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Urine Drug Testing Practices

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Executive Summary

Laboratory testing for drug use by workers in government and industry has been implemented in many countries over the past 25 – 30 years. Testing urine for drug consumption is one objective indicator of recent drug use. Urine drug testing, however, does not measure drug related impairment of a worker but does provide an indication of recent drug use. These programmes have a very specific drug testing menu and are not used to screen for all drugs which may be in a donor's urine specimen.

Each of the components of a workplace drug testing programme have been developed in a legally defensible manner, from the specimen collection site, transportation of the specimens to the testing laboratory, receipt of specimen at the forensic laboratory, individual donor demographics, actual testing - screening and confirmation for drugs and/or metabolites.

The technical aspects of urine drug testing has a solid scientific basis and forensic laboratories performing workplace drug testing are certified by an external governmental agency in the US which provides workplace laboratory certification in the US and Canada. Rigorous quality assurance and on-site inspection teams visiting laboratories every six months ensures reliability of the testing. All aspects of this testing follows the standard approach used in forensic testing programmes of initially employing a screening test (designed to detect a specific drug or drug class) and a second (confirmation) test for all specimens that screen positive in the initial testing.

The drugs or drug classes that are generally part of workplace testing programmes include cannabinoids (marijuana), cocaine, opiates – codeine, morphine and heroin metabolite, phencyclidine and amphetamines. It is recommended that the Canadian Nuclear Safety Commission (CNSC) include these drugs or drug classes with the following exception. It is recommended that the CNSC not include phencyclidine in the testing programme due to low prevalence of this drug in Canada. Two additions to the testing programme are recommended. It is recommended that the CNSC have a broader testing menu in the opiates sub-category including - hydromorphone, hydrocodone, oxycodone and methadone. In addition, it is strongly recommended that the prescription medications – the benzodiazepines be incorporated in the workplace testing programme. The CNSC should develop a process to revise the drug menu for the drug testing programme periodically.

Due to the widespread use of drugs of abuse in our society, it is strongly recommended that CNSC develop a workplace drug testing programme as a deterrent to inappropriate drug use/abuse and to provide an objective indicator of drug use by workers in the industry.

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Urine Drug Testing Practices

1. Introduction

Over the past 25-30 years, many countries including Canada, Australia, New Zealand, Switzerland and the USA have developed forensic urine drug testing programmes for use in the workplace. These programmes were developed to aid in the detection of illicit drug use in various workplace settings and to act as a deterrent to drug use by current workers and/or applicants applying to work in an industry or field. The same scientific and forensic principles of workplace drug testing have been applied to specific populations such as military personnel in different countries, offenders living in correctional facilities or living in the community, and in child welfare cases where there are concerns about inappropriate drug use by a parent and/or guardian of children.

The objective of this report is to provide an overview of forensic urine drug testing as used specifically in the workplace setting. These drug testing programmes are based on laboratory analysis of urine specimens for specific drugs and/or drug metabolites found in urine. These programmes have a very specific drug testing menu and are not used to screen for all drugs which may be in a donor's urine specimen. All aspects of this testing follows the standard approach used in forensic testing programmes of initially employing an immunoassay screening test (designed to detect a specific drug or drug class) and a second (confirmation) test for all specimens that screen positive in the initial testing. In each of these programmes, laboratories apply administratively defined cut-off or threshold values in the initial screening tests and in the confirmation tests.

The detection of a drug or drug metabolite by an immunoassay screening test above the cut-off value is designed to 'rule out' drug negative urine specimens as shown for the Correctional Service of Canada (CSC) testing programme (Table 1). The majority of screening tests performed in the workplace are negative so these specimens do not require confirmation testing and are reported as drug negative. All urine specimens that initially screen positive (considered 'presumptive positive') are then analyzed by a second more sensitive and specific method(s) which are gas chromatography-mass spectrometry (GC-MS) or liquid chromatography mass spectrometry/mass spectrometry (LC-MSMS) for confirmation of presumptive immunoassay positive urine specimens at defined cut-off values as shown for CSC (Table 2). A urine specimen is considered positive for drug a, b, or c, etc. only after the screening and confirmation test results are equal to or exceed the cut-off concentrations of a drug or metabolite in that urine specimen and all quality assurance indicators of acceptable analyses are met.

Point of Care or On-Site drug testing has grown in popularity over the past decade. This testing approach is not recommended for drug screening of workers for several reasons. The tests available to screen for a drug or group of drugs is dependent on the test panels sold via the vendor. Secondly, these types of tests are generally not read objectively by an instrument but by visual examination only. Thirdly, testing for urine dilution by analysis of creatinine and specific gravity are not incorporated into On-Site testing devices.

The screening and confirmation cut-off values used in different programmes are not always identical and these cut-off values are modified over time as new information about drug metabolism and excretion is published and technological advances are available in the laboratory. It is important to appreciate that setting a screening and/or confirmation cut-off is an administrative decision set by the testing laboratory, the corporation introducing drug testing in the workplace or by a laboratory accrediting agency/governmental agency, etc. The initial screening and confirmation cut-off values promulgated in the late 1980s in the US by the National Institute on Drug Abuse (NIDA) and Substance Abuse and Mental Health Services Administration (SAMHSA) programme (Table 3) were not based on urine drug metabolite excretion patterns in clinical research studies. The cut-off values set at that time were based on the ability of the screening and confirmation testing systems in the laboratory being able to perform these tests in an acceptable and consistent standard over time. These cut-off values have been developed and modified based on over three decades of forensic toxicology research. One goal in all forensic drug testing programmes is that everyone participating in urine drug testing is subject to the identical testing standards. For example, one laboratory may be able to perform testing at a lower cut-off concentration than other laboratories. Organizations or corporations want all their workers and or job applicants subject to testing in one laboratory have comparable quality assurance testing standards as found in a second or third laboratory, etc.

The actual drugs or drug metabolites included in a forensic workplace drug testing programme are fixed and are not modified in an ad hoc manner based on a company's perception of impairment of an individual worker. Organizations that certify workplace testing laboratories such as SAMHSA have a process in place to review the current test menu and follow steps to modify the testing scheme when indicated.

Drug testing in urine is unable to measure the level of immediate drug impairment, since the active drug is often completely out of the blood circulation and most metabolites analysed in urine are not pharmacologically active. Secondly, it is impossible to reliably estimate the drug dose consumed and to accurately determine the time of last drug intake from a urine test even if the testing is performed quantitatively. Opponents of workplace drug testing may state that urine drug testing is 'unreliable'. It is helpful to understand what is meant when someone states that urine drug testing is 'unreliable'. If one expects a urine drug testing programme to measure drug related impairment, that expectation is not correct. That does not make urine drug testing 'unreliable'. Urine drug testing is highly reliable at determining if drug exposure/consumption occurred in the recent past and acts as a deterrent to illicit drug consumption in individuals subject to urine drug testing. Scientifically, test result reliability is based on rigorous quality assurance programmes in the laboratories offering this service using the latest scientific methods for drug screening and confirmation in biological fluids.

Table 1: Immunoassay Screening Cut-Off Concentrations in the CSC Programme

Drug/Drug Class	Cut-Off Value (ng/mL)
Cocaine Metabolite (Benzoylecgonine)	150
Opiates	300
6-Acetylmorphine	10
Phencyclidine (PCP)	25
Amphetamines	500
Cannabinoids (THC-Carboxylic acid)	50
Benzodiazepines	100
Methadone Metabolite (EDDP)	100

Table 2: GC-MS and LC-MSMS Confirmation Cut-off Concentrations in CSC Programme

Drug(s)	Cut-Off Value (ng/mL)
Amphetamines (Amphetamine, Methamphetamine, MDMA, MDA, MDEA)	250
Cannabinoids (as 11-nor-Δ-9 THC COOH)	15
Cocaine Metabolite (Benzoylecgonine)	100
Phencyclidine (PCP)	25
Methadone Metabolite (EDDP)	100
Opiates:	
Morphine, Codeine	300
Hydromorphone, Hydrocodone, and Oxycodone	300
6-monoacetylmorphine (6-AM, heroin metabolite)	10
Benzodiazepines (LC-MSMS):	
Oxazepam, Temazepam, Diazepam, Nordiazepam	50
Alprazolam, Lorazepam, Triazolam, Clonazepam	50
Bromazepam, Flurazepam	50

Table 3: Confirmation Cut-off Concentrations in the SAMHSA Programme

Drug(s)	Cut-Off Value (ng/mL)
Amphetamines (Amphetamine, Methamphetamine, MDMA, MDA, MDEA)	250
Cannabinoids (as 11-nor-Δ-9 THC COOH)	15
Cocaine Metabolite	150
Phencyclidine (PCP)	25
Opiates :	
Morphine, Codeine	2000
6-monoacetylmorphine (6-AM)	10

2. Individual Drugs or Drug Classes Commonly Included in Workplace Drug Testing Programmes:

2.1 Cannabinoids (Marijuana)

Marijuana refers to the dried leaves, stems, seeds and/or flowers of the hemp plant or Cannabis sativa that grows worldwide in temperate and tropical climates in addition to green houses in colder climates. Cannabis contains over 400 chemical compounds including 60 cannabinoids that contain pyran and phenolic ring structures. Marijuana has been stated to be useful therapeutically for the control of acute glaucoma and nausea that often accompanies chemotherapy for various forms of cancer. Clinical research studies are investigating the use of marijuana therapeutically using routes of drug administration other than by smoking.

