LTSN Physical Sciences Development Project: Final Report

Development of a Synthetic Blood Substitute for use in Forensic Science Teaching

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Aim

To develop an inert and non-toxic blood substitute that accurately mimics the dynamics and flow characteristics of equine blood, for use in the teaching of blood pattern analysis and reconstruction.

Rationale

Scenes of violent crime often necessitate the analysis of blood patterns using string reconstruction, or more recently, computer aided software. It is a common feature of crime scene processing to reconstruct observed blood patterns in a sterile laboratory environment in order to discern information such as blood source movement and method of deposition. Accurate interpretation of bloodstain morphology, geometry and directionality will allow the calculation of an angle of impact and blood source position within a 3-dimensional space. Reconstructive experiments are often completed using animal-derived blood, or screened human blood. Animal-derived blood, such as equine blood, is selected primarily because it physically mimics human blood, and because its zoonoses load is low. The health and safety implications of such experiments are clear, but with the use of appropriate personal protective equipment, controlled experimental conditions and adherence to standard operating procedures, the aerosolisation of animal-derived blood can be controlled and the risks to the operator minimised. The risks, however, still remain and in reconstruction involving the expiration of liquid blood, the requirement of operator-donated blood is added.

With the growing number of university courses in forensic science, the teaching of blood pattern analysis and reconstruction of crime scenes using liquid blood becomes essential. Not only is blood pattern science included in curricula across the UK, it forms the basis for a large number of national and international training courses. With evidence of the advantages of experiential learning, the involvement of students in the reconstruction of blood patterns is clear. To circumvent the health issues raised with the use of body fluids, some institutions elect to teach crime scene science using physically unrealistic substitutes such as theatrical blood. However, the potential health risks could be negated with the development of a blood substitute that realistically mimics liquid blood for use in teaching situations. In the current educational climate where permission to use animal-derived products in research is progressively more difficult to obtain, a risk free substitute would be beneficial. Expand this to the presentation of blood pattern evidence to the court, and the development of an inert blood substitute could be indispensable.

Research Framework

The developmental work presented here was undertaken by two undergraduate students at London Metropolitan University as part of the research programme in Forensic Science and was presented in thesis form for assessment as part of the modular scheme that was core to their graduation. A suite of research in forensic science was presented to students, but this project was unique in that it was supported by LTSN funding, a budget which was partially devolved to participants through their development of the blood substitutes. It also provided a project which was novel in its scope and which nurtured a great deal of enthusiasm, motivation and dedication from those involved. This student centred approach to research at undergraduate level, unveiled a new approach to research within the department. This demonstrably allowed students to develop autonomy within the project and had a marked influence on their personal and professional development. Completion of this research represents a milestone for undergraduate research at London Metropolitan University and I believe that it should be encouraged in the formulation of undergraduate research programmes across academia, particularly those which expand their curricula to forensic science.

Market Research: *commercially available blood substitutes*

Theatrical Bloods

Theatrical bloods are widely available and stocks can be easily developed and maintained through the use of Internet formulations (e.g. http://www.firstaid.co.uk). From a forensic science perspective these are not scientifically accredited and probative trials performed for this study quickly determined that these products were not suitable for studies in blood dynamics. Formulations which were sourced through media suppliers had been developed to mimic specific physiological blood states e.g. clotted, pools, scabs and were therefore not suitable for use in the investigation of blood dynamics. Additionally, artificial blood composites which were commercially available were often issued with toxicity warnings and were prohibitively expensive.

Synthetic Medical Substitutes e.g. Oxycyte©

The properties of medically available blood substitutes such as Oxycyte© are intrinsic to their medical application i.e. oxygen carrying, intravenous emulsions, and are also prohibitively expensive; importantly for these purposes, they are unsuitable for use in the simulation and investigation of blood patterns.

A general review of the non-biological blood substitutes that are currently available has shown that they do not accurately reflect the dynamics of human blood and that, in general, they are visually unrealistic. Although the aesthetic properties are not the primary focus of substitute development, the development of a composite, which is visually realistic, would be beneficial. It is clear that, at present, there are no suitable synthetic products that can be used in the field of blood pattern analysis.

Methodology

Bloodstain Reference Dataset

Sterile oxalated equine blood (TCS Biosciences) whose coagulation is arrested through precipitative removal of Ca^{2+} ions was used to develop a reference bloodstain set. Bloodstains of fixed drop volume (1ml disposable pipette; 1.5mm f_{int}/ Bibby Sterilin) were produced at variable drop heights (0.1m – 2.0m) and at impact angles (10^{o} – 90^{o}) onto a fixed target surface (supported white card; 180gms).

Measurement of Resultant Stain Dimensions

Measurement of the resultant stains was in accordance with published methods on bloodstain reconstruction. Maximum stain width and elliptical length (Figure 1) was recorded using a loupe, 0.1mm accuracy (available through Jessops Camera Company). Stain morphology and appearance was also noted and digitally recorded with a scale reference.

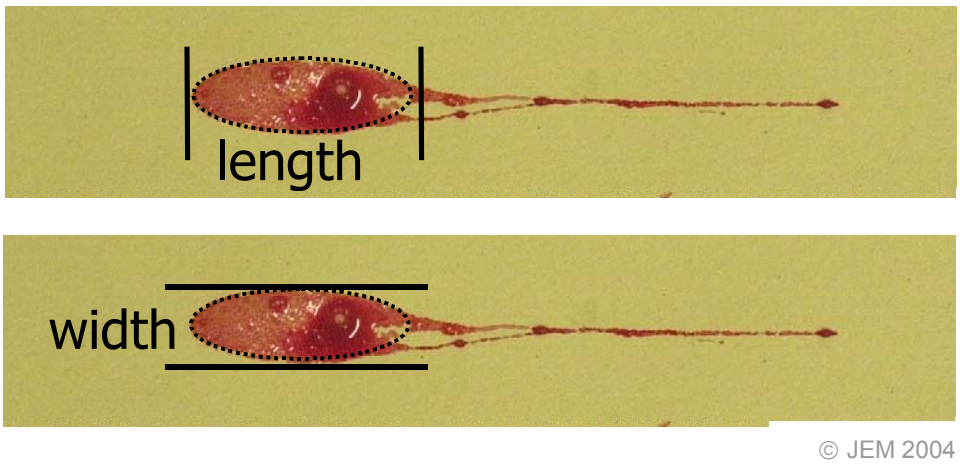


Figure 1 Measurement of resultant stain dimensions.

Formulation of Blood Substitutes

The formulation of each blood substitute was informed by published liquid blood data (viscosity, biochemical composition), but cognisance was given to the fact that in biological systems the viscosity of blood does not remain constant. It is influenced, not exhaustively, by haematocrit values, temperature etc. (Wonder, 2001). The developments were therefore informed more generally by case-based observations and experience, and also on practical observations throughout the trial. The substitutes developed, and which are presented here, represent a proportion of formulations whose development was continued. Many were regarded as unsuitable liquid blood mimics due to their substandard properties (e.g. consistency, high viscosity). Substitutes that were continued were subject to trials based on fixed target impacts and their characteristics recorded. Most formulations were given a nominal colour, with the addition of scarlet food dye, for the purpose of development of a substitute base that would significantly mimic equine and therefore human-derived blood. Manipulation of the colour of the base was the focus of a concurrent study.

Components that were used were purchased through the University technical department or through supermarket chain stores; a list of products and their source is provided. It was the aim of the study to use

components, which were readily available in the academic forum, and as such the recipes listed below should be fulfilled using dedicated suppliers.

Finally, substitutes that were found to mimic the reference data set were used to reconstruct composite blood distributions. Blood patterns prepared have been presented here for consideration.

Oscillating Drop Characteristics

The dimensions and characteristics of a resultant stain are dependent upon many parameters such as target surface, drop volume, force causing drop formation, environmental factors. Controlled conditions for each trial therefore were maintained through out. An area that was sheltered from air currents was utilised; target surface, drop volume, blood supply were fixed. The issue of drop sphericity at the target surface, which is dependent on fluid properties, was also addressed. Published data on oscillating drop characteristics indicates that liquid blood exhibits negligible distortion after ~1m freefall. Water exhibits negligible distortion after 1.4m freefall (Raymond *et al.*, 1996), after which it is assumed that the drop is stable and that the stain at the target surface is produced from the impact of a spherical drop.

