Presumptive and Confirmatory Forensic Tests

Alyson Grine
Defender Educator
UNC School of Government
agrine@sog.unc.edu

Sarah Rackley
Forensic Resource Counsel
Office of Indigent Defense Services
Sarah.H.Rackley@nccourts.org

Outline

1) **Presumptive and confirmatory tests**
   - What is the difference?
   - What do I do if only presumptive tests were done?

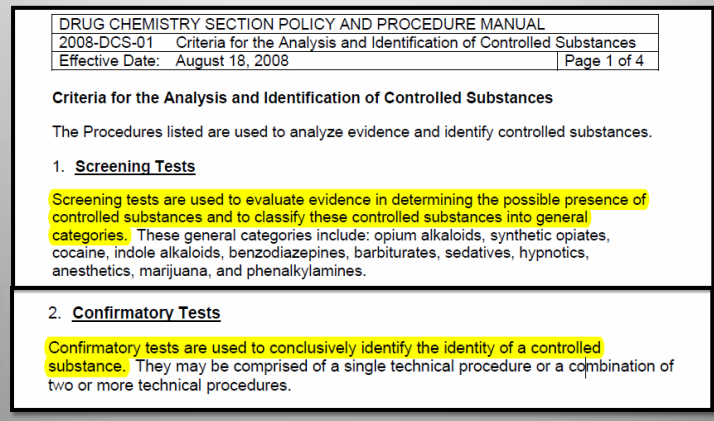
2) **State Crime Laboratory**
   - What tests are used?
     - Serology
     - Drug Chemistry

Definitions

- **Presumptive tests**
  - Can establish the possibility that a specific bodily tissue or fluid is present
- **Confirmatory tests**
  - Can identify a specific biological material

--NFSTC, DNA Analyst Training, Presumptive v. Confirmatory Tests

Lab Protocols



DRUG CHEMISTRY SECTION POLICY AND PROCEDURE MANUAL	
2008-DCS-01 Criteria for the Analysis and Identification of Controlled Substances	
Effective Date: August 18, 2008	Page 1 of 4

Criteria for the Analysis and Identification of Controlled Substances

The Procedures listed are used to analyze evidence and identify controlled substances.

1. **Screening Tests**

Screening tests are used to evaluate evidence in determining the possible presence of controlled substances and to classify these controlled substances into general categories. These general categories include: opium alkaloids, synthetic opiates, cocaine, indole alkaloids, benzodiazepines, barbiturates, sedatives, hypnotics, anesthetics, marijuana, and phenalkylamines.

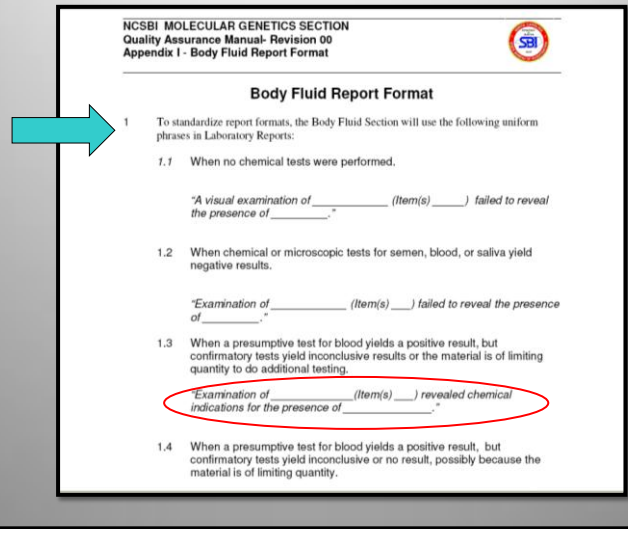
2. **Confirmatory Tests**

Confirmatory tests are used to conclusively identify the identity of a controlled substance. They may be comprised of a single technical procedure or a combination of two or more technical procedures.

Pros and Cons

Presumptive	Confirmatory
○ Narrows possibilities and helps decide which test to do next ○ Can use on larger areas ○ Can locate evidence not visible to naked eye ○ Sensitive ○ Risk of false positives	○ Conclusively identify a substance ○ Smaller risk of false positives ○ Cost more ○ Require additional equipment ○ Take longer

Reporting Results



NCSBI MOLECULAR GENETICS SECTION
Quality Assurance Manual- Revision 00
Appendix I - Body Fluid Report Format

Body Fluid Report Format

1 To standardize report formats, the Body Fluid Section will use the following uniform phrases in Laboratory Reports:

1.1 When no chemical tests were performed.

"A visual examination of _____ (Item(s) _____) failed to reveal the presence of _____."

