Welcome to the forensic toxicology podcast. My name is Dr. Sanela Martic. I am an assistant professor in the Department of Forensic Science at Trent University. Today's podcast is on the topic of sample collection and storage. In this podcast you're going to hear about CanLII cases where sample collection and storage play a critical role. More specifically, you will see cases where a toxicologists, clinicians, and other experts have to really care about drugs stability and degradation in various samples and varied biological fluids. And they also have to think about how sample storage periods affect the drug concentrations and their interpretation. Let's look at this and listen to the case: R. versus Sukhdeo 2019 ON-CJ-150. This case, you'll see that clearly the sample selection and sample collection is really important and it can certainly interfere with the interpretation. One has to be mindful of the storage periods that we call which are time intervals between the incident collection analysis, and post analysis, or re-analysis. All of these time windows, time periods could affect the concentrations of the drugs in the fluid. And of course, that could also affect the interpretation. In this particular case, Mr. Palmentier, who was a CFS toxicologist, testified that given the process of drug elimination from the body, that drug concentrations in the body change over time. Drugs present earlier may not be present or detected at the time of sample collection. So that means that the time between incident and sample collection is quite long and that time has allowed for drug clearing out of the system, for example. Some drugs are more volatile and maybe eliminated from the body very quickly before a tox sample has been gathered. The situation depends upon the drug, the concentration of the drug, and the type of sample collected from the subject. Some drugs are not eliminated from the body through urine. The longer the period of time between the incident and sample collection, the more likely that these effects are to be present. Now, Mr. Palmentier testified that once collected, so now you are at the collection stage, the drug concentration in samples can also change over time. So now we're looking at the storage period too, which is period between the collection of the sample and analysis of the sample. Now even within that window, the changes to the drugs and chemicals in those samples will be taking place. The potential for this occurrence is dependent upon the type of drug and maybe minimized by some of the appropriate forensic practices. However, the concentration of drug may decrease between the time of collection of sample and the time that the analysis was performed. In addition, while CFS has extensive analytical capabilities, it is not possible for any lab to detect all the drugs. That means that their screens are really focused and they're not inclusive. Scientifically validated method testing for specific drugs may not be available at the time of testing. Screening methods for some drugs may not be sufficiently sensitive at the time of testing. And, in addition, the type and volume of the sample, type of volume of the biological fluid collected may also limit possible analysis where you could be able to do one analysis but you've run out of sample and you can't do multiple other types of techniques. So in this case you can perfectly here the stability of drugs and how time periods between incident collection analysis and maybe even re-analysis can influence the concentration of the drug and the interpretation. Let's consider this next case, R. versus Vanlerberghe 1993 CanLII 231 (BC SC). Unlike the previous case, that talked about general principles of drug degradation and how drug degradation can take place after the incident, after the collection, even after analysis of our sample is sitting in that fluid. This case here talks about very specific drug, cocaine. The peak blood levels attained after intranasal or intravenous injections of low to moderate amounts of cocaine range from 90 to 470 nanograms of cocaine per mil. of blood. After that ingestion of cocaine, either by snorting, injection, smoking or so on, the cocaine disappears from the body within three to ten hours, on average five hours. And that really depends on the amount of cocaine taken as well as the individual involved. Because we know that the metabolism of drugs is really based on the enzymes that are being, that are being expressed or present. And so the enzyme levels and maybe enzyme mutations between individuals may result in them metabolizing drugs in a slightly different rates. Cocaine may break down upon even storage after sampling. And so that really

highlights that second period, you know, after sample collection, if you let the sample sit there prior to analysis, that storage period two is really critical. As such, the blood cocaine levels reported may be lower than those which were present at the time of sampling and collection. The cocaine actually falls. apart into several metabolites and benzoylecgonine arises in the body from the metabolism or cocaine, as well as upon storage from just decomposition of cocaine. So that tells us that even when the cocaine is sitting in a solution, biological liquid where there are enzymes, it could be hydrolyzed into benzoylecgonine. But even if there are no enzymes, let's say, there's chemical hydrolysis that could take place. Methylecgonine arises in the body also has a metabolite from metabolism of cocaine. basically. Neither of these metabolites, benzoylecgonine, methylecgonine are pharmacologically active, while of course the parent drug is. The presence of those two metabolites indicates prior use of cocaine, but it is not possible to accurately