Nano-Check™ DAT 10M Test

THC / OPI / COC / mAMP / PCP / BAR / AMP / MDN / TCA / BZO

For in vitro Diagnostic Use

One–Step Immunochromatographic Assay for the Detection of Cannabinoids, Opiate, Cocaine, Methamphetamine, Phencyclidine, Barbiturates, Amphetamine, Methadone, Tricyclic Antidepressants, Benzodiazepines in Human Urine

1. INTENDED USE

The **Nano-Check™ DAT 10M THC/OPI/COC/mAMP/PCP/BAR/AMP/MDN/TCA/BZO (Nano-Check™ DAT 10M)** test is a rapid, self-controlled, immunoassay for the qualitative detection of Cannabinoid, Opiate, Cocaine, Methamphetamine, Phencyclidine, Barbiturates, Amphetamine, Methadone, Tricyclic antidepressant, and Benzodiazepines compounds and their metabolites in human urine. The detection limits (Cut off concentrations) of this test are THC metabolite at 50 ng/ml for THC, Morphine at 300 ng/ml for OPI, Benzoylecgonine at 300 ng/ml for COC, Methamphetamine at 1000 ng/ml for mAMP, Phencyclidine at 25 ng/ml at PCP, Secobarbital at 300 ng/ml for BAR, D-Amphetamine at 1000 ng/ml for AMP, Methadone at 300 ng/ml at MDN, Nortriptyline at 1000 ng/ml at TCA, Oxazepam at 300 ng/ml for BZO. This assay is intended for professional and *in-vitro* use only. The test provides only a preliminary test result. A more specific alternative testing method should be used to confirm the immunoassay result. Gas chromatography/mass spectrophotometer (GC/MS) analysis is the recommended confirmatory method of the National Institute on Drug Abuse (NIDA). Clinical consideration and professional judgment should be applied to any drug of abuse test results, particularly when positive results are observed.

2. SUMMARY AND EXPLANATION OF THE TEST

THC: THC (Δ^9-tetrahydrocannabinol) is the primary active compound in Marijuana and other cannabinoid products**.** Marijuana is the most commonly used hallucinogenic illicit drug derived from the hemp plant. Street terms for marijuana are grass, pot, weed, bud, Mary Jane, dope, indo, and hydro. Marijuana is usually smoked as a cigarette (called a joint) or in a pipe or bong. Inhalation of marijuana affects the central nervous system causing a euphoric effect, altered mood and sensory perception, hallucinations and increased heart rate. Chronic smokers of marijuana experience impaired memory and learning, anxiety, panic attacks, tolerance, and physical dependence and may have many of the same respiratory problems as tobacco smokers including daily cough and phlegm, chronic bronchitis symptoms, and frequent chest colds; chronic abuse can also lead to abnormal functioning of lung tissues. When marijuana is inhaled, the major metabolite excreted in urine is 11-nor-Δ^9 tetrahydrocannabinol-9-carboxylic acid. The metabolite compounds can be found within hours of inhalation and remain detectable for 3-10 days after smoking.

Opiate: Opiates are a class of compounds occurring naturally in opium that is present in the poppy plant, *papaver somniferum*. Opioid analgesics comprised of a large group of substances such as morphine, codeine, heroin and semi synthetic forms of morphine that control pain by depressing the central nervous system. The principal effects of opiate intoxication include miosis, analgesia, sedation, respiratory depression, and confusion. In patients receiving morphine, initially 25-35% of total amount of morphine present in urine is free morphine but this ratio declines after 12 hours to an average of 6% of total morphine. The remainder of the dose is excreted in the conjugate form such as morphine 3-glucuronide. Morphine and morphine 3-glucuronide are detectable in urine for 1-3 days after the opiate dose.

Cocaine: Derived from the leaves of coca plant, cocaine is a potent central nervous system stimulant as well as a local anesthetic. The most common psychological effects induced by cocaine are euphoria, confidence and a sense of increased energy accompanied by an increased heart rate, dilation of the pupils, fever, and tremors and sweating. Continued ingestion of cocaine could induce tolerances and physiological dependency, which leads to its abuse. Cocaine can be ingested intravenously, nasally, orally or by smoking and is excreted in the urine primarily as benzoylecgonine in a short period. Benzoylecgonine has a biological half-life of 5 – 8 hours, which is much longer than that of cocaine (0.5 – 1.5 hours), and can be generally detected 12 – 72 hours after cocaine use or exposure.

