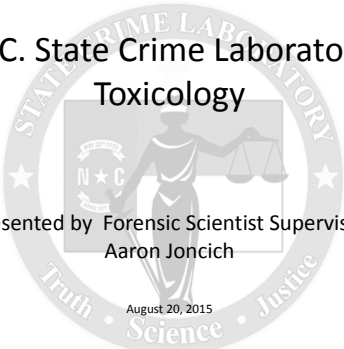


**N.C. State Crime Laboratory
Toxicology**

Presented by Forensic Scientist Supervisor
Aaron Joncich


August 20, 2015



NCSCCL – Toxicology

Presenter's Background


- Aaron Joncich
 - B.S. Degree in Chemistry with an emphasis in Biochemistry
 - Certificate of Training in Toxicology
- Worked at the N.C. State Crime Lab since 1994
 - Three years spent working Drug Chemistry cases and Clandestine Drug Labs
 - 18 years working Toxicology Cases
- Previous work experience at the San Diego County Medical Examiner's Toxicology Lab and in Pharmaceutical manufacturing
- ABFT Certified in Forensic Alcohol Analysis
- Chemical Analyst permit from the NC DHHS



NCSCCL – Toxicology

**North Carolina State Crime Lab
(NCSCCL)**

- The main Crime Laboratory location is in Raleigh.
- Satellite Labs in:
 - Asheville / Skyland (Western Lab)
 - Greensboro (Triad Lab)



NCSCCL – Toxicology

What happens when evidence is received at the State Crime Lab

- Evidence is received in the Evidence Control Unit
- A case is started in the Crime Lab's computer database
 - The database is a Laboratory Information Management System (LIMS) called Forensic Advantage (FA)
 - Information supplied by the officer on a "Request for Laboratory Examination" form is entered into the database.
 - Each case is given a unique Laboratory Case Number
 - A chain of custody for the evidence is recorded in the LIMS database
 - The persons handling the evidence are required to enter their personal password to record an entry into the chain of custody
- *Toxicology evidence is stored in secure refrigerators*



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Case Analysis Record-Keeping

- All information related to the analysis of evidence is kept in a computer database
 - LIMS (Laboratory Information Management System)
 - The NCSCCL uses a LIMS called Forensic Advantage (FA)
- What is kept in FA - examples
 - Chain of custody
 - Submission forms, notes, etc.
 - Communication logs
 - Analysis data, notes, and documentation
 - Analysis reports
 - Resources used



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Types of Testing in a Toxicology case


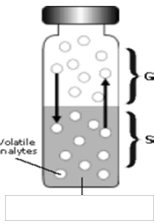
- **Blood Alcohol Concentration (BAC)** - if requested
 - Analyzed using an instrument called a Headspace Gas Chromatograph (GC)
- Analysis for **drugs in blood** - if requested
 - An initial panel of 12 screening tests called ELISA tests are performed
 - ELISA is an immunoassay type test, similar to what hospitals and workplace drug testing labs use to test for drugs
 - Drugs are extracted from blood evidence, for analysis on confirmatory instruments
 - Each drug requires one of four different extraction procedures
 - Confirmatory instruments:
 - Gas Chromatograph / Mass Spectrometer (GC-MS)
 - Liquid Chromatograph / Tandem Mass Spectrometer (LC-MS-MS)
- Not all drugs can be tested for – if a specific drug is requested, it will be addressed on the report




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Blood Alcohol Concentration (BAC) analysis

- Henry's Law: the partial pressure of a gas is proportional to its molar concentration.
- Conditions: equilibrium is reached at a constant temperature in a sealed container


<http://schoolworkhelper.net/wp-content/uploads/2011/01/Headspace-vial.gif>




NCSCL – Drug Chemistry/Toxicology

Blood Alcohol Concentration (BAC) analysis

- 200 μ L of sample are pipetted with 1,800 μ L of Internal Standard (IS) using a liquid handler/ pipettor
- Two separate blood samples are prepared









