15/0180/44

Evaluation of Methamphetamine Immunoassay Rapid Test Kits Used in Thailand

การประเมินชุดน้ำยาตรวจยาบ้าด้วยหลักการอิมมูโนแบบรวดเร็วที่ใช้ในประเทศไทย

Veeravan Lekskulchai[1] Chintana Mokkhavesa[2] Narumol Junvimaluang[2] Anoot Keawpoompuang[2]

บทคัดย่อ : วีรวรรณ เล็กสกุลไชย[1] จินตนา โมกขะเวส[2] นฤมล จันทร์วิเมลือง[2] และ อานุช เขียวพุ่มพวง[2]. 2544. การประเมินชุดน้ำยาตรวจยาบ้าด้วยหลักการอิมมูโนแบบรวดเร็วที่ใช้ในประเทศไทย. วารสารวิจัยวิทยาศาสตร์การแพทย์ 15(1) : 1-12.

ชุดตรวจยาบ้าด้วยหลักการอิมมูโนแบบรวดเร็วสองชนิดได้รับการประเมิน ชนิดแรกคือ PointeStep Meth-amphetamine test เป็นผลิตภัณฑ์นำเข้าจากประเทศสหรัฐอเมริกา ชนิดที่สองคือ Bioline Methamphetamine test ซึ่งผลิตภายในประเทศจึงมีราคาถูกกว่า Bioline มีขายทั้งแบบเป็นแผ่นทดสอบและแถบทดสอบ ชนิดที่เป็นแถบทดสอบไม่ต้องมีการถ่ายตัวอย่างจากภาชนะที่บรรจุ อย่างไรก็ดี ผลที่ได้อาจผิดพลาด หากจุ่มแถบทดสอบจนระดับปัสสาวะเกินขีดที่กำหนดบนแถบ ถึงแม้ว่า PointeStep จะมีราคาแพง แต่มีความไวต่อปริมาณเมทแอมเฟตามีนสูงกว่า และให้ผลการตรวจที่ชัดเจน อ่านง่ายกว่า ระดับตัดสินผลบวก-ลบ ของ PointeStep และ Bioline คือ 500 และ 1,000 นาโนกรัม/มิลลิลิตร ตามลำดับ แต่ Bioline ให้ผลไม่ชัดเจนที่ระดับเมทแอมเฟตามีน 1,000-5,000 นาโนกรัม/มิลลิลิตร นอกจากนี้ PointeStep ยังมีความไวต่อสารกลุ่มแอมเฟตามีนตัวอื่นสูงกว่า Bioline ได้แก่ ethylamphetamine, MDMA และ MDEA ทำให้ PointeStep สามารถใช้เป็นชุดตรวจกลุ่มยาบ้าและยาอนุพันธ์ของยาบ้าที่ต้องสงสัยได้พร้อมกัน การวิจัยนี้ยังสนับสนุนแนวคิดที่ว่า น้ำยาตรวจทางอิมมูโนที่มีความจำเพาะต่อเมทแอมเฟตามีน จะให้ผลบวกกับอนุพันธ์ยาบ้าที่เป็น secondary amines ด้วย

คำสำคัญ : เมทแอมเฟตามีน สารกระตุ้นกลุ่มแอมเฟตามีน การวิเคราะห์ทางอิมมูโน ชุดตรวจภาคสนาม

[1]Department of Pathology, Faculty of Medicine, Srinakharinwirot University, Bangkok
ภาควิชาพยาธิวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ กรุงเทพมหานคร
[2]Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok
ภาควิชาพยาธิวิทยา คณะแพทยศาสตร์ โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล กรุงเทพมหานคร

Abstract : Veeravan Lekskulchai, Chintana Mokkhavesa, Narumol Junvimaluang and Anoot Keawpoompuang. 2001. Evaluation of methamphetamine immunoassay rapid test kits used in Thailand. Thai J Hlth Resch 15(1) : 1-12.