Methamphetamine: Methamphetamine, commonly referred to on the street as *speed*, *meth*, *ice*, *crystal*, or *glass*, is a synthetically produced central nervous system stimulant that produces effects similar to cocaine. Because it metabolizes much slower than cocaine, methamphetamine has longer lasting effects. The acute effect of methamphetamine generally last 2-4 hours, and half-life of the drug in the body is 9-24 hour. It produces a number of dose-related effects including increased alertness and euphoria, as well as increases in heart rate, blood pressure, respiration, and body temperature. Agitation, tremors, hypertension, memory loss, hallucinations, psychotic episodes, paranoid delusions, and violent behavior can result from chronic abuse. Withdrawal from high doses of methamphetamine often produces severe depression. Methamphetamine may be injected, ingested orally, snorted, or smoked. Methamphetamine is excreted in the urine as amphetamine, deaminated and/or oxidized derivatives, but 10-40% of methamphetamine is excreted in the urine as unchanged molecules. Methamphetamine is generally detectable in the urine for 3-5days after use.

Phencyclidine: PCP (Arylcyclohexylamine) is mostly used as an animal anesthetic drug. PCP, when abused is a hallucinogen which interacts with the dopamine, cholinergic and adrenergic systems. It has dose dependent stimulant, depressant, hallucinogenic and psychological effects. PCP is mostly administered orally or intravenously. Even moderate amounts of PCP, from 5ng/ml to 100ng/ml, can result in psychotic, violent and self-destructive behavior. At high doses, from 100ng/ml to 500ng/ml, PCP can cause convulsions, hypertension, prolonged coma, absent peripheral sensation and even death. PCP is metabolized via hydroxylation, oxidation, and conjugation with glucuronic acid in the liver. About 10% of the dose is excreted in urine as a parent compound, Phencyclidine, which can be detected in the urine for 7 to 8 days after drug administration. For chronic users, PCP may persist in urine for 2 to 4 weeks.

Barbiturates: Barbiturates (Secobarbital) are a class of organic compounds that were developed in the late 19th century for the treatment of anxiety and insomnia. Barbiturates traditionally have been classified into 4 categories according to their duration of action: ultra–short-acting barbiturates (eg, thiopental, thiamylal, methohexital), short-acting barbiturates (eg, secobarbital, pentobarbital), intermediate-acting barbiturates (eg, amobarbital, butalbital, butabarbital, aprobarbital), and long-acting barbiturates (eg, phenobarbital, mephobarbital). Among them, short-acting barbiturates, pentobarbital and secobarbital, are the preferred drugs of abuse. Common street names, depending on the barbiturate, are blue birds (amobarbital), red devils, pink ladies, goofballs (secobarbital) and yellow jackets (pentobarbital). Barbiturates are dangerous drugs, with a narrow therapeutic index between the dose required for sedation and the dose that will cause coma and death. They are physiologically addictive if taken in high doses over 1 month or more, and the abstinence syndrome can be life-threatening. Barbiturates suppress the activity of all excitable tissue, including the CNS, the peripheral nervous system, and the cardiovascular system. They also depress gastrointestinal function and have a number of effects on hepatic function. Their effects on the respiratory system are due mainly to CNS inhibition. Short-acting barbiturates are metabolized entirely by the liver and are excreted in the urine as hydroxylated, dealkylated and conjugated metabolite, with only 3-6% remaining active before excretion. Detection times in the urine vary depending on the ingested barbiturate from a day or less to weeks. Intermediate and short acting barbiturate can be detected for 2-4 days after ingestion.

