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Interference by Drugs Contained in Over-the-counter Cold Syrups on Methamphetamine Immunoassay Test Kits Used in Drug Abuse Assessment

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ABSTRACT

Eight commercial methamphetamine test kits : AbuSign, accuPINCH,AccuSign, I.D. BLOCK, Medi-Mate, QuikPac Ⅱ, SureStep and Visualine Ⅱ were evaluated for their interferences by twelve drugs commonly found in over-the-counter cold syrups: aspirin, brompheniramine, chlorpheniramine, ephedrine, ethenzamide, guaifenesin, l-phenylephrine, methylephedrine, pheniramine, phentermine, phenylpropanolamine, and pseudoephedrine; and by clobenzorex, ranitidine which have been found to interfer in some amphetamine immunoassays. These fourteen drugs were evaluated by using measured concentrations ranging from 1 mg/L to 1000 mg/L. Results indicate that ephedrine and pseudoephedrine demonstrate a significant interference in most of test kits, while most analgesics, antihistamines and antitussives show no interference with the exception of brompheniramine. Furthermore, phenylpropanolamine, phentermine, phenylephrine, ranitidine and methylephedrine all show some degree of interference in Quickpac Ⅱ, SureStep and Visualline Ⅱ. While accuPINCH was shown to be the most specific test kit, SureStep and QuikPac Ⅱ, which produced false positive results when tested at concentrations of 1 mg/L of ephedrine or pseudoephedrine, and thus demonstrate less specificity than all the other test kits.

Key words: Methamphetamine, test kit, interference, cold syrup.

INTRODUCTION

Cases of drug abuse with metamphetamine and heroin have significantly increased in Taiwan since 1991[1]. Presently these two drugs are periodically tested in both foreign laborers and drug addicts. Amphetamine-like compounds, such as phenylpropanolamine, ephedrine, pseudoephedrine, which often appear in over-the-counter(OTC) cold syrups, may produce false positive results in some commercial immunoassay systems for amphetamines. Therefore, it is necessary for a laboratory to have a sound, properly validated confirmation assay to identify the drugs of interest. The Mandatory Guidelines for Federal Workplace Drug Testing[2] recommended by the Department of Health and Human Service(DHHS) in U.S.A. regulate an initial test that is based on an immunoassay to eliminate negative urine specimens from further consideration and a confirmatory test that requires the use of a gas chromatography/mass spectrometry

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Table 1. Specificity data of commonly used sympathomimetics in OTC cold syrups on package insert of different test kits

Unit:mg / L

Test Kit	Ephedrine	Pseudo-ephedrine	Phenylpro-panolamine	Methyl-ephedrine	Phenyl-ephrine	Phenter-mine
AbuSign	+ / 90	+ / 60	−	✳	−	−
accuPINCH	− / 100	− / 100	− / 100	− / 100	✳	− / 100
AccuSign	✳	✳	−	✳	−	−
I.D. BLOCK	+ / 10	✳	✳	✳	✳	+ / 10
Medi-Mate	+ / 5	+ / 20	−	✳	−	−
QuikPac Ⅱ	✳	✳	✳	✳	✳	✳
SureStep	✳	✳	✳	✳	✳	✳
Visualine Ⅱ	+ / 100	+ / 10	− / 100	✳	✳	✳

Notes: + / 100 : produce interference when tested at concentration of 100 mg / L
− / 100 : do not produce interference when tested at concentration of 100 mg / L
− : produce interference when tested (did not report its concentration)
✳ : No specificity data on package insert.

(GC/MS) method if a specimen is identified as positive on the initial test. In our country, the guidelines of drug testing also follow the similiar regulations as those in U.S.A.

Recently, problems have been reported in the analysis of methamphetamine. Specifically, several specimens have been reported by drug testing laboratories in Taiwan to be positive for methamphetamine which were subsequently confirmed to be false positive. These specimens have several common characteristics, including not only the absence of methamphetamine but also the presence of extremely high concentrations of ephedrine or pseudoephedrine[3]. These problems had previously appeared in the United States, and lead the DHHS to require an amphetamine concentration greater than 200 mg/L in addition to a methamphetmaine concentration greater than 500 mg/L before a sample is reported as positive for methamphetamine[2]. Because the general populace of Taiwan are accustomed to buying and self-administering OTC cold syrups from drug stores whenever they contract a cold, the problem mentioned above seem more formidable in Taiwan than in the United States.

