Final Technical Report

Report Title: Bloodstain Patterns on Textile Surfaces: A Fundamental Analysis

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Abstract

Bloodstain pattern analysis, BPA, on hard surfaces (such as walls, tables, appliances, hardwood floors, etc.) has grown into a science-based investigative tool that can help determine scenarios that are consistent with or counter to the events described by witnesses or suspects. At the vast majority of crime scenes involving a bloodletting event, textiles are present as apparel, household textiles (sheets, towels), upholstery, carpets, and so forth. Yet, the science of BPA is not able to render the same level of confidence in the analysis as on hard surfaces due to the complex structure of textiles and their ability to wick liquids. In the work described herein, a detailed examination of factors that affect BPA on two textile fabrics, an unbalanced 130 x 70 plain woven 100% cotton bed sheeting fabric (often referred to as a 200 thread count bed sheet) and a 100% cotton jersey knit T-shirt fabric.

During this study, both porcine blood and several synthetic blood recipes were used. The dynamic impact tests (time after impact < 100 ms) used porcine blood, while most wetting and wicking experiments employed synthetic blood (time after impact > 100 ms). Most of the synthetic blood recipes examined performed badly. Either they would not dry or they did not wick into the fabrics, but remained on the surface. A synthetic blood recipe from the American Society for Testing Material (ASTM test method F1819-07) performed well, but its viscosity and surface tension were both lower than typical human blood. Thus, this recipe was modified to lie within the range of surface tension and viscosity of human blood. It was used for the majority of wicking and wetting experiments. In a preliminary comparison, it was found that synthetic blood SB5 behaved similarly to porcine blood in many aspects, but the SB5 stains were significantly larger than the porcine bloodstains. We attributed this difference to the presence of red blood cells, which behave as particles, as well as plasma, which behaves as a liquid, in porcine blood. SB5 is an aqueous solution and behaves entirely as a liquid.

Using porcine blood, it was found that bloodstain development had two clear timescales, unlike many hard surface events. For times less than 100 ms after impact, the dynamics of impact and the interaction of the spreading drop led to distinctive bloodstain patterns, similar to bloodstains on hard surfaces but with characteristic differences attributed to the surface texture of the fabric and due to the energy absorption by the fabric and the presence of any supporting material in contact with the fabric. Using synthetic blood, it was found that if the fabric was lying on a hard surface, the initial pattern resembled a bloodstain on a hard surface, but more extreme. On the other hand, if the fabric was lying on a soft surface, much of the impact energy was absorbed and only a small (synthetic) bloodstain pattern occurred. In a third scenario, the fabric was suspended across two objects or held tautly between them. If suspended, the fabric absorbed much of the energy and only the highest impact velocities led to a stain with spines or satellite stains. However, if it was held taut, it acted as a drumhead and tossed the drop back off the surface, which led to stains with irregular geometries. These findings indicate that where there are bloodstain or bloodstain pattern evidence located on fabrics, it is important for the BP analyst to

note and document any surrounding or backing textile surfaces that may have come into contact with the bloodstains or bloodstain patterns that were observed. Examples include a bloodied bed sheet lying on top of a bedding fabric at a crime scene or a bloodied shirt over a T-shirt worn by the suspect. In both these scenarios, the shape, size and distribution of the observed bloodstains or bloodstain patterns may be distorted and the final appearance which is observed by the BP analyst will look different compared to if these stains are deposited onto a hard surface.

After the initial impact, the wicking within the fabric takes over. Asymmetry in the fabric structure can lead to an asymmetric synthetic bloodstain patterns. These asymmetric patterns can lead to a stain that appears to come from an angle, even when a drop impacted the surface at right angles. For a drop that impacts the fabric from a known angle, it can lead to a pattern that appears to originate from a different angle or even from a different mechanism. This might lead to misinterpretation, for example, a passive drop falling onto an unbalanced woven fabric may be wrongly classified as a projected bloodstain. Furthermore, different fabric constructions and even different yarn manufacturing processes can lead to very different wicking behavior. This is exemplified in a synthetic blood transfer experiment that showed that when synthetic blood was applied to a *woven* fabric in a stack with a knit fabric, the larger stain occurred on the *knit* fabric.

In addition to these factors, it was observed that wicking resulted in a much larger stain than the initial drip stain that occurs immediately upon drop deposition. As the stain grew over the next 2 – 5 minutes, it spreads and masks the initial bloodstains, eliminating any of the initial spines and many of the nearby satellite stains.

Based on these studies, a bloodstain analyst should approach the interpretation of bloodstains and bloodstain patterns on textiles with great caution and be aware of the textile factors that can influence the appearance of blood on fabrics; as what they observe may not resemble the initial stain or the actual mechanism of deposition of the blood. This study has demonstrated that fabric may interact with, distort and alter the stain in many different and complex ways. Considerable additional research is needed to bring BPA on textiles up to the standards expected for BPA on hard surfaces.

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Executive Summary

Bloodstain pattern analysis, BPA, on hard surfaces (such as walls, tables, appliances, hardwood floors, etc.) has grown into a science-based investigative tool that can help determine scenarios that are consistent with or counter to the events described by witnesses or suspects. At the vast majority of crime scenes involving a bloodletting event, textiles are present, either as apparel, household textiles (sheets, towels), upholstery, carpets, and so forth. Yet, the science of BPA on textiles is not able to render the same level of confidence in the analysis as on hard surfaces due to the complex structure of textiles and their ability to absorb some of the energy of an impact and to wick liquids. In the work described herein, a detailed examination of factors that affect BPA on two textile fabrics, an unbalanced 130 x 70 plain woven 100% cotton bed sheeting fabric (often referred to as a 200 thread count bed sheet) and a 100% cotton jersey knit T-shirt fabric. Due to the large number of samples tested, we began by searching for a suitable synthetic blood.

The BPA literature has several synthetic blood recipes, ranging from water and cornstarch to more complicated recipes. We found that, although these recipes may give patterns that resemble real bloodstains on hard surfaces, they had several serious deficiencies. Some would not dry while others sat on the fabric surface and did not wick into the fabric. Still others would flake off, destroying any evidence that they had even been present. After several attempts, we settled on a recipe from the American Society for Testing Materials, ASTM. This recipe was developed to mimic the penetration of blood through protective hospital gowns, but has a lower viscosity and a lower surface tension than human blood. To better represent bloodstains on textiles, we modified this synthetic blood recipe to increase its viscosity and surface tension. We found this synthetic blood to give stains that appeared realistic, that readily wet and wicked into the fabrics. Even one of our safety professionals thought we were working with real blood.

It has been known (or well documented) that when a drop of blood falls passively onto a hard surface hitting it at right angles, the initially spherical drop flattens and spreads out radially. It may then retreat back into a spherical cap resting on the surface after a few oscillations, or it may begin to distort at its rim, forming a crown with spines. These spines may break up into additional satellite drops that fall back onto the substrate some distance from the initial impact site resulting in satellite stains. All this occurs in less than 100 ms, as shown by high-speed video, and leads to the characteristic bloodstains and bloodstain patterns that have assisted investigators in analyzing or reconstructing a crime scene. However, when the same event occurs on a fabric surface, what is observed can vary from that described for hard surfaces and produce a very different bloodstain and bloodstain pattern stain due to the interaction of the blood drop with the fabric and due to wicking of the blood into the fabric. We found wicking to occur over a time span of several minutes, thus bloodstain pattern development on fabrics has two clear timescales, unlike many hard surface events. For times less than 100 ms, the dynamics of the blood drop impacting the textile surface and the interaction of the spreading drop with the fabric lead to distinctive bloodstain patterns, similar to bloodstains on hard surfaces but with characteristic differences attributed to the surface texture of the fabric and due to the energy absorption by the fabric and the presence of any supporting material in contact with the fabric. Using high-speed video to capture the impact of porcine blood on bed-sheeting and T-shirt knit fabrics lying on a poster board, it was found that the drop spreading and breakup into satellite stains resembled a bloodstain on a hard surface, but with more satellite stains, especially for the

knit fabric. The T-shirt knit fabric generated a large number of very small satellite stains. Similar results were found for synthetic blood falling onto these fabrics when they were lying on a hard laboratory benchtop.

On the other hand, when either fabric was placed on a soft surface, much of the energy of the drop impacting onto the fabric was absorbed. Using synthetic blood only a small synthetic bloodstain pattern occurred with very few spines and very few satellite stains. In a third scenario, each fabric was placed either loosely or tautly in an embroidery hoop. Each case led to a different pattern. If the fabric was loosely held, again, most of the energy of the falling drop was absorbed by fabric deformation and only the highest impact velocities led to synthetic bloodstains with spines or satellite stains. However, if the fabric was held taut, it acted as a drumhead and tossed the drop back off the surface, leading to unusual stains.

After the initial impact of a drop on a fabric (i.e. for times >100 ms), wicking within the fabric took over and the fabric construction played a key role in stain development. The first fabric tested was a plain-woven bed sheet fabric, which was asymmetric with 130 ends per inch and 70 picks per inch. In addition, the ends (the warp yarns) were a different size and had a different twist level than the picks (weft yarns). This asymmetry in the fabric construction led to an asymmetric synthetic bloodstain, extending more in the warp direction than in the weft or cross direction. The resultant bloodstain was elongated and rough at the edges and appeared to originate from a drop that impacted the fabric at an oblique angle, even when the impact occurred at right angles to the fabric surface. Furthermore, a drop that impacted the fabric from an oblique angle could lead to a synthetic bloodstain that appears to come from a different angle or even from a different mechanism. Fully developed synthetic bloodstains were also several times larger than their initial size as the synthetic blood wicked away from the impact location. In many cases, the original pattern had all the characteristics of a hard surface synthetic bloodstain pattern, but after a couple of minutes, the enlarged stain that developed from the spreading drop completely masked the original spines and many of the satellite stains. Wicking continued for about 10 minutes, at which point the synthetic blood was fully adsorbed into the capillary channels between the fibers within the yarns.

When similar experiments were performed on a jersey knit fabric (T-shirt material), the synthetic blood quickly filled in the knit loops then wicked into the yarns, while draining the loops. Similar behavior was observed for the channels between the yarns within the woven fabric, but the process was much less dramatic and occurred over a much greater time. Wicking into the knit fabric was very rapid, completing in about 2 minutes. The wicking distance in the knit fabric was much shorter than for the woven fabric. In addition, stains on knit fabric were slightly oval with a smooth perimeter while those on the woven fabric were highly distorted.

The wicking properties of individual yarns within each fabric were also evaluated. The warp yarns of the woven fabric wicked synthetic blood approximately twice as fast as the weft or filling yarn, which would account for the asymmetric synthetic bloodstain. The synthetic blood simply moved faster and further in the yarns for which wicking was fastest. Since the volume of the synthetic blood drop limited the extent of wicking, wicking ceased when the supply of synthetic blood was exhausted. This may also explain the jagged synthetic bloodstains in the woven fabric since there was large yarn-to-yarn variation in the wicking rate. Thus, synthetic

blood would wick rapidly in one yarn, but more slowly in an adjacent yarn, creating an uneven stain shape.

On the other hand, wicking of the knit yarn was 7x faster than for the woven warp yarn. This may account for the much faster wicking in the knit fabric. In addition, its packing factor (the amount of solid fibers within the yarn) was much lower for the knit yarn. This means that there was much more space for synthetic blood within the yarn and thus the pattern that developed was much more compact than for the woven fabric. The difference in the fabric construction between the knit and the woven fabric also accounts for some of the differences in wicking behavior. In the woven fabric, there are two different types of yarn and they run perpendicular to each other, thus leading to asymmetric wicking. In the knit structure, only a single type of yarn was used. Each yarn runs across the fabric forming loops. Although the synthetic blood wicks rapidly along the yarn and more slowly from yarn-to-yarn, the large void space within the knit yarn limits the spreading of the synthetic blood and thus limits the asymmetry of the synthetic bloodstain.

In another set of experiments, wicking in a layered fabric structure was measured. One fabric was placed directly on top of another of the same or different construction. A single drop of synthetic blood was placed gently on the top fabric. Wicking was recorded using a video-microscope. When the woven fabric was placed on top of another layer of woven fabric, wicking occurred slowly. With time, the synthetic blood migrated through the upper fabric and, upon coming into contact with the lower woven fabric, the synthetic blood wicked into both fabrics at about the same rate. In this case, the upper fabric had a slightly larger stain than the lower fabric since it had more time to wick into the structure before contacting the lower fabric. When the knit fabric was placed on top of another knit fabric, the same behavior occurred, but much faster. When the knit fabric was placed on top of the woven fabric, it wicked into the knit fabric quickly, leaving only a small stain on the woven fabric. In this case, the synthetic blood was nearly fully entrained within the knit fabric before coming into contact with the woven fabric. Once trapped within the knit yarn, it did not seem to transfer to the woven fabric.

Surprisingly, when the woven fabric was placed on top of the knit fabric, the synthetic blood slowly wicked through the woven fabric until it contacted the knit fabric, as before. However, immediately upon touching the knit fabric, the synthetic blood was rapidly drawn into the knit and developed a large synthetic bloodstain in the knit fabric, leaving only a small stain on the woven fabric, which had developed before the synthetic blood wicked through to the knit fabric. In this case, the larger stain occurred on the lower fabric.

Similar experiments were performed where a woven or knit fabric was placed on top of a polyester film, to simulate a fabric lying on a smooth non-absorbent surface, such as a countertop or floor. In this case, when synthetic blood was placed on the fabric, it would wick into the fabric, but upon contacting the film, a capillary bridge formed between the fabric and the film. This accelerated the wicking rate, but reduced the extent of wicking since the synthetic blood could occupy the space within the yarn and between the fabric and the film.

Based on these studies, a bloodstain analyst should approach the interpretation of bloodstains and bloodstain patterns on textiles with great caution and be aware of the textile factors that can influence the appearance of blood on fabrics; as what they observe may not resemble the initial

stain or the actual mechanism of deposition of the blood. The fabric may interact with, distort and alter the stain in many different and complex ways. The studies conducted to date have demonstrated that bloodstains and bloodstain patterns on textiles may be altered by fabric construction, yarn construction, surface finishes applied to the fabric, materials in contact with the fabric or a combination of all of these factors. It is important for crime scene investigators to carefully document any surrounding or backing textile surfaces that may have come into contact with the bloodstains or bloodstain patterns.

It is also important to recognize that the results reported herein are specific to the particular fabrics studied and to the particular synthetic blood used. In the case where porcine blood was used, it is important to recognize that porcine blood is also not human blood. Likewise, textiles are dynamic materials whose behavior changes due to laundering, wear, body oils, and so forth. Nevertheless, wetting and wicking within textiles is important for comfort as well as for forensics. Thus, the extensive literature in wicking and wetting can be leveraged to better understand bloodstains on textiles.

The authors believe that, in the future, BPA on textiles will be as scientifically rigorous as it is currently on hard, non-absorbent surfaces and it can also be a science-based forensics tool. However, considerable work remains to interrogate and better understand the complex interactions between human blood and textiles, noting that there is a diverse range of textile materials and surface treatments. In research on synthetic or real blood on textiles, it is essential that researchers carefully document the complete details of the fabric structure and properties, that the experimental conditions are fully reported, and that the properties of the synthetic or real blood be carefully compared and determined. Using the approach above, the authors believe that the true underlying features of bloodstains on textiles will be amenable to rigorous forensics analysis.

Although it is currently beyond the capability of most crime labs to completely document the fabric structure and properties, it is essential for the BP analyst to be aware and understand the factors and variables that can influence BPA on textiles as it may assist in verifying or excluding scenarios put forward by persons of interest or witnesses of a crime.

I. Introduction:

One of the most common body fluids encountered at a crime scene particularly if it is of violent nature is blood. The forensic examination of bloody clothing and other textiles such as sheets, towelling and upholstery may help forensic scientists to reconstruct a crime, and in some cases may support or refute specific version of event(s) given by complainant, suspect or eyewitness. Deoxyribonucleic acid (DNA) evidence which is deemed "the gold standard" of Forensic Science may link a person to a particular bloodstain, but it does not indicate how it got there and more importantly what had happened. Bloodstain pattern analysis (BPA) on the other is an important forensic tool that can provide useful information that may help fill the gaps in the investigation of the crime.

The examination of bloodstains or bloodstain patterns on clothing may provide information about the position, activity and movements of the wearer during and after the bloodshed event. In the past few years there have been several high profile homicide trials in which bloodstain pattern evidence have been at the center of controversial arguments relating to the explanation of the mechanisms that produce very small bloodstains on clothing (e.g. [1]). At the heart of these controversies is the observation that many mechanisms produce bloodstain patterns that do not have significant or sufficient individual characteristics to distinguish how the pattern was produced. For example, *impact, expiration, and transfer* mechanisms (see [2] for definitions) can each produce blood spatter comprised of very small bloodstains and can be confused with one another, especially on fabric.

Although bloodstain pattern analysis of small stains on hard, nonabsorbent surfaces can provide valuable insight into the impact velocity and impact angle of a blood drop as well as its volume, the same level of confidence does not exist when the drop is found on a textile surface. One reason for the lack of clarity on textiles is the wide diversity of textile materials, which include woven, knitted, nonwoven, and braided fabrics as well as ropes and carpets, and their absorbency and surface texture often results in a distortion in the appearance of bloodstains on clothing. For example, liquid wicking (definition: [3]) into fabrics should be symmetric if the yarn sizes and types are the same in the warp and weft directions and if the fabric count is the same in both directions. However, it is common for the warp and weft yarns to be different and for the fabric count to be different in these two directions, e.g. in percale bed sheets [4] and denim [5]. This can result in asymmetric wicking patterns, flowing more in one direction than another. Hence, conventional methods used in the examination of bloodstains on smoother, less absorbent surfaces may not be applicable.

Because of its ubiquitous presence, the wicking behavior of liquids into textiles has been extensively studied. Although these studies provide a good basis for a fundamental understanding of blood wicking into a textile, they have severe limitations for the analysis of bloodstain patterns. First, most of these tests have been performed with large quantities of liquid, while bloodstains often involve less than 1 mL of fluid. Second, blood is a complex fluid that includes liquids, proteins, and cells, while fabrics can filter blood cells and/or alter the fluid path. Thus, the complex nature of textiles combined with the complex nature of blood coupled with the complex interaction between them limits the bloodstain pattern analyst's ability to fully account for the events that led up to the formation of any bloodstain or bloodstain patterns on textiles. To reduce this effort to a tractable problem, the work reported herein has focused on the

impingement of porcine blood or an optimized synthetic blood onto textiles and the wicking of porcine blood and synthetic blood into plain-woven bed sheeting and into jersey knit T-shirt fabric, both made from 100% cotton. In future studies, the affect of red blood cells and other blood components will be incorporated into the analysis.

1. **Statement of the problem:**

The value of Bloodstain Pattern Analysis (BPA) is well documented in forensics literature [6,7] and its probative value is well known in courtrooms around the country. In some trials BPA is pivotal in deciding the guilt or innocence of the individual charged. For example, if bloodstains found on an accused person were caused by spatter from a gunshot wound, this evidence might be incriminating. If however there was evidence that these same bloodstains were instead caused by direct contact or transfer between the bloody victim and the accused, the findings may help exonerate the accused. Because of the frequency of bloodshed at scenes of violent crime and the value of the bloodstain pattern analysis, BPA evidence is routinely used in modern criminal cases.

BPA research has undergone resurgence in the last few years. However most of the research effort has focused on bloodstains on hard, non-porous and non-absorbent surfaces of the sort commonly encountered at crime scenes. This partly reflects the fact that bloodstains on these surfaces are generally easier to analyze. However, bloodshed invariably involves people and therefore, their clothing, as well as upholstery, bed sheets, etc. Since textiles can wick liquids into their structure, when blood deposition processes occur on textile fabrics a far more complex analytical problem arises. *Surprisingly, to date, no comprehensive and fundamental research on bloodstain patterns on textile materials has been undertaken*. This is despite the fact that thousands of items of bloodstained clothing and other textile products are examined in forensic laboratories around the world every year. The study described herein is therefore both imperative and urgent.

2. **Statement of hypothesis or rationale for the research:**

The overall goal of this study is: To provide a fundamental understanding of the complex *interactions between individual drops of blood and textile materials*. The fundamental understanding of bloodstain pattern formation processes on textiles is an essential first and substantial step towards reliable classification of the resulting bloodstain patterns on textiles. A further goal for the study is for the establishment of an effective forensic science protocol for BPA on textiles. To achieve these goals, work has been undertaken to achieve the following objectives.

Objective: To develop an in-depth understanding of the development of bloodstain patterns on fabric surfaces incorporating:

a) textile engineering variables in the most common fabric structures (woven and knit),
b) the molecular level physico-chemical surface properties of the textile substrate,
c) the physical properties of blood, and
d) vector-based dynamic forces leading to blood transfer onto/within the interstices of textiles.

Literature Review

Limited studies on small, ink-jet drops for printing textiles showed that wetting from small drops is more complicated than from an infinite pool [8]. However, there is a dearth of prior art directly concerning bloodstains on textiles. The majority of information available is in BPA textbooks [6,7] and concentrates on the proper collection, examination and documentation of bloody clothing.

The first systematic experiments of the effect of textile surfaces on bloodstain formation were published by Balthazard et al. in 1939 [9]. The authors observed that bloodstains on textile surfaces were distorted by "retraction, enlargement, diminishing, misshaping and amputation" when compared to the bloodstains on a control surface. They also observed that the distortion was different on each of the different textile surfaces. White [10] observed similar behavior; he dropped two different volumes of blood from different heights and angles onto a variety of fabrics using cardboard as a control surface. He too observed that the bloodstains were distorted to different degrees on the different fabric surfaces when compared to the control.

In 1999 Slemko [11] assessed bloodstains as a function of blood droplet velocity, fabric structure and absorbance properties. He found that the distortion in the appearance of bloodstains on fabrics is dependent on fabric absorbency and texture, and that angle of impact calculations for bloodstains on fabric requires extreme caution due to the numerous variables involved.

Karger et al. in 1998 [12] used small blood drops (0.1 -10 μL) to generate a series of *transfer* stains on a variety of fabrics and compared them to a series of *drip* and *spatter* stains (see [2] for definitions) of similar size generated on the same fabrics. Their results showed that *transfer* stains tended to impregnate the weave of the fabric whereas the *drip* and *spatter* droplets remained on the surface of the weave. In spite of Karger et al.'s findings, there appears to be a general consensus that the difference in appearance between bloodstains caused by an impact event and those caused by transfer is the level of penetration into the weave [6, 13-15]. The velocity behind *impact spatter* is thought to cause the blood to be projected into the weave, whereas with *transfer* stains the blood only stains the upper portion of the fabric. The latter characteristic was observed by Pex and Vaughan during their research into backspatter from gunshot wounds [16]. As case based studies with case facts will exceed any study, the study presented below has been limited to furthering our fundamental understanding of complex interactions that occur between blood and fabric. Since these studies represent the heart of the research into bloodstains on fabric surfaces, it is clear that the lack of science in this area highlights the urgent need for foundational research, which is reflected in two of current BPA research priorities published by SWGSTAIN [17]:

"1. RESEARCH THAT WOULD MINIMIZE AMBIGUITY IN THE CHARACTERIZATION OF SMALL STAIN BLOOD SPATTER PATTERNS. BLOOD SPATTER PATTERNS CONSISTING OF SMALL STAINS CREATED BY DIFFERENT MECHANISMS CAN APPEAR SIMILAR. RESEARCH IS NEEDED TO DEVELOP METHODS THAT PROVIDE MORE DISCRIMINATION BETWEEN THESE PATTERNS.

2. RESEARCH TO EVALUATE THE BEHAVIOR OF BLOOD ON FABRIC SURFACES. A GREAT MANY BPA INVESTIGATIONS INVOLVE THE EXAMINATION OF BLOODSTAINED CLOTHING. RESEARCH IS NEEDED TO DEVELOP AN UNDERSTANDING OF THE COMPLEX PROBLEMS OF HOW BLOOD TRANSFERS TO, INTERACTS WITH AND PERSISTS ON FABRIC SURFACES."

The Basic Phenomena Underlying Blood Interactions with Textile Materials: When blood spatters on a flat surface, the blood can dry in place or flow, but otherwise it does not move on its own. However, on textiles such as bedding, towels, and apparel, the interaction of the drops with the textile plays a major role in the final bloodstain shape. A drop may remain on the surface of polypropylene (indoor-outdoor carpet, sportswear) or it may wick into the textile (cotton shirt, towel, pants). If pressure is applied to the drop holding a bloody fabric against another object, a capillary bridge will form between them. The amount of blood transferred depends on the surface energy and construction of the two materials [18]. If the blood wicks into the textile, several additional factors should be considered. For example, the shape of the bloodstain will be changed by the wicking pattern, which is controlled by the spacing between yarns and capillaries formed between fibers [19]. Fabrics are also often used as filters to remove particles, including cells. Thus, cells could be filtered out and only blood serum could penetrate into the yarn. The tightness of a weave or knit could greatly affect the migration of cells. These factors may contribute to the difficulty in characterizing and understanding BPA on textile surfaces.

The most common types of fabric found at crime scenes are woven and knit fabrics, and carpets. Woven fabrics consist of three main types, plain weaves, twills (pants), and the less common satins (formal wear). More complicated weave structures are used in upholstery, but their main features are similar to plain weaves. In woven fabrics, the fibers are held in place by twist within the yarns and the yarns are held in place by the interlacing of the yarns that make up the fabric. Typically, 80-95% of a woven fabric consists of air spaces [20] where blood can pool and wick into the fabric. However, the typical spacing between fibers within a yarn is less than the diameter of a red blood cell. Even when large amounts of blood pools on a woven fabric and the fabric is saturated, the red blood cells may lie in the regions between yarns while only the serum may be found within the yarn.

The simplest weaves, plain weaves, are used in bedding and woven shirts. They are characterized by the 1x1 alteration of warp (lengthwise) and filling (widthwise) yarns on the fabric surface. This deceptively simple structure is complicated by construction variables. The warp and filling yarns may be of different sizes, different fiber blends, different twist levels, and different yarn construction. In addition, the thread count is often different in the warp and filling directions. Water that wicks into plain weaves is found to follow capillaries within the yarn structure [19]. Although the vast amount of literature on wicking discusses the effects of yarn construction, yarn size and fabric construction, it has almost exclusively been limited to large reservoirs of liquid and thus is not directly applicable to BPA. Nevertheless, some important conclusions can be made: (1) wicking is usually asymmetric [8]; (2) the largest *amount* of liquid moves in the largest capillaries; and (3) the largest wicking *pattern* is caused by the smallest capillaries [21].

Denim slacks, a twill weave, are often present at a crime scene in the form of blue jeans. The average consumer in the United States owns an average of 7-8 denim jeans [22]. Many jeans are a 3/1 twill in which the warp yarns pass over three filling yarns, tuck under one, and then move back to the surface. This results in a twill line, a line that moves diagonally across the fabric where the filling yarns appear briefly on the fabric surface [5]. There are usually many more warp yarns than filling yarns, which reside primarily on the inside of the jeans. The yarn sizes in denim are often quite large and may even be larger than a drop of blood. When all these factors are considered, bloodstain patterns on twill fabrics and plain-woven fabrics could be expected to differ greatly. Larger drop volumes may follow the twill line while smaller drops may follow the threads [8].

Unlike woven fabrics, knit fabrics (T-shirts, golf shirts, sweaters, etc.) are usually made with yarns that are less tightly twisted and, instead of the yarns crossing each other at 90° angles, they form intermeshing loops. Knits bend and stretch much more easily than woven fabrics and their yarns can easily open up and snag [20]. Blood may penetrate these dynamic fabrics more easily than woven fabrics, but may not spread as far. If a drop of blood on a loosely knitted sweater is pressed against another surface, the drop may enter the fabric without significant spreading.

We postulate that development of a fundamental understanding of the interaction of blood, and its components, with textile structures under different scenarios typical of a crime scene will ultimately enable development of an expert protocol for characterizing bloodstains on textiles. The studies reported below primarily examine wicking effects of a synthetic blood. Replication studies with fresh human blood will need to be performed to verify the conclusions drawn below.

II. Methods

This section describes the materials used in this project, the development of our synthetic or surrogate blood and the methods implemented in order to test the relevant variables including the fabric characterization, the yarn analysis, and the construction of a drop tower to drip individual drops onto the fabric while video recording the motion of the drop after impact.

II.1 Fabric

The fabrics used were 100% cotton fabrics from Test Fabrics, Inc. One fabric was a plain-woven percale sheeting fabric that resembles bed sheets (product code 439XW). It was finished with an optical brightener and had a basis weight of 120 grams/meter^2, a width of 110 inches (279 cm), a nominal thread count of 130 epi (ends per inch) × 70 ppi (picks per inch) and a measured thread count of 135 epi x 65 epi. The other fabric was bleached cotton Jersey-knit T-shirt fabric (product code 437-60) with a basis weight of 124 grams/meter^2 and a width of 60 inches (152 cm). There were 52 courses per inch (cpi) and 35.5 wales per inch (wpi). Initial experiments showed that the sheeting material repelled water, unlike natural cotton; washing the fabric once greatly improved wetting and wicking of the fabric while washing the fabric ten times did not improve the behavior beyond a single wash.

Thereafter, all fabrics were washed according to AATCC standard laboratory practice for home laundering [23]. In this procedure, the fabric was added to the washer and the washer was filled with water at 60 ± 3°C, and a lukewarm rinse setting of 29 ± 3°C was selected. TIDE (a registered trademark of Procter & Gamble Co., Cincinnati OH 45217) detergent (66 ± 1 gram)

was added to the washer and the wash timer was set for a 12-minute cycle. Upon completion, the load was transferred to a dryer at the high temperature setting for a 45-minute cycle. After running this laundry sequence, 100 fabric samples of dimensions 5” × 3” and 11” × 11” were cut and stacked for subsequent experimental use. All experiments used these fabrics unless otherwise specified. Washing the fabric once also provides a realistic condition of clothing worn during the commission of a crime, therefore, any bloodstains formed on the washed experimental fabrics will be similar to the appearance of bloodstains encountered on forensic fabric items.

For some experiments, a polyester transparent film for photocopiers was used to provide a smooth, non-porous surface.

II.2 Temperature of blood at impact

Since the viscosity of blood varies rapidly with temperature, it is very important to know whether the 10-100 µL droplets leave the body and equilibrate to room temperature prior to impact or start wicking at body temperature itself in order to determine the proper viscosity for synthetic blood [24]. When inside the body, blood flows at 37°C, whereas most crime scenes occur at room temperature of around 20°C. To determine the temperature at which a drop impacts the fabric in our experiments, a small gauge thermocouple (tip volume ~1/1000 volume of drop) was placed at the point of impact as shown in Figure II.1. The synthetic blood was heated to 37°C along with the pipet tip and was dripped immediately from a height of 0.3 m onto the thermocouple, which was positioned just above the fabric, and the temperature recorded. For synthetic blood volumes of 20 – 80 µL, the temperature at impact ranged from 20 – 23°C as shown in Table II.1. In other words, the blood drop temperature equilibrated to ambient temperature very quickly for such small drops while wicking occurred over several minutes (see Results and Discussion). Thus, the viscosity of blood at 20°C was used for the viscosity of the synthetic blood.

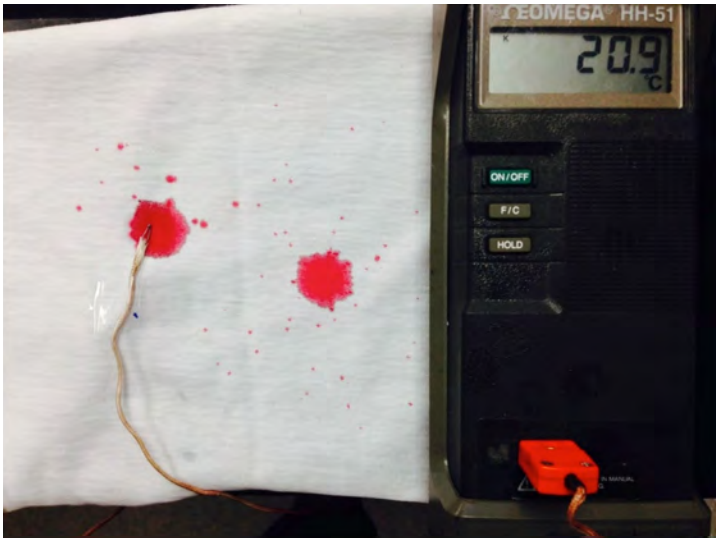


Figure II.1. Blood temperature measurement at impact after heating it to 37°C and dripping it 0.3 m onto the thermocouple and fabric.

TABLE II.1: Temperature (°C) at impact of 37°C synthetic blood drops falling from 0.3 m through air onto plain-woven fabric.

Size of drop (μL)	Temp. 1	Temp. 2	Temp. 3	Average ± Standard Deviation
20	20	21	21	20.7 ± 0.6
40	21	22	20	21.0 ± 1.0
60	22	20	22	21.3 ± 1.2
80	21	22	23	22.0 ± 1.0

II.3 Synthetic blood

Due to university restrictions, many experiments were performed using a surrogate blood, or synthetic blood. We tested several types of synthetic blood [25-26] and found that several recipes for synthetic bloods either would not dry, or they did not wick into the fabric, but rather sat on top. These recipes were determined to be unsuitable for the tests described below. Although early experiments used recipes that were found to be inadequate, for the bulk of our work, we used a recipe patterned after the ASTM test method F1819-07 synthetic blood [26]. The synthetic blood obtained using this test method is referred to as SB3 below. It resulted in a synthetic blood whose viscosity was significantly lower than values reported for human blood [24], and thus it was modified to increase the viscosity as described below. ASTM standard uses distilled water, red dye and Acrysol G110 as a thickening agent. To achieve a higher viscosity synthetic blood recipe, Acrysol 8306 was substituted for Acrysol G110.

Thus, a 1% dye solution was prepared by mixing 1 g of Direct Red 81 in 100 mL of deionized water. Acrysol 8306 was diluted 15x with distilled water. This solution was stirred for one hour until all the entrapped air bubbles had escaped the solution and a translucent homogenous solution was obtained. Next, 20 mL of dye solution was added to the diluted translucent thickener solution. Finally, the surface tension and viscosity were measured as described below and the solution was adjusted to achieve the desired viscosity and surface tension by adding the dye solution or thickener solution. From the temperature results above, the viscosity was adjusted to 3.5 ± 0.29 cSt since human blood at 25°C has a kinematic viscosity of 3.5 ± 0.29 centistokes (cSt) [24]. (At 37°C the viscosity is 2.44 ± 0.09 cSt [24]). The synthetic blood recipe SB4 was measured and adjusted to this range each time prior to use.

Likewise, the surface tension of human blood at 37°C is 42 mN/m and at 20°C it is 50 mN/m [27,28]. The surface tension of the synthetic blood was measured by the capillary rise method as described below. The solution was adjusted to achieve 50 mN/m as follows. If the capillary height was less than desired, then 1 ml of dye solution was added. This was done gradually until a height of 1.33 cm was attained. On the other hand, to lower the height of capillary rise, Acrysol 8306 was added in small amounts. Viscosity measurements were remeasured to check whether the solution was within the acceptable operating range. Usually, the solution was found to be stable for a period of 5-7 days if it was stored in an airtight container. This synthetic blood was used for drip stain measurements and is referred to as SB4.

After the majority of the experiments were complete, new values for the density = 1.060 g/mL, the viscosity = 6.3 x10^{-3} kg/ms (6.3 cP), and the surface tension = 61 mN/m at 20°C were found [29]. For the subsequent fabric sandwich experiments, the synthetic blood recipe modified from

ASTM F1819-07 [26] was adjusted for these new values as follows. A 0.5% dye solution was prepared by mixing 0.1 g of Direct Red 81 in 20 mL of deionized water. Next, Acrysol 8306 was diluted 40x by putting 40 mL distilled water into 1.0 g thickening agent and stirred for one hour until a homogeneous solution was obtained. Then the dye solution was injected into the thickening solution to get 60 mL synthetic blood.

Finally, the density, viscosity and surface tension of the optimized synthetic blood were measured. The density was measured using a graduated cylinder and adding 10 mL of solution and determined to be 0.988 g/mL. The surface tension and viscosity were measured using Ostwald method (section II.4) and pendant drop method (section II.5) respectively. The viscosity was found to 6.5 $x10^{-3}$ kg/ms (6.5 cP) and the surface tension was 68 mN/m. The measurements were carried out at room temperature, ~ 20^{o}C. The resulting values compare well with the literature values of reference [28], so this synthetic blood recipe was used for fabric sandwich wicking studies and referred to as SB5.

The synthetic blood recipes for all of the synthetic bloods used are given in the appendices. Synthetic blood types SB1 and SB2 were taken from reference [25], SB3 was taken from reference [26], while SB4 and SB5 were patterned after SB3, but modified to obtain the desired viscosity and surface tension.

II.4 Viscosity of synthetic blood

The kinematic viscosity was measured using an Ubbelohde Viscometer (Cannon Instrument Company, PA 16803) for the kinematic viscosity in the range of 2-10 cSt. The viscometer was calibrated as per Standard Test ASTM D 445 [30] using water. The filled viscometer was allowed to equilibrate in a water bath at 20°C for 20 minutes prior to measurement. The kinematic viscosity was converted to dynamic viscosity by dividing by the measured density of 0.988 g/mL. For SB4, the viscosity was adjusted to 3.5 ± 0.29 cP, while for SB5, the viscosity was adjusted to 6.3 cP to match the more recent values given in reference [29].

II.5 Surface tension of synthetic blood

The surface tension of blood also varies with temperature. At 37°C it is 42 mN/m and at 20°C it is 50 mN/m [27,28], or 6.1 mN/m [29]. In the initial tests, the surface tension was measured using the Young Laplace equation for the rise of a liquid column within a capillary [31]:

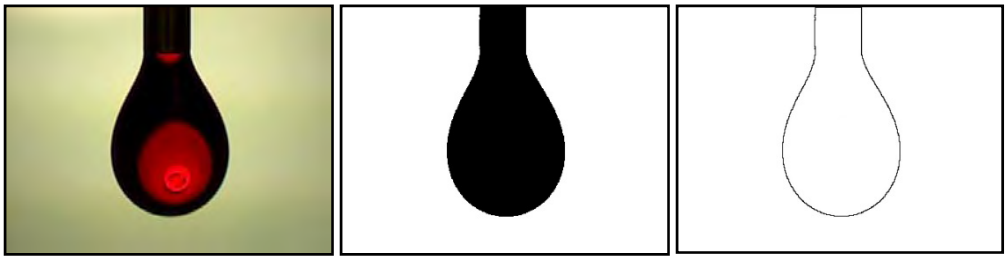
$$\Delta p = \frac{2\gamma}{r} = \rho g h \qquad \text{(II.1)}$$

where Δp is the Laplace pressure, γ is the liquid-vapor surface tension of the solution, r is the radius of the capillary tube. But the Laplace pressure is also equal to the difference in the pressure at the bottom and top of the liquid column (right hand side of the equation), where ρ is the density of the solution, g is the acceleration due to gravity, and h is the capillary rise in the tube.

Synthetic blood type SB4: In these experiments, r ~ 0.7 mm, g = 980 cm/s^2, density of the synthetic recipe = 0.988 g/mL and γ = 50 mN/m. The height of capillary rise obtained by substituting these values in the above equation was 1.45 cm. The surface tension of each batch of synthetic blood was measured and the surface tension adjusted until it fell within the accepted range. First the tube diameter was measured by measuring the capillary rise of water and its

known surface tension and density. Then the capillary tube was placed in a pool of synthetic blood and the height of liquid rise in the capillary tube was recorded. The density of the batch of synthetic blood was measured by weighing a know volume of the blood. The surface tension of the solution was adjusted as described above.

Synthetic blood type SB5: The pendant drop method to measure the surface tension where a pendant drop is imaged as shown below.



(a) (b) (c)

Figure II.2. A pendant drop of synthetic blood is (a) photographed, (b) converted to a binary image, and (c) the perimeter is found. Image processing software Image J was used to obtain the surface tension from the drop picture.

II.6 Ensuring that only a single drop of blood of known volume was dripped onto the fabric

In mimicking drip stains, it is important to assure that only the effect of a single drop of known volume fell onto the fabric. To confirm the absence of an accompanying drop a high-speed video camera captured the falling drop when dripped from a pipet tip, as shown in Figure II.3. The tip was modified as describe below to ensure that a single drop fell. The weight of the drop was measured to calibrate the tip and at least ten independent measurements were made to calibrate the tip.

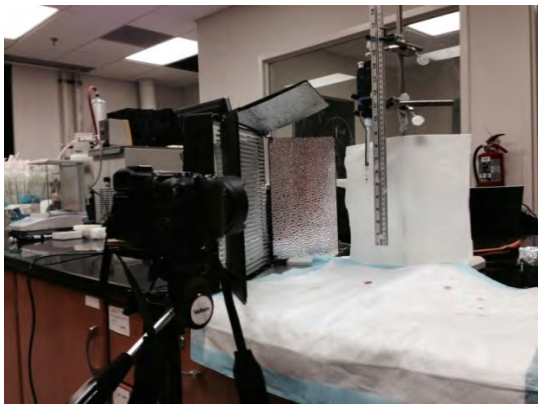


Figure II.3. High-speed video imaging experimental set-up to determine that only a single drop with no accompanying drop fell onto the fabric.

A digital single-lens reflex camera (digital SLR or DSLR) was set on a tripod stand at an optimum height to image the drop from the pipette. Then the mode dial of the camera was set to shutter priority with a shutter speed of 1/2000 of a second to avoid motion blur of the drop in

flight. For the current set of experiments, the distance between the photographic lens and the drop was 23.5 cm and an external set of light was projected allowing the camera to capture clear images at very low exposure time. Figure II.4 shows that a single drop was created.

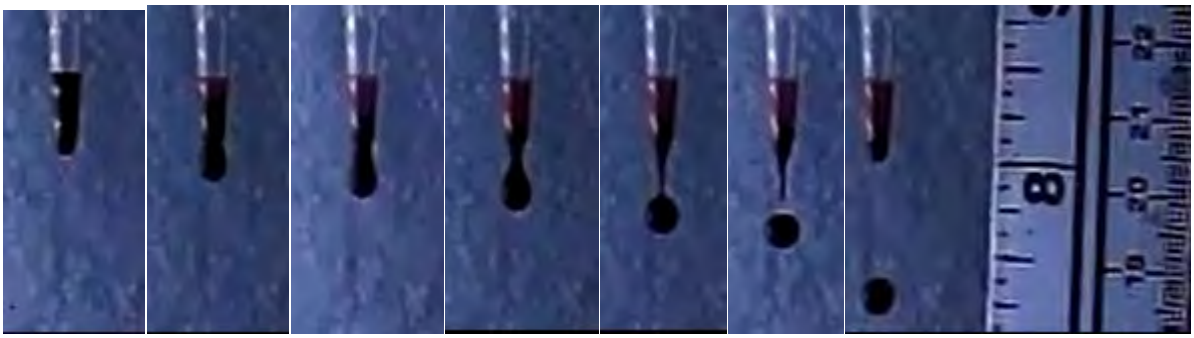


Figure II.4. Drop formation and dispensing action as a single drop.

