deionised water using a volumetric pipette. The samples are allowed to stand for 5 minutes before gentle mixing for at least 20 minutes. External Quality Assessment samples are analysed by ion trap LCMS, even if the Olympus screening results do not indicate that this is required. This is to ensure that all drugs that are looked for in the laboratory are also looked for in the EQA samples. Failure to follow this procedure may give a false negative result for weak positive morphine, quinine or cyclizine, among others.

METHOD

Worksheet Creation

- i) In Instrument Manager, go to specimen management and click on SM Workspace. From the dropdown menu, select Tox-LCMS. Highlight all samples that are to be analysed and click 'manually download' icon . Close CDM and open sequence import folders from desktop. Open Xcaliber 2 and double click on the latest excel file, sort into numerical order, close file and click yes to 'save changes'.
- ii) Open the following folder: G:\Chem\Toxicology\Charts & Forms\Drugs\FTP.
- iii) Double click on 'LCMS sequence import'. The computer will need to have the macro settings set to medium (in Excel go to Tools, Options, Security tab, Macro Security, and select Medium), on subsequent returns to LCMS sequence import, a security warning will appear, click on enable macros.
- iv) Select the "FULL WORKLIST" button to import the worklist into Excel. Select the correct Excel file for that days worklist (from sequence import folders) and click open. It will prompt you to enter the correct worklist number-this will usually be correct as it counts on from the previous worksheet that was created. Add any repeats from the previous day at the bottom of the list and put them in italics and right aligned. Print worksheet.
- v) Select the "Export Sequence" button. When prompted, click ok to any boxes that appear. Close Excel and click yes to 'save changes'.
- vi) Insert memory stick (there is a Toxicology one if you do not have your own) and type in password. Right click on the correct worklist number in the FTP folder and 'Send to' memory stick.


Sample Preparation

If labels have been saved by person running Olympus:

Collect labels and monovettes containing that days urines and take to sample preparation area.


If labels have not been saved by person running Olympus:

Log on to WinPath, open Results Entry and enter first number on worklist into 'Enter Lab


No' box and click Go. Click on down arrow on  icon. Click on General Labels, select correct printer number (40 or 12 for those in Toxicology Sample Reception) and click Start. Repeat for all samples on worksheet.

- i) Centrifuge the monovettes containing the samples that are to be analysed at 36,000 rpm for 5 minutes.
- ii) Pipette 25 µL of internal standard solution into correctly labelled glass HPLC vial.
- iii) Pipette 975 µL of urine sample into appropriate glass HPLC vial.
- iv) Cap the HPLC vial.
- v) Load vials into the appropriate position in the autosampler tray. Repeat for all samples.

Run Preparation (Xcalibur)

- i) Select Xcalibur from the Start menu or desktop
- ii) From the Xcalibur Roadmap, select SEQUENCE SETUP.
- iii) Select FILE and IMPORT from the drop down menu. The shortcut for this is CTRL+I
- iv) Select the correct worklist file on the memory stick
- v) Once imported, the sequence file can be edited if required e.g. adding any repeats from previous day.
- vi) The instrument method should already be set to the following path file: C:\LCMSData\LCMSTurboflowDOAConfirmationMethods\TurboflowMassSpecMethod, the Processing Method should be C:\LCMSData\ToxID. If this is not the case, right mouse click on the appropriate column and select the correct file path. Highlight entire column and click on  to fill down.
- vii) Ensure that the correct autosampler tray is selected e.g. CStk1-03.
- viii) Save the sequence in the C:\LCMS Data\YEAR\MONTH\RUN folder. The sequence name should match the Work list name, with a .SLD extension.

Run Preparation (Aria)

- i) Open Aria and select "Batch Editor"
- ii) From the File menu, select Import and Xcalibur SLD. Select the file just created.
- iii) Ensure that AS Method is set to:
C:\LCMSData\LCMSTurboflowDOAConfirmationMethods\TurboflowASMethod
- iv) Ensure that LC Method is set to:
C:\LCMSData\LCMSTurboflowDOAConfirmationMethods\CycloneMAXTurboflowLCMethod
- v) Ensure that Acq Method is set to:
C:\LCMSData\LCMSTurboflowDOAConfirmationMethods\TurboflowMassSpecMethod
- vi) Edit the tray and vial positions for each sample as appropriate. Fill down and Increment can be used to make this stage quicker (highlight vial column and click on , click increment). Cutoff and QC's are kept in tray CStk1-01 and patient samples usually start in CStk1-03 and carry on to CStk1-04 if necessary.
- vii) Select Edit, Customise and the AS/FC tab, tick the AS_injector box and click OK. Highlight the AS_injector column, click with the right mouse button, and select TX. Highlight the AS_TrayType column, click with the right mouse button, and select VT54.
- viii) When editing the sequence is complete, save the batch template as LCMSXXXX in the correct folder as specified at stage vi) above.
- ix) Select Submit (or Submit Selected). Click OK to the list of errors that appear. Check that the path and batch number is correct for the data files and amend if necessary by selecting the icon with the yellow folder. If this is the second time part of this batch has been run, a suffix will be needed to allow submission.

