

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

**PREPARATION OF QUALITY CONTROL
for
ENZYME-LINKED IMMUNOSORBENT ASSAY
in
WHOLE BLOOD, SERUM AND VITREOUS HUMOR**

ANALYTES

Enzyme-linked immunosorbent assays (ELISA) are routinely used to test whole blood, serum or vitreous humor for the presence of **opiates, cocaine metabolite (benzoylecgonine), amphetamines, barbiturates, benzodiazepines, cannabinoids, methadone and oxycodone**. 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

Human Whole Blood and Serum are purchased from UTAK Laboratories, Inc., Valencia, CA. The Whole Blood and Serum are validated as negative as follows: methadone by GC, opiates, benzoylecgonine, oxycodone by LC/MS, amphetamines, benzodiazepines by GC/MS and barbiturates by HPLC. It is also run by all available ELISA assays.

CALIBRATORS*

*The print outs from 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 (NCAL/NEG1): UTAK Negative Blank Whole Blood, UTAK Serum or Vitreous Humor
2. Positive Calibrator (HP1): Positive Whole Blood, Serum or Vitreous Humor Calibrator prepared or purchased to contain:

Amphetamine	200 ng/mL (d-amphetamine)
Methamphetamine	200 ng/mL (d-methamphetamine)
Barbiturates	200 ng/mL (secobarbital)
Benzodiazepines	200 ng/mL (oxazepam)

Cocaine Metabolite	100 ng/mL (benzoylecgonine)
Opiates	100 ng/mL (morphine)
Cannabinoids	60 ng/mL (11-nor-carboxy-delta-9-thc) except vitreous humor
Methadone	50 ng/mL (methadone)
Oxycodone	40 ng/mL (oxycodone)

Note: Vitreous humor calibrators and controls do NOT contain cannabinoids

To 200 mL of certified negative whole blood, serum or vitreous humor, add the indicated amounts of a 1.0 mg/mL reference solution to achieve the final concentration below:

- 40 ng/mL - 8 µL of 1.0 mg/mL Oxycodone reference solution
- 50 ng/mL - 10 µL of 1.0 mg/mL Methadone reference solution
- 60 ng/mL - 12 µL of 1.0 mg/mL 11-nor-carboxy-delta-9-thc reference solution
- 100 ng/mL - 20 µL of 1.0 mg/mL Morphine reference solution
- 100 ng/mL - 20 µL of 1.0 mg/mL Benzoylecgonine reference solution
- 200 ng/mL - 40 µL of 1.0 mg/mL Oxazepam reference solution
- 200 ng/mL - 40 µL of 1.0 mg/mL Secobarbital reference solution
- 200 ng/mL - 40 µL of 1.0 mg/mL d-Amphetamine reference solution
- 200 ng/mL - 40 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 March 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 Whole Blood, Serum or Vitreous Humor Calibrator prepared or purchased to contain:

Amphetamine	100 ng/mL (d-amphetamine)
Methamphetamine	100 ng/mL (d-methamphetamine)
Barbiturates	100 ng/mL (secobarbital)
Benzodiazepines	100 ng/mL (oxazepam)
Cocaine Metabolite	50 ng/mL (benzoylecgonine)
Opiates	50 ng/mL (morphine)
Cannabinoids	30 ng/mL (11-nor-carboxy-delta-9-thc) except vitreous humor
Methadone	25 ng/mL (methadone)
Oxycodone	20 ng/mL (oxycodone)

To a 200 mL volumetric flask, add 100 mL from positive calibrator above. Add 100 mL of certified negative whole blood, serum or vitreous humor. Add a magnetic stirrer and mix for 1 hr.

Assign a lot number, e.g. 012513C2 for a positive calibrator (C2) prepared on March 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) Whole Blood, Serum or Vitreous Humor Calibrator prepared or purchased to contain:

Amphetamine	50 ng/mL	(d-amphetamine)
Methamphetamine	50 ng/mL	(d-methamphetamine)
Barbiturates	50 ng/mL	(secobarbital)
Benzodiazepines	50 ng/mL	(oxazepam)
Cocaine Metabolite	25 ng/mL	(benzoylecgonine)
Opiates	25 ng/mL	(morphine)
Cannabinoids	15 ng/mL	(11-nor-carboxy-delta-9-thc) except vitreous humor
Methadone	12.5 ng/mL	(methadone)
Oxycodone	10 ng/mL	(oxycodone)

To a 200 mL volumetric flask, add 100 mL from cutoff calibrator above. Add 100 mL of certified negative whole blood, serum or vitreous humor.

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

Validated Whole Blood, Serum or Vitreous Humor 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 negative calibrator in use.

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

OraSure Negative Serum/Oral Fluid Calibrator

Supplied with kit for plate validation. Negative for drugs.

OraSure Cutoff Serum/Oral Fluid Calibrator

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	30 ng/mL (11-nor-carboxy-delta-9-thc)
Methadone	5 ng/mL (methadone)

Note: there are no OraSure kit calibrators for the Oxycodone kit

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 negative control (Utak Whole Blood or Serum, or collected vitreous humor) 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 Control (PCTRL2X): Positive Whole Blood, Serum or Vitreous Humor Calibrator prepared or purchased to contain:

Amphetamine	200 ng/mL (d-amphetamine)
Methamphetamine	200 ng/mL (d-methamphetamine)
Barbiturates	200 ng/mL (secobarbital)
Benzodiazepines	200 ng/mL (oxazepam)
Cocaine Metabolite	100 ng/mL (benzoylecgonine)
Opiates	100 ng/mL (morphine)
Cannabinoids	60 ng/mL (11-nor-carboxy-delta-9-thc) except vitreous humor
Methadone	50 ng/mL (methadone)
Oxycodone	40 ng/mL (oxycodone)

The in assay positive control is prepared as follows:

To 100 mL of certified negative whole blood, serum or vitreous humor, add the indicated amount of a 1.0 mg/mL reference solution to achieve a final concentration below:

40 ng/mL - 4 μ L of 1.0 mg/mL Oxycodone reference solution
50 ng/mL - 5 μ L of 1.0 mg/mL Morphine reference solution
60 ng/mL - 6 μ L of 1.0 mg/mL 11-nor-carboxy-delta9-thc reference solution
100 ng/mL - 10 μ L of 1.0 mg/mL Morphine reference solution
100 ng/mL - 10 μ L of 1.0 mg/mL Benzoyllecgonine reference solution
200 ng/mL - 20 μ L of 1.0 mg/mL Oxazepam reference solution
200 ng/mL - 20 μ L of 1.0 mg/mL Secobarbital reference solution
200 ng/mL - 20 μ L of 1.0 mg/mL d-Amphetamine reference solution
200 ng/mL - 20 μ L 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, LC/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 1 year.

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

This procedure also applies to vitreous humor. Vitreous humor is pooled from previously analyzed negative cases for which toxicological analysis has been completed and case closed.