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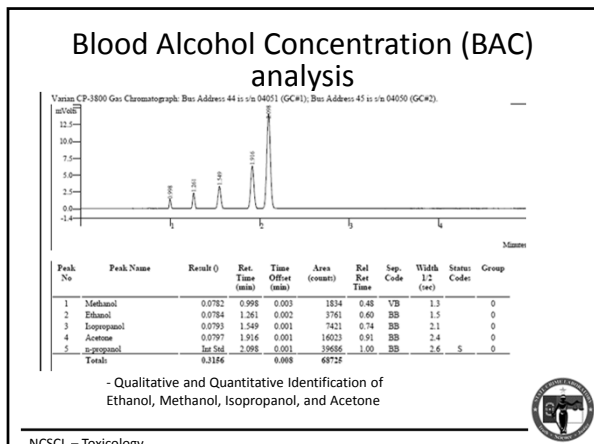
Blood Alcohol Concentration (BAC) analysis

- **Head-Space Gas Chromatograph (HS-GC) instrument used**
- A measured sample of the blood is put in a sealed vial along with a reference standard (internal standard)
- The vial is heated to make the chemicals that evaporate easily, like alcohols, move into the air in the vial (head-space)
- The GC uses a syringe through the rubber seal on the vial, to sample the head-space containing the alcohols
- The head-space sample is analyzed twice by the GC



NCSCL – Toxicology



ELISA - Drug Screening

Immunoassay test

Enzyme-linked immunosorbent assay (ELISA)

Hospitals and work-place drug testing uses this, or a similar test, for drug testing.

NCSCL - Toxicology

ELISA - Drug Screening

ELISA drug screening test - 12 panels

1. Cocaine Metabolite (Benzoyllecgonine)
2. Opiates
3. Barbiturates
4. Benzodiazepines
5. Methadone
6. Carisoprodol/Meprobamate
7. Cannabinoids
8. Amphetamine/MDA
9. Methamphetamine/MDMA
10. Tramadol
11. Zolpidem
12. Oxycodone/Oxymorphone

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ELISA - Drug Screening

- Some ELISA tests are specific for a single drug (e.g. methadone)
- Some ELISA tests are for a related group of drugs (e.g. opiates), and are referred to as "class assays"
- The test produces data in the form of a number
 - The lower the number, the more drug is present
 - A solution with a known concentration of a drug is tested to determine a number that represents a "cut-off" value for evaluating case samples
 - ELISA tests with a resulting number below the cut-off are considered to indicate the presence of the drug, at a concentration greater than in the cut-off

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Screening test (ELISA) positive results that are not confirmed

- Each ELISA test has a "cut-off" concentration, and the follow up test for confirmation has a limit of detection (LOD) concentration for each specific drug
 - The "cut-off" for the ELISA may be lower than the LOD of the confirmatory test (i.e. the first test may be more sensitive than the second test)
- Some ELISA tests are designed for "classes" of drugs (e.g. opiates)
 - If there are multiple opiates in the blood, they will add up to reach the cut-off concentration
 - Confirmatory testing identifies the specific drugs in the samples, which may be below the individual drug's LOD

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Screening test (ELISA) positive results that are not confirmed

ELISA "cut-off" at 50 ng/ml – test is POSITIVE

Drug	Concentration (ng/ml)
Morphine	25
Hydrocodone	25
Codeine	15
Total	65

The GC/MS limit of detection at 30 ng/ml means that none of the drugs are identified.

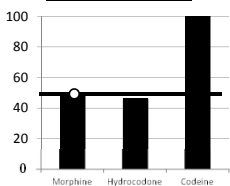
Drug	Concentration (ng/ml)
Morphine	25
Hydrocodone	25
Codeine	15

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Screening test (ELISA) positive results that are not confirmed

Cross-reactivity

The ELISA response for each drug at the same concentration



- In a "class" assay, the test has a different sensitivity to each drug - referred to as "Cross-reactivity"
- The "cut-off" is set using one drug at a specific concentration
- Therefore, the "cut-off" concentration can not be used to estimate the concentration of a "class" assay drug
- Example: cut-off is set at 50ng/ml with morphine, but 50 ng/ml of hydrocodone will give a negative result



