COLA Clinical Laboratory Accreditation

COLA Primers Accreditation

COLA PRIMER 56

Urine Drug Screening

• Introduction • test

The opioid crisis in the United States has brought Urine Drug Screening (UDS) into focus, as the cost in lives and healthcare dollars is astounding. Because of the personal, occupational, and legal implications that accompany drug testing, laboratories that perform UDS must be confident in their methodology and their ability to interpret screening results and respond appropriately.

• What methodologies are used for UDS? •

Immunoassay for initial screening and liquid chromatography with tandem mass spectrometry (LC-MS/MS) for confirmatory testing are the methods most commonly utilized to test for drugs. Any credible drug screening program will involve a two-step process that incorporates both methods. Using a combination of both tests allows a high level of sensitivity and specificity and a low chance for false positives or false negatives.

There are various immunoassay methods. The most common is enzymatic immunoassay (EIA), based on the principle of competitive binding of an enzyme labeled drug in the reagent competing with the drug in the sample for limited specific antibody sites. Enzyme activity decreases upon binding of the labeled drug in the reagent to the antibody, so drug concentration can be measured in terms of enzyme activity. There are many manufacturers that offer instruments for this type of testing.

Enzyme immunoassays are intended only for the qualitative screening of drugs in human urine. This is a unique competitive inhibition and does not build a curve that is linear as would be needed for quantitative results. In general, EIA screening tests for classes of drugs, but cannot positively identify the presence of a specific drug or metabolite in the urine.

The immunoassay is performed first and is often used as an initial screening method. If the immunoassay is negative, no further action may be required. In some cases, it may be necessary to perform additional testing, if the suspect drug(s) cannot be identified in the screening assays.

If the sample is positive, an additional confirmatory LC-MS/MS analysis is performed on a separate aliquot of the biological sample, either by the same laboratory or at a reference laboratory. The more specific LC-MS/MS is used as a confirmatory test to identify individual drug substances or metabolites and quantify the amount(s) present. Confirmatory tests, such as LC-MS/MS, should be utilized prior to reporting positive drug test results. False positive samples from the screening test will usually be negative on the confirmation test. Samples testing positive for both screening and confirmation tests are reported as positive to the entity that ordered the test. Most laboratories save positive samples for a period of months or years in the event of a disputed result or lawsuit.

This Primer will focus on the initial urine drug screening, usually performed by immunoassay.

The following guidance is divided into two sections:

1. Guidance for qualitative Toxicology screening methods that are FDA approved and unmodified; and
2. Guidance for qualitative Toxicology methods that:
    - Are FDA-approved but modified by the laboratory
    - Are developed by the laboratory OR
    - Utilize a reagent/instrument pairing that is not listed on the FDA CLIA database

● Verification of performance of qualitative Toxicology screening methods that are FDA-approved and unmodified ●

The CLIA regulation for verification of performance can be found at CFR 42 493.1253

This section covers qualitative screening for drug classes, using instrument/reagent pairings that are listed on the FDA CLIA database. No values/concentrations are included in the patient report. Test results are typically reported as positive or negative or present or absent.

Note that because these tests are typically reported as positive or negative based on a concentration cutoff value, **accuracy, precision, and reportable range must be verified.**

It is not required to verify the reference range (normal values) for Toxicology screening assays, as long as both positive and negative samples are included in the verification studies. Known negative samples would represent the population that is not taking the specific drug class, and if the results obtained are negative as expected, this is considered acceptable as verification of the "reference" range.

Verification studies must be performed by the lab's own personnel. Your laboratory should define acceptability criteria for both accuracy and precision before you begin the studies.
Use samples that have a matrix as close as possible to patient specimens. The first choice for clinical tests is patient samples, followed by control material and reference solutions (calibrators, etc.).

All studies must be reviewed for acceptability, approved, and signed by the Laboratory Director or designee prior to beginning patient testing. The delegation of this responsibility to a qualified person, such as the Technical Consultant or Technical Supervisor, must be in writing.

Accuracy

Definition: How close the measured value to the "true" value. You must verify that the test gives the correct results in your laboratory. Accuracy can be verified by testing samples with known values and comparing them to the results that you obtain with your method.

To verify Accuracy:

The laboratory should run a variety of samples with known values and compare the results. The study must include both positive and negative samples. It is recommended that the study include a minimum of 20 samples.