Marijuana is abused due to its' euphoric effects, followed by drowsiness/relaxation. Clinical effects of marijuana include tachycardia, conjunctivae infection, dry mouth and throat, increased appetite, decreased respiratory rate, etc. Intoxication results in temporarily impaired concentration, learning and perceptual motor skills.

In research studies performed on experienced airline pilots, individuals may be impaired while performing complex tasks on flight simulators up to 24 hours after smoking a social marijuana dose (long after the individual is aware of any of the drug's euphoric effect). This could indicate a level of impairment in the workplace where a worker no longer perceives him/her feeling any effect from earlier drug consumption.

From drug excretion studies, it is well known that approximately 20% of a dose of smoked marijuana is excreted within five days in the urine. Delta-9 tetrahydrocannabinol (Δ9-THC, the active component of marijuana) is extremely fat-soluble and accumulates in adipose (fat) tissue with chronic drug use. Δ9-THC metabolites are slowly released over time and can be detected in the urine for several days or weeks following cessation of chronic marijuana use. Recent studies by Cone and Huestis indicated that after smoking one low dose marijuana cigarette (in controlled experiments in human volunteers), the time period that random urine specimens remained positive for cannabinoids ranged from 6.4 to 45 hours (h) (average time: 26h). After smoking one higher dose marijuana cigarette, the longest time period before urine specimens were negative for cannabinoids was 44.8 to 54 hours (average time: 49h). These studies were performed in volunteers smoking one marijuana cigarette only. Based on this study, however, one can conclude that whenever a urine drug test for cannabinoids is positive for marijuana in the "occasional" user using the SAMHSA defined cut-off values (50 ng/mL for screening and 15 ng/mL for confirmation testing of Δ9-THC carboxylic acid (COOH)), one can state that the individual was probably smoking marijuana/hashish within 48 hours prior to urine collection.

Passive inhalation of marijuana smoke is a frequently used explanation given when an individual is faced with a positive marijuana drug test. The possibility of passive drug inhalation has been studied extensively over the past 20 to 25 years. Some passive drug (marijuana or cocaine) inhalation does occur but the amount of marijuana/hashish inhaled is not sufficient to produce a positive urine test result with the current cannabinoid screening cut-off of 50 ng/mL and confirmation cut-off value of 15 ng/mL for the major marijuana metabolite (Δ9-THC COOH).

In the late 1990s, several investigators reported the possibility of a positive cannabinoid drug test following consumption of commercially available hemp seed oil. Hemp is a plant, Cannabis sativa, cultivated for its fibre and oil and is low in cannabinoid compounds content. In order to obtain approval for these products to be available in the Canadian market the producers must establish that there is no practical presence of Δ9-THC in their products. A number of products advertised as "Hemp" products are now on the North American market. Health food stores and nutritionists market hemp products as a source of essential amino acids and fatty acids. At this time, the scientific studies performed have not established with certainty the amounts of hemp products required to give a true positive cannabinoid drug test using the SAMHSA screening cut-off of 50 ng/mL and confirmation cut-off of 15 ng/mL. The drug testing program identifies and measures only a human metabolite of Δ9-THC COOH, a compound not present in the hemp plant. Therefore, it should be impossible to exceed the fairly high cut-off concentration of this metabolite when ingesting a reasonable quantity of hemp oil based products.

The following prescription drugs contain cannabinoids: Dronabinol (Marinol®) is a synthetic Δ9-THC available as gelatine capsules may be used for stimulating appetite and preventing weight loss in patients with a confirmed diagnosis of AIDS and treating the nausea and vomiting associated with cancer chemotherapy. Marinol use will give a true positive drug test for cannabinoid use (marijuana or hashish). This drug is no longer sold in Canada.

Delta-9-tetrahydrocannabinol (Sativex®) is a synthetic Δ9-THC and cannabidiol mixture marketed in Canada since 2005. It is indicated for the relief of the symptoms of neuropathic pain in multiple sclerosis in adults. The drug is administered as a spray on the wall of the mouth (buccal mucosa). Sativex® use will give a true positive drug test for cannabinoid use (marijuana or hashish).

Another synthetic product which is often compared to cannabinoids is nabilone (Cesamet®). Cesamet® use, however, will not give a positive drug test for cannabinoid use because the chemical structure is very different from Δ-9-tetrahydrocannabinol.

Several issues concerning marijuana/hashish use often arise in workplace drug testing. These issues include passive inhalation of marijuana smoke and time since last drug use. Individuals faced with a positive drug test result may state that they are not actively smoking marijuana but were in a car or other enclosed area where others were using the drug. This assertion to explain a positive drug test finding has been made numerous times but research studies on volunteers reported in the toxicology literature indicate that one does not obtain true positive marijuana findings with a screening cut-off value of 50 ng/mL and confirmation findings for Δ9-THC-COOH at or above 15 ng/mL with passive marijuana smoke inhalation. The second issue which arises is when someone states they last smoked marijuana or hashish several weeks or months prior to the most recent drug test. There are scientific valid means of reviewing a series of positive marijuana drug test results in an individual by incorporating the urine specimen concentration to help determine whether there was on-going drug use or not.

2.2 Opiate Drug Class

Opiate analgesic drugs are among the most effective medications for treatment of moderate to severe pain. These drugs, however, are often abused due to their desirable central nervous system (CNS) effects, especially euphoria. Many opiates are highly addictive, leading to physical and psychological dependence. Drugs classified as opiate analgesics may be naturally occurring, semi-synthetic or wholly synthetic chemical substances. The naturally occurring opiates (morphine and codeine) are obtained from the opium plant. Among the many alkaloids of opium, only morphine and codeine have psychoactive properties. Semi-synthetic narcotic analgesics such as heroin (diacetylmorphine) or hydrocodone are derived by chemically modifying either morphine or codeine. The synthetic agents (known as opioids) include methadone and meperidine (pethidine). These drugs mimic the effects of opiates but are not prepared chemically from opium.

a) Codeine

Codeine is a naturally occurring substance of the opium poppy, Papaver somniferum. Codeine's euphoric and analgesic effects are mild; its clinical uses are in the management of mild to moderate pain and the control of cough. In Canada, codeine is the only narcotic analgesic that can be obtained without a prescription (in small amounts per unit dose in combination with other drugs such as acetaminophen or salicylates) directly from a pharmacist. This possibility of having a true positive for codeine and/or morphine from taking low dose codeine highlights the importance of workers always providing a complete medication history (including over the counter medications) as part of a workplace drug testing programme.

Following consumption of codeine, one may detect in the urine codeine only, morphine only or codeine and morphine depending on multiple factors such as time of last dose, individual differences in drug metabolism and excretion, etc. Hydrocodone is a minor metabolite of codeine and may also be detected following heavy use of codeine. The time to a negative urine drug test after last codeine use is highly variable. In general, urine specimens are negative for codeine 48 hours after last drug use.

Codeine and morphine have been reported from consumption of poppy seeds from foods such as desserts containing a large quantity of poppy seeds. Experience has shown that this possibility has never been a limitation of opiate drug testing in Canada. In the US SAMHSA programme, one of the considerations in setting the opiate screening and confirmation cut-off value at 2000 ng/mL was the possibility of having a food consumption related positive opiate result with a lower cut-off value (300 ng/mL).

b) Heroin

Heroin (also called diamorphine and diacetylmorphine) is a powerful semi-synthetic narcotic analgesic produced by chemical modification of morphine. Because of the potency of its' euphoric and analgesic effects, heroin has the greatest potential for producing dependence of any of the common narcotic analgesics.

Following heroin use, one will usually detect only morphine in the urine. If a urine specimen is collected within a few hours after last heroin use (~10 – 12 hours), the laboratory may detect a unique heroin metabolite 6-monoacetylmorphine (also called 6-MAM). 6-MAM is routinely

screened for by immunoassay and confirmed by GC-MS confirmation. Unchanged (non-metabolized) heroin is very rarely found in a urine specimen, even in heroin overdose fatalities.

c) Morphine

Morphine is a naturally occurring substance in the opium poppy, Papaver somniferum. It is a potent analgesic and its' primary clinical use is in the management of moderately severe to severe pain. Morphine has the one of the greatest abuse liability of the narcotic analgesic after heroin.

