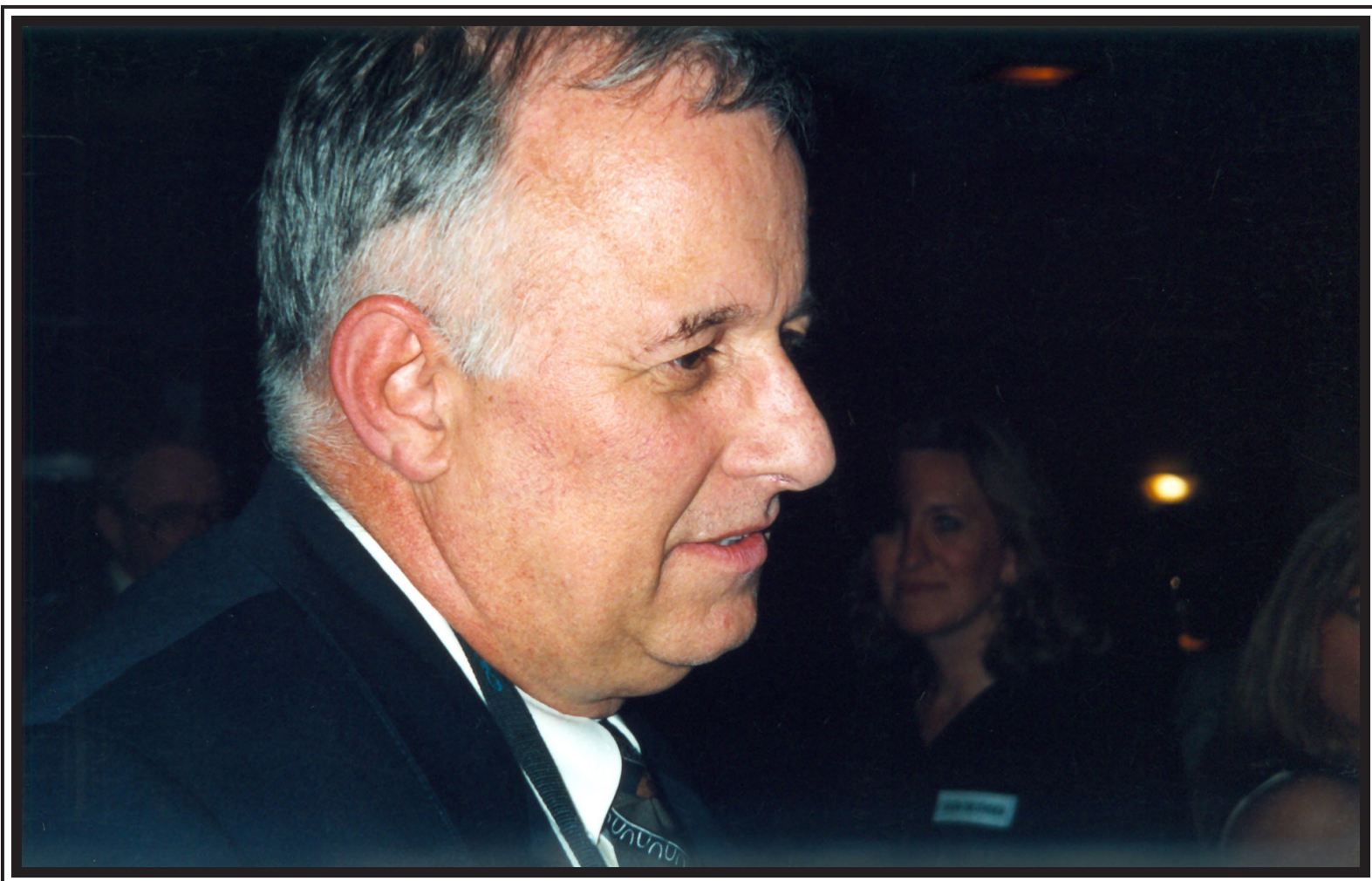


The **CACNews**

News of the California Association of Criminalists • First Quarter 2021



Frederic Andre Tulleners
1940—2020

The President's Desk

jamie
Lajoie



CAC President

**Great job everyone
so far that has
contributed to our
online presence.
I really can't
thank you enough
for helping the
organization to
move forward in
this medium.**

Positive Affirmations

Here we are again. The light at the end of the tunnel seems to be zooming out away from us. I know that seems bleak, but I think there are some things I have learned from this extended period.

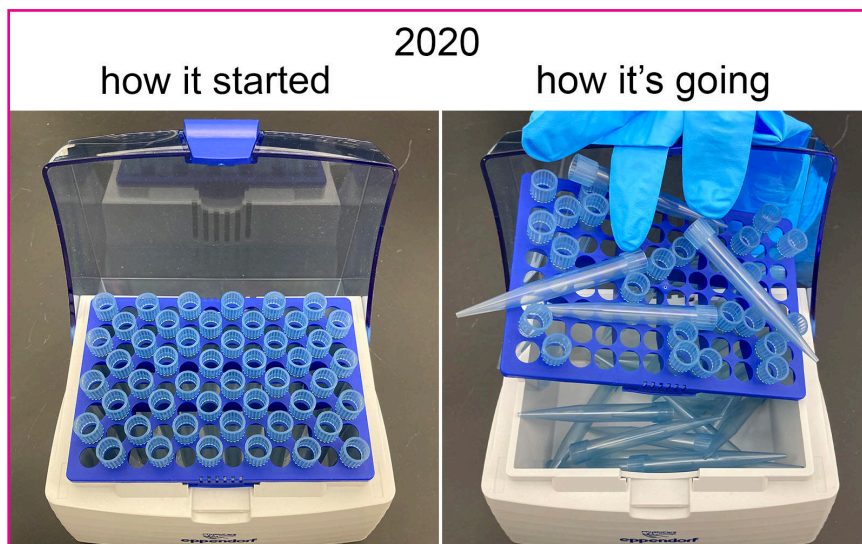
PROFESSIONALLY

Our comfort zone has been officially challenged and so we keep stepping up. Prior to this pandemic, many of us did not have much experience with meetings conducted in a virtual setting unlike other industries or perhaps the private sector. Due to this, the mechanics of putting on virtual workshops and presentations has had a steep learning curve. Most of us have fine-tuned in-person presentation personas but we are still mastering our online personalities. Trying to convey yourself as a good speaker through the online media is a new animal. Prior to this we never had to worry about positioning the height of your camera, making sure your audio is good, your internet service is strong, and that your ring light is charged; New and interesting challenges.

On the organization side there have also been new skills to learn including linking everyone to the workshops and multiple study groups, checking people in electronically, keeping an eye on the chat box, making sure people stay muted, and verify participants participation. Thankfully we have some awesome CAC webmasters; Steve, Bonnie, and Regina. These three are the unsung heroes of our organization this year. Thank you for all you do.

