Improved micro-sampling methodology enables accurate platelet function analysis

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Improved micro-sampling methodology enables accurate platelet function analysis

Tyler L. Horn*, Abhishek Sadhu*, Hind O. Alosaimi*, Chris I. Jones‡, and Craig E. Hughes‡

Institute for Cardiovascular and Metabolic Research, School of Biological Sciences, University of Reading, Reading, UK

ABSTRACT

Micro-sampling for platelet function assays offers a minimally invasive alternative to venipuncture that may facilitate point-of-care testing. We have developed a new micro-sampling methodology for human and mouse and evaluated these methods for platelet function analysis. Blood was collected by venepuncture or our new finger-prick sampling method in humans, and by terminal bleeding or a modified tail vein bleeding method in mice. Platelet activation was assessed by flow cytometry, measuring fibrinogen binding and P-selectin exposure in response to ADP, CRP-XL or TRAP-6 in humans ($n = 14$) and fibrinogen binding in response to ADP, CRP-XL in mice ($n = 5$). In humans, platelet sensitivity (EC_{50}) and capacity (maximal minus minimal response) showed no significant differences between capillary and venous samples, except for a higher EC_{50} of P-selectin exposure to CRP-XL in capillary samples (0.179 µg/mL), compared to venous samples (0.026 µg/mL). This may be clinically relevant but requires further investigation. In mice, no significant differences were observed in EC_{50} or capacity in response to ADP or CRP-XL between the terminal bleeding techniques and our modified tail vein method. Our new finger-prick sampling and tail vein bleeding methods are viable alternatives to the standard methods used for platelet function analysis. They maintain assay accuracy while improving accessibility and reducing and refining the use of animal models.

PLAIN LANGUAGE SUMMARY

What is the context?:

- Venepuncture is the standard blood collection method for platelet function testing both clinically and for research; however, assays such as flow cytometry can result in a large volumes of wasted blood.
- Animal blood sampling methods for platelet function testing are currently terminal procedures due to the volumes of blood extracted. This increases the number of mice required in a study.
- Currently, micro-sampling is not seen as a viable method of blood draw for platelet function testing as there are fears that platelets would be pre-activated

What is new?:

- In this study, we have developed a modified micro-sampling method usingfinger prick sampling in humans or tail vein bleeding in mice, which provides resting, activatable platelets for use in platelet function testing.
- Validation of this method indicates that comparing the sensitivity (EC_{50}) and capacity (maximal minus minimal response) suggests that the methods are highly comparable.
- The only statistically significant difference was a small increase in sensitivity of P-selectin exposure in response to CRP-XL

What is the impact?:

- This study provides a new micro-sampling method for use in both humans and mice which maintains assay accuracy and reproducibility
- Micro-sampling in humans mitigates needle phobia as a barrier to entry for study recruitment and opens the door for introducing platelet function testing as a point of care assay
- Tail vein sampling in mice for platelet function assays drastically reduces the numbers of animals required for studies and refines the platelet function data produced as multiple samples can be taken from the same animal.

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CONTACT Craig Hughes c.e.hughes@reading.ac.uk Institute for Cardiovascular and Metabolic Research, School of Biological Sciences, University of Reading, Health and Life Sciences Building, Reading RG6 6AS, UK.

*Joint first authors
‡Joint senior authors

Introduction

Platelets are anuclear blood cells that are key for maintaining hemostasis and preventing unwanted thrombosis.[1] The dysfunction of these cells can lead to both bleeding disorders or thrombotic complications such as myocardial infarction and ischemic strokes.[1,2] Therefore, understanding platelet function is paramount for understanding these disease pathologies.

Venepuncture is the standard blood collection method used in platelet diagnostics and research. However, it requires trained phlebotomists, which limits accessibility and the expansion of point-of-care platelet testing. Additionally, in groups such as the elderly, neonates, and individuals with obesity, blood collection via venepuncture can be challenging due to difficult venous access, and it can lead to complications such as hematoma formation, nerve damage, infection, iatrogenic anemia, arterial puncture, allergies, and unwanted thrombi.[3–7]

In clinical settings, micro-blood sampling via finger-prick, which samples from a capillary bed rather than directly from a vein, is already conventionally used in many assays such as blood glucose level and C-reactive protein monitoring for disease management.[8–10] This sampling is simple, minimally invasive and can be taken via the use of a lancet-style device on the finger without the need for clinical personnel such as specialist phlebotomists. Recent work has shown that previous capillary sampling methods can be used in routine coagulation assays but not for full blood counts.[11,12] Full blood counts compared between both methods show that the number of platelets is significantly reduced in capillary blood compared to venous blood, which may be due to the adhesion of platelets to the site of the skin puncture.[11–14] Therefore, further method development and investigation are required to determine whether capillary sampling is a viable sampling method for platelet function assays.

Platelet function is typically measured using techniques like VerifyNow, PFA-100 and light transmission aggregometry (LTA),[15–19] which require relatively large volumes of blood. For example, LTA, considered to be the gold standard for platelet function testing, requires 180–500 µL of platelet-rich plasma (PRP) per cuvette; therefore, a minimum of 10 mL of whole blood is needed for a series of tests, which cannot be achieved by micro-sampling. However, flow cytometry, whereby fluorescently tagged antibodies are bound to platelet surface receptors, and the level of fluorescence is measured in individual cells,[20–25] only requires a few microliters of blood per sample. When analyzing platelet function by flow cytometry, <100 µL of blood, which can be achieved by micro-sampling, is enough to perform a series of tests with multiple different agonists to produce full-dose response curves.[21,25]

Mouse models of thrombosis and hemostasis are widely used to study human physiology and pathophysiology of platelets.[26–29] Using mouse models is advantageous as they closely mimic human physiology, and their genome can be manipulated to be representative of human diseases such as hemophilia A, immune thrombocytopaenia, and an αIIbβ3 knockout model of Glanzmann's thrombasthenia.[28,30–32] A common blood collecting method from mice for conventional platelet function testing is terminal bleeding, for example, by cardiac puncture, as platelet function testing demands a large blood volume. Bleeding by cardiac puncture can yield approximately 1 mL of blood depending on the age and size of the mouse which, which as with human venepuncture samples, will be sufficient for a limited set of LTA samples.[33] However, this volume remains a large excess beyond what is needed for a comprehensive flow cytometry screen of platelet function.[34]

The total blood volume (TBV) of a laboratory mouse is approximately 6–8% of their total body weight according to the National Centre of Replacement, Refinement & Reduction of Animal in Research (NC3Rs). It is recommended not to remove more than 10% of the TBV during any single, non-terminal sampling. Removing more than 25% TBV is lethal and considered terminal bleeding.[34,35] Contrastingly, noninvasive, tail vein bleeding methods are easily recoverable and can yield up to 150 µL for a single sample or ≤50 µL of blood (based on UK animal legislation) for repeat blood collection which is sufficient for flow cytometric screening of platelet function.[36,37]

Other, repeat bleeding methods include retro orbital bleeding (more common outside of the UK), or bleeding from saphenous, mandibular or sublingual veins, or via blood vessel cannulation; however, these methods are relatively invasive compared to tail vein bleeding and for some, namely bleeding from the mandibular or sublingual veins, are significantly more stressful and harmful to the animal.[38–41] These blood collection methods also require sedation, and these anaesthetic agents may also be a factor that can impact

platelet function.[42,43] Moreover, in the context of Reduction, Refinement and Replacement (the 3Rs), the use of micro-sampling from the tail vein for platelet function studies would be both a refinement and a reduction approach as it is non-terminal, noninvasive and sampling can be repeated longitudinally over multiple time points, unlike some of the alternative methods.[34,35] So, as with human capillary sampling, micro-sampling from the tail vein should be investigated for platelet function studies.

