BEST PRACTICES IN FORENSIC DNA SAMPLE COLLECTION AND ANALYSIS

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Introduction:

The science (and art) of forensic identification has been transformed by the power and utility of forensic DNA (deoxyribonucleic acid) evidence. Never before has DNA enjoyed such a first class treatment outside the research laboratory, and rightfully so. For close to 30 plus years now, ever since Sir Alec Jeffreys set us on this course, significant advancements and innovations continue to bolster our ability to use this "molecule of life" in the quest to preserve the liberty of the innocent and punish the guilty for their crimes. Concurrent with these advancements are efforts to assure the public and the judicial system that DNA evidence is based on robust science, sound methodologies, and sufficient education of those who steward the forensic evidence from collection to final lab report. Every crime scene is unique: members of law enforcement and personnel involved in crime scene processing must be versed in multiple disciplines, forensics and otherwise. They undergo rigorous education and training that is coupled with hands-on experience and field work. They are also exposed to a healthy dose of best practices and proper methodologies that have been developed throughout the years by pioneers in the field. Keeping up with this education and best practices is paramount as DNA evidence becomes more and more indispensible as a forensic tool.

The popularity of DNA evidence lies within its power of determining not only the presence of biological material from a human source but also the identity of the contributor of that material. Some forensic textbooks refer to this as "individualization" or "individual characteristic", as opposed to a "class characteristic" such as determining that a paint chip is from an automotive source and not from another industry. If a perpetrator is being sought, DNA evidence offers the shortest line between point A (evidence) and point B (suspect identity), and can lead to successful outcomes when other techniques fail.

DNA evidence, in many ways, is easier to work with than, say, determining the pattern of blood spatter or obtaining a usable cast from a shoe imprint in the snow. However, the challenges lie in

preserving the biological samples to the extent possible and minimizing the chances of cross contamination. While the laboratory environment offers the most suitable conditions for preserving DNA evidence, (most) crime scenes do not happen in the laboratory. Biological evidence is most vulnerable prior to packaging. Therefore, the collection and preservation of items that are destined for forensic DNA processing will require the mindset that Edmond Locard's exchange principle is still working, but on a much smaller scale, down to invisible or submicroscopic level. To be successful in that endeavor, we must, to the extent possible, extrapolate the laboratory environment and best practices to the crime scene and throughout the chain of custody. The literature is packed with tried and true recommendations and guidance on such best practices. The aim here is to provide members of law enforcement and personnel involved in crime scene processing a concise survival guide of the most important items that should be kept in mind while dealing with DNA evidence.

First, Some Basics:

What is DNA and where does it come from?

DNA is a biological molecule (a polymer) found in every living cell (with very few exceptions), whether it is a bacterial cell, a cell from a tree leaf, or a cell from a human liver, skin, or hair follicle. DNA orchestrates the activity of the cell, thereby allowing the organism to carry out all the functions associated with life, from food digestion and energy conversion to responding to disease and other environmental pressures. DNA is packed inside a cellular structure called the nucleus. One human cell's worth of DNA carries the entire human genome made up of 46 chromosomes. In theory, one cell carries all the information needed to generate a forensic DNA profile from a person. However, technical limitations preclude us from doing so, at least for the time being.

What is forensic DNA analysis?

In a nutshell, forensic DNA analysis (also known as STR analysis: STR stands for Short Tandem Repeats) entails harvesting DNA from cells, a process known as DNA extraction, and subjecting that DNA to three additional steps; quantitation, amplification, and electrophoresis. DNA extraction must be performed regardless of the origin of the biological sample. This process releases the DNA from its compartment (the nucleus), and combines the DNA from all the cells collected from, say, a swab or a stain. There is a DNA extraction method that is suitable for biological samples such as blood, skin, and semen. Other extraction protocols are appropriate for bone, teeth or hair. During the quantitation step, the amount of DNA obtained from a biological sample is measured. This measurement ensures that the amount of DNA used in the subsequent steps of the analysis falls within an optimum range. As importantly, this step allows the forensic analyst to determine early on whether a sample contains a sufficient amount of DNA to generate a full profile. Low DNA amounts will often result in partial profiles with limited or no forensic value. Quantitation will also alert the analyst to the potential presence of DNA profiling inhibitors in the sample. This early sign will allow the analyst some room to mitigate or troubleshoot this inhibition.