NCSCL – Toxicology

Solid-Phase Extractions (SPE)



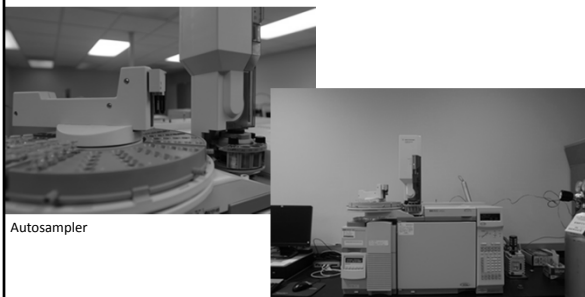
Positive Pressure Manifold for SPE

- Extraction = separating something from the whole (the rest)
- In a solid phase extraction, chemical compounds are removed from a liquid flowing through a solid sorbent by retention on the sorbent. This is achieved based on the solubility of the compounds in different solvents and the pH and pKa's.



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GC/MS



Autosampler

Gas Chromatograph Mass Spectrometer



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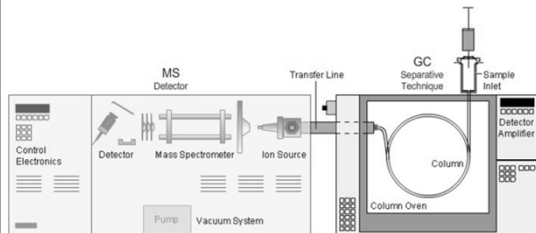
GC-MS

- The Gas Chromatograph – Mass Spectrometer (GC-MS) is actually two instruments connected together
- The GC is the same type of instrument used for BAC analysis
 - The GC separates substances in a mixture
 - The data from the GC is called a Retention Time
- The MS produces a different type of data for each substance in a sample
 - The data is called a Mass Spectrum



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GC/MS



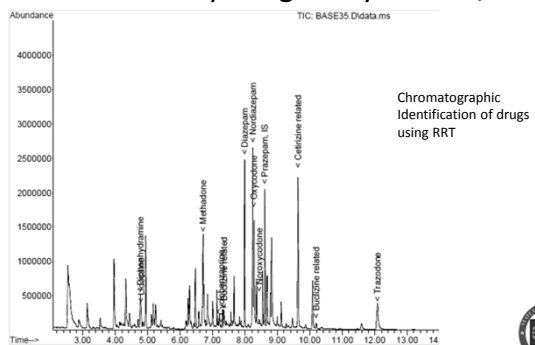
GC/MS diagram

www.chromacademy.com



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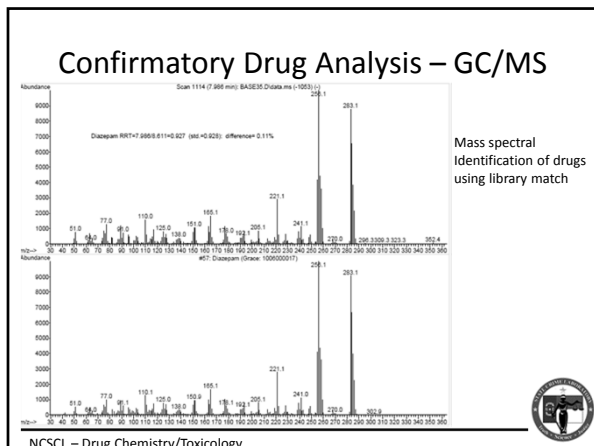
Confirmatory Drug Analysis – GC/MS

