HemaScein™

Discovery and Testing for Human Blood

by

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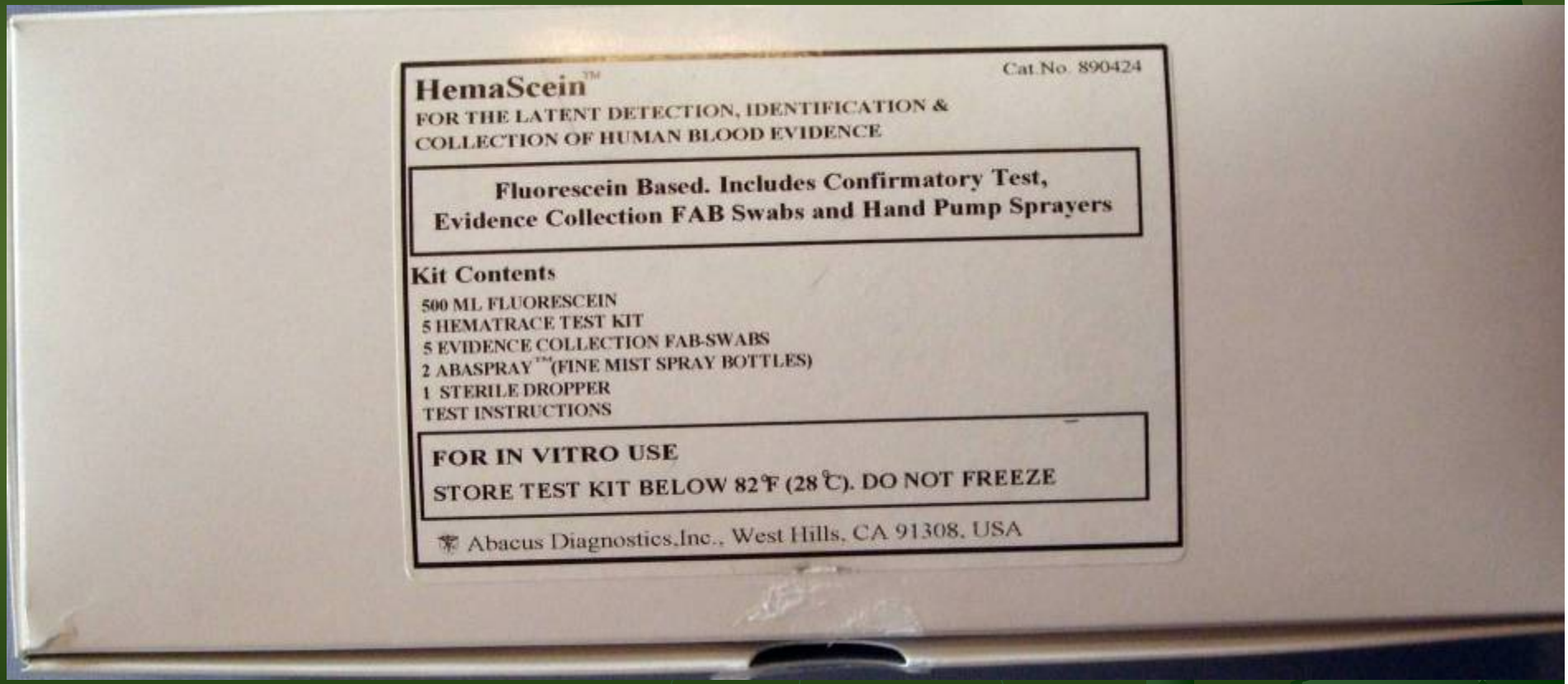
Purpose: Evaluate HemaScein™

- An easy to use kit for the presumptive discovery of human blood and the confirmation of human blood has long been needed for crime scene investigations. Abacus Diagnostics has developed the HemaScein™ kit to address this need.
- The purpose of this report is to present the results of the use of HemaScein™ by college students.

Abacus Diagnostics

HemaScein™

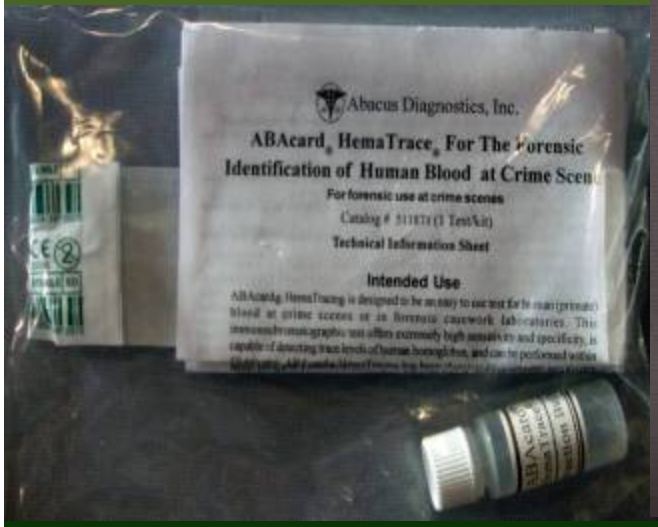
(http://www.abacusdiagnostics.com/hemascein.htm)



HemaScein™

HemaTrace Components:

The Hematrace components consist of five Hematrace cassettes, swabs for collecting blood for testing, and swabs for collecting for DNA. A positive reaction is confirmatory for human blood.



Hemascein Components: To prepare add distilled water to the fluorescein formulation product vial and shake. Allow the vial contents to settle. Add distilled water to one of the ABA Spray containers. Draw off 2 ml of the vial contents liquid and add to the ABA sprayer. Fill the sprayer to 200 ml. Fill the other sprayer with 3 % hydrogen peroxide.

The Nature of the Problem

- The location of blood is a critical component of a crime scene investigation. In many cases the blood is readily visible to the unaided eye (patent stains). In other cases there is a need to enhance blood images (patent stains plus possible latent stains), and in the third case there is a need to discover blood not visible to the unaided eye (latent stains).
- Fluorescein, luminol, BLUESTAR®FORENSIC and other products react with blood to produce a reaction that gives the appearance of emission of light. All are useful for the latent discovery of human blood, and for the presumptive identification of human blood.

