



OFFICE OF THE DEPARTMENT OF DEFENSE COORDINATOR
FOR DRUG ENFORCEMENT POLICY AND SUPPORT

1510 DEFENSE PENTAGON
WASHINGTON DC 20301-1510



17 SEP 1996

MEMORANDUM FOR LIEUTENANT COLONEL AARON JACOBS, MS, USA
COMMANDER MARILYN PAST, MSC, USN
LIEUTENANT COLONEL JAMES A. SWABY, USAF, BSC

SUBJECT: Transition to the New Roche On-Line THC Immunoassay Reagents

Attached is a letter from Roche Diagnostic Systems containing information important to the transition to the new THC immunoassay Reagent Kits on the Olympus AU800. The letter contains general information regarding receipt and shipping of the new THC immunoassay reagent kits.

A copy of the new THC reagent package information insert and a copy of the Olympus AU800 test parameters for the new THC reagent are attached to the Roche letter. **Please note that there are significant changes in the THC AU800 test parameters which must be followed. These parameters only apply to the new THC reagents (THC Order Number 47510) to be received on or about October 1, 1996 and are not to be used with the current THC reagents (THC Order Number 43428).** Please ensure that the correct parameters have been entered when transitioning to the new THC reagent.

For additional information or comment, please contact Captain John Jemionek, MSC, USN, who may be reached at (703) 693-1917.


Lennard J. Wolfson
Director, Demand Reduction and Systems

Attachment:
As stated

CC:
Commander FTDTL Fort Meade, ATTN: LTC Armitage
Commander FTDTL Tripler AMC, ATTN: MAJ Lukey
Commanding Officer Great Lakes, ATTN: CDR Thomas
Commanding Officer Jacksonville, ATTN: LCDR McWhorter
Commanding Officer San Diego, ATTN: LCDR Vias





Roche Diagnostic Systems

A Member of the Roche Group

Roche Diagnostic Systems, Inc.
Branchburg Township
1000 U.S. Highway 202
Somerville, New Jersey 08876-3771

Direct Dial (908) 253-7550
Fax (908) 253-7645

September 13, 1996

Captain John F. Jemionek, USN
The Pentagon, Room 2E549
Washington, DC 20301-1515

Dear Capt. Jemionek,

I am pleased to inform you that the initial lot of 90 mL kit of Abuscreen ONLINE[®] Cannabinoids-ES 50/100, Order No. 47510 will be received by all DoD testing facilities by October 1, 1996, with 4 month dating as per our discussions. This product will replace the current 90 mL kit of Abuscreen ONLINE THC, Order No. 43428, previously used at DoD testing sites.

It is our desire to insure that the change to the new product go as smoothly and uneventfully as possible. I am enclosing a copy of the package insert for the new Abuscreen ONLINE Cannabinoids-ES 50/100, Order No. 47510, as well as the instrument parameters for the Olympus AU800 should you wish to distribute them from your office to the testing facilities.

With your approval, I would like to have Kurt Moore, your Roche Diagnostic Systems Key Account Manager speak with each of the facility directors to confirm that the new parameter settings have been programmed into their instruments. Kurt will also confirm that all new orders placed for Abuscreen ONLINE Cannabinoids-ES 50/100 reference Order No. 47510 to avoid possible confusion in the future. To further insure that proper parameter settings have been programmed into the AU800 instruments prior to initiating use of the product, each new kit of Abuscreen ONLINE Cannabinoids-ES 50/100 will be labeled with a sticker alerting the user to change instrument parameters.

Roche Diagnostic Systems has truly enjoyed the scientific relationship we have developed with all individuals involved in the DoD drug testing program. We feel privileged to have been selected as the vendor supplying your program with non-RIA drug testing products and will continue to make ever effort to provide "Best in Class" service and products to meet your evolving needs.

If I can be of any further service to you or any of the facility directors please feel free to contact me at the numbers listed above.

