1.0 PRINCIPLE

This procedure describes a rapid screening/confirmation method for GHB and/or GBL in biological fluids and liquids by GC Headspace. GHB present in biological aqueous samples is converted to the corresponding lactone by treatment with acid. GBL is then extracted by dichloromethane, and analyzed by Headspace-GC. A vapor-phase sample aliquot is injected into the Headspace-GC, split onto two columns with distinct stationary phase, each with separate FID detector. GBL present in the sample aliquot is identified by retention time, on both columns and quantitated by FID response. Quantitation is based on a 5-point calibration curve, 5, 10, 20, 40, and 80 mg GHB/mL. Internal standard is □-methylene-□-butyrolactone.

2.0 SPECIMEN

Specimens requiring analysis for GHB/GBL, are listed in LIMS. Any aqueous sample may be suitable for this analysis including but not limited to blood, urine, vitreous humor, gastric contents and tissue homogenates. Samples will normally be submitted in gray-top tubes. Aqueous biological samples not stored in gray-top tubes may be subject to post-sampling generation of GHB. Samples should be sealed and stored in appropriate airtight glass or polypropylene containers. If not analyzed immediately, preserved liquid samples should be refrigerated at 4 degrees C^{o}.

2.1 All evidence transfers, either between individuals or between an individual and a storage location must be documented on the Chain of Custody for the case, either in the LIMS, or on hard-copy COC document maintained in the Case Jacket.

2.2 When not in the sampling or aliquot process, samples in the toxicology section must be stored in a secure and locked area.

2.3 Samples must be maintained in such a manner so that they are protected from contamination or deleterious change. Depending on the nature of the sample, this may mean refrigeration or freezing when not in the analytical process.

2.4 When analysis is complete, samples in the toxicology section must be maintained "Under Proper Seal." This is interpreted to mean that the sample, or a container in which the sample is kept is sealed with tamper-evident tape, with the initials and date of person placing the seal on the seal as well.

2.5 Samples are stored in the Toxicology Section, for 8 weeks following completion of the analytical process, in the absence of notification of any legalaction, or other

reason to maintain the samples. After this period, samples are discarded in the appropriate medical waste disposal container. Sample from homicides, or cases with pending legal action and requests for retention are maintained by the laboratory. Samples from Sexual Assualt cases are returned to the submitting Agency after analysis.

3.0 EQUIPMENT:

3.1 MATERIALS AND EQUIPMENT:

Shimadzu GC-2010 Headspace Gas Chromatograph with AOC-5000 autoinjector, equipped with Rtx-BAC1 and Rtx-BAC2 30m capillary columns and Lab Solutions GC Solution software.
20 mL Headspace vials
Tube Rotator
Vortex mixer
General Laboratory Glassware and equipment

3.2 REAGENTS:

3.2.1 Reagents available as stock items:

Deionized water (DIW; Millipore or equivalent In-House supply)
GHB (Gamma Hydroxybutyrate)
Internal standard (AMGB – □□Methylene-□□Butyrolactone)
Methanol (HPLC grade or equivalent)
Drug free urine
Drug free Blood (Oxalate & Fluoride preserved)
SuLfuric acid (Conc. Reagent grade or equivalent)
Methylene Chloride (HPLC grade or equivalent)

Note: Reagent Preparation and Validation is documented in the Toxicology "Reagent Preparation/Validation Logbook" maintained in the Toxicology section. Validation is addressed in section 3.2.4, below.

3.2.2 Calibrators and Internal Standard Stock Solutions:

Gamma Hydroxybutyrate (GHB; 1 mg/mL)
To a 10 mL volumetric flask, add 12.1 mg GHB-sodium salt. QS with DIW, refrigerate. Stable for 1 year.

□-Methylene-□-Butyrolactone (AMGB; 10 mg/mL)
To a 25 mL volumetric flask, add 250 mg AMGB. QS with DIW, refrigerate. Stable for 1 year.

a-Methylene-g-Butyrolactone (AMGB; 1 mg/mL)
To a 10 mL volumetric flask, add 1.0 mL stock AMGB. QS with DIW, refrigerate. Stable for 1 year

3.2.3 Working Solutions:
GHB Calibration Standards
0 ug/mL; no stock addition to blank matrix
5 ug/mL; add 5 uL GHB stock (1 mg/mL) to 1 mL blank matrix
10 ug/mL; add 10 uL GHB stock (1 mg/mL) to 1 mL blank matrix
20 ug/mL; add 20 uL GHB stock (1 mg/mL) to 1 mL blank matrix
40 ug/mL; add 40 uL GHB stock (1 mg/mL) to 1 mL blank matrix
80 ug/mL; add 80 uL GHB stock (1 mg/mL) to 1 mL blank matrix