Amphetamine: Amphetamine is commonly referred to on the street as speed, ice, or glass, is a synthetically produced central nervous system stimulant that produces effects similar to but longer lasting than cocaine. From a pure chemical standpoint, amphetamine is structurally similar to other biogenic amines, which account not only for its biological activity but also for some of the analytical challenges it poses. A number of precursor compounds, which can be used for legitimate reason, are metabolized by the body to amphetamine or methamphetamine. The acute effect of amphetamine generally lasts 2-4 hours, and half-life of the drug in the body is 9-24 hours. It produces a number of dose-related effects including increased confidence, exhilaration, and alertness, along with increased aggression, irritability and feelings of paranoia. High doses of amphetamine, especially if repeated frequently over a period of several days, can produce delirium, panic, hallucinations and

feelings of persecution. Withdrawal from high doses of methamphetamine often produces severe depression. Amphetamine may be injected, ingested orally, snorted, or smoked. Significant amounts of amphetamine are converted to phenylaceton, followed by oxidation to benzoic acid, part of which conjugates to form hippuric acid. Glucuronide conjugate of benzoate and hydroxyl forms of the conjugate are also formed and excreted in the urine. Normally about 30 % of amphetamine is excreted in the urine as unchanged molecules and generally detectable in the urine for 3-5 days after use.

Methadone: Methadone was synthesized by German scientists during World War II because of a shortage of morphine. Although chemically unlike morphine or heroin, methadone produces many of the same effects. Introduced into the United States in 1947 as an analgesic (Dolophinel), it is primarily used today for the treatment of narcotic addiction. It is available in oral solutions, tablets, and injectable Schedule II formulations, and is almost as effective when administered orally as it is by injection. Methadone's effects can last up to 24 hours, thereby permitting once-a-day oral administration in heroin detoxification and maintenance programs. High-dose methadone can block the effects of heroin, thereby discouraging the continued use of heroin by addicts under treatment with methadone. Most of individuals give positive result for methadone has prescription or under methadone maintenance program. Chronic administration of methadone results in the development of tolerance and dependence. Over dosage effects of Methadone is similar to Opiates intoxication, including respiratory depression, hypotension circulatory collapse, and coma. The withdrawal syndrome develops more slowly and is less severe but more prolonged than that associated with heroin withdrawal. Major urinary excretion compounds of Methadone ingestion are Methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrroline (EDDP) and 2-ethyl-,5-methyl-3,3-diphenylpyrroline (EMDP). The ratio of these excretion compounds in urine is highly variable depending on the urine pH, dose, and volume of individual variation. In methadone maintenance patients, 24 hours after receiving Methadone, 5-50% of total dose of methadone is present in urine as parent molecules. The remainder of dose is excreted in the urine EDDP (3-35% of total dose) and EDMP (less than 1% of total dose).

Tricyclic Antidepressants: Tricyclic Antidepressants (TCAs) historically have been the drugs of choice for the treatment of major depressive illness. TCAs are used for the treatment of a variety of adult conditions (eg, depression, chronic pain disorders) and pediatric conditions (eg, enuresis, attention deficit hyperactivity disorder). The report of data from the American Association of Poison Control Centers (AAPCC) lists TCA as the leading cause of death in the United States. Cyclic antidepressants are classified according to their aromatic ring structure. TCAs contain a 3-ring aromatic nucleus and include amitriptyline, nortriptyline, imipramine, desipramine, clomipramine, and doxepin. Amoxapine, a tricyclic dibenzoxazepine, and maprotiline, a tetracyclic, vary structurally but retain a similar pharmacologic profile to TCAs. Although the exact mechanism by which TCAs exert their antidepressant effects is unknown, the ability of TCAs to block reuptake of centrally active neurotransmitters, such as norepinephrine and serotonin, is believed to be of primary importance in the antidepressant effects. The progressive symptomatology of TCA includes agitation, confusion, hallucinations, hypertonicity, seizures, and EKG changes. The half-life of TCA varies from few hours to few days.

