LSD

cobas®

Lysergic Acid Diethylamide

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
20763284 122	Abuscreen OnLine LSD (200 tests)	System-ID 07 6328 4	Roche/Hitachi **cobas c** 501/502
20766356 122	Abuscreen OnLine LSD Calibration and Control Pack LSD Calibrator 1 × 5 mL LSD Positive Control 2 × 4 mL LSD Negative Control 2 × 4 mL	Code 640	

English

System information

For **cobas c** 501 analyzer:

LSD: ACN 784: for qualitative assay

For **cobas c** 502 analyzer:

LSD: ACN 8784: for qualitative assay

Intended use

Lysergic acid diethylamide (LSD) assay is an in vitro diagnostic test for the qualitative detection of LSD and its metabolites in human urine on Roche/Hitachi **cobas c** systems at a cutoff concentration of 0.5 ng/mL.

The LSD assay 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 or Liquid Chromatography coupled with Mass Spectrometry or Tandem Mass Spectrometry (GC-MS, GC-MS/MS, LC-MS or LC-MS/MS) is the preferred confirmatory method.[1,2] Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

Summary

Lysergic acid diethylamide (LSD, lysergamide, Delysid) is an ergot alkaloid that is a natural product of the rye fungus Claviceps. However, most of the LSD consumed illicitly is derived by synthesis involving the reaction of lysergic acid with diethylamine. [3,4] LSD is a hallucinogenic drug that can cause severe altered states of consciousness, can induce psychotic reactions characterized by hallucinations, and can cause other perceptual disturbances that mimic functional psychosis.[4,5] LSD is thought to exert its psychotomimetic effects through antagonism of serotonin (5-hydroxytryptamine) activity in the brain stem neurons.[4,5,6] Of the four possible synthetic diastereomers of LSD only *d*-LSD has mind altering properties. The *d*-LSD is one of the most potent hallucinogenic agents known to man, but it has a remarkably low acute toxicity.[3,5,6] LSD is usually administered by drug abusers as the tartrate salt in oral doses of 25 to 250 µg.[3] This drug undergoes such rapid and extensive metabolism that only a small fraction of a dose is excreted in human urine as unchanged LSD. Urine from suspected drug abusers contain multiple metabolites including (in order of decreasing concentration) 2-oxo-3-hydroxy-LSD, LSD-o-glucuronide, 2-oxo-LSD, LSD, N-desmethyl-LSD (nor-LSD).[1,2,3] Although iso-LSD is not a metabolite of LSD, it is a major contaminant in many illicit LSD preparations and hence is frequently detected in the urine and other body fluids from LSD abusers.[1]

Test principle

Abuscreen OnLine automated assays are based on the kinetic interaction of microparticles in a solution (KIMS)[7,8] 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. As the aggregation reaction proceeds in the absence of sample drug, the absorbance increases.

When a urine sample contains the drug in question, 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. 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.

Reagents - working solutions

Sample Diluent (SD)	**R1**	Buffer; stabilizer; 0.09 % sodium azide
Antibody Reagent (AB)	**R2**	LSD antibody (goat polyclonal); buffer; 0.09 % sodium azide
Microparticle Reagent (MP)	**R3**	Conjugated LSD derivative microparticles; buffer; 0.09 % sodium azide

R1 is in position A, R2 is in position C and R3 is in position B.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

Reagent handling

Ready for use

Carefully invert reagent container several times prior to use to ensure that the reagent components are mixed.

Storage and stability

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label
On-board in use and refrigerated on the analyzer:	8 weeks

Do not freeze.

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Urine: Collect urine samples in clean, amber glass or non-transparent plastic containers. 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. As a precaution against photo decomposition of LSD, urine samples should be protected from exposure to light.[1] 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 5 days of collection.[9] For prolonged storage, freezing of samples is recommended.

Centrifuge highly turbid specimens before testing.

