HSA Forensic Laboratories have been accredited since 1996 and are currently accredited under the ANSI National Accreditation Board (ANAB) Forensic Testing Laboratory Programme.

All procedures and methods performed in the laboratories (as set out in this Primer) are validated to conform to international best practices and standards.

A Guide to Forensic Analysis

Analytical Toxicology Laboratory - Drug Abuse Testing Unit

The analysis of urine samples for controlled drugs/metabolites, as stipulated under Section 31 (Urine tests) of the Misuse of Drugs Act 1973, is conducted using internationally recognised techniques and validated instrumentation.

The analytical process begins with an initial test to detect the presence of controlled drug(s) or their metabolite(s) or both. Urine samples that are tested positive are subjected to confirmatory tests, which involves sample preparation followed by qualitative or quantitative test using instrumental analysis.

HSA
Health Sciences Authority

Urine Sample Submission & Handling

Two urine samples from each subject are submitted to the laboratory by law enforcement officers in two separate locked security boxes (Photo 1). The boxes are unlocked separately (using a key securely stored in HSA) to retrieve the samples (Photo 2).

Photo 1: Two separate locked security boxes

Two analysts independently examine and verify the seal's integrity and details of the labels on the urine bottles against the information transmitted from the law enforcement agency's system to **HSA's Laboratory Information Management System (LIMS).**

A **unique sample number** is automatically assigned to each urine sample by LIMS. Once verified, the **two urine samples are unsealed and processed independently by two different teams of laboratory officers (LO).**

