MAYO CLINIC LABORATORIES

Test Definition: BNZX

Benzodiazepines Confirmation, Chain of Custody, Random, Urine

Overview

Useful For

Detecting drug use involving benzodiazepines such as alprazolam, chlordiazepoxide, clonazepam, diazepam, midazolam, oxazepam, temazepam, clobazam, flunitrazepam, flurazepam, lorazepam, prazepam, triazolam, and zolpidem, in urine specimens handled through the chain-of-custody process

Providing chain of custody for when the results of testing could be used in a court of law. Its purpose is to protect the rights of the individual contributing the specimen by demonstrating that it was always under the control of personnel involved with testing the specimen; this control implies that the opportunity for specimen tampering would be limited.

Additional Tests

Test Id	Reporting Name	Available Separately	Always Performed
COCH	Chain of Custody Processing	No	Yes
ADLTX	Adulterants Survey, CoC, U	Yes	Yes

Testing Algorithm

Testing for adulterants will be performed on all chain-of-custody urine samples per regulatory requirements.

Method Name

Immunoassay/Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

NY State Available

Yes

Specimen

Specimen Type

Urine

Specimen Required

Supplies: Chain of Custody Kit (T282)
Container/Tube: Chain of custody kit containing the specimen containers, seals, and documentation required
Specimen Volume: 5 mL
Collection Instructions: Collect specimen in the container provided, seal, and submit with the associated documentation to satisfy the legal requirements for chain-of-custody testing.
Additional Information: Submitting less than 5 mL will compromise the ability to perform all necessary testing.

Forms

1. Chain of Custody Request is included in the Chain of Custody Kit (T282).
2. If not ordering electronically, complete, print, and send a Therapeutics Test Request (T831) with the specimen.

Specimen Minimum Volume

1 mL

Reject Due To

Gross hemolysis	Reject
Gross icterus	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Urine	Refrigerated (preferred)	7 days	
	Frozen	14 days	

Clinical & Interpretive

Clinical Information

Benzodiazepines are any of a group of compounds having a common molecular structure and acting similarly as depressants of the central nervous system. As a class of drugs, benzodiazepines are among the most prescribed drugs in the western hemisphere because of their efficacy, safety, low addiction potential, minimal side effects, and high public demand for sedative and anxiolytic agents.

Chain of custody is a record of the disposition of a specimen to document each individual who collected, handled, and performed the analysis. When a specimen is submitted in this manner, analysis will be performed in such a way that it will withstand regular court scrutiny.

Reference Values

Negative
Positive results are reported with a quantitative result.

Cutoff concentrations:

Immunoassay screen:
100 ng/mL

Liquid chromatography tandem mass spectrometry:
Alprazolam: 10 ng/mL
Alpha-hydroxyalprazolam: 10 ng/mL

Chlordiazepoxide: 10 ng/mL
Clonazepam: 10 ng/mL
7-Aminoclonazepam: 10 ng/mL
Diazepam: 10 ng/mL
Nordiazepam: 10 ng/mL
Midazolam: 10 ng/mL
Alpha-hydroxy midazolam: 10 ng/mL
Oxazepam: 10 ng/mL
Temazepam: 10 ng/mL
Clobazam: 10 ng/mL
N-Desmethylclobazam by LC-MS/MS: 10 ng/mL
Flunitrazepam: 10 ng/mL
7-Aminoflunitrazepam: 10 ng/mL
Flurazepam: 10 ng/mL
2-Hydroxy ethyl flurazepam: 10 ng/mL
Lorazepam: 10 ng/mL
Prazepam: 10 ng/mL
Triazolam: 10 ng/mL
Alpha-hydroxy triazolam: 10 ng/mL
Zolpidem: 10 ng/mL
Zolpidem phenyl-4-carboxylic acid: 10 ng/mL

Interpretation

Benzodiazepines are extensively metabolized, and the parent compounds are not detected in urine. This test screens for (and confirms) the presence of:
-Alprazolam
-Alpha-hydroxyalprazolam (metabolite of alprazolam)
-Chlordiazepoxide
-Clonazepam
-7-Aminoclonazepam (metabolite of clonazepam)
-Diazepam (separate prescribable drug and metabolite of medzazepam)
-Nordiazepam (metabolite of clorazepate, halazepam, prazepam, diazepam and medazepam)
-Midazolam
-Alpha-hydroxy midazolam (metabolite of midazolam)
-Oxazepam (separate prescribable drug and metabolite of clorazepate, halazepam, prazepam, medazepam, temazepam, and diazepam)
-Temazepam (separate prescribable drug and metabolite of medazepam and diazepam)
-Clobazam
-N-Desmethylclobazam (metabolite of clobazam)
-Flunitrazepam
-7-Aminoflunitrazepam (metabolite of flunitrazepam)
-Flurazepam
-2-Hydroxy ethyl flurazepam (metabolite of flurazepam)
-Lorazepam