Adulteration or dilution of the sample can cause erroneous results. If adulteration is suspected, another sample should be collected. Specimen validity testing is required for specimens collected under the *Mandatory Guidelines for Federal Workplace Drug Testing Programs*.[10]

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

See “Order information” section

General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator’s manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

LSD

Lysergic Acid Diethylamide

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Application for urine

Deselect Automatic Rerun for these applications in the Utility menu, Application screen, Range tab.

cobas c 501/502 **test definition**

	Qualitative	
Assay type	2-Point End	
Reaction time / Assay points	10 / 37-70	
Wavelength (sub/main)	– /600 nm	
Reaction direction	Increase	
Unit	mAbs	
Reagent pipetting		Diluent (H_2O)
R1	65 µL	–
R2	50 µL	–
R3	40 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	27 µL	–	–
Decreased	27 µL	–	–
Increased	27 µL	–	–

Calibration

Calibrator	S1: Abuscreen OnLine LSD Calibrator, 0.5 ng/mL
	The drug concentration of the calibrator has been verified by LC-MS/MS or GC-MS.
Calibration K Factor	For the qualitative application, enter the K Factor as -1000 into the Calibration menu, Status screen, Calibration Result window.
Calibration mode	Linear
Calibration frequency	Blank Calibration
	- after reagent lot change
	- as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against a primary reference method (LC-MS/MS).

Quality control

For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

Drug concentrations of LSD Positive and Negative Controls have been verified by LC-MS/MS or GC-MS.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Results

The cutoff calibrator is used as a reference in distinguishing between preliminary positive and negative samples. Samples producing a positive or "0" absorbance value are considered preliminary positive. Preliminary positive samples are flagged with >Test. Samples producing a negative absorbance value are considered negative. Negative samples are preceded by a minus sign.

Use caution when reporting results as there are various factors that influence a urine test result, such as fluid intake and other biological factors.

As with any sensitive test for drugs of abuse on automated clinical chemistry analyzers, the possibility exists for analyte carry-over from a sample with an extremely high concentration to a normal (negative) sample which immediately follows it.

Confirm all preliminary positive results by another method.

Limitations - interference

See the "Specific performance data" section of this document for information on substances tested with 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).

A preliminary positive result with this assay indicates the presence of LSD and/or its metabolites in urine. It does not measure the level of intoxication.

Interfering substances were added to drug free urine at the concentration listed below. These samples were then spiked to 0.5 ng/mL using a LSD stock solution. Samples were tested in triplicate (n = 3) on a Roche/Hitachi **cobas c** 501 analyzer. The median % recoveries were calculated and are listed below.

Substance	**Concentration Tested**	**% LSD Recovery**
Acetone	1 %	82
Ascorbic acid	1.5 %	107
Bilirubin	0.25 mg/mL	116
Creatinine	5 mg/mL	134
Ethanol	1 %	82
Glucose	2 %	99
Hemoglobin	0.1 g/L	65
Human albumin	0.5 %	105
Ibuprofen	0.5 mg/mL	58
Ofloxacin	0.1 mg/mL	117
Oxalic acid	2 mg/mL	93
Sodium chloride	0.5 M	94
Sodium chloride	1 M	83
Urea	6 %	105

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD-SMS-SmpCln1+2-SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is not required.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Expected values

Results of this assay distinguish preliminary positive (≥ 0.5 ng/mL) from negative samples only. The amount of drug detected in a preliminary positive sample cannot be estimated.

Specific performance data

Representative performance data on a Roche/Hitachi analyzer are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined in an internal protocol by running a series of calibrator and controls (repeatability n = 20, intermediate precision n = 100). For this assay, the S1 Abs was set to zero and a K factor of 1000 was entered. This caused the results to be reported as mAbs. Calibration was not performed. The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.

