Final Report to Beat Drugs Fund Association

Project title

頭髮驗毒平台的多向發展：支援本地社福機構及復康計劃

Multi-directional Optimization of Hair Drug Testing Platform:

Rehabilitation Services Support for Local communities

(BDF101014)

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Introduction

Hong Kong has undergone rapid developments in its economy and technology in the past decades. However, some social and educational problems still exist and jeopardize the mental and physical health of our next generation. Drug abuse has been one of those unresolved problems. According to the Central Registry on Drug Abuse of the Hong Kong Government, there is an increasing trend for young drug abusers aged under 21, an increase of 53% was observed from 2005 to 2008 [1]. The Hong Kong Government has been trying to tackle this problem since 2007. In October 2007, a high level inter-departmental task force (the Task Force) led by the Secretary for Justice was formed and is responsible for making recommendations on how to tackle the youth drug abuse problem. In November 2008, the Task Force proposed to have school drug testing as one of the anti-drug activities and in July 2009, a Trial Scheme on School Drug Testing in the Tai Po District was launched. In November 2010, the corresponding evaluation research report recommended that the drug test scheme be extended to all schools in Hong Kong [2]. More recently, drug driving-related accidents have shown a rapid increase and the Hong Kong Government is considering setting new laws to give the Police the authority to run drug tests. Even there was a representative drop of 77% in young drug abusers from 2008 to 2014 [1], unquestionably, there is a need for a more efficient drug testing system in Hong Kong.

According to the Task Force, drug testing may serve the objectives of monitoring and deterrence, early identification, preventing drug abuse and crime investigation and prevention [3]. Besides, parents, teachers and principals of schools under the Trial Scheme of School Drug Testing have all agreed that drug testing is beneficial and could prevent teenagers from drug abuse [2]. In fact, running drug tests in schools is not new in Hong Kong or overseas. In Hong Kong, some schools take drug testing as a condition for school admission or a regular activity in their education programs on substance abuse [2]. Drug testing in schools is also common in countries such as United States, United Kingdom and Australia. Even though it is still controversial to make drug testing a compulsory activity in schools, research findings do support that drug testing is effective in reducing drug abuse on campus. According to the Office of National Drug Control Policy in US, random drug testing was effective in reducing and deterring drug use among adolescents. Drug testing led to a reduction in cannabis use among teenagers from 18.5% to 11.8% [4]. On the other hand, when the drug testing program was suspended, there was an increase in drug use among students [5]. Although there is a concern regarding privacy and labeling effect on students having positive test result, drug testing in schools seems to be one of the most possible and effective ways of finding the “hidden” drug abuser and preventing teenagers from taking drugs.

A survey in 2008/2009 indicated that a significant number of secondary schools in Hong Kong reported with drug-taking students [6]. However, there is no confirmed positive case found among 1975 students in the Trial Scheme School Drug Test in Tai Po [2]. As suggested by the evaluation report, it is possible that students may change their drug abuse pattern in order to avoid the drug test. They may take drugs during holidays and stop only a few days before they return to schools. On the other hand, it is also possible that urine, as the testing material in the Trial Scheme, is not sensitive enough to detect drug abusers. Several human specimens including urine, hair, blood, sweat and saliva have been used in determining the residues of abused drugs, and each has its own strengths and weaknesses. In contrast to most of the specimens, hair gives a longer detection window (more than 1 year, if the hair is longer than 12 cm), which enables retrospective investigation of the past drug consumption. In addition, hair samples can be easily obtained and is difficult to adulterate, and can be stored and transported without specific precautions owing to its stability [7]. Another advantage of hair-drug testing is that the segmental hair analysis might help to determine the time of drug exposure [8]. Moreover, the stable nature of hair also allows retests of the same sample when doubt or dispute over a positive test result arises. More importantly, the detection limit of hair drug testing is in the range of nanograms which is much more sensitive than those of typical urine testing.

Apart from the aforementioned advantages, hair drug testing has another unique and irreplaceable role in rehabilitation programs. Rehabilitation is a continuous process to show support and care to drug abusers and at the same time to monitor and prevent them from taking drugs again. Hair drug test, but not other drug tests, provides a continuous record of drug consumption across the rehabilitation programs which not only provides important information for program evaluation, but also encourage drug abusers and their families that the rehabilitation program is working well for them. HKUST has been cooperating with various schools and NGOs in the past year, providing them with more than 700 free drug testing services on hair samples. With this valuable experience and feedback from schools and NGOs, we are able to better understand the limitations of the existing drug testing platforms and optimize the drug testing platform to best suit the society.

Objectives

1. To optimize the existing drug testing platform;
2. To include the most updated drug residues in the testing platform;
3. To provide free testing service to schools and NGOs;
4. To provide tailor-made support and facilitate the corresponding rehabilitation programs;
5. To evaluate the cut-off value of ketamine in the hair drug tests.

Research methodology

1. To optimize the existing drug testing platform

1.1 Reduction in the number of hairs needed in the drug tests

According to the evaluation report of the Trial Scheme on School Drug Test, many of the students were not in favor of hair testing because they feared that cutting hair would affect their appearances [2]. And our experience with schools and NGOs also show that teenagers are reluctant to provide hairs for drug testing because of similar reasons. Therefore, reducing the hair amount is definitely a good way to increase the acceptance of hair drug testing. Normally, about 40 to 50 strands of hair are required for the drug testing. Our laboratory at HKUST has tried to reduce the amount to around 20 strands and currently we can detect over 10 drug resides with just 20 strands of hair (~3 cm in length). All experiments were carried out by using an Agilent 6410Btriple quadrupole mass spectrometer (QQQ-MS/MS) with ESI source (Agilent Technologies, Waldbronn, Germany) coupled with Agilent 1290 Infinity Binary Liquid Chromatography Systems. Agilent Mass Hunter ChemStation software (version B01.03) was used for data acquisition and processing.

