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# The effect of the digital printing of fabric on the morphology of passive bloodstains

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ABSTRACT

Bloodstained fabrics found at crime scenes are likely to have had processing treatments, such as dyeing or printing, but the effect of the treatments on bloodstain morphology is not always considered. In order to study the effect of digital printing on bloodstain morphology, drip stains were created from five impact velocities (1.9–5.4 $ms^{-1}$) on three different mass per unit areas (88–226 g/m²) of 100% cotton calico which had been digitally printed using reactive dye. Across all three printed fabrics, the bloodstains appeared visually similar, and no correlation was found between the dry bloodstain area and the impact velocity. When comparing the bloodstains on the printed fabric to those which had been created previously on the same fabric in a dyed and not-coloured state, the dry bloodstains on the printed fabric were statistically significantly larger (e.g. for the calico with the lightest mass per unit area, mean dry bloodstain area was 126.6, 64.4 and 44.3 mm² for the printed, dyed and not-coloured fabrics respectively). Examination of the larger bloodstains on the printed calico with the micro computed tomography scanner and scanning electron microscope, suggested that the printing process increased the wettability of the fabric, so the blood could spread more easily on the surface. This allowed the blood to coat the yarns, and wick into them before wicking along the intra-yarn spaces. The results presented in this paper showed that care must be taken when examining bloodstains at crime scenes. Depending on the fabric and the processing of the fabric the size of the blood stains may not increase with impact velocity as wicking may result in a larger bloodstain from a lower velocity. The bloodstain on the penetrated face of the fabric may be larger than on the impacted face and the same fabrics with different processing will produce different blood stain sizes and shapes.


## 1. Introduction

An area of interest within bloodstain pattern analysis (BPA) is the interaction of blood and fabrics, as bloodstained clothing and household textiles are frequently encountered at crime scenes [1]. The interaction of blood and fabric was found to be more complex than that of blood on non-absorbent surfaces in the first comprehensive study of bloodstains on fabrics in 1986 [2]. Since then, with the reliability and scientific integrity of BPA under question [3], further research has been undertaken to improve the understanding of the interaction of blood and fabrics [4–10]. A number of variables have been found to affect the resultant bloodstains on fabrics. These include fibre content and fabric structure [4,5], the mounting method of the fabric [6], impact angle [5], mass per unit area and thickness [7] and yarn structure [7]. However, an area which has not been thoroughly investigated in BPA is the effect of finishing treatments on the interaction of blood and fabric. When blood impacts a fabric at a crime scene, it is unlikely the fabric in question will be loom-state. More likely, it will have undergone a number of processing stages including bleaching, scouring, dyeing and/or printing to produce the end-use fabric. Therefore, investigating the effects of processing is important to BPA.

Work which has been carried out within textile science can be used to understand what is occurring following the impact of a blood drop onto fabric. Some experimentation has been undertaken within textile science to assess the effect of treatment methods on yarn wicking. Differences in cotton knitted fabrics were seen to occur following bleaching, dyeing and softening [11]. Dyeing affected the rigidity, mass per unit area and geometric roughness of the fabrics as a result of the shrinkage and disruption of the fabric during

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dyeing. Cotton yarns with a yarn count of 30 tex and various twist levels (350 and 550 turns per metre) were subjected to bleaching, prewashing and plasma treatment to assess the effect of these treatments on the wicking of the yarns [12]. Both prewashing and plasma treatment improved the amount of wicking from none, owing to the hydrophobicity of the raw fibre, to 0.5 cm $s^{-1}$ after plasma treatment due to the increased wettability of the cotton fibre. This previous work [11,12] showed that processing treatments affected the structure and wettability of yarns and fabrics. This suggests that the bloodstains on fabrics which have been subjected to processing will differ depending on the treatments.

The effect of reactive dye on drip stains on three 100% cotton fabrics with different mass per unit areas (91, 171 and 243 g/m²) has been investigated [13]. The bloodstains on the dyed fabric were statistically significantly larger than the equivalent bloodstains on the not-coloured fabric, owing to the intra-yarn spaces becoming a more optimum size for wicking as a result of the dyeing process. Therefore, BPA research which has been undertaken on bleached and/or optically brightened textiles [6,8,14,15], while providing a basis for understanding the interaction of blood and fabric, cannot be directly applied to fabric which has been dyed [13]. Each of the various methods of colouring fabrics needs to be examined individually to assess the effect on the resultant bloodstains.

An area of increasing growth within the textiles market is that of digitally printed textiles. Although in 2016 digitally printed textiles accounted for only 2.8% of the textile market, this is predicted to grow by 12.3% every year until 2021, much higher than the industry average growth of 3% [16]. Digitally printed textiles can be used for anything from cushions, tea towels and table cloths to bespoke clothing fabrics [17]. Therefore, the likelihood of encountering a blood-soaked fabric at a crime scene which has been digitally printed will increase, and therefore an understanding of how this method of dyeing affects the interaction of blood and fabric is important.

The aim of the current work was to test the hypothesis that the digital inkjet printing of fabric altered the blood stain morphology of drip stains. To do this, drip stains were created on three different mass per unit areas of inkjet printed, 100% cotton calico fabric. The resulting blood stains were compared to previous drip stains generated on dyed [13] and not-coloured [7] fabrics. To compare the bloodstains on the printed, dyed and not-coloured fabric the bloodstains were analysed with the use of micro computed tomography (μCT) and scanning electron microscopy (SEM). This allowed a detailed examination of the internal structure of the bloodstains.

## 2. Materials and method

To study the effect of digital printing on bloodstain morphology, horse blood was dripped onto digitally printed 100% calico cotton and examined using a CT scanner and Scanning Electron Microscope.

### 2.1. Materials

The fabrics used in this research were three different mass per unit areas of calico, a 100% cotton plain woven fabric, (88 g/m² (light), 165 g/m² (medium) and 226 g/m² (heavy)). An SEM image of each printed fabric at 50x and 250x magnification is given in Fig. 1. These fabrics have been previously used to study bloodstains in both a not-coloured [7] and dyed [13] state.

All fabrics were inkjet printed using the drop on demand technique by Magic Textiles.[2] The inks used were Huntsman reactive dyes[3] designed for digital textile printing. The side of the fabric which was printed was the technical face. The printed fabrics were prepared as per Dicken et al. [7]. The mass per unit area, thickness and sett of the printed fabrics are given in Table 1, along with those of the dyed and not-coloured fabrics [7,13].

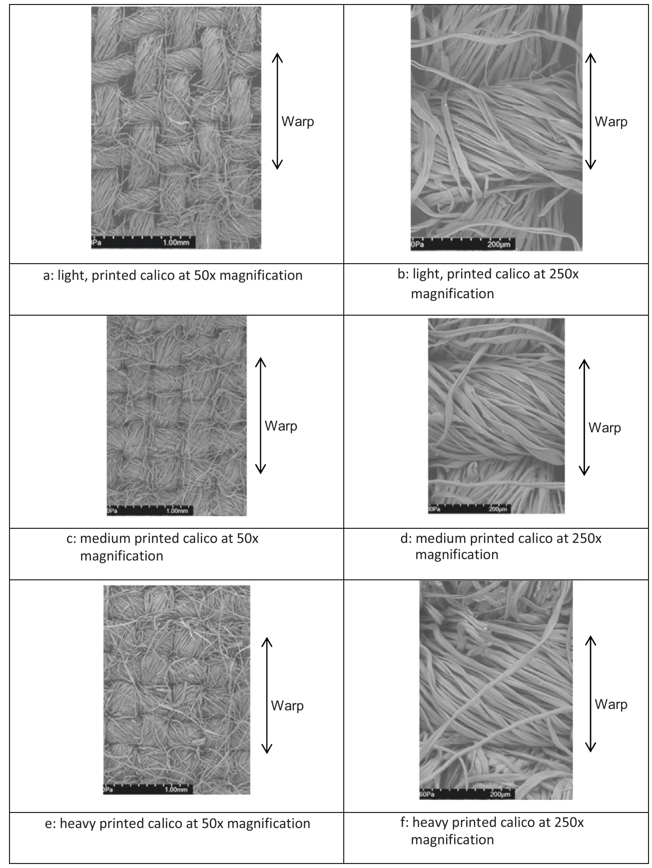


**Fig. 1.** example SEM images of the printed calicos at 50x and 250x magnification.

Defibrinated horse blood was used to create the bloodstains, sourced from Southern Group Laboratory.[4] The blood was stored below 4 °C until required and was used within one week of delivery to ensure use before the use by date.

### 2.2. Method

The horse blood was heated to 37 °C prior to use to simulate a blood-letting event at human body temperature. A Pasteur pipette was used to drop the blood onto the printed face (PF) of the fabric from five different heights (Table 2) to create drip stains. All blood drops were filmed using a Phantom V12 high-speed video (1280 ×800 resolution, 6273 fps, exposure 70 μs). The high-speed videos were analysed using Phantom Camera Control software[5] to measure droplet diameter and impact velocity (Table 2). The mean droplet diameter was 3.5 ± 0.2 mm. Five repeats were undertaken at drop heights of 200 – 1500 mm, and three at 2500 mm, resulting in 69 specimens.