The Crime Scene Investigator

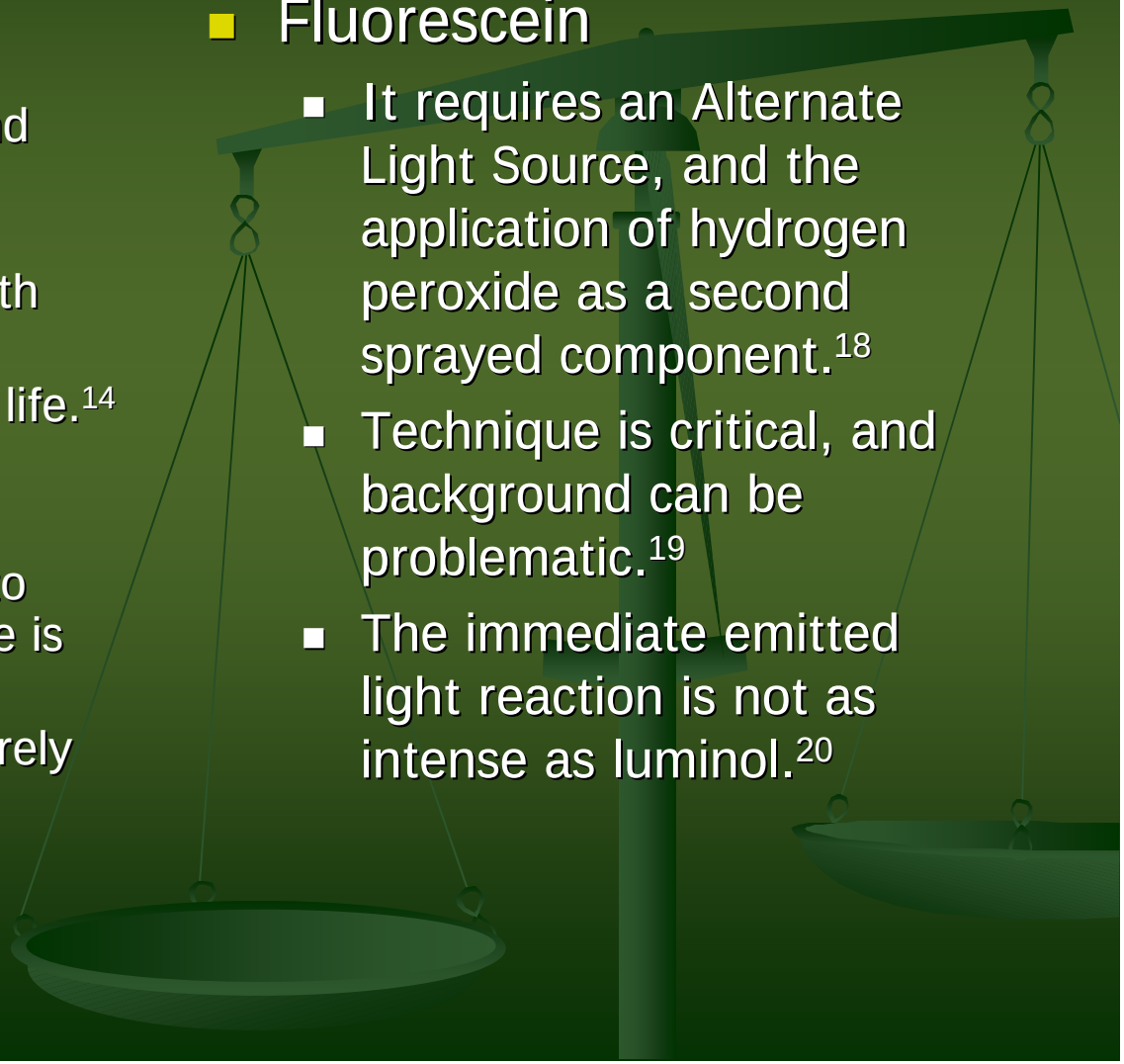
- It is important to the crime scene investigator to have a process that is easy to store, easy to prepare, and easy to apply for the detection of latent bloodstains.
- It is important that the detection process can be readily documented through photography and visualization.
- It is important that the process presents minimal physical hazard to the crime scene investigator and any subsequent persons who might come in contact with any target area.

Literature Review: Positive Features

- Luminol
  - Preparation is commercially available, only water is required to mix, no background staining, sensitivity on non-absorbent surface at 1:100,000 and on absorbent surface at 1:100.[1]
  - It does not interfere with STR analysis of DNA.[2]
  - It produces an immediate bright reaction to undiluted blood, and a faster reaction to old blood.[3]
  - BLUESTAR®FORENSIC produced an immediate bright reaction.[4]
- Fluorescein
  - Preparation is commercially available and only water is needed to mix.
  - It does not interfere with STR Analysis of DNA.[5]
  - It can be used in a less than complete dark environment, and the light emission is longer.[6]
  - The sensitivity is 2-5 times greater than luminol. Protein blood enhancement stains and dusting for fingerprints can be used after application.[7]
  - It has a low hazard to humans.[8]
  - It has a long shelf life.[9]
  - Real time photography can be accomplished with digital imaging techniques.[10]
  - Class and individual characteristics are possible end products.[11]

Literature Review: Negative Features

- Luminol
  - The reaction must be observed in the dark, and there is a short reaction time.[12]
  - It poses a potential health hazard and a liability.[13]
  - It has a very short shelf life.[14]
  - Preparation time is long term.[15]
  - Photography is difficult to do.[16] Light emission time is very short.
  - Impression detail can rarely be photographically captured.[17]
- Fluorescein
  - It requires an Alternate Light Source, and the application of hydrogen peroxide as a second sprayed component.[18]
  - Technique is critical, and background can be problematic.[19]
  - The immediate emitted light reaction is not as intense as luminol.[20]



