Blood Experiments

Why is the blood so red? Can you make a blood smear that is similar to the evidence in question?

Observation of blood stain evidence (in the case of Steven Avery) did not appear natural; stains were a bright, vivid red, and the pattern of smear itself was questionable. With the allegation that blood could have been planted making those smears, it raised the questions: What causes a blood stain to change colors? Does EDTA preserved blood have a different appearance in dried bloodstains than from active bleeding? If there is a visual difference, what makes it different? What could be used to make a visually similar blood smear?

Factors that influence color changes of blood.

Normal blood coagulation during and after a blood loss incident is a very detailed and intricate process in the body. Damage to endothelial cells triggers the coagulation cascade, with varying factors contributing to clot formations. One of those factors is Calcium. It is reported to play a role in the oxidation reduction of hemoglobin, which is normally contained in erythrocytes (red blood cells/ RBC's), and gives blood its red color. After a blood loss incident, the hemoglobin contained in blood outside the body begins an oxidation process which causes the reduction of oxyhemoglobin (oxygen rich hemoglobin) to the Fe (III) state, meaning it can no longer be an oxygen carrier. In the normal intravascular environment, cytochrome b5 reductase would be present, to halt the auto oxidation of oxyhemoglobin to hemichrome. With this element missing in vitro the ferric oxidation process becomes irreversible, leading to the complete decay/ degradation process of blood. One of the end products from the oxidation process is Methemalbumin, also known as Fairley's Pigment, along with water volume loss due to drying, is what changes the color of dried blood from red to brown. These changes are known historically to be biphasic and very rapid in the first few hours after blood deposition and then more slow and steady in the second phase. The speed of the process is influenced highly by temperature and to some degree by humidity.

Preservatives used for blood collection and the changes that occur to blood samples.

When blood is taken for lab work or other analysis, a preservative is added to the collection tube to prevent coagulation. The most common are EDTA, Citrate, and Heparin. Because of the action of these preservatives, and what tests need to be done, one could be a better choice than another. EDTA and Citrate both work due to their Calcium binding ability. Calcium is pivotal to blood coagulation at multiple points in the process; it also binds to Albumin, a protein that is reduced in the formation of Methemalbumin, the brown pigment in degraded blood. Therefore, it can be theorized the in vitro (outside body) oxidation of hemoglobin byproducts would be interrupted, thus preventing some of the brown pigmentation of blood stains. EDTA is used regularly for basic labs and DNA analysis as it is reported to cause less morphological changes to cells. Another factor in preserved blood that would affect color is hemolysis. Hemolysis is a breakdown of red blood cells causing the hemoglobin within the cell to become free. Most laboratory and blood bank workers can visually recognize a hemolised sample because of its pink red pigment. The usual cause of hemolysis is damage to RBC's during phlebotomy, transport, or storage.

Illustration of hemolised samples for consideration of color. Image from sagepub.com

Haemoglobin added (g/dl)	0	0.05	0.1	0.25	0.5	1
Haemolysis index	< 2	46	97	227	446	907
Sample frequency (%)	*84.4*	*8.5*	*4.6*	*2*	*0.5*	

Theory for Color of Blood/ Smear Patterns:

1- EDTA+ samples will maintain a red color after drying, EDTA free blood will oxidize appearing a brown color.

2.Smear pattern may be due to placement with a swab.

After doing research, I have theorized the most logical explanation of the unnatural color of the blood smears found in the evidence pictures to be caused by a combination of factors. The blood coagulation interruption caused by Calcium, in addition to probable hemolysis of stored sample blood, could be a reason for the blood smear in question to be a bright, vivid color. This would support the idea that the smears originated from a stored blood sample and not from an active bleeding incident.

The visualization of smear pattern and color could only be supported by additional similarities in new smears, still being variable beyond scientific certainty. By attempting new smears in similar conditions with both EDTA free blood and EDTA preserved blood, it would support or discredit certain theories. It would also give a visual basis for drawing a conclusion, and the need for further attention or testing to the validity of whether or not these theories are possible at all. It would be a starting point.