As for all the presenters, the courage to be recorded should be acknowledged. I hated reviewing the business meeting video where I had to watch the recording of myself. I couldn't stop thinking, "I might need to get a blow out and a better ring light for the next business meeting." So to all those presenters who have allowed us to record you so members will have the opportunity to review the presentations later, we say "Thank you". But, who knows, maybe this will inspire someone to be a CSI Instagram influencer, a toxicologist podcaster, a trace YouTuber, a firearms Snapchatter, a drug analyst twitterer, DNA Facebook Live streamer or blood alcohol TikTokker. And if you do become inspired to be a forensic social media influencer, please let me know! Great job everyone so far that has contributed to our online presence. I really can't thank you enough for helping the organization to move forward in this medium.

cont'd



FIRST QUARTER 2021

The CACNews

www.cacnews.org



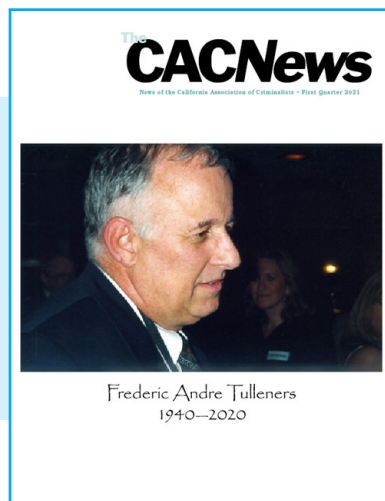
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On the cover:
*We remember Fred
Tulleners...*

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Submissions should be made in the form of Windows compatible files on CD or by e-mail. Alternatively, text files may be saved as plain ASCII files without formatting codes, e.g. bold, italic, etc. Graphics, sketches, photographs, etc. may also be placed into articles. Please contact the editorial secretary for details.

The deadlines for submissions are: December 1, March 1, June 1 and September 1.

INSIDE

CACBits.....	4
Editor's Desk: "Opportunities" <i>Jonathan Charron</i>	5
Remembering Fred Tulleners.....	6
The recent CAC Awards.....	10
Investigating the Effects of Temperature on Ethanol Concentrations in Preserved and Unpreserved Blood Ethanol Samples <i>Kristine E. Myhre and Allyson S. Avina</i>	12
Abstracts from the December DNA Virtual Workshop	17

The President's Desk

PERSONALLY

Believe it or not, I used to be quite athletic and had played sports most my life. One thing a sport can teach you is that the game is often a lot more mental than physical. Yes, you can be physically talented but if you don't have the mental toughness to push through being tired, sore, or being behind you can easily give up. We are all in for a long haul with this pandemic and with this our mental toughness is being tested. So, let's remember to breath, say some positive affirmations, and find a good place to let out those screams.

In fact, let us take a moment right now to say some positive affirmations together?

"Hold up your head! You were not made for failure, you were made for victory!"—Anne Gilchrist

"Failure is just another way to learn how to do something right!"

—Marian Wright Edelman

"You must do the things you think you cannot do."—Eleanor Roosevelt

"We must accept finite disappointment, but never lose infinite hope."

—Martin Luther King Jr.

"My ability to conquer my challenges is limitless; my potential to succeed is infinite."—Dr. Carmen Harra

"I am a good criminalist and I can do this job!"

—Unknown author (This one I use quite often!)

Now take that deep breath! And repeat these or any other positive affirmation when the stress starts to build up. Don't be like me and let yourself be caught off guard at work or at home not realizing your stress level was at a boiling point. It is easy for the pressures and stresses to sneak up on you so remember to give yourself some time to re-set.

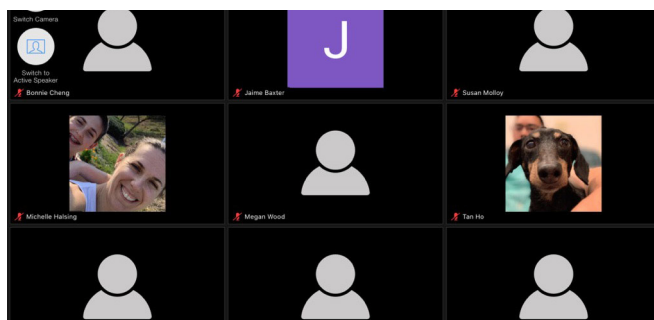
Stay safe and stay sane.




Code of Conduct for CAC Virtual Study Groups & Workshops

Due to some recent activities the CAC Board would like to reiterate and further define some code of conduct for our virtual meetings.

Registration is required to attend the meeting, to receive a Certificate of Attendance, and to receive the Zoom meeting ID code and password. Do not share the code and password. An email with instructions will be sent prior to the meeting



date. When joining the Zoom meetings you must use a real first and last name for CAC to verify your attendance. If your attendance can not be verified, you may be removed from the virtual meeting.

The virtual business meetings, study groups, and workshops that are presented on Zoom and archived on the CAC member website are considered the property of the CAC. These material are being provided on a need-to-know basis only and unauthorized distribution of its contents is strictly prohibited. The following actions, including but not limited to, viewing by individuals without a valid CAC registration, screen shot captures, recording of presentations, and the printing of any screens, are expressly forbidden and not authorized without the prior written approval of the CAC.

We highly encourage you to test joining the meeting in advance with the computer you intend on using. If you get a message saying the meeting has not yet started, you'll be good to go. Please contact the Webmasters if you have any technical difficulties. Thank you!

Please note: At this time, the CAC does not have a way to give on-demand video access to non-members. Access to any workshop or study group taken by a non-CAC member will be available in real-time only.

We encourage anyone that would like access to the recorded study groups and/or workshops to apply for membership. Not all study groups and/or workshops will be recorded. Recording will be done on the basis of instructor and/or presenters' consent. All participants will be warned before a study group and/or workshop begins on whether a recording will be done.

Wanted: CAC Webmaster

The CAC is looking for another webmaster to join our current team! The purpose of the CAC webmaster positions is to develop and maintain the CAC website www.cacnews.org. This position encompasses many tasks including identifying and securing hosting service, server maintenance, updating content, maintaining the membership database, fixing broken links and images, adding page animations, new pictures, new events, and event registration.

If the CAC Board or Publications Committee chair desire to make changes to the website, the webmasters work to implement those required changes. For a detailed description of the many tasks the webmasters handle, visit the committee manual at cacnews.org.

A good base knowledge of computers is desired along with a minimum of a two year commitment. Training for specific tasks can be provided. Please email the editorial secretary at editor@cacnews.org or the president at president@cacnews.org if you are interested in serving!

jonathan
Charron



CAC Editorial Secretary

**As I look back at my
calendar of events
for this past year, I
saw many trips lined
out next to a sad
face emoji.**

Opportunities

Another year is ending, and I know I am not alone in being happy about saying goodbye to 2020. There has been constantly changing restrictions, mandates, and challenges we have all had to adapt to in both our personal lives as well as how we conduct business. Many of us at this point have known a close friend who has gotten sick with COVID-19 or had the strain of dealing with a positive diagnosis ourselves. And while these pressures of the world we live in bear down on us, we are still relentlessly tackling the necessary, but endless, influx of work our careers demand. This is why I sense so many of us are looking forward to the new year, but we would be remiss in not taking a moment to reflect on some of the positive things that have come out of this pandemic-stricken world we have had to adapt to.

As I look back at my calendar of events for this past year, I saw many trips lined out next to a sad face emoji. Yet, as those handful of trips became suddenly impossible, the empty blocks of my weeks began to populate with Zoom ID numbers and passwords. Though being packed close together into a large room for a presentation has become “so 2019”, the opportunity for learning and sharing our knowledge with each other has only seemed to become more vibrant with the added restrictions. The CAC has always provided diverse and numerous opportunities throughout the year for learning, sharing, and growing. I am proud to say that 2020 has not been able to change that, and in my opinion, the opportunities and our reach to the membership has only grown this year.