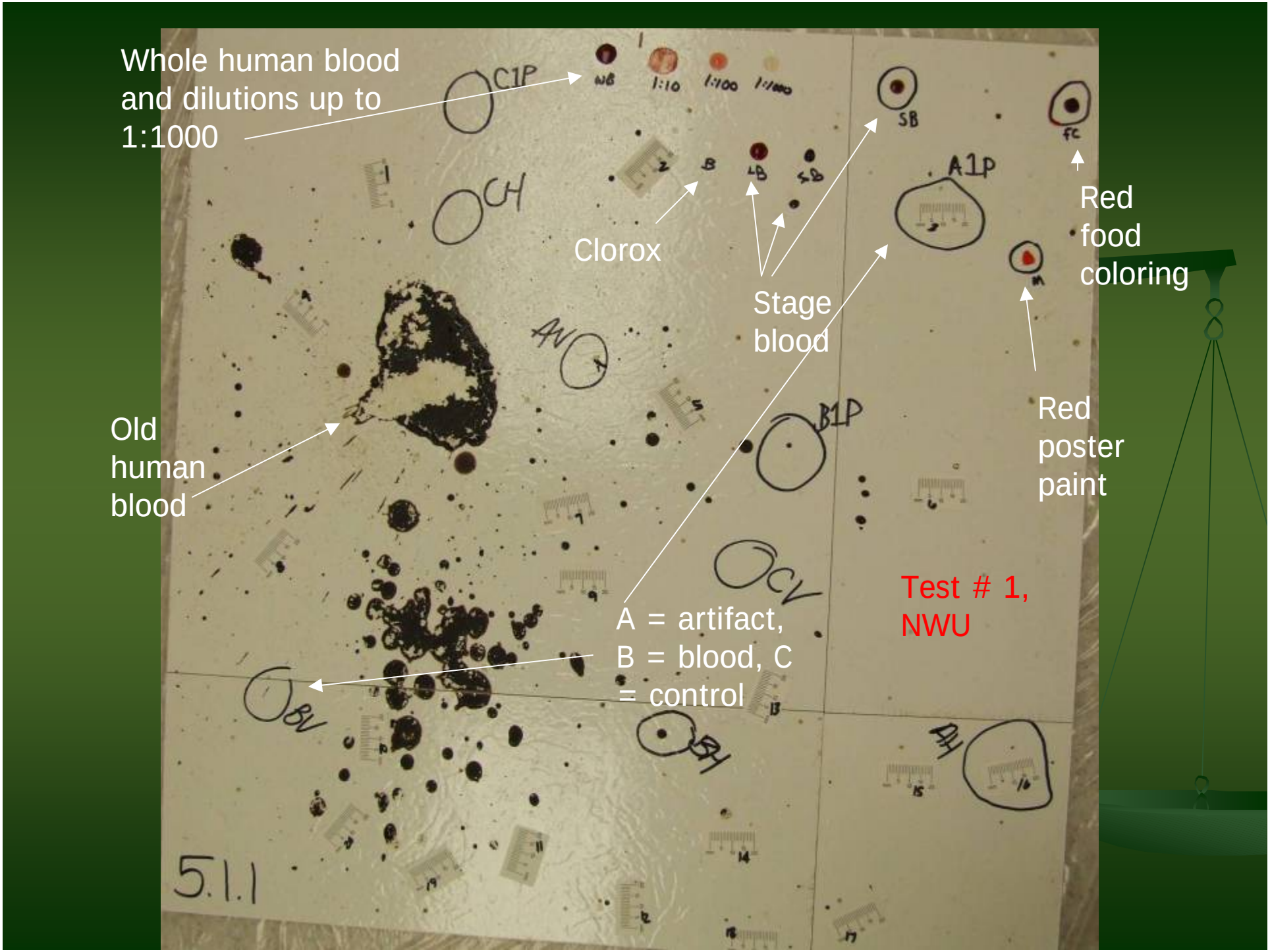
Literature Review: False Positives

- Luminol can react with cupric sulfate, ferric sulfate, and nickel chloride, but not with 5% bleach, saliva, nor potato, as examples. BLUESTAR®FORENSIC can react with potato, tomato, red onion, kidney bean, horseradish, ascorbic acid, 5% bleach, cupric sulfate, ferric sulfate, and nickel chloride.[21] Fluorescein can react with potato, non-human blood, and some oils as examples.[22]
- The products are presumptive only and some false positives are presumptive. A comprehensive and exclusive list on false positives warrants further research. See http://www.bluestar-forensic.com/gb/compare.php for additional information.