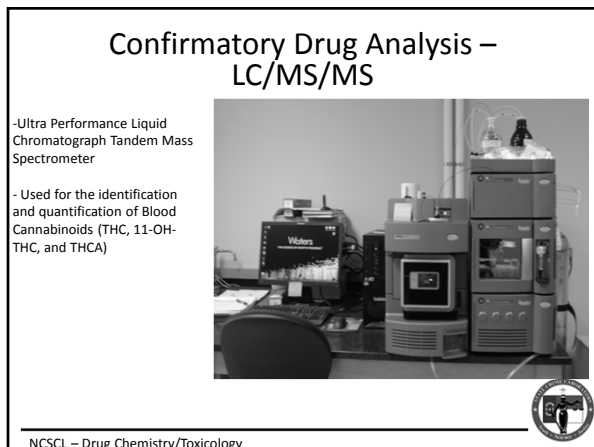


Chromatographic
Identification of drugs
using RRT



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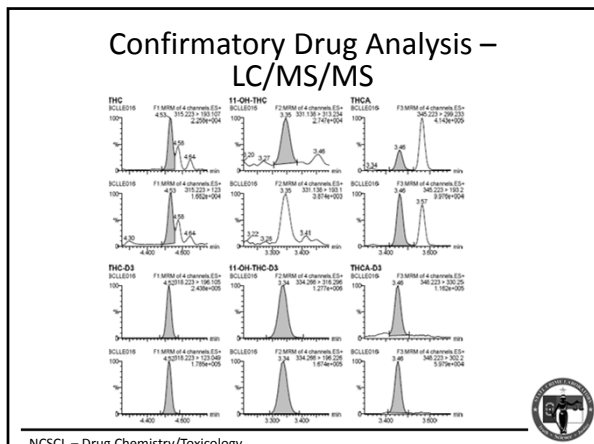




LC/MS/MS

- The Liquid Chromatograph – Tandem Mass Spectrometer (LC/MS/MS) is actually three instruments connected together
- The LC is somewhat similar to the GC used in BAC analysis and the GC/MS
 - The LC separates substances in a mixture
 - The data from the LC is called a Retention Time
- The LC/MS/MS uses two mass spectrometers in series to “clean up” the mass spectrum data, which increases sensitivity and selectivity
 - The MS/MS data is referred to as Transitions

NCSCL – Drug Chemistry/Toxicology



Quality Assurance/Quality Control

- All samples of the evidence and documentation are labeled with the unique Crime Lab Case Number
- Nothing can be reported unless at least two tests indicate that drug
 - A separate sample of the evidence is required for each test
 - Medical records or similar information can be used
- Each report and the data supporting the results are reviewed by a second Forensic Scientist
- Each type of test has multiple quality control checks

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Example of Quality Control Checks in a BAC analysis

- Instrument Calibration
 - Retention times must be within a defined time window (0.05min. +/- 0.5%) for the four components in each of the 15 calibration standards and the 12 verification standards
 - Requirement on how well the points fit the curve (R²>0.995)
- Calibration Verification
 - Analyze purchased certified reference solutions of 3 different concentrations, each containing 3 alcohols and acetone at specific concentrations – 3 samples prepared
 - 1 prepared-in-lab verification solution of 3 alcohols and acetone at specific concentrations – 3 samples prepared
 - The testing instrument analyzes each sample twice
 - Each measured value must be within an acceptable range
 - Sub-total of 336 quality control checks during the calibration of the GC instrument
- Daily Quality Control Checks
 - A minimum of three negative and three purchased Positive Quality Control standards analyzed before and after cases
 - The Positive Quality Control standards are evaluated as the Calibration Verifications are
 - Sub-total of 78 quality control checks during the daily checks
- During the analysis of an case containing ethanol
 - Two samples of the blood are prepared, and each sample is analyzed twice
 - All four results must identify ethanol
 - All four determined concentrations must be consistent with each other (±5% of the mean)
 - Sub-total of 8 quality control checks during analysis of a case sample
- **Grand total = 402 quality control checks are performed before an alcohol concentration is reported**

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Forensic Toxicologist Training

- Minimum of a B.S. degree in Chemistry or related scientific field
- NCSCCL Blood Alcohol Training
 - Effects of alcohol on a person at different concentrations
 - Absorption
 - Distribution
 - Metabolism (how the body gets rid of Alcohol)
 - Elimination
 - BAC retrograde calculations
 - Effects of blood collection and storage
 - The instruments used to test for alcohol



NCSCCL – Toxicology

Forensic Toxicologist Training

Continued...