Our regional directors have always organized and advocated for nearby training opportunities in both northern and southern regions of the state. This year has seen the possibility of what happens when these two programs join forces to provide these study group opportunities for everyone throughout the state to participate together. Having our study group meetings via Zoom has allowed our fantastic speakers to reach a larger audience and allowed participants to safely learn from the comforts of their home. This socially distanced existence will not last forever, so the question of what we do moving forward to maintain these levels of participation and outreach must be asked. So, we asked. A recent survey we sent out asked if this upcoming seminar should still be held in person as planned. Instead of a lot of “yes” or “no”, I saw many responses speak about possibilities. 2020 has been a year of opportunity to change the way things have been done out of necessity. And now with those molds broken of how things have always been done, 2021 will bring us possibility with how we reshape our world going forward.

Does this possibility include in-person meetings soon? I think it is fair to argue that while a virtual setting can get the job done in many instances, there is something that just doesn't replace the experience of attending a meeting in person. The smell emanating from that giant coffee urn, the sound of someone hitting their knee on the leg of the deceptively short conference table, or that feeling of anticipation at the sight of a presenter walking up to the lectern to give their talk is something that cannot be experienced in a virtual setting. I believe that the experience of attending an in-person meeting or conference is absolutely in our future, but that there are also lots of possibilities that can be explored. Is that a conference that is attended both virtually and in person the future? Maybe a new virtual platform that allows for socialization and vendor visits the next step? Or does possibility include going back to how we have always done things in the past.

I don't have that answer for you. But I would love to hear your ideas, suggestions, and visions of what possibility means to you. Email me your ideas to editor@cacnews.org and we can include this as a topic at our next Board meeting. I am proud of what the CAC has been doing this past year to keep you all engaged, informed, and involved. The possibilities for the future are endless and limited only by the imagination and innovation of the membership, and with members like you, all things are possible.

A handwritten signature in dark ink, appearing to read "Jonathan Charron".

Remembering Fred Tulleners



FREDERIC ANDRE TULLENERS passed away in Sacramento, California, from complications due to cancer on November 24, 2020. He is survived by his wife, Faye Springer Tulleners, of 45 years, four children and six grandchildren.

In December, 1949, Fred and his family immigrated from the Netherlands to the United States, and finally settled in Costa Mesa, California. Fred had strong bonds with his family. They all cherished his witty humor, his zest for life and adventure, and his thirst for knowledge.

Fred served in the Navy as both enlisted seaman and a commissioned officer. He honorably retired from the Navy reserve as a Commander in 1994. He obtained a BS degree at CSULA in Chemistry and Master's Degree from UC Irvine in Radiation and Physical Chemistry. He spent most of his profession career in the forensic sciences.

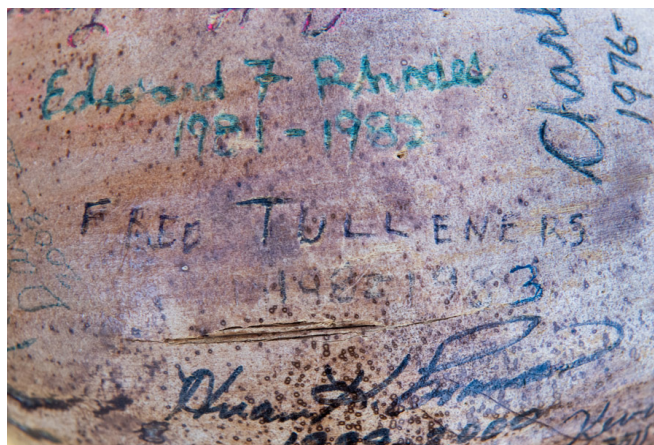
Fred's career started as a criminalist at the Los Angeles Police Department in the Scientific Investigation Division. After two years, he moved to Riverside, California, to work as a Criminalist for the California Department of Justice. It was here that he met and married his wife, Faye Springer. While in Riverside, he became a Supervising Criminalist. In 1988, they both moved to the California Department of Justice in Sacramento. He became a Program Manager for the California Criminalistics Institute where he developed the training courses for trace evidence. He also wrote grants to obtain funds for training and research and did legislative reviews of all firearms related bills for California. In 2000, he became laboratory manager at the California Department of Justice Sacramento and Santa Rosa Laboratories while still doing special projects related to firearms legislation. Later he became the Laboratory Manager of the California Criminalistics Institute. In 2003, he retired from the State of California and became the Director of Forensic Science Graduate program at the Univer-

sity of California in Davis. Fred loved doing research, teaching, and working with students. He finally retired in 2017.

During his career in forensic science, Fred made numerous contributions to the field of forensic science through his research and over 40 publications. He has also presented numerous oral papers to the California Association of Criminalists, Association of Firearms and Toolmark Examiners, Inter/Miro, American Academy of Forensic Sciences, and many other organizations. Many of the publications and presentations dealt with ballistic imaging, statistical evaluations of physical matching of duct tape and glass, quantitative evaluation of firearms and toolmark stria, micro marked firing pins, quantitative evaluation of blood spatters, and many other subjects.

His wife says that Fred is the only person she knows that will have a glass of scotch, read a statistics book and watch homesteading in Alaska at the same time. Burial will be at the Sacramento Valley National Cemetery in Dixon.

Fond Memories from Friends



Fred Tulleners' coconut signature as CAC President 1982-1983

...as a Mentor

When I think of Fred, I think of him antiquing with Mike Giusto, his gravelly voice, and his zest and love for life. I will forever be indebted to Fred, for he helped me get my first job in Forensics. I met him while taking forensic classes through the California Criminalistics Institute (CCI), before there was a graduate program at UC Davis. Once that officially began, I was one of the first students enrolled in the program in January, 2003.

After I completed my thesis and graduated in December 2005, I started to apply for various jobs. I got an interview with Contra Costa Sheriff's Office for a laboratory aide position in their Firearms Section. I had no experience in Firearms, but I knew Fred worked in that area for many years. I called him up and asked him to help me prepare for this interview. I believe we met at an IHOP and just went over potential questions and general information regarding Firearms. His help was invaluable. I got the job and within a year was promoted to a deputy sheriff criminalist.

I spent 10 and-a-half years at that agency with some of the best people around. I feel incredibly lucky and blessed

I worked there, and without Fred, I wouldn't have experienced one of the best periods of my life. Fred helped so many people, wanting people to succeed, pushing them to achieve greatness. He will be sorely missed. He had a presence that could be felt, and I am deeply saddened I won't see him again in the halls at CCI. I pray for Faye and the rest of his family, that they feel the collective grief of his loss in the Forensics community, and the comfort that the same community will provide to them. Godspeed, Fred. We've got it from here.

—Catherine Currier

...as a Researcher

As a Criminalist who started his career in 2000, I became aware of Fred through my professional interactions within the CAC and other California forensic professional activities. However I did not really get to know Fred until I started attending the UC Davis Masters in Forensic Science program in 2005. At the time the UC Davis program was relatively new and Fred was instrumental in getting the program off the ground. I think it was critical that he was involved because he ensured that much of the coursework was relevant to practitioners by including coursework that was hands-on, taught by senior criminalists. In 2008 my career had a dramatic shift and I changed disciplines: I moved from DNA to Firearms Examination. At UC Davis (which I was doing part-time), I was on the "DNA track" including working on DNA research. Fred helped me find a new advisor and when I proposed a research topic, he immediately put me in contact with his contacts at NIST. From this initial contact, NIST, Fred and myself published (in the *Journal of Forensic Sciences*) 3D-based analysis on consecutively manufactured firearms. I think this research was one of the earlier papers in this field, and helped kick-start an ever-growing body of literature that involves 3D/algorithm analysis of firearm ammunition.