say at what time or to what extent based on their presence alone. And so this is the situation that toxicologists find themselves where while they can detect metabolites that indicate prior drug use, it's really not possible to determine how much was used or when it was used and so on. Most often that's not really feasible to be determined. I think one drug or toxic and that you could backtrack from your measurement to estimate how much was taken would be ethanol. We know really ethanol very well. We understand its elimination rates and so toxicologists could, you know, measure ethanol levels, let's say blood alcohol levels, and they could backtrack from that number. in terms of time to incident time, for example, they could tell, or estimate, how much alcohol was ingested at the time of collection or even at the time of accident. And so that's very unique to ethanol. Most other drugs have this characteristic that we don't have a simple formula to extrapolate how much was used and when it was used, simply based on measurements of their metabolites in biological fluids. In this last case, in sample collection and storage, Layes versus Bowes 2020 NSSC 345 [?]. This case is trying to determine the cause of death, and it also describes the issues that toxicologists faced in terms of drug stability. But it also used very unique matrix, biological matrix, specifically hair. We find that hair as a sample, does not necessarily undergo all the biochemical reactions that take place in urine or blood, for example. It's a very unique biological matrix that does not necessarily suffer from challenges that other matrices suffer from. In addition, the hair, the length of the hair is actually a time profile of the drugs and chemicals that have entered the body over several months or a year, even depending of course, on my hair length available. And so the part of the hair closest to the scalp, if you were to analyze the chemistry there, it will tell you about her recent use in the last month, or two, or three. And then if you look at the length of the part of the hair further away from the skull, and depending on the length of that hair, you can start getting some other information such as drug use, or levels six months ago, or nine months ago even. And so it's really unique matrix. So let's jump into it. In this case, Kevin Shanks is highly qualified forensic toxicologist who is an expert on both the forensic toxicology side, but also as the overall effect that the passage of time has on the spoilation of drug metabolites in all various matrices capable of being examined in criminal forensic tox labs. Here is the sentence on the topic of passage of time, or a storage time. will tell us about effect of that time has on the stability of the drug. As we know, certain drugs decompose over time, and their decomposition is really dependent on the biological matrix they're in. Drugs are present in bodily tissue and they deteriorate over time, so that the detectable concentrations decrease mostly. This means that drugs that were in the same tissue at the time of death maybe detectable in a lower concentration, or maybe not even detect it at all after sufficient time has passed. So to avoid further loss of evidence and to avoid drug decomposition, basically, the post-mortem analysis in this case should be carried out immediately, either to take specimens immediately, or to preserve the body and preserve the specimen separately. And any drugs within it until legal authorization is there to collect and analyze them. So in this case, there were quite a few tox reports going on. And one tox report has found that Mr. Shanks'

tox analysis was allegedly using over diluted samples and that could have skewed his interpretation. Now, despite this, the specific norketamine drug found was also contradicting the Exhibit D results where that has shown that Exhibit D was presented and stated that the presence of norketamine on those kinds of drugs were due to exogenous contamination, meaning that the hair was simply exposed environmentally to the drug, rather than actually drug was taken by the person. But the fact that norketamine was found in the tox screen rules out this exogenous contact as a probable reason for the drug being present in the hair strands. And the other segmental hair drug results reported by forensic toxicologist are also in favor of this, but these drugs really came through ingestion and over approximately three month period before death. And so here is an example of how hair can be used to estimate how long it has one person been exposed to a chemical x. In this case, for example, ketamine. or norketamine. So, the detection of metabolites of the drug ketamine in the analysis of the hair samples clearly states that this is irrefutable evidence that ketamine was administered to the deceased and it was not medically prescribed to him, and it was not accidentally, environmentally absorbed on the hair. But it was really kind of a wrongful drug dosing of the individual that resulted in death. This case really nicely tells you about issues of drug decomposition in biological fluids, and how hair when possible, could be used unequivocally to determine presence of drugs in the system due to exposure either accidental or purposeful. And that clearly underlines how hair is very different from other biological samples. You have now heard about several CanLII cases where sample collection and storage played a critical role. You've heard about possibility of drug decomposition and degradation over time, and how sample storage time period could affect concentrations measured as well as interpretation. This was a forensic toxicology podcast on sample collection and storage. Thank you for joining me.