

# **Blood clotting, serum halo rings, and the bloodstains on the Shroud of Turin**

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## **Abstract**

One of the most interesting characteristics of the bloodstains on the Shroud is the presence of serum “halos/rings” surrounding various wounds in the ventral and dorsal portions of the image. These areas were noted in the 1978 STURP investigation during examination of the cloth under ultraviolet light. The presence of such markings led to the interpretation that clotted blood was transferred to the cloth, and thus, could not have been fabricated by the direct addition of whole blood. Relatedly, the improbability that a forger would have added the detail of “halos/rings” in anticipation of their eventual discovery by the then unknown method of ultraviolet detection has also been commented on relative to the direct addition of blood to the cloth. Here, we have investigated various characteristics of blood clotting and accompanying serum halos/rings and discuss these findings in relation to what is present on the Shroud.

## **Introduction**

Whole blood consists of a cellular portion, which is approximately 45%, and a liquid portion, plasma, which is approximately 55%. The plasma contains various blood clotting enzymes, which function in a cascade fashion to help blood to coagulate following injury. Once clotting has occurred, the liquid portion of the blood is referred to as serum; serum is essentially plasma minus the clotting factors (1). Numerous factors may influence the clotting and drying time of

blood *ex vivo*, including blood volume, relative humidity and temperature, and surface material (2).

The Shroud of Turin is an approximately 14 feet by 3.4 feet linen cloth containing the faint ventral and dorsal images of a man, with wounds corresponding to scourging and crucifixion. In 1978, a series of studies were performed on the Shroud as an initial attempt to characterize its basic features and the images upon it. To date, this investigation remains the main depository of information about the object, particularly in relation to the bloodstains. Multiple blood components were chemically demonstrated to exist within the Shroud bloodstains, including hemoglobin, bilirubin, and albumin (3-5). Interestingly, for many of the wounds examined, a fluorescent border was noted under ultraviolet (uv) light, which was suggested to represent serum “halos” or “rings” at the periphery of the bloodstain (6). The main body of the bloodstain was highly absorbing, in agreement with what has been reported for blood analysis under uv (6,7). Proponents of the Shroud’s authenticity argue that the presence of serum contraction rings support the hypothesis that the cloth once wrapped a body, and that blood was imprinted from actual wounds. Skeptics, on the other hand, might suggest that the blood was directly added to the cloth by an artist.

In searching the literature for information about formation of serum “halos/rings” and their detection using uv light, it was surprising that relatively little information that exists on this topic. To better understand what has been described on the Shroud, and to evaluate the properties of formation/detection of serum contractile rings in general, we performed a series of studies under a variety of conditions. Of particular interest was to evaluate the similarities and differences of transfer of clotted blood to material, versus blood which was directly added.

## Materials and Methods

**Blood, plasma, and serum.** Human blood was obtained from healthy volunteers by the finger stick method using a Health Lancing device (CVS pharmacy, USA) fitted with a micro lancet (CVS Pharmacy, USA). For transfer experiments, blood was added to parafilm “M” laboratory film (Bemis, Amazon.com, USA) or other materials and filter paper or cloth placed directly onto the blood pool with very little pressure. Twenty to 25 microliters of blood was typically used for each group. For purification of blood serum and cellular fractions, whole blood was spun in a microcentrifuge, the supernatant was removed and then transferred to a new tube. This cycle was repeated 2x for the supernatant until no red material was visible. Whole blood and serum from Gunn rats was obtained from RRRC, University of Missouri, USA. The Gunn rat contains a spontaneous mutation in the UDP-glucuronosyltransferase (*ugt*) gene and expresses high levels of bilirubin (8). Whole blood and serum from *ugt* <sup>-/-</sup> and *hugt* <sup>-/-</sup> mice was kindly provided by Drs. Robert Tukey and Nghia Nguyen, University of California, Sand Diego, USA. In *ugt* <sup>-/-</sup> mice, the UDP-glucuronosyltransferase gene has been genetically disrupted; these mice express extremely high (lethal) levels of bilirubin. In *hugt* <sup>-/-</sup> mice, the UDP-glucuronosyltransferase gene has been genetically disrupted and replaced with a human counterpart; these mice express high levels of bilirubin (9).

**Linen and Filter Paper.** Linen of various sources was used, including that which was purchased commercially or woven from natural, unprocessed flax. Filter paper sources were Whatman filter paper 1mm and 3mm (Whatman, Amazon.com, USA).

**Ultraviolet light detection.** Ultraviolet light systems were used throughout these studies at an output of ~365 nm. A special thanks to Dr. Pellicori who provided excellent advice on a modern, miniaturized version of the system that was used in the previous study of Shroud.

## **Results**

### **UV detection of blood components**

In our initial set of experiments, we examined the detection of plasma and serum using ultraviolet light. As shown in Figures 1a,b, whole blood and the cellular fraction showed high absorbance (no fluorescence), whereas serum and plasma were the opposite (Figures 1a and 1b). Fluorescence of plasma and serum were of relatively similar intensities (Figures 1a and 1b). Next, we examined the fluorescence of serum absorbed onto different materials, including linen from various sources. As demonstrated, serum fluorescence was variable among different linen samples (Figure 2); a feature we have noted using over twenty different types from multiple sources. Thus far, we have not been able to isolate a specific characteristic (color, washing, dyeing) that is directly related to optimal fluorescent detection. We have also observed an influence of oven baking of linen to simulate aging on the fluorescence of serum under uv (data not shown).