We have developed a modified micro-sampling method for both human (capillary sampling by finger-prick bleeding) and mice (tail vein bleeding). These methods allow for repeated, noninvasive sampling of both mice and humans for platelet function testing. This paves the way for the development of point-of-care testing (POCT) for platelet function in humans. Additionally, in mice, this allows for repeated platelet function testing across different experimental setups, subsequently refining the bleeding method as well as reducing the number of animals required.

Methods

Blood sampling from human donors

Healthy, drug-free donors aged 18 years or older provided non-fasted venous and capillary blood. The study was approved by the University of Reading Research Ethics Committee.

Venepuncture for the collection of venous blood was performed using a standard vacuum extraction system. A tourniquet was applied to the arm, and a 20 G butterfly needle was inserted into either the median cubital or cephalic vein. The tourniquet was then released, the first 3 mL of blood was discarded, and subsequent blood was collected into a vacutainer containing sodium citrate (3.2%). The blood was rested in a water bath at 30°C for 20 minutes prior to use. Bleeding was stopped by applying pressure to the area[7].

Capillary Sampling was performed as shown in Figure 1. The donor's hand was briefly warmed for one minute using warm tap water (<50°C to avoid scalding) to ensure capillary dilation and maximal blood flow. The area was then cleaned using an antibacterial wipe prior to using a Unistik 3 Normal Lancet, 23 G, 1.8 mm to pierce the finger. The first drop of blood was discarded, and subsequent drops were collected onto a piece of parafilm prior to being immediately mixed with sodium citrate (3.2%) in a 1:9 (v/v) ratio.[44,45]

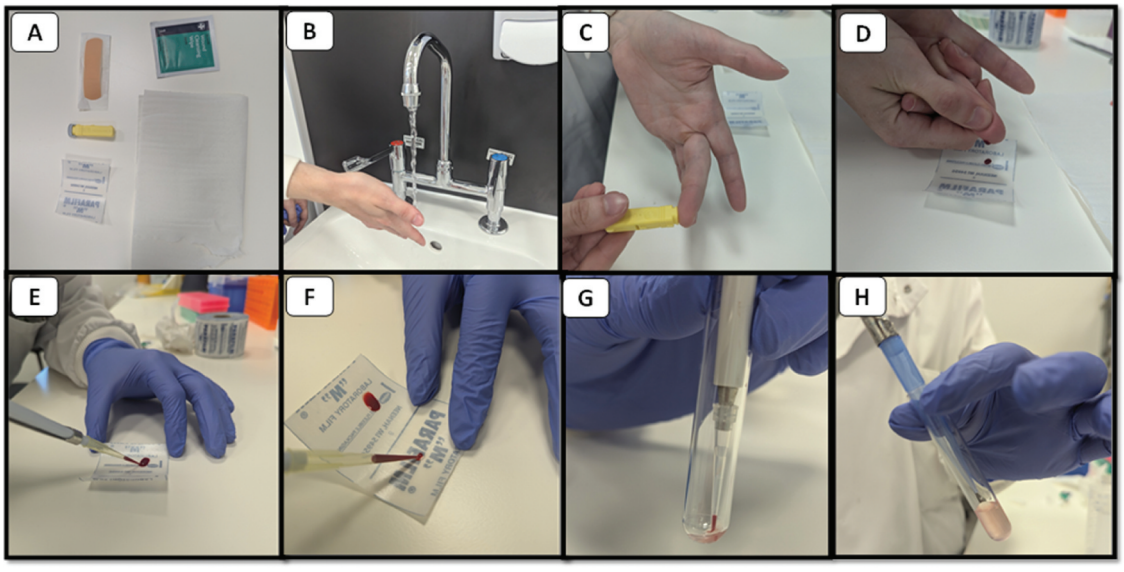

Figure 1. Procedure for finger-prick sampling. (A) Equipment required including alcohol wipe, Unistik 3 Normal lancet, 23 G, 1.8 mm, parafilm, tissue to remove the first drop of blood and a plaster. (B) Finger warmed using a hot tap. (C) Finger pierced using a lancet device and first drop wiped away. (D) Approximately 4 drops of blood were dropped directly onto the parafilm and the gentle pressure applied to stop any further bleeding. (E) Exactly 54 µL of blood is then added to 6 µL of sodium citrate. (F) Blood and citrate gently mixed by pipetting up and down twice. (G) 2 µL of the citrated blood was then added to the reaction mixture, mixed and incubated in the dark for 20 minutes. (H) 1 mL of 0.2% fixative was the added prior to analyzing on the flow cytometer.

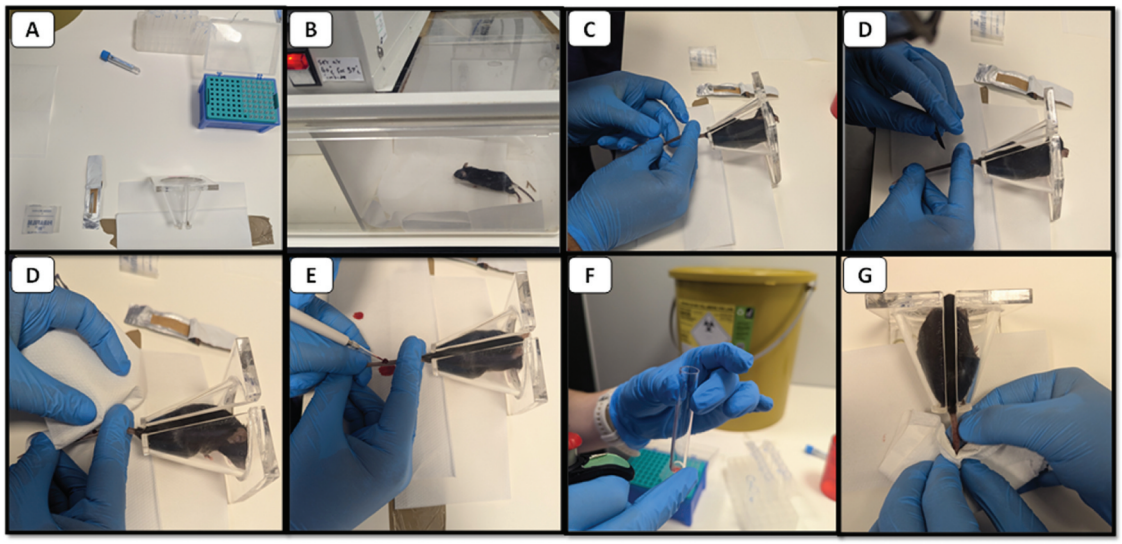

Figure 2. Procedure for tail bleed sampling. (A) Equipment required including surgical blade, parafilm with pre-dispensed sodium citrate, tissue to remove first drop of blood and a mouse restraint. (B) The mouse is warmed using a hot box set to 40°C. (C) Locating the lateral caudal vein. (D) Lateral caudal vein was incised with a surgical blasd and the first drop of blood was wiped away. (E) Blood was collected using a pipette to transfer the blood to the parafilm and exactly 36 µL of blood was immediately mixed with 4 µL of pre-dispensed sodium citrate to achieve a 1:9 (v:v) ratio to prevent clotting. (F) 2 µL of citrated blood was added directly into the reaction mixture and incubated in the dark for 20 minutes prior to adding 2 mL 0.2% formyl saline solution. (G) Gentle pressure was then applied to the incision site on the mouse until the bleeding ceased.