Sincerely,

Alan Ridzinski
Marketing Manager

Enclosure

Abuscreen

ONLINE[®]

Automated Assays for Drug Abuse



THC
50/100

90 mL Kit for
Cannabinoids-ES 50/100

U.S. Order No. 47510
Art. No. 07 6413 2

INTENDED USE

Abuscreen ONLINE[®] for Cannabinoids-ES 50/100 is an in vitro diagnostic test for the qualitative and semi-quantitative detection of cannabinoids in human urine at 50 and 100 ng/mL cutoffs. Semi-Quantitative test results may be obtained that permit laboratories to assess assay performance as part of a quality control program.

Abuscreen ONLINE for Cannabinoids-ES 50/100 provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.¹ Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

SUMMARY AND EXPLANATION OF TEST

The principal psychoactive component of the hemp plant, *Cannabis sativa*, is generally accepted to be Δ^9 -tetrahydrocannabinol (Δ^9 -THC), although other cannabinoids may contribute to the psychological and physiological actions of marijuana. The acute effects of marijuana use, concomitant with the desired "high," are memory impairment, time confusion, interference with learning, impaired motor skills and depersonalization.^{2,3,4} These effects are also manifested in chronic users in addition to cardiovascular, pulmonary, and reproductive effects.

Marijuana is usually smoked, but may be ingested, either incorporated into food or as a liquid extract (tea). It is rapidly absorbed from the lungs into the blood with rapid onset of effects; the onset is slower but prolonged when ingested. The natural cannabinoids and their metabolic products are fat soluble and are stored in the body's fatty tissues, including brain tissue, for prolonged periods after use.⁵

Cannabinoid metabolites are found in blood, bile, feces, and urine and may be detected in urine within hours of exposure. Because of their fat solubility, they also remain in the body's fatty tissues with slow release and subsequent urinary excretion for days, weeks, and even months after the last exposure, depending on the intensity and frequency of use.¹ The prominent Δ^9 -THC metabolite, 11-nor- Δ^9 -THC-9-carboxylic acid (Δ^9 -COOH THC), is the primary urinary marker for detecting marijuana use.

PRINCIPLE OF PROCEDURE

Abuscreen ONLINE Automated Assays are based on the kinetic interaction of microparticles in a solution (KIMS)[®] as measured by changes in light transmission. In the absence of sample drug, free antibody binds to drug-microparticle conjugates causing the formation of particle aggregates. When a urine sample containing the drug in question is present, this drug competes with the particle-bound drug derivative for free antibody. Antibody bound to sample drug is no longer available to promote particle aggregation, and subsequent particle lattice formation is inhibited.

As the aggregation reaction proceeds in the absence of sample drug, the absorbance change increases. Conversely, the presence of sample drug diminishes the increasing absorbance in proportion to the concentration of drug in the sample. Sample drug content is determined relative to the value obtained for a known cutoff concentration of drug.⁷

REAGENTS

Each Abuscreen ONLINE for Cannabinoids-ES 50/100 kit contains:

1. Antibody Reagent: Cannabinoids monoclonal antibody (mouse) in buffer with protein and 0.09% sodium azide.
2. Microparticle Reagent: Conjugated cannabinoid derivative microparticles in buffer and 0.09% sodium azide.
3. Sample Diluent: Buffer and 0.09% sodium azide.

Warnings and Precautions

1. For in vitro diagnostic use.

2. Reagents from different kit lots must not be interchanged. Reagents within kit lots have been matched to ensure optimum test performance. A new calibration curve must be generated whenever reagent lots are changed.
3. Specimens containing human-sourced materials should be handled as if potentially infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories* (HHS Publication Number [CDC] 88-8395).
4. These reagents contain sodium azide as a preservative. Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build up.

Instructions for Reagent Handling

Before use, the Antibody Reagent must be diluted with an equal volume of Diluent and mixed thoroughly but gently. These reagents have different viscosities, and thorough mixing is required to ensure homogeneity and realize expected reagent performance. This mixture is stable for 1 month when refrigerated (2 – 8°C). The Antibody Reagent may be poured directly into the oversized Diluent bottle.

Storage and Stability

Store all reagents upright and tightly capped at 2 – 8°C. Do not freeze reagents. Reagent that has been frozen should be discarded.