1.2 Accreditation of hair-drug testing

The necessary step for drug testing in human hair is to standardize all the operation procedures, equipment and documentation systems, so that the drug test being conducted by our center is qualified and internationally accepted. Therefore, we had apply for accreditation from HOKLAS. HOKLAS is an accreditation scheme operated by the Hong Kong Accreditation Service (HKAS). The scheme is open to voluntary participation from any Hong Kong laboratory that performs objective testing and calibration, falling within the scope of the Scheme and meeting the HOKLAS criteria of competence. The aims of HOKLAS are: 1) to upgrade the standard of testing and management of Hong Kong laboratories, 2) to identify and officially recognize competent testing and calibration laboratories in Hong Kong, and 3) to promote the acceptance of test and calibration data from accredited laboratories, both locally and internationally. Specifically, we will apply for ISO/IEC 17025 (general requirements for the competence of testing and calibration laboratories). Accreditation is recognition of a laboratory's capability to perform specific tests. Laboratories accredited or seeking accreditation under HOKLAS are required to have their testing and measuring equipment regularly calibrated by a competent calibration organization to obtain measurement traceability to the International System of Units. According to our established protocol for drug testing in human hair, a variety of laboratory equipments must be validated and calibrated, including

pipetman, refrigerators, shakers, incubators, sonicators, and so on. In addition, a complete filing and document system for data recording must also be established. Currently, accreditation of our laboratory has been achieved, and then we are providing qualified service to the public.

2. To include the most updated drug residues in the testing platform

According to the Central Registry of Drug Abuse, the number of young drug abusers aged under 21 reached a record high of 3474 in 2008 [1]. Although the number dropped to 800 in 2014, among the commonly abused psychotropic substances, ketamine was the major type of drugs being abused during 2005 to 2014 [1]. It is not surprising that drug abusers would switch to a different drug from time to time, either for the purpose of experiencing a different kind of stimulation or to avoid from being identified in a drug test. Obviously, there is an urgent need to update and extend the drug residues being identified in the drug testing system. In fact, in the past year, we have been receiving requests from NGOs regarding the detection of newly available drugs in the public, and we have successfully developed detection protocols for additional drug residues including benzodiazepines (triazolam, midazolam and zopiclone), synthetic cannabinoids (JWH-018), piperazine derivatives (benzylpiperazine), tobacco (nicotine and cotinine) and barbiturates (secobarbital).

3. To provide free testing service to schools and NGOs

Apart from schools and the Government, drug testing also serves as an important tool in rehabilitation programs among NGOs. Currently hair drug testing services in Hong Kong are very limited, even though it is commonly agreed that the tests are good supplements for other testing methods, with regards to its high sensitivity, long history of detection, no adulteration and strong scientific evidence. We have established a very good network and working platform with 12 NGOs and rehabilitation units. In response to the social need, we offered not less than 3000 free drug tests to NGOs in Hong Kong. The free testing service offered to NGOs has been designated as one of our major objectives in the project. Free test service to school was not covered in this project since hair test was conducted by Government Laboratory in the School Drug Testing Scheme which was arranged by the school drug testing team from NGO.

4. To provide tailor-made support to schools and NGOs

4.1 Quota for urgent test report

Test reports in our laboratory usually take 7 days to issue. Priority would be given and the time of report issuing was reduced to 3 days. In fact, the request of urgent report is quite common. We see a need in the society regarding the flexibility of report issuing and we aim to provide

1000 quota out of the 3000 free testing services for the urgent report. We are open to rearrange our resources to serve NGOs in the best way so that our society could get the most advantage out of the drug test.

4.2 Free consultations and on-site visits

Hair Drug Testing is a relatively new idea for NGOs and the general public. We provided leaflet regarding the instructions of hair sample collection, storage and transportation. Second, we made phone calls to them regarding the small details of hair sample collection. Third, we provided on-site visit, demonstrating the whole procedure of hair sample collection. Fourth, we provided free consultations regarding the results of test reports to NGOs. Fifth, we also welcomed the public to visit, enabling us to share and transfer the technology of hair drug test to the society.

4.3 A tailor-made service of the drug testing

Hair samples from NGOs are often collected from clients of rehabilitation programs. Test reports could provide valuable information about the drug consumption habits of clients before, during and after the rehabilitation program. With this information, NGOs would have an idea about how long and how heavily the clients commit to drug abuse, and hence, will be able to choose the best rehabilitation program to begin with. Meanwhile, the results of drug tests could be used to monitor the effectiveness of the rehabilitation program and help NGOs to make corresponding adjustments. Finally, test reports also help to determine the duration of rehabilitation programs; a continuous negative result might suggest the successful quitting of drug abuse and provide an idea of completion of rehabilitation programs. Consumption habit of drug abusers varies; we often receive requests in making adjustments in the testing period of the hair drug test. Social workers sometimes want to know the latest consumption habit of their clients (i.e. the latest one month) or make a comparison between two periods of time (i.e. compare the latest one month with the earlier 3 months). Because of this important demand, our research team will continue to provide this service by dividing the whole strand of hair into different segments according to the expected testing time periods. This tailor-made service is most welcomed by social workers, as similar kinds of tailor-made service are not available elsewhere in Hong Kong. Therefore, we provided segmental analysis that hair can be analyzed with the desired hair section which indicates the particular detection period. Usually the first 3-cm from the hair scalp representing for the last 3-month period. Since the average growth rate is approximately at 1-cm per month, if the hair is long enough (e.g.12-cm), 1-year of drug history can be revealed.