"Fred had strong bonds with his family. They all cherished his witty humor, his zest for life and adventure, and his thirst for knowledge"

After publishing the paper, I remember Fred once said to me that he thought our paper was going to have a lasting effect on the discipline and he was really proud of the work. We'll see if that's true, but so far I think Fred was right. I am forever grateful for Fred's help in my own career.

—Todd Weller

...as a Teacher

I remember the time Fred shot a laser out of a gun during my research project. He had gotten a laser that could loosely fit down the muzzle of a barrel so he could line up the gun better with our target (he did most of our firing). The bullet was supposed to go through a blood-soaked sponge and a piece of butcher paper behind it. He tried using it a few times and it seemed to help him hit the target a bit more consistently in the center. Then, after this one round, the sponge flew off its pedestal (somewhat violently) and we saw a huge hole in the paper. None of us knew what to think at the time on how that could have happened until we reviewed the high speed video and saw a mangled laser flying through the frame. Fred had forgotten to take the laser out of the barrel before firing! It ended up in a twisted form at the end of the range. Once we realized what had happened, we all cracked up laughing because we couldn't believe he had forgotten to take it out. He said he wanted to save that video for his retirement, but I don't know if anyone ever saw it after that. (I think I still have the video somewhere.)

That's the story that stands out the most for me. But in general, through working with him on my thesis project, I just remember his kindness and how he was always curious and wanting to learn more despite all the experience he already had. He was always forward thinking.

—Sonya Siu



Fred with his wife, Faye Springer Tulleners

...as a Colleague

Fred had a penchant for trying to save money, often by salvaging tired, unwanted stuff. One of his findings was some used 4" x 4" boards he thought he could use. The Riverside lab had a seldom-used table saw in the "garage" area so Fred decided to use the saw during lunch hour to trim the boards to slightly smaller dimensions to expose fresh clean wood underneath the weathered exterior. I don't recall that he confided to anyone what he was doing but soon he came back through the office area moving towards the hall where the single, shared restroom was. He was rubbing his upper chest / neck area and I heard him say something like, "Man! Those wood splinters really hurt!"

It appeared he had some small cuts or scratches and there was a small spot of blood visible so several folk followed him to the restroom to get a band aid and help him bind his saw dust wounds. The secretary had cleaned away the blood and was trying to position a small butterfly bandage over what looked like a small cut. She said she was having trouble getting the small, narrow bandage to hold the "wound" closed. I remember leaning in and looking at the defect and thinking, "That looks like something penetrated the skin."

Fred stood and started toward the door to leave, slowed, stood stock still for a moment, then fell back in a dead faint! The secretary caught him and prevent his head from slamming into the tile floor. As he lay on the floor, I looked again at the small, gapping 1/4 inch hole near his neck and began to wonder. Literally, at a full run, I went through the office area, a small conference area and into the garage. The sawdust covered table saw with partially sawed wood was in some disarray. The insert that normally surrounds the blade was slightly askew. I pulled it out of the way and carefully rotated the blade and immediately determined that a number of the blade's carbide teeth were missing. Reversing my run back to the clerical area, I said "Call an ambulance! Quick!"

It was a while after Fred was whisked away that the crime scene reconstruction revealed what apparently had occurred. As Fred sawed thin layers of wood off the surface of each board, one thin scrap slipped down next to the blade, and, caught by the rotating saw blade, was kick upwards, dislodging the metal insert that surrounded the blade. The metal insert revealed gouges where it was caught by the blade, in turn dislodging a number of the rapidly moving carbide teeth, one of which struck Fred, penetrating into his neck fractions of an inch from his carotid artery! It was so close to the artery, that the doctors left it there rather than risk the minor surgery so close to the critical blood vessel.

So, we generally agreed that the incident was best described as a firearm examiner shot in the neck by a table saw. Of course, being dedicated forensic folk, we considered the wound ballistics, and from the table saw blade RPM and radius, attempted to calculate a "tooth" exit velocity and mass to evaluate the energy transfer and to await details of the depth of penetration of the not so aerodynamic projectile. Fred was lucky. A few millimeters might have made a Fred adventure much more serious...but no less memorable.

Its hard to remember after all these years, but I don't think this shooting was ever written up in the AFTE Journal.

—Torrey Johnson

"Fred helped so many people, wanting people to succeed, pushing them to achieve greatness."



...as a Friend

I first met Fred when he and John Murdock travelled to Sydney to present their week long "Conservative Objective Criteria for Identification" Course from 15 to 18 November 2001. Firearms examiners from all around Australia attended and everyone enjoyed it immensely. The methodology of applying CMS (Consecutive Matching Striae) to striated toolmarks was still relatively new for us in Australia. However it was accepted in this country as a useful (albeit optional) tool to use in firearm and toolmark casework, without the division that I saw was occurring in the USA between the "line-counters" and those that used traditional pattern matching. Fred was a great guy with a quirky sense of humour and the class managed to have some enjoyable social events with both Fred and John whilst they were teaching, where we got to know them on a more social level and we got to show them a bit of Sydney.

Several years later when I was visiting California on a combined work and holiday trip, I spent time with Fred at the DOJ lab in Sacramento and I recall having a very enjoyable BBQ at his place one afternoon. Fred struck me as passionate and committed to his work and he was very knowledgeable. Although I had great respect for him regarding his firearm and toolmark expertise, I was also aware he was skilled in many other areas as well. I also had the chance to spend time chatting with him at the annual AFTE (Association of Firearm and Toolmark Examiners) Seminars I occasionally managed to attend every few years in different cities in the USA. It was always enlightening to discuss identification philosophy with him, let alone socialising on a personal level and I always enjoyed his company.

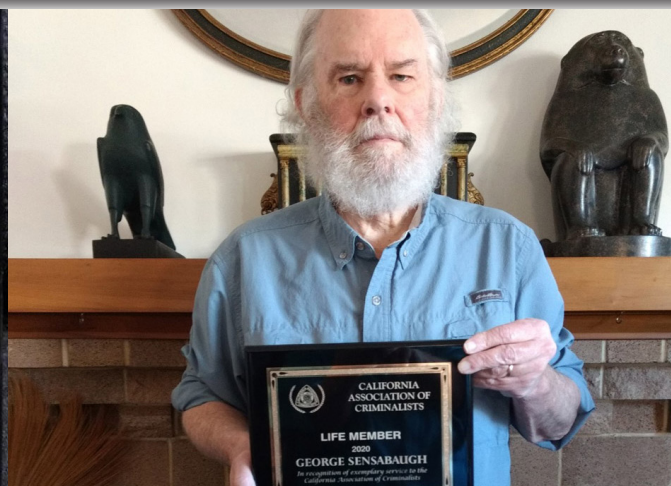
I was quite sad to hear of his recent passing; the profession is now the poorer for it. As we would say in Australia about those we like and respect, Fred was a 'top bloke'! Rest easy my friend.

—Gerard Dutton



IMAGINE YOUR NAME HERE...