Two on-site methamphetamine immunoassay kits were evaluated. PointeStep Drug Screen Meth-amphetamine test was imported from the USA. Bioline Methamphetamine test was manufactured in Thailand. Therefore, it is considerably less expensive than the former one. Bioline is available in slide or card and strip form. The strip seemed to be readily to use, not requiring sample cup. Error occurred when the urine level surpassed the maximum level. Despite the large cost per test differential of these two kits, PointeStep showed several advantages over the Bioline kit. First, the cutoff of PointeStep was 500 ng/ml while the cutoff of Bioline was 1,000 ng/ml, making PointeStep more sensitive to methamphetamine. The Bioline showed ambiguous results, interpreted as borderline, at methamphetamine concentration 1,000 to 5,000 ng/ml. Thus, its sensitivity is still in doubt. Although both test kits showed cross-reactivity with ethylamphetamine, MDMA, and MDEA, PointeStep showed greater cross-reactivity to these analogs and can be used for amphetamine-type stimulants screening when the use of these analogs is suspected. The results from this study confirmed that immunoassays specific to methamphetamine most likely presented cross-reactivity to analogs that are also secondary amines such as MDMA, MDEA, and ethylamphetamine.

Key words : methamphetamine, amphetamine-type stimulants, immunoassay, on-site test kit

---

Introduction

Amphetamine and methamphetamine are sympathomimetic amines that were first clinically used for treatment depression, narcolepsy, and obesity (Prinzmetal and Bloomberg, 1935; Cho, 1990). They became abused drugs because of their stimulant effects including inducing euphoria (feeling of well-being), increasing alertness, and decreasing appetite (Miller, *et al.*, 1989). They are among the most frequency abused drugs world wide probably due to their widespread availability and low cost (Rasmussen, *et al.*, 1989). In Thailand, addiction of "Ya Ba" or amphetamine and methamphetamine is a growing serious problem. They are classified as the narcotics of Category I (The Narcotics Act, B.E. 2522). They were detected in urine of college students, truck drivers, farmers, industrial workers, and athletes (Pidetcha, *et al.*, 1995). Emergence of chemical analogs or "designer drugs" to amphetamine and methamphetamine has complicated the problem. Manufacturers, distributors and users are switching to these drugs in order to avoid the legal actions of controlled substance act. Although the controlled substance act has up-dated to cover these designer drugs, the detection of these drugs is still rarely possible by methods currently available. Thus, they can be used without interference from law enforcement agencies (Cody and Schwarzhoff, 1993).

Several amphetamine analogs have been seen on the illicit market with increasing frequency. Ethylamphetamine is the N-ethyl amine analog of amphetamine. It was widely known as an anorectic agent, Apetinil. Currently, although it was banned from the market and classified as a controlled substance schedule I, it is periodically detected in urine of suspected amphetamine abusers (Nagai, *et al.*, 1997). "Ya E" or 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') and its structural analogs, 3,4-methylenedioxyamphetamine (MDA), and 3,4-methylene dioxyethylamphetamine (MDEA, 'Eve') are ring-substituted analogs of amphetamine. Widespread use of MDMA began in the early 1980s, before it became controlled. MDEA is the newest recreational drug and was just listed as a schedule I controlled substance few years ago. MDA, although a popular drug of abuse itself, is also a metabolite of MDMA and MDEA produced in vivo by the N-dealkylation, in the same manner that methamphetamine undergoes N-demethylation to its metabolite, amphetamine (Hegadoren, *et al.*, 1995). They are also becoming popular drugs in Thailand especially in teenagers.