## **Formation and detection of serum halos/rings**

To evaluate the presence of serum “halos/rings” resulting from blood transfer, whole blood was placed on parafilm, and clotting allowed to proceed before transfer to filter paper (Figure 3). This system is similar to that described by Lavoie except that in his studies, blood was added to saran wrap (10); and the system described here is performed in microscale using relatively small volumes of blood. As shown in Figure 4, in samples that had undergone clotting prior to transfer, a serum edge was present at the periphery (Figure 4, arrow); similar results were obtained when linen was used (Figure 5). The appearance of serum edges in blood imprints was time-dependent, occurring optimally at about 40 minutes (Figure 6); imprinting was performed while blood was in the gelatinous (moist) phase of clotting. Serum edges were also detected when blood was placed onto skin, and clotting allowed to proceed prior to transfer (Figure 7). In contrast, no serum edge was evident when blood was directly added to filter paper (Figure 8), even when a variety of applicators was used (Figure 9). Similar results were obtained when blood was directly added to linen (data not shown). Blood that had clotted for several hours was vigorously stirred and added directly, yet no serum edge was evident (Figure 10). Relatedly, blood that had clotted and dried for several days was ground in a mortar and pestle, rehydrated and directly added with a similar result, i.e. no serum ring was visible (Figure 10). When blood was allowed to clot for 10, 20, 30 minutes, mixed, and then added directly to material, no serum edge was visible (data not shown). Taken together, these results demonstrate that blood added directly to material did not result in the presence of a fluorescent serum edge/halo detectable by uv light. Serum edges/halos were only observed only when blood was imprinted during the clotting process.

We next investigated whether it was possible, under any conditions, to create bloodstains with a “serum edge” by adding blood directly, i.e. blood plus certain additives. Citric acid is commonly used as an anticoagulant during modern blood collection, and may be found in the liquids of many common fruits. Art Lind has previously commented on the use of lemon juice as an anticoagulant in studies on the painting of blood onto various materials (11). As shown in Figure 11, juice from multiple fruits showed a similar uv fluorescence as serum (Figure 11), as did honey and collagen (gelatin) (Figure 11). This was also the case with connective tissue and bone that had been ground and hydrated (Figure 11). Figure 12 shows the uv fluorescence of juice from various regions of pineapple (Figure 12); pineapple is particularly interesting in that it contains bromelain, a compound that is effective at disrupting clotted blood. Next, a composite of four samples of blood is featured (Figure 13), one of which represent a (normal) clotted imprint, the other three were created by direct addition of blood containing some of the substances shown in Figure 11. In Figure 14, it can be seen that when a mixture of blood and alcohol/honey or blood and fruit juice was added directly to material, a peripheral edge was observed, similar in appearance to that seen in transfers of clotting imprints (Figure 14).

### **Bilirubin and serum fluorescence**

Regarding natural blood components that might affect fluorescence, we evaluated human serum plus excess bilirubin added *in vitro* (Figure 15), and serum from rodent strains with endogenously high levels of serum bilirubin (Figure 16). As demonstrated, increased amounts of bilirubin was associated with increased fluorescence under uv, in both a simulacrum with human blood, as well as rodent strains with hyperbilirubinemia (Figures 15,16). In all cases, bilirubin fluorescence diminished with time, even in the presence of little to no light (data not shown).

It is often proposed that the high bilirubin levels that have been reported for Shroud bloodstains are the result of a severe physiological trauma. Several years ago, Dr. David Lee's lab made the surprising discovery that certain plants also express bilirubin, bilirubin that is chemically indistinguishable from that found in humans (12,13). Conceivably, a skeptic might argue that an artist chose a plant with an orange/red pigment to mix with blood for coloration, and unknowingly also added bilirubin (in a relatively concentrated form). This is an intriguing idea as plant and human bilirubin are chemically identical. It is curious that bilirubin, a molecule that is extremely light sensitive, is found in the flowers and fruits of plants, an organism whose survival depends upon efficient light collection. Perhaps there is an associated molecule in plants that helps to confer stability of bilirubin, although this is entirely speculation.

#### **A few final comments on the blood first/image second hypothesis**

Finally, one of the most clever and intriguing experiments that Adler performed is the digestion of Shroud fibers from various locations with protease enzyme. First, he found that fibers from non-image areas were unaffected as were those from image locations (Figure 17). This is important as it argues against a protein binder being involved in the creation of the image. Second, when fibers from bloodstains were digested, no coloration (image) was found underneath. These results have been used to support the blood first/image second hypothesis, that is, that bloodstains initially present on the cloth protected the fibers underneath from receiving the image during the successive image formation process. Blood first/image second fits best with an authentic scenario; it is somewhat difficult to imagine a forger applying the bloodstains first and then creating the image around it.