Drip stains were also created by dropping blood from three heights (Table 2) onto the not-printed face (NPF) of the fabric. The mean droplet diameter was 3.6 ± 0.1 mm. Five repeats were

[2] Unit 1 A, Churnet Works, James Brindley Rd, Leek, Staffordshire, ST13 8YH https://www.magictextiles.co.uk/ (accessed 11th April 2022).

[3] http://www.huntsman.com (accessed 11th April 2022)

[4] E-H Cavendish Courtyard, Sallow Road, Weldon Industrial Estate, Corby, Northants. www.sglab.co.uk (accessed 11th April 2022).

[5] https://www.phantomhighspeed.com/resourcesandsupport/phantomresources/pccsoftware (accessed 11th April 2022).

**Table 1**
mean fabric properties and standard deviation.

| | | Thickness (mm) | Mass per unit area (g/m²) | Sett (yarns per 10 mm) |
|---|---|---|---|---|
| Light calico | Printed | 0.35 ± 0.02 | 88 ± 2.3 | 27 × 23 |
| | Dyed* | 0.39 ± 0.02 | 91 ± 1.84 | 27 × 23 |
| | Plain+ | 0.38 ± 0.03 | 85 ± 1.54 | 27 × 23 |
| Medium calico | Printed | 0.45 ± 0.01 | 165 ± 2.6 | 25 × 26 |
| | Dyed* | 0.51 ± 0.01 | 171 ± 3.71 | 25 × 27 |
| | Plain+ | 0.46 ± 0.02 | 164 ± 2.26 | 25 × 26 |
| Heavy calico | Printed | 0.53 ± 0.005 | 226 ± 3.1 | 26 × 26 |
| | Dyed* | 0.6 ± 0.04 | 243 ± 1.77 | 26 × 26 |
| | Plain+ | 0.56 ± 0.03 | 225 ± 1.56 | 26 × 26 |

References: * [13] + [7]

**Table 2**
the mean and standard deviation impact velocity resulting from each drop height.

| Drop Height (mm) | Impact velocity and standard deviation ($ms^{-1}$) drops onto Printed Face | Impact velocity and standard deviation ($ms^{-1}$) drops onto Not – Printed Face |
|---|---|---|
| 200 | 1.9 ± 0.07 | 1.7 ± 0.03 |
| 500 | 3 ± 0.1 | |
| 1000 | 4.1 ± 0.1 | 4.1 ± 0.05 |
| 1500 | 5 ± 0.2 | 4.8 ± 0.1 |
| 2500 | 5.4 ± 0.1 | |

undertaken on each fabric at each of the three drop heights, resulting in 45 specimens.

The terms technical face and technical rear will not be used to describe the side of the bloodstain being examined, as the technical face is determined by the side of the fabric which was printed on. When the bloodstains were therefore created on the NPF of the fabric, the 'technical rear', the use of the terms technical face and technical rear could lead to some confusion as to which side of the bloodstain was being examined. Therefore, to describe bloodstains on the side of the fabric which the blood drop impacted, 'impact face' will be used. For bloodstains on the opposite side of the fabric, 'penetrated face' will be used.

Further experiments simultaneously filmed the technical face and the technical rear of the fabric for drop impacts on the printed face as per Dicken et al. [18] for a limited number of impact velocities. Three different heights were used; 200 mm, 1000 mm and 2000 mm resulting in impact velocities of 1.8 $ms^{-1}$, 4.3 $ms^{-1}$ and 5.7 $ms^{-1}$. The blood drop diameter was 3.7 ± 0.15 mm². Two repeats were undertaken on each fabric from each height, resulting in 18 specimens.

All subsequent analysis was undertaken as per Dicken et al. [7]. Briefly, the wet impact face and dry impact face and penetrated face bloodstains were photographed. The bloodstain areas were measured from the photographs with the use of the inbuilt tools in ImageJ.[6] All 69 specimens from the initial experiment were scanned in a Nikon XTH225 micro computed tomography (µCT) scanner, the parameters, which differed from Dicken et al. [7], can be seen in Table 3. Two specimens on each fabric from each of the five velocities were examined using a Hitachi SU3500 SEM with the EDAX TEAM microanalysis system[7] (15 kV, 60 Pa).

Analysis of variance (ANOVA) (IBM SPSS statistics version 22)[8] was used to assess whether drop impact velocity or fabric type had a statistically significant effect on the wet and dry impact face and dry penetrated face bloodstain area for both the PF and NPF, as well as dry impact face area among the PF, dyed and not-coloured fabrics. Tukey's HSD analysis identified which variables contributed to any significant effects. An independent samples t-test was used to assess whether there were any statistically significant differences between wet and dry impact face and dry penetrated face bloodstains between the PF and NPF. Repeated measures ANOVA was undertaken to assess whether there were statistically significant differences between the impact face and the penetrated face of the bloodstains. Equality of variances and normality of data were checked.

## 3. Results and discussion

The photographs of the wet and dry bloodstains, the high speed videos and the µCT and SEM images were examined to study the effects of fabric mass per unit area and impact velocity on the bloodstain area of the printed fabrics.

The results and discussion will be separated into four sections:

- The trends seen in the bloodstains from blood drops on the PF.
- Comparison between the bloodstains from blood drops on the PF and NPF.
- The effect of the printing on the fabric.
- Comparison to not-coloured [7] and dyed [13] calicos.

### *3.1. Bloodstains from blood drops on the Printed Face, PF*

When the blood initially impacted the printed face of calico, the amount of lateral spreading which occurred on the surface of the fabric increased with the impact velocity for all three fabrics (Fig. 2). The maximum lateral spread on the surface of medium and light calicos and was reached by between 1.6 and 2.6 ms after impact (Fig. 2). No blood remained pooled on the surface of the heavy calico for impact velocities ≥ 3 $ms^{-1}$ and all medium and light calicos (Fig. 3) as the blood was able to wet and swiftly spread over the surface of the fabric.

The wet stain area for the PF face generally showed a decrease in area with an increase in fabric mass per unit area (Fig. 4). The light fabric had a significantly larger stain area than the medium and heavy calicos (Table 4). The variation in stain area with velocity showed some statistically different stain areas as the velocity increased but not always (Table 4, Fig. 4).

The light and medium calicos have a lower thickness and yarn linear density compares to the heavy calico (Table 1) and in the light and medium calicos the blood could wick through to the penetrated face of the fabric across the entire bloodstain by 20 ms and 50 ms after impact respectively (Fig. 5d and e). For the heavy calicos the blood could not reach the penetrated face of the heavy calico within the 50 ms recorded of the high speed video (Fig. 5 f), however the blood did reach the penetrated face of the heavy calico before drying (Fig. 6c).

Very little, if any, pooled blood dried on the surface of the fabric. The dry impact face bloodstains (Fig. 6a-c) had even colouration across the majority of the bloodstain, with a very small area of darker blood surrounding the bloodstain. The particulates were spread evenly throughout the bloodstains CT scans (Fig. 7) with only a small area around the edge of the bloodstain where the blood appeared denser, indicative of the coffee ring effect [19] (Fig. 7, marked 'C').

In both the warp (marked 'A′) and weft (marked 'B′) directions the yarns were soaked in blood for all three fabrics (Fig. 7d-i). For the heavy calico this occurred to a greater extent on the impact face than the penetrated face, as the blood was denser towards the impact face. Blood-soaked yarns were present across the entire bloodstain for all three fabrics (Fig. 10a-c). Even in these areas of denser blood, where the coffee ring occurred, there were no inter-yarn spaces filled for any of the three fabrics as shown on the CT scans (Fig. 7a-c,

6 https://imagej.nih.gov/ij/ (Accessed 11th April 2022)
7 http://www.edax.com/products/eds/team-eds-system-for-the-sem (Accessed 5th September 2018)
8 https://www.ibm.com/uk-en/analytics/spss-statistics-software (Accessed 5th September 2022)

**Table 3**
µCT scanner parameters.

| Scanning values | | | | | | Reconstruction | |
|---|---|---|---|---|---|---|---|
| Target | Voltage (kV) | Current (µA) | Exposure (ms) | Projections | Frames per projection | Beam hardening | Noise reduction |
| Tungsten | 50 | 160 | 500 | 1080 | 2 | 1 | 1 |