Benzodiazepaines: Benzodiazepines are among the most frequently prescribed of all drugs and have been used for their anxiolytic, anticonvulsant, and sedative/hypnotic properties. More than two dozen varieties of benzodiazepines are available by prescription in the USA. Even though the benzodiazepines are rarely used as "party" or "good time" drugs, their usefulness is limited by a broad range of side effects comprising sedation, ataxia, amnesia, alcohol and barbiturate potentiation, tolerance development and abuse potential. Overdosage of the drug produces effects similar to ethanol intoxication, including drowsiness, ataxia, dysphoria, hypnosis and coma. If benzodiazepine is taken together with other depressants or alcohol, it can cause serious problems including coma or death. Benzodiazepine can be present in the urine as the parent form, dealkylated, hydroxylated or conjugated metabolites. Most of the long acting benzodiazepine including diazepam, nordiazepam and chlordiazepam are excreted in urine as parent molecules and oxazepam. A detection time for benzodiazepines in urine is highly variable depending on the compound structure. Long acting benzodiazepine (diazepam, nordiazepam, and oxazepam) can be detectable in the urine for weeks to months after chronic use. Short acting benzodiazepines (alprazolam, triazolam) might only be detected for few days.

3. PRINCIPLE

The Nano-Check™ DAT 10M test is a one step, immunochromatographic assay for the qualitative detection of Cannabinoid, Opiate, Cocaine, Methamphetamine, Phencyclidine, Barbiturates, Amphetamine, Methadone, Tricyclic antidepressant, Benzodiazepines compounds and their metabolites. The Nano-Check™ DAT 10M test device contains a membrane strip on which either antibodies against drug or drug conjugate to protein are immobilized at each specific test line. The colored indicator antibody or antigen coupled with gold colloidal particles is placed at the end of the membrane. When the test urine is applied to the sample well of the device, the colored indicator particles move along with urine sample across the membrane by the capillary action. If any of the specified drugs is in the urine specimen, they compete with either the colored indicator drug conjugate or drug antibodies for the limited amount of antibodies or drug conjugates immobilized on the membrane. If an amount equal to or greater than the cut off concentration of drug or its metabolite is present in the urine sample, the drug compounds will prevent the binding of drug conjugate to the target antibody. Absence of colored band at a specific drug line indicates a positive result, while the presence of colored band indicates a negative result for the specific drug. The control line is present for a self-procedure validation control. If the control line does not appear, the test result is not valid.

4. REAGENT AND MATERIALS

Provided

1. Test device containing membrane strip
2. One disposable sample dropper
3. Instruction for use

Required but not provided

1. Urine collection cup
2. Timer

Optional

1. Nano-Checker™ 710 Reader

5. STORAGE AND STABILITY

The test kit should be stored at 2°C to 30°C in the original sealed pouch for the duration of the shelf life.

6. PRECAUTIONS

1. For *in vitro* diagnostic use only
2. For professional use only
3. Urine specimens may be potentially infectious. Proper handling and disposal methods should be established.
4. Avoid cross-contamination of urine samples by using a new specimen collection container and specimen pipette for each urine sample.
5. Do not use test kit if the pouch is damaged or improperly sealed.
6. Do not use test kit beyond expiration date.
7. Test kit does NOT contain any materials used for pre-drug or drugs of abuse.

7. SPECIMEN COLLECTION AND PREPARATION

The Nano-Check™ DAT 10M test is formulated for use with urine specimens. Fresh urine does not require any special handling or pretreatment but the urine specimen should be collected in a clean plastic or glass container. Approximately 80 ul of urine sample is required for each well. The specimen may be refrigerated at 2°C to 8°C for 2 days, or frozen at - 20°C for a longer period of time. Specimens that have been refrigerated or frozen must be equilibrated to room temperature prior to testing. Urine specimens showing large amount of precipitate or turbidity should be clarified by centrifugation or allowed to settle before testing.

Note: Urine specimens and all materials contacted with urine specimens should be handled and disposed of as if it is infectious and capable of transmitting infection. Avoid contact with skin by wearing gloves and proper laboratory attire.

8. TEST PROCEDURE AND PROTOCOL

1. Review "Specimen Collection" instructions. Test device and patient's samples should be brought to room temperature (20°C to 30°C) prior to testing. Do not open pouches until ready to perform the assay.