The ratio was achieved by using a pipette to transfer 54 µL of blood to a pre-dispensed 6 µL sodium citrate (3.2%) drop on the parafilm and gently mixed by pipetting up and down once. Bleeding was stopped by applying pressure to the injury site.

Blood sampling from mice

C57BL/6 mice were purchased from Envigo and maintained under standard laboratory conditions with a 12-hour light/dark cycle and *ad libitum* access to food and water. Mice were bled by two methods, non-terminal blood sampling from the tail vein, or terminal bleeding via cardiac puncture. Procedures were approved by the University of Reading Animal Welfare and Ethical Review Board and the UK Home Office (PPL number: PP5275821).

Non-terminal tail blood sampling was performed on the mice prior to their terminal cardiac procedure (Figure 2). The mouse was warmed to 40°C in an air-heated box for 10 minutes to ensure capillary dilation and increased blood flow. The mouse was then restrained in a VetTech front-open cone restrainer with direct access to the tail, and the caudal vein identified. An incision was made in the mid-upper area of the dorsal side of the tail using a carbon steel #10 surgical blade, and the first droplet of blood was discarded. The blood was pipetted from the incision site, being careful that the tip did not contact the skin of the mouse or the incision site to avoid unnecessary stress to the mouse and collected on parafilm prior to immediate mixing with sodium citrate. As with human blood, a 1:9 (v/v) ratio was achieved by using a pipette to transfer 36 µL of blood to a pre-dispensed 4 µL sodium citrate (3.2%) drop on the parafilm and gently mixed by pipetting up and down once. Bleeding was stopped by applying pressure to the incision site.

Terminal bleeding was performed by exposing mice to a gradual increase in CO_2 for two minutes, and death was confirmed by snipping the tip of the tail to ensure the cessation of bleeding and loss of circulation. Blood was collected with a 23 G, 5/8" hypodermic needle attached to a 1 mL syringe containing 1:9 (v/v) 3.2% sodium citrate. For cardiac puncture, the needle was inserted directly into the heart, aiming toward the left ventricle, and blood was withdrawn. For bleeding from the inferior vena cava (IVC), the vessel was exteriorized by transverse laparotomy and the needle inserted into the vessel. Blood was withdrawn until the vessel collapsed, pausing to allow it to refill and then repeated until refilling ceased. For both methods, cervical dislocation was then performed.[33,35]

Platelet activation by flow cytometry

Platelet activation was measured by flow cytometry using a BD Accuri C6. For humans, samples were dual stained with FITC labeled polyclonal rabbit anti-human fibrinogen antibody (Dako) and PE-Cy5 labeled polyclonal mouse anti-human P-selectin antibody (BD Pharminogen). For mice, samples were stained with FITC labeled polyclonal rabbit anti-human fibrinogen antibody (Dako). Human blood (2 µL) was added to a reaction mixture containing 38 µL HEPES Buffered Saline (HBS) at pH 7.4, 5 µL of agonist (ADP/CRP-XL /TRAP-6) at stated concentrations and 2 µL of each antibody. Similarly, 2 µL of mouse blood was stimulated by adding to 38 µL Tyrode's with glucose at pH 7.4, 5 µL of agonist (ADP/CRP-XL) at stated concentrations and 2 µL of antibody. The reaction mixtures were then incubated in the dark for 20 minutes at room temperature prior to being fixed using 1 mL (for humans) or 2 mL (for mice) of 0.2% formyl saline solution and analyzed in the flow cytometer. The platelet population was identified by FSC vs SSC, and 10 000 platelet events were collected.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 10.1.2 software. Linear regressions are presented as mean values ± standard deviation of the mean. Linear regressions were analyzed by two-way ANOVA with matched values both stacked and spread across a row with Sidak's multiple comparisons test with single pooled variance. The EC_{50} comparisons are expressed as donor-matched sampling showing individual points per donor. These pairs were analyzed via paired, parametric multiple t-tests. Normal distributions were determined by the Shapiro–Wilk test and significance was analyzed via one-way ANOVA.

Results

The impact on platelet function of both standard venepuncture and our novel capillary sampling method using blood from the finger-prick was determined by flow cytometry. The level of platelet activation was assessed by measuring fibrinogen binding and P-selectin exposure following stimulation with a range of concentrations of three key agonists: cross-linked collagen-related peptide (CRP-XL), adenosine diphosphate (ADP), and thrombin-related activatory peptide (TRAP-6).

Fibrinogen binding and P-selectin expression in resting platelets were equally low in both sampling methods (Figure 3(A–F)). All three agonists induced dose-dependent increases in fibrinogen binding, generating classical sigmoidal curves for both venepuncture and capillary blood-taking methods (Figure 3 (A–C)). ADP induced activation showed no significant differences between either blood draw method (Figure 3(A)). Following stimulation with higher concentrations CRP-XL there was no significant difference in fibrinogen binding (Figure 3(B)). There was, however, a small, statistically significant decrease in fibrinogen binding for finger-prick blood at low concentrations of CRP-XL (Figure 3B). Conversely, following TRAP-6 stimulation, the two methods showed similar results at low concentrations, but fibrinogen binding significantly decreased, albeit to a small extent, in capillary blood samples at the highest concentrations (Figure 3(C)). These classical responses were largely mirrored when measuring platelet activation by P-selectin; however, in this case, ADP demonstrated the largest difference between the existing venepuncture and our novel capillary sampling method (Figure 3(D–F)). It should be noted, however, that these experiments were performed in the absence of extracellular calcium, and so responses may be altered slightly with different anticoagulants or with calcium added back in. Despite these small differences, it is important to note that differences in the dose-response curves did not translate to significant differences in the overall capacity of platelet response (maximal minus minimal response) nor the sensitivity (EC_{50}) (Figure 3(G–J)) except for a reduction in platelet sensitivity to CRP-XL, measured by P-selectin exposure, from 0.026 µg/mL in venous blood to 0.179 µg/mL following finger prick sampling (Figure 3(H)). These data show that regardless of whether the blood was taken by venepuncture or finger-prick, the platelets remain resting and activatable with no significant effect on their overall response to agonists.

To confirm that the method of blood draw does not impact the relative platelet reactivity in blood donors, we analyzed the correlation between the two methods for donor capacity (Figure 4(A–C)) and for sensitivity (Figure 4(D–F)). In response to ADP, CRP-XL and TRAP-6, there was a significant, positive correlation of