In order to obtain single drops, the pipette tip needed to be trimmed as shown in Figure II.5. ZAP (Zero Aerosol Pipetting) tips from VWR, designed to dispense 10-100µl were attached to the pipette. The entire volume of the pipette, ranging from 10-90 µL, was to be pipetted out as a single drop of blood. To accomplish this, the tip was cut with a razor blade in 1 mm increments to widen the tapering tip diameter until a single drop fell, as determined by high-speed video imaging. If an accompanying drop was observed, the tip was cut an additional one mm to widen the tip diameter further. This procedure was repeated until the tip delivered a single drop of blood. Once a single drop was obtained, the delivered volume was determined by weighing each of ten drops and taking their average weight. The dispensed weight (volume) was reproducible to ± 5%. Each tip was individually calibrated, after which it could be used for several hours as long as it was kept clean. With these trimmed tips, we could routinely deliver single drops in the volume range of 10 – 90 µL.

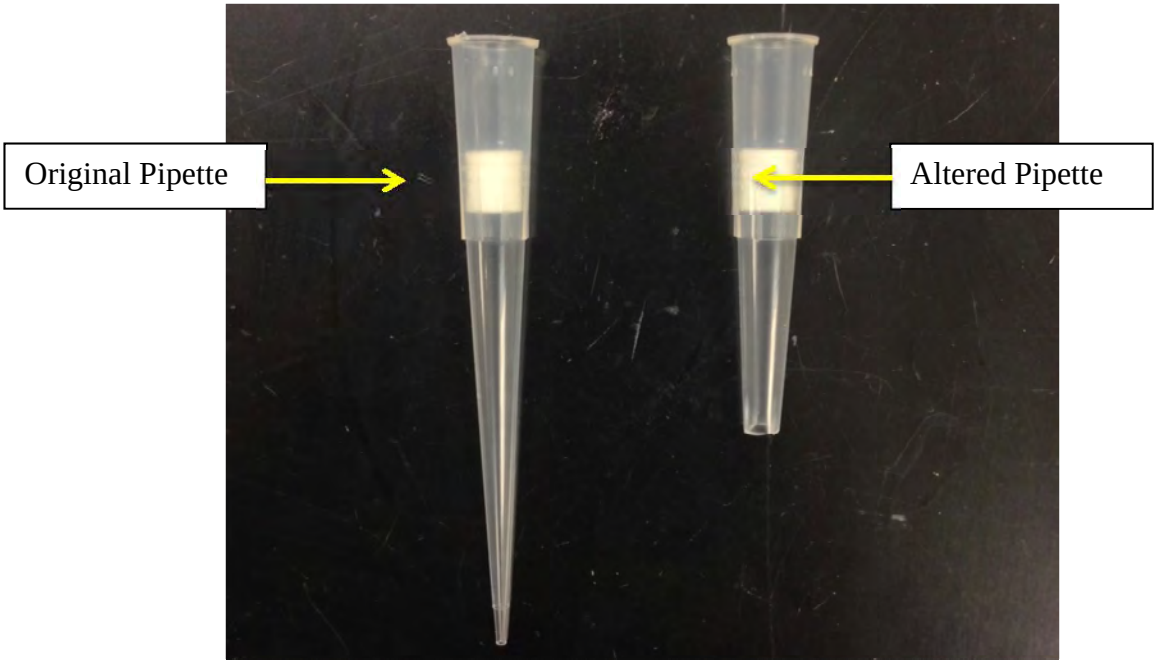


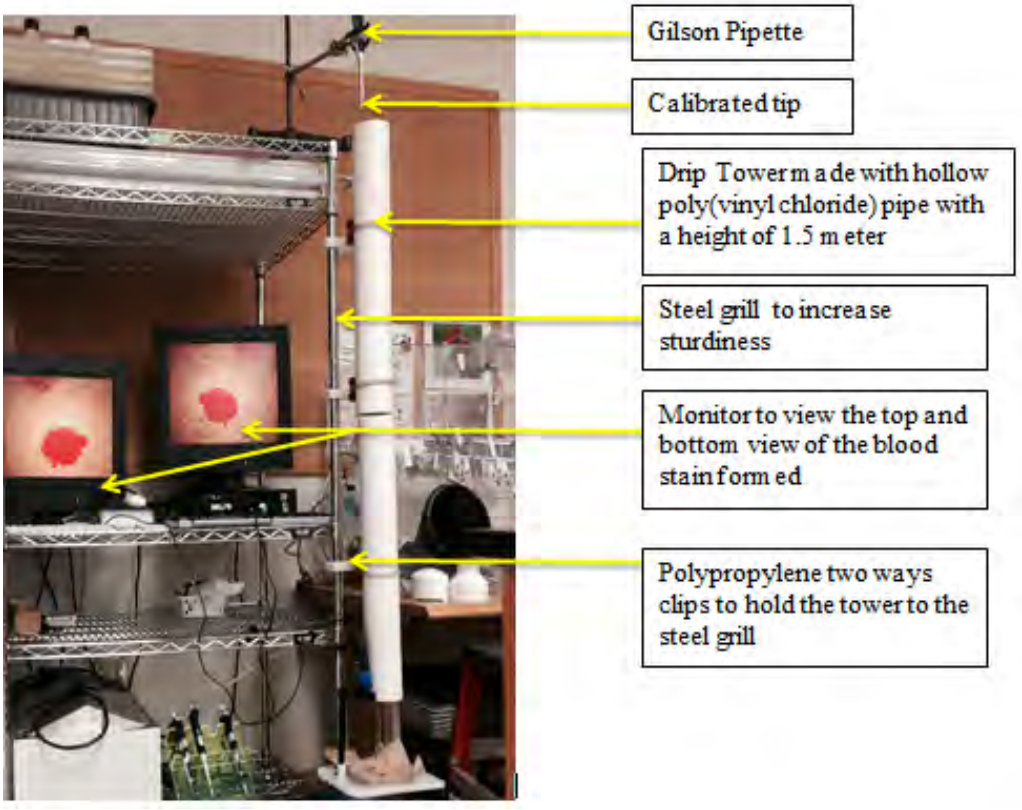
Figure II.5. Pipette tip prior to and after trimming to ensure single drop dispensing.

II.7 Design and Construction of Drop Tower

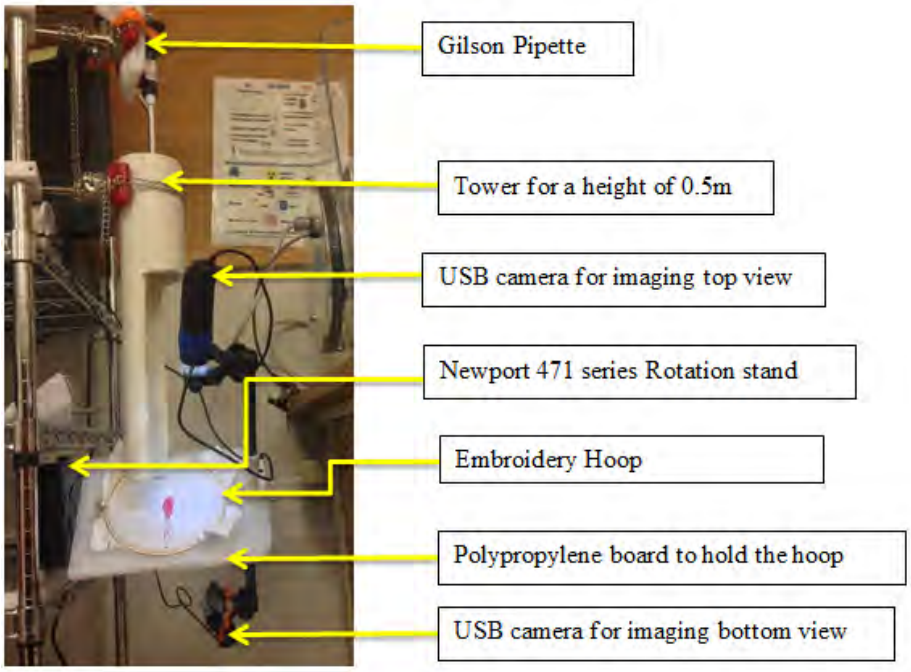
The goal of this portion of the work is to determine the physical appearance of drip stains created when single blood drops fall onto fabric from different heights and angles. After preliminary experiments using synthetic blood types SB1 and SB2, it was determined that a drop tower was needed in which drops of known volume could fall onto fabric mounted on a support. The design of the drip tower is shown in Figure II.6. The essential features are a dispensing pipet that could be mounted at two chosen heights, 0.5 m (approximate knee height of an adult) and 1.5 m (approximate chin height of an adult), a sample rotation stage that could hold the fabric and tilt it to different angles relative to vertical, and two video cameras, one monitoring the impact side of the fabric and one monitoring the back. The cameras were mounted such that they do not interfere with the falling drop and such that both cameras view the surfaces at the same point from the same angle and at the same distance from the fabric to assist in comparisons. In addition, they should rotate with the fabric so that they always monitor the fabric from the same orientation to allow for corrections to the shape due to the necessary offset of the cameras.

Figure II.6 shows a Gilson Pipette with a working range of 001-900 µL fitted with a calibrated tip. During a drip experiment, this tip was immersed in a pool of synthetic blood and the required volume was drawn into the pipette. At the appropriate time, the blood was released from the pipette and the drop fell through the white plastic tube, which limited disruption of the drop path due to air currents within the laboratory. In Figure II.6a, the pipette is mounted 1.5 m above the fabric sample, while in Figure II.6b, it is mounted at 0.5 m. Fabric samples were carefully cut and placed in 3-inch embroidery hoops in earlier experiments. Later the drop tower was modified to accommodate 6-inch embroidery hoops to accommodate the larger area needed for impacts at steep angles. These hoops were then placed in a cutout in a polypropylene plate that was

mounted on a Newport 471 series rotating stage. This allowed the fabric to be supported only at its perimeter. The polypropylene plate along with the embroidery hoop and fabric could be rotated through required angles of interest (0-90°). This arrangement also allowed the angle between the warp (machine) direction of the fabric to be rotated about the blood impact plane. (This relationship will be discussed further below.) The two USB video microscopes with adjustable focus and zoom were mounted on the holder and adjusted so that both cameras had the same magnification and viewed the same sample area, but from opposite sides of the fabric. The video images were collected simultaneously using two personal computers for analysis at a later time.



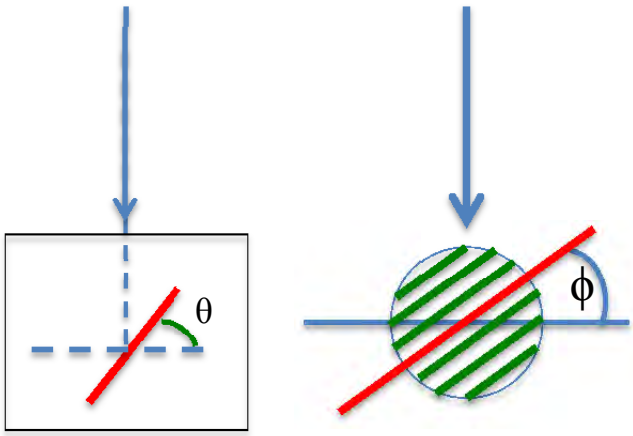
(a)



(b)

Figure II.6. Drip tower apparatus (a) with the dispensing pipette located 1.5 m and (b) at 0.5 m above the fabric. In (b) the fabric mounted in an embroidery hoop and placed in the cutout in the polypropylene plate is shown tilted to 60° from horizontal by the Newport rotation stage.

Samples were identified by the fabric type, the drip height, the drop volume, the impact angle, θ – 90° (impact perpendicular to the surface), 60°, and 30° (as shown in Figure II.6b), and the angle of the fabric warp direction relative to the tilt rotation axis, ϕ. A 90° warp angle was defined to be when the warp direction coincided with the tilt rotation axis for the fabric, as shown in Figure II.7



(a) (b)

Figure II.7 Coordinate system for fabric drip tests where (a) the red line represents the plane of the fabric, the arrow indicates the falling direction of the drop and θ is the impact angle. In (b) the green lines represent the orientation of the fabric warp direction. The blue line indicates the tilting axis of the polypropylene supporting board, while the red line indicates the rotation of the warp direction relative to the tilting axis and given by angle ϕ.

During a single drip experiment, the pipette was placed at the desired height. The fabric was neatly placed in the embroidery hoop and the surface was fixed while taking care to avoid stretching the fabric. The fabric could be pulled taut in the hoop or draped loose across the inner hoop before tightening the outer ring of the embroidery hoop, as desired. Then the sample in the embroidery hoop was placed into the polyproylene mounting board with the warp direction aligned in the desired direction. The Newport 471 series rotation stage was then used to tilt the polypropylene board and the fabric to the desired impact angle. In case of the plain woven, the warp direction was marked before starting the experiment or after drying the sample. The two USB video cameras were adjusted so that the fabric was in good focus. The pipette was charged with required volume of the synthetic blood. A trial sample was obtained to confirm that ejecting the fluid from the pipette landed on the fabric in a region which could be captured by the two microscopes. After making 5 alignment samples, video capture on the two computers was intiated and the drip experiments were run. Using ZipScope software, the two computers recorded the time series of the synthetic bloodstain development using the two USB cameras. This allowed for the recording and processing of the stain pattern formation and to capture the output of individual video frames. Each sample picture was saved with a unique identification tag. Following these protocols helps to make the study more reliable and makes it easy to change one parameter at a time. The pipette tips were changed and calibrated after every 3 hours of continuous use. At least three replicas were performed for each set of the experiments naming them as r1, r2, r3 etc. The entire bloodstained fabric with its embroidery hoop was carefully removed from the board and allowed to dry. Once dried, it was placed back in the board and the final image of the dried sample was captured. All samples were properly photographed and documented and then placed in a transparent plastic bag. Care was taken to not fold any package that was still in a wet condition. The same steps were followed after alterating any one parameter in the experiment. Care was taken to not change two parameters at a time as this would create difficulty in understanding the final pattern.

II.8 Fabric test parameters

Due to the potentially large number of experimental values, it was decided to limit these tests to three fabric types (plain woven – balanced (60x60 epi x ppi), plain woven – unbalanced (130 epi x 70 ppi), and jersey knit (44 cpi x 32 wpi). In addition, the drop height was restricted to 0.15, 0.30, 0.50, and 1.5 m; the fabric inclination angle was 0°, 30° or 60°; and the warp angle was 0° or 90°. In addition, the drop volume was varied from 20 to 100 μL; the fabric was either laundered prior to the tests or used as received from the manufacturer; and the fabric was either unbacked (unsupported), or backed (supported) by a hard substrate (the lab bench) or a soft substrate (an absorbent pad.) This resulted in over 1000 possible combinations. In addition, at least three replicates were performed for each set of conditions. To simplify data analysis, only one variable was manipulated at a time and, when possible, less important parameters were eliminated to reduce the sample set.

II.9 Yarn characterization and properties

Blood and other liquids wick through fabric structures by going through the spaces between yarns or the spaces between the fibers within the yarn. The spaces or capillaries between the yarns are larger than those within the yarn, so they are capable of carrying or storing larger quantities of blood. However, the smaller capillaries between the fibers have a higher capillary pressure and thus wick the blood further. In order to understand wicking in the fabric, one must understand wicking within and between the yarns. To accomplish this, the yarn physical properties and their wicking behavior were characterized.

II.9.1 Yarn physical properties

Wicking properties of the yarn depend on their chemical surface treatment, the yarn size (count and linear density), the yarn diameter, and the yarn twist level. Since these fabrics were believed to be 100% cotton with no surface treatment, the chemical surface properties were not measured. From the linear density and the yarn diameter, the yarn packing factor can be determined, which indicates the amount of open space within the yarn in which the liquids are able to move. Thus, these properties were measured using standard techniques. In addition, the yarn structure was observed using both optical and scanning electron microscopy, as described below.

The yarn linear density is the weight per unit length, usually in units of denier (g/9000 m) or dtex (g/10,000 m). The yarn count in the English system (Ne) is the number of 840 yd lengths that weigh one pound. Thus both the linear density and the yarn count can easily be determined by measuring a known length of yarn. To accomplish this, 12” lengths of yarn were extracted from the plain-woven fabric in both the warp direction and the weft direction. For the knit, a 12” yarn was extracted by unraveling a course. The yarn was stretched carefully to straighten it, but not extend it, and 10” was marked. The yarns were then cut to exactly 10” and weighed on a Mettler AE 163 balance to the nearest 0.00001 g (10 μg). Twenty yarns each from the warp, from the weft, and from the knit fabric were measured and the average and standard deviation were recorded.

In staple yarns, such as cotton, the yarn twist affects the yarn strength and its packing factor. Low twist results in a weak yarn with large open capillaries. Medium twist give a strong yarn with much smaller capillaries, while a highly twisted yarn produces a weak yarn due to fiber damage, but results in very small capillaries. The yarn twist is also related to the yarn diameter,

density, and hairiness. Test method ASTM D1422-99(2008) [32] was used to determine the twist in a single spun yarn by the untwist-retwist method. This method has been found satisfactory for all ring-spun and 100% cotton open-end yarns. The twist of all three types of yarns used in this work (warp, weft, knitted) were measured. A single yarn was removed from the fabric and placed in the rotating grip of a Model D 8000 power-driven Twist Tester, Figure II.8. The other end of the yarn was inserted into the canteleverd grip and pulled until the distance between the grips was exactly 10". The rotatable grip was made to rotate either clockwise or counter clockwise to untwist the yarn, thus lengthening the distance between the grips. The rotation of this grip continued until the cantilever passed through the maximum separation (fully untwisted yarn) and returned to the initial grip separation distance (re-twisted yarn). A counter on the rotating grip indicated the number of revolutions to untwist and re-twist the yarn. The turn per inch were determined by:

$$T = \frac{R}{2L} \tag{II.2}$$

where, T= turns per inch (tpi), R = total number of turns to untwist and re-twist the yarn from the counter and L (10") is the distance between grips in inches. The factor of two is used to account for untwisting and then re-twisting the yarn. The twist was measured for each of twenty yarns extracted from the warp and twenty from the weft directions of the woven fabric as well as twenty yarns extracted from the knit fabric.

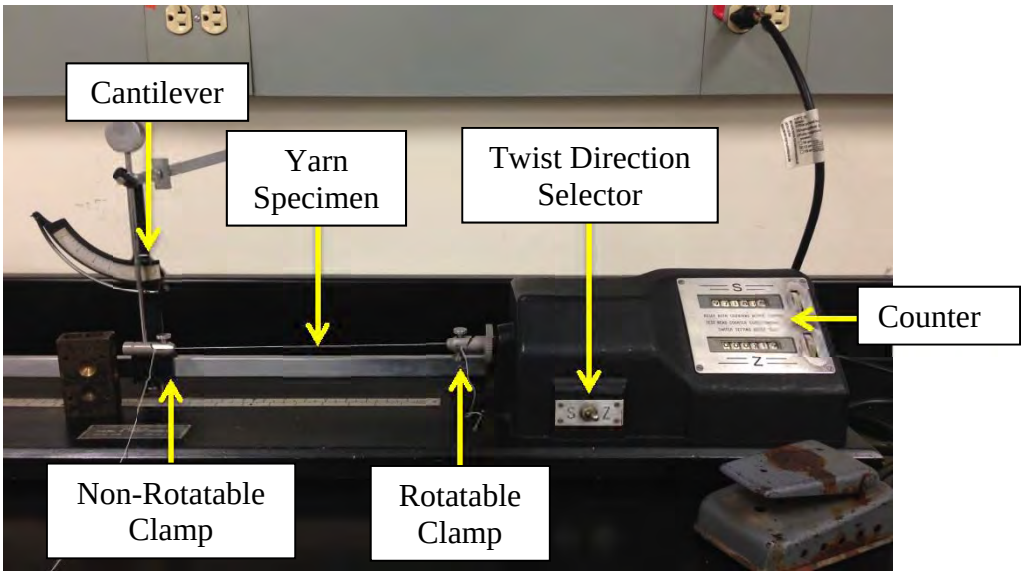


Figure II.8. Model A 8000 power-driven Twist Tester with counter display. A yarn is mounted between the two grips and the rotatable grip was turned until the cantilever moved the maximum distance to the left and returned to its original position. The counter was then read to determine the twist level.

II.9.2 Yarn wicking properties

Most wicking experiments described in the literature have been performed on strips of fabric dipped into a large pool of liquid (infinite bath). However, these tests do not indicate the wicking behavior within the yarns, which the authors of this report believe is critical for understanding the wicking of individual drops of blood. Furthermore, it is more relevant to examine the wicking behavior for a limited or small volume of liquid blood as they are the type of bloodstains that can be deposited on surfaces at scenes or on articles of clothing examined in forensic investigations. To determine wicking within single yarns, a new test method was developed. Using the Twist Tester described above, yarns were mounted in the grips and a video microscope was mounted above the yarn. A ruler was mounted behind the yarn and very close to it, but not touching it to enable measuring wicking distance with the video microscope. The yarn was either used directly, or it was untwisted and re-twisted to the desired twist level. Then a 1 μL drop of synthetic blood type SB4 was placed on the yarn and its wicking rate recorded, as shown in Figure II.9.

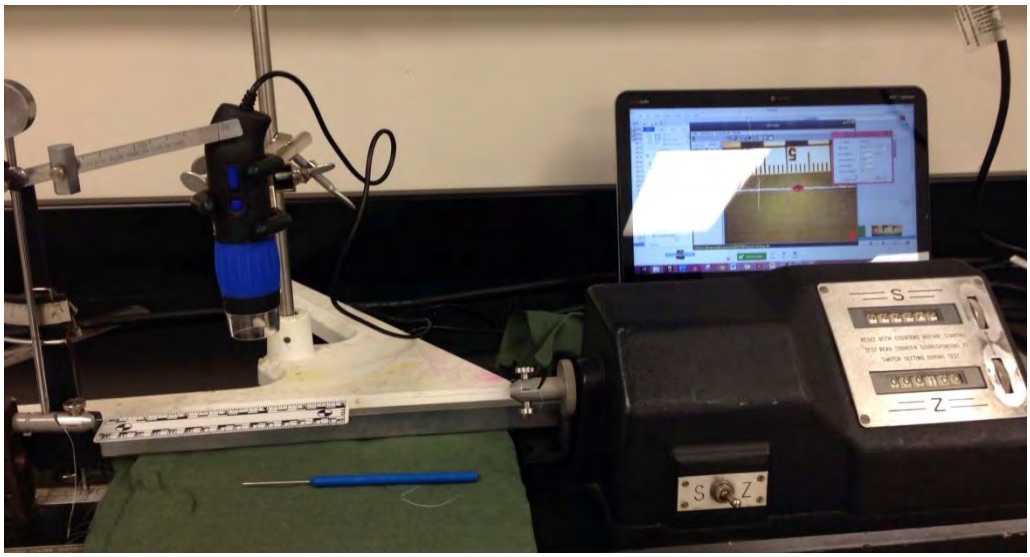


Figure II.9. Apparatus for measuring wicking in yarns at different twist levels is shown. A yarn is mounted in the twist tester, a ruler was mounted nearby and a video microscope was used to monitor wicking.

II.10 Yarn and fabric morphology

The morphology of both the yarns and the fabric can also contribute to wicking. Optical microscopy (conventional and inverted) and scanning electron microscopy, SEM, were used to image the structural features of the yarns extracted from the fabrics. For optical microscopy, the specimen (fabric or yarn) was first mounted on a microscope slide using tape; the slide was placed on the microscope stage, the microscope was focused and the image captured with a high-resolution camera.

A Scanning Electron Microscope (SEM) was used to determine the spinning technology used to produce the yarns. Pieces of conductive, two sided adhesive carbon tape were stuck onto standard stubs. Yarn samples that were ~5 mm long were placed on the carbon tape. The stubs

were carefully put in a coating chamber and the yarns were metalized using a gold-palladium alloy to dissipate charge during imaging. The stubs with the coated yarns were then placed in the sample chamber of the SEM (JEOL JSM – 5900 LV) and high-resolution images were acquired. The electron beam was operated at an accelerating voltage of 15 kV and focused by to a spot of 0.4 nm to 5 nm in diameter. Images at different magnifications were taken to allow the examination of yarn morphology and determine of yarn and fiber diameter.

II.11 Fabric wicking and drying of placed drops

When small drops impact onto a fabric, the drop wicks into the fabric and simultaneously dries. To isolate the wicking and drying events from the impact event, a new test protocol was developed. The fabric or film to be tested was mounted in a 3" embroidery hoop. The hoop was then placed on the stage of a precision balance. A USB video microscope was mounted above the sample to constantly monitor the growing wicking pattern, while the balance constantly monitored the sample weight. (See Figure II.10.) Next, the balance was tared and a drop of synthetic blood (SB5) of known volume was allowed to drop directly onto the fabric from a height of approximately one cm. Video recording and the recording of the weight were commenced immediately. Drying was obtained from the residual weight of the drop, while spreading was obtained from the video images. Note that an image of a ruler was also captured and the images were calibrated for each frame that was analyzed.

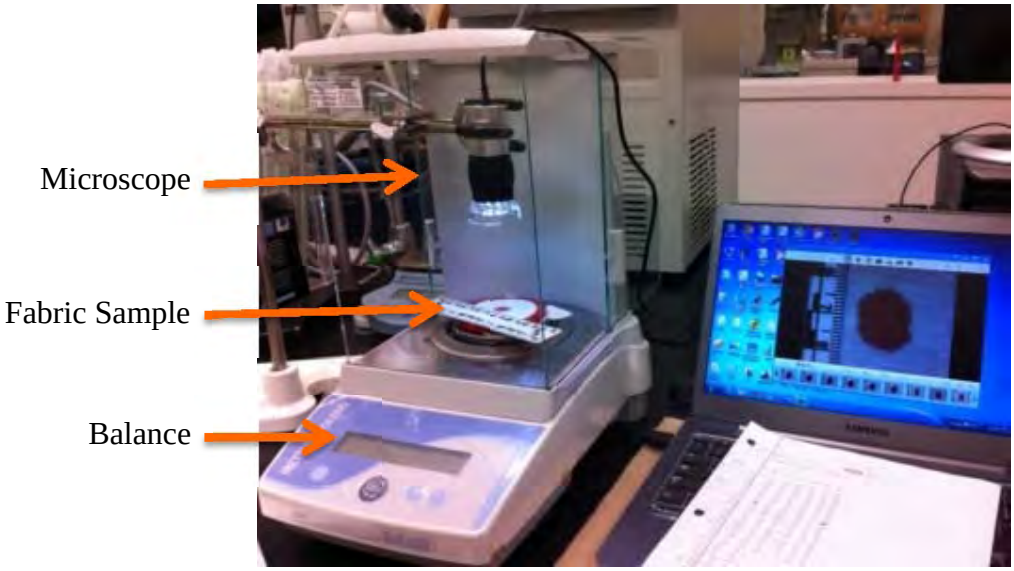
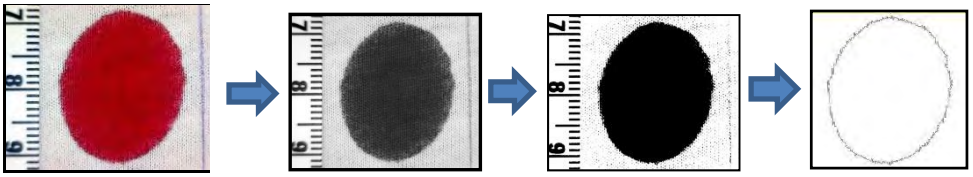


Figure II.10. Photograph of the instrumental setup to observe the synthetic blood spreading and drying process when the synthetic blood droplet was dropped one centimeter onto the fabric sample.

II.12 Area, perimeter and circularity of drop on fabric

In the images presented in the Results section below, the bloodstains often exhibited rough edges and non-circular shapes. In order to get a clear impression of bloodstains on fabrics, the area, perimeter, and circularity of the wicking pattern was obtained through image processing using Image J. Image J was developed by the National Institutes of Health has been validated by many researchers; it is freely available on the internet. Thus bloodstains using SB5 could be obtained

objectively and quantitatively. Single video frames were captured, as shown in Figure II.11a. They were then converted to a gray scale and, using the threshold command converted to a binary image (Figure II.11b and c, respectively). Finally, the perimeter was identified as the boundary between black and white.



(a) (b) (c) (d)

Figure II.11. The image processing process to measure the area of a bloodstain, where (a) shows the original bloodstain image; (b) the grey scale image; (c) the binary image and (d) the perimeter of the bloodstain pattern.

The area *A* of the bloodstain pattern was calculated by counting the number of pixels that the stain image includes. The length of the perimeter *P* was calculated by counting the number of pixels that construct the borderline of the stain image. Finally, the circularity *C* was calculated using equation II.3:

$$C = 4\pi \frac{A}{P^2} \tag{II.3}$$

The value of Circularity ranges from 0.0 to 1.0, with a value of 1.0 indicating a perfect circle.

II.13 Transfer of blood from one fabric to another

Often two fabrics will be layered on top of each other at a crime scene, for example an upper and lower bed sheet, a woven shirt over a T-shirt or two layers of knit fabrics. In other cases, the victim may have bloodied clothing and the clothing on someone rendering assistance or on the perpetrator may come into contact with the victim's clothing. In this case, blood may also be transferred from one fabric to another simultaneously or after some time delay. A protocol was developed, as shown in Figure II.12 to determine the transfer process when both fabrics are in contact with each other at the moment synthetic blood is applied to the first fabric. No studies were performed in which there was a delay between blood being applied to the first fabric and contact with the second fabric.

The apparatus shown in Figure II.10 was used. A 3" embroidery hoop was placed onto the balance pan and fabric 2 was draped gently over the embroidery hoop. Next, fabric 1 was laid gently onto of fabric 2. Finally, a 30 µL synthetic blood drop (SB5) was gently placed onto fabric 1 and began to spread and diffuse until it dried. Upon completion of wicking and drying, the two fabrics were gently separated and imaged.

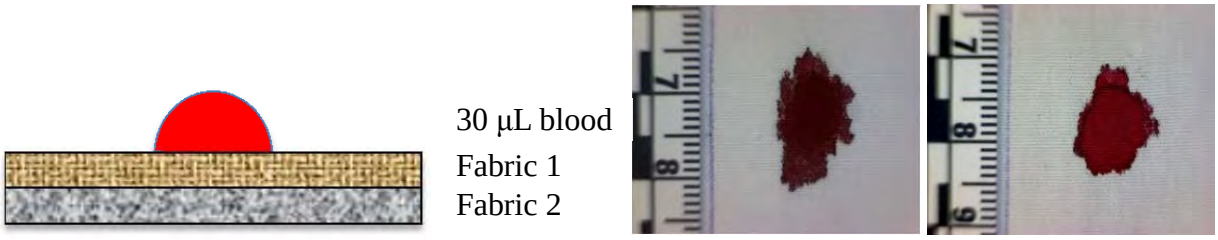


Figure II.12. Transfer of blood from one fabric to a second fabric that is in direct contact with it. (a) The experimental arrangement is shown along with (b) a photograph of the bloodstain on fabric 1 and (c) on fabric 2.

II.14 Porcine blood drop breakup on fabric surfaces

In the experiments described above the primary studies involved the longer term spreading and wicking of synthetic blood. In the following experiments, porcine blood droplets were used since the initial bloodstain formation is expected to depend on the non-Newtonian viscosity of the blood, while the synthetic bloods were not designed or evaluated for this purpose. Therefore, porcine blood was released onto horizontal fabric targets. Two high-speed video cameras were used to film blood droplet flight and impact. Trials were carried out on the bleached cotton T-shirt jersey knit fabric and plain woven percale bed sheeting fabric.

Fabrics were cut into 100 mm x 100 mm squares and stapled to cardboard sheets. These were mounted on A4 or A3 sheets of paper to capture any secondary spatter that was projected beyond the fabric square. For each trial, these targets were positioned on a flat surface in the view of the two cameras. A cardboard test surface was also used for comparison purposes.

Two blood droplet sizes (‘small’ and ‘large’) were selected and these were dropped from a height of 500 mm using an Eppendorf Xplorer 200 µL motorized pipette. Small drops (2.6 mm diameter, approximately 11 µL) were produced using a Labcon specialty pipette-tip (catalogue number 1039-800). The pipette was set with a dispensing volume of 20 µL and a dispensing and aspiration speed of 7. To produce the large drops (5 mm diameter, approximately 65 µL), the same specialty pipette-tip was used with 20 mm cut off the dispensing end. The pipette was set with a dispensing volume of 70 µL and aspiration and dispensing speed of 7. A new pipette tip was used for each test.

This gave droplets with impact Reynolds numbers of between 1727 (small drops) and 3598 (large drops) and impact Weber numbers of between 359 (small) and 806 (large). Ambient room temperature was kept constant at 21°C.

A Photron® SA-X2 high-speed digital video camera with a Tamron® 90 mm macro lens, set at 45° to the target surface, was used to film blood droplet impact and spreading. A Photron® SA1 high speed video camera with a Nikkor 50 mm lens, set parallel to the target surface, was used to film droplet flights. Camera settings and image resolution are given in Table II.2. Droplets in flight were backlit by a Kaiser 1000 W video light. Incident lighting was provided by two 1000 lumen LED arrays. Bloodstained fabric surfaces were photographed 24 hours after impact, using a Nikon D7000 SLR digital camera.

Table II.2: Camera settings

Camera	*Frame rate (fps)*	*Shutter speed (µsec)*	*f-stop*	*Image resolution (Pix/mm*
SA-X2	4000	33	8	8.6 (horizontal)
SA1	4000	50	5.6	13.1

Porcine blood at room temperature was used in all experiments in this section. Blood was collected from an abattoir using the method described by Williams et al. [33] and preserved using aqueous acid citrate dextrose (ACD) anticoagulant (12.5 % of blood volume). Experiments were undertaken within two days of collection and blood was stored overnight in a refrigerator at 4°C. Blood was removed from the refrigerator four hours before testing commenced and allowed to warm to room temperature. Prior to each experimental set, blood was gently agitated for 10 minutes to ensure a homogenous mixture. Blood was kept on a magnetic stirrer throughout the duration of the experiment to prevent erythrocyte sedimentation.

Blood droplet diameter and impact velocity recorded with the SA1 camera were measured using in-house MATLAB® particle identification tracking software. Stain area, stain morphology and the number of satellite stains on each fabric target recorded in static photographs were measured using in-house MATLAB® particle identification software.

II.15 Relative contributions of inertial and wicking forces

It was found that two major time scales and their associated processes were involved in the bloodstains, the inertial forces and the wicking forces. In this set of experiments, an attempt was made to determine the relative amount of each factor.

Specifically, 30 µL of porcine blood was dispensed from a micro-pipette from heights of 100, 200 and 300 mm forming drops of between 3.2 mm and 3.8 mm diameter and impact velocities of approximately 1.4 m/sec (100 mm drip height), 1.9 m/sec (200 mm) and 2.3 m/sec (300 mm). These were allowed to fall under gravity onto horizontal fabric targets. A Photron® SAX2 with a Tamron® 90 mm lens was positioned parallel to the target at a height of 300 mm to film drop impact and spread. A Photron® SA1.1 with a 120 mm Nikkor lens was positioned at 90 degrees to the target, at a horizontal distance of 800 mm, to film the drop flight. Drops in flight were backlit by a Kaiser 1000 W video light. Incident lighting was provided by one 8,800 lumen LED array. Ambient room temperature was kept constant at approximately 21°C. Blood was warmed to room temperature prior to testing and was kept on a magnetic stirrer for the duration of the experiment. Trials were carried out on the same two cotton fabrics (knit and plain woven) used throughout this project.

Fabric samples were mounted on embroidery hoops (Figure II.13) to eliminate any effect of the blood spreading as the result of any underlying surface.

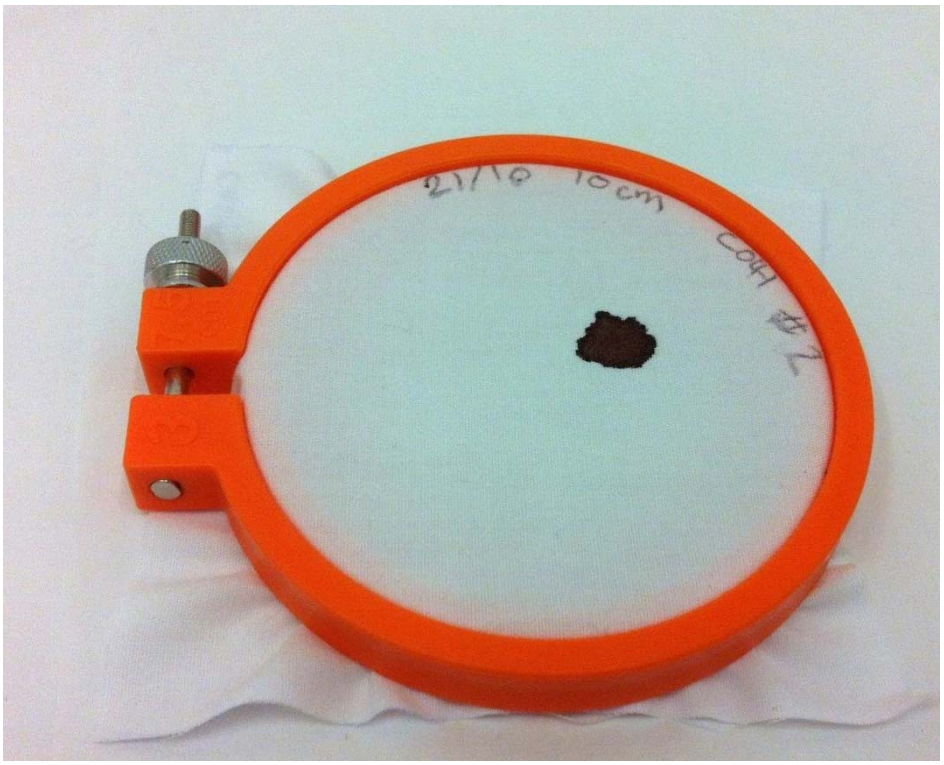


Figure II.13: Fabric mounted in an embroidery hoop

Typical drop impact velocities, Weber and Reynolds impact numbers in this experiment are given in Table .

Table II.3 Porcine blood drop properties for drip stain formation

Height	Impact Velocity (m/s)	We**ber Number (We)at** Impact	Re**ynolds Number (Re) at** Impact
300 mm	2.3	347	2083
200 mm	1.88	219	1607
100 mm	1.38	110	1098

II.16 Spatter stains on fabric surfaces using porcine blood drops

In addition to the drip stains described above, a few experiments were performed using porcine blood to generate spatter stains. Small fast-moving blood drops were generated using a rotating mechanical device and projected horizontally onto the two study fabrics. The fabrics were stapled to Foamcore cards. Drop flights and impacts were filmed using two high speed video

cameras. Comparisons were made between drop impact and spreading dynamics on the different surfaces. These results were also considered in comparison with existing studies relating blood drop size to the resulting stain size [31].

Blood drops were projected horizontally at a range of velocities using a motorized blood drop generation device (BDGD) [33] creating bloodstains on vertically-orientated target surfaces. The BDGD comprised a 600 mm aluminium rotating disc attached to a small electronic motor ('A' in Figure II.13). Porcine blood was applied to the disc via a diaphragm pump. Blood beaded off the edge of the disc into drops, consistent with the principle of rotary atomization. The disc velocity and the volume of blood applied to the disc could be finely controlled in order to create drops of particular sizes and velocities. Blood drop flight and impact dynamics were recorded using two high speed digital video cameras.

A purpose-built target frame was constructed to hold Foamore® target supports, which could be easily swapped for subsequent experiments. The frame was designed to be easily repositioned for experiments requiring it to be different distances from the disc ('B' in Figure II.13). An SAX2 Photron® high speed video camera was positioned parallel to the target to record drop flight prior to impact ('C' in Figure II.13). An Infinity K2/DistaMax long distance video microscope lens was used to magnify the drops in flight and so minimize the uncertainty in the measurement of drop size and velocity. An SA5 Photron® high speed video camera with a Tamron 90 macro lens was positioned approximately 80° to the target ('D' in Figure II.13), offset to accommodate the disc. This camera was used to record drop impact, spreading and wicking of the blood on the fabric. A diffuser screen was positioned 55 mm behind the plane of the disc to accommodate backlighting for the SAX2 ('E' in Figure II.13). Table 2 shows three arbitrarily chosen categories of spatter based on combinations of drop size and impact velocity that were possible with this drop-generating device.