Reference Bloodstain Data

Oxalated equine blood was used to provide the reference data set. Drops of fixed volume were dripped onto a fixed white cardboard target at 90°, for variable drop heights, and at variable impact angles. Angles were controlled with the development of an angled board which presented target slots at fixed angles: secured wood struts with slots at measured angles. Each card target was supported with MDF board to prevent bending at the periphery.

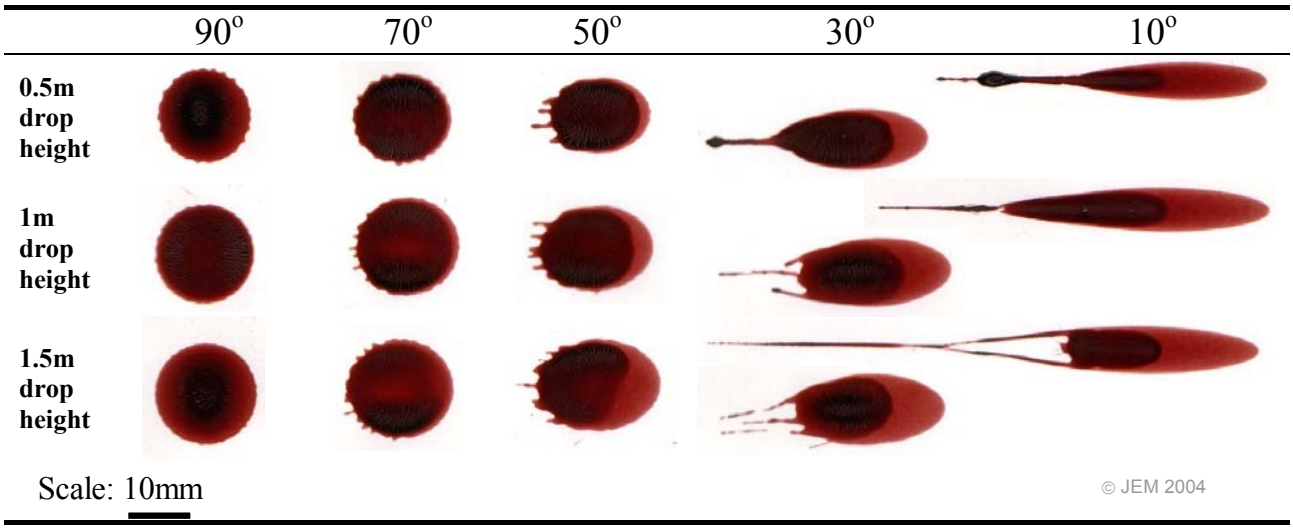


Figure 2 Resultant bloodstains for oxalated equine blood at impact angles of 90°, 70°, 50°, 30° and 10°.

Figure 2 visually demonstrates the trend that with decreased angle of impact there is an elongation in the resultant stain. In some cases, the stain will exhibit wave cast off and the appearance of winging can be observed. Characteristics such as these formed the basis of the physical characteristics, which were recorded for each trial. At a perpendicular angle, the resultant stain tends to circular with a 1:1 ratio of width to length, as expected.

Substitute Development

The formulations for each of the developed substitutes are given in table 1. Further details on the source and provider for the components are given below.

Brand name details for domestic components:

Birds sugar free strawberry flavour jelly mix (2 x 12g sachets)

Supercook red food colouring (38ml)

Supercook green food colouring (38ml)

Supercook glycerine (38ml)

Waitrose superfine self-raising flour (500g)

Waitrose superfine plain flour (500g)

Lyles strawberry flavour syrup (400g)

The formulations listed represent those that were found to mimic the reference set most closely and define the base formulation. This can be manipulated through the addition of differing proportions of food dye colour within the volume of food dye specified in order to mimic the required blood state (Figure 3). Stains generated using each of the substitutes were digitally scanned, measured and recorded. The data was analysed with comparison to the reference data set and significance ascribed.

Ten of the formulations demonstrated significant similarities to equine blood and by association to human-derived blood. These substitutes were the focus of further developmental and validation trials which addressed reproducibility and precision.

Manipulation of Substitute Colour

Substitute colour can be controlled through the addition of commercially available food dye. This facilitates the manipulation of colour and hue to represent varying physiological blood states; old vs. fresh blood, liquid vs. dried blood. The colour chart produced (Figure 3) shows the range of colours that can be achieved through the addition of drops of food dye in the ratios specified.

The addition of food dye facilitates the simulation of fresh oxalated and aged oxalated blood colour. Fresh oxalated blood tone can be achieved with the addition of food dye in a 2 red: 1 green drop ratio. Increasing the addition of red, green and black for the reconstruction of degraded samples i.e. 4 red: 1 black: 1 green. If these ratios are reflected in the volume of food dye added to the recipes in table 1, the colour can be manipulated to meet the desired requirement.

Substitute Code	*Component*	*Quantity*	*Comments*
A	D(+) Glucose Anhydrous (GPR™)	2g	Mix to a homogeneous constituency and store at room temperature
	Methyl Cellulose (Sigma)	2g	
	Sterile Distilled Water	25ml	
	Scarlet Food Colouring (Supercook)	1ml	
B	Plain Flour (Waitrose)	10g	Mix to a homogeneous constituency and store at room temperature
	Distilled Water	20ml	
	Scarlet Food Colouring (Supercook)	2ml	
C	Sodium Chloride (AnalaR)	0.9g	Mix to a homogeneous constituency and store at room temperature
	Distilled Water	90ml	
	Scarlet Food Colouring (Supercook)	2ml	
	D(+) Glucose Anhydrous (GPR™)	2g	
	Alpha Cellulose (Sigma)	2g	
	Corn Starch (Sigma)	5g	
	Superfine Plain Flour (Waitrose)	2g	
	Glycerol (Supercook)	4ml	
D	Golden Syrup (Sainsbury's)	9g	Mix to a homogeneous constituency and store at room temperature
	Sterile Distilled Water	90ml	
	Scarlet Food Colouring (Supercook)	2ml	
	D(+) Glucose Anhydrous (GPR™)	5g	
	Methyl Cellulose (Sigma)	5g	
	Glycerol (Supercook)	2ml	
E	Sterile Distilled Water	80ml	Mix to a homogeneous constituency and store at room temperature
	Scarlet Food Colouring (Supercook)	2ml	
	Golden Syrup (Sainsbury's)	15g	
F	Sterile Distilled Water	90ml	Mix to a homogeneous constituency and store at room temperature
	Scarlet Food Colouring (Supercook)	2ml	
	Strawberry Delight Dessert Mix (Birds)	5g	
G	Superfine Plain Flour (Waitrose)	31.4g	Mix to a homogeneous constituency and store at room temperature
	D(+) Glucose Anhydrous (GPR™)	4g	
	Bovine Serum Albumin (Sigma)	0.5g	
	Sterile Distilled Water	120ml	
	Scarlet Food Colouring (Supercook)	6ml	
H	Superfine Plain Flour (Waitrose)	39.6g	Mix to a homogeneous constituency and store at room temperature
	D(+) Glucose Anhydrous (GPR™)	5g	
	Glycerol (Supercook)	2ml	
	Sodium Chloride (Sigma)	0.7g	
	Bovine Serum Albumin (Sigma)	1.5g	
	Sterile Distilled Water	125ml	
	Scarlet Food Colouring (Supercook)	2ml	
I	Superfine Self-raising Flour (Waitrose)	13g	Mix to a homogeneous constituency and store at room temperature
	Sodium Chloride (Sigma)	1g	
	Glycerol (Supercook)	1ml	
	Strawberry Sugar Syrup (Lyles)	1ml	
	Scarlet Food Colouring (Supercook)	1ml	
	Sterile Distilled Water	183ml	
J	Superfine Self-raising Flour (Waitrose)	39g	Mix to a homogeneous constituency and store at room temperature
	Sodium Chloride (Sigma)	1g	
	Glycerol (Supercook)	1ml	
	Strawberry Sugar Syrup (Lyles)	1ml	
	Scarlet Food Colouring (Supercook)	1ml	
	Sterile Distilled Water	183ml	

Table 1 Substitute formulations.

