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Toxicology Section

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Toxicology Procedural Manual
Section Five
Quality Assurance
5.7 Review of Toxicology Proficiency and Competency Tests

Revision #	Issue Date	History
0	04-25-02	Original Issue

Approval

Technical Leader: S.C. Williamson **Date:** 04/25/02

Issuance

QC Manager: Rick D. Groff **Date:** 5-2-02

Issued 04-25-02
Reissued 05-24-2006

Idaho State Police
Forensic Services
Toxicology Section

Section Five
Quality Assurance

5.7 Review of Toxicology Proficiency and Competency Tests

5.7.1 SCOPE

As described in section 11 of the *Quality Manual*, proficiency testing is an integral part of a quality program. This procedure describes the evaluation of the results obtained for competency and proficiency testing for the toxicology program.

5.7.2 BACKGROUND

In accordance with the *Toxicology Training Manual*, upon the completion of training, the trainee will complete a competency test consisting of ≥six (6) specimens which contain representative commonly encountered parent drug and drug metabolites. After successful completion of the competency test, the trainee will participate in the next appropriate proficiency test. This must be completed within one year of being approved for casework analysis. For urine and blood drug testing the trainee should complete an ASCLD/LAB approved proficiency test. For blood alcohol analysis, the trainee should perform the Department of Transportation (DOT) proficiency test or the proficiency test approved by ASCLD/LAB should that be different than the DOT proficiency test.

As described in section 5.6 of this manual, to perform alcohol determinations for legal purposes, a laboratory must take part in an Idaho State Police Forensic Services (ISP-FS) recognized, semiannual proficiency testing program and be approved by the ISP-FS Laboratory for start-up or to continue analysis of samples for alcohol content. To comply with ASCLD/LAB requirements it is only necessary for an analyst to successfully complete one test annually. If an ISP-FS laboratory has more than one trained alcohol analyst, then the responsibility can be shared. If only one analyst is available, then the analyst must complete both DOT proficiency tests each year.

5.7.3 PROCEDURE

5.7.3.1 Alcohol Analysis

5.7.3.1.1 Competency Test

5.7.3.1.1.1 The competency test can be ordered through a reliable vendor.

5.7.3.1.1.2 The acceptable alcohol concentration range is determined from the target

value provided by the manufacturer of the competency test.

5.7.3.1.1.3 Reported values must fall within ±10% of the target value reported by manufacturer.

5.7.3.1.1.4 If the value reported do not fall within the allowable range, analysis procedures will be reviewed and additional training/retraining may be required as deemed appropriate by the Toxicology Program Technical Leader. The analyst will be required to perform an additional competency test.

5.7.3.1.2 Proficiency Test

Refer to section 5.6 Criteria for *Site Approval to Perform Legal Alcohol Determinations.*

5.7.3.2 Qualitative Urine and Blood Analysis

5.7.3.2.1 Competency Testing

5.7.3.2.1.1 The competency test will so designed that appropriate SOPs and all necessary standards will be available for the analyst to pursue all requisite analysis.

5.7.3.2.1.2 The competency test can be prepared by the Toxicology Program Technical Leader, her/his designee, or ordered through a reliable vendor.

5.7.3.2.1.3 If the analyst does not report all appropriate analytes, the analyst's training will be reviewed and additional training/retraining may be required as deemed appropriate by the Toxicology Program Technical Leader. The analyst will be required to complete additional competency test samples. The number of samples will be determined by the nature of the discrepancy.

5.7.3.2.2 Proficiency Testing

5.7.3.2.2.1 Only analytes that are tested for with current ISP-FS SOPs and approach to analysis will be evaluated.

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Revision #	Issue Date	History
0	04-25-02	Original Issue

Approval

Technical Leader: _____ **Date:** _____
S.C. Williamson

Issuance

QC Manager: _____ **Date:** _____
Rick D. Groff

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Fo Issued 3-09-2005
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Revision #	Issue Date	History
0	10-18-2002	Original Issue
1	04-16-2003	Clarifications, Updated.
2	07-23-2003	Clarification of authentication process.
3	03-09-2005	Reformatted, scope broadened.