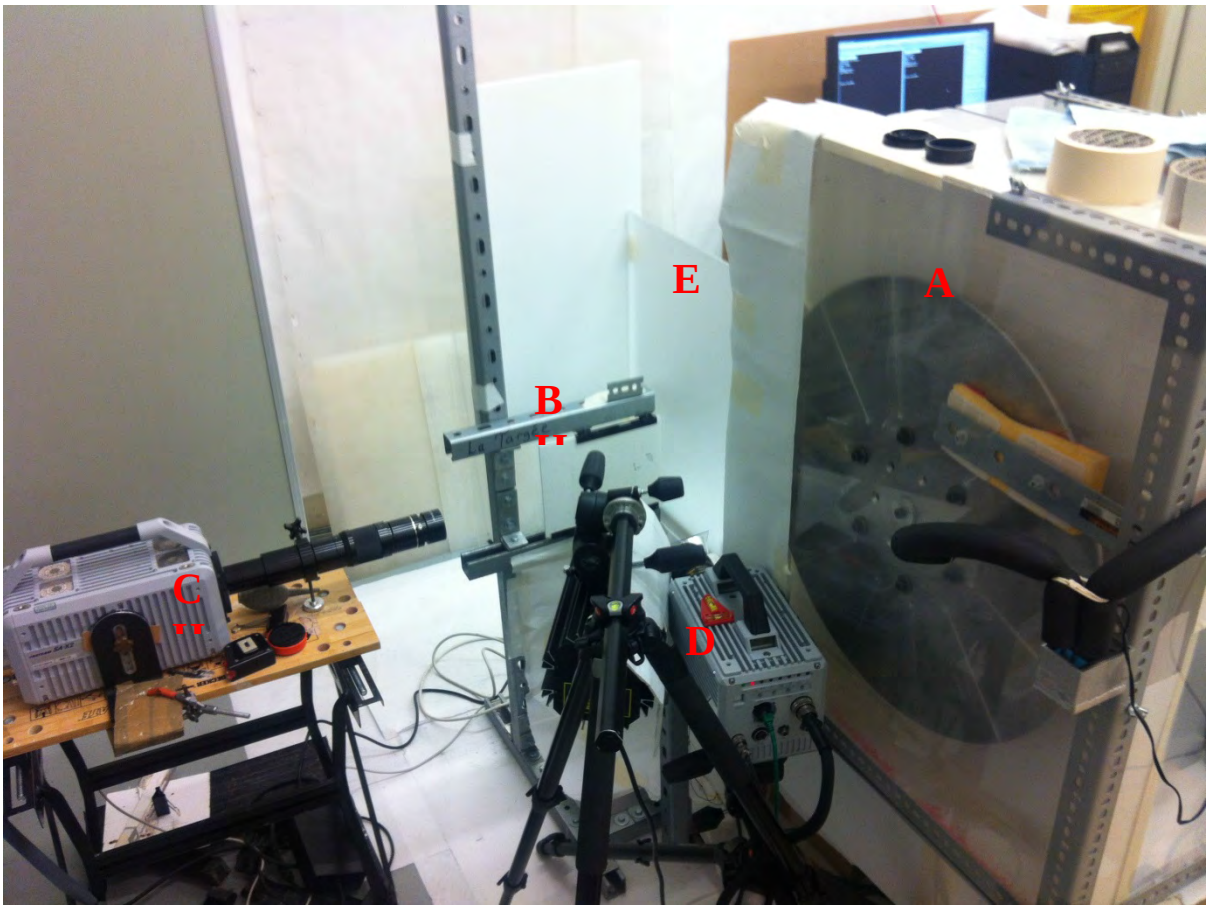


Figure II.13 Set up showing machine disc, SA5 and SA-X2 high speed video cameras, lighting and target.

Table II.3 Drop sizes and velocities for spatter drops generated using the BDGD

Drop Diameter (mm)	Disc Velocity (m/s)	Drop Impact Velocity (m/s)
0.5 to 1	10 to 14	8 to 8.5
0.8 to 1.5	5, 6, 7	4 to 5
0.5 to 1	5 to 6	1 to 2

II.17 Comparison of SB5 and porcine blood stains on fabric surfaces

The previous transfer stain tests were performed using synthetic blood SB5. A set of matched experiments were performed in the same manner as described in section II.13, however, both SB5 synthetic blood and porcine blood were used and the results compared (see section III.26). Fabrics (100% cotton jersey knit and 100% cotton plain woven bed sheeting) were mounted in 3 embroidery hoops and placed on a precision balance. A USB video microscope was positioned

directly above the fabric and a drop of SB5 or porcine blood was placed on the fabric. The spreading and wicking of the drop was recorded along with the weight over times of several minutes. Finally, the video frames were analyzed using ImageJ to generate the stain area, perimeter, and circularity as a function of time. The results for the two fabrics and for the two liquids were compared and are discussed in section III.26.

III. Results

This study has been carried out to set base parameters for conducting bloodstain experiments on textile surfaces and investigating the effects of various parameters on the final bloodstain patterns. Each section will focus on its usefulness, application and results obtained from the authors' perspective. Some concepts are straightforward while reverse engineering was useful in grasping others. The authors remind the reader that each experiment was repeated at least three times, and some experiments many more than that. The images shown below are single examples of the observations for illustrative purposes while the results reported are based on the full set of observations for all repeats.

III.1 Fabric down selection

There are hundreds of types of fabrics with a multitude of yarn variations. Thus it was imperative to start by selecting a small set of materials to enable comparisons and to understand their differences in bloodstains and bloodstain patterns. The most common types of fabrics at a crime scene are plain-wovens (bed sheets, table clothes), jersey knits (T-shirts), denim and carpets. In addition, cotton is the most prevalent fiber due to its perceived comfort. Therefore, only 100% cotton plain-woven and jersey knit fabrics were studied. In addition, to compare the different fabrics, similar basis weight fabrics were used for both fabric types. Thus, 100% cotton percale bed sheeting with a basis weight of 120 g/m^2 made from ring spun, combed cotton yarn, and a 100% jersey knit T-shirt material with a basis weight of 124 g/m^2, which was believe to be made from a ring spun, combed cotton yarn were used. Both fabrics were purchased from Test Fabrics.

III.2 Laundering

To aid manufacturing, yarns and fabrics are often sized or waxed. These treatments can alter the wicking behavior. Since fabrics at a crime scene are likely to have been washed at least once, our first experiments evaluated the effect of washing on the synthetic bloodstains. Thus, we compared bloodstains on the percale bed-sheeting fabric as received, after washing them once according to the process described in AATCC Monograph M7 standard and after washing them 10 times using this procedure [23]. Figure III.1 shows a time series of the wicking for the non-laundered and laundered fabrics. The wicking rate and extent was considerably larger for the washed samples. No difference was seen in the wicking behavior between the single washing and the multiple washing experiments. Therefore, all subsequent samples were washed once using the AATCC method prior to testing.

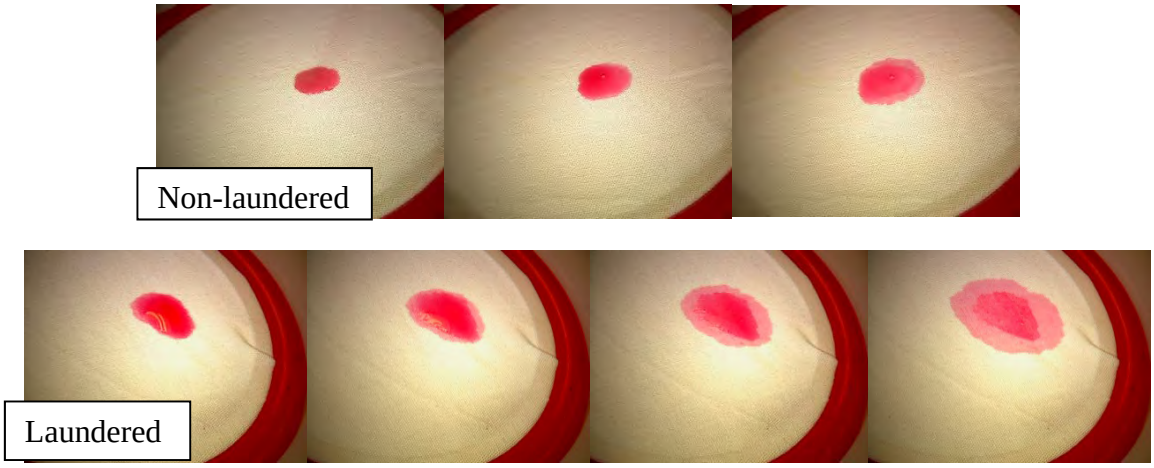
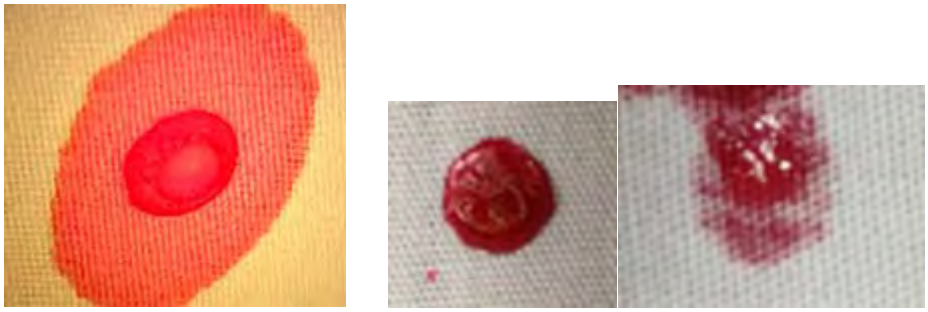


Figure III.1. Time series images (top view) of a single synthetic blood drop (SB1) wicking into the fabric as received (non-laundered) and after one laundry cycle. The time between frames was 10 seconds in both cases. Samples are shown mounted in a 3" embroidery hoop.

III.3 Blood recipe development

As described above, there are over 1000 combinations of primary experimental parameters that may have to be analyzed to understand the behavior of blood on the two fabrics mentioned above. Therefore, it is logistically practical and imperative that the bulk of these studies be carried out using a suitable synthetic blood. In the search for a suitable recipe, several criteria were defined. First, the bloodstain pattern on textiles should resemble real bloodstains and the blood should wick into the textile. Second, the recipe should be reproducible. Third, the bloodstain should dry over time. Fourth, the physical properties as they relate to wetting should mimic real blood, e.g. the viscosity and surface tension.

The first two types of synthetic blood tested, were defined as SB1 and SB2 (see Appendix for recipe). These recipes used cornstarch, corn syrup, and red food coloring along with other ingredients. The resultant synthetic bloods were pleasing to the eye, but were found to unsuitable, as shown in Figure III.2. In case of SB1, the stain formed a globule over the surface and only a portion of the blood would wick into the fabric structure, whereas the solids in the recipe remained on the surface. The place containing the solid globule (dark red spot in the figure) would flake off if the fabric was not handled carefully.



Type 1 Type 2

Figure III.2. Synthetic blood types 1 (SB1) and 2 (SB2) on textiles after being allowed to wick into the fabric and drying overnight.

In the case of SB2, the stain appeared gel-like. It barely wicked into the fabric and would completely flake off if not handled properly, leaving almost no trace of its former presence. After overnight drying of larger volumes, the remaining drop had a slimy appearance and formed a thin, hard, shiny film (see Figure III.2).

Type 3 (SB3) recipe followed the ASTM test method F1819-07 synthetic blood [26] using guar gum as a thickener. It was found to be more consistent in its performance. The viscosity and surface tension were adjusted to lie within the working range of viscosity and surface for the solution.

The Type 4 (SB4) recipe was finally settled upon. It closely followed the ASTM test method F1819-07 synthetic blood [26], but modified to increase the viscosity (using a different thickener than for SB3) and surface tension to lie near the midpoint of the values reported for human blood. The ASTM test method was developed to determine whether blood would penetrate through surgical gowns. This is facilitated by a viscosity and surface tension at the bottom end of the range for human blood. However, in the current research, values closer to the midpoints are more relevant. Thus, the suggested recipe was modified to use a higher viscosity thickener. This blood type was used in most of the research reported below. However, initial studies were performed with synthetic bloods SB1 and SB2 to determine key parameters prior to settling on an appropriate artificial blood. Some of these experiments were not repeated with synthetic blood SB4 as it was deemed unnecessary.

III.4 Effect of different plain woven fabric construction

Early in this research, single, 50 μL drops of SB1 were dropped from a height of 6” at 0° fabric angle (perpendicular to fabric surface) onto a balanced plain weave (60 epi x 60 ppi) and an unbalanced plain weave (130 x 70), as shown in Figure III.3. The bottom camera was used to obtain these pictures to eliminate distortion due to off-axis viewing by the top camera in these early experiments. The dark red portion of the image shows the location of the original drop application and the wider ring shows the wicking of the drop into the fabric. In the case of the balance weave, the pattern is nearly circular, except where the two drops merged. On the other hand, in the unbalanced weave, the stain is elongated in the direction of the fabric warp. On a hard, non-porous surface, both of these stains would be circular. However, the unbalanced

structure of the unbalanced weave distorted the pattern due to preferential wicking in the warp direction.

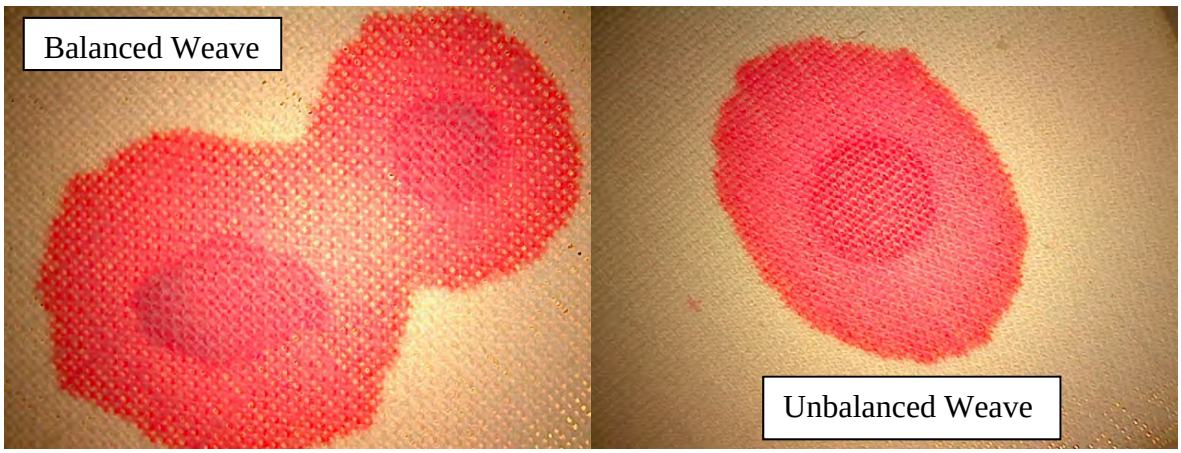


Figure III.3. Synthetic bloodstains using SB1 of two drops (left) on a balanced plain weave and a single drop (right) on an unbalanced plain weave are shown. Both images show the underside of the fabric. On the balanced weave (left) the patterns are nearly circular, while on the unbalanced weave (right), the pattern is elongated in the warp direction.

III.5 Determination of the minimum volume for complete penetration

In printing of textiles, there is a certain minimum drop size required to penetrate through the fabric onto the backside. By analogy, there will be a minimum blood drop volume required to penetrate all the way through. To determine this volume, 5, 20 and 50 µL drops of synthetic blood SB2 were dripped from a height of 6" onto 130 × 70 plain woven cotton fabric samples. The 5 µL blood drop did not penetrate all the way through the fabric although the location of the drop was easily observed. The 20 µL drop seeped into the interstices of the weave and penetrated through in a few locations. The 50 µL drop penetrated completely through the fabric and even formed a suspended drop.

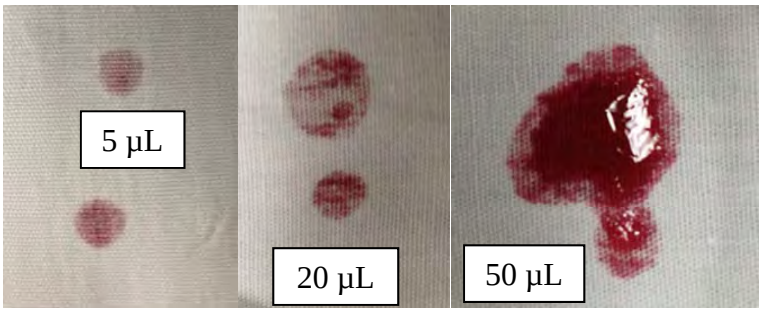


Figure III.4. Bottom view of synthetic blood SB2 drops on the unbalanced plain woven fabric (130 x 70). Drop volumes are given for each image. The smaller drops did not penetrate completely through the fabric.

However, we also note that this synthetic blood, SB2, did not spread or wick through the fabric even when it was allowed to dry overnight. In later experiments (see Section III.8), it was found

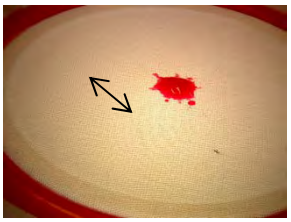
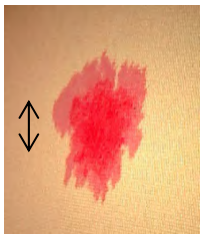
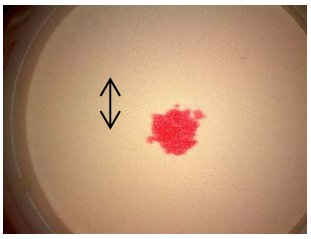
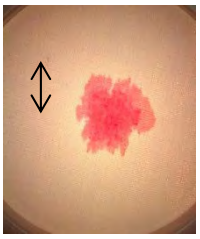
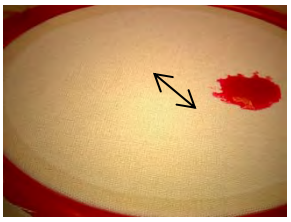
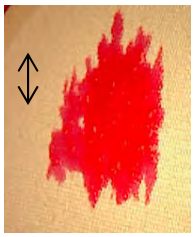
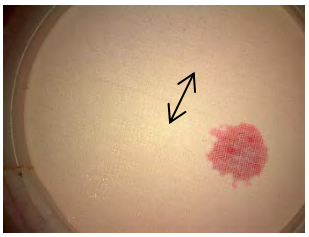
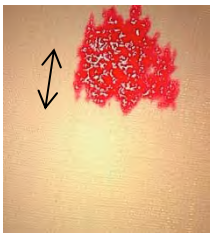
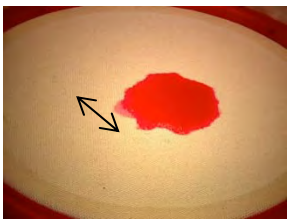
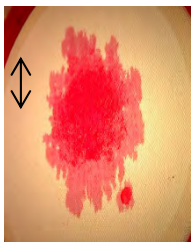
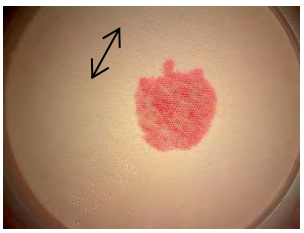
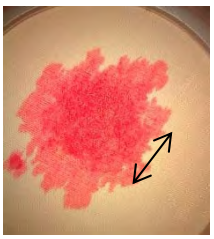
that even satellite drops from a 20 μL drop of SB4 penetrated completely through the fabric. These satellite drops appear to be less than 1 μL, which implies that even very small drops of a suitable synthetic blood will wick all the way through the plain woven fabric used in this study. This indicates that the modified synthetic blood recipe SB4 resembles blood and is suitable for studying bloodstains on textiles.

III.6 Effect of drop volume on bloodstain pattern

In traditional wicking experiments, one end of a fabric is placed into a container containing the desired test liquid, usually water, and the distance that the liquid wicks is measured as a function of time. The fabric is cut into strips with their length in the warp or weft direction. These "infinite bath" wicking experiments give only the rate of wicking. However, in the case of bloodstains, single drops of blood impact onto the fabric and wick in all directions at rates dependent on the local structure. In addition, wicking stops along with the stain development when the liquid volume is fully entrained within the yarns. Therefore, the extent of wicking and the stain that develops should be dependent on the drop volume.

Table III.1 shows images of 20, 50 and 100 μL drip stains formed by dripping of synthetic blood SB4 onto the plain woven (130 x 70) fabric from a height of 6", impacting the fabric with an inclination angle of 0° (i.e. the fall direction is perpendicular to the fabric surface or angle of impact is 90°.) The 0^{th} second images show that the initial stain shape is circular, as expected. However, after several seconds to minutes, the SB4 wicks into the fabric, altering or distorting the initial stain shape. There are several things to note. First, wicking of the 20 μL drop is limited by the small drop volume, which prevents the shape from fully developing. It largely retains its initial shape, with some wicking evident along the yarns, resulting in a rough perimeter. The 50 μL SB4 drop wicks further into the fabric and the pattern is more developed along the warp direction. The perimeter is quite rough and there are even some areas within the stain that appear unstained. At 100 μL, the SB4 drip stain pattern is even more developed, with very convoluted edge characteristic and wicking in the warp direction is very evident, while wicking in the weft direction is less pronounced. From these results, it appears that the 20 μL drop results in a final drip stain that is less sensitive to the fabric structure while the 100 μL drop results in a final drip stain that is very sensitive to the fabric structure. This suggests that small drops may be more useful and reliable for the forensic scientist in the analysis of bloodstains and bloodstain patterns. Much more work is required with both synthetic and real blood to determine if this is a fruitful approach.

Table III.1. The effect of volume on SB4 synthetic bloodstains and bloodstain patterns for drip stains are shown just after impact and after drying for 24 hrs. Drip height was 6".[†]

Time	Top View (Distorted Shape)[††]		Bottom View (True Shape)[††]	
Drop Size	0th Second	After 24 hrs.	0th Second	After 24 hrs.
20 µL				
50 µL				
100 µL				

[†]Arrows mark the warp direction of the fabric.
[††]The top view is distorted due to camera position, which was placed off-axis to allow the drop to fall unimpeded. The bottom image gives the true shape since the lower camera was placed directly below the center of the fabric.

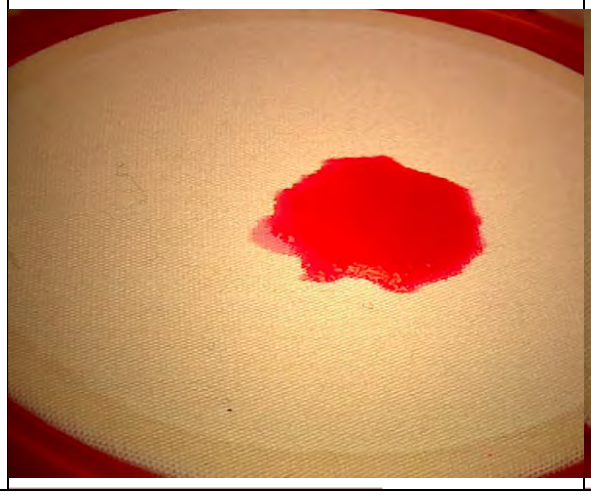
III.7 Effect of drop height on bloodstain pattern

It is generally accepted that, in small, passive drip stains on hard, irregular, nonporous materials, the bloodstain or bloodstain pattern that an analyst observes is the same as that which was present less than a second after the drop impacted the surface unless it was disturbed by some other event. It is this property that provides the great evidentiary value to BPA. Performing a similar experiment on fabric results in similar behavior immediately after impact, as shown in Table III.2 (see also section III.23).

For a 20 µL SB4 drop, dripped from a height of only 6", the initial stain pattern is compact with a few, short spines and only a few satellite stains, which are located very close to the parent

stain. At a drip height of 12”, there are still only a few spines, but there are several satellite stains located further away from the parent stain than for the 6” drip height. For larger drop volumes, 50 and 100 μL SB4 drops, the stains that occur at a drip height of 6” exhibit few or no spines or satellite stains. However, there is a marked change when the drip height is increased to 12”. In this case, there are multiple spines and many satellite stains located at a substantial distance from the parent stain. The behavior is quite similar to bloodstains on hard surfaces. At low drip heights, there is simply not enough impact energy to overcome the liquid surface tension so there is little or no satellite stains or spines. At higher drip heights, there is more impact energy, which results in a larger number of spines and satellite stains.

Table III.2. Drip stain patterns formed by dripping SB4 drops onto the plain-woven bed sheet fabric from 6” and 12” are shown as they appeared immediately after the synthetic blood drop impacted the target surface.

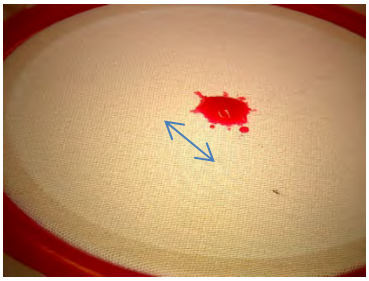
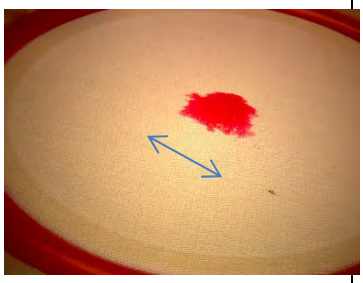
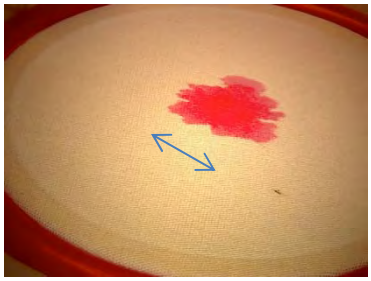
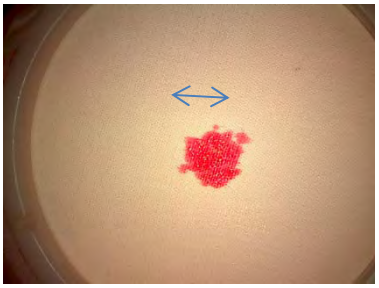
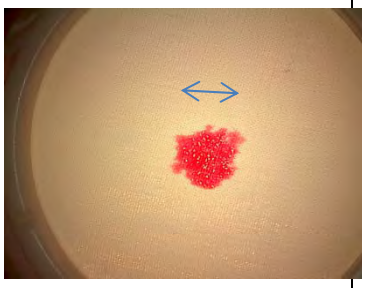
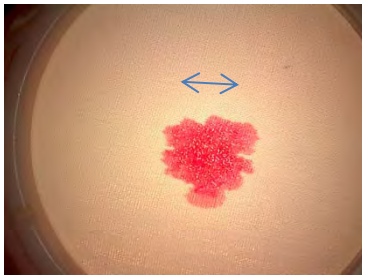
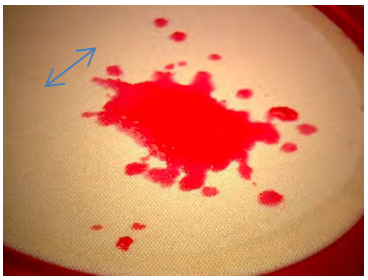
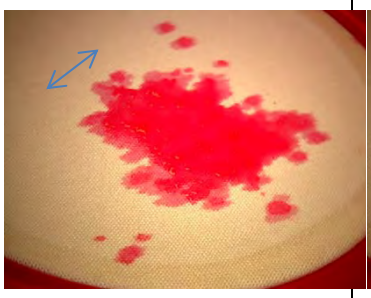
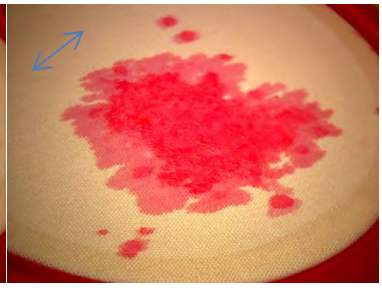
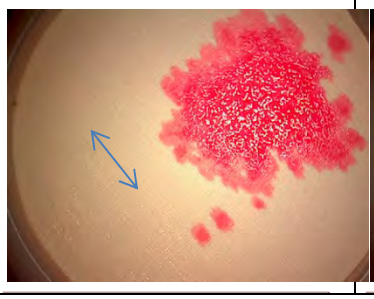
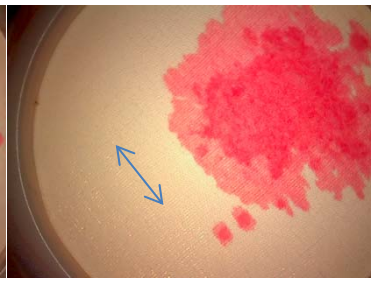
	6”	12”
20 µL		
50 µL		
100 µL		

III.8 Time evolution of bloodstains and bloodstain patterns

As shown above, the (synthetic) bloodstain and bloodstain patterns that occur immediately after impact for fabrics are similar to those on hard, irregular surfaces. However, textiles are well known for their wicking behavior, unlike hard, non-porous materials. This results in a change in the stain with time for fabric that simply does not occur on hard, non-porous surfaces. The evolution of SB4 stains for a 20 μL drops falling from 6" and a 90 μL drops falling from 12" are shown in Table III.3. Note that even though the 0^{th} second patterns are quite similar to what would be observed on a hard, irregular surface, the 40^{th} second patterns are quite different.

For the smaller drop (20 μL, SB4), dripped from a height of only 6", the initial stain is compact with few, short spines and only a few satellite stains, which are located very close to the parent stain. After only 20 seconds, the spines have been masked and the satellite stains now appear to touch the edge of the growing stain. After 40 seconds, no evidence remains of the initial spines or the satellite stains due to wicking by the fabric. Wicking of SB4 has masked the initial pattern completely. The larger drop (90 μL, SB4), dripped from a 12" height exhibits several satellite stains (>15) and multiple short spines immediately after impact. After 20 seconds, many of the nearby satellite stains have merged with the edge of the parent stain and the short spines have been masked due to wicking by the spreading stain. After 40 seconds, all of the nearby satellite stains and the spines have been completely masked by wicking of the parent stain in addition to their own wicking. This results in a jagged perimeter, but the initial stain cannot be discerned from the stain after only 40 seconds. Only the satellite stains that are located well away from the parent stain remain, but even their shapes have been distorted due to wicking. This demonstrates that by the time an analyst examine bloodstains present on a textile, they may be quite different from the initial stain generated by the bloodletting event and quite different from what would be observed on a hard nonporous surface.

Table III.3. Time evolution of passive drip stains of SB4 drops falling onto the plain-woven fabric from a height of 6” or 12” with a fabric inclination angle = 0°.[†]

Time	0 seconds	20 seconds	40 seconds
Top View[††] 20µl Drip Height 6”			
Bottom View 20µl Drip Height 6”			
Top View[††] 90 µL Drip Height 12”			
Bottom View 90 µL Drip Height 12”			

[†]Arrows mark the warp direction of the fabric.

III.9 Effect of warp angle on final bloodstain pattern for woven fabric

If a blood drop falls on a horizontal, flat, non-porous hard surface, the stain observed does not depend on how the surface is oriented (rotated about the vertical axis). However, for a textile whose structure may be different in different directions, the relative orientation may alter the

observed stain. Figure III.5 shows SB4 drip stains on the unbalance percale plain woven bed sheeting (130 epi x 70 ppi), with fabric inclination angle of 0°; the SB4 drop volume was 60 μL, the drip height was 6", and the images were taken after the stain had dried overnight. For these tests, the warp direction of the fabric was rotated about the vertical axis such that the warp angle (see Figure II.7) of 0°, 45°, and 90°. Note that the pattern rotates as the fabric is rotated in the plane of the fabric. The pattern is clearly elongated along the warp direction, with multiple regions of extended wicking. This would not occur for a similar experiment on non-porous hard surfaces.

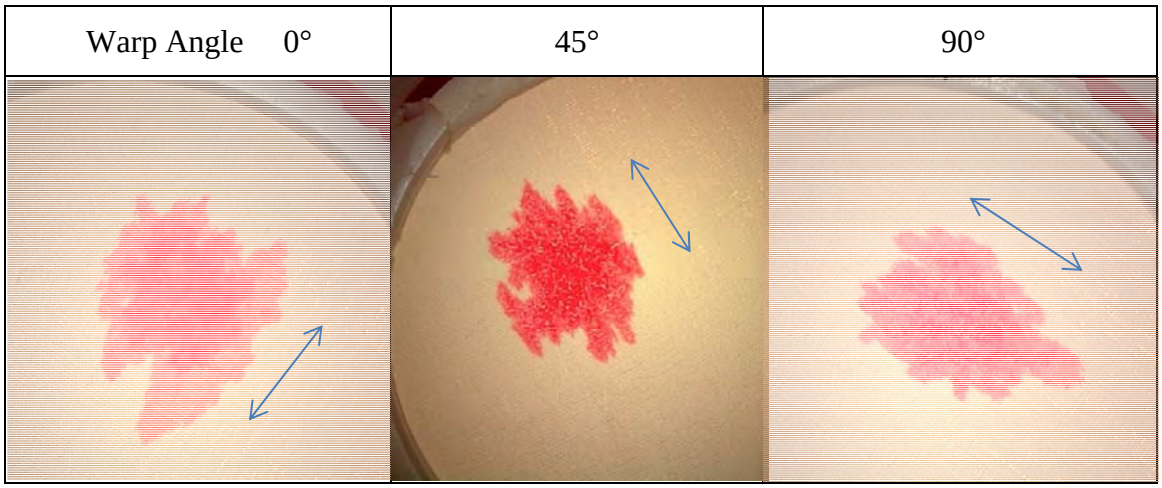
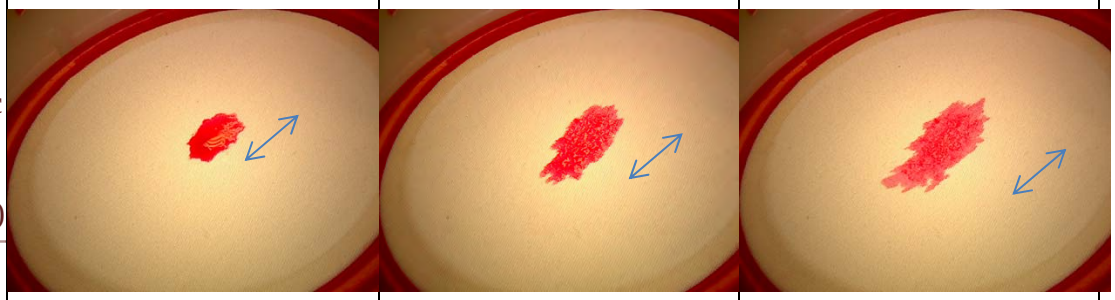
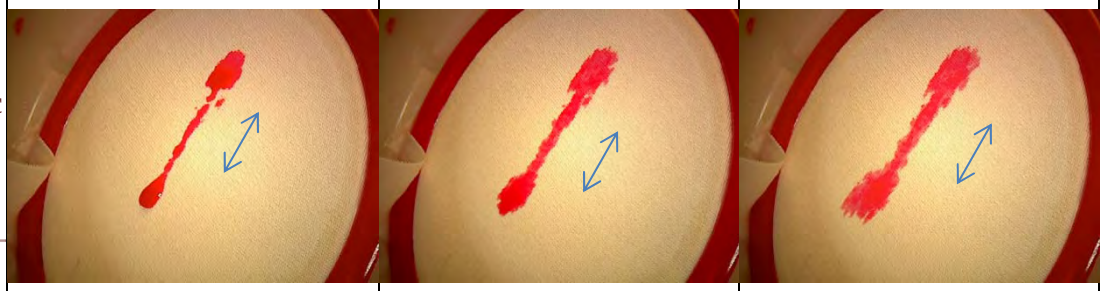
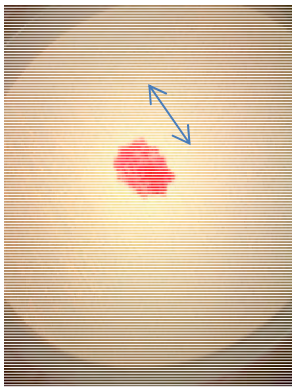
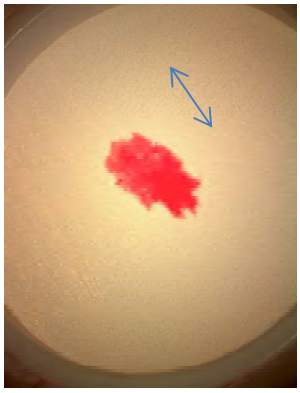
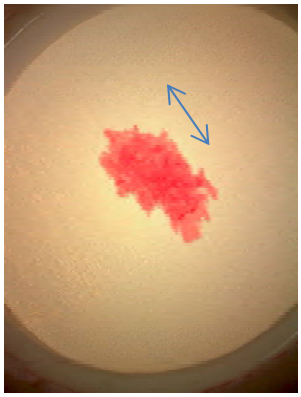


Figure III.5. Bottom view of SB4 drip stains spreading on 130 x 70 plain-woven percale bed sheeting as the warp angle was rotated about the vertical axis. Impact angle was 0°, impact height was 6" and drop volume was 60 μL. Arrows mark the warp direction of the fabric.

III.10 Effect of fabric inclination on final stain pattern for woven fabric

In all of the above work, the synthetic blood drops fell onto fabrics that were horizontal, i.e. at 0° inclination angle and perpendicular to the path of the falling drop (impact angle is 90°). However, at crime scenes, the target surfaces are not always perpendicular to the source of blood; rather a drop may impinge on the fabric from some other angle. Table III.4 shows the time evolution of SB4 drops falling on the fabric at two different angles. At a high angle of impact (60°) and at 0 time, the drop is slightly elongated in the impact direction. The drop wicks into the fabric, which, in this case, elongates the stain. This may be due to gravity or due to the warp direction of the fabric. At a low angle of impact (30°) and 0 time, the drop runs down the surface and finally stops. The stain has a large spot at the point of impact and a large spot at the bottom of the stain. There are also occasional skips in the stain trail as the drop bounces off the surface. This bounce is not always seen. Over time, the stain and trail wick into the fabric, broadening it significantly and masking much of the characteristics of the initial pattern. The images from the bottom side of the fabric enhance the skipping and the wicking images. Furthermore, immediately after impact, it was obvious which end of the fabric was up. However, in the final pattern after wicking has occurred, it is quite difficult to be sure of the fabric orientation.

Table III.4. Time evolution is shown for SB4 drip stains on 130 x 70 percale bed sheeting tilted at 30° and 60° from the horizontal. The fabric warp direction lies in the plane of motion of the drop.†

Top	0th Sec	20th Sec	60th Sec
Angle of Impact = 60° (Fabric tilted 30° from Horizontal)			
Angle of Impact = 30° (Fabric tilted 60° from Horizontal			
Bottom	0th Sec	20th Sec	60th Sec
Angle of Impact = 60° (Fabric tilted 30° from Horizontal)			

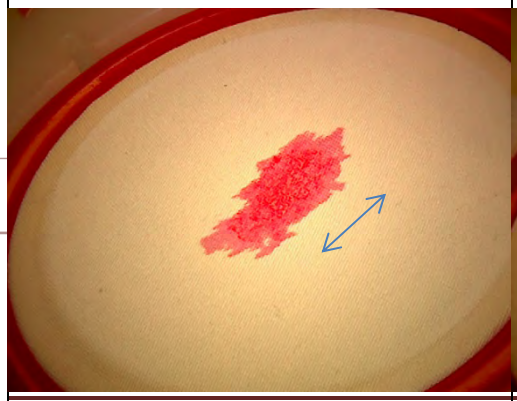
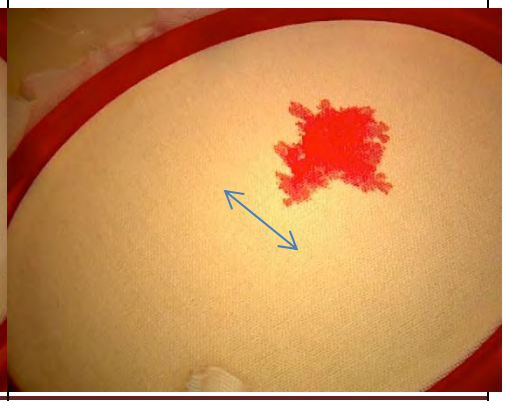
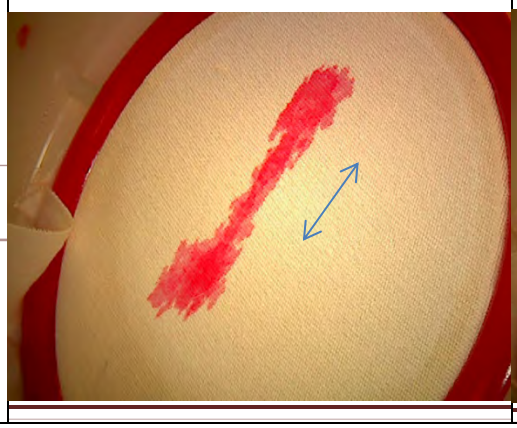
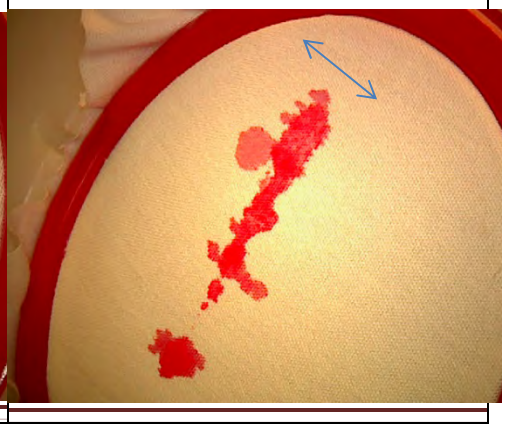
Angle of Impact = 60° (Fabric tilted 30° from Horizontal)			

†Arrows mark the warp direction of the fabric.

III.11 Combined effect of fabric inclination and warp orientation on final bloodstain

Both the warp orientation angle and the fabric inclination angle affect the SB4 stain, but in different ways. Table III.5 shows the combined effect of these orientation factors. Figure III.5 showed that, for the unbalanced 130 x 70 plain woven fabric, wicking occurred more rapidly in the warp direction and thus the stain was elongated in the warp direction. Table III.4 showed that the fabric tilt angle caused the stain to be elongated in the vertical direction. The images in the second column of Table III.5 are the same images as in Table III.4. The stain is elongated in the warp and tilt directions, and the final pattern is more diffuse than the initial stain due to wicking. However, the patterns shown in the third column of Table III.5 are quite distinct. For a fabric tilt angle of 30°, the pattern should be elongated from the upper right to the lower left, the downward direction of the fabric. However, the pattern is elongated more in the cross direction due to the preferential wicking in the warp direction. An analyst would be hard pressed to define from which direction the blood drop came. The situation for the steeper fabric inclination angle is only superficially clearer. In this case, there is a clear line from the upper right to lower left. In addition, there was a large skip in the pattern at the bottom of its travel as well as a couple of smaller skips above it. However, the preferential wicking in the warp direction suggests that this pattern may have originated from many smaller drops moving from the upper left to the lower right, therefore leading to a possible incorrect interpretation on the shape, size, distribution, and even directionality of the bloodstains. This was a single drop impact that occurred in the upper right side of the pattern.

Table III.5. The combined effect of impact of an SB4 drop dripping on the fabric at an inclination angle and with the warp direction parallel (0°) or perpendicular (90°) to the impact direction.

Fabric Inclination Angle \ Warp Angle	0°	90°
30°		
60°		

†Arrows mark the warp direction of the fabric.

III.12 Fabric tautness on final bloodstain

In the examples presented above, all of the fabrics were held taut in the embroidery hoop, i.e. similar to a drumhead. However, at a crime scene, a fabric such as a bed sheet will often be loosely draped across a bed, therefore unstretched. The test shown in the last row of Table III.5 was repeated for a loose fabric and the results are shown in Figure III.6. The patterns observed are quite different from above. When the warp angle was 0°, the stain was clearly elongated with the larger stain at the top and trailing down the fabric before wicking into the fabric, thus blurring the edges of the stain. On the other hand, when the warp angle was 90°, the stain is still elongated in the vertical direction, but it is now much more diffuse and appears broader throughout with no distinct larger stain at the top. Again, it would be difficult for the analyst to

state that these two stains were formed under identical conditions except for the orientation of the fabric warp.