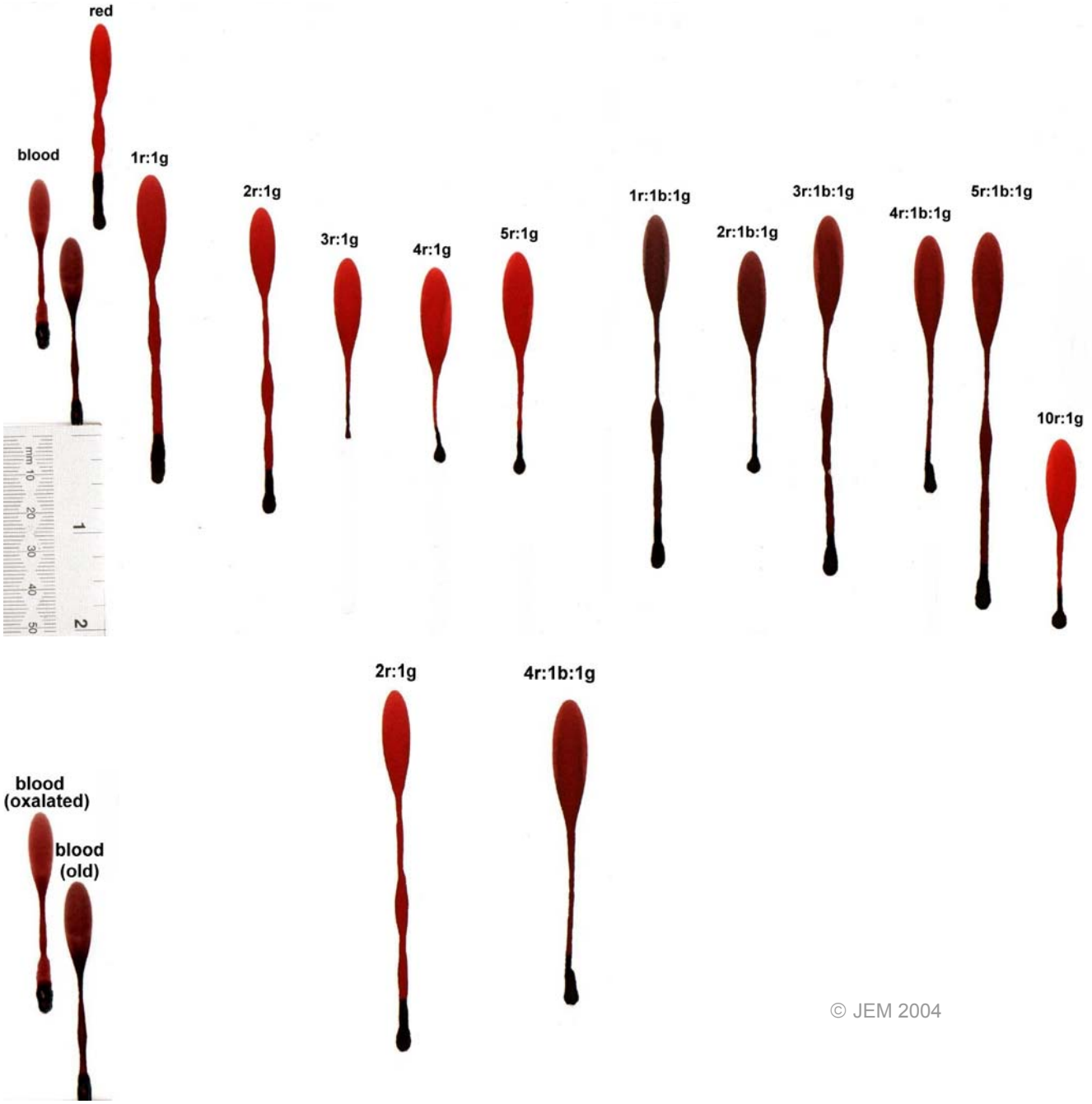


Figure 3 Manipulation of substitute colour with addition of food dye. (r=red, b=black, g=green). Reference bloodstains provided for comparison.

Results

Two approaches were adopted to investigate blood dynamics under controlled conditions. The first looked at influence of drop height on resultant stain morphology, the second, influence of impact angle. These approaches are based on the published scientific understanding of blood dynamics (e.g. Willis *et al*., 2001). The results presented here are derived from two concurrent undergraduate projects which developed independently.

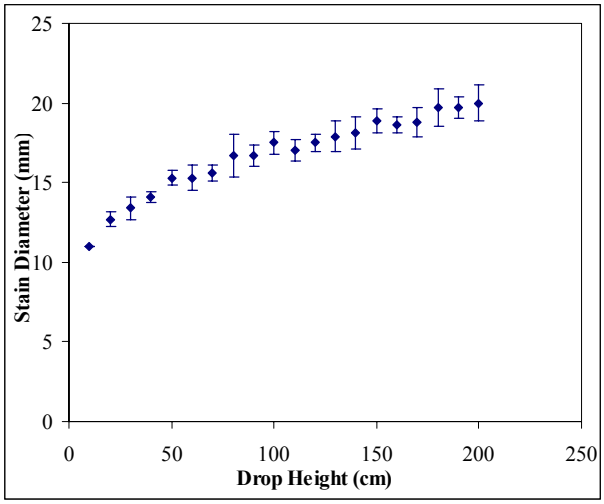
Drop Height Trials

Fixed volume drops of oxalated equine blood were presented to a fixed target surface at an impact angle of 90^{o} and at variable vertical drop heights. Several bloodstains for each trial were produced allowing calculation of mean values and the range represented by $\pm$ the standard deviation (Figure 4). The trend reflects that found within published figures: an increasing stain diameter is exhibited as drop height increases. The trend asymptotes at a stain diameter representing the height required for a drop to reach terminal velocity. The logarithmic nature of the relationship between drop height and stain diameter is also demonstrated. With repeated trials the data was accepted as a robust reference set.

Substitutes A through J were subjected to the drop height trial. Overview analysis of the experimental data compared to the reference data (Figure 5, 6) shows that the substitutes, in general terms, exhibit a similar relationship between drop height and stain diameter. Resultant stains however, are proportionately larger or smaller in diameter, visually, which is further demonstrated as data falls above or below the reference data set. In summary for most substitutes, the trend is reflected; overall size is not. Substitute D, which lies within the range of the reference set, cannot be statistically distinguished from the reference stains.

Simple regression analysis was performed on the reference and all substitute data. The behaviour of stain morphology with increasing drop height assumes a logarithmic relationship. Stain diameter is proportional to the natural logarithm of drop height: a significant linear relationship is verified ($P < 0.001$, $R^2 = 0.8899$). The substitutes also demonstrated similar significant linear relationships between stain diameter and the natural logarithm of drop height (Figure 7).

The drop height – stain diameter data was examined using simple correlation between the observed substitute results and the reference linear regression (Table 2). This provided a good indication of a strong linear association between each of the substitutes and the reference, with results mostly strong ($r > 0.9$). An F-test was also performed to assess the similarity of variance within the reference dataset in comparison to the substitutes. The resulting F-test *P-values* (Table 2) indicate that the variance in substitutes A, C, D, E, G, H, I and J are not significantly different (at the 99% confidence level) from the variance in the reference data set. Substitutes B and F were significantly different at the 99% confidence level.



