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Evaluation of Five Commercial Amphetamines and Opiates Immunoassay Test Kits in Taiwan

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ABSTRACT

Five commonly used commercial amphetamines (AMs) and opiates (OPs) immunoassay test kits: HYCOR accuPINCH™, Drug Screening System (DSS) MACH TV® Screen™, Princeton BioMeditech (PBM) Biosign™ and International Diagnostic Systems I.D. Block™ were evaluated for their cutoff verification and assay performance with standardized and confirmed urine specimens. Cutoff verification was evaluated at several cutoffs for commercial AMs and OPs test kits. The assay performance was evaluated with authentic urine specimens confirmed by GC/MS. Results indicated that the real cutoffs for most AMs or OPs test kits were lower than those reported. accuPINCH™, AbuSign™ and I.D.Block™ were better than MACH IV® Screen™ and ONTRAK ™ for AMs test kits; accuPINCH™, AbuSign™, I.D.Block™ and ONTRAK™ were better than MACH IV® Screen™ for OPs test kits.

Key words : Methamphetamine, Morphine, Immunoassay, Test kits

INTRODUCTION

Cases of drug abuse with methamphetamine(MA) and heroin have vastly increased in Taiwan area since 1991[1]. MA is a synthetic derivative of ephedrine and other phenethylamines and is a potent central nervous system stimulant. Heroin is an opiate which is a synthetic derivative of morphine(MP) that extracted from the seed pod of the opium poppy. Heroin and MA have been banned since 1956 and 1990 respectively in Taiwan, R.O.C. because of their toxicity and addiction potential.

Analytical methods for urine drug screening include high performance liquid chromatography (HPLC), gas chromatography(GC), thin-layer chromatography(TLC) and immunoassays[2-3]. Most screening procedures except immunoassays require sophisticated instrumentation and are labor-intensive. An immunoassay screening test kit for the detection of drug abuse provides a handy, rapid method requiring no sophisticated instrumentation; such a kit is commonly used as the initial test in urine drug testing.

The Mandatory Guidelines for Federal Workplace Drug Testing[4] recommended by the Department of Health and Human Service (DHHS) of U.S.A. regulate a initial test which requires the use of an immunoassay to eliminate negative urine specimens from further consideration, and a confirmatory test which requires the use of Gas Chromatography/Mass Spectrometry (GC/MS) methods if a specimen was identified as positive on the initial test. Each of the tests mentioned above has their threshold levels(cut-

offs). Various test kits meet the regulations of DHHS guidelines. They must reliably differentiate specimens at or above specific threshold levels from specimens containing durgs below the cutoff concentrations. For OPs testing, the immunoassay must detect specimens containing opiate metabolites at or above 300ng/ml. For AMs testing, the immunoassay must detect specimens containing AMs at or above 1000ng/ml. Presumptive positive specimens must be further confirmed by GC/MS for the presence of OPs or AMs.

The urine drug testing for arrestees in the Taiwan area follows DHHS guidelines. More than five commercial kits of AMs and OPs are available and used in initial test of urine samples in the Taiwan area. Assessment of the validity of those kits is of particular importance because of the specific characteristics of the immunoassay antibody and the number of commercial kits available. Different commercial kits have differnt cutoffs, and different lots may lead to differences in the same kit when antibody lots are changed. Because of the dynamic nature of immunoassay kits, evaluation of kits needs to be performed periodically. David and John[5] have evaluated the screening for Drugs of Abuse with Roche ONTRAK test kits, and G. Anderson et. al.[6] have evaluated the accuPINCH kits. Some literature which has evaluated commonly used preliminary methods[7-15] focused mainly on one drug or immunoassays for automatic analysis only. In this context cutoff verification and assay performance have been evaluated for five common commercial test kits with standardized urine samples and confirmed urine samples. The cutoff verificaton for kits was determined by testing duplicates of a sequence of cutoff concentrations for those kits simultaneously. For assay performance assessment, forty confirmed urine samples were tested and compared with the results obtained by using GC/MS.

MATERIALS AND METHODS

I. *Materials*

Drugs used in testing were obtained from the following sources: Methamphetamine Hydrochloride, (Sigma Chemical Co.), Morphine Hydrochloride (U.S.P.). All standards were Reagent Grade.