1.2 When chemical or microscopic tests for semen, blood, or saliva yield negative results.

"Examination of _____ (Item(s) ___) failed to reveal the presence of _____."

1.3 When a presumptive test for blood yields a positive result, but confirmatory tests yield inconclusive results or the material is of limiting quantity to do additional testing.

"Examination of _____(Item(s) ___) revealed chemical indications for the presence of _____."

1.4 When a presumptive test for blood yields a positive result, but confirmatory tests yield inconclusive or no result, possibly because the material is of limiting quantity.

Serology Evidence

- Blood
- Semen
- Saliva

NCSBI Forensic Biology Section	Body Fluid Identification SOP	Effective Date: Dec 7, 2009
Title: **Technical Procedures Manual: Body Fluid Identification**		Revision 06

Body Fluid Identification Procedures Index

(Listed by Section)

1. Aseptic Technique and Contamination Control
   - 1.1 Flame Sterilization
   - 1.2 Sonication Procedure
   - 1.3 Sterilization of Bench Top
   - 1.4 Aseptic Techniques While Handling Evidence
2. Blood Analysis.
   - 2.1 Kastle Meyer Test (Phenolphthalein test)
   - 2.2 RSID Blood Test
   - 2.3 ABA card Hematrace
   - 2.4 Report wording for samples requiring DNA analysis
   - 2.5 Luminol Test

2. **BLOOD ANALYSIS**

2.1 **Kastle Meyer Test (Phenolphthalin Test)**

The Kastle Meyer Test is a presumptive test for blood and can give reactions for substances other than blood.

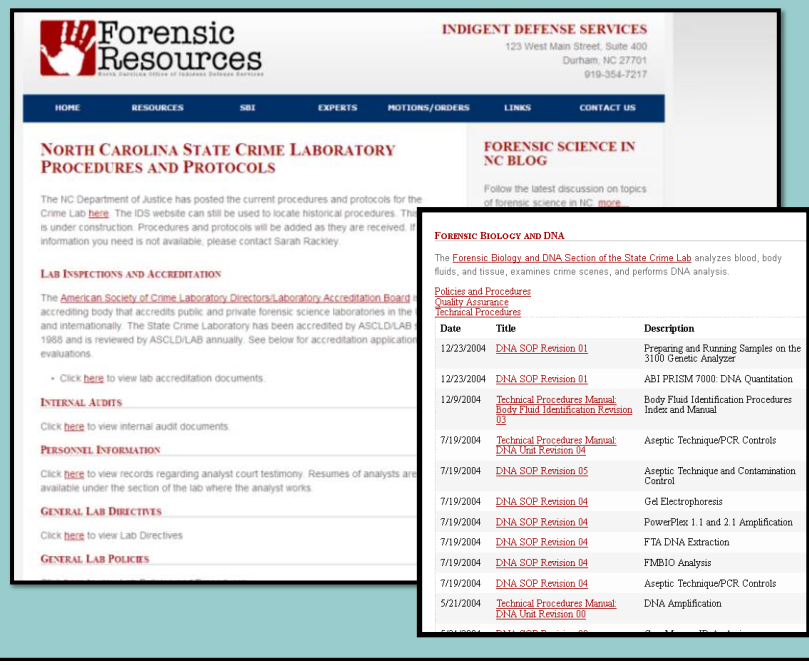
Standards and controls

Standards will include a known blood stain (positive control) and a known blood-free sample (negative control). These controls will be tested prior to analysis on a daily basis and the results will be recorded in the laboratory notes.

Procedure

2.1.1 Rub the suspected stain with a folded piece of filter paper or a clean cotton swab. Add the following reagents onto the filter paper or swab in order; one drop of ethanol, one drop of phenolphthalein, and one drop of 3% H_2O_2 onto the sample rubbing. A positive reaction is indicated by the development of a pink color within 5 seconds of adding the H_2O_2. Reactions occurring after 5 seconds or before the addition of the hydrogen peroxide are inconclusive.