SPECIMEN COLLECTION

Abuscreen ONLINE for Cannabinoids-ES 50/100 is formulated for use with urine specimens. Fresh urine specimens do not require any special handling or pretreatment, but an effort should be made to keep pipetted samples free of gross debris. Samples should be within the normal physiological pH range of 5 – 8. No additives or preservatives are required. It is recommended that urine specimens be stored at 2 – 8°C and tested within 3 days of collection. For prolonged storage, freezing of samples is recommended. It has been reported that THC and its derivatives may absorb onto plastics used for sample collection containers, effectively lowering the drug concentration of the sample.⁸

PROCEDURE

Materials Provided

- | | |
|-------------------------------|-----------|
| 1. Antibody Reagent (AB) | 1 × 90 mL |
| 2. Microparticle Reagent (MP) | 1 × 80 mL |
| 3. Sample Diluent (DIL) | 1 × 90 mL |

Materials Required But Not Provided

1. Validated chemistry analyzer meeting the following general specifications:
Wavelength: 450 – 600 nm; Temperature: 37°C
2. Abuscreen ONLINE Cannabinoids Calibration Pack (U.S. Order No. 42349, Art. No. 07 3812 3)
3. Abuscreen ONLINE Positive Control (U.S. Order No. 42320, Art. No. 07 3504 3)
4. Abuscreen ONLINE Negative Control (U.S. Order No. 42321, Art. No. 07 3505 1)

Instructions for Use

1. Transfer samples, calibrators, and controls to the appropriate sample cup.
2. Before use, the Antibody Reagent must be diluted with an equal volume of Diluent and mixed thoroughly by gentle swirling. Place the reagents in the appropriate container/position on the analyzer.
3. Ensure that the analyzer is programmed with the correct parameters.
4. The analyzer pipettes all samples, calibrators, controls, and reagents and performs the assay according to the programmed parameters.
5. Refer to the appropriate Abuscreen ONLINE applications sheet or call the Roche Response Center[®] at 1-800-526-1247 (or your local Roche Representative if outside the US) for detailed information on the performance of this assay on commercially available analyzers.

Calibration

Instrument calibration is performed using the Abuscreen ONLINE Cannabinoids Calibrators. Calibration must be performed upon initial set-up of the system or upon the use of a new lot of reagent. Intermittent calibration of the system is necessary to ensure accurate and reliable results. As individual runs are of various lengths depending on a given laboratory work load, it is recommended that the frequency of calibration be determined by individual laboratories based on the performance of the Abuscreen ONLINE Negative and Positive Controls. Inconsistent control results indicate the necessity for test system calibration. Shown below are typical absorbance values obtained on an Olympus AU800.

THC Calibrator (ng/mL)	Absorbance
0	0.0187
50	0.4729
100	0.6788
150	0.7323

Quality Control

It is recommended that each test run include an Abuscreen ONLINE Positive and Negative Control. Drug concentrations of the Abuscreen ONLINE Cannabinoids Calibrators and Abuscreen ONLINE Controls have been verified by GC/MS.

RESULTS

Results equal to or greater than the concentration of Δ⁹-COOH-THC in the cutoff calibrator are considered positive. Alternatively, a flag system may be used for result interpretation where any result that is equal to or greater than the concentration of Δ⁹-COOH-THC in the cutoff calibrator is identified as positive.

Although the current National Institute on Drug Abuse (SAMHSA) guideline for a cannabinoids screening assay cutoff is 50 ng/mL, the Abuscreen ONLINE for Cannabinoids-ES 50/100 reagents can be used at cutoff points of 50 ng/mL and 100 ng/mL. Based upon specific laboratory needs, another cutoff between 50 and 100 ng/mL may be used. If an alternate cutoff is used, care should be taken to ensure that the performance characteristics of the product at that cutoff meet that laboratory's established criteria.

PROCEDURAL LIMITATIONS

See PERFORMANCE CHARACTERISTICS for information on substances tested for cross-reactivity in this assay. There is the possibility that other substances and/or factors may interfere with the test and cause erroneous results (e.g., technical or procedural errors). Adulteration of the sample can cause erroneous results. If adulteration is suspected, another sample should be collected. For information on how adulterants may affect the performance of this assay, contact the Roche Response Center at 1-800-526-1247 (or your local Roche Representative if outside the US).