Although the cross-reactivity toward over-the-counter phenylethylamines is undesirable, cross-reactivity of antibody toward amphetamine analogs is particularly useful for drug screening when and where the positive results for the analogs are expected. Analysis of samples from individuals who have consumed a drug may or may not reveal its presence in urine, depending on the dose administered, interindividual variations in drug metabolism and body clearance, and sample collection time (Armbruster, *et al.*, 1995). Clearly, cases in which a larger dose and/or a

shorter interval between ingestion and subsequent sampling will result in larger concentration of ingested drug presenting in the urine and higher chance of detection. In order to decrease time interval between ingestion and sample collecting and transporting, tests that can detect at the site of sample collection are needed. New assays have been developed that allow visual detection by mean of an immunochemical reaction and, therefore, do not require any instrumentation and can be used by non-laboratory staff outside a traditional laboratory environment. Undoubtedly, this form of on-site testing has a number of advantages over laboratory-based testing, including the rapid availability of results. Such tests are useful for on-site drug abuse screening even when the abusers are still under the influence of the drug. The presence of drug(s) in urine from individuals who have consumed some of drug also depends on the analytical procedures used. Despite the advantages above, the reliability and accuracy of the on-site test kits still need to be investigated before practically used, particularly when the results can lead to legal action. Likewise, the cross-reactivity of each test kit must be known before the interpretation. In this study, the reliability, accuracy, and cross-reactivity toward amphetamine analogs of two on-site methamphetamine immunoassay test kits used in Thailand were investigated. This knowledge will enable drug-testing agencies to choose a suitable on-site amphetamine or methamphetamine kit and to more accurately evaluate results from the assay.

Materials and Methods

Drug free urines were supplied by staff volunteers. Standard drugs; i.e., d-amphetamine, d,l-amphetamine, d-methamphetamine, d,l-methamphetamine, d,l-MDA, d,l-MDMA, d,l-MDEA, and d,l-ethlyamphetamine, were purchased from Radian Corp.(Austin, Texas). Stock (100,000 ng/ml free base equivalent) standards of drugs were prepared in methanol and stored in the freezer. These solutions were diluted in drug free urine to the given concentrations; 50,000, 25,000, 12,500, 10,000, 5,000, 3000, 1,250, 1,000, 750, and 500 ng/ml, respectively. Double analyses were done for each concentration.

PointeStep™ Drug Screen Meth-amphetamine Test (Pointe Scientific, Licon Park, Michigan, USA.) was given by Apex Diagnostic Co., Ltd. (Bangkok, Thailand). It is a slide test, which screens urine qualitatively for the presence of methamphetamine. The test was based on competition for binding antibody between drugs coated on a membrane and drugs in urine specimen. From the manufacturer's insert, the test contains methamphetamine polyclonal antibody that will bind to methamphetamine in the sample. The test pouch of PointeStep was opened, and the test was removed. Five drops of urine were pipetted into the test sample cavity marked with an S (figure 1). According to the manufacturer's instruction, results should be read between 3-10 min. Two color bands in the C and T regions indicate a negative result; one color band in the C region

indicates a positive result. A control band appears in all tests unless the test has failed. The presence of an additional red band in the test area (T); due to binding of non-bound antibody-dye conjugate to the immobilized drug in this area, indicates a negative result. Whilst the absence of this band indicates that methamphetamine is present in the urine at a concentration above the test threshold (500 ng/ml). Each test card is supplied in a sealed packet under dry conditions and should be stored at 4°C to 30°C.

Bioline Methamphetamine Strip (Pacific Biotech Co., Ltd., Bangkok, Thailand) was given by Bangkok Radioimmunoassay Center (Bangkok, Thailand). It was available in both the slide and dip-strip form. An advantage of the dip-strip is that no sample transferring process is needed. In this study, the dip-strip was used. The principle of detection is based on a competitive immunoassay. The membrane is precoated with colloidal gold-conjugated anti-methamphetamine monoclonal antibodies. The end of the strip was placed into the urine in an upright position until the urine was seen to move up through the test area. The colored bands developed in the control (C) and test (T) area (figure 2) over a short period of time. The procedure and interpretation for dipstick are similar to that of PointeStep Meth-amphetamine slide test. Each dipstick and test card is supplied in a sealed packet under dry conditions and should be stored at 4°C to 30°C.