Following use of morphine, one may detect morphine in the urine. Hydromorphone is a minor metabolite of morphine and may be detected following heavy use of morphine. The time to a negative drug test after last morphine is highly variable. In general, urine specimens are negative for morphine 48 hours after last drug use.

d) Hydromorphone

Hydromorphone (dihydromorphinone) is a powerful semi-synthetic narcotic analgesic. Its primary clinical uses are relief of severe pain and suppression of severe cough. Because of its relatively easy availability in prescription cough syrup and tablets and low cost, hydromorphone is popular among narcotic drug abusers. The most common trade name for hydromorphone is Dilaudid®.

Following use of hydromorphone, one detects only hydromorphone in urine. The time to a negative result by urinalysis is highly variable. In general, random urine specimens are negative for hydromorphone 48 hours after last drug use.

e) Hydrocodone

Hydrocodone (dihydrocodeinone) is a synthetic narcotic analgesic. Its' primary clinical uses are for suppression of a severe cough (antitussive) but this drug also has powerful analgesic properties. Because of its relatively easy availability in prescription cough syrup and tablets and low cost, hydrocodone is popular among narcotic drug abusers.

Following use of hydrocodone, one detects hydrocodone only, hydromorphone only or hydrocodone and hydromorphone in the urine. Hydrocodone is also a minor metabolite of codeine and could be found in urine of heavy codeine users. In those cases codeine or codeine/morphine are also found. The time to a negative urine test result after last use of hydrocodone is highly variable. In general, random urine specimens are negative for hydrocodone 48 hours after last drug use.

f) Oxycodone

Oxycodone is a semisynthetic narcotic analgesic derived by chemical modification to codeine. It produces potent euphoria, analgesic and sedative effects and has a dependence liability similar to morphine.

This drug has a very high abuse potential because:

1. it is highly effective when taken orally
2. it is often easily available and widely prescribed

3. it has a high degree of consistent potency

Following use of oxycodone, one generally detects oxycodone only, oxycodone and occasionally oxymorphone in the urine. The time to a negative result by urinalysis after last use of oxycodone is highly variable. In general, random urine specimens are negative for oxycodone 48 hours after last drug use.

g) Methadone

Methadone is a synthetic narcotic analgesic similar in potency to morphine. The advantages of methadone over morphine and heroin include:

1. Its' effects are longer lasting and can therefore be administered less frequently
2. It is highly effective when administered orally

Unfortunately, methadone also has a high dependence liability. Following use of methadone, one may detect methadone only, methadone and demethylated metabolite (EDDP), or the two demethylated metabolites (EDDP and EMDP) without any unchanged methadone in the urine. Most drug testing programmes screen for the major methadone metabolite (EDDP) rather than the unchanged drug – methadone in urine.

Urine drug testing programmes for opiate type drugs often use several different testing approaches. Many commercial immunoassays used in workplace drug programmes were designed to optimally detect codeine and morphine and not other drugs classified as opiates. Separate screening tests are often used to detect the unique heroin metabolite (6-acetylmorphine), oxycodone and methadone. Many workplace programmes only include codeine, morphine and 6-acetylmorphine in their opiate drug testing menu. Due to the widespread use/abuse of many different opiates in our society, it is recommended to have a workplace drug testing programme that includes the different opiates described in the section – codeine, morphine, heroin metabolite, hydromorphone, hydrocodone, oxycodone and methadone.

2.3 Cocaine

Cocaine is an alkaloid extracted from the leaves of the plant, Erythroxylon coca, grown primarily in the northern Andes Mountains of South America. This drug has been used for centuries by the Incas of Peru who chewed the leaves in religious ceremonies and elsewhere as a stimulant. Cocaine was once found in many tonics sold in North America including Coca-Cola in the late 19^{th} to early 20^{th} century. Cocaine became a controlled substance in the early 1900s as abuse became a public health concern after two epidemics of widespread abuse.

This drug was used therapeutically by physicians in Canada and the USA as a vasoconstrictive anesthetic for opthalamoscopic, otolargyngological, and trauma surgery. In North America, cocaine is the most commonly abused drug after ethyl alcohol and marijuana. It has acquired numerous street names including "blow, coke, crack (free base cocaine), dust, flake, lady, nose, snow, stardust, toot, and white", etc. The behavioural effects of cocaine are mediated by its' ability to block reuptake of dopamine and facilitate its release in the central nervous system. Desirable effects of cocaine for the abuser include euphoria, self-confidence, anorexia, hyperactivity, and profound sexual excitement. The central stimulatory effects caused by

cocaine are followed by depression. The positive reinforcement of the “rush” versus the negative reinforcement of the “crash” is felt to be the principal reason for the development of chronic abuse, especially after the use of “crack” cocaine.

Cocaine is rapidly metabolized in the blood and liver to benzoylecgonine and ecgonine methyl ester and is excreted in the urine primarily as these two metabolites. Laboratory analysis by immunoassay screening and GC-MS confirmation is directed toward detection of the major metabolite benzoylecgonine. The time to a negative result by urinalysis is generally 48-72 hours after last drug use although some investigators reported a longer time period until the urine is drug free in chronic cocaine users.

Individuals may offer another explanation for a positive drug test for cocaine metabolite other than use of cocaine. Absorption of cocaine through the skin and “passive inhalation” of cocaine have been used as explanations for urinary cocaine metabolites in medical and law enforcement personnel who claimed exposure to cocaine in the workplace. Laboratory studies, however, do not support this claim. In a study of 11 otolaryngologists who allowed a 4% solution of cocaine to dry on their hands or who administered a 2 second spray of cocaine to a patient did not produce any positive urine drug test for benzoylecgonine (150 ng/mL cut-off) in these physicians. In another study, urine specimens were collected over a 24-hour period from two subjects who had handled paper money completely covered with powdered coca paste. When urine specimens were analyzed with a cut-off value of 150 ng/mL, all specimens collected from the two subjects were negative. A further study examined the breath of subjects and room air concentrations of cocaine after smoking of crack cocaine in a controlled setting. In a study performed by Dr. Ed Cone of the Addiction Research Center, National Institute on Drug Abuse, in Baltimore, Maryland, US, subjects were exposed to cocaine vapour at high doses of cocaine (up to 200 mg) in a controlled environment and urine specimens were collected following exposure. It was found that cocaine metabolite was detected in some urine specimens but the amounts present were below defined cut-off screening cut-off of 150 ng/mL and confirmation cut-off of 150 ng/mL. In another study by Cone, research staff working with subjects smoking “crack” cocaine at three doses had their urine specimens collected for ~24 hours after first exposure. In the urine specimens collected from the research staff members, cocaine metabolite was detectable in some specimens but the amounts measured were well below the defined cut-off concentrations. Cone concluded that passive exposure to cocaine vapor resulted in absorption of small but detectable amounts of cocaine. When subjects were exposed to very high cocaine concentrations, cocaine and cocaine metabolite was detected in the urine but below the cut-off concentrations. The conclusion of all these studies is that passive inhalation of cocaine vapour resulted in minor exposure to cocaine but the exposure would not result in positive urine test findings for cocaine metabolite at a cut-off of 150 ng/mL.

2.4 Amphetamines

Amphetamines are a class of phenethylamine compounds that have varying degrees of potency as sympathomimetic drugs. This type of drug mimics the action of normal endogenous neurotransmitters that stimulate the sympathetic nervous system. Amphetamine, methamphetamine (speed), MDMA (Ecstasy), MDA and MDEA are all central nervous system stimulants. Tolerance can develop to the effects of amphetamine, methamphetamine, MDMA, MDA and MDEA. Abusers inject the drug intravenously, sometimes intranasally "snorting" or by smoking. Lethargy, drowsiness, hyperphagia, vivid dreams and mental depression may

persist for a few days to weeks after abrupt termination of repeated high amphetamine or methamphetamine doses.

Amphetamine is excreted in the urine as both unchanged amphetamine and as metabolites. The urinary excretion rate of amphetamine increases significantly when the urine is acidic (low pH). High dose amphetamine or methamphetamine abusers may have positive urine drug tests for 48 - 96 hours after last drug use.

Trade names of prescription medications products available in Canada that contain amphetamine (or which metabolize to amphetamine) include:

1. Dexedrine®,
2. Adderall XR (mixed salts amphetamine)
3. Vyvanse (lisdexamfetamine dimesylate)

The following prescription drugs available in Canada also metabolize into amphetamine: Selegiline (l-deprenyl HCl), also available as Apo-Selegiline, Gen-Selegiline, Novo-Selegiline and Nu-Selegiline.