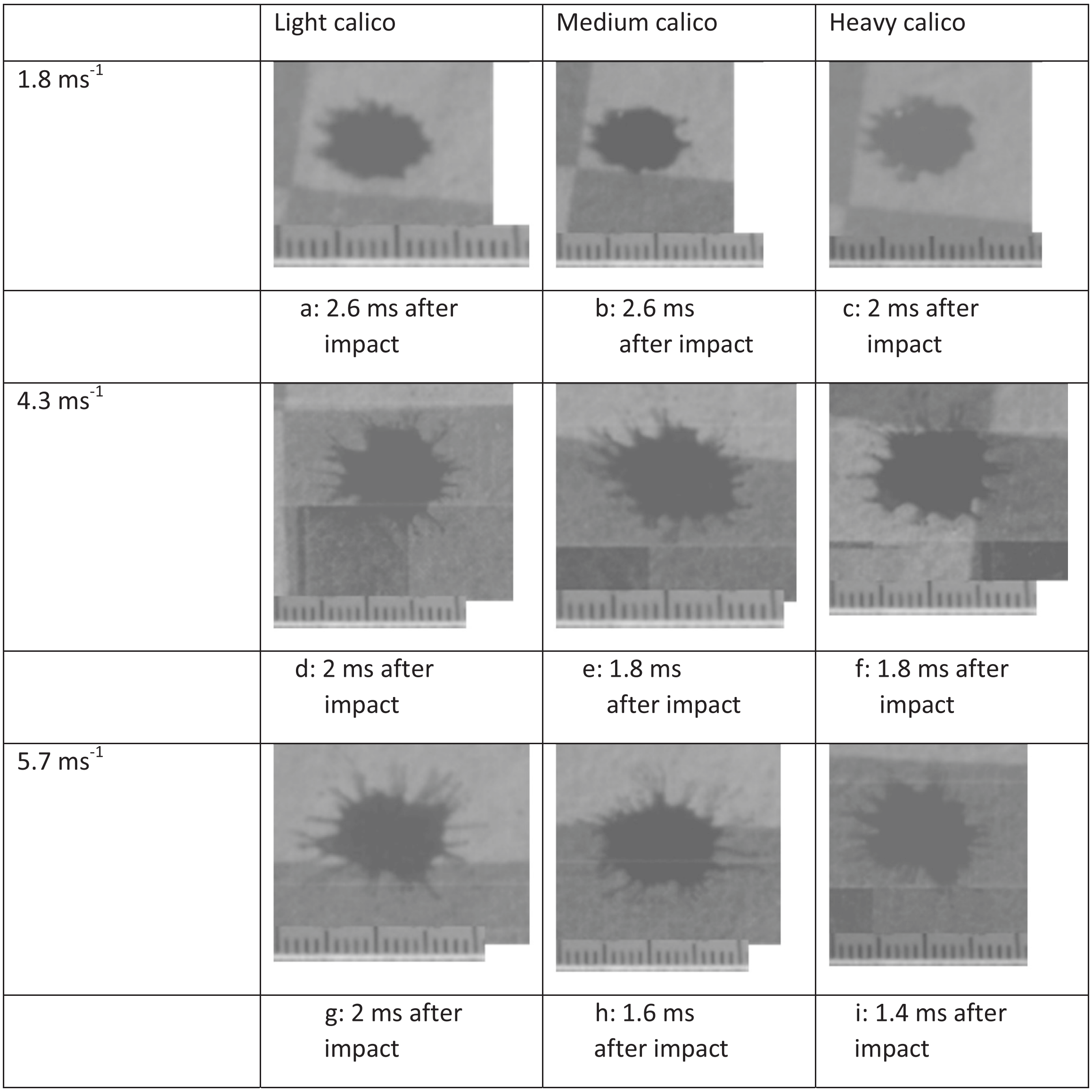


**Fig. 2.** high speed video stills of the maximum spread of the blood drop following impact onto each fabric from each velocity. Scale is 20 mm.

marked ‘C′). SEM images taken also showed no blood in the inter-yarn spaces.

Generally, the light calico had the largest and the heavy calico the smallest dry impact face bloodstains (Fig. 8). However, there was large variation and overlap in the data. For example there was a difference of 55.4 mm² between the largest (126.7 mm²) and smallest (71.3 mm²) dry bloodstain area for the medium calico specimens from an impact velocity of 3 $ms^{-1}$ (a coefficient of variation (CV) of 21%). The low yarn linear density of the light calico allowed the blood to more easily coat the yarns and wick into them. The low yarn linear density also meant there was less volume in each yarn for the blood to fill, and therefore the blood could wick further along the yarns. In contrast the greater yarn linear density of the heavy calico meant the blood could not penetrate the fabric across the entire bloodstain (Fig. 6c). The blood was able to penetrate to the centre of the yarns which lay on the impact face of the fabric (Fig. 7f and i). As the heavy calico yarns were a high yarn linear density there were a greater number of intra-yarn spaces for the blood to fill than in the lower density yarns of the medium and light calico. Therefore, to wick into the centre of the heavy calico yarns a large volume of blood would be required. Owing to the finite amount of blood on each specimen (the blood drop volume), the more blood which was required to completely penetrate the yarns, the less blood which was available for wicking along the intra-yarn spaces. This resulted in the heavy calico having the smallest mean dry bloodstain area at all but one velocity (Fig. 8).

Looking at the dependency of velocity on bloodstain size, the only impact face dry bloodstain areas which were statistically

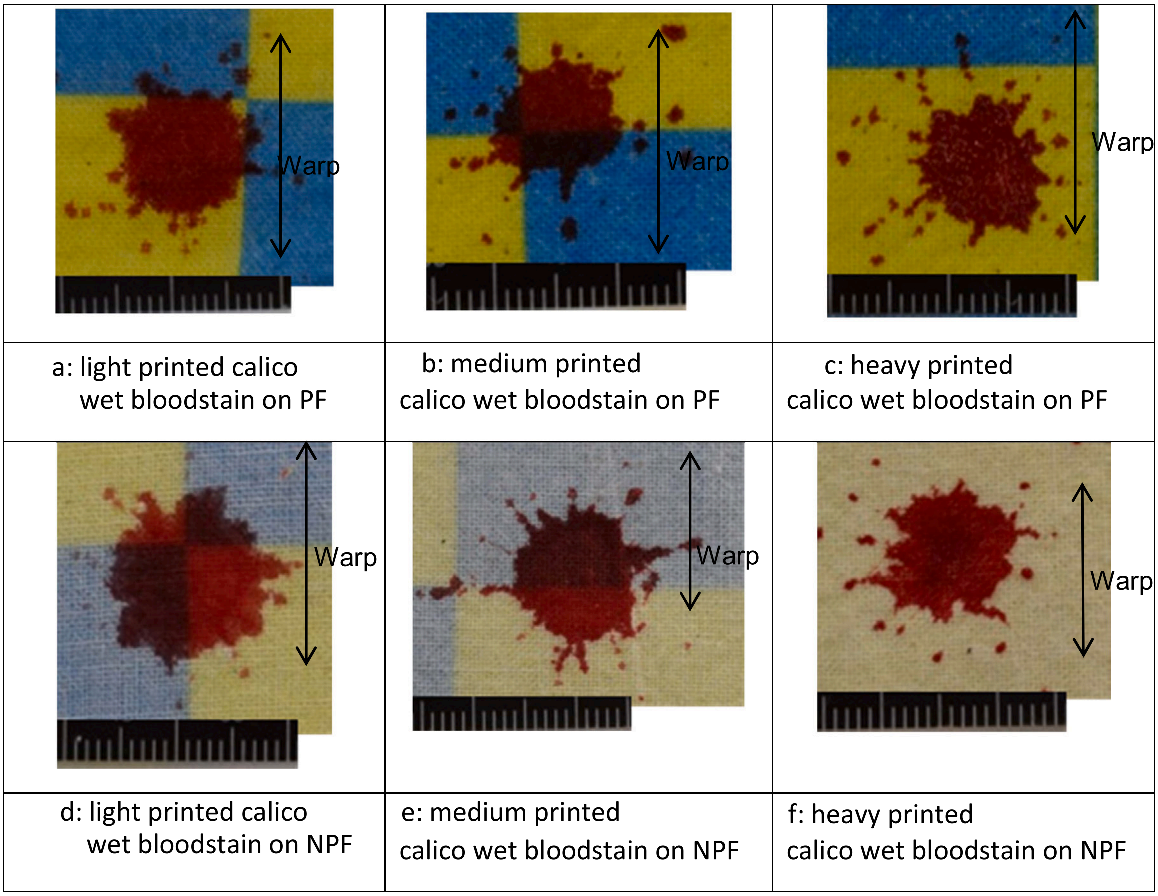


**Fig. 3.** an example wet impact face bloodstain on each of the three printed fabrics from drops onto both the PF and NPF from 4.1 $ms^{-1}$. Scale is 20 mm.

