1.0 Principle;

It is the intent and purpose of the Toxicology Laboratory to report only quality analytical data that can withstand rigorous forensic challenge. An important basis for the ability of data to meet such criteria is the use of validated standard and control materials to calibrate and evaluate each analytical batch. This procedure outlines the processes by which standard materials, including control materials are evaluated and documented for use in the Toxicology Laboratory. The purpose of this procedure is to ensure that all qualitative and quantitative analyses are carried out using validated reference materials as the basis for those analyses. Standard materials, used either for the preparation of calibrators or controls, must be validated prior to use, by one of the methods described below. Documentation of validation is maintained within the section, as appropriate in the "Validated Standards" notebook.

2.0 Standard Material Retrieval

2.1 The storage, record keeping and control of scheduled standards has been designed to meet the requirements set forth by the state of Connecticut in the Connecticut Comprehensive Drug Laws. Laws relating to this include but may not be limited to chapter 420b sections 21a-262-1, 21a-262-2, 21a-262-7 and 21a-254. A combination safe and a padlocked refrigerator containing all the scheduled standards are located in the Controlled Substance Evidence Room.

2.2 To retrieve a scheduled standard from the storage location the analyst must be accompanied by a Controlled Substance section analyst. Only Controlled Substance section analysts and the Laboratory Director have access to the safe and standard refrigerator.

2.3 Scheduled Standard Retrieval (Performed with Witness)

2.3.1 Locate the appropriate inventory paper work ('Controlled Substance Activity Record/Inventory') which corresponds to the item being removed. (located in one of two inventory books; "Standard Safe", and "Refrigerator"). Each book is divided with schedule I and II items separated from schedule III, IV and V items. (Per requirements of State of Connecticut Comprehensive Drug Laws.)

2.3.2. Open the appropriate storage location with a witness. (Note; The key for entry into the refrigerator is located in a lock box, CS analysts have

access to this lock box).

2.3.4. Locate and remove the needed standard(s), close and re-lock Safe or refrigerator, with appropriate annotation in the Safe or Refrigerator Entry log book as follows;
Name of person opening the storage location,
Date and time of action.
Name and lot number of item(s) removed.
Witness signature and date.

2.3.5. Weigh out the amount of material needed, and complete the 'Controlled Substance Activity Record/Inventory' as follows;
Weight of container with substance,
direct weight of substance taken,
weight of container with substance after removing needed amount.

Note #1: *** If, upon taking the initial weight of the container with the substance there is greater than a 5% variation since the last use, immediately notify the Laboratory Director, Quality Manager or Supervisor.

Note #2: Variations >5% need to be investigated. Any documentation for discrepancies will be attached to the "Controlled Substance Activity Record/Inventory' form. If applicable notations can be made directly on the paperwork itself.

2.3.6 Using a witness, return the substance to the storage location, and annotate that process in the Safe or Refrigerator log book, with witness initials.

Note #1: Standards removed from their storage locations for use must be returned immediately after use; no standards are to remain in an analyst's possession overnight.

Note #2: A 'Disposition Record' form must be completed for standards made in concentrations greater than 1mg/ml, or when a portion of standard (in whatever form it is supplied in) is isolated for continuous use (such as with the cocaine isolated for the monthly IR validation). When this form is created it stays with the solution/substance until it is disposed of. The record is then stored with the QA records.

Note #3: Each analyst has the responsibility to make sure all paperwork is completed fully and accurately and that as supplies are low they inform the Laboratory Director or the Quality Manager.

2.3.8 Witness Responsibilities:

2.3.8.1 Observe the opening or closing of the storage location and verify what is being removed or returned.

2.3.8.2 Initial the Safe or Refrigerator Entrance log book as witness to what was removed.

2.3.8.3 Verify that the lock of the storage location is secure when work in the area is completed.

2.4 Retrieval of Non-Scheduled Standards; either solid dose substances stored in within the Toxicology Laboratory or DEA exempted liquid standards stored in refrigerators in the laboratory section; these are retrieved by simply removing them from their storage location and using them as needed.

3.0 Validation of Standard and Control Materials;

Standard Materials (other than DEA exempt standards with corresponding COAs) are required to be validated prior to use as the basis for reporting of results. This validation may occur by one of the methods noted below (in order of preference). However, where possible, both UV spectroscopy and GC/MS will be used. This will enable the analysts to evaluate the concentration while also verifying that there are no other UV absorbing compounds in the standard which may skew the UV results. Preparation and validation of standard and control materials is documented on a "Standard Preparation Form" and by a "Green Sticker" (green circular dot label) affixed to the standard bottle itself, indicating the date of validation.

