References

1. Fairlight Consulting. http://www.fairlite.com/ocd/articles/ tricyclic.shtml

2. Bickel MH. Poisoning by Tricyclic Antidepressant Drugs. *Int. J. Clinical Pharmacol.* 11 (1975) 145-176 (No. 2).

3. Baselt RC. *Disposition of Toxic Drugs and Chemicals in Man.* 2nd ed., Davis, CA: Biomedical Publ.;1982;pp. 30-34, 223-225, 394-397, 568-571.

Symbols Key

	Instructions For Use (Read)
REF	Item Number
30°C / 2°C	Store At
	Expiration Date
CONT	Contents
IFU	Instructions For Use
PIP	Transfer Pipette
IVD	For In Vitro Diagnostic Use
LOT	Lot Number
	Manufacturer
MF	Manufactured For
EC REP	Authorized Representative
CE	CE Mark

Printed in U.S.A.
P-58109-F
232-9/13/12

CE

EC REP
MT Promedt Consulting GmbH
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Manufactured by
PBM
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New Jersey 08852, U.S.A.
1-732-274–1000 www.pbmc.com

MF Manufactured for:
lifeSign
A PBM Group Company
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Skillman, NJ 08558
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www.lifesignmed.com

P-58109-F

Status DS

One-Step TCA Assay

For *In Vitro* Use Only

Simple One-Step Immunoassay for the Qualitative Detection of Tricyclic Antidepressants in Urine

LifeSign, LLC

Item No. 20910 10 Test Kit
Item No. 20935 35 Test Kit

Intended Use

Status DS TCA is a simple, one-step immunochromatographic assay for the rapid, qualitative detection of tricyclic antidepressants (TCAs) . The test is standardized to detect nortryptiline at a cutoff concentration of 1000 ng/mL in human urine.

The ***Status DS*** TCA test is a qualitative screening test, and provides only a preliminary analytical result. A negative result does not eliminate the possibility of the presence of tricyclic antidepressants (TCAs) in the urine specimen at concentrations below the cutoff. A positive result may be due to the sum of the reactivities of more than one tricyclic antidepressant and/or their metabolites (see Table 2: Specificity). To obtain a confirmed analytical result, a more specific alternative method should be used, e.g., high performance liquid chromatography (HPLC) or gas chromatography, mass spectrometry (GC/MS). Clinical consideration and professional judgment should be applied to any drug test result, particularly when preliminary positive results are used.

Summary and Explanation

Tricyclic antidepressants (TCAs) are a type of prescription drug intended for clinically depressed patients. Unfortunately, they are becoming more frequently abused and are now one of the leading causes of death by drug overdose in the United States. There are two broad chemical classes of TCAs. The tertiary amines— amitryptiline, imipramine, trimipramine and doxepin—boost serotonin levels and are prescribed for insomnia, irritability and overstimulation. The secondary amines—nortryptiline, desipramine and protryptiline—enhance nore- pinephrine levels and are prescribed for opposite types of symptoms, such as excessive fatigue, withdrawal and inertness.[1] Abuse of TCAs may lead to coma, respiratory depression, convulsions, blood pressure deviations, hyperprexia and severe cardiac conditions. TCAs are excreted in urine mostly in the form of metabolites for up to ten days.[2,3]