5. To determine the cut-off value for ketamine detection in hair

Cut-off value of a particular drug residue is an important piece of information to determine whether a person has committed to drug abuse. In Hong Kong, ketamine was one of the most commonly abused drugs. About 47% of reported young drug abusers aged under 21 abused ketamine in 2014 [1]. Ketamine was originally used as a dissociative anesthetic drug, but it is currently abused as a recreational drug in "rave" parties. It induces hallucination and increases the incidence of crime. Ketamine has been regulated in Schedule III of the United States Controlled Substance Act in 1999 and Schedule 1 of Hong Kong Dangerous Drugs Ordinance (Chapter 134) in 2000. After entering the body, ketamine is N-demethylated to norketamine (NK), and then converted to dehydronorketamine (DHNK) and other metabolites through the liver. So far there is very limited information about the metabolism of ketamine, and unfortunately there is no information regarding the cut-off values for ketamine or its metabolite. According to the Vice-President of Society of Hair Testing, the best we can do at the moment is to take the cut-off value of amphetamine as a reference point to determine the cut-off value of ketamine due to the similar chemical properties of these two chemicals.

NGOs have often raised concerns regarding the correlation between the detected value of ketamine and the corresponding drug consumption habits. The accumulated sample number of ketamine positive sample has reached over 1000. We obtained the statistical data via the survey including an updated statistic data regarding gender, age of drug abusers and types of consumed drugs. The survey could provide information of the most up-to-date trend of drug consumption habit. We could determine the cut-off value of ketamine among drug abusers in Hong Kong. The concentrations of ketamine and norketamine were evaluated from these ketamine-positive samples. The proposed cut-off value is the concentration of hair ketamine when over 90% of samples are being detected with the presence of norketamine, which is a proof of ketamine abuse. This value could be applied as a screening or occupational cut-off for reference.

Results

1. To optimize the existing drug testing platform

Reduction in the number of hairs needed in the drug tests

To reduce in the number of hairs needed in the drug tests, the amount of hair specimen has been reduced to 5mg (about 20 strands of hair). Method accuracy and precision were determined. Accuracy of the method (i.e. bias or systematic error) was determined by the recovery test on sample with known amount of analyte. Six replicates of spiked samples at low (200 pg/mg), medium (2000 pg/mg) and high concentration (10000 pg/mg) were analysed. The mean accuracy values for each analyte were calculated by the average of analysis results. The mean accuracy values for the low, medium and high concentrations should be within 30% from the true values. Precision refers to the closeness of the series of replicate measurements to each other. It is the measure of the spread or dispersion of a set of results. Precision study was performed by analysis of six replicates of spiked samples at low (200 pg/mg), medium (2000 pg/mg) and high concentration (10000 pg/mg). The mean precision values for the low, medium and high concentrations were within 20% Relative Standard Deviation (RSD). The required hair amount provided an acceptable accuracy and precision among 16 commonly abused drugs and its metabolites.

Summary Table of Accuracy

Analyte	Accuracy (%)		
	Low Level (200 pg/mg)	Medium Level (2,000 pg/mg)	High Level (10,000 pg/mg)
6-Acetylmorphine	-15.14	-11.33	-2.94
Amphetamine	-9.99	-8.70	-3.15
Benzoylecgonine	-13.60	-12.47	-6.94
Cocaethylene	-6.65	-11.31	-3.99
Cocaine	-3.99	-0.90	9.66
Codeine	-9.71	-18.73	-13.15
Ketamine	-11.94	-14.05	-7.69
MDA	-18.32	-18.52	-13.97
MDEA	-8.35	-1.06	11.18
MDMA	-10.59	-5.81	3.88
Methadone	-13.52	-5.80	5.42
Methamphetamine	-13.39	-17.77	-9.90
Morphine	-26.10	-29.66	-23.66
Norcocaine	-6.02	-11.30	-3.06
Norketamine	-1.51	-8.39	-1.66
PCP	-11.21	-17.18	-7.78

Table 1. Summary table of accuracy

Summary Table of Intra-batch Precision

Analyte	Precision (RSD%)		
	Low Level (200 pg/mg)	Medium Level (1,000 pg/mg)	High Level (10,000 pg/mg)
6-Acetylmorphine	8.55	6.93	4.01
Amphetamine	2.16	4.98	3.76
Benzoylecgonine	4.67	3.45	4.61
Cocaethylene	1.60	4.89	4.07
Cocaine	2.00	4.31	4.25
Codeine	4.07	4.68	2.73
Ketamine	1.62	3.81	4.28
MDA	4.14	4.34	3.35
MDEA	3.44	3.93	4.59
MDMA	3.08	5.33	3.37
Methadone	3.40	6.50	6.53
Methamphetamine	3.92	4.34	4.27
Morphine	12.35	7.91	2.95
Norcocaine	2.55	5.55	3.48
Norketamine	4.74	3.85	2.90
PCP	2.07	3.53	2.93

Table 2. Summary table of precision

Accreditation of hair-drug testing

In May 2014, our laboratory (Laboratory for Molecular Testing, The Hong Kong University of Science and Technology) has obtained HOKLAS accreditation of ISO17025 on testing drug of abuse in human hair (HOKLAS registration number 238). The scope of abuse drugs in human hair, covering 16 analytes of 10 commonly abused drugs and their metabolites (cocaine, norcocaine, benzoylecgonine, cocaethylene, morphine, 6-Acetylmorphine, codeine, methadone, amphetamine, methamphetamine, MDMA, MDEA, MDA, phencyclidine (PCP), ketamine and norketamine). The laboratory information and the scope can be viewed in HOKLAS website as below.