The Shroud image is believed to result from oxidative/dehydration, conversion of single bonds to double bonds that result in the coloration of fibers. Heller and Adler reported that they could, in fact, reverse the fiber coloration by treatment with a strong oxidizing or strong reducing agent (3,4). As food for thought, consider the following idea which is compatible with either an authentic or nonauthentic scenario: something is present in the blood that acts as an oxidizing/reducing agent, and that is why there is no image underneath (Figure 18). This could be an endogenous blood component (either behaving normally or in an altered state following the imaging event), or an added component. Several of the compounds mentioned previously, such as citric acid, are known reducing agents; whether strong enough to affect fiber coloration is unknown. This idea provides an alternative to a physical blocking mechanism, a molecular basis and chemical explanation as to why fibers underneath bloodstains are devoid of coloration. Adler's observation is very important and worth further investigation to confirm and extend these findings. Indeed, it is unknown if this property is true for the majority of bloodstained areas on the cloth.

## **Discussion**

The current study was motivated by the observation that serum contractile rings were reported for various wounds on the Shroud of Turin, originally visualized under ultraviolet light. This characteristic is often mentioned in support of the authenticity of the Shroud, and the conclusion that the cloth once wrapped a dead body. Adler proposed that a medieval forger would have had to anticipate the forthcoming discovery of uv light, and to "to paint a contractile ring [of serum] around every wound" to achieve a similar result (5). The results in this study indicate that this is not necessarily the case. While our data support the idea that imprints of clotting blood showed

serum edges/rings, whereas blood that was directly added does not; evidence was also included to show that serum edges/rings may be created by inclusion of certain additives, known to inhibit/retard coagulation. A forger could have perhaps used such additives for their ability to retard clotting or as a blood “thinner”, without realization of their fluorescent properties. The current study represents a preliminary investigation into the formation and detection of serum halo/rings in a microscale environment. Future efforts will involve investigation using increased blood volumes and parameters that affect textile surface properties.

An association between bilirubin levels and increased fluorescence was noted, with the caveat that fluorescence fades over time, even in little to no light. It was also pointed out that certain plants express bilirubin which is chemically indistinguishable from human bilirubin. The suggestion was made that perhaps plants contain an associated molecule that helps confer bilirubin stability as plant life is typically dependent upon light collection and exposure.

Finally, an alternative explanation was put forth for the blood first/image second hypothesis, based on previous protease digestion studies by Adler. Namely, the idea was suggested that an oxidizing/reducing agent is present in blood (either naturally or added) that reverses the color of the image fibers underneath. This is entirely speculation at this point, but was proposed as food for thought, as an alternative to a physical blocking mechanism for protection of fibers underneath bloodstains.

## **Acknowledgements**

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As always, thank you to my wife Kathy, for everything.

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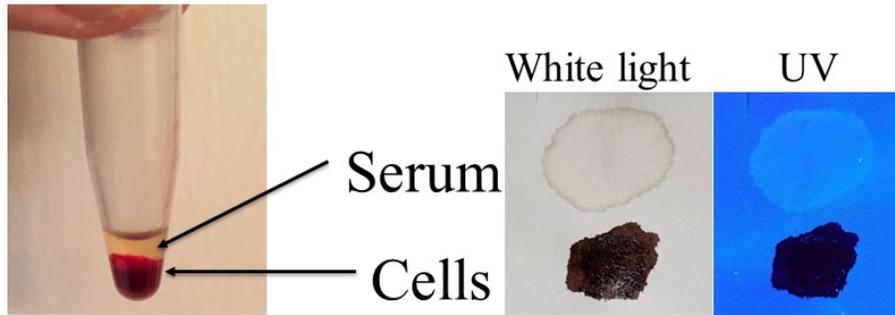
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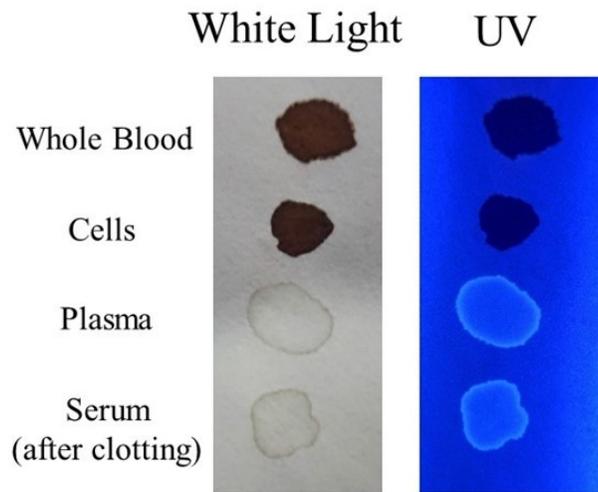
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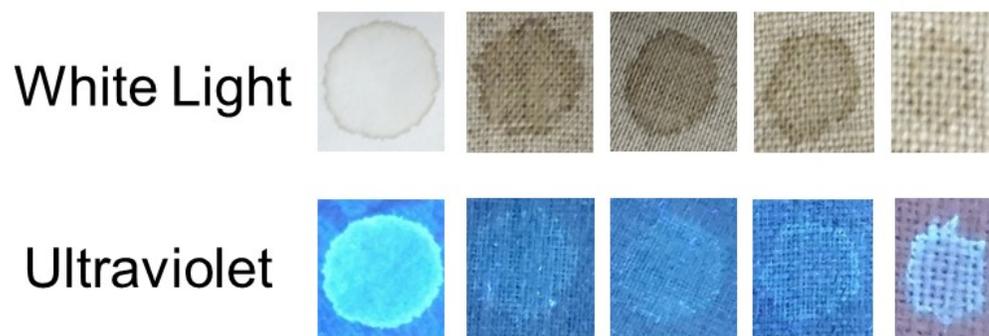
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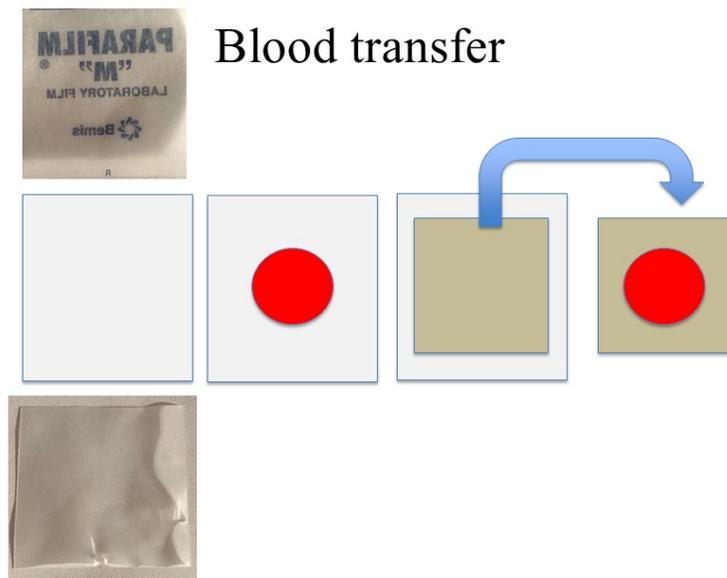
**Figure 1a. Separation of blood components and their detection by uv.** Clotted blood was spun in a microcentrifuge and separated into cellular and serum fractions. Samples were added to filter paper and examined under normal (white) light and ultraviolet (uv).