Using these samples in a method comparison experiment for accuracy is best over five days. It is best that side-by-side testing be done to ensure sample stability will not be affected. If this is not possible, refrigerating or freezing samples between testing may preserve the sample. Always take into account any freeze/thaw cycle limitations the respective method may have.

Document the results of the new method, comparing the known values from the reference sources, another CLIA-licensed laboratory results, or results from the current method. It is acceptable to include both reference samples, such as QC, and patient samples, but patient samples should be given priority. If possible, involve various routine testing personnel.

Calculate the percent of positive, negative, and total accuracy, by dividing observed results over known results, multiplied by 100.

Accuracy Example:

Sample	Day	Known value	New method result	Expected result? Y/N
1	1	Pos	Pos	Y
2	1	Pos	Pos	Y
3	1	Pos	Pos	Y
4	1	Neg	Neg	Y
5	2	Neg	Neg	Y
6	2	Neg	Neg	Y
7	2	Pos	Pos	Y
8	2	Pos	Neg	N
9	3	Neg	Neg	Y
10	3	Pos	Pos	Y
11	3	Neg	Neg	Y
12	3	Pos	Pos	Y
13	4	Pos	Pos	Y
14	4	Neg	Neg	Y
15	4	Neg	Neg	Y
16	4	Neg	Neg	Y
17	5	Neg	Neg	Y
18	5	Pos	Pos	Y
19	5	Pos	Pos	Y
20	5	Neg	Neg	Y

Percent positive accuracy: 9/10 X 100 = 90%
Percent negative accuracy: 10/10 X 100 = 100%
Total accuracy: 19/20 X 100 = 95%

Precision

Definition: Also known as Reproducibility – defines how consistent the results are when running the same sample multiple times.

How to verify Precision:
For quantitative tests, precision is typically evaluated using the coefficient of variation, or CV. However, for qualitative tests, a CV cannot be calculated. In the case of qualitative tests, we are asking this question: If I run the same specimen multiple times, will I get the same result?

You should select a minimum of two known positive samples and two known negative samples and run these each five times on two different days. You should expect that the two known positive samples result in positive answers for each of the 10 replicates; you should expect that the two known negative samples result in negative answers for each of the 10 replicates. If you do not achieve these results, then you will need to determine the cause of the discrepancy, implement a corrective action plan, and rerun the study.

Precision Example:

Sample	Day	New method result	Expected result? Y/N
Neg sample #1	1	Neg	Y
Neg sample #1	1	Neg	Y
Neg sample #1	1	Neg	Y
Neg sample #1	1	Neg	Y
Neg sample #1	1	Neg	Y
Neg sample #2	1	Neg	Y
Neg sample #2	1	Neg	Y
Neg sample #2	1	Neg	Y
Neg sample #2	1	Neg	Y
Neg sample #2	1	Neg	Y
Neg sample #1	2	Neg	Y
Neg sample #1	2	Neg	Y
Neg sample #1	2	Neg	Y
Neg sample #1	2	Neg	Y
Neg sample #1	2	Neg	Y
Neg sample #2	2	Neg	Y
Neg sample #2	2	Neg	Y
Neg sample #2	2	Neg	Y
Neg sample #2	2	Neg	Y
Neg sample #2	2	Neg	Y

Do the same with at least two known positive results.

Reportable range

Definition: CLIA defines this as the highest and lowest test values that can be analyzed while maintaining accuracy.

For tests that are reported qualitatively based upon a concentration cutoff, you will need to verify that known negative samples obtain negative results and samples with concentrations of the drug class just above the cutoff obtain positive results, and that samples with increasingly higher concentrations of the drug class also obtain positive results.

How to verify reportable range:
To verify the reportable range, test at least five negatives, five low positives (just above the cutoff) and five high positive samples once. These studies can be combined with accuracy/precision studies to save time and resources.