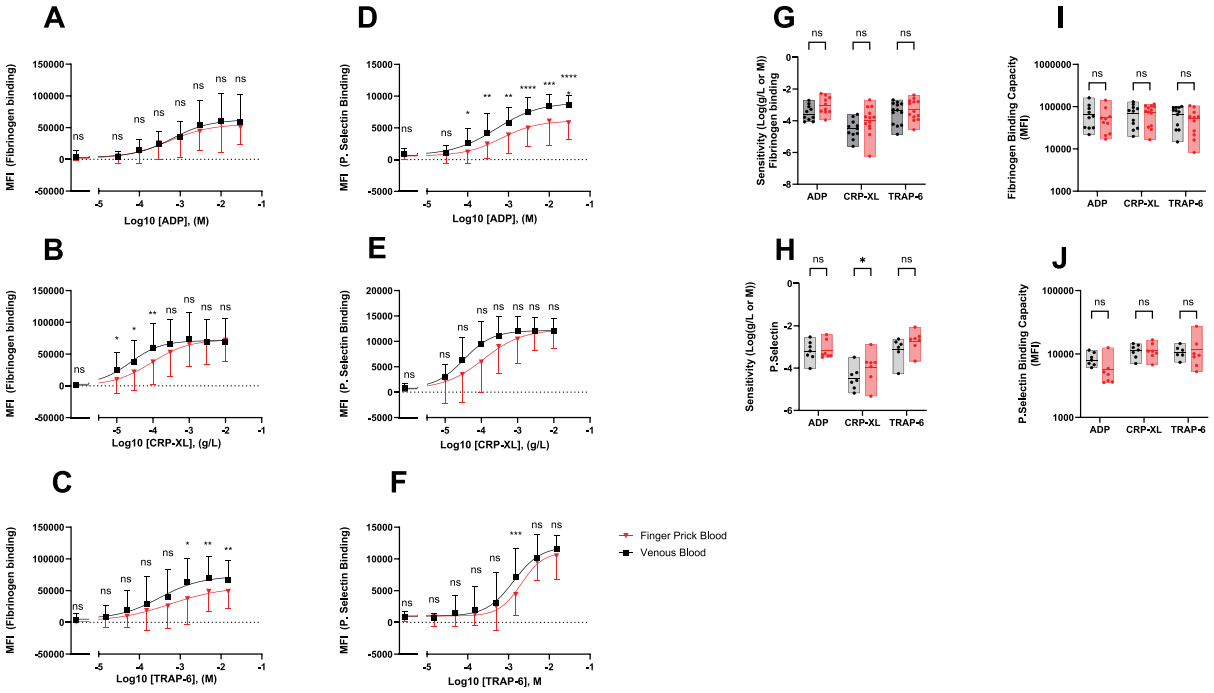

Figure 3. Blood sampling location has limited effects on platelet function analysis. A–F: Whole blood co-stained with FITC labeled anti-human fibrinogen and PE-Cy5 labeled P-selectin then stimulated by ADP, CRP-XL or TRAP-6 for 20 minutes before being fixed and analyzed on the flow cytometer and events recorded. Nonlinear regression produced from the data where Black shows venous blood activation and Red shows capillary blood activation. Error bars show standard deviation and changes in between FP and VB as determined by a one way ANOVAshown above as ns (non-significant), *($p = < 0.05$), **($p = < 0.01$), ***($p = < 0.001$) and ****($p = < 0.0001$). G,H: Changes in sensitivity to agonists, individual EC_{50} data points show, change in significance as determined by a multiple paired t-test highlighted above. I,J: Changes in platelet activation capacity to agonists, individual data points shown, changes to significance as determined by a multiple paired t-test, highlighted above.

fibrinogen-binding capacity between venous and finger-prick blood (Figure 4(A–C)). The same was true for platelet sensitivity in response to ADP and TRAP-6, but not CRP-XL (Figure 4(D–F)). This suggests that inter-individual variation in agonist response is maintained in both sampling methods, and that blood donors with a high or low response can be identified by both sampling methods.

When using venipuncture as the standard blood draw method, there is typically a delay between blood taking and platelet function analysis, for example, due to the time taken between the blood draw and its arrival in the laboratory. Because of the future potential for point-of-care use, where finger-prick sampling may not experience any delay to processing we tested platelet reactivity, with different resting times over a 30-minute period. We found no significant effect on resting time, with similar responses in blood processed within 5 minutes of finger-prick and blood which was rested for up to 30 minutes post-finger-prick (Figure 5). This suggests that platelets in a capillary sample retain reactivity and are comparable to standard venepuncture techniques.

Moreover, this non-significant variation in platelet function between the two blood sampling methods led us to investigate the variability in the assay technique to ensure that platelet function analysis using our novel technique was reproducible. We performed a coefficient of variance of the capillary assay whereby we performed five replicates of the assay using a single donor. This demonstrated low variation with each of the agonists, ranging from 2.07% (TRAP-6; sensitivity) to 10.59% (TRAP-6; capacity) (Table 1).

We extended this work to mice by comparing cardiac puncture (CP) and a similarly modified tail vein (TV) bleeding assay for platelet function testing. The level of platelet activation was assessed by measuring fibrinogen binding following stimulation with a range of concentrations of CRP-XL and ADP.

Normal dose-dependent response curves were observed for both sampling methods following stimulation by CRP-XL and ADP (Figure 6(A,D)). There was no significant difference in platelet responses to CRP-XL between the two blood draw methods (Figure 6(A)). Furthermore, as with human samples, platelet