Experience has shown that one regularly finds amphetamine in urine specimens collected from individuals prescribed amphetamine for Attention Deficit Hyperactivity Disorder (ADHD) such as Dexedrine or Vyvanse. When drug testing reports indicate positive tests for both amphetamine and methamphetamine, this indicates consumption of methamphetamine (Ecstasy) and is not due to consumption of any prescription medication available in Canada that contains amphetamine only.

Methamphetamine metabolizes into amphetamine and drug testing programmes require confirmation of both methamphetamine and amphetamine in all specimens reported positive for methamphetamine.

2.5 Benzodiazepines

Benzodiazepines are among the most widely prescribed drugs in North America. This class of therapeutic agent was first approved for use following the synthesis of chlordiazepoxide (Librium®) by Hoffman LaRoche in 1957. These drugs are available only by prescription in Canada and are used clinically as anti-anxiety agents, sedative hypnotics, muscle relaxants, for treatment of panic disorders, in anaesthesia and as seizure control (anticonvulsant) agents. When first introduced to the market, benzodiazepines offered several advantages over earlier medications for treatment of anxiety and sleeping disorders. In the 1980s, benzodiazepines were the most highly prescribed central nervous system (CNS) active drugs in the world. In the past 20 years, scientists, psychiatrists and regulatory authorities in many countries have carefully scrutinized benzodiazepine usage. These concerns arose due to multiple reports of psychological and physiological addiction, misuse, abuse, and adverse effects associated with long term use and/or withdrawal of benzodiazepines, especially in the elderly.

The benzodiazepines alprazolam, lorazepam and clonazepam are often among the most popular prescribed generic medications in North America. Diversion of these drugs is also significant since these benzodiazepines are often found in drug seizures as reported in crime laboratory statistics.

All drugs in this category exert some pharmacological activity as hypnotic agents, anxiolytic activity, anticonvulsant action, muscle relaxant activity and amnesic effects. There are major differences in potency and half-life of pharmacological effects due to varying benzodiazepine receptor binding affinity and rates of metabolism and excretion, etc.

There are currently 14 different benzodiazepines available in Canada ranging from ultra-short acting drugs such as triazolam (Halcion®) to many long acting drugs such as diazepam Valium®).

The benzodiazepines available in Canada in 2014 include:

1. alprazolam
2. bromazepam
3. chlordiazepoxide
4. clobazam
5. clonazepam
6. clorazepate
7. diazepam
8. flurazepam
9. lorazepam
10. midazolam
11. nitrazepam
12. oxazepam
13. temazepam
14. triazolam

Certain benzodiazepines (such as alprazolam and diazepam) are more subject to abuse than other benzodiazepines. These drugs are often abused along with other drugs of abuse (such as methadone) and/or ethyl alcohol.

Factors that play a role in the development of benzodiazepine dependence include:

1. Drug dose and duration of drug use
2. Pharmacological differences between different benzodiazepines
3. Individual characteristics

Individuals at increased risk of becoming dependent on a benzodiazepine include:

1. Persons with current or prior dependence on sedative hypnotics, including alcohol and previous benzodiazepines
2. Persons who have chronic medical or psychiatric illness
3. Persons who have personality disorders
4. Persons with chronic difficulties with sleeping

There are very limited clinical situations where an individual would require a prescription for more than one benzodiazepine at the same time.

Detection times for benzodiazepines and metabolites in the urine are extremely variable. Longer acting benzodiazepines (diazepam, nordiazepam, chlordiazepoxide, oxazepam, etc.) are given in

large doses and can be detected in the urine for several days to one to two weeks after cessation of chronic use. Short acting benzodiazepines (such as triazolam) are only detectable for one to two days after use and sometimes for only a few hours.

To complicate interpretation of benzodiazepine excretion in urine specimens, several different benzodiazepines excrete the identical metabolite(s). As an example, the following benzodiazepines are converted to nordiazepam as a metabolite prior to being excreted in the urine: diazepam, chlordiazepoxide and clorazepate.

The following benzodiazepines are converted to oxazepam as a metabolite that is excreted in urine: diazepam, chlordiazepoxide, clorazepate, temazepam and oxazepam. It is often challenging to determine which specific benzodiazepine was consumed based on the number of different metabolites found in urine specimens which are collected at varying times after the last drug dose consumed, etc.

Regulated workplace testing programmes such as the Substance Abuse and Mental Health Services Administration (SAMHSA) in the US do not include benzodiazepines in their laboratory drug testing menu. There are many other forensic drug testing programmes, however, that include benzodiazepines in their programmes such as criminal justice settings and child welfare programmes. It is recommended that benzodiazepines be included in the workplace drug testing programmes required by the CNSC.

3. Urine Collection and Transportation to the Laboratory

One of the key components of successful workplace drug testing programmes is urine specimen collection, specimen integrity, chain of custody and transportation of specimens in a forensically rigorous manner to a certified laboratory.

One of the reasons why this component is essential to the integrity of the entire programme is that one does not have an objective means of knowing with certainty if a collector is following the policies as developed. There are no video records of this key activity and it is virtually impossible to establish a 'blind' programme to monitor the collector's activities.

Many companies have security personnel escort workers to a urine collection site at workplace settings. How the worker arrives at a testing facility is not specifically addressed by the SAMHSA programme.

Key components of this activity must have very specific policies on:

1. The duties of the collector
2. Policies on collector and collection site records
3. Specific requirements of the actual collection site
4. When and where the donor has access to their personal effects and water, etc.
5. Requirements for when a urine collection is handled in a controlled environment and when the circumstances require an actual observed collection compared to an indirect collection
6. Specific details on donor identity verification

7. Type of containers used for specimen collection
8. Single vs. double specimen collections
9. Recording of urine temperature on collection bottles
10. Use of tamper evident seals on collection containers
11. Development of procedures for circumstances where the donor does not provide the minimum specimen volume or does not provide any urine specimen
12. Requirement that the donor initial each specimen bottle seal verifying that was their specimen(s)
13. Document any unusual characteristics of the specimen: unusual colour, presence of a foreign body, unusual odour (bleach aroma as an example), signs of adulteration (such as excessive foaming when shaken, etc.).
14. Establish what errors made during urine collection that are correctable if found in the laboratory. These may include adding a correct date or contact number, etc.
15. Establish what errors made during the urine collections that are not correctable at a later time (non-recoverable, fatal error). An example is not having donor initials on the specimen bottle labels.
16. Have a secure site to store specimens prior to pick-up by a courier service.
17. Establish a maximum time that specimens can be stored prior to courier pick-up

Organizations have to decide whether they will require two separate specimen containers for each collection or only one specimen container. The World Anti-Doping Agency (WADA) and Canadian Centre for Ethics in Sport (CCES) require two urine specimens for each case. The SAMHSA programme in the US also requires two specimens for each collection. It is recommended that two separate specimen containers be collected for each urine specimen collection. In the Correctional Service of Canada (CSC) drug testing programme and other programmes, only one urine specimen container is required.

The CSC has had a forensic urine drug testing programme for over 20 years. Some issues that have arisen about specimen collections within that organization include the following:

1. Collection of specimens at remote locations with limited courier service to the laboratory.

   One has to define the maximum number of days specimens are acceptable for testing from collection date to receipt in the laboratory (such as over a 4-day week-end holiday as an example). CSC has a maximum time period of six days including weekends and holidays, etc. The ideal time period from specimen collection to receipt at the laboratory is <48 hours. The maximum time period from specimen collection to receipt at the laboratory is six calendar days. Any specimen that arrives later than six days is not processed and would require a new specimen collection from the worker.
2. Maximum time period and conditions that specimens can be stored until shipment to the laboratory
3. Custody and Control forms for each specimen that are incomplete when shipped with specimens to the laboratory
4. CSC gives donors advance notice (2 hours) that they are required to come to the collection site to provide a specimen. The consequences for missing the appointment have to be made to donors given advanced notice prior to specimen collection. CCES

uses monitors who accompany athletes from time of notice for specimen collection until specimens are collected.

5. Donors may not provide the minimum urine volume required by the programme policy. The donor has to understand the consequences of not providing sufficient urine specimen for testing (30 to 50 mL are typical specimen volumes).

The SAMHSA urine specimen collection manual is very thorough and addresses all the matters that need to be addressed when setting up a workplace drug testing collection protocol.

It is recommended that workers be given up to 2 hours to provide sufficient urine for both urine collection containers. Workers should have access to fluids (maximum of 500 mL) while waiting to provide the urine specimen. CNSC should require the development of a shy bladder protocol for cases where workers may hesitate voiding into a container in the presence of a company representative.