To become a **Life Member** of the California Association of Criminalists is to receive one of the highest honors the Association can bestow upon someone. Life Members are recognized as criminalists who have dedicated much of their professional lives toward serving criminalistics and the CAC.

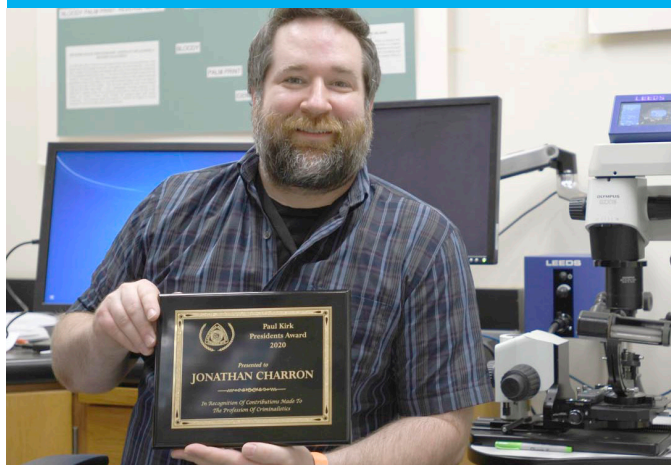
FULL DESCRIPTIONS
OF ALL AWARDS:

www.cacnews.org/awards/awards_dates.shtml

The purpose of the **Edward F. Rhodes Memorial Award** is to give a CAC member who is preparing for a career in criminalistics or is newly employed (less than three years) in the field of criminalistics the opportunity to attend one major forensic or scientific meeting of benefit to forensic practitioners.



The **Paul Kirk Presidents Award** was established in 1994 to encourage a collegial relationship between the CAC and the Chartered Soc. of Forensic Sciences in England by promoting scientific exchange and fellowship between members. The award is granted in even numbered years to a young forensic scientist who is sponsored to attend a meeting in the UK.



The **W. Jack Cadman Award** recognizes Full Members who have contributed a substantial amount of time and effort to the Association. The Immediate Past-President will track the information necessary to receive the award and will verify the self-nominations from Full Members who believe that they have met the criteria.



DEADLINES FOR THE CAC AWARDS

- **ABC Examination Award**—Jul. 1 - Dec. 31, Self-nomination
- **Alfred A. Biasotti Most Outstanding Presentation**—N/A , 3-Judge panel
- **Anthony Longhetti Distinguished Member**—Jan.1 - Jun.30, Any member
- **Best Poster**—Each Seminar , 3-Judge panel
- **Edward F. Rhodes Memorial**—Jul. 1 - Dec. 31, Self-nomination

- **Full Member Seminar Lottery**— ~60 Days Prior to Each Seminar. Full members enter themselves
- **Paul Kirk and Presidents** (even years)—Jan.1 - Jun.30, Any member
- **Roger Sherman Greene III Memorial**—Anytime , Any member
- **W. Jack Cadman**—Anytime , Self-nomination
- **AAFS Regional**—Decided by AAFS, CAC Board of Directors

Investigating the Effects of Temperature on Ethanol Concentrations in Preserved and Unpreserved Blood Ethanol Samples

Kristine E. Myhre¹, Allyson S. Avina¹

Abstract

The purpose of this study is to determine how varying storage conditions, such as temperature and time of storage, will affect the ethanol concentration of blood vials with and without preservative. Blood samples were collected into numerous tubes from drinkers at varying blood alcohol concentrations. One group of samples was collected into 10 mL gray top tubes each containing 20 mg of potassium oxalate (anticoagulant) and 100 mg of sodium fluoride (preservative). The other group of samples was collected into 10 mL red top tubes that had 0.01 mL of water saturated with potassium oxalate added. The tubes were assigned a storage condition and timeframe to be opened and analyzed, as well as marked with the name of the person donating the blood. The samples were analyzed using headspace gas chromatography with flame ionization and mass spectrometry detection (HS-GC-FID/MS). The alcohol containing samples were stored at 35°F, 71°F, and 100°F for up to eight weeks. Analysis was performed hours after collection, after three days, one week, two weeks, four weeks, and eight weeks. Alcohol negative samples were stored at the same temperatures and were analyzed after four weeks and eight weeks to monitor any possible ethanol fermentation. No ethanol formation occurred in any of the alcohol negative tubes with or without preservative over the eight-week period. In all ethanol containing samples, the overall ethanol concentration decreased at every storage temperature. Ethanol dissipated more rapidly in vials containing sodium fluoride when compared to vials without with the greatest rate of decrease occurring at 100°F. When calculating in percentage, this was an ethanol loss of 35.4%. The sample quality also appeared to degrade faster in the gray top tubes and at an increased rate as the storage temperature increased. The color of the blood changed from red to brown to green and the sample texture became grittier.

Background

In 2019, a recall for BD Vacutainer® Fluoride Tubes for Blood Alcohol Determination occurred due to a portion of a lot sold being confirmed to have had no potassium oxalate (anticoagulant) or sodium fluoride (preservative) within the tubes (1). A tube without anticoagulant would result in a clotted sample and would be visibly unsuitable for analysis. The recall letter claims that reliable results would be yielded from a sample without the preservative in the tube if the sample was stored at room temperature for no longer than two days.

¹Sacramento County District Attorney's Laboratory of Forensic Services, 4800 Broadway, Suite 200, Sacramento CA 95820.

We gratefully acknowledge the assistance of two peer reviewers in the preparation of this paper.

The letter goes on to say that in the event it is stored for longer than two days, the alcohol determination could be falsely high or falsely low (2). Rodd et al (2020) found that the possibility of reporting falsely increased ethanol concentrations in samples collected into tubes lacking preservative and anticoagulant are low (3). While there have been many studies on the effect of storage conditions on ethanol concentrations as well as the absence or presence of preservative, these studies compared different types of tube material as well as different types of anticoagulant (4-8). There are also studies on the effects of ethanol concentrations in samples inoculated with *Candida albicans* but, considering that formation of ethanol is not inhibited in samples inoculated or contaminated with *Candida albicans*, the expected effect would be the same in both sets of tubes, so this was not investigated in this study (9).

Objectives

To determine if the ethanol concentration of a blood sample is affected by varying storage conditions or the presence of the preservative sodium fluoride within the tube.