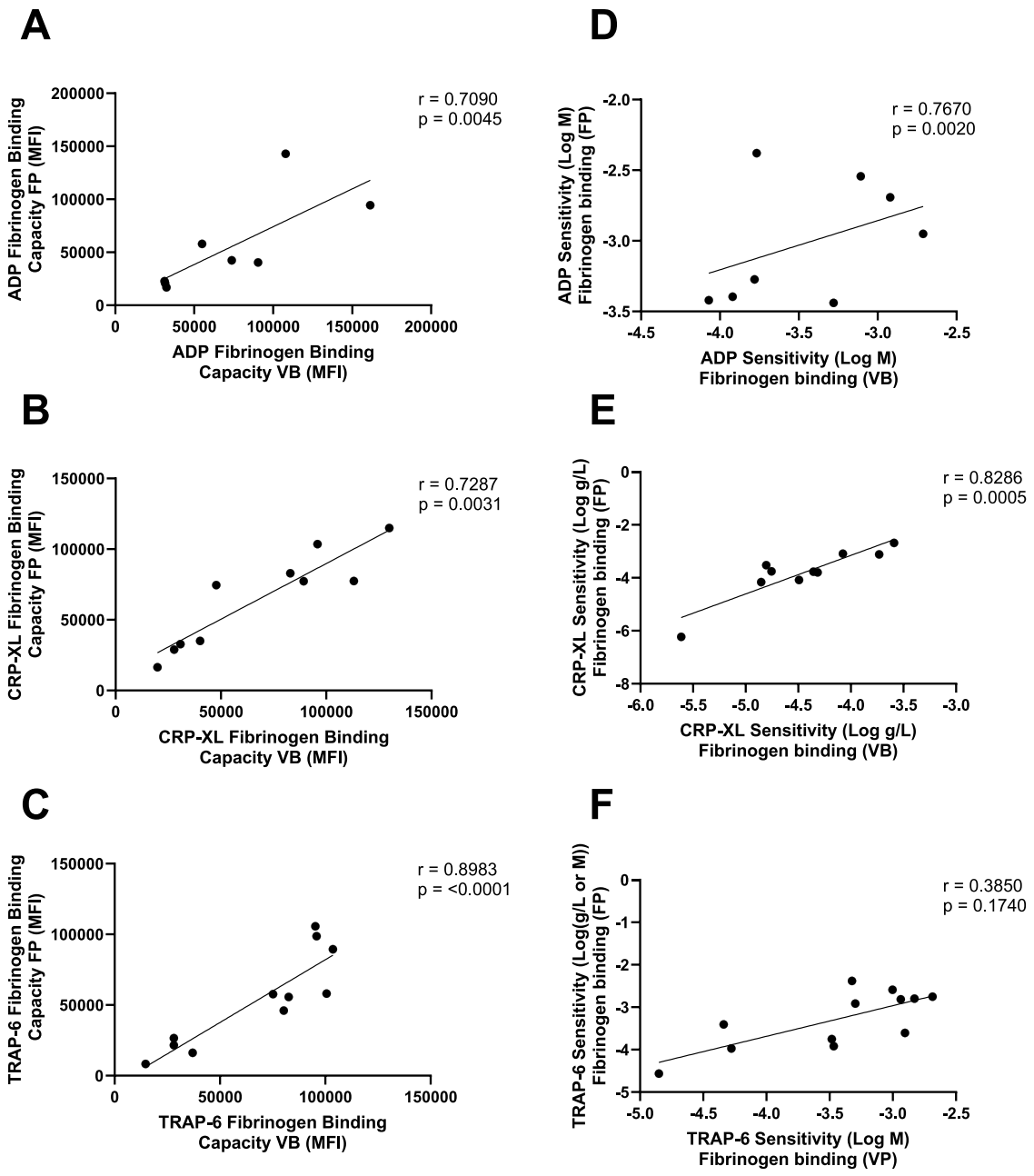

Figure 4. Platelet response measured following different blood sampling methods is highly correlated. A–F: the capacity of the platelets to bind fibrinogen in response to ADP, CRP-XL and TRAP-6. G–L: the sensitivity of the platelets to ADP, CRP-XL and TRAP-6 was determined by their anti-fibrinogen antibody binding sensitivity. Correlations were peerformed using a Spearmans non-parametric, two-tailed correlation in GraphPad prism.

response from the CP and TV blood draw methods for both ADP and CRP-XL were similar in sensitivity (EC_{50}) and capacity (Figure 6(H–I)). Again, these experiments were performed in the absence of extracellular calcium.

Fibrinogen binding in response to several ADP concentrations was lower when sampling by cardiac puncture. As resting levels of fibrinogen binding were the same in both methods (Figure 6(G)), and responses to CRP-XL were unchanged, we concluded that this was not due to hyper-responsive platelets in tail vein samples. Furthermore, the plateauing of the ADP dose response curve at a lower level following cardiac puncture indicates that platelets stimulated following cardiac puncture are refractory to further stimulation, which may be indicative of desensitization.

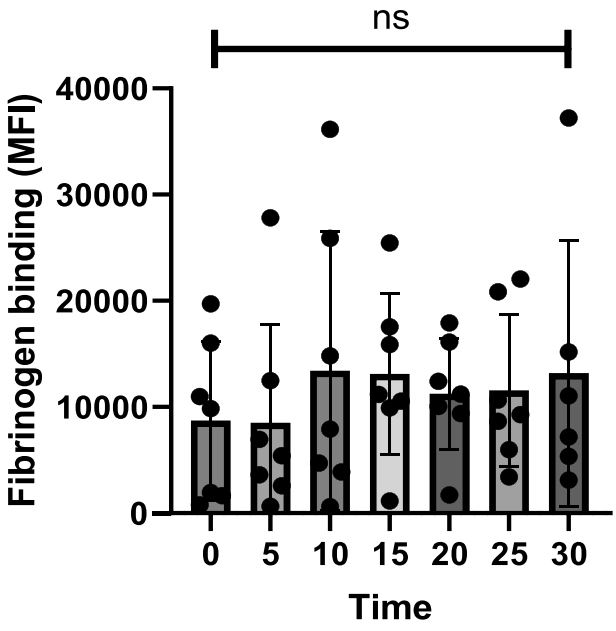

Figure 5. Time from capillary sampling to analysis does not impact platelet reactivity. A time series of 0 to 30 minutes to evaluate platelet activation. Normality was determined by the Shapiro-Wilk test, and significance was determined by one-way ANOVA with multiple comparisons. $p = <0.0001$.

Table 1. The coefficient of variance remains low for the micro-sampling method.

Antibody	Agonist	Sensitivity CV (%)	Capacity CV (%)
Fibrinogen Binding	ADP	5.51	5.66
P-Selectin exposure	ADP	3.05	5.22
Fibrinogen Binding	CRP-XL	7.21	5.50
P-Selectin exposure	CRP-XL	7.84	2.48
Fibrinogen Binding	TRAP-6	5.22	10.59
P-Selectin exposure	TRAP-6	2.07	3.98

Sensitivity CV: variance in the platelets sensitivity. Capacity CV: variance in the platelet capacity.

To confirm these results, we performed experiments with blood drawn from the inferior vena cava (IVC); another terminal bleeding method similar to cardiac puncture. The results demonstrate that IVC and tail vein bleeding yield highly comparable platelet responses to both ADP and CRP-XL (Figure 6(A–F)). In contrast, cardiac puncture samples showed a marked reduction in ADP responsiveness compared to IVC samples (Figure 6(F)) while maintaining similar CRP-XL responses (Figure 6). These findings support our conclusion that the modified tail vein method does not produce hyper-responsive platelets, and that the lower ADP response in cardiac puncture samples likely reflects reduced sensitivity specific to that collection method.

Discussion

Platelet function analysis has typically been performed using relatively large blood samples from either venepuncture in humans, or terminal bleeding in mice.[19,33] This has been driven by the requirement of most platelet assays for blood volumes greater than 1 mL. Therefore, almost all data, such as normal ranges of platelet reactivity, is based on blood drawn using these standardized methods. The use of flow cytometry, however, has removed the requirement for large volumes of blood due to its ability to measure individual platelets.[20,21] Blood withdrawal with less invasive methods, such as finger-prick sampling in humans and tail vein bleeding in mice, yields blood volumes too small for LTA, but an adequate volume for flow cytometry. In this study, we optimized these blood-taking methods and demonstrated that they yield platelets in a resting and activatable state, suitable for platelet function analysis.