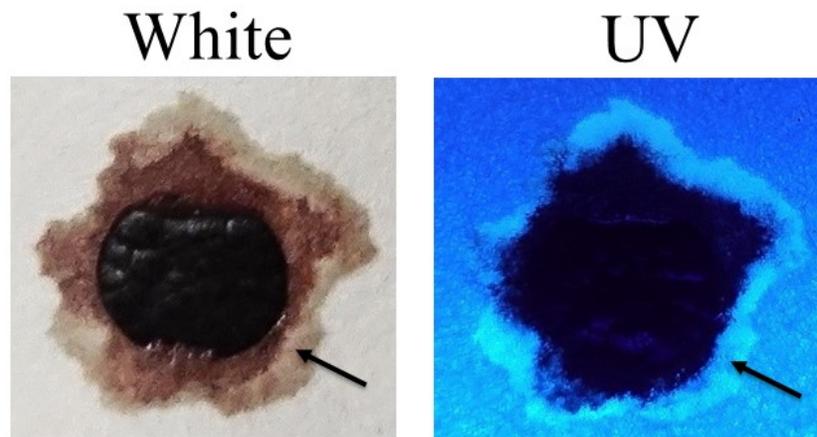
**Figure 1b. Uv detection of blood components.** Blood fractions were added to filter paper and examined under normal (white) light and ultraviolet (uv).



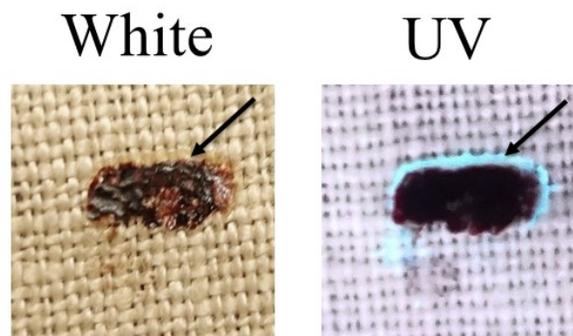
**Figure 2. Uv fluorescence of serum on various linen samples.** Purified serum was added to filter paper (far left) and various linen types, and examined under normal (white) light and ultraviolet (uv).



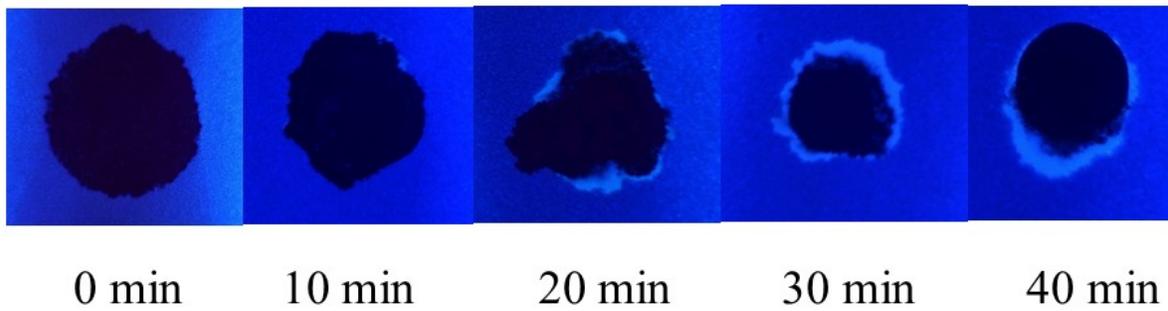
**Figure 3. Blood imprinting/transfer system.** Blood was added to parafilm and after a certain period of time, filter paper or linen was placed on top, and allowed to dry. Following drying, filter paper or linen was removed and evaluated.