A positive result with this assay indicates the presence of cannabinoids in urine, but does not reflect the degree of intoxication. Significant increases in urinary levels of cannabinoids from passive inhalation have been reported to occur only after exposure to extremely high concentrations of marijuana smoke in small unventilated areas.⁹

These extreme exposure conditions are not typical of the usual situations in which the drug is used. More recent reports indicate that urine cannabinoid concentrations resulting from passive inhalation are not likely to exceed 20 ng/mL.^{10,11}

PERFORMANCE CHARACTERISTICS

Accuracy

Two hundred twenty urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel were evaluated with Abuscreen RIA for Cannabinoids and Abuscreen ONLINE for Cannabinoids-ES 50/100. All 220 of the samples were negative relative to the 50 and 100 ng/mL cutoffs.

One hundred urine samples, obtained from a clinical laboratory where they screened positive with a commercially available immunoassay and were subsequently confirmed positive by GC/MS (15 ng/mL cutoff), were evaluated with Abuscreen RIA for Cannabinoids (50 and 100 ng/mL cutoffs) and Abuscreen ONLINE for Cannabinoids-ES 50/100 (50 and 100 ng/mL cutoffs). As shown below, the results demonstrate the expected increase in detection rate at the lower cutoff.*

		50 ng/mL Cutoff (RIA)		100 ng/mL Cutoff (RIA)	
50 ng/mL Cutoff (ONLINE)	+	94	0	62	3
	-	0	6	0	35
		15 ng/mL Cutoff (GC/MS)		15 ng/mL Cutoff (GC/MS)	
50 ng/mL Cutoff (ONLINE)	+	94	0	65	0
	-	3	3	32	3

*The number of positive results at each cutoff varies because of samples with intermediate cannabinoids concentrations.

Precision

The precision of Abuscreen ONLINE for Cannabinoids-ES 50/100 was determined by running a series of calibrators and controls in replicates of 20 five times. The following results were obtained:

Cutoff	Concentration	Qualitative Precision		Confidence Level
		Number Tested	Correct Results	
50 ng/mL	40 ng/mL	100	100	>95% negative reading
	60 ng/mL	100	100	>95% positive reading
	80 ng/mL	100	100	>95% negative reading
100 ng/mL	80 ng/mL	100	100	>95% negative reading
	120 ng/mL	100	100	>95% positive reading

Quantitative Precision (50 ng/mL cutoff)*

Within-run			
Concentration (ng/mL)	40	50	60
Mean (ng/mL)	40	50	61
SD	2	2	2
CV%	6	4	3
Run to run			
Concentration (ng/mL)	40	50	60
Mean (ng/mL)	40	52	61
SD	3	3	2
CV%	7	5	3

Quantitative Precision (100 ng/mL cutoff)*

Within-run			
Concentration (ng/mL)	80	100	120
Mean (ng/mL)	85	102	136
SD	2	2	3
CV%	2	2	2
Run to run			
Concentration (ng/mL)	80	100	120
Mean (ng/mL)	85	102	135
SD	2	4	2
CV%	2	4	2

*Results are to be used in QC programs

Sensitivity

The sensitivity of Abuscreen ONLINE for Cannabinoids-ES 50/100 was determined by performing 25 replicate assays on the 0 ng/mL calibrator. Two standard deviations above the mean yields an analytical sensitivity of less than 6 ng/mL Δ⁹-COOH-THC.

Specificity

The specificity of Abuscreen ONLINE for Cannabinoids-ES 50/100 for various cannabinoids and cannabinoid metabolites was determined by generating inhibition curves for each of the compounds listed and determining the approximate quantity of each compound that is equivalent in assay reactivity to the 50 and 100 ng/mL Δ⁹-COOH-THC assay cutoffs. The results obtained with both cutoffs were substantially equivalent. The following results were obtained at the 50 ng/mL cutoff.