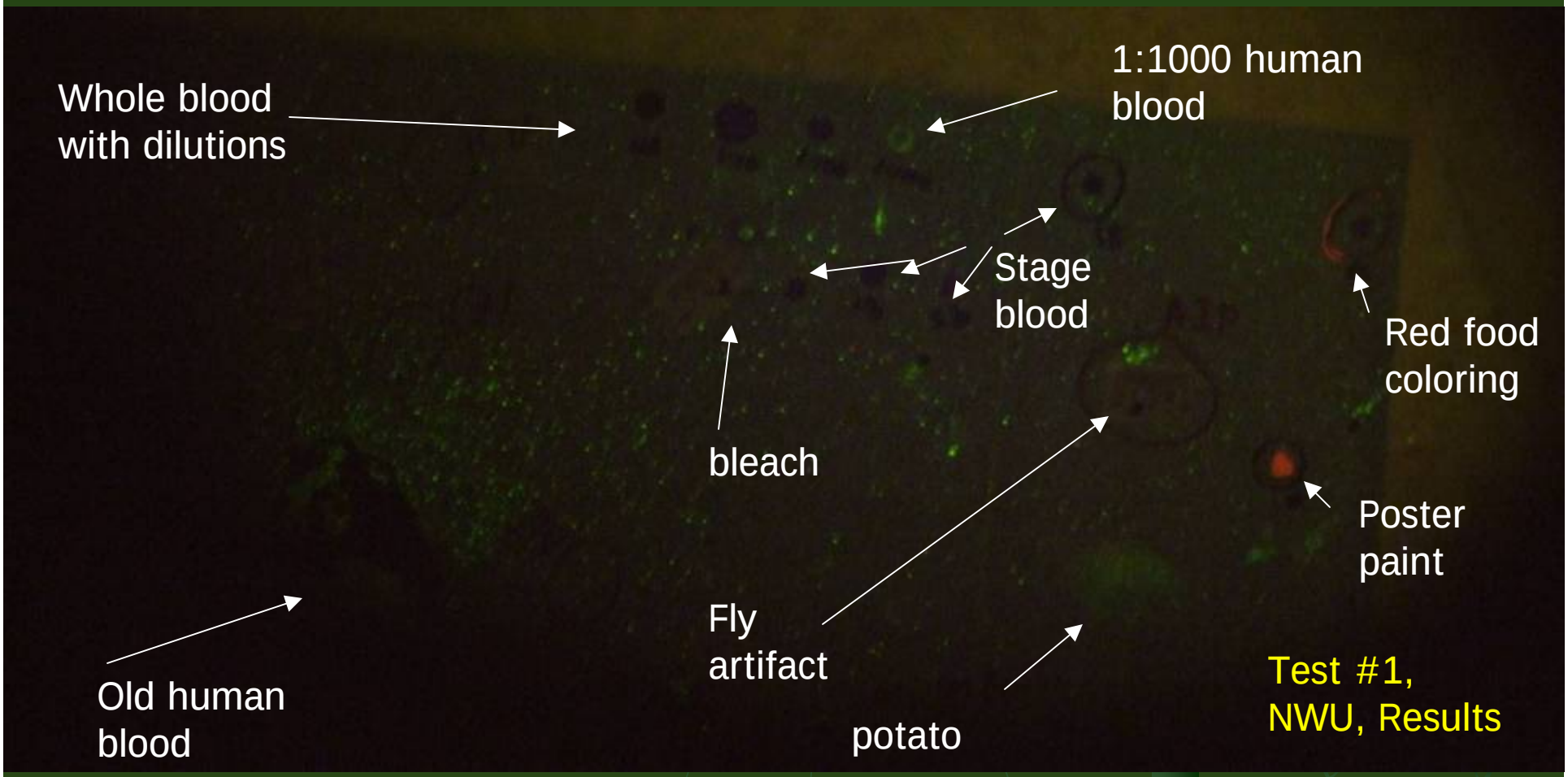
Method 1

- Nebraska Wesleyan University (NWU) Forensic Science Graduate students in an Advanced Bloodstain Pattern Analysis seminar were provided two HemaScein™ kits. They were given directions to prepare exemplars with human blood dilutions, commercial stage blood products, red food coloring, Clorox, and red poster paint. They were assigned to prepare the fluorescein product via the kit instructions, apply the fluorescin to their exemplars, and document and observe the reactions on the exemplar. They were to test known whole blood using the HemaTrace components of the kit.

- The exemplars were pieces of white “sheetrock” that had been used in bloodstain research involving fly artifacts. The exemplar came to the students with old human blood and fly artifacts.