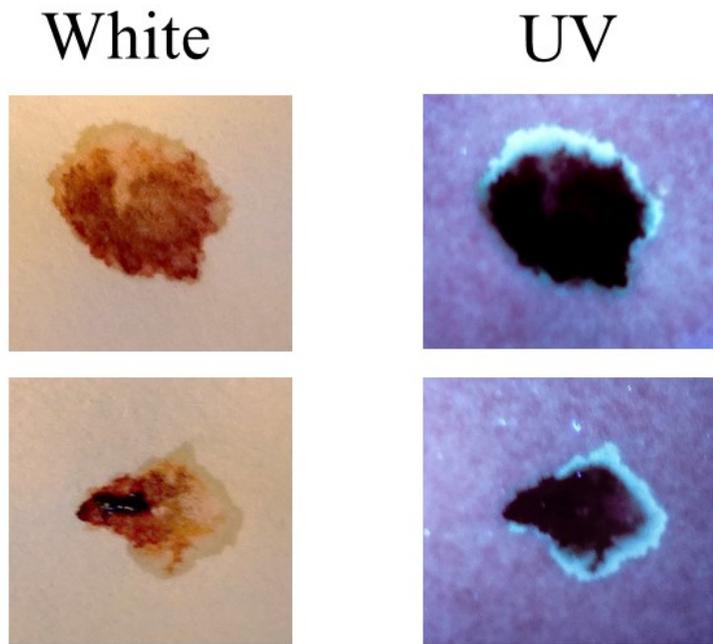
**Figure 4. Evaluation of clotted blood using direct and uv light.** Blood was applied to parafilm and allowed to clot for ~40 minutes, then filter paper placed on top. Following drying, filter paper was removed and examined using normal (white) light and ultraviolet (uv).



**Figure 5. Evaluation of clotted blood using direct and uv light.** Blood was applied to parafilm and allowed to clot for ~40 minutes, then linen placed on top. Following drying, linen was removed and examined using normal (white) light and ultraviolet (uv).



**Figure 6. Time dependence of serum halo/edge detection using uv light.** Blood was applied to parafilm and allowed to clot for the time period indicated. At that time filter paper was placed on top, and the sample allowed to dry. Following drying, filter paper was removed and examined using ultraviolet (uv) light.

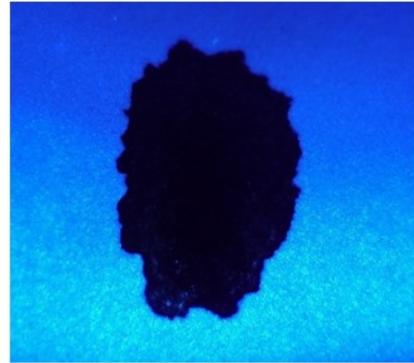


**Figure 7. Evaluation of serum halo/edges formed on skin prior to imprinting.** Blood was applied to skin and allowed to clot for ~ 40 minutes; at that time filter paper was placed on top, and the sample allowed to dry. Following drying, filter paper was removed and examined using white and ultraviolet (uv) light.

