# ABSTRACTS

### Friday, the 13th of June 2024 – Scientific Session I (13:30 – 14:30)

# Abstract 1: 13:30 – 13:40 Persistence of Biological Traces in Gun Barrels Following the Use of Different 9 mm Parabellum Cartridges

C. Hartmann, Institute of Forensic Medicine, Bern

Background: Biological traces within the barrel of a firearm following contact shots are of consid-erable forensic relevance, particularly for victim identification and reconstruction of the shooting event. While the detectability of such traces using standard 8 g full metal jacket (FMJ) bullets is well established, systematic investigations into the per-sistence of traces following the use of subsonic cartridges or deformation bullets are still lacking.

Method: A standardized ballistic model (gelatin block with an embedded reservoir of hepa-rinized blood–acrylic mixture) was used to conduct contact shots with four different types of 9 mm FMJ cartridges. Three shots were fired per cartridge type (n = 12), complemented by single shots using deformation bullets (n = 5). After each shot, endoscopic documentation was performed, followed by separate sampling from the anterior and posterior barrel sections. A subsequent shot using the same cartridge type was carried out to evaluate trace persistence. DNA quantities were quantified via qPCR and, where possible, typed using short tandem repeat (STR) analysis.

Results: In most samples, human DNA of usable quality was detected after the initial shot. Even following the subsequent shot, DNA traces suitable for typing were still pre-sent in a relevant proportion of cases. Overall, DNA concentrations were markedly higher in the anterior barrel section. The morphological findings did not suggest any systematic influence of internal or terminal ballistic parameters on trace formation. The observed differences in molecular genetic detectability are likely attributable to other factors.

Conclusion: This study confirms the fundamental detectability and partial persistence of biologi-cal traces within the barrel, even when using cartridges with varying gas pressure characteristics. Further research may enable a more differentiated interpretation of DNA traces in forensic casework. Future investigations should focus on qualitatively and quantitatively assessing the factors influencing the removal of DNA from gun barrels.

### Abstract 2: 13:40 – 13:50

# Detection of gunshot residues using infrared photography: Influence of ammunition type, surface color and blood contamination

J. Bottoni, Institute of Forensic Medicine, Basel

Background: Detecting gunshot residues (GSR) plays a crucial role in forensic science and forensic medicine by providing important insights into the shooting distance, the shooter, as well as the type of weapon and ammunition used. Detection of GSR on dark surfaces is often impossible on site, and traditional methods such as tape-lift techniques with adhesive films or scanning electron microscope tabs might destroy the GSR pattern at the crime scene during their application. Infrared (IR) photography has proven particularly effective in detecting GSR on dark surfaces, enabling the preservation of the GSR pattern before applying destructive methods.

Method: This study aimed to examine how the type of ammunition and the presence of bloodstains affect GSR detection and differentiation using IR photography. 15 types of leaded and 5 types of lead-free 9 mm Luger ammunition were fired onto white cotton fabric and dark-blue denim fabric using the same firearm model, with an additional 14 samples being stained with blood.

Results: Resulting GSR patterns varied depending on the ammunition type and fewer GSR particles were visible on denim fabric, even in IR photography, compared to white cotton fabric. IR photography effectively confirmed GSR on dark fabric, thereby achieving reliable results comparable to the standard tape-lift method.

Conclusion: In conclusion, IR photography could successfully distinguish GSR from overlapping bloodstains and detect GSR from lead-free ammunition on dark surfaces. Therefore, IR photography provides a robust, easy-to-use and non-destructive tool for the spatial allocation of GSR.

#### Abstract 3: 13:50 - 14:00

### **Gunshot Trauma to the Head: Impression Fractures**

S. Kissling, Institute of Forensic Medicine, Basel

Gunshot injuries are a fundamental aspect of forensic medicine, playing a critical role in both living and postmortem examinations. While morphological characteristics of skin wounds are often key in the assessment of gunshot trauma, skeletal evidence - particularly cranial fractures - can provide additional valuable insights into the mechanics of the injury. The large variability of possible findings results from factors including shooting distance, entry site, firearm type and ammunition.

Two forensic cases involving gunshot-induced head injuries will be presented. In both similar cranial impression fractures were observed, offering an interesting comparison of skeletal findings in different contexts. Additionally, the presented cases demonstrate significant variation in other forensic details, identified with the help of additional radiological and technical examinations. These findings underscore the importance of a multidisciplinary approach in legal medicine, where detailed analysis of both skin and skeletal injuries plays a pivotal role in reconstructing the events surrounding gunshot trauma.

## Abstract 4: 14:00 – 14:10

### 'Explosion' of a truck wheel rim

A. Schuff, Department of forensic medicine, Laboratoire national de santé (LNS), Dudelange, Luxembourg

Fatal accidents at work are not uncommon in routine forensic autopsies. In the forensic autopsy reports of the Grand Duchy of Luxembourg, fatal accidents at work account for approximately 5% of cases. The case presented here is a special constellation involving unusual force during a tyre change on a truck rim. While demounting a two-part rim with the truck tyre still correctly mounted, the demounted part of the rim detached explosively, causing severe and fatal open head trauma to the mechanic. The cause of this explosive detachment of the rim part was that the air had obviously been forgotten to be released from the truck tyre before the rim was removed. The presentation will outline the technical circumstances and the consequences of the force exerted by the projectile-like rim part on the mechanic's skull and also on the adjacent parts of the building.

#### Abstract 5: 14:10 – 14:20

# Sudden unexpected cardiac death among the young (5 to 40 years) in Switzerland between 2011 and 2019: incidence and autopsy rate *C. Frigerio, University Center of Legal Medicine, Geneva*

Sudden cardiac death (SCD) is generally defined as a sudden unexpected death from cardiovascular cause or a sudden unexpected death without any structural heart anomalies and no obvious extra cardiac causes have been identified by post-mortem examination, occurring within 1 hour after the onset of the symptoms or if unwitnessed within 24h of last being seen alive. Globally, the exact incidence of SCD in the young is unclear and shows great variability between studies, ranging from 0,4 to 13,6/100000 person-year. The autopsy is considered the gold standard to establish the cause of death and enables clinical management of families with hereditary cardiac disease The data for Switzerland are missing. Therefore, the aims of this study were to determine the incidence of SCD and to evaluate the autopsy rate of SCD in Switzerland, for individuals aged from 5 to 40 years of age. Statistical data was extracted from the Swiss Statistics Office (OFSP) in all Swiss cantons, from 2011 to 2019, according to the International Classification of Diseases 10th Revision (ICD-10) codes. During the period of the study, there were 2283 deaths, from which 494 deaths corresponding to the ICD-10 codes "diseases of the cardiovascular system" (100-199) and 828 deaths, corresponding to the ICD-10 codes "unknow death" (R95-R99). National incidence of SCD in Switzerland has been evaluated to be 4,13/100000 person-year, showing a great variability between Swiss cantons, possibly suggesting different cantonal approaches in managing SCD cases. Autopsy rates for SCD cases in Switzerland have been calculated to be 30% with differences between cantons. A homogenous management of SCDs deaths, an increased autopsy rate and a better management of the results of autopsies are strongly advised.