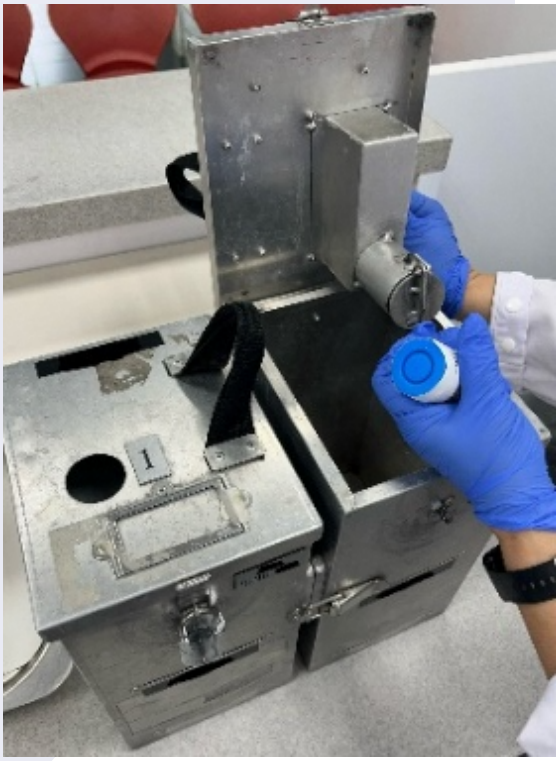

Photo 2: Retrieval of a urine sample from one of the security boxes

Initial Test

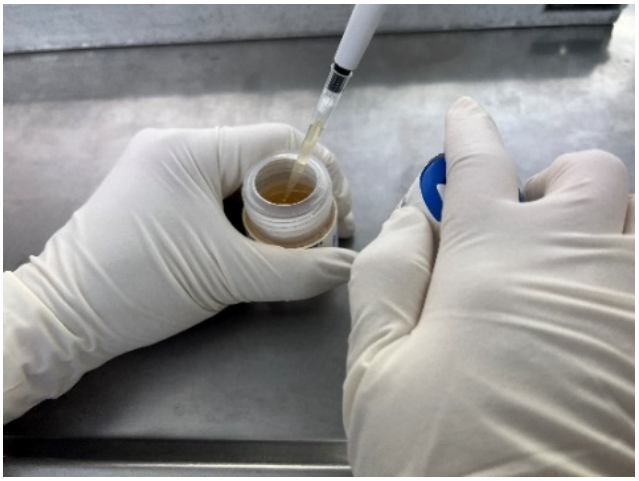

Photo 3: Sampling of urine from a urine bottle

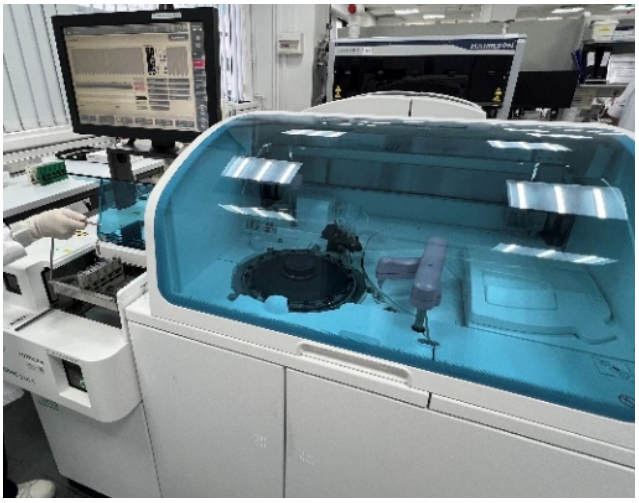

Photo 4: Loading of samples into an instrument to perform immunoassay

Photo 5: LC-HRMS instrument for initial test

A portion (aliquot) of urine is taken from each sample (Photo 3) for an initial test to detect the potential presence of controlled drug(s) or their metabolite(s) in the urine sample.

Depending on the drug test(s) requested, either an immunoassay or a liquid chromatography–high-resolution mass spectrometry (LC-HRMS) technique is used.

I**mmunoassay is a presumptive/screening test designed to detect structurally related compounds within specific drug classes**, including amphetamines, cannabinoids and opiates (Photo 4). A urine sample is positive when the drug classes are detected above their respective screening cut-off concentrations as stated in Table 1.

Table 1: Screening cut-off concentrations for amphetamines, cannabinoids and opiates in urine

Class of drugs	Cut-off concentration (ng/mL)
Amphetamines	500
Cannabinoids	50
Opiates	300

LC-HRMS is employed (Photo 5) to detect other drugs, including ketamine, LSD, fentanyl and new psychoactive substances (NPS). The use of LC-HRMS for initial test involves the separation of the drugs/metabolites and other substances by a chromatographic column based on their chemical and physical properties, producing a retention time for each individual drug/metabolite.

Each separated drug/metabolite is then introduced into the high-resolution mass spectrometer (HRMS), where its accurate mass is measured, and subsequently, undergo fragmentation, producing a characteristic fragmentation pattern that is specific to a drug/metabolite.

Positive identification is indicated when the retention time (RT), accurate mass and its characteristic fragmentation pattern correspond to a drug/metabolite's retention time and spectrum in the library (Note: The library is constructed using drug/metabolite standards).

Confirmatory Test

When tested positive by the initial test, the same urine sample will proceed for a confirmatory test. **The confirmatory test is a specific and sensitive technique used to confirm the presence of drug/metabolite in a urine sample.**

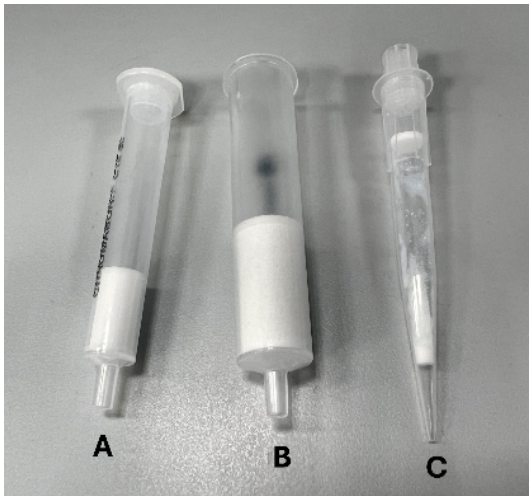

Photo 6:
A: SPE extraction cartridge
B: SLE extraction cartridge
C: LLE extraction cartridge

Sample Preparation

A portion (aliquot) of urine is withdrawn from the same sample for extraction of the drug/metabolite. The drug/metabolite is separated from the urine using an extraction method, resulting in a sample extract.

