Xylazine Rapid Test Panel (Urine)

Package Insert

A rapid test for the qualitative detection of xylazine in human urine.
For forensic use only, not for in vitro diagnostic use.

【INTENDED USE】

The Xylazine Rapid Test Panel (Urine) is a rapid chromatographic immunoassay for the detection of xylazine in human urine at a cut-off concentration of 1,000ng/mL. This test will detect other related compounds, please refer to the Analytical Specificity table in this package insert. This assay provides only a qualitative, preliminary 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. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

【SUMMARY】

Xylazine is a veterinary tranquilizer, which is not approved for human use in the United States, but is commonly used for sedating large animals. Although human intoxication with xylazine has been reported sporadically over the past several decades in a number of case studies. It was also noted in the literature describing drug overdose deaths in Philadelphia as early as 2006, yet it did not appear in high prevalence at that time. However, since the mid-2010 s, xylazine has been noticed by people who inject drugs (PWID) and public health practitioners as an increasingly commonplace additive in the street opioid supply of Philadelphia. Further, recent reports from Connecticut implicated xylazine in a rising fraction of overdose deaths in 2019–2020.[1]

In Puerto Rico, heroin is commonly adulterated with xylazine. Also, xylazine is frequently found in speedball (a cocaine and heroin mixture). Xylazine has also been reported to be misused as a horse doping agent, a drug of abuse, a drug for attempted sexual assault, and as source of accidental or intended poisonings. From human reported cases, central nervous system depression, respiratory depression, bradycardia, hypotension, and hyperglycemia were observed. Literature shows some similar pharmacologic effects between xylazine and heroin in humans.[2]

The Xylazine Rapid Test Panel (Urine) is a rapid urine-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 Xylazine Rapid Test Panel (Urine) yields a positive result when the xylazine in urine exceed 1,000ng/mL.

【PRINCIPLE】

The Xylazine Rapid Test Panel (Urine) is an immunoassay based on the principle of competitive binding. Drugs which may be present in the urine specimen compete against the drug conjugate for binding sites on the antibody.

During testing, a urine specimen migrates upward by capillary action. Xylazine, if present in the urine specimen below 1,000 ng/mL, will not saturate the binding sites of the antibody in the test. The antibody coated particles will then be captured by immobilized Xylazine-protein conjugate and a visible colored line will show up in the test line region. The colored line will not form in the test line region if the Xylazine level exceeds 1,000 ng/mL because it will saturate all the binding sites of anti-Xylazine antibody.

A drug-positive urine specimen will not generate a colored line in the test line region because of drug competition, while a drug-negative urine specimen or a specimen containing a drug concentration less than the cut-off will generate a line in the test line region. To serve as a procedural control, a colored line will always appear at the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

【REAGENTS】

The test contains rabbit polyclonal anti-Xylazine antibody coupled particles and Xylazine-protein conjugate. A goat antibody is employed in the control line system.

【PRECAUTIONS】

- For forensic use only, not for *in vitro* diagnostic use.
- Do not use after the expiration date.
- The test should remain in the sealed pouch until use.
- All specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- The used test should be discarded according to local regulations.

【STORAGE AND STABILITY】

Store as packaged in the sealed pouch either at room temperature or refrigerated (2-30°C). The test is stable through the expiration date printed on the sealed pouch or label of the closed canister. The test must remain in the sealed pouch or closed canister until use. **DO NOT FREEZE.** Do not use beyond the expiration date.

NOTE: Once the canister has been opened, the remaining test(s) are stable for 50 days only.

【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 2-8°C for up to 48 hours prior to testing. For prolonged storage, specimens may be frozen and stored below -20°C. Frozen specimens should be thawed and mixed before testing.