#### Abstract 6: 14:20 – 14:30

## Myocarditis: new diagnostic criteria at autopsy

K. Michaud, University Center of Legal Medicine, Lausanne-Geneva

The diagnosis of myocarditis is important in routine pathological practice in the clinical setting when interpreting endomyocardial biopsies (EMBs) and during post-mortem examination of the heart at autopsy. The Dallas Criteria were established in 1987 to codify the histologic findings of myocarditis at EMB for consistent interpretation across centers. Histologic assessment of a specimen from an EMB allows to identify subtypes of myocarditis, such as lymphocytic, eosinophilic, and giant-cell myocarditis and cardiac sarcoidosis, which have specific prognostic and therapeutic implications. Although these criteria have been introduced for EMB, they were also used for other surgical, and autopsy specimens. New diagnostic approaches for EMB were proposed in 2011 and 2013 including immunochemistry and molecular viral analyses on heart and blood samples. The need to update clinical and pathological criteria has become obvious with the appearance of new form of myocarditis as immune checkpoint inhibitor myocarditis and SAR-CoV-2 related myocarditis and with the discrepancies observed between the pathological and clinical diagnosis of myocarditis.

The lack of consensus diagnostic criteria for myocarditis at autopsy and in surgical specimens other than EMB has been noted for years, several pathological studies have postulated systems to diagnose and grade myocarditis at autopsy, based mostly on quantified level of inflammatory cells. However, until now there has been no reproducible method for diagnosing myocarditis in the post-mortem context, nor any systematic approach to determining whether inflammation of the heart was the cause of death or simply an incidental finding.

In March 2025, the Society for Cardiovascular Pathology (SCVP) and the

Association for European Cardiovascular Pathology (AECVP) agreed new working criteria for the diagnosis of lymphocytic myocarditis, one for EMB and the other for autopsy. The aim of this presentation is to outline the new diagnostic criteria established for autopsy.

### Friday, the 13th of June 2024 – Scientific Session II (15:00 – 15:50)

### Abstract 7: 15:00 – 15:10

Forensic tissue mechanics – validation of diabetic brain induration J. Zwirner, Institute of Legal Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, Department of Oral Sciences, Otago, Dunedin, New Zealand

Forensic tissue mechanics refers to the application of the biomechanical properties of human tissues in estimating the time since death and assisting in postmortem diagnostics. However, its potential as a validation tool for haptic diagnoses during autopsy - widely used for descriptive purposes - remains largely unexplored. This study aims to validate the assessment of brain induration during autopsy through a concurrent analysis of biomechanical properties and postmortem biochemistry, with a specific focus on diabetes mellitus.

Conical samples (1 cm in diameter) were obtained from routinely prepared brain slices at autopsy from 49 human cadavers and biomechanically tested using a standard rheometer. The storage modulus was analyzed, with higher values indicating stiffer brain tissue. Six brain regions were investigated, including predominantly gray matter (frontal cortex, anterior and posterior deep brain) and white matter (frontal white matter and medulla oblongata) samples, as well as the cerebellum. Autopsy reports were reviewed for documentation of brain induration based on haptic examination, cerebrospinal fluid (CSF) dipstick analysis results, brain weight, and the deceased's history of diabetes. Concurrently, the Traub formula was calculated from CSF and vitreous humor, and the HbA1c concentration was determined from venous blood. Microscopic features of brain edema were assessed using HE-stained slides from all cases and brain regions.

Brain induration was noted in only one of the 49 autopsy reports, where the CSF dipstick analysis showed a strongly positive result for glucose (4+). This 75-year-old case ranked in the top 5 for storage modulus values across all measured brain regions and second to last in terms of brain weight among the adults. The 57-year-old deceased with the highest Traub value (458 mg/dL) in CSF ranked among the top 4 for storage modulus values across all brain regions but had the lowest brain weight among adults. In contrast, strongly edematous brains (e.g., a 53-year-old with fatal TBI after a 2-day survival, and a septic 2-year-old) exhibited a broader distribution of storage moduli, ranging from the 2nd to 43rd highest values and the 1st to 13th highest values, respectively.

Forensic tissue mechanics is a valuable tool for validating haptic diagnoses during autopsy through objective measurements. The results suggest that dehydration contributes to diabetic brain induration, which shows a homogeneous storage modulus across regions. In contrast, brain edema significantly contributes to brain induration, resulting in a less homogeneous storage modulus across regions. Subjective brain palpation during autopsy has low sensitivity in detecting brain induration and appears to be influenced by the results of CSF dipstick analysis and the known pre-existing conditions.

#### Abstract 8: 15:10 – 15:20

## Drowning site inference through diatom metabarcoding

E. Rossini, Institute of Ecology and Evolution, Bern; Institute of Forensic Medicine, Bern

Diatoms are photosynthetic unicellular algae that inhabit almost all aquatic environments and moist terrestrial habitats. These organisms have several forensic applications, such as post-mortem interval (PMI) estimation, drowning diagnosis, and locating drowning sites. Due to their high sensitivity to environmental conditions, diatom communities are locally shaped by physiochemical and biological factors providing information on the specific location of the drowning medium. Previous studies based on morphological identification of taxa highlighted the usefulness of diatoms in identifying different freshwater reservoirs, as well as distinguishing between freshwater and seawater after the river mouth. However, because of the high taxonomic expertise required for species identification under the microscope, in recent years efforts have been made to employ molecular techniques such as metabarcoding. In this project, we investigate the spatial and temporal distribution of diatom communities using metabarcoding, with a focus on the river Aare system between lake Thun and lake Biel. Preliminary results from high-throughput sequencing of the rbcl gene on a small subset of samples (10 water samples, 9 epilithic samples) allowed us to identify 1,534 different ASVs classified in the Bacillariophyta phylum comprising 45 genera and 73 distinct diatom species. We see clear differences in diatom assemblages between connected water bodies (lake Thun, river Aare, lake Biel), underscoring the potential use of diatom communities in distinguishing drowning in these lakes from drowning in stretches of the river. The spatial and temporal information on diatom communities will allow us to generate a diatomological map and ideally infer the drowning site by comparing it with the diatoms identified in drowning victims' tissues.

#### Abstract 9: 15:20 – 15:30

# Myositis ossificans as a component of morphologic wound age determination

C. Süss, Institut für Rechtsmedizin, St. Gallen

Forensic wound age determination of injuries follows a known phased course of the healing process in the tissue, which is characterized by the chronological, partially overlapping occurrence of a large number of histologically detectable individual changes in the injured tissue. Taking into account possible influencing factors, conclusions can be drawn about the time at which an injury occurred. In some cases, the wound healing of muscular injuries is accompanied by reactive, progressive cartilage and bone formation (myositis ossificans).

We present the case of a 21-year-old woman who died after a physical confrontation with her partner despite resuscitation measures by the emergency services, with the partner claiming a staircase fall as the cause of death. The autopsy revealed numerous blunt force traumas to the extremities and trunk with the development of myositis ossificans in the leg muscles. On the basis of the histological determination of the age of the wound, it was possible to prove that there had been recurrent, considerable violence over several weeks, which had ultimately led to the young woman's death due to internal blood loss. The case was classified as homicide.

#### Abstract 10: 15:30 – 15:40

# Mobile application to estimate time of death based on body temperature and postmortem changes for forensic investigations

C. Berger, Institute of Forensic Medicine, Basel

Introduction: During legal inspections, the time of death in the early postmortem interval is estimated based on early postmortem changes, i.e. body temperature, mechanical and electrical excitability of the skeletal muscles, livor mortis and rigor mortis. This estimation is calculated manually on-site, which limits precision and complicates the reliable integration of corrective factors, enabling the time of death estimation based on the body temperature to be as precise as possible. Corrective factors account for deviations from the standard cooling conditions established by Henssge and are included later, typically once the investigators return to the workplace. To simplify the procedure of the time of death estimation, including all early postmortem changes and easily incorporating the corrective factors of the body cooling on-site, we developed a mobile application called "ToD" (time of death) allowing the time of death estimation digitally.