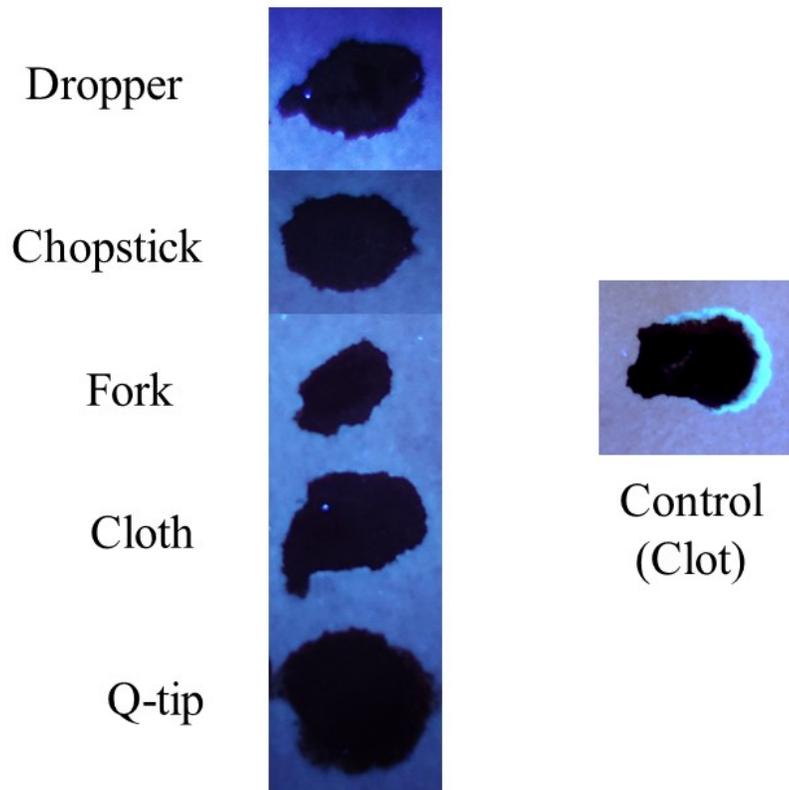
White



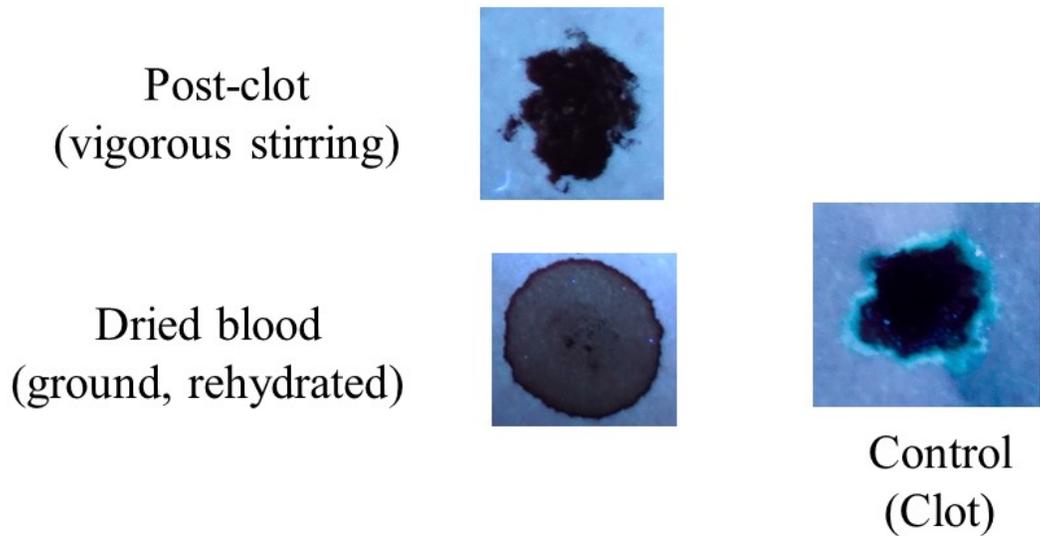
UV



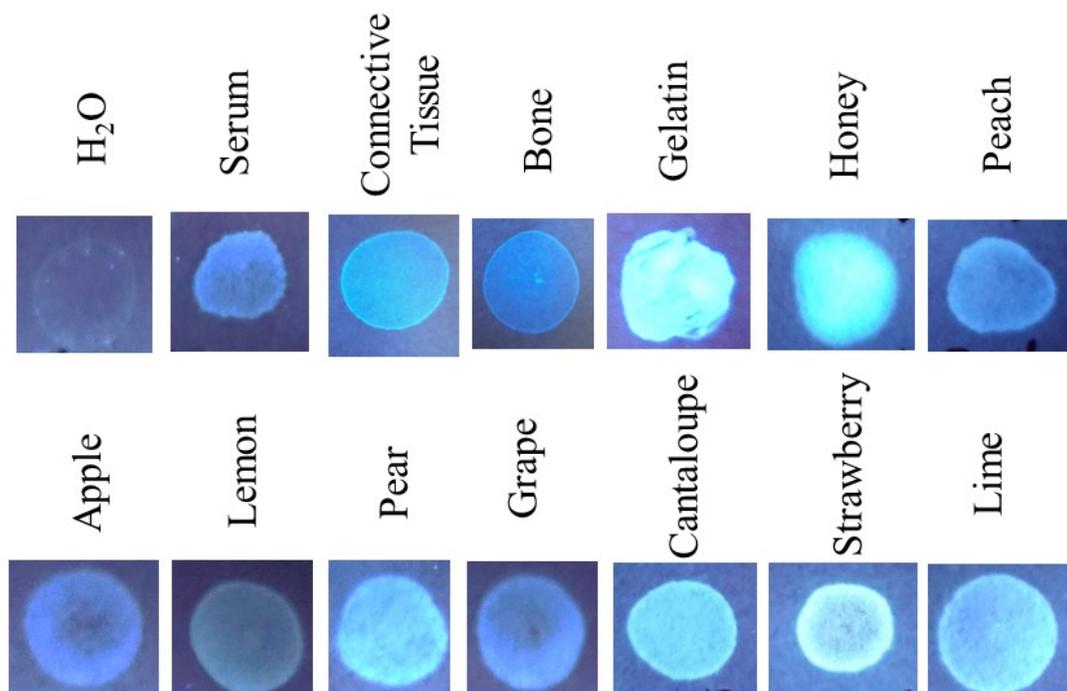
**Figure 8. Evaluation of serum halo/edges in blood added directly to filter paper.** Blood was added directly to filter paper, allowed to dry, and examined using normal (white) light and ultraviolet (uv).



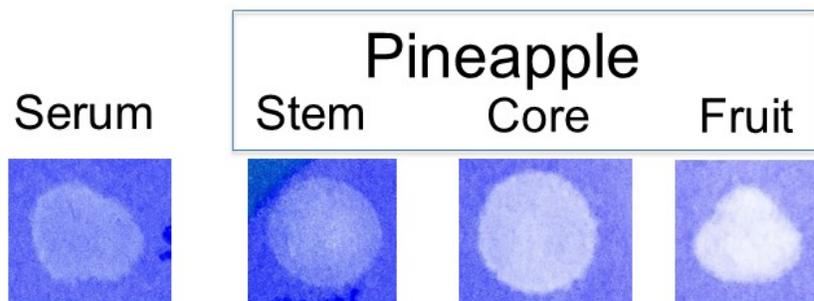
**Figure 9. Evaluation of serum halo/edges in blood added directly to filter paper.** Blood was added directly to filter paper using the indicated applicator, allowed to dry, and examined using ultraviolet (uv) light. Clotted blood is shown as a control.