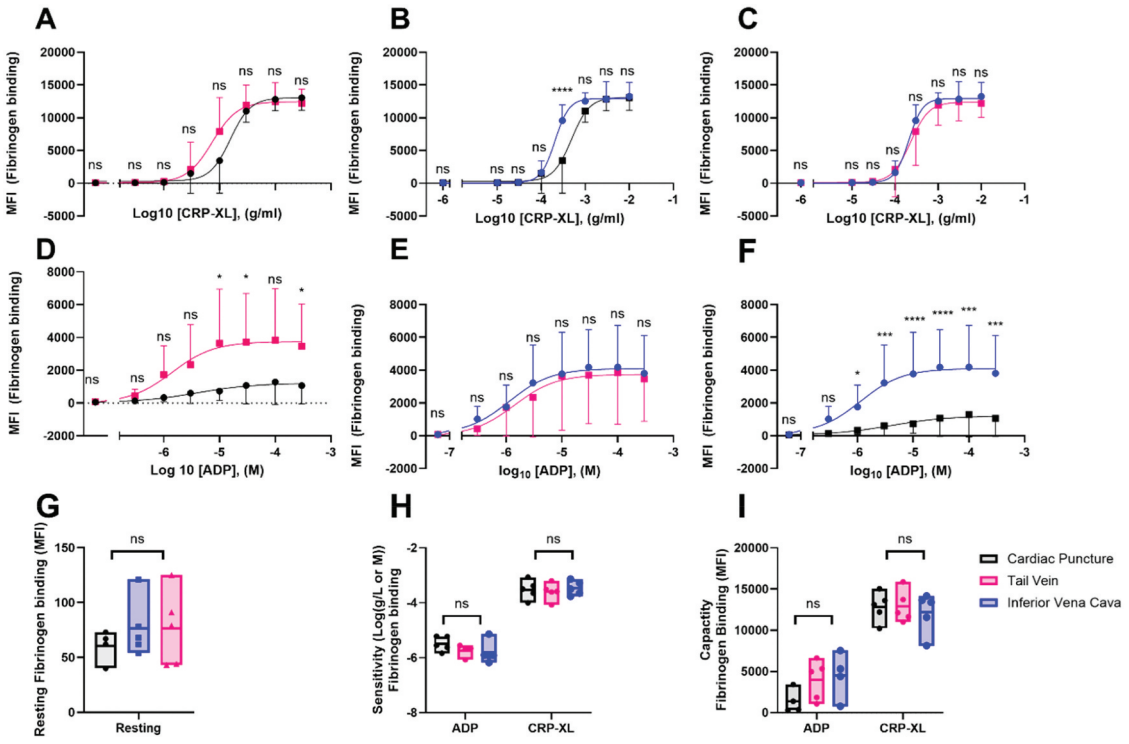

Figure 6. Blood sampling location has limited effects on platelet function analysis in mice. A–F: Whole blood was stimulated by ADP or CRP-XL for 20 minutes before being fixed and analyzed on the flow cytometer, events recorded with FITC labeled anti-fibrinogen antibody. Nonlinear regression produced from the data with cardiac puncture platelet activation is shown in Black, tail vein platelet activation is shown in Pink and inferior vena cava shown in Blue. Standard deviation shown as error bars and changes in significance between tail vein and cardiac puncture as determined by one-way ANOVA shown above as ns (non-significant), *($p = < 0.05$), **($p = < 0.01$), ***($p = < 0.001$) and ****($p = < 0.0001$).
G: Variation in non-stimulated platelet activation for all blood draw methods. H: Changes in platelet sensitivity to agonists, individual EC_{50} data points shown and changes in significance as determined by a multiple paired t-test highlighted. I: Changes in platelets capacity individual mfi capacity data points are shown, and the significance determined by a multiple paired t-test.

Sensitivity to agonist (EC_{50}) and activation capacity (maximal minus minimal response) are key metrics of the platelet response.[20,24] In humans, while we found minor statistical differences in the dose-response curves with some of the agonists tested, these differences did not significantly impact the overall capacity or sensitivity of the platelets, with one exception: sensitivity to CRP-XL measured by P-selectin exposure. Furthermore, the blood draw method did not impact the relative platelet reactivity in blood donors, suggesting that both sampling methods can identify blood donors with a high or low platelet response. In addition, finger-prick sampling showed low intra-sample variability and can be used either immediately post-collection or after a 30-minute period without impacting the results. The finger prick sampling method developed here, therefore allowed similar platelet function analysis compared to venepuncture, with good reproducibility and flexibility, suggesting that this method may be used in future point-of-care applications.

Capillary sampling, in humans, via finger prick bleeding, has the further advantage that it bypasses the requirement for trained phlebotomists. A further barrier, alleviated by finger-prick sampling, is the stigma of needles, which is removed by using a lancet-style device, as shown in a previous study where 49% of people avoided blood donation due to fear of needles.[46]

Similarly, when comparing cardiac puncture and a modified tail vein bleeding assay in mice, we found that the differences between the two methods were mostly non-significant, except for large differences in ADP capacity. In mice, platelets responded weakly to ADP following standard cardiac puncture sampling compared to our novel tail bleeding samples. ADP desensitization seems a likely explanation here as CRP-XL-mediated platelet activation is similar for both blood draw methods.[47–50] Moreover, when compared to terminal bleeding from the inferior vena cava, our modified tail vein sampling had similar platelet responses

to ADP. This further suggests that the issue is a reduced response to ADP in cardiac puncture samples rather than hyper-reactivity in tail vein samples (Figure 6(E,F)).

Our modified tail vein sampling enables both the reduction and refinement of mouse use in platelet research, allowing platelet function studies using potentially fewer animals through non-terminal, repeated blood sampling from individual mice.[34,36,37] This could, allow for longitudinal studies in a single mouse where multiple mice may have been required.[51] United Kingdom legislation states that repeat blood sampling must be less than 10% TBV taken at any sampling occasion and no more than 1% TBV for daily sampling (capped at 15% TBV for a given month).[34,52] The method presented here requires only two drops of blood (<50 µL), remains well below the weekly guideline of 10% of the total blood volume of adult mice (approximately 100 µL) permitted for non-terminal blood collection in the UK.[14,15] However, in experimental setups involving fewer tests or single-concentration analyses, this method could be used for more frequent sampling. The presence of two parallel caudal veins in the mouse tail enables collection from different veins on separate days, making it feasible to obtain samples twice within a week. Recovery from the small-volume collection is typically rapid, but re-sampling from the same vein is best avoided for at least a week. This approach can therefore support twice-weekly platelet function testing without exceeding monthly limits.[34,41,53] Outside of the UK it may be possible to take repeated samples of resting platelets using other bleeding methods.

A key difference with the modified tail vein sampling method presented here, is that it is less invasive compared to other non-terminal blood sampling procedures. Facial vein bleeding requires manual handling and has a high failure rate.[54–56] Other methods such as saphenous vein sampling are also technically difficult in comparison and can cause further stress for the animal. These techniques are also associated with longer recovery times and increased pain, distress, potential nerve and tissue damage, a higher risk of hemorrhage, and reduced post-procedural survival rates, which are not observed in tail vein bleeding.[53,57,58]

Other commonly used bleeding methods, such as retro-orbital bleeding, are considered less invasive and cause less tissue damage and mortality than facial vein puncture. However, the use of retro-orbital bleeding is less widespread and not permitted as a non-terminal method in countries such as the UK. Our modified tail vein bleeding technique therefore offers a suitable, less invasive, and refined alternative for repeated blood collection.[58]

Our modified micro-sampling method is relatively straightforward and easy to train and perform compared to these terminal and non-terminal alternatives.[36] Furthermore, over-citration of the blood is unlikely to occur as the blood is mixed with the anticoagulant by pipette following blood collection, so the exact citrate and blood volumes are controlled. In contrast, cardiac puncture relies on pre-loading a syringe with anticoagulant, yet the volume of blood withdrawn may be variable depending on the age, size and stress level of the animal. Additionally, handler proficiency may also affect the blood volume withdrawn and therefore the blood-citrate ratio is not guaranteed, which may impact platelet function[42,59]

In conclusion, we have established that micro-sampling blood for platelet function testing via finger prick in humans or tail vein bleeds in mice is an effective tool for producing accurate dose-response curves to measure platelet function. These methods are viable alternatives to the standard methods used for platelet function analysis, maintaining assay accuracy and sensitivity while improving accessibility and aligning with the ethical principles of reduction, refinement, and replacement for animal usage.

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T.L.H., A.S. and H.O.A. designed and performed the experiments, analysed the data and wrote the manuscript. C.I. J. and C.E.H. designed the study, analysed the data and wrote the manuscript.

Disclosure statement

C.I.J. CTO and Co-Founder of HaemAnalytica. All other authors report no competing interests.

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Ethics approval

Peripheral and venous blood collected from healthy adult donors with their informed consent. The study was approved by the University of Reading Research Ethics Committee (UREC).

Animal handling and working procedures were approved by University of Reading Animal Welfare, Ethical Review Board and the UK Secretary of State for the Home Department. PPL number: PP5275821 under the UK Animal Act 1986.

