

**FORENSIC TOXICOLOGY LABORATORY  
OFFICE OF CHIEF MEDICAL EXAMINER  
CITY OF NEW YORK**

**PREPARATION OF QUALITY CONTROL  
for  
ENZYME-LINKED IMMUNOSORBENT ASSAY IN URINE**

**ANALYTES**

Enzyme-linked immunosorbent assays (ELISA) are routinely used to test urine for the presence of **opiates, cocaine metabolite (benzoylecgonine), amphetamines, benzodiazepines, barbiturates and cannabinoids**. Each batch must include at least one negative control and one positive control for each 22 unknown specimens and a negative and a positive control at the end of the assay. External or internal controls may be used. All controls must be validated prior to use.

**EXTERNAL QUALITY CONTROL MATERIALS**

Urine is collected from an in-house volunteer. The urine is validated as negative for opiates, benzoylecgonine, amphetamines and benzodiazepines by GC and GC/MS and barbiturates by HPLC analyses.

**CALIBRATORS\***

\*The print outs the DYNEX DSX instruments label the calibrators and controls as follows:

Negative Calibrator:	NEG1
50% of Cutoff Calibrator:	LP1
Cutoff Calibrator:	POS1
Positive Calibrator:	HP1

These are hardwired and cannot be changed.

1. Negative calibrator (NEG1): URINE Negative Blank Urine
2. Positive Calibrator (HP1): ELISA Cutoff Urine Calibrator prepared or purchased to contain:

Amphetamine	1000 ng/mL (d-amphetamine)
Methamphetamine	1000 ng/mL (d-methamphetamine)
Barbiturates	400 ng/mL (secobarbital)
Benzodiazepines	400 ng/mL (oxazepam)
Opiates	400 ng/mL (morphine)
Cocaine Metabolite	200 ng/mL (benzoylecgonine)
Cannabinoids	100 ng/mL (11-nor-carboxy-delta-9-thc)

To 100 mL of certified negative urine, add the indicated amounts of a 1.0 mg/mL reference solution to achieve the final concentration below:

- 100 ng/mL- 10 µL of 1.0 mg/mL 11-nor-carboxy-delta-9-thc reference solution
- 200 ng/mL- 20 µL of 1.0 mg/mL Benzoylecgonine reference solution
- 400 ng/mL- 40 µL of 1.0 mg/mL Morphine reference solution
- 400 ng/mL - 40 µL of 1.0 mg/mL Oxazepam reference solution
- 400 ng/mL - 40 µL of 1.0 mg/mL Secobarbital reference solution
- 1000 ng/mL - 100 µL of 1.0 mg/mL d-Amphetamine reference solution
- 1000 ng/mL - 100 µL of 1.0 mg/mL d-Methamphetamine reference solution

Add a magnetic stirrer and mix for 1 hr.

Assign a lot number, e.g. 012513C3 for a positive calibrator (C3) prepared on January 25, 2013.

Aliquot 1 mL after completion of step 3 below into 12 x 75 mm labeled culture tubes and store in the freezer until needed.

3. Cutoff Calibrator (POS1): ELISA Cutoff Urine Calibrator prepared or purchased to contain:

Amphetamine	500 ng/mL (d-amphetamine)
Methamphetamine	500 ng/mL (d-methamphetamine)
Barbiturates	200 ng/mL (secobarbital)
Benzodiazepines	200 ng/mL (oxazepam)
Opiates	200 ng/mL (morphine)
Cocaine Metabolite	100 ng/mL (benzoylecgonine)
Cannabinoids	50 ng/mL (11-nor-carboxy-delta-9-thc)

To a 100 mL volumetric flask, add 50 mL of positive calibrator above. Add 50 mL of certified negative urine. Add a magnetic stirrer and mix for 1 hr.

Assign a lot number, e.g. 012513C2 for a cutoff calibrator (C2) prepared on January 25, 2013.

Aliquot 1 mL after completion of step 4 below into 12 x 75 mm labeled culture tubes and store in the freezer until needed.

4. 50% of Cutoff Calibrator (NCTRL/LP1): Negative (1/2 cutoff) Urine Calibrator prepared or purchased to contain:

Amphetamine	250 ng/mL (d-amphetamine)
Methamphetamine	250 ng/mL (d-methamphetamine)
Barbiturates	100 ng/mL (secobarbital)
Benzodiazepines	100 ng/mL (oxazepam)
Opiates	100 ng/mL (morphine)
Cocaine Metabolite	50 ng/mL (benzoylecgonine)
Cannabinoids	25 ng/mL (11-nor-carboxy-delta-9-thc)

To a 100 mL volumetric flask, add 50 mL of cutoff calibrator above. Add 50 mL of certified negative urine. Add a magnetic stirrer and mix for 1 hr.