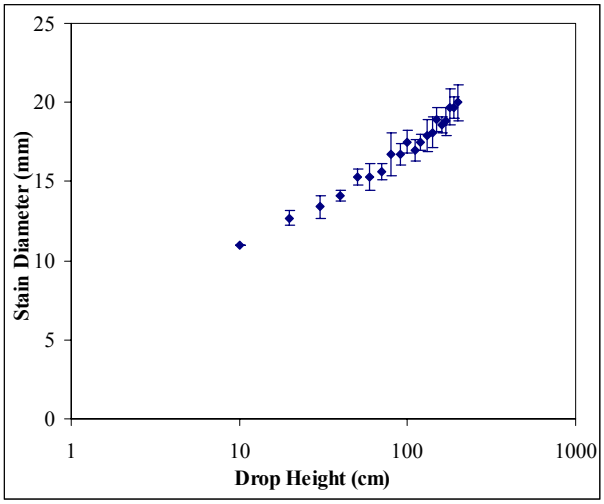
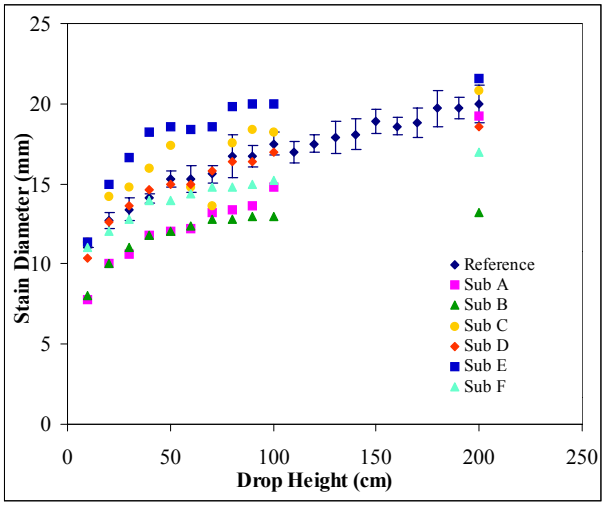


Figure 4 Reference blood data set, fixed volume drops, impact angle 90°, variable drop heights. Error bars represent standard deviation. Actual and log scale representations.



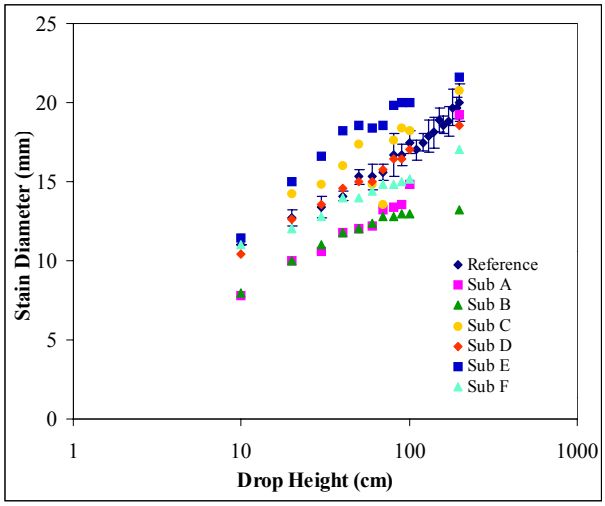
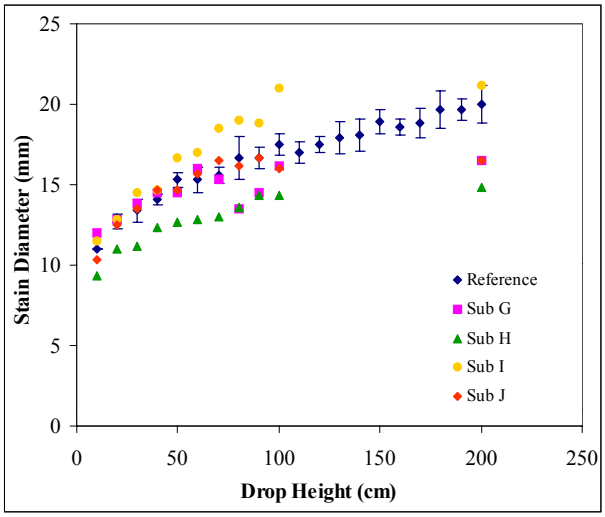


Figure 5 Substitute A-F data set, fixed volume drops, impact angle 90°, variable drop heights. Error bars represent standard deviation. Actual and log scale representations.



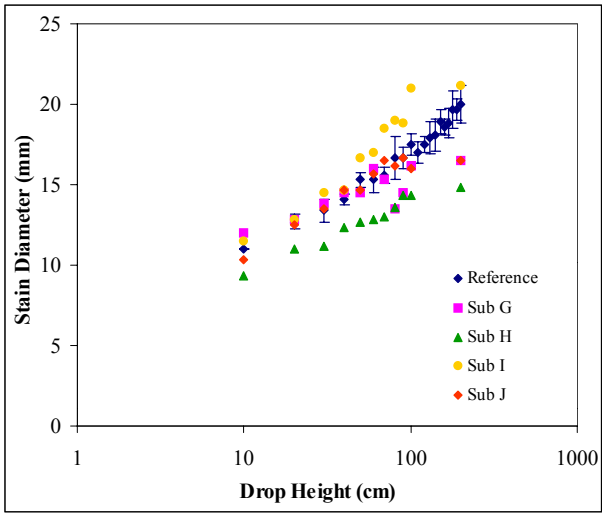


Figure 6 Substitute G-J data set, fixed volume drops, impact angle 90°, variable drop heights. Error bars represent standard deviation. Actual and log scale representations.

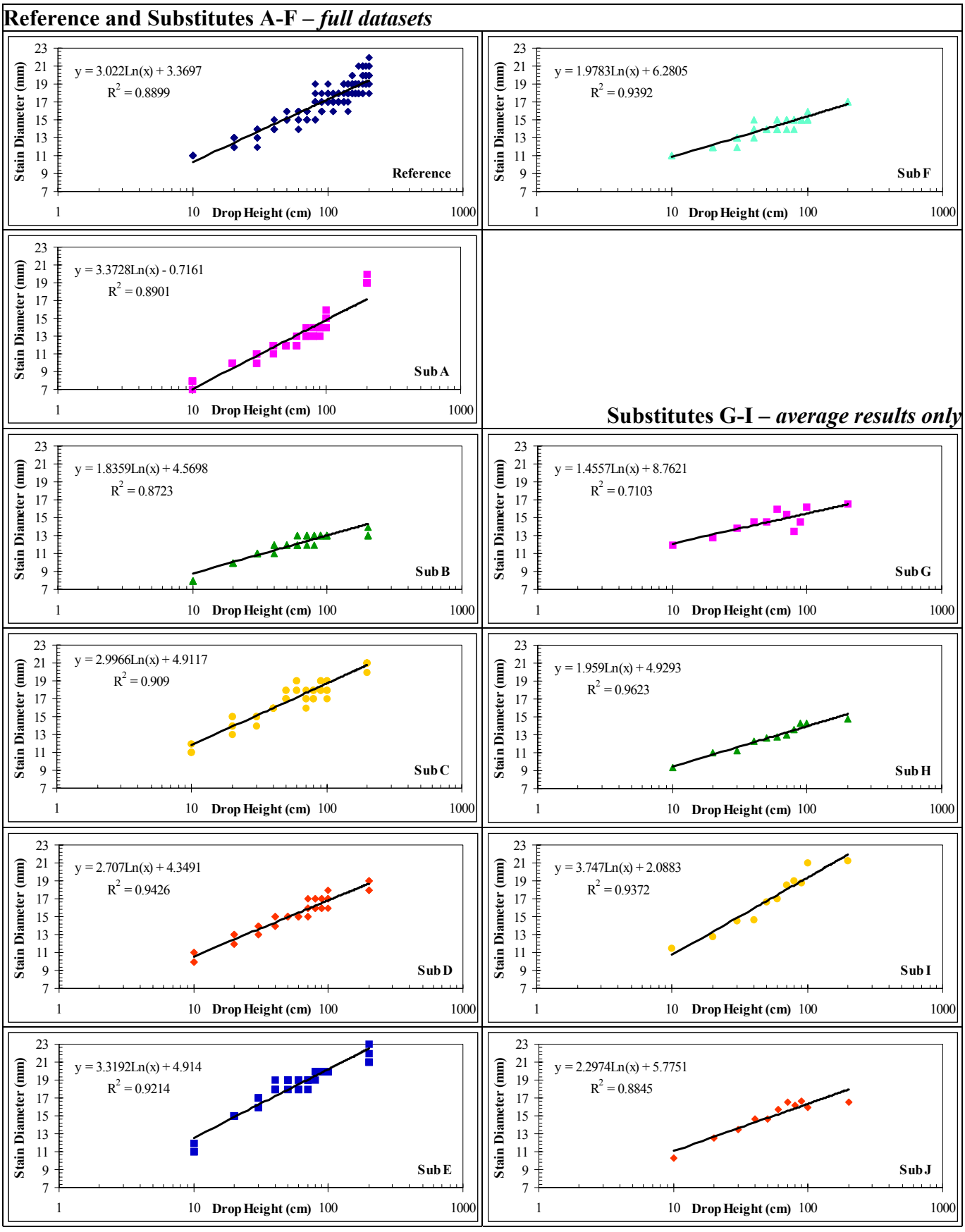


Figure 7 Simple linear regression analysis of the relationship between drop height and stain diameter, applied to the reference blood and all substitutes. Trends are supported by $P < 0.001$ in all cases.