Approval

Technical Leader: Susan C. Williamson **Date:** 09 MARCH 2005

Issuance

QC Manager: Richard D. Groff **Date:** March 9 2005

Idaho State Police
Forensic Services
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Section Five
Quality Assurance

5.8 Quality Assurance Measures - Urine and Blood Toxicology

5.8.1 BACKGROUND

The quality assurance measures applied towards analysis utilizing a gas chromatograph equipped with a mass selective detector (GC-MSD) promote confidence in results. The requirements apply to all data obtained by GC-MSD. This SOP was created so that the shared requirements did not have to be included in every SOP addressing GC-MSD analysis.

5.8.2 SCOPE

This SOP addresses qualitative and quantitative authentication of reference material and general acceptance requirements for analysis data obtained through analysis with a GC-MSD. Reference materials include both analytical reference standards and commercially obtained matrix controls. Requirements for analysis with other instrumentation are addressed in relevant SOPs.

5.8.3 EQUIPMENT AND SUPPLIES

5.8.3.1 Tube Rocker (Fisher Scientific or equivalent)
5.8.3.2 Laboratory Centrifuge (Fisher Marathon or equivalent)
5.8.3.3 Waterbath (Fisher or equivalent)
5.8.3.4 Drybath (Fisher or equivalent)
5.8.3.5 Evaporative Concentrator (Zymark TurboVap or equivalent) equipped with nitrogen tank.
5.8.3.6 Glassware
Refer to appropriate SOP for extraction glassware.
GC/MS ALS vials (HP 5182-0865 or equivalent)
GC/MS vial microinsert (HP 5183-2088 or equivalent)
5.8.3.7 Gas Chromatograph equipped with a mass selective detector (HP 6890/5973 or equivalent) and a HP-5MS Ultra low bleed (5%-Diphenyl-95%-Dimethylsiloxane co-polymer) capillary column (25M) or equivalent.

5.8.4 REAGENTS

5.8.4.1 Refer to appropriate SOP and manual sections 2.6 and 3.8 for solution preparation instructions.

5.8.5 REFERENCE MATERIAL AUTHENTICATION

5.8.5.1 General

5.8.5.1.1 Appropriate authentication should be documented for reference materials prior to official use.

5.8.5.1.2 Reference materials applied for qualitative purposes must have their chemical identity well established.

5.8.5.1.3 The manufacturer of reference standards used for quantitative purposes should utilize balances calibrated with weights traceable to National Institute of Standards and Technology (NIST) standards. The certificate of analysis should be consulted to verify compliance with this requirement.

5.8.5.1.4 When a standard or control contains more than one constituent, only the compound(s) of interest need be authenticated.

5.8.5.1.5 When available, reference materials used for quantitation should be analyzed against existing calibration standards.

5.8.5.1.6 Whenever possible, source of reference standard used to prepare matrix controls should differ from that used to prepare calibration standards. If different vendors are not available, the standards and controls should be prepared separately.

5.8.5.1.7 For qualitative authentication, evaluate a single GC-MSD analysis obtained in full scan mode.

5.8.5.1.8 For quantitative authentication, a minimum of three determinations, in a single analysis run, should be evaluated.

5.8.5.1.9 For quantitative determinations, utilize SOP and GC-MS conditions optimized for the analyte under evaluation.

5.8.5.1.10 Whenever possible, the GC-MSD data should be compared to a previous lot of reference material.

5.8.5.1.11 The mean quantitative concentration should fall within 10% of the target value listed on *Certificate of Analysis* for standards or *Package Insert* for matrix controls. The precision between replicates should be ≤ 5%.

5.8.5.1.12 Certificate of Analysis (COA) for all standards and package inserts for commercially obtained matrix controls, will be stored centrally in the laboratory performing the authentication.

5.8.5.2 Reference Standard Authentication

5.8.5.2.1 Reference standards are used for both qualitative and quantitative purposes.

5.8.5.2.2 Whenever possible, qualitative authentication is accomplished comparing the instrumental data obtained through the instrumental analysis of the new standard with data from a peer reviewed scientific journal, reference standard compendium, instrumental data and/or library searches in conjunction with the data provided on the COA.

5.8.5.2.2.1 Comparison should result in no significant differences.

5.8.5.2.3 When comparison to a journal, compendium or other document, is not an option, mass spectral interpretation may be used in conjunction with the COA. This would apply in cases where instrumental data for a drug metabolite is not yet published but a structurally similar compound is available to assist with interpretation.