Not all the DNA from the cell is needed for profiling. Thus, during the amplification step, specific areas of the genome (known as loci or markers) are copied millions of times to yield a detectable amount of only those specific markers. Typically, anywhere from eight to 16 markers are analyzed. However, when it comes to criminal cases, the goal is to analyze a minimum of 13 markers, also known as the CODIS (Combined DNA Index System) core markers. At the final step, or electrophoresis, the amplified sections of the genome (or STRs) are separated by size. At the end of the analysis, this size is depicted numerically for each marker. For instance, at marker TPOX, the result could be (6, 7). The individual from which this DNA sample was obtained is said to have alleles 6 and 7 at marker TPOX. Based on the laws of genetics, this individual inherited allele 6 from one parent, and allele 7 from the

other parent. Another individual's DNA profile could yield (9, 10) at the same market. Again, this person inherited allele 9 from one parent, and allele 10 from another parent. What about an individual's DNA profile that shows (9, 9) at this marker? This individual inherited allele 9 from one parent and allele 9 from another parent. In summary, the uniqueness of a DNA profile lies within the combination of numbers that are obtained from analyzing a set of markers for a given biological sample or evidence item. This combination of numbers is known as the DNA profile. A DNA profile is also developed from a suspect or a list of suspects (usually from a mouth "buccal" swab or a blood sample) and serves as a reference or standard for comparison. A match is declared between the evidence item and potential suspect if the DNA profiles from both have the exact combination of numbers.

Dealing with Biological Samples Destined for DNA Analysis

DNA analysis is extremely sensitive. Although it is theoretically possible to detect the presence of DNA from one cell, technical limitations require that a minimum of 20 to 50 cells be analyzed for a more reliable outcome. Nonetheless, this number of cells is invisible to the naked eye, and can be easily transferred by touching a surface, or by two items touching each other (remember Locard's exchange principle!). Furthermore, humans shed thousands of dead skin cells every hour. While not all of these cells will contain DNA, some do, and remember, your body, clothes, hands, hair, perspiration, personal equipment like radios, pens, cameras and flashlights, all contain YOUR DNA at some level. When collecting samples for DNA analysis, the main objective is to avoid cross contaminating the items and the scene through primary or secondary transfer. Your goal is to also keep your DNA from contaminating the evidence.

Wear Gloves, and wear them properly! At the crime scene, anything and any surface can contain biological material which might be of forensic value. It can also contain hazardous infectious disease. Wear your gloves (and if needed, other Personal Protective Equipment or PPE) before commencing any interaction with that scene. Wearing gloves (as an act) means nothing if the gloves are

not worn and used properly. Always pick fresh gloves from the glove box by the cuff and avoid touching the finger and palm portions of the outer surface of the gloves with your bare hands. Once worn, if your gloves touch one item of evidence, it is time to change them before touching anything else. Take your gloves off starting at the cuff of one glove, then the other cuff. Done appropriately, you should be able to take both gloves off without ever touching the outer surface of either glove with your bare skin.

Remember, after wearing a pair of gloves for a while your hands will sweat. At this point, be extra careful when reaching for the glove box for a new pair. This is prime opportunity for leaving your biological material on other surfaces. Furthermore, sweaty hands are difficult to glove, especially if using powder-free gloves. That is one of the reasons why healthcare professionals and laboratory personnel resort to double-gloving, especially at times when frequent glove changing is expected. Here is a scenario: you double-glove and enter the crime scene. While processing an evidence item with potential bodily fluid, you decide to jot down a note for documentation. Your pen and note pad are tucked in your pocket. What do you do? Take off the outer pair of gloves, get your documentation done, put on a new pair of gloves, and proceed with your work. In doing so, you have avoided transferring biological material from the evidence to your pen (and vice versa). Also, with the existing inner glove, your hands are ready for a fresh pair of gloves immediately. Remember; always think of the potential transfer of biological material before going into your next task. While at the scene, wearing full protective clothing such as sterile suits/coveralls, head and shoe covers is highly recommended and should be mandated, regardless of your role at that scene.