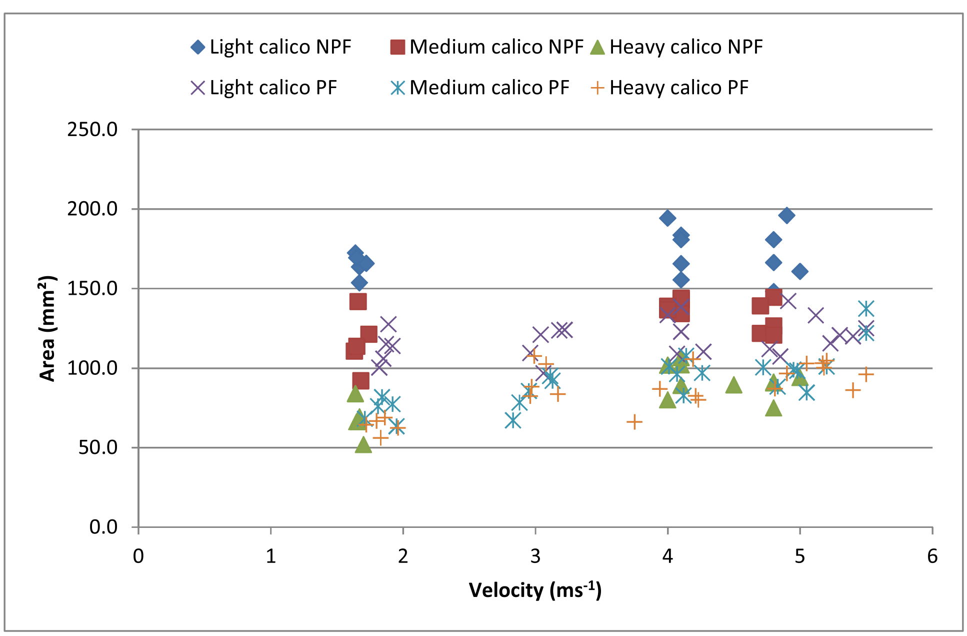


**Fig. 4.** the wet bloodstain areas from the blood drops on the PF and NPF of the light, medium and heavy calicos.

significantly different were the specimens from an impact velocity of 1.9 $ms^{-1}$, which were significantly smaller than all other velocities (Table 5). This is because the amount of spreading at impact was considerably less than for the other velocities (Fig. 2a-c). A least squares fit to the data gave no correlation between dry bloodstain area and velocity for any of the printed fabrics (Fig. 9). As discussed earlier at impact the amount of lateral spreading which occurred varied depending on the impact velocity (Fig. 2). However, the amount of wicking which occurred following this was not dependent on the impact. Instead how much wicking can occur within a fabric varies with any irregularities within the yarns and fabric [20]. This causes some of the large variation in bloodstain size within any

**Table 4**
results of ANOVA on the wet impact face bloodstain area.

| | | F statistic and significance | Tukey's HSD |
|---|---|---|---|
| PF | Among fabrics | $F_{14,54}$ = 53.627, $p \leq 0.01$ | Light larger than medium and heavy calicos. |
| | Among velocities | $F_{14,54}$ = 12.894, $p \leq 0.01$ | 1.9 $ms^{-1}$ smaller than all other velocities, 3 $ms^{-1}$ smaller than 5.4 $ms^{-1}$. |
| NPF | Among fabrics | $F_{8,36}$ = 181.944, $p \leq 0.01$ | Light larger than medium and heavy, medium larger than heavy calico. |
| | Among velocities | $F_{8,36}$ = 10.914, $p \leq 0.01$ | 1.7 $ms^{-1}$ smaller than 4.1 and 4.8 $ms^{-1}$. |

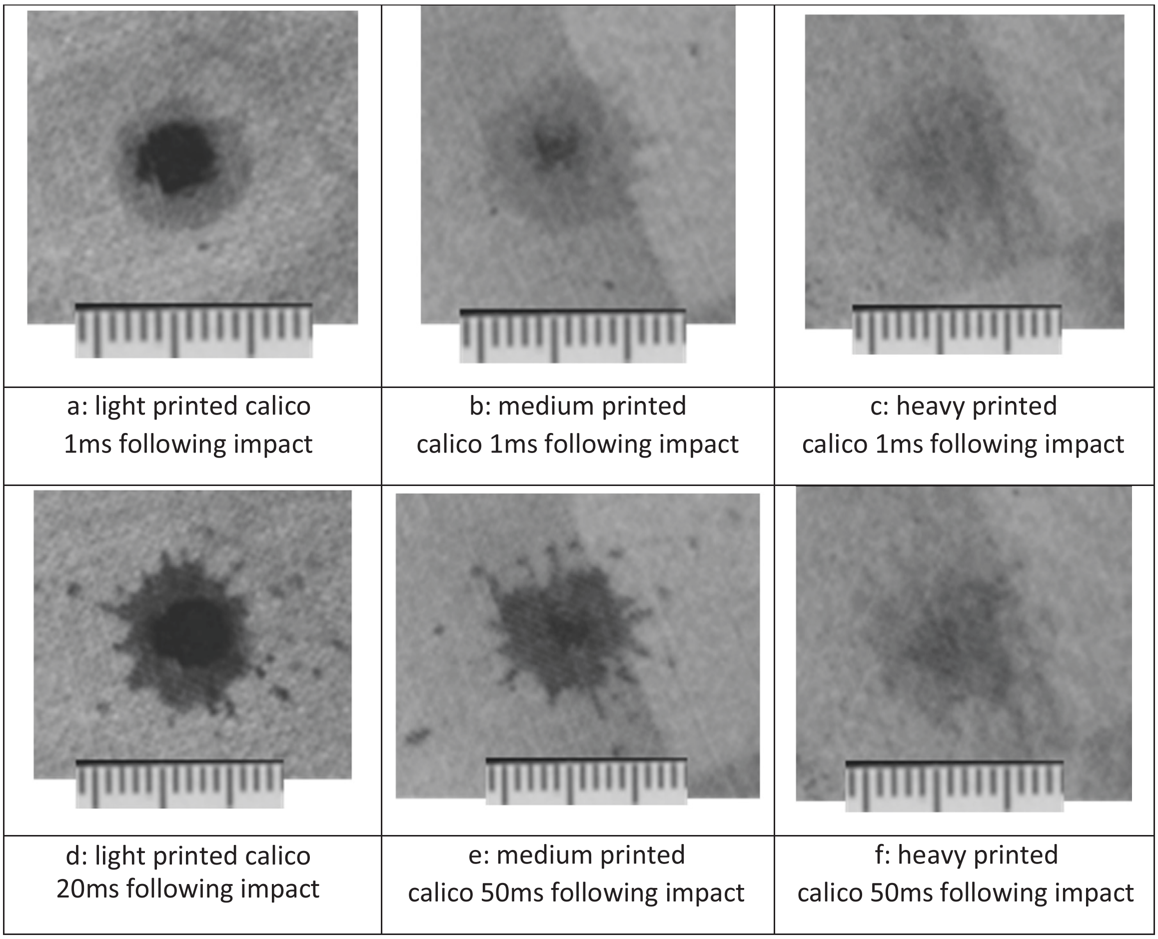


**Fig. 5.** stills from filming the penetrated face of the fabric at impact on each printed fabric PF following impact from 4.3 $ms^{-1}$. Scale is 15 mm.

given velocity, and the very small difference in dry bloodstain area among the velocities for all three fabrics (Fig. 8). As a result, in a crime scene situation, it could not be assumed that a larger bloodstain seen on a printed fabric impacted at a higher velocity than that of a smaller bloodstain.

### *3.2. Comparison between bloodstains from blood drops on the Printed Face, PF and Not Printed Face, NPF*

For three velocities (1.7, 4.1 and 4.8 $ms^{-1}$) blood was also dropped onto the NPF of each of the three calicos.

Visually, as with the PF (Fig. 3a-c) the wet bloodstains from the blood drops on the NPF (Fig. 3d-f) face had a similar consistent colour, and therefore density, of blood across the bloodstain. As for the PF, no blood remained pooled on the surface of the light or medium calico specimens. For the heavy printed calico, as for the PF, blood only remained pooled on the surface of the specimens from 1.9 $ms^{-1}$ impacts Fig. 10.

Unlike the PF face where the light fabric had a significantly larger stain area than the medium and heavy calicos (Table 4) for the NPF the light calico wet stain area was statistically larger than the medium, which was statistically larger than the heavy calcio (Table 4). The variation in stain area with velocity for NPF, as for PF, showed some statistically different stain area as the velocity increased, but not always (Table 4).

Comparing NPF and PF wet bloodstain areas (Fig. 4), drops on the NPF were statistically significantly larger than those on the PF for the light calico ($t_{28}$ = 10.36, $p \leq 0.01$) and the medium calico ($t_{28}$ = 7.81, $p \leq 0.01$). For the heavy calico, however, there was overlap between the wet bloodstain areas from the blood drops onto both the PF and NPF with no statistically significant difference. The heavy calico NPF bloodstains also overlap with those on the PF of the medium calico; these three groups produced the smallest wet bloodstains.

Like the dry impact face bloodstains (Fig. 6a-c) from drops on the PF, the dry impact face bloodstains on the NPF had even colouration across the majority of the bloodstain, with a very small area of darker blood surrounding the bloodstain (Fig. 6d-e).