Methodology

Tubes with and without sodium fluoride (NaF) were utilized while staying consistent in using the same anticoagulant (potassium oxalate) and tube material (glass). 10 mL BD Vacutainer® gray top tubes containing 20 mg potassium oxalate (anticoagulant) and 100 mg sodium fluoride (preservative) as well as 10 mL Coviden red top tubes with 0.01 mL of water saturated with potassium oxalate added were used. After the addition of approximately 2.5 mL of blood, the expected concentration of potassium oxalate in the treated red top tubes was 1.44 mg/mL, which was confirmed through additional testing to be sufficient enough to prevent clotting in samples left at room temperature for over a week. To ensure adequate vacuum for blood collection was maintained, the tubes were not opened to add powdered potassium oxalate. Instead, the red top tubes had 0.01 mL of saturated potassium oxalate in water added. This amount was decided upon to minimize the chances of sample dilution and was added using a U40 syringe a few hours prior to meeting with the subjects. To assure that the alcohol concentration of each subject was varied, each of the five subjects provided a breath sample using a Dräger Alcotest 9510 instrument prior to blood sample collection. Blood was drawn from the same isopropanol sterilized site until all the blood tubes were collected. Approximately 80 mL of blood was collected and thoroughly mixed into a total of 32 tubes (sixteen 10 mL preservative tubes and sixteen 10 mL no preservative tubes with about 2.5 mL in each tube) for each subject. The 2.5 mL of blood was chosen to simulate a scenario with more headspace. This presents the opportunity for ethanol loss through evaporation within tubes (10) as well as to limit the overall amount of blood taken from each subject. One vial with preservative and one without were analyzed using HS-GC-FID/MS shortly after collection for their alcohol concentration (g/dL). The remaining tubes were then divided evenly and stored at 35°F, 71°F, and 100°F. One tube with and without preservative from each temperature group was analyzed after being stored for three days, one week, two weeks, four weeks, and eight weeks. Each tube was only analyzed once to maintain the vacuum and decrease potential ethanol loss from evaporation of opened tubes during storage. Each tube was also analyzed in compliance with Title 17 of the California Code of Regulations (CCR) (11) as well as the laboratory's

approved procedures for analyzing volatile compounds. This includes the sample being analyzed in duplicate and the average of the duplicate determinations being calculated to the fourth decimal place and then truncated to the third decimal for reporting. The duplicate determinations must also agree within 5% of the mean.

Additionally, blood from a subject who did not consume alcohol was collected into three preserved and three unpreserved tubes, with each tube containing approximately 2.5 mL of blood. These samples were used to monitor for ethanol production during storage and were analyzed shortly after the blood draw, at four weeks, and at eight weeks.

The measurement of uncertainty for the samples is determined by using the root sum squares (RSS) method which is consistent with the laboratory's procedure for uncertainty of measurement. To comply with the RSS method, each batch of case samples includes multiple quality control samples. The results for the low quality control samples are entered into the Multi-QC database as a percentage, truncated to the third decimal place. The relative standard deviation of the low quality control data as well as other sources of uncertainty such as reference material purity, volumetric delivery, and calibrator preparation are combined. The resulted overall uncertainty for the two HS-GC-FID/MS instruments utilized is an approximate 99.7% level of confidence using a coverage factor of $k=3$ (12). The samples were reported using the standard laboratory protocol for uncertainty and it was not calculated specifically for this study.

Results

Isopropanol was not detected or identified using HS-GC-FID/MS in any of the samples indicating that a draw site sterilized with isopropanol did not have an effect on ethanol concentration. There was no ethanol formation in the negative control samples (subject A) for the tubes with and without preservative at each of the storage conditions.

Subject B had the greatest difference between BAC values in the preservative tube stored at 100°F. The initial ethanol concentration was 0.197 g/dL. There was significant loss by week four with a concentration of 0.164 g/dL, and then the sample appeared to stabilize yielding a concentration of 0.166 g/dL at eight weeks. This is an approximate 17% ethanol loss. When calculating as a percentage, Subject D had the largest decrease in alcohol concentration in the preservative tube stored at 100°F. The initial ethanol concentration was 0.079 g/dL with a concentration of 0.051 g/dL by week eight. This is an approximate 35.4% ethanol loss.

Overall, it was observed that the ethanol concentration decreased in every sample at every storage temperature regardless of the presence or absence of preservative. There was a more rapid decrease in the gray top tubes with the greatest rate of decrease occurring at the storage temperature of 100°F. The sample quality also appeared to degrade faster in the gray top tubes and at an increased rate as the storage temperature increased. The color of the blood changed from red to brown to green and the sample became grittier.

Table 1 – Ethanol concentration (g/dL) of samples from subject B

Initial Concentration		Storage Temp.	3 Days		1 Week		2 Weeks		4 Weeks		8 Weeks	
+NaF	-NaF		+NaF	-NaF	+NaF	-NaF	+NaF	-NaF	+NaF	-NaF	+NaF	-NaF
0.197	0.199	35°F	0.185	0.195	0.189	0.195	0.185	0.193	0.185	0.191	0.187	0.193
		71°F	0.191	0.196	0.191	0.198	0.187	0.196	0.188	0.194	0.185	0.194
		100°F	0.184	0.190	0.182	0.189	0.178	0.188	0.164	0.183	0.166	0.185

Figure 1 – Change in ethanol concentration of samples from subject B

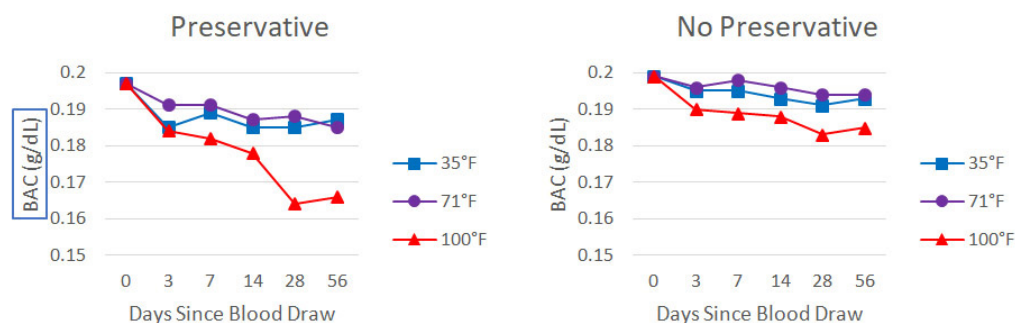


Table 2 – Ethanol concentration (g/dL) of samples from subject C

Initial Concentration		Storage Temp.	3 Days		1 Week		2 Weeks		4 Weeks		8 Weeks	
+NaF	-NaF		+NaF	-NaF	+NaF	-NaF	+NaF	-NaF	+NaF	-NaF	+NaF	-NaF
0.049	0.048	35°F	0.046	0.045	0.047	0.048	0.046	0.046	0.045	0.045	0.047	0.046
		71°F	0.047	0.047	0.046	0.048	0.047	0.047	0.045	0.045	0.041	0.046
		100°F	0.045	0.048	0.042	0.046	0.036	0.045	0.037	0.043	0.033	0.045

Figure 2 – Change in ethanol concentration of samples from subject C

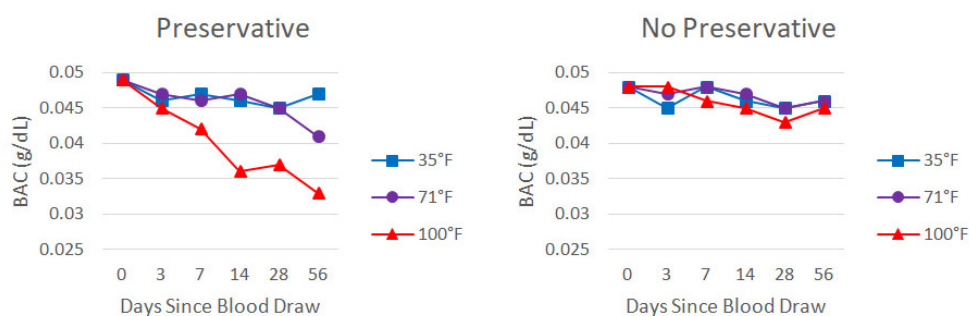


Table 3 – Ethanol concentration (g/dL) of samples from subject D

Initial Concentration		Storage Temp.	3 Days		1 Week		2 Weeks		4 Weeks		8 Weeks	
+NaF	-NaF		+NaF	-NaF	+NaF	-NaF	+NaF	-NaF	+NaF	-NaF	+NaF	-NaF
0.079	0.080	35°F	0.077	0.077	0.076	0.080	0.076	0.079	0.074	0.077	0.076	0.078
		71°F	0.076	0.080	0.075	0.080	0.074	0.077	0.072	0.074	0.068	0.070
		100°F	0.073	0.076	0.063	0.070	0.057	0.069	0.053	0.068	0.051	0.068