Generally there are sixteen drugs commonly found in OTC cold syrups[4] including acetaminophen, aspirin, brompheniramine, chlorpheniramine,dextromethorphan, codeine, ephedrine, ethenzamide, guaifenesin, ibuprofen, l-phenylephrine, methylephedrine, pheniramine, phentermine, phenylpropanolamine, and pseudoephedrine. These drugs can be categorized into four classes: sympathomimetics : ephedrine, pseudoephedrine, phenylpropanolamine, methylephedrine, phenylephrine, phentermine; analgesics : acetaminophen, ibuprofen, ethenzamide, aspirin; antihistamines : chlorpheniramine,

brompheniramine, pheniramine; antitussives : dextromethorphan, codeine, guaifenesin. Two other drugs : clobenzorex, ranitidine, which are not routinely added to cold syrups have also been reported to interfer some immunoassays[5,6]. Therefore, a total of eighteen drugs were assessed in the present study.

Specificity data of commonly used drugs are often reported on the package insert of each test kit[7~14] and are summerized in Table 1～5. Table 1 lists the specificitiy data for the sympathomimetics; Table 2 for the analgesics; Table 3 for the antihistamines; Table 4 for the antitussives; Table 5 for clobenzorex and ranitidine. Table 1

Table 2. Specificity insert of commonly used analgesics in OTC cold syrups on package insert of different test kits

unit:mg / L

Test kit	Acetaminophen	Ibuprofen	Ethenzamide	Aspirin
AbuSign	–	–	–	–
accuPINCH	– / 100	– / 100	✳	✳
AccuSign	–	✳	✳	✳
I.D. BLOCK	✳	✳	✳	✳
Medi-Mate	–	✳	✳	✳
QuikPac Ⅱ	✳	✳	✳	✳
SureStep	✳	✳	✳	✳
Visualine Ⅱ	– / 100	– / 100	✳	✳

Note: – / 100 : do not produce interference when tested at concentration of 100 mg / L
– : produce interference when tested (did not report its concentration)
✳ : No specificity data on package insert.

Table 3. Specificity insert of commonly used antihistamines in OTC cold syrups on package insert of different test kits

unit:mg / L

Test Kit	Chlorpheniramine	Brompheniramine	pheniramine
AbuSign	–	✳	✳
accuPINCH	✳	✳	✳
AccuSign	–	✳	✳
I.D. BLOCK	✳	✳	✳
Medi-Mate	–	✳	✳
QuikPac Ⅱ	✳	✳	✳
SureStep	✳	✳	✳
Visualine Ⅱ	✳	– / 100	✳

Note: – / 100 : do not produce interference when tested at concentration of 100 mg / L
– : produce interference when tested (did not report its concentration)
✳ : No specificity data on package insert.

Table 4. Specificity insert of commonly used antitussives in OTC cold syrups on package insert of different test kits

unit:mg / L

Test Kit	Dextromethorphan	Codeine	Guaifenesin
AbuSign	—	—	✳
accuPINCH	✳	— / 100	✳
AccSign	—	—	✳
I.D. BLOCK	✳	✳	✳
Medi-Mate	—	—	✳
QuikPac Ⅱ	✳	✳	✳
SureStep	✳	✳	✳
Visualine Ⅱ	✳	✳	✳

Note: — / 100 : do not produce interference when tested at concentration of 100 mg / L
— : produce interference when tested (did not report its concentration)
✳ : No specificity data on package insert.

shows that ephedrine and pseudoephedrine are apt to produce cross reactions, and other drugs either show no cross reaction, or do not have their specificity data. Because some package inserts, such as those of QuikPac Ⅱ, SureStep, do not contain specificity data related to these drugs, and no test kit has complete specificity data for these sympathomimetics, it is necessary to evaluate the overall specificity data of these sympathomimetics.

Aside from sympathomimetics, other drugs, such as analgesics(Table2), antihistamines(Table 3), antitussives(Table 4), either show little or no specificity data or do not produce interference according to their package inserts. Table 2 and Table 4 show that acetaminophen, ibuprofen, dextromethorphan, and codeine did not produce any significant interference. Therefore, these four drugs were not included in this study.