The linear trends calculated for each substitute was compared to the linear relationship between drop height and stain diameter represented by the reference, in order to examine if the substitutes demonstrated comparable behaviour. The regression analysis provides significance indicators for intercept and slope (Table 2): $P < 0.01$ denotes a significant difference (99%) in intercept (or slope) between the reference and substitute. The results illustrate that substitutes F and G behave significantly differently (at 99%, $P < 0.01$) to the reference in terms of both intercept and slope; substitutes A, B, H and J exhibit a significant difference in either intercept or slope. Substitutes C, D, E and I, however **cannot** be statistically discriminated ($P > 0.01$) from the reference in terms of the characteristics of the linear relationship between drop height and stain diameter. This infers that substitutes C, D, E and I display a similar behaviour to the reference in this trial.

In summary, the drop height – stain diameter results and selected statistics indicate that substitutes C, D, E and I cannot be statistically differentiated from the results from the reference dataset and therefore are considered to behave similarly to the reference blood.

Substitute		**Comparison with Reference Linear Trend**		**Regression Analysis**		
		Correlation Coefficient (r)	*F-test P-value*		*P-value*	*Test Result*
A	*full dataset*	0.943	0.110	*intercept*	< 0.001	s
				slope	0.037	**ns**
B		0.934	0.001	*intercept*	0.059	**ns**
				slope	< 0.001	s
C		0.953	0.389	*intercept*	0.021	**ns**
				slope	0.874	**ns**
D		0.971	0.278	*intercept*	0.121	**ns**
				slope	0.040	**ns**
E		0.960	0.163	*intercept*	0.021	**ns**
				slope	0.065	**ns**
F		0.969	0.002	*intercept*	< 0.001	s
				slope	< 0.001	s
G	*averages only*	0.843	0.046	*intercept*	< 0.001	s
				slope	< 0.001	s
H		0.981	0.104	*intercept*	0.058	**ns**
				slope	< 0.001	s
I		0.968	0.224	*intercept*	0.291	**ns**
				slope	0.016	**ns**
J		0.940	0.257	*intercept*	0.034	**ns**
				slope	0.009	s

Table 2 Regression and correlation statistics for substitutes with respect to the reference [ns = intercept/slope IS NOT significantly different, s = intercept/slope IS significantly different].

Impact Angle Experiments

Fixed volume drops of oxalated equine blood were presented to a fixed target surface at variable impact angles (10^o - 90^o) at a fixed vertical drop height. Several bloodstains for each trial were produced allowing calculation of mean values and the range represented by $\pm$ the standard deviation. The trend reflects the general behaviour of blood drops under these conditions found within published figures. An elongation of the resultant stain is observed with a reduction in the angle of impact. A trigonometric relationship exists between the angle of impact and stain dimensions such that:

$$\sin \vartheta = \frac{width}{length}$$

where ϑ is the angle of impact (refer to figure 1 for instructions on stain measurement).

Impact angle was calculated from sin function of width and length. It was assumed that calculation of impact angle from stain dimensions would result in the accurate calculation of impact angle and therefore experimental data is referenced to the theoretical. It is also recognised that at higher impact angles, ~60^o – 90^o, the practitioner is unable to significantly distinguish between bloodstains that have impacted at these high angles due to the sensitivity of near circular stains to errors in measurement. Inherent limitations in resultant stain measurement result in a significant influence on calculated impact angle. Automated digital measurements are more precise, but still fail to recognise differences between stains impacting at 80^o and 90^o. A 0.1mm error in estimation of width, for example (the accuracy limit of the loupe), accounts for <2% error in width measurement, which at 90^o results in >6^o error in impact angle calculation.

This illustrates that at higher impact angles the formula is not an accurate predictor of impact angle and it is important that the user is cognisant of these limitations. It is therefore preferable that the substitutes concur with the reference set at angles of 60^o and less rather than at angles that tend toward the perpendicular. With repeated trials the data was accepted as a robust reference set.

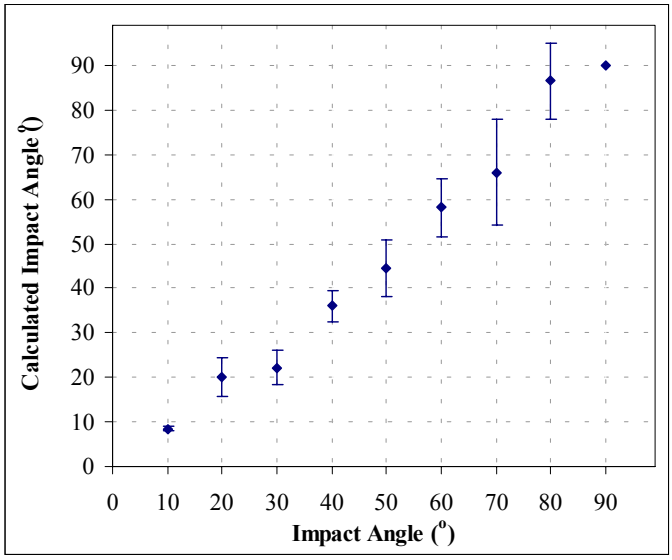


Figure 8 Reference data set, fixed volume drops, fixed drop height, variable impact angle 10-90°. Error bars represent standard deviation.

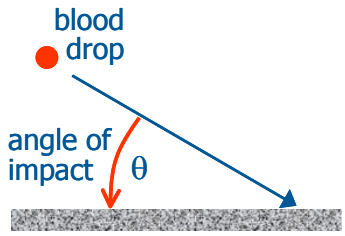


Figure 9 Representation of impact angle.

Experimental Trials

Under the same conditions, the selected substitutes were subjected to the impact angle protocol. Eight of the substitute formulations were progressed through this trial. These exhibited physical properties that were visually similar to the reference blood. A similarity between calculated impact angle compared to actual was generally observed, and most substitutes exhibited properties that were representative of the reference set. Development of two substitutes was suspended due to their unfavourable consistency. Regression analysis was not performed as only average stain measurements had been provided.

Initial examination of the impact angle results indicated that substitutes E and G were inconsistent in their behaviour and calculated angles (from measured widths and lengths) and were notably different from the reference and actual (observed) angles (Figure 10). Removal of these data from the simple comparison graphs in Figure 10 reveals considerable consistency in the behaviour of the other substitutes and the reference, particularly at low impact angles (Figure 10: right). The result at 70° for substitute C is considered to be an anomaly.

