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Maximizing DNA Recovery

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Mini-Popule Developed to Maximize DNA Recovery for Robotic Forensic Analysis

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As the field of forensics evolves, vendors are joining with forensics professionals to create or redesign products for specific new uses – and better science.

Traditionally, cotton swabs have been used by forensic investigators for the collection of biological material, such as body fluids, found at crime scenes or on items submitted to forensic laboratories for more detailed examination. The double swabbing technique is commonly used to collect trace, or contact, DNA from shed skin cells left behind by somebody holding or touching an item, such as a knife handle. Using this method, DNA is collected by using a cotton swab moistened with sterile water and then applying it to the area that needs to be sampled. Immediately following the wet swab, a dry cotton swab is used to collect any moisture remaining that may contain DNA missed by the first swab. The cotton swabs are then extracted as one and DNA profiles are generated from the material collected on the swabs. In 2001, Forensic Science SA (FSSA), a forensic laboratory located in Adelaide, South Australia, observed that some cotton swab samples from good DNA sources, such as bloodstains, were occasionally failing to generate expected DNA profiles. It was decided to try some new sampling options and to test these against the traditional cotton collection swab.

IDENTIFYING DNA SWABS

Ted Silenieks, a senior technical officer who works in the Biology Evidence Recovery Section at FSSA, accessed several different foam swab types, including various hydrophobic and hydrophilic foam popules. The popules appeared to be an ideal trace DNA collection device. They had a larger head than cotton swabs that would allow for easy sampling of larger areas, which would maximize the collection of skin cells needed for DNA analysis. Popules are self-saturating, foam swabs produced by Puritan Medical Products Co. The foam heads are attached to a hollow handle filled with 91% isopropanol. When the handle is squeezed, a seal inside the foam head is broken which allows the isopropanol to flow into the foam. We provided swabs for projects conducted by FSSA, which included two sizes of hydrophobic swab samples, one type of hydrophilic swab sample, and a knitted polyester swab. The cotton swabs and nylon flocked swabs were sourced from another manufacturer (See Table 1).

Silenieks needed to determine, under controlled conditions, which of the swab head media produced the best samples of trace DNA. Results of preliminary testing indicated that the hydrophobic foam popule swab had potential for the collection of trace DNA from touched items. He was encouraged by the early DNA extraction results from the hydrophobic swabs, but the unit costs were high and the heat-sealed, foam heads were difficult to remove from the handles. The first popules used were not sterile, supplied in bags of 1000 units, and had large foam heads that had to be cut into smaller pieces for DNA extraction, which increased extraction times and added to potential user contamination. These facts proved to be barriers, preventing the new popule swabs from being used on a routine basis, particularly by local crime scene examiners.

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SUPERIOR DNA RECOVERY

In 2002, a university student working in conjunction with FSSA, completed a Ph.D. thesis investigating the recovery of trace DNA using different types of foam populе swabs containing different solvents, comparing them to the recovery from cotton swabs.[1] The student found that the hydrophobic populе that contained 91% isopropanol inside its hollow stem consistently recovered the most DNA of all the swabs tested in the study. Following this study, populе swabs were introduced into FSSA casework in 2003 for a six month trial period. They were used to sample trace DNA from items submitted to FSSA by local Crime Scene Investigators (CSIs), such as screwdrivers and other tools, which are commonly used in volume crimes (i.e. house break-ins and auto theft). The results were compared to cotton swab samples taken from similar items by the CSIs at crime scenes during the same six-month period. Results indicated that the populе samples were generating more DNA profiles and had a higher success rate than that of cotton. This was the beginning of a long process to develop a swab that was compatible with DNA robotics, increased the amount of DNA collected, and yielded more full DNA profile results.