Experiment Materials:

1. Willing blood donor. No written consent needed as I am the donor.
2. Crime scene vehicle, 1997 Ford F150, grey colored dash.
3. Digital camera, Kodak, I phone 5 + (camera built in) for images
4. Winged "butterfly" type needle used for venipuncture, 21 gauge, with 12 inch tubing and luer lock adapter
5. Vacutainer blood collection tube (purple top) with EDTA K2 powder preservative
6. Two Culturette swabs with plastic sheath, culture medium removed
7. 1 ml syringes with 31 gauge needles, "insulin" type
8. Small plastic dropper
9. Household cotton swabs
10. Cloth "work" type gloves
11. Nitrile blue disposable gloves
12. Sterile water
13. Ziploc type plastic bags

All dates in experiment are precise, times are approximate.

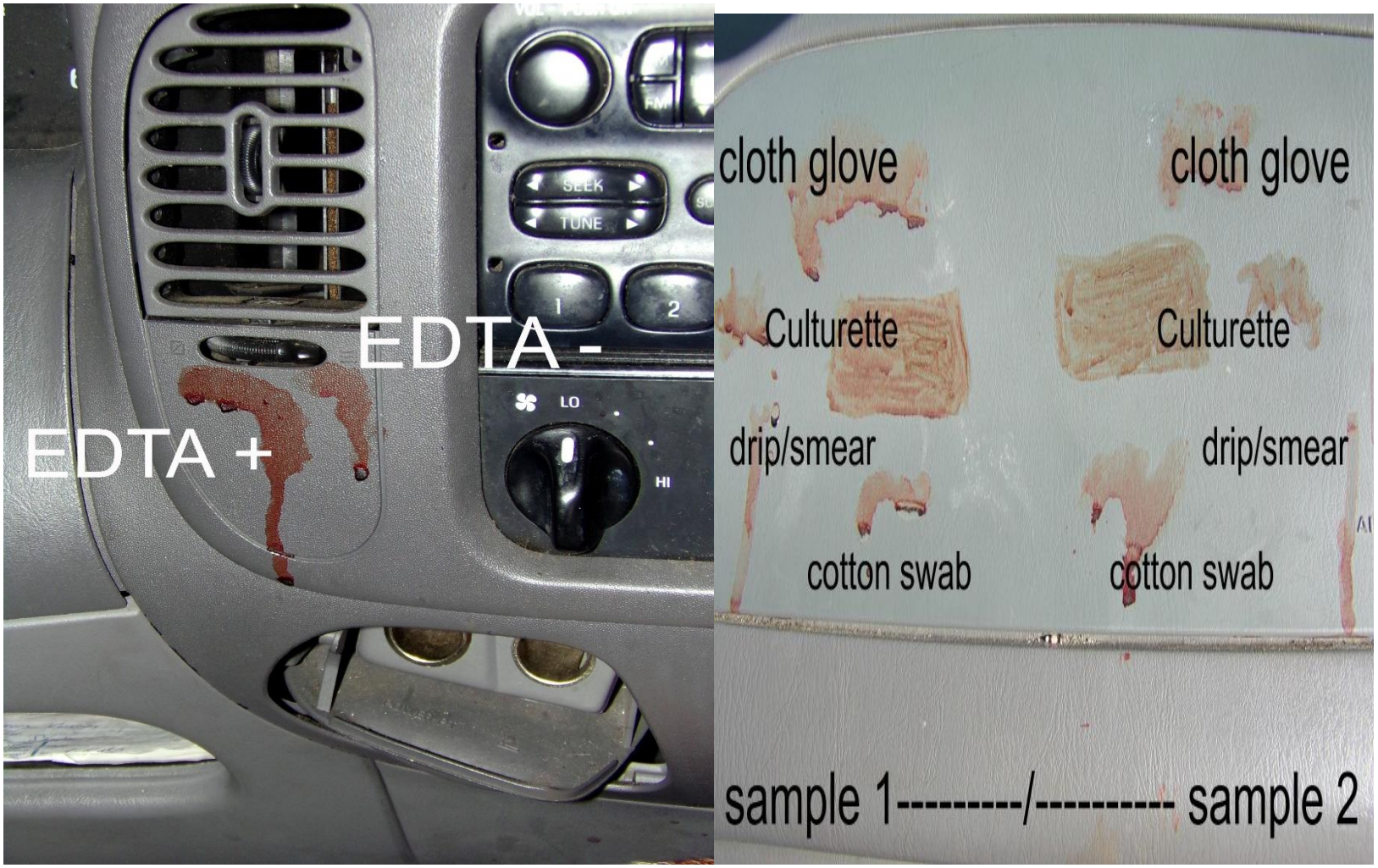
Preparation and Gathering Materials

January 29th 2016 a venipuncture was performed with winged needle 21 gauge to fill evacuated blood collection tube to appropriate volume. Once completed, a 31 gauge needle syringe was used to withdraw blood left in tubing. The blood withdrawn from tubing will be sample 2 EDTA free blood sample. An additional syringe is used to withdraw blood from tube, this will be sample 1 EDTA + blood sample. Blood is then applied to culturette swabs for sample 1 and 2 respectively.