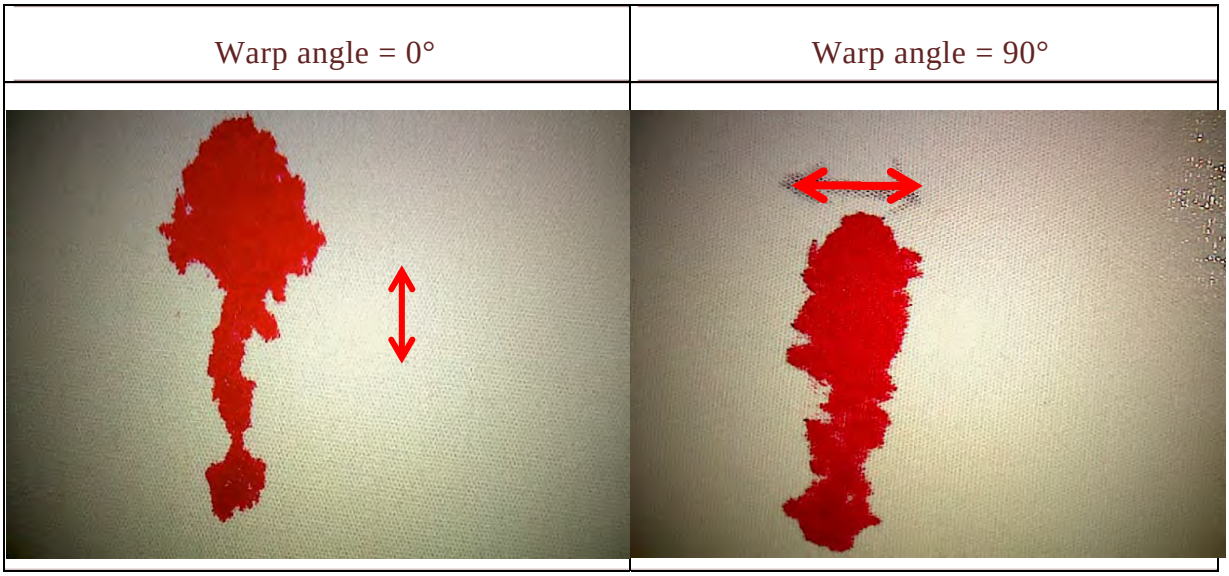


Figure III.6. A SB4 drip stain on unstretched fabric at an inclination angle of 60° is shown. The drip height = 0.5 m, drop volume = 30 µL, impact angle = 30° and the warp direction marked with the arrow.

After observing the effect of the experimental parameters given above and how each parameter played an important role in forming the final stain or stain pattern, new parameters were identified that affect the final synthetic bloodstains and bloodstain patterns:

1) Type of fabric: Knit and woven
2) Drip height: 0.5 m and 1.5 m
3) Drop volume: 30 µL and 80 µL
4) Angle of Impact: 30°, 60° and 90° - Angle between the plane of target and the area of origin.
5) Warp angle: 0° and 90° with respect to axis of rotation of the fabric.

It should be noted that all experiments performed to test the above parameters were done in three replicates and data was recorded with only one parameter altered at a time.

III.13 Type of Fabric

Although bed sheets and similar plain-woven fabrics are common at crime scenes, another common fabric is T-shirt material. In the US, the most common T-shirt is a cotton single jersey knit. This fabric structure is formed of intermeshed loops and results in a fabric that is much softer and stretches more easily than plain-woven fabric. Figure III.7 compares SB4 stains and stain patterns on the bed sheet fabric and T-shirt jersey fabric for a 30 µL SB4 synthetic blood drop falling onto a horizontal fabric from a height of 0.5 m, which is a higher drip height than in the studies shown above. The angle of impact was 90° and the warp angle was 90°. The images were captured 10 seconds after impact. There is a large differnce observed between the stains on the two fabrics. When the drop impacted the plain woven fabric, it broke up and formed

pronounced spines as well as satellite stains on the plain woven fabric. However, on the knit, the stain a nearly circular stain with a smooth periphery was obtained. When the synthetic blood impacted onto the knit fabric, it rapidly wicked into the fabric and formed a very neat, uniform, well defined boundary. It is interesting to note that the plain weave has a simpler construction but led to a complex stain. On the other hand, the knitted structure is a more complex fabric but it led to a simple stain.

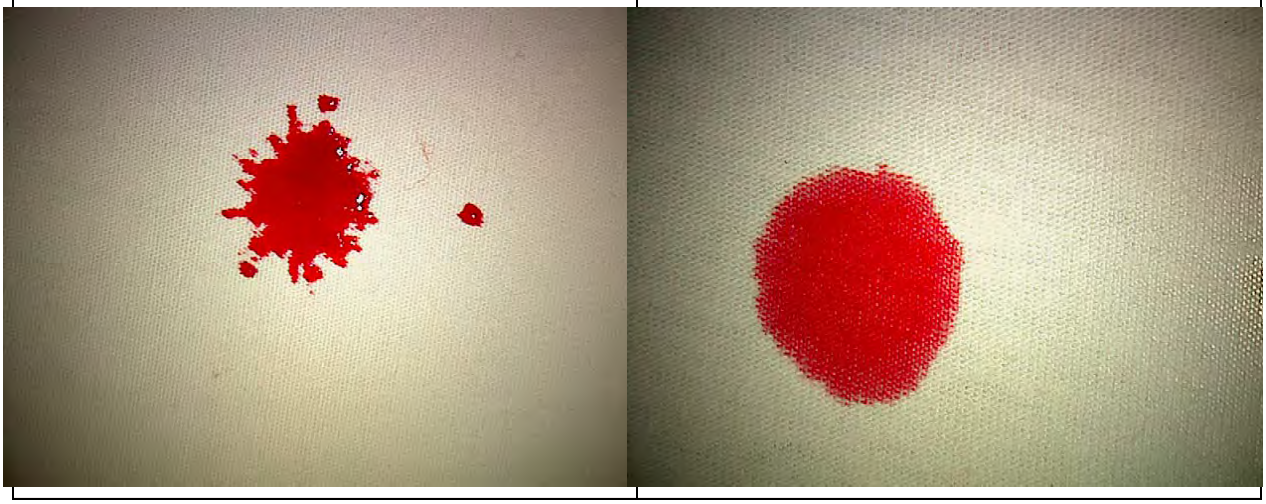
Plain Woven	Knitted
	

Figure III.7. SB4 drip stain is shown for 30 μL drops that fell 0.5 m onto plain woven and jersey knit fabrics with an angle of impact of 90°. Note: the magnifications are different in the two photographs such that the size of the stains cannot be compared.

Figure III.8 shows the pattern development of a drip stain on the jersey knit when 30 μL of SB4 was dripped from a height of 1.5 m onto a fabric inclined at 30° (angle of impact = 60°). In the first image, there is clear downward movement of the drip with a few spines and satellite stains. After only one second, the edges of the drip are diffuse and the detail close to the parent stain is beginning to be masked. After three seconds, the spines have been completely masked and the close-in satellite stains have merged with the parent stain. Only the most distant satellite stain remains as a separate stain, but it is nearly touching the parent stain and is quite diffuse. Furthermore, the drop has completely wicked into the jersey knit within five seconds, while it took more than two minutes for the same size drop to wick into the plain woven.

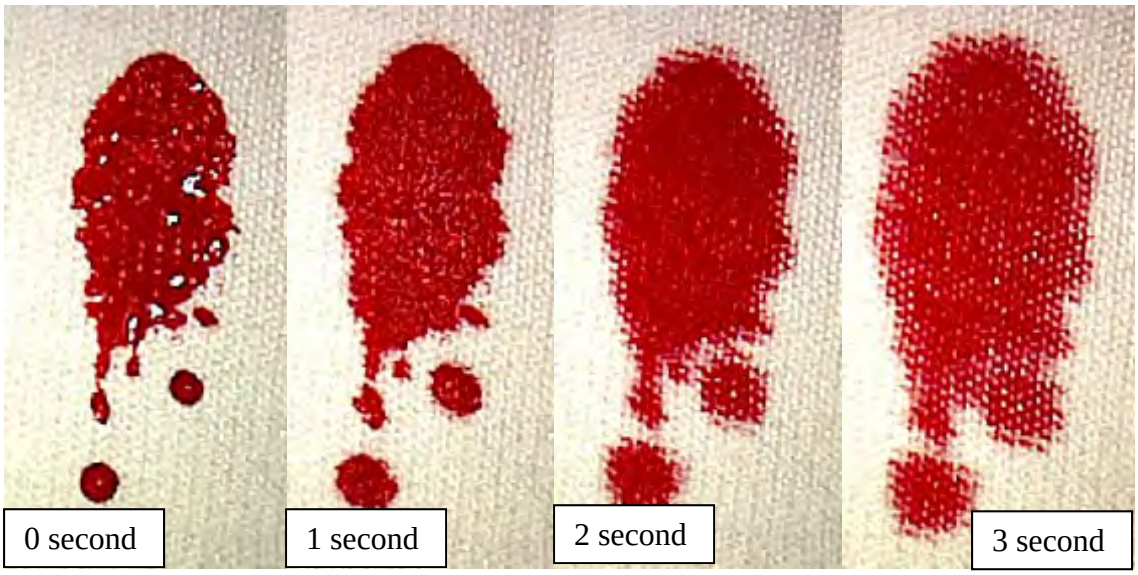


Figure III.8. Evolution of a SB4 drip on a jersey knit from 0 – 3 seconds from the time of the drop impacting onto the fabric.

In addition,

- if the plane of fabric was perpendicular to the area of origin (angle of impact = 90°), and only the height was altered, the number of spines and satellite stains increased as the height increased;
- if the fabric plane was inclined and the height of impact was increased, the drop had more energy to travel along the direction of inclined plane and formed a larger stain;
- for knitted fabric, a satellite stain was not produced at a height of 0.5 m but produced a satellite stain at heigts ≥ 1 m .

III.14 Effect of backing material

At a crime scene, a fabric may be stretched taut, or lie loosely. In section III.12, it was seen that the tautness of the fabric affected the stain that developed. However, a fabric may also lie on a hard surface, e.g. a table, or on a soft surface, i.e. a bed, couch, easy chair. Figure III.9 shows a single piece of knit fabric with two drip stains (30 μL, drip height 1.5 m, SB4). The left side of the fabric was placed over an absorbent pad similar to an incontinence pad, while the right side was placed on a stone laboratory bench. Both stains exhibited satellite stains, but their characteristics are quite different. The stain on the soft backing had a few, large satellite stains, which were much closer to the parent stain than those on the right. The stain on the right has a large number of satellite stains, each of which is much smaller than those on the left and travel much larger distances from the parent stain. Thus, at a crime scene, it will be relevant for the bloodstain analyst to note whether a bloodied fabric is taut or loose, and whether it lies on a soft or a hard surface.

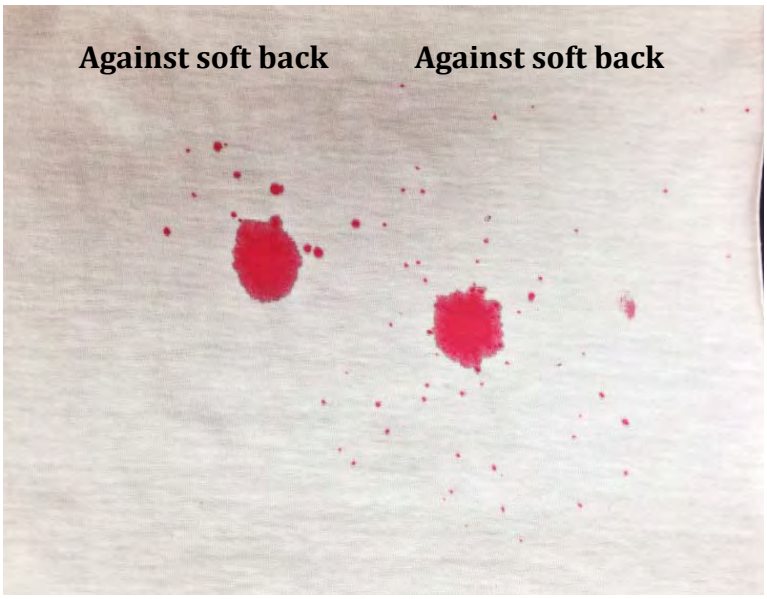


Figure III.9. SB4 stains and stain patterns for a drop impacting on a knit fabric that rests on (left) a soft backing or (right) a hard backing. These stains were photographed after wicking was complete and the stains had dried.

Similar results are found on impact for the woven fabric (Figure III.10, top), but much of the pattern is masked after wicking takes place (Figure III.10, bottom) as discussed above.

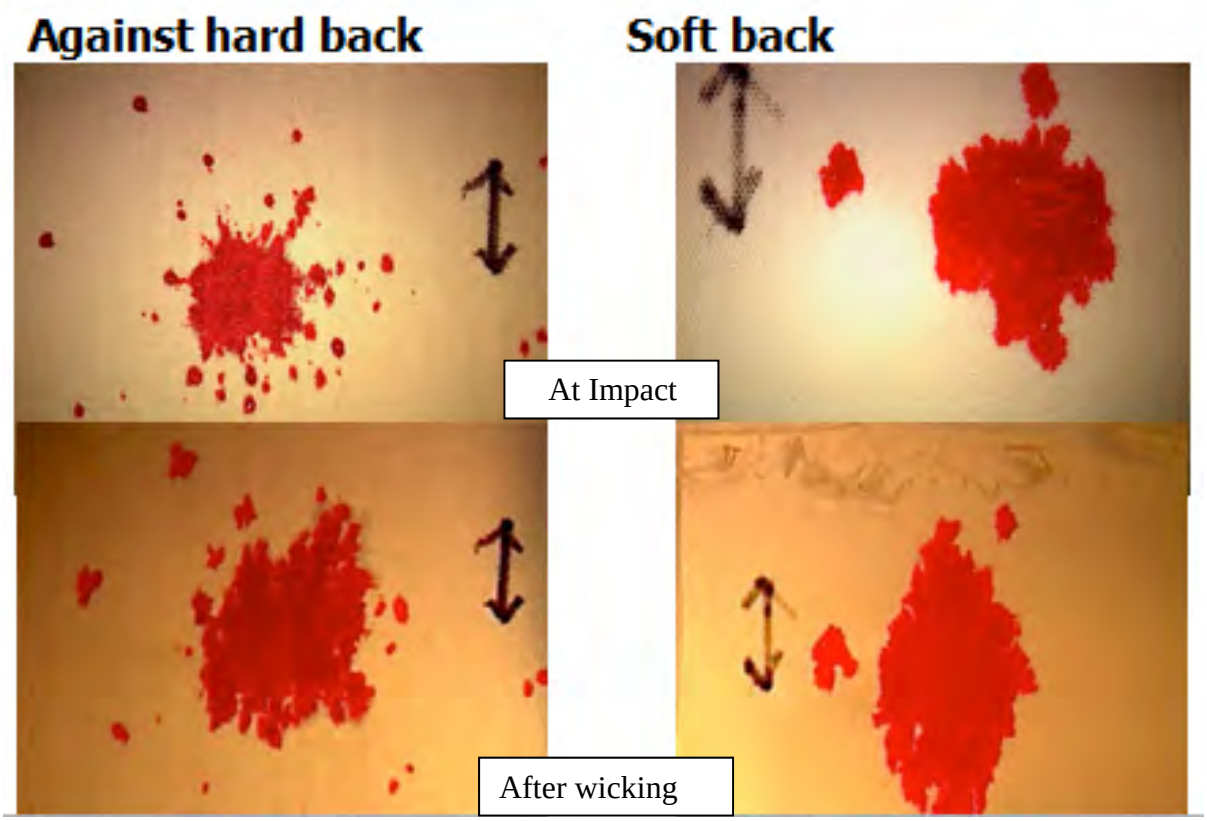


Figure III.10. SB4 drip stains on a plain-woven material with different backing materials. Left images were obtained with the fabric lying on a stone laboratory bench while the images on the right were obtained with the fabric lying on a soft pad.

To better understand the process leading to these two distinctively different patterns, high-speed video imaging was used to photograph a time series of an SB4 drop impacting the fabric, which was resting on a stone lab bench (Figure III.11) or on a soft absorbent pad (Figure III.12). When the drop hit the fabric lying on the hard surface, the drop flattened, expanded radially, and broke up into many small droplets, which subsequently fell back onto the fabric resulting in numerous small and widely dispersed satellite stains. On the other hand, when the drop hit the fabric lying on the soft surface, the fabric and backing absorbed much of the impact energy and only a few, larger satellite drops were generated and these landed very close to the parent stain since they did not have the kinetic energy to travel further.

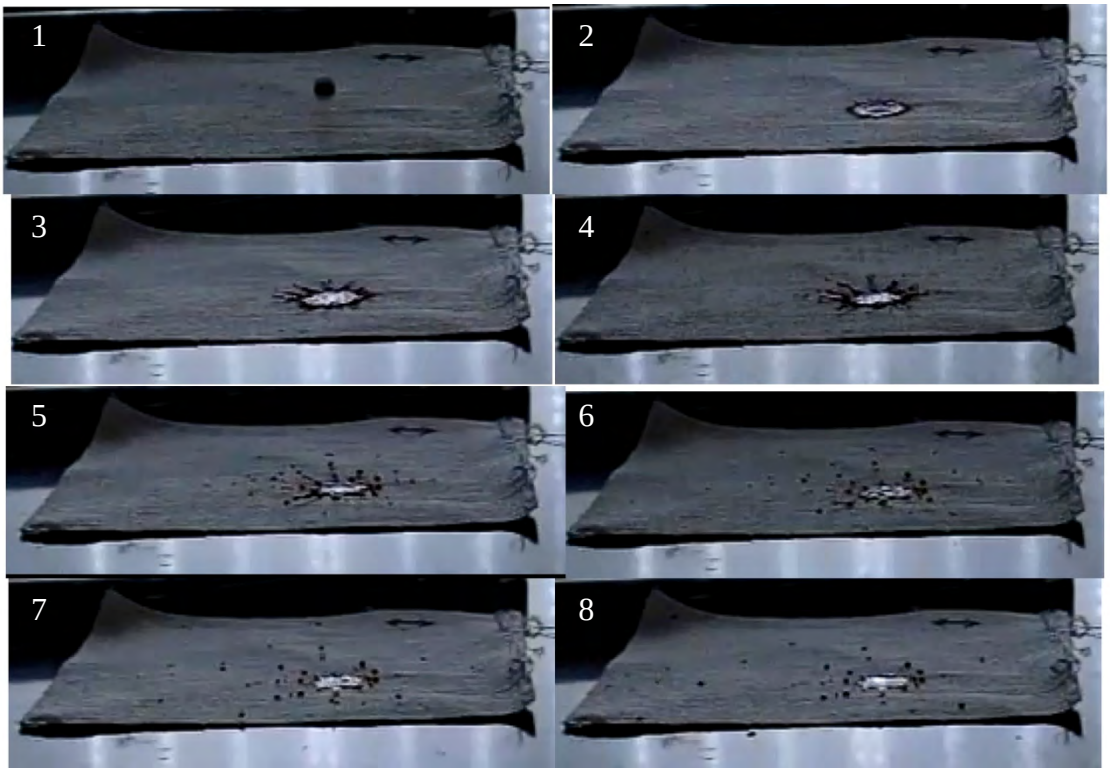


Figure III.11. High-speed video images of an SB4 drop impacting onto a knit fabric resting on a hard table. The drop height was 1.5 m.

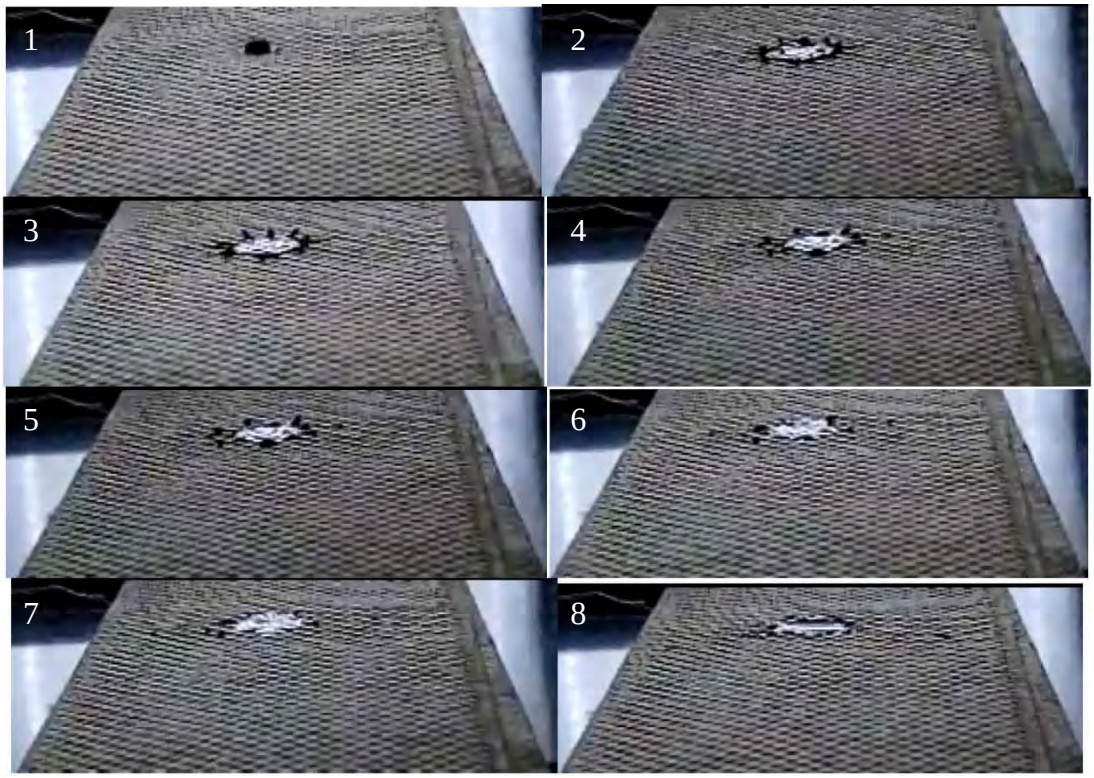


Figure III.12. High-speed video images of as SB4 drop impacting onto a knit fabric resting on two layers of soft absorbent padding. The drop height was 1.5 m.

III.15 Fabric defect on final bloodstain

Fabrics are made at high speed on sophisticated machinery using yarns produced on equally complicated machinery. Inevitably, there are occasional defects, as shown in Figure III.13 of the plain-woven (130 x 70) bed sheeting. The fabric pattern is the characteristic “over-under” pattern of a plain weave. The yarns running horizontally in this picture (fabric warp direction, 130 epi) are spaced more closely than those running vertically (fabric weft direction, 70 ppi). The arrow points to a thin end (warp yarn) lying next to what appears to be a thick end just above it. This defect lead to the unusual pattern shown in Figure III.14 that developed when a single SB4 drop fell onto the fabric from 6”. Thus an analyst must also examine areas of the fabric which displayed bloodstains for any potential defects that could alter a bloodstain or bloodstain pattern when blood is deposited on the affected area.

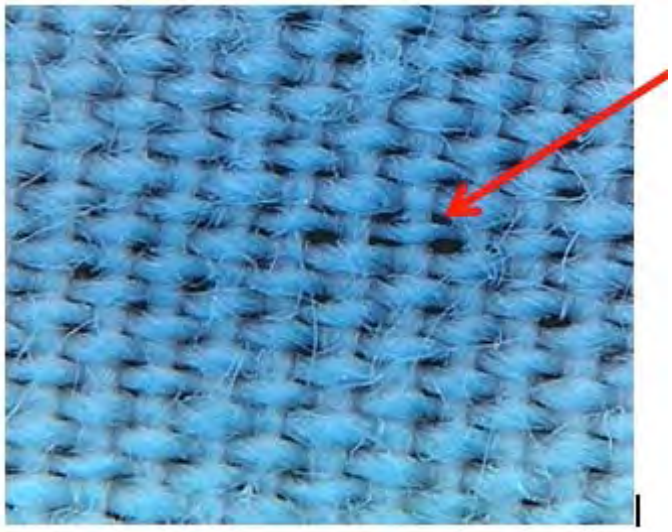


Figure III.13. Warp defect (indicated by the red arrow) observed in the bed sheet fabric, which leads to the unusual stain observed in Figure III.14.

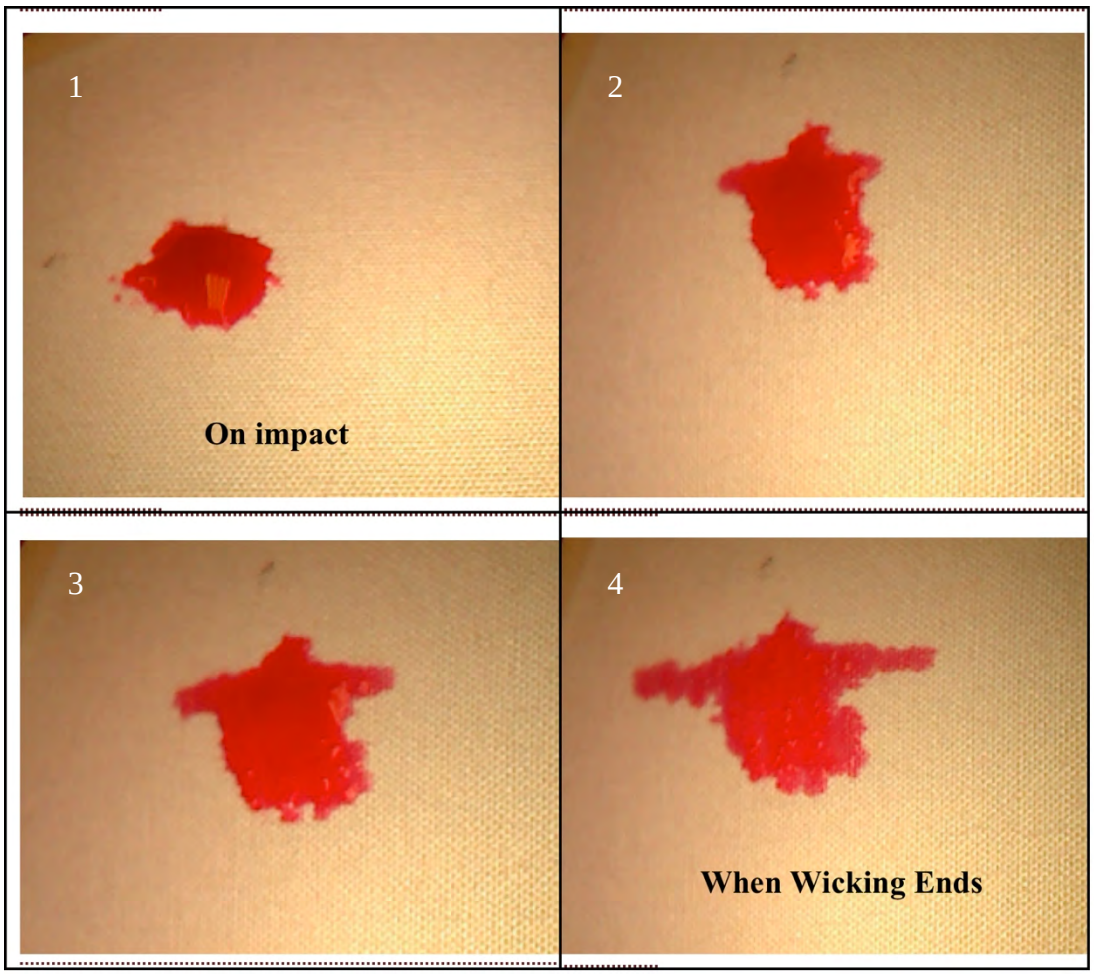


Figure III.14. Time evolution of an SB4 drop wicking into an area of a plain woven fabric which encountered a fabric defect.

This result, along with the difference between the warp and weft yarns in the plain woven fabric, suggests that the wicking performance of the yarns may also contribute significantly to the stain that develops over time. A yarn that has little space for blood, but wicks well may result in a very diffuse pattern while a yarn capable of absorbing a lot of blood may result in a compact, less diffused pattern. Thus, a series of tests were conducted to evaluate the wicking behavior of individual yarns.

III.16 Characterization of the yarns

Five yarns were extracted from the warp direction and five from the weft direction of the plain woven fabric, as well as five yarns from the knit fabric to measure the linear density and twist level. Ten yarns were also extracted to measure the yarn diameters. The results of these measurements are given in Table III.6 along with the cotton count, the twist multiplier, the yarn packing-factor and the percentage of the yarn cross-section occupied by air. The packing factor is just the fraction of the cross-sectional area of the yarn that is occupied by the (solid) fibers. The remainder is air resulting in capillaries through which liquid can wick.

Table III.6. Physical properties of woven and knit yarns.[†]

Property	Knit	Warp	Weft
Linear Density (Denier)	174.5 ± 2.5	131.2 ± 2.1	123.9 ± 4.3
Cotton Count (Ne)	30.5 ± 0.4	40.5 ± 0.7	42.9 ± 1.5
Twist Per Inch (TPI)	22.4 ± 0.4	25.0 ± 0.4	17.2 ± 0.2
Twist Multiplier[††]	4.07	3.93	2.63
Yarn Diameter[†††] (mm)	0.16 ± 0.01	0.12 ± 0.01	0.12 ± 0.02
Yarn Packing Factor	0.59	0.81	0.76
% Air within Yarn	41	19	24

[†] Numbers are averages of five yarns ± the standard deviation.
[††] The twist multiplier is the twist per inch divided by the square root of the cotton count.
[†††]Diameter average and standard deviation are from 10 yarn specimens.

The linear density of the knit yarn is somewhat larger than that of the warp or weft yarns of the plain woven fabric. This means that the yarn is larger and, therefore, has more capillaries for wicking blood. In addition, the % air within the knit fabric yarns is approximately twice as large as for the yarns of the plain-woven fabric. Thus, not only are there more capillaries within the knit yarn, but they are also larger. These yarn characteristics may explain the smaller stain size (see section III.19) for synthetic blood SB4 when dropped onto a knit fabric as opposed to a woven fabric.

The driving force for wicking is just the Laplace pressure. Due to the highly variable shape of the capillaries within the yarns, the capillaries are treated as equivalent circular capillaries with the Laplace pressure Δp given by:

$$\Delta p = \frac{2\gamma \cos\theta}{R} \qquad \text{(III.1)}$$

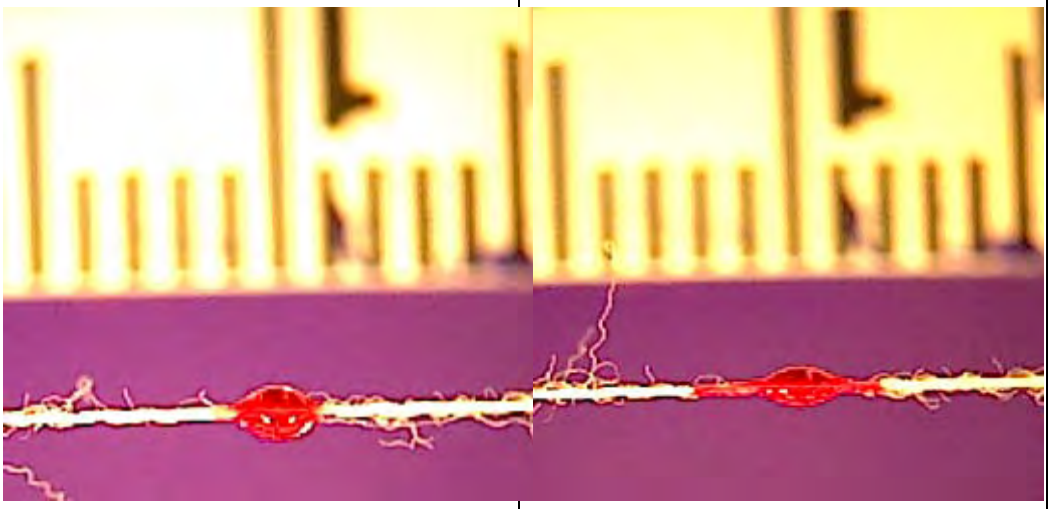
where γ is the liquid-vapor surface tension, θ is the contact angle between the fiber and the liquid, and R is the radius of the equivalent cylindrical capillary. Thus, as the capillary radius increases, the Laplace pressure decreases, which is the driving force for wicking. In addition, the viscous force resisting flow decreases as the capillary radius increases. So as the capillary radius increases, both the resistance to flow and the driving force for flow decrease. Although it is difficult to say how wicking should depend on these construction factors, the experiments above suggest that wicking in the knit yarn is fast, but the synthetic blood does not go far. On the other hand, wicking within the plain-woven fabric is slow, but wicks into an extended stain. To get a better understanding of the wicking behavior, the rate of wicking was measured for each of the yarns.

III.17 Wicking behavior of the yarns

Figure III.15 shows the wicking of a single, 5 μL SB4 drop into a warp yarn. The time for a 1 μL drop to wick 3 mm was measured for each of the plain woven yarns and for 4 mm for the knit yarn for several different twist levels. (The knit yarn wicked faster so a longer wicking distance was used to facilitate measurement.) The results of these measurements for five replicates are shown in Figure III.16 for the plain-woven yarns and in Figure III.17 for the knit yarns. The following observations were noted.

- As the twist increased in the woven fabric yarns, the time required to wick 3 mm decreased, while for the knit fabric yarns, the wicking time increased as twist increased. This result was surprising.

- The time scale for wicking in the woven yarns was 4 – 18 minutes, while for the knit fabric, it was only 13 – 36 seconds, i.e. it took approximately seven times longer for a drop to wick into the woven yarns than for the knit yarn (both at ~22 tpi). These differences were baffling since it was believed that all three yarns were made with similar processes. Therefore, the structure of the yarns was examined in detail and is reported in the following section.



0^{th} Minute | 4 Minutes

Figure III.15. Wicking of a 5 μL SB4 drop into a warp yarn with 25 TPI is shown after the yarn was extracted from the plain woven fabric.

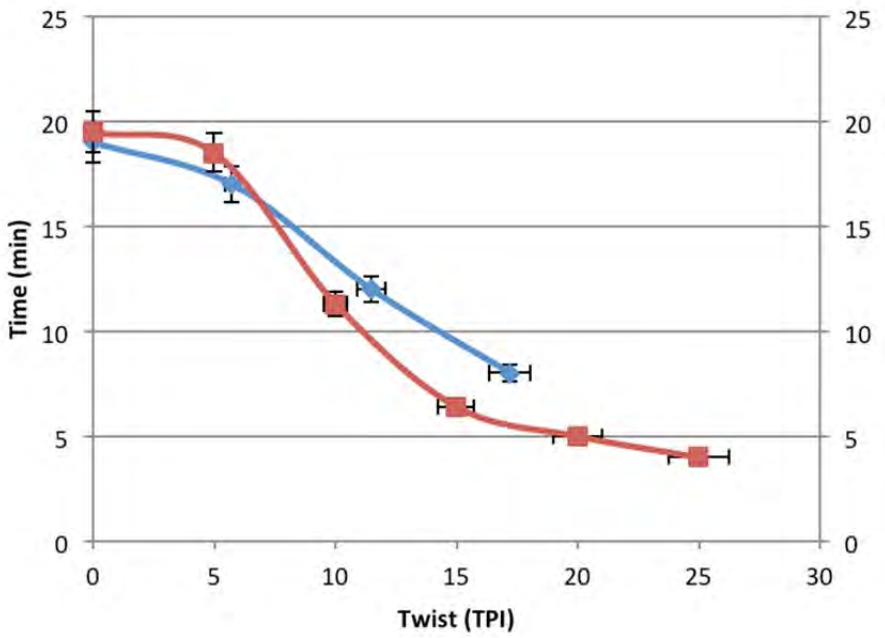


Figure III.16. Time v. twist level for warp (red) and weft (blue) yarns extracted for the plain woven fabric.

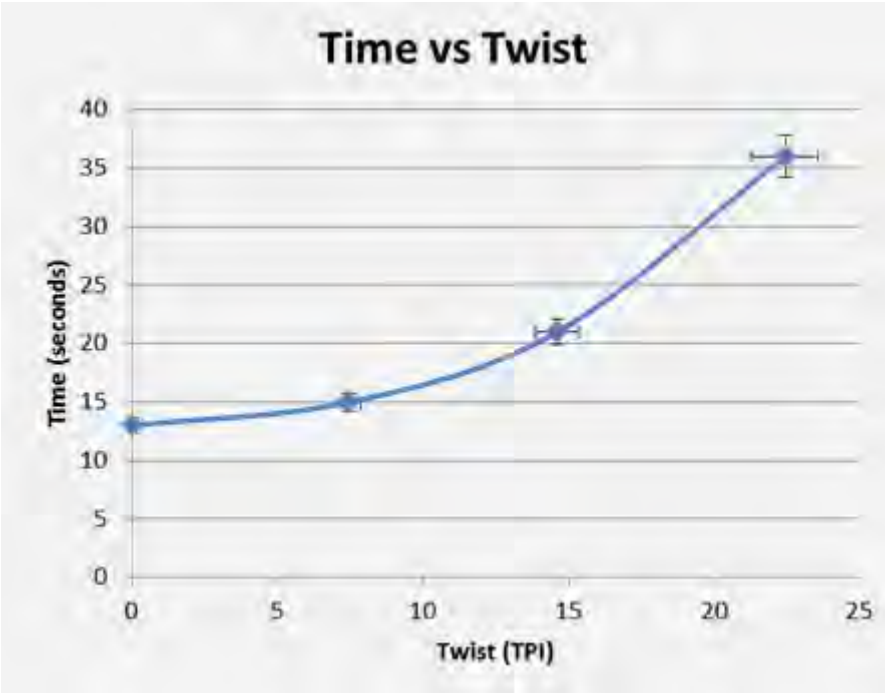


Figure III.17. Time v. twist for yarns extracted from the knit fabric.

III.18 Morphological assessment of the yarns

For a better understanding of the yarn and fabric structure, the yarns were examined on an optical microscope and a scanning electron microscope, SEM; the images are shown in Tables III.7 and III.8. The warp and weft yarns were confirmed to be combed, ring-spun yarns, as expected. Ring spun yarns get their strength from twist, which compresses the yarn, and thus reduces the size of the capillaries. However, the knit yarn was found to have a different structure, and is believed to be a Murata vortex spun yarn, which is characterized as having wrapping fibers on the outside, but more parallel fibers in the center. These yarns are held together by the wrapper fibers which compress the yarn, but only in a few places. Thus most of the yarn remains open with relatively large spaces between the fibers.

Table III.7. Optical microscopy images of the fabric yarns.

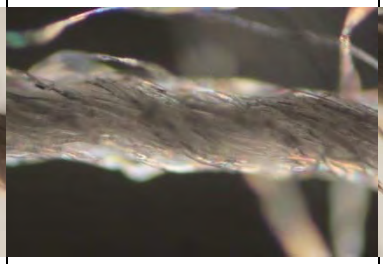
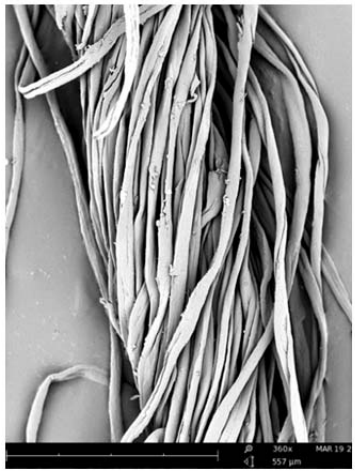
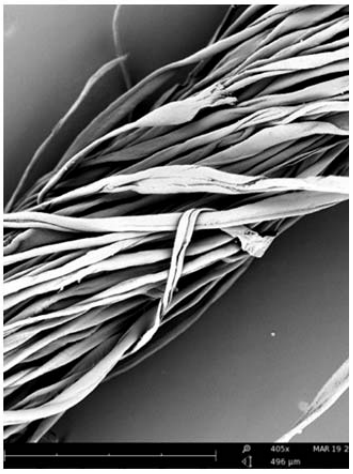
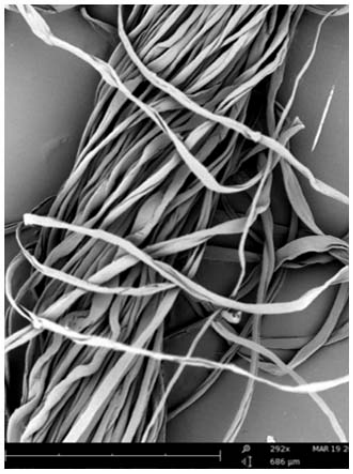
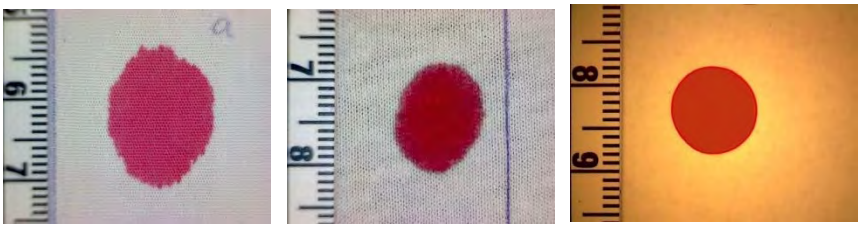
Warp Yarn	Weft Yarn	Knit Yarn
		
		

Table III.8. Scanning electron microscopy (SEM) images of fabric yarns.

Warp Yarn	Weft Yarn	Knit Yarn
		

III.19 Bloodstain area and circularity on single layer fabric

In order to get more quantitative measures of the SB4 wicking, the stain area, perimeter, circularity and drying time were measured. Preliminary experimental results showed that SB4 stains formed on fabric samples exhibited clearly different stains than on PET films, as shown in Figure III.18. The area, perimeter, and circularity were determined and are given in Table III.9. To avoid the complications from spines and satellite stains, the drops were allowed to fall from a height of only 1 cm. This is referred to as a "placed drop". (Circularity is defined as the perimeter squared divided by 4π times the area. For a perfect circle, the circularity = 1.)



(a) (b) (c)

Figure III.18. Pictures of typical SB4 stains resulting from a 20 µL SB4 droplet placed onto (a) plain woven cotton fabric, (b) plain jersey knit fabric, and (c) PET film.

Table III.9. Characteristics of SB4 stains resulting from a 20 µL SB4 droplet placed on woven fabric, knit fabric, and PET film.