5.8.5.2.4 For the quantitative authentication of the concentration of a component of a reference standard, evaluate gas chromatography-mass spectrometry (GC-MS) data in conjunction with the certificate of analysis (COA) provided by the manufacturer.

5.8.5.2.5 Deuterated internal standards may also be evaluated in SIM mode prior to use.

5.8.5.3 Matrix Control Authentication

5.8.5.3.1 Matrix controls are analyzed in parallel with casework samples to demonstrate that a procedure performed as intended.

5.8.5.3.2 Matrix controls serve to validate a calibration curve.

5.8.5.3.3 Matrix controls may be prepared with authenticated reference standards and appropriate matrix or obtained through a vendor.

5.8.5.3.4 The chemical identity of component(s) in a commercially obtained matrix control should be based on the package insert. If the analyst is unfamiliar with the MS of the component, reference materials should be consulted as described in 5.8.5.2.2.

5.8.5.3.5 To authenticate the concentration of a component of a commercially obtained matrix control, evaluate gas chromatography-mass spectrometry (GC-MS) data in conjunction with the package insert provided by the manufacturer.

5.8.5.3.6 Controls in use prior to the start date of this SOP revision can be used until consumed.

5.8.5.4 Authentication Documentation

5.8.5.4.1 A coversheet providing the information necessary for authentication will be prepared and placed with the MSD data. The coversheet for qualitative validation should, at a minimum, list the lot number, vendor, date of analysis, analyst, and mode of authentication. For quantitative authentication, the coversheet should include an evaluation of quantitative data.

5.8.5.4.2 Coversheet and GC-MSD data should be initialed and stored centrally in a designated location.

5.8.5.4.3 The container for the standard or control will be designated as "authenticated" after the authenticity of the standard has been validated.

5.8.5.4.4 It is the responsibility of each analyst to verify that each standard or control used has been properly authenticated.

5.8.6 ANALYSIS QUALITY ASSURANCE

5.8.6.1 Qualitative Analysis

5.8.6.1.1 Non-extracted Standards (NES)

5.8.6.1.1.1 Standards must be prepared and analyzed as designated in appropriate SOP.

5.8.6.1.1.2 Acquired data should be comparable to authentication data. No significant differences in GC-MS data should be apparent.

5.8.6.1.2 Matrix Controls

5.8.6.1.2.1 Controls should be prepared and analyzed as designated in the appropriate SOP.

5.8.6.1.2.2 Positive controls should exhibit proper retention time and mass spectral characteristics for compounds of interest.

5.8.6.1.2.3 Negative controls should be examined for compound(s) of interest and interfering substances.

5.8.6.1.3 Solvent Blanks

5.8.6.1.3.1 An appropriate solvent blank should be run between sample extracts.

5.8.6.1.3.2 If the solvent blank contains a reportable analyte of interest, the corrected area of the analyte peak must be a minimum of 10 times stronger than the corresponding peak in the blank preceding it. Ideally, no contamination should be apparent.

5.8.6.1.3.3 Reportable is defined as a complete fragmentation pattern at the appropriate retention time. Analytes of interest include, but are not limited to, analytes routinely reported.

5.8.6.1.3.4 If significant contamination is present, as discussed in 5.8.6.1.3.2, evaluate the analysis of a newly obtained solvent blank and the sample extract in question. If the contamination is still apparent, troubleshoot the instrument to determine the source of contamination. In addition, the sample in question should be reextracted prior to reanalysis on rectified instrument.

5.8.6.2 Quantitative Analysis

Quality measures are optimized for the analytes in question and are addressed in each individual quantitative SOP.

5.8.6.3 Distribution of Quality Data

5.8.6.3.1 Originals of casework standards and matrix controls will be stored in a designated central location in the laboratory where the analysis was performed.

5.8.6.3.2 Copies of all quality assurance control data need not be placed in each case file except those required under 5.8.6.3.3.

5.8.6.3.3 Copies of analytical standards used to substantiate the identification of each drug compound must be included in each case file if not otherwise indicated in the relevant SOP.

5.8.7 REFERENCES

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5.8.7.3 SOFT/AAFS Forensic Toxicology Laboratory Guidelines, 2002

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5.8 Evaluation of Quality Assurance Measures

Revision #	Issue Date	History
0	10-18-2002	Original Issue
1	04-16-2003	Clarifications, Updated.
2	07-23-2003	Clarification of authentication process.
3	03-09-2005	Reformatted, scope broadened.

