E45 An Evaluation of the Effects of Various Digest Times on the DNA Yield for Differential Separations at the West Virginia State Police Forensic Laboratory

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Learning Overview: The goal of this presentation is to communicate the findings that 60 minutes is the ideal digest time for epithelial cell lysis in differential samples containing sperm from sexual assault cases. Attendees will learn that heating differential samples at 56°C for 60 minutes yields the best male DNA yield while maintaining the integrity of the DNA.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by improving the method for processing sexual assault samples in order to obtain higher male DNA yields and better forensic male DNA profiles.

Every year, thousands of sexual assaults occur in the United States.[1] Sexual assaults make up a large percentage of the cases handled by forensic DNA laboratories and adopting an optimized procedure for processing these cases is of the utmost importance.

Differential extraction is the process used to separate epithelial cells (e-cell) and sperm cells during DNA extraction in sexual assault cases.[2] Samples are heated while suspended in digest reagents, with the goal of separating the sperm cells from the e-cells.[3] This isolated sperm can then be used to develop a male DNA profile.

The current procedure for differential extraction at the West Virginia State Police Forensic Laboratory requires sample digestion at 56°C for 30 minutes to two hours.[2] The purpose of this study was to determine the ideal incubation time within this range in order to optimize the DNA yield in the e-cell and sperm fractions of differential samples. Five digest times were evaluated in this study: 30 minutes, 45 minutes, 60 minutes, 90 minutes, and 120 minutes.

To prepare for this study, a three-fold serial semen dilution was prepared for a series of six semen dilutions (A-F). Female saliva was obtained and diluted (1:2) with nuclease-free water. Six sets of samples (A-F) were prepared by adding 25µL of a respective semen dilution and 25µL of saliva dilution to sterile cotton swabs and dried. Swabs were nutated in 1,000µL of nuclease-free water for one hour, transferred to spin baskets, and centrifuged at 13,000 RPMs for five minutes. The resulting liquid was added back to the sample tube and re-centrifuged for an additional five minutes to concentrate the cellular material into a pellet. Approximately 920µL of water was removed and discarded, and 500µL of digest mix (475µL Sarkosyl buffer + 25µL Proteinase K) was added to the remaining pellet for each sample.

Samples were digested on the 56ºC heat block for each of the incubation times and transferred to the QIAGEN® QIAcube® for separation, using Protocol 12A/12B. A sperm-lysis solution was prepared for a pipet volume of 145µL per sample (7.25µL ProK + 7.25µL DTT + 130.5µL Sarkosyl buffer) and loaded into the QIAcube® in position A to be added to the sperm fraction after the wash steps. Samples were extracted on the QIAGEN® EZ1® using Large Volume protocol for the e-cell fraction and Trace protocol for the sperm fraction; samples were eluted in 40µL of TE buffer.

All samples were quantitated using Quantifiler® Trio on the Applied Biosystems® 7500 Real-Time PCR System and amplified using GlobalFiler™. Capillary electrophoresis was performed on the Applied Biosystems® 3500 Genetic Analyzer, and analysis was performed using GeneMapper® IDX.

Results for each semen dilution (A-F) indicate that a 60-minute digest time yields the highest male DNA quantitation values. The second highest male DNA yields were seen at 90 minutes or 45 minutes. The data for each semen dilution graphically follows a normal distribution pattern, with the ideal digest time being 60 minutes. Both the human and male DNA quantitation values for the 120-minute samples had the lowest quantitation values for all semen dilutions. The data suggests that 30 or 45 minutes is not enough time for the digest mix to lyse the e-cells.

The results generated from this study support the conclusion that 60 minutes is the ideal digest time. The DNA profiles from the 60-minute digest time samples had the purest and most robust sperm cell fractions, as they produced single-source profiles with alleles above the analytical threshold. The sperm fraction of samples digested at 30 and 45 minutes contained female DNA, likely due to undigested epithelial cells. The sperm fraction samples subjected to 90- and 120-minute digest times had good separation but showed evidence of degraded DNA due to lower allele peak heights.

While male DNA profiles can be generated from samples heated for 45–90 minutes, the ideal digest time for differential samples is 60 minutes. Implementing a strict 60-minute digest time into the differential separation protocol could lead to better separation of epithelial and sperm cells, while maintaining the integrity of the male DNA.

Reference(s):

1. Sexual Assault Forensic Examination (SAFE) Commission Annual Report. *West Virginia Division of Justice and Human Services*, (September 1, 2016-August 31, 2017): http://www.wvlegislature.gov/legisdocs/reports/agency/J03_G_2017_13749.pdf.
2. WVSPFL Quality Assurance Board. *The West Virginia State Police Forensic Laboratory DNA Analysis Procedures Manual.* (July 2018), South Charleston, WV.
3. Marshall University Forensic Science Center DNA Laboratory. *Internal Validation of the QIAcube for Differential Separation*. (May 2013), Huntington, WV.

Differential Extraction, Sexual Assault, Digest Time