Substrate	Plain Woven	Plain Jersey Knit	PET Film
Area (mm^2)	171.9	106.8	80.0
Perimeter (mm)	97.2	64.1	33.6
Circularity	0.23	0.62	0.89

The size and shape of SB4 stains on both plain woven and jersey knit fabrics are different from the stain on PET film. Stains on the woven and knit fabrics have larger areas, are more elongated, and much longer perimeters than the one on the hard surface (PET film). On smooth, non-porous surfaces like PET film, SB4 drops form a compact spherical cap with a contact angle appropriate for the solid and liquid surface energies. Other than shrinking, the SB4 drops retain that shape until they have completely dried, see Figure III.19. On fabric surfaces, SB4 drops first pooled on the surface and spread slightly while filling large voids in the fabric structure. Simultaneously, SB4 wicked into the yarns within the fabric and continued to spread via wicking until either the SB4 drop dried, or its supply was exhausted. The stains on the fabrics had much lower circularity than on PET films. This can be easily explained by the anisotropic structure of the fabrics both in-plane and through the thickness. Thus, we drew an initial conclusion that liquids traveling within the yarns in a fabric are directionally dependent. In addition, the apparent rough edges of SB4 stains on the two fabrics could be due to the in-plane non-uniformity of the yarns.

To determine the relationship of the SB4 stain areas on fabrics with respect to the volume of the SB4-drop, the areas of each stain on the PET film, the plain-woven cotton bed sheeting, and the knit cotton T-shirt fabric were measured for drop volumes of 20, 30, 40, 50, 60, and 80 µL. Each experiment was repeated ten times and the images were analyzed using Image J software. Figure III.19 shows the images of the stains on PET film, and similar images are shown for the woven fabric (Figure III.20) and the knit fabric (Figure III.21). The area v. volume curves for all three materials are shown in Figure III.22.

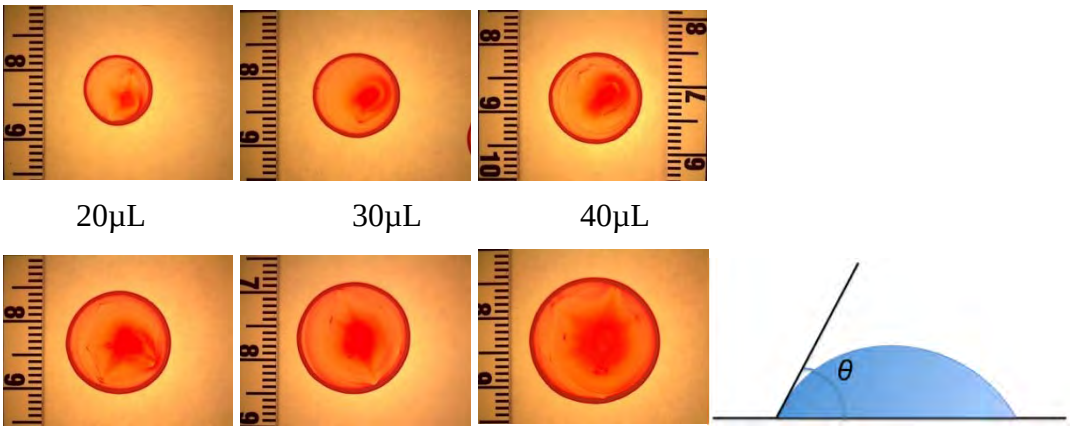
The area A of a perfectly circular ring of an SB4 drop or of blood on the non-porous PET flat film should be given by

$$A = \pi R^2 \tag{III.2}$$

where the radius R of the spot for volume V is:

$$R = \left(\frac{3V}{4}\right)^{1/3} \frac{1}{\left(2 - 3\cos\theta + \cos^3\theta\right)} \tag{III.3}$$

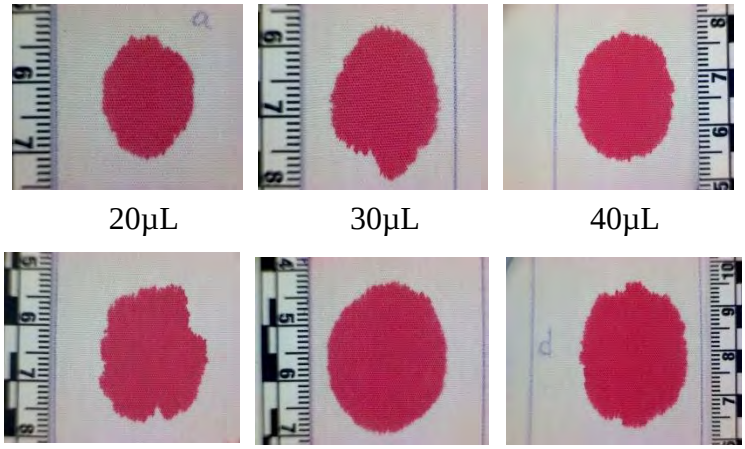
where θ is the contact angle where the edge of the drop meets the PET film, as shown in Figure III.19. This type of relationship cannot be given for stains on fabric since the liquid wicks into the fabric.



20µL 30µL 40µL

50µL 60µL 80µL

Figure III.19. Typical SB4 stains of 20-80 µL blood drops placed on PET films and allowed to dry. The right hand drawing depicts the contact angle between the drop and the film for an ideal spherical cap.



20µL 30µL 40µL

50µL 60µL 80µL

Figure III.20. Typical SB4 stains of 20-80 µL drops on plain-woven cotton bed sheet. Note, the solid lines are parallel to the warp direction of the fabric. [Note: stain sizes may appear different due to different magnifications. The centimeter ruler within the image indicates the true scales.]

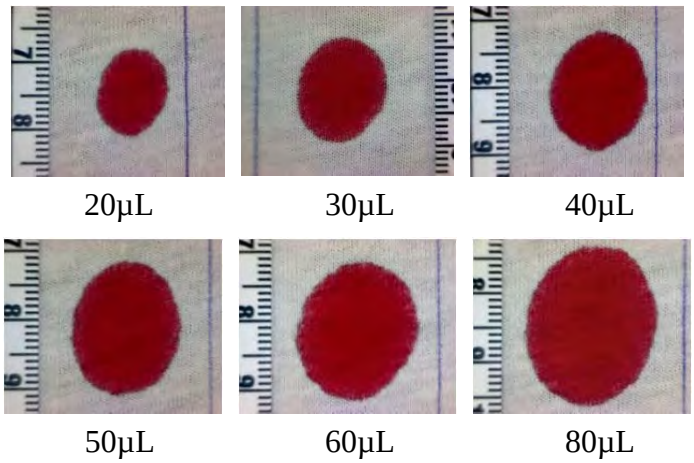


Figure III.21. Typical SB4 stains for 20-80 µL drops on T-shirt plain jersey knit fabrics. The lines show the direction of the wales (the lengthwise direction of the fabric). [Note: stain sizes may appear different due to different magnifications. The centimeter ruler within the image indicates the true scales.]

The stain area on the films was smallest since the films do not wick liquids. Rather, the drop dries in place. The stains on the knit fabric expand more than on the film, but less than on the woven fabrics. The stains also spread more quickly than on the woven fabric. Finally, wicking into the woven fabric was slower than for the knits and the stains are less uniform, however, the stained areas on the plain woven fabric are larger than on the knit fabric or the film. In each case, the stained areas are nearly linear with volume. Since the fabric thickness is much thinner than the wicking radius, the blood migrates through the entire fabric thickness and thereafter spreads only in the plane of the fabric. Thus the volume of the stain is just the fabric thickness times the stain area. This volume contains the synthetic blood, fibers, and residual air.

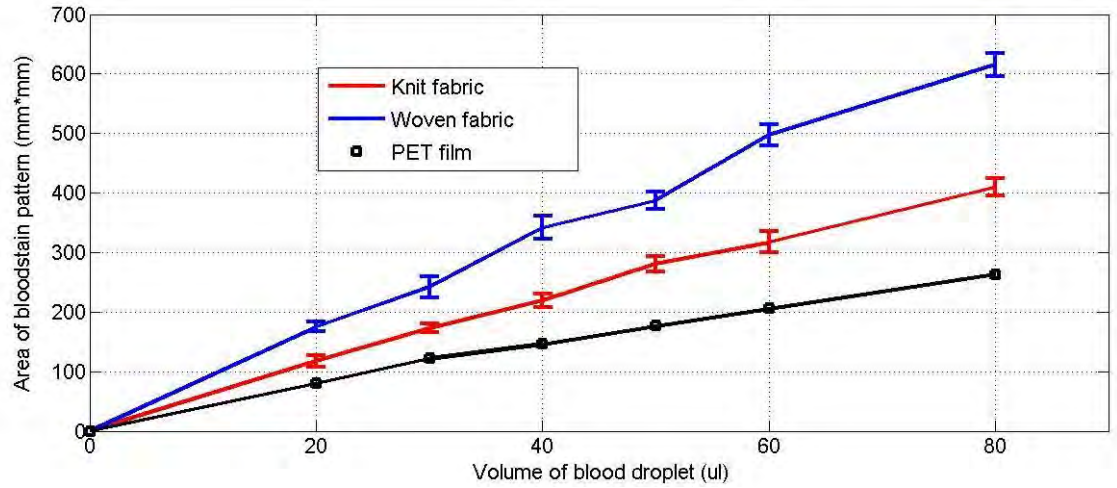


Figure III.22. The relationship of SB4 stain areas and drop volumes are shown for PET films, jersey knit cotton fabrics and plain-woven cotton fabrics, respectively.

In conclusion, from the stains formed on woven fabric, knit fabric, and PET film as described above, it was found that stains exhibit characteristic patterns on certain substrates and stain areas are nearly linear dependent on the volume of the drop for each substrate studied, yet the slopes of area-volume curves vary between different materials. These observations show that the type, construction, composition and finishing of the fabric and yarn play an important role in determining the stains and stain patterns, that is, the analyst must not only be alert to the fabric involved in the bloodstain formation, but also to understand the wicking behavior of the fabrics.

III.20 Growth of bloodstain area with time

Another aspect of bloodstain development is the time evolution of the stain, as shown qualitatively in section III.8. Figure III.18 shows a selection of images of the growth of a 40 µL SB4 stain on polyester film while Figure III.19 shows the stain area growth for 20 and 30 µL drops over time where the area was determined for every 0.2 s after the drop was applied.

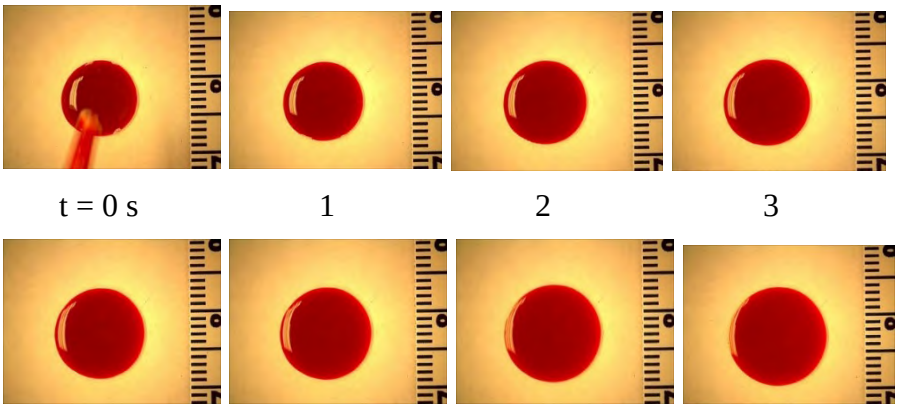


Figure III.18. Growth of SB4 stains of a 40 µL SB4 drop on PET film.

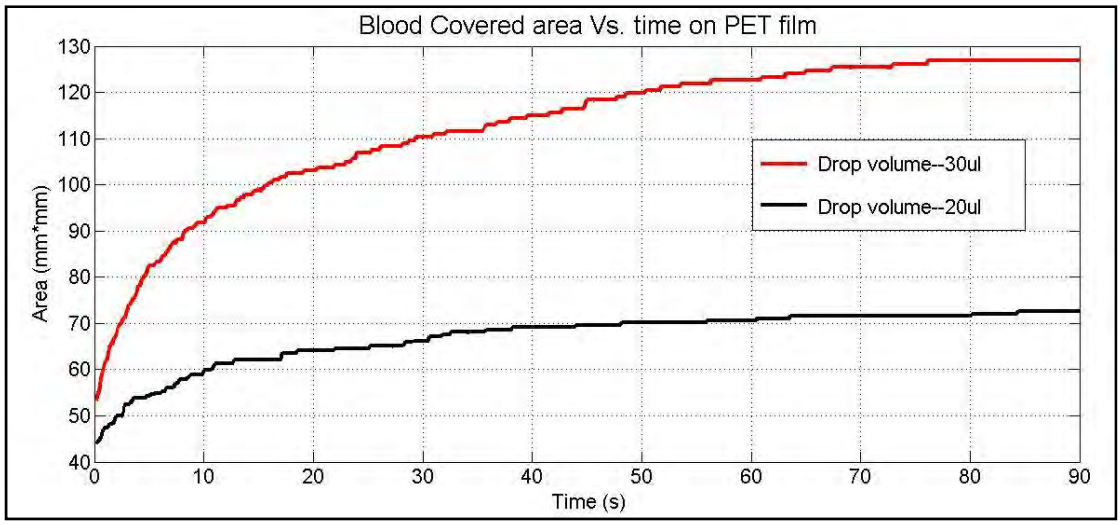


Figure III.19. SB4 covered area vs time on PET film.

Initially the SB4 drop spreads rapidly on the PET film, but slows until the drop reaches a steady state. Similar behavior is observed for both the knit and the woven fabrics, but with very different kinetics, as shown in Figures III.20 – 24.

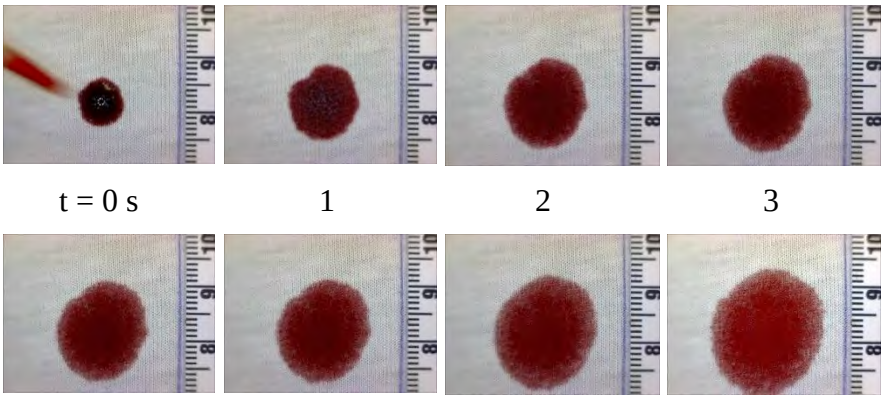


Figure III.20. Growth of SB4 stain patterns of a 30 µL SB4 drop on T-shirt cotton knit fabric.

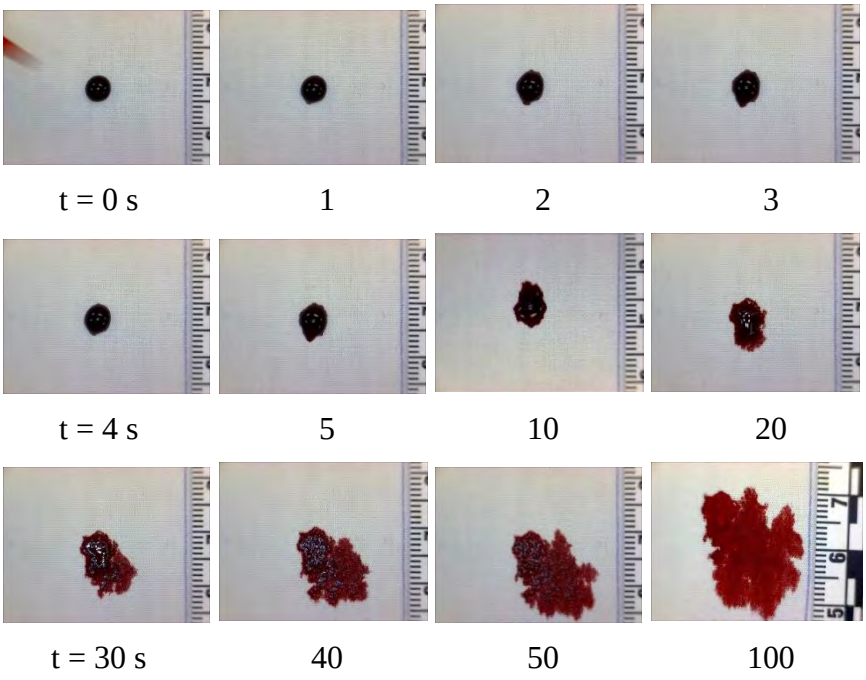


Figure III.21. Growth of SB4 stain patterns of a 30 µL SB4 drop on plain woven cotton bed sheet.

By comparing the successive images of Figures III.20 and III.21, it was seen that knit fabrics absorb the drop nearly simultaneously with its spreading. The stains on knit fabrics quickly reach their final patterns with a well-defined, relatively smooth perimeter while SB4 spreading on woven fabric takes much longer, has a jagged perimeter, and each drop results in a unique pattern. The stain area vs time curves area shown in Figures III.22 – 23, and to make comparison

easier, the area vs time curves for a 30 µL drop on all three materials are combined in Figure III.24.

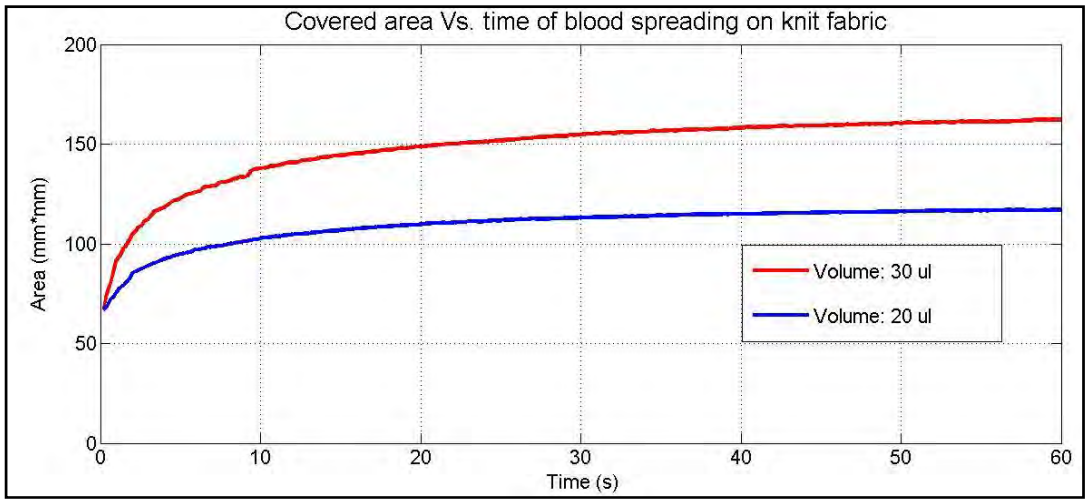


Figure III.22. SB4 stained (with volume 20µL and 30µL) area vs. time on knit fabrics

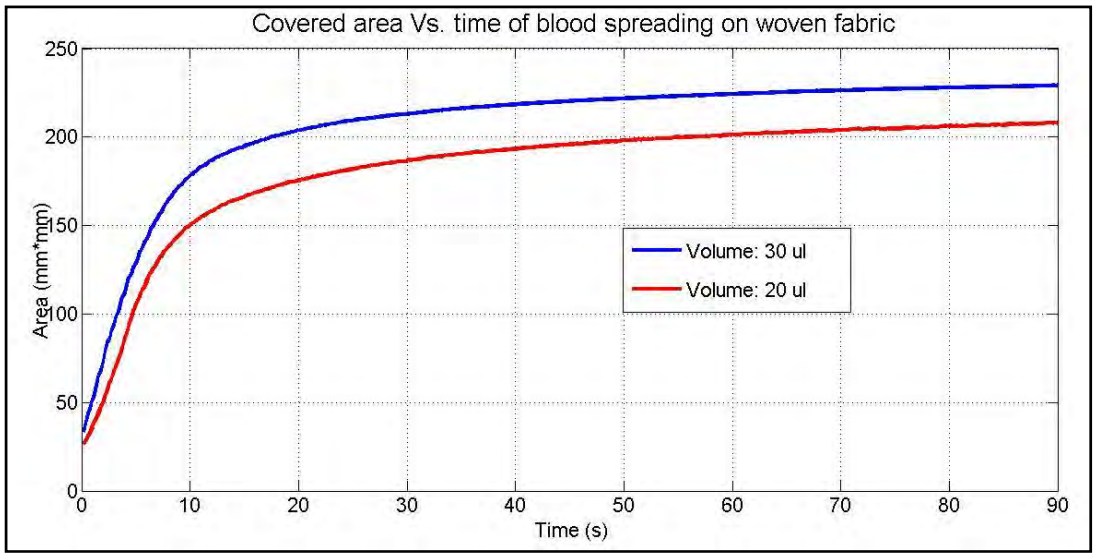


Figure III.23. SB4 stained (with volume 20µL and 30µL) area vs. time on plain woven fabrics.

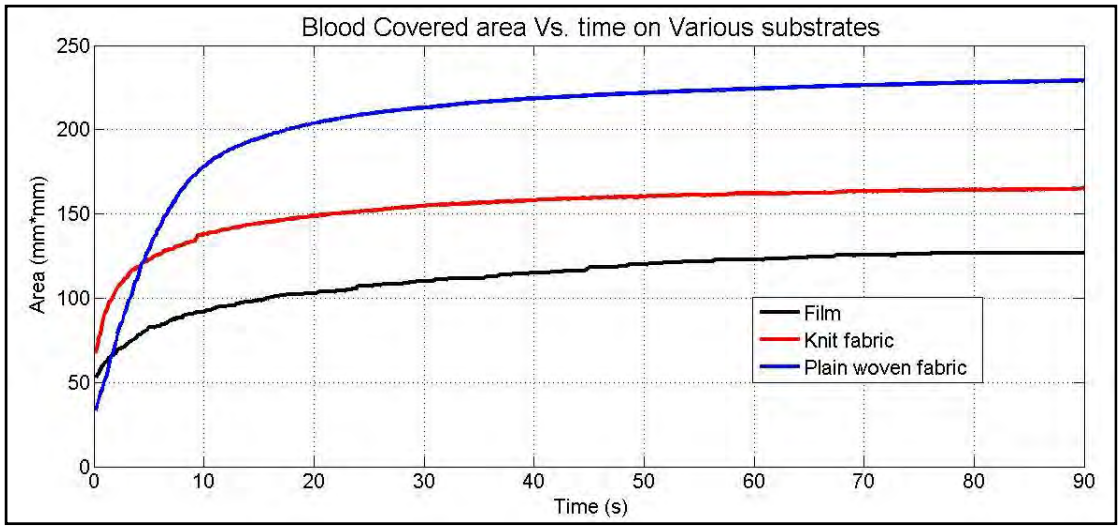


Figure III.24. Development of SB4 stain area from a 30 µL SB4 drop on three kinds of target materials: PET film, jersey knit fabric and plain woven fabric.

The stain area for the knit fabric and the film follow similar curves, increasing quickly at first, then slowing until the pattern is fully developed in ~60 s. On the other hand, the woven fabric pattern is still developing 100 s after the drop was placed on the surface. In addition, the growth of the stain area on the knit fabric and the film progress uniformly while the stain on the bed sheet grows slowly at first as it spreads on the surface, then accelerates as it is drawn into the fabric by the capillary action associated with wicking, and slows again as the wicking distance increases.

III.21 Bloodstain drying on single layer fabric

Spreading of a bloodstain ceases when there is no more blood to spread. This can occur by two mechanisms, either the blood has filled the capillaries and none is left or the blood has dried or clotted. Understanding the blood drying process on fabrics is important since the viscosity of blood changes as water evaporates or as the blood clots, thus the spreading on the surfaces will change accordingly. The instrument used to trace the drying process of synthetic blood drops on fabrics was shown in Figure II.9. Figure III.25 shows a 20 µL SB4 droplet drying during stain formation.

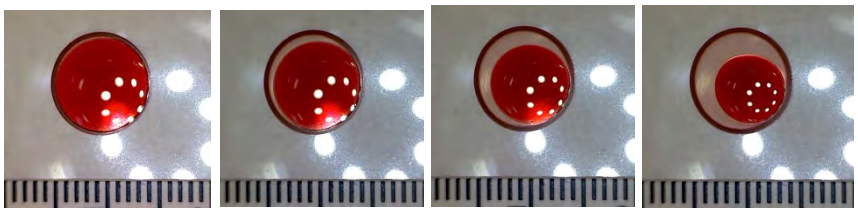


Figure III.25. SB4 drop (with original volume 20 µL) shrinks on PET film during the stain forming and drying process.

On a fabric, the synthetic blood drop does not just sit on the surface, but is drawn into the fabric by capillary action, where it spreads through wicking and dries. Thus drying is masked and cannot be observed visually. To overcome this, drying of a blood droplet in a fabric was obtained by measuring the weight loss of SB4 drops using an accurate electronic balance. Drying curves were obtained by recording the weight of the drop at 10 s time intervals throughout the first 20 minutes, and every 30 s thereafter. The experiments were carried out in the laboratory environment for which the temperature was 20^{o}C and relative humidity was 30%. Due to the sensitivity of this setup, environmental disturbances, such as air flow and building vibrations, the data was smoothed using a Savitzky-Golay FIR smoothing method. The recorded data is shown in Figure III.26 and the smoothed data in Figure III.27 for a 30 µL drop on the plain woven fabric. Although the area of the stain on the cotton plain woven fabric is larger than on the PET film, the drying time is approximately the same on the two materials.

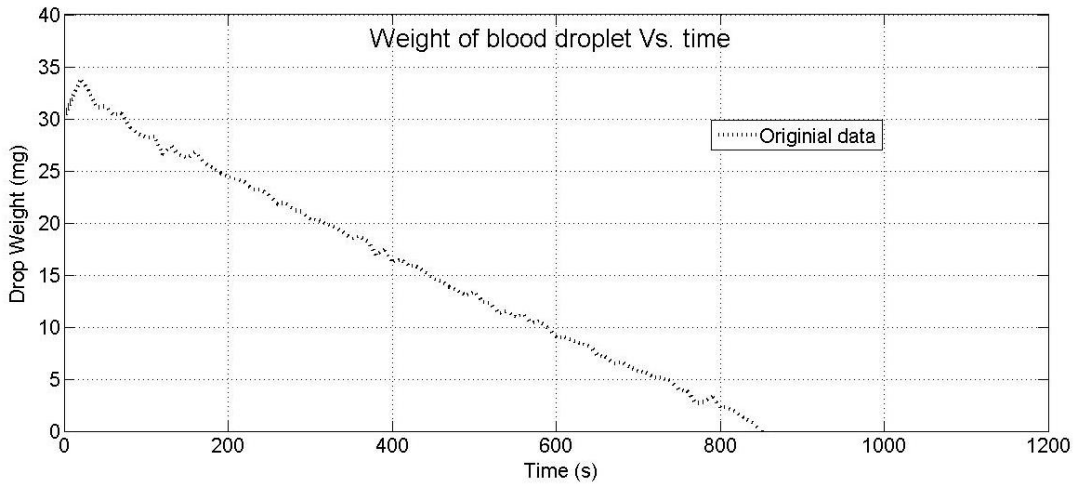


Figure III.26. Original data of the weight loss vs. time curve for a 30µL SB4 droplet on plain woven fabric.

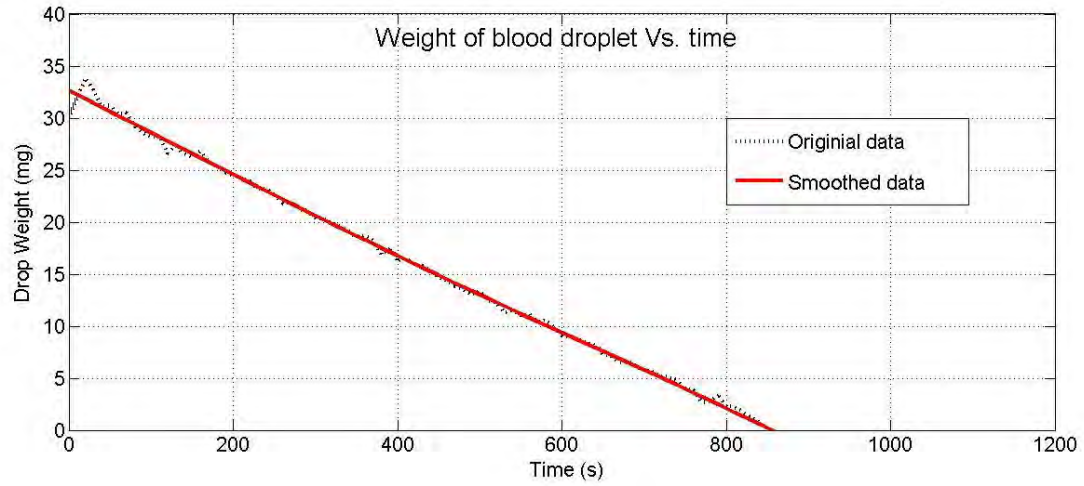
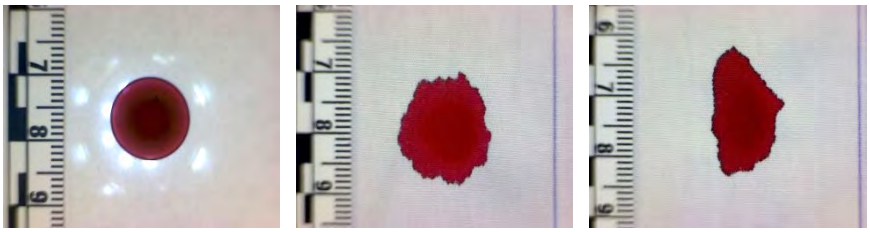


Figure III.27. Smoothed weight loss curve of Figure III.26 using a Savitzky-Golay FIR smoothing method. (step length=20)

A 30µL SB4 droplet was placed respectively on a PET film, on a plain woven fabric sheet, and on a plain woven fabric placed directly on a PET film. The stain patterns that developed are shown in Figure III.28, while the drying curves are given in Figure III.29 for a 30 µL drop.



(a) on PET film (b) on woven fabric (c) on woven fabric & film

Figure III.28. SB4 stain patterns of 30µL SB4 droplets on PET film, plain woven fabric, and plain woven fabric placed on top of and in contact with PET film.

The corresponding areas of the above images were: (a) 123.2 mm^2, (b) 207.4 mm^2, and (c) 174.2 mm^2. It is clear that SB4 drops of the same volume produced much larger stained areas on the plain woven fabric than on PET film sheet, which agrees with our above experiments.

When the plain woven fabric was placed on the PET film, the area of the SB4 stain produced lies between the above single layer cases, as discussed further in the next section. The capillary bridges that formed between the woven fabric layer and the film layer apparently restricted the blood from diffusing to the same extent as for the fabric alone. The weight loss curves for these samples are shown in Figure III.29. These curves show that the weight loss process was nearly the same for the individual fabric and the fabric & film samples, but the individual film sample dried a little slower than the other two. On the film layer, the SB4 drop was confined to the surface in a limited area by the liquid-film surface tension, and only a small area of the blood was exposed to air. However, in the other two substrates, the SB4 drop was guided or driven by capillary action to relatively far distances, and a rather large liquid area was exposed to air. Thus the stains on these two substrates dried faster than the single film substrate.

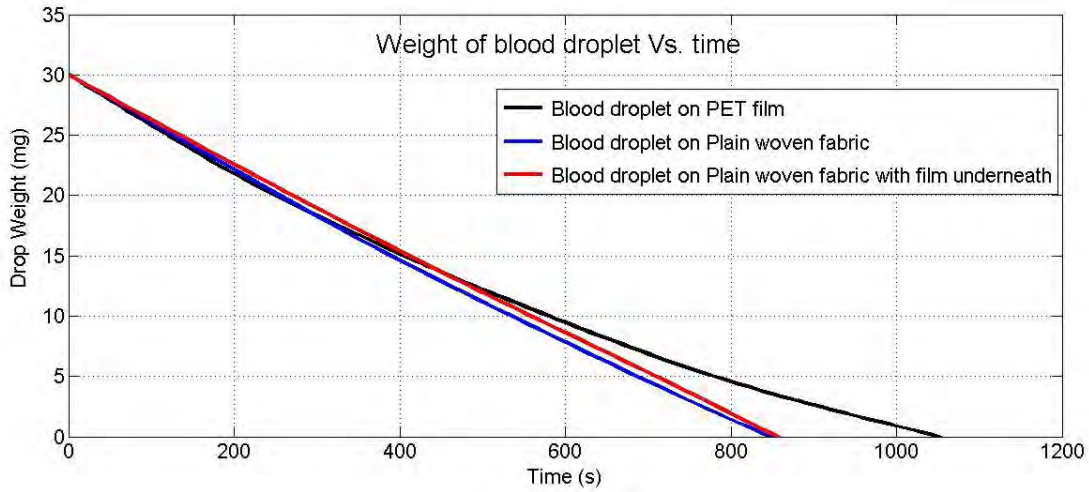
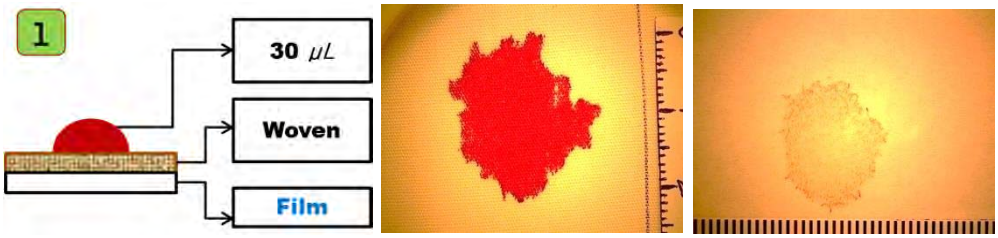


Figure III.29. Savitzky-Golay smoothed weight loss curve of 30 µL SB4 droplets on PET film, plain woven fabric, and plain woven fabric on film combination. Each test was repeated 12 times and these curves are representative of these results.

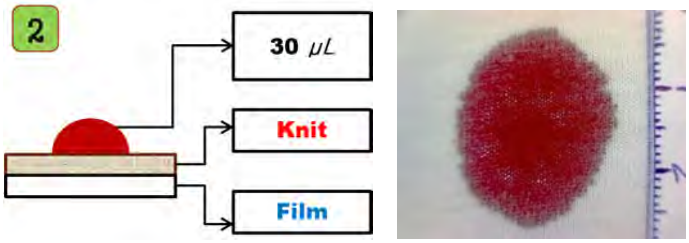
III.22 Blood transfer from upper fabric to lower substrate (fabric or film)

Often two fabrics will be layered on top of each other, for example an upper and lower bed sheet, a woven shirt over a T-shirt or two layers of knit fabrics, or the fabric may lie on a smooth surface, e.g. a table or a person's skin. In order to understand how blood volumes are distributed between two fabrics or between a fabric and a smooth surface in a layer-to-layer blood transfer, a set of experiments with different combinations was performed, as shown in Figures III-30 – 35. Schematics of the drop and fabric arrangements are also given. In each combination, a 30 µL SB4 drop was placed on the top fabric and imaged using a video microscope while simultaneously measuring the weight. When the stain had fully dried, the layers were carefully separated and photographed.



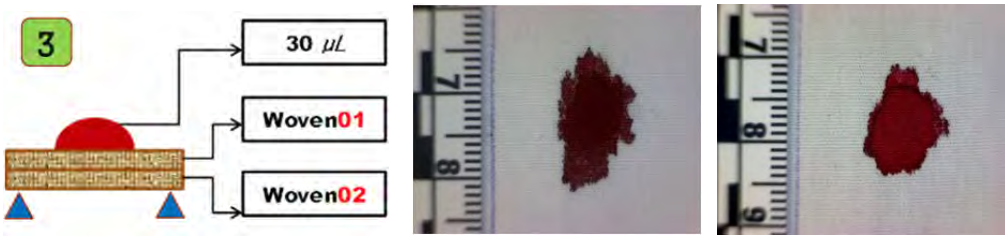
(a) (b) (c)

Figure III.30. Spreading and drying of a 30 µL SB4 drop placed on a woven-film sandwich, where (a) is the schematic experimental design, (b) is the image of the top layer and (c) the bottom layer.



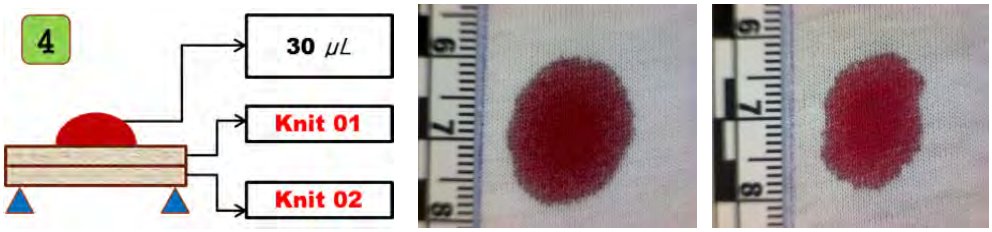
(a) (b) (c) Image could not be seen.

Figure III.31. Spreading and drying of a 30 µL SB4 drop placed on knit-film sandwich, where (a) is the schematic experimental design, (b) is the image of the top knit fabric layer. In this configuration, no image could be seen on the PET film.



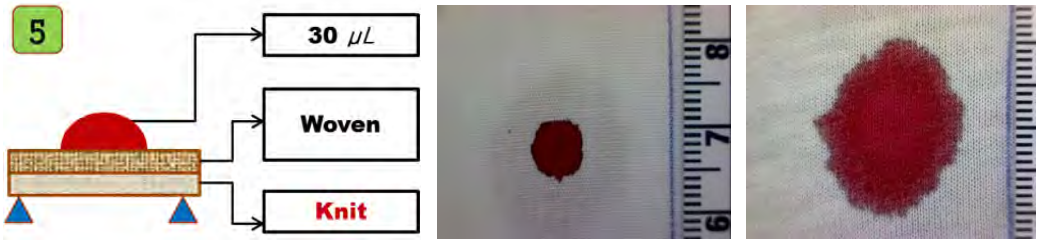
(a) (b) (c)

Figure III.32. Spreading and drying of a 30 µL SB4 drop placed on a woven-woven sandwich, where (a) is the schematic experimental design, (b) is the image of the top layer and (c) the bottom layer.



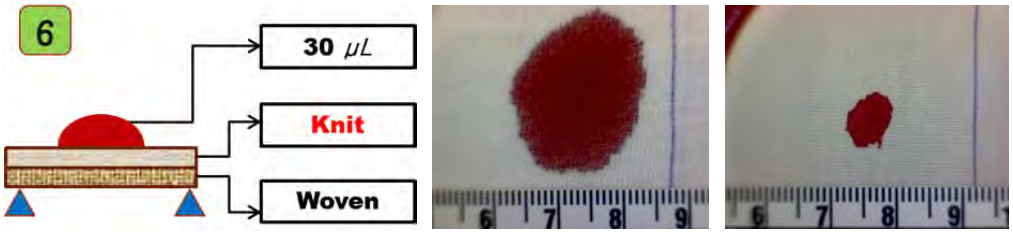
(a) (b) (c)

Figure III.33. Spreading and drying of a 30 µL SB4 drop placed on a knit-knit sandwich, where (a) is the schematic experimental design, (b) is the image of the top layer and (c) the bottom layer.



(a) (b) (c)

Figure III.34. Spreading and drying of a 30 µL SB4 drop placed on a woven-knit sandwich, where (a) is the schematic experimental design, (b) is the image of the top layer and (c) the bottom layer.



(a) (b) (c)

Figure III.35. Spreading and drying of a 30 µL SB4 drop placed on a knit-woven sandwich, where (a) is the schematic experimental design, (b) is the image of the top layer and (c) the bottom layer.

When the top and bottom fabrics are the same, the stain on the top layer is slightly larger than the bottom layer, but the shape of the stain on the two layers are similar. However, when there is a woven and a knit fabric, the stain in the knit fabric is always much larger than in the woven fabric, regardless of whether the knit is the top or bottom layer (see Figures III.34 – 35.)

In Figure III.36, the increase in area over time of a knit-film sandwich and of a knit fabric alone are compared. The time evolution of the drops spreading on the two materials parallel each other, but the area of stain on knit fabric was obviously larger than that on knit-film sandwich. This indicates that some of the synthetic blood formed a capillary bridge between the fabric and the film, and thus was not available for wicking.

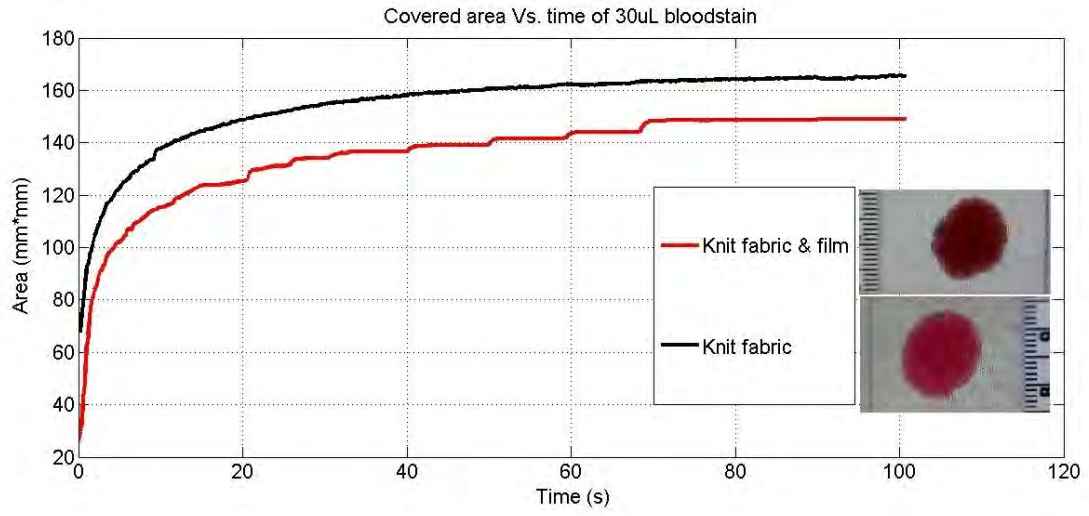


Figure III.36. Area of a 30 µL SB4 drop spreading on knit fabric or on a knit-film sandwich.