No recommendation is being made whether a Third Party collection agency should be used to collect and transport urine specimens.

4. Dilution and Forensic Urine Drug Testing

4.1 Identification of Dilute Urine Specimens for Additional Testing

The CSC developed a process called the “Dilution Protocol” many years ago in response to a very high percentage of urine specimens being extremely dilute when collected for urine testing in their drug testing programme. An inherent assumption in urine drug testing is that the urine specimens being analysed are ‘normally concentrated’. Whenever there are any punitive consequences associated with a positive drug test result, urine donors often consume large volumes of fluids prior to providing a specimen in an attempt to flush the system or have the drug at a non-detectable value in their urine.

To address this matter, CSC developed a dilution protocol where the drug screening and confirmation testing was modified in urine specimens considered very dilute. It is recommended that CNSC include requirements for a dilution protocol in the worker drug testing programme.

Creatinine is an endogenous metabolite derived from muscle metabolism of creatine and is found in the urine of all normal healthy people, generally in proportion to their body muscle mass. Urine specimens will typically contain creatinine at concentrations much greater than 20 mg/dL. Each urine specimen found to contain creatinine <20 mg/dL of creatinine in the dilution protocol is subjected to follow up ‘specific gravity’ testing. Specific gravity is a density test and serves to measure the urine specimens’ similarity to water. Specific gravity measurements provide a second indicator of urine dilution in addition to creatinine testing. Normal urine from healthy people will typically have a specific gravity >1.003 g/L. Scientific studies indicate that a urine specimen obtained under normal conditions will not provide “positive" (i.e. dilute) results for both creatinine and specific gravity tests. In the CSC dilution protocol, all urine specimens that

are identified as "dilute" (specific gravity ≤1.003 and creatinine <20mg/dL) will be treated in an alternate manner as outlined below.

4.2 Initial Immunoassay Screening of "Dilute" Urine Specimens

Any urine specimen that is considered dilute and that test 'presumptively positive' using the standard immunoassay screen cut-off values, are referred for confirmation by GC-MS for the appropriate drug or drug class using the regular cut-off values for all presumptively positive screening tests. If the drug or drug metabolite concentration is above the standard confirmation cut-off values, those specimens are reported as drug positive in the usual manner. If the drug or drug metabolite concentration is less than the GC-MS or LC-MSMS confirmation cut-off value, the Lower Limit of Quantitation (LLOQ) cut-off for that drug is used instead. In these instances, it is recorded on the laboratory report that the specific gravity and creatinine were out of "normal urine' range (very low) but testing was performed in accordance with the routine procedure or the LLOQ was used as the confirmation cut-off value.

Any specimen that is considered dilute by the above criteria and tests negative in the initial immunoassay cut-off values will be subjected to the lower screening and confirmation cut-off values (Table 4) using a combination of immunoassay screening and GC-MS or LC-MSMS confirmatory testing if the lower screening result is 'presumptive positive'. Any specimen that tests positive above the lower screening cut-off value is then sent for GC-MS or LC-MSMS confirmation testing using the LLOQ for that drug class as the confirmation cut-off value for a positive test result.

Table 4: Dilution Protocol Cut-Off Concentrations

Drug/Metabolite	Screening Cut-Off Value (ng/mL)	Confirmation Cut-Off Value (ng/mL)
Amphetamine / Methamphetamine	100	100
Benzodiazepines	50	50
Cannabinoids	20	6
Cocaine Metabolite	15	15
Opiates (Codeine and Morphine only)	120	120
Methadone Metabolite	50	50

In the CSC statistical summaries of drug testing results each year since introduction of the dilution protocol, many of the dilute urine specimens (4-6% of all specimens submitted for testing) would have been reported as 'no drugs detected' if testing was only performed using the standard screening and confirmation cut-off values. The dilution protocol has demonstrated that additional drug use can be identified in very dilute specimens when using lower test cut-off values.

5. Tampering and Adulteration of Urine Specimens

5.1 Adulterants

Several years ago, individuals wanting to "beat the urine drug test" would drink a large volume of fluids immediately prior to providing a urine specimen in hopes of avoiding drug detection by intentionally diluting their urine. Testing laboratories started analyzing all urine specimens for

dilution by analysis of a normal urine constituent (creatinine) and/or measuring the specific gravity of urine. Creatinine is a normal by-product of muscle metabolism found in all urine specimens and specific gravity measures the "density" of urine. Creatinine and specific gravity measurements are routinely performed in forensic drug testing laboratories today. The CSC Dilution Protocol was discussed previously.

Chemical adulteration of urine specimens occurs with the addition of commonly available household products such as bleach, vinegar, liquid soap, ammonia, or strong chemicals such as sodium hydroxide as found in products such as Drano are added to the urine specimen. These adulterants were added directly to the urine specimen, not consumed orally by the individual. In most circumstances, these adulterated specimens are easily detected by the appearance or odour of the urine specimen at the collection site or when opened in the laboratory.

Recently, adulterants have become more sophisticated and a wide variety of products are sold specifically to individuals who want to make a drug positive specimen into a "clean" urine specimen on the Internet.

When forensic drug testing laboratories and commercial suppliers develop methods to detect an adulterant, there continues to be proliferation of new chemical mixtures (often sold via the Internet to individuals willing to try anything to beat a urine test). Publications such as 'High Times' magazine often have advertisements for urine adulteration products.

Agencies that certify drug testing laboratories (such as SAMHSA) require that laboratories have systems in place to test for chemical adulterants. The actual type of adulterants and processes are changing continually as new products reach the market and laboratories become aware of such adulterants.

a) Surfactants

Surfactants such as detergents are known to act by inhibiting detection of marijuana (cannabinoid metabolites) in urine. Surfactants can cause urine specimens with low concentrations of marijuana metabolites to screen negative by immunoassay testing. Laboratory staff often observes excessive foaming (like soapsuds) in these specimens. Whenever these soapy like specimens screen positive, the surfactant in the specimens has no effect on GC-MS confirmation procedures.

b) Glutaraldehyde

Glutaraldehyde (commonly marketed as UrinAid) inhibits the enzymes in many screening assays containing an enzyme. The chemical agent glutaraldehyde has a strong odour that is readily detected by laboratory staff. The odour is similar to that of overripe fruits and vegetables such as squash, pumpkins or apples. GC-MS confirmation methods are not affected by the presence of glutaraldehyde.

c) Acids

Many products contain strong acids such as hydrochloric acid (Amber 13, THC Free and earlier versions of Urine Luck). Hydrochloric acid interferes with enzyme based immunoassays and

result in negative screening tests. All laboratories routinely screen all urine specimens for acidity (pH test). According to SAMHSA guidelines, a urine specimen is defined as adulterated if the pH is less than or equal to 3 or greater than 11.

d) Nitrites

Nitrite containing products such as Klear did not affect the screening tests for marijuana but the extraction recovery percentage (in the GC-MS confirmation method for cannabinoids) is very low. Nitrites act by eliminating the major marijuana metabolite from urine. Toxicologists have reported methods to remove nitrite interference but most laboratories routinely screen for nitrites by commercially available methods.

e) Pyridinium Chlorochromate

Pyridinium chlorochromate is often sold as Urine Luck. This adulterant is an oxidizing agent that chemically converts alcohols into a ketone. This means that pyridinium chlorochromate oxidizes carboxy THC. This means that urine specimens adulterated with pyridinium often screen positive for cannabinoids but generally do not give a positive test in the GC-MS confirmation method. There are commercially available test strips for detecting the presence of pyridinium. In addition, the characteristic odour of pyridine is often detected due to pyridinium ion converting to pyridine.

f) Chromium

Chromium based adulterants such as potassium dichromate do not affect cannabinoid screening tests but the GC-MS confirmation procedure is generally negative. Since 1999, reagent manufacturers have developed kits for the detection of chromium in urine specimens which are often used in forensic drug testing laboratories.

6. Immunoassay Screening for Drugs and Drug Metabolites

Reagents systems used for initial screening for drugs of abuse classes in the laboratory are commercially available immunoassay products. One popular product is the Cloned Enzyme Donor Immuno Assay (CEDIA) immunoassay reagents manufactured by Thermo Scientific (Microgenics, Inc). Other manufacturers of similar drug screening products include Roche Diagnostics, Abbott Laboratories and Siemens (EMIT®). These biochemical tests measure the concentration of a substance in a fluid such as urine using a reaction between an antibody or antibodies toward an antigen (drug or drug metabolite). Antibodies are a type of protein produced by the immune system in response to the presence of a foreign substance (antigen). Antibodies bind to the antigen responsible for their production in the immune system. Antibodies are prepared to recognize specific drugs/metabolites or drug classes based on their three-dimensional shape and the charge of drugs/metabolites. Detection of the amount of drug present is based on competition between the drug present in the specimen being analysed and a drug tracer added to each specimen. The tracer tag is an enzyme, fluorescent label or a particle. When these drug/metabolites interact with these antibodies, one obtains a measurable chemical response from the tracer tag which is proportional to the amount of drug/metabolite present in each urine specimen. Specificity in immunoassays is defined as the affinity of an immunoassay for the target drug or metabolite. Specificity is measured by cross-reactivity which is the response exhibited when an immunoassay reacts with a substance other than the target drug or

metabolite. Because no immunoassay is 100% specific for a certain drug or metabolite, one cannot report an immunoassay result without confirmatory testing in a forensic setting. Specificity for a drug within a drug class varies with the manufacturer of the immunoassay reagent system.

