



A132 Optimizing Sperm Cell Recovery From Cotton Swabs Prior to Christmas Tree Staining and p30 Test

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After attending this presentation, attendees will be able to utilize the method presented in order to recover an abundant amount of sperm cells from cotton swabs for the Christmas Tree stain and p30 test.

This presentation will impact the forensic science community by enabling analysts to recover an ample amount of sperm cells from cotton swabs to perform the Christmas Tree stain, and therefore, confirm the presence of sperm.

The Texas Department of Public Safety Houston Crime Laboratory's confirmatory test for the presence of semen is microscopic visualization of the sperm after an extraction and staining. Many laboratories require this confirmatory test before sending evidence onto DNA analysis; otherwise, it is under the serologist's discretion to send the sample for further testing. Therefore, an optimal recovery method must be utilized prior to staining and microscopic viewing.

Previous studies performed by others suggest using different detergents to remove sperm cells from the substrate. Other detergents considered are sodium dodecyl sulfate (SDS) solution, a sarkosyl solution, and a Triton-X 100 solution. Garvin, et al. (2009) found Triton-X 100 to be the best detergent for DNA extraction. According to Norris, et al. (2007) 2% SDS was best for cell recovery. Current methods at the Texas Department of Public Safety Houston Crime Laboratory use ultrapure deionized (DI) water for removal of sperm from substrates.

A recovery method for sperm cells on a cotton swab was developed by determining the best detergent and the optimal working concentration of the detergent. The detergents used were an SDS solution, a sarkosyl solution, and a Triton-X 100 solution. The concentrations ranged from 0.5% to 20%. After determining the optimal concentration of each detergent, diluted semen samples subjected to the removal process to determine the best detergent. 0.5% SDS yielded the most sperm cells recovered at all diluted concentrations (lowest being 1:1000). The extraction method was performed according to the Texas Department of Public Safety Houston Crime Laboratory Standard Operating Procedure for all analyses. Following the extraction, the supernatant was removed for p30 testing and replaced with DI water; this reduced any soapy residue. The pellet was introduced onto a microscope slide and Christmas Tree stained. The slides were evaluated microscopically (400x) and the sperm present were manually counted.

The supernatant is used for p30 testing on an ABA card at the Texas Department of Public Safety Houston Crime Laboratory. Although SDS recovered the most sperm cells, a positive p30 was not obtained. If SDS is to be used as the recovery method for sperm cells, then two separate extractions would have to be conducted (one for the microscopic examination with the SDS solution and one for the p30 test with DI water).

Sperm Cell Recovery, Christmas Tree Stain, Sperm From Swab