Principle

The ***Status DS*** TCA test uses solid-phase chromatographic membrane immunoassay technology for the qualitative detection of tricyclic antidepressants. The test is based on the principle of the highly specific immunochemical reactions between antigens and antibodies which are used for the analysis of specific substances in biological fluids. The test relies on the competition between the drug conjugates and the drugs which may be present in the urine sample, for binding to antibodies. In the test procedure, a sample of urine is placed in the Sample Well of the device and is allowed to migrate upward. If the drug is present in the urine sample, it competes with the drug conjugate bound to the dye, for the limited antibodies immobilized on the membrane. If the level of drug or drug metabolite is above the cutoff level, the drug will saturate the antibodies, thus inhibiting the binding of the dye coated with drug conjugates to the antibodies on the membrane. This prevents the formation of a line on the membrane. Therefore, a drug-positive urine sample will not generate a line at Test (T) position in the Result Window, indicating a positive result from positive drug competition. A negative urine sample will generate a line at T position in the Result window, indicating a negative result from an absence of competition with free drugs.In addition to the Test line that may appear in the Result window, a Control line is present to confirm the viability of the test. This Control line (validation line) should always appear if the test is conducted properly. Polyclonal sheep anti-mouse IgG antibody is immobilized on the control line. The monoclonal antibody-dye conjugates that pass the line will be captured and produce a colored line at the Control position (C). This works as a procedural control, confirming that proper sample volume was used and the reagent system at the Control line and the conjugate-color indicator worked properly. If insufficient sample volume is used, there may not be a Control line, indicating the test is invalid.

Materials Provided

The ***Status DS*** TCA test kit contains all the reagents necessary to perform the tests.

- ***Status DS*** TCA device. The test device contains a membrane strip coated with polyclonal anti-nortryptiline antibody and a pad containing drug-dye conjugate in a protein matrix.
- Disposable specimen pipette.
- Instructions for use.

Precautions

- For *in vitro* diagnostic use only.
- Avoid cross contamination of urine samples by using a new urine specimen container and pipette for each urine sample.
- The test kit does not contain any HIV or hepatitis infective components.
- Urine specimens are potentially infectious. Proper handling and disposal methods should be established according to good laboratory practices.
- The ***Status DS*** device should remain in its original sealed pouch until ready for use. Do not use the test if the pouch is damaged or the seal is broken.
- Do not use the test kit after the expiration date.

Storage and Stability

The ***Status DS*** TCA test kit should be stored at 2–30°C (35–86°F) in the original sealed pouch. The expiration dating was established under these storage conditions.

Specimen Collection and Preparation

Approximately 110 µL of urine sample is required for each test. Fresh urine specimens do not require any special handling or pretreatment. Specimens should be collected in a clean glass or plastic container. If testing will not be performed within 2 hours, specimens should be refrigerated (2–8°C) for up to 48 hours. If longer storage is required, specimen may be stored frozen (-20°C or colder). Specimens should be brought to room temperature before testing. Specimens containing a large amount of particulate matter may give inconsistent test results. Such specimens should be clarified by centrifuging or allowing to settle before testing.

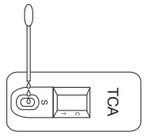
Test Procedure

The test procedure consists of adding the urine sample to the Sample Well of the device and watching for the appearance of colored lines in the Result Window.

Test Protocol

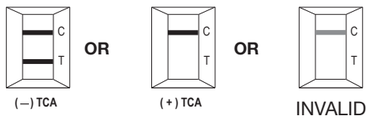
1. For each test, open one ***Status DS*** pouch and label the ***Status DS*** device with the patient ID.

2. Holding the pipette vertically, dispense 3 full drops (110 µL) of the urine sample into the Sample Well.



3. Read the results after 3 minutes, but within 10 minutes of sample application.

Interpretation of Results



Negative: The appearance of a reddish-purple Control line (C) and a line for a specific drug indicates a negative test result; i.e., no drug above the cutoff level has been detected. The color intensities of the Control line and a specific drug line may not be equal. Any faint line in the Result window, visible in 10 minutes, should be interpreted as negative. A negative test result does not indicate the absence of drug in the sample; it only indicates the sample does not contain drug above the cutoff level in qualitative terms.

Positive: The appearance of a reddish-purple Control line and no distinct line next to a specific drug name indicates the test result is positive for that drug (i.e., the specimen contains the drug at a concentration above the cutoff level). A positive test result does not provide any indication of the level of intoxication or urinary concentration of the drug in the sample; it only indicates the sample contains drug above the cutoff level in qualitative terms.

Invalid: A distinct Control line (C) should always appear. The test is invalid if no Control line forms at the C position. Such tests should be repeated with a new ***Status DS*** test device. There are other possible redults depending on the combination of drugs in the urine sample.