The spreading time and the drying time of the synthetic blood droplet was also recorded in each experiment. The spreading time is defined as the length of time from the point when a drop touches the target until it stops spreading, obtained from the video images. The drying time is the time from when the drop was place on the material until there was no more weight loss. Table III.15 summarized the spreading time and the drying time for these sandwich structures.

Table III.15. Spreading time and drying time of a 30 µL SB4 drop on each kind of two-layer sandwich.

Substrates	Spreading time (s)	Drying time (min)
Woven-Film- 	407	28
Knit-Film- 	103	15
Woven-Woven- 	546	25
Knit-Knit- 	62	25
Woven-Knit- 	474	30
Knit-Woven- 	104	29

The drying time for all samples was between 25 – 30 minutes except for the knit-film sandwich, which dried much faster. The spreading times ranged from 62 – 546 seconds (1 minute for 'knit-on-knit' to 9 minutes for 'woven-on-woven'). However, the spreading times for sandwich structures where the knit fabric was the top layer were much shorter than when the woven fabric was the top layer. When the woven fabric was the top layer and the knit the bottom layer, the blood would slowly wick through the fabric thickness. However as soon as the drop contacted the knit layer, the rapid wicking due to the knit fabric would pull the synthetic blood through the woven fabric and spread rapidly in the knit fabric. This can be seen clearly in Figure III.34, where the synthetic blood in the knit fabric can be seen to have a much wider stain than on the woven fabric. This is also probably the reason that this combination dried more slowly since the synthetic blood is held in the lower knit layer and is not directly exposed to air.

III.23 Porcine blood impacting onto fabric

When a blood drop falls onto a smooth surface, the kinetic energy from the fall deforms the drop and the blood flows outward and forms a concave disk. If there is sufficient energy, ligaments may form as well as droplets if the ligaments break. Figure III.37 shows a sequence of images from a 5 mm diameter (~65 µL volume) porcine blood drop falling 50 cm at 90° onto a smooth cardboard surface. As the drop impacted the surface it deformed and spread producing a stain that became wider than the diameter of the original drop. During spreading a wave within the fluid expanded outwards and the liquid formed a concave disk. For lower Weber and Reynolds number impacts, surface tension forces can cause a retraction of this disk. In the example shown in Figure III.37, however, the inertial force and internal pressure generated at impact are

significantly larger than the surface tension forces. Ligaments (spines) began to form on the rim and depending on the opposing forces (inertial force vs surface tension) these eventually broke-up to splash satellite spatter around the parent stain.

Figures III.38 and III.39 show similar sized drops falling 50 cm onto the surface of the two fabrics of choice for this study and that were mounted on the white cardboard backing. The drip stain formation dynamics were similar to those observed for the cardboard surface, except that the more pronounced irregularities in the fabric surfaces led to a dramatic difference in drop splashing on impact. Essentially drop spread was limited and rim instability and ligament formation were significantly accentuated. As a consequence there were many more satellite stains produced. Figures III.40 – III.42 show a similar set of images for 2.6 mm (~ 9 μL volume) porcine blood drops.

While both fabric surfaces produced more satellite spatter stains than the smooth cardboard surface, the knit surface produced more than the plain woven (Figure III.43). This appears to be related to the greater surface irregularity of the knit fabric compared with that of the plain woven. The average size of the satellite stains on the knit fabric surface, however, was smaller than the plain woven surface (Figure III.44). In other words the knit surface on average produced more and smaller satellite stains. Other factors, such as the compressibility of the fabrics and their wetting properties, have not been evaluated at this stage.

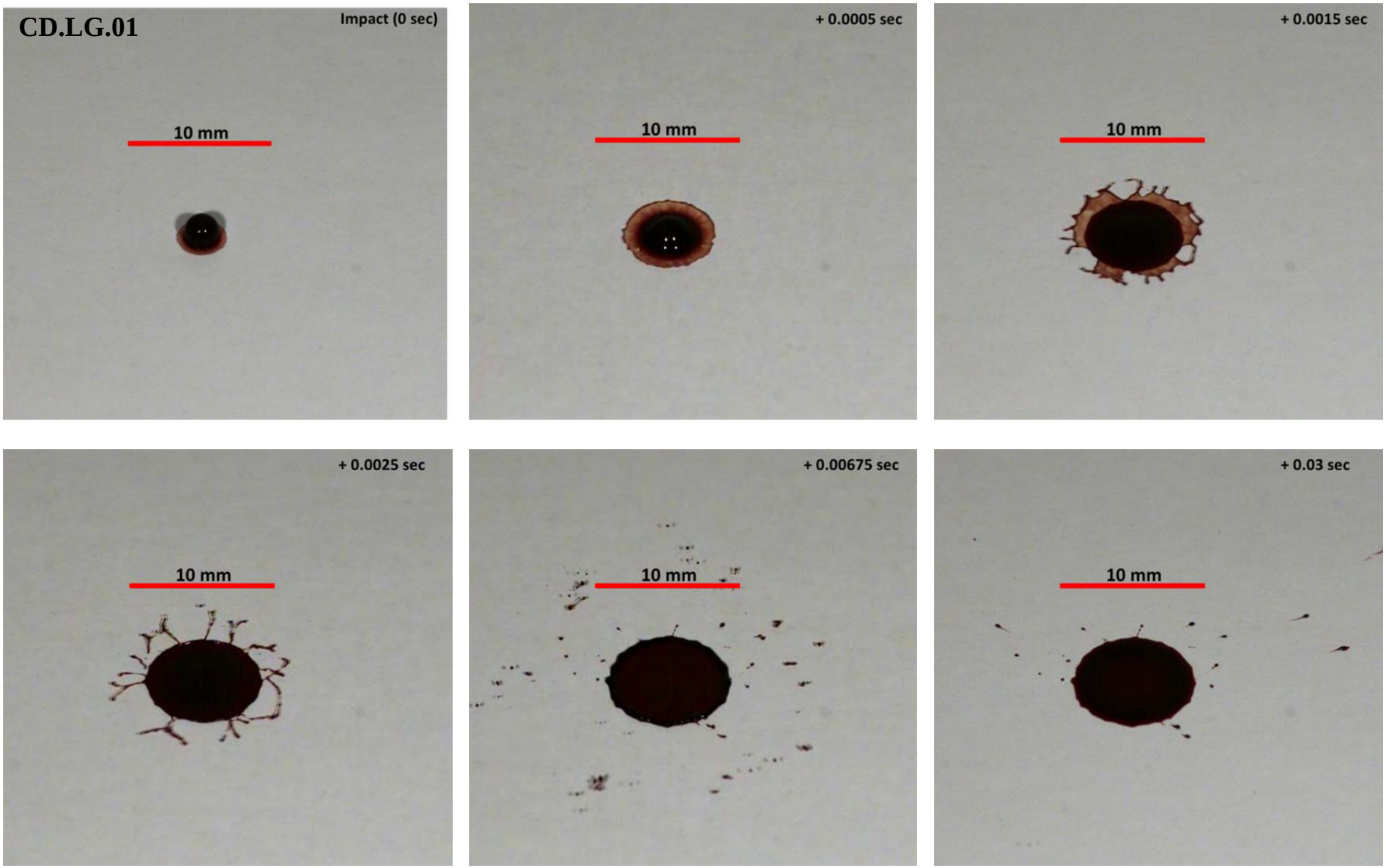


Figure III.37. A 5 mm (~65 µL) porcine blood drop impacting a plain cardboard surface.

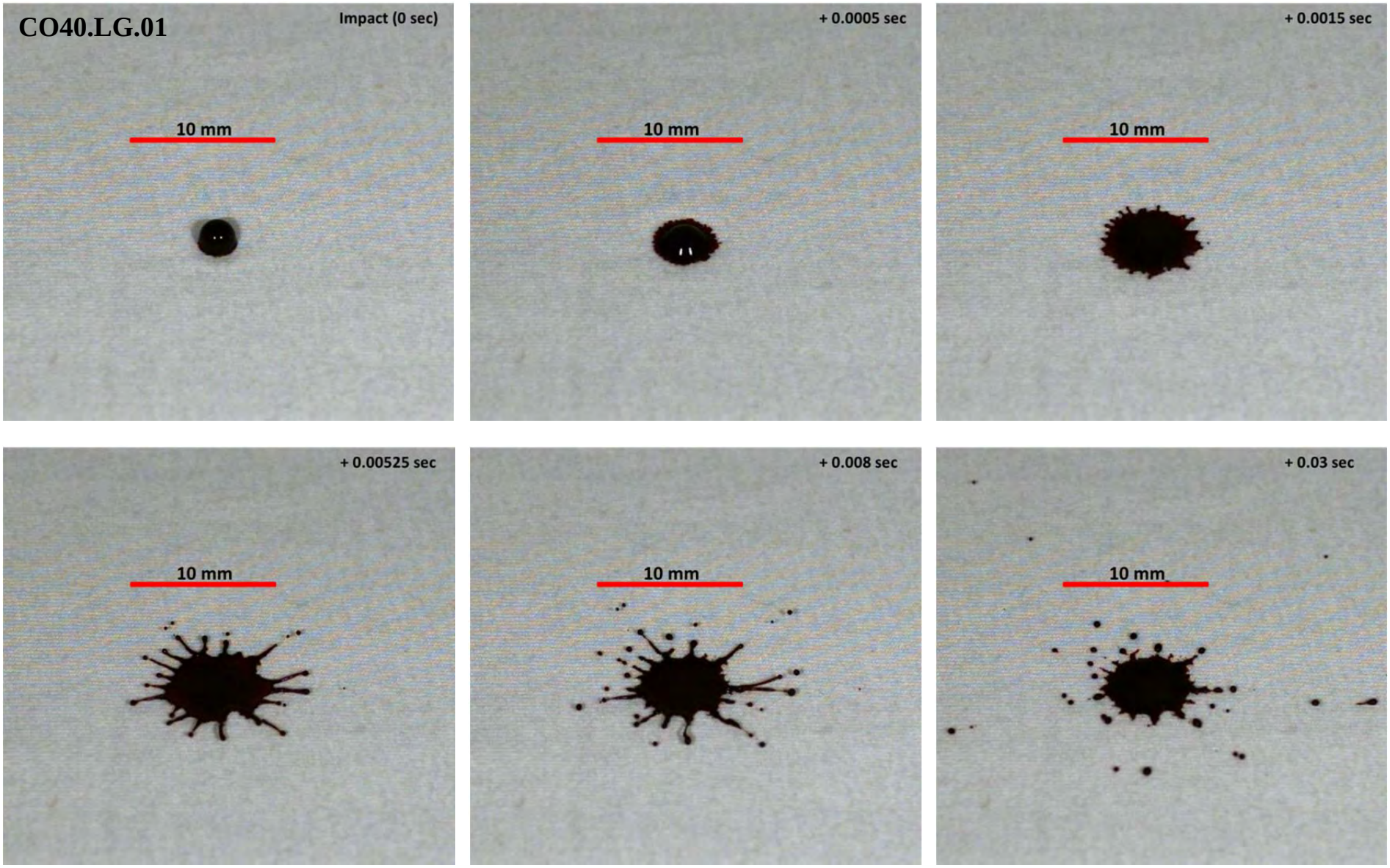


Figure III.38. A 5 mm (~65 µL) porcine blood drop impacting the cotton knit fabric surface.

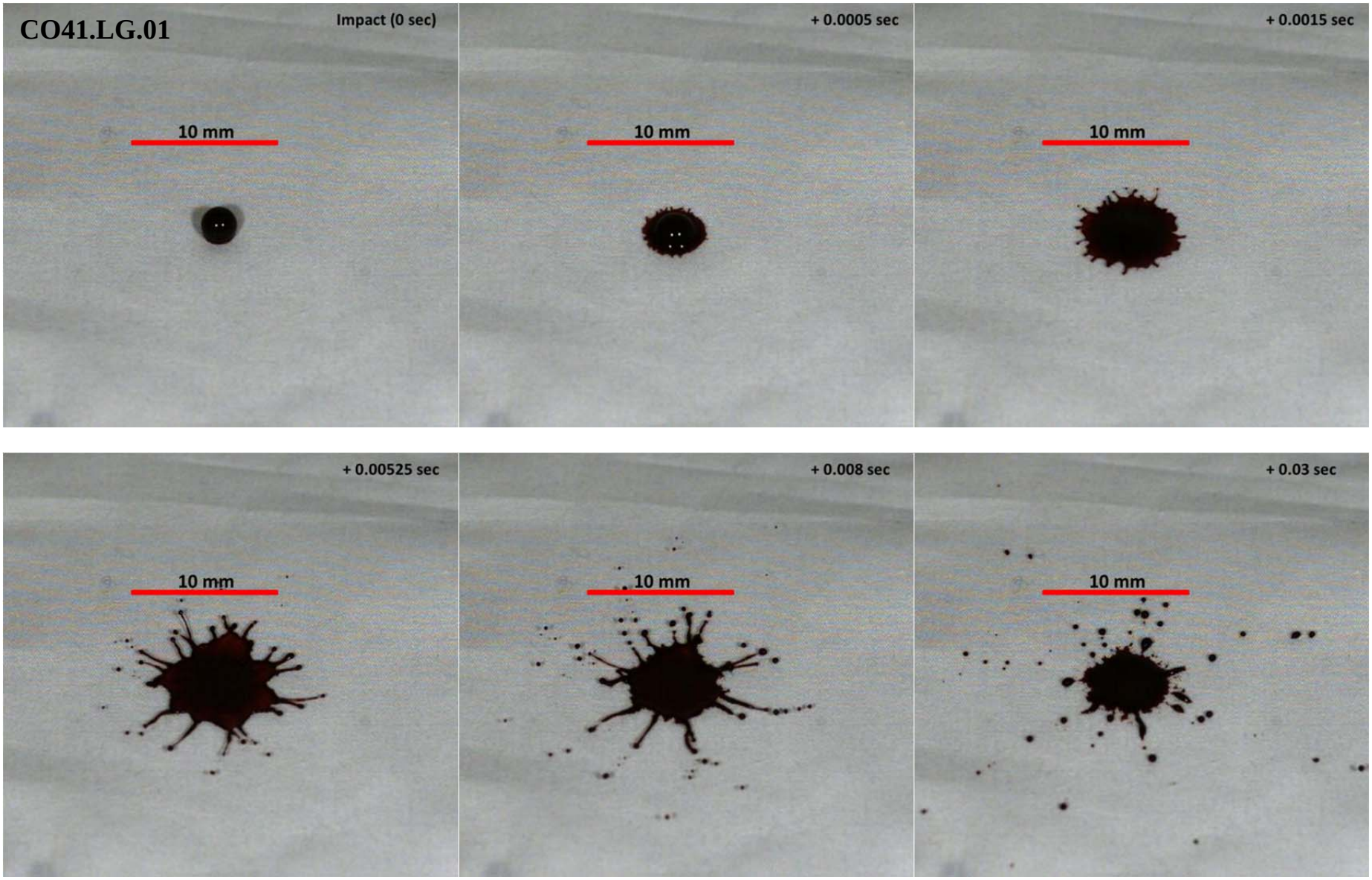


Figure III.39. A 5 mm (~65 µL) porcine blood drop impacting the cotton plain woven fabric surface.

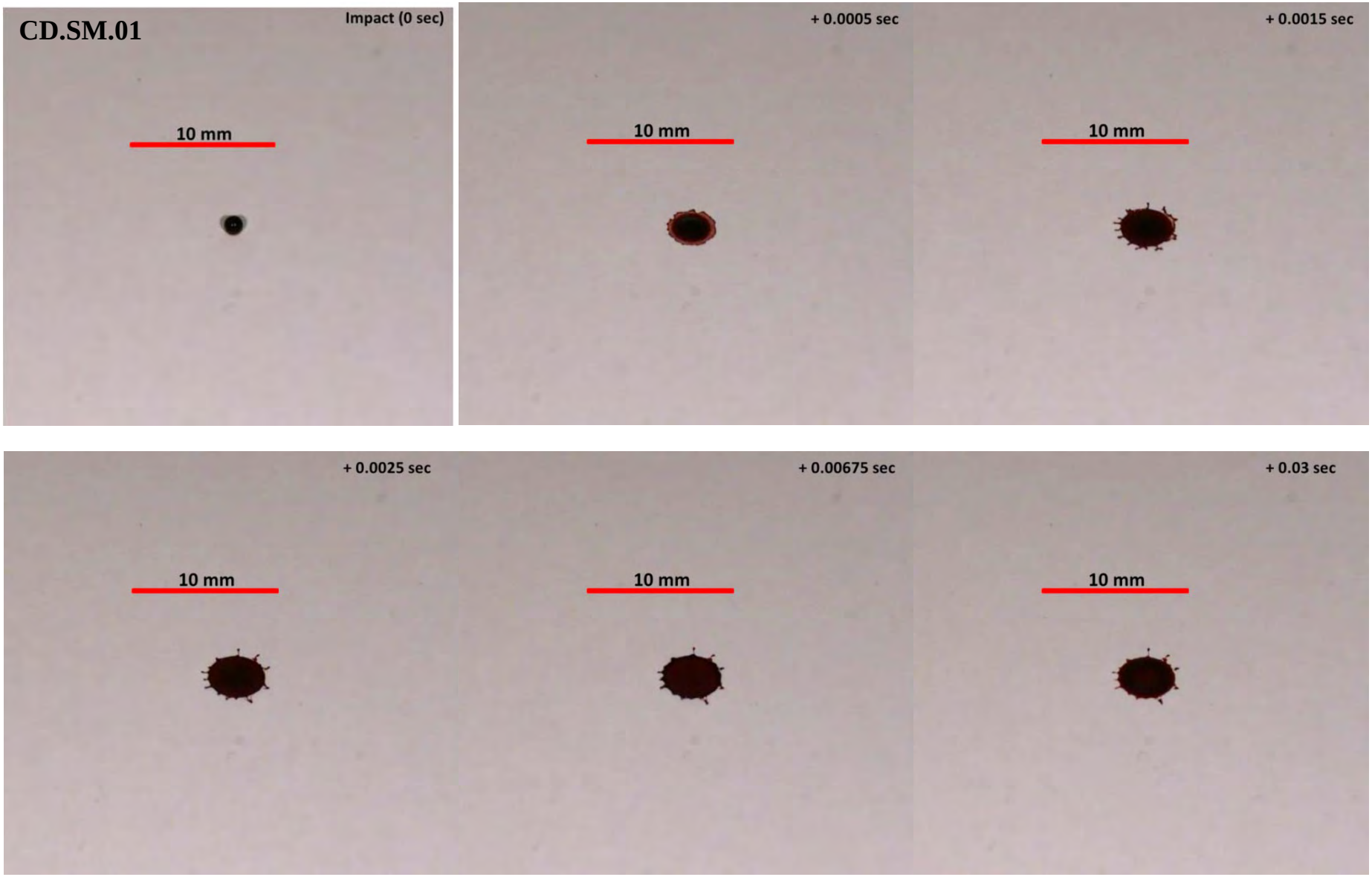


Figure III.40. A 2.6 mm (~ 11 µL) porcine blood drop impacting a plain cardboard surface.

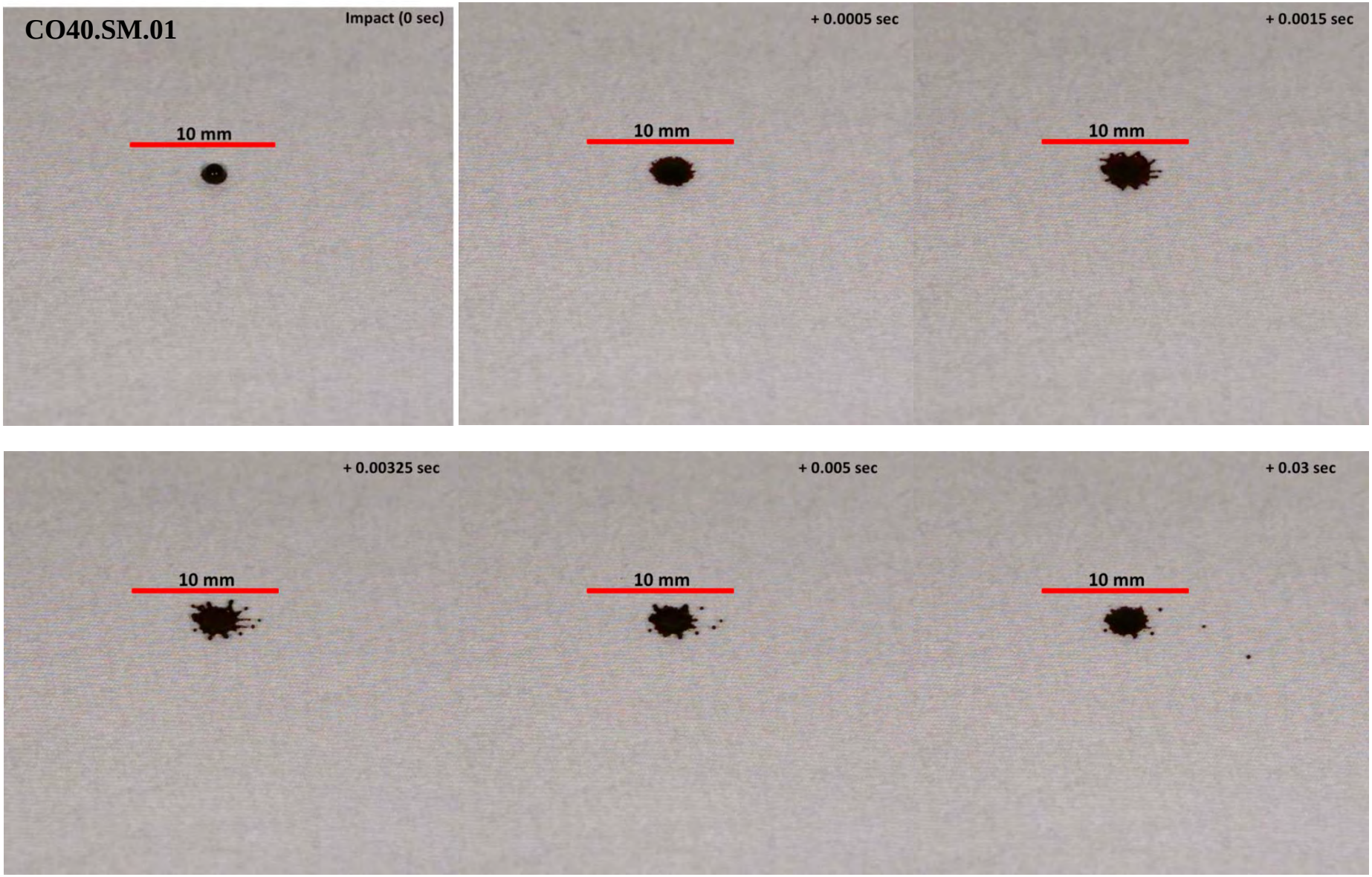


Figure III.41. A 2.6 mm (~ 11 μL) porcine blood drop impacting the cotton knit fabric surface.

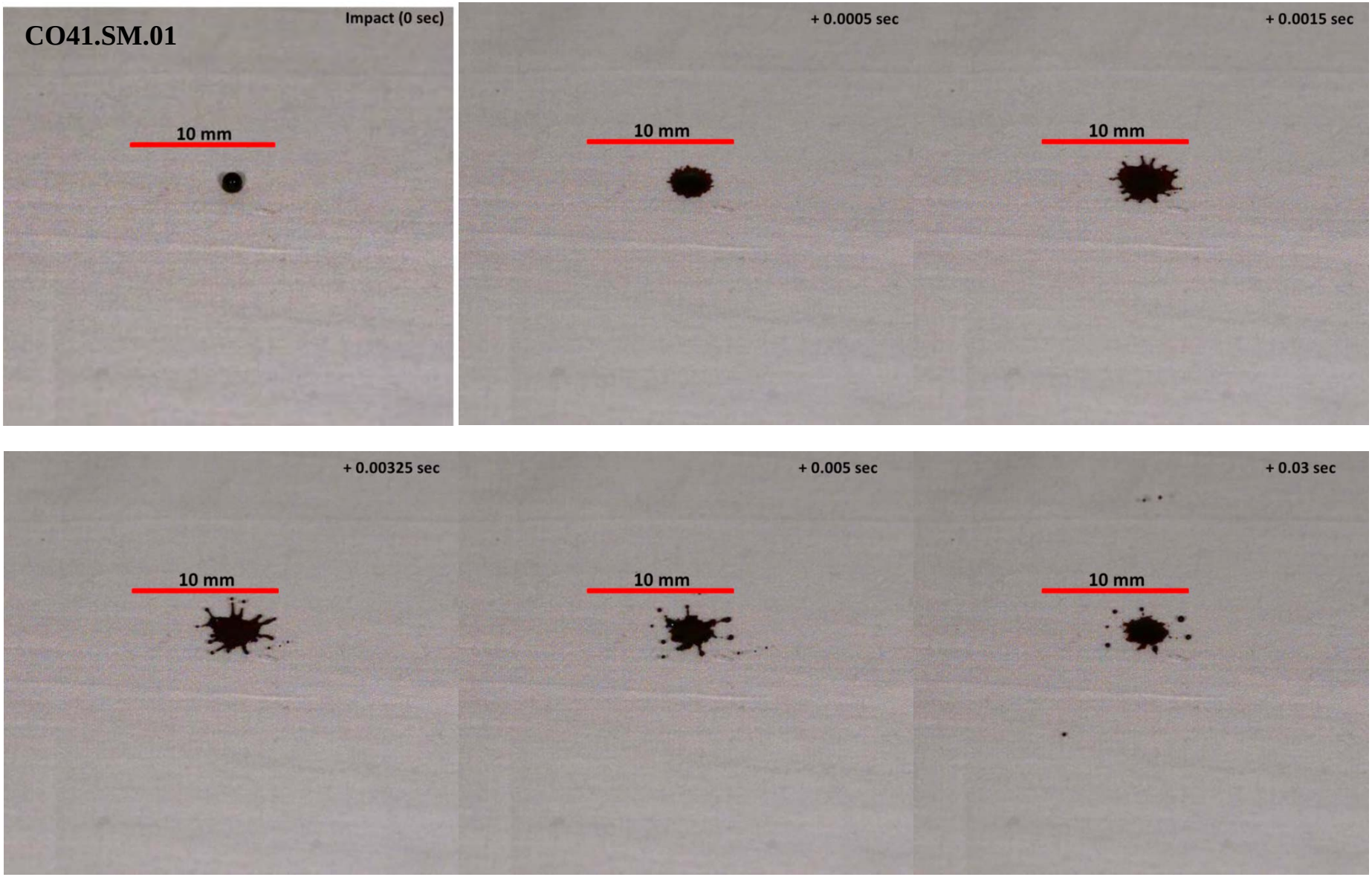


Figure III.42. A 2.6 mm (~ 11 µL) porcine blood drop impacting the cotton plain woven fabric surface.

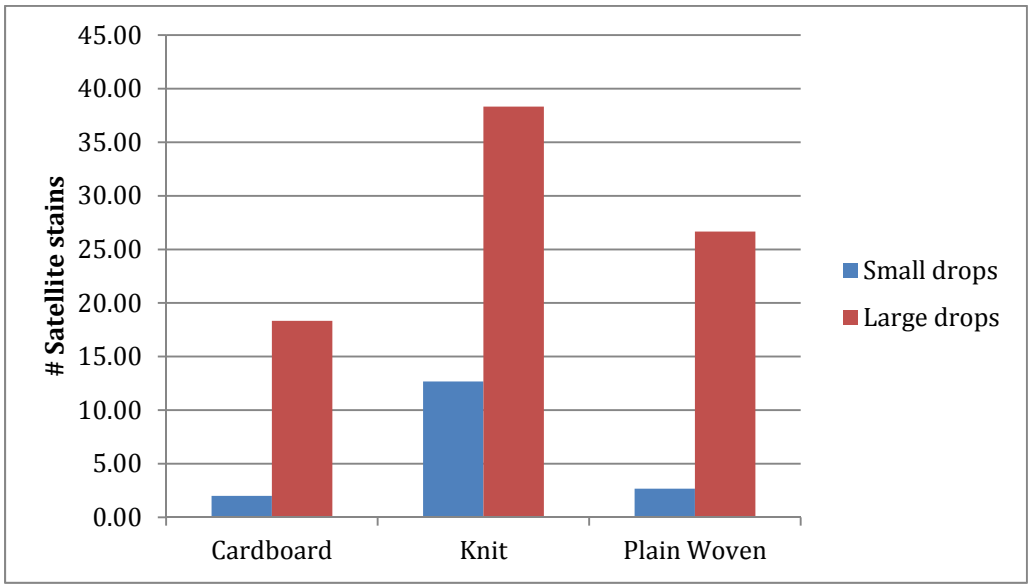


Figure III.43. Satellite stain formation on target surfaces: number of stains formed. Small drops are approximately 2.6 mm diameter (~9 µL volume) drops while the large drops are approximately 5 mm diameter (~65 µL volume).

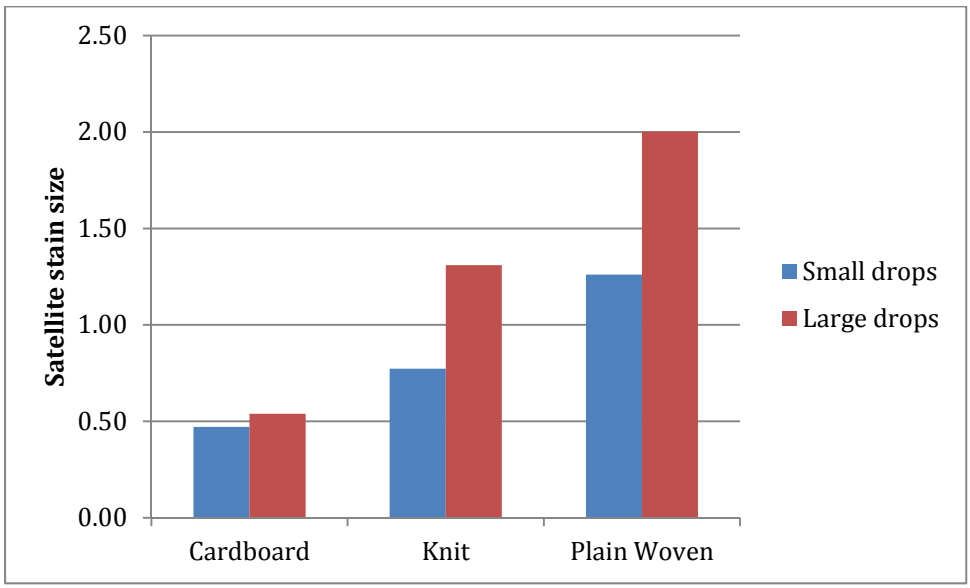


Figure III.44. Satellite stain formation on target surfaces: average stain size. Small drops are approximately 2.6 mm diameter (~9 µL volume) drops while the large drops are approximately 5 mm diameter (~65 µL volume).

III.24 Spreading and Wicking of Porcine Blood Impacting onto Fabric from Low Heights
As shown above, when a drop impacts a fabric, it spreads, may break up, and then wicks into the fabric. In the study described below, 30 μL droplets of porcine blood impacted different fabrics from a selection of low heights: 100, 200 and 300 mm in order to differentiate the spread of blood due to inertial effects and that due to wicking.

Four distinct stages of spreading were identified for porcine blood droplets impacting both the knit and plain woven fabrics when dropped from low heights (100, 200 and 300 mm):

1. **Inertial spread** – following impact, blood drops collapsed, spread laterally outward and formed an uneven raised rim. Where the inertial forces were sufficient, each ligament (spine) on the rim will break-up and detach forming satellite spatter that splash around the parent stain.

2. **Retraction** – under the influence of the surface tension forces of the blood, the bulk liquid then retracted back towards the center.

3. **Initial absorption** – following retraction, the stained area remained relatively constant for approximately 0.5 second. It is surmised that during this period blood was soaking vertically into the fabric structure.

4. **Wicking** – at approximately 0.5 to 1 second from contact, wicking occurred and the stain area increased.

Figures III.45-46 show the differences between the knit and plain woven fabrics during droplet spreading for a 30 μL porcine droplet falling from 200 mm. It was evident that the plain woven fabric gave rise to a greater maximum spread area than the knit in the inertial spread stage. No splashing of blood was observed for this combination of drop height and volume.

These results show that the inertial spread of a drop falling on the plain woven fabric exhibits a greater initial maximum spreading area than the knit. This was also seen in the results from different drop heights for knits (Figures III.47-48) and for plain woven fabrics (Figures III.49-50). Figures III.45-46 also show the final stain area on the plain woven fabric in this example to be greater than the knit due to the greater extent of wicking in the woven fabric. On the other hand, the rate of wicking was faster in the knit than in the plain woven fabric.

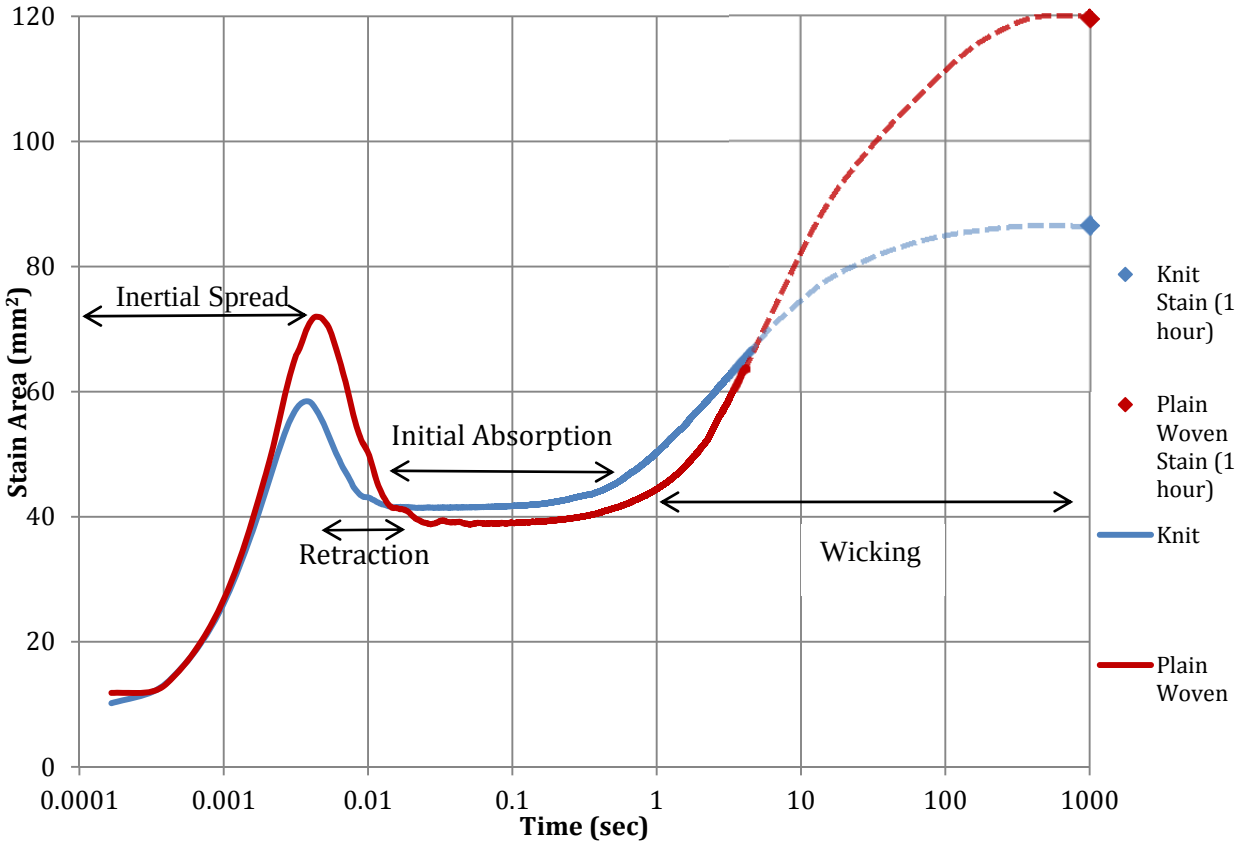


Figure III.45. Plot showing the different phases of droplet spreading (stain area vs time) and the dried stain area for 30 µL porcine blood droplets released at a height of 200 mm, onto cotton jersey knit and plain woven fabrics.

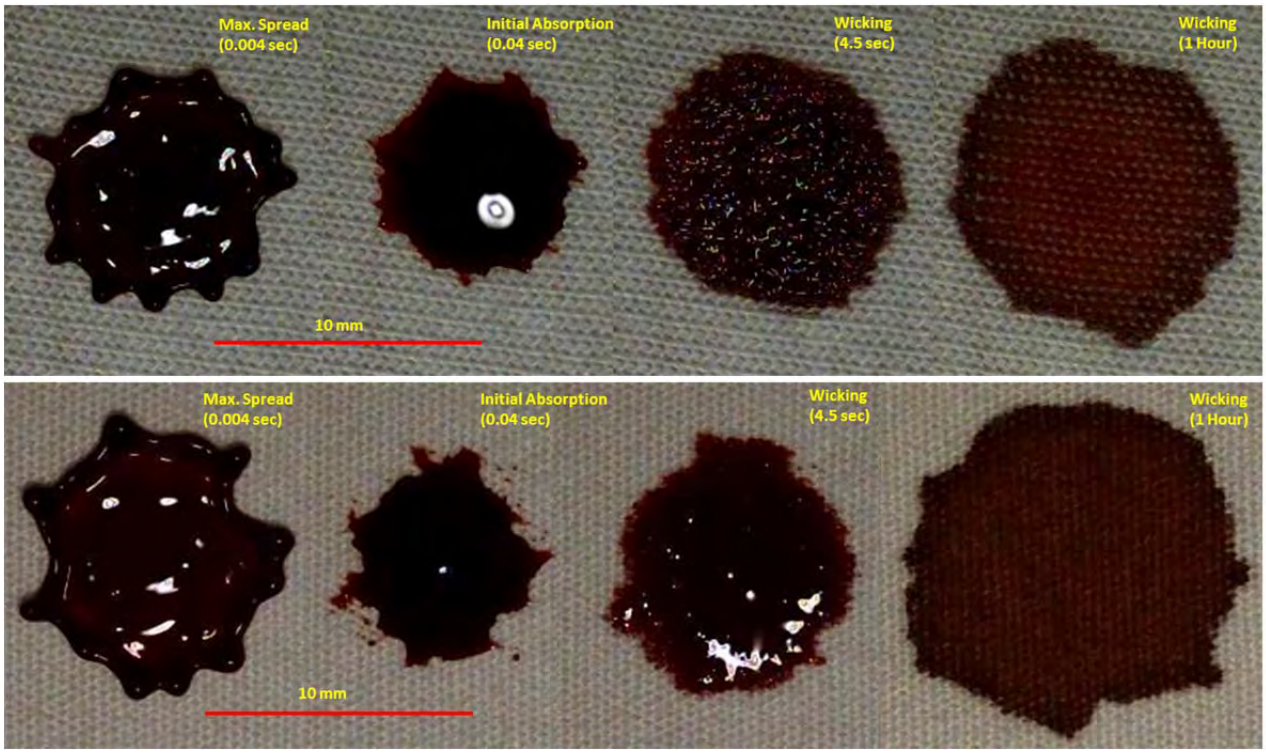


Figure III.46. Still images showing different stages of the spread of a 30 µL porcine blood droplet falling on knit fabric (top, warp direction is horizontal) and plain woven fabric (bottom, warp direction is vertical) from a height of 200 mm.

In Figure III.47, the area vs time after impact is shown for 30 µL porcine blood drops falling from 100, 200 and 300 mm onto the cotton jersey knit fabric. It can be seen that the initial inertial spreading of the drop increased with increasing drop height. Furthermore, as the initial inertial impact force increases, the final size of the resulting stain also increases. Still images of the corresponding stains are given in Figure III.48.

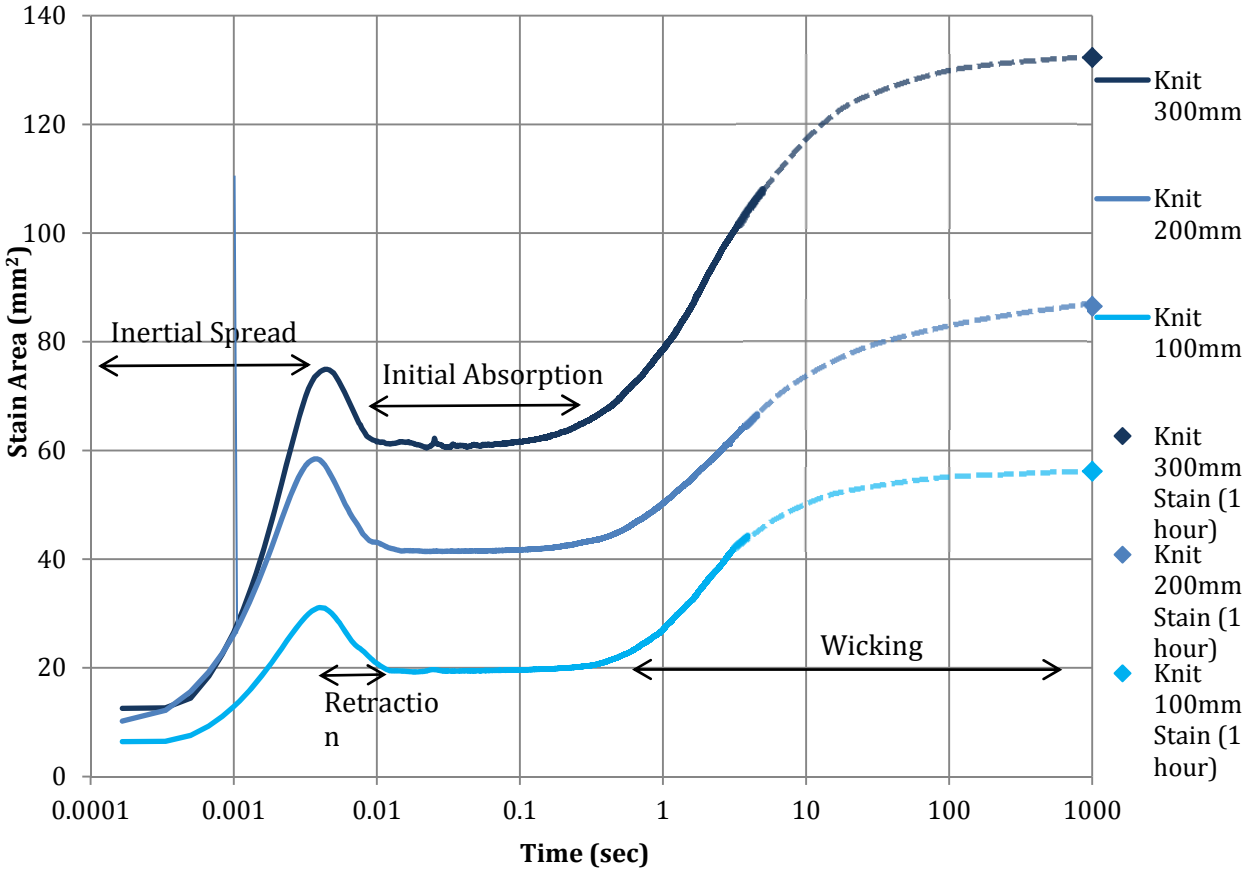


Figure III.47. Plot showing the different phases of droplets spreading for 30 µL porcine droplets released from heights of 100, 200 and 300 mm onto the knit fabric, and the size of the final stain after 1 hour. Note: the dashed lines indicate the gap in time between the last high speed video image to the final still photograph of the stain and does not show the true time evolution during this period.