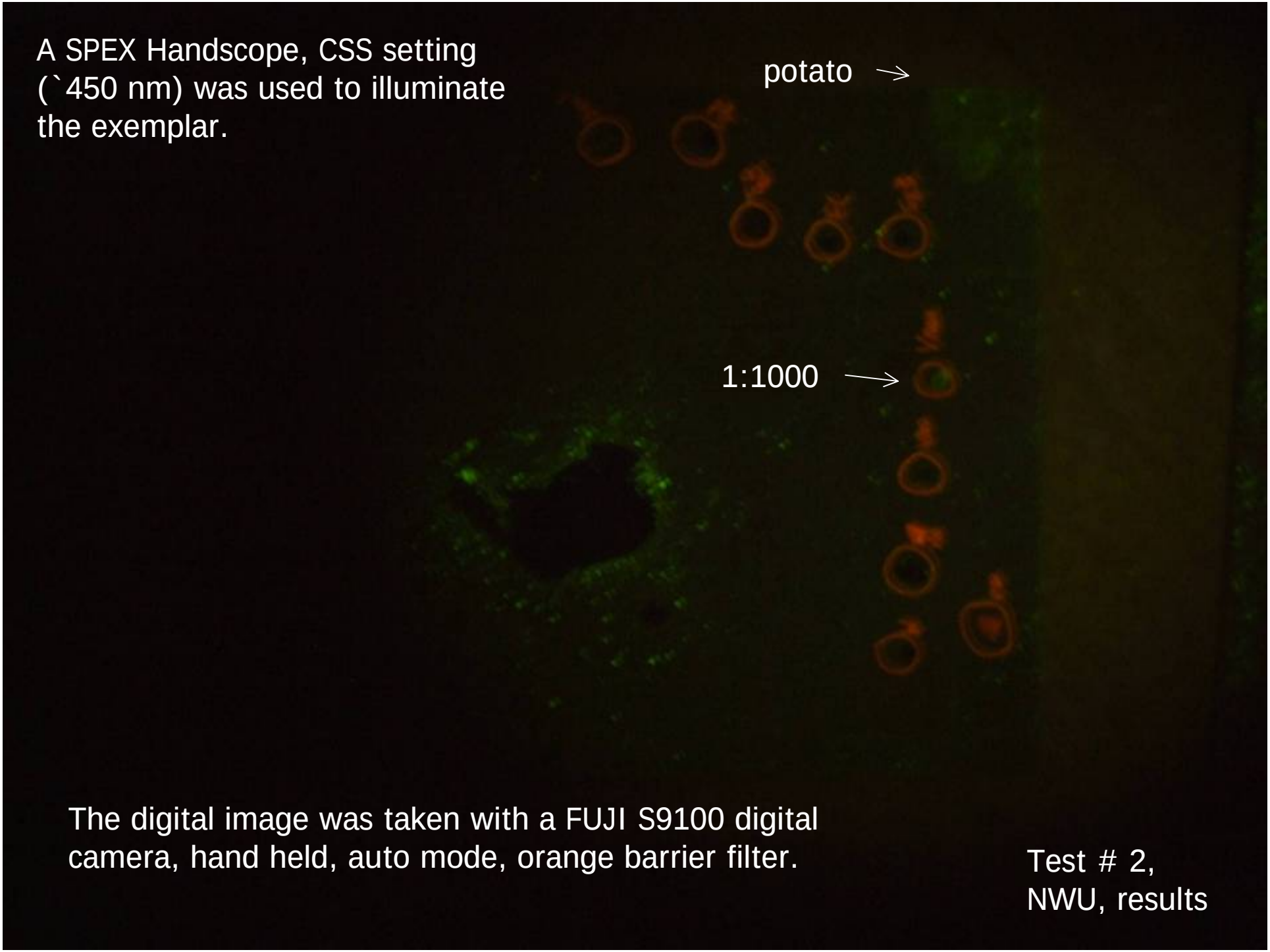

Whole human blood and dilutions up to 1:1000
Clorox
Stage blood
Red food coloring
Red poster paint
Old human blood
A = artifact, B = blood, C = control
Test # 1, NWU
5.1.1

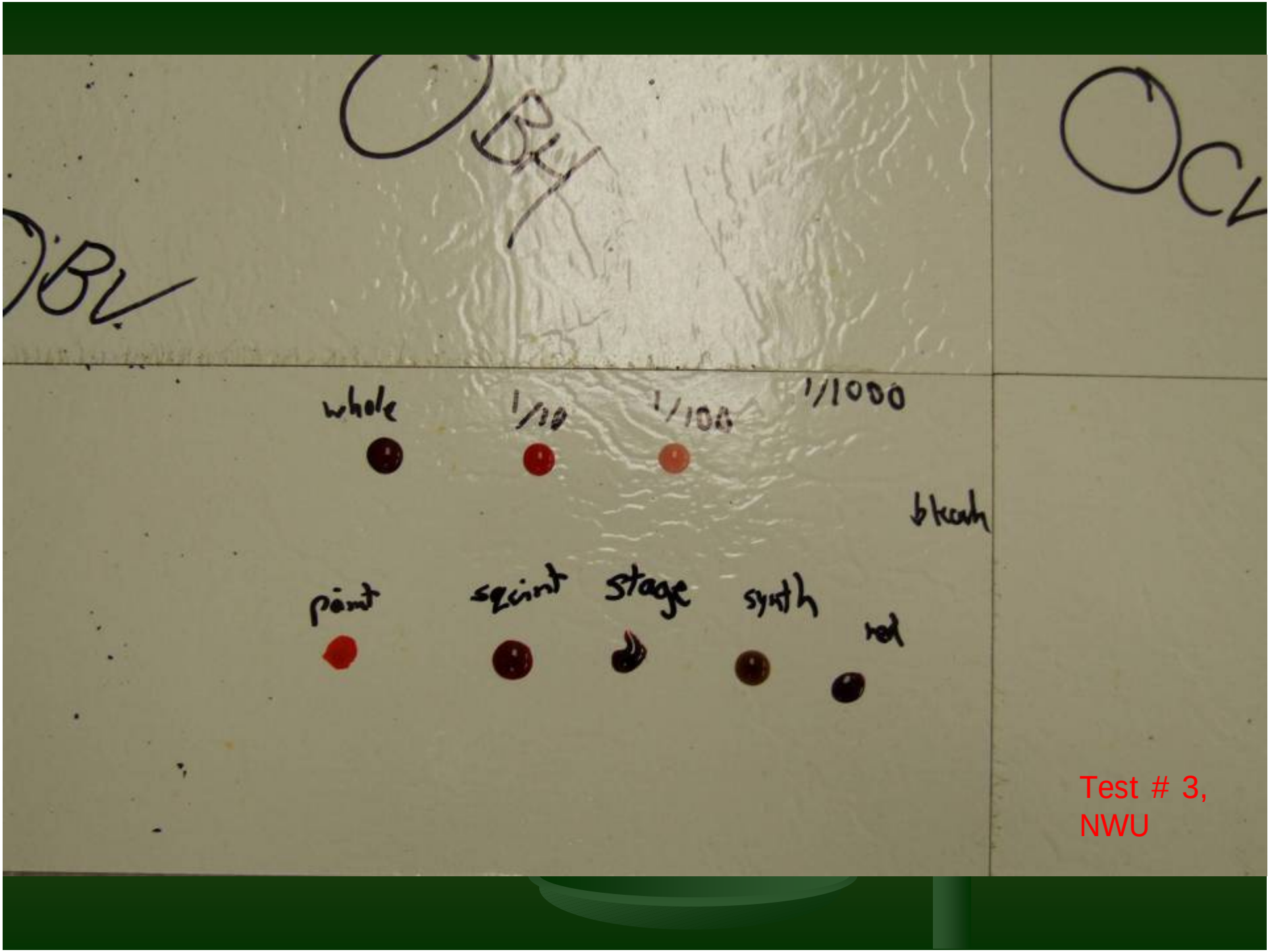
A SPEX Handscope, CSS setting (`450 nm) was used to illuminate the exemplar.