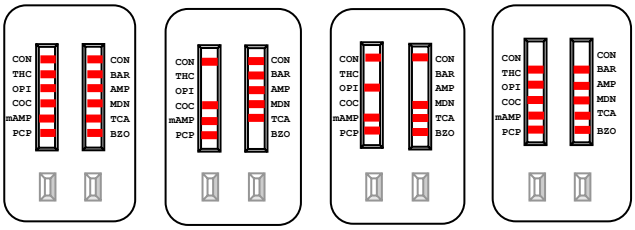
2. Remove the test device from the sealed pouch immediately before use. Label the device with patient or control identification.

3. Holding the dropper vertically and firmly squeeze the top bulb, place tip in the sample and release to draw specimen into the pipette. Excess specimen will spill into the second bulb.

4. Firmly squeeze the top bulb to dispense 80µL of the urine sample into each sample well. The excess amount in the second bulb will not be dispensed.

5. More than one colored band(s) will appear depending on the concentration of target drugs in the test specimen in 5 to 10 minutes. Negative results may be observed in as short as 2 minutes but must wait 10 minutes to confirm positive results. Do not interpret results after 10 minutes. Avoid cross-contamination of urine samples by using a new specimen collection container and specimen pipette for each urine sample.

9. INTERPRETATION OF RESULTS



The lines in the test region (THC, OPI, COC, mAMP, PCP, BAR, AMP, MDN, TCA, BZO) are drug probed. A red band in control region (CON) is used to indicate the validity of the test performance.

***Negative*:** The appearance of a red band at the control line and at the drug probed line(s) indicate a negative result for specific target drug(s). The color intensity of the test line may be weaker or stronger than that of the control line. Any faint visible bands at the drug probed lines at 10 minutes after urine sample application should be considered negative.
A negative result does not mean the absolute absence of drugs in the specimen, it only indicates that the specimen does not contain drugs at concentrations equal to or above the cut off level in qualitative terms.

***Positive*:** Absence of a red band at the drug probed line and presence of a red band at the control line indicates a positive result for a specific drug. In other words, the specimen may contain the drug or its metabolites at a concentration above the cutoff level. Examples of positive results are shown in the figure.

***Invalid*:** Under no circumstances should a positive sample be identified until the control line appears in the test window. If the colored line does not appear in the control region (CON), the test result is invalid and inconclusive. The assay should be repeated with a new test strip.

Read the results at 10 minutes with the naked eye or by using the Nano-Checker™ 710 Reader. Follow the procedure in the Nano-Checker™ 710 Reader user manual.

10. QUALITY CONTROL

The test kit has a built-in control. A red colored line in the control region is a positive procedural control and the clear background in the test window after performing the test is a negative procedural control. Good laboratory practice recommends the use of control materials to ensure proper test performance. Quality control specimens are available from commercial sources. Controls should be tested at intervals using a more confirmative method other than immunoassay. When testing the positive and negative controls, use the same assay procedure as with a urine specimen.

11. LIMITATIONS OF PROCEDURE

- The assay kit is designed to be used for drugs of abuse test with unadulterated human urine only.
- Adulterant compounds may produce erroneous results regardless of the method of analysis procedure. If adulteration is suspected, the test should be repeated with fresh samples.
- A positive result with any of the tests indicates the presence of a drug/metabolite at the concentration at or above cutoff level only and does not indicate or measure intoxication.
- There is a possibility that technical or procedural error as well as other substances or factors not listed may interfere with the test and cause false results. See the **CROSS-REACTIVITY SECTION** for lists of substances that may produce positive results, or that do not interfere with test performance.
- If it is suspected that the sample has been mislabeled or tampered with, the test should be repeated with a new specimen.