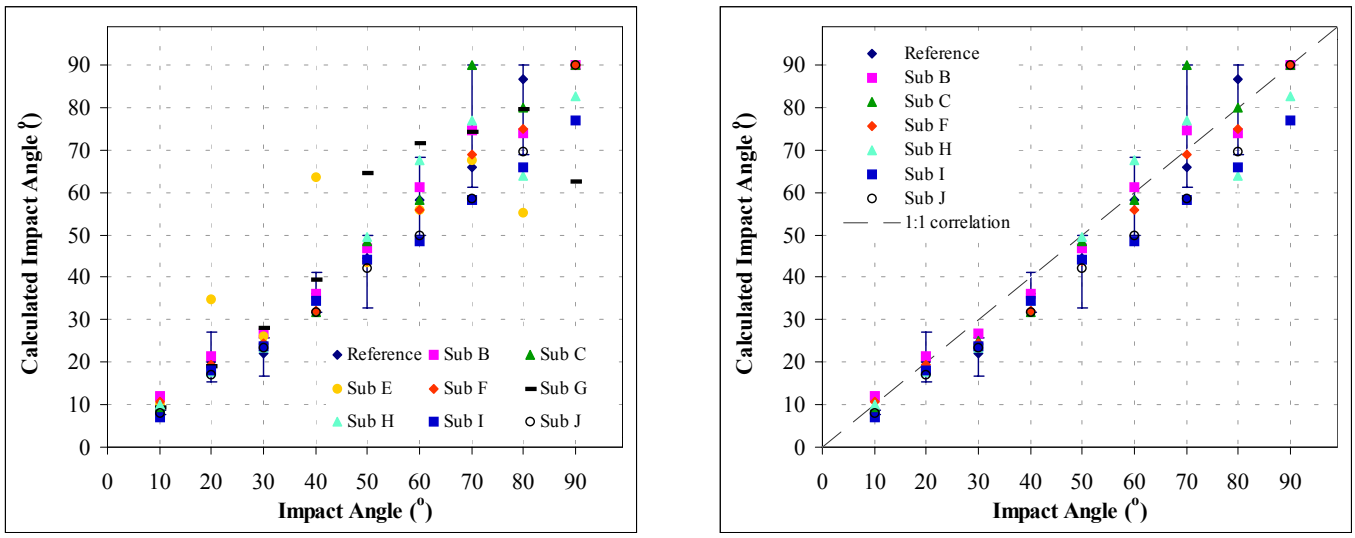


Figure 10 Substitute data set, fixed drop volume, fixed drop height, variable impact angle 10-90°. Substitutes E and G have been removed from the right hand graph, which also provides a 1:1 trend line. Error bars give the standard deviation of the reference results.

Observed Angle	*Predicted Angles*								
	Reference	*Sub B*	*Sub C*	*Sub E*	*Sub F*	*Sub G*	*Sub H*	*Sub I*	*Sub J*
10	8.48	12.20	9.26	10.07	10.77	9.39	9.99	6.91	7.88
20	20.16	21.24	18.50	34.85	19.47	19.07	17.65	18.04	16.90
30	22.22	26.88	25.03	26.01	24.36	27.97	23.57	23.58	23.27
40	36.00	36.03	31.72	63.71	32.23	39.40	34.38	34.37	31.83
50	44.43	46.79	48.59	43.63	43.81	64.57	49.38	44.12	42.20
60	58.13	61.31	58.29	55.81	55.91	71.57	67.55	48.64	49.71
70	66.02	74.50	90.00	67.67	68.78	74.24	77.08	58.07	58.45
80	86.49	74.06	79.86	55.31	74.78	79.71	63.89	65.75	69.66
90	90.00	90.00	90.00	90.00	90.00	62.67	82.46	77.02	90.00

Table 3 Predicted impact angle calculated from width and length measurements compared to actual (observed) impact angle.

Further examination of the results shows that the predicted impact angles often only differs from the actual by a few degrees (Table 3). Although individual results may be well correlated, the substitute needs to perform well at all impact angles, and therefore the disparities between calculated and observed were examined using a simple residual analysis. The sum of squares of residuals (SS_{res}) is a common technique associated with simple linear regression, where the measure is used as a relative indicator of goodness of fit: improved association between two sets of data is assumed given a decrease in the SS_{res}. The ranking of SS_{res}. provides some indication of which substitutes deliver calculated angles that are best associated with the actual impact angles used: in this case, the lowest SS_{res} figures are obtained for substitutes B and F, both of which are at least comparable to the reference (Table 4). It is clear from these results that substitutes E and G do not behave as blood. A correlation analysis of the calculated impact angles again highlights the inadequacies of substitutes E and G, and suggests that the behaviour of substitutes B, F and I is best representative of the reference blood (Table 4).

	Comparison with of Calculated (measured) with Actual (observed) Angles [10-90°]			**Comparison with of Calculated (measured) with Actual (observed) Angles [10-50°]**		
	Sum of Square Residuals (SS_{res})	*SS_{res} Rank*	*Correlation Coefficient (r)*	*Sum of Square Residuals (SS_{res})*	*SS_{res} Rank*	*Correlation Coefficient (r)*
Reference	**171.4**		**0.992**	**109.9**		**0.984**
Sub B	**99.5**	1	0.992	**42.2**	1	0.995
Sub C	501.0	3	0.972	**98.1**	3	0.980
Sub E	1471.8	8	0.869	839.1	8	0.759
Sub F	**176.8**	2	0.994	**131.3**	5	0.991
Sub G	1117.0	7	0.904	218.1	7	0.971
Sub H	502.5	4	0.963	**79.0**	2	0.983
Sub I	764.0	6	0.998	**120.9**	4	0.996
Sub J	533.3	5	0.989	187.0	6	0.997

Table 4 Basic statistical analysis of the predicted (calculated) impact angle and actual (observed) impact angles: sum of square residuals and correlation coefficients.

It is clear, from all calculation of impact angles data, that problems of consistency are notably apparent at high impact angles, specifically 60-90°. This has significant implications for the use of width-length measurements as the basis for crime scene reconstruction (Willis *et al*., 1997), and blood itself is often found to produce spurious results at these angles. It is worth considering therefore, the results of the substitutes the low angles specifically, where these inherent problems have the least impact on the results obtained. The residual and correlation analysis was repeated solely for angles 10-50° (Table 4). Substitutes B, F and I behave as well as the reference, but by discounting the high angles, substitutes C and H also perform favourably; with low SS_{res} and high correlation coefficients.

In summary therefore, the impact angle trials infer that substitutes B, F and I perform agreeably over the full range of impact angles. Disregarding the problematic high impact angles then indicates that substitutes C and H are also comparable to blood.

Despite agreement with the mathematical relationship between stain dimensions and impact angle, morphologically, some of the resultant substitute stains showed visual differences. Substitute C for example (Set 1) had a glutinous texture that was reflected in the resultant stain (Figure 11). The reflection of wave cast, which is not a defining characteristic of bloodstain morphology but which is a characteristic worthy of note, was demonstrated in some trials. Set 2 (substitute F) illustrates that viscosity can impede the elongation of the stain and lead to the formation of a bulbous 'tail'. In these instances the main body of the stain, from which measurements of width and length derive, were concordant with the reference set. It is the judgement of the user to decide if aesthetics are more important than stain dimensions. Substitute selection can therefore be informed by the practical exercises that have been defined in the laboratory. Set 3 shows substitute I whose colour has been manipulated in line with recommendations to reflect aged oxalated equine blood.