2.1.2 Perform the Kastle Meyer Test on any stains that visually appear to be blood even if blood analysis is not requested.



Blood

Presumptive

- Phenolphthalein Test
  - AKA Kastle Meyer Test
- Luminol Test
  - AKA Albrecht Reaction
- Alternate Light Sources

Confirmatory

- Takayama Test
  - No longer listed in State Crime Lab protocols
- RSID Test for Human Blood
- ABA Card Hematrace
  - Can cross react with some animal blood
  - "Consistent for presence of human blood")

DNA: Not confirmatory!

Individualization

- After blood confirmed, lab may perform tests to determine source.
- **Current Test**
  - DNA
- **Past Tests**
  - Ouchterlony
    - Species of origin test
  - ABO typing
    - Identifies a person's blood type
  - Hematrace cards

Saliva

Presumptive

- Phadebas Test

Confirmatory

- Phadebas Test **+**
  - RSID Test for Human Saliva

Semen

Presumptive

- Acid Phosphatase Test
  - AKA Walker Test
- Alternative Light Sources
- Prostate Specific Antigen
  - Not currently used by State Crime Lab

Confirmatory

- Christmas Tree Stain
  - Sperm Identification
- RSID test for Semen

Drug Chemistry

Drug Chemistry

- **Drug Analysis**
  - Analysis of a suspected controlled substance
- **Toxicology**
  - Analysis of blood, urine or hair sample to determine whether a substance has been consumed

Drug Analysis

Presumptive

- Color tests/spot tests
- Microcrystalline tests
- Ultraviolet spectroscopy
- Infrared spectroscopy
- Microscopic examinations
- Thin layer chromatography (TLC)

Duquenois-Levine Test:

No scientific acceptance as a reliable and accurate means of identifying marijuana.

-*State v. Tate,* 300 N.C. 180 (1980)

Confirmatory

- Gas Chromatograph/Mass Spectrometer
- Infrared Spectrophotometry

Tests Required

Minimum Criteria for the Identification of a Controlled Substance

Categories of Analytical Techniques
Listed in order of decreasing discriminatory power from A to C:

Category A	Category B	Category C
Infrared Spectroscopy	Gas Chromatography	Color Tests
Mass Spectroscopy	Liquid Chromatography	Immunoassay
	Microcrystalline Tests	Ultraviolet Spectroscopy
	Pharmaceutical Identifiers	
	Thin Layer Chromatography	
	Cannabis Only: Macroscopic Examination Microscopic Examination (Counts as one each)	

Toxicology

Presumptive

- Alco-sensor
- EMIT Analyzer
- ELISA Immunoassay

Confirmatory

- Headspace Gas Chromatography

Challenging the Failure to Perform Confirmatory Testing

- **Pretrial Motion to Exclude**
- **Motion to Limit Testimony**
- **Cross-Examination**
- **Jury Instructions**

Motion to Exclude

- Lack of Reliability (Rule 702)
- More Prejudicial than Probative (Rule 403)
- Due Process
- Caselaw
- Lab Procotols
- National Standards (Scientific Working Groups)
- NAS Report

"[J]urors may ascribe so much authority to such a noteworthy expert that in forensic chemistry that they treat his testimony as infallible and automatically accept his opinion on the chemical composition of a substance, without properly appreciating--even with vigorous cross-examination and proper jury instructions--that the expert chemist never even performed a scientific, chemical analysis."

-*State v. Ward*, 364 NC 133, 146 (2010).

Motion to Limit Testimony

that you've indicated did show positive results for the presence of blood, what did you do after finding in your expert opinion there was blood on those items?

A. With reference to the shoes, there was some blood stains on both the sides of the shoes, on both the left outside of the shoe as well as the right shoe on the outside. I took sterile swabs and put water on them and then attempted to remove as much of the stain off as I could.

