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IMPROVING EXTRACTION, PURIFICATION AND CONCENTRATION PROTOCOLS FOR TRACE AMOUNTS OF DNA

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Trace amounts of human cellular material are encountered during the investigation of volume crimes. Unfortunately, the chance of generating an informative DNA profile cannot be guaranteed when using current forensic techniques. It is therefore desirable to minimize DNA loss when extracting, purifying and concentrating trace amounts of DNA. A comparison of the following DNA extraction methodologies, Organic, Chelex®, Invisorb® Forensic Kit (Invitek), QIAamp® DNA Micro Kit (Qiagen), DNA IQTM Kit (Promega) and the ChargeSwitch® Technology (CST®) Forensic DNA Purification Kit (Invitrogen), showed that Chelex® extraction performed best for dealing with trace amounts of naked DNA (50 - 0.1 ng), as well as with fresh saliva (10, 3 or 1 uL) air-dried onto cotton cloth. There was negligible DNA loss of the naked DNA with this Chelex® method; however, only 55% of the available DNA from the air-dried saliva on cotton cloth was recovered. Modifications to the cellular lysis incubation time, and presence or absence of sarkosyl and dithiothreitol during Chelex® extraction, did not improve retrieval of DNA from saliva cells, but the presence of proteinase K and sodium dodecyl sulphate improved the retrieval of DNA from saliva cells. Use of the QIAquick® PCR Purification Kit and the QIAamp® DNA Micro Kit protocols to purify forensic samples showed that they can lose up to 85% of the DNA. Concentration of DNA extracts with Microcon® YM-100 columns reduces the DNA yield, by approximately 30%. The loss of DNA was however able to be significantly reduced when using a co-precipitant such as Poly-A RNA. Protocols currently used by some within the forensic community are not ideal for trace amounts of DNA. In a bid to maximize the chances of generating informative DNA profiles effective modifications or alternatives to current extraction, purification and concentration protocols need to be sought.