II. *Immunoassay Kits*

HYCOR accuPINCH™ Mehtamphetamine Test and Opiates Test were obtained from Miles -Sankyo Co., Ltd. (Hycor Biomedical Inc.), Abuscreen® ONTRAK™ for Amphetamine and for Morphine were obtained from Roche Products Ltd. (Roche Diagnostics), MACH TV® Screen™ For Methamphetamine Metabolites and For Opiates Metabolites were obtained from Hanson Biomedical Co. (Drug Screening Systems, Inc.), Methamphetamine and Morphine /Opiates I.D. Block™ Detection kit were obtained from Hope Wang Enterprises Co. Ltd. (International Diagnostic Systems), BioSign™ MET one Step Methamphetamine Test and AbuSign™ MOP Opiate/Morphine Test were obtained from Charng Ching Health Diagnostic Co. (Princeton BioMeditech)[16-23].

III. *Specimens*

Lyphochek Urine Toxicology Control Screen was obtained from BIO-RAD Laboratories. Authentic urine samples of arrestees were obtained from local police stations in the Taiwan area.

IV. *Kit Characteristics*

Manufacturer's stated sample volume, quality controls, kit/reagents storage conditions, operation time for every kit were evaluated.

V. *Cutoff Verification*

The stated cutoff values of AMs and OPs for five common commercial test kits are listed in Table 1. The cutoffs for BioSign™ and MACH IV® Screen™ MA test kit are lower

Table 1. Manufacturer's stated cutoff(ng/ml) of AMs and OPs for five commercial immunoassay kits available in Taiwan

kit	AMs	OPs
DHHS	AMs 1000	Ops 300
accuPINCH	MA 500	OPs 300
MACH IV	MA 1000	OPs 300
BioSign	MA 500	OPs/MP 300 (Abusign)
ONTRAK	AMs 1000	MP 30
I.D.Block	MA 1000	MP 300

*MA : Methamphetamine
AMs : Amphetamines
OPs : Opiates
MP : Morphine

than those required by the DHHS for AMs. A sequence of spiked and multiconstituent controls were duplicately tested for AMs and OPs respectively because of various cutoff values. The concentrations (ng/ml) of MA were prepared as 1200, 1000, 500, 300, 100 and blank respectively. The concentrations (ng/ml) of MP were prepared as 360, 300, 100 and blank respectively.

VI. *Assay Performance*

The assay performance for AMs and OPs test kits evaluated against 40 authentic urine specimens collected from local police stations and confirmed to be MA or MP positive by GC /MS. Definition of terms used were:

TP=True Positives
=positive screening results found to be positive by GC/MS.
TN=True Negatives
=negative screening results found to be negative by GC/MS.
FP=False Positives
=positive screening results found to be negative by GC/MS.
FN=False Negatives
=negative screening results found to be positive by GC/MS.
%Predictive Value of Positive Results
=TP/(TP+FP)×100
%Predictive Value of Negative Results
=TN/(TN+FN)×100
%Accuracy
=(TP+TN)/(TP+TN+FP+FN)

RESULTS

Kit Characteristics.

Maunfacturer's specifications of sample volume, quality controls, kit/reagents storage conditions, estimated operation time for five common commercial kits are summarized in Table 2. Most test kits should be refregerated prior to testing except Biosign, and all kits should be used for testing at room temperature. Test kits should be taken out from the refregerator before testing and standed until they reach room temperature; this might mean a 30-to-45 minute wait prior to use. The expiration periods for the test kits are from 6 to 12 months after manufacture. The ONTRAK™ test kit used only 11 μl urine and the least amount of sample volume. The AbuSign™ and MACH IV® Screen™ don't have any quality control urines; ONTRAK™ have negative control urine; and accuPINCH™ useds positive control urine only. Testing with all test kits can be finished within 3 to 15 minutes.

Cutoff Verification.