Weather reports for Wisconsin and Tennessee showed the average temps in November 2005 Manitowoc, Wisconsin and Nashville, Tennessee now to be within 5 degrees of each the highs and lows respectively, although when date specified more, the temperatures in Tennessee were a few degrees higher than those averages. With what is known about the oxidation process, this would speed up the process, as higher temperatures have been shown to.

Day 1 Through Day 8 Pictures- Final Data Pictures

- Day 1
- Day 2
- Day 3
- Day 4
- Day 5
- Day 6
- Day 7/8
- Final Look: What was left out.



Method for placement of each individual smear.

Day 1

Blood was drawn and applied to dash with very small amounts applied to Culturette swabs- 0.3 ml. As I applied the first smear to dash, I realized both blood samples were drying much faster than I had expected. The reason the first smear became a rectangle was it would have been indistinguishable to the naked eye that there was a smear in that area at all. Particularly true of sample 2, which was drying almost as soon as I got done applying. The applications of these first smears made it clear that a larger amount would be needed.

Day 2

With the use of the same sample 1 from the purple top tube, and a fresh actively bleeding finger tip via a lancet prick for sample 2, new smears were attempted to mimic theories that have been discussed. Smears were attempted with a household cotton swab, cloth work glove, and drips then smears with nitrile gloves. The exact location of each type of smear is illustrated in the above images. Using larger amounts of blood, the smears have dripped down the dash, making the smears lighter except on the bottom where the drops are pooling. They do not have the appearance of density that the evidence smear does. When applying enough blood to make the color visually similar, the dripping increases, in turn making them lighter as I said before. With these examples, it is now obvious that the evidence

smear was either from coagulating blood that had been exposed to air for an amount of time, or swabs that were saturated with EDTA + tube blood that was exposed to air for an amount of time.

Day 3

Smears that were applied on days 1 and 2 are showing a brown color. EDTA + smears appear to be less brown. After coming to the conclusion that the smears in evidence were higher in density, now to test that theory. On the morning of day 3, using a medicine cup and a household cotton swab, I then saturated the swab with EDTA + blood and left it to sit in room air until the night of day 3. I then applied a new blood smear using that swab. Although this new smear is still wet, it is clear that the evidence smears are more consistent with this dense appearance of a water volume loss blood smear.

Day 4

Smears that were applied on days 1 and 2 are showing a stable color change now. They have all browned to some degree, but the EDTA + smears still maintain a more vivid red color. It appears the smears are staying steady near the end of phase 2, the slow structural changes that occur with the decay process, and the changes are not as

obvious as they were within the first day. It was decided to use sterile water and swabs to remove the first smears from the dash. There is a notable difference in color between swabs with EDTA + and EDTA free blood. The swabs that removed the EDTA + blood are still red, unlike the EDTA free swabs that are more evenly brown in color. The EDTA + smear applied on day 3 is now dry. To make a comparison, I have performed an additional fingerstick with a lancet to obtain an actively bleeding blood sample. I then let the finger bleed into the med cup, “milking” the finger to produce a larger sample. 15 minutes elapsed. Then using a cotton swab, applied a new smear to dash with EDTA free blood.

Day 5

Both the new smears are dry on dash. The red color of both smears are more vivid than earlier smears applied to dash. The sample 1 EDTA + smear is brighter- almost a magenta undertone can be seen. The sample 2 EDTA free smear has a dull brown undertone. After a suggestion by a colleague that has been examining the details of this experiment, one last smear will be attempted with a cotton swab, plastic bag with zipper closure, and the purple top EDTA + blood. I opened the purple top blood tube, with a swift motion, dipped two swabs into the EDTA + blood. Those swabs were then put into the plastic bag. Plastic bag is sealed. We will wait at least 24 hours before applying a new smear to dash with these swabs. The theory is that losing water volume in this smaller amount will reproduce a visually similar smear in color to the evidence smear, without the dripping that we have seen in previous smears.

