Rapid Response™

Xylazine Test Strip
(Urine)

REF XYL-1S27-100 Product Insert

A rapid test for the qualitative detection of Xylazine (XYL) in human urine.

For forensic use only.

Intended Use

The Rapid Response™ Xylazine Test Strip (Urine) is a rapid chromatographic immunoassay for the detection of xylazine in urine at the following cut-off concentration:

Parameter	Calibrator	Cut-off(ng/mL)
XYL (Xylazine)	Xylazine	100

This assay provides only a qualitative, 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) or Liquid Chromatography mass spectrometry (LC/MS) is the preferred confirmatory method. Clinical consideration and professional judgment should be applied to any drug test result, particularly when preliminary positive results are used.

Summary

Xylazine was synthetized in 1962 and found to be a potent central α_2 adrenergic receptor agonist. The drug causes sedation and anesthesia, respiratory depression, slow heart rate and muscle relaxation. It also causes significant slowing of the heart rate and low blood pressure. Due to these side effects, it was not FDA approved for human use. Xylazine has found use as an animal "takedown" agent and anesthetic and is known by the trade name Rompun™. In the early 2000s, xylazine became an illicit drug in Puerto Rico and was added to heroin or included in "speedballs" as an addition to or as a substitute for heroin. Xylazine was called anesthesia de caballo (horse anesthesia) on the street. Since it has many of the same effects as opioids, it could be substituted for the opioid or the two together have additive effects. Since then, the drug has shown up intermittently with the National Forensic Lab Information System and between 2006-2018; the Philadelphia Medical Examiners Office recorded increasing incidence of cases. [1,2]
The Rapid Response™ Xylazine Test Strip (Urine) is a rapid Drug screening test that can be performed without the use of an instrument. The test utilizes the antibody to selectively detect elevated levels of xylazine in urine. The Rapid Response™ Xylazine Test Strip (Urine) yields a positive result when the xylazine in urine exceeds the cut-off level.

Principle

The Rapid Response™ Xylazine Test Strip (Urine) detects drugs through visual interpretation of color development on the test strip. Drug conjugates are immobilized on the test region of the membrane. During testing, the specimen reacts with antibodies conjugated to colored particles and precoated on the sample pad. The mixture then migrates through the membrane by capillary action and interacts with reagents on the membrane. If there are insufficient drug molecules in the specimen, the antibody-colored particle conjugate will bind to the drug conjugates, forming a colored band at the test region of the membrane. Therefore, a colored band appears in the test region when the urine is negative for the drug. If drug molecules are present in the urine above the cut-off concentration of the test, they compete with the immobilized drug conjugate on the test region for limited antibody binding sites. This will prevent attachment of the antibody-colored particle conjugate to the test region. Therefore, the absence of a colored band at the test region indicates a positive result. The appearance of a colored band at the control region serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

Reagents

The test contains mouse monoclonal anti-XYL antibody-coupled particles and XYL-protein conjugate. A goat anti-rabbit IgG and rabbit IgG are used in the control line system.

Precautions

- There are no direct therapeutic or diagnostic claims being made for this product. These tests are not involved in diagnosing, treating, mitigating, or preventing a disease, disorder or symptom in human being, nor do they restore, modify or correct a body structure, function of the human body.
- Do not use after expiration date indicated on the package. Do not use the test if its foil pouch is damaged. Do not reuse tests.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest or inhale).
- Read the entire procedure carefully prior to performing any tests.
- Do not eat, drink or smoke in the area where the samples and kits are handled. It is recommended to wear protective clothing such as disposable gloves and eye protection when handling harmful substances.
- Humidity and temperature can adversely affect results.
- The used testing materials should be discarded in accordance with local, state and/or federal regulations.
- This test has been tested for extreme shipping conditions and its performance has not been impacted.
- The kit should be stored at 36-86°F (2-30°C) until the expiry date printed on the sealed pouch.
- For individually packed test, the test should remain in the sealed pouch until use.
- If any serious incident has occurred in relation to this test shall be reported to us and the competent authority of the Member State in which the user and/or the patient is established.

Storage and Stability

- The kit should be stored at 36-86°F (2-30°C) until the expiry date printed on the sealed pouch.
- The test must remain in the sealed pouch until use.
- **Do not freeze.**
- Care should be taken to protect the components of the kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

Specimen Collection and Preparation

Urine Assay

The urine specimen must be collected in a clean and dry container. Urine collected at any time of the day may be used. Urine specimens exhibiting visible particles should be centrifuged, filtered, or allowed to settle to obtain a clear specimen for testing.

Specimen Storage

Urine specimens may be stored at 36-46°F (2-8°C) for up to 48 hours prior to testing. For prolonged storage, specimens may be frozen and stored below -4°F (-20°C). Frozen specimens should be thawed and mixed before testing.