The cutoff verification results are listed in Table 3(for AMs) and Table 4 (for OPs). The results for test kits of AMs indicated that most kits were positive at and above MA 1000 ng/ml except the ONTRAK™. The accuPINCH™ and I.D.Block™ were still positive at 300ng/ml, and all kits still showed presumptive positive at 100 ng/ml except ONTRAK™. The results of OPs

Table 2. Operation characteristics of kits

Item	ONTRAK	I.D.Block	MACH IV	AbuSign	accuPINCH
Storage Condition	2-8°C	2-8°C	2-8°C	R.T.*	2-8°C
Operation Temperature	R.T. (17-29°C)	R.T. (23-29°C)	R.T. (<27°C)	R.T.	R.T.
Sample Volume	11 μl	100 μl	5 drops	4 drops	Specimen Pipette
Negative Control (when)	Yes (Per batch)	Yes (Per batch)	—	—	—
Positive Control (When)	—	—	—	—	Yes (Use new reagent)
Operation Time(Min)	3-5	5-10	5-10	5-10	10-15

*R.T. : Room Temperature

— : Not available

Table 3. Cutoff verification for AMs test kits

Kit	Concentration (ng/ml)						Stated Cutoff
	1200	1000	500	300	100	0	(ng/ml)
accuPINCH	+	+	+	+	±	−	MA* 500
MACH IV	+	+	±	±	±	−	MA 1000
BioSign	+	+	±	±	±	−	MA 500
ONTRAK	−	−	−	−	−	−	AMs** 1000
I.D.Block	+	+	+	+	+	−	MA 1000

*MA : Methamphetamine

**AMs : Amphetamines

+ : Response greater than or equal to kit's cutoff.

− : Response less than kit's cutoff.

± : Response near kit's cutoff.

indicated that all test kits were positive at and above morphine 300ng/ml. All test kits were still positive at morphine 100ng/ml except the ONTRAK™.

Assay Performance.

Test results are listed in Table 5(for AMs) and Table 6 (for OPs), and the assay performance results are listed in Table 7 (for AMs) and Table 8(for OPs). Forty urine specimens were tested for AMs and OPs respectively. A total of 34 urine samples of AMs gave positive results by Emit® d.a.u.™ and one sample was confirmed by GC/MS as false positive; a total of 33 urine samples of OPs gave positive results by Emit® d.a.u.™ and 6 samples were confirmed by GC/MS as false positive. The accuPINCH™,

Table 4. Cutoff (ng/ml) verification for OPs test kits

Kit	Concentration (ng/ml)				Stated Cutoff
	360	300	100	0	
accuPINCH	+	+	+	−	OPs* 300
MACH IV	+	+	+	−	OPs 300
AbuSign	+	+	+	−	OPs/MP** 300
ONTRAK	+	+	−	−	MP 300
I.D.Block	+	+	+	−	MP 300

*OPs : Opiates

**MP : Morphine

+ : Response greater than equal to kit's cutoff.

− : Response less than kit's cutoff.

BioSign™ and I.D.Block™ agreed in all cases with Emit® d.a.u.™ for AMs testing and for OPs testing. There were 33 positives, 6 negatives and 1 false positive for AMs testing, and 27 positives, 7 negatives and 6 false positives for OPs testing. The MACH IV® Screen™ gave 6 false positives from 27 positives for OPs testing. The ONTRAK™ gave 11 false negatives for AMs testing, but there were no false positive for AMs testing and fewer positives than other test kits for OPs testing.

For AMs testing, the results indicated that the predictive value of positive results for ONTRAK™ was 100%; for accuPINCH™, BioSign™, and I.D.Block™ was 97%; and for MACH IV® Screen™ was 85%. The predictive value of negative results for all test kits was 100% exept for ONTRAK™ (39%). Therefore, the accuracy for all AMs test kits were 98% except for ONTRAK™ and MACH IV® Screen™.

For OPs testing, the results indicated that the predictive value of positive results for ONTRAK™ was 84%; for accPINCH™, BioSign™, and I.D.Block™ was 82%; and for MACH IV® Screen™ was 79%. The predictive value of negative results for all test kits except MACH IV® Screen™ (64%) was 100%. Therefore, the accuracy for ONTRAK™ test kit was 88%; for accuPINCH™, BioSign™ and I.D.Block™ was 85%; and for MACH IV® Screen™ was 75%.