Comparing PF and NPF bloodstains, (Fig. 8), the dry bloodstain areas were largest on the light calico from blood drops on the NPF. As for the wet bloodstain areas, the dry bloodstains from the blood

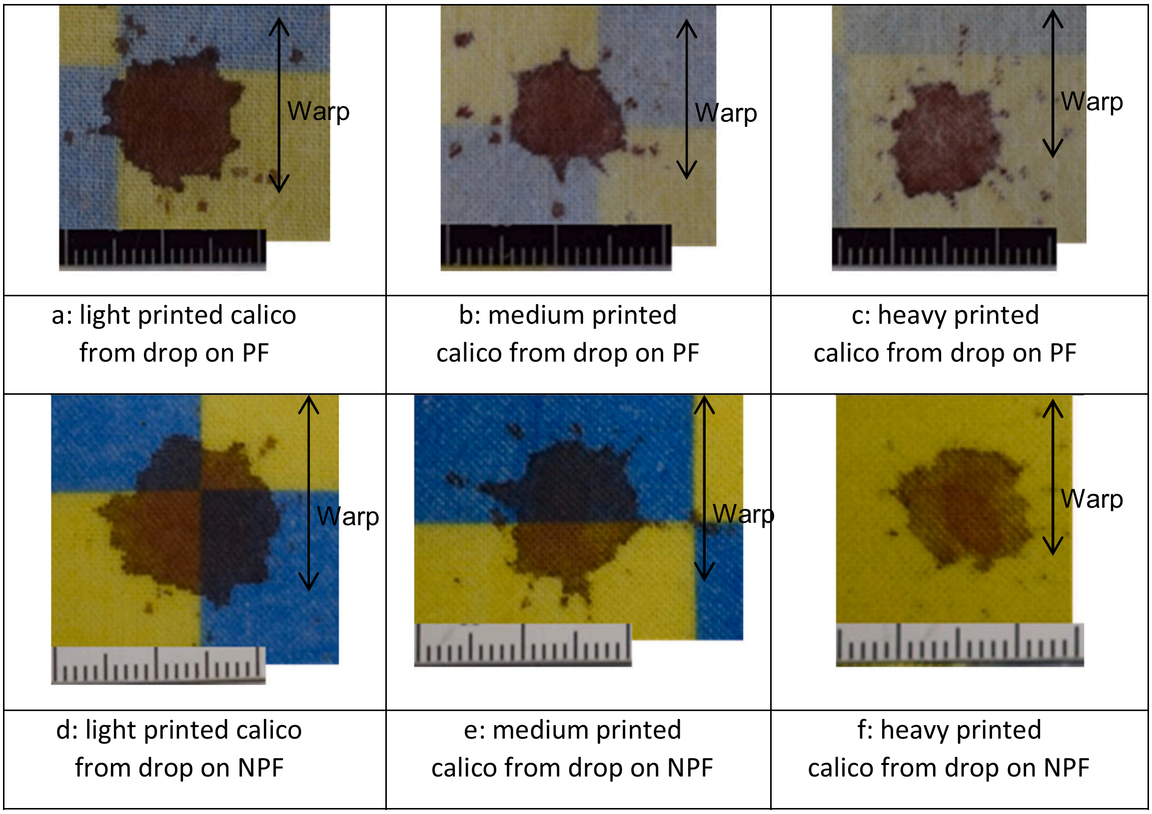


**Fig. 6.** an example dry penetrated face bloodstain on each of the three printed fabrics from drops onto both the PF and NPF from 4.1 $ms^{-1}$. Scale is 20 mm.

drops on the NPF of the medium and light calico were larger than their counterparts on the PF of the fabric. Again, there was overlap for the heavy calico between the dry bloodstain areas of the blood drops onto both the PF and NPF of the fabric. The dry bloodstain area from drops on the NPF was statistically significantly larger than those on the PF for the light calico ($t_{28}$ = 13.526, $p \leq 0.01$) and the medium calico ($t_{28}$ = 9.336, $p \leq 0.01$). There was no statistically significant difference for the heavy calico. As with the PF face, a least squares fit to the data gave no correlation between dry bloodstain area and velocity for the NPF (Fig. 8).

### 3.3. Dry penetrated face bloodstain

Looking at the results for the penetrated face for both the PF and NPF, visually, bloodstains on the penetrated face from the drops on the NPF (Fig. 9d-f) appeared patchier than those from the drops on the PF (Fig. 9a-c) with the amount of blood penetrating through the fabric decreasing from the light calico to the heavy calico (Fig. 9) as the blood has not penetrated through the fabric across the entire bloodstain for the medium and heavy calicos.

For the medium and heavy calcio, the impact face bloodstain was statistically significantly larger than the penetrated face bloodstain (medium (PF: $F_{4,18}$ = 29.077, $p \leq 0.01$; NPF: $F_{2,12}$ = 73.63, $p \leq 0.01$) and heavy (PF: $F_{4,18}$ = 130.645, $p \leq 0.01$, NPF: $F_{2,12}$ = 132.846, $p \leq 0.01$)).

On the light calico for blood drops impacting the PF both the penetrated face and impact face bloodstains had consistent colouring with a slightly denser area around the edge (Fig. 9a and 3a). For all but one specimen, the penetrated face bloodstain was larger than the impact face bloodstain. The penetrated face bloodstain was superimposed on top of the impact face bloodstain (Fig. 10) to show the similarity in shape and area between the two bloodstains. Repeated measures ANOVA from blood drops on the PF of the light calico showed the penetrated face bloodstain (135.9 $mm^2$) was statistically significantly larger than the impact face bloodstain (126.6 $mm^2$) ($F_{4,18}$ = 49.976, $p \leq 0.01$). Without any contextual clues at a crime scene, such as the way in which the fabric was lying, it would be extremely difficult to ascertain on this particular printed fabric on which side the blood drop impacted. This could, therefore, lead to misinterpretation of the evidence seen, for example whether an item of clothing was being worn at the time of bloodshed, or whether it was discarded inside out.

When the blood drops impacted the NPF, the penetrated face bloodstains on the light calico (Fig. 6d) were statistically smaller ($F_{2,12}$ = 41.414, $p \leq 0.01$) and patchier than the impact face bloodstains (Fig. 3d). This suggests less wicking was occurring as the blood moved through to the penetrated face. Together, these points imply the blood was able to wick more easily along the yarns on the NPF of the fabric than the PF.

The penetrated face dry bloodstain area from drops on the NPF were statistically significantly larger than those on the PF for the light calico ($t_{28}$ = 9.643, $p \leq 0.01$) and the medium calico ($t_{28}$ = 8.969, $p \leq 0.01$), (Fig. 8). The larger bloodstains suggests the blood was able to wick along the intra-yarn spaces with greater ease when the blood impacted the NPF than when it impacted the PF.

There were no statistically significant differences for the heavy calico between the bloodstains created from drops on the PF and NPF. The volume of blood which was required to create the penetrated face bloodstains on the heavy calico (Fig. 9c and f) was greater than for either the medium or light calico owing to the greater thickness (0.53 mm). Therefore, the volume of blood available for wicking in the heavy calico would be depleted owing to the volume required to reach the penetrated face of the fabric. The similarity in the dry bloodstain area for drops on both the PF and NPF of the heavy calico was due to there being not enough blood available for further lateral wicking to occur following blood drop impacts on the NPF.

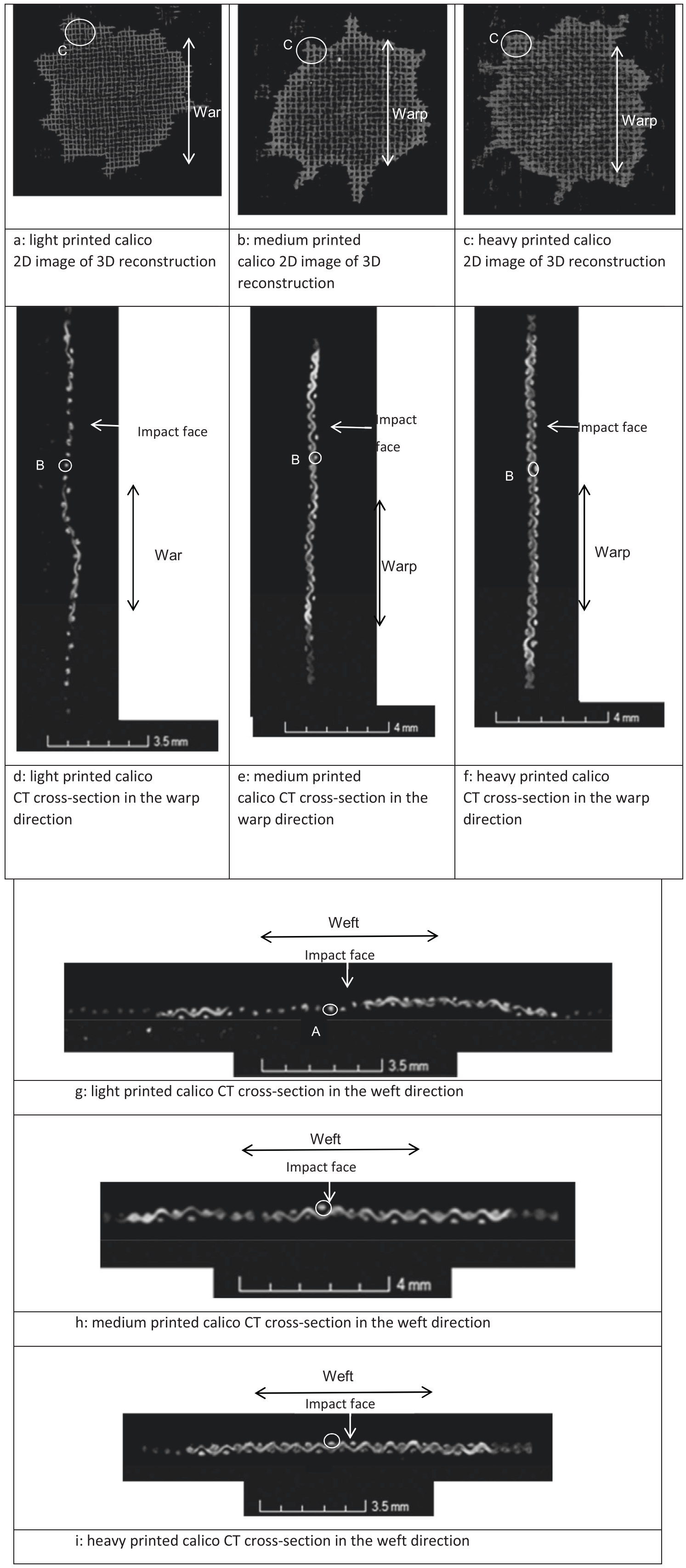


**Fig. 7.** A 2D image of the complete 3D reconstruction and cross-sections from a specimen from the printed calico from 4.1 $ms^{-1}$. 'A′ is a blood-soaked warp yarn, 'B′ a blood-soaked weft yarn. 'C′ is an example of the denser blood at the edge of the bloodstain.