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CLIENTELE **服務對象**	:	Public 公眾
ACCREDITED TEST CATEGORIES **認可測試類別**	:	Chemical Testing 化學測試
SCOPE OF ACCREDITATION **認可範圍**	:	Accredited activities are shown on the following pages 認可項目詳見後頁

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The Hong Kong University of Science & Technology – Laboratory for Molecular Testing
香港科技大學 – 分子檢測實驗室

TEST CATEGORY 試驗類別	ITEMS, MATERIALS OR PRODUCTS TESTED 試驗項目、物料或產品	SPECIFIC TESTS OR PROPERTIES MEASURED 特定試驗或量度的特性	SPECIFICATION, STANDARD METHOD OR TECHNIQUE USED 規範、標準方法或應用技術
CHEMICAL TESTING 化學測試	Human hair	Test for drugs of abuse:- - Ketamine - Norketamine - Cocaine - Norcocaine - Benzoylecgonine - Cocaethylene - Codeine - 3,4-Methylenedioxymethamphetamine (MDMA) - 3.4-methylenedioxyethylamphetamine (MDEA) - 3,4-Methylenedioxyamphetamine (MDA) - Amphetamine - Methamphetamine - Morphine - 6-monoacetylmorphine - Phencyclidine - Methadone	In-house Method LP101 (LC-MS/MS)

2. To include the most updated drug residues in the testing platform

The detection protocols for additional drug residues including benzodiazepines (triazolam, midazolam and zopiclone), synthetic cannabinoids (JWH-018), piperazine derivatives (benzylpiperazine), tobacco (nicotine and cotinine) and barbiturates (secobarbital) have been developed. The conditions for mass spectrometry (except midazolam and triazolam) were optimized as follow: drying gas (10 L/min nitrogen, 325C); capillary voltage (4000 V); scan mode (positive). Experiments for midazolam and triazolam were carried out by using an Agilent 6410Btriple quadrupole mass spectrometer with an HPLC-Chip cube interface (Agilent Technologies, Waldbronn, Germany) coupled with a set of nano liquid chromatography (LC). Nano-LC was performed by using an Agilent 1200 instrument consisting of a nanoflow pump with a degasser, a capillary pump with a degasser, and a thermostat microwell-plate sampler. The following components were integrated into a microfluidic chip (G4240-65010-Agilent Technologies): a 25 mm, 500 nL enrichment column packed with ZORBAX 80 SB-C18 with 5 μm particle size, a 150 mm×75 μm separation column packed with ZORBAX 80 SB-C18 with 5 μm particle size, and a nanospray emitter. The microfluidic chip was inserted into the HPLC-Chip cube interface, which was mounted directly on the MS source and included a valve for flow switching and a miniature camera for spray visualization. The elutes from nanospray emitter were injected into an Agilent 6410B-QQQ equipped with an ESI source. An Agilent Mass Hunter ChemStation software (version B01.03) was used for data acquisition and processing. The mass spectrometric conditions and sensitivity including Limit of Detection (LOD) and Limit of Quantification (LOQ) were investigated (Table 3). Sensitivity was evaluated by the limit of detection (LOD) and limit of quantification (LOQ). A series of decreasing concentrations of drug-spiked hair was evaluated to determine the LOD and LOQ. LOD was determined as the concentration with a signal-to-noise ratio (S/N) of at least 3; while LOQ was the lowest concentration with a S/N of at least 10. In general, LODs were ranging from 1 to 50 pg/mg while LOQs were ranging from 1 to 100 pg/mg.

Analytes	Ion mode	Precursor [m/z]	Fragmentor energy (V)	Collision energy (V)	Product ion	LOD (pg/mg)	LOQ (pg/mg)
Benylpiperazine	positive	177.1	100	21	91	5	50
			100	49	65		
Benylpiperazine-d7	positive	184.2	100	25	98		
			100	53	70		
Secobarbital	positive	237.12	100	57	149.5	20	100
			100	33	119.8		
Secobarbital-d5	positive	242.15	100	33	199.1		
			100	70	240.2		
Nicotine	positive	163.1	100	21	130	20	100
			100	29	117		
Nicotine-d4	positive	167.1	100	13	136.1		
			100	21	134.1		
Cotinine	positive	177.1	150	21	98	20	100
			150	25	80		
Cotinine-d3	positive	180.1	100	21	101		
			100	25	80		
Midazolam	positive	326.1	100	25	291.1	1	1
			100	41	249.1		
Midazolam-d4	positive	330.1	100	25	295.2		
			100	41	253.1		
Triazolam	positive	343.1	100	29	315	1	1
			100	25	308.1		
Triazolam-d4	positive	347.1	100	29	319.1		
			100	25	312.1		
Zopiclone	positive	389.1	100	13	245	5	50
			100	33	217		
Zopiclone-d4	positive	393.1	100	5	349.2		
			100	13	245.1		
Nimetazepam	positive	296.11	100	21	250.1	50	50
			100	33	221.1		
JWH-018	positive	342.2	150	49	127.0	1	1
			150	21	155.0		

Table 3. spectrometric conditions and sensitivity (LOD and LOQ)

3. To provide free testing service to schools and NGOs

There were a total of 14 units including NGOs, rehabilitation units and private clinics used our hair test service since 2012 (Table 4). A total of 1771 samples were tested for the accredited scopes covering 16 analytes of 10 commonly abused drugs and their metabolites (cocaine, norcocaine, benzoylecgonine, cocaethylene, morphine, 6-Acetylmorphine, codeine, methadone, amphetamine, methamphetamine, MDMA, MDEA, MDA, phencyclidine, ketamine and norketamine). 651 HOKLAS-endorsed test reports were issued since accreditation was granted in May 2014 and 12198 test items were performed. Three clients were from private clinics.

Name	Number of samples
NGOs and rehabilitation units	
1. Caritas Wong Yiu Nam Centre 明愛黃耀南中心	539
2. ELCHK Enlighten Centre 基督教香港信義會天朗中心	880
3. Hong Kong Christian Service Jockey Club Lodge of Rising Sun 香港基督教服務處賽馬會日出山莊	2
4. Caritas Lok Heep Club (Kowloon Centre) 明愛樂協會(九龍中心)	200
5. Caritas Lok Heep Club (Hong Kong Centre) 明愛樂協會(香港中心)	
6. HKFYG Sai Kung & Wong Tai Sin Outreaching Social Work Team 香港青年協會西貢及黃大仙外展社會工作隊	20
7. Barnabas Charitable Service Association Limited 基督教巴拿巴愛心服務團	5
8. The Hong Kong Federation of Youth Groups 香港青年協會	33
9. Hong Kong Sheng Kung Hui Welfare Council Neo-Horizon 香港聖公會福利協會新念坊	13
10. The Christian Home of Faith & Grace 基督教恩信之家	14
11. Society for Community Organization 香港社區組織協會	47
Private Clinics:	
1. Quality Healthcare Medical Services 卓健醫療服務有限公司 2. Dr. Cheung Kin Leung Ben 張建良醫生 3. Smile & Face Medical Centre	18
Total	**1771**

Table 4. Sources of hair sample

4. To provide tailor-made support to schools and NGOs

Quota for urgent test report

A total of 41 HOKLAS-endorsed test reports have been issued for urgent cases within 3-5 working days.