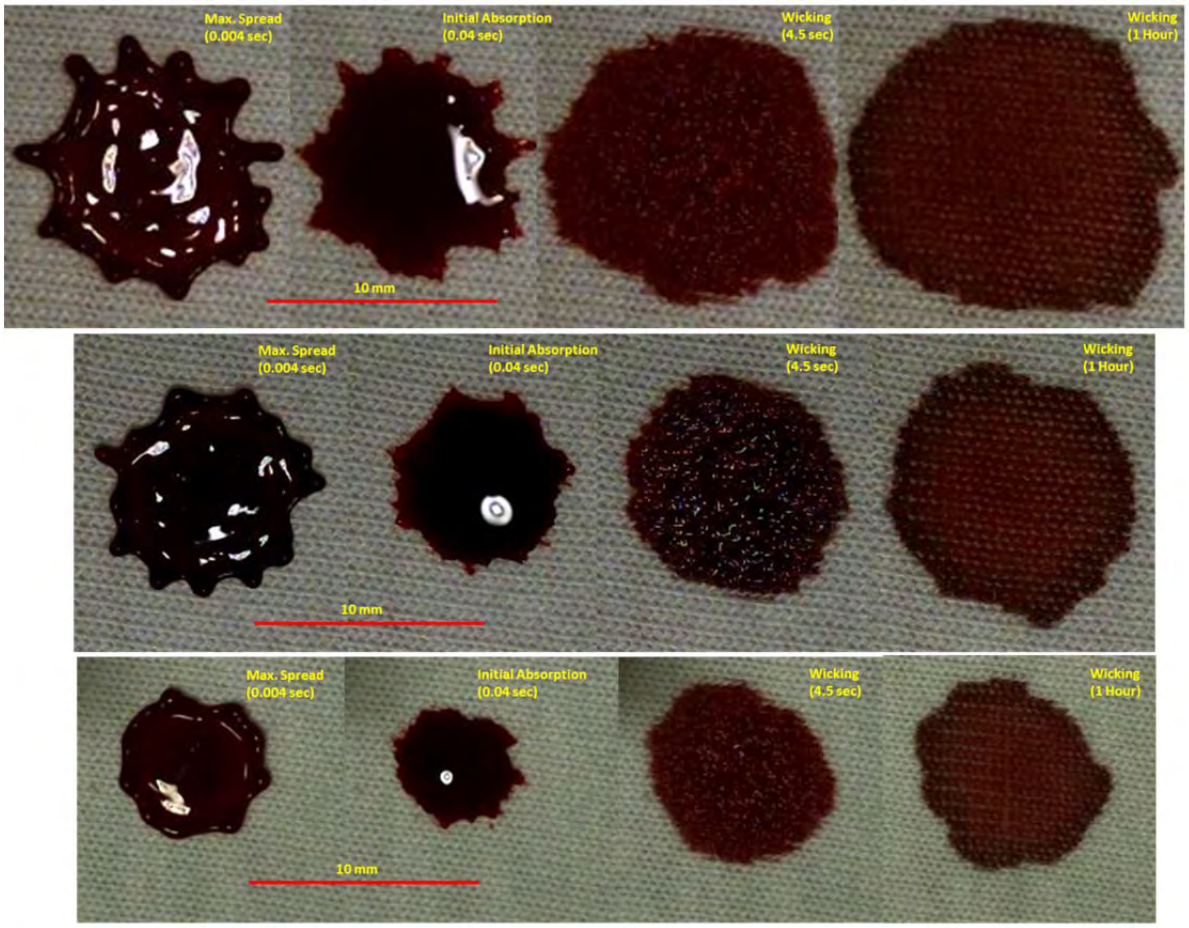


Figure III.48. Still images showing different stages of the spread of 30 μL porcine blood droplets falling onto the knit fabric from heights of 300 mm (top), 200 mm (middle) and 100 mm (bottom). (Fabric warp direction is horizontal.)

For the knit fabric, the still images in Figure III.48 show the presence of spines protruding from the parent in the maximum spread image of the droplet released from 300 mm. These are observed to settle around the perimeter of the parent stain in the initial absorption phase and then are covered by the bulk liquid as it spreads out in the wicking phase. While spines are present in the maximum spread phase in the droplet released from 200 mm, these appear largely to retract back into the bulk liquid. In the droplet released from 100 mm, a raised outer rim was observed in the maximum inertial spread phase; however the bulk liquid retracts back towards the center where it settles. The inertial forces are not strong enough in this instance to overcome the adhesive surface tension force. From this data it appears that for the 100 mm release height, wicking rather than inertial force is more dominant in the droplet spreading. Between release heights of 200 and 300 mm, both the inertial force and wicking play important roles in droplet spread on the knit fabric.

A different set of impact dynamics were observed in the plain woven fabric as opposed to the knit. Figure III.50 shows the final stain size of the droplet released from a height of 200 mm to be larger than for the droplet released from a height of 300 mm. This is despite the fact that the

initial inertial spread area was much greater for the 300 mm drop height. In Figure III.50, it can be seen that the 300 mm droplet undergoes significant splashing on impact, resulting in loss of volume of the parent droplet.

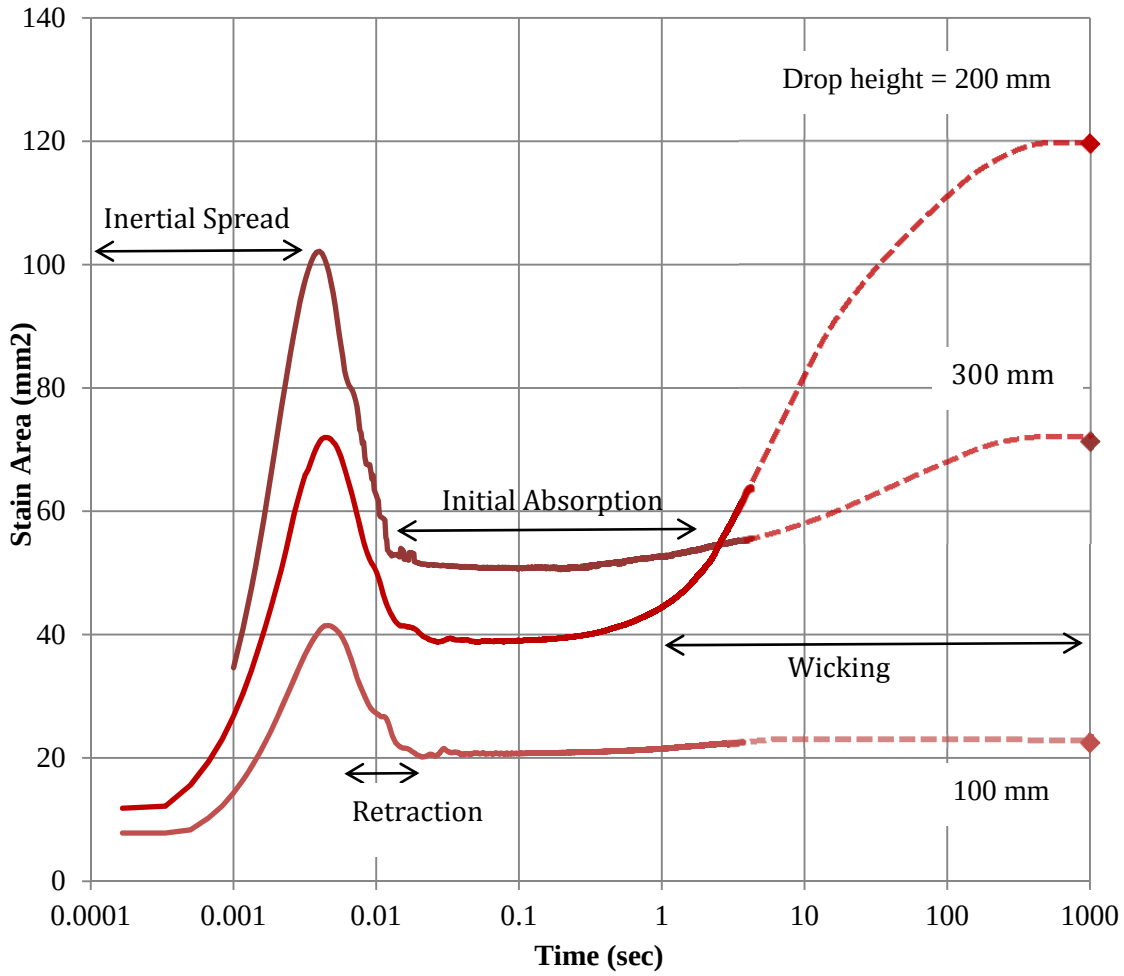


Figure III.49: Plot showing the different phases of droplets spreading for 30 µL porcine droplets released from heights of 100, 200 and 300 mm onto the plain woven fabric, and the size of the final stain after 1 hour. Note: the dashed lines indicate the gap in time between the last high speed video image to the final photograph of the stain and does not show the true time evolution during this period.

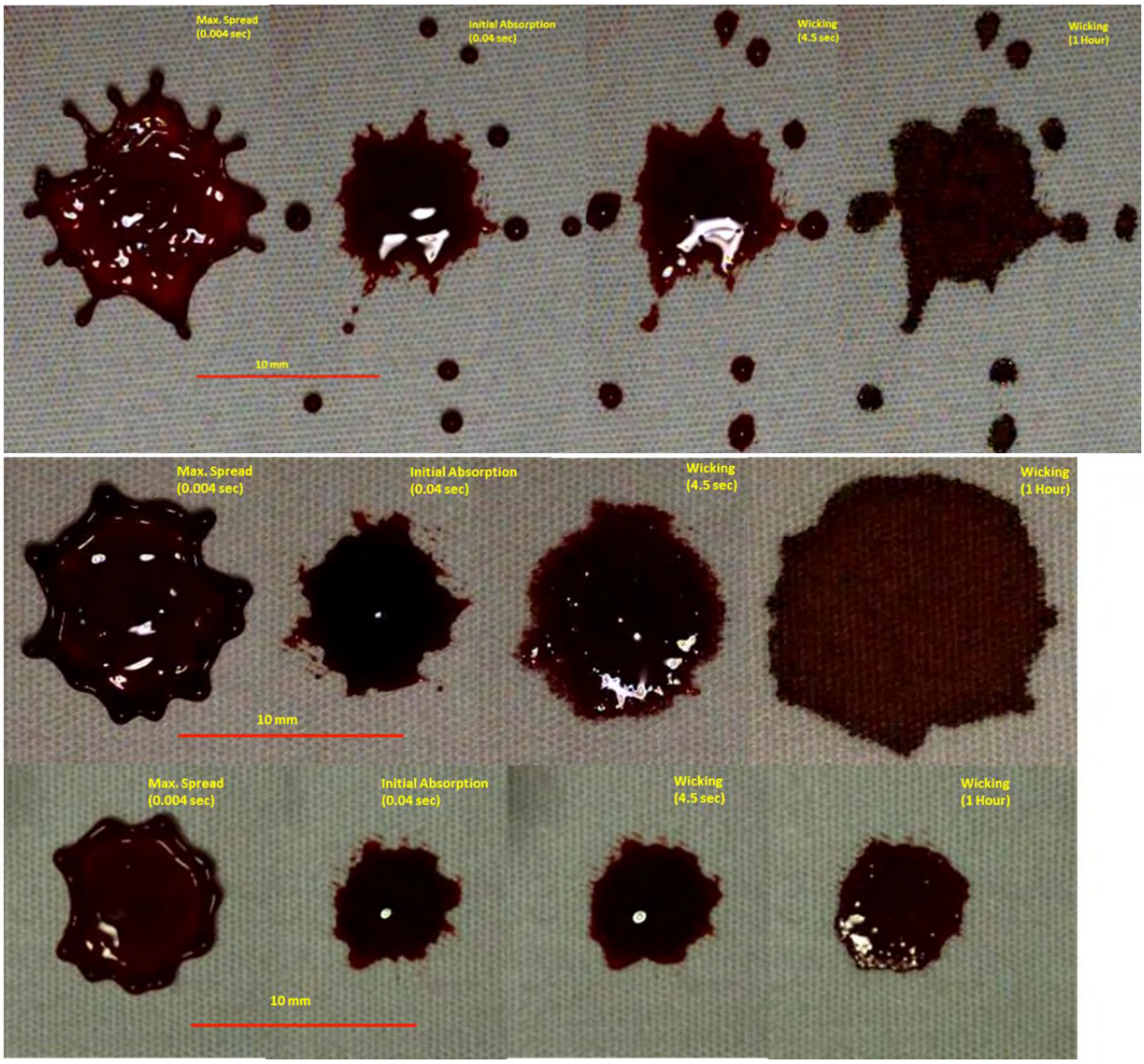


Figure III.50: Still images showing different stages of the spread of 30 μL porcine blood droplets falling onto the plain woven fabric (warp direction vertical) from heights of 300 mm (top), 200 mm (middle) and 100 mm (bottom).

Clear differences can be seen between the 100 and 200 mm droplets throughout the different phases. The initial inertial spread is significantly greater in the 200 mm droplet; however, the greatest difference is at the wicking phase where the sizes of these two droplets do not increase in proportion to one another (see Figure III.49). The 200 mm droplet appears to soak into the fabric and give a dry appearance after one hour. Conversely, the size and appearance of the 100 mm droplet at the initial absorption phase is similar to the subsequent image; 4.5 seconds after initial contact. The appearance of the stain after an hour is not dry like droplets released from greater heights. It may be that for a 30 μL droplet to penetrate the fabric, inertial forces greater than that of the 100 mm droplet are required.

From this series of experiments, we note that

- Wicking of porcine blood into the knit fabric appeared to be faster than for the plain woven, in agreement with our findings for SB4 synthetic blood drops.
- For each given fabric, the timing of the different phases was similar for the different release heights.
- In low-inertia droplets (drop height = 100 mm) on the plain woven fabric the stain area did not appreciably increase following the initial absorption phase. The bulk liquid appeared not to soak into the fabric and wick along the fibers as it did with the knit. This is indicative of a minimum inertial force required for small blood droplets (high surface tension) to penetrate the fabric.
- The 300 mm droplets impacting on the plain woven fabric splash, with satellite stains separating from the parent stain. On the knit, spines can be seen but retract back into the bulk liquid and do not separate. The splash parameter is different for these two fabrics. The absorptive capacity of the knit may reduce splashing.

III.25 Spatter stains on fabric surfaces using porcine blood drops

Although the drip stain experiments highlight difficulties in analyzing bloodstains on textiles, it was felt that small drops, such as observed for spatter stains, might produce different results. Thus, a set of spatter stain trials were performed and provided a total of 150 drops with corresponding stains on three surfaces; Foamcore, jersey knit fabric and plain woven fabric. These were created at disc velocities of between 6 and 14 m/s. Figures III.51 and II.52 show typical examples of spatter stains created on the two study fabrics.

Figure III.53 shows two examples of the spreading of spatter drops on each of the two study fabrics. Details of the properties of these drops are given in Table III.16. The phases of spreading observed for the drip stains are apparent in spatter stains as well. However the inertial spreading phase occurs much more rapidly for the spatter stains, reflecting the higher impact velocities involved in the formation of a spatter stain compared with a drip stain (6 – 7 m/sec for the spatter drops, 1 - 2 m/sec for the passive drops) (Figure III.54).

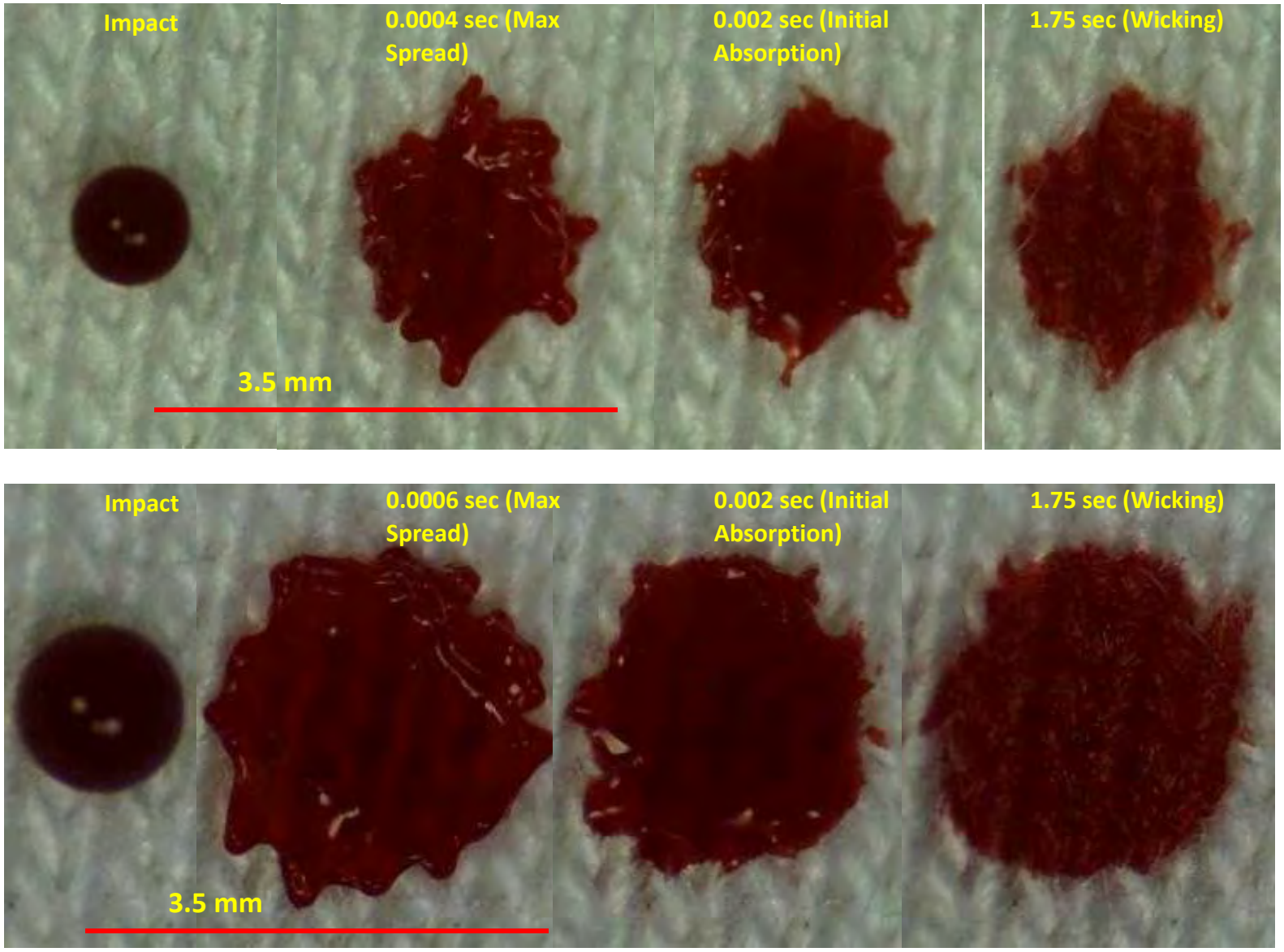


Figure III.51. Example of a spatter stain on the knit fabric (top: a 0.93 mm drop impacting at 7.6 m/s, bottom: a 1.23 mm diameter drop impacting at 6.8 m/s).

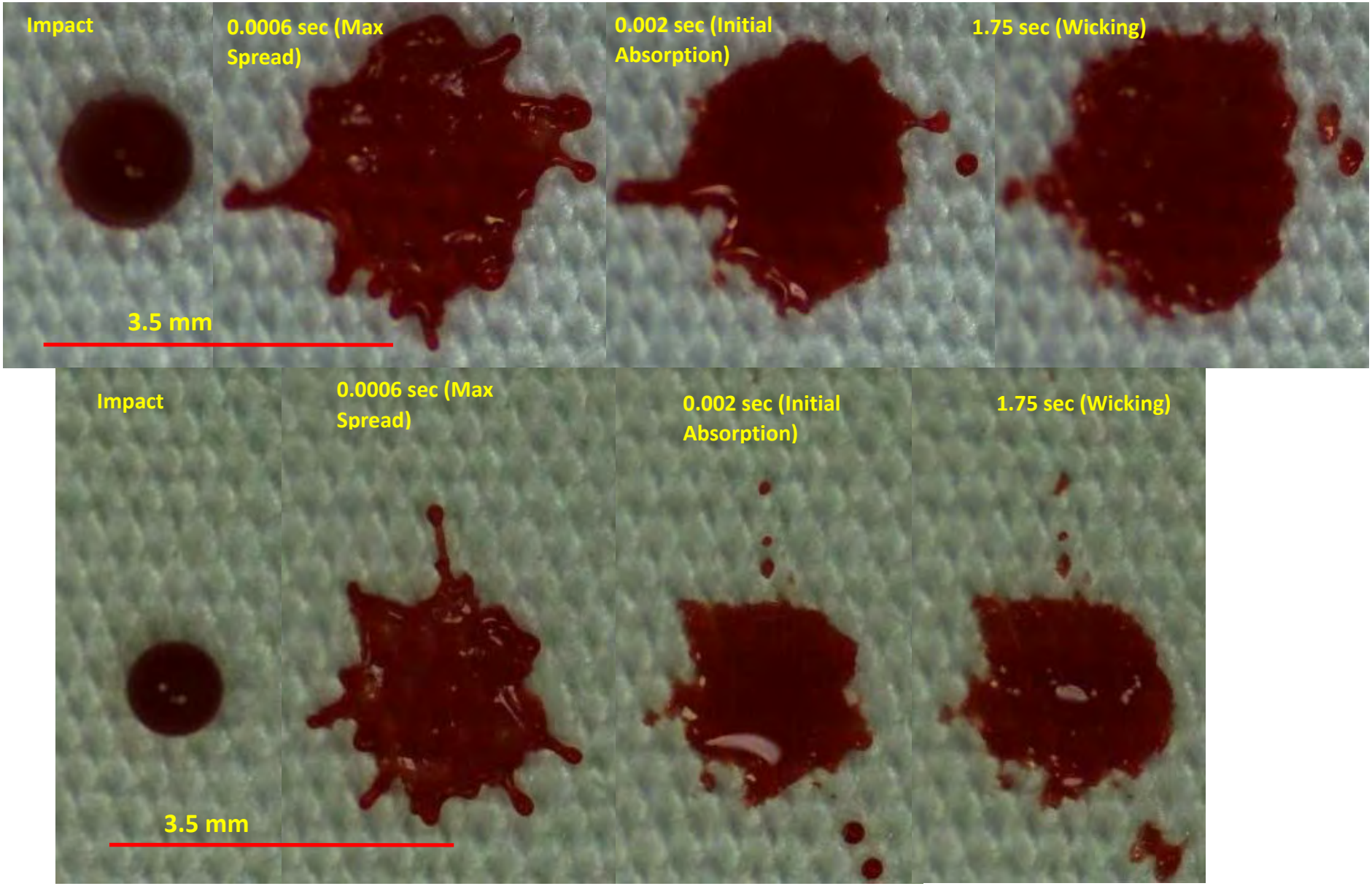


Figure III.52. Example of a spatter stain on the plain woven fabric (top: 0.95 mm drop impacting at 7.4 m/s, bottom: 1.22 mm diameter drop impacting at 5.9 m/s).

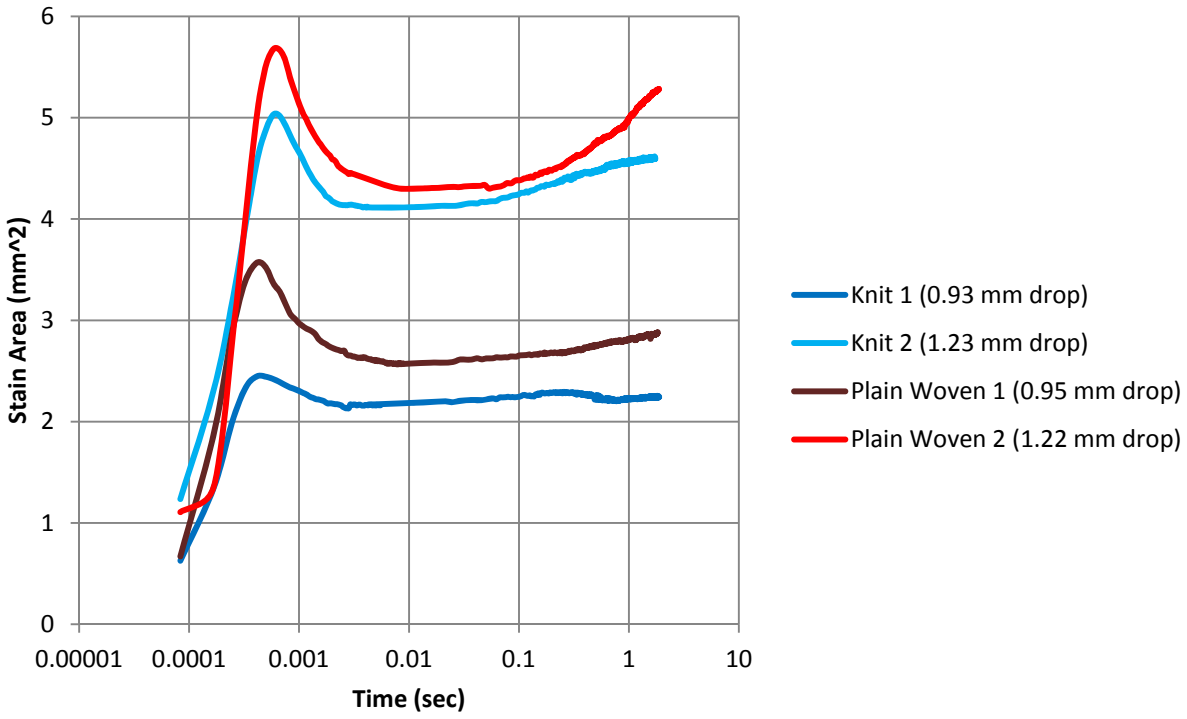


Figure III.53. Plot showing examples of the spreading of small drops (0.93 – 1.23 mm diameter) onto the knit and plain woven fabrics.

Table III.16 Blood drop properties for spatter stain formation.

	Drop diameter (mm)	Impact velocity (m/sec)	Re (impact)	We (impact)
Knit specimen 1	0.93	7.57	1646	904
Knit specimen 2	1.23	6.78	1939	955
Woven specimen 1	0.95	7.37	1634	873
Woven specimen 2	1.22	5.92	1689	725

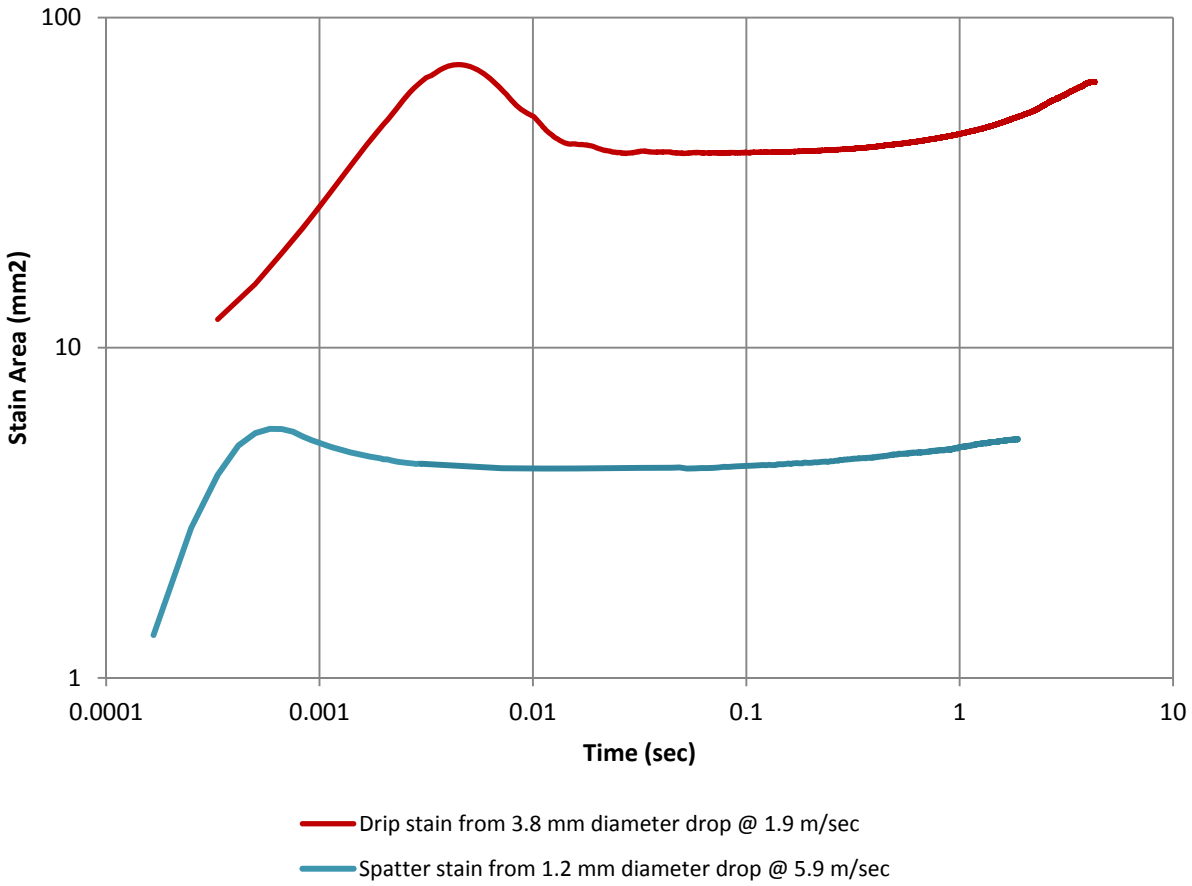


Figure III.54. The spreading of a typical drip stain compared with a spatter stain, both on the plain woven fabric.

An empirical relationship between stain diameter and drop diameter has been suggested for blood drops on a range of surfaces [35,36]. Under conditions where inertial forces dominate and surface tension effects are negligible (which occur for many drip stain and spatter stain drop impacts), drop spread is a function of the Reynolds number only, which in turn depends mainly on the drop speed and its diameter. One useful relationship, derived by Pasandideh-Fard [36] from a conservation of energy balance, is the relationship between the ratio of stain diameter (D) to drop diameter (d) and Re:

$$D/d = 0.5\ Re^{0.25}$$

Figure III.55 shows the results for all drops in this experiment relative to the energy balance equation proposed by Pasandideh-Fard. The parameters presented are the Reynolds number (Re) and the final stain diameter (D) divided by the drop diameter (d). Drop spread for the fabric surfaces is less than that for a hard surface and less than the energy-balance model predicts. This is to be expected where there is likely to be energy loss associated with the spread of the fluid across a textured surface.

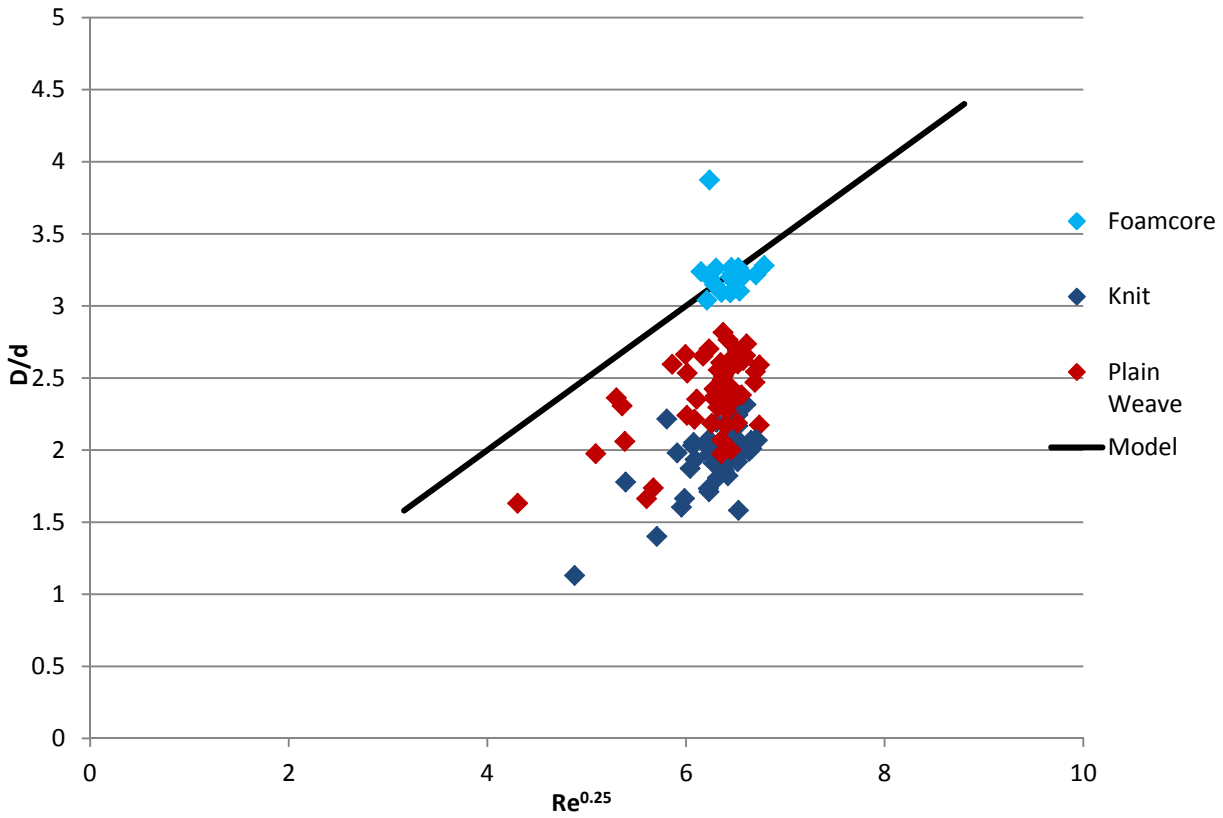
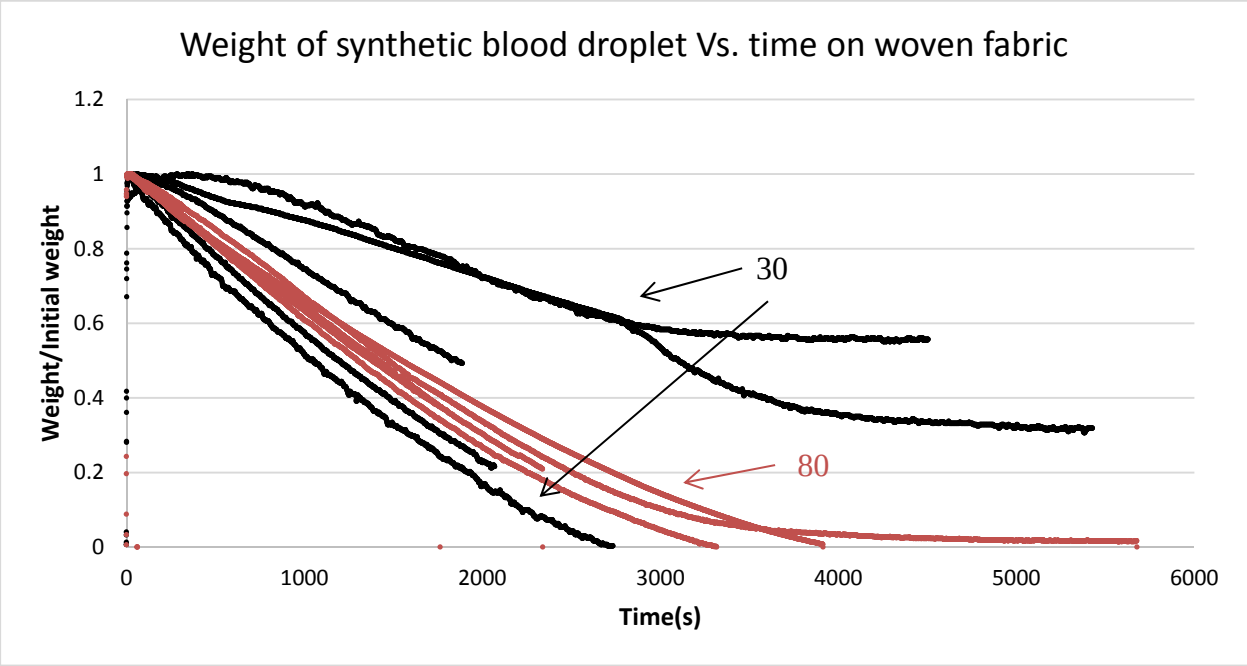


Figure III.55: $Re^{0.25}$ vs D/d for Foamcore, knit and plain woven fabrics for spatter experiments, relative to the energy balance model proposed by Pasandideh-Fard [36].

III.26 Comparison of SB4 stains and porcine blood stains

When a very large number of samples need to be examined, it is challenging to use real blood. However, the use of synthetic blood can only be justified if stains using the synthetic blood mimic the essential features of real blood. Thus, in this section, stains were produced using both porcine blood and synthetic blood SB5.

In Figures III.56 and III.57, the drying process of the synthetic blood SB5 and porcine blood are shown for 30 and 80 μL drops placed on plain woven and jersey knit fabrics. In these curves, the weights were normalized by dividing each point of the drying curves by the weight of the drop that was placed on the fabric. The 80 μL drying curves were uniform for both fabric types and for both the porcine blood and SB5 synthetic blood. In addition, drying times for the plain woven and knit fabrics were in the range 3500 – 4500 seconds. For the porcine blood, the weight stopped going down at a relative weight of 20 – 45%, which is in the range of the hematocrit. We interpret this point as being when the blood was dry and only the cells remained. The time to reach this point was also in the range of 3000 seconds. For the 30 μL drops, the drying times were quite erratic for both fabrics and both the porcine and synthetic blood. Thus, for the purposes of studying the drying times, SB5 mimicked porcine blood well. The main discrepancy is due to the red blood cells present in the porcine blood, which do not participate in drying.



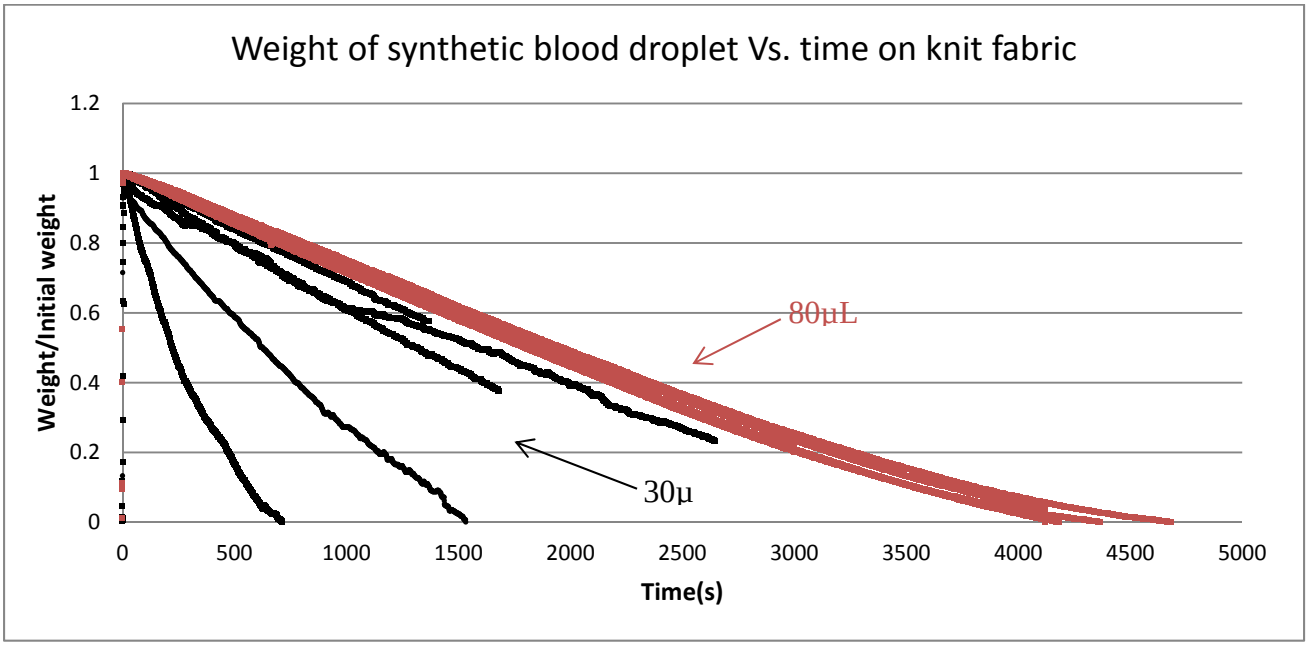
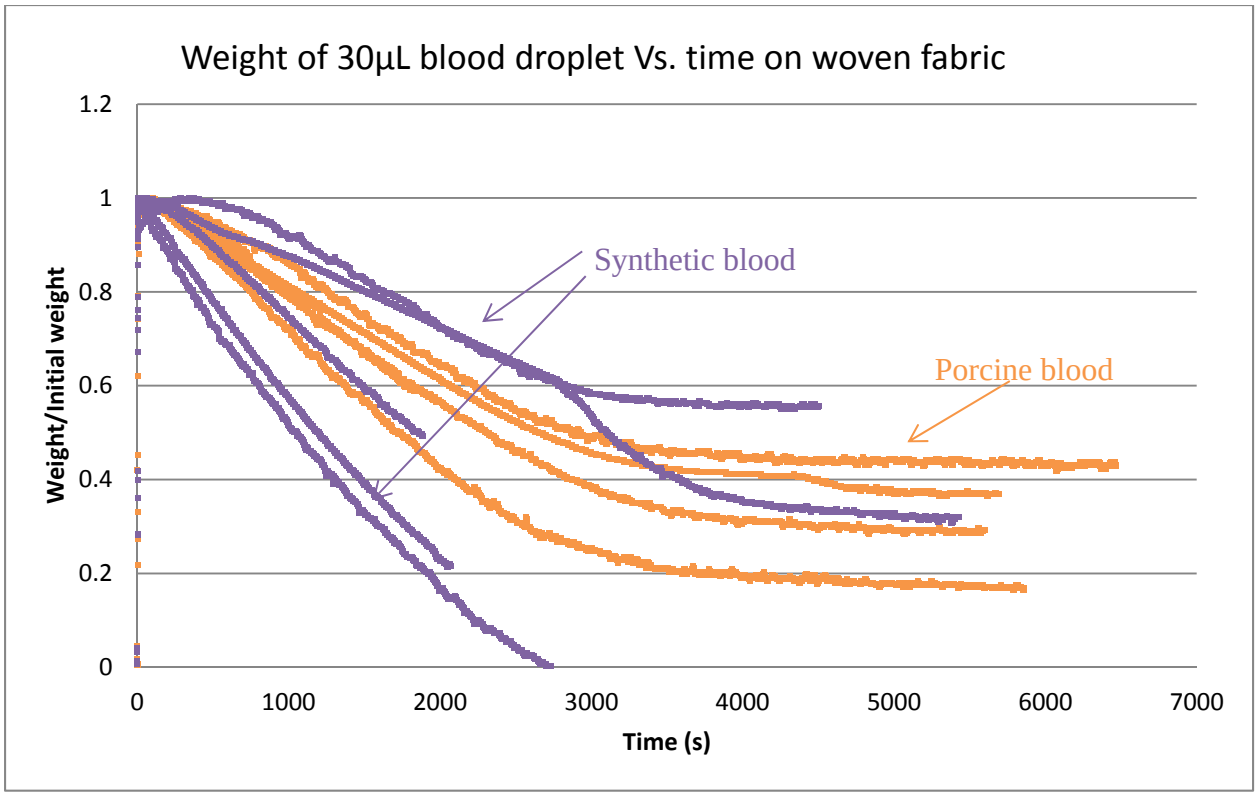


Figure III.56 Normalized drying times for 30 and 80 µL SB5 drops placed onto plain woven (upper) and jersey knit (lower) fabrics.