Note: DEA-Exempt 1 ml vials of defined concentration may be used as supplied. Associated manufacturer's documentation should be filed.

3.1 UV Spectroscopy; (See Appendix 1)

3.1.1 An appropriate dilution of the standard material is examined by UV spectroscopy both for qualitative confirmation of structure, and for a quantitative evaluation, using a published, or traceable extinction coefficient, spectra and documentation of the standard material

concentration calculation should be appended to the standard preparation form.

3.1.2 The concentration of prepared solutions shall be within 5% of their theoretical or supplied value. In the event that standard materials fail to meet their quantitative target by UV within 5%, the Director and/or Quality Manager should be notified prior to the use of such materials in the preparation of calibrators or controls.

3.2 Gas Chromatography/Mass Spectrometry;

3.2.1 The material is examined by GCMS to ensure purity, and qualitative identification. A scan-type analysis is run, with the purity determined by the analyst as a function of number of peaks observed, and agreement of the spectra with literature or reference spectra. Based on this evaluation, and literature provided by the supplier, the concentration of the material may be accepted. Data and documentation of the review process should be documented on, and appended to, the standard preparation form.

3.2.2 All documentation used to prove the qualitative and/or quantitative validity of a standard will be filed in the Standard Validation log book for the specific section.

3.2.3 Literature:

3.2.3.1 Clark's Isolation and Identification of Drugs in pharmaceuticals, body fluids, and post-mortem materials.

3.2.3.2 In the absence of a published extinction coefficient, or a compound that does not readily chromatograph, the manufacturers literature may serve as documentation for validation; (subject to approval by the Director or Quality Manager).

3.3 Other Methods:

Depending on circumstances, other procedures may be used to validate standard materials. Examples might be (1) sending the material out for analysis (2) quantitative evaluation vs. defined standards borrowed from other laboratories (3) evaluation of PT materials, etc. Documentation of the validation process should be documented on, and

appended to, the standard preparation form; this must be approved by the Director or the Quality Manager.

3.4 Validation of Controls;

3.4.1 Externally Purchased Controls:

Externally purchased controls may be validated by multiple analyses in the target procedure. Ideally, this would consist of 10 or more separate analyses, on 5 or more independent analytical runs. The mean, sd and cv are then calculated, and that mean should be within 20% of the target value, with a CV of <10%. Control materials may be considered validated with fewer data points, or with a greater difference from target value, or with a cv >10% if, in the judgment of the Director and or Quality Manager, acceptance of such a control does not jeopardize the validity of any analysis in which that control might be utilized.

Note: EMIT controls are evaluated on a "Worked as expected" basis, as positive/negative only.

3.4.1.1 The acceptance range for Control Materials in analytical processes is the Validation Mean, +/- 20%, except as noted below:

3.4.1.2 When PT materials are being utilized as "emergency" controls. (See "C", below) In this case, the target value and acceptance ranges are those of the PT materials (e.g. CAP).

Note: The Director may approve alternate acceptance ranges, or target validation mean. The reasoning behind such a decision should be clearly documented on the Control Preparation/Validation Form. Further; specific procedures may detail and specify alternate control ranges.

The process by which validation of a specific control is carried out, including either the validation values, or reference to the location thereof, is documented in the "Validation Details" section of the Standard Preparation Form. Validation data and performance of controls in particular analyses are maintained in the QC logbook.

3.4.2 Internally Prepared Controls;
"Internally Prepared" controls may exist in two formats:
"Spiking Solutions," or Prepared Controls.

Both types of controls must undergo validation as described in "A",above, prior to use.

3.4.3 Spiking Solutions;
These solutions are used to prepare "spiked" controls on a per-run basis. The preparation of such a solution is detailed on a "Standard Preparation Form." The details of preparation are reviewed for mathematical accuracy, and the (prepared) target value checked and signed off in the "Validation Details" section. Validation is as described in "A" above, including the establishment of target value and acceptable ranges.

3.4.3 Prepared Controls;

These solutions are prepared from routine blank sample matrix (e.g. blood or urine), to contain a defined quantity of target analyte. Preparation details, including the sources of all materials used for the preparation of controls are detailed on the Standard Preparation Form. Validation consists of ten quantitative determinations, performed on 5 or more analytical runs. Interim validation may be generated with Directors and/or Quality Managers approval, with fewer data points or runs. Validation data and performance of controls in particular analyses are maintained by the QC director in the QC logbook.

3.4.4 PT Materials Utilized as Controls;

PT Materials may be utilized as controls on an "emergency" basis, without receiving the usual validation. In such a case, the documentation provided as a function of the PT report may be accepted as its validation. The target value is the reference value provided, and acceptance ranges are those of the PT test.