Assign a lot number, e.g. 012513C1 for a negative (1/2 cutoff) calibrator (C1) prepared on January 25, 2013.

Aliquot 1 mL into 12 x 75 mm labeled culture tubes and store in the freezer until needed.

Validate in triplicate in three separate assays by ELISA and in triplicate by GC/MS, LC/MS and HPLC. Single THC analysis is performed by NMS.

Positive Calibrator, Cutoff Calibrator, 50% Cutoff Calibrator and Negative Calibrator are stable for 6 months.

Validated Urine calibrators must meet the following criteria:

1. New calibrators must meet the Orasure displacement requirements.
2. The cutoff calibrator must give an OD reading with  $\pm$  20% of the cutoff calibrator in use.
3. The new negative control (NCTRL) must give a negative response and the OD reading must be within  $\pm$  20% of the negative control in use.
4. The new positive calibrator (PCTRL) must give a positive response and the OD reading must be within  $\pm$  20% of the positive calibrator in use.

If any of these criteria are not met, consult a supervisor.

OraSure Negative Calibrator (Urine)

Supplied with kit for plate validation. Negative for drugs.

OraSure Cutoff Calibrator (Urine)

Supplied with kit for plate validation. Drugs concentrations as follows:

Amphetamine	100 ng/mL (d-amphetamine)
Methamphetamine	100 ng/mL (d-methamphetamine)
Barbiturates	100 ng/mL (secobarbital)
Benzodiazepines	100 ng/mL (oxazepam)
Cocaine Metabolite	100 ng/mL (benzoylecgonine)
Opiates	100 ng/mL (morphine)
Cannabinoids	50 ng/mL (11-nor-carboxy-delta-9-thc)

Stated to be  $\pm$  10% of target concentration by GC/MS.

## IN ASSAY QUALITY CONTROL

In addition to the reagents above which are routinely run at the beginning of the assay, positive and negative controls from different sources are run after every 22 unknown samples and/or at the end of the assay.

### Negative In Assay Control

The urine negative control is validated in-house as negative. The control should, if possible, be from a different lot than the calibrators.

### Positive In Assay Control (2x cutoff):

Positive urine control prepared or purchased to contain:

Amphetamine	1000 ng/mL (d-amphetamine)
Methamphetamine	1000 ng/mL (d-methamphetamine)
Barbiturates	400 ng/mL (secobarbital)
Benzodiazepines	400 ng/mL (oxazepam)
Opiates	400 ng/mL (morphine)
Cocaine Metabolite	200 ng/mL (benzoylecgonine)
Cannabinoids	100 ng/mL (11-nor-carboxy-delta-9-thc)

The in assay positive control is prepared as follows:

To 100 mL of certified negative urine, add the indicated amount of a 1.0 mg/mL reference solution to achieve a final concentration below:

- 100 ng/mL - 10 uL of 1.0 mg/mL 11-nor-carboxy-delta-9-thc reference solution
- 200 ng/mL - 20 uL of 1.0 mg/mL Benzoylecgonine reference solution
- 400 ng/mL - 40 uL of 1.0 mg/mL Morphine reference solution
- 400 ng/mL - 40 uL of 1.0 mg/mL Oxazepam reference solution
- 400 ng/mL - 40 uL of 1.0 mg/mL Secobarbital reference solution
- 1000 ng/mL - 100 uL of 1.0 mg/mL d-Amphetamine reference solution
- 1000 ng/mL - 100 uL of 1.0 mg/mL d-Methamphetamine reference solution

Add a magnetic stirrer and mix for 1 hr. Aliquot 1 mL into 12 x 75 mm labeled culture tubes and store in the freezer until needed. Validate in triplicate in three separate assays by ELISA and in triplicate by GC/MS and HPLC. Single THC analysis is performed by NMS.

Assign a lot number, e.g. 0126132x for a positive control (2X) prepared on January 26, 2013.

The frozen control is stable for 6 months.

Results must be positive by ELISA and within  $\pm 20\%$  from target concentration by GC, GC/MS, HPLC and reference lab results. If any result is negative consult a supervisor.