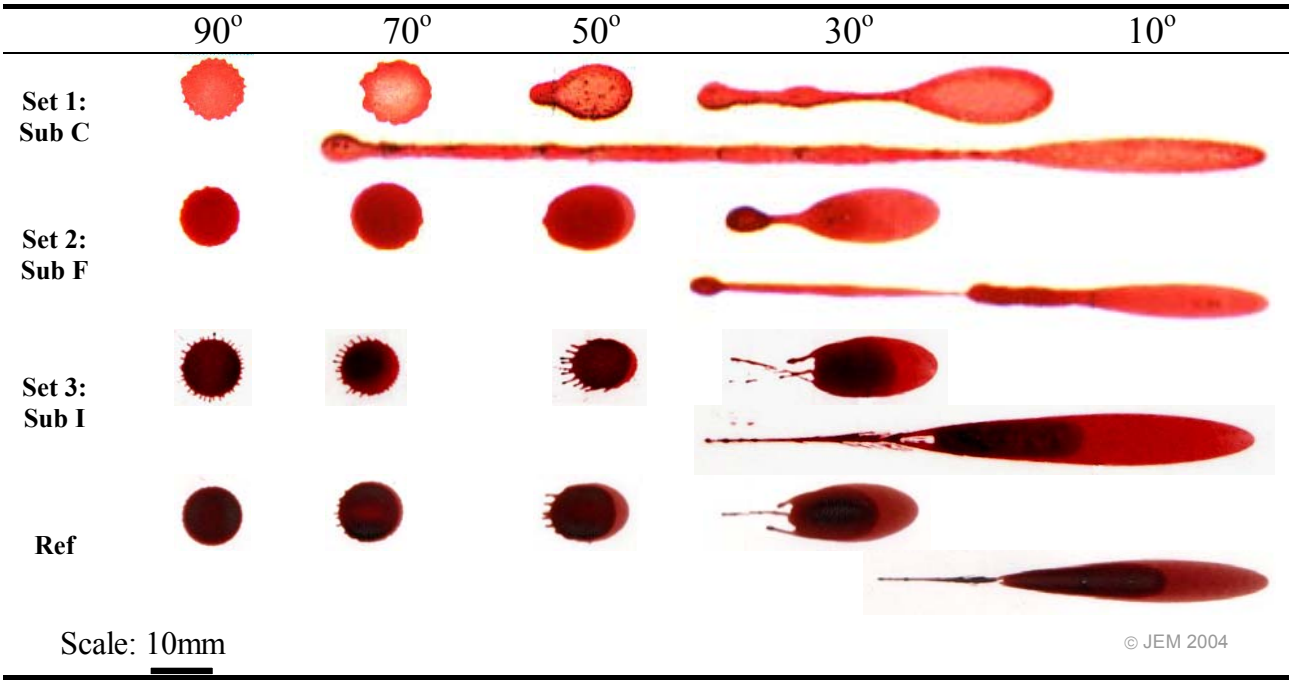


Figure 11 Digital representation of resultant substitute stains at impact angles described. Set 3 = substitute I with colour manipulation to reflect old blood (addition of black, green and red food dye as defined).

Reconstruction of Composite Stain Patterns

Several trials were completed to demonstrate the behaviour of substitute I (Figure 11), colour manipulated to reflect aged oxalated blood. Boards which demonstrate exhalated blood, blood projected from a syringe, composite blood into blood and cast-off spatter were reconstructed. The results are presented for visual comparison.

Exhalated Blood

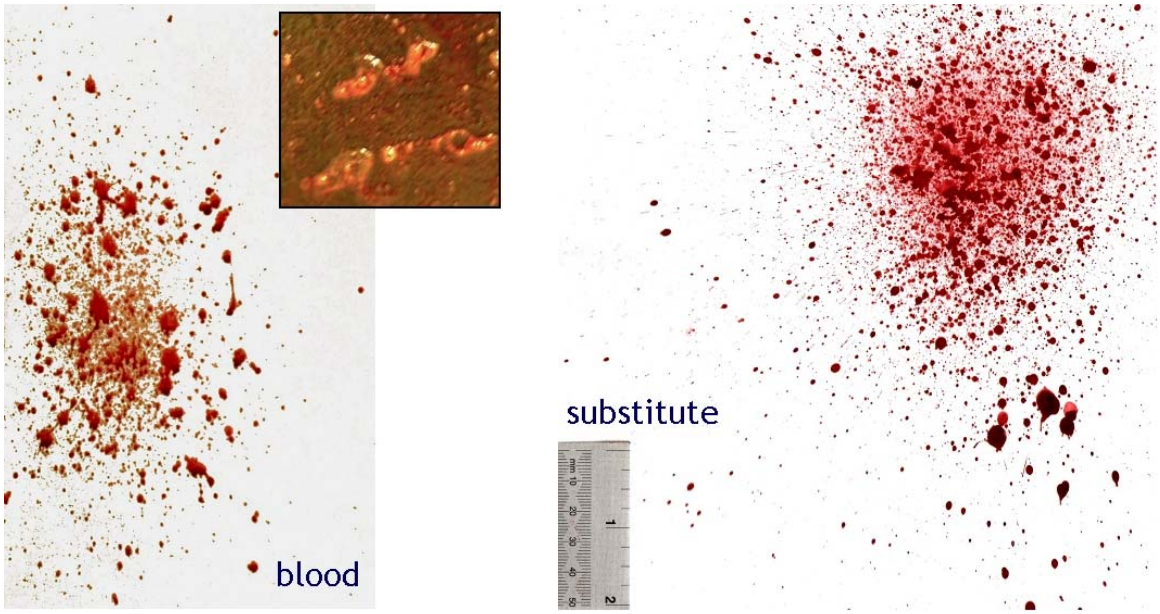


Figure 12 Exhalated blood vs. exhalated substitute blood pattern.

Projected Blood

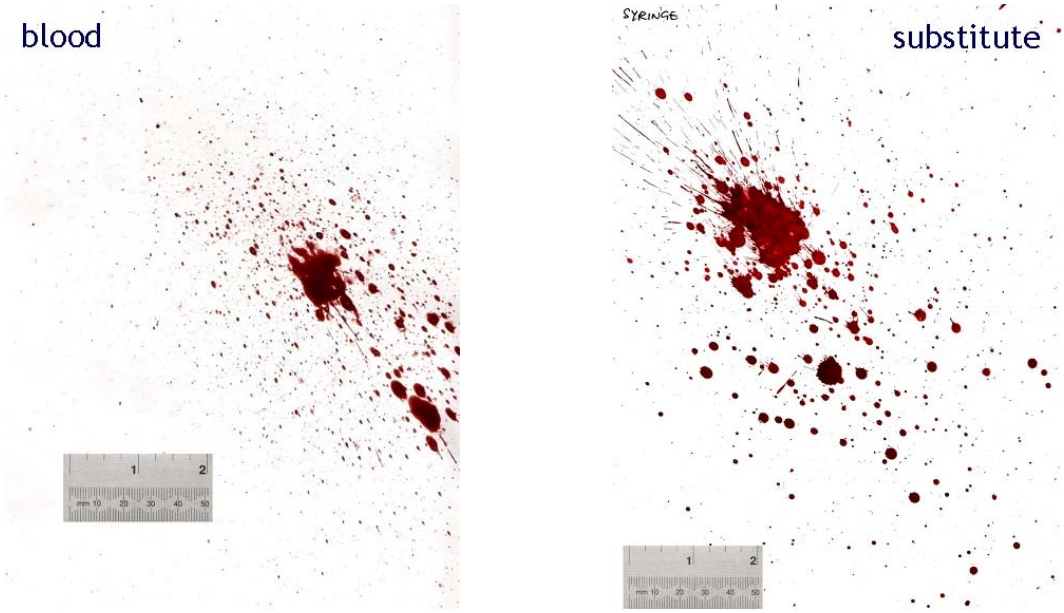


Figure 13 Projected blood vs. projected substitute blood pattern.

Blood into Blood

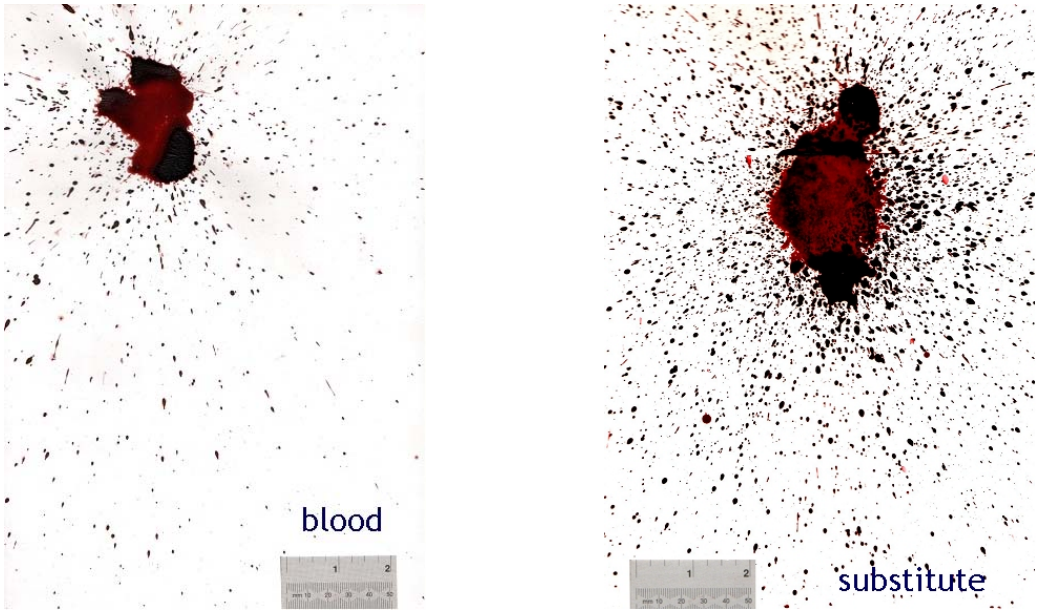


Figure 14 Blood into blood vs. substitute 'blood into blood' pattern.