Reportable Range Example:

Cannabinoids screen, cutoff is 50 ng/mL

Sample	Known concentration	Result obtained	Acceptable Y/N
Negative sample #1	10 ng/mL	Negative	Y
Negative sample #2	25 ng/mL	Negative	Y
Negative sample #3	40 ng/mL	Negative	Y
Negative sample #4	Absent	Negative	Y
Negative sample #5	Absent	Negative	Y
Low positive sample #1	55 ng/mL	Positive	Y
Low positive sample #2	60 ng/mL	Positive	Y
Low positive sample #3	70 ng/mL	Positive	Y
Low positive sample #4	75 ng/mL	Positive	Y
Low positive sample #5	80 ng/mL	Positive	Y
High positive sample #1	150 ng/mL	Positive	Y
High positive sample #2	200 ng/mL	Positive	Y
High positive sample #3	250 ng/mL	Positive	Y
High positive sample #4	300 ng/mL	Positive	Y
High positive sample #5	500 ng/mL	Positive	Y

Specimen Integrity

The Laboratory must follow all manufacturer requirements pertaining to safeguarding the integrity of the specimen. The laboratory cannot test samples that have not been stored at the prescribed temperature and cannot test samples past the manufacturer's published timeframes for each environment (room temperature, refrigerated, frozen).

If circumstances require that specimens be tested after being stored for longer periods of time than prescribed by the manufacturer, the laboratory must undertake ***extensive*** studies to demonstrate that the specimen integrity is maintained. This procedure must be thoroughly documented and approved by the Laboratory Director. This practice is discouraged, as doing so may result in reclassification of the test to a modified FDA-approved procedure, thereby requiring high complexity personnel and establishment of all performance specifications.

Verification Summary

When the verification studies have been completed, a summary of all results must be approved by the Laboratory Director or designee. The laboratory must clearly state the purpose of the verification, what platform/method and the number of samples for each study. Any discrepant results should be investigated and explained in the summary. Test results that show sample problems such as contamination and degradation, should not be used in the assessment, but still listed with a detailed explanation.

The summary should also contain a conclusion stating whether the verification studies met the acceptance criteria and indicate if the method is suitable for use in the laboratory. If the study results fail to meet pre-established criteria, the test(s) methodology cannot be implemented for use in the laboratory until problems that led to the failures are corrected and the studies repeated and found to be acceptable.

The completed acceptable verification studies must be reviewed, approved, signed and dated, by the Laboratory Director, **PRIOR** to any patient testing being reported.

All verification data, including the raw instrument data, calculations, and summary, must be maintained for as long as the method is used in the laboratory, and two years thereafter.

IMPORTANT NOTES:

- If the laboratory has multiples of the same analyzer, each analyzer must have its own respective verification studies performed.
- If two separate licensed laboratories (separate CLIA numbers) share the same physical location and share instrumentation, each laboratory must perform its own verification studies.
- If the instrument is moved to a new location and the environment or testing personnel will be different at the new location, performance specifications must be re-verified.
- If the instrument is moved to a new location and the environment and the testing personnel will be the same, the Laboratory Director can define an abbreviated re-verification procedure, to make sure that the instrument and personnel can still achieve the expected performance specifications.

Additional requirements for verification of performance for unmodified FDA-approved procedures

Calibration Verification and Cutoff Verification

The requirements and exceptions for calibration verification are described in COLA criterion CA 2. However, for tests that are reported qualitatively, based upon a concentration cutoff, the requirement for calibration verification is considered met if the laboratory performs a cutoff verification every six months, as described in COLA criterion CA 2.1.

Validity Testing

Validity testing is testing that is performed exclusively to determine whether a specimen is acceptable for testing. This includes adulterant testing, such as specific gravity, urine creatinine and oxidants, to name a few. Labs must follow all manufacturer instructions for the performance and acceptability of validity testing and must include the required validity testing in their laboratory procedure. For all validity testing, the procedure must include criteria for acceptance of the specimen.

Validity testing, if reported simply as acceptable/unacceptable OR pass/fail, is not subject to the same regulatory scrutiny as other routine laboratory tests. However, it is very important that labs follow their procedures by rejecting specimens that, according to their procedures and according to the manufacturer's instructions, fail validity testing.

Validity testing reported as negative/positive or normal/abnormal IS subject to all CLIA regulations, including PT, QC, and performance verification requirements.

If validity testing is reported as numeric data compared to a reference range OR normal/abnormal, then this testing IS subject to all CLIA regulations, including requirements for PT, QC, and performance verification.

It should be emphasized that no matter how the validity testing is reported, labs must still follow all manufacturer requirements for the test.

