B73 A New High Throughput Easy to Use Differential Extraction Method

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After attending this presentation, attendees will to introduce to the forensic community a new differential extraction method that can rapidly and efficiently separate sperm and epithelial cells in a high throughput format.

This presentation will impact the forensic community and/or humanity by demonstrating a new differential extraction method that will significantly reduce the amount of time needed to process a major fraction of violent crime sample types thus allowing more cases to be solved without increasing personnel. The high throughput method will allow the use of automation and will finally provide a means to significantly reduce the large backlog of sexual assault evidence.

One of the main reasons for the large backlog of sexual assault samples is the difficultly in working with the evidentiary material. Typical vaginal swabs contain a mixture of victim epithelial cells in large excess over sperm cells. Unprocessed, these samples can only be analyzed using male specific markers that provide important evidence but are of limited use in searching national databases due to the inheritance and nonrecombinatorial nature of the Y chromosome.

In 1985, Gill *et al.* developed a method to enrich for sperm cells in the presence of an excess of epithelial cells. This process relies on the fact that sperm structural proteins as opposed to epithelial cells contain a large amount of disulfide bonds that inhibit the proteolysis of these proteins. After a controlled proteolysis in the absence of a reducing agent, the sample is centrifuged in a spin basket to remove from the solid matrix intact sperm and solution containing the DNA from lysed epithelial cells. Because the resulting sperm pellet contains loose cell debris a considerable amount of contaminating solution is left and must be diluted out with serial washings and centrifugations. This process is time consuming and results in loss of sperm and variability between examiners.

The authors have developed a new differential extraction method that takes advantage of the nearly two decades of experience using the standard differential extraction. After a standard Proteinase K digestion of the sample, the solid support and DNA-containing solution are centrifuged through a special material that effectively separates the sperm from soluble DNA and cell debris. The samples are washed once without centrifugation to remove any remaining soluble DNA in the sperm fraction. DNA IQ™ Lysis Buffer containing DTT is then added to the epithelial and sperm fractions. This buffer effectively lyses the sperm without need for further Proteinase K digestion. The total time for separating the sperm from epithelial cells following addition of the sample to the Proteinase K Digestion Solution is approximately 1 hour 20 minutes which includes the 1-hour Proteinase K digestion. The purification of the DNA requires 40 minutes so the total separation and purification can be accomplished in 2 hours.

Because the same standard Proteinase K digestion and initial centrifugation is used to help remove the sperm from the solid support and to lyse the epithelial cells, the efficiency of these steps will be identical to what is currently available. However, only one centrifugation is required for efficient separation so the sperm recovery is better. In addition, the hands on time as well as the overall time needed to do the separation has been greatly reduced from the current method. Data will be presented on the sensitivity and successful processing of old samples.

Although the new differential extraction method significantly reduces the time to process samples, there is significant time spent in transferring samples to new tubes following the digestion step. The centrifugation of samples in a single tube format also does not mesh with current automation methods. To increase the throughput of this method plasticware has been developed that allows the incubation and subsequent centrifugation of samples in the same 96 deepwell format. This eliminates transfer steps where contamination can occur and makes the method fully compatible with current robotic methods. The use of this plasticware also allows the efficient extraction of reference samples on buccal swabs and blood cards to be performed with the same chemistry in the same plate as the sexual assault samples if desired. The time to process a full 96 well plate of samples using this new differential extraction method coupled with automated DNA IQ™ purification on a Biomek® 2000 workstation is approximately 4 hours of which only a small fraction of this time requires manual intervention.

Differential Extraction, High Throughput, Automation

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