Because of variability in commercial immunoassay kits, a comprehensive and periodic evaluation of immunoassay characteristics is required for selecting an appropriate initial test for the drug of abuse. The cross-reactivity of amphetamine analogs or other interferring compounds have already been evaluated in several immunoassays[15~24]. However, most reports evaluated either automatic analyzers only, such as EMIT[15~16], TDX[17], their method comparison[18~20], or the immunoassay test kits from the same manufacturers[21~24]. Furthermore these papers mainly focused on amphetamine analogs only, and they did not pay much attention to the other compounds in OTC syrups. In 1992, Jeffrey et al.[20] examined six commercial immunoassays for their cross-reactivity to ephedrine, pseudoephedrine, and phenylpropanolamine, and their results showed that all specimens spiked at 100

Table 5. Specificity insert of two cross-reactive drugs on package insert of different test kits

unit:mg / L

Test Kit	Clobenzorex	Ranitidine
AbuSign	✳	—
accuPINCH	✳	— / 100
AccSign	✳	—
I.D. BLOCK	✳	✳
Medi-Mate	✳	—
QuikPac Ⅱ	✳	✳
SureStep	✳	✳
Visualine Ⅱ	✳	✳

Note: + / 100 : produce interference when tested at concentration of 100 mg/L
— / 100 : do not produce interference when tested at concentration of 100 mg / L
— : produce interference when tested (did not report its concentration)
✳ : No specificity data on package insert.

Table 6. Test results of sympathomimetics commonly used in OTC cold syrups on eight test kits

Unit:mg / L

Test Kit	Ephedrine	Pseudo-ephedrine	Phenylpro-panolamine	Methyl-ephedrine	Phenyl-ephrine	Phenter-mine
AbuSign	± / 10	± / 100	− / 1000	− / 1000	± / 10	− / 1000
accuPINCH	− / 1000	± / 100	− / 1000	− / 1000	− / 1000	− / 1000
AccuSign	+ / 50	+ / 500	− / 1000	− / 1000	− / 1000	− / 1000
I.D. BLOCK	+ / 100	± / 50	± / 500	− / 1000	± / 10	± / 500
Medi-Mate	+ / 10	± / 50	− / 1000	− / 1000	+ / 500	− / 1000
QuikPac Ⅱ	+ / 1	+ / 1	± / 100	± / 500	+ / 50	± / 100
SureStep	+ / 1	+ / 1	± / 500	± / 500	+ / 100	+ / 500
Visualine Ⅱ	+ / 100	+ / 10	± / 1000	± / 1000	± / 1000	+ / 1000

Notes: + / 50 : response greater than or equal to 1 mg / L when tested at concentration of 50 mg / L
− / 1000 : response less than 1 mg / L when tested at concentration of 1000 mg / L
± / 1000 :response near 1mg / L cutoff when tested at concentration of 1000 mg / L

mg/L were negative as judged by all immunoassays tested. Because these drugs are sold in some OTC cold preparations and are excreted into urine largely as the parent drugs, it is possible that their concentrations in the range of 1000 mg/L might be encountered in urine specimens, and thus evaluation of higher concentrations is also necessary. Therefore, concentrations of drugs in OTC cold syrups ranging from 1 mg/L to 1000 mg/L in urine were included in the study.

MATERIALS AND METHODS

Ⅰ. *Materials*

Aspirin, brompheniramine maleate, chlorpheniramine maleate, ephedrine HCl, guaifenesin, methamphetamine HCl, l-phenylephrine HCl, methylephedrine, pheniramine maleate, phentermine, phenylpropanolamine HCl, pseudoephedrine HCl and ranitidine HCl were purchased from Sigma Chemical Co.. Ethenzamide were purchased from Aldrich Co.. Clobenzorex is a reference standard of our laboratory. All standards were reagent grade.

Ⅱ. *Immunoassay Kits*

HYCOR accuPINCH™ Methamphetamine Test test kits[7] (Lot. No. 35178C3) were obtained from Hycor Biomedical Inc., Methamphetamine I.D. BLOCK™ Detection Kit test kits[8] (Lot. No. 13994-MAB-1) were obtained from International Diagnostic Systems, AbuSign™ MET One-Step Methamphetamine Test test kits[9] (Lot. No.856401) were obtained from Princeton BioMeditech, AccuSign™ MET New One-Step Methamphetamine Test test kits[10] (Lot. No. 858101) were obtained from Bio AccuTech Inc., Medi-Mate™ MET One Step Methamphetamine Test test kits[11] (Lot. No. 857201) were obtained from Medi-Mate Technologies Inc., QuikPac Ⅱ - One Step Urine Amphetamine Test test kits[12] (Lot. No. 010743) were obtained from Syntron Bioresearch, Inc. SureStep™ Drug Screen Methamphetamine test kits[13] (Lot. No. 201) were obtained from Applied Biotech, Inc., Visualine Ⅱ ™ Methamphetamine Test One-Step Urine Drug Screening test kits[14] (Lot. No.