Immunoassay drug screening is performed semi-quantitatively; applying administratively defined cut-off values or concentrations for each drug/drug class screened. It is always challenging scientifically to develop immunoassays that provide comparable performance (affinity or cross-reactivity toward all the drugs or drug metabolites) in a specific drug class such the opiates or the benzodiazepines, etc. For the opiate drug class, many laboratories use one immunoassay system to test for codeine, morphine, etc., a second immunoassay to test for 6-acetylmorphine and a third immunoassay designed to optimally screen for oxycodone. Many commercially available immunoassays for opiates are unable to reliably detect drugs such as hydromorphone and hydrocodone unless the drug concentration is much higher than the cut-off values. For the benzodiazepines, there are large variations in immunoassay cross-reactivity for the various benzodiazepine metabolites found in urine specimens. The regular dose of various benzodiazepines varies widely as does the relative amounts of these drugs excreted in urine of users. The challenge of varying immunoassay cross-reactivities is also an issue with the amphetamine class of drugs since laboratories screen for amphetamine, methamphetamine, methylenedioxymethamphetamphetamine (MDMA), methylendioxyamphetamine (MDA) and methylenedopxyethylamphetamine (MDEA) in urine specimens. The ideal situation would be to have sufficient systems used to optimally detect all drug/metabolites included in the drug test menu. This ideal situation may not be realistic in a large commercial laboratory driven by cost containment to succeed in the marketplace.

These immunoassay screening techniques are fast and efficient, but are not always specific for one drug or drug class (Table 5). Immunoassays are designed to eliminate drug negative specimens from other testing by more specific, technically challenging and expensive confirmation methods such as GC-MS or LC-MSMS. The cut-off concentrations used in the initial screening assays were set high enough to not detect one-time drug users and/or individuals exposed to second hand smoke (such as marijuana and/or cocaine smoke as discussed previously). Different drug testing programmes or the organizations certifying laboratories may specify a certain cut-off concentration. For example, the opiate cut-off value is lower in the CSC programme (300 ng/mL) compared to 2,000 ng/mL in many workplace programmes such as the SAMHSA programme (Table 5). It is recommended that the CNSC use a 2000 ng/mL opiate screening and confirmation cut-off value for codeine and morphine which are identical to the SAMHSA values. The major support for the 2000 ng/mL cut-off is that this value avoids the possibility of a food consumption (poppy seeds) related positive test result. For oxycodone and hydromorphone, a 300 ng/mL screening and confirmation is recommended.

Table 5: Immunoassay Screening Cut-Off Concentrations

Drug/Drug Class	Cut-Off Value (ng/mL)
Cocaine Metabolite (Benzoylecgonine)	150 (CSC, SAMHSA)
Opiates	300 (CSC) and 2,000 (SAMHSA)
6-acetyl morphine	10 (CSC, SAMHSA)
Phencyclidine (PCP)	25 (CSC, SAMHSA)
Amphetamines (D Methamphetamine equivalents) Methamphetamine, MDMA, MDA, MDEA	500 (CSC, SAMHSA)
Cannabinoids (THC-Carboxylic acid)	50 (CSC, SAMHSA)
Benzodiazepines (Oxazepam equivalents)	100 (CSC)
Methadone Metabolite (EDDP)	100 (CSC)

6.1 False Positives and False Negatives in Immunoassay Screening

When considering the role of immunoassays in a forensic drug testing programme, it is universally accepted that a positive immunoassay test result is a 'presumptive' or preliminary positive only. In no circumstance would an immunoassay result be reported without confirmatory testing by GC-MS or LC-MSMS. Therefore, the incidence of 'false positives' in a forensic drug testing programme should not be an issue for an organization considering implementation of a workplace drug testing programme. A higher frequency of 'false positives' by immunoassay would lead to a higher percentage of specimens referred for confirmation. This results in more expense and operational costs for the laboratory but does not lead to a false positive result being reported to an agency since the confirmation method also has to be positive at or above the cut-off value prior to being reported.

Instead of false positives, organizations should be aware that immunoassay systems used by their contract laboratory may not be able (false negatives) to always detect certain drugs in a drug class (such as hydromorphone in the opiates drug class or clonazepam and lorazepam in the benzodiazepine drug category).

7. Gas Chromatography Mass Spectrometry (GC-MS)

7.1 Confirmation by Gas Chromatography–Mass Spectrometry (GC-MS)

All "presumptive" positive results from the initial screening process are confirmed by GC-MS, which has long been considered the "Gold Standard" for drug testing throughout the world. GC-MS eliminates the possibility of false positives that may be found in immunoassay screening tests. The specificity of GC-MS in drug analysis is because there are two distinct analytical methods associated with GC-MS confirmation methods. First, the high-resolution gas chromatograph analytical column separates drugs and metabolites extracted from urine from each other and from other impurities. The time window at which the drugs or drug metabolites elute from the gas chromatograph column into the mass spectrometer is called the "retention time". Retention times serve as a very reproducible identifier for a particular drug or drug metabolite since different drugs/metabolites generally elute at different retention times. The second component of GC-MS analysis is the mass spectrometer. The mass spectrometer "breaks down" the drug molecules into fragments, which are unique for every drug/metabolite, analogous

to a “fingerprint” being unique to an individual. The computer generated mass spectra are essentially fingerprints of different drugs/metabolites. The intensities of these mass fragments measured are directly proportional to the quantity of the analyzed drug in a particular specimen. Calibrating (or standardizing) the GC-MS with varying concentrations of drug standards allows for a very sensitive, specific, precise and accurate quantitation of drugs and/or drug metabolites in urine specimens. To make GC-MS confirmation methods even more precise and accurate, isotopically labelled drugs (deuterium atoms are substituted for hydrogen atoms) are used as “internal standards” for drug quantitation. Mass spectrometers recognize the heavier fragments of deuterium labelled drugs, even though they are removed from urine specimens by extraction in an identical manner as the non-deuterium labelled drugs during the extraction process. By adding the same amount of internal standards to all urine specimens prior to extraction, the extraction recoveries are monitored for every drug test. As is the case with immunoassay screening, the confirmation cut-off concentrations are designed to separate positive and negative specimens. Urine specimens in which the drug concentration is less than the cut-off concentration are reported as negative, even though some drug/metabolite may be present below the cut-off concentration. GC-MS methods can detect much lower concentrations of drug and drug metabolites than the cut-off concentrations used. The smallest amount of drug/metabolite that can be reliably measured is called the Lower Limit of Quantitation (LLOQ). In the case of confirmation of drugs in dilute urine specimens such as the CSC dilution protocol, the LLOQ is used as the cut-off value. Drugs or metabolites may be reliably identified at lower concentrations than the LLOQ. The limit of reliable detection is referred to as the Lower Limit of Detection (LLOD). The cut-off concentrations of abused drugs in urine range from 15-1000 nanograms (10^{-9} gram) per millilitre (10^{-3} litre) range. Modern GC-MS techniques can measure drugs concentrations down to picogram and femptogram ranges (10^{-12} to10^{-15} gram; one nanogram is equal to 0.000,000,001 gram).

When performing GC-MS confirmation testing, a second portion of urine is removed from the original specimen bottle for this purpose. This specimen aliquot is removed in the presence of a second individual who monitors this process, under the chain of custody protocol. The urine donor (worker) cannot state "It was not my urine sample” as a valid explanation for the positive test result when confronted with a positive drug test result. The specimen handling process is fully computerized and specimen tubes are bar code labelled to eliminate any clerical and transcription errors. The final review of test results is performed by the most experienced staff (called certifying scientists) that reviews all data generated for that specimen, including review of all open and blind quality control (QC) samples that are analyzed with every batch of tests. If the QC samples fall outside of very narrow acceptable ranges, the whole batch of specimens being tested must be repeated. There is a minimum of 10% open (or known) quality control QC samples analyzed with every batch. Positive employee urine specimens are kept for a year to thirteen months in a locked freezer, unless they are challenged in a legal proceeding (in which case they are kept indefinitely until the case is settled). If required, the positive specimens can be referred, under chain of custody documentation, to another certified laboratory for re-analysis.