Figure 3 – Change in ethanol concentration of samples from subject D

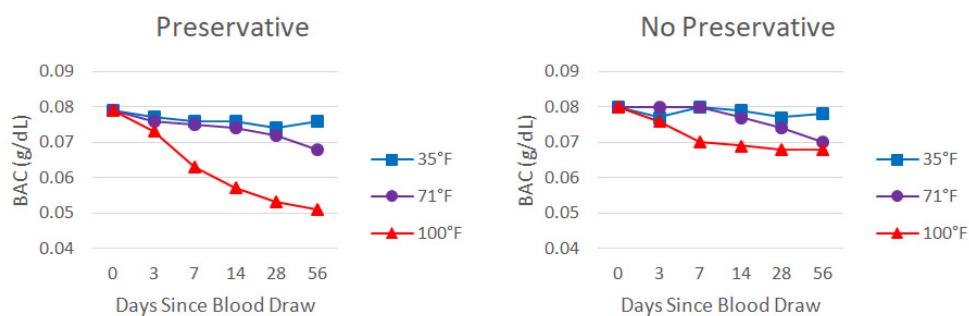


Table 4 – Ethanol concentration (g/dL) of samples from subject E

Initial Concentration		Storage Temp.	3 Days		1 Week		2 Weeks		4 Weeks		8 Weeks	
+NaF	-NaF		+NaF	-NaF	+NaF	-NaF	+NaF	-NaF	+NaF	-NaF	+NaF	-NaF
0.093	0.094	35°F	0.087	0.090	0.088	0.089	0.088	0.090	0.086	0.088	0.089	0.090
		71°F	0.091	0.090	0.088	0.090	0.089	0.091	0.085	0.088	0.082	0.090
		100°F	0.087	0.089	0.082	0.086	0.078	0.085	0.074	0.084	0.074	0.085

Figure 4 – Change in ethanol concentration of samples from subject E

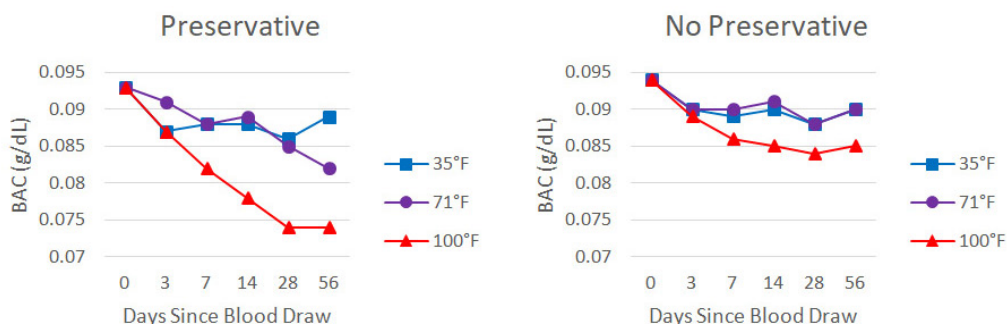
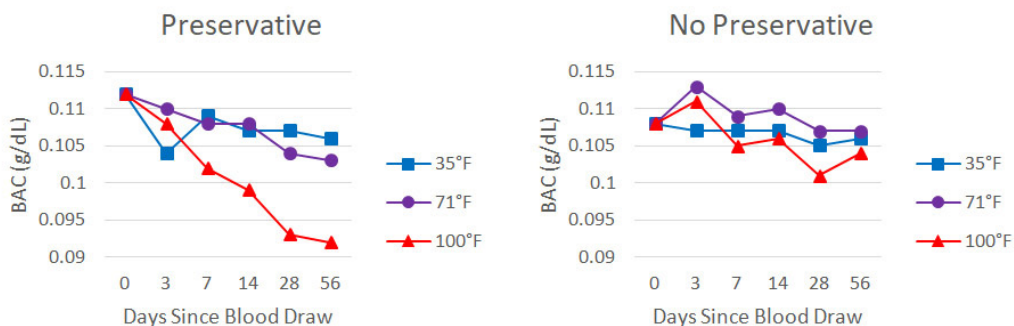


Table 5 – Ethanol concentration (g/dL) of samples from subject F

Initial Concentration		Storage Temp.	3 Days		1 Week		2 Weeks		4 Weeks		8 Weeks	
+NaF	-NaF		+NaF	-NaF	+NaF	-NaF	+NaF	-NaF	+NaF	-NaF	+NaF	-NaF
0.112	0.108	35°F	0.104	0.107	0.109	0.107	0.107	0.107	0.107	0.105	0.106	0.106
		71°F	0.110	0.113	0.108	0.109	0.108	0.110	0.104	0.107	0.103	0.107
		100°F	0.108	0.111	0.102	0.105	0.099	0.106	0.093	0.101	0.092	0.104

Figure 5 – Change in ethanol concentration of samples from subject F



Discussion

Isopropanol was used to sterilize the draw site which was not in compliance with Title 17 of the CCR which states that "alcohol or other volatile organic disinfectant shall not be used to clean the skin where a specimen is to be collected (11)." If, however, isopropanol was present in the blood sample, it would be observed in the sample chromatogram and mass spectra. Analyzing the samples with instrumentation that can differentiate between ethanol and isopropanol showed that there was no isopropanol detected in any of the samples.

While there was no formation of ethanol within the negative control, some subjects yielded ethanol results that were higher than the initial results. These values fell within the measurement of uncertainty and indicate that the sample was not increasing in alcohol content.

The ethanol concentration of samples with preservative decreased at a faster rate than the samples with no preservative. While some loss is expected due to evaporation from the tube as well as the hemoglobin catalyzed oxidation of alcohol, the greater loss in the samples with preservative may be explained by a salting-out effect, especially since a blood volume of less than 9 mL was used (8, 13). The salting-out of nonelectrolytes from aqueous solutions, which is well known and studied, can be summarized as a decrease in the solubility of a nonelectrolyte from additions such as salt (14, 15). The internal standard n-propanol, which was utilized in this study, salts-out more effectively than ethanol which means the relative concentration of n-propanol in the equilibrated headspace will increase compared to the concentration of ethanol (13, 14). It has been shown that measured ethanol levels decrease as the amount of sodium fluoride in the blood sample increases due to the salting-out effect (16).

As with all studies, this study is subject to limitations. The main limitations include the small number of subjects used and the possibility of varying quantities of saturated potassium oxalate and volume of blood within each of the tubes due to human error. The U40 syringes aided in more accurately measuring out 0.01 mL of the saturated potassium oxalate; however, there was still the opportunity for slight variation depending on the individual adding anticoagulant to the tubes. The blood tubes were marked to indicate approximately 2.5 mL of blood; however, the volume of blood may have varied slightly. Additional limitations include the testing frequency of the samples and the difference in the amount of potassium oxalate within the tubes with and without preservative. Potassium oxalate shrinks red blood cells and studies suggest that cell shrinkage may be greater with higher concentrations of potassium oxalate (17). The effect of red blood cell shrinking would need to be investigated more deeply to determine if this would influence ethanol concentration.