Limitations

- If inadequate sample is dispensed into the sample well (less than 3 full drops or 110 µL), the sample may not migrate in the device. Should no migration be observed within the first minute after addition of the sample, the user may dispense another drop of the urine sample into the device. This process may be repeated a second time. If migration does not occur after the second sample addition the test should be repeated with a new device.

- The test is designed for use with unadulterated urine only.

- There is a possibility that factors such as technical or procedural errors, as well as other substances in the urine sample which are not listed in Table 3 below, may interfere with the test and cause erroneous results.

- Adulterants, such as bleach and/or alum, in urine specimens may produce erroneous results regardless of the method of analysis. If adulteration is suspected, the test should be repeated with a new sample.

- The test result read after 10 minutes may not be consistent with the original reading obtained within the 10 minute reading period. The test must be read within 10 minutes of sample application.

User Quality Control

Internal Control: Each ***Status DS*** TCA test device has built-in controls. The Control line is an internal positive procedural control. A distinct reddish-purple Control line should always appear at the C position, if the test procedure is performed properly, an adequate sample volume is used, the sample and reagent are wicking on the membrane, and the test reagents at the control line and the conjugate-color indicator are reactive. In addition, if the test has been performed correctly and the device is working properly, The background in the result window will become clear and provide a distinct result. This may be considered an internal negative procedural control.

The positive and negative procedural controls contained in each ***Status DS*** TCA test device satisfy the requirements of testing a positive control and a negative control on a daily basis. If the Control line does not appear at the Control position, the test is invalid and a new test should be performed. If the problem persists, contact LifeSign for technical assistance.

External Control: External controls may also be used to assure that the reagents are working properly and the assay procedure is followed correctly. It is recommended that a control be tested at regular intervals as good laboratory testing process. For information on how to obtain controls, contact LifeSign's Technical Services.

Expected Values

Status DS TCA is a qualitative assay. The amount of nortryptiline present in the urine cannot be estimated by the assay. The assay results distinguish positive from negative samples. Positive results indicate the samples contain nortryptiline above the cutoff concentration.

Performance Characteristics

The ***Status DS*** TCA test has been shown to detect nortryptiline at an average cutoff of 1000 ng/mL in urine. The accuracy of ***Status DS*** TCA was evaluated in comparison to commercially available immunoassay, Triage®. A total of 203 samples was tested by both procedures. Complete agreement was observed in 99% of the samples as shown below (Table 1).

Table 1. Accuracy: Comparison of ***Status DS*** TCA with Triage®

		Triage®		
		Positive	Negative	TOTAL
Status DS (TCA)	Positive	103	2	105
	Negative	0	98	98
TOTAL		103	100	203

Precision and Accuracy

The precision of the ***Status DS*** TCA assay was determined by carrying out the test with serially diluted standard drug solutions. About 95% of the samples containing nortryptiline concentrations 25% over the cutoff level consistently showed positive results. The study also included over 40 samples ± 25% cutoff level. These results were found to be consistently in agreement with expected test results.

Distribution of Random Error:
Twenty (20) blind samples prepared by spiking various concentrations of nortryptiline were separately tested by two operators. The test results from the two operators showed complete agreement.

Reproducibility

The reproducibility of the test results of the ***Status DS*** TCA assay was examined at three different sites using a total of 15 blind controls, consisting of 5 negative samples, 5 moderately positive samples (2000-3000 ng/mL nortryptiline), and 5 strongly positive samples (4000-8000 ng/mL nortryptiline). The results obtained at these three sites with these controls demonstrated 100% agreement with each other.

Specificity

Compounds that are detected by the ***Status DS*** TCA test are listed below. The specificity of the ***Status DS*** TCA test was determined by adding the drugs and drug metabolites listed to drug-negative urine specimens and testing with the ***Status DS*** TCA test kit. The results are expressed in terms of the concentration required to produce a positive result (Table 2).

