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

```
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 phenethylamines.

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
- 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

Confirmatory
- Conclusively identify a substance
- Smaller risk of false positives
- Cost more
- Require additional equipment
- Take longer

Reporting Results

Body Fluid Report Format

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

1. When no chemical tests were performed.
   "A visual examination of ______ (type(s)) failed to reveal the presence of ______.

1.2 When chemical or microscopic tests for semen, blood, or saliva yield negative results.
   "Examination of ______ (type(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 ______ (type(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

<table>
<thead>
<tr>
<th>NCBA Forensic Biology Section</th>
<th>Body Fluid Identification SOP</th>
<th>Effective Date: Dec 7, 2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Fluid Identification Procedures Index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Listed by Section)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Aseptic Techniques and Contamination Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.1 Flame Sterilization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.2 Sanitization Procedure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.3 Sterilization of Bench Top</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.4 Aseptic Techniques While Handling Evidence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Blood Analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1 Kastle Meyer Test (Phenolphthalein test)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.2 RSTD Blood Test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.3 ABA card Hematrace</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.4 Report wording for samples requiring DNA analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5 Livitred Test</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. BLOOD ANALYSIS

2.1 Kastle Meyer Test (Phenolphthalein 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 5% H2O2 onto the sample swab. A positive reaction is indicated by the development of a pink color within 5 seconds of adding the H2O2. 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

<table>
<thead>
<tr>
<th>Presumptive</th>
<th>Confirmatory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color tests/spot tests</td>
<td>Gas Chromatograph/Mass Spectrometer</td>
</tr>
<tr>
<td>Microcrystalline tests</td>
<td>Infrared Spectrophotometry</td>
</tr>
<tr>
<td>Ultraviolet spectroscopy</td>
<td></td>
</tr>
<tr>
<td>Infrared spectroscopy</td>
<td></td>
</tr>
<tr>
<td>Microscopic examinations</td>
<td></td>
</tr>
<tr>
<td>Thin layer chromatography (TLC)</td>
<td></td>
</tr>
</tbody>
</table>

**Duquenois-Levine Test:**
No scientific acceptance as a reliable and accurate means of identifying marijuana.

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

<table>
<thead>
<tr>
<th>Category A</th>
<th>Category B</th>
<th>Category C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infrared Spectroscopy</td>
<td>Gas Chromatography</td>
<td>Color Tests</td>
</tr>
<tr>
<td>Mass Spectroscopy</td>
<td>Liquid Chromatography</td>
<td>Immunocassay</td>
</tr>
<tr>
<td>Microcrystalline Tests</td>
<td>Ultraviolet Spectroscopy</td>
<td></td>
</tr>
<tr>
<td>Pharmaceutical Identifiers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thin Layer Chromatography</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cannabis Only:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microscopic Examination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microscopic Examination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Counts as one each)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 Protocols
- National Standards (Scientific Working Groups)
- NAS Report

"[J]urors may ascribe so much authority to such a noteworthy expert 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."

Motion to Limit Testimony

Q. 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 possiblly 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@ncourts.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)

<table>
<thead>
<tr>
<th>PROS</th>
<th>CONS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PRESUMPTIVE</strong></td>
<td>Narrow possibilities and help decide which test to do next</td>
</tr>
<tr>
<td>Can use on larger areas</td>
<td>Risk of false positives</td>
</tr>
<tr>
<td>Can locate evidence not visible to naked eye</td>
<td></td>
</tr>
</tbody>
</table>

| **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.)
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, Porassum 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.)