Conclusion

This study has demonstrated that, regardless of storage temperature and duration, ethanol concentrations decreased in samples collected into tubes with and without preservative; however, the decrease of ethanol concentration was greater in the tubes containing the preservative. The study has also demonstrated that ethanol formation did not occur in any of the preserved or unpreserved tubes regardless of the storage temperature and duration.

This study suggests that analyzing samples that are drawn from sites having been sterilized with isopropanol will not affect blood ethanol concentrations. It also suggests that

analyzing samples collected into tubes known not to contain the preservative sodium fluoride, will still provide an ethanol concentration. This information may be useful in the event that Title 17 of the CCR (11) for collection and preservation of blood samples is not followed precisely.

The data indicates that the most likely effect on samples not containing sodium fluoride would be a decrease in their ethanol concentrations.

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CAC Virtual DNA Workshop

Dec. 2, 2020 - Abstracts

Justice Delayed: The Use of DNA testing and Genetic Genealogy to Exonerate Ricky Davis

Missy O'Connell and Linda Starr (Northern CA Innocence Project), and Ricky Davis

In 2005, Ricky Leo Davis was convicted of the 1985 murder of Jane Hylton, a colleague of Davis' grandmother. In July 1985, Hylton was found brutally murdered in 20-year-old Davis' home which he shared with his mother and his girlfriend. After several suggestive and coercive interrogations by law enforcement, Davis' girlfriend implicated him in the murder leading to his conviction. Years later, DNA evidence led to Davis' exoneration. Davis' case puts a spotlight on the dangers of certain police interrogation methods and the power of DNA in innocence work. Davis' exoneration also highlights the critical need for continuing advancements in science; but for genetic genealogy, Davis might not be free today. Davis is only the second person in the nation to be exonerated based on the use of genetic genealogy.

Beyond Buccal Swabs: The efficacy of the ANDE 6C Rapid DNA platform on generating data from single source case-type samples

Dr. Lilliana Moreno (FBI)

Rapid DNA is a technology originally designed to enable point-of-contact collection, processing and searching of arrestee buccal exemplars in the booking station. Given this, most Rapid DNA initiatives have heavily focused on the processing of single source buccal samples. As the technology matures and public interest grows, companies have introduced cartridges and modified their expert system parameters to enable the generation of data from other types of single source samples, to include bone, tissue, and other common crime scene evidence samples. This presentation will summarize the findings of some internal work being conducted at the FBI laboratory as it pertains to the success of the ANDE 6C Rapid DNA system and its associated Investigative biochip when processing single source samples other than buccal swabs.

Proteomic Sex Estimation in Degraded or Juvenile Human Skeletons

Dr. Glendon Parker (U.C. Davis) -

Accurate sex estimation is a starting point for the developing and addressing basic questions about the forensic context of unidentified human remains. Historically skeletal markers have been used to estimate biological sex, but in the case of degraded or partial skeletons, or with infants and juveniles, skeletal markers are missing or undeveloped. This resulted in a large portion of skeletons being unsexed. The invention of DNA-based methods allowed, for the first time, a means to sex skeletons including juvenile and degraded, partial remains. Many of these methods, such as STR-typing protocols, focus on the amelogenin gene family that are located on each of the sex-chromosomes. The success of DNA-based methods has limitations however: it requires stringent confirmation to control for environmental contamination, and DNA may degrade as a result of environmental conditions, temperature or time. Our laboratory has developed a third method of biological sex estimation. The most characterized sex-specific genes, amelogenins, are expressed as protein in enamel, the most robust human tissue. These peptides are highly stable and persist over an archaeological time frame, they have been used to successfully estimate sex in fetal, infant juvenile and cremated skeletons, as well as skeletons over 9000 years old. To validate the method we conducted a side-by-side comparison with osteologic, genomic and proteomic sex estimation on fifty-five archaeological skeletons, ranging in age from 100 to 2400 years. Sex estimation was possible in 100% of this burial sample using proteomics, in 91% using genomics, and in 51% using osteology. Agreement between the methods was high, however conflicts did occur. Genomic sex estimates were 100% consistent with proteomic and osteological estimates when DNA reads were above 100,000 total sequences. However, more than half the samples had DNA read numbers below this threshold, producing high rates of conflict with osteological and proteomic data where nine out of twenty conditional DNA sex estimates conflicted with proteomics. This introduces a third limitation for genomic sex estimation: higher errors rates occur when DNA is degraded. Proteomic sex estimation therefore may work when DNA-based fail to provide a sex estimate or potentially provide an estimate that is prone to error.



NIST Video includes Lindbergh Case

A scientist and a historian at NIST team up to discover the mostly forgotten history of Wilmer Souder, a scientist who worked at the National Bureau of Standards (now NIST) from 1911 to 1954. Souder was an early expert in the field of forensic science. His careful analysis of evidence and his expert testimony sent to prison countless murderers, bootleggers, gangsters, and thieves. The most famous case he worked on was the Lindbergh kidnapping case, and this video reveals that his involvement in that case was much greater than previously known.

<https://www.nist.gov/video/detective-x-re-discovering-wilmer-souder>

(l-r) Robert Thompson, Kristen Frederick-Frost, John Butler

Maxprep™: Automation Made Easy

Jessica Seifarth (Promega Corporation)

Automation in a forensic laboratory has not been an easy transition. The amount of time and dedication spent towards implementation and validation has, at times, outweighed the benefits of using an automated platform. The Maxprep™ Liquid Handler has changed all of that by taking the burden off the laboratory. This modular system comes with methods designed to process samples through the casework and database workflow. Seamlessly integrating the Promega Portal software allows for sample tracking and run-specific information to be transferred between the Maxprep™ Liquid Handler and supported Maxwell® Instruments. This demonstration will show you how easy automation can really be.

Validation and implementation of the Hamilton AutoLys STAR for the automation of the DNA differential extraction

Sonja Klein and Chris Tanforan (CA DOJ Jan Bashinski DNA Laboratory)

This two-part presentation discusses the validation and implementation of an automated differential extraction pro-

cedure using the Hamilton AutoLys STAR liquid handling system. This work is a continuation of previously published work (FSIG 2018: 34: 170-177; FSIG 2019: 40: 96-104) which integrates a modified standard differential extraction procedure with robotic processing. The first part of the presentation describes the method and details some of the validation results. The second part discusses the incorporation of the automated differential extraction procedure into the existing workflow of the Casework section of the Jan Bashinski DNA Laboratory and its effectiveness.

Gaining Workflow Efficiency Through Lab Automation

Nick Andrews (ThermoFisher)

Many labs face bottlenecks in their workflow that can be overcome with automation solutions. Within this talk, we will look at the different bottlenecks a lab might face and the automation solutions, such as the ID NIMBUS™ Presto System using the Applied Biosystems™ PrepFiler™ Automated Forensic DNA Extraction Kit, address bottlenecks and improve efficiency for each step in DNA processing.

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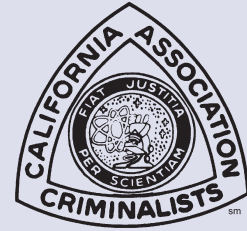


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