Compound	Approx. ng/mL Equivalent to 50 ng/mL Δ ⁹ COOH-THC	Approx. Percent Cross-reactivity
8-α-hydroxy-Δ ⁹ -THC	94	53
11-hydroxy-Δ ⁹ -THC	56	90
Δ ⁹ -THC	625	8
8-β-11-dihydroxy-Δ ⁹ -THC	122	41
11-hydroxycannabinol	500	10
Cannabinol	8333	0.6
Cannabidiol	>100,000	<0.002%

Cross-reactivity with Unrelated Drugs

The following compounds were added to aliquots of pooled normal human urine at a concentration of 100 000 ng/mL. None of these compounds gave values in the assay that were equal to or greater than 0.02% cross-reactivity.

Acetaminophen	Diphenylhydantoin	α-Methamphetamine
Acetylsalicylic acid	Dopamine	Methaqualone
Aminopyrine	Ecgonine	Methyprylon
Amitriptyline	Ecgonine methyl ester	Morphine
Amobarbital	Ephedrine	Naloxone
Amphetamine	Epinephrine	Naltrexone
Ampicillin	Erythromycin	Naproxen
Ascorbic acid	Estriol	Niacinamide
Aspartame	Fenopropfen	Norethindrone
Atropine	Furosemide	Oxazepam
Benzocaine	Gentisic acid	Penicillin G
Benzoyllecgonine (cocaine metabolite)	Glutethimide	Pentobarbital
Benzphetamine	Guaiaicol glycerol ether	Phencyclidine
Butabarbital	Hydrochlorothiazide	Phenobarbital
Caffeine	5-Hydroxyindole-3-acetic acid	Phenothiazine
Calcium hypochlorite	5-Hydroxyindole-2-carboxylic acid	Phenylbutazone
Chlordiazepoxide	Ibuprofen	Phenylpropranolamine
Chloroquine	Imipramine	Procaine
Chlorpheniramine	Isoproterenol	Promethazine
Chlorpromazine	Ketamine	Quinidine
Cocaine	Lidocaine	Quinine
Codeine	LSD	Secobarbital
Dextromethorphan	Melanin	Sulindac
Dextropropoxyphene	Meperidine	Tetracycline
Diazepam	Methadone	Tetrahydrozoline
Diphenhydramine		Trifluoperazine
		Verapamil

Adulteration of reagents or the use of instruments without appropriate capabilities can affect the performance characteristics and stated or implied labelling claims. Any modification of the instructions as set forth in this labelling requires validation by the laboratory performing this assay.

REFERENCES

1. Hawks RL, Chiang CN, eds. Urine testing for drugs of abuse. *National Institute on Drug Abuse (NIDA) Research Monograph 73*. 1986.
2. Tinklenberg JR, Darley CF. Psychological and cognitive effects of cannabis. In: Connell H, Dom N, eds. *Cannabis and Man: Proceedings of Third International Cannabinoids Conference, London, 1975*. London: Churchill Livingstone; 1975.
3. Klonoff H. Marijuana and driving in real-life situations. *Science*. 1974;186:317 - 324.
4. Meiges FT, Tinklenberg JR, Holkster LE, Gillespie HK. Temporal disintegration and depersonalization during marijuana intoxication. *Arch Gen Psychiatry*. 1970;23:204 - 210.
5. Lemberger L, et al. Delta-9-tetrahydrocannabinol metabolism and disposition in long-term marijuana smokers. *Science*. 1971; 173:72 - 74.
6. Adler FL, Liu CT. Detection of morphine by hemagglutination-inhibition. *J Immunol*. 1971;106(6):1684 - 1685.
7. Antonian E, McNally AJ, Ng C, Slemmon P, Twarowska B, Salamone SJ. An Abuscreen® Immunoassay for THC metabolites in urine on the Olympus AU5000 Series Clinical Analyzers. In: American Academy of Forensic Sciences. *Program: The Forensic Sciences and Government*. 1991:177. Abstract.
8. Decker WJ. Laboratory support of drug abuse control programs: An overview. *Clinical Toxicology*. 1977;10(1):28.
9. Cone EJ, Johnson RE, Darwin WD, et al. Passive inhalation of marijuana smoke: Urinalysis and room air levels of Δ^9 -tetrahydrocannabinol. *J Anal Toxicol*. 1987;11:89 - 96.
10. Perez-Reyes M, Di Guseppi S, Mason AP, Davis KH. Passive inhalation of marijuana smoke and urinary excretion of cannabinoids. *Clin Pharmacol Ther*. 1983;34(1):36 - 41.
11. Mulé SJ, Lomax P, Gross SJ. Active and realistic passive marijuana exposure tested by three immunoassays and GC/MS in urine. *J Anal Toxicol*. 1988; 12:113 - 116.