Methods: The application was developed using an iterative approach, guided by forensic medical user requirements. An Excel sheet was first created to define the necessary parameters and variables for estimating the time of death, providing a reference for data input, calculations, and output. The open-source framework Flutter, along with the Dart programming language, was chosen for its cross-platform compatibility, ensuring efficient real-time processing. The system was designed to accept one or multiple types of inputs of postmortem changes for estimating time-of-death. Rigorous user testing by forensic pathologists on-site ensured error handling, input validation, and usability enhancements.

Results: The ToD application estimates the time of death by analyzing early

postmortem changes. Each case is assigned a unique, non-personalized ID, and essential forensic data is securely stored locally to ensure privacy and accessibility. Users must input the date and time of examination, which remains adjustable for forensic accuracy.

The time of death estimation algorithm adapts to available data, supporting calculations based on:

Algor mortis (body temperature), based on Henssge

Livor mortis, documented via structured input options

Rigor mortis, with dropdown menus for different progression stages

Supravital reactions, assessing electrical and mechanical excitability of skeletal muscles.

For user experience, the app features real-time error detection highlighting divergent results and multilingual support (German, French, Italian, English, Croatian, and Spanish). Users can export results as a standardized PDF report, ensuring secure documentation and case-sharing capabilities.

Discussion: Estimating the time of death is a critical aspect of forensic investigations, relying on early postmortem changes such as livor mortis, rigor mortis, supravital reactions, and body temperature cooling. The ToD mobile application was developed to enhance accuracy and improve on-site convenience by integrating these factors into a single tool. Unlike existing internet-based software, this app functions offline and is available for both, iOS and Android using the Flutter framework. It further includes, besides body temperature, also livor mortis, rigor mortis and supravital reactions for the time of death estimation. The proposed formula by Henssge remains the gold standard for temperature-based time of death estimation, but manual calculations struggle to account for non-standard conditions. The ToD app automates these corrections, applying corrective factors to refine estimations even in fluctuating environmental conditions. Additionally, the app detects inconsistencies in entered data, allowing forensic experts to reassess and

correct input errors in real time. By enhancing computational power on-site, the app improves the precision and reliability of time of death estimation compared to traditional manual methods.

## Abstract 11: 15:40 – 15:50 Injuries and anatomical variations of the female genitalia L. D. Martin. Institute of Forensic Medicine. Basel

Background: Female genital injuries are often underreported or misinterpreted, particularly in cases of sexual violence. Most research has focused on injuries resulting from sexual intercourse, while less attention has been given to injuries arising from daily activities or anatomical variations. This study aims to document genital injuries in healthy women, independent of sexual assault, to contribute to a foundational understanding of genital injury patterns and improving forensic interpretations.

Methods: A monocentric observational study was conducted with 100 healthy women (aged 20–70) who underwent standardized external genital examinations. Data on demographics, medical history, and lifestyle factors were collected via questionnaires. Injuries were categorized as erosions, lacerations, petechiae, and ecchymoses, excluding redness and swelling. Statistical analysis included T-tests.

Results: Genital injuries were observed in 30% of participants, with erosions and petechiae being the most common (12% each). The clitoris (32.4%) and the labia majora and minora (23.5% each) were most frequently affected. Anatomical variations, particularly in the labia minora, were noted.

Conclusion: This study provides valuable insights into the prevalence, distribution, and characteristics of genital injuries occurring in daily life, independent of sexual assault. Exposed structures, such as the clitoris

and labia, are more susceptible to external trauma compared to more anatomically protected areas like the vaginal introitus. By establishing a baseline understanding of genital injuries, this research contributes to the understanding of genital injury patterns and in the future improving forensic assessments, particularly in cases of sexual violence.

### Saturday, the 14th of June 2024 – Scientific Session III (08:30 – 10:00)

### Abstract 12: 08:30 - 08:40

Think forensic, act forensic, feel forensic- Qualitative descriptive recording of forensic nurses' experiences in German- Speaking Switzerland

M. Vetter, Institute of Forensic Medicine, Zurich

This study examines the evolving practice of forensic nursing (FN) in Germanspeaking Switzerland, particularly in light of the implementation of the Istanbul Conventions. The aim of this study was to explore the role of FNs in Germanspeaking Switzerland and the barriers and facilitators they encounter in implementing this new role.

The qualitative descriptive research involved ten expert interviews with FNs. The core findings indicated that FNs main aim is to improve the care for survivors. They have different responsibilities in direct contact with survivors and in the interprofessional team. They feel secure with their responsibilities. However, they face challenges such as insufficient awareness of their role among other professionals and a need for clearer role definitions, especially in emergency departments where most of the FNs are nurses with an additional FN role.

The study also shows that interprofessional collaboration is not yet fully

developed but has a significant impact. The integration of FN into the Swiss healthcare system is influenced by various factors, including political pressure, professional interest, and funding.

This study highlights the need for improved communication, better exchange between FNs and more research about the FNs practice in Switzerland. It suggests that Swiss FNs need to overcome challenges to adapt to international practices and to fully realise the potential of forensic nursing within the national framework.

### Abstract 13: 08:40 – 08:50

3D-Surface Scanning and Photogrammetry at the University Center of Legal Medicine Lausanne-Geneva: a retrospective study over 8 years *G. Gobat, University Center of Legal Medicine, Lausanne* 

During the last decade, 3D surface scanning combined with photogrammetry has become an integral tool in forensic medicine. Compared to other forensic imaging methods, such as computed tomography and magnetic resonance imaging, this method has also been integrated into police practices. This method can be very useful, especially for the reconstruction of traffic accidents, in cases of homicides, and to compare the morphology of lesions with objects that could have been involved in a crime, especially in cases where autopsy findings or clinical examinations cannot provide all the answers.

Since 2016, the University Center of Legal Medicine Lausanne-Geneva (CURML) has been regularly conducting 3D documentations of living and post-mortem cases. The equipment used includes structured light surface scanners (ATOS Compact Scan 5M, GOM, Brauschweig, Germany; Go!Scan50 and Go!Scan20, Creaform Ametek, Lévis, Canada), CT-scanner (64-row LightSpeedVCT & CT750HD, GE Healthcare, Milwaukee, USA), and

photogrammetry software (Agisoft Metashape) for documenting the surface of bodies, internal lesions (principally fractures), and involved objects. In some cases, the 3D data acquired by the police concerning scenes and vehicles were also used. Data analysis and reconstruction were performed with different software (e.g. GOM Inspect, CloudCompare, Autodesk 3ds Max). Between 2016 and 2024, 444 documented cases and more than 50 expert reports have been performed at the request of the public prosecutor. These expert reports mainly included 3D reconstructions of different types of traffic accidents and morphometric comparisons between specific type of lesions, particularly wounds and bruises, and associated objects.

This presentation will show the advantages of the 3D scanning, combined with photogrammetry, for forensic cases through specific examples. Moreover, the limitations and perspectives of this method will be discussed to show how this technique can be effectively integrated into routine forensic practice.

#### Abstract 14: 08:50 – 09:00

## Photogrammetry in Forensic Medicine: Comparing Methods and Exploring Visualization Techniques

C. Lux, Institute of Forensic Medicine, Zurich

Introduction: In forensic medicine, photographic documentation of relevant findings on the body has traditionally relied on 2D overview and close-up images. With the advent of modern 3D measurement techniques and powerful visualization software packages it is now possible to capture and represent findings in a three-dimensional and highly realistic manner. To achieve high-quality 3D reconstructions, both, technical and environmental factors must be taken into account. Building on a previous study that focused on optimizing photogrammetric imaging methods in the autopsy room, a collaborative project

between the Institute of Forensic Medicine Zurich and the Zurich Forensic Science Institute—within the framework of the 3D Center Zurich—shifted focus to lighting setups and various camera and flash systems. The goal was to further refine the methodology for 3D documentation and to evaluate its practical application in forensic contexts.