In January 2005, Silenieks co-supervised a FSSA Research and Development project on the "Comparison of Swab Types for the Recover of Trace DNA in Forensic Investigations."[2] The study assessed several different swab types, such as knitted polyester, nylon flocked, cotton, different sized populе swabs, and their ability to recover trace DNA. The design mimicked the collection of trace DNA by applying diluted saliva to the surface of latex gloves, a particularly difficult medium to sample. Saliva dilutions used contained similar levels of DNA that could be found in trace DNA samples. 200µL of diluted saliva was applied to the outer palm area of powder-free latex gloves, and allowed to dry at room temperature for 24 hours. The areas containing the dried saliva were then swabbed using the different sample swabs and analyzed. Saliva was chosen as an amount containing a known quantity of DNA could be applied to the glove surface, and the DNA recovery rate for the swabs used could then be determined.

SWABBING DEVICES	SWAB HEAD	HANDLE
Large hydrophobic foam populе	14mm x 25mm	Hollow tubular handle 85mm x 7mm. Filled with 1.4 mL 91% isopropanol
Hydrophobic foam mini-populе	10mm x 23mm	Hollow tubular handle 85mm x 7mm. Filled with 1.4 mL 91% isopropanol
Hydrophilic foam mini-populе	10mm x 23mm	Hollow tubular handle 85mm x 7mm. Filled with 1.4 mL 91% isopropanol
Nylon flocked swab	5mm x 16mm	Blue cap/handle measures 33mm x 11mm; plastic stem is 120mm x 3mm
Knitted polyester swab	Consists of inner and outer layer of knitted polyester 8mm x 17mm	135mm x 3mm
Cotton swab in clear, sterile plastic tube with paper label	Tightly wound cotton 4mm x 12mm	Red cap/handle measures 33mm x 11mm; wooden stem 120mm x 2mm

SWABBING TECHNIQUES

The latex gloves were swabbed with populеs until no liquid remained in the handle. The wet/dry swabbing technique was used for the nylon flocked, knitted polyester, and cotton swabs. Two different solvents, water and isopropanol, were used in conjunction with the wet/dry swabbing technique. The wet swab was wiped on the glove by rotating the swab and covering small sections at a time. The dry swab was used to sample the wet area and absorb any residual solution.

Standard DNA extractions were performed on all the sample swabs, followed by a second extraction to determine the amount of residual DNA remaining in the swab material after the first extraction. The total manual extraction times were also noted, with the large populе consuming the most time, 3.5 hours; the two mini-populеs requiring 3.25 hours; the nylon flocked and knitted polyester requiring 3.0 hours; and the cotton requiring 2.0 hours for extraction.

Of the swabs tested, Silenieks *et al* found that "trace DNA could be recovered from all of the swabbing devices tested but the quality and intensity of the profiles generated was superior for large and mini-populеs." The results showed that the two sizes of hydrophobic populе swabs and the hydrophilic swab all recovered full profiles in the 1/100 sample dilution, and recovered most of the DNA in the first extraction. The nylon flocked, knitted polyester and cotton resulted in "low" performance and retained more DNA in the heads after the first extraction. Results of this study were presented in March 2006 at the 18th Australian and New Zealand International Symposium on the Forensic Sciences, held in Fremantle, Western Australia.

In 2005, FSSA purchased a Perkin Elmer Multiprobe II Forensic Workstation, with the intention of using robotic analysis to generate DNA profiles from all reference DNA samples, and eventually the majority of casework samples. The specific demands of the machine required that collection devices used for DNA collection from casework items be compatible with the robotic techniques. This presented new challenges in optimizing the trace collection devices, particularly populеs, used in South Australia.

CUSTOMIZATION OF THE POPULES

Silenieks contacted us and we began what would be an 18-month trans-global dialogue between our Guilford, Maine, headquarters and Forensic Science South Australia. Silenieks explained his requirements for a populе swab that would maximize FSSA's ability to extract full DNA profiles using their robotic systems, minimize lab staff labor, and enable technicians in the field to easily collect DNA samples with a minimum of potential contamination.

The first challenge was to overcome problems presented by the robotic system. Unlike the cotton swabs, the foam populеs didn't get snagged on the robotic needles during the DNA extraction phase, and their research had shown that the amount of DNA recovered from initial extraction was higher than cotton. Initial test results showed the amount

of trace DNA recovered by robotic extraction from cotton swabs had decreased and the cotton swab heads were not interfacing well with the robotic needles. To overcome this, cotton swabs needed to be shaved from the handles using scalpels, increasing labor and potential contamination problems. It was found that the extraction vials used by the robot only had enough room for a single cotton swab, which meant the double-swabbing technique was not compatible with the robot.