12. PERFORMANCE CHARACTERISTICS

1. Sensitivity

The Nano-Check™ DAT 10M test can detect the listed compounds in human urine at the following cut-off concentrations in **Table 1.** The cutoff value was determined using control urines spiked with the specified drugs at the indicated concentration; 0, 25%, 50 % below cut off level, and 25%, 50% above cut off level. The test results are listed in **Table 2.**

Table 1 Cut off level of Tested Drugs

Compounds Name	Cut off level
11-nor-Δ^9 THC-9 COOH (**THC**)	50 ng/ml
Morphine (**OPI**)	300 ng/ml
Benzolecognine (**COC**)	300 ng/ml
D-Methamphetamine (**mAMP**)	1000 ng/ml
Phencyclidine (**PCP**)	25 ng/ml
Secobarbital (**BAR**)	300 ng/ml
D-Amphetamine (**AMP**)	1000 ng/ml
Methadone (**MDN**)	300 ng/ml
Nortriptyline (**TCA**)	1000 ng/ml
Oxazepam (**BZO**)	300 ng/ml

Table 2 Cut off Validation Study

Drug	Level (ng/ml)	Negative	Positive	Coincidence (%)
THC	0	25	0	100
	25	25	0	100
	37.5	20	5	80
	50	15	10	N/A
	62.5	1	24	96
	75	0	25	100
OPI (Morphine)	0	25	0	100
	150	25	0	100
	225	25	0	100
	300	22	3	N/A
	375	3	22	88
	450	0	25	100
COC (Benzoylecgonine)	0	25	0	100
	150	25	0	100
	225	21	4	84
	300	11	14	N/A
	375	1	24	96
	450	0	25	100
mAMP (Methamphetamine)	0	25	0	100
	500	25	0	100
	750	25	0	100
	1000	14	11	N/A
	1250	3	22	88
	1500	0	25	100

PCP (Phencyclidine)	0	25	0	100
	12.5	25	0	100
	18.5	20	5	80
	25	10	15	N/A
	31.5	1	24	96
	37.5	0	25	100
BAR (Secobarbital)	0	25	0	100
	150	25	0	100
	225	22	3	88
	300	18	7	N/A
	375	4	21	84
	450	0	25	100
AMP (Amphetamine)	0	25	0	100
	500	25	0	100
	750	23	2	92
	1000	19	6	N/A
	1250	5	20	80
	1500	0	25	100
MDN (*Methadone*)	0	25	0	100
	150	25	0	100
	225	22	3	88
	300	15	10	N/A
	375	3	22	88
	450	0	25	100
TCA (Nortriptyline)	0	25	0	100
	500	25	0	100
	750	23	2	92
	1000	17	8	N/A
	1250	4	21	84
	1500	0	25	100
BZO (Oxazepam)	0	25	0	100
	150	25	0	100
	225	21	4	84
	300	16	9	N/A
	375	4	21	84
	450	0	25	100

2. Cross Reactivity

The following compounds were tested to determine cross reactivity at the cut off concentration of target drug using Nano-Check™ DAT 10M test. The test results are summarized in **Table 3**.