Cast-off

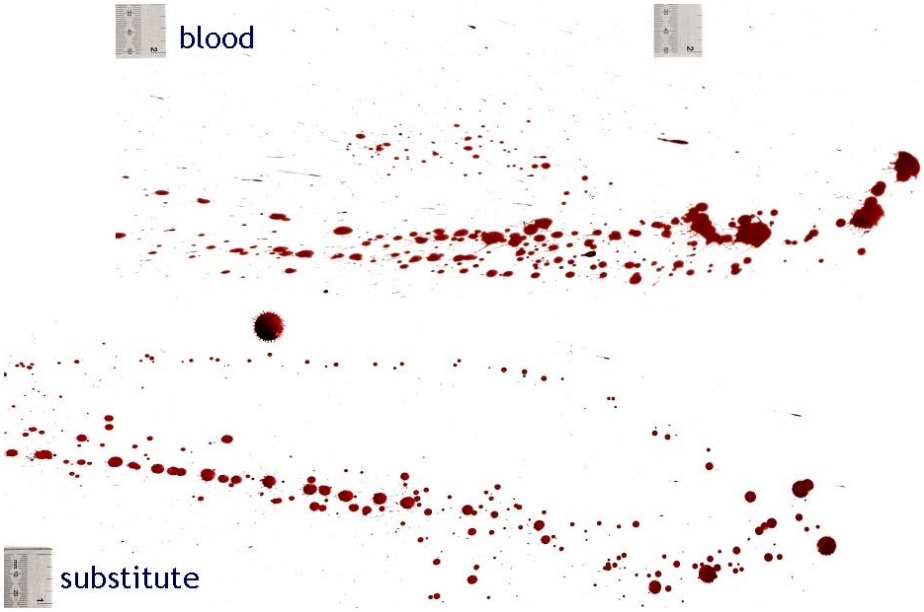
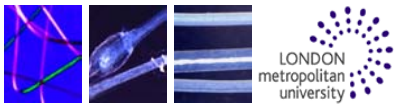


Figure 15 Cast-off blood vs. cast-off substitute blood pattern.



Conclusion

The developmental work presented here provides a suggested template for the formulation of a blood substitute that can be produced to mimic equine blood and therefore blood which is human derived. The blood substitutes that have been developed in the laboratory, and which have progressed to the final stages, are as inert as feasible and are cheap to reproduce. In preparation, and particularly in the reconstruction of exhalated blood patterns, the user needs to be aware of the published safety data sheets for the consumables that are being used. For this purpose, those substitutes that are composed of edible consumables and which closely reflect animal derived blood should be used. The ingredients that have been listed are commercially available and accessible within the academic and scientific market. This increases their availability to the operator, minimises the cost of production and avoids the need for specialist supply.

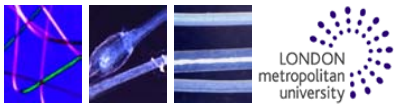
Pedagogical Context and Benefits

London Metropolitan University emerged as an institution that was strategically well positioned to diversify its prospectus to include forensic science in September of 2001. The initial framework for forensic science teaching was based on the existing biological modules in the department of Health and Human Sciences. These presented a strong analytical base on which future curriculum development in forensic science could be based, although it was acknowledged that the scope of the existing practical exercises had to be broadened to accommodate forensic aspects.

The research programme in forensic science was initiated at a time when students, who would participate in the research module, were enrolled in non-forensic disciplines such as biomedical sciences and biochemistry. The research programme which evolved, and which included the development of a blood substitute as defined through the remit of this research, proved to be a challenging and worthwhile exercise for the participants and formed a framework on which they could develop their own scientific investigation and research strategy. This particular research was novel and unique, and did not conform to the typical framework of research usually offered to undergraduate/ novice researchers. However, the subject was motivating and was met with the anticipated verve of enthusiastic nominees and allowed students to develop their scientific and research skills.

The introduction of experiential learning (Knowles, 1984) and the presentation of scenarios that encourage problem-based learning [PBL](Biggs, 1996; Shuell, 1986) has been recognised as a key objective of curriculum development in forensic science. PBL is identified as a tool that can be used to motivate students outside their field of study and can increase tacit practical skills (Albanese & Mitchell, 2000). The presentation of novel research, which was uniquely accompanied with dedicated resources, to novice researchers was challenging at first. Strategic differences in their scientific approach highlighted the importance of active and explicit supervision particularly in the early stages. Dynamic sessions were key in the discussion and evolution of the project as this was novel undergraduate territory; there were relatively few references on which to base the approach.

For a module in its infancy, the evaluation of a new innovation is difficult when the framework to which it is being applied is itself evolving. However, qualitative testing and the collection of feedback from students and staff who experience it first hand can be used to further evaluate its efficiency and continue development. Personal experience shows that PBL can be successfully applied in a teaching environment and that this is applied daily in an analytical and professional context.



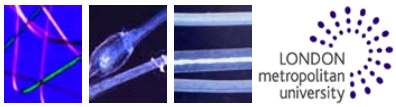
For educational programmes that are developing in this field, or which seek to expand beyond the traditionalist framework, I recommend that practitioners veer away from the tried and tested and embrace the challenges which prevail from the introduction of novel research at undergraduate level, and do not save such projects exclusively for postgraduate research. In a climate where the number of undergraduate degree and ancillary programmes in forensic science is expanding I believe that it is critical that the research programme which accompanies this expansion reflects the true nature of the science and investigates the novel approaches of this discipline. A balance between the components of research and teaching is essential in order to motivate students, stimulate their participation and satisfy educational guidelines. Ultimately, enhanced motivation will engender an increase in student retention and more broadly, support the social inclusivity agenda of the University. Additionally, and perhaps more importantly, in a time when research at functional forensic laboratories is being outsourced, or indeed sidelined to satisfy operational needs, it would be pertinent for local academic institutions to partner operational laboratories and offer research initiatives. Not only does this maintain local and national links, it explicitly demonstrates the ambition of the institution and its commitment to forensic science. An action which may allay the fears that are commonly voiced by the professional community and the media which attack the motivating factor behind an institutions decision to diversify its prospectus to forensic science.

Future Strategy

This LTSN development project has allowed extensive research to be completed in a novel field. As such the research is not exhaustive and although the grant has supported immense progress, there is the potential for this to be developed further. Further studies are recommended into substitute longevity and storage properties. Indications at this stage suggest that the final blood substitute is stable when dry, but that the properties are maximised when the formulation is freshly prepared and used over the short-term. Investigation into environmental influences, humidity and temperature, all of which affect liquid blood properties, could be investigated. Current prototypes have taken on a cherry red colouration that, on paper, reflects dilute blood. The stated aim of this project was to produce a realistic blood substitute and therefore the physical properties and resultant stain morphology were the priority, but it is acknowledged that colour matching will increase the aesthetics of the final product. The colour chart that has been presented can be used to support the substitute formulation, but budgetary and time constraints on this project prevented their full validation. This work is proposed for completion in the future at London Metropolitan University as part of the continued and expanding undergraduate research programme in forensic science.

Dissemination of Results

Dissemination of these results to teaching institutions is welcomed, as are collaborative proposals. Requests for information should be addressed to the author.



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