-Prazepam
-Triazolam
-Alpha-hydroxy triazolam (metabolite of triazolam)
-Zolpidem
-Zolpidem phenyl-4-carboxylic acid (metabolite of zolpidem)

The clearance half-life of long-acting benzodiazepines is more than 24 hours. It takes 5 to 7 half-lives to clear 98% of a drug dose. Therefore, the presence of a long-acting benzodiazepine greater than the limit of quantification indicates exposure within a 5 to 20-day interval preceding specimen collection. Following a dose of diazepam, the drug and its metabolites appear in the urine within 30 minutes. Peak urine output is reached between 1 and 8 hours. For additional information including metabolism, clearance (half-life), and approximate detection times, see Optimize Urine Drug Monitoring for CNS Depressants.

Cautions

No significant cautionary statements

Clinical Reference

1. Gudin JA, Mogali S, Jones JD, Comer SD. Risks, management, and monitoring of combination opioid, benzodiazepines, and/or alcohol use. Postgrad Med. 2013;125(4):115-130. doi:10.3810/pgm.2013.07.2684
2. Dowell D, Ragan KR, Jones CM, Baldwin GT, Chou R. CDC Clinical Practice Guideline for Prescribing Opioids for Pain - United States, 2022. MMWR Recomm Rep. 2022;71(3):1-95
3. Baselt RC. Disposition of Toxic Drugs and Chemical in Man. 12th ed. Biomedical Publications; 2020
4. Langman LJ, Bechtel LK, Holstege C. Clinical toxicology. In: Rifai N, Chiu RWK, Burnham CD, Wittwer CT, eds. Tietz Textbook of Laboratory Medicine. 7th ed. Elsevier; 2023:chap 43

Performance

Method Description

Preliminary screen is performed by immunoassay.

The benzodiazepine assay is based on the kinetic interaction of microparticles in a solution as measured by changes in light transmission. In the absence of sample drug, soluble drug conjugates bind to antibody-bound microparticles 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 drug derivative conjugate for microparticle-bound 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.(Package insert: BNZ2. Roche Diagnostics; V 2.0, 04/2024)

Benzodiazepines are extensively metabolized by the liver and subsequently exist in the urine primarily as conjugated esters (-glucuronides). The conjugated metabolites are cleaved during a mild hydrolysis utilizing the enzyme

glucuronidase. Stable isotope forms of the compounds are added as internal standards to account for extraction losses. An aliquot of the hydrolyzed sample is diluted and the analytes are separated by liquid chromatography tandem mass spectroscopy and analyzed by multiple reaction monitoring.(Unpublished Mayo method)

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
3 to 6 days

Specimen Retention Time
14 days

Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Superior Drive

Fees & Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

Test Classification
This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information
80347
G0480 (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
BNZX	Benzodiazepines Conf, CoC, U	90890-5

Result ID	Test Result Name	Result LOINC® Value
608452	Benzodiazepines Immunoassay Screen	14316-4
608280	Alprazolam by LC-MS/MS	59615-5

608281	Alpha-Hydroxyalprazolam by LC-MS/MS	16348-5
608282	Chlordiazepoxide by LC-MS/MS	20522-9
608283	Clonazepam by LC-MS/MS	16229-7
608284	7-aminoclonazepam by LC-MS/MS	51776-3
608285	Diazepam by LC-MS/MS	16227-1
608286	Nordiazepam by LC-MS/MS	16228-9
608287	Midazolam by LC-MS/MS	16233-9
608288	Alpha-Hydroxy Midazolam by LC-MS/MS	59590-0
608289	Oxazepam by LC-MS/MS	16201-6
608290	Temazepam by LC-MS/MS	20559-1
608291	Clobazam by LC-MS/MS	59635-3
608292	N-Desmethylclobazam by LC-MS/MS	97160-6
608293	Flunitrazepam by LC-MS/MS	20528-6
608294	7-aminoflunitrazepam by LC-MS/MS	51777-1
608295	Flurazepam by LC-MS/MS	16231-3
608296	2-Hydroxy Ethyl Flurazepam by LC-MS/MS	97159-8
608297	Lorazepam by LC-MS/MS	17088-6
608298	Prazepam by LC-MS/MS	17479-7
608299	Triazolam by LC-MS/MS	16232-1
608300	Alpha-Hydroxy Triazolam by LC-MS/MS	49876-6
608301	Zolpidem by LC-MS/MS	72770-1
608302	Zolpidem Phenyl-4-Carboxylic acid by LC-MS/MS	72768-5
608449	Benzodiazepines Interpretation	69050-3
608450	Chain of Custody	77202-0