THE EFFICACY OF HAIR WASHING PRIOR TO SUBMISSION FOR NUCLEAR DNA ANALYSIS

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BACKGROUND

Before nuclear DNA analysis of hair roots, it is not uncommon for the hair to be washed. In theory, washes are performed in order to remove extraneous biological material from the surface of the hair to ensure that any DNA profile obtained is from the hair itself and not from debris on the shaft. Additionally, the washed hair portion is divided into a root and shaft section. The shaft section acts as a control used to ensure that the washing process is effective. No nuclear DNA profile is expected on the shaft section. The washing process requires substantial manipulation of the hair, introducing numerous opportunities for the hair to be lost, for contamination to occur, or for the tissue to become dislodged.

The literature is fairly silent on hair washing in relation to nuclear DNA analysis, and queries posed to DNA and trace groups provided minimal yet varied responses with regard to current protocol. While some type of washing makes logical sense, there is not data to support the need for it or if it is worth the possibility of contamination and loss that additional manipulation creates. Analyst observations and anecdotal data collection within the trace section of the OSP Portland Metro lab suggest that the current procedure should be evaluated and optimized or eliminated.

OSP's washing procedure consists of sequential washes in xylene, ethanol, and water. Historically, the xylene wash was done in cases where hair had been mounted in order to remove the mounting medium. On at least one occasion, a DNA profile was obtained from a shaft control of a hair with no observed shaft contaminant where xylene was left out of the washing procedure. One explanation for the profile was that it could be due to a build up of natural oils or other non-water soluble surface contaminant. The analyst thought that it may be beneficial to wash all hairs in xylene. Xylene may act as a degreaser, thus potentially removing non-water soluble compounds (e.g., oils, sebum, etc.) from the hair shaft. Since that time, shaft profiles have been rare. However, it is unknown if this is due to the success of the washing procedure, because there is nothing on the shaft to begin with, or if the biological material on the shaft is below the detection threshold of current technology. Therefore, it is unclear if the necessity of washing hairs before nuclear DNA analysis is supported by actual data. The role of xylene as a degreaser has not been supported by data. Additionally, the reason that the ethanol step comes before the water step has been unclear and should be evaluated.

PURPOSE

A multi-phased experiment was designed to assess the efficacy of the current OSP washing procedure through answering the following questions:

- Does the current wash procedure remove surface contaminants (water soluble and non-water soluble)?
- Does the order of the washes make a difference?
- What solvent and/or solvent combination is the most efficacious at removing water soluble compounds from the surface of hair shafts?
- Does xylene remove natural oils or other non-water soluble surface contaminants?

EXPERIMENTAL CONSIDERATIONS

Initial considerations:

- Whole blood was used to intentionally contaminate hairs and represent water soluble compounds (e.g., semen, saliva).
- In Experiments A through E hair was freshly cleansed to limit contamination of samples by oily surface residues.
- Hair and blood samples were collected from different individuals.
- The preparer/sample handler did not donate hair or blood.
- Washes were retained.
- References to "dirty" hair pertain to hair samples collected from individuals that refrained from scalp/hair cleansing for the purpose of increasing the retention potential for oily deposits on the hair shafts. (Experiments F & G)
- Only shaft portions were analyzed in all experiments except Experiment G.

Criteria used in the assessment of nuclear DNA profile data:

- The OSP DNA Unit categorizes nuclear DNA results based upon the number of STR loci obtained at genotyping.
- No data at any STR locus is described as "No DNA profile" in the results.
- Allelic information at one to three STR loci is deemed insufficient for comparison by our interpretation guidelines; however, for the purposes of this study, the data falling into this category was evaluated to determine whether the genetic information obtained was consistent with either the hair donor or the blood donor. It is described as "Uninterpretable DNA profile" in the results.
- STR data at four or more loci has been determined to constitute a DNA profile, and is appropriate for comparison to reference standards. It is described as "Interpretable DNA profile" in the results.