Table 7. Test results of analgesics, antihistamines, and antitussives commonly used in OTC cold syrups on eight test kits
unit: mg / L

Test Kit	Ethenza-mide	Aspirin	Chlorphe-niramine	Bromphe-niramine	Phenira-mine	Guaifene-sin
AbuSign	− / 1000	− / 1000	− / 1000	− / 1000	− / 1000	− / 1000
accuPINCH	− / 1000	− / 1000	− / 1000	− / 1000	− / 1000	− / 1000
AccuSign	− / 1000	− / 1000	− / 1000	− / 1000	− / 1000	− / 1000
I.D. BLOCK	− / 1000	− / 1000	− / 1000	+ / 1000	− / 1000	− / 1000
Medi-Mate	− / 1000	− / 1000	− / 1000	− / 1000	− / 1000	− / 1000
QuikPac Ⅱ	− / 1000	− / 1000	− / 1000	− / 1000	− / 1000	− / 1000
SureStep	− / 1000	− / 1000	− / 1000	− / 1000	− / 1000	− / 1000
Visualine Ⅱ	− / 1000	− / 1000	− / 1000	− / 1000	− / 1000	− / 1000

Notes: + / 1000: response greater than or equal to 1 mg / L when tested at concentration of 1000 mg / L
− / 1000: response less than 1 mg / L when tested at concentration of 1000 mg / L

82509) were obtained from Hanson Hong Biomedical Co. Ltd.

Ⅲ. *Control and Standard Urines*

Methamphetamine control urines, Amphetamines positive specimen (Lot. No.57702) and negative specimen (Lot. No. 57711), were purchased from BIO-RAD Laboratories. Methamphetamine standard urine at concentration of 1 mg/L cutoff was prepared from spiking methamphetamine HCl in aforementioned negative specimen.

Ⅳ. *Preparations of Drugs in Urine*

The test solutions of aspirin, brompheniramine, chlorpheniramine, clobenzorex, ephedrine, ethenzamide, guaifenesin, l-phenylephrine, methylephedrine, pheniramine, phentermine, phenylpropanolamine, pseudoephedrine and ranitidine were prepared in a serial concentrations ranging from 1000, 500, 100, 50, 10, to 1 mg/L, if necessary, by spiking each of these drugs in negative specimen respectively.

Ⅴ. *Procedure*

Each drug was tested in duplicate for every test kit by the following procedure. Firstly, each drug was tested at concentration of 1000 mg/L. Then we either continued to evaluate the test kits at lower concentrations in sequence of 500, 100, 50 ,10,1 mg/L if their results were positive, which mean the response was greater than or equal to that of 1 mg/L cutoff, or until the result was negative and then recorded the previous concentration, which means the response was less than that of 1 mg/L cutoff.

RESULTS

Ⅰ. *Sympathomimetic Amines*

The results of interference by sympathomimetics commonly used in cold syrups in eight methamphetamine immunoassay test kits are listed in Table 6. The table clearly indicates that many sympathomimetics are apt to produce cross reactions in these immunoassay test kits at specified concentrations. Pseudoephedrine is the most

Table 8. Test results of clobenzorex, ranitidine on eight test kits

unit:mg / L

Test kit	Clobenzorex	Ranitidine
AbuSign	− / 1000	− / 1000
accuPINCH	− / 1000	− / 1000
AccuSign	− / 1000	− / 1000
I.D. BLOCK	± / 1000	+ / 1000
Medi-Mate	− / 1000	− / 1000
QuikPac Ⅱ	− / 1000	± / 50
SureStep	− / 1000	± / 50
Visualine Ⅱ	− / 1000	± / 1000

Notes: + / 1000 : response greater than or equal to 1 mg / L when tested at concentration of 1000 mg / L
− / 1000 : response less than 1 mg / L when tested at concentration of 1000 mg / L
± / 1000 :response near 1 mg / L cutoff when tested at concentration of 1000 mg / L

cross-reactive drug producing positive response even at a concentration of 1 mg/L in the SureStep test kit and the QuikPac Ⅱ test kit. Ephedrine also produces significant interference in these test kits with the exception of the accuPINCH test kit. Phenylephrine also demonstrates little intrerference in test kits with the exception of the accuPINCH and AccuSign test kit. In addition, phenylpropanolamine produces little interference in the Visualine Ⅱ, SureStep, QuicPac Ⅱ, and the I.D.Block test kits; Phentermine produces little interference in the Visualine Ⅱ, SureStep, QuikPac Ⅱ, and the I.D.Block test kits. Methylephedrine also demonstrates little interference in the Visualine Ⅱ, SureStep, and the QuikPac Ⅱ test kits. The latter drug is the least cross-reactive sympathomimetic that only produces false positive results at concentrations above 500 mg/L. Table 6 clearly shows that ephedrine and pseudoephedrine are more cross-reactive than all the other sympathomimetics.