The extraction method used is either solid-phase extraction (SPE), or supported-liquid extraction (SLE), or liquid-liquid extraction (LLE), which are standard extraction methods adopted by toxicology laboratories worldwide.

Photo 6 shows the examples of the extraction cartridges used for the different extraction methods.

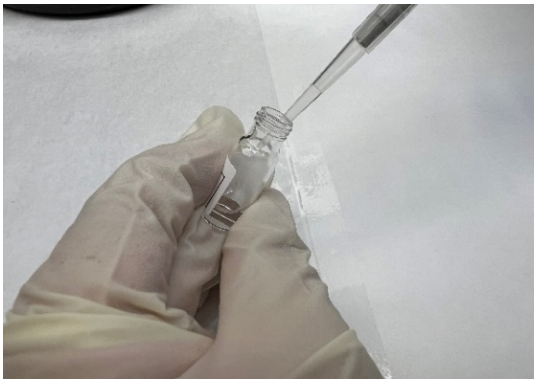

Photo 7: Transferring of a sample extract into a vial for instrumental analysis

Instrumental Analysis

The sample extract is transferred into a vial (Photo 7) for analysis. In our laboratory, gas chromatograph-mass spectrometer (GC-MS), liquid chromatograph-tandem mass spectrometer (LC-MS/MS) and LC-HRMS are the techniques used for analysing the sample extracts. They provide unequivocal identification of the drug/metabolite, if present in the sample extracts. The choice of instrument for analysis depends on the type of drugs to be tested, and whether quantification is required.

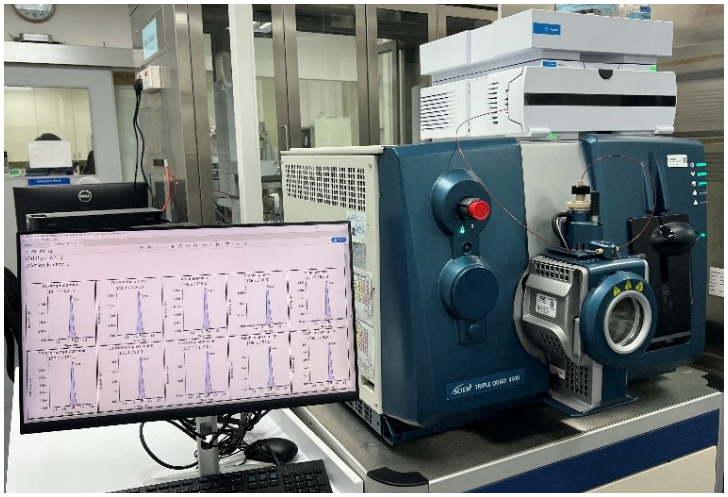

Photo 8: LC-MS/MS

Quantitative Analysis

LC-MS/MS and GC-MS techniques (Photos 8 and 9) operate on a general principle whereby drugs/metabolites and other substances in the drug extract are separated by a chromatographic column based on their chemical and physical properties to produce a retention time for each individual drug/metabolite. The separated drugs/metabolites then undergo fragmentation in the mass spectrometer to produce fragments specific to each drug/metabolite.

Positive identification of a drug/metabolite is achieved by comparing the retention time and characteristic fragmentation pattern against a drug standard analysed under identical conditions. Quantification is achieved by measuring the signal intensity of a specific fragment and comparing it to that of a drug standard with a known concentration. A list of confirmation cut-off concentrations for drugs in urine are stated in Table 2.