Table 2. Specificity:

Compound	Concentration (ng/mL)
Amitryptiline	800
Chlorpromazine	100,000
Clomipramine	5,000
Cyclobenzaprine	2,500
Desipramine	1,500
Diphenhydramine	>100,000
Dothiepin	2,000
Doxepin	1,500
Imipramine	1,000
Norclomipramine	850
Nordoxepin	5,000
Nortriptyline	1,000
Perphenazine	41,000
Promazine	5,000
Protryptiline	2,000
Trimipramine	3,000

The following compounds show no cross-reactivity when tested with ***Status DS*** TCA at a concentration of 100 µg/mL (Table 3).

Table 3. Non Cross-Reacting Compounds:

4-Acetamidophenol
Acetophenetidin (Phenacetin)
N-Acetylprocainamide
Acetylsalicylic acid
Aminopyrine
Amitryptyline
Amobarbital
Amoxapine
Amoxicillin
D,L-Amphetamine
l-Amphetamine
Apomorphine
Aspartame
Atropine
Benzilic acid
Benzoic acid
Benzphetamine
Butabarbital
Cannabidiol
Chloralhydrate
Chloramphenicol
Chlordiazepoxide
Chlorothiazide
Chlorpromazine
Chlorquine
Cholesterol
Clomipramine
Clonidine
Codeine
Cortisone
(–) Cotinine
Creatinine
Deoxycorticosterone
Dextromethorphan
Diazepam
Diclofenac
Diethylpropion
Diflunisal
Digoxin
Diphenhydramine
Domperidone
Doxylamine
(+) Ephedrine
(±) Ephedrine
(–) Ephedrine
(–) Ψ Ephedrine
Erythromycin
ß-Estradiol
Estrone-3-sulfate
Ethyl-p-aminobenzoate
Fenoprofen
Furoxmide
Gentisic acid
Glucuronide
Glutethimide
Guaifenesin
Hippuric acid
Hydralazine
Hydrochlorothiazide
Hydrocodone
Hydrocortisone
Hydromorphone
O-Hydroxyhippuric acid
3-Hydroxytyramine
Ibuprofen
Imipramine
Iproniazid
(–) Isoproterenol
Isoxsuprine
Ketamine
Ketoprofen
Labetalol
Levorphanol
Lidocaine
Loperamide
Loxapine succinate
Maprotiline
Meperidine
Meprobamate
Methadone
p-Hydroxymethamphetamine
Methaqualone
Methoxyphenamine
(±) 3,4-Methylenedioxyamphetamine
(±) 3,4-Methylenedioxymethamphetamine
Methylphenidate
Methyprylon
Morphine-3-ß-Dglucuronide
Nalidixic acid
Nalorphine
Naloxone
Naltrexone
Naproxen
Niacinamide
Nifedipine
Norcodein
Norethindrone
Noroxymorphone
D-Norpropoxyphene
(–) Norpseudoephedrine
Noscapine
Nylidrin
D,L-Octopamine
Oxalic acid
Oxazepam
Oxolinic Acid
Oxycodone
Oxymetazoline
Oxymorphone
Papaverine
Penicillin-G
Pentazocaine
Pentobarbital
Perphenazine
Phencyclidine
Phendimetrazine
Phenelzine
Phenobarbital
Phentermine
Phentoin
L-Phenylephrine
ß-Phenylethylamine
Phenylpropanolamine
Prednisolone
Prednisone
Procaine
Promazine
Promethazine
D,L-Propanolol
Propiomazine
D-Propoxyphene
D-Pseudoephedrine
Quinidine
Quinine
Rantidine
Salicylic acid
Secobarbital
Serotonin
Sulfamethazine
Sulindac
Temazepam
Tetracycline
Tetrahydrocortisone
Tetrahydrozoline
Thebaine
Thiamine
Thioridazine
D,L-Thyroxine
Tolbutamide
Triamterene
Trifluoperazine
Trimethoprim
Trimipramine
Tryptamine
D,L-Tryptophan
Tyramine
D,L-Tyrosine
Uric acid
Verapamil
Zomepirac