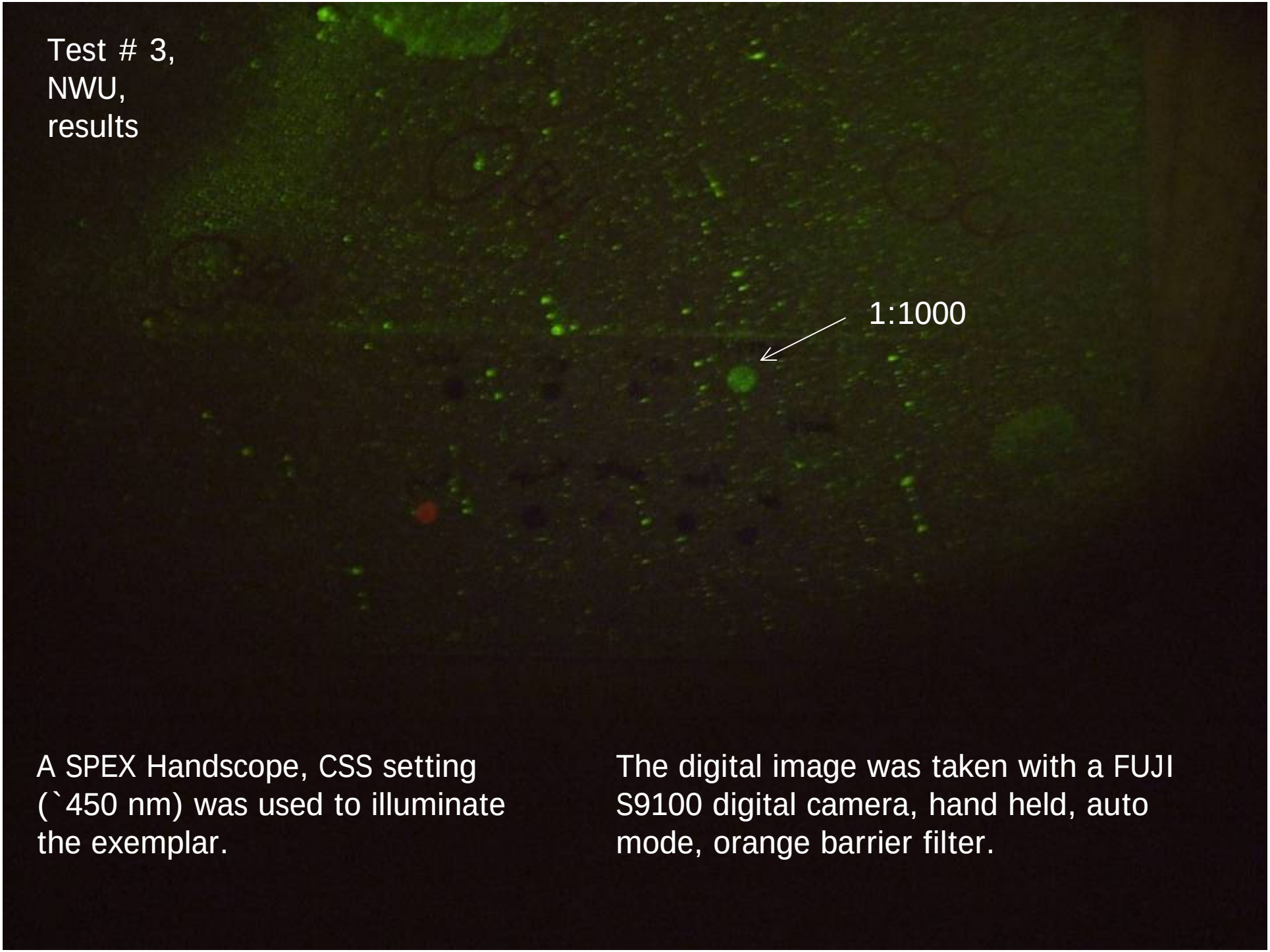
The digital image was taken with a FUJI S9100 digital camera, hand held, auto mode, orange barrier filter.

Clorox
potato
Test # 2,
NWU


A SPEX Handscope, CSS setting (`450 nm) was used to illuminate the exemplar.
potato
1:1000
The digital image was taken with a FUJI S9100 digital camera, hand held, auto mode, orange barrier filter.
Test # 2, NWU, results



Test # 3, NWU


Test # 3,
NWU,
results
1:1000
A SPEX Handscope, CSS setting (`450 nm) was used to illuminate the exemplar.
The digital image was taken with a FUJI S9100 digital camera, hand held, auto mode, orange barrier filter.

Method 2

- University of Nebraska – Lincoln (UNL) undergraduate forensic science students in a crime scene investigation class were provided a **HemaScein™**. They were given directions to prepare exemplars with human blood dilutions, commercial stage blood products, red food coloring, Clorox, and red poster paint. They were to prepare the fluorescein product via the kit instructions, apply the fluorescin to their exemplars, and document and observe the reactions on the exemplar. They were also to prepare luminol from raw materials, and BLUESTAR®FORENSIC from a commercial kit, and apply to the exemplars.

Method 2 Exemplars

- The exemplars were pieces of white “sheetrock” that had been used in bloodstain research involving fly artifacts. The exemplar came to the students with old human blood and old fly artifacts.
- Dilutions were prepared from freshly drawn human blood. Additional test spots were several commercial synthetic blood, stage blood, red food coloring, red poster paint, Clorox, and potato.
- The students didn’t photograph the reactions.

University of Nebraska Exemplar

University of Nebraska Student Observations

- Hemascein took longer to react, but lasted longer.
- Luminol reacted very fast. BLUESTAR®FORENSIC reacted fastest, and was brighter than stock luminol and lasted a little longer than stock luminol.
- Hemascein lasted longest of the three.
- All three reacted up to 1:1,000,000.

Method 3

- Nebraska Wesleyan University students were asked to test the components of the HemaScein™ kit to test for human blood. They were charged with reading the kit instructions, following the instructions, and performing a confirmatory test.
- University of Nebraska students were charged with the same process, but they were to test a known fly artifact.

Method 3 Observations

- Nebraska Wesleyan University students reported positive results with swabs from the known, old, human blood using HemaTrace. Several students commented that the instructions should be written in more clear language.
- Four University of Nebraska student groups got positive results with known, old, fly artifacts. One group got negative results. This was using HemaTrace for confirmation of human blood.

Hemascein: Findings and Observations

- Students at both universities were able to follow the kit instructions and successfully apply the Hemascein. Positive results were observed at 1:1,000 (highest level tested) for human blood (NWU), and the 1:1,000,000 (highest level tested) for human blood (UNL).
- Hemascein took some time to react. Once it reacted the emitted light presented for sufficient time to take real time digital images using the Fuji digital camera auto setting in a hand held mode. Students noted that they had to spray the surface several times with Hemascein. This was a confirmation of the ability of the sprayers to prevent over spraying of Hemascein.
- The fluorescein reaction never totally took place with all aspects of the known whole human blood.
- Hemascein clearly worked best in the student exercises with more dilute bloodstains.