Proficiency Testing (PT)

Most Toxicology drug screening tests are “unregulated,” and as such, PT is not ***required,*** however PT is strongly encouraged. If you do not enroll in PT, you must implement a split-sample protocol using a minimum of five samples, twice per year.

If you enroll in PT, but the PT module does not include all drug classes that you report, you must either enroll in another PT module that does include those drug classes OR implement a split-sample testing protocol for those analytes, using a minimum of five samples twice per year, and including both positive and negative samples. Your written procedure for split-sample testing must include your lab’s acceptability criteria.

• Establishment of performance of qualitative methods when the method:

- Is FDA approved but modified by the laboratory
- Is developed by the laboratory
- Utilizes a reagent/instrument pairing that is not listed on the FDA CLIA database

Laboratories that modify an FDA-cleared or approved test system, or introduce a test system not subject to FDA clearance or approval (including methods developed in-house and standardized methods such as text book procedures), or use a test system in which performance specifications are not provided by the manufacturer must, before reporting patient test results, establish test system performance specifications for the following performance characteristics, as applicable:

- Accuracy
- Precision
- Sensitivity
- Specificity to include evaluation of interfering substances
- Reportable Range
- Reference Range*
- Specimen Integrity and
- Any other performance characteristics relevant to the test(s).

The CLIA regulation regarding the establishment of performance can be found at CFR 42 493.1253

For immunoassay drug screening, the most common reason that labs are required to ***establish*** performance specifications is that they are using instrument/reagent pairings that are not specifically listed on the FDA CLIA database. The tests are therefore considered high complexity and must be treated the same as a non-FDA-approved method, even though the instrument and/or reagents may have clearance separately from the FDA.

In these circumstances, even though the laboratory will be establishing performance specifications, it is still required to follow all manufacturer instructions as written in the reagent package insert AND the instrument operator's manual.

In general, you will need to use a higher number of study samples when ***establishing*** performance specifications, as opposed to when you are simply verifying the performance of an unmodified FDA-approved method.

**It is not required to establish a reference range (normal values) for Toxicology screening assays, as long as both positive and negative samples are included in the verification studies. Known negative samples would represent the population that is not taking the specific drug class, and if the results obtained are negative as expected, this is considered acceptable as the establishment of the "reference" range.*

Performance establishment studies must be performed by the lab's own personnel. Your laboratory should define acceptability criteria for both accuracy and precision before you begin the studies.
Use samples that have a matrix as close as possible, to patient specimens. The first choice for clinical tests is patient samples, followed by control material and reference solutions (calibrators, etc.).

All studies must be reviewed for acceptability, approved, and signed by the Laboratory Director or designee prior to beginning patient testing. The delegation of this responsibility to a qualified person, such as the Technical Consultant or Technical Supervisor, must be in writing.

Accuracy

Definition: How close the measured value to the "true" value. You must verify that the test gives the correct results in your laboratory. Accuracy can be established by testing samples with known values and comparing them to the results that you obtain with your method.

To establish Accuracy:

The laboratory should run a variety of samples with known values and compare the results. The study must include both positive and negative samples. It is recommended that when establishing accuracy, the study include a minimum of 30 samples.

Using these samples in a method comparison experiment for accuracy is best over five days. It is best that side-by-side testing be done to ensure sample stability will not be affected. If this is not possible, refrigerating or freezing samples between testing may preserve the sample. Always take into account any freeze/thaw cycle limitations the respective method may have.

Document the results of the new method, comparing the known values from the reference sources, another CLIA-licensed laboratory results, or with results from the current method. It is acceptable to include both reference samples, such as QC, and patient samples, but patient samples should be given priority. If possible, involve various routine testing personnel.

Calculate the percent of positive, negative, and total accuracy, by dividing observed results over known results, multiplied by 100, as in the example provided in the first section of this guide.

Precision

Definition: Also known as Reproducibility – defines how consistent the results are when running the same sample multiple times.

How to establish Precision:
For quantitative tests, precision is typically evaluated using the coefficient of variation (CV). However, for qualitative tests a CV cannot be calculated. In the case of qualitative tests,

we are asking this question: If I run the same specimen multiple times, will I get the same result?