Liquid chromatography mass spectrometry mass spectrometry or LC-MSMS is a comparable analytical methodology to GC-MS and is the preferred methodology for certain drug classes such as the benzodiazepines and opiates. LC-MSMS is quickly becoming a second ‘gold standard’ drug testing method in forensic laboratories around the world.

7.2 False Positives and False Negatives in GC-MS

When GC-MS and LC-MSMS systems are operated according to the strict protocols mandated by laboratory certification organizations in the forensic setting, the possibility of a true false positive drug result is extremely rare. This is because GC-MS and LC-MSMS systems incorporate two different confirmations with each specimen being analysed. The gas chromatographic column and the liquid chromatographic column are the first confirmations which are followed by either a single mass spectrometer analysis in a GC-MS system or dual mass spectrometer analysis in the LC-MSMS systems. In addition, no urine specimen is referred for confirmation unless the initial test reads as a 'presumptive positive' by immunoassay.

One is always aware that a false negative can occur. To reduce the possibility of a false negative result, laboratory quality assurance policies require that rigorous quality control systems are part of each analytical run and laboratories are required to include 'content blind' samples into their daily operations. In addition, there are commercial organizations that market "content blind' urine specimens to companies using drug testing programmes where these 'content blind' samples are submitted to the laboratory as if they are authentic urine specimens from an employee. All of these quality assurance systems serve to very much reduce the possibility of a true false positive drug report to an infinitesimally small number or zero frequency.

8. Specimen Flow in a Forensic Drug Testing Laboratory

The following flow chart illustrates the progression of the urine specimens through the laboratory processes in the CSC drug testing programme. This process is recommended for the CNSC worker testing programme.

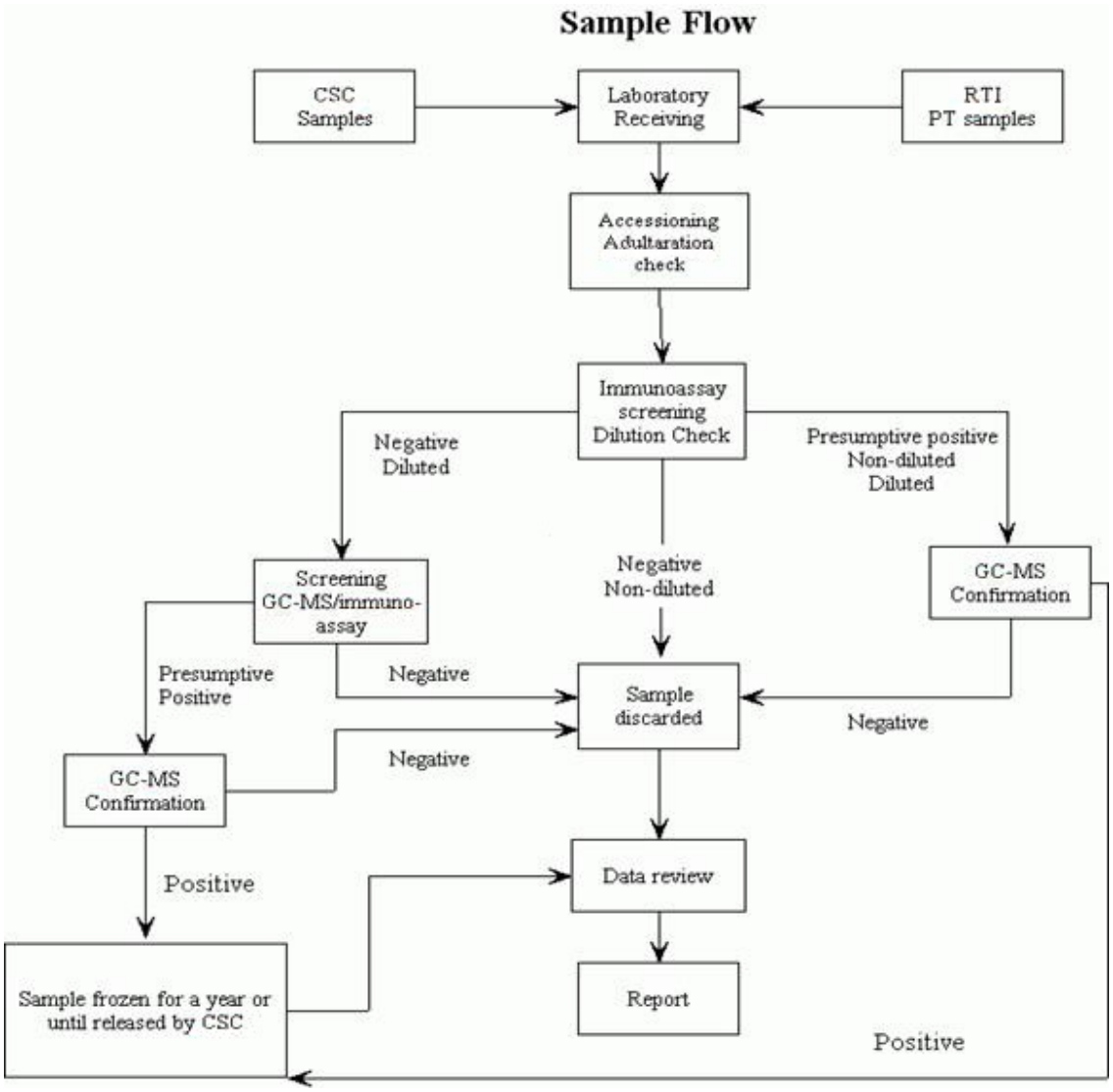

Figure 1. ***Sample Flow in a Forensic Drug Testing Laboratory***

It is important to appreciate that urine specimens shipped from various collection sites to a laboratory are stored in a secure accessioning area of the laboratory. Technologists and scientists working in the immunoassay screening area and confirmation area do not have access to the original urine specimens. Aliquots of the original specimens are transferred in a secure manner to the immunoassay and confirmation areas of the laboratory. Secondly, the final data review is performed by individuals who are called negative and positive certifying scientists. These individuals review all the data from other technologists in the laboratory. The accessioning staff also introduce internal laboratory 'blind urine specimens' into the test batches. The technologists that performed the testing do not know the position or content of any blind specimens provided to them for analysis. The certifying scientist is able to review whether the report for each blind specimen is correct. In addition, there are commercial vendors that market external blind controls that arrive to the laboratory along with the actual workplace urine collections. The laboratory is not aware of these external blind controls unless notified by the corporation or contract responsible for the external blind control specimens.

Each area of the laboratory is secure such that no one can walk freely from one area to another area without authorization (card key access). A list of personnel with approved access is posted at every entrance. In principle, the only individuals with access to an area are required to have a specific duty to perform in that area or be a laboratory inspector from an outside agency such as CSC or SAMHSA, etc.

One area of the laboratory is not shown in the above figure. This is the long term storage freezer(s) where positive urine specimens are kept for a minimum of twelve to thirteen months after the report is issued. These specimens are stored indefinitely if the specimen is being challenged by the urine donor or in a hearing, etc.

9. Drug Test Result Interpretation

In the SAMHSA programme in the US, the laboratory does not interpret the test findings. The final reports are sent to a physician who is designed as a "medical review officer" or MRO. This approach is used in the US Department of Transportation (DOT) programme and in all SAMHSA certified laboratories for workplace drug testing. In the vast majority of forensic laboratory settings, however, the scientist who oversees the testing or performs the testing also interprets the analytical findings.

In other programmes such as the CSC and Canadian Armed Forces programmes, drug test reports are not submitted to an MRO. In the CSC, the test reports are sent to the parole officer or staff person overseeing drug testing for the offender tested. Individual parole officers may contact the regional urinalysis coordinator or a CSC consultant toxicologist for assistance if needed to interpret test finding, especially when comparing test results to the offender's prescription medications. CSC toxicologists have also developed a frequently asked questions document which is available to CSC staff to assist in drug test result interpretation.

Either a MRO programme or a team (involving a pharmacist, forensic toxicologist and physician working as a team on behalf of the Commission) could carry out the role of drug test result interpretation for all urine test reports. The US based MRO system has a training and certification programme for physicians who want to become MROs. These trained MROs are educated in the current drug test menu used in the SAMHSA or DOT programmes. Organizations such as CSC have a much broader drug testing menu than SAMHSA and information about interpretation of test results for benzodiazepines, etc. is not part of the US based MRO training programme.