Materials and Methods: During ten autopsies, different combinations of camera systems (DSLR, mirrorless) and external flash systems (ring flash, studio flashes with reflectors and softboxes) were tested under varying lighting conditions. High-powered flashes (for indirect lighting) were used for full-body documentation, while ring flashes served as a low-budget alternative for capturing specific findings (e.g., head, extremities). A controlled high-frequency image capturing setup, with defined exposure time and activated autofocus, was used to assess the efficiency and level of detail of the imaging processes.

Results: The use of four external studio flashes proved particularly effective for achieving consistent and well-balanced lighting of the body—even under challenging conditions such as shiny skin surfaces or deep skin folds. With this setup, several hundred high-resolution images could be taken within just a few minutes (approx. 1–4 minutes), resulting in high-quality, metrically reliable 3D models. For focused documentation of specific findings (e.g., gunshot wounds to the head), the ring flash offers a simple, cost-effective, and still highly detailed alternative. Visualization options include: (1) two-dimensional static representations of findings, optionally with labels to complement written documentation; (2) animated three-dimensional representations; and (3) detailed morphological assessment of wounds or injuries, made possible by the high resolution of the data, both during initial analysis and in secondary expert reviews.

Outlook: In addition to improving imaging techniques in terms of both image quality and the fast creation of image sequences, this presentation places

particular emphasis on the practical uses of the generated 3D models in forensic settings. Example applications will be demonstrated, including formats used in courtroom presentations and scientific talks—among them immersive visualizations in virtual reality environments.

#### Abstract 15: 09:00 - 09:10

# Pars basilaris size to estimate fetal and young infant age using forensic post mortem CT imaging

W. Schweitzer, Institute of Forensic Medicine, Zurich

Background: estimating the age of a fetus or infant based on its remains can be performed based on the pars basilaris of the occipital bone. Advancements in post-mortem computed tomography (PMCT) imaging have enabled the use of pars basilaris biometry for age estimation through statistically validated regression models based on its maximum length (ML) and maximum width (MW).

Methods and Material: a retrospective case series was examined using all nine (9) fetal and neonate/stillbirth cases in our database that had undergone PMCT scans. Their ages were known or estimated through prior investigations. The pars basilaris was visualized and measured in the PMCT scans. Age predictions with 95% confidence intervals were calculated using published regression equations based on both MW- and ML-derived statistical models. Additionally, a combined model was developed to provide a single age estimate with a corresponding 95% confidence interval.

Results: in all nine cases, the predicted 95% confidence age intervals included the known or previously estimated age. Certain maternal diagnoses, such as alcoholism and diabetes mellitus, correlated with larger- or smaller-thanaverage sizes of the pars basilaris. Outlook: This method is straightforward and can be implemented using standard PMCT protocols. Further studies are required to evaluate its applicability across different populations and forensic contexts.

### Abstract 16: 09:10 - 09:20

## **Designer-benzodiazepine and sodium nitrite intoxication: a case report** *V. Hofmann, Institute of Forensic Medicine, Basel*

Background: In the cours of the autopsy of a 30-year-old male, methemoglobinemia was suspected due to the appearance of the body and its blood. Following the quantification of methemoglobin levels in the blood and the completion of an immunochemical and general unknown screening in the urine, the positive immunochemical result for benzodiazepines could not be confirmed by blood analysis. However, during legal inspection a vial lablelled "Bromazolam" and a minigrip bag marked "NaNO2" were found. These findings provided the initial indication of which substances could have been ingested by the deceased prior to death. The use of a newly developed LC-MS/MS method incorporating a wider range of benzodiazepines facilitated the detection and quantification of bromazolam in blood and the identification of the cause of death.

Methods: A simplified extraction method for blood samples was developed. The procedure was performed by transferring blood-aliquots of 50  $\mu$ L from each case to Micronic® tubes. The samples were extracted with 150  $\mu$ L methanol containing internal standards such as bromazolam-d5. Afterwards, the samples were mixed for 10 minutes and centrifuged for 30 minutes at 4000 rpm. A volume of 5  $\mu$ L supernatant was injected into the LC-MS/MS system (UHPLC: Shimadzu Nexera series, tandem mass spectrometer: Sciex API 6500+ QTrap) operated with positive electrospray ionization in the multiple

reaction monitoring mode.

The chromatography procedure was executed using an Accucore biphenyl column, with water (solvent A) and methanol (solvent B) as mobile phases that were supplemented with 0.1% formic acid. The analysis of methemoglobin was performed at the university hospital in Basel with a Radiometer ABL 90 series.

Results: A definitive cause of death could not be established after a full autopsy, but there was considerable evidence to suggest that the individual had died from intoxication. Forensic toxicological analysis resulted in a methemoglobin level of approximately 56% and a bromazolam blood concentration of approximately 140  $\mu$ g/L. Additionally, citalopram and its active metabolite, desmethylcitalopram, were detected in the deceased individual.

Conclusion: After performing all forensic-toxicological and clinical toxicological analysis the cause of death could be described as a central regulatory failure due to the toxic but not compelling lethal methemoglobin amount in the analyzed blood in combination with the determined bromazolam concentration. As cause of the methemoglobinemia the uptake of sodium nitrite was considered but until now the route of administration is not clear.

## Abstract 17: 09:20 - 09:30

Beyond Immunoassays: Can high-resolution mass spectrometry provide a more effective approach for drug screening?

U. Duthaler, Institute of Forensic Medicine, Basel

Background: Forensic toxicologic assessments often involve screening for drugs of abuse in urine samples, a critical task in both clinical and legal contexts. An effective urine screening method must balance sensitivity, specificity, practicality, cost-effectiveness, and the ability to detect a broad range of both licit and illicit substances. Currently, immunoassays (IA) are the gold standard for initial drug screening due to their widespread use and ease of implementation. However, these methods have limitations, particularly in terms of cross-reactivity and the scope of substances they can detect. Liquid chromatography high-resolution mass spectrometry (LC-HRMS) presents a powerful alternative, offering greater selectivity and, therefore, higher detection confidence. The aim of this study is to evaluate whether LC-HRMS can effectively replace immunoassay-based approaches for the routine screening of drugs in urine samples.

Methods: An easy extraction protocol was developed for LC-HRMS analysis, involving a deconjugation step, followed by methanol extraction and dilution with water, all performed in a 96-well format. The data were processed using a streamlined evaluation template, bypassing in-depth data analysis. The method was validated in accordance with regulatory guidelines, including cross-validation with the established cloned enzyme donor immunoassay (CEDIA) using 252 authentic samples. Additionally, the method comparison was supported by a comprehensive analysis of both marketed and designer benzodiazepines.

Results: The limit of identification (LOI) for 108 compounds, including marketed drugs and common drugs of abuse, was assessed using LC-HRMS. The LOI ranged from 0.5 to 50 µg/L, with a median value of 2.5 µg/L (interquartile range (IQR): 1 - 10 µg/L). The average %RSD at the LOI was 12.5% (n = 7 replicates). For benzodiazepines (n = 33), the LOI determined by the CEDIA immunoassay ranged from 50 to 250 µg/L (with Z-drugs > 1 mg/L), with a median of 75 µg/L (IQR: 50 - 100 µg/L). In contrast, LC-HRMS analysis showed a lower median LOI of 5 µg/L (IQR: 2.5 - 10 µg/L). Analysis of authentic samples revealed almost perfect agreement between the methods for benzodiazepines ( $\kappa = 0.89$ ), cannabinoids ( $\kappa = 0.95$ ), cocaine ( $\kappa = 0.93$ ), opiates ( $\kappa = 0.91$ ), methadone ( $\kappa = 0.92$ ), and ethylglucuronide ( $\kappa$ 

= 0.88). However, there was slight to no agreement for buprenorphine ( $\kappa$  = 0.13), LSD ( $\kappa$  = 0.25), and amphetamines ( $\kappa$  = 0.28). This can be attributed to cross-reactivities affecting the immunoassay results, with LSD assays showing interference from fentanyl and buprenorphine assays cross-reacting with opiates such as codeine and morphine. For amphetamines, endogenous interferences in the urine matrix led to false positive results in LC-HRMS automated analysis of phenylethylamines, such as MDA.