SUMMARY OF FINDINGS

- **Extraneous DNA can be detected from unwashed hair shafts.**
- **Water soluble surface contaminants are most effectively removed by washing first with water then with ethanol.**
- **Xylene does not appear to effectively remove non-water soluble surface contaminants.**
- **Shaft controls appear unnecessary for routine casework samples.**

EXPERIMENT A

Goal: attempt to determine if the sensitivity of current nuclear DNA analysis techniques will detect DNA on hair shafts known to be contaminated with blood

Potential outcomes:

- If no DNA was present, washing would appear to be unnecessary.
- If DNA was detected, washing was necessary and the current procedure was evaluated.

Method:

- Ten hairs were soaked in whole blood and allowed to dry overnight in a weighboat.
- One ~1cm shaft portion from each was placed into separate tubes without washing and analyzed.

Results/Conclusions:

	No DNA profile	Uninterpretable DNA profile	Interpretable DNA profile
No wash	**40%**	**20%**	**40%**

- The sensitivity of the current nuclear DNA analysis process can detect water soluble surface contaminants on hair.
- Notes:
  - When removing the hairs from the weighboat, the blood was flakey such that many flakes came off of the shafts while some remained. In attempts to make the coating as homogenous as possible, the flakes were manually removed, leaving residual blood. The removal also is more reflective of casework, as visible blood would be removed prior to submission for nuclear DNA analysis.
  - A hair prepared in the same manner, but not used in the washing project, was swabbed 5 consecutive times. Each swab gave a positive presumptive result for blood indicating that even though blood was not visible, a detectable amount remained.
  - The flaking of the blood suggested the need to use a different procedure for the application of blood on the hair shaft and may be the reason four of the hairs had no detectable DNA on them.

EXPERIMENT B

Goal: determine if the current washing procedure (xylene, ethanol, water) effectively removes blood from the hair surface

Potential outcomes:

- Regardless of whether or not DNA was detected in this second phase, the washing procedure was evaluated to optimize effectiveness.
- If DNA was present, optimization included a variation on the order and/or type of washing solvents.
- If no DNA was present, further study was done in an attempt to determine if all washing steps were needed to remove the extraneous DNA.

Method:

- A second ~1cm shaft portion from each of the ten previously blood soaked hairs of Experiment A was prepared following the current washing protocol.

Results/Conclusions:

	No DNA profile	Uninterpretable DNA profile	Interpretable DNA profile
xylene/ethanol/water	**100%**	**0%**	**0%**

- No DNA was detected on the hair shafts that were washed using the current procedure of xylene, ethanol, and water.
- The current procedure works on some level at removing surface contaminants.
- Notes:
  - From the unwashed hair data (Experiment A), it is possible that 40% of these hairs were not adequately spiked with blood, thus this data set may be of limited value to assess success of the current washing procedure.

EXPERIMENT C

Goal: begin to optimize washing procedure through the evaluation of single washes.

Potential outcomes:

- If DNA was present, then the wash was not successful.
- If no DNA was present then the wash was successful. The successful washes were evaluated for greatest success.

Method:

- Ten hairs were dredged in whole blood and strung to dry.
- Four ~1cm shaft portions were cut from each hair and prepared as follows:
  - no wash
  - xylene
  - ethanol
  - water

Results/Conclusions:

- Xylene alone does not remove blood from hair surfaces efficiently.
- Ethanol and water appear to be equally successful at removing blood from hair, although the fact that red material was visually observed being removed mechanically during the ethanol wash suggests that it may not be as effective as a solvent in which the contaminant is soluble (such as water).
- Data and observations made during preparation suggest that the removal of blood from the shaft is due to the combination of mechanical manipulation and solubility in the solvent. Data and observations made during preparation suggest that blood is insoluble to sparingly soluble in xylene calling into question its relevance in a routine washing procedure.

	No DNA profile	Uninterpretable DNA profile	Interpretable DNA profile
no wash	**30%**	**0%**	**70%**
xylene	**20%**	**10%**	**70%**
ethanol	**60%**	**20%**	**20%**
water	**60%**	**30%**	**10%**

- The efficacy of removal of surface contaminants with water and ethanol needs to be explored further.
- Because no DNA was detected in 30% of the unwashed hairs sample set, the technique for applying blood to the shaft was reevaluated.

EXPERIMENT D

Goal: continue to optimize washing procedure through the evaluation of different wash combinations using water and ethanol.