A tailor-made service of the drug testing

We provided segmental analysis that hair could be analyzed with the desired hair section which indicates the particular detection period. For example, the subject (Sample no. 19667) who was over 30 years-old female and self-reported with methamphetamine and ketamine use over 5 years. Assuming the average growth rate is approximately at 1-cm per month, results indicated that the concentration of ketamine was reducing in the past 12 months; however, the methamphetamine showed an increasing trend. Segmental analysis provided supporting information in the historical use of drug.

Sample no.: 19667					
Period (month)	Length (cm)	[Ketamine] (pg/mg)	[Norketamine] (pg/mg)	[Amphetamine] (pg/mg)	[Methamphetamine] (pg/mg)
0-3	0-3	3486.9	1098.8	1151.9	11028.2
3-6	3-6	7880.2	1697.3	797.9	8142.5
6-9	6-9	13346.0	1626.8	517.3	5901.3
9-12	9-12	14912.6	1312.8	317.9	3562.5

Table 5. Example of segmental analysis

5. To determine the cut-off value for ketamine detection in hair

Hair ketamine cut-off value

The presence of metabolite is a proof of drug abuse and distinguishing that from passive exposure. Norketamine is the major metabolite of ketamine. Ketamine is being biotransformed to norketamine by N-demethylation in liver. The detection of ketamine and its metabolite norketamine, and 977 ketamine-positive hair samples were identified out of total 1371 samples during 2012–2014. Based on 997 ketamine-positive samples, we have proposed the cut-off value for hair ketamine. The proposed cut-off value is the concentration of hair ketamine when over 90% of samples are being detected with the presence of norketamine, which is a proof of ketamine abuse. Our results showed that there were about 90% of ketamine samples containing norketamine, giving that the cut-off value of 400 pg/mg was assigned. In other words, 10% of ketamine samples not containing norketamine were defined as negative or non-ketamine users. These subjects might be considered as ‘‘not detected’’ at this assigned cut-off value. Article entitled as “Determination of hair ketamine cut-off value from Hong Kong ketamine users by LC-MS/MS analysis.” has been published on 21 Dec 2015 in Forensic Science International (259:53-58). (Appendix)

Survey Data Analysis

Survey was conducted on the drug taking habit of drug addicts, especially in the cases involving the use of ketamine. Different kinds of information such as gender, age, types of drug addicted, frequency and dosage of consumption and drug types detected were collected from the tested samples during Jan 2012 – Mar 2016.

Subject information

Among 1771 hair samples, 1543 survey forms were returned. Over two-third of subjects were male. Over half of subjects were in the age group of 22 to 30 (61.4%). Age above 30 was 20% whereas about 18.6% subjects were below 21 (Table 6).

	Number	**%**
Gender		
Male	1188	77.2
Female	351	22.8
subtotal	**1539**	
Not provided	232	
Total	1771	
Age group		
below 18	90	5.9
18 to 21	193	12.7
22 to 30	933	61.4
30 above	304	20.0
subtotal	**1520**	
Not provided	251	
Total	**1771**	

Table 6. Subject information

Drug types

Subjects self-reported their drug use history (time was not specified). Among 1771 subjects, over 80% of subject claimed as ketamine abusers. About 40% of subjects reported taking cocaine and 26.5% of subjects were methamphetamine users. Others substances included heroin, Ecstasy, cannabis, cough medicine and other substances (Table 7).

Drug types	**Yes**		**No**		**Subtotal**	**Not provided**
	Number	**%**	**Number**	**%**	**Number**	**Number**
Heroin	70	4.8	1374	95.2	1444	327
Methamphetamine	387	26.5	1072	73.5	1459	312
MDMA	259	17.8	1200	82.2	1459	312
Cocaine	600	40.8	872	59.2	1472	299
Cannabis	359	24.6	1098	75.4	1457	314
Cough medicine	147	10.1	1305	89.9	1452	319
Ketamine	1234	81.6	279	18.4	1513	258
Others	95	6.6	872	60.3	1446	319

Table 7. Self-report substance use

Ketamine use behavior

Among 1234 reported ketamine users, over half users (62.7%) reported having ketamine use history of over 5 years. About 14.4% of subjects reported with 3 to 4 years of ketamine use history whereas about 22.8% were new users (within 2 years) (Table 8). In terms of the ketamine use frequency, among 1234 ketamine users, 51% of them used ketamine with 23 to 30 days per month. About 23.9% of ketamine users took drug from 1 to 7 days per month (Table 9). There was insufficient information of the ketamine dose intake by the users since the "units" of amount reported by the users were not consistent.