- x) Close batch editor.

Starting the Run

Xcalibur:

- i) Check under the Vacuum tab (if not visible, click on LCQ Fleet MS) that the Ion Gauge Pressure is less than 2 and that the Convection Gauge Pressure is greater than 1.

Source	Vacuum	Syringe	Analog	State
Ion Gauge Pressure (E-5 Torr):				1.00
Convection Gauge Pressure (Torr):				1.24
Turbo Pump:				
Life (hours):				4293
Speed (Hz):				750
Power (watts):				62
Temperature (°C):				57

- ii) The ESI probe should be set at position C.

Aria

- i) If not already open, open the System 1 window by clicking on the Logic States tab, and then click on the dark grey rectangle in the Turboflow Pump System box. In the System 1 window, click on the green 'Play' button to start the pumps.
- ii) After 20-30 seconds, the pumps will have stabilised. Click on the Start Analysis button from the Batch Info tab. Check that the autosampler drawers are closed and select OK.

Ensure that the daily maintenance sheet (located in the Ion Trap Maintenance folder) is filled in before the run is started.

ToxID software

ToxID software is used to identify and report drugs on the ion trap. This identifies a drug based on the parent ion mass, the principal daughter ion intensity and the library match scores. The output is a .PDF file and a .CSV file for each sample.

ToxID can be accessed manually from the start menu, and has a number of options:

- Source File** This is the .RAW data file that is to be processed
- Config File** This file configures ToxID with general and drug specific parameters. See below for operation
- Summary Report** Selecting short report gives an abbreviated report with up to 8 analytes per page. This is the one used by default in the Toxicology Unit. There are 2 options within this, whether to View or Print the .PDF file created. Leaving both checkboxes blank will create the .PDF & .CSV files only
- Long report** The long report gives a single page report for each analyte, containing the chromatogram, acquired and library spectra, difference spectrum and library structure (if known). As for the short report, there are checkboxes for viewing and printing reports

Save Selecting this button saves the current setup without processing the .RAW file
Process This button causes ToxID to process the .RAW file using the specified configuration file.

Configuration File

The ToxID configuration file is a .CSV file that is best edited in Excel. It can be accessed as follows: C:\LCMS Data\ToxID OPIATES_Turboflow. The top 19 lines contain required general information, such as the laboratory name & logo, the mass and retention time windows to use, search libraries and the header row for the analyte list.

The rows below the header row should contain the following information for each drug:

Analyte The analyte list shows each drug name with identification parameters for each. To enable correct operation, the drug name must match that found in the MS spectra library.

Parent Mass This tells ToxID which MS2 scan filter to search

Id Parent drug, Internal Standard or Metabolite

Product mass This tells ToxID the mass of the most intense daughter ion, used to draw the chromatogram and used for the intensity measurement


Retention Time The retention time \pm the window specified in the header identifies where in the chromatogram to look for a peak

Intensity Only if the daughter ion intensity is above the specified intensity threshold will a positive result be recorded

SI The Search Index is a calculated score of the similarity of the acquired spectrum with the library spectrum. The specified value must be exceeded for a positive result to be recorded

RSI The reverse search index is the similarity between the library spectrum and the acquired spectrum. This value must be exceeded for a positive result to be recorded

Processing LCMS Data

- i) Select Xcalibur from the Start menu
- ii) Select SEQUENCE SETUP
- iii) Select FILE – OPEN – select the last sequence file (if not already open)
- iv) Select BATCH REPROCESS . Select the required lines to reprocess, and select the “Programs” checkbox.
- v) Select OK to finish.
- vi) Click on the Start menu, select the ‘Current Month’ folder and then select the correct folder for the worklist you are processing.
- vii) Click the date modified heading to put the files into a more appropriate order. Go through the worklist and for each sample double click on the PDF file to view the report.

REQUESTING AND REPORTING OF RESULTS

Interpreting results

ToxID gives a summary report for each patient sample. Negative results are not shown, and all identified drugs or metabolites listed should be considered as potentially positive.

Examine the results from the cutoff calibrator and the QC samples. This should give an estimate of the intensity threshold for positive results.

For each result in the ToxID report, check that the following applies:

- i) The intensity of the analyte is similar to or above the intensity of the cutoff calibrator
- ii) The chromatogram shows a peak, and is not either flat or random noise
- iii) The SI/RSI values are similar and at least 400 each

In general, a drug is considered POSITIVE if the parent drug or primary metabolite pass the above criteria, and the immunoassay result (if available) gives a positive result.