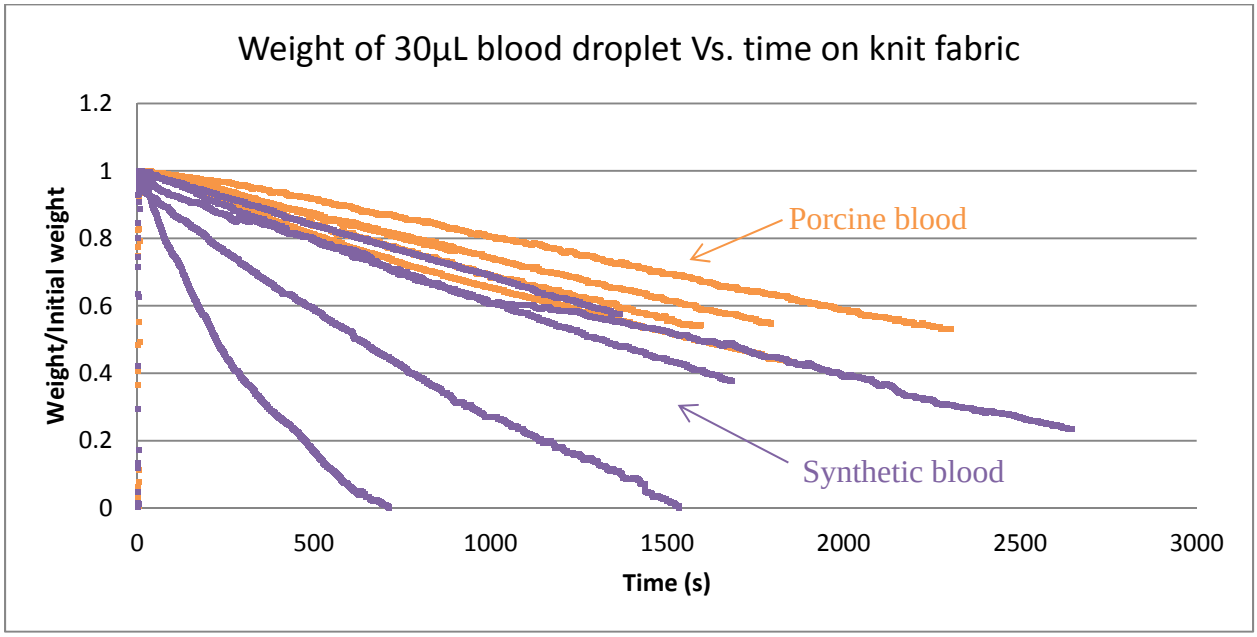


Figure III.57. Normalized drying times for 30 and 80 µL porcine blood drops placed onto plain woven (upper) and jersey knit (lower) fabrics.

Wicking Curves

Figures III.58 (SB5) and III.59 (porcine blood) show the growth of the stain area on plain woven and knit fabric. The area growth curves have similar shapes for SB5 and porcine blood on the plain woven fabric and on the knit fabric. However, the stains on the knit fabric grow differently than on the plain woven fabric. In addition, the area of the SB5 stains are significantly larger than for the porcine blood stains.

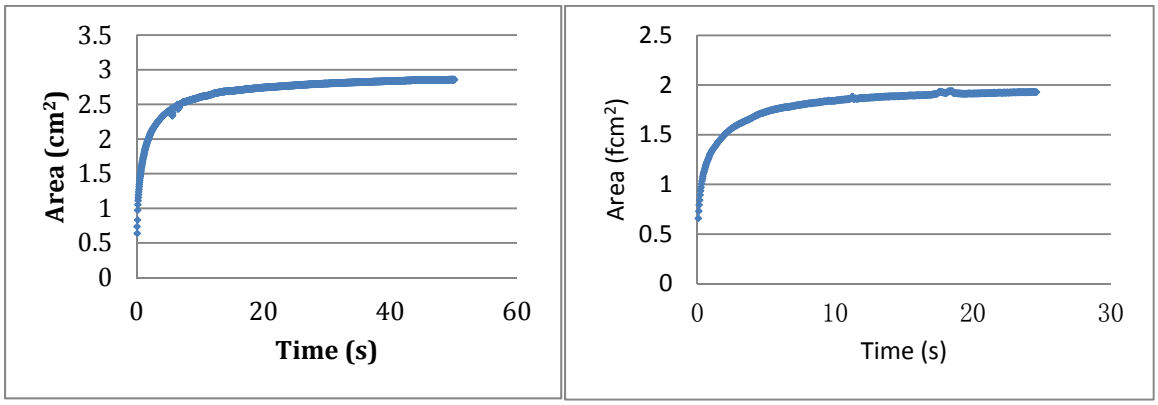


Figure III.58 The growth of the stain area for a 30 SB5 stain is shown on the plain woven fabric (left) and knit fabric (right).

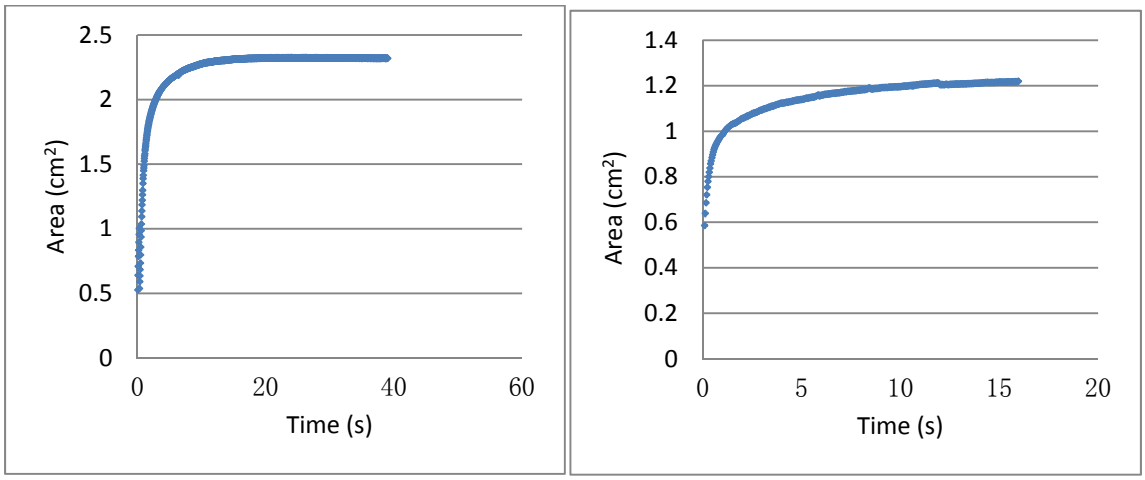


Figure III.58 The growth of the stain area for a 30 porcine blood stain is shown on the plain woven fabric (left) and knit fabric (right).

To better see the change in the area with time, typical curves of the time derivative of the stain area are shown in Figures III.60 (SB5) and III.61 (porcine blood) on the plain woven and knit fabrics. The behavior of SB5 and porcine blood is similar on both fabric types.

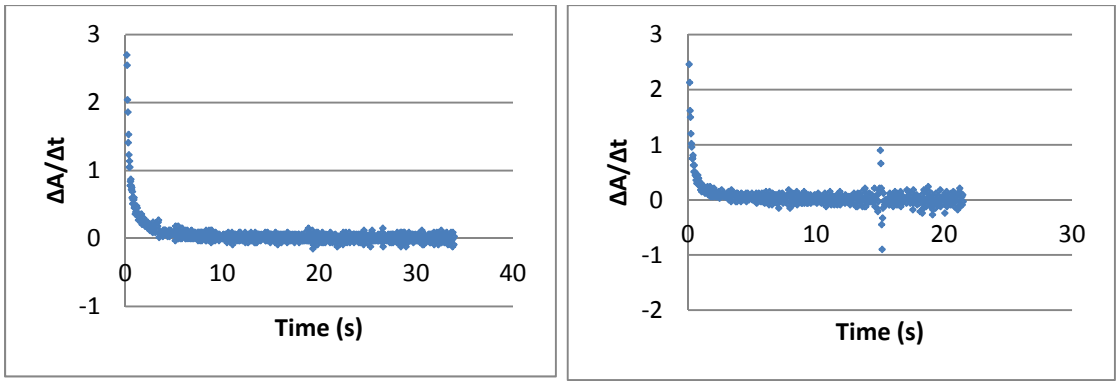


Figure III.60 The time derivative of the stain area for a 30 μL SB5 stain is shown on the plain woven fabric (left) and knit fabric (right).

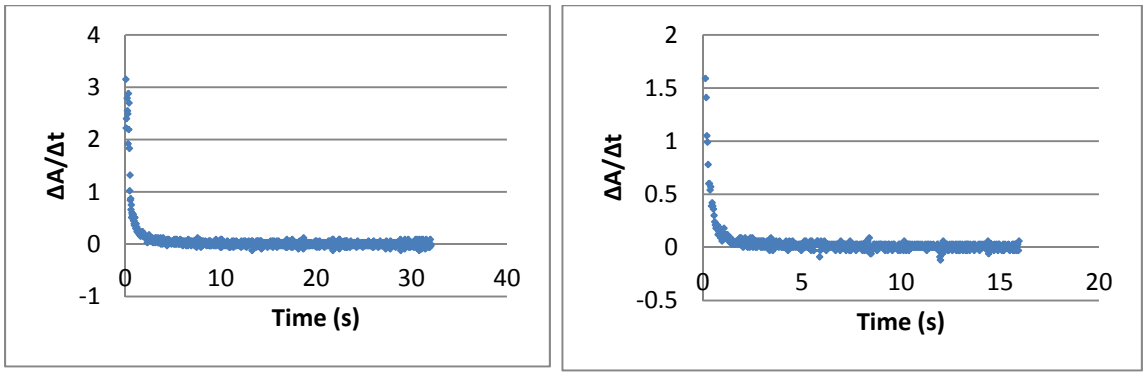


Figure III.61 The time derivative of the stain area for a 30 μL porcine blood stain is shown on the plain woven fabric (left) and knit fabric (right).

Next the perimeter of the stains was measured for 30 μL SB5 and porcine blood drops, as shown in Figures III.62 and III.63. For the plain woven fabric, the perimeter of the SB5 stain takes slightly longer to stabilize than the porcine blood. However, the differences are less than the variability from drop to drop. Similar behavior is observed for the knit fabric, although the time evolution of the perimeter is more uneven as the stain fills in as it grows.

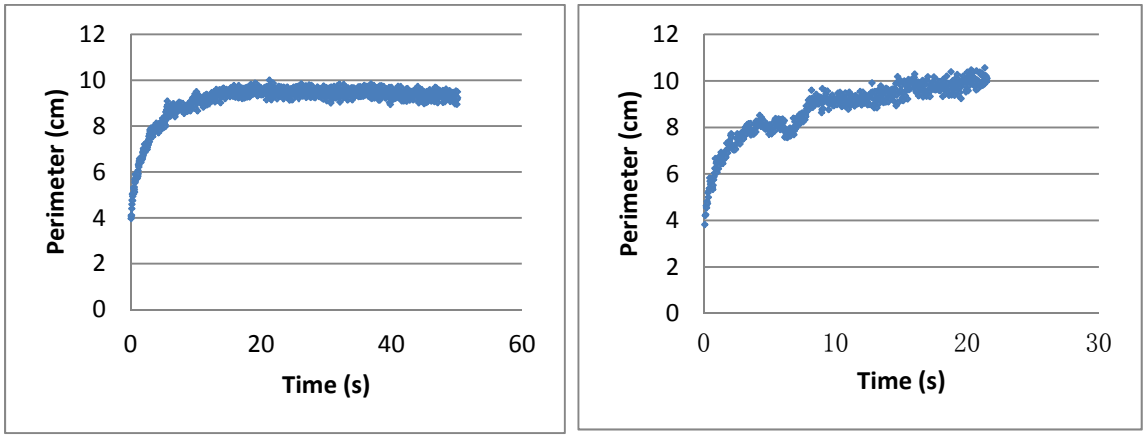


Figure III.62 The perimeter of the stain for 30 μL SB5 stains are shown on the plain woven fabric (left) and knit fabric (right).

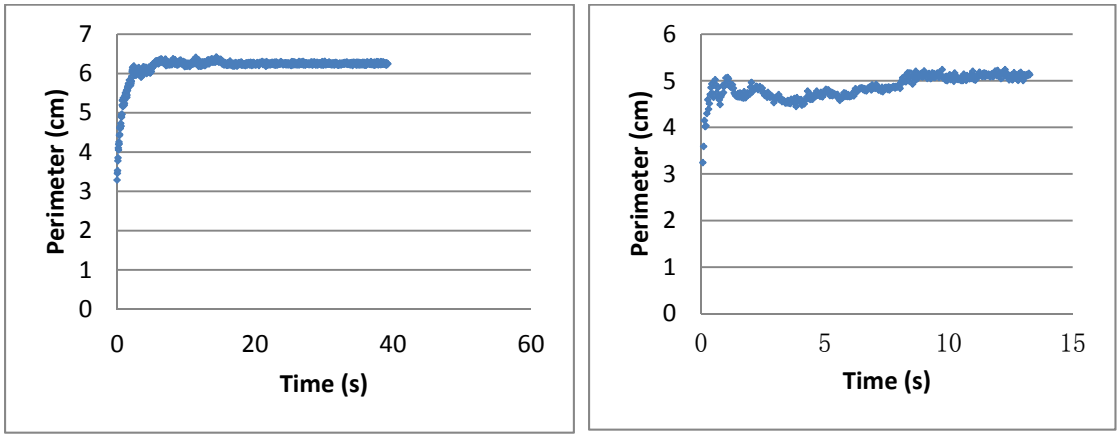


Figure III.63 The perimeter of the stain for 30 μL porcine blood stains are shown on the plain woven fabric (left) and knit fabric (right).

The circularity C of the stains, defined as $C = 4\pi \frac{A}{p^2}$, was measured for 30 μL SB5 and porcine blood drops, as shown in Figures III.64 and III.65. In these curves, the SB5 stains do not match the porcine stains as well as above. The circularity time evolution is more varied on both types of fabrics as the stain grows first in one area making the perimeter larger and the circularity smaller, the fills in gaps, thus reducing the perimeter and increasing the circularity. Due to the large variability in the time evolution of these curves, more work is needed to understand these behaviors.

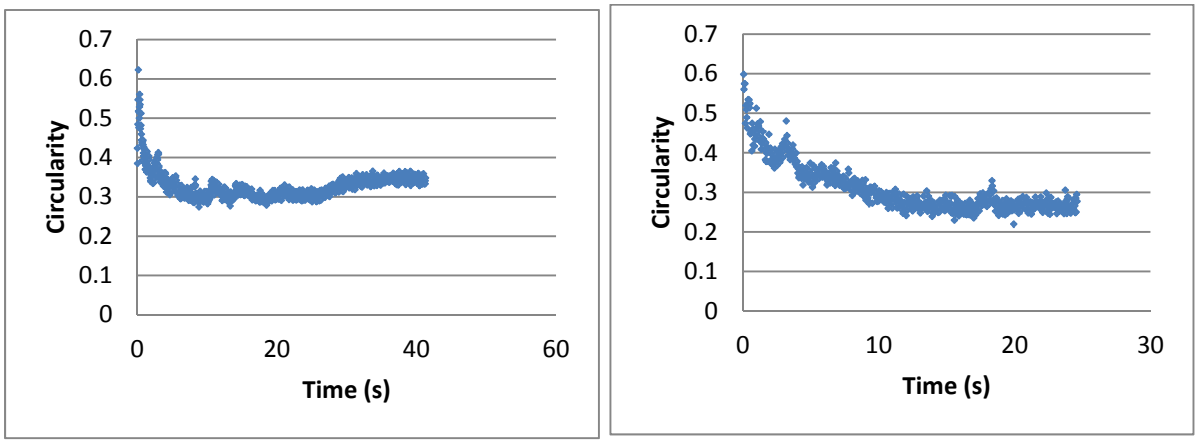


Figure III.64 The circularity of the stain for 30 μL SB5 stains are shown on the plain woven fabric (left) and knit fabric (right).

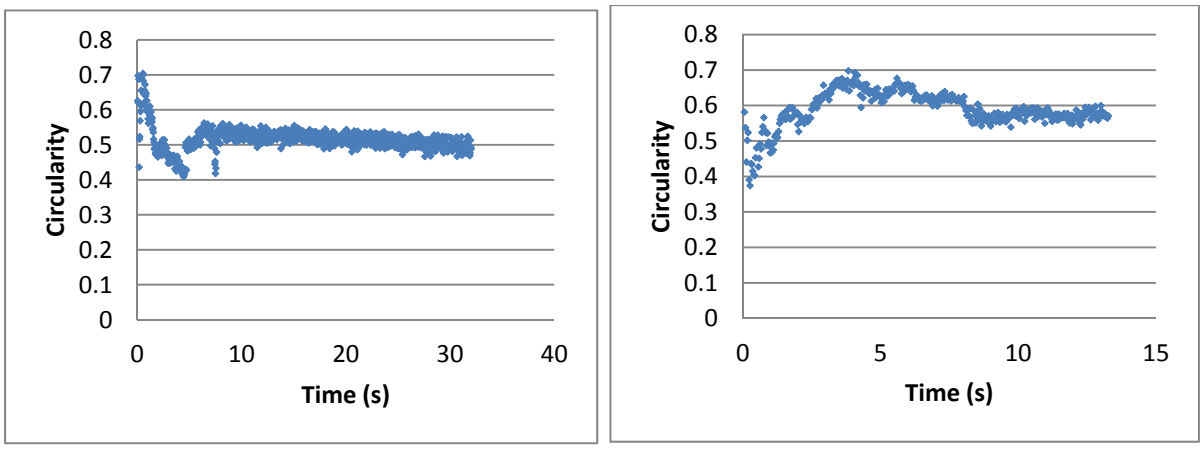


Figure III.65 The circularity of the stain for 30 μL porcine blood stains are shown on the plain woven fabric (left) and knit fabric (right).

Table III.17 gives the average values of the area, perimeter and circularity of SB5 and porcine blood drops on the plain woven and knit fabrics. In each case, the value for SB5 is much larger than for the porcine blood. It is interesting to note that the area of the porcine blood stain is 62-77% of the area of the SB5 stain. This is consistent with the amount of plasma in porcine blood (100% - hematocrit). It is unclear at this time whether the proportion of red blood cells is responsible for this difference and more work needs to be done.

These results show that synthetic blood SB5 behaves similarly to porcine blood, but there are important differences, especially in the size of the stains generated.

Table III.17 Summary stain area, perimeter and circularity of 30 µL SB5 and porcine blood stains.

Label	Repeat number	Area (cm^2)	mean area (cm^2)	s.d	Perim. (cm)	mean perimeter (cm)	s.d	circularity	mean circularity	s.d
synthetic blood 30 µL on woven fabric	1	2.90			11.57			0.27		
	2	3.01			13.81			0.20		
	3	2.94			9.65			0.40		
	4	2.75			10.80			0.30		
	5	2.65	2.85	0.14	11.53	11.47	1.52	0.25	0.28	0.07
porcine blood 30 µL on woven fabric	1	2.16			5.96			0.77		
	2	2.33			6.24			0.75		
	3	1.84			6.05			0.63		
	4	2.58			6.49			0.77		
	5	2.19	2.22	0.27	6.52	6.25	0.25	0.65	0.71	0.07
synthetic blood 30 µL on knit fabric	1	2.07			6.34			0.65		
	2	2.04			6.65			0.58		
	3	1.88			6.30			0.60		
	4	2.09			6.79			0.57		
	5	2.07	2.03	0.09	6.46	6.51	0.21	0.62	0.60	0.03
porcine blood 30 µL on knit fabric	1	1.21			5.26			0.55		
	2	1.28			5.88			0.47		
	3	1.35			5.99			0.47		
	4	1.21			4.72			0.68		
	5	1.25	1.26	0.06	4.79	5.33	0.59	0.69	0.57	0.11
synthetic blood 80 µL on woven fabric	1	10.46			23.61			0.24		
	2	10.53			25.02			0.21		
	3	10.37			26.58			0.18		
	4	10.05			22.80			0.24		
	5	10.34	10.35	0.18	24.94	24.59	1.45	0.21	0.22	0.02
synthetic blood 80 µL on knit fabric	1	7.14			19.03			0.25		
	2	6.09			14.93			0.34		
	3	6.25			17.07			0.27		
	4	6.22			15.15			0.34		
	5	6.75	6.49	0.44	17.98	16.83	1.78	0.26	0.29	0.05

III.27 Custom yarns and fabrics

In the research described in the previous sections, we found that both the fabric construction and the yarn construction affected the final stain. However, it was not possible to determine the relative contribution of each factor. To separate the effects of yarn and fabric, we obtained cotton

yarns made from the same bale of cotton but by three different yarn spinning techniques: ring spinning, open-end spinning and Murata vortex spinning. Each yarn had the same nominal size, Ne 36/1, and the same nominal twist multiplier, NM = 3.8.

These yarns were then converted into three types of fabric: a 100x100 plain woven, a 130x70 plain woven and a jersey knit with the same nominal basis weight (g/m^2). The fabrics were then washed, bleached, dried, and ironed under identical conditions. In ongoing research, these fabrics will be tested with drip stains and by direct application of drops of SB5 synthetic blood and with porcine blood. The resulting stains will be compared and analyzed for their area, perimeter, and circularity.

IV. Conclusions

At a crime scene where a bloodletting event has occurred, there are frequently many types of textiles that may have been stained. Although there are many types of fabrics, two of the most common are jersey knits used to make T-shirts and plain woven fabrics, such as bed sheets or table clothes. Both T-shirts and bed sheets usually are made of cotton. Therefore, we restricted our study to two types of fabric, a plain woven 200 thread count percale bed sheet fabric and a jersey knit T-shirt material, both made of 100% cotton. We studied the development of drip stains as a function of drop volume, drop height, impact angle, fabric orientation (warp angle), and the backing material (none, hard surface, soft surface). We also studied the effect of different yarn construction parameters and transfer of blood from one fabric to another. Finally, we examined the impact of blood onto these fabrics. We used both synthetic blood patterned after ASTM F1819-07.

Dynamic drop breakup. We found that there was a clear separation in the time at which different processes occurred. First, the impact of blood onto the fabric surface, inertial spreading, subsequent drop breakup, and retraction due to surface tension occurred in less than 100 ms. Using high-speed video, the breakup dynamics of blood drops were observed. Compared to white cardboard, drops impacting a woven fabric mounted on the cardboard led to more spatter than the cardboard alone. Drops impacting onto a knit fabric, also mounted on cardboard led to even more spatter of very fine drops. It appears that the fabric roughness, which occurs on a scale of about $1/10^{th}$ of the drop diameter, greatly alters the expanding drop ring leading to enhanced breakup. However, other studies showed that if the backing material was soft or if the fabric was suspended loosely, spatter was greatly reduced, or even absent. This was attributed to the fabric being able to absorb a large portion of the impact energy such that little energy remained to cause drop breakup.

Wetting and wicking. After the initial 100 ms, wetting of the surface occurs over the next few seconds followed by wicking into the fabric, which became the dominant process. Since fabric construction leads to many different arrangements of yarns and different interstial spaces, wetting and wicking can lead to different patterns than on a non-absorbing surface. At the same time that wicking is taking place, water is evaporating from the blood, thus increasing its viscosity and reducing the amount of liquid that can be wicked. The complex interplay of these properties was evaluated. On the unbalanced woven bed sheeting, the synthetic bloodstains were elongated along the warp direction and had jagged edges. This uneven wicking pattern was shown to alter the apparent direction of the impact of synthetic blood onto the fabric. In addition,

the extended wicking would often mask spines and secondary spatter that landed close to the parent drop. Even when the initial synthetic bloodstain pattern had characteristics similar to a similar impact on a hard surface, once wicking had occurred, much of this detail could no longer be seen. On the other hand, the knit fabric we studied wicked synthetic blood in very quickly, but only extended the stain a small amount compared to the woven fabric. The synthetic bloodstain pattern was also more symmetric. Although there was some masking of the secondary spatter, it could be observed more easily.

We also studied the transfer of synthetic blood from one fabric to another when the two fabrics formed a sandwich structure. We found that, regardless of which fabric the blood was placed on, the larger stain occurred on the fabric with the fastest wicking. Thus, placing a synthetic blood drop on the woven fabric lying on a knit fabric, the stain on the woven fabric was quite small, while the stain on the knit fabric was more than twice as large. This means that the size of the stain cannot be used to determine which fabric first received the (synthetic) blood.

Based on the results above, we also decided to evaluate the wicking rate within the yarn. Although all yarns were thought to have been made by the same ring spun process, the knit fabric consisted of yarn made using the Murata vortex spinning. Its blood wicking rate was about 10x faster than for the similar ring spun yarn. It is not known at this time whether the faster wicking of the knit fabric compared to the woven fabric is primarily due to the different fabric structure or the different yarn structure. In addition, in the woven fabric, wicking occurred faster in the warp direction than in the weft direction. The warp yarn wicked synthetic blood slightly faster than the weft yarn, but there were also almost twice the number of warp yarns than weft yarns. Both of these factors could be responsible for this anisotropy.

Finally, it was found that, under our laboratory conditions, the synthetic blood SB5 behaved similarly to porcine blood, but there were important differences, especially in the area of the stain. We also found that the synthetic blood dried within approximately 30 minutes in one laboratory, but it took several hours in a nearby laboratory. If the fabric was lying on a flat surface, drying was slower than if it was held off the surface. Clearly, this has important ramifications for a crime scene investigation and needs to be investigated further.

This study has demonstrated that extreme care and consideration must be used in analyzing blood on textiles. First, at the moment of impact, the bloodstain that occurs depends strongly on what the fabric is backed by. The bloodstain patterns on the fabric may appear to be from a much higher velocity event due to the fabric roughness or from a much lower velocity event if the fabric is backed by a soft surface, such as a pillow or even a person’s skin. Furthermore, since the analyst will not see the fabric until after substantial wicking has occurred, the pattern seen may hardly resemble the initial pattern. Substantial masking and alteration of the stain may occur. If the blood impacts the fabric perpendicularly, the pattern may appear to have originated at some other angle. If the blood impacts the fabric at an inclined angle, the pattern may appear to have originated from a different angle or be made so diffuse that it appears to have originated from multiple drops or from a smear. Thus the analyst must take great care to avoid misinterpreting the bloodstains and the mechanisms of the bloodletting event.

One surprise that occurred in this research was the extreme differences in wicking within the two types of yarns, which could be even more important than the fabric analysis. To alleviate this situation, fabrics made from identical yarns have been made by each of several spinning methods. Analysis of these fabrics is underway.

Further research on transfer on fabric is also needed. We observed that the wicking process on the knit was complete in just a few minutes, while on the woven fabric it was complete in 10 minutes, even though it took 30 minutes to dry. Once the (synthetic) blood has wicked into the fabric, it may no longer be able to transfer to another fabric unless great pressure is applied. This could mean that a transfer stain for small drops may only be possible in the first 5-10 minutes, i.e. the transfer stain recipient had to be at or very close to the scene when it occurred. Studies of the time interval between drop impact and transfer at different contact pressures are underway to determine how easily fabric to fabric transfer occurs, if at all.

V. References

1) R v Jenkins, Central Criminal Court, London 2005, Indiana v David Camm, Indiana Supreme Court 2009, California v. Phillip Spector, Los Angeles Supreme Court 2010.
2) SWGSTAIN, *Recommended Terminology*. Forensic Science Communications, 2009. **11**(2):
   Impact Definition: A **bloodstain pattern** resulting from an object striking liquid blood.
   Expiration Definition: A **bloodstain pattern** resulting from blood forced by airflow out of the nose, mouth, or a wound.
   Transfer Definition: A bloodstain resulting from contact between a blood-bearing surface and another surface.
   Drip Definition: A bloodstain that formed as the result of a falling drop that formed due to gravity.
3) The Dictionary of Fiber Science and Technology, (Hoechst Celanese, Charlotte, NC, c. 1990) defines wicking as the dispersion or spreading of liquid through capillary action.
4) http://www.cottoninc.com/corporate/Pressroom/PressReleases/2008/item4050.cfm downloaded April 15, 2012.
5) "Denim Fabric Manufacturing" Technical Bulletin ISP 1010, c. 2004, Cotton, Inc. Cary, NC.
6) Bevel, T. and R.M. Gardner, *Bloodstain Pattern Analysis: With an Introduction to Crime Scene Reconstruction 3rd Ed*. Third ed. 2008, Boca Raton: CRC Press. 440.
7) James, S.H., P.E. Kish, and T.P. Sutton, *Principles of Bloodstain Pattern Analysis: Theory and Practice*. 1st ed. 2005, Boca Raton, Fla: CRC Press. 532.
8) Erik Kissa, Wetting and Wicking, Textile Research Journal 66(10), 660-668 (1996).
9) Balthazard, V., et al. *Etude des giuttes de sang projecte*. in *22nd Congress of Forensic Medicine*. 1939. Paris, France.
10) White, B., *Bloodstain patterns on fabrics: the effect of drop volume, dropping height and impact angle.* Canadian Society of Forensic Science Journal, 1986. **19**(1): p. 3-36.
11) Slemko, J., Bloodstains on fabric, the effect of droplet velocity and fabric composition. *Int. Assoc. Blood Pattern Anal. Newsl*, 2003, 3-11.
12) Karger, B., S.P. Rand, and B. Brinkmann, *Experimental bloodstains on fabric from contact and from droplets.* International Journal of Legal Medicine, 1998. **111**: p. 17-21.

13) Raymond, T., *Crime Scene Reconstruction from Bloodstains.* Australian Journal of Forensic Sciences, 1997. **29**(2): p. 69-78.
14) van Stratton, M.J. and T.J. Griffin, *Examination of Bloodstained Clothing*, in *Rocky Mountain Association of Bloodstain Pattern Analysts*. 2002.
15) Holbrook, M., *Evaluation of blood deposition on fabric: Distinguishing spatter and transfer stains.* International Association of Bloodstain Pattern Analysis News, 2010. **26**(1): p. 3-12.
16) Pex, J.O. and C.H. Vaughan, *Observations of high velocity bloodspatter on adjacent objects.* Journal of Forensic Sciences, 1987. **32**(6): p. 1587-1594.
17) SWGSTAIN. *Current Research Needs for Bloodstain Pattern Analysis.* 2011; Available from: https://5914991469299414462-a-swgstain-org-s-sites.googlegroups.com/a/swgstain.org/public/documents/SWGSTAINResearchTopics.pdf?attachauth=ANoY7cqao7WJvOUqitpy0qQ61MEMj7rdnprZkkitQViXSE4PNHAVsyRVL6FAi2JFdaQZ6VrqM7oN0SvKfxs1nJVaz4NsywXGukGTXV6lAyVPm2PJDOo9xldk8yJOImoJP56pd9oai-RxLMLfQjKk60oLhja8qVgB7yEJT0tPaZucc3lfnVyh_zbsvOzQJaii-_nM-G7-l3B0Q3EH0L67bOX98WI1fJkmIQ%3D%3D&attredirects=0.
18) Y. Wang, S. Michielsen, and H.J. Lee, unpublished results.
19) Nyoni, A.B., and D. Brook, *Wicking mechanisms in yarn-the key to fabric wicking performance*, J. Textile Institute, 2005, **97**: p. 119-128.
20) Hatch, K. L., *Textile Science*, West Publishing, Minneapolis/St. Paul, MN, c. 1993.
21) Patnaik, A., R. S. Rengasamy, V. K. Kothari, and A. Ghosh, *Wetting and Wicking in Fibrous Materials*, Textile Progress, (2006), **39**(1): p. 1-105.
22) http://time.com/money/3108285/cheap-jeans-sale-yoga-pants-leggings/ (Aug. 13, 2014) accessed September 11, 2014.
23) AATCC *Standardization of Home Laundry Test Conditions, Rev. 2005*Stammers, Alfred H., et al. "Quantification of the effect of altering hematocrit and temperature on blood viscosity."
24) Stammers, A. H., Vang, S.N., Mejak, B.L. Rauch, E.D., *Quantification of the Effect of Altering Hematocrit and Temperature on Blood Viscosity*, Journal of Extracorporeal Technology, 2003, **35**: 143-151.
25) Blood Spatter Fake Blood Recipes, Forensic Investigations FSB06. http://www.clt.uwa.edu.au/__data/assets/pdf_file/0018/2301651/fsb06.pdf. Last accessed on 04/11/2014.
26) Standard Test Method for Resistance of Materials Used in Protective Clothing for Penetration of Synthetic blood Using a Mechanical Pressure technique, *ASTM International, Designation : F1819-07* (Reapproved 2013)
27) Raymond, M. A., E. R. Smith, and J. Liesegang. "The physical properties of blood–forensic considerations." *Science & Justice* 36.3 (1996): 153-160.
28) Harkins, H. N., & Harkins, W. D. (1929). The surface tension of blood serum, and the determination of the surface tension of biological fluids. *Journal of Clinical Investigation*, *7*(2), 263.
29) Attinger, D., Moore, C., Donaldson, A., Jafari, A., & Stone, H. A. (2013). Fluid dynamics topics in bloodstain pattern analysis: Comparative review and research opportunities. Forensic Science International, 231(1), 375-396.
30) ASTM D446-12 Standard Specifications and Operating Instructionf for Glass Capillary Kinematic Viscometers.

31) Adamson, Arthur W., and Alice Petry Gast. "Physical chemistry of surfaces." (1967): 400-408.
32) ASTM D1422-99. Standard Test method for Twist in single spun yarns by the untwist-retwist method. http://database.texnet.com.cn/db-stan/view--401.html . Last accessed on 04/07/2014.
33) Williams, E, Neumann, E, Taylor M C, *The Development of a Passive, Closed-System Pig Blood Collection Apparatus for Bloodstain Pattern Analysis Research and Crime Scene Reconstruction*. Journal Bloodstain Pattern Analysis (2012), 28(2): 11-18
34) AATCC Monograph M7, http://www.aatcc.org/testing/resources/docs/208-M7-StdPrac.pdf. Last Accessed on 04/05/2014.
35) Hulse-Smith, L., Mehdizadeh, N. Z., & Chandra, S. (2005). Deducing drop size and impact velocity from circular bloodstains. J Forensic Sci, 50(1), 54-63.
36) Pasandideh-Fard, M., et al., *Capillary Effects during Drop Impact on a Solid Surface.* Physics of Fluids, 1996. **8**(3): p. 650-659.

VI. Dissemination of Research Findings

Presentations:

"Bloodstain Pattern Analysis on Textiles: A Fundamental Analysis," by Stephen Michielsen, Institute for Environmental Science and Research, Christchurch, New Zealand, June 4, 2013.

"Bloodstain Pattern Analysis on Textiles: A Fundamental Analysis," by Stephen Michielsen, IABP, San Diego, CA, October 2, 2013.

"Bloodstain Pattern Analysis on Textiles: A Fundamental Analysis," by Feng Ji, Namrata Parekh, Michael Taylor, Stephen Michielsen, World Forensics Festival, Seoul, Republic of Korea, October 15, 2014.

VII. Appendix

Various Blood types used in the early experiments

As the project proceeds we have tried several blood recipes to mimic real blood, given below.

Blood Type 1 (SB1): [25]
Corn starch (44 g) and 80 mL of deionized water were added to a 500 mL beaker and stirred thoroughly. While stirring, 160 mL of corn syrup was added along with 8 drops of red food coloring and 4 drops of green food coloring.

Blood Type 2 (SB2): [25]
A poly(acrylic acid) aqueous solution was made by adding 1 g of poly(acrylic acid) to 100 mL of deionized water. The mixture was stirred overnight until a uniform solution was formed. Corn starch (44 g) and 80 mL of the poly(acrylic acid) aqueous solution were added to a 500 mL beaker and stirred thoroughly. While stirring, 160 mL of corn syrup was added along with 12 drops of red food coloring and 5 drops of green food coloring. Finally, 20 mL of hot deionized water was added to blend all ingredients well.

The stability of Blood Type 2 was greater than Blood Type 1 as polyacrylic acid has bio-adhesive properties like blood and can easily absorb any salts present in the system giving a thicker recipe than the one obtained from blood type 1.

Blood Type 3 (SB3): [26]
Distilled water (550 mL) was boiled and allowed to cool to room temperature. Then a paste of 12.5 g of guar gum was made by stirring a small amount of the boiled water into the guar gum. This paste was stirred into the remaining the boiled water and mixed for one hour. Finally, 5 g or Red Dye 81 was stirred into the solution for an additional 1 ½ hrs until a homogeneous solution was formed.

This synthetic blood type was then standardized according to the ASTM method by adjusting the surface tension to 0.042 +- 0.002 N/m. This surface tension resembles the surface tension of blood and this recipe was used only after the corrected surface tension was achieved.

Blood Type 4 (SB4): [26]

Modified ASTM
A 1% dye solution was prepared by mixing 1 g of Direct Red 81 in 100 mL of deionized water. Acrysol 8306 was diluted 15x with distilled water and a magnetic stirring bar was placed in the mixture. This solution was stirred for one hour until all the entrapped air bubbles had escaped the solution and a translucent homogenous solution was obtained. Next, 20 mL of dye solution was added to the diluted translucent thickener solution. Finally, the surface tension and viscosity were measured as described below and the solution was adjusted to achieve the desired viscosity and surface tension by adding the dye solution or thickener solution. Human blood at 25°C has a kinematic viscosity of 3.5 ± 0.29 Centistokes (CST) [24] and at 37°C the viscosity range is 2.44 ± 0.09 CST [24]. From the temperature results above, we chose our target viscosity to be 3.5 ± 0.29 CST. Thus, the synthetic blood recipe was measured and adjusted to this range each time prior to use.

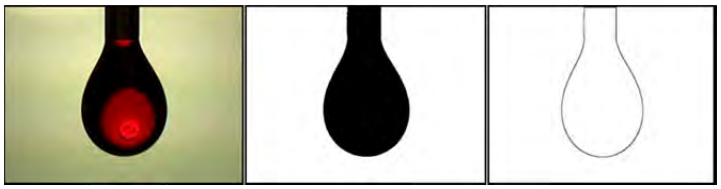
Likewise, the surface tension of human blood at 37°C is 42 mN/m and at 20°C it is 50 mN/m [27,28]. The surface tension of the synthetic blood was measured by the capillary rise method as described below and the solution was adjusted to achieve 50 mN/m as follows. If the capillary height was less than desired, then 1 ml of dye solution was added. This was done gradually until a height of 1.33 cm was attained. On the other hand, to lower the height of capillary rise, rheology modifier solution of Acrysol 8306 was added in small amounts. Viscosity measurements were made to check whether the solution was within the acceptable operating range. Usually, the solution was found to be stable for a period of 5-7 days if it was stored in an air-tight container.

Surface tension of blood and this recipe was used only after the corrected surface tension was achieved.

Blood Type 5 (SB5):
SB5 was similar to SB4, but modified to have a higher viscosity. In addition, the surface tension was measured using a pendant drop rather than capillary rise. Specifically, a 0.5% dye solution was prepared by mixing 0.1 g of Direct Red 81 in 20 mL of deionized water. Next, Acrysol 8306

was diluted 40x by putting 40 mL distilled water into 1.0 g thickening agent and stirred for one hour until a consistent and homogeneous solution was obtained. Then the dye solution was injected into the thickening solution to get 60 mL synthetic blood SB5.

Finally, the synthetic blood was tested to ensure proper density, viscosity and surface tension. The density was measured using a graduated cylinder and a balance and found to be 0.988 g/mL The viscosity and surface tension were measured using Ostwald method and pendant drop method (see Figure A.1), respectively. The surface tension was 68 mN/m and the viscosity was 6.5×10^{-3} Pa-s. These results are in acceptable agreement with the density (1.060 g/mL), viscosity (6.3×10^{-3} Pa-s) and surface tension (61 mN/m) of human blood at 20^{o}C. The experiments were performed at room temperature of around 20^{o}C and 50 % R.H.



Pendant blood drop binary image perimeter

Figure A.1. Surface tension test of the synthetic blood using pendant drop method and the image treatment process, where (a) shows the original drop photo, (b) is the corresponding binary image, and (c) is the borderline of the pendant drop.