Conclusions: LC-HRMS is a highly reliable screening method that outperforms immunoassays in terms of both sensitivity and specificity. Moreover, the full scan trace allows retrospective identification of unknown compounds without re-extraction or re-analysis. However, the lack of specificity in immunoassays can be beneficial, as it increases the likelihood of detecting structurally related unknown psychoactive substances (e.g. new synthetic opioids).

#### Abstract 18: 09:30 - 09:40

## A Metabolomics Approach to Screen for Endogenous Biomarkers Indicating Sample Integrity of Blood Specimens

A. Bovens, Institute of Forensic Medicine, Zurich

Background and Aims: Some drugs (of abuse) like cocaine are known to undergo rapid degradation in blood particularly at room temperature (RT) which can influence toxicological interpretation. Thus, proper sample handling and storage are crucial to ensure reliable (quantitative) results. However, storage conditions (e.g., time at RT) until samples arrive in the toxicological laboratory are usually not known. This study aimed to screen for endogenous surrogate markers which correlate to the degradation of drugs (of abuse), using cocaine as a model. Such a stability marker could improve the interpretation of forensic toxicology results by providing information about sample integrity and post-collection handling (e.g. prolonged storage at RT).

Methods: Whole blood from healthy volunteers (n=5) was collected in fluoridestabilized tubes. Aliquots of all blood samples were divided into two sets; one set was spiked to have a cocaine blood concentration of 640 ng/mL, while the other set served as a control group. All aliguots were stored at RT and protein precipitation was performed after 0 h, 6 h, 12 h, 24 h, 2 d, 3 d, 9 d and 14 d, followed by storage at -80° C before measurement with the Impact II TargetScreener® QTOF from Bruker with ESI+ in data dependent acquisition mode. Cocaine concentration changes over time were quantified using Bruker's TASQ® software against an external eight-point calibration curve. Untargeted data analysis for endogenous features (unknown substance with a distinct retention time and mass combination) was done with MSDIAL (version 4.9). In R (version 4.4.1), probabilistic quotient normalization was used and features showing a linear behavior  $\leq 0.6$  across diluted pool samples were excluded. Spearman correlations between cocaine degradation and individual feature behavior over time were calculated. For feature identification, SIRIUS (version 6.1.0) was used against the bio database which combines several major databases like COCONUT, HMDB and PubChem.

Results: 79 features (with a spearman correlation  $\geq 0.5$  to cocaine degradation) showed an identification confidence score  $\geq 0.5$  in SIRIUS. So far, arginine ( $\rho$ =0.50), glutathione ( $\rho$ =0.98) and inosine ( $\rho$ =0.69) could be identified using a reference standard (matching retention times, precursor mass accuracy  $\leq$ 10 ppm, two MS/MS fragments with a ppm error  $\leq$  20).

Conclusion and Outlook: An endogenous surrogate marker for blood sample integrity could improve the interpretation of (quantitative) results in forensic toxicology. Using a metabolomics approach, arginine, glutathione and inosine could be identified as first, potentially promising markers, showing similar degradation behavior to cocaine at RT. These potential biomarkers for sample integrity for forensic toxicological routine applications, need to be further ANNUAL MEETING 2025

validated in a larger sample cohort to level out expected endogenous interindividual variability. Also, their behavior and reproducibility under other whole blood stabilizing agents and varied storage temperatures need to be tested.

### Abstract 19: 09:40 - 09:50

# Metabolic Fate of $\Delta 8$ -, $\Delta 9$ -, and $\Delta 10$ -THC: The Case of the Missing Carboxylic Acid

M. C. Monti, Division of Clinical Chemistry and Pharmacology, Linköping, Sweden; Institute of Forensic Medicine, Basel

Background and Aim: With the emergence of semi-synthetic cannabinoids (SSCs), analogues and isomers of  $\Delta$ 9-tetrahydrocannabinol ( $\Delta$ 9-THC) made an appearance on the recreational drug market.  $\Delta$ 8-THC was amongst the earliest SSCs to emerge. Due to its close structural resemblance to  $\Delta$ 9-THC,  $\Delta$ 8-THC poses various analytical challenges; Forensic toxicological laboratories are advised to check and adapt their analytical methods to prevent misclassification of  $\Delta$ 8-THC and its metabolites as  $\Delta$ 9-THC. Sporadic reports from the US imply that also the isomer  $\Delta$ 10-THC is sold as a new alternative. To decipher the metabolism of these new SSCs, we investigated the in vitro metabolism of  $\Delta$ 8-THC and  $\Delta$ 10-THC in comparison to  $\Delta$ 9-THC using human hepatocytes.

Materials and Methods:  $\Delta 8$ -,  $\Delta 9$ -, and  $\Delta 10$ -THC were incubated with human hepatocytes for 1 h and 3 h. The samples were measured using high-performance liquid chromatography coupled to time-of-flight mass spectrometry (HPLC-QToF; Agilent Technologies). Metabolites were identified using an in-house database and fragmentation patterns were investigated for structure elucidation. For  $\Delta 8$ - and  $\Delta 9$ -THC the in vitro generated metabolites were additionally compared to metabolites detected in authentic casework urine samples from Sweden.

Results and Discussion: Eight metabolites were identified for  $\Delta$ 8-THC with  $\Delta$ 8-THC-COOH being the major metabolite, which is in close agreement with  $\Delta$ 9-THC's well-established metabolism. The major metabolites were also detected in the authentic urine samples (in vivo), confirming these results. Interestingly,  $\Delta$ 10-THC metabolized considerably different compared to its isomers  $\Delta$ 8-THC and  $\Delta$ 9-THC, as the carboxylic acid was only detected in trace amounts. The main metabolite for  $\Delta$ 10-THC resulted from dehydration paired with monohydroxylation at the benzopyran ring (positions 7 – 10). Assessment of the reactive centers for  $\Delta$ 10-THC imply that whilst the methyl group at the 9 position is prone for metabolism for  $\Delta$ 8-THC and  $\Delta$ 9-THC, this site is sterically unfavorable for  $\Delta$ 10-THC. For  $\Delta$ 10-THC, it is proposed that in an initial reaction the reactive tertiary carbon at position 9 is oxidized, followed by conjugation and water loss. This intermediate metabolite is then oxidized again, leading to the observed main metabolite.

Conclusion: The structural similarity of  $\Delta 8$ -,  $\Delta 9$ -, and  $\Delta 10$ -THC might suggest comparable metabolic pathways; however, this study showed that the distinct steric properties of  $\Delta 10$ -THC favor a different pathway and that  $\Delta 10$ -THC-COOH is therefore not recommended as the target analyte.

#### Abstract 20: 09:50 - 10:00

# Dose-dependent increase of Phosphatidylethanol concentration and its detection window in whole blood

M. Bantle, Institute of Forensic Medicine, Bern

Aims: Phosphatidylethanol (PEth) can be used to detect alcohol consumption and is also increasingly being used in different applications such as driving aptitude assessment or abstinence control. However, dose-dependent characteristics of PEth are still not fully known. This overview shall summarize the findings of three studies regarding the minimal amount of alcohol needed to reach a quantifiable PEth concentration, the effect of repeated intake of low doses of alcohol on PEth, and the elimination of PEth after chronic excessive alcohol consumption.