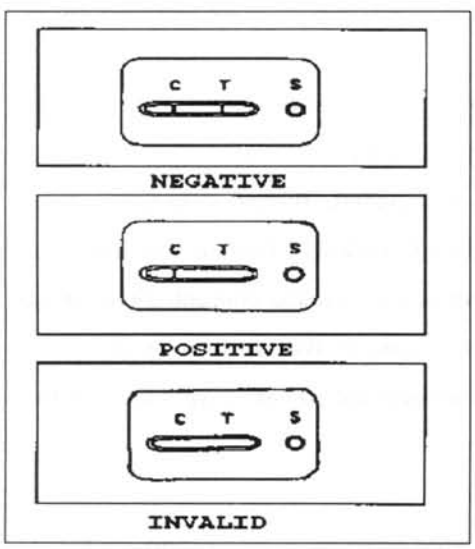


Figure 1 PointeStep slide test

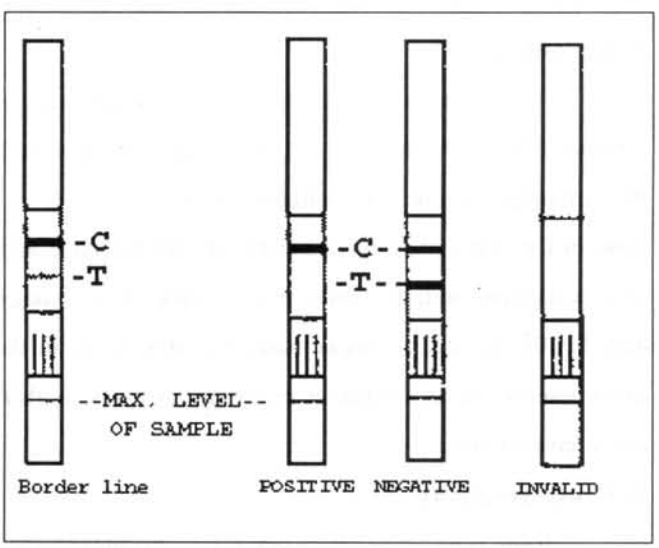


Figure 2 Bioline dip-strip

Results

1. Cost

PointeStep drug screen Meth-amphetamine test was imported from the USA. The price per test for this test kit was about $4.00, which was high for Thailand (equivalent to 160 bahts by

2000 exchange rates). The Bioline methamphetamine strip is a new product, developed and produced by a Thai company. This product is one result from the Thai government's efforts to develop a domestically produced amphetamine/ methamphetamine test kit so as to lower the cost of drug testing. The price for one Bioline test strip is 40 baht, less than $2. If this product development is successful, it will reduce the need for certain imports and subsequently provide a more economic solution for Thailand.

2. Simplicity

The slide tests of PointeStep and Bioline have similar procedure. Sample must be transferred to the slide, wait for 3-10 min before reading. The Bioline strip does not need sample transferring; however, error easily occurs if the strip is immersed into the sample excess the maximum level. Some users preferred to transfer urine into a small sample cup before dipping the strip to avoid the error.

3. Reading Time

The time for reading the results of both PointeStep and Bioline were recommended to be within 3-10 min after adding sample. In this study, no band appeared after 3 min of adding sample. The control band appeared after at least 8 min of adding urine. From both manufacturers' inserts, the extra band was expected to be occurred after 10 min. But the results showed that not extra band occurred even left for hours.

4. Reliability

The PointeStep gave clear bands that were easily read. At methamphetamine concentrations between 500 to 50,000 ng/ml, no weak band appeared in the test zone of PointeStep (figure 1). At methamphetamine concentrations between 1,000 to 5,000 ng/ml, Bioline strip gave a weak band in the test zone, which ideally should not have appeared (figure 3). Thus, it was interpreted as a borderline and; for sure, it was reported as a negative result. At the concentrations greater than 5,000 ng/ml, no weak band was developed in the test zone of Bioline strip so that it was easily interpreted as a positive result. In all cases, both PointeStep and Bioline gave a clear redline in the control zone.