Findings and Observations: HemaTrace

- Students at both universities reported some difficulty in following the instructions for confirmatory testing using HemaTrace.
- They were eventually able to follow the instructions and to successfully complete the test.
- One test on fly artifacts was negative. This was using HemaTrace.[23]
- The availability of HemaTrace in HemaScein™ was considered a positive feature of HemaScein™.

Conclusion

- Fluorescin solution as presented in the form of Hemascein is viable for detecting human blood dilutions. Hemascein reacted with potato for a false positive. It didn't seem to react with synthetic blood, stage blood, food coloring, poster paint or bleach.
- Once the Hemascein reaction took place it lasted sufficient time for real time digital imaging without long term camera exposure.
- The HemaTrace tests worked as designed on known human blood when instructions were followed as written. This allows on scene positive identification of human blood.
- HemaScein™ is a positive one stop kit for the discovery of latent blood and the confirmatory testing of blood.
- All tests (fluorescein products and luminol products) are only presumptive catalytic (peroxidase/reduction) reactions. They are subject to similar false positives variables like concentration and substrate background.

Conclusion (cont.)

- Current literature does not indicate destruction of DNA with application of fluorescin nor the amounts of hydrogen peroxide needed for the fluorescein reaction. The Hemascein formulation should present no threat to recovery of DNA.
- HemaScein™ addresses the issue of health hazards and liability with the fluorescein product as the primary blood detection product.
- The fine spray should reduce aesthetic and clean-up issues when spraying the interior of vehicles.
- HemaScein™ sprayers worked as designed. In some cases students had to spray several times to get a reaction started. The sprayers effectively addressed the issues of over spraying of fluorescin solutions causing running of the bloodstains and over spraying of fluorescin solutions causing background interference.

Epilogue: Author's Observations

- Fluorescein discovery and presumptive testing has an experiential application curve. For those used to using luminol and luminol based products the immediacy and light intensity associated with fluorescein is not as great. It takes some getting used to knowing what you should see. Once you become accustomed to what to look for, fluorescein becomes a workable product for discovery of latent blood either singularly or as an extension of patent stains.

- Fluorescin does not react quickly with whole blood. This is a positive aspect for crime scene investigators. That is, if it is observable why test for latent materials? If there is a need for enhancement, use one of the protein stains. Fluorescein and luminol products are for what you cannot see. (Fluorescein is the material added to make the solution. Fluorescin is the material in solution. Fluorescin goes back to fluorescein when reacting with blood components).

- Real time or very short time exposures are possible with fluorescein. This is due to the long reaction time and the use of the alternate light source. Photography has always been an issue with luminol and similar products due to the need for long time exposures and continuous application to sustain the light emission. The constant needed to apply luminol products often causes running of the latent blood materials and loss of detail. Heamscein offers a distinct advantage for capturing image detail by lessening the problem of overspray and maintaining a long lasting light emission.

Epilogue 3: Author Observations

- Application of fluorescein has always been an issue. Due to the longer reaction time, crime scene investigators seem to want to overspray. This can be a definite problem. Abacus has developed a superior sprayer. It is such that some students thought the sprayer was not working. The Abacus sprayer puts out a very fine low volume mist. This is a positive feature. Crime scene investigators not experienced with a fluorescein product will need to get used to using a finely engineered sprayer. It will keep them from over spraying, but they might have to make several applications. The sprayer for the hydrogen peroxide is a fine mist sprayer. This is a positive feature. It will prevent the historical issue of over spraying of the fluorescin.

- The Abacus sprayer should work very well for small areas. A motor vehicle interior, footwear, clothing, weapons, and similar targets should be the featured target for the Abacus sprayer. For large areas such as a living room of a house or a large carpet area, a larger volume sprayer would be needed for ease of application and timely results. The author most often uses a sprayer similar to a household cleaning product hand squeeze sprayer.

- Fluorescein works on Cold Cases.[24]

Epilogue 4: Author Observations

- The liability issue has loomed large in the authors experience. A luminol application created substantial liability issue in a claim to purchase a vehicle and the payment of medical bills of an adult and children who were affected by the residue luminol left behind in the application to a vehicle interior. The author is aware of similar experiences by other law enforcement agencies. Hemascein does not present this liability issue of a residual hazardous material left behind at the scene.

- The search for a product to replace luminol, based on the health hazard issues, is what lead to the authors discovery of luminol as a possible product. This came about through the published research of Rob Cheeseman and his training programs. Since the late 90's there has been a need to take fluorescein based products to the next level. Particularly to the level of addressing the issues of background and over spraying the solution. See www.rcforensic.com for additional information.

- Abacus HemaScein™ is a solution, in the author's opinion, that meets the need for a better application of fluorescein products. Hemascein is the fluorescein based product in HemaScein™. The inclusion of the Hematrace test is an added feature that should take question out of the issue of human blood. HemaScein™ as an example, could be the standard for bloodstain pattern analysis in terms of discovery and confirmation of human blood.

Epilogue 5: Author Observations

- See http://www.abacusdiagnostics.com/hemascein.htm for detailed information Hemascein.
- See http://www.bluestar-forensic.com/gb/compare.php for the PowerPoint of Jason Guffey on luminol, bluestar, and fluorescein. This site also provides information on on false positives.

End Notes

- 1. Bruce Budowle, PhD., et.al. "The Presumptive Reagent Fluorescein for Detection of Dilute Bloodstains and Subsequent STR Typing of Recovered DNA." J Forensic Sci. 2000; 45(5), p. 1091.
- 2. Cathy J. Jakovich. "STR Analysis Following Latent Blood Detection by Luminol, Fluorescein, and BlueStar." J Forensic Ident. 2007; 57(2), PP. 196-197. Budowle, et. al. reports similar results with luminol and fluorescein.
- 3. Barksdale, L. Personal Experience. This is based on personal observations by the author during teaching exercises with college students and in-service crime scene investigators. This has taken place over the past 30 years. Seven year old blood on a brown shag carpet, as an example, produced a bright blue luminol reaction within one minute. Fluorescin took over 1 hour to produce the greenish-yellow glow associated with fluorescein.
- 4. Tina Young. "Comparison of Luminol, Fluorescein, and Bluestar." J. Forensic Ident. 2006; 56(60: p. 91.
- 5. Ibid., 196-197, STR Analysis Following Latent Blood Detection...