Methods: In the first study, participants were recruited at the University of Freiburg (Germany). After an abstinence period of four weeks, the participants administered alcohol to reach a blood alcohol concentration of 0.6 g/kg or 0.75 g/kg. Blood was collected as dried blood spot samples on each day of drinking as well as on the following three days. In a pilot study (second study), a healthy volunteer consumed moderate amounts of alcohol and recorded the amount of alcohol consumed over three months. Blood samples were collected as dried blood spots on multiple time points. In the third study, PEth concentrations from 49 patients undergoing treatment for alcohol use disorder were monitored over time. The samples were analysed with a validated method by LC-MS/MS.

Results and Discussion: After the abstinence period, all participants of the first study had initial PEth concentrations below 20 ng/mL. In the first drinking trial (0.6 g/kg), PEth concentrations in nine out of twelve participants reached a maximum of more than 20 ng/mL with a maximum concentration of 28.8 ng/mL, whereas in the second trial (0.75 g/kg), PEth concentrations in seven out of twelve participants reached more than 20 ng/mL with a maximum of 44.9 ng/mL. Most of the maximum concentrations (19/24) were achieved on the day of drinking. PEth concentrations rapidly decreased afterwards leading to values < 20 ng/mL within one to three days in most of the samples (21/24). In the second study, the maximum daily amount of ethanol consumed was 38 g. The PEth concentrations ranged from < LOQ (10 ng/mL) to 22.5 ng/mL. In the third study, initial PEth concentrations ranged from < 20 to > 2500 ng/mL. Depending on the initial concentration, the duration until the PEth

concentrations fell below 20 ng/mL ranged from 1-2 weeks to more than six weeks.

Conclusion: Formation of PEth with concentrations exceeding the quantification limit of 10 ng/mL was observed after the ingestion of a single dose of alcohol leading to blood alcohol concentrations of 0.47 and 0.68 g/kg. Repeated consumption of low or moderate doses of alcohol lead to formation of PEth between 10 and 20 ng/mL. For individuals with chronic excessive consumption habits, an abstinence period of four weeks might not be sufficient to reach PEth concentrations below the cut-off concentration of 20 ng/mL. These findings make PEth a promising marker to monitor controlled moderate consumption of alcohol as well as for abstinence control, with limitations regarding the short detection window after single drinking events.

Key words: Phosphatidylethanol, dried blood spots, alcohol consumption, abstinence monitoring.

## Saturday, the 14th of June 2024 – Scientific Session IV (10:20 – 12:10)

#### Abstract 21: 10:20 – 10:30

Ethyl glucuronide (EtG) in blood is applicable for abstinence control *W. Weinmann, Institute of Forensic Medicine, Bern* 

Ethyl glucuronide (EtG) is considered a sensitive marker for the detection of alcohol consumption. In contrast to ethanol itself, which is only detectable in the body for a few hours, EtG remains detectable in the blood/serum for much longer. EtG in blood" has not yet been used for abstinence control in Switzerland, although EtG in blood would certainly be suitable for this purpose. The prerequisite for EtG being detectable in blood for even longer time than in urine - namely 1 to 4 days - is the lowering of the analytical limit of

quantification for EtG detection in blood. Neumann et al. already achieved this in 2020. A comparison of different alcohol consumption markers in clinical samples can be found in Neumann et al. 2021.

We adapted the published method in 2022 and were able to use it reproducibly for EtG detection from dried-blood spots with a detection limit of 5 ng/mL EtG in blood using a QTrap 5500 LC-MS/MS. Using a more sensitive mass spectrometer, this detection limit can be further reduced so that a detection limit of 1 ng/mL can be achieved, as in Neumann et al.

Based on the data collected by Neumann et al., it can already be concluded that 'EtG in blood' can therefore be used for abstinence monitoring, provided that explorants are called in promptly (24-48 h) before the sample is taken. Such a short appointment period will also be mandatory for phosphatidylethanol (PEth) for abstinence testing in the new edition of the 'Assessment Criteria' for Germany. The introduction of EtG in blood could eliminate the need for manipulable urine samples. This would provide another complementary method to EtG hair analysis if, for example, hair is not available.

### Abstract 22: 10:30 - 10:40

# Driver Sleepiness Detection in Biofluids via Metabolic Signatures and Machine Learning

M. Scholz, Institute of Forensic Medicine, Zurich

Drowsy driving causes more fatalities than drunk driving. However, due to the lack of specific biomarkers, police and forensic experts must rely on selfreports when assessing an operator's ability to drive. To remedy this situation, we aimed to identify sleepiness in biofluids via changes in metabolism. In a randomised clinical trial, we quantified the effects of sleep restriction

(SR) and sleep deprivation (SD) on metabolism, performance, and subjective

perception, compared to controlled sleep. 20 participants performed goldstandard tests for vigilance and attention (Psychomotor Vigilance Test, d2-Test of Attention), subjective sleepiness (Karolinska & Stanford Sleepiness Scales), and a state-of-the-art driving simulation task at regular intervals. Oral fluid specimens were repeatedly collected to monitor sleepiness effects on the metabolism. These biological samples were analysed via liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) in an untargeted metabolomics approach. The resulting table of molecular features was used as input for machine learning classification algorithms.

Both interventions (SR and SD) resulted in a significant decrease in psychomotor vigilance (reaction time, lapses), attention and concentration (processing speed, rule compliance), and driving performance (lane keeping, microsleep episodes), despite limited subjective perception. The negative effects were often more pronounced after sleep deprivation. The LC-HRMS analyses revealed a unique metabolic signature of sleep deprivation and, to a lesser extent, sleep restriction. With the use of predictive machine learning models, sleep-deprived individuals could be identified precisely (F0.5 scores 0.87-0.90) based on 10 to 12 biological markers (i.e., metabolic features). Conveniently, these methods do not require a reference sample.

This study revealed a metabolic signature of sleep loss in oral fluid under realistic conditions. The result holds promising potential for future applications in forensic roadside driver testing.

# Abstract 23: 10:40 – 10:50 Loss of control at the steering wheel: An uncommon cause *C. Lanz, Solothurner Spitäler, Solothurn*

A 69 year old man lost control of his car while driving home. He found himself

in the yard of a house in the immediate neighbourhood to his address. He had no apparent explanation for this loss of consciousness. He told the first medical examiner that he was reaching for the command of the garage door. In his past medical history a Sleep Apnea Disorder was noted, he was under CPAP therapy. With the exception of Rosuvastatin, the patient was not taking any medication. On the day in question, he was returning from a day repairing his boat and had been driving for one hour. He claimed to have no sleep deprivation. The physical exam produced results within normal limits. The patient got 12/18 points in the DemTect exam; he completed the Trail A test within 52 seconds. He made three errors in the Trail B test while completing it within 120 seconds.

He subsequently underwent an MRI Scan of his brain. This exam revealed subcortical micro-hemorrhages in both hemispheres, however more on the right side. Focal hemosiderin deposits were observed in the occipital region. The suspected diagnosis was cerebral amyloid angiopathy. No haemorrhagic or ischemic lesions were noted.

A comprehensive neuropsychological evaluation resulted in the diagnosis of a mild cognitive impairment with deficits in all tested domains (executive functions, attention, visual reconstruction, mnestic functions.)

Cerebral Amyloid Angiopathy CAA is characterised by amyloid deposits in the small and medium calibre arteries of the brain without presence of systemic amyloidosis. The clinical manifestation of CAA may be that of progressive dementia or of recurrent cerebral haemorrhages.