5. Cross-reactivity

Both tests were designed for methamphetamine. The cutoff was set at 500 ng/ml for PointeStep and 1,000 ng/ml for Bioline. According to the manufacturers' inserts, both tests were not sensitive to amphetamine. Table 1 shows the concentrations of amphetamine and its analogs that gave positive results by PointeStep and Bioline test kits.

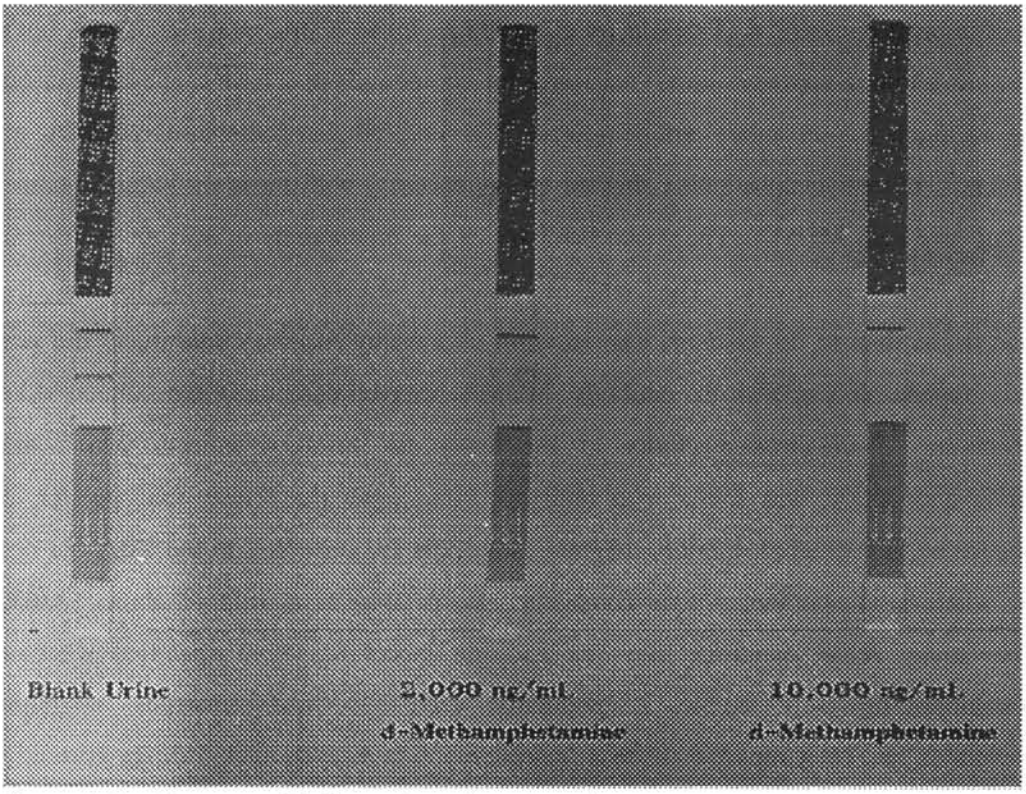


Figure 3 Results seen on Bioline methamphetamine strip

Table 1 Cross-reaction of amphetamine and its analogs analyzed by PointeStep and Bioline test kits

Drugs	Concentrations (ng/ml) giving positive result by PointeStep	Concentrations (ng/ml) giving positive result by Bioline
Amphetamine	≥ 25,000	> 50,000
Methamphetamine	≥ 500	≥ 5,000
MDMA	≥ 750	≥ 10,000
MDEA	≥ 3,000	≥ 25,000
Ethylamphetamine	≥ 3,000	≥ 25,000
MDA	≥ 25,000	> 50,000