Table 3 Cross Reactivity Study

Test	Compounds	Cut-off (ng/ml)	Cross Reactivity (%)
THC	11-Nor-Δ^9-THC-2-COOH	50	100
	11-Nor-Δ^8-THC-2-COOH	75	67
	Δ^9-Tetrahydrocannabinol	>100,000	<0.05
	Δ^8-Tetrahydrocannabinol	>100,000	<0.05
	11-Hydroxy-Δ^9-THC	>10,000	<0.5
	Cannabinol	>100,000	<0.05
OPI	Morphine	300	100
	Codein	300	100
	Hydrocodone	500	60
	Hydromorphone	500	60
	Morphine-3β-D-glucuronide	5000	6
	Nalorphine	500	60
	Oxycodone	1000	30
	Procaine HCl	>100,000	<0.3
	Oxymorphone	5000	6
	Ofloxacin	>100,000	<0.3
	6-Acetylmorphine	500	60
COC	Benzoylecgonine	300	100
	Cocaine HCl	250	120
	Ecgonine	25,000	1.2
mAMP	d-Methamphetamine	1,000	100
	d-Amphetamine	>100,000	<1
	dl-Amphetamine	>100,000	<1
	l-Amphetamine	>100,000	<1
	(±) Ephedrine	>100,000	<1
	(-)Ephedrine	>100,000	<1
	Methylenedioxymethamphetamine(MDMA)	1,000	100
	Methylenedioxyamphetamine(MDA)	>100,000	<1
PCP	Phencyclidine	25	100
	Ibuprofen	>100,000	<0.025
	Thienylcyclohexylpiperidine(TCP)	4,000	0.6
	Hydromorphone	50,000	0.05
BAR	Amobarbital	5,000	6
	Butabarbital	2,000	15
	Pentobarbital	2,000	15
	Phenobarbital	8,000	3.75
	Secobarbital	300	100
AMP	d-Amphetamine	1,000	100
	dl-Amphetamine	5,000	20
	l-Amphetamine	100,000	1
	(±) Ephedrine	>100,000	<1
	(-)Ephedrine	>100,000	<1
	Methylenedioxymethamphetamine(MDMA)	>100,000	<1
	Methylenedioxy-amphetamine (MDA)	2,500	40
MDN	EDDP	500	60
	EMDP	100,000	0.3
	LAAM	500	60
	Meperidine	5,000	6
	Methadone	300	100
TCA	Amitriptyline	2,500	40
	Chlorpromazine	5,000	20
	Clomipramine	5,000	20.0
	Desipramine	1,000	100
	Diphenhydramine	>100,000	<1.0
	Dothiepin	2,500	40
	Doxepin	5,000	20
	Imipramine	1,000	100
	Nortriptyline	1,000	100
	Protriptyline	7,500	13.3
	Trimipramine	10,000	10
BZO	Alprazolam	1,250	24.0
	Bromazepam	1,000	30.0
	Chlordiazepoxide	100,000	0.3
	Clobazam	1,000	30.0
	Clonazepam	3,000	10.0
	Diazepam	500	60.0
	Estazolam	1,000	30.0
	Flurazepam	>100,000	<0.3
	Flunitrazepam	500	60.0
	Lorazepam	1,250	24.0
	Lometazepam	2,000	15.0
	Midazolam	10,000	3
	Nitrazepam	100	300
	Nordiazepam	500	60
	Oxazepam	300	100
	Prazepam	100,000	0.3
	Temazepam	100	300
	Triazolam	2,000	15.0

3. Accuracy

Clinical samples containing Cannabinoid, Opiate, Cocaine, Methamphetamine, Phencyclidine, Barbiturates, Amphetamine, Methadone, Tricyclic antidepressant, and Benzodiazepines confirmed by GC/MS were analyzed using the Nano-Check ™ DAT 10M test panel.
The results are shown in **Table 4.**

Table 4 Accuracy study

		GC/MS	
		Positive	Negative
Nano-Check™ DAT 10M, THC	Positive	80	5
	Negative	3	262
	Total	83	267
	Agreement	96.4%	98.1%
	Total agreement	97.7%	
Nano-Check™ DAT 10M, OPI	Positive	82	1
	Negative	3	264
	Total	85	265
	Agreement	96.5%	99.6%
	Total agreement	98.9%	
Nano-Check™ DAT 10M, COC	Positive	86	4
	Negative	1	259
	Total	87	263
	Agreement	98.9%	98.5%
	Total agreement	98.6%	
Nano-Check™ DAT 10M, mAMP	Positive	76	2
	Negative	2	270
	Total	78	272
	Agreement	97.4%	99.3%
	Total agreement	98.9%	
Nano-Check™ DAT 10M, PCP	Positive	50	1
	Negative	1	298
	Total	51	299
	Agreement	98.0%	99.7%
	Total agreement	99.4%	

Nano-Check™ DAT 10M, BAR	Positive	75	8
	Negative	3	293
	Total	78	301
	Agreement	96.2%	97.3%
	Total agreement	97.1%	
Nano-Check™ DAT 10M, AMP	Positive	59	7
	Negative	3	310
	Total	62	317
	Agreement	95.2%	96.5%
	Total agreement	97.4%	
Nano-Check™ DAT 10M, MDN	Positive	68	6
	Negative	3	302
	Total	71	308
	Agreement	95.8%	98.1%
	Total agreement	97.6%	
Nano-Check™ DAT 10M, TCA	Positive	49	6
	Negative	3	321
	Total	52	327
	Agreement	94.2%	98.2%
	Total agreement	97.6%	
Nano-Check™ DAT 10M, BZO	Positive	71	6
	Negative	4	298
	Total	75	304
	Agreement	94.7%	98.0%
	Total agreement	97.4%	

13. INTERFERENCE TEST

The following substances did not interfere with the Nano-Check™ DAT 10M test.