Approval

Technical Leader: _____ **Date:** _____
Susan C. Williamson

Issuance

QC Manager: _____ **Date:** _____
Richard D. Groff

Approved 5-20-04
Replaced 5-4-2006

Idaho State Police
Forensic Services
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Section Two
Urine Toxicology

2.4 Liquid-Liquid Extractio
2.4.2 Qualitative Co **te (GHB)**
in Urine Utilizin

Revision #	Issue Date	History
1	02/05/02	Original Issue in SOP Format
2	10/18/02	Refinements
3	05/20/04	Refinements Renamed from 2.4.2.1 Extraction option two moved to 2.4.6 GHB products moved to SOP 6.1 and 6.2 No extraction method modifications therefore no revalidation pursued.

Approval

Discipline Leader

S. C. Williamson — 05/20/04
Date

Issuance

Quality Assurance Manager

Richard D. Groff — May 20, 2004
Date

Idaho State Police
Forensic Services
Toxicology Section

Section Two
Urine Toxicology

2.4 Liquid-Liquid Extraction Methods for GC/MSD Confirmation

2.4.2 Qualitative Confirmation of Gamma-Hydroxybutyrate (GHB) in Urine Utilizing TOXI-B Extraction Tubes

2.4.2.1 BACKGROUND

GHB occurs naturally in minute quantities as a result of the metabolism of the inhibitory neurotransmitter, GABA.[6,8] The drug GHB is a potent tranquilizer that was previously used as an anesthetic and as a treatment for major depressive illnesses, alcohol withdrawal, and narcolepsy.[1-8] Legitimate use is limited due the side effects associated with the drug. GHB can produce visual disturbances, nausea, vomiting, drowsiness, dizziness, severe respiratory depression, unconsciousness and involuntary muscle spasms.[1,5,6] Overdoses can require emergency medical treatment including intensive care due to the respiratory depression, bradycardia and coma.[5,6,8]

The use of GHB has been increasing since the 1980s and currently is popular among body builders, teenagers and on the club/dance scene. Body builders use the drug due its alleged role as a growth hormone releasing agent to enhance muscle growth.[5,6,8] GHB has not been proven to possess any anabolic effects.[6] GHB has gained notoriety as a date rape drug due to its ability to produce short-term amnesia and decreased inhibitions.[1-8] The FDA banned the use of GHB in 1990 except for FDA approved physician supervised protocols.[6,8]

A dose of GHB consists of a capful that is usually approximately one teaspoon. This results in a dose anywhere from 2.5 to 4.0 grams of GHB. The taste of GHB has been described as salty or soapy, the odor is said to be mothball-like.[5,6] Due to the short half-life of GHB (0.3 to 1.0 hours[5], 27 ± 5 minutes[7]) the person will re-administer every 45 minutes to 1 hour. The onset of effects is 15 to 60 minutes. The effects of the drug will be detectable during a DRE exam for 4 to 6 hours. GHB is classified as a central nervous system depressant. The observed effects include horizontal and vertical nystagmus, lack of convergence, body tremors, and slowed breathing. The person will also exhibit a lowered pulse, blood pressure, and body temperature. In addition, the muscle tone will exhibit flaccidity and the person may be in a trance-like state, the pupils will exhibit a lack of reaction to light.[5] Lower doses will promote an agitated, combative state however their pulse and other vitals will be depressed.[5,6] Combining GHB with alcohol plus a stimulant or marihuana allows the user to remain conscious during use. This allows them

to experience the euphoric "buzz" that is the desired effect of its abuse. The desired effect is a state of relaxation and tranquility, a pleasant drowsiness, mild euphoria, hallucinations and a release of inhibitions. Combining GHB with alcohol or other central nervous system depressant will provide an additive depressant effect.[5]

GHB is detectable in blood for up to eight hours and in urine for up to 12 hours [5,6,7]. Peak plasma levels are obtained in 20 to 45 minutes.[7] Peak urine GHB concentrations on the order of 1100 µg/mL are observed within the first four hours after a 100 mg/kg oral dose.[3,7]

GHB is manufactured by reacting butyrolactone with sodium hydroxide in an aqueous solution in the presence of a low molecular weight alcohol (methanol, ethanol).[5,6] The average yield of GHB is 70%. If the yield of the manufacturing process is low, there will be significant amounts of the lactone present in the product. Abuse of this compound will continue due to its relatively simple synthesis and the availability and low cost of starting materials.[5] Users can ingest Gamma butyral lactone (GBL), a degreaser and floor stripper, and it is converted in vivo into GHB. GBL is therefore diverted from legitimate sources to either be taken directly or converted to GHB.