- NCSCCL Blood-Drug Training
 - Drug categories (by structure and use)
 - Slightly different than DRE training
 - NC Controlled Substance schedules (Ch. 90)
 - The general effects of specific drugs, their uses, and categories
 - Pharmacokinetics (e.g. - how fast the drug is eliminated from a person's blood)
 - The instruments used to test for the drugs



NCSCCL – Toxicology

Can the Toxicology Lab determine if a person was impaired?

No

- Impairment is affected by several factors that are unknown, including:
 - How much drug was used
 - When was it used
 - What is the person's tolerance to the drug
 - How long have they used it
 - How much do they regularly use
 - Combined effects with other drugs
 - There may be drug present that the lab cannot test for
 - Daily/hourly variations in the person's health
- Example: methadone concentrations can reach 1100 ng/ml in the blood of someone who has taken it for a long time, and they can display very little impairment. There are reports of methadone overdose fatalities at only 400 ng/ml.



NCSCCL – Toxicology

Can the Toxicology Lab determine if a person was impaired?... No

What can the Lab's Forensic Toxicologist testify to about impairment?

- We can identify drugs that are in the blood
- We can tell if the drugs have the potential to be impairing, and generally how they impair
- We can give ranges of how long some drugs can be detected in blood, with an average use



NCSCL – Toxicology

Uncertainty of Measurement

- BAC result example - "0.080 ± 0.004 grams per 100 milliliters"
- Can also be referred to as the doubt that exists for the measurement
- Calculated based on factors that can affect the measurement, and a statistical analysis of past measurements at the Crime Lab
- What does it mean?
 - Any measurement will not be exactly the same every time you perform the measurement, if you look close enough.
 - The confidence interval range represents a statistical analysis of how much your result can vary.
 - The measured value, at the center of the range, is the most probable "true" value.
 - Values at the edges of the range are possible, but increasingly less likely as you go further from the center of the range.



NCSCL – Toxicology

Proficiency Testing

- All analysts at the NCSCL are proficiency tested in their discipline annually
- In the Toxicology section, double-blind Blood Alcohol and Blood-Drug samples are purchased for proficiency testing



NCSCL – Toxicology

Certification / Permits

- The NCSCCL is dual-accredited by:
 - ASCLD-LAB (American Society of Crime Lab Directors – Laboratory Accreditation Board)
 - ANAB (ANSI-ASQ National Accreditation Board)
 - The Crime Lab was certified by FQS, which was incorporated into ANAB
- Analysts in the Toxicology section are individually certified, when eligible
- Analysts in the Toxicology section are issued Chemical Analyst permits by the NC DHHS, authorizing them to test blood for the presence of alcohol or other impairing substances



NCSCCL – Toxicology

Rush Requests

- The District Attorney’s offices can request a “Rush” on their cases via a special web-site or phone call
- The rush requests are evaluated and triaged by a lab manager



NCSCCL – Toxicology

Subpoenas Received by the Crime Lab

- The labs have a person responsible for managing subpoenas for the analysts
 - The “Court Coordinator”
 - Notifies analysts when they are requested to appear
- The subpoenas are prioritized based on the type of court, and then the date received
 - Federal court has priority over Superior court, which has priority over District court
- A DA’s office with a “lower” priority subpoena must contact the DA’s office with the “higher” priority subpoena to “clear”/release the analyst from that county




NCSCCL – Toxicology

General Misconceptions

“The Lab’s BAC test is affected by how the blood sample is collected”
There are different types of tests for alcohol and drugs, and they each have different attributes

<p>BAC by Immunoassay (used by some hospitals)</p> <ul style="list-style-type: none"> • Uses serum (processed portion of blood) <ul style="list-style-type: none"> – Causes an BAC result that is higher than when testing whole blood – A calculation can be used to convert the SAC to a BAC value • Damage to red blood cells can affect results, and is caused by... <ul style="list-style-type: none"> – Shaking the blood samples too much – Use of a small needle to collect the blood – Older samples • Cannot tell the difference between different types of alcohol 	<p>BAC by HS-GC (used by the NCSCl)</p> <ul style="list-style-type: none"> • Uses whole blood <ul style="list-style-type: none"> – As defined by 20.4.01.(1a).a. • The test is not affected by damaged blood cells • Identifies the different types of alcohol and other volatiles <ul style="list-style-type: none"> – Ethanol (drinking alcohol) – Methanol (wood alcohol) – Isopropanol (rubbing alcohol) – Acetone (may be elevated in diabetics) – 1,1-Difluoroethane (duster spray) – Ether
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NCSCl – Toxicology