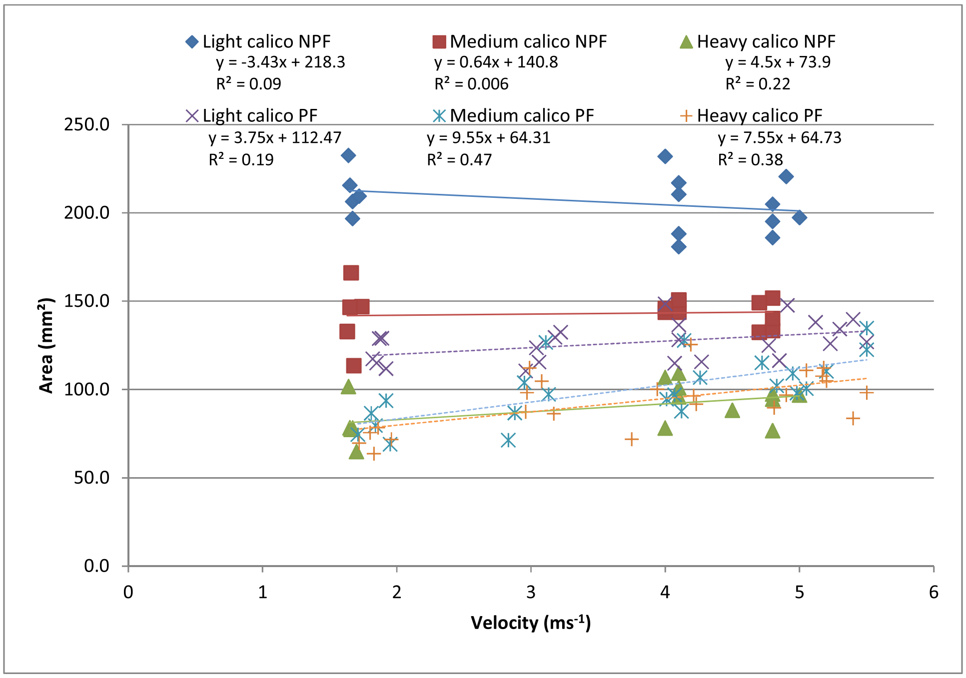


**Fig. 8.** the dry bloodstain areas from the blood drops on the PF and NPF of the light, medium and heavy calicos.

**Table 5**
results of ANOVA on the dry impact face bloodstain area.

| | | F statistic and significance | Tukey's HSD. |
|---|---|---|---|
| PF | Among fabrics | $F_{14,54} = 44.551$, $p \leq 0.01$ | Light larger than medium and heavy calicos. |
| | Among velocities | $F_{14,54} = 8.773$, $p \leq 0.01$ | 1.9 $ms^{-1}$ smaller than all other velocities. |
| NPF | Among fabrics | $F_{8,36} = 277.571$, $p \leq 0.01$ | Light larger than medium and heavy, medium larger than heavy calico. |
| | Among velocities | Not significant. | |

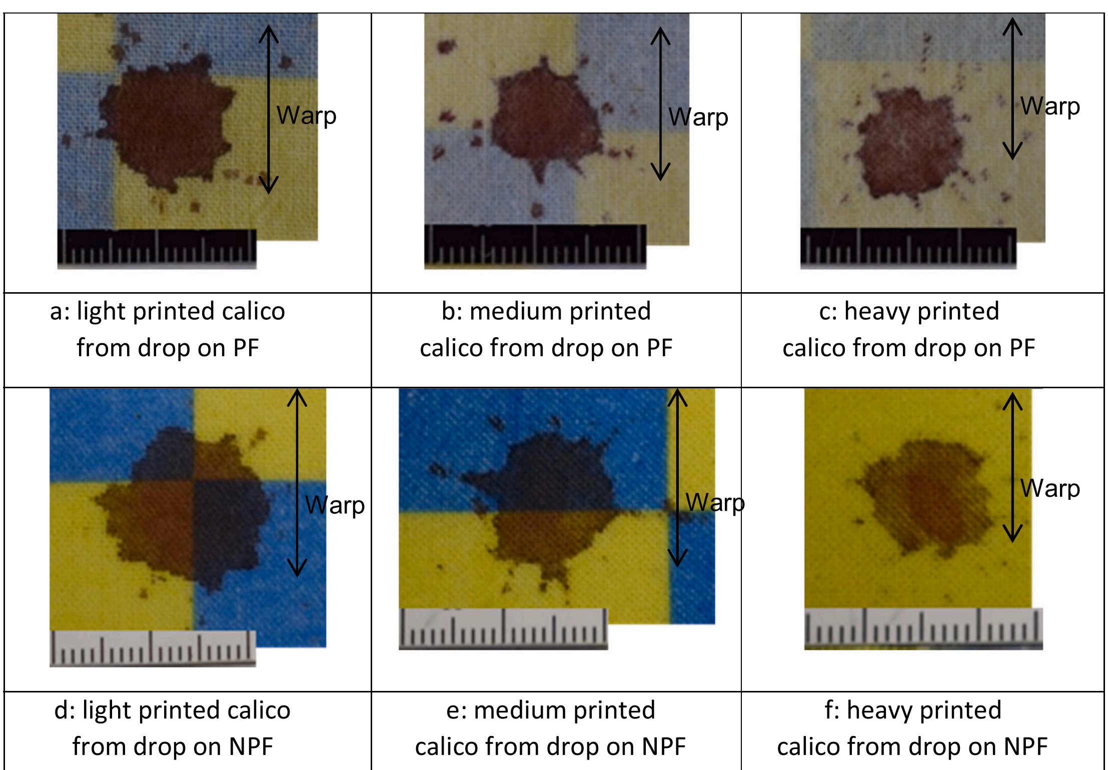


**Fig. 9.** an example dry penetrated face bloodstain on each of the three printed fabrics from drops onto both the PF and NPF from 4.1 $ms^{-1}$. Scale is 20 mm.

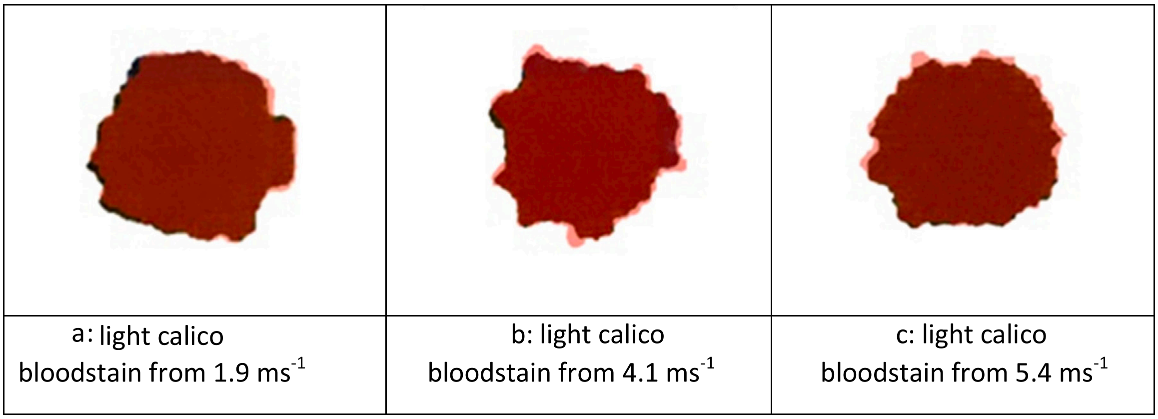


**Fig. 10.** a typical example of a light calico (PF) penetrated face bloodstain (translucent, red) superimposed on the equivalent impact face bloodstain (opaque, black) from 1.9 $ms^{-1}$, 4.1 $ms^{-1}$ and 5.4 $ms^{-1}$ showing the similarity in shape between the two bloodstains.

### 3.4. Effect of printing on the bloodstains

The blood drops on the NPF of the calico resulted in larger dry bloodstains than the PF (Fig. 8) suggesting a greater amount of wicking occurred in the former. Therefore, it is not the addition of the dye which increased the wetting and wicking properties of the fabric, but something else within the inkjet printing process. The inkjet dye itself slightly decreased the ability of the blood to wick along the intra-yarn spaces evidenced by the smaller impact face bloodstains on the PF than the NPF. This possibly occurred as a result of the dye filling the intra-yarn spaces slightly and therefore reducing them to smaller than optimum size for wicking.