End Notes (cont.)

- 6. Ibid., 1090, The Presumptive Reagent Fluorescein for Detection of Dilute Bloodstains ...
- 7. Cheeseman, R. “Direct Sensitivity Comparison of the Fluorescein and Luminol Bloodstain Enhancement Techniques.” J Forensic Ident 1999; 49(3): 261-268.
- 8-11. Ibid. Personal Experience. The author over the past 11 years in using fluorescein has never received a complaint from a citizen or investigator of direct health issues. Luminol and luminol based products are well known to cause high level health risks. The author has used a five year old fluorescin preparation. It is not uncommon to use a 1-2 month preparation. Luminol rarely last more than 1 hour. All preparations, including fresh preparations, should be tested on a known exemplar prior to scene application. The long lasting fluorescence from fluorescein and the alternate light source intensity combine to allow immediate digital imagining of fluorescein reactions. Thus, additional materials not required to maintain a reaction as it is in the case of luminol. This allow a higher probability of detail with fluorescein reactions. The author has captured numerous shoe wear impressions that provided class characteristic comparison.

End Notes (cont.)

- 12. Ibid., idem, 1090-1091, The Presumptive Reagent Fluorescein for Detection of Dilute Bloodstains ...
- 13. Ibid., Personal Experience. The author has been involved in several cases in which investigators reported burning to their hands and face after using luminol. Two cases have been adjudicated in which private citizens filed a claim and received compensation directly due to health issues arising from the use of luminol. The white residue left by luminol can cause burning, redness, and rash if contacted by human skin. In both cases the jurisdiction of record bought motor vehicles that had been processed with luminol.
- 14. Ibid., idem, The author has conducted personal research using bloodstain footwear impressions on white linoleum and on concrete. Luminol provided a nearly useless reaction 30 minutes after preparation. Fluorescin can still be effective years after preparation.
- 15. Ibid., idem, Luminol preparation from raw chemicals typically takes about one hour to go into solution. Commercially prepared products are much faster.

End Notes (cont.)

- 16-17. A typical luminol photographic technique is a 15 – 30 second shutter speed with an f 2.0 aperture. It is usually necessary to keep refreshing the target with luminol. This increases the probability for loss of image detail.

- 18. Ibid., idem, 1090-1091, The Presumptive Reagent Fluorescein for Detection of Dilute Bloodstains ...

- 19-20. Ibid., idem, Personal Experience. The author has noted a tendency for investigators to overspray. Fluorescein requires spraying above the target, as an example, and allowing the product to drift onto the target. Old blood can take up to one hour to react. Fresh and diluted blood typically reacts in less than one minute. The slower reaction time for those used to using luminol seems to spur a need for repeated spraying in the anticipation of a reaction. The yellowish-green fluorescein reaction is not as intense and aesthetic as that of luminol. This causes some investigators to feel the fluorescein is not working and to apply more material. There is a learning and experience curve associated with fluorescein recognition. This seems to be particularly so for those used to using luminol. Fluorescein has a tendency to produce background fluorescence. This can particularly be a problem with over spraying.

End Notes (cont.)

- 21. Tobe SS, Watson N, Daeid NN. "Evaluation of Six Presumptive Tests for Blood, Their Specificity, Sensitivity, and Effect on High Molecular-Weight DNA." J Forensic Sci 2007; 52(1): 106.

- 22. Ibid., idem, Personal Experience. The author has observed fluorescein reactions with potato, bovine blood, and motor vehicle oil. Luminol will react violently with Clorox. The author often uses Clorox as a "gee whiz" demonstration with students. Luminol and fluorescein will react with rust from lawn furniture left on concrete. Fluorescein, Luminol, and Bluestar will react with fly spots, cockroach stains, and bug splatters (not spatters) on a car windshield. See www.bluestar-forensics.com for information on false positives.

- 23. The instructor observed that several of the reactions from the fly artifacts were rather slow. Several students initially reported a negative and left their test. After walking around to look at other students results they came back and noticed theirs had a weak reaction. It may be that the one groups did not properly follow instructions, or they may have given up on their test. Further research is in order with fly artifacts and HemaTrace.

- 24. A piece of red clothing had been processed by a state forensic lab and a federal forensic lab. Both labs reported no presence of blood on the clothing. The clothing was taken from storage after about ten years from the time of the original examination and examined with the application of fluorescin. There was a reaction area. This area was swabbed and sent for DNA analysis. A full DNA profile was gotten from this swab.

References

- Barksdale, LE. "Observations of reactions times luminol and fluorescein on bloodstains." Personal Experience. 1977 - 2009.
- Budowle B, Leggitt JL, Defenbaugh DA, Keys KM, Malkiewicz SF. "The Presumptive reagent fluorescein for detection of dilute bloodstains and subsequent SSTR typing of recovered DNA. J Forensic Sci 2000; 45(5): 1090-1092.
- Cheeseman R, DiMeo LA. "Fluorescein as a field worthy latent bloodstain detection system." J Forensic Ident 1995; 45(6): 631-646.
- Cheeseman, R. "Direct sensitivity comparison of the fluorescein and luminol bloodstain enhancement techniques." J Forensic Ident 1999; 49(3): 261-268.
- Gardner, R. Practical Crime Scene Processing and Investigation. Boca Raton: CRC Press, 2005.

References (cont.)

- Jakovich CJ, "STR Analysis Following Latent Blood Detection by Luminol, Fluorescein, and Bluster." J Forensic Indent 2007; 57(2): 193-198.
- Tobe, SS, Watson, N, Daeid, NN. "Evaluation of Six Presumptive Tests for Blood, Their Specificity, Sensitivity, and Effect on High Molecular-Weight DNA." J Forensic Sci 2007; 52(1): 102-109.
- Young, T. "A Photographic Comparison of Luminol, Fluorescein, and Bluestar." J Forensic Ident 2006; 56(6): 906-912.