There is one area of concern with the US SAMHSA/DOT programme drug test interpretation that should be highlighted. Actual urine drug concentrations are often being reported on all positive reports to MROs in workplace urine specimens positive for a drug such as morphine or codeine, etc. Forensic drug testing laboratories are testing urine specimens at random times during the day where each specimen may have widely varying urine concentration/dilution compared to other specimens. The differences in urine concentration from one specimen to another can be up to a 12-15 fold difference in concentration based on creatinine measurements. Urine concentration is related to the time period that urine is in the bladder prior to voiding and is dependent on fluid consumption, etc. A highly coloured yellowish urine specimen would typically have a higher creatinine value compared to a very pale appearing specimen with the

appearance of clear water.

As discussed previously, drug analysis is focussed on using set cut-off values for screening and confirmation. Laboratories are required to perform regular quality monitoring at these cut-off values and control materials from -75% to 125% of the actual cut-off values. A positive test report is made when the unknown specimen screens at or above the screening cut-off value and the confirmation result is equal to or exceeds the confirmation cut-off value.

The question arises: Why do forensic drug testing laboratories not provide drug metabolite concentrations in all their reports?

There are several valid scientific reasons for not reporting drug metabolite concentrations in random urine samples:

1. Unlike blood, the "concentration of urine" can vary normally by 10 – 15 times (1000 – 1500%). A first morning specimen (strong yellowish colour) may have a creatinine reading of 275 – 300 mg/dL whereas another urine sample (having the appearance of water) collected from the same individual may have a creatinine value of 25 mg/dL after drinking two litres of fluid as an example.
2. The drug metabolites measured in urine samples have no impairing effect on the individual who submits the specimen. One can have an extremely high cocaine metabolite in urine concentration and no pharmacologically active drug - cocaine in blood.
3. The forensic drug testing processes are focussed on having very accurate and precise results around the defined screening and confirmation cut-off values. Every SAMHSA certified laboratory uses the same cut-off values so someone tested in a distant laboratory should always have the identical test results.

There is a vast scientific literature developed over 3 decades on the drug testing cut-off values used today. For example, a GC-MS positive test for cocaine metabolite near the cut-off value (150 - 200 ng/mL for cocaine metabolite by GC-MS) with a low creatinine of 25 mg/dL has the same urine drug metabolite ratio to creatinine as another urine specimen with a cocaine metabolite concentration of 2000 ng/mL and a corresponding creatinine concentration of 250 mg/dL. If one corrects or 'normalizes' the drug metabolite concentrations to account for the differences in specimen concentration/dilution, both cocaine metabolite results are identical!

In summary, the regulation that SAMHSA/DOT certified laboratories report actual urine drug concentrations to an MRO to aid in interpretation is not supported. Based on research on urine specimens carried out in the laboratory, any request for an actual drug concentration value in urine cannot be supported without normalizing the urine drug concentration by dividing the drug concentration by the creatinine concentration. A serum or blood drug concentration is often helpful in a clinical patients care context but not a urine drug concentration (such as 540 ng/mL cocaine metabolite, etc.) unless the individual interpreting the test findings can account for the urine specimen dilution and how that impacts the actual drug concentration on a urine volume basis.

10. Drugs and Human Performance

There is a very large peer reviewed literature on drug use and impairment often including studying complex attention and psychomotor skills while driving. Literature references related to the impacts on human performance are provided in the reference section of this report by drug category.

11. Summary and Conclusion

Based on the information presented in this document, it is recommended that the CNSC have very specific criteria for drug testing including drug/drug metabolite cut-off values.
The recommended screening and confirmation cut-off values for the CNSC are found in Tables 6 to 8.

It is recommended that the CNSC develop and establish a process where new drugs can be added to the testing menu when indicated over time. There are many reasons why CNSC should have a process in place to modify the drug testing menu of tests and cut-off values over time. This includes the fact that new drugs of abuse will inevitably emerge in Canada and elsewhere in the future. For example, in the CSC and other drug testing programmes, there are concerns about current drug use which is undetected since the newer drugs are not part of the drug testing menu. Examples include synthetic cannabinoids such as K-2 and Spice, pain medications such as fentanyl (this potent drug is often eluted from fentanyl patches), newer designer amphetamine type drugs and novel benzodiazepines such as diclazepam (CAS 2894-68-0), otherwise known as chlorodiazepam. In addition, some of the current drugs on the test menu may not be a concern about use in the future. The technology of drug testing is always evolving and changes in the testing processes may be needed as the testing technology changes in the future. It is always challenging for the forensic laboratory to develop new methods to screen and confirm each novel substance as they emerge.

The CNSC could use worker urine specimens (after testing is complete) to study the prevalence of another drug not on the testing menu in an anonymous blind manner where the identity of the donor would not be revealed. Based on test findings in 250 - 500 urine specimens, the CNSC would have objective data on the use of another drug consumed by workers which would go undetected unless the drug(s) was added to the test menu. Another drug may or may not be added to the test menu based on incremental cost, technical requirements of testing, additional urine specimen volume requirement, etc. In 2014, adding testing for substances such as anabolic steroids is very expensive and steroid testing is unavailable in the majority of forensic workplace drug testing laboratories in Canada and the US.

Although the major biological specimen used for forensic drugs of abuse testing is urine, the technology and application of other biological specimens for drug detection continually moves forward based on research studies. Recently, a Swedish scientist published an article on the use of exhaled breath for drugs of abuse testing in the criminal justice setting in Sweden. Alternate matrices such as breath are of interest currently but this novel technical approach has not be validated extensively in any centre by the spring of 2014.

This forensic toxicology report provides an overview of technical aspects of a forensic urine drug testing programme applicable in a workplace setting. Specific recommendations to the Canadian Nuclear Safety Commission are provided related to the technical aspects needed to ensure the development of a rigorous urine drug testing programme.

Table 6: Immunoassay Screening

Drug/Drug Class	**Cut-Off Value (ng/mL)**
Cocaine Metabolite (Benzoylecgonine)	150
Opiates :	
Morphine, Codeine	2000
Hydromorphone, Hydrocodone, and Oxycodone	300
6-Acetylmorphine	10
Amphetamines	500
Cannabinoids	50
Benzodiazepines	100
Methadone Metabolite (EDDP)	100

Table 7: GC-MS and LC-MSMS Confirmation

Drug/Drug Class	**Cut-Off Value (ng/mL)**
Amphetamines (Amphetamine, Methamphetamine, MDMA, MDA, MDEA)	250
Cannabinoids (as 11-nor-Δ-9 THC COOH)	15
Cocaine Metabolite (Benzoylecgonine)	100
Methadone Metabolite (EDDP)	100
Opiates:	
Morphine, Codeine	2000
Hydromorphone, Hydrocodone, and Oxycodone	300
6-monoacetyl morphine (6-AM, heroin metabolite)	10
Benzodiazepines (LC-MSMS):	
Oxazepam, Temazepam, Diazepam, Nordiazepam	50
Alprazolam, Lorazepam, Triazolam, Clonazepam	50
Bromazepam, Flurazepam	50

Table 8: Recommended Dilution Protocol Cut-Off Concentrations

Drug/Drug Class	**Screening Cut-Off Value (ng/mL)**	**Confirmation Cut-Off Value (ng/mL)**
Amphetamine/ Methamphetamine	100	100
Benzodiazepines	50	50
Cannabinoids	20	6
Cocaine Metabolite	15	15
Opiates (Codeine and Morphine only)	120	120
Methadone Metabolite	50	50

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Glossary

6-AM – 6-acetylmorphine (heroin metabolite)

ADHD - Attention Deficit Hyperactivity Disorder

CCES - Canadian Centre for Ethics in Sport

CEDIA – Cloned Enzyme Donor Immuno Assay

CNS – Central Nervous System

CNSC – Canadian Nuclear Safety Commission

CSC – Correctional Service of Canada

DOT – Department of Transportation, US

EDDP – Methadone Metabolite

EMIT – Enzyme Multiplied Immunoassay Test

GC-MS - Gas Chromatography Mass Spectrometry

HHS - Health and Human Services

LC-MSMS Liquid Chromatography Mass Spectrometry/Mass Spectrometry

LLOD – Lower Limit of Detection

LLOQ – Lower Limit of Quantitation

MDA – Methylenedioxyamphetamine

MDEA – Methylenedioxyethylamphetamine

MDMA – Methylenedioxymethamphetamine

MRO – Medical Review Officer

NIDA – National Institute on Drug Abuse

PCP – Phencyclidine

PT- Proficiency Testing

QC – Quality Control

RTI – Research Triangle Institute, North Carolina, USA

SAMHSA – Substance Abuse and Mental Health Services Administration, US

THC-Carboxylic Acid – Δ9-THC-COOH (Major Cannabinoid Metabolite in Urine)

WADA - World Anti-Doping Agency

Δ9-THC – Marijuana Active Drug