Ketamine use history, year(s)	**Number**	**%**
below 1 year	126	11.2
1 to 2 years	130	11.6
3 to 4 years	162	14.4
over 5 years	704	62.7
subtotal	**1122**	
Not provided	112	
Total	**1234**	

Table 8. Ketamine use history

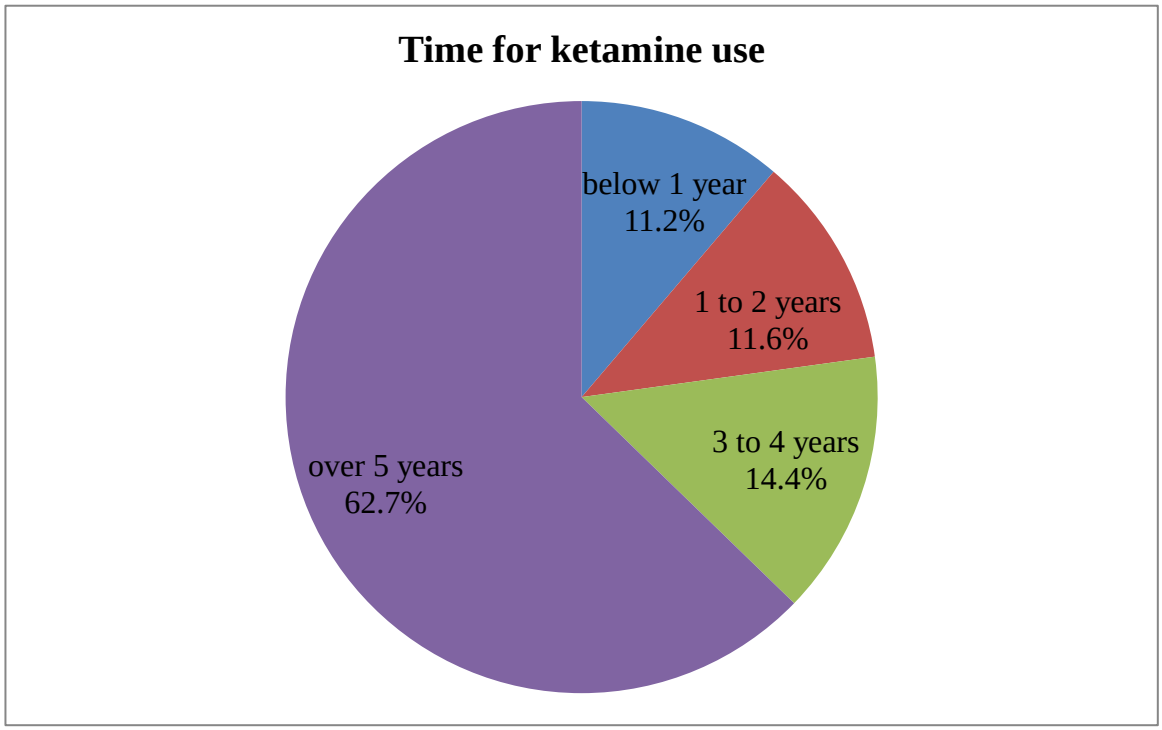

Figure 1. Ketamine use history

Monthly ketamine use frequency, day(s)	Number	%
Less than 1	1	0.1
1 to 7	257	23.9
8 to 15	149	13.8
16 to 22	121	11.2
23 to 30	549	51.0
subtotal	**1077**	
Not provided	157	
Total	**1234**	

Table 9. Monthly ketamine use frequency.

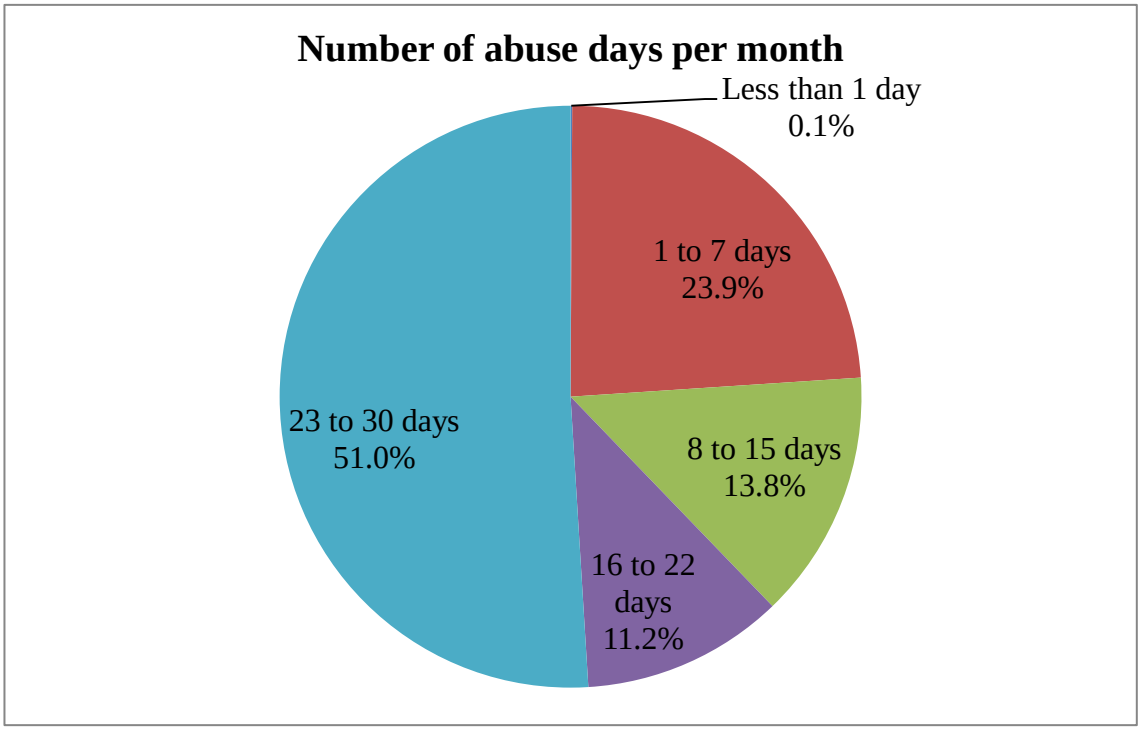

Figure 2. Monthly ketamine use frequency.

Ketamine dependence

About 67.6% of ketamine users (750 out of 1110) reported with drug dependence (psychological or physiological) (Table 10, Figure 3).