DISCUSSION

In the present study, the cutoff verification and accuracy of five commonly used commercial immuoassay test kits(accuPINCH™, MACH IV® Screen™, BioSign™, ONTRAK™ and I.D.Block™) for AMs or OPs in urine were evaluated with stadardized urine containing MA and MP. Each test kit except ONTRAK™ demonstrated the repuired sensitivity for detection of MP or MA at levels substantially below those mandated by DHHS guidelines. The ONTRAK™ exhibited the required sensitivity for detection of MP at prescribed cutoffs, but did not meet the required sensitivity for detection of MA at 1000 ng/ml. According to the newly amended DHHS guidelines[24], a specimen reported as positive for only MA in the amphetamine class of drugs must also contains the metabolite amphetamine at a concentration equal to or greater than 200 ng/ml. Therefore, it is inappropriate for taking ONTRAK™ to detect AM only in the initial test for MA addicts.

The accuracy of accuPINCH™, BioSign™, and I.D.Block™ MA test kitys was much better than that of ONTRAK™ and MACH IV®

Table 5. Test results of AMs test kits

Test result	+ (TP)	− (FN)	+ (FP)	− (TN)
accuPINCH	33	0	1	6
MACH IV	33	0	6	1
BioSign	33	0	1	6
ONTRAK	22	11	0	7
I.D.Block	33	0	1	6
Emit d.a.u.	33	0	1	6
GC/MS	33	0	0	7
		(Positive)	(Negative)	

Table 6. Test results of OPs test kits

Test result	+ (TP)	− (FN)	+ (FP)	− (TN)
accuPINCH	27	0	6	7
MACH IV	23	4	6	7
AbuSign	27	0	6	7
ONTRAK	27	0	5	8
I.D.Block	27	0	6	7
Emit d.a.u.	27	0	6	7
GC/MS	27	0	0	13
		(Positive)	(Negative)	

Table 7. Assay performance for AMs kits

Item	accuPINCH	MACH IV	BioSign	ONTRAK	I.D.Block
Predictive Vlaue of Positive Results (%)	97	85	97	100	97
Predictive Value of Negative Results (%)	100	100	100	39	100
Accuracy (%)	98	85	98	72	98

Table 8. Aassay performance of OPs kits

Item	accuPINCH	MACH IV	BioSign	ONTRAK	I.D.Block
Predictive Vlaue of Positive Results (%)	82	79	82	84	82
Predictive Value of Negative Results (%)	100	64	100	100	100
Accuracy (%)	85	75	85	88	85

Screen™; and the accuracy of accuPINCH™, BioSign™, ONTRAK™ and I.D.Block™ of OPs test kits were better than that of MACH IV® Screen™. For MA assay, the ONTRAK™ was found to have the lowest degree of predictive value of negative results and thus the lowest accuracy. For OPs assay, the MACH IV® Screen™ was found to have the lowest degree of predictive value of negative results and positive results and thus the lowest accuracy, but the ONTRAK ™ test kit was found to have the highest degree of predictive value of positive results and thus the highest accuracy. Because more false positive samples were evaluated, the OPs test kits had lower specifcity and lower accuracy than AMs test kits.

This study demonstrated that, in addition to ONTRAK™, four commonly used commercial immunoassay test kits for AMs and OPs exhibited the required sensitivity for MP or MA detection at cutoffs as prescribed by DHHS guidelines. THE MACH IV® Screen™ test kit was less accurate than the other three kits. Because of the changing nature of commercial immunoassay kits, periodic evaluation of assay characteristics is needed for appropriate interpretation of testing results.

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市售尿液中安非他命類及鴉片類藥物簡易檢測套組之評估

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摘　要

本研究係針對常用之市售尿液中安非他命類及鴉片類藥物免疫學分析套組，如accuPINCH™, ONTRAK™, MACH IV®, I.D. Block™ 及 BioSign™五種套組進行精密度及準確度之評估，以期選出適用之套組，供作例行尿液檢體分析的初步篩選試驗方法。

精密度之評估係以一系列不同之甲基安非他命或嗎啡濃度分別進行測試，測試結果顯示，甲基安非他命部分除ONTRAK™於濃度1200 ng/ml時無法檢出外，其餘所有套組之最低檢出量均低於其Cutoff值。準確度之評估則以經GC/MS確認之尿液檢體進行測試，測試結果顯示，甲基安非他命部分中除MACH IV®及ONTRAK™較差外，其餘套組尚可，而嗎啡部分中除MACH IV®較差外，其餘套組尚可。

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