For establishing precision, you should select a minimum of five known positive samples (include samples with both high and low amounts of the drug) and five known negative samples and run these each five times on two different days. You should expect that the five known positive samples result in positive answers for each of the 10 replicates; and you should expect that the five known negative samples result in negative answers for each of the 10 replicates. If you do not achieve these results, then you will need to determine the cause of the discrepancy, implement a corrective action plan, and rerun the study.

Reportable Range

Definition: CLIA defines this as the highest and lowest test values that can be analyzed while maintaining accuracy.

For tests that are reported qualitatively based upon a concentration cutoff, you will need to establish that known negative samples obtain negative results and that samples with a concentration of the drug class just above the cutoff obtain positive results, and that samples with increasingly higher concentrations of the drug class also obtain positive results.

How to establish reportable range:
To establish the reportable range, test at least five negatives, five low positives (just above the cutoff) and five high positive samples once. These studies can be combined with the accuracy/precision studies to save time and resources.

Using a similar example as in the first section of this guide:

Cannabinoids screen, cutoff is 50 ng/mL

Sample	Known concentration	Result obtained	Acceptable Y/N
Negative sample #1	5 ng/mL	Negative	Y
Negative sample #1	10 ng/mL	Negative	Y
Negative sample #2	25 ng/mL	Negative	Y
Negative sample #3	40 ng/mL	Negative	Y
Negative sample #5	Absent	Negative	Y
Low positive sample #1	55 ng/mL	Positive	Y
Low positive sample #2	60 ng/mL	Positive	Y
Low positive sample #3	70 ng/mL	Positive	Y
Low positive sample #4	75 ng/mL	Positive	Y
Low positive sample #5	80 ng/mL	Positive	Y
High positive sample #1	150 ng/mL	Positive	Y
High positive sample #2	200 ng/mL	Positive	Y
High positive sample #3	350 ng/mL	Positive	Y
High positive sample #4	500 ng/mL	Positive	Y
High positive sample #5	1,000 ng/mL	Positive	Y

Based upon the results of this study, you have established that this qualitative assay is accurate when the raw numeric result is from 5 ng/mL to 1,000 ng/mL.

Sensitivity

Definition: In the context of a qualitative test, Sensitivity is the percent of known positive samples that yield positive results when tested with the method.

How to establish Sensitivity:
Sensitivity can be calculated by dividing the number of true positives by the sum of the number of true positives plus the number of false negatives and multiplying by 100. [TP/(TP+FN)] x 100 = Estimated Sensitivity. Use a minimum of 30 samples, including known negative samples, and samples with varying amounts of the analyte present.

Example: Cannabinoids, cutoff 50 ng/mL

Sample	Known value	Result
1	Absent	Neg
2	Absent	Neg
3	5 ng/mL	Neg
4	10 ng/mL	Neg
5	12 ng/mL	Neg
6	15 ng/mL	Neg
7	15 ng/mL	Neg
8	20 ng/mL	Neg
9	20 ng/mL	Neg
10	25 ng/mL	Neg
11	30 ng/mL	Neg
12	35 ng/mL	Neg
13	40 ng/mL	Neg
14	45 ng/mL	Pos
15	45 ng/mL	Neg
16	50 ng/mL	Pos
17	55 ng/mL	Neg
18	60 ng/mL	Pos
19	70 ng/mL	Pos
20	75 ng/mL	Pos
21	80 ng/mL	Pos
22	100 ng/mL	Pos
23	150 ng/mL	Pos
24	200 ng/mL	Pos
25	250 ng/mL	Pos
26	300 ng/mL	Pos
27	400ng/mL	Pos
28	500 ng/mL	Pos
29	600 ng/mL	Pos
30	750 ng/mL	Pos

From this data, you can construct a simple truth table:

Your results	Know positives (15)	Known negatives (15)
Positive	TP = 14	FP = 1
Negative	FN = 1	TN = 14

TP= True positive
FN = False negative
FP = False positive
TN – True negative
Using the formula: [TP/(TP+FN)] x 100 = the sensitivity is 14/(14+1) x 100, or 93%.

Specificity

Definition: In the context of a qualitative test, Specificity is defined as the percent of known negative samples that yield negative results when tested with the method.

How to establish Specificity:
Specificity can be expressed by dividing the number of true negatives by the sum of the number of true negatives plus the number of false positives and multiplying by 100. [TN/(TN+FP)] x 100 = Estimated Specificity. Use a minimum of 30 samples, including known negative samples, and samples with varying amounts of the analyte present.