For technical assistance in the US, call the Roche Response Center® at 1-800-526-1247. For assistance outside the US, contact your local Roche Representative.

Roche Diagnostic Systems

A Member of the Roche Group

Roche Diagnostic Systems, Inc.
Branchburg Township
1080 US Highway 202
Somerville, NJ 08876-3771

Olympus AU800 Test Parameters for ONLINE Cannabinoids-ES 50/100, Order #47510

*** INDIVIDUAL TEST PARAMETERS ***

PAGE 1/2

SELECT NO. > __

1. TEST1-THC1(SERUM) > __

	SERUM	URINE		SERUM	URINE
2. SAMPLE VOLUME	[12] > __	0	9. OD VALUE RANGE		
3. SAMPLE VOLUME FOR REPEAT RUN	[3] > __	0	MAX [] >		
4. REAGENT VOLUME	R1 [180] > __		MIN [] >		
DILUENT VOLUME	[0] > __		10. % VARIANCE	[] >	
	R2 [80] > __		11. NO-LAG-TIME	[] >	
DILUENT VOLUME	[0] > __		12. DATA CHECK1	[0] > __%	
5. WAVELENGTH	1 [570] > __		Px[0] > __		
	2 [] > __		Py[0] > __		
6. METHOD	[END] > __		Pz[0] > __		
7. REACTION SLOPE	[--] >		13. DATA CHECK2	[0] > __%	
8. MEASURING POINT	S1 [8] >		Px[0] > __		
	E1 [14] >		Py[0] > __		
	S2 []		Pz[0] > __		
	E2 []				

*** INDIVIDUAL TEST PARAMETERS ***

PAGE 2/2

SELECT NO. > __

1. TEST 1-THC1(SERUM) > __

	SERUM	URINE
14. NORMAL VALUE	H [49] > _____	0
	L [0] > _____	0
15. DYNAMIC RANGE	H [1000] > _____	0
	L [-200] > _____	0
16. PANIC VALUE	H [1000] > _____	0
	L [-200] > _____	0
17. REAGENT OD RANGE		
FIRST POINT	H [2.5000] > _____	
	L [-2.0000] > _____	
LAST POINT	H [2.5000] > _____	
	L [-2.0000] > _____	
18. DECISION LEVELS FOR REPEAT RUN		
	H [999999] > _____	0
	L [-999999] > _____	0

Olympus AU800 Test Parameters (cont.) for ONLINE Cannabinoids-ES 50/100, Order #47510

*** CALIBRATION PARAMETERS ***

TEST NO. [1] > _
 # CALIBRATION TYPE NO. [5AB] > _
 # FORMULA NO. [3] > _
 # SELECT NO. > _

TEST = 1-THC1 NO. POINT	CAL NO.	OD	FACTOR RANGE		L	FACTOR
			CONC	H		
1. OD0	_	0.0000	[0]			
2. POINT-1	[1]	[-----]	[0]	[999999.00]	[-99999.00]	[139.50]
3. POINT-2	[2]	[-----]	[25]	[999999.00]	[-99999.00]	[139.50]
4. POINT-3	[3]	[-----]	[50]	[999999.00]	[-99999.00]	[302.66]
5. POINT-4	[4]	[-----]	[100]	[999999.00]	[-99999.00]	[204.91]
6. POINT-5	[5]	[-----]	[150]	[999999.00]	[-99999.00]	[245.09]
7. POINT-6	[----]	[-----]	[-----]	[-----]	[-----]	[-----]
8. POINT-7	[----]	[-----]	[-----]	[-----]	[-----]	[-----]

<< 1 POINT CAL >>

OPERATION	CAL NO.	CONC	COEFFICIENT K
9. [NO] > _	[----] > _	[-----] > _____	[-----] > _____