Q. Okay. What did you do in regards to the folding knife, I think State's Eighty-Five?

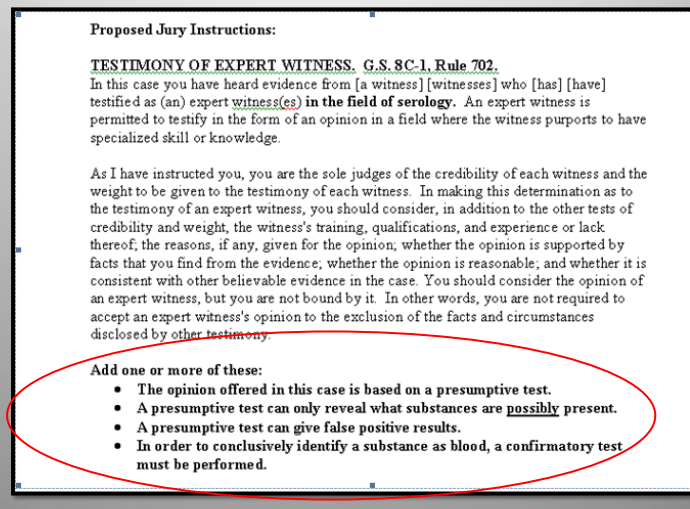
A. With reference to the folding knife, again, after finding the chemical indication for the presence of blood -- The blood that was found on the handle of this knife, if you

Cross-Examination

- Lab protocols
- SWGDRUG standards

- SWGDRUG stands for Scientific Working Group on the Analysis of Seized Drugs.
- SWGDRUG is a working group of scientists that was formed by the FBI.
- SWGDRUG's mission is to establish guidelines and standards for the forensic examination of controlled substances.
- SWGDRUG makes recommendations for the minimum criteria to identify a controlled substance.
- SWGDRUG makes recommendations about when it is appropriate to use a color test.
- The Marquis reagent is a type of color test.
- SWGDRUG recommends that color tests be used **only** in conjunction with a confirmatory test.
- According to the SWGDRUG standards, the Marquis Reagent should **not** be used alone to identify a controlled substance.

Jury Instructions



Proposed Jury Instructions:

TESTIMONY OF EXPERT WITNESS. G.S. 8C-1, Rule 702.
In this case you have heard evidence from [a witness] [witnesses] who [has] [have] testified as (an) expert witness(es) **in the field of serology.** An expert witness is permitted to testify in the form of an opinion in a field where the witness purports to have specialized skill or knowledge.

As I have instructed you, you are the sole judges of the credibility of each witness and the weight to be given to the testimony of each witness. In making this determination as to the testimony of an expert witness, you should consider, in addition to the other tests of credibility and weight, the witness's training, qualifications, and experience or lack thereof; the reasons, if any, given for the opinion; whether the opinion is supported by facts that you find from the evidence; whether the opinion is reasonable; and whether it is consistent with other believable evidence in the case. You should consider the opinion of an expert witness, but you are not bound by it. In other words, you are not required to accept an expert witness's opinion to the exclusion of the facts and circumstances disclosed by other testimony.

Add one or more of these:

- **The opinion offered in this case is based on a presumptive test.**
- **A presumptive test can only reveal what substances are possibly present.**
- **A presumptive test can give false positive results.**
- **In order to conclusively identify a substance as blood, a confirmatory test must be performed.**

Summary

- Presumptive tests can only establish the **possibility** that a particular substance is present
- Confirmatory tests can identify a specific material
- A lab report or affidavit may **not** reveal what type of test was done
- Testimony at trial may be misleading, ie, "blood, blood, blood"
- Defenders must obtain the underlying notes and reports of the analyst
- Defenders should raise a challenge where:
  - Only a presumptive test was done
  - The mandated combination of tests was not done
  - Testing protocol was not followed
- And seek to:
  - Limit misleading testimony
  - Instruct the jury, ie, on the limitations of a presumptive test in the absence of a confirmatory test.

Questions

Sarah Rackley
Forensic Resource Counsel
Indigent Defense Services
Sarah.H.Rackley@nccourts.org
(919) 354-7217

Alyson Grine
Defender Educator
UNC School of Government
agrine@sog.unc.edu
(919) 966-4248

Presumptive and Confirmatory Forensic Tests

Definitions:

- **Presumptive tests** - can establish the possibility that a substance, bodily tissue or fluid is present
- **Confirmatory tests** - are used to conclusively identify a substance or specific biological material

References: President's DNA Initiative (for serology tests)
State Crime Lab procedures (for drug chemistry tests)

	PROS	CONS
PRESUMPTIVE	Narrow possibilities and help decide which test to do next	Sensitive
	Can use on larger areas	Risk of false positives
	Can locate evidence not visible to naked eye	
CONFIRMATORY	Conclusively identify a substance	Cost more
	Smaller risk of false positives	Require additional equipment
		Take longer

Reporting results:

Reading the lab's reporting procedures can clarify what a certain conclusion in a lab report means. The State Crime Lab's Body Fluid Report Format and STR Interpretation guidelines (for DNA evidence) are available. Be sure to refer to the reporting procedures that were in effect when the evidence in your case was analyzed.