Materials

Materials provided

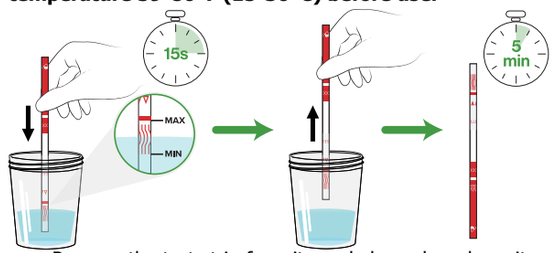
- Test Strip
- Package insert

Materials required but not provided

- Specimen collection container
- Timer

Directions for Use

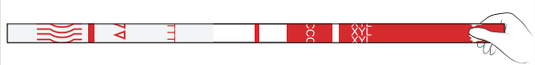
Bring tests, samples, buffer and/or controls to room temperature 59-86°F (15-30°C) before use.



1. Remove the test strip from its sealed pouch and use it as soon as possible. For best results, the test should be performed within one hour.
2. Hold the strip by the end, where the product name (XYL) is printed. To avoid contamination, do not touch the strip membrane (the white section of the strip).
3. Holding the strip vertically, dip the test strip in the liquid for at least 10-15 seconds. Immerse the strip where the wavy lines are, but not above the solid (maximum) line on the test strip.
4. Remove the strip from the sample and place it on a non-absorbent flat surface. Start the timer and wait for the colored line(s) to appear.

Interpretation of Results

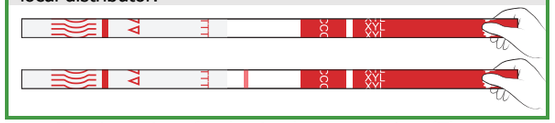
Positive
One colored line appears in the control line region (C). No line appears in the test line region (T). This positive result indicates that the xylazine concentration exceeds the detectable cut-off level.



Negative
Two lines appear. One colored line should be in the control line region (C), and another apparent colored line should be in the test line region (T). This negative result indicates that the xylazine concentration is below the detectable cut-off level.

Invalid

Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit immediately and contact your local distributor.



1. The intensity of color in the test region (T) may vary depending on the concentration of analytes present in the sample. Therefore, any shade of color in the test region should be considered negative. Note that this is a qualitative test only and cannot determine the concentration of analytes in the sample.
2. Insufficient sample volume, incorrect operating procedure or expired tests are the most likely reasons for control line failure.

Quality Control

Internal Procedural Controls

Internal procedural controls are included in the test. A colored band appearing in the control region (C) is considered an internal positive procedural control, confirming sufficient specimen volume and correct procedural technique.

External Positive and Negative Controls

External controls are not supplied with this kit. It is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

Limitations

1. The Rapid Response™ Xylazine Test Strip (Urine) provides only a qualitative, preliminary analytical result. A secondary analytical method must be used to obtain a confirmed result. Gas chromatography mass spectrometry (GC/MS) or Liquid Chromatography mass spectrometry (LC/MS) is the preferred confirmatory method.[3,4]
2. It is possible that technical or procedural errors, as well as other interfering substances in the urine specimen may cause erroneous results.
3. Adulterants, such as bleach and/or alum, in urine specimens may produce erroneous results regardless of the analytical method used. If adulteration is suspected, the test should be repeated with another urine specimen.
4. A positive result indicates presence of the drug or its metabolites but does not indicate level of intoxication, administration route or concentration in urine.
5. A negative result may not necessarily indicate drug-free urine. Negative results can be obtained when drug is present but below the cut-off level of the test.
6. Test does not distinguish between drugs and certain medications.

Expected Values

A negative result indicates that the xylazine concentration is below the detectable level.
Positive result means the concentration of xylazine is above the level.

Performance Characteristics

Analytical Sensitivity

A drug-free urine pool was spiked with xylazine at the following concentrations: 0% cutoff, -50% cutoff, -25% cutoff, cutoff, +25% cutoff, +50% cutoff and 3X cutoff. The result demonstrates >99% accuracy at 50% above and 50% below the cut-off concentration. The data are summarized below:

Xylazine Concentration	n	Strip	
		Negative	Positive
0% cutoff	30	30	0
-50% cutoff	30	30	0
-25% cutoff	30	27	3
Cut-off	30	17	13
+25% cutoff	30	4	26
+50% cutoff	30	0	30
3X cutoff	30	0	30

Analytical Specificity

The following table lists compounds that are positively detected in urine by the Rapid Response™ Xylazine Test Strip (Urine) at 5 minutes.

Compound	Concentration (ng/mL)
Xylazine	100 (100%)
4-Hydroxy Xylazine	5 (2000%)
3-Hydroxy Xylazine	5 (2000%)
Cimetidine	100,000 (0.1%)
(1R, 2S) - (-)-Ephedrine	100,000 (0.1%)
Clonidine	100,000 (0.1%)
Benztropine	100,000 (0.1%)
Nimesulide	100,000 (0.1%)
Tetrahydrozoline	100,000 (0.1%)
Codeine	100,000 (0.1%)
Levorphanol	100,000 (0.1%)
(-) Epinephrine	100,000 (0.1%)
Heroin	100,000 (0.1%)
D, L-Homatropine Hydrobromide	100,000 (0.1%)
Gatifloxacin	100,000 (0.1%)
S-(-)-Nicotine	100,000 (0.1%)