Ⅱ. *Analgesics, Antihistamines, and Antitussives*

The results of interference by the analgesics, antihistamines, and antitussives commonly used in cold syrups in eight methamphetamine immunoassay test kits are listed in Table 7. The table clearly indicates that most of these drugs do not effect any significant levels of interference in these test kits except brompheniramine in the I.D. block test kit at 1000 mg/L.

Ⅲ. *Clobenzorex, Ranitidine*

The results of interference by clobenzorex, ranitidine in eight methamphetamine immunoassay test kits are listed in Table 8. The table clearly indicates that clobenzorex does not produce a significant interference except for the I.D. block test kit, while ranitidine produces little interference in the I.D. block, QuikPac Ⅱ, SureStep, and the Visualine Ⅱ test kit, of which the QuikPac Ⅱ and the SureStep test kits produce false positives at about 50 mg/L of ranitidine.

DISCUSSION

Cross-reactivity patterns with the sympathomimetic amines, clobenzorex, and ranitidine for methamphetamine test kits differed among these immunoassays. The sympathomimetic amines,

especially pseudoephedrine and ephedrine, are more cross-reactive than all the other drug classes. Clobenzorex and ranitidine also exhibits a degree of interference on some of these test kits, but the analgesics, the antihistamines, and the antitussives do not effect any significant interference except brompheniramine in the I.D. block test kit at 1000 mg/L.

This study demonstrates that the QuikPac Ⅱ and SureStep test kits have a greater chance of producing false positive amphetamine screening results due to the presence of a high concentration of the sympathomimetic amines, such as pseudoephedrine, ephedrine, phenylpropanolamine, phentermine, methylephedrine, and phenylephedrine in OTC cold syrups. These sympathomimetic amines may also produce false positive results for other test kits, including the AbuSign, AccuSign, I.D. block, MediMate, and the Visualine Ⅱ test kits. In contrast, the accuPINCH test kit, which produces false positive result only at 100 mg/L of pseudoephedrine, is the most specific among these test kits.

Because some drugs in OTC cold syrups can cause interference in methamphetamine immunoassay test kits, a urine drug testing laboratory should establish a routine procedure that includes at least one immunoassay served as the initial screening method and a second nonimmunoassay method which confirmed the putative positive result from the initial test. Information from this study may be of use in selecting a method to ensure that the combination of the two methods does not produce a false positive result in routine work.

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國內常見感冒糖漿劑成分對尿液中甲基安非他命篩檢之影響

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摘　要

本計畫係針對感冒製劑成分，對尿液中甲基安非他命在免疫學分析套組試驗中所可能產生的交叉反應進行評估，以期瞭解各套組之特異性，供作選用尿液檢體初步篩選用檢測套組之參考。

本計畫評估之免疫學分析套組有：AbuSign, accuPINCH, AccuSign, I.D. BLOCK, Medi-Mate, QuikPac Ⅱ, SureStep及Visualine Ⅱ等八種，列入評估之藥物有：aspirin, brompheniramine, chlorpheniramine, clobenzorex, ephedrine, ethenzamide, guaifenesin, ℓ-phenylephrine, methylephedrine, pheniramine, phentermine, phenylpropanolamine, pseudoephedrine及ranitidine等十四種。

結果顯示，ephedrine及pseudoephedrine產生交叉反應之濃度最低，即上述藥物於1 μg/ml時，以QuikPac Ⅱ及SureStep檢測即呈偽陽性反應。若以藥物濃度為1000 μg/ml時，仍不呈陽性或疑似陽性反應為判定交叉反應之界限，則pseudoephedrine對八種套組均呈交叉反應。ephedrine對於AccuPINCH外之套組，均呈交叉反應。Phenylephrine對除accuPINCH, Accusign外之套組，均呈交叉反應。Phentermine及phenylpropanolamine對除Abusign, AccuPINCH, Accusign, Medi-Mate外之套組，均呈交叉反應。phentermine及phenylpropanol amine對除Abusign, AccuPINCH, Accusign, Medi-Mate外之套組，均呈交叉反應。Methylephedrine對QuikPac Ⅱ，SureStep及Visualine Ⅱ套組呈交叉反應，對其他不呈交叉反應。此外ethenzamide, aspirin, chlorpheniramine, pheniramine及guaifenesin對八種套組均不呈交叉反應；而brompheniramine及clobenzorex只對I.D. Block套組產生交叉反應；ranitidine對除Abusign, AccuPINCH, Accusign及Medi-Mate外之套組均呈交叉反應。