【MATERIALS】

Materials Provided

- Test panels
- Package insert

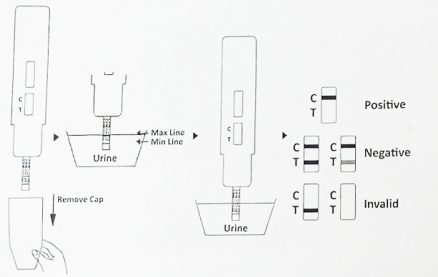
Materials Required but Not Provided

- Specimen collection containers
- Timer

【DIRECTIONS FOR USE】

Allow the test, urine specimen and/or controls to reach room temperature (15-30°C) prior to testing.

1. Bring the pouch to room temperature before opening it. Remove the test panel from the sealed pouch and use it within one hour.
2. Remove the cap.
3. With the arrow pointing toward the urine specimen, immerse the test panel vertically in the urine specimen for at least **10 to 15 seconds**. Immerse the strip to at least the level of the wavy lines, but not above the arrow on the test panel.
4. Replace the cap and place the test panel on a non-absorbent flat surface.
5. Start the timer and wait for the colored line(s) to appear.
6. The result should be read at **5 minutes**. Do not interpret the result after 10 minutes.



【INTERPRETATION OF RESULTS】

(Please refer to the illustration above)

NEGATIVE:* Two colored lines appear. One colored line should be in the control line region (C) and another colored line should be in the test line region (T). This negative result indicates that the Xylazine concentration is below the detectable level (1,000 ng/mL).

***NOTE:** The shade of color in the test line region (T) may vary, but it should be considered negative whenever there is even a faint colored line.

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 level (1,000 ng/mL).

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.

【QUALITY CONTROL】

A procedural control is included in the test. A colored line appearing in the control line region (C) is considered an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.

Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as good laboratory testing practice to confirm the test procedure and to verify proper test performance.

【LIMITATIONS】

1. The Xylazine Rapid Test Panel (Urine) provides only a qualitative, preliminary result. A secondary analytical method must be used to obtain a confirmed result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.
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 of abuse and certain medications.

【EXPECTED VALUES】

This negative result indicates that the Xylazine concentration is below the detectable level of 1,000ng/mL. Positive result means the concentration of Xylazine is above the level of 1,000ng/mL. The Xylazine Rapid Test Panel (Urine) has a sensitivity of 1,000ng/mL.

【PERFORMANCE CHARACTERISTICS】

Analytical Sensitivity

A drug-free urine pool was spiked with Xylazine at the following concentrations: 0ng/mL, 500ng/mL, 750ng/mL, 1,000ng/mL, 1,250g/mL, 1,500ng/mL and 3,000ng/mL. The result demonstrates >99% accuracy at 50% above and 50% below the cut-off concentration. The data are summarized below:

Xylazine Concentration (ng/mL)	Percent of Cut-off	n	Visual Result	
			Negative	Positive
0	0	30	30	0
500	-50%	30	30	0
750	-25%	30	28	2
1,000	Cut-off	30	15	15
1,250	+25%	30	4	26
1,500	+50%	30	1	29
3,000	3X	30	0	30

Analytical Specificity

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

Compound	Concentration (ng/mL)
Xylazine	1,000

Effect of Urinary Specific Gravity

Fifteen urine specimens of normal, high and low specific gravity ranges were spiked with 500ng/mL and 1,500ng/mL of Xylazine. The Xylazine Rapid Test Panel (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 500ng/mL and 1,500ng/mL. The spiked, pH-adjusted urine was tested with the Xylazine Rapid Test Panel (Urine) in duplicate. The results demonstrate that varying ranges of pH do not interfere with the performance of the test.

【BIBLIOGRAPHY】

1. Joseph Friedman, Fernando Montero, Phillippe bourgois. Xylazine spreads across the US:A growing component of the increasingly synthetic and polysubstance overdose crisis. Drug and Alcohol Dependence,Volume 233, April 2022,109380.
2. Kazandra Ruiz-Colón, Carlos Chavez-Arias, José Enc Díaz-Alcalá, María A. Martínez Xylazine intoxication in humans and its importance as an emerging adulterant in abused drugs: A comprehensive review of the literature. Forensic Science InternationalVolume 240, 2014, PP 1-8