

USE OF PCR CONTROLS IN FORENSIC DNA LABORATORIES: STANDARDS, PRACTICES AND LIMITATIONS

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Standards for DNA testing of forensic samples using the polymerase chain reaction (PCR) provide guidance on many aspects of testing. Included in the Standards, are requirements for qualifications of personnel, components of a quality assurance system, evidence control, validation and analytical procedures. While all labs submitting data to NDIS are required to follow the applicable Standards as they are delineated in the Quality Assurance Audit for Forensic DNA and Convicted Offender DNA Databasing Laboratories, the Standards do not provide thorough descriptions or guidelines of best practices for some criteria. The Analytical Procedures section requires the use of reagent blanks (extraction controls) as well as negative and positive amplification controls. These controls are designed to monitor for contamination introduced during laboratory testing and for the proper functioning of the amplification reagents and equipment. They may also detect contamination in laboratory consumables and problems with software used in profile analysis.

Laboratories are allowed significant latitude regarding how these controls are processed and interpreted. However, the methods used to process these controls can affect how well the controls function in detection of contamination. Since many evidence samples contain very small amounts of DNA and mixtures, in particular, may contain very small amounts of minor components, the design of the reagent blank control and negative control relative to the sample can be critical. This is of increasing concern as laboratories introduce low copy number protocols.

We will discuss the purpose of the positive and negative controls used in forensic laboratories and describe the theoretical concepts which must be considered to ensure that these controls serve their intended function. A significant focus will be a discussion of the steps in the analysis process that affect the laboratory's ability to detect and identify the source of contaminating DNA in the reagent blank, negative control and samples taking into account the threshold of detection of the contaminating DNA at the amplification and at the analysis stages. Steps in sample processing that may create differences in the handling of controls and their corresponding evidence samples due to procedure modification, batch processing of samples through the extraction and concentration steps, alteration of sample volumes or reagent concentrations and the use of sequential tests (e.g., STRs and Y-STRs) will be discussed. Solutions to common PCR control handling issues in the laboratory will be outlined as they relate to typical samples versus samples containing low amounts of DNA or mixtures.