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Qualitative precision

Repeatability	*Mean mAbs*	*SD mAbs*	*CV %*
Level 1 (0.25 ng/mL)	584	20.3	3.5
Level 2 (0.5 ng/mL)	375	11.0	2.9
Level 3 (1.0 ng/mL)	175	4.3	2.4

Intermediate precision	*Mean mAbs*	*SD mAbs*	*CV %*
Level 1 (0.25 ng/mL)	608	26.1	4.3
Level 2 (0.5 ng/mL)	375	12.8	3.4
Level 3 (1.0 ng/mL)	175	5.3	3.0

Cutoff (0.5)	Number tested	Correct results	Confidence level
0.5x	100	100	> 95 % negative reading
2x	100	100	> 95 % positive reading

Lower detection limit of the test

0.03 ng/mL

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 2 standard deviations above that of the lowest standard (standard 1 + 2 SD, repeatability, n = 21).

Accuracy

100 urine samples, that screened negative in a drug test panel, were evaluated with the LSD assay. 100 % of these normal urines were negative relative to a 0.5 ng/mL cutoff. 54 individual human urine samples were spiked with LSD to preliminary positive drug levels and were subsequently quantified by LC-MS/MS to be positive at drug levels between 0.54 ng/mL and 3.83 ng/mL. These samples were evaluated with the LSD assay and 100 % of these samples were positive relative to a 0.5 ng/mL cutoff. In addition, 10 additional individual human urine samples were spiked with LSD around the negative control and were confirmed by LC-MS/MS to have levels between 0.24-0.30 ng/mL. These 10 samples gave negative results relative to the 0.5 ng/mL cutoff. Data from the accuracy studies described above that fell within the near cutoff value ranges were combined with data generated from positive samples. The following results were obtained with the LSD assay on the Roche/Hitachi **cobas c** 501 analyzer relative to the LC-MS/MS values.

LSD Clinical Correlation (Cutoff = 0.5 ng/mL)

		Negative Samples	LC-MS/MS values (ng/mL)		
			Near Cutoff		
			0.24-0.30	0.54-1.02	1.04-3.83
cobas c 501 analyzer	+	0	0	12	42
	–	100	10	0	0

Samples were evaluated with this assay on a Roche/Hitachi **cobas c** 501 analyzer and a COBAS INTEGRA 700 analyzer. 100 urine samples, that screened negative in a drug test panel, were evaluated with the LSD assay. 100 % of these normal urines were negative relative to the COBAS INTEGRA 700 analyzer. 54 individual human urine samples were spiked with LSD to preliminary positive drug levels and were subsequently quantified by LC-MS/MS to be positive at drug levels between 0.54 ng/mL and 3.83 ng/mL. 100 % of the samples were positive on both the Roche/Hitachi **cobas c** 501 analyzer and the COBAS INTEGRA 700 analyzer.

LSD Correlation (Cutoff = 0.5 ng/mL)

		COBAS INTEGRA 700 analyzer	
		+	-
cobas c 501 analyzer	+	54	0
	-	0	100

Analytical specificity

The specificity of this assay 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 0.5 ng/ml LSD assay cutoff. The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.

Compound	**ng/mL Equivalent to 0.5 ng/mL LSD**	**Approximate % Cross-reactivity**
Nor-LSD (N-desmethyl-LSD)	2	33
2-oxo-3-hydroxy-LSD	4	12
Iso-LSD	14	3.6
Lysergic acid N-(methyl-propyl) amide	15	3.3
Methysergide maleate	2904	0.017
Ergonovine maleate	10769	0.0046
2-Bromo-α-ergocryptine	31724	0.0016
Ergotamine tartrate	36296	0.0014
d-Lysergic acid	113636	0.0004
d-Tryptophan	166667	0.0003
Serotonin	238095	0.0002
α-Ergocryptine	1000000	0.0001

Drug interference

The following compounds were added to aliquots of pooled normal human urine at a concentration of 100000 ng/mL. None of these compounds gave values in the assay that were greater than 0.0004 % cross-reactivity.