Ketamine dependence (psychological or physiological)	**Number**	**%**
Yes	750	67.6
No	360	32.4
subtotal	**1110**	
Not provided	124	
Total	**1234**	

Table 10. Ketamine dependence of drug users

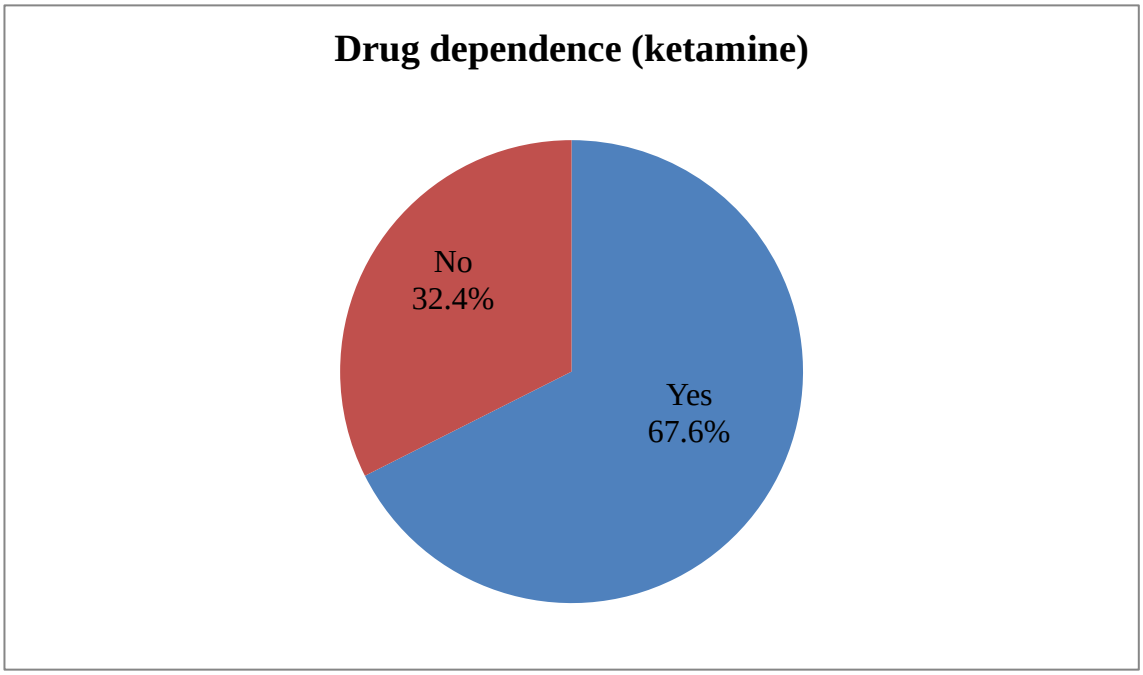

Figure 3. Ketamine dependence of drug users

Drugs detected in hair samples

16 analytes of 10 commonly abused drugs and their metabolites (cocaine, norcocaine, benzoylecgonine, cocaethylene, morphine, 6-Acetylmorphine (heroin's metabolite), codeine, methadone, amphetamine, methamphetamine, MDMA, MDEA, MDA, phencyclidine, ketamine and norketamine) were analysed in 1771 hair samples. Samples detected above the assigned cut-off value will be identified as drug positive. Except for ketamine and methadone, the cut-off concentrations followed the Mandatory Guidelines for Federal Workplace Drug Testing Programs published by the Substance Abuse and Mental Health Services Administration (SAMHSA) [9]. Since there were no cut-off values available for ketamine and methadone, the cut-off value for ketamine was assigned by our proposed cut-off at 400 pg/mg. The cut-off value for methadone followed the cut-off value of opiates such as morphine, 6-Acetylmorphine and codeine at 200 pg/mg (Table 11). 689 samples were not detected with the above drugs while 1082 samples were drug positive which detected with the drugs in the test panel.

Among all the tested samples including drug negative samples (1771 samples), 48.9% of samples detected with ketamine. Cocaine was the second frequently detected drug which was about 13%. The third frequently detected drug was methamphetamine which was about 10%. Codeine and amphetamine were about 4%. Opiates including morphine, 6-acetylmorphine and methadone were less than 1%. If the tested samples detected any drug(s) above the corresponding cut-off values were considered as drug positive, there were 1082 drug positive samples among 1771 tested samples. Ketamine, cocaine and methamphetamine were about 80%, 21.3% and 16.5% respectively. It indicated that the major source of sample was ketamine users (Table 11).

When the self-reported drug types by the abusers compared to the drug types identified by our test service, there were significant discrepancy observed. The numbers of drug positive samples (above the reference cut-off or proposed in-house cut-off) were far less than those from self-report (Table 12). In case of ketamine, the self-reported number of ketamine was 1234 but only 726 samples were identified as positive. In those ketamine-negative samples (508), about 86.2% (438) were not detected with any drug from our list. Another example, the self-reported number of methamphetamine users was 387 but only 116 samples were identified as positive. About 31.7% of methamphetamine-negative samples were detected with other drugs. Moreover, only 1 case was identified with MDMA from a total of 259 self-reported cases; however, over half of MDMA-negative samples (53.1%) were detected with other drugs (Table 12). Such discrepancies with low identification rate could be explained by the following situations. First, the testing period cannot reflect the self-reported drug use history over a long period. Our hair test service measures the pass 3-month period but the subject might report drug use habit

beyond the testing windows or over the year. Second, subjects had started drug withdrawal program. Since most of the subjects were from NGOs and rehabilitation units, these subjects had started the treatment program during the residential period (e.g. Caritas Lok Heep Club Wong Yiu Nam Centre). If the treatment period covers the whole detection period, the drug residues may not be detected in the hair. Third, abused drug may mix with other toxic substances. Taking Ecstasy as an example, the main ingredient of Ecstasy pills is MDMA. However, some Ecstasy pills on the market were not pure MDMA but mixing a wide variety of drugs or other toxic substances and stimulants including ketamine and amphetamine [10], some even contains no MDMA at all. The subjects believed that they were taking Ecstasy pills which were pure MDMA. This may contribute for the low identification rate for MDMA (0.4%). Forth, the testing analyte of our service is just one of the possible ingredients in the abused substance. In case of cough medicine, codeine was the main active ingredient in the traditional cough medicine. Over the years, codeine had been replaced with dextromethorphan as cough suppressant. Our test service only covers codeine as the target analyte, therefore, it accounts for the discrepancy in the self-reported number and the identified drug positive number. Fifth, the cut-off values assigned in the proposal may be only applicable to regular and frequent abusers but not to the occasional users. These occasional users with low drug taking frequency and dosage may escape from the assigned cut-off values.