References

1. Holinstat M. Normal platelet function. Cancer Metastasis Rev. 2017. 36(2):195–198. doi: 10.1007/s10555-017-9677-x.
2. Sang Y, Roest M, de Laat B, de Groot PG, Huskens D. Interplay between platelets and coagulation. Blood Rev. 2021; 46:100733. doi: 10.1016/j.blre.2020.100733.
3. Dhawan H, Arora S, Sachdev S, Sharma R, Marwaha N. Allergic contact dermatitis to antiseptic medicated dressing applied on phlebotomy site. Asian J Transfus Sci. 2010;4(2):133. doi: 10.4103/0973-6247.67020.
4. Morris W, Heong Tay M. Strategies for preventing peripheral intravenous cannula infection. Br J Nurs. 2008; 17 (Sup8):S14–S21. doi:10.12968/bjon.2008.17.Sup8.31470.
5. Stefanini M. Iatrogenic anemia (can it be prevented?). J Thromb Haemostasis. 2014; 12(10):1591. doi: 10.1111/jth.12642.
6. Ohnishi H, Watanabe M, Watanabe T. Butterfly needles reduce the incidence of nerve injury during phlebotomy. Arch Pathol Lab Med. 2012; 136(4):352–352. doi: 10.5858/arpa.2011-0431-LE.
7. Neelam D. WHO guidelines on drawing blood: best practices in phlebotomy. Safe Injection Global Network. World Health Organization; 2010. 109. https://www.who.int/publications/i/item/9789241599221.
8. Topping J, Reardon M, Coleman J, Hunter B, Shojima-Perera H, Thyer L, Simpson P. A comparison of venous versus capillary blood samples when measuring blood glucose using a point-of-care, capillary-based glucometer. Prehosp Disaster Med. 2019;34(5):506–509.
9. Otten AT, van der Meulen HH, Steenhuis M, Loeff FC, Touw DJ, Kosterink JGW, Frijlink HW, Rispens T, Dijkstra G, Visschedijket MC, et al. Clinical validation of a capillary blood home-based self-sampling technique for monitoring of infliximab, vedolizumab, and C-reactive protein concentrations in patients with inflammatory bowel disease. Inflamm Bowel Dis. 2024;30(3):325-335. doi: 10.1093/ibd/izad103.
10. Butler CC, Gillespie D, White P, Bates J, Lowe R, Thomas-Jones E, Wootton M, Hood K, Phillips R, Melbye H, et al. C-reactive protein testing to guide antibiotic prescribing for COPD exacerbations. NEJM Evid. 2019;381 (2):111–120.
11. Fliervoet LAL, Tiel Groenestege WM, Huisman A. Comparison of capillary and venous blood sampling for routine coagulation assays. Clin Biochem. 2022;104:30–35. doi: 10.1016/j.clinbiochem.2022.01.010.
12. Bates-Fraser LC, Moertl KM, Stopforth CK, Bartlett DB, Ondrak KS, Jensen BC, Hanson ED. A practical approach for complete blood count analysis following acute exercise: capillary vs. venous blood sampling. Adv Exercise Health Sci. 2024;1(1):43–50. doi: 10.1016/j.aehs.2024.01.002.
13. Tai DY, Chan KW, Chee YC, Mak KH. Comparison of platelet counts in simultaneous venous and capillary blood samples using an automated platelet analyser. Singapore Med J. 1995;36(3):263–266.
14. Feusner JH, Behrens JA, Detter JC, Cullen TC. Platelet counts in capillary blood. Am J Clin Pathol. 1979;72 (3):410–414. doi: 10.1093/ajcp/72.3.410.
15. Kang M, Bresette CA, Ku DN. Advancing microfluidic point-of-care platelet function tests: opportunities and challenges from bench to market. Front Bioeng Biotechnol. 2024;12. doi: 10.3389/fbioe.2024.1507972.
16. Lordkipanidzé M, Pharand C, Schampaert E, Palisaitis DA, Diodati JG. Evaluation of the platelet count drop method for assessment of platelet function in comparison with "gold standard" light transmission aggregometry. Thromb Res. 2009;124(4):418–422. doi: 10.1016/j.thromres.2009.02.002.
17. Harrison P. Platelet function analysis. Blood Rev. 2005;19(2):111–123. doi: 10.1016/j.blre.2004.05.002.
18. Würtz M, Hvas AM, Christensen KH, Rubak P, Kristensen SD, Grove EL. Rapid evaluation of platelet function using the Multiplate® analyzer. Platelets. 2014;25(8):628–633. doi: 10.3109/09537104.2013.849804.
19. Le Blanc J, Mullier F, Vayne C, Lordkipanidzé M. Advances in platelet function testing—light transmission aggregometry and beyond. J Clin Med. 2020;9(8):2636. doi: 10.3390/jcm9082636.
20. McKinnon KM. Flow cytometry: an overview. Curr Protoc Immunol. 2018;120(1). doi: 10.1002/cpim.40.
21. van Velzen JF, Laros-van Gorkom BAP, Pop GAM, van Heerde WL. Multicolor flow cytometry for evaluation of platelet surface antigens and activation markers. Thromb Res. 2012;130(1):92–98. doi: 10.1016/j.thromres.2012.02.041.
22. Jones CI, Tucker KL, Sasikumar P, Sage T, Kaiser WJ, Moore C, Emerson M, Gibbins JM. Integrin-linked kinase regulates the rate of platelet activation and is essential for the formation of stable thrombi. J Thromb Haemostasis. 2014;12(8):1342–1352. doi: 10.1111/jth.12620.