Precision

A study was conducted at three labs by untrained operators using three different lots of products to demonstrate the within run, between run and between operator precision. An identical panel of coded specimens containing, no Xylazine, 25%Xylazine above and below the cut-off and 50% Xylazine above and below the cut-off was provided to each site. The following results were tabulated:

Xylazine Concentration	n per Site	Site A		Site B		Site C	
		-	+	-	+	-	+
0% cutoff	10	10	0	10	0	10	0
-50% cutoff	10	10	0	10	0	10	0
-25% cutoff	10	9	1	9	1	9	1
+25% cutoff	10	1	9	1	9	2	8
+50% cutoff	10	0	10	0	10	0	10

Effect of Urinary Specific Gravity

Fifteen urine specimens of normal, high, and low specific gravity ranges were spiked with -50% cutoff and +50% cutoff of xylazine. The Rapid Response™ Xylazine Test Strip (Urine) was tested in duplicate using the fifteen neat and spiked urine specimens. The results demonstrate that varying ranges of urinary specific gravity do not affect the test results.

Effect of Urinary pH

The pH of an aliquoted negative urine pool was adjusted to a pH range of 5 to 9 in 1 pH unit increments and spiked with xylazine to -50% cutoff and +50% cutoff. The spiked, pH-adjusted urine was tested with the Rapid Response™ Xylazine Test Strip (Urine) in duplicate. The results demonstrate that varying ranges of pH do not interfere with the performance of the test.

Cross-Reactivity

A study was conducted to determine the cross-reactivity of the test with compounds in either drug-free urine or xylazine positive urine. The following compounds show no cross-reactivity when tested with the Rapid Response™ Xylazine Test Strip (Urine) at a concentration of 100 µg/mL.

Acetaminophen	Isoxsuprine
Acetone	Kanamycin
Acetophenetidin	Ketoprofen
Aspirin	Labetalol
Albumin	Lidocaine
Amoxapine	Lindane
Amoxicillin	Loperamide
Ampicillin	Methoxyphenamine
Ascorbic acid	Metoprolol
Aspartame	Nalidixic acid
Atropine	(+)-Naproxen
Benzoic acid	Norethindrone
Bilirubin	Noscapine
(+/-) Brompheniramine	Niacinamide
Benzocaine	Norephedrine
Buspirone	Orphenadrine
Caffeine	Oxalic acid
Chloramphenicol	Oxolinic acid
Chloroquine	Oxymetazoline
(+/-)-Chlorpheniramine	Papaverine
S- (+)-Chlorpheniramine maleate salt	Pemoline
Chlorpromazine	Penicillin-G
Chlorprothixene	Perphenazine
Clomipramine	Phenelzine
Creatine	Pheniramine
Cyclobenzaprine	Phenothiazine
Dextromethorphan	β-Phenylethylamine
Diclofenac	Procaine
Dicyclomine	Promethazine
Diflunisal	Quinacrine
Digoxin	Quinidine
4-Dimethylaminoantipyrine	Ranitidine
Diphenhydramine	Riboflavin
5,5-Diphenylhydantoin	Sodium chloride
Disopyramide	Sulfamethazine
Doxylamine	Sulindac
Dopamine	Temazepam
Erythromycin	Tetracycline
Ethanol (Ethyl alcohol)	Thebaine
Etodolac	Theophylline
Famprofazone	Thiamine
Fenoprofen	Thioridazine
Fluoxetine Hydrochloride	Tolbutamide
Furosemide	Trazodone
Gentisic acid	Triamterene
D (+) Glucose	Trifluoperazine
Guaiacol Glyceryl Ether	Trimethoprim
Hemoglobin	Trimipramine
Hydralazine	Tryptamine
Hydrochlorothiazide	Tyramine
Hydroxyzine	Uric acid
Imipramine	Verapamil
Isoproterenol hydrochloride	Zomepirac

Bibliography

1. M Colić, V Cupić, L Pavicić, et al. Xylazine, an alpha 2-adrenergic agonist, modulates proliferation of rat thymocytes in vivo and in vitro[J]. Methods Find Exp Clin Pharmacol, 2000, 22(7):557-562.
2. K Ruiz-Colón, Chavez-Arias C , JE Díaz-Alcalá, et al. Xylazine intoxication in humans and its importance as an emerging adulterant in abused drugs: A comprehensive review of the literature[J]. Forensic Science International, 2014, 240(240C):1-8.
3. Lane JC, Tennison MB, Lawless ST, et al. Movement disorder after withdrawal of fentanyl infusion. J Pediatr, 1991, 119(4):649-651
4. Dominguez KD, Lomako DM , Katz RW , et al. Opioid withdraw in critically ill neonates. Ann Pharmacotherm, 2003, 37(4):473-477

Glossary of Symbols

Consult instructions for use
Test per Kit
Do not use if package is damaged
Store between 36°F - 86°F (2-30°C)
Use by
Do Not Reuse
LOT Lot Number
REF Catalogue #

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