Within a typical inkjet printing process, a number of stages occur: singe > desize > scour > bleach > mercerise > dry > print > steam > wash > roll [21]. A number of these stages (scouring, bleaching, mercerising) are undertaken in order to remove the hydrophobic wax layer from the cotton fibres, increasing the wettability of the fabric and therefore the dye uptake. Although these methods are undertaken in order to improve the dye uptake within the yarns, they will also have a longer term effect on the wettability of the fabric, and have increased the ease with which the blood drop spread on the surface of the fabric. Larger dry impact face bloodstains were seen from blood drops on the NPF of the fabric, where the processing altered the fabric, but dye was not added.

### 3.5. Comparison to not-coloured and dyed calico

The three mass per unit areas of calico investigated in the current work had previously been subjected to drip blood drops from the same drop heights when they were not-coloured [6] and following reactive dyeing [13].

The amount of lateral spreading at impact did not alter between the dyed and not-coloured calicos [13]. However, when comparing the heavy printed and heavy dyed calico from a drop impact of 1.8 $ms^{-1}$, the maximum area of spreading was greater, 62 $mm^2$, for the printed calico compares to only 43 $mm^2$ for the dyed calico (Fig. 11).

Differences were seen in the wet impact face bloodstains (Fig. 12a-c) amongst the not-coloured [7], dyed [13] and printed fabrics. For the medium and heavy not-coloured calico, blood was pooled on the surface of the fabric up to and including the specimens from 4.1 $ms^{-1}$ [7]. On the medium and heavy dyed calico, blood was only pooled on the surface of the fabric up to and including the specimens from 2.8 $ms^{-1}$ [13]. The only pooling of wet blood which occurred on the PF and NPF of the printed calico was the specimens on the heavy calico from 1.9 $ms^{-1}$.

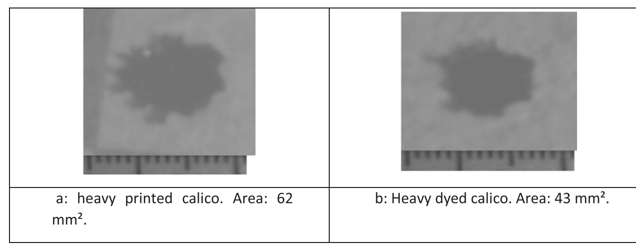


**Fig. 11.** High speed video stills showing the maximum area of spreading on the dyed (image taken for research done in [13] but not used in publication) and printed heavy calico from a drop impact at 1.8 $ms^{-1}$. Scale: 15 mm.

The blood which remained on the surface of the not-coloured calico dried in the manner of the coffee ring effect [7]. The large area of dense blood surrounding the central area of the bloodstain is indicative of the blood which remained on the surface of the fabric (Fig. 12d). Less pooling of blood was seen on the dyed than the not-coloured calico, with less evidence of the coffee ring effect on the surface of the fabric as a result [13] (Fig. 12e). The almost complete lack of pooling of blood on the surface of the printed calico resulted in the lack of a ring of dense blood surrounding the dried bloodstain (Fig. 12 f).

The dry impact face bloodstain areas for the blood drops onto both the PF and NPF of the printed fabric, dyed [13] and not-coloured [7] fabrics are shown in Fig. 13. Although there was overlap in bloodstain area between the dyed and not-coloured fabrics, there was very little overlap between these and the printed fabrics. The not-coloured medium and heavy calicos were the only sets of specimens where the dry bloodstain area was correlated with velocity [7]. The dry impact face bloodstains on the PF of the printed calico were statistically significantly larger than those on the not-coloured and the dyed calicos across the light ($F_{2,68}$ = 351.909, $p \le 0.01$), medium ($F_{2,68}$ =151.822, $p \le 0.01$) and heavy ($F_{2,68}$ = 93.025, $p \le 0.01$) fabrics.

The amount of blood which penetrated through the fabric differed among the different treatments (Fig. 9 printed calico, Fig. 14 not-coloured and dyed calico). Owing to the different yarn linear densities, the light calico consistently had the largest penetrated face bloodstain area and the heavy calico the smallest across all treatments (Table 6). For all three fabrics, the largest penetrated face bloodstains were seen from blood drops on the NPF of the printed calico, and the smallest on the not-coloured fabric.

The blood drops on the light, printed calico PF produced the only specimens where the mean penetrated face bloodstain was larger than the mean impact face bloodstain. On each of the not-coloured [7] and dyed [13] light calicos, there were only two specimens across

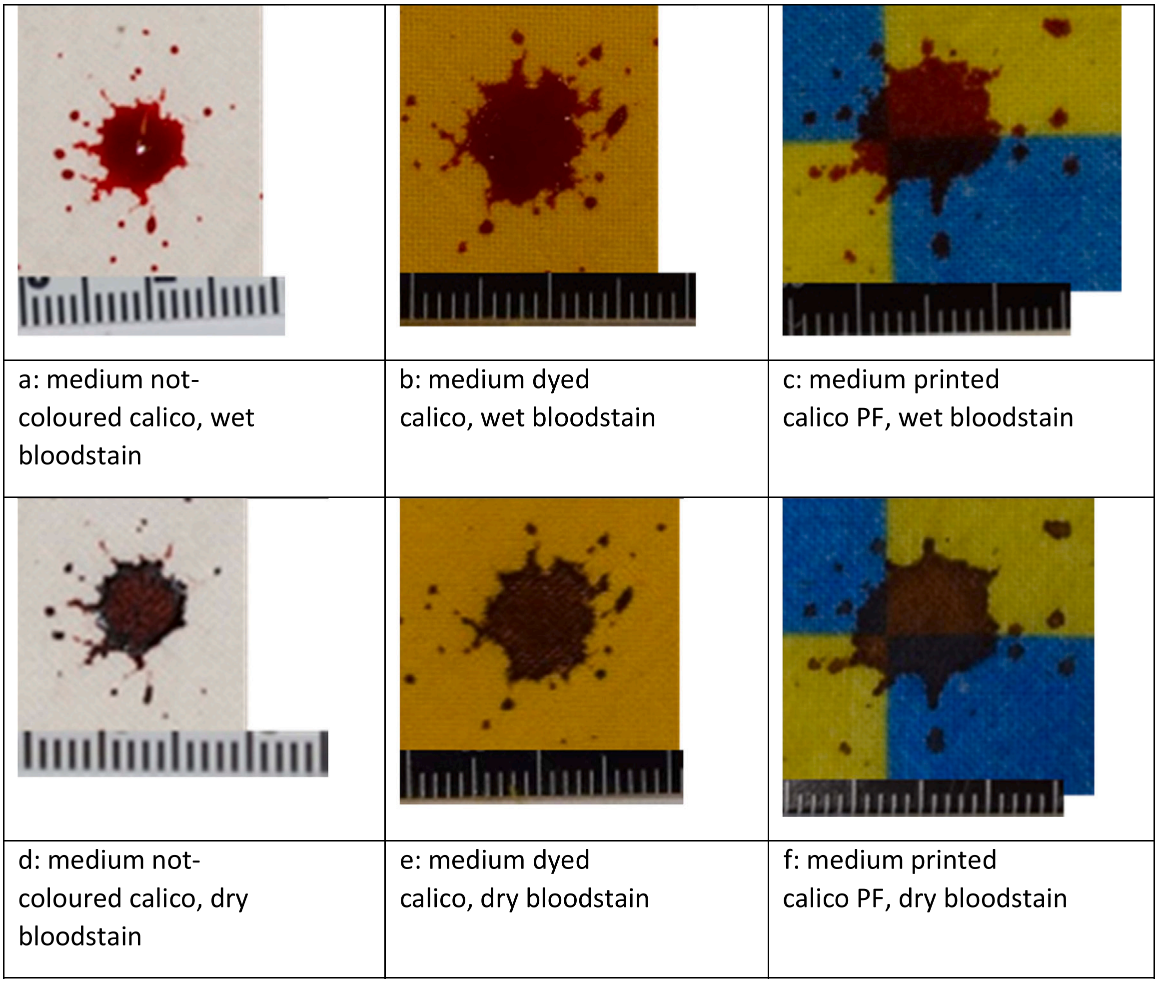


**Fig. 12.** a typical example of a wet and dry bloodstain on each of the medium not-coloured and dyed calicos from 4.1 ms to 1 [7,13]. Scale 20 mm.