In the present case, the loss of control of his car was most probably explained by a seizures due to hemosiderin deposits in the brain. The fitness to drive was denied on this ground and due to the neuropsychological deficits.

#### Abstract 24: 10:50 – 11:00

Towards correlating levels of ADHD-medication and its metabolites in blood with those in non-invasive samples and with driving performance during monotonous driving task – preliminary data from a lisdexamfetamine treated volunteer

S. Lakämper, Institute for Forensic Medicine, Zurich

Untreated attention deficit hyperactivity disorder (ADHD) leads to an increased risk of road accidents due to loss of attention and higher distractibility. This risk can be mitigated by stimulant treatment, as could be shown indirectly in driving simulation studies. However, affected drivers might want to take socalled drug holidays once in a while: that is, they interrupt their medication, for example, over the weekend. The effect of such short-term treatment interruptions on driving performance has not been studied yet. Also, the driving performance has not yet been directly linked to measured medication levels in blood. We will address both problems in a cross-over randomized controlled trial (RCT) starting to recruit on May 1st, 2025. This study will include non-invasive specimens suitable for roadside testing, such as saliva, dried blood spots, and exhaled breath samples. As a first step, we want to explore whether concentrations of ADHD drugs or their metabolites in blood correlate with those in dried blood spots, saliva, and exhaled breath. For this - and to potentially correlate stimulant blood concentrations directly with driving performance for the first time – we obtained samples from an ADHD patient treated with lisdexamfetamine during a full day. Performance during a 40-minute monotonous nighttime scenario was acquired twice (morning and late afternoon) to test the ability to maintain focus during driving. During driving eye-tracking parameters and single-channel EEG recordings were additionally obtained. We will present preliminary analyses and results.

### Abstract 25: 11:00 – 11:10

# Assessing the influence of CYP2C9 and CYP2C19 genotypes on the metabolism of CBD-cannabis in DUID cases

J. Schulte, Institute of Forensic Medicine, Basel

Interest has been steadily increasing in pharmacogenetic analysis and its implications for both optimal drug dosing and minimizing dose-related adverse drug reactions. Recent publications indicate that its application in the forensic field could serve as a supportive tool for the comprehensive interpretation of toxicological findings and reporting, which so far has been performed without consideration of genetic factors. Investigating biological characteristics, such as an individual's genotype for drug-metabolizing enzymes or transporters, may help explain interindividual variations in pharmacokinetics and dynamics. This information can be relevant in both post-mortem analysis and driving under the influence of drugs (DUID) cases. In parallel, the availability of cannabis for both recreational and medical purposes has increased substantially, making it the most widely used recreational drug globally and the most frequently detected substance in road traffic control samples. In this context, genetic variations in genes encoding for polymorphic cannabinoid-metabolizing enzymes are of high relevance. However, these factors are still poorly studied, and their effects on plasma concentrations of tetrahydrocannabinol (THC), cannabidiol (CBD), and their metabolites remain unclear.

In 2019, the Institute of Forensic Medicine Basel conducted a prospective, placebo-controlled, double-blinded, randomized crossover study to evaluate cannabinoid concentrations in blood and driving-related impairment, after vaporization of cannabis high in CBD and containing less than 1% THC, thus being legally available. Participants were healthy volunteers who were between 18 and 65 years old, without psychiatric or physical diseases, in possession of a valid driver's license, and experienced in smoking (tobacco

and/or cannabis). The follow-up study presented here focused on the potential impact of genotype-predicted phenotypes of CYP2C9 and CYP2C19 on pharmacokinetics -and dynamics of cannabinoids after the intake of CBDcannabis. DNA was extracted from heparin-blood samples (n = 30) and analyzed by allele specific real-time PCR to detect function-impairing variants in CYP2C9 and CYP2C19. The analysis revealed that 40% of participants carried function-impairing alleles in CYP2C9 and about 57% in CYP2C19. Subsequently, genetic and demographic data were correlated and statistically analyzed alongside blood concentrations of THC, CBD, and their metabolites. In conclusion, the study provides insights into the correlation of CYP2C9 and CYP2C19 gene variations and cannabinoid plasma concentrations in DUID cases. However, to confirm that genetic variability contributes to interindividual differences in cannabinoid blood levels, a larger controlled study is needed. To this end, we plan to conduct a clinical trial with inhaled THC-rich cannabis products, focusing on time-related detectability across different consumption patterns to determine the role of function-impairing genotypes in forensic assessments.

### Abstract 26: 11:10 – 11:20

# From wild to tame: The challenge(s) of differentiating dogs and wolves *N. V. Morf, Institute of Forensic Medicine, Zurich*

Currently there are over 35 wolf packs (231 identified individuals) living in Switzerland, and these have been responsible for an estimated 1000 livestock deaths (mainly sheep) in 2024. Affected farmers are generally compensated for their loss by the government. To receive such compensation, it must be proven that the livestock died as a result of a wolf. However, it is not only wolves that can cause such harm to livestock; also unsupervised dogs can attack and kill livestock, in which case the dog owners are responsible for the compensation. Hence, the first step after an attack is to identify the actual attacker. If a dog was responsible for such an attack, it is very important that the police act in a timely manner; for instance, to find witnesses of the attack or other crucial evidence. To aid in such investigations, we evaluated several genetic markers for their ability to differentiate between wolves and dogs, with the aim to offer a fast but nevertheless accurate method to support law enforcement. We tested 13 short tandem repeats (STRs), eleven single nucleotide polymorphisms (SNPs) and one gene, which shows copy number variations (CNVs) between dogs and wolves. In order to use STR or SNP markers to assign an individual to either the dog or wolf population, it is important to have a reference database containing genetic data from individuals of both subspecies. Establishing such reference databases is challenging, especially when a CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) protected subspecies like the wolf is involved. Once such a database is established, statistical programs can be used to assign an individual to a specific population, based on allele frequencies of the analyzed STR or SNP markers. In contrast, the approach with the gene exhibiting CNV, the AMY2B gene, does not need such a database, but unfortunately our results show reduced specificity when compared to the performance of the STR or SNP methods. The advantages of SNP over STR markers lie in their amplicon length, which is generally shorter and therefore facilitates the amplification of possibly degraded DNA. Despite their longer amplicons, STR markers offer the possibility to identify subspecies and individual at the same time. The decision to use SNPs or STRs or even a combination of both is dependent on the quality of the sample and if the identification of a specific individual is crucial. Either way canine STR and SNP markers are a promising and vital tool to support law enforcement.

### Abstract 27: 11:20 – 11:30

# DNA typing of trees for forensic use: tracing origins based on population clustering and individual matching

S. Riverendo, Universität Bern

Forensic botany offers promising tools to support criminal investigations. This study investigates the potential of genetic fingerprinting in trees using two complementary approaches: one focusing on population-level clustering, the other on individual-level matching. Analyses were conducted on Betula spp. (birch) and Quercus spp. (oak).

The population clustering approach included 79 B. pendula samples from Switzerland and three other European countries, as well as 78 Quercus spp. samples from various Swiss regions. Despite broad geographic sampling, no significant genetic structure was detected between populations by microsatellite markers, limiting the ability to infer sample origin based on clustering alone.

In contrast, the individual matching approach, applied to 373 Betula spp. trees in the city of Biel, allowed for the identification of single trees. Both B. pendula and B. pubescens were genotyped and mapped in a searchable QGIS-integrated database. Single leaves could be matched to specific trees with high accuracy, supported by low random match probabilities. A separate temporal analysis of the urban tree population revealed subtle changes in genetic diversity over time.

When tree leaves are secured as forensic evidence, the potential for the identification of individual trees is high when the crime scene is known. However, tracing the geographic origin of plant material without additional case-related context remains a challenge.