**Figure 10. Evaluation of serum halo/edges in blood added directly to filter paper.** Top: Blood was allowed to clot for several hours, and the clot disrupted by vigorous stirring with a micro pipet tip. Blood was then transferred directly to filter paper and examined using uv light. Bottom: Clotted blood that had dried for several days was ground using a mortar and pestle, rehydrated with a small volume of distilled water, and added directly to filter paper. Samples were examined using ultraviolet (uv) light. Clotted blood is shown as a control.

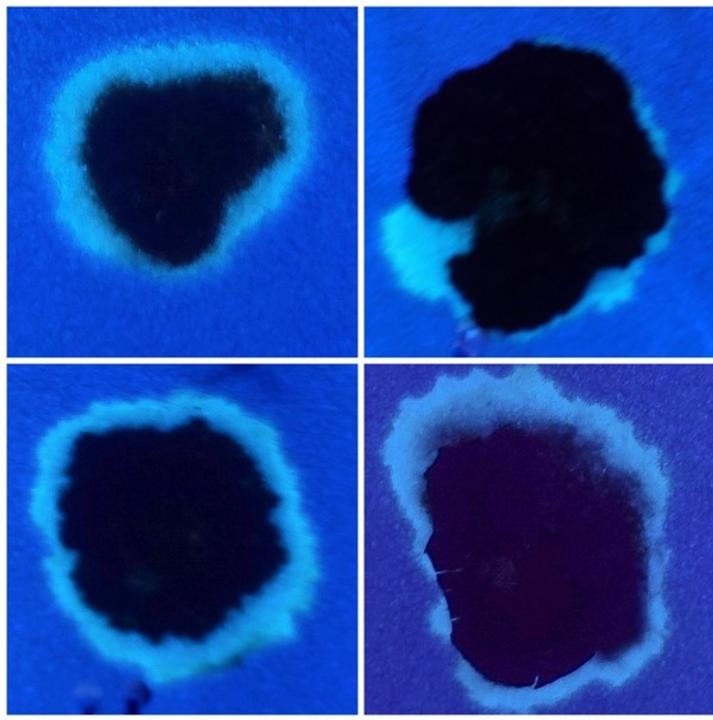


**Figure 11. Fluorescence of various solutions under ultraviolet light.** Approximately 10 microliters of the indicated solution was added to filter paper and evaluated under uv. Water (far left) is included as a wetting control, showing that merely wetting the filter paper did not result in a similar fluorescence. All juices were freshly squeezed.

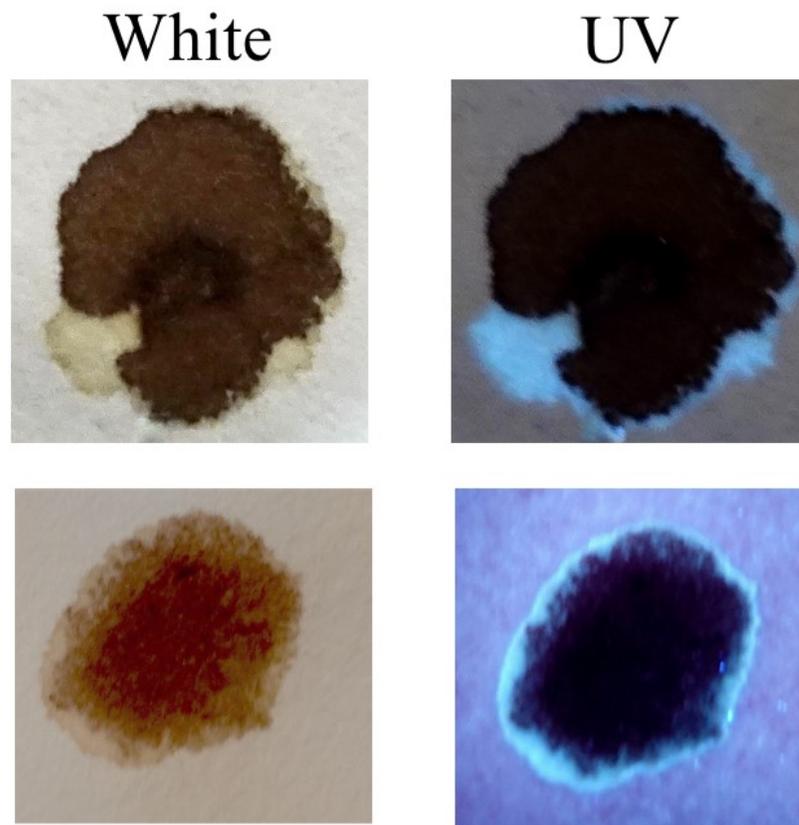


**Figure 12. Fluorescence of juice from various pineapple regions under ultraviolet light.**

Approximately 10 microliters of the indicated solution was added to filter paper and evaluated under uv. All juices were freshly squeezed.

