Technology Transition Workshop| *Robert O'Brien*

Rules Concerning Low Level Samples and CODIS Entry

Low Copy Number (LCN) Samples and Entry into CODIS

- This presentation covers:
  - Low level samples
  - What cannot be entered into the NDIS system
  - How techniques such as MinElute® Post-PCR Cleanup can be used to help develop investigative leads
- The following topics will be discussed:
  - What is LCN?
  - What effect does MinElute® have on LCN samples?
  - How can LCN samples that undergo MinElute® still be used to solve cases?

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What is LCN?

- There are several definitions of LCN samples:
  - LCN typing is typing of samples containing less than 100 pg of DNA
    - Gill, P., et al. *An Investigation of the Rigor of Interpretation Rules for STRs Derived from Less than 100 pg of DNA*. Forensic Science International 112: 17 – 40.
  - The analysis of any results below the stochastic threshold for normal interpretation
    - Budowle, B., et al. *Low Copy Number – Consideration and Caution*. Laboratory Division of the FBI, Pub. No. 01-26.
  - More recently it was defined as typing any sample below 200 pg

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What is LCN?

- Still others define LCN as an increase in amplification cycles
- If the decision is to set a specific quantity for LCN samples, then the question of how this quantity was achieved has to come up
- Previously we have seen that problems with the quantitation systems can make it difficult to get an accurate quantity reading

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What is LCN?

- Therefore one laboratory's 200 pg may be another laboratory's 100 pg
- When auditing these laboratories to determine if they are in compliance, would the accuracy of the quantitation system be taken into account?
- An increase in amplification cycles can also vary
  - Applied Biosystems recommends 28 amplification cycles for their kits
  - Promega has a range from 28 to 32 cycles

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What is LCN?

- Does this mean that if the cycles are increased in an Applied Biosystems kit from 28 to 32 cycles we have now crossed over to LCN typing?
- Also, if a laboratory typically uses 28 cycles with a Promega kit, but increases cycles to 32 for low level samples, are they now performing LCN analysis?
- Or since Promega allows for a range in amplification cycles, would the increase to 32 cycles not be considered LCN testing?

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What is LCN?

- Also as technology of instrumentation and amplification kit chemistry improves, analysts are able to detect full profiles at lower and lower levels
- Yet with the quantity definition this ability would not matter since the data may not be eligible for NDIS

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What is LCN?

- The stochastic threshold must be looked at
  - Should be determined based on validations performed in the laboratory
  - May differ from one amplification kit to the next or even between instruments
  - Accordingly stochastic threshold should be set by the individual laboratory
- These validations can then be repeated as kit chemistries and/or instruments are improved

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Effect of MinElute® on LCN Samples

- As previously shown in results, MinElute® Post-PCR Cleanup is able to improve the signal from DNA fragments by cleaning up excess primers and dyes
- For LCN samples, MinElute® will simply raise the peak heights of low level samples
  - MinElute® will not correct any problems associated with the amplification of low level DNA
- Raising these peak heights may cause the stochastic threshold to rise

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Effect of MinElute® on LCN Samples

- Using MinElute® on low level samples will impact the definition of 200 pg being the cut off point for LCN
- On testing I performed, a concentration of 125 pg was targeted
- At this level I had data with RFUs well into the hundreds with no stochastic effects that affected the interpretation of data

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Effect of MinElute® on LCN Samples

- There were differences in peak height ratios
- No allelic drop out or drop in was observed
- The profiles after the cleanup process could be easily interpreted
- With tools such as MinElute®, setting a specific quantity of DNA to identify samples as LCN would not be appropriate
  - When using MinElute® many factors can affect the increase in sample peak heights (number of washes, volume, etc.)

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Effect of MinElute® on LCN Samples

- When electing to use MinElute®, a laboratory must rely on their validations to determine the correct threshold for the system
  - Ultimately, the threshold will be affected by the specifics of the protocol the laboratory decides to adopt based on what best fits their needs

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Using LCN Samples as Investigative Leads

- Despite rulings on what is allowed into NDIS, laboratories can still use the MinElute® system to increase peak heights of low level samples
- On a state level the CODIS administrator can allow the entry of LCN data to assist in searches at the state and local levels

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Using LCN Samples as Investigative Leads

- One concern:
  - If NDIS won't allow this data to be entered, could a defense attorney successfully argue the court should not consider the data?
- Resolution of this concern would have to go back to the validation performed
- As long as the laboratory can show the results are accurate, consistent and reliable, then the data should hold up in the court system despite rulings by NDIS

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Questions?

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