Acetaminophen
Acetylsalicylic acid
Aminopyrine
Amitriptyline
Amobarbital
d-Amphetamine
l-Amphetamine
Ampicillin
Ascorbic acid
Aspartame
Atropine
Benzocaine
Benzoylecgonine
(cocaine metabolite)
Benzphetamine
Brompheniramine
Butabarbital
Caffeine
Calcium hypochlorite
Chlordiazepoxide
Chloroquine
Chlorpheniramine
Chlorpromazine
Clemastine
Cocaine
Codeine
Lidocaine
MDA
MDMA
Melanin
Meperidine
Methadone
d-Methamphetamine
l-Methamphetamine
Methapyrilene
Methaqualone
Methylphenidate
Methyprylon
Morphine
Naloxone
Naltrexone
Naproxen
Niacinamide
Nordiazepam
Norethindrone
l-Norpseudoephedrine
Nortriptyline
Oxazepam
Penicillin G
Pentazocine
Pentobarbital
Phencyclidine

LSD

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Desipramine	*β*-Phenethylamine
Dextromethorphan	Phenobarbital
Dextropropoxyphene	Phenothiazine
Diazepam	Phentermine
Diphenhydramine	Phenylbutazone
Diphenylhydantoin	*d*-Phenylpropanolamine
Dopamine	*d,l*-Phenylpropanolamine
Doxepin	Procaine
Ecgonine	Procyclidine
Ecgonine methyl ester	Promethazine
d-Ephedrine	*d*-Pseudoephedrine
d,l-Ephedrine	*l*-Pseudoephedrine
l-Ephedrine	Quinidine
Epinephrine	Quinine
Erythromycin	Secobarbital
Estriol	Sulindac
Fenoprofen	Tetracycline
Flumazenil	Δ^9 THC-9-carboxylic acid
Furosemide	Tetrahydrozoline
Gentisic acid	Thioridazine
Glutethimide	Trifluoperazine
Guaiacol glycerol ether	*d,l*-Trihexyphenidyl
Hydrochlorothiazide	Trimipramine
p-Hydroxyamphetamine	Tripelenamine
Ibuprofen	Tyramine
Imipramine	Verapamil
Isoproterenol	Zolpidem
Ketamine	Zopiclone

References

1 Reuschel SA, Eades D, Foltz RL. Recent advances in chromatographic and mass spectrometric methods for determination of LSD and its metabolites in physiological specimens. J Chromatogr B Biomed Sci Appl 1999;733(1-2):145-159.

2 Poch GK, Klette KL, Hallare DA, et al. Detection of metabolites of lysergic acid diethylamide (LSD) in human urine specimens: 2-oxo-3-hydroxy-LSD, a prevalent metabolite of LSD. J Chromatogr B Biomed Sci Appl 1999;724(1):23-33.

3 Baselt RC. Disposition of Toxic Drugs and Chemicals in Man. 7th ed. Foster City, CA: Biomedical Publications 2004.

4 Caldwell J, Sever P. The biochemical pharmacology of abused drugs: Amphetamine, Cocaine, and LSD. Clin Pharmacol Ther 1974;16(4):625-638.

5 Hardman JG, Limbird LE, Gilman A, eds. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 10th ed. New York, NY: McGraw Hill Pub Co. 2001.

6 Boakes RJ, Bradley PB, Briggs I, et al. Antagonism of 5-hydroxytryptamine by LSD-25 in the central nervous system: a possible neuronal basis for the actions of LSD-25. Br J Pharmacol 1970;40(2):202-218.

7 McNally AJ, Goc-Szkutnicka K, Li Z, et al. An OnLine immunoassay for LSD: comparison with GC-MS and the Abuscreen RIA. J Anal Toxicol 1996;20(6):404-408.

8 Armbruster DA, Schwarzhoff RH, Pierce BL, et al. Method comparison of EMIT II and ONLINE with RIA for drug screening. J Forensic Sci 1993;38:1326-1341.

9 Toxicology and Drug Testing in the Clinical Laboratory; Approved Guideline. 2nd ed. (C52-A2). Clinical and Laboratory Standards Institute 2007;27:33.

10 Mandatory Guidelines for Federal Workplace Drug Testing Programs. Fed Regist 2008 Nov 25;73:71858-71907.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see https://usdiagnostics.roche.com for definition of symbols used):

CONTENT	Contents of kit
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

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