Example:

From the same data and simple truth table above, used to establish Sensitivity, using the formula [TN/(TN+FP)] x 100, the specificity is 14/14+1 x 100, or 93%.

Interfering Substances

The laboratory must demonstrate that structurally related compounds are not interfering with the accuracy of the test.

How to evaluate Interfering Substances:
If a laboratory, for example, is performing screening for Amphetamines and Opiates, the laboratory should test several samples from patients who have not taken amphetamines but who have taken over-the-counter (OTC) Amphetamine-like substances, to show that structurally related compounds are not interfering with the analyte. The laboratory may also test several samples from patients who have not taken opioids but have taken OTC cough syrups that contain Opiate-like substances. It is not practical to test for every possible interfering substance. The Laboratory Director is responsible for determining what potential interfering substances should be evaluated for each analyte.

Specimen Integrity

The laboratory must establish specimen requirements for acceptability, including storage, transportation, temperature and specimen age requirements, prior to testing. There must be specimen rejection criteria included in the procedure for each analyte.

How to establish Specimen Integrity:
The laboratory must perform a study on multiple fresh samples which have been tested via the lab's routine procedure. The samples must then be aliquoted and stored at room temperature, refrigerated temperature, and freezer temperature at increasing intervals. The container or containers, in which the samples would be collected, transported, or stored, must be used in the specimen integrity study. The sample containers can affect the accuracy of results, by absorbing certain drug analytes. The study must be performed for each analyte that the laboratory plans to report. Use a minimum of five negative and five positive samples.

Example – this example is abbreviated and simplified for demonstration purposes. You will likely test additional intervals for each temperature.

Cannabinoids

Sample #	Fresh	RT 6 hrs	RT 12 hrs	RT 24 hrs	RT 48 hrs	Frig 24 hrs	Frig 48 hrs	Frig 72 hrs	Frig 96 hrs	Freez 3 days	Freez 6 days	Freez 10 days
1	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
2	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
3	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
4	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
5	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
6	Pos	Pos	Pos	Pos	Neg	Pos	Pos	Neg	Neg	Pos	Pos	Neg
7	Pos	Pos	Pos	Pos	Neg	Pos	Pos	Neg	Neg	Pos	Pos	Neg
8	Pos	Pos	Pos	Pos	Neg	Pos	Pos	Neg	Neg	Pos	Pos	Neg
9	Pos	Pos	Pos	Pos	Neg	Pos	Pos	Neg	Neg	Pos	Pos	Neg
10	Pos	Pos	Pos	Pos	Neg	Pos	Pos	Neg	Neg	Pos	Pos	Neg

From this data, you can conclude that for Cannabinoids, the specimen is acceptable for testing up to:

- 24 hours after collection at RT
- 48 hours after collection refrigerated
- 6 days after collection frozen

Establishment of Performance Summary

When the establishment studies have been completed, a summary of all results must be approved by the Laboratory Director or designee, such as the Technical Supervisor. The laboratory must clearly state the purpose of the studies, what platform/method and the number of samples for each study. Criteria for acceptability of the studies must be determined prior to performance of the studies.

The summary should also contain a conclusion stating the results of each performance parameter, and acceptance by the Laboratory Director or designee, based upon the predetermined acceptability criteria. If the study results fail to meet pre-established criteria, the test(s) methodology cannot be implemented for use in the laboratory until problems that led to the failures are corrected and the studies repeated and found to be acceptable.

All performance establishment testing must be performed by the laboratory's personnel. The completed acceptable performance establishment studies must be reviewed, approved, signed and dated, by the Laboratory Director, or designee, PRIOR to any patient testing being reported.

All verification data, including the raw instrument data, calculations, and summary, must be maintained for as long as the method is used in the laboratory, and two years thereafter.

IMPORTANT NOTES:

- If the laboratory has multiples of the same analyzer, each analyzer must have its own respective establishment studies performed.
- If two separate licensed laboratories (separate CLIA numbers) share the same physical location and share instrumentation, each laboratory must perform its own establishment studies.
- If the instrument is moved to a new location and the environment or testing personnel will be different at the new location, performance specifications must be re-established.
- If the instrument is moved to a new location and the environment and the testing personnel will be the same, the Laboratory Director can define an abbreviated procedure for verifying that the established level of performance can still be achieved at the new location.
- If the method is changed after implementation, such as a change in cutoff or a change in specimen type, performance specifications must be re-established.

