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What is LCN? Definitions and Challenges

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Low copy number (LCN) testing has been a commonly used term in forensic DNA testing for much of the past decade. But what does the term “LCN” really mean? “LCN” associated with other words (such as DNA, testing, low template DNA) has been used in different contexts with different meanings amongst different practitioners. The existence of multiple definitions and multiple modifying words within the forensic DNA testing community has created some confusion regarding the definition of LCN both in the forensic science and legal communities. During the same period of time, laboratories have experimented with various procedures that aid in increasing the sensitivity of the PCR testing processes as examination and testing of evidence has expanded to a wider variety of samples having limiting amounts of DNA. The addition of some of these processes alone or in combination may cause a particular sample to fall into the realm of “LCN testing” by some or all definitions.

The courts in the United States have seen minimal challenges to DNA testing in criminal cases over the past decade as laboratories have established routine testing procedures with few or inconsequential modifications introduced. As a result, admissibility hearings have become a thing of the past with prosecutors and DNA analysts settling into fairly routine presentations of DNA test results and conclusions to juries and the court. However, several recent challenges to DNA testing have arisen in the United States with a focus on the definition of LCN, the procedures used and the validity of the DNA testing results obtained. Similar challenges are likely to become more prevalent in the near future. These challenges will require the laboratories and attorneys seeking to admit DNA testing performed on samples with small amounts of DNA to address the scientific and legal questions regarding admissibility of evidence in criminal courts and to perhaps participate in a *Frye* or *Daubert* admissibility hearing to determine if the scientific evidence can be legally presented to the trier-of-fact. A brief historical perspective of the definitions of LCN will be presented along with a description of some procedures that may increase the sensitivity of DNA testing using the PCR. Recommendations for laboratories and attorneys to prepare for the scientific challenges and for the possible admissibility hearings will be presented.

Low Copy Number DNA

- Low Copy Number DNA
- Low Copy Number DNA Testing
- Low Copy Number Testing
- Low Template DNA (LTDNA)
- Low Template DNA Analysis

- What do they all mean?

Low Copy Number DNA

- Historically, referred to small amounts of recovered DNA and the procedures being used to increase sensitivity
  - Usually referred to increased amplification cycles used for samples with small amounts of DNA (e.g., Forensic Science Service Lab)

Low Copy Number DNA

Definitions based on Quantitation

- Small amounts of DNA recovered from evidence = low level of template DNA
- <200 pg of DNA
- <100 pg of DNA

Low Copy Number DNA

Definitions based on Procedures

- Increased amplification cycles (28 – 34)
- Any procedure used to increase sensitivity (e.g., injection time, reduced amplification sample volume, increased sample volume for injection, post-amp “clean-up” of products, high purity formamide, other)

Low Copy Number DNA

Definition based on the DNA Profile

- Any sample showing **Stochastic Effects**
- Any DNA sample where the results are below the stochastic threshold

Low Copy Number DNA

All definitions:

- Relate to limited amount of template DNA available for amplification
- Due to small amount recovered, degradation, inhibition affecting amplification, etc.
- Insufficient quantity to generate a complete profile

Low Copy Number DNA

- Other definitions presented to the courts
  - Quantity of DNA on slot blot
  - Any amount of DNA used below the kit manufacturer's recommended range

Low Copy Number DNA

- Many different uses of the term and the abbreviation “LCN”
- Amount of DNA (Quantitative definition) vs. Technology/Process definition vs. Stochastic Effects (Qualitative definition)
- Imperative to clarify the definition when using the term “LCN”
- Or use more descriptive terminology

Legal Issues

- Multiple varying definitions create confusion in the scientific community which leads to confusion in the courtroom
- Is there lack of scientific consensus suggesting lack of reliability?
- Issues of “LCN” are scientific
- Scientists need to define “LCN” – not the courts

Court

- When do you cross the line between a scientific modification that requires an admissibility hearing vs. a reasonable modification to an existing procedure?
- Some current issues in court regarding DNA testing are likely issues of weight not admissibility
- However, admissibility of a DNA profile can still be challenged even if the technology is accepted

Court

- Admissibility hearings for use of LCN technologies and DNA results in criminal cases
- Hearing to decide if an admissibility hearing is needed – what is definition of "LCN"? Was "LCN" done?
- Different jurisdictions have different legal requirements (Frye vs. Daubert)

Court

- Many crime labs in US have never gone through an admissibility hearing
- Many prosecuting and defense attorneys in US have never gone through an admissibility hearing

Are you prepared?

Recommendations for Laboratory

Note:

All of the following recommendations hold for all laboratory testing,

NOT just “LCN” issues

Recommendations for Laboratory

- Perform **Validation Studies** – with appropriate range of studies for **all** procedures used in the laboratory to define limitations of the systems used
  - Sensitivity Studies
  - Mixture Studies
  - "Stochastic Threshold"
- Develop and use SOPs that are **very** closely aligned with validation studies
- Make SOPs and validation studies available through discovery for review

Recommendations for Laboratory

- Develop interpretation guidelines and policies that accurately reflect the data obtained and the limitations of the test system
  - What data are sufficient for interpretation
  - Criteria needed to report an exclusion
  - Criteria needed to report an inclusion
  - What does “inconclusive” mean and what are the reasons for declaring a result “inconclusive”

Recommendations for Laboratory

- Report what can be defended scientifically
- Use report wording that **accurately** reflects the data obtained without bias
  - "consistent with" – what does this mean?
  - "cannot be excluded"
- Statistics
  - Assign appropriate statistical frequency to the profile with clarity for what question the calculation is addressing

Recommendations for Laboratory

- Adequate training in data interpretation for profiles with limitations
  - Validation studies
  - Case work samples
    - Use sexual assault cases where the contributor of the samples are “known”
    - Evaluate the partial profiles, mixtures, peak heights, etc. to see how DNA from real samples perform in “known” mixtures

Recommendations for Laboratory

Victim's reference profile:

- Compare to the non-sperm fraction profile
- Compare to sperm fraction profile

Sperm fraction profile:

- Compare to the non-sperm fraction profile

Recommendations for Laboratory

- Agree to Disagree – discuss sample results with colleagues in and outside of laboratory
- Dispute is OK
- Differences of opinions are OK
- Use challenges to improve

Recommendations for Laboratory

- Labs will need to assist attorneys with documentation for legal challenges
- Help attorneys and court understand definitions of “LCN” and whether it is applicable to the case
- Are processes able to produce accurate results?
- Can results be relied upon?

Reminders for Analysts/Witnesses

- Analysts have 'need' to get interpretable results
- Expected to exclude or include → power of DNA testing
- We are all reluctant to have "inconclusive" results
- Caution to not "over interpret" the data
- Disservice to community if falsely exclude or falsely include

Reminders for Analysts/Witnesses

- ALWAYS be a proponent of good science and **neutral** to the case – no bias
- Obtain the experience and knowledge to defend your work
- If it is good, support it. If it isn't, do not go forward with it.