Analytes such as morphine glucuronide and codeine glucuronide should ideally have an MS3 spectrum. If not, they may be reported as positive if another analyte is present to confirm it i.e. morphine glucuronide (MS2) may be reported as positive if one of the following analytes is also detected: morphine, morphine glucuronide (MS3), 6-monoacetyl-morphine, codeine, codeine glucuronide.

Extra certainty about a result may be achieved through identification of several metabolites, for example, cocaine, cocaethylene and benzoylecgonine may all be present after cocaine use.


Record the results on the worklist using a + to show that one of the analytes is present. If you are uncertain whether or not the peak intensity is high enough, it is better to write down the intensity value in the box instead of a +. If an analyte is present in the MS3 spectrum, record it as +³.

For the cutoff and QC's, the intensity values should be written in the boxes rather than just a +.

Street heroin metabolites usually seen are hydroxypapaverine, hydroxypapaverine glucuronide, dihydroxypapaverine glucuronide, noscapine, meconine or 6-acetylcodeine. To record street heroin as positive, one of the following criteria must be matched: (i) hydroxypapaverine glucuronide is present in the MS3 spectrum, (ii) at least two of these analytes are present at an intensity of greater than 100, or (iii) one of the analytes is present at an intensity greater than 400. If there is only one present or if they are less than 100 in intensity, they should be written down (with intensity next to them) in the Others box.

Additional analytes that should be recorded under Others include buprenorphine glucuronide, norbuprenorphine glucuronide, methamphetamine, MDMA, ketamine, norketamine, cocaethylene, dihydrocodeine and dihydrocodeine glucuronide.

Entering Results

- i) Log on to WinPath and go to Results Entry. Enter the laboratory accession number and click Go.
- ii) LCMS results are recorded on the LCMS data line. Record the results from the Worklist. This line is for internal lab use only, so acceptable shorthand is used.
e.g. m- mg+ c- cg+ 6m- her+ eddp+ co-
If the confirmation was for amphetamine class, the following should also be used:
a- mea- mdma+
- iii) Enter either Positive or Negative on the appropriate lines for the opiate confirmation section and for the amphetamine confirmation where necessary.
- iv) Occasionally it may be necessary to add extra tests if they have been detected on the LCMS i.e. dihydrocodeine. If this is the case, whilst on the Results Entry screen, press Ctrl + Q which will bring up a text box to add the appropriate test. If the code is known this can be entered, otherwise it can be selected from a list by clicking .
- v) If a buprenorphine result on the Olympus is less than 50 ng/mL but greater than 5 ng/mL or if cocaine metabolite is less than 1000 but greater than 300 ng/mL, they will be recorded in WinPath as Immunoassay Positive. When the results have been obtained from the LCMS, these results can be changed either to 'Positive, previous result confirmed by LCMS', or 'Negative. Previous result not confirmed by LCMS' as appropriate. This is done by clicking on the result, pressing Ctrl + O and then press F7 twice. This brings up a list of comments that can be chosen from, click on Toxicology and OK and then select the appropriate comment. However, if buprenorphine was recorded as Immunoassay Positive but then not detected on the LCMS, leave it as Immunoassay Positive.
- vi) All patient results are entered onto the LIMS system, including research work and external quality assessment. Quality control results are recorded on the LCMS, and this data must be kept for at least 10 years.

Interferences

Due to the nature of the Olympus immunoassay, it is not 100 % selective for all analytes, hence the need for the confirmation method for opiates and amfetamines. There are some known interferences (and some unknown) for most of the analytes that are measured on the Olympus. There are three that are particularly important to be aware of in the Drugs of Abuse unit.

The first is the interaction between the amfetamine immunoassay and mebeverine, an antispasmodic used for the relief of stomach cramps. Mebeverine is commonly prescribed to those people undergoing opiate withdrawal treatment and those patients will often test positive for amfetamine class on the Olympus as a result of mebeverine. Therefore when these samples are analysed using LC-MS they will usually be negative for amfetamine, methamfetamine and ecstasy. When entering the results into WinPath, the amfetamine class lines can be filled in with Negative and then whilst the cursor is in the ecstasy results line, press Ctrl + A, this adds an extra text line. Next, press F7 twice which brings up the comments box, click on Toxicology and OK and then click on the amfetamine comment and OK. This will add the following comment to the results:

Amfetamine class substance detected by immunoassay. Specific analysis for Amfetamine, methamfetamine and ecstasy did NOT detect their presence. Mebeverine is known to interfere with the class analysis. Please ring the laboratory on 0203 299 5881 if further advice is required.