#### Abstract 28: 11:30 - 11:40

# Telomere length in blood and saliva cells: a parameter to investigate the individual age

M. Bottinelli, Forensic Genetics, Lugano

Genomic DNA is under constant attack from both, endogenous and exogenous, damaging agents. In 1986 Cooke and Smith associated telomeres directly to aging by noticing that the length of telomere repeats, capping sex chromosomes in sperm cells, was longer than in adult sperm. In humans, telomeres consist of thousands of repetitive DNA non-coding sequences, 5'-TTAGGG-3', that cap both ends of linear chromosomes. Telomeres shorten with age and with each round of mitosis because of the inability of the DNA replication machinery to read and copy up to the ends of linear chromosomes. In this perspective, telomeres act as an expendable DNA to prevent the loss of coding DNA. When telomere lengths drop below a certain threshold, the cell decreases the proliferative potential and may undergo cellular senescence. The rate of telomere shortening can be increased by oxidative stress and other epigenetic factors. Telomere length is maintained by telomerase activity, a reverse transcriptase enzyme that adds telomeric repeats de novo after each cell division discovered in 1984 by Greider and Blackburn, however the expression of telomerase is strictly controlled and practically non expressed in fibroblasts, a cell type that could be used to correlate with human ageing.

In our study we performed telomeres length in both blood and saliva human samples and compared them with human chronologic age. Telomeric sequences were PCR-amplified along with a single copy gene and the ratio of these two gene expressed as function of age.

Results show that telomere length can be used as a complementary tool to investigate the age of an individual based on a simple mouth smear. Additional tests are needed to optimize the correlation in terms of accuracy. Furthermore, this test could be compared to other pre-existing methods for biological age determination like mouth X-ray analysis.

#### Abstract 29: 11:40 – 11:50

# Performance testing of four commercially available adhesive films and stamps on different textile surfaces for touch DNA collection

S. Egger, Institute of Forensic Medicine, Basel

A considerable portion of the evidence collected today comprises skin contact traces, known as touch DNA, which are transferred from individuals to other objects or subjects via physical contact. Collecting touch DNA at a crime scene is widely recognized as difficult due to its in-visible nature and small initial quantities. Depending on the type of substrate and its properties (e.g., texture), various methods and techniques are available, with contact traces on textiles are often secured by adhesive films and stamps. However, performance studies on adhesive collection tools are rare. Thus, this study aimed to investigate the applicability of commercially available and frequently used adhesive tapes and stamps on common textile surfaces for touch DNA collection.

Touch DNA was applied by a healthy volunteer with good DNA shedding properties on three distinct textiles (cotton shirt, jeans, and fleece) in two independent rounds and with ten repli-cates each (n = 240). DNA collection was performed with four adhesive films and stamps: (1) DNA Tape (Coloprint, Hilden, Germany), (2) Scene Safe Fast<sup>™</sup> Minitapes (Voigtländer, Blum-berg, Germany), (3) Force-Forex Adhesive Stamp (Helling GmbH, Heidgraben, Germany), and (4) DNA Stub Recording Stamp (Coloprint, Hilden, Germany). As both tapes protruded above the liquid level of 400 µL in the tube prior to DNA extraction, protocol adjustments were made regarding the volume of

lysis buffer to 500  $\mu$ L (n = 60) and 600  $\mu$ L (n = 60), respectively.

The performance of the adhesive tapes and stamps will be presented based on the results of DNA quantity and STR profile quality. In addition, the respective adhesive tape and stamp handling in terms of collection and lab-based processing (e.g., extraction) will be discussed, with the overall intention to provide a recommendation for tape usage that may benefit police and forensic genetic laboratories

### Abstract 30: 11:50 – 12:00

# Clustering of DEPArray<sup>™</sup>-derived single-cell data to generate consensus DNA profiles for autosomal haplotypes

J. Berger, Institute of Forensic Medicine, Basel

Forensic single-cell analysis is crucial for resolving complex DNA mixtures, especially when a suspect's minor DNA contribution is masked by multiple contributors. By isolating specific cell types prior to DNA analysis, it becomes possible to deconvolute (complex) heterogenous and homogeneous mixtures and link individual single-cell electropherograms (scEPGs) to particular persons of interest (POIs). However, interpreting scEPGs remains challenging due to the limited amount of DNA and associated stochastic effects such as peak imbalance, allele drop-in or dropout. Clustering multiple partial scEPGs from the same donor by using (dis-)similarities in allele information and combining them into a consensus profile can reveal consistent genetic signals. In contrast to diploid cells, sperm cells possess only one allele per locus, with individual scEPGs from the same donor exhibiting a high number of various allele combinations due to random meiotic recombination, necessitating an adapted clustering approach. This study aimed to group scEPGs from diploid and haploid cells, leading to more reliable and comprehensive donor profiles

while assessing the number of contributors, without the need of reference profiles.

We previously developed a unique and easy-to-use scoring-based algorithm for clustering scEPGs from diploid cells, which groups them with high accuracy. To extend the clustering approach to challenging haploid sperm cells, we employed a graph theory-based approach that allows accounting for the inherent full range haplotypes of one POI: from complete identical to entirely different haplotypes. For this, each sperm cell is represented as a point (node), while they are connected with lines (edges) if they share common genetic features (alleles). Hence, allele-sharing relationships of neighboring nodes are repre-sented by a set of edges, while non-neighboring nodes can be genetically diverse but still linked within the set through the neighbored nodes. Therefore, the network structure enables the linkage of scEPGs originating from a POI, by building bridges between nodes, even when they share only a few or even no alleles. The graph theory-based approach helps group the cells into clusters, by applying a clustering algorithm directly on the resulting graph structure.

We used empirical data from five donors, recovered using the DEPArrayTM technology, and generated artificial data representing 1000 donors based on the observed empirical parameters (e.g., dropout rate). Best to our knowledge, the developed method comprises a completely new approach to tackle scEPG clustering of haploid data, while being developed on simulated datasets and validated on both artificial and real data. Performance was evaluated in terms of profile completeness of combined profiles and the accuracy of reconstructing contributor genotypes in mixture samples with unknown composition.

### Abstract 31: 12:00 - 12:10

## The Potential of the ForenSeq Kintelligence Kit SNPs for Biogeographical Ancestry Inference

P. Resutik, Institute of Forensic Medicine, Zurich

The resolution of the "Golden State Killer" case has powerfully demonstrated the potential of forensic investigative genetic genealogy (FIGG). FIGG is particularly valuable in serious criminal cases where no matches are found in national forensic DNA databases. In such cases, investigators turn to publicly accessible genealogical DNA databases containing millions of genetic profiles and markers. By reconstructing familial relationships, these databases can provide investigative leads into the identity of unknown individuals who left DNA at crime scenes.

In response to these developments, QIAGEN developed the ForenSeq Kintelligence Kit, which targets approximately 10,000 carefully selected SNPs. This kit is specifically optimized for forensic casework—especially for samples with highly degraded or limited quantities of DNA. The selected markers ensure compatibility with GEDmatch PRO, a genealogical DNA database designed specifically for forensic use. While FIGG is already widely used in the United States, it currently lacks a legal framework in Switzerland. In this study, we investigate whether Kintelligence profiles can also be used for biogeographical ancestry (BGA) inference. Our analysis is based on a comprehensive reference dataset comprising genotype and ancestry information from 3,957 individuals, which we compiled in a previous study. We assess the accuracy of Kintelligence-based biogeographical ancestry inference against self-reported ancestry. In addition, we compare its predictions to those of the VISAGE Basic Tool (115 SNP markers), the current standard for BGA inference in our laboratory.



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