Swabbing surfaces, items, or suspects:

As stated before, collecting biological evidence is relatively easy. The ultimate objective is to harvest biological material from an item or surface, and then package that material for delivery to the lab. The sterile cotton swab has served this purpose well for decades. And while the swab continues to work well, recent innovations in synthetic materials have made it possible to replace cotton with

superior materials that not only can collect biological tissue more efficiently but also offer some protection to the biological material in transit. The effectiveness of the "double-swab" technique has been well appreciated. Biological materials/tissues (cells, body fluid, etc.) lose moisture relatively quickly and dry on surfaces with some tenacity. This can be easily reversed by re-hydrating this tissue with clean, sterile water. Hence, the double swab technique is done by simply using the first swab (moistened with water) to rub the biological material gently, followed by the second dry swab to pick up the moist tissue. Both wet and dry swabs will contain a portion of that biological material, and therefore, should be packaged together and sent to the lab. Under normal conditions, the lab will combine the material from both swabs for maximum tissue recovery. Do keep in mind though that prolonged storage of moist evidence can lead to degradation. Most commercially available swabs now come with packaging and shipping contraptions that allow moisture to escape. Dry biological evidence can last for centuries or longer (remember, DNA has been extracted from mummies and semi-fossilized tissue). Moisture is a perfect environment for fungus and bacterial growth, both of which can cause the rapid degradation of DNA samples.

In the past decade, and mainly due to the enhancements in DNA analysis technologies, the "buccal" swab has almost replaced the need to collect blood samples for the purpose of developing reference DNA profiles from suspects (or other references/exemplars with some exceptions). Buccal swabs (named after the buccal cavity in the cheek area) are obtained by rubbing the inside cheek pouch of an individual with a clean/sterile swab. Done properly, the swab will yield enough DNA to generate an individual's profile many times over. This process is simple, but you will have to approach this collection with the same mindset as working a crime scene. Again, wear gloves properly and keep an eye out for potential contamination. Make sure that the swab is new and sealed prior to cutting into the package. Inspect the suspect's mouth for any foreign objects. Once collected, the swab should be resealed immediately in a secure package and the chain of custody documentation initiated. If delivery

to the laboratory is delayed, protect the swab package from environmental factors to the extent possible, particularly heat, humidity, chemicals and damaging radiation.

So where does DNA evidence come from? DNA profiles can be developed from biological tissue such as blood, saliva, skin, semen, or any biological fluid that comes from a human body. Hair follicles are also a good source of DNA, especially hairs that are in the Anagen phase of growth (the first of three stages of hair growth). In recent years a new term, "Touch DNA", has been introduced to the forensic lexicon to describe the recovery of low-level DNA from a surface. Suppose a suspect touched a door handle in the course of committing a robbery. There is a chance that the suspect will leave on that door handle some skin cells that could be swabbed for DNA analysis. It turns out, skin cells are not the only source of that DNA. Sweat and oils from the skin also contain DNA (also known as "Free DNA"). A swab can then be sent to laboratory for testing with a request by the officer or crime scene technician to process for "Touch DNA". It is important to note that the laboratory will conduct the exact same test on this sample like any other swab with biological material. The only difference is, the laboratory will know beforehand to expect a sample with low DNA yield, and therefore, can adjust the extraction protocol accordingly if need be. Not surprisingly, the analysis of "Touch DNA" samples often results in weak data (partial DNA profiles or complex mixtures that cannot be interpreted), or outright no data at all. Do keep in mind that "Touch DNA" does not always mean that someone actually touched a surface. Touch is presumed, not confirmed. DNA analysis will not provide any information on how or when that "Touch DNA" sample got there in the first place.

Summary Points and Best Practices:

- Forensic DNA analysis is here to stay. Members of law enforcement and crime scene processing are becoming well versed in handling this type of evidence through proper education and training.
- Proper collection and preservation of biological evidence is critical.
- The goal is to avoid cross-contaminating the biological evidence, and to keep your DNA out of those items.

-Contamination can happen very easily if you don't think ahead of your actions.

-Don't talk, sneeze, or cough over the evidence. All those actions can generate minute aerosolized droplets containing your DNA.

-Wear gloves, wear them properly, and change them frequently.

-Keep your glove box in a sealed container away from sources of potential contamination.

-Don't touch your skin or clothes with your gloves.

-Equipment that you touch with your bare hands must not be touched with the same gloves that are used for evidence collection. Consider the double-glove technique.

-Use clean equipment and keep your equipment clean.

-Fingerprint brushes, tweezers, and other crime scene tools can be the source of cross contamination. Consider using disposable single-use equipment and tools when able.

-Biological evidence can be invisible. Treat all biological evidence with that mindset, and protect yourself from potential biohazards and infectious diseases.

-If you think you contaminated an item, inform the laboratory as soon as the item is submitted for analysis.

-Evidence preservation must not stop at the crime scene. It needs to be practiced throughout the chain of custody, even when the evidence may be displayed during court proceedings.