This compound has been referred to by a variety of names as listed in the table below.

Street Names	Marketing Names
"G"	Revitalize
"G" caps	Rejuvenate
Liquid X	Renewtrient
Soap	Revivarant
Easy Lay	Blue Nitro
Georgia Home Boy	Thunder Nectar
G-riffic	Rest-Eze
Grievous Bodily Harm	Energy Drink

There are thousands of documented GHB overdoses with numerous deaths. The danger in GHB use stems from its steep dose-response curve. A small increase in dose can create a dramatic difference in adverse effects. This makes the potential of overdosing with GHB very high. This is compounded by the fact that GHB effects users so differently. A dose that one individual uses could adversely effect another, thus word of mouth is a poor determiner of how much of the substance to use.

2.4.2.2 SCOPE

This method provides an efficient qualitative analysis option for the liquid-liquid extraction of urine samples suspected of containing γ-Hydroxybutyrate/γ-Hydroxybutyric Acid (GHB). GHB is isolated from an

acidified solution into methylene chloride and heptane with zinc chloride to facilitate the extraction process. The extraction is achieved with an Ansys Toxi-B extraction tube. The extraction is followed by the creation of a di-TMS derivative of GHB. The derivative is analyzed by full scan GC/MS in EI mode. This method may not provide adequate sensitivity for weaker concentrations of GHB. This method should only be used for driving under the influence of drugs (DUID) situations where GHB is suspected. SOP 2.4.7 should be used for drug facilitated sexual assault (DFSA) cases.

2.4.2.3 EQUIPMENT

2.4.2.3.1 Tube Rocker (Fisher Scientific or equivalent)
2.4.2.3.2 Evaporative Concentrator (Zymark Turbo-Vap or equivalent)
2.4.2.3.3 Laboratory Centrifuge (Fisher Marathon or equivalent)
2.4.2.3.4 Glassware
- 2.4.2.3.4.1 Tapered tip 16X144 centrifuge tubes (Fisher catalog 05-538-41C or equivalent)
- 2.4.2.3.4.2 Snap caps (Fisher 05-538-41N or equivalent)
- 2.4.2.3.4.3 GC/MS vials (HP 5182-0865 or equivalent)
- 2.4.2.3.4.4 GC/MS vial microinserts (HP 5183-2088 or equivalent)

2.4.2.3.5 Gas Chromatograph equipped with a mass selective detector (HP 6890/5973 or equivalent) and a nonpolar capillary column with a phase composition capable of efficiently separating GHB and its analogs in toxicological specimens (e.g. 100%-dimethylpolysiloxane or 95%-dimethyl-polysiloxane with 5%diphenyl)

2.4.2.4 REAGENTS

2.4.2.4.1 ANSYS TOXI-TUBES B (109B-100)
2.4.2.4.2 Silylating Agent (select from)
MSFTA (Pierce #48910 or equivalent)
BSTFA with 1%TMCS (Pierce #38831 or equivalent)

2.4.2.5 REFERENCE MATERIAL

2.4.2.5.1 GHB Stock Solution [1.0mg/mL]
Cerilliant #G-001 or equivalent.

2.4.2.5.2 GHB Spiked Urine Positive Controls
- 2.4.6.5.2.1 **100µg/mL**
  Add 100uL of GHB 1mg/mL stock to 950uL negative urine. Vortex.
- 2.4.6.5.2.2 **200µg/mL**

Add 200uL of GHB 1mg/mL stock to 800uL negative urine. Vortex.

2.4.2.5.3 Non-Extracted GHB Standard [100µg]
Place 100uL of GHB stock into taped-end centrifuge tube.

2.4.2.5.4 Negative Control
Negative Urine (Ansys 170A, Utak 88121-CDF (L) or equivalent.)

2.4.2.6 PROCEDURE

2.4.2.6.1 Initial set-up

2.4.2.6.1.1 Label TOXI-TUBES B for positive control(s), negative control and case samples.

2.4.2.6.1.2 Label tapered-bottom centrifuge tubes and GC/MS vials for positive controls, negative control, case samples and non-extracted standard.