3.2.4 GHB Controls:
15 mg/L; add 15 uL GHB stock (1 mg/mL) to 1 mL blank matrix
30 mg/L; add 30 uL GHB stock (1 mg/mL) to 1 mL blank matrix

Notes:
1. Controls are spiked by an individual other than the analyst.
2. Acceptable control performance is target value +/- 20%.
3. Each run must incorporate a high and low control for each matrix.
4. Blood samples must be evaluated against blood-based calibrators, however, urine controls may be evaluated against a blood or urine calibrators.

3.2.5 Validation of Reagents:
Validated reagents are Marked with a green dot, detailing the specific procedure for which the reagent was validated, and the batch on which that process was documented. Newly prepared reagents may be evaluated for validity on an analytical batch, prior to any consideration of sample resuLts. Acceptable performance of all batch control materials and overall batch acceptability (although individual samples may fail) is considered as validation of reagents. Reagents so validated are marked with a green sticker as noted above. Preparation of reagents, and their validation is documented in the Toxicology Section Reagent Preparation Validation Logbook, Maintained in the Toxicology laboratory.

4.0 PROCEDURE

Note: Departure from procedures as specified in this SOP is not anticipated. Should an issue arise that may require such a departure, the issue must be raised with Deputy Director. If the proposed change will not present a change of a magnitude that would require validation, the change may be approved, and the Director will modify and re-issue the SOP accordingly. Significant method changes require customer approval, prior to sample analysis.

Any such procedural changes would be subject to the review process afforded by the quality control measures of the analytical scheme described herein. Hence, any modification or change that produces an unexpected deleterious effect on the analytical procedure wouLd be expected to trigger analysis or batch failure in the QC review stages.

4.1 Label small test tubes with case numbers for all samples (in duplicate), calibrators, blanks and controls.

4.2 Add 1 mL of negative urine, blood or other matrix as appropriate to each control, calibrator and blank sample tube.

4.3 Add 50 uL of working internal standard (AMGB; 1 mg/mL) to each calibrator, blank, control and sample tube.

4.4 Add the appropriate amount of GHB (1 mg/mL) to each calibrator and control tube, as per 3.2.3 and 3.2.4 above (control addition to be performed by a separate analyst).

4.5 Add 150 uL of concentrated sulfuric acid to each calibrator, blank, control and sample. Vortex and let stand for five minutes.

4.6 Add 5 mL of methylene chloride to each tube and rotate for 15 min.

4.7 Centrifuge for 5 minutes.

4.8 Transfer lower organic layer to a labeled 5 mL conical tube.
(*Take care to not transfer any aqueous phase!*)

4.9 Concentrate each aliquot to about 100 uL.
(*Take care to not let the sample evaporate completely!*)

4.10 Transfer ~75 uL of concentrated aliquot to a 20 mL headspace vial, cap, seal and reserve for analysis.

5.0 HEAD SPACE CHROMATOGRAPHY

5.1 Instrument and Setup:

GC Headspace/Autosampler: (Shimadzu GC-2010 Gas Chromatograph, AOC 5000 Autosampler)
Column: Rtx-BAC1 and Rtx-BAC2 30m capillary columns
Inj. Temp. 110 ^{o}
Det. Temp. 250 ^{o}
Oven (init.): 90^{o} (1.2 min hold) 9 ^{o} /min to 160 ^{o}, (5.0 min hold).
13.98 min total run time
0.8 minutes injection

Note: Method Details Appended (Appendix I)

5.2 Injection/Batch sequence
Analytical batches for GHB confirmation should follow the format indicated below:
Note: Samples are prepared and analyzed in duplicate.
Conditioner (any level control)
Calibrator low
Calibrator medium
Calibrator High
Matrix blank
Control High
Control Low
Matrix blank
Sample 1 rep 1
Sample 1 rep 2
Samples 2-? Rep 1 and 2 (Max 10 samples between controls)
Control High

5.3 DETECTION AND IDENTIFICATION

A sample is considered positive for GHB if the following criteria are met:
- The calibration curve is linear with a correlation coefficient of 0.98 or greater
- GBL is detected in the acid-hydrolyzed sample

- The concentration of GBL is greater than 10 microgram/mL

Note 1: GHB may be distinguished from GBL by re-analysis of the original sample in the absence of the acidification. If GHB was present in the initial sample, there will be no GBL signal in the extract. If GBL was present in the sample, it will again appear in the extract.