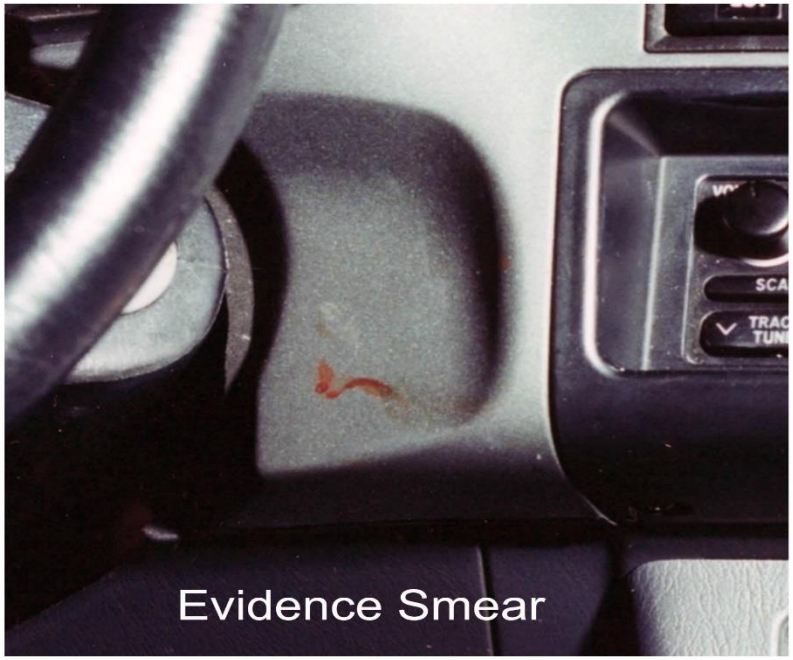
Day 6

The two remaining smears continue to degrade and lose color, although the EDTA + smear has maintained a vivid red in comparison to the EDTA free smear. On the evening of day 6, the swabs that were contained in the plastic bag with EDTA + blood were used to make a new smear on dash. This smear has been the most visually similar smear to the evidence smear to date. It has the dense red color, and it also had very minimal dripping on dash.

Day 7/8

The remaining three blood smears are dry on the dash. The first two of the three smears have lost very little of their red quality. The last smear of the EDTA + blood that was applied after 1.5 days in the plastic bag has a bright vivid red color still. It did not drip or pool near the bottom of the smear. It continues to be the most visually similar smear applied throughout this experiment.

Color difference of swabs used to remove both samples.



Side by side comparison of evidence blood smear and blood smear applied to dash of experiment crime scene using household cotton swabs dipped in EDTA+ purple top tube blood, left in plastic ziploc type bag for 1.5 days, then used to make a blood smear.

In Conclusion

At the conclusion of this experiment, some very interesting observations have been noted. It does not appear that a smear visually similar to the evidence smear could be made with fresh blood, or preserved blood that had not been decreased in volume due to water loss. Any smear attempted under these conditions made pooling and dripping under the smear. However, when a smear was attempted after a significant water volume loss it was more consistent in appearance to the evidence smears. Most visually similar was the last smear with the swabs left in plastic bag made with EDTA + blood. Even though our hypothesis was not proved to any degree of scientific certainty, it does beg to question if the smears in evidence were of a natural source. I have faith that science will give us a definitive answer one day, but today we can be sure that we raised the question if the information given to us in this case was honest and truth seeking, or possibly devious and self serving. I enjoyed learning about the scientific method and I hope all of you will enjoy reading this. I have taken a huge lesson from this; that is the need to question things that I do not understand, learn about them, figure out why, and then try to find the truth. As long as we are honest and truthfully searching we will find our answers.

Links to Reports, Articles, And Various Sources

- Wintrobe's Clinical Hematology
- Historical Weather- Nashville. TN
- EDTA Information, BD Vacutainer
- Heparin Action
- Methemalbumin
- Hemolyzed Specimens
- Effect of time, temperature and anticoagulants on in vitro complement activation: Consequences for collection and preservation of samples to be examined for complement activation
- In Vitro Hemolysis
- Albumin
- Hypocalcemia
- Chelators for Iron Overload
- NIR Raman spectra of whole human blood: Effects of laser-induced and in vitrohemoglobin denaturation

- Biphasic Oxidation of Oxy-Hemoglobin in Bloodstains
- Age estimation of bloodstains using smartphones and digital image analysis
- Differences in CMYK and RGB Color Scapes
- Color Differences