Potential outcomes:

- If DNA was present, then the wash was not successful.
- If no DNA was present then the wash was successful. The successful wash combinations were evaluated for greatest success.

Method:

- Ten hairs were strung across a box, swabbed with a whole blood soaked swab, and allowed to air dry.
- Four ~1cm shaft portions were cut from each hair and prepared as follows:
  - ethanol/water
  - water/ethanol
  - water/water
  - ethanol/ethanol

Results/Conclusions:

- The results of the data are mixed. While it appears that washing with water before ethanol is more effective than the other wash types, the number of washed samples in all series resulting in an interpretable DNA profile indicates that blood is remaining on too many of the hairs for this procedure to be adequate for routine removal of surface contaminants.
- The swabbing of the hairs with blood is an abrasive application technique and is not reflective of what would be seen in typical casework samples. It is likely that the washes were not able to fully remove the blood from under the scales of the cuticle. This theory is also supported by the comparatively effective single washes of ethanol and water employed in Experiment C which involved a non-abrasive application technique.
  - 90% of hairs washed in a single water wash resulted in either no DNA profile or a profile that is uninterpretable versus 50% of those washed in two water washes
  - 80% of hairs washed in a single ethanol wash resulted in either no DNA profile or a profile that is uninterpretable versus 10% of those washed in two ethanol washes

	No DNA profile	Uninterpretable DNA profile	Interpretable DNA profile
ethanol/water	**20%**	**20%**	**60%**
water/ethanol	**50%**	**20%**	**30%**
water/water	**40%**	**10%**	**50%**
ethanol/ethanol	**10%**	**0%**	**90%**

- This portion of the experiment should be repeated using a less abrasive application technique and include samples sets with no wash, single solvent washes, and combination washes to provide for more comparable data.
- However, the data does suggest that a washing series that includes water followed by ethanol is most effective.
- Notes:
  - Red areas were still noticeable visually and stereoscopically on several of the hairs for the double ethanol wash while no red areas were noticeable with washes that included water.

EXPERIMENT E

Goal: continue to optimize washing procedure through the evaluation of different variations of washes

Potential outcomes:

- If DNA was present, then the wash was not successful.
- If no DNA was present then the wash was successful. The successful washes were evaluated for greatest success.

Method:

- Ten hairs were strung across a box, swabbed with a whole blood soaked swab along the direction of the scales (proximal to distal), and allowed to air dry.
- Eight ~1cm shaft portions were cut from each hair and prepared as follows:
  - no wash
  - ethanol
  - water
  - ethanol/water
  - water/ethanol
  - water/water
  - xylene/ethanol/water
  - xylene/water/ethanol

Results/Conclusions:

	No DNA profile	Uninterpretable DNA profile	Interpretable DNA profile
no wash	**0%**	**0%**	**100%**
ethanol	**30%**	**20%**	**50%**
water	**80%**	**20%**	**0%**
ethanol/water	**40%**	**10%**	**50%**
water/ethanol	**100%**	**0%**	**0%**
water/water	**70%**	**30%**	**0%**
xylene/ethanol/water	**30%**	**30%**	**40%**
xylene/water/ethanol	**70%**	**0%**	**30%**

- It has been demonstrated that hairs should be washed before DNA extraction.
- Washes that do not seem adequate regardless of sample prep include:
  - Washes that only use ethanol
  - Sequential wash of ethanol then water
  - Washes that include xylene, regardless of whether it is combined with water or ethanol
- Washes that seem adequate at removing surface contaminants include:
  - A single water wash
  - A sequential water wash
  - A water then ethanol wash
- While no interpretable data resulted from these washes, only the hairs washed in a series of water then ethanol resulted in no detectable DNA. In the other washes, trace DNA was detected at 1-3 loci in at least two samples.
- Therefore, it appears that the wash series consisting of water then ethanol is the most effective.
- If a single wash is to be selected, water may be the best solvent to choose; however, data and preparer observation is suggestive that a multi-step wash is advantageous.

EXPERIMENT F

Goal: attempt to determine if the sensitivity of current nuclear DNA analysis techniques will detect extraneous DNA on dirty hair shafts

Potential outcomes:

- If no DNA was present, washing would appear to be unnecessary.
- If DNA was detected, washing was necessary and the current procedure was evaluated for the ability to remove non-water soluble surface contaminants

Method:

- Donors did not cleanse their hair for at least 3 days.
- One ~1cm portion of a shaft section near the root from each was placed into separate tubes without washing and analyzed.