Discussion

The sensitivity and the cross-reactivity of an immunoassay are very important factors and are mainly dependent on the nature of the antibody (Aoki, 1996). There are several different binding sites present on the antibodies in the assay, which have different affinities for amphetamine and/or methamphetamine, resulting in differential binding characteristics and the resultant variations in apparent cross-reactivity. In order to use the assay most effectively for an intended purpose, a complete understanding of relative cross-reactivity to

the drugs of interest is essential (Przekop, *et al.*, 1991). From a structural standpoint, both studied test kits showed increased cross-reactivity for compounds that have a substitution on the amine nitrogen or secondary amine (figure 4); i.e., ethylamphetamine, MDMA or MDEA. MDEA and ethylamphetamine showed cross-reactivity with these methamphetamine test kits, but less than MDMA. The stereochemistry of the compounds is also an important factor in its cross-reactivity. The reagents showed greater reactivity toward d-methamphetamine than to d,l-isomers. The addition of a methylenedioxy substituent did not significantly impede binding with the antibody. MDMA behaved essentially the same as d,l-methamphetamine. Poor cross-reactivity of the assay for primary amines, which are amphetamine and MDA, was found with both test kits. Since these tests are specific to methamphetamine, not to amphetamine, they are useful kits for screening in the USA but not in Europe where amphetamine, not methamphetamine, is used as a drug of abuse (Aoki, 1996). For many Asian countries like Thailand, no report has been specified whether methamphetamine or amphetamine is popularly used in these countries although methamphetamine has been reported to be a major drug found in smuggled drugs currently (Palanuvej, *et. al*, 1997). It has the advantage of increased cross-reactivity to some of the more common illicit analogs like ethylamphetamine, MDA, MDMA, and MDEA, thereby allowing the identification of analogs without loss of the ability to identify the primary analyte. Because of the increased use of these compounds, it is important to have the ability to detect these drugs, particularly for cases in which their use is suspected (Cody, 1990).

While Bioline methamphetamine reagent reacted with ethylamphetamine, MDMA and MDEA, the response was not enough to make it of practical value when compared with response to methamphetamine. Because of the lack of substantial cross-reactivity to many of the amphetamine analogs studied, use of the Bioline testing methodology is limited. Use of two or more reagent systems increases the capability of identifying amphetamine, methamphetamine, and their analogs in urine. Alternative methods should be investigated for detecting those analogs that did not demonstrate significant cross-reactivity with this test kit. For PointeStep slide test, MDMA, MDEA, and ethylamphetamine have substantially higher cross-reactivity. Therefore, the likelihood of getting a positive response (at or above 1000 ng/ml) is high, making it a useful test kit for on-site screening for methamphetamine and these analogs, which also have a high abuse potential.

It is important to keep the cross-reactivity of the amphetamine analogs in mind when interpreting the results of amphetamine/methamphetamine immunoassays. Positive results may be caused by the presence of an amphetamine analog. Likewise, a negative

immunoassay result does not indicate that there was no amphetamine analog present (Cody and Schwarzhoff, 1993). There are many factors that influence the final urinary excretion of a given substance, including the dose administered, interindividual variations in drug metabolism and body clearance, and sample collection time (Armbruster, *et al.*, 1995, de la Torre, *et al.*, 1996). Clearly, cases in which a larger dose and/or a shorter interval between ingestion and subsequent sampling will give a high concentration of the parent drug in urine and a positive response with the assays. Thus, when methamphetamine reagents like these two studied test kits are used, MDMA was easily detected and the ethylamphetamine and MDEA would likely identify users for a reasonable length of time.

