Name of Procedure:

Toxicology
ELISA Screen for Methamphetamine and 3,4-Methylenedioxymethamphetamine (MDMA) Using the Immunalysis Methamphetamine Direct ELISA Kit

Suggested Uses:

This procedure does not cover every aspect of the instruments used. The operator of the instrument should read the manual for the instruments before using this procedure.

The procedure can be used to screen blood and urine samples for methamphetamine and 3,4-Methylenedioxymethamphetamine (MDMA).

The cutoff for blood case samples is 100 ng/ml d-methamphetamine, and the cutoff for urine case samples is 200 ng/ml d-methamphetamine.

This is only a screening test. Any positive result must be confirmed using a more specific analysis, such as gas or liquid chromatography/mass spectrometry before it can be reported. There are some cases of uncommon substances causing false positives using this test, which must be kept in mind when evaluating the results of this test.

Items Used to Perform Procedure:

Reagent Kit for Methamphetamine
- 96 well coated Microplates
- 50 ng/ml Urine d-Methamphetamine Standard
- Urine Negative
- Enzyme Conjugate
- TMB Substrate Reagent
- Stop Reagent

Automatic pipets, 1 mL, 100 uL, 0.010 - 1.0 mL adjustable
Test tubes
Adjustable microchannel pipet (15-200 uL)
Volumetric flasks 100 mL
Interval Timer
Microplate Washer
Microplate Reader capable of reading at 450 nm and 630 nm

Reagents Used in Procedure:

Analytical Grade Drug Standard - Methamphetamine, 1 mg/mL
Methanol

1,000 ng/ml Stock Methamphetamine Verification Standard:

To a 100 mL volumetric flask, add 100 microliters of a 1 mg/ml methamphetamine standard and dilute the flask to volume with methanol. Label the solution. Include on the label initials, the date prepared, and an expiration date determined by the expiration date of the acquired drug standard.

100 ng/ml Working Methamphetamine Verification Sample:

Prepare a 100 ng/ml working methamphetamine verification sample by adding 100 microliters of the 1,000 ng/ml stock methamphetamine verifier solution to a clean test tube. Add 0.90 mL of blood to the test tube and vortex. This sample should be prepared fresh on the same day that it is used for the assay.

Microplate Washer:

1. Read and follow all manufacturer instructions for use of the microplate washer.

Microplate Reader:

1. Read and follow all manufacturer instructions for use of the microplate reader.

Application of Procedure on Evidence:

Allow all reagents and samples to equilibrate to room temperature before use.

1. Urine case sample must be diluted with 3 volumes of deionized water or negative urine to one part urine case sample before their use in this procedure.
2. Add 10 microliters of the of the urine negative, 50 ng/ml d-methamphetamine urine standard, working verification sample, and case sample(s) to each well in duplicate.
3. Add 100 microliters of the enzyme conjugate to each well. Keep the time differences in the addition of enzyme conjugate to each well at a minimum. Tap the sides of the plate holder to ensure proper mixing.
4. Incubate the plate for 60 minutes after addition of enzyme conjugate to the last well. Incubate at room temperature in the dark.
5. Wash each well 6 times with 350 microliters of deionized water.
6. Invert the wells and vigorously slap dry on absorbent paper or towel to ensure that all residual moisture is removed.

7. Add 100 microliters of the TMB substrate reagent to each well and tap sides of the plate holder to ensure proper mixing. Keep the time differences in the addition of substrate reagent to each well at a minimum.
8. Incubate the plate for 30 minutes after addition of the substrate reagent to the last well. Incubate at room temperature in the dark.
9. Add 100 microliters of the Stop Solution to each well. Keep the time differences in the addition of stop solution to each well at a minimum. The color should change from blue to yellow.
10. Measure the absorbance at a dual wavelength of 450 nm (measuring filter) and 630 nm (background reference filter).

Results:

A reciprocal relationship exists between absorbance and concentration.

The adjusted negative control value is defined as the average value of the negative controls run minus 0.300.

Blood case samples that have at least one absorbance value at or below the average value of the 100 ng/ml blood working methamphetamine verification standards are considered positive. Blood case samples that have all absorbance values above the average value of the 100 ng/ml blood working methamphetamine verification standards, but are at or below the adjusted negative blood control are considered elevated. Blood case samples that are above the adjusted negative blood control will be considered negative.

Diluted urine case samples that have at least one absorbance value at or below the average value of the 50 ng/ml urine working d-methamphetamine standards (200 ng/ml cutoff) are considered positive. Diluted urine case samples that have all absorbance values above the average value of the 50 ng/ml urine working methamphetamine standards, but are at or below the adjusted negative urine control are considered elevated. Diluted urine case samples that are above the adjusted negative urine control will be considered negative.

Data Record Keeping:

The following information must be recorded for each sample that was run using this procedure.

1. Lot numbers of reagents and 50 ng/ml d-methamphetamine urine standard.

2. Expiration dates of reagents, 50 ng/ml d-methamphetamine urine standard, and the stock methamphetamine verification standard.
3. Results of the urine negative, 50 ng/ml d-methamphetamine urine standard, working methamphetamine verification sample, and case sample(s).

Safety Concerns:

When working with biohazardous samples use protective measures, such as gloves, eye protection, and work with the samples in a biosafety hood.

The stop reagent is corrosive.

Maintenance:

Perform the rinse procedure on the microplate washer after each use.

Literature Reference:

Methamphetamine Direct ELISA Kit Insert, Immunalysis Corporation, Version 5/2001.