Results/Conclusions:

No wash	No DNA profile	Uninterpretable DNA profile	Interpretable DNA profile
group 1 (4 hairs)	**100%**	**0%**	**0%**
group 2 (4 hairs)	**100%**	**0%**	**0%**
group 3 (3 hairs)	**100%**	**0%**	**0%**
group 4 (4 hairs)	**75%**	**0%**	**25%**
group 5 (5 hairs)	**60%**	**40%**	**0%**
group 6 (30 hairs)	**73%**	**20%**	**7%**
group 7 (20 hairs)	**65%**	**25%**	**10%**

- DNA can be detected on unwashed shafts of dirty hair.
- Overall, DNA profile information was detected in 26% of the samples though only 7% of all of the samples resulted in interpretable data.
- Note:
  - Hairs were taken from multiple donors. Body chemistry and activity level differences between donors may affect oil production and resulting oily deposits on their hair shafts.

EXPERIMENT G

Goal: determine if the optimized washing procedures effectively remove non-water soluble (e.g., oily deposits) and water soluble contaminants from the hair surface

Method:

- For the duration of the experiment, hair shaft sections have been used in sampling. However, casework samples predominantly involve hair roots with apparent tissue. To further test the efficacy of wash options, whole blood was applied to 20 dirty hairs with anagen roots. An ~1cm root section and an ~1cm adjacent shaft section were washed.
  - 10 hairs washed with water/ethanol
  - 10 hairs washed with xylene/water/ethanol

Potential outcomes:

- If no DNA was present on the shaft sections, the washing procedure effectively removed both water and non-water soluble surface contaminants.
- If DNA from the hair donor was detected on the shaft sections, the washing procedure should be further evaluated for the removal of non-water soluble surface contaminants.
- If DNA was detected from the whole blood donor on any sample, the washing procedure should be further evaluated for the removal of water soluble surface contaminants.
- If DNA was detected from both the whole blood and hair donors on the shaft sections, the washing procedure should be further evaluated for the removal of water and non-water soluble surface contaminants.

Results/Conclusions:

	No DNA profile	Uninterpretable DNA profile		Interpretable DNA profile	
		blood donor	hair donor	blood donor	hair donor
water/ethanol (shaft)	**60%**	**0%**	**30%**	**0%**	**10%**
water/ethanol (root)	**0%**	**0%**	**0%**	**0%**	**100%**
xylene/water/ethanol (shaft)	**80%**	**0%**	**10%**	**0%**	**10%**
xylene/water/ethanol (root)	**0%**	**0%**	**0%**	**0%**	**100%**

- All of the hairs with anagen roots spiked with whole blood resulted in full profiles that matched the profile for the hair donor regardless of the wash type (water/ethanol vs. xylene/water/ethanol).
- No DNA information related to the donor of the whole blood or from the preparer was detected in either the root or shaft portions.
- Profile information related to the donor of the hair was obtained in 40% of the adjacent shaft portions in the wash without xylene and 20% of the samples where xylene was included. As shown in the unwashed shafts of dirty hair samples (Experiment F), DNA profile information is obtained 26% of the time. Thus, it cannot be assumed that extraneous DNA due to oily deposits was removed from the remaining washed shaft sections since it may not have been there to be removed in the first place.
- Therefore, it does not appear that xylene adequately removes oily deposits from the hair surface nor does it have an additive effect on the efficacy of the washing procedure.
- Also important to mention, out of the over 300 hair samples extracted for this project, only two samples resulted in profile information that did not belong to the donor of the hair or donor of the whole blood. This fact indicates that surface contaminants due to oily deposits are most likely from the donor of the hair and not from an extraneous source. Furthermore, because an effective washing procedure has been proven in its ability to remove blood (and thus other water soluble surface contaminants), the need for shaft controls in the course of routine casework samples appears unwarranted. In certain instances (e.g., roll over accident, visible surface contaminant, etc.), however, good laboratory practice may necessitate a shaft control.