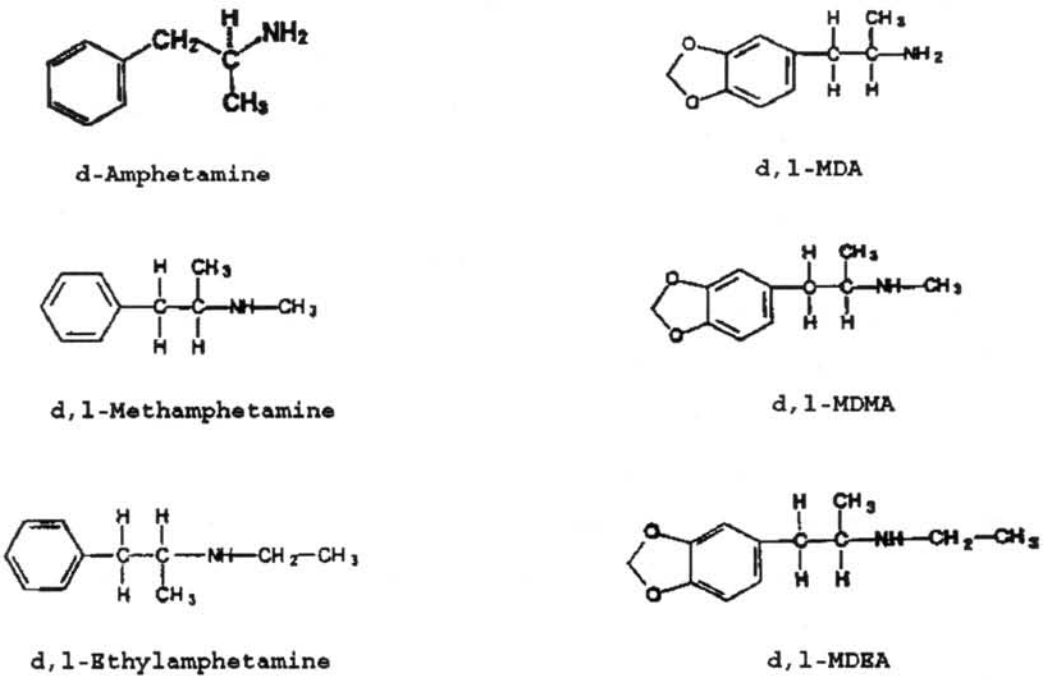


Figure 4 Molecular structures of drugs studied (Baselt and Cravey, 1995)

MDMA is excreted in urine principally in the unchanged form with small amount appearing as MDA. It is likely that MDEA will be metabolized and excreted in a similar manner (Verebey, *et al.*, 1988). The mean MDMA concentration was 13,400 ng/ml with a range of values from 380 to 96,200 ng/ml. The mean MDA concentration was 1,600 ng/ml with range of values from 150 to 8,600 ng/ml. There appears to be no clear

relationship between the MDMA concentration and MDA concentration (Kunsman, 1996). For the newest analogs, ethylamphetamine and MDEA, there is no report on how much their concentrations may be found in urine of abusers. However, for heavy or chronic users, large amounts of these drugs could be found in urine and are most likely to give positive results by cross-reactivity of PointeStep antibody.

Although these on-site test kits are simply used and of value when a quick result is needed and when an answer to the question of whether large quantities of a drug of abuse are present or absent is the sole prerequisite, they only give a result as positive or negative: nothing can be concluded about the amount of drug present in the urine (Ros, *et al.*, 1998). Interpretation is subjective, but for PointeStep it was unambiguous. PointeStep test; therefore, could have a useful role in general practice and may be of value in some laboratories, e.g. using the methamphetamine test to check for MDMA. However, the test is more expensive than the reagent costs for most laboratory-preliminary-screening-procedures (not including the cost of instrument) and confirmation by laboratory methods may be required.

The ambiguous results were found with Bioline test. The intensity of the red detection bars showed a dependence upon the concentration of the drug involved. In some cases, at low concentrations faint bars appeared in the test zone., and were classified as borderline and reported as negative. This will ensure that all the results achieved are unequivocal. The borderline was found even in case of d-methamphetamine at the concentration between 1,000 to 5,000 ng/ml, making the test poor sensitive to the drug of testing. Both manufacturers recommended that results should be read between 3-10 min but it was found that the control band was clearer by leaving cards/strips for at least 8 min. In addition, colored bands appeared to be stable and no extra band appearing for several days.

References

Aoki K., Yoshida T. and Kuroiwa Y. 1996. Forensic immunochemistry. Forensic Sci Inter. 80: 163-173.