The second common interference is between cocaine and quinine. Quinine is often prescribed in patients going through opiate withdrawal treatment to relieve nocturnal leg cramps. These patients will often test positive on the Olympus for cocaine metabolite but because it will show as a very low concentration, the result will say Immunoassay Positive and it will automatically be put onto the LCMS confirmation worklist. Once the presence of benzoylecgonine has been confirmed as positive or negative on the LCMS, on Results Entry in WinPath, click on the cocaine metabolite result line and press Ctrl + O and then press F7 twice which brings up the comments box, click on Toxicology and OK and then click on either the immunoassay negative comment or the immunoassay positive comment and click OK. This will add one of the following comments to the results:

NEGATIVE. Previous result not confirmed by LCMS.

POSITIVE. Previous result confirmed by LCMS.

The third interference that may be seen in the Drugs of Abuse unit is between opiates and buprenorphine. If a patient has particularly high concentrations of opiates in their system, this may interact with the buprenorphine immunoassay and give a result of Immunoassay Positive. If this is the case and the confirmation method does not show that buprenorphine is present then the results should be left as Immunoassay Positive and the following comment may be added:

High concentrations of opiates are known to cross react with the buprenorphine immunoassay. If the patient is not prescribed/known to take buprenorphine, consider this a possibility.

If buprenorphine glucuronide or norbuprenorphine glucuronide is detected on the LCMS, in WinPath click on the buprenorphine result line and press Ctrl + O and then press F7 twice which brings up the comments box, click on Toxicology and OK and then click on the immunoassay positive comment and click OK. This will add the following comment to the results:

POSITIVE. Previous result confirmed by LCMS.

REFERENCE RANGES

There is no numerical reference range for drug screening, as the results are reported as positive or negative and will vary according to the patient's medication. However, drug users tend to keep taking the same drugs, and where a result does not fit in with previous drug use, immunoassay results, or where the clinical details do not match the results, extra care should be taken in interpreting the results, or repeat sample analysis to check.

LIMITATION OF THE ASSAY

Many different compounds will be detectable by LCMS, and their identities may only be safely determined with reference to a matching standard. The retention time windows and library searching should ensure that only correct analytes are reported.

The nature of the samples being tested and the reasons for the testing mean that it is probable that some patients will try to adulterate their samples to give false results. Any sample which does not look or behave as expected for urine must be considered suspect and be brought to the attention of senior staff.

The cutoff levels are more sensitive than those used in the immunoassay, and are set at the following approximate values.

Morphine/Morphine glucuronide	500ng/mL
Codeine/ Codeine glucuronide	500ng/mL
Dihydrocodeine	500ng/mL
Cocaine metabolite	150ng/mL
Methadone	200ng/mL
Methadone metabolite	200ng/mL
Amfetamine	250ng/mL
Metamfetamine	250ng/mL

INTERPRETATION OF RESULTS

Drugs will remain detectable in the urine for a varying period of time depending on the drug, the amount taken and a variety of other factors.

As a general guide, morphine, codeine and cocaine metabolites can usually be detected in urine for 3-5 days following use, although this gives no indication about the amount of drug taken.

Morphine is the main active metabolite of heroin, but is also prescribed and used itself. It is also a minor metabolite of codeine and pholcodine

6-monoacetyl morphine is the primary metabolite of heroin, but has a short half life and can normally only be detected in the urine for 12-24 hours after heroin use. Heroin (diacetylmorphine) has a half life in blood of around 2 minutes, so is very unlikely to be detected.

Codeine can be present when heroin is taken, as codeine is a common adulterant or contaminant, but it can also be taken as a medicine, available over the counter without prescription.

Dihydrocodeine is not metabolised to morphine, and the presence of morphine in a client given dihydrocodeine is an indication of extra use of opiates. Dihydrocodeine is now available as an over the counter medicine.

ANALYTES SEEN IN QUALITY CONTROL MATERIAL

ANALYTE	Negative	C2	C3	C4
Amfetamine	NEG	POS	POS	POS
Metamfetamine	NEG	POS	POS	POS
MDEA	NEG	NEG	NEG	POS
MDMA	NEG	NEG	NEG	POS
Cocaine-Benzoyllecgonine	NEG	POS	POS	POS
Methadone	NEG	NEG	POS	POS
Codeine	NEG	POS	POS	POS
Morphine-3-glucuronide	NEG	POS	POS	POS
PPX	NEG	NEG	POS	POS

REFERENCES

Clarke's Analysis of Drugs and Poisons 3rd Ed. (2004) Ed. AC Moffat, MD Osselton, B Widdop

Xcalibur software manual

PROCEDURE AMENDMENT FORM

Number	Date	Page No	Amendment	Authorised by:
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

- The amendment must be authorised by the author of the procedure
- The amendment must be underlined and an asterisk written in the margin along side the change
- Up to ten minor amendments can be made before the procedure is revised
- Major changes must result in the immediate review of the procedure

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