General Misconceptions

“The collection tube was past its expiration date”

- The collection tubes (Vacutainers® - BD)
 - The Vacutainer contains a vacuum (negative pressure), designed to “suck” a blood sample into the tube
 - The Vacutainer also may contain additives – usually a blood preservative and/or anti-coagulant
 - The color of the rubber stopper on the tube indicates what is added to the tube during its manufacture
 - The expiration date applies only to the vacuum in the tube, not the additives
 - When the Vacutainer expires, the seal of the rubber stopper may have failed and allowed air into the Vacutainer
 - The Vacutainer may not “suck” in all the blood it was designed to






NCSCl – Toxicology

NCSCl Toxicology Improvements

as of August 2015

- In 2004 there were 4 Toxicologists
- Currently there are:
 - 2 supervisors
 - 1 trainer
 - 7 analysts with more that 1 year of experience
 - 5 analysts with less than 3 months of experience
 - 5 within a month of completing training
 - 4 beginning training
- Re-staffing the Triad Toxicology Lab (eventually 4 analysts)
- Opening the Western Toxicology Lab (eventually 10 analysts)
- Lean Six Sigma
- Task Oriented Analysis (TOA)



NCSCl – Toxicology

NCSCCL TOXICOLOGY
Process
Improvements


Task Oriented Analysis
“TOA”



NCSCCL – Drug Chemistry/Toxicology

PAST

- Prior to 2013
- Before Lean Six Sigma came to the North Carolina State Crime Laboratory under the direction of Director Judge John
- 5 Total analysts
- 3 of the 5 were Supervisors with many other responsibilities
- “Cradle to Grave” type analytical approach to case work




NCSCCL – Drug Chemistry/Toxicology

IMPROVEMENTS BEGIN

- Still “Cradle to Grave” analysis
- Reviews were bottlenecked in a Push system
- No predictability to the process for management or the customer

↓

- 2013 Team of Toxicology employees begin Lean Six Sigma training
- Stopped case work and caught up the reviews



NCSCCL – Drug Chemistry/Toxicology

THE TRANSITION

"Cradle to Grave" analysis continues

Each analyst still maintained long term custody of all the evidence they analyzed

6 new analysts in the process of completing full Toxicology analysis (BAC and Blood Drug)

Early 2013: "Batch" style analysis began

Early "Batching" for Blood Drug Analysis

- Each analyst prepared the aliquots of their evidence for analysis and process worksheets were incorporated to document the batch work
- Analysts were assigned an analytical process to perform for the team (i.e. ELISA drug screen, base extractions, etc.)
- Each analyst processed all the data for their evidence

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TASK ORIENTED ANALYSIS

- Introduced September 2014
- To make this work, Toxicology had to move to the General Storage system for all evidence, no more individual, long term custody.
- Toxicology analysis now became 3 separate processes dependent on the customer request:
 - 1: BAC analysis
 - 2: Blood Drug Screening
 - 3: Blood Drug Confirmations
- Along with getting new analysts established in working cases, over 11,000 cases were transitioned into the new system over a period of 4 months

NCSCL – Drug Chemistry/Toxicology

BENEFITS

- Analytical work is able to continue when an analysts gets called away to court
- Processor stays with a process for about 3 months

increased proficiency and more alert to trends with the process

- More predictability for the customer
- Lessens employee job dissatisfaction and frustrations
- Utilizes equipment and supply resources more efficiently
- Predictable resource consumption
- *Higher throughput*

NCSCL – Drug Chemistry/Toxicology

Final Words

- The Toxicology Lab is continually looking to improve our service to the judicial system.
- The processes discussed in this presentation will change over time to improve our product.



NCSCL – Drug Chemistry/Toxicology