PT Materials may also undergo the routine validation process in the laboratory. In this case, the target value and acceptance ranges become as noted in 3.A., above. PT samples validated by this means are documented as per the process noted above.

APPENDIX I: **ANALYSIS OF STANDARD OR REFERENCE MATERIALS BY UV-VISABLE SPECTROSCOPY**

1.0 PRINCIPLE:

The acceptability of quantitative standard solutions is determined (when the analyte has a usable UV absorbance), using a UV spectrophotometer to determine the true concentration of the solution. The validation will be performed when the solution is prepared; prior to use with case materials. The specific wavelength and absorptivity of the substance will be determined utilizing a reference such as Clark's Isolation and Identification of Drugs. Absorptivity (A11) is defined as "the absorbance of a 1%w/v solution in a cell of 1cm path-length".

2.0 INSTRUMENTATION:

Shimadzu UV-2401PC, UV-VIS Recording Spectrophotometer with UV Probe version 2.1 software.

3.0 SAFETY:

The UV spectrophotometer utilizes a light source which should not be viewed. As with any electrical device there is a chance of electrical shock if not handled properly, do not perform maintenance on this instrument unless trained to do so.

4.0 Reagents:

Aqueous acid solution (1% HCl): 1 ml of Hydrochloric acid into 100 ml of deionized water

Aqueous alkaline solution: (0.05M Borax buffer): dissolve 19.97 g. of Borax in deionized water, bring to 1000 ml

Other matrix solutions prepared as the absorbtivity reference data requires

5.0 Sample Preparation:

5.1 Based on the reference data for absorptivity, quantitative dilutions will be prepared from the stock standard solutions which will give an UV absorbance in a range of 0.5 au to 2.0 au. The solutions are to be made in aqueous acid or aqueous alkaline, according to the reference data.

5.2 For standards that are made in a solvent that is not miscible with the needed UV matrix; evaporate the amount needed for the appropriate dilution and quantitatively reconstitute with the needed matrix.

5.3 Prepare a Matrix Blank: this is the aqueous acid or aqueous alkaline solution used to prepare the dilution of the standard combined with the solvent used to prepare the standard (prepared in the same ratio).

6.0 CONTROLS:

Instrument check (run upon instrument start-up).
Baseline blank (run prior to each set of samples).
Matrix blank (run with the sample/test solutions to correct for any matrix absorbance.

7.0 Procedure:

7.1 Instrument Set-Up

7.1.1 Turn on instrument, and select "UV probe" software.

7.1.2 Click the connect icon to initiate the communication. An 'instrument check' will run automatically. (If unacceptable, refer to Section Supervisor, Quality Manager or Director).

7.1.3 Run a Baseline Scan, with an air blank

7.1.4 Set up the method under the "Spectrum" program.

7.1.5 Set wavelength scan range as appropriate (In general, run 50 nm above and below the target absorbance wavelength.)

7.1.6 Under "Method" enter weight, volume, dilution, path length and additional information as necessary.

7.1.7 Fill a quartz cuvette with the blank solution (sample matrix) and place this in the back cell holder. Put the standard in a quartz cuvette and place this in the front cell holder.

7.1.8 Run the method

7.1.9 Click the peak point icon to label the peaks

7.1.10 Go to the Reporting software open the stndval.rpt report format and print the report.

7.1.11 Calculation:

$$\text{Concentration (mg/ml)} = \frac{\text{(absorbance (au) x 10mg/ml)}}{\text{Absorptivity (au)}}$$

absorbance is the reading at the specified peak
10mg/ml factor is based on the fact that the (A11) information is based on 1%w/v solution.
absorptivity is from the reference material

7.2 Reporting: The 'stndval' format in the reporting software is set up to provide spaces for all the needed data.

7.2.1 Fill in the needed information and calculate the concentration.

7.2.2 Have the Quality Manager, the Section Supervisor or the Laboratory Director review and sign off on the information.

7.3 POTENTIAL SOURCES OF ERROR:

7.3.1 Placing the sample in the wrong position in the sample chamber

7.3.2 Not using a matrix blank or using the wrong matrix blank

7.3.3 Preparing the sample in the wrong matrix (aqueous alkaline instead of aqueous acid)

7.3.4 Applying the calculation incorrectly

7.3.5 Incorrect dilution factors

7.3.6 Incorrect absorptivity reference data

REFERENCES:

Shimadzu UV-2450/2550 Instruction Manual

Shimadzu Instruction Manual UV-2401PC/2501PC User's System Guide

Clark's Isolation and Identification of Drugs in pharmaceuticals, body fluids, and post-mortem materials, The Pharmaceutical Society of Great Britain.