The second challenge was to optimize the size of the populе head to provide a maximum surface area for collecting the DNA, but not so large that it required extra labor and time — and the risk of sample contamination — to cut the large populе head to fit within the narrow tubes in the forensic workstation. The foam head size was reduced and the new mini-populеs had evolved. These mini-populеs fitted into the extraction vials and generated good DNA profiles.

Finally, the populе head needed to detach easily from the hollow handle after sampling, but maintain its integrity while swabbing. We worked with FSSA and provided several different options for assessment by Silenieks before the final version was decided upon. In 2006, Silenieks co-supervised a second FSSA Research and Development project, "Evaluation of Sampling Techniques for Trace DNA with Robotic Considerations."[3] In this study, mini-populеs were again compared to cotton swabs. The swabbing techniques for the cotton swabs included single and double swabbing, and using a water solvent and an isopropanol solvent. Results of this study again showed that mini-populеs were the "superior swabbing devices for the recovery of trace DNA" and that the mini-populеs were compatible with robotic trace DNA extraction techniques. It is hoped that the results of this project will be presented at the 19th Australian and New Zealand International Symposium on the Forensic Sciences, to be held in Melbourne, Australia, in October 2008.

Silenieks and I corresponded over the year and a half reviewing changes to the populеs, sizing the populе swab head such that it worked with the robotics and allowed for a maximum swabbing area. Getting the attachment and detachment properties just right seemed to be the biggest challenge. Most of the time, we develop a product so that the head stays firmly in place. FSSA's request for an easily detachable head had our Research and Design team working on different solutions.

CASEWORK RESULTS

The final design of the Puritan hydrophobic mini foam populе has now been completed. The populеs are available as sterile, single packaged-swabs that meet forensic requirements and supplied with user instructions. Hydrophobic foam populеs have been in use in the Forensic Science South Australia Biology Lab since 2003, during which time we fine-tuned and developed the hydrophobic mini-populе swab to better interface with the robotic technology and to reduce the unit costs. In the real world application, Silenieks explained, you often sample very low levels of DNA material, so the ability to collect samples without having to add sterile water and increase the chance for contamination is regarded as a real plus. An added bonus of using populеs is that, unlike cotton swabs, populеs do not have to be dried to prevent bacterial degradation of the collected DNA. The isopropanol actually helps preserve the DNA and prevents degradation by bacteria.

Silenieks has seen many successful DNA profiles generated from hydrophobic mini-populеs used in casework in South Australia, with success from both non-porous and semi-porous surfaces. In one instance, a perpetrator had licked a sexual assault victim, and the area was swabbed with a populе generating a full, clean DNA profile, which was a good result for prosecutors. In an unusual case, an offender went on a rock-throwing crime spree, throwing large rocks at vehicles traveling at high speed along freeways, endangering life and causing considerable damage to vehicles. A populе swab sample taken from one of the rocks thrown through a car windscreen was tested by FSSA, generating a DNA profile. Police investigators eventually arrested a suspect and his DNA profile matched the one generated from the rock populе sample.

An interesting application has also surfaced with drug enforcement agents in South Australia. Investigators have used the mini-populе to swab the hands of suspected cannabis growers for traces of THC, the oil in marijuana. The cannabis oil is readily soluble in the isopropanol.

"Now that we've been able to reduce the cost of the mini-populе, it's become widely accepted within South Australian law enforcement," Silenieks noted. The neighboring state of Queensland has begun testing the populеs and is hoping to introduce them into their routine crime scene sample collection, he added.

As the field of forensics evolves, many manufacturers are joining with forensics professionals to create or redesign products for specific new uses, with the anticipated result of better science and improved profiling. In this instance, it is also worthwhile to note that controlled studies and research can result in the establishment of new protocols for forensics departments that should withstand the scrutiny of the legal system.

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