In contrast, some cases were detected drug positive in those claimed not taking the drugs (Table 13). The detection rate for drug users claiming not take the drugs ranged from 0.3% for MDMA to 5.7% for ketamine. The reason for the low MDMA detection rate for and relatively high for ketamine in those users claiming not taking the drug might be explained by the fact that ketamine and the other stimulants were added into the Ecstasy pills to replace MDMA. Therefore, the Ecstasy pill is not always MDMA. Other substances may be mixed in the pills for the persistent hallucination effect.

Drug Type	Cut-off (pg/mg)	Number	%	%
Ketamine	400	866	48.9	80.0
Cocaine*	500	231	13.0	21.3
Methamphetamine*	300	178	10.1	16.5
Amphetamine*	300	85	4.8	7.9
Codeine*	200	74	4.2	6.8
Morphine*	200	12	0.7	1.1
6-Acetylmorphine*	200	12	0.7	1.1
Methadone	200	10	0.6	0.9
MDMA*	300	6	0.3	0.6
PCP*	300	0	0.0	0.0
* cut-off from SAMHSA		Total	All samples (1771)	Drug positive (1082)

Table 11. Drug types detected in hair samples

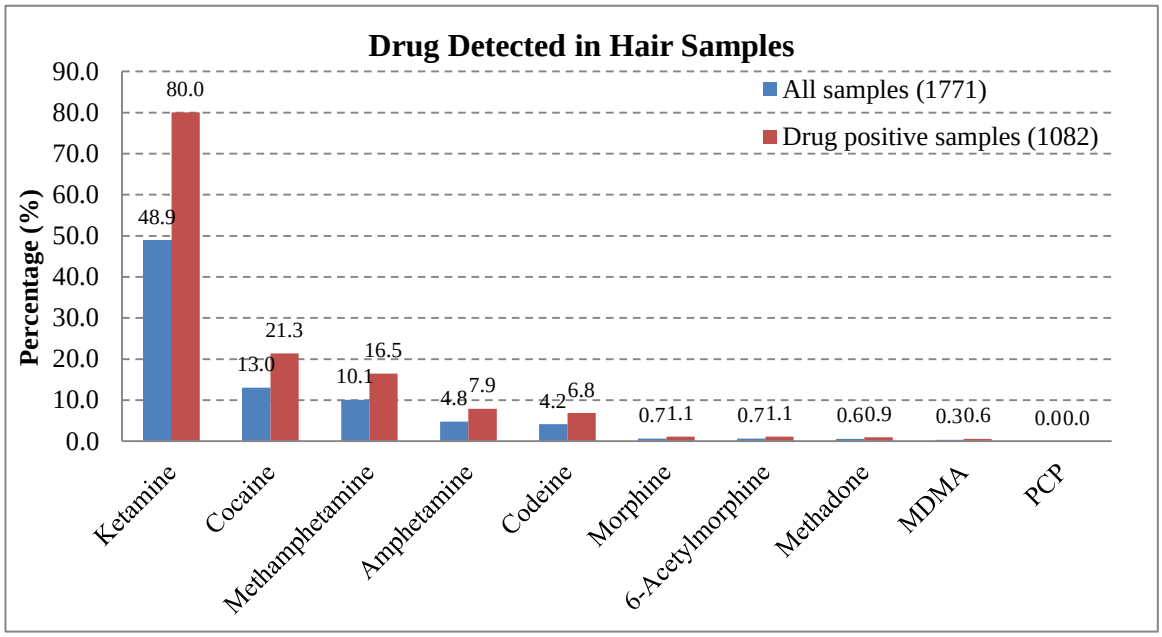

Figure 4. Drug types detected in hair samples.

Drug types	Self-reported number	Number of drug positive*	Identification rate (%)	Number of drug negative	
				Other drugs identified	No drugs identified
Ketamine	1234	726	58.8	70 (13.8%)	438 (86.2%)
Cough medicine	147	46	31.3	32 (31.7%)	69 (68.3%)
Methamphetamine	387	116	30.0	86 (31.7%)	185 (68.3%)
Cocaine	600	151	25.2	212 (47.2%)	237 (52.8%)
Heroin	70	11	15.7	31 (52.5%)	28 (47.5%)
MDMA	259	1	0.4	137 (53.1%)	121 (46.9%)
Cannabis	359	Not tested	N.A	N.A	N.A
Others	95	Not tested	N.A	N.A	N.A

* detected drug concentration above the cut-off shown in Table 11

Table 12. Identification rate from self-reported abusers

Drug types	Claimed not taking the specific drug in self-report (Number)	Above cut-off value* (Number)	Detection rate (%)
Ketamine	279	16	5.7
Methamphetamine	1072	37	3.5
Cocaine	872	30	3.4
Heroin	1374	11	0.8
Cough medicine	1305	18	1.4
MDMA	1200	3	0.3

* detected drug concentration above the cut-off shown in Table 11

Table 13. Identification rate from self-reported abusers claiming not taking the drugs

Conclusion

With the support of Beat Drug Fund Association, we have optimized our hair drug test service platform and provided free accredited hair drug test services for the rehabilitation units. We have built the communication and logistics in the drug test service to our clients with satisfactory results. Our proposed cut-off hair ketamine value could act as a useful reference value with international acceptability.

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