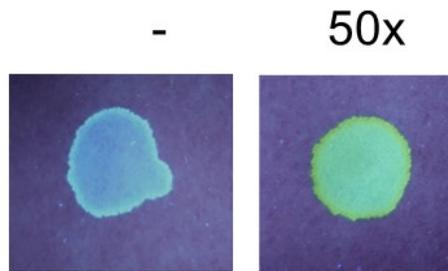


**Figure 13. Composite showing serum halo/edges formed in clotted blood and blood (plus additives) transferred directly to filter paper.** One of the pictures depicts clotted blood transferred to filter paper; the other three show fresh blood (plus additives) transferred directly to filter paper. After drying, samples were evaluated under ultraviolet (uv) light.

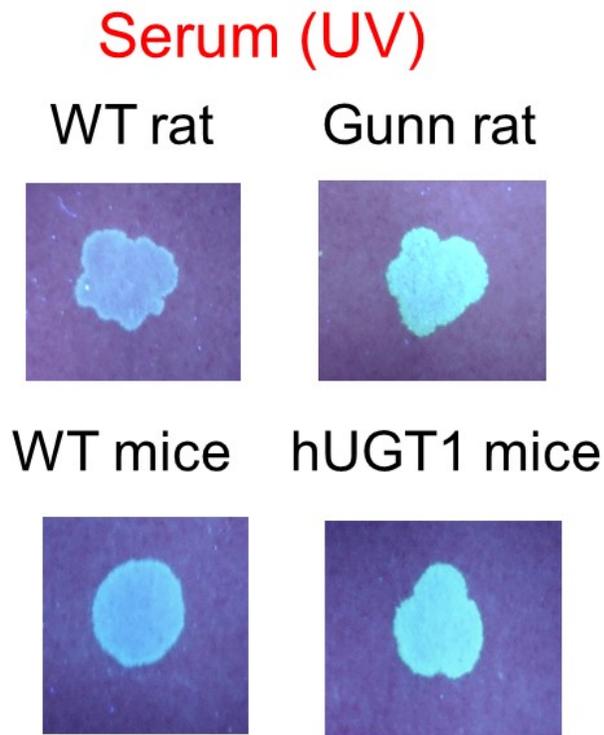


**Figure 14. Evaluation of serum halo/edges in blood added directly to filter paper.** Top row: Blood plus a honey/alcohol mixture was added directly to filter paper and allowed to dry. Sample were examined using normal (white) light and ultraviolet (uv). Bottom row: Blood plus lemon juice was added directly to filter paper and allowed to dry. Sample were examined using normal (white) light and ultraviolet (uv).

## Human Serum plus Bilirubin



**Figure 15. Effect of bilirubin on ultraviolet fluorescence of human serum.** Human serum containing normal or 50x elevated amounts of bilirubin was added to filter paper and evaluated under ultraviolet light. Serum containing excess bilirubin was created as an *in vitro* simulacrum.



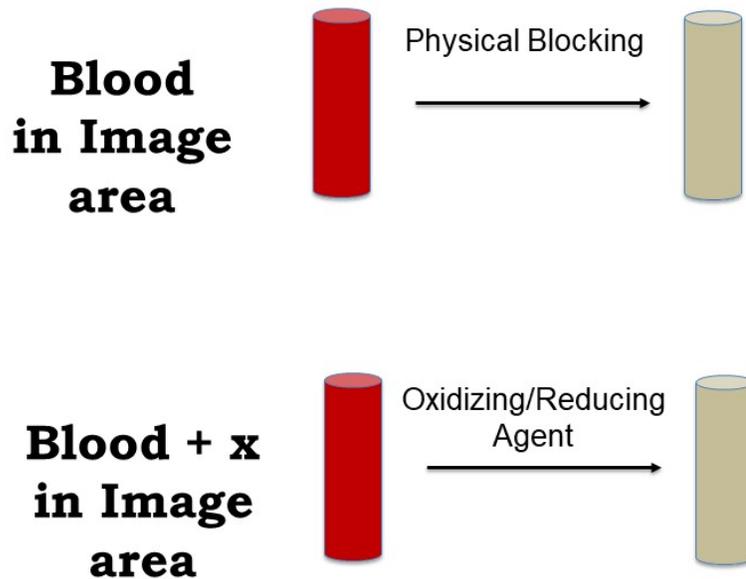
**Figure 16. Effect of bilirubin on ultraviolet fluorescence of rodent serum.** Top row: serum from wild type (WT) and Gunn rats was added to filter paper and evaluated under ultraviolet light. Gunn rats contain high levels of bilirubin in their serum. Bottom row: serum from wild type (WT) and hUGT1 mice was added to filter paper and evaluated under ultraviolet light. hUGT1 mice contain high levels of bilirubin in their serum.

## Protease Treatment



**Figure 17. Adler's protease digestion experiment.** Fibers from three different areas of the Shroud were treated with protease enzymes and evaluated for coloration. See text for details.

## Protease Treatment



**Figure 18. Two hypotheses to explain the results of Adler's protease treatment**

**experiments.** In the top drawing, bloodstains physically block the fibers (underneath the blood coating) from the imaging event. When samples are digested with protease, the fibers are shown to be uncolored (no image) underneath. In the bottom drawing, a substance in blood (x) functions as an oxidizing/reducing agent to reverse the coloration of fibers that has occurred during the imaging event. The top drawing is primarily favored with an authentic viewpoint, blood first/image second; the bottom drawing is compatible with either an authentic or nonauthentic viewpoint and provides an alternative explanation for the obtained results.