The Swecker-Wolf Report, An Independent Review of the SBI Forensic Laboratory, examines the reporting practices of the Forensic Biology Section of the State Crime Lab.

SEROLOGY EVIDENCE

(See State Crime Lab Body Fluid Procedures for a description of each procedure)

1. Blood

Presumptive

- Phenolphthalein Test (aka Kastle Meyer Test)
- Luminol Test (aka Albrecht Reaction)
- Alternate Light Sources

Confirmatory

- Takayama Test (no longer listed in State Crime Lab procedures)
- RSID Test for Human Blood
- ABA Card Hematrace (note: can cross react with some animal blood. Results will be reported as "consistent for presence of human blood")

After blood confirmed, a lab may perform tests to determine the source, a process called "**individualization**." Current test used is DNA analysis. Previous tests: Ouchterlony test (species of origin test), ABO typing (identifies a person's blood type) or Hematrace cards.

Saliva

2. Saliva

Presumptive

- Phadebas Test

Confirmatory

- Phadebas Test + RSID Test for Human Saliva

See "Forensic Tests for Saliva: What you should know" on the Forensic Science in North Carolina blog for more information about these tests.

3. Semen

Presumptive

- Acid Phosphatase Test (aka Walker Test)
- Alternative Light Sources
- Prostate Specific Antigen (not currently used by State Crime Lab)

Confirmatory

- Christmas Tree Stain (for sperm identification)
- RSID test for Semen

(See "Forensic Tests for Semen: What you should know" on the Forensic Science in North Carolina blog for more information about these tests. Also,NCAJ has an online training on crime scene investigation and serology evidence available here. Dr. Marilyn Miller covers evidence collection techniques and presumptive and confirmatory tests for blood, saliva and semen.)

DRUG CHEMISTRY AND TOXICOLOGY EVIDENCE

1. Drug Analysis: An analysis of a suspected controlled substance, e.g., to determine whether a white powder is cocaine.

Presumptive

- Color tests/spot tests - Marquis reagent, Duquenois-Levine, Cobalt Thiocyanate Reagent, Ferric Chloride Reagent, Koppanyi Reagent, Porassium Permanganate Reagent, p-Dimethylaminobenzaldehyde Reagent (PDMAB), Froehde's Reagent, Mecke's Reagent, Silver Nitrate Reagent, Zwikker Reagent), Secondary Amine Reagent #1, Secondary Amine Reagent #2,Barium Chloride Reagent, Methanolic Potassium Hydroxide Reagent
- Microcrystalline tests
- Ultraviolet spectroscopy
- Infrared spectroscopy
- Microscopic examinations
- Thin layer chromatography (TLC)

Confirmatory

- Gas Chromatography/Mass Spectrometry
- Infrared Spectrophotometry (FTIR)

(See State Crime Lab Preliminary Tests procedure for a description of how to perform each presumptive test. See the Drug Chemistry Section Policy and Procedure Manual – Criteria for the Analysis and Identification of Controlled Substances for the limitations of screening tests. See also the Scientific Working Group on the Analysis of Seized Drugs (SWGDRUG) standards.)

2. Toxicology: An analysis of blood, urine or hair sample to determine whether a substance has been ingested

Presumptive

- Alco-sensor
- EMIT Analyzer
- ELISA Analyzer

Confirmatory

- Headspace Gas Chromatography (see the NACDL amicus brief in Bullcoming v. New Mexico for a thorough explanation of this technique

(See State Crime Lab Toxicology Procedures for a description of how to perform each test. NCAJ has an additional online training on drug analysis available here. Forensic chemist Dana Way covers presumptive and confirmatory tests used in drug analysis and toxicology.)