A35 Optimized Centrifugal Methods for Separation of Semen From Superabsorbent Polymers for Forensic Analysis

Lucy Camarena, BS, 220 Three Rivers Dr, El Paso, TX 79912; Bailey K. Glasscock, BS, 1020 W Main St, Box 843079, Richmond, VA 23284-3079; Nicolle Hardell, BS, 1020 W Main St, Box 843079, Richmond, VA 23284-3079; and Sarah J. Seashols, MS, Virginia Commonwealth Univ, Dept of Forensic Science, PO Box 843079, Richmond, VA 23284-3079*

After attending this presentation, attendees will gain an understanding of the challenges in separating cellular material from the Superabsorbent Polymer (SAP) materials and the fibrous matrices found in evidence such as diapers, sanitary napkins, absorbent medical pads, and other related forensic evidence, and a protocol that can successfully separate the cellular material from the substrate.

This presentation will impact the forensic science community by describing best practices for isolating semen from evidence containing absorbent and superabsorbent materials, and the impact that SAPs has on downstream DNA analysis.

The best evidence to connect a perpetrator to a sexual assault is the confirmed presence of semen, which through forensic examination can prove sexual contact by verifying ejaculation and/or penetration. Sexual assault cases can involve evidentiary items such as sanitary napkins or diapers, which contain superabsorbent polymers (SAPs). SAPs are cross-linked polymeric materials that can absorb and maintain large amounts of aqueous solutions without compromising structure. Ejaculation of semen onto these SAP-containing substrates results in cellular material, including spermatozoa, becoming entangled in the SAP gel-like mesh. Thus, separation is difficult and the likelihood of a typable spermatozoa yield is questionable. Additionally, visualization and proper identification of spermatozoa through microscopic examination can become problematic when SAPs are present; therefore, common practice involves working with only the top cotton or paper layers of these types of evidence to avoid SAPs whenever possible.

The success rate of obtaining DNA profiles from SAP-containing evidence has not been evaluated in a systematic fashion. It has historically been assumed that residual SAPs in the purified DNA extract can interfere with and create complications for downstream forensic applications. Therefore, an evaluation on the effects of SAPs on forensic DNA processes was necessary, as was the development of a simple, efficient protocol for separating cellular material from evidence containing SAPs. A number of centrifugal-filtration methods were evaluated to determine best practices for isolating biological materials from SAP-containing evidence. In order to select the most effective filtration device, excisions of commonly used sanitary napkins, diapers, and adult incontinence products containing 100 µL depositions of 1:5 human semen were filtered utilizing five different types of centrifugal filters. The selection of the best filter was based on spermatozoa yield, DNA yield, and ease of use. The resulting filtrates were microscopically examined for presence of spermatozoa and percent yields were calculated and compared. DNA from each sample type was isolated using a differential organic extraction method and was evaluated for DNA yield and quality. Shifts in Internal Positive Control (IPC) cycle thresholds and melt curves were assessed to address possible inhibition caused by the presence of SAPs. Results indicate that polyester fabric-layered basket filters were simplest to use and resulted in significantly higher spermatozoa and DNA yields than other centrifugal filter methods. No indication of significant inhibition by residual SAPs was observed in any of the filtered DNA extracts.

A sampling comparison was made between the top cotton or paper layer of the SAP-containing substrates versus taking the entire substrate excision. This set of experiments sought to determine the best sampling method as determined by spermatozoa visualization and DNA yield. Results indicated that sampling the entire excision of the SAP-containing substrates yielded a significantly higher quantity of visualized spermatozoa and DNA yield than just sampling the top layer of the evidence. As no PCR inhibition from the SAPs was observed in the entire excision samples, it is therefore recommended that the full depth of SAP-containing evidence be sampled for spermatozoa identification and DNA analysis in forensic casework.

In conclusion, filtering samples taken from evidence containing SAPs greatly improves the screening process of spermatozoa identification and makes these samples easier to work with for downstream processes. The optimized filtration method also allows for subsequent separation of the sperm and non-sperm fractions, and processing of biological samples using a variety of validated forensic DNA isolation protocols. There is a significant increase in sperm and DNA yield when the entire excision is filtered in comparison to the common practice technique of taking only the top layer in order to avoid the SAP gel portion. The yields of spermatozoa and DNA purified from these filtered samples show promise that usable STR profiles can be obtained.

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 ** Presenting Author*