Glucose	2000mg/dl
Human albumin	2000mg/dl
Human hemoglobin	10mg/dl
Urea	4000mg/dl
Uric acid	10mg/dl

14. CROSS ACTIVITY

Following listed compounds were not detected by the Nano-Check™ DAT 10M Test at a concentration of 100 µg/ml.

(-)-Arterenol
(-)-Cotinine
(-)-Deoxyephedrine
(-)-Isoproterenol
(-)-Scopolamine
(+)-Amethopterin
(+)-Brompheniramine
(+)-Chlorpheniramine
(+)-Isoproterenol
(+)-Propoxyphene
(S)-6-Methoxy-d-Methyl-2-Naphthaleneacetic Acid
Chlorpheniramine
Metoprolol
Phenylpropanolamine
Verapamil
2-Methyl-3-(3,4-Dihydroxyphenyl)-dl-Alanine
2-Methyl-3-(3,4-Dihydroxyphenyl)-l-Alanine
2-Propylpentanoic Acid
4-Dimethylaminoantipyrine
5,5-Diallylbarbituric Acid
5,5-Diphenylhydantoin
5-Hydroxyindole-2-Carboxylic Acid
5-Hydroxyindole-3-Acetic Acid
Acetaminophen
Acetone
Acetylsalicylic Acid (Asprin)
Albumin
Amikacin
Aminopyrine
Amoxicillin
Ampicillin
Apomorphine
Aspartame
Atropine
Benzoic Acid
Benztropine Methane Sulfonate
Betethal
Bilirubin
Bromocriptine Mesylate
Caffine
Cannibidiol
Cephalexin
Chloramphenicol
Chloroquine
Chlorpropamide
Chlorprothixene
Cimetidine
cis-Thiothixene
Clemastine
Clomipramine
Clonidine
Creatinine
Cyclizine
Cyclosporin A
Cyproheptadine
d-Aspartic Acid
Diflunisal
Digoxin
Diphenoxylate
dl-Aminoglutethimide
dl-Aspartic Acid
dl-Glutethimide
dl-Trihexyphenidyl
Erythromycin
Estriol
Estrone-3-Sulfate
Ethanol
Ethosuximide
Ethylenediaminetetraacetic Acid
Ethyl-p-Aminobenzoate
Fenfluramine
Fenoprofen
Fentanyl
Furosemide
Gentamicin
Gentisic Acid
Glucose
Griseofulvin
Guaiacol Glyceryl Ester
Human Hemoglobin
Hydrochlorothiazide
Indole-3-Acetic Acid
Indole-3-Butyric Acid
Indomethacin
Isoxsuprine
Kanamycin
Ketamine
Ketoprofen
l-Ascorbic Acid (Vitamin C)
l-Aspartic Acid
Lidocaine
Lithium Carbonate
l-Phenylalanine
l-Phenylephrine
Melanin
Mescaline
Methylphenidate
Methyprylon
Metoclopramide
N-Acetylprocainamide
Nafcillin
Naloxone
Naphazoline
Naproxen
Niacinamide
Nialamide
Nicotinic Acid
Nifedipine
Nomifensine
Nylidrin
o-Hydroxyhippuric Acid
Orphenadrine
Oxalic Acid

Papaverine
Penicillin G
Pentazocine
Phenelzine
Pheniramine
Phenothiazine
Phentermine
Phenylacetone
Pheylbutazone
Piroxicam
Potassium Chloride
Prednisolone
Primidone
Procainamide
Pyrilamine
Quinidine
Quinine
Riboflavin
Salicylic Acid
Sodium Chloride
Terbutaline
Tetracycline
Tetraethylthiuram Disulfide (Antabuse)
Tetrahydrozoline
Thioridazine
Tobramycin
Triamterene
Trimethoprim
Trimipramine
Vancomycin
Zomepirac

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