Additional requirements for establishment of performance (for modified methods, LDTs, and reagent/instrument pairings not listed in the FDA CLIA database)

Calibration Verification and Cutoff Verification

The requirements and exceptions for calibration verification are described in COLA criterion CA 2. However, for tests that are reported qualitatively, based upon a concentration cutoff, the requirement for calibration verification is considered met if the laboratory performs a cutoff verification every six months, as described in COLA criterion CA 2.1.

Validity Testing

Validity testing is testing that is performed exclusively to determine whether a specimen is acceptable for testing. This includes adulterant testing, such as specific gravity, urine creatinine and oxidants, to name a few. Labs must follow all manufacturer instructions for the performance and acceptability of validity testing and must include the required validity testing in their laboratory procedure. For all validity testing, the procedure must include criteria for acceptance of the specimen.

Validity testing, if reported simply as acceptable/unacceptable OR pass/fail, is not subject to the same regulatory scrutiny as other routine laboratory tests. However, it is very important that labs follow their procedures by rejecting specimens that, according to their procedures and according to the manufacturer's instructions, fail validity testing.

Validity testing reported as negative/positive or normal/abnormal IS subject to all CLIA regulations, including PT, QC, and performance verification requirements.

If validity testing is reported as numeric data compared to a reference range, then this testing IS subject to all CLIA regulations, including requirements for PT, QC, and performance verification.

It should be emphasized that no matter how the validity testing is reported, labs must still follow all manufacturer requirements, AND the lab's own procedure for the test.

Proficiency Testing (PT)

Most Toxicology drug screening tests are "unregulated," and as such, PT is not ***required,*** however PT is strongly encouraged. If you do not enroll in PT, you must implement a split-sample protocol using a minimum of five samples, twice per year.

If you enroll in PT, but the PT module does not include all drug classes that you report, you must either enroll in another PT module that does include those drug classes, OR implement a split-sample protocol for those analytes, using a minimum of five samples twice per year, and including both positive and negative samples. Your written procedure for split-sample testing must include your lab's acceptability criteria.

Important information on COLA requirements pertaining to UDS

Please reference the COLA Accreditation Manual for a comprehensive list of requirements. Below are some requirements that are particularly important for COLA labs performing UDS.

QA 6.1

Does the laboratory have a process for monitoring the integrity of all specimens received for testing, specifically for specimen age, storage, and transport temperature?

This is a significant quality monitor, particularly for labs that receive specimens from other locations and those that perform batch testing. Specimens that are not received or tested within

the lab's established acceptability criteria must be rejected. Rejected specimens should be logged and monitored for patterns. Patterns related to submission are addressed with the submitting client(s). Patterns related to delays in testing are addressed by laboratory management. The process can be written or verbally explained, but rejected specimens must be documented and monitored. All corrective action and follow-up QA activities must be documented.

MSPEC 6

If the laboratory uses a cutoff value for reporting positive or negative, do the quality control materials used for each analyte include one with an expected result that is below the positive cutoff value and one with an expected result that is above the positive cutoff value?

In order for QC to be relevant, materials that challenge the positive cutoff or decision point, on both sides, should be used. This criterion also applies to other methods that have a positive cutoff value.

CA 2.1

For screening assays that are reported by the laboratory as qualitative (e.g. positive or negative) based upon a cutoff or threshold, has the laboratory verified the accuracy of the assay at the cutoff level at least every six months?

This requirement satisfies calibration verification for this type of test. Rather than verifying the reportable range at the low, mid-point, and high levels, the lab is required to verify values at the cutoff, and slightly below and above the cutoff, according to a procedure and acceptability requirements approved by the Lab Director. Materials used for this purpose cannot be the same materials used for daily Quality Control. Calibration requirements for the assay must always be met (see CA 1). If calibration includes a calibrator at the cutoff level, this requirement is considered met if calibrated at least every six months. This requirement does not apply to tests that cannot be calibrated by the user.

A note about Quality Assessment: QA must be performed and documented. A well-defined QA plan and implementation is necessary for monitoring and improving your lab's service. QA must consist of periodic review of the pre-analytical, analytical and post-analytical phases of the testing.