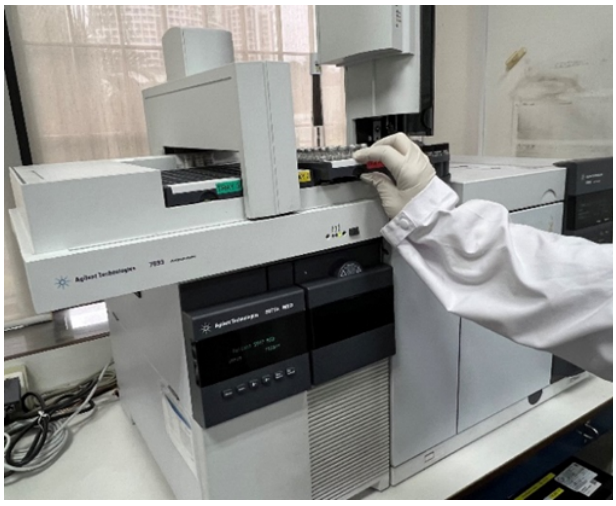

Photo 9: Loading of sample extracts into GC-MS

Table 2: Confirmation cut-off concentrations for drugs in urine

Reported Drug	Confirmation Cut-off Concentration (ng/mL)
Methamphetamine	500
MDMA	500
Norketamine	100
Monoacetylmorphine	10
Morphine	500
THC-COOH*	15
Benzoylecgonine	300

* 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid

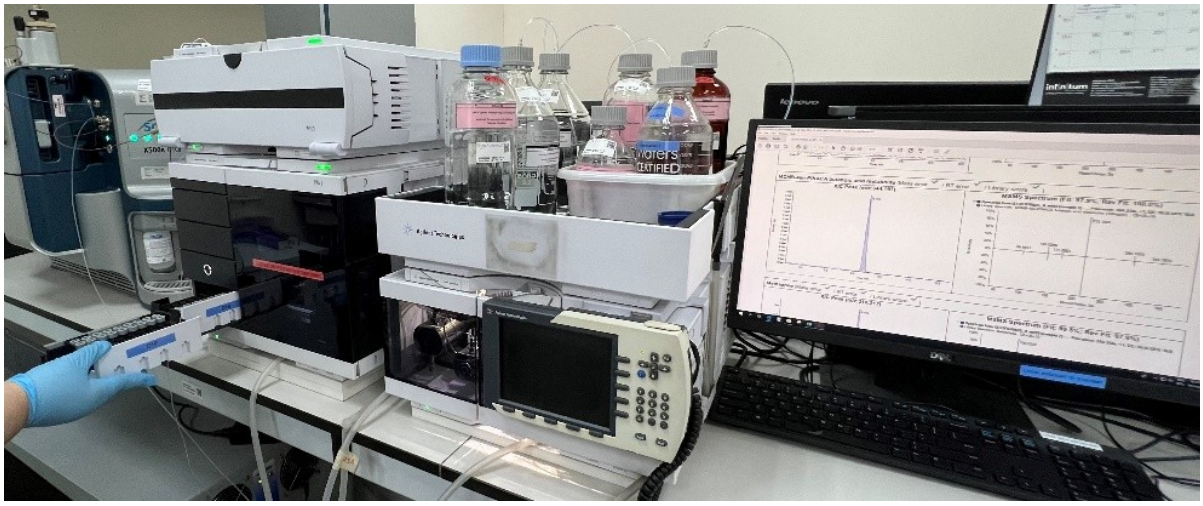

Photo 10: Loading of sample extracts into LC-HRMS for confirmatory test

Qualitative Analysis

In using LC-HRMS (Photo 10) for qualitative analysis, drugs/metabolites and other substances are separated by a chromatographic column based on their chemical and physical properties, producing a retention time for each individual drug/metabolite.

Each separated drug/metabolite is then introduced into the HRMS, where its accurate mass is measured, and subsequently, undergo fragmentation, producing a characteristic fragmentation pattern that is specific to a drug/metabolite.

Identification of a drug/metabolite is confirmed by comparing the retention time (RT), accurate mass and its characteristic fragmentation pattern to a drug standard analysed under identical conditions.

Quality Assurance

All instruments in the laboratory are **calibrated with reference to a drug standard solution,** and the **calibration is verified using quality control samples analysed together** with the drug extracts from urine samples to ensure accuracy of the results.

Report Generation

After the initial and confirmatory tests are completed for both urine samples, two analysts review and interpret independently the records and data from the respective analyses before drawing a conclusion for each urine sample. **A certificate under Section 16 of the Misuse of Drugs Act 1973 for each urine sample is then prepared by each of the analysts.**

Technical Review

The test records, data and certificates pertaining to the two urine samples **are reviewed by another analyst** before the certificates are issued to the law enforcement agency.