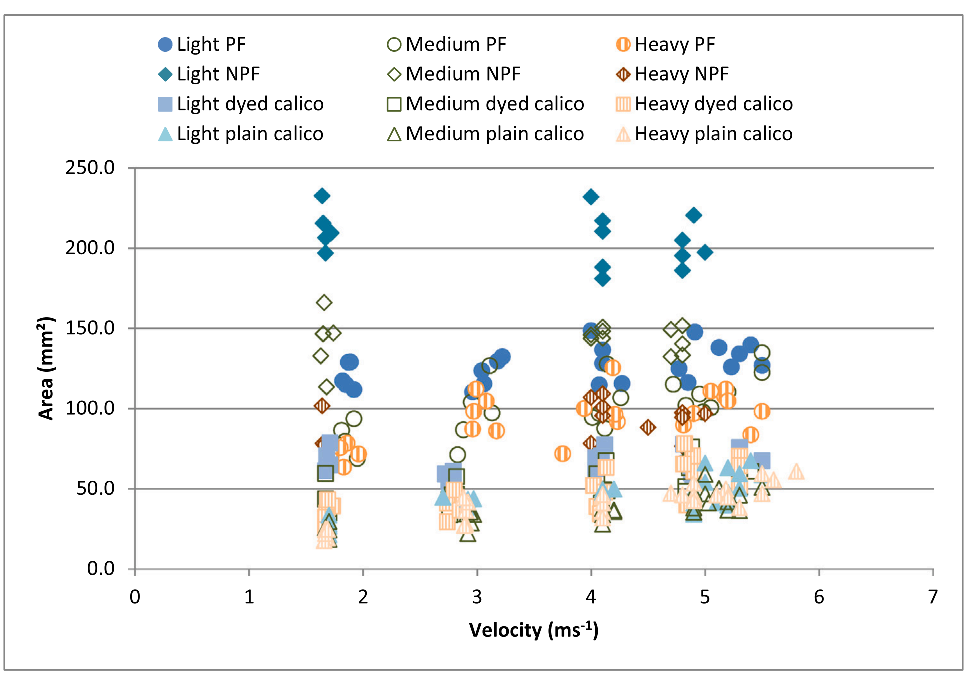


**Fig. 13.** the dry impact face bloodstain areas for all specimens from blood drops on the PF and NPF of the printed calico, dyed [13] and not-coloured [7] calicos.

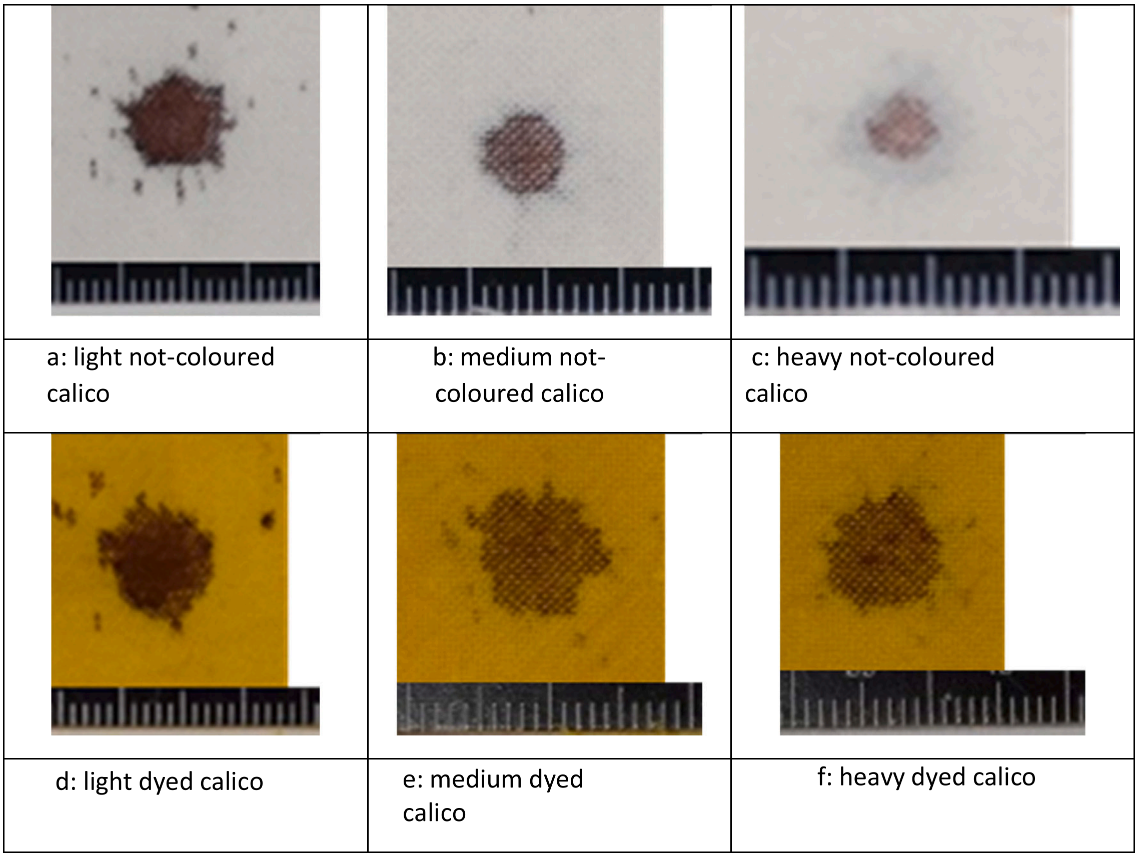


**Fig. 14.** a typical example of a penetrated face bloodstain from an impact velocity of 4.1 $ms^{-1}$ on each mass per unit area and treatment of fabric [7,13] (Fig. 6-15d not used in publication). Scale 20 mm.

**Table 6**
the mean penetrated face bloodstain area for each fabric state and each mass per unit area [7,13].

| Fabric state | Light | Medium | Heavy |
|---|---|---|---|
| PF | 136 mm² | 95 mm² | 72.5 mm² |
| NPF | 194 mm² | 134.7 mm² | 79.7 mm² |
| Dyed | 62.3 mm² | 40.9 mm² | 26.1 mm² |
| Not-coloured | 37.9 mm² | 15.2 mm² | 14.9 mm² |

all velocities where the penetrated face bloodstain on the light calico was larger than the impact face bloodstain. For blood drops on the NPF of the light, printed calico, only one specimen across all velocities had a larger penetrated face than impact face bloodstain.

The differences in the wet and dry impact face and dry, penetrated face bloodstains among the four groups of specimens were indicative of differences in the interaction of the blood and the fabric. The blood drops on the printed fabric initially spread on the surface of the fabric to a greater extent than on the dyed calico (Fig. 12). This was indicative of the blood being able to wet the surface of the fabric to a greater extent for the printed calico than for the dyed calico. This was a result of the procedures in the inkjet printing process (e.g. scouring, bleaching, mercerising) which removed the hydrophobic layer from the cotton yarns in order to increase the dye uptake.

The printed calico (PF and NPF) had larger wet and dry bloodstains than the dyed calico which had larger bloodstains than the not-coloured calico. This indicates that the blood was able to wick into and along the yarns to a greater extent for the printed calico. This led to no correlation with velocity being seen for either of the printed (PF and NPF) or dyed fabrics, as variations within the fabrics and yarns themselves would have had an effect on the amount of wicking which occurred [20]. This increased the variability of dry bloodstain area among specimens. The correlation between dry bloodstain area and velocity which was seen for the heavy and medium not-coloured calico was a result of the smaller amount of wicking which occurred for these fabrics. The primary mechanism which created the dry bloodstain area for the medium and heavy not-coloured fabrics was the increase in lateral spreading with increase in impact velocity [7].

## 4. Conclusions

Drip bloodstains were created on the printed face (PF) from five different impact velocities (1.9, 3, 4.1, 5 and 5.4 $ms^{-1}$) on three different mass per unit areas (light: 88 g/m², medium: 165 g/m² and heavy 226 g/m²) of 100% cotton plain woven calico which had been digitally printed with reactive dye. Three impact velocities (1.7, 4.1 and 4.8 $ms^{-1}$) were also used to create drip bloodstains on the not printed face (NPF) of the fabrics. The dry impact face bloodstains appeared visually similar for all velocities, mass per unit areas and the PF and NPF. The bloodstains had even colouration across the majority of the bloodstain, with a very small area of darker blood surrounding the bloodstain. The dry impact face bloodstains on the NPF of the medium and light calicos were statistically significantly larger than those on the PF. Owing to the lower yarn linear density the largest dry impact face bloodstains were seen on the light calico, and the smallest on the heavy calico with the highest yarn linear density.

The amount of wicking which occurred following impact was not dependent on the impact velocity, but the irregularities within the yarns and fabric. This caused a large variation in bloodstain size within any given velocity, and very small differences in dry bloodstain area among the velocities for all three fabrics, resulting in no correlation between impact velocity and bloodstain size.

For the light calico from blood drops on the PF, but not the NPF, the mean penetrated face bloodstain was larger than the mean impact face bloodstain. This may make it difficult to ascertain onto which side the blood drop impacted.

When comparing the dry bloodstains between the printed calico and those which had been previously produced on dyed [13] and not-coloured [7] fabrics, those on the printed fabrics were statistically significantly larger as a result of the increased wicking seen in the printed fabrics.

The results presented in this paper show that digital inkjet printing does alter the bloodstain morphology of drip stains and that care must be taken when examining bloodstains at crime scenes. Depending on the fabric, the processing of the fabric and the face impacted:

the same fabrics with different processing will produce different blood stain sizes and shapes.

the size and shape of the blood stains may or may not depend on the impact velocity as wicking may result in a larger bloodstain from a lower velocity.

the bloodstain on the penetrated face of the fabric may be larger than on the impacted face.

## CRediT authorship contribution statement

Dr Clare Knock: (Bloodstains and overall project) Supervision, Writing – review and editing, Conceptualization; Dr Lisa Dicken: Investigation, Methodology, Formal analysis, Writing – original draft; Dr Sophie Beckett: (Use of CT scanner) Analysis, Supervision, advice; Dr Debra Carr: (Fabrics) Supervision, Methodology, Writing – review and editing.

## Conflicts of interest

Declarations of interest: None.

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