23. Mitchell JL, Dunster JL, Kriek N, Unsworth AJ, Sage T, Mohammed YMM, De Simone I, Taylor KA, Bye AP, Ólafsson G, et al. The rate of platelet activation determines thrombus size and structure at arterial shear. J Thromb Haemostasis. 2023;21(8):2248–2259. doi: 10.1016/j.jtha.2023.03.044.
24. Dunster JL, Bye AP, Kriek N, Sage T, Mitchell JL, Kempster C, Batista J, McKinney H, Thomas P, Jones CI, et al. Multiparameter phenotyping of platelet reactivity for stratification of human cohorts. Blood Adv. 2021;5(20):4017–4030.
25. Jourdi G, Ramström S, Sharma R, Bakchoul T, Lordkipanidzé M. Consensus report on flow cytometry for platelet function testing in thrombocytopenic patients: communication from the SSC of the ISTH. J Thromb Haemostasis. 2023;21(10):2941–2952. doi: 10.1016/j.jtha.2023.07.006.
26. Eitzman DT, Westrick RJ, Nabel EG, Ginsburg D. Plasminogen activator inhibitor-1 and vitronectin promote vascular thrombosis in mice. Blood. 2000;95(2):577–580. doi: 10.1182/blood.V95.2.577.
27. Gordy D, Swayne T, Berry GJ, Thomas TA, Hudson KE, Stone EF. Characterization of a novel mouse platelet transfusion model. Vox Sang. 2024;119(7):702–711. doi: 10.1111/vox.13642.
28. Stefanini L, Lee RH, Paul DS, O'Shaughnessy EC, Ghalloussi D, Jones CI, Boulaftali Y, Poe KO, Piatt R, Kechele DO, et al. Functional redundancy between RAP1 isoforms in murine platelet production and function. Blood. 2018;132(18):1951–1962. doi: 10.1182/blood-2018-03-838714.
29. Perlman RL. Mouse models of human disease: an evolutionary perspective. Evol Med Public Health. 2016;eow014. doi: 10.1093/emph/eow014.
30. Doering C, Parker ET, Healey JF, Craddock HN, Barrow RT, Lollar P. Expression and characterization of recombinant murine factor VIII. Thromb Haemost. 2002;88(3):450–458. doi: 10.1055/s-0037-1613237.
31. Jirouskova M, Shet AS, Johnson GJ. A guide to murine platelet structure, function, assays, and genetic alterations. J Thromb Haemostasis. 2007;5(4):661–669. doi: 10.1111/j.1538-7836.2007.02407.x.
32. Li X, Wang SW, Feng Q, Hou Y, Lu N, Ma CH, Gao CJ, Hou M, Peng J. Novel murine model of immune thrombocytopaenia through immunized CD41 knockout mice. Thromb Haemost. 2019;119(3):377–383.
33. Hughes CE. How to perform aggregometry and lumi-aggregometry in mouse platelets. Platelets. 2018;29(7):638–643. doi: 10.1080/09537104.2018.1478074.
34. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. J Pharmacol Pharmacother. 2010;1(2):87–93.
35. Hoggatt J, Hoggatt AF, Tate TA, Fortman J, Pelus LM. Bleeding the laboratory mouse: not all methods are equal. Exp Hematol. 2016;44(2):132–137.e1.
36. Dürschlag M, Würbel H, Stauffacher M, VON Holst D. Repeated blood collection in the laboratory mouse by tail incision—modification of an old technique. Physiol Behav. 1996;60(6):1565–1568. doi: 10.1016/S0031-9384(96)00307-1.
37. Sadler AM, Bailey SJ. Validation of a refined technique for taking repeated blood samples from juvenile and adult mice. Lab Anim. 2013;47(4):316–319. doi: 10.1177/0023677213494366.
38. Hem A, Smith AJ, Solberg P. Saphenous vein puncture for blood sampling of the mouse, rat, hamster, gerbil, guinea pig, ferret and mink. Lab Anim. 1998;32(4):364–368. doi: 10.1258/002367798780599866.
39. Bardelmeijer HA, Buckle T, Ouwehand M, Beijnen JH, Schellens JHM, van Tellingen O. Cannulation of the jugular vein in mice: a method for serial withdrawal of blood samples. Lab Anim. 2003;37(3):181–187. doi: 10.1258/002367703766453010.
40. Heimann M, Roth DR, Ledieu D, Pfister R, Classen W. Sublingual and submandibular blood collection in mice: a comparison of effects on body weight, food consumption and tissue damage. Lab Anim. 2010;44(4):352–358. doi: 10.1258/la.2010.010011.
41. Meyer N, Kröger M, Thümmler J, Tietze L, Palme R, Touma C. Impact of three commonly used blood sampling techniques on the welfare of laboratory mice: taking the animal's perspective. PLOS ONE. 2020;15(9):e0238895. doi: 10.1371/journal.pone.0238895.
42. Dwyer SD, Meyers KM. Anesthetics and anticoagulants used in the preparation of rat platelet-rich-plasma alter rat platelet aggregation. Thromb Res. 1986;42(2):139–151. doi: 10.1016/0049-3848(86)90290-2.
43. Sashindranath M, Sturgeon SA, French S, Craenmehr DD, Selan C, Freddi S, Johnson C, Cody SH, Nesbitt WS, Hamilton JR, et al. The mode of anesthesia influences outcome in mouse models of arterial thrombosis. Res Pract Thromb Haemost. 2019;3(2):197–206.
44. Tekkeşin N, Esen ÖB, Kilinç C, Eviyaoğlu O. Discard first tube for coagulation testing. Blood Coagul Fibrinolysis. 2012;23(4):299–303. doi: 10.1097/MBC.0b013e328351ebbf.
45. Meites S. Skin-puncture and blood-collecting technique for infants: update and problems. Clin Chem. 1988;34(9):1890–1894. doi: 10.1093/clinchem/34.9.1885.
46. Alsbrooks K, Hoerauf K. Prevalence, causes, impacts, and management of needle phobia: an international survey of a general adult population. PLOS ONE. 2022;17(11):e0276814. doi: 10.1371/journal.pone.0276814.
47. Brake MA, Ivanciu L, Maroney SA, Martinez ND, Mast AE, Westrick RJ. Assessing blood clotting and coagulation factors in mice. Curr Protoc Mouse Biol. 2019;9(2):e61. doi: 10.1002/cpmo.61.
48. Elaïb Z, Adam F, Berrou E, Bordet JC, Prévost N, Bobe R, Bryckaert M, Rosa J–P. Full activation of mouse platelets requires ADP secretion regulated by SERCA3 ATPase–dependent calcium stores. Blood. 2016;128(8):1129–1138. doi: 10.1182/blood-2015-10-678383.

49. Foster CJ, Prosser DM, Agans JM, Zhai Y, Smith MD, Lachowicz JE, Zhang FL, Gustafson E, Monsma FJ, Wiekowski MT, et al. Molecular identification and characterization of the platelet ADP receptor targeted by thienopyridine antithrombotic drugs. J Clin Invest. 2001;107(12):1591–1598.
50. Alshehri OM, Hughes CE, Montague S, Watson SK, Frampton J, Bender M, Watson SP. Fibrin activates GPVI in human and mouse platelets. Blood. 2015;126(13):1601–1608. doi: 10.1182/blood-2015-04-641654.
51. Hollander CF, Solleveld HA, Zurcher C, Nooteboom AL, Van Zwieten MJ. Biological and clinical consequences of longitudinal studies in rodents: their possibilities and limitations. An overview. Mech Ageing Dev. 1984;28 (2–3):249–260. doi: 10.1016/0047-6374(84)90025-3.
52. Teilmann AC, Kalliokoski O, Sørensen DB, Hau J, Abelson KSP. Manual versus automated blood sampling: impact of repeated blood sampling on stress parameters and behavior in male NMRI mice. Lab Anim. 2014;48 (4):278–291. doi: 10.1177/0023677214541438.
53. Li Z, LoBue A, Heuser SK, Li J, Engelhardt E, Papapetropoulos A, Patel HH, Lilley E, Ferdinandy P, Schulz R, et al. Best practices for blood collection and anaesthesia in mice: selection, application and reporting. Br J Pharmacol. 2025;182(11):2337–2353.
54. Kim S, Foong D, Cooper MS, Seibel MJ, Zhou H. Comparison of blood sampling methods for plasma corticosterone measurements in mice associated with minimal stress-related artefacts. Steroids. 2018;135:69–72. doi: 10.1016/j.steroids.2018.03.004.
55. Matsuhisa F, Kitamura N, Satoh E. Effects of acute and chronic psychological stress on platelet aggregation in mice. Stress. 2014;17(2):186–192. doi: 10.3109/10253890.2014.888548.
56. Sandrini L, Ieraci A, Amadio P, Popoli M, Tremoli E, Barbieri SS. Apocynin prevents abnormal megakaryopoiesis and platelet activation induced by chronic stress. Oxid Med Cell Longev. 2017;2017(1). doi: 10.1155/2017/9258937.
57. Moore ES, Cleland TA, Williams WO, Peterson CM, Singh B, Southard TL, Pasch B, Labitt RN, Daugherity EK. Comparing phlebotomy by tail tip amputation, facial vein puncture, and tail vein incision in C57BL/6 mice by using physiologic and behavioral metrics of pain and distress. J Am Assoc Lab Anim Sci. 2017;56(3):307–317.
58. Regan RD, Fenyk-Melody JE, Tran SM, Chen G, Stocking KL. Comparison of submental blood collection with the retroorbital and submandibular methods in mice (Mus musculus). J Am Assoc Lab Anim Sci. 2016;55 (5):570–576.
59. Krisdiyanto, Bin Raja Ghazilla RA, Azuddin M, Bin Ahmad Hairuddin MKF, Risdiana N. An analysis of the effect of syringe barrel volume on performance and user perception. Medicine. 2023;102(23):e33983. doi: 10.1097/MD.0000000000033983.