2.4.2.6.2 Extraction procedure

2.4.2.6.2.1 Extract 4.5 mL of specimen, negative or spiked urine in TOXI-TUBE B (acidic extraction @pH=4.5).

2.4.2.6.2.2 Rock TOXI-TUBE for 15 minutes.

2.4.2.6.2.3 Centrifuge TOXI-TUBE at 2500 rpm for 15 minutes.

2.4.2.6.2.4 Transfer solvent from TOXI-TUBE into tapered-end centrifuge tube.

2.4.2.6.2.5 Evaporate solvent to approximately 50µL with nitrogen at 40°C in TurboVap apparatus.

2.4.2.6.3 Derivatization Procedure

2.4.2.6.3.1 Add 40µL silylating agent to evaporated extracted samples, spiked control(s) and non-extracted standard. Cap tube with snap cap.

2.4.2.6.3.2 Vortex tube.

2.4.2.6.3.3 Place tube in 60°C sandbath for 15 minutes.

2.4.2.6.3.4 Remove tube from sandbath. Allow sample to cool. Transfer derivative to labeled GC/MS ALS vial for analysis.

2.4.2.6.4 Gas Chromatography/Mass Spectrometry (GC/MS) Parameters

2.4.2.6.4.1 Key parameters are specified below. Parameters not specified are at the discretion of

the analyst and should be optimized for the both gas chromatographic and mass spectral characteristics of an instrument. Refer to GC/MS METHOD printout for current parameters for analysis. Each laboratory should maintain a centrally stored current METHOD printout.

2.4.2.6.4.2 **ALS Parameters**
Injection Volume: 1µL (1 stop)
Viscosity Delay: A minimum of 3 seconds
Solvent Washes (A & B): A minimum of 4 pre- and post-wash rinses.

2.4.2.6.4.3 **Acquisition Mode**
Sample should be analyzed full scan acquisition mode.

2.4.2.6.5 Detection and Identification Criteria

2.4.2.6.5.1 **Chromatographic Criteria**
The retention time of the analyte should fall within ±2% of the retention time exhibited by GHB standard and control(s).

2.4.2.6.5.2 **Mass Spectral Criteria**
Full scan mass spectral data should be compared against within run GHB standard and control(s). No significant differences should be apparent.

2.4.2.7 QUALITY ASSURANCE

2.4.2.7.1 General

2.4.2.7.1.1 Urine samples should be kept frozen until checked out for analysis. Samples thereafter should be stored under refrigeration until completion of analysis and long-term frozen storage.

2.4.2.7.1.2 Refer to toxicology manual section 5.3.1 for GC-MSD maintenance information.

2.4.2.7.2 Per Analysis Run Control and Standard Requirement

2.4.2.7.2.1 Each run should include, at a minimum, a 100µg/mL or 200µg/mL GHB control, a negative control and a non-extracted GHB standard.

2.4.2.8 REFERENCES

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2.4.2.8.7 Determination of Gamma-Hydroxybutyric Acid by GC/MS, Dade County Medical Examiner's Toxicology Lab SOP.

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2.4.2.8.11 Goldberger, B.A., Huestis, M.A., Wilkins, D.G. *Commonly Practiced Quality Control and Quality Assurance Procedures for Gas Chromatography/Mass Spectrometry Analysis in Forensic Urine Drug-Testing Laboratories,* Forensic Sci Rev, 1997, 9(2):59-79.

Idaho State Police
Forensic Services
Toxicology Section

Section Two
Urine Toxicology

2.4 Liquid-Liquid Extraction Methods for GC/MSD Confirmation
2.4.2 Qualitative Confirmation of Gamma-Hydroxybutyrate (GHB) in Urine Utilizing TOXI-B Extraction Tubes

Revision #	Issue Date	History
1	02/05/02	Original Issue in SOP Format
2	10/18/02	Refinements
3	05/20/04	Refinements Renamed from 2.4.2.1 Extraction option two moved to 2.4.6 GHB products moved to SOP 6.1 and 6.2 No extraction method modifications therefore no revalidation pursued.

Approval

Discipline Leader

S. C. Williamson _____ Date _____

Issuance

Quality Assurance Manager

Richard D. Groff _____ Date _____