Armbruster DA., Hubster ED., Kaufman MS. and Ramon MK. 1995. Cloned enzyme donor immunoassay (CEDIA) for drug-of-abuse screening. Clin Chem. 41: 92-98.

Baselt RC. and Cravey RH. Disposition of Toxic Drugs and Chemicals in Man, 4th ed., Chemical Toxicology Institute, California, 1995.

Cho AK. 1990. Ice: A new dosage form of an old drug. Science. 249: 631-634.

Cody JT. 1990. Detection of d,l-amphetamine, d,l-methamphetamine, and illicit amphetamine analogs using diagnostic products corporation's amphetamine and methamphetamine radioimmunoassay. J. Anal. Toxicol. 14: 321-324.

Cody JT. and Schwarzhoff R. 1993. Fluorescence polarization immunoassay detection of amphetamine, methamphetamine, and illicit amphetamine analogs. J. Anal. Toxicol. 17: 26-30.

de la Torre R., Badia R., Gonzalez G., *et al.* 1996. Cross-reactivity of stimulants found in sports drug testing by two fluorescence polarization immunoassays. J Anal Toxicol. 20: 165-170.

Hegadoren KM., Baker GB. and Coutts RT. 1995. Analysis of the enantiomers of 3,4-methylenedioxy-N-ethylamphetamine (MDE, "Eve") and its metabolite 3,4-methylenedioxyamphetamine (MDA) in rat brain. J. Pharmacol. Toxicol. Methods. 34: 117-123.

Kunsman GW., Levine B., Kuhlman JJ., *et al.* 1996. MDA-MDMA concentrations in urine specimens. J Anal Toxicol. 20: 517-521.

Miller NS., Millman RB. and Gold MS. 1989. Amphetamine: Pharmacology, abuse, and addiction. Adv. Alcohol Subst. Abuse. 8: 53-69.

Nagai T., Kanaya H., Matsushima K. and Kamiyama S. 1997. Time-lapse changes of d- and l-enantiomers of racemic (dl)-ethylamphetamine in human urine. J. Anal. Toxicol. 21: 112-115.

Palanuvej C., Issaravanich S., Rungphitackchai B., Chinveschakitvanich V. and Danthamrongkul V. 1997. Chemical analysis of illicit amphetamines. Thai J Hlth Resch. 11: 73-87.

Pidetcha P., Putriprawan T., Suwanton L., Congpuong P., Rekakanahul. and Tantrarongroj S. 1995. Screening for urinary amphetamine in truck drivers and drug addicts. J. Med. Assoc. Thai. 78: 554-558.

Prinzmetal M. and Bloomberg W. 1935. The use of Benzedrine for treatment of narcolepsy. J. Am. Med. Assoc. 105: 2051-2054.

Przekop MA., Manno JE., Kunsman GW., Cockerham KR. and Manno BR. 1991. Evaluation of the Abbott ADx amphetamine/methamphetamine II abused drug assay: Comparison to TDx, EMIT, and GC/MS methods. J Anal Toxicol. 15: 323-326.

Rasmussen S., Cole R. and Spiehler V. 1989. Methamphetamine in ante mortem blood and urine by radioimmunoassay and GC/MS. J. Anal. Toxicol. 13: 263-267.

Ros JJW., Pelders MG. and Egberts ACG. 1998. Performance of Abusign™ drugs-of-abuse slide tests with particular emphasis on concentrations near the cutoff: Comparison with FPIA-ADx and confirmation of results with GC-MS. J Anal Toxicol. 22: 40-44.

The Narcotics Act, B.E. 2522. Notification of the Ministry of Public Health, B.E. 2539, published in the Government Gazette Volume 113 Part special 23 ngor of 16th August B.E. 2539.

Verebey KV., Alrazi J. and Jaffe JH. 1988. The complications of 'ecstasy' (MDMA). J Am Med Assoc. 259: 1649-1650.