Note 2: This procedure is considered a screen for the presence of GHB in a biological sample. Definitive identification and quantitation of GHB is described in SOP TX-22.

5.4 CALIBRATION AND QUANTITATION

Calibration for the purpose of quantitation is done on a per-run basis. Calibration is accomplished by the incorporation into the sample work-up of GHB calibrators. The response system to the calibrators defines a run-specific standard curve that is used as the basis for the sample calculation. Quantitation is accomplished by the comparison of the response ratio of the analyte in a specific sample to the response ratio of the calibrators as expressed on the standard curve. The concentration of the analyte in the sample is the extrapolated from the standard curve and corrected for any dilution that may have been performed to facilitate the analysis of relatively concentrated samples.

5.5 QUALITY CONTROL AND RUN EVALUATION

5.5.1 Vial sequence is verified prior to, and after sample injection
5.5.2 Chromatographic quality is evaluated for each peak
5.5.3 Potential for carryover is evaluated on a case-specific basis When a question of potential carryover exists, the potentially affected samples are repeated
5.5.4 Control Results: For the batch to be considered acceptable both control results for that analyte must be within 20% of the target value. Positive samples bracketed with acceptable controls are considered valid. Negative samples bracketed by one acceptable control and one failing control may be considered reportable, but such cases should be considered with the supervisor and/or director.
5.5.5 Internal Standard: For any individual injection to be acceptable, the internal standard abundance must within least 20% of the corresponding abundance
In the calibrator, and greater than the minimal abundance noted above.

5.6 LINEARITY

Linearity of the calibration curve is demonstrable in each batch, for each analyte as a function of quantitative resuLts of control materials. Acceptable linearity is greater than or equal to 0.98.

5.7 SENSITIVITY- LIMITS OF DETECTION (LOD) and QUANTITATION (LOQ)

For the purposes of this procedure, the LOD and LOQ are defined as equal to the lowest concentration of the lowest control. Qualitative Identification and/or Quantitative analysis below the concentration of the low control may be accepted on a case by case basis with the concurrence of the analyst, technical reviewer, Deputy Director and/or Quality Manager.

5.8 ACCURACY AND PRECISION

Precision of the procedure is evaluated on a yearly basis, by repeat analyses of control or PT materials. Accuracy is expressed as a mean (absolute value) percentage difference between mean quantitative value of 10 reps of the specific control, and the target value. Precision is expressed as the CV of that value.

6.0 REPORTING OF RESULTS

Qualitative identification in the absence of relative retention time information from standard materials is considered presumptive, unless the identification is of particular high quality, and is consistent with case history. Runs are performed as part of GC batches containing controls and calibrators. Specific chromatograms for each case are filled in the appropriate case file. The GC run is reviewed and signed off by a reviewer distinct from the operator.

7.0 QUALITY ASSURANCE

A Batch summary sheet will be produced with each batch. Data on each batch should include fields such as: Sample name, Batch ID (Date of Batch), analysts who generated data, matrix, analyte found (and concentration if applicable), controls run with the batch and results obtained.

7.1 Quality Assurance is provided by the multiple layers of checks that are performed both during and after analysis. Specifically:

7.1.1 The GC run is thoroughly checked by the operator, including vial position on the autosampler, both prior to and following the injection of samples.

7.1.2 The GC run is reviewed and signed off by a reviewer distinct from the operator, with this review including an evaluation of qualitative and quantitative (where applicable) results, including:

a. Control Results

b. Chromatographic Characteristics

7.1.3 The results, as transcribed in the Batch Summary Form are checked against the original run summary sheet during the process of report preparation, and during the administrative review of case results. If controls do not meet the criteria, the batch can be rejected as a whole or by a case by case basis. The supervisor is notified and proper action is taken to correct any problem. Batches and/or cases shall be repeated as needed.

7.1.4 The original run is compared to the Final Report during the Final review, prior to case sign-off.

8.0 SOURCES OF ERROR:

Quantitative uncertainty is determined in the following manner; For single analyte procedures, the average percent difference from target value is determined for 20 control replicates, along with the associated standard deviation. Uncertainty for the procedure is then defined as the larger value of two standard deviations, expressed as a percentage, plus the uncertainty associated with the standard material from which the calibrators were prepared, or 20%, the acceptance range for batch co-extracted controls, consistent with accepted practice. When uncertainty is reported on quantitative values, the format will be "Determine Value, units